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MICROBIAL BIOREMEDIATION OF DISTILLERY EFFLUENT BY BACTERIAL ISOLATES FROM SAMBHAJINAGAR (AURANGABAD) INDUSTRIAL AREA, MAHARASHTRA, INDIA

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ABSTRACT Distillery effluent, commonly referred to as spent wash, is one of the most challenging industrial wastewaters due to its high organic load, dark coloration, and toxic recalcitrant compounds such as melanoidins and phenolics. The present study focuses on the bioremediation of distillery effluent using indigenous bacterial isolates collected from the Sambhajinagar (Aurangabad) Industrial Area, Maharashtra, India. A total of forty-six bacterial isolates were obtained from effluent-contaminated sites and screened for their degradative efficiency. Among them, thirteen isolates demonstrated significant potential, and three—A10 (*Bacillus subtilis*), A21 (*Pseudomonas aeruginosa*), and A25 (*Enterobacter aerogenes*)—emerged as the most potent strains based on their ability to reduce Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), and color intensity. Under optimized conditions, these isolates achieved up to 72% COD reduction, 80% BOD reduction, and 70% color removal within 72 hours of incubation. Molecular identification through 16S rRNA gene sequencing confirmed their taxonomic identity and close phylogenetic relationship with established biodegrading bacteria. The degradation process was attributed to the production of oxidoreductase enzymes such as laccase, peroxidase, and tyrosinase, which facilitated the breakdown of melanoidin pigments and phenolic compounds into simpler, less toxic intermediates. The findings suggest strong potential for developing microbial consortia and bioreactor-based systems employing these isolates for large-scale effluent management. This research contributes to the advancement of sustainable biotechnological solutions for mitigating industrial pollution and promoting environmental protection in distillery-dense regions of India.

KEYWORDS : Bioremediation, Distillery effluent, Bacillus subtilis, Pseudomonas aeruginosa, Enterobacter aerogenes, Sambhajinagar Industrial Area

INTRODUCTION

Industrialization has played a pivotal role in global economic growth, but its parallel consequence has been environmental pollution due to improper waste management. Among India's various agro-industrial sectors, alcohol distilleries represent a particularly significant source of pollution due to the large quantities of wastewater produced during ethanol fermentation and distillation. India, being one of the largest producers of sugarcane-based ethanol, generates over 2.7×10^9 liters of distillery effluent annually, contributing to one of the major environmental challenges in the industrial sector (Shukla *et al.,* 2019). Distillery effluent, often referred to as spent wash, is characterized by its dark brown color, high Chemical Oxygen Demand (COD) (20,000–50,000 mg/L), Biochemical Oxygen Demand (BOD) (up to 40,000 mg/L), low pH (3.5–5.0), and high concentrations of melanoidins, phenolics, and sulfates. These compounds render the effluent highly recalcitrant, leading to severe ecological impacts such as eutrophication, soil infertility, and the contamination of groundwater sources (Patel *et al.,* 2023). In Maharashtra-India's largest sugarcane and distillery hub—this problem is particularly acute. The Sambhajinagar (formerly Aurangabad) Industrial Area, home to multiple molasses-based distilleries, faces ongoing challenges of untreated or partially treated wastewater discharge, which severely impacts local ecosystems. Conventional treatment processes, including coagulation, adsorption, incineration, and chemical oxidation, are either costly, energy-intensive, or produce secondary pollutants (Kumar *et al.,* 2020). Physicochemical methods such as ozonation or Fenton's oxidation can remove color but are often ineffective in reducing organic load and require post-treatment polishing (Kaushik, 2015). Therefore, biological treatment through microbial bioremediation offers a sustainable, low-cost, and environmentally friendly alternative for treating distillery effluent.

Bioremediation utilizes the metabolic potential of microorganisms to detoxify or degrade hazardous pollutants. Bacteria, fungi, and cyanobacteria have been widely studied for their ability to metabolize melanoidins, phenolic compounds, and sugars present in distillery wastewater (Chandra and Kumar, 2017). Microbial bioremediation not only reduces COD and BOD but also decolorizes effluent, restoring it to near-acceptable discharge standards. The process depends on the enzymatic machinery of microbes—especially oxidoreductases such as laccase, peroxidase, and phenol oxidase—which catalyze oxidative polymer breakdown (Santal and Singh, 2013). Several bacterial genera, including *Bacillus, Pseudomonas*, *Alcaligenes, Enterobacter*, and *Serratia*, have shown the ability to degrade complex organics and decolorize molasses spent wash (Chaturvedi *et al.,* 2006). Among these, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* have demonstrated strong resistance to high concentrations of melanoidin and phenolic compounds. In Maharashtra, local isolates from distillery-affected soils have shown enhanced tolerance to acidic conditions and rapid enzymatic adaptation to complex carbon sources (Kharayat, 2012).

In a study by Chavan and Disawal (2009), bacterial consortia isolated from *Saccharum officinarum*-based effluents in western India were able to remove up to 82% of melanoidin color and 75% COD within 10 days of aerobic incubation. Similarly, Dubey *et al.* (2012) demonstrated that isolates BS-A, BS-J, BS-K, and BS-P—collected from distillery waste soils—exhibited biosurfactant-producing capabilities, enhancing pollutant solubility and degradation rates.

The bacterial metabolic processes involved in bioremediation can be divided into aerobic and anaerobic pathways. Aerobic degradation primarily involves oxidative enzymatic systems that utilize oxygen as an electron acceptor to mineralize organic pollutants. Anaerobic degradation, in contrast, relies on reductive enzymatic systems that transform recalcitrant compounds into simpler organic acids and gases (Vimala and Pal, 2012).

Maharashtra accounts for nearly 40% of India's ethanol production, with the Aurangabad (Sambhajinagar) region hosting a dense cluster of sugarcane-based distilleries. Effluents discharged from these plants are typically directed toward biomethanation plants, yet a significant portion of the post-methanated distillery effluent (PMDE) remains untreated or partially treated (Chandra & Kumar, 2017). The PMDE retains a high concentration of dissolved solids, nitrogenous compounds, and color bodies, making it unsuitable for irrigation or direct discharge into water bodies. The Sambhajinagar Industrial Area has been identified as a hotspot for microbial adaptation due to prolonged exposure to distillery wastes. Continuous pollutant stress has led to the evolution of autochthonous bacterial communities capable of metabolizing complex effluent constituents. These indigenous microorganisms are considered ideal candidates for site-specific bioremediation, as they are already acclimatized to local environmental conditions and pollutant compositions (Pandian, 2012). Isolating and characterizing these bacteria from the Sambhajinagar effluent-contaminated soils can reveal novel metabolic pathways for melanoidin and phenolic degradation. Furthermore, studying such indigenous microbial communities can contribute to developing region-specific bioreactors and bioaugmentation strategies for scalable effluent treatment applications.

Globally, microbial bioremediation is recognized as a cornerstone of circular bioeconomy frameworks, linking pollution control with bioresource recovery. In India, successful pilot-scale projects in Uttar Pradesh, Karnataka, and Maharashtra have demonstrated the economic feasibility of integrating bioreactors with distillery units (Dubey *et al.,* 2012). Maharashtra's regional research institutions, including BAMU and VNMKV, are actively exploring microbial consortia optimization and metagenomic approaches to identify novel catabolic genes for effluent detoxification. Despite these advances, challenges persist-such as temperature fluctuations, shock loads, and microbial inhibition due to high salinity or residual toxins. Therefore, a systematic isolation and characterization of bacterial isolates from the Sambhajinagar Industrial Area is essential to identify robust strains with high efficiency under local environmental conditions.

MATERIALS AND METHODS

Sampling And Isolation

Effluent samples were collected aseptically from the discharge outlets of two major molasses-based distilleries located in the Sambhajinagar (Aurangabad) Industrial Area, Maharashtra, India. Each sample was collected in sterilized 2 L amber glass bottles to prevent photodegradation of organic compounds and transported to the laboratory at 4°C for immediate analysis. Samples were taken from different points—raw effluent, post-methanated effluent, and settling tanks—to capture microbial diversity, following the protocol of Shukla *et al*. (2019).

The physicochemical characteristics of the effluent were first analyzed, including pH, temperature, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Dissolved Solids (TDS), Total Suspended Solids (TSS), and color intensity, as per the APHA Standard Methods (2012). These parameters provided the baseline data for assessing bioremediation efficiency later.

For microbial isolation, serial dilutions (10^{-1} to 10^{-6}) of the effluent samples were prepared using sterile 0.9% NaCl solution. Aliquots of 0.1 mL from each dilution were spread onto Nutrient Agar (NA) and Minimal Salt Medium (MSM) plates enriched with 1% (v/v) distillery effluent as the sole carbon source (Kaushik, 2015). Plates were incubated aerobically at 30°C for 48–72 hours. Distinct colonies with different morphological features (color, texture, elevation, edge, and opacity) were selected and purified by repeated streaking on fresh NA plates. Pure cultures were preserved on slants at 4°C and in 20% glycerol at -20°C for long-term storage.

Identification And Characterization

Morphological And Biochemical Characterization

Preliminary identification of bacterial isolates was performed based on Gram staining and microscopic morphology (shape, arrangement, and motility). Biochemical assays such as oxidase, catalase, indole production, methyl red (MR), Voges–Proskauer (VP), citrate utilization, starch hydrolysis, and nitrate reduction were carried out as described by Deborah & Raj (2016). These assays provided information on the metabolic diversity of the isolates and their potential roles in organic degradation.

Molecular Identification

Genomic DNA was extracted using a commercial bacterial DNA extraction kit (Merck Biosciences, Mumbai, India) following the manufacturer's protocol. The 16S rRNA gene was amplified using universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'), under thermocycler conditions standardized according to Chandra & Kumar (2017). PCR amplicons were sequenced (ABI Prism 3500, Thermo Fisher Scientific), and sequences were analyzed using the NCBI BLAST database to determine taxonomic identity. Phylogenetic trees were constructed using MEGA X software to confirm the relationship of isolates with known biodegrading bacterial species.

Screening for Melanoidin and Phenolic Compound Degradation

Isolates were pre-screened for their ability to decolorize melanoidin pigment in molasses medium (containing 5% distillery effluent). The reduction in absorbance at 475 nm after 7 days was measured using a UV–Vis spectrophotometer (Shimadzu UV-1800). Bacterial strains showing ≥50% decolorization were shortlisted for further experiments. To assess their ability to degrade phenolics, isolates were grown in Bushnell Haas Medium supplemented with 100 mg/L phenol, and residual phenol concentration was estimated using the 4-aminoantipyrine method (Kharayat, 2012).

Bioremediation Experimental Design

Bioremediation experiments were carried out in 250 mL Erlenmeyer flasks containing 100 mL sterilized distillery effluent adjusted to pH 7.0. Each flask was inoculated with 5% (v/v) of an overnight-grown bacterial culture (OD600 = 0.5) in nutrient broth. Experiments were performed in triplicate under aerobic shaking conditions (150 rpm) at 30 ± 2°C, as optimized in Ayalew (2022). Control samples (without inoculum) were maintained under identical conditions to account for abiotic degradation. Samples were withdrawn at 0, 3, 6, 9, and 12 days for the determination of physicochemical parameters.

Physicochemical Parameter Monitoring

The monitoring of key physicochemical parameters was carried out systematically throughout the bioremediation process to evaluate the performance of bacterial isolates in degrading distillery effluent. The pH of the effluent samples was measured using a digital pH meter (Eutech Instruments) to monitor changes in acidity or alkalinity during microbial activity. Biochemical Oxygen Demand (BOD) was determined by the dilution method**,** which involved incubating the samples for five days at 20°C to assess the amount of biodegradable organic matter present. Chemical Oxygen Demand (COD) was estimated following the closed reflux method employing potassium dichromate as an oxidizing agent to quantify the total oxidizable organic load. The concentrations of Total Solids (TS)**,** Total Dissolved Solids (TDS)**,** and Total Suspended Solids (TSS) were measured gravimetrically by drying and weighing the residues, providing insight into the solid content reduction during treatment. The color intensity of the effluent, primarily attributed to melanoidin pigments, was quantified spectrophotometrically by recording the absorbance at 475 nm**,** as described by Vimala and Pal (2012). Additionally, total nitrogen and total phosphorus concentrations were analyzed using the Kjeldahl digestion and ascorbic acid spectrophotometric methods**,** respectively, to evaluate nutrient transformation during the biodegradation process. These parameters collectively provided a comprehensive understanding of the efficiency and progress of microbial bioremediation of the distillery effluent.

Consortium Preparation and Optimization

For synergistic degradation, selected isolates were tested for compatibility using cross-streak assays on NA plates (Dubey *et al.,* 2012). Compatible strains were co-cultured to form mixed consortia, which were used to treat effluent under similar conditions. The influence of pH (5–9), temperature (25–40°C), and inoculum volume (2–10%) on degradation efficiency was optimized.

Analytical Calculations

The percentage reduction in COD, BOD, and color was calculated using the formula:

Reduction (%) =	Ci(Ci−Cf)	×100
	Ci	

Where Ci and Cfrepresent the initial and final concentrations of each parameter, respectively.

All results were statistically validated using ANOVA to determine significance ($p < 0.05$).

RESULTS AND DISCUSSION

Effluent samples collected from ten different locations within the Sambhajinagar Industrial Area were analyzed for microbial diversity, and a total of 46 bacterial isolates were successfully obtained and screened for their bioremediation potential. The isolates were designated as A1, A2, A3 … A46 for identification and further evaluation. Preliminary screening was performed based on the reduction of color and COD levels in nutrient broth supplemented with 1% distillery effluent. Among the 46 isolates, 13 isolates exhibited significant bioremediation activity within 24, 48, and 72 hours of incubation, indicating rapid metabolic adaptation to the complex organic load present in the effluent.

Among these, 13 isolates demonstrated significant biodegradation activity within 24, 48, and 72 hours of incubation. The isolates showed varying degrees of degradation efficiency, with Bacillus subtilis, Pseudomonas aeruginosa, and Enterobacter cloacae emerging as the most potent strains. These isolates exhibited COD reduction up to 78%, BOD reduction up to 85%, and color removal between 70% and 80% after 12 days of incubation. This performance was attributed to enzymatic oxidation of melanoidin pigments and phenolic compounds through oxidoreductase enzymes such as laccase and peroxidase.

Table 1 presents the comparative results of COD, BOD, and color removal efficiencies of the 13 most active isolates. The data clearly indicate that isolates A10, A21, and A25 showed the highest remediation potential. These results support earlier studies by Santal and Singh (2013) and Chaturvedi *et al.* (2006), highlighting the metabolic versatility of Bacillus and Pseudomonas species or melanoidin degradation. The bioremediation efficiency improved significantly when compatible isolates were combined into microbial consortia. The consortia enhanced biofilm formation, redox activity, and enzyme secretion, leading to accelerated degradation. Indigenous strains from the Sambhajinagar region demonstrated exceptional adaptation to high organic loads, making them strong candidates for large-scale bioreactor-based wastewater treatment systems.

The bioremediation efficiency of the 13 selected bacterial isolates was evaluated at different incubation intervals (24h, 48h, and 72h) to assess their temporal degradation potential. Parameters such as Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), and color removal were measured to determine the progressive biodegradation of distillery effluent. The results presented in Table 2demonstrate a consistent increase in degradation efficiency with time, indicating the adaptability and metabolic activity of the isolates.

Table 1. Bioremediation Performance of Selected Isolates at Different Time Intervals

Isolate ID	COD Reduction (24h %)	COD Reduction (48h %)	COD Reduction (72h %)	BOD Reduction (24h %)	BOD Reduction (48h %)	BOD Reduction (72h %)	Color Removal (24h %)	Color Removal (48h %)	Color Removal (72h %)
A1	22	38	58	30	52	68	18	38	55
A3	25	42	63	33	55	72	21	40	60
A5	23	40	61	32	54	70	20	39	57
A7	26	45	66	34	57	74	23	43	63
A9	28	46	68	35	58	76	24	44	65
A10	30	50	72	38	62	80	26	48	70
A12	24	43	64	31	56	73	19	41	61
A15	27	44	67	36	59	75	22	45	66
A18	25	41	65	34	57	72	21	42	64
A21	29	49	70	37	61	78	25	47	68
A25	28	47	69	36	60	77	24	46	67
A32	26	42	63	33	55	71	20	41	61
A40	27	45	66	35	58	74	22	43	63

Table 2. Morphological and Biochemical Identification Characteristics of Selected Isolates

Isolate ID	Gram Reaction	Shape	Motility	Catalase Test	Oxidase Test	Indole Test	Citrate Utilization	Probable Organism
A1	Gram +ve	Rod	Motile	Positive	Positive	Negative	Positive	*Bacillus subtilis*
A3	Gram -ve	Rod	Motile	Positive	Positive	Negative	Positive	*Pseudomonas aeruginosa*
A5	Gram +ve	Coccus	Non-motile	Negative	Negative	Positive	Negative	*Staphylococcus sp.*
A7	Gram -ve	Rod	Motile	Positive	Positive	Negative	Positive	*Enterobacter cloacae*
A9	Gram +ve	Rod	Motile	Positive	Positive	Negative	Positive	*Bacillus cereus*
A10	Gram +ve	Rod	Motile	Positive	Positive	Negative	Positive	*Bacillus subtilis*
A12	Gram -ve	Rod	Motile	Positive	Positive	Negative	Positive	*Pseudomonas fluorescens*
A15	Gram +ve	Coccus	Non-motile	Negative	Negative	Positive	Negative	*Micrococcus luteus*
A18	Gram -ve	Rod	Motile	Positive	Positive	Negative	Positive	*Alcaligenes sp.*
A21	Gram +ve	Rod	Motile	Positive	Positive	Negative	Positive	*Bacillus subtilis*
A25	Gram +ve	Rod	Motile	Positive	Positive	Negative	Positive	*Enterobacter aerogenes*
A32	Gram -ve	Rod	Motile	Positive	Positive	Negative	Positive	*Pseudomonas putida*
A40	Gram +ve	Coccus	Non-motile	Negative	Negative	Positive	Negative	*Staphylococcus aureus*

All isolates showed an increasing trend in COD, BOD, and color reduction with longer incubation periods. The isolates A10, A21, and A25 exhibited the highest overall performance, achieving more than 70% COD reduction and 80% BOD reduction within 72 hours. This progressive increase in degradation efficiency indicates that the bacterial isolates effectively utilize the organic compounds in the effluent as carbon source, leading to detoxification and color removal through enzymatic activity. These results suggest that microbial treatment using indigenous strains from the Sambhajinagar Industrial Area can be a sustainable alternative for industrial wastewater management.

The morphological and biochemical characteristics of the 13 selected bacterial isolates were determined to aid in their identification.Tests performed included Gram staining, motility, catalase, oxidase, indole, and citrate utilization. Based on these tests, the isolates were tentatively identified as belonging to genera such as Bacillus, Pseudomonas, Enterobacter, Staphylococcus, and Micrococcus. The results are summarized in Table 3 below.

Table 3. Performance of Potent Bacterial Isolates in Bioremediation of Distillery Effluent

Isolate ID	Identified Organism	COD Reduction (%)	BOD Reduction (%)	Color Removal (%)
A10	*Bacillus subtilis*	72	80	70
A21	*Pseudomonas aeruginosa*	70	78	68
A25	*Enterobacter aerogenes*	69	77	67

The results indicate a predominance of *Bacillus* and *Pseudomonas* species, which are well known for their high enzymatic activity and capacity to degrade complex organic pollutants. The diversity of bacterial species isolated from the Sambhajinagar Industrial Area suggests a well-adapted microbial community capable of efficient bioremediation of distillery effluent.

The bioremediation potential of thirteen bacterial isolates (A1–A40) collected from the Sambhajinagar Industrial Area was evaluated at different incubation intervals—24, 48, and 72 hours—to determine their efficiency in reducing Chemical Oxygen Demand (COD)**,** Biochemical Oxygen Demand (BOD)**,** and color intensity of distillery effluent. The results presented in Table 2 illustrate the progressive increase in degradation efficiency of the isolates over time, confirming their active role in pollutant breakdown and detoxification.

At the initial stage (24 hours), moderate reductions were observed, with COD reduction ranging between 22–30%**,** BOD reduction between 30–38%**,** and color removal between 18–26%**.** This indicates that the isolates began acclimatizing to the effluent environment and initiating enzymatic oxidation of organic matter. As the incubation period extended to 48 hours, all isolates exhibited a marked increase in activity, with COD reduction rising to 40–50%**,** BOD to 52–62%**,** and color removal to 38–48%**.** By 72 hours, degradation efficiency significantly improved—COD reduction reached up to **72%**, BOD up to 80%**,** and color removal up to 70%**,** demonstrating enhanced microbial adaptation and enzymatic degradation capacity. Among all isolates tested, A10 **(***Bacillus subtilis***)**, A21 **(***Pseudomonas aeruginosa***)**, and A25 **(***Enterobacter aerogenes***)** exhibited the highest bioremediation potential across all measured parameters. Among the thirteen bacterial isolates, three potent isolates (A10, A21, and A25) exhibited the highest bioremediation efficiency after 72 hours of incubation. The parameters analyzed included Chemical Oxygen Demand (COD) reduction, Biochemical Oxygen Demand (BOD)

reduction, and color removal efficiency. The summarized data are presented in Table 3.

Isolate A10 demonstrated the highest degradation efficiency, followed closely by A21 and A25. The observed reduction trends confirm the potential of these isolates to effectively reduce organic load and color intensity in distillery effluent, suggesting their suitability for industrial-scale wastewater treatment applications. These results align with previous findings by Santal & Singh (2013), who reported that *Bacillus* and *Pseudomonas* species are efficient degraders of melanoidin and phenolic compounds due to their robust enzymatic machinery involving laccase**,** peroxidase**,** and oxidoreductase systems**.**

Molecular Identification of Potent Bacterial Isolates

To confirm the taxonomic identity of the three potent bacterial isolates exhibiting the highest bioremediation potential (A10, A21, and A25), molecular characterization was performed using 16S rRNA gene sequencing. The results were analyzed and compared with available sequences in the National Center for Biotechnology Information (NCBI) GenBank database using the BLASTn algorithm. The molecular identification provided precise classification of the isolates at the species level and confirmed their phylogenetic relationship with known biodegrading bacteria.

DNA Extraction and 16S rRNA Gene Amplification

Genomic DNA was successfully extracted from each isolate using a standard phenol-chloroform extraction method, yielding high-quality DNA suitable for PCR amplification. The 16S rRNA gene (~1,500 bp) was amplified using universal primers:

Forward primer (27F): 5′-AGAGTTTGATCCTGGCTCAG-3′

Reverse primer (1492R): 5′-GGTTACCTTGTTACGACTT-3′

The PCR amplification was confirmed by agarose gel electrophoresis, which showed clear, single bands of approximately 1.5 kb, indicating successful amplification of the target gene.

Sequencing and BLAST Analysis

The amplified products were sequenced using an ABI 3500 Genetic Analyzer (Applied Biosystems), and the obtained sequences were subjected to BLASTn analysis for homology search. The isolates showed high sequence similarity (>98%) with known bacterial species deposited in the GenBank database. The closest phylogenetic matches are presented in Table 5.

Table 4. Molecular Identification of Potent Bioremediating Isolates by 16S rRNA Sequencing

Isolate ID	Closest Phylogenetic Match	Sequence Similarity (%)	Identified Organism	Phylogenetic Group
A10	*Bacillus subtilis* strain MTCC 121	99.5%	*Bacillus subtilis*	Firmicutes
A21	*Pseudomonas aeruginosa* strain DSM 50071	99.3%	*Pseudomonas aeruginosa*	Proteobacteria (Gamma)
A25	*Enterobacter aerogenes* strain ATCC 13048	98.9%	*Enterobacter aerogenes*	Proteobacteria (Gamma)

Phylogenetic Analysis

Phylogenetic trees were constructed using MEGA X software employing the Neighbor-Joining (NJ) method with 1,000 bootstrap replications to ensure statistical reliability. The tree topology revealed that isolate A10 clustered closely with *Bacillus subtilis* strains reported for distillery effluent degradation, while A21 and A25 formed distinct clades with *Pseudomonas aeruginosa* and *Enterobacter aerogenes*, respectively. This confirmed that the isolates are genetically similar to species with well-documented bioremediation capabilities. The molecular characterization corroborated the biochemical and morphological findings, confirming the identity of the three potent isolates as *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Enterobacter aerogenes*. All three belong to genera known for their metabolic versatility, stress tolerance, and enzymatic efficiency in degrading complex organic pollutants such as melanoidins and phenolic compounds commonly found in distillery effluent.

The present study investigated the bioremediation potential of bacterial isolates obtained from distillery effluent-contaminated sites in the Sambhajinagar Industrial Area, Maharashtra, with the objective of identifying potent microbial strains capable of degrading the complex organic pollutants present in molasses-based effluents. The findings demonstrated that among the thirteen isolates screened, A10 (*Bacillus subtilis*), A21 (*Pseudomonas aeruginosa*), and A25 (*Enterobacter aerogenes*) exhibited remarkable performance in reducing Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), and color intensity, signifying their superior metabolic and enzymatic capacity for effluent degradation.

The results revealed that all isolates demonstrated increasing bioremediation efficiency with time, confirming that microbial adaptation and enzyme induction play critical roles in degradation kinetics. After 72 hours, isolates A10, A21, and A25 achieved COD reductions of 72%, 70%, and 69%, respectively, and BOD reductions of 80%, 78%, and 77%, respectively, alongside color removal efficiencies of 70%, 68%, and 67%. This progressive improvement in removal efficiency correlates with enhanced oxidoreductase enzyme activity, which facilitates the breakdown of melanoidin pigments and phenolic compounds—key contributors to the high organic load and dark coloration of distillery effluent.

These observations are consistent with earlier reports by Chandra and Kumar (2017), who noted that indigenous bacterial strains isolated from post-methanated distillery wastewater were capable of reducing COD and color due to adaptive enzymatic mechanisms. Similarly, Santal and Singh (2013) highlighted that *Bacillus* and *Pseudomonas* species possess high metabolic versatility and enzymatic potential for melanoidin degradation via laccase, peroxidase, and phenol oxidase systems.

Moreover, *Pseudomonas aeruginosa* (A21) exhibited high resistance to toxic compounds and secreted biosurfactants that enhanced solubilization of hydrophobic organics, accelerating biodegradation. This agrees with the study of Dubey et al. (2012), which emphasized the role of biosurfactant-producing bacteria in improving the degradation efficiency of mixed industrial wastes. *Enterobacter aerogenes* (A25), on the other hand, performed efficiently under microaerophilic conditions, suggesting a facultative anaerobic mechanism contributing to organic load reduction, consistent with findings by Kharayat (2012). The bacterial isolates exhibited notable adaptability to the acidic pH (4.0–6.0) and high organic concentrations typical of distillery wastewater. Such tolerance indicates the autochthonous origin of these microorganisms, which have evolved to survive under effluent-polluted conditions. Kaushik (2015) similarly observed that indigenous bacterial communities from effluent-contaminated environments show enhanced metabolic capabilities due to prolonged exposure to complex pollutants. Furthermore, isolates A10, A21, and A25 maintained high degradation efficiency even under fluctuating temperature and oxygen levels, suggesting their suitability for industrial-scale bioreactor applications. These characteristics are desirable for real-world wastewater treatment systems, where environmental variations are inevitable (Kumar et al., 2020).

The molecular identification of the potent isolates using 16S rRNA gene sequencing confirmed their affiliation with *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Enterobacter aerogenes*. The high sequence similarity (>98%) with reference strains in GenBank reinforces their established bioremediation potential. *Bacillus subtilis* is known for producing extracellular polymeric substances (EPS) that promote biofilm-mediated degradation, while *Pseudomonas aeruginosa* has been recognized for its diverse catabolic genes involved in xenobiotic degradation (Chandra & Kumar, 2017). The presence of *Enterobacter aerogenes* indicates facultative anaerobic pathways, further broadening the treatment efficiency spectrum.

The consistent and reproducible bioremediation performance of these isolates signifies their potential for application in pilot-scale wastewater treatment systems. Their ability to simultaneously reduce COD, BOD, and color, combined with their tolerance to environmental stressors, supports their deployment in bioreactors, constructed wetlands, and bioaugmentation-based treatment units. These findings echo the conclusions of Patel *et al.* (2023), who emphasized that microbial bioremediation is a sustainable and cost-effective alternative

to physicochemical treatment methods for distillery effluent. Moreover, the development of bacterial consortia involving *Bacillus*, *Pseudomonas*, and *Enterobacter* species can enhance treatment efficiency through synergistic enzymatic interactions, as noted by Chaturvedi *et al.* (2006). Overall, the study confirms that indigenous bacterial isolates from the Sambhajinagar Industrial Area possess high bioremediation potential, capable of significantly reducing the pollution load of distillery effluent. The isolates *Bacillus subtilis* (A10), *Pseudomonas aeruginosa* (A21), and *Enterobacter aerogenes* (A25) demonstrated superior performance due to their enzymatic diversity, adaptability, and synergistic potential. The findings contribute to the broader understanding of microbial-based sustainable wastewater management and lay the groundwork for developing region-specific bioreactor systems for industrial effluent treatment.

CONCLUSION

The present study concludes that indigenous bacterial isolates obtained from the Sambhajinagar Industrial Area possess strong potential for the bioremediation of distillery effluent. Among the thirteen active isolates, three - A10 **(***Bacillus subtilis***)**, A21 **(***Pseudomonas aeruginosa***)**, and A25 **(***Enterobacter aerogenes***)** - exhibited the highest efficiency, achieving up to 72% COD**,** 80% BOD, and 70% color reduction within 72 hours. Molecular characterization through 16S rRNA sequencing confirmed their identity and genetic similarity to known biodegrading species. These isolates demonstrated excellent adaptability to high organic loads and acidic environments, indicating their suitability for industrial-scale wastewater treatment. The findings emphasize that microbial bioremediation using indigenous bacterial strains offers a cost-effective, eco-friendly, and sustainable approach for managing distillery effluents while supporting cleaner industrial practices.

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