

# Microbial Degradation and Decolorization of Acid Orange Dye by Anaerobic /Aerobic Sequential Process

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**Abstract** This study investigates the anaerobic treatability of Acid orange dye in an anaerobic/aerobic sequential process. Laboratory scale anaerobic baffled reactor and fixed activated sludge reactor were operated at different organic loadings and hydraulic retention times. The effects of shock dye concentration on the chemical oxygen demand and color removal efficiencies were investigated in the anaerobic baffled reactor. The effect of hydraulic retention time on the color and chemical oxygen demand removal efficiencies were also investigated in the aerobic reactor. The studies were carried out in continuous mode and the effluent of the anaerobic baffled reactor was used as feed for the fixed activated sludge reactor. Chemical oxygen demand removal efficiency of 54.5% was obtained at HRT =1 day in the anaerobic reactor. The average color removal was 89.5%. Chemical oxygen demand removal efficiency of 69% was obtained at HRT =7 h in the aerobic fixed activated sludge reactor. A slight decrease of the color was also observed in the aerobic reactor. This investigation has shown that successful treatment of a highly colored wastewater is possible in the anaerobic baffled reactor. Also the results showed that, anaerobic biological system has higher efficiency in dye removal than fixed activated sludge system, while aerobic system has higher efficiency in chemical oxygen demand removal comparing with the anaerobic baffled reactor.

**Keywords:** azo (reactive dye), acid orange, anaerobic baffled reactor, aerobic fixed activated sludge reactor, sequential process

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## 1. Introduction

Decolorization of azo dyestuffs by sequential reactions involves the degradation of azo dye by reduction or cleavage of azo bond by anaerobic digestion and ultimate biotransformation of aromatic amines in aerobic conditions. The limited past investigations have shown that azo dyes can be completely decolorized and some intermediates such as aromatic amines with side groups (–SO<sub>3</sub>, –OH, –COOH, –Cl, –N) containing metabolites were quantitatively detected (O'Neill et al., 2000; Luangdilok and Panswad, 2000; Van Der Zee *et al.*, 2003). The successful application of anaerobic technology for the treatment of industrial wastewaters depends on the development of high-rate bioreactors, which achieve a high reaction rate per unit reactor volume by retaining the biomass in the reactor for long periods of time. The anaerobic baffled reactor (ABR) is a high-rate reactor that contains between 3 and 8 compartments in which a series of vertical baffles force the wastewater to flow under and over the mass it passes from inlet to outlet (Barber and Stuckey, 1999; Sponza and Isik, 2002). The bacteria within the reactor gently risen, settle due to flow characteristics and gas production in each compartment

but move horizontally down the reactor at a relatively slow rate giving rise to cell retention time (CRT) of 100 day at 20 h hydraulic retention time (HRT); therefore the wastewater can come into intimate contact with large amounts of active biomass as it passes through the ABR with short HRT (6-20 h), while the effluent remains relatively free of biological solids (Grobecki and Stuckey, 1991). In a study performed by Sponza and Isik (Sponza and Isik, 2002) the color and chemical oxygen demand (COD) removal efficiencies were investigated using anaerobic/aerobic reactors (UASB, CSTR) for treatment of 100 mg/L of Reactive Black 5. A total of 67% COD and 95% color removal efficiencies were obtained while glucose was used as carbon source. O'Neill demonstrated 75% color removal efficiency, in an upflow anaerobic sludge blanket (UASB)/activated sludge reactor system treating reactive Red 141 (O'Neill et al., 1999). In a study performed by An et al, 83% color and 69% COD removal efficiencies were obtained in anaerobic (HRT =6–10 h) and aerobic (HRT =6.5 h) sequential reactors degrading acid yellow and basic Red dyes at organic loading rate of 5.3 kgCOD/m<sup>3</sup> day and a COD concentration of 1200 mg/L (An et al., 1996) Bell and Buckley obtained 86% decolorization efficiency in an anaerobic baffled reactor at an organic loading rate of 4g COD/L using sucrose as carbon source. It was observed that 250 mg/L of reactive

Red 141 was degraded with 90% COD removal efficiency in anaerobic reactor (Bell and Buckley, 2004). Probably the most significant advantage of ABR is its ability to separate acidogenesis and methanogenesis longitudinally down the reactor. The latter causes shift in bacterial populations allowing increased protection against toxic materials and higher resistance to changes in environmental parameters such as pH and temperature (Barber and Stuckey, 1999). The objective of this study was to investigate the color as well as organic carbon measured in terms of chemical oxygen demand (COD) removal efficiencies in anaerobic/aerobic sequential reactors. In this study the effect of operational conditions (shock dye concentration, HRT and pH) on the treatability of Acid orange dye were investigated in an anaerobic ABR and aerobic fixed activated sludge (FAS) reactors.

## 2. Materials and Methods

### 2.1. Reactor

Continuously fed anaerobic ABR and aerobic FAS reactors were used in sequence for the experimentation. A 5-compartment laboratory-scale ABR was set up in a constant room temperature of 35°C. The ABR reactor had an effective volume of 13 L; the FAS reactor consisted of an aerobic tank (effective volume =3.2 L and fix bed volume =0.8 L) and a settling compartment (effective volume =1.6 L). A schematic drawing of the lab- scale sequential ABR and FAS reactor used in this study. The effluent of anaerobic ABR reactor was used as the influent of aerobic FAS reactor.

### 2.2. Seed

The reactor (ABR) was seeded with 8.2 L (1.64 L/compartments) of screened digester sludge taken from a south wastewater plant in Isfahan (total solids=35 g/L, volatile solids=25 g/L). The sludge was left to settle for one week before feeding began. Activated sludge culture obtained from the wastewater plant in Isfahan (total solids=12 g/L, volatile solids=8 g/L) was used as seed for the aerobic FAS reactor

### 2.3. Composition of Synthetic Wastewater

Acid orange dye was obtained from a textile factory in Ankleshwar, India. The synthetic feed containing (per liter) glucose =4 g; peptone=0.89 g; meat extract=0.4 g;  $K_2HPO_4$ =0.08g;  $NaHCO_3$ =1.5 g; and also the trace minerals  $COCl_2 \cdot 6H_2O$ ,  $FeCl_2 \cdot 4H_2O$ ,  $MnCl_2 \cdot 4H_2O$ ,  $Na_2MoO_4 \cdot 2H_2O$  and  $NiCl_2 \cdot 6H_2O$  were fed to the reactor during startup (Bell and Buckley, 2003). Once the reactor had reached steady state, at a 50 HRT, Acid orange dye was added to the feed solution. The COD concentration to the reactor was maintained at 4 g COD/L, which represented the COD of waste streams produced in the dyeing process; the COD of the dye was negligible. To achieve acclimation, the concentration of Acid orange dye was increased stepwise from 30 mg/L, to 100 mg/L on day =83, to 250 mg/L on day =112, to 350 mg/L on day =133. Throughout the experimental period, the reactor was supplied with a constant COD loading of 5 g COD/L.d of the synthetic feed cosubstrate.

## 2.4. Analytical Methods

COD in influent and effluent samples were determined by closed reflux colorimetric method (Lenore et al., 2005). Color measured in the influent and effluent sample of anaerobic and aerobic reactor were measurement by ADMI. ADMI color was determined with a spectrophotometer (DR 5000 uv-vis spectrophotometer) in accordance with the ADMI test method 2120D detailed in standard methods (Lenore et al., 2005). The ADMI color values were calculated with a computer program developed by Mr. Jako (national Sun Yat –Sun University, Taiwan) (Kao et al., 2001). The spectrophotometer was calibrated before each use with standard platinum cobalt color solution of 50, 100, 200, 250 and 500 ADMI color units (Lenore et al., 2005). Also results were measured at a wavelength of 534 nm in which maximum absorbance was obtained in UV-vis spectrophotometer. The samples were centrifuged at 4000 rpm for 5 min and the supernatant liquor was filtered through glass –fiber filters (0.45  $\mu$ m). The samples were diluted 1 in 5 with distilled water and then the absorbance value of supernatants was determined (Bell and Buckley, 2003).

## 2.5. Operating Conditions

Fifty days after the start up period, approximately 50% COD removal efficiency was obtained. COD concentration increased from 1000 to 5000 mg/L per day during the next 51 days. The ABR reactor was brought into operation with 30 mg/L Acid orange dye. Value of the operating parameters for 150 days of operation is summarized in Table 1. Organic loading was increased from 1 to 5 KgCOD/m<sup>3</sup> per day following the 50 days of startup period. The ABR reactor was operated at loading rate between 4-5 KgCOD/m<sup>3</sup> per day between days 50 and 150, while HRT decreased from 4 days to 1 day in ABR reactor following the 50 days of start up period.

## 3. Results

### 3.1. COD Removal

According to the obtained results, the average percentage of COD removal on anaerobic baffle reactor was 54.5% and 66% in FAS reactor. Figure 1 shows the average COD removal percentage in different ABR reactor compartments. In this figure compartment 1 represents the reactor soluble COD by the reactor over time. feed and compartment 7 represents the reactor effluent. Figure 2 and Table 1 show the soluble COD influent in ABR and FAS reactor and also the removal percentage of the soluble COD by the reactor over time.

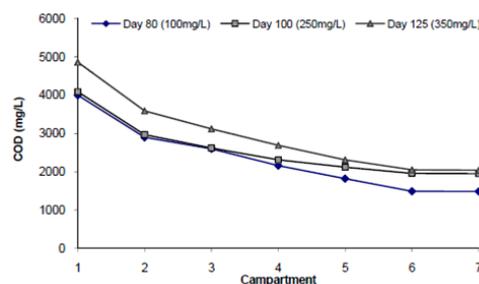


Figure 1. The COD reduction profile during the experimental period

Table 1. Characteristic ABR reactor during the experimental period

Operations day	OLR KgCO D/m <sup>3</sup> (day)	H RT day	COD remov al efficie ncy (%)	Alkali mity Mg/L Caco3	Dye concentra tions Mg/L	Compartment 1			Compartment 2			Compartment 3			Compartment 4			Compartment 5		
						Removal efficiency			Removal efficiency			Removal efficiency			Removal efficiency			Removal efficiency		
						CO D (%)	dy e (%)	p H												
5-15	0.5	3.3	44	3117	0	14.4	0	6.9	13.2	0	5.9	5.3	0	5.9	4.9	0	5.9	4.4	0	5.9
16-30	1	1.95	47	3467	0	22.4	0	6.7	10.8	0	6.5	8.5	0	6.3	5	0	6.7	7.6	0	7
31-59	2.5	1.25	51	3807	0	30.8	0	7.3	13	0	7.2	4	0	7.2	4.5	0	5.3	3	0	7.5
50-60	3	1.35	57	3750	30	25.5	24	6.6	14.7	40	6.4	5	11	6.5	5	11	6.7	5.1	4.2	6.9
61-80	3	1.2	62	5187	50	21	26.4	6.4	13	39	6.4	9.7	16	6.5	8.3	4	6.6	9.9	4.8	6.8
81-100	4	1	61.5	3475	100	21.7	20.7	6.1	8	42	5.9	15.5	22	6.2	8.4	5	6.5	7	2.8	6.8
101-125	4	1	51	3460	520	27.5	24.3	5.9	8.3	44	5.7	7.9	2	6	5.6	1.7	6.1	4.3	1	6.2
126-150	4.5	1	61.6	1787	350	28	13.5	6.5	5.3	39	6.4	11	17	6.5	10	3	6.7	9	3	6.8
	5	1	52	2551	350	23	13	6.9	15	42	6.9	6.4	18	7	4	3	7	3	4	7.2

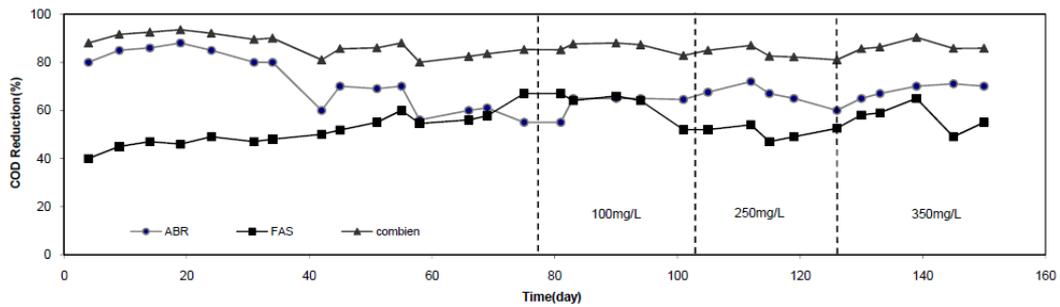


Figure 2. COD reduction profiles during the experimental period

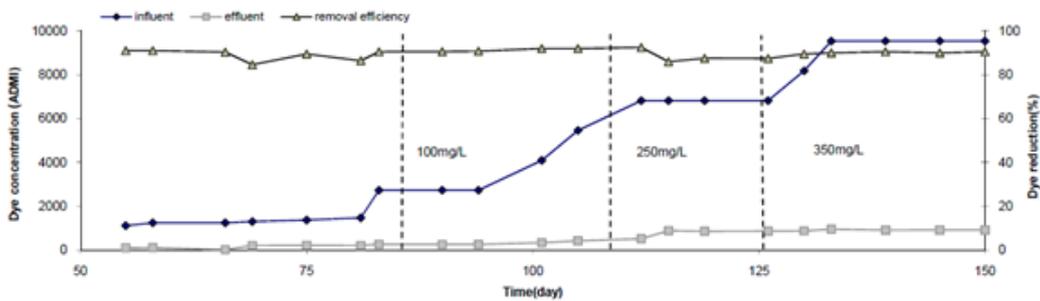


Figure 3. Dye reduction in ABR reactor

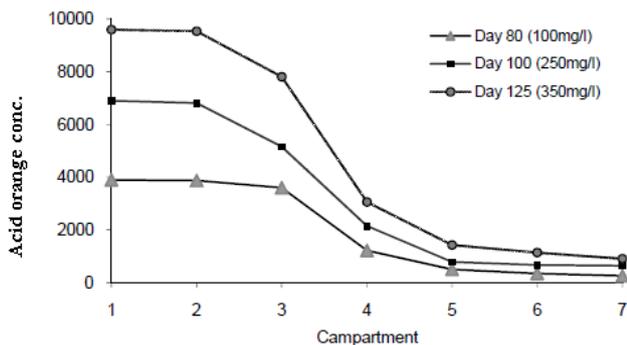


Figure 4. Color reduction profile during the experimental period

### 3.2. Color Removal

Figure 3 and Table 1 show the color reduction over time. These results show efficient decolorization of the Acid orange dye waste stream. The color reduction averaged 87 % with 100 mg/L dye concentration in the feed and 85 % for the 250 mg/L concentration. The color removal dropped to 65 % when the dye concentration was increased to 350 mg/L (day=127). This was the lowest color removal achieved throughout the duration of the test, then increased to 80 % in one HRT. Figure 4 shows the average color removal percentage in different ABR compartments at different time periods during the experiment.

### 3.3. pH Profile

The pH of ABR reactor was variable but when the reactor was stabilized, the changes of the reactor reached

their minimum. Figure 5 shows the pH measured in compartments 2 and 5 of the ABR. The dotted lines indicate the changes in dye concentration in the feed. The compartment 1 equalized the influent feed.

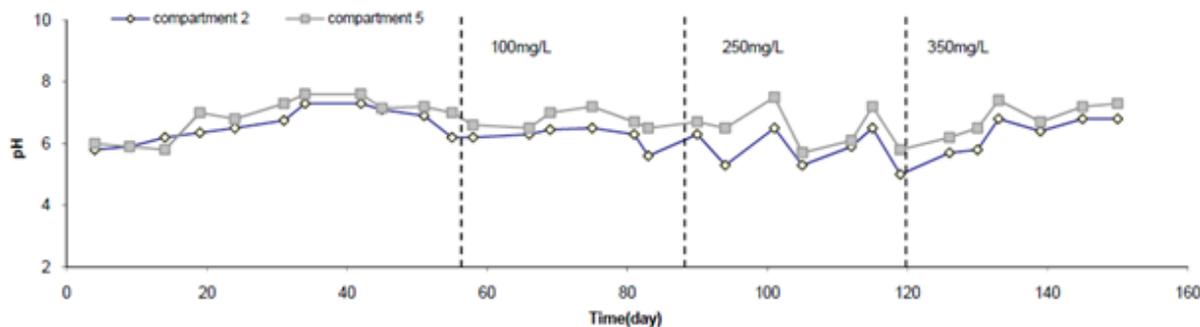


Figure 5. pH profiles in ABR reactor

## 4. Discussion

The profiles (Figure 1) show that the majority of the COD was reduced in the first three compartments of the reactor. The profiles also show very little COD reduction in the last two compartments of the reactor; this was substantiated by the biogas results and the population characterization experiments. The COD removal during start up, or before the addition of the dye to the feed stream was maximum 60% on day 50. Acid orange was added to the feed stream at a concentration of 30 mg/L on day 50. This resulted in a slight decrease in the COD reduction with removal efficiency of 54%. The COD reduction stabilized within 9 HRT to give an effluent COD concentration of 1170 mg/L. The average COD reduction for the complex feed containing 100 mg/L dye concentration was 62%. This average was 51% for the 250 mg/L concentration and 58% for the 350 mg/L concentration dye. Thus, there was a slight decrease in the COD removal efficiency with each increase in the dye concentration. This showed low metabolic activity in these compartments. The concentration of Acid orange in the Influent increased periodically after at least 20 hydraulic retention times (HRT =24 h) and when more than 90% removal of the dye had been achieved. In a study performed by, Bell and Buckley (2003), ABR reactors have been used in laboratory for removing a mixture of food-based colors, which correlates with the results of this study. Bell and Buckley obtained 86% decolorization efficiency in an 8-compartment anaerobic baffled reactor at an organic loading of 4 g COD/L using sucrose as carbon source. It was observed that 250 mg/L of reactive Red 141 was degraded with a COD efficiency removal of 90% in 24 hours, in anaerobic reactor(Bell and Buckley, 2003) The reason for the greater COD removal in current research can be attributed to the larger number of compartments in reactor. In a research by Kong and Wu, an ABR reactor was used in laboratory system for measuring the removal of textile dye with HRT in 8 h. The findings showed 58% COD removal efficiency and also 85% color removal at a temperature of 30°C (Kong and Wu, 2008). It is worth mentioning that in FAS reactor more COD removal efficiency was observed because this reactor was aerobic. The COD removal efficiencies were

40–60% at organic loading rates of 1–5 kg/m<sup>3</sup> per day. About 100 mg/L Acid orange and 3000 mg/L of glucose–COD containing wastewater was treated with a maximum COD removal efficiency of 96% and the effluent COD was reduced to 120 mg /L in the combined system. Color removal was efficient with an average color removal of 86 % over the whole test period. The minimum dye concentration achieved in the effluent was 5 mg/L, on day 91; however, this concentration is still significant since color is visible at concentrations < 1 mg/L. The effluent would require further treatment before discharge to a water source. If this color reduction was achieved by pretreatment at the factory, further aerobic reduction of the aromatic amines could be achieved by conventional treatment at a wastewater treatment works. As for FAS reactor the color removal would not happen and sometimes an increase of color would even be seen due to the autoxidation process. In comparison to research done by Bill and Buckley in which the food and CI reactive Red 141 color removal efficiencies were 86% on average, in this research a greater amount of Acid orange color was removed. But in comparison with research by Sponza (96%) the efficiency of color removal was less. That is due to the combination of systems used by Sponza (Sponza and Isik, 2005). These data show that changes in the dye concentration had a slight effect on the reactor pH and also illustrate the horizontal separation of acid genesis and methanogenesis through the ABR (Bell and Buckley, 2002; Ujang and Henze, 2004). The findings have shown that the increase of color has no influence on the process activity of these bacteria during acid production. Also results pointed out the fact that an increase of Acid orange concentration dye in effluent wastewater has less influence on different pH compartments of the reactor. The obtained results in color removal with the use of ABR reactor have shown that the methane bacteria are more sensitive than acidgenes bacteria. The findings of this study have shown that the sequential anaerobic/aerobic system was efficient in the degradation of azo dye Acid orange. In this experiment with partially digested sludge cultures and with glucose as cosubstrate, Acid orange dyestuff was almost decolorized under anaerobic conditions. Up to 90% of the color of the azo dyestuff was removed in the anaerobic stage. The released intermediates were mineralized in the aerobic part of the two stage system. It was observed that 85–95% of the

remaining COD was removed in the aerobic stage. No color removal occurred aerobically. The use of a digested sludge containing ABR reactor has proven the capability of resistance to shock organic loading. The addition of ABR reactor systems prior to conventional activated sludge systems, which are in use in many industries in Iran, will result in 90% color removal efficiency, particularly in textile and dye industries.

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