Desalination and Water Treatment
www.deswater.com

doi: 10.1080/19443994.2015.1063089

57 (2016) 14077–14082
June

Taylor & Francis
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Biochemical degradation of Methylene Blue using a continuous reactor packed with solid waste by *E. coli* and *Bacillus subtilis* isolated from wetland soil

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Received 27 October 2014; Accepted 6 June 2015

ABSTRACT

Effluent from textile industries contain various azo dyes. Methylene Blue is one of the most predominant one which is hazardous for the environment and has to be degraded chemically or biochemically. A pure culture of *Bacillus subtilis* and *E. coli* cells which were already acclimatized to phenol as the sole carbon source were taken for the purpose of the present work. The rate of degradation of MB for *E. coli* had increased at a much slower rate than that of *B. subtilis* over the entire degradation period. Hence, in the batch process, overall removal efficiency of Methylene Blue by *B. subtilis* is higher than that of *E. coli*. Thus, *B. subtilis* was taken for further biodegradation of Methylene Blue studies in a continuous process in a counter current packed bed reactor packed with clay chips. In addition to a packing material, the clay chips also act as a whole cell immobilizing matrix for the *Bacillus* cells. The effect of Methylene Blue flow rate and air flow rate on percentage Methylene Blue removal efficiency was observed. The optimum air flow rate and substrate flow rate for maximum removal efficiency of Methylene Blue were 2.0 lpm and 0.5 mL/min, respectively. Maximum removal efficiency under these conditions was 84.7%.

Keywords: Methylene blue; Continuous packed bed reactor; *E. coli*; *B. subtilis*; Clay chips

1. Introduction

The environment is gradually but inevitably polluted by increasing industrialization and urbanization. The industrial effluents from various industries contain various organic and inorganic pollutants which are detrimental to the environment and our health. Among various industries, effluent from textile industry contains various azo dyes like derivatives of benzene, toluene, naphthalene, phenol and aniline. These are xenobiotic compounds which are recalcitrant and persist in the wastewater. Methylene Blue (MB) is one of the heterocyclic cationic azo dyes extensively used in textile industries. It is also used as an indicator and antiseptic agent in clinical therapy. The molecular formula is $C_{16}H_{18}N_3SCl$. At room temperature it appears as a solid, odourless, dark green powder that yields a blue solution when dissolved in water with maximum absorption at 670 nm [1,2].

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MB obstructs the penetration of dissolved oxygen (DO) into the natural water bodies [3]. It is hazardous to aquatic life by affecting photosynthetic activity in aquatic plants. Besides it is carcinogenic as well as mutagenic [4,5]. MB causes haemolytic anaemia, hyperbilirubumia and acute renal failure in humans [6]. Therefore, it is important to treat wastewater from dye industry as well as textile industry before it is discharged into the primary water resource like rivers and seas. Wastewater treatment for removal of dyes or colouring agents are done both chemically as well as biochemically. Physicochemical methods like coagulation, ultrafiltration, electro-chemical adsorption, sonochemical degradation and photocatalytic degradation can remove dyes from wastewater [7–15].

As opposed to chemical breakdown biodegradation of MB is preferred. Chemical treatment often results in the formation of toxic intermediates which are recalcitrant to further degradation [16]. Though slower than chemical methods, biodegradation of MB leads to complete mineralization of the organic compounds to nontoxic products. Both anaerobic and aerobic micro-organisms can be used for this purpose. Most of the reports available have worked with anaerobic culture [17,18]. The present study looked into the response of the aerobic cultures *Bacillus* sp., isolated from East Calcutta Wetlands, to MB. On successful completion of batch kinetic studies, the process was upgraded to a continuous reactor at various process variables.

2. Materials and methods

2.1. Bacterial strain used and maintained in laboratory

A pure culture of *Bacillus subtilis* and *E. coli* cells which were already acclimatized to phenol as the sole carbon-source were taken for the purpose of the present work.

The strain of *B. subtilis* and *E. coli* isolated from the soil sample collected from East Calcutta Wetlands was maintained in laboratory in mineral salt agar containing 500 mg/L of phenol as the sole source of carbon. Composition of the media (for 1L) is KH_2PO_4: 0.68 g, K_2HPO_4: 1.73 g, $FeSO_4$: 0.03 g, NH_4NO_3: 0.1 g, $MgSO_4$: 0.10 g, $CaCl_2$: 0.02 g, $MnSO_4$: 0.03 g, pH maintained at 7.0. 24 h old culture grown in mineral broth containing 1% glucose was used as an inoculum for further studies. Since the bacteria were initially found to degrade phenol, therefore, they were maintained in phenolic media before introducing to Methylene Blue.

2.2. Chemical and reagents

The heterocyclic aromatic compound MB containing synthetic mineral salt (MS) media used in the experimental study is prepared from analytical grade chemicals procured from Merck, India. Phenol and glucose were used as carbon source in the media were of reagent grade and were supplied by Merck, India.

2.3. Methylene Blue degradation in the batch process

About 100 mL of 0 mg/L, 50 mg/L, 100 mg/L, 200 mg/L and 500 mg/L of MB in MS media were prepared in Erlenmeyer flasks and a standard curve was prepared of absorbance vs. concentration of MB using a spectrophotometer (Shimadzu UV1700). 50 mg/L of MB in MS media was prepared in four Erlenmeyer flasks. These media were inoculated with *E. coli* and *B. subtilis* in duplicate. The absorbance of the media was measured at 610 nm at 0 h and at regular intervals of 2 h till MB concentration in the media became negligible. The kinetic parameters were calculated for both the cultures. The *Bacillus* culture showed better MB degradation rate and thus was chosen for further studies in a continuous packed bed reactor.

2.4. Packed bed reactor system

The packed bed reactor was a bench scale reactor of volume 1,000 mL. The reactor had an inlet port 110 mm from the base of the reactor. There were two outlet ports at 25 and 50 mm from the base of each PBR, respectively. Oxygen was supplied from the lower end of the reactor from a compressed air source at varying flow rates from 0 to 2.5 Lpm. The air flow rate was controlled and measured by a rotameter. The rate of aeration was sufficient to maintain saturated DO level (4.5 mg O_2/L) in the reactor medium so as to assume growth not to be limited by DO level. The column reactor made of Borosil glass had an aspect ratio 10:1 (Fig. 1).

The clay chips used as packing material were derived from earthen tea cups used in West Bengal, a state in eastern India. These tea cups were disposable but nonbiodegradable. They, thus, produce a huge bulk of solid waste. Recycling them for other uses would help to reduce the bulk solid waste. The used tea cups were collected from the vendors, washed and dried under the sun. Then they were broken into smaller pieces. The broken pieces were sifted and similar sized pieces were selected for packing the column reactor. The bed volume was 80% of the total reactor volume.

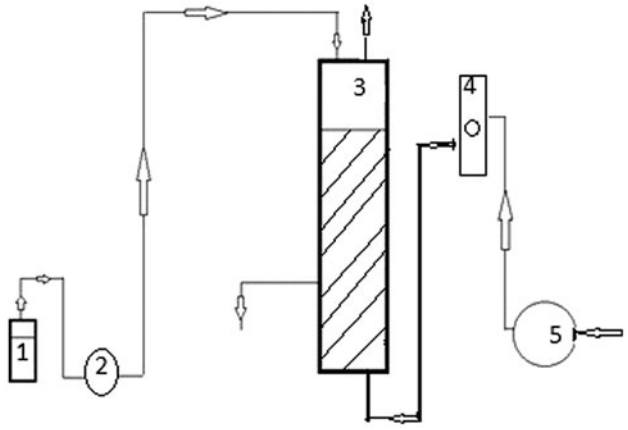

Fig. 1. Schematic of the countercurrent packed bed reactor: (1) Substrate, (2) Peristaltic pump, (3) Bioreactor, (4) Rotameter and (5) Compressor.

The *Bacillus* cells were immobilized on the clay chips to 70% cell loading. Above 70% cell loading on the immobilizing matrix, the rate of Methylene Blue degradation decreased due to diffusion limitation of the substrate into the biofilm. The synthetic media containing 500 mg/L of MB was introduced into the reactor with the help of a peristaltic pump. Air was sparged into the reactor in counter current direction to the substrate. The steady state system was studied at various substrate flow rates and air flow rates at room temperature. The rate of removal of MB from the synthetic media was measured spectrophotometrically. Then, further studies were done on the performance of the continuous reactor system in the removal of MB. The reaction conditions were optimized based on the removal efficiency of MB at room temperature. Then the removal efficiency of the packed bed reactor was studied under different optimized substrate and air flow rates.

3. Results and discussion

3.1. Batch kinetic study of Methylene Blue degradation by B. subtilis cells

The study of the MB degradation was done in batch process in Erlenmeyer flasks. The initial concentration of MB taken in the synthetic media (MS) for the degradation study was 50 mg/L. The MB degradation time profile was as given in Fig. 2. MB was the sole source of carbon for the *Bacillus* cells. Thus, the degradation of MB occurred mainly in the exponential phase of the growth cycle. The specific rate of MB degradation was calculated from the time profile as $1/X(-\mathrm{d}S/\mathrm{d}t)$. The specific degradation rate of MB

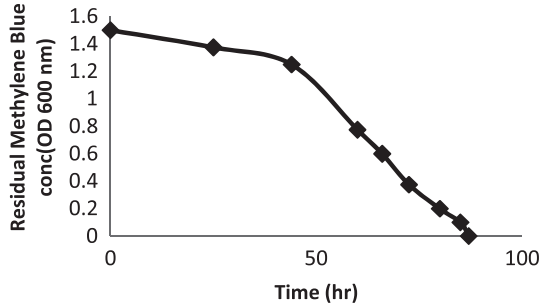

Fig. 2. Time profile of degradation of Methylene Blue by *B. subtilis* in batch culture.

increased with time till MB concentration depleted considerably (Fig. 3). The maximum specific rate of degradation of MB was determined from data of batch experiments as shown in Fig. 3. The maximum specific rate of degradation of MB was 1.6 h^{-1}. Complete degradation of MB was observed at 90 h (Fig. 2). Percentage degradation was much better than that reported by previous researchers. Fulekar et al. observed 69% degradation of 20 mg/L MB in 7 d by a *Pseudomonas* sp. isolated from soil [19].

3.2. Batch kinetic study of MB degradation by E. coli cells

The study of the MB degradation was done in the batch process in Erlenmeyer flasks with an initial concentration of 50 mg/L. Since MB was the sole source of carbon for the *E. coli* cells, therefore, the degradation of MB occurred mainly in the exponential phase of the growth cycle (Fig. 4). The specific degradation rate of MB $[1/X(-\mathrm{d}S/\mathrm{d}t)]$, as calculated from the time profile, increased with time till MB concentration depleted considerably (Fig. 5). The maximum specific rate of degradation of MB was 3.85 h^{-1} (Fig. 5). Complete degradation of MB was observed at 85 h (Fig. 4).

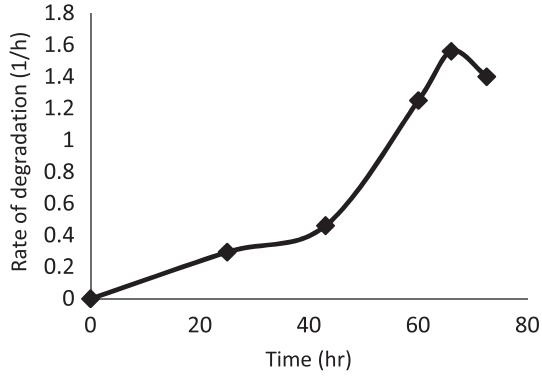

Fig. 3. Rate of degradation of Methylene Blue by *B. subtilis*.

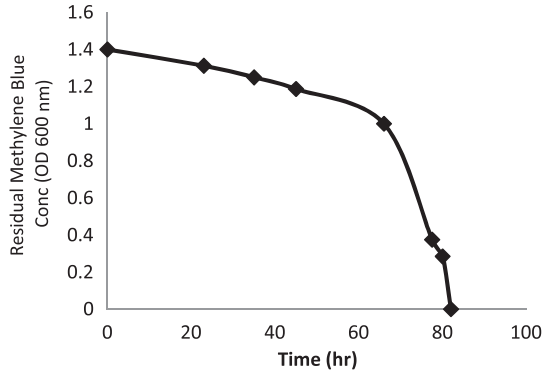

Fig. 4. Time profile for degradation of MB by *E. coli.*

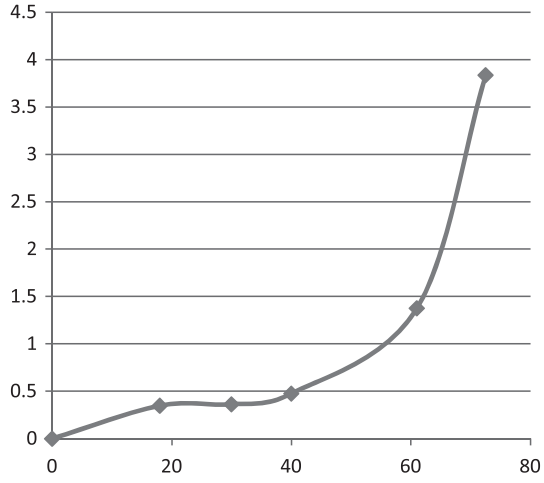

Fig. 5. Rate of degradation of MB by *E. coli.*

3.3. Performance study of continuous packed bed reactor in MB biodegradation

It was observed from the batch kinetic study that both *B. subtilis* and *E.coli* were able to degrade and utilize MB as the sole source of carbon. From Figs. 3 and 5, it could be deduced that the rate of degradation of MB for *E. coli* had increased at a much slower rate than that of *B. subtilis* over the entire degradation period. Hence, the overall efficiency of *B. subtilis* is higher than *E. coli*. Therefore, though the maximum specific degradation rate for *E. coli* was higher than *B. Subtilis* (as shown in Figs. 3 and 5), *B. subtilis* was selected for further study of biodegradation of MB in a counter flow continuous packed bed reactor. The removal efficiency of MB was measured at different substrate flow rates and air flow rates.

3.4. Effect of substrate flow rate on MB removal efficiency

Residence time of the substrate in the reactor is directly proportional to the efficiency of MB removal. At low substrate flow rate, the cells get more time to interact with MB and thus remove MB more efficiently. As the MB (substrate) flow rate increases the residence time decreases thus leading to inefficient MB removal as shown in Tables 1–3. The highest removal efficiency of MB was at the substrate flow rate of 0.5 mL/min. Substrate flow rates lower than 0.5 mL/min was not viable for efficient removal of MB. It was observed from Fig. 6 that the removal efficiency increased as hydraulic retention time (HRT = V/Q, where V = effective volume of reactor in mL and Q = substrate flow rate in mL/min) increased for relatively lower HRT of substrate, but at higher HRT values the removal efficiency decreased. This

Table 1
Experimental data for the calculation of % removal efficiency of MB when substrate flow rate (2 mL/min)

Substrate flow rate (mL/min)	Air flow rate	MB in	MB out	% removal efficiency
2	0	0.87	0.39	55.172
2	1	0.82	0.38	53.658
2	1.5	0.84	0.24	71.428
2	2	0.80	0.17	78.75
2	2.5	0.80	0.16	80

Table 2
Experimental data for the calculation of % removal efficiency of MB when substrate flow rate (1 mL/min)

Substrate flow rate (mL/min)	Air flow rate (Lpm)	MB in	MB out	% removal efficiency
1	0	0.88	0.18	79.545
1	1	0.88	0.21	76.136
1	1.5	0.88	0.17	80.681
1	2	0.88	0.16	81.818
1	2.5	0.88	0.18	79.545

Table 3
Experimental data for the calculation of % removal efficiency of MB when substrate flow rate (0.5 mL/min)

Substrate flow rate (mL/min)	Air flow rate (Lpm)	MB in	MB out	% removal efficiency
0.5	0	0.85	0.16	81.176
0.5	1	0.85	0.17	80
0.5	1.5	0.85	0.14	83.529
0.5	2	0.85	0.13	84.705
0.5	2.5	0.85	0.16	81.176

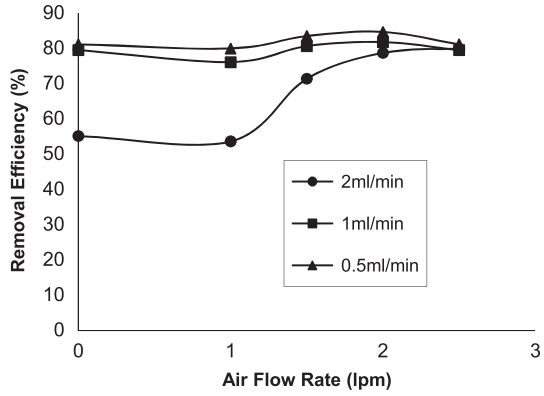

Fig. 6. Percentage (%) removal efficiency of MB by *B. subtilis* at different substrate flow rates.

could be because of an existence of an external diffusion layer and also saturation of cellular metabolism. The rate of transfer of substrate through the diffusion was inversely proportional to the thickness of this layer. On the other hand, the thickness of this layer was inversely proportional to the substrate flow rate through the reactor [20–22]. The hydraulic retention time of the substrate in the reactor was inversely proportional to substrate flow rate.

Thus, the rate of diffusion of substrate to the *E. coli* cells decreased at higher hydraulic retention time [21]. As a result, the removal efficiency of MB effectively decreased at higher HRT. The maximum removal efficiency was 84% with a substrate flow rate of 0.5 mL/min (Fig. 6).

3.5. Effect of air flow rate on MB removal efficiency

B. subtilis cells are aerobic in nature. Air is sparingly soluble in water or in aqueous medium. As a result, especially in case of reactor with high aspect ratio, surface aeration might be insufficient for the cells to carry out their metabolic activities at the highest rate. Thus, external aeration was required. In the present study, air was sparged in counter current direction into the reactor. The removal efficiency of the *Bacillus* cells was higher at lower air flow rate as compared to higher air flow rates after the process, which became independent of rate of aeration. With further increase in aeration rate, it was found that the removal efficiency reduced due to excessive foaming in the reactor leading to insufficient gas–liquid interfacial diffusion [23]. In addition to that foaming caused changes in both the size and composition of the air bubbles and altered the concentration of DO in the medium due to heterogeneous dispersion throughout the volume of the reactor. Due to foaming, the residence time of the air bubbles increased in the reactor. It resulted in the depletion of oxygen content in the reactor and increase in concentration of CO_2 by trapping it within the foam and consequently metabolic activities of the bacterial cells were inhibited [23]. Thus, accumulation of CO_2 in the reactor space due to foaming decreased the removal efficiency of MB by *B. subtilis* cells. The maximum MB removal efficiency of 84% was observed at an air flow rate of 2 lpm and substrate flow rate of 0.5 mL/min as shown in Fig. 6.

4. Conclusion

Both *B. subtilis* and *E. coli* isolated from East Calcutta Wetlands can utilize Methylene Blue as the sole source of carbon. The removal efficiency of Methylene Blue was better for *B. subtilis* than *E. coli* in batch culture. The reactor study was carried out in a packed bed reactor with *B. subtilis* immobilized on clay chips at room temperature. The optimum air flow rate and substrate flow rate for maximum removal efficiency of Methylene Blue were 2.0 lpm and 0.5 mL/min, respectively. Maximum removal efficiency under these conditions was 84.7%.

References

[1] M. Wainwright, K.B. Crossley, Methylene blue—A therapeutic dye for all seasons? J. Chemother. 14 (2002) 431–443.
[2] F.R. Frankenburg, R.J. Baldessarini, Neurosyphilis, malaria and the discovery of antipsychotic agents, Harv. Rev. Psychiatry 16 (2008) 299–307.
[3] B. Manu, S. Chaudhari, Anaerobic decolorisation of simulated textile wastewater containing azo dyes, Bioresour. Technol. 82(3) (2002) 225–231.
[4] R.G. Tantiado, Comparative mutagenic effects of laboratory dyes on *Drosophila melanogaster*, Int. J. Biosci. Biotechnol. 4(4) (2012) 55–61.
[5] R. Gong, X. Liu, M. Feng, J. Liang, W. Cai, N. Li, Comparative study of methylene blue sorbed on crude and monosodium glutamate functionalized sawdust, J. Health Sci. 54(6) (2008) 623–628.
[6] M. Albert, M.S. Lessin, B.F. Gilchrist, Methylene blue: Dangerous dye for neonates, J. Pediatr. Surg. 38 (2003) 1244–1245.
[7] N. Kannan, M.M. Sundaram, Kinetics and mechanism of removal of methylene blue by adsorption on various carbons—A comparative study, Dyes Pigm. 51 (2001) 25–40.
[8] F. Kargi, S. Ozmıhcı, Biosorption performance of powdered activated sludge for removal of different dyestuffs, Enzyme Microb. Technol. 35 (2004) 267–271.
[9] K.G. Bhattacharyya, A. Sharma, Kinetics and thermodynamics of methylene blue adsorption on neem (*Azadirachta Indica*) leaf powder, Dyes Pigm. 65 (2005) 51–59.

[10] M. Srinivasan, T. White, Degradation of methylene blue by three-dimensionally ordered macroporous titania, Environ. Sci. Technol. 41(12) (2007) 4405–4409.
[11] L. Wang, J. Zhang, A. Wang, Removal of methylene blue from aqueous solution using chitosan-g-poly(acrylic acid)/montmorillonite superadsorbent nanocomposite, Colloids and Surf., A 322(1–3) (2008) 47–53.
[12] J. Yao and C. Wang, Decolorization of methylene blue with sol via UV irradiation photocatalytic degradation, Int. J. Photoenergy (2010), doi: 10.1155/2010/643182 (Article ID 643182).
[13] Q. Shen, F. Du, S. Huang, P. Rodriguez, L.T. Watts, T.Q. Duong, Neuroprotective efficacy of methylene blue in ischemic stroke: An MRI study, PLoS One 8 (11) (2013) e79833, doi: 10.1371/journal.pone.0079833.
[14] C. Minero, M. Lucchiari, D. Vione, V. Maurino, Fe (III)-enhanced sonochemical degradation of methylene blue in aqueous solution, Environ. Sci. Technol. 39(22) (2005) 8936–8942.
[15] M. Bielska, J. Szymanowski, Removal of methylene blue from waste water using micellar enhanced ultrafiltration, Water Res. 40 (2006) 1027–1033.
[16] M.A. Mohammed, A. Shitu, A. Ibrahim, Removal of methylene blue using low-cost adsorbent: A review, Res. J. Chem. Sci. 4(1) (2014) 91–102.
[17] Ong Soon-An, Toorisaka Eiichi, Hirata Makoto, Hano Tadashi, Biodegradation of redox dye methylene blue by up-flow anaerobic sludge blanket reactor. J. Hazard. Mater., B 124 (2005), 88–94.
[18] C.M. Carliell, S.J. Barclay, N. Naidoo, C.A. Buckly, D.A. Mulholland, E. Senior, Microbial decolorisation of a reactive azo dye under anaerobic conditions, Water SA 21 (1995) 61–69.
[19] M.H. Fulekar, S.L. Wadgaonkar, A. Singh, Decolourization of dye compounds by selected bacterial strain isolated from dye-stuff industrial area, Int. J. Adv. Res. Technol. 2(7) (2013) 182–192.
[20] M.D. Lilly, W.E. Hornby, E.M. Crook, The kinetics of carboxymethyl cellulose-ficin in packed beds, Biochem. J. 100(3) (1966) 718–723.
[21] F.N Kök, M.Y. Arıca, C.Halıcıgil, G.Alaeddinoğlu, V.Hasırcı, Biodegradation of aldicarb in a packed-bed reactor by immobilized Methylosinus, Enzyme Microb. Technol. 24(5–6) (1999) 291–296.
[22] B. Chakraborty, S. Indra, D. Hazra, R. Betai, L.G. Ray, S. Basu, Performance study of chromium(VI) removal in presence of phenol in a continuous packed bed reactor by *Escherichia coli* isolated from east Calcutta Wetlands, Biomed. Res. Int. (2013), doi: 10.1155/2013/373412 (Article ID 373412).
[23] P.F. Stanbury, A. Whitaker, S.J. Hall, Principle of Fermentation Technology, Butterworth-Heinemann, second ed., 2008.