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ORIGINAL PAPER

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

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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.[9, 10] 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.[11, 12] Physicochemical methods like coagulation and flocculation are expensive and discharge large amounts of sludge after treatment which must be safely disposed.[13-15] Adsorption and membrane filtration methods generate secondary waste streams which need additional treatment. Nevertheless, biological removal by microorganisms is widely employed due to cost-effective, easy operation, high efficiency, less sludge production and compatibility with the environment.[15-17] Different groups of microorganisms such as bacteria, fungi, yeast, algae are capable of degrading dyes in aerobic and anaerobic conditions.[18-20] Among these microorganisms, bacteria are more appropriate for dye removal due to their greater number, activity, compatibility and short growth cycle.[21] In studies by El-sersy *et al* and Chen *et al*, different species of *Bacillus thuringiensis* were used for the biodegradation of MB.[22, 23] 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.[24]

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).[25] 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.

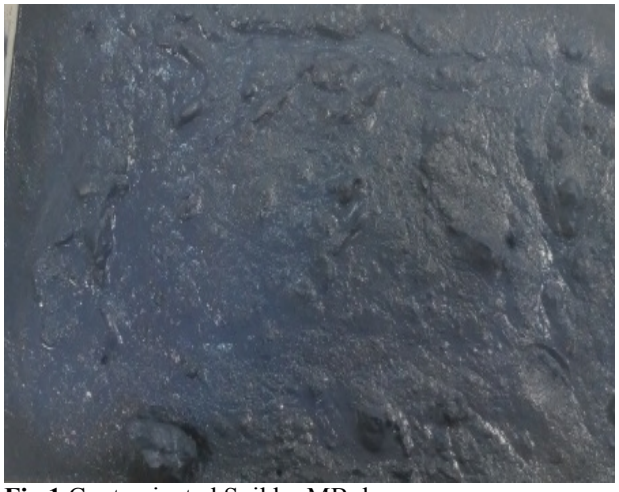


Fig.1 Contaminated Soil by MB dye

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 innoculated 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 innoculated 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.[25, 26]

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 KH_2PO_4, 50 mg $MgSO_4$-$7H_2O$ and 300 mg K_2CO_3 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

in a shaker incubator (100 rpm) at 30°C for 24 h. Finally, dye removal results were analyzed at 665 nm by UV/Vis spectrophotometer, SP-3000 PLUS, OPTIMA Co.

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).

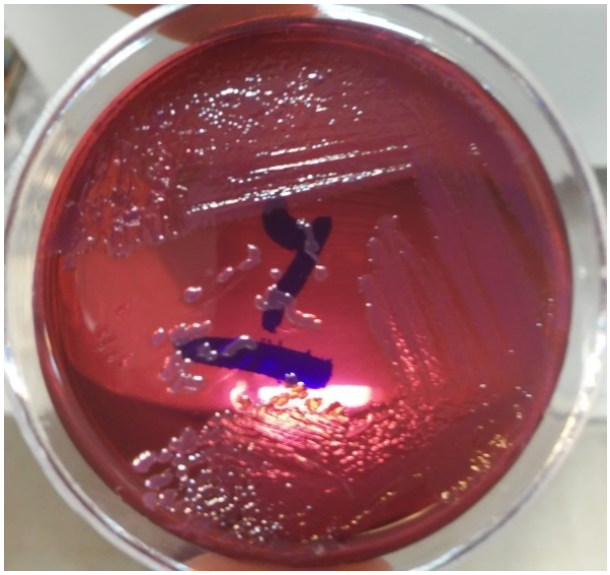


Fig.2 Grown colonies on EMB medium

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

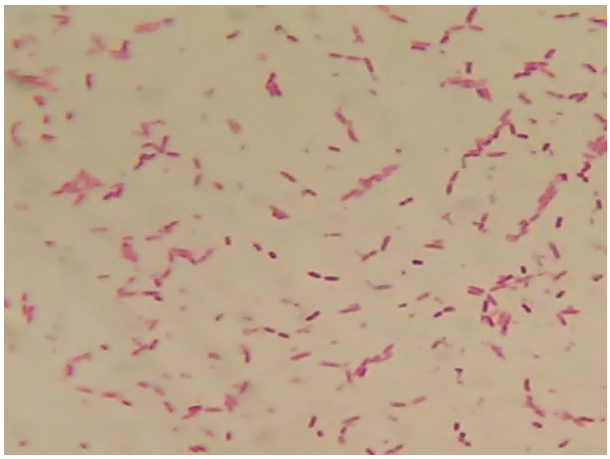


Fig.3 Optical microscope image of rod-shaped Bacillus

biodegradation.[22, 24] 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.

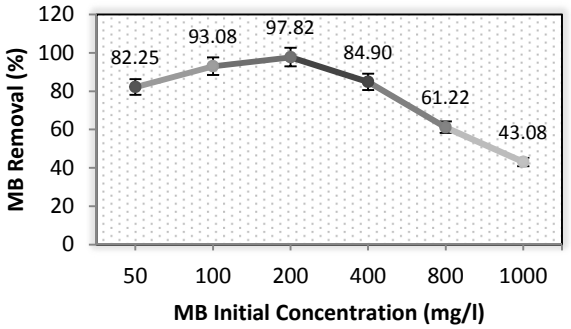


Fig.4 The removal efficiency of MB by bacteria at different concentrations

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.[27] Researches have shown that *P. aeruginosa* is capable of using azo dyes as the sole source of

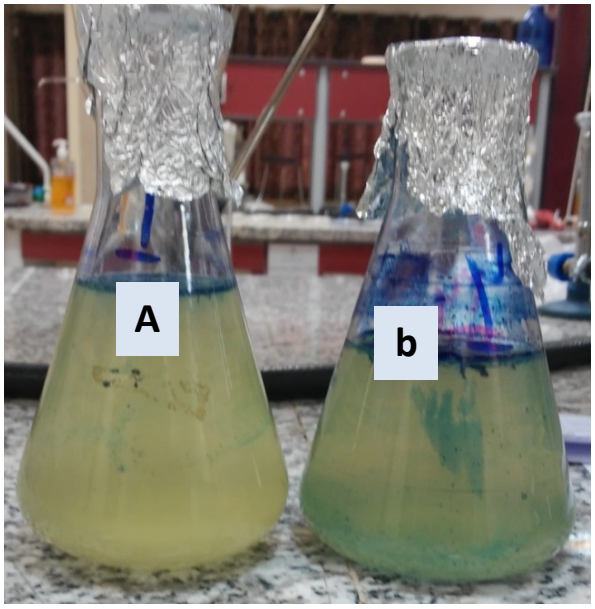
carbon; these bacteria can break -N = N- bonds and use amines as carbon and energy sources.[28]

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. 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. 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 alkaliphilic 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.[31] 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 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 luteus* bacteria was generally decreased by increasing the initial dye concentration. However, *M. luteus* bacteria can tolerate all concentrations with the same efficiency. The dye removal efficiency of *Bacillus pumilus* bacteria in concentrations of 29, 58, 78 and 98 mg/l, was 71, 74.3, 67.2 and 69.9%, respectively.[32]

Table 1 The initial and final concentration of MB in the presence of bacteria

Run	initial concentration of MB (mg/l)	Final concentration of MB after 24h at 30°C (mg/l)
1	50	8.87
2	100	6.92
3	200	4.36
4	400	60.38
5	800	310.25
6	1000	569.23



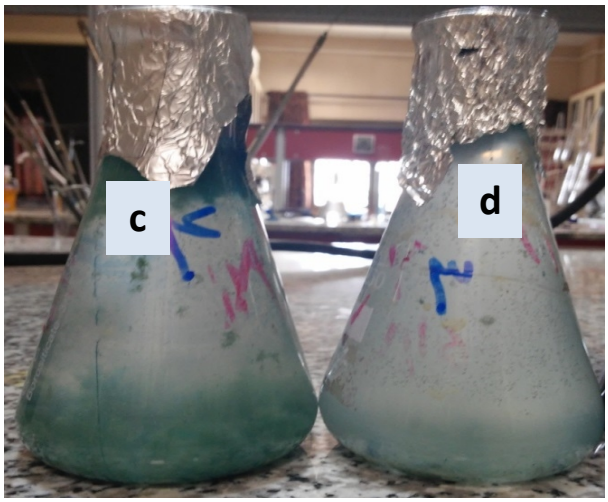


Figure5. Erlenmeyer containing inoculum and MB after removal, initial concentrations of MB a) 50, b) 100, c) 200 and d) 400 mg/l

Conclusion

According to the results, by increasing MB concentration, dye biodegradation efficiency of *P. 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

1. Shahi DH, Eslami H, Ehrampoosh MH, Ebrahimi A, Ghaneian MT, Ayatollah S, et al. Comparing the efficiency of Cyperus alternifolius and Phragmites australis in municipal wastewater treatment by subsurface constructed wetland. Pak J Biol Sci 2013;16(8):379-384.
2. Eslami H, Hematabadi PT, Ghelmani SV, Vaziri AS, Derakhshan Z. The Performance of Advanced Sequencing Batch Reactor in Wastewater Treatment Plant to Remove Organic Materials and Linear Alkyl Benzene Sulfonates. Jundishapur Journal of Health Sciences 2015;7(3):33-39.
3. Xiao Y, Liu X, Wang D, Lin Y, Han Y, Wang X. Feasibility of using an innovative PVDF MF membrane prior to RO for reuse of a secondary municipal effluent. Desalination 2013;311:16-23.
4. Yagub MT, Sen TK, Afroze S, Ang HM. Dye and its removal from aqueous solution by adsorption: a review. Adv Colloid Interface Sci 2014;209:172-184.
5. Yao Y, Xu F, Chen M, Xu Z, Zhu Z. Adsorption behavior of methylene blue on carbon nanotubes. Bioresour Technol 2010;101(9):3040-3046.
6. Singh RL, Singh PK, Singh RP. Enzymatic decolorization and degradation of azo dyes–A review. Int Biodeterior Biodegrad 2015;104:21-31.
7. Ghanizadeh G, Asgari G. Removal of methylene blue dye from synthetic wastewater with bone char. Iranian Journal of Health and Environment 2009;2(2):104-13.[In persion]
8. Yang C, Li L, Shi J, Long C, Li A. Advanced treatment of textile dyeing secondary effluent using magnetic anion exchange resin and its effect on organic fouling in subsequent RO membrane. J Hazard Mater 2015;284:50-57.
9. Özer A, Dursun G. Removal of methylene blue from aqueous solution by dehydrated wheat bran carbon. J Hazard Mater 2007;146(1):262-9.
10. Dos Santos AB, Cervantes FJ, van Lier JB. Review paper on current technologies for decolourisation of textile wastewaters: perspectives for anaerobic biotechnology. Bioresour Technol. 2007;98(12):2369-2385.
11. Kalaivani GJ, Suja S. TiO2 (rutile) embedded inulin—A versatile bio-nanocomposite for photocatalytic degradation of methylene blue. Carbohydr Polym. 2016;143:51-60.
12. Karagozoglu B, Tasdemir M, Demirbas E, Kobya M. The adsorption of basic dye (Astrazon Blue FGRL) from aqueous solutions onto sepiolite, fly ash and apricot shell activated carbon: Kinetic and equilibrium studies. J Hazard Mater 2007;147(1-2):297-306.
13. Gupta V. Application of low-cost adsorbents for dye removal–A review. J Environ Manage. 2009;90(8):2313-42.
14. Santos SC, Vilar VJ, Boaventura RA. Waste metal hydroxide sludge as adsorbent for a reactive dye. J Hazard Mater 2008;153(3):999-1008.
15. Zodi S, Merzouk B, Potier O, Lapicque F, Leclerc J-P. Direct red 81 dye removal by a continuous flow electrocoagulation/flotation reactor. Sep Purif Technol 2013;108:215-22.
16. Forgacs E, Cserhati T, Oros G. Removal of synthetic dyes from wastewaters: a review. Environ Int 2004;30(7):953-71.
17. Gopinath KP, Murugesan S, Abraham J, Muthukumar K. Bacillus sp. mutant for improved biodegradation of Congo red: random mutagenesis approach. Bioresour Technol 2009;100(24):6295-6300.
18. Baldev E, MubarakAli D, Ilavarasi A, Pandiaraj D, Ishack KSS, Thajuddin N. Degradation of synthetic dye, Rhodamine

B to environmentally non-toxic products using microalgae. Colloids Surf B Biointerfaces 2013;105:207-14.
19. Lv G-Y, Cheng J-H, Chen X-Y, Zhang Z-F, Fan L-F. Biological decolorization of malachite green by Deinococcus radiodurans R1. Bioresour Technol 2013;144:275-280.
20. Tan L, Ning S, Zhang X, Shi S. Aerobic decolorization and degradation of azo dyes by growing cells of a newly isolated yeast Candida tropicalis TL-F1. Bioresour Technol 2013;138:307-313.
21. Pearce C, Lloyd J, Guthrie J. The removal of colour from textile wastewater using whole bacterial cells: a review. Dyes and pigments 2003;58(3):179-196.
22. Chen Z, Chen H, Pan X, Lin Z, Guan X. Investigation of methylene blue biosorption and biodegradation by Bacillus thuringiensis 016. Water, Air, Soil Pollut 2015;226(5):1-8.
23. El-Sersy NA. Bioremediation of Methylene Blue by Bacillus thuringiensis 4 G 1: Application of Statistical Designs and Surface Plots for Optimization. Biotechnology 2007;6(1):34-39.
24. Noraini CHC, Morad N, Norli I, Teng TT, Ogugbue CJ. Methylene blue degradation by Sphingomonas paucimobilis under aerobic conditions. Water, Air, Soil Pollut 2012;223(8):5131-5142.
25. Sarvi Moghanlo V, Chorom M, Motamedy H, Pourzamani H, Falah M. Identification of Hydrocarbon Degrading Bacteria Genus from Oil-Crude Contaminated Soils. J Health Syst Res 2012;8(6):1098-106.[In Perstion]
26. Kafilzadeh F, Hoshyaripour F, Afrough R, Jamali H, Allahverdi G. Isolation and Identification of Pyrene-degrading Bacteria from Soils around Landfills in Shiraz and Their Growth Kinetic Assay. Journal of Fasa University of Medical Sciences 2011;1(3):154-9. [In Perstion]
27. Jing W, Byung-Gil J, Kyoung-Sook K, Young-Choon L, Nak-Chang S. Isolation and characterization of Pseudomonas otitidis WL-13 and its capacity to decolorize triphenylmethane dyes. J Environ Sci (China) 2009;21(7):960-4.
28. Pandey A, Singh P, Iyengar L Bacterial decolorization and degradation of azo dyes. Int Biodeterior Biodegrad 2007;59(2):73-84.
29. Núñez SC, Garcez AS, Kato IT, Yoshimura TM, Gomes L, Baptista MS, et al. Effects of ionic strength on the antimicrobial photodynamic efficiency of methylene blue. Photochemical & Photobiological Sciences 2014;13(3):595-602.
30. Perni S, Piccirillo C, Pratten J, Prokopovich P, Chrzanowski W, Parkin IP, et al. The antimicrobial properties of light-activated polymers containing methylene blue and gold nanoparticles. Biomaterials 2009;30(1):89-93.
31. Lalnunhlimi S, Krishnaswamy V. Decolorization of azo dyes (Direct Blue 151 and Direct Red 31) by moderately alkaliphilic bacterial consortium. Braz J Microbiol 2016;47(1):39-46.
32. Karatay SE, Kılıç NK, Dönmez G. Removal of Remazol Blue by azoreductase from newly isolated bacteria. Ecol Eng 2015;84:301-4.