Desalination and Water Treatment
www.deswater.com

doi: 10.1080/19443994.2013.782255

51 (2013) 7621–7630
December

Taylor & Francis
Taylor & Francis Group

Decolorization of methyl orange using upflow anaerobic sludge blanket (UASB) reactor—An investigation of co-substrate and dye degradation kinetics

V. Murali[a], Soon-An Ong[a,*], Li-Ngee Ho[b], Yee-Shian Wong[a]

[a]*School of Environmental Engineering, University Malaysia Perlis, Arau, 02600 Perlis, Malaysia*
Tel. +60 4 9798986; Fax: +60 4 9798636; email: saong@unimap.edu.my
[b]*School of Materials Engineering, University Malaysia Perlis, Arau, 02600 Perlis, Malaysia*

Received 20 November 2012; Accepted 18 February 2013

ABSTRACT

Decolorization of a synthetic wastewater containing Methyl Orange (MO) azo dye was performed in an upflow anaerobic sludge blanket (UASB) reactor. Color removal efficiencies approaching 94, 90 and 96% were obtained with influent MO concentrations of 50, 100 and 150 mg l^{-1} respectively. COD removal decreased with respect to the increasing of color concentration. Maximum COD removal was attained 69% in 50 mg l^{-1} of MO. Cleavages of azo bond tends to the accumulation of aromatic amines which contributed COD in the effluent. Zero-, first-, and second-order reaction kinetics were used to find out the suitable COD removal and decolorization kinetics. The COD removal process was suitable to second-order reaction kinetic, and the degradation of MO approximates was suitable to the first-order kinetic model. The regression coefficient (R^2) for both decolorization and COD degradation was around 0.9 which ensures the high-degree linear relationship between the concentration and time.

Keywords: UASB reactor; Methyl Orange; Azo dye; Color removal; Kinetic model

1. Introduction

Azo dyes are playing a major role in industries such as the textile, paper, printing, food, leather, pharmaceutical and cosmetic. These industrial discharges cause impacts to the environment and human health. Textile azo dye processing plant effluents are mutagenic and carcinogenic if there are not properly treated [1]. Sulfonated azo dye affects the nitrogen transformation processes in soil [2]. Treatment is the option to overcome these issues due to azo dye discharges. Many attempts were made in physical and chemical treatments for different azo dyes [3–6]. Adsorption, chemical precipitation, photo-catalytic, and electrochemical treatment are some of physical/chemical treatment methods for the removal of dyes from wastewater, which encounters the following drawbacks such as being economically unfeasible, unable to completely remove the azo dyes, generating a significant amount of sludge that may cause secondary pollution problems and involving complicated procedures. Transform the pollutants into less harmful forms, being cost-competitive, producing less sludge and being eco-friendly are the merits of biological system over the others [7]. Thus, the best choice for the

---

*Corresponding author.

azo dye treatment is using biological process. This study deals about decolorization of mono azo dye (Methyl Orange) in a laboratory-scale upflow anaerobic sludge blanket (UASB) reactor.

Limited studies have been performed to investigate the decolorization of Methyl Orange (MO) using biological processes. Cai et al. [8] investigated the decolorization of Methyl Orange by a specific anaerobic microorganism (Shewanella oneidensis MR-1) and reported 80% removal efficiency. Adsorption in anaerobic sludge and kinetic study of MO degradation in the anaerobic sequential batch reactor was studied by Yu et al. [9]. Several studies have reported that UASB reactor is suitable for the degradation of azo dye among various anaerobic biological processes. Isik and Sponza [10] performed a study about the effects of substrate concentrations and alkalinity on the decolorization of Congo Red in UASB reactor. They observed 100% color removal by using the UASB reactor. Anaerobic process is more efficient than aerobic process for azo dye removal, and it was depicted in the study conducted by Ong et al. [11]. The degradation performance of Orange II by UASB reactor and sequential batch reactor for was evaluated and high efficiency was obtained in UASB reactor [11]. The same author also performed a study on the degradation of redox dye Methylene Blue by UASB reactor [12]. Co-substrate plays an important role in the azo dye treatment using UASB reactor [13]. To strengthen the above statement, Baêta et al. [14] investigated the anaerobic degradation of the azo dye Drimaren blue HFRL in a bench-scale UASB reactor and reported color removal increased from 62 to 93% due to increasing co-substrate from 100 to 500 $mg\,l^{-1}$. All the above investigations ensures that the followings; MO can be treatable using anaerobic microorganisms, UASB reactor is one of the best among the various biological anaerobic system for azo dye degradation and co-substrate is essential to enhance the efficiency. From the literature review, the biodegradation of MO using UASB reactor is not documented. This kind of attempt may bring out the treatability of MO using UASB reactor and provide the scope of treating real wastewater along with MO. The aim of this study is to investigate the treatment performance of UASB reactor on mono azo dye MO containing synthetic wastewater. The co-substrate employed in this study were sodium benzoate and sodium acetate. The effects of MO concentrations on decolorization and COD removal rates using UASB reactor were investigated. Kinetic for decolorization of the MO and degradation of organic contents was evaluated using the zero-, first-, and second-order kinetic model.

2. Materials and methods

2.1. Chemicals

Methyl Orange ($C_{14}H_{14}N_3NaO_3S$) was supplied by Sigma Aldrich. The MO is a mono azo dye with λ_{max} at 460 nm, and the chemical structure is shown in Fig. 1. The synthetic wastewater consisted of organic carbon, nutrients, and buffer solution. The composition of synthetic wastewater is (concentration in $mg\,l^{-1}$): C_6H_5COONa (107.1), CH_3COONa (204.9), NH_4Cl (95.18), NaCl (7.0), $MgCl_2{\cdot}6H_2O$ (3.4), $CaCl_2{\cdot}2H_2O$ (4.0), $K_2HPO_4{\cdot}3H_2O$ (36.7) giving COD concentration 326 $mg\,l^{-1}$, T-P 5.0 $mg\,l^{-1}$, and NH_3-N 24.58 $mg\,l^{-1}$. Sodium benzoate and sodium acetate are the carbon sources in the synthetic wastewater, and the other compositions are acting as nutrient and buffer solution. All other chemicals were of analytical grade.

2.2. Experimental setup and operating conditions

A laboratory scale UASB reactor had 2.5 l of working volume with a diameter of 7.2 cm and 70 cm height (Fig. 2). The gas–liquid–solid separator was fixed at the top, consisting of an inverted funnel for the collection of biogas, and a gas collection bag was connected to it. An experiment was conducted as a semi-batch with a 24 h retention time at room temperature. The reactor inoculated using sludge collected from the biological wastewater treatment plant of SHORUBBER (M) SDN BHD, Malaysia. Magnetic stirrer was kept under the reactor to maintain the sludge under suspension. Initial run was started with synthetic wastewater as the influence of 326 $mg\,l^{-1}$ COD and operated until the stabilization of COD removal. After the 28-day initial run, 50 $mg\,l^{-1}$ MO was introduced along with synthetic wastewater which noted as phase 1. Duration of phase 1 was 35 days and phase 2 executed with MO concentration of 100 $mg\,l^{-1}$ for 32 days. Phase 3 was conducted up to 36 days with MO concentration of 150 $mg\,l^{-1}$. In each phase, three sets of hourly data were collected from 0 h to 14 h for kinetic study.

2.3. Analytical procedures

The effluent from the UASB reactor was analyzed for COD, concentration of MO, aromatic amines, and ammonia nitrogen (NH_3-N) after filtering through a

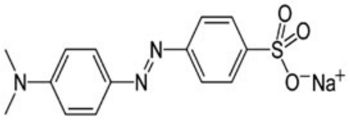

Fig. 1. Chemical structure of Methyl Orange.

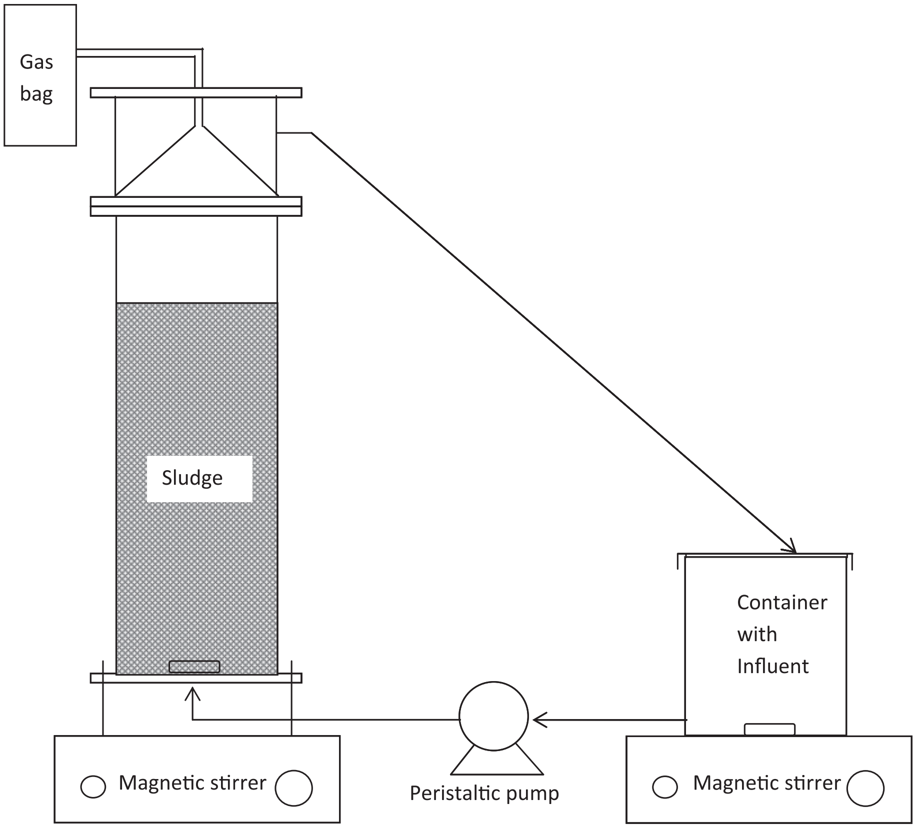

Fig. 2. Schematic diagram of upflow anaerobic sludge blanket reactor.

membrane filter of 0.45 μm. Analyses were carried out by duplicated samples. Concentration of COD and NH_3-N were determined using HACH DR2800 spectrophotometer. The MO concentration was estimated from the standard curve of dye vs. optical density at its maximum absorption wavelength ($\lambda_{max} = 460$ nm) using a UV–vis spectrophotometer (HITACHI U-2800, Japan). The aromatic amines were determined using UV–vis spectrophotometer (HITACHI U-2800, Japan) at wavelength 254 nm. pH, dissolved oxygen (DO) and oxidation reduction potential (ORP) were monitored for entire study with pH meter (MARTINI, Mi 151), DO meter (HANNA, HI9146) and ORP meter (HANNA, HI98201), respectively. For kinetic study, samples were collected at every hour interval until the 14th hour of operation. All the hourly samples were filtered through a membrane filter of 0.45 μm before estimating the COD and color concentrations.

3. Results and discussion

3.1. Treatment performance of the UASB reactor

3.1.1. COD removal

The performance of the UASB reactor was evaluated based on the removal efficiency of the COD, color and aromatic amines. COD contributed by the synthetic wastewater and concentration of MO. The variation of COD concentration and MO concentration from phase 1 to 3 was depicted in Fig. 3. In phase 1 (35 days), the average influent, effluent and percentage removal of COD were 385.6 mg l^{-1}, 120.6 mg l^{-1} and 68.7%, respectively. Standard deviation (SD) in phase 1 for effluent COD concentration and COD removal percentage was higher than the other two phases which was ascribed to the adaptation of microorganisms to the MO containing wastewater influent. During phase 2 (32 days), the average influent, effluent, and percentage removal of COD were 425.5, 150.5 mg l^{-1}, and 64.6%, respectively. In phase 3 (36 days) the average influent, effluent and percentage removal of COD was depicted as 487.1, 254.1 mg l^{-1} and 47.8%, respectively. The COD concentration in the influent was due to the co-substrate (sodium benzoate and sodium acetate), MO and aromatic amines. The sodium benzoate and sodium acetate are more biodegradable than aromatic amines. The degradation of this co-substrate releases the electron that support to azo bond cleavages in the decolorization process. Increasing MO concentration from phase 1 to 3 was responsible for the increasing aromatic amines in the

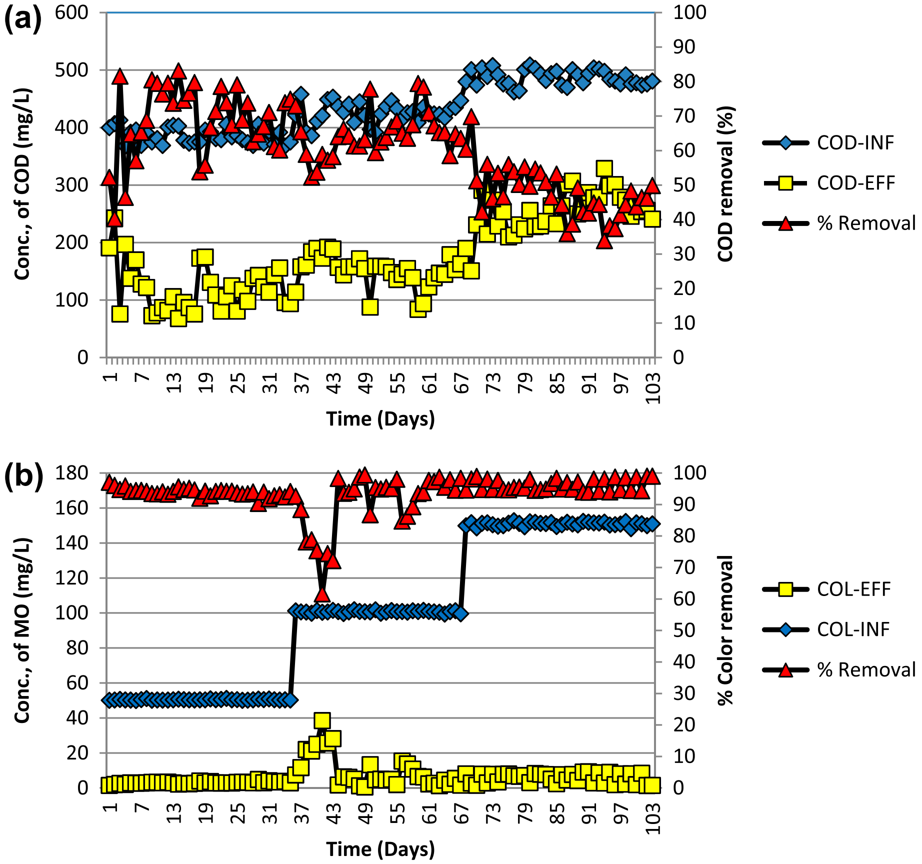

Fig. 3. COD concentration (a) and concentration of MO (b) in influent, effluent and% removal from Phase 1, 2 and 3.

effluent. The cleavage of azo bond in the MO results the formation of aromatic amines [15]. As shown in Table 1, the aromatic amines were increased from phase 1 to 3 and the accumulation of these aromatic amines caused the increase in COD concentration in effluent. This is in agreement with Spagni et al. [16] who reported COD removal was affected by the presence of aromatic amines from cleavages of azo bond. Wijetunga et al. [17] reported that the COD concentration in effluent represents the dye metabolites or unconsumed co-substrate. Brás et al. [18] also observed the COD removal deteriorated with the increase in dye concentration due to the metabolites of dye in effluent. In this study, the degradation of acetates was not affected by the presence of azo dye. Fig. 4 shows the hourly changes in UV–vis spectrum of the effluent. In zero hour three peaks were noticed which two peaks at 460 nm and 249 nm are due to the azo linkages and aromatic amines in MO. The third peak at 222 nm in the UV region represents the sodium benzoate. The peak at 222 nm was disappeared in the first hour of operation and this shows the degradation of sodium benzoate in short time by microorganisms in the UASB reactor. The azo peak at 460 nm decreased with respect to operation time and simultaneous increase the aromatic amine peak at 249 nm which depicts the cleavage of azo bond tends to the formation of aromatic amines. Effluent water sample (24 h) contains only one highest peak at 249 nm shows the accumulation of aromatic amines which contributed COD in the effluent.

3.1.2. Color removal

Some of the recent researches on the decoloraization of azo dyes were evaluated under anaerobic, aerobic, and sequential anaerobic–aerobic environments [19–23]. Anaerobic environment of this study was confirmed by the parameters like ORP and NH_3-N. The average ORP in effluent was noted as −158.2 mV, −200.4 mV, and −172.0 mV in phase 1, 2, and 3, respectively. Maas and Chaudhari [24] experienced decoloration of dye initiated at redox potential around −150 mV. In contradict; Coughlin et al. [25] reported the complete mineralization of mono azo dye Acid Orange 7 was attained in aerobic environments. Since

Table 1
Treatment performance of UASB reactor in Phase 1, 2, and 3

Phase	Duration	Parameters	Influent			Effluent			% Removal		
			Min	Max	Avg ± SD	Min	Max	Avg ± SD	Min	Max	Avg ± SD
1	Days 1–35	COD[a]	368	413	385.63 ± 13.65	68	243	120.66 ± 41.22	40.3	83.1	68.7 ± 10.25
		MO[a]	49.98	51.21	50.46 ± 0.28	1.476	4.884	3.04 ± 0.64	90.39	97.06	93.98 ± 1.26
		AA[b]	–	–	–	0.375	1.701	1.23 ± 0.23	–	–	–
		ORP[c]	–	–	–	−207	−103	−158.2 ± 26.46	–	–	–
2	Days 36–67	COD	386	458	425.56 ± 18.05	84	192	150.59 ± 27	52.3	79.5	64.6 ± 6.38
		MO	99.44	101.89	100.77 ± 0.61	0.609	38.508	9.64 ± 9.5	61.66	99.4	90.44 ± 9.43
		AA	–	–	–	1.488	2.31	1.98 ± 0.27	–	–	–
		ORP	–	–	–	−384	−112	−200.4 ± 54.87	–	–	–
		NH3–N[a]	21.6	23	22.12 ± 0.39	16.8	19.8	17.79 ± 0.81	13.91	25	19.56 ± 3.51
3	Days 68–103	COD	462	509	487.17 ± 12.96	151	329	254.17 ± 36.09	33.94	69.86	47.82 ± 7.32
		MO	148.26	152.76	150.94 ± 1.05	1.44	9.24	5.71 ± 2.77	93.92	99.04	96.22 ± 1.83
		AA	–	–	–	2.102	4.00	2.57 ± 0.4 1	–	–	–
		ORP	–	–	–	−268	−101	−171.97 ± 43.2	–	–	–
		NH_3-N	21.2	22.9	22.12 ± 0.45	17.2	20.8	18.81 ± 1.14	4.17	21.36	14.96 ± 4.95

[a] Unit for COD, MO & NH3-N—mg l-1, [b] Unit for AA—Absorbance, [c] Unit for ORP — mV.

this ORP results (less than −100 mV) show the highly reductive conditions were developed that confirm the anaerobic environment in the system [26].

The percentage removal of NH_3-N in phase 2 and 3 was 19.5 and 14.9%, respectively. Nitrification may take place to oxidize the NH_3-N into other forms of nitrogen in an aerobic environment [27]. A minimum amount of nitrite is required for the anaerobic ammonium oxidation (anammox) reaction to convert NH_3-N into nitrogen gas [28]. The nutrient used in this research contains nitrogen only in the form of ammonium chloride so that very less chance for anammox reaction which results very less percentage removal of NH_3-N. In addition to that Martin et al. [29] reported NH_3 removal affected due to azo dye (acid black 1) in a sequencing batch reactor.

The concentration of MO in influent, effluent, and removal efficiency throughout the study was depicted in the Fig. 3(b). The average percentage color removal in phase 1, 2, and 3 were 93.9, 90.4, and 96.2%, respectively. In phase 1, the color removal ranges from 90 to 97% and not much variation in the removal efficiency. MO concentration other than adsorbed on anaerobic sludge experiences biodegradation in this phase. Yu et al. [9] reported saturated MO adsorption on anaerobic sludge on certain period. After the adsorption saturated, the entire concentration of MO undergoes biodegradation. This may cause gradual increase in MO concentration for biodegradation in phase 1. In transition from phase 1 to 2 experience the sudden increase in MO concentration for biodegradation which tends to fluctuation in color removal and it was noticed in the starting period of Phase 2. Adaptability of the microorganisms at higher concentration of MO could be one of the reasons in the variation of color removal during the starting period of phase 2. Maximum color removal was reported as 99% in phase 2 and 3 which was higher than the phase 1 which indicated that the microorganism convenience with the environment. Azo bond and aromatic amines are the two major components in MO. The azo bond cleavage results the accumulation of aromatic amines in the UV region [30].

Fig. 5 shows the variation of ratio between the absorbance of aromatic peak and azo peak noted from hourly samples. The values depicted are directly proportional to the aromatic amines and inversely proportional to the azo bond. The graph shown a raising trend ensured the decreasing peak in azo and increasing in aromatic amines. In the zero hour, ratio of aromatic and azo peak was nearly same in all three phases. From first hour onwards, the degradation started and stabilized after 11th hour. In 12th, 13th, and 14th, hour data show the increase with respect to

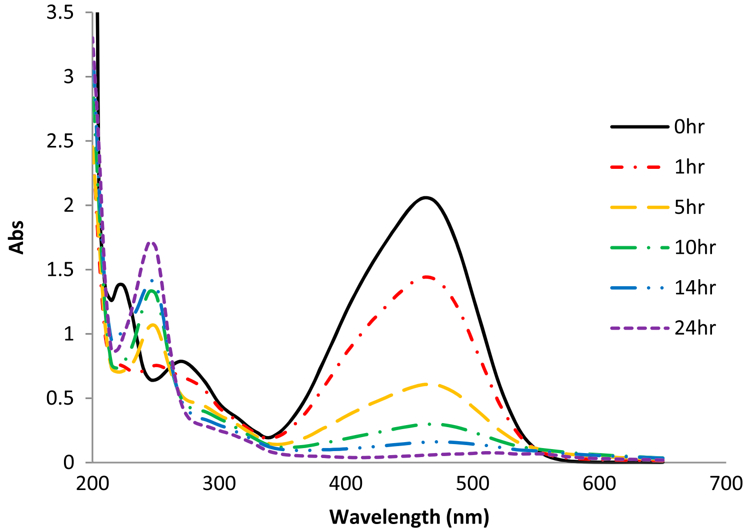

Fig. 4. Hourly changes in UV–vis spectrum of MO samples.

MO concentration which ensures that higher concentration of MO responsible for higher aromatic amines accumulation. From UV–vis analysis, only the aromatic peak was observed in the water samples of effluent (Fig. 4). The area of aromatic peak increased with respect to time and reached maximum in the effluent which confirm not complete mineralization of aromatic amine was carried out in the system. Higher color removal was observed in the effluent due to the complete cleavage of azo bond. Cleavage of azo bond with respect to time in the visible region of wavelength scan for hourly samples (Fig. 4). Most of the researches for azo dye degradation in anaerobic reported that the decolorization rather than complete mineralization in an anaerobic environment [10–12,15].

3.2. Kinetic studies on COD and color degradation

Fig. 6(a) and (b) shows the hourly monitoring of COD and MO in phase 1, 2 and 3. The removal rates of COD and MO under UASB reactor operation were evaluated using zero-, first- and second-order kinetic models. For the COD degradation kinetic, the obtained experimental data were plotted by S vs. time, $\ln S$ vs. time, and $1/S$ vs. time using the following Eqs. (1), (2), and (3), respectively [31].

$$S_t = S_0 - K_0 t \tag{1}$$

$$S_t = S_0 e^{-K1t} \tag{2}$$

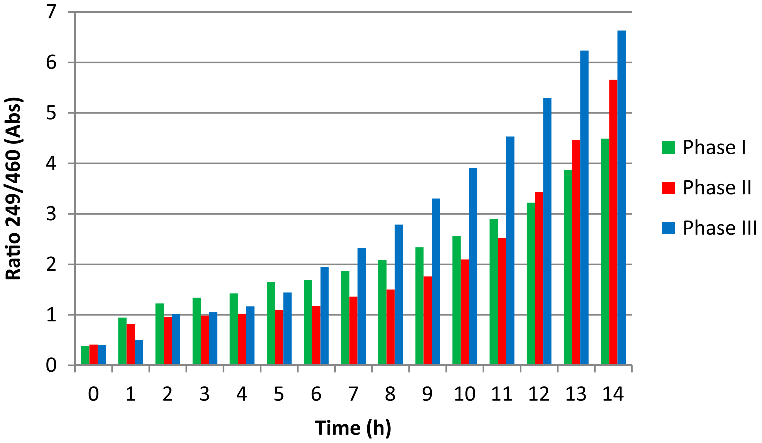

Fig. 5. Ratio of absorbance between 249 nm and 460 nm.

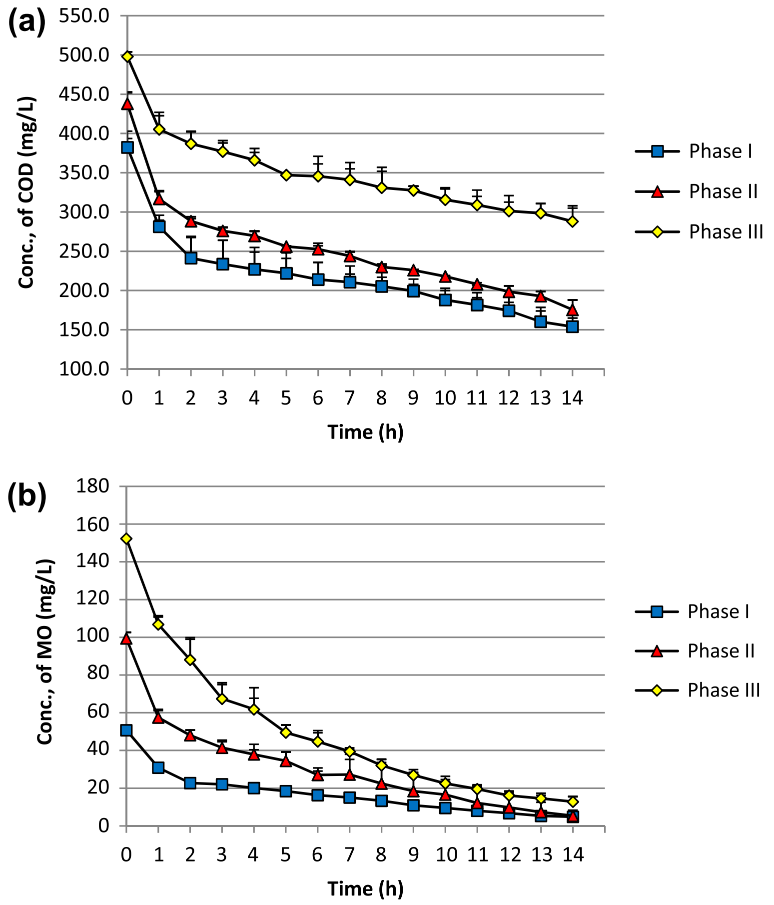

Fig. 6. (a) Hourly COD degradation in phase 1, 2 and 3 and (b) Hourly MO removal in phase 1, 2, and 3.

$$1/S_t = 1/S_0 + K_2t \qquad (3)$$

where S_t—residual COD concentration at selected time (t) (mg l^{-1}), S_0—COD concentration at beginning of the reaction time (mg l^{-1}). K_0—zero-order rate constant through COD removal (mg l^{-1} h^{-1}), K_1—first-order rate constant through COD removal (h^{-1}), K_2—second-order rate constant through COD removal (l mg^{-1} h^{-1}).

Similarly, for the MO degradation kinetic, the obtained experimental data were plotted by C vs. time, $\ln C$ vs. time and $1/C$ vs. time using the following Eqs. (4–6), respectively [31].

$$C_t = C_0 - k_0t \qquad (4)$$

$$C_t = C_0e^{-k1t} \qquad (5)$$

$$1/C_t = 1/C_0 + k_2t \qquad (6)$$

where C_t—residual MO concentration at selected time (t) (mg l^{-1}), C_0—color (MO) concentration at beginning of the reaction time (mg l^{-1}). k_0—zero-order rate constant through decolorization (mg l^{-1} h^{-1}), k_1—first-order rate constant through decolorization (h^{-1}), k_2—second-order rate constant through decolorization (l mg^{-1} h^{-1}).

The kinetic rate constant and regression coefficient (R^2) relevant to the zero, first and second orders were depicted in Table 2. For the COD degradation rate, the R^2 of second-order kinetic model was around 0.9 in all phases. In zero and first order, the R^2 value noticed as around 0.7 and 0.8, respectively. Regression coefficient values of second order ensured the high-degree linear relationship between the concentration and time. Moreover, the rate constant in second-order shows the decreasing trend from phase 1 to 3 which was experienced by other researchers [32–34]. In all the three phases, second-order rate constant (K_2) and its R^2 values showed that COD was removed according to the second-order kinetic. Some of the researchers reported the second-order kinetic for the substrate (COD) removal. Sarioglu and Bisgin [32] reported the second-order kinetic for the degradation of COD and Maxilon Yellow GL using anaerobic mixed culture in

Table 2
Zero-, first-, and second-order kinetic constants obtained in UASB reactor during COD and MO degradation

Kinetic	Constant	COD degradation rate			Constant	MO degradation rate		
		Phase 1	Phase 2	Phase 3		Phase 1	Phase 2	Phase 3
Zero order	K_0 (mg l^{-1} h^{-1})	10.396 ± 0.539	12.934 ± 3.17	10.967 ± 1.850	k_0 (mg l^{-1} h^{-1})	2.488 ± 0.438	5.053 ± 0.559	8.163 ± 0.580
	R^2	0.786 ± 0.137	0.712 ± 0.118	0.797 ± 0.062	R^2	0.767 ± 0.021	0.809 ± 0.013	0.816 ± 0.051
First order	K_1(h^{-1})	0.047 ± 0.004	0.052 ± 0.007	0.03 ± 0.005	k_1 (h^{-1})	0.163 ± 0.039	0.221 ± 0.051	0.170 ± 0.018
	R^2	0.880 ± 0.083	0.877 ± 0.021	0.861 ± 0.05	R^2	0.963 ± 0.014	0.952 ± 0.0096	0.983 ± 0.011
Second order	K_2 (l mg^{-1} h^{-1})	0.0002333 ± 0.0000057	0.0002 ± 0	0.00008 ± 0.000015	k_2(l mg^{-1} h^{-1})	0.016 ± 0.007	0.015 ± 0.011	0.005 ± 0.001
	R^2	0.926 ± 0.045	0.943 ± 0.019	0.910 ± 0.041	R^2	0.882 ± 0.058	0.664 ± 0.042	0.926 ± 0.049

a batch experiment. Second-order reaction kinetic constant (k_2) values decreased from 0.000003 to 0.00000031 mg^{-1} h^{-1}, as the MY GL dye concentration increased from 0 to 1,000 mg l^{-1}. Sandhya and Swaminathan (2006) [33] also reported the second-order kinetic for the COD degradation in a hybrid column upflow anaerobic fixed bed reactor with textile wastewater and noticed that the k_2 value fluctuated with varying influent concentration. Ong et al. [34] used reactive black 5 with synthetic wastewater in a biofilm sequencing batch reactor and reported the COD degradation fit to the second-order kinetic. The rate constant value decreased from 0.002 to 0.0011 mg^{-1} h^{-1} with respect to the increasing reactive black 5 concentrations from 100 to 200 mg l^{-1}. Similar decreasing trend in rate constant value from phase 1 to 3 (0.0002333, 0.0002 and 0.00008 l mg^{-1} h^{-1}) with respect to increasing MO concentration (50, 100, and 150 mg l^{-1}) observed in the present study. In contradict, Sponza and Isik (2004) [31] reported first-order kinetic suitable to the COD removal. Since, the rate constant values decreased with increasing trend in dye (Direct Black 38) concentration was noticed.

MO degradation rate fit to the first-order kinetic, which was confirmed by the R^2 and reaction rate constant values. Among the data obtained (Table 2), the R^2 was more than 0.9 in all phases ensured the high degree linear relationship between the concentration and time. First-order reaction kinetic to dye degradation was reported by some researchers. Yu et al. [9] reported decolorization kinetic of MO by anaerobic sludge suitable by the pseudo-first-order kinetic. First-order rate constant sharply decreased from 1.68 to 0.93 h^{-1}, when the influent increased from 100 to 200 mg l^{-1}. Isik and Sponza [35] also conducted a comparative study on Monod, zero-, first-, and second-order reaction kinetic models and reported the decolorization (Reactive Black 5 and Direct Brown 2) and substrate removal process approximates to first-order kinetic model. Sponza and Isik [31] tested Monod, zero-, first-, and second-order kinetic to determine the most suitable substrate and color removal. First-order kinetic suitable for higher concentration (1,600 and 3,200 mg l^{-1}) of dye (Direct Black 38) and second-order kinetic fit with lower dye concentrations (200, 400, and 800 mg l^{-1}) were reported. In contradict, Hsueh and Chen [36] used Monod kinetic model to compare the degradation of different azo dye including MO (400 ppm). In our study, the k_1 values were observed 0.163, 0.221, and 0.17 h^{-1} in phase 1, 2, and 3 respectively. The k_1 value increase from phase 1 to 2 and decrease from phase 2 to 3. Since the phase 3 k_1 value was higher than the phase 1.In color degradation, decreasing trend of k_1 value intimating the inhibition due to the intermediate products in the degradation process. As per our results, there was not much inhibition due to aromatic compounds from phase 1 to 2. Since the value decrease from phase 2 to 3 indicated the inhibition due to the aromatic amines resulted from azo bond cleavages.

4. Conclusion

The result of this study showed that synthetic wastewater containing azo dye (MO) could be treated effectively by UASB reactor at different concentration varying from 50 to $150\,\mathrm{mg\,l^{-1}}$. Color removal was noticed as 94, 90, and 96% in phase 1, 2, and 3 respectively. COD removal percentage in phase 1, 2 and 3 were 69, 65, and 49%, respectively. Since the COD removals decreasing from phase 1 to 3 due to the accumulation of aromatic amines, kinetic study depicted the decolorization of MO and degradation of organic content followed first-order and second-order kinetic model respectively. The regression coefficient (R^2) for MO degradation in phase 1, 2, and 3 were 0.96, 0.95, and 0.98. In COD degradation, 0.93, 0.94, and 0.91 were the R^2 in phase 1, 2, and 3, respectively, which ensures the high-degree linear relationship between the concentration and time. Complete mineralization of MO not achieved in this anaerobic environment due to the presence of aromatic amines in the effluent. Sequential anaerobic–aerobic system may attain the complete mineralization of MO.

References

[1] R.O.V. Alves de Lima, A.P. Bazo, D.M.E.V. Salvadori, C.I.M. Rech, D. de Palma Oliveira, G. Aragão Umbuzeiro, Mutagenic and carcinogenic potential of a textile azo dye processing plant effluent that impacts a drinking water source, Mutat. Res-Gen. Tox. En. 626 (2007) 53–60.

[2] F.O. Topaç, E. Dindar, S. Uçaroğlu, H.S.S. Başkaya, Effect of a sulfonated azo dye and sulfanilic acid on nitrogen transformation processes in soil, J. Hazard. Mater. 170 (2009) 1006–1013.

[3] H. Zhu, M. Zhang, Y. Liu, L. Zhang, R. Han, Study of congo red adsorption onto chitosan coated magnetic iron oxide in batch mode, Desalin. Water Treat. 37(1–3) (2012) 46–54.

[4] M. Belhachemi, F. Addoun, Adsorption of congo red onto activated carbons having different surface properties: studies of kinetics and adsorption equilibrium, Desalin. Water Treat. 37(1–3) (2012) 122–129.

[5] M.C. Shih, Kinetics of the batch adsorption of methylene blue from aqueous solutions onto rice husk: effect of acid-modified process and dye concentration, Desalin. Water Treat. 37(1–3) (2012) 200–214.

[6] S.A. Ong, L.N. Ho, Y.S. Wong, O.M. Min, L.S. Lai, S.K. Khiew, V. Murali, Photocatalytic mineralization of azo dye Acid Orange 7 under solar light irradiation, Desalin. Water Treat. 48(1–3) (2012) 245–251.

[7] R.G. Saratale, G.D. Saratale, J.S. Chang, S.P. Govindwar, Bacterial decolorization and degradation of azo dyes: A review, J. Taiwan Inst. Chem. E. 42 (2011) 138–157.

[8] P.J. Cai, X. Xiao, Y.R. He, W.W. Li, J. Chu, C. Wu, M.X. He, Z. Zhang, G.P. Sheng, M.H.W. Lam, F. Xu, H.Q. Yu, Anaerobic biodecolorization mechanism of methyl orange by Shewanella oneidensis MR-1, Appl. Microbiol. Biot. 93 (2012) 1769–1776.

[9] L. Yu, W.W. Li, M.H.W. Lam, H.Q. Yu, Adsorption and decolorization kinetics of methyl orange by anaerobic sludge, Appl. Microbiol. Biot. 90 (2011) 1119–1127.

[10] M. Işik, D.T. Sponza, Effects of alkalinity and co-substrate on the performance of an upflow anaerobic sludge blanket (UASB) reactor through decolorization of Congo Red azo dye, Bioresource Technol. 96 (2005) 633–643.

[11] S.A. Ong, E. Toorisaka, M. Hirata, T. Hano, Decolorization of Orange II using an anaerobic sequencing batch reactor with and without co-substrates, J. Environ. Sci. 24 (2005) 291–296.

[12] S.A. Ong, E. Toorisaka, M. Hirata, T. Hano, Biodegradation of redox dye Methylene Blue by up-flow anaerobic sludge blanket reactor, J. Hazard. Mater. 124 (2005) 88–94.

[13] B. Donlon, E. Razo-Flores, M. Luijten, H. Swarts, G. Lettinga, J. Field, Detoxification and partial mineralization of the azo dye mordant orange 1 in a continuous upflow anaerobic sludge-blanket reactor, Appl. Microbiol. Biot. 47 (1997) 83–90.

[14] B.E.L. Baêta, S.F. Aquino, S.Q. Silva, C.A. Rabelo, Anaerobic degradation of azo dye Drimaren blue HFRL in UASB reactor in the presence of yeast extract a source of carbon and redox mediator, Biodegradation 23 (2011) 199–208.

[15] B. Manu, S. Chaudhari, Anaerobic decolorisation of simulated textile wastewater containing azo dyes, Bioresource Technol. 82 (2002) 225–231.

[16] A. Spagni, S. Grilli, S. Casu, D. Mattioli, Treatment of a simulated textile wastewater containing the azo-dye reactive orange 16 in an anaerobic-biofilm anoxic aerobic membrane bioreactor, Int. Biodeter Biodegr. 64 (2010) 676–681.

[17] S. Wijetunga, X.F. Li, C. Jian, Effect of organic load on decolourization of textile wastewater containing acid dyes in upflow anaerobic sludge blanket reactor, J. Hazard. Mater. 177 (2010) 792–798.

[18] R. Brás, A. Gomes, M.I.A. Ferra, H.M. Pinheiro, I.C. Gonçalves, Monoazo and diazo dye decolourisation studies in a methanogenic UASB reactor, J. Biotechnol. 115 (2005) 57–66.

[19] X. Wang, X. Cheng, D. Sun, Interaction in anaerobic biodecolorization of mixed azo dyes of Acid Red 1 and Reactive Black 5 under batch and continuous conditions, Colloid. Surf. A 379 (2011) 127–135.

[20] E. Hosseini Koupaie, M.R. Alavi Moghaddam, S.H. Hashemi, Post-treatment of anaerobically degraded azo dye Acid Red 18 using aerobic moving bed biofilm process: Enhanced removal of aromatic amines, J. Hazard. Mater. 195 (2011) 147–154.

[21] G.N. Buitrón, M. Quezada, G. Moreno, Aerobic degradation of the azo dye acid red 151 in a sequencing batch biofilter, Bioresource Technol. 92 (2004) 143–149.

[22] D. Cui, G. Li, D. Zhao, X. Gu, C. Wang, M. Zhao, Microbial community structures in mixed bacterial consortia for azo dye treatment under aerobic and anaerobic conditions, J. Hazard. Mater. 221 (2012) 185–192.

[23] M. Jonstrup, N. Kumar, M. Murto, B. Mattiasson, Sequential anaerobic aerobic treatment of azo dyes: Decolourisation and amine degradability, Desalination 280 (2011) 339–346.

[24] R. Maas, S. Chaudhari, Adsorption and biological decolourization of azo dye Reactive Red 2 in semicontinuous anaerobic reactors, Process Biochem. 40 (2005) 699–705.

[25] M.F. Coughlin, B.K. Kinkle, P.L. Bishop, Degradation of acid orange 7 in an aerobic biofilm, Chemosphere 46 (2002) 11–19.

[26] D. Georgiou, C. Metallinou, A. Aivasidis, E. Voudrias, K. Gimouhopoulos, Decolorization of azo-reactive dyes and cotton-textile wastewater using anaerobic digestion and acetate-consuming bacteria, Biochem. Eng. J. 19 (2004) 75–79.

[27] H. Xiao, H. Peng, P. Yang, Performance of a new-type integrated biofilm reactor in treating high concentration organic wastewater, Proc. Environ. Sci. 11 (2011) 674–679.

[28] K. Isaka, T. Sumino, S. Tsuneda, High nitrogen removal performance at moderately low temperature utilizing anaerobic ammonium oxidation reactions, J. Biosci. Bioeng. 103 (2007) 486–490.

[29] R.W. Martin, Jr., C. Robert Baillod, J.R. Mihelcic, Low-temperature inhibition of the activated sludge process by an industrial discharge containing the azo dye acid black 1, Water Res. 39 (2005) 17–28.

[30] E. Hosseini Koupaie, M.R. Alavi Moghaddam, S.H. Hashemi, Investigation of decolorization kinetics and biodegradation of azo dye Acid Red 18 using sequential process of anaerobic sequencing batch reactor/moving bed sequencing batch biofilm reactor, Int. Biodeter. Biodegr. 71 (2012) 43–49.

[31] D.T. Sponza, M. Işik, Decolorization and inhibition kinetic of Direct Black 38 azo dye with granulated anaerobic sludge, Enzyme Microb. Techn. 34 (2004) 147–158.

[32] M. Sarioglu, T. Bisgin, Removal of Maxilon Yellow GL in a mixed methanogenic anaerobic culture, Dyes Pigments 75 (2007) 544–549.

[33] S. Sandhya, K. Swaminathan, Kinetic analysis of treatment of textile wastewater in hybrid column upflow anaerobic fixed bed reactor, Chem. Eng. J. 122 (2006) 87–92.

[34] S.A. Ong, L.N. Ho, Y.S. Wong, K. Raman, Performance and kinetic study on bioremediation of diazo dye (Reactive Black 5) in wastewater using spent GAC–biofilm sequencing batch reactor, Water Air Soil Poll. 223 (2012) 1615–1623.

[35] M. Işık, D.T. Sponza, A batch kinetic study on decolorization and inhibition of Reactive Black 5 and Direct Brown 2 in an anaerobic mixed culture, Chemosphere 55 (2004) 119–128.

[36] C.C. Hsueh, B.Y. Chen, Comparative study on reaction selectivity of azo dye decolorization by Pseudomonas luteola, J. Hazard. Mater. 141 (2007) 842–849.