



Innovative Biodegradation of Methylene Blue Dye Coupled with Bioelectricity Generation by *Bacillus cereus* SVN1

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Abstract

This study investigates the capacity of the ligninolytic bacterium *Bacillus cereus* SVN1 to degrade methylene blue dye while generating electricity in a microbial fuel cell (MFC). Screening techniques confirmed *Bacillus cereus* SVN1, demonstrating its ability to decolorize methylene blue and produce lignin peroxidase enzyme. Quantitative analysis revealed that the optimal dye concentration for MFC operation was 40 mg/L, with a decolorization rate of 61.21%. During MFC operation, the highest power density achieved was 0.1455 W/m² at the 30th hour, with a 52% decolorization rate by the 48th hour. UV-vis spectrophotometer analysis confirmed dye degradation by SVN1. Despite slightly lower decolorization rates compared to other studies, SVN1 ability to degrade methylene blue while generating electricity underscores its potential for sustainable wastewater treatment and bioenergy production. These findings shed light on the simultaneous processes of dye degradation and electricity generation in MFCs using ligninolytic bacteria, advancing our understanding of their environmental remediation capabilities.

Keywords: Methylene blue, Decolorization, Biodegradation, *Bacillus cereus*, Microbial Fuel Cell, Bioremediation, Wastewater treatment.

Introduction

The textile industry is a major contributor to environmental pollution, particularly through the discharge of wastewater containing a complex mixture of toxic recalcitrant dyes, dissolved solids, colorants, and heavy metals. Among these pollutants, dyes are the primary source of contamination in water bodies. Annually, approximately 280,000 tons of dyes are released into aquatic environments worldwide, posing significant ecological and health risks (Kishor et al., 2021). Methylene Blue (MB), a widely used dye in various industries including textiles, tannery, plastics, cosmetics, food, paper, and medicine, is of particular concern due to its persistence and potential toxicity (Luo et al., 2020). MB is a synthetic, recalcitrant, and heterocyclic aromatic dye widely used in the dyeing of cotton, wool, leather, and paper, leading to the release of approximately 70% of unfixed dyes into water bodies such as rivers, lakes, streams, and ponds. Additionally, Methylene Blue (MB) is widely used in microbiological laboratories for several purposes. It acts as an antifungal agent, preventing the growth of fungi in cultures. Furthermore, MB serves as a crucial staining agent, aiding in the classification and identification of microorganisms under a microscope. Beyond these primary uses, MB finds application in a variety of other laboratory techniques and procedures, demonstrating its versatility and importance in microbiological research and diagnostics (Bharti et al., 2019).

Wastewater containing MB dye has detrimental effects on water and soil ecosystems, as well as living organisms. In aquatic systems, MB dye reduces photosynthetic activity and decreases dissolved oxygen (DO) levels, leading to elevated biochemical oxygen demand (BOD) and chemical oxygen demand (COD) values. This disturbance negatively impacts both aquatic fauna and flora. In soil, MB dye accumulates over time, increasing soil salinity and disrupting microbial communities, contributing to soil pollution. Furthermore, MB dye poses carcinogenic, mutagenic, and allergenic risks to all life forms (Bharti et al., 2019; Kishor et al., 2021). Consequently, wastewater containing MB dye must be thoroughly treated and detoxified before being discharged into the environment (Bilińska et al., 2019; Sun et al., 2020).

Traditional methods for dye removal, such as chemical oxidation, coagulation, and adsorption, often face limitations related to cost, efficiency, and secondary pollution. Various physico-chemical methods, including adsorption, coagulation, flocculation, and oxidation, have been reported for wastewater treatment. However, these methods typically convert pollutants from one form to another and generate large amounts of toxic sludge, posing significant environmental and public health risks (Bharti et al., 2019; Bilińska et al., 2019). Membrane treatment technologies, such as nanofiltration (NF), reverse osmosis (RO), microfiltration (MF), and ultrafiltration (UF), have also been used for wastewater treatment (Upendar et al., 2017). Despite their effectiveness, these methods require high pressure, expensive materials, and produce undesired by-products (sludge). Advanced oxidation processes (AOPs), including fenton, photo-fenton, photocatalytic, sonocatalytic, electro-coagulation, and electro-oxidation methods, are known for their rapid and efficient treatment of dyes (Luo et al., 2020). However, they are often costly, may result in incomplete mineralization, and can generate carcinogenic and mutagenic intermediates. Consequently, there is a growing interest in developing sustainable

and effective bioremediation strategies to treat dye-contaminated wastewater. Biodegradation, which utilizes the metabolic capabilities of microorganisms, presents a highly promising solution for the removal of synthetic dyes from wastewater. This process involves harnessing various bacteria, fungi, and algae to break down complex dye molecules into simpler, less harmful compounds. Through enzymatic reactions, these microorganisms can effectively degrade and detoxify a wide range of synthetic dyes, converting them into environmentally benign substances. Biodegradation not only offers an eco-friendly alternative to traditional chemical and physical methods but also ensures that the treated water is safer for discharge into natural water bodies or for reuse. The adaptability and efficiency of microbial communities in degrading diverse dye structures underscore the potential of biodegradation as a sustainable approach to wastewater treatment. Recent studies have shown that various microorganisms can decolorize a broad range of textile dyes, with bacteria demonstrating significant potential due to their adaptability and diverse metabolic pathways. While fungi, yeasts, and algae have been explored for their potential in azo dye decolorization, the effectiveness of fungal decolorization is often hindered by several limitations. Fungal decolorization processes tend to be relatively slow and are usually confined to specific fungal species capable of breaking down azo dyes. Furthermore, these fungi often fail to target and decompose the chromophore center of the dye molecule, which is the part responsible for its color. This incomplete degradation can result in insufficient decolorization and the persistence of potentially harmful dye residues in the treated water. Due to these constraints, fungal-based methods are generally considered unsuitable for large-scale industrial applications, where rapid and comprehensive dye removal is essential. As a result, alternative microbial or chemical methods are typically preferred for the efficient and effective treatment of dye-contaminated wastewater on a commercial scale (Kishor et al., 2021).

In contrast, bacterial decolorization offers a higher degree of color removal and complete mineralization of dyes under optimal conditions (ARIFFIN and ANUAR, 2022). Bacteria have emerged as effective candidates for dye decolorization by breaking down organic pollutants and utilizing them as carbon and energy sources for growth. Many bacterial strains exhibit high tolerance to extreme toxic conditions and have short growth cycles, making them highly efficient for this purpose. Different microbial biomasses, whether live or dead, under various conditions (aeration, temperature, pH, salinity, and pressure) have shown significant contributions to dye degradation (Karim et al., 2018). Several bacterial genera, including *Bacillus*, *Pseudomonas*, *Staphylococcus*, *Acinetobacter*, and *Raoultella*, as well as fungi like *Aspergillus* and *Galactomyces*, have demonstrated the ability to degrade methylene blue (MB) (Pham et al., 2022). These microbial groups are capable of complete dye mineralization, making them promising agents for sustainable and effective bioremediation strategies in dye-contaminated wastewater.

Microbial fuel cells (MFCs) offer a sustainable solution to the problem of dye pollution by utilizing bacteria to degrade dyes and generate electrical energy simultaneously. The degradation of dyes in MFCs is achieved through the activity of the microbial community on the anode. The bacteria on the anode use the

organic matter in the dye as a carbon source and release electrons to the electrode, which generates electrical energy. The microbial community can be optimized to degrade specific types of dyes by selecting appropriate bacterial species and adjusting the operating conditions of the MFC. Several studies have investigated the use of MFCs for the degradation of different types of dyes. MFCs offer several advantages over conventional methods of dye degradation. Firstly, MFCs are a sustainable technology that generates electrical energy while degrading pollutants, which can help to offset the costs of wastewater treatment. Secondly, MFCs can be used to treat dye-contaminated wastewater directly, without the need for pre-treatment or additional chemicals. Thirdly, MFCs can be used to degrade a wide range of pollutants, including dyes, without the need for specific enzymes or catalysts. The use of MFCs for the degradation of dyes offers a sustainable and efficient solution to the problem of dye pollution. The technology is still in its early stages of development, and further research is needed to optimize the performance of MFCs for the degradation of different types of dyes and to scale up the technology for industrial applications. Nevertheless, the potential of MFCs for the treatment of dye-contaminated wastewater is promising and could contribute to a more sustainable and cleaner environment (Santoro et al., 2017).

This study aims to investigate the capability of *Bacillus cereus* SVN1 in simultaneously degrading Methylene Blue dye and generating electricity. By integrating biodegradation with MFC technology, this research seeks to offer a sustainable and cost-effective method for wastewater treatment. The findings from this study could pave the way for innovative applications in environmental biotechnology, combining the benefits of pollution mitigation with energy recovery.

2. Methodology

2.1 Microorganism

The bacterium SVN1 was obtained from the Genetic Engineering and Regenerative Biology Laboratory, Department of Biotechnology, Manonmaniam Sundaranar University for use in this biodegradation study. It was maintained on luria bertani (LB) agar plates.

2.2 Qualitative assessment of Methylene blue dye decolorization

Bacterial strain was inoculated onto an LB-MB modified agar plate and incubated at 30 °C to assess its potential for decolorizing MB dye. A clear decolorization zone formed around the colony, demonstrating the strain's ability to decolorize the MB dye (Wu et al., 2022).

2.3 Screening for lignin peroxidase enzyme production

To screen for lignin peroxidase enzyme activity in the isolated bacterium SVN1, an LB agar medium supplemented with 0.002% w/v Azure B dye was prepared. The bacterial culture was carefully streaked onto the prepared LB agar plates and then incubated for 72 hours at a constant temperature of 30 °C. Following the incubation period, the plates were examined for signs of decolorization in the medium surrounding the bacterial colonies. The presence of a clear, decolorized zone around the colonies indicated the activity of lignin peroxidase enzyme produced by bacterium SVN1 (Kameshwar et al., 2017).

2.4 Molecular identification

The genomic DNA of bacterium SVN1 was isolated using a HiPura bacterial genomic DNA purification kit. To amplify the 16S rRNA gene, universal primers 27 F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492 R (5'-CGGTTACCTTGTTACGACTT-3') were utilized. The PCR reactions included denaturation, annealing, and extension steps. The resulting PCR product was sequenced with a DNA analyzer (Applied Biosystems, model no. 3730xl), and the obtained sequences were analyzed using BLAST on the NCBI website (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to identify the closest homologous bacteria (Altschul et al., 1997).

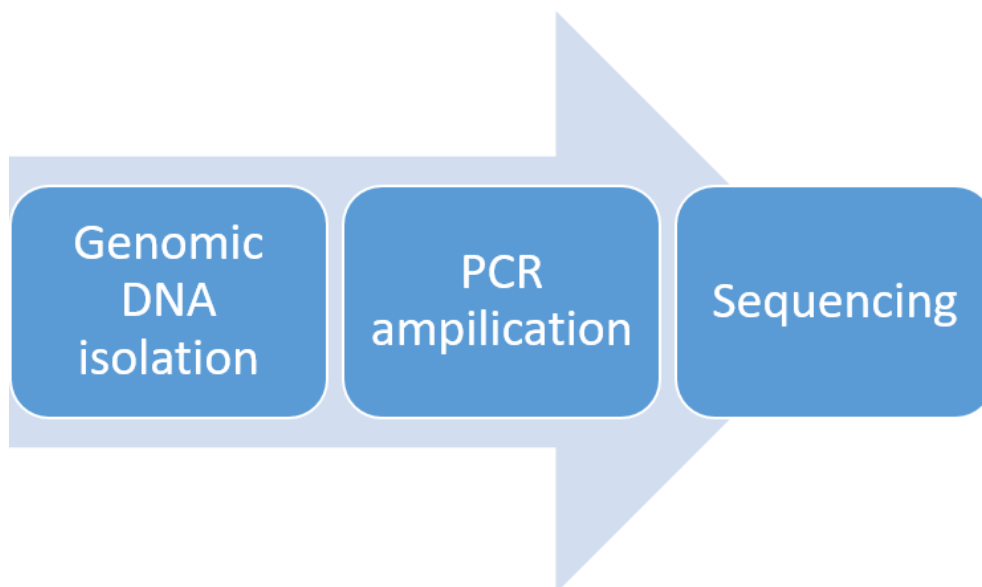


Fig. 1 Workflow for the 16s rRNA sequencing

For phylogenetic analysis, sequence alignment was initially performed using the Clustal W tool. A phylogenetic tree was subsequently constructed using Molecular Evolutionary Genetics Analysis (MEGA) software, employing the neighbor-joining method for the analysis (Poomani et al., 2023; Saitou and Nei, 1987; Tamura et al., 2004). The sequences were submitted to the GenBank database to obtain the accession number for bacterium SVN1.

2.5 Quantitative analysis of MB dye decolorization

The decolorization experiment was conducted using a 150 mL Erlenmeyer flask containing 50 mL of NaCl-Yeast extract medium composed of NaCl (4 g/l), and yeast extract (5 g/L) (Bharti et al., 2019). SVN1 was cultured in 50 mL of Luria-Bertani (LB) broth at 30 °C for 18 hours to serve as the inoculum for subsequent decolorization studies. The bacterial cell suspension was then inoculated into 50 mL of sterile decolorization medium containing 40 mg/L of methylene blue (MB) dye to evaluate the decolorization capability of SVN1. After the decolorization process, 2 mL aliquots were extracted from the sample and centrifuged at 10,000 RPM for 10 minutes at 4 °C for further analysis (Wu et al., 2022). This was assessed based on the capacity for dye decolorization. The supernatant was collected for measuring the absorbance in double beam UV-vis spectrophotometer at $\lambda_{\max} = 665$ nm, and the decolorization percentage of MB was calculated according to the following formula:

$$\text{Decolourization (\%)} = [(A_0 - A_t)/A_0] \times 100$$

Where A_0 is the absorbance value of the initial dye concentration, and A_t is the absorbance value of the dye concentration at time t (Kishor et al., 2021).

2.6 Effect of different concentration of MB dye on decolourization

For analysis the effect of different concentration dye decolorization, the bacterial strain SVN1 was inoculated into test tubes containing NaCl-Yeast extract medium supplemented with different concentrations of methylene blue (MB) dye. The specific dye concentrations selected for this study were 20 mg/L, 40 mg/L, 60 mg/L, 80 mg/L, and 100 mg/L. After the bacterial inoculation, the test tubes were incubated at a temperature of 35 °C for a period of 48 hours.

2.7 UV–vis spectroscopy study

The Biodegradation of MB was investigated using UV-visible spectrophotometer. Following the decolorization experiment, a 2.0 ml aliquot of the solution was extracted from the flasks. This aliquot was then centrifuged at 10,000 RPM and a temperature of 4°C for 10 minutes to separate any solid particles from the solution. The extent of decolorization was measured by analyzing the absorbance of the centrifuged samples over a wavelength range of 400 to 800 nm using the UV–vis spectrophotometer. This spectral analysis allowed for precise monitoring of the reduction in dye concentration, confirming the progress and effectiveness of the decolorization process (Kishor et al., 2021).

2.8 MFC construction and electricity generation

A dual chambered microbial fuel cell (MFC) was constructed for the current study using glass bottles with a total capacity of 500ml each for the anode and cathode chambers. Each chamber had two openings: one for electrode insertion and another for connecting the proton exchange membrane, which was made using an agar salt bridge composed of Potassium chloride, agar, and water. For its preparation, 50 ml of 1M KCl solution was mixed with 3% agar and boiled for 10 minutes. The mixture was then poured into a glass rod and allowed to solidify before being soaked in 1M KCl solution overnight. The agar salt bridge was then tightly fitted into the chamber and sealed with parafilm to prevent leakage. Graphite sheets served as the anode and cathode, connected by copper wires and a digital multimeter for measuring electrical parameters. The anolyte consisted of 200 ml NaCl-Yeast extract medium with 40 mg/l Methylene blue dye, while the catholyte comprised 200 ml of 50mM Potassium ferricyanide in 50mM Phosphate buffer. The anode chamber was aseptically inoculated with 1 OD of overnight-grown SVN1 culture, and the setup was operated at room temperature under static conditions for 48 hours to assess both electricity generation and dye decolourization (Thiruppathi et al., 2021).

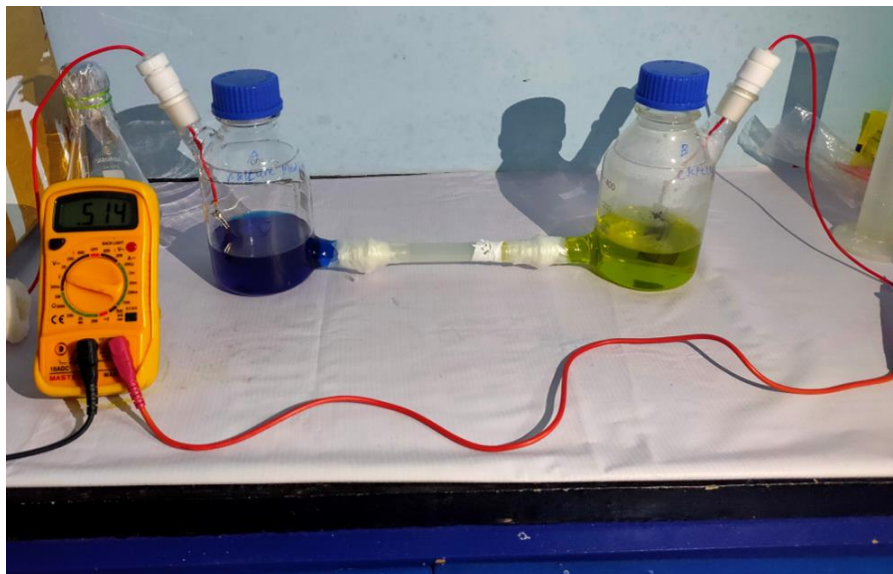


Fig 2 Microbial Fuel Cell setup

Open circuit voltage, indicating the potential difference between the two chambers, and current, indicating the flow of electrons from anode to cathode, were measured using a digital multimeter. Power density is the measure of power output obtained per unit area of the anode. Power density obtained during operation was calculated using the below mentioned formula (Choudhury et al., 2021).

$$\text{Power density (W/m}^2\text{)} = \text{Current} * \text{voltage} / \text{anode surface area}$$

3. Result and discussion

This study explores the dual capability of *Bacillus cereus* SVN1 to degrade methylene blue dye and generate electricity in a Microbial Fuel Cell (MFC), offering a novel approach to sustainable wastewater treatment and bioenergy production. The research examines various aspects, including bacterial performance, dye degradation efficiency, electricity generation, and comparative analysis with other studies.

3.1 Initial screening for MB dye degradation and ligninolytic activity

The SVN1 bacteria (Fig. 3 (C)) which isolated from mangrove soil samples is used for the present study. Initially the SVN1 bacteria, was revealed from the glycerol stock and maintained in LB agar plate (Fig. 3(A)). The initial analysis of MB dye degradation involved plating the strains on a new LB agar medium with methylene blue (25 mg/l), followed by a 3-day incubation at 35 °C (Kishor et al., 2021). After the incubation period, the SVN1 bacteria (Fig. 3 (B)) exhibited significant decolorization near bacterial growth, indicating MB degradation and ligninolytic activity.

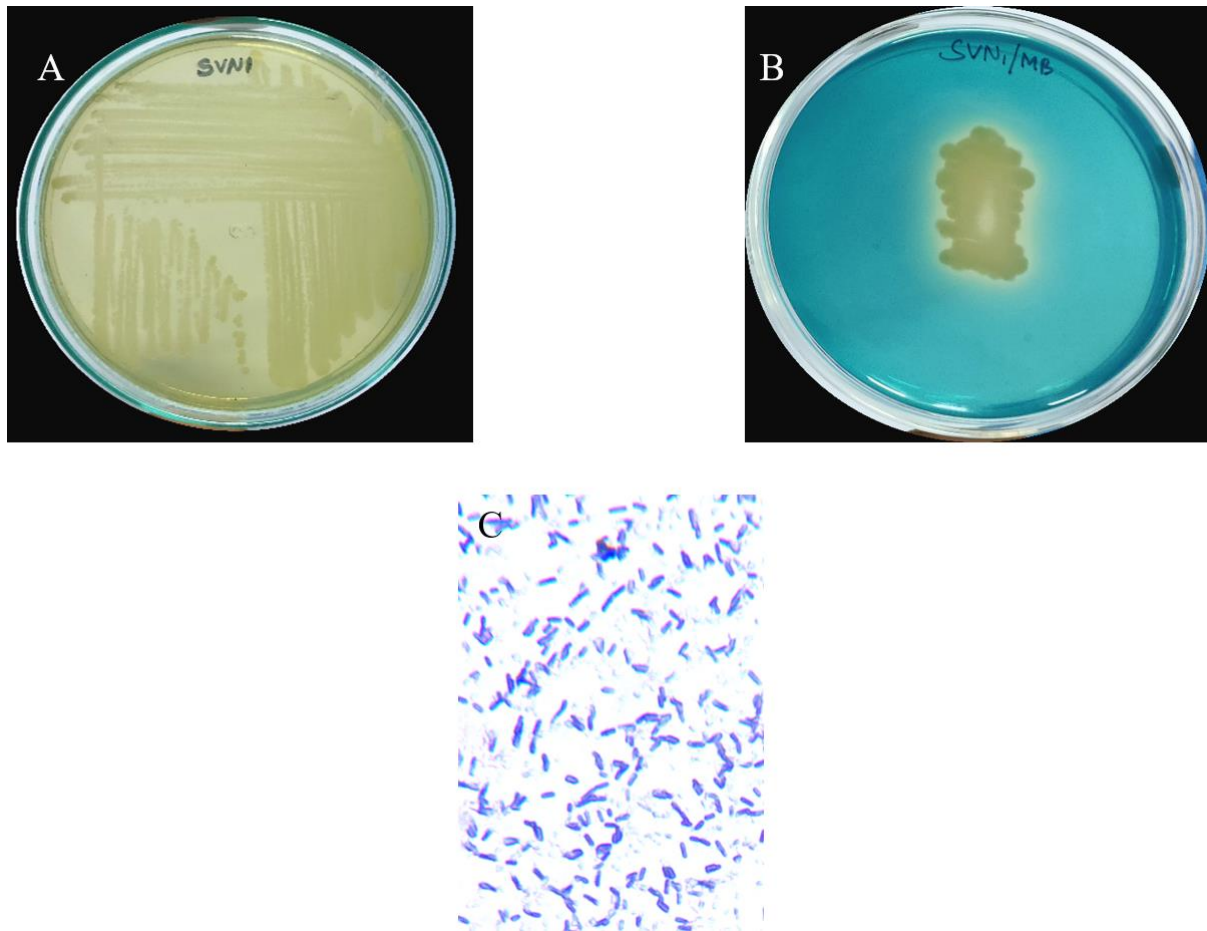


Fig. 3(A) SVN1 on LB agar, Fig. 3(B) Dye decolorization of SVN1 on MB incorporated LB agar, Fig. 3(C) Gram staining image of SVN1

3.2 Screening for ligninolytic enzyme activity

The screening of ligninolytic enzyme production, specifically lignin peroxidase, by the SVN1 strain included a screening process using azure B. Examination of the azure B-incorporated plate (Fig. 4) revealed the emergence of a clearly defined clearance zone around the bacterial growth. The decolorization of azure B dye indicating the production of lignin peroxidase by the bacterial strain (Kameshwar et al., 2017). The production of lignin peroxidase enzyme by SVN1 plays a crucial role in the biodegradation process, breaking down complex dye molecules into simpler, non-toxic compounds. This enzymatic activity underscores the potential of SVN1 in bioremediation applications, particularly in environments contaminated with synthetic dyes.

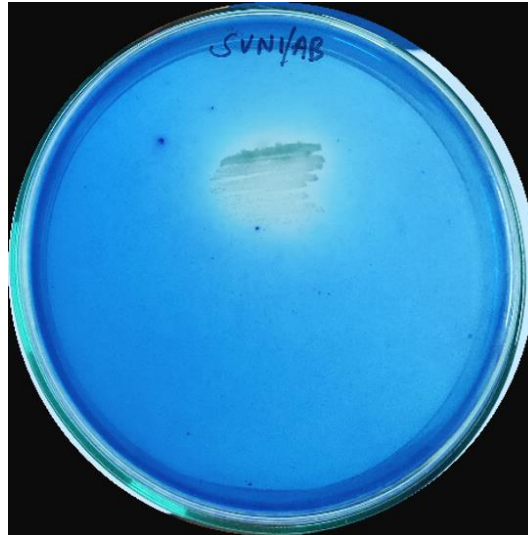


Fig 4 Azure B incorporated plates by SVN1

3.3 Phylogenetic analysis of SVN1

To determine the phylogenetic classification of strain SVN1, a detailed analysis of the 16S rRNA gene sequence, which is 1477 base pairs long, was conducted. The gene sequence was compared to known sequences in the database, revealing exceptionally high similarities with several *Bacillus cereus* strains. Specifically, the sequence showed a 99.86% similarity to both *Bacillus cereus* ATCC 14579 and *Bacillus cereus* CCM 2010. To further validate these findings, a neighbor-joining phylogenetic tree was constructed (Figure 5). This tree clearly positioned strain SVN1 within the cluster of the *Bacillus* genus, which corroborates its classification as *Bacillus cereus*. The 16S rRNA gene sequence of strain SVN1 was subsequently submitted to the NCBI GenBank database, where it was assigned an accession number for future reference and accessibility. The obtained the accession number was PP859884.

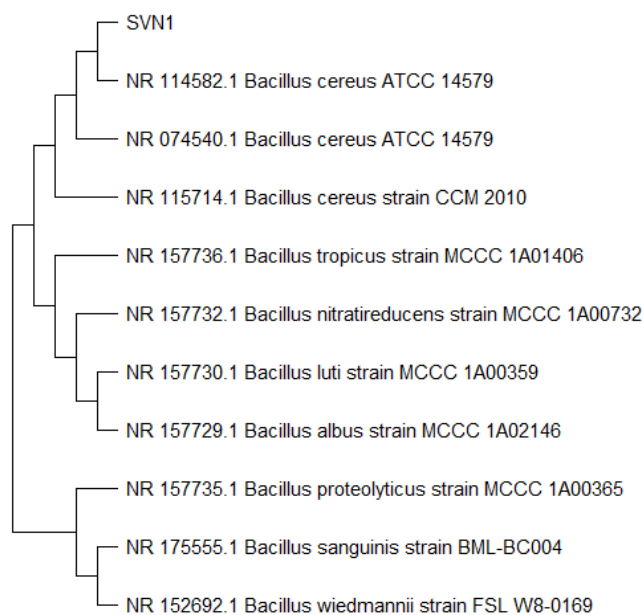


Fig. 5 Phylogenetic tree for SVN1 bacterial strain

It is notable that *Bacillus cereus* is capable of degrading various classes of organic compounds. For instance, a *Bacillus cereus* strain has been demonstrated to break down the reactive textile dye Novacron Super Black G, along with other aromatic compounds such as anthracene. In contrast to the numerous reports on *Bacillus cereus*'s ability to degrade different organic pollutants, there is only one recent study highlighting the use of immobilized *Pseudomonas guariconensis* for degrading the Reactive Red 120 dye (Alhefeiti et al., 2021; Reddy and Osborne, 2020).

3.4 Optimal Dye Concentration

The decolorization and degradation of methylene blue (MB) have been reported in green algae, fungi, and bacteria. Fungi remove dyes from wastewater through biosorption and biodegradation; biosorption involves interactions between dyes and functional groups on the fungi, while extracellular oxidoreductases catalyze the breakdown of complex dye molecules. Green algae use functional groups on their cell wall surfaces to bind dye molecules, enhancing adsorption during the decolorization process. Bacterial degradation of dyes involves oxidoreductase systems and other enzymes. Compared to fungi and algae, bacteria are more widely used due to their high activity, adaptability, and efficient degradation rates. This positions *Bacillus cereus* SVN1 as a highly effective agent for wastewater treatment and electricity production (Wu et al., 2022).

The ability of various microorganisms to decolorize methylene blue (MB) has been well-documented. Fungi, such as *Phanerochaete chrysosporium*, can decolorize 84.8% of 400 mg/L MB within 22 days. Green algae, specifically *Desmodesmus* sp., achieve a 98.6% decolorization rate of 20 mg/L MB in 6 days. Among bacteria, *Escherichia coli* decolorizes 71.26% of 50 mg/L MB in just 5 hours. *Arthrobacter globiformis* manages a 95% decolorization rate of 0.2 mM MB in 72 hours. *Bacillus subtilis* MTCC 441 can decolorize 91.68% of 20 mg/L MB in 6 hours, while *Bacillus thuringiensis* achieves a 95% decolorization rate of 25 mg/L MB in 70 hours. *Alcaligenes faecalis* decolorizes 96.2% of 150 ppm MB in 5 days, and *Bacillus albus* shows a remarkable 99.27% decolorization rate of 100 mg/L MB in just 6 hours. These findings underscore the efficiency and rapid action of bacteria, particularly *Bacillus* species, in the decolorization of methylene blue dye compared to fungi and algae (Al-Fawwaz and Abdullah, 2016; Wu et al., 2022).

The dye decolorization efficiency of *Bacillus cereus* SVN1 was evaluated at various concentrations of methylene blue (MB) (Fig. 6 and 7). At a concentration of 20 mg/L of MB, *Bacillus cereus* SVN1 achieved a decolorization percentage of 69.34%. When the concentration of MB was increased to 40 mg/L, the decolorization percentage decreased to 61.21%. At a further increased concentration of 60 mg/L, the decolorization percentage dropped significantly to 7.79%. As the concentration reached 80 mg/L, the decolorization percentage was 6.68%. Finally, at the highest tested concentration of 100 mg/L, *Bacillus cereus* SVN1 demonstrated a decolorization percentage of just 1.02%. The study determined that a methylene blue dye concentration of 40 mg/L was optimal for MFC operation, achieving a decolorization rate of 61.21%. This concentration likely provides a balance between sufficient substrate availability for bacterial metabolism and avoidance of toxic effects that higher dye concentrations might impose on bacterial activity. The efficiency at

this concentration indicates that *Bacillus cereus* SVN1 can maintain metabolic activity and decolorization capacity without being hindered by substrate toxicity.

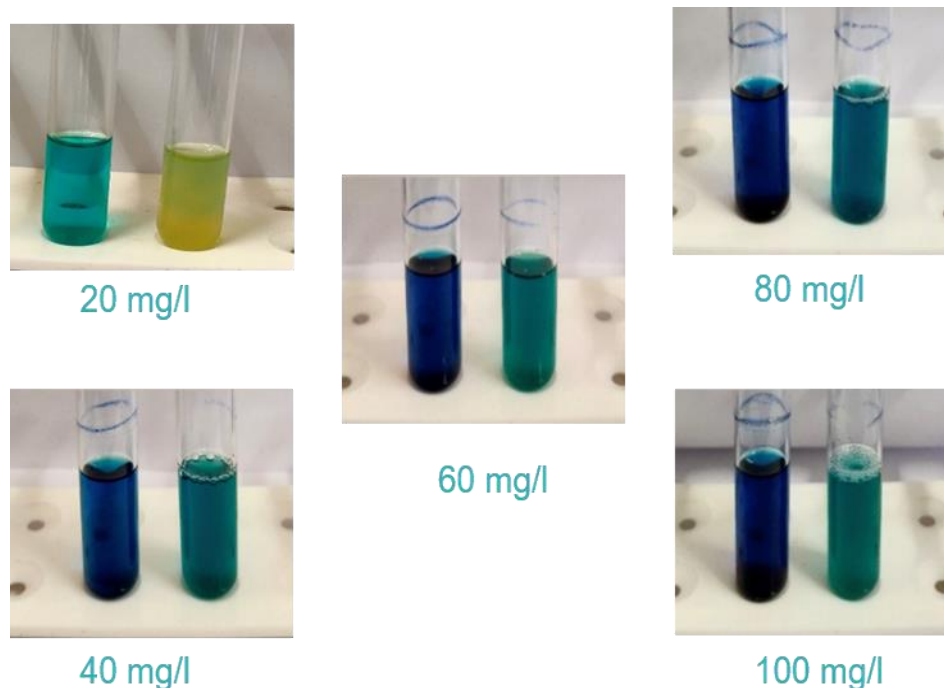


Fig. 6 Effect of different MB dye concentration

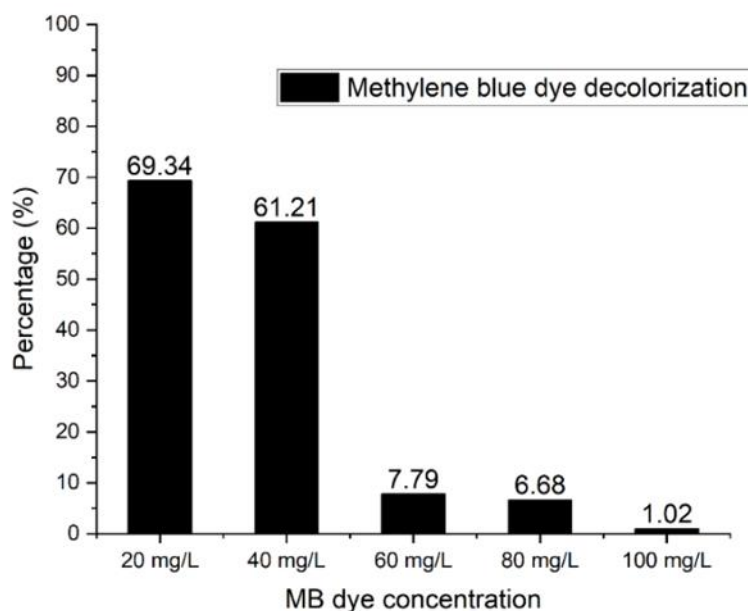


Fig. 7 Bar diagram for different concentrations of MB dye decolorization by SVN1

3.5 Simultaneous Electricity Generation and dye decolorization

The ability of *Bacillus cereus* SVN1 to concurrently generate electricity and degrade methylene blue dye represents a significant advancement in the field of microbial fuel cells (MFCs). The recorded highest power density of 0.1455 W/m² at the 30th hour (Fig. 8) of the experiment demonstrates the potential of SVN1 in bioelectricity generation. While this power density may appear modest compared to some other bacterial

strains, such as *Geobacter* sp. T32 and *Enterobacter cloacae* IIT-BT 08, which have reported higher power densities of 0.486 W/m² and 0.236 W/m² respectively, it's noteworthy that SVN1 possesses ligninolytic capabilities (Mohan et al., 2008; Nien et al., 2011). This aspect makes it particularly valuable for sustainable wastewater treatment applications. Additionally, the achieved decolorization rate of 52% by the 48th hour (Fig. 9) indicates the efficient degradation of methylene blue dye by SVN1. This dual functionality underscores the versatility of *Bacillus cereus* SVN1 in MFC applications, highlighting its potential for both environmental remediation and bioenergy production. Further research could focus on optimizing the operational parameters of MFCs to enhance the power output of SVN1 and its efficacy in dye degradation, paving the way for its practical implementation in wastewater treatment systems.

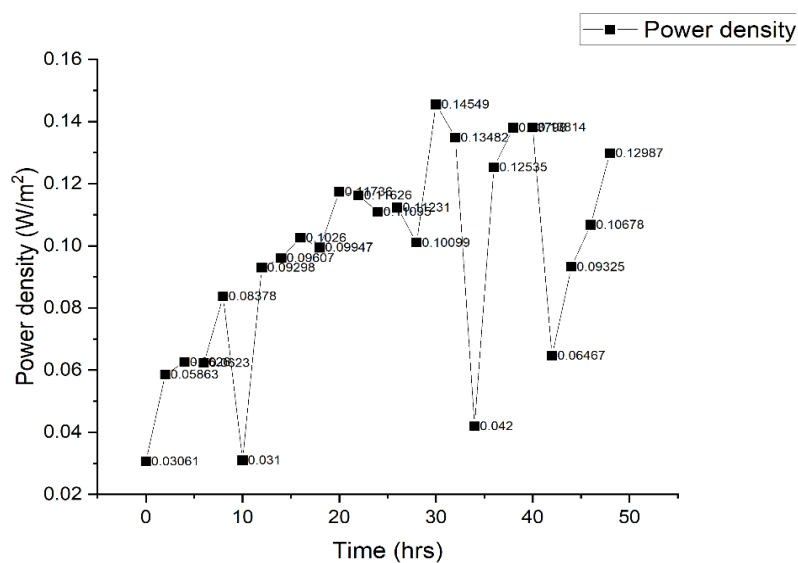


Fig. 8 Power density of SVN1 in MB dye incorporated medium

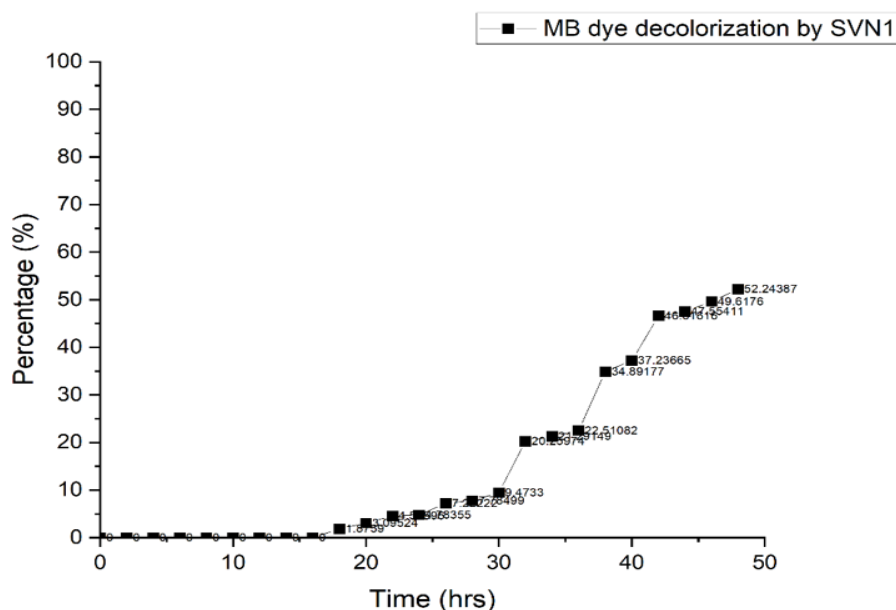


Fig. 9 MB dye decolourization of SVN1 during MFC operation

The electricity generation process in *Bacillus cereus* SVN1 is facilitated by its metabolic activities, which involve transferring electrons to the anode of the MFC, generating an electric current. This dual capability of dye degradation and electricity generation highlights the multifunctional potential of *Bacillus cereus* SVN1 in MFC applications, emphasizing its role in both environmental remediation and sustainable energy production.

3.5 Dye Degradation study through UV spectroscopy

UV-vis spectrophotometer analysis (Fig. 10) provided conclusive evidence of methylene blue dye degradation by *Bacillus cereus* SVN1. The reduction in absorbance at specific wavelengths corresponding to methylene blue confirmed the breakdown of the dye molecules. This analytical technique is essential for verifying the extent of dye degradation and ensuring that the process results in the removal of the dye from the solution.

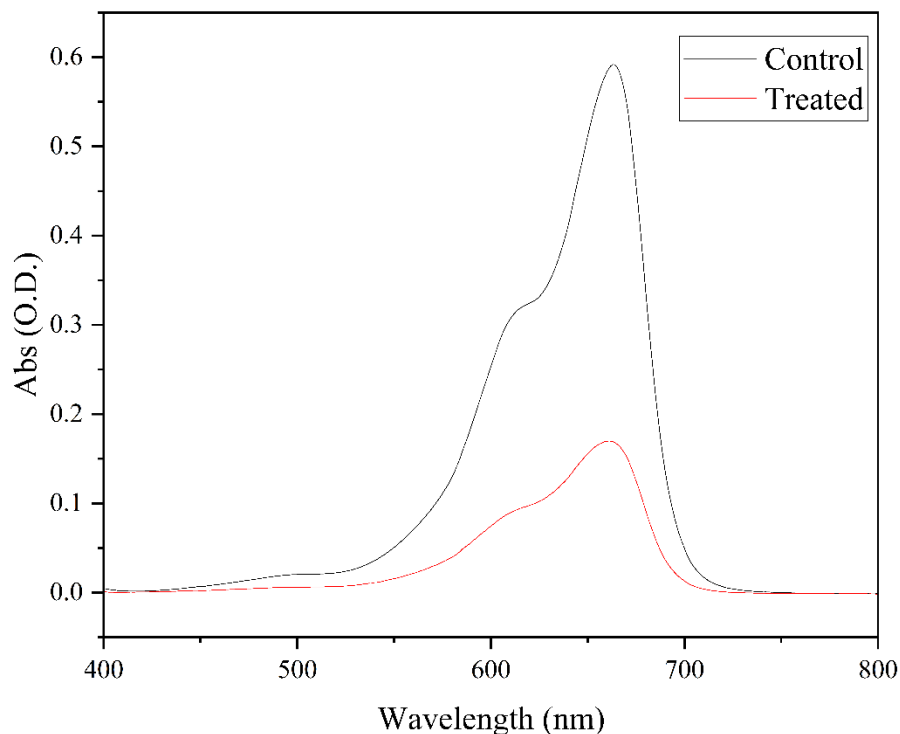


Fig. 10 UV Vis Spectroscopic analysis

On comparing to other studies, the decolorization rates achieved by *Bacillus cereus* SVN1 are slightly lower. However, it is important to note that the focus of this study is not solely on dye degradation but also on the concurrent generation of electricity. Other studies may have reported higher decolorization rates but did not explore the aspect of electricity generation. Therefore, the dual functionality of SVN1 in this study represents a significant advancement in the field of wastewater treatment and bioenergy production. The ability of *Bacillus cereus* SVN1 to degrade methylene blue dye while generating electricity has profound implications for sustainable wastewater treatment. Traditional wastewater treatment methods often involve energy-intensive processes and chemical additives. In contrast, the use of MFCs with ligninolytic bacteria like SVN1 offers a greener alternative, leveraging biological processes to achieve pollutant degradation and energy recovery

simultaneously. This approach aligns with the principles of sustainability and circular economy, where waste is not merely treated but also converted into useful by-products such as bioelectricity. Future research should focus on optimizing the operational parameters of MFCs to enhance both dye degradation and electricity generation. Investigating the effects of different dye concentrations, types of dyes, and environmental conditions on the performance of *Bacillus cereus* SVN1 can provide deeper insights into its capabilities. Additionally, scaling up the MFC system and integrating it with real wastewater streams will be crucial for assessing the practical applicability of this technology.

4. Conclusion

In conclusion, this study highlights the promising potential of *Bacillus cereus* SVN1 in the dual role of degrading methylene blue dye and generating electricity in microbial fuel cells (MFCs). With optimal decolorization at 40 mg/L and a peak power density of 0.1455 W/m², SVN1 demonstrates significant potential for sustainable wastewater treatment and bioenergy production. Phylogenetic analysis confirmed SVN1 as a *Bacillus cereus* strain, known for its robust biodegradation capabilities. Although decolorization rates were slightly lower compared to other studies, the concurrent electricity generation underscores SVN1's unique multifunctionality, offering a greener alternative aligned with sustainable and circular economy principles. These findings contribute to the growing body of knowledge on sustainable wastewater treatment technologies and pave the way for future innovations in the field. Future research should focus on optimizing MFC parameters and testing in real wastewater conditions to fully realize this technology's practical applications.

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