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Decolorization of Orange II using an anaerobic sequencing batch reactor with and without co-substrates

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Abstract

We investigated the decolorization of Orange II with and without the addition of co-substrates and nutrients under an anaerobic sequencing batch reactor (ASBR). The increase in COD concentrations from 900 to 1750 to 3730 mg/L in the system treating 100 mg/L of Orange II-containing wastewater enhanced color removal from 27% to 81% to 89%, respectively. In the absence of co-substrates and nutrients, more than 95% of decolorization was achieved by the acclimatized anaerobic microbes in the bioreactor treating 600 mg/L of Orange II. The decrease in mixed liquor suspended solids concentration by endogenous lysis of biomass preserved a high reducing environment in the ASBR, which was important for the reduction of the Orange II azo bond that caused decolorization. The maximum decolorization rate in the ASBR was approximately 0.17 g/hr in the absence of co-substrates and nutrients.

Key words: anaerobic reduction; azo dye; decolorization rate; endogenous lysis; Orange II

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Introduction

Many dyes are difficult to decolorize due to their complex structure and synthetic origin. The chemical dyes employed most frequently on an industrial scale are azo, anthraquinone, sulfur, indigoid, triphenylmethyl (trityl), and phthalocyanine derivatives (Forgacs et al., 2004). The presence of very low concentrations of dyes in effluent is highly visible and undesirable because of their impact on photosynthesis of aquatic plants, the carcinogenic nature of many of these dyes and their break-down products (Nigam and Marchant, 1995; Weisburger, 2002). Numerous physical and chemical treatment techniques, including coagulation, flocculation, precipitation, electrolysis, oxidation, adsorption and membrane filtration, have been used for decolorization of dyeing effluents. However, these treatment techniques have significant disadvantages including sludge generation, costly, adsorbent regeneration and membrane fouling.

Current studies on biological treatment of textile wastewater have focused on anaerobic biotechnology to achieve color removal (Spagni et al., 2010; Işk and Sponza, 2005; Nuttapun et al., 2004; Ong et al., 2010). The recalcitrant nature of azo dyes together with their toxicity to microorganisms makes aerobic treatment difficult. Under anaerobic conditions, azo dyes are readily cleaved via a four electron reduction through the linkage of azo bonds generating aromatic intermediates (Işk and Sponza, 2004, 2005). The use of microorganisms for the removal of synthetic dyes from industrial effluents offers considerable advantages. The process is relatively inexpensive, running costs are low and the end products of complete mineralization are not toxic. Biodegradation of azo dyes is only feasible if the azo bond is first reduced with electrons from a co-substrate. It has been reported that azo dye reduction is a capacity of many microorganisms under anaerobic conditions (Carliell et al., 1995; Donlon et al., 1997; Razo-Flores et al., 1997a, 1997b; Sponza and Işk, 2004; Ong et al., 2008a, 2008b). Anaerobic cleavage of the azo bond results in permanent decolorization of the dye but the intermediates can be re-oxidized to colored by-products (Wuhrmann et al., 1980).

Azo dye reduction can result from biological processes, either as a direct enzymatic reaction or a reaction mediated by biologically regenerated enzyme cofactors or other electron carriers (Stolz, 2001). It has also been reported that the carbon source fed to a bacterial culture could affect the decolorization process. Most azo reduction to amine occurs during active bacterial growth. The addition of electron donors such as glucose or acetate ion apparently stimulates the reduction cleavage of azo bonds (Bras et al., 2001). The co-substrate is an alternate growth substrate which when supplied to a bioreactor can enhance the degradation of some wastes or pollutants that cannot support microbial growth alone (Atlas, 1993). It has been reported that 1000 mg/L of glucose can increase the color reduction efficiency of an anaerobic biomass inoculated in

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a serum bottle (Carliell et al., 1995), and that co-substrates are important for reduction of azo bond. Generally, the addition of external carbon sources results in much higher dye decolorization rates. The aim of this study was to investigate the behavior of Orange II biodegradation under anaerobic sequencing batch reactor (ASBR) operation with and without the presence of external carbon sources.

1 Materials and methods

1.1 Basal medium and chemicals

Azo dye Orange II was supplied by Tokyo Kasei Kogyo Co. Ltd., Japan. Orange II is a mono-azo compound with λ_{max} at 480 nm. Synthetic wastewater consisting of a base mix of bacto-peptone, sucrose, nutrients and buffer solution of the following composition (concentrations in mg/L): bacto-peptone (188), sucrose (563), NH_4Cl (344), $MgSO_4$ (49), $FeCl_3$ (11.3) and KH_2PO_4 (318) giving a COD of 800–850 mg/L. All chemicals were analytical grade.

1.2 ASBR operation

A plexiglass reactor with total volume of 5 L was used to simulate the activated sludge process. The ASBR was operated with 30 min fill, 21.5 hr react, 1 hr settle, 45 min draw and 15 min idle. A mixer was used during the fill and react modes to provide efficient mixing in the ASBR system. The system received 3 L of synthetic wastewater consisting of substrates, phosphorus, nitrogen and micronutrients for each batch. The activated sludge seed was obtained from a municipal wastewater treatment plant and was acclimatized in the laboratory by synthetic wastewater feeding. To investigate the effects of co-substrates on the COD and color removal rates, three different COD dosages were tested (900, 1750, and 3730 mg/L). For COD dosage, the ASBR system was operated for about 20 days and the mean COD and color removal values were calculated. In all cases, concentrations of the mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) in the reactor were 5500–6500 mg/L and 5000–6000 mg/L, respectively. To study the sludge auto-lysis products as carbon and nutrient sources for stabilization of Orange II under the ASBR process, synthetic wastewater with co-substrates- and nutrients-free was used. The concentrations of Orange II tested were in the range of 100 to 600 mg/L.

1.3 Analytical procedures

The effluent of the ASBR system was collected at the end of the draw mode and analyzed for COD and Orange II concentrations immediately. The COD, MLSS and MLVSS were measured according to standard methods. For COD and Orange II measurements, the samples collected from anaerobic reactor were filtered through a 0.45-μm membrane filter. The Orange II concentrations were estimated from the standard curve of dye concentration versus optical density at its maximum absorption wavelength (λ_{max} 480 nm) using an UV-Vis spectrophotometer (UV-1200, Shimadzu Co., Ltd., Japan). The ORP (oxidation-reduction potential) was monitored with an ORP meter (RM-20P, Japan).

2 Results and discussion

2.1 Effects of COD dosages on color and COD removal

As shown in Fig. 1, the increase in COD concentrations from 900 to 1750 to 3730 mg/L in the system treating 100 mg/L of Orange II-containing wastewater enhanced color removal efficiency from 27% to 81% to 89%, respectively. Azo dye Orange II as a non-growth substrate was decolorized by anaerobic microbial community. The reducing equivalents from sucrose as electron donors were transferred to the Orange II during the cleavage of the azo bond. Azo dye reduction by bacteria under anaerobic conditions is often a co-metabolic reaction, in which the reducing equivalents produced during the anaerobic oxidation of a co-substrate are used to break the azo bonds. Azo dyes act as terminal electron acceptors in the respiratory electron transfer chain (Brigé et al., 2008). The presence of external carbon sources is favorable for the rate process (Carliell et al., 1995) because the oxidation of these compounds yields electrons used for the formation of reduced cofactors (FAD, FMN, NADH). In general, the decolorization rate showed a tendency to increase with increasing initial co-substrate concentration (Bras et al., 2001). Chinwekitvanich et al. (2000) reported that the addition of tapioca as a co-substrate increased decolorization efficiency by up to 70% in textile wastewater treatment by UASB systems. However, the excessively high concentration of tapioca did not enhance process capability in terms of color removal efficiency. Previous research has reported on the increase in the decolorization rate by the addition of readily biodegradable carbon (glucose) under an upflow anaerobic sludge blanket (Somasiri et al., 2008), where anaerobic degradation of azo dye Orange II in an ASBR utilizing sucrose as an external supply of substrate (electron-donor) lead to high decolorization of the dye.

Oxidation-reduction potential (ORP) monitoring was

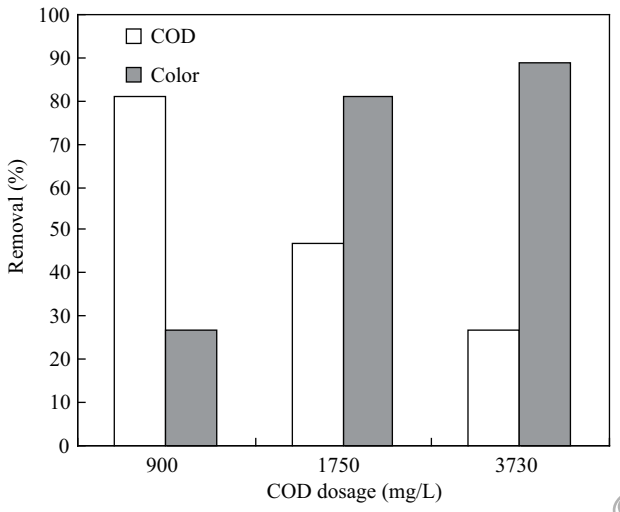

Fig. 1 Color and COD removal at various COD dosages under anaerobic degradation.

carried out in the ASBR with and without the presence of co-substrates and nutrients. As shown in Fig. 2, the redox potential in the anaerobic reactor was in the range of –250 to –450 mV. Redox potentials greater than 100 mV commonly indicate an aerobic environment, whereas those with potentials less than –100 mV indicate an anaerobic environment (Suthersan, 2002). It was observed that the reducing environment in the ASBR with the presence of co-substrates and nutrients (day 1 to day 60) was higher than the system with co-substrate- and nutrient-free conditions (day 61 to day 155). This shows the importance of co-substrates to preserve highly reducing environments in the anaerobic bioreactor. The low ORP values in the bioreactor indicate a highly active anaerobic biomass (Georgiou et al., 2004; Chinwekitvanich et al., 2000). The co-substrate used in the study (sucrose) was oxidized by anaerobic microbes and the reducing environment prevailing in the bioreactor caused the reduction of the azo bond in Orange II. This agreed with observations of Beydilli et al. (1998), where reduction of dye took place when low redox conditions prevailed in the bioreactor. Manu and Chaudhari (2002) also observed ORP values in the range of –299 to –368 mV for the decolorization of Orange II and Reactive Black 3HN.

On the other hand, the increase of COD dosages significantly deteriorated COD removal efficiency. Most COD was contributed by sucrose and bacto-peptone, which are considered aerobically and anaerobically biodegradable. In this study, however, these substrates were not degraded completely by anaerobic microbes and had pH (4.5) in the reactor, which was too low for the microbes to perform. These results showed that the ASBR system could decolorize Orange II effectively but the case was opposite for organic substrates degradation, especially under high COD dosages.

2.2 Decolorization of Orange II under co-substrates and nutrients-free condition

Anaerobic biomass was acclimatized to co-substrate and nutrient-free conditions by feeding with Orange II solution only. The concentration of Orange II tested was in the range of 100 to 600 mg/L. Figure 3 shows almost complete decolorization was achieved under Orange II addition up to 400 mg/L and 30 hr of operation. The decolorization rate tended to decline for higher concentrations. This clearly shows that the acclimatized anaerobic biomass reduced Orange II without the presence of co-substrates and nutrients. Color removal was achieved by biodegradation, adsorption of dye into microbial granules, and adsorption followed by biodegradation (Wijetunga et al., 2008, 2010). The deterioration in color removal efficiency at Orange II concentration at and above 500 mg/L may be due to exceeding the bioreactor's degradation capacity or by causing toxicity to the anaerobic biomass (Ong et al., 2008b).

It is essential to provide a co-substrate to donate electrons for breaking down the azo bond leading to decolorization (Carliell et al., 1995; Chinwekitvanich et al., 2000; Bras et al., 2001; Wijetunga et al., 2010; Senthilkumar et al., 2011). Under anaerobic conditions, in the presence of co-substrate, decolorization of azo dyes is achieved with cleavage of the azo bond, thus rendering the azo dye colorless, with formation of corresponding aromatic amines (Carliell et al., 1995). However, results from the present study show the ability of an anaerobic reactor in the degradation of Orange II without the presence of co-substrates and nutrients.

Figure 4 shows the UV-Vis spectrum for the influent and effluent wastewater collected from the ASBR system. The molecular structure of azo dye Orange II consists of an azo bond, a benzene ring, and a naphthalene ring, which all exhibit different absorbance peaks. The azo bond absorbs in the visible region (480 nm), while the benzene ring (226 nm) and the naphthalene group (310 nm) absorb in the UV region. The disappearance of the absorbance peak at 480 nm in the effluent was an unequivocal signal of almost complete decolorization and the breakdown in the chromophoric group. Although the cleavage of the azo bond in Orange II removes the visible problem, there remains the problem of the aromatic amines. The absorbance at wavelength 248 nm for the effluent shows the presence of intermediate aromatic amines.

Without the addition of co-substrates, the endogenous lysis of biomass plays an important role in the decol-

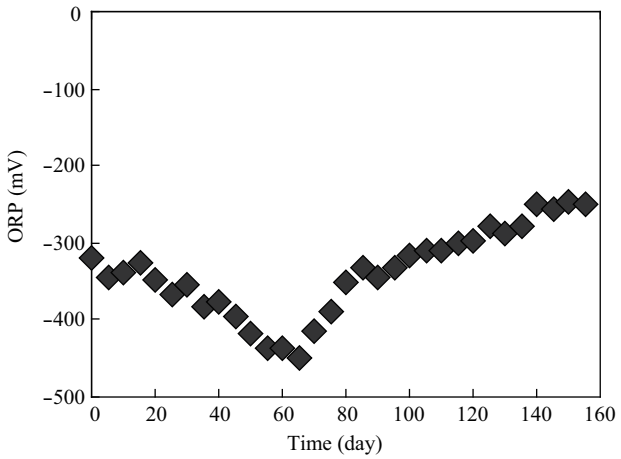

Fig. 2 ORP monitoring in ASBR with and without co-substrates and nutrients.

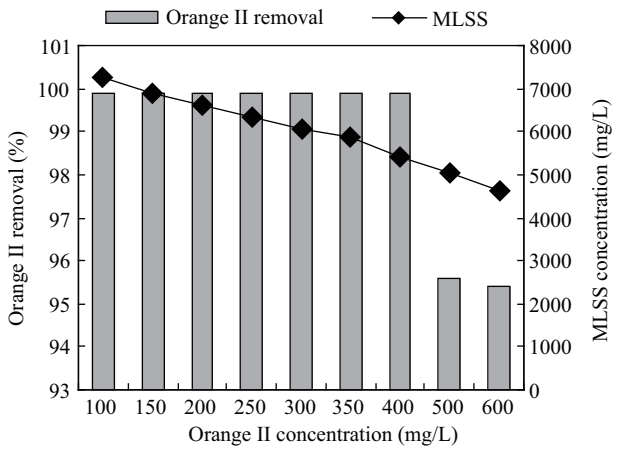

Fig. 3 Color removal and changes of MLSS in co-substrates and nutrients-free operation.

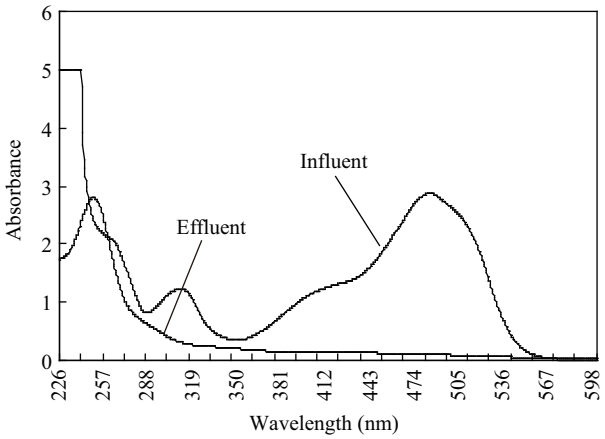

Fig. 4 UV-Vis spectrum of influent and effluent ASBR system.

orization of Orange II. In addition, the aromatic amines generated from the reduction of Orange II were used as co-substrates by anaerobic microbes and preserved the reducing condition in the ASBR. In the absence of external carbon sources and nutrients, the endogenous substrates from the biomass would supply the reducing equivalents needed for the reduction of the dye as indicated by low ORP values in the range of –250 to –450 mV. As shown in Fig. 3, the MLSS in the anaerobic reactor decreased gradually from 7200 to 4700 mg/L, which indicates the endogenous lysis of sludge was performed by anaerobic microbes to generate the reducing environment in the bioreactor for decolorization.

Studies performed by Razo-Flores et al. (1997a, 1997b) and Beydilli et al. (1998) showed that decolorization of dyes did not depend on COD since the dye itself can be used as a carbon source by the anaerobic microorganisms. It is generally assumed that the aromatic amines generated from the linkage of azo bound compounds are not further degraded under anaerobic conditions (Brown and Hamburger, 1987; Haug et al., 1991). However, several studies have demonstrated that aromatic amines can be fully or partially biodegraded in anaerobic environments and serve as a carbon and energy source for anaerobic microbes (Razo-Flores et al., 1997a, 1997b; O'Connor and Young, 1993; Işk and Sponza, 2004, 2005; Kalyuzhnyi et al., 2000). As a result, aromatic amines can be utilized as co-substrates by anaerobic microbes and can maintain the reducing environment for the reduction of the azo bond in Orange II.

Endogenous lysis caused about 5% reduction of sludge in each Orange II dosage in present study. Kim et al. (2008) investigated the effects of a reductant and carbon source on the decolorization of diazo dye C.I. Reactive Black 5 in an anaerobic sludge system. They found that the decolorization rates were about 40% without additional substrate and about 20% with methanol (10^{-3} mol/L), which were 2- to 3-fold lower than the rates obtained with the anaerobic culture in the presence of glucose as a fermentative substrate. They postulated that endogenous respiration occurred in the absence of the co-substrate and that the anaerobicconsortia used in their experiment were insufficiently acclimated to methanol (Kim et al., 2008).

2.3 Effects of initial Orange II concentration on decolorization

Figure 5 shows the effects of initial Orange II concentration on the specific decolorization rate under ASBR operation without the presence of co-substrates and nutrients. The specific decolorization rate increased with the increase of initial Orange II concentration. The correlation between the specific decolorization rate and initial Orange II concentration can be expressed by the Michaelis-Menten model as below:

$$r_{dye} = r_{dye,max} C_{dye} / (C_{dye} + K_m) \quad (1)$$

where, r_{dye} (g/hr) is the specific decolorization rate; $r_{dye,max}$ (g/hr) is the limiting decolorization rate; C_{dye} (mg/L) is the initial Orange II concentration and K_m (mg/L) is the Michaelis constant.

As shown in Fig. 5, the maximum specific decolorization rate ($r_{dye,max}$) and the value of the apparent Michaelis constant (K_m) estimated from the experiment data were 0.17 g/hr and 180 mg/L, respectively. Orange II did not exhibit significant inhibitory effects on the acclimatized anaerobic microbes when at concentrations up to 600 mg/L. We also observed that the ASBR could treat high strength azo dye-containing wastewater even in the absence of co-substrates and nutrients.

2.4 Kinetic study in COD removal

As shown in Fig. 6, the COD removal rate was high at the beginning of the reaction then decreased gradually until a quasi-state was achieved after 25 hr of operation. In the system without co-substrates and nutrients addition, the residual COD after ASBR treatment indicated the presence of organic compounds in the bio-reactor, which may be due to intermediate products from the biodegradation of Orange II by anaerobic microbes (Fig. 4). These aromatic amines did not degrade completely under anaerobic conditions but the accumulated aromatic amines were further mineralized under aerobic conditions (Ong et al., 2005). However, a few aromatic amines in the presence of hydroxyl and/or carboxyl groups could be mineralized anaerobically (Razo-Flores et al., 1997a). Thus, initial

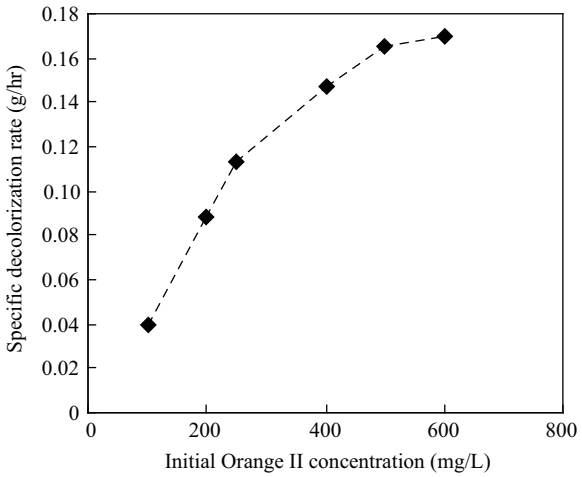

Fig. 5 Effect of initial Orange II concentration on the specific decolorization rate.

Table 1 Kinetic constant for COD removal in the system with and without the presence of co-substrates and nutrients

Kinetic	Constant	Without co-substrates		With co-substrates
		Orange II 400 mg/L	Orange II 600 mg/L	Orange II 0 mg/L
Zero	k_0 (mg/(L·hr))	7.038	7.545	41.796
	r^2	0.478	0.476	0.321
First	k_1 (hr^{-1})	0.0121	0.0095	0.1143
	r^2	0.583	0.546	0.478
Second	k_2 (L/(mg·hr))	0.00002	0.00001	0.00040
	r^2	0.676	0.598	0.640
	R_{max} (mg/(L·hr))	7.82	6.11	76.00
Monod	K_s (mg/L)	909	1111	625
	r^2	0.984	0.997	0.988

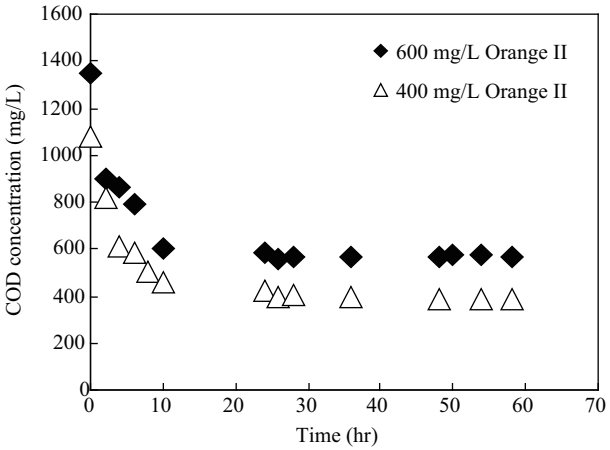

Fig. 6 COD removal curves in ASBR system in the absence of co-substrates and nutrients.

COD in the bio-reactor was contributed by Orange II and the balance of intermediate aromatic amines from previous batch. As a result, in the absence of co-substrates and nutrients, metabolism of aromatic amines by microbes additional to the endogenous substrates from the biomass supplied reducing equivalents for the reduction of the azo bond in Orange II. Işk and Sponza (2005) reported that the complete decolorization of Congo Red under co-substrate free operation attributed to total aromatic amines metabolism, which provided the electrons required for the cleavage of the azo bond in Congo Red in the UASB reactor.

The substrate removal rate in a batch reactor can be expressed by the Monod equation as follows:

$$-\mathrm{d}S/\mathrm{d}t = (R_{max}S)/(K_s+S) \quad (2)$$

After integral Eq. (2),

$$(\ln(S/S_0))(1/t) = -(R_{max}/K_s) + (1/K_s)((S_0-S)/t) \quad (3)$$

where, S (mg/L) is the substrate concentration expressed by COD, R_{max} (mg/(L·hr)) is the maximum substrate removal rate and K_s (mg/L) is the half-velocity coefficient for the substrate. Some studies have shown that removal of co-substrate through decolorization of azo dyes might follow zero-, first- and second-order reaction kinetics, which can be expressed by Sponza and Işk (2004):

$$S_t = S_0 - k_0 t \quad (4)$$

$$S_t = S_0 e^{-k_1 t} \quad (5)$$

$$1/S_t = 1/S_0 - k_2 t \quad (6)$$

S_t (mg/L) is the substrate concentration at time t (hr), S_0 (mg/L) is the initial substrate concentration, and k_0 (mg/(L·hr)), k_1 (hr^{-1}) and k_2 (L/(mg·hr)) are the zero-, first- and second-order kinetic constants, respectively.

Table 1 shows the Monod kinetic model provided the best description for COD removal with correlation coefficients of more than 0.98 in the decolorization of Orange II with and without the presence of co-substrates and nutrients. In the system with co-substrates and nutrients addition, the R_{max} was significantly higher than the system without co-substrates and nutrients whereas K_s showed the contrary trend. The co-substrates used were more biodegradable than Orange II or intermediate products (aromatic amines) by anaerobic microbes. Higher K_s values in the system with Orange II addition and co-substrates and nutrients-free operation can be characterized as a system with more inhibition relatively.

3 Conclusions

Increased co-substrate concentrations enhanced Orange II decolorization rates but COD removal deteriorated under ASBR operation. In the co-substrate and nutrient-free system, the endogenous lysis of sludge provided the required amount of reducing equivalents for the reduction of Orange II. Over 95% of decolorization was achieved in the ASBR system with the addition of 600 mg/L of Orange II. The maximum specific decolorization rate estimated from the Michaelis-Menten model was about 0.17 g/hr, which showed the potential of the ASBR system in treating high strength dye-containing wastewater.

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