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Environmental Impact Reduction of Synthetic Dyes Through Marine Bacterial Biodegradation

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Abstract

Azo dyes are widely used in industrial applications and contribute significantly to environmental pollution due to their persistence and toxicity. This study focuses on the biodegradation of synthetic azo dyes and antibacterial potential of halophilic bacteria isolated from water samples collected along the east coast of Tamil Nadu, India. The isolates were identified through morphological, biochemical, and molecular (16S rRNA) methods. The selected halophilic strains were evaluated for dye degradation under optimized conditions. Protein estimation and SDS-PAGE analysis were performed to study protein expression associated with biodegradation. Further characterization of efficient isolates was carried out to understand their functional properties. Additionally, waterborne pathogenic bacteria were isolated and identified to assess the antimicrobial activity of the halophilic strains. The results demonstrated effective dye degradation and significant antibacterial activity, highlighting the dual potential of halophilic bacteria in environmental bioremediation and antimicrobial applications.

Keywords

Azo dye biodegradation; Halophilic bacteria; Bioremediation; Antibacterial activity; Waterborne pathogens; SDS-PAGE

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1. Introduction:

The treatment of contaminated wastewater has become a major global concern over the past few decades due to rapid industrialization, population growth, and the limited availability of freshwater resources. Among industrial pollutants, untreated or partially treated effluents released from textile industries pose a serious environmental threat to aquatic ecosystems and human health [1]. Textile effluents are a major source of water pollution and represent one of the most hazardous environmental contaminants, primarily due to their high color intensity, toxicity, and resistance to degradation.

Textile dyes are chemically diverse and are classified into several categories, including reactive, azo, heterocyclic, triphenylmethane, and polymeric dyes. Among these, azo dyes are the most extensively used in the textile industry owing to their cost-effectiveness, color stability, and wide color range [2]. Azo dyes contain one or more azo (–N=N–) bonds linking aromatic rings and represent a major class of synthetic dyes widely used in industries such as textiles, paper, leather, food, and cosmetics. In India alone, textile dyes are estimated to contribute up to 40% of dye-related pollution, while textile and garment exports remain a major source of foreign exchange for the country [3].

Azo dyes and their breakdown products are associated with serious environmental and health hazards, including carcinogenicity, mutagenicity, chromosomal aberrations, hepatotoxicity, kidney damage, and allergic reactions [4]. Methyl Red, a mono-azo dye lacking sulfonate groups, is reported to be mutagenic [5], while Methylene Blue is a redox-active dye known to inhibit monoamine oxidase and cause toxic effects at elevated concentrations [6]. Due to their high solubility, these dyes can easily enter aquatic systems and accumulate through the food chain, posing long-term ecological risks.

Conventional physical and chemical treatment methods for dye removal are often costly, inefficient, and generate secondary pollutants. In contrast, biological treatment approaches are considered more economical, environmentally friendly, and effective, as microorganisms can degrade dyes into less toxic or non-colored compounds [7]. Microbial degradation of azo dyes is primarily mediated by enzymes such as azoreductases, oxidases, and peroxidases, which cleave the azo bond and facilitate dye mineralization.

In recent years, halophilic bacteria have gained attention for their potential application in dye decolorization, particularly in saline textile effluents where conventional microorganisms fail to survive. Halophilic bacteria possess unique metabolic pathways and enzyme systems that remain active under high salt concentrations and other extreme environmental conditions [8]. Their ability to tolerate salinity, heavy metals, and toxic compounds makes them suitable candidates for bioremediation of saline industrial wastewater. Despite this potential, limited studies have focused on the application of halophilic bacteria for textile dye degradation [9]. Therefore, the present study aimed to isolate and characterize halophilic bacteria from the marine environment of the Bay of Bengal, Chennai, India, and to evaluate the dye decolorization efficiency of the identified strain, *Virgibacillus salexigens*, against commonly used azo dyes. This study highlights the potential application of halophilic bacteria in the sustainable treatment of saline textile wastewater.

2. Materials and Methods:

1. Sample Collection:

Marine sediment samples were collected from different locations along the east coast region. All samples were aseptically collected using sterile polythene bags and immediately transported to the laboratory. The collected samples were preserved at 4 °C until further processing. The measured pH of the sediment samples ranged from 8.1 to 8.5, and the ambient temperature during sampling was approximately 23–24 °C.

2. Isolation and Screening of Halophilic Bacteria:

For the isolation of halophilic bacteria, 1 g (wet weight) of each sediment sample was suspended in 4 g of sterile 5% (w/v) sodium chloride solution and mixed thoroughly. Serial dilutions were prepared, and 10 µL aliquots from the 10^{-2} and 10^{-4} dilutions were spread evenly onto modified nutrient agar plates containing peptone (1%), beef extract (1%), NaCl (5%), and agar (1.5%). The inoculated plates were incubated at 37 °C for 72 h. Following incubation, colony-forming units were enumerated, and morphologically distinct colonies were selected. The isolates were further subcultured on nutrient agar medium supplemented with varying sodium chloride concentrations ranging from 5% to 20% (w/v) to assess salt tolerance. Isolates exhibiting growth at higher salinity levels were identified as halophilic bacteria and selected for subsequent studies. For long-term storage, the cultures were preserved in glycerol (20%, v/v) at −80 °C [10].

3. Identification of Dye-Degrading Halophilic Bacteria:

Biochemical and physiological analyses were performed to determine the morphological characteristics and taxonomic nature of the dye-degrading halophilic bacterial isolates [11]. The optimized bacterial culture was centrifuged, and the cell-free supernatant was collected and employed for the decolorization studies using Methyl red and Methylene blue dyes. The isolates exhibiting efficient dye degradation were selected and used for subsequent investigations [12].

For molecular identification, genomic DNA was isolated from the selected bacterial isolate using the BioLit Genomic DNA Extraction Kit following the manufacturer's instructions. The extracted DNA was subjected to polymerase chain reaction (PCR) amplification of the 16S rRNA gene using universal primers: forward primer (5′-AGAGTTTGATCCTGGCTAG-3′) and reverse primer (5′-TCTACGCATTTCACCGCTAC-3′). The PCR-amplified products were purified using the SMART Prime PCR Purification Kit and sequenced by the Sanger sequencing method. The obtained nucleotide sequence was analyzed using the BLAST tool for sequence similarity search, and a phylogenetic tree was constructed using MEGA-X software employing the neighbor-joining method [13].

4. Effect of Dye Decolorization:

The decolorization efficiency of the selected halophilic bacterial isolate was evaluated using the optimized inoculum. The bacterial culture was centrifuged at 10,000 rpm for 10 min in a refrigerated centrifuge to obtain a cell-free supernatant. To this supernatant, Methyl red and Methylene blue dyes were added separately at a final concentration of 0.01%. The reaction mixtures were incubated, and dye degradation was monitored over 36hrs. The extent of dye decolorization was determined at regular intervals using UV–visible spectrophotometry by measuring the decrease in absorbance of the respective dyes [14].

5. Characterization of Dye Degradation:

The degradation of dyes was confirmed using UV–visible spectrophotometry and Fourier Transform Infrared (FTIR) spectroscopy. In UV–visible spectrophotometric analysis, untreated Methylene blue and Methyl red solutions served as controls. The dye-degraded samples with higher concentrations were selected for analysis and scanned over a wavelength range of 200–1000 nm. Changes in the absorption spectra before and after decolorization were recorded to confirm dye degradation.

Functional group alterations resulting from dye degradation were further analyzed using FTIR spectroscopy. The degraded dye suspension was centrifuged at 5,000 rpm for 10 min using a refrigerated centrifuge. The collected supernatant was lyophilized to obtain dried samples. Untreated Methylene blue and Methyl red dyes were used as controls for comparative FTIR analysis. The resulting spectra were analyzed to identify structural changes in functional groups, confirming the degradation of dyes [15].

3. Results and Discussion:

Halophilic bacteria, particularly moderately halophilic groups, have gained increasing attention due to their ecological significance and wide-ranging biotechnological applications, especially in environmental remediation processes [16]. These microorganisms are well adapted to saline habitats and play a vital role in maintaining ecological balance in coastal and marine ecosystems.

Tamil Nadu is characterized by an extensive coastline along the Bay of Bengal to the east and the Indian Ocean to the south. The surface salinity of the Bay of Bengal typically ranges between 30 and 33%, which is comparatively lower than that of the Indian Sea and the Arabian Sea. This reduced salinity is largely attributed to substantial freshwater inflow from rivers, seasonal monsoon rainfall, and land runoff. Such fluctuations in salinity strongly influence the composition and distribution of microbial communities in coastal regions. Variations in rainfall patterns across different states further contribute to changes in salinity levels, thereby affecting the diversity and abundance of resident microorganisms [17].

In the present study, halophilic bacteria were successfully isolated from marine sediment samples collected from different coastal locations. The samples were properly preserved and processed to ensure the viability of microbial populations. Colony enumeration revealed the presence of diverse halophilic bacterial populations, indicating that the coastal sediments provide a favorable environment for the growth and survival of salt-tolerant microorganisms. These findings highlight the ecological adaptability of halophilic bacteria in coastal marine environments and support their potential application in biotechnological processes such as dye degradation and bioremediation.

1. Isolation of Halophilic Bacteria:

Distinct bacterial colonies obtained from spread plate cultures were initially subcultured on nutrient agar supplemented with 5% NaCl. The successfully grown isolates were subsequently transferred to nutrient agar media containing progressively higher sodium chloride concentrations, up to 20% (w/v), to evaluate their salt tolerance. Among the isolates obtained, six bacterial strains demonstrated the ability to grow at extreme salinity levels of up to 20% NaCl. These salt-tolerant isolates were selected for further experimental studies. Previous studies have reported that halophilic bacteria exhibit diverse ecological, taxonomic, and phylogenetic characteristics and possess significant biotechnological potential, including applications in textile dye degradation and bioremediation [18].

2. Identification of Dye-Degrading Halophilic Bacteria:

Based on the dye decolorization assay, only one out of the three halophilic bacterial isolates demonstrated the ability to degrade both Methylene Blue and Methyl Red dyes when their cell-free supernatants were treated with 0.01% dye concentration. These efficient dye-degrading isolates were identified, while the remaining isolates showed negligible or no degradation activity. Consequently, strain 2 were selected for further characterization and advanced studies.

Comparable findings have been reported in earlier studies, where Methyl Red degradation was achieved using *Bacillus circulans* NPP1, and *Proteus mirabilis* [19]. Similarly, Methylene Blue degradation has been reported using *Bacillus thuringiensis*, *Staphylococcus aureus*, and *Pleurotus* sp. [20], supporting the dye-degrading potential of halophilic and salt-tolerant microorganisms.

3. Physiological and Biochemical Characterization:

The physiological and biochemical characteristics of the dye-degrading halophilic isolate. The isolate was found to be Gram-positive bacteria. Morphological examination revealed that strain formed rod-shaped cells, The isolate tested positive for glucose fermentation, citrate utilization, and catalase activity.

Motility tests indicated that strain was motile, and oxidase activity was detected positive. Carbohydrate utilization tests revealed that positive in sucrose, whereas negative in lactose fermentation. Fructose utilization was positive only in isolated halophilic bacteria.

4. Molecular Identification and Phylogenetic Analysis:

For molecular identification, the dye-degrading halophilic isolate were subjected to 16S rRNA gene sequencing. Genomic DNA was successfully isolated and amplified using universal 16S rRNA primers, and the presence of PCR amplicons was confirmed. The obtained sequences were analyzed using the BLAST tool of the GenBank database to determine sequence similarity and taxonomic affiliation.

Phylogenetic analysis revealed that strain was closely related to *Virgibacillus* salexigens. The phylogenetic trees constructed using the neighbor-joining method in MEGA software illustrated the evolutionary relationships among the isolates and their closest relatives (Figure:1). Bootstrap analysis with multiple replicates was employed to validate the reliability of the phylogenetic clustering. The trees were drawn to scale, with branch lengths representing evolutionary distances, and all evolutionary analyses were performed using NCBI-supported tools [21].

5. Effect of Dye Degradation:

The dye decolorization efficiency of the halophilic bacterium *Virgibacillus salexigens* was evaluated using two azo dyes, namely Methyl Red and Methylene Blue. The strain exhibited noticeable decolorization activity within 24 h of incubation, and a significant increase in dye degradation was observed with prolonged incubation. Maximum decolorization was achieved within 36 h, indicating the rapid metabolic capability of *V. salexigens* toward azo dye degradation.

The results demonstrated that *Virgibacillus salexigens* achieved a degradation efficiency of 76% for Methyl Red and 73% for Methylene Blue within 36 h (Figures: 2-3). The progressive reduction in absorbance confirmed the effective breakdown of dye molecules, as monitored by UV–visible spectrophotometry. The higher degradation efficiency observed for Methyl Red compared to Methylene Blue suggests differential enzymatic affinity toward the dye structures.

Comparable studies have reported efficient azo dye degradation by various bacterial species. Previous reports indicate successful Methyl Red degradation under laboratory conditions using UV–visible spectroscopic analysis [22]. Similarly, studies have shown that halophilic and salt-tolerant bacteria are capable of degrading textile dyes effectively under saline environments [23]. The rapid degradation observed in the present study highlights the superior efficiency of *Virgibacillus salexigens* within a shorter incubation period. Overall, the findings of this study demonstrate that *Virgibacillus salexigens* possesses strong potential for the biodegradation of azo dyes commonly used in the textile industry. Its ability to achieve high decolorization efficiency within 36 h under saline conditions suggests its suitability for application in the treatment of saline textile wastewater.

6. Characterization of Dye Degradation:

The degradation of Methyl Red and Methylene Blue dyes by *Virgibacillus salexigens* was confirmed using UV–visible spectrophotometry and Fourier Transform Infrared (FTIR) spectroscopy. UV–Vis spectral analysis revealed distinct changes in the absorbance profiles of both dyes before and after biodegradation. Prior to treatment, Methylene Blue exhibited a characteristic absorption peak in the visible region at 660 nm. Following biodegradation, this peak was completely absent, indicating effective decolorization of the dye. Similarly, untreated Methyl Red showed a prominent absorption peak at 420 nm, which disappeared after biodegradation. The UV–Vis spectra recorded before and after treatment demonstrated a significant reduction in absorbance intensity in the visible region without any shift in the λmax values, confirming complete dye decolorization (Figures: 4-5).

Further confirmation of dye degradation was obtained through FTIR spectral analysis of Methyl Red and Methylene Blue before and after treatment. The biodegradation efficiency of *Virgibacillus salexigens* was evident from the marked differences observed between the FTIR spectra of untreated dyes and their degraded metabolites. In the FTIR spectrum of untreated Methyl Red, a characteristic absorption peak at 1450.49 cm^{-1} corresponding to the asymmetric stretching of the azo bond (–N=N–) was observed. This peak disappeared after degradation, indicating cleavage of the azo linkage, which is primarily responsible for dye coloration. Additionally, the absence of peaks at 1219.99 cm⁻¹ (C–N stretching) and 1322.22 cm⁻¹ (phenolic O–H stretching) in the degraded samples further confirmed structural breakdown of the dye molecule. The disappearance of these functional group peaks suggests azo bond reduction, likely mediated by azoreductase activity. Significant differences in functional group composition were observed between untreated and degraded Methyl Red, with no prominent absorption peaks detected after decolorization, confirming complete dye degradation [24].

The FTIR spectrum of Methylene Blue degradation products also revealed substantial structural alterations. Untreated Methylene Blue exhibited characteristic peaks at 903.6 cm⁻¹ corresponding to C=O stretching of inorganic carbonates, 1328 cm⁻¹ representing C–N stretching of amide III bonds, and 1483.2 cm⁻¹ associated with aromatic C=C stretching. These peaks were absent or significantly reduced in the degraded samples. Furthermore, noticeable shifts were observed in the broad peak at 3373.55 cm⁻¹, corresponding to O–H stretching of amines and amides, along with the disappearance of the aromatic C–H stretching peak at 3070.72 cm⁻¹. The loss of these peaks indicates cleavage of chromophoric groups responsible for dye color formation. The pronounced differences between the FTIR spectra of control dyes and the degradation products confirm that *Virgibacillus salexigens* effectively biodegraded both Methyl Red and Methylene Blue into intermediate metabolites. The observed peak shifts and disappearance of functional groups suggest disruption of the original dye structures, validating the biodegradation process [25] (Figures: 6-9).

4. Conclusion:

The halophilic bacterial strain isolated and identified in this study demonstrated the ability to grow across a wide range of salinity conditions and exhibited strong potential for the biodegradation of textile dyes under varying environmental parameters. The results clearly indicate that this halophilic bacterium functions as an effective biological agent for textile dye bioremediation. Efficient dye decolorization was achieved under optimized conditions, highlighting the adaptability and metabolic efficiency of the strain.

The observed decolorization capability confirms that halophilic bacteria can tolerate high salt concentrations while effectively degrading dye pollutants. This characteristic offers a significant advantage for the treatment of saline textile wastewater, where conventional microbial treatment methods often fail. Overall, the findings suggest that this halophilic bacterium holds considerable promise for application in the bioremediation of wastewater contaminated with azo dyes from the textile industry.

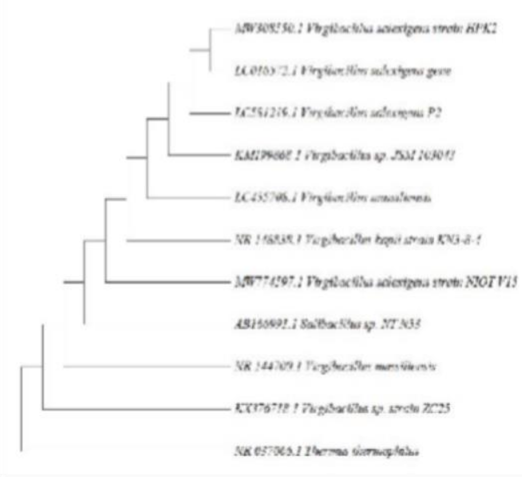

Figure 1: Phylogenetic tree constructed using neighbour-joining method based on the nearly complete 16S rRNA gene sequences of *Virgibacillus salexigens* was used as the outgroup.

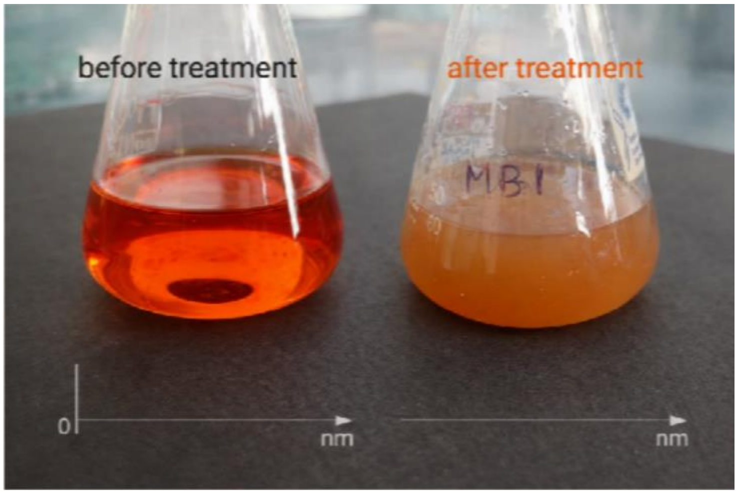

Figure-2: Before and After Treatment of 0.1% Methyl red dye using halophilic bacteria *Virgibacillus salexigens*

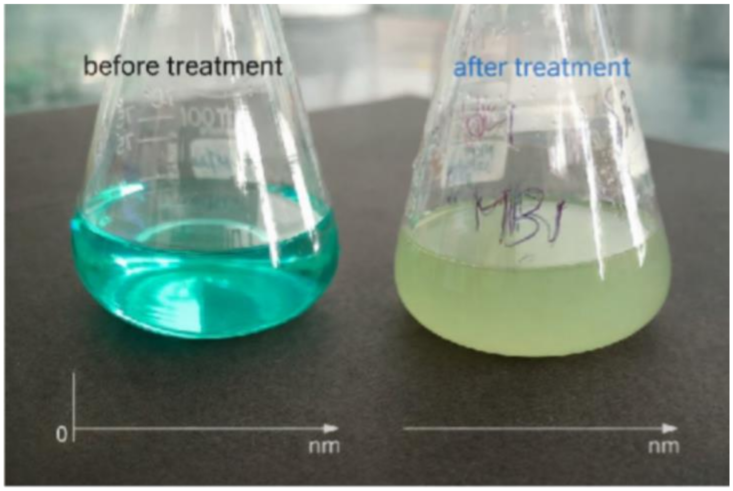

Figure-3: Before and After Treatment of 0.1% Methylene blue dye using halophilic bacteria *Virgibacillus salexigens*

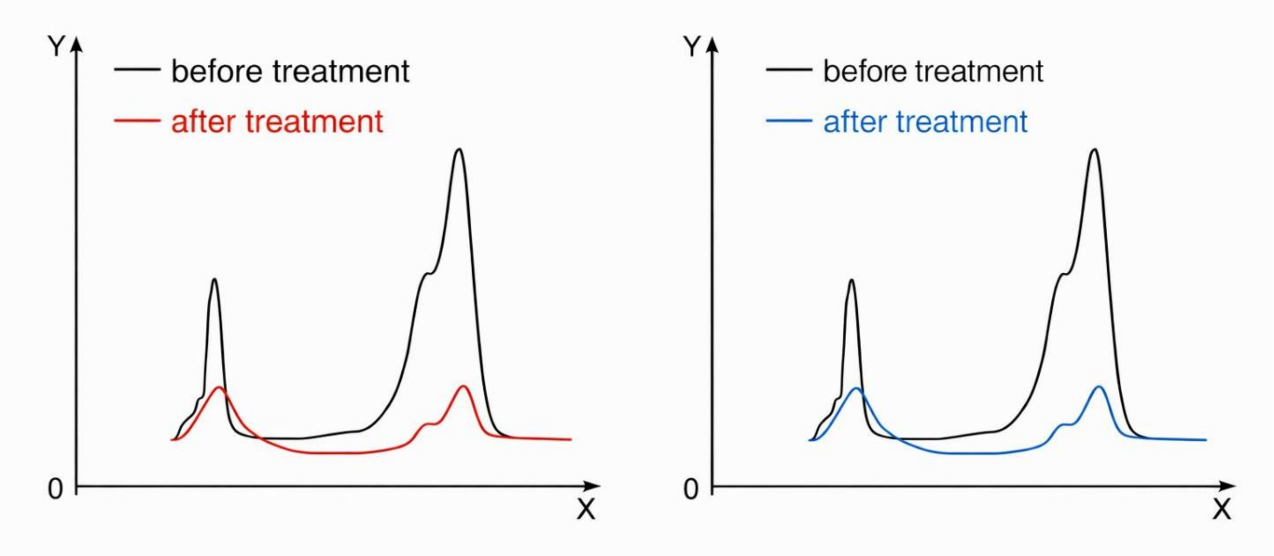

Figure-4: UV-Vis Spectrophotometry analysis of Methyl red dye before and after treating

With halophilic bacteria *Virgibacillus salexigens*

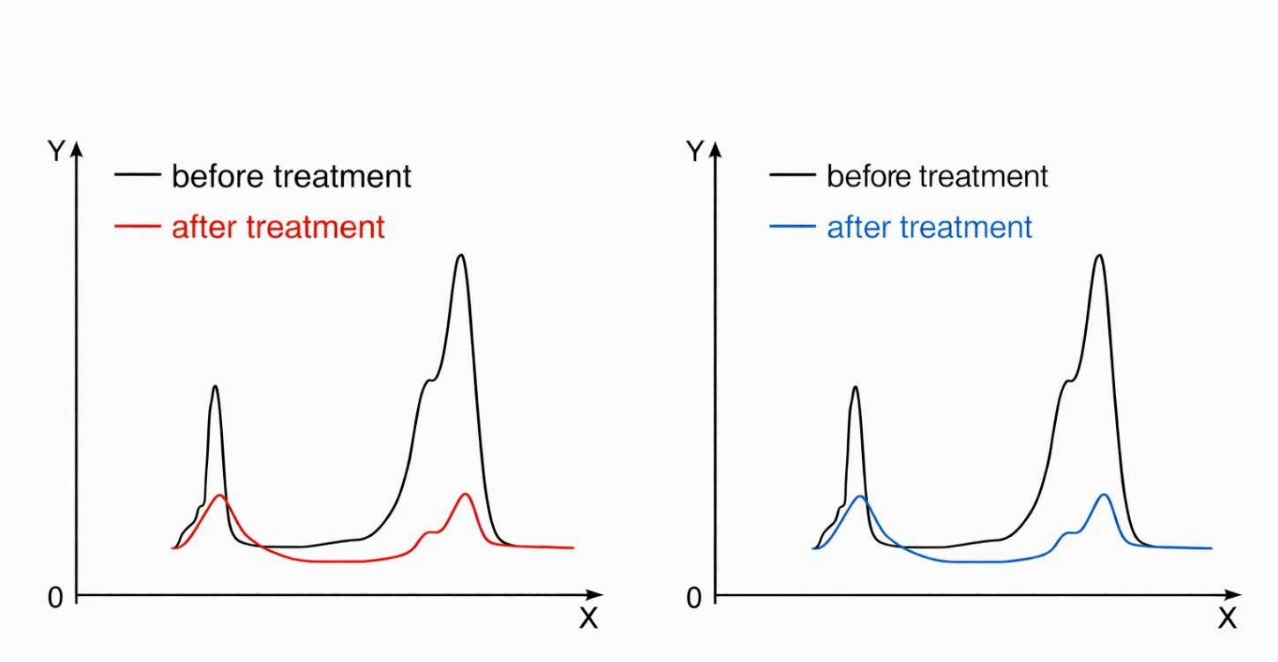

Figure-5: UV-Vis Spectrophotometry analysis of Methylene blue dye before and after treating With halophilic bacteria *Virgibacillus salexigens*

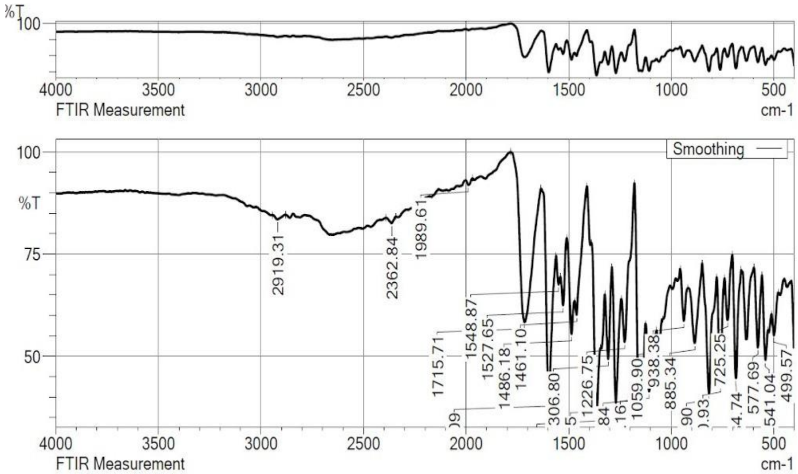

Figure-6: FTIR analysis of Methyl red dye before treating

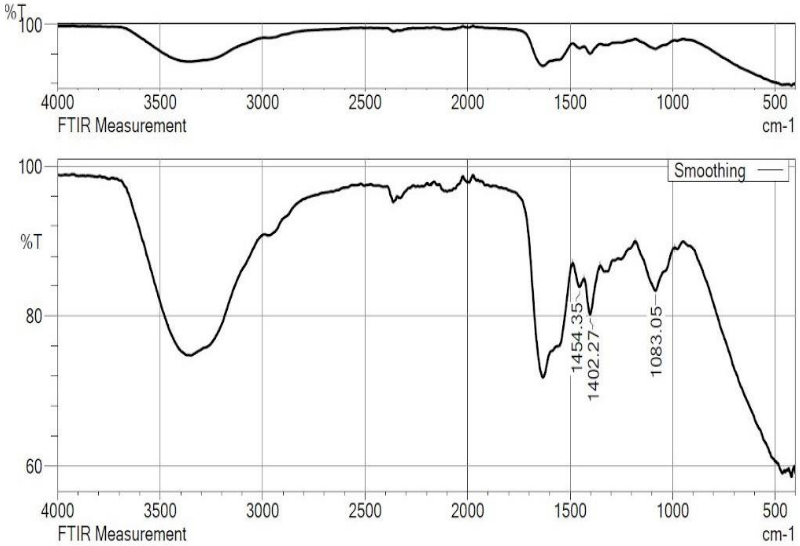

Figure-7: FTIR analysis of Methyl red dye after treating with halophilic bacteria *Virgibacillus salexigens*

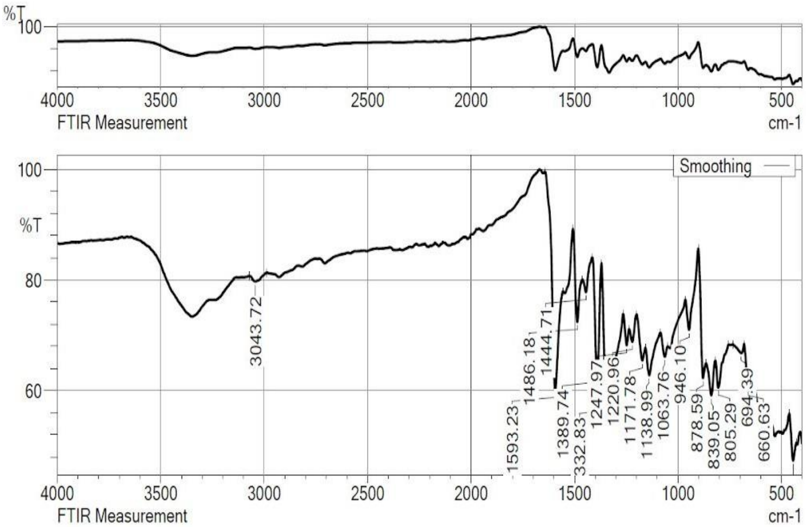

Figure-8: FTIR analysis of Methylene Blue dye before treating

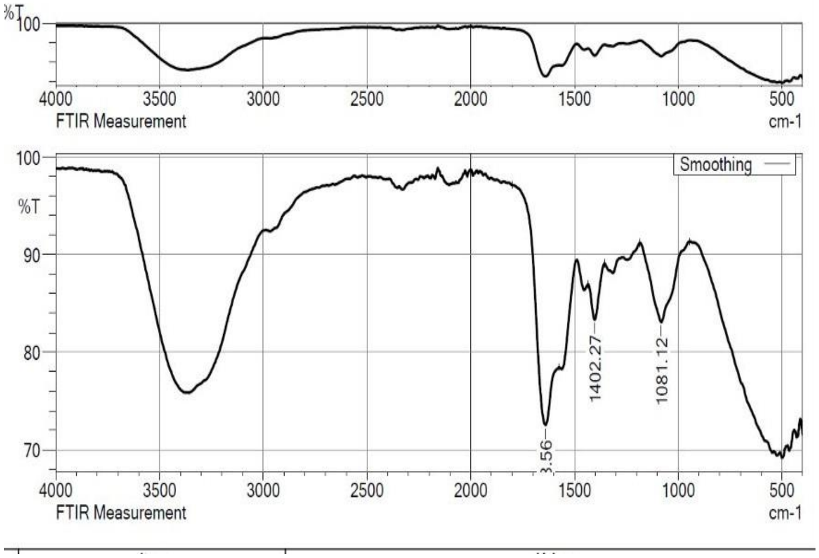

Figure-9: FTIR analysis of Methylene Blue dye after treating with halophilic bacteria *Virgibacillus salexigens*

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