Research Article

Investigation of Physicochemical Characteristics, Elemental Composition and GCMS Studies on Steel Plant Effluent Treated with Indigenous Bacteria

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ABSTRACT

Aim/ Background: Steel plant effluents pose significant environmental challenges due to the high concentrations of contaminants such as heavy metals and organic compounds. This study aimed to evaluate the potential of indigenous bacteria, specifically *Shewanella* sp. strain DADJ and *Bacillus licheniformis*, as bioremediation agents for the treatment of steel plant effluent. **Materials and Methods:** Two indigenous bacterial strains, *Shewanella* sp. strain DADJ and Bacillus licheniformis, were isolated and exposed to steel plant effluent for a 15-day period. The physicochemical properties of untreated and treated effluent, including pH, Total Dissolved Solids (TDS), Chemical Oxygen Demand (COD), and Biochemical Oxygen Demand (BOD), were analyzed. Additionally, the reduction of iron and other heavy metals (cadmium, arsenic, chromium, lead, and mercury) in the effluent was assessed using ICP-OES spectroscopy. Organic compounds in the effluent were analyzed both before and after treatment using Gas Chromatography-Mass Spectrometry (GC-MS). **Results:** The results showed that the untreated effluent had high concentrations of iron and heavy metals. After treatment with the bacterial strains, the iron content was drastically reduced to 18.12 ± 3.15 µg/mL and 23.08 ± 0.61 µg/mL in the effluents treated with *Shewanella* sp. strain DADJ and *Bacillus licheniformis*, respectively. The ICP-OES analysis revealed significant reductions in cadmium, arsenic, chromium, lead, and mercury. GC-MS analysis indicated that the organic compounds, including 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester, and Silane, dimethyl(2-naphthoxy) heptyloxy, were present only in the treated effluent, suggesting the transformation of existing chemical compounds into novel ones. **Conclusion:** The Indigenous bacterial strains *Shewanella* sp. strain DADJ and *Bacillus licheniformis* demonstrated effective bioremediation potential for treating steel plant effluent by significantly reducing iron, heavy metals, and organic contaminants.

Keywords: Bacteria, Bioremediation, Effluent, Heavy metals, Steel plant, Wastewater.

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INTRODUCTION

The steel industry remains an integral part of contemporary society, providing the energy needed to build important structures as well as the machinery required to advance industrialization.[1] The massive volume of water required for cooling, cleaning and processing that comes from the manufacturing of steel creates a substantial effluent stream that is full of contaminants. This effluent contains a variety of contaminants, including suspended particles, heavy metals and organic compounds, all of which have detrimental impacts on the environment and public health.[2] Heavy metals including lead, chromium and mercury as well as organic pollutants like Volatile Organic Compounds (VOCs) and Polycyclic Aromatic Hydrocarbons (PAHs) can be found among these contaminants. Moreover, oils, dust, greases and

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suspended solids are frequently found in the effluent of the steel sector.[3]

Steel factory effluent, which is either untreated or not sufficiently treated, is released into water bodies, where it contaminates aquatic habitats and threatens the delicate equilibrium of aquatic life.[4] Lead, chromium and cadmium are prominent instances of heavy metals that can build up in sediments and biota, causing bioaccumulation and biomagnification up the food chain that ultimately puts aquatic species and people at risk that depend on these environments for survival.[5] The production of steel also leads to the generation of effluents that contain harmful substances such as cyanide, ammonia, metal particles and acids. These substances have contaminated water bodies and adjacent land areas, making them unsuitable for human usage. The blood, kidneys, bones and liver are among the essential organs impacted by heavy metals and so this wastewater must be treated effectively prior to discharge.[6]

It is critical to address the problem of wastewater discharge from steel plants in light of these widespread and serious environmental and public health concerns.[7] The treatment of effluent from the steel industry is a confluence of challenges and opportunities. The treatment process must effectively and precisely negotiate a maze of pollutants, from the removal of suspended particles and heavy metals to the mitigation of organic compounds and pH adjustment.[8]

A variety of technologies and methodologies are at the forefront of wastewater treatment for steel plants, each specifically designed to meet the demands of industrial activities and the distinct composition of effluent streams.[9] The eradication of suspended particles and particulate matter is facilitated by physical processes like sedimentation, filtration and centrifugation, while chemical approaches like coagulation, flocculation and oxidation aid in the precipitation and neutralization of contaminants.[10]

In contrast, biological treatment techniques use the ability of microbial communities to break down organic materials and lower pollution levels. These microbes have evolved the capacity to defend against the harmful effects of heavy metals through a variety of processes, including reduction, adsorption, absorption, methylation and oxidation.[11] Heavy metals can be eliminated from aqueous solutions through many methods, with the aid of microbes and higher plants. Bioremediation is one of the waste management strategies that can be applied *ex situ* or *in situ* that employs primary microbes to decompose or neutralize contaminants. It can be used on steel industrial waste that is solid, liquid, or gaseous state.[12]

Biological treatment is less expensive, more environmentally friendly and reduces the concentration of inorganics as well as the organic loadings of COD and BOD. Microbial action uses electron acceptors including sulfate, nitrate and carbon dioxide to transform colloidal and dissolved organic materials into stable solids.[13] Microbes isolated from contaminated sources are used to bioleach and biosolubilize heavy metal-loaded effluents, eliminating heavy metals more effectively than non-polluted sources.[14] Therefore, our study aims to evaluate the potential of indigenous microbes as a potential bioremediation agent for the steel plant effluent treatment.

MATERIALS AND METHODS

Sample collection

The effluent was collected at the discharge pipe from the Steel manufacturing industry, Salem. The effluent sample was collected in sterile glass bottles and it was tightly sealed. The collected sample was promptly moved to the laboratory and stored at 4ºC. Physiochemical parameters such as pH, TDS, EC, salinity, salinity specific gravity, resistivity, ORP, nitrates, nitrites, ammonia, chloride, sodium, sulphide, total phosphates, inorganic phosphate, organic phosphate and calcium present in the effluent were examined and recorded in Table 3.

Enumeration and Isolation of microbes

Serial dilution method was performed to isolate the microbes present in the effluent sample. The collected effluent sample was serially diluted to 10^{-8} and 1 mL of each dilution was aseptically plated in the nutrient agar and potato dextrose agar. The petri plates were incubated for 24 hr at 37ºC. The microbial count of the collected effluent was analyzed and represented as Colony Forming Unit per mL (CFU/mL). Random colonies were selected from each dilution and seeded to the fresh nutrient agar and potato dextrose agar plates. The plates were incubated at 37ºC overnight and the colony morphology of the selected isolates was studied.

Screening of microbes for bioremediation

In the present study, COD and BOD were taken as a parameter to select the microbes. All the isolated microbes were subjected to degradation studies for 24 hr. The degradation experiment was carried out using the shake flask method. The collected steel industrial effluent (100 mL) was introduced to each of the volumetric flasks and pre-incubated 1% of selected bacterial and fungal isolates for 24 hr with continuous agitation at the rate of 180-200 rpm. The treated effluent

was utilized to determine BOD and COD levels after incubation.[15]

Identification of Strain by Sequencing and NCBI Submission

The process began with PCR amplification using universal primers and conditions specified by Weisburg *et al.* (1991). The resulting DNA fragments were sequenced using the dideoxy chain termination method, with sequencing services provided by Macrogen, Inc. in South Korea. Subsequently, we analyzed the DNA sequences for similarity using the BLAST search tool from the National Center for Biotechnology Information (NCBI), utilizing the GenBank database as described by.[16]

Bioremediation using selected microbes

1% of selected bacterial isolates were inoculated to the flasks containing effluent samples respectively and it was incubated for 15 days.[17] After incubation, the effluent treated using *Shewanella* sp. strain DADJ and *Bacillus licheniformis* bacterial strains were tested for physiochemical parameters.

Physiochemical analysis

Physiochemical parameters such as pH, TDS, EC, salinity, salinity specific gravity, resistivity, ORP (Oxidation-Reduction Potential), nitrates, nitrites, ammonia, chloride, sodium, sulphide, total phosphates, inorganic phosphate, organic phosphate and calcium present in the effluent treated with bacterial strains *Shewanella* sp. strain DADJ and *Bacillus licheniformis* were examined and compared with untreated effluent.[18]

Determination of Iron

The Ferrozine method was used to determine the iron content in the untreated as well as *Shewanella* sp. strain DADJ and *Bacillus licheniformis* treated effluent according to the protocol of.[19] The cuvette was filled with 1 mL of each HCl-fixed sample and 0.1 mL of ferrozine solution, which reacts with Fe^{2+} to generate a pink complex. After 15 min of treatment, the absorbance was measured at 562 nm spectrometerically. The optical densities of the samples were compared to a calibration curve made from the standard iron solutions to ascertain the amount of iron present in the effluent samples.

Analysis of elemental composition

ICP-OES (PerkinElmer Optima 5300 DV, Germany) was used to analyze element concentrations present in the untreated as well as *Shewanella* sp. strain DADJ and *Bacillus licheniformis* treated effluent, including cadmium, arsenic, chromium, lead and mercury. Samples were digested and filtered before ICP-OES analysis. The ICP-OES was directly aspirated to analyze the target elements.[20]

GCMS analysis

GCMS analysis for the untreated effluent and the effluent treated with *Shewanella* sp. strain DADJ and *Bacillus licheniformis* were performed using the GCMS Agilent Technologies (GC: 8890; MS: 7000D) equipped with 30 mX250 mX0.25 m HP 5MS Ultra inert capillary column. Helium (UHP Grade) served as the carrier gas and it flowed at a rate of 1.516 mL per minute. The recorded chromatogram was compared with the NIST library of MS spectra to identify the compounds that existed in the samples.

RESULTS

Enumeration and Isolation of microbes

In the present study, some regular and spherical-shaped bacterial and fungal colonies were obtained. A total of 5 bacterial strains and 3 fungal individual colonies were isolated from the effluent sample. The selected bacterial isolates were named B1, B2, B3, B4 and B5, whereas the fungal isolates were labelled as F1, F2 and F3. The colony morphological characteristics were studied for the selected bacterial and fungal isolates (Table 1). The colony forming unit per ml calculated for bacteria and fungi were $2.43x10^{-4}$ and $1.76x10^{-2}$ respectively.

Screening of microbes for bioremediation

The BOD and COD of the untreated effluent and the effluent treated with isolated bacterial and fungal strains were analyzed and the results were provided in Table 2. The BOD and COD of the untreated effluent were found to be 280.4 mg/L and 388.0 mg/L respectively. The maximum reduction of BOD and COD was exhibited by the bacterial isolate B1, followed by the bacterial isolate B3. The BOD of the B1 and B3 treated effluent was calculated as 83.95 and 76.02 mg/L respectively, whereas the COD ranges 135.44 mg/L for B1 and 76.02 mg/L for isolate B3. However, the BOD and COD levels of the effluent treated with fungal isolates didn't show any considerable reduction, which was rather equal to the untreated effluent. Thus, further studies were conducted with the bacterial isolates B1 and B3.

Identification of Strain by Sequencing and NCBI Submission

In this study, universal primers were used to amplify the 16S rRNA gene fragment from selected bacterial strains. The resulting PCR products were successfully

Table 1: Colony morphological characteristics.

Microbes	Isolates	Shape	Pigmentation	Texture	Elevation
Bacteria	B1	Spherical	Off-white	Smooth	Convex
	B2	Spherical	Slight yellowish	Smooth	Convex
	B3	Spherical	Off-white	Smooth	Convex
	B4	Irregular	Off-white	Smooth	Flat
	B5	Spherical	Whitish	Smooth	Flat
Fungi	F1	Filiform	Blue-green	Smooth	Umbonate
	F2	Filiform	Black	Rough	Umbonate
	F3	Filiform	Orange	Smooth	Crateriform

Table 2: BOD and COD value of isolated species.

Sample	BOD (mg/L)	COD (mg/L)
Untreated effluent	280.4	388.0
Effluent treated with B1	83.95	135.44
Effluent treated with B2	130.00	233.01
Effluent treated with B3	76.02	163.03
Effluent treated with B4	110.49	359.0
Effluent treated with B5	123.8	260.41
Effluent treated with F1	270.8	380.09
Effluent treated with F2	266.76	374.98
Effluent treated with F3	258.73	376.65

amplified and sequenced. These sequences were then submitted to the NCBI database for further analysis. The GenBank accession numbers for these sequences PQ215959.1 and OM349563.1

Phylogenetic analysis was conducted using BLAST (Basic Local Alignment Search Tool) to determine the identities of the isolated bacterial strains are illustrated in Figures 1 and 2. The partial 16S rRNA gene sequences from the bacterial strains were found to have significant similarity with known bacterial species. Specifically, the sequences showed high homology with *Shewanella* sp. strain DADJ and *Bacillus licheniformis*. This indicates that the isolated strains are closely related to these species, confirming their identities as *Shewanella* sp. and *Bacillus licheniformis*, respectively.

Bioremediation using selected microbes

The steel plant effluent was mixed with the 1% bacterial isolate *Shewanella* sp. strain DADJ and *Bacillus licheniformis* and the bioremediation process was carried out for 15 days. After 15 days of incubation, the treated effluent samples were taken for further studies.

Physiochemical analysis

The results of physical parameters exhibited that the pH, conductivity, total dissolved solids, specific gravity and alkalinity of *Shewanella* sp. strain DADJ and *Bacillus licheniformis* treated effluent were significantly lower than untreated effluent. Likewise, the chemical parameters such as nitrate, nitrite, phosphate, copper, ammonia, sodium and sulphide were also analyzed (Table 3). The reduction that occurred in the level of physicochemical parameters in both treated effluents can be taken as an indicator of the effective bioremediation process.

Determination of iron

The concentration of iron content determined using the ferrozine method is given in Table 4. The iron

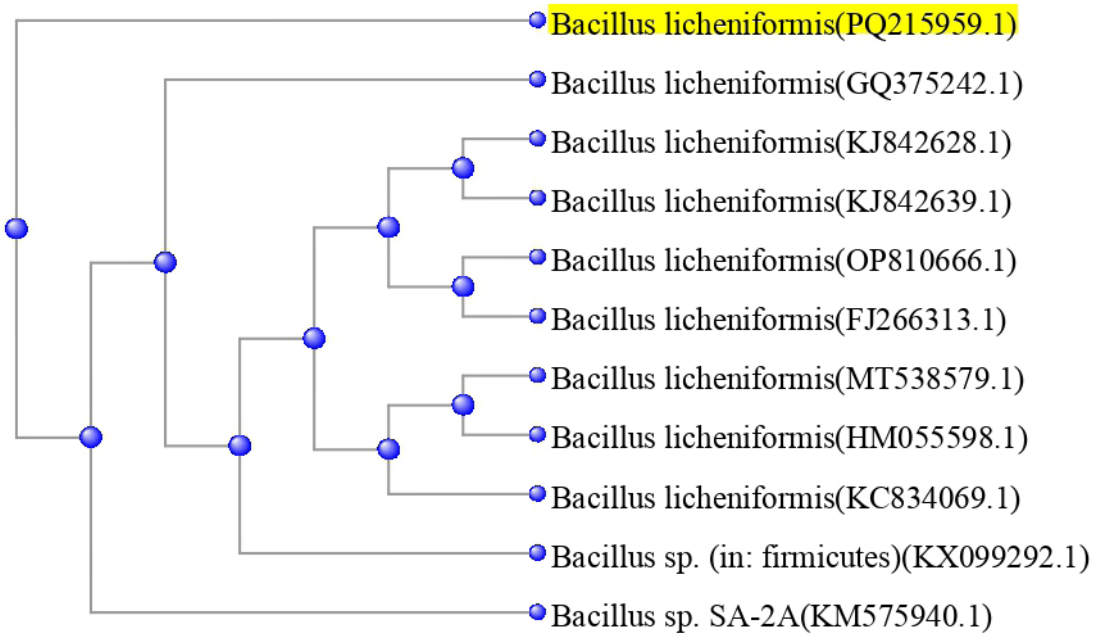

Figure 1: The phylogenetic tree of 16S rRNA of *Bacillus licheniformis* isolate with the closely related sequences.

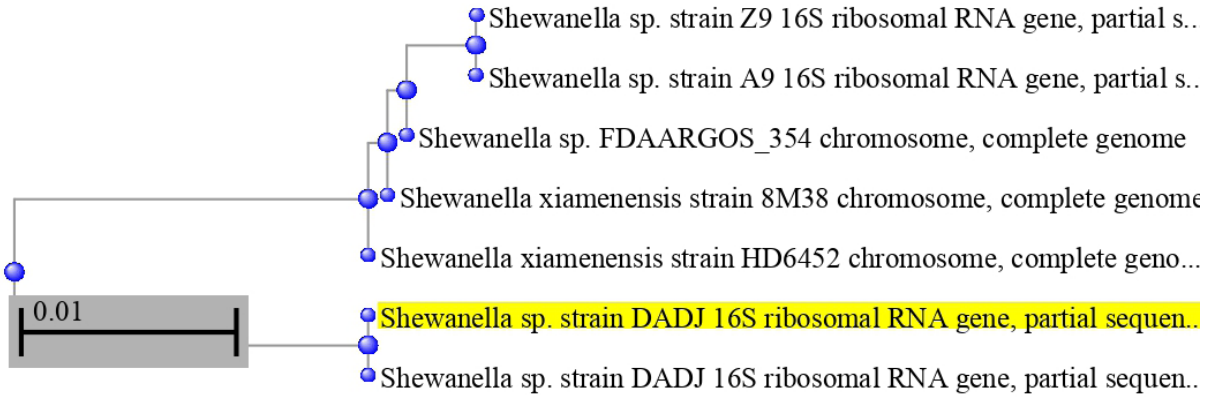

Figure 2: The phylogenetic tree of 16S rRNA of *Shewanella* sp. isolate with the closely related sequences.

Table 3: Physicochemical parameters.

Parameters	Untreated Effluent	B1 treated effluent	B3 treated effluent
pH	8.76	7.76	7.11
TDS (ppm)	1.389	836	493
Electrical Conductivity (µs/cm)	791	417	438
Salinity (%)	0.03	0.03	0.03
Specific gravity	1	1	1
Resistivity (KΩ)	19.27	2.546	2.629
ORP (mv)	36	175	155
Colour	Colourless	Colourless	Colourless
Odour	No significant odour	No significant odour	No significant odour
Alkalinity (mgL^{-1})	161.08	186.01	135.08
Nitrate (mgL^{-1})	92.00	47.22	29.14
Nitrite (mgL^{-1})	69.18	79.18	75.79
Sodium (mgL^{-1})	31.50	12.00	28.03
Copper (mgL^{-1})	19.00	7.49	8.33
Ammonia (mgL^{-1})	56.23	43.81	71.19
Organic Phosphate (mgL^{-1})	72.39	87.00	84.7
Inorganic Phosphate (mgL^{-1})	41.62	67.38	49.27
Total Phosphate (mgL^{-1})	159.55	182.15	170.51
Chloride (mgL^{-1})	45.95	24.10	39.49
Sulphide (mgL^{-1})	31.10	17.93	21.02

content present in the untreated effluent was found to be 41.54±5.75 µg/mL, which was considerably reduced in the *Shewanella* sp. strain DADJ and *Bacillus licheniformis* treated effluent. The concentration of iron in the *Shewanella* sp. strain DADJ and *Bacillus licheniformis* treated effluent samples was calculated to be 18.12±3.15 and 23.08±0.61 µg/mL respectively. The iron content was significantly reduced in the B1-treated effluent than the B2-treated effluent.

Table 4: Determination of iron.

Sample	Concentration of Iron (µg/mL)
Untreated effluent	41.54±5.75
B1 treated effluent	18.12±3.15
B3 treated effluent	23.08±0.61

Values are mean±SD.

Analysis of elemental composition

The elemental composition of the untreated and treated effluent is provided in Table 5. A total of five heavy metals were analyzed in all samples. However, chromium was only detected in all samples and the amount of chromium found in the untreated effluent was 0.082 mg/L. But, the level of chromium and iron was abruptly reduced in the *Shewanella* sp. strain DADJ (0.051 mg/L) and *Bacillus licheniformis* treated effluent (0.046 mg/L). Therefore, the reduction in the levels of chromium can be taken as a sign of the bioremediation carried out by the isolated strains *Shewanella* sp. strain DADJ and *Bacillus licheniformis*.

GCMS analysis

The GCMS analysis of the untreated effluent is given in Table 6 and Figure 3. The highest peak was obtained by the compounds such as benzene, nitro-, benzaldehyde, 3,5-dimethyl- and 1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester for all three tested samples. Some of the compounds that existed in the untreated effluent

Table 5: Analysis of elemental composition.

Elements	Concentration (mg/L)		
	Untreated effluent	B1 treated effluent	B2 treated effluent
Cadmium	Not detected	Not detected	Not detected
Arsenic	Not detected	Not detected	Not detected
Chromium	0.082	0.051	0.046
Lead	Not detected	Not detected	Not detected
Mercury	Not detected	Not detected	Not detected

Table 6: GCMS analysis of untreated effluent.

Sl. No.	Compound name	Retention time	Peak area (%)
1	Octacosane	5.431	0.82
2	Benzene, nitro-	5.920	43.14
3	Benzaldehyde, 3,5-dimethyl-	7.220	17.70
4	Pentadecane	7.653	1.20
5	Decane, 6-ethyl-2-methyl-	8.086	0.95
6	1-Undecene, 4-methyl-	8.175	0.52
7	Decane, 2,3,5-trimethyl-	8.253	0.47
8	1-Iodo-2-methyl-undecane	9.564	1.95
9	Dodecane, 4-methyl-	9.642	0.33
10	Eicosane	9.942	2.49
11	2-Bromo dodecane	10.019	0.47
12	Pentadecane, 2,6,10-trimethyl-	10.097	0.51
13	Heneicosane	10.197	0.37
14	Hexadecane	10.275	0.34
15	Hexadecane	11.242	1.98
16	Heneicosane	11.308	0.37
17	2-Bromo dodecane	11.564	2.17
18	Eicosane, 10-methyl-	11.642	0.59
19	Decane, 2,3,5-trimethyl-	11.708	0.75
20	Heneicosane	11.775	0.47
21	Hexadecane, 2,6,11,15-tetramethyl-	12.752	1.96
22	Octadecane, 2-methyl-	13.041	1.96
23	Heptadecane, 9-octyl-	13.086	0.70
24	Octadecane, 1-iodo-	13.152	0.50
25	2-Bromo dodecane	13.208	0.41
26	Octadecane, 1-iodo-	13.297	0.42
27	Octadecane	13.341	0.37
28	Heptacosane	14.108	1.09
29	Docosane	14.152	0.44
30	Hentriacontane	14.319	0.44
31	Octacosane	14.363	1.47
32	Pentacosane	14.408	0.56
33	Heneicosane	14.463	0.42
34	Octadecane, 1-iodo-	14.508	0.52
35	Heptacosane	14.586	0.62
36	Tetratriacontane	15.352	0.59
37	Octadecane, 1-iodo-	15.530	0.34
38	Heptacosane	15.574	0.98
39	1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester	16.452	8.28
40	Heptacosane	16.696	0.38

were Benzene, nitro-, Benzaldehyde, 3,5-dimethyl-, Heptacosane, Octacosane and Heneicosane. The GCMS analysis of *Shewanella* sp. strain DADJ and *Bacillus licheniformis* treated effluent also revealed 40 compounds (Figures 4 and 5). The bioactive compounds found in the *Shewanella* sp. strain DADJ treated effluent were Docosane, 3-Ethyl-3-methylheptane, Undecane, 4,6-dimethyl-, 2-Bromotetradecane and Eicosane, 2-methyl- (Table 7). Decane, 2,3,7-trimethyl- and Docosane, 11-butyl- were found only in *Bacillus licheniformis* treated effluent (Table 8). Likewise, the compounds such as 1-Undecene, 4-methyl-, Octadecane, 1-iodo-, 1-Iodo-2-methylundecane and Pentadecane, 2,6,10-trimethyl- were observed only in effluent sample. The chromatogram of *Shewanella* sp. strain DADJ and *Bacillus licheniformis* treated effluent exhibited numerous peaks and reduction of retention time and peak area percentage corresponds to the formation of new metabolites, as the result of conversion of organic compounds by the bacteria isolates. Therefore, those compounds might be utilized by the *Shewanella* sp. strain DADJ and *Bacillus licheniformis* isolates for their growth and metabolism, which can be taken as a confirmation of the bioremediation.

DISCUSSION

Industrial effluents are the primary source of harmful pollutants in any environment. The presence of different microbial communities in steel industry effluent demonstrates the possibility of biological treatment solutions to reduce environmental pollution.[21] Furthermore, isolating and characterizing microbial strains from industrial effluents aids in the construction of microbial repositories for biotechnology applications. These isolates can be used to select microbial consortia that target certain pollutant breakdown pathways.[22] Most organisms that are capable of surviving and continuing to function in extreme conditions can be found and possibly selected for bioremediation.[23] The isolated indigenous species showed an array of reactions to heavy metal variety and levels in the prior investigation.[24] Therefore, we have isolated the microbes from the collected effluent sample and used the selected isolates as a bioremediation agent for the steel plant effluent treatment. This study uncovered an assortment of microbial populations living in steel industry effluent, including bacteria and fungi. The microbial species have been shown to exhibit diverse metabolic capacities, including the breakdown of organic

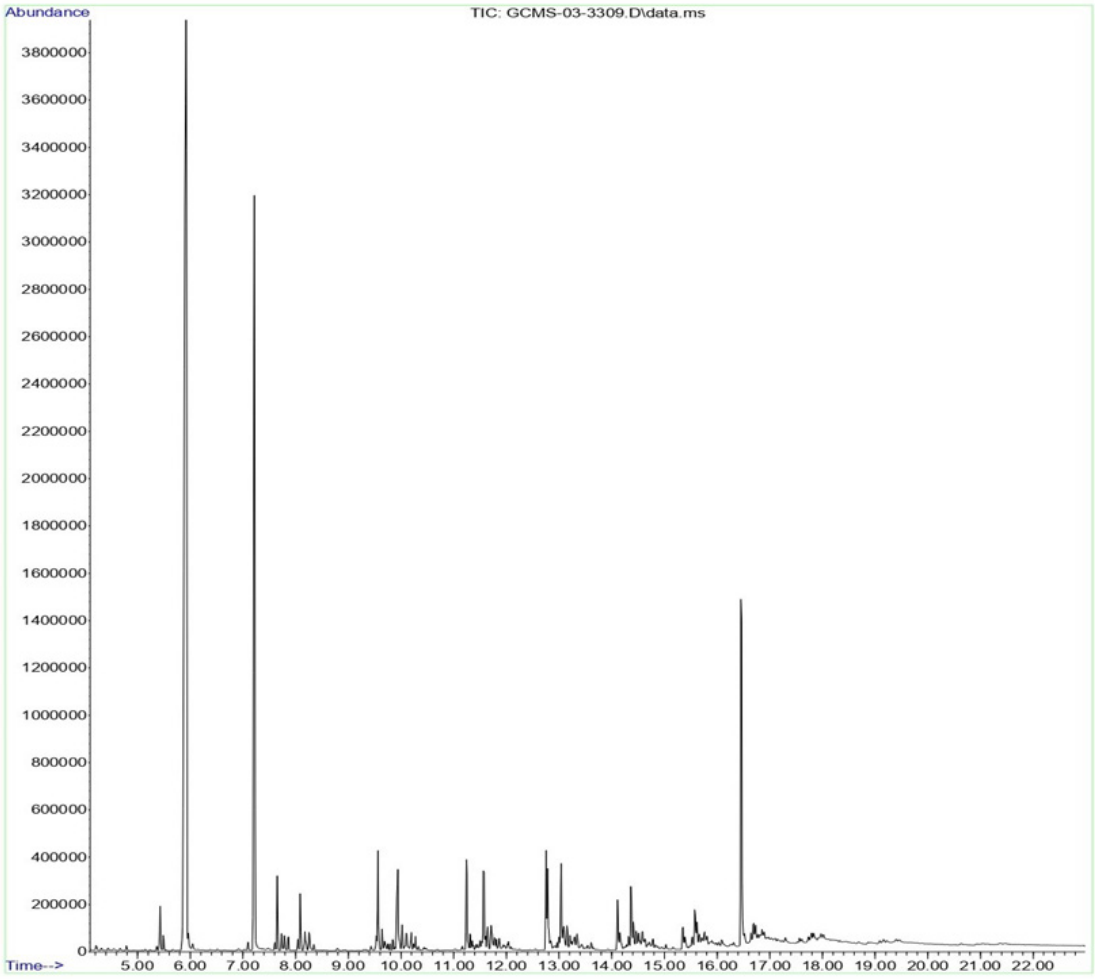

Figure 3: GCMS analysis of untreated effluent.

contaminants often found in steel industry effluents.[25] Due to a lack of extensive studies on the bioremediation of effluent from steel plants using indigenous microbes, comparisons were drawn with related industrial sectors. The pH is a measure of hydrogen ion concentration (pH=-log [H+]), the acidic nature of the effluents was most likely caused by an elevated level of Hydrogen ions [H+] in the effluents. It is an essential factor in the long-term viability of aquatic species.[26] which can directly relate to the presence or absence of certain ionic components.[27] The pH of steel plant effluent was found to be 8.76, reflecting an alkaline level. The release of effluent into water bodies may induce a reduction or elevation in pH values depending on the size and activity of the microbial community.[28] Electrical Conductivity (EC) is typically linked to the concentration of dissolved particles or minerals. It refers to the ability of water to conduct electricity (The work done by.[29] revealed that the electrical conductivity of the effluent from the textile industry ranges from 2.12 to 5.79 mS/cm.

The two primary ways to quantify organic materials are BOD (Biochemical Oxygen Demand) and COD (Chemical Oxygen Demand), whereas the inorganic matter includes heavy metals, sulfate, chloride and ammonium.[30] The most often used metric for determining an effluent's strength is BOD, which is typically provided for a five-day incubation period.[31] The elevated levels of organic matter in the effluent can result from excessive BOD and COD values, which indicates the toxic nature of the effluent. COD and BOD levels assess the relative oxygen loss from polluted waste.[32] The BOD and COD obtained after the bioremediation of sugar industry effluent using the isolated bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) was 28.9 mgL^{-1} and 50 mgL^{-1} respectively.[33] (The bioremediation of petrochemical effluent treatment using bacterial consortia resulted in the reduction of BOD levels.[34] Likewise, a study revealed that the BOD and COD of the brewery effluents using indigenous isolates varied by 94.85% and 93.25% respectively.[35]

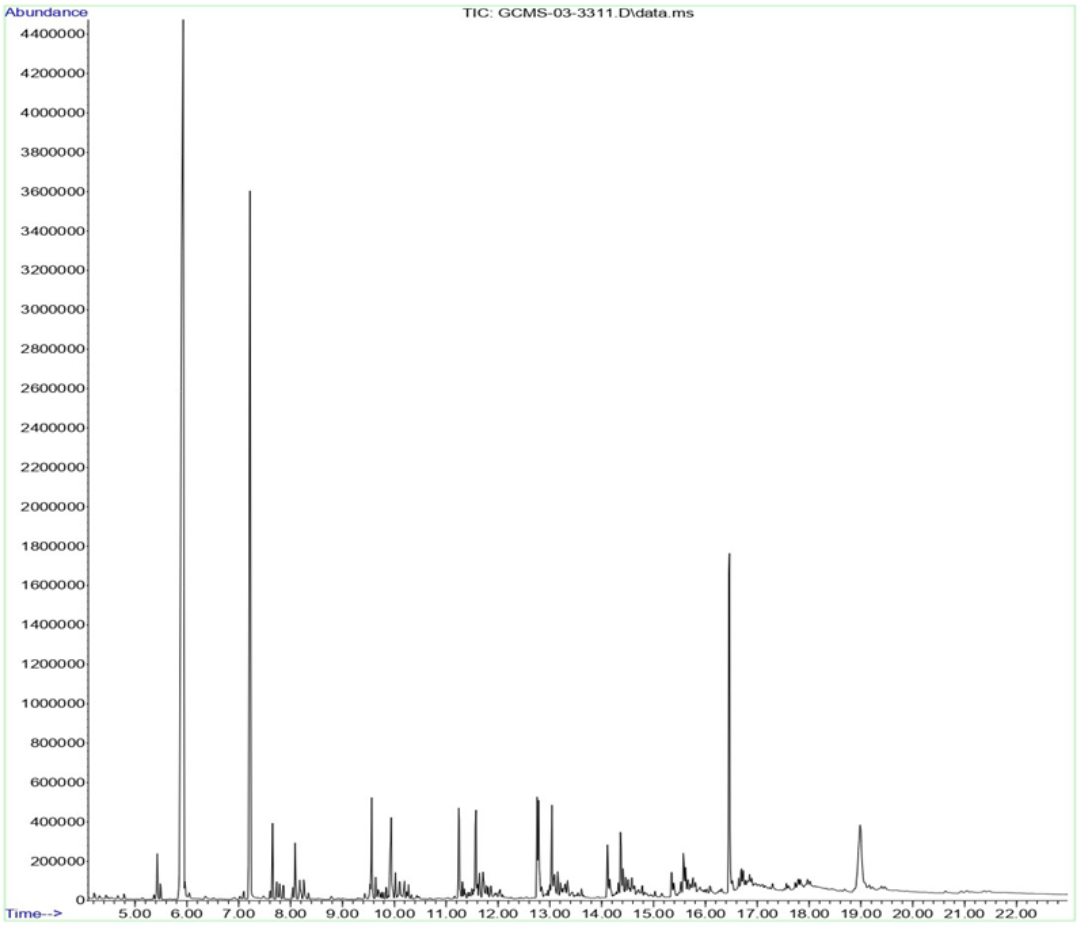

Figure 4: GCMS analysis of B1-treated effluent.

In the current study, the BOD and COD were remarkably reduced by the isolated strains B1 and B3 as they may use the dissolved organic compounds to promote their growth.

High concentrations of minerals, acids, alkalis and metallic ions in the dissolved form are indicated by high TDS (Total Dissolved Solids) concentrations. Interestingly, the isolated B1 and B3 have reduced the TDS levels. Previous research has shown that bacteria use the dissolved and suspended organic components in wastewater as a means of growth and development.[36] The TDS of industrial effluent was reported to be 5758-6672 mg/L. The high concentration of TSS and TDS could be caused by the insoluble organic and organic materials found in the wastewater.[37] The main constituents of total dissolved solids include nitrogen, calcium, sodium, potassium, iron, sulphates, bicarbonates, chlorides, sulphates, phosphates and nitrates.[38] (Excessive amounts of these minerals may negatively impact aquatic species by creating algal blooms and reducing oxygen levels in the water.[39]

Excessive sulphates can harm public health by producing diarrhea, especially in infants, the elderly and those with pre-existing diseases. Chlorine poses environmental risks and harms aquatic and soil life.[40] Likewise, the amount of organic matter present in the effluent is primarily from the breakdown of nitrogenous substances and proteins, which is expressed by the nitrate concentration.[41] Red blood cell mobility is impeded by nitrate because it cannot be acted upon in the human gut when it reaches hazardous levels.[42] Significantly, the isolated strains *Shewanella* sp. strain DADJ and *Bacillus licheniformis* have effectively reduced the nitrate, nitrates, sodium, phosphates, ammonia, copper and chloride present in the effluent.

The metal industries like iron and steel frequently use processes like acid pickling to eliminate impurities, rust, crusts and oxide layers from metals. This results in wastewater with high concentrations of heavy metals like Cu, Ni, Zn and Cr.[43] Heavy metals are non-biodegradable and have long-lasting environmental effects. It can build up in food and vegetables, which when consumed by humans, pose a serious health danger. Cardiovascular disease, neurological damage, gastrointestinal issues, kidney impairment and carcinogenic consequences are some

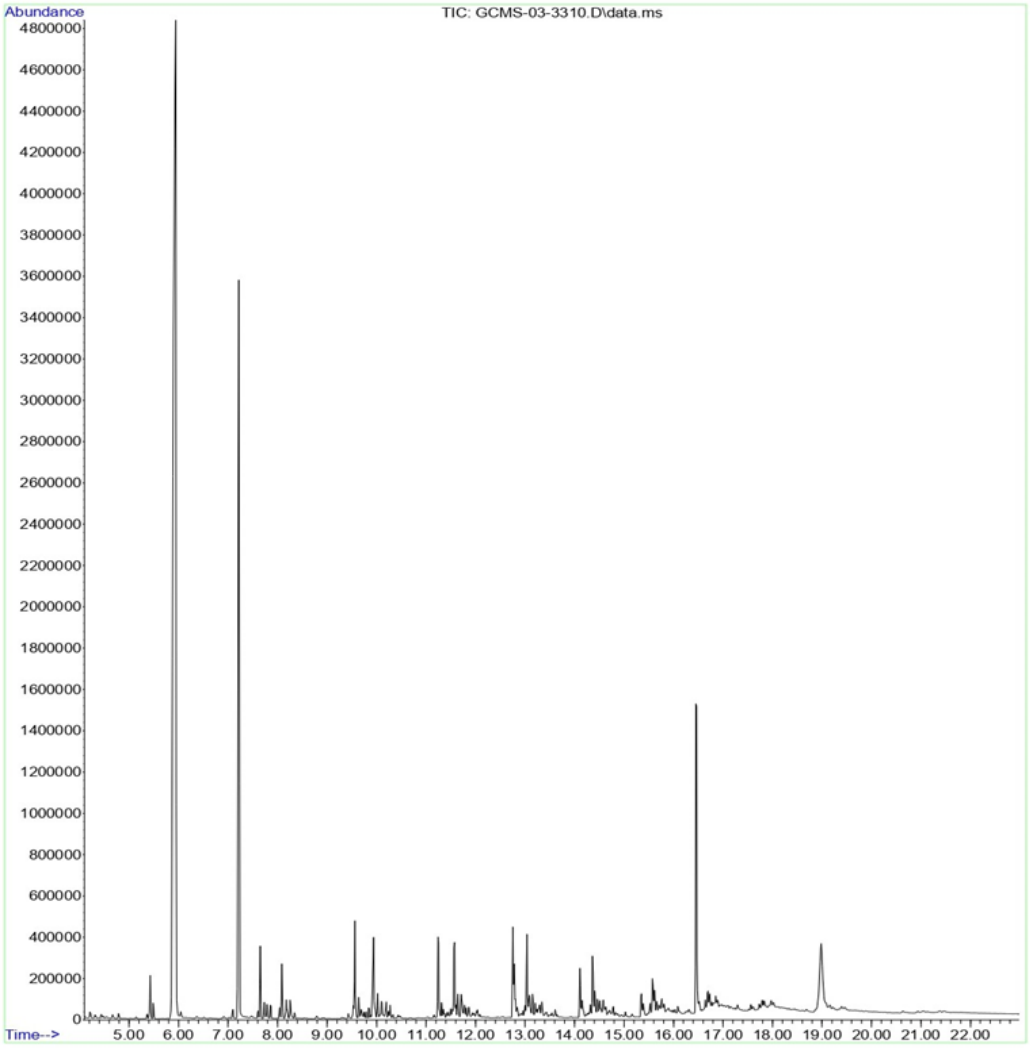

Figure 5: GCMS analysis of B3-treated effluent.

of the health dangers linked to heavy metal toxicity.[44] Heavy metals in soil and effluent samples can be reduced by microorganisms using a variety of influx and efflux approaches in addition to metal complexation.[45] The isolated bacteria toward heavy metal determination in the present research revealed that the test organisms demonstrated numerous strategies to lower high heavy metal concentrations. Chromium is a highly dangerous contaminant commonly found in industrial wastewater. In the present study, chromium detected in the untreated effluent was effectively reduced by the bacterial isolates B1 and B3. Similarly, according to,[46] chromium present in tannery effluent was reduced by bacterial species such as *Microbacterium arborescens* HU33, *Enterobacter* sp. HU38 and *Pantoea stewartii* ASI11.

Environmental protection needs to remediate industrial effluents, especially those contaminated with harmful metals.[46] Iron is one of the most important nutrients for human nutrition, yet excessive amounts of it in ecosystems can lead to serious pollution and health issues for humans, including diarrhea, vomiting and heart attacks.[47] The iron content in the cassava mill effluent treated with *Saccharomyces cerevisiae* was found to be 63.780±12.080 mg/kg.[48]

GCMS-identified compounds such as hexacosane, octacosane, dodecane, docosane and eicosane were reported in the previous study by.[49] Likewise, the compounds such as 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester and Silane, dimethyl(2-naphthoxy) heptyloxy were detected only in the effluent sample treated with B1 and B3 respectively. These substances may be the consequence of microbial activity during effluent treatment, which can break down or change complex substances into novel compounds.[50] GC-MS investigation of *Bacillus albus* strain VKDS9 treated wastewater revealed the removal of chemical pollutants and the formation of novel metabolites as end products of biodegradation.[51] Additionally

Table 7: GCMS analysis of B1-treated effluent.

Sl. No.	Compound name	Retention time	Peak area (%)
1	Decane, 4-ethyl-	5.431	0.79
2	Benzene, nitro-	5.931	40.16
3	Benzaldehyde, 3,5-dimethyl-	7.220	16.02
4	Pentadecane	7.653	1.16
5	Tetradecane	8.086	0.91
6	3-Ethyl-3-methyl heptane	8.175	0.50
7	Undecane, 4,6-dimethyl-	8.253	0.45
8	Dodecane, 4-methyl-	9.564	1.87
9	2-Bromo dodecane	9.942	2.25
10	Heptadecane, 2,6,10,15-tetramethyl	10.020	0.44
11	Dodecane, 2,6,11-trimethyl-	10.097	0.48
12	Hexadecane	10.197	0.36
13	2-Bromotetradecane	11.242	1.90
14	Heneicosane	11.308	0.37
15	Heptacosane	11.575	2.09
16	Eicosane, 2-methyl-	11.642	0.57
17	Octacosane	11.708	0.73
18	Docosane	11.775	0.45
19	Heptadecane	12.753	1.96
20	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	12.786	1.72
21	Heptadecane, 9-octyl-	13.041	2.05
22	Heptadecane, 9-octyl-	13.086	0.79
23	Tetratriacontane	13.153	0.58
24	Heneicosane	13.208	0.51
25	Hentriacontane	13.297	0.52
26	Heneicosane	13.341	0.47
27	Tetracosane	14.108	1.11
28	Heptadecane, 9-octyl-	14.152	0.45
29	Tetracosane	14.319	0.47
30	Heptadecane, 9-octyl-	14.363	1.40
31	Docosane	14.408	0.52
32	Pentacosane	14.463	0.39
33	Heptacosane	14.508	0.47
34	Tetratriacontane	14.586	0.54
35	Pentacosane	15.352	0.63
36	Tetracosane	15.530	0.38
37	Docosane	15.574	0.89
38	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	16.463	7.38
39	Tetratriacontane	16.697	0.37
40	Silane, dimethyl(2-naphthoxy) heptyloxy	18.985	4.89

Table 8: GCMS analysis of B3-treated effluent.

Sl. No.	Compound name	Retention time	Peak area (%)
1	Decane, 4-ethyl-	5.431	0.69
2	Benzene, nitro-	5.942	47.27
3	Benzaldehyde, 3,5-dimethyl-	7.220	15.30
4	Pentadecane	7.653	1.04
5	Decane, 6-ethyl-2-methyl-	8.086	0.82
6	Decane, 2,3,7-trimethyl-	8.175	0.45
7	Decane, 2,3,5-trimethyl-	8.253	0.40
8	Eicosane	9.564	1.64
9	Octacosane	9.642	0.28
10	Pentacosane	9.942	2.00
11	Heptacosane	10.020	0.37
12	Dodecane, 2,6,11-trimethyl-	10.097	0.40
13	Heptacosane	10.197	0.31
14	Hexadecane	10.275	0.28
15	Octacosane	11.242	1.63
16	Heptacosane	11.308	0.31
17	Eicosane, 10-methyl-	11.575	1.79
18	Eicosane	11.642	0.49
19	Heptacosane	11.708	0.63
20	Hexadecane	11.775	0.39
21	Heptadecane	12.753	2.48
22	Heptadecane, 9-octyl-	13.041	1.66
23	Heptadecane, 9-octyl-	13.086	0.63
24	Octadecane	13.152	0.44
25	Tetratriacontane	13.297	0.37
26	Heneicosane	13.341	0.32
27	Heneicosane	14.108	0.93
28	Docosane, 11-butyl-	14.152	0.39
29	Octacosane	14.319	0.40
30	Tetracosane	14.363	1.21
31	Pentacosane	14.408	0.45
32	Pentacosane	14.463	0.34
33	Heptacosane	14.508	0.41
34	Heneicosane	14.586	0.42
35	Octacosane	15.352	0.52
36	Tetratriacontane	15.530	0.31
37	Heptacosane	15.574	0.76
38	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	16.452	6.69
39	Heneicosane	16.696	0.32
40	Silane, dimethyl(2-naphthoxy) heptyloxy	18.985	4.44

Tripathi S,[52] also documented that metabolites were formed during the microbial treatment of distillery effluent, along with the degradation and modification of organic and organometallic contaminants. Hence, in view of the preceding findings, it has been determined that indigenous bacterial isolates *Shewanella* sp. strain DADJ and *Bacillus licheniformis* may be a promising choice for the development of bioremediation solutions for effluent produced by the steel industry.

CONCLUSION

This study revealed the efficacy of utilizing isolated indigenous bacterial strains *Shewanella sp. strain DADJ* and *Bacillus licheniformis* in the treatment of steel plant effluent for 15 days. Significant increases in the quality of the treated effluent were seen compared to the untreated effluent, as determined by extensive physicochemical studies, including assessments of iron content and heavy metal concentration. The results of the physicochemical study of the treated and untreated steel plant effluents indicated that the treated effluent has much lower concentrations of EC, TDS, chlorides, sulphates, BOD, COD, sodium, calcium, nitrates, phosphates, etc., Spectroscopy and GCMS tests revealed additional information about the removal or decrease of numerous pollutants, emphasizing the isolated strains' potential for mitigating environmental toxins. These findings highlight the importance of bioremediation technologies in combating industrial wastewater contamination and pave the way for more sustainable effluent treatment practices. Moving forward, further study into the enhancing microbial treatment technologies via optimization studies and their application in industrial settings will be critical for encouraging environmental conservation and guaranteeing the long-term viability of industrial activities.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

TDS: Total Dissolved Solids; **COD:** Chemical Oxygen Demand; **BOD:** Biochemical Oxygen Demand; **GCMS:** Gas Chromatography-Mass Spectrometry.

SUMMARY

This study investigates the potential of indigenous bacteria, *Shewanella* sp. strain DADJ and *Bacillus licheniformis*, for bioremediation of steel plant effluent, which is typically rich in contaminants like heavy metals and organic compounds. Over 15 days, the bacteria were exposed to untreated effluent and changes in physicochemical parameters such as pH, TDS, COD and BOD were measured. Notably, iron content was significantly reduced in treated effluents. ICP-OES and GC-MS analyses revealed a decrease in heavy metals and the transformation of organic compounds into novel forms, confirming the effectiveness of these bacterial strains in reducing pollutants and enhancing effluent quality.

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