Anaerobic Treatment of Synthetic Textile Wastewater Containing a Reactive Azo Dye

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Abstract: In this study, anaerobic treatment of synthetic textile wastewater containing a reactive azo dye, namely, Remazol Brilliant Violet 5R, was investigated. A fluidized bed reactor (FBR) was used in the study. Before the operation period, start-up of the FBR was completed in 128 days with an immobilized microorganism level of 0.069 g volatile suspended solids per g support material (pumice). Anaerobic treatment of synthetic textile wastewater revealed that 300 mg/L dye was removed in the FBR system. Chemical oxygen demand (COD) and color reduction in the system were approximately 60 and 94%, respectively. Under anaerobic conditions, formation of two sulfonated aromatic amines (SAAs) was detected due to anaerobic reduction of the dye. The SAAs were not degraded under anaerobic conditions. In addition to the anaerobic treatment, the effectiveness of aerobic treatment was investigated in order to further reduce the COD after the anaerobic treatment.

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Introduction

Textile and dyestuff manufacturing industries are the major sources of dye that is released to the environment. Dye is the most difficult constituent of the textile wastewater to treat. Dyes are largely synthetic, typically derived from coal tar and petroleum-based intermediates (EPA 1997). Over 7×10^7 tons of synthetic dyes are produced annually worldwide. Azo dyes are the most widely used dyes in industry (Fitzgerald and Bishop 1995) with a world market share of 60–70%. These dyes are characterized by nitrogen to nitrogen double bonds (N═N). The color of azo dyes is due to the azo bond and associated chromophores. Azo dyes can be classified in several ways. The most commonly used dyes today are reactive azo dyes for cotton dyeing. They react with fiber molecules to form covalent bonds and color the fiber with a fixation rate between 60 and 90%. Reactive dyes are easily hydrolyzed, resulting in a high portion of unfixed dyes that have to be washed off during the dyeing process. As much as 50% of the initial dye load is present in the dye bath effluent (Shore 1995).

In recent years there has been a tendency to use biological systems to treat dye-bearing wastewaters. The strong electron absorbing character of the azo group stabilizes the aromatic pollutants against conversion by oxygenases; hence azo dyes are resistant to aerobic degradation by bacteria (Razo-Flores et al. 1997). The widely used reactive dyes are poorly removed in activated sludge systems (Huren et al. 1994).

A wide range of azo dyes is decolorized anaerobically (Huren et al. 1994; Seshadri et al. 1994; Carliell et al. 1995; Basibüyük and Foster 1997; Razo-Flores et al. 1997; Lourenço et al. 2000; O'Neill et al. 2000; Panswad and Luangdilok 2000). Under anaerobic conditions, azo dyes are readily cleaved via a four-electron reduction at the azo linkage, generating aromatic amines. The required electrons are provided by electron donating carbon sources, which can be volatile fatty acids (VFAs) or glucose. In addition, methanogenic and acetogenic bacteria in anaerobic sludge contain unique reduced enzyme cofactors, such as F_{430} and vitamin B_{12}, that could also potentially reduce azo bonds (Zaoyan et al. 1992; Razo-Flores et al. 1996). Although these processes the remove color of the dyes, they do not completely mineralize the aromatic amines generated in the anaerobic environment (Brown and Laboureur 1983; Kool 1984; Zeyer et al. 1985) with few exceptions (O'Connor and Young 1993; Razo-Flores et al. 1996). Unfortunately, treatment of the aromatic amines is essential, since they are suspected mutagens and carcinogens. It is known that some of the aromatic amines can be biodegraded under aerobic conditions (Brown and Hamburger 1987; Seshadri et al. 1994; Carliell et al. 1995).

Recent studies have indicated the success of sequential biological systems in achieving the complete biodegradation of azo dyes (Huren et al. 1994; Basibüyük and Forster 1997; Lourenço et al. 2000; O'Neill et al. 2000; Panswad and Luangdilok 2000). Several high-rate anaerobic reactor configurations have been developed for treating wastewaters at relatively short hydraulic retention times. Of these, the anaerobic fluidized bed reactor (FBR) is one of the technological advances. It has been successfully employed in a broad spectrum of wastewaters including both readily biodegradable wastes and those resistant to biodegradation (Hickey and Owens 1981; Henze and Harremoes 1983; Denac and Dunn 1988). Although many high-rate anaerobic reactors such as upflow anaerobic sludge blanket reactors and anaerobic filter reactors have been used in recent studies on the anaerobic treatment of textile dyes or wastewater, there are only a few stud-

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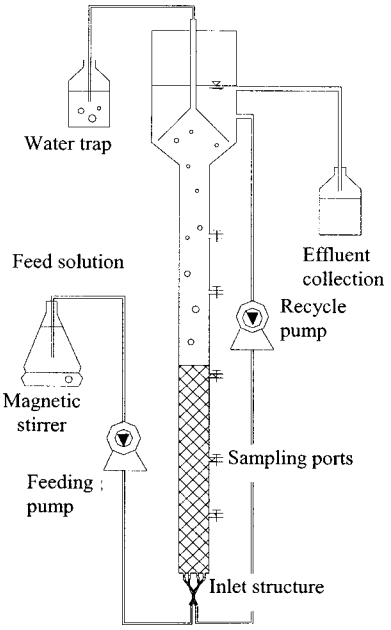

Fig. 1. Schematic diagram of anaerobic fluidized bed reactor system

ies using FBRs in textile dye treatment (Seshadri et al. 1994).

The objective of this study was to investigate the treatability of synthetic textile wastewater including a reactive azo dye in an anaerobic system using an anaerobic fluidized bed reactor and to obtain preliminary data for the effectiveness of aerobic treatment of the intermediates and therefore further chemical oxygen demand (COD) reduction.

Materials and Methods

Fluidized Bed Reactors

A 5.2 cm inner diameter, 73 cm long plexiglass tube was fused to a 15 cm inner diameter, 25 cm long tube to form the 4 L reactor body (Fig. 1). The enlarged top section was used as a gas-solid separator. The bottom of the reactor was flat with four symmetrically placed pores through which flow was equally distributed into the reactor. Five sampling ports were installed on the reactor wall to obtain solid samples. Effluent was collected by gravity through a closed loop connected to a port on the top section of the reactor. The recycle flow was drawn from the top section (5 cm below the free liquid surface) using a peristaltic pump and then fed upward into the reactor at the bottom. Gas produced in the reactor went through a water trap to prevent the intake of oxygen from the atmosphere. The reactor was placed in a temperature-controlled room at $35\pm2°C$. Pumice (HESS Pumice Products, Inc., Malan, Id.) with a diameter of 0.25–1.4 mm and a particle density of 1,764 kg/m^3 was used as support material in the FBR. The experiments conducted in this study were divided into two types: anaerobic and aerobic.

Anaerobic Experiment

The anaerobic experiment was conducted in two periods: the start-up and the operation period. The aim of the start-up period was to achieve biofilm formation on the support material. A mixed anaerobic culture that had mixed liquor suspended solid (MLSS) and mixed liquor volatile suspended solid (MLVSS) concentrations of 72.7 ± 6.8 and 26.03 ± 1.37 g/L, respectively, was obtained from the anaerobic sludge digesters of the Ankara wastewater treatment plant. Feed (COD of approximately 5,000 mg/L) including glucose, methanol, yeast extract, and basal medium (BM) was used for the start-up period. Basal medium contains all the necessary micro- and macronutrients for optimum anaerobic microbial growth (Demirer and Speece 1998), including (mg/L) NH_4Cl (1,200), $MgSO_4\cdot7H_2O$ (400), KCl (400), $Na_2S\cdot9H_2O$ (300), $CaCl_2\cdot2H_2O$ (50), $(NH_4)_2\cdot HPO_4$ (80), $FeCl_2\cdot4H_2O$ (40), $CoCl_2\cdot6H_2O$ (10), KI (10), $MnCl_2\cdot4H_2O$ (0.5), $CuCl_2\cdot2H_2O$ (0.5), $ZnCl_2$ (0.5), $AlCl_3\cdot6H_2O$ (0.5), $NaMoO_4\cdot2H_2O$ (0.5), H_3BO_3 (0.5), $NiCl_2\cdot6H_2O$ (0.5), $NaWO_4\cdot2H_2O$ (0.5), Na_2SeO_3 (0.5), cysteine (10), and $NaHCO_3$ (3000). The pH of the BM was 8.5. Yeast extract was used at 20 mg/L and the remaining COD was supplied by methanol and glucose at different ratios in the start-up mixture.

Table 1. Organic Load and Percentage of Methanol, Glucose + Yeast, and Ammonium Chloride during Start-Up

Time (days)	Load (kg COD/m$^3\cdot$d)	Methanol (% COD)	Glucose + yeast (% COD)	NH_4Cl[a]
0–24	0–3.75	50	50	50
25–37	3.75–10	25	75	75
38–45	10–15	12.5	87.5	100
46–128	15–22	0	100	100

[a]% of its value at the end of the start-up.

Start-up of each anaerobic reactor was achieved using a procedure involving incremental increases in COD loading and substrate replacement by methanol. The loading increase was implemented by increasing the feed rate while keeping the feed COD at around 5,000 mg/L. During the period of initial biofilm development, up to half of the COD provided by glucose was replaced by methanol to encourage the growth of *Methanosarcina*. In addition, NH_4Cl concentration was decreased by 50% during this period in order to increase the C/N ratio and encourage extracellular polymer production, which aids bacterial attachment on solid surfaces. Table 1 shows the organic loading and percentage of methanol and NH_4Cl during the start-up.

In the operation period, the reactor was fed with synthetic textile wastewater containing a hydrolyzed reactive azo dye, Remazol Brilliant Violet 5R (Fig. 2), hydrolyzed starch solution, acetic acid, and BM with 2 g/L $NaHCO_3$. Starch and acetic acid were used as carbon sources because the COD in real textile wastewater is mainly contributed from sizing agents (60%) such as starch and some additives like acetic acid. In order to better simulate the real case condition, hydrolyzed starch and dye were used. To prepare hydrolyzed starch solution, 100 g starch and 40 g sodium hydroxide were dissolved in distilled water and stirred for 15 hours at room temperature, then neutralized to pH 7 with 37% HCl, and diluted to 1 L with distilled water. For preparation of hydrolyzed dye solution, 5 g dye was dissolved in distilled water and the pH was adjusted to 12 with 1 M NaOH solution and

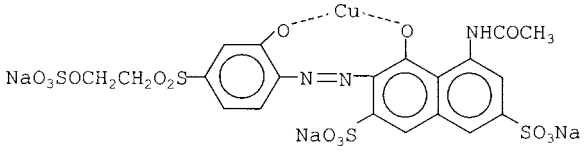

Fig. 2. Chemical structure of Remazol Brilliant Violet 5R

Table 2. Various Basal Medium Compositions and Dye Concentrations Fed to Anaerobic Fluidized Bed Reaction in Operation Period

Stage	Operation days	OLR (kg COD/m^3·d)	Hydraulic retention time (h)	Feed Composition	
				Basal medium	Dye (mg/L)
1	1–46	1	24	100% basal medium	25
2	47–62	1	24	100% basal medium except NH_4Cl (120 mg/L)	25
3	63–68	1	24	10% of basal medium	25
4	69–80	1	24	10% of basal medium	50
5	81–94	1	24	10% of basal medium	100
6	95–115	1	24	10% of basal medium	200
7	116–129	1	24	10% of basal medium	300

then stirred for 1 hour at 80°C. After cooling to room temperature, the solution was neutralized to pH 7 with 37% HCl and diluted to 1 L with distilled water (Lourenço et al. 2000). In the operation period, the FBR was operated under seven different operational conditions in 129 days. These different operational conditions are given in Table 2.

Aerobic Experiment

The effluent of the anaerobic FBR for particular days was aerobically treated in 500 mL batch reactors to obtain preliminary data about the effectiveness of aerobic treatment of aromatic amines and therefore further COD reduction. Effluent samples at days 124 and 125 were used in the batch aerobic experiments. Nitrogen and phosphate concentrations and COD of the effluent were measured before conducting the batch aerobic experiment to determine if there was nutrient deficiency. After the addition of required nutrients, aerobic microorganisms from laboratory-scale semibatch aerobic reactors were added, to result in a MLVSS concentration of 2 g/L in the reactors. Then wastewater-containing flasks were placed in a 20°C water bath and aerated with air pumps with a flow rate of 1,300 mL/min, ensuring that dissolved oxygen was not limiting. The aerobic batch reactors were operated for 15 days.

Analytical Methods

Measurements of pH were performed with a pH meter (Model 2906, Jenway Ltd., Essex, U.K.) and a pH probe (G-05992-55, Cole Parmer Instrument Co., Vernon Hills, Il.). Chemical oxygen demand was measured with a Hach spectrophotometer (Model P/N 45600-02) and vials for 0–1,500 mg/L COD. Suspended solids and volatile suspended solids were measured as described in *Standard Methods* 2540 D,E (*Standard Methods* 1998). Total phosphorus and total Kjeldahl nitrogen concentrations were also determined by *Standard Methods* 4500-P-E and 4500-N_{org}, respectively. To measure the immobilized biomass, samples from the expanded bed material were collected in a ceramic dish through a sampling port (5–10 mL). Suspended biomass in the mixed liquor was removed by gentle wash, then it was dried at 105°C for 24 h. The dried sample was then muffled at 600°C for 1 h. The difference between the two dried weights yields the weight of biomass immobilized as attached volatile solids (AVS).

Color was measured with a UV-visible spectrophotometer (Varian Cary 100 Conc, Mulgrave, Victoria, Australia) at the Remazol Brilliant Violet 5R peak absorption wavelength (560 nm). Before analysis, samples were filtered through 0.45 μm filters to remove suspended matter. Dye removal was measured by high performance liquid chromatography (HPLC) (Shimadzu Co., Kyoto, Japan). The UV-visible detector was set at 560 nm. The HPLC column was a reverse-phase Nucleosil C18 column; the mobile phase used was a mixture of acetonitrile and 20 mM ammonium acetate buffer (pH 9) in a 1:1 ratio. The flow rate was 0.75 mL/min. Before injection, samples were filtered through 0.45 μm filters. Aromatic amine formation was monitored by HPLC. The mobile phase used for separation of aromatic amines was a mixture of acetonitrile and water in a 3:7 ratio. The flow rate was 1 mL/min. The UV-visible detector was set at 254 nm (Macherey-Nagel 1990).

Results and Discussion

Anaerobic Experiment

The start-up period was completed in 128 days. Table 3 indicates the operational parameters obtained at the end of the start-up period. During the operation period, bed expansion was increased and kept between 35 and 40%, which is in the typical range (Stronac et al. 1987; Balaguer et al. 1991). When attached volatile solid values were compared with the corresponding literature values (Tseng and Lin 1994; Garcia et al. 1996; Shieh and Hsu 1996; Farhan et al. 1997; Perez et al. 1999), it was seen that the AVS values attained in this study were within the typical range reported in the literature.

In the operation period, synthetic textile wastewater treatment was studied in the reactor. The corresponding effluent pH, VFA, and alkalinity values, influent and effluent COD values, COD removal, influent and effluent colors and color removal, influent and effluent dye concentrations, and dye removals are depicted in Fig. 3. The optimum reactor operation conditions are achieved in anaerobic systems when pH and alkalinity values are in the ranges of 6.5–8.5 and 800–1,500 mg/L (as $CaCO_3$), respectively and when VFA concentration is not higher than 1,000 mg/L. The effluent pH, VFA, and alkalinity values [Figs. 3(a, b, and c)] indicated that the reactor was operating properly in most of the experimental period.

Table 3. Operational Parameters at End of Start-Up Period for Fluidized Bed Reactor

Operation parameter	Fluidized bed reactor
Hydraulic retention time (h)	5.7
Upflow velocity (m/h)	19
$Q_{recycle}/Q_{feed}$	295
Expansion (%)	18
Volume of expanded bed (cm^3)	690
$M_{support}$ (gram)	600
g VSS/g support	0.068
Total VSS (g)	41.2

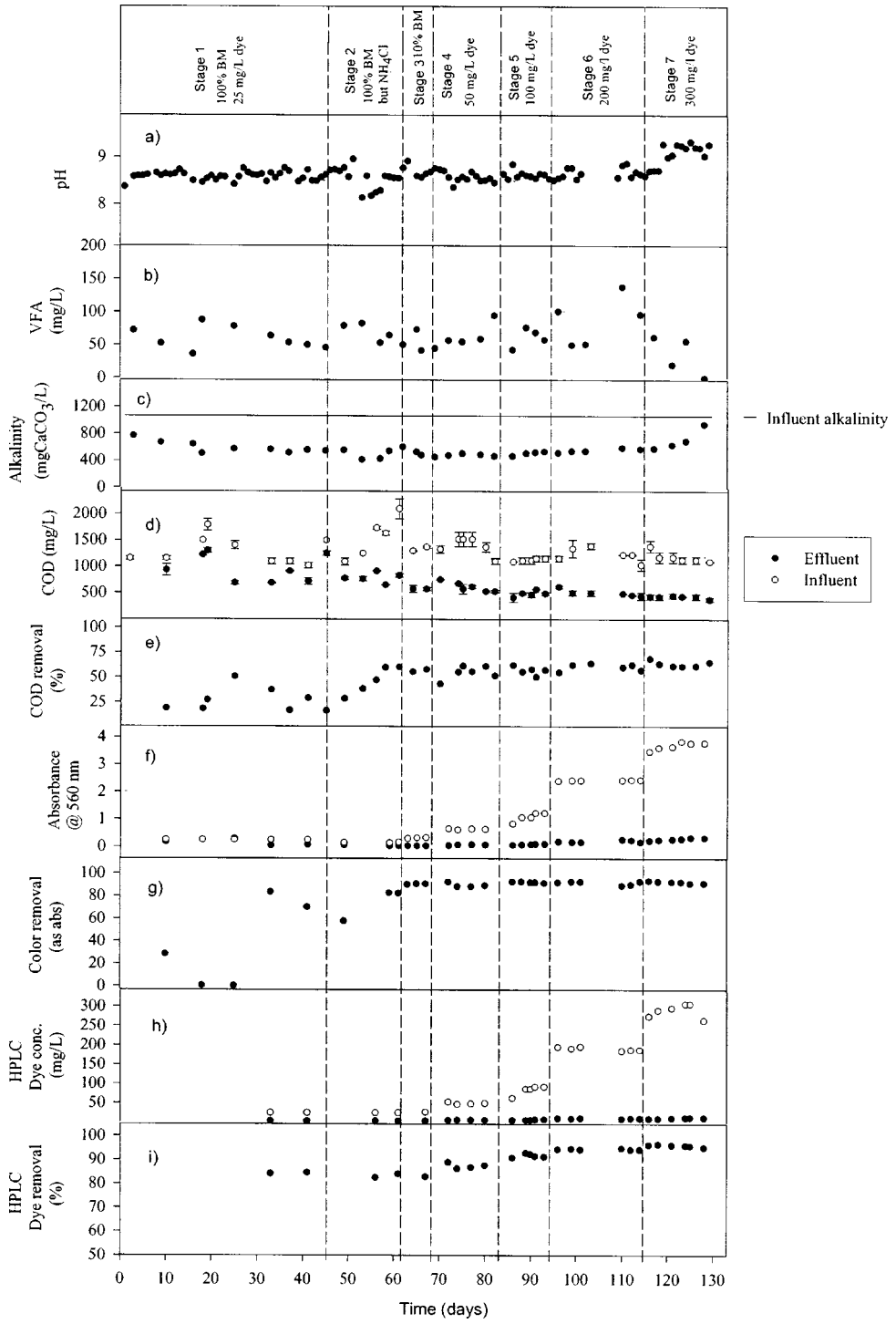

Fig. 3. Chemical oxygen demand, dye, and color removals from synthetic textile wastewater in anaerobic fluidized bed reactor and pH, volatile fatty acids and alkalinity in effluent

Stage 1

In Stage 1 (days 1–46), the synthetic textile wastewater was composed of BM, 25 mg/L hydrolyzed dye, and acetic acid and hydrolyzed starch (85% and 15% on a COD basis, respectively). On day 10 of the operation, the color removal rate was determined as 29%, and then it dropped to zero [Figs. 3(f and g)]. During the first 10 days no biotransformation occurred; dye was adsorbed to biomass gradually until the equilibrium concentration was reached. As stated by Huren et al. (1994), in continuous systems, adsorption reached equilibrium in about 10 days. After the adsorption equilibrium was reached, no dye removal was observed. After a lag phase of 25–30 days, the color removal rate increased to 80% on day 33. The HPLC measurements [Figs. 3(j and k)], which indicated 84% dye removal, also supported effective dye removal. At the end of this period, COD removal was less than 25% on average, which is quite low. Possible reasons for low COD removal were considered and operational conditions were changed accordingly in Stage 2.

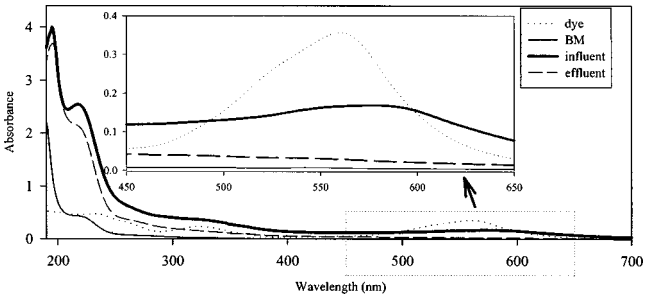

Fig. 4. Ultraviolet-visible scan of constituents of synthetic textile wastewater

Stage 2

Initially, the synthetic textile wastewater composition was thought to be responsible for the low color removal. The NH_4Cl concentration in the BM was 1,200 mg/L, which is abundant for 1,000 mg/L of COD in an anaerobic treatment with an optimum COD:N:P ratio of 100:1:0.2 (Chinwetkitvanich et al. 2000). Therefore, the NH_4Cl concentration was reduced to 10% of its initial value, or 120 mg/L. As a result, both COD and color removal rates increased [Figs. 3(e and g)]. The COD removal increased from 25 to 60%, while color removal (as absorbance) increased from 50 to 80%. According to the HPLC measurements [Figs. 3(h and i)], the dye removal efficiency was around 85%.

Stage 3

In Stage 3 (days 63–68), the concentration of each component the BM was reduced to 10% of its original value. The reason for this change was that a simple analysis was conducted to see clearly which constituent of the synthetic textile wastewater resulted in which peak, and if the resultant UV-visible scan pattern of the synthetic textile wastewater was cumulative of its constituents' scan patterns during Stage 2. The results are given in Fig. 4. The 25 mg/L dye solution showed a peak in the visible range at 560 nm; the synthetic textile wastewater, which also contained 25 mg/L dye solution, on the other hand, did not show that peak. Based on this observation it was concluded that $Na_2S \cdot 9H_2O$, which gave the BM a black color, masked the dye's brilliant appearance and did not allow it to show the peak at the usual wavelength. To eliminate any possible errors in the color measurements, the concentrations of all the constituents of the BM (except $NaHCO_3$) were reduced by 90%. Even after this change, the nutrient concentrations in the BM were still adequate for an influent COD concentration of around 1,000 mg/L as supported by Razo-Flores et al. (1997), O'Neill et al. (2000), and Panswad and Luangdilok (2000). Fig. 5 shows an UV-visible scan of the dye solution and synthetic textile wastewater after reducing the BM constituent concentrations by 90%. In Stage 3, although a steady COD removal of 55–60% was observed, better color removal was recorded with respect to absorbance measurements (90%). According to HPLC measurement results, dye removal was 83% at the end of Stage 3.

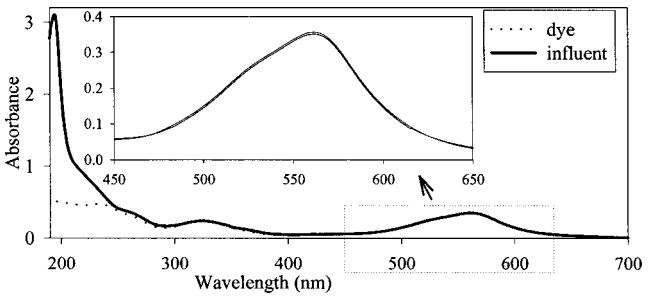

Fig. 5. Ultraviolet-visible scan of dye solution and synthetic textile wastewater after reducing basal medium composition by 90%

Stage 4

In Stage 4 (days 69–84), the dye concentration in the synthetic textile wastewater was increased to 50 mg/L and the system performance was monitored. While COD removal in the reactor did not change considerably, 92% of color was removed effectively and 89% of dye.

Stage 5

In this stage (days 85–94) only the dye concentration in the influent was increased to 100 mg/L; the other constituents were the same. The COD removal efficiency in the reactor was in the range of 55–58%. Color removal was 92% and 91% as absorbency and HPLC measurement results, respectively. Therefore, it can be stated that 100 mg/L dye was effectively decolorized in the system.

Stage 6

In Stage 6 (days 95–115), the dye concentration in the influent was further increased to 200 mg/L. The COD removal rate remained constant around 60%. Color removal achieved in the reactor was 93% and 94% as absorbency and HPLC measurement results, respectively. The effluent dye concentration was 11 mg/L.

Stage 7

In Stage 7 (days 116–129), the dye concentration in the influent was increased to its final value of 300 mg/L in order to observe the effect of further increase in dye concentration on color and COD removal. The COD removal rate remained between 61 and 63%. Color removal achieved in the reactor was 92% and 96% as absorbency and HPLC measurement results; respectively. The effluent dye concentration was 13 mg/L. As a result, it can be concluded that 300 mg/L dye was decolorized in the system effectively without affecting the COD and color removal performance.

Aromatic Amine Production

From Remazol Brilliant Violet 5R, the formation of two sulfonated aromatic amines is expected under anaerobic conditions. Production of the sulfonated aromatic amines was analyzed with HPLC. The first peak obtained was due to a benzene-based aromatic amine and the second peak to a naphthalene-based amine (through comparison of retention times with amine standards in Macherey-Nagel 1990). With the increase in dye concentration given to the reactor, aromatic amine production also increased. It must be noted that this was only a qualitative analysis and quantification of these aromatic amines was not within the scope of the study.

Aerobic Experiment

Under aerobic conditions, COD values were reduced from 440 $\pm$ 2.1 to about 261.7 $\pm$ 28, by about 40%, after a 6 h running time. After that time there was an increase in COD values with time. However these values were fluctuating, which could be due to experimental errors in the COD measurement. The COD reduction in a 6 h running time was probably due to degradation of readily degradable compounds in the form of acetic acid and

starch that were present in the anaerobic effluent of the synthetic textile wastewater treatment. In a 6 h period, the readily degradable compounds were exhausted. Therefore, microorganisms which could not easily use sulfonated aromatic amines as carbon source began an endogenous decay.

Degradation of Aromatic Amines

According to the results of HPLC analysis, the aerobic phase introduced an alteration in the chromatograms, mainly that the benzene-based sulfoamine was partially converted. The area under the peak in the HPLC measurements corresponding to the benzene-based sulfoamine decreased by 40% after aerobic treatment. However, the naphthalene-based amine peak remained almost unchanged after the aerobic phase.

Difficulties with the mineralization of sulfonated aromatic amines under aerobic conditions after anaerobic reduction of azo dyes were also encountered by Tan et al. (1999). However, Brown and Laboureur (1983) indicate that many aromatic amines are readily degraded under aerobic conditions. This study suggests that degradation of sulfonated aromatic amines (SAAs) is difficult with unacclimated aerobic cultures. With cultures adapted to SAAs, on the other hand, complete mineralization and therefore further COD reduction might be achieved (Fiegel and Knackmuss 1988).

Conclusions

The results of this research indicate that FBRs could successfully be applied for the treatment of textile industry wastewater with a low hydraulic detention time, and stable operation and high color removal efficiency could be achieved. The following specific conclusions can be made based on the experimental outputs of this study.

- Three hundred mg/L Remazol Brilliant Violet 5R was decolorized successfully in the FBR system without negatively affecting the COD and color removal performance. The color and COD removal rates attained in the anaerobic reactor were about 94 and 60%, respectively.
- Formation of two SAAs was detected due to the reduction of Remazol Brilliant Violet 5R under anaerobic conditions. Removal of these amines was not observed in the anaerobic reactor.
- Under aerobic conditions, SAAs could not be completely mineralized with an unacclimated culture and therefore considerable COD reduction was not achieved.
- Although color was removed satisfactorily under anaerobic conditions, the effluent COD was still high for discharge. Therefore, a two-stage configuration consisting of anaerobic and aerobic (as a polishing step) reactors should be used in order to lower the effluent COD of textile wastewaters for discharge into receiving water bodies.

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