Biodegradation of methylene blue from aqueous solution by bacteria isolated from contaminated soil

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ABSTRACT

The use of biodegradation methods by microorganisms in the removal of industrial dyes are widely considered owing to their high efficiency and compatibility to the environment. Therefore, this study aims to evaluate the biodegradation of methylene blue (MB) from aqueous solution by bacteria isolated from contaminated soil. This study was performed in laboratory scale on bacteria isolated and purified from contaminated soil with methylene blue. Initially, the bacteria was isolated from contaminated soil. Thereafter, medium containing 50, 100, 200, 400, 800 and 1000 mg/l of methylene blue, 50 ml of salt medium with glucose and 2.5 ml of Brain-heart infusion (BHI) medium containing bacteria were prepared. The results of dye removal were analyzed using UV/Vis spectrophotometer at 665 nm. The results of purification and identification of the bacterial species which degrade methylene blue indicated that Pseudomonas aeruginosa was the dominant bacteria. In this study, the removal efficiency of bacteria was attained from 82.25 to 97.82% with an increase in initial MB concentration from 50 to 200 mg/l. Nevertheless, with increase in MB concentration from 200 to 1000 mg/l, removal efficiency was reduced to 43.08%. The optimum concentration of MB removal was 200 mg/l. It is evident from the results that the bacteria had used methylene blue as an auxiliary source of carbon apart from glucose. Finally, it can be concluded that P. aeruginosa is an appropriate candidate for the removal of methylene blue from the environment.

Keywords: Biodegradation; Methylene blue; Bacteria; Environmental Pollution

Introduction

Nowadays, different types of pollutants and synthetic chemicals are encountered in the environment due to population growth and industrial development.¹⁻³ Synthetic dyes like of conventional wastewater treatment systems methylene blue (MB) are chemical pollutants which are widely employed in textile, paper, leather, cosmetic, plastic, printing and food industries.⁴⁻⁶ Textile industries consume large volume of water during dying process. Consequently, large volume of dyed wastewater is produced. This wastewater must be treated according to environmental regulations until dye concentration is reduced to an acceptable level in effluent.⁷,⁸ Dyed materials of industrial wastewaters are important due to toxicity on aquatic organisms, disorder in the performance and aesthetic environment. Given that
discharging dyed industrial wastewater in receiving water resources can lead to eutrophication and disturbance in ecology, removal of dye from wastewater and aquatic environments is essential for protection of human health and the environment. Conventional methods for the removal of dyed compounds such as MB include adsorption, photocatalyst, ozonation, biodegradation, coagulation and flocculation, ion exchange, chemical oxidation and electrochemical treatment. Physicochemical methods like coagulation and flocculation are expensive and discharge large amounts of sludge after treatment which must be safely disposed. Nevertheless, biological removal by microorganisms is widely employed due to cost-effective, easy operation, high efficiency, less sludge production and compatibility with the environment. Different groups of microorganisms such as bacteria, fungi, yeast, algae are capable of degrading dyes in aerobic and anaerobic conditions. Among these microorganisms, bacteria are more appropriate for dye removal due to their greater number, activity, compatibility and short growth cycle. In studies by El-sersy et al and Chen et al, different species of Bacillus thuringiensis were used for the biodegradation of MB. In another study by Noraini et al, Sphingomonas paucimobilis was used for the biodegradation of MB. The results showed that after 5 days retention time, 85% of dye was removed with 1000 mg/l concentration. According to the importance of removing MB from the environment, the aim of this study was to investigate the biodegradation of MB using isolated bacteria from contaminated soil.

**Materials and Methods**

This is an experimental study performed in laboratory scale. To identify growing microorganisms in the presence of MB, 2 kg soil was mixed with 5 g MB dye and water and then placed in environmental conditions for 20 days while only moisture was added (Figure 1). Thereafter, 1 g of contaminated soil with MB was mixed with 9cc Brain-heart infusion (BHI enriched medium and placed in an incubator at 30°C for 24 h.

**Purification and identification of MB degrading bacteria**

For purification and identification of bacteria, the samples from the Erlenmeyer flask including inoculum with different concentrations of dye and grown bacteria were inoculated on Eosin methylene blue (EMB) agar (for the growth of Gram-negative bacteria) and blood medium (for the growth of gram-negative and gram-positive bacteria) by a sterile loop. The plates were incubated for 24 h at 35°C, then the colonies were stained while catalase and oxidase tests were performed. To identify the bacterial species, colonies were inoculated in Indol test, Methyl red test, Voges-Proskauer test, Citrate utilization test (IMViC) and Oxidation and Fermentation (OF) glucose mediums, respectively. Finally, colonies were cultured in Mueller-Hinton agar to detect species.

**Batch MB removal experiments**

To determine the capability of the grown bacteria on medium for the removal of MB, salt medium containing 50 mg KCl, 100 mg KH₂PO₄, 50 mg MgSO₄-7H₂O and 300 mg K₂CO₃ was used. The salt medium attained a volume of 1 L and then was placed in an autoclave for sterilization. Thereafter, 5 g of glucose was added to salt medium and then 50 cc of this mixture medium was poured in250 cc Erlenmeyer flask. In the next step, to add MB, 1 g of stock solution was prepared and added to Erlenmeyer flask containing medium and glucose at concentrations of 50, 100, 200, 400, 800 and 1000 mg/l. Then, 2.5 cc from enriched medium including dye and contaminated soil was poured in each Erlenmeyer flask and placed
Results and Discussion
In this study, after the combination of 1 g of contaminated soil with 9 ml BHI enriched medium, the results revealed that the MB was removed while sludge was formed in the test tube (Figure 2).

Purification of bacterial species
Nowadays, the use of pure microbial cultures to remove environmental pollutants has gained significant recognition. Noraini et al (2015) and Chen et al (2013) used bacterial species for MB biodegradation. The results showed that no bacterium has grown in the blood medium; however, colonies have grown on EMB medium. Figure 3 shows grown bacteria on EMB medium. Figure 4 shows the results of gram-staining and indicates that the bacteria are rod-shaped bacillus.

![Fig.2 Grown colonies on EMB medium](image)

![Fig.3 Optical microscope image of rod-shaped Bacillus](image)

IMViC Tests
The study indicated positive result for Citrate utilization test, Methyl Red test was positive and Voges-Proskauer test was negative. Indol test (Sulfide-Indole-Motility), urease, glucose and lactose tests were also negative. These results indicated Non-fermenting Gram-negative Pseudomonas. Moreover, oxidase and catalase tests results revealed that the samples were positive for oxidase and catalase. Oxidative Fermentative (OF) test indicated that the genus of the grown bacterial strains in various concentrations is Pseudomonas. For detection of bacterial species, Mueller-Hinton agar medium was used. The results revealed that the species produced green pigment. According to this result, the species was aeruginosa. Therefore, according mentioned data, the intended bacteria was P. aeruginosa. Many bacteria have been studied for degradation and removal of dyed compounds from aquatic solutions. The results of biochemical tests showed that P. aeruginosa (a heterotrophic bacterium) is the only resistant species which can be involved in the biodegradation of cationic MB. Different studies have shown that P. aeruginosa is capable of degrading and removing various pollutants such as dyed compounds. Jing et al (2009) used isolated Pseudomonas otitidis from sludge of dyeing wastewater treatment plant for the degradation of triphenylmethane dye. Researches have shown that P. aeruginosa is capable of using azo dyes as the sole source of

![Fig.4 The removal efficiency of MB by bacteria at different concentrations](image)
carbon; these bacteria can break \(-N = N-\) bonds and use amines as carbon and energy sources.\(^{28}\)

**Methylene blue removal efficiency**

Evaluating the effect of dye concentrations on removal efficiency revealed that by increasing initial MB concentration from 50 to 200 mg/l, removal efficiency by \(P. \text{aeruginosa}\) increased from 82.25 to 97.82\% (Table 1 and Figure 5). With increase in the concentration of MB dye to 200 mg/l, \(P. \text{aeruginosa}\) used MB as an auxiliary source of carbon besides glucose and began to grow up and with the growth of this bacteria, removal efficiency was also increased. Furthermore, given that the color of bacterial biomass was blue, it can be concluded that both biodegradation and biosorption processes are involved in the removal of MB.\(^{22}\) However, removal efficiency was decreased by increasing the initial concentration of MB up to 200 mg/l. Removal efficiency decreased from 97.82\% at a concentration of 200 mg/l to 43.08\% at an initial concentration of 1000 mg/l of MB. This could be attributed to antibacterial properties of MB in high concentrations,\(^{29}, \ 30\) and decrease of microbial biomass in proportion to the increased concentration of MB (bacteria proportion to the dye molecules). Also, it was due to blocking of active sites of degrading enzyme by different structures of MB.\(^{24}\) Therefore, the initial concentration of 200 mg/l MB is determined as the optimum concentration. The results of this study are in line with those of other studies such as Lalnunhlimi et al (2016). Lalnunhlimi et al used the alkalophilic bacterial consortium for the removal of Direct Blue 151 and Direct Red 131. It was found that decolorization was increased by increase of initial dye concentration from 100 to 200 mg/l. Furthermore, decolorization was increased by increase in the dye concentration to 250 mg/l. This increasing trend continued until concentration was up to 300 mg/l. In another study by Karatay et al (2015), three different bacterial species were isolated for the removal of Remazol Blue. The results revealed that by increasing the dye concentration from 29 mg/l to 58 and 78 mg/l, \(Bacillus \text{megaterium}\) could tolerate the removal efficiency of 50\%. However, by increasing concentration to 98 mg/l, removal efficiency was reduced to 31.7\%. Removal efficiency of \(Micrococcus \text{luteus}\) bacteria was generally decreased by increasing the initial dye concentration. However, \(M. \text{luteus}\) bacteria can tolerate all concentrations with the same efficiency. The dye removal efficiency of \(Bacillus \text{pumilus}\) bacteria in concentrations of 29, 58, 78 and 98 mg/l, was 71, 74.3, 67.2 and 69.9\%, respectively.\(^{32}\)

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**Conclusion**

According to the results, by increasing MB concentration, dye biodegradation efficiency of \(P. \text{aeruginosa}\) was increased (50-200 mg/l).
Therefore, the bacteria along with glucose used the carbon from the MB dye as an auxiliary source and removal efficiency was increased. The results showed that biodegradation and biosorption have an important role in the removal of MB. Optimum concentration of dye removal was 200 mg/l. Biodegradation efficiency decreased at concentrations more than 200 mg/l of MB due to the antibacterial properties of MB, reduction in the bacterial number, and decrease of bacterial biomass. Thus the rate of biodegradation was reduced. Finally, it can be concluded that *P. aeruginosa* is an appropriate alternative for removal of MB from the environment.

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**Reference**


