Tropical Agricultural Research & Extension 8,2005

Biological Decolourization of Simulated Textile Wastewater in UASB Reactor System by Anaerobic Granular Sludge

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Accepted 5th August 2005

ABSTRACT

The textile wastewater, containing three acid dyes belonging to different chemical groups; Acid Blue 204 (AB204), Acid Red 131 (AR131) and Acid Yellow 79 (AY79), was studied in UASB (Upflow Anaerobic Sludge Blanket) reactor system from 10 mg/l to 300 mg/l dye concentrations with COD (Chemical Oxygen Demand) of 2000 mg/l as co-substrate. The hydraulic retention time (HRT) of 24 h was maintained. The decolourization achieved was in the range of 73±13.4% to 95%. At 300 mg/l dye level, 89% decolourization was attained. The study suggested that decolourization was due to the combination of processes of adsorption and biodegradation. The UV-Visible spectrum of influents and effluents showed significant changes confirming the degradation of dyes. Acid Blue 204 was decolouriszed by adsorption into microbial granules while other dyes were biodegraded. The COD removal efficiency was over 85% during all dye concentrations indicating no severe toxicity caused to microorganisms by dye mixture. The VFA (volatile Fatty Acids) and alkalinity in effluents were well below 61 mg/l and 1733 mg/l, respectively, confirming the reactor operated properly. If proper adaptation and maintenance of the UASB reactor system are practiced, the decolourization of textile wastewater containing acid dyes could be achieved via anaerobic microorganisms

Keywords: Decolourization, Degradation, Anaerobic, UASB, Textile waste water, Acid dyes

INTRODUCTION

Wastewater generated from various industries creates a major environmental detrimental effect, leading to imbalance of bio-systems. Textile industry, which is one of the largest water consumers in the world, produces the wastewater comprising various recalcitrant agents such as dye, sizing agents, and dying aids. Therefore, it has to be really concerned in releasing these types of wastewater to the environment. Colour is very important in the disposal of textile wastewater due to aesthetic deterioration as well as the obstruction of penetration of dissolved oxygen and sun light into natural water bodies. The reduction of dissolved oxygen and sunlight penetration into natural water sources seriously affect aquatic life. Besides, the dye precursors and dye degradation products have proven to be carcinogenic and mutagenic in nature. The different types of dyes are used for the colourization of textiles in the production process. Most of these dyes presently used in

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the world are synthetic in nature, and generally have a complex chemical structure, unfamiliar to natural environment and hence persist in nature.

The largest class of dyes used in the world is referred to as acid dyes (Color Index, 1998). Acid dyes are anionic compounds mainly used for dyeing nitrogen containing fabrics like wool, polyamine and silk. Most of acid dyes belong to azo and anthraquinone groups.

Azo dyes, characterized by their typical azo bond (R_1-N=N-R_2), are more popular group of dye used, basically, in textile industry. More than one million tons of dyes annually produced in the world are azo dyes representing 70 % by weight (Hao *et al.*, 2000). Azo dyes, due to their poor exhaustion properties as much as 30 % of initial dye applied remain unfixed and end up in effluents (Manu and Sanjeev, 2003). Anthraquinone dyes are one of the largest classes of dyes used in textile industry and their characteristic chromophore is the carbonyl group, and this may be present once or several times. It is estimated that 10-20 % of dyes are lost into wastewater during dyeing process (Graça *et al.*, 2001). Therefore, the proper treatment technologies should be used before discharging the textile wastewater into natural biotic environment to avoid hazards created by direct disposal.

The possibility of using various physical and chemical methods for the treatment of textile wastewater has been reported. The existing physical and chemical methods proven to be effective such as advanced oxidation process like use of Fenton's regent (H_2O_2 & Fe^{2+}), H_2O_2, and Ozonation are costly in terms of operation costs; coagulation flocculation using lime, alum, polyelectrolyte and ferrous salts produce large amount of sludge giving handling and disposal problems; the adsorption of dyes using activated carbon and various other adsorbents are also costly; and photochemical oxidation of dyes using UV and sunlight with oxidation agents like catalytes, H_2O_2 are also not economically viable (Tanja *et al.*, 2003; Stanislaw and Monika, 1999; Huseyin, 2005; Georgiou *et al.*, 2003; Daneshvar *et al.*, 2003; Zhemin *et al.*, 2001; Muruganandham and Swaminathan, 2004).

The biological treatment methods have proven that they can be used for various types of wastewaters, including recalcitrant chemicals containing wastewater also. Even though, earlier, it was assumed that the biological methods could not be used for the decolourization of azo dye containing wastewater due to recalcitrant nature, nowadays it has been proven that decolourization could be achieved through biological processes providing low cost and effective means for the treatment of textile wastewaters (Pearce *et al.*, 2003).

Azo dye wastewater treatment has been studied in aerobic as well as anaerobic treatment processes and it cannot be degraded leading to decolourization by aerobic microorganisms due to electron withdrawing nature of azo bond, while anaerobic microorganisms have shown that decolourization is possible through cleavage of azo bond, producing corresponding amines (Knackmuss, 1996; Georgiou *et al.*, 2003, Mendez-Paz *et al.*, 2005).

On the other hand, the products released by the cleavage of azo bond by anaerobic microorganisms, aromatic amines that are considered as carcinogenic compounds, are quite stable in anaerobic environment. Further mineralization of these compounds has been reported under aerobic conditions, although auto oxidation can also occur (Kudlich *et al.*, 1999; Stolz, 2001).

On the contrary, aromatic amines having

hydroxyl and carboxyl groups can be fully mineralized under anaerobic methanogenic conditions (Razo-Flores *et al.*, 1996, 1997). It is reported that anthraquinone dyes are also very resistant to biodegradation (Stanislaw *et al.*, 2001; Yang *et al.*, 2004).

Upflow Anaerobic Sludge Blanket (UASB) reactor, developed in 1970's and considered as a high rate bioreactor, is generally more resistant to toxic compounds as a result of structure of formed granular sludge with good settling properties and mechanical strength, and suitable for the treatment of wastewaters containing xenobiotic and recalcitrant compounds, and it promotes adaptation of bacteria to the presence of toxic compounds, as well as it could be used for treatment of wastewaters previously considered unsuitable for anaerobic treatment (Jantsch *et al.*, 2002; Harada *et al.*, 1996; Van Lier *et al.*, 2001; Donlon *et al.*, 1997).

The studies on degradation or the decolourization of textile wastewater containing mixture of dyes belong to different chemical classes in anaerobic systems are inadequate in literature and little has been done on decolourization of acid dyes using biological methods. Therefore, the purpose of the present work was to evaluate the decolourization of mixture of three commercial acid dyes containing simulated textile wastewaters by a UASB reactor system with un-acclimated anaerobic granular sludge.

MATERIALS AND METHODS

Digester Design

A laboratory scale (UASB) reactor was used in the study. The effective volume of the reactor, having 230 mm height and 84 mm internal diameter, was 1.25 l. The flow distributor to distribute influent evenly from the bottom, and

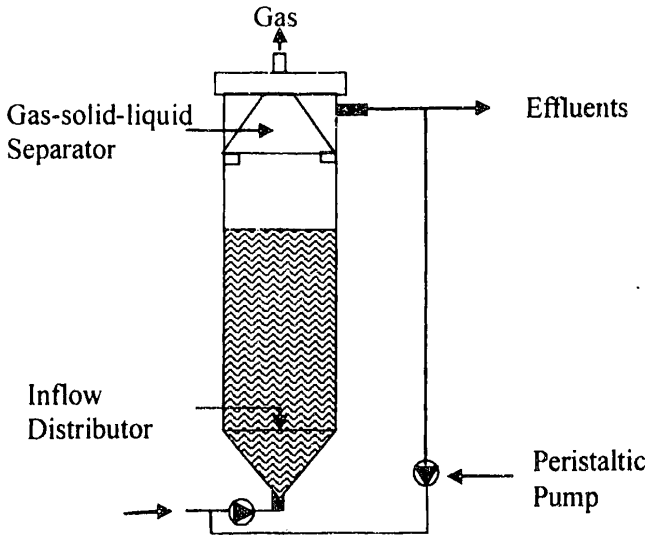

Figure 1. Diagram of the UASB reactor

solid-gas-liquid separator to prevent loss of granules from the reactor and for the easy release of produced gas, was placed at the bottom and the upper part of the reactor, respectively. The detailed diagram of the reactor is shown in Figure 1. The peristaltic pump (BT-200, Shanghai Hu Xi Analysis Instruments Factory Co. Ltd, P.R China), which can discharge constant flow rate, was used to pump substrate into the reactor. The flow rate of the pump is in between 1-6800 ml/h. The reactor was operated in temperature controlled room at $35 \pm 3\ ^{0}C$.

Preparation of Basal Medium

The basal medium was prepared according to Ergüder *et al.*, (2003), who used this basal medium to treat toxic compounds, with some adjustments. The medium composed of macro and micro nutrients essential for microbial growth. The composition of the medium used was (in mg/l): NH_4Cl (1200), $MgSO_4.7H_2O$ (400), KCl (400), $CaCl_2.4H_2O$ (50), $(NH)_2HPO_4$ (170), $FeCl_2.4H_2O$ (40), $CoCl_2.6H_2O$ (10), KI (10), $MnCl_2.4H_2O$ (0.5),

$CuCl_2.2H_2O$ (0.5), $ZnCl_2$ (0.5), $AlCl_3.6H_2O$ (0.5), $Na_2MoO_4.2H_2O$ (0.5), H_3BO_3 (0.5), $NiCl_2.6H_2O$ (0.5), $NaWO_4.2H_2O$ (0.5), Na_2SeO_3 (0.5). To maintain sufficient buffering capacity in the reactor, $NaHCO_3$ was used at the concentration of 4500 mg/l. The substrate was prepared separately such as macro, micro, buffering solution and carbon source, once every six days and stored below 4 °C to prevent premature degradation. The concentrated substrate solutions were diluted according to the nutrient requirements of the microorganism when preparing the simulated textile wastewater.

Reactor Startup

The reactor was seeded, up to 1/3 of the reactor height, with anaerobic granular sludge obtained from another UASB reactor running with glucose containing synthetic wastewater. Total Suspended Solid (TSS) and Total Volatile Suspended Solid (TVSS) of granules were 52±0.76 g/l and 41.69±0.69 g/l, respectively. Initially, the reactor was fed with synthetic wastewater containing basal medium and glucose as carbon source (~1000 mg COD/l) at 1 kg/m3-day of organic loading rate (OLR) and increased COD concentration up to 2000 mg/l (OLR is 2 kg/m^3-day) within three weeks achieving over 90 % COD removal.

Analytical Methods

Total alkalinity, pH, TSS and TVSS were determined according to the procedure outlined in standard methods (APHA, 1985). The Chemical oxygen demand was determined spectrometrycally by 5B-1 Quick COD analyzer (The LianHua Environmental Instrument Institute, Langzhou, PR China). Volatile fatty acids (VFA) in effluents were determined by Shimadzu GC-14A gas chromatography (GC) with PEG-20M (SGE International, Australia) capillary column, 30m length and internal diameter 0.53 mm. Prior to injection, sample were acidified with 1M H_2SO_4, centrifuged 15 min at 13000 rpm, and filtered through 0.22 M filter (Tahar and Sami, 2005). The column temperature was initially set at 100 °C for 1 min and then increased at a rate of 5 °C per minute up to 24 °C and held for 20 min. The injector and detector temperature were set at 24 °C and 25 °C, respectively. Nitrogen was used as carrier gas at the flow rate of 1.5 ml/min. The colour of influent and effluent was determined spectrophotometrically at maximum absorbance (552 nm) by UV-visible spectrometer (Shimadzu UV-2450). The samples were filtered by microfiber filter and centrifuged at 7000 rpm for 10 min prior to absorbance measurements (Delia and Mustafa, 2004). The residual dyes in effluents were quantified by standard curve (R2=0.99) prepared at maximum absorbance. Colour removal was determined according to Eq. (1).

$$\frac{A_0 . A_1 \times 100}{A_0} \qquad (1)$$

where A_0 is absorbance in influent and A_1 is absorbance in effluent.

All chemicals used for analysis were in AR grade except GC, for which GC grade was used. Dyes; Telon Red M-3B (C.I. Acid Red 131; Azo Dye), Telon Blue M-RLW (C.I. Acid Blue 204; Anthraquinone Dye) and Telon Yellow M-4GL (C.I. Acid Yellow 79; Chemical class has not been disclosed) used for wastewater preparation, were in commercial grade and used without further purification.

Experimental Procedure

All experiments were carried out under the steady state conditions of UASB reactor. The steady state can be defined as a state which can be maintained indefinitely without system failure, during which the variation in bioreactor performance parameters in last consecutive measurements are relatively constant (<10%) (Trnovec and Britz, 1998; Ayoob et al., 2003). Hence, the length of each phase of the steady state was based on the stability of the bioreactor effluent pH, colour and COD removal. The reactor was operated in effluent recycled mode of which one influents batch (1.25 l) was recycled for 24 h. The flow rate and HRT during operation period was 1.8 l/h and 24 h, respectively.

After reaching the steady state with synthetic wastewater without dye (>90% COD removal), dye containing simulated textile wastewater was introduced. The simulated textile wastewater was prepared by mixing, three dyes in equal quantity. Initially, 10 mg/l concentration dye containing wastewater was fed and then 25, 50, 100, 150, 300 mg/l concentrations were introduced, respectively, after reaching the steady state in each stage.

The study was continued for about three months with three steady state runs in each concentration of different dye containing simulated textile wastewater. The reported values are the mean values of three different steady state runs having less than 5% of standard deviation and otherwise stated.

RESULTS AND DISCUSSION

Colour Removal in the Reactor

The study was conducted, keeping the co-substrate, glucose, concentration at relatively constant level throughout the study period.

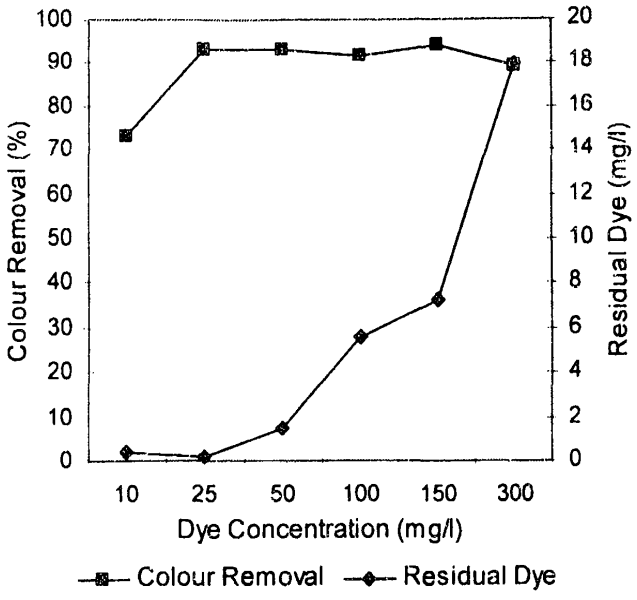

Figure 2. Colour removal and residual dye after treatment at different dye concentrations

Since the dye mixture used in the experiment contains azo dye as well, it was essential to have co-substrate to provide required electron donation for breaking down of azo bond for decolourization (Georgiou *et al.*, 2004). The dye concentration was stepwise increased from 10 mg/l to 300 mg/l after reaching the steady state in each dye level. Such a gradual increased administration procedure of dyes was preferred to acclimatize the granules to toxicant and recalcitrant compound containing wastewaters. It avoids the possible inhibitory effects to biological activities at high concentrations (Ergüder *et al.*, 2003).

Figure 2 shows the colour removal and the residual colour in effluents. At 10 mg/l concentration of dye, the colour removal was 73±13.4% and it was the lowest removal observed. The maximum colour removal, 94%, was attained at 150 mg/l dye concentration. At the same time, when the dye concentration was increased up to 300 mg/l, colour removal appeared to be reduced down to 89%. But, the

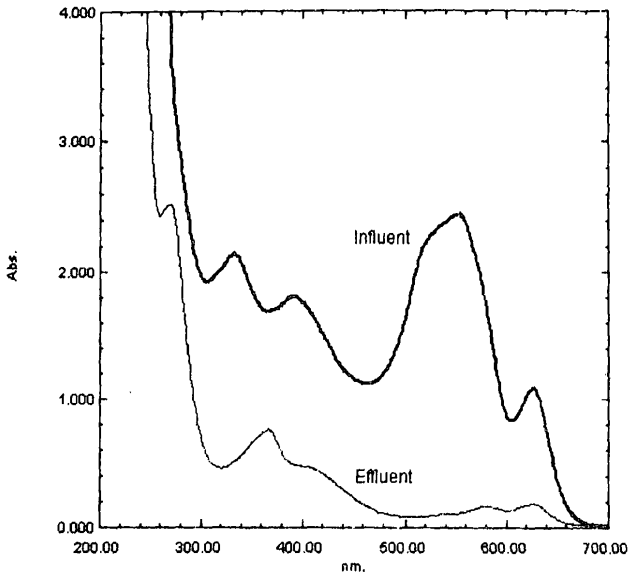

Figure 3. UV-Visible spectrum of influents and effluents

overall removal efficiency of colour during entire study period was over 89%, except at initial study period of 10 mg/l concentration.

The colour removal could be achieved through biodegradation, the adsorption of dye onto microbial granules, and adsorption, followed by biodegradation. The lower colour removal at the initial stage of the experiment, 10 mg/l dye concentration, seems to be due to the low adsorption on to granules; when the dye concentration is increased, the adsorption is also increased with the simultaneous increase of biodegradation. Even though decolourization was high (approximately 90%) at increased dye concentrations, the higher amounts of residual dyes were also noticed in effluents. The residual dye in effluent was 0.4 mg/l at 10 mg/l dye concentration while 18 mg/l remained at 300 mg/l dye concentration.

The remarkable changes of the UV-Visible spectra in influents and effluents can be seen and explained by the structural modifications of the dye molecules by means of anaerobic microorganisms (Manu and Sanjeev, 2003; Rui *et al.*, 2001; Pinheiro, 2004; Robert and Sanjeev, 2005; Brás *et al.*, 2005). The influent spectrum in the visible region (400 800 nm) has two prominent peaks at 625 and 552 nm and effluent spectrum shows two peaks at 625 and 580 nm (Figure 3). The peak at 552 nm in influents disappeared in effluent spectrum and at the same time new two peaks in UV region emerged at 270 and 366 nm. According to Pinheiro and co-workers (2004), the peak at 270 nm is probably due to aromatic amines. The previous studies showed that anaerobic degradation of azo dyes produce relevant aromatic amines by cleavage of azo bond (Méndez-Pas *et al.*, 2005; Stolz, 2001; André *et al.*, 2004; Georgiou *et al.*, 2003). Therefore, peak at 270 nm gives evidence of degradation of AR131 by anaerobic granules. The absorbance peak at 625 nm can be seen in both influents and effluents but peak in effluent is relatively very small compared to influent peak. The UV-Visible spectrum obtained in single dye solutions showed that this peak is created by AB204, anthraquinone dye. At the same time, this dye makes another peak, relatively small than previous one, at 580 nm. And in the effluent spectrum, this peak can also be properly observed. Therefore, it seems that AB204 dye was not biologically degraded but it is adsorbed into microbial granules leading to decolourization. Malpei and co-workers (1998) have studied the degradation of Brillant Red Resolin BLS, anthraquinone type dye, under batch anaerobic conditions using UASB granules obtained from soft drink wastewater treatment system and they hardly found any degradation. It is reported that biodegradation of Acid Blue 40, anthraquinone dye, is difficult but pretreatment by advanced oxidation can increase the biodegradation. The sole removal of dye without pretreatment by microbes is due to bio-sorption (Stanislaw *et al.*, 2001).

Thongchai and Worrawit (2000) reported that anthraquinone dye, Remazol Blue R and Cibacron Blue CR, concentration of 20 mg/l can be decolourized by anaerobic aerobic sequential batch reactor system up to 64-66%. They concluded that entire decolourization achieved is not due to microbial degradation but through the adsorption on to floc materials.

The dyes, AY79 and AR131, showed their maximum absorbance at 403 and 548 nm (visible region), respectively. In the effluents, any absorbance peaks can not be seen relevant to these two dyes. Therefore, these two dyes have gone through microbial degradation leading to decolourization. The peak at 270 nm in effluents due to aromatic amines produced from AR131 by anaerobic degradation and peak at 366 nm, possibly, due to degradation products of AY79 or AR131 or both. The mixture of dyes studied has shown higher decolourization under anaerobic UASB reactor system. The primary mechanisms for decolourization of these dyes appear to be adsorption of dyes onto microbial granules and followed by biodegradation.

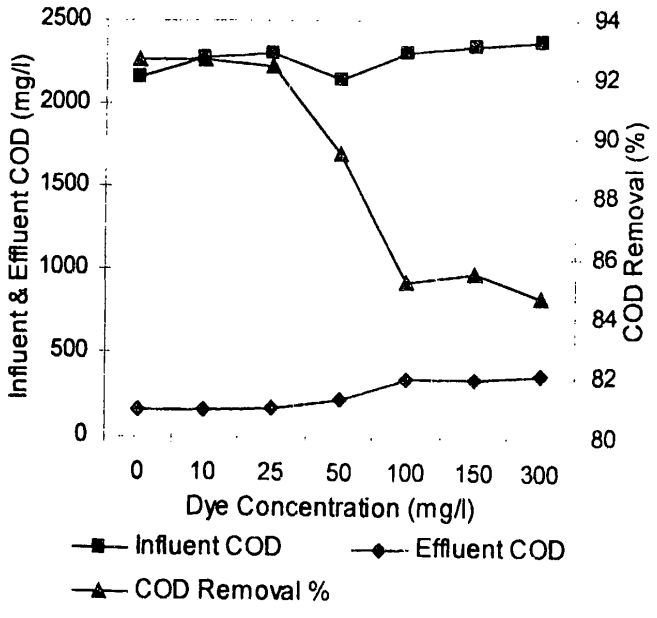

Figure 4. The influent and effluent COD and COD removal

COD Removal in the Reactor

The COD removal at the steady state condition without dye was 93%. At the dye concentration of 10 mg/l and 25 mg/l, over 90% COD removal was achieved. But, when dye concentration was increased from 25 mg/l to 100 mg/l (in two steps), the overall COD removal decreased from 92% to 85% and it continued up to 300 mg/l dye concentration in influent. During study period, the COD removal efficiency in the reactor system achieved was more than 85% and it was obtained operating the reactor in 24 h HRT.

The COD removal observed in the present research was higher than those reported by Brás and co-workers (2005), who used Acid Orange 7 up to 300 mg/l with co-substrate of sodium acetate, 1925±133 mg/l COD, in their UASB system. They further stated that COD removal decreased from 92±3% at 60 mg/l to 67±2% at 300 mg/l. However, the overall acetate based COD removal was more than 90%, indicating that there was no toxicity effects for COD removal to microorganisms up to 300 mg/l. The remaining dye or dye metabolites in effluents was the reason to reduce the overall COD removal in the system. In our study, it seems that some of metabolites of dye degradation were mineralized and it led to higher COD removal. However, decreased COD removal from 93% without dye to 85% with 300 mg/l dye indicated that further remaining dye or dye metabolites in effluents have caused to reduce COD removal. The UASB system treating only azo dye (PROCion Red H-E7B) containing wastewater with co-substrate of modified starch achieved over 60% COD removal (O'Neill et al., 2000). They also

further reported that azo dye or its metabolites contibuted to higher COD in effluents. Sponza and lisk (2002) stated that they obtained maximum of 60% of COD removal at organic loading rate of 5 kg/m^3-day when treating simulated textile wastewater containing azo dye, 100 mg/l of Reactive Black 5, with glucose as co-substrate in UASB system. When the treating of simulated textile wastewater with Direct Red 28, banned azo dye, in UASB system, the maximum COD removal of approximately 84%was obtained at 3000 mg/l and 1500 mg/l COD supplied with glucose (Mustafa and Delia, 2005).

Most of the previous studies used single azo dye containing wastewaters. However in our study, mixture of dyes belong to different chemical groups was used for better simulation of textile wastewater. Therefore, combined effects of dyes, configuration of reactor and operating parameters may be the reason for higher COD and Colour removal in this study.

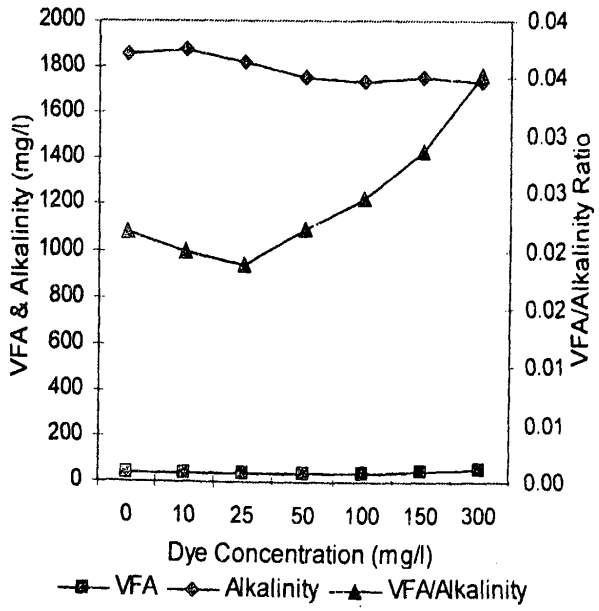

Figure 5. Alkalinity, VFA/Alkalinity ration in Effluents

Process Stability of the Reactor

It should be noted that for an optimum operational performance or optimum anaerobic treatment, pH should be in the range of 6.5 < pH < 8.2 (Speece, 1996; Rajeshwari *et al.*, 2000). Under stable operating conditions, H_2 and acetic acid formed by acidogenic and acetogenic microbes are used by methanogenic microbes and converted to methane. So the VFA concentration is typically very low, carbonate alkalinity is not consumed, and pH is stable. On the other hand, under overload or in the presence of toxicants or inhibitory substances, the activity of methanogenic and acetogenic populations was reduced causing an accumulation of VFA which, in turn, increased the total acidity reducing pH. The extent of the pH drop depends on the alkalinity concentrations (Ori *et al.*, 2002).

During the experimental period, it was observed that pH is in the range of 8.30-8.55 and it was slightly beyond the desirable limits reported. This observation agrees with Erüder and co-workers (2003). They observed that when treating inhibitory substances in UASB system, pH was shifted to in between 8.3-9.1 and it is beyond the recommended limits.

The maximum VFA and alkalinity observed was 61 mg/l and 1856 mg/l, respectively (Figure 5). The VFA concentration was slightly increased at 300 mg/l dye concentration and conversely, alkalinity was reduced down to 1733 mg/l indicating that alkalinity had been consumed by VFA. The optimum VFA for better operation of anaerobic system should be below 250 mg/l and alkalinity should be in the range of 1000-5000 mg/l (Speece, 1996). Even though pH was maintained beyond the recommended values, VFA and alkalinity values were well within the optimum values, indicating that the system operated well. The VFA and alkalinity, separately, are not good

indicators for evaluating the process stability of the anaerobic reactor since total alkalinity reflects both levels of VFA and bicarbonate, and under unstable conditions, increased VFA reduced the bicarbonate resulting in constant total alkalinity. It is reported that the ratio of VFA to alkalinity is the best option to monitor process stability in anaerobic systems to overcome above mentioned misunderstandings (Sánchez *et al.*, 2005; Zhao and Viraraghavan, 2004). If the ratio of VFA to alkalinity exceeds 0.8, the inhibition of methanogens occurs and process failure was apparent and an increase above 0.3-0.4 indicated the system instability which need immediate corrective actions. A proper ratio is between 0.1 and 0.2 (Zhao and Viraraghavan, 2004). On the contrary, Sánchez and co-workers (2005) and Malpei and co-workers (1998) have stated that optimum ratio of VFA to alkalinity should be less than 0.3 or 0.4. The Figure 5 shows that the VFA to alkalinity ratio is well below 0.04, indicating the overall process stability in all dye concentrations studied. The ratio VFA to alkalinity shows an increasing trend from low dye concentration to high dye concentrations but it does not indicate any sign of process failure since values are very low. The reasons for optimum performance of the reactor may be prolonged HRT and the stepwise increase of dyes. The long HRT can facilitate microorganisms to acclimate toxicants, and stepwise increase of dye is also required to proper acclimate microorganisms to dye mixture. And all these steps can lead to proper process stability.

CONCLUSIONS

The textile wastewater prepared using three acid dyes was treated using UASB reactor system achieving maximum decolourization up to 89% at 300 mg/l dye concentration. At 10 mg/l dying wastewater, colour removal was low compared to other dye concentrations studied. This phenomenon could be explained by the low adsorption of dye onto granules and less acclimate time at initial stages, and this may have led to poor degradation. The colour removal was not solely due to adsorption onto microbial granules. It suggests that decolourization was due to the combination of processes of adsorption and adsorption followed by microbial degradation. According to UV-Visible spectrum, AB204 removal was attributed to adsorption and other two dyes were decolourized by microbial degradation. The COD removal was reduced from 93% to 85% at 10 mg/l and 300 mg/l dye concentrations, respectively. The reactor performance stability of UASB system was properly maintained showing desirable values of VFA, alkalinity and VFA to alkalinity ratio. Finally, it could be concluded that UASB systems can treat textile wastewater containing acid dye; AB204, AR131 and AY79, without any system failure up to 300 mg/l dye concentration, when proper adaptation and operation conditions are maintained. This study showed the possibility of using anaerobic digestion for the treatment of textile wastewaters, which is considered to be difficult to treat via biological systems, without any failure of the UASB reactor system. The possibilities of using real textile wastewater for the UASB reactor system for the decolourization are being investigated in our laboratory as a continuation of this study.

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