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Comparison on biodegradation of anionic dye orange II and cationic dye methylene blue by immobilized microorganisms on spent granular activated carbon

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ABSTRACT

Activated carbon adsorption and biological degradation are two important methods used in the treatment of industrial wastewater among others. The objective of this study is to investigate the biodegradation of anionic dye orange II and cationic dye methylene blue by anaerobic microbes immobilized on spent granular activated carbon through attachment and under packed column operation. The maximum biodegradation rate of orange II and methylene blue was 0.38 g/h and 0.56 g/h, respectively, with initial dye concentration of 1150 mg/l. Results revealed that the difference in the biodegradation rate of methylene blue and orange II was influenced by the molecular structure of the dye. Adsorption study showed that methylene blue was adsorbed more readily by biofilm than orange II and subsequently contributed to higher removal rate of methylene blue than orange II in GAC-biofilm packed column operation.

Keywords: Immobilization; Orange II; Methylene blue; Granular activated carbon; Biodegradation

1. Introduction

There are more than 10,000 types of synthetic dyes employed for various applications in industries and the classification of dyes can be based on their applications or molecular structures. The dye molecules and the intermediate products after partial treatment are toxic to living organisms and could upset the biological activities in an ecosystem. Most of the dyes are designed to resist light, chemicals, and microbes, and thus they are difficult to degrade once released into aquatic systems [1]. There are various researches on the physical, chemical, and biological treatments for removing the color from industrial effluents. Some of the treatment techniques proved to be efficient, but there are still a lot of drawbacks in each treatment which should be solved, such as operational cost, sludge formation, biomass accumulation, knowledge of technology, etc. [2–5].

Research on sequential anaerobic process followed by aerobic process for removing azo dyes has been investigated extensively. Various reactor configurations

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were used for both aerobic and anaerobic systems, from suspension to attached systems [6,7]. Most of the research outcomes showed that anaerobic process was responsible for color removal due to the breakdown of azo bond and the aromatic amines generated resisted further anaerobic degradation, but these intermediate products could be mineralized aerobically [7–9]. The use of granular activated carbon as a medium for immobilization of microbes has been proven to be more resistant to toxic compounds in water or wastewater treatment, and the performance was greater in suspended cells [10,11].

The objective of this study was to compare the decolorization of anionic dye orange II and cationic dye methylene blue by dye-degrading microbes immobilized on spent granular activated carbon through attachment. In our study, the concentration of dyes in feed solution was increased to up to 1,300 mg/l to examine the feasibility of the bioreactor to treat high-strength dyes containing wastewater.

2. Materials and methods

Two parallel GAC-biofilm packed columns, namely Column-A and Column-B, with 4 cm diameter × 105 cm height were developed and used for the treatment of orange II- and methylene blue-containing wastewater, respectively. The experimental setup for the GAC-biofilm packed column is shown in Fig. 1.

The source of dye-degrading microbes was an anaerobic sequencing batch reactor (ASBR). The ASBR was used for the treatment of orange II-containing wastewater in our previous study. Spent GAC was immersed in the ASBR for more than a month in order to immobilize the dye-degrading microbes on its surface. Then, the developed GAC-biofilm was packed into a column as shown in Fig. 1. About 3 l of dye-containing wastewater was introduced into the column daily. The effluent from the column was collected in a collection tank and circulated between the column and collection tank for 24 h [12]. The amount of orange II and methylene blue used in this study is shown in Table 1. For each run, the study was repeated for five times and the average results were reported. A redox meter was installed in the collection tank to measure the oxidation reduction potential of the wastewater. The temperature and the influent pH were set approximately at 28°C and 7, respectively. A mixer was also installed to well mix the treated water discharged from the column.

The dyes (methylene blue and orange II) were obtained from Chroma Ltd. and the granular activated carbon was supplied by Wako Pure Chemical Industries Ltd. The synthetic wastewater consisted of bacto-peptone (188), sucrose (563), NH_4Cl (344), $MgSO_4$ (49), $FeCl_3$ (11.3), and KH_2PO_4 (318) giving a chemical oxygen demand of 800–850 mg/l. All of these chemicals were of analytical grade. Water sample in the collection tank was collected daily and analyzed for dye concentration. Sample was prepared by filtering through a 0.45-μm membrane filter. The orange II and methylene blue concentrations were measured with UV–vis spectrophotometer (UV-1,200, Shimadzu, Japan) at λ_{max} 480 nm and 661 nm, respectively.

3. Results and discussion

3.1. Performance of GAC-biofilm packed column in dye removal

Fig. 2 depicts the correlation between the biodegradation rate and the initial concentration of orange II and methylene blue employed in each run. It was found that the biodegradation rate of dyes in GAC-biofilm packed column increased as the initial dye concentration increased up to 1,100 mg/l. The biodegradation rate for orange II and methylene blue at the optimum point with initial dye concentration of 1,150 mg/l was 0.38 g/h and 0.56 g/h, respectively, and tended to decline beyond this initial concentration. The GAC-biofilm packed column showed high tolerance to dye concentration up to 1,000 mg/l. When the concentration of orange II and redox dye

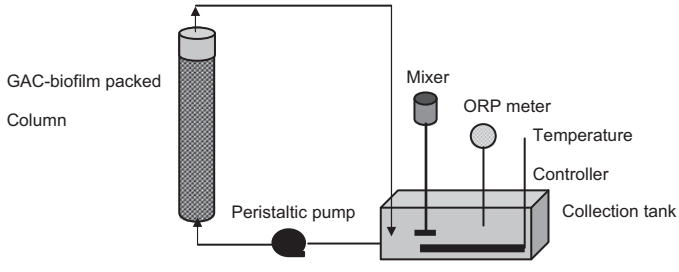

Fig. 1. Schematic diagram of GAC-biofilm packed column.

Table 1
Dyes concentration tested in the study

Run	Methylene blue con. (mg/l)	Orange II con. (mg/l)
1	100	50
2	200	100
3	300	200
4	500	300
5	600	400
6	800	500
7	950	700
8	1,150	900
9	1,350	1,100
10	–	1,300

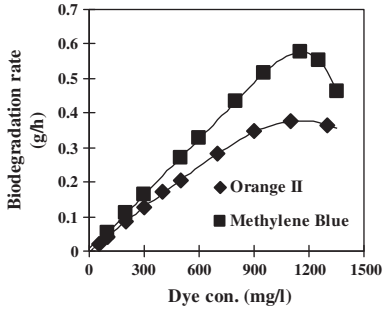

Fig. 2. Biodegradation rates of dyes using GAC-biofilm packed column.

methylene blue increased up to 700 mg/l and 800 mg/l, respectively, complete decolorization was achieved. The high removal efficiency of GAC-biofilm packed column could be due to the simultaneous adsorption and biodegradation of dyes by the microbes immobilized on the spent GAC. The immobilized microbial system has been reported to improve the performance of the bioreactor significantly and has the potential to degrade toxic chemicals faster than conventional wastewater treatment systems [13–15]. The biodegradation rate of methylene blue was significantly higher than that of orange II, which can be ascribed to the different molecular structures between the dye molecules. Overall, the result shows that the GAC-biofilm packed column could be employed for the treatment of high-strength dye containing wastewater compared to the conventional suspended biological wastewater treatment system.

3.2. Adsorption capacity of virgin GAC and biofilm-GAC

The adsorption capacity of virgin GAC and biofilm-GAC (collected from Column A and B) was analyzed in batch study. As shown in Table 2, the maximum adsorption capacity of virgin GAC on orange II and methylene blue was 116 and 139 mg/g, respectively, whereas for the biofilm-GAC it was about 7 and 13 mg/g, respectively. The GAC used in the present study was the spent GAC, which was used in our previous study in the sequential anaerobic–aerobic SBR system. Most of the pores and the surface area of the spent GAC was exhausted and covered by the biofilm; thus, the adsorption capacity of the GAC-biofilm was much lower than that of the virgin GAC. The GAC-biofilm exhibited maximum adsorption capacity of about 7–13 mg/g according to the Langmuir isotherm. The biofilm may contain anionic and cationic exchange sites, such as amino, phosphoryl, sulfhydryl, carboxylic, and hydroxyl groups, that contribute to the binding contaminants in the adsorption process [16]. The anions and cations at the binding sites may determine the net charge carried by the cells. Generally, the cells are assumed to carry a net negative charge [17].

Fig. 3 shows the molecular structure for methylene blue and orange II. methylene blue is a basic cation dye and it will be dissociated to the cation (chromophore of the dye) and the anion, Cl^-, in aqueous solution. Thus, it can be represented by Dye^+Cl^- [18]. On the other hand, orange II is an acid dye and can be represented as Dye^-Na^+. Since the biofilm is assumed to carry a net negative charge, methylene blue can be adsorbed onto the biofilm more readily than orange II through the interaction of the positive charge of the chromophore dye and the negative charge of the biofilm. This was shown by the slightly higher adsorption capacity of the GAC-biofilm

Table 2
Adsorption capacity of virgin GAC and biofilm-GAC

Adsorbent	Adsorbate	Maximum adsorption capacity (mg/g)
Virgin GAC	Methylene blue	139
	Orange II	116
Biofilm-GAC	Methylene blue	13
	Orange II	7

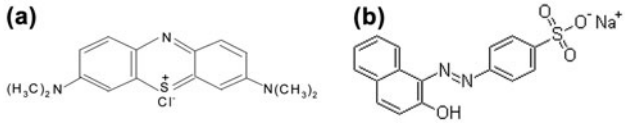

Fig. 3. Structural molecules for (a) Methylene blue and (b) orange II.

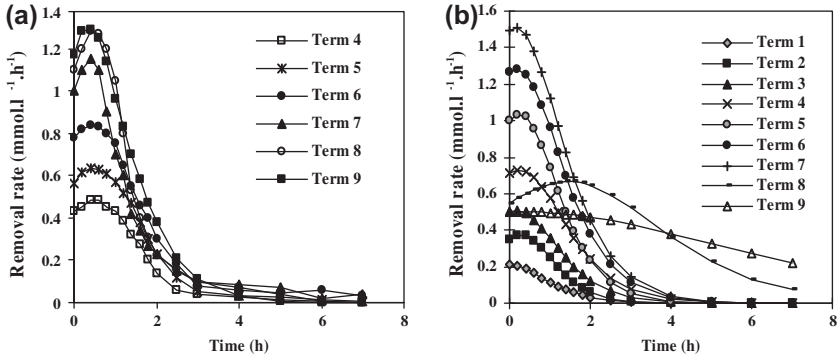

Fig. 4. Orange II (a) and Methylene blue (b) removal rate profiles calculated from the autocatalytic kinetic model.

on methylene blue than on orange II. The removal of dyes by GAC-biofilm packed columns was due to simultaneous adsorption and biodegradation processes. The dyes were adsorbed on the biofilm at the initial stage, followed by biodegradation by microbes in biofilm. The higher adsorption on methylene blue can be one of the reasons that contributed to the higher removal of methylene blue in the GAC-biofilm packed column.

3.3. Biodegradation behavior of dye

In biological reactions under anaerobic condition, the reduction of dye can be either via enzymatically catalyzed reaction or through reduced enzyme co-factors. It has been reported that the reduction of azo bond in orange II by anaerobic microbes leads to the formation of sulfanilic acid and 1-amino-2-naphthol [19–22]. Previous studies on the biodegradation of orange II under anaerobic conditions showed that 1-amino-2-napthol can act as a redox mediator [19,21]. The indirect biological reduction (redox mediator-catalyzed) of the orange II with 1-amino-2-naphthol, which acts as a redox mediator, may cause the enhancement of the biodegradation rate. The data obtained in the present study were analyzed with the autocatalytic model. As shown in Fig. 4(a), the removal rate increased as the initial concentration of orange II increased. The maximum removal rate was achieved after the system was operated for about half an hour, which indicates that the intermediate aromatic amine 1-amino-2-naphthol might act as a redox mediator to catalyze the removal of orange II.

Methylene blue is widely used as a redox indicator in analytical chemistry. It could be observed that the color of methylene blue disappeared as the dissolved oxygen was entirely consumed by the microbes in the bioreactor. However, the color appeared again when it was oxidized by oxygen or air. The redox potential of methylene blue might enable it to play an important role in the transport of electron to the dye itself, thus making the whole process autocatalytic. The autocatalysis behavior of methylene blue under up-flow anaerobic sludge blanket operation was reported in our previous study [23]. As shown in Fig. 4(b), the removal rate increased as the initial methylene blue concentration increased from 100 to 950 mg/l. Beyond this concentration, the removal rate drastically dropped and the time taken to achieve maximum removal rate became slightly longer as the added methylene blue concentration was 1,150 mg/l. Compared to the biodegradation of orange II, the time taken to achieve the maximum removal rate for methylene blue was about 5–10 min, which is slightly faster than orange II. This could be due to the readiness of methylene blue as a redox mediator to transfer the electron, compared to orange II that required to be reduced to form 1-amino-2-naphthol as a redox mediator.

4. Conclusions

The GAC-biofilm packed column showed the ability to treat high-strength dyes containing wastewater. The optimum biodegradation rate of orange II and methylene blue was 0.38 g/h and 0.56 g/h, respectively, under GAC-biofilm packed column operation. The different degradation efficiencies of methylene blue and orange II were greatly influenced by the molecular structure of the dyes. The adsorption study showed that methylene blue was adsorbed more readily by the biofilm than orange II, which have might have contributed to the higher removal rate of methylene blue than orange II in the GAC-biofilm packed column. The readiness of methylene blue as a redox mediator to transfer the electron compared to orange II might have also influenced the biodegradation rate of these dyes.

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