Biodegradation of organo-metallic pollutants in distillery wastewater employing a bioaugmentation process

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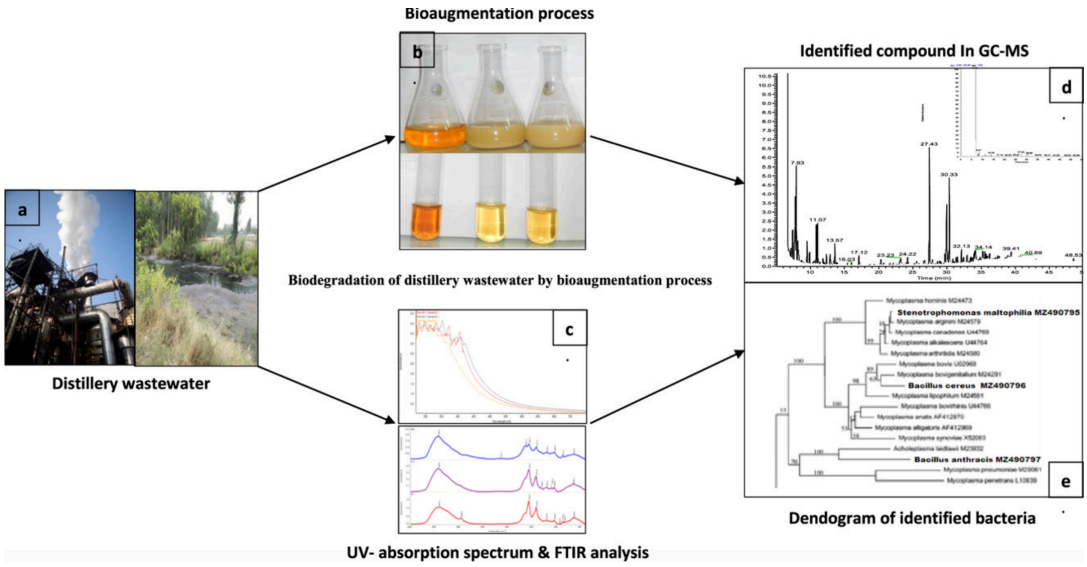
Highlights

- Distillery wastewater is dark in color and heavily polluted with organometallic pollutants.
- The constructed bacterial consortium provides effective treatment of distillery wastewater.
- The consortium biosorbed and biotransformed the metal and organic pollutants, respectively.

Graphical abstract



Abstract

This objective of this work was to study the potential of a constructed bacterial consortium (comprising strains of *Stenotrophomonas maltophilia*, *Bacillus cereus*, and *Bacillus thuringiensis*) to treat distillery wastewater via the bioaugmentation process. The discharged wastewater showed elevated total ammonium nitrogen (195.0 ± 1.24 mg L^{-1}), total dissolved solids (25980.6 ± 8.09 mg L^{-1}), chemical oxygen demand (20534.5 ± 3.12 mg L^{-1}), and biological oxygen demand (20534.5 ± 3.12 mg L^{-1}). High concentration of heavy metals, phenolic and organo-metallic compounds were also detected. Results showed that growing the bacterial consortium in the distillery wastewater at 37 ºC supplemented with 1% glucose achieved the best colour reduction (up to 90 %) in 144 h. The physico-chemical quality of the treated wastewater also improved by 50-70 %. Furthermore, many of the major organic pollutants present in the distillery wastewater were degraded by the constructed consortium to below detection limit via active biotransformation and biodegradation. Heavy metals were biosorbed by the bacterial consortium, and the ligninolytic enzymes such as Lip and MnP played an important role in the degradation of the organo-metallic pollutants. The constructed bacterial consortia therefore offered a sustainable and effective solution to treat distiller wastewater.

1. Introduction

Industrialization has been the major driver in the economic development of the world. Industrial processes improved productivity and allowed for mass production, which has increased standards of living. However, many industries are also the main sources of environmental pollution. In particular, industrial wastewaters have been identified as the primary sources of environmental pollution that threaten the environment and human health (Goutam et al., 2018; Jiang et al., 2019; Sharma et al., 2021a). For example, distillery wastewater is a significant source of economic activity, but it is also a significant source of pollution due to the discharge of large volumes of black wastewater.

There are 319 distilleries in India, producing approximately $3.25 \times 10_9$ liters of alcohol and $40.4 \times 10_{10}$ liters of wastewater annually (Tripathi et al., 2021b; Chandra et al., 2018a). The dark-colored wastewater contains high levels of melanoidins, the high molecular weight products of Maillard reaction that cause oxygen depletion and increase BOD (Chowdhary et al., 2018). In addition, distillery wastewater has very high total solids (TS), biological oxygen demand (BOD) and chemical oxygen demand (COD). The excess sulfate, phosphate, and nitrogen contents of the distillery wastewater also known to cause eutrophication in water resources (Mahimaraja and Bolan, 2004). Elevated levels of phenolics, heavy metals (Fe, Ni, Cu, Cr, Pb, Cd, Zn), and toxic organic compounds (such as di-n-octyl phthalate, di-butyl phthalate, benzenepropanoic acid, and 2-hydroxysocaproic acid) are frequently detected in distillery wastewater that caused severe groundwater pollution and health risks (Arora et al., 2018; Chandra et al., 2018a,b; Sharma et al., 2021b,c). Many of these pollutants are also known to be carcinogenic, mutagenic, genotoxic, and endocrine disruptors (Tripathi et al., 2021a; Dixit et al., 2015; Tamanna and Mahmood, 2015; Arimi et al., 2015). Endocrine disrupting chemicals (EDCs) can alter hormonal activities, such as metabolism, sexual growth, development and activity, stress response, and reproductive functions (Kabir et al., 2015). As a result of their high toxicity, many of the distillery pollutants have been classified as priority by the US Environmental Protection Agency (USEPA), the World Health Organization (WHO), and the Agency for Hazardous Substances and Disease Registry (ATSDR). Bacteria have excellent bio-sorbent ability due to the high surface ratio and active chemisorptions sites in their cell wall (Delil et al., 2020; Tripathi et al., 2021c). They have significant capacity to reduce environmental toxicity (Sharma and Singh, 2021; Sharma and Rath, 2021; Sharma, 2021; Raimondo et al., 2020a,b). Biological agents are considered to be an efficient alternative

to physicochemical treatment approaches that have high energy cost and carbon footprint and may produce secondary pollution. The US Environmental Protection Agency has accepted bioremediation as an environmentally friendly waste management plan. Bioremediation via bioaugmentation is a promising treatment technique (Alessandrello et al., 2017). Bioaugmentation is the introduction of exogenous pollutant-degrading microbes to enhance contaminant degradation. It has been established that a defined microbial consortium performed better than pure monoculture, since the diversity within the consortium increased the number of catabolic pathways available for biodegradation, thus, enhanced the toxin removal efficiency (Fuentes et al. 2014; Cuevas-Díaz et al., 2017). However, the natures of these mechanisms are still not fully understood.

In this work, the performance of a constructed bacterial consortium in treating distillery wastewater was evaluated. The consortium was constructed based on the capabilities of the strains to degrade distillery pollutants. In addition, the biochemical mechanisms involved in the bioremediation process by the consortium was also examined.

2. Materials and methods

2.1 Site study and sample collection

Distillery wastewater was collected from M/s Unnao distillery industry, situated in Uttar Pradesh, Unnao, India (26°320" N, 80°30′0"E). This industry uses sugarcane molasses as feedstock which undergoes fermentation, and a series of distillation and redistillation to produce pure alcohol. The spent wash generated after the first distillation process is treated via a methanogenesis process in the effluent treatment stage before being discharged into the receiving water. Freshly discarded distillery wastewater sludge (approximately 20 L) was collected in clean, pre-sterilized polythene bags from the distillery plant's dumping site which was situated inside the industry's premises (Tripathi et al., 2021b).

2.2 Bacterial consortium preparation

Indigenous bacterial strains were isolated from the distillery wastewater using serial dilution and the plate-streak method on Nutrient Agar (NA) plate. Bacterial colonies were isolated and purified by passaging on NA agar. The procedures in the Cowan and Steels Manual were used to preliminary identify the dominant isolated bacteria strains (Barrow and Feltham, 1993) and selected strains were further identified using 16sRNA sequencing. Two indigenous bacteria

Stenotrophomonas maltophilia (RCS-1, MZ490795) and *Bacillus cereus* (RCS-2, MZ490796) were selected to construct the aerobic bacterial consortium together with a strain of *Bacillus anthracis* (RCS-3, MZ490797) previously isolated from the same site (Chandra et al., 2018a,b,c). The constructed bacterial consortium was used to examine their ability to degrade pollutants present in the distillery wastewater.

2.3 Experiment setup

Each distillery wastewater sample (250 mL) was supplement with 1 g of glucose as carbon source and 0.5 g of nitrogen source and autoclaved at 121 °C. The sterilized wastewater was incubated at 37 °C at the 150-rpm speed for 48h in a rotary shaking incubator (New Brunswick Scientific) to ensure there was no microbial growth. An overnight culture of the bacteria consortium (2mL) was added to the wastewater and incubated at 37 °C at 150-rpm up to 144 h. The cells were harvested at time 0 and then 24h intervals by centrifugation at 10,000 x g for 20 min for further experimentation (Chandra et al., 2018a,b,c).

To optimize the bioremediation process, the following experimental parameters were investigated: temperatures between 25–50 °C, pH between 4–12, and shaking speeds between 100–220 rpm. In addition, the effects of different nutrient source on the growth of the bacterial consortium were also examined: ammonium chloride, beef extract, sodium sulfate, yeast extract, peptone, and urea at 0.5% (w:v).

2.4 Physico-chemical and heavy metals analysis

The physico-chemical properties of the distillery wastewater were analyzed according to Tripathi et al. (2021a). The pH, sodium, nitrate, chloride, and potassium ions of the wastewater sample were measured using an Orion ion meter (Model 960) and selective electrodes. The COD was determined using an open reflux method and the BOD by a 5-day method. Total nitrogen (TN) was quantified using Kieldahl digestion method whereas total organic carbon (TOC) and total solid (TS) were estimated using a VCSH analyzer (Shimadzu, Japan). The amount of sulfate was assessed using the vanadomolybdo–phosphoric acid colorimetric method and the $BaCl_2$ precipitation method, respectively. The heavy metal concentrations in wastewater were determined using an ICP-OES spectrometer after acid digestions (Thermo Electron; Model IRIS Intrepid II XDL, USA).

2.5 Estimation of total proteins, colony-forming unit, and biomass

The total extra cellular protein of the augmented samples and the control were analyzed using the Lowry method (Lowry et al., 1951). Total viable count of the bacteria in the bioaugmentation process was carried out using the plate counting method, the Nutrient Agar plates were incubated at 37 °C for 24 h (Chandra et al., 2018a,b,c). A single colony was picked and re-suspended in 1 mL sterile distilled water in a pre-weighed Eppendorf tube, the tube was centrifuged and dried at 50 °C until constant weight. The weight loss equaled to the bacterial biomass per mL.

2.6 Estimation of enzyme assay

The estimation of enzymatic activity of manganese peroxide (MnP) and laccase during the bioaugmentation process was carried out using a method described by Arora et al. (2018). The cells were harvested using centrifugation at 6500 g x for 10 min at 4 °C, the supernatant was retained for enzyme analysis.

2.7 Fourier transform-infrared spectrophotometry and UV–Vis spectrophotometer analysis

Fourier transform-infrared spectrophotometry analysis of the purified extract was performed using a spectrophotometer (Nexus890, Thermo Electron Co., Yokohama, Japan) in order to identify the chemical nature of wastewater. The purified samples were dispersed in spectral-grade KBr (Merck, Darmstadt, Germany) and made into pellets by applying 5–6 tons cm^{-2} of pressure for 10 min using a hydraulic pressure (Specac, United Kingdom) instrument. The spectrum was generated in the range of 400 to 4,000 cm^{-1} with a resolution of 4 cm^{-1} for all samples to determine the degradation and decoloration in the bioaugmentation process sample (maximum absorption). The effectiveness of the constructed bacterial consortium to decolourize distillery wastewater was measured using a UV–Vis spectrophotometer at the wavelength range 200–700 nm (Sharma et al., 2021d).

2.8 Characterization of organic compounds

The optimal organic solvent for extracting organic contaminants was ethyl acetate. The distillery wastewater sample (10 mL) was measured, mixed with 10 mL of ethyl acetate solvent in an Erlenmeyer (250 mL) flask and stirred constantly for 5 h. The extraction of the organic

pollutants was performed successively three times. After bioremediation, a similar procedure was used to detect metabolic products using GC–MS analysis.

2.9 Statistical analysis

Each experiment was carried out in triplicates. The standard deviation (SD) was determined with a Mean ±SD value from Microsoft Excel (ver. 2007, Microsoft®, USA). The experimental data was further analyzed using Student t-test.

3. Results and discussion

3.1 Identification of isolated bacterial strains

16S rRNA sequencing was used to identify the purified isolated and autochthonous bacterial strains. The parameters of the Gamma model were determined to within 0.1000000000 logarithmic probability units of accuracy. Two isolated *Stenotrophomonas maltophilia* (RCS1) and *Bacillus cereus* (RCS 2), as well as bioaugmented strain *Bacillus anthracis* (RCS-3), were deposited to the NCBI database based on the 16S rRNA sequencing data.

3.2 Physico-chemical analysis

The physico-chemical properties of the control (distillery wastewater), and bioaugmentation samples (at Time 0 and 144 h) are shown in Table 1. The control samples showed higher pH, TDS, BOD, COD, and EC values. The elevated pH of the control sample may be attributed to the binding of melanoidin with the high levels of soluble products and heavy metals in the distillery wastewater. The Time 0 samples showed that after autoclave some chemical characteristics had been altered but the extend was not significant except for the chloride content. McGuire and Judd (2020) demonstrated there were significant positive relationship between organic matter and Cl^- retention, it was likely as a result of the thermal treatment chloride was released from the bound organic matter.

Significant reduction in the pollution criteria in the wastewater was observed at the end of the bioaugmentation period (Table 1). The elevated values of COD and BOD of the untreated wastewater (control) indicated the existence of greater amounts of organic and inorganic compounds. Metals may be released during the fermentation and distillation processes of fermented sugarcane molasses in distilleries, resulting in post-methanation distillery effluent (PMDE) with an alkaline pH that is eventually discharged. At Time 0, small reduction in metal

content was observed, probably as a result of passive adsorption onto the dead bacterial cells, which were removed after the centrifugation and filtration process. In comparison, after 144 h of incubation, the treated bioaugmented samples showed a significant reduction in all of different pollution parameter except for Cl^-. This suggested that the constructed bacterial consortium was actively biodegrading and bio transforming various organic and inorganic contents. The pH of the medium was initially reduced to 4.47 during melanoidin degradation, but after 144 h of treatment, the pH steadily increased. The development of organic acids such as phosphoric acid, acetic acid, octadecanoic acid, and ethanedioic acid, can caused a reduction in pH during the early stages of bacterial growth. However, examination of BOD and COD values at different periods indicated a steady decline in BOD and COD values as the percentage of decolorization increased.

Table 1. Physico-chemical analysis of discharged distillery wastewater collected from M/s Unnao Distillery Pvt. Ltd. Unnao, Uttar Pradesh, India, and wastewater underwent bioaugmentation at Time 0 h and Time 144 h.

Parameters	Distillery wastewater	Bioaugmentation At Time = 0h	Bioaugmentation at Time = 144h	Permissible Limit (CPCB 2012)
pH	8.00 ± 0.01	7.20 ± 0.12	8.05 ± 0.09ˆa	7.54± 0.01
Electric conductivity	3.6 ± 3.211	2.9 ± 2.21	1.9 ± 2.21ˆa	-
Ions				
Na^+	65± 5.298	45.230 ± 0.97	12.453 ± 0.80ˆa	-
Cl^-	2021 ±0.901	9788.9 ± 0.12	5543.000 ± 565.4ˆns	11.82± 0.01
NO^{3-}	201 ±3.14 l	120.916 ± 1.30	47.295 ±0.90ˆa	47.00± 0.00
Ammoniacal nitrogen	195 ± 1.657	123.111 ± 0.11	70.000 ± 0.11ˆa	9.90± 0.00
Total dissolve solid	25980.6 ± 8.09	14987.9± 1.03	3541 ± 90.29ˆa	152± 0.01
Biological oxygen demand	20534.5 ± 3.12	10987.2 ± 7.90	2111.990 ± 1.78ˆa	47.00± 0.00
Chemical oxygen demand	41980.9 ± 0.043	20981 ± 1.32	4000.111 ± 1.23ˆa	79.00± 0.01
Total organic carbon	20.225 ± 0.112	14.211 ± 0.98	6.321 ± 0.89ˆa	11.82± 0.01
Total Sulphate	5.111 ± 0.000	2.000 ±0.09	1.111 ± 0.87ˆb	-
Heavy metals				
Iron (Fe)	2598 ± 7.000	1235.88 ± 3.00	852.528 ± 1.05ˆa	-
Zinc (Zn)	386.12 ± 6.787	85.111 ± 0.32	23.456 ± 1.67ˆa	0.15
Copper (Cu)	85.21 ± 5.212	54.658 ± 1.20	12.435± 1.02ˆa	0.01
Chromium (Cr)	24.987 ± 0.321	11.432 ± 0.90	7.800 ±0.43ˆa	1.28
Cadmium (Cd)	3.213± 1.981	0.420 ± 0.98	0.10 ± 0.09ˆc	0.19
Manganese (Mg)	222.23 ± 0.345	80.760 ± 2.87	46.140 ± 0.34ˆa	1.45
Nickel (Ni)	16.789 ± 0.932	6.432 ± 0.89	3.750 ± 0.11ˆa	0.02
Lead (Pb)	20.66 ± 1.11l	11.999 ± 8.76	6.111 ± 0.11ˆa	BDL

All the values are Mean SE. (n=3); Unit of all parameters is in (mgL) except pH, color (Co-Pt. Unit) and EC (mhoscm); Students t test (two tailed as compared to pre-treated sludge); Highly significant at $p<0.001$; Significant at $p<0.01$; Significant at $p<0.05$; Non-significant at $p>0.05$; BDL: Below detection limit.

3.3. Optimisation of the bioaugmentation process

For a 144-hour bacterial incubation period, distillery wastewater degradation was assessed using different glucose concentrations. Results showed that the consortium's efficiency increased as the glucose concentration increased from 0.1 to 1% (w/v), achieving maximum decolorization of 70%–90%. Further increase in the glucose levels did not encourage the growth or decolorization of melanoidins by bacteria. However, the combination of glucose and peptone failed to stimulate the bacterial consortium, which only achieved up to 50%–60% of melanoidins decoloration. This agreed with the previous observation that the increased peptone concentrations reduced the decoloration effectiveness of bacterial consortium (Sharma et al., 2020a,b, 2021c), probably as a result of preferential utilization of the nitrogen source in peptone by the consortium over the complex nitrogen source present in the distillery wastewater. The pH and temperature are significant factors in melanoidin decolorization and bacterial community biochemical performance. At pH 8.1, the bacterial consortium recorded higher decolorization (79%) due to the combined action of the bacterial enzymes and the loosening of conjugated bonds of Maillard reaction products. The bacterial consortium depolymerized melanoidins at high pH, causing the color to fade. The optimized bacterial consortium also showed maximum decolorization (81%) at 37°C, while increasing the temperature to 45°C harmed the bacterial consortium's growth and decolorization capacity. Moreover, the impact of shaking speed revealed that 180 rpm resulted in the best degradation of organic pollutants by a potential bacterial consortium.

3.4. Performance of total proteins, CFU, and biomass

At different incubation times, the increasing total protein content correlated positively with the trends in oxidative enzymes. The analyzes of total protein, CFU and biomass revealed a constant increase in the bioaugmentation samples compared to the untreated control and the samples from Time 0. Periodic SEM assessments of the treated samples displayed an improvement in bacterial population. It was noted that after sterilizing the wastewater, there was reduction in the color compared to the control, which could be attributed to the thermal destruction of some of the polymers. However, the decolorization was much more pronounced in the bioaugmentation samples, suggesting active degradation of melanoidins by the constructed bacterial consortium.

The optimization process indicated the best decolorationof melanoidins (90%) was obtained after 144 h. The biomass reached 5.22 gL^{-1} and the final bacterial count of 10^6 $CFUmL^{-1}$. This is confirmed by the denser bacteria cell observed using the SEM.

3.5 Enzyme activity during degradation of bioaugmentation process

Ligninolytic enzymes play a major role in decolorization of melanoidins, and promote the development of microorganisms in mixed pollutants (Bonugli-Santos et al., 2012). Laccase and MnP are substrate oxidizing enzymes that can serve a wide variety of chemical bonds found in recalcitrant compounds, including phenolic and non-phenolic compounds. MnP and laccase development may be attributable to the depolymerization of melanoidins. MnP is the dominant enzyme at the initial stage of melanoidin degradation during the bioaugmentation process. MnP activity was highest at 144h (3.8 U $mL^{-1}min^{-1}$) whereas maximum laccase activity was detected at 144h (2.39U $mL^{-1}min^{-1}$). This suggested MnP was directly involved with the degradation of melanoidins resulting in the production of phenolic compounds that triggered the laccase enzyme production.

3.6 Metabolites assessment FTIR analysis and UV Vis-Scanning

The FTIR analysis confirmed degradation of the organo-metallic compounds in the bioaugmentation bacterial treatment (Fig. 1). FTIR analysis showed that the thermal treatment altered the functional groups compared to the control and further changes were caused by the bioaugmentation process. The UV–Vis spectrophotometry scanning of the sterilised sample (Time 0) revealed very similar pattern to the untreated control while the bioaugumented samples showed peak shifting, reduction in height of peaks, and the detection of new chemical bonds and metabolites (Fig. 1a, b), demonstrating the consortium's ability to decolorize and degrade distillery wastewater by biotransformation and biodegradation of distillery wastewater into various metabolites (Table 3).

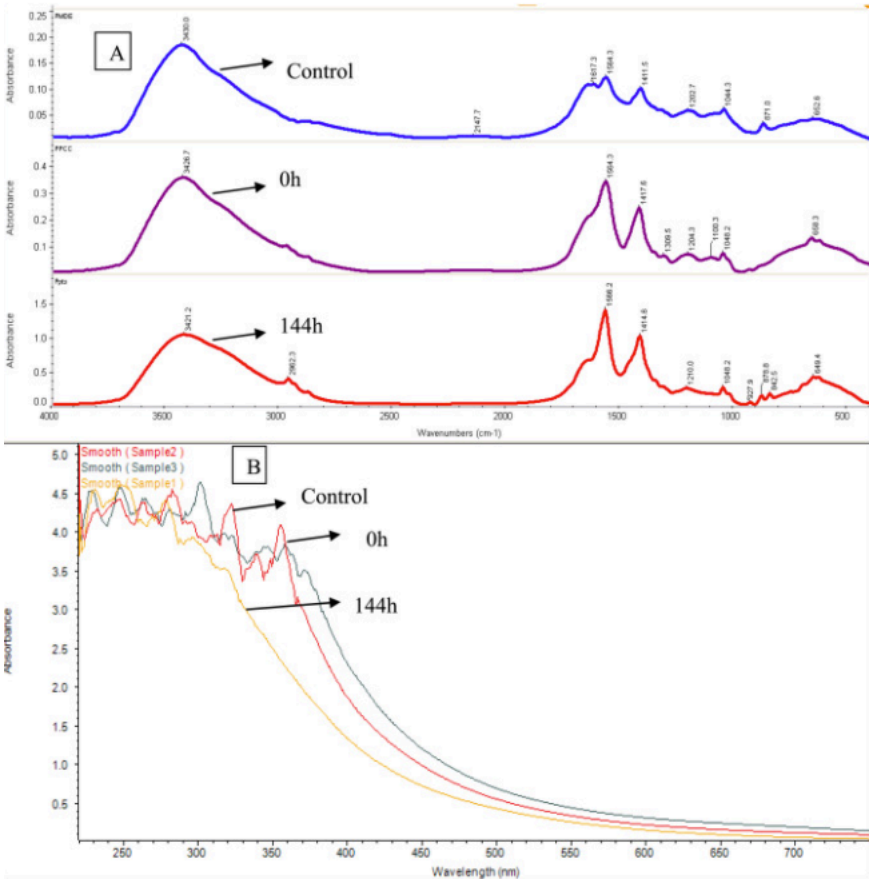


Fig. 1. Chemical, visual and microscopic analysis of the untreated distiller wastewater (control), wastewater treated by the constructed consortium at time (T) 0 and 144h. (a)FTIR analysis; (b) UV Vis scanning analysis.

3.7 Metabolites identification after the bioaugmentation process

The metabolites produced by the constructed bacterial consortium were identified using GC-MS (Table 2). The untreated distillery wastewater containing a large number of organic compounds that were absent in the treated wastewater. The main metabolic products identified as phenol and phenolic compounds in the control were classified as pyridine, 2-methyl-3-[TMS ester]-4,5-bis-[(TMS ester)methyl], 12-methoxy-2trimethylsilyloxy-19-nor-5β-podocarpa-1,3,8,11,13-pentane, 3-benzyl-1,4-diazo-2,5-dioxobicyclo[4,3,0] nonane, hexadecanoic acid, 2,3-bis[(TMS)oxy]propyl ester[CAS], (Ss*,S*)-phenyl[((2,4,6-TM phenyl) sulfinyl)phenyl) methanol, 2-monostearin TMS ether, 1,3-dimethyl-6-TMS oxymethyl-uracil. These products demonstrated that the 2-methoxyphenol group was present in their structures but had some replacements on their aromatic rings. A number of the organo-metallic compounds were thermally degraded after autoclave but a number of thermal stable compounds remained (Table 2). However, after the bioaugmentation process, all these

compounds were either degraded below the detection limit or greatly reduced in quantity, confirming again that active biotransformation and biodegradation had taken place. It was interesting to note that butanoic acid, bis [(TMS) ester was detected in the bioaugmented samples only and was absent in the untreated and thermally treated samples, indicating the production of a new metabolite.

Table 2. Identification of residual organic pollutants in distillery industry wastewater before and after bioaugmentation process by GC-MS analysis.

S.No.	RT	Compound name	Control	0hr	144hr
1.	7.96	Propanoic acid,3 –[(trimethylsilyl)oxy], TMS ester [CAS]	+	+	-
2.	8.20	1- TMS-TRICYCLO [2.2.1.2(2,6)] HEPTANE	+	-	-
3.	10.40	BUTANOIC ACID,2-[(TMS]OXY]-T TMS ESTER	+	-	-
4.	10.83	3-Hydroxy- 2-butanone TMS ether	+	-	+
5.	10.87	t- Butyldimethyl[2-styryl [1,3] dithian-2-yl]silane	+	+	-
6.	11.06	Butane-1,3diol, 1-methyl,bis [trimethylsilyl] ether+	+	-	-
7.	11.18	3-Hydroxy- 2-butanone, trimethylsilyl ether	+	+	-
8.	12.02	2- hydroxyisocaproic acid, TMS ether, TMS ester	+	-	-
9.	12.33	Silanol,trimethyl-,benzoate [CAS]	+	+	-
10.	12.78	2-Methyl-2,3-dihydro-1Hbenz[g] indole	+	-	-
11.	12.97	Silane,trimethyl[1-methyl butoxy]	+	+	+
12.	13.05	Silane,trimethyl[1-methyl butoxy]	+	-	-
13.	13.45	2,3-BUTANDIOL,BIS-0-[TMS]	+	-	-
14.	13.57	2-METHYL-1,-PROPANEDIOL 2 TMS	+	+	-
15.	14.95	D-[-1]-Lactic acid, trimethylsilyl ether, TMS ester	+	+	+
16.	16.03	Silane,[(1-methyl-1,3-propanediyl]Bis(oxy]) Bis[(TMS)	+	-	-
17.	16.03	DITHIOTHREITOL 4 TMS	+	-	-
18.	17.42	TRIMETHYLSILYL-3 HYDROXYISOVALERIC ACID	-	+	+
19.	17.75	3,7-dioxa-2,8-disilanonane,2,2,8,8-TM-5-[(TMS)oxy]	+	-	-
20.	18.29	Butanoic Acid, Bis[(TMS) ester	-	-	+
21.	19.33	Hexadecanoic acid, TMS ester	+	+	-
22.	20.83	Trans-9- Octadecanoic acid, TMS ester	+	-	-
23.	22.65	Octadecanoic acid, trimethylsilyl ester	-	+	-
24.	24.92	Pyridine, 2-methyl-3-[TMS ester]-4,5-bis-[(TMS ester)methyl]	+	-	-
25.	27.43	12-Methoxy-2 trimethylsilyloxy-19-nor-5β-podocarpa-1,3,8,11,13-pentane	+	+	+
26.	29.52	1,,3-DIMETHYL-6- TMS OXYMETHYL-URACIL	+	-	-
27.	29.80	3-cyano-5,6-dihydro-2-Methyl-4-(methylthio)-[1]benzoxepino	+	-	-
28.	29.87	9,2- Octadecanoic acid, [Z,Z]- TMS [CAS]	+	-	+
29.	29.97	Trans-9-Octadecanoic acid, TMS ester	+	+	+
30.	30.33	[+]-,11,12-cis-10,11-dihydro-12-hydroxy-4-propyl-6,6,11-trimethyl	+	-	-
31.	32.03	3-benzyl-1,4-diazo-2,5-dioxobicyclo[4,3,0] nonane	+	-	-
32.	33.73	Hexadecanoic acid,4-[(TMS)oxy] butyl ester [CAS]	+	+	+
33.	33.94	Hexadecanoic acid, 2,3-bis[(TMS)oxy]propyl ester[CAS]	+	-	-
34.	34.32	(Ss*,S*)-Phenyl[((2,4,6-TM phenyl) sulfinyl)phenyl) methanol	+	-	-
35.	35.27	2-Monostearin TMS ether	+	+	-
36.	39.40	Silane, [[(3β)-cholest-5-en-3-yl]oxy]trimethyl-[CAS]	+	-	-
37.	42.94	Silane, trimethyl[[(3β)-stigmast-5-en-3-yl-]oxy]-[CAS]	+	-	-

Key: present; - absent or below detection limit.

Table 3. Summary of identified FTIR band observed in sediments collected from distillery wastewater discharged site at different time intervals in bioaugmentation process.

Control	Functional Group	Intensity	0h	Functional Group	Intensity	144h	Functional Group	Intensity
3610.8	Alcohol O-H	Strong	3431.8	Amine, N-H	Medium	3409.7	Amine, N-H	Medium
3434.7	Amine, N-H	Medium	2922.2	Amine, N-H	Medium	2924.4	Amine, N-H	Medium
2923.8	Carboxylic Acid, O-H	Strong	2853.1	Carboxylic Acid, O-H	Strong	2855.0	Carboxylic Acid, O-H	Strong
2854.0	Carboxylic Acid, O-H	Strong	2032.1	Alkyne, C≡C	Variable	1710.3	Ketone C=O	Strong
2031.0	Alkyne, C≡C	Variable	1709.7	Ketone C=O	Strong	1663.3	Amide C=O	Strong
1737.5	Ketone C=O	Strong	1663.9	Amide C=O	Strong	1459.3	Benzene Ring C=C	Strong
1638.4	Amide C=O	Strong	1461.9	Benzene Ring C=C	Strong	1367.2	Alkane -C-H	Medium
1576.9	Benzene Ring C=C	Strong	1370.6	Alkane -C-H	Medium	1234.6	Acid C-O	Strong
1462.6	Benzene Ring C=C	Strong	1297.7	Acid C-O	Strong	1187.5	Acid C-O	Strong
1378.8	Alkane -C-H	Medium	1225.9	Acid C-O	Strong	1080.3	Ester, C-O	Strong
1294.1	Acid C-O	Strong	1121.1	Ester, C-O	Strong	969.2	Alkene, =C-H	Medium
1211.3	Acid C-O	Strong	1081.4	Ester, C-O	Strong	892.7	Alkene, =C-H	Medium
1077.8	Ester, C-O	Strong	1033.6	Ester, C-O	Strong	826.3	Alkene, =C-H	Medium
1035.1	Ester, C-O	Strong	967.9	Alkene, =C-H	Medium	716.7	Alkyl Halide, C-Cl	Strong
968.5	Alkene, =C-H	Medium	721.5	Alkyl Halide, C-Cl	Strong	602.3	Alkyl Halide, C-Cl	Strong
892.7	Alkene, =C-H	Strong	494.8					
724.0	Alkyl Halide, C-Cl	Strong						

3.8 Practical applications of bioaugmentation of distillery wastewater

As the value of the environmental parameters and metal content detected in the discharged distillery wastewater were considerably higher than the permissible limit prescribed by the CPCB (2012) (Table 1), it suggested strongly that the secondary treatment process was not adequate in treating the effluent before it was discharged into the receiving water. Similar observations also indicate it is a widespread problem associate with various industries in India (Chandra et al., 2018a,b,c; Yadav and Chandra, 2018; Sharma et al., 2020b) and additional treatment is needed to safeguard the environment and human health. Compared to chemical and physical remediation methods, biological treatment processes are very economical and efficient options when the wastewaters contain biodegradable pollutants. This study showed that the use of bioaugmentation to treat industrial effluent could be a sustainable and effective (tertiary) treatment method to enhance the removal of organo-metallic and refractory organic pollutants. However, despite of a number of encouraging results in laboratory scales, this approach has not translated to full scale wastewater treatment. Further work to understand and optimize the bioaugmentation process, such as substrates concentration, temperature changes, pH, nutrient limitations, competition between introduced and indigenous microorganisms, pollutant load, grazing by protozoa, sequential operation of the bioreactors, could further improve the performance of the bacterial consortium and provide valuable information for scale-up. Engagement with relevant stakeholders (e.g., industry owners, policy makers and

environmental engineers) will be paramount in order to transfer this process to a higher technology readiness level (TRL).

4. Conclusion

Our findings revealed that distillery wastewater was highly contaminated with elevated BOD, COD, TSS, TDS, organo-metallic complex compounds (such as EDCs), and heavy metals. Distillery wastewater could be successfully treated by a constructed bacterial consortium using a bioaugmentation process in 144h through active biotransformation and biodegradation of the pollutants. Heavy metals were biosorbed by the bacterial consortium, and the ligninolytic enzymes such as Lip and MnP played an important role in the degradation of the organo-metallic pollutants. The constructed bacterial consortia *Stenotrophomonas maltophilia* (RCS1) *Bacillus cereus* (RCS 2), and *Bacillus anthracis* (RCS-3) therefore can offer a sustainable solution to treat distillery wastewater. However, despite significant reductions in the pollution parameters by the constructed bacterial consortium, the resulting wastewater still contained levels of pollution above permissible limit published by the CPCB, therefore, further research is needed to optimize the system to maximize the consortium's remediation potential.

Credit authorship contribution statement

Sonam Tripathi: Sample collection, Experiment plans, Writing - original draft, Writing - review & editing. **Pooja Sharma:** Sample collection, Experiment plans, Writing - original draft, Writing - review & editing. **Diane Purchase:** Data analysis, Writing - review & editing. **Madhu Tiwari:** Bacterial sequence analysis. **Debasis Chakrabarty:** Bacterial sequence analysis. **Ram Chandra:** Funding acquisition, Experiment plans.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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