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Biodegradation of redox dye Methylene Blue by up-flow anaerobic sludge blanket reactor

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Abstract

The objective of this study is to evaluate the decolorization of Methylene Blue (MB) by an up-flow anaerobic sludge blanket (UASB) reactor. The UASB reactor was operated under batch condition with total treatment volume of 3 l and operation time of 24 h per batch. It was found that the color of MB disappeared within a few minutes after entering into the UASB reactor due to reduction by anaerobic biomass. However, the reduced MB was re-oxidized again by air after discharged from the reactor and thus caused low color removal efficiency. The presence of suitable amount of organic content (sucrose and peptone) as an electron donor played an important factor for color removal. It was observed that more than 90% of color removal efficiency was achieved in the UASB reactor with 0.627 mmol l^{-1} of MB concentration and the presence of low amount of organic content (< 0.45 g COD/(l d)). Biological dye reduction kinetics depends on the concentration of dye and reducing equivalents. The kinetic behavior of MB biodegradation by microbes was also investigated to determine the model involved in the process.

Keywords: Methylene Blue; Autocatalysis; UASB; Anaerobic reduction; Dye

1. Introduction

One of the main problems associated with the treatment of textile wastewater is the removal of dyes. Apart from the aesthetic deterioration and obstruction for penetration of dissolved oxygen into the natural water bodies caused by the presence of color, some of the dyes, dye precursors and dye degradation products are carcinogenic and mutagenic in nature [1]. Azo and reactive dyes are electron deficient in nature and this property makes them less susceptible to oxidative catabolism [2]. They are also hydrophilic in nature and hence pass through the conventional aerobic process untreated. However, under strict anaerobic conditions, decolorization of dye can be gratuitously achieved and is well documented [3].

Addition of electron-donating primary substrates generally resulted in much higher dye decolorization rates because the oxidation of these compounds produced electrons used for the formation of reduced cofactors (FAD, FMN and NADH). It has been reported that the addition of external carbon sources such as glucose and acetic acid apparently stimulates the reduction of azo dyes [3–5]. The basal dye decolorization activity was supported by endogenous substrate in the sludge, possibly associated with hydrolysis of sludge biomass. Only a small portion of the sludge would have to be consumed to supply the required amount of reducing equivalents [6].

Some researchers had observed that the decolorization of azo dyes follows the first-order kinetic model [3,4,7–9]. The course of the decolorization process approximates the first-order kinetics with respect to the dye concentration [4,7,10,11], whereas other researchers found zero-order kinetics [12–14]. Besides, the autocatalytic behavior was also reported by some researchers in the anaerobic biological degradation and chemical reduction of Acid Orange 7. The autocatalytic nature is related to the generation of 1-amino-2-naphthol, an intermediate produced after anaerobic

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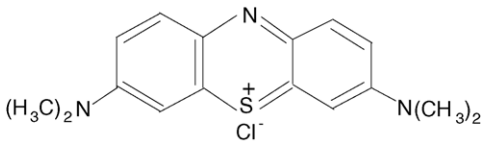

Fig. 1. Structure of Methylene Blue.

breakdown of dye, which acts as a redox mediator favoring the reduction of the dye [15,16].

In this study, the biodegradation behavior of Methylene Blue (MB) by microbes in up-flow anaerobic sludge blanket (UASB) and the kinetic model involved were investigated. The bio-chemical reactions between MB and activated sludge microbes were also discussed.

2. Materials and methods

The Methylene Blue used as a model for evaluation in this study was obtained from Chroma Ltd. and the molecular structure is shown in Fig. 1. A lab-scale UASB reactor (4 cm Ø × 80 cm H) was fed with 3 l of synthetic wastewater per batch per day. For Terms 1–2, the composition and concentration of the synthetic wastewater used were (mg/l): bacto-peptone (188), sucrose (563), NH_4Cl (344), $MgSO_4$ (49), $FeCl_3$ (11.3) and KH_2PO_4 (318), whereas the concentration of synthetic wastewater used in Terms 3–4 was reduced to about 0.15 times from Terms 1–2. As a result, the organic loading rate used in Terms 1–2 and Terms 3–6 was 2.88 and 0.45 g COD/(l d), respectively. The reactor was seeded using activated sludge from a municipal wastewater treatment plant. The mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) at start up were about 5100 and 3400 mg/l, respectively. When the activated sludge was acclimatized to the synthetic wastewater, MB in various concentrations was added into the synthetic wastewater. The operating condition studied in this work is shown in Table 1. The effluent from reactor was collected daily and analyzed for COD and MB concentrations following the Standard Methods. The MB concentrations were measured by UV–vis spectrophotometer (UV-1200, Shimadzu Co. Ltd.) at λ_{max} 661 nm. The determination of suspended solids, mixed liquor suspended solids and mixed liquor volatile suspended solids concentrations followed the Standard Methods.

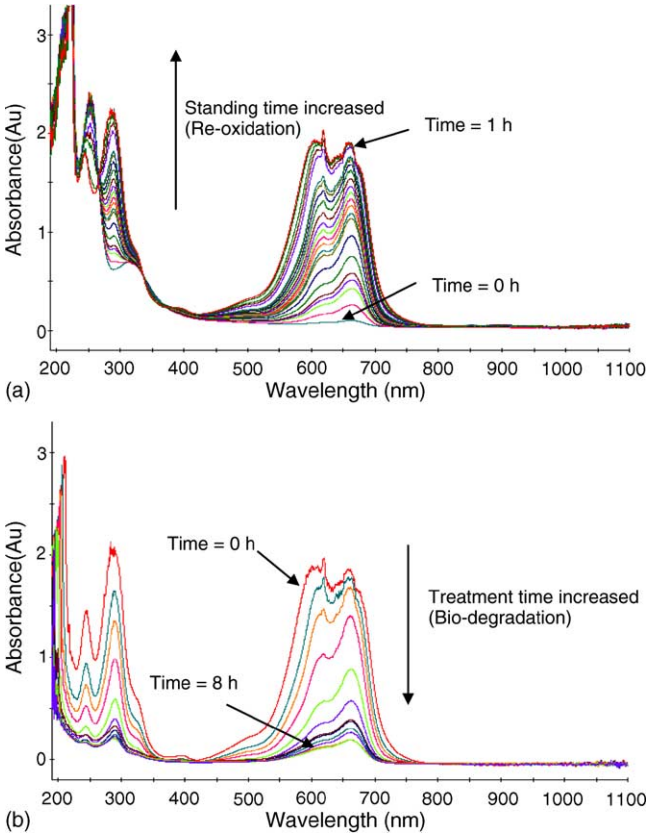

Fig. 2. (a) Re-oxidation of MB, (b) biodegradation of MB in UASB system.

3. Result and discussion

3.1. Bio-chemical reaction of MB in UASB reactor

Methylene Blue is one of phenothiazine dyes with a planar structure. Its formal potential, E_o, is between −0.1 and −0.4 V in solutions with pH between 4 and 11 [17]. The redox potential of MB might enable it to play an important role in the transport of electron to the dye itself, thus giving the whole process as autocatalytic. By passing the MB-containing wastewater through UASB reactor, it was observed that the color of MB disappeared quickly once it entered the reactor. However, the wastewater was slowly turned into blue when it discharged from the reactor. To investigate this behavior, the colorless wastewater was collected from the top of the reactor and analyzed with UV–vis. Fig. 2(a) shows the change of UV–vis spectrum of MB with

Table 1
Treatment conditions in UASB system

Term	MB loading rate (mmol l^{-1})	Organic loading rate (g COD/l d)	Operation time (h/batch)
1	0.062	2.88	24
2	0.157	2.88	24
3	0.157	0.45	24
4	0.313	0.45	24
5	0.470	0.45	24
6	0.627	0.45	24

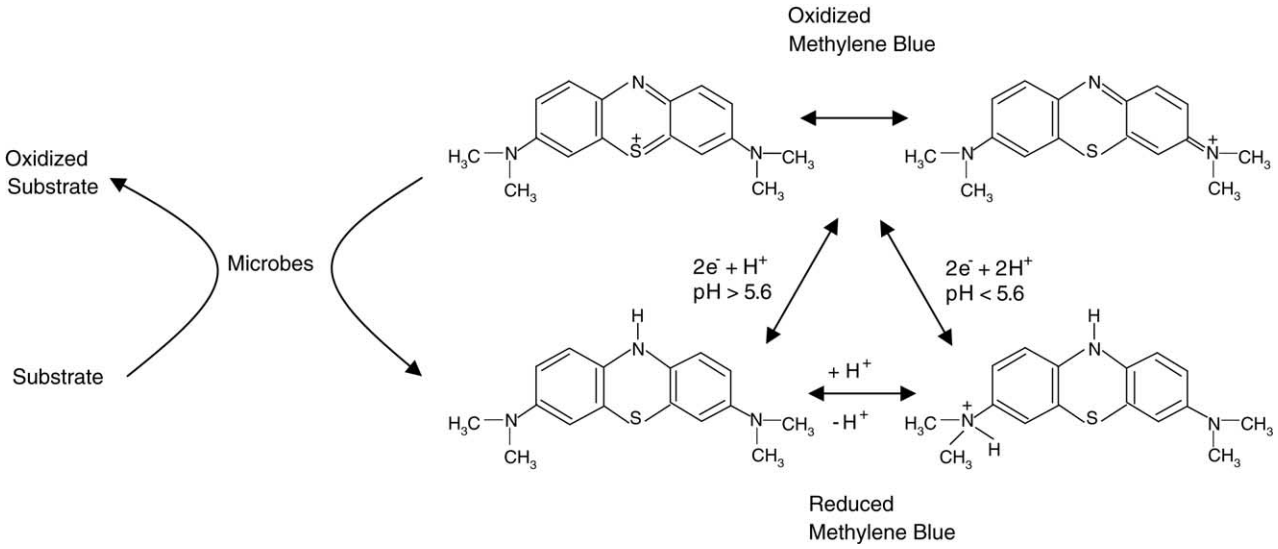

Fig. 3. Scheme depicting the redox mediation of Methylene Blue.

a prominent absorption peak at around 660 nm (monomer) and a hump at around 610 nm (dimer). The colorless sample collected from the top of the reactor was exposed to air for about 1 h and the spectrum was monitored at certain time interval. As the standing time proceeded (0–1 h), the peaks at 280, 610 and 660 nm were increased, whereas the peak at 250 nm was in contrary. This indicated that there were some bio-chemical reactions involved between the MB and microbes in the reactor. Fig. 3 shows the schematic represent the way of MB can be reduced in the experiment system used in this study. The substrates (organic content) used were sucrose and peptone. When the microbes oxidized the substrates, electron produced will be accepted by MB (oxidized form) and the reduced form of MB is colorless. As the colorless wastewater was exposed to atmosphere, the reduced MB was re-oxidized by air and the color appeared gradually as time proceeded. A redox mediator should be cycled from its oxidized and reduced states and thus should be very effective at catalytic concentrations [18]. Thus, the capability of MB being reduced and oxidized in the UASB reactor might enable it to play as a redox mediator such as riboflavin, anthraquinone-sulfonate and anthraquinone-disulfonate, in the biodegradation of dye. Decolorization in anaerobic sludge environments is a combined process of biotic and abiotic reactions. Azo dyes can be reduced in a direct chemical reaction with bulk biogenic reducing agents, but they can also be reduced by biological reactions, either directly as an enzymatically catalyzed reaction or indirectly via reduced enzyme cofactors [19]. The indirectly biological reduction (redox mediator catalyzed) of the dye, MB, which acts as a redox mediator, may cause the increment of the biodegradation rate. Fig. 2(b) shows the UV–vis spectrum for MB, which monitored as a function of treatment time (0.0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 h). As the biodegradation time proceeded (0–8 h), all of the peaks at 250, 280, 610 and 660 nm were decreased gradually indicating decolorization. Fig. 2(a and b) very clearly demonstrate that the spectrum of biodegradation and re-oxidized of MB was different.

3.2. Treatment performance in UASB reactor

Fig. 4 depicts the COD and MB removals in the UASB reactor after 24 h of treatment. In Terms 1 and 2, the COD and MB removals were low as compared to Terms 3–6. This could be due to the organic loading rate (2.88 g COD/(l d)) used in Terms 1 and 2 was too high compare to the MB concentration. The high concentration of readily biodegradable carbon source induced the microbes to degrade the sucrose and peptone for energy. As a result, the organic loading rates added to the UASB reactor decreased daily and it was found that the MB removal efficiency was improved. At 0.45 g COD/(l d) of organic loading rate, the MB and COD removals were optimum. As shown in Fig. 4, the average of COD and MB

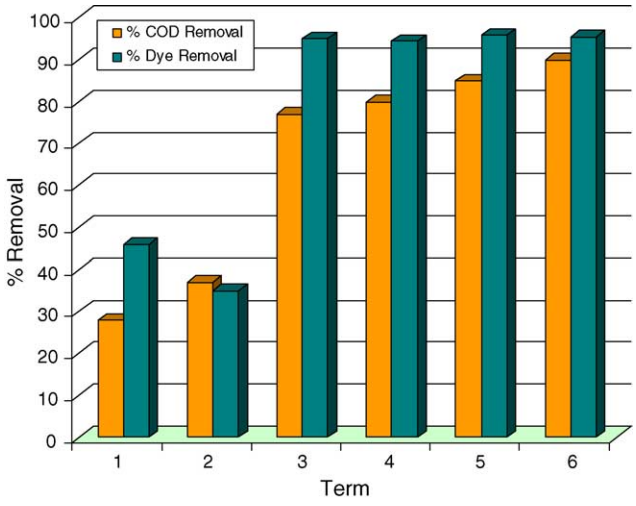

Fig. 4. Treatment performance in UASB system.

removal efficiencies with 0.45 g COD/(l d) of organic loading rate was 83 and 95%, respectively. This result shows that the presence of suitable amount of organic content (sucrose and peptone) as an electron donor played an important factor for color removal efficiency. Once the external carbon source was terminated, the MB removal efficiency decreased gradually until about 25–30%. Although without external electron donor, the reaction depended fully on the production of endogenous reducing power [6]. Thus, over or under dosage of organic content would have adverse effect on COD and dye removals.

3.3. Kinetic study

Several studies indicate that the main limiting factor of the reduction of azo dyes by anaerobic sludge is electron transfer. In order to accelerate the reduction of azo dyes by microorganisms in the sludge, redox-mediating compounds

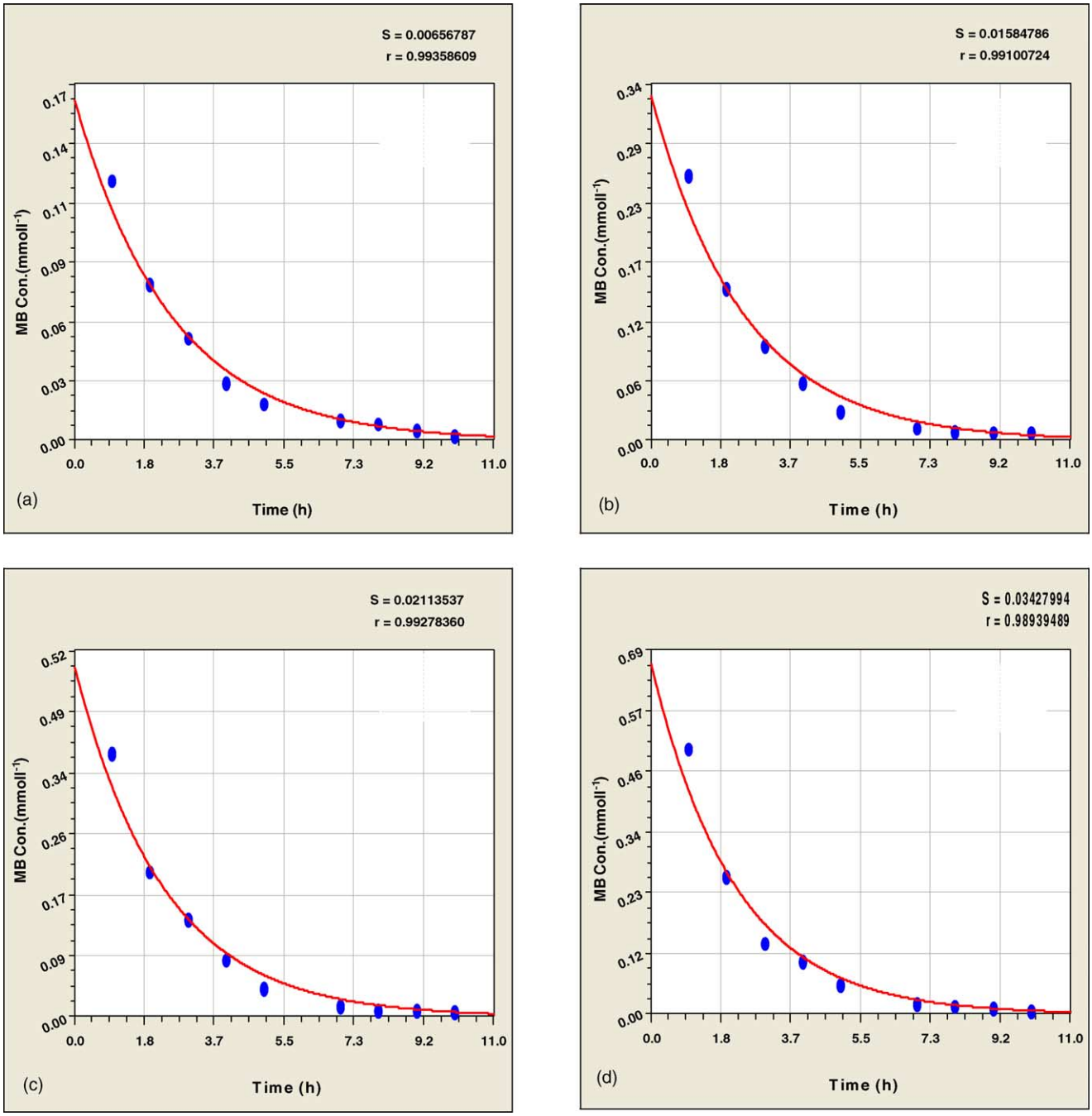

Fig. 5. MB concentrations decay curve in UASB system. Experimental data (●) and calculated lines based on the first-order model (−). (a) 0.157 mmol l^{-1}; (b) 0.313 mmol l^{-1}; (c) 0.470 mmol l^{-1}; (d) 0.627 mmol l^{-1}.

can be considered. Previous works have explored the use of quinine compounds and flavins in accelerating the rate of azo dye reduction [6,7,16,18,20,21]. As discussed in previous section, the MB used in this study can act as a redox mediator, thus giving to the whole process an autocatalytic in nature. The kinetic models used to describe the experimental data obtained were the first-order and autocatalytic kinetic models. The autocatalytic model was previously used by Van der Zee et al. [16] and Mendez-Paz et al. [15] to study the autocatalytic role in the chemical reduction and anaerobic biodegradation of Acid Orange 7.

The first-order decolorization rates were calculated by using equation stated below:

$$r = \frac{-\mathrm{d}C}{\mathrm{d}t} = kC \quad (1)$$

$$C_t = C_0 \mathrm{e}^{-kt} \quad (2)$$

where k is the first-order rate constant (per day), C_t the concentration at time t and C_0 is the initial concentration. Fig. 5 depicts the MB concentration decay curve in UASB system. It was observed that the concentration of MB decreased along with reaction time and almost 95% removal efficiency was

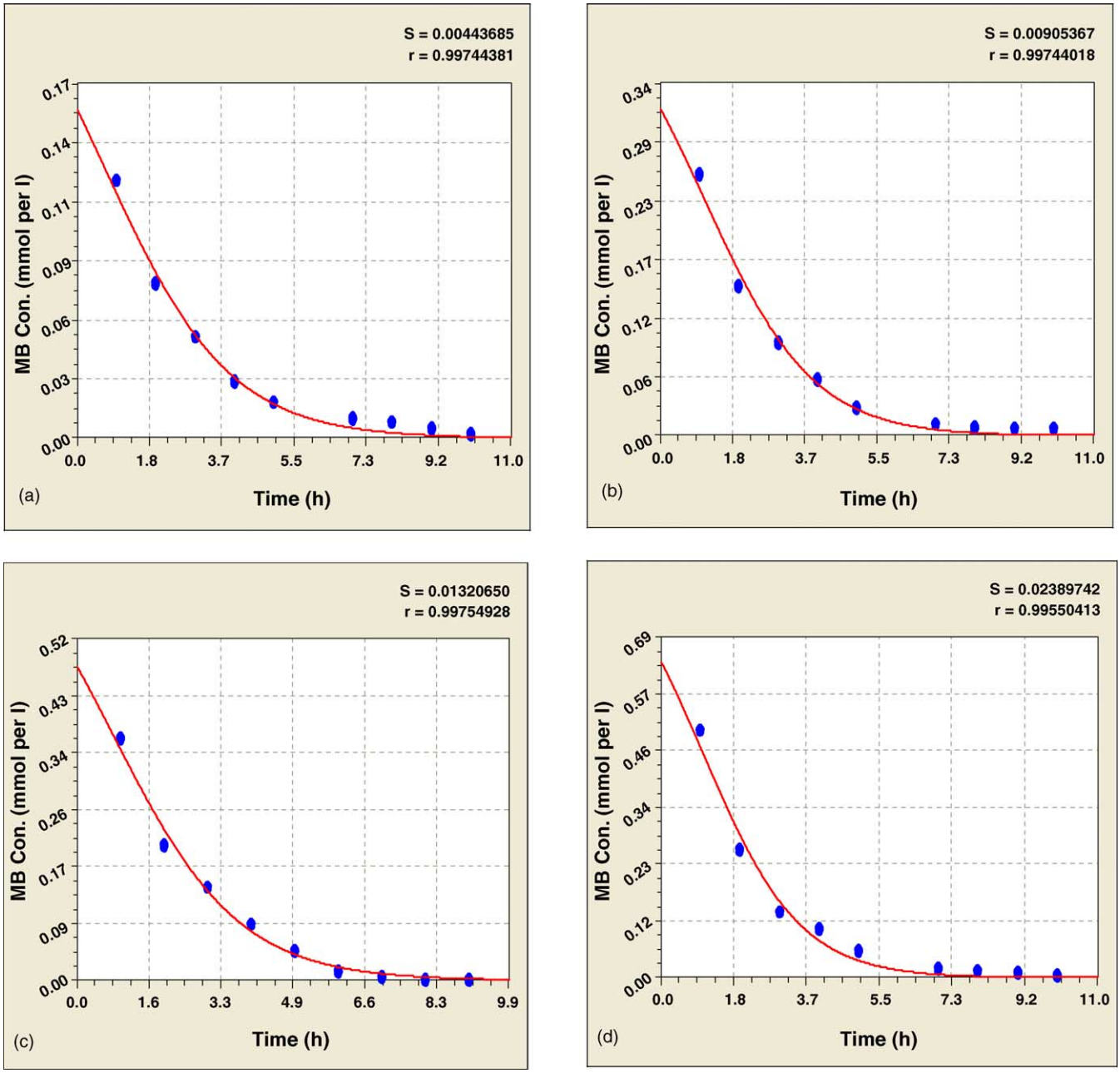

Fig. 6. MB concentrations decay curve in UASB system. Experimental data (●) and calculated profiles (−) with the autocatalytic kinetic model. (a) 0.157 mmol l^{-1}; (b) 0.313 mmol l^{-1}; (c) 0.470 mmol l^{-1}; (d) 0.627 mmol l^{-1}.

Table 2
First-order kinetic constants and correlation factor obtained

Term	k (h^{-1})	R^2
3	0.396	0.994
4	0.412	0.991
5	0.428	0.993
6	0.455	0.989

achieved after 8 h of operation time in all cases. The first-order reaction rate constants were evaluated and the data are shown in Table 2. The result showed that the increase of MB concentration from 0.157 to 0.627 mmol l^{-1} enhanced the kinetic constant from 0.396 to 0.455 h^{-1} or about 15% improvement.

The autocatalytic model was previously used by Mandez-Paz et al. [15] and Van der Zee et al. [16] in the modeling of Acid Orange 7 reduction.

$$r = -\frac{dC}{dt} = k_1 C + k_2 C(C_0 - C)^a \quad (3)$$

Where k_1 is first-order kinetic constant and k_2 is correlated to autocatalytic constant. With the factor a equal to 1 as stated by Mandez-Paz et al. [15], Eq. (4) is obtained after integration.

$$C_t = \frac{C_0(k_1 + k_2 C_0)e^{-(k_1+k_2C_0)t}}{(k_1 + k_2 C_0 e^{-(k_1+k_2C_0)t})} \quad (4)$$

The maximum removal rate (r_{max}), as shown in Eq. (5), can be calculated by derivation of Eq. (3) equal to 0.

$$r_{max} = \frac{k_1(k_1 + k_2C_0)}{2k_2} + \left(\frac{1}{2}\right)(k_1 + k_2C_0) \times \left[\frac{C_0 - (k_1 + k_2C_0)}{2k_2}\right] \quad (5)$$

As shown in Table 3 and Fig. 6, the data obtained can be fitted very well with the autocatalytic model with $R^2 > 0.995$ in all cases. The successful in the application of the autocatalytic kinetic model indicated that the autocatalytic process occurred and played an important role in the biodegradation of MB in UASB reactor. The increase of MB concentration in UASB reactor had resulted in the increase of maximum removal rate, which is calculated from Eq. (5). Fig. 7 shows the removal rate of MB, which was calculated from the autocatalytic model. It was observed that the maximum removal rate was achieved after 1 h treatment by UASB reactor.

Table 3
Kinetic constants, correlation factor and maximum removal rates calculated with the autocatalytic model

Term	k_1 (h^{-1})	k_2 (l mmol^{-1} h^{-1})	R^2	r_{max} (mmol l^{-1} h^{-1})
3	0.249	2.375	0.997	0.0407
4	0.221	1.596	0.997	0.0813
5	0.253	0.933	0.998	0.1282
6	0.241	0.910	0.996	0.1809

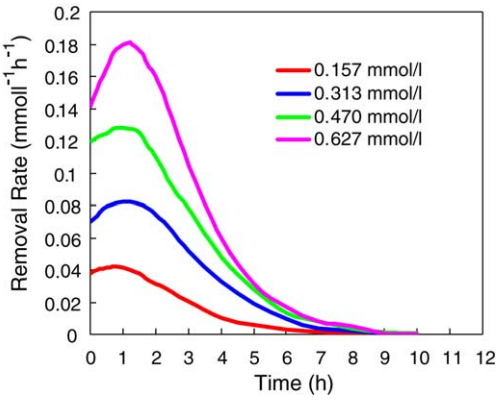

Fig. 7. MB removal rate profiles calculated from the autocatalytic kinetic model. (a) 0.157 mmol l^{-1}; (b) 0.313 mmol l^{-1}; (c) 0.470 mmol l^{-1}; (d) 0.627 mmol l^{-1}].

4. Conclusion

- In the presence of suitable amount of organic content (sucrose and peptone), the COD and MB removal efficiencies in UASB reactor were able to achieve about 83 and 95%, respectively. Over or under dosage of organic substrates would have adverse effect on the treatment efficiency.
- The ability of MB being reduced and oxidized enabled it to play as a redox mediator in biodegradation of dye itself, thus giving to the whole process an autocatalytic in nature.
- The successful in the application of autocatalytic kinetic model with the data obtained in kinetic study indicated that the autocatalytic process occurred and played an important role in the biodegradation of MB in UASB reactor.
- The increase of MB concentration had resulted in the increase of maximum removal rate of dye. From the result obtained, the maximum removal rate of MB was achieved after 1 h treatment by UASB reactor.

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