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*Full Length Research Paper*

# Decolorization of reactive dyes under batch anaerobic condition by mixed microbial culture

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**Decolorization of reactive dyes, which are used in textile industry, under batch anaerobic conditions by mixed microbial culture was investigated in this study. Decolorization of C.I. Reactive Black 5 (RB 5), C.I. Reactive Red 24 (RR 24) and C.I. Reactive Blue 49 (RB 49) with initial concentrations ranging from 150 to 2400 mg/L was investigated. Decolorization efficiencies obtained were 93.4% for RB 5 and 98.9% for RR 24 both with initial concentration of 2400 mg/L after 24 h incubation period. However, decolorization was lower for the dye of RB 49 than other two dyes in all concentrations despite 72 h incubation period by mixed anaerobic culture. All of the three dyes correlated with 1$^{st}$ order reaction kinetic with respect to decolorization kinetics. The results of the study demonstrated that high decolorization was obtained under anaerobic condition depending on chemical structure of the dye.**

**Key words***:* Reactive dyes, decolorization, anaerobic condition, mixed culture, kinetics.

## INTRODUCTION

Synthetic dyes are widely employed in textile, coating, paper and printing industry (Claus et al., 2002). Currently, more than 100,000 types of synthetic dyes are used commercially and 700,000 tons of dyes are manufactured in the world annually (Dansehvar et al., 2005; Selvam et al., 2003). Decolorization process of many dyes found in textile industrial wastewater is difficult due to their complex chemical structures and origins. Dyes may have various structures such as acid, reactive, direct, basic, disperse, mordant and metal complex (Clarke and Anliker, 1980; Hao et al., 2000).

The past investigations have shown that azo dyes can be completely decolorized and some intermediates such as aromatic amines with side groups (-$SO_3$, -OH, -COOH, -Cl, ≡N) containing metabolites were quantitatively detected (O'Neill et al., 2000). A mechanism for azo reduction was proposed by Gingell and Walker (1971) involving a two stage reduction of the azo bond as given below reactions (1) and (2). The intermediate product of reaction (1) is an unstable colorless compound and the azo bond can reformed upon oxidation regaining the color.

$$2e^- + 2H^+ + (R–N=N–R^1) \rightarrow (R–NH–NH–R^1) \text{ color.} \quad (1)$$

$$2e^- + 2H^+ + (R–NH–NH–R^1) \rightarrow (R–NH_2) + (R^1–NH_2) \quad (2)$$

Where R and $R^1$ are variously substituted phenyl and naphthol residues. Carriers in the electron transport chain utilize azo compounds as terminal electron acceptors, thus regenerating themselves and fortuitously reducing azo bond, breaking the dyes chromophore. However, this process is inhibited by the presence of oxygen, which is more energetically favorable oxidizing agent, and also a readily degradable carbon source is required for metabolism to produce the electron carriers (Carliell et al., 1995; Sponza and Işık, 2002).

Various chemical, physical and biological techniques may be employed for the decolorization of dyes found in wastewater, but each method has technical and economic limitations (Clarke and Anliker, 1980; Vandevivere and Bianchi, 1998; Robinson et al., 2001). Many physicochemical decolorization methods are not ideal because they are expensive, have restricted usage areas, interfere with other wastewater components, or cause wastes that require retreatment. An alternative to the physicochemical methods is the relatively inexpensive biological treatment method, which may be preferred for decolorization (van der Zee and Villaverde, 2005).

Most of the researches have investigated decoloriza-

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tion of azo dyes under batch anaerobic condition. Işık and and Sponza (2003) studied decolorization of Congo Red and Direct Black 38 *Escherichia coli* and *Pseudomonas* sp. cultures in anaerobic, aerobic, and microaerophilic condition. They reported that anaerobic conditions were more favourable than other conditions for decolorization. Setiadi and Van Loosdrecht (1997) demonstrated about 70% color removal efficiency, in an anaerobic reactor treating reactive azo dye. Some previous batch studies, following decolorization efficiencies were obtained for reactive Black-5; 75 and 95% color removal in 10 and 30 h, respectively (Işık and Sponza, 2004), 95% in 48 h (Oxspring et al., 1996) and 79% in 10 h (Beydilli et al., 1998). Zero order reaction kinetic with respect to dye concentration has also been reported by several researchers (Brown, 1981), whereas some others der Zee et al., 2001; Işık and Sponza, 2004).

The aim of this study was to investigate decolorization levels of three synthetic textile dyes (C.I. Reactive Black 5, C.I. Reactive Red 24, C.I. Reactive Blue 49) found in textile industrial wastewaters with different initial concentrations and Vanderbilt minerals under batch anaerobic conditions by using mixed microbial culture. Furthermore, decolorization reaction kinetics of selected dyes was also investigated.

## MATERIALS AND METHODS

### Dyes and chemicals

Dyestuffs used in the study were C.I. Reactive Black 5, C.I Reactive Red 24 and C.I. Reactive Blue 49 obtained from Kucuker. Co. Textile industry, Denizli, Turkey. The detail information of used dyes was given in Table 1. The media components and chemicals were purchased from dealer of Merck or Sigma in Turkey. All chemicals used in this study were of analytical grade.

Laboratory-scale batch anaerobic reactor and synthetic dye Wastewater studies were performed in 120 ml capacity dark glass serum The, bottles. They were sealed with 5 mm diameter butyl rubber septum and kept in place by a screw cap. Each serum bottle contained 4,000 mg/L of partially granulated anaerobic sludge obtained from the UASB reactor treating the wastewaters of Frito Uzay Chips Industry in Izmir, Turkey. Table 2 describes incubation bottles; macro- and micro-nutrients (Speece, 1996 for Vanderbilt minerals), carbon source (3000 mg COD/L glucose), etc. The dyes/synthetic wastewater contains reactive dye 150, 300, 600, 1200, 2400; $NH_4Cl$, 400; $MgSO_4 \cdot 7H_2O$, 400; KCl, 400; $Na_2S \cdot 9H_2O$, 300; $(NH_4)_2HPO_4$, 80; $CaCl_2 \cdot 2H2O$, 50; $FeCl_3 \cdot 4H_2O$, 40; $CoCl_2 \cdot 6H_2O$, 10; KI, 10; $(NaPO_3)_6$, 10; l-cysteine, 10; $AlCl_3 \cdot 6H_2O$, 0.5; $MnCl_2 \cdot 4H_2O$, 0.5; $CuCl_2$, 0.5; $ZnCl_2$, 0.5; $NH_4VO_3$, 0.5; $NaMoO_4 \cdot 2H_2O$, 0.5; $H_3BO_3$, 0.5; $NiCl_2 \cdot 6H_2O$, 0.5; $NaWO_4 \cdot 2H_2O$, 0.5 and $Na_2SeO_3$, 0.5 mg/L (Vanderbilt minerals). The anaerobic conditions were maintained by adding 667 mg/L of sodium thioglycollate that is proposed for (w/w) 0.01 – 0.2% anaerobic conditions (Sponza and Isık, 2004). The alkalinity and neutral pH were adjusted by addition of 5,000 mg/L $NaHCO_3$. According to Sponza and Isık (2004) study, 3,000 mg/L of glucose was used as co-substrate for COD providing reducing equivalents with electron fission. All incubations were carried out in a temperature controlled incubator at 35 ± 1°C. The bottles were vigorously shaken at certain intervals and the liquid samples were taken from the supernatants with a syringe for analyses.

### Analytical methods

Total suspended solid (TSS) in granulated sludge was determined by the filtration technique using membrane filters with 0.45 µm pore sized (APHA, AWWA, WPCF, 1989). Color density was measured spectrophotometrically (Dr. Lange UV 200 model) at the wavelength corresponding to the maximum absorbance of the dyes (Table 1). The samples were centrifuged at 7,000 rpm for 10 min in a centrifuge (Hettich EBA III model) and the absorbance values of supernatants were measured. The calculation of color removal efficiency after anaerobic treatment was performed using this formula:

$$CR(\%) = \frac{Do - D}{Do} * 100 \quad (3)$$

Where $D_0$ and D are concentrations (mg/L) of dye before and after anaerobic treatment, respectively. Each experiment was replicated three times, and the mean values used in the results. Statistical analyses showed that standard errors were lower than 0.01 ($p<0.01$) for three replicates; if higher than $p<0.01$, experiments were repeated.

## RESULTS AND DISCUSSION

### Effect of initial dye concentration on decolorization

Abiotic tests performed with autoclaved anaerobic partially granulated sludge showed that the microbial decolorization was preceded primarily by biological degradation. Decolorizations, which ranged from only 1 to 3.5%, were obtained under abiotic conditions during incubation period as shown in Figures 1 and 2. Compared to more than 95% color removal under biotic incubation condition, abiotic conditions led to only 3.5% color removal, which was probably due to physical adsorption of dye molecules by the cells dying or by auto-oxidation (Brás et al., 2001). This also implies that the physical adsorption of dye molecules on cell mass was a negligible mechanism in color removal (Sponza and Işık, 2004).

Decolorization percentages for C.I. Reactive Black 5 having initial concentrations ranging from 158.6 to 2405.2 mg/L after 24 h are seen in Figure 1. At the end of the 24 h incubation period, decolorizations ranging from 99.4 to 93.4% were obtained in all dye concentrations.

Decolorization of C.I. Reactive Red 24 having initial concentrations ranging from 147.4 to 2391.5 mg/L after 24 h incubation by mixed anaerobic cultures were investigated (Figure 2). At the end of the 12 h incubation period, decolorizations ranging from 80.37 to 71.34% were obtained. In all concentrations of RR 24, decolorizations obtained were more than 95% after 24 h incubation period. For C.I. Reactive Blue 49,at the initial decolorization efficiencies obtained rangedfrom17.5 to 12.6%concentrations of 165.3 and 2406.2 mg/L, respectively, after 24 h incubation period. For all concentrations of RB 49, decolorization efficiencies ranging from 16.51 to 22.9% after 72 h incubation period were obtained (Figure 3).

**Table 1.** Characteristics of the dyes.

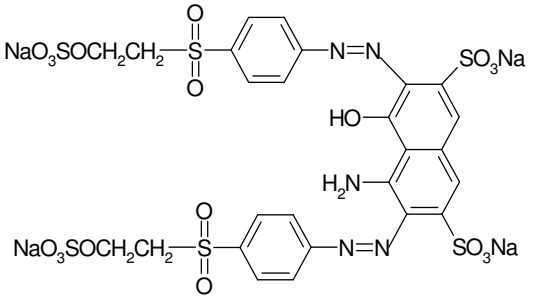
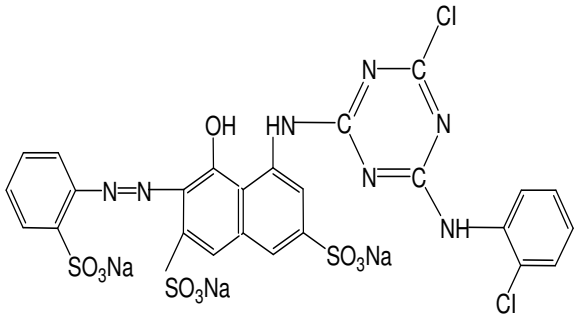
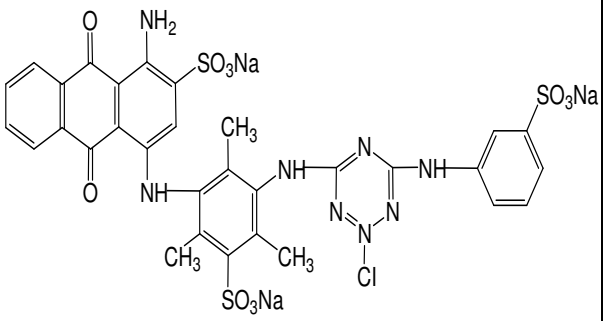
| Open formulas | Maximum wavelength (nm)$\lambda_{max}$ | COD value of dye of 1000 mg/L (mg/L) |
|---|---|---|
|  Reactive black 5 C.I. 20505 | 598 | 782 |
|  Reactive red 24 C.I. ( -- ) | 514 | 789 |
|  Reactive blue 49 C.I. ( -- ) | 586 | 718 |

**Table 2.** Protocol for decolorization and kinetics throughout batch tests

| Stock | Abiotic control (ml) | Dye containing bottles (ml) | Resulting concentrations (mg/L) |
|---|---|---|---|
| Sludge (60 g/L) | - | 5 | 4000 |
| Glucose(30 gCOD/L) | 7.5 | 7.5 | 3000 |
| Vanderbilt mineral medium | 7.5 | 7.5 | Desired composition |
| $NaHCO_3$ (50 g/L) | 7.5 | 7.5 | 5000 |
| Sodyum Tiyoglikolat ($C_2H_3NaO_2S$) (50 g/L) | 1 | 1 | (w/w) % 0.067 |
| Dye (9 g/L) | 1.25 | 1.25, 2.5, 5, 10, 20 | 150, 300, 600, 1200, 2400 |
| Total volume (ml) | 75 | 75 | - |

Decolorization efficiencies obtained with different initial concentrations after 24 - 72 h incubation are seen in Table 3. For di azo(RB 5) and azo (RR 24) dyes having chromophore group by anaerobic mixed culture, despite increasing in dye concentrations, high decolorization efficiencies were obtained. However, in case of RB 49

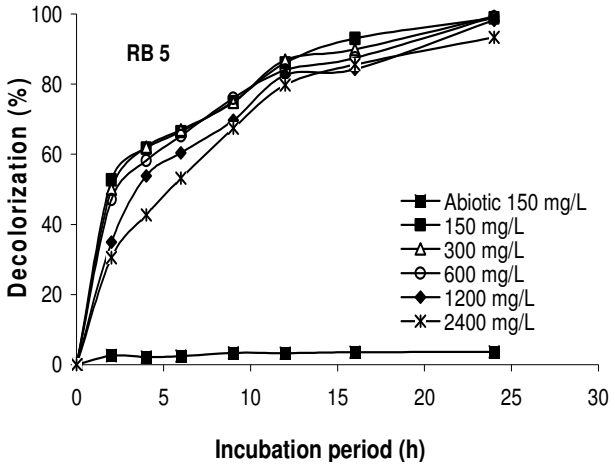



**Figure 1.** Effect of initial dye concentration on decolorization of RB 5.

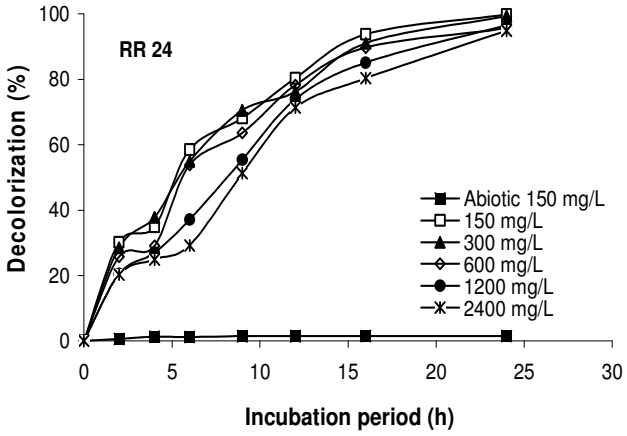



**Figure 2.** Effect of initial dye concentration on decolorization of RR 24.

having anthraquinone (R=O) chromophore group, decolorization efficiency remained lower even after 72 h incubation. As implied by this result, decomposition of dyes having anthraquinone structure as chromophore group by micro organisms is more difficult compared to those having mono-azo (-N=N-) and di-azo groups.

## Reaction kinetics of decolorization

Experimental data obtained from the batch reactor tests were plotted for tree reaction kinetics order 0, 1st and 2nd; dye concentration (C) versus time (t), ln C versus time and 1/C versus time according to Eq. (4) to (6), respectively. The zero, first, and second order rate constants ($k_0$, $k_1$ and $k_2$), through color removals of all dyes are listed in Table 4. The first order reaction rate constants ($k_1$) resulting from the fitting Eq. (5) to the whole curve are listed in Table 4.

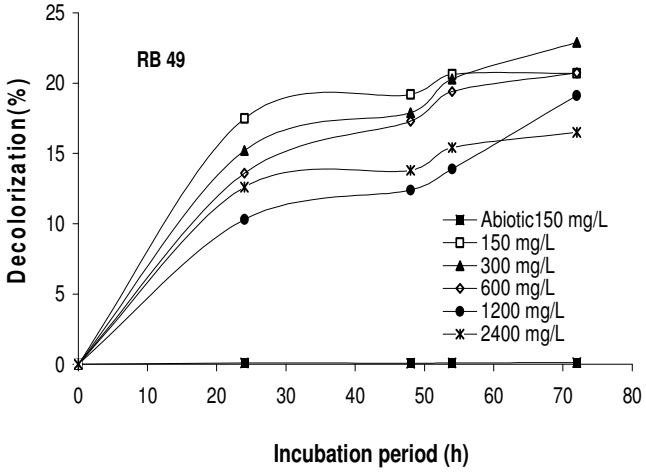



**Figure 3.** Effect of initial dye concentration on decolorization of RB 49.

**Table 3.** Decolorization of different initial dye concentrations during incubation period.

| $C_o$ (mg/L) | Decolourisation (%) | Incubation period (h) |
|---|---|---|
| **Reactive black 5** | | |
| 158.6 | 98.9 | 24 |
| 326.3 | 99.4 | 24 |
| 617.8 | 99.2 | 24 |
| 1264.5 | 98.3 | 24 |
| 2405.2 | 93.4 | 24 |
| **Reactive red 24** | | |
| 147.4 | 99.8 | 24 |
| 286.2 | 99.4 | 24 |
| 593.0 | 95.6 | 24 |
| 1234.1 | 96.6 | 24 |
| 2391.5 | 94.9 | 24 |
| **Reactive blue 49** | | |
| 165.3 | 20.7 | 72 |
| 347.5 | 22.9 | 72 |
| 612.3 | 20.7 | 72 |
| 1239.8 | 19.1 | 72 |
| 2406.2 | 16.5 | 72 |

$$C_t = C_0 - k_0 t \quad (4)$$

$$C_t = C_0 e^{-k_1 t} \quad (5)$$

$$\frac{1}{C_t} = \frac{1}{C_0} + k_2 t \quad (6)$$

All dyes were correlated with 1st order kinetics. However,

**Table 4.** The rate constants through decolorization of all azo dyes.

| Dye | | Constants | 150 mg/L | 300 mg/L | 600 mg/L | 1200 mg/L | 2400 mg/L |
|---|---|---|---|---|---|---|---|
| RB 5 | 0. | $k_0$ (mg/L.h) | 4.9 | 9.8 | 19.8 | 41.8 | 84.3 |
| | | $R^2$ | 0.69 | 0.69 | 0.72 | 0.78 | 0.84 |
| | 1. | $k_1$ (1/h) | 0.173 | 0.190 | 0.177 | 0.153 | 0.110 |
| | | $R^2$ | 0.97 | 0.93 | 0.93 | 0.95 | 0.99 |
| | 2. | $k_2$ (L /mg.h) | 0.021 | 0.019 | 0.007 | 0.0017 | 0.0007 |
| | | $R^2$ | 0.69 | 0.61 | 0.62 | 0.66 | 0.66 |
| RR 24 | 0. | $k_0$ (mg/L.h) | 6.0 | 11.7 | 23.6 | 49.1 | 96.6 |
| | | $R^2$ | 0.85 | 0.86 | 0.86 | 0.93 | 0.94 |
| | 1. | $k_1$ (1/h) | 0.248 | 0.199 | 0.135 | 0.139 | 0.123 |
| | | $R^2$ | 0.91 | 0.93 | 0.99 | 0.97 | 0.97 |
| | 2. | $k_2$ (L /mg.h) | 0.129 | 0.018 | 0.001 | 0.0009 | 0.0003 |
| | | $R^2$ | 0.59 | 0.62 | 0.85 | 0.74 | 0.76 |
| RB 49 | 0. | $k_0$ (mg/L.h) | 0.4 | 1.0 | 1.7 | 2.9 | 5.2 |
| | | $R^2$ | 0.75 | 0.88 | 0.89 | 0.94 | 0.81 |
| | 1. | $k_1$ (1/h) | 0.003 | 0.0034 | 0.0032 | 0.0027 | 0.0023 |
| | | $R^2$ | 0.77 | 0.91 | 0.91 | 0.94 | 0.84 |
| | 2. | $k_2$ (L /mg.h) | 0.00002 | 0.00001 | 0.000006 | 0.000002 | 0.000001 |
| | | $R^2$ | 0.77 | 0.92 | 0.93 | 0.93 | 0.82 |

decolorizations of RB 5 and RR 24 occurred more rapidly compared with RB 49. This could be attributed to the groups in the composition of azo dyes, and intermetabolites produced could not be completely degraded and exhibited inhibition as reported by Chung and Stevens (1993). The decolorization rates decreased slightly with the increases in dye concentrations for all dyes (Table 3). For instance, the decolorization constants were found to be 0.173 and 0.110 1/h for RB 5 and 0.248 and 0.123 1/h for RR 24 dye concentrations of 150 and 2,400 mg/L, respectively, while the rate constants of RB 49 dye were 0.003 and 0.0023 1/h at concentrations of 150 and 2,400 mg/L, respectively.

The course of the decolorization process approximates first order kinetic with respect to the dye concentration. Similarly, first order kinetic with respect to dye concentration has also been reported by some researchers (Wuhrmann et al., 1980; Weber and Wolfe, 1987; Weber, 1991; Carliell et al., 1995; van der Zee et al., 2001; Işık and Sponza, 2004a), but some others found zero order kinetic (Dubin and Wright, 1975; Brown, 1981). A probable explanation for these contradictory observations is that the rate-limiting step in the reduction of azo dyes may differ between the different experimental conditions studied (van der Zee et al., 2001). Furthermore, the decolorization of 20 selected dyes by granular sludge was studied by van deer Zee et al. (2001). They found that decolorization reactions followed by first order kinetic with respect to dye (0.3 mM) concentrations. Decolorization rates of 97 and 96% were obtained in azo dyes Direct Black 19 and Reactive Black 5, while the first order rate constants were 3 and 5 1/day, respectively. Yoo (2002) found that the decolorization of C.I. Reactive Orange 96 shows a first order kinetic (0.12 mM/min) with respect to both dye (0.1 M) and sulfide concentrations (0.01 M). Batch decolorization studies performed by Işık and Sponza (2004a) found that the decolorization rate constant decreased from 0.098 to 0.070 1/h while the C.I. Reactive Black 5 concentration increased from 200 to 400 mg/L. In their study, 400 mg/L of RB 5 decolorized approximately two times lower than our result with 0.070 1/h of rate constant in anaerobic conditions with granular sludge. This could be due to the extremely dense and compact structure of granules providing very high metabolic activity under anaerobic reduced conditions resulting in very high decolorization rates.

Azo-reductase enzyme systems help bacteria to decolorization high concentrations of azo-dyes with co-substrate under anaerobic conditions (Carliell et al., 1995; Yoo, 2002). Especially, aromatic amines, which form as result of decomposition of azo colors, produced during anaerobic treatment and can be reduced aerobically; however, they have treatment-resistance and a toxic effect on microorganisms at anaerobic conditions (O' Neill et al., 2000a; Ganish et al., 1994).

## Conclusion

Generally, wastewater of Turkish textile industry is treated in active sludge systems under aerobic condition. Decolorization operation may not be carried out in this

type of treatment system. Treatment efficiencies of dye wastewater in conventional aerobic system decreased due to toxic effects of dye components on aerobic micro organisms. In our study, decolorization removal efficiencies differed depending on chemical structures of dyes. Decolorization removal efficiency of dyes containing azo and di azo chromophore groups increased up to 98% after 24 h incubation period even at very high dye concentrations. Decolorization efficiencies of the dye containing anthraquinone chromophore group was about 20% even after 72 h incubation period, and decreased to about 15% with increasing concentrations. Reaction kinetic of the all dyes used in this study was in the 1st order. However, decolorization rates of RB 5 and RR 24 were higher than RB 49.

Mostly, aromatic amines are produced during decomposing dyes under anaerobic conditions, and they may not be removed and accumulated in the anaerobic incubation medium, but aerobic bacteria may easily remove the aromatic amines in aerobic conditions (Knackmuss, 1996). When the anaerobic and aerobic treatment systems were performed subsequently, dyes may be completely mineralized in sequential systems (Brown and Hamburger, 1987; Field et al., 1995; Razo-Flores et al., 1997). There was no removal inhibition of azo dyes containing samples in lower concentrations. In practice, the observed inhibition may not be important because real textile wastewater contains only 100 – 500 mg/L of these dyes (Sponza and Isık, 2004). There may not be inhibition of the degradation of organic matter but it is important in the decolorization of textile wastewaters containing these azo dyes. This study only shows that color from azo dyes in textile wastewater may be removed by anaerobic reactor before present conventional aerobic reactor.

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