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INVESTIGATION OF POLLUTANT REMOVAL PROCESSES WITHIN A COMBINED WETLAND/AERATED POND SYSTEM FOR THE TREATMENT OF AIRPORT RUNOFF

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AIMS AND OBJECTIVES OF STUDY

The Heathrow treatment facility (HTF) represents a novel research facility for the study of the different treatment processes for the control of glycol and other airport runoff pollutants from the commencement of operation in 2002 The research findings will contribute significantly towards achieving the optimum operating conditions which are consistent with providing the maximum treatment efficiency In addition the results will inform the design of future systems both in terms of the individual components and their combination to produce an overall scheme This research will progress current scientific understanding of the processes involved in this innovative overall procedure for the treatment of airport runoff The main objectives of this study are summarised below

- To investigate the removal potential of a combined aeration pond/reedbed treatment system with respect to hydrocarbons in particular glycol, in airport runoff
- To assess the impact of the possible controlling/interfering factors (such as dissolved oxygen levels nutrient levels and Fe concentrations and species) on the biodegradation of glycols
- To fully understand the kinetics of glycol biodegradation within both aqueous and substrate-based systems and to assess the impact of seasonal factors on decay rates
- To isolate the microbial consortia which most readily support glycol biodegradation and to determine their efficiencies in controlled laboratory tests

OPTIMISATION OF THE TREATMENT SYSTEM

The Reedbeds

The design of the floating reedbeds (FLRB) and sub-surface reedbeds (SSF) systems and the estimation of their performance efficiency has been based on the results obtained from the pilot scale beds which were constructed adjacent to the Eastern Balancing Reservoir (EBR) and studied by Middlesex University between 1995 and 1998 The experiments conducted on the pilot scale beds involved exposing both systems to water taken directly from the EBR and to artificial dosing with mixtures of three glycols (ethylene glycol diethylene glycol and propylene glycol) The data obtained from these experiments were scaled-up to establish the required design sizes and flow rates in the reedbeds for the treatment system It is proposed to fully analyse the field data from the relevant BiOX instruments (automated devices placed adjacent to each unit of the treatment system for the determination of the BOD levels) to assess the validity of the scaling–up process and to compare the efficiencies of the different reedbed systems under different prevailing conditions (e g temperature dissolved oxygen level conductivity, nutrient composition) The results will facilitate the refinement of the

operational procedures proposed at the design stage and will also contribute to the future design of constructed wetlands for the treatment of airport runoff

Aerated Ponds and Reservoirs

Three water bodies, with associated aeration are incorporated into the design of the HTF EBR Mayfield Farm Reservoir (MFR) and Mayfield Farm Balancing Pond (MFBP) The predicted treatment to be provided by each of these components has been based on the theoretical understanding of the microbiological breakdown of biodegradable material at different water temperatures The kinetics employed were based on a predicted biodegradation rate for Kilfrost (propylene glycol) and therefore did not take account of the presence in the real situation of additional glycols (ethylene glycol and diethylene glycol) with different decay rates and also the presence of other pollutants (e g oils) which might interfere with the biodegradation process Additionally the impact of seasonal variations in parameters such as temperature and nutrient levels will be assessed A full analysis of BIOX field data will enable the design criteria to be authenticated (or otherwise) and hence allow the optimisation of the treatment system in terms of factors such as storage times and aeration rate requirements Refined aeration predictions will benefit future designs of treatment systems using aeration and storage for the biodegradation of organic pollutants

INVESTIGATION OF FACTORS INFLUENCING THE BIODEGRATION RATE

The efficient operation of the HTF is critically dependent on the preservation of effective biodegradation activity within the aeration ponds and the reedbeds Temperature is an acknowledged controlling factor for this process but others include oxygen supply the presence of effective microbial populations and efficient mixing particularly in large water bodies An unexpected occurrence in the early operational stages of the HTF has been the development of high ferric compound concentrations and a perceived reduction in biodegradation efficiency The combined effects of these different factors will be investigated through a series of experiments designed to simulate the characteristics of the real system These experiments will explore the relationship between the Fe^{2+}/ Fe^{3+} equilibrium and the depletion of dissolved oxygen in the presence of different glycol (i e BOD) concentrations and for different starting concentrations of Fe^{2+} The effects of re-aeration after the establishment of anoxic conditions will also be investigated Given the potential flocculating properties of Fe^{3+} compounds it will be important to determine the impact of this at different Fe concentrations on the biodegradation process in the presence of varying dissolved oxygen saturation levels and the existence or otherwise of fully mixed conditions

DEVELOPMENT OF THE TREATMENT SYSTEM

Field based studies

An important pollutant removal mechanism in both the reedbeds and the aeration ponds will be microbiologically induced biodegradation It is proposed to follow the development and

adaptation of the microbial populations as each component of the treatment system matures following repeated exposures to glycol-contaminated runoff Representative samples will be collected and subsequently analysed for fungi bacteria and actinomycetes species Sampling of the SSF will concentrate on the interstitial waters and the substrate adjacent to the plant roots Within the floating reedbed sampling will take place around the root systems and in both cases microbiological techniques previously developed during the pilot plant experiments will be adapted appropriately Duplicate sampling will be practiced from all locations including waters collected from the three aeration ponds (EBR MFR and MFBP), and will be carried out routinely for laboratory analysis The collection of samples for microbiological analysis will be accompanied by *in-situ* determination of temperature and dissolved oxygen and the taking of water samples for BOD and nutrient analysis The scientific knowledge obtained from this part of the study will contribute to the optimisation of the microbial decay processes which will ensure that the system is operating at maximum efficiency

Laboratory based studies

The field based studies, described above will be supported by laboratory studies which will enable the kinetics of the glycol degradation processes in each of the treatment components to be fully investigated In the case of the aeration ponds this will be achieved by incubating collected water samples under different controlled temperature conditions in the laboratory By collecting samples representative of different glycol loading conditions it will be possible to fully establish rate constants for the degradation process These findings will inform the operating parameters which are currently in place and also contribute to the design of future systems In the case of the sub-surface bed it will be necessary to remove a portion of the substrate adjacent to the root system together with the plant for relocation to the laboratory Careful application of glycol dosing conditions will enable the effect of plants on the overall biodegradation process to be determined For the sub-surface planted situations, experiments will be needed to establish the appropriate methodology required to accurately simulate field conditions

The biodegradation experiments will be refined by confirming the presence of the consortia of microorganisms in the HTF which have the particular ability to degrade glycols This will be achieved by isolating microorganisms from the different treatment components and testing them for tolerance to glycols and ability to facilitate effective biodegradation This will lead to a better understanding of the microbial populations which are particularly active in glycol degradation and may lead to the identification of strains which could be cultured and returned to the treatment system to improve its efficiency

CONFERENCES AND MEETINGS ATTENDED

- Institute of Social and Health Research Postgraduate Summer Conference (London 18 June 2004)-*Relevance of Research in the Real world*- Oral Presentation
- Fourth World Wide Workshop for Young Environmental Scientists (Paris France 10-13 May 2005)-*Urban waters Resource or Risk*- Oral Presentation
- 1st National Sustainable Urban Drainage Systems Network (SUDSnet) Student Conference (Coventry, 22 June 2005)- Oral Presentation
- Institute of Social and Health Research Postgraduate Summer Conference (London 24 June 2005)-*Research in Practice*- Oral Presentation
- EPSRC Sustainable Drainage Systems (SUDS) Masterclass (Oxford 9 February 2006)-Participant
- A Sustainable Energy Policy for the UK (London 28 February 2006)-Participant
- 10th International Conference on Wetland Systems for Water Pollution Control (Lisbon Portugal 23-29 Sept 2006)-Oral Presentation

PEER-REVIEWED PUBLICATIONS

- Adeola S O (2005) *Pollutant removal processes within a combined wetland/aerated pond system for the treatment of airport runoff* Journal of Health and Environmental issues JHSEI
- Adeola S O Revitt M Shutes B Garelick H and Jones C (2006) *A Combined Constructed Wetland and Aerated ponds system for the treatment of airport runoff Operational issue* Proceedings of the 10th International Conference on Wetland Systems for Water Pollution Control
- Adeola, S O Revitt M Shutes B , Garelick H and Jones C (2007) *Phytoremediation of Deicing Agents by a Constructed Wetland and Aerated Pond System at London Heathrow Airport* In Abstract Book Workshop of WG2 and WG4 and Management Committee Meeting-Fate of Pollutants in Plant/Rhizosphere System Fundamental Aspects and their Significance for Field Applications-Prospect and Research needs

ABSTRACT

An integrated system of aerated ponds together with rafted and horizontal flow sub-surface reedbeds has been installed to treat the glycol-contaminated runoff from two of Heathrow Airport s susceptible catchment areas the Southern Catchment and the Eastern Catchment The original plan was to transfer pre-treated water from the latter through the existing fire main which runs around the airport perimeter to the Mayfield Farm Treatment Facility (MFTF) which receives runoff from the Southern Catchment However this part of the overall Heathrow treatment scheme for surface runoff has not yet been put into operation Results from monitoring of the treatment system has shown significant reductions in Biological Oxygen Demand (BOD) throughout the system with levels decreasing by 76 6±10 5% across the constructed wetland cells at the MFTF following high airport deicing fluids applications However continued exposure to BOD concentrations exceeding the design target of 110 mg/l has resulted in anaerobic conditions being established in the wetland

Background nitrate and phosphate levels observed in two aerated ponds at the MFTF which are used for storage and initial treatment of airport runoff were low Initial results from laboratory experiments conducted to assess the ideal nutrient levels required to support glycol biodegradation in the aerated ponds showed efficient BOD removal (within 5 days) after repeated glycol additions There were also increases in bacteria populations in aerated pond water samples from average background levels of 10^7 to 10^{10} Colony forming units (CFU) per litre Based on the interpretation of the laboratory experiments a nutrient dosing protocol commenced on 12 November 2004 at the MFTF to ensure that nutrient levels in the aerated ponds were appropriate to treat the contaminated airport runoff received during the winter months Although the BOD levels recorded in the aerated ponds were still high after the commencement of the nutrient dosing regime in November 2004 the BOD removal efficiency of the aerated reservoir following the influx of high winter BOD load increased to 47 5% (February 2005) from 25 5% recorded in March 2004

In microbiological analyses conducted on the water samples from the MFTF only 18 strains of bacteria, 3 fungal strains and 2 actinomycetes strains were culturable in the laboratory A combination of API biochemical tests and PCR (Polymerase chain reaction) analyses showed that at least six of these bacteria were *Pseudomonas* species which have been widely reported to have high potential for biodegradation of organic pollutants However biodegradation experiments conducted using the consortium of the bacteria fungi and actinomycetes isolates in sterile distilled water dosed with glycol showed that they lacked the ability to efficiently remove the BOD despite the continuously high BOD reductions recorded in some of the natural systems they were isolated from

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CHAPTER 1 INTRODUCTION

The increasing risks associated with urban runoff such as that generated from airport activities during wet weather conditions have given reasons for much concern in recent years due to the possible adverse impacts on local water courses The implementation of the EC Water Framework Directive (WFD) on 22 December 2000 (establishing a new integrated approach to the protection, improvement and sustainable use of Europe's rivers, lakes estuaries coastal waters and ground water) mean that appropriate conditions have to be placed on surface water discharges in order to meet the increasing stringent water quality standards The need for careful management activities to minimise water quality impacts has made the establishment of adequate surface water management an inevitable requirement for many dischargers of potentially polluted water With this goal in mind London Heathrow Airport one of the largest international airports in the world commissioned the construction of an innovative treatment system as part of their strategy for sustainable management of surface water The treatment system an integration of wetlands and aeration ponds has been designed to solve the potential problems associated with runoff from the airport and commenced operation in the winter of 2002

London Heathrow Airport Limited considered a number of options including carbon filtration, modified cellulose filtration, reverse osmosis UV catalytic oxidation and a number of bioremediation techniques (including constructed wetlands) to achieve an appropriate technology able to deal with the problems posed by the airport runoff (Revitt *et al* 2001) A growing number of studies have provided evidence that wetlands can provide an effective means of treating many types of wastewater including airport runoff (Ellis *et al* 1994 Hatano *et al* 1994 Zhang and Feagley, 1994 Revitt *et al* 1997) Results from pilot scale constructed wetlands (developed as part of the pollution control strategy for London Heathrow Airport Ltd) have shown that most wetland plants and micro-organisms are not adversely affected by exposure to high pollutant loads similar to those found in airport runoff (Chong *et al* 1999)

The characteristics of airport runoff its potential impact on receiving waters and the different treatment options available are discussed in the following section The roles of the different types of wetlands and aerated ponds in the effective management of polluted runoff are also discussed

1 1 AIRPORT RUNOFF

The constituents of airport runoff are similar to those found in most urban and highway runoff and are known to include contaminants such as vehicular oil and

lubricants suspended solids biochemical oxygen demand (BOD) and heavy metals (Chong *et al* 1999) In addition airport runoff contains considerable quantities of anti-icing and de-icing fluids (ADFs) used in the winter months for the safe operation of airport activities as well as other products associated with airport operations such as aircraft fuelling and maintenance Some of the most common contributors to airport runoff are identified below

a) Aircraft fuel

Aircraft fuelling is a daily activity at airports and accidental spillages may occur due to human error faulty valves or fuel venting Although most aircraft stands have adequate spill containment equipment there are cases where the spillage is washed off with other pollutants as part of the surface runoff during storm events

b) Oils

Inappropriate handling storage or disposal of oil containers can result in pollution of the surface water Oils used during routine aircraft maintenance can also be introduced directly into the runoff or washed off from surfaces during storm events

c) Herbicides and pesticides

The need to keep the airport's paved areas free of weeds for safety reasons and the preservation of structural integrity makes the use of herbicides necessary in airport operations Some of these chemicals, used as part of weed control operations may be eventually removed in the surface runoff

d) Vehicular sources

A significant contribution to airport runoff comes from ground transportation which operates in areas including car parks, roads bus stops and bus stations Vehicular activities are associated with a number of pollutants such as fuel, gasoline and diesel (from leakages) oils and grease (from leaks or improperly discarded used containers) and a range of heavy metals and polyaromatic hydrocarbons (from car exhaust, worn tyres engine parts break pads and car body corrosion) Surface accumulation of such substances can eventually be removed in surface run off

e) De-icing and anti-icing agents

De-icing and anti-icing activities are essential for the safe operation of most airport activities particularly in the winter months De-icing fluids are used on impermeable airport surfaces e g runways Anti-icing agents are used for the prevention of the build-up of ice on aircraft wings and fuselage The application rates of de-icing and anti-icing agents can result in ADF loadings as high as 175 000 kg at major airports during severe winters (Revitt *et al* 2001) It has been reported that approximately

80% of the ADFs used on aircraft end up on airport surfaces (O Connor and Douglas 1993) The composition biodegradability and toxicity of ADFs have been widely studied to assess the potential risks of their presence in airport runoff

1 1 1 Chemical composition of ADFs

In the UK de-icing agents are mainly formulated from ethylene (EG) and di-ethylene (DEG) glycol mixtures (trade name *Konsin*) or potassium acetate (trade name *Clearway*) Anti-icing agents contain propylene glycol (PG) (trade name *Kilfrost*), as an active component In the United States there are several types of ADFs depending on the purpose of use Type I fluids are anti-icers generally used to remove ice from the wings and fuselage of aircraft and they contain approximately 80% glycol by weight Types II and IV are de-icers used to prevent the formation of ice and snow on surfaces They contain at least 50% glycol by weight (Corsi *et al* 2001 Switzenbaum *et al* 2000) Each ADF formulation contains different additives, many of which are proprietary, so that the identification of the chemicals present is not always easy The proprietary mix of these additives accounts for between 1% and 2% of ADFs and they are collectively referred to as Adpack in the USA (Cornell *et al* 1998) The main types of commonly used additives are listed below

- **Surfactants (wetting agents)**

Surfactants reduce the surface tension of de-icing fluids hence aiding the adherence to aircraft surfaces They may comprise between 0 4 and 0 5% by volume of anti-icing fluids Of the surfactants identified in ADFs only the alkylphenol ethoxylate group has been reported to pose a significant environmental threat The most prevalent member of this family is nonylphenol ethoxylate It is a low cost non-ionic organic compound characterised by a relatively low foaming potential The nonylphenyl backbone is the hydrophobic component of the molecule which is para-substituted with a hydrophilic ethoxylate chain attached by an ether linkage at the phenolic oxygen (Corsi *et al* 2003)

Alkylphenol ethoxylates have been widely reported to demonstrate acute toxicity to aquatic organisms even at low concentrations with the toxicity increasing with the length of the hydrophobic chain Studies on the alkylphenol ethoxylate group have shown that they generally biodegrade into less-biodegradable and more toxic products Alkylphenol mono- and di-ethoxylates alkylphenoxy acetic acid alkylphenoxy-poly-ethoxy acetic acids and alkylphenols are all metabolites of alkylphenol ethoxylates which are capable of accumulating in organisms with bio-concentration factors varying from the tens to thousands depending on the species, metabolite and organ (Warhurst, 1995) There are also reports that alkylphenols are capable of mimicking the activity of oestradiol a hormone that influences the

development and maintenance of female sex characteristics and the maturation and function of accessory sex organs (Alberts *et al* 1983 Warhurst 1995)

- **Corrosion inhibitors and flame retardants**

Corrosion inhibitors protect the aircraft components to which anti-icing fluids have been applied from corroding while flame retardants reduce the flammability hazards created when the fluids are applied to vulnerable sections of the aircraft They may comprise up to 0 5 % by volume of ADFs and are present at concentrations of approximately 100 to 300 mg/l A mix of 4- and 5-methylbenzotriazoles (4- and 5-MeBT), a weak hydrophobic organic acid which complexes strongly with many metals is commonly used as a combined corrosion inhibitor and flame retardant 5-MeBT is biodegradable while 4-MeBT is a recalcitrant compound The hydrophobic characteristics, metal-binding properties and recalcitrant nature of 4-MeBT enables the chemical to accumulate in sediments with possible severe consequence (Cornell *et al* 1998) The tolerance limit of MeBT for bluegills and minnows has been reported as 27 5 mg/l after 48 hours and 25 mg/l after 96 hours exposure For trout the tolerance limit was reported as 15 mg/l after 48 hours and 12 mg/l after 96 hours The higher fish mortality rate recorded after 96 hours suggests MeBT has a cumulative toxic effect (Wu *et al* 1998)

Cancilla *et al* (2003) reported the median lethal concentrations (LC50) of 5-MeBT for *Pseudomonas promelas* and *Ceriodaphnia dubia* to be 22 0 mg/l and 81 3 mg/l, respectively In the same study, the 25% inhibition concentration (IC25) of 5-MeBT for the green alga *Selenastrum capricorntum* was found to be 23 2 mg/l, while the average median effective concentration (EC50) determined using the Microtox test was 4 25 mg/l MeBT has been identified in ground water below a major airport at concentrations 25 times greater than the reported acute EC50 values (Cancilla *et al* 2003) There are also reports of an ecological toxicity effect for MeBT to *Lepomis macrochirus* (96-h LC50 of 31 mg/l) and *Daphnia magna* (48-h LC50 of 74 mg/l) (Cornell *et al* 1998) Another type of corrosion inhibitor used in ADFs are the phosphate esters produced from the reaction of phosphoric acids and an alkyl or aryl alcohol They have varying toxicities and normally comprise up to 0 125 % by volume of the ADFs Sodium nitrate sodium benzoate and borax have also been used as corrosion inhibitors

- **Colorants or dyes**

Colorants or dyes are chemicals usually organic in nature, used to make ADFs visible so that de-icing personnel can easily see where the fluids have been applied They typically comprise less than 0 25 % by volume In the United States

Types II and IV fluids are coloured but there have been no reports on the toxicity produced by the addition of colorants and dyes

1 1 2 Biodegradability of ADFs

Although the toxicity of ADFs through the additives they contain has been documented in some instances little is known about the impact of these additives on the biodegradation process The successful treatment of airport runoff depends on the reduction or complete removal of ADFs from surface waters before discharge to receiving waters The proprietary nature of these fluids makes it difficult to ascertain the extent to which these additives affect the degradation of ADFs

There are two main pathways by which ADFs can be biodegraded, through anaerobic methanogenisis or by aerobic biodegradation

1 1 2 1 Anaerobic biodegradation

Anaerobic biodegradation of ADFs is achieved by a consortium of microorganisms capable of degrading glycols to methane (CH_4) and carbon dioxide (CO_2) The methane producing microorganisms (methanogens) play the key role in the complete degradation of glycols and are supported by fermentative and hydrogen producing microorganisms The methanogens are strictly anaerobes and have a high degree of substrate specificity (Table 1 1)

Table 1 1 Methanogens and their substrate (adapted from Johnson *et al*, 2001)

Species	Substrate utilised
Methanosaeta concilli	Acetate only
Methanosarcina mazeii	Acetate, formate methanol, methylamine H_2/CO_2 (only reluctantly)
Methanosarcina strain TM-1	Acetate formate methanol methylamines CO_2
Methanosarcina barkeri	Acetate methanol methylamine CO_2 H_2/CO_2
Methanobacterium	H_2/CO_2
Methanococcus	H_2/CO_2 formate
Methanomicrobium	H_2/CO_2 formate
Methanogenium	H_2/CO_2 formate
Methanospirillum	H_2/CO_2, formate

Previous studies have shown that both ethylene glycol and polyethylene glycol can be completely degraded under anaerobic conditions (Figures 1 1a and 1 1b) These pathways suggest that there is a highly syntrophic association among the microorganisms in the consortium (Dwyer and Tiedje 1983 Veltman *et al*, 1998)

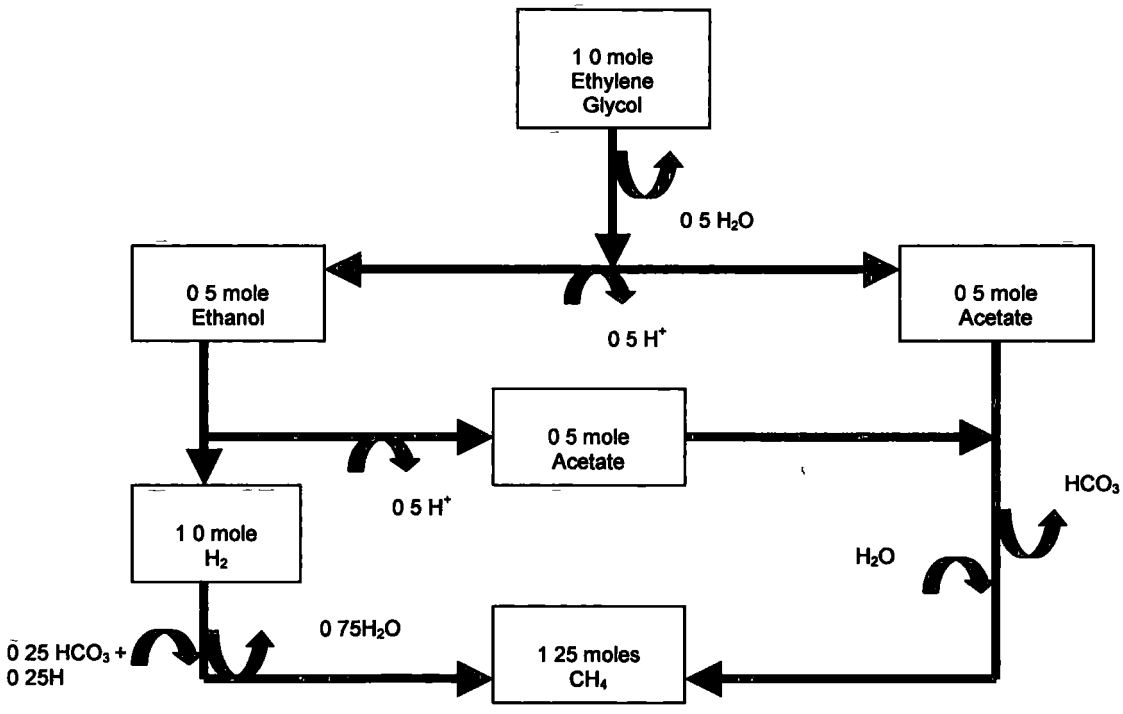


Figure 1 1(a) Pathways for the anaerobic degradation of ethylene glycol as modified by Veltman *et al* (1998)

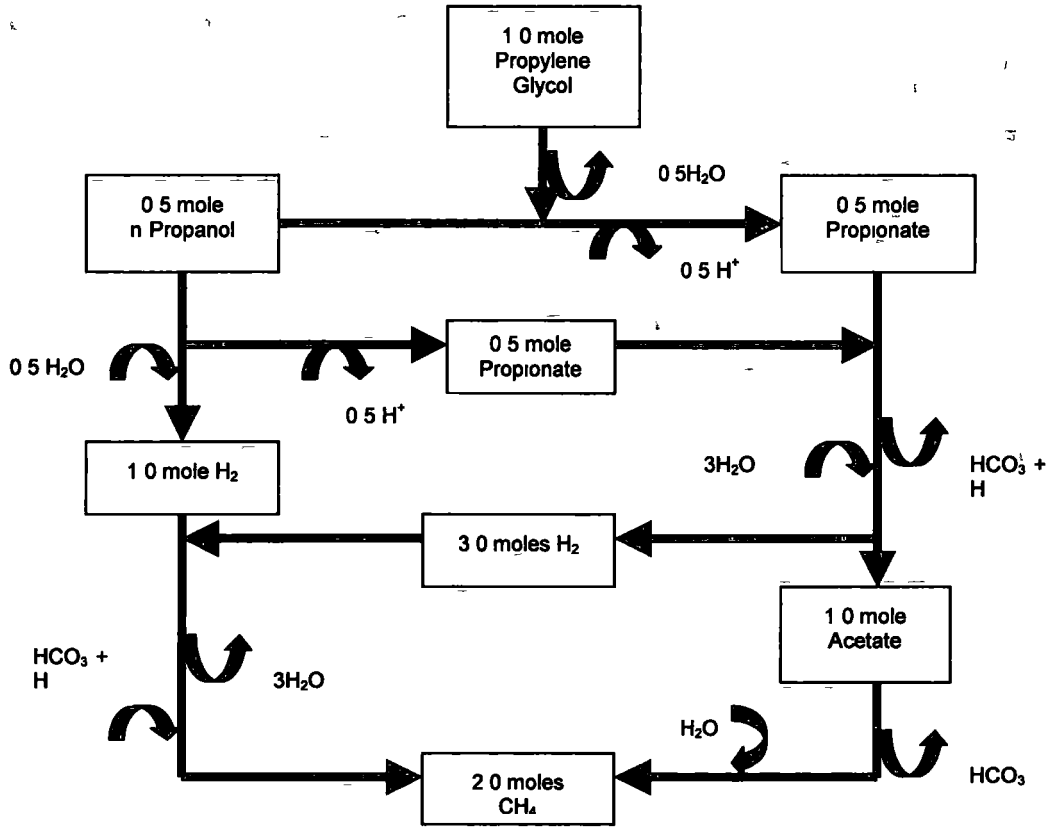


Figure 1 1(b) Pathways for the anaerobic degradation of propylene glycol as modified by Veltman *et al* (1998)

The initially formed alcohols ethanol (Figure 1 1a) and propanol (Figure 1 1b), are oxidised to volatile fatty acids by a group of bacteria in the consortium The bioconversion of propionate to methane (Figure 1 1b) is a very important step in the biodegradation of propylene glycol It has been reported that the presence of hydrogen (H_2) and increases in the concentrations of propionate and acetate can be inhibitory to the process (Fukuzaki *et al* 1990) The bioconversion of acetate to CO_2 and H_2O occurs via acetoclastic methanogenesis a significant ecological process which many studies have found to be inhibited by a number of toxic compounds Amongst these compounds are

- Crude oil (Bekins *et al* 1999)
- Chloroform bromoethanesulfonic acid, trichloroacetic acid and formaldehyde (Hickey *et al* 1987)
- Benzene ring compounds (Patel *et al* 1991)
- Monosubstituted benzenes chlorobenzene and benzaldehyde (Sierra-Alvarez and Lettinga, 1991)
- Chlorinated and fluorinated low molecular weight aliphatic and aromatic compounds (Colleran *et al* 1992)
- Chlorophenols and chloroanilines (Davies-Venn *et al* 1992)
- Pentachlorophenol (Van Beelen and Fleuren-Kemila , 1993)
- N-substituted aromatics (Donlon *et al* , 1995)

1 1 2 2 Aerobic biodegradation

Aerobic biodegradation of ethylene glycol (EG) and propylene glycol (PG) occurs through aerobic respiration an oxidation-reduction process in which molecular oxygen serves as the electron acceptor (Veltman *et al* , 1998) Aerobic bacteria require an extensive supply of oxygen in order to efficiently degrade EG and PG which they use as sources of carbon and energy There are claims that certain bacteria present on airport surfaces are capable of promoting the biodegradation of glycols prior to their transport to receiving waters The biodegradability of propylene glycol in the presence of *Pseudomonas* and *Aerobacter* was demonstrated by Raja *et al* (1991) in an experiment in which 90% of the BOD exerted by a mixture of propylene oxide propylene glycol and associated polyols was removed

In field studies carried out in a lysimeter trench to examine the transport and degradation of propylene glycol and potassium acetate the degradation rate constant for propylene glycol after an initial application was calculated as 0 015 day^{-1} increasing to 0 047 day^{-1} after the second application a year later The corresponding value for potassium acetate was estimated to be 0 02 day^{-1} (French *et al* 2001) In another experiment conducted on the biodegradation of glycol-

based and acetate-based de-icing agents on airport surfaces, biodegradation rate constants were found to decrease with decreasing temperatures The propylene glycol based anti-icer (Kilfost) had a lower potential for biodegradability reducing from 0 081 day ^{1} at 8°C to 0 045 day ^{1} at 1°C The biodegradability potential of an ethylene glycol based de-icer (Konsin) was slightly higher reducing from 0 091 day ^{1} 8°C at to 0 064 day ^{1} at 1°C (Revitt *et al* 2003) Previous evidence suggests that the higher biodegradability of Konsin is due to the presence of ethylene glycol which has been shown to be capable of providing the only source of carbon and energy for a consortium of bacteria (McVicker *et al* 1998 Nikitin *et al* 1999)

1 1 3 Pollution impact of ADFs on receiving waters

During winter months, airport runoff generated by heavy rainfall may contain significant amounts of ADFs washed off impermeable surfaces and aircraft surfaces The solubilities of these fluids and the quantities applied make them the dominant source of BOD in airport runoff with the potential to cause severe impacts on receiving waters There are reports of airport runoff with BOD values as high as 4 500 mg/l and total glycols of 6 000 mg/l (Ellis *et al*, 1997) Glycol concentrations during storm events have been reported to rise with increasing storm flow volumes with up to 99% of the applied ADFs capable of being delivered to receiving waters (Corsi *et al* 2001) Winter storm runoff has been shown to cause acute toxicity to both fathead minnows (*Pimephales promelas*) and the daphnid (*Daphnia magna*) with LC50 values of 1 0% of the effluent (Fisher *et al* 1995 Cancilla *et al* 2003) Laboratory results have demonstrated that both anti-icers (propylene glycol-based) and de-icers (ethylene glycol-based) are toxic with the former exhibiting an enhanced toxicity of two orders of magnitude more (Hartwell *et al* 1995) Koryak *et al* (1998) also reported the presence of dense biological slimes on streambeds and severely stressed invertebrate and fishery communities downstream of an airport runoff discharge

In addition to the acute toxic effect of airport runoff to aquatic organisms in receiving waters it can also place a high demand on the dissolved oxygen present when discharged untreated Dissolved oxygen concentration is an important indicator of stream, river and lake quality Its presence or absence in a water body determines to a large extent the prevailing physicochemical characteristics in such an environment Pure water at 20°C contains a maximum concentration of 9 2 mg/l of dissolved oxygen which represents the value in equilibrium with the oxygen content of the atmosphere This also corresponds to 100% dissolved oxygen saturation at 20°C but other factors such as the concentration of dissolved salts and barometric pressure can influence the dissolved oxygen concentration Fish invertebrates plants and aerobic bacteria all require oxygen for respiration

The potential of wastewater to utilise dissolved oxygen is measured in the laboratory by the reduction in this parameter over a five day period and is known as the five-day biochemical oxygen demand (BOD_5) The approximate BOD_5 concentration for a healthy river should not exceed 10 mg/l, whereas raw sewage may reach 500 mg/l and polluted ADF-laden airport runoff up to 200 000 mg/l (Zitomer 2001) Other measures used are the ultimate biochemical oxygen demand (BOD_u) and the chemical oxygen demand (COD) The BOD_u is the biochemical oxygen demand of a water sample for up to 20 days while the COD is a measure of the quantity of oxygen used in the non-biological oxidation of the constituents of the water sample Unlike the BOD_5 and BOD_u tests which take days COD results can be obtained in hours

In natural waters the dissolved oxygen utilised by microbial activities can be transferred from air to water This process is called re-aeration and it increases with increasing degree of contact and mixing between air and water In healthy streams rivers and lakes a balance exists between re-aeration and biological oxygen uptake The average rate of re-aeration must be equal or less than the average rate of dissolved oxygen consumption for the balance to be maintained The high dissolved oxygen demand placed on water bodies by polluted runoff such as that obtained from airports cannot be satisfied by natural aeration The potential risks to receiving waters associated with the direct discharge of airport runoff makes adequate pre-treatment imperative

1 1 4 Collection, transportation and treatment of airport runoff

Despite the severe consequences which discharges of airport runoff can have on receiving waters only a few airports have developed efficient recovery systems or adequate treatment facilities for the glycol laden runoff (Sabeh and Narasiah 1992) Some airport operators still discharge surface runoff directly into receiving waters and hence risk jeopardising national environmental standards One practice common in some airports in North America is the use of surface sealed and drained de-icing pads for the collection and removal of the glycols used on aircraft (Switzenbaum *et al* 2000) There are however claims that the bulk of the glycols accumulated on these pads eventually enter the stormwater runoff along with other chemicals as the ice melts (Higgins and Maclean, 2002)

There are several alternative approaches which have been used for the treatment and disposal of airport runoff These can be divided into three main groups off-site on-site and recovery options The off-site category consists of treatment options involving discharges of airport stormwater into a collection system or the conveyance of the stormwater to a local treatment or disposal plant Some airport

operators use drains ditches and sewers to transport the runoff into one or more detention ponds or flow balancing tanks (Higgins and Maclean 2002) Such units usually require lining to prevent infiltration into groundwater There are also reports of the use of lined lagoons glass fused to steel storage tanks and concrete tanks for the storage of airport runoff (Switzenbaum *et al* 2000) The various types of storage and collection systems used for surface runoff will be discussed later in this chapter The on-site category could consist of an aerobic or anaerobic treatment facility (in some cases both) constructed within the airport perimeter for the pre-treatment or treatment of the runoff The recovery category utilizes filtration reverse osmosis and distillation to recover glycol from runoff (Switzenbaum *et al* 2000)

The varying nature of airport runoff in terms of temperature differing quantities of contaminants and intermittent supply will inevitably cause hydraulic and, in some cases chemical shocks to any treatment process adopted Table 1 2 which is adapted from Worrall *et al* (2002), outlines the relative merits and demerits of a number of treatment options

Table 1 2 The relative merits and demerits of airport treatment options

Approach	Key factors for or against the option
Anaerobic digestion	There is a high energy cost associated with running such a system and the inability of such a system to deal with shock loads associated with the runoff poses a major problem for successful management
Bacterial remediation	Requires a high level of experience and maintenance
Carbon filtration	An adequate maintenance and management routine is required for the successful operation of such a system
Modified cellulose filtration	Energy costs associated with the operation is high and there is the potential problem of finding an appropriate means of disposing the waste generated
UV catalytic oxidation	Very expensive process requiring a great deal of expertise
Constructed wetlands	This option is cost effective if land is available and it gives the opportunity for sustainable development

1 2 NATURAL AND CONSTRUCTED WETLANDS FOR THE TREATMENT OF POLLUTED WATERS

Natural wetlands are usually described as areas of land in which the water table is at or above the ground surface long enough each year to maintain saturated soil conditions and related vegetation They are also described as representing a transition between terrestrial and aquatic systems where water is the dominant factor determining the development of soils and associated biological communities (Reed *et al*, 1988) There are other general definitions of wetlands based on scientific classifications Swamps for example are in general shallow water saturated areas occupied by water tolerant woody plants Marshes are slightly different because they are predominantly occupied by soft-stemmed plants In general wetlands are areas that fulfil one or more of the following three conditions

a) support predominantly hydrophytes at least periodically
b) consist of predominantly un-drained hydric soils which are wet for a period of time long enough to produce anaerobic conditions that limit the types of plant that can be supported
c) consist of a non-soil substrate such as rock or gravel that is saturated or covered by shallow water at some time during the growing season with an average depth of ≤ 2 m (Cowardin *et al* 1979)

Wetlands are found in every continent apart from the Antarctic and they exist under most climatic conditions Maltby and Turner (1983) estimated that about 8 6 million km^2 of land surface is composed of wetland, representing 6% of the world s land surface area About 56% of this area is in the tropical and sub-tropical regions of the world (Vymazal 1998) Matthews and Fung (1987) estimated that the total area occupied by wetlands was smaller (5 3 million km^2) and that most of this consisted of boreal wetlands Aselman and Crutzen (1989, 1990) estimated that wetlands covered a surface area of 5 6 million km^2, with a higher percentage in the temperate regions of the world Their source was regional wetland surveys and monographs rather than maps as in the two previous estimates

Swamps, marshes fens peatlands bogs shallow lakes, flood plains riparian zones and other freshwater wetlands and shallow continental water bodies further increase the total area occupied by wetlands to some 11 65 million km^2, which is 7 7% of the total land area in the world All the conflicts surrounding the nature and context of a wetland make it extremely important to have a clear definition of the exact composition All over the world the existence and types or names given to wetlands differ Some of the known ones are

- In humid, cool regions of the world they are referred to as bogs fens and tundra
- Along rivers and streams they are often referred to as riparian wetlands seasonally flooded forests and back-swamps
- Along temperate, sub-tropical and tropical coastlines the descriptions salt marshes mud flats and mangrove swamps are used
- In arid regions of the world they are referred to as inland salt flats seasonal playas and vernal pools

Wetlands have also been described in different contexts

- Spatial context wetlands lie between dry land and coastal open water around inland lakes and rivers or as mires arranged across the landscape
- Ecological context intermediates between terrestrial and aquatic ecosystems
- Temporal context destined either to evolve into dry land as a result of lowered water tables sedimentation and plant succession or to be submerged by rising water tables associated with relative sea level rise as the climate changes
- Geological context ephemeral component of landscape, highly dependent on disturbance They can evolve from long term large scale tectonic forces or localised events like annual or daily flooding and drying fire or storm Without tectonic or hydrologic disturbance, wetlands gradually progress through a succession of stages to relative dry upland type ecosystems (Hammer and Bastian 1989)

1 2 1 Wetland Hydrology

Wetland hydrology is the single most important factor that affects the ecological, physical and chemical characteristics of a wetland system (Gosselink and Turner 1978, Novitzki, 1978) It is this unique factor that allows a clear distinction between deep water aquatic systems and well drained terrestrial systems The movement of both energy and nutrients from and to the wetland is achieved through hydrological pathways Other factors that characterise wetlands depend largely on the hydrology of the system Neither the characteristic substrate nor the characteristic biota can develop or function in the absence of specific hydrological conditions Alteration to the biota or substrate can only produce a wetland in which the characteristic substrates or organisms are absent at least temporarily Elimination of the characteristic hydrology of a wetland will most definitely eliminate the wetland even though the characteristic substrate and biota may persist for some time after the change It then follows that the presence of certain organisms and

substrate that characterise a wetland at a specific period is not necessarily indicative of a wetland particularly after any alteration in the hydrology of the wetland (Lewis 1995)

1 2 2 Wetland Vegetation

Ecosystems predominantly occupied by aquatic macrophytes are reported to be the most productive in the world This can be attributed to the presence of ample light water, nutrients and the presence of plants with developed morphological and biochemical adaptation enabling them to optimise to the prevailing conditions Wetland vegetation consists of plants which have evolved functional mechanisms tailored to deal with environmental stresses According to Mitch and Gosselink (1986), there are two classifications of these adaptations known as Tolerators (which allow organisms to tolerate stress created in the ecosystem) and Regulators (which allow organisms to regulate the stress)

Aquatic plants can be divided into two broad forms, free floating and rooted with the latter sub-divided into three classes based on their morphology and physiology As the name suggests, the first group of macrophytes are not rooted to the substrate but float freely in or on the water column They are often restricted to non-turbulent sections of water bodies Two common examples of such macrophytes are *Spirodela polyrhiza* and *Eichhornia crassipes* The rooted plants are equipped with elaborate root structures that can avoid root anoxia (Mitch and Gosselink 1986) The presence of air spaces in their stems and roots allows oxygen diffusion from aerial portions of the plants The diffused oxygen is vital in rhizosphere oxygenation as well as the movement of soluble phytotoxins present in high concentrations in either the water or the soil (Gambrell and Patrick, 1978) These plants are capable of diffusing enough oxygen to supply the roots for these activities and also oxidise adjacent anoxic regions The degree of oxygenation in wetlands is dependent on the chemical and biochemical oxygen demand of the system as well as oxygen release from the roots which differs from plant to plant

- **Emergent macrophytes**

This group of plants grow in water saturated or submersed soils in situations where the water table is about 0 5 m below the soil surface to where the sediment is covered with approximately 1 5 m of water Examples are *Acorus calamus Carex rostrata Phragmites australis Scirpus lacustris,* and *Typha latifolia*

- **Floating- leaved macrophytes**

These are rooted plants in submersed sediments in water depths of approximately 0 5-3 m possessing either floating or aerial leaves Examples are *Nymphaea odorata* and *Nuphar lutea*

- **Submergent macrophytes**

There are two types of macrophytes in this group which can exist at considerable depths in the water and at all levels of the photic zone The first group the non-vascular macroalgae can exist at water depths of 200 m at the lower limits of the photic zone A common example of such macrophytes is *Rhodophyceae* otherwise known as the red algae Macrophytes in the second group are generally referred to as vascular angiosperms examples of which are *Myriophyllum spicatum* and *Ceratophyllum demersum* They can be found at water depths of up to 10 m in the water

1 2 3 Wetland microorganisms

The diversity of physical and chemical interactions in wetlands provides ideal environmental conditions for the growth and reproduction of a variety of microbial species These organisms normally establish interspecific functions which result in a greater diversity, more complete utilisation of energy inflows and the emergence of certain properties of the wetland ecosystem (Kadlec and Robert, 1996) The genetic and functional responses of wetland microbes are limitless and as such allow the system to adapt to changing environmental conditions created by the presence of a pollutant They use these responses for growth and reproduction thereby mediating physical chemical and biological transformations and consequently modifying the water quality

Bacteria and fungi are the two main groups of microorganisms found in wetlands They play a key role in the assimilation transformation and recycling of a wide range of chemical constituents present in wastewater The two groups are the first group of microbes to colonise and decompose solids in wastewater They also have access to dissolved constituents in wastewater by either sorption/transformation or by symbiotically living with other species as they capture dissolved elements and make them accessible to their hosts (Kadlec and Robert 1996) Table 1 3 (adopted from Kadlec and Knight 1996) outlines the attributes of some of the most common bacteria found in wetlands

Table 1 3 Classification of important bacteria in wetland treatment systems

Group	Representative Genera	Comments
Phototrophic bacteria	*Rhodospirillum* *Chlorobium*	Non-symbiotic bacteria N fixers
Gliding bacteria	*Beggiatoa Flexibacter* *Thiothrix*	Filamentous implicated in reduced sludge formation *Beggiatoa* oxides H_2S
Sheathed bacteria	*Sphaerotilus*	Filamentous Found in sewage treatment plants and common polluted waters
Methane producing bacteria	*Methanobacterium*	Anaerobic bacteria found in sediments of wetlands converting carbonate to methane
Budding and/or appendaged bacteria Gram-negative aerobic rods and cocci	*Caulobacter* *Hyphomicrobium* *Pseudomonas* *Zooglea* *Azotobacter Rhizobium*	Aquatic bacteria which grow firmly attached to surfaces *Pseudomonas* spp denitrifies NO_2 to N_2 under anaerobic conditions and can oxidise hydrogen gas *P aeruginosa* causes a variety of infections in humans *Azotobacter* spp is a non-symbiotic N fixer *Rhizobium* is a symbiotic N fixer
Endospore-forming rods and cocci	*Clostridium Bacillus*	*C botulinum* survives in soils and bottom sediments of wetlands and causes avian botulism some *Clostridium* spp are nonsymbiotic N fixers *B thuringiensis* is an insect pathogen *B licheniformis* denitrifies NO_2 to N_2O
Gram negative facultative anaerobic rods	*Escherichia Shigella Salmonella Klebsiella Enterobacter Aeromonas* and *K pneumoniae*	*E coli* is the predominant coliform in faeces *Salmonella spp* cause food poisoning and typhoid fever *Shigella spp* cause bacillary dysentery species in the genera Klebsiella and Enterobacter are nonsymbiotic N fixers and are in the total coliform group K pneumoniae is important in human and industrial waste
Gram negative chemolithotrophic bacteria	*Nitrosomonas* *Nitrobacter* *Thiobacillus*	*Nitrosomonas* catalyse the conversion of NH_4^+ to NO_2 *Nitrobacter* oxidises NO_2 to NO_3 *T ferrooxidans* oxidize iron sulphides producing Fe^{3+} and SO_4^{2}
Gram positive cocci	*Streptococcus*	Faecal streptococci include human species (*S faecalis* and *S faecium*) and animal species (*S bovis S equinus S avium*)
Endospore-forming rods and cocci	*Clostridium Bacillus*	*C botulinum* survives in soils and bottom sediments of wetlands and causes avian botulism some *Clostridium* spp are nonsymbiotic N fixers *B thuringiensis* is an insect pathogen *B licheniformis* denitrifies NO_2 to N_2O

1 2 4 Functions of wetlands

Natural wetlands are known to possess the ability to remove a range of pollutants from wastewater This is achieved by a combination of processes including natural filtration sedimentation physical and chemical immobilisation vegetative uptake and microbial interactions Organic matter (BOD) is removed by microbial degradation, suspended solids by sedimentation and filtration nitrogen compounds by volatilization as ammonia nitrification denitrification and plant uptake phosphorus compounds by precipitation, adsorption on soil colloids and plant uptake, trace elements by precipitation and adsorption and microorganisms through die-off sedimentation radiation, desiccation and adsorption The ability of wetlands to improve water quality has been at the centre of considerable research in recent years Over the years there has been a huge improvement in the knowledge of wetland applications and their impact on the environment The benefits of wetlands in a variety of applications have been identified in several areas including

- recharge of ground water aquifers, drinking water, irrigation, flood control water quality and wastewater treatment
- use of wetland plants as a staple food grazing land timber, paper production, roofing agriculture horticulture
- wild life e g breeding grounds for water fowls preservation of flora and fauna fish and invertebrates (shrimps oysters crabs mussels) integrated systems and aquaculture (fish cultivation combined with rice production)
- erosion control
- gene pools and diversity
- energy (hydroelectricity, gas, solid and liquid fuel)
- educational and training recreation and reclamation (Hollis *et al* 1988)

Although wetlands have been used to treat wastewater for over a century it wasn t until the 1950 s that the quality of water was monitored and the potential of wetlands for purification purposes emerged Results showed significant improvements in the quality of wastewater and studies of natural wetlands have led to a greater understanding of their potential to assimilate pollutants This has also helped in the planned and controlled use of wetlands for wastewater treatment as well as the preservation of natural wetlands and indeed the construction of more wetlands for the same purpose

1 2 5 Pollutants in wetlands and their removal mechanisms

- **Organic compounds**

The removal of organic pollutants associated with settleable particulates in wetland systems occurs through deposition and filtration under quiescent conditions

Soluble organic pollutants are removed by the suspended biomass and that attached to the roots and substrates by biodegradation with the required oxygen for aerobic processes being supplied from the atmosphere by diffusion or by oxygen leakage through the roots in the rhizosphere (Cooper *et al* 1996) The complete aerobic biodegradation of an organic pollutant (represented as a simple sugar) by heterotrophic bacteria can be summarised by the following reaction

$(CH_2O)_n + O_2 \longrightarrow nCO_2 + nH_2O$ *equation 1 1*

Anaerobic degradation is a multi-stage process which occurs in the absence of dissolved oxygen and is not as fast as aerobic degradation Anaerobic degradation predominates in any system with a high organic loading and where oxygen is a limiting element (Cooper *et al* 1996) Facultative or obligate anaerobic heterotrophic bacteria are responsible for the processes which can be summarised by the following equations

$C_6H_{12}O_6 = 3\ CH_3COOH + H_2$ *equation 1 2*

$C_6H_{12}O_6 = 2\ CH_3CHOHCOOH$ *equation 1 3*

$C_6H_{12}O_6 = 2\ CO_2 + 2\ CH_3CH_2OH$ *equation 1 4*

$CH_3COOH \longrightarrow 2\ CO_2 + 2\ H_2O$ *equation 1 5*

$CH_3COOH + 4H_2 \longrightarrow CH_4 + 2\ H_2O$ *equation 1 6*

$4H_2 + CO_2 = CH_4 + 2\ H_2O$ *equation 1 7*

The initial products formed in the anaerobic degradation in most flooded sediments are acetic acid (equation 1 2) lactic acid (equation 1 3) alcohols (equation 1 4) and carbon dioxide and water (equation 1 5) Sulphate reducing bacteria which are strictly anaerobic and methane forming bacteria (equations 1 6 and 1 7) depend on a complex consortium of fermentative bacteria to utilise the product of the first stage degradation for their metabolic activities Decomposition of organic matter and carbon recycling in wetlands is strongly influenced by the sulphate reducing and methane forming bacteria (Vymazal 1995) Whilst the acid forming bacteria are quite adaptable to their environment the over-production of acid could completely halt the activities of the more sensitive methane-formers which only operate optimally in the pH range, 6 5 to 7 5 Over-production of acid by the acid-

formers rapidly reduces the pH, stops the action of the other group of bacteria present and results in the formation of odorous compounds

- **Suspended solids**

Settleable suspended solids are removed from wetlands through sedimentation and filtration during the prolonged hydraulic residence time which is common to most wetlands The non-settling colloidal solids are partially removed through a combination of bacteria growth and adsorption onto other surfaces of plants suspended solids or the bottom sediment Bacterial growth results in the microbial decay of some of the particles and the eventual settling of others (Stowell *et al* 1980)

- **Nitrogen**

Studies have suggested that the major removal mechanisms for nitrogen are nitrification and denitrification Other processes such as plant uptake, volatilisation (common in wetland systems with free flowing macrophytes) ammonification and matrix adsorption also contribute to nitrogen removal in wetland systems

(i) Nitrification and Denitrification

Nitrification is a chemo-autotrophic process which involves sequential oxidation of ammonium to nitrate via nitrite with the energy being used for the synthesis of new cells (Vymazal 1998) The two step oxidation process of ammonium is summarised as

$NH_4^+ + 1\,5\,O_2 = NO_2 + 2\,H^+ + H_2O$ *equation 1 8*

$NO_2 + 0\,5\,O_2 = NO_3$ *equation 1 9*

$NH_4^+ + 2\,O_2 = NO_3 + 2\,H^+ + H_2O$ *equation 1 10*

The first stage of the process (equation 1 8) is believed to be mediated by a group of bacteria known as *Nitrosospira (N briensis)* So far *Nitrosovibrio (N tenius) Nitrosococucus (N nitrosus) and Nitrosomonas (N europaea)* have all been identified Hauck (1984) summarised the sequential stages of the conversion of ammonia to nitrite as follows

$(NH_3/NH_4 \longrightarrow NH_2OH \longrightarrow NOH \longrightarrow NO_2\,NH_2OH \longrightarrow NO_2)$

The intermediate products hydroxyl-amine nitroxyl and nitro-hydroxyl-amine are involved in the electron transfer at each oxidation stage

The second stage (equation 1 9) involving oxidation of nitrite to nitrate is mediated by facultative chemotrophs which are capable of generating energy for growth from organic matter in addition to nitrate Some of them are able to grow in the absence of oxygen The group of bacteria in this category are called *Nitrobacter*

Nitrification is influenced by a number of factors including temperature pH oxygen availability concentration of ammoniacal nitrogen and the microbial population The minimum temperatures for the growth of *Nitrosomonas* and *Nitrobacter* are 5°C and 4°C respectively Approximately 4 3 mg of oxygen is needed for the complete oxidation of 1 mg of ammoniacal nitrogen to nitrate (Cooper *et al* 1996)

Denitrification is the bacteria mediated reduction of nitrate to molecular nitrogen in a system depleted of oxygen It is an irreversible bacterial process in which oxides of nitrogen serve as terminal electron acceptors, in place of oxygen, for respiratory electron transport the electron donor in most cases is an organic substrate Denitrifying bacteria utilise the energy generated, in the form of ATP to support respiration (Hauck 1984) The entire process can be summarised as

$$6(CH_2O) + 4\,NO_3 = 6\,CO_2 + 2\,N_2 + 6\,H_2O \quad \textit{equation 1 11}$$

There is evidence from pure culture studies that denitrification can occur in the presence of oxygen This explains the reduction of nitrate observed in waterlogged soils before the complete depletion of dissolved oxygen has occurred (Laanbroek, 1990)

There are 17 commonly reported genera of bacteria mostly chemotrophs which are believed to have demonstrated denitrifying capabilities *Pseudomonas Aeromonas* and *Vibrio* are the most widely reported genera in aquatic environments Other less common genera which have been reported are *Achromobacter Aerobacter Alcaligenes Azospirillum, Brevibacterium Flavobacterium Spirillum* and *Thiobacillus*

Denitrification is mediated almost exclusively by facultative anaerobic bacteria that utilise oxidised nitrogen in place of oxygen for respiration under anoxic conditions These bacteria are also capable of utilising oxygen for respiration under aerobic conditions, an ability that allows denitrification to progress at a significant rate soon after the system becomes anoxic without any changes in the original microbial population (Hauck 1984)

Factors that affect the rate of denitrification in a system are temperature, pH, absence of oxygen presence of denitrifying bacteria soil type organic matter and the nature of the overlying substrate The presence of dissolved oxygen in the system actually suppresses the production of enzymes required for denitrification to occur (Cooper *et al* 1996) Denitrification proceeds at a very slow rate at temperatures under 5°C with the actual process itself resulting in an increase in the pH of the system

It is generally believed that nitrification and denitrification occur simultaneously in flooded wetlands where the aerobic layer lies immediately above the anaerobic layer or the aerobic rhizosphere microsites are in contact with anaerobic substrate (Reddy and Patrick, 1984) The combination of these processes can be summarised as follows

$$24\ NH_4^+ + 48\ O_2 = 24\ NO_3 + 24\ H_2O + 48\ H^+$$ *equation 1 12*

$$24\ NO_3 + 5\ C_6H_{12}O_6 + 24\ H^+ = 12\ N_2 + 30\ CO_2 + 42\ H_2O$$ *equation 1 13*

$$24\ NH_4^+ + 5\ C_6H_{12}O_6 + 48\ O_2 = 12\ N_2 + 30\ CO_2 + 66\ H_2O + 24\ H^+$$ *equation 1 14*

(ii) Plant uptake

The net productivity, i e the growth rate, and the concentration of nutrient in plant tissue determines the potential rate of uptake by any given plant As a result desirable characteristics for an ideal wetland plant are rapid growth rate, high tissue nutrient content and a high biomass per unit area (DeBusk and Reddy 1987) Water hyacinth (*Eichhornia crassipes*) is reported to have a very high uptake capacity i e the amount of biomass that can be harvested with an estimated value of 6000 kg N ha^{-1} a^{-1} Emergent macrophytes have uptake capacities ranging between 1000 and 2500 kg N ha^{-1} a^{-1} which is lower than the water hyacinth but higher than the uptake capacity of 700 kg N ha^{-1} a^{-1} in submerged macrophytes (Brix 1994 Vymazal 1995)

The amount of nutrient that could possibly be harvested in plants used for treatment is quite insignificant when compared to the background nutrient levels of wastewaters particularly in wetlands characterised by emergent macrophytes (Brix 1994) Average nitrogen removals under optimum conditions are reported to be between 10 and 16% (Gersberg *et al* 1985 Herskowitz 1986) With time a large amount of the nutrient incorporated into the plant tissues is returned to the wastewater through decomposition unless the plants are harvested (Brix, 1994)

(iii) Ammonification

The mineralization of complex organic nitrogen compounds into simpler inorganic nitrogen compounds is called ammonification The rate of ammonification in wetlands reduces with decreasing dissolved oxygen concentration in the transition from aerobic to anaerobic conditions Other factors that affect the rate of ammonification are temperature substrate structure N/P ratio available nutrient and the pH value (Reddy and Patrick 1984) Literature compilations suggest that the rate of ammonification doubles with every 10°C rise in temperature (Reddy *et al* 1974) The optimal pH range for ammonification is between 6 5 and 8 5 Ammonification however causes a decrease in pH level in well drained systems as a result of the accumulation of nitrate and hydrogen ions This process is uncommon in saturated systems as when the pH is buffered around neutrality (Patrick and Wyatt, 1964)

(iv) Ammonia volatilisation

Volatilisation is one of the physical processes involved in pollutant removal in wetlands It is the conversion of pollutants in liquid or solid forms to the gaseous form i e vapour The most common pollutant lost in wetlands through volatilisation is ammonia which occurs under alkaline conditions Attainment of alkaline conditions in wetlands is quite rare and only occurs during alga blooms when the removal of ammonia from the water column is assisted (Cooper *et al* 1996)

$$NH_{3\,(aq)} + H_2O = NH_4^+ + OH \qquad \textit{equation 1 15}$$

Ammonia losses through volatilisation occur when the pH in flooded soils and sediments rises to 9 3 This condition is often created by algal and macrophyte photosynthesis and increases with decreasing concentrations of CO_2 At lower pH values of just below 8, losses are not as significant and below 7 5 ammonia losses become insignificant (Reddy and Patrick 1984) On the whole the rate of ammonia volatilisation is controlled by the NH_4^+ concentration in the wetland, wind velocity nature and variety of aquatic plants present the capacity of the wetland system to change the pH level during diurnal cycles and the temperature (Vymazal 1995)

- **Phosphorus**

Phosphorus can be found in three main forms in wastewaters orthophosphate dehydrated orthophosphate or poly-phosphate and organic phosphorus Removal often occurs through adsorption plant absorption complexation or precipitation (Watson *et al* 1989) Studies have shown that sediments of terrestrial ecosystems act as a better long term sink for phosphorus than aquatic systems (Richardson 1985) The most stable form of phosphorus found in sediments is the +5 oxidation state lower oxidation states are thermodynamically unstable No changes are

experienced in the oxidation state of phosphorus during decomposition of the organic form by microorganisms or biotic assimilation of the inorganic form (Lindsay 1979)

The adsorption and retention of phosphorus in wetlands is controlled by the interaction of redox potential pH value the presence of Fe Al Ca minerals and background phosphorus content of the sediment (Lindsay 1979, Faulkner and Richardson 1989 Richardson and Vaithiyanathan, 1995) Removal of phosphate in a wetland is largely dependent on the condition of the substrate Removal of inorganic phosphorus has been related to the high content of Al, Fe and Ca in sediments or soils It has also been shown to be dependent on the oxalate-extractable (amorphous) aluminium content of the sediment or soil (Richardson 1985) In acidic systems removal is through adsorption onto hydrous oxides or precipitation as insoluble phosphates of either Fe or Al At pH values higher than 7 calcium phosphate is a more likely end product (Qualls and Richardson 1995)

Flooding causes a reduction in redox potential and the transformation of crystalline minerals of Al and Fe to their amorphous forms The amorphous forms of both metals have larger numbers of singly-coordinated surface hydroxyl ions hence a higher phosphorus sorption capacity than their crystalline counterparts (Patrick and Khalid 1974) There are also claims of ligand exchange reactions where phosphate displaces the water or hydroxyl from surfaces of hydrous oxides of Al and Fe Monodentate binuclear complexes within the coordination spheres of hydrous oxides of Al and Fe are formed in this process (Faulkner and Richardson 1989)

- **Metals**

Metals in wastewater are either particulate associated or in solution the distribution between the two being determined by certain physico-chemical processes, including sorption precipitation complexation sedimentation erosion and diffusion Metal removal processes in wetlands involve sedimentation filtration adsorption complexation precipitation cation exchange plant uptake and microbially-mediated reactions particularly oxidation (Watson *et al* 1989) Factors affecting the sediment-water partitioning of metals include the flow/suspended solids ratio ionic strength oxic/anoxic conditions dissolved and particulate organic content pH value organic and inorganic ligand concentrations and metal mobilisation by biochemically mediated processes

(i) Adsorption and cation exchange

Adsorption is the binding of dissolved substances onto sites on plant or matrix surfaces The binding of positively charged metal ions to negatively charged sites

on the surface of the adsorption material is referred to as cation exchange This exchange is brought about by electrostatic forces which are dependent on a number of factors Cation exchange occurs when the electrostatic force of attraction of a particular site for a given metal ion in solution is higher than the existing binding force between the surface and the metal ion residing on that surface The cation exchange capacity (CEC) of a given material is a measure of the number of binding sites per given volume or mass (Cooper *et al* 1996)

The CEC value of most macrophytes is quite high due the presence of the carboxyl function group (-COOH) in the humic acids of plant cellular tissue CEC values have been shown to be the same in both live and dead wetland plant tissues Both adsorption of metal ions and cation exchange are influenced by the vegetation surface area (Vymazal *et al* 1998)

(ii) Microbially mediated processes

Two main types of bacteria commonly found in wetland sediments, remove dissolved metals from wastewater as precipitates The first group called metal-oxidising bacteria, are found in the aerobic zones and cause the precipitation of metal oxides The other group of bacteria are the sulphur-reducing bacteria which are found in the anaerobic zones and are responsible for the precipitation of metal sulphides (Vymazal *et al* 1998)

Microbial precipitation of iron and manganese has been reported in wetlands Iron is found in wetlands in its soluble more bioavailable form as ferrous iron (Fe^{2+}) The precipitation of iron as Fe^{3+} is achieved by a confined group of bacteria through alteration of pH value and E_h which eventually leads to the chemical oxidation of Fe^{2+}

Another way iron is removed from wetlands is through the activities of certain chemo-lithotrophic bacteria *Thiobacillus ferooxidans Sulfolobus* spp and *Gallionella* spp are capable of oxidising iron to form insoluble oxides of Fe^{3+} Many studies have shown *Thiobacillus ferooxidans* to be the most common iron oxidising bacteria known to exist in a variety of systems The process can be summarised as follows

$$4\ FeSO_4 + 2\ H_2SO_4 + O_2 = 2\ Fe_2(SO_4)_3 + 2\ H_2O \quad \textit{equation 1 16}$$

$$2\ Fe_2(SO_4)_3 + 12\ H_2O = 4\ Fe(OH)_3 + 6\ H_2SO_4 \quad \textit{equation 1 17}$$

It is believed that other metals including nickel copper lead zinc and gold follow the same oxidation pathways (Cooper *et al* 1996) The precipitation of ferric

oxyhydroxides (and oxyhydroxides of other transitional metals) is brought about by the steep redox gradient created by the radial oxygenation of the rhizosphere by the roots of the macrophytes in wetland systems The precipitated iron forms a plaque matrix on the plant roots which aids the adsorption and immobilisation of other metals (Taylor and Crowder 1983, Liu *et al* 2004) and in some cases nutrients (Hupfer and Dollan 2003 Heal *et al* 2005)

There are three known strains of sulphate reducing bacteria belonging to the genera *Desulfovibrio Desulfotomaculum* and *Desulfuromonas* (Zehnder and Zinder 1980) They are classified into two groups the first group, found in marine and fresh waters is capable of oxidising organic compounds to fatty acids The second group restricted to marine waters oxidise organic matter completely to carbon dioxide (Laanbroek 1990) These bacteria remove pollutants from the water column by precipitation of associated metals as sulphides under anaerobic conditions

$2CH_2O + SO_4^{2} = H_2S + 2HCO_3$ *equation 1 18*

$M^{2+} + S^{2} =$ MS (insoluble metal sulphide) *equation 1 19*

The activities of sulphate reducing bacteria produce hydrogen sulphide and bicarbonate which increases the alkalinity of the system The hydrogen sulphide is soon ionised to sulphide (S^2) which forms precipitates with a range of metals (Cooper *et al* 1996) The precipitation of insoluble metal sulphides is of great potential in the treatment of contaminated water and as such has been receiving increasing attention in recent years The sulphides formed are less susceptible to resuspension

1 2 6 Constructed versus natural wetlands

Wetland hydrology is one key factor that distinguishes natural wetlands from constructed wetlands Natural wetlands, particularly those in tropical regions, experience a wide range of hydrological changes and suffer from a number of operational limitations Constructed wetlands represent emerging ecotechnological treatment systems which are designed to overcome the disadvantages of natural wetlands by better hydraulic control and management of the vegetative and other components of the system They are engineered systems designed to utilise the natural processes involving wetland vegetation substrate and their associated microbial assemblages to assist in treating polluted water They can be built with a greater degree of control and as such allow the establishment of experimental treatment with well defined substrate composition, vegetation type, flow patterns

and hydraulic rates (Vymazal 1998) There is also a great deal of flexibility with the site location sizing water retention time and hydraulic pathways

The first experimental scale constructed wetland was set up at the Max Planck Institute in 1952 (Seidel 1955) The original focus was on the ability of a wetland to remove nutrients from wastewater by plant uptake as a measure of the pollutant removal capability The first operational full scale constructed wetland was later built in Othfresen Germany in 1974 for municipal sewage treatment (Kickuth 1976) Today over three decades after the first operational constructed wetland was built there are reported uses of constructed wetlands for the treatment of a variety of wastewater types Below is a list of the documented usage of constructed wetlands

1952-phenol waste water-Experimental (Seidel 1955)
1956-dairy waste water-Experimental (Seidel 1976)
1956-livestock wastewater-Experimental (Seidel 1961)
1973-textile wastewater-Experimental (Widyanto 1975)
1974-municipal sewage-Operational (Kickuth 1976)
1975-oil refinery wastewaters-Operational (Litchfield and Schatz 1989)
1975-photograhic laboratory wastewaters-Experimental (Wolverton and McDonald 1976)
1976-textile mill wastewaters-Operational (Kickuth 1976)
1978-acid mine drainage-Experimental (Huntsman *et al* 1978)
1981-heavy metals removal-Experimental (Gersberg *et al* 1985)
1982-agricultural drainage effluents-Experimental (Reddy *et al* 1982)
1982-urban stormwater runoff-Operational (Silverman, 1989)
1983-pulp/paper mill wastewater-Experimental (Thut 1989)
1985-diary wastewaters-Operational (Brix and Schierup 1989)
1987-thermally affected wastewater-Operational (Ailstock, 1989)
1988-livestock wastewater-Operational (Hammer 1989 Hammer, 1992)
1989-agricultural runoff-Operational (Higgins *et al* 1993)
1989-chicken manure-Experimental (Vymazal 1998)
1991-highway runoff-Operational (Ellis *et al* 1994)
1992-bakery wastewater-Operational (Vymazal 1998)
1994-abbatoir wastewater-Operational (Vymazal 1998)
1994-glycol contaminated runoff-Operational (Worrall 1995)
1994-urban surface water outfalls-Operational (Scholes *et al* 1995)
1994-airport runoff-Experimental-(Chong *et al* 1999)
1995-swine lagoon wastewater-Operational (Stone *et al* 2004)
1996-drainage from a cropland-Operation (Luckeydoo *et al* 2000)

1997-effluent from a natural gas compressor station-Operational (Johnson *et al* 1999)
1999- atrazine in nursery irrigation runoff-Operational (Runes H B 2003)
2000-airport runoff-Operational (Higgins and Maclean 2002)
2001-partially nitrified liquid swine manure-Experimental (Poach *et al* 2003)
2001-treatment of airport runoff-Operational (Worrall *et al* 2002)

1 2 7 Types of constructed wetlands

There are three main classifications of constructed wetlands for wastewater treatment based on the root system of the dominant macrophytes

- **Free-floating macrophyte-based systems**

Free-floating macrophyte-based systems are made up of diverse types of plants ranging from those with well-developed submerged roots (e g *Eichhornia crassipes* (water hyacinth) *Pistia stratiotes* (water lettuce) and *Hydrocotyle umbellate* (pennywort)) to tiny surface floating plants possessing little or no root system such as Duckweed (*Lemna* spp) (Brix and Schierup, 1989)

Water hyacinth can be used for the treatment of raw wastewater and primary effluent or for upgrading existing secondary treatment and advanced secondary or tertiary systems all depending on the organic loading into the system (Reed *et al* 1988) The well developed root structures trap suspended solids in the wastewater which eventually settles under gravity in quiescent water beneath the plants The root structure also provides a huge surface area for attachment of microorganisms hence increasing the potential for microbial decomposition of organic waste The ability of water hyacinth to transport oxygen from foliage to the rhizosphere enables the efficient removal of BOD and also provides the right conditions for microbial nitrification (Reed *et al* , 1988)

Low temperatures, below 10°C greatly reduce the efficiency of the water hyacinth system and therefore its successful use is restricted to tropical and subtropical regions of the world There are reports of successful use of such systems in greenhouses and outdoors during the summer in the Czech Republic (Vymazal 1998)

- **Submerged macrophyte-based systems**

Submerged macrophyte-based systems are characterised by plants with entirely submerged photosynthetic tissues Mineral uptake in the plants is often through the roots and shoots (Vymazal 1995) Light penetration is important to support plant photosynthetic activities in such systems They can only grow well in oxygenated waters and as such they are not efficient in the treatment of wastewaters with a

high level of readily biodegradable organic matter where anoxicity is possible (Brix 1994)

There are also reports of other submerged macrophyte-based systems *Elodea nuttallii* (waterweed) for polishing secondary treated wastewater (Bishop and Eighmy 1988) *Egeria densa* (dense waterweed), *Elodea canadensis* (waterweed) *Ceratophyllum demersum* (coontail) *Hydrilla verticillata* (hydrilla) *Cabomba caroliniana* (fanwort) *Myriophyllum heterophyllum* (water milfoil) and *Potamogeton* spp (pondweeds) for wastewater treatment (McNabb 1976 Reed *et al*, 1988)

The presence of submerged macrophytes is believed to deplete dissolved inorganic carbon in water and elevate dissolved oxygen concentrations, particularly during photosynthesis This also increases the pH value resulting in the chemical precipitation of phosphorus the mineralization of organic matter and the volatilisation of ammonia (Brix 1994)

- **Rooted emergent macrophyte-based systems**

There are four broad classifications of systems with emergent plants depending on the water flow pattern through the system

- Surface flow systems
- Horizontal subsurface flow systems
- Vertical subsurface flow systems
- Hybrid systems i e combinations of the other systems

(i) Surface flow systems

Surface flow systems are characterised by shallow water flowing through channels or basins at low flow velocities with soil or a similar medium preventing seepage and at the same time supporting the emergent vegetation (Figure 1 2) The main features present in this kind of system ensure that plug flow conditions are maintained (Reed *et al* 1988) In municipal treatment systems most of the solids are filtered and settled within a short distance from the inlet (Watson *et al* 1989) This is due to the lowered flow velocities and sheltering of the water column from wind Dissolved pollutants are sorbed by soils, plant tissues and active microbial populations before entering into the overall mineral cycle of the ecosystem (Kadlec and Knight 1996)

Surface flow systems have been in operation in the Netherlands for over 30 years Over a hundred systems are reportedly in use in North America for water quality improvement, one of which is a 16 000 ha system used for the treatment of agricultural wastewater in the south of Florida (Kadlec and Knight 1996)

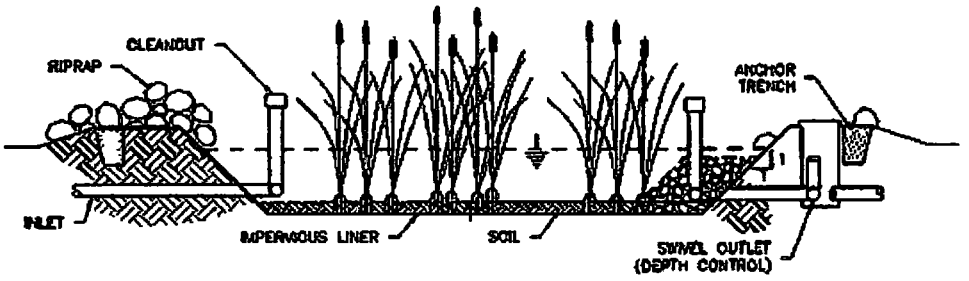


Figure 1 2 Diagrammatic representation of a Surface Flow Wetland (source OWDP, Onsite Wastewater Demonstration Project, 2002 http //www cet nau edu/Projects/WDP/)

(ii) Horizontal subsurface flow systems

In a horizontal subsurface flow system (HSF), the wastewater flow rate is reduced as it passes through the porous substrate medium The flow proceeds in a horizontal manner until it reaches the outlet and then leaves the system through a level control arrangement (Figure 1 3) Microbial degradation filtration and sedimentation occur during the passage of the wastewater through the rhizosphere The most common type of horizontal subsurface flow system in Europe is the Reed Bed Treatment System (RBTS) adopted from the name of the macrophytes the common reed (i e *Phragmites australis*), used (Vymazal, 1998)

The aerobic and anaerobic degradation of organic compounds in HSF systems is mainly carried out below the water surface by resident media surface bacteria and those associated with the roots and rhizomes Anaerobic degradation plays a key role in the decomposition of organic pollutants in HSF systems because the oxygen transport capacity of the reeds is insufficient to sustain aerobic degradation Insufficient oxygen supply is another reason for incomplete removal of nitrogen in HSF systems Because the common media used in most HSF systems do not contain large amounts of metals, such as Fe Al or Ca removal of phosphorus through ligand exchange reactions is inadequate

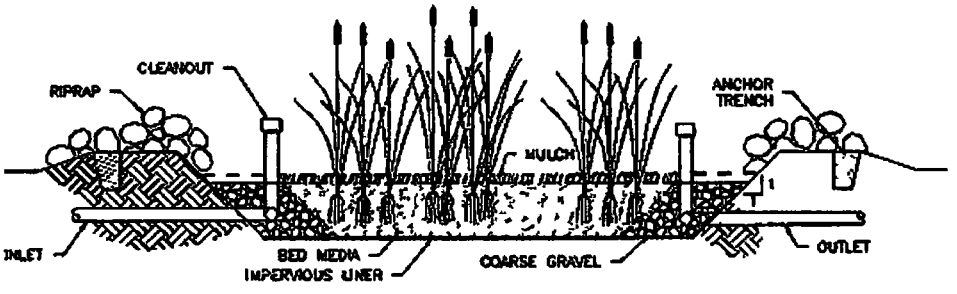


Figure 1 3 Diagrammatic representation of a Horizontal Sub-surface Flow Wetland (Source OWDP, Onsite Wastewater Demonstration Project, 2002 http //www cet nau edu/Projects/WDP/)

(iii) Vertical subsurface flow systems

Vertical subsurface flow systems (VSF) have been in operation in Europe for well over 25 years They were often referred to as infiltration fields' in The Netherlands The configuration of VSF systems can be similar to that employed in HSF systems, the difference is the vertical flow of wastewater through the substrate Loading of wastewater into the VSF system is usually intermittent and discontinuous and not continuous as in HSF systems The vegetative species utilized are usually the same as those utilized in horizontal flow systems

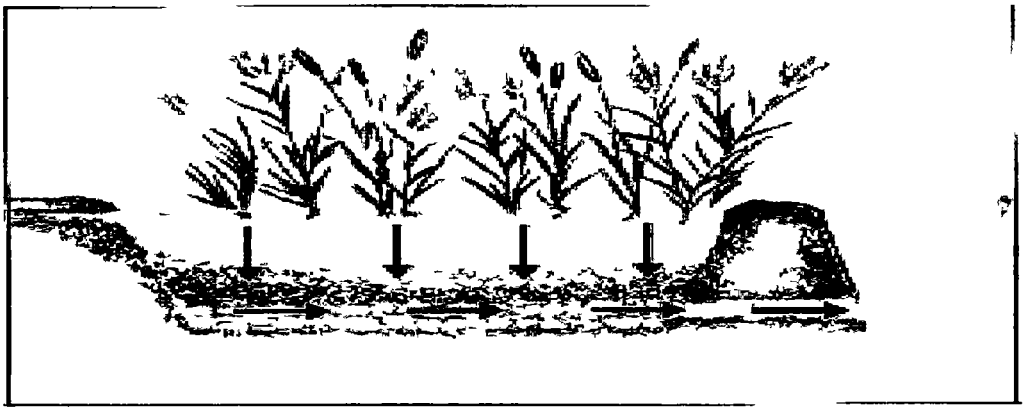


Figure 1 4 Diagrammatic representation of a Vertical Sub-surface Flow Wetland (Source IRIDRA, 2005)

Hydraulic retention times in VSF systems are usually short with the superficial top layer of sand lessening the velocity of the flow This has been found to favour processes like denitrification and phosphorus removal in the system

(iv) Hybrid flow systems

Examples of hybrid flow systems include a combination of horizontal subsurface flow and vertical subsurface flow systems arranged in a staged manner The horizontal flow beds are less aerobic and are good for suspended solids removal and moderate BOD reductions In contrast the vertical flow beds are more aerobic hence better for nitrification and BOD removal The actual arrangement required for a hybrid flow treatment plant depends on the skill of the designer in using the advantages of a particular system type to compensate for the disadvantages of the other Good examples of this technique are the treatment plants in Denmark (Johansen and Brix 1996) and Poland (Ciupa, 1996) where large horizontal-flow systems are followed by a vertical-flow stage In this set up nitrification takes place in the vertical flow stage which is at the end of the process sequence The wastewater can then be pumped back to the less aerobic horizontal flow stage for denitrification using the raw feed as the carbon source

1 2 8 Pollutant Removal Mechanisms in Constructed Wetlands

Pollutant removal in constructed wetlands involves a combination of physical chemical and biological processes including sedimentation precipitation adsorption and assimilation by plant tissue and microbial transformation Wetlands also serve as a sink for a number of pollutants and in some cases as a biological filter Many of these processes require further studies to provide a full understanding of the actual mechanisms involved and the extent of pollutant removal

- **Physical processes**

Through gravitational settling, particulate pollutants are removed from the water column by the sedimentation process This process helps to clear the water of particulate pollutants which would have hindered light penetration into the water column It also aids in the removal of suspended solid associated BOD, nutrient microbial biomass and heavy metals Factors such as dense stands of reeds and reduction in flow rates reduction in turbulence or any wind generated waves have been shown in the past to aid sedimentation (Kadlec and Knight 1996) The pollutant removal abilities of wetlands have been linked with the sediment removal by the litter layers which trap pollutants as incoming wastewater enters at reduced flow rates (Brix, 1994)

- **Chemical processes**

The two main chemical processes that aid pollutant removal in wetlands are specific adsorption and precipitation of insoluble compounds (Hemond and Benoit 1988) Specific adsorption is the adherence of pollutant species or substances through chemical bonds resulting in the formation of stable chemical complexes Both iron and aluminium oxides are known adsorption sites for phosphates and a host of heavy metals Clays are also good sites for ligand exchange and chelation A range of pollutants in the water column are further removed by ionic hydrate adsorption of solids which is then followed by sedimentation

Phosphates and heavy metals in water columns are also precipitated out of solution through the formation of insoluble complexes with iron aluminium or calcium all of which are abundant in wetland associated clay particles (Hemond and Benoit, 1988) Other insoluble complexes can be precipitated out of the water column as hydroxides carbonates and phosphates In most of these cases precipitation of complexes is followed by sedimentation into the sediment sink where other transformations and/or conversions may take place

- **Biological processes**

Wetland plants and microorganisms have been shown to store a range of pollutants including heavy metals (Zhang *et al* 1998) and phosphorus and nitrogen (Brix 1994 Jayaweera and Kasturiarachchi 2004 Kuusemets and Lohmus 2005) Pollutants are removed from the sediments by emergent plants from the water column by floating plants or from both by submerged plants (Barko and Smart, 1990)

Pollutants are stored in different parts of the plants depending on the type of plant Submergent plants store most of the pollutants in roots and rhizomes which is the same for emergent plants with smaller amounts stored in the leaves Plant uptake occurs more during periods of active growth Because of seasonal variations, certain parts of the world, particularly the temperate regions do not have efficient pollutant uptake during the winter months The pollutants particularly nutrients and heavy metals bioaccumulated over the active growing season (summer and spring) are translocated from the leaves to the roots and rhizomes where they are stored for growth in the next season Most of the heavy metals however are moved from the root section and become incorporated into the sediments over time (Kadlec and Knight, 1996)

Unlike plant uptake which is seasonal microbial activities are not necessarily reduced during winter months Most of the microbes remain in the system in association with appropriate attachment sites Very little has been done to assess the seasonal impact of microbial populations on pollutant uptake accumulation or removal

1 3 LAGOONS AND PONDS

Lagoons and ponds are two broad classifications for man-made pits or basins constructed in or on the ground surface with earthen dikes (or other man-made material) for water retention Lagoons and ponds have been used to treat different forms of wastewater ranging from stormwater runoff to industrial effluent They are mainly used for suspended solids BOD and nutrient removal although there are cases where they are also used for pathogen removal Natural stabilization processes occur with the required oxygen coming through atmospheric diffusion photosynthetic and/or mechanical sources In certain cases ponds are used as the only means of treatment prior to discharge to receiving waters while in other cases they act as a storage facility prior to treatment or discharge The role they play in surface runoff management determines their location size and to an extent the name by which they are designated Some of the types commonly used are discussed below

a) Detention and retention ponds

The increase in impervious area due to urbanisation has made structures like detention and retention ponds a key feature in the successful management of increasing surface runoff volumes The main role of detention ponds and retention ponds is to control the water flow during storm events but they also improve stormwater quality by detaining the stormwater to allow pollutants that are suspended in the runoff to settle out

Detention ponds are designed to stay dry until a significant storm event occurs Retention ponds on the other hand, have a permanent pool of water into which stormwater is directed and treated before being washed out during subsequent storm events Detention ponds have been reported to be effective for the removal of pollutants such as nutrients faecal bacteria and heavy metals (Mallin *et al* 2002) Enhanced treatment can be achieved by extending the retention time or through the use of aquatic plants around the pond (Figure 1 5) Examples of such applications are retention ponds located on the Surrey section of the London Orbital M25 motorway (Hares and Ward 1999) and the Ann McCrary pond in Burnt Mill creek watershed North Carolina (Mallin *et al* 2002)

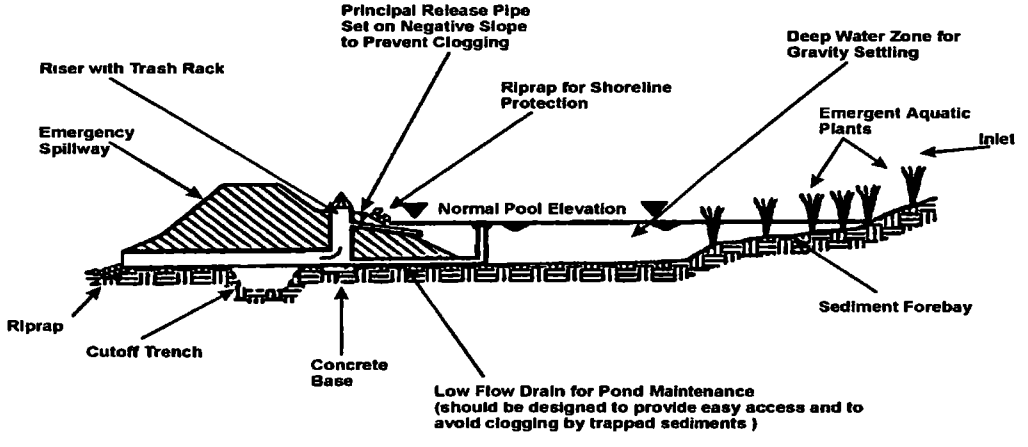


Figure 1 5 Typical layout of a retention pond (source Maryland Department of the Environment, 1986 http //www epa gov/owm/mtb/wetdtnpn pdf)

b) Aerobic ponds

Aerobic ponds are mainly used for the primary treatment of soluble organic waste present in water They usually contain bacteria and algae in suspension under aerobic conditions In certain aerobic ponds the objective is to increase the algae population while in others the objective is to increase the dissolved oxygen levels The general operating depth in the former is between 0 15 m and 0 45 m This encourages the growth of rooted aquatic plants In the latter the operating depth is

usually around 1 5 m and such ponds are usually equipped with pumps or surface aerators to achieve adequate mixing and an efficient oxygen supply

c) Anaerobic ponds

Anaerobic ponds are used for the treatment of high strength wastewater (usually BOD greater than 500 mg/l) and for situations in which a high quality effluent is not required They are generally deep around 9m to conserve heat and maintain anaerobic conditions The upper layer (surface zone) of the pond is usually aerated through atmospheric diffusion with the rest of the pond being anaerobic Pollutant removal in anaerobic ponds is carried out by a combination of anaerobic metabolism and precipitation, the acid-forming bacteria being the dominant microorganisms in the system The release of odours during the reduction of sulphate compounds to hydrogen sulphide makes it imperative that such systems are sited in remote locations

d) Facultative ponds

Facultative ponds operate through a combination of aerobic and anaerobic mechanisms and are very commonly used for the treatment of domestic waste and a variety of industrial wastes They are made up of three zones containing different types of organisms (Figure 1 6)

- The surface zone receives atmospheric oxygen by diffusion allowing algae and bacteria to thrive symbiotically Aerobic bacteria utilise the oxygen provided by the algae growing on the surface to oxidise soluble organics yielding carbon dioxide The carbon dioxide is then used by the algae as a carbon source
- The aerobic-anaerobic zone in the middle of the pond contains facultative bacteria which convert waste through oxidisation
- The anaerobic zone at the bottom of the pond is where accumulated sludge and organics are converted by the anaerobic bacteria The products of this conversion CH_4 CO_2 and H_2S are either oxidised by aerobic bacteria in the pond or released directly to the atmosphere

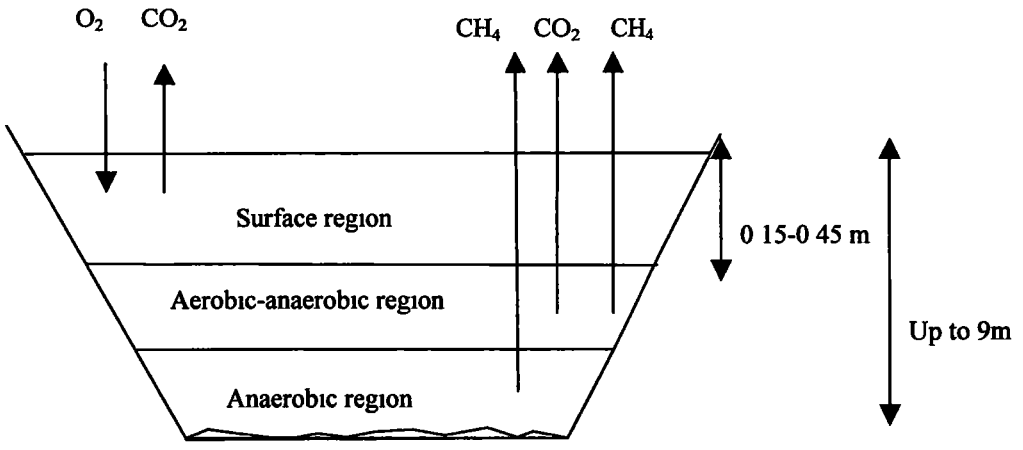


Figure 1 6 Facultative pond strata, showing the three depth regions

e) Aerated lagoons

Aerated lagoons (also referred to as aerated ponds) are systems in which biodegradable organic matter is broken down by aerobic processes with the necessary oxygen supplied by mechanical aeration The presence of mechanical aerators leads to a marked change in the ecology with replacement of algae associated with wastewater ponds and lagoons by a mixed heterotrophic bacterial community The operation of aerated lagoons exclude algae because the turbulence created by the aeration keeps the biomass suspended which in turn provides the turbidity that restricts penetration of any form of light that would encourage algal growth in the water column The turbulence is also expected to assist the conveyance of oxygen at the surface to the lower layers and also to reduce thermal stratification

Aerated lagoons are usually constructed with sloping walls lined with materials such as bentonite clay asphalt synthetic membranes and concrete to prevent any form of infiltration The inlet and outlet pipes are usually placed at diagonally opposite corners Badly positioned inlet and outlet pipes could lead to the direct passage of untreated wastewater to the outlet section (i e short-circuiting) especially if the outlet end of the pond is located downwind Baffles are usually placed within the ponds to ensure that this does not occur The last factor the retention time varies for different treatment facilities depending on the type of wastewater being treated and the effluent quality requirement For most domestic wastewater the retention time varies from 3 to 6 days (Liu and Liptak 2000)

1 3 1 The ecology of lagoons and ponds

The diverse growth environment found in ponds supports a diversity of microorganisms which are different from those observed in many other biological treatment processes Aerobic and anaerobic bacteria as well as algae and some higher life forms such as protozoans rotifers daphnia and insect larvae have been reported to be present in lagoons and ponds

1 3 1 1 Aerobic bacteria

Aerobic bacteria found in lagoons and ponds are similar to those found in most treatment processes such as activated sludge The main functional groups found are

- Single dispersed bacteria
- Floc-forming bacteria
- Filamentous bacteria

These bacteria function by utilising organic carbon i e BOD to produce carbon dioxide and new cells The single dispersed bacteria are the most common of the three types found in wastewaters because they grow quite well in ponds with high organic loadings and low dissolved oxygen They are quite effective in BOD degradation Their dispersed nature however makes retention in the pond difficult as most of the biomass is passed along in the effluent stream as part of the suspended solids loads Floc-forming bacteria on the other hand grow in large aggregates which degrade BOD settling at the end of the process to yield a low TSS (total suspended solid) effluent The pathway followed by aerobic bacteria in the degradation of BOD has been explained earlier in this chapter Another group of aerobic bacteria that has been reportedly found in lagoons are nitrifying bacteria These are strict aerobes and are capable of oxidising ammonia via nitrite to nitrate The activities of this group have been fully discussed earlier in this chapter Aerobic bacteria are effective in BOD removal over a wide pH range (6 5-9 0) and at temperatures from 3-4°C to 60-70°C There is however, a rapid decline in BOD removal performance if the temperature falls below 3-4°C

1 3 1 2 Anaerobic bacteria

Anaerobic bacteria that are commonly found in lagoons are mainly involved in methane formation (the acid-forming and methane bacteria) and sulphate reduction (sulphate reducing bacteria) These process pathways have been explained earlier in this chapter Generally anaerobic bacteria are capable of hydrolysing proteins fats and polysaccharides present in wastewater to smaller compounds like amino acids, short-chain peptides, fatty acids, glycerol, and mono- and disaccharides

They are known to have a wider range of environmental tolerance to pH and temperature variation compared to aerobic bacteria

Another group of anaerobic bacteria found in almost all lagoons and ponds are the photosynthetic anaerobic sulphur bacteria, generally grouped into the red and green sulphur bacteria category and represented by about 28 genera (Ehrlich 1991) They oxidise reduced sulphur containing compounds using light energy to yield sulphur and sulphate The most common species are *Chromatium Thiocystis* and *Thiopedia* which can grow in profusion and give a lagoon a pink or red colour Their presence is most often an indication of organic overloading and the occurrence of anaerobic conditions in an intended aerobic system

1 3 1 3 Algae

Algae are usually the providers of some of the oxygen required by bacteria for waste stabilisation There are three classifications of algae based on their chlorophyll type

- brown algae
- green algae
- red algae

The prevailing growth conditions particularly temperature organic loading oxygen status, nutrient availability and predation pressures in a lagoon determine the predominant algal species at any given time A fourth group of algae called the blue-green algae are also found in lagoons They are slightly different from the first three because of their ability to fix atmospheric nitrogen Blue green algae thrive in lagoons with poor supporting conditions such as high water temperature low light levels low nutrient availability (many fix nitrogen) and high predation pressure Common species that have been reportedly found in ponds are *Aphanothece Microcystis Oscillatoria and Anabaena*

Generally algae grow more efficiently at warmer temperatures with longer detention times and when the inorganic minerals needed for growth are in excess Although the operation of certain systems like aerated lagoons and ponds does not encourage algal growth within the system they are still able to survive in such an environment

1 4 ENUMERATION, ISOLATION AND IDENTIFICATION OF MICROORGANISMS

In nature the microbial populations do not segregate themselves by species they usually exist in a mixture of several other cells The composition of every mixture is dependent on the nature of the environment to which it is exposed Microorganisms thrive differently in different environments and this to a large extent determines which cells will be predominant The accurate enumeration of any cell in a particular medium depends on its culturability in the laboratory Certain bacteria especially Gram-negative bacteria sometimes enter a viable but non-culturable (VBNC) state, during which they cease growing conventionally whilst retaining their existence In this state, these cells are reduced in size, become ovoid and do not grow on standard laboratory media so that enumeration results become flawed This phenomenon is generally regarded as being analogous to sporulation which is common in Gram-positive bacteria like *Bacillus subtilis* The emergence of this concept in the 1980s led to considerable research into the existence and significance of such bacteria in the environment (Yokomaku *et al* 2000)

It was initially reported that when cells loose their ability to grow on laboratory medium they also forfeit the ability to incorporate substrates such as glucose (Barcina *et al* 1990) Results from tests conducted on *V cholerae* and *E coli* however show reduced substrate uptake and metabolic activities only and not a total cessation for both cells in their VBNC state The uptake by *V cholerae* improved significantly after a temperature upshift from 4°C to 30°C (Chowdhury *et al* 1994) which agrees with the findings of Nilsson *et al* (1991) in which *V vulnificus* was resuscitated after an increase in temperature In another study *Salmonella enteritidis* regained its culturability on addition of nutrient (Roszak *et al* 1984) which demonstrates that culturability can be regained On the other hand *Pseudomonas flourescens* became VBNC when the temperature was increased from 23°C to 35°C and 37°C (Bunker *et al* 2004) and *Bradyrhizobium japonicum* exposed to elevated temperature in the soil also became VBNC (Kennedy and Wollum 1998)

In a number of other studies, freshwater and marine bacteria were found to enter the VBNC state under different conditions (Xu *et al* 1982 Roszak *et al* 1984, Rollins and Colwell 1986, Nilsson *et al*, 1991) while they remained active *Escherichia coli* have also been shown to be capable of entering into a VBNC state in response to adverse environmental conditions (Xu *et al*, 1982 Colwell *et al* 1985 Roszak and Colwell *et al* 1987) There are further claims that some Gram-negative bacteria loose there colony-forming ability once inside the soil microcosm e g *Alcaligene eutrophus* (Pedersen and Jacobsen 1993) *Pseudomonas flourescens* (Binnerup *et al*, 1993 Troxler *et al* 1997) *Flavobacterium sp*

(Heijnen *et al* 1995) *Salmonella typhimurium* (Troxler *et al* 1997) All these results suggest that abiotic stresses contribute to the entry of many cells into the VBNC state Colwell *et al* (1985) reported that despite being in the VBNC state *V cholerae* remains potentially pathogenic It would appear from all these results that the VBNC state exists as a response to stress The subjecting of culturable cells to prolonged starvation in a sterile laboratory microcosm has been shown to lead to cell count declines over time (Bogosian and Bourneuf 2001)

The Kogure s protocol is one of several proposed to ascertain whether cells unable to grow on a routine culture media are viable It is based on the assumption that viable cells can respond to the presence of a nutrient but do not necessarily form colonies on the plate The test is performed using nalidixic acid (at a standard concentration of 10 0 mg/l), which inhibits the growth and reproduction of both gram-positive and gram-negative bacteria, by blocking the bacterial DNA synthesis and interfering with cell division The viable cells usually enlarge without division distinguishing them from the non-viable ones which will remain small It is not entirely certain that all viable cells will respond positively to this technique although there are reports that this is the case (Bottomley and Maggard 1990 Heijnen *et al* 1995 Troxler *et al* 1997)

The entry of certain bacteria, particularly Gram-negative ones into the VBNC state has an important implication for the understanding of the actual pathways followed in the degradation of pollutants such as glycol The need to identify a medium that would sustain the growth of such cells during this state so as to conduct further tests cannot be over-emphasised The difficulty in obtaining such cells for more detailed tests (API biochemical tests and the Polymerase chain reaction PCR) makes it even more difficult to fully understand the microbial processes involved in pollutant removal in systems made up of a large diversity of bacteria

1 4 1 Enumeration of microorganisms

The techniques employed for the enumeration of microorganisms in the environment fall into two main categories direct and indirect methods The direct technique as the name suggests involves actual counting of bacteria cells using a number technique It basically equates viability with culturability The conventional plate count method is the most widely used technique and has been in use for a long time in the applied microbiology field However this method has a few inherent disadvantages The main disadvantage is that results are not obtained immediately as it takes at least 2 or 3 days before a realistic count can be made There is also the problem of under-estimation of the population due to uneven distribution or the formation of clumps or chains Auty *et al* (2001) also claimed that oxidative killing of anaerobic bacteria such as *Bifidobacterium* during plating

could also result in an under-estimation of the actual population Colony counts tend to under-estimate the actual bacteria diversity a case indicated by microscopic counting of cells after immunofluorescence (IF) staining (Bottomley and Maggard 1990) The spiral plate and petrifilm techniques are also popular and have both been in use for a long time Other more recent techniques are

-Hydrophobic grid membrane filtration (HGMF)
-Direct staining microscopy
-Direct epifluorescent filter technique (DEFT)
-Microcolony DEFT

The indirect technique is basically a means of enumeration based on the reaction of the microbial population It is merely estimates a constituent or product of the microorganisms e g measurement of the utilisation of pyruvate or adenosine triphosphate (ATP) by the cells Other methods that fall into this category are -

-Limulus amoebocyte lysate (LAL)
-Radiometry
-Flow cytometry
-Gas chromatography
-Pyrolysis
-Turbidity
-Catalase production

The advantages and disadvantages of both techniques are listed below

Table 1 4 The advantages and disadvantages of the direct and indirect method of enumeration of microorganisms

Direct method	Indirect method
Advantages	
• Rapid • Minimal and inexpensive equipment • Immediate determination of cell morphologies	• Accurate results • Requires only a small volume of innoculum
Disadvantages	
• Counts all cells both dead and living • The use of a small volume of sample magnifies interference • Slow	• Requires media and equipment • Accuracy depends on growth and activity of microorganism

1 4 2 Isolation of Microorganisms

In order to assess the morphological and biochemical attributes of a given microorganism the cells are usually separated into pure cultures containing only one organism type represented by a discrete colony Colonies are the individual,

macroscopic visible mass of microbial growth on a solid medium For successful isolation of a particular microorganism the number of cells on the innoculum is reduced ensuring that that the individual cells are sufficiently far apart on the surface of the medium There are three techniques commonly used for the isolation of discrete colonies

- **Streak-plate technique**

This is a rapid qualitative isolation technique which involves the spreading of a loopful of culture over the surface of a suitable agar plate By doing this, millions of cells are spread over the surface of the solid medium with some individual cells deposited distinctly from the others The cells on the plate grow and reproduce forming isolated colonies, some well separated from others These colonies usually serve as a good source for a pure culture

- **Spread-plate technique**

This technique involves the use of a sterile L-shaped bent rod to spread a previously diluted solution of microorganisms over a solid agar surface The solution is left to absorb into the agar before being incubated at an appropriate temperature with the plates in inverted positions Depending on the dilutions used, discrete colonies appear on the surface of the agar after between 1 and 3 days

- **Pour-plate technique**

The pour-plate technique requires the use of solution of microorganisms which are then diluted serially usually using a loop or pipette The diluted innoculum is transferred in a molten agar medium to a Petri dish where it is mixed and allowed to solidify When the agar has solidified, the dishes are inverted and incubated at the appropriate temperature As for the spread-plate technique discrete colonies are observed in the media after between 1 and 3 days depending on the nature of the microorganism

1 4 3 Identification of microorganisms

The traditional methods of identifying microorganisms are usually based on the morphological characteristics of the cells and antigen detection In recent years polymerase chain reactions (PCR) and API-biochemical techniques have gained popularity both in the clinical industrial and environmental arenas There are also other automated identification methods which have been developed The prerequisite for all these methods is the need to obtain a pure culture Some of the identification techniques widely used are listed below

- **Vitek**

This technique was initially introduced as an automated system by BioMerieux in 1976 It is based on the detection of certain microbial growth in microwells built into plastic cards which had previously been widely used for tests on urine isolates The system is an integrated modular set-up consisting of a filler-sealer unit reader incubator computer and printer which may be interfaced with other laboratory information management systems The innoculum is automatically transferred to the test cards before being incubated at 35°C The underlying principle of the device is based on changes in the optical density which is an indication of the response of the innoculum to the different antimicrobial susceptibility tests One major disadvantage of the device is the time taken to identify non-glucose fermenting Gram-negative bacilli Gram-positive bacteria and yeast There are suggestions that further modifications are required for the identification of some staphylococcus and streptococcus species (Collins *et al* 1995)

- **API**

The API system consists of between 20 and 32 biochemical tests which are based on the average 29 to 32 tests believed to be required for the reliable identification of most bacteria strains The system comprises of a densitometer inoculator reader and data handler One unique feature of this technique is the output index produced which provides the user with culture identification The index also indicates if the isolate is typical or atypical compared with the base profile of the test species Different API strips have been developed over the years for more closely related species Some of the common ones are -

- API20E for the identification of enteric bacteria
- APIStrep for the identification of streptococcus bacteria
- APIStaph for the identification of staphylococcus bacteria
- ID32staph which is used for a more comprehensive identification of staphylococcus bacteria

- **Biolog**

The Biolog system is a computer based system consisting of a manual 8-channel repeating pipettor, a turbidometer and a microplate reader which is made up of 96-well microtitre plates in which test organisms are inoculated to assess their ability to reduce tetrazolium violet to purple formazan Each well contains a carbon source which remains colourless along with the control well when not used by the test organism The resulting profile produced is referred to as a metabolic fingerprint which is then used to identify the organism through the Biolog Gram-positive or Gram-negative database Unknown profiles can be compared with both databases to create a user-defined file

- **Cobas Bact**

This is an automated system first introduced for the susceptibility testing of nonfastidious Gram-negative and Gram-positive bacteria The more recent version of the device is made up of a disposable rotor with 16 peripheral cuvettes containing dehydrated biochemical substrates an incubator a spectrophotometer reader and a computer module which is used for interpretation and printing of results It is quite accurate in the determination of clinically important Gram-negative rods but not so good with non-fermenting bacteria (Collins *et al* 1995)

- **DNA Probe**

DNA probes are generally used to find specific pieces of DNA called target DNA in a test sample A DNA probe is a short single-stranded piece of DNA molecule that is denatured by heating into a single strand before been radioactively labelled with phosphorus The radioactive phosphorus is built into the phosphate group of each nucleotide of the DNA This is then incorporated into the backbone of the DNA strands through incubation with a polymerase enzyme creating a short piece of radioactively labelled DNA with known sequence This DNA sequence is then used to hybridise with any complementary nucleic acid strands in the mixture of other single stranded DNA molecules in the innoculum The position of the hybridisation probe is determined by creating an image on a phosphorus sensitive screen, which is similar to developing an x-ray image There are several commercial DNA probes used both in clinical and environmental practices

- **PCR (Polymerase Chain Reaction)**

PCR technology is the best-established DNA amplification technique and is one of the most widely used techniques in molecular biology It is a rapid inexpensive and simple means of producing large numbers of copies of DNA molecules from a small source in a thermal cycler The components of the process are -

- A DNA template which contains the region of the DNA fragment to be amplified
- Two primers which specify the front and end of the region to be amplified
- Taq polymerase which copies the region to be amplified
- Deoxynucleotides-triphosphate (dNTP) from which the Taq polymerase builds the new DNA
- The buffer solution which provides the suitable chemical environment for the DNA polymerase

PCR is based on the repetitive cycling of three main reactions which form the basis of the whole technique. The first of the three reactions is the denaturing of the double stranded DNA. This involves heating the DNA to temperatures as high as 96°C to ensure that the strands melt and are denatured. The next stage of the process is the annealing phase which takes place at a lower temperature of between 40-65°C. A series of strong and weak ionic bonds are formed between the single stranded primers and templates that match, with the stronger bonds remaining stable for a longer period of time. During this period the polymerase attaches itself to the double stranded DNA (template and primer) and starts copying the template. This forms bases with strong ionic bonds which are not easily broken. The elongation stage is the last part of the overall reaction. It occurs at a temperature of around 72°C which is ideal for the polymerase. It is at this stage that the action of the polymerase kicks in as it extends fragments with strong ionic bonds which are already built into the base, forming DNA fragments of defined lengths. To visualise the DNA fragments formed, the PCR products are loaded onto an agarose gel (containing 0.8-4.0% ethidium bromide) using an appropriate molecular-weight marker and run through an electrophoresis unit. Under ultraviolet illumination, the size of the DNA fragments formed with the primers can be determined by comparing with the bands from the known molecular-weight markers on the ladder. The ladder is a mixture of fragments with known size to compare with the PCR fragments. The distance between each fragment on the ladder is usually represented logarithmically as show below.

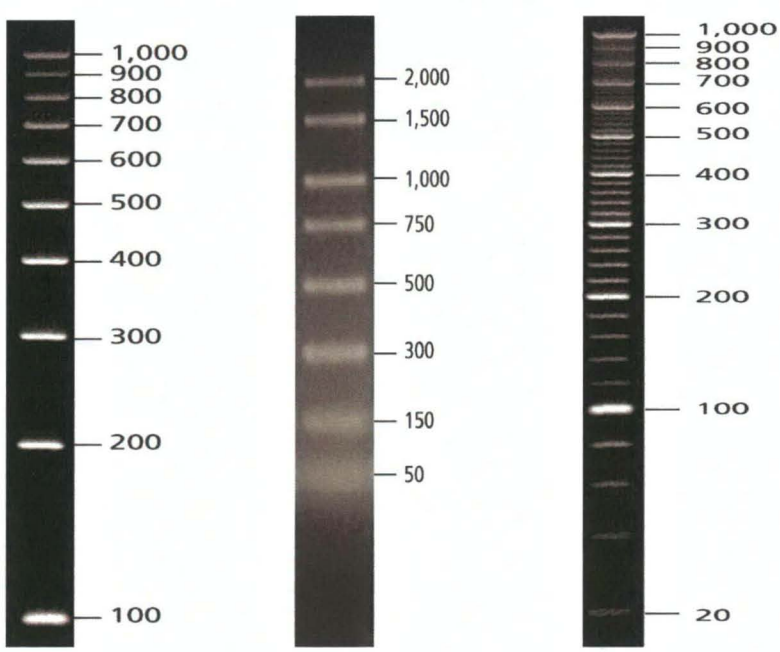


Figure 1.7 DNA ladder markers with base pairs of different sizes (Koneman *et al.*, 1997)

Several difficulties arise during the identification of typical strains of microorganisms using most of the discussed techniques These problems arise when a typical strain or rare or newly described species does not match any of those held on the respective database This problem may be compounded when the strains are mis-identified rather than unidentified and so there is a need to be familiar with taxonomic reference texts and journals that publish papers on new species There is also a need to take adequate measures to avoid contamination of the innoculum as this could seriously jeopardise the results

CHAPTER 2 SITE DESCRIPTION AND METHODOLOGY

2 1 THE HEATHROW TREATMENT FACILTY (HTF)

The Heathrow treatment facility (HTF) is an integration of wetlands and aeration ponds divided into two parts, Mayfield Farm (identified on the left hand side of Figure 2 1) and the Eastern Balancing Reservoir (shown diagrammatically on the right hand side of Figure 2 1) It has been designed to treat high-strength ADF-enriched effluent which is periodically washed off the surfaces of two of the airport s largest catchment areas The first of these is the Southern Catchment which covers an estimated 290 ha land area (78% of which is impermeable) incorporating Terminal 4 runways and cargo areas The second is the Eastern Catchment covering Terminals 1 2 and 3 maintenance areas and runways with an estimated surface area of 309 ha of which 80 % is impermeable

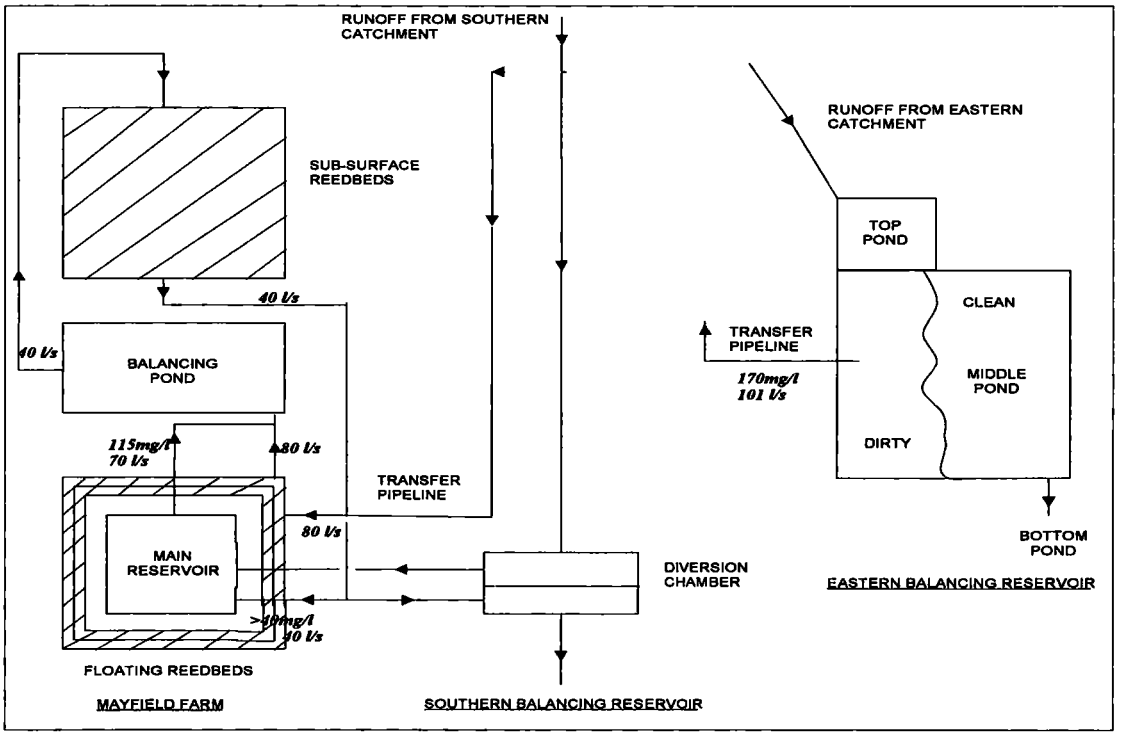


Figure 2 1 Schematic flow diagram for the HTF

2 1 1 Runoff from the Southern Catchment

Surface runoff from the Southern Catchment with a BOD concentration above 40 mg/l (the consent level allowed for direct discharge) is diverted to the Mayfield Farm part of the HTF, which is located 2 km south of the airport BOD diversions to the treatment facility are controlled automatically based on BOD readings recorded continuously by a BIOX analyser (Figure 2 2) located adjacent to the Mayfield Farm

diversion chamber. Other BiOX analysers are located at different positions within the Mayfield Farm treatment system (Table 2.1) to provide, in principle, real time BOD concentrations which can be used to determine the BOD removal performances of the different treatment components. The monitored BOD concentrations are also used to initiate water transfer between different components of the system.

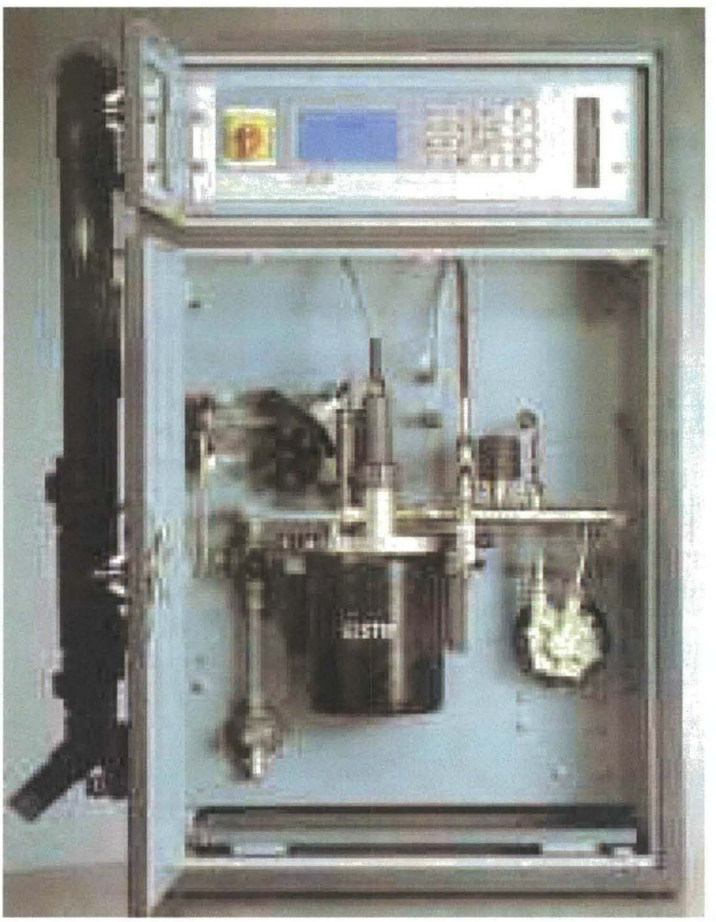


Figure 2.2: Photograph of a BiOX instrument showing the internal components and the readout panel

Table 2 1 Location and functions of the BIOX analysers within the Mayfield Farm treatment system

BIOX identification code	Location	Function
CK5	Mayfield Farm diversion chamber	Used to monitor the BOD concentrations in the runoff from the Southern Catchment The decision to divert runoff from the Southern Catchment to treatment is based on a pre set threshold value of 40 mg/l at the diversion chamber BIOX analyser
CK6	Pumping station outlet for the Mayfield Farm Reservoir (MFR)	Used to monitor the BOD concentrations within the MFR for transfer to the Mayfield Farm Balancing Pond (MFBP)
CK7	Within the Mayfield Farm Balancing Pond (MFBP)	Presently used to monitor the BOD concentration within the MFBP It was initially designed to assess the BOD concentration of the mixture of the partially treated runoff from the MFR and the Floating Reed Bed system (FLRB)
CK8a	Mayfield Farm return flow ditch	Used to monitor the BOD concentrations prior to discharge from the treatment system The water is re-diverted into the MFR if the BOD remains above 40 mg/l
CK8b	Outlet of the Floating Reed Bed system (FLRB) into the MFR	Serves the purpose of providing continuous information on the treatment efficiency achieved by the floating reedbeds relative to the water quality arriving from the Eastern Reservoir This BIOX is not currently operational

BOD concentrations are generated by the BIOX analysers based on the activities of micro-organisms present in a bioreactor Thousands of small plastic rings in the bioreactor provide a growth area for the biomass which is in constant contact with the polluted water Before reaching the bioreactor, the wastewater is diluted with oxygen-saturated dilution water by a gear pump located in the instrument The continuous inflow of the waste sample allows the biomass to adapt to the variations in the wastewater in the same manner as the activated sludge system in a biological wastewater treatment plant This water serves as a continuous source of organic material and nutrient for the sustenance of the biomass The respiration rate of the microorganisms is automatically maintained at a constant level by a feedback loop that varies the dilution ratio Increasing the concentration of the wastewater increases the respiration rate, which in turn increases the dilution rate At low BOD concentrations, the respiration rate falls and so does the dilution rate As the oxygen consumption in the bioreactor varies the volume of wastewater pumped into the chamber is controlled so as to maintain a target rate of 3 mg/l O_2 consumption The required ratio of dilution water to wastewater sample is used to determine the BOD

Polluted runoff with a BOD concentration above the consent level of 40 mg/l is directed to the Mayfield Farm Reservoir (MFR), an aerated storage reservoir with a holding capacity of 45,000 m^3 (Figure 2.3) via three discharge pipes (Figure 2.4). Primary treatment, in the form of biodegradation of organic contaminants, commences here supported by compressed air bubble-stream aeration.

Figure 2.3: View of the MFR when approximately half full

Figure 2.4: Discharge pipes delivering contaminated surface runoff to the MFR following operation of the diversion chamber

2 1 2 Runoff from the Eastern Catchment

Runoff from the Eastern Catchment of the airport discharges initially to the top pond at the Eastern Reservoir (ER) (Figure 2 1) This is the first of three ponds which have an overall holding capacity of 227 000 m^3 but only the middle pond is equipped with surface aerators This pond is divided into two sections by a floating flexible butyl curtain with the function of separating the incoming water into dirty' and clean' compartments according to the BOD concentration The flexible butyl curtain enables the capacity of the dirty side to be maximised for winter storage (up to 109 000 m^3) BOD concentrations of the airport runoff entering the top pond are monitored for diversion into either

(a) the clean side when the BOD is less than 100 mg/l in the winter or less than 50 mg/l in the summer where it is treated by aeration before discharge into the River Crane via the bottom pond

or

(b) the 'dirty side when it is more polluted (BOD higher than 100 mg/l in the winter or higher than 50 mg/l in the summer) where it is stored and aerated until the BOD concentration reduces to a threshold value of 170 mg/l

The diversion and transfer of water is based on the BOD concentrations registered by the BIOX analysers (discussed in Section 2 1 1) which are located at different points within the ER (Table 2 2)

Table 2 2 Location and functions of the BIOX analysers at the ER

CK1	Inlet discharge pipes to the ER	Used to control the distribution of runoff between the dirty' and clean sides of the reservoir
CK2	At the ER transfer pumping station	Used to monitor the BOD concentrations within reservoir
CK4	Adjacent to the ER outfall channel	Used to monitor the BOD concentrations at the outfall of the ER

The original plan was to transfer the pre-treated water from the dirty side of the middle pond of the Eastern Reservoir through the existing fire main which runs around the airport perimeter to the Mayfield Farm treatment facility a distance of approximately 3 km However this part of the overall Heathrow treatment scheme for surface runoff has not yet been put into operation At the moment the water on

the 'dirty' side is been aerated and monitored for BOD before discharge into the River Crane via the bottom pond.

2.1.3 Mayfield Farm treatment facility

The transferred water from the ER would have been directed into two canals of rafted reedbeds, each 10 m wide and approximately 500 m in circumference and covering a total surface area of 1.2 ha. These reedbeds have not been operational but modifications are currently being carried out to allow them to receive runoff from the Southern Catchment after treatment in the MFR.

Currently, the partially treated runoff from the Southern Catchment is transferred from the MFR to the MFBP (Figure 2.5). This provides further aeration within a holding capacity of 19,000 m^3 using compressed air bubble-stream aeration. The MFBP was originally designed to receive partially treated water from both the Southern and Eastern Catchments for hydraulic balancing. The installed aeration system in the system is required to reduce the predicted maximum mixed BOD concentration of 110 mg/l to 108.1 mg/l (in 0.15 days at 6°C or 0.06 days at 20°C) before subsequent transfer to the Sub-Surface Flow Reedbed (SSF) system (Revitt *et al.*, 2001).

Figure 2.5: View along the length of the Mayfield Farm Balancing Pond; aerators not operational

The SSF system is a network of twelve gravel filled reedbeds of different sizes, planted with *Phragmites australis* (common reed), with an estimated total surface area of 2.08 ha. Each bed is filled with gravel (10 mm sub-angular flint) to a depth

of 600 mm and contains hydraulically discrete cells, lined with an impermeable bentonite liner to prevent water loss or ingress to the reedbeds. There are open water channels (Figure 2.6) measuring 20 m x 2 m at the front and end of the beds and between the cells of each bed. The front and end channels (Figure 2.7) are for distribution and collection of water flowing through the beds. The channels between the cells reduce the occurrence of channelisation and short-circuiting along the whole length of the beds.

Figure 2.6: View across the sub-surface flow reedbed system showing the open water channels located between the beds

Figure 2.7 View along an exit channel for the sub-surface flow reedbed system showing the outlet flow control mechanisms

2 2 MONITORING OF THE HEATHROW TREATMENT FACILITY (HTF) COMPONENTS

The performances of the individual components of the HTF have been monitored by following the monthly changes in the BOD levels and microbial populations in water samples collected from within the individual components of the treatment facility The dissolved oxygen levels (DO) and temperatures of the water in each component were also determined *in-situ* each month (more frequently in the winter when there were increased de-icing and anti-icing activities at the airport) (Table 2 3)

Table 2 3 Details of water sample collections for laboratory analyses from the different components of the HTF (*in-situ* determinations of DO and temperature were also carried out on these dates)

Date	Eastern Reservoir		Mayfield Farm				
	Top Pond	Middle Pond	MFR	MFBP	SSF	FLRB	EXIT
29 01 04	×	×	✓	✓	✓	×	✓
02 02 04	×	×	✓	✓	✓	×	✓
06 02 04	×	×	✓	✓	✓	×	✓
10 02 04	×	×	✓	✓	✓	×	✓
13 02 04	×	×	✓	✓	✓	×	✓
19 02 04	×	×	✓	✓	✓	×	✓
27 02 04	×	×	✓	✓	✓	×	✓
18 03 04	×	×	✓	✓	✓	×	✓
25 03 04	×	×	✓	✓	✓	×	✓
07 04 04	✓	✓	✓	✓	✓	×	✓
21 04 04	✓	✓	✓	✓	✓	×	✓
04 05 04	✓	✓	✓	✓	✓	×	✓
26 05 04	✓	✓	✓	✓	✓	×	✓
15 06 04	✓	✓	✓	✓	✓	×	✓
12 07 04	✓	✓	✓	✓	✓	×	✓
18 08 04	✓	✓	✓	✓	✓	×	✓
14 09 04	✓	✓	✓	✓	✓	×	✓
18 10 04	✓	✓	✓	✓	✓	×	✓
10 11 04	✓	✓	✓	✓	✓	×	✓
22 11 04	✓	✓	✓	✓	✓	×	✓
29 11 04	✓	✓	✓	✓	✓	×	✓
13 12 04	✓	✓	✓	✓	✓	×	✓
07 01 05	✓	✓	✓	✓	✓	×	✓
21 01 05	✓	✓	✓	✓	✓	×	✓
08 02 05	✓	✓	✓	✓	✓	×	✓
16 02 05	✓	✓	✓	✓	✓	×	✓
28 02 05	✓	✓	✓	✓	✓	×	✓
07 03 05	✓	✓	✓	✓	✓	×	✓
24 03 05	✓	✓	✓	✓	✓	×	✓
14 04 05	✓	✓	✓	✓	✓	×	✓
29 04 05	✓	✓	✓	✓	✓	×	✓
20 05 05	✓	✓	✓	✓	✓	×	✓
15 06 05	✓	✓	✓	✓	✓	×	✓
26 08 05	✓	✓	✓	✓	✓	×	✓
28 09 05	✓	✓	✓	✓	✓	×	✓
27 10 05	✓	✓	✓	✓	✓	×	✓
23 11 05	×	×	✓	✓	✓	✓	✓
09 12 05	✓	✓	✓	✓	✓	✓	✓
10 02 06	✓	✓	✓	✓	✓	✓	✓
16 03 06	✓	✓	✓	✓	✓	✓	✓
27 04 06	✓	✓	✓	✓	✓	✓	✓

Key × = tests not conducted at this site on this date

✓ = tests conducted at this site on this date

The nutrient levels and their impact on bacteria populations and BOD removals in the aerated ponds have also been monitored following the commencement of a nutrient (nitrate in the form of calcium nitrate fertiliser and phosphate in the form of Triple Phosphate fertiliser) dosing regime on 12 November 2004 The performances of the system components particularly the MFR (which was further dosed with nitrate in December 2004 January February and March 2005) were assessed based on BOD removal and bacterial growth following the addition of nutrient The nitrate and phosphate levels in the system were closely monitored to ensure that no detrimental environmental effects occurred through the discharge of nutrient rich effluent

2 2 1 Sample collection and field measurements

Water samples were collected from the different components of the treatment system using pre-washed plastic sample bottles Restricted access to the aerated ponds meant samples could only be collected from the corners and approximately halfway to the bottom of the ponds The collected water samples were stored in an ice box for the journey back to the laboratory and immediately analysed in duplicates for the following parameters

- **BOD**

BOD tests were carried out in duplicate on collected water samples by using an appropriate dilution ratio determined by the BOD reading given by the relevant BIOX analyser The dilution water was made up by dissolving one sachet of HACH BOD Nutrient Buffer pillow in 3 litres of fully aerated demineralised water Each sachet contains -

i ammonium chloride
ii calcium chloride
iii ferric chloride
iv magnesium sulphate
v potassium phosphate
vi sodium phosphate

The DO content of the diluted sample was immediately determined using a Checkmate CIBA Corning DO meter ® and the process repeated after incubation at 20°C for five days in a sealed 250 ml BOD bottle The BOD of the sample was calculated from the difference between the DO readings and the dilution factor used

- **Micro-organism counts**

Water samples (1 ml) collected from each component of the treatment system were diluted by factors of between 10^{1} and 10^{-6} A sample (0 1 ml) from each serial dilution was then separately spread-plated onto the following media (contained in 90 mm petri-dishes) -

i tryptic soya agar (TSA-Oxoid) - for bacteria detection
ii sabouraud's dextrose agar (SDA-Difco) - for fungi detection
iii glycerol yeast extract agar (GYEA-prepared from base compounds) - for actinomycetes detection

Following incubation at a constant temperature of 20°C for 5 days the colonies on each plate were counted using a Gallenkamp colony counter® After taking account of the dilution factor the counts were reported as colony forming units per litre (CFU per litre)

- **Relevant chemical parameters**

Chemical tests were also conducted on the collected water samples to determine the concentrations of nitrate, phosphate ferrous ions and total iron The following tests were conducted using a HACH DR2000® spectrophotometer

i Cadmium reduction method for the determination of nitrate-nitrogen using commercial powder pillows This method is based on the reduction of nitrates to nitrites by cadmium metal An intermediate diazonium salt is formed when the nitrite ion reacts with sulfanilic acid giving an amber-coloured product

The programmed number for high ranges of nitrate nitrogen (0 1 to 30 mg/l) was entered into the HACH DR2000® spectrophotometer by pressing '355 READ/ENTER and setting the wavelength to 500 nm A sample cell was filled with 25 ml of collected water sample and the contents of one NitraVer 5 nitrate reagent powder pillow were added The sample cell was shaken vigorously for one minute before allowing a reaction time of five minutes A second sample cell was filled with 25 ml of water sample to serve as the blank This was placed in the cell holder and the ZERO key pressed until the display read 0 0 mg/l N NO_3 The blank was replaced with the prepared sample and the READ/ENTER key pressed to give the nitrate concentration in the water sample

An instrument accuracy check was carried out before the commencement of each series of tests using five prepared nitrate concentrations (potassium nitrate KNO_3) (2 mg/l, 4 mg/l, 5mg/l 8 mg/l and 10 mg/l)

ii Orthophosphate method for determination of phosphate using commercial powder pillows This method is based on the reaction of orthophosphate with molybdate in an acid medium to produce a phosphomolybdate complex This complex is reduced by ascorbic acid (present in the commercial powder pillows) to give an intense molybdenum blue colour

The stored program number (490) for phosphate determination using the HACH DR2000® spectrophotometer was entered and the wavelength set to 890 nm A sample cell was filled with 25 ml of collected water sample and the contents of one PhosVer phosphate powder pillow were added The sample cell was swirled immediately to achieve full mixing and a two-minute reaction time was allowed A second sample cell was filled with 25 ml of water sample to serve as the blank This was placed in the cell holder and the ZERO key pressed until the display read 0 0 mg/l PO_4^{3-} The blank was then replaced with the prepared sample and the READ/ENTER key pressed to give the PO_4^{3-} concentration in the water sample

An instrument accuracy check was carried out before the commencement of each series of tests using four prepared phosphate concentrations (potassium di-hydrogen phosphate KH_2PO_4) (1 mg/l, 2 mg/l 3mg/l and 4 mg/l)

iii 1, 10 phenanthroline method for determination of ferrous iron using commercial powder pillows In this technique, 1 10 phenanthroline acts an indicator forming an orange colour with an intensity proportional to the iron concentration

The stored program number (255) for ferrous iron determination using the HACH DR2000® spectrophotometer was entered and the wavelength set to 510 nm A sample cell was filled with 25 ml of collected water sample and the contents of one ferrous iron reagent powder pillow were added and swirled to mix fully A three-minute reaction time was allowed A second sample cell was filled with 25 ml of water sample to serve as the blank This was placed in the cell holder and the ZERO key pressed until the display read 0 0 mg/l Fe^{2+} The blank was then replaced with the prepared sample and the READ/ENTER key pressed to give the Fe^{2+} concentration in the water sample

An instrument accuracy check was carried out before the commencement of each series of tests using five prepared concentrations of Fe^{2+} ions as ferrous sulphate (1 mg/l 2 mg/l 3mg/l 4 mg/l and 5 mg/l)

iv FerroVer method for determination of total iron using commercial powder pillows The FerroVer reagent reacts with all soluble iron and most insoluble forms of iron (Fe^{2+} and Fe^{3+}) present in the water sample with 1 10 phenanthroline serving as an indicator

The stored program number (265) for total iron determination using the HACH DR2000® spectrophotometer was entered and the wavelength set to 510 nm A sample cell was filled with 25 ml of collected water sample and the contents of one FerroVer iron reagent powder pillow were added and swirled to mix fully A three-minute reaction time was allowed A second sample cell was filled with 25 ml of water sample to serve as the blank This was placed in the cell holder and the ZERO key pressed until the display read 0 0 mg/l Fe FV The blank was then replaced with the prepared sample and the READ/ENTER key pressed to give the total iron concentration in the water sample

2 2 2 BIOX data analysis

Because the BIOX meters play an important role in the efficient operation of the HTF it was necessary to validate the authenticity of the readings obtained Validation was also carried out to assess the BIOX instrument performance when exposed to different levels and ranges of glycol induced BOD as well as to variable concentrations of other pollutants (e g ammonia hydrocarbons and nutrients) which may influence the BIOX response A calibration protocol for each BIOX instrument in the Heathrow treatment system was established by comparing the instrument BOD readings with the corresponding laboratory BOD_5 values for water samples collected adjacent to each of the instruments

2 3 LABORATORY BASED STUDIES

The laboratory based studies discussed in this section were designed to replicate the prevailing conditions at the treatment facility in a controlled environment and to assess the impact of such conditions on the overall performance of the system

2 3 1 Assessment of the impact of iron on the biodegradation of glycol in aerated pond water samples

Following the observation of an ochrous colouration in the water bodies at the Mayfield Farm side of the HTF in February 2003 and a repeat occurrence in February 2004 biodegradation tests were conducted on water samples collected from the MFR The depletion in dissolved oxygen (DO), which is indicative of the biodegradation process was monitored with each experiment designed to investigate different aspects that could possibly hinder the biodegradation performance in the system Unless indicated the experiments were conducted in BOD bottles which have been acid-washed and left to dry before use Water

samples used in each experiment were prepared in duplicates with the content of the extra BOD bottle used to compensate for the volume loss during immersion of the DO probe and the Fe^{2+}/Fe^{3+} determination The DO concentration was determined using a Checkmate CIBA Corning DO meter® while the Fe^{2+}/Fe^{3+} concentration was determined using the 1 10 phenanthroline technique described previously (Section 2 2 1) Readings were obtained in duplicates to minimise experimental errors

2 3 1 1 Impact of the Fe^{2+}/Fe^{3+} equilibrium on BOD removal in MFR water samples in enclosed systems at 20°C

This experiment was designed to assess the impact of the Fe^{2+}/Fe^{3+} equilibrium on BOD removal (in the form of Kilfrost, a form of glycol) in MFR water sample within an enclosed system An enclosed system was used to minimise the influence of atmospheric oxygen in the oxidation of Fe^{2+} This was achieved by using sealed BOD bottles which were placed in an incubator at a constant temperature of 20°C In the experiments with added BOD a concentration equivalent to 350 mg/l was produced by dissolving 748 mg/l of propylene glycol (Kilfrost) in the water sample This is equivalent to the BOD concentration observed in the aerated ponds when the ochrous colouration was initially observed The starting Fe^{2+} concentration (when present) was 3 mg/l (equivalent to the average Fe^{2+} levels recorded at the treatment facility during the occurrence of the ochrous colouration see Chapter 4 for more details) Four separate samples containing varying components as described below, were prepared in duplicates and fully saturated with air at the start of the experiment using a portable pump The compositions of the water samples were as follows

Sample A Water/Glycol/Fe^{2+} (3 mg/l Fe^{2+} and 350 mg/l BOD)
Sample B Water/Glycol (350 mg/l BOD)
Sample C Water/Fe^{2+} (3 mg/l Fe^{2+})
Sample D Water (Blank)

The DO and the Fe^{2+} concentrations were monitored daily over a period of 3 days in all the water samples obtaining duplicate readings in each case

2 3 1 2 Impact of the Fe^{2+}/Fe^{3+} equilibrium on BOD removal in MFR water samples with different background concentrations of Fe^{2+} in enclosed systems at 20°C

This experiment was designed to assess the impact of different initial concentrations of Fe^{2+} (ranging from 1 mg/l to 4 mg/l) on the biodegradation of glycol in aerated pond water samples This experiment was expected to give an

indication of the effect of varying Fe^{2+}/Fe^{3+} concentrations on the performance of the system (in terms of BOD reduction) The compositions of the different systems were as follows

Sample 1 1mg/l of Fe^{2+} in MFR water sample containing 350 mg/l BOD
Sample 2 2mg/l of Fe^{2+} in MFR water sample containing 350 mg/l BOD
Sample 3 3mg/l of Fe^{2+} in MFR water sample containing 350 mg/l BOD
Sample 4 4mg/l of Fe^{2+} in MFR water sample containing 350 mg/l BOD

Initially all samples were fully saturated with air and then allowed to go anoxic as the biodegradation of the glycol proceeded during incubation at 20°C On achieving anoxic conditions after 8 days the samples were re-aerated to saturation and each transferred in into sealed BOD bottles in duplicates which were returned to the incubator The DO and Fe^{2+} concentrations were monitored daily (where feasible) over 7 days for each bottle

2 3 1 3 Impact of BOD (350 mg/l) on the inter-conversion of $Fe^{2+}$and Fe^{3+} in MFR water samples containing 3 mg/l of Fe^{2+} in enclosed systems at 20°C for an extended period of time

This experiment was designed to assess the effect of a prolonged anoxic phase on the inter-conversion of $Fe^{2+}$and Fe^{3+} in aerated pond water samples with different components The prolonged anoxic condition represented similar conditions to those at the MFR when the ochrous colouration was observed The composition of the water samples used were as follows

Sample A 3 mg/l of Fe^{2+} in MFR water sample containing 350 mg/l BOD
Sample B 3 mg/l of Fe^{2+} in MFR water sample

Duplicate samples of A and B were kept in an incubator at 20°C whilst monitoring the DO and the conversion of Fe^{2+} to Fe^{3+} until Sample A became anoxic The total conversion of Fe^{2+} to Fe^{3+} was adjudged to be at the point when the concentration of Fe^{2+} in Sample A was approximately zero The loss of water during the determination of Fe^{2+} concentrations and during the immersion of the DO probe was compensated for by refilling with the content of an extra BOD bottle Following the total conversion of Fe^{2+} to Fe^{3+} and the complete depletion of the DO Sample A was re-aerated to saturation and then returned to the incubator Thereafter the Fe^{2+}/Fe^{3+} inter-conversion was monitored by measuring the concentration of Fe^{2+} in the system (Sample A) along with the DO concentration This experiment was repeated using water samples containing 1000 mg/l BOD (in the form of glycol) to assess the impact of a higher BOD concentration on the inter-conversion of $Fe^{2+}$and Fe^{3+} in aerated pond water samples

2 3 1 4 Impact of stirring a Fe^{3+} precipitate at a concentration of 3 mg/l in MFR water samples with varying added components on the degradation processes in enclosed systems at room temperature

This experiment was designed to test the influence of the agitation of a Fe^{3+} precipitate on the biodegradation of glycol in aerated pond water The systems were set up in duplicates as follows

System 1 A continuously stirred system consisting of the MFR water sample dosed with 350 mg/l BOD and 3 mg/l Fe^{2+}

System 2 A replica of System 1 to provide duplicate results

System 3 A non-stirred MFR water sample system dosed with 350 mg/l BOD and 3 mg/l Fe^{2+}

System 4 A continuously stirred system of the MFR water sample dosed with 3 mg/l Fe^{2+} only

Each of the systems containing 3 mg/l Fe^{2+} was left exposed to the atmosphere until complete oxidation to Fe^{3+} had occurred Each solution was fully aerated and then glycol added to Systems 1, 2 and 3 (350mg/l as BOD) By adding the glycol directly to the Fe^{3+} any oxygen depletion due to biodegradation of glycol was not influenced by the oxidation of Fe^{2+} to Fe^{3+} A magnetic stirrer was used for the agitation in Systems 1 and 2 The DO and Fe^{2+}/Fe^{3+}concentrations were monitored over three days This experiment was repeated using a higher concentration of a Fe^{3+} precipitate (10 mg/l) in order to assess the impact of stirring a higher concentration of a Fe^{3+} precipitate within an enclosed system (BOD bottles) on the biodegradation process at room temperature

2 3 2 Assessment of the biodegradation potential of water samples from Mayfield Farm and the Eastern Reservoir

Laboratory tests were carried out to investigate the biodegradation potential of water samples collected from the Eastern Reservoir (ER) and the three operating components of the Mayfield Farm treatment system i e the Main Reservoir (MFR) the Balancing Pond (MFBP) and the Sub-surface reedbeds (SSF) by monitoring the changes in the DO levels in each sample with time Water samples (in duplicates) from each component were initially saturated with air at 6°C and 20°C and the DO concentrations determined before they were transferred to BOD bottles which had been pre-washed with acid before use The DO concentration in each system was monitored daily (where feasible) obtaining duplicate readings Water loss by immersion of the DO probe was compensated by topping-up with the content of an extra BOD bottle for each sample

2 3 3 Assessment of the Impact of 5-methyl benzotriazole (MeBT) on the biodegradation of glycol and biomass growth rates in water samples from the MFR

The impact of MeBT a corrosion inhibitor present in ADF at a typical concentration of 1% w/w (Grunden and Hernandez 2002) on the biodegradation potential of water samples collected from the MFR was assessed at 6°C and 20°C All water samples were prepared in duplicates and initially saturated with air The constituents of each sample were as follows

Sample A MFR water sample (control)
Sample B MFR water sample containing 350 mg/l BOD (in the form of glycol)
Sample C MFR water sample containing 350 mg/l BOD and 1% w/w of laboratory grade MeBT (equivalent to 7 48 mg/l based on 748 mg/l of glycol being equivalent to 350 mg/l BOD)

The DO and biomass population (using the TSA plate count technique described previously in Section 2 2 1) in each sample was monitored and reported daily at 6°C and 20°C, when feasible Loss of water through the immersion of the DO probe was compensated by topping-up with the content of an extra BOD bottle

2 3 4 Assessment of the impact of nutrient addition on the biodegradation potential of aerated pond water samples

Laboratory based experiments were conducted on aerated pond water samples collected from the Heathrow Treatment Facility (HTF) to investigate the effect of the addition of various forms of nutrient on biomass growth and BOD removal in the system

2 3 4 1 Impact of the addition of two different levels of nutrients (nitrate as KNO_3 and phosphate as KH_2PO_4) on bacteria growth rates and BOD reductions in aerated pond water samples

In this experiment the effects on bacteria growth rates and BOD reduction of two different levels of nutrients (nitrate as KNO_3 and phosphate as KH_2PO_4) in aerated pond water samples were investigated Nitrate and phosphate salts were used at two different concentration levels referred to as high and moderate The high concentration level was based on a carbon (C) nitrogen (N) phosphorus (P) molar ratio of 60 5 1 being required for the efficient mineralisation of organic compounds (McGahey and Bouwer 1992) With a BOD equivalent of 350 mg/l (in the form of propylene glycol Kilfrost) the equivalent nitrate and phosphate concentrations were 130 7 mg/l and 17 7 mg/l respectively The moderate concentration level was

set using the 50 mg/l recommended levels for nitrate in drinking water as a guideline (European standards for drinking water 1970) The equivalent moderate phosphate concentration based on the above ratio was of 6 8 mg/l The water samples were incubated at 20°C and monitored daily (where feasible) over 7 days for BOD concentrations and biomass population using the TSA plate count technique (details in Section 2 2 1)

2 3 4 2 Impact of nutrient addition (moderate level) on BOD reduction in aerated pond water samples

This experiment was designed to assess the impact of the addition of nutrients in the form of nitrate and phosphate (KNO_3 and KH_2PO_4) on BOD removal and bacteria growth rates in MFBP water samples at 6°C and room temperature Aerated pond water samples were dosed with

- 50 mg/l nitrate (as KNO_3),
- 6 8 mg/l phosphate (KH_2PO_4) and
- 350 mg/l BOD (748 mg/l of propylene glycol i e Kilfrost)

The moderate nutrient levels (also referred to as the HTF recommended nutrient level) were used in this experiment as results from the previous experiment (Section 2 3 4 1) showed no marked difference in bacteria growth rates associated with using higher nutrient levels The DO, BOD nitrate and phosphate levels and the bacteria CFU counts were monitored over time in sealed BOD bottles (in duplicates) Water samples were aerated daily for five minutes (through plastic tubes passed through the lid of the BOD bottles) using a portable automated air pump set on a timer This was to prevent the development of anoxic conditions in the sample bottles Water loss through the chemical analyses and the immersion of the DO probe was compensated for by topping up with the contents of extra BOD bottles

In later experiments conducted using the same procedure, an additional dose of 350 mg/l BOD (in the form of glycol) was introduced into the water samples 7 days after the experiment commenced as tests results showed reductions in BOD concentrations in the system with time As the nutrient levels in the system fell a further additional BOD dose (equivalent to 350 mg/l) as well as a nutrient dose (nitrate 50 mg/l and phosphate 6 8 mg/l) was introduced into the system on Day 20 This was done to assess the impact of the presence of additional nutrient and BOD on the performance of the system Full details of modifications to the different experiments are discussed in Section 5 3 1 of Chapter 5 (Experiments 1-5)

2 3 5 Assessment of BOD reduction in aerated pond water sample spiked with different concentrations of activated sludge

The aim of this experiment was to investigate the role which a different and more diverse microbial consortium (present in the activated sludge) has on the BOD reductions in aerated pond water samples at 6°C and 20°C The compositions of the different water samples used were

Sample A MFBP water sample dosed with 0 5% activated sludge and 350 0 mg/l BOD
Sample B MFBP water sample dosed with 1 0% activated sludge and 350 0 mg/l BOD
Sample C MFBP water sample dosed with 2 0% activated sludge and 350 0 mg/l BOD
Sample D MFBP water sample dosed with 5 0% activated sludge and 350 0 mg/l BOD
Sample E MFBP water sample dosed with 350 0 mg/l BOD only (Control)

Each of the systems was made up in duplicate and aerated daily for five minutes through plastic tubes passed through the cover of the sample bottles to prevent them from becoming anoxic The BOD levels in each of the water samples at the start of the experiment and on Days 8 15 and 24 were measured Water losses to the BOD tests were compensated for by the content of an extra BOD bottle

2 3 6 Assessment of BOD reduction in a river water sample dosed with 350 mg/l BOD in the form of glycol and nutrient (50 mg/l nitrate and 6 8 mg/l phosphate

Using an identical experimental set up to that described in Section 2 3 5 the BOD levels in water samples from the Pymmes Brook in North London dosed with 350 mg/l BOD in the form of glycol were monitored at 6°C The aim of the experiment was to ascertain if the Mayfield Farm aerated ponds contained contaminants which were hindering the biodegradation of glycol in the collected water samples This experiment was also designed to assess the ability of the biomass population in the river to degrade glycol The water sample was aerated daily for five minutes in a similar manner to that described in Sections 2 3 5 A control experiment using MFBP water instead of Pymmes Brook water sample was set up for comparison The components of the two systems (in duplicate) used are described below

A river water sample dosed with 350 mg/l BOD 50 mg/l nitrate and 6 8 mg/l phosphate

B aerated pond water sample dosed with 350 mg/l BOD 50 mg/l nitrate and 6 8 mg/l phosphate

The changes in BOD nutrients and bacteria population concentrations were monitored on Days 0 4 7 11 15 20 and 27 of the experiment Water losses during the measurements were compensated for by the content of an extra bottle

2 3 7 Assessment of BOD reduction in water samples collected from the Eastern Reservoir (ER) and different forms (filtered and un-filtered) water samples obtained from washing the roots of plants from the Mayfield Farm subsurface flow reed bed system (SSF)

The BOD reductions in water samples collected from the ER and in filtered and unfiltered water samples obtained by thoroughly washing (with sterile distilled water) the roots of plants from the SSF were monitored using identical experimental set ups to those discussed in the previous sections (Sections 2 3 5 and 2 3 6) The aim of the experiment was to assess the ability of the biomass population present in an environment different to the aerated ponds to degrade glycol Details of the different experiments conducted are discussed below

2 3 7 1 Comparison of the BOD reduction in filtered and un-filtered water samples obtained from the washing of plants' roots from the SSF

The BOD reductions in filtered and un-filtered water samples collected by thoroughly washing the roots of plants from the SSF with sterile distilled water were monitored The aim of the experiment was to establish if the unfiltered debris associated with the roots and sediments had any significant effect on the BOD reduction achieved in the SSF Water samples from washing the plant roots with sterile distilled water were collected in a beaker During the washing process, the roots were shaken vigorously to assist the release of attached sediment and adsorbed materials The resultant washings were divided into two equal portions in two separate 250 ml conical flask Debris dead roots and sediments were removed from one half of the water sample by filtering through a 100 µm filter paper In order to assess the extent of the influence of the un-filtered material, both filtered and unfiltered water samples were subjected to further dilutions (1 100 and 1 1000) using sterile distilled water and dosed with 350 mg/l BOD in the form of glycol The components of the different system used are summarised below

A_0 Unfiltered water sample obtained from root washings dosed with 350 mg/l BOD and the HTF recommended nutrient levels

A_1 1 in 100 dilution of unfiltered water sample from root washings dosed with 350 mg/l BOD and the HTF recommended nutrient levels

A_2 1 in 1000 dilution of unfiltered water sample from root washings dosed with 350 mg/l BOD and the HTF recommended nutrient levels

B_0 Filtered water sample from root washings dosed with 350 mg/l BOD and the HTF recommended nutrient levels

B_1 1 in 100 dilution of filtered water sample from root washings dosed with 350 mg/l BOD and the HTF recommended nutrient levels

B_2 1 in 1000 dilution of filtered water sample from root washings dosed with 350 mg/l BOD and the HTF recommended nutrient levels

The filtered and un-filtered water samples were aerated daily for five minutes using a portable automated pumping device to prevent them from becoming anoxic The BOD levels and the biomass populations were monitored in both samples on Days 0, 7, 14 and 21 of the experiment

2 3 8 Assessment of BOD reductions in sterile distilled water samples inoculated with the different bacteria strains from the Mayfield Farm aerated ponds, Eastern Reservoir and the Mayfield Farm Sub-surface reedbed system

The different bacteria strains observed in the course of the biodegradation experiments described so far were isolated using the streak-plate technique (Sections 1 4 1 and 2 2 1) From a mixture of colonies cultured on TSA plates, a total of 3 bacteria strains from the Mayfield Farm aerated ponds (MFR and MFBP) and 13 bacteria from the root washings of plants from the Mayfield Farm Sub-surface reedbed systems (SSF) were isolated as pure colonies from the water samples These colonies were differentiated by their sizes colours and shapes (more details in Chapter 7) The different experiments discussed in the sections that follow were conducted using these isolated strains

2 3 8 1 The impact of two different nutrient levels on the bacteria growth rates and BOD removal potentials in sterile distilled water by bacteria strains isolated from the Mayfield Farm aerated ponds and the Sub-surface reedbed system

This experiment involves the 3 different strains of bacteria which were isolated from the Mayfield Farm aerated ponds water samples using cultured laboratory media The effects of two nutrient levels the moderate nutrient levels used above (Section 2 3 4 2) and those provided by minimum medium on the biodegradation process were investigated The composition of the minimum medium was

- 7 0 g/l K_2HPO_4
- 2 0 g/l KH_2PO_4

- 1 0 g/l $(NH_4)_2SO_4$
- 0 5 g/l sodium citrate
- 0 1 g/l $MgSO_4$
- 3 0 ml of at trace element solution made up of
    - 30 0 mg/l H_3BO_3
    - 20 0 mg/l $CoCl_2$ $6H_2O$
    - 10 0 mg/l $ZnSO_4$ $7H_2O$
    - 3 0 mg/l $MnCl_2$ $4H_2O$
    - 3 0 mg/l $NaMoO_4$ $2H_2O$
    - 2 0 mg/l $NiCl_2$ $6H_2O$ $6H_2O$
    - 0 79 mg/l $CuCl_2$ $6H_2O$

The aim of this experiment was to assess the ability of the each of the strains to remove BOD (in the form of glycol) in sterile distilled water samples One pure colony of each strain was isolated from the respective laboratory media using a sterilised loop before inoculating 100 ml of sterile distilled water samples The water samples were then dosed with 350 mg/l BOD (in the form of glycol) and two different nutrient levels (minimum medium and the moderate nutrient levels 50 mg/l nitrate and 6 8 mg/l phosphate) These samples were prepared in triplicate and placed in a shaking incubator at 20°C to prevent the water samples from going anoxic The BOD concentrations and biomass populations in the samples were monitored obtaining duplicate readings where feasible Water losses from the test samples during the BOD analyses and biomass monitoring were compensated for by topping with the contents of extra bottles

This experiment was repeated using a combination of the first two bacteria strains a third strain isolated from the diversion chamber and a combination of all 3 isolates The entire procedure was repeated in a follow-up experiment using a lower BOD concentration of 50 mg/l in order to compare the performance of the strains in the presence of lower BOD concentrations Details of other modifications to each experiment are discussed in Section 5 7 1 (Experiments 1-4) Identical experiments were carried out using systems containing another 13 bacteria strains isolated from the SSF alone and in combination with the first 3 isolates using only the moderate nutrient levels (50 mg/l nitrate and 6 8 mg/l phosphate) in sterile distilled water samples dosed with 350 mg/l BOD in the form of glycol (Experiment 5 in Section 5 7 1)

2 3 8 2 Investigation of the significance of sediments on BOD reduction in a water sample inoculated with isolated bacteria strains from the washing of SSF plant roots

The experiment was designed to investigate why the rapid BOD reductions recorded in the water samples collected from plant root washings were not repeated in systems inoculated with bacteria isolates from the SSF The experiment also aims to assess the possible role played by the particulate materials present in the system The BOD reduction in a sterile water sample (containing 350 mg/l BOD in the form of glycol) inoculated with all 13 bacteria strains isolated from the roots of plants from the SSF were monitored in systems containing 100 mg of sterilised sediments also obtained from the SSF The sterilisation was carried out using the autoclave which is ideally capable of destroying resident microorganisms in the sediments which might interfere with the performance of the inoculated strains The components of the systems used are summarised below

Sample A-sterile water sample dosed with 350 mg/l BOD and the HTF recommended nutrient levels containing sterile sediments and placed in a shaking incubator

Sample B-sterile water sample dosed with 350 mg/l BOD and the HTF recommended nutrient levels with no sediments and placed in the shaking incubator-control experiment

Sample C-sterile water sample dosed with 350 mg/l BOD and the HTF recommended nutrient levels containing sterile sediments with regular aeration through a pump

Sample D-sterile water sample dosed with 350 mg/l BOD and the HTF recommended nutrient levels with no sediments using regular aeration through a pump-control experiment

The BOD concentration in the water was monitored in a shaking incubator (to ensure that the water samples do not become anoxic) at 20°C obtaining duplicate readings on days 0, 5, 12 19 and 23

2 3 9 Assessment of the role of SSF plant root exudates on BOD reduction in sterile distilled water sample

In this experiment the possible role played by the presence of root exudates (mixtures of complex sugars vitamins amino acids purines nucleosides inorganic ions, gaseous molecules, enzymes and root border cells) on the BOD reduction in sterile distilled water sample dosed with 350 mg/l BOD (in the form of glycol) were investigated Plant roots from the SSF were thoroughly washed with

sterile distilled water to remove all associated sediments For root exudate collection the washed roots of plants from the SSF were left to photosynthesise in 2000 ml of sterile deionised water in a beaker for 7 days before dosing with 350 mg/l BOD in the form of glycol Half (1000 ml) of the water sample was poured into another beaker and then autoclaved (to kill off any living microorganisms present) Both water samples (autoclaved and non-autoclaved) were then further subdivided into 500 ml in separate beakers, each with one portion being inoculated with the 13 strains of bacteria and the other not inoculated The compositions of the resulting water samples were as follows

Sample A Un-sterilised water sample (non-autoclaved) dosed with 350 mg/l BOD and inoculated with 13 strains

Sample B Un-sterilised water sample (non-autoclaved) dosed with 350 mg/l BOD only

Sample C Sterilised water sample (autoclaved) dosed with 350 mg/l BOD and inoculated with 13 strains

Sample D Sterilised water sample (autoclaved) dosed with 350 mg/l BOD only

Each system was placed in a shaking incubator at 20°C and the BOD levels monitored over time, obtaining duplicate values on Days 0 5 7 and 14

2 3 10 Enumeration of bacteria, fungi and actinomycetes in the washings of sediments and plant roots from the SSF and an assessment of their ability to reduce BOD in water samples dosed with glycol

In order to identify the biomass population responsible for the rapid BOD reductions recorded in systems associated with the roots and sediments from the SSF the presence of bacteria, fungi and actinomycetes in different water samples were observed using the respective nutrient media (see details in Section 2 2 1) Different washing technique intensities were used in order to compare the degree of microbial attachments and associated diversity Sterile distilled water was used for the washing in each case The different techniques used are summarised below

Sample 1 Light washing of the roots of plants from the SSF This was achieved by gently running 100 ml of sterile distilled water over the plant roots from the SSF

Sample 2 Vigorous washing of the roots of plants from SSF The plant roots from the SSF were washed and shaken vigorously in 200 ml of sterile distilled water to release any attached sediment or substance

Sample 3 Half of Sample 2 (100 ml) was filtered using a 100 µm filter paper to exclude associated solids

Sample 4 100 ml of sterile distilled gently run over approximately 100 mg of sediments collected from the SSF

Sample 5 About 100 mg of sediments from the SSF vigorously washed in 200 ml of sterile distilled water

Sample 6 Half of Sample 5 (100 ml) was filtered using a 100 µm filter paper to exclude associated solids

2 3 10 1 Assessment of the ability of all the isolated microorganisms to reduce BOD in sterile distilled water samples dosed with BOD (in the form of glycol) and nutrient (50 mg/l nitrate and 6 8 mg/l phosphate)

In this experiment the biodegradation abilities of the 3 fungi and 2 actinomycetes strains along with the 13 bacteria strains isolated from the roots and sediments from the SSF through vigorous washing (as described in Section 2 3 10) were investigated The aim of the experiment was to investigate the ability of these isolates to remove BOD in the form of glycol in sterile distilled water samples In systems similar to the ones already described (section 2 3 7) sterile distilled water samples containing 350 mg/l BOD (in the form of glycol) were inoculated with the pure strains of the isolates which were pre-washed in sterile distilled water to exclude any external medium 25ml of each solution was then placed in a shaking incubator at 20°C in duplicates The BOD levels in the water samples were then monitored in each of these samples obtaining duplicate readings on Days 0 5 7, 14 and 21 The compositions investigated in terms of the different strains were

Sample 1 sterilised water sample dosed with 350 mg/l BOD and containing all 3 fungi strains

Sample 2 sterilised water sample dosed with 350 mg/l BOD and containing all actinomycetes strains

Sample 3 sterilised water sample dosed with 350 mg/l BOD and containing all actinomycetes and fungi strains

Sample 4 sterilised water sample dosed with 350 mg/l BOD and containing a consortium of all bacteria strains from the aerated ponds and the plants roots from the SSF as well as the all fungi and actinomycetes from the roots and sediments from the SSF (including the 13 bacteria strains initially isolated from the plant roots the 3 fungi and 2 actinomycetes strains)

2 4 PILOT SCALE VERTICAL FLOW REEDBED COLUMN EXPERIMENT

These experiments were designed to simulate the removal processes prevalent within the Heathrow treatment facility (HTF) wetlands but under controlled dosing

conditions. Eight small scale vertical flow systems were designed and constructed to assess the variations in BOD removal during the exposure of planted columns and substrate only columns to different de-icant, nutrient and ferrous iron dosing regimes. Each column was 0.75 m high, with outer and inner diameters of 0.18 m and 0.14 m respectively and an overall holding capacity of 6.5 litres (Figure 2.8). The columns were filled with gravel substrate collected from the upper, middle and lower layers of one of the beds at the Mayfield Farm sub-surface flow reedbed system (SSF) on 20 August 2004 and transferred in the same order to the columns. One carefully collected *Phragmites* plant from the SSF, was planted in each of four columns while the remaining four were filled with gravel substrate only.

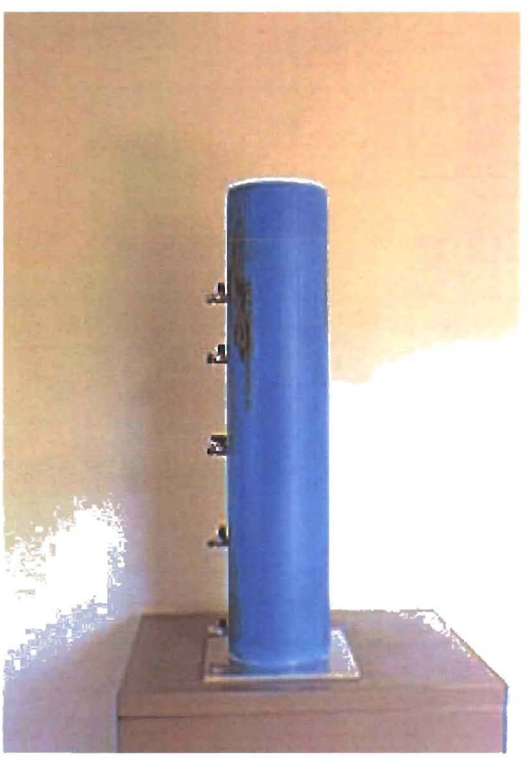


Figure 2.8 Vertical column used in the pilot scale vertical flow reedbed experiments

The columns were conditioned by filling each initially with 5 litres of Mayfield Farm aerated pond water. The water was left standing in each column for up to 5 days before draining the columns through the outlet pipes located at different depths (Figure 2.8). The columns were then refilled with aerated pond water and the above procedure repeated for three weeks. This was to allow the plants to adapt to their new environment and encourage the root structures to develop within the substrate column. Each column was then fed in batches with 5 litres of Mayfield

Farm aerated pond water from overhead containers containing the different components listed in Table 2 4 The responses of the columns to different dosing regimes were followed by monitoring the concentrations of BOD nutrients and iron as well as the bacteria populations at different depths in the columns along with the temperatures at intervals of 7 and 21 days after the commencement of each test 100 ml water samples were collected in sample bottles from the different depths of each column on Days 7 and 21 and the respective laboratory tests conducted using the different techniques described earlier in Section 2 2 1 Initial results showed that there were no marked differences in the values obtained for these parameters at the different depths of the columns As a result subsequent sample collections focused on the use of the outlet pipe at the bottom of the columns

Table 2 4 The different dosing solutions used for the planted and substrate-only columns in the pilot scale vertical flow reedbed experiment

Column components	Constituents of dosing solution
Planted	350 mg/l BOD (as Kilfrost) in Mayfield Farm aerated pond water spiked with 50 mg/l of nitrate (as KNO_3) and 6 8 mg/l of phosphate (as KH_2PO_4) to assess the impact of nutrient addition on the BOD removal capacities in the columns
Substrate-only	
Planted	350 mg/l BOD in Mayfield Farm aerated pond water spiked with 3 mg/l Fe^{2+} (as $FeSO_4$) to investigate the impact of the presence of Fe^{2+}/Fe^{3+} equilibrium reactions on BOD removal to ascertain to what extent the high concentrations of iron observed in the reedbed system particularly in the outlet channel affect its performance
Substrate-only	
Planted	BOD and additive-free Mayfield Farm aerated pond water to serve as a control
Substrate only	
Planted	350 mg/l BOD in Mayfield Farm aerated pond water to assess the BOD removal capacities in the columns planted and substrate-only when free of any additional nutrient and iron
Substrate only	

The vertical flow reedbed experiments were repeated seven times between September 2004 and September 2005 (Table 2 5) to represent the conditions prevalent at different times of the year and to record any seasonal variations in BOD reductions in the columns The columns were re-conditioned with additive-free Mayfield Farm aerated pond water for at least a week before the commencement of each new experimental run The water in each column was then released through the outlet pipes of the columns This was to ensure that the effect

of any residual constituents (BOD nitrate phosphate or iron) from a previous test were minimised with respect to the new test

Table 2 5 Identification of the dates and durations of the 7 pilot scale vertical flow reedbed column experiments

Experiments	Start and Finish date
A	20 September 2004 to 11 October 2004
B	25 October 2004 to 15 November 2004
C	29 November 2004 to 20 December 2004
D	25 January 2005 to 15 February 2005
E	25 May 2005 to 15 June 2005
F	28 June 2005 to 19 July 2005
G	2 September 2005 to 23 September 2005

2 5 IDENTIFICATION OF MICROORGANISMS FROM THE DIFFERENT COMPONENTS OF THE HEATHROW TREATMENT FACILITY (HTF)

The successful operation of the HTF depends on the existence and ideally the enhancement of the microbial consortium in the system, which is capable of effective BOD reduction The main aim of the experiments described in this section is to establish the identity of the species capable of doing this Using the streak-plate technique already described (Section 1 4 2) bacteria fungi and actinomycetes from the different parts of the HTF were isolated using the TSA, SDA and GYEA agar media respectively Different biochemical tests were conducted on each of the 13 different bacteria strains from the plant roots and another 3 isolated from the aerated ponds and the diversion chamber PCR (Polymerase chain reaction) analyses were also carried out on all these bacteria along with the actinomycetes and fungi isolated from the SSF plant roots

Gram Staining

The Gram-staining test was used to separate the bacteria cells into two broad groups (Gram-positive and Gram-negative) based on the chemical and physical properties of their cell walls A black-blue or purple colour indicates that the test organism is Gram-positive while a pink colour is an indication that the organism is Gram-negative The Gram-staining tests were performed on pure colonies of bacteria strains isolated from the aerated ponds and those from the roots of plants and sediments from the SSF The test which is in four parts involves the use of certain dyes to make a bacterial cell stand out against its background

The test colony was picked from the TSA culture plate using a sterilised loop This was then smeared in a drop of sterile water on to a sterile slide The suspension

produced was dried and fixed on the sterile slide by passing the slide over a Bunsen burner flame Care was taken not to hold the slide too long over the flame The reagents used to perform the test were

- Crystal Violet solution (the Primary Stain)
- Iodine solution (the Mordant)
- Decolorizer (ethanol)
- Safranin (the Counterstain)
- Distilled water

The test was conducted by performing the following steps

Step 1

The slide containing the bacteria colony was placed on a clean slide holder over the sink and flooded completely with the crystal violet solution After allowing to stand for about 60 seconds the slide was washed with sterile distilled water for 5 seconds

Step 2

Immediately after washing with sterile distilled water the specimen was flooded with the iodine solution and left to stand for about one minute before washing with water for 5 seconds and immediately proceeding to step 3

Step 3

At this stage the decolouriser ethanol was added to the specimen in a dropwise manner until the blue-violet colour was no longer emitted from the specimen, leaving a dull blue-violet mark on the slide This step was somewhat subjective because adding too much decolouriser could result in a false Gram-negative result and not using enough may yield a false Gram-positive result

Step 4

The fourth and final step involved flooding the slide with Safranin before allowing it to stand for about one minute to allow the bacteria to incorporate the reagent The Gram-positive cells are able to incorporate little or no counter-stain (Safranin) and remain blue-violet in appearance while the Gram-negative bacteria take on the pink colour of the Safranin Any excess dye was removed from the slide by rinsing with water for 5 seconds before drying carefully with filter paper and then viewing under the microscope

2 5 1 API biochemical test

The next stage of identification tests carried out on the bacteria isolates were the API biochemical tests The tests are a combination of between 20 and 32 different biochemical tests used for the identification of most bacteria strains selected as colonies from the surface of solid nutrient agar Four different API tests kits were used in this study Each test kit is made up of a plastic strip containing microtubes with different substrates a reaction plastic case with a lid and different test reagents In each case the strip of microtubes containing different substrates were inoculated with the bacterial suspension (obtained by suspending a reasonably sized colony in 1 ml sterile distilled water) and then incubated at the specified temperature (between 25-30°C) The strips were placed on incubation boxes (tray and lid) with honey-combed wells containing sterile distilled water to keep the reaction box moist whilst in the incubator During incubation the metabolic activities of the added bacteria yield colours either spontaneously or after the addition of a reagent Table 2 6 is a summary of the tests conducted in each of the four tests used The response of each test and the implication (positive or negative) is also shown The output index generated by the individual reactions in each test is recorded on the reaction slip provided with the kit The profile created is used to match the respective reference database to provide an identification of the bacteria species

Table 2 6 The reading table for the different API biochemical tests showing the implications of the results of each test

TESTS	SUBSTRATES	REACTIONS AND ENZYMES	RESULTS		API20E	APIStaph	APIStrep	ID32Staph
			NEGATIVE	POSITIVE				
ONPG	Ortho-nitro phenyl B D galactopyranoside	Beta-galactosidase	Colourless	Yellow	√	X	X	X
ADH	Arginine	Arginine dihydrolase	Yellow	Red / orange	√	√	√	√
LDC	Lysine	Lysine decarboxylase	Yellow	Orange	√	X	X	X
ODC	Ornithine	Ornithine decarboxylase	Yellow	Red / orange	√	X	X	√
CIT	Sodium citrate	Citrate utilisation	Pale green/yellow	Blue green/blue	√	X	X	X
H_2S	Sodium thiosulphate	H_2S production	Colourless/grey	Black deposit/thin line	√	X	X	X
URE	Urea	Urease	Yellow	Red/orange	√	√	X	√
TDA	Tryptophane	Tryptophane deaminase	Yellow	Dark brown	√	X	X	X
IND	Tryptophane	Indole production	Yellow then colourless	Red then pink	√	X	X	X
VP	Sodium pyruvate	Acetoin production	Colourless	Pink/red	√	√	X	√
GEL	Kohn s gelatin	Gelatinase	No diffusion of black pigment	Diffusion of black pigment	√	X	X	X
GLU	Glucose	Fermentation/oxidation	Blue/blue-green*/Red**	Yellow/grey yellow	√	√	X	√
MAN	Mannitol	Fermentation/oxidation	Blue/blue green*/Red**	Yellow	√	√	√	√
INO	Inositol	Fermentation/oxidation	Blue/blue-green	Yellow	√	X	X	X
SOR	Sorbitol	Fermentation/oxidation	Blue/blue-green	Yellow	√	X	√	X
RHA	Rhamnose	Fermentation/oxidation	Blue/blue-green	Yellow	√	X	X	X
SAC	Sucrose	Fermentation/oxidation	Blue/blue-green*/Red**	Yellow	√	√	X	√
MEL	Melibiose	Fermentation/oxidation	Blue/blue green*/Red**	Yellow	√	√	X	X
NO_3 NO_2	GLU tube	NO_2 production and reduction to N_2	Yellow	Red	√	√	X	√
PAL	B naphthyl phosphate	Alkaline phosphate	Yellow*/colourless**	Violet	X	√	√	X
RAF	D raffinose	Acidification	Red	Yellow	X	√	√	√
XLY	D xylose	Acidification	Red	Yellow	X	√	X	X
MDG	Methyl-αD glucopyranoside	Acidification	Red	Yellow	X	√	X	X
NAG	N-acetyl glucosamine	Acidification	Red	Yellow	X	√	X	X

* colour response of inoculum to API20E test

** colour response to APIStaph test

*** colour response to APIStrep test

Table 2 6 (continued) The reading table for the different API biochemical tests showing the implications of the results of each test

TESTS	SUBSTRATES	REACTIONS AND ENZYMES	RESULTS		API20E	APIStaph	APIStrep	ID32Staph
			NEGATIVE	POSITIVE				
HIP	Hippuric acid	Hydrolysis	Colourless	Black	X	X	√	X
ESC	Esculin ferric citrate	Hydrolysis	Colourless	Black	X	X	√	√
PYRA	Pyroglutamic acid	Arylamidase	Colourless/pale orange	Orange	X	X	√	√
αGAL	αGalactopyranosi de	αGalactosidase	Colourless	Violet	X	X	√	X
*B*GUR	Glucuronic	Glucuronidase	Colourless/pale orange	Blue	X	X	√	√
*B*GAL	*B*Galactopyranosi de	*B*Galactosidase	Colourless/pale orange	Violet	X	X	√	√
LAP	Leucine-naphthylamide	L Aminopeptidase	Colourless/pale orange	orange	X	X	√	X
RIB	D ribose	Acidification	Red	Yellow	X	X	√	√
INU	Inulin	Acidification	Red	Yellow	X	X	√	X
AMD	Starch	Acidification	Red	Yellow	X	X	√	X
GLYG	Glycogen	Acidification	Red	Yellow	X	X	√	X
CEL	D-cellobiose	Fermentation	Red	Yellow	X	X	X	√
NAG	N acetyl glucosamine	Fermentation	Red	Yellow	X	X	X	√
TUR	D turanose	Fermentation	Red	Yellow	X	X	X	√
MOB	API M medium	Motility	Non motile	Motile	√	X	X	X
McC	McConkey medium	Growth	Absence	Presence	√	X	X	X
OF F	Glucose	Fermentation under mineral oil and oxidation in air	Green	Yellow	√	X	X	X
OF O					√	X	X	X
FRU	D fructose	Acidification	Red	Yellow	X	√	X	√
MNE	D mannose	Acidification	Red	Yellow	X	√	X	√
MAL	D-maltose	Acidification	Red	Yellow	X	√	X	√
LAC	D-lactose	Acidification	Red	Yellow	X	√	√	√
TRE	D trehalose	Acidification	Red	Yellow	X	√	√	√
XLT	Xylitol	Acidification	Red	Yellow	X	√	X	X
AMY	Amygdalin	Fermentation/oxid ation	Blue/blue-green	Yellow	√	X	X	X
ARA	Arabinose	Fermentation/oxid ation	Blue/blue-green	Yellow	√	X	√	√
OX	On filter paper	Cytochrome oxidase	Colourless	Violet	√	X	X	X

* colour response of inoculum to API20E test

** colour response to APIStaph test

*** colour response to APIStrep test

2 5 2 Polymerase Chain Reaction (PCR)

The PCR technique was used to confirm the group to which the isolates used in this study belong through the use of the commercially available universal primers shown in Table 2 7

Table 2 7 Genus specific universal primers used for the detection of the different isolates

Target	Primer forward and reverse sequence	Size (bp)
Pseudomonas	5 -*GACGGGTGAGTAATGCCTA-3*	618
	5 -*CACTGGTGTTCCTTCCTATA-3*	
Staphylococcus	5 -*GGCCGTGTTGAACGTGGTCAAATCA-3*	370
Specific	5 -*TIACCATTTCAGTACCTTCTGGTAA-3*	
Enterococcus	*5 -TACTGACAAACCATTCATGATG-3*	110
Specific	5 - *AACTTCGTCACCAACGCGAAC-3*	
Actinomycetes	5 -*GGATGAGCCCGCGGCCTA-3*	nd
	5'-*CCGCGGCTGCTGGCACGTA-3*	
Fungi	*5 - GGAAGTAAAAGTCGTAACAAGG-3*	333
	5 - TCCTCCGCTTATTGATATGC-3	

nd not defined by the manufacturer

2 5 2 1 Colony PCR Protocol

Reasonably sized colonies of bacteria and actinomycetes isolates were chosen from the surface of the respective solid agar plate using a sterilised loop which was then suspended in 100 µl double-distilled sterile water In the case of fungi approximately 25 mg of fungal element was obtained from the SDA plates and then suspended in 100 µl double-distilled sterile water These were thoroughly mixed on a vortex before they were used for PCR Pure colonies of *Pseudomonas putida Enterococcus faecalis Staphylococcus aureus Streptomyces* and *Fonsecaea sp* were also resuspended in 100 µl double-distilled sterile water to be used as positive controls The amplification of the targeted DNA was conducted using 25 µl reaction volumes each containing the following

-1 0 µl of colony suspension
-2 5 µl of PCR buffer solution (200 mM Tris-HCl 25 ml EDTA 0 5% wt/vol sodium dodecyl sulphate and 250 mM NaCl)
-0 3 µl of deoxyribonucleotide triphosphate (dNTP) solution

-2 5 µl of $MgCl_2$ solution
-1 0 µl of the first part of the reference primer template
-1 0 µl of the other part of the reference primer template
-0 2 µl of Taq polymerase
-16 5 µl of sterile double distilled water

The PCR was performed using a Thermal Cycler a laboratory device in which the DNA amplification is carried out This device contains a thermal block with holes in which the tubes containing the PCR reaction mixtures are inserted The temperature of this block is raised and lowered in discrete pre-programmed steps The hot bonnet a heated plate that presses against the lids of the reaction tubes prevents condensation of water from the reaction mixtures to the inside of the lids The following program sequence was used to conduct the PCR reaction

- 95°C for 5 minutes (denaturing)
- 30 cycles consisting of
  - -95°C for 40 seconds
  - -55°C for 40 seconds and
  - -72°C for 1 minute (annealing),
- 72°C for 10 minutes (elongation) after which the reaction was left on hold at 4°C overnight

After thermal cycling 5 0 µl of the amplified product was subjected to electrophoresis using 2 0% agarose gel containing 1 0% ethidium bromide and the loading dye along with the 100 bp ladder in the electrophoresis chamber for 45 minutes at 100 volts Two gels were run simultaneously in order to accommodate all the PCR products Each gel was made up of 3 rows of 16 wells in which the PCR products were inoculated The gels were then visualised using UV light before the images were captured on photographic plates The sizes of the DNA fragments formed with the primers were determined by comparing the observed bands with those from the 100 bp molecular-weight markers on the ladder

CHAPTER 3 AN ASSESSMENT OF THE PERFORMANCE OF THE HEATHROW TREATMENT FACILITY

The measurement of the BOD levels temperature DO nitrate and phosphate concentrations and biomass populations for the different components of the HTF (Heathrow Treatment Facility) between January 2004 and April 2006 have been used to assess the overall performance of the system The seasonal variations in these key parameters and their implications for the performance of the system are discussed in this chapter The authenticity of the BIOX meters is also assessed by comparing laboratory BOD results with the field-based readings registered by these devices

3 1 VARIATIONS IN TEMPERATURE, BOD CONCENTRATIONS AND DO LEVELS IN THE INDIVIDUAL COMPONENTS OF THE HTF

3 1 1 Discussion of trends observed in each component

The Mayfield Farm Main Reservoir (MFR)

The results of routine laboratory BOD tests conducted on water samples collected from the MFR between January 2004 and April 2006 are shown in Figure 3 1 along with the in-situ temperature and DO levels for the water during each visit Laboratory BOD results showed that the BOD level in the MFR immediately after the de-icing events in January 2004 was 295 0 mg/l (Figure 3 1) The DO concentration in the MFR during the 2 February 2004 visit was 1 4 mg/l which increased throughout February reaching 9 4 mg/l by 27 February 2004 BOD levels in the MFR remained well above 200 0 mg/l for most of February apart from two occasions (10 and 27 February) when they fell to 85 0 mg/l and 92 5 mg/l respectively The average temperature during the month of February was 7 8$\pm$2 2°C In March 2004 with the average temperature at 7 9$\pm$1 0°C the BOD level remained fairly constant with values of 255 0 mg/l and 267 5 mg/l being recorded on the 18 and 27 March followed by a gradual fall to 190 0 mg/l towards the end of April 2004 when the average temperature had risen to 11 4$\pm$2 7°C Coinciding with the average temperature rising to 15 7$\pm$4 3°C during May 2004 the BOD level in the MFR had fallen dramatically to 64 0 mg/l and remained low throughout the entire summer The DO level in the MFR also remained relatively low for most of the summer because the aerators in the system were not fully operational until November when an immediate rise to 10 0 mg/l was observed on 10 November 2004 (Figure 3 1)

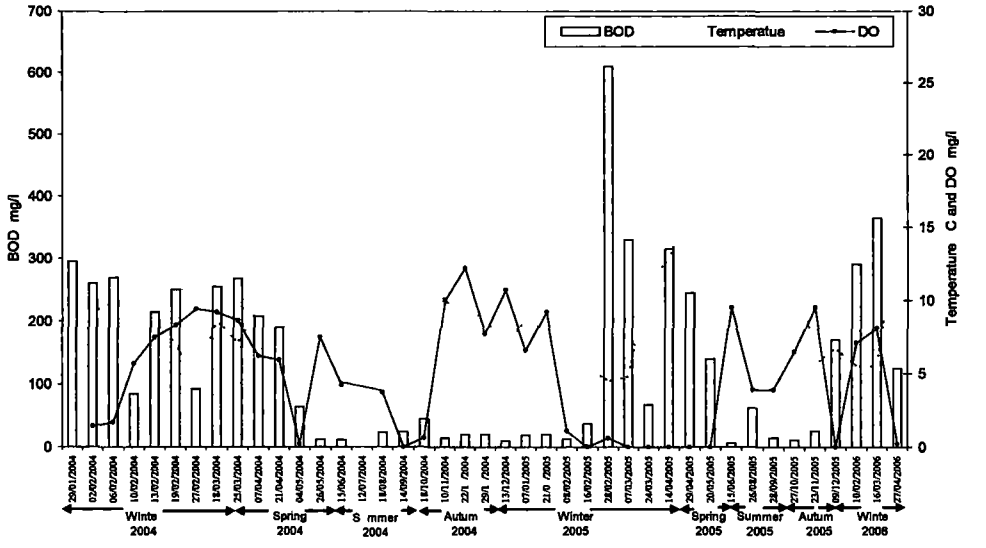


Figure 3 1 Variations in BOD concentrations, DO levels and temperature in the MFR between January 2004 and April 2006

During the winter of 2004-2005 de-icing activities did not commence at Heathrow until late February 2005 after which the BOD level in the MFR reached the elevated value of 610 0 mg/l on 28 February 2005 (Figure 3 1) Unlike the previous winter when the DO remained relatively high in the MFR due to continuous aeration intermittent aeration between February and April 2005 and a malfunction in the aeration system later in April (observed during the 29 April visit) meant the DO level in the MFR remained zero for most of this period (Figure 3 1) The average temperature recorded in February 2005 (5 8±1 5°C) was lower than that for the corresponding month of the previous winter (7 8± 2 2°C) The BOD level on the next visit (7 March 2005) had fallen to 330 0 mg/l before reaching 67 5 mg/l 17 days later due to what appears to have been a direct effect of dilution from incoming runoff with a lower BOD concentration The BOD level subsequently rose to 315 0 mg/l by mid-April 2005 before falling to 140 0 mg/l towards the end of the same month The average temperature in April 2005 was 14 8±2 8°C and as the water temperature rose the BOD levels in the MFR remained low into the summer of 2005

The BOD level in the MFR following the first de-icing event of the 2005/2006 winter in December 2005 was 171 0mg/l With the water temperature stabilised close to 5 5°C throughout the winter period the BOD level increased to 290 7 mg/l in February 2006 rising even further to 365 0 mg/l 16 March 2006 As with previous seasons the BOD level fell to 125 0 mg/l by April 2006 as the temperature increased to 15 5°C The DO

levels recorded in the MFR during the visits to the HTF in the months prior to the winter of 2005/2006 were not particularly stable The highest DO (9 5 mg/l) was recorded on 23 November 2005 Although the DO in the MFR was zero on 9 December 2005 (the first visit after the de-icing event of the 2005/2006 winter) continuous aeration throughout this period had a positive impact as the levels increased to 7 1 mg/l and 8 1 mg/l during the following visits on 10 February and 16 March 2006 respectively (Figure 3 1) By 27 April 2006 a malfunction in the aerator system resulted in the DO level in the water drop ping to 0 20 mg/l

The Mayfield Farm Balancing Pond (MFBP)

The BOD level in the MFBP during the first routine visit to the HTF on 29 January 2004 was 5 0 mg/l before higher values of 125 0 mg/l 352 5 mg/l 82 5 mg/l 242 5 mg/l 217 5 mg/l and 260 0 mg/l were recorded on the 2 6 10 13 19 and 27 February 2004 respectively (Figure 3 2) The BOD levels remained high during March and most of April, dropping to 90 0 mg/l during sampling on 21 April 2004 and 39 0 mg/l on 4 May 2004 (Figure 3 2)

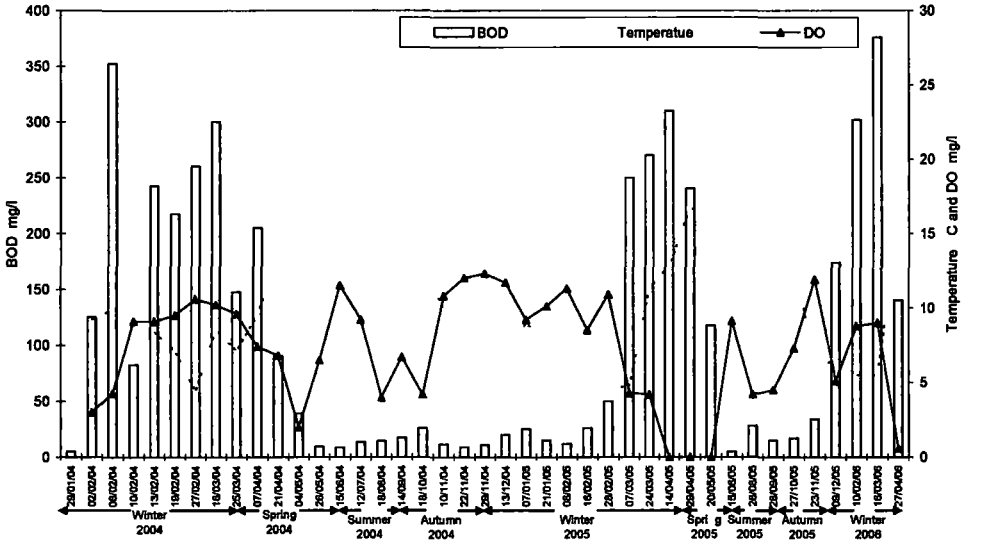


Figure 3 2 Variations in BOD concentrations, DO levels and temperature in the MFBP between January 2004 and April 2006

Within the same period the average temperature increased gradually from 7 8±2 2°C in February to 7 9±1 1°C in March 11 4±2 7 °C in April and 15 8±4 3 °C in May (Figure 3 2) The MFBP always demonstrated a positive supply of DO between January 2004 and February 2005 with the lowest recorded level being 2 0 mg/l on 4 May 2004 All through this period there was no recorded incidence of prolonged shut down of the

aerators apart from during the summer of 2004 The DO level soon peaked at 10 8 mg/l when the aerators were put on in November 2004 falling gradually into the winter with the arrival of high organic load from the MFR (Figure 3 2) By April 2005 the DO level in the MFBP was zero during the two visits (14 and 29 April) and later in the month of May (Figure 3 2) With continuous aeration in the MFBP the DO observed during subsequent visits in June 2005 and up until March 2006 was relatively high with a minimum value of 4 2 mg/l Due to the problems encountered with the aeration system in April 2006 the DO in the MFBP dropped sharply to 0 6 mg/l

As for the MFR the BOD level in the MFBP stayed low throughout the summer autumn and early winter periods with a rising trend commencing at the end of February 2005 as the average temperature dropped to 5 0±0 6°C (Figure 3 2) Following the major de-icing event in February 2005 the BOD levels in the MFBP rose progressively as determined during the routine visits to 310 0 mg/l by mid-April 2005 The arrival of higher BOD load in the MFBP also coincided with a fall in the average DO level from 10 2±1 5 mg/l in February to 4 3±0 1 mg/l in March before reaching zero during the visits in April and May 2005 The average temperature during this period increased gradually from 8 1±4 6°C in March to 14 8±2 8°C in April (Figure 3 2) Subsequent visits indicated a reduction in BOD to typical background summer values less than 50 0 mg/l The repeated winter peak BOD values commenced on 9 December 2005 with the falling limb having been detected commencing on 27 April 2006 (Figure 3 2)

The outlet of the Sub Surface Flow Reedbed System (SSF)

The BOD level recorded at the outlet of the SSF on 29 January 2004 was low at 26 3 mg/l but on subsequent visits the BOD concentrations were 113 8 mg/l 176 3 mg/l 80 0 mg/l and 136 3 mg/l on 2 6 10 and 13 February 2004 (Figure 3 3) The average temperature recorded during this period was 9 2±0 8°C The highest recorded BOD value during spring 2004 was 187 5 mg/l on 7 April 2004 (Figure 3 3) The DO levels at the inlet of the SSF in the months immediately after the de-icing activities in January were generally high with a maximum concentration of 11 3 mg/l during the 5 March 2004 visit to the HTF (Figure 3 3) In contrast the DO levels at the outlet during each visit within the same period were generally low with the DO level of 4 5 mg/l recorded in early February 2004 being by far the highest recorded (Figure 3 4)

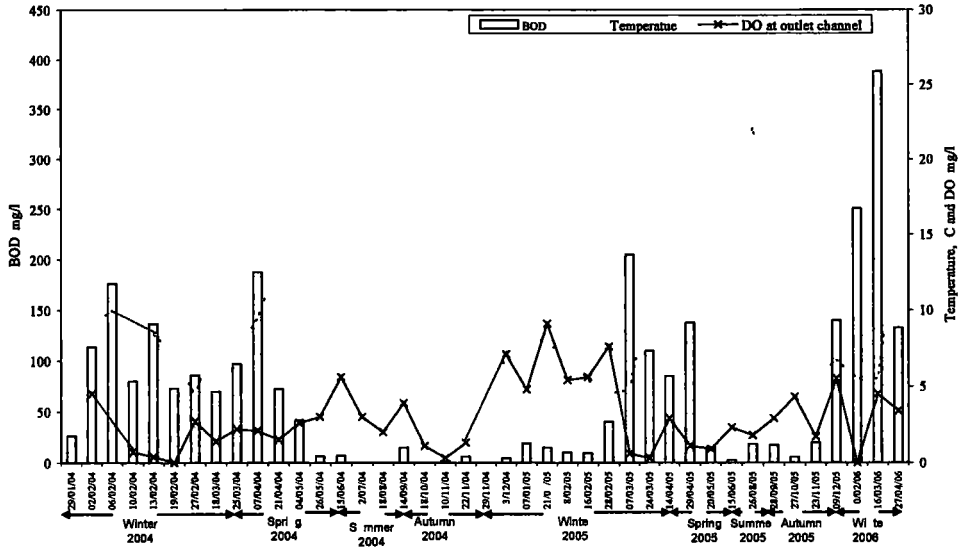


Figure 3 3 Variations in BOD concentrations, DO levels and temperature at the outlet of the SSF between January 2004 and April 2006

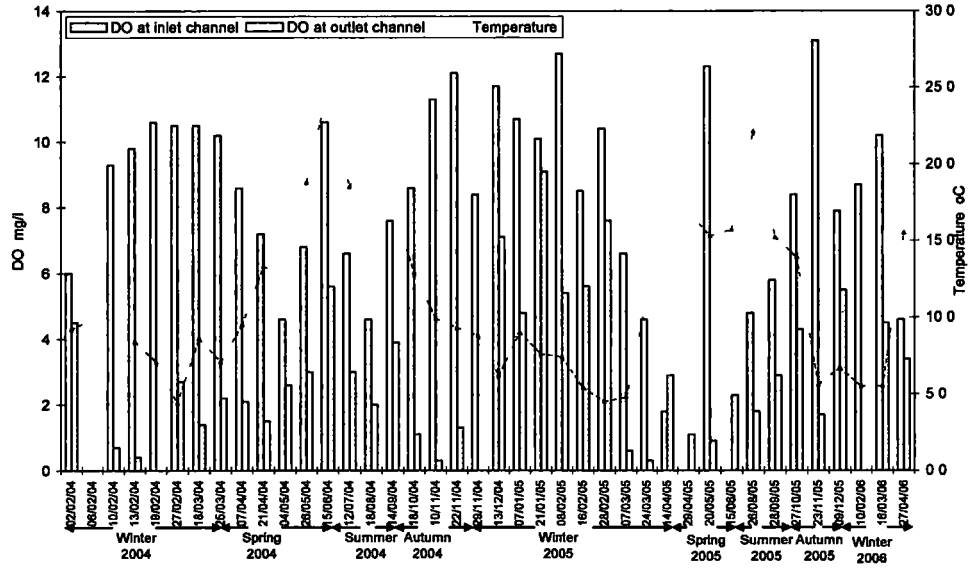


Figure 3 4 Comparison of the DO levels and temperature at the inlet and outlet of the SSF between January 2004 and April 2006

During the 7 March 2005 visit to the HTF the BOD level in the SSF 205 0 mg/l was the first major increase following the background BOD concentrations which were

maintained throughout the June 2004 to February 2005 period During this period the temperature dropped from 24 9°C recorded on the 15 June 2004 visit to an average of 5 8+1 5°C during the three visits in February 2005 (Figure 3 3) The subsequent BOD levels recorded later in March and in April although lower did not reduce significantly until May 2005 when the BOD level in the SSF had fallen to 15 0 mg/l as the temperature rose to 15 3°C (Figure 3 3)

Over the summer 2004 period the DO levels in the inlet and outlet channels remained above zero until 7 and 24 March 2005 when the DO concentrations measured at the outlet were 0 6 mg/l and 0 3 mg/l respectively (Figure 3 3) The situation improved during the summer 2005 months although the DO recorded at the outlet never rose above the 5 5 mg/l recorded during the 9 December 2005 visit (Figure 3 4) After the de-icing activities of the 2005/2006 winter the BOD level in the SSF during the 10 February 2006 visit was 250 8 mg/l reaching a maximum level of 388 0 mg/l by mid-March 2006 The final BOD level recorded in the SSF in April 2006 was 132 5 mg/l as the temperature rose from 5 5°C recorded in February and March to 15 5°C in April 2006 (Figure 3 3)

The Eastern Reservoir (ER)

Due to the difficulties in gaining access to all parts of the Eastern Reservoir only the results from the middle pond will be discussed in this chapter The laboratory BOD analysis did not commence until April 2004 while in-situ DO level determination started in November 2004 The BOD level recorded for the 7 April 2004 visit was 132 5 mg/l which was lower than the BOD levels in the different components of the Mayfield Farm (i e MFR MFBP and the SSF) at the same time During the next visit on 21 April 2004 the background BOD level of 10 0 mg/l was much lower than the levels recorded at Mayfield Farm The temperature had also increased from 9 5°C recorded early in April to 13 3°C on 21 April 2004 (Figure 3 5) The BOD levels recorded remained low for all subsequent visits until after the de-icing activities of the 2004/2005 winter when maximum BOD levels for the ER of 160 0 mg/l and 180 0 mg/l were recorded on 14 and 29 April 2005 respectively The corresponding temperatures during these visits were 12 8°C and 16 8°C respectively (Figure 3 5) By the next visit in late May 2005 the BOD level in the ER had decreased to 25 0 mg/l and subsequently remained low until the 9 December 2005 visit The highest observed BOD concentration during this period was 174 0 mg/l in mid-March 2006 and this had dropped to just 5 0 mg/l by 27 April 2006 as the temperature increased from 5 5°C to 15 5°C (Figure 3 5)

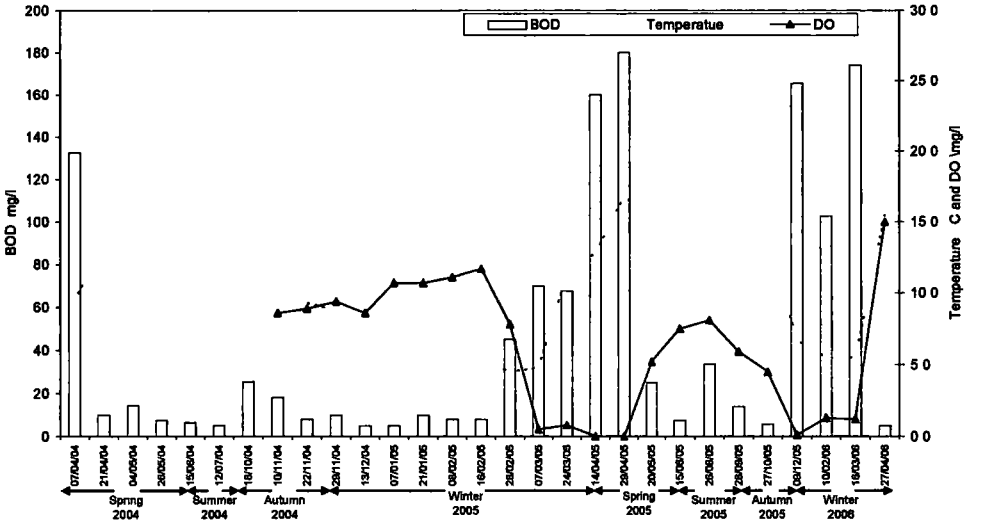


Figure 3 5 Variations in BOD concentrations, DO levels and temperature at the ER (middle pond) between April 2004 and April 2006

The DO levels in the ER clearly mirror the inverse trends of BOD concentrations with the initial high values rapidly decreasing following the deicing activities in late February 2005 with DO at or near zero until the end of April 2005 The situation subsequently improved during the summer months with the DO staying well above zero until the next de-icing event in December 2005 when very low levels were again recorded (Figure 3 5)

3 1 2 Deductions from the trends observed in BOD and DO levels

There is clearly a seasonal pattern in the variation of the BOD levels in all four components of the HTF High BOD levels are observed over the winter months following de-icing activities at the airport whereas lower BOD levels are recorded in spring as the temperature increases The elevated BOD levels recorded during the winter months are an indication of the influx of ADF laden runoff into the system During the first winter of this study (2003/2004) the average BOD level recorded in the MFR between late January and early February 2004 was 275 0±18 0 mg/l Although the aerators in the MFR were on the presence of a high organic load appeared to have produced a significant drop in DO with levels of 1 4 mg/l and 1 7 mg/l being observed on 29 January and 2 February 2004 respectively Apart from the 10 and 27 February 2004 visits when lower BOD levels of 85 0 mg/l and 92 5 mg/l respectively were recorded in the MFR The average BOD level in the MFR during this winter was 230 8±30 9 mg/l and lasted until late April 2004 While the two occasions on which lower BOD levels were recorded may indicate how much removal has occurred within

the system it could also be a direct effect of dilution resulting from the influx of a large volume of less polluted runoff

The high BOD levels in the MFBP between February and early April 2004 were a direct result of the transfers from the MFR The continuous transfer results in the initial BOD level of 5 0 mg/l in late January 2004 increasing to 125 0 mg/l on 2 February 2004 before reaching 352 5 mg/l 4 days later The consistently high BOD levels recorded in the MFBP during winter 2003/2004 highlight the inability of the MFR to effectively remove the incoming BOD before transfer into the MFBP Despite the high average DO concentrations (8 2±2 5 mg/l) existing during this period in the MFBP there were no signs of any significant BOD reductions The average temperature during this period was 7 8±2 2°C With both the MFR and MFBP not performing as effectively as would be expected during the winter period, the SSF was continually exposed to BOD levels higher than the 110 0 mg/l level it was designed to handle There were only two occasions during the 2003/2004 winter/spring (10 February and 21 April 2004) when the recorded BOD concentration in the MFBP was below this level Prolonged exposure of the SSF to high organic loads in the winter place a demand on the DO and the absence of any aeration within the SSF resulted in an inability of the DO levels in the water channels within the SSF to recover from zero or near zero levels throughout the winter months

In a study conducted between February and August 2004 the DO levels were monitored along one of the cells of the SSF as shown in Figure 3 6 Each cell incorporates 4 plant beds and 5 open water channels with additional channels located before and after each bed making a total of 7 open water channels Figure 3 6 shows a schematic representation of an individual cell with tabulated DO levels within the water channels on different sampling dates The effect of continuous exposure of the SSF cells to high organic loads is clearly shown in Figure 3 6 The drop in DO levels across the first bed is evident with the influx of high BOD load from 15 February 2004 until 7 April 2004 After this date the incoming BOD loads starts to decrease and the DO drop becomes less exaggerated The DO concentration in one of the inner open water channels (labelled channel c) was zero on 19 February and for most of the following winter period as the system was continually exposed to high organic loads The rapid depletion of the DO in the cells is in itself an indication of the active microbial activities within the bed resulting in a high utilisation of DO and influencing the efficiencies of the following wetland cells The prolonged discharge of high BOD loads from the aerated ponds thus contributes to the development of anoxic conditions in the 2nd 3rd and 4th beds and hence an absence of aerobic biodegradation McGahey

and Bouwer (1992) have implicated insufficient DO caused by high organic loads in the incomplete biodegradation of glycols observed in sealed batch microcosms The situation at the HTF could be rectified by the direct aeration of the relevant intermediate channels enabling aerobic biodegradation to continue throughout the entire length of each reedbed Although the anaerobic biodegradation of glycol-based Type I ADFs has been reported by Schoenberg *et al* (2001) the optimal temperature of 35°C at which this occurs is much higher than the typical winter temperature of 6 5±0 7 °C recorded in the SSF

The high BOD level (610 0 mg/l) recorded in the MFR on 28 February 2005 is an indication of the influx magnitude of polluted water that can result from the deicing activities at the airport The lower BOD levels recorded in the MFBP and SSF during the same visit indicate that no transfers had been initiated from the MFR For most of the 2004/2005 winter season the aerated ponds (MFR and MFBP) acted as the holding points for the incoming runoff so as not to expose the SSF to high organic loads The subsequent higher BOD levels observed in the MFBP between March and April 2005 are an indication of transfers from the MFR Although the high BOD levels persisted in the MFR and MFBP for most of the winter the 67 5 mg/l BOD level recorded in the MFR on the 24 March 2005 visit suggests that the holding technique employed in the aerated ponds was effective There is also a possibility that the reduction recorded is partly due to the influx of less polluted water into the MFR as its initial content was transferred into the MFBP

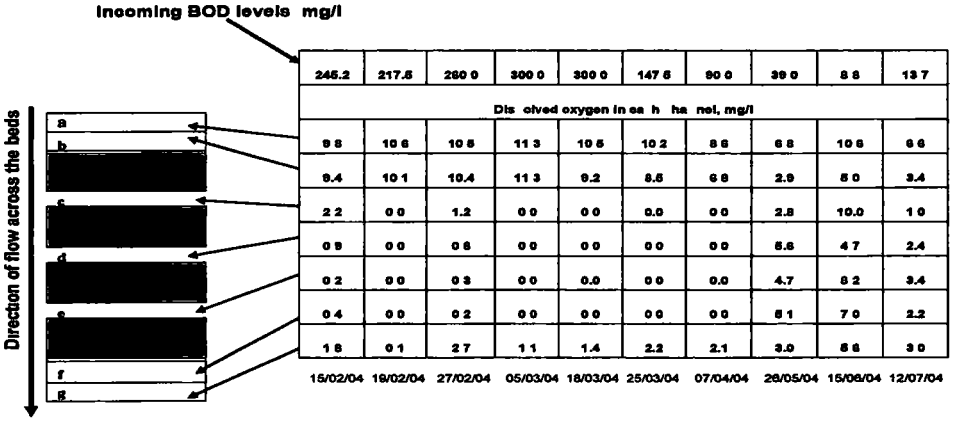


Figure 3 6 Variations in DO levels within the SSF bed between 15 February and 12 August 2004

During the 2005/2006 winter period high BOD levels were recorded in the MFR MFBP and the SSF on the 9 December visit suggesting that water from deicing activities had been transferred throughout the system The BOD level recorded at the exit of the SSF (139 7 mg/l) is an indication that this part of the treatment system has been exposed to high organic loads from the aerated ponds The higher BOD levels recorded in the MFR and MFBP later in the winter (February and March 2006) is again directly reflected in the high BOD levels recorded at the outlet of the SSF Although the continuous aeration of ponds in the months prior to the winter sustained the DO levels in the MFR and MFBP for most of the 2005/2006 winter the high BOD levels recorded at the exit to the SSF and the critically low DO levels recorded within the SSF channels suggest that the performance of the SSF was again hampered by its exposure to high organic loads It still remains unclear why the high DO level recorded in both ponds was not sufficient to assist reduction of the BOD levels due to biodegradation

In all three winters covered in this study the peak BOD level recorded in the MFR was much higher than the levels recorded in the ER During the 2003/2004 winter the highest BOD level recorded in the ER was 132 5 mg/l compared to 207 5 mg/l and 205 mg/l recorded in the MFR and MFBP respectively during the same visit The BOD level in the ER later dropped to 10 0 mg/l during the next visit (two weeks later) The 92 5% BOD reduction achieved within this time is better than the 8 4% and 56 1% reductions recorded in the MFR and MFBP respectively In the following winter (2004/2005) the 180 0 mg/l BOD level recorded in the ER on 29 April 2005 was well below the peak value of 610 0 mg/l recorded in the MFR on 2 February 2005 The BOD level in the ER during the 2 February visit was 45 0 mg/l The trend remained the same during the 2005/2006 winter with the highest BOD level recorded in the ER (174 0 mg/l) being much lower than the 365 0 mg/l and 376 0 mg/l values recorded in the MFR and MFBP respectively on 16 March 2006 By the next visit on 27 April 2006 (6 weeks later) the BOD level in the ER had fallen to 5 0 mg/l (equivalent to a 97 1% reduction) The BOD levels in the MFR and MFBP fell to 125 0 mg/l and 140 0 mg/l respectively (equivalent to 65 8% and 62 8% BOD reduction respectively) within the same period

The higher peak BOD levels recorded during the winter in the MFR and MFBP compared to the ER could be a result of the more frequent use of ADF at Southern catchment from which the MFR receives polluted water With an estimated 80% of the ADFs applied to an aircraft ending up on airport surfaces either through overspray or drippage (O Connor and Douglas 1993) and another 15-20% lost during taxiing and takeoff (Gallagher 1998) a large proportion of the ADF makes it way into the

stormwater delivered to the treatment facility The high composition of ADF in the water also means that the water is composed of a high proportion of the additives associated with ADFs most of which are reported to have inhibitory effects on microbial activities (Cancilla *et al* 2003) This may contribute to the poor performances recorded in the MFR and MFBP There are also indications that the presence of vegetation around the sides of the ER could have created a more natural state in the system encouraging more efficient microbial activities and consequently higher BOD reductions Detailed microbial analysis of the different components of the HTF showed that some of the bacteria isolates found in the SSF (which has a high potential for rapid BOD reduction) were also found in the ER (more details in Chapters 5 and 7)

The performance of the MFR although not as effective as expected is consistent with observations at the Dane County Regional Airport where delays of up to three months following de-icing activities have been noted for any significant BOD reduction to occur (Gallagher 1998) The BOD decrease was normally associated with April when the temperature was warm enough to support continuous microbial activities in the water In a pilot scale study in which a dual-tank bioreactor was used for the treatment of water collected from the deicing pads of the Dane County Regional Airport the BOD level in the treatment system was found to drop from over 1000 mg/l to less than 50 mg/l over a period 3 days when the operating temperature was maintained at 30°C (Gallagher 1998) While this demonstrates that an increase in temperature will enhance BOD reduction in the MFR and MFBP the inability of both ponds to hold the total volume of stormwater generated during the winter and the high energy cost that would be involved in the operation of such a heated system make this impracticable Therefore the emphasis in this study is shifted to other possible factors which may contribute to the effective reduction of incoming elevated BOD levels

3 2 VARIATION IN NITRATE AND PHOSPHATE CONCENTRATIONS IN THE COMPONENTS OF THE HTF

Based on the results of a series of laboratory experiments conducted to assess the benefit of nutrient addition to BOD removal in aerated pond water samples (see Chapter 5) a nutrient dosing regime commenced at the Mayfield Farm aerated ponds (MFR and MFBP) in November 2004 Prior to this the nutrient levels in the HTF had not been monitored until June 2004 in the MFBP and September 2004 in the rest of the system The objective nutrient concentrations in the MFR were set at 50 mg/l for nitrate (11 3 mg N/L) and 6 8 mg/l for phosphate (2 26 mg P/L) in order to prevent the

discharge of nitrate levels which could be harmful to the receiving water Although nitrates and phosphates are essential in microbial metabolic processes, elevated nutrient levels in the water could lead to eutrophication This situation promotes excessive algal growth which reduces the amount of sunlight available to water organisms having a serious impact on aquatic ecosystems (Painting *et al* 2007) The excessive growth of algae increases the competition for the available inorganic compounds such as CO_2 NH_4^+ NO_3 and PO_4^{3-} and other food sources

The nutrient levels used are based on a nitrogen (N) phosphorus (P) molar ratio of 5 1 adapted from the original 60 5 1 carbon (C) N P molar ratio which is required for the complete mineralisation of organic compounds (McGahey and Bouwer 1992) The nitrate based fertiliser used was calcium nitrate while superphosphate was used as the phosphate source The following calculations are based on nutrient additions to the MFR (45 000 m^3) followed by circulation around FLRB (the floating reedbeds system) and then into MFBP

- **Required Nitrate-N using Ca $(NO_3)_2$ (Calcium Nitrate fertiliser)**

Calcium nitrate contains 15 5% N by weight

-Based on the nitrate-N level of 1 24 mg/l in the MFBP in September 2004 10 06 mg N/L would be required to increase the nitrate-N level to 11 3 mg/l

-10 06 mg N/L in 45 000 m^3 ≡ 453 kg N ≡ 2923 kg calcium nitrate

-Approximate cost of calcium nitrate in 1993 = £11 80 / 50 kg

-At this price the cost of raising the nitrate level in MFR to 50 mg/l (11 3 mg N/L) would be £690

- **Required Phosphate-P $Ca_3(PO_4)_2$ (Triple Superphosphate fertiliser)**

Triple Superphosphate contains 20 0% P by weight

-Based on the phosphate-P level of 0 20 mg/l in the MFBP in September 2004 2 06 mg P/L would be required to increase the phosphate-P level to 2 26 mg/l

-2 06 mg P/L in 45 000 m3 ≡ 92 7 kg P ≡ 464 kg superphosphate

-Approximate cost of superphosphate in 1993 = £3 94 / 40 kg

-At this price the cost of raising the phosphate level in the MFR to 6 8 mg/l (2 26 mg P/L) would

be £46

The plan was to commence nutrient dosing gradually prior to a de-icing event in order to increasing the levels of nitrate and phosphate within the MFR to 10 00 mg/l (2 26 mg N/L) and 1 35 mg/L (0 45 mg P/L) These levels will be diluted as the water passes through the system (FLRB MFBP and the SSF) and with subsequent dosing the

nutrient levels is expected to reach the maximum calculated levels (50 mg/l for nitrate and 6 8 mg/l for phosphate) It is also expected that biomass growth would be enhanced during retained time within the system The resulting dilution should ensure no detrimental effects are caused by the nutrient levels associated with the discharged water After the nutrient dosing the treatment system would be operated in the recycling mode and the nitrate and phosphate levels (as well as BOD) would be monitored at the outlet position to determine when release of treated water was appropriate

The dosing regime commenced on 12 November 2004 using the above calculations as a guide for nutrient addition The insolubility of the phosphate fertiliser posed an operational difficulty and as such only nitrate dosing was carried out during the subsequent nutrient additions on 14 January 4 February and 25 February 2005 The trends observed across the system following the commencement of the nutrient dosing regime are discussed in the sections that follow

3 2 1 Discussion of observed trends in the different treatment components

Following the commencement of the nutrient dosing regime on 12 November 2004 the concentration of nitrate in the MFBP increased rapidly from an average background level of 3 86±2 67 mg/l recorded in the months prior to the commencement of the nutrient dosing (between 15 June and 10 November 2004) to 15 95 mg/l during the 22 November 2004 visit (Figure 3 7) Although an initial increase in the concentration of nitrate from 1 70 mg/l to 9 75 mg/l had been recorded between 18 October and 10 November 2004 in the MFR the 7 53 mg/l recorded on 22 November was an increase from the average background level of 5 12±4 16 mg/l prior to the nutrient dosing (Figure 3 8) The concentration of phosphate in the MFBP also increased from the average background level (0 37±0 16 mg/l) before nutrient dosing to 0 95 mg/l recorded on 22 November 2004 (Figure 3 7) There was no increase recorded in the concentration of phosphate in the MFR as the average phosphate level (0 39±0 18 mg/l) before dosing was higher than the 0 21 mg/l recorded on 22 November 2004 (Figure 3 8) While the background nitrate concentration in the SSF increased from 2 29±2 03 mg/l recorded in the visits prior to nutrient dosing in the MFR and MFBP to 5 32 mg/l on 22 November 2004 the phosphate concentration actually dropped from the average background concentration of 0 31±0 21 mg/l to 0 11 mg/l on the same visit (Figure 3 9)

Prior to the commencement of nutrient dosing the concentrations of nitrate in the MFR (5 12±4 16 mg/l) and MFBP (3 86±2 67 mg/l) were lower than the 10 63 mg/l value

observed in the ER (Figure 3 10) On the first visit after nutrient dosing commenced in the MFR and the MFBP the concentration of nitrate recorded in the ER (12 40 mg/l) remained higher than the 7 53 mg/l recorded in the MFR but lower than the 15 95 mg/l in the MFBP (Figure 3 10) During the same visit the phosphate concentration in the ER (0 35 mg/l) was also higher than the level observed in the MFR (0 21 mg/l) but lower than the 0 95 mg/l observed in the MFBP (Figure 3 10) Prior to this time the average background levels of phosphate in the MFR (0 39±0 18 mg/l) and MFBP (0 37±0 16 mg/l) were higher than the 0 20 mg/l recorded in the ER (Figure 3 10) The average concentrations of the nutrients nitrate and phosphate observed in the different components of the HTF together with the minimum and maximum levels observed throughout this study are summarised in Table 1 Appendix 3A

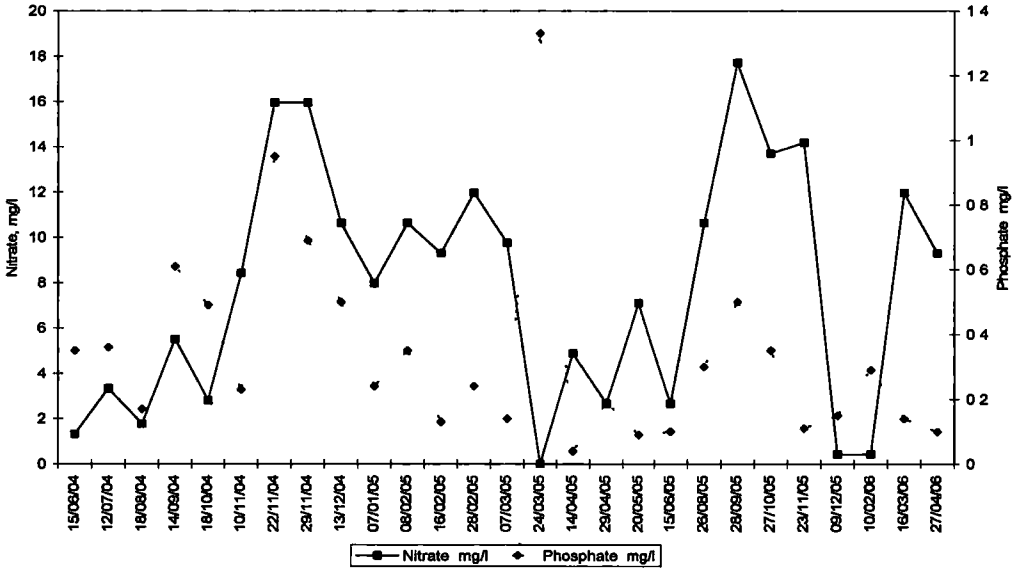


Figure 3 7 Variations in nitrate and phosphate concentrations in the MFBP between June 2004 and April 2006

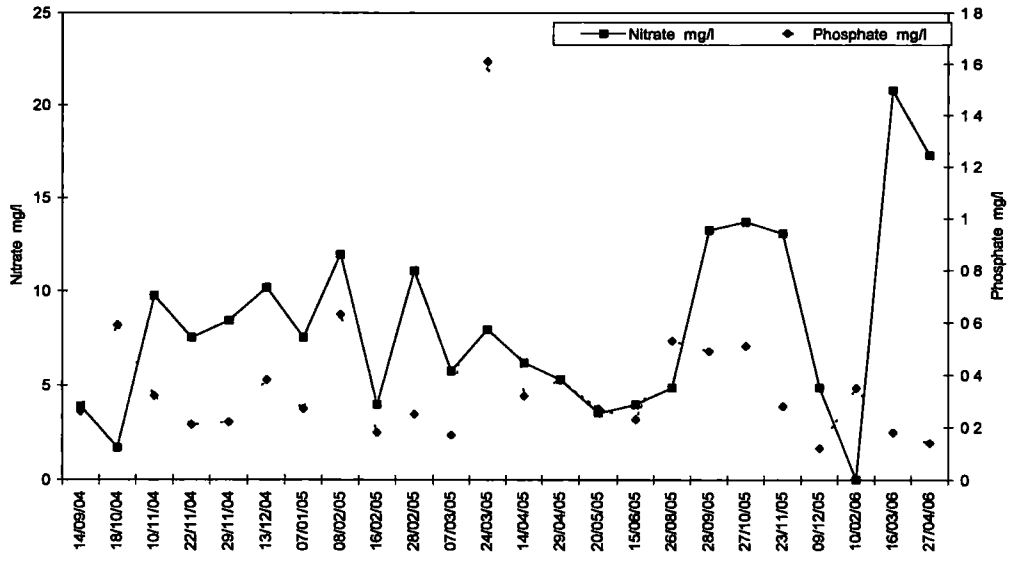


Figure 3 8 Variations in nitrate and phosphate concentrations in the MFR between September 2004 and April 2006

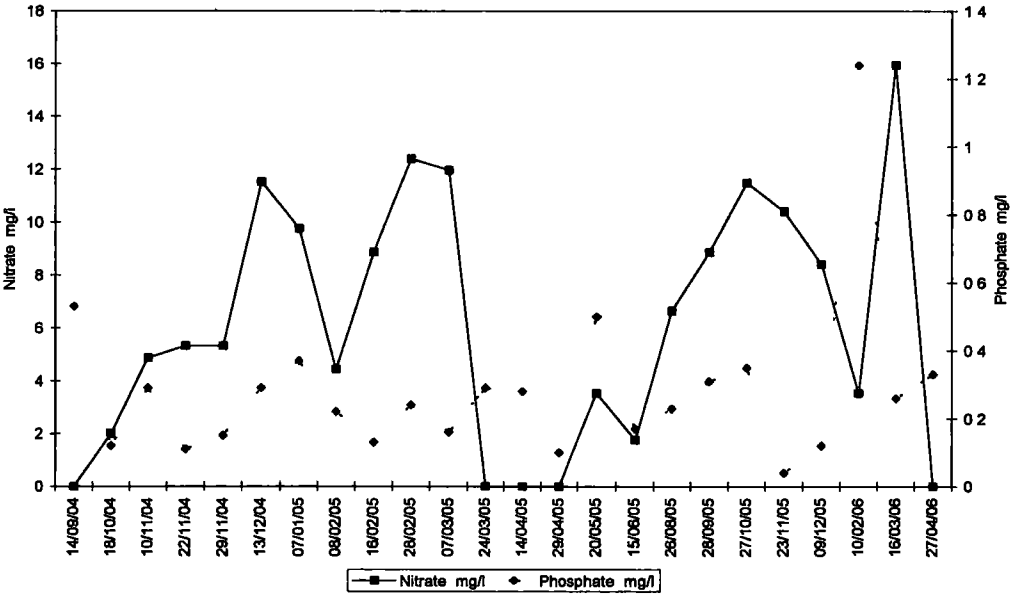


Figure 3 9 Variations in nitrate and phosphate concentrations in the SSF between September 2004 and April 2006

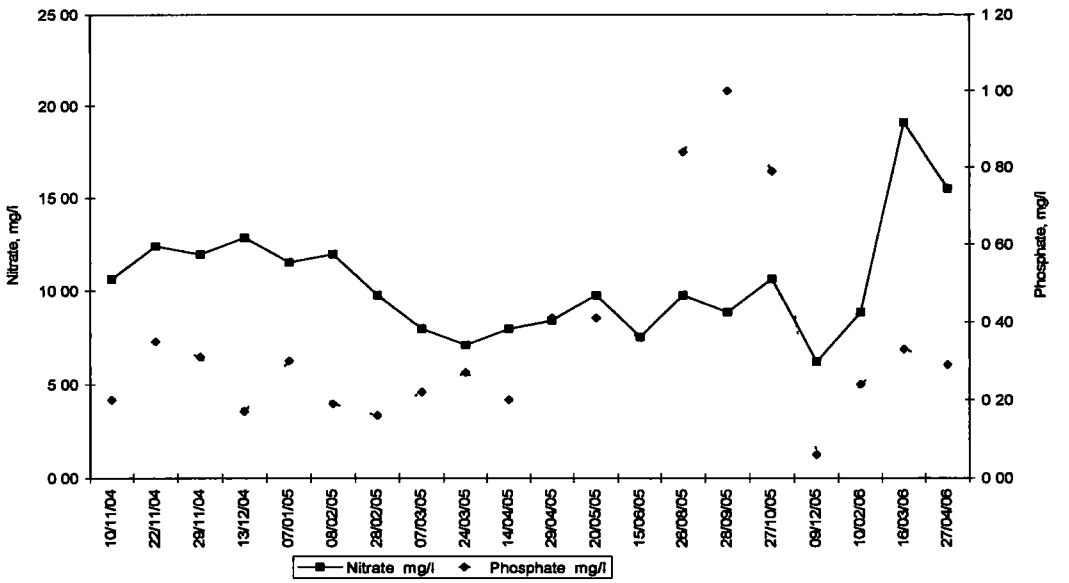


Figure 3 10 Variations in nitrate and phosphate concentrations in the ER between November 2004 and April 2006

3 2 2 Deductions from observed trends

Based on the results observed from monitoring the nutrient levels in the MFR and MFBP before and after the commencement of the nutrient dosing regime the application of nutrient increased the background concentration of nutrients in the MFR and MFBP immediately after dosing (apart from phosphates in the MFR) The effect of the nutrient addition was also evident in the increase recorded in nitrate concentrations at the outlet of the SSF The increases observed in the average background concentrations of nitrate in the MFR and MFBP from 5 12±4 16 mg/l and 3 86±2 67 mg/l to 8 06±2 47 mg/l and 9 70± 4 78 mg/l respectively in the winter months immediately after dosing commenced (22 November 2004 to 14 April 2005) clearly demonstrate the impact of nutrient addition The equivalent increases in phosphate concentrations (0 39±0 18 mg/l to 0 42±0 44 mg/l and 0 37±0 16 mg/l to 0 46±0 41 mg/l in the MFR and MFBP respectively) over the same period further highlight this

The nutrient levels observed in the ER on the other hand suggest there are possible nutrient contributions from the surface runoff arriving from the Eastern catchment of the airport where most of the maintenance activities at Heathrow airport are carried out Some of the pollutants generated from these activities are oils soils and residues from cleaning products which are likely to contain nitrates and phosphates Phosphate

compounds are key ingredients in detergents used for washing aircraft Surface runoff from agricultural landscapes (employing nitrate and phosphate fertilisers) is another likely source of the nutrients detected in the ER The higher average background nitrate concentrations observed in the ER over the autumn/winter months (10 98±3 46) compared to the levels observed over the spring/summer months (9 16±1 11) agree with the claims of Harrison *et al* (1996) that the solubility of nitrate compounds makes them more easily available in water flowing over agricultural soils particularly in the autumn/winter months

Due to the nutrient applications to the MFR and MFBP in November 2004 it was difficult to tell if there were any nutrient contributions from surface runoff during the subsequent months Although there were occasional peaks in nitrate concentrations in both the MFR and MFBP in the months after the nutrient dosing commenced the concentrations of nitrate observed in the MFR on 16 March 2006 (20 80 mg/l) and 27 April 2006 (17 28 mg/l) are indications that there are also contributions from incoming runoff during the winter months In contrast the close association of phosphates with soil particles makes them less detectable in surface waters and as such it is difficult to ascertain how much is contributed by the surface runoff The higher concentration of phosphates observed in the ER (0 64±0 27 mg/l) over the summer compared to the 0 24±0 08 mg/l in the winter agrees with the claim that sediments washouts during high surface flow particularly in the autumn and winter increases the levels during the spring/summer months (Frank and Reay 2006) There were no seasonal trends recorded in the MFR The highest level recorded in March 2005 (1 61 mg/l) occurred after a series of three dosing events (14 January 4 February and 25 February 2005) making it difficult to tell if this increase was caused by the resuspension of phosphates accumulated sediments in the preceding months

The average nutrient levels observed at the outlet to the SSF (6 68±4 80 mg/l for nitrate and 0 28±0 25 mg/l for phosphate) after the nutrient dosing commenced indicate that the concentrations of the nutrients in the water leaving the system were well below the 50 00 mg/l and 6 80 mg/l targets set for nitrate and phosphate respectively in other to avoid any detrimental effect on receiving waters once released from the system The results observed also showed that the concentrations of phosphate attained in the MFR (0 21 mg/l) and MFBP (0 95 mg/l) were below the target (1 35 mg/l) set for the system before the deicing events (see Section 3 2) The situation was the same for the level of nitrate in the MFR as the 7 53 mg/l recorded on 22 November 2004 was below the 10 00 mg/l target, although this may be a result of

dilution caused by the arrival of nutrient-deficient runoff In contrast the 15 95 mg/l level recorded in the MFBP on the same visit was in excess of the nitrate target The average nutrient levels recorded in the MFR and MFBP after the commencement of deicing activities at the airport in late February 2005 were below the 50 0 mg/l and 3 38 mg/l target set for nitrate and phosphate respectively even after subsequent dosing on 14 January 4 February and 25 February 2005 Based on the average BOD levels in the MFR and MFBP in the winter months immediately after the deicing events (313 5 mg/l and 240 0 mg/l in February and April 2005 respectively) the average nitrate concentrations of 8 06±2 47 mg/l and 9 70±4 78 mg/l and the average phosphate levels of 0 42±0 44 mg/l and 0 46±0 41 mg/l in the MFR and MFBP respectively are well below the 60 5 1 C N P ratio required for effective mineralisation of the BOD as recommended by McGahey and Bouwer (1992) The effect of the nutrient addition on the performance of the system and the biomass population is discussed later in this chapter

3 3 VARIATION IN BIOMASS POPULATION THE COMPONENTS OF THE HTF

The main aim of commencing a nutrient dosing regime in the MFR and MFBP on 12 November 2004 was to assess the impact of nutrient addition on the bacteria populations in the aerated ponds Initial laboratory studies had shown increases in bacteria populations from 10^7 to 10^{10} CFU per litre on addition of nutrient with evidence of utilisation of the nutrient (see Section 5 3 in Chapter 5) This was a further reason for initiating a nutrient dosing regime at the HTF Following dosing the biomass populations in water samples collected from different components of the HTF were monitored in the laboratory using three different media

- Tryptic soya agar (TSA-Oxoid) for detection of bacteria
- Sabouraud s dextrose agar (SDA-Difco) for detection of fungi
- Glycerol yeast extract agar (GYEA-prepared from base compounds) for detection of actinomycetes

Full details of the relevant experimental procedures have been discussed in Section 2 3 1 of Chapter 2

While the initial tests (29 January 2004 to 19 February 2004) showed moderately high levels of bacteria in the water samples from the MFR and MFBP the populations of actinomycetes and fungi were more or less negligible (1 CFU/ml) hence only results

from enumeration tests conducted using TSA are referred to as the biomass populations in this chapter The biomass populations recorded in each component of the HTF between January 2004 and April 2006 are shown in Figure 3 9

3 3 1 Discussion of trends recorded in the different components

During the first visit to the HTF on 29 January 2004 the biomass population in the MFBP was 1 75 x 10^8 CFU/l increasing to 6 00 x 10^6 CFU/l by the next visit on 2 February 2004 (Figure 3 11) Although a gradual drop in the bacteria population was observed in the MFBP towards April 2004 the average population throughout the winter months (until April) was around 10^7 CFU/l The situation was similar in the MFR with the biomass level recorded during 2 February 2004 visit being 1 38 x 10^9 CFU/l The bacteria population had fallen to 1 30 x 10^7 CFU/l by the next visit on 10 February 2004 reaching 2 50 x 10^7 CFU/l on 21 April 2004 When sampling started at the ER in April 2004 the initial biomass population observed was 3 00 x 10^6 CFU/l which is lower than the levels observed in both the MFR and MFBP during the same period

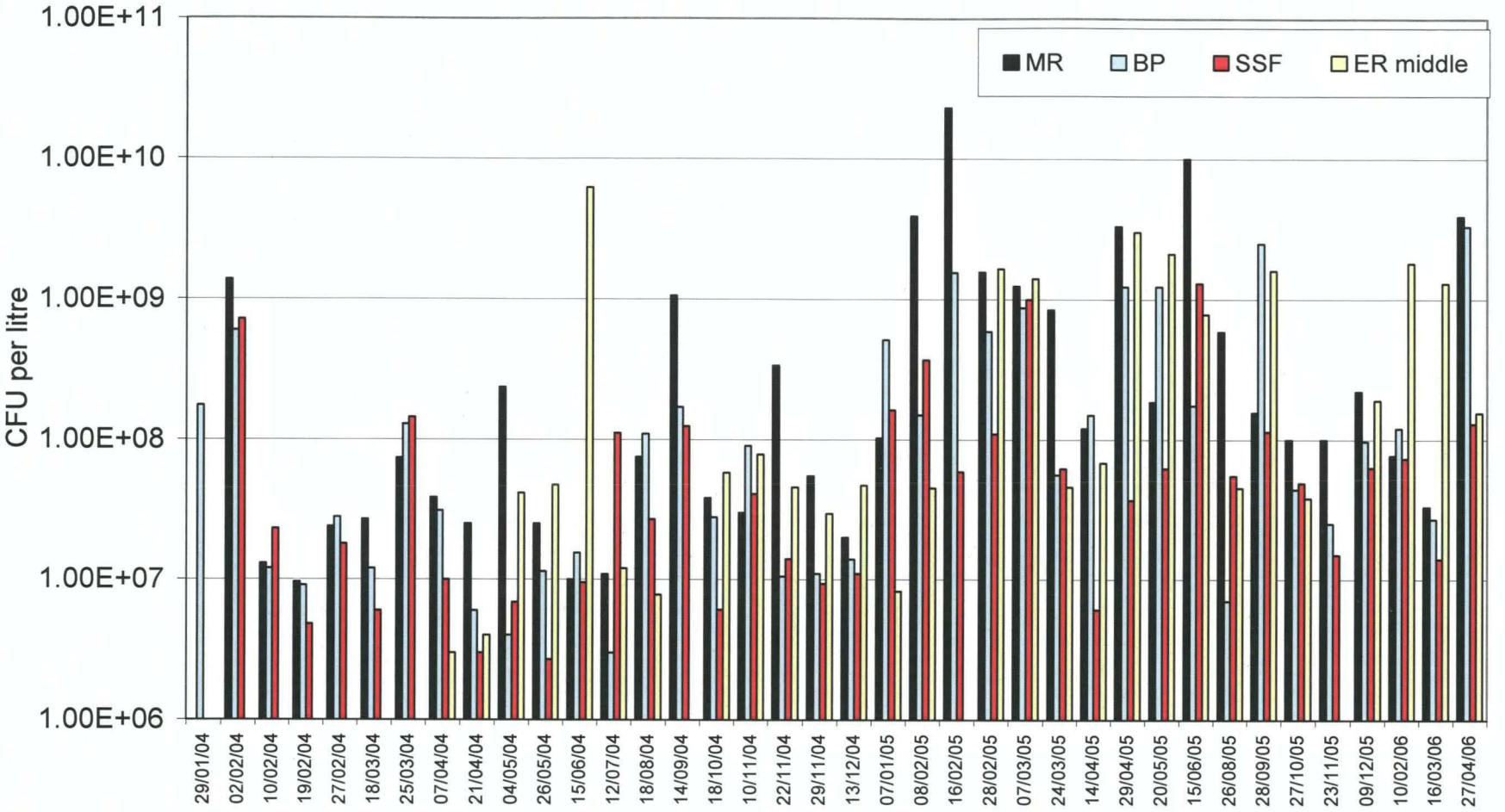


Figure 3.11 The trend in bacteria biomass population in the MFR, MFBP, SSF and ER between January 2004 and April 2006

After the commencement of the nutrient dosing regime in November 2004 no immediate increases were recorded in biomass populations until 7 January 2005 when the levels were 1 03 x 10^8 CFU/l 5 15 x 10^8 CFU/l and 1 62 x 10^8 CFU/l in the MFR MFBP and SSF respectively During the same visit the biomass population in the ER (which had not been dosed with any nutrient) was lower at 8 20 x 10^6 CFU/l (Figure 3 11) By 16 February 2005 the biomass population in the MFR had reached 2 30 x 10^{10} CFU/l finally dropping to 7 70 x 10^7 CFU/l and 3 30 x 10^7 CFU/l on 10 February and 16 March 2006 respectively (Figure 3 11) The maximum level recorded in the MFBP immediately after nutrient dosing was 1 55 x 10^9 CFU/l (on 16 February 2005) although higher levels of 2 49 x 10^9 CFU/l on 28 September 2005 and 3 30 x 10^9 CFU/l on 27 April 2006 were attained (Figure 3 11) The average biomass populations average bacteria population in the MFR MFBP and SSF before and after the nutrient dosing commenced on 12 November are compared with the levels observed in the ER over the same period in Table 2 Appendix 3A

3 3 2 Deductions from trends observed

Based on results of the laboratory tests conducted on water samples collected from the MFR MFBP SSF and ER there are no definite seasonal trends in biomass populations in any of the components of the HTF The average biomass populations in the MFR during the winter months were not particularly different from the levels observed in the summer The average biomass population in the MFR was 2 55 x 10^8 CFU/l during the 2003/2004 winter while in the following summer months it was 1 55 x 10^8 CFU/l The situations in the MFBP and SSF were slightly different as the average biomass levels fell in both components from winter levels of 1 38 x 10^8 CFU/l and 1 53 x 10^8 CFU/l to 1 55 x 10^7 CFU/l and 3 42 x 10^7 CFU/l respectively This suggests that the glycol-laden runoff from the airport does not exert an obvious adverse effect on the biomass population as earlier speculated This also agrees with findings from Chong *et al* (1999) in which it was shown that most wetland plants and micro-organisms are not adversely affected by exposure to high pollutant loads similar to those found in airport runoff

The higher biomass counts recorded in water samples collected from both the MFR and MFBP in the months after the nutrient dosing commenced (Table 3 2) suggests that nutrient addition enhances biomass growth in the aerated ponds The average biomass level in the MFR was 2 37 x 10^9 CFU/l an increase from the previous average level of around 1 92 x 10^8 CFU/l (Table 3 2) With further nutrient dosing of the MFR on 14 January 4 February and 25 February 2005 the bacteria level increased reaching

10^{10} CFU/l once during the winter (16 February 2005) and later in the summer (15 June 2005) The average biomass level for the remaining of the summer was 1 82 x 10^9 CFU/l

Although there was an increase in average biomass population in the MFBP after the nutrient dosing commenced it is not clear why the overall increase over the winter that followed was not as significant as recorded in the MFR The average biomass level in the MFBP over the 2004/2005 winter (immediately after the nutrient dosing commenced) was 6 22 x 10^8 CFU/l compared to the 5 11 x 10^9 CFU/l recorded in the MFR The presence of nutrient in the MFBP appears to have contributed to the maintenance of the average biomass population of 6 69 x 10^8 CFU/l over the following spring and summer months The population in the MFR over the same period dropped slightly to 1 82 x 10^9 CFU/l Although no nutrient addition was carried out at the ER the biomass population recorded in the water was comparatively high This is consistent with the high nitrate and phosphate levels recorded in the ER The average biomass population over the 2004/2005 winter months was 6 3 x 10^8 CFU/l increasing to 1 09 x 10^9 CFU/l over the summer months

The low nutrient levels observed in the MFR and MFBP between December 2005 and February 2006 (Figures 3 7 and 3 8 appear to have had an adverse impact on the biomass populations in both ponds as the levels in the MFR fell to 7 70 x 10^7 CFU/l in February and 3 30 x 10^7 in March 2006 This was further highlighted in the MFBP and SSF with the biomass populations dropping to 10^7 CFU/l in both units during the same period However the effect of increased nutrient levels on the biomass population was demonstrated by increases recorded in all units towards the end of April 2006 (3 89 x 10^9 CFU/l and 3 30 x 10^9 CFU/l in the MFR and MFBP respectively and 1 30 x 10^8 in the SSF) which followed the occurrence of elevated levels of nitrate in the system during 16 March 2006 visit The impact of the biomass population on BOD reduction recorded in the system is discussed later in the chapter

3 4 THE EFFECT OF NUTRIENT ADDITION, TEMPERATURE AND DO LEVELS ON BOD REDUCTION AND BIOMASS POPULATION IN THE MAYFIELD FARM AERATED PONDS

The commencement of a nutrient dosing regime at the Mayfield Farm in November 2004 was based on recommendations arising from results obtained from laboratory experiments (See Chapter 5 for full details) The overall aim of the nutrient addition was to increase the background microbial population in the aerated ponds by

achieving the right balance between the available carbon source and the added nutrient in the form of nitrate and phosphate The objective was to achieve the maximum biodegradation of glycol Safferman *et al* (2002) had shown that a slow-release fertilizer containing nitrogen and phosphorus provides the macronutrient required for the biodegradation of organic pollutants in stormwater originating from airports Initial laboratory tests in this study showed evidence of nutrient utilisation particularly nitrate in the aerated pond water samples The utilisation of phosphate was moderate and considerably lower than that for nitrate The BOD exerted by glycol was found to be more efficiently removed when a plentiful supply of nitrate was available There were also clear signs that the addition of nutrient increased the biomass population Details of the results obtained in these tests are discussed in Chapter 5

A key site operational issue that was addressed in more detail after the first year of study was the aeration process in the system Initially the aerators were operated only after the influx of polluted water into the system Following a series of consultations it was suggested that the aerators in the two ponds (MFR and MFBP) should operate on a continuous basis for at least two months prior to the first deicing event Results from routine site monitoring showed that this was effective as the DO in the ponds remained fairly high before the arrival of the first high organic load (Figures 3 1 and 3 2) The DO levels in the MFR and MFBP late in November 2004 were 12 2 mg/l and 12 0 mg/l falling to 9 2 mg/l and 10 1 mg/l later in January 2005 These high levels were maintained more efficiently in the MFBP as the DO remained as high as 10 9 mg/l even after the deicing event late in February 2005 The effects of these factors on the BOD reduction in the system together with the biomass population are discussed in this section

3 4 1 Effect of nutrient addition on BOD reduction and biomass populations in the MFR and MFBP

The addition of nutrient to the MFR and MFBP was not as straightforward in practice as it was on the laboratory scale Whilst it was crucial to maintain the right balance between the organic load and the added nutrient it was also important to keep the nutrient concentrations below the consent nitrate and phosphate level for discharge into receiving water so as to avoid eutrophication (see full details in section 3 2 2) With this in mind the dosing of both aerated ponds was carried out in a controlled manner (see previous calculations) The impact of dosing on the average nutrient levels in the ponds was immediate with increases being observed in the weeks immediately

following the first dosing event on 12 November 2004 Prior to this the average nitrate concentrations in the MFR and MFBP were 2 80±1 56 mg/l and 2 95±1 63 mg/l The average background levels of nitrate between November and December 2004 were 8 97±1 22 mg/l and 12 74±3 82 mg/l respectively in the MFR and MFBP Within the same period the average phosphate concentration increased only slightly from 0 40±0 17 mg/l to 0 59±0 30 mg/l in the MFBP The average phosphate level in the MFR prior to the dosing was higher at 0 43±0 23 mg/l compared to 0 28±0 08 mg/l recorded in the weeks immediately after the dosing (between November and December 2004) A comparison of the average biomass levels in the MFR and MFBP before and after the commencement of the nutrient addition showed increases from 1 92 x 10^8 CFU/l and 8 44 x 10^7 CFU/l to 2 37 x 10^9 CFU/l and 6 03 x 10^8 CFU/l in the MR and BP respectively (Table 2 Appendix 3A)

While the increased levels of nutrients appear to have enhanced the biomass growth in the aerated ponds the inconsistency in operation techniques at the HTF makes it difficult to ascertain if the improved BOD reduction recorded in the ponds in the 2004/2005 winter was a direct result of the increased nutrient levels Following the application of nutrient to the MFR and MFBP in November 2004 the BOD level in the MFR fell gradually from a peak level of 610 0 mg/l in late February (after the deicing event) to 315 0 mg/l in mid April 2005 eventually reaching 140 0 mg/l towards the end of May 2005 This is equivalent to an initial reduction of 48 4% over a 6 week period and an overall reduction of 77 1% over a total of 10 weeks During the same period the biomass population was stable at 1 20 x 10^8 CFU/l which represented a decrease from the value of 1 56 x 10^9 CFU/l recorded late in February A subsequent slight increase was observed towards the end of May 2005 with a final level of 1 85 x 10^8 CFU/l When part of the partially treated water was eventually transferred from the MFR in March 2005 the peak BOD level recorded in the MFBP on 14 April 2005 was 310 0 mg/l At this time the biomass population was 5 90 x 10^8 CFU/l Subsequently the biomass population increased reaching 1 23 x 10^9 CFU/l by the next visit on 29 April 2005 (two weeks later) and the BOD level in the MFBP dropped to 245 0 mg/l reaching 117 5 mg/l in 5 weeks (towards the end of May 2005) equivalent to a BOD reduction of 62 1%

In the previous winter (prior to the commencement of the nutrient dosing regime) the peak BOD level recorded in the MFR in late January 2004 (after deicing event) when monitoring started was 295 0 mg/l and the biomass population was 1 38 x 10^9 CFU/l Six weeks after this only 13 6% of the BOD had been removed and the biomass level

had fallen to 2 70 x 10^7 CFU/l In total it took over 12 weeks for the BOD level to be reduced significantly to 64 0 mg/l by the first week of May 2004 representing a BOD reduction of 78 3% The biomass level in the MFR in May 2004 was 2 35 x 10^8 CFU/l The peak BOD level recorded in the MFBP was 352 5 mg/l in early February 2004 (after the transfer of pre-treated dirty water that arrived in the MFR late in January 2004) and the biomass population was 6 00 x 10^8 CFU/l It also took about 12 weeks for the BOD level to drop considerably to 90 0 mg/l a level which is ideal for transfer into the SSF for further treatment However unlike the MFR where the biomass population remained around 10^8 CFU/l the biomass population in the MFBP after the 12 week period fell to 6 00 x 10^6 CFU/l

While it is evident that the BOD reduction attained in the MFR and MFBP during the 2004/2005 winter was better than that recorded in the 2003/2004 winter it is important to state that the operational procedures for the aerated ponds during the two occasions were very different During the 2004/2005 winter the MFR and MFBP were operated independently of each other for most of the winter The nutrient dosing was carried out separately in the different aerated ponds with no transfer initiated either remotely or manually at the start of the winter This is very different from the manner in which the system was operated during the 2003/2004 winter when the waters from the aerated ponds were being re-circulated round the entire system until the BOD level was low enough for discharge With the water moving through the system in the 2003/2004 winter the BOD in the aerated ponds remained consistently above 250 0 mg/l for most of the winter before showing signs of reduction in late April 2004 This also coincided with the disappearance of the ochrous coloration which had developed across the entire system when the re-circulating of the water commenced There were suggestions at the time that the presence of the ochrous coloration was responsible for the inability of the system to reduce the organic load Laboratory tests later showed this was not the case Details of the results of tests conducted to investigate the impact of the presence of the Fe^{2+}/Fe^{3+} equilibrium on BOD reduction are discussed in Chapter 4

The performance of the system during the winter of 2006 further highlights the role elevated pond nutrient levels on the BOD reductions The high nutrient levels recorded in both ponds prior to the winter particularly between September and November 2005 and later between March and April 2006 had an immediate impact on the biomass population The biomass populations remained at around 10^8 CFU/l and 10^7 CFU/l in the MFR and MFBP respectively between September and December 2005 before

increasing to higher levels of 10^9 CFU/l in both units as the nutrient levels increased in March and April 2006 The biomass populations had initially fallen to 3 30 x 10^7 CFU/l and 2 70 x 10^7 CFU/l in March 2006 in the MFR and MFBP after the nutrient levels dropped between December 2005 and February 2006 The BOD levels in the MFR and MFBP dropped from peak levels of 365 0 mg/l and 376 0 mg/l to 125 0 mg/l to 140 0 mg/l respectively within this period This is equivalent to BOD reductions of 65 8% and 62 8% in the MFR and MFBP respectively within six weeks This is comparable with the BOD reductions achieved within six weeks of deicing activities in the previous year when nutrient dosing was first employed

The operation of the system during the 2005/2006 winter was a combination of the techniques used in the two previous winters The first flush of polluted water was stored and treated in the MFR before transfer was initiated into the MFBP The capacity of the two aerated ponds was not sufficient to retain the high volume of water arriving at the facility over the 2005/2006 winter and hence it was necessary to transfer polluted water into the SSF With the performance of the SSF being jeopardised by the rapid reduction in DO levels in the front-end beds the system had to be operated in a re-circulating mode until the BOD was adequately removed towards the end of April 2006 As for the 2003/2004 winter the commencement of water re-circulation coincided with the development of an ochrous coloration throughout the system which eventually disappeared in late April when the BOD dropped The actual source of the ochrous coloration is not known but a series of possibilities and the relationship with BOD levels in aerated pond water samples are discussed in Chapter 4

3 4 2 Effect of DO levels and temperature on BOD reduction and biomass population

Although the successful operation of an aerated treatment system is believed to be highly dependent on an efficient aeration system the relatively high DO levels recorded in both the MFR and MFBP during the 2003/2004 winter failed to achieve an efficient BOD reduction in the ponds The average DO levels in the MFR and MFBP over a six week period (between late January and mid-March 2004) were 6 8$\pm$3 6 mg/l and 8 2$\pm$2 5 mg/l respectively and yet only 13 6% and 14 9 % of the BOD in the MFR and MFBP were removed during that period It took over 12 weeks for the BOD to be removed efficiently despite the corresponding average DO levels being 6 3$\pm$3 5 mg/l and 7 6$\pm$3 0 mg/l in the MFR and MFBP respectively During the 2004/2005 winter the DO levels in both the MFR and MFBP reached zero due to a mechanical fault in the aeration system In spite of this better BOD reductions were achieved taking 6

weeks to attain 48 4% reduction and about 10 weeks to reach 77 1% reduction in the MFR In the MFBP 62 1 % BOD reduction was attained between mid-April and the third week in May 2005 even though the DO level was zero throughout this period In the winter of 2005/2006 DO levels in the MFR and MFBP fell from 8 1 mg/l and 9 0 mg/l to 0 2 mg/l and 0 6 mg/l respectively between mid-March and late April 2006 and yet the BOD reductions attained within this period were 65 8 % and 62 8 % respectively

There are indications that the depletion of the DO levels in the MFR and MFBP is associated with the reduction in the BOD levels recorded in both ponds between mid-March and late April 2006 The decomposition of organic matter has been consistently shown to be associated with a decline in DO levels (McCartney *et al* 2003) The observation in the MFR and MFBP is consistent with results obtained from the Dane County Regional Airport pilot-scale dual-tank bioremediation system The average DO level in the system was 0 43±0 21 mg/l during the 3 day period in which 71 4% of the BOD was reduced (Gallagher 1998) Strong-Gunderson *et al* (1995) using respirometry data also reported high DO consumption during the biodegradation of a mixture of deicing products including propylene glycol by a consortium of bacteria All these systems suggest there is an inverse relationship between DO levels and the BOD concentrations under conditions in which intrinsic biodegradation is occurring indicating that microbes deplete the DO during the biodegradation processes On the other hand the non-utilisation of the DO in the system during the 2003/2004 winter is reflected in the high DO levels maintained throughout the winter and the prolonged period for which the BOD levels in the ponds remained high Due to the mechanical fault experienced with the aeration system in the MFR and MFBP during the 2004/2005 winter it is impossible to ascertain if the rapid depletion in DO between February and April 2005 was due to biodegradation

The results show the expected indications that the increasing temperature during the early spring contributes to the BOD reduction typically recorded at this time Aerobic degradation of glycols is claimed to be temperature dependent (Kent *et al* 1999) In a study of water samples from four different rivers Evans and David (1974) reported 100% BOD reduction in all water samples within 3 days at 20°C compared to less than 20% reduction in two of the four samples in over 14 days at 4°C Results from laboratory experiments conducted using aerated pond water samples have shown more efficient BOD reduction in water samples at higher temperatures (details in Chapter 5) Predictions from the HTF pilot scale study had shown improved BOD

reductions in the different components as the temperature increased from 6°C to 20°C (Revitt *et al*, 2001) During the 2003/2004 winter no significant BOD reduction was recorded in the MFR and MFBP between late January and early April 2004 when the average temperature was 8 0±1 8°C By late April 2004 when the temperature had increased to 13 3 °C the BOD in the MFR and MFBP had fallen by 35 6% and 74 4% respectively This pattern was repeated during the following winter when no significant reduction in the BOD level was recorded between mid-February and most of March 2005 (average temperature 4 9±0 5°C) The first sign of any BOD reduction was in April when the temperature had risen to 12 8°C By the third week in May 2005 with the temperature rising further to 15 3°C the overall BOD reductions attained in the MFR and MFBP were 77 1% and 53 0% respectively In the following winter (2005/2006) the overall BOD reductions in the MFR and MFBP reached 65 8 % and 62 8 % respectively as the temperature increased from an average winter level of 5 8±0 6 °C to 15 5°C on 27 April 2006

3 4 3 Relationships between BOD concentrations and the biomass populations in the MFR and MFBP

Amongst the factors that influence microbial viability none are as directly related to the population size as inorganic nutrient and organic carbon availability (Shane 1999) While the impact of nutrient addition is reflected in the biomass increases recorded in the MFR and MFBP it is difficult to establish the exact relationship between the BOD concentrations and the biomass populations in both ponds (MFR and MFBP) During the 2003/2004 winter the presence of a high BOD load between 29 January 2004 and 25 March 2004 corresponded to an initial decrease in biomass population from 1 38 x 10^9 CFU/l to 1 30 x 10^7 CFU/l in the MFR While the initial decrease in biomass population could be a result of the shock effect caused by the sudden introduction of the high organic load the average biomass population of around 10^7 CFU/l throughout the winter and the eventual increase to 2 35 x 10^8 CFU/l as the BOD level dropped from 207 5 mg/l to 64 0 mg/l between 7 April 2004 and 4 May 2004 could be an indication of the utilisation of the organic load for growth and sustenance This is consistent with claims by Shane (1999) that indigenous microbes use ambient inorganic nutrients and organic carbon to proliferate and maintain their cell tissues which is consequently reflected in an increase in population A similar trend was observed in the MFBP The arrival of a high organic load on 6 February 2004 resulted in a decrease in the observed biomass population from 1 75 x 10^8 CFU/l to 9 10 x 10^6 CFU/l on 19 February 2004 before stabilising at around 10^7 CFU/l until early April 2004 However unlike the MFR the biomass population in the MFBP dropped further

to 6 00 x 10^6 CFU/l as the BOD concentration dropped to 39 0 mg/l While this appears to contradict the expected trend the lower population recorded may be an indication of the lack of substantial organic load to support microbial population

In the 2004/2005 winter the arrival of the first high organic load (represented by a BOD concentration of 610 0 mg/l) into the MFR on 28 February 2005 was followed by a decrease in biomass population from 2 30 x 10^{10} CFU/l to 8 45 x 10^8 CFU/l as the BOD was initially reduced to 67 5 mg/l on 24 March 2005 Following a further increase the BOD fell from 315 0 mg/l to 6 25 mg/l (between 14 April 2005 and 15 June 2005 and the biomass population increased from 1 20 x 10^8 CFU/l to 1 00 x 10^{10} CFU/l) The initial trend in the MFBP was similar with a drop in biomass population from 5 90 x 10^8 CFU/l to 5 60 x 10^7 CFU/l when the BOD level increased from 50 0 mg/l on 28 February 2005 to 270 0 mg/l on 24 March 2005 As the BOD level increased further to 310 0 mg/l on 14 April 2005 the biomass population increased to 1 50 x 10^8 CFU/l increasing even further to 1 23 x 10^9 CFU/l as the BOD level dropped to 117 5 mg/l on 20 May 2005 suggesting that the initial decline in biomass population in the presence of high BOD was a response to the shock effect By 15 June 2005 when the BOD level in the MFBP had gone down to 5 0 mg/l a decline was recorded in biomass population reaching an average of around 10^7 CFU/l in the summer/autumn as the BOD concentration remained low While a decline in biomass population (from 10^8 CFU/l to 10^7 CFU/l) was observed in the MFR between November 2005 and February 2006 as the BOD concentration increased from 25 65 mg/l to 290 7 mg/l the increase in BOD concentration in the MFBP (from 34 2 mg/l to 302 0 mg/l) was followed by a corresponding increase in biomass population from 2 50 x 10^7 CFU/l to 1 20 x 10^8 CFU/l

In all three winters the initial introduction of high organic load (high BOD levels) appears to cause a decrease in the biomass population of the ponds Although the responses of the biomass population beyond the initial increase in BOD level varied widely in both systems there are indications that the subsequent increase in biomass population is a result of the utilisation of the organic load in the ponds In the next section the statistical relationships between the biomass populations and other factors already discussed on the BOD concentrations in the ponds are explored

3 4 4 Statistical analysis of the effects of the biomass population, temperature, DO and nutrient levels on the BOD concentration in the aerated ponds

Initial statistical analyses of the identified parameters in the aerated ponds between January 2004 and April 2006 showed no definite relationships with the BOD concentration Figures 1 and 2 Appendix 3B are matrix scatter-plots for all the parameters monitored in the MFR and MFBP during this period In Figure 1 none of the parameters appear to show any relationships with the BOD concentrations (for all three winters) in the MFR hence no detailed linear regression analysis was conducted Temperature and DO levels showed the most tendency to correlate with BOD concentrations in the MFR with p values of $p < 0\ 038$ and $p < 0\ 039$ respectively The R-squared value for the relationship was however low at 27 8% which implies that the variation in BOD levels in the MFR are explained by changes in the temperature and DO levels less than 30 0 % of the time between January 2004 and April 2006

The situation in the MFBP was slightly different with Figure 2 showing that the DO level and temperature appear to have a better relationship with BOD levels Linear regression analysis showed that both parameters were slightly more correlated with the BOD levels in the MFBP than in the MFR with a p value of $p < 0\ 005$ The biomass population in the MFBP also showed a good correlation with the BOD level at $p < 0\ 02$ with a R-squared value of 53 35%

3 5 COMPARISON OF THE LABORATORY BOD CONCENTRATIONS WITH BIOX BOD LEVELS RECORDED DURING ROUTINE SITE VISITS TO THE DIFFERENT COMPONENTS OF THE TREATMENT SYSTEM

Due to the crucial role played by the BIOX analysers in initiating water transfer between the different components of the system, based on pre-determined BOD levels it was considered important to establish the accuracy of these devices The recorded BOD levels were compared with the laboratory BOD results for water samples collected from the corresponding components of the HTF The aim was to ascertain how well the BOD readings registered by each BIOX analyser agree with the measured (laboratory) BOD concentrations The BOD levels from the comparative studies of the Mayfield Farm Diversion Chamber (DC) the Mayfield Farm Main Reservoir (MFR) and Balancing Pond (MFBP) and the exit channel of the system are summarised in the Tables that follow in each section

3 5 1 The Mayfield Farm Diversion Chamber (DC)

Under ideal operating conditions runoff from the Southern Catchment with a BOD concentration above 40 0 mg/l is diverted into the MFR This is initiated by the BOD concentration registered by the BIOX device located adjacent to the DC at the entrance to the Mayfield Farm section of the HTF The laboratory BOD concentrations in water samples collected from the DC were compared with the BOD level registered by the BIOX during visits between 21 April 2004 and 27 April 2006 Due to frequent operational faults with the BIOX analysers comparative BOD studies could only be conducted at the DC on 17 occasions and the results are summarised in Table 3 2 Although there are clearly differences between the BOD levels recorded in the laboratory test and those registered by the BIOX the increasing (attributed to the arrival of high organic load in the winter) or decreasing BOD trend in the water is detected by both systems There are no indications from the results that the BIOX analyser at the DC is unable to register BOD levels above 40 0 mg/l which is the trigger level for initiating the transfer of polluted water into the treatment system According to the laboratory tests the BOD concentrations arriving at the DC were observed to be higher than the 40 0 mg/l mark on five occasions but only three of these gave readings this level on the BIOX There are no indications from the laboratory BOD concentrations obtained for the water samples on the other two occasions that the failure of the device would have had severe consequence on the receiving water when discharged The results also show that the BIOX at the DC is unlikely to initiate unnecessary diversion of water as there was no single record of the BIOX analyser registering a BOD level above 40 0 mg/l when the laboratory tests showed otherwise i e the BIOX instrument tended to read consistently lower (Table 3 Appendix 3A)

3 5 2 The Mayfield Farm Main Reservoir (MR) and Balancing Pond (BP)

The diverted water from the Southern Catchment is stored and treated in the MFR before being transferred into the MFBP This transfer is initiated by the BOD reading registered by the BIOX analyser at the outfall of the MFR Transfer from the MFBP to the SSF is initiated similarly The laboratory BOD concentrations and the corresponding BOD levels recorded by the BIOX analyser at the MFR and the MFBP between March 2004 and April 2006 are compared in Table 4 Appendix 1 Between March 2004 and late April 2006 the BIOX analyser adjacent to the MFR recorded a wide range of BOD concentrations The lowest level registered was 10 0 mg/l on 26 May 2004 while the highest level (1060 0 mg/l) was recorded on 25 March 2004 (Table 3 4) The corresponding laboratory BOD values on these two occasions were 9 5 mg/l

and 267 5 mg/l the latter being considerably lower than the BIOX value The results also show that the BOD levels recorded by the BIOX device were higher than the laboratory values 67 8% of the time with the results being identical on only two occasions (Table 4 Appendix 3A) Of the four winter readings taken between March 2004 and April 2004 the laboratory BOD level was higher than the BIOX reading only on one occasion (7 April 2004) During the following winter periods the BOD levels recorded by the BIOX were higher on three of the five measurements made between February 2005 and April 2005 and on two of the four measurements made between December 2005 and April 2006 Although the BOD levels recorded by the device during the summer were mostly higher than the laboratory BOD concentrations the differences observed were not as significant as observed in the winter

The highest reading recorded by the BIOX adjacent to the MFBP between March 2004 and April 2006 was 505 0 mg/l (on 10 February 2006) The corresponding laboratory BOD concentration was 302 0 mg/l (Table 4 Appendix 3A) The lowest BOD reading recorded by the device was 7 0 mg/l with a corresponding laboratory BOD concentration of 11 0 mg/l The BOD levels recorded by the BIOX at the MFBP were higher than the laboratory levels for 60 0% of the measurements results shows that the BOD levels in the latter were higher on three of the four measurements made in March and April 2005 However the BIOX BOD readings were higher than the laboratory BOD values once during the four measurements in March and April 2004 and twice during the four measurements between December 2005 and April 2006 The BIOX BOD readings during the summer were generally higher than the levels recorded in the laboratory

3 5 3 The Mayfield Farm exit channel

Treated water from the SSF leaves the HTF through the exit channel which is equipped with a BIOX analyser The BOD level registered by the device at this point determines the fate of the treated water either to be discharged or re-circulated This is arguably the most important BIOX analyser at the Mayfield Farm section of the HTF as the release of water above the consent level of 40 mg/l BOD could have serious consequences for the receiving water It also serves as a measure of the overall performance of the treatment system The laboratory BOD values and the BOD levels registered by the BIOX at the exit channel between 4 May 2004 and 27 April 2006 are summarised in Table 5 Appendix 3A

Both the laboratory BOD concentrations and the levels registered by the BIOX throughout the monitoring period were as expected lower than those observed in the

other parts of the HTF Because of the lower recorded BOD values typically below 40 0 mg/l the differences in BOD levels determined by the two methods were not as significant as recorded in the MFR and MFBP particularly in the winter The BOD levels registered by the BIOX were higher than the laboratory BOD levels for 51 9% of the entire monitoring period Unlike the other BIOX analysers the BOD levels registered by the BIOX adjacent to the exit channel were lower than the laboratory BOD measurements made for most of the winter months (Table 5 Appendix 3A) Between February 2005 and April 2005 the laboratory BOD measurements were higher in three of five measurements made in all but one of the measurements taken between December 2005 and April 2006 and on two occasions between 4 May 2004 and 9 December 2005 During the May 2004 measurement the BOD level registered by the BIOX was 26 5 mg/l while laboratory tests showed a concentration of 45 0 mg/l With the BOD at this concentration, it is unlikely that the discharged water would pose any significant adverse effect to the receiving water For the December 2005 measurement the laboratory BOD concentration was 125 0 mg/l while the BIOX reading was 19 0 mg/l The implication of this is the possible discharge of water above the set consent of 40 0 mg/l It is not clear how long the water was discharged at this level but this occurrence could have been responsible for problems with DO levels which developed downstream later that winter There were newspaper reports at the time of the fouling of the local stream downstream of the HTF caused by the possible discharge of polluted water from the treatment system

3 5 4 Implications of the results obtained from the comparative study of the BIOX BOD levels and the laboratory BOD result and the laboratory BOD results

Statistical analysis of the BOD data obtained from the comparative study conducted between the readings of the BIOX analysers and laboratory BOD_5 results shows there is a positive correlation However the differences in the BOD results from the two techniques become generally more pronounced during the winter months as the influx of high organic load into the system increases This suggests that the accuracy of the BIOX device is adversely affected by high BOD levels during the winter This was highlighted for the MFR when the BIOX registered a BOD value of 1060 0 mg/l against a laboratory measurement of 267 5 mg/l on 25 March 2004 The anomaly in the BIOX was also shown in the MFBP on 7 March 2005 when it recorded 39 1 mg/l against a laboratory measurement of 250 0 mg/l Even the BIOX device at exit channel, which was generally exposed to lower BOD levels showed signs of malfunction on 9

December 2005 when it registered a BOD level of 19 0 mg/l against a laboratory measured level of 125 0 mg/l

It is not entirely clear why there is a disparity between the BIOX BOD readings and the laboratory measured levels particularly during the winter There is however a possibility that the high volume of water diverted into the system during winter event increases the build-up of solids in the device which would inevitably affect the accuracy of the device At the high flow rates there is likelihood that the microorganisms in the BIOX are unable to maintain the required ratio of dilution water to wastewater sample in order to accurately determine the BOD (see Section 2 1 1 of Chapter 2) During the pre-commissioning tests conducted on the BIOX analysers the blockage of the suction pump responsible for the transfer of water into the device was found to lead to erratic and un-reliable outputs Rectifying this problem often required dismantling the whole device this was laborious and could take several hours As a result a rigorous maintenance regime was put in place by the operators It is not clear from the results obtained if this has been particularly successful as faults were still reported in the devices during routine site visits particularly during the winter The higher reliability of the BIOX device at lower BOD levels is clearly an advantage at the Diversion Chamber (DC) because it means there is less possibility of water below the consent BOD level of 40 0 mg/l level being unnecessarily diverted into the system giving room for the storage and treatment of more polluted water in the MFR This is also the case at the exit channel where the ability of the device to register lower BOD levels means the discharge of water below the consent level (40 mg/l) would be effectively initiated

Statistically, the performance of the BIOX analyser at the DC (in comparison to laboratory BOD determinations) is the best with a correlation value of 0 92 The performance of the BIOX at the exit of the system was also impressive with a correlation value of 0 86 The correlations between the BOD levels recorded by the BIOX and the laboratory measured BOD levels for the MFR and MFBP were 0 76 and 0 80 respectively The p-values for these correlations were all significant at $p < 0\ 005$ Although this suggests there is a good degree of agreement between the laboratory measured BOD levels and the BIOX BOD readings the differences recorded during routine visits to the site clearly show that the devices were not reliable during winter events Consequently the unreliability of a device at higher BOD levels would adversely affect transfers within the system particularly between the aerated ponds (MFR and MFBP) and from the MFBP to the SSF making it impossible to truly assess the performance of each unit of the entire system

CHAPTER 4 ASSESSMENT OF THE IMPACT OF IRON ON THE BIODEGRADATION OF GLYCOL

Following the observation of a pronounced reddish brown colouration (later found to be caused by the presence of ferric iron, Fe^{3+}) in the Mayfield Farm aerated ponds during February 2003 a series of experiments were conducted to assess the impact of the presence of iron on the biodegradation performance of the system The reddish brown coloration remained for about eight weeks and simultaneously the BOD concentrations in the aerated ponds recorded by the BIOX analysers were static at around 350 mg/l for the entire period (February to mid-April 2003) Laboratory analysis of grab samples collected in March 2003 showed lower BOD values (<100 mg/l) although COD values were elevated (400-500 mg/l) Initial tests carried out in March 2003 also showed the concentration of total dissolved iron in the aerated ponds to be 2 6 mg/l predominantly in the form of Fe^{3+} The source of the iron compounds remains unknown although a number of possibilities have been proposed These include ingress of iron contaminated groundwater via the underground pipes transporting runoff from the airport contributions from materials (e g gravel substrate, brick gabions) within the treatment system and runoff derived sources from the airport surfaces The estimations of the large amount of iron in the entire treatment system were however not supportive of any of these theories

These estimates were based on the assumption that the overall Fe loading within the treatment system was composed of three main Fe phases, soluble Fe Fe associated to sediments and Fe deposited in the basal sediment There was the possibility that the estimated level of Fe deposited in the basal sediment when determined in June 2003 could have given rise to an over-estimation of the total iron in the system as it had previously existed as soluble/insoluble Fe in March 2003 However any over-estimation was believed to have been compensated for by the under-estimation in two other parts of the system, the return flow ditch where it was not possible to determine the Fe loading and the Sub-surface flow reedbeds (SSF) in which there was a predominance of soluble ferrous ions in most of the cells when samples were collected for analyses in March 2003

Estimation of iron loading in the aerated ponds (MFR and MFBP) and the Sub-surface flow reedbeds (SSF)

1 The Mayfield Farm Main Reservoir (MFR)

a) Total dissolved Fe

The concentration of total dissolved Fe in the MFR in March 2003 was 2 6 mg/l With the water level in the reservoir at 75% of its total capacity of 45 000 m^3 this was equivalent to 33 750 m^3 giving a total weight of dissolved Fe of 87 8 kg

b) Total suspended solid associated Fe

The concentration of total suspended solids in the MFR in March 2003 was 18 5 mg/l The total weight in 33 750 m^3 (75% of the full capacity) was 624 4 kg The concentration of Fe in dry solids collected from the sides of the reservoir in June 2003 which was likely to have been initially present as suspended solids (SS) was determined as 79,230 mg/kg Hence the weight of Fe attached to the SS in the MFR was calculated as 624 4 x 79 230 = 49 5 kg

c) Fe in basal sediment

From the sample of basal sediment collected from the MFR on 15 July 2003, the top 10 mm of the sediment was identified as the part containing most of the Fe Of this a section measuring 60 mm x 70 mm x 10 mm weighed 62 07 g which is equivalent to a density of 1 478 kg/m^3 The surface area of the sediment was estimated to occupy 6,800 m^2 (i e 80 m x 85 m the dimension of the MFR)

Volume of the Fe containing layer = 6,800 x 0 01 = 68 m^3
Weight of the Fe containing layer = 68 x 1 48 = 100 5 kg
Concentration of Fe in the surface sediment in June 2003 = 23 440 mg/kg
Total weight of Fe in basal sediment = 100 5 x 23 440 = 2 36 kg

Total estimated weight of Fe in the MFR = 87 8 + 49 5 + 2 36 = 139 7 kg

2 The Mayfield Farm Balancing Pond (MFBP)

a) Total dissolved Fe

The concentration of total dissolved Fe in the MFBP in March 2003 was 0 021 mg/l The total weight of dissolved Fe in the water at the time (the MFBP was 60% full which is equivalent to 11 400 m^3) was estimated as 0 24 kg

b) Total suspended solid associated Fe

In March 2003, the concentration of SS in the MFBP was 12 5 mg/l The total weight in 11 400 m^3 water was calculated as 142 5 kg Assuming the concentration of Fe in the dry solids collected from the sides of the MFR (79 230 mg/kg) is appropriate to estimate the concentration of SS in the MFBP the weight of Fe attached to the SS in the MFBP = 142 5 x 79 230 = 11 3 kg

c) Fe in basal sediment

The surface area of the basal sediment was estimated as 5400 m^2 (180 m x 30 m)
Volume of the Fe containing layer = 5,400 x 0 01 = 54 m^3
Weight of the Fe containing layer = 54 x 1 48 = 79 8 kg

Using the concentration of the Fe in the surface sediment (June 2003) obtained from the sides of the MFR (23 440 mg/kg) to estimate the concentration of Fe in the basal sediment of the MFBP the weight of Fe in the basal sediment = 79 8 x 23 440 = 1 87 kg

Total estimated weight of Fe in the MFBP = 0 24 + 11 3 + 1 87 = 13 4 kg

3 Sub-surface flow reedbeds (SSF)
The total surface area of the SSF = 2 08 ha = 20 800 m^2
Depth of the beds is 0 6 m and approximately a third of the whole volume within the SSF was occupied by water giving a volume of 4 000 m^3
There is approximately 600 m of open water channels in the SSF each 1 m wide
With a depth of 0 6 m each the overall water volume occupied = 360 m^3
Therefore, the total water volume within the SSF = 4000 + 360 = 4360 m^3

Front-end SSF cells
12 of the total 58 cells within the SSF are considered to be associated with the front-end of the system which were characterised by high soluble Fe concentrations in March 2003

a) Total dissolved Fe
The concentration of total dissolved Fe in the front-end of the SSF in March 2003 was 3 7 mg/l
This is contained in 12/58 x 360 = 74 5 m^3 of water
Therefore the total weight of dissolved Fe in the front-end part of the SSF = 0 28 kg

b) Particulate associated Fe
During sampling in March 2003, the high water level in SSF made it impossible to retrieve any SS for estimation Therefore the SS concentration in the MFBP (12 5 mg/l) which transfers directly to the SSF has been used

74 5 m^3 of water containing SS at a concentration of 12 5 mg/l gives a total sediment weight of 0 93 kg

Using the same Fe sediment concentrations as in the MFBP i e 79 230 mg/kg the estimated weight of Fe in the solids in the front-end of the SSF would be 74 2 x 79 230 = 5 9 kg

Non-front-end SSF cells

a) Total dissolved Fe

The concentration of total dissolved Fe in the non-front-end of the SSF in March 2003 was 0 026 mg/l

This is contained in (46/58 x 360) + 4000 = 4,285 5 m^3 of water

Therefore the total weight of dissolved Fe in the non-front-end part of the SSF = 0 11 kg

b) Particulate associated Fe

4 285 5 m^3 of water containing SS at 12 5 mg/l gives a total sediment weight of 53 6 kg

Assuming an Fe sediment concentration of 79,230 mg/kg (as above), the estimated weight of Fe in the solids in the non-front-end of the SSF was 4 285 5 x 79 230 = 339 5 kg

Total estimated weight of Fe in the SSF = 0 28 + 5 9 + 0 11 + 339 5 = 345 8 kg

Neglecting the return flow ditch the total estimated weight of Fe in all three components (MFR MFBP and SSF) = 498 9 kg

Groundwater which was identified as one possible source of Fe influx into the system was estimated to have a potential Fe contribution of up to 3 mg/l The volume of water required to produce the estimated load of 498 9 kg would be 166,300 m^3, such a contribution is not feasible given that the combined capacity of the three storage/treatment units at the Mayfield Farm is 68 360 m^3

Consecutive analyses of filtered water samples collected from the substrate area of the SSF exhibited BOD concentrations above 200mg/l with realistic COD concentrations of >300 mg/l By mid-April 2003 the reddish-brown colouration within the water bodies had disappeared leaving reddish-brown stains on the sides of the aerated ponds and the substrate region of the reedbeds as the only remaining evidence Almost immediately the BIOX analysers registered a significant drop in BOD The most obvious conclusion at the time was that the sudden disappearance of the brown colouration (due to Fe^{3+}) observed in the aerated ponds and the consequent drop in BOD registered by the BIOX meters were linked

Laboratory experiments were conducted on water samples taken from the aerated ponds to investigate the impact of the existence of an Fe^{2+} / Fe^{3+} aqueous equilibrium on the biodegradation performance of a treatment system involving

aeration ponds The results are discussed in this chapter Realistic attempts have been made to recreate the conditions existing in the aerated ponds in terms of BOD levels (through the addition of commercially available propylene glycol trade name Kilfrost) oxygen supply iron concentrations (through the addition of hydrated ferrous sulphate $FeSO_4\ 7H_2O$) turbulence and temperature The impacts of different BOD levels have also been assessed in terms of how these affect the oxidation/reduction of iron complexes in the system The latter part of this chapter discusses the effect that stirring through the generation of turbulence in the system will have on the biodegradation of glycols

4 1 IMPACT OF BOD (350 mg/l) ON THE FORMATION OF Fe^{3+} IN MAYFIELD FARM AERATED POND WATER SAMPLES IN ENCLOSED SYSTEMS AT 20°C

This experiment was conducted to assess the impact of the presence of BOD in the form of glycol on the Fe^{2+}/Fe^{3+} equilibrium in aerated pond water samples This was done by comparing the rate of oxidation of Fe^{2+} to Fe^{3+} in water samples with and without glycol The initial dose of Fe^{2+} in the water sample was set at 3 mg/l as more recent tests showed this to be a typical level found in the aerated ponds Details of the experiment are discussed in Section 2 3 1 1 of Chapter 2 The degradation of glycol (indicated by the depletion in DO concentrations) in water samples initially containing Fe^{2+} was also monitored after the water samples were fully saturated with air at the onset of the experiment The compositions of the water samples used are summarised below

System A Pond water/Glycol/Fe^{2+} (350 mg/l BOD and 3 mg/l Fe^{2+})
System B Pond water/Glycol (350 mg/l BOD)
System C Pond water/Fe^{2+} (3 mg/l Fe^{2+})
System D Pond water (Blank)

The results obtained from tests extending over a period of 8 days are summarised in Appendix 4A, Table 1 Figure 4 1 shows the percentage conversion of Fe^{2+} to Fe^{3+} in samples A and C over the same period

4 1 1 Discussion of trends observed in the experiment

DO depletion was recorded in each of the four systems but with greater decreases in samples A and B possibly as a consequence of the biodegradation of the added glycol which was not present in samples C and D The trends in DO depletion in Samples A and B are somewhat similar the only difference being the sharp drop in DO between Days 2 and 3 (3 70 mg/l to 1 30 mg/l) in sample B (Table 4 1) Both systems became anoxic by Day 6 Although these observations are indicative of

biodegradation activities in the water samples, the DO depletions recorded are not as rapid as those observed by Gallagher (1998) in which complete DO depletion was achieved within 2-3 days during the biodegradation of glycol at 20°C The higher DO depletion rates observed in Samples A and B compared to C suggest that the added BOD and not the oxidation of Fe^{2+} to Fe^{3+} places more demand on the DO The utilisation of DO to convert Fe^{2+} to Fe^{3+} is however indicated from a comparison of the behaviours of Samples C and D (both not containing additional BOD) This probably also explains the higher intermediate DO depletion rate observed in sample A compared to Sample B although it is unclear, at this point what the exact impact of Fe^{2+}/Fe^{3+} is on the biodegradation of glycol

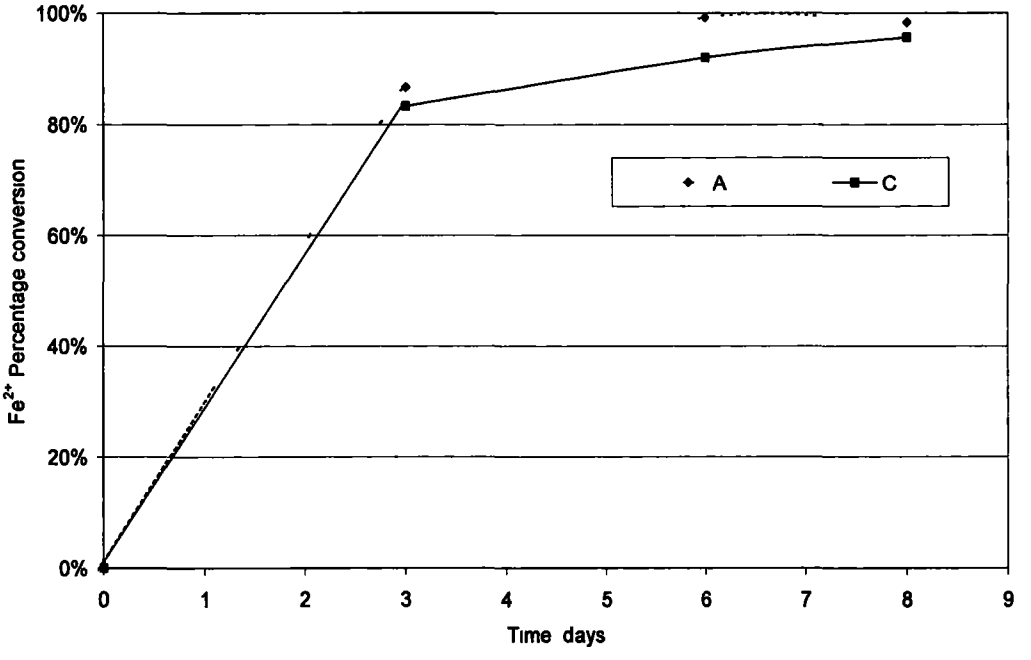


Figure 4 1 Temporal changes in the percentage conversion of Fe^{2+} to Fe^{3+} in an aerated pond water sample dosed initially with 3 mg/l Fe^{2+}

The conversion rate of Fe^{2+} to Fe^{3+} in sample A was slightly higher than in sample C (Figure 4 1) On Day 3, 86 7% of the Fe^{2+} had been converted to Fe^{3+} compared to 83 3% in Sample C The difference became more significant by Day 6 with 99 0% (near complete conversion) in sample A compared to 92 0% in sample C At this point Sample A was anoxic and as a consequence a subsequent small reduction in Fe^{3+} concentration from 2 98 mg/l to 2 95 mg/l was recorded This indicates the potential for the reduction of Fe^{3+} to Fe^{2+} under anoxic conditions with a possibility of further reduction if this condition is prolonged Such a phenomenon was reported by Ehrenreich and Widdel (1994) in their study of iron-rich sediments The concentration of Fe^{3+} continued to increase in Sample C after Day 6 towards a 100% conversion rate due to the continued presence of DO in this solution

4 2 IMPACT OF BOD (350 mg/l) ON THE FORMATION OF Fe^{3+} IN THE MAYFIELD FARM AERATED POND WATER SAMPLES WITH DIFFERENT BACKGROUND CONCENTRATIONS OF Fe^{2+} IN ENCLOSED SYSTEMS AT 20°C

The DO levels and Fe^{3+} concentrations in four systems containing different starting concentrations of Fe^{2+} (ranging from 1 mg/l to 4 mg/l) were monitored to assess the impact of different concentrations of Fe^{2+}/Fe^{3+} on the biodegradation of glycol in aerated pond water samples (see also Section 2 3 1 2 of Chapter 2) The results obtained from these tests are reported in Table 2 Appendix 4A The temporal relationships between the DO and the Fe^{3+} concentrations are shown in Figures 4 2-4 5 In these diagrams Days 0-8 represent the initial biodegradation to anoxic conditions followed by re-aeration at Day 8 and then further biodegradation through to Day 19 Systems 1 2, 3 and 4 were dosed with 1, 2 3 and 4 mg/l of Fe^{2+} respectively and all contained 350 mg/l BOD in the form of glycol Each system was allowed to go anoxic as the biodegradation of the glycol proceeded during incubation at 20°C All four samples were re-aerated on Day 8 (previous experiments had suggested total conversion of Fe^{2+} to Fe^{3+} and the development of anoxic conditions occur within 8 days)

4 2 1 Discussion of trends observed in the experiment

The highest drop in DO concentration upon re-aeration on Day 8 was recorded in System 4 dropping to 0 8 mg/l and then 0 2 mg/l on Days 9 and 10 respectively (Table 3 Appendix 4A) A rapid fall in DO in the first 24 hours after re-aeration was also observed in the other three systems By Day 10, 48 hours after re-aeration the DO levels were 0 7 mg/l 0 4 mg/l and 0 35 mg/l for Systems 1 2 and 3 respectively suggesting a higher utilisation/depletion rate of dissolved oxygen with increasing concentrations of Fe^{3+} under similar conditions All four systems became completely anoxic by Day 11 (Table 2 Appendix 4A)

There were marked increases in Fe^{3+} concentrations following re-aeration on Day 8 in all four systems implying that the oxidation of Fe^{2+} to Fe^{3+} re-commenced following this process and increasing Fe^{3+} concentrations continued until Day 12 in Systems 3 and 4 (Table 4 2 and Figures 4 4 and 4 5) The highest increase in Fe^{3+} concentration following re-aeration was in System 4, increasing from 1 29 mg/l to 3 51 mg/l between Days 9 and 10 before reaching a maximum concentration of 3 93 mg/l (representing a conversion of 98 3% of the original Fe^{2+} concentration) on Day 12 A similar pattern was repeated in System 3 with the Fe^{3+} concentration increasing from 1 31 mg/l on Day 9 to 2 71 mg/l on Day 10 reaching a maximum concentration of 2 77 mg/l (representing a conversion of 92 3% of the original Fe^{2+} concentration) by Day 12

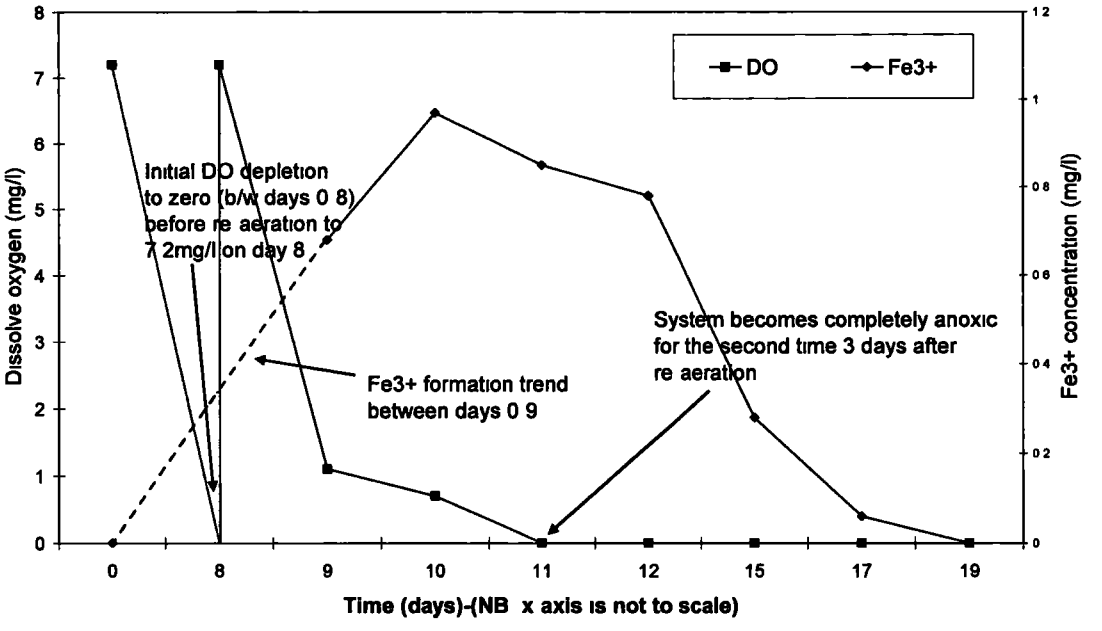


Fig 4 2 Temporal changes in Fe^{3+} and DO concentrations for 1 mg/l Fe^{2+} in an aerated pond water sample

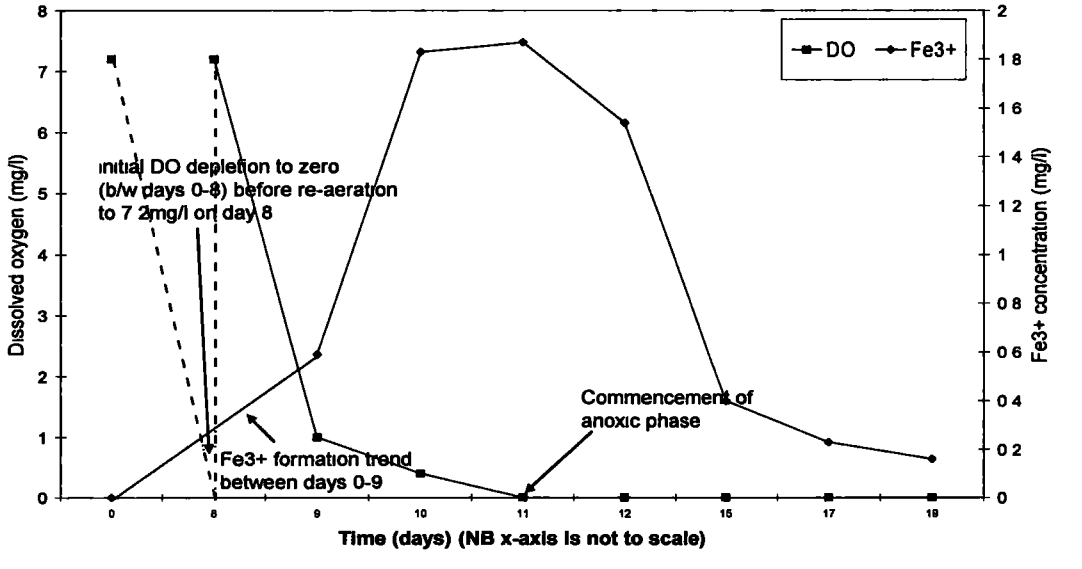


Fig 4 3 Temporal changes in Fe^{3+} and DO concentrations for 2 mg/l Fe^{2+} in an aerated pond water sample

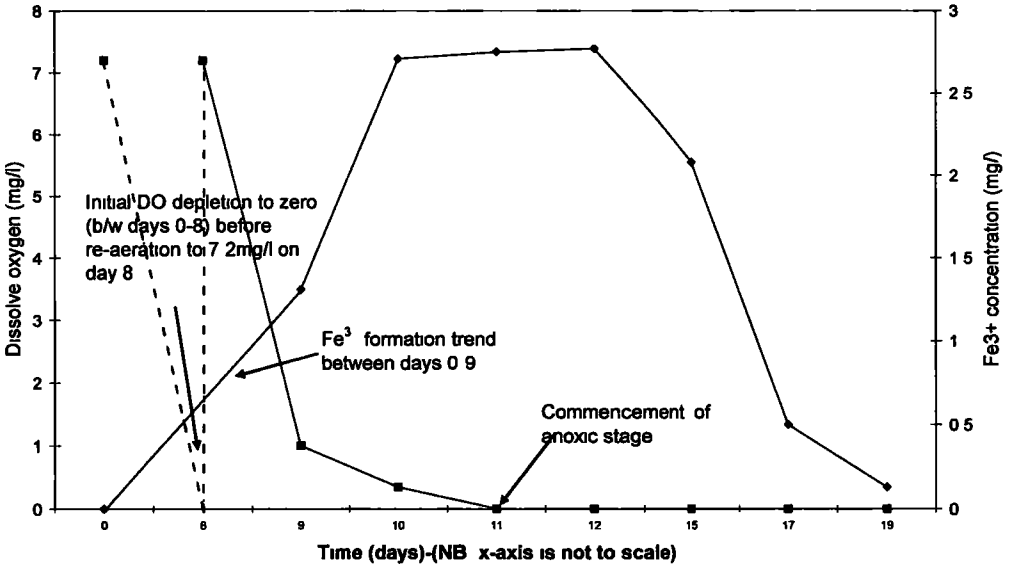


Fig 4 4 Temporal changes in Fe^{3+} and DO concentrations for 3 mg/l Fe^{2+} in an aerated pond water sample

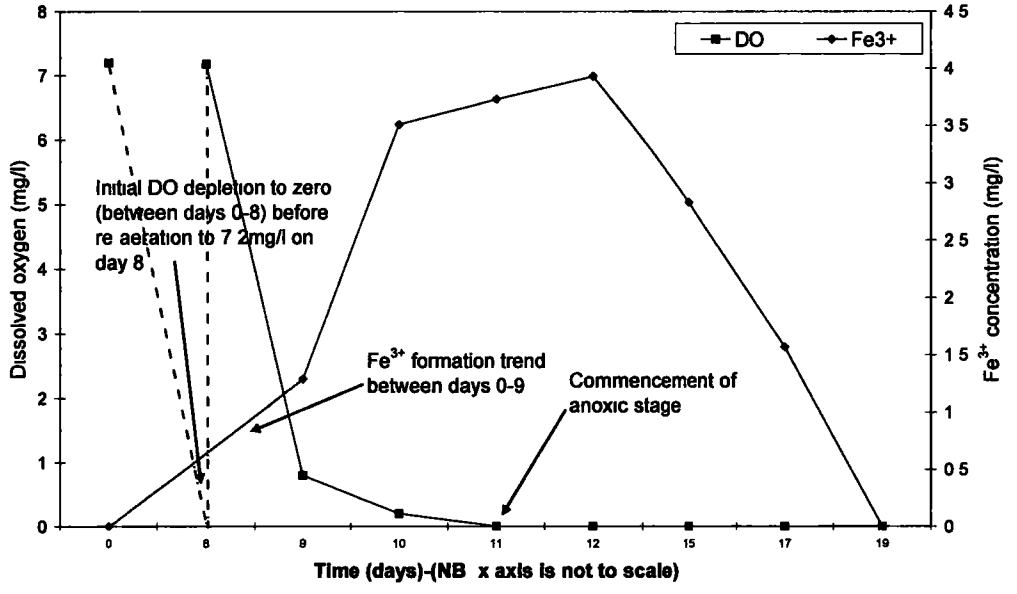


Figure 4 5 Temporal changes in Fe^{3+} and DO concentrations for 4 mg/l Fe^{2+} in an aerated pond water sample

There were no decreases observed in the concentrations of Fe^{3+} in both Systems 3 and 4 during the first day of the anoxic stage (Day 11) but subsequent decrease

did occur The concentration of Fe^{3+} in System 3 dropped from 2 71 mg/l (92 0% conversion) to 2 08 mg/l (69 0% conversion) on Day 15 (4 days into the commencement of the second anoxic stage) The overall conversion recorded by Day 19 (8 days into the commencement of the second anoxic stage) was 95 7% (Table 2 Appendix 4A) The Fe^{3+} concentration in System 4 dropped from 3 93 mg/l to 2 83 mg/l in the same period of the anoxic phase corresponding to a reduction from 98 3% to 70 8% in the conversion of Fe^{3+} to Fe^{2+} with a complete regeneration of Fe^{2+} by Day 19 (Table 2 Appendix 4A)

In System 2 the maximum Fe^{3+} concentration attained was 1 87 mg/l (representing a conversion of 93 5% of the original Fe^{2+} concentration) on Day 11 The reduction of Fe^{3+} to Fe^{2+} commenced in System 2 during the first day of the anoxic phase reaching 1 54 mg/l This decrease continued gradually so that by Day 15 (4 days into the anoxic phase) only 20 0% of the produced Fe^{3+} remained The overall Fe^{3+} to Fe^{2+} conversion by Day 19 was 92 0% The only increase in Fe^{3+} concentration recorded in System 1 following re-aeration was between Days 9 and 10 with an increase in Fe^{3+} concentration from 0 68 mg/l to 0 97 mg/l (97 0% of the original Fe^{2+} concentration) The reduction of Fe^{3+} commenced on Day 11 reaching 72 0% Fe^{3+} to Fe^{2+} conversion on Day 15 and 100 0% by Day 19 There is a need to investigate the effect of a prolonged anoxic phase on the Fe^{3+}/Fe^{2+} concentrations and the next experiment has been designed to investigate this as well as the role of BOD

With all four systems becoming anoxic 3 days after re-aeration (Day 11), it appears the reduction of Fe^{3+} to Fe^{2+} commences once the maximum Fe^{3+} concentration is attained (Figures 4 2-4 5) The responses of Systems 3 and 4 to the prevailing anoxic conditions (indicated by the reduction of Fe^{3+} to Fe^{2+}) which was not as rapid as observed in System 1 suggests that the oxidation/reduction equilibrium depends on the concentration of Fe^{2+}/Fe^{3+} in the system In both systems, the higher concentration of Fe^{2+} initially present appears to serve as a potential for Fe^{3+} formation even after the systems became anoxic

4 3 IMPACT OF BOD (350 mg/l) ON THE INTER-CONVERSION OF Fe^{2+} AND Fe^{3+} IN AERATED POND WATER SAMPLES CONTAINING 3 mg/l OF Fe^{2+} IN ENCLOSED SYSTEMS AT 20°C FOR AN EXTENDED PERIOD OF TIME

The DO and Fe^{3+} concentrations in two enclosed systems were monitored over a extended period of time to assess the effect of a prolonged anoxic phase on the inter-conversion of Fe^{2+} and Fe^{3+} in aerated pond water samples with different

components Details of the experiments are in Section 2 3 1 3 of Chapter 2 The components of the two water samples were as follows,

Sample A 3 mg/l of Fe^{2+} in MFR water sample dosed with 350 mg/l BOD
Sample B 3 mg/l of Fe^{2+} in MFR aerated pond water sample

Sample A was re-aerated after it became completely anoxic on Day 10 and the Fe^{2+}/Fe^{3+} inter-conversion was monitored throughout the 17-day experiment The DO and Fe^{3+} concentrations observed in both systems are reported in Table 4 3 The relationships between these trends are shown in Figure 4 6

4 3 1 Discussion of trends observed in the experiment

The variations observed during the prolonged monitoring of the DO and the Fe^{3+} concentrations in Samples A and B resemble the trends observed in the previously described experiments The DO in Sample A was completely utilised within 8 days having dropped from an initial level of 6 4 mg/l Anoxic conditions could have been established prior to this as no readings were available for Days 5-7 Sample A was re-aerated on Day 10 and the DO level subsequently fell from 5 7 mg/l to 2 3 mg/l in the following 24 hours The second anoxic stage was achieved within 4 days of re-aeration During the first 8 days of the experiment, only 36 8% of the DO in Sample B was used up as the concentration decreased to 4 3 mg/l from an initial level of 6 8 mg/l The DO subsequently remained fairly constant with a final value (after 17 days) of 3 6 mg/l

The complete depletion of the DO in Sample A on two occasions is clearly due to the presence of the additional biochemical demand exerted by the presence of glycol and this did not appear to be hindered by the presence of Fe^{2+}/Fe^{3+} The reasons for the different rates of achievement of the first and second anoxic states are not immediately clear From Figure 4 6, it can be seen that during the second decrease in DO (following re-aeration) the Fe^{2+}/Fe^{3+} ratio is predominantly in favour of Fe^{3+} (98% to 94% of total Fe content during Days 10 to 14) In the development of the first anoxic stage (Days 0 to 8), the Fe^{2+} is undergoing oxidation and the Fe^{3+} composition varies from 0-97% Hence it is tempting to hypothesise that it is the presence of soluble Fe^{2+} ions compared to the precipitated Fe^{3+} ions that is more likely to reduce the rate of biodegradation and therefore the rate of dissolved oxygen utilisation However, an added factor will be the possible increases over time in the active microbial populations enabling a more rapid biodegradation during the formation of the second anoxic phase Ehrenreich and Widdel (1994) have reported the enrichment of anoxygenic phototrophic bacteria using ferrous iron as the sole electron donor for photosynthesis in anoxic sediments In Sample B there is never a complete loss

of DO and that which does occur is concentrated during the first 4 days of the experiment and is used to support the oxidation of Fe^{2+} to Fe^{3+} (70 6% conversion at Day 4) for which the rate subsequently decreases (Figure 4 6)

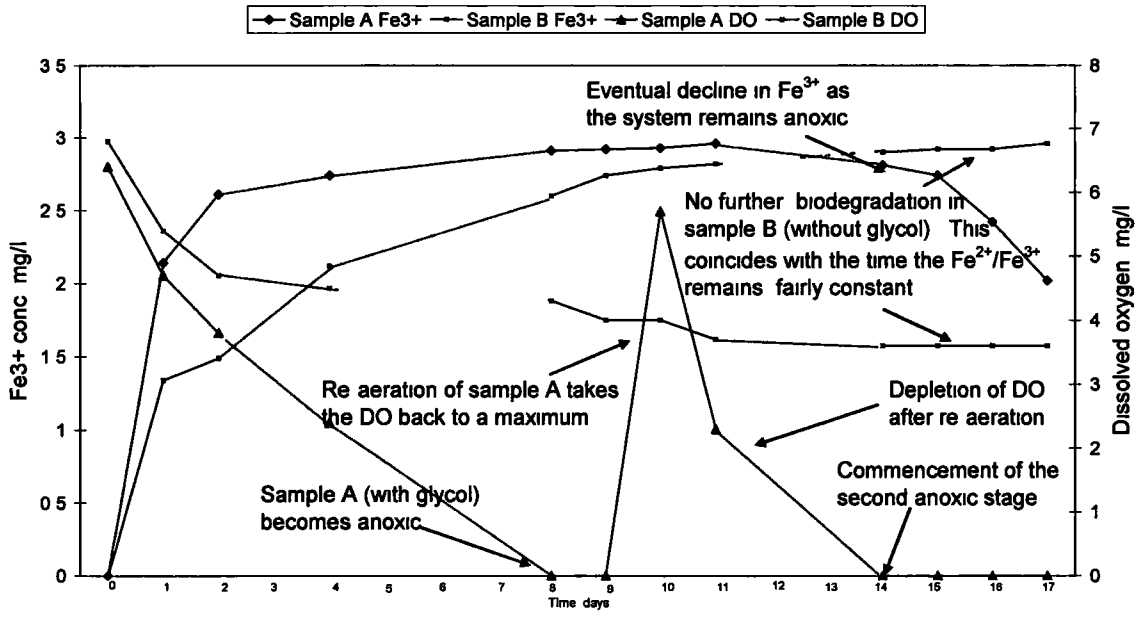


Figure 4 6 Temporal changes in DO and Fe^{3+} concentrations for 3mg/l Fe^{2+} in an aerated pond water sample with and without glycol (BOD 350mg/l) incubated at 20°C

The initial Fe^{2+} conversion rates were higher in Sample A compared to Sample B with a 71 3% conversion rate after 1 day which slows down as the system reaches a maximum Fe^{3+} concentration of 2 96 mg/l (98 6% conversion of the total Fe^{2+}) on Day 11 (a day after re-aeration and two days after the system became anoxic) After attaining maximum conversion to Fe^{3+}, the concentration of the oxidised form dropped gradually as the system became anoxic again resulting in 32 6% of the Fe^{3+} being reduced to Fe^{2+} in 6 days Figure 4 6 shows that the rate of reduction of Fe^{3+} to Fe^{2+} under anoxic conditions is slower than the rate of oxidation of Fe^{2+} to Fe^{3+} during the same time interval However this effect was not so obvious in the other experiments The concentrations of $Fe^{2+}$and Fe^{3+} observed throughout this experiment give a clearer picture of what happens to the Fe^{2+}/Fe^{3+} equilibrium in oxic and anoxic environments Both experiments indicate that there is a lag phase for the commencement of the reduction of Fe^{3+} to Fe^{2+} after the establishment of prolonged anoxic conditions

4 4 IMPACT OF STIRRING A Fe^{3+} PRECIPITATE AT A CONCENTRATION OF 3 mg/l IN AERATED POND WATER SAMPLES (WITH VARYING COMPONENTS) ON THE DEGRADATION PROCESSES IN ENCLOSED SYSTEMS AT ROOM TEMPERATURE

The DO concentrations in four different systems were monitored to assess the influence of a constantly mixed Fe^{3+} precipitate on the biodegradation of glycol in aerated pond water samples at room temperature (see Section 2 3 1 4 of Chapter 2 for experimental details) The components of the four systems used were as follows

System 1 A continuously stirred system consisting of the aerated MFR water sample dosed with 350 mg/l BOD and 3 mg/l Fe^{2+}

System 2 A replica of System 1 to provide duplicate results

System 3 A non-stirred aerated MFR water sample system dosed with 350 mg/l BOD and 3 mg/l Fe^{2+}

System 4 A continuously stirred system consisting of the aerated MFR water sample dosed with 3 mg/l Fe^{2+} only

Each of the pond water samples was dosed with 3 mg/l Fe^{2+} and then left exposed to the atmosphere for 8 days (by which time previous experiments have shown that the oxidation would have produced maximum Fe^{3+} concentrations) Each solution was fully aerated and then 350mg/l BOD in the form of glycol was added to Systems 1 2 and 3 By adding the glycol directly to the Fe^{3+} any oxygen depletion due to biodegradation of glycol was not influenced by the oxidation of Fe^{2+} to Fe^{3+} The daily variations in DO concentrations for all four systems after the introduction of glycol into 3 of the water samples are shown in Figure 4 7

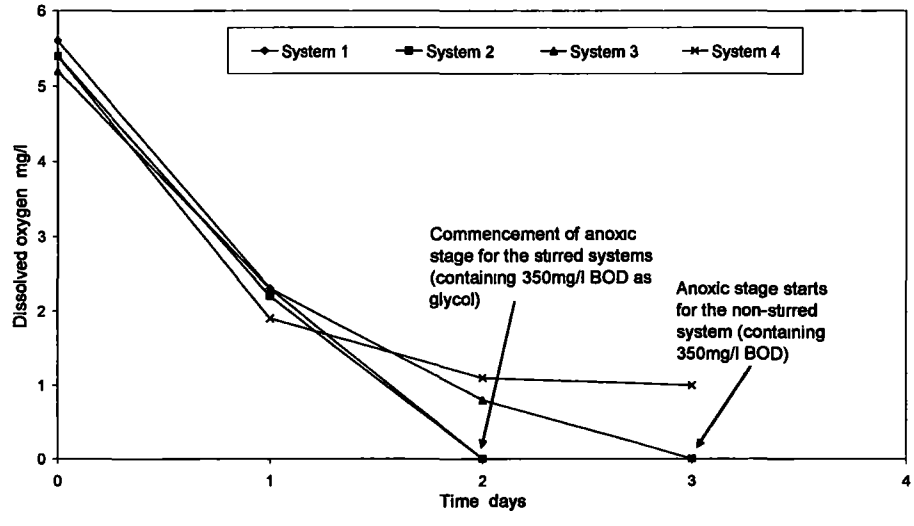


Figure 4 7 Effect of stirring a Fe^{3+} precipitate (approximately 3 mg/l) on the depletion of dissolved oxygen in enclosed systems at room temperature after the addition of BOD

4 4 1 Discussion of trends observed in the experiment

A rapid depletion of the DO (in the water samples) occurred within System 1 reaching zero DO in just two days after the addition of BOD (Figure 4 7) The DO levels in the non-stirred comparable system reached zero on Day 3 The slightly higher DO depletions observed over the first 2 days in the continuously stirred systems suggest that the turbulence created by the stirring may have some effect on the biodegradation process in the system There is a possibility that the stirring process ensures a fully mixed system which enhances the bioactivity of microorganisms attached to the Fe^{3+} flocs in the water sample Suzuki *et al* (2003) in a study of pollutant removal in two forms of bioreactors recorded higher biodegradation rates in a more turbulent air-sparged system compared to the rotating biological contactors In the absence of stirring the Fe^{3+} flocs (precipitates) can more efficiently settle out (flocculation technique used in water treatment) carrying the microbes with them so that their effectiveness is reduced However, these effects are not consistent with the short-term study of the second stage biodegradation in this experiment Therefore, it is unclear from the levels of Fe^{3+} precipitate used in this experiment whether significant numbers of microorganisms were available to have a significant impact on biodegradation under stirred and non-stirred conditions A higher level of Fe^{3+} is used in the next experiment

4 5 IMPACT OF STIRING A Fe^{3+} PRECIPITATE (AT A CONCENTRATION OF 10 mg/l) IN AERATED POND WATER SAMPLES ON THE BIODEGRADATION PROCESSES IN ENCLOSED SYSTEMS AT ROOM TEMPERATURE

The DO levels in four fully saturated enclosed systems containing varying components were monitored over six days to assess the impact of stirring a higher concentration of Fe^{3+} precipitate (10 mg/l hydrated ferric chloride, $FeCl_3\,6H_2O$) on the biodegradation of glycol at room temperature The experimental set-up is the same as described in the previous section Full experimental details are provided in Section 2 3 14 The components of each system were as described below

System 1 A continuously stirred system containing aerated pond water sample dosed with 350 mg/l BOD and 10 mg/l Fe^{3+}

System 2 A non-stirred system containing aerated pond water sample with 10 mg/l Fe^{3+}

System 3 A continuously stirred system containing aerated pond water sample dosed with 350 mg/l BOD

System 4 A continuously stirred system containing aerated pond water sample with 10 mg/l Fe^{3+}

The trends in DO concentrations observed for the different systems are illustrated in Figure 4 8 Figures 4 9 and 4 10 compare the results from this experiment with those from previous experiments

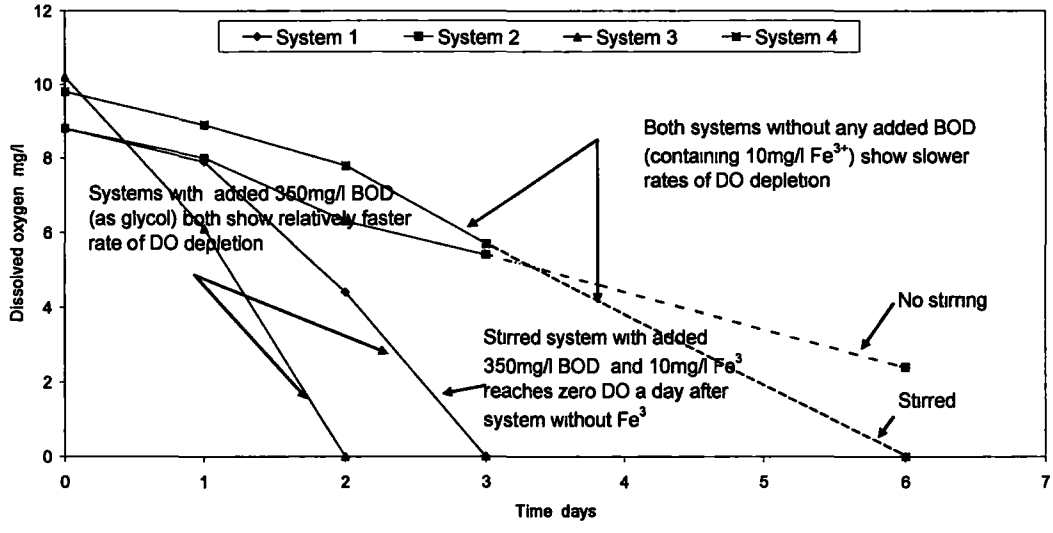


Fig 4 8 Temporal changes in DO concentrations in aerated pond water samples due to the presence of a precipitate of Fe^{3+} (10 mg/l) under different controlling conditions

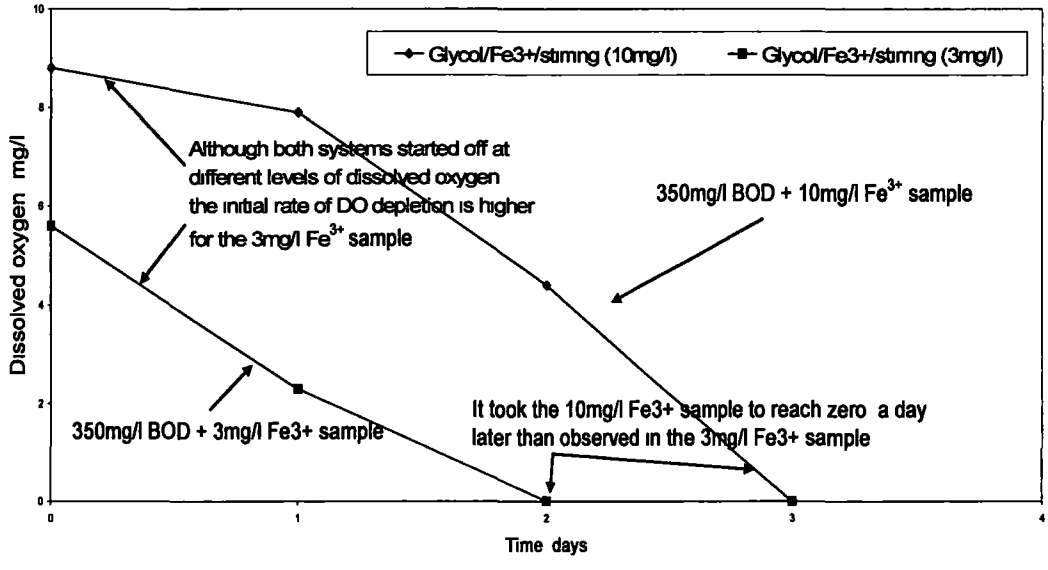


Fig 4 9 Comparison of the dissolved oxygen variations for stirred aerated pond water samples containing glycol and different concentrations of Fe^{3+}

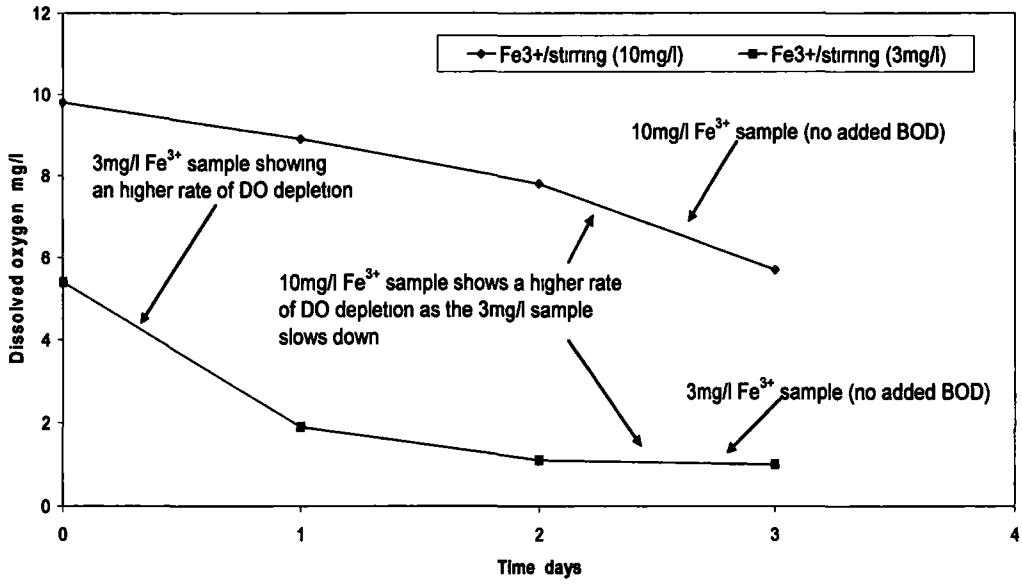


Fig 4 10 Comparison of the DO variations for stirred aerated pond water samples containing different concentrations of Fe^{3+}

4 5 1 Discussion of trends observed in the experiment

The trends in DO depletion observed for the four systems tend to fall into two patterns, depending on the presence or absence of added BOD (Figure 4 8) The highest rate of DO depletion was observed in System 3 with a 40 2% drop in the first 24 hours (from 10 0 mg/l to 6 1 mg/l) before falling to zero in just two days The DO depletion rate in System 1 was initially slower but increased by Day 2 with a decrease from 7 9 mg/l to 4 4 mg/l Anoxic conditions were established by Day 3 (Figure 4 8) The complete depletion of DO in Systems 1 and 3 confirms the fact that the added glycol has a significant role to play in the process and is consistent with the results from previous experiments where the systems containing glycol attained the anoxic state most rapidly Trends in the dissolved oxygen depletion in Systems 1 and 3 (Figure 4 8) suggest that the presence of a Fe^{3+} precipitate in System 1 may be responsible for the overall slightly slower rate observed

The complete depletion in DO observed in System 4 (becoming anoxic after six days) was unexpected given the absence of any added BOD However it confirms the presence of organic material within the MFR water sample and the susceptibility of this to decay via the resident microbes The relatively higher DO depletion rates observed in System 4 compared to System 2 and the results from the previous experiment indicate that stirring assists the bio-degradation process

Comparison of the DO depletion rates for the 10 mg/l Fe^{3+} and the 3 mg/l Fe^{3+} water samples which are both continuously stirred and contain 350mg/l added

BOD (Figure 4 9) shows that the lower Fe^{3+} concentration enables a total depletion of the dissolved oxygen to be achieved by Day 2 (dropping from 5 6 mg/l to zero DO) In the same time period the system containing 10 mg/l Fe^{3+} showed a DO reduction from 8 8 mg/l to 4 4 mg/l attaining anoxic condition after three days (Figure 4 9) The DO depletion rates were also higher in the stirred system containing 3 mg/l Fe^{3+} compared to the stirred system containing 10 mg/l Fe^{3+} in the absence of any added BOD (Figure 4 10) The initial 1 day drop in the former was 64 8% (5 4 mg/l to 1 9 mg/l) compared to only 9 2% (9 8 mg/l to 8 9 mg/l) in the 10 mg/l Fe^{3+} water sample Neither solution had reached anoxicity under the test conditions but by Day 3 the 3 mg/l Fe^{3+} sample had used up 81 5% of the available DO compared to 41 8% in the 10 mg/l Fe^{3+} sample The results indicate that increased concentrations of a Fe^{3+} precipitate may have a restricting influence on the biodegradation process but as shown in System 4 (Figure 4 8) an Fe^{3+} concentration of 10 mg/l is not sufficient to completely inhibit microbial activities even when minimal quantities of biodegradable material are available

4 6 INVESTIGATION OF THE IMPACT OF BOD (1000 mg/l) ON THE FORMATION OF Fe^{3+} IN AERATED POND WATER SAMPLES IN ENCLOSED SYSTEMS AT 20°C

The DO and Fe^{3+} concentrations in two systems were monitored to assess the impact of higher BOD concentrations on the Fe^{2+}/Fe^{3+} Equilibria in aerated pond water samples Details of the experiment have been discussed in Section 2 3 1 1 of Chapter 2 The components of the two systems were as follows

Sample A Aerated pond water sample dosed with 1000 mg/l BOD and 3 mg/l Fe^{2+}
Sample B Aerated pond water sample with 3 mg/l Fe^{2+}

The trends followed by both samples with respect to Fe^{3+} and DO concentrations are shown in Table 4, Appendix 4A These results are compared with those from the previous experiments in Figure 4 11

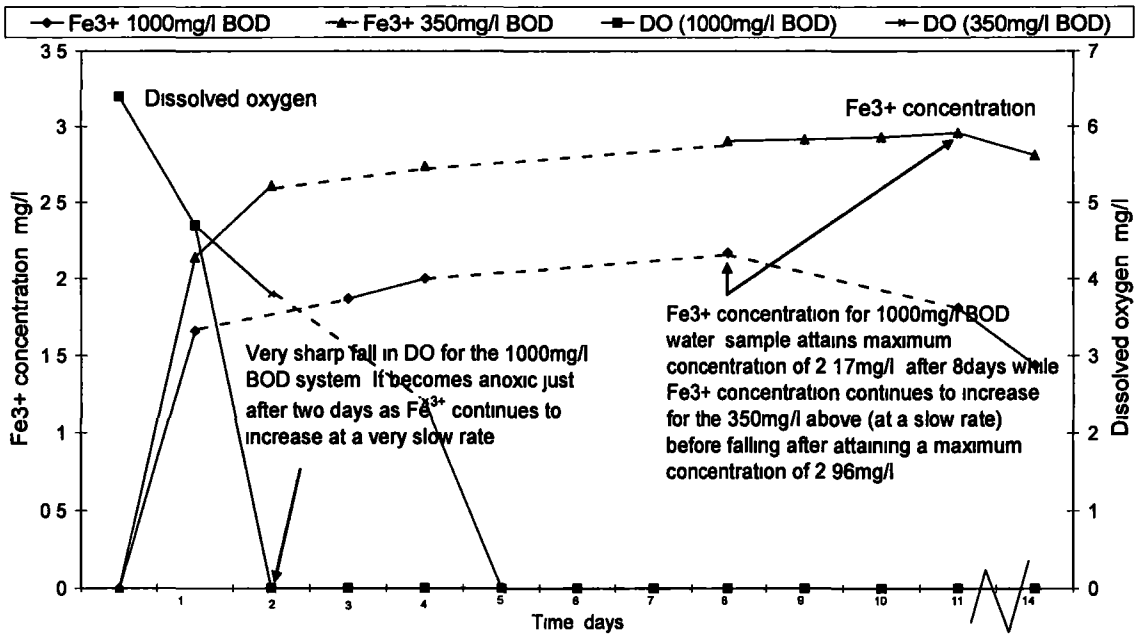


Fig 4 11 Temporal changes in Fe^{3+} and DO concentrations for 3mg/l of Fe^{2+} aerated pond water samples with 350mg/l and 1000mg/l BOD

4 6 1 Discussion of trends observed in the experiment

The trends observed in Fe^{3+} and dissolved oxygen concentrations in Sample A (Table 4, Appendix 4A) are similar to those observed in water samples with added BOD in previous experiments The initial one day fall in DO in Sample A was 31 3% before becoming anoxic after three days The DO depletion in Sample B was much slower with an average daily drop of approximately 6% over the 11 day monitoring period as the DO fell from 6 3 mg/l to 2 2 mg/l

Initial oxidation rates of Fe^{2+} to Fe^{3+} in both systems were higher than at any other point in the experiment (Table 4 Appendix 4A) and there was more efficient oxidation in the presence of glycol The oxidation process appeared to continue in Sample B whilst it ceased in Sample A on Day 8 five days after it became anoxic This is a trend that has been demonstrated in previously monitored systems When this trend is compared to the results for a solution containing 350 mg/l BOD (Figure 4 11) it is clear that a lower Fe^{2+}/Fe^{3+} conversion was ultimately obtained in the 1000mg/l BOD sample Similarly the anoxic phase in the 350 mg/l BOD system commenced 3 days later than in the 1000 mg/l BOD system (Figure 4 11) The existence of a lag time during the maintained anoxic phase before reduction of Fe^{3+} back to Fe^{2+} was once again observed

4 7 DEDUCTIONS FROM ALL THE TESTS CONDUCTED

Based on the depletion in dissolved oxygen (indicative of active biodegradation) observed in all the experiments reported in this Chapter it is clear that the presence of Fe^{2+}/Fe^{3+} does not adversely affect the biodegradation process in the aerated ponds While the results show that a higher concentration of Fe^{3+} precipitate (10 mg/l) (Section 4 5) reduces the DO depletion rate more significantly than a lower Fe^{3+} concentration (3 mg/l) (Section 4 4) there are no indications that the Fe^{3+} levels observed during the winter event (February/March 2003) in the different treatment units of the Mayfield Farm were sufficient to put the performance of the entire system at risk

The ease of oxidation of Fe^{2+} to Fe^{3+} to give the associated reddish brown colouration under different conditions and for different levels of Fe^{2+} has been indicated clearly (Section 4 2) Results from the visual observation of the laboratory experiments have shown that the intensity of this colouration is dependent on the concentration of Fe^{3+} in the system Similar trends observed in both the DO and Fe^{2+}/Fe^{3+} concentrations under different experimental conditions (including variable BOD concentrations [Section 4 6]) suggest that both processes (DO depletion and oxidation of Fe^{2+} to Fe^{3+}) are independent of one another The prevailing redox conditions in the system have been shown to play a key role in the Fe^{2+}/Fe^{3+} equilibrium The results provided by the experiments in Sections 4 1 4 2 and 4 6 all show the reduction of Fe^{3+} to Fe^{2+} as the system becomes and stay anoxic Visual observation of water samples in each case shows a connection between the reduction process and the disappearance of the reddish brown colouration This offers a tentative explanation for the observations in the different components of the HTF after the winters of 2003/2004 and 2005/2006 (see Chapter 3 for more details)

CHAPTER 5 THE BIODEGRADATION EXPERIMENTS

The results of laboratory studies conducted to assess the biodegradation potential of water samples from the Heathrow Treatment Facility (HTF) are reported and discussed in this chapter These experiments were designed to replicate the different prevailing conditions at the HTF using controlled environments to assess the impact of imposed conditions on the biodegradation of glycol which is reflected in the BOD reductions recorded The impacts of different levels of nutrient sources on BOD reductions in different water samples are also reported Further biodegradation experiments conducted using isolates from the HTF are also discussed

5 1 ASSESSMENT OF THE BIODEGRADATION POTENTIAL OF WATER SAMPLES FROM THE HTF

Following the estimation of the microbial populations in the different components of the HTF (see details in Chapter 3), it is important to assess the biodegradation potential of these microorganisms and hence estimate the overall BOD reduction potential of the HTF In order to assess the potential for the biodegradation of glycol by these microorganisms the changes in the dissolved oxygen (DO) concentrations in additive-free water samples collected from four different components of the HTF were monitored at two different temperatures 6°C and 20°C While the lower temperature was chosen to replicate the conditions in the water during the winter, the higher temperature would assess the effect of increasing the temperature on the biodegradation process Water samples used for these experiments were collected from the following parts of the treatment system during the 10 February 2004 visit

MFR Mayfield Farm Main reservoir
MFBP Mayfield Farm balancing pond
SSF Mayfield Farm subsurface flow reedbed system
ER Eastern reservoir

Theoretically the depletion of DO in water samples can be expressed exponentially as -

$$C = C_o e^{kt} \qquad \textit{equation 5 1}$$

Where

C = Final concentration of dissolved oxygen
C_o = Initial concentration of dissolved oxygen
t = Time (days)
k = Biodegradation rate constant (day ^{1})

Assuming a constant biomass population in the water samples the biodegradation rate constant k has been determined for each system at 6°C and 20°C Figures 5 1 to 5 4 illustrate how this has been achieved for oxygen saturated water samples from each of the four treatment system components Using the nearest-fit exponential plots the curves derived from the DO depletion recorded in the systems are shown below The deviations of the recorded trends from the exponential curves can be attributed to the natural state of the water samples used for these experiments

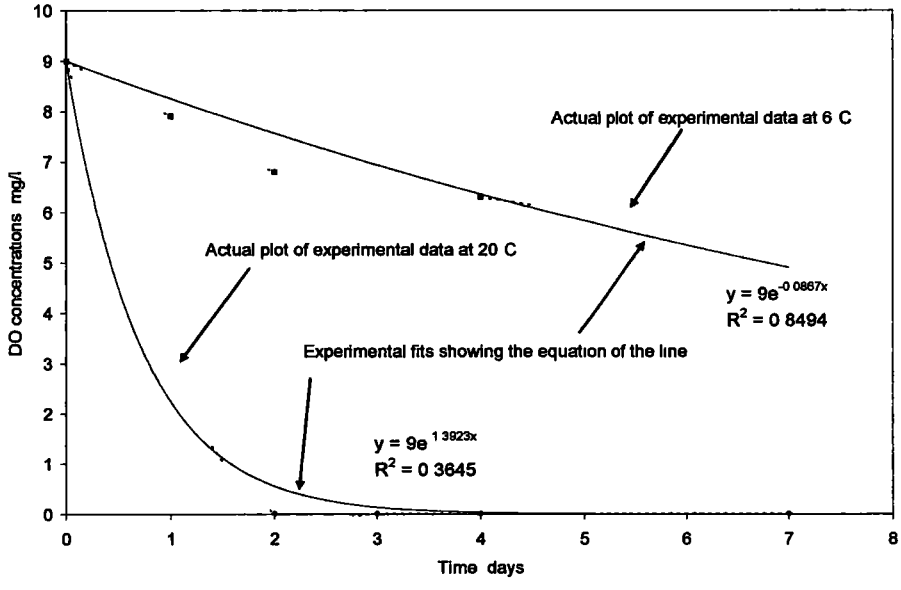


Figure 5 1 Comparison of the changes in DO concentrations for the water samples from the MFR at 6°C and 20 °C

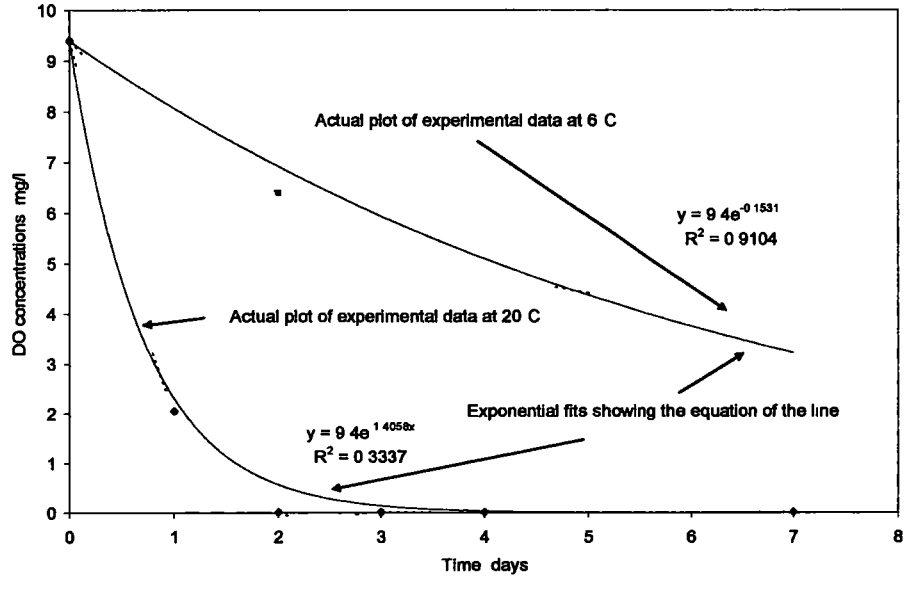


Figure 5 2 Comparison of the changes in DO concentrations for the water samples from the MFBP at 6°C and 20 °C

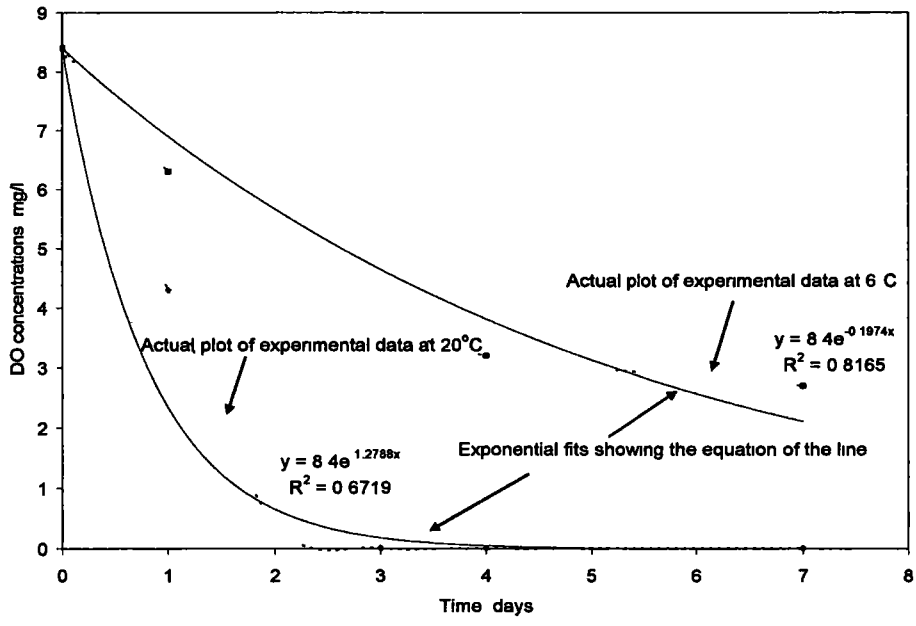


Figure 5 3 Comparison of the changes in DO concentrations for the water samples from the SSF at 6°C and 20 °C

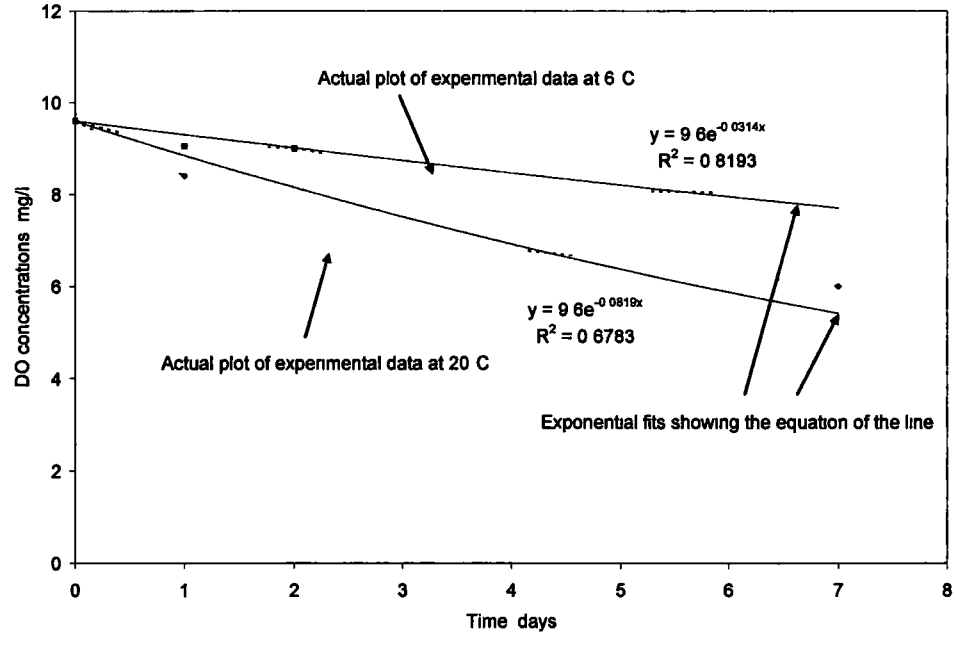


Figure 5 4 Comparison of the changes in DO concentrations for the water samples from the ER at 6°C and 20 °C

Derivation of the biodegradation rate in the water samples

The exponential decay equations derived for the different water samples at both temperatures in (Table 1, Appendix 5A) correspond to the predicted exponential decrease in DO which is expressed in equation 5 2 The biodegradation rate constant (k) was not obtained directly from the respective exponential equations which are based on the actual experimental plots

$$e^{kt} = \frac{C_t - C_u}{C_o - C_u} \qquad \textit{equation 5 2}$$

Where C_t = DO concentration after time t

C_o = Initial DO concentration

C_u = Ultimate DO concentration (indicative of equilibrium DO concentration reached after prolonged biodegradation)

Using the exponential decay equation established for MFR at 6°C the DO concentration after any time (C_t) can determined by substituting a value for t into the Equation 5 1

Hence for t = 5 days

Then $C_t = C_5 = 9\ 0\ e^{-0\ 0867 \times 5}$

$C_5 = 9\ 0\ e^{-0\ 4335}$

$C_5 = 5\ 83$ mg/l

If the initial DO concentration (C_o) has decreased by 90 0% of its original value to reach C_u (as seen in Figures 5 1 to 5 4) the latter will have a value of 0 1 C_o i e 0 9 mg/l The time it takes to attain this concentration in the water sample can be calculated using Equation 5 2, where $C_t = C_5$ and t = 5 days the biodegradation rate can be determined as

$$e^{5k} = \frac{5\ 83 - 0\ 90}{9\ 00 - 0\ 90}$$

$e^{5k} = 0\ 6086$ k = 0 099 day ^{1}

Determination of the potential BOD reduction in the water sample

The reduction in the amount of biodegradable material associated with the water samples by the microbial activities can be calculated from the biodegradation rates using Equation 5 3

$$BOD_t = L_u (1 - e^{kt}) \qquad \textit{equation 5 3}$$

Where

BOD_t = Biochemical oxygen demand (BOD) after time t in days

L_u = ultimate oxygen demand

k = biodegradation rate (day^{-1})

t = time in days

The fraction of the initial BOD concentration removed in any given water sample relative to the ultimate value after time t can be expressed as

$$\frac{BOD_t}{L_u} = 1 - e^{kt} \qquad \textit{equation 5 4}$$

The reduction in BOD concentration in the MFR water sample at 6°C after a day (t = 1) using equation 5 4 is

$$\frac{BOD_t}{L_u} = 1 - e^{-kt} = 1 - e^{0\,099 \times 1} = 1 - 0\,9048$$

$$= 0\,0952 \ (9\,52\%)$$

The time taken for half (50%) of the ultimate BOD to be removed ($T_{1/2}$) can be determined using the above expression where

$$0\,5 = \frac{BOD_t}{L_u} = 1 - e^{-0\,10 \times T1/2}$$

$$0\,5 = 1 - e^{-0\,10 \times t0\,5}$$

$$e^{-0\,099 \times T1/2} = 0\,5$$

$$-0\,099\ T_{1/2} = -0\,6932$$

$$T_{1/2} = 7\,0 \text{ days}$$

Using the same approach for the other water samples the derived values for biodegradation rates and predicted times to remove 50% of the original BOD were also obtained (Table 2, Appendix 5A)

5 1 1 Discussion of trends observed in the different water samples

There was evidence of utilisation of the DO in all four water samples within the first twenty-four hours of the experiment at both temperatures The highest drop in the DO concentration was recorded in the MFBP water sample at 20°C falling from 9 4 mg/l to 2 1 mg/l (Figure 5 2) The drop in DO concentration in the MFR within the same period was from 9 0 mg/l to 2 85 mg/l (Figure 5 1) The DO in the MFR and

MFBP was completely depleted by Day 2 of the experiment at 20°C At the same temperature the DO concentration in the SSF dropped from 8 4 mg/l to 4 3 mg/l in the first 24 hours and then to 0 4 mg/l on Day 2 before becoming completely depleted 3 days after the experiment started (Figure 5 3) In the ER water sample the DO dropped from 9 6 mg/l to 8 4 mg/l in the first 24 hours and continued to decrease slowly throughout the experiment reaching 6 0 mg/l after 7 days at 20°C (Figure 5 4) As expected the rate of DO utilisation in the water samples at 6°C was not as rapid as that recorded at 20°C The depletion in DO concentration over the 7 days of the experiment was more noticeable in the MFBP and SSF where the DO concentration dropped from 9 4 mg/l to 3 65 mg/l and 8 4 mg/l to 2 7 mg/l respectively Within the same period the DO concentration in the MFR dropped from 9 0 mg/l to 5 3 mg/l As observed at 20°C the DO depletion rate in the water samples from the ER was the slowest with the DO concentration dropping from 9 6mg/l to only 8 0 mg/l in 7 days (Figure 5 4)

Comparison of the biodegradation rates in all water samples indicates that the SSF had the highest biodegradation potential at 6°C with a biodegradation decay rate of 0 239 day ^{1} with 50 0 % depletion in DO concentration in 2 9 days (Appendix 5A Table 2) The lowest biodegradation rate (0 043 day ^{1}) at 6°C was recorded in the ER taking 17 3 days for half of the background BOD concentration to be removed The biodegradation rates at 20°C in the MFR MFBP and SSF were similar with values of 0 440 day ^{1} 0 442 day ^{1} and 0 443 day ^{1}, respectively It took approximately 1 6 days for the removal of 50% of the initial BOD concentration The biodegradation rate of 0 091 day ^{1} calculated for the ER at 20°C was the lowest recorded at 20°C in all the systems and it was comparable with the rates observed in the MFR and MFBP at the lower temperature of 6°C

5 1 2 Deductions from experiments conducted

The decreases in DO concentrations recorded in all the water samples with time demonstrate clearly that all the components of the HTF had the potential for biodegradation The higher biodegradation rates observed at 20°C are consistent with the findings of Revitt *et al* (2002) in which increases in the biodegradation rates were observed in dosed water samples collected from airport surfaces when the temperature was increased from 1°C to 8°C This emphasises the role temperature plays in microbial biodegradation At 6°C the SSF demonstrated the greatest potential for biodegradation with a rate of 0 239 day ^{1}, which is higher than the 0 081 day ^{1} reported by Revitt *et al* (2002) for airport paved surface water samples dosed with different types of ADF at a slightly higher temperature of 8°C The results obtained for the SSF water sample at 6°C confirms the presence of

active microbial processes in the system and is consistent with the observations of rapid DO depletion in the front-bed of the SSF system at the HTF (see Chapter 3)

The complete depletion of the DO in the MFR, MFBP and SSF at 20°C within 2-3 days is consistent which the observation of Gallagher (1998) in which the DO in a bioreactor designed for the treatment of airport runoff was completely used up within 2 days The biodegradation rates of 0 099 day^{1} and 0 181 day^{1} observed in the water samples from the MFR and MFBP respectively at 6°C are also comparable with the rates (0 073 day^{1} and 0 081 day^{1}) observed in water samples from airport paved surface between 4°C and 8°C, respectively (Revitt *et al* , 2002) The time taken (6 9 days for the MFR and 3 9 days for the MFBP) to achieve a potential 50 0% reduction in the BOD concentration is however inconsistent with the observations at the HTF, where it has been shown that between six and ten weeks are required for any considerable BOD reduction in the aerated ponds This is in contrast to the performance of the ER in which a 98 3% BOD reduction was recorded over approximately 35 days during the 2004/2005 winter The BOD reduction over 14 days during the following winter (2005/2006) was 92 5% (see Chapter 3) It is however unclear at this stage why the biodegradation rates recorded in the ER at both 6°C and 20°C are lower than those recorded in the rest of the system

It is not clear why the higher biodegradation rates observed in water samples from the MFR and MFBP are not replicated on the field There have been suggestions that the recalcitrant nature of some of the components present in ADF products may be responsible Methyl benzotriazole (MeBT) for example, has been shown to be inhibitory to microbial processes (Cancilla *et al* 2003) In the next section the results of experiments carried out to investigate the effect of MeBT on the biodegradation of the propylene glycol based ADF (Kilfrost) in MFR water samples are discussed

5 2 ASSESSMENT OF THE IMPACT OF 5-METHYL BENZOTRIAZOLE (MeBT) ON THE BIODEGRADATION DECAY RATES OF AERATED POND WATER SAMPLES

Methyl benzotriazole has been implicated in the lowering of biodegradation potentials in ADF polluted water samples (Cancilla *et al* 2003, Corsi *et al* 2003) The impact of 5-methyl benzotriazole (MeBT) a weak hydrophobic organic acid which complexes strongly with many metals on the biodegradation rates in water samples from the MFR was assessed by monitoring the DO depletion rates at 20°C and 6°C (see details of experiment in Section 2 3 3 of Chapter 2) The components of the different water samples used in the experiments were

A MFR water sample (control)

B MFR water sample dosed with 350 mg/l BOD as Kilfrost

C MFR water sample dosed with 350 mg/l BOD as Kilfrost and 7 48 mg/l MeBT (equivalent to the 1% concentration (w/w) typically formulated in ADF)

The DO depletion in the water samples was monitored over time and the exponential plots matched to the DO depletion trends in each system are shown in Figures 5 5 to 5 7

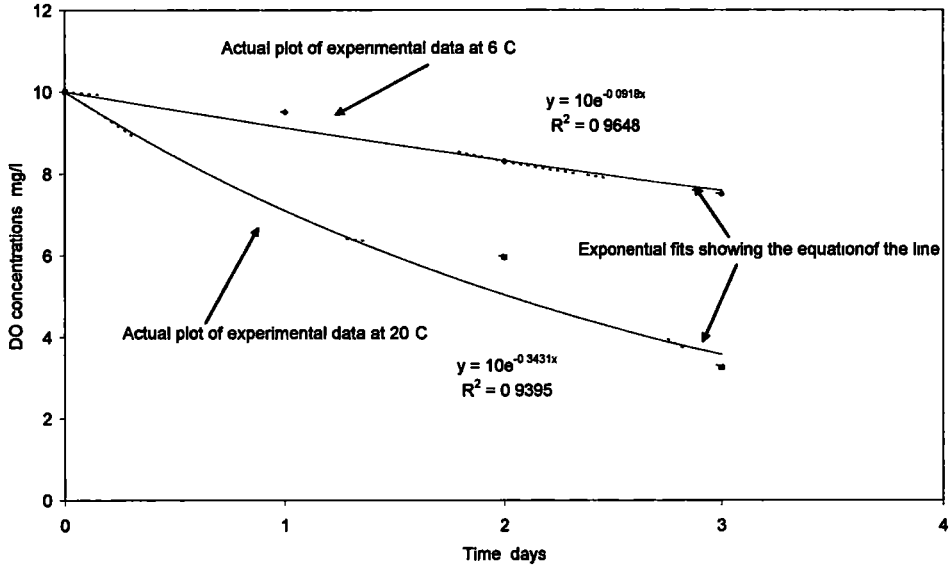


Figure 5 5 Comparison of the changes in DO concentrations for the water samples from the MFR (control) at 6°C and 20 °C

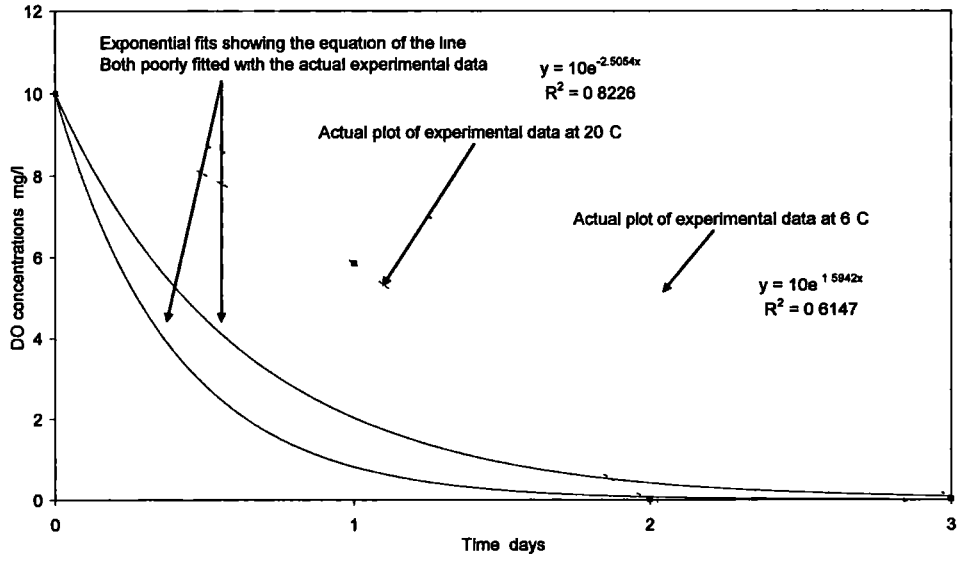


Figure 5 6 Comparison of the changes in DO concentrations for the water samples from the MFR dosed with 350 mg/l BOD as Kilfrost at 6°C and 20 °C

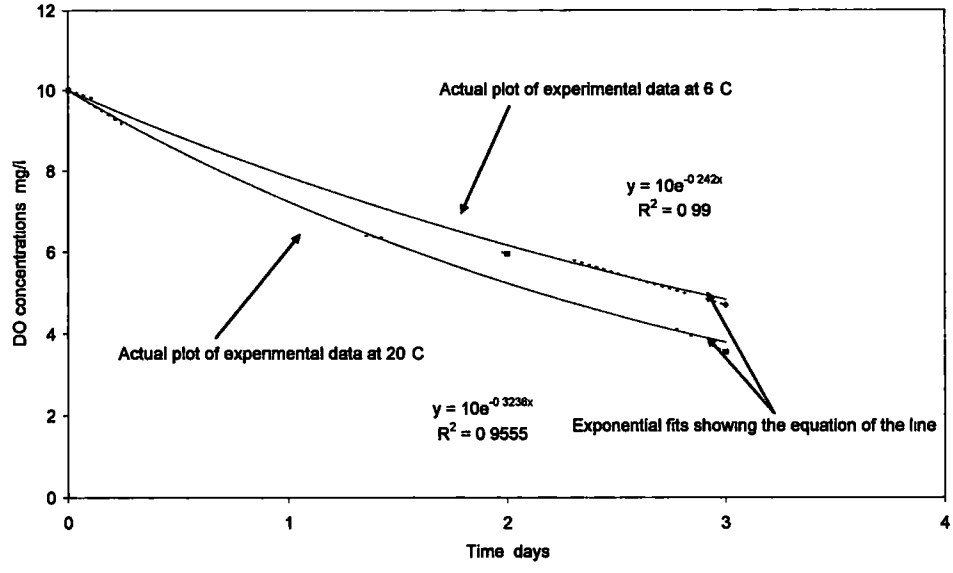


Figure 5 7 Comparison of the changes in DO concentrations for the water samples from the MFR dosed with 350 mg/l BOD as Kilfrost and 7 48 mg/l MeBT at 6°C and 20 °C

Using the approaches described in the previous section, the biodegradation rate BOD fraction removed after one day and the time taken to remove half of the BOD in the different systems at 6°C and 20°C were also calculated (Table 3 Appendix 5A)

5 2 1 Discussion of trends observed in the different water samples

The highest rates of depletion in DO concentrations were observed in Sample B with complete depletion being achieved on Days 2 and 3 at 20°C and 6°C respectively (Figure 5 6) The biodegradation rates 1 093 day^{-1} at 20°C and 1 081 day^{-1} at 6°C, are higher than the rates recorded in MFR water samples in the previous experiment (Section 5 1) The reduction in DO concentration in the control (Sample A) was gradual with the system at 20°C showing a higher depletion rate reaching 3 3 mg/l after 3 days compared to 7 5 mg/l at 6°C (Figure 5 5) The biodegradation rate of 0 093 day^{-1} obtained at 6°C is comparable to the 0 099 day^{-1} rate observed in the additive-free water sample collected from the MFR in the experiments discussed in Section 5 1 Although the reduction in DO concentration in Sample C (dosed with MeBT) was not as rapid as recorded for Sample B the rate observed was faster than that observed for the control particularly at 6°C with

a potential to attain 50 0% BOD reduction in 2 8 days (2 1 days at 20°C) despite the presence of MeBT in the water sample (Figure 5 7)

5 2 2 Deductions from experiments conducted

Based on the biodegradation rates recorded in Sample C (containing MeBT and Kilfrost) there are no indications that the presence of MeBT adversely affects the biodegradation of propylene glycol (Kilfrost) Although the biodegradation rates recorded in Sample C (containing MeBT and Kilfrost) are lower than those recorded in Sample B (dosed with Kilfrost only) at 6°C and 20°C, there are no indications from the estimated time taken for the removal of half of the BOD concentration in the water sample (2 8 days at 6°C and 2 1 days at 20°C) that the presence of MeBT adversely affected the biodegradation of the added glycol This clearly contradicts the claims of Wu *et al* (1998) that MeBT inhibits microbial biodegradation The rates attained in the water sample are also higher than previously reported for the biodegradation of commercial propylene glycol (Kilfrost) on airport paved surfaces 0 073 day ^{1} at 4°C and 0 081 day ^{1} at 8°C (Revitt *et al* 2002) The biodegradation rate at 6°C (0 247 day ^{1}) is also higher than the rate recorded by McVicker *et al* (1998) (0 091 day ^{1}) for ethylene ethylene/diethylene glycol (Konsin) at a higher temperature of 8°C

Studies of the role of MeBT conducted to date have focused mainly on its toxicity effects on organisms (Fisher *et al* 1995 Hartwell *et al*, 1995 Cancilla *et al* 1997 Cancilla *et al* 1998 Cornell *et al* 1998, Wu *et al* 1998 Pillard *et al* 2000) An additional experiment was therefore conducted to assess the impact of the presence of MeBT on bacterial growth rates in MFR water samples at 6°C Three samples (A, B and C) containing the components previously described showed an initial decrease in biomass population in the first 24 hours (Figure 5 8) The highest subsequent biomass count of 1 80 x 10^{8} recorded after 6 days was in the water dosed with MeBT The fall in biomass population in the first 24 hours in Sample C represents only a slight drop from the initial count of 2 35 x 10^{8} CFU/litre compared to drop in population to 10^{7} CFU in Samples A and B (Figure 5 8) The higher final biomass population recorded suggests that the presence of MeBT in the MFR water sample does not have any long term detrimental effect on the bacteria growth rates This is consistent with results from the HTF where there was no indication of any adverse effects due to the ADF laden polluted water on the biomass population Chong *et al* (1998) in a pilot scale study, also recorded no adverse effects of airport runoff on the microbial populations in an experimental reedbed system

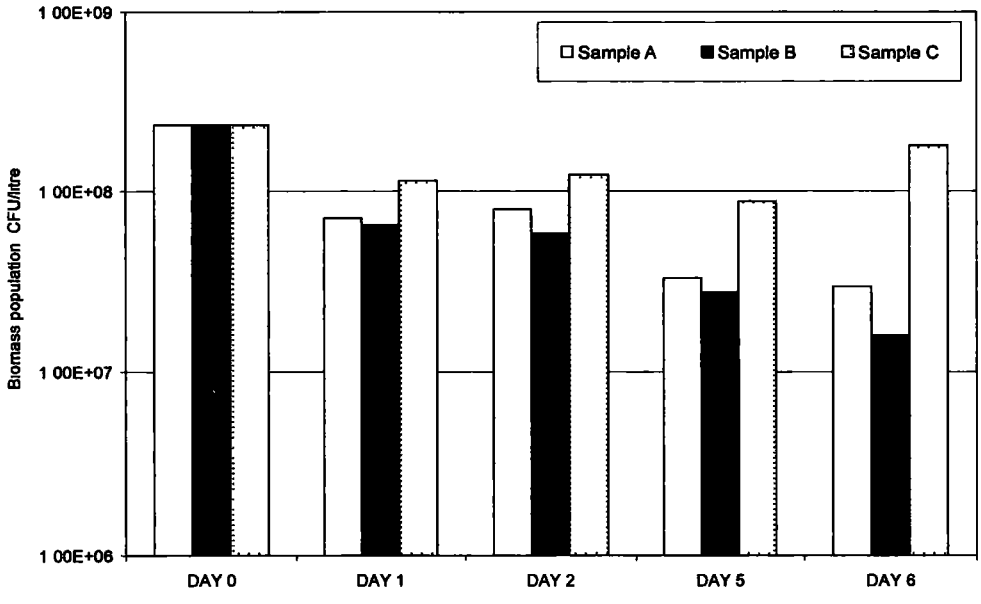


Figure 5 8 Changes in biomass population CFU/l observed at 6°C in MFR water samples dosed with different components

The biomass populations recorded in the water samples (generally above 10^7 CFU/l) and the utilisation of DO suggests that the MFR and MFBP contain microbial populations with a potential for the biodegradation of glycol (in terms of the population present and not necessarily with respect to the species present) Although there are no indications from the results obtained from field samples that the aerated ponds do not support a sufficiently large microbial population (see details in Chapter 3) there are possibilities that they are hampered by the absence of essential components such as nutrients In the next section the effect of the addition of nutrient (nitrate and phosphate) on microbial BOD reduction in MFR water samples is assessed

5 3 IMPACT OF THE ADDITION OF NUTRIENTS (NITRATE AS KNO_3 AND PHOSPHATE AS KH_2PO_4)ON BACTERIA GROWTH RATES AND BOD REDUCTIONS IN AERATED POND WATER SAMPLES

Due to the low BOD reductions recorded within the Mayfield Farm aerated ponds (MFR and MFBP) (see Chapter 3) particularly during the winter months a number of options were considered regarding how to improve the biodegradation performance of the aerated ponds One of these options was supplementing the nutrient levels in the ponds with additional nitrogen (N) in the form of nitrate and phosphorus (P), in the form of phosphate The phosphorus requirement for biomass growth is one-fifth that of nitrogen which, in turn is one-twelfth of the amount of carbon present on a mass basis (McGahey and Bouwer, 1992) A

typical Mayfield Farm water sample with a BOD of 350 mg/l contains 748 mg/l of propylene glycol (Kilfrost) which can be equated to the corresponding mass of carbon

1 mole (76 g) of propylene glycol contains 36 g of carbon
0 748 g (748 mg) of propylene glycol will contain
36 x 0 748/76 = 0 354 g of carbon

N ($1/12^{th}$ of carbon) = 0 03 g (30 mg/l) equivalent to 130 7 mg/l as nitrate

P ($1/5^{th}$ of nitrogen) = 0 006 g (6 mg/l) equivalent 17 7 mg/l as phosphate

The average concentrations of nitrate and phosphate in the MFR prior to the commencement of the nutrient dosing regime were 4 30 mg/l and 0 39 mg/l, respectively (see details in Chapter 3) The levels in the MFBP were 3 40 mg/l nitrate and 0 44 mg/l phosphate These levels were therefore well below the required N and P levels for optimal biomass growth and mineralisation of glycol particularly at the concentrations recorded in the winter months

An initial comparative study conducted using the above calculated nutrient levels and lower concentrations of 50 mg/l for nitrate (11 3 mg N/l) and 6 8 mg/l for phosphate (2 26 mg P/l) i e maintaining the N P mass ratio of 5 1 showed that there was no marked difference in the impact on biomass growth Consequently, the objective concentrations were set at the lower values bearing in mind the need not to produce nitrate levels which could be harmful to the receiving water if discharged at the dosed level Using water samples collected from the Mayfield Farm aerated ponds the BOD removal nutrient utilization and biomass growth were monitored in biodegradation experiments The experiments were designed to allow for 5 a minute supply of dissolved oxygen every twenty-four hours in order to prevent the development of anaerobic or near-anaerobic conditions (see details in Section 2 3 4 of Chapter 2) Results from each of these experiments are discussed in the sections that follow

5 3 1 Discussion of the trends observed in the different experiments

Experiments 1- 5 were designed to assess the impact of the addition of nutrients in the form of nitrate and phosphate on BOD removal and bacteria growth rates in MFBP water samples at 6°C and 20 °C as specified in the different experiments Details of the experimental designs have been discussed in Section 2 3 4 2 of Chapter 2

Experiment 1

In the first biodegradation experiment conducted in August 2004 a significant drop in BOD level was recorded in the water sample within the first 4 days of the experiment The BOD concentration fell from 350 0 mg/l to 35 0 mg/l (Figure 5 5) The BOD concentration after 7 days was however higher at 66 0 mg/l Beyond Day 7 BOD levels in the water sample remained fairly constant at around 50 0 mg/l with a final concentration of 52 0 mg/l after 23 days (Figure 5 9) There were signs of rapid nitrate utilisation with 55 7% reduction within the first 4 days The final nitrate concentration after 23 days was 5 54 mg/l (Figure 5 9) representing an overall reduction of 88 9%

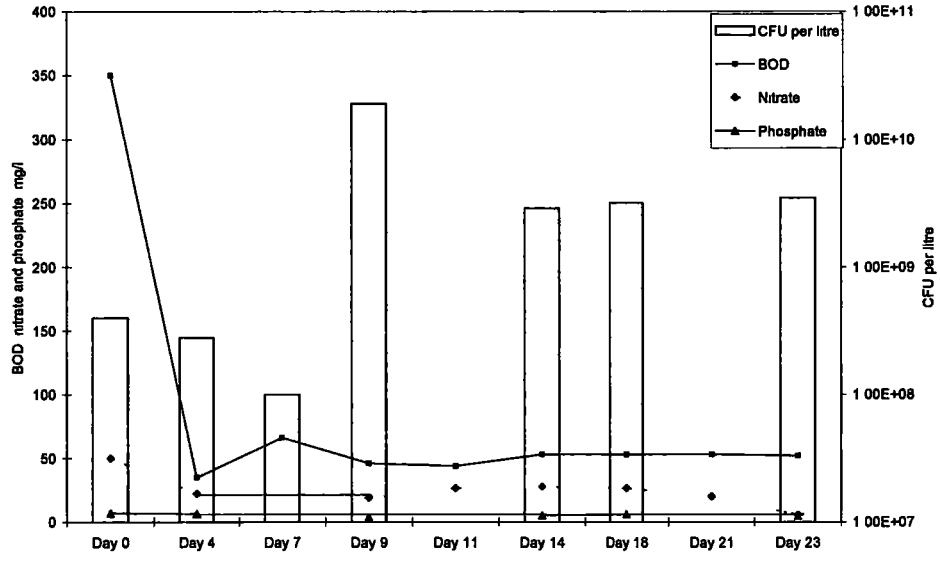


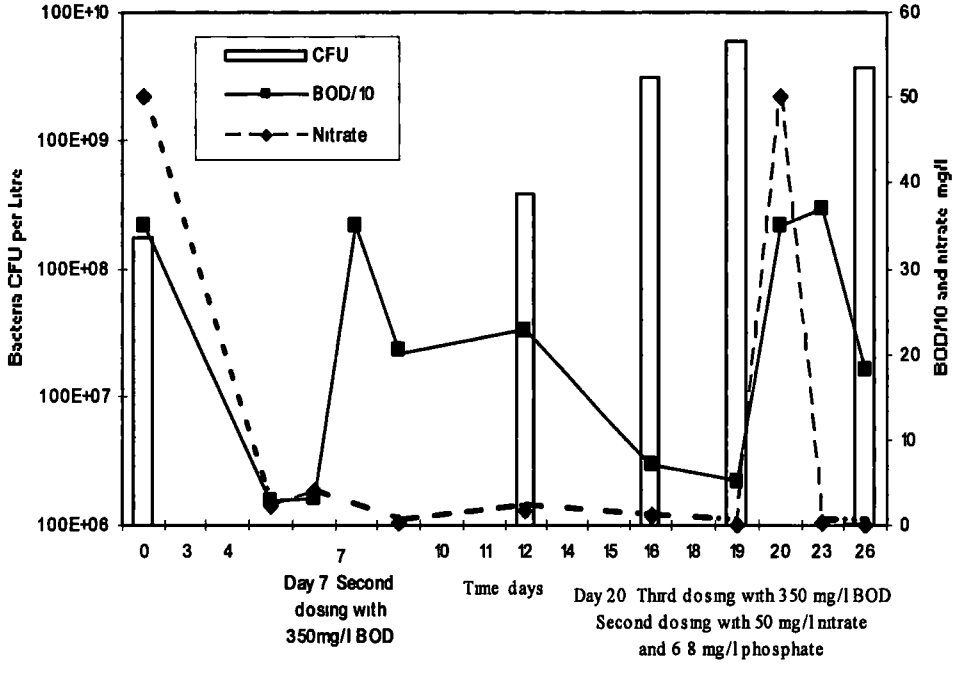
Figure 5 9 Temporal variations in BOD, nitrate, phosphate and bacteria CFU levels in aerated pond water samples at 6°C

The utilisation of phosphate in the system was relatively moderate and considerably lower than that recorded for nitrate The concentration of phosphate decreased from 6 80 mg/l to 6 36 mg/l in the first 4 days and reached 4 68 mg/l after 23 days (an overall reduction of 31 2%) The biomass population was fairly constant at 10^8 CFU per litre before rising to a maximum concentration of 1 9 x 10^{10} CFU per litre on Day 9 The final biomass population after 23 days was 3 50 x 10^9 CFU per litre (Figure 5 9)

Experiment 2

In the second experiment, which was a modification of the first additional doses of 350mg/l BOD were re-introduced on Days 7 and 20 while more nutrients (50 mg/l nitrate and 6 8 mg/l phosphate) were also added on Day 20 This was done to assess the impact of the presence of additional nutrient and BOD on the performance of the system The trend in BOD reduction recorded in the early

stages was similar to that observed in the first experiment The BOD dropped to 27 5 mg/l over the first 4 days (Figure 5 10) representing a 92 1% reduction There was however a marginal increase in the BOD concentration in the system on Day 7, reaching a final concentration of 32 5 mg/l (Figure 5 10)



NB Axis not linear

Figure 5 10 Temporal variations in BOD concentrations, bacteria colony forming units and nitrate levels in aerated pond water samples at 6°C

The BOD reduction recorded in the system after re-dosing with 350mg/l BOD on Day 7 was not as rapid as initially recorded The BOD concentration on Day 12 was 227 0 mg/l representing a reduction of 35 1% compared to over 90 0% recorded during the same period in the first phase (Figure 5 10) The BOD reduction achieved by Day 19 12 days after re-dosing was 85 0% At this stage there had been a complete utilisation of nitrate but the level of phosphate in the system still remained at 4 50 mg/l, representing a fall of only 33 8% from the original concentration of 6 80 mg/l (Figure 5 10) The more efficient utilisation of nitrate compared to phosphate was consistent with the results of the previous experiment

There was a further decline in BOD reduction rate during the final stages of the experiment (beyond Day 20) The BOD level on Day 26, 6 days after the re-dosing was 182 5 mg/l which represented a 50 7% reduction The utilisation of nitrate during the same stage of the experiment was rapid with 99 6% of the nitrate being

used up within 3 days following the second addition The utilisation of phosphate still remained low while the final biomass population was 3 74 x 10^9 CFU per litre (Figure 5 10) which represents a 93 3% increase over the 26 days of the experiment

Experiment 3

Two different sources of BOD glycol (System A) and clearway (System B) were used in Experiment 3 This was done to compare the BOD reduction observed in a water sample containing a carbon source (clearway) other than the one used in the experiments conducted so far Additional BOD and nutrient were introduced into both systems on Day 7 The BOD reductions observed at the onset in both systems were not as rapid as recorded in the first two experiments The BOD level in System A fell from 405 0 mg/l to 150 mg/l in 5 days before reaching 80 0 mg/l after 7 days (Figure 5 11) which represented a reduction of 80 2% Although the initial drop in BOD concentration in System B 410 0 mg/l to 210 0 mg/l (Figure 5 12) was less pronounced than in System A the BOD reduction after 7 days (80 0%) was close to the level attained in System A (80 2%) within the same period

The BOD concentration in System A dropped to 295 0 mg/l by Day 12 5 days after re-dosing with 350 0 mg/l BOD and eventually to a final concentration of 167 5 mg/l after a further 10 days (Figure 5 11) The situation in System B was quite different There were no further reductions recorded in BOD levels after the re-dosing with BOD on Day 7 The final BOD concentration for the remaining 16 days of the experiment was consistent at 370 0 mg/l (Figure 5 12)

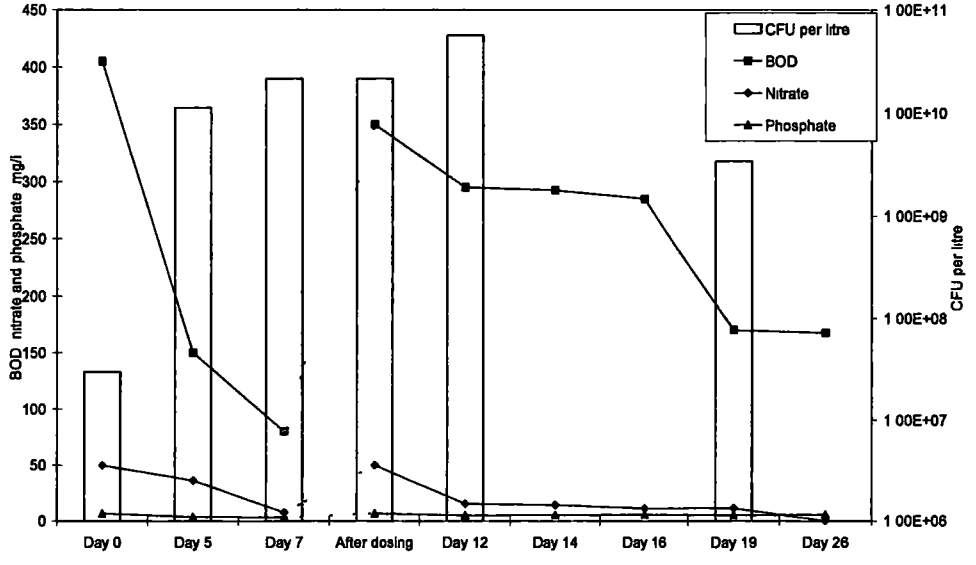


Figure 5 11 Temporal variations in BOD, nitrate, phosphate and bacteria CFU in system A at 6°C

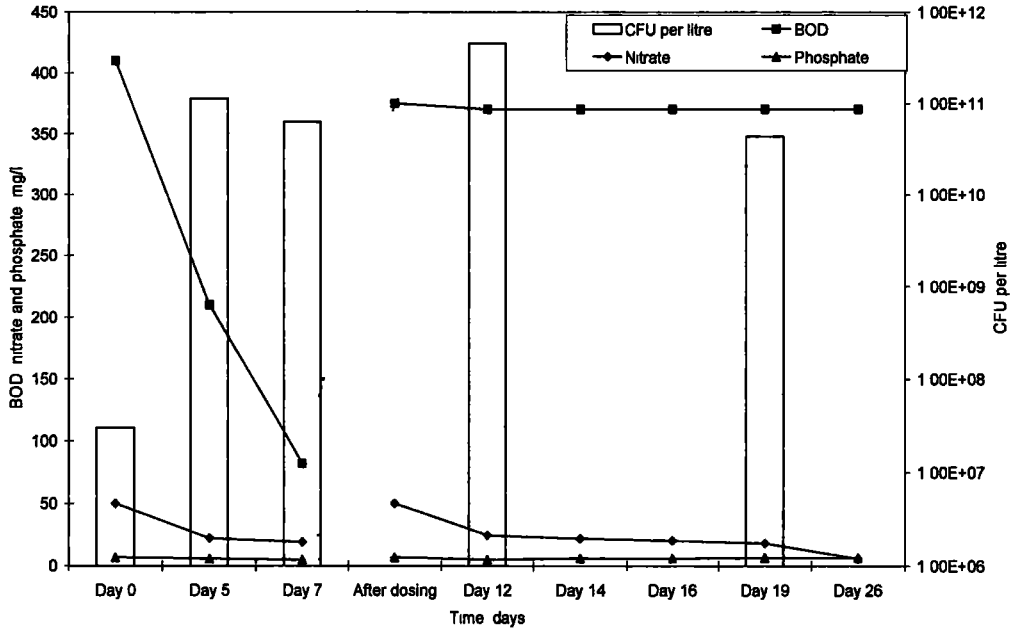


Figure 5 12 Temporal variations in BOD, nitrate, phosphate and bacteria CFU in system B at 6°c

Again, the utilisation of nutrient particularly nitrate was rapid The nitrate concentration reached within the first 7 days was lower in System A (7 53 mg/l) than in System B (19 05 mg/l) (Figures 5 11 and 5 12) The levels on Day 12 5 days after re-dosing with nutrients were also lower in System A (15 51 mg/l) compared to System B (24 50 mg/l) There was complete utilisation of nitrate in System A after 26 days while the residual concentration in System B was 5 76 mg/l Phosphate utilisation was slightly higher in System A with the concentration decreasing from 6 80 mg/l to 3 20 mg/l in the first 7 days and then remaining at 5 70 mg/l until Day 26 after re-dosing on Day 7 (Figure 5 11) Initially the concentration of phosphate in System B fell to 5 72 mg/l on Day 7 followed by fluctuations during the rest of the experiment before reaching a final concentration of 6 60 mg/l on Day 26 (Figure 5 12)

The biomass levels in both systems rose significantly from 3 0 x 10^7 CFU per litre to 1 12 x 10^{10} CFU per litre and 1 13 x 10^{10} CFU per litre in Systems A and B respectively within the first 7 days of each experiment (Figures 5 11 and 5 12) Further increases were observed in both systems immediately after re-dosing on Day 7 with a maximum level of 4 55 x 10^{11} CFU per litre being reached in System B (Figure 5 12) There were however declines in biomass population in both systems after 19 days with the populations decreasing to 3 40 x 10^9 CFU per litre and 4 35 x 10^{10} CFU per litre in Systems A and B respectively (Figures 5 11 and 5 12)

Experiment 4

Unlike the previous experiments it took up to 14 days for any noticeable BOD reduction to be recorded in the system Consequently the system was not re-dosed with BOD or nutrients The initial BOD concentration at the start of the experiment was 442 0 mg/l It remained as high 400 0 mg/l into Day 12 of the experiment before falling to 300 0 mg/l on Day 14 (Figure 5 13) The BOD reduction beyond Day 14 was however more rapid decreasing to 110 0 mg/l by Day 21 (Figure 5 13) The utilisation of nitrate in this experiment was considerably less rapid compared to the previous experiments where complete utilisation occurred within 7 days The concentration of nitrate after 7 days was 24 81 mg/l decreasing further to 8 86 mg/l by Day 21 (Figure 5 13) The utilisation of phosphate on the other hand improved significantly with a final concentration of 2 51 mg/l after 21 days representing a 63 1% reduction which was the highest recorded in all the experiments so far As with previous experiments the biomass population increased from an initial level of 5 0 x 10^{7} CFU per litre to 2 10 x 10^{11} CFU per litre within 12 days reaching 2 23 x 10 ^{11} CFU per litre after 21 days (Figure 5 13)

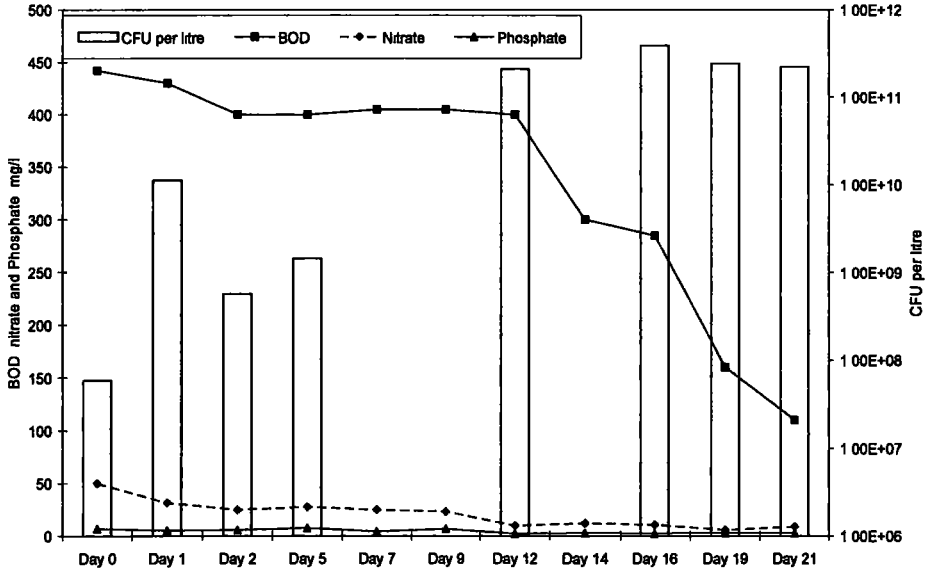


Figure 5 13 Temporal variations in BOD, nitrate, phosphate and bacteria CFU in aerated pond water samples at 6°C

Experiment 5

Following the poor performance recorded in the water samples in Experiment 4 the BOD reduction at a higher temperature of 20°C was monitored using glycol as the only BOD source The experimental set up used is the same as with the

previous experiment (Experiment 4) with no introduction of either BOD or nutrient in the course of the experiment Another experiment was set up alongside at 6°C in other to compare the BOD reduction at the different temperatures Initial results showed better BOD reduction at 20°C with a decrease in the BOD concentration from 405 5 mg/l to 287 5 mg/l (29 1%) after 8 days At 6°C on the other hand the BOD level within the same period remained at 405 5 mg/l (Figure 5 14) The most significant reduction in BOD at 6°C occurred between Days 8 and 15 during which 80 3% of the BOD was removed in 7 days (Figure 5 14) There was complete utilisation of nitrate by Day 5 at 20°C and Day 8 at 6°C (Figure 5 14) There was an overall increase in the biomass population at both temperatures with an increase in the initial level of 1 55 x 10^9 CFU per litre to 8 9 x 10^{10} CFU per litre and 1 30 x 10^{11} CFU per litre in 23 days at 6°C and 20°C respectively (Figure 5 14)

In a repeat experiment carried out using the same set up the BOD reductions at both temperatures were high (although not as high as recorded in Experiments 1 2 and 3) There were initial decreases in BOD concentrations within the first 9 days of the experiment from 400 0 mg/l to 280 0 mg/l (30 0%) and 157 5 mg/l (60 6%) at 6°C and 20°C respectively The final BOD concentration after 23 days at 6°C was 131 3 mg/l (67 2%) compared to a much lower value of 22 5 mg/l (94 4%) at 20°C (Figure 5 15)

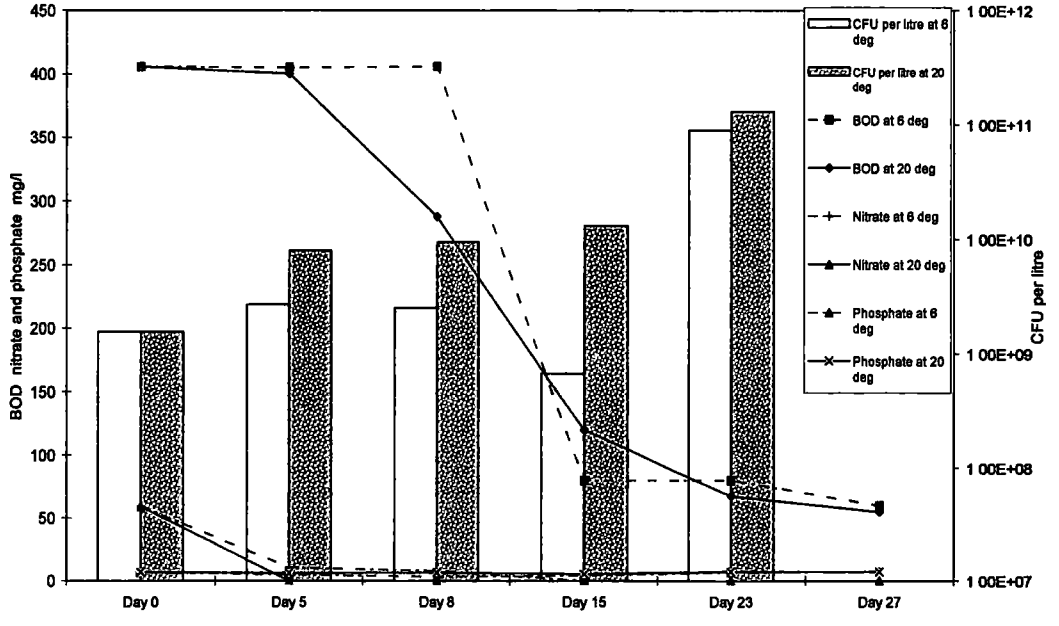


Figure 5 14 Temporal variations in BOD, nitrate, phosphate and bacteria CFU in aerated pond water samples at 6°C and 20 °C

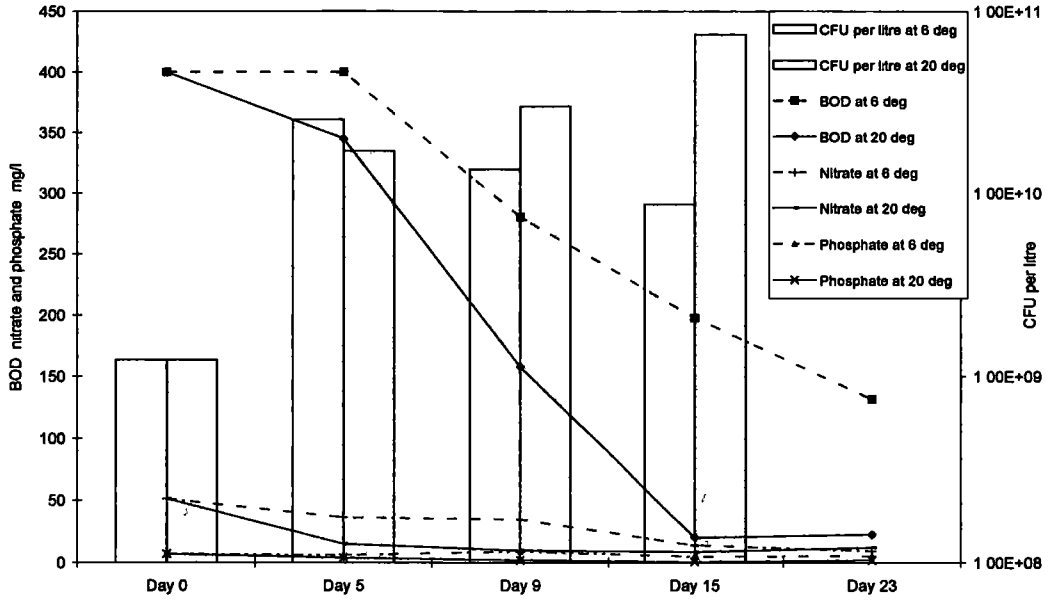


Figure 5 15 Temporal variations in BOD, nitrate, phosphate and bacteria CFU in aerated pond water samples at 6°C and 20 °C

The trends observed in the biomass growth and nutrient utilisation (Figure 5 15) are not significantly different from those observed in the other experiments The biomass population in both systems increased from an initial concentration of 1 23 x 10^9 CFU per litre to 2 54 x 10^{10} CFU per litre and 1 71 x 10^{10} CFU per litre within 5 days falling slightly to 8 65 x 10^9 CFU per litre at 6°C and increasing to 7 44 x 10^{10} CFU per litre at 20°C after 15 days (Figure 5 15) While the overall phosphate utilisation at 20°C after 23 days (74 75%) was higher than the 26 11% observed at 6°C, nitrate utilisation was better at the lower temperature (81 85% compared to 76 66 %) within the same period (Figure 5 15)

5 3 2 Deductions from Experiments 1, 2, 3, 4 and 5

The rapid BOD reduction recorded in the first 5 days of the first three biodegradation experiments particularly Experiments 1 and 2, and the rate of increase in biomass population in all the experiments, suggest that the addition of nutrient enhances BOD reduction in the aerated pond water samples There were however some inconsistencies recorded in BOD reductions in subsequent experiments particularly in Experiments 4 and 5 where up to 14 days were required for any marked reductions in BOD concentrations to be recorded The 90 0% and 92 1% BOD reductions observed within 4 days in Experiments 1 and 2 are consistent with the high biodegradation rates initially observed in the MFR water samples dosed with 350 mg/l Kilfrost when 50 0% BOD reduction was recorded in 0 6 days at 6°C (Table 5 3) The biodegradation rates of 0 581 day^{-1} and 0 642 day^{-1} recorded in Experiment 1 and the first stage of Experiment 2 (using Equation 5 2) respectively although lower than the 1 081 day^{-1} recorded in Section

5 2 at 6°C are desirable operationally as they exceed the actual BOD reductions recorded in the aerated ponds in practice (details in Chapter 3) The lower biodegradation rates observed in the second and third stages of Experiment 2 (0 091 day^{-1} and 0 162 day^{-1} respectively) despite increasing nutrient utilisation and biomass growth, suggest that the continuous exposure of the system to high organic loads had an effect on the BOD reduction potential achieved with time

The relative lower biodegradation rate (0 233 day^{-1}) observed in the first stage of Experiment 3 although impressive is a decline in performance from the first two experiments (0 581 day^{-1} and 0 642 day^{-1}, respectively) The decreased biodegradation activity may be an indication of a change in the microbial population contained in the MFR water samples used in the experiment McCartney *et al* (2003) have reported that microbial populations vary frequently in aquatic environments with changing temperature and nutrient availability The lower biodegradation rate of 0 054 day^{-1} recorded during the second phase of Experiment 3 supports the initial claim that the shock caused by further exposure of the system to further organic loads reduces the potential for biodegradation This is further highlighted by the performance of the second system in Experiment 3 (dosed with Clearway) The final biodegradation rate of 0 003 day^{-1} recorded in the later stages of the experiment is much lower than the initial 0 233 day^{-1} recorded within the first 7 days of the same system It is not clear why the rapid BOD reduction observed in the early experiments were not repeated in subsequent tests (Experiments 4 and 5) The biodegradation rates recorded in both experiments 0 022 day^{-1} and 0 041 day^{-1} at 6°C although lower than those observed in first three experiments are consistent with levels recorded on airport paved surfaces at 1°C (Revitt *et al* 2002) Even at a higher temperature of 20°C the biodegradation rates recorded in Experiment 5 (0 073 day^{-1} and 0 112 day^{-1}) were still lower than the typical rates observed in MFR water samples at a similar temperature

The benefits of nutrient addition were demonstrated repeatedly in all the experiments by the increase in background biomass population from around 10^7 CFU per litre to 10^{10} CFU per litre within the first 7 days in most cases which is consistent with claims by Tate and Terry (1980) that nutrient enrichment increases biomass population Although the utilisation of the nutrient appears to aid the biodegradation processes in the first three experiments there was no evidence of this in the later experiments It remains unclear why no rapid BOD reduction was recorded in the later experiments (Experiments 4 and 5) despite an increase in biomass population There are indications from the BOD results that the consortia responsible for the rapid reductions initially recorded were lacking from the water samples used in the later stages This would explain why the increase in biomass

population in the water sample had no significant effect on the BOD reduction This observation is consistent with observations at the MFR and MFBP where despite the presence of a large biomass population adequate BOD reductions were not recorded (see details in Chapter 3) The isolation of only two dominant strains of bacteria in the MFR and MFBP (see details in Chapter 7) is a contrast to the diverse microbial population reported in marine and lake water systems by Scala and Kerkhof (2000) and Jiang *et al* (2006), respectively In the next section results from tests conducted to investigate the impact that a varied bacteria population could have on BOD reductions in aerated water samples are discussed

5 4 ASSESSMENT OF BOD REDUCTIONS IN AERATED POND WATER SAMPLES SPIKED WITH DIFFERENT LEVELS OF ACTIVATED SLUDGE

Following the inconsistencies and delays recorded in the BOD reductions in several biodegradation experiments the impact of a different and more diverse microbial consortium on BOD reductions in aerated pond water samples was investigated using activated sludge systems (see details in Section 2 3 5 of Chapter 2) Switzenbaum *et al* (2001) have suggested that the activated sludge system represents a biological technique which is capable of the effective treatment of airport runoff In this experiment aerated pond water samples were dosed with different levels of activated sludge as described below

A MFBP water sample dosed with 0 5% activated sludge and 350 0 mg/l BOD
B MFBP water sample dosed with 1 0% activated sludge and 350 0 mg/l BOD
C MFBP water sample dosed with 2 0% activated sludge and 350 0 mg/l BOD
D MFBP water sample dosed with 5 0% activated sludge and 350 0 mg/l BOD
E MFBP water sample dosed with 350 0 mg/l BOD only (control)

The trends in BOD reductions, monitored at 6°C and 20°C are shown in Figures 5 16 and 5 17 respectively

5 4 1 Discussion of the Trends observed in the different experiments

The highest BOD reduction recorded in the first 8 days at 6°C was in D with a 38 1% reduction equivalent to a drop from 621 3 mg/l (high BOD value due to the presence of 5 0% activated sludge) to 384 8 mg/l (43 8%) (Figure 5 16) The BOD reduction in C during the same period was 24 4% (from 464 5 mg/l to 351 0 mg/l) and 17 7% in B (from 412 3 mg/l to 339 2 mg/l) The BOD reduction in A was least (3 3 %) with a decrease from 386 1 mg/l to 373 4 mg/l in 8 days The final BOD reduction in D was 77 1% after 24 days (Figure 5 16) with a final BOD concentration of 142 5 mg/l (the lowest level recorded in the experiment) The final

BOD reductions in A B and C were 32 1% (final BOD concentration of 262 0mg/l), 46 8% (final BOD concentration of 219 5 mg/l) and 54 6% (final BOD concentration of 210 9 mg/), respectively (5 16)

There were improvements in the BOD reductions within the first 8 days in the water samples at 20°C The highest BOD reduction of 43 8% was recorded in D (Figure 5 17) with a drop in initial concentration from 621 3 mg/l to 349 1 mg/l The BOD reduction within the same period in C was 34 0 % (falling from 464 5 mg/l to 306 4 mg/l) The BOD reduction in A (0 3%) was marginally lower than the 1 1 % reduction recorded in the control (E) There were further BOD reductions in all water samples by Day 15 The highest BOD reduction of 84 8% was recorded in D followed by C (72 4%), A (43 1%) and the control (27 8%) (Figure 5 17) The final BOD reductions after 24 days in A, C and D were 54 2%, 73 6% and 84 9% respectively with the control unchanged at 27 8%

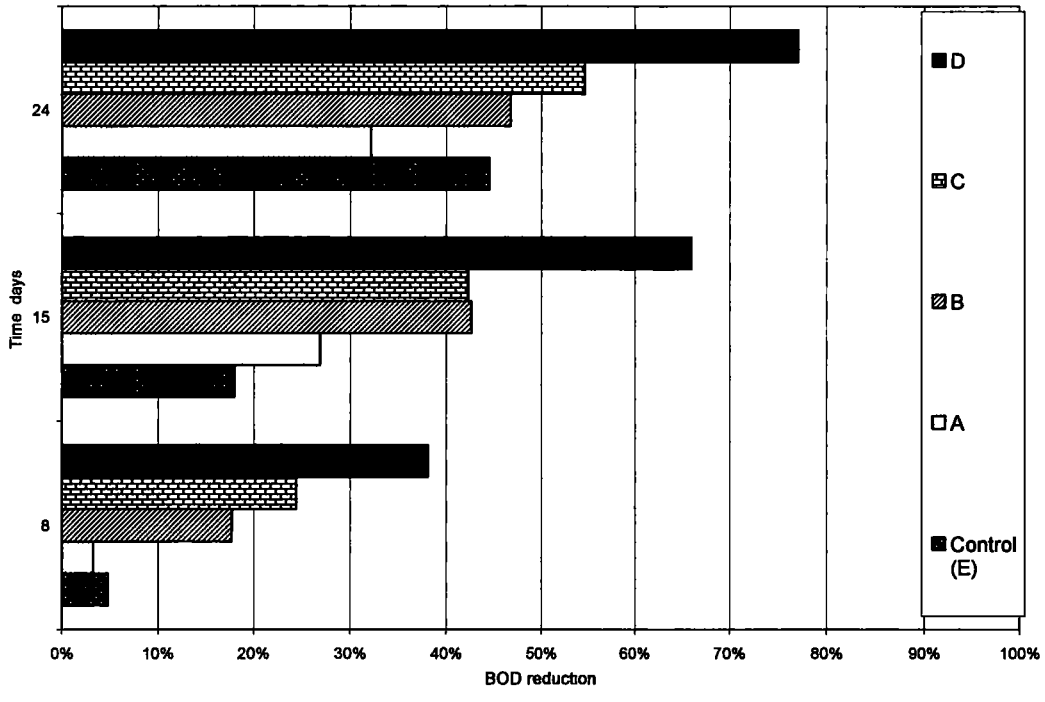


Figure 5 16 Percentage BOD reductions in aerated pond water samples dosed with different levels of activated sludge at 6°C

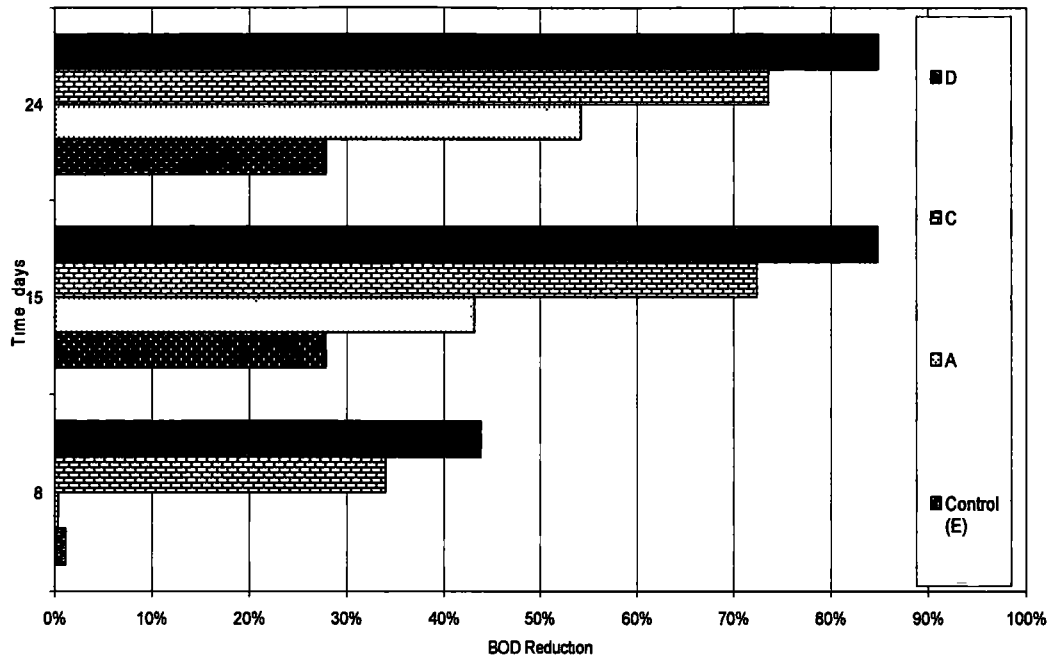


Figure 5 17 Percentage BOD reductions in aerated pond water samples dosed with different levels of activated sludge at 20°C

5 4 2 Deductions from experiments conducted

The monitored BOD reductions in water samples spiked with activated sludge were not as rapid as expected given its diverse microbial species composition (Smalla *et al* 1998 Tanaka *et al* 2003) and established BOD reduction rates of over 90 0% within 6-7 days in an activated sludge system (Liu *et al* 2000) The overall performance at 6°C was slightly better than observed in the later biodegradation experiments conducted using MFR water samples (Experiments 4 and 5) The calculated biodegradation rates recorded were 0 022 day^{1} 0 031 day^{1} and 0 063 day^{1} in systems B C and D respectively These levels are comparable with the 0 022 day^{1} and 0 041 day^{1} values recorded in the MFR water samples at the same temperature Although the biodegradation rates recorded in C and D at 20°C (0 053 day^{1} and 0 073 day^{1}, respectively) were better than recorded at 6°C they were lower than the rates initially observed in the first three experiments conducted using the MFR water samples This implies that the consortia of bacteria present in the activated sludge are unable to degrade glycol in aerated pond water samples as rapidly as initially thought In the next section results of tests conducted to assess the ability of the consortia of bacteria present in river water samples to biodegrade glycol are discussed This would also help ascertain if the poor biodegradation recorded so far is in anyway related to the characteristics of the aerated pond water used

5 5 ASSESSMENT OF BOD REDUCTIONS IN RIVER WATER SAMPLES DOSED WITH BOD (IN THE FORM OF GLYCOL) AND NUTRIENT (NITRATE AND PHOSPHATE)

These experiments were conducted to assess the impact of a different microbial consortium on BOD reduction in a system dosed with BOD in the form of glycol at 6°C The changes in BOD and biomass concentrations in water samples collected from the Pymmes Brook in North London dosed with 350 mg/l BOD in the form of glycol was monitored A control experiment was also set up using a MFBP water sample to compare the BOD reductions in both systems (see Section 2 3 6 of Chapter 2 for more details) The components of the two systems used in this experiment are described below

A river water sample dosed with 350 mg/l BOD 50 mg/l nitrate and 6 8 mg/l phosphate
B aerated pond water sample dosed with 350 mg/l BOD, 50 mg/l nitrate and 6 8 mg/l phosphate

The changes in BOD, nutrient and bacteria population concentrations are summarised in Figure 5 18

5 5 1 Discussion of the trends observed in the different experiments

Although the BOD reduction in A appears to be marginally better the overall trend in both systems were the same (Figure 5 18) While there were no signs of any BOD reduction in B before Day 15 the BOD concentration in A by Day 15 had dropped from 442 0 mg/l to 381 0 mg/l equivalent to a 13 8% reduction This was followed by a rapid BOD reduction phase during which the BOD concentration in A dropped to 128 5 mg/l (70 9%) in 5 days (between Days 15 and 20) before reaching a final concentration of 99 8 mg/l (77 4%) after 27 days (Figure 5 18) The final BOD reduction in the control (B) was 75 1%, with the BOD falling from 416 0 mg/l to 103 8 mg/l in 27 days (Figure 5 18) The utilisation of nutrient in B was not particularly rapid compared to the trends observed in experiments in A The concentration of nitrate in the system fell by 78 9% after a slow start, dropping from an initial concentration of 58 40 mg/l to 12 32 mg/l compared to 10 19 mg/l in B (Figure 5 18) The utilisation of phosphate in Sample A was unusually high dropping to 1 32 mg/l (83 8% reduction) after 20 days before attaining a final concentration of 2 03 mg/l after 27 days Although the final concentration in B (0 18 mg/l) was lower the trends in both systems were similar

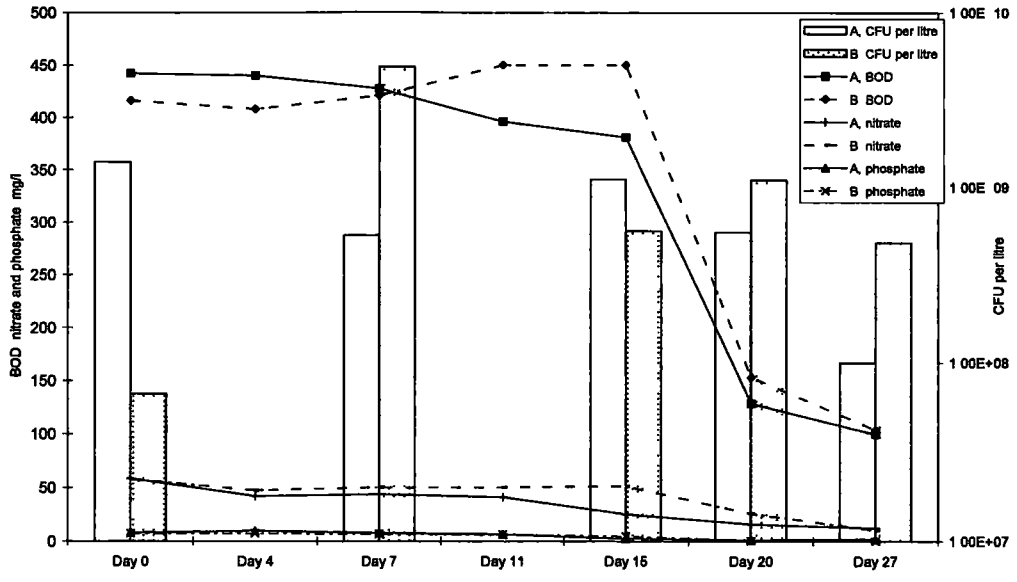


Figure 5 18 Temporal changes in BOD, nitrate, phosphate and bacteria CFU concentrations in aerated pond and river water samples at 6°C

Unlike previous biodegradation experiments, there were no increases in biomass populations in the river water sample (A) In fact the converse was true with population initially falling from 1 40 x 10^9 CFU per litre to 5 30 x 10^8 CFU per litre after 7 days before reaching a final level of 1 00 x 10^8 CFU per litre after 27 days (Figure 5 18) The biomass population in B on the otherhand increased significantly from the initial level of 6 70 x 10^7 CFU per litre to 4 9 x 10^9 CFU per litre after 7 days before dropping to 4 8 x 10^8 CFU per litre after 27 days which still represents an increase from the starting population (Figure 5 18) This is consistent with the observation in all aerated pond water samples

5 5 2 Deductions from experiments conducted

The lower rate of utilisation of the added nitrate and the response of the microbial population in terms of negative growth particularly in the first 7 days of the experiment for the river water sample is an indication of a shock effect on the microbial population Based on the results obtained from this test, there is no marked difference in the overall BOD reductions recorded in the river water sample when compared to the performances recorded in previous experiments (which have been very variable) The calculated biodegradation rate of 0 063 day^{-1} at 6°C is identical to the rate recorded in the MFR water sample dosed with 5 0% activated sludge This is also comparable with the rate observed in the control experiment (0 056 day^{-1}) and generally falls within the same range as the rest of the water samples used in the later experiments (Experiments 4 and 5) at 6°C

5 6 ASSESSMENT OF BOD REDUCTIONS IN WATER SAMPLES COLLECTED FROM THE EASTERN RESRVOIR (ER) AND FROM WASHING OF PLANT ROOTS FROM THE MAYFIELD FARM SUB-SURFACE FLOW REEDBED (SSF)

To compliment the biodegradation experiments conducted using microbial communities from outside the HTF a further series of tests were carried out using water samples from different components of the treatment system namely the ER and the SSF Results from monitoring the performance of the HTF suggest the presence of active microbial populations in both units which are capable of rapid BOD reduction (see details in Chapter 3) The BOD concentrations and the biomass population in water samples from the ER and from the washings (using sterile distilled water) of the roots of plants from the SSF were monitored at 6°C and 20°C The water samples were initially dosed with 350 mg/l BOD in the form of Kilfrost (propylene glycol) 50 0 mg/l nitrate and 6 8 mg/l phosphate (see full experimental details in Chapter 2) The results obtained are shown in Figures 5 19 and 5 20

5 6 1 Discussion of the trends observed in the different experiments

The BOD reductions in the water samples prepared from SSF plant root washings were higher than recorded in the more recent biodegradation experiments conducted using MFR water samples (in which the average BOD reduction was barely up to 10 0% in the first 5 days of the experiment particularly at 20°C) The BOD concentrations in the plant root washings water sample fell from an initial concentration of 415 0 mg/l to 262 5 mg/l (equivalent to 36 75% BOD reduction) and 270 0 mg/l (equivalent to 34 9% BOD reduction) at 20°C and 6°C, respectively in the first 5 days (Figure 5 19) There were further reductions in BOD concentrations beyond Day 5 reaching 157 5 mg/l (62 1%) at 20°C and 215 0 mg/l (48 2%) at 6°C on Day 14 The final concentrations of 62 5 mg/l (84 9%) and 130 0 mg/l (68 7%) were attained at 20°C and 6°C respectively after 21 days

The biomass populations at both temperatures increased gradually throughout the experiment from an initial concentration of 7 82 x 10^8 CFU per litre to 1 10 x 10^9 CFU per litre and 3 35 x 10^9 CFU per litre after 7 days at 6°C and 20°C, respectively There were further increases to 3 04 x 10^{10} CFU per litre and 1 25 x 10^{10} CFU per litre after 21 days at 6°C and 20°C, respectively (Figure 5 19) It is not clear why the final bacterial biomass population after 21 days at 6°C was higher than recorded at 20°C A similar trend in BOD reduction was observed in the dosed ER water sample with the BOD concentration after 5 days at 20°C dropping from 445 0 mg/l to 282 5 mg/l (36 5%) compared to 350 0 mg/l (21 4%) at 6°C (Figure 5 20)

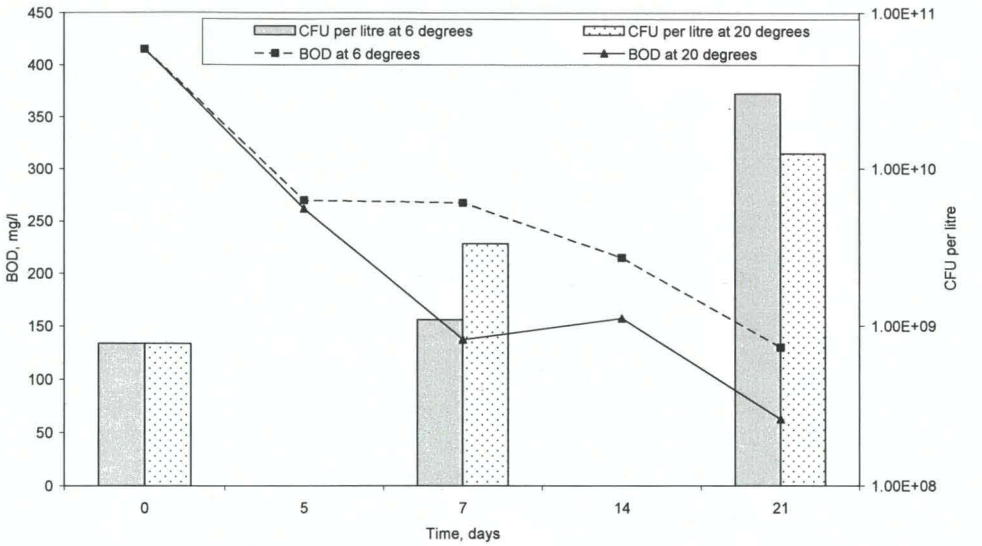


Figure 5.19 Temporal changes in BOD and bacteria CFU concentrations in dosed water samples collected by washing the roots of plants from the SSF at 6°C and 20°C

There were further BOD reductions in both systems to 157.5 mg/l (64.6%) and 217.5 mg/l (51.2%) at 20°C and 6°C, respectively by Day 7 (Figure 5.20). The final BOD concentrations after 21 days were 42.5 mg/l (90.5% reduction) and 165.0 mg/l (62.9%) at 20°C and 6°C, respectively. The biomass populations increased at both temperatures from an initial level of 1.01×10^8 CFU per litre to 1.04×10^{10} CFU per litre and 7.70×10^9 CFU per litre after 21 days at 6°C and 20°C, respectively (Figure 5.20), although it is not clear why the increase observed after 7 days was higher at 6°C.

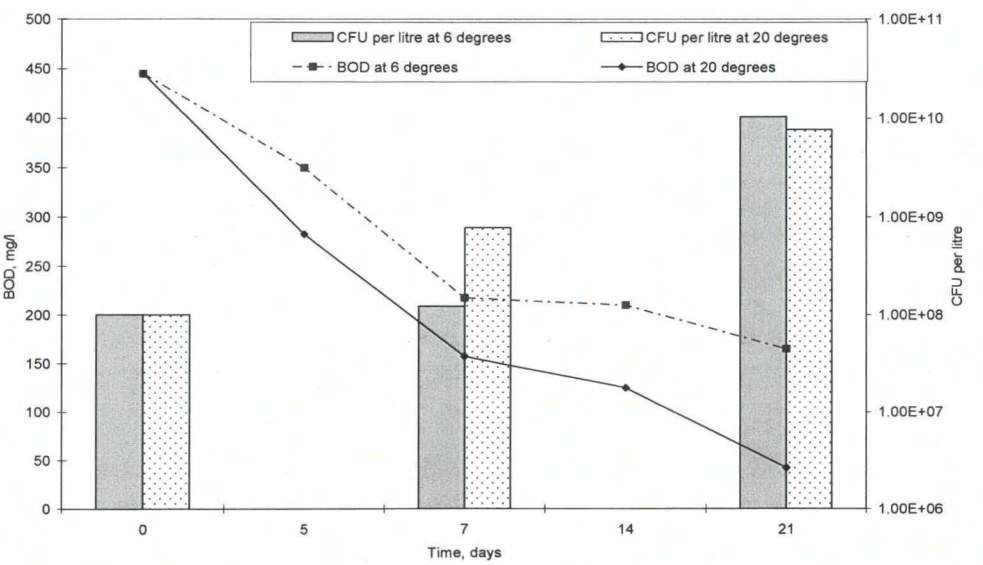


Figure 5.20 Temporal changes in BOD and bacteria CFU concentrations in dosed ER water sample at 6°C and 20°C

5 6 2 Deductions from experiments conducted

The high BOD reductions recorded at the early stages (within the first 5 days) in the root washings sample and the ER water sample are an indication of the possible presence of a microbial consortium capable of rapid glycol degradation This characteristic has been lacking in most of the more recent systems explored so far (Experiments 4 and 5 aerated pond water samples dosed with activated sludge and the river water systems) apart from the aerated pond water samples used at the early stages of this work (Experiments 1, 2 and 3) The biodegradation rates of 0 094 day ^{1} and 0 091 day ^{1} calculated for the SSF root washings at 20°C and 6°C suggest that temperature does not affect the microbial activities in the root system In contrast the significance of temperature was further demonstrated by the higher biodegradation rate recorded for the ER water sample at 20°C (0 094 day ^{1}) compared to the value (0 052 day ^{1}) at 6°C

The BOD reductions recorded for the root washings and the ER water samples within the first 5 days at 6°C (34 9% and 21 3% respectively) are higher than the average BOD reduction of 3 1±4 1% attained in the aerated ponds water samples and the 0 5% BOD reduction achieved in the river water samples (Figure 5 21) Between Days 7-9, the BOD reductions recorded in the root washings and the ER water samples still remained higher than recorded in the rest of the systems (apart from Experiments 1 2 and 3 which showed good biodegradation) with values of 51 1% and 35 5% respectively compared to the average level of 8 9% and 20 9% in the aerated pond and activated sludge systems (Figure 5 21) Although the BOD reductions attained between 14-17 days in the ER water sample (52 8%) and the root washings (48 2%) were both higher than the river water sample (13 8%) and the average levels in the aerated pond (38 4±32 0) and the activated sludge systems (44 5±16 1%) the BOD reductions (66 1%) attained in L (aerated pond water sample dosed with 5 0% activated sludge) and E (aerated pond experiment conducted in February/March 2005) were better than recorded in the ER and root washings water samples (Figure 5 21) The average BOD reduction of 71 3±6 7% recorded in the aerated pond water sample between Days 20-24 at 6°C was higher than the levels attained in both the root washings system (68 7%) and the ER water sample (62 9%) (Figure 5 21) Despite the overall higher BOD reductions attained in the ER and root washings water samples at the early stages of the experiments (Days 3-5 and 7-9) the later trends in BOD reductions suggests that retention time is less beneficial (in terms of BOD reduction) to both systems at 6°C

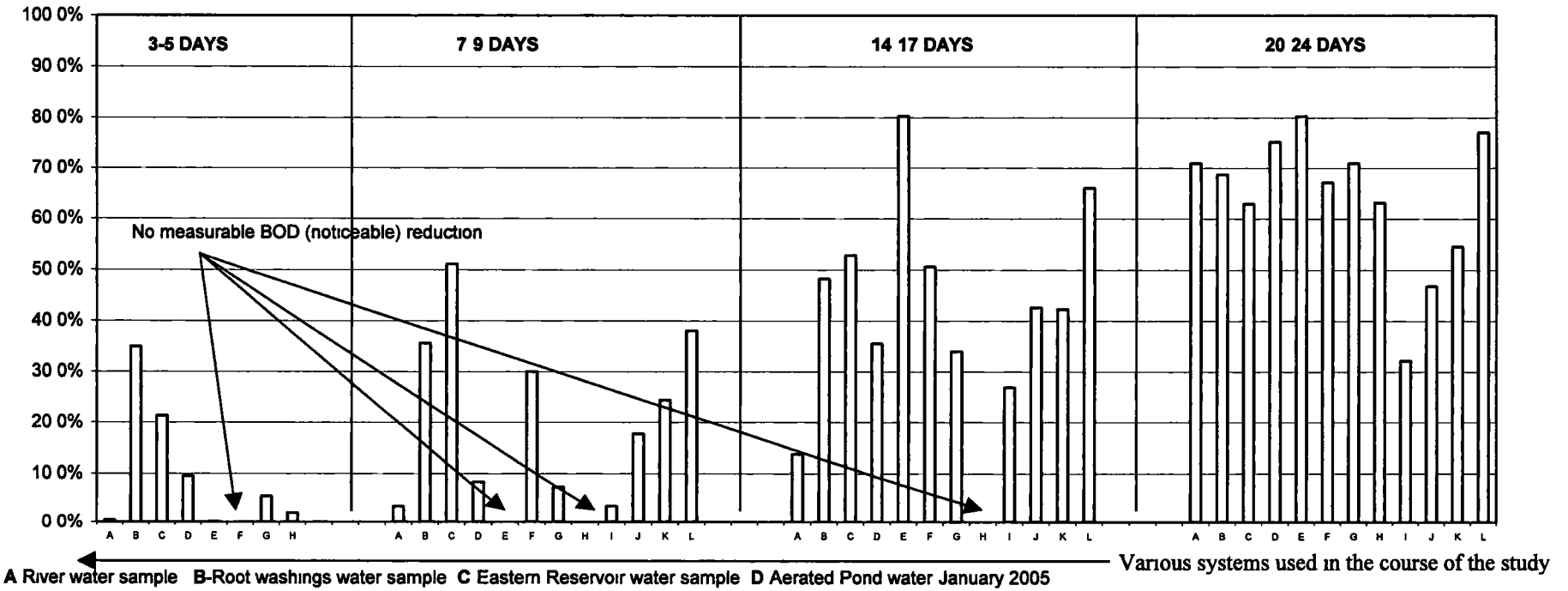


Figure 5 21 Different BOD reductions attained at different stages for the different water samples used in the biodegradation experiments at 6°C

The performance of the ER and the root washings systems at 20°C were similar to the trends recorded at 6°C The BOD reductions within the first 5 days in the ER and the root washings systems were 36 9% and 36 5% (Figure 5 22) compared to an average BOD reduction of 6 9±6 3% recorded in the aerated pond water samples Although slight improvements were recorded in the aerated pond systems after Day 7 the average BOD reduction attained (36 6±21 3%) was lower than the 64 6% and 66 9% recorded in the ER and the root washings systems respectively (Figure 5 22) The average BOD reduction in the activated sludge system within the same period was 26 0±22 8% The role of retention time was further highlighted after 14 days as the average BOD reductions in the aerated pond water samples (71 5±23 0%) was only marginally lower than attained in the ER (71 9%) and higher than recorded in the root washings (62 0%) at 20°C (Figure 5 22) This trend continued into the later stages of the experiments as the average BOD reduction recorded in the aerated pond water samples (86 1±7 3%) was marginally better than recorded in the root washings (84 9%) and only lower than attained in the ER water sample (90 4%) The comparable average BOD reduction in the activated sludge systems was 70 9±15 5%

There are indications that the different trends in BOD reductions recorded particularly in the aerated ponds when compared to the ER and root washing water samples is due to the varying nature of the dominant microbial population in each system It therefore becomes imperative to identify the microbial populations associated with each component in order to establish a better understanding of the BOD removing potential of the entire system Details of the microbial isolation techniques and the different biochemical tests conducted on the different isolates from the treatment system are discussed in Chapter 7 In the next section the results of biodegradation tests conducted using some of these isolates are discussed

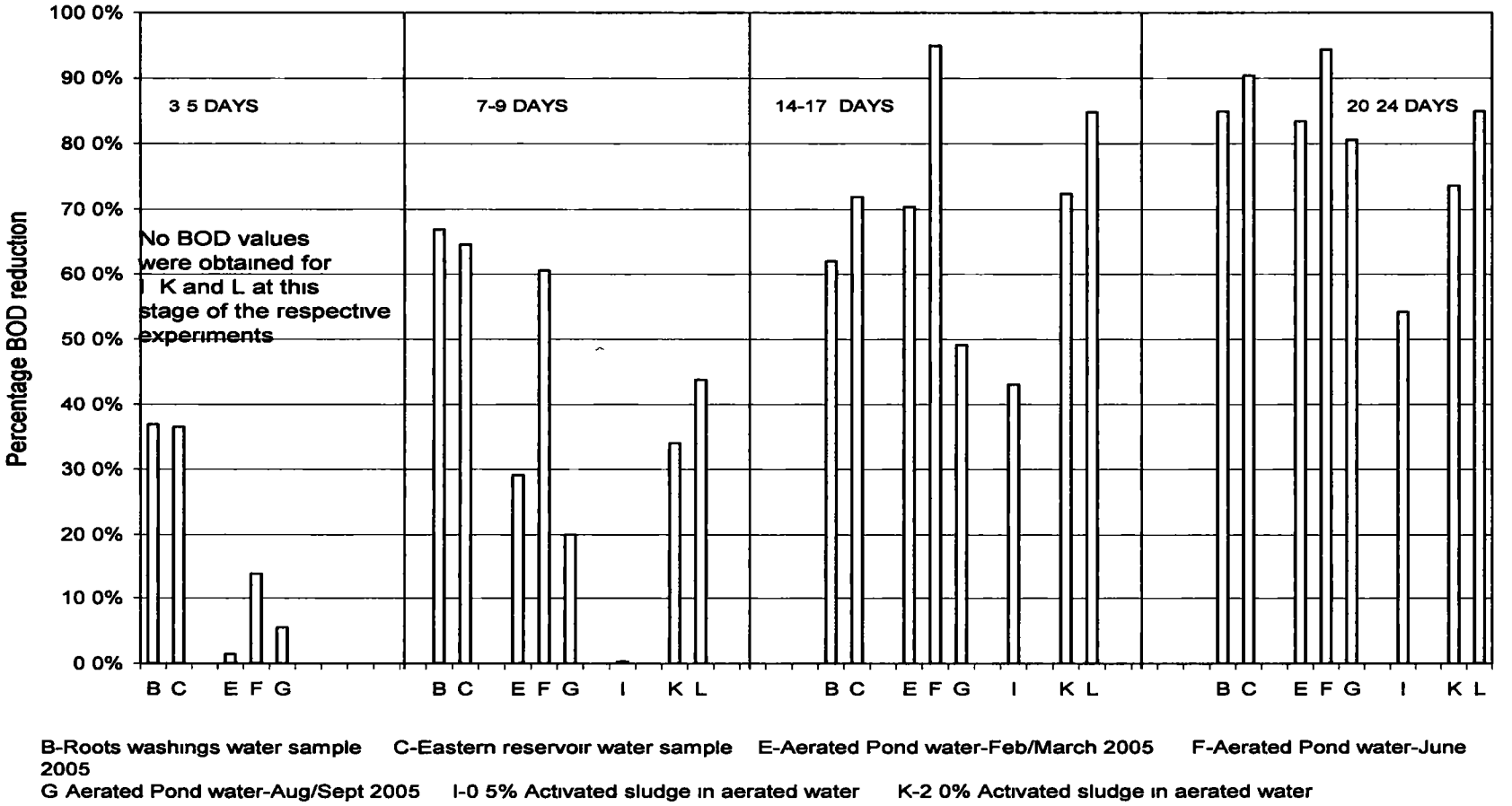


Figure 5 22 Different BOD reductions attained at different stages for the different water samples used in the biodegradation experiments at 20°C

5 7 ASSESSMENT OF BOD REDUCTIONS IN STERILE DISTILLED WATER SAMPLES INNOCULATED WITH THE DIFFERENT BACTERIA STRAINS FROM THE AERATED PONDS, EASTERN RESERVOIR AND THE ROOTS OF PLANTS FROM THE MAYFIELD FARM SUB-SURFACE FLOW REEDBED SYSTEM

Following the isolation of the different bacterial strains from the different components of the HTF the biodegradation potentials of these strains suspended in sterilised distilled water dosed with 350 mg/l BOD were assessed The origin and description of the different bacterial strains used in the different experiments have been summarised in Table 4 Appendix 5A (see Chapter 2 for the isolation techniques used) Two different nutrient levels (the recommended 50 mg/l nitrate and 6 8 mg/l phosphate nutrient levels used during the nutrient dosing regime at the HTF) and minimum medium levels (represents a higher concentration of nitrate and phosphate see details in Chapter 2) were used in tests conducted to assess the impact of the nutrient level on biomass growth and BOD reduction The minimum medium nutrient level was used in order to assess the effect of the presence of a higher and more diverse nutrient source has on the performance of the bacterial strains

The initial tests carried out showed that the presence of glycol and the different nutrient levels had no adverse effects on the growth of these bacteria strains This is consistent with the findings of Chong *et al* (1999), in which no adverse impacts to the biomass population were observed following exposure to varying concentrations of glycol in surface runoff from the Heathrow airport Experiments were also conducted with a lower BOD dosed concentration of 50 mg/l compared to the normal 350 mg/l to determine if an optimal BOD concentration range exists for microbial performance Unless specified all water samples were kept in a shaking incubator at 20°C (see Chapter 2 for full details of the experiment) The results of the individual experiments are described below

5 7 1 Discussion of the trends observed in the different experiments

Experiment A

The BOD concentrations and growth rates of bacteria Strain *1* monitored in sterilised distilled water samples dosed with 350 mg/l BOD and two different nutrient levels (minimum medium level (A) and the HTF recommended nutrient level (B)) at 20°C are shown in Figure 5 23 The monitoring system was designed to enable the biodegradation and bacterial growth trends over the short term to be assessed

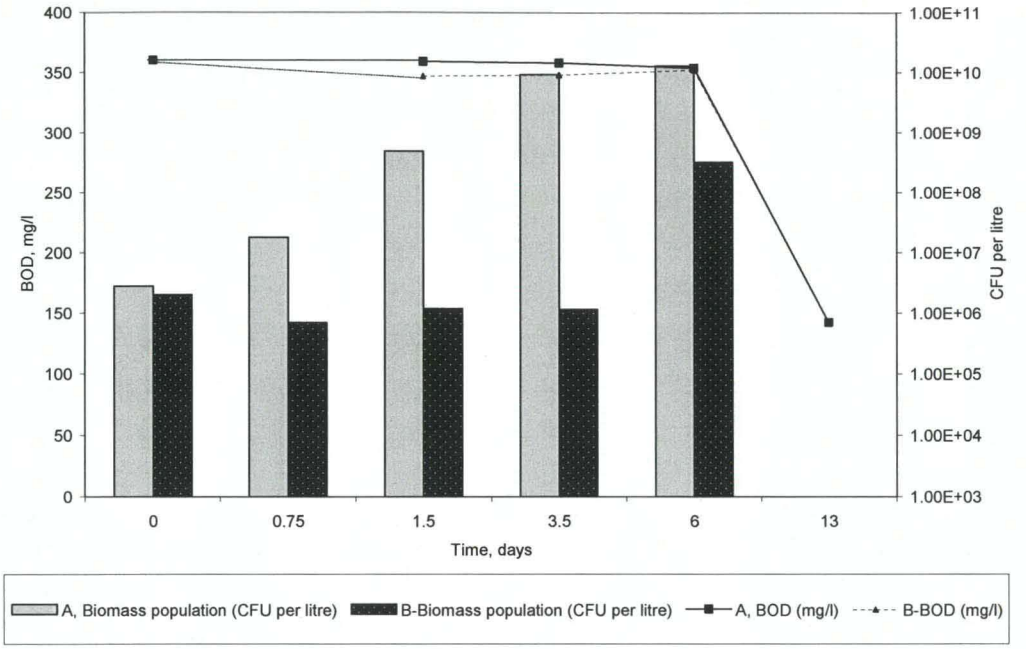


Figure 5.23 Temporal changes in BOD and biomass concentrations in sterilised distilled water dosed with 350 mg/l BOD containing two different nutrient levels and inoculated with Strain 1 at 20°C

No significant BOD reduction was recorded in either of the two systems until between 6 and 13 days, when the BOD reductions in both systems were approximately 60.0%, due to a decrease from the initial 360.0 mg/l to 142.0 mg/l (Figure 5.23). The biomass growth in the minimum medium sample (A) was higher than recorded in the HTF recommended nutrient sample (B). The growth rate in the former was rapid with an initial increase from 2.8×10^6 CFU per litre to 4.94×10^8 CFU per litre in 36 hours, before reaching a maximum concentration of 1.30×10^{10} CFU per litre in 6 days. The highest level attained within the same period in B was 3.20×10^8 CFU per litre from an initial population of 2.00×10^6 CFU per litre (Figure 5.23).

Experiment B

In the second experiment, the BOD reductions in sterilised distilled water samples inoculated solely with either Strain *1* (System A) or Strain *2* (System B) or a combination of both strains (System C), dosed with the HTF recommended nutrient level at 20°C were monitored. The objective of this experiment was to compare the BOD reduction and biomass population changes in systems containing either of the two strains with that containing both strains. The BOD and biomass levels recorded are shown in Figure 5.24.

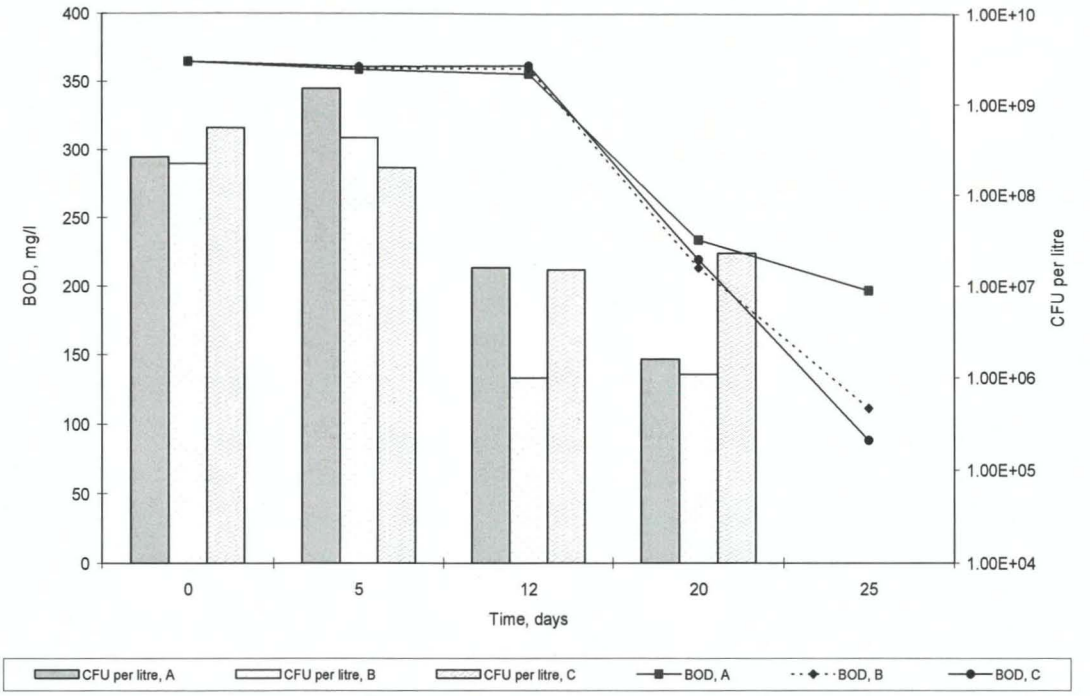


Figure 5.24 Temporal changes in BOD and biomass concentrations in sterilised distilled water dosed with 350 mg/l BOD and inoculated with Strain 1 or Strain 2 or both at 20°C

There were no signs of any BOD reductions in all three systems until after Day 12, when the BOD concentration in A dropped from 356.0 mg/l to 233.8 mg/l. The BOD concentrations in B and C were slightly lower at 213.3 mg/l and 219.4 mg/l, respectively (Figure 5.24). These concentrations are equivalent to BOD reductions of 35.9%, 41.5% and 39.9% and biodegradation rates of 0.041 day^{-1}, 0.050 day^{-1} and 0.043 day^{-1}, in A, B and C respectively. The final BOD concentrations recorded in the three systems after 25 days were 196.8 mg/l (46.0% BOD reduction) in A, 111.3 mg/l (69.5% BOD reduction) in B and 88.3 mg/l (75.8% reduction) in C. There were increases in the biomass populations of A and B within the first 5 days before a gradual fall in populations was observed as the test continued (Figure 5.24). In contrast, there was an initial decline in the population of C over the first 12 days of the experiment before a slight increase was recorded during the closing stages of the experiment (Figure 5.24).

Experiment C

In this experiment, tests were carried out on a third isolate, Strain 3, found in water samples taken from the diversion chamber at the Mayfield Farm treatment system, prior to entry into the MFR. The changes in BOD concentrations and biomass populations with time are shown in Figure 5.25.

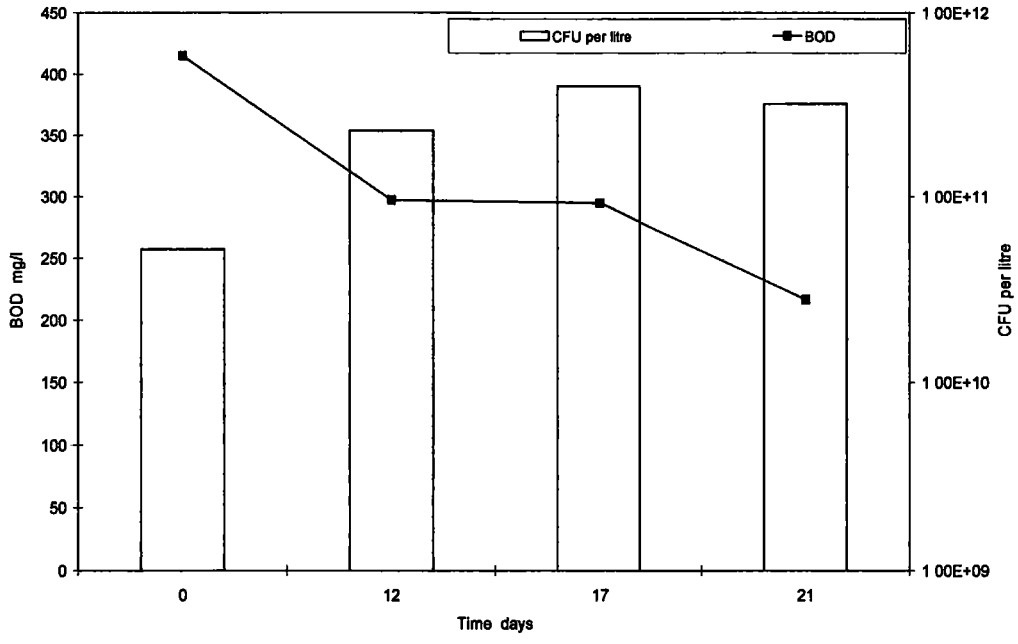


Figure 5 25 Temporal changes in BOD and biomass concentrations in sterilised distilled water dosed with 350 mg/l BOD and inoculated with strain 3 at 20°C

Improved BOD reductions were observed in this experiment compared to Experiments A and B with an initial drop in BOD concentration from 415 0 mg/l to 297 5 mg/l in the first 12 days (Figure 5 25) This is equivalent to a biodegradation rate of 0 030 day^{-1} The final BOD concentration after 21 days was 216 7 mg/l representing a total reduction of 47 8% There was an initial increase in biomass population from 5 2 x 10^{10} CFU per litre to 2 3 x 10^{11} CFU per litre after 12 days The final biomass population after 21 days was 3 2 x 10 ^{11} CFU per litre (Figure 5 25)

Experiment D

In this experiment a lower BOD level of 50 mg/l was used in systems inoculated with Strain 1 (System A) or Strain 2 (System B) or a combination of the two strains (System C) (full experimental details in Chapter 2) The BOD concentrations in Systems A, B and C after 5 days were 45 6 mg/l (20 0% BOD reduction), 48 5 mg/l (14 9% BOD reduction) and 42 8 mg/l (24 9% BOD reduction) respectively (Figure 5 26) The biodegradation rates recorded in Systems A, B and C were 0 052 day^{-1} 0 030 day^{-1} and 0 061 day^{-1} respectively It took up to 15 days for there to be any substantial decrease in the BOD concentrations in all three systems The BOD level attained in System C (17 1 mg/l representing a 70 0% BOD reduction) was the lowest of all three systems after 15 days (Figure 5 26) The BOD levels in Systems A and B within the same period were 22 8 mg/l (60 0% reduction) and 31 4 mg/l (45 0%) respectively

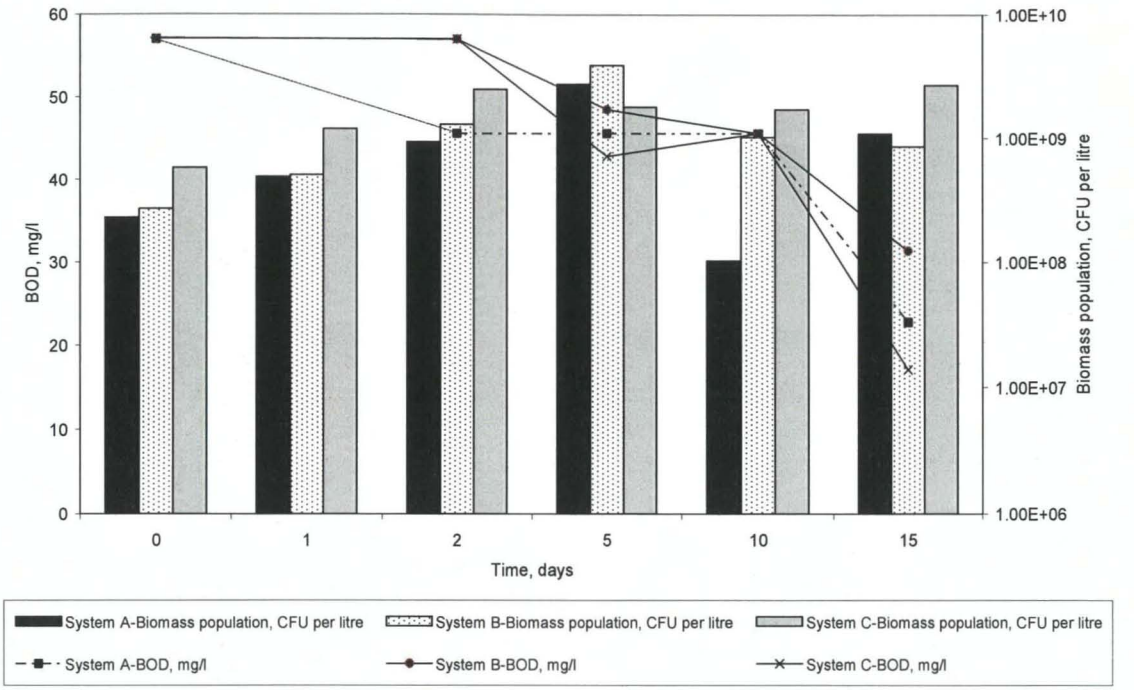


Figure 5.26 Temporal changes in BOD and biomass concentrations in sterilised distilled water dosed with 50 mg/l BOD and inoculated with strain 1 or strain 2 or both at 20°C

The biomass growth in all three systems was gradual, increasing from an initial level of 2.30 x 10^8 CFU per litre to 2.75 x 10^9 CFU per litre in System A; 2.70 x 10^8 CFU per litre to 3.90 x 10^9 CFU per litre in System B and 5.80 x 10^8 CFU per litre to 1.80 x 10^9 CFU per litre in System C after 5 days (Figure 5.26). There were declines in all systems on Day 10, System A recording the largest drop as the biomass population fell to 1.03 x 10^8 CFU per litre (Figure 5.26). The equivalent levels in Systems B and C were 1.02 x 10^9 CFU per litre and 1.71 x 10^9 CFU per litre (Figure 5.26). As observed in Experiment 2, the biomass population reached in System C (2.70 x 10^9 CFU per litre) was higher than observed in the other two systems (Figure 5.26).

Experiment E

In experiment E, each of the 13 isolates (A-M) obtained from the ER and the roots of plants from the SSF were assessed for their ability to reduce 350.0 mg/l BOD, in the form of glycol, in sterile distilled water samples at 20°C. The HTF recommended nutrient levels for nitrate and phosphate were also added (see Chapter 2 for details of the experiment). The BOD reductions at different stages of the experiment were monitored and the percentage reductions are is shown in Figures 5.27-5.30, which also show the results for the combined isolates (referred to as 'ALL')

As for most of the previous biodegradation experiments in which bacteria isolates have been used no significant BOD reductions were observed within the first 5 days (Figure 5 27) Isolate *E* recorded the highest individual BOD reduction (37 5%) after 7 days (Figure 5 28) The BOD reductions for the other individual isolates apart from *B* (29 4%) were below 20 0% The combined isolate experiment demonstrated a BOD reduction of 23 8% within the same period increasing to 33 8% after 14 days (Figure 5 29) and 34 4% after 21 days (Figure 5 30) This was outperformed by isolate *E* which attained an overall BOD reduction of 40 0% over 21 days (after an initial dip to 32 5% on Day 14) The final BOD in those systems containing isolates *B H* and *K* were the only other systems that recorded BOD reductions above 20 0% by the end of the test (Figure 5 30)

All 14 systems, apart from isolate *J*, recorded increases in the biomass populations during the first 5 days (Figure 5 31) The biomass level in the system inoculated with isolate *J* dropped from 8 90 x 10^6 CFU per litre to 1 00 x 10^5 CFU per litre over 5 days rising to 1 00 x 10^7 CFU per litre after 7 days before finishing at 1 00 x 10^6 CFU per litre after 21 days (Figure 5 31) Within the same period the highest biomass increase was recorded by isolate *E* increasing to 2 03 x 10^{10} CFU per litre in 5 days from the initial level of 2 00 x 10^6 CFU per litre before eventually falling to 4 20 x 10^8 CFU per litre after 21 days (Figure 5 31) The lowest biomass count recorded in any system was in isolate *D* where there was a decline in population from 8 30 x 10^7 CFU per litre to 7 00 x 10^4 CFU per litre between Days 5 and 7 (Figure 5 31)

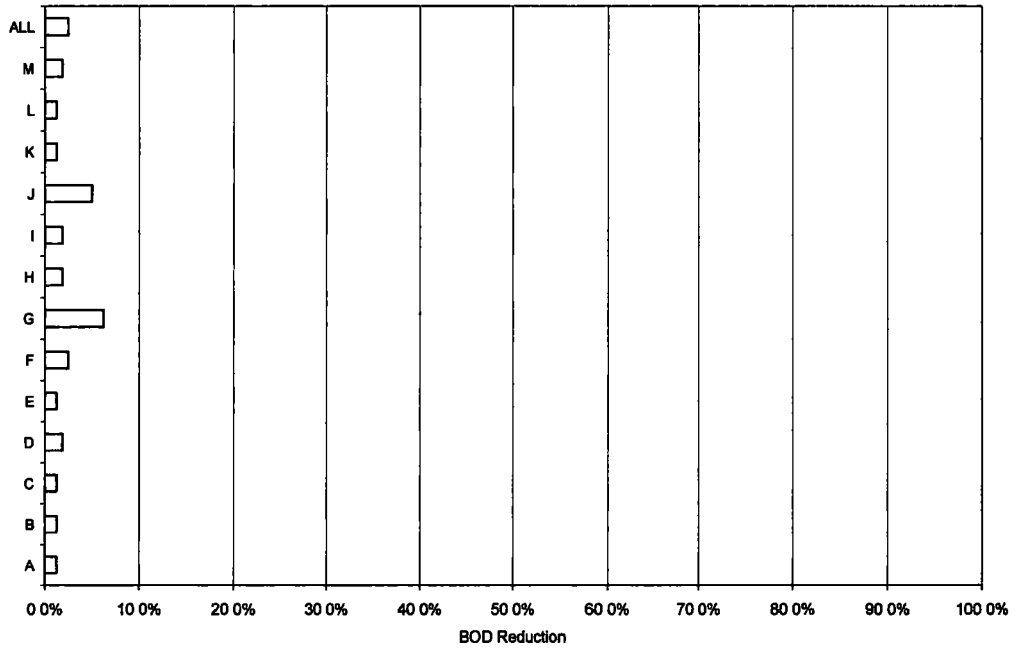


Figure 5 27 The percentage BOD reductions attained by the different isolates after Day 5 in sterilised distilled water dosed with 350 mg/l BOD and nutrients at 20°C

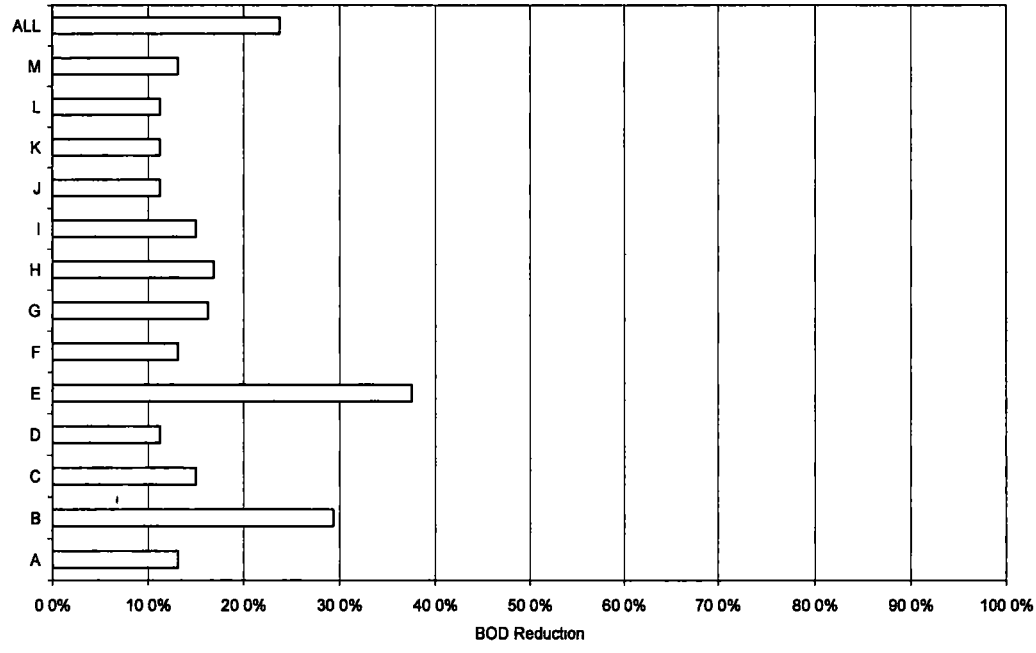


Figure 5 28 The percentage BOD reductions attained by the different isolates after Day 7 in sterilised distilled water dosed with 350 mg/l BOD and nutrients at 20°C

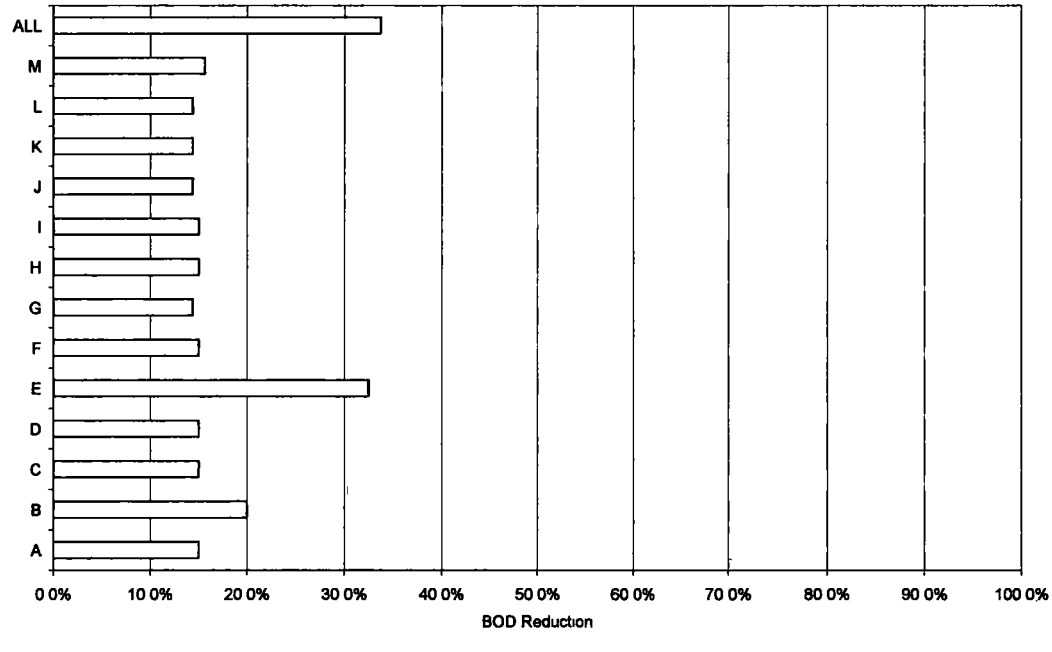


Figure 5 29 The percentage BOD reductions attained by the different isolates after Day 14 in sterilised distilled water dosed with 350 mg/l BOD and nutrients at 20°C

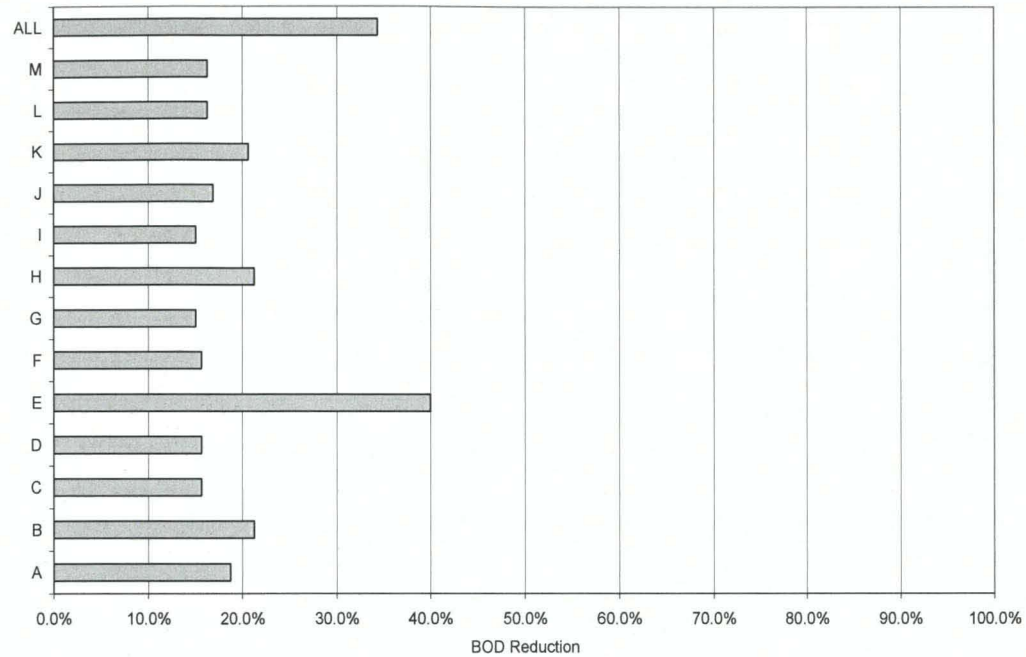


Figure 5.30 The percentage BOD reductions attained by the different isolates after Day 21 in sterilised distilled water dosed with 350 mg/l BOD and nutrients at 20°C

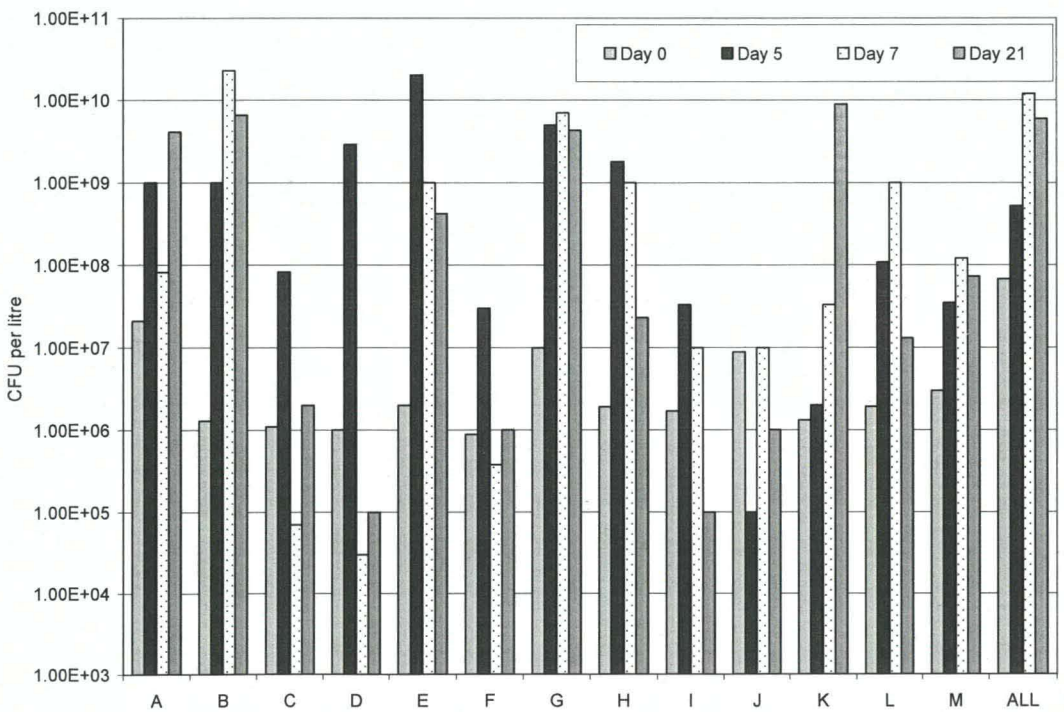


Figure 5.31 Temporal changes biomass populations in sterilised distilled water dosed with 350 mg/l BOD and inoculated with the different bacteria strains at 20°C

5 7 2 Deductions from experiments conducted on isolates

There are no indications from the BOD reductions observed in Experiment A that the presence of a higher nutrient level (i e minimum medium) has any significant impact on BOD reduction in the system Although the higher nutrient levels in the minimum medium compared to the HTF recommended nutrient levels enhanced the biomass population in Experiment A the BOD reductions recorded in both systems were identical Although the rate of BOD reductions observed in all the three systems in Experiment B were lower than observed in Experiment A the final BOD reductions achieved between Days 20 and 24 suggest that retention time plays a discriminating role in influencing the overall performance of the different strains

The biodegradation rate recorded by Strain *2* (0 050 day^{-1}) and the 0 043 day^{-1} recorded by Strain *1* and a combination of both strains is comparable to the rates recorded in the later experiments (Experiments 4 and 5) conducted using MFR water samples at the same temperature (20°C) In Experiment D where a lower BOD concentration (50 mg/l) was used the biodegradation rates of 0 052 day^{-1} 0 030 day^{-1} and 0 061 day^{-1} recorded in Systems A B and C respectively are comparable with the rates observed when higher starting BOD concentrations were used This suggests that the rate of BOD reduction in the water samples is not dependent on the initial BOD concentration

The trends observed in all four experiments involving the two strains from the Mayfield Farm aerated pond suggest that a lag phase exists before the commencement of any considerable degradation of glycol It is not clear what exactly is responsible for the existence of this lag phase but the performance of most of the strains isolated from the water samples collected from washing the root of plants from the SSF system also showed this trend in Experiment E The highest BOD reduction rate was observed for Strain *E* and the biodegradation rate (0 072 day^{-1}) was closest to the 0 094 day^{-1} and 0 091 day^{-1} value calculated for the SSF root washings sample at 20°C and 6°C (Section 5 6) The biodegradation rate recorded in the system containing all the isolates (0 040 day^{-1}) is lower than this value and is only comparable to the typical rates recorded by the individual strains from the MFR A comparison of the BOD reductions produced by all the different isolated bacteria strains shows that BOD reductions during the early stages (between Days 3 and 5) of the experiments were typically below 10 0% The only exceptions were for Experiment D where the combination of Strains *1* and *2* produced a BOD reduction of 24 9% within 5 days and the individual strains achieved BOD reductions of 20 0% and 14 9% for Strains *1* and *2*, respectively

The highest level of BOD reduction (37 5%) recorded between Days 7 and 9 was by Strain *E* followed by the 29 4% recorded by Strain *B* During the later stages of the experiments between Days 14 and 17 the BOD reduction in systems containing the two isolates from the MFR (Strains *1* and *2*) outperformed those from the ER and the SSF roots The highest BOD reduction (70 0%) was by the combination of Strains *1* and *2* used in Experiment D This was followed by the 60 4% reduction recorded by Strain *1* in Experiment A The highest BOD reduction achieved by isolates from the roots within the same period was 33 8% in the system containing all the isolates The highest BOD reduction recorded between Days 20 and 25 was the 75 8% attained in Experiment B in the system containing Strains *1* and *2* This is considerably higher than the highest BOD reduction of 40 0% attained by isolate *E* (from the roots) The BOD reduction for the system containing all isolates from the roots within the same period was 34 4% compared to the 68 7% (at 6°C) and 84 9% (at 20°C) reduction recorded at the same stage in the root washings water sample (Section 5 6) from which all the strains had been isolated

The inability of the isolates from the root to perform as efficiently as they did in the water samples collected from the roots prior to isolation suggests there are factors besides the presence of these bacteria isolates contributing to the overall BOD reduction recorded in the system There are possibilities that the laboratory isolated strains of bacteria used in the biodegradation tests which represent only a small proportion of the entire population, do not fully constitute the consortium that is responsible for the rapid degradation initially recorded Staley and Konopka (1978) Bottomley and Maggard (1989) Kell *et al* (1998) and Oliver (2005) have all reported that a higher percentage of the microbial population that exists in environmental samples may not be culturable on laboratory prepared media Lehman *et al* (2001) also reported microbial compositional differences in subsurface water systems which differed according to the carbon source utilization between those attached to solid substrates and unattached community In the next section the role sediments in BOD reduction in the water samples is investigated

5 8 COMPARISON OF THE BOD REDUCTION IN FILTERED AND UNFILTERED WATER SAMPLES FROM THE ROOT WASHINGS OF PLANTS FROM THE SUB-SURFACE FLOW REED BED SYSTEM (SSF)

The aim of the experiments discussed in this section was to assess some of the possible factors affecting bacteria activities in terms of BOD reduction in water samples obtained from washing the roots of plants from the SSF The roles of sediments and other associated solids in BOD reduction in the water sample collected from the washing of SSF plant roots have been investigated by comparing the BOD removals in filtered and unfiltered water samples Full

experimental details have been discussed in Chapter 2. The water samples were diluted serially, using 1 in 100 and 1 in 1000 dilutions in order to compare the BOD reduction in systems with varying sediment and associated solids composition. Each sample was then dosed with 350 mg/l BOD, in the form of glycol, and the HTF recommended nutrient levels. The components of the different systems used are summarised below

A_0 Unfiltered water sample obtained from root washings dosed with 350 mg/l BOD and the HTF recommended nutrient levels

A_1 1 in 100 dilution of unfiltered water sample from root washings dosed with 350 mg/l BOD and the HTF recommended nutrient levels

A_2 1 in 1000 dilution of unfiltered water sample from root washings dosed with 350 mg/l BOD and the HTF recommended nutrient levels

B_0 Filtered water sample from root washings dosed with 350 mg/l BOD and the HTF recommended nutrient levels

B_1 1 in 100 dilution of filtered water sample from root washings dosed with 350 mg/l BOD and the HTF recommended nutrient levels

B_2 1 in 1000 dilution of filtered water sample from root washings dosed with 350 mg/l BOD and the HTF recommended nutrient levels

The temporal changes in BOD concentrations and biomass populations in all 6 systems were monitored at 20°C. The results obtained are shown in Figures 5.32 and 5.33.

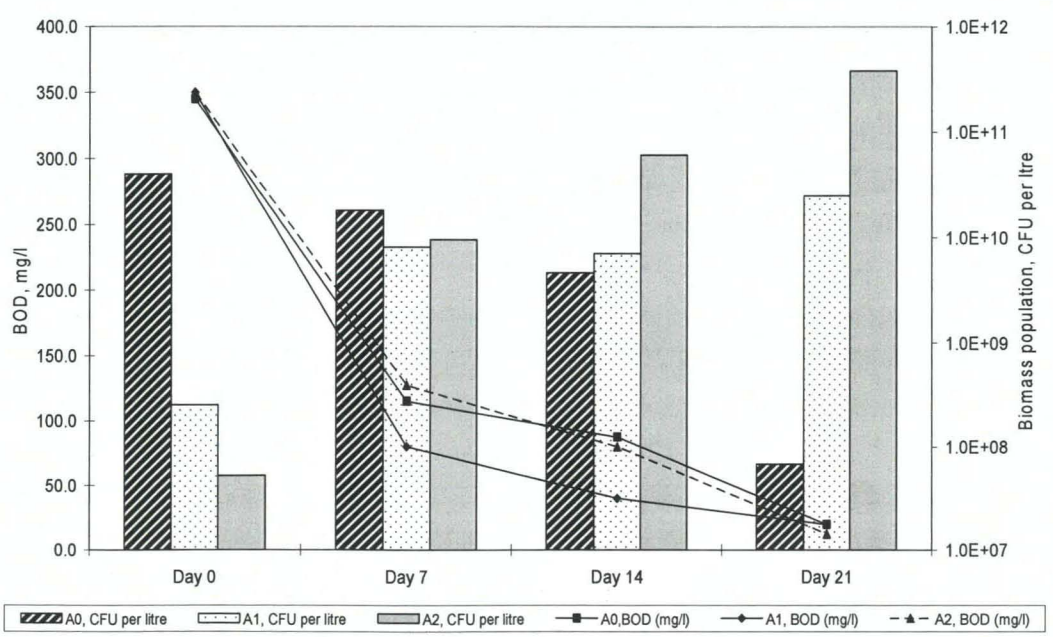


Figure 5.32 Temporal changes in the BOD concentrations and the biomass populations in the different dilutions of unfiltered water samples

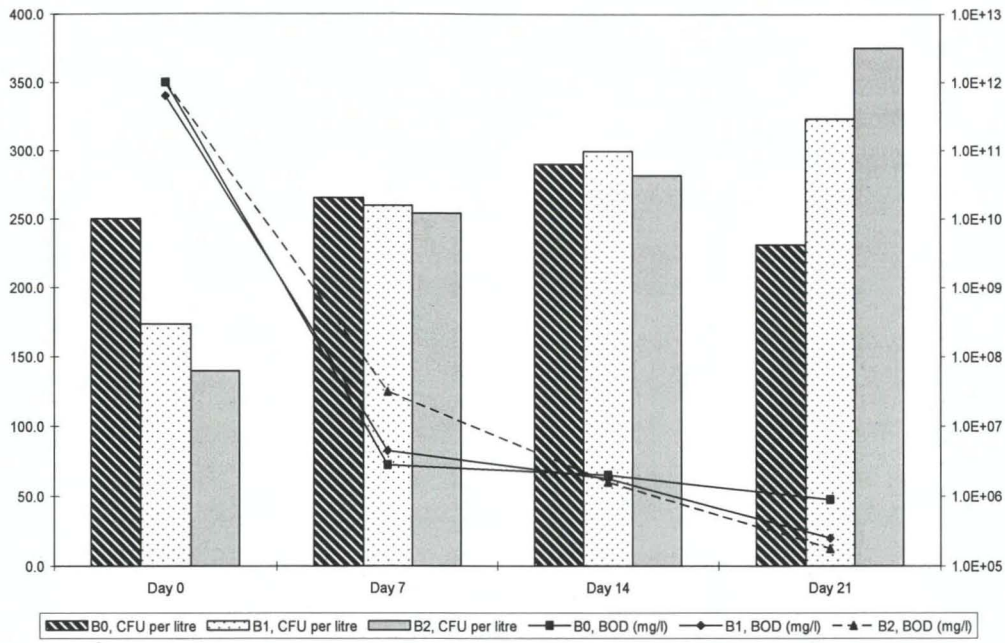


Figure 5.33 Temporal changes in the BOD concentrations and the biomass populations in the different dilutions of filtered water samples

5.8.1 Discussion of the trends observed in the experiments

Rapid BOD reductions were recorded in all 6 water samples within the first 7 days of the experiment with typical BOD reductions of over 60.0%. The lowest initial BOD reduction (63.6%) was recorded in A_2 with the BOD level dropping from 350.0 mg/l to 127.5 mg/l (Figure 5.32). The highest comparable BOD reduction (75.7%) was observed for B_1 with the BOD concentration falling from 340.0 mg/l to 82.5 mg/l (Figure 5.33). Further BOD reductions were recorded in all systems by Day 14 such that the lowest BOD concentration (40.0 mg/l) was found in A_1, representing a 88.6% BOD reduction (Figure 5.32). The BOD concentration in A_0 was the highest at that point (87.5 mg/l), equivalent to a BOD reduction of 74.6%. The BOD concentration in all the systems continued to decrease up to Day 21 with a final average BOD level of 22.1 mg/l being attained (Figures 5.32 and 5.33).

Increases were recorded in the biomass populations in all but one (A_0) of the 6 systems within the first 7 days of the experiment (Figures 5.32 and 5.33). The highest increase was recorded in B_2, where the biomass population rose from 6.3 x 10^7 CFU per litre to 1.2 x 10^{10} CFU per litre in 7 days before reaching a maximum concentration of 3.2 x 10^{12} CFU per litre on Day 21 (Figure 5.33). There was a steady but gradual fall in bacteria population in system A_0, dropping from an initial concentration of 4.0 x 10^{10} CFU per litre to 6.8 x 10^7 CFU per litre over 21 days (Figure 5.32)

5.8.2 Deductions from the experiments conducted

The higher average BOD reduction of 73.1±7.8% observed in the filtered water sample within the first 7 days compared to 69.1±7.1% (Figure 5.34) in the unfiltered water sample within the same period suggests there are no benefits associated with the presence of solids in the water sample. The unfiltered systems were however slightly more efficient in the closing stages of the experiments with average final BOD reductions of 95.0±1.3% compared to 92.3±7.8% (Figure 5.34) for the filtered systems. There are indications that the presence of debris, dead roots and organic sediments may have contributed to the slightly higher BOD concentrations initially recorded within the first 7 days in the unfiltered systems. The higher biodegradation rates recorded in these systems (0.192 day^{-1} and 0.170 day^{-1} in the filtered and unfiltered water samples, respectively) compared with the 0.094 day^{-1} value initially recorded in water samples from the roots (Section 5.6) is an indication of the presence of a more active microbial population. However, microbial analysis showed no difference in the diversity of the culturable bacteria (details in Chapter 7).

The increases in biomass populations observed in most of the systems despite the decreasing BOD levels suggest that the components of the root washings, filtered or unfiltered, can support biomass growth in either of the two systems. In the next section results from tests conducted to assess the influence of the presence of autoclaved sediments and debris from the SSF on the performance of the bacteria population isolated from the roots in a sterile environment are discussed.

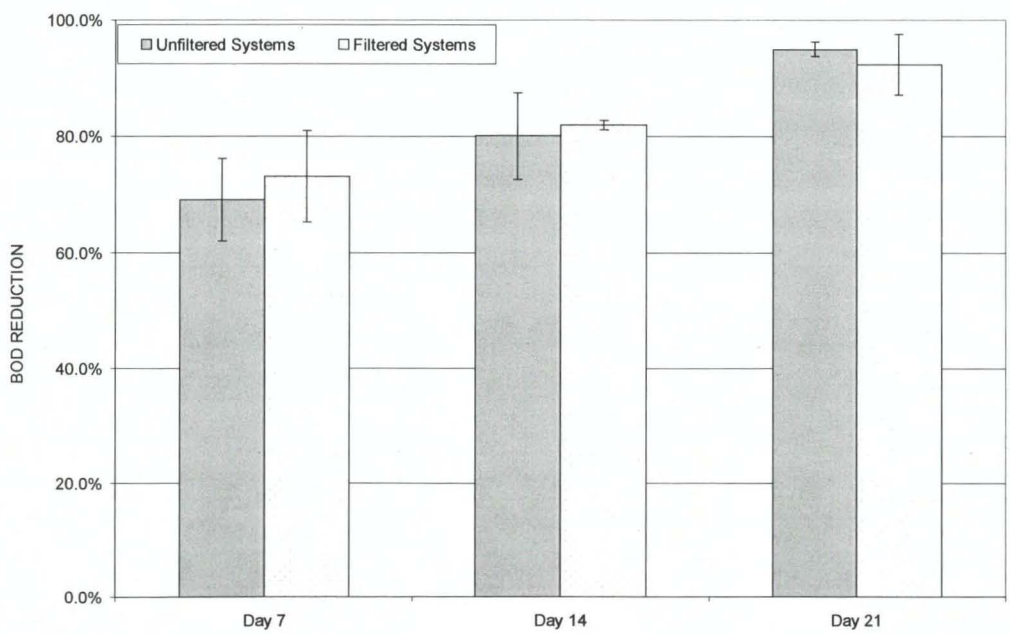


Figure 5.34 Average BOD reductions in unfiltered and filtered water samples with different levels of associated sediments

5.9 ASSESSMENT OF THE SIGNIFICANCE OF SEDIMENTS IN BOD REDUCTION IN WATER SAMPLES INNOCULATED WITH ISOLATES FROM THE ROOTS OF PLANTS FROM SSF

The main aim of this experiment was to assess the impact the presence of sediments from the SSF have on the performance (BOD reduction) of the bacteria strains obtained from the roots of plants from the SSF. This was done by monitoring the BOD reductions in four separate water samples dosed with 350.0 mg/l BOD and the HTF recommended nutrient levels for nitrate and phosphate (see details of the experiment in Chapter 2). Each sample was then inoculated with the 13 isolates from the roots of the SSF. Sterile distilled water was used in the experiment to exclude the influence of any other microbial population other than those introduced into the systems. The other components of the different systems are as follows:

A-containing sterile sediments and placed in a shaking incubator (undisturbed sediments)
B-control experiment (i.e. no sediments) placed in the shaking incubator
C-containing sterile sediments and with regular aeration using a pump (disturbed sediments)
D-control experiment (i.e. no sediments) with regular aeration using a pump

Sterile sediments were used in A and C to exclude any interference that could be caused by the presence of a microbial population other than the ones the systems were inoculated with. The BOD and biomass levels in both systems at 20°C were monitored and the results obtained are summarised in Figure 5.35.

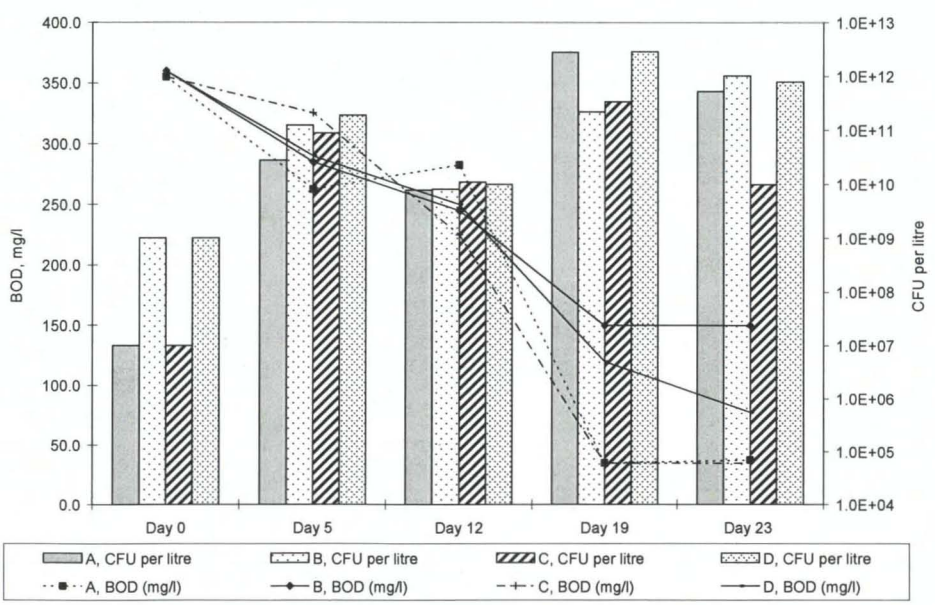


Figure 5.35 Temporal changes in BOD levels and the biomass populations in water samples with and without sterilised sediments, inoculated with the 13 isolates

5 9 1 Discussion of the trends observed in the experiments

There were signs of BOD reduction in all four systems within the first 5 days The highest reduction (26 1%) was recorded for A with the initial BOD level dropping from 355 0 mg/l to 262 5 mg/l (Figure 5 35) The BOD concentrations in B and D fell from an initial concentration of 360 0 mg/l to 285 0 mg/l (20 8%) and 290 0 mg/l (19 4%) respectively within the same period (Figure 5 35) The worst performance over the first 5 days was demonstrated by C (8 3%), with the BOD concentration dropping from 355 0 mg/l to only 325 5 mg/l Further BOD reductions were recorded in all the systems with the BOD concentrations in both A and C reaching 35 0 mg/l representing a 90 1% reduction after 19 days The final BOD levels in B and D were 150 0 mg/l (58 3%) and 77 5 mg/l (78 5%) respectively after 23 days (Figure 5 35) All four systems recorded biomass growth over the first 5 days with the final biomass population reaching a maximum of 1 0 x 10^{12} CFU per litre in A after 23 days from an initial level of 1 0 x 10^{7} (Figure 5 35) The lowest final biomass count was recorded in C reaching 1 0 X 10^{10} CFU per litre from 1 0 X 10^{7} CFU per litre

5 9 2 Deductions from the experiments conducted

The higher BOD reductions of 89 4% and 90 1% recorded after 23 days in A and C (the two systems with sterile sediments) compared to 58 3% and 78 5% in B and D respectively are an indication of the long term benefit of the presence of sediments in the system The lower average BOD reductions (18 7±7 5%) recorded in the systems inoculated with the bacteria isolates with or without sediments in the early stages of the experiment compared to the average levels attained in the filtered and unfiltered system (73 1±7 8% and 69 1±7 8% respectively) (Section 5 8) suggests that the sterile systems lack several forms of nutrient and dead cells, which have been shown to support microbial metabolic activities (Chard *et al*, 2001 Fan *et al* 1997 Marschner 1995)

The rhizosphere is also known to contain plant-derived organic compounds called exudates which are made up of low molecular weight organic acids such as lactic acetic butyric, oxalic tartaric succinic, glutaric maleic propionic and valeric acids (Young *et al*, 1998) These acids play a key role in making nutrients like P and metals like Fe readily available for microorganisms present in the rhizosphere (Munch *et al* 2006) They also contain mixtures of complex sugars vitamins amino acids purines nucleosides, inorganic ions gaseous molecules enzymes and root border cells (Dakora *et al* 2002) which serve as substrates for microbial metabolism and intermediates for biogeochemical reactions Results of tests conducted to assess the effect of the presence of root exudate on BOD reduction in water samples inoculated with the 13 bacteria isolates are described in the next section

5.10 ASSESSMENT OF THE ROLE OF ROOT EXUDATES ON THE BOD REDUCTION IN WATER SAMPLE COLLECTED BY WASHING ROOTS OF PLANTS FROM THE SSF

In order to ascertain whether or not the presence of root exudates affects the BOD reduction in a water sample dosed with BOD in the form of Kilfrost, plant roots were washed thoroughly and then suspended in sterile distilled water for 7 days after which the resulting water sample was separated into four different portions to prepare the following samples:

A. Sterile distilled water (containing root exudates) inoculated with the 13 isolates and dosed with 350 mg/l BOD and the HTF recommended nutrient levels for nitrate and phosphate
B. Sterile distilled water (containing root exudates) dosed with 350 mg/l BOD and the HTF recommended nutrient levels for nitrate and phosphate
C. Autoclaved sterile distilled water (containing root exudates) inoculated with the 13 isolates and dosed with 350 mg/l BOD and the HTF recommended nutrient levels for nitrate and phosphate
D. Autoclaved sterile distilled water (containing root exudates) dosed with 350 mg/l BOD and the HTF recommended nutrient levels for nitrate and phosphate

Full details of the experiment have been discussed in Chapter 2. The variations in BOD and biomass levels in each of the four systems are shown in Figure 5.36

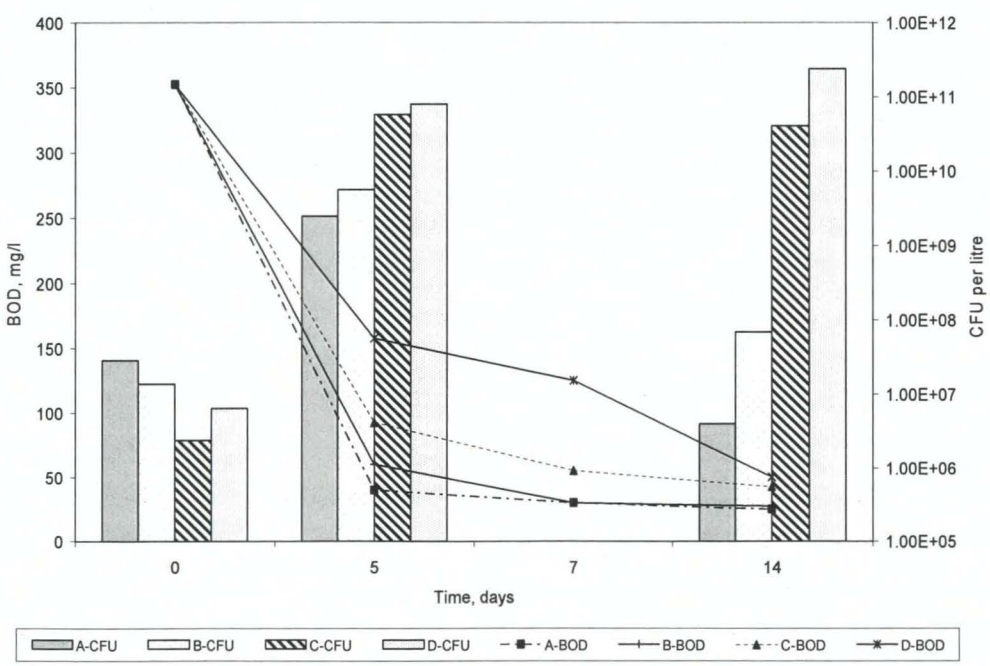


Figure 5.36 Temporal changes in BOD levels and the biomass population in water samples containing root exudates

5 10 1 Discussion of the trends observed in the experiments

The BOD reductions recorded in all four samples were rapid particularly in Systems A and B where the BOD concentration dropped from 352 5 mg/l to 40 0 mg/l (88 7%) and 60 0 mg/l (83 0%) respectively in the first 5 days of the experiment (Figure 5 36) The comparable reductions in BOD concentrations in the other two systems were also high falling to 92 5 mg/l (73 8%) and 157 5 mg/l (55 3%) in C and D respectively (Figure 5 36) By Day 14, the BOD concentrations had fallen to 25 0 mg/l (92 9%) 27 5 mg/l (92 2%) 42 5 mg/l (87 9%) and 50 0 mg/l (85 8%) in A B C and D respectively Both Systems B and D which were not inoculated with any of the 13 isolates were found to contain biomass populations of 1 4 x 10^7 CFU per litre and 6 5 x 10^6 CFU per litre respectively (Figure 5 36) These levels were comparable with the populations detected in A (2 9 x 10^7 CFU per litre) and C (2 4 x 10^6 CFU per litre) which were originally inoculated with the 13 isolates There were noticeable increases in biomass populations in all four systems after Day 5 reaching levels of 2 5 x 10^9 CFU per litre in A 5 7 x 10^9 CFU per litre in B 5 8 x 10^{10} CFU per litre in C and 8 0 x 10^{10} CFU per litre in D At the end of the experiment (14 days) the biomass levels in A B C and D were 4 0 x 10^6 CFU per litre, 6 9 x 10^6 CFU per litre 4 1 x 10^6 CFU per litre and 2 4 x 10^6 CFU per litre respectively (Figure 5 36)

5 10 2 Deductions from the experiments conducted

The high BOD reductions recorded in all four systems within the first 5 days of the experiment are an indication that the components of roots exudates enhance the biodegradation process The biodegradation rates recorded in A B and C (0 443 day^{-1}, 0 351 day^{-1} and 0 270 day^{-1}, respectively) are comparable with the rates initially recorded in the neat water sample from the SSF (0 443 day^{-1}) and the high rates recorded in the first two biodegradation experiments conducted using aerated pond water samples (0 581 day^{-1} and 0 642 day^{-1}) The rate observed in System D (0 164 day^{-1}) though not inoculated with any of the isolates is comparable with the rates observed in the filtered/unfiltered water samples from the SSF (0 192 day^{-1} and 0 170 day^{-1}) The slightly higher BOD reduction recorded within 5 days in A (88 7%) compared to B (82 9%) suggests that the presence of the isolates in A benefited the system The BOD reduction in B is clearly an indication of the presence of an active microbial population which is possibly due to the presence of the root exudates

The BOD reductions observed in C and D (73 8% and 55 3% respectively) within the first 5 days of the experiment although lower than observed in A and B are high enough to suggest the existence of an active microbial population despite autoclaving both samples The detection of a diverse microbial population in B and D, increasing from the start of the experiment at 1 4 x 10^7 CFU per litre and 6 5 x

10^6 CFU per litre to 5 7 x 10^9 and 8 0 x 10^{10} CFU per litre respectively within 5 days of starting the experiment, supports the claim of the existence of active microbial populations in both systems Further analysis of the microbial population in all four systems showed that they also support fungi and actinomycetes species which may have contributed to the observed BOD reductions Although the growth of fungi (separately and in coexistence with other bacteria species) has been reported in litter associated with Phragmites the specific role played during biodegradation is unclear (Romani *et al* 2006) Actinomycetes on the other hand have been identified as one of the groups of microorganisms that form part of the microbial community responsible for nutrient recycling in natural systems such as would be found in roots (McCarthy 1987) Details of the biodegradation experiments conducted using these species are discussed in Section 5 11

The advantage provided by the presence of the 13 isolates in the water samples are further highlighted by the higher BOD reduction of 73 8% recorded in C (autoclaved sample inoculated with 13 isolates) after 5 days compared to 55 3 % attained in D within the same period A comparison of the BOD reduction in C with a similar system in Section 5 7 where sterile distilled water was inoculated with the same 13 isolates shows that the root exudates present in C enhance BOD reduction The high BOD reductions in C and D despite autoclaving suggest that autoclaving could have broken down some of the complex components of the root exudates into smaller more usable units This in combination with the dead bacteria cells encourages rapid biomass growth hence the BOD reductions recorded The survival of these species in the autoclave is supported by claims that certain microorganisms like *Pseudomonas aeruginosa* (commonly found around the rhizosphere) can form biofilms which make them resistant to antibacterial treatments or attacks (Walker *et al* 2004)

5 11 ASSESSMENT OF THE BIODEGRADATION POTENTIAL OF FUNGI AND ACTINOMYTES OBTAINED FROM ROOT WASHINGS

Following the detection of fungi and actinomycetes species in water samples collected from washing of the roots of plants from the SSF appropriate techniques were used to isolate these different species (details in Chapter 2) Table 5 5 is a summary of the different isolates shapes and their origin The abilities of the individual isolates as well as in combination with other isolates were assessed in sterile water samples dosed with 350 mg/l BOD and the HTF recommended nutrient levels for nitrate and phosphate This allows a comparison of the BOD reduction achieved with those of the previous systems in which bacterial isolates have been used The different systems were placed in a shaking incubator at 20°C

(see Chapter 2 for a full description of the experiment) and the BOD levels in each system monitored after inoculation with the following isolates (more details in Table 5, Appendix 5A):

- Fungi F1
- Fungi F2
- Fungi F3
- Actinomycetes A1
- Actinomycetes A2
- Isolate F/A
- All Fungi (i.e. F1 + F2 + F3 + F/A)
- All Actinomycetes (i.e. A1 + A2 + F/A)
- All Fungi and Actinomycetes (i.e. F1 + F2 + F3 + A1 + A2 + F/A)
- All Fungi, Actinomycetes and the 13 bacteria isolates from the plant roots

The temporal changes in BOD concentrations for the different experiments are shown in the figures that follow.

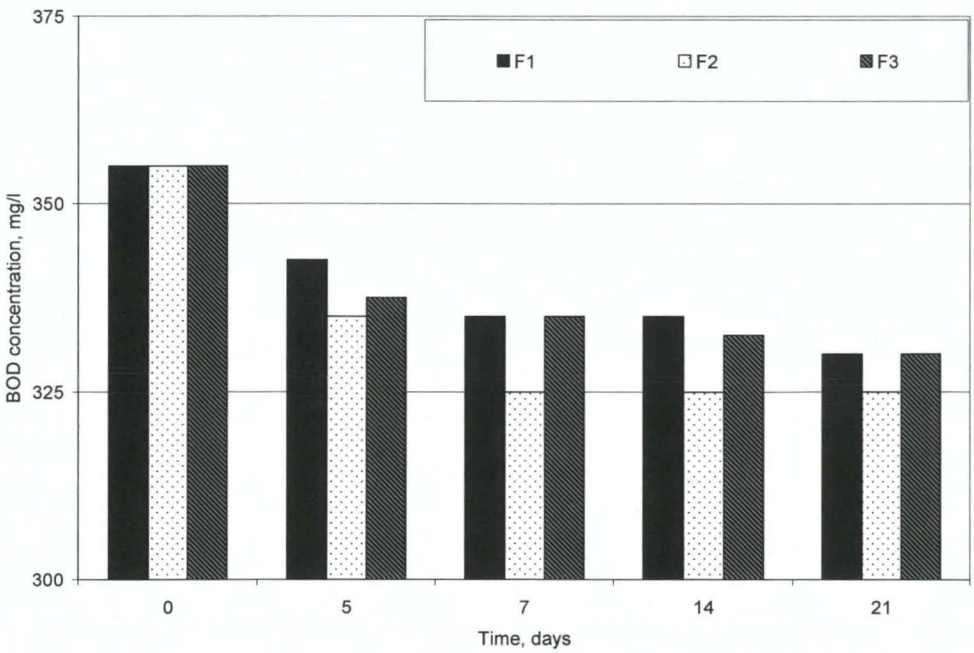


Figure 5.37 Changes in BOD levels in systems inoculated with different individual fungi isolates

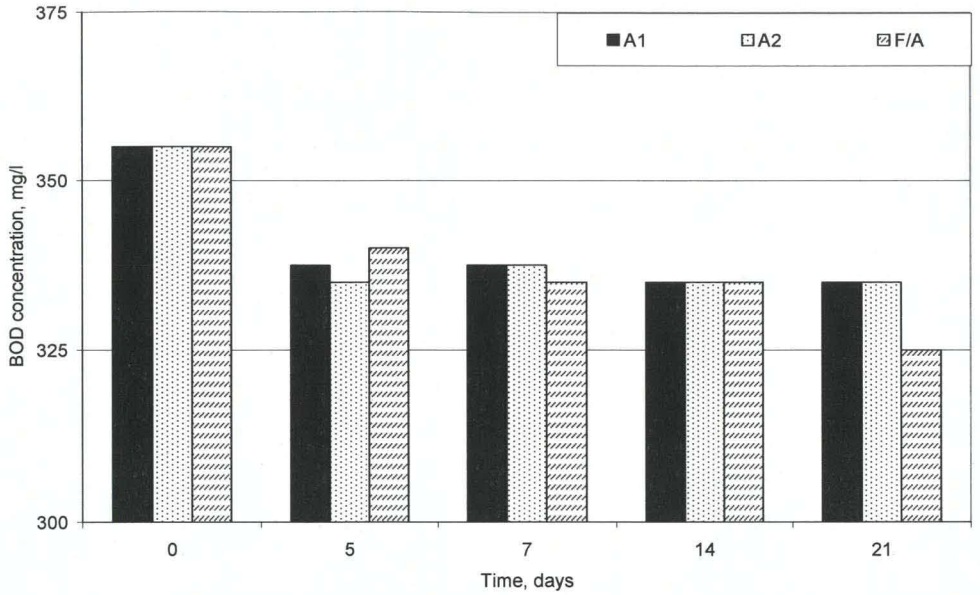


Figure 5.38 Changes in BOD levels in systems inoculated with different actinomycetes isolates

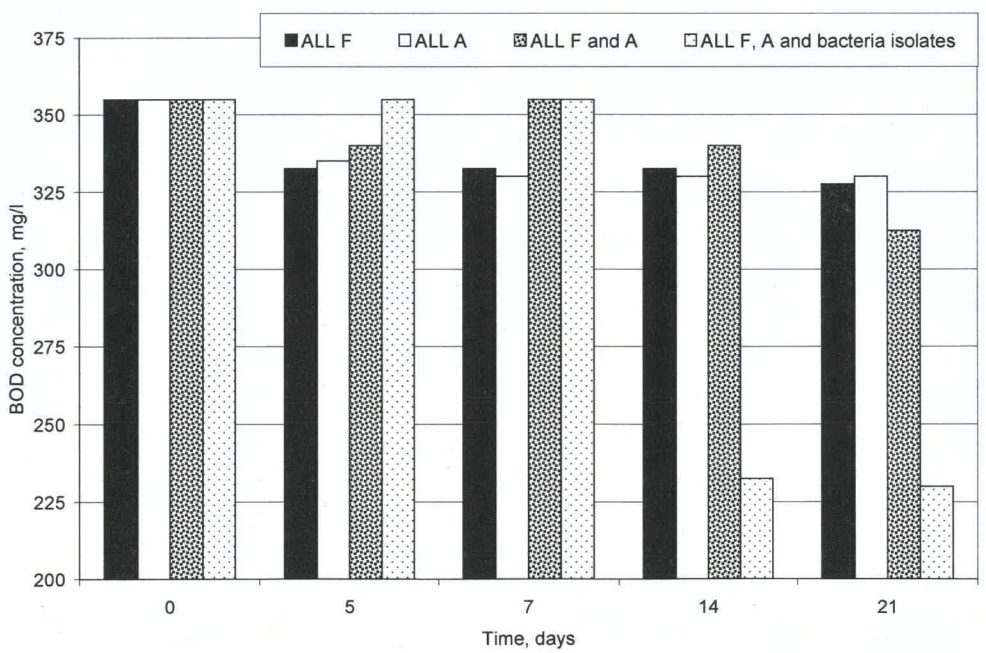


Figure 5.39 Changes in BOD levels in systems inoculated with different combinations of all the isolates

5.11.1 Discussion of the trends observed in the experiments

The initial decreases in BOD concentrations in all 10 systems were quite slow with the highest drop over the first 5 days (from 355.0 mg/l to 332.5 mg/l (6.3%)) recorded in the system inoculated with all the fungi isolates (Figure 5.39). This is

equivalent to a biodegradation rate of 0 022 day^{-1} The lowest BOD reduction (3 7%) from 355 0 mg/l to 342 5 mg/l) was observed for *F1* (biodegradation rate of 0 007 day^{-1}) Throughout the experiment, no substantial reduction was recorded in any of the systems apart from that containing all the bacteria isolates (including Strains *1 2* and *3*) the actinomycete and fungi isolates The BOD level in this system fell to 232 5 mg/l by Day 14 and 230 0 mg/l by Day 21 (Figure 5 39), equivalent to BOD reductions of 34 5% and 35 2% respectively

5 11 2 Deductions from the experiments conducted

Although the biodegradation of organic matter by fungi (Field *et al* 1992 Bennet et al 2002 Hamman 2004 Wohl and McArthur 2004 Romani *et al* 2006) and actinomycetes (McCarthy, 1987, Wohl and McArthur, 2004) has been reported the performances of the systems containing both types of microorganisms showed no indication of effective BOD reduction under the sterile conditions created in this experiment The slightly better performance recorded in the fully combined system containing all bacteria, fungi and actinomycetes isolates demonstrates the advantage created by a diverse consortia even though such a system lacks the initial rapid BOD reduction recorded in any of the water samples collected by washing the roots The 0 040 day^{-1} biodegradation rate recorded in this system is identical with the rates recorded in the sterile system made up of all the bacteria strains isolated from the roots There are indications from these results that despite the presence of the culturable bacteria fungi and actinomycetes strains factors responsible for the rapid BOD reduction recorded in the water samples containing root exudates are lacking in these systems Although it is not entirely clear what exactly these factors are there have been reports that the interaction of bacteria-fungi-actinomycetes in systems where effective decomposition has occurred are facilitated by the presence of plant-derived easily utilisable carbon sources and inducible extracellular enzymes (McCarthy 1987)

5 12 IMPLICATIONS OF BOD RESULTS IN ALL THE BIODEGRADATION EXPERIMENTS

The BOD reductions recorded in the different biodegradation experiments discussed in the previous sections clearly show that all the systems demonstrate a potential for the biodegradation of glycol While some of the systems particularly sterile systems inoculated with the different isolates (bacteria actinomycetes and fungi) performed poorly the BOD reductions recorded in systems associated with the SSF plant roots were generally higher The BOD reductions attained by the individual isolates at different stages of the experiments (Days 3-5 7-9 14-17 and 21-25) are shown in Figures 5 40a-d The comparable BOD reductions in systems containing different combinations of the isolates are also shown in Figures 5 41a-d

The different combinations of isolates used in the systems are represented as follows

AA All bacteria isolates
BB Bacteria isolates *1* and *2*
CC All fungi isolates
DD All actinomycetes isolates
EE All fungi and actinomycetes isolates
FF All fungi actinomycetes and bacteria isolates
GG All fungi, actinomycetes and bacteria isolates with sterile sediments
HH All fungi, actinomycetes and bacteria isolates with root exudates
II All fungi actinomycetes and bacteria isolates with autoclaved root exudates

In the figures that follow (Figures 5 42a-d) the comparable BOD reductions recorded during the same period for all the non-sterile field samples used are also shown The different samples have been represented as follows

JJ Filtered root washings
KK Un-filtered root washings
LL Distilled water sample containing root exudates
MM Distilled water sample containing autoclaved root exudates
NN Aerated ponds (MFBP) water samples
OO Aerated ponds (MFBP) water samples spiked with activated sludge
PP River water sample
QQ Eastern reservoir water sample

The BOD reduction recorded by the individual isolates in sterile systems (sterile distilled water dosed with BOD and nutrients) was generally low with the highest attained within the first 3-5 days being 6 3% (bacteria isolate *G*) This was followed by fungi isolate *F2* (5 6%) bacteria isolates *J* (5 0%) and *F3* (4 9%) (Figure 5 40a) The comparable BOD reductions within the same period in sterile systems containing different combinations of the bacteria actinomycetes or fungi isolates were equally poor The highest BOD reduction attained (6 3%) in the system containing all the fungi isolates was identical to the reduction achieved by bacteria isolate *G* (Figure 5 40a) The significance of sediment was highlighted by the BOD reduction (25 5%) attained in the system containing all the isolates in the presence of sterile sediments The comparable BOD reduction in the same system without the sterile sediments was 0 0% (Figure 5 41a)

The BOD reductions in the systems containing root exudates which were inoculated with the different isolates were even much higher than recorded in any

of the systems containing a combination of different isolates used with or without sterile sediments The BOD reductions in the systems containing all the isolates in the presence of root exudates (88 7%) and autoclaved root exudates (73 8%) were by far the highest attained within the first 3-5 days (Figure 5 41a) The comparable BOD reductions in the other non-sterile systems (Figure 5 42a) were 0 5% (river water sample) 36 5% (eastern reservoir water sample) and 36 9% (root washings water sample) Although the average BOD reduction recorded in the aerated ponds (6 9±6 3%) was also lower than recorded in the root exudates systems the BOD reductions initially recorded in the pond water samples in the first two experiments (90 0% and 92 1% respectively) were higher (Section 5 3)

Further BOD reductions were attained in the sterile systems inoculated with individual isolates after 7-9 days the highest was by isolates *B* (29 4%) and *E* (37 5%) (Figure 5 40b) The average BOD reduction attained after 7-9 days in the systems inoculated with the different individual isolates was 6 1±1 3% The highest BOD reduction attained by any combination of the different isolates was 23 8% in the sterile distilled water sample containing all the bacteria isolates (Figure 5 41b) This was higher than the BOD reduction (19 9%) recorded in the same system in the presence of sterile sediments (5 41b)

The BOD reductions attained in systems containing the isolates in the presence of root exudates (91 5% and 84 4%) were much higher than attained in the sterile distilled water sample with or without sediments (5 42b) The comparable BOD reductions in the non sterile systems from which these isolates were obtained (36 6% in aerated pond water sample, 64 6% in eastern reservoir water sample 69 1% in filtered root washings and 73 1% in un-filtered root washings water samples) were lower than observed for the root exudates systems The sterilised water samples inoculated with all the isolates in the presence of root exudates continued to outperform the rest of the systems The 92 9% and 87 9% BOD reductions attained in the sterile and autoclaved water samples containing root exudates after 14-17 days were significantly higher than the 34 5% recorded in the same system without root exudates (Figure 5 41c) The highest BOD reduction by any individual isolate within the same period was by *E* (32 5%) (Figure 5 40c) which was actually a drop in the level attained between Days 7-9

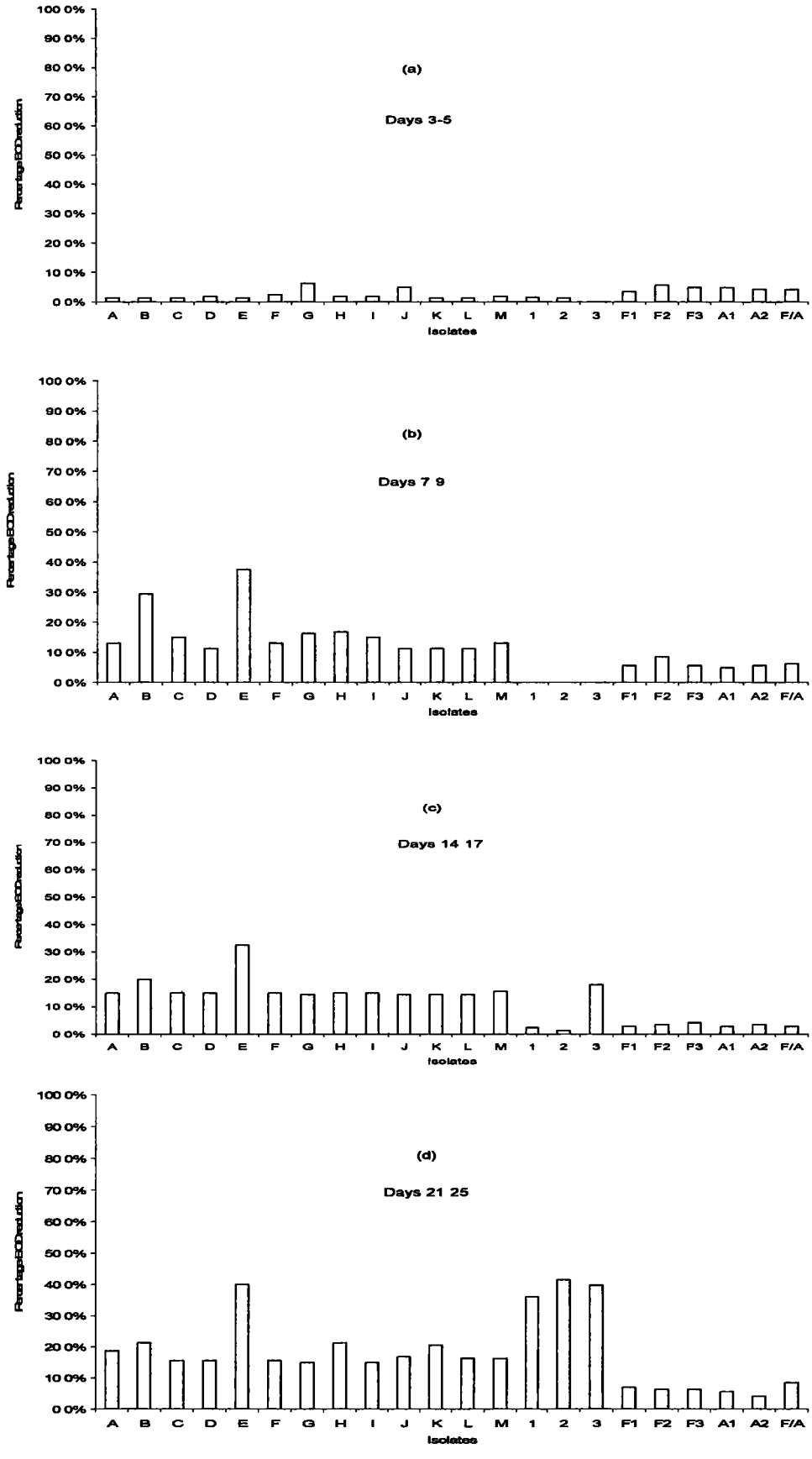


Figure 5 40 (a-d) Comparison of the BOD reductions in sterile water samples inoculated with different bacteria, actinomycetes and fungi isolates

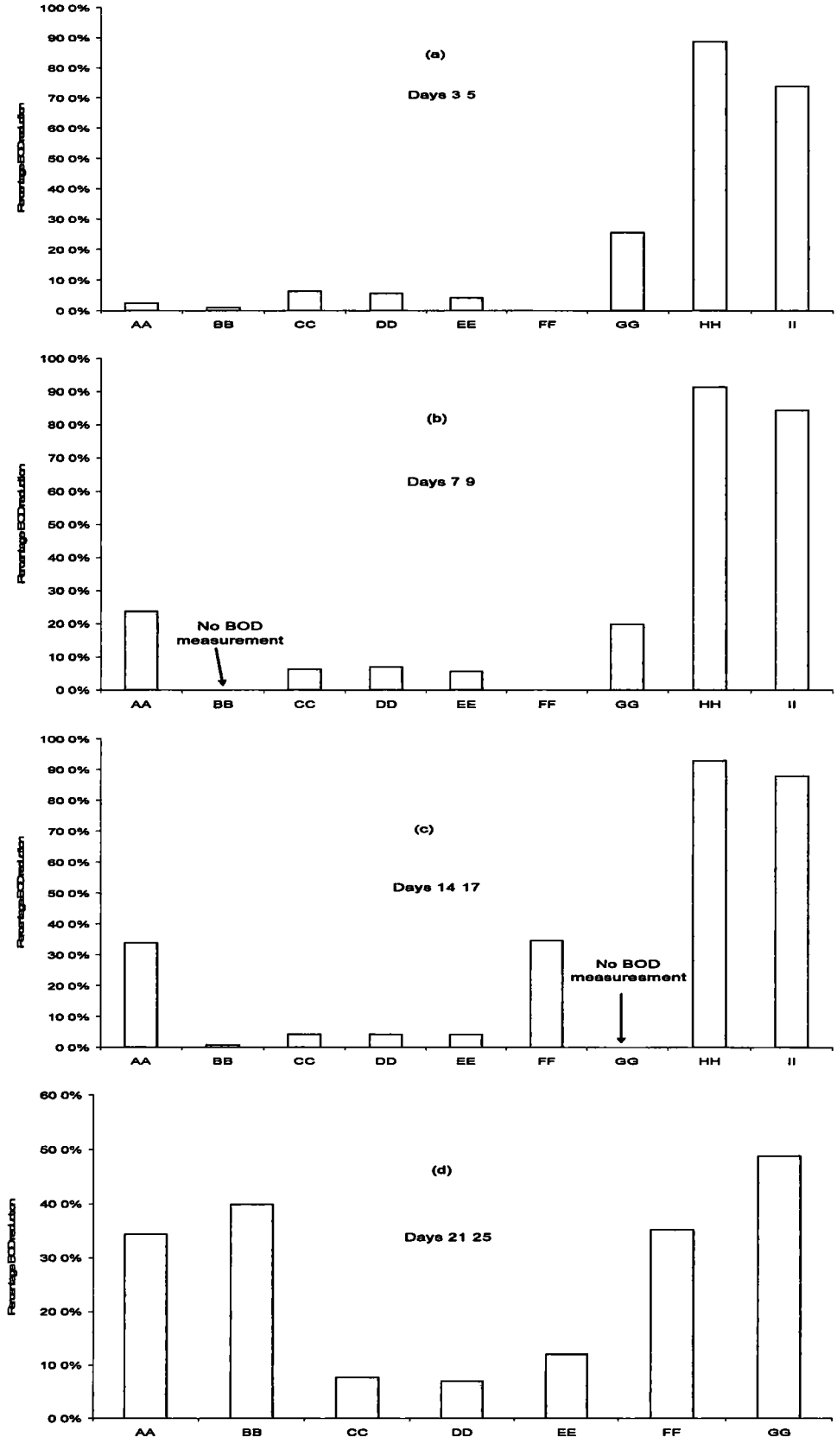


Figure 5 41 (a-d) Comparison of the BOD reductions in sterile water samples inoculated with different combinations of bacteria, actinomycetes and fungi isolates

Significant improvements were observed in the rest of the non sterile systems between Days 14-17 with the aerated pond system recording an average BOD reduction of 71 5±23 0% The comparable BOD reduction in the aerated pond water samples dosed with activated sludge was 66 8±21 4% (Figure 5 42c) Further BOD reductions were also recorded in both the filtered and un-filtered root washings between Days 14-17 with the average BOD reductions reaching 82 0±0 8% and 80 1±7 4% respectively The BOD reduction in the river water sample remained low at 13 8% (Figure 5 42c)

In the closing stages of the experiments (Days 21-25) the BOD reductions in sterile systems inoculated with individual isolates remained generally low the highest attained was by bacterial isolate *2* (41 5%) This was closely followed the BOD reductions attained by *E* (40 0%) and *3* (39 8%) (Figure 5 40d) The closest comparable BOD reduction in systems with a combination of the isolates was recorded by isolates *1* and *2* (39 9%) The BOD reduction in the sterile systems containing all the isolates was 35 2% (Figure 5 41d) The benefit of sediment to the isolates was once again demonstrated as the BOD reduction in the system containing all isolates in the presence of sterile sediments reached 48 9% (Figure 5 41d) Further BOD reductions were recorded in the non-sterile systems the highest (95 0±1 3%) was recorded in the un-filtered root washings water sample (Figure 5 42d)

The inability of the consortium of isolated bacteria fungi and actinomycetes isolates used in this study to efficiently remove the BOD in the water samples despite the high BOD reduction recorded in the natural systems they were isolated from suggests that they merely constitute a part of the entire consortium This is consistent with the claim that only a small proportion of the total microbial population in environmental samples is culturable on laboratory prepared media (Staley and Konopka, 1978 Bottomley and Maggard, 1989 Kell 1998 Oliver, 2005) Because some of these microorganisms are culture-specific and as such are non-culturable through conventional laboratory techniques (Szewzyk *et al* 2006) there is a need to use a variety of growth media in order to isolate and identify the consortia of microorganisms responsible for the efficient BOD reduction recorded in the presence of roots and root extracts The consistently high BOD reduction recorded in the systems containing root exudates (and those associated with plant roots) highlights the role of vegetation in the providing the right synergy required for adequate BOD removal in the system

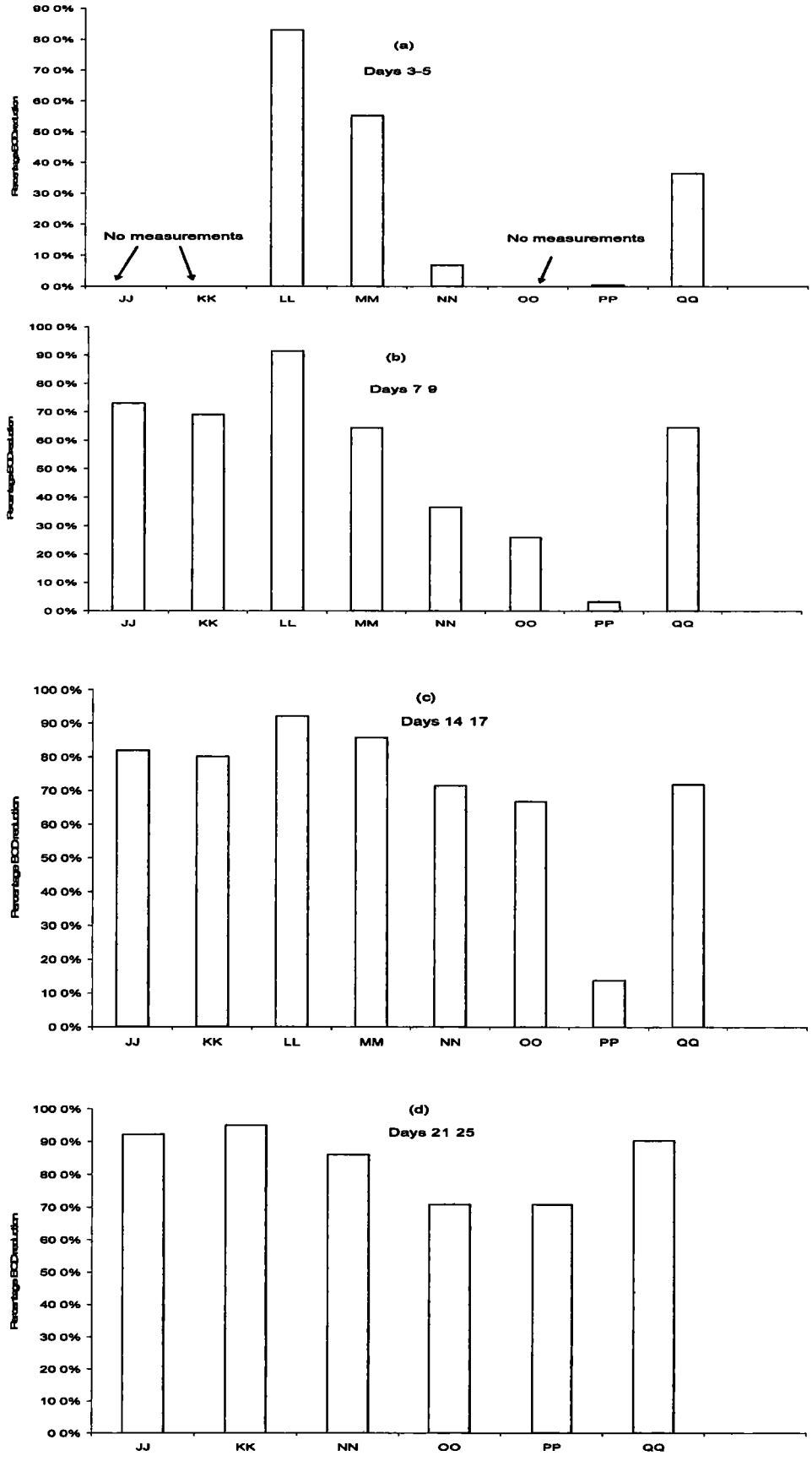


Figure 5 42 (a-d) Comparison of the BOD reductions in various systems

CHAPTER 6 THE VERTICAL FLOW COLUMN PILOT SCALE STUDY

Despite the presence of active microbial degradation in the Sub-surface flow reedbeds (SSF) at the Mayfield Farm (demonstrated by the rapid depletion of the dissolved oxygen half-way through the first set of reedbed cells) the BOD levels recorded at the exit of the system remained higher than the consent level (40 0 mg/l) particularly in the winter (details in Chapter 3) A series of tests were conducted on pilot scale vertical flow reedbed systems to assess the conditions that could affect the performance of the SSF (details in Chapter 2) The impact of the addition of nutrient on the BOD reduction in the columns was investigated by comparing the BOD reductions in planted and substrate-only columns containing nitrate in the form of KNO_3 and phosphate as KH_2PO_4 with nutrient-free planted and substrate-only columns The impact of the presence of the Fe^{2+}/Fe^{3+} equilibrium on BOD reduction in the columns was also investigated by monitoring the BOD reduction in planted and substrate-only columns with and without the Fe^{2+}/Fe^{3+} ions in the initial form of $FeSO_4$ Details of the experiments and the dosing used have been discussed earlier in Chapter 2 The temporal changes in BOD nitrate and phosphate levels, iron concentrations DO levels and the average temperature in all the columns fed with aerated water samples from the Mayfield Farm Balancing pond (MFBP) were monitored 7 and 21 days after dosing with the different solutions as described in Table 6 1 A total of 7 sets of experiments were conducted between August 2004 and September 2005 to assess the impact of seasonal changes on the BOD removal process (Table 1 Appendix 6A)

Initially water samples for analysis were collected from three different heights within the experimental columns (top middle and bottom) using the specially installed taps However no significant differences in the BOD reductions were observed for samples collected at the different depths Consequently, water samples were only collected from the outlet pipe at the bottom of each column in the remaining experiments The results obtained from the control columns, which have been fed with additive-free MFBP water samples confirmed the presence of active microbial processes within the planted and substrate-only columns as the background BOD concentration in both columns reduced from 35 0 mg/l to 32 5 mg/l and 27 5 mg/l after 7 days and finally to 10 0 mg/l and 7 5 mg/l respectively after 21 days The effects of each of the added components (nutrient and ferrous ions) on BOD reductions in the rest of the columns are reported and discussed in the following sections In particular the influences of retention time and whether the column was planted or not are assessed

Table 6 1 Details of dosing solutions applied to both planted and substrate-only columns

Dosing agent	Planted column	Substrate-only column
MFBP water only	✓ (control)	✓ (control)
MFBP water sample dosed with 350 mg/l BOD (in the form of glycol)	✓ (Section 6 1/6 3)	✓ (Section 6 2/6 4)
MFBP water sample dosed with 350 mg/l BOD (in the form of glycol) 6 8 mg/l phosphate (in the form of KH_2PO_4) and 50 mg/l nitrate (in the form of KNO_3)	✓ (Section 6 1)	✓ (Section 6 2)
MFBP water sample dosed with 350 mg/l BOD (in the form of glycol) and 3 mg/l Fe^{2+} (in the form of $FeSO_4\ 7H_2O$)	✓ (Section 6 3)	✓ (Section 6 4)

✓ Indicates that the respective dosing agent is discussed in the specified section

6 1 EFFECT OF NUTRIENT ON BOD REMOVAL IN PLANTED COLUMNS

6 1 1 Trends observed in the different experiments

Based on the BOD concentration measurements in the columns the percentage BOD reduction in Experiment A after 7 days in the nutrient-dosed column was 97 1% compared to 51 2% in the nutrient-free column (Figure 6 1) However, the differences in BOD reductions became insignificant by day 21 when the BOD removal percentage in the nutrient free column rose to 94 8% compared to 93 4% in the nutrient dosed column (Figure 6 2) Experiment A was conducted between August and September 2004 when the average temperature in the columns over 21 days was 20 5±0 2°C Rapid utilisation of nutrient was also recorded in the nutrient-dosed column 81 5% for nitrate and 91 2% for phosphate in 7 days (Tables 2 and 3 Appendix 6A) There were however increases in both the nitrate and phosphate concentrations in the columns between days 7 and 21 (9 25 mg/l to 11 80 mg/l and 0 60 mg/l to 1 20 mg/l respectively) After the maximum column retention time the DO concentration was 0 20 mg/l in the nutrient-dosed column and zero in the nutrient-free planted column (Table 4 Appendix 6A)

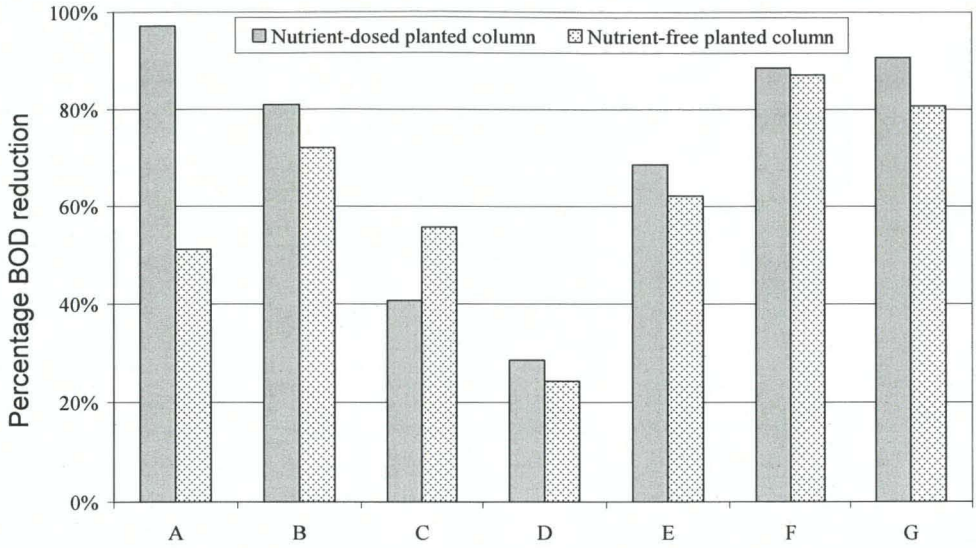


Figure 6.1 Comparison of BOD reductions after 7 days in the nutrient-dosed and nutrient-free planted columns at different times of the year

BOD reductions during the first 7 days of Experiment B in both the nutrient-dosed and the nutrient-free columns were high at 81.0% and 72.1%, respectively (Figure 6.1). There were further BOD reductions after 21 days with the columns recording final BOD removal percentages of 95.4% and 94.0%, respectively (Figure 6.2). The nutrient utilisation over 21 days was also high reaching 100% for nitrate and 94.1% for phosphate (Tables 2 and 3, Appendix 6A). The DO levels recorded in both columns after 21 days were zero (Table 4, Appendix 6A) and the average temperature throughout the experiment was 15.9±4.0°C.

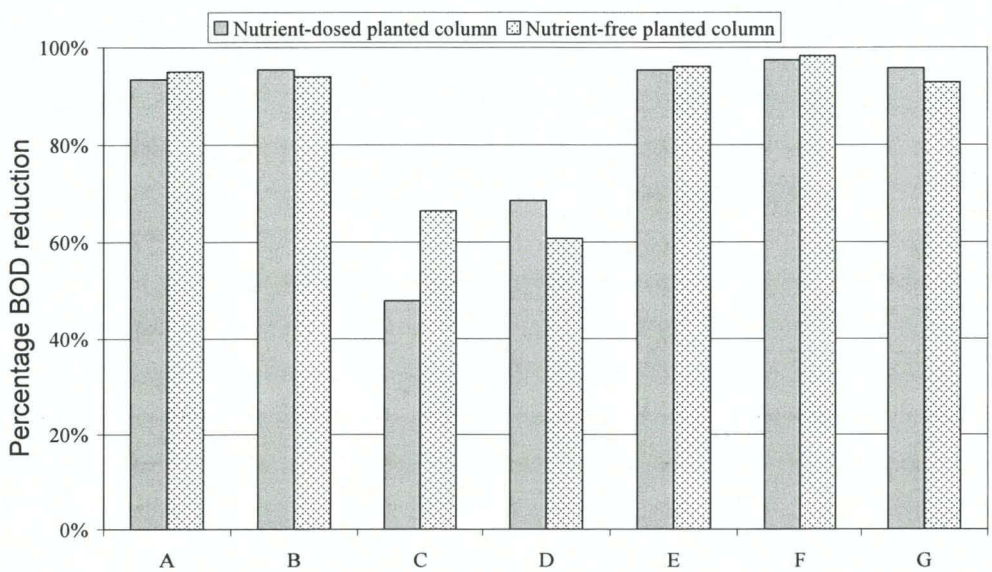


Figure 6.2 Comparison of BOD reductions after 21 days in the nutrient-dosed and nutrient-free planted columns at different times of the year

The BOD reductions in Experiment C in both the nutrient-dosed planted and the nutrient-free planted columns were lower than previously recorded in the first two experiments The initial BOD reduction observed in the nutrient-free column (55 7%) was higher than the 40 7% attained in the nutrient-dosed column (Figure 6 1) As for the second experiment there were further although less pronounced reductions in BOD after 21 days with the overall removal in the nutrient-free column rising to 66 4% but only to 47 9% in the nutrient- dosed column (Figure 6 2) Nutrient utilisation after 21 days in the nutrient-dosed column remained high with values of 79 6% for nitrate and 98 8% for phosphate (Tables 2 and 3 Appendix 6A) The DO was effectively used up in both columns with 0 50 mg/l remaining in the basal water from the nutrient-dosed column and 0 20 mg/l from the nutrient-free column after 21 days (Table 4 Appendix 6A) The average temperature in the columns throughout the experiment was 8 0±2 8°C, which is representative of the time of the year (November/December) during which this experiment was conducted

Further initial declines in percentage BOD reductions in both columns were observed in Experiment D Within the first 7 days BOD reductions recorded in the nutrient-dosed and nutrient-free planted columns were 28 6% and 24 3% (Figure 6 1) rising to 68 6% and 60 7% respectively after 21 days (Figure 6 2) Overall nitrate utilisation in the nutrient-dosed column (71 6%) was slightly lower than observed in previous experiments in contrast with the 97 6% reduction for phosphate (Tables 2 and 3, Appendix 6A) The DO levels in both columns were completely depleted after 7 days (Table 4 Appendix 6A) The average temperature in the columns was 6 5±3 5°C over the 21 day winter period

Improvements in BOD reductions (68 6% and 62 1% within the initial 7 days in the nutrient dosed and nutrient free columns respectively) were observed in the columns during Experiment E (Figure 6 1) Further BOD reductions to 95 4% in the nutrient-dosed column and 96 1% in the nutrient-free column (Figure 6 2) occurred by Day 21 The average temperature in the columns over the 21-day period was 17 6±0 6°C There was complete utilisation of nitrate in the nutrient-dosed planted column after 7 days with only 7 6% of the added phosphate remaining after 21 days (Tables 2 and 3 Appendix 6A) At the end of the experiment the DO concentrations in both columns were low (0 8 mg/l in the nutrient-dosed column and 0 7 mg/l in the nutrient-free column) (Table 4 Appendix 6A)

The average temperature in the columns during Experiment F rose sharply to 26 7±2 6°C BOD reductions in the nutrient-dosed planted column and the nutrient-free column after 7 days were similar at 88 6% and 87 1% respectively (Figure

6 1) Increased reductions to 97 4% and 98 3% respectively occurred in both columns after 21 days (Figure 6 2) Over the duration of the experiment 79 4% of the applied phosphate was utilised and there was complete utilisation of nitrate in the nutrient-dosed column (Tables 2 and 3, Appendix 6A) Final DO levels of 1 2 mg/l in the nutrient-dosed column and 1 0 mg/l in the nutrient-free column were recorded (Table 4 Appendix 6A)

In Experiment G the initial BOD reductions were also high with values of 90 7% and 80 7% in the nutrient-free column (Figure 6 1) Further reductions to 95 7% and 92 8% respectively were recorded after 21 days (Figure 6 2) There was efficient utilisation of both nutrients (96 0% for phosphate and 100% for nitrate) (Tables 2 and 3 Appendix 6A) The average temperature in the columns throughout the experiment was 22 9±6 6°C and anoxic conditions were recorded in the water eluting from the bottom of both columns after 7 days (Table 4, Appendix 6A)

6 1 2 Discussion of trends observed in the different experiments

The results from the seven completed experiments indicate that the addition of nutrients (nitrate and phosphate) to the planted columns enhances applied BOD reductions within the first 7 days There is however no clear evidence of any significant long-term impact (beyond 7 days) of the addition of nutrients on the BOD removal in the planted columns The initial average BOD removal after 7 days for the nutrient-dosed planted column was 70 8±26 5% compared to 61 9±21 1% in the nutrient-free planted column The overall average BOD removal percentages after 21 days were however slightly higher in the nutrient-free column (86 2±15 6%) compared to the nutrient dosed column (84 8±19 2%) A distinct benefit of nutrient addition in the summer months was demonstrated by an average BOD removal over 7 days of 85 2±10 9% for the relevant experiments (Experiments A B, E, F and G) Such rapid BOD reductions in the planted columns during this period may be attributed to the fact that the tests were conducted between July and November which corresponds to the growing season for *Phragmites australis* (Diggs *et al* 1999) The comparable BOD reductions in the nutrient-free column produced an average removal efficiency of 70 7±14 4%

Performances of the planted columns during the winter months were lower than observed in the summer The average BOD reduction after 7 days was 34 6±8 6% (Experiments C and D) in the nutrient-dosed planted column with a slightly higher value of 40 0±22 2% in the nutrient-free planted column, rising to 58 2±14 7% and 63 6±4 0% respectively after 21 days There is a possibility that higher BOD levels were measured in the nutrient-dosed planted column due to contributions from

decaying leaves and shoots (rich in nutrient accumulated over time) during the winter months This would result in lower overall BOD reduction which was further highlighted by the higher overall BOD reduction of 75 4±10 6% recorded after 21 days in the nutrient-dosed substrate-only column (Section 6 2) when compared to the 58 2±14 7% recorded in the nutrient-dosed planted column The overall BOD reductions in the nutrient-free columns planted and substrate-only, were equivalent (63 6±4 0% and 63 6±20 2% respectively)

The reduction in DO concentrations recorded in the columns in the different experiments (Table 4 Appendix 6A) is an indication of the presence of active utilisation of DO for BOD reduction All systems, regardless of the time of the year, recorded almost complete depletion of DO by Day 21 suggesting that seasonality does not affect the utilisation of DO in the columns There is also no evidence from the results that the presence of the roots in the planted columns benefited the system by providing additional oxygen

Nutrient utilisation was efficient in all the experiments The overall average utilisations of phosphate and nitrate over 21 days were 91 5±7 6% and 89 7±13 1%, respectively This is consistent with previously reported uptake values of up to 99 0% for phosphate and 98 0% for nitrate by wetland plants (Spieles and Mitsch 2000) The average nitrate utilisation was higher over the summer months (95 3±10 6%) compared to 75 6±5 6% recorded over the winter months The high nitrate consumption observed in the summer is most likely the consequence of high biological activity and high nitrogen requirement for growth Phosphate utilisation on the other hand was higher during the colder weather at 98 2±0 8% compared to 88 9±7 5% during the summer months, the reverse of the trend in nitrate uptake This is consistent with the findings of McCartney *et al* (2003) in which low phosphate concentration were attributed to high level of biological activity in maturing reedbed systems during the winter

It is not clear why the measured nitrate and phosphate concentrations after 21 days were higher than those recorded after 7 days in some of the experiments (Experiments A and D for nitrate Experiments A B D and F for phosphates) It is probable that the high utilisation rates observed in the columns are attributable to the high level of biological activity Statistical analyses however showed no strong correlation between nutrient utilisation and BOD reductions in the columns

6 2 EFFECT OF NUTRIENT ON BOD REMOVAL IN SUBSTRATE-ONLY COLUMNS

6 2 1 Trends observed in the different experiments

The BOD reduction in the nutrient-dosed column of Experiment A after 7 days was 63 3% compared to 51 4% in the nutrient-free column (Figure 6 3) There were further reductions to 75 7% and 77 1% respectively after 21 days (Figure 6 4) The nutrient utilisation over 21 days was also high 88 2% for nitrate and 86 6% for phosphate (Tables 2 and 3, Appendix 6A) The DO level recorded in both columns after 21 days was zero (Table 4, Appendix 6A)

In Experiment B, the trend in BOD reductions were similar but higher with values after 7 days of 83 4% in the nutrient-dosed column and 72 2% in the nutrient-free column (Figure 6 3) There were further reductions to 91 4% and 90 6%, respectively after 21 days (Figure 6 4) There was efficient utilisation of the nutrients (99 2% for nitrate and 86 3% for phosphate) over the 21-day duration of the experiment (Tables 2 and 3 Appendix 6A) The DO in both columns was completely used up over the same period (Table 4 Appendix 6A)

BOD reductions within the first 7 days of Experiment C were lower than observed in Experiments A and B (the same trend was observed for the planted columns) with measured values of 47 9% in the nutrient-dosed column and 40 7% in the nutrient-free column (Figure 6 3) The overall BOD reduction after 21 days in the nutrient-dosed column was enhanced to 67 9% compared to a percentage increment of only 8 5% in the nutrient-free column (Figure 6 4) Nutrient utilisation over the duration of the experiment was high 80 5% for nitrate and 95 6% for phosphate (Tables 2 and 3 Appendix 6A) The DO was also effectively used up in both the nutrient-dosed and nutrient-free columns reaching minima of 0 4 mg/l and 0 2 mg/l respectively, after 21 days (Table 4 Appendix 6A)

The BOD reduction after 7 days in the nutrient-free column of Experiment D was the lowest measured and considerably less (32 9%) than the 51 4% observed for the nutrient-dosed column (Figure 6 3) There were substantial improvements in BOD reductions in both columns after 21 days reaching 82 9% in the nutrient-dosed column and 77 9% in the nutrient-free column (Figure 6 4) The utilisation of phosphate after 21 days was higher (94 1%) than that for nitrate (71 6 %) (Tables 2 and 3 Appendix 6A) The DO was completely used up in both columns after 7 days (Table 4 Appendix 6A)

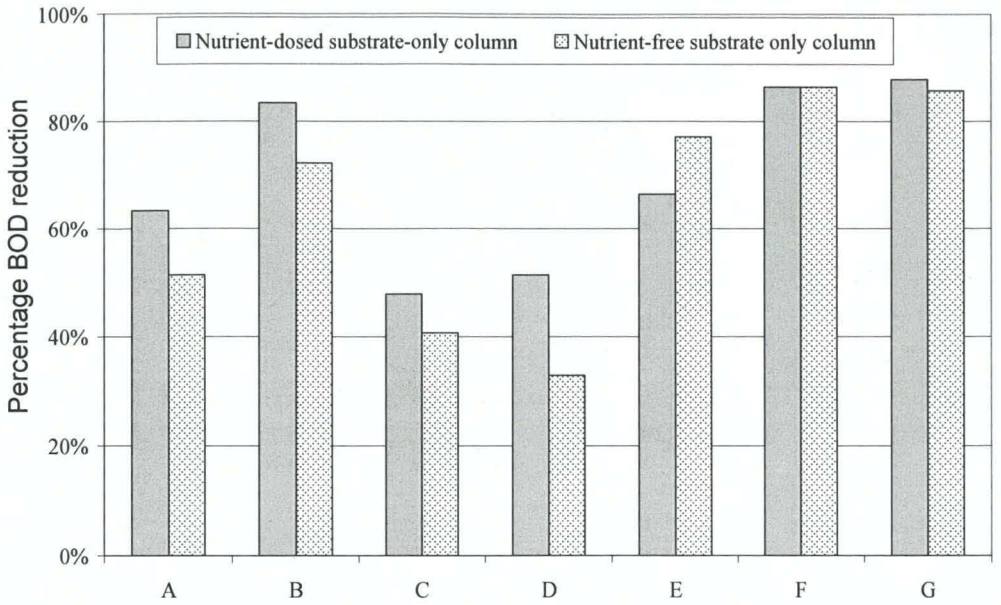


Figure 6.3 Comparison of BOD reductions after 7 days in the nutrient-dosed and nutrient-free substrate-only columns at different times of the year

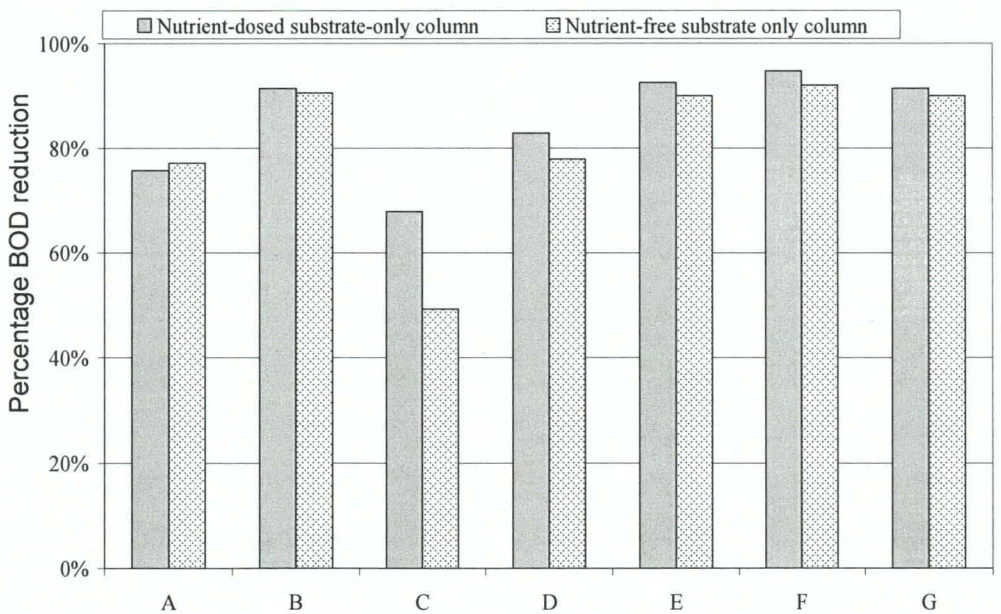


Figure 6.4 Comparison of BOD reductions after 21 days in the nutrient-dosed and nutrient-free substrate-only columns at different times of the year

Experiment E was the only experiment in which, after 7 days, the BOD reduction in the nutrient-free column was greater (77.1%) than that recorded in the nutrient-dosed column (66.4%) (Figure 6.3). However, this trend was reversed after 21 days with a higher BOD reduction in the nutrient-dosed column (92.5%) compared to the nutrient-free column (90.0%) (Figure 6.4). There was complete utilisation of

nitrate in the nutrient-dosed column and only 13 2% of the added phosphate was unused after 21 days (Tables 2 and 3 Appendix 6A) The DO in both columns was effectively used over the 21 days of the experiment, reaching 0 7 mg/l in the nutrient-dosed column and 0 8 mg/l in the nutrient-free column (Table 4, Appendix 6A)

BOD reductions in Experiment F in both columns after 7 days were the same (86 4%) (Figure 6 3) The overall BOD reductions after 21 days were 94 7% and 92 0% in the nutrient-dosed and nutrient-free columns respectively (Figure 6 4) There was complete utilisation of the added nitrate after 21 days in the nutrient-dosed column while only 16 3% of the phosphate remained unused over the same period (Tables 2 and 3 Appendix 6A) DO levels in both columns were the same (1 3 mg/l) after 21 days (Table 4, Appendix 6A)

The BOD values measured during Experiment G followed the general observed pattern with 7 day reductions being higher in the nutrient-dosed column (87 9%) than recorded in the nutrient-free column (85 7%) (Figure 6 3) and being followed by further BOD reductions to 91 4% and 90 0% respectively after 21 days (Figure 6 4) As with the two previous experiments, there was complete utilisation of nitrate in the nutrient-dosed column after 21 days Only 6 1% of the added phosphate remained after the same period (Tables 2 and 3 Appendix 6A) The DO in both columns was completely used up after 21 days (Table 4, Appendix 6A)

6 2 2 Discussion of trends observed in the different experiments

The results from the seven experiments show that the addition of nutrient to the substrate-only column enhances BOD reductions The average BOD reduction after 7 days in the nutrient-dosed column was 69 5±16 6% compared to 63 8±21 9% in the nutrient-free substrate-only column, rising to 85 2±10 2% and 81 0± 15 3% respectively after 21 days The average BOD reduction recorded in the nutrient-dosed column after 7 days in the summer (Experiments A B, E, F and G) was only slightly higher (77 5±11 7%) than observed in the nutrient-free column (74 6±14 3%) Comparable BOD reductions after 21 days were 89 2±7 63% and 87 9±6 1% respectively Although the average BOD reductions recorded after the first 7 days in both columns in the winter (Experiments C and D) were lower (49 6±2 5% and 36 8±5 6% in the nutrient-dosed and nutrient-free columns respectively) than those observed in the summer the overall BOD reductions of 75 4±10 6% recorded after 21 days in the nutrient-dosed column compared to 65 6±20 2% in nutrient-free column further demonstrates the long term benefit of nutrient addition While the BOD reductions achieved after 21 days in the columns (nutrient-dosed and nutrient-free) in the summer are similar, (89 2±7 63% and

87 9±6 1%), the greater difference in BOD reductions between both columns in the winter (75 4±10 6% and 65 6±20 2%) over the same exposure period highlights the role that nutrient addition can play in the winter months

There was efficient utilisation of added nutrients 91 4±11 6% and 89 5±4 8% for nitrate and phosphate, respectively after 21 days The average utilisation of nitrate after 21 days was higher in the summer (97 5±5 2%) compared to the winter (76 1±6 3%) The average phosphate utilisation was however higher in the winter (95 2±0 5%) compared to 87 3±3 4% recorded in the summer This is consistent with reported values for the nutrient-dosed planted column (section 6 1 2), suggesting temperature has an influence on nutrient removal in the columns It is not clear why there was an increase in nitrate level recorded between days 7 and 21 in Experiment D Statistically no strong correlations were found between nutrient utilisation and BOD reductions in the substrate-only column

6 3 EFFECT OF Fe^{2+}/Fe^{3+} EQUILIBRIUM ON BOD REMOVAL IN PLANTED COLUMNS

6 3 1 Trends observed in the different experiments

In Experiment A, the BOD reduction achieved after 7 days in the iron-dosed column was higher (57 1%) than recorded in the iron-free column (51 2%) (Figure 6 5) The overall BOD reduction after 21 days in the iron-free column was however substantially higher at 94 8% compared to 87 1% in the iron-dosed column (Figure 6 6) The DO levels in both columns were completely depleted over 21 days (Table 5 Appendix 6A) The Fe^{2+} concentration in the water sample collected from the column after 21 days was higher (4 22 mg/l) than the 3 00 mg/l concentration in the feed stream at the start of the experiment (Table 6 Appendix 6A)

Compared to Experiment A, there were improvements in the BOD reductions recorded in both columns after 7 days of Experiment B (81 7% and 72 1%) (Figure 6 5) rising to 96 5% and 94 0% after 21 days in the iron-dosed and iron-free columns, respectively (Figure 6 6) The DO concentrations in both columns after 21 days were zero (Table 5 Appendix 6A) and over the same period the Fe^{2+} concentration at the base of the iron-dosed column was 3 10 mg/l (Table 6 Appendix 6A)

In Experiment C the BOD reduction after 7 days in the iron-dosed column was lower (40 7%) than the 55 7% value achieved in the iron-free column (Figure 6 5) The difference however became insignificant after 21 days as the BOD reduction in both columns rose to 66 4% (Figure 6 6) The DO levels in both columns were low

after 21 days 0 3 mg/l in the iron-dosed column and 0 2 mg/l in the iron-free column The concentration of Fe^{2+} in a water sample collected from the base of the column after 21 days had increased to 4 75 mg/l (Table 6, Appendix 6A)

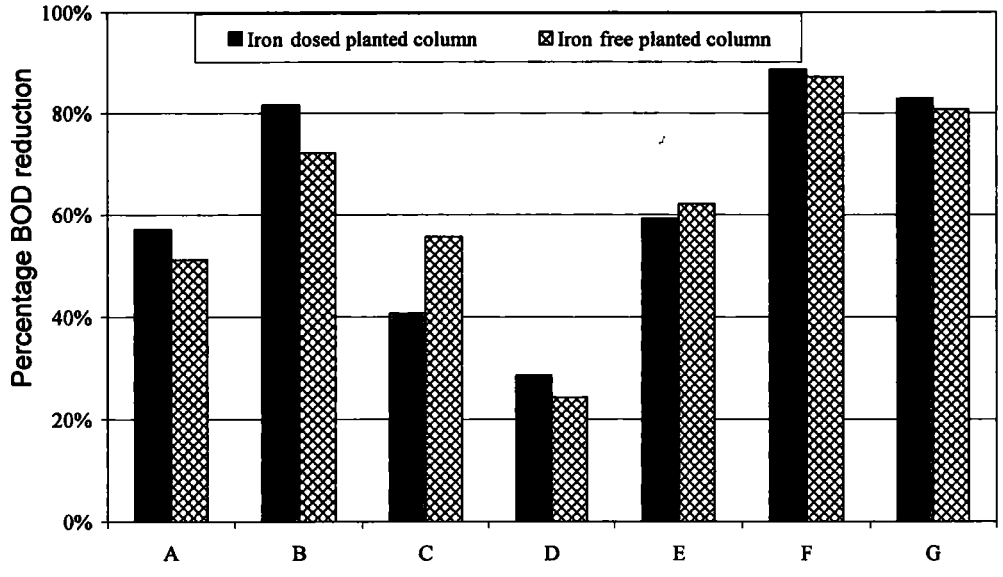


Figure 6 5 Comparison of BOD reductions after 7 days in the iron-dosed and iron-free planted columns at different times of the year

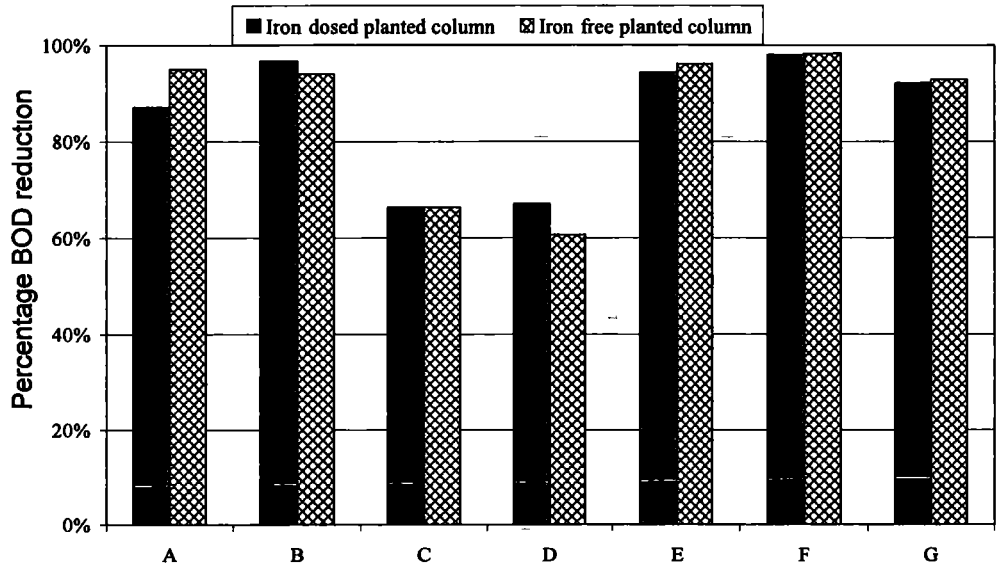


Figure 6 6 Comparison of BOD reductions after 21 days in the iron-dosed and iron-free planted columns at different times of the year

The BOD reductions after 7 days of Experiment D in both the iron-dosed and iron-free columns were much lower than for all experiments at 28 6% and 24 3% respectively (Figure 6 5) There were significant improvements in BOD reductions after 21 days with the iron-dosed column recording a higher level of 67 1% compared to 60 7% achieved in the iron-free column (Figure 6 6) The DO in both columns was completely depleted earlier on in the test i e day 7 (Table 5, Appendix 6A) In this experiment the final Fe^{2+} concentration after 21 days in the column was 1 77 mg/l (Table 6, Appendix 6A)

In Experiment E the BOD reductions after 7 days were similar with values of 62 1% in the iron-free column and 59 3% in the iron-dosed column (Figure 6 5) This pattern was maintained after 21 days as the overall BOD reductions increased to 96 1% and 94 3% respectively (Figure 6 6) The DO levels after 21 days were 0 7 mg/l and 1 2 mg/l in the iron-free and iron-dosed columns, respectively (Table 5 Appendix 6A) The concentration of Fe^{2+} in the column after 21 days (6 40 mg/l) was substantially higher than the initial concentration of 3 00 mg/l in the water sample fed into the column (Table 6 Appendix 6A) BOD reductions in the columns after 7 days of Experiment F were the highest of all the experiments at this stage with values of 88 6% and 87 1% in the iron-dosed and iron-free columns respectively (Figure 6 5) Subsequent increased BOD reductions after 21 days were to 98 0% and 98 2%, respectively (Figure 6 6) The DO concentration after 21 days was 1 2 mg/l in the iron-dosed column and 1 0 mg/l in the iron-free column The concentration of Fe^{2+} in the column at the end of the experiment was 2 74 mg/l

The initial BOD reduction during the first 7 days of Experiment G was 82 9% in the iron-dosed column and 80 7% in the iron-free column (Figure 6 5) There were further increases in BOD reductions to 92 1% and 92 9% respectively after 21 days (Figure 6 6) The DO in both columns had been completely depleted within the initial 7-day period The concentration of Fe^{2+} in the column at the end of the experiment showed little overall change at 3 25 mg/l

6 3 2 Discussion of trends observed in the different experiments

There are indications from the results of the seven experiments that the presence of Fe^{2+}/Fe^{3+} does not have an adverse effect on BOD reductions in the planted column There was no substantial difference in the average BOD reductions in the iron-free column (62 7±22 8%) and iron-dosed column (61 9±21 1%) after 7 days The overall BOD reductions achieved after 21 days were also almost identical at 86 0±13 6% in the iron-dosed and 86 2±15 6% in the iron-free columns

In the winter months, the average BOD reduction achieved in the iron-dosed column after 7 days was lower (36 6±8 6%) than recorded in the iron-free column

(40 0±22 2%) The overall average BOD reduction after 21 days in colder conditions was however higher (66 8±0 5%) compared with 63 6±4 1% attained in the iron-free column There were also no signs of any hindrance to BOD reductions in the iron-dosed planted column in the summer The average BOD reduction within the first 7 days in the iron-dosed column was 73 9±14 6% compared to 70 7±14 4% achieved in the iron-free column The overall BOD reductions after 21 days in both columns were enhanced to high values of 93 7±4 3% in the iron-dosed column and 95 2±2 1% in the iron-free column which is consistent with the findings of Heal *et al* (2005) Liu *et al* (2004) and Zhang *et al* (1999) all of whom reported that iron had no adverse impact on pollutant uptake in wetland plants In a related study of 44 different wetland plant species by Snowden and Wheeler (1995) there were no reports of any adverse effects on plant growth despite the occurrence of an ochrous precipitate (probably hydrated ferric hydroxide) and a pale yellow solid (possibly ferric phosphate) on the roots of the plants Statistical analyses showed that the presence or absence of iron in the planted columns had no significant effect on BOD removal

6 4 EFFECT OF Fe^{2+}/Fe^{3+} EQUILIBRIUM ON BOD REMOVAL IN SUBSTRATE-ONLY COLUMNS

6 4 1 Trends observed in the different experiments

The initial BOD reduction within the first 7 days of Experiment A was higher in the iron-dosed column (69 0%) compared to the iron-free column (51 4%) (Figure 6 7) There were further reductions to 84 3% and 77 1% respectively after 21 days (Figure 6 8) The DO in both columns was completely utilised after 21 days (Table 5, Appendix 6A) and the final Fe^{2+} concentration in the iron-dosed column was 0 54 mg/l Initial Fe^{2+} concentration was 3 10 mg/l (Table 6 Appendix 6A)

In Experiment B the initial BOD reduction after 7 days was 83 6% in the iron-dosed column and 72 2% in the iron-free column (Figure 6 7) increasing to 87 6% and 90 6% respectively after 21 days (Figure 6 8) The DO levels in both columns after 21 days were zero (Table 5, Appendix 6A) while the Fe^{2+} concentration in the iron-dosed column after the same retention time was 2 10 mg/l an increase from the 0 09 mg/l initially recorded on Day 7

The BOD reduction achieved after 7 days of Experiment C was higher in the iron-dosed column (54 3%) than recorded in the iron-free column (40 7%) (Figure 6 7) This pattern was maintained after 21 days but with only small increases in the reduction levels to 57 9% and 49 2% (Figure 6 8) The DO in both columns was effectively utilised, reaching a consistent concentration of 0 2 mg/l over 21 days

(Table 6 6) The initial Fe^{2+} concentration in the iron-dosed column was 2 40 mg/l after 7 days increasing to 3 90 mg/l by Day 21 (Table 6 Appendix 6A)

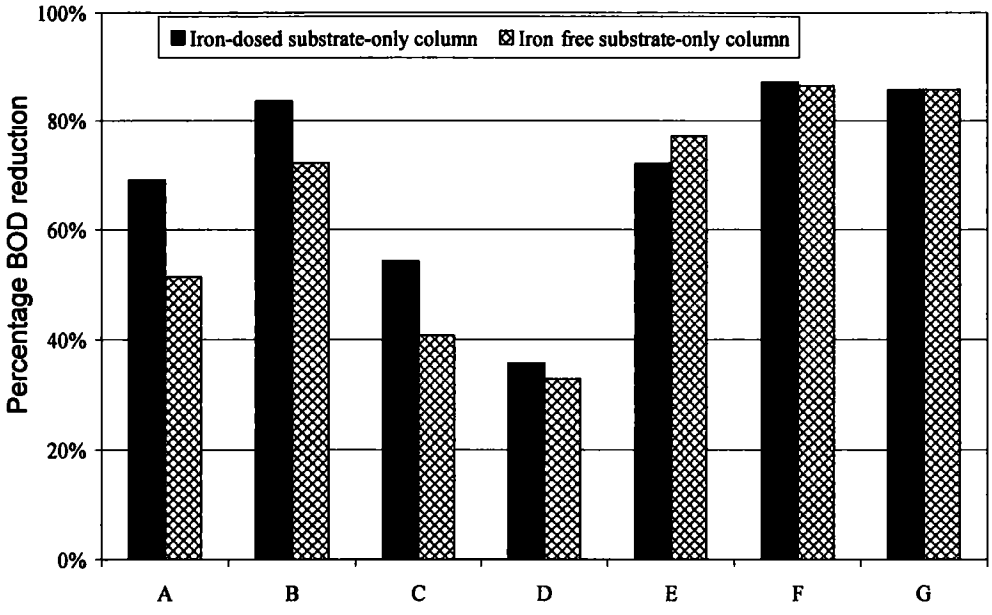


Figure 6 7 Comparison of BOD reductions after 7 days in the iron-dosed and iron-free substrate-only columns at different times of the year

Further declines were recorded in the BOD reductions in both columns within the first 7 days of Experiment D with values of 32 9% in the iron-free column and 35 7% in the iron-dosed column (Figure 6 7) The BOD reductions however increased substantially to 72 0% and 77 9% respectively after 21 days (Figure 6 8) The DO levels in both columns decreased rapidly and anoxic conditions had been established after 7 days The concentration of Fe^{2+} in the iron-dosed column on Day 7 was unusually high (12 60 mg/l) dropping remarkably to 2 00 mg/l after 21 days (Table 7 Appendix 6A)

There was a marked improvement in BOD reductions achieved in both columns within the first 7 days of Experiment E The initial BOD reductions in the iron-free and iron-dosed columns were 77 1% and 72 1% (Figure 6 7) increasing to 90 0% and 90 4%, respectively after 21 days (Figure 6 8) The DO concentrations after 21 days were 0 80 mg/l in the iron-free column and 0 60 mg/l in the iron-dosed column The Fe^{2+} concentration at the base of the iron-dosed column was 9 52 mg/l at the completion of this experiment

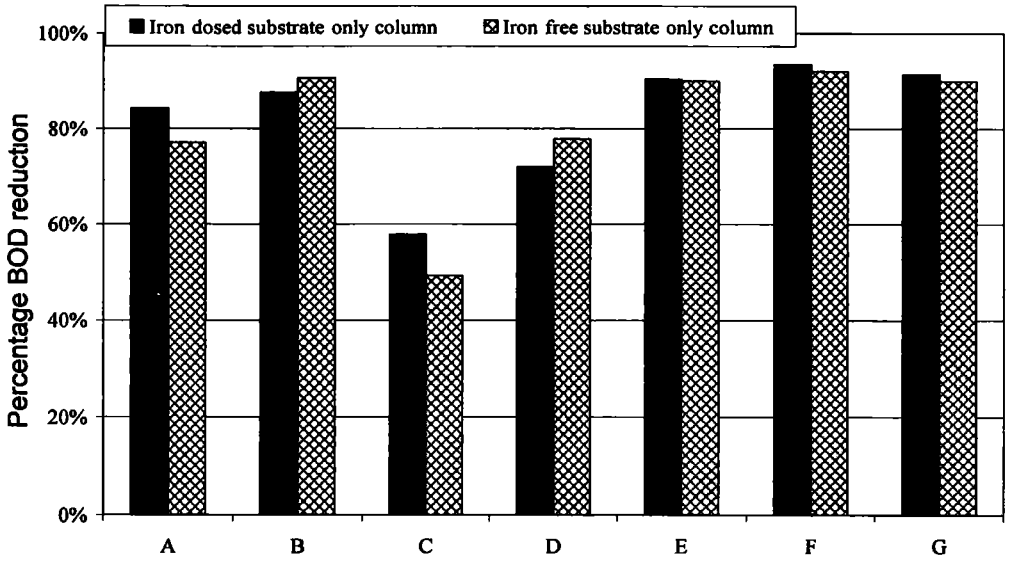


Figure 6 8 Comparison of BOD reductions after 21 days in the iron-dosed and iron-free substrate-only columns at different times of the year

In Experiment F the initial BOD reductions in both columns, within the first 7 days were high at 87 1% and 86 4% in the iron-dosed and iron-free columns respectively (Figure 6 7) The overall BOD reductions after 21 days were 93 4% and 92 0%, respectively (Figure 6 8) The DO levels after 21 days were 1 00 mg/l in the iron-dosed column and 1 30 mg/l in the iron-free column After the same treatment period the Fe^{2+} concentration in the iron-dosed column was 5 38 mg/l

The BOD reduction achieved in both columns after the first 7 days of Experiment G was the same (85 7%) (Figure 6 7) The overall BOD reduction after 21 days was 91 4% in the iron-dosed column and 90 0% in the iron-free column (Figure 6 8) The DO in both columns was completely depleted after 7 days (Table 5, Appendix 6A) The concentration of Fe^{2+} in the column after the same period was 2 15 mg/l (Table 6 Appendix 6A)

6 4 2 Discussion of trends observed in the different experiments

The results obtained from of all seven experiments suggest that the addition of Fe^{2+} and hence the presence of Fe^{2+}/Fe^{3+} equilibrium reactions has no adverse effect on BOD reductions in the substrate-only column The overall average BOD reduction for the iron-dosed substrate-only column in the first 7 days was 69 7±19 0% compared to 63 8±21 9% in the iron-free column There were further increases in the average reductions to 82 4±13 0% and 81 0±15 3% respectively after 21 days A similar trend in favour of the iron-dosed column was observed in the BOD reductions achieved in both columns in the summer and winter seasons The initial average BOD reduction after the first 7 days in the iron-dosed column in

the winter was 45 0±13 1% compared to 36 8±5 6% in the iron-free column Further increases in BOD reductions to 64 9±10 0% and 63 6±20 2% were achieved after 21 days, respectively In the summer the initial average BOD reduction after 7 days in the iron-dosed column was 79 5±8 3% compared to 74 6±14 3 in the iron-free column increasing to 89 4±3 6 and 87 9±6 1%, respectively after 21 days

The BOD reductions achieved in the iron-dosed substrate-only column show that wetland substrates play a significant role in overall BOD reduction The varying concentration of Fe^{2+} observed in all the experiments is an indication that the abiotic reduction/oxidation along with other pollutant removal processes like sedimentation adsorption precipitation volatilisation and microbial activities associated with the wetland systems occur within a substrate-only system The difference in BOD reductions achieved in the iron-dosed planted and substrate-only columns will be discussed later in this chapter Statistical analyses showed that the presence or absence of iron in the column had no significant effect on BOD reduction

6 5 EFFECT OF RETENTION TIMES ON BOD REDUCTIONS IN PLANTED AND SUBSTRATE-ONLY COLUMNS

Enhanced BOD reductions over the 21 day period compared with the 7 day period were recorded in all columns during all of the experiments (Figures 6 9 6 10 6 11 and 6 12) In the nutrient-dosed and nutrient-free planted columns the average BOD reduction increased from 70 75±27 99% and 61 90±24 72% on Day 7 to 84 83±22 05% and 86 19±17 84% on Day 21 respectively (Figure 6 9) A similar trend was observed in the nutrient-dosed and nutrient-free substrate-only columns The BOD reduction increased from 69 54±18 82% and 63 79±25 78% on Day 7 to 85 21±11 03% and 80 98±17 97% on Day 21 in the nutrient-dosed and nutrient-free columns respectively (Figure 6 10) The increases in BOD reductions in the iron-dosed and iron-free planted columns were from 62 67±25 98% and 61 90±24 72% on Day 7 to 85 98±15 98% and 86 19±17 84% on Day 21 respectively (Figure 6 11) The BOD reduction increased from 69 67±21 93% and 63 79±25 78% on Day 7 to 82 43±15 55% and 80 98±17 97% on Day 21 in the iron-dosed and iron-free substrate-only columns (6 12)

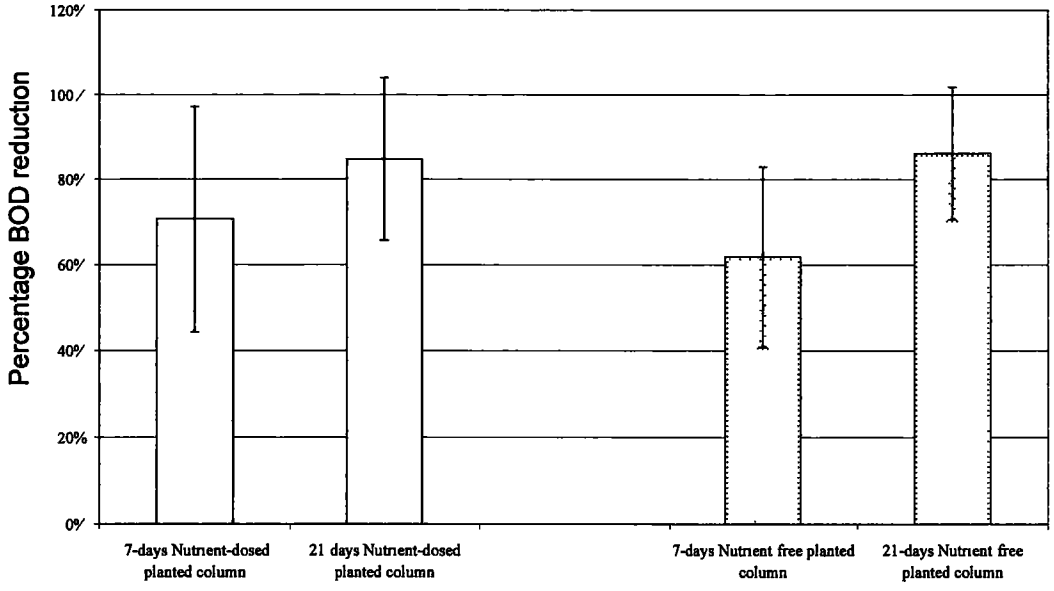


Figure 6 9 The average BOD reductions in nutrient-dosed and nutrient-free planted columns on days 7 and 21

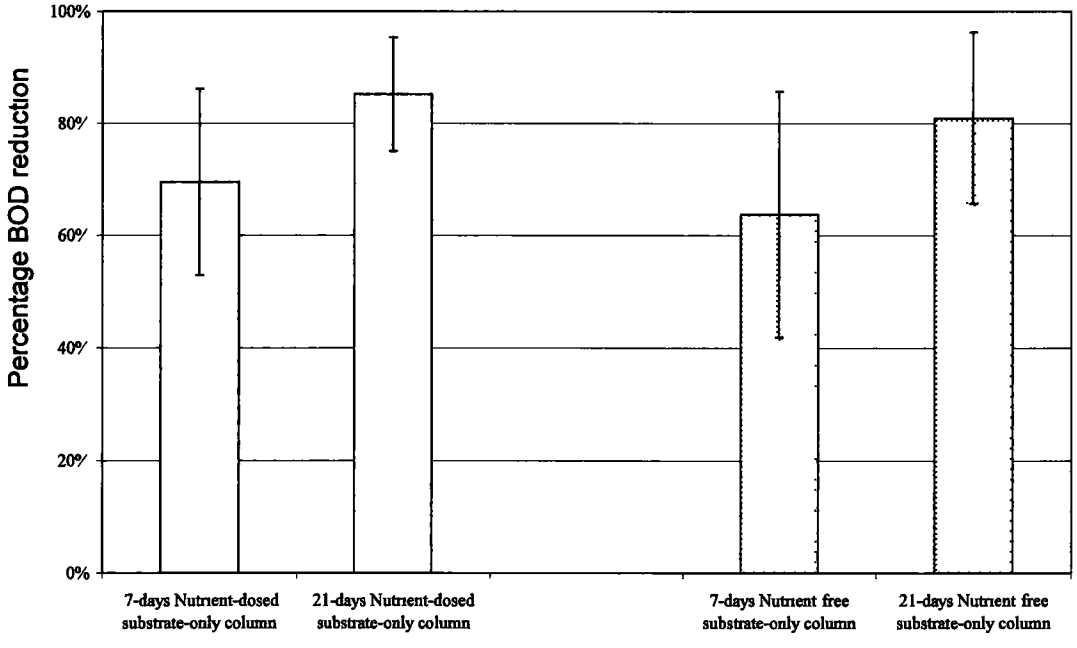


Figure 6 10 The average BOD reductions in nutrient-dosed and nutrient-free substrate-only columns on days 7 and 21

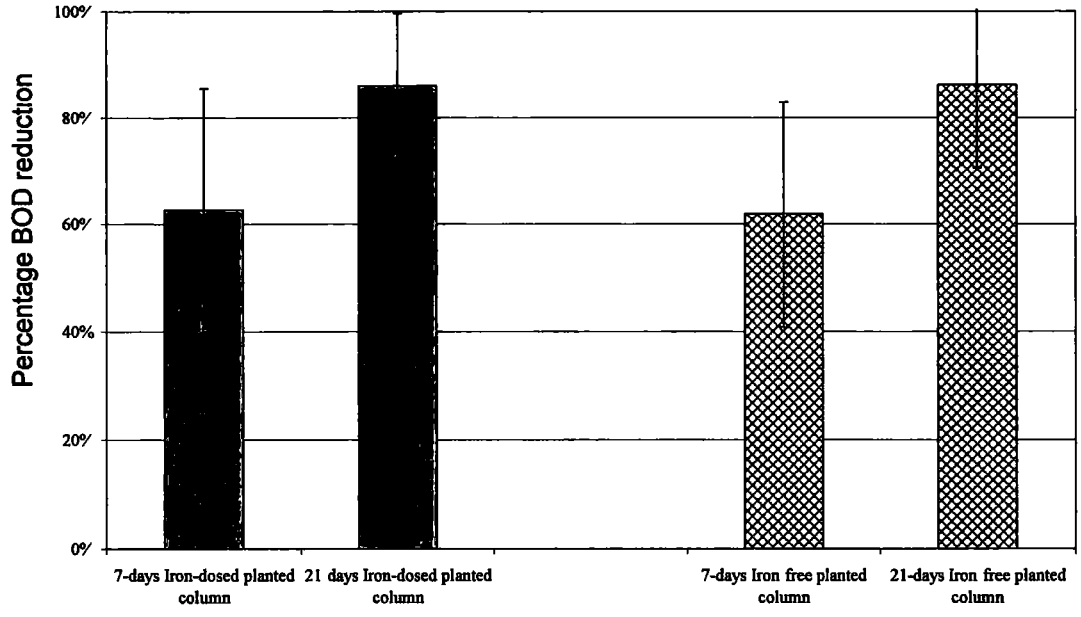


Figure 6 11 The average BOD reductions in iron-dosed and iron-free planted columns on days 7 and 21

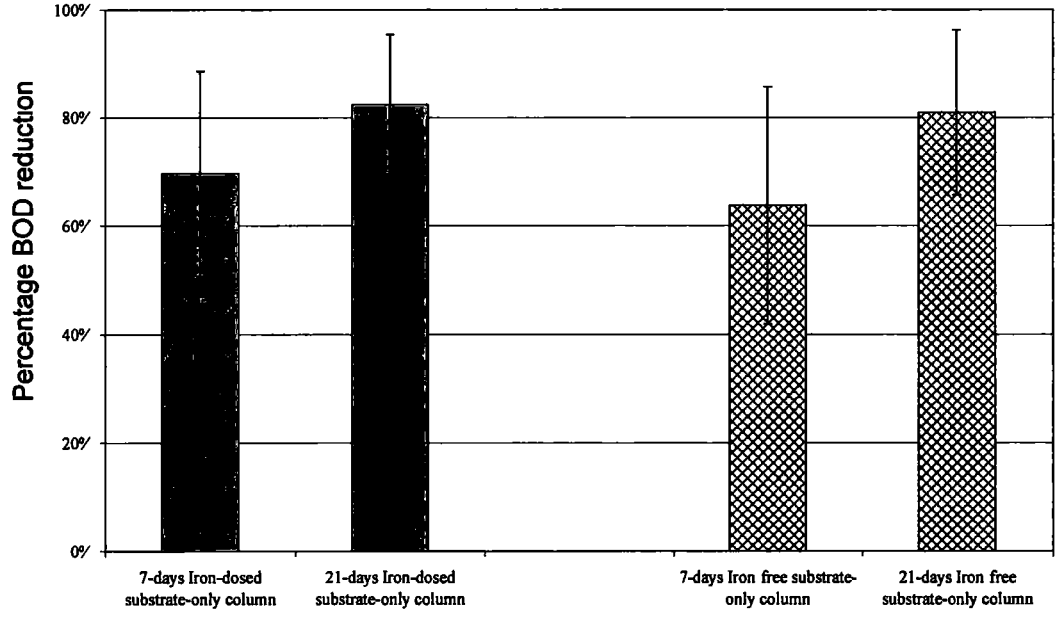


Figure 6 12 The average BOD reductions in iron-dosed and iron-free substrate-only columns on days 7 and 21

Generally, the increase in BOD reduction between Days 7 and 21 was slightly more evident in the planted columns with an average further BOD reduction of 20 6±11 2% The corresponding value achieved in the substrate-only columns over

the same period was 15 2±11 6% The benefit of a longer column retention time becomes even more evident in the winter There were further BOD reductions of 26 4±16 8% and 24 1±19 0% in the 14 day period between Days 7 and 21 of the experiments in the planted and substrate-only columns, respectively The corresponding values for the summer experiments were 18 2±9 7% in the planted columns and 11 6±6 5% in the substrate-only columns In general BOD reductions achieved between Days 7 and 21 were higher in the winter (25 3±6 5%) than in the summer (14 9±7 4%) Statistical analyses showed that retention time had a significant effect on BOD reductions in both the planted and substrate only columns ($p < 0\ 0001$)

6 6 SEASONAL VARIATIONS IN BOD REDUCTIONS IN PLANTED AND SUBSTRATE-ONLY COLUMNS

Of the 7 experiments conducted Experiments A B E F and G were carried out during summer months while the remaining two (Experiments C and D) were conducted during the winter All the columns planted and substrate-only exhibited a typical seasonal trend in BOD reductions with higher reductions in the summer (Figures 6 13 to 6 16) In general the overall average BOD reduction in the columns after 21 days in the summer was higher (91 8±5 1) than achieved in winter (63 9±8 7) The highest BOD reduction recorded in the planted columns in the summer was in the nutrient-dosed planted column 85 2±10 9% within the first 7 days (Figure 6 13) increasing to 95 5±1 4% after 21 days (Figure 6 14) The average BOD reductions in the same column over the winter were 34 6±8 6% (Figure 6 13) after 7 days and 58 2±14 7% (Figure 6 14) after 21 days The best performance in the winter was recorded in the nutrient-free planted column with an average BOD reduction of 40 00±22 22% in the first 7 days (Figure 6 13) increasing to 63 57±4 04% after 21 days (Figure 6 14)

The highest BOD reduction recorded in the substrate-only columns in the summer was in the iron-dosed column with an average BOD reduction of 79 54±8 33% (Figure 6 15) after 7 days increasing to an overall BOD reduction of 89 43±3 55% (Figure 6 16) after 21 days The performance of the column although lower than the summer level was also the best within the first 7 days with an average BOD reduction of 54 29±35 71% (Figure 6 15) The BOD reduction in the column after 21 days was 64 93±10 00% (Figure 6 16)

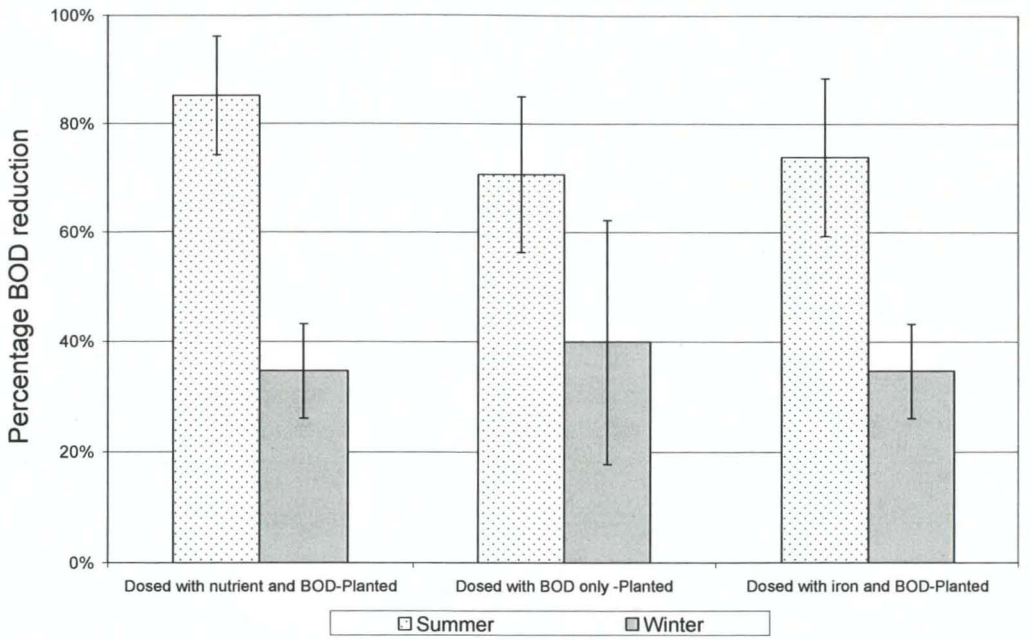


Figure 6.13 The average BOD reduction recorded in the planted columns during the summer and winter after 7 days

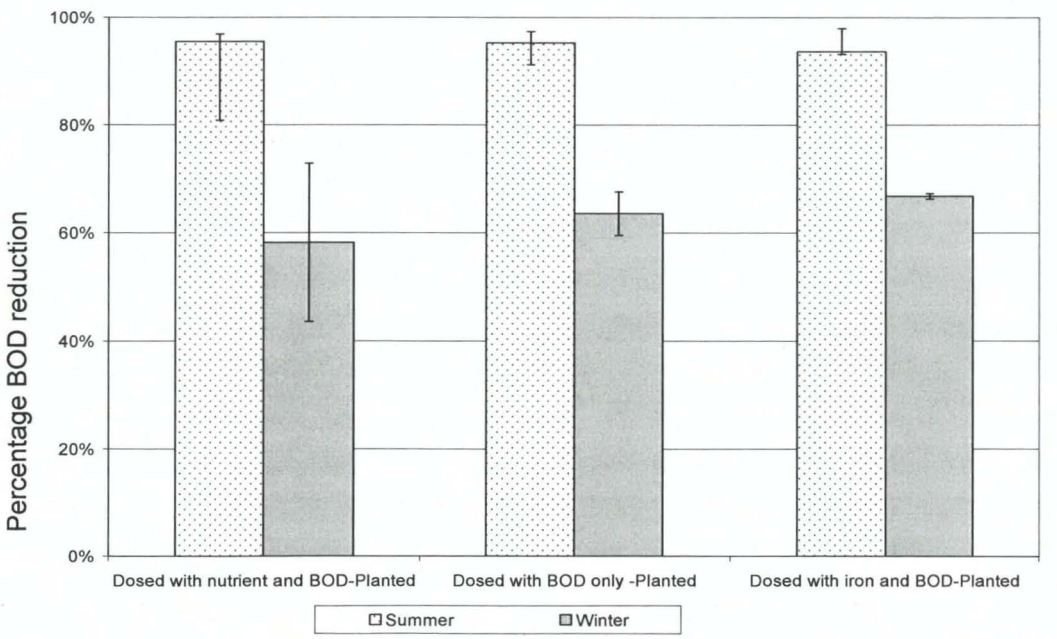


Figure 6.14 The average BOD reduction recorded in the planted columns during the summer and winter after 21 days

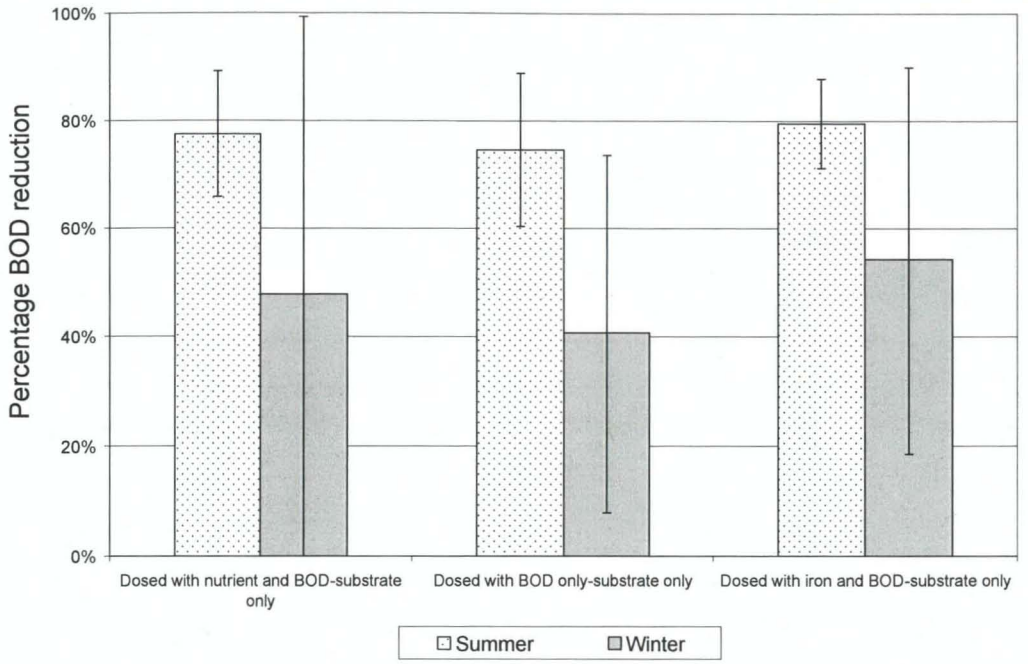


Figure 6.15 The average BOD reduction recorded in the substrate-only columns during the summer and winter after 7 days

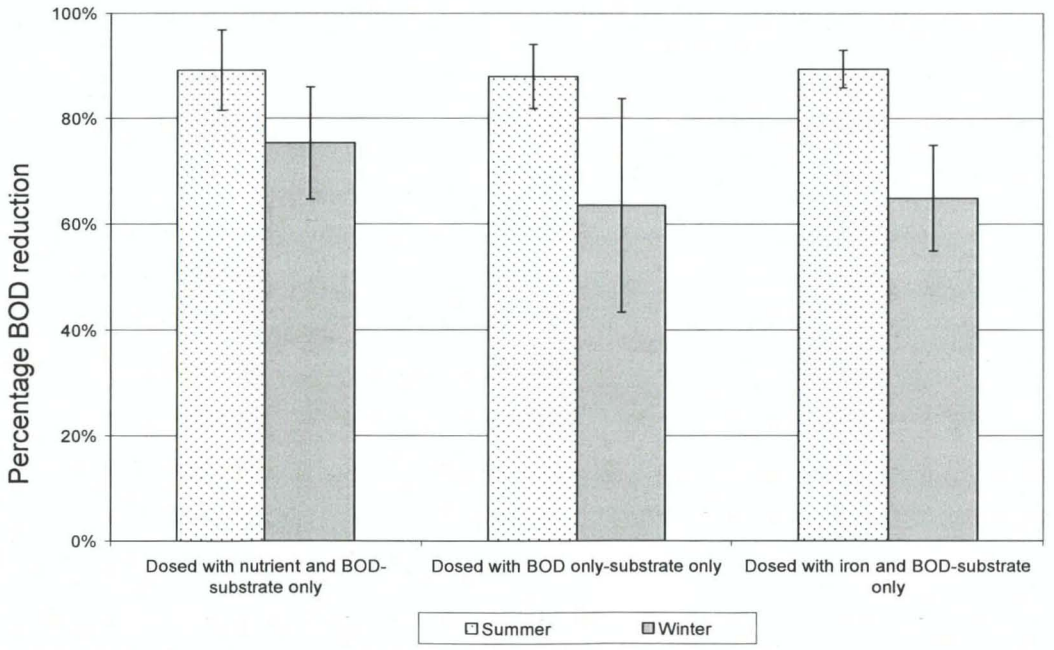


Figure 6.16 The average BOD reduction recorded in the substrate-only columns during the summer and winter after 21 days

In general, the planted columns outperformed the substrate-only columns in the summer with an overall average BOD reduction of 94.8±2.3% after 21 days

compared to the 88 8±5 6% achieved in the substrate-only columns during the same period This is consistent with results from a series of studies conducted by Picard *et al* (2005) on planted and non-planted microcosms It is also supported by findings of Juwarkar *et al* (1995) Zhu and Sikora, (1995) Hunter *et al* (2001) and Fraser *et al* (2004) all of whom reported better nutrient removal in planted microcosms The planted microcosms were more effective in pollutant removal particularly in the summer The effect of the presence of a plant on BOD reductions in a column is discussed in the next section Statistically there was a strong correlation between the BOD reductions recorded in the columns and the temperature ($p < 0\ 0001$)

6 7 EFFECTS OF PLANTING ON BOD REDUCTIONS IN THE DIFFERENT COLUMNS

The results from the 7 conducted experiments do not clearly show any statistically significant effect due to the presence of plants on the BOD reductions in the columns, particularly in the first 7 days The average BOD reductions within the first 7 days in the nutrient-dosed columns were similar 70 8±28 0% (planted) and 69 5±18 8% (substrate-only) rising to 84 8±22 2% and 85 2±11 0%, respectively after 21 days (Figure 6 17) The average BOD reductions within the first 7 days in the iron-dosed columns were actually higher in the substrate-only column at 69 7±21 9% compared to 62 7±26 0% recorded in the planted column (Figure 6 18) The overall BOD reduction after 21 days was however higher in the planted column 86 0±15 5% compared to 82 4±15 6% in the substrate-only column (Figure 6 18) In the nutrient/iron-free column, the average BOD reduction after 7 days was marginally higher in the substrate-only column, 61 9±24 7% compared to 63 8±25 8% recorded in the planted columns (Figure 6 19) This pattern was reversed after 21 days with the overall average BOD reduction being higher in the planted column (86 2±17 8% compared to 81 0±18 0%) (Figure 6 19) These results suggest that a longer column retention time plays a significant role in the overall BOD reduction in a planted column

Although the overall BOD reduction in winter was higher in the substrate-only columns 64 9±10 0% compared to the 62 9±3 7% achieved in the planted columns the possible benefit of the presence of a plant on BOD reduction in a column was highlighted in the summer The overall average BOD reduction after 21 days in the planted column was 94 8±2 25% compared to 88 6±5 6% in the substrate-only column The higher BOD reductions recorded in the planted columns over the summer may be directly due to the rapid plant growth observed during this period

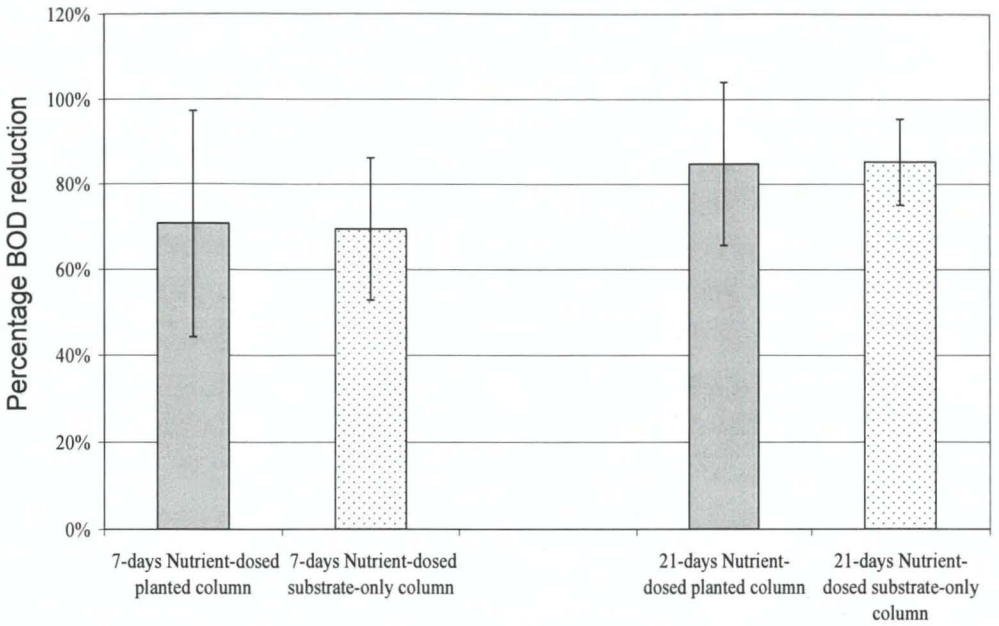


Figure 6.17 Average BOD reductions in nutrient-dosed planted and substrate-only columns on days 7 and 21

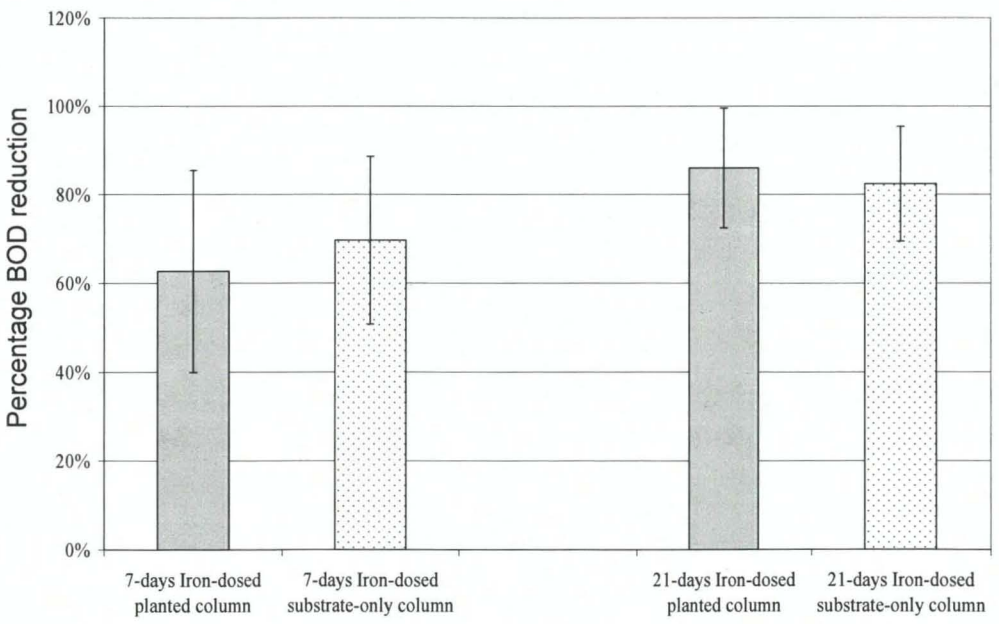


Figure 6.18 Average BOD reductions in iron-dosed planted and substrate-only columns on days 7 and 21

The occurrence of some higher BOD reductions in the substrate-only columns, compared to the planted columns, suggest that processes (e.g. microbial degradation) other than plant uptake play a significant part in BOD reductions in wetlands. Pollutant removal processes like sedimentation, adsorption, precipitation, volatilisation and biological uptake associated with the wetland system involve the

transfer or storage of pollutants from the water component to the fixed component of the wetland system (i.e. wetland substrate, vegetation and biofilms). Of all the main removal mechanisms identified in wetlands systems (details in Chapter 1), plants are only known to be directly associated with two of these mechanisms (plant uptake and microbially-mediated reactions). The majority of the other mechanisms involve fixed surfaces which can be provided by the wetland substrates.

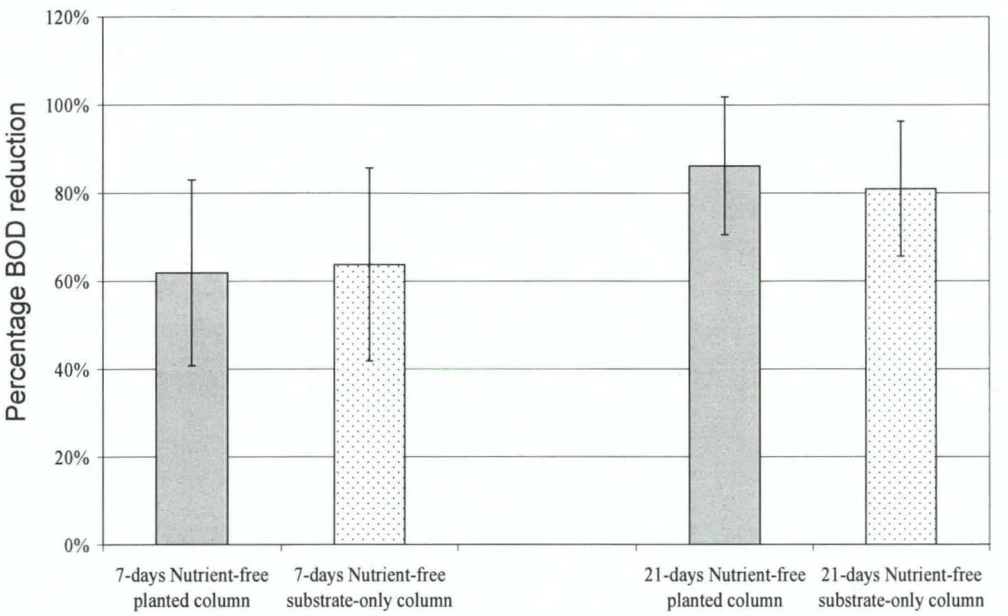


Figure 6.19 Average BOD reductions in nutrient/iron-free planted and substrate-only columns on days 7 and 21

There is a substantial amount of research supporting the claim that microbial processes, which occur within the water column and on surfaces, provide important pathways for pollutant removal in wetlands (Tanner *et al.*, 1995; Tanner *et al.*, 2001; Ottova *et al.*, 1997; Werker *et al.*, 2002). Biofilms, which consist of an interaction of bacteria, algae and microfauna, have been reportedly found not only on plant roots but also on substrate surfaces (Costerton *et al.*, 1995; Costerton and Stewart, 2001, Fujishige *et al.*, 2006). Theoretically, the biodegradation processes which occur within the wetland system are facilitated by the aerobic and anaerobic microorganisms associated with the biofilms. These microorganisms are capable of utilising processes like nitrification (and the subsequent denitrification) to release nitrogen as gas to the atmosphere and the co-precipitation of pollutants like phosphorus with metals such as iron, aluminum and calcium (Bhamidimarri *et al.*, 1991). Statistical analyses showed no strong correlation between the presence of a plant in a column and the BOD reduction.

CHAPTER 7 MICROBIAL ANALYSIS OF ISOLATES FROM THE HEATHROW TREATMENT FACILITY (HTF)

Techniques used for isolation and identification of clinical and environmental microorganisms have historically been based on a combination of phenotypic characteristics including a range of morphological features and biochemical reactions, some of which are time-consuming and imprecise (Bourne *et al.*, 2001). Results from the microbiological experiments conducted on the different microbial isolates from the HTF are discussed in this chapter. Three main nutrient media; tryptic soya agar (TSA) for bacteria, sabouraud's dextrose agar (SDA) for fungi and glycerol yeast extract agar (GYEA) for actinomycetes were used for the isolation and enumeration of the respective microorganisms (see Chapter 2 for the methodology). Two main identification techniques have been used during this study, the API biochemical tests (for preliminary identification) and the Polymerase Chain Reaction (PCR). In the preliminary analysis of water samples from the different components of the Mayfield Farm Reservoir (MFR) and Mayfield Farm Balancing Pond (MFBP), 5 bacterial strains (identified as Strains *1, 2, 3, 4* and *5* in Table 1, Appendix 7A) were cultured and isolated on TSA plates. Later in the study, 13 other strains (identified as Strains *A, B, C, D, E, F, G, H, I, J, K, L* and *M* in Table 1, Appendix 7A) were cultured and isolated from the roots of plants in the SSF on TSA plates.

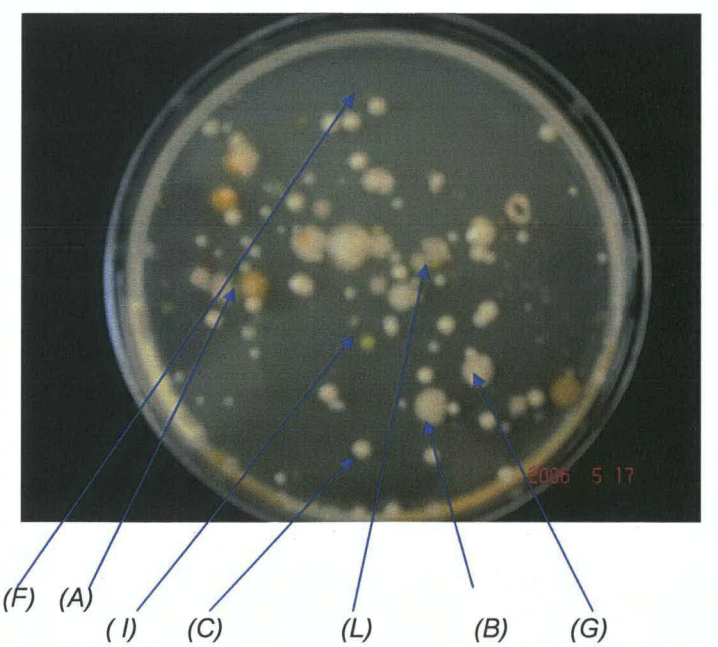


Figure 7.1 Some of the bacteria isolates from the roots and sediments of the SSF on a TSA plate. The letters refer to some of the different bacteria strains.

During further tests conducted to assess the biodegradation potential of microorganisms from the roots of plants from the SSF, SDA and GYEA plates were also used. In contrast to the aerated pond waters, 3 fungal strains and 2 actinomycetes strains were isolated from the plant roots from the SSF (identified as Strains *F1, F2, F3, A1* and *A2*, respectively in Table 1, Appendix 7A). A third strain (*F/A*), with similar characteristics to a fungus was also observed on the GYEA plates. Pictures showing the appearance of these colonies on the respective plates are illustrated in the figures that follow.

Figure 7.2 A TSA plate showing the domination by the brown colonies of bacteria isolate *1* from a MFR water sample

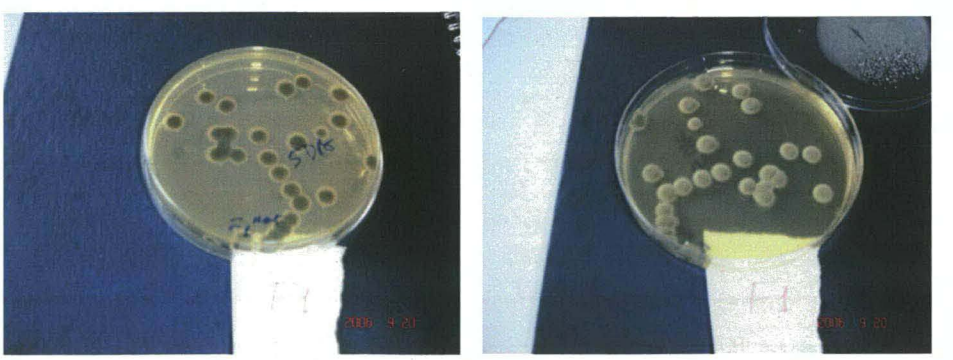


Figure 7.3 Jet black reversed side (left) and the top side of pure colonies of a fungi isolate (F1) on SDA plates

Figure 7.4 Pure colonies of more fungi isolates (F1, F2 and F3) on SDA plates

Figure 7.5 Mixed cultures of fungi isolates (F1, F2, F3 and F/A) found in water sample collected from vigorous washing of the plant roots on SDA plates

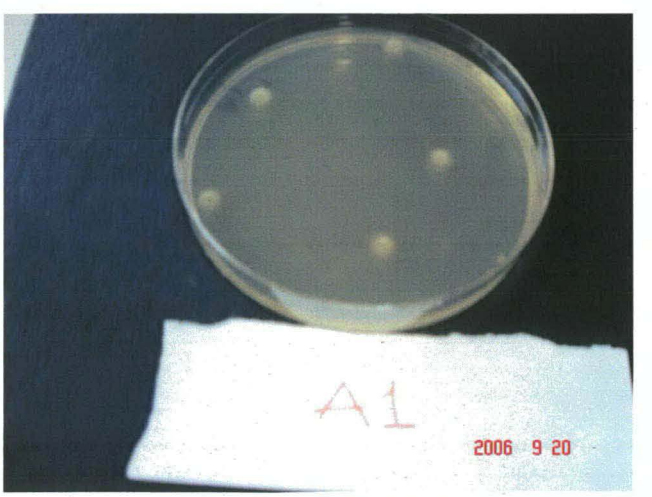


Figure 7.6 Pure colonies of actinomycetes isolate (A1) on a GYEA plate

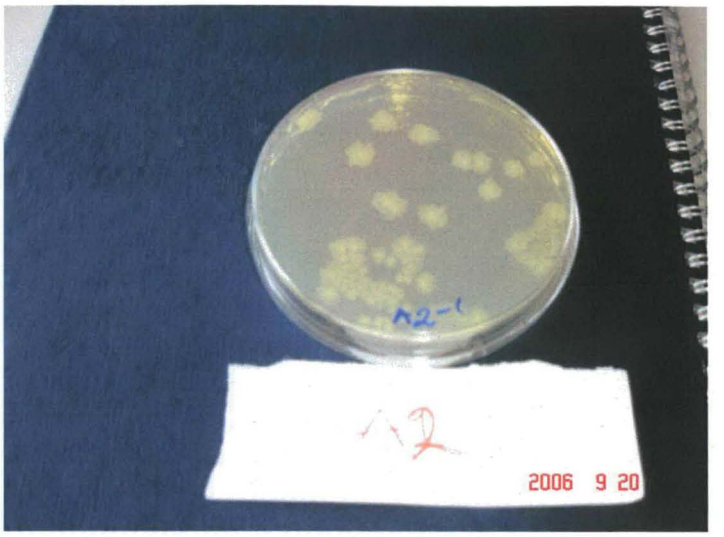


Figure 7.7 Pure colonies of actinomycetes isolate (A2) on GYEA plate

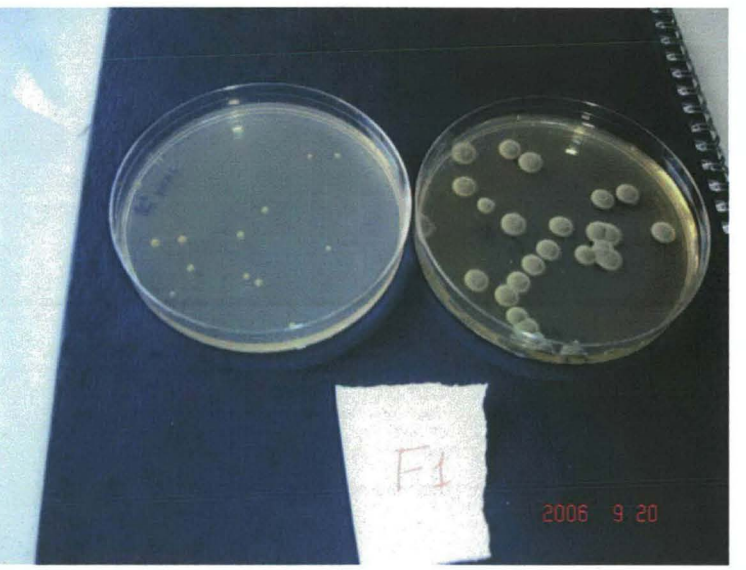


Figure 7.8 Pure isolates of a third isolate (F/A) on a SDA plate (left) and a GYEA plate (right)

7.1 THE API-BIOCHEMICAL TESTS

Prior to the detection of microorganisms suspected to be fungi and actinomycetes in water samples collected from washing the roots of plants from the SSF, API biochemical tests were conducted on all the pure bacteria isolates grown on TSA plates. The results from a selection of API biochemical tests kits (API20E, APIStaph, APIStrep and ID32Staph) are summarised in the Table 2, Appendix 7A and an example presented in Figure 7.9.

Figure 7.9 Examples of the API biochemical test columns used for the preliminary identification of the bacteria isolates

7.1.1 Discussion of the results of the morphological tests and API biochemical tests

- **Gram staining**

The Gram-staining test is mainly used to separate the bacterial cells into two broad groups (Gram-positive or Gram-negative) based on the chemical and physical properties of their cell walls. A black-blue or purple colour indicates that the test organism is Gram-positive while a pink colour is an indication that the organism is Gram-negative. Results from the Gram staining tests indicate that all but one of the initial 5 bacteria strains isolated from the MFR and MFBP were Gram-positive, the exception being Strain *4*. Of the other 13 bacteria isolates from the roots of plants from

the SSF and the ER only 3 strains *D F and J* were Gram-positive (Table 2 Appendix 7A) The result of test conducted on strain *I* was inconclusive

- **Catalase production**

The production of catalase by test bacteria is an indication of their ability to convert hydrogen peroxide (H_2O_2) to hydrogen and water This ability is possessed by most aerobic and facultative bacteria excluding the *Streptococci sp* (Bergey Manual 1994) Strains *1 3, A, B, C, H I, J, L* and *M* all tested positive to catalase production while isolates *2, D E F G* and *K* gave negative results (Table 2 Appendix 7A)

- **Beta-galactosidase production**

Microorganisms that produce the enzyme beta-galactosidase have the ability to hydrolyse beta-galactosides, a disaccharide into monosaccharides (Bergey Manual 1994) Strains *2, A B, H I J K, L* and *M* all tested positive for the possession of this enzyme (Table 2, Appendix 7A)

- **Arginine dihydrolase production**

Arginine dihydrolase is the enzyme responsible for the hydrolysis of arginine a naturally occuring amino acid which plays an important role in cell division, into smaller protein units The possession of the enzyme is also believed to prolong motility in bacteria under anaerobic conditions Arginine is originally synthesized from citrulline (a protein in the urea cycle) by the sequential conversion of two enzymes, arginninosuccinate and arginninosuccinate lyase The possession of the enzyme arginine dihydrolase has proven to be valuable for differentiating members within the *Enterobacteriaceae* family and also aerobic *Pseudomonas* from other Gram-negative *Bacilli* (Bergey Manual, 1994) Only four of the Strains *1 2 3* and *5* tested positive for the possession of this enzyme (Table 2 Appendix 7A)

- **Lysine decarboxylase production**

Lysine decarboxylase is the enzyme responsible for the decarboxylation of lysine which is one of the 20 amino acids usually found in protein molecules Along with arginine and histidine, lysine is classed as a basic amino acid essential for cell growth (Bergey Manual, 1994) All the tested strains apart from Strain *1* gave a negative response to this test Results of tests on Strains *2 3* and *4* were inconclusive (Table 2 Appendix 7A)

- **Ornithine decarboxylase production**

The decarboxylation of ornithine is catalysed by the enzyme ornithine decarboxylase producing diamine putrescine, a polyamine compound required for cell division All the strains apart from *1 2* and *3* tested negative for the possession of this enzyme (Table 2, Appendix 7A)

- **Citrate utilisation**

This test basically assesses the ability of certain bacteria to utilise citrate as a sole carbon source for energy (citrate is one of the metabolites in the Krebs cycle) The test in itself is used as a main form of identification for *Enterobacteriaceae* It is composed of citrate anion as the sole carbon source and ammonium phosphate as the sole nitrogen source Microorganisms capable of utilising citrate can also extract nitrogen from the ammonium salt forming an alkaline by-product to give a positive result for the test (Bergey Manual 1994) Strains *1* and *2* showed evidence of citrate utilisation while isolates *A-M* gave negative responses to the test (Table 2 Appendix 7A)

- **Hydrogen sulphide (H_2S) production**

The production of H_2S by certain organisms is a demonstration of their ability to liberate sulphur from sulphur-containing compounds as an energy source (Bergey Manual, 1994) None of the isolates showed any evidence of this ability (Table 2, Appendix 7A)

- **Production of urease**

The production of the enzyme urease is a characteristic common to many species of microorganisms although some produce the enzyme more rapidly than others (Bergey Manual 1994) The enzyme is required to hydrolyse amides to ammonia and carbon dioxide forming alkaline ammonium carbonate in solution Only Strains *1 3 4 5* and *L* gave positive results to the test (Table 2 Appendix 7A)

- **Indole production/ Tryptophan production**

Production of indole (one of the metabolic degradation products of amino acids) is an indication of the presence of the enzyme tryptophanase This enzyme is capable of hydrolysing and deaminating trytophan to produce indole, pyruvic acid and ammonia The test is particularly useful in separating *Enterobacter* (for which the test is negative) from *E coli* (for which the test is positive) (Koneman *et al* 1997) All the isolates apart from one (Strain *2*) tested negative for indole production although this isolate and Strain *1* both tested positive for tryptophan production (Table 2, Appendix 7A)

- **Acetoin conversion to di-acetyl**

The conversion of acetoin to di-acetyl in the presence of potassium hydroxide and atmospheric oxygen is the underlying reaction in the Voges-Proskauer test On addition of α-naphthol and creatine, the formation of a red complex implies a positive result The formation of acetoin is an alternative pathway in the metabolism of pyruvic acid and is an ability demonstrated by some *Enterobacteriaceae* (Koneman *et al* 1997) This group of bacteria yield acetoin as the chief and final product of glucose fermentation Apart from Strains *3* and *4*, all the other bacteria isolates from the aerated ponds and the roots demonstrated the ability to convert acetoin to diacetyl (Table 2, Appendix 7A)

- **Gelatinase production**

Gelatinase is an extracellular enzyme which allows the test organisms to hydrolise gelatin into smaller polypeptides peptides and amino acids These are then assimilated into the cell membrane and utilised by the organism These tests check if the bacteria strain possesses the different enzymes required for the acidification of carbon sources with varying complexities (Koneman *et al* 1997) Strains *1 2 A C F G H I* and *M* all tested positive for gelatinase production (Table 2 Appendix 7A)

- **Fermentation of Glucose, Arabinose, Melibiose, Sucrose and Amygdalin**

The oxidation of simple sugars by non-fermenting bacteria produces weak acids which are usually unable to cause a sufficient drop in pH to initiate a colour change in the medium (Bergey Manual 1994) There was evidence of glucose fermentation in systems inoculated with Strains *1 2 3* and *5* All the isolates apart from *1, 3 4* and *5* tested negative for melibiose utilisation Isolates *1 2* and *D* tested positive for the utilisation of arabinose while Strains *2 3 5 L* and *M* were able to utilise sucrose Only isolates *2* and *M* showed signs of fermentation in amygdalin a glycoside which is a form of polysaccharide media (Table 2 Appendix 7A)

- **Acidification of Maltose, Lactose, Trehalose and Fructose**

In the acidification tests for maltose lactose, trehalose and fructose acidification tests Strains *3* and *4* gave negative results for all three tests while Strains *1* and *2* gave positive results for both the maltose and lactose tests and negative results for the trehalose tests with Strain *5* giving a positive result for the trehalose test Only Strains *3* and *5* tested positive for fructose (Table 2 Appendix 7A) The acidifications tests were not included in the test kits used for the remaining isolates

- **Utilisation of alcohols and polyols (Mannitol, Inositol, Xylitol and Sorbitol)**

These tests assess the potential of test organisms to mineralise alcohols to simpler forms producing carbon dioxide and water (Bergey Manual 1994) Strains *2 4, 5 A L* and *M* all gave a positive response to mannitol while none gave a positive result to inositol and sorbitol (Table 2, Appendix 7A) Of the four isolates tested with xylitol Strains *4* and *5* gave positive results while Strains *1* and *2* were negative

- **Utilisation of naturally occurring sugars (Rhamnose and Mannose)**

In these tests the microorganisms' ability to degrade rhamnose and mannose both naturally-occurring sugars were assessed All the isolates tested negative with rhamnose with Strain *1* recording the only positive result (Table 2, Appendix 7A) Strains *1 2* and *3* also gave positive results with mannose

- **Oxidase production**

The oxidase test is very useful in screening isolates suspected of being *Enterobacteriaceae* which would give a negative response, and identifying those suspected of belonging to other genera like *Pseudomonas Aeromonas Campylobacter* and *Pasteurella* all of which will give a positive result (Bergey Manual 1994) The presence of the oxidase enzyme in organisms allows them to supply cytochromes (iron-containing hemoproteins) which act as the last link in the chain of aerobic respiration, forming water as electrons are transferred to the oxygen molecules (Bergey Manual 1994) This process occurs in aerobic microaerophilic and facultative organisms Strains *1 2 B C D, E, G H J K* and *M* all gave positive results while A F H and L tested negative to oxidase production (Table 2 Appendix 7A)

- **Alkaline phosphatase production**

Alkaline phosphatase is the enzyme responsible for the removal of phosphate from the 3- and 5- positions in molecules such as nucleotides proteins and alkaloids located outside the cell and are resistant to inactivation denaturing and degradation (Bergey Manual, 1994) Phosphate uptake by bacteria is facilitated by the production of these enzymes particularly during phosphate starvation although some mutant *E coli* lacking the enzyme survive well as do mutants unable to shut off production The presence of phosphate groups usually prevents the passage of organic molecules through the cell membrane (Koneman *et al* 1997) Of the all isolates tested only Strain *3* tested positive for the possession of the enzyme alkaline phosphatase (Table 2 Appendix 7A)

7 1 2 Interpretation of the API biochemical tests results

The profiles generated from duplicate results of tests conducted on all the isolates were compared with the profiles of known organisms using the different API databases available A summary of the preliminary identifications made are summarised below

- Using the analytical profile index (available with each test kit) Strains *A* and *I* had profiles which closely matched *Sphingomonas paucimobilis* a Gram-negative bacteria which forms yellow colonies on TSA plates This group of bacteria were formerly known as *Pseudomonas paucimobilis* which are enteric in nature although some have been known to be associated with plant roots (Southern and Kutscher 1981)

- Strains *B C D E F G J* and *K* all had similar profiles and were all found to be closely related to *Pseudomonas sp* (API20E profile index)

- Based on the profiles generated as a result of the tests conducted on Strain *H*, it was initially identified as *Sphingobacterium multivorum*, a Gram-negative bacillus which has been reportedly found in aquatic environments (Kampfer *et al.*, 2005).

- Strain *L* had a profile which closely matched those exhibited by *Enterococcus agglomerans* (API20E Ref 20 190, 1994)

- The profile generated by Strains *M, 3, 4* and *5* could not be matched with any of the organisms in the entire range of API databases.

- Results from the API database suggested that the profiles of Strains *1* and *2* match those of a *Staphylococcus xylosus* and *Enterococcus faecium*. Results of further presumptive tests conducted showed that both strains had the ability to grow on sheep blood agar with rapid growth recorded by Strain *1* within 24 hours (Figure 7.10). This test is based on the ability of bacterial colonies grown on agar plates to break down red blood cells in the culture.

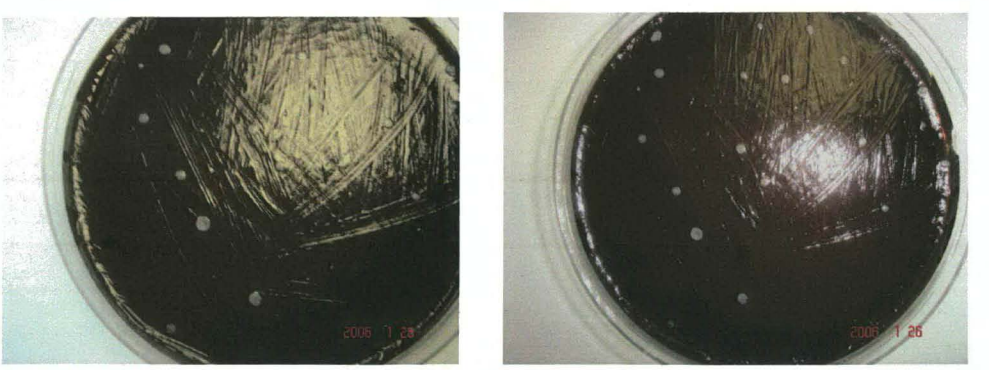


Figure 7.10 Sheep blood agar plates showing distinct colonies formed by Strains *1* (left) and *2* (right)

Based on all the results obtained so far, a summary of all the respective presumptive identification test results are outlined and compared with those of known species in the tables that follow.

7.1.3 Deductions from results obtained

Although a comparison of the profiles generated by some of the bacteria with those of known species show some similarities in key characteristics, the degree of variability exhibited by some of the isolates highlights the challenges of using the API biochemical tests as a means of conclusive identification. Based on the results of the API biochemical tests and the other morphological tests conducted, most of the

bacteria isolates fall into three main genuses (genera) *Staphylococcus Enterococcus* and *Pseudomonas* The growth of Strains *1* and *2* on sheep blood agar is an indication that both strains are closely related to *Staphylococcus* and *Enterococcus* genus Apart from being Gram-positive, the appearance of both strains on TSA plates as single colonies pairs clusters and chains agrees with the description given for *Staphylococcus* and *Enterococcus* species in the Bergey Manual (1994) The formation of distinctive colonies of Strain *1* within 24 hours on sheep blood agar is consistent with the characteristics of *Staphylococcus* species (Rowlinson *et al* 2006) The positive response of Strains *1* and 3 to the catalase and glucose fermentation tests (Table 3 Appendix 7A) is another characteristic common to *Staphylococcus* species (Koneman *et al* 1997) Of the five strains isolated from the aerated pond Strain *1* appears to be the only isolate with characteristics more closely related to *Staphylococcus* species None of the five isolates had key characteristics that were consistently related to *Enterococcus* species (Table 4 Appendix 7A)

The profiles of most of the isolates from the roots of plants from the SSF closely match the general profile of *Pseudomonas sp* reported in the Bergey Manual (1994) *Pseudomonas sp* are generally Gram-negative non-fermenters that test positive to the cytochrome oxidase reaction (Koneman *et al* 1997) All the 13 strains apart from *D F* and *J* were Gram-negative and all apart from *C* and *L* are non-fermenters giving negative results in most of the fermentation tests although there were occasional positive responses recorded (Table 2 Appendix 7A) None of the 13 strains showed any ability to ferment inositol and rhamnose (Strain *2* had a similar response) Strains *L* and *M* showed evidence of fermentation in sucrose mannitol and glucose media As expected for *Pseudomonas sp* all the isolates apart from Strains *A F, I* and *L* tested negative to the oxidase cytochrome experiment

Another distinguishing characteristic of non-fermenting *Pseudomonas sp* is the weak decarboxylation activity they undergo following inoculation and incubation at 35°C (Bergey Manual 1994) All the 13 isolates from the roots showed no evidence of decarboxylation in the lysine and ornithine decarboxylase test (Table 5, Appendix 7A) The positive responses of Strains *1 2 3* and *4* with respect to the possession of arginine decarboxylase is on the other hand a characteristic consistent with *Enterobacteriaceae* (Koneman et al 1997) The 100% negative responses of all the 13 isolates to the indole production test is further prove of their association with non-fermenting *Pseudomonas sp* known to be weak producers of indole (Bergey Manual 1994)

The API biochemical tests mainly depend on the production of metabolites by the test organisms and as such are prone to errors from factors such as the innoculum density incubation time and contamination which could all influence the outcome of the tests

(Tillotson *et al* 1988) The API 20E test strips have been reportedly used to identify bacteria species such as *Pseudomonas sp* with 37% accuracy (Popovic *et al* 2004) In another study Cunha *et al* (2004) reported 2 2% 25% 37 5% and 47 1% inaccurate identification for *Staphylococcus epidermidis*, *Staphylococcus hominis* *Staphylococcus haemolyticus* and *Staphylococcus warneri* using the APIStaph test strip Hauschild and Schwarz (2003) recorded almost 100% accuracy using the ID32Staph test kits for identification of *Staphylococcus sciuri* strains in rodents and insectivores Tillotston *et al* (1988) reported good consistency in a study using the APIStrep identification system with three supplementary tests (catalase production, urease production and nitrate reduction) for the identification of clinical isolates Although the API biochemical tests used in this study assisted in narrowing down the identification of some of the test species there is still a need to confirm the identities of these isolates using a more accurate system that will compensate for the inadequacies of the API technique

7 2 POLYMERASE CHAIN REACTIONS (PCR)

Although the genotypic nature of the PCR technique has made it more reliable for species identification taxonomical complexity within some groups of organisms has made accurate identification a challenging task Despite this it has been successfully used by many researchers in the identification of a wide range of microorganisms Martineau *et al* (2001) employed the PCR technique for the successful identification of *staphylococcus* at the genus level in clinical and environmental samples Tsuchizaki and Hotta (2000) used PCR for rapid amplification of DNA from microbial colonies of *Staphylococcus* and *Enterococcus species* A host of *pseudomonas species* were also identified in soil samples by Kuske *et al* (1998) Shepard and Gilmore (1999), in a study conducted on aerobically and anaerobically induced genes identified *Enterococcus faecalis* in environments related to commensal or environmental colonisation and infection sites Ke *et al* (1999) studied clinical enterococci isolates using the PCR technique and efficiently identified all 159 isolates tested

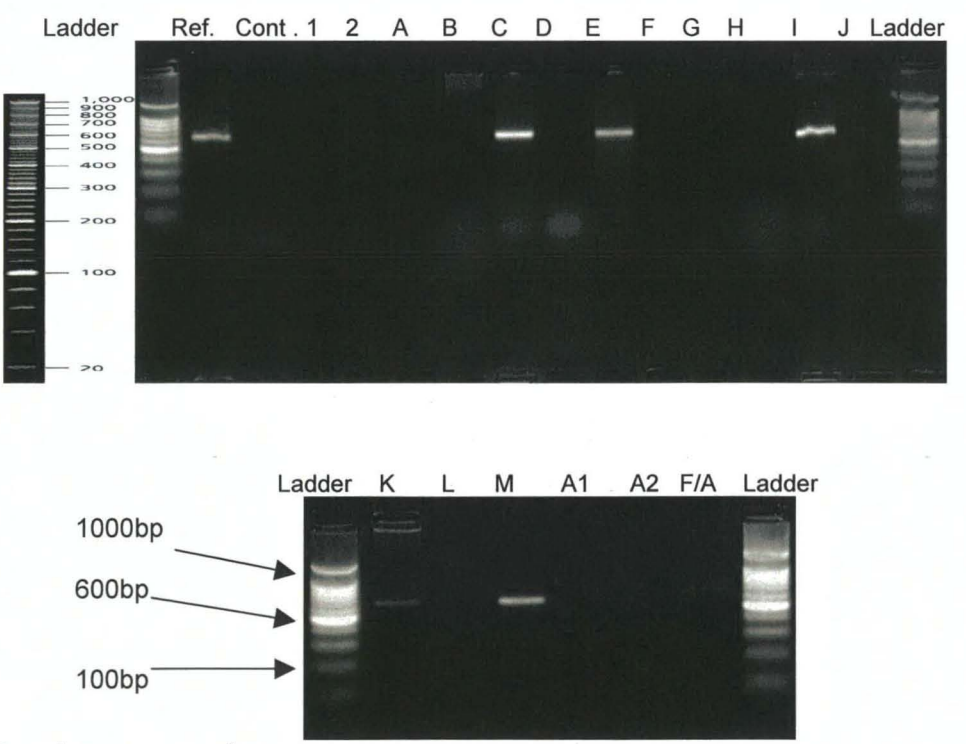
Based on the results of the API identification tests initially carried out PCR-based assays were used to test the different isolates at their respective genus levels *Staphylococcus Enterococcus Actinomycetes and Pseudomonas species* PCR-based assays developed by Martineau *et al* (2000) Ke *et al* (1999) Thirup *et al* (2000) and Spilker *et al* (2004) respectively were used to target complimentary DNA sequences in Strains 1 2 A-M and three additional Strains (A1 A2 and F/A) cultured on GYEA plates Strains 3 4 and 5 were not included in the PCR analyses because all three lost their culturability on laboratory media at the time the PCR tests commenced Using the standard colony PCR technique (see Chapter 2 for details) the

photographic UV images of the agarose gels for each of the targeted DNA sequences in the isolates were obtained and these are discussed in the following section.

7.2.1 Discussion of results

PCR analysis using *Pseudomonas* genus-specific primers

The pair of *Pseudomonas* genus-specific forward and reverse primers used in this analysis were designed based on the alignment of 16S rDNA sequences available in the GenBank. They are intended to target and amplify the DNA sequences *GACGGGTGAGTAATGCCTA* and *CACTGGTGTTCCTTCCTATA* in test isolates belonging to the *Pseudomonas* genus (Spilker *et al.*, 2004). Results from the PCR assays employing these primers produced DNA bands of the predicted size (618 bp) in the lanes containing the reference strain (*Pseudomonas aeruginosa*) and five other isolates, namely *C, E, I, K* and *M*, although the photographic image for *K* is very faint (Figure 7.11). None of the other isolates tested produced any DNA bands even though the results from the API biochemical tests conducted on these isolates suggested that most were either *Pseudomonas sp.* or closely related species. The DNA band produced in the lane containing isolate *M* is a positive identification of a strain with no matching biochemical profile in the API database. Isolates *1* and *2*, the only surviving strains from the aerated ponds also showed no signs of DNA products for this analysis.

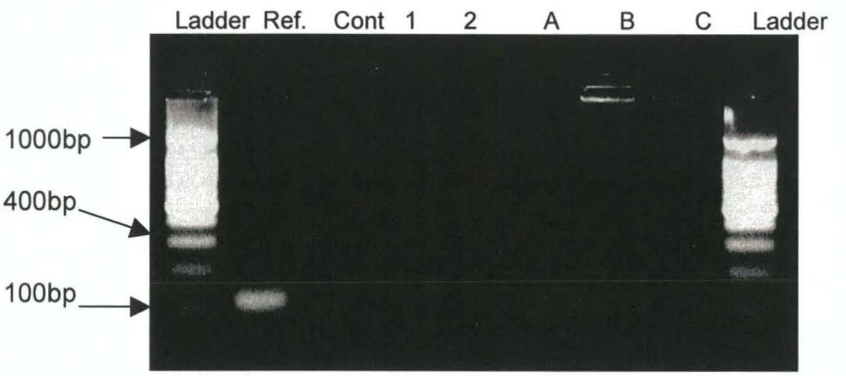


Ref: *Pseudomonas aeruginosa*

Figure 7.11 PCR analysis of the isolates using the *Pseudomonas* genus-specific primers

PCR analysis using *Enterococcus* genus-specific primers

Apart from the DNA band (around the suggested size of 112 bp) observed in the lane containing the reference strain (*Enterococcus faecalis*), no comparable DNA bands were observed for any of the other isolates. The pair of *Enterococcus* genus-specific forward and reverse primers used, *TACTGACAAACCATTCATGATG* and *AACTTCGTCACCAACGCGAAC* which were designed to target the elongation factor Tu gene (*tuf gene*) at genus levels (Ke *et al.,* 1999) failed to form amplicons even with Strains *2* and *L*, which were initially thought to be *Enterococcus faecium* and *Enterococcus agglomerans*, respectively (Figure 7.12).



Ref: *Enterococcus faecalis*

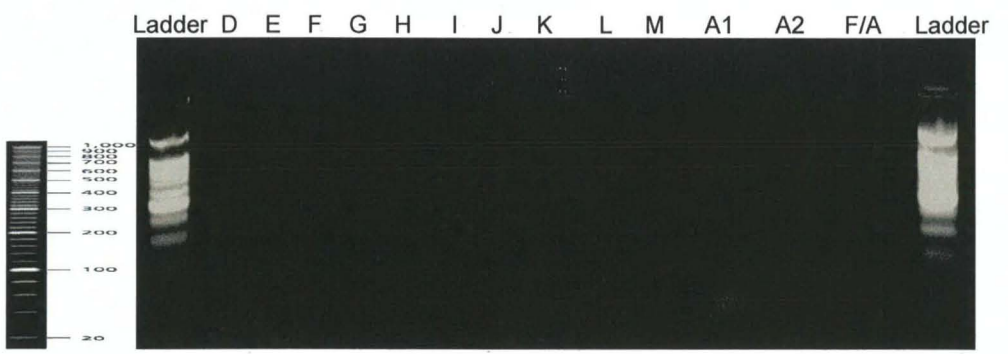
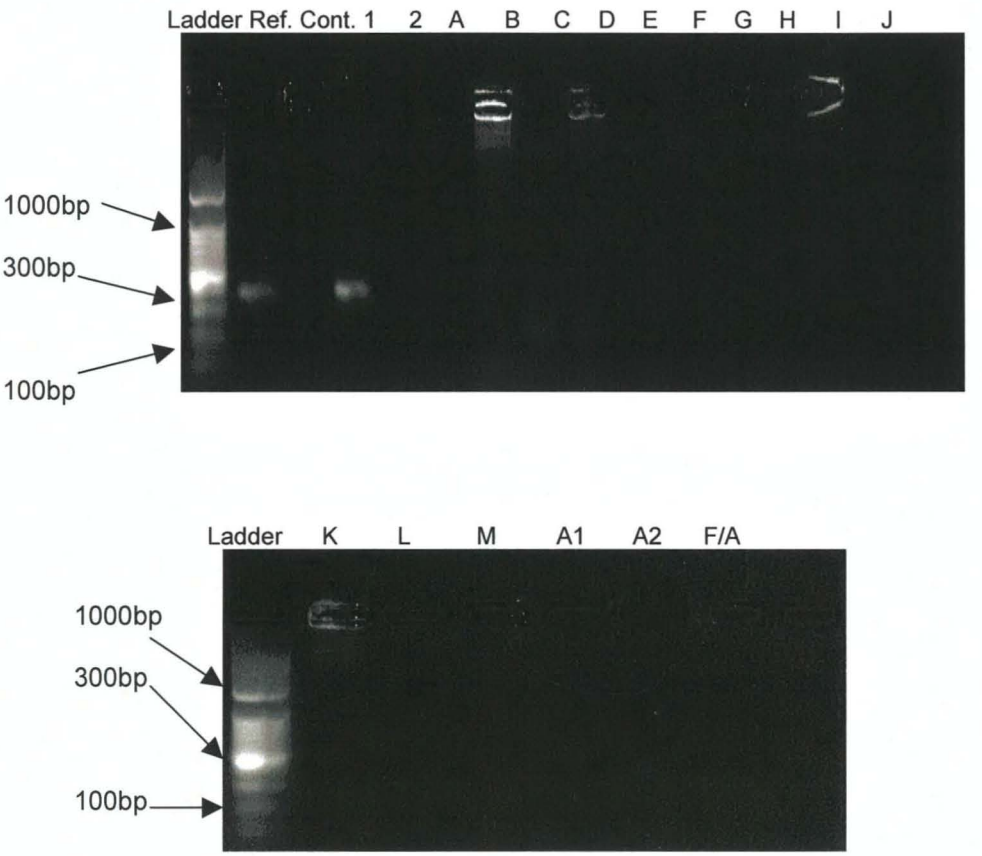


Figure 7.12 PCR analysis of the isolates using the *Enterococcus* genus specific primers

PCR analysis using *Staphylococcus* genus-specific primers

The only DNA bands formed with the *Staphylococcus* genus-specific primers *GGCCGTGTTGAACGTGGTCAAATCA* and *TIACCATTTCAGTACCTTCTGGTAA* (apart from the one observed in the lane containing DNA from *Staphylococcus aureus,*

the reference strain used as a positive control) was found in the lane containing DNA from Strain *1* (Figure 7.13). The size of the DNA produced (370 bp) was consistent with that produced by the reference strain used (Figure 7.13). The other suspected *Staphylococcus* strain (Strain 2) formed no comparable DNA bands.



Ref: *Staphylococcus aureus*

Figure 7.13 PCR analysis of the isolates using the *Staphylococcus* genus-specific primers

PCR analysis using *Actinomycetes* genus-specific primers

Strains *B*, *D* and *I* formed DNA bands with the *Actinomycetes* genus-specific primers *GGATGAGCCCGCGGCCTA* and *CCGCGGCTGCTGGCACGTA*. There are also indications of the formation of several DNA bands in the lane containing isolate *K* (Figure 7.14). The molecular sizes of the DNA bands formed by the reference strain (*Streptomyces*) and isolates *B* and *I* are all close to 255 bp as suggested by the designers of the primers (Thirup *et al.*, 2000). The products from strains *D* and *K* are clearly different in molecular size from that formed by the reference strain (Figure 7.14).

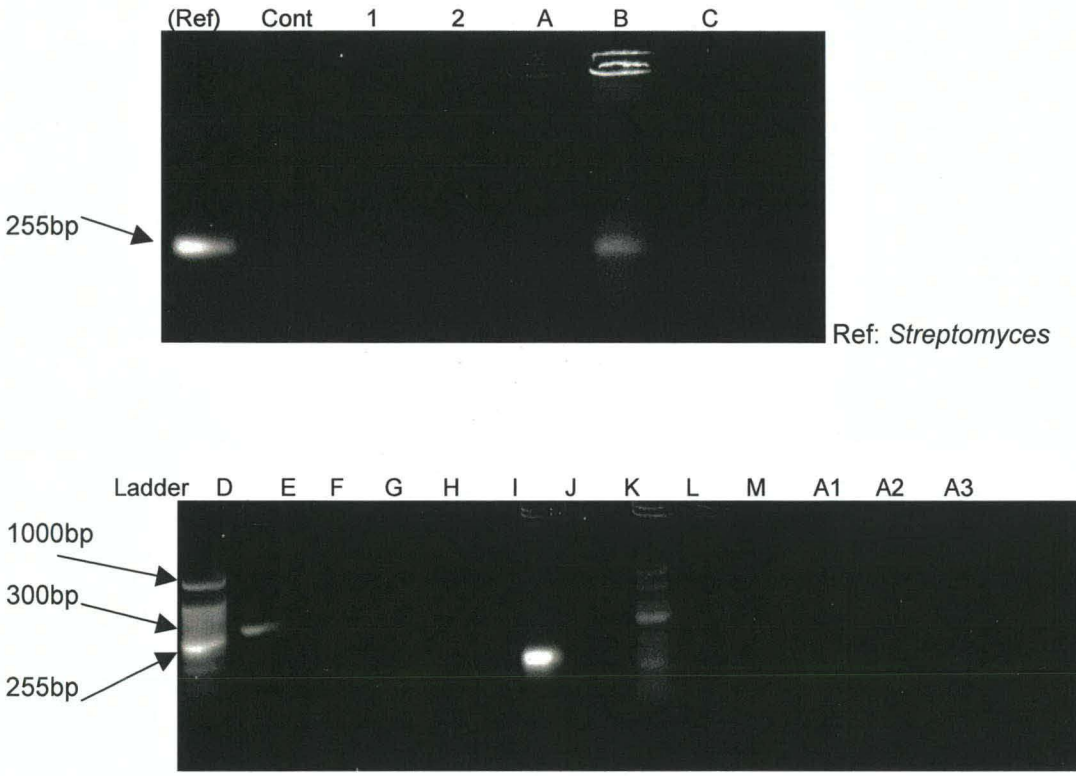


Figure 7.14 PCR analysis of the isolates using the *Actinomycetes* genus-specific primers

7.2.2 Deductions from the PCR results observed

The results of the PCR analysis indicate that the specificity of each of the genus-specific universal primers used is accurate, forming appropriate DNA bands in the lanes containing DNA from the reference strains in each case (Figures 7.11-7.14). Unfortunately, the degree of specificity has been limited to genus level in this study and as such no definitive specie identification was made using this technique. The analysis conducted using the *Pseudomonas* genus-specific primers was the most successful with six positive identifications up to genus level (Figure 7.11). The specificity and sensitivity of these primers have been successfully tested with 42 culture collections of *Pseudomonas* strains, including *Pseudomonas putida*, *Pseudomonas flourescens*, *Pseudomonas stutzeri* and *Pseudomonas syringae*, all of which are common environmental species (Spilker *et al.,* 2004). Although the results of API biochemical tests are generally regarded as not being as reliable as the PCR technique, five of the six strains identified by PCR technique were positively identified as *Pseudomonas sp.* (C = *Pseudomonas sp*, E = *Pseudomonas sp*, *I* = *Pseudomonas paucimobilis*, K = *Pseudomonas sp*) using the API20E strip tests.

The failure of the *Enterococcus* genus-specific primers to produce DNA bands with any of the test isolates (Figure 7 12) despite the results of the API biochemical tests is an indication of the possible mis-identification by the API system The primers used in this study had been successfully used for the amplification of 159 *Enterococcus* species including the reference strain (*Enterococcus faecalis*) and the supposed Strain *2* (*Enterococcus faecium*) However many of these were clinical isolates (Ke *et al* 1999) The results obtained do not preclude the occurrence of *Enterococcus* species in the tested systems of roots and aerated pond water Johnston and Jaykus (2004) have isolated a number of enteric species from environmental sources such as soil surface water plants and animal products There are claims that the intrinsic nature of these species allows them to develop antibiotic resistance by the transfer of plasmids and chromosomal exchanges This often causes mutation which, in itself, presents a significant challenge for successful identification using PCR techniques (Johnston and Jaykus 2004)

The formation of a DNA bands by Strain *1* in the presence of *Staphylococcus* genus-specific primers (Figure 7 13) is consistent with the initial identification made using the API biochemical tests Strain *1* was initially identified as *Staphylococcus xylosus* a Gram positive cocci usually found in the environment and on animal skins It is a biochemically highly active organism which produces a wide variety of acids with carbohydrates (Koneman *et al*, 1997) The specificity of the primers used for the detection of this specie has been tested using 27 known *Staphylococcus* species (Martineau *et al* 2001) Like the Enterococcus primers they were designed to target the elongation factor Tu gene (*tuf gene*) at genus levels Results from the study showed that the PCR primers complementary to the defined region could amplify specifically and efficiently DNA fragments of all the 27 isolates tested The sequence was further verified using all 27 American Type Culture Collection (ATCC) *Staphylococcal* reference strains and 307 clinical *Staphylococci* isolates from the Quebec City region (Martineau et al 2001)

The anomalies observed in the formation of DNA bands with the actinomycetes primers (Figure 7 14), particularly for Strains *I* and *K* can be explained by the specificity of the pair of primers used The forward primer (*GGATGAGCCCGCGGCCTA*) is reported to be specific in targeting *Actinomycetes* while the reverse primer (*CCGCGGCTGCTGGCACGTA*) is less specific and as such targets other species as well (Thirup *et al* 2001) It is therefore not surprising that Strain *I* which had been originally identified as belonging to the *Pseudomonas* genus formed DNA bands with these primers Thirup *et al* (2000) reported no mismatches using these primers with *Streptomyces* (the reference strain used) as has been demonstrated in this study However he claimed there was at least one mismatch

within the *Actinomycetes genera* It is still not clear why Strain *K* appears to have several DNA bands along the lane (Figure 7 14)

7 3 IMPLICATION OF IDENTIFICATION RESULTS

It has been suggested that the applicability of the API strip test systems for the identification of microorganisms is successful only at genus levels with further morphological and physiological tests required for identification at specie level (Juang and Morgan, 2001) Results from this study support this claim particularly in the analysis conducted on the isolates from the root system The reliability of the PCR technique has been proven in many arenas for the detection of specific bacteria from human pathogens in food to microorganisms from the environment (Yamamoto and Harayama 1995) suggesting that the results of the PCR analyses in this study are likely to be more reliable in identifying the types of microorganisms present in the different analysed components of the HTF

The detection of *Pseudomonas* species within the root systems of the SSF is consistent with reports of these species in environments associated with root systems by Yeung *et al* (1989) Troxler *et al* (1997), Gleba *et al* (1999), Thirup *et al* (2001) and Walker *et al* (2004) In another study, Liste and Felgentreu (2006) associated the high degradative root activities with what was regarded as a particularly large population of rhizosphere bacteria' including *Pseudomonas* species (*Pseudomonas putida Pseudomonas flourescens Pseudomonas stutzeri and Pseudomonas syringae*) along with other known hydrocarbon degraders The profile of the Gram-negative rod shape isolates found in soil which readily degraded high concentrations of glycol as reported by Strong-Gunderson *et al* (1995) matches that of a *Pseudomonas specie* The rapid BOD reduction recorded in water samples associated with the root systems in particular plant root washing is an indication of the presence of these species

Pseudomonas species are claimed to be generally located in biofilms (Costerton *et al* 1995) which are highly structured surface-attached communities of cells encased within a self-produced extra cellular polymeric matrix which remains intact and withstands microbiocidal attacks (Walker *et al*, 2004) The nature of biofilms formed by these species offers an explanation for the rapid BOD reduction recorded in autoclaved water samples collected from washings of the roots of plants from the SSF (Chapter 5) Dakora and Phillip (2001) claim that plant associated microbial populations such as *pseudomonas sp* which resides in or near the roots system also utilise root exudates as mediators of mineral acquisition This not only serves as a source of carbon for microbial growth but also contain chemicals that promote the movement of soil microbes to the rhizosphere hence the detection of other species like actinomycetes and fungi in the rhizosphere This is highlighted by the isolation of

fungi and actinomycetes as well as 13 bacteria strains from the roots compared to the two strains that were predominantly found in the aerated ponds

Although the total number of microbial species (13 bacteria strains, 3 fungi strains and 2 actinomycetes strains) isolated from the SSF plant roots are higher compared to the 2 bacteria strains predominantly found in the aerated pond systems the observed range and variation is below that expected Vymazal *et al* (2001) reported the occurrence of over 70 species of bacteria amoebae, ciliates rotifers, cyanobacteria and algae in a constructed wetland system with 45 species cultured on plates There are however claims that considerably more microbes exist than can be cultured on laboratory prepared media (Staley and Konopka 1978 Bottomley and Maggard 1989 Kell 1998 Oliver 2005) Some of these microorganisms referred to as viable but nonculturable (VBNC) though viable in the indigenous environments are culture-specific and as such are nonculturable through conventional laboratory techniques (Szewzyk *et al*, 2006) This phenomenon has been reported for several Gram-negative bacteria found in surface runoff and wastewater (Xu *et al* 1982 Colwell *et al* 1985 Roszak and Colwell 1987) Szewzyk *et al* (2006) also reported the same occurrence in indicator bacteria found in wetland systems This perhaps offers an explanation as to why the rapid BOD reduction repeatedly recorded in water samples associated with roots of plants from the SSF could not be repeated in sterile water samples containing a different mixed population of the bacteria fungi and actinomycetes isolated from the same systems (see details in Chapter 5) The average BOD reductions after 7-9 days in sterile water samples containing all isolates with and without sterile sediments were 0 0% and 19 0% compared to 71 0% recorded in the root washings from which they were isolated (Chapter 5) While resolving the concept of VBNC may remain a daunting task, there are possibilities that the use of a variety of different nutrient media would yield a more diverse microbial population Chazarenc *et al* (2006) in a study of the microbial communities in constructed wetlands reported different trends in biomass growth in the 31 different carbon sources used Adoption of a similar technique could lead to the isolation and identification of other microbes in the consortium which are responsible for the efficient BOD removal that has been observed in some of the systems

CHAPTER 8 CONCLUSIONS AND RECOMMENDATION

The following conclusions have been made from the results of laboratory based studies conducted using water samples collected from the HTF and from observation of the events recorded during various sites visits between January 2004 and April 2006 For the purpose of this thesis the conclusions have been divided into sub-headings highlighting the key scientific and project specific findings of this study

8 1 THE PERFORMANCE OF THE HEATHROW TREATMENT FACILITY (HTF)

The results from the monitoring the performance of the Heathrow treatment facility (HTF) show that despite high biomass populations recorded in the aerated reservoirs the BOD reductions recorded during the winter months were below the designed levels However the increasing temperature during the early spring contributes to the BOD reduction recorded in the system (55 0±27 4% in April 2004 65 1±17 0% in April/May 2005 and 64 3±2 1% in April 2006 as the temperatures increased from typical winter values of less than 10°C to 13 3°C 15 3°C and 15 5°C respectively) This offers an operational benefit for the operators of the HTF as holding back the treatment of the polluted water collected over the winter till early spring would save cost of energy used for the aeration of the reservoir in the winter

Although the results of a pilot scale study of the sub-surface flow reedbed (SSF) demonstrated the potential of the system for effective BOD reduction prolonged exposure to high organic loadings from the aerated reservoirs particularly in the winter places a demand on the DO concentration which jeopardises its ability to perform efficiently The critically low DO levels recorded within the SSF channels during the winter months show that the performance of the SSF is hampered by its exposure to high organic loads This also shows that the microbial population present in the system rely significantly on the availability of dissolved oxygen for efficient reduction of BOD in the system

Typical BOD removal observed in the aerated reservoirs (MFR and MFBP) in the HTF between January 2004 and April 2006 although not as effective as expected is consistent with observations at the Dane County Regional Airport where delays of up to three months following de-icing activities have been noted for any significant BOD reduction to occur (Gallagher 1998) Significant BOD reductions are generally recorded towards the month of April when the water temperature becomes warm enough to support continuous microbial activities Gallagher further demonstrated the

impact of increasing temperature on BOD removal in a pilot scale study in which a dual-tank bioreactor was used for the treatment of water collected from the de-icing pads of the Dane County Regional Airport The BOD level in the treatment system was found to drop from over 1000 mg/l to less than 50 mg/l over a period 3 days when the operating temperature was maintained at 30°C (Gallagher 1998)

The findings of over 3 years monitoring period of the HTF shows that the system has the potential to effectively remove high BOD from incoming polluted runoff there is however a need for some operational modifications in order to maximise this potential The rapid depletion of the DO in the front-end of the SSF coupled with the rapid BOD reduction recorded in water samples associated with the roots of plants from the SSF is perhaps the most obvious proof of the existence of the microbial population capable of achieving the desired performance The improved BOD reduction as the water temperature increases is a clear indication of positive response of this microbial population to the changes in the system It however remains a challenge for the operators of the HTF to hold up the polluted water in the system till the temperature rises in April especially as the combined capacity of the aerated reservoirs (64 000 m^3) at the Mayfield Farm is inadequate to hold the total volume of stormwater generated during the winter On the other hand the high-energy cost that will be associated with the operation of a heating system to increase the water temperature to levels observed in the spring and summer makes this an impracticable option

8 2 THE EFFECT OF AN Fe^{2+}/Fe^{3+} EQUILIBRIUM ON THE PERFORMANCE OF THE HTF

Although the source of the ochrous coloration caused by the presence of elevated levels of Fe^{3+} in the system could not be ascertained in this study the occurrence was found to coincide with the commencement of water re circulation around the system during the winter months The results of biodegradation experiments conducted using varying concentrations of Fe^{3+} precipitate (Section 4 5) also showed that a higher concentration (10 mg/l) reduces the DO depletion rate more significantly than a lower Fe^{3+} concentration (3 mg/l) (Section 4 4) There are however no indications from this study that the typical Fe^{3+} levels (approximately 3 0 mg/l) observed during the different winter events at the HTF was sufficient to adversely affect the performance of the entire system The results from the pilot scale study of the SSF further showed that the BOD reductions of 61 9±21 1% and 69 7±19 0% achieved after 7 days in the iron-dosed planted and substrate-only columns respectively, were within the same range

with the levels attained in the iron-free planted (62 7±22 8%) and substrate-only (63 8±21 9%) columns

Based on the results of experiments conducted and the observations during site visits to the HTF there is evidence of rapid oxidation of Fe^{2+} to Fe^{3+} to give the associated reddish brown coloration (ochre) for different levels of Fe^{2+} Visual observations of the laboratory experiments have shown that the intensity of this coloration is dependent on the concentration of Fe^{3+} in the system Laboratory studies have also shown that the prevailing redox conditions in the system play a key role in the Fe^{2+}/Fe^{3+} equilibrium with evidence of a connection between the reduction process and the disappearance of the reddish brown coloration

From the operational point however this study has clearly shown that recirculation of the polluted water during the winter creates an unfavorable aesthetic challenge with the presence of the ochrous coloration along the walls of the reservoirs and on the roots of the plants in the SSF The precipitation of this ochre when the dissolved oxygen in the system falls creates another problem accumulating at the bottom of the reservoir and then coming back to the surface of the waters when aerations commence in the reservoirs Overall this study has also shown that recirculation has no operational benefit as there were no signs of any significant reductions in the BOD concentration in the system during these periods

8 3 THE IMPACT OF NUTRIENT ADDITION ON THE PERFORMANCE OF THE HTF

The benefits of nutrient addition to the system were demonstrated by bacteria population increases in the aerated ponds from an average background level of 10^7 to 10^{10} CFU per litre following the commencement of the nutrient dosing regime in November 2004 However the inconsistency in operational techniques at the HTF makes it difficult to ascertain if the improved BOD reduction recorded (increasing from 25 5% in March 2004 to 47 5% in February 2005) was a direct result of the increased nutrient levels Although there were also some inconsistencies recorded in the BOD reductions observed in the biodegradation experiments conducted using aerated pond water samples dosed with nutrients (up to 90 0% BOD reductions observed within 5 days in the first sets of experiments and then taking between 14-20 days to record any marked BOD reductions in later experiments) the benefits of nutrient addition were demonstrated repeatedly in all the experiments by increases in background biomass populations from around 10^7 CFU per litre to 10^9 CFU per litre within the first 7 days

The benefit of nutrient dosing was further highlighted in the pilot study of the SSF with the BOD reduction increasing from 61 9±21 1% to 70 8±26 5% on addition of nutrient Although no marked improvements in bacteria populations were observed microbial analyses of the plant roots of the SSF showed that the biomass population in the SSF was significantly different from that in the aerated reservoirs The difference in BOD reductions recorded in these systems is indicative of the varying nature of the dominant microbial population present in each

The positive impact of nutrient addition indicated by the increase in bacteria population and improved BOD reduction in the pilot scale studies clearly shows that a potential nutrient source capable of improving the performance of the system was identified Although the overall desired result was not attained the results from both field and laboratory studies prove that the microbial population in the HTF responded positively to the nutrient addition regime The inability of these increases to be accompanied by efficient BOD reduction in the reservoir as in some of the simulated systems in particular those associated with the roots of plants of the SSF suggests that phosphate and nitrate are only part of the synergy required by the microbial population in the reservoir to attain the desired level of reduction The outstanding performance of the simulated systems containing root exudates further demonstrates this Based on this evidence it is key for the operators of HTF to incorporate the nutrient-dosing regime as part of the operation of the system looking for other complimentary nutrient sources that could improve the performance of the system

8 4 THE ABILITY OF THE DIFFERENT MICROORGANISMS, ISOLATED FROM THE HTF, TO REMOVE BOD

Although the applicability of the API strip test in this study was limited to the identification of the isolated microorganisms at genus levels the genotypic nature of the PCR technique (which makes it a more reliable technique) was successfully used to confirm the presence of one *Staphylococcus* and six *Pseudomonas* species in the HTF water samples The presence of these species however few in number and the isolation of fungus and actinomycetes species in the water samples collected from the wetland section of the HTF is a clear indication of the possibility of the existence of a diverse microbial population in the entire system The low number of microbial population isolated in this study when compared to the 70 species reported by Vymazal *et al* (2001) in a constructed wetland system further demonstrates the limitation of the use of laboratory-cultured media for the isolation of microorganisms

from water samples as claimed by Staley and Konopka (1978) Bottomley and Maggard (1989) Kell (1998) and Oliver (2005)

There is evidence from this study of the existence of a high potential for BOD removal in the system particularly in the wetland section of the system as demonstrated repeatedly in water samples associated with the wetland plants The inability of the individual isolates from these plants and the different laboratory-synthesised consortia used to effectively reduce the BOD present in the replicated water samples further highlights the limitation of the use of laboratory-prepared media for the isolation of the diverse microbial population that could be present in system It also suggests that the isolates used during this study merely constitute a part of the entire consortium responsible for the high BOD reductions recorded in the natural systems they were detached from The increase in BOD reduction from 23 8% to 88 7% within 5 days by the same consortium following the addition of root exudates from the SSF suggests the existence of a microbial/plant synergy in the reedbed which is absent in the aerated reservoirs This supports Dakora and Phillip (2001) claim that plant associated microbial populations such as *pseudomonas sp* which reside near the roots system use additional media such as root exudates for mineral acquisition and other metabolic activities

For the operators of the HTF these findings indicate that a considerable portion of the BOD concentration in the system is more likely to be removed in the wetland section of the system

8 5 RECOMMENDATIONS FOR MODIFICATIONS TO THE HTF AND FOR FURTHER RESEARCH

Based on the results of this study there is evidence that an integrated system of aerated reservoir and wetlands such as the HTF offers a potential for the effective treatment of glycol-laden airport runoff There are however a number recommendations and modifications required in order for such a system to operate effectively particularly during the winter months These are identified below

- The high potential for BOD reduction demonstrated repeatedly by the reedbed section of the system clearly shows it has a crucial role to play in the overall performance of the system As a result emphasis should be placed on enhancing the performance of the reedbed The introduction of an aeration system within the cells of the SSF offers a potential solution to the critically low

DO levels observed in the reedbed cells during the winter months The direct aeration of the relevant intermediate channels would enable aerobic biodegradation to continue throughout the entire length of each reedbed This could be achieved by delivering air to the intermediate channels of the SSF via a compressor

- The diverse microbial population exhibited by the SSF also suggests that the introduction of more vegetation into the overall treatment system would promote microbial degradation to the level which prevails in the SSF Incorporating the dormant floating reedbed system (FLRB) (originally designed to receive pre-treated water from the dirty side of the middle pond of the Eastern Reservoir through an existing fire main running around the airport perimeter) as part of the treatment stream would provide a viable alternative as this requires no additional construction but only an alteration to the flow delivery system

- Although there are limitations in using laboratory prepared media for identification of microbial species the use of a variety of media types in the microbial analysis of water samples from the HTF is recommended in order to determine a wider range of species Because some of these microorganisms are culture-specific and as such are non-culturable using conventional laboratory techniques there is a need to use a variety of growth media in order to isolate and identify the consortia of microorganisms responsible for efficient BOD reduction While using this method to determine the actual consortium required may be challenging it would have the advantage of identifying a range of microorganisms closer to that which exists in the field

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APPENDIX 1

Table 1 A comparison of average, minimum and maximum nutrient levels observed in the MFR and MFBP with the levels in the ER

	Before nutrient dosing (Prior to the 22 November 2004 visit)			Winter period immediately after first nutrient dosing (22 November 2004 14 April 2005)			Post nutrient dosing period (22 November 2004 27 April 2006)		
	MFR	MFBP	ER*	MFR	MFBP	ER*	MFR	MFBP	ER*
Average Concentration Nitrate	5 12	3 86	10 63	8 06	9 70	10 39	8 63	8 94	10 42
Phosphate (mg/l)	0 39	0 37	0 20	0 42	0 46	0 24	0 37	0 33	0 36
Maximum Concentration Nitrate	9 75	3 33	n/a	11 96	15 95	12 85	20 80	17 72	19 09
Phosphate (mg/l)	0 59	0 36	n/a	1 61	1 33	0 35	1 61	1 33	1 00
Minimum Concentration Nitrate	1 79	1 33	n/a	3 99	0 00	7 10	0 00	0 00	6 20
Phosphate (mg/l)	0 18	0 17	n/a	0 17	0 04	0 16	0 12	0 04	0 06

* No nutrient addition was carried out at the ER
n/a Not applicable because only one record of the nutrient levels in the ER was available prior to 22 November 2004

APPENDIX 2

Table 1 BOD levels observed in the different units of the Heathrow treatment facility (HTF)

BIOX/Laboratory BOD (mg/l)	29/01/04	02/02/04	06/02/04	10/02/04	13/02/04	19/02/04	27/02/04	18/03/04	25/03/04	07/04/04
Diversion Chamber	nd	nd	nd	nd	Nd	nd	nd	nd	nd	nd
MFR	295	260	270	85	215	250	92 5	435/255	1060/ 267 5	130/207 5
MFBP	5	125	352 5	82 5	242 5	217 5	260	192/300	305/147 5	328/205
SSF	26 3	113 8	176 3	80	136 3	73	86	70	97	187 5
Exit	6	47 5	82 5	46 5	78	141 3	66	86 25	225	172 5
ER	nd	nd	nd	nd	Nd	nd	nd	nd	nd	30/132 5

Table 1 (continued) BOD levels observed in the different units of the Heathrow treatment facility (HTF)

BIOX/Laboratory BOD (mg/l)	21/04/04	04/05/04	26/05/04	15/06/04	12/07/04	18/08/04	14/09/04	18/10/04	10/11/04
Diversion Chamber	12 5/17 6	9 1/27 5	8 6/12 1	8 7/12 3	nd	22/31	nd	26/36 7	9 6/13 5
MFR	258/190	64	13 5/12 25	11 25	Nd	23/10 6	19 5/23 75	44 5/20 5	14 5/14 5
MFBP	139/90	93/39	10/9 5	8/8 75	13 7/13	14 3/10 7	12 6/17 5	26/19 5	12/11 5
SSF	72 5	41 5	6 5	7	Nd	nd	15	nd	1 5
Exit	62 5	26 4/45	5 8/8 25	6 6/6 25	2 1/1	10/3 7	11/15 7	6/2 2	6 6/7 1
ER	10	14 5/14 5	3 5/7 5	4 3/6 5	17 3/5 1	nd	nd	25 4/8 2	nd/18 1

Table 1 (continued) BOD levels observed in the different units of the Heathrow treatment facility (HTF)

BIOX/Laboratory BOD (mg/l)	22/11/04	29/11/04	13/12/04	07/01/05	21/01/05	08/02/05	16/02/05	28/02/05	07/03/05	24/03/05	14/04/05
Diversion Chamber	15 1/21 3	22 5/46 8	nd	10 8/6	12 9/15	17 7/12	11 3/11	103/150	37 4/30	38 2/62 5	nd
MFR	nd/20	20 7/20	11 3/10	28 9/18 5	15 5/20	51/13	42 9/37	501/610	433/330	190/67 5	330/315
MFBP	nd/9	7//11	20 7/20	45 8/25	13 1/15	nd/12	12 3/26	28 8/50	39 1/250	341/270	130/310
SSF	6	nd	4 4	19	15	10	9 5	40	205	110	85
Exit	nd/8	5 5/5	8 3/5	20 1/4 25	nd/10	6 9/9	6 6/9	5 8/25	163/40	98/56 25	4 9/25 0
ER	16 4/8	15 9/10	nd/5	nd/5	nd/10	11 8/8	11 1/8	108/45	271/70	nd/67 5	nd/160 0

Table 1 (continued) BOD levels observed in the different units of the Heathrow treatment facility (HTF)

BIOX/Laboratory BOD (mg/l)	29/04/05	20/05/05	15/06/05	26/08/05	28/09/05	27/10/05	23/11/05	09/12/05	10/02/06	16/03/06	27/04/06
Diversion Chamber	nd	nd	nd	nd	nd	Nd	nd	488 00	85/71 25	20 10	44/47 5
MFR	230/245	140/140	14 6/6 25	NA/62 5	16 3/15	11 1/11 4	25 65	104/171	407/290 7	589/365	123/125
MFBP	193/240	150/117 5	9 8/5 0	30 3/28	25 6/15	18 8/17 1	34 2	94/174	505/302	360/376	159/140
SSF	137 5	15	2 5	18 5	17 5	5 7	20	139 7	250 8	388	132 5
Exit	7 9/16 25	4 7/1 25	6 2/1 25	13 5/29 5	12 7/ 13 75	12 8/5 7	14 25	19/125 4	298/225	269/368	42 2/55
ER	nd/180	54/25	19 0/7 5	nd/33 5	nd/13 75	Nd/5 7	nd	165 3	224/102 6	174	7 7/5

Table 2 DO levels observed in the different units of the Heathrow treatment facility (HTF)

DO mg/l	02/02/04	06/02/04	10/02/04	13/02/04	19/02/04	27/02/04	05/03/04	18/03/04	25/03/04	07/04/04
MR	1 4	1 7	5 7	7 5	8 3	9 4	10 9	9 2	8 6	6 2
BP	3	4 3	9 1	9 1	9 5	10 6	10 3	10 2	9 6	7 4
SSFinlet	6	0	9 3	9 8	10 6	10 5	11 3	10 5	10 2	8 6
SSFoutlet	4 5		0 7	0 4	0	2 7	1 1	1 4	2 2	2 1
Exit	8 1	8 5	7 2	6 5	6 6	6 9	6	6 1	7 8	5 8
ER	nd	nd	nd	nd	Nd	nd	nd	nd	nd	nd

Table 2 (continued) DO levels observed in the different units of the Heathrow treatment facility (HTF)

DO mg/l	21/04/04	04/05/04	26/05/04	15/06/04	12/07/04	18/08/04	14/09/04	18/10/04	10/11/04	22/11/04
MR	5 9	0 2	7 5	4 2	nd	3 7	0	0 6	10 00	12 20
BP	6 8	2	6 5	11 5	9 2	4	6 7	4 2	10 80	12 00
SSFinlet	7 2	4 6	6 8	10 6	6 6	4 6	7 6	1 1	0 30	1 30
SSFoutlet	1 5	2 6	3	5 6	3	2	3 9	8 6	11 30	12 10
Exit	5	5 2	6 5	13 5	4 2	5 7	8 2	11 4	12 50	13 80
ER	nd	nd	nd	nd	nd	nd	nd	nd	8 60	8 90

Table 2 (continued) DO levels observed in the different units of the Heathrow treatment facility (HTF)

DO mg/l	29/11/04	13/12/04	07/01/05	21/01/05	08/02/05	16/02/05	28/02/05	07/03/05	24/03/05	14/04/05
MR	7 70	10 70	6 60	9 20	1 10	0 00	0 60	0 00	0 00	0 00
BP	12 30	11 70	9 20	10 10	11 30	8 50	10 90	4 30	4 20	0 00
SSFinlet	nd	11 70	10 70	10 10	nd	nd	10 40	6 60	0 30	1 80
SSFoutlet	8 40	7 10	4 80	9 10	12 70	8 50	7 60	0 60	4 60	2 90
Exit	16 30	9 80	9 40	7 10	10 20	10 60	11 30	6 90	1 50	0 10
ER	9 40	8 60	10 70	10 70	11 10	11 70	7 80	0 50	0 80	0 00

Table 2 (continued) DO levels observed in the different units of the Heathrow treatment facility (HTF)

DO mg/l	29/04/05	20/05/05	15/06/05	26/08/05	28/09/05	27/10/05	23/11/05	09/12/05	10/02/06	16/03/06	27/04/06
MR	0 00	0 00	9 50	3 90	3 90	6 50	9 50	0 00	7 10	8 10	0 20
BP	0 00	0 00	9 10	4 20	4 50	7 30	11 90	5 10	8 80	9 00	0 60
SSFinlet	0 00	12 30	0 00	4 80	5 80	8 40	1 70	7 90	8 70	10 20	4 60
SSFoutlet	1 10	0 90	2 30	1 80	2 90	4 30	13 10	5 50	0 00	4 50	3 40
Exit	7 50	0 00	1 00	5 40	5 20	6 80	14 40	7 30	7 00	6 80	5 30
ER	0 00	5 20	7 50	8 10	5 90	4 50	nd	0 10	1 30	1 20	15 00

Table 3 Biomass populations observed in the different units of the Heathrow treatment facility (HTF)

Bact CFU/Lit	29/01/04	02/02/04	10/02/04	19/02/04	27/02/04	18/03/04	25/03/04	07/04/04	21/04/04	04/05/04
MR	nd	1 38E+09	1 30E+07	9 60E+06	2 40E+07	2 70E+07	7 40E+07	3 85E+07	2 50E+07	2 35E+08
BP	1 75E+08	6 00E+08	1 20E+07	9 10E+06	2 80E+07	1 20E+07	1 29E+08	3 10E+07	6 00E+06	4 00E+06
SSF	nd	7 20E+08	2 30E+07	4 80E+06	1 80E+07	6 00E+06	1 44E+08	1 00E+07	3 00E+06	6 90E+06
exit	nd	1 20E+08	1 40E+07	8 10E+06	1 20E+07	1 60E+07	6 60E+07	3 00E+06	1 70E+07	6 90E+06
ER	nd	nd	Nd	nd	Nd	nd	nd	3 00E+06	4 00E+06	4 15E+07

Table 3 (continued) Biomass populations observed in the different units of the Heathrow treatment facility (HTF)

Bact CFU/Lit	26/05/04	15/06/04	12/07/04	18/08/04	14/09/04	18/10/04	10/11/04	22/11/04	29/11/04	13/12/04
MR	2 50E+07	1 00E+07	1 09E+07	7 50E+07	1 06E+09	3 82E+07	3 00E+07	3 37E+08	5 50E+07	2 00E+07
BP	1 14E+07	1 55E+07	3 00E+06	1 10E+08	1 71E+08	2 78E+07	9 00E+07	1 05E+07	1 10E+07	1 39E+07
SSF	2 70E+06	9 50E+06	1 11E+08	2 70E+07	1 25E+08	6 10E+06	4 10E+07	1 40E+07	9 35E+06	1 10E+07
exit	8 00E+06	5 40E+07	2 00E+07	3 20E+07	7 00E+07	6 00E+06	6 00E+07	3 00E+06	1 24E+07	1 65E+07
ER	4 75E+07	6 20E+09	1 20E+07	7 80E+06	nd	5 80E+07	7 85E+07	4 55E+07	2 95E+07	4 70E+07

Table 3 (continued) Biomass populations observed in the different units of the Heathrow treatment facility (HTF)

Bact CFU/Lit	07/01/05	21/01/05	08/02/05	16/02/05	28/02/05	07/03/05	24/03/05	14/04/05	29/04/05	20/05/05
MR	1 03E+08	nd	3 90E+09	2 30E+10	1 56E+09	1 24E+09	8 45E+08	1 20E+08	3 31E+09	1 85E+08
BP	5 15E+08	nd	1 50E+08	1 55E+09	5 90E+08	8 70E+08	5 60E+07	1 50E+08	1 23E+09	1 23E+09
SSF	1 62E+08	nd	3 70E+08	5 90E+07	1 10E+08	1 00E+09	6 20E+07	6 10E+06	3 70E+07	6 20E+07
exit	9 85E+07	nd	1 90E+07	1 33E+08	1 27E+08	1 60E+09	2 70E+07	2 20E+07	2 48E+09	1 10E+08
ER	8 20E+06	nd	4 50E+07	Nd	1 65E+09	1 40E+09	4 60E+07	6 80E+07	3 02E+09	2 10E+09

Table 3 (continued) Biomass populations observed in the different units of the Heathrow treatment facility (HTF)

Bact CFU/Lit	15/06/05	26/08/05	28/09/05	27/10/05	23/11/05	09/12/05	10/02/06	16/03/06	27/04/06
MR	1 00E+10	5 85E+08	1 56E+08	1 00E+08	1 00E+08	2 20E+08	7 70E+07	3 30E+07	3 89E+09
BP	1 74E+08	7 00E+06	2 49E+09	4 40E+07	2 50E+07	9 70E+07	1 20E+08	2 70E+07	3 30E+09
SSF	1 30E+09	5 50E+07	1 14E+08	4 90E+07	1 50E+07	6 30E+07	7 30E+07	1 40E+07	1 30E+08
exit	1 10E+09	5 50E+07	9 35E+07	7 50E+07	2 60E+08	6 10E+07	7 30E+07	5 75E+06	5 70E+07
ER	7 80E+08	4 50E+07	1 60E+09	3 80E+07	nd	1 90E+08	1 80E+09	1 30E+09	1 55E+08

Table 4 Nitrate levels observed in the different units of the Heathrow treatment facility (HTF)

Nitrate, mg/l	10/11/04	22/11/04	29/11/04	13/12/04	07/01/05	21/01/05	08/02/05	16/02/05	28/02/05	07/03/05	24/03/05
Div Chamber	nd	nd	nd	nd	nd	6 16	4 47	5 20	6 16	4 47	2 18
MR	9 75	7 53	8 42	10 19	7 53	8 86	11 96	3 99	11 08	5 76	7 97
BP	8 42	15 95	15 95	10 63	7 97	5 32	10 63	9 30	11 96	9 75	0 00
SSF	4 87	5 32	5 32	11 52	9 75	9 30	4 43	8 86	12 40	11 96	0 00
Exit	6 20	8 86	7 09	11 08	7 97	9 30	7 97	9 75	9 30	7 97	2 66
ER	10 63	12 40	11 96	12 85	11 52	16 39	11 96	11 52	9 75	7 97	7 10

Table 4 (continued) Nitrate levels observed in the different units of the Heathrow treatment facility (HTF)

Nitrate mg/l	14/04/05	29/04/05	20/05/05	15/06/05	26/08/05	28/09/05	27/10/05	23/11/05	09/12/05	10/02/06	16/03/06	27/04/06
Div Chamber	nd	0 44	Nd	nd	12 4	nd	nd	nd	nd	nd	nd	0 00
MR	6 20	5 32	3 54	3 99	4 87	13 26	13 70	13 07	4 87	0 00	20 80	17 28
BP	4 87	2 66	7 09	2 66	10 63	17 72	13 70	14 18	0 44	0 44	11 96	9 30
SSF	0 00	0 00	3 54	1 77	6 65	8 86	11 50	10 41	8 42	3 54	15 95	0 00
Exit	2 22	1 33	2 66	7 09	7 97	8 86	11 08	10 19	5 32	0 00	4 43	3 54
ER	7 97	8 42	9 75	7 53	9 75	8 86	10 63	nd	6 2	8 86	19 09	15 50

Table 5 Phosphate levels observed in the different units of the Heathrow treatment facility (HTF)

Phosphate, mg/l	10/11/04	22/11/04	29/11/04	13/12/04	07/01/05	21/01/05	08/02/05	16/02/05	28/02/05	07/03/05	24/03/05
Div Chamber	nd	nd	nd	nd	Nd	0 18	0 26	0 15	0 23	0 18	0 31
MR	0 32	0 21	0 22	0 38	0 27	0 34	0 63	0 18	0 25	0 17	1 61
BP	0 23	0 95	0 69	0 50	0 24	0 16	0 35	0 13	0 24	0 14	1 33
SSF	0 29	0 11	0 15	0 29	0 37	0 22	0 22	0 13	0 24	0 16	0 29
Exit	0 25	0 32	0 20	0 19	0 36	0 33	0 21	0 18	0 34	0 84	0 26
ER middle	0 20	0 35	0 31	0 17	0 30	0 44	0 19	0 17	0 16	0 22	0 27

Table 5 (continued) Phosphate levels observed in the different units of the Heathrow treatment facility (HTF)

Phosphate, mg/l	14/04/05	29/04/05	20/05/05	15/06/05	26/08/05	28/09/05	27/10/05	23/11/05	09/12/05	10/02/06	16/03/06	27/04/06
Div Chamber	nd	0 35	Nd	nd	0 81	nd	nd	nd	nd	nd	nd	2 02
MR	0 16/0 48	0 38	0 27	0 23	0 53	0 49	0 51	0 28	0 12	0 35	0 18	0 14
BP	0 00/0 04	0 19	0 09	0 10	0 30	0 50	0 35	0 11	0 15	0 29	0 14	0 10
SSF	0 28	0 10	0 50	0 17	0 23	0 31	0 35	0 04	0 12	1 24	0 26	0 33
Exit	0 28	0 37	0 37	0 28	0 22	0 22	0 27	0 05	0 12	0 41	0 14	0 19
ER middle	0 20	0 41	0 41	0 36	0 84	1 00	0 79	nd	0 06	0 24	0 33	0 29

Table 6 Ferrous iron levels observed in the different units of the Heathrow treatment facility (HTF)

Ferrous iron, mg/l	29/01/04	06/02/04	19/02/04	27/02/04	18/03/04	25/03/04	07/04/04	21/04/04	14/09/04	10/11/04	21/01/05	08/02/05	16/02/05
Diversion Chamber	nd	nd	nd	Nd	nd	nd	nd	nd	nd	nd	nd	0 00	0 00
MR	0 01	0 015	0	0	0 03	0 03	0	0	0 03	0 01	0 95	0 11	0 06
BP	0 01	0 02	0	0	0 02	0 03	0	0	0 04	0	0 30	0 02	0 01
SSF	nd	0 02	0 01	0	0 05	0 04	0 02	0 07	nd	nd	0 35	0 02	0 01
Exit	nd	nd	0	0 52	0 12	0 05	0 02	0 14	0 01	nd	0 25	0 00	0 01
ER middle	nd	nd	nd	Nd	nd	nd	nd	nd	nd	nd	0 00	0 04	0 01

Table 6 (continued) Ferrous iron levels observed in the different units of the Heathrow treatment facility (HTF)

Ferrous iron, mg/l	28/02/05	07/03/05	24/03/05	14/04/05	29/04/05	20/05/05	15/06/05	26/08/05	28/09/05	27/10/05	23/11/05	09/12/05	10/02/06	16/03/06	27/04/06
Diversion Chamber	0 25	0 00	0 00	nd	0 01	0 00	nd	nd	nd	nd	nd	nd	nd	nd	0 08
MR	0 02	0 07	2 19	0 02/0 11	1 20	2 23	0 01	0 01	0 01	0 00	0 01	0 01	0 00	0 11	2 09
BP	0 04	0 00	0 02	0 03/0 46	0 32	0 51	0 01	0 01	0 00	0 00	0 02	0 01	0 00	0 16	0 01
SSF	0 01	0 00	0 04	0 00	0 01	0 00	0 00	0 00	0 00	0 02	0 01	0 02	0 25	2 05	0 30
Exit	0 02	0 02	0 02	0 00	0 01	0 03	0 01	0 00	0 00	0 02	0 01	0 01	0 11	1 85	0 01
ER middle	0 01	0 02	0 00	0 05	0 02	0 09	0 01	0 00	0 00	0 01	NA	0 01	0 31	0 06	0 02

Table 7 Total iron levels observed in the different units of the Heathrow treatment facility (HTF)

Fe-total	29/01/04	02/02/04	06/02/04	10/02/04	13/02/04	19/02/04	27/02/04	18/02/04	25/03/04	07/04/04	21/04/04
Diversion Chamber	nd	nd	nd	nd	Nd	nd	nd	nd	nd	nd	nd
MR	1 12	3 26	1 42	2 17	3 43	5 18	2 4	1 67	2 49	2 29	2
BP	1 03	1 86	2 22	1 15	1 94	1 72	1 98	2 12	2 78	1 33	1 54
SSF	nd	nd	nd	14 48	13 04	8 06	3 74	8 84	5 29	4 26	3 59
Exit	nd	nd	2 1	4 32	4 7	4 75	3 44	5 56	3 63	3 4	2 91
ER	nd	nd	nd	nd	Nd	nd	nd	nd	nd	nd	nd

Table 7 (continued) Total iron levels observed in the different units of the Heathrow treatment facility (HTF)

Fe-total	29/04/05	20/05/05	15/06/05	26/08/05	28/09/05	27/10/05	23/11/05	09/12/05	10/02/06	16/03/06	27/04/06
Diversion Chamber	0 01	0 01	Nd	nd	nd	nd	nd	nd	nd	nd	6 00
MR	1 82	7 12	0 21	0 23	nd	0 33	0 52	1 36	3 80	3 03	5 68
BP	0 60	1 31	0 22	0 22	nd	nd	0 17	0 51	3 48	2 86	2 52
SSF	0 11	2 17	2 47	0 25	nd	0 14	0 42	2 04	3 48	6 56	4 76
Exit	0 21	3 76	0 88	0 71	nd	nd	0 12	1 70	4 70	5 80	3 62
ER	0 45	0 18	0 00	0 01	nd	0 31	nd	0 48	0 44	0 34	0 04

APPENDIX 3A

Table 1 A comparison of average, minimum and maximum nutrient levels observed in the MFR and MFBP with the levels in the ER

	Before nutrient dosing (Prior to the 22 November 2004 visit)			Winter period immediately after first nutrient dosing (22 November 2004-14 April 2005)			Post nutrient dosing period (22 November 2004 27 April 2006)		
	MFR	MFBP	ER*	MFR	MFBP	ER*	MFR	MFBP	ER*
Average concentration Nitrate	5 12	3 86	10 63	8 06	9 70	10 39	8 63	8 94	10 42
Phosphate (mg/l)	0 39	0 37	0 20	0 42	0 46	0 24	0 37	0 33	0 36
Maximum concentration Nitrate	9 75	3 33	n/a	11 96	15 95	12 85	20 80	17 72	19 09
Phosphate (mg/l)	0 59	0 36	n/a	1 61	1 33	0 35	1 61	1 33	1 00
Minimum concentration Nitrate	1 79	1 33	n/a	3 99	0 00	7 10	0 00	0 00	6 20
Phosphate (mg/l)	0 18	0 17	n/a	0 17	0 04	0 16	0 12	0 04	0 06

* No nutrient addition was carried out at the ER
n/a Not applicable because only one record of the nutrient levels in the ER was available prior to 22 November 2004

Table 2 Summary of the average biomass populations and ranges observed in the MFR, MFBP and SSF before and after nutrient dosing commenced at Mayfield Farm

	Before nutrient dosing (Prior to the 22 November 2004 visit)				Post nutrient dosing period (22 November 2004-27 April 2006)			
	MFR	MFBP	SSF	ER*	MFR	MFBP	SSF	ER*
Average biomass population (CFU/l)	$1\ 92 \times 10^{8}$	$8\ 44 \times 10^{7}$	$7\ 86 \times 10^{7}$	$7\ 17 \times 10^{8}$	$2\ 37 \times 10^{9}$	$6\ 03 \times 10^{8}$	$1\ 77 \times 10^{8}$	$7\ 56 \times 10^{8}$
Minimum biomass population (CFU/l)	$9\ 60 \times 10^{6}$	$3\ 00 \times 10^{6}$	$2\ 70 \times 10^{6}$	$3\ 00 \times 10^{6}$	$2\ 00 \times 10^{7}$	$7\ 00 \times 10^{6}$	$6\ 10 \times 10^{6}$	$8\ 20 \times 10^{6}$
Maximum biomass population (CFU/l)	$1\ 38 \times 10^{9}$	$6\ 00 \times 10^{8}$	$7\ 20 \times 10^{8}$	$6\ 20 \times 10^{9}$	$2\ 30 \times 10^{10}$	$3\ 30 \times 10^{9}$	$1\ 39 \times 10^{9}$	$3\ 02 \times 10^{9}$

*ER was not dosed with nutrient

Table 3 Comparisons of the laboratory measured BOD concentrations with the BiOX analyser results for Diversion Chamber samples

Date	BiOX mg/l	Laboratory mg/l
21/04/04	12 50	17 60
04/05/04	9 10	27 50
26/05/04	8 60	12 10
15/06/04	8 70	12 30
18/08/04	22 00	31 00
18/10/04	26 00	36 70
10/11/04	9 60	13 50
22/11/04	15 10	21 30
29/11/04	22 50	46 80
07/01/05	10 80	15 00
08/02/05	17 70	12 00
16/02/05	11 30	11 00
28/02/05	103 00	150 00
07/03/05	37 40	30 00
24/03/05	38 20	62 50
10/02/06	85 00	71 25
27/04/06	44 00	47 50

Table 4 Comparisons of the laboratory measured BOD concentrations with the BIOX analyser results for MFR and MFBP samples

Date	MFR		MFBP	
	BIOX BOD mg/l	Laboratory BOD mg/l	BIOX BOD mg/l	Laboratory BOD mg/l
18/03/04	435 0	255 0	192 0	300 0
25/03/04	1060 0	267 5	305 0	147 5
07/04/04	130 0	207 5	328 0	205 0
21/04/04	258 0	190 0	139 0	90 0
04/05/04	*NW*	64 0	93 0	39 0
26/05/04	10 0	9 5	10 0	9 5
15/06/04	*NW*	11 3	8 0	8 8
18/08/04	23 0	10 6	14 3	10 7
14/09/04	19 5	23 8	12 6	17 5
18/10/04	44 5	20 5	26 0	19 5
10/11/04	14 5	14 5	12 0	11 5
22/11/04	*NW*	20 0	*NW*	9 0
29/11/04	20 7	20 0	7 0	11 0
13/12/04	11 3	10 0	20 7	20 0
07/01/05	28 9	18 5	45 8	25 0
21/01/05	15 5	20 0	13 1	15 0
08/02/05	51 0	13 0	*NW*	12 0
16/02/05	42 9	37 0	12 3	26 0
28/02/05	501 0	610 0	28 8	50 0
07/03/05	433 0	330 0	39 1	250 0
24/03/05	190 0	67 5	341 0	270 0
14/04/05	330 0	315 0	130 0	310 0
29/04/05	230 0	245 0	193 0	240 0
20/05/05	140 0	140 0	150 0	117 5
15/06/05	14 6	6 3	9 8	5 0
26/08/05	*NW*	62 5	30 3	28 0
28/09/05	16 3	15 0	25 6	15 0
27/10/05	11 1	11 4	18 8	17 1
09/12/05	104 0	171 0	94 0	174 0
10/02/06	407 0	290 7	505 0	302 0
16/03/06	589 0	365 00	360 00	376 0
27/04/06	123 0	125 00	159 00	140 0

NW Device not working

Table 5 Comparisons of the laboratory measured BOD concentrations with the BIOX analyser results for Mayfield Farm exit channel samples

Date	BIOX BOD level mg/l	Laboratory BOD level mg/l
04/05/04	26 5	45
26/05/04	5 8	8 3
15/06/04	6 6	6 3
12/07/04	2 1	1
18/08/04	10	3 7
14/09/04	11	15 7
18/10/04	6	2 2
10/11/04	6 6	7 1
29/11/04	5 5	5
13/12/04	8 3	5
07/01/05	20 1	4 3
08/02/05	6 9	9
16/02/05	6 6	9
28/02/05	5 8	25
07/03/05	163	40
24/03/05	98	56 3
14/04/05	4 9	25
29/04/05	7 9	16 3
20/05/05	4 7	1 3
15/06/05	6 2	1 3
26/08/05	13 5	29 5
28/09/05	12 7	13 75
27/10/05	12 8	5 7
09/12/05	19	125
10/02/06	298	225
16/03/06	269	368
27/04/06	42 2	55

APPENDIX 3B

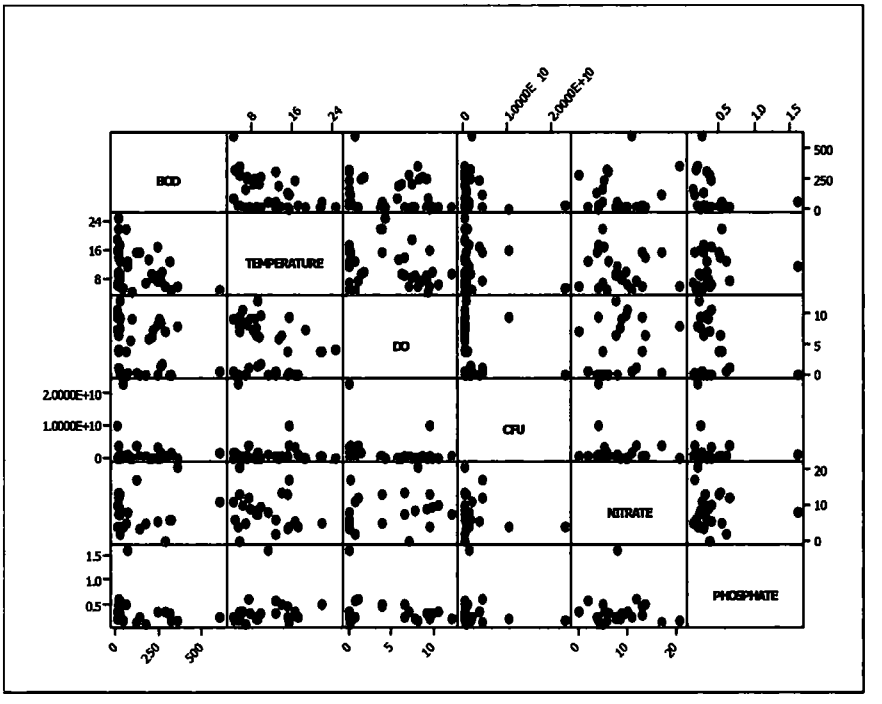


Figure 1 Matrix plots of BOD, DO, nitrate and phosphate levels and the biomass populations in the MFR

Figure 2 Matrix plots of BOD, DO, nitrate and phosphate levels and the biomass populations in the MFBP

APPENDIX 4A

Table 1 Temporal Changes in DO and Fe^{2+}/Fe^{3+} concentrations for aerated pond water samples containing different components

Time, days		0	1	2	3	6	8
System A	DO (mg/l)	7 20	3 70	3 65	3 35	0 00	0 00
	Fe^{2} (mg/l)	3 00	nd	nd	0 40	0 03	0 05
	Fe^{3+} (mg/l)	0 00	nd	nd	2 60	2 97	2 95
	% conv Fe^{2+}	0 00	nd	nd	86 7	99 0	98 3
System B	DO (mg/l)	7 20	4 10	3 70	1 30	0 00	0 00
System C	DO (mg/l)	7 20	3 70	4 50	4 40	3 40	3 00
	Fe^{2} (mg/l)	3 00	nd	nd	0 50	0 24	0 13
	Fe^{3} (mg/l)	0 00	nd	nd	2 50	2 76	2 87
	% conv Fe^{2}	0 00	nd	nd	83 3	92 0	95 7
System D	DO (mg/l)	7 20	4 20	5 20	5 10	4 40	4 40

nd not determined due to equipment malfunction

Table 2 Variations in dissolved oxygen and Fe^{3+} concentrations (mg/l) for initial concentrations of 1, 2, 3 and 4 mg/l Fe^{2+} in aerated pond water samples

Time days		0	Re-aeration for approximately 30 minutes on Day 8	9	10	11	12	15	17	19
Dissolved oxygen mg/l	System 1	7 20		1 10	0 70	0 00	0 00	0 00	0 00	0 00
	System 2	7 20		1 00	0 40	0 00	0 00	0 00	0 00	0 00
	System 3	7 20		1 00	0 35	0 00	0 00	0 00	0 00	0 00
	System 4	7 20		0 80	0 20	0 00	0 00	0 00	0 00	0 00
Fe^{2} concentration mg/l	System 1	1 00		0 32	0 03	0 15	0 22	0 72	0 94	1 00
	System 2	2 00		1 41	0 17	0 13	0 46	1 60	1 77	1 84
	System 3	3 00		1 69	0 29	0 25	0 23	0 92	2 50	2 87
	System 4	4 00		2 71	0 49	0 27	0 07	1 17	2 43	4 00
Time days		0		9	10	11	12	15	17	19
Fe^{3+} concentration mg/l	System 1	0 00	Re-aeration for approximately 30 minutes on Day 8	0 68	0 97	0 85	0 78	0 28	0 06	0 00
	System 2	0 00		0 59	1 83	1 87	1 54	0 40	0 23	0 16
	System 3	0 00		1 31	2 71	2 75	2 77	2 08	0 50	0 13
	System 4	0 00		1 29	3 51	3 73	3 93	2 83	1 57	0 00
Percentage Fe^{3+} Concentration	System 1	0 0		68 0	97 0	85 0	78 0	28 0	6 0	0 0
	System 2	0 0		29 5	91 5	93 5	77 0	20 0	11 5	8 0
	System 3	0 0		43 7	90 3	91 7	92 3	69 3	16 7	4 3
	System 4	0 0		32 2	87 7	93 2	98 2	70 7	39 2	0 0

Table 3 Variations in Fe^{3+} and DO concentrations for aerated pond water represented by Samples A and B incubated at 20°C

	Sample	Day 0	Day 1	Day 2	Day 4	Day 8	Day 9	Day 10	Day 11	Day 14	Day 15	Day 16	Day 17
DO levels (mg/l)	A	6 4	4 7	3 8	2 4	0 0	0 0	0 0/5 7	2 3	0	0	0	0
	B	6 8	5 4	4 7	4 5	4 3	4 0	4 0	3 7	3 6	3 6	3 6	3 6
Fe^{3+} concentration (mg/l)	A	0	2 14	2 61	2 74	2 91	2 92	2 93	2 96	2 81	2 74	2 24	2 02
	B	0	1 34	1 49	2 12	2 60	2 71	2 79	2 82	2 90	2 92	2 92	2 96

Table 4 Variations in Fe^{3+} and DO concentrations for aerated pond water dosed with 1000 mg/l BOD and 3 mg/l Fe^{2+} (Sample A) and 3 mg/l Fe^{2+} only (Sample B) incubated at 20°C

	Sample	0	1	3	4	7	8	11
DO (mg/l)	A	6 40	4 40	0 00	0 00	0 00	0 00	0 00
	B	6 30	5 47	4 13	3 73	3 20	2 63	2 20
Fe^{3} (mg/l)	A	0 00	1 66	1 87	2 00	2 14	2 17	1 81
	B	0 00	1 11	1 19	1 24	1 84	2 11	2 49

APPENDIX 4B

Figure 1 A Section of the MFBP showing the brown coloration of the water caused by the presence of Fe^{3+}

APPENDIX 5A

Table 1 Summary of equations and correlation coefficients for the DO exponential decay process in the different water samples at 6°C and 20°C

Components	Temperature	Exponential Equation	R^2 value
MFR	6°C	$y = 9.0e^{-0.0867x}$	0.8494
	20°C	$y = 9.0e^{1.3923x}$	0.3645
MFBP	6°C	$y = 9.4e^{-0.1531x}$	0.9104
	20°C	$y = 9.4e^{1.4058x}$	0.3337
SSF	6°C	$y = 8.4e^{-0.1974}$	0.8165
	20°C	$y = 8.4e^{1.2788x}$	0.6719
ER	6°C	$y = 9.6e^{-0.0314x}$	0.8193
	20°C	$y = 9.6e^{-0.0819x}$	0.6783

Table 2 Summary of the experimentally determined biodegradation rates (day^{-1}), percentage BOD reduction after a day and the time taken to remove half of the BOD in the different water samples at 6°C and 20°C

Components of the HTF	Biodegradation rates (day^{-1})		Percentage BOD reduction after 1 day		Time taken to remove 50% of initial BOD concentration, (days)	
	6°C	20°C	6°C	20°C	6°C	20°C
MFR	0.099	0.440	9.5%	35.6%	7.0	1.6
MFBP	0.181	0.442	16.5%	35.6%	3.9	1.6
SSF	0.239	0.443	21.3%	35.6%	2.9	1.6
ER	0.043	0.091	3.9%	8.6%	17.3	7.7

Table 3 Summary of the experimentally determined biodegradation rates (day^{-1}), percentage BOD reduction after a day and the time taken to remove half of the BOD in MFR water samples with different components at 6°C and 20°C

Water samples	Biodegradation rates (day^{-1})		Percentage BOD reduction after 1 day		Time taken to remove 50% of initial BOD concentration, (days)	
	6°C	20°C	6°C	20°C	6°C	20°C
A	0 093	0 352	8 6%	29 5%	7 7	2 0
B	1 081	1 093	66 0%	66 4%	0 6	0 6
C	0 247	0 332	22 1%	28 1%	2 8	2 1

Table 4 A brief description of the different bacteria isolates from the HTF

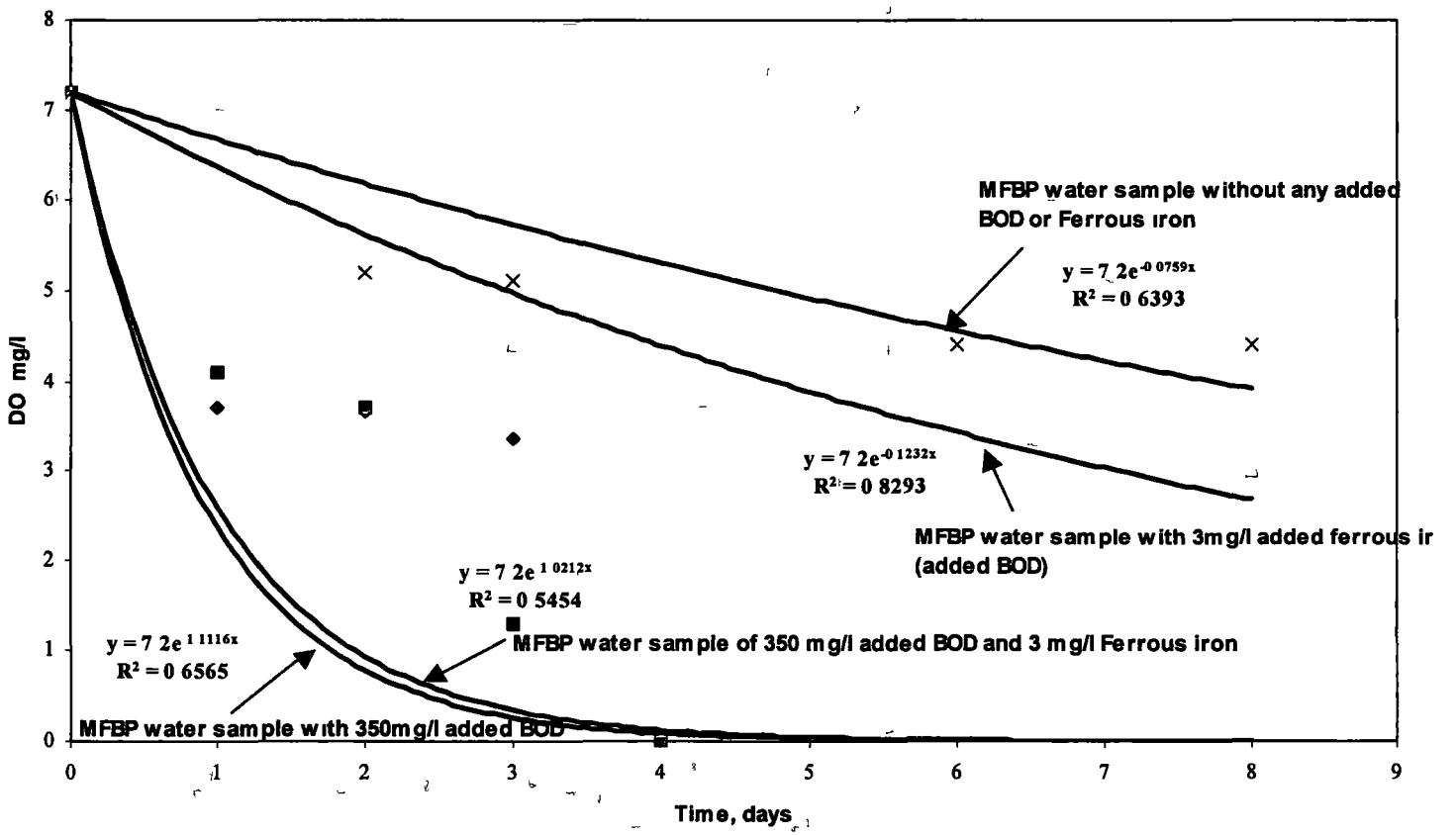
Bacterial strains	Location	Description
1	Mayfield Farm aerated ponds, roots and ER	Round brown colonies
2	Mayfield Farm aerated ponds	Large round beige colonies
3	Mayfield Farm aerated ponds, roots and ER	Round whitish colonies
4	Mayfield Farm aerated ponds	Deep brown colonies, slightly large
5	Mayfield Farm aerated ponds	Large beige colonies with brown in the middle
A	Plant roots and ER	Dark brown round colonies
B	Plants roots	Beige-Brown colonies
C	Plants roots	Small beige round colonies
D	Plants roots	Very light brown round colonies
E	Plants roots	Rough light brown colonies
F	Plants roots	Large dark brown round colonies
G	Plant roots	Transparent beige-brown colonies
H	Plant roots and ER	White sponge like colonies
I	Plant roots and ER	Small yellow colonies
J	Plant roots and ER	Dark brown colonies
K	Plant roots and ER	Transparent brown, slightly rough colonies
L	Plant roots	Whitish rough colonies
M	Plant roots and Diversion chamber	Round whitish colonies very similar to 3

Table 5 Brief descriptions of the different fungi and actinomycetes from the HTF

Isolate	Location	Description of colony observed on agar plate
A1	Plant roots and substrate of the SSF	Round-shaped slightly-raised beige colonies
A2	Plant roots and substrate of the SSF	Unusually shaped dark beige colonies
F/A	Plant roots and substrate of the SSF	Small light-beige solid colonies
F1	Plant roots and substrate of the SSF	Large colonies with jet-black reversed side
F2	Plant roots and substrate of the SSF	Large mouldy colonies
F3	Plant roots and substrate of the SSF	Slight green-black moulds covering plate

APPENDIX 5B

BIODEGRADATION CURVES FOR MFBP WATER SAMPLES WITH VARYING COMPONENTS UNDER THE SAME CONDITIONS (20 degrees cent)



APPENDIX 6A

Table 1 Identification of the individual column experiments relative to date carried out

Experiment	Duration of Experiment
Experiment A	20 September 2004 to 11 October 2004
Experiment B	25 October 2004 to 15 November 2004
Experiment C	29 November 2004 to 20 December 2004
Experiment D	25 January 2005 to 15 February 2005
Experiment E	25 May 2005 to 15 June 2005
Experiment F	28 June 2005 to 19 July 2005
Experiment G	2 September 2005 to 23 September 2005

Table 2 Variations in nitrate concentrations in nutrient-dosed planted and substrate-only columns (starting nitrate concentration of 50 mg/l)

Experiments	A	B	C	D	E	F	G
	Nitrate concentrations mg/l						
Nitrate concentration after 7 days in planted column dosed with nutrient	9 25	0 25	12 85	0 00	0 00	7 97	11 96
Nitrate concentration after 21 days in planted column dosed with nutrient	11 80	0 00	10 19	14 18	0 00	0 00	0 00
Nitrate concentration after 7 days in substrate-only column dosed with nutrient	5 30	2 00	11 96	4 87	0 00	5 76	13 29
Nitrate concentration after 21 days in substrate-only column dosed with nutrient	5 90	0 40	9 75	14 18	0 00	0 00	0 00

Table 3 Variations in phosphate concentrations in nutrient-dosed planted and substrate-only columns (starting phosphate concentration of 6 8 mg/l)

Experiments	A	B	C	D	E	F	G
	Phosphate concentration mg/l						
Phosphate concentration after 7 days in planted column dosed with nutrient	0 60	0 17	0 08	0 11	0 77	0 54	0 46
Phosphate concentration after 21 days in planted column dosed with nutrient	1 20	0 40	0 08	0 16	0 52	1 40	0 24
Phosphate concentration after 7 days in substrate-only column dosed with nutrient	0 92	1 12	0 56	0 14	1 35	0 84	0 91
Phosphate concentration after 21 days in substrate-only column dosed with nutrient	0 91	0 93	0 30	0 35	0 90	1 11	0 48

Table 4 DO concentrations in nutrient-dosed and nutrient-free planted and substrate-only columns 7 and 21 days after the commencement of each experiment

Experiments	A	B	C	D	E	F	G
	Dissolved oxygen levels mg/l						
DO after 7 days in planted column dosed with nutrient and BOD	(7 30) 6 60	(7 10) 2 80	(7 90) 1 40	(8 00) 0 00	(7 10) 0 50	(7 20) 1 10	(7 10) 0 00
DO after 21 days in planted column dosed with nutrient and BOD	0 20	0 00	0 50	0 00	0 80	1 20	0 00
DO after 7 days in substrate-only column dosed with nutrient and BOD	(7 30) 1 90	(7 10) 3 00	(7 90) 1 20	(8 00) 0 00	(7 10) 3 00	(7 20) 1 30	(7 10) 0 00
DO after 21 days in substrate-only column dosed with nutrient and BOD	0 00	0 00	0 40	0 00	0 70	1 30	0 00
DO after 7 days in planted column dosed with BOD	(7 20) 0 10	(7 10) 2 30	(7 80) 1 10	(8 00) 0 00	(7 10) 0 50	(7 20) 1 80	(7 10) 0 00
DO after 21 days in planted column dosed with BOD	0 00	0 00	0 20	0 00	0 70	1 00	0 00
DO after 7 days in substrate only column dosed with BOD	(7 20) 2 40	(7 10) 2 70	(7 80) 1 10	(8 00) 0 00	(7 10) 1 30	(7 20) 1 50	(7 10) 0 00
DO after 21 days-in substrate-only column dosed with BOD	0 00	0 00	0 20	0 00	0 80	1 30	0 00

Key () = DO concentration of water sample fed into the columns

Table 5 The DO concentrations in iron-dosed and iron-free planted and substrate-only columns 7 and 21 days after the commencement of each experiment

Experiments	A	B	C	D	E	F	G
	Dissolved oxygen levels mg/l						
DO after 7 days in planted column dosed with iron and BOD	(7 20) 2 20	(7 10) 3 50	(7 90) 1 80	(8 00) 0 00	(7 10) 0 90	(7 20) 2 10	(7 10) 0 00
DO after 21 days in planted column dosed with iron and BOD	0 00	0 00	0 30	0 00	1 20	1 20	0 00
DO after 7 days in substrate-only column dosed with iron and BOD	(7 20) 2 70	(7 10) 2 80	(7 90) 1 10	(8 00) 0 00	(7 10) 0 60	(7 20) 2 20	(7 10) 0 00
DO after 21 days in substrate-only column dosed with iron and BOD	0 00	0 00	0 20	0 00	0 60	1 00	0 00
DO after 7 days in planted column dosed with BOD	(7 20) 0 10	(7 10) 2 30	(7 80) 1 10	(8 00) 0 00	(7 10) 0 50	(7 20) 1 80	(7 10) 0 00
DO after 21 days in planted column dosed with BOD	0 00	0 00	0 20	0 00	0 70	1 00	0 00
DO after 7 days in substrate-only column dosed with BOD	(7 20) 2 40	(7 10) 2 70	(7 80) 1 10	(8 00) 0 00	(7 10) 1 30	(7 20) 1 50	(7 10) 0 00
DO after 21 days-in substrate-only column dosed with BOD	0 00	0 00	0 20	0 00	0 80	1 30	0 00

Key () = DO concentration of water sample fed in the columns

Table 6 Variations in Fe^{2+} ions concentrations in iron-dosed planted and substrate-only column (starting Fe^{2+} ion concentration of 3 mg/l)

Experiments	A	B	C	D	E	F	G
	Fe (II) concentrations mg/l						
7 days-Iron-dosed planted column	4 43	0 19	2 05	13 1	3 07	3 37	1 59
21 days-Iron-dosed planted column	4 22	3 10	4 75	1 77	6 40	2 74	3 25
7 days-Iron-dosed substrate-only column	3 10	0 09	2 40	12 60	5 66	6 16	1 58
21 days- Iron-dosed substrate-only column	0 54	2 10	3 90	2 00	9 52	5 38	2 15

APPENDIX 6B

Figure 1 Section of the Pilot-Scale Vertical flow column during the winter

APPENDIX 7A

Table 1 A brief description of the different isolates from the HTF

Isolate	Location	Description of colony observed on agar plate
1	Mayfield Farm aerated ponds roots and ER	Round brown colonies
2	Mayfield Farm aerated ponds	Large round beige colonies
3	Mayfield Farm aerated ponds roots and ER	Round whitish colonies
4	Mayfield Farm aerated ponds	Deep brown colonies, slightly large
5	Mayfield Farm aerated ponds	Large beige colonies with brown in the middle
A	Plant roots and ER	Dark brown round colonies
B	Plants roots	Beige-Brown colonies
C	Plants roots	Small beige round colonies
D	Plants roots	Very light brown round colonies
E	Plants roots	Rough light brown colonies
F	Plants roots	Large dark brown round colonies
G	Plant roots	Transparent beige brown colonies
H	Plant roots and ER	White sponge like colonies
I	Plant roots and ER	Small yellow colonies
J	Plant roots and ER	Dark brown colonies
K	Plant roots and ER	Transparent brown slightly rough colonies
L	Plant roots	Whitish rough colonies
M	Plant roots and Diversion chamber	Round whitish colonies very similar to 3
A1	Plant roots and substrate of the SSF	Round-shaped slightly-raised beige colonies
A2	Plant roots and substrate of the SSF	Unusually shaped dark beige colonies
F/A	Plant roots and substrate of the SSF	Small light beige solid colonies
F1	Plant roots and substrate of the SSF	Large colonies with jet-black reversed side
F2	Plant roots and substrate of the SSF	Large mouldy colonies
F3	Plant roots and substrate of the SSF	Slight green black moulds covering plate

*Plant roots refers to plants from the Sub-surface Flow Reedbeed (SSF)

Table 2 Characterisation of the different bacteria isolates based on some of the API biochemical tests

Tests/Strains	1	2	3	4	5	A	B	C	D	E	F	G	H	I	J	K	L	M
Gram staining	+	+	+		+				+		+			+/	+			
Catalase production	+		+	nd	nd	+	+	+					+	+	+		+	+
Beta-galactosidase production		+	nd	nd	nd	+	+						+	+	+	+	+	+
Arginine dihydrolase production	+	+	+		+													
Lysine decarboxylase production	+		nd	nd	nd													
Ornithine decarboxylase production	+	+	+															
Citrate utilisation	+	+	nd	nd	nd													
H_2S production			nd	nd	nd												-	
Production of urease	+		+	+	+												+	
Tryptophane production	+	+	nd	nd	nd													
Indole production		+	nd	nd	nd													
Acetoin production	+	+			+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gelatinase production	+	+	nd	nd	nd	+		+			+	+	+	+				+
Glucose fermentation	+	+	+		+												+	+
Fructose acidification			+		+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Arabinose fermentation	+	+						+										+
Melibiose fermentation	+		+	+	+													
Maltose acidification	+	+			+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Lactose acidification	+	+				nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Trehalose acidification					+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Sucrose fermentation		+	+		+												+	+
Amygdalin fermentation		+	nd	nd	nd													+
Mannitol fermentation		+		+	+	+											+	+
Inositol fermentation			nd	nd	nd													
Xylitol acidification			nd	+	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Sorbitol fermentation			nd															+

Table 2 (continue) Characterisation of the different bacteria isolates based on some of the API biochemical tests

Tests/Strains	1	2	3	4	5	A	B	C	D	E	F	G	H	I	J	K	L	M
Rhamnose fermentation	+		nd	nd	nd													
Mannose acidification	+	+	+			nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Oxidase production	+	+	nd	nd	nd	-	+	+	+	+		+	+		+	+		+
Denitrification	+		+	+		nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Alkaline phosphate production			+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

- +/- Partly positive partly negative result (inconclusive result)
- + Positive result (or Gram positive) - Negative result (or Gram negative)
- nd Not determined because test was not available on the API strip used (also used to indicate that the test is inconclusive)

Underlined Where the name of the test is underlined it indicates that the test was conducted under anaerobic conditions

Table 3 Comparison of some key characteristics of a known *Staphylococcus species* with those of Strains *1-5*

Bacteria Strains	Alkaline phosphate	Arginine dihydrolase	Ornithine decarboxylase	Acetoin Production	Urease production	Beta-galactosidase	Denitrification	Catalase production	Acidification of									
									Glucose	Maltose	Fructose	Sucrose	Lactose	Mannitol	Mannose	Trehelose	Xylitol	Arabinose
Staphylococcus aureus	+	+		+	v		+	+	+	+	+	+	+	+	+	+		
Strain *1*		+	+	+	+		+	+	+	+			+		+			+
Strain *2*		+	+	+		+			+	+		+	+	+	+			+
Strain *3*	+	+	+		+	nd	+	+	+		+	+			+		nd	
Strain *4*	nd				+	nd	+	nd						+			+	
Strain 5	nd	+		+	+	nd		nd	+	+	+	+		+		+	+	

v variable response
nd not determined

Table 4 Comparison of some key characteristics of two known *Enterococcus species* with those of Strains *1-5*

Bacteria Strains	Arginine dihydrolase	Beta-galactosidase	H_2S Production	Melibiose fermentation	Acidification of					
					Glucose	Lactose	Sorbitol	Sucrose	Trehalose	Xylitol
Enterococcus faecalis	+				+	+	+	+	+	
Enterococcus faecium	+			+	+	+			+	
Strain *1*	+			+	+	+				
Strain *2*	+	+			+	+		+		
Strain *3*	+	nd	nd	+	+		nd	+		nd
Strain *4*		nd	nd	+						+
Strain 5	+	nd	nd	+	+			+	+	+

Table 5 Comparison of some key characteristics of know *Pseudomonas species* with those of Strains *A-M*

Bacteria Strains	Indole production	H_2S production	Lysine decarboxylase production	Ornithine decarboxylase	Mannitol fermentation	oxidase	Gelatinase production	Arginine dihydrolase
Pseudomonas aeruginosa						+	+	+
Strain *A*							+	-
Strain *B*						+		
Strain *C*						+	+	
Strain *D*						+		
Strain *E*						+		
Strain *F*					-		+	
Strain *G*						+	+	
Strain *H*						+	+	
Strain *I*							+	
Strain *J*						+		
Strain *K*						+		
Strain *L*					+			
Strain *M*					+	+	+	-

v variable response, nd not determined

APPENDIX 7B

Figure 1 Mix population of bacteria, fungi and actinomycetes grown on laboratory prepared culture