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Research Article

COMPARATIVE ANALYSIS OF MARINE BACTERIA FOR THE DEGRADATION OF TEXTILE EFFLUENT DYES

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ABSTRACT

The discharge of textile dye effluents into aquatic environments poses a major ecological threat due to the toxic, persistent, and non-biodegradable nature of synthetic dyes. Biological treatment using marine bacteria has emerged as an eco-friendly and cost-effective approach for the remediation of dye-contaminated wastewater, especially under saline conditions. The present study aimed to isolate and evaluate potential marine bacterial strains for the degradation of textile dyes. Marine water and sediment samples were collected from coastal regions, and bacterial isolates were obtained using standard serial dilution and enrichment techniques. The isolates were screened for dye-degrading ability against azo dyes, particularly Mordant Black 17, under laboratory conditions. Among the four isolates obtained, two promising strains, A1 and A2, exhibited significant decolorization efficiency and were selected for further study. Based on morphological and biochemical characterization, the isolates were identified as *Halomonas* sp. and Bacillus cereus. At 100 ppm dye concentration, A1 showed a maximum decolorization efficiency of 70.0%, while A2 exhibited 63.2%. Optimization studies revealed that pH 7, temperature 40°C, and 10% inoculum size were optimal for enhanced dye degradation, achieving up to 98% decolorization under static conditions. The results demonstrate that marine bacterial strains, particularly *Halomonas* sp., possess strong potential for biodegradation of textile dyes and can be effectively applied for the treatment of saline textile wastewater in an environmentally sustainable manner.

Keywords: Dye, Marine Bacteria, Serial dilution, *Halomonas* sp. and *Bacillus cereus.*

INTRODUCTION

The discharge of synthetic dyes from textile and industrial processes has become a significant environmental concern due to their toxicity, persistence, and potential to cause mutagenic and carcinogenic effects. Traditional chemical and physical dye removal techniques are either costly, ineffective, or produce additional contaminants. On the other hand, biological treatment with dye-degrading bacteria provides a more affordable and environmentally beneficial option. Some bacterial species have the enzymatic machinery necessary to decompose complicated dye compounds into non-toxic constituents. In addition to cleaning up the environment, this process known as biodegradation helps manage wastewater sustainably. One of the most dangerous environmental contaminants endangering our biodiversity is water pollution, which is primarily caused by effluents from dye-based sectors like the textile industry (Cripps, C., *et al.*, 1990). Textile dyes are typically categorized as azo, reactive, triphenylmethane, heterocyclic, or polymeric. Azo dyes are most suited for usage in the textile sector. They are resistant to light, water, heat, bleach, detergents, and perspiration due to their high quality and unique π-conjugated azo bond structure. During the dyeing process, around 5-10% of colors are discharged into textile wastewater streams and end up in natural water bodies without proper treatment (Youssef, A. S *et al.*, 2008).

The discharge of untreated dye effluent into water resources, such as rivers and lakes, increased the pH level, leading to improved BOD, COD, and TOC measurements (Lade, H. S. *et al.*,2012). Large amounts of synthetic dyes have been released into aquatic environments as a result of the textile industry's explosive rise. These dyes can lead to serious ecological imbalances and are resistant to

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degradation. Due to its affordability and environmental friendliness, biological therapy using bacteria has drawn interest.

Nature recycles garbage by breaking down organic matter into nutrients that other species can consume. This process is known as biodegradation. "Degradation" refers to decay, and the prefix "bio-" indicates that a wide variety of bacteria, fungus, insects, worms, and other organisms that consume dead matter and recycle it into new forms are responsible for the decay. Since everything in nature is recycled, there is no waste. One organism's waste product serves as food for other organisms, breaking down the waste organic matter and supplying nutrients and energy. All organic compounds will eventually decompose, albeit some will do so far more quickly than others. People can lessen trash and remove some environmental pollutants by utilizing these natural biodegradation processes. Composting speeds up organic waste's natural biodegradation and turns it into a useful resource. Additionally, the natural processes of biodegradation are accelerated by wastewater treatment. In this instance, the goal is to decompose organic waste so that when the water is released into the environment, pollution issues won't arise. Microorganisms are utilized in bioremediation to remove organic pollutants and oil spills. Composting and bioremediation provide many possibilities for student research.

The release of textile azo dyes to the environment is an issue of health concern, while the use of microorganisms has proved to be the best option for remediation (Hunger, K. (Ed.). 2007). Azo-dyes in textiles can be difficult to decompose using traditional physicochemical methods, including coagulation, flocculation, reverse osmosis, and activation (Naresh, B., *et al.*, 2013 and Mortadi, A., 2020). Among various microbial systems, bacteria have emerged as efficient agents for dye degradation because of their rapid growth rate, adaptability, and diverse metabolic capabilities. However, most studies have focused on freshwater or terrestrial bacterial strains, which often show limited efficiency under high salinity and extreme conditions typically found in textile effluents. Textile wastewater frequently contains high concentrations of salts, heavy metals, and complex organic compounds, creating a harsh environment that restricts the activity of conventional microorganisms (McMullan, G *et al.*, 2001).

Marine bacteria represent a promising yet underexplored resource for dye degradation. These microorganisms are naturally adapted to saline environments and possess unique physiological and metabolic traits that enable them to survive and function under extreme conditions, including high salinity, variable pH, and the presence of toxic substances. Marine bacteria such as species of *Pseudomonas, Bacillus, Halomonas, and Marinobacter* have demonstrated significant potential in the degradation of various dyes. Their ability to produce extracellular enzymes, including azoreductases, laccases, and peroxidases, plays a crucial role in breaking down complex dye molecules into simpler, less toxic forms (Saratale, R. G., *et al.*, 2011; Solis, M., *et al.*, 2012). The mechanism of dye degradation by marine bacteria typically involves an initial adsorption of dye molecules onto the bacterial cell surface, followed by enzymatic reduction or oxidation. For instance, azo dyes, which constitute a major class of textile dyes, are degraded through the cleavage of azo bonds (-N=N-) by azoreductase enzymes under anaerobic conditions, leading to the formation of aromatic amines (Iravani, S. *et al.*, 2011). These intermediate compounds are further degraded aerobically into non-toxic end products such as carbon dioxide, water, and inorganic salts. This sequential anaerobic-aerobic process enhances the overall efficiency of biodegradation (Ahmed, S., *et al.*, 2016).

The advantages of microbial degradation, certain limitations such as slow reaction rates, incomplete degradation, and sensitivity to environmental fluctuations still exist. To overcome these challenges, recent research has focused on integrating nanotechnology with biological systems. Nanoparticles, particularly metal nanoparticles such as silver, iron, zinc oxide, and titanium dioxide, have attracted attention due to their high surface area, catalytic properties, and ability to enhance redox reactions. When synthesized through biological routes using microorganisms, these nanoparticles offer additional benefits, including reduced toxicity, eco-friendliness, and cost-effectiveness (Sharma, K., *et al.*, 2022). The combination of marine bacteria and biologically synthesized nanoparticles represents a novel and promising approach for enhanced dye degradation. In such systems, nanoparticles can act as catalysts, accelerating the breakdown of dye molecules, while bacteria facilitate enzymatic degradation and mineralization (Zheng, X., 2026). This synergistic interaction can significantly improve the efficiency, speed, and stability of the degradation process. Moreover, marine bacteria are particularly suitable for nanoparticle-assisted systems due to their inherent tolerance to metal ions and harsh environmental conditions. In addition to improving degradation efficiency, it is essential to evaluate the detoxification of treated effluents, as the breakdown products of dyes may still pose environmental risks. Therefore, comprehensive studies involving both decolorization and toxicity assessment are necessary to ensure the effectiveness and safety of the treatment process.In this context, the present study focuses on the isolation and characterization of marine bacterial strains from coastal environments and their application in dye degradation. Furthermore, the study explores the synergistic effect of biologically synthesized nanoparticles and marine bacteria in enhancing dye removal efficiency. This integrated approach aims to provide a sustainable, efficient, and environmentally friendly solution for the treatment of textile wastewater (Kumari, S *et al.*, 2024).

Traditional treatments have problems such as superfluous chemicals, increased energy consumption, sludge production, and secondary contamination. Azo dyes can be degraded by many microorganisms, including bacteria, fungi, yeast, and algae. The mechanics, important bacterial strains, and possible uses of microbial dye degradation in environmental biotechnology are examined in this article. Numerous techniques, such as chemical degradation and physical methods, are used to recycle these effluents. Physicochemical techniques such as adsorption, irradiation, ion exchange, oxidative process, and coagulation have been employed to decolorize textile effluent. These techniques, however, are expensive, ineffective, and may result in dangerous byproducts. The ecosystem is also impacted by the deterioration process (Praveen, S., 2009). Although studies on the decolorization and degradation of biological dye effluent have produced promising results, they have mostly concentrated on single-model compounds (Shirkoohi, M. G *et al.*, 2022).

MATERIAL AND METHODS

Sample collection

The textile industry's effluent sample was gathered, brought to the lab in a hidden, airtight container, and kept at 4°C for experimental analysis. Samples of soil and wastewater from the dye factory near the Vaadipatti River in Madurai were gathered and transported to the lab in sterile bottles.

Isolation of bacteria from marine

Marine water and sediment samples were collected aseptically from coastal regions using sterile containers and transported to the laboratory for analysis. The collected samples were serially diluted with sterile saline solution up to 10^{-6} dilution, and aliquots from each dilution were spread onto Marine Agar plates. The plates were incubated at 37°C for 24-48 hours for bacterial growth. Distinct colonies were selected based on morphological characteristics and purified by repeated streaking on fresh agar plates. The purified isolates were screened for dye-degrading ability using agar plates supplemented with textile dyes such as Congo red and Methylene blue. After incubation for 48-72 hours, the formation of clear zones around the colonies indicated dye degradation activity. Potential isolates were further subjected to secondary screening in dye-containing broth under shaking conditions. Decolorization efficiency was determined by measuring the reduction in absorbance using a UV-Visible spectrophotometer at specific wavelengths. Finally, efficient dye-degrading bacterial isolates were characterized by morphological, biochemical, and molecular identification methods.

Morphological characterization of dye degrading marine bacteria

The purified isolates were screened for dye-degrading ability using agar plates supplemented with textile dyes such as Congo red and Methylene blue. Incubation for 48-72 hours.

Effect of strains on Azo dye

After 72 hours of incubation, the impact of the inoculum and starting dye concentration on the rate of decolorization was assessed. After 72 hours, an aliquot of the decolorized media was collected and centrifuged for 10 minutes at 10,000 rpm in order to separate the bacterial cell mass. The decolorization at the absorbance of 590 nm was measured using the supernatants (Mohmood, I., *et al.*, 2015).

Enhancement of the decolorization procedure

Two different incubation conditions were used for the tests: static (no shaking) and shaking to optimize the decolorization conditions. The tests for pH, temperature, and inoculum volume were performed correctly.

Effect of pH on dye degradation

The pH of the broth medium was adjusted to 3, 5, 7, and 9 in a static condition to examine how pH affected the selected strains' capacity to decolorize. Optical density was then used to quantify decolorization (Afrin, S. *et al.*,2021).

Effect of temperature on dye degradation

The combination was incubated at various temperatures (20, 30, 40, and 60°C) in a static environment to examine how temperature influences the selected strains' capacity to decolorize. By measuring OD, decolorization was evaluated (Afrin, S. *et al.*,2021).

Effect of inoculum size on dye degradation

The effects of initial dye concentration and inoculum volume on the rate of decolorization were tested using various inoculum volumes (5%, 10%, 15%, and 20%). After that, the samples were cultured for 72 hours. An aliquot of the decolorized liquid was obtained after 72 hours, and it was centrifuged for 10 minutes at 10,000 rpm to separate the bacterial cell mass. The supernatants were used to measure the decolorization at the absorbance of 590 nm (Afrin, S. *et al.*, 2021).

RESULTS AND DISCUSSION

In the present study, the effluent samples were collected from the textile dye industry at two different selected pockets in Vaadipatti River in Madurai, Tamil Nadu, and brought to the laboratory for experimental analysis. Isolation of bacteria from marine soil was carried out by enrichment culture technique using four different azodyes studied as a source of carbon and energy. The effluent samples were serially diluted and spread-plated on a nutrient agar medium (Figure 1). The colonies exhibiting different morphological characters were picked up and sub-streaked on nutrient agar plates as pure culture which is kept for degradation assay.

Screening experiments were carried out for different marine bacterial isolates collected from coastal samples. Among the four azodye-degrading marine bacterial isolates obtained, two isolates, A1 and A2, showed the highest efficiency and were selected for further degradation studies. Both isolates demonstrated significant growth in medium containing Mordant Black 17, indicating their potential for dye degradation under marine conditions. A2 showed good growth in the dye-containing medium, and a similar observation was recorded for A1. Thus, among the marine bacterial strains identified as *Halomonas sp. and Bacillus cereus*, isolates A1 and A2 were found to be the most versatile organisms for effective decolorization of the azo dye (Amoozegar, M. A., 2011). The biochemical characteristics of the marine isolates were presented in Table 2 and Figure 3. Based on the biochemical tests, isolate A1 was identified as *Halomonas sp*. and isolate A2 as Bacillus cereus, and both were further evaluated for their dye-decolorizing ability in saline conditions. Among the tested isolates, A1 exhibited superior decolorization efficiency. The A1 strain showed positive reactions for catalase, oxidase, citrate utilization, urease, nitrate reduction, glucose utilization, and gelatin liquefaction tests. The A2 strain showed positive reactions for VP, citrate, TSI, and H_2S production tests, confirming their adaptability and biodegradation potential in marine environments.

Figure 1. Azo dye effluent from industry.

Table 1. Physicochemical characterization of textile dye effluent samples.

S. No.	Name of the parameters	Results
1.	Temperature	28-36 degrees Celsius
2.	pH	6.9
3.	Color	Reddish yellow
4.	Odor	Unpleasant
5.	Total suspended solids	201
6.	BOD	178
7.	COD	563
8.	Dissolved oxygen	110
9.	Chloride	1124

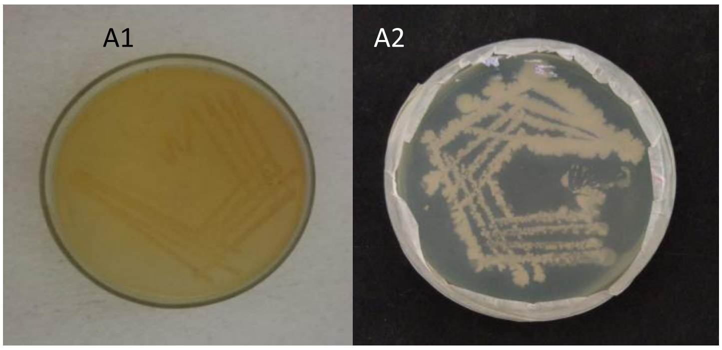

Figure 2. Pure culture of the marine microorganism (A1) and (A2).

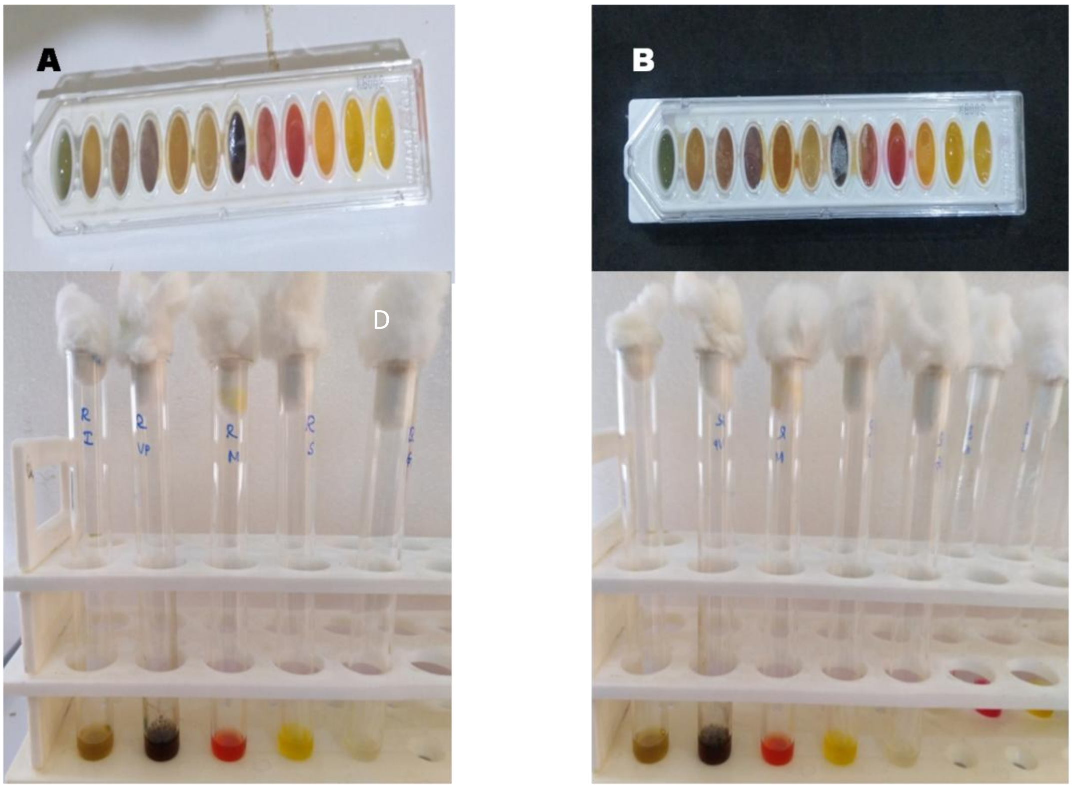

Figure 3. Biochemical test for azo dye degrading microorganism -A1 (A) and Biochemical test for azo dye degrading microorganism -A2 (B).

Table 2. Biochemical characterizations of screened bacterial isolates.

S. No	Tests	Organisms	
		A1 ***Halomonas sp***	**A2** ***Bacillus cereus***
1	Gram reaction	Gram-negative	Gram-positive
2	Motility	Motile	Motile
3	Shape	Rod shape	Straight rod
4	Spore	Negative	Positive
5	Catalase	Positive	Positive
6	Oxidase	Positive	Negative
7	Indole production	Negative	Negative
8	Methyl red	Negative	Negative
9	Voges-Prosakauer	Negative	Positive
10	Citrate	Positive	Positive
11	Urease	Negative	Negative
12	Starch hydrolysis	Negative	Positive
13	Nitrate reduction	Positive	Positive
14	Glucose utilization	Positive	Positive
15	Lactose utilization	Negative	Negative
16	Gelatin	Negative	Negative
17	H_2S	Negative	Positive
18	TSI	Alkaline slant	Positive

Table 3 shows the degradation efficiency of various marine bacterial strains at varying doses of the azo dye (100 ppm). Under maritime conditions, the chosen marine isolates, A1 and A2, showed efficient degradation of the azo dye Direct Blue. Analysis of the tested isolates revealed that A1 had a higher decolorization efficiency (70.0% degradation) than A2 (63.2% degradation). These findings suggest that the marine bacterial isolates have a significant capacity to biodegrade azo dyes in salty conditions (Figure 4) (Asad, S., *et al.*, 2007).

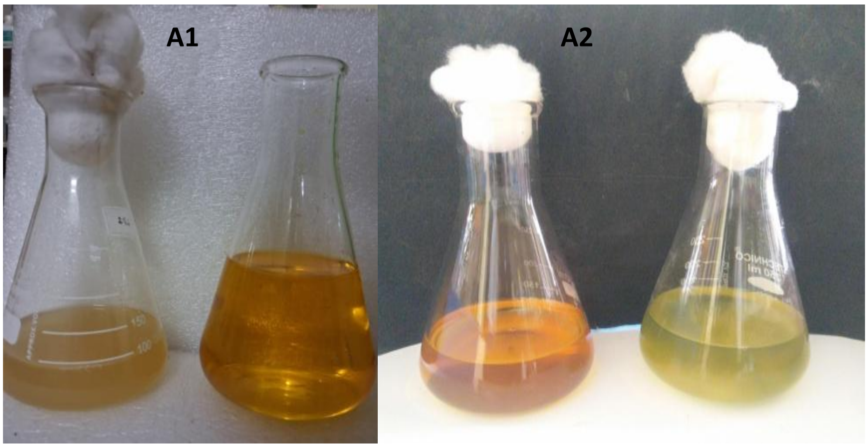

Figure 4. Degradation of azo dyes observed in *Halomonas sp*(A1) and *Bacillus cereus* (A2).

Table 3. Degradation of decolorization efficiency of different strains exposed to different dyes (100 ppm).

Azodyes	**Decolourization efficiency of the marine isolates (%)**	
	***Halomonas sp* (A1)**	***Bacillus cereus* (A2)**
Mordant black 17	70.0%	63.2%

The impact of *Halomonas sp*. (A1) cell mass concentration on azodye Mordant Black 17 degradation was found to be 19 at 1% inoculum. As the inoculum size rose, so did the degradation efficiency. *Bacillus cereus* (A2) showed an enhanced degradation efficiency of 63.2%, whereas Mordant Black 17 showed an enhanced efficiency of 70.0%. The decolorization effectiveness of marine bacterial isolates under various incubation circumstances, such as pH, temperature, and inoculum size, is shown in Figures 5, 6, and 7. The findings demonstrated that as the temperature rose above 40 degrees Celsius, the rate of decolorization increased. The decolorization ability was greatly reduced at 44 degrees Celsius. At 40°C, the greatest decolorization of 98.2% was observed (Figure 6). The study showed that decolorization efficiency decreased as dye concentration increased (Figure 7). For both A1 and A2, 97% decolorization was achieved in 10% (v/v) of bacterial inoculum following 72 hours of static incubation at 37°C (Figure 7). The most efficient pH for dye decolorization was 7, and the effectiveness changed with pH. However, at both extremely acidic and alkaline pH levels, the rate of decolorization decreases. The results of the current investigation were comparable to those of some previous studies (Al-Tohamy, R., 2020 and Mohanty, S. S., 2021). According to the same study, development and decolorization were negatively impacted by temperatures exceeding 40 degrees Celsius. This might be because the bacterial culture is developing in ideal conditions for dye decolonization and is generating additional enzymes. This might be because the bacterial culture is developing in ideal conditions for dye decolonization and is generating additional enzymes. Decolorizing activity was significantly reduced at 44 degrees Celsius, which may be due to the deactivation of the decolorizing enzymes or a loss of cell viability (Al-Tohamy, R., 2020 and Mohanty, S. S., 2021). The degree of decolorization rose with the inoculum's size, and 10% (v/v) of the inoculum achieved 97.4% decolorization. The bacteria's ability to decolorize decreased as the inocula volume rose. Increasing the initial dye concentration decreased decolorization efficiency, according to similar studies (Mishra, S. *et al*., 2019 and Maiti, A., *et al*., 2020). The harmful effects of dye and its metabolites grew predominant with successive increases in dye concentration, preventing decolorization. The toxic effect of the dye, a lack of biomass to absorb the higher dye concentration, and the enzyme's ability to detect the substrate effectively at very low concentrations are the reasons for the decrease in color removal efficiency with increasing dye concentration (Al-Tohamy, R., 2020).

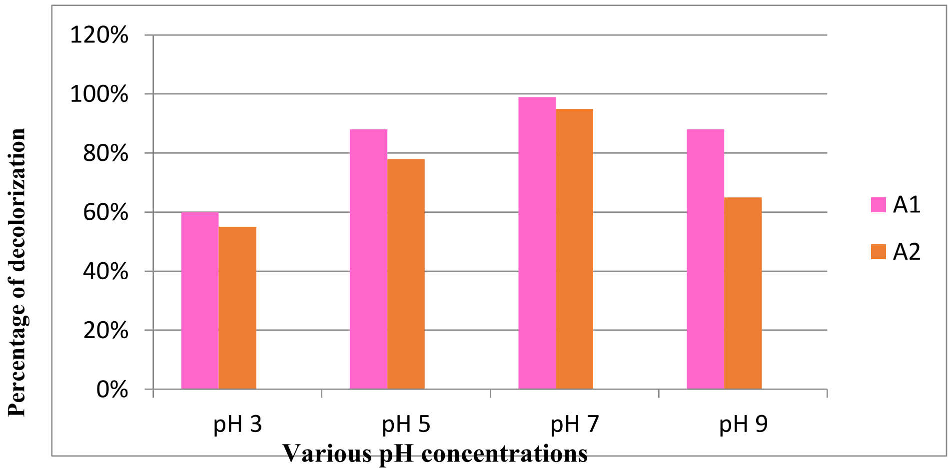

Figure 5. Effect of pH on dye degradation.

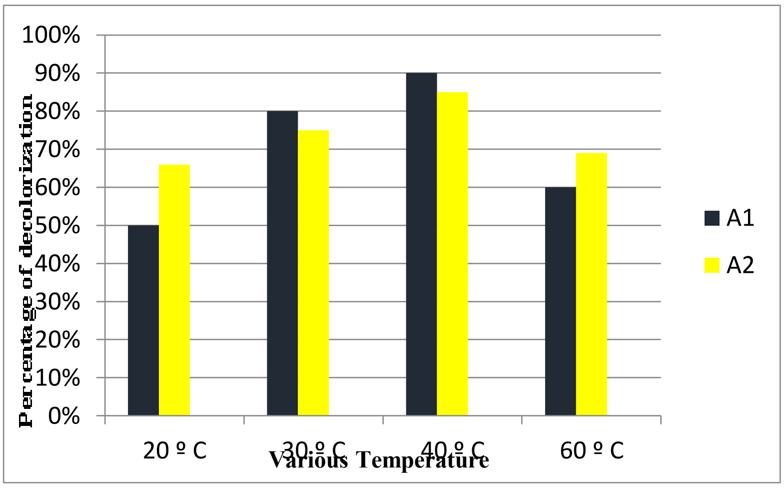

Figure 6. Effect of temperature on dye degradation.

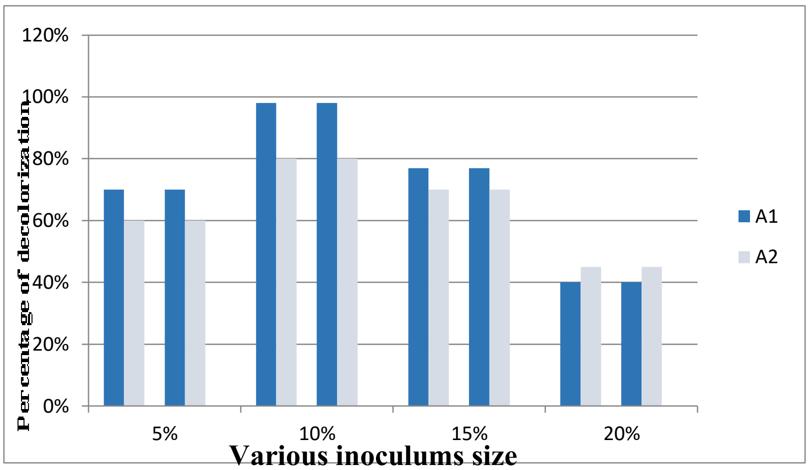

Figure 7. Effect of inoculum size on dye degradation.

CONCLUSION

Wastewater tainted with dyes has been released into aquatic ecosystems as a result of rapid industrialization, seriously polluting the environment and upsetting ecological equilibrium. The hunt for efficient and sustainable ways to handle textile dye effluents has been prompted by this growing concern. The current study shows that marine bacteria are effective biological agents for removing textile dyes from wastewater. *Halomonas sp* and *Bacillus cereus*, two marine bacterial isolates, exhibited the greatest decolorization at 40°C in static aerobic circumstances. When compared to individual strains, the marine bacterial consortium demonstrated improved azo dye degradation with shorter incubation times. The breakdown products were not harmful to microorganisms that are beneficial to agriculture, according to a toxicity assessment of the treated dye samples. These results imply that marine bacterial species, such as *Halomonas sp*, *Bacillus cereus*, and their consortium, have a great deal of potential for effectively decolorizing textile colors while reducing toxicity. Additionally, the metabolites produced by marine bacteria during dye decolorization may function as promising bio-agents for the biodegradation of textile dyes in industrial and marine wastewater systems.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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