Nanotechnology Perceptions
ISSN 1660-6795
www.nano-ntp.com

Sustainable Bioremediation of Leather Tannery Effluents Using N- TE2A2B36 Bacterial Consortium for Environmental Protection

J. Raveena Jayam, K.J. Sharmila*

Department of Biotechnology, Dr M.G.R. Educational and Research Institute, India
Email: sharmila.ibt@drmgrdu.ac.in

The discharge of untreated or partially treated effluent from leather tanning industries poses a severe threat to water and soil resources. To mitigate this environmental hazard, it is crucial to properly treat and detoxify tannery effluent (TE) before its environmental release. This study demonstrated effective degradation of actual TE using a newly developed bacterial consortium, N-TE2A2B36, over a 120-hours. ICP-OES, HP-LC, FT-IR analyses revealed that the bacterial consortium completely mineralized or degraded most organic contaminants in the untreated TE, resulting in new degradation products. The process was optimized at pH 7, with 0.5% glucose and ammonium chloride, 130 rpm agitation, and 15 mL inoculum volume. The efficacy of the bacterial treatment was further validated through phytotoxicity tests using Phaseolus mungo L as a terrestrial model organism. The results indicated a significant reduction in the toxicity of the treated TE, with 70% seed germination observed, confirming successful detoxification. In summary, the bacterial consortium N-TE2A2B36 exhibited remarkable ability in efficiently treating and detoxifying leather TE, thereby contributing to environmental protection.

1. Introduction

Increased pollution of the land, air, and water environments as well as overuse of readily available resources are issues brought on by the population's rapid growth and the growing need for industrial facilities to support human requirements(Kumari et al. 2024). The economic value of heavy metal is important in industrial use and has grown to be a major global environmental concern. contamination of the environment by large. In an ecosystem, metals are now a major hazard to living things(Kookhaee et al. 2022).(Environmental Protection Agency (EPA), 2010) defines parameterization as a spontaneous process where the microbiological process is utilized to break down, degrade, or cure or remove and get rid of toxins from environmental media by changing dangerous contaminants into less harmful or nontoxic forms(Arti and Mehra 2023). Through their metabolic activities, microorganisms consume chemical contaminants as a source of energy throughout the microbiological procedures. However, microbial suppression is brought on by an overabundance of inorganic nutrients in soil(Kalsoom et al. 2023).Tannery workers were previously found to have a

significant prevalence of scabies (73.9%), gastrointestinal issues (71.7%), diarrhea (71.7%), asthma (49.9%), eye issues (46.7%), and high blood pressure (52.2%)(Mohanta et al. 2023).

Cr (VI) is poisonous and extremely soluble in water, it can quickly cross cell membranes and eventually come into contact with proteins and nucleic acids(Kookhaee et al. 2022; López Arias et al. 2024). Thus, cancer, mental retardation, renal malfunction, and other abnormalities are caused by the buildup of toxic heavy metals in humans(Manzoor et al. 2024). Out of all the industrial wastes, tanning effluents are the most polluting. They contribute significantly to the pollution caused by chromium. Water supplies and agricultural land have been widely contaminated as a result of the long-term disposal of tannery waste(Kalsoom et al. 2023). When waste from tanneries is It has an impact on the soil's fertility when it is released into agricultural areas or utilized for irrigation(Lejri et al. 2024). Urban regions frequently experience land pollution due to industry and municipal wastes, which are the main environmental pollutants. The tannery industries, which turn hides and skins into leather, produce the most of these wastes(Maoya et al. 2024).

While the majority of wastewater is recycled during the tanning process, only a small portion is comprising high levels of chemical oxygen demand (COD), biochemical oxygen demand (BOD), total dissolved solids (TDS), salt, and other contaminants(Shah 2021). The land, rivers, and oceans are contaminated by chromium, sulphate, synthetic tannins, and azo dyes. When tannery wastewater containing these harmful pollutants is released into the environment, it causes significant soil and water pollution, which further compromises human and other species' health live things (Naguib et al. 2024; Pasciucco et al. 2024).The diverse composition of organic and inorganic compounds combined with high pollution concentrations presents a significant challenge to remediation techniques(Chau et al. 2023).Numerous physical and chemical procedures, including enhanced oxidation and ultra-filtration, have been developed over time; nonetheless, they are costly, complex, and inefficient. Because of this, bioremediation techniques have emerged as a potential, safe, and economical solution to these issues(Dubey et al. 2024).

Indigenous microorganism-based technology is extensively used for waste management. These creatures are a collection of naturally occurring microbial consortiums that live in soil and on living things' surfaces and are capable of biodegradation, bioleaching, and biocomposting. Therefore, using these naturally occurring microbes is a naturally appealing and effective method of environmental protection. Therefore, to determine how well the native bacterial consortium obtained from chromepet tannery regions can bioremediate and decrease the toxicity of all effluents, we have conducted this study. Furthermore, utilizing a toxicity assay Phaselous mungo as a model organism, the bacterial isolates' capacity for bioremediation and their decrease in pollutant levels were confirmed.

2. MATERIALS AND METHODS

Heavy metal stock solutions

Individual stock solutions of heavy metals, such as cadmium nitrate, lead nitrate, copper nitrate, nickel chloride, and chromium trioxide, were added to distilled water. A 0.22µm

membrane filter was used to sterilize the solutions before they were used in subsequent tests(Kookhaee et al. 2022).

Collection of Samples and Bacterial Isolation

Samples of tannery effluent were taken from the Chromepet Chennai, which is home to the tannery industry. For physicochemical parameters, such as BOD, COD, pH, TDS, electrical conductivity, and bacteriological tests, samples were gathered in sterile bottles and aseptically transported to the lab with an ice box (Kirkinci et al. 2021). For 15 minutes, the nutritional agar was sterilized at 121°C. On separate spread and pour plates, 100 µl of serial dilutions ranging from 10^{-1} to 10^{-7} were incubated for 24 hours at 37°C. The plates were checked for dominant colonies following incubation(Kookhaee et al. 2022; Vijayaraj et al. 2018).

Minimum Inhibitory Concentration

Isolates were streaked on nutrient agar plates supplemented with several heavy metals, including Cr, Cu, Pb, Ni, and Cd, at increasing concentrations ranging from 50 ppm to 10,000 ppm, to screen. The isolates were cultured for three days at 37°C for 72 hours. Each isolated heavy metal resistance capacity was assessed following incubation(Kookhaee et al. 2022; Vijayaraj et al. 2018; Elahi et al. 2022).

Identification of potential strains

For bacterial identification, the strains were described using their morphological, cultural, and biochemical traits. Isolates' oxidase, catalase, and gram staining activities were biochemically described. After an overnight growth in nutrient broth, the bacterial isolate genomic DNA was isolated using a DNA purification kit. Following the extraction procedure outlined in the kit handbook, the sample was frozen at -4 °C until a PCR reaction could be performed. The PCR thermocycler was set up for 30 cycles of initial denaturation at 95°C for 5 minutes, denaturation at 95°C for 1 minute, annealing at 55°C for 1 minute, extension at 72°C for 2 minutes, and final extension at 72°C for 10 minutes. To get rid of impurities, the PCR amplicon was cleaned. By running the PCR result on a 1% agarose gel, the size of the DNA was evaluated. Using universal primers(27F&1492R),5'-AGAGTTTGATCCTGGCTCAG-3',5'-GGTTACCTTGTTACGACTT-3', the PCR product was sequenced using the BDT V3.1 Cycle sequencing kit ABI 3730xl Genetic Analyzer DNA sequences. Genomic characterization was done using 16S sequence analysis. Multiple alignments with the sequences of the most closely related individuals were carried out using BioEdit, and the degrees of sequence similarity were computed. To enable comparison, 16S rRNA gene sequences were obtained from the National Centre for Biotechnology Information database(Hossan et al. 2020).

Phylogenetic Analysis

The NCBI database's BLAST tool was utilized to identify sequences. The top ten sequences, selected based on their highest identity scores, were then aligned using Clustal W, a multiple sequence alignment program. MEGA 10 software was employed to construct a distance matrix and phylogenetic tree. The sequences were then submitted to GenBank, and the NCBI was subsequently contacted to acquire the accession number(Hasintha Kumari et al., 2021).

Bio reduction Experiment

For the bio reduction process, bacterial isolates were cultivated in NB broth medium within

500mL Erlenmeyer flasks. The flasks were subjected to various conditions: temperatures (37°C, 45°C, 55°C), incubation periods (48, 96, 144 h), and pH levels (5, 7, 9), while being shaken at 150 rpm in a rotary incubator. Upon reaching an OD of 0.2, 100 ppm of sterilized heavy metals (Cr, Cu, Cd, Ni, and Pb) were introduced, and incubation continued. Bacterial growth was monitored over 24 hours by measuring absorbance (at λmax = 600 nm) using a spectrophotometer. The incubation lasted for 6 days, with samples extracted every 48 hours. These samples were centrifuged at 6000 rpm for 10 minutes, and the resulting supernatant was analyzed using ICP-OES(Pinki et al., 2021).

$$\text{Reduction capacity \%} = \frac{A-B}{A} \quad (1)$$

A - Initial Concentration of sample(ppm); B – Final Concentration of sample(ppm)

Formulation and assessment of bacterial consortium N-TE2A2B36 performance

During bio interaction trials, a new bacterial consortium labelled NC-TE2A2B36 emerged, composed of promising strains N2A, N2B, NB3, NB6. This consortium was developed based on the mono-culture performance in TE bioremediation studies. To prepare the consortium, researchers aseptically transferred a loopful of each pure bacterial strain into 250 mL Erlenmeyer flasks containing 50 mL of Nutrient Broth (pH 7.0). These flasks underwent incubation at 35°C with 130 rpm agitation for 18 hours. Following this, 15 mL of the newly formed bacterial consortium was added to 85 mL of undiluted TE (pH 7.0) in 500 mL Erlenmeyer flasks. To evaluate TE bioremediation efficiency, the flasks were incubated for five successive days at 35°C and 120 rpm in an incubator shaker. Samples of the bacterially treated TE were extracted every 24 hours, subjected to centrifugation at 10,000 rpm for 10 minutes, and the resulting supernatant was utilized to measure the COD parameter(Ahmed et al., 2021).

Bioremediation experiment

The bioremediation experiments were conducted using the optimized concentration (%, v/v) of the established bacterial consortium NC-TE2A2B36, which was refreshed with undiluted real TE in 500 mL Erlenmeyer flasks. The flasks were then placed in an incubator shaker for a total of five days, along with appropriate abiotic factor controls, under suitable environmental conditions (pH and temperature) and agitation (rpm). Throughout the study, bacterial growth was monitored by measuring absorbance (at λmax = 600 nm) using a spectrophotometer. Every 24 hours, samples of the bacterially treated TE were extracted, subjected to centrifugation at 10,000 rpm for 10 minutes, and the resulting supernatant was used for COD measurements(Singh et al., 2019; Zhou et al., 2023).

$$\text{Removal Efficiency (\%)} = C_0\text{-}C_t / C_0 \times 100 \quad (2)$$

[Where C_0 = initial concentration of contaminants (mgL^{-1}) in the untreated TE; C_t = final concentration of contaminants (mgL^{-1}) in the TE after biotreatment].

Inductively Coupled Optical Emission Spectroscopy (ICP-OES)

ICP-OES analysis was employed to quantify heavy metals in the experimental sample

(Nutrient broth-treated) and in both untreated and treated tannery effluent using bacterial consortia NC-TE2A2B36. The entire culture underwent centrifugation, and the resulting supernatants were combined with double-concentrated HNO3. The mixtures were heated on a hotplate stirrer at 100°C for acid digestion until the volume reduced to the initial supernatant volume. The extract was filtered through Whatman filter paper, collected in a flask, and subsequently diluted. This prepared extract was then analyzed using ICP-OES(Plestenjak et al., 2022).

Fourier transform-Infrared (FT-IR) analysis

FT-IR analysis was utilized to identify the functional groups of hazardous organic compounds in tannery effluent (TE) before and after treatment with bacterial consortia NC-TE2A2B36. A 10 mL filtered TE sample was oven-dried at 100°C, then mixed with FT-IR grade-KBr (1:30 ratio, 99% purity). This mixture was ground and compressed into a thin pellet (13 mm diameter, 1 mm thickness) under vacuum using a PCI Analytics Cast Steel IR Hydraulic Press (10-ton capacity). The absorption spectrum was recorded using a Nicolet TM 6700 FT-IR spectrometer. Spectra were obtained by scanning the mid-IR range (4000 - 400 cm -1, 4 cm -1 resolution) in ambient air against a pure KBr background spectrum. The spectrum was processed using OMNIC™ software (v7.4), and absorption peaks in the FT-IR spectrum were assigned according to standard reference text(Xue et al., 2020).

Phytotoxicity experiment

The OECD Safety Guidelines (http://www.oecd.org/chemicalsafety/testing/33653757. pdf) were adhered to in evaluating the phytotoxicity of TE both before and after treatment with the bacterial consortium. Phaseolus mungo (black urad) is a terrestrial model implemented in toxicity evaluation (Hossan et al. 2020). Using 10 healthy, surface-sterilized seeds (thrice rinsed with distilled water before eradicating the seed-borne fungus with a mixture of 2.0% $HgCl_2$), a seed propagation test was performed in three duplicates to evaluate the phytotoxicity of TE Phaseolus mungo, a natural model employed in toxic assessments, was used to evaluate the phytotoxicity of TE before and after treatment with bacterial consortia NC-TE2A2B36 pursuant with the OECD Safety Guidelines. Phaseolus mungo seeds which were properly maintained and surface-sterilized (by applying a mixture of 2.0% HgCl2 to kill the seed-borne fungus proceeded by three cleanings with distilled water) have been utilised in a three-replica seed production test to assess the toxic effects of TE (Kumar et al. 2022; Nirmala 2021).

Statistical Analysis

A statistical analysis was carried out using the isolate's degradation percentage at different temperatures, pH levels, and incubation times. Both GraphPad Prism and Microsoft Excel were used in the analysis of all the data. Comparative analyses were performed by Tukey's Test analysis by two-way ANOVA method (Zhou, Zhang, and Wang 2023).

3. Results and Discussion

Characterization of bacterial strains

Tannery effluent sample was collected from the tannery industry was supplied by Chromepet. The sample concentrations were determined to be 2218CFU/ml after serial dilution. For

further examination, five distinct bacterial colonies were selected and streaked. The bacterial cultures underwent salt tolerance testing. Eight bacterial isolates (N2A, N2B, N3, N4A, N4B, N5A, N5B, N6) from initial screening were individually grown on nutrient agar plates containing various metals (Cr, Cu, Cd, Ni, and Pb) at concentrations ranging from 50 ppm to 300 ppm at 37 °C. Growth was monitored for three days. The minimum inhibitory concentration (MIC) determines the increase in heavy metal concentration required to inhibit bacterial growth. Isolates N2A, N2B, N3, N6 were demonstrated tolerance to all concentrations, exhibiting significant resistance at 10,000 ppm. The bacterial isolates were characterized based on biochemical, morphological, and cultural characteristics. Tests included catalase oxidase, urease, nitrate, colony color, shape, texture, and gram staining. Four potential bacterial strains were ultimately chosen and identified through various morphological and biochemical tests, determined by COD removal efficiency, temperature, and salt tolerance tests. Strains N2A exhibited rod shaped, tough texture and protective endospore. N3 showed rough, opaque, and irregular shape and texture. N2B had round or irregular, with undulate or fimbriate margins. N6 depicted as rough, opaque, fuzzy white shape. N2A, N2B, N3, N6 showed gram positive. Potential bacterial isolates underwent 16S rRNA sequencing amplification, and a phylogenetic tree was constructed. Strains N2A, N2B, N3, N6 were identified in the NCBI GenBank database as Bacillus subtilis JC43 (OR740579), Bacillus licheniformis NWPZ-62 (OR740683), Bacillus subtilis DCK5 (OR741746), Bacillus subtilis MK729017.1 (OR741754). The phylogenetic tree, generated using MEGA 10 software, revealed that the species belong to the Bacillaceae family and Bacillus genus. Furthermore, bacterial strains D1, D3, and D12A exhibited the highest tolerance to salt (NaCl) concentrations up to 3%, 6%, and 9% (w/v), indicating their halotolerant nature and suitability for treating or detoxifying TE. The evolutionary tree was constructed using the neighbor-joining approach (consensus test) in MEGA software (v10). These findings are consistent with research demonstrating that Bacillus subtilis JC43, Bacillus licheniformis NWPZ-62, Bacillus subtilis DCK5, Bacillus subtilis MK729017.1 can decompose and restore industrial waste and environmental pollutants (Bolonhesi et al., 2023; Marzan et al., 2017).

Analysis of tannery effluent properties

Standard methods were employed to assess the physiochemical attributes (Table 1). The effluent exhibited an acidic pH of 3.42, dark green, and an offensive smell. Sodium levels were elevated high at 2056 mg/L. The effluent's total dissolved solids (TDS) were notably high at 5837 mg/L. Electrical conductivity reached an exceptionally high value of 9120 µs/cm. The effluent showed moderate levels of chemical oxygen demand (COD) and biological oxygen demand (BOD). COD measurements were recorded at 10398 and 1720 mg/L, surpassing BOD levels. The study also examined other parameters and heavy metals, including chromium, nickel, copper, lead, and cadmium. The findings indicated that the deliming effluent was contaminated, necessitating the implementation of technology capable of breaking down mixed tannery effluent. Untreated tannery effluent (TE) displayed extremely high levels of BOD (1720 mg L−1), TDS (5837 mg L−1), total suspended solids (TSS) (1254 mg L−1), phosphate (15 mg L−1), sulphate (21 mg L−1), nitrate (14 mg L−1), and phenol (0.9 mg L−1). Significant concentrations of heavy metals were also present in untreated TE, including Ni (6.14), Cu (1.68), and Cr (4.24 mg L−1). All pollutant parameters examined in this study

exceeded the standard limits for wastewater discharge. The presence of sulfate was linked to the foul odor in untreated TE. The dark green TE was attributed to azo dye chemicals used in leather coloring processes (Kiraye et al., 2018). The high BOD, indicating low dissolved oxygen in the effluent, may be due to the substantial organic matter content in untreated TE. The elevated COD and TDS levels in the collected effluent could be attributed to the presence of dissolved salts, minerals, and persistent organic contaminants in the untreated TE(Bharagava & Saxena, 2020; Saxena et al., 2017).

Table 1 Physiochemical characteristics of Tannery effluent

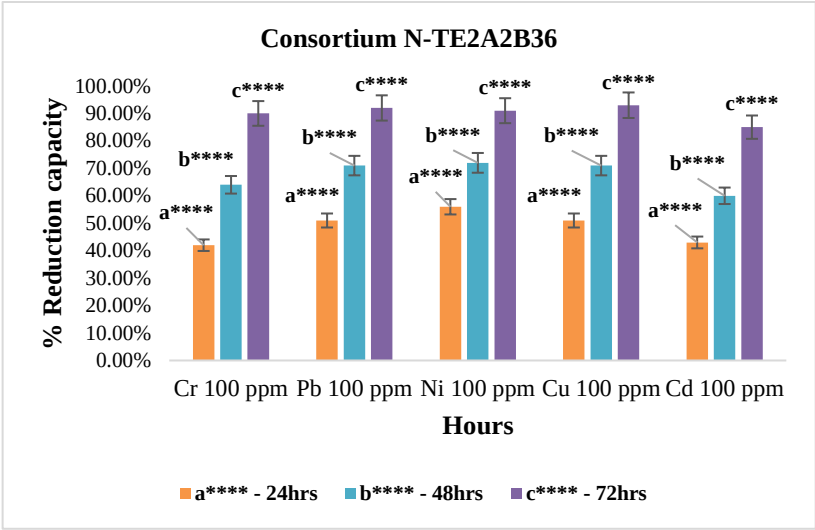
Physiochemical Parameters	Effluent Discharge limits	Tannery Effluent
Color	-	Dark green
Odor	-	Unpleasant
pH@25 (℃)	6.0-9.0	3.42
Temperature (℃)	>35	37
EC (µs/cm)	-	9120
Potassium (mgL^{-1})	-	85
Calcium (mgL^{-1})	785	280
Magnesium (mgL^{-1})	123	196
Sodium(mgL^{-1})	258-14056.45	2056
SS (mgL^{-1})	35–100	621
TS (mgL^{-1})	-	170
TDS (mgL^{-1})	2100	5837
TSS (mgL^{-1})	100	1254
DO (mgL^{-1})	6.5-8	10
BOD (mgL^{-1})	20	1720
COD (mgL^{-1})	250	10398
Sulphate	1500	21
NH_3(mgL^{-1})	10.0	18
Total Nitrogen (mgL^{-1})	1-2	25
Phenol (mgL^{-1})	1.0	0.9
Nitrate(mgL^{-1})	10.0	14
Total Phosphates	5.0	15
Total Alkalinity (mgL^{-1})	-	150
Turbidity (TNU)	-	852
As(mgL^{-1})	0.2	0.1
Cd(mgL^{-1})	0.05	0.56
Pb(mgL^{-1})	0.1	12.28
Ni(mgL^{-1})	3.0	6.14
Cr(mgL^{-1})	2.0	4.24

Cu(mgL^{-1})	3.0	1.68
Fe(mgL^{-1})	3.0	1.09
Mn(mgL^{-1})	2.0	0.8

EC: Electrical conductivity; BOD: Biochemical oxygen demand; COD: Chemical oxygen demand; DO: Dissolved Oxygen; SS: Suspended Solids; TS: total solids; TDS: total dissolved solids; TSS: Total suspended solids; BQL: Below Quantification limit; ND: Not detected; UT-TE: Untreated tannery effluent; *As per Central Pollution Control Board (2024); Ministry of Environment, (2024), India. ** Pollutant removal efficiencies (%) were calculated according to Eq 2.

Bio Reduction Experiment

A growth curve is meant to show the population of bacteria in a culture as a function of time. The lag, exponential, stagnant, and death phases are the four stages of a normal growth curve. The amount of time it takes for bacteria to reach a stage when they may proliferate and develop quickly is known as the "lag phase"(Pinki et al., 2021). Among the phases seen in a bacterial growth curve is the "log phase." Other names for it include the logarithmic phase and the exponential growth phase. The characteristic that makes this phase unique is cell doubling. When cells cease to develop yet maintain an active metabolism, this is known as the stationary phase. These phases are fascinating to study since they entail a variety of chemical and physical changes(Yadav et al., 2021). The variety of physical and chemical changes that occur during this phase makes them fascinating to study. The production of unique proteins during the stationary phase is crucial to the bacteria's ability to survive(Plestenjak et al., 2022). The investigation looked at the impacts of several heavy metals introduced at 0.6 OD at log Phase after they had been sterilised to a concentration of 100 ppm. Remarkably, (Fig.1) shows a considerable reduction and tolerance to all heavy metals. In two days, Consortium N-TE2A2B36 shown reduction efficiency of 42, 51, 56, 43, 51% in Cr, Pb, Ni, Cu, and Cd; in four days, it demonstrated remarkable efficiency of 64,71,72,71, 60%. The consortium demonstrated ****$p < 0.0001$ and achieved greater efficiency in 6 days, reducing by 90, 92, 91, 93, 85%.



Bioremediation of tannery effluent using bacterial consortium N -TE2A2B36

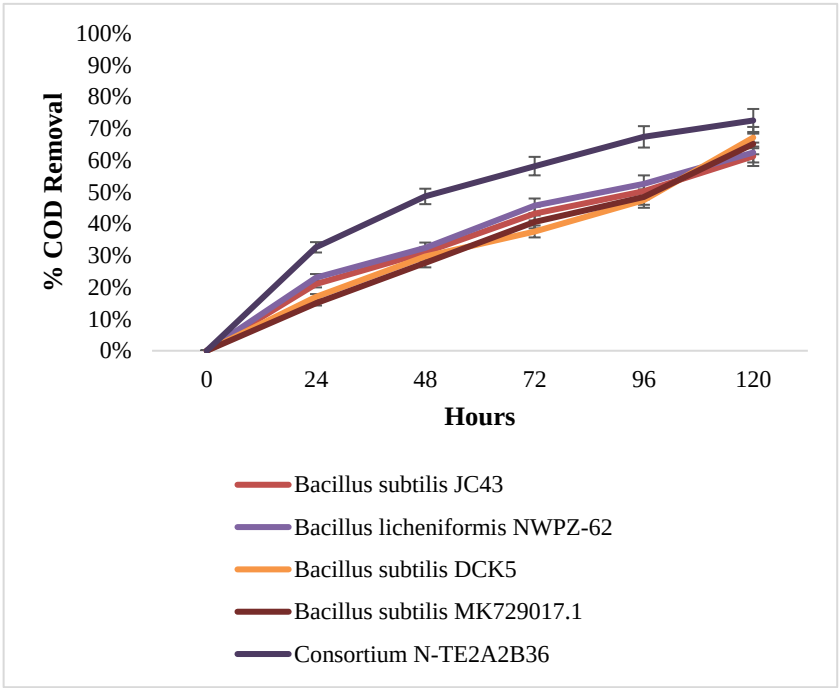


Figure 2 Bacterial consortia N - TE2A2B36 as well as individual bacteria, removed COD untreated TE - pH 7, temp - 35°C, speed of 130 rpm – represented by error bars.

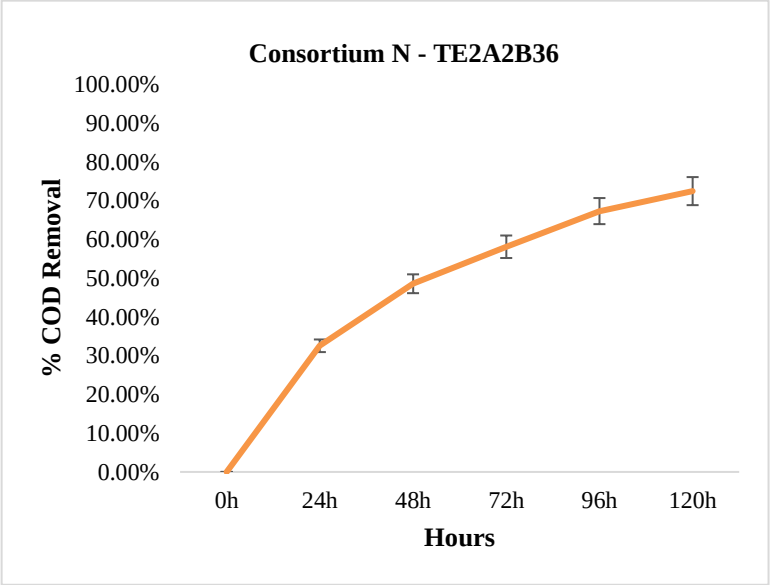


Figure 3 Effect of inoculum concentration on COD removal from real TE using newly developed bacterial consortium N-TE2A2B36. Error bars represent the standard deviation calculated from at least three independent experiments (performed at the optimized conditions: 7 pH, 35 ◦C, 130 rpm, 15 mL inoculum volume, and 0.5 %, w/v glucose and NH_4Cl).

The treatment and detoxification of tannery effluent (TE) is essential for environmental protection due to its composition of heavy metals and potentially harmful organic substances(Bharagava et al., 2018). Chemical Oxygen Demand (COD) is considered a key indicator of pollution and a standard measure for assessing wastewater and sewage contamination levels(Tufail et al., 2022). Aquatic ecosystems are negatively affected by industrial effluents with high COD, disrupting natural processes for plants and animals(Shah, 2022). Thus, COD removal from TE is crucial for safeguarding public health and the environment(Okina Solomon et al., 2024). In this research, the primary metric for monitoring the biological removal of TE is COD reduction, as shown in Eq.2. Bioremediation studies on actual TE demonstrated COD elimination rates of 61.23, 62.42, 67.12, 65.12, 72.51% by Bacillus subtilis JC43, Bacillus licheniformis NWPZ-62, Bacillus subtilis DCK5, Bacillus subtilis MK729017.1 respectively, over 120 hours at 35°C and 130 rpm (Fig. 2). Microbial consortia comprising potential strains are considered more effective for wastewater treatment and bioremediation compared to individual bacterial strains(Khanam et al., 2024). This is because the intermediate compounds produced by one strain's catabolic pathway can be further broken down by another strain's suitable catabolic pathway, effectively treating and detoxifying persistent industrial effluents(Prasad et al., 2021). However, the extent of pollutant exploitation limits high COD clearance(Zhou et al., 2023).

Characterization of organic pollutants and contaminants

Using HP-LC, FT-IR techniques, researchers analyzed organic contaminants and their breakdown products during the bioremediation of genuine leather TE. This study employed a

novel bacterial consortium, N-TE2A2B36. HP-LC examination of untreated TE showed several organic pollutants, evidenced by multiple peaks at various retention times (RT: 1.51, 1.90, 2.37, 3.91) (Fig. 4). In contrast, bacteria-treated TE exhibited distinct peaks (at RT: 1.09, 1.83, 2.30, 3.87), which were subsequently confirmed through FT-IR and HPLC analyses.

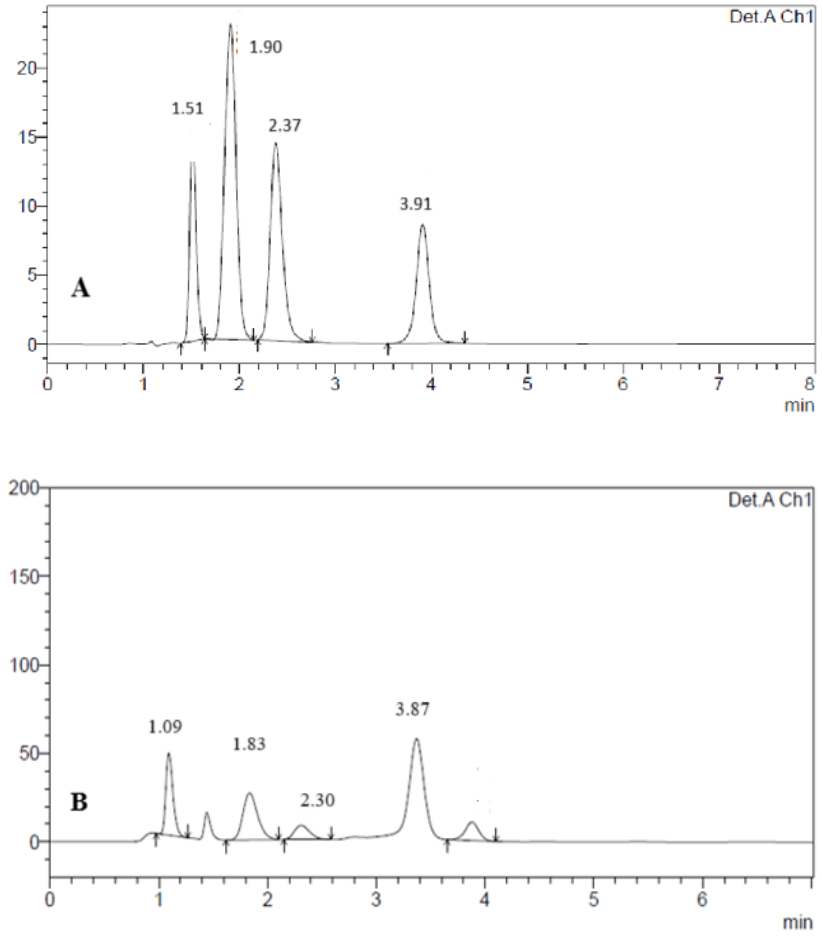


Figure 5 HPLC spectrum of untreated tannery effluent (A) Tannery effluent (B) Newly developed consortium N-TE2A2B36

These findings strongly indicate the biodegradation or biotransformation of organic contaminants and the formation of new metabolic byproducts. The observed reduction in peak area further supports this conclusion, indicating a decrease in pollutant concentration. The analysis revealed a significant medium peak near 3329.14 cm^{-1}, indicating N-H stretching associated with aliphatic primary amine groups. A weak bond at 2113.98 cm^{-1} represents the triple stretching of the alkyne group (C ≡ C), while the strong bond at 1637.56 cm^{-1} corresponds to C=C double bond stretching, indicative of alkene groups. Additionally, a medium peak at 3327.21 cm^{-1} corresponds to N-H stretching from secondary amine groups. In summary, the untreated sample (TE) is suspected to contain phenols, azo dyes, alcohols,

carboxylic acids, surfactants, and aliphatic amines. However, the appearance of additional peaks and the disappearance of some prominent peaks associated with functional groups of hazardous compounds suggest that the organic pollutants in the bacterially treated sample have either been broken down or transformed.

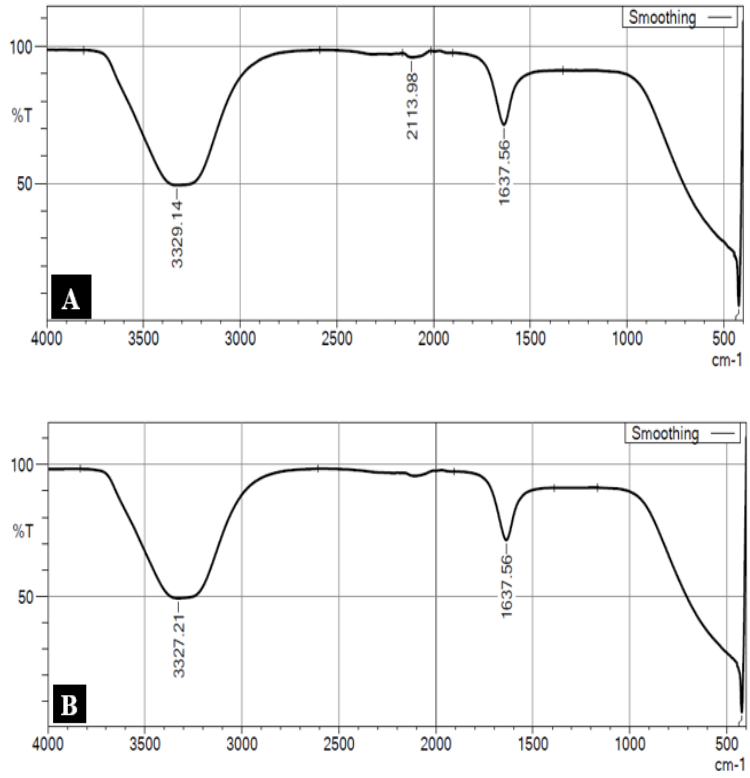


Figure 6 FT-IR spectrum of untreated tannery effluent (A) Tannery effluent (B) Newly developed consortium N-TE2A2B36

Phytotoxicity Test

The excessive release of TE from LTs into the receiving environment (soil/water) has a negative impact on the ecology and causes high pollution levels in Chennai, India. endanger the local wildlife and vegetation, hence this effort is of top priority in the current situation. According to the current investigation, the untreated TE was extremely hazardous and reduced the physiological factors that are important for Phaseolus mungo L. seed germination and seedling growth were greatly enhanced when The created bacterial consortium, N-TE2A2B36 was used to irrigate seeds with TE (Table 2, Fig.7). Maybe this was because metal pollutants and toxic organic compounds identified in TE were degraded or detoxified by bacteria, as verified by physico-chemical characterisation (Table 1) as well as HP-LC and FT-IR assessments. The seeds irrigated with different concentrations (12, 25, 50, 75, 100%, v/v) of untreated TE showed a much lower germination percentage (GP) than the control (TP: tap water). All seeds treated with varying concentrations of TE (25–100%, v/v) treated with the

bacterial consortia, however, showed a considerable improvement in GP. The phytotoxicity percentage (PP) of untreated TE ranged from 72.1% to 31.2% at greater and lower concentrations (100 % to 25%, v/v). The high salt load and harmful organic pollutants and metals in the untreated TE may have caused this tendency. These substances used to create an anaerobic environment and high osmotic pressure, which led to the uptake of poisonous metals in plants and their harmful effects(Hossan et al., 2020). In addition, seeds treated with the bacterial consortia, which broke down and detoxified the organic compounds and harmful metals in the TE, showed a significant improvement in PP when irrigated with varying concentrations of TE (100 percent to 25 percent, v/v).

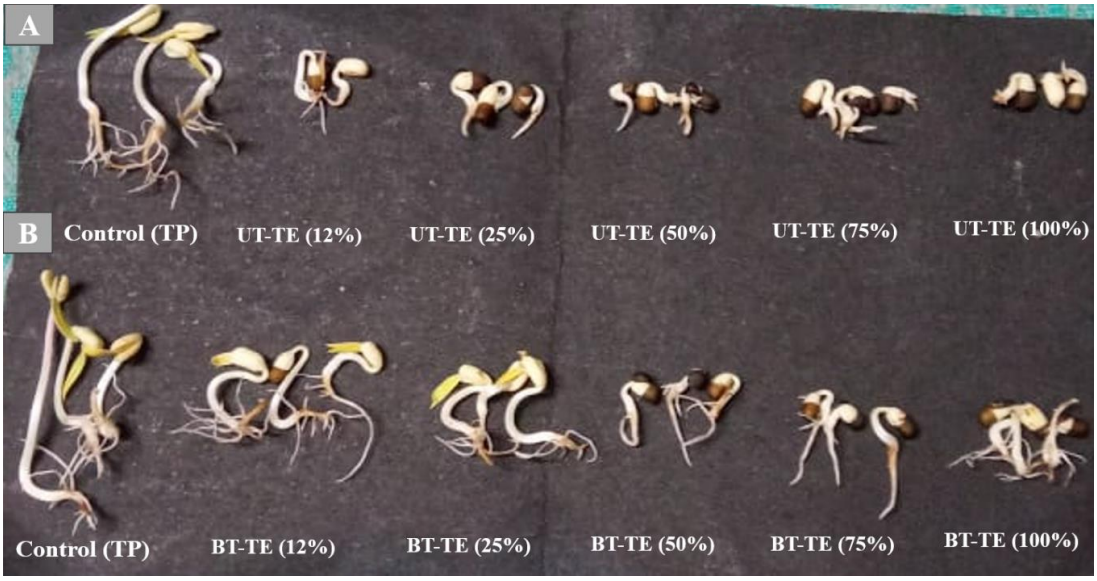


Figure 7 Day 5 Growth Observation (A) Untreated tannery effluent (B) Bacterial treated tannery effluent consortium N-TE2A2B36

Table 2: Phytotoxicity Test

TE%	GP%	SLM%	GI%	SL (cm)	RL (cm)	RSR	SVI	PP	α - amylase activity
Control	100	0	100	6±0.02	4.7 ±0.03	0.68±0.01	812	40.6	0.68±0.13
UT-TE (12%)	100	25	51.2	5.1±0.43	3.18±0.1	0.67±0.05	752	31.2	0.62±0.01
BT-TE (12%)	100	0	79.3	5.32±0.29	4.12±0.21	0.62±0.02	789	43.7	0.60±0.01
UT-TE (25%)	90	15	40.07	3.10±0.01	3.17±0.12	0.65±0.05	524	30.6	0.63±0.02
BT-TE (25%)	100	0	61.52	4.17±0.12	4.21±0.11	0.62±0.01	594	65.4	0.60±0.02
UT-TE (50%)	75	35	23.33	1.2 ±0.1	2.21±0.20	0.58±0.03	335.3	43.5	0.53±0.05

BT-TE (50%)	100	0	48.4	3.02±0.01	3.23±0.12	0.51±0.02	412	54.9	0.50±0.05
UT-TE (75%)	55.3	46.27	12.03	1.1±0.2	1.08±0.10	0.55±0.04	198.2	67.4	0.54±0.07
BT-TE (75%)	90	10	35.15	1.4±0.13	2.10±0.05	0.49±0.02	239.7	56.4	0.41±0.07
UT-TE (100%)	37.5	54.2	5.27	0.4±0.1	0.5±0.1	0.45±0.01	40.31	72.1	0.45±0.02
BT-TE (100%)	79	30 .6	20.34	1±0.10	1.18±0.04	0.30±0.01	165.8	69.5	0.33±0.01

Nevertheless, the detrimental effects of industrial effluents on the physiological factors important for seed or seedling germination and growth can differ depending on the concentration and kind of crops that are irrigated(Kumar et al., 2022).Gibberellins, cytokinins, auxins, and other phytohormones that are essential for seed and seedling germination and growth have been shown to be inhibited by high concentrations of salts and unpleasant organic and inorganic pollutants (metals)(Nirmala, 2021). Seedling mortality (SLM), which peaked at 66.34 percent at a higher effluent concentration (100 percent, v/v) in comparison to control (TP, 0.00 percent), further demonstrated the toxicity of untreated TE. However, upon treatment with the bacterial consortia, the SLM significantly decreased from higher to lower concentrations (100 % to 25%, v/v) of TE(Bharagava et al., 2018). The α-amylase enzyme activity observed in the germinating seeds further demonstrated the harmful effects of untreated TE on seed/seedling germination and growth (Table 3). A maximum α-amylase activity of 0.62 ±0.04 Unit grain 1 was observed in seeds watered with 25% concentration (v/v) of untreated TE. Subsequently, it steadily declined at progressively larger volumes (i.e., 50% – 75%, v/v) of the TE. Salts, harmful heavy metals, and stubborn organic pollutants have been shown to suppress α-amylase activity, which is essential for converting starch to sugars and is hence helpful for seed germination(Vijayaraj et al., 2018). In comparison to leather TE that had not been treated, the outcomes at 25% and 50% concentrations (v/v) of bacterially treated TE were consistently superior. Furthermore, these bacterially treated TE concentrations (25 and 50%, v/v) may function similarly to a liquid fertiliser and be non-toxic to plant growth and development; as a result, they might be utilised to irrigate agricultural crops properly diluted.

4. Conclusion

The goal of this study was to create a new bacterial consortia N-TE2A2B36 for the detoxification and breakdown of genuine leather TE as well as the assessment of its phytotoxicity for environmental safety. A new bacterial consortium was emerged using four potential pollutants-degrading bacterial strains N2A, N2B, N3, N6 that were isolated from Pre tanning TE in the current study. These strains were able to remove COD from real leather TE up to 61.23, 62.42, 67.12, 65.12%, respectively and could consortium 72.51%. Additionally, these bacterial strains were determined to be Bacillus subtilis JC43, Bacillus licheniformis

NWPZ-62, Bacillus subtilis DCK5, Bacillus subtilis MK729017.1, respectively by a combination of identification and morphological characterizations as well as 16S rRNA gene sequence analysis. The physico-chemical analysis of leather tannery effluent (TE) used in bioremediation studies revealed elevated levels of COD, BOD, TDS, phenol, and total chromium, which also exhibited phytotoxic effects on Phaseolus mungo L., a terrestrial model organism. These results demonstrate its strong potential for treating and detoxifying leather TE. The optimal conditions for the bioremediation process were identified as a pH of 7, a temperature of 35°C, an inoculum volume of 15 mL, and an agitation rate of 120 rpm. Analysis using FT-IR, HPLC, techniques confirmed the complete removal of organic contaminants from untreated TE and the formation of new metabolic products in the bacterially treated TE. Phytotoxicity studies further revealed that the toxicity of the treated TE was significantly reduced, enabling 70% seed germination compared to seeds irrigated with untreated TE (100%, v/v). This suggests that bacterially treated TE could potentially be used as a liquid fertilizer for irrigating agricultural crops after suitable dilution. Overall, this study highlights the efficacy of the bacterial consortium in the treatment and detoxification of leather TE, contributing to environmental sustainability and protection.

Declaration of Competing Interest The authors report no declarations of interest

Acknowledgement

I express my earnest and deep sense of gratitude to my supervisor and to my college Dr M.G.R Educational and Research Institute, Maduravoyal, has always been a moral support.

Author contribution Raveena Jayam. J - Investigation, Review of literature, Writing-original draft, review and editing, Supervision- K.J. Sharmila.

References

1. Ahmed, S. F., Mofijur, M., Nuzhat, S., Chowdhury, A. T., Rafa, N., Uddin, M. A., Inayat, A., Mahlia, T. M. I., Ong, H. C., Chia, W. Y., & Show, P. L. (2021). Recent developments in physical, biological, chemical, and hybrid treatment techniques for removing emerging contaminants from wastewater. Journal of Hazardous Materials, 416. https://doi.org/10.1016/J.JHAZMAT.2021.125912
2. Bharagava, R. N., & Saxena, G. (2020). Progresses in Bioremediation Technologies for Industrial Waste Treatment and Management: Challenges and Future Prospects. In Bioremediation of Industrial Waste for Environmental Safety (pp. 531–538). Springer Singapore. https://doi.org/10.1007/978-981-13-3426-9_21
3. Bharagava, R. N., Saxena, G., Mulla, S. I., & Patel, D. K. (2018). Characterization and Identification of Recalcitrant Organic Pollutants (ROPs) in Tannery Wastewater and Its Phytotoxicity Evaluation for Environmental Safety. Archives of Environmental Contamination and Toxicology, 75(2), 259–272. https://doi.org/10.1007/s00244-017-0490-x
4. Hasintha Kumari, W. M. N., Chandrasekharan, N. V., & Wijayarathna, C. D. (2021). Characterization of a Multi-Metal Resistant Bacillus cereus Strain TWSL_5 and it's Bioremediation Potential for Cr, Pb, Zn and Fe. American Journal of Environmental Sciences, 17(3), 49–62. https://doi.org/10.3844/ajessp.2021.49.62
5. Hossan, S., Hossain, S., Islam, M. R., Kabir, M. H., Ali, S., Islam, M. S., Imran, K. M., Moniruzzaman, M., Mou, T. J., Parvez, A. K., & Mahmud, Z. H. (2020). Bioremediation of hexavalent chromium by chromium resistant bacteria reduces phytotoxicity. International Journal of Environmental Research and Public Health, 17(17), 1–19. https://doi.org/10.3390/ijerph17176013
6. Khanam, R., Al Ashik, S. A., Suriea, U., & Mahmud, S. (2024). Isolation of chromium resistant

bacteria from tannery waste and assessment of their chromium reducing capabilities – A Bioremediation Approach. Heliyon, 10(6). https://doi.org/10.1016/j.heliyon.2024.e27821

7. Kumar, S., Yadav, A., Maurya, A., Pratap, S. G., Singh, P. K., & Raj, A. (2022). Characterization of tannery effluents by analyzing the recalcitrant organic pollutants and phytotoxicity assay. Journal of Applied Biology and Biotechnology, 10, 91–99. https://doi.org/10.7324/JABB.2022.10s210
8. Nirmala, A. (2021). On the Phytotoxicity of Waste-Water from Textile Industry on Selected Crop Seed Germination and its Treatment Using Bacteria with Zinc Oxide Nanoparticles. Bioscience Biotechnology Research Communications, 14(4), 1564–1570. https://doi.org/10.21786/bbrc/14.4.30
9. Okina Solomon, N., Simpa, P., Adeolu Adenekan, O., Chinenye Obasi, S., & Author, C. (2024). Sustainable nanomaterials' role in green supply chains and environmental sustainability. Engineering Science & Technology Journal, 5(5), 1678–1694. https://doi.org/10.51594/estj/v5i5.1136
10. Pinki, S. A., Karim, M. R., Dewanjee, D., Bhuiyan, H. R., Al Masud, H. M. A., & Hoq, M. I. (2021). Microbial reduction and detoxification of chromium from tannery effluent by natural inhabitants. Nature Environment and Pollution Technology, 20(3), 1369–1380. https://doi.org/10.46488/NEPT.2021.V20I03.051
11. Plestenjak, E., Kraigher, B., Leskovec, S., Mandic Mulec, I., Marković, S., Ščančar, J., & Milačič, R. (2022). Reduction of hexavalent chromium using bacterial isolates and a microbial community enriched from tannery effluent. Scientific Reports, 12(1). https://doi.org/10.1038/s41598-022-24797-z
12. Prasad, S., Yadav, K. K., Kumar, S., Gupta, N., Cabral-Pinto, M. M. S., Rezania, S., Radwan, N., & Alam, J. (2021). Chromium contamination and effect on environmental health and its remediation: A sustainable approaches. Journal of Environmental Management, 285, 112174. https://doi.org/10.1016/J.JENVMAN.2021.112174
13. Saxena, G., Chandra, R., & Bharagava, R. N. (2017). Environmental pollution, toxicity profile and treatment approaches for tannery wastewater and its chemical pollutants. In Reviews of Environmental Contamination and Toxicology (Vol. 240). Springer New York LLC. https://doi.org/10.1007/398_2015_5009
14. Shah, M. P. (2022). Chemistry in the Environment Series No. 5 Biological Treatment of Industrial Wastewater Edited. www.rsc.org
15. Singh, S. K., Mangotra, A., & Murali, R. S. S. (2019). BIOREMEDIATION OF INDUSTRIAL EFFLUENTS: A COMPREHENSIVE REVIEW AND PERSPECTIVES. https://www.researchgate.net/publication/343125709
16. Tufail, M. A., Iltaf, J., Zaheer, T., Tariq, L., Amir, M. B., Fatima, R., Asbat, A., Kabeer, T., Fahad, M., Naeem, H., Shoukat, U., Noor, H., Awais, M., Umar, W., & Ayyub, M. (2022). Recent advances in bioremediation of heavy metals and persistent organic pollutants: A review. Science of The Total Environment, 850, 157961. https://doi.org/10.1016/J.SCITOTENV.2022.157961
17. Vijayaraj, A. S., Mohandass, C., Joshi, D., & Rajput, N. (2018). Effective bioremediation and toxicity assessment of tannery wastewaters treated with indigenous bacteria. 3 Biotech, 8(10). https://doi.org/10.1007/s13205-018-1444-3
18. Xue, J., Wu, Y., Fu, X., Li, N., Sun, J., & Qiao, Y. (2020). Study on degradation characteristics and bacterial community structure changes of immobilized cells in straw-alginate beads in marine environment. Bioresource Technology Reports, 10. https://doi.org/10.1016/j.biteb.2020.100402
19. Yadav, P., Yadav, A., Srivastava, J. K., & Raj, A. (2021). Reduction of pollution load of tannery effluent by cell immobilization approach using Ochrobactrum intermedium. Journal of Water Process Engineering, 41. https://doi.org/10.1016/j.jwpe.2021.102059
20. Zhou, B., Zhang, T., & Wang, F. (2023). Microbial-Based Heavy Metal Bioremediation: Toxicity and Eco-Friendly Approaches to Heavy Metal Decontamination. In Applied Sciences (Switzerland) (Vol. 13, Issue 14). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/app13148439