Anaerobic decolorisation of simulated textile wastewater containing azo dyes

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Abstract

This study deals with the decolorization of the commercially important azo dyes, Orange II (C.I. Acid Orange 7) and Reactive Black 3HN (C.I. Reactive Black 8) under anaerobic conditions in wastewater. Laboratory scale semicontinuous studies were conducted using simulated cotton dyeing wastewater at ambient temperatures (24–28 °C) by maintaining a HRT of 10 days. The dye concentration in wastewater was maintained at 100 mg/l. The reactors were operated for 58 days and Orange II and Black 3HN were easily decolorized under the experimental conditions employed. The performance of the bioreactors was evaluated by monitoring oxidation–reduction potential (ORP) in the reactor, color and Chemical oxygen demand (COD) removal. Color removal of >99% was achieved in both the dye-containing reactors. COD removals of up to 95%, 92% and 94% were achieved in control, orange- and black dye-containing reactors, respectively. Effect of dyes and salts present in textile wastewater on methanogenesis was evaluated based on maximum methane production and methanogenic activity. Based on the maximum methane production data, no inhibition of methanogenesis was observed for dye concentrations of up to 400 mg/l for both the dyes. However from the methanogenic activity data, it was observed that the black dye concentration of 400 mg/l seemed to cause inhibition of methanogenesis.

Keywords: Anaerobic decolorisation; Simulated textile wastewater; Azo dyes; Methanogenic activity test

1. Introduction

Azo dyes are extensively used for dyeing of cotton and constitute about 60–70% of total dyes produced. They are characterised by their typical –N=N–nature and this is the most common chromophore of reactive dyes. About 1000 mg/l of dye is present in a typical dyebath (Ince and Tezcanli, 1999). However, due to the poor exhaustion properties of reactive dyes, as much as 40% of the initial dye remains unfixed and ultimately ends up in the dyebath effluent (Shah, 1998). Dyes are usually made resistant to biological attack, light, heat and oxidation. In a textile industry about 40–65 l of wastewater is generated per kg of cloth produced. Dyeing, desizing and scouring processes are the major sources of water pollution in a textile industry. Wastewaters from a textile industry are characterized by their highly visible color (3000–4500 ADMI units), Chemical oxygen demand (COD) (800–1600 mg/l), al-
floculation using lime, alum, polyelectrolyte and ferrous salts produce huge amount of sludge which pose handling and disposal problems. Adsorption of dyes using activated carbon and various other adsorbents are also quite uneconomical. Electrochemical oxidation of dye wastewaters is a slow process and process knowledge is not fully understood. Photochemical oxidation of dyes using a UV source and in the presence of oxidizing agents like hydrogen peroxide and catalysts are also slow and costly (Uygur, 1997). However, biological processes hold promise in providing a low cost and efficient means to treat the textile effluent (Beydilli et al., 1998; Laszlo, 1997). Decolorisation of dyes using pure (algal, fungal and bacterial) cultures is impractical as most of the isolated cultures are dye specific and hence their application on to large scale is impractical and not possible, because to maintain the pure form in wastewater treatment plant is difficult (Coughlin et al., 1997). Also, biodegradation of dyes can be accomplished when catabolic activities, present in mixed microbial communities, complement each other (Knackmuss, 1996). Azo and reactive dyes are electron deficient in nature and this property makes them less susceptible to oxidative catabolism (Knackmuss, 1996). They are also hydrophilic in nature and hence pass through the conventional aerobic process untreated. However under strict anaerobic conditions, decolorisation of dye can be gratuitously achieved and is well documented (Carliell et al., 1995). The rates of dye decolorisation are also enhanced in the presence of quinones (Zee et al., 2000). The presence of competitive electron acceptors in the anaerobic environment is also a rate-controlling factor, with denitrification occurring preferentially to the reduction of the reactive azo dye (Carliell et al., 1995). Several researchers have reported that a low redox condition maintained in the bioreactor by the methanogenic culture is responsible for color removal (Beydilli et al., 1998; Carliell et al., 1995). However, Chinwetkitvanich et al. (2000) did not find any clear relationship between ORP and color removal. Zee et al. (2000) have reported that transfer of reducing equivalents rather than their production is the rate-limiting factor in dye reduction. Generally, during the anaerobic process reduction in COD of up to 60–70% can also be achieved (Zaoyan et al., 1992). Hence it seems that an anaerobic process holds promise in effective treatment of textile wastewaters.

Most of the earlier studies were conducted using pure dye solutions. Experiments were carried under controlled temperatures (26 and 36 °C) and in batch modes, so few reports are available on continuous/semi-continuous treatment of synthetic/actual textile wastewaters, which actually describe the field operation. Also the complex nature of the textile wastewater containing dye and various auxiliaries, salts and sulfates is not taken into account. Salts and sulfates might have an inhibitory effect on the anaerobic decolorisation. Correlation of various parameters in better understanding of dye reduction is missing in earlier studies. Mostly, the anaerobic reactors in the field are operated under submesophilic temperatures (20–27 °C). Therefore this study aimed at better understanding of the dye reduction phenomena by correlating various parameters during anaerobic digestion. Experiments were conducted using a bench-scale, semi-continuous reactor with simulated cotton textile dyeing wastewater containing azo dyes, under reducing conditions at ambient temperatures (24–28 °C).

2. Methods

Commercially important and commonly used azo dyes for cotton dyeing Orange II (C.I. Acid Orange 7) and Black 3HN (C.I. Reactive Black 8), were purchased from the local market and used for the studies without any further purification. Seed sludge for the semi-continuous anaerobic reactors was collected from the primary settling tank of the domestic sewage treatment plant of I.I.T. Bombay, Powai, Mumbai, India.

2.1. Experimental procedure

The lab-scale model anaerobic reactors consisted of 5 l glass aspirator bottles. Each reactor was initially filled with 1 l (TS: 70 and VSS: 30 g/l) of seed sludge sieved with a 250 μm sieve.

2.1.1. Start-up and steady-state operation

Initially, as startup all the reactors were fed with tap water containing 1 g/l of starch and 1.5 g/l of NaHCO₃. Every alternate day 1 l of effluent was withdrawn and 1 l of feed was added to the reactors. The reactors were operated for a period of up to 14 days with this influent until reduction in COD of up to 92% was achieved on three consecutive days in all the reactors. From then onwards the reactors were fed with the simulated cotton dyeing effluent (STE). The STE was prepared according to the procedure outlined in O’Neill et al. (2000). The feed contained in g/l: NaCl: 0.15, acetic acid: 0.53, (NH₄)₂SO₄: 0.28, NH₄Cl: 0.23 and Na₂HPO₄: 0.038. Stock starch solution (20 g/l) was prepared by hydrolyzing the solution in 4% NaOH solution, heated for 2 h at 80 °C. Stock dye solution (10 g/l) was prepared by adjusting the solution to pH 12 using NaOH (4%) and then heating the solution at 80 °C for 2 h. Trace metal solution was prepared according to the composition mentioned in Prakash and Gupta (2000), but with an extra addition of calcium chloride (5 g/l). It contained in g/l: MgSO₄·7H₂O: 0.5, FeCl₃·4H₂O: 0.6, COCl₂: 0.88, H₂BO₃: 0.1, ZnSO₄·7H₂O: 0.1, CuSO₄: 0.05, NiSO₄·6H₂O: MnCl₂·5, (NH₄)₆Mo₇O₂₄·4H₂O: 0.64 and CaCl₂·
2H2O:5. The influents to the reactors were prepared by diluting the stock solutions to the desired values, adding sodium carbonate 1.5 g/l, pH adjusted to 7.0–7.2 and then feeding to the reactors. Initial dye concentration used for the studies was 100 mg/l. The system was operated at 10-d HRT by removing 1 l of liquid and adding 1 l of fresh feed every two days, and, under ambient temperatures (24–28 °C). Table 1 gives the description of different reactors in terms of dye present and their influential characteristics.

2.2. Batch anaerobic inhibition

Anaerobic toxicity assays were conducted for orange and black dye in order to determine the inhibitory effects of dyes, if any, to the methanogenic sludge. The test protocol followed was according to the methanogenic activity test described in Isa et al. (1993). Four concentrations of dye were chosen for the toxicity assay: 100, 200, 300 and 400 mg/l. A dye concentration of 400 mg/l was thought to be the highest dye concentration to be present in textile wastewaters. Control bottles contained no dye. The anaerobic toxicity assay was performed using 1-l bottles and at ambient temperatures (19–21 °C). A known amount of sludge from the respective semi-continuous reactor with estimated VSS was transferred to a 1-l bottle to give 1–2 g VSS/l. Simulated textile wastewater with sufficient quantity of NaHCO3 to buffer the system and appropriate dye concentration was added to obtain initial COD levels in the range 2000–2500 mg/l. The bottles were capped and connected to the liquid displacement system containing 5% w/v NaOH. The contents of the bottles were mixed by swirling manually. A short time interval (0.5–2 h) was selected for noting gas production in the first 12 h and longer intervals (3 h or more) up to 48 h after feeding. After every reading, contents of the bottles were mixed by swirling manually. After recording the gas production for the first feeding, the supernatant of the bottles was decanted. A similar quantity of substrate as in the case of 1st feeding was added and the bottles were capped and connected to the liquid displacement system. A similar gas recording procedure as to the first feeding was followed. The procedure was repeated for a third feeding. The maximum slope among the cumulative methane production vs the time graphs for different feedings yielded the methanogenic activity of the sludge expressed as kg CH4—COD/kg vss day.

2.3. Analytical methods

Total alkalinity, COD: closed reflux titrimetric method, TS, mixed liquor volatile suspended solids (MLVSS) were measured according to the procedure outlined in standard methods (APHA, 1989). Absorbance of the dye-containing solution was measured at their respective \( \lambda_{max} \) values using a UV-Visible recording spectrophotometer (Shimadzu-260, Japan). It appeared that effluent from the bioreactors contained not only undegraded but also colloidal organic matter. The colloidal matter in the anaerobic effluent interfered with measurement of color. Therefore, to estimate color removal by the bioreactor, the absorbance of the anaerobic effluent at \( \lambda_{max} \) was applied a correction by deducting the absorbance of the effluent from the control reactor (containing no dyes). pH was measured using a digital pH meter (Control Dynamics, India). Oxidation–reduction potential (ORP) was measured using a pocket ORP meter (Model 108, Orion Research, USA). The anaerobic effluent sample was dried at 104–105 °C and the residue’s FTIR spectra was obtained from Nicolet Impact/400 FT/IR.

3. Results and discussion

Semi-continuous reactors were operated for a period of 58 days. The performance of the control reactor containing simulated cotton-dyeing wastewater but with no dye is depicted in Fig. 1. pH of the reactor effluent remained fairly constant throughout the study period and was within the acceptable range for an anaerobic treatment system i.e. 6.8–7.2 (Ross et al., 1992). Effluent alkalinity content of the reactor increased (220–320 mg CaCO3/l) and it is known that reduction of sulfate to sulfide generates alkalinity in an anaerobic digester (McCartney and Oleszkiewicz, 1991). The anaerobic effluent had a typical smell of H2S. COD removal was

<table>
<thead>
<tr>
<th>Reactor no.</th>
<th>Dye present</th>
<th>Influent characteristics</th>
<th>OLR (kgCOD/m³ d)</th>
<th>SLR (kgCOD/ kgVSS d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>pH</td>
<td>Alkalinity (mg/l) as CaCO3</td>
<td>COD (mg/l)</td>
</tr>
<tr>
<td>R0 (Control)</td>
<td>None</td>
<td>7.0–7.2</td>
<td>980–1140</td>
<td>1440–2000</td>
</tr>
<tr>
<td>R1</td>
<td>Orange II</td>
<td>7.0–7.2</td>
<td>***</td>
<td>1280–2080</td>
</tr>
<tr>
<td>R2</td>
<td>Black 3HN</td>
<td>7.0–7.2</td>
<td>***</td>
<td>980–2080</td>
</tr>
</tbody>
</table>

***Could not be measured due to the color interference. OLR: Organic loading rate. SLR: Sludge loading rate.
reactor operation. From then on, there was a slight increase in COD removal rates.

The observed decrease in COD removal efficiencies could also be due to the formation of sulfides in such concentration, which was inhibiting the methanogenesis, and when the trace metal solution was added, sulfides got precipitated out, thus reducing the effect of sulfides and hence improvement in COD removal was observed.

ORP values were in the range −299 to −366 mV. Average methane production in control reactor in 48 h was 253 ml. It is 35–38% less than that in the reactors that contained dyes. It may be due to toxicity of salts present in the simulated textile effluent.

3.1. Decolorisation of Orange II dye

The performance of the reactor treating the orange dye is shown in Fig. 2. The initial high color removal efficiencies observed could be attributed to adsorption of dye on to the biomass. But after the 10th day onwards there was a decrease in color removal efficiencies and then it started to increase once again. This nature of color removal pattern shows that dye degradation was occurring in the bioreactors. This can be well supported by comparing the spectra of the influent and the effluent.

more than 90% for the first 3–4 days. Afterwards the COD removal started decreasing. On 14th day it was less than 80%. Initially the trace metal solution was not added to the reactor. But due to the decrease in COD removal, it was thought that the sodium (2.65 g/l) and sulfates (0.1 g/l) salts present in the simulated textile effluent were inhibiting the methanogens, therefore trace metal solution at the rate of 2 ml/l of feed was added from 16th day onwards for two days and then continued with 1 ml/l in the feed. Similar results have been reported by Feijoo et al. (1995), where sodium concentrations of 3–16 g/l caused 50% inhibition of methanogenesis in the absence of any nutrients or any other cations. De Baere et al. (1984) have reported that a sodium concentration in the range of 3.5–5.5 g/l causes moderate inhibition. After the addition of the trace metals COD removal efficiencies improved. It may have been due to the addition of ferrous, calcium and magnesium salts present in the trace metal solution. Similar observations have been reported by Feijoo et al. (1995). However from the 34th day onwards, once again there was decrease in COD removal. It was felt that the trace nutrients addition was not sufficient and therefore the addition of trace metals solution was increased from 1 to 2 ml/l from 40th day and this rate maintained till the end of the
The influent spectrum had a peak in the visible region at 480 nm and a peak in the UV region i.e. at 304 nm. In the anaerobic treated-sample spectrum, the peak at 480 nm had disappeared, indicating the cleavage of the azo bond, which thus made the dye colorless. In the ultraviolet region of the effluent spectrum the absorbance values were out of the range. Once the dye was broken down into simpler colorless aromatic compounds, these compounds could have given peaks in the ultraviolet region, hence the effluent samples were diluted up to 5 times using distilled water. In the 1:5 diluted effluent spectrum two peaks were observed; one at 339 nm and the other at 249 nm. It was felt that the peak at 249 nm would be due to the nondegraded byproducts after anaerobic treatment. The biodegradation pathway proposed under anaerobic conditions mentions that Orange II dye upon reduction yields sulfanilic acid and 1-amino-2-naphthol (Zee et al., 2000; Coughlin et al., 1997). But 1-amino-2-naphthol could not be detected in the treated effluent, as it is unstable. The absorbance spectrum of sulfanilic acid compared well with the Orange II spectrum after anaerobic treatment, suggesting that sulfanilic acid was present in the effluent. The FTIR spectrum (not shown) of the dried effluent powder also confirmed the presence of sulfanilic acid in the anaerobic treated effluent. It is interesting to note that average methane production was 388 ml in 48 h i.e. 35% more than the control reactor. This could have been due to the reduced inhibition effect of sodium and sulfate salts in the presence of dye. Similar results have been reported by Yerkes et al. (1997) where the addition of a small quantity of betaine (1 mM) to the anaerobic systems treating wastewaters, which contained high sodium concentrations, improved their treatment efficiency. Also researchers have reported comparatively more methane production in the dye-containing reactor than the control reactor during experiments conducted using pure dye solutions. It could be due to the degradation of dye and some of the dye intermediates could be used as a carbon source by the methanogens.

ORP values were in the range of –318 to –368 mV. It could be observed that color removal did not correlate with COD removal. It seems that the reducing environment prevailing in the bioreactor might have caused color removal as indicated by lower ORP values. This is in agreement with the observation made by Beydilli et al. (1998), where reduction of dye took place when low redox conditions prevailed in the bioreactors irrespective of the culture activity level. Knapp et al. (1997) have reported 98% decolorisation of Orange II dye (1000 mg/l dye conc.) using an aerobic fungus F29. Seshadri et al. (1994) could achieve up to 90% Orange II dye transformation in an anaerobic, fluidized bed reactor (AFBR). Laszlo (1997) has reported complete decolorisation of Orange II dye under reducing conditions using borohydride solution. Coughlin et al. (1997) have achieved a maximum of 99% Orange II dye color removal using an aerobic bacterial culture M12 in 122 h.

3.2. Decolorisation of Black 3HN dye

Effluent pH of the black dye reactor was in the range of 6.8–7.2, which is considered optimal for anaerobic treatment. There was an increase in effluent alkalinity (60–280 mg CaCO₃/l) content during the study period. The COD and color removal were in the range of 78–94% and 61–100%, respectively (Fig. 3). Some correlation was observed in COD and color removal which may have been due to the fact that when reducing equivalents had been produced in sufficient quantity by the degradation of starch, then only dye reduction took place. ORP values were in the range of –331 to –372 mV. There was no significant change in the ORP values throughout the study period. Hence it seems that color removal was independent of the ORP values for this dye. Average methane production in 48 h was 406 ml i.e. 38% more than the control reactor.

The influent spectrum had a peak in the visible region at 589 nm. Two peaks were observed in the UV region, at 387 and 304 nm. The peak in the visible region had disappeared from the anaerobic treated-sample spec-

![Graph](image-url)  
Fig. 3. Performance of the reactor treating black dye.
trum, rendering the dye colorless. However, in the 1:5 diluted spectrum, one peak in the UV region could be observed at 255 nm, which could correspond to an n-substituted benzene. Feng et al. (1999) have reported of up to 40% decolorisation of C.I. Reactive Black 8 dye under both UV light and sunlight in 10 min, while carrying out decolorisation experiments in UV/ferricoxide system and sunlight/ferricoxide system.

3.3. Batch anaerobic inhibition

Inhibition of methanogenesis by dyes on to anaerobic sludge was assessed from the total methane production data in the control and the dye amended reactors. From the maximum rate ratio (MRR) value inhibition could be calculated. The inhibition was also calculated from rate of methane production per unit weight of biomass.

Maximum methane production in different reactors, MRR and methanogenic activities for inhibition assays conducted for orange and black dye are presented in Table 2.

From Table 2, it seemed that Orange II did not cause any inhibition to methanogenesis for dye concentrations of up to 400 mg/l. However, from the methanogenic activity data of Black 3HN dye it was observed that dye concentration of 400 mg/l seemed to cause inhibition of methanogenesis.

4. Conclusions

- Azo dyes, Orange II and Reactive Black 3HN were gratuitously decolorized under experimental conditions employed.
- From the methane production data of the control reactor in which no dye was present, it seems that salts present in textile effluent inhibited methanogenesis to a limited extent.
- From the maximum methane production rates, it seems that both orange and black dye concentrations of up to 400 mg/l causes no inhibition to methanogenesis.
- With Orange II dye, COD removal did not correlate with color removal. It seems that reducing environment prevailing in the bioreactor might have caused color removal as indicated by low ORP values.
- In case of Black 8 dye, some correlation was observed between COD and color removal. Hence, it seemed that microbial activity was responsible for dye decolourisation.

References


<table>
<thead>
<tr>
<th>Dye conc. (mg/l)</th>
<th>Maximum methane production (ml)</th>
<th>MRR</th>
<th>Methanogenic activity (KgCH₄/COD/KgVSS d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Orange</td>
<td>Black</td>
<td>Orange</td>
</tr>
<tr>
<td>0</td>
<td>47</td>
<td>48</td>
<td>1.00</td>
</tr>
<tr>
<td>100</td>
<td>81</td>
<td>84</td>
<td>1.72</td>
</tr>
<tr>
<td>200</td>
<td>100</td>
<td>68</td>
<td>2.13</td>
</tr>
<tr>
<td>300</td>
<td>91</td>
<td>98</td>
<td>1.94</td>
</tr>
<tr>
<td>400</td>
<td>98</td>
<td>83</td>
<td>2.09</td>
</tr>
</tbody>
</table>

MRR: Maximum rate ratio is the ratio of maximum rate of gas production to the average rate of gas production in control over the same time period.