AZO DYE REDUCTION AND BIODEGRADABILITY MECHANISMS DURING AN ANAEROBIC PROCESS

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Abstract

An anaerobic process for azo dye treatment had a high performance in organic removal with values reaching 85.51% and 90.29% for total chemical oxygen demand (TCOD) and soluble chemical oxygen demand, respectively. At the same time, the process was also capable of color removal. The absorption peak of anaerobic effluent appeared in wavelengths ranging between 195 nm to 246 nm which is identified as being in the aromatic amine regions. The Fourier transform infrared spectrum of anaerobic effluent represented the aromatic region. Therefore, azo dyes were degraded to aromatic compounds during the anaerobic process. The anaerobic influent contained a very high soluble inert chemical oxygen demand (S_I) fraction with 60.68% of TCOD. However, the S_I fraction was reduced by the anaerobic process at 93.46% removal. It is possible that the SI fraction was adsorbed on the microorganism surface and/or the dead cell composition. The maximum specific growth rate (μ_{max}) and sludge yields were found in the anaerobic influent fed condition that had higher values than in the effluent fed condition. That caused a high organic concentration in the influent. However, the μ_{max} of the heterotroph microorganism was still high at the anaerobic effluent fed condition. This enabled the aerobic treatment to be capable of aromatic amine removal at S_0/X_0 ratio 0.151-0.156.

Keywords: Azo dye reduction, biodegradability kinetics, COD fraction

Introduction

Azo dyes represent the largest class of dyes that are applied in textile processing which produces large volumes of effluents containing mineral salts and which are highly colored. These dyes are toxic to aquatic biota and are considered carcinogenic and mutagenic to humans (Weisburger, 2002; Puvaneswari *et al*., 2006). Several physical, chemical, and biological techniques were applied to remove dyes from wastewater. Most physicochemical dye removal methods have drawbacks because they are expensive, have limited versatility, greatly interfere with other wastewater constituents, and/or generate waste products that require further handling. Alternatively, biological treatment presents a relatively inexpensive way to remove dyes from wastewater (Robinson *et al*., 2001;

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Van der Zee and Villaverde, 2005). Generally, bacterial azo dye biodegradation proceeds in 2 stages. The first stage involves reducing the cleavage of the dyes' azo linkages, resulting in the formation of generally colorless but potentially hazardous aromatic amines. The second stage involves degradation of the aromatic amines. Azo dye reduction usually requires anaerobic conditions, whereas bacterial biodegradation of aromatic amines is an almost exclusively aerobic process. A wastewater treatment process in which anaerobic and aerobic conditions are combined is therefore the most logical concept for removing azo dyes from wastewater (Işik and Sponza, 2004; Van der Zee and Villaverde, 2005). Specifically, anaerobic azo dye reduction should be accepted for being a non-specific and presumably extracellular process, in which the reducing equivalents from either the biological or chemical source are transferred to the dye. The transfer of the reducing equivalents from a primary electron donor (co-substrate) to a terminal electron acceptor (azo dye) generally acts as the process rate limiting step in anaerobic azo dye reduction (Laowansiri *et al.*, 2008). Research on aromatic amine biodegradation has been usually conducted with relatively stable, not easily autoxidizing aromatic amines, representing only a part of the aromatic amines from azo dyes. Aromatic amines from azo dye reduction will be highly reactive in the presence of oxygen (Van der Zee and Villaverde, 2005). Therefore, much attention has been focused on the careful reduction of the dyes discharged from the dyeing and textile industries, since some of these dyes or their metabolites may be mutagenic or carcinogenic. Furthermore, color has been recognized as a harsh contaminant in these industries and must be removed before being discharged into the environment. Meanwhile, the effect of biodegradability kinetics for aerobic post treatment should be raised as an issue for investigation as well as in order to improve the underlying mechanisms during the anaerobic process. This paper presents the investigation of an anaerobic process treatment for azo dye wastewater by characterization of carbonaceous materials, the potential of aromatic amine generation, and biodegradability kinetics.

Materials and Methods

Synthesis of Dyeing Wastewater

Synthetic dyeing wastewater was prepared from the desizing, scouring, bleaching, mercerizing, dyeing, and finishing processes in the textile industry. The components of the synthetic dyeing wastewater contained 100 mg/L Reactive Red 141 dye, 900 mg/L starch, 150 mg/L polyvinyl alcohol, 50 mg/L polyacrylic acid, 110 mg/L NaOH, and nutrients: 67 mg/L KH_2PO_4, 26 mg/L $CaCl_2{\cdot}2H_2O$, 28 mg/L $MgSO_4{\cdot}7H_2O$, 6 mg/L $FeCl_3{\cdot}6H_2O$, and 1 ml/L of a trace element solution containing 5000 mg/L $FeSO_4{\cdot}7H_2O$, 392 mg/L $CuSO_4{\cdot}7H_2O$, 248 mg/L $Co(NO_3)_2{\cdot}6H_2O$, 177 mg/L $NaB_4O7{\cdot}10H_2O$, 100 mg/L $MnCl_2{\cdot}4H_2O$, 25 mg/L $NiCl_7{\cdot}6H_2O$, and 11 mg/L $ZnSO_4{\cdot}7H_2O$.

Anaerobic Unit Experimental

The experimental set-up consisted of continuous stirred tank reactors, as shown in Figure 1. A pilot reactor with a capacity of 20 L was used, which had a diameter of 0.20 m. and a length of 0.8 m. The reactor has an agitation system (60 rpm) for the purpose of mass transference. The anaerobic process was operated using 24 h hydraulic retention time (HRT) and 1 kg chemical oxygen demand (COD)/m^3-d of organic loading over a period of 120 days after the steady state condition (Table 1). The process for treatment of the azo dyes' wastewater was synthesized by values of 1000 mg/L of COD and 100 mg/L of reactive red 141 dye concentrations.

Sampling and Analytical Methods

The influent and effluent samples of the anaerobic process were analyzed following the standard methods for the examination of water and wastewater (APHA, 2005) including COD, total Kjeldahl nitrogen, and

total phosphorus. Dissolved oxygen (DO) concentrations were continuously measured during the oxygen uptake rate (OUR) experiment with a DO meter (Eutech Instruments PD650, Eutech Instruments Pte. Ltd., Singapore). Biochemical oxygen demand (BOD) was determined with an OxiTop®-C measuring pressure head instrument (Expotech USA Inc., Houston, TX, USA). The carbonaceous material characterizations were measured in terms of the COD parameter subdivided into a number of fractions following Wentzel *et al*. (1999). Also, the UV-Vis absorbance spectra were evaluated for azo dye during the anaerobic process (Pinherio *et al*., 2004). Fourier transform infrared (FTIR) spectroscopy was done in the mid infrared (IR) region of 400-4000 per cm with 16 scan speeds by a Perkin Elmer model Spectrum GX (Perkin Elmer, Inc., Waltham, MA, USA). The sample was fixed in a sample holder and analyses were carried out.

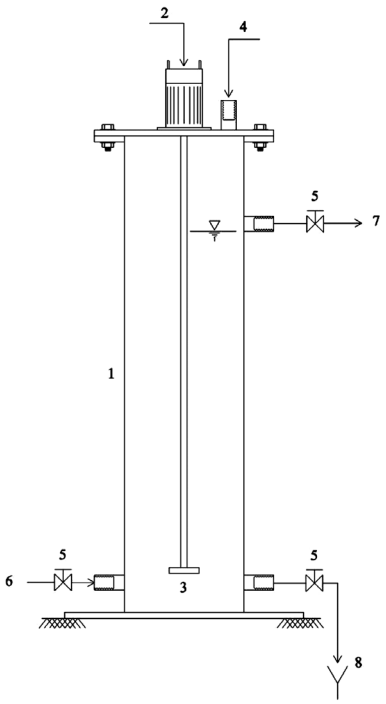

Figure 1. Anaerobic unit: 1) Anaerobic reactor, 2) Agitator, 3) Paddle, 4) Air outlet, 5) Ball valve, 6) Inlet, 7) Outlet, and 8) Drain

Biodegradability Kinetic Evaluations

The OUR experiments were conducted to determine the biodegradability kinetic coefficients of the aerobic heterotrophs via the procedure of Ekama *et al*. (1986). The batch tests shown in Figure 2 were maintained at a temperature 30±0.5°C and a pH 7±0.2 and with added suppressing nitrification of 70 mg N-ammonia/L. The sludge samples were obtained from a municipal activated sludge process. The initial mixed liquor volatile suspended solids (MLVSS) concentration (X_0) in the sludge samples was brought to 400 mg/L for the batch tests and the various initial substrate concentrations (S_0) that govern the quality of the batch respirometric tests. Two initial substrates in the batch experiment, the influent and effluent of the anaerobic process, were analysed for influences on the biodegradability kinetic coefficients. The OUR results were used for calculating the maximum specific growth rates (μ_{max}), substrate utilization rate (r_x), half-velocity constant (K_S), and sludge yield coefficient

Table 1. Experimental conditions

Parameters	Unit	Condition value
Anaerobic digester reactor	liter	20
Hydraulic retention time (HRT)	hour	24
Organic loading rate (OLR)	kgCOD/m^3-d	1
Mixed liquor suspended solid (MLVSS)	g VSS/L	30
Alkalinity	mg/L as $CaCO_3$	≈ 500

(Y) based on Monod kinetics by regression analysis.

Results and Discussion

Anaerobic Process Performances

The overall results of the anaerobic process performances are illustrated in Table 2. The anaerobic process had high performances of organic removal with values up to 85.51% and 90.29% for total chemical oxygen demand (TCOD) and soluble chemical oxygen demand, respectively. The process also had a high capability in color removal.

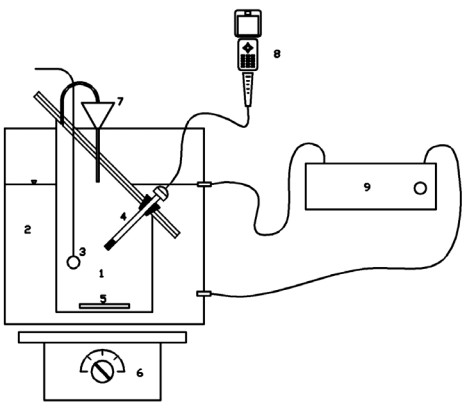

Figure 2. Respirometer: 1) Respiration cell, 2) Water jacket, 3) Air diffuser, 4) DO probe, 5) Magnetic bar, 6) Magnetic stirrer, 7) Expansion funnel, 8) DO meter, and 9) Water bath

Azo Dye Reduction through Anaerobic Process

The structure of the Reactive Red 141 dye showing the constituent aromatic amine is illustrated in Figure 3. The potential of azo reduction to aromatic amines was evaluated by major visible light absorbance peak disappearance and/or new peak appearance by the distribution of absorbencies within 200 nm to 1100 nm of UV spectra. The results were shown in Figure 4. The absorption peak of the anaerobic influent appeared in the wavelength ranging between 504 nm to 553 nm and the maximum value (λ_{max}) was found at 544 nm. The second absorbance values were found at 217 nm and 289 nm of UV wavelengths. However, the absorption peak of the anaerobic effluent appeared in the wavelength ranges between 212 nm to 246 nm. These regions of UV spectra absorbencies were identified as the aromatic amines range (Pinheiro *et al*., 2004). This confirmed that the azo dyes were degraded to aromatic amines during the anaerobic process (Gottlieb *et al*., 2003). Azo dye can break down during the anaerobic process. The product is aromatic amine. The aromatic amines are relatively stable and do not easily autoxidize. In many cases, the aromatic amine from azo dye reduction was found to be

Table 2. Experimental results

Parameters	Influent (mg/L)	Anaerobic effluent (mg/L)	% Removal
TCOD	1000.00	144.92	85.51
SCOD	713.93	69.30	90.29
COD/BOD	0.15	0.43	-
Total Kjeldahl Nitrogen (TKN)	9.66	7.28	24.73
Total phosphorus	3.62	2.64	42.79
Color	2070.02[a]	1096.04[a]	47.05
Color (λ_{max} = 544 nm)	100.00	26.20	73.80

Remark: [a] ADMI unit

highly reactive in the presence of oxygen. In aerobic bioreactors, autoxidation and, possibly, reactions with compounds within the sludge matrix will compete with biodegradation. The limited amount of data on these chemical oxidation processes, in combination with analytical problems, makes it difficult to predict the fate of aromatic amines during anaerobic-aerobic treatment of azo dyes (Zille *et al.*, 2005).

Fourier Transform Infrared Spectroscopy (FTIR)

To investigate the textile wastewater degrading mechanisms, FTIR analysis was carried out. A comparison of the FTIR spectrum between the textile raw wastewater and the wastewater treated with the anaerobic digestion membrane bioreactorsystem was shown in Figure 5. The peak in the textile wastewater spectrum represented the stretching vibrations of N=N stretching at 1644 cm^{-1} (azo dye group), -SO_3- stretching at 1261 cm^{-1} (sulfonate group), -C-Cl stretching at 1018 cm^{-1} (benzene ring), and -N-H stretching at 3401 cm^{-1} (amine) (Telke *et al.*, 2008). The FTIR spectrum of the anaerobic effluent showed a significant change when compared with the textile wastewater. Anaerobic effluent was observed in new bands, at above 3000 cm^{-1} attributed to =C-H stretching and N-H stretching and at lower than 1704 cm^{-1} associated with =C=C aromatic stretching, N-H bending, and C-N stretching, which is the aromatic region. A new peak at 2061 cm^{-1} represented =C=C aromatic stretching. Therefore, reducing cleavage of the dyes' azo linkages caused generally colorless but potentially hazardous aromatic compounds. However, several researches recorded that azo dyes were reduced to aromatic amine such as Reactive Red 141 cleavage, the products being 1, 3, 5 triazine 2, 4 diol, p-dinitro benzene, naphthalene diazonium, and 2-nitroso naphthol (Telke *et al.*, 2008).

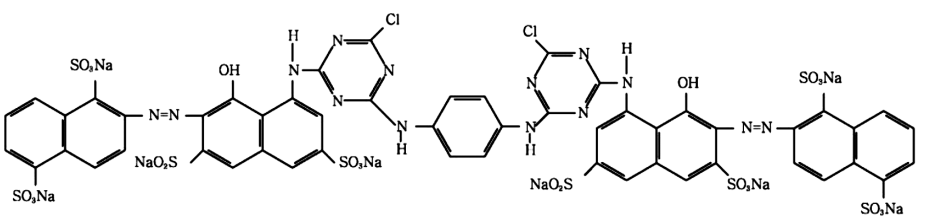

Figure 3. The structure of Reactive Red 141 indicates the diazo and sulfonategroup (Telke *et al.*, 2008)

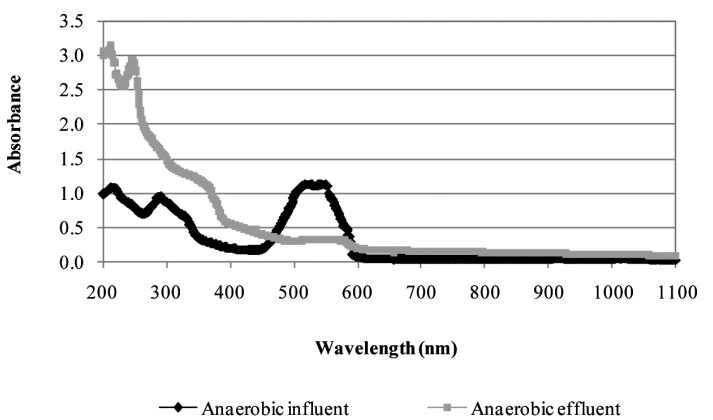

Figure 4. Absorbance spectra in influent and effluent anaerobic process at HRT 24 h

COD Fractionations

COD fractionations were evaluated for the biodegradability potential with results as illustrated in Table 3. The anaerobic influent contained a very high soluble inert COD (S_I) fraction with 60.68% of TCOD. This caused the azo dyes' characteristic to be highly dissolvable in water solution. The structure of azo dyes is more complex with large molecules which have low biodegradability by heterotroph bacteria. However, the S_I fraction was reduced by the anaerobic process with 93.46% removal of S_I. This is the potential of the S_I fraction adsorbed on a microorganism surface and/or dead cell composition (Ng *et al.*, 1994) and degraded under anaerobic conditions because the major visible light absorbance peak completely disappeared (Figure 2) (Sponza and Işik, 2005). As well, the particulate inert COD (X_I) was removed through the anaerobic process by natural settling. Even the proportion of X_I / C_T is higher than in the influent because the particulate residual products' generation increases in the wastewater composition, which has more complex organic compounds (Orhon *et al.*, 1999). However, the slowly biodegradable COD (X_S) of the influent was significant at the same value as the effluent. This can be explained by the potential of the azo dye that was reduced to aromatic amines during the anaerobic process (Gottlieb *et al.*, 2003).

Biodegradability Kinetics

The biodegradability kinetics during the anaerobic degradation was studied. Two substrates of anaerobic influent (azo dye wastewater) and anaerobic effluent were used for biokinetic evaluation. The initial substrate concentration and the initial biomass

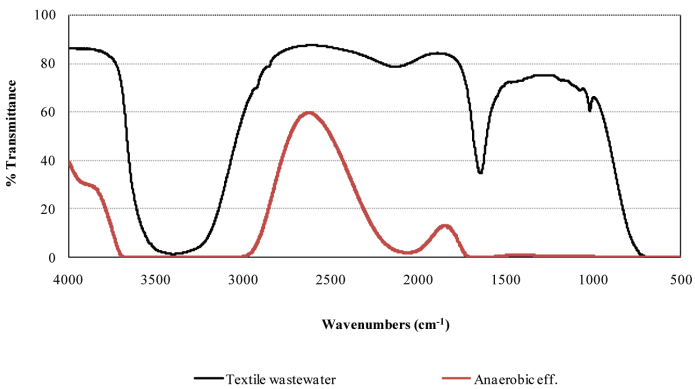

Figure 5. FTIR spectra of textile wastewater and anaerobiceffluent

Table 3. COD fractions of the influent and effluent

COD fractions	Anaerobic influent		Anaerobic effluent	
	Concentration (mg/L)	% of C_T	Concentration (mg/L)	% of C_T
Readily biodegradable COD, S_S	107.09	10.71	29.60	20.43
Slowly biodegradable COD, X_S	42.91	4.29	32.30	22.29
Soluble inert COD, S_I	606.84	60.68	39.70	27.39
Particulate inert COD, X_I	243.16	24.32	43.32	29.89

concentration had a significant effect on the reaction rate and on the biomass yield coefficient (Y). The maximum specific growth rate (μ_{max}) and Y were found when the anaerobic influent was fed as illustrated by the results in Table 4. This caused a high organic concentration in the influent with the COD value about 107 mg/L of ready biodegradation. However, several researches recorded that azo dyes were reduced to aromatic amine (Telke *et al.*, 2008), being not readily biodegradable and toxic to microorganisms (Gottlieb *et al.*, 2003). On the other hand, biodegradation of the aromatic amines has been effective in activated sludge systems (Işik and Sponza, 2004). This is confirmed by the details of the aerobic biodegradability kinetics that were shown in Table 5. The μmax of the heterotroph microorganism was still high in the anaerobic effluent fed condition. This shows the potential for aerobic treatment to be capable of aromatic amine removal (Van der Zee and Villaverde, 2005).

Effect of S_0/X_0 Ratio on the Biodegradation

The ratio of initial substrate concentration (S_0) to initial biomass concentration (X_0) indicates the availability of a carbon source (Liu *et al.*, 2005) and inhibition for microbial growth. The textile wastewater showed a significant inhibitory effect on decreasing the specific growth rate of microorganisms at a higher concentration (Figure 6), which was the sole carbon source for maximum specific growth rate up to a maximum S_0/X_0 ratio 0.215-0.265. Anaerobic biodegradation of azo dyes' cleavage of the azo bond generates aromatic amines which are more toxic than azo dyes (Gottlieb *et al.*, 2003). Table 3 shows

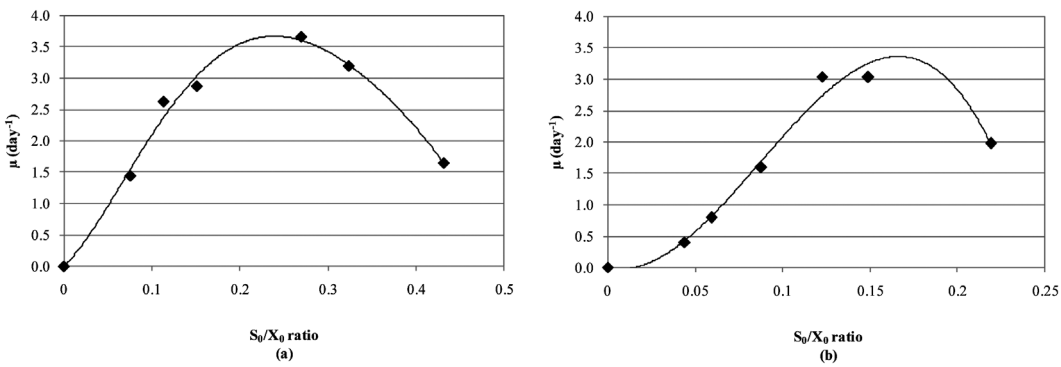

Figure 6. Relation between μ and S_0/X_0 ratio a) Anaerobic influent and b) Anaerobic effluent

Table 4. Biokinetic experimental data of mixed bacterial sludge with anaerobic influent

Substrate (mg COD/L)	F/M	r_X (mg COD/mg MLVSS.h)	Y (mg MLVSS/ mg COD)	μ (day^{-1})	μ_{max} (day^{-1})	K_S (mg COD/L)
30.19	0.0755	0.0897	0.6718	1.4460	4.00	40.62
45.29	0.1132	0.1548	0.7089	2.6328		
60.38	0.1510	0.1725	0.6950	2.8781		
107.81	0.2695	0.2180	0.7007	3.6670		
129.38	0.3234	0.1854	0.7194	3.2009		
172.50	0.4313	0.0963	0.7144	1.6513		

Table 5. Biokinetic experimental data of mixed bacterial sludge with anaerobic effluent

Substrate (mg COD/L)	F/M	r_X (mg COD/mg MLVSS.h)	Y (mg MLVSS/ mg COD)	μ (day^{-1})	μ_{max} (day^{-1})	K_S (mg COD/L)
17.52	0.0438	0.0425	0.3895	0.3972	3.25	34.00
23.65	0.0591	0.0751	0.4401	0.7937		
35.03	0.0876	0.1022	0.6496	1.5936		
49.04	0.1226	0.1801	0.7044	3.0455		
59.55	0.1489	0.1890	0.6685	3.0314		
87.58	0.2189	0.1179	0.6987	1.9773		

the comparison that, for the textile wastewater and anaerobic effluent, microorganisms can degrade the effluents of the anaerobic process less than the influents can. This is because the microorganisms degrade the anaerobic effluent as a carbon source for maximum growth rate up to range of S_0/X_0 ratio 0.151-0.156, which is lower than the influent. Furthermore, the maximum specific growth rate of the anaerobic effluent is lower than the influent, as shown in Tables 4 and 5. Therefore, this indicated that the anaerobic effluent is more toxic to microorganisms than the influent.

Conclusions

The anaerobic process was effective for organic and color removal. However, the azo dye was reduced to aromatic amine during the anaerobic condition, which was monitored by UV-Vis and FTIR analysis. These are potentially hazardous products. The soluble inert COD (S_I) fraction was reduced by being adsorbed on the microorganisms' surface and/or dead cell composition during the anaerobic process. However, the slowly biodegradable COD (X_S) cannot be removed by anaerobic treatment. This can be explained by the potential of the azo dye which was reduced to aromatic amines during the anaerobic process. Moreover, the high value of the maximum specific growth rate (μ_{max}) and sludge yield (Y) were found when the anaerobic influent was fed that caused its high organic concentration content. Similarly, the μ_{max} of the heterotroph microorganism is still high in the anaerobic effluent fed condition. This created the potential for the aerobic treatment being capable of aromatic amine removal. However, microorganisms are able to metabolize into aromatic amine to achieve an optimal growth rate at an S_0/X_0 ratio less than the azo dye wastewater. It was demonstrated that the aromatic amine in the anaerobic effluent is more toxic than the azo dye wastewater.

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