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Mineralization of Methyl Orange-containing wastewater by integrated anaerobic and aerobic processes using spent granular activated carbon–biofilm under sequencing batch reactor operation

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ABSTRACT

Mineralization of azo dye required combination of anaerobic and aerobic processes either in two stages or single stage of treatment. The aim of the present study was to evaluate the treatment performance in the decolorization of Methyl Orange (MO)-containing wastewater and mineralization of intermediate aromatic amines using an integrated anaerobic and aerobic processes by spend granular activated carbon–biofilm under sequencing batch reactor operation (spent GAC–biofilm-SBR). The spent GAC–biofilm-SBR was operated with FILL, REACT, DRAW, and IDLE modes in a time ratio of 2:20:1.5:0.5 for a cycle time of 24 h. The bioreactor was fed with 1 L of MO-containing wastewater daily. The high reductive condition at the bottom layer of spent GAC–biofilm-SBR was responsible for the biological reduction of azo bond and this led to decolorization. Meanwhile, low reductive conditions at the top layer of spent GAC–biofilm-SBR promote the growth of aerobic microbes and caused the mineralization of intermediate aromatic amines.

Keywords: Spent GAC; Biofilm; SBR; Methyl Orange; Aromatic amines

1. Introduction

Azo dyes are extensively used in industrial applications such as textile, tannery, pulp and paper, printing, and pharmaceutical. The discharge of azo dyes from these industries into surface waters is problematic not only for esthetic reasons, but also because azo dyes and their intermediate aromatic amines are carcinogenic and mutagenic. There are numerous physiochemical methods, such as coagulation, flocculation, adsorption, membrane filtration, and advanced oxidation processes, have been used to remove azo dyes-containing wastewater. However, these methods suffer the disadvantages of sludge generation, adsorbent regeneration, membrane fouling, economically unviable, and some of these methods are environmentally unfriendly [1–6]. Comparatively, biological processes are effective, economical, and environmentally friendly.

A wide range of azo dyes is stable to aerobic biodegradation, whereas it can be easily reduced under anaerobic condition with the elimination of color but with the formation of potentially harmful aromatic

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amines [7–9]. These intermediate aromatic amines are difficult to be biodegraded under anaerobic conditions. The toxic inhibition by aromatic amines especially with high concentration of azo dyes in influent may deteriorate the performance of anaerobic biological systems in terms of COD removal and decolorization. Moreover, even though aromatic amines have been considered easily biodegradable under aerobic conditions, some researchers have found evidence of the low biodegradability of the sulfonated aromatic amines formed during the azo bond cleavage of certain azo dyes [10,11]. Since aerobic degradation is capable of mineralizing these intermediate products, it is possible that combined anaerobic–aerobic treatment can efficiently remove color and at the same time completely degrade organic matter [12–14]. This combination of anaerobic–aerobic treatment can be achieved by employing a two stage anaerobic–aerobic process; one anaerobic reactor for decolorization and another one for mineralization of intermediate aromatic amines. One stage anaerobic–aerobic processes for decolorization of azo dye and mineralization of aromatic amines also can be realized in a single bioreactor using of mixed microbial populations.

The attached-growth biofilm systems have shown to be more drastic than suspended-growth processes for the removal of compounds which are difficult to degrade [15]. It has also been reported that the biofilm cells are more resistant to toxicity than freely suspended ones [16]. Immobilization of microflora on granular activated carbon (GAC) particles as biofilm provides some advantages, such as high biomass hold up enables the process to be operated at a higher organic loading rate, and GAC can act as a buffer to reduce the concentration of toxic and recalcitrant compounds such as dye [17,18]. Biofilm configuration coupled with sequencing/periodic discontinuous batch mode operation appears to be a promising option for the effective treatment of complex industrial wastewater containing poorly degradable compounds [19]. On the other hand, the immobilized microbial consortium through encapsulation technique demonstrated high performance in the decolorization of azo dye after short incubation time. The specific decolorization rate of the immobilized microbial consortium was accelerated in high azo dye concentrations and the maximal rate occurred at 1000 mg/L of dye 4BS [20]. The aim of the current study is to investigate the mineralization of azo dye Methyl Orange-containing wastewater by integrated aerobic–anaerobic processes by spent granular activated carbon–biofilm under sequencing batch reactor operation (spent GAC–biofilm-SBR).

2. Materials and methods

2.1. Chemicals

Methyl Orange, MO, ($C_{14}H_{14}N_3NaO_3S$) was supplied by Sigma Aldrich and the molecular structure is shown in Fig. 1. The MO is used directly without further purification. The UV–Vis spectrum of MO was scanned from 200 to 800 nm using a UV–Vis spectrophotometer (Hitachi U-2800, Japan) and it was observed that the maximum absorbance wavelength (λ_{max}) of MO was at 460 nm. The spent GAC was obtained from a factory where it was used as adsorbent in a water treatment plant. The composition of synthetic wastewater was as follows (concentration in mg/L): C_6H_5COONa (107.1), CH_3COONa (204.9), NH_4NO_3 (176.1), NaCl (7.0), $MgCl_2{\cdot}6H_2O$ (3.4), $CaCl_2{\cdot}2H_2O$ (4.0) and $K_2HPO_4{\cdot}3H_2O$ (36.7) giving COD 326 mg/L, T-N 62 mg/L, and T-P 5.0 mg/L. All other chemicals were of analytical grade.

2.2. Spent GAC–biofilm-SBR setup

Activated sludge from a municipal wastewater treatment plant was used as inoculum and was acclimatized in the laboratory for a month by feeding with synthetic wastewater containing MO under anaerobic condition. The spent GAC with average sizes of 3 mm that collected from a factory's water treatment plant was immersed in the activated sludge for a month in order for immobilization of azo dye-degrading microbes onto the surface of spent GAC. The azo dye-

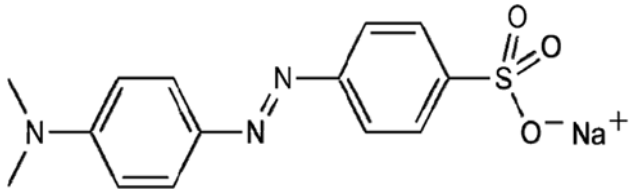

Fig. 1. Molecular structure of Methyl Orange.

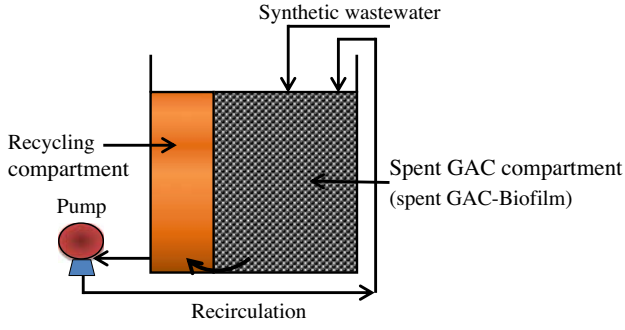

Fig. 2. Schematic diagram of spent GAC–biofilm SBR.

degrading microbes were immobilized on the spent GAC through attachment and the biofilm was developed on the surface of spent GAC. Then, the spent GAC–biofilm was transferred to a reactor with dimensions 17 cm × 17 cm × 17 cm ($L \times W \times H$) (Fig. 2). The bioreactor was used to simulate the biological process for treating MO-containing wastewater and under sequencing batch reactor (SBR) operation. The developed spent GAC–biofilm-SBR has two compartments, namely GAC compartment and recycling compartment. The bottom of the wall that separated the bioreactor into two compartments was not sealed and the little spacing allowed the water to flow underneath between GAC and recycling compartments. The 1.8 L of spent GAC–biofilm was loaded into the GAC compartment. The spent GAC–biofilm-SBR consisted of four operating modes controlled by timer: FILL (2.0 h), REACT (20.0 h), DRAW (1.5 h), and IDLE (0.5 h) modes for a cycle time of 24 h. The spent GAC–biofilm-SBR was filled with 1 L of MO-containing wastewater daily.

The synthetic wastewater consisted of organic carbon, nutrients, and buffer solution, and MO was added to the synthetic wastewater to provide a constant concentration of 75 mg/L (phase 1) and 175 mg/L (phase 2) to the influent of the bioreactor. During FILL modes, the MO-containing wastewater was introduced into GAC compartment and the wastewater infiltrate downward through the spent GAC–biofilm. The partially treated MO-containing wastewater which flows underneath from GAC compartment to recycling compartment will be recycled back to the GAC compartment during REACT mode with a flow rate of 15 mL/min for further treatment before being discharged after 24 h of treatment time.

2.3. Adsorption studies

The 100 mg/L of Methyl Orange was prepared in the volume of 500 mL in a beaker. The 10 g of spent GAC was added into the beaker and then the prepared sample was stirred on a magnetic stirrer. Sampling was collected at selected time intervals and filtered samples were analyzed with a UV–Vis spectrophotometer (Hitachi U-2800, Japan).

2.4. Analytical procedures

Water samples for influent and effluent were collected and were analyzed to examine the treatment performance of the bioreactor. The effluent from spent GAC–biofilm-SBR was collected during DRAW mode and analyzed for COD, MO, and aromatic amines. The water samples were prepared by filtering through a membrane filter of 0.45 μm. Concentration of COD was determined using a HACH DR/2800 portable colorimeter. Concentrations of the MO in the samples were determined using a UV–Vis spectrophotometer (Hitachi U-2800, Japan) at wavelength 460 nm. The aromatic amine was determined using a UV–Vis spectrophotometer (Hitachi U-2800, Japan) at wavelength 250 nm. Oxidation–reduction potential (ORP) was monitored in the spent GAC–biofilm-SBR with HANNA ORP meter.

The efficiency of decolorization was calculated as follows:

$$\text{Decolorization efficiency } (\%) = (C_0 - C_t)/C_0 \times 100\% \quad (1)$$

where C_t is the MO concentration at reaction time t (min) and C_0 is the initial MO concentration.

3. Results and discussion

The average COD, T-N, T-P, NH_4–N, and NO_3–N in the synthetic wastewater containing 75 mg/L MO were 419, 55, 6.7, 35, and 30 mg/L, respectively. As the MO concentration increased to 175 mg/L during phase 2, the COD influent was increased to 541 mg/L, and the nutrients were remaining unchanged. The temperature monitored along the height of bioreactor was almost the same, with an average value of 24 ± 1°C and most of the reported anaerobic reactors were operated under sub-mesophilic temperatures (20–27°C) [21]. The spent GAC–biofilm-SBR was operated for 58 days and 26 days with a concentration of MO of 75 mg/L (phase 1) and 175 mg/L (phase 2), respectively, in order to evaluate the feasibility of the bioreactor for decolorization of the MO and mineralization of intermediate aromatic amines. Fig. 3 shows the COD and MO monitoring throughout the study and the performance of bioreactor in terms of COD, color, and aromatic amines removals are summarized in Table 1. As shown in Table 1, the average COD concentrations in effluent for phases 1 and 2 were 71 and 76 mg/L, respectively. The COD removal efficiency in phase 2 (86%) was slightly higher than phase 1 (83%). On the other hand, it was observed that the MO removal efficiency for both phases was approximately 99%.

In adsorption study, 10% removal of 100 mg/L MO was obtained with 10 g of spent GAC being used. The adsorption capacity of spent GAC was about 0.5 mg/g which indicated the adsorption process for removing MO can be ignored. Thus, the micro-organisms that growing in the spent GAC–biofilm-SBR was

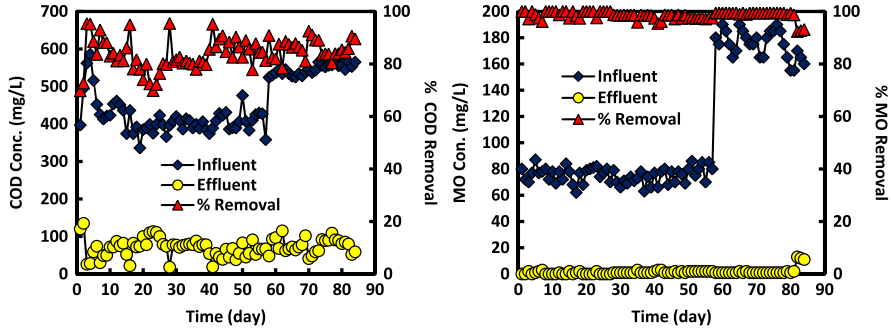

Fig. 3. COD (a) and MO (b) removal efficiency by spent GAC–biofilm-SBR.

Table 1
Treatment performance of MO-containing wastewater in phases 1 and 2

Duration	Samples	COD (mg/L)	Parameters	
			MO (mg/L)	Abs at 250 nm
Phase 1				
Days 1–56	Influent	419 ± 30	75 ± 7	–
	Effluent	71 ± 26	1.1 ± 1.5	0.58
	% Removal	83 ± 7	98.5 ± 1.45	–
Phase 2				
Days 57–84	Influent	541 ± 32	175 ± 4	–
	Effluent	76 ± 9	2.4 ± 9.2	0.82
	% Removal	86 ± 3	98.6 ± 4.8	–

responsible for removing the organic compounds and MO. The biological reactions in the bioreactor are supposed to be due to the microbial activity through the biofilm formation on the spent GAC. Organic compounds and MO are degraded both aerobically and anaerobically by the heterotrophic micro-organisms in the spent GAC–biofilm-SBR depending on the oxygen concentration within the bioreactor. From the ORP analysis on the surface, middle and bottom of the spent GAC–biofilm-SBR, the ORP values observed were approximately −80, −180, and −320 mV, respectively. According to Suthersan [22], redox potentials greater than 100 mV are commonly interpreted to indicate an aerobic environment, whereas ones less than −100 mV are to indicate an anaerobic environment.

There are a number of reports on the decolorization of dyes with the redox potential developed in the bioreactor. Redox potential has an influence on the decolorization rate under methanogenic conditions as reported in the batch assays [23,24]. Besides, laboratory-scale semicontinuous studies were conducted using simulated cotton dyeing wastewater at ambient temperatures (24–28˚C) by Manu and Chaudhari [25]. They observed that the reducing environment prevailing in the bioreactor (−299 to −360 mV) might have caused the color removal in Orange II and Black 3HN. In the present study, the high reductive and almost oxygen-free condition at the bottom GAC compartment might be suitable for the growth of anaerobic microbes, which were responsible for azo bond reduction in the MO and led to decolorization. As proposed by Parshetti et al. [34], the degradation of MO by *Kocuria rosea* formed 4-amino sulfonic acid and N,N-dimethyl p-phenylenediamine through cleavage of its azo bond by azo reductase. These intermediate compounds generally could not be further degraded under anaerobic condition and may contribute to the COD concentration in the effluent. Meanwhile, the low reductive condition at the top layer of GAC compartment might promote the growth of aerobic microbes responsible for organic compounds removal and mineralization of intermediate aromatic amines which formed from the reduction of the azo bond in MO. Besides, anaerobic and aerobic microniches may develop at the inner and outer biofilm of spent GAC surfaces that responsible for decolorization and mineralization of intermediate aromatic amines.

Decolorization of azo dyes-containing wastewater is not successfully performed under aerobic biological process because of their strong electron-withdrawing azo linkages, which protects them from attack by the oxygenases [26]. However, it is well known that azo bond is readily reductively cleaved under anaerobic conditions by micro-organisms [27,28]. The anaerobic condition at the lower layer of spent GAC–biofilm-SBR mainly responsible for the cleavage of azo bond in MO and the aromatic amines generated could be further degraded under aerobic condition at the upper layer of bioreactor. As a result, integrated anaerobic and aerobic conditions that developed in the spent GAC–biofilm-SBR were suitable for decolorization and mineralization of aromatic amines in textile wastewater.

The reduction of azo dyes by anaerobic consortia is often a co-metabolic reaction. The azo dyes are non-growth substrates and it can be decolorized by anaerobic consortia through the cleavage of the azo bonds. The reducing equivalents generated from various carbon sources are transferred to the dye [29]. In this co-metabolic reaction, azo dyes act as the terminal electron acceptors in the respiratory electron transfer chain [30]. However, the quantity of co-substrates such as sucrose, glucose, acetate, etc. which required for generating reducing equivalents is just a little. In the present study, the mass quantities of sodium benzoate and sodium acetate used as co-substrates were 0.107 g and 0.205 g, respectively, and giving 326 mg/L of COD. It was observed that the MO removal efficiency with this quantity of co-substrates was 98% for both phases.

The UV–Vis spectrum analysis was conducted for influent and effluent and the result is illustrated in Fig. 4. It can be seen that there are two absorption peaks in the spectrum of MO (270 nm and 460 nm) in the influent. The absorption peak in the visible region 460 nm for MO is associated with the azo bond (–N=N–) whereas the absorption peak in UV region 270 nm is referred to the aromatic compounds [31–33]. Parshetti et al. [34] observed that the absorbance at maximum wavelength (460 nm) was decreased with small shift towards lower wavelength after degradation of Methyl Orange by *Kocuria rosea* (MTCC 1532), which indicates the formation of other metabolites. In the present study, the absorption peak at 460 nm was disappeared and a new absorption peak at wavelengths around 250 nm in the effluent water sample was appeared (Fig. 4). According to previous reports [35,36], the decolorization of dyes can be due to the adsorption to the biomass or biodegradation. If the dye removal is attributed to the biodegradation, either the major absorbance peak in visible region will disappear or a new peak will appear in UV region. The diminished of absorbance peak at 460 nm and appearance of new peak at 250 nm in present study indicating the azo dye MO has been removed corresponding to the biodegradation and forming intermediate aromatic amines. From the literature, the UV–Vis peaks near to 250 nm could be caused by p-phenylenediamine group and sulfanilate ion. Parshetti et al. [34] reported that the initial step in the decolorization of MO involved symmetrical cleavage of azo bond and resulted in the formation of 4-amino sulfonic acid and N,N-dimethyl p-phenylenediamine.

The biological reduction of azo bond in MO caused the formation of intermediate aromatic amines [34,36] and it can be monitored on the absorbance at 250 nm. The intensity of absorbance at 250 nm was monitored daily in the effluent for phases 1–2, and the result is shown in Fig. 5. It was observed that the intensity of absorbance at 250 nm in effluent for both phases was considered low but it still contributed to the COD in effluent. Generally, the intermediate aromatic amines from anaerobic decolorization of azo dye resist further anaerobic degradation. Due to the non-degradative nature of these intermediate products, the COD concentration persists in the effluent [13,37]. The aerobic condition at the upper layer of spent GAC–biofilm-SBR could mineralize the intermediate aromatic amines that generated from the reduction of azo bond of MO at the lower layer of bioreactor. In order to remove all of the intermediate aromatic amines, supplementary aeration can be applied to the bioreactor. Thus, the integrated anaerobic and aerobic processes in the developed spent GAC–biofilm-SBR not only can remove the color but also able to mineralize the intermediate aromatic amines. Ong et al. [38] reported the using of GAC

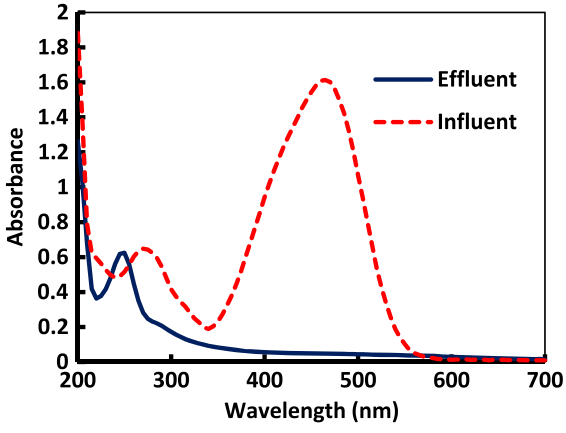

Fig. 4. UV–Vis spectrum analysis of influent and effluent.

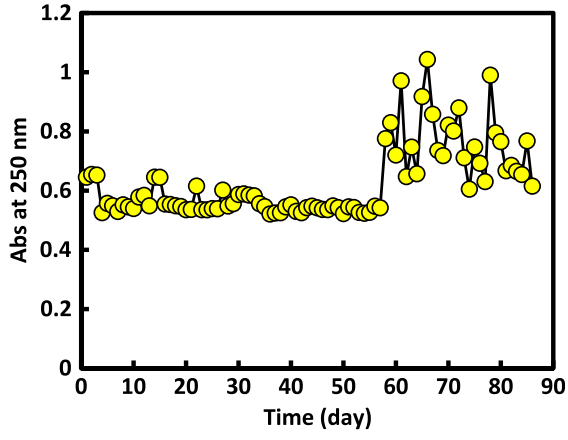

Fig. 5. Aromatic amines monitoring at absorbance 250 nm.

immobilized with azo dye-degrading microbes through attachment technique could nearly complete mineralized the Acid Orange 7-containing wastewater under SBR operation with initial Acid Orange 7 concentrations of 625 mg/L. The high performance in the decolorization of azo dye and mineralization of intermediate aromatic amines by spent GAC–biofilm-SBR shows the promising of this bioreactor in treating azo dyes-containing wastewater.

4. Conclusions

The spent GAC–biofilm-SBR showed high performance in color and organic compounds removal. With influent 541 mg/L COD and 175 mg/L MO, the bioreactor could remove 86% and 99% of COD and MO, respectively. The formation of intermediate aromatic amines after the reduction of azo bond can be observed with the appearance of new absorption peak in UV region through UV–Vis spectrum analysis. The anaerobic and aerobic regions that developed in the lower and upper layer of spent GAC–biofilm-SBR responsible for decolorization of the MO and mineralization of intermediate aromatic amines simultaneously in a single bioreactor.

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