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Bioremediation of Methylene Blue Dye by a Soil Bacterium

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ABSTRACT

Biodegradation techniques employing microorganisms to remove industrial dyes are highly effective and environmentally friendly, and thus are being extensively explored. This research aims to evaluate the capacity of soil bacterium isolate a1 for degrading methylene blue (MB), specifically isolated from contaminated soil. The study was conducted in a laboratory setting using bacteria isolated from polluted soil. The focus was on assessing the influence of initial dye concentration and solution pH on the biodegradation process. The results demonstrated that an increase in the initial MB concentration from 50 to 400 mg/L resulted in a reduction in bacterial clearance efficiency from 90.41% to 71.28%. Additionally, the effect of pH on dye degradation was significant: raising the pH from 5 to 7 increased the percentage of methylene blue decolorization from 57.32% to 75.89%. However, further increasing the pH from 7 to 9 led to a decrease in decolorization efficiency from 75.89% to 46.17%. This study highlights the potential of soil bacterium isolate a1 for bioremediation of methylene blue in contaminated environments and underscores the importance of optimizing conditions such as dye concentration and pH to enhance biodegradation efficiency. The findings contribute valuable insights into the application of microbial bioremediation for industrial dye pollution.

INTRODUCTION

One of the textile dyes that is frequently utilized in the textile coloring business is methylene blue. Pollution of the environment may result from the release of textile wastewater into the environment that contains leftover textile coloring agents. Therefore, employing bacterial strains for bioremediation can help lower dye pollution [1]. In 1876, methylene blue was first created as an aniline-based dye for the textile industry [2]. However, researchers like Paul Ehrlich and Robert Koch soon realized that methylene blue could also be used as a stain for microscopy [3]. Aniline-based dyes have been tested against tropical diseases as a result of the finding of selective staining and inactivation of microbial species [3]. The first such substance to be given to people was methylene blue, which has been demonstrated to be useful in treating malaria [4].

Methylene blue was also the first antiseptic color to be used therapeutically and the first synthetic substance to be utilized as an antiseptic in clinical medicine. Actually, before sulfonamides and penicillin were developed, methylene blue and its derivatives were widely used [3]. Recent studies have shown that methylene blue exists as numerous distinct hydrates rather than as a trihydrate [5]. The Working Group observed that regardless of hydration state, the majority of scientific literature simply mentions "methylene blue." Commercial methylene blue is usually sold as the hydrate due to its hygroscopic nature, yet it is occasionally presented mistakenly as the trihydrate [6]. N,N-dimethyl-phenylenediamine is oxidized with sodium dichromate ($Na_2Cr_2O_7$) in the presence of sodium thiosulfate ($Na_2S_2O_3$) to produce methylene blue in the commercial sector [7]. This oxidation is then repeated in the presence of N,N-dimethylaniline. After adding 30% hydrochloric acid and a saturated common salt solution to the dye solution, methylene blue hydrochloride is separated; the product is then filtered and cleaned with a 2% common salt solution. Instead of sodium dichromate, manganese dioxide, and catalytic quantities of copper sulfate can be employed for the oxidation [2].

High-purity methylene blue can be made by acidifying the aqueous solution, isolating the dye, and then using chloroform to remove contaminants from raw dye solutions in borate buffer at a pH of 9.5–10 [2]. Methylene blue is a biological stain and disinfectant [7,3]. Methylene blue is marketed to end users as an aquarium fungicide and disinfectant [8]. Methylene blue has most recently been employed in photoelectrochromic imaging, as

an optical probe in biophysical systems, as an intercalator in nanoporous materials, and as a redox mediator [7]. In addition to coloring paper and office supplies, methylene blue is also used to lighten the colors of silk [2]. Methylene blue is used in analytical chemistry to identify anionic surfactants, sometimes known as "methylene blue active substances" [9]. Redox indicator reagents and pH indicators also contain methylene blue [10].

The food processing, paper, dye manufacture, leather, textile, and dyeing sectors all produce hazardous dye effluents that are a major cause of pollution for living things. Decolorization of dyes is thus a crucial step in wastewater treatment before to disposal. It is necessary to separate, characterize, and assess the function of bacteria as a biological instrument for dye bioremediation.

MATERIALS AND METHODS

Sampling location

Samples of dirt polluted with engine oil were collected from the mechanic's location at mile 3 in the Nigerian state of Gombe. The samples were stored in sterile plastic bags until they were needed. After that, samples were delivered to the Gombe State University department of biochemistry laboratory, where the study was conducted.

Making the methylene blue solution

In order to prepare a stock solution with a concentration of 1000 ppm, precisely 1 gram of methylene blue powder is dissolved in 1000 milliliters of distilled water. The stock solution is subsequently diluted with double distilled water to obtain the required concentrations, which fall within the range of 50-400 ppm.

Growth Medium

The nutrient agar and broth were prepared following the instructions provided by the manufacturer and subjected to autoclaving at a temperature of 121°C for a duration of 15 minutes to guarantee complete sterilization. A total of five sets of petri dishes were employed for the purpose of isolating the bacterium. A volume of 1 milliliter of the inoculum with a concentration of 10^6 colony-forming units per milliliter (CFU/mL) was introduced into the petri plate. Place the sterilized agar nutrient medium onto the petri plate and distribute it evenly. The inoculum is evenly dispersed by rotating the petri dish, and this entire procedure is conducted under a UV lamp. After the media was made solid, each petri dish was placed in an incubator and kept at a temperature of 37 degrees Celsius for a period of 24 hours.

Nutrient agar and broth were prepared according to the manufacturer's instructions, typically involving the dissolution of 28 grams of nutrient agar powder in 1 liter of distilled water and 13 grams of nutrient broth powder in 1 liter of distilled water. The prepared media were thoroughly mixed and heated to dissolve completely. The pH of the medium was adjusted to 7.0 ± 0.2, ensuring optimal conditions for bacterial growth. Once prepared, both media were dispensed into appropriate containers and autoclaved at a temperature of 121°C for a duration of 15 minutes to ensure complete sterilization.

The inoculum was evenly dispersed across the agar surface using a sterile glass spreader, employing the spread plate technique to ensure uniform distribution of bacterial colonies. The incubation period lasted 24 hours, allowing sufficient time for the colonies to develop to a countable size, while preventing overgrowth that could result in confluent colonies and difficulty in isolation [13].

Bacteria Identification

After agar was removed, the bacteria cells were spread out and left to dry on a glass slide. The substance was heated gently using a Bunsen burner, without the use of a slide. After a one-minute interval of crystal violet staining, the specimen was washed with water. Acetone was used to eliminate the color from the stain until it was entirely free of pigmentation. After applying safarine as a counterstain, distilled water was used to rinse it. After the drying process was finished, the specimen was carefully scrutinized using a microscope. A 10 ml solution containing nutritional broth and a specific amount of dye (50–400 ppm) was sterilized using an autoclave at 15 lb pressure and 121°C for 20 minutes.

The purpose was to study the behavior of the bacterium in eliminating methylene blue. Subsequently, it was cooled and exposed to ultraviolet radiation. The study investigated the influence of different factors, such as the initial dye concentration (ranging from 50 to 400 ppm), on the percentage of decolorization and the pH of the solution (with values of 5, 7, and 9) at an initial concentration of 50 mg/l. The UV spectrophotometer was employed to assess each sample at a specific wavelength of 665 nm. The subsequent equation is employed to ascertain the proportion of decolorization:

$$\text{Percentage of dye decolorization } (\%) = \frac{A-B}{A} \times 100 \quad \text{(Eqn. 1)}$$

Where A is the beginning concentration and B is the final concentration.

RESULTS

Bacterial isolate characterization

The morphological trait of the bacterium is shown in **Table 1**.

Table 1. Morphological trait of the soil bacterium.

Test	Result
Cell shape	Rod shape
Gram staining	-
Spore	Non sporing

Methylene blue dye removal efficiency

Fig. 1 evaluates the impact of initial dye concentration on removal efficiency. The results demonstrate that as the initial dye concentration increased, the proportion of decolorization dropped. The percentage of decolorization dropped from 90.41 to 71.28% with an increase in the starting concentration of dye from 50 to 400 mg/L, correspondingly.

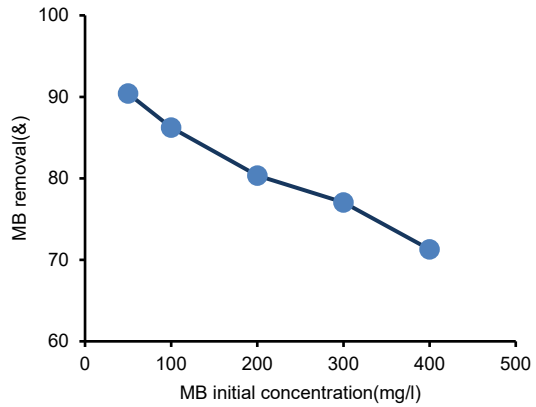

Fig. 1. The removal efficiency of MB by E.coli at different concentrations.

Effect of pH on MB removal

Fig. 2 illustrates the impact of pH on the extraction of methylene blue from its aqueous solution using *E. coli* and the outcomes that were attained. When the pH was raised from 5 to 7, the percentage of methylene blue decolorization first increased from 57.32 to 75.89%, then when the pH was raised from 7 to 9, it reduced from 75.89 to 46.17%.

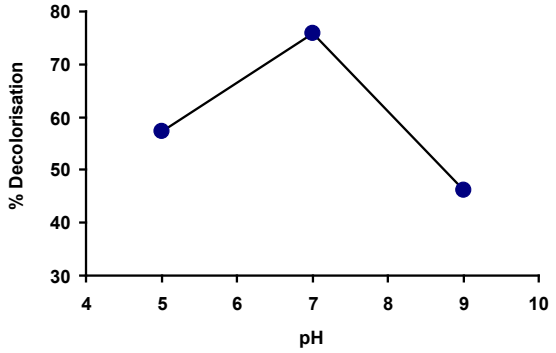

Fig. 2. Effect of the pH on the biodegradation of methylene blue (MB) dye at the initial MB concentration (50 ppm).

DISCUSSION

Fig. 1 examines the effect of initial dye concentration on removal efficiency, revealing a critical relationship between concentration and microbial decolorization activity. Similar patterns have been reported in the bacterial decolorization of various azo dyes, as detailed by [11]. High concentrations of azo dyes tend to suppress cell growth and inhibit nucleic acid synthesis, negatively impacting microbial activity and decolorization performance [12].

These findings emphasize the importance of considering dye concentration in practical applications, as it directly affects organismal growth and efficiency. Research by [13] demonstrates that increased dye concentrations reduce decolorization efficiency, likely due to the inhibitory effects on microbial enzymatic activity. Similar trends were observed in the microbial degradation of reactive azo dyes, further corroborating the impact of dye concentration on bioremediation processes [14-16]. Additionally, studies have identified a significant reduction in microbial activity when higher concentrations of reactive azo dyes contain sulfonic acid (SO3H) groups on their aromatic structures, which enhance their toxicity and environmental persistence [17,18]. This underlines the need for optimized strategies to manage dye concentrations in wastewater treatment.

Notably, [19] reported that employing bacterial co-culture systems rather than pure cultures alleviates the inhibitory effects associated with high dye concentrations. The synergistic interactions between different bacterial species in co-culture enhance enzymatic activities and microbial resilience, thereby improving overall decolorization efficiency. This finding highlights the potential of co-culture systems as a promising approach to address challenges posed by high dye concentrations in industrial wastewater, paving the way for more effective and sustainable bioremediation solutions.

Fig. 2 showcases the influence of pH on the extraction and decolorization of methylene blue from an aqueous solution using soil bacterium isolate a1. The analysis indicates that the decolorization efficiency is markedly affected by the medium's pH, with a peak efficiency observed at a neutral pH of 7. Under these conditions, the rate of color removal is significantly enhanced, whereas it diminishes at pH levels above 7. This suggests that the decolorization capability of isolate a1 is closely linked to its active metabolic processes and optimal cell growth conditions. The findings underscore that isolate a1 achieves its maximum decolorization performance at a neutral pH, outperforming its activity in both acidic and alkaline settings. Consequently, maintaining the operational pH at 7 is advantageous, as it aligns with the natural growth conditions of the bacterium and negates the need for additional pH adjustments or investments. This is in contrast to previous studies, such as [20], which recommended an optimal pH of 8 for similar processes.

Notably, the thriving state of isolate a1 at pH 7 not only facilitates efficient dye degradation but also supports the bacterium's robustness in neutral environments, which are less demanding in terms of pH control. A comparative analysis in **Table 1** highlights other microorganisms' methylene blue degradation capabilities, along with their unique characterizations, illustrating isolate a1's competitive advantage in neutral pH conditions for effective and sustainable dye removal.

Table 1. MB-degrading microorganisms.

Microorganism	Optimum pH	Optimum Temperature (°C)	Level of Methylene Blue Degraded	References
Escherichia coli	7	37	75.89% at 200 mg/L	[13]
Pseudomonas aeruginosa	7.5	30	98% at 100 mg/L	[14]
Bacillus subtilis	8	35	92% at 150 mg/L	[15]
Klebsiella pneumoniae	6.5	30	85% at 50 mg/L	[16]
Staphylococcus aureus	7	32	80% at 100 mg/L	[17]
Enterobacter cloacae	7	37	70% at 200 mg/L	[18]
Lactobacillus delbrueckii	6	30	65% at 200 mg/L	[19]
Aeromonas hydrophila	7.5	28	95% at 100 mg/L	[20]

The observations underscore the pivotal role of environmental parameters, particularly dye concentration and pH, in shaping the efficacy of microbial decolorization processes. The inverse correlation between dye concentration and removal efficiency corroborates earlier findings on azo dye degradation, which consistently reveal that elevated dye concentrations

adversely affect microbial growth and metabolic functions. This impact arises primarily from the toxic effects on cellular mechanisms, including nucleic acid synthesis and enzyme activity [21]. Such inhibition is especially pronounced in dyes possessing reactive groups like sulfonic acid, which exhibit strong interactions with microbial cells, further reducing decolorization efficiency.

In the context of wastewater treatment, these insights emphasize the necessity of optimizing dye concentrations to achieve a delicate balance—ensuring effective treatment while minimizing inhibitory effects on microbial communities. Additionally, the critical role of pH in influencing decolorization efficiency becomes evident, with marked declines observed at pH levels beyond the optimal range. This reinforces the notion that key enzymatic activities and cellular processes essential for dye breakdown are intrinsically pH-dependent [22].

Practical applications of these findings are significant. By maintaining an operational pH within the optimal range, microbial activity can be maximized, mitigating the need for frequent pH adjustments. This not only reduces costs but also enhances the sustainability of bioremediation processes. Moreover, these results provide valuable guidance for designing strategies that enhance microbial efficiency under varying environmental conditions. Such informed approaches are integral to advancing bioremediation technologies, ensuring they remain both effective and adaptable across diverse wastewater treatment scenarios..

CONCLUSION

The bacterium identified in this study is rod-shaped, Gram-negative, and non-spore-forming, showing considerable potential for efficiently removing methylene blue dye from aqueous solutions. Its decolorization capability is influenced by both the initial dye concentration and the pH of the solution. At a lower concentration of 50 mg/L, the bacterium achieved a high removal efficiency of 90.41%. However, as the dye concentration increased to 400 mg/L, the removal efficiency dropped to 71.28%, likely due to the inhibitory effects of higher dye concentrations on bacterial metabolic processes. The optimal pH for decolorization was found to be 7, with a removal efficiency of 75%. Conversely, in acidic conditions (pH 5) and strongly alkaline conditions (pH 9), the efficiency declined significantly. This suggests that the bacterium's dye-degrading enzymatic activity is most effective at neutral pH levels. Such findings underscore the importance of maintaining appropriate environmental conditions to optimize bacterial performance. These results have practical implications for the application of this bacterium in environmental remediation, particularly in treating dye-contaminated industrial wastewater. Adjusting the operational conditions, such as dye concentration and pH, can enhance the bacterium's efficiency, making it a valuable tool for sustainable and effective wastewater treatment. The study highlights the potential for deploying microbial systems to address pollution in a cost-effective and environmentally friendly manner.

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