Journal of Environmental Chemical Engineering 13 (2025) 118150

Contents lists available at ScienceDirect

Journal of Environmental Chemical Engineering

journal homepage: www.elsevier.com/locate/jece

Harnessing coastal halophiles for azo dye biodegradation: Phylogenetic and spectroscopic evidence of efficient bioremediation

Pavithran Kumar [a], Pasiyappazham Ramasamy [b,c,*], Manjunathan Jagadeesan [a,**]

[a] Department of Biotechnology, School of life Sciences, Vels Institute of Science Technology and Advanced Studies (VISTAS), Pallavaram, Chennai, Tamil Nadu 600117, India

[b] Marine Biopolymer Research Laboratory, Centre for Marine and Aquatic Research (CMAR) Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu 602105, India

[c] Department of Prosthodontics and Implantology, Saveetha Dental College & Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu 600077, India

ARTICLE INFO

Keywords:
Halophilic bacteria
Methyl red
Methylene blue
Decolourization

ABSTRACT

Halophilic bacteria from the east coast of Tamil Nadu, India, demonstrated efficient decolorization of methyl red and methylene blue azo dyes. They are of special importance because the hydrolytic enzymes they produce are usually stable, specific, and useful in conditions of high salinity and tidal variations. Many applications, particularly bioremediation, use them due to their exceptional performance in a variety of harsh environments. From the east coast of Tamil Nadu, India, halophilic bacteria were identified. Optimization of pH, temperature, and salt concentration for growth was performed on the isolated halophilic bacteria. The decolorization of methyl red and methylene blue azo dyes by identified halophilic bacteria was studied. The capacity of three of the six halophilic bacterial strains that were isolated from marine sediments to decolorize the commonly used azo dyes was impressive. Phenotypic description and phylogenetic research based on comparisons of 16S rRNA sequences identified these strains as members of *Salinicoccus roseus*, *Bacillus altitudinis*, and *Staphylococcus warneri.* Azo dye Decolorization and degradation were possible for three of six isolated strains, methyl red, and methylene blue azo dyes after 5 days of incubation in static culture. UV-Visible spectroscopy and FT-IR analyses of methyl red and methylene blue, conducted before and after decolorization, confirmed both decolorization and structural degradation. The results indicate that degradation primarily occurred through azoreductase-mediated cleavage of the azo bonds.

1. Introduction

The discharge of untreated textile effluents, particularly azo dyes in saline waste streams, poses a significant environmental challenge. The problem is compounded by the ecosystem's limited supply of fresh water and its constantly expanding population. Living things are seriously threatened by the release of contaminated water from industrial sources, which poses a major environmental risk. Industrial textile effluent is treated little or not at all before being released [1,8]. The interaction between nitrite-oxidizing bacteria (NOB) and ammonia-oxidizing bacteria (AOB) is crucial to the nitrification process and is the primary bioremediation by microbial bacteria. The study aims to shed more light on the function of nitrifying bacteria found in membrane bioreactor activated sludge and examine strategies for lowering biofouling through nitrifying bacteria enrichment. In order to investigate fouling and treatment effectiveness based on zero to 100 % amounts of nitrifiers, the nitrifying community was enhanced in the activated sludge [30]. A cleaner method based on microalgae could take the role of the aeration system in traditional nitrification processes, improving nutrient removal, increasing carbon capture, decreasing metabolite formation,

Abbreviations: NADH, Nicotinamide Adenine Dinucleotide NAD+ Hydrogen H; NADH-DCIP, Nicotinamide Adenine Dinucleotide NAD+ Hydrogen H –Dichlorophenolindophenol; NADPH, Nicotinamide Adenine Dinucleotide Phosphate; NaCl, Sodium Chloride; PCR, Polymerase Chain Reaction; SMART, Simple Modular Architecture Research Tool; BLAST, Basic Local Alignment Search Tool; FTIR, Fourier Transform Infrared Spectroscopy.

* Corresponding author at: Marine Biopolymer Research Laboratory, Centre for Marine and Aquatic Research (CMAR) Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu 602105, India.

** Corresponding author.

E-mail addresses: ramkanth281@gmail.com (P. Ramasamy), jmanjunathan@gmail.com (M. Jagadeesan).

https://doi.org/10.1016/j.jece.2025.118150
Received 14 May 2025; Received in revised form 15 July 2025; Accepted 16 July 2025
Available online 17 July 2025

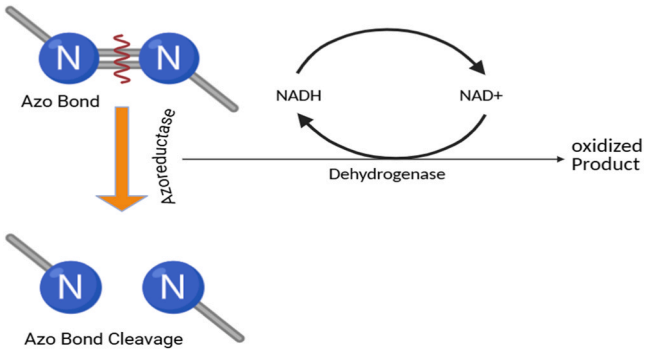

Fig. 1. Mechanism of Azo dye degradation.

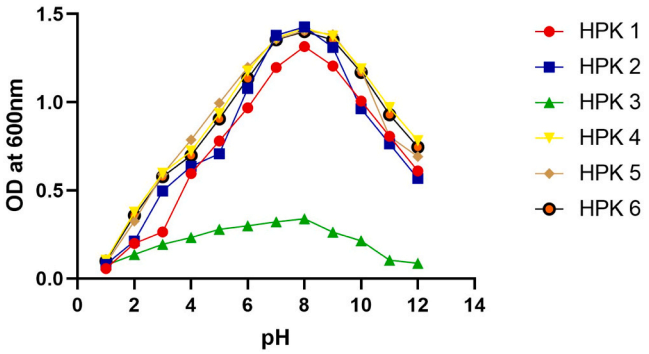

Fig. 2. Optimization - Growth at different pH of halophilic bacteria.

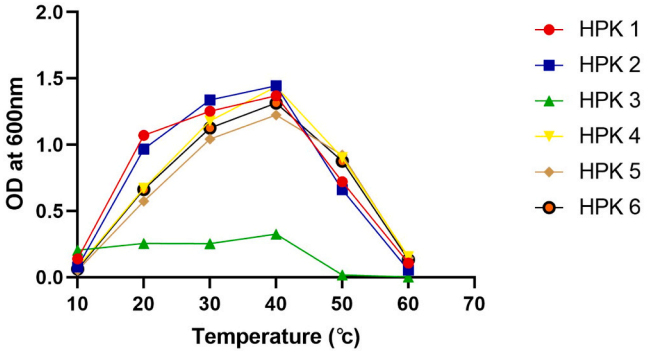

Fig. 3. Optimization - Growth at different temperature of halophilic bacteria.

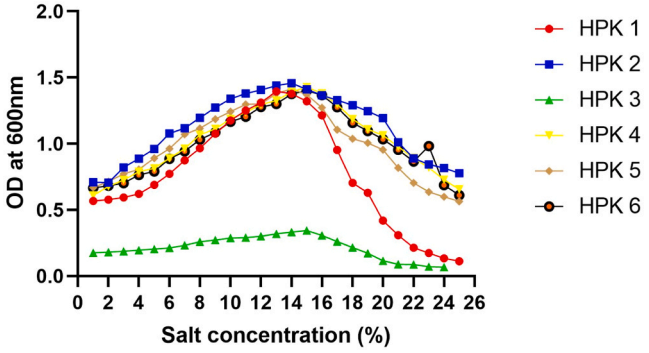

Fig. 4. Optimization - Growth at different salt concentration of halophilic bacteria.

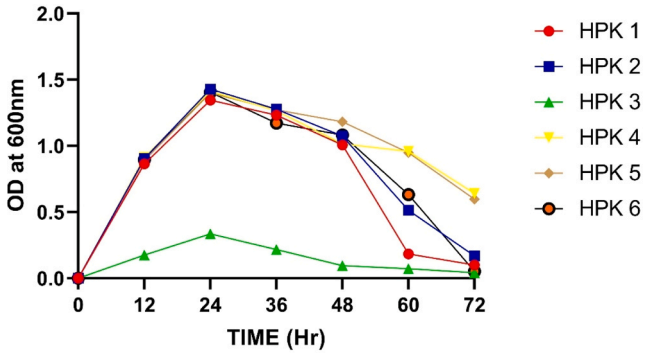

Fig. 5. Growth curve at different time interval of halophilic bacteria.

Table 1
Physiological and biochemical characterization of halophilic bacteria.

Characterization	HPK1	HPK3	HPK4
Colony	Flat	Rod	Cocci
Gram staining	+	+	+
Motility	-	+	+
Catalase	+	+	+
Indole	-	+	+
Citrate	+	+	+
Urease	-	+	+
Coagulase	-	+	+
Oxidase	+	+	-
Sucrose	-	+	+
Glucose	+	+	+
Fructose	-	+	-
Lactose	-	+	+

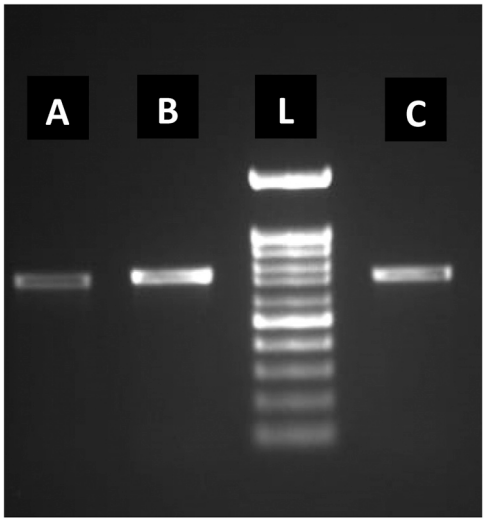

Fig. 6. Agarose gel electrophoresis 16S rRNA PCR products (Lanes: A-HPK1 PCR product, B-HPK3 PCR product, C-HPK4 PCR product & L- 1kbp DNA Ladder).

and producing less extra sludge [31,32]. The main source of water pollution, which is most alarming environmental pollutants endangering our biodiversity, is effluents from dye-based industries, such as the textile sector. Textile dyes often fall into one of several categories, including reactive, azo, heterocyclic, triphenylmethane, and polymeric compositions. The most appropriate textile dyes for usage in the textile industry are azo dyes [10,13].

Azo dyes are characterized by the presence of one or more azo bonds (-N = N-) linking aromatic rings. These aromatic compounds also belong to a significant family of synthetic dyes that are used in many different

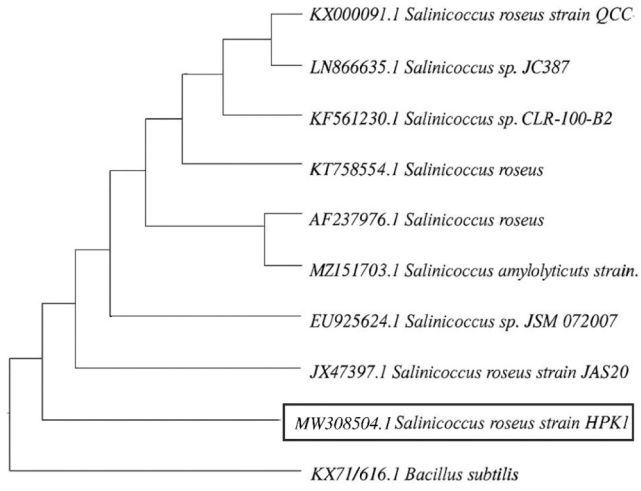

Fig. 7. Phylogenetic tree constructed using neighbour-joining method based on the nearly complete 16S rRNA gene sequences of *Salinicoccus roseus* was used as the outgroup.

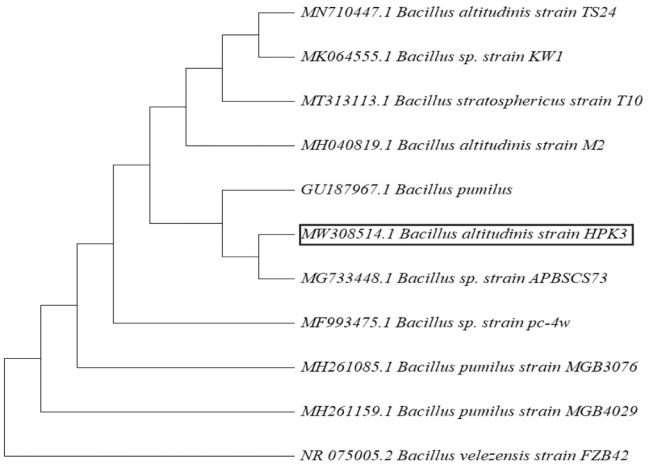

Fig. 8. Phylogenetic tree constructed using neighbour-joining method based on the nearly complete 16S rRNA gene sequences of *Bacillus altitudinis* was used as the outgroup.

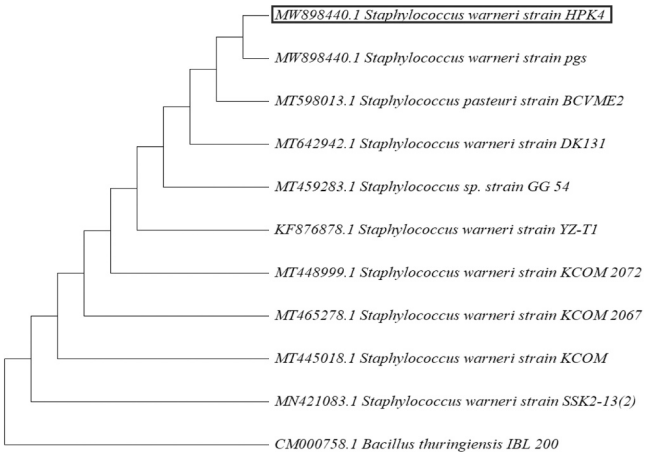

Fig. 9. Phylogenetic tree constructed using neighbour-joining method based on the nearly complete 16S rRNA gene sequences of *Staphylococcus warneri* was used as the outgroup.

commercial applications. Numerous businesses, including the food, paper, leather, and fabric sectors, use these artificially produced coloured compounds. They are also utilized in other industries as a component of other products. In India, these dyes might account for as much as 40 % of the dying pollution. The primary source of foreign exchange income for the country is the export of textiles and apparel [39]. Bacterial enzymes, particularly azoreductases, play a key role in breaking down azo dyes via reductive cleavage of the azo bond. The most important enzyme in the decolorization of azo dyes is azoreductase, which cleave azo via NADH-dependent reduction. Under aerobic conditions, several bacteria have been shown to destroy dyes [18]. Azo dyes have numerous negative consequences on people, including hepatocarcinoma, chromosomal abnormalities in cells, cancer, eye discomfort, and instabilities in the nucleus in experimental animals. These colours can cause fever, cramping, and kidney damage due to their high water solubility and ability to enter the human body through the food chain [19]. These dyes cause carcinogenesis, mutagenesis, and teratogenicity. It is believed that methyl red dye is mutagenic. It has the mono-azo bond and is devoid of a sulphonated group [22,36].

Methylene blue, a common cationic thiazine dye used in textiles [37]. There are a number of substances that can degrade azo dye and change it into non-toxic forms. Compare to physical and chemical techniques, best approach is biological treatment since it can break down dye. These techniques are both economical and environmentally friendly. Because they are non-toxic and naturally present in the environment, microorganisms make the process environmentally pleasant. In addition to being more affordable than other treatment techniques, biological treatments are versatile and effective in a variety of ways because of different pH values, temperatures, and dye concentrations can all be adapted to by microorganisms [38]. Halophilic bacterial strains have demonstrated promise in dye decolorization in recent years Potential bacterial possibilities have recently been shown to be essential to the biological breakdown of dyes by means of extracellular or intracellular enzymes or the entire bacterial cell. Particularly, the metabolic pathways of halophilic bacteria that create many enzymes have drawn a lot of attention. These Halophilic bacteria's remarkable qualities are used in bioremediation techniques, such as dye decolorization. They can withstand stress, and in some environmental circumstances, they can mineralize synthetic colours whole or partially or break them down into non-coloured molecules Kangli [20].

The high salinity of textile effluents frequently poses challenges for traditional biological treatments, which are often inhibited by high salinity. Halophilic bacteria, however, can tolerate. They are therefore used in the bioremediation of oxyanion and oil pollution, but there has been very little evidence of their ability to decolorize textile effluents [7]. As compared to typical bacteria in tests on dye degradation, The potential of marine halophilic bacteria from the Bay of Bengal ecosystem for synthetic textile dye degradation remains underexplored, highlighting their environmental significance and novelty for bioremediation research. The goal of this work is to use the marine sources of halophilic bacteria from the Bay of Bengal region to manage ecosystems sustainably by utilizing the diversity of local microbes. The east coast of Tamil Nadu, which is situated along the Bay of Bengal, is a significant industrial region in India. It is home to numerous textile manufacturers, many of which release their wastewater into the ocean. For region-specific bioremediation techniques, it is essential to investigate the synthetic azo dye degradation potential of halophilic bacteria in this area. By assessing the azo dye decolorization and synthetic dye degradation potential of halophilic bacteria in the Bay of Bengal, this study aimed to fill this gap. Thus, the purpose of this investigation was conducted to examine how Halophilic bacteria contribute to the decolorization of synthetic textile effluents, specifically within the marine environments of the Bay of Bengal near Chennai, India.

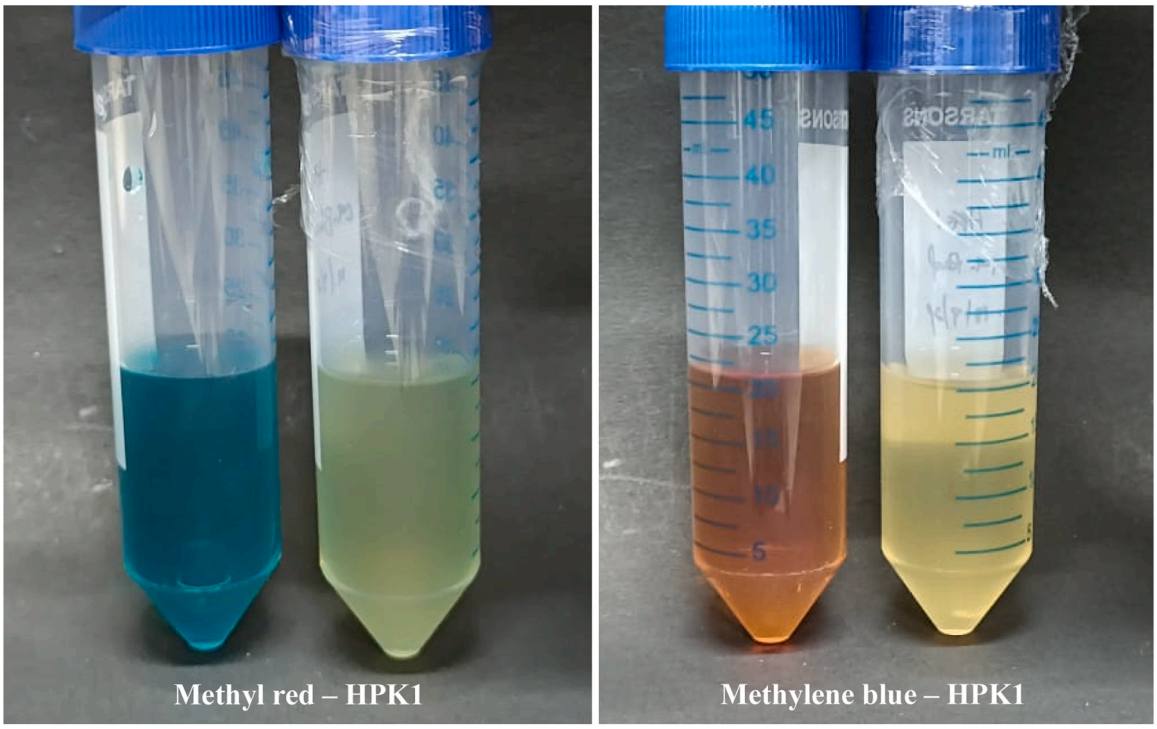

Fig. 10. Decolourization of Methyl red and Methylene blue using *Salinicoccus roseus* HPK1 strain.

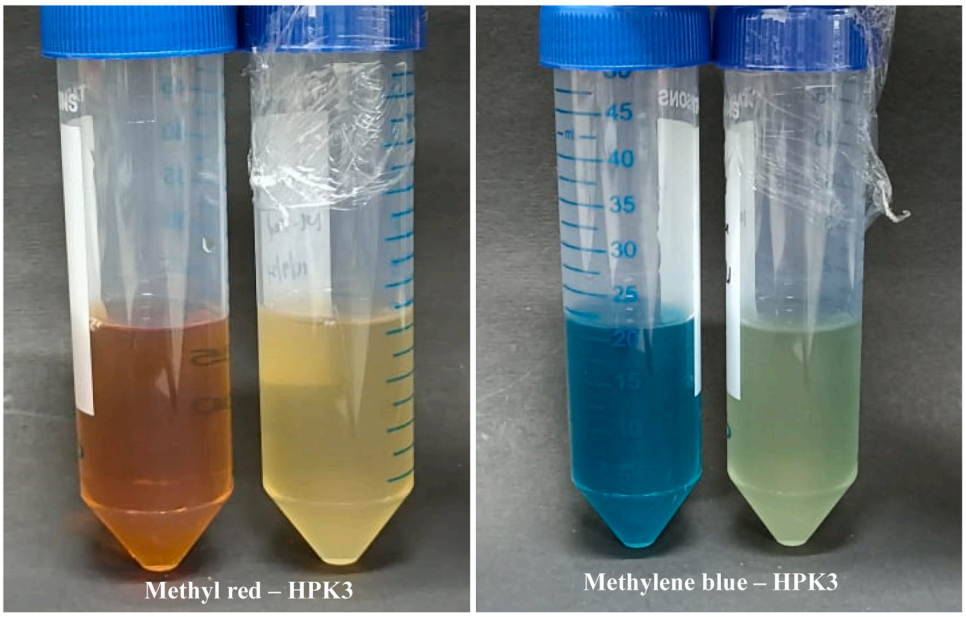

Fig. 11. Decolourization of Methyl red and Methylene blue using *Bacillus altitudinis* HPK3 strain.

1.1. Mechanism of azoreductase

Reductases can aid in the breakdown of methyl red and methylene blue azo dyes by breaking down azo bonds. The enzymes that have been identified the most in dye degradation investigations include azoreductase and NADH-DCIP reductase. These reductases are proteins that contain flavodoxin and use NADH as an electron donor to break the azo bonds during the biological textile wastewater treatment process. They are essential for the catalysis of flavin and azo reduction in both methyl red and methylene blue azo dyes.

The cytoplasm and membrane of halophilic bacteria contain azo reductases. Decolorization occurs when methyl red and methylene blue azo dyes with aromatic amine metabolite production have their azo bonds reduced by NADPH. Both inside and outside of the cell, reducing molecules can participate in azo-bond cleavage and serve as electron donors in this process. A redox mediator or chemical reduction by biogenic reductants are the two methods used to break down the methyl red and methylene blue azo bonds. This cleaves the azo linkage and causes decolorization of methyl red and methylene blue. Nevertheless, not all azo dyes may be readily broken down by these reductases, and some could need particular substrates in order to break down. The chromophore groups of the dyes are often reduced by reductive

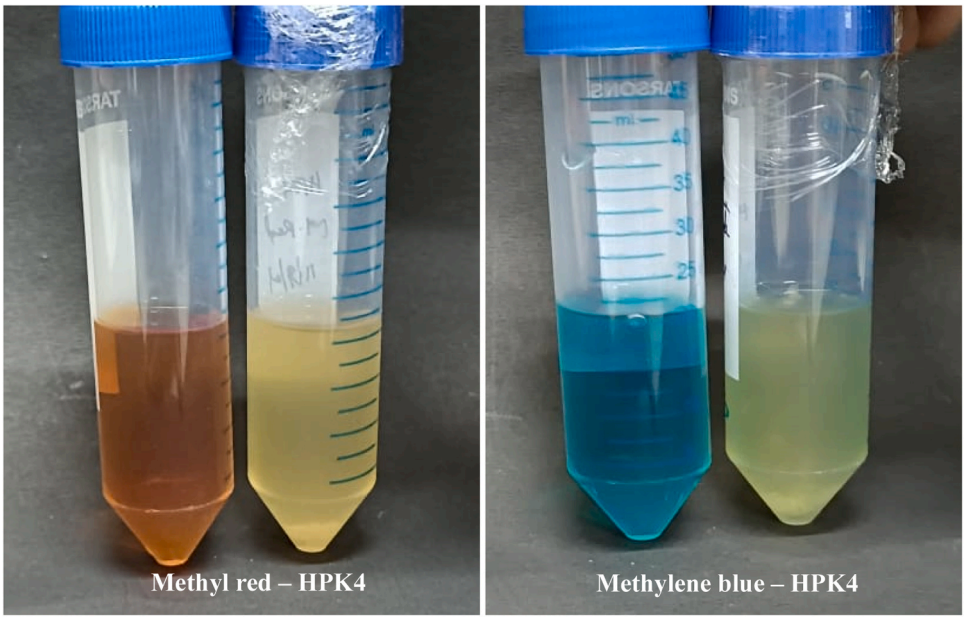

Fig. 12. Decolourization of Methyl red and Methylene blue using *Staphylococcus warneri* HPK4 strain.

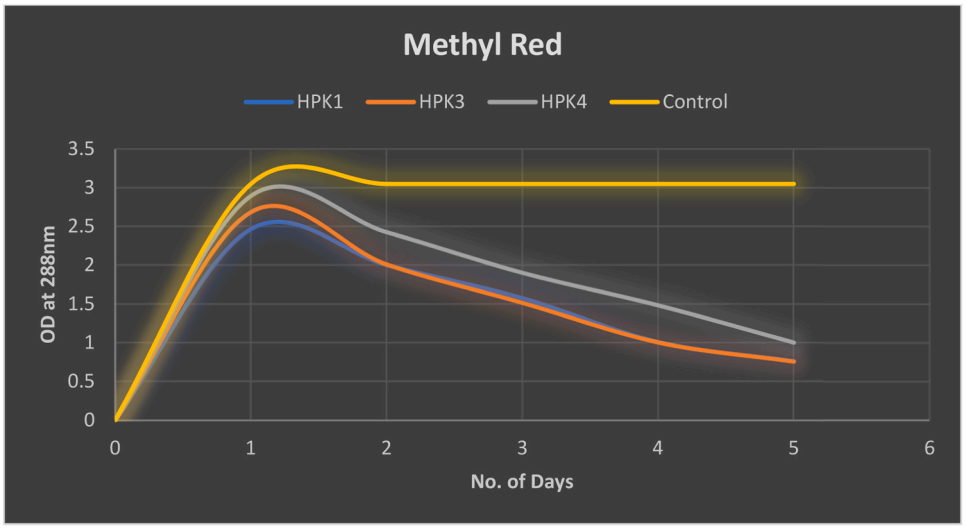

Fig. 13. Characterization of Methyl red Dye degradation from day 1–5 interval period.

enzymes, primarily azoreductase and NADH-DCIP reductase. When azo dyes are broken down, these enzymes are significantly expressed. Therefore, halophilic bacteria will create reductive enzymes like azo reductase enzyme for the breakdown of methyl red and methylene blue azo dyes. Additionally, because the organisms are extremely adaptive in adverse environmental conditions, they will readily break down the azo bonds. Aragaw [4,14,23] (Fig. 1).

2. Materials and methodology

2.1. Collection of samples

Halophilic bacteria isolated from coastal sediment samples namely HPK1 (13°04'59.53"N, 80°28'24.02"E), HPK3 (E 12° 97'58.10" N) (N 80° 26' 44.80" E), and HPK4 (E 12° 96'11.20" N) (N 80° 26' 37.30" E) were collected from different region of East coastal marine region in sterile polythene bags and stored at 4°C. The pH was measured between 8.1 – 8.5 and temperature- 23°C – 24°C of all the samples respectively.

2.2. Isolation of halophilic bacteria

One gram of sample (wet weight) was dispersed into 4 gm of sterilized 5 % NaCl solution and were serially diluted. 10 µl of 10^{-2} and 10^{-4} were spread plated on modified nutrient agar medium (peptone- 1 %, Beef extract- 1 %, NaCl- 5 % and Agar 1.5 %) and incubated at 37°C for 3 days. The number of colonies were calculated in each sample and inoculated in nutrient agar supplemented with 5–20 % (w/v) sodium chloride. Based on high salt concentration growth the halophilic bacteria were selected for further studies. Glycerol stock (20 %, v/v) for long-term storage at −80°C [27].

2.3. Effect of pH, salt & temperature

The pH optimum was found at a variety of pH levels ranging from 2.0 to 12.0. Salt concentrations (0 % - 25 % NaCl) in the bacterial mixture were varied to investigate the effect of salt on halophilic bacteria. The experiment was carried out at various temperatures (10.0 ± 0.5) °C to

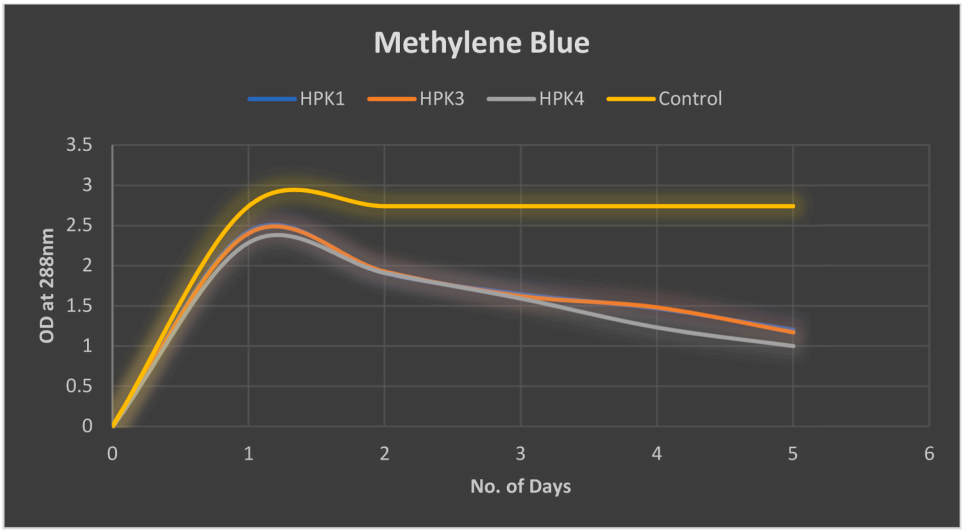

Fig. 14. Characterization of Methylene blue Dye degradation from day 1–5 interval period.

Table 2
Percentage of methyl red dye degradation from day 1–5 interval time.

Days	HPK1	HPK3	HPK4
1	**19.03 %**	**11.81 %**	**4.95 %**
2	**34.23 %**	**34 %**	**20.24 %**
3	**48.24 %**	**50.24 %**	**37.51 %**
4	**66.81 %**	**66.98 %**	**51.29 %**
5	**74.93 %**	**75.12 %**	**67.08 %**

Table 3
Percentage of methylene blue dye degradation from day 1–5 interval time.

Days	HPK1	HPK3	HPK4
1	**11.17 %**	**12.12 %**	**16.35 %**
2	**30.30 %**	**29.57 %**	**30.19 %**
3	**39.75 %**	**40.7 %**	**41.84 %**
4	**46.33 %**	**45.81 %**	**54.94 %**
5	**55.93 %**	**57.17 %**	**63.38 %**

(60.0 ± 0.5) °C to determine the temperature optimization of halophilic bacteria. Finally, Growth curve was carried out in different time interval period between 12 and 72 h. The optimized strains were carried out for dye degradation studies. Based on stranded value graphs have been plotted [5].

2.4. Identification of dye degrading halophilic bacteria

Biochemical and physiological characterization were conducted to identify the morphology and type of dye degrading bacteria [28]. The optimized Bacterial inoculum was centrifuged and the supernatant was used for dye decoloring the methyl red and methylene blue dyes. The dye degrading bacteria was used for further studies [1]. For molecular identification, genomic DNA was extracted using BioLit Genomic DNA extraction Kit. The extracted genomic DNA was amplified using 16 s rRNA universal primers: forward primer (5' AGAGTTTGATCCTGGCTAG 3') and reverse primer (5' TCTACGCATTTCACCGCTAC 3'). The amplified PCR product was purified using SMART prime PCR purification kit and sequenced by Sanger sequencing. The sequence was analyzed using BLAST search and the phylogenetic tree was constructed using MEGA-X software neighborhood joining method [16].

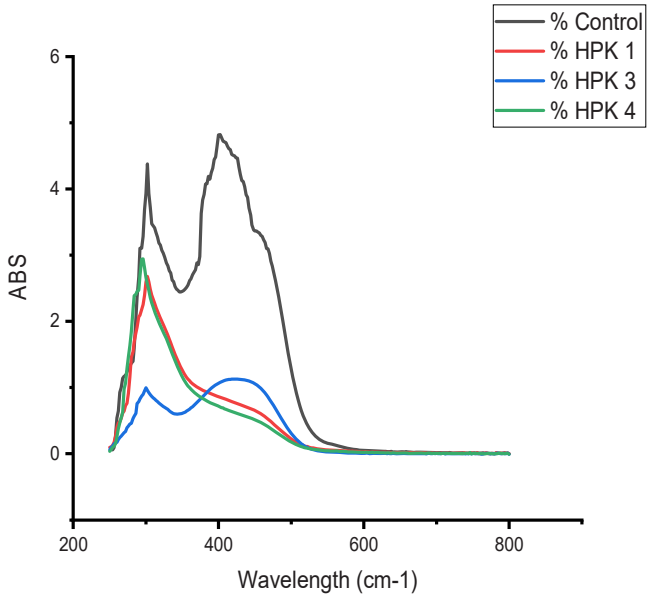

Fig. 15. Characterization of Methyl red Dye degradation before and after treatment under UV- Vis Spectrometry.

2.5. Effect of dye decolorization

The optimized inoculum has been utilized to decolorize methyl red and methylene blue dyes. In a cooling centrifuge, the optimized inoculation was centrifuged for 10 min at 10,000 rpm. In order to decolorize the cell-free aqueous supernatant, 0.01 % methylene blue and methyl red dyes were added. The dye degradation period has been established by UV spectrophotometry to be between 0 and 5 days [24]. Control was used as 0.01 % methylene blue and methyl red dyes without bacterial samples. The percentage decolorization was calculated using the formula: [3]

$$\% \text{ Decolorization} = [(\text{Initial OD} - \text{Final OD}) / \text{Initial OD}] \times 100$$

where OD is the optical density at the λmax of the respective dye.

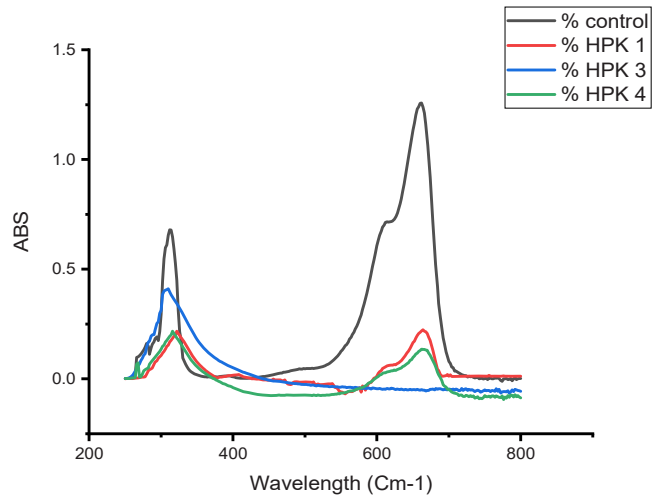

Fig. 16. Characterization of Methyl red Dye degradation before and after treatment under UV- Vis Spectrometry.

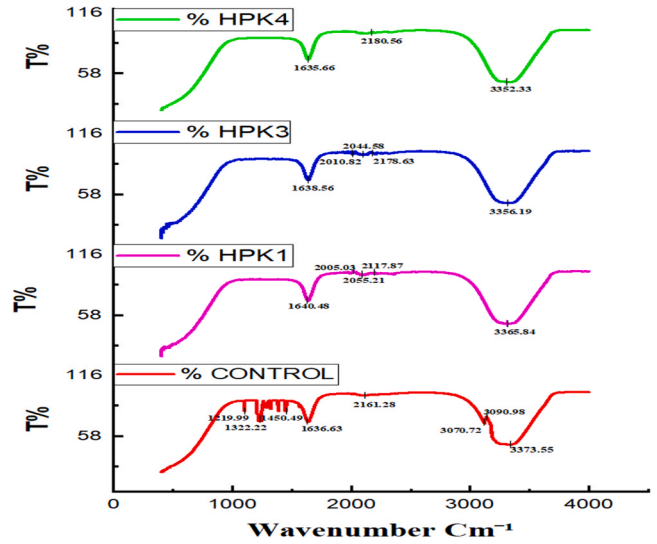

Fig. 17. FTIR spectrum of Methyl red Dye degradation before and after treatment.

2.6. Characterization of dye degradation

Dye degradation was confirmed using UV spectrophotometry UV-1900i Shimadzu instrument and FTIR Shimadzu IR-spirit-lx instrument. In UV spectrophotometry, methylene blue and methyl red used as a control where, the higher concentrated degraded solution was chosen for analysis and scanned between 200 and 1000 nm. Before and after decolorization peaks have been recorded. The decoration functional groups were analyzed in FTIR spectroscopy. The degraded suspension was centrifuged at 5000 rpm for 10 min in cooling centrifuge. The supernatant solution was lyophilized and methylene blue & methyl red dyes used as control for FTIR analysis [29].

2.7. Statistical analysis

All data are presented as means ± standard deviation (SD). Data were analyzed using analysis of variance ($p < 0.05$), and means were differentiated by Duncan's multiple range test. The outcomes were analyzed utilizing SPSS version 23.

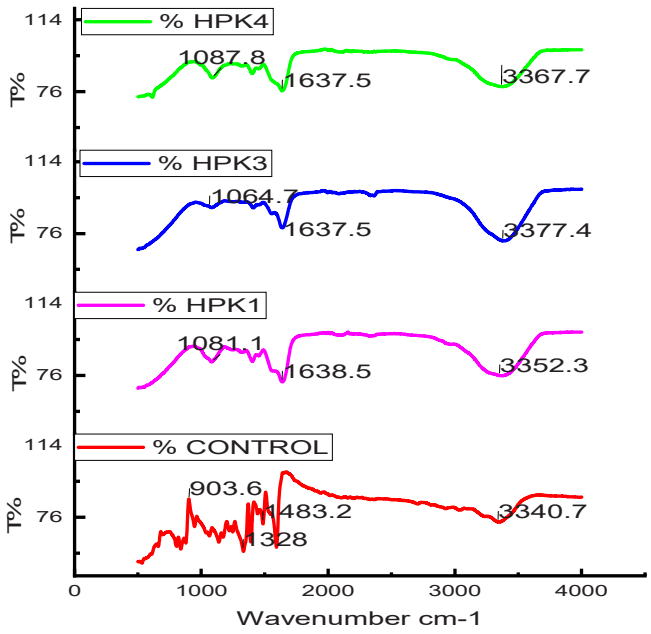

Fig. 18. FTIR spectrum of Methylene blue Dye degradation before and after treatment.

3. Result and discussion

Six halophilic bacterial strains (HPK1-HPK6) tolerant to up to 20 % NaCl were isolated from Bay of Bengal sediments near Chennai. Numerous investigations have been carried out about their ecological and phylogenetic traits, as well as their biotechnological uses, such as bioremediation [12]. Tamil Nadu is bordered by coastal regions that face the Indian Ocean to the south and the Bay of Bengal to the east. The Bay of Bengal's waters have an average surface salinity of 30 %–33 %, which is noticeably lower than that of the Indian and Arabian Seas. Salinity trends and resident microbial communities are significantly impacted by the wide variations in rainfall patterns among states. Generally speaking, these variations result from a significant input of freshwater brought on by the waters salinity and the expanding microbial community [28]. Halophilic bacteria were isolated and counted from marine water samples that were obtained from various locations and preserved appropriately.

3.1. Isolation of halophilic bacteria

From spread plate, the different colonies were inoculated in the 5 % NaCl nutrient agar and grown bacteria were inoculated in increased NaCl concentrations up to 20 %. Totally six bacteria are capable to grow in extreme salt concentration up to 20 % of NaCl. These six isolates were labelled as HPK1 to HPK6 and used for further studies. The ecology, taxonomy, and phylogeny of halophilic bacteria, as well as their biotechnological uses such as textile dye degradation, have been the subject of several investigations [19].

3.2. Optimization of pH, temperature & salt concentration

When cells are cultivated in higher concentrations of salts, metabolic activity is enhanced; this could be because enzyme activity is raised. pH is typically regarded as one of the primary elements influencing the bacteria stability since it is essential for the passage of cell components across the membrane [17]. The halophilic bacteria in this investigation showed a broad range of pH adaption. It's essential to investigate the salt tolerance of halophilic bacteria because osmotic pressure influences bacterial efficiency and because bacteria often possess a high salt

content [21]. Therefore, this study assessed the consequences of varying salt levels on the halophilic bacterial isolates which resulted different optimized salt concentration in all the isolates. Bacterial metabolism is significantly influenced by temperature. While high temperatures can damage the enzyme's active core and prevent it from functioning, low temperatures can result in insufficient enzyme activity [11]. Thus, one of the primary factors influencing the bioremediation of halophilic bacteria in our investigation was temperature.

Optimal growth conditions were determined as follows: *Salinicoccus roseus* HPK1 - pH 8.0, 40°C, 13 % NaCl; *Bacillus altitudinis* HPK3 - pH 8.0, 40°C, 15 % NaCl; *Staphylococcus warneri* HPK4 - pH 8.0, 40°C, 15 % NaCl. The Growth curve of all the strains are highest peak values are obtained in 24 h. When isolates were cultivated on a medium with an optimal pH, salt and temperature level based on their isolates, it was shown that cell growth decreased as the quantity of salt increased. Optimal growth conditions for dye degradation studies were determined to be pH 8.0, 40°C, and 13–15 % NaCl depending on the strain (Figs. 2–5).

3.3. Identification of dye degrading halophilic bacteria

According to the characterization of dye degrading halophilic bacteria, only three of the six isolates were able to breakdown both methylene blue and methyl red after their supernatant was inoculated with 0.01 % of the dyes. The dye degrading bacteria are HPK1, HPK3 and HPK4. These three halophilic bacteria that break down dye are used in future research. The similar study reported in methyl red dye degradation using actinobacterium *Zhihengliuella* sp. Takkar et al., [37], and methylene blue dye degradation using *Bacillus thuringiensis* [38]. And a study reported that degradation of methyl red using *Bacillus circulans* NPP1, *Proteus mirabilis* [2,26] and for methylene blue dye degradation using *Staphylococcus aureus* and *Pleurotus sp*. Silva et al., [6,34].

The following Table 1 lists the physiological and biochemical characteristics. The three dye degrading strains underwent additional biochemical investigation. The flat colony HPK1 strain, the bacillus (rod-shaped) HPK3 strain and the cocci colony HPK4 strain. Biochemical characterization confirmed the isolates as Gram-positive bacteria (Table 1). HPK1 (*Salinicoccus roseus*) was non-motile and oxidase positive, HPK3 (*Bacillus altitudinis*) was motile and oxidase positive, while HPK4 (*Staphylococcus warneri*) was motile but oxidase negative.

For molecular identification, all the three halophilic isolates underwent phylogenetic analysis using the 16S ribosomal RNA gene sequencing technique. The genomic DNA was isolated and was used for 16S rRNA sequencing, using universal primer the PCR product was identified (Fig. 6). The evolutionary distance is shown along with the isolates' phylogenetic relationship and sequence comparison with the GenBank database using the Basic Local Alignment Search Tool (BLAST) tool. The strains are identified as HPK1 - *Salinicoccus roseus* (MW308504), HPK3 - *Bacillus altitudinis* (MW308514), and HPK4 - *Staphylococcus warneri* (MW308518). To examine their evolutionary relationships, the 16S rRNA of one of the halophilic bacterial strains was sequenced. A phylogenetic tree indicates the function of halo bacterial isolates. Using bootstrap consensus for multiple replicates and the neighbour-joining technique, the evolutionary history of the taxa was deduced. With branch lengths matching the evolutionary distances required to make conclusions, the phylogenetic tree is depicted to scale. NCBI tools were used to conduct evolutionary investigations [9] (Figs. 7–9).

3.4. Effect of dye degradation

The strain's capacity to decolorize was tested by screening it with a methyl red and methylene blue of azo dyes. The effect of both textile dye degradation in different interval time period, the methylene blue and methyl red could start able to degrade in 24 h. Continuously both the dye increases in decolorizing day by day, the efficiency of dye degrading increase in percentage. After 5 days, *Salinicoccus roseus* HPK1 degraded 74.93 % of methyl red and 55.93 % of methylene blue. *Bacillus altitudinis* HPK3 degraded 75.12 % of methyl red and 57.17 % of methylene blue. *Staphylococcus warneri* HPK4 degraded 67.08 % of methyl red and 63.38 % of methylene blue (Figs. 10–14, Tables 2–3). Similarly, the study was to determine how successfully the *Pseudomonas putida* strain could degrade methyl red in a laboratory setting. Methyl red, an azo colour, and the following circumstances were both better broken down by the chosen bacterial strain. UV–vis spectroscopy were employed to examine the deterioration of methyl red dye [33]. Comparability a recent study, *Rhodopseudomonas palustris* can degrade methyl red in 10 days [35]. In this study, a strain of *Salinicoccus roseus* was able to break down 55.93 % of the methylene blue in just five days. In five days, the *Bacillus altitudinis* and *Staphylococcus warneri* strains can degrade methylene blue by 57.17 % and 63.38 %, respectively. Comparability methylene blue can be broken down by the *Bacillus licheniformis* genus partially, according to a study and *Pseudomonas aeruginosa* bacteria will degrade of methylene blue [25]. According to this study, halophilic bacteria may effectively cure azo dyes used in the textile sector (Figs. 10–14) (Tables 2–3).

3.5. Characterization of dye degradations

The presence of spectral alteration was confirmed by degradation experiments of methyl red and methylene blue using UV–VIS spectrophotometry UV-1900i Shimadzu instrument. Before treatment, the methylene blue sample showed a prominent peak in the visible area at 660 nm. More than 90 % of the methylene blue dye was used to reduce the peaks at 660 nm following treatment with halophilic bacterial strains of *Salinicoccus roseus*, *Bacillus altitudinis*, and *Staphylococcus warneri*, showing significant decolorization. The spectral peaks were compared with before and after treatment. For methyl red the prominent peak visible in 420 nm before treatment. After methyl red degradation, all of the bacterial sample peaks were visibly absent. UV-Vis spectroscopic study of the two azo dyes (before and after treatment) shows that the solution (methylene blue and methyl red) was completely decolorized, as evidenced by the decrease in absorbance peaks in the visible area without any change in λ-max (Figs. 15–16). The methyl red and methylene blue azo dyes under study's FTIR spectra Shimadzu IR-spirit-lx instrument both before and after decolorization. The biodegradation capacity of the parent dye molecule by *Salinicoccus roseus*, *Bacillus altitudinis,* and *Staphylococcus warneri* strains is evident from the comparison of the FTIR spectra of the recovered metabolites during decolorization with the FTIR spectra of the control dye (before to degradation). In methyl red, A shifted band at 1640.18 cm^{-1} replaces the Azo bond peak at 1636.63 cm^{-1} in *Salinicoccus roseus* HPK 1, suggesting cleavage and potential transformation to amide/ketone. The absence of the aromatic C═C peak at 1450.49 cm^{-1} indicates that the aromatic ring has been disrupted. The newly produced polar functionalities or nitrile may be represented by the new peak that occurs at 2005.03 cm^{-1} . These modifications validate that the *Salinicoccus roseus* HPK1 strain has broken down the azo linkage and aromatic structure in methyl red. *Bacillus altitudinis* HPK3 exhibits structural change of azo or neighboring conjugated systems, as evidenced by the replacement of the original azo/aromatic band at 1636.63 cm^{-1} by 1638.56 cm^{-1} . Aromatic ring cleavage is suggested by the disappearance of the 1332.22 cm^{-1} band and the aromatic C–H band at 3070.72 cm^{-1} . The production of degradation byproducts such as nitriles or other unsaturated intermediates is suggested by the appearance of a new 2044.58 cm^{-1} peak. Significant dye structure degradation by *Bacillus altitudinis* HPK3 is confirmed by changes and the removal of aromatic and azo signals. A new band at 1638.66 cm^{-1} replaces the azo-associated peak (1636.63 cm^{-1}) in *Staphylococcus warneri* HPK4, indicating azo bond cleavage or conversion. Aromatic ring loss is shown by the disappearance of the aromatic C═C (1450.49 cm^{-1}) and aromatic C–H (3070.72 cm^{-1}) peaks. A new peak at 2180.56 cm^{-1} appears, which is

probably related to degradation intermediates of the nitrile type. The structural breakdown of methyl red by *Staphylococcus warneri* HPK4 is confirmed by the formation of additional peaks and the elimination of core aromatic characteristics. The peaks that cause the cleavage of various bonds and functional groups were generally found and matched. By employing distinct bacteria, methyl red was similarly decolorized to distinguish between treated and untreated methyl red [35]. Halophilic bacterial strains-degraded product's FTIR spectrum after being isolated from methylene blue dye exhibited peaks, In *Salinicoccus roseus* HPK1, A shifted band at 1640.18 cm^{-1} replaces the azo/thiazine-associated peak at 1636.63 cm^{-1} , indicating chromophore structural breakdown. The absence of aromatic C═C at 1450.49 cm^{-1} and C–N at 1332.22 cm^{-1} suggests that the thiazine ring structure has disintegrated. New bands between 2005.03 and 2117.87 cm^{-1} imply the creation of intermediates that resemble nitrile. *Salinicoccus roseus* HPK 1's degradation of methylene blue is confirmed by a significant chromophoric and ring system disintegration. A change in the core dye structure is shown by the replacement of the azo/conjugated peak at 1636.63 cm^{-1} with 1638.56 cm^{-1} in *Bacillus altitudinis* HPK3. Degradation of the aromatic thiazine ring is indicated by the loss of peaks at 1332.22 cm^{-1} (C–N) and 1450.49 cm^{-1} (C═C). Additionally, the appearance of 2044.58 cm^{-1} and 2175.63 cm^{-1} points to the formation of intermediate functional groups (such as nitriles and isocyanates). Significant breakdown by *Bacillus altitudinis* HPK3 is confirmed by the loss of core dye signals and the development of additional peaks. The degradation-supporting 1636.63 cm^{-1} azo/thiazine peak in *Staphylococcus warneri* HPK4 is replaced by a new band at 1638.66 cm^{-1} . The collapse of the thiazine chromophore is shown by the disappearance of 1450.49 cm^{-1} and 1332.22 cm^{-1} . Breakdown intermediates of the nitrile type or a similar kind are indicated by a strong new band at 2180.56 cm^{-1} . The structural breakdown of methylene blue by *Staphylococcus warneri* HPK4 is confirmed by the spectrum changes and peak loss.

The breakdown of the dye into intermediate compounds is indicated by changes in the peaks of the supernatant following dye decolorization as compared to the original dye [15]. The shift in the peak points to a possible breakdown of functional groups that altered the dyes' initial structure in the test samples that were decolorized (Figs. 17 -18)

4. Conclusion

Three halophilic bacterial strains - *Salinicoccus roseus* HPK1, *Bacillus altitudinis* HPK3, and *Staphylococcus warneri* HPK4 - isolated from the Bay of Bengal demonstrated significant potential for decolorizing methyl red (67–75 %) and methylene blue (56–63 %) under optimized conditions (pH 8.0, 40°C, 13–15 % NaCl) within 5 days. Achieving up to 75 % decolorization, highlighting their effectiveness as bioagents for saline textile effluent bioremediation. The observation demonstrated that the halophilic bacteria decolorize pollutants and are adaptable in nature. The strains had a benefit for treating wastewater from the textile sector since it can withstand and decolorize textile dyes at high concentrations. While effective under static conditions, further research is needed to evaluate performance in dynamic systems simulating industrial wastewater flow and to scale up the process. Toxicity assessment of degradation metabolites is also essential before field application.

CRediT authorship contribution statement

K. Pavithran: Writing – original draft, Methodology, Investigation. **Manjunathan Jagadeesan:** Visualization, Validation, Conceptualization. **Pasiyappazham Ramasamy:** Writing – review & editing, Supervision, Project administration.

Ethics declaration

Not applicable.

Funding declaration

No funding.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors are grateful to the authorities of the Vels Institute of Science, Technology, and Advanced Studies (VISTAS), Chennai and Saveetha Dental College & Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, for providing the necessary facilities and support.

Data availability

Data will be made available on request.

References

[1] S. Afrin, H.R. Shuvo, B. Sultana, F. Islam, A.A. Rus'd, S. Begum, M.N. Hossain, The degradation of textile industry dyes using the effective bacterial consortium, Heliyon 7 (2021) e08102, https://doi.org/10.1016/j.heliyon.2021.e08102.
[2] I. Ahmed, F. Haque, M.A.T.M.T. Rahman, M.A.K. Parvez, T.J. Mou, Screening of methyl red degrading bacteria isolated from textile effluents of Savar area, Dhaka, Bangladesh, Adv. Biosci. Biotechnol. 11 (2020) 301–318, https://doi.org/10.4236/abb.2020.117022.
[3] A.A. Algarni, Combining of molecular 16S rRNA gene and metabolic fingerprinting through biolog system for the identification of streptomycetes in Saudi Arabia, J. King Saud. Univ. Sci. 34 (2022) 101889, https://doi.org/10.1016/j.jksus.2022.101889.
[4] T.A. Aragaw, Potential and prospects of reductases in azo dye degradation: a minireview, Microbe (2024) 100162, https://doi.org/10.1016/j.microb.2024.100162.
[5] S. Asad, M.A. Amoozegar, A.A. Pourbabaee, M.N. Sarbolouki, S.M.M. Dastgheib, Decolorization of textile azo dyes by newly isolated halophilic and halotolerant bacteria, Bioresour. Technol. 98 (2007) 2082–2088, https://doi.org/10.1016/j.biortech.2006.08.020.
[6] L. Ayed, K. Bekir, C. Jabeur, Modeling and optimization of biodegradation of methylene blue by *Staphylococcus aureus* through a statistical optimization process: a sustainable approach for waste management, Water Sci. Technol. 86 (2022) 380–394, https://doi.org/10.2166/wst.2022.211.
[7] M.S. Ayilara, O.O. Babalola, Bioremediation of environmental wastes: the role of microorganisms, Front. Agron. 5 (2023) 1–15, https://doi.org/10.3389/fagro.2023.1183691.
[8] S.P. Bera, M.P. Shah, M. Godhaniya, Microbial remediation of textile dye acid orange by a novel bacterial consortium SPB92, Front. Environ. Sci. 10 (2022) 1–8, https://doi.org/10.3389/fenvs.2022.930616.
[9] G. Bianchini, P. Sánchez-Baracaldo, TreeViewer: flexible, modular software to visualise and manipulate phylogenetic trees, Ecol. Evol. 14 (2024) 1–16, https://doi.org/10.1002/ece3.10873.
[10] J. Chanwala, G. Kaushik, M.A. Dar, S. Upadhyay, A. Agrawal, Process optimization and enhanced decolorization of textile effluent by *Planococcus sp.* isolated from textile sludge, Environ. Technol. Innov. 13 (2019) 122–129, https://doi.org/10.1016/j.eti.2018.11.008.
[11] H. Chen, Y. Teng, S. Lu, Y. Wang, J. Wang, Contamination features and health risk of soil heavy metals in China, Sci. Total Environ. 512–513 (2015) 143–153, https://doi.org/10.1016/j.scitotenv.2015.01.025.
[12] K.C. Chen, J.Y. Wu, D.J. Liou, S.C.J. Hwang, Decolorization of the textile dyes by newly isolated bacterial strains, J. Biotechnol. 101 (2003) 57–68, https://doi.org/10.1016/S0168-1656(02)00303-6.
[13] C. Cripps, J.A. Bumpus, S.D. Aust, Biodegradation of azo and heterocyclic dyes by *Phanerochaete chrysosporium*, Appl. Environ. Microbiol 56 (1990) 1114–1118, https://doi.org/10.1128/aem.56.4.1114-1118.1990.
[14] S. Das, L. Cherwoo, R. Singh, Decoding dye degradation: microbial remediation of textile industry effluents, Biotechnol. Notes 4 (2023) 64–76, https://doi.org/10.1016/j.biotno.2023.10.001.
[15] H. Eslami, S. Sedighi Khavidak, F. Salehi, R. Khosravi, R.A. Fallahzadeh, R. Peirovi, S. Sadeghi, Biodegradation of methylene blue from aqueous solution by bacteria isolated from contaminated soil, J. Adv. Environ. Heal. Res. (2017), https://doi.org/10.22102/jaehr.2017.46690.
[16] S. Gupta, P. Sharma, K. Dev, M. Srivastava, A. Sourirajan, A diverse group of halophilic bacteria exist in Lunsu, a natural salt water body of Himachal Pradesh, India, Springerplus 4 (2015), https://doi.org/10.1186/s40064-015-1028-1.

[17] M. Ikram, M. Naeem, M. Zahoor, M.M. Hanafiah, A.A. Oyekanmi, R. Ullah, D.A. A. Farraj, M.S. Elshikh, I. Zekker, N. Gulfam, Biological degradation of the Azo dye Basic Orange 2 by *Escherichia coli*: a sustainable and ecofriendly approach for the treatment of textile wastewater, Water (Switz.) 14 (2022), https://doi.org/10.3390/w14132063.

[18] R. Jamee, R. Siddique, Biodegradation of synthetic dyes of textile effluent by microorganisms: an environmentally and economically sustainable approach, Eur. J. Microbiol. Immunol. 9 (2019) 114–118, https://doi.org/10.1556/1886.2019.00018.

[19] J. John, R. Dineshram, K.R. Hemalatha, M.P. Dhassiah, D. Gopal, A. Kumar, Bio-decolorization of synthetic dyes by a halophilic bacterium *Salinivibrio sp.* Front. Microbiol 11 (2020) https://doi.org/10.3389/fmicb.2020.594011.

[20] Kangli Wu, Meiling Shi, Xiaomei Pan, Jianqiang Zhang, Xiaomeng Zhang, Tong Shen, Y.T, Decolourization and biodegradation of methylene blue dye by a ligninolytic enzyme-producing *Bacillus thuringiensis*: degradation products and pathway, Enzym. Microb. Technol. (2022), https://doi.org/10.1016/j.enzmictec.2022.109999.

[21] R.M. Martínez-Espinosa, Halophilic archaea as tools for bioremediation technologies, Appl. Microbiol. Biotechnol. 108 (2024), https://doi.org/10.1007/s00253-024-13241-z.

[22] A. Mishra, B. Gupta, N. Kumar, R. Singh, A. Varma, I.S. Thakur, Synthesis of calcite-based bio-composite biochar for enhanced biosorption and detoxification of chromium Cr (VI) by *Zhihengliuella sp.* ISTPL4, Bioresour. Technol. 307 (2020) 123262, https://doi.org/10.1016/j.biortech.2020.123262.

[23] G. Mustafa, M.T. Zahid, M.B. Kurade, S.M. Patil, F.R. Shakoori, Z. Shafiq, S. Ihsan, Y. Ahn, A.A. Khan, A. Gacem, B.H. Jeon, Molecular characterization of azoreductase and its potential for the decolorization of Remazol Red R and Acid Blue 29, Environ. Pollut. 335 (2023) 122253, https://doi.org/10.1016/j.envpol.2023.122253.

[24] N.G. Osborne, Mycoplasma, Infectious Diseases in Obstetrics and Gynecology, sixth ed. (2008), https://doi.org/10.5005/jp/books/12721_22.

[25] B.Y. Patel, H.K. Patel, Current approaches toward the removal of methylene blue dye from synthetic textile effluent using bacterial treated agricultural waste absorbent through statistical design, Heliyon 9 (2023) e19857, https://doi.org/10.1016/j.heliyon.2023.e19857.

[26] N.P. Patil, A.D. Bholay, B.P. Kapadnis, V.B. Gaikwad, Biodegradation of model azo dye methyl red and other textile dyes by isolate *Bacillus circulans* npp1, J. Pure Appl. Microbiol 10 (2016) 2793–2800, https://doi.org/10.22207/JPAM.10.4.38.

[27] V.H.T. Pham, J. Kim, S. Chang, D. Bang, Investigating bio-inspired degradation of toxic dyes using potential multi-enzyme producing extremophiles, Microorganisms 11 (2023) 1–15, https://doi.org/10.3390/microorganisms11051273.

[28] D. Rathakrishnan, A.K. Gopalan, Isolation and characterization of halophilic isolates from Indian salterns and their screening for production of hydrolytic enzymes, Environ. Chall. 6 (2022) 100426, https://doi.org/10.1016/j.envc.2021.100426.

[29] D.C. Roy, S.K. Biswas, A.K. Saha, B. Sikdar, M. Rahman, A.K. Roy, Z.H. Prodhan, S. S. Tang, Biodegradation of crystal violet dye by bacteria isolated from textile industry effluents, PeerJ 2018 (2018) 1–15, https://doi.org/10.7717/peerj.5015.

[30] A. Sepehri, M.H. Sarrafzadeh, Effect of nitrifiers community on fouling mitigation and nitrification efficiency in a membrane bioreactor, Chem. Eng. Process. Process. Intensif. 128 (2018) 10–18, https://doi.org/10.1016/j.cep.2018.04.006.

[31] A. Sepehri, M.H. Sarrafzadeh, Activity enhancement of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria in activated sludge process: metabolite reduction and CO2 mitigation intensification process, Appl. Water Sci. 9 (5) (2019) 131, https://doi.org/10.1007/s13201-019-1017-6.

[32] A. Sepehri, M.H. Sarrafzadeh, M. Avateffazeli, Interaction between Chlorella vulgaris and nitrifying-enriched activated sludge in the treatment of wastewater with low C/N ratio, J. Clean. Prod. 247 (2020) 119164, https://doi.org/10.1016/j.jclepro.2019.119164.

[33] K. Sharma, S. Pandit, A.S. Mathuriya, P.K. Gupta, K. Pant, D.A. Jadhav, Microbial electrochemical treatment of methyl red dye degradation using co-culture method, Water (Switz.) 15 (2023) 1–17, https://doi.org/10.3390/w15010056.

[34] A.S.V.D. Mass, N.J.R.D. Silva, A.S.V. da Costa, A.R. Barros, C.A. Bomfeti, The degradation of methylene blue dye by the strains of *Pleurotus sp.* with potential applications in bioremediation processes, J. Appl. Sci. (2018) 13, https://doi.org/10.4136/ambi-agua.2247.

[35] S. Sma-Air, R.J. Ritchie, Methyl red dye decolourization by the photosynthetic bacteria, *Rhodopseudomonas palustris* and Afifella marina, Int. Biodeterior. Biodegrad 196 (2025) 105915, https://doi.org/10.1016/j.ibiod.2024.105915.

[36] R.K. Sonwani, G. Swain, B.S. Giri, R.S. Singh, B.N. Rai, Biodegradation of Congo red dye in a moving bed biofilm reactor: performance evaluation and kinetic modeling, Bioresour. Technol. 302 (2020), https://doi.org/10.1016/j.biortech.2020.122811.

[37] S. Takkar, B. Tyagi, N. Kumar, T. Kumari, K. Iqbal, A. Varma, I.S. Thakur, A. Mishra, Biodegradation of methyl red dye by a novel actinobacterium *Zhihengliuella sp.* ISTPL4: kinetic studies, isotherm and biodegradation pathway, Environ. Technol. Innov. 26 (2022) 102348, https://doi.org/10.1016/j.eti.2022.102348.

[38] H.C. Wang, Y.Q. Wang, X. Wang, W.X. Yin, T.C. Yu, C.H. Xue, A.J. Wang, Multimodal machine learning guides low carbon aeration strategies in urban wastewater treatment, Engineering 36 (2024) 51–62, https://doi.org/10.1016/j.eng.2023.11.020.

[39] S. Zafar, D.A. Bukhari, A. Rehman, Azo dyes degradation by microorganisms – an efficient and sustainable approach, Saudi J. Biol. Sci. 29 (2022) 103437, https://doi.org/10.1016/j.sjbs.2022.103437.