

Research article

Wastewater Management Towards Microbial Degradation of Synthetic Azo Dyes from Tannery Effluent

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Abstract

Industrial waste has the potential to contaminate the soil and groundwater. Globally, the tanning industry is regarded as a potential source of environmental pollution. Inorganic amines are abundant in the tannery industry's by-products. Azo dyes are the primary components of tannery effluent, which negatively impacts aquatic and terrestrial flora and fauna. Therefore, the elimination of these dye pollutants is crucial for preventing the contamination of our vulnerable resources. Our research work focused on an efficient way to clean and regulate azo dye pollution emerged in recent years. Azo dye degrading bacteria were isolated from tannery effluent solid waste and employed for decolorizing azo dye. The isolated strains of bacteria were identified as *Bacillus tropicus* (OQ799318) and *Bacillus paramycoides* (OQ799314). The novel bacterial strain exhibited rapid decolorization activity, removing 91% of the yellow azo dyes and 90% of the brown azo dye. The optimum conditions for azo dye degradation was at 10% inoculum concentration, pH 7.0, temperature 37°C, and 48 h. *Bacillus tropicus* and *Bacillus paramycoides* can be used as eco-friendly biological expedients for the bioremediation and detoxification of azo dyes used in industries.

Keywords: azo dye; degradation; tannery effluent; biological treatment; *Bacillus*

1. Introduction

Human activities are the leading cause of environmental pollution, with humans polluting the biological system in numerous ways. On the one hand, the development of various types of industries contributes significantly to the country's economic growth. On the other hand, these industries generate a substantial amount of liquid and solid refuse that is released into the environment (Stapleton & Singh, 2002). The tannery industry plays a crucial role in the discharge of vast quantities of waste in the form of effluent and sludge (Framis, 2018). The industry generates waste containing organic and inorganic substances that are highly toxic, carcinogenic, and mutagenic. Industrial waste management is a formidable obstacle, particularly in developing nations. Large amounts of industrial waste

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are being dumped onto soil surface, resulting in adverse environmental effects. Due to the high cost of treatment, little or no attention is being paid to the appropriate disposal of accumulated waste (Hussain et al., 2020). In addition, to affecting soil flora and fauna as well as the aquatic environment, such industrial discharge causes secondary infections in humans, posing grave environmental concerns (Sanjay et al., 2020).

Tanning is an age-old practice in the leather industry to preserve animal products and transform them into usable goods (Fathima et al., 2012). Unfortunately, the tannery industry's improper disposal of effluents has led to the conversion of fertile agricultural land into infertile fields. The waste generated by tanneries depends on the leather processing method. Over 10,000 tonnes of synthetic dyes are utilised by the tannery industries, out of the approximately 7×10^7 tonnes that are produced globally each year. Dyes are commonly classified into various categories based on their source, composition, and usage. The tannery industries utilize azo, direct, reactive, mordant, acid, basic, dispersion, and sulfide dyes (Mahmood et al., 2013). The discharge of sediment and effluent from tanneries and dyeing industries is often highly polluted with a variety of contaminants, including sulfides, azo dyes, and heavy metals such as cadmium, lead, and arsenic (Verma & Sharma, 2023). By raising the biological and chemical oxygen demand, tanning dyes also reduce the aesthetic value of water bodies. This can lead to a variety of negative effects, including toxicity, mutagenicity, and carcinogenicity, as well as difficulties with photosynthesis, plant growth inhibition, entry into the food chain, recalcitrance, and bioaccumulation (Anjaneya et al., 2011).

Organic molecules with the functional group $R-N=N-R'$, where R and R' are typically aryl and substituted aryl groups, are known as azo dyes. These are azo compounds, or compounds with the C-N=N-C connection, that are significant to the commercial community (Mahmood et al., 2011). Azo pigments are chemically related derivatives of azo dyes that are insoluble in water and other solvents. The increased salinity can make it difficult for plants to grow, and the high BOD/COD levels can lead to oxygen depletion in waterways, causing fish and other aquatic life to die (Erkurt, 2010).

In addition to the environmental and health risks posed by tannery industries, there are also social and economic consequences to consider. These industries often operate in developing countries, where regulations and enforcement may be lacking, and the negative effects on local communities can be severe. On the economic side, the negative environmental and health impacts can harm tourism and other industries that rely on clean water and a healthy environment (Sivaram & Barik, 2019).

Tannery residues mainly consist of azo dyes, which can pose significant environmental risks. These industries generate a significant amount of waste, with a large proportion containing chromium and various azo dyes. Unfortunately, up to 2-50% of pigments and 40% of the applied chromium end up in effluent and sludge, which may have a direct impact on the environment. Since both azo dyes and chromium are highly toxic, the discharge of tannery waste into wastewater streams and soil causes severe environmental issues (Rajwade et al., 2022).

Azo dyes are the most widely used synthetic dyes in commercial applications and account for over 50% of global dye production (Saratale et al., 2011). With more than 2,000 structurally distinct azo dyes currently in use, they are highly persistent and xenobiotic in natural environments, causing acute toxicity to ecosystems. There are reports of their mutagenic and genotoxic effects on living organisms, making sludge and effluents contaminated with azo dyes a serious concern for water ecosystems and human health (Deng et al., 2020). The persistence of azo dyes in the environment is attributed to their complex molecular structures, such as fused aromatic rings and stable $-N=N-$ azo linkages, which make them resistant to microbial degradation. Additionally, their high water

solubility limits their interaction and uptake by microbial cells, further inhibiting biodegradation (Feng et al., 2012). Although various physiochemical techniques can remove azo dyes from effluent, these methods are often expensive and generate large quantities of chemical waste. This increases the overall process cost and makes transporting the waste to a suitable location difficult (Hussain et al., 2018).

Biological treatment methods, such as microbial degradation and phytoremediation, have emerged as promising alternatives. These methods are not only environmentally friendly but also cost-effective, making them attractive for large-scale wastewater treatment applications. However, more study is needed to optimize these methods for the removal of azo dyes and to overcome their limitations (Rave et al., 2018). The use of biotechnology for environmental remediation has gained significant attention in recent years due to its cost-effectiveness and eco-friendliness (Selvaraj et al., 2019). Bioremediation is a microbial technology that has shown potential for removing target compounds from polluted sites and treating sludge and wastewater. Various microorganisms, including fungi, yeasts, bacteria, algae, and actinomycetes, have demonstrated the ability to decolorize azo dyes, with bacteria being the most effective and extensively studied group for azo dye degradation (Paul et al., 2013). In the field of bioremediation, microorganisms have been extensively researched and utilized for their ability to break down pollutants (Saxena & Bharagava, 2020). Bacterial communities are effective in removing heavy metals from contaminated soil and water, e.g., *Oceanobacillus profundus*, *Staphylococcus epidermidis*. This is because some bacteria can naturally sequester and transform heavy metals through processes such as biosorption, biomineralization, and bio-reduction. Moreover, some bacteria such as *Chromobacterium violaceum*, *Serratia marcescens*, and *Streptomyces coelicolor* can produce harmful by-products during the bioremediation process. Therefore, the selection of an appropriate bacterial strain for a specific bioremediation application is crucial. Recent advances in molecular biology techniques have provided new tools for the selection and optimization of bacterial strains for bioremediation (Dubey et al., 2010).

Brown and yellow are frequently utilized azo dyes in the tannery industry's manufacturing unit. The objective of the current study was to isolate and characterize bacterial strains to decolorize/degrade four azo dyes used in the leather industry. Since the bacterial isolates originated from the tannery industry, they were readily acclimatized to the prevalent local environment. Therefore, the use of these bacteria is an effective biological method for azo-dye-contaminated wastewater that results in zero sludge disposal.

2. Materials and Methods

2.1 Collection of samples from tannery effluent

From a leather tanning industry site at Latitude, 12° 58' 8.81"N. Longitude, 80° 8' 15.93"E, tannery effluent samples were collected in sterile reagent bottles and brought to the laboratory.

2.2 Isolation characterization and identification of bacteria from tannery effluent sample

Bacterial strains from tannery effluent samples were isolated using the spread plate technique. Serial dilutions were done. From each dilution, 0.1mL was spread over a solid plate containing nutrient agar. Then, the Petri plates were incubated at 37°C for 24 h. Well-

grown bacterial colonies were picked and further purified by streaking. The isolated strains were maintained on nutrient agar slants. Isolates were characterized by a four-step approach. The four-step approach in the characterization of bacterial isolates included the study of colony features, microscopic characteristics, biochemical tests and identification of bacterial isolates.

2.2.1 Catalase test

A loopful of bacterial culture was placed on a dry slide using a sterile inoculating loop. A few drops of hydrogen peroxide were then added. The immediate generation of bubbles at this stage was a positive indication of the presence of catalase in the culture. No bubble formation indicated a negative for catalase (Rave et al., 2018).

2.2.2 Spore stain test

Spore staining was carried out on all the bacterial strains (Sunantha et al., 2025).

2.3 Preparation of tannery azo dye solution

Synthetic azo dye stock solutions were prepared by dissolving 0.01g of each dye in 1 mL of distilled water in a 10 mL conical flask. The dyes used in this study included: tartrazine yellow and direct brown, which were obtained from a major tannery industry in Chennai. Each solution was mixed thoroughly and sterilized by autoclaving at 121°C for 15 min. After sterilization, the solutions were stored at room temperature in amber glass bottles to protect them from light degradation. These stock solutions were used for subsequent dye degradation experiments.

2.4 Screening of effective azo dye decolorizing bacterial isolates

The screening was done to enhance the effective bacterial strains capable of degrading dyes using LB broth. For this purpose, test tubes were prepared in which LB broth (4.5 mL), dye (4.5 mL) and isolate (1 mL) were added. The tubes were then incubated at 37°C. The decolorization was determined by spectrophotometer (JASCO 700) using absorbance at 450 nm for the tannery brown dye, and 520 nm wavelength for tannery yellow dye. The readings were taken at the time intervals of the 0th h, 12th h, 24th h, 36th h, and 48th h, respectively. Uninoculated blanks were run to determine abiotic decolorization. Isolates that produced favorable results throughout the screening process were chosen and stored at 4°C before further investigation (Hussain et al., 2018).

2.5 The decolorizing activity of bacterial isolates

The decolorizing activity of the bacterial isolates was determined by measuring the percentage reduction in dye concentration. Uninoculated LB broth containing the respective dyes served as the abiotic control. Absorbance measurements were taken at 420 nm for the yellow dye and 450 nm for the brown dye at time intervals of 0, 12, 24, 36, and 48 h (Anjaneya et al., 2011). Percentage decolourization was calculated using the formula:

$$\text{Percentage decolorization (\%)} = \frac{A_0 - A_t}{A_0} \times 100$$

where A_0 is the initial absorbance of the dye solution and A_t is the absorbance at time t .

2.6 Identification of the selected isolates

Identification was based on Gram staining, spore staining and the catalase test according to Bergey (1994). After the DNA was purified from the isolated organism, in order to amplify 16S DNA using PCR, the sample was first subject to a 5 min initial denaturation and final extension at 94°C and 72°C, respectively. These were followed by 35 cycles of annealing at 50°C for 45 s, denaturing at 94°C for 60 s, and extending at 72°C for 1 min as part of the polymerase chain reaction. The finished product was examined in an agarose gel electrophoresis and was visualized under a UV lamp. The Sanger sequencing technique was used to sequence the 16S rRNA. Following the receipt of the organism's nucleotide sequence, the query sequence and reference sequences from public databases were aligned and compared (e.g. by BLAST), and a phylogenetic tree was constructed to determine the closest taxonomic match.

Based on the aligned sequences, a phylogenetic tree was constructed using the MEGA X program with the Maximum Likelihood method. The Neighbor-Joining method with 1,000 bootstrap replicates was applied to assess the reliability of the branching. The phylogenetic analysis determined the relationship between the isolated bacterium and closely related organisms obtained from the NCBI database. Following identification, the bacterial nucleotide sequence was submitted to NCBI GenBank and assigned an accession number. The phylogenetic tree was then used to place the isolate within its evolutionary context relative to closely related taxa.

2.7 Optimization of physical factors for efficient decolorization

The high percentage of degradation and ideal growing conditions for the chosen isolates, variables including as temperature, time, pH, and inoculum concentration were optimized through testing. To find the ideal temperature for the isolates, LB broth was inoculated with isolates and incubated at 16°C, 37°C, and 60°C. Before inoculation, 0.1 N HCl and 0.1 N NaOH were used to adjust the pH of LB broth, with pH values of 5, 7, and 9 to establish the ideal pH for the development of isolates. To determine the optimal inoculum concentration for isolate growth, the isolates were added at 1%, 5%, 10%, and 15% concentrations (Mahmood et al., 2011).

2.8 Instrumental analysis

Instrumentation analyses such as UV-vis spectrophotometry and FTIR were done to analyse the degrading potential of the isolates.

2.8.1 UV-spectrophotometry

Most compounds absorb UV radiation, making UV-vis spectrophotometry the most recent analytical method. The degradation of the azo dye compound by the isolated organism was identified, described, and analysed using UV-vis spectrophotometry. The test and control were examined every 12 h for tannery acid azo yellow dye absorbance at 420 nm and

tannery azo brown dye absorption at 450 nm. The percentage of degradation was confirmed by this analysis. This was performed using a multimode spectrophotometer at the School of Life Sciences, B S Abdur Rahman Crescent Institute of Science and Technology.

2.8.2 FT-IR analysis

The FT-IR method uses infrared light to scan test samples and observe chemical properties. Powdered form of the control, tannery azo yellow dye, and tannery acid azo brown dye was examined to confirm the breakdown of complex azo dye compounds by the isolated organisms. The FT-IR analysis was performed at the Department of Chemistry, B S Abdur Rahman Crescent Institute of Science and Technology.

FTIR analysis, which is used to identify and characterize unknown materials, can also be used as a quantitative tool to quantify specific functional groups. Sample preparation needs to be performed before conducting an infrared spectroscopy (IR) study. Furthermore, the carrier used in preparing the sample should be optically transparent in the IR region (4000-400 cm^{-1}). For the KBr pellet method, it is essential to pulverize the KBr powder into a 200-mesh size followed by drying at 110°C to remove any bound water molecules. For a sample pellet of 13 mm diameter, 0.1 to 1% of the sample is mixed with 200 to 250 mg of powdered KBr.

2.8.3 Statistical analysis

Triplicate measurements were done in all cases during the observation and assessment of bacterial growth. Data were captured into Microsoft Excel Software, version 2010 which was used to calculate means and standard deviations. Standard deviation was applied to confirm that the observed changes were statistically significant.

3. Results and Discussion

3.1 Isolation and identification of the bacterial isolate

The colonies on the plate were observed to have irregular or yellow borders, were creamy to white, and were opaque and transparent (Anjaneya et al., 2011). The isolates were revealed to be Gram-positive rod-shaped bacteria. Biochemical characterization was performed (Bergey, 1994). The presence of catalase enzyme in bacteria can be determined by catalase test. The bactericidal effects of hydrogen peroxide are countered by the catalase enzyme. The enzyme catalase accelerates the conversion of hydrogen peroxide (H_2O_2) into oxygen and water.

3.2 Identification of azo dye degrading bacteria by 16S rRNA sequencing analysis

The sequences of the bacterial strains shared a high degree of similarity (99%) with the genus *Bacillus*. The evolutionary history of the bacterial strains is represented in Figure 1 and Figure 2. Phylogenetic trees reveal the species' closest relative (Ranjani et al., 2021). Our strains were more closely related to *Bacillus paramycoides* and *Bacillus tropicus*, which are members of the *Bacillus* genus. The isolated strains displayed the highest degree of sequence similarity in 16S rRNA sequencing. Additionally, the isolated strains

shared a distant genetic relationship with other *Bacillus* strains. In addition, after submission to the sequence to GenBank, the strains were assigned accession numbers for *Bacillus paramycoides* as OQ799314 and *Bacillus tropicus* as OQ799318, respectively.

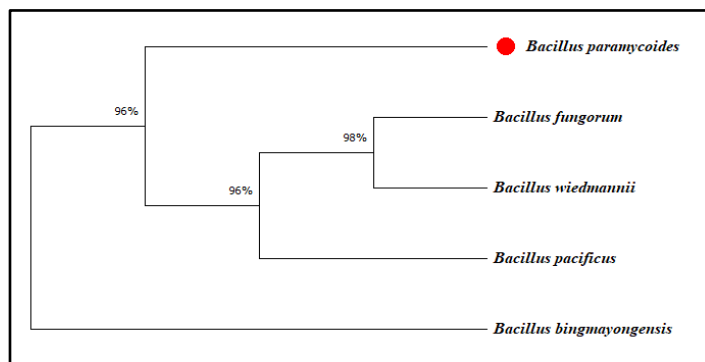


Figure 1. Phylogenetic tree of bacteria *Bacillus paramycoides* (OQ799314). The percentage indicates *Bacillus paramycoides* (OQ799314) are closely related organisms obtained from NCBI determined by the phylogenetic tree.

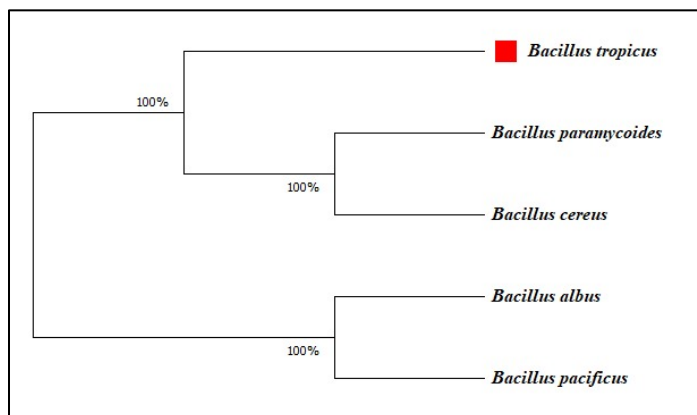


Figure 2. Phylogenetic tree of bacteria *Bacillus tropicus* (OQ799318). The percentage indicates *Bacillus tropicus* (OQ799318) are closely related organisms obtained from NCBI determined by the phylogenetic tree.

3.3 Screening of azo dye degradation by bacterial isolates

In the screening test, major dyes used in the tannery industry were used for screening analysis. Using the isolated microorganisms *Bacillus paramycoides* (OQ799314) and *Bacillus tropicus* (OQ799318), the tannery azo yellow and brown dyes were degraded (Figures 3a and 3b). After incubation at 37°C for 24 to 48 h, the decolorization percentages of 87% for the yellow dye, and 89.9% for the brown dyes were found. The decolorization results are shown in Figure 4. Further spectroscopic analysis was done to examine the degradation potential of bacteria *Bacillus paramycoides* (OQ799314) and *Bacillus tropicus* (OQ799318) and the results are represented in Figure 5.

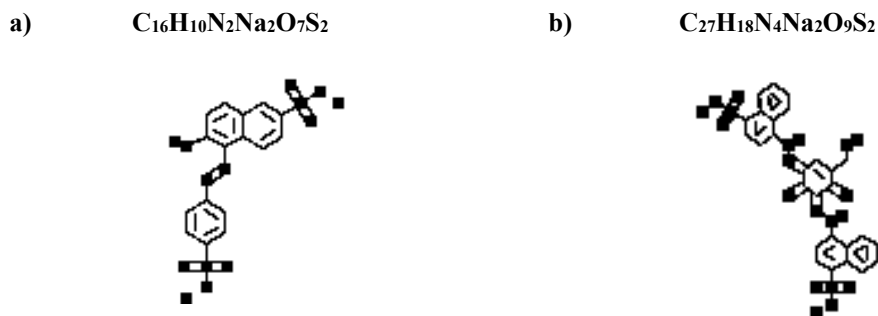


Figure 3. Chemical structure of azo yellow (3a) and brown dye (3b)

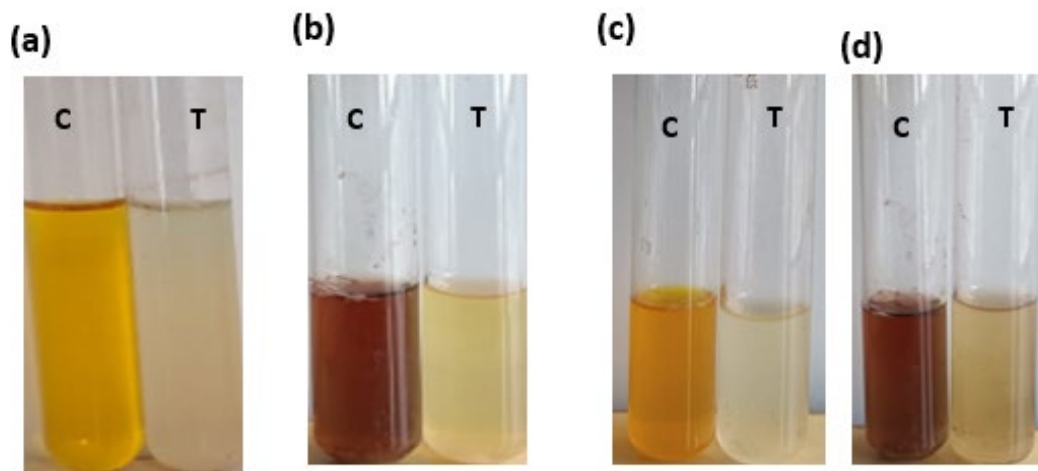


Figure 4. Decolorization of azo dyes by bacteria *Bacillus paramycoides* (OQ799314) and *Bacillus tropicus* (OQ799318); C: control; T: test; (a) Degradation of tannery acid azo yellow dye by *Bacillus paramycoides*; (b) Degradation of tannery acid azo brown dye by *Bacillus paramycoides*; (c) Degradation of tannery acid azo yellow dye by *Bacillus tropicus*; (d) Degradation of tannery acid azo brown dye by *Bacillus tropicus*

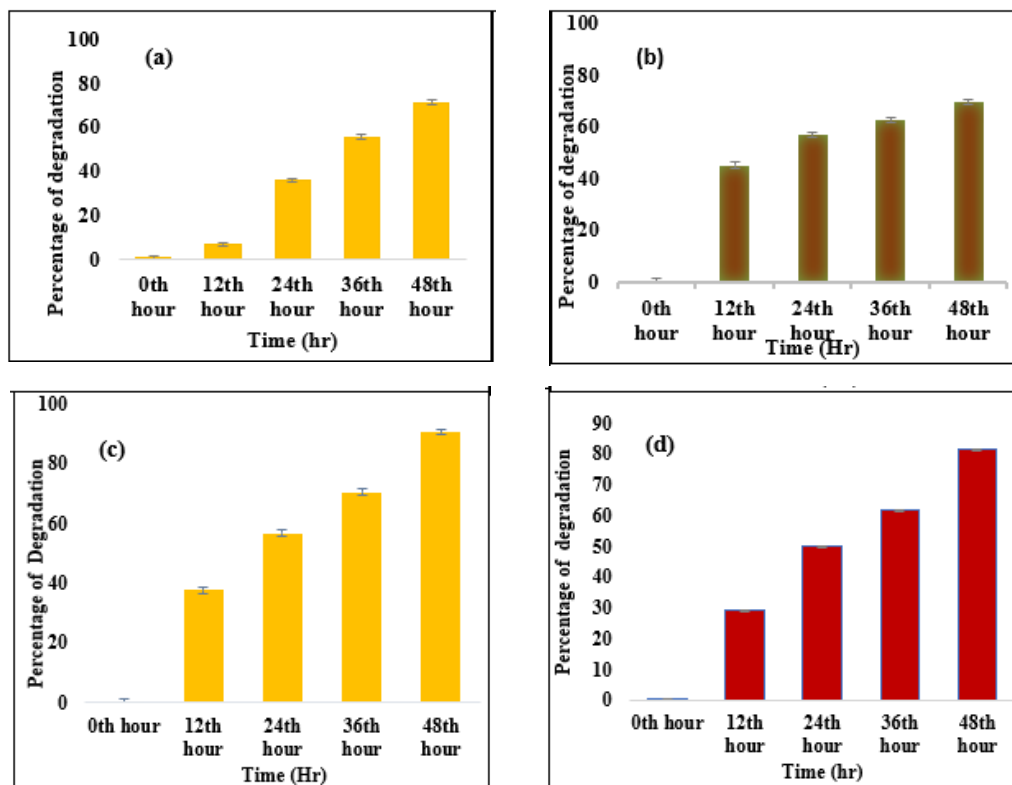


Figure 5. Percentage of degradation of azo dyes by *Bacillus paramycoides* (OQ799314) and *Bacillus tropicus* (OQ799318); (a) Percentage of degradation of tannery acid azo yellow dye by bacteria *Bacillus paramycoides*; (b) Percentage of degradation of tannery acid azo brown dye by bacteria *Bacillus paramycoides*; (c) Percentage of degradation of tannery acid azo yellow dye by bacteria *Bacillus tropicus*; (d) Percentage of degradation of tannery acid azo brown dye by bacteria *Bacillus tropicus*

3.4 Factors influencing the dye degradation process

Temperature, time, pH, and inoculum concentration are factors that can have a significant impact on the dye degradation process.

3.4.1 Effect of temperature

Temperature is a key environmental parameter that influences both the growth rate of bacteria and the rate of biodegradation. To assess this effect, we evaluated the dye-degrading ability of *Bacillus tropicus* and *Bacillus paramycoides* across a temperature range of 16°C to 60°C. We found that both strains exhibited optimal degradation activity at 37°C. The degradation results are summarized in Figure 6.

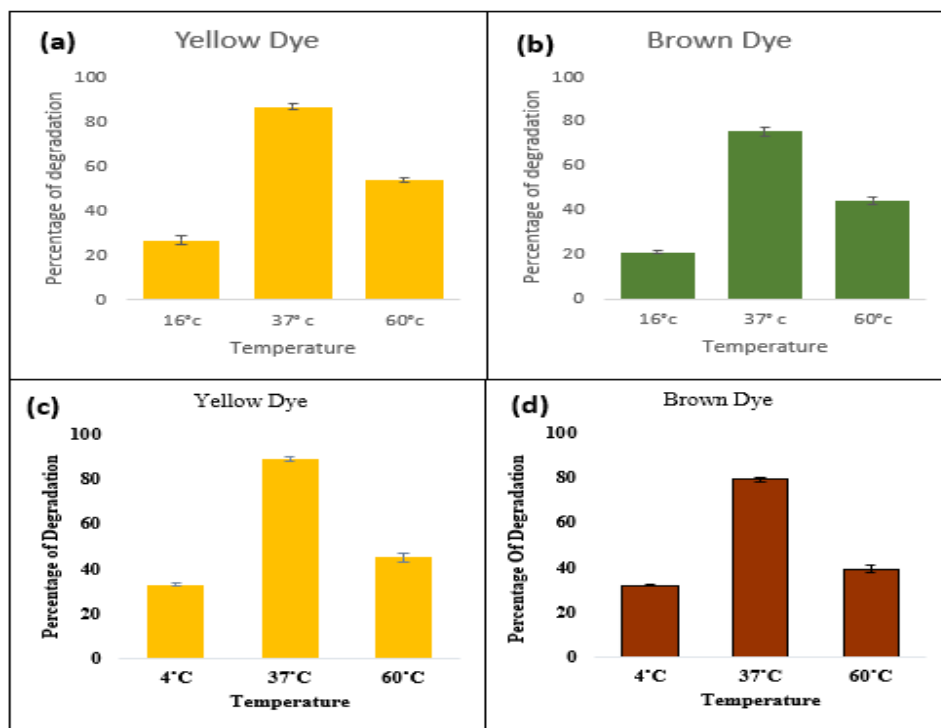


Figure 6. Effect of temperature on decolourization of the azo dyes by bacteria *Bacillus paramycoides* (OQ799314) and *Bacillus tropicus* (OQ799318); (a) Percentage of degradation of tannery acid azo yellow dye by bacteria *Bacillus paramycoides*; (b) Percentage of degradation of tannery acid azo brown dye by bacteria *Bacillus paramycoides*; (c) Percentage of degradation of tannery acid azo yellow dye by bacteria *Bacillus tropicus*; and (d) Percentage of degradation of tannery acid azo brown dye by bacteria *Bacillus tropicus*

3.4.2 Effect of pH

pH influences the ionic properties of bacterial cells and thereby affects their growth rate and metabolic activity. While many bacteria grow optimally near neutral pH (around 7), some can tolerate more acidic or alkaline environments. In our experiments, we adjusted the medium's pH using HCl or NaOH, inoculated it with bacterial cultures under different pH conditions along with the dye, and after incubation measured degradation. We found that *Bacillus paramycoides* (accession OQ799314) and *Bacillus tropicus* (accession OQ799318) degraded all the tested azo dyes (specifically, yellow and brown) most effectively at pH 7 (Figure 7).

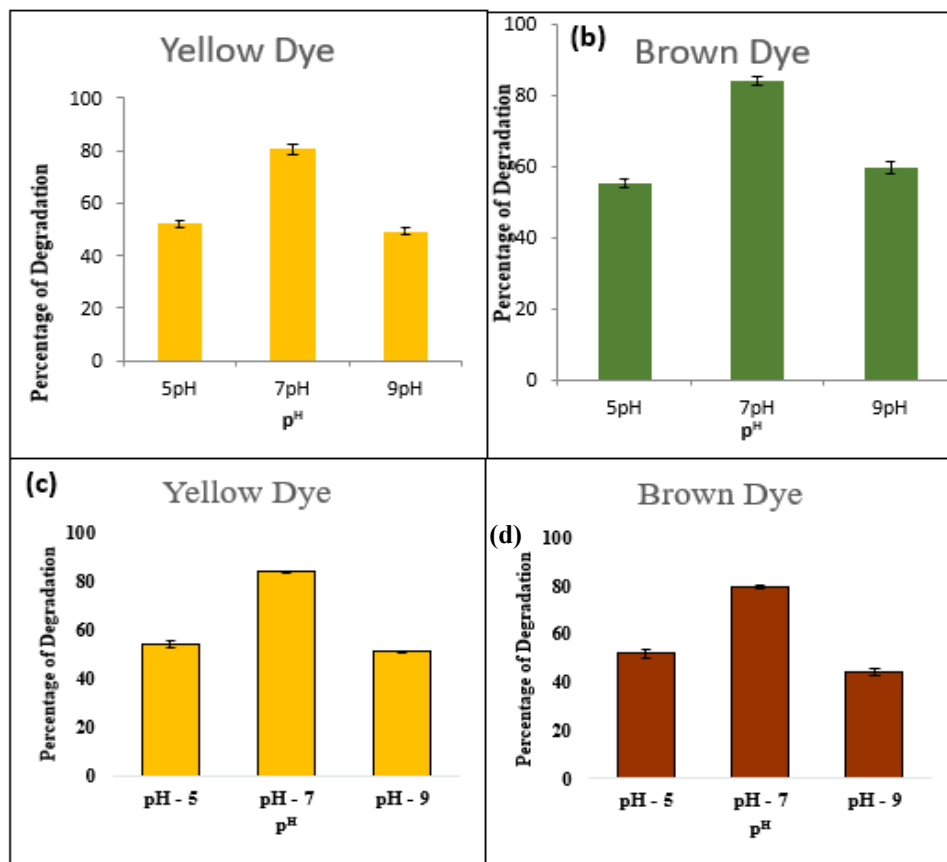


Figure 7. Effect of pH on decolorization of the azo dyes at pH 5, 7 and 9 by bacteria *Bacillus paramycoides* (OQ799314) and *Bacillus tropicus* (OQ799318); (a) Percentage of degradation of tannery azo yellow dye by bacteria *Bacillus paramycoides*; (b) Percentage of degradation of tannery azo brown dye by bacteria *Bacillus paramycoides*; (c) Percentage of degradation of tannery azo yellow dye by bacteria *Bacillus tropicus*; and (d) Percentage of degradation of tannery azo brown dye by bacteria *Bacillus tropicus*

3.4.3 Effect of inoculum concentration

A critical culturing parameter refers to the initial ratio of the microorganism relation to the total working volume. It has commonly been noted that the efficiency of dye decolorization relate to dye concentration. The bacteria *Bacillus paramycoides* (OQ799314) and *Bacillus tropicus* (OQ799318) were inoculated in the LB broth along with various inoculum concentrations (1%,5%,10% and 15%) along two different colored azo dyes. After inoculation and incubation, the optimum inoculum concentration of the selected bacterial strain was observed. *Bacillus paramycoides* (OQ799314) and *Bacillus tropicus* (OQ799318) were best able to degrade both dyes well at the inoculum concentration of 10%, as shown in Figure 8.

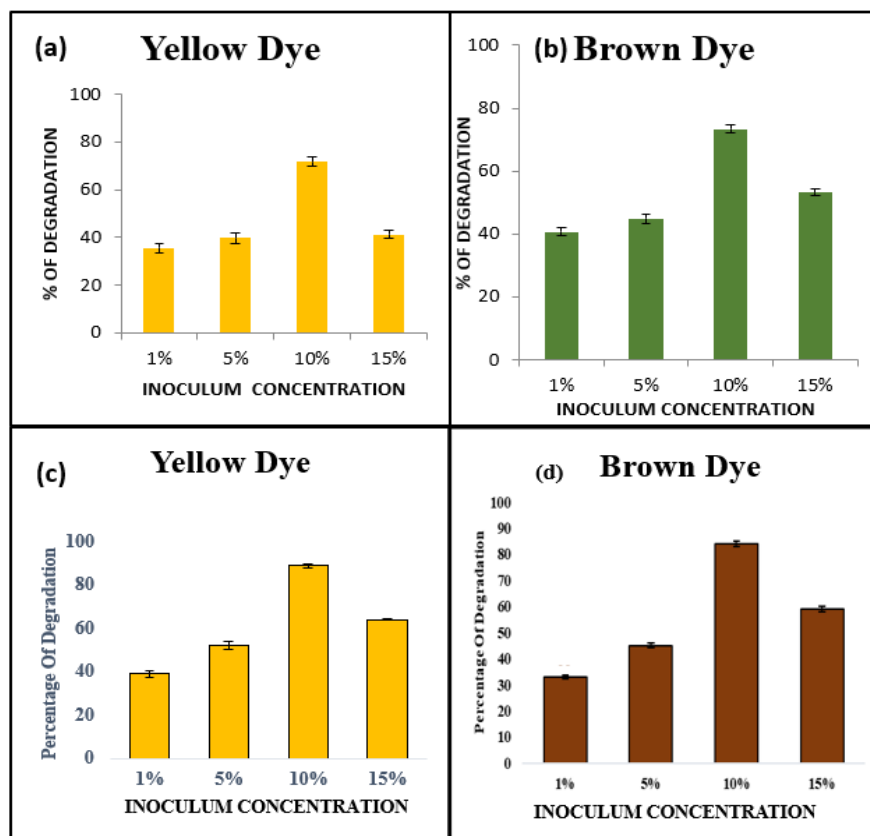


Figure 8. Effect of inoculum concentration (1%,5%,10% and 15%) on decolorization of azo dyes by *Bacillus paramycoides* (OQ799314) and *Bacillus tropicus* (OQ799318); (a) Percentage of degradation of tannery acid azo yellow dye by *Bacillus paramycoides*; (b) Percentage of degradation of tannery acid azo brown dye by *Bacillus paramycoides*; (c) Percentage of degradation of tannery acid azo yellow dye by *Bacillus tropicus*; and (d) Percentage of degradation of tannery acid azo brown dye by *Bacillus tropicus*

3.4.4 Effect of the time

The isolated bacteria *Bacillus paramycoides* (accession OQ799314) and *Bacillus tropicus* (accession OQ7993180) were inoculated into LB broth and incubated. After incubation, dye degradation was observed. The percentage of dye degradation increased over time, and the results are presented in Figure 9.

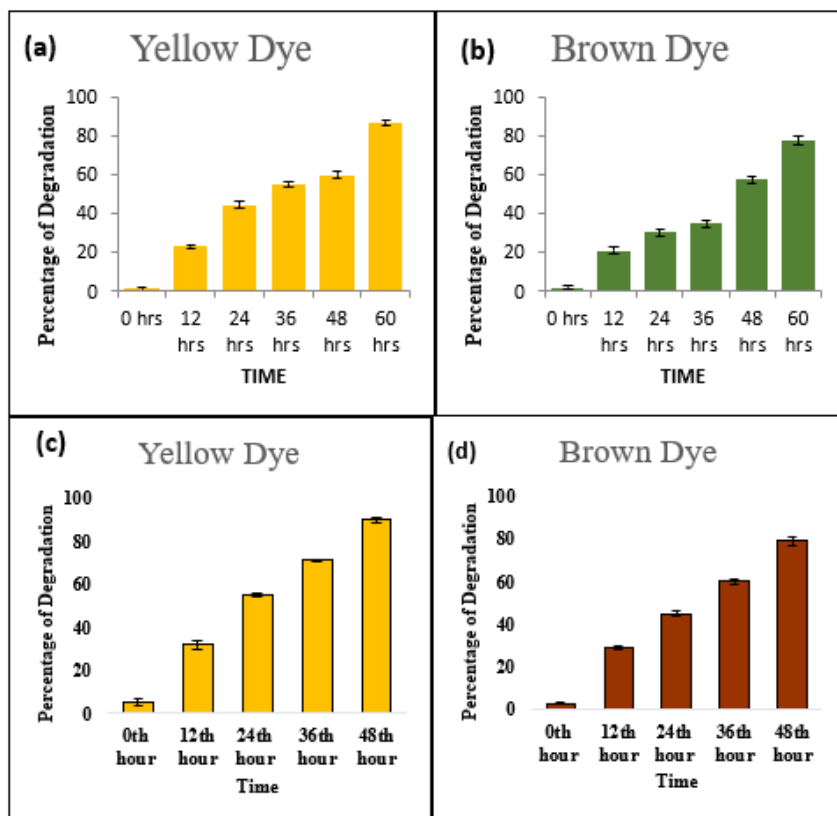


Figure 9. Effect of time (0th h, 12th h, 24th h, 36th h, 48th h, and 60th h) on decolorization of azo dyes by *Bacillus paramycoides* (OQ799314) and *Bacillus tropicus* (OQ799318); (a) Percentage of degradation of tannery acid azo yellow dye by *Bacillus paramycoides*; (b) Percentage of degradation of tannery acid azo brown dye by *Bacillus paramycoides*; (c) Percentage of degradation of tannery acid azo yellow dye by *Bacillus tropicus*; and (d) Percentage of degradation of tannery acid azo brown dye by *Bacillus tropicus*

3.5 FT-IR analysis

FT-IR (Fourier-transform infrared) analysis was carried out to monitor structural changes in the dye compounds after biodegradation by *Bacillus paramycoides* (accession OQ799314). The degraded samples were compared with an untreated control. In our spectra, we observed prominent broad absorption bands attributable to –OH groups, indicating the presence of hydroxyl moieties. To prepare samples for FT-IR, the supernatant containing degraded dye products was first separated (e.g. by centrifugation), then dried (for example by lyophilization or oven drying) to yield a powdered form. This dried residue was then typically mixed with KBr to make a pellet (or pressed disc) for FT-IR measurement. This dried-form preparation helps minimize interference from water (which strongly absorbs in the IR) and ensures sharper, more interpretable spectra.

The degradation of the yellow dye by *Bacillus paramycoides* was evaluated by comparing FT-IR spectra of the control dye (Figure 10a) and the treated sample (Figure 10b). The broad O–H (or N–H) stretching band observed at $\sim 3275\text{ cm}^{-1}$ in the control shows a slight shift to 3273 cm^{-1} and a reduction in intensity in the treated sample, indicating partial modification of hydroxyl/amine-containing moieties. The C–H deformation band at 1450 cm^{-1} in the control is shifted and markedly decreased in intensity (appearing near 1404 cm^{-1}) after treatment, consistent with alteration of aliphatic groups. The C–N stretching band at 1323 cm^{-1} in the control is attenuated and slightly shifted to 1321 cm^{-1} in the treated sample, suggesting partial modification of amine-containing structures. A C–O stretching feature near 1107 cm^{-1} is present in the treated sample, while a nearby C–O/C–O–C (aliphatic ether) band at 1085 cm^{-1} is visible in the control, indicating changes in oxygenated functional groups. A band at $\sim 518\text{ cm}^{-1}$, assignable to C–I stretching of halo-organic moieties, appears in the treated sample. Overall, the observed reductions, shifts, and disappearance of characteristic peaks support the conclusion that *Bacillus paramycoides* affected chemical modification and partial degradation of the complex functional groups in the yellow dye.

The degradation of the brown azo dye by *Bacillus paramycoides* (OQ799314) was then demonstrated by a comparison of the FT-IR spectra of the control brown dye (Figure 11a) and test brown dye sample (Figure 11b). Alcohol groups likely caused the O–H bending at 1402 cm^{-1} , which is visible in peaks in the control spectrum. O–H bending is absent in the test sample indicating the bacteria were able to degrade the alcohol group. The N–O stretching band observed at 1637 cm^{-1} in the control spectrum (indicating the presence of nitro compounds in the dye) shifted to 1554 cm^{-1} . The presence of nitro compound was verified by the N–O stretching band at 1539 cm^{-1} . The peak's presence in the control and the test both supported dye degradation. The secondary alcohol was verified by the existence of C–O stretching at 1105 cm^{-1} . The peak's presence in the control and the peak's removal in the test both supported dye degradation. The sulfonyl chloride group likely caused the S=O stretching at 1321 cm^{-1} , which is visible in the test spectrum. Halo compound caused the C–I stretching at 520 cm^{-1} , which is visible in peaks in the test spectrum. Carboxylic acid caused the O–H stretching at 3273 cm^{-1} , which is visible in peaks in the test spectrum. Alkane groups caused the C–H stretching at 2954 cm^{-1} , which is visible in peaks in the test spectrum. From these results, we can conclude that the bacteria *Bacillus paramycoides* are able to degrade the alcohol, nitro, and secondary alcohol groups present in the brown dye.

The FT-IR analysis of *Bacillus tropicus* inoculated into yellow dye (Figure 12) demonstrated its ability to degrade some of the tannery azo yellow dye's components. By comparing the FT-IR spectra of the control yellow dye (Figure 12a) and that of the treated tannery azo yellow dye (Figure 12b), it was found that the peak at wavelength 2956 cm^{-1} , which corresponded to C–H stretching and indicated the presence of an alkane group, was absent in the treated dye but present in the control.

The C–H bending was visible at 1450 cm^{-1} in the control spectrum and shifted to 1404 cm^{-1} in the treated sample. The S=O stretching, indicated the presence of sulfone groups appeared at 1398 cm^{-1} in the control, and was reduced to 1321 cm^{-1} in the treated sample. Furthermore, halo compounds exhibited C–I stretching at 521 cm^{-1} in the control, which shifted slightly to 518 cm^{-1} in the treated sample. These spectrum changes suggest that *Bacillus tropicus* is capable of degrading the alkane related functional group present in the control.

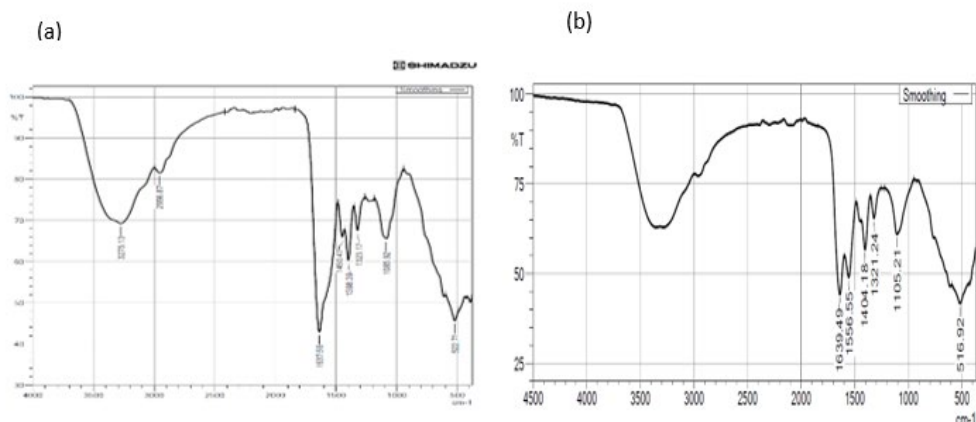


Figure 10. FTIR spectrum of azo yellow dye by *Bacillus paramycoides* (OQ799314) (a) Control (b) Test.

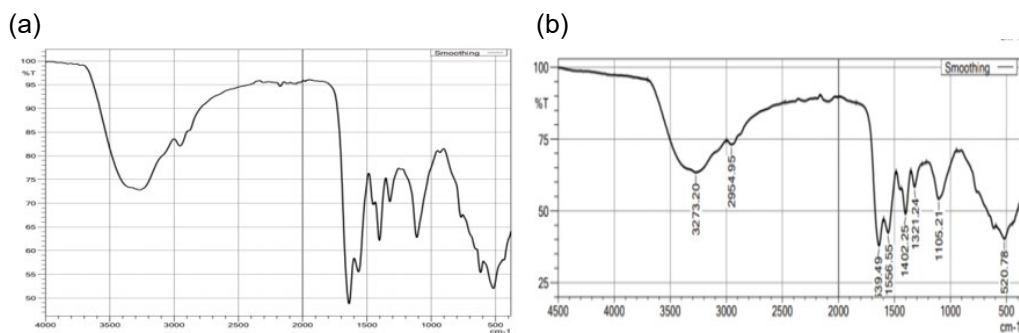


Figure 11. FTIR spectrum of azo brown dye by *Bacillus paramycoides* (OQ799314) (a) Control (b) Test

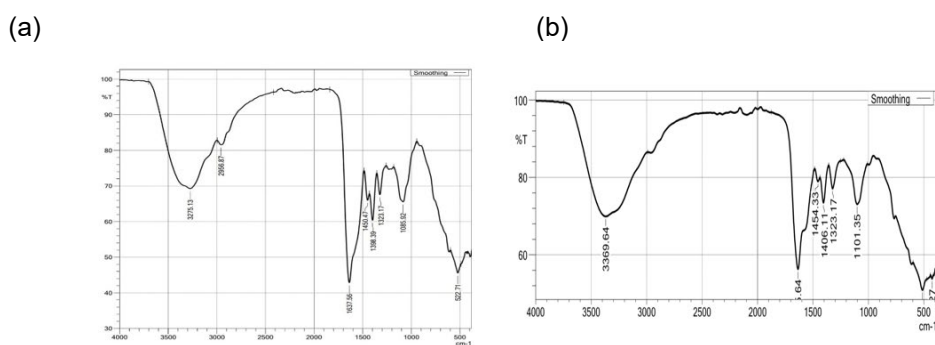


Figure 12. FTIR spectrum of tannery azo yellow dye by *Bacillus tropicus* (OQ799318) (a) Control (b) Test

Figure 13 presents the FT-IR spectra of *Bacillus tropicus* inoculated in azo brown dye, showing evidence of dye degradation. The comparison between the control dye spectrum (Figure 13a) and the spectrum of the treated sample (Figure 13b) revealed significant structural modifications in the dye molecules. In the control spectrum, characteristic peaks were observed at 2950 cm^{-1} (C–H stretching of alkanes), 2210 cm^{-1} (C≡C stretching of alkynes), 1560 cm^{-1} (C=N stretching, possibly associated with imine or azo linkages), and 900 cm^{-1} (C–H out-of-plane bending of alkenes). These peaks disappeared in the treated sample, indicating that *Bacillus tropicus* effectively degraded the corresponding functional groups. Additional characteristic absorption bands present in the control but absent or diminished in the treated sample include 3350 cm^{-1} (O–H stretching, alcohols or phenols), 1600 cm^{-1} and 1450 cm^{-1} (C–H bending), 1400 cm^{-1} reduced to 1323 cm^{-1} (O–H bending), 1350 cm^{-1} slightly shifted to 1348 cm^{-1} (C=N stretching), 1100 cm^{-1} (C–O stretching), 600 cm^{-1} (C=C bending), and 500 cm^{-1} shifted to 336 cm^{-1} (C–I stretching of halo compounds). The disappearance, attenuation, or shifting of these bands indicates that *Bacillus tropicus* enzymatically cleaved or transformed multiple functional groups within the dye structure, confirming its ability to biodegrade complex azo dye molecules. Proper disposal of chemicals used in leather manufacturing is essential, as untreated dye effluents can be hazardous to both human health and the environment. The demonstrated biodegradation capability of *Bacillus tropicus* suggests that this bacterium could serve as a promising agent for the bioremediation of azo dye contaminated industrial effluents.

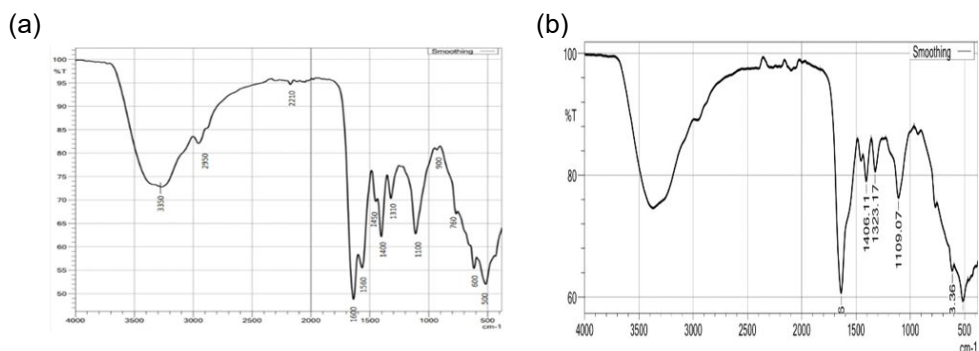


Figure 13. FT-IR spectrum of brown azo dye by *Bacillus tropicus* (OQ799318)
(a) Control (b) Test

4. Conclusions

Relevance of traditional sludge and wastewater treatment requires enormous cost and continuous input of chemicals which becomes expensive and causes further secondary pollution. Disposing of the chemicals used in the tannery industry is challenging; they cannot be disposed of easily and the industry dumps them into land, where they are hazardous to people and to the environment. Hence, economical and eco-friendly techniques using bacteria (*Bacillus paramycoides* and *Bacillus tropicus*) could be applied for the refining of wastewater and sludge treatment, which resulted in zero sludge disposable. Interestingly, the bacterial species used in the decolorization of azo dye in this study (*Bacillus paramycoides* and *Bacillus tropicus*) were isolated from the waste sludge of dye used in the tannery industry. Various bacterial strains were isolated from the sludge

sample, among which two strains *Bacillus paramycoides* and *Bacillus tropicus* effectively degraded two azo dyes (yellow, brown). These two isolates demonstrated the ability to degrade and decolorize the azo dyes present in tannery effluent within 48 h, as confirmed by the degradation profile. Furthermore, the factors influencing degradation were successfully optimized. Based on the positive findings and ongoing developments, microbiological treatment holds great promise as a predominant approach for the removal of dyes from tannery effluent.

5. Conflicts of Interest

The authors declare that there are no conflicts of interest.

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