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Bio-decolourization of azo-dye under anaerobic batch conditions[#]

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Abstract: Anaerobic treatment system was used for determination of colour removal under batch conditions. Azo-dye (Reactive Black 5, RB 5) with glucose as a carbon source was used as a synthetic wastewater. The decolourization process follows first order kinetics with respect to RB 5 azo-dye concentration in batch conditions. Decolourization rate constant (k_1) value for dye concentration of 150 mgL^{-1} was 0.1751 h^{-1} in the batch experimental study performed with RB 5. Maximum Reactive Black 5 removal efficiency was obtained after 24 hour (99%) under batch experimental condition.

Keywords: Batch condition, anaerobic decolourization, Azo dye, reactive black 5, kinetics

Introduction

The textile industry is one of the largest water users and polluters that still threaten the environment with its highly coloured discharge [1]. Discharge of azo-dyes is undesirable, not only for aesthetic reasons, but also because many azo-dyes and their breakdown products are toxic toward aquatic life and mutagenic for humans [2]. Above 60-70% of more than 10.000 dyes used in the textile industry are azo dyes [3]. Approximately 10-15% of dyes are released into the environment during dyeing of different substrates, such as, synthetic an natural textile fibres, plastics, paper, leather, mineral oils, waxes, and even foodstuffs and cosmetics [4]. They have one or more azo groups (R_1-N=N–R_2) having aromatic rings mostly substituted by sulfonate groups. These complex aromatic substituted structures make conjugated system and are responsible for intense colour, high water solubility and resistance to degradation of azo-dyes under natural conditions [5-7]. High rate anaerobic treatment systems have been considered for the treatment of azo-dyes in textile industry wastewater [8-10]. The colour removal is believed to be due to chemical reductive cleavage of azo bonds within dye molecules under the anaerobic conditions [11].

Most researches have investigated decolourization of azo-dyes under batch anaerobic condition. In a study performed by Işık and Sponza [12] for decolourization of Congo Red and Direct Black 38 was investigated using *Escherichia coli* and *Pseudomonas* sp. cultures in anaerobic, aerobic, and microaerophilic condition. They reported that anaerobic conditions were more favourable than other conditions for decolourization. Setiadi and Van Loosdrecht [13] demonstrated about 70 % colour removal efficiency, in a anaerobic reactor treating reactive azo-dye. Some previous batch studies, the following decolourisation efficiencies were obtained for Reactive Black-5; 75 and 95% colour removal in 10 and 30 h, respectively [14], 95% in 48 h [15] and 79% in 10 h [16]. Batch decolourisation studies performed by Brás et.al.[17] that the decolourisation rate constant was decreased from 0.04 to 0.016 h^{-1} while the Acid Orange concentration increased to 300 mg/L. The decolourisation of 20 selected dyes by granular sludge was studied by van der Zee *et a*l. [18] that decolourisation reactions were in first-order kinetic.

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The colour substances used in textile industry, in cases where chemical coagulation and flocculation processes are used in single way, are removed with low treating efficiency, and inert KOI and colour removal from the wastewater containing inert colour substances are not able to perform adequately by conventional aerobic activated sludge systems [19]. Alternative treatments may be used for colour removal from these waters depend on the character of wastewater [20]. The objective of this study was investigation of anaerobic decolourisation kinetics with 150 mgL^{-1} of azo dye (Reactive Black 5) under batch conditions.

Material and Methods

Dyes and chemicals

The azo-dye C.I. Reactive Black 5 (Fig. 1), commercially important and commonly used in local textile processing industries was obtained from Kucuker Co. Textile industry, Denizli, Turkey. The media components and chemicals were purchased from Merck and Sigma (Konya, Turkey). All chemicals used were analytical grade.

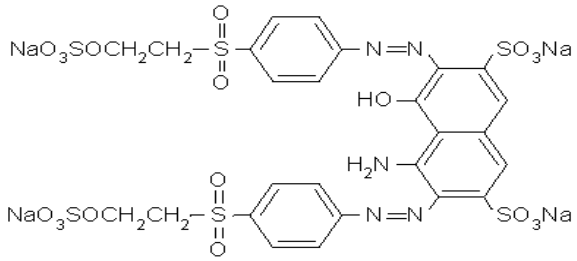

Figure 1: Chemical structure of azo dye (Reactive Black 5,C.I. 20505; λ_{max} 598 nm; 1000 mgL^{-1} of Reactive Black 5 equal to 782 mgL^{-1} solution COD)

Experimental lab-scale batch anaerobic reactor and synthetic dye wastewater

The studies were performed in 120 ml capacity dark glass serum bottles with 3.0 g of volatile suspended solids (VSS) per litre and the reaction liquid volume of 75 ml. The basic composition of dye synthetic wastewater was containing reactive azo-dye 150, 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 (mgL^{-1}) [21]. The anaerobic conditions were maintained by adding 667 mgL^{-1} of sodium thioglycollate (0.067%) that is proposed between (w/w) 0.01–0.2 percent for anaerobic conditions. The alkalinity and neutral pH were adjusted by addition of 5,000 mgL^{-1} NaHCO3. For COD, 3,000 mg/L of glucose was used as co-substrate providing reducing equivalents with electron fission. All incubations were carried out in a temperature controlled incubator at 35 ^oC. The bottles were vigorously shaken at certain intervals and the liquid samples were taken from the supernatants with a syringe for analyses.

Analytical methods

Volatile suspended solids (VSS), total suspended solids (TSS) were determined by standard methods [22]. Colour density was measured spectrophotometrically at the wavelength corresponding to the maximum absorbance of the dye ($\lambda_{max:}$, 598 for Reactive Black 5). 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. Absorbance measurements were done by using a Dr. Lange UV 200 model spectrophotometer. The calculation of colour removal efficiency after anaerobic treatment was performed using this formula:

$$CR(\%)=\frac{Do-D}{Do}*100 \quad (1)$$

where D_0 and D are concentrations of dye before and after anaerobic treatment in mg/L, respectively.

Result and Discussion

The decolourisation of an azo-dye C.I. Reactive Black 5 was studied using anaerobic batch reactor. The obtained results for the treatment of RB 5 are shown in Figure 2.

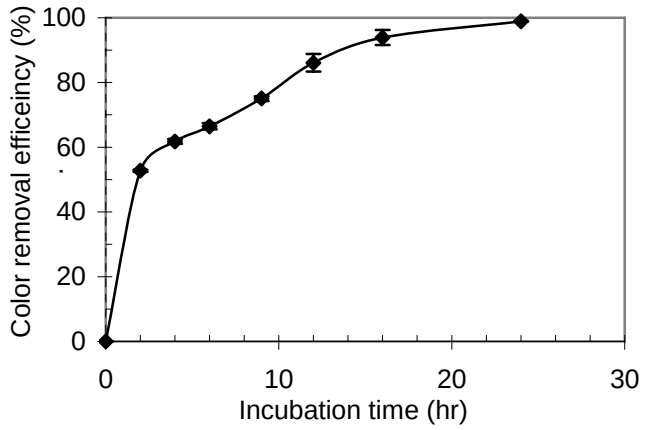

Figure 2: Removal percentage of colour RB 5 throughout the 24 hours incubation period

This figure indicates to the removal of colour percentage for azo-dye RB 5, during the 24 h of incubation period. The colour removal was about 50% in batch reactor containing 150 mgL^{-1} initial concentration of RB 5 azo-dye in the first 2 hours, and followed by significant decolourisation in the next 12 hours resulting in 86% colour removal. At the end of the incubation period of 24 hours, 99% colour removal efficiency obtained (Fig. 2).

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 (t) and 1/C versus time (t) respectively. Figure 3 shows afore mentioned plots in order to determine the kinetic constants for dye sample. The kinetic coefficients and correlations relevant to the zero, first and second orders summarized Table 1. The kinetic data obtained in this study showed that first order rate constant for RB-5 concentration 150 mgL^{-1}. In this study, the decolourisation constant was found to be 0.1751 h^{-1} for first order kinetic.

Azo-reductase enzyme systems help bacteria to decolorize high concentrations of azo-dyes with co-substrate under anaerobic conditions [3, 23]. Especially, aromatic amines, which form as result of decomposition of azo colours produced during anaerobic treatment and can be reduced aerobically; however, they have treatment-resistance and a toxic effect on microorganisms at anaerobic conditions [24, 20].

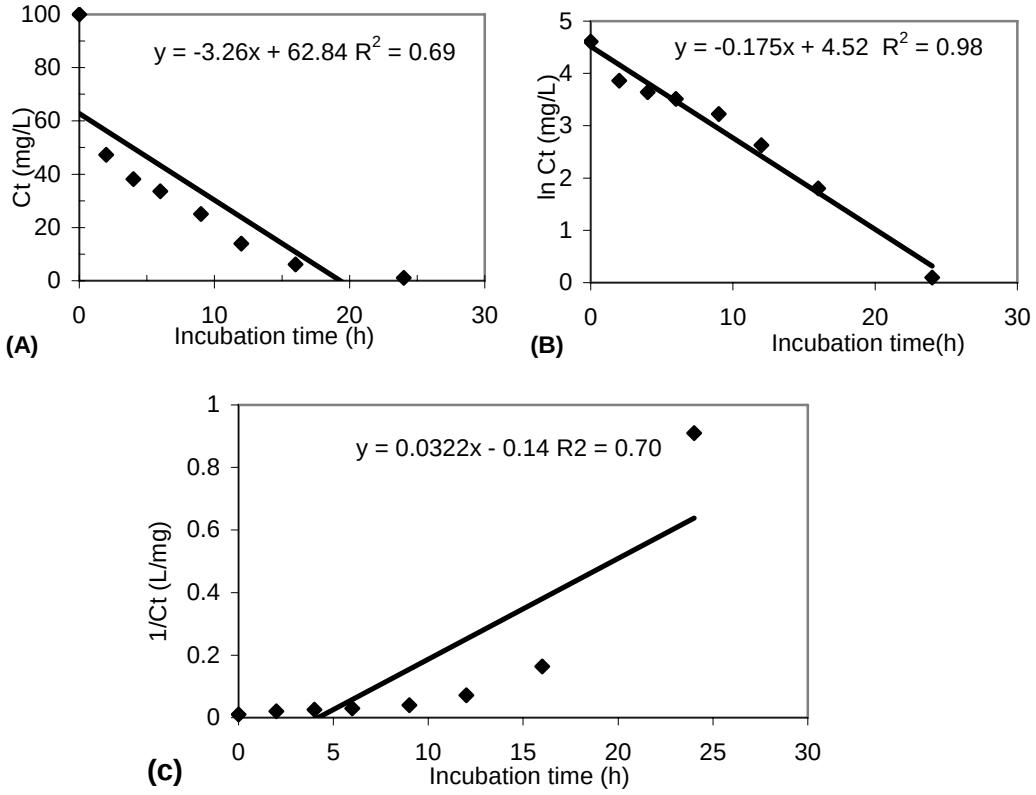

Figure 3: Reaction kinetics for 150 mg/l of RB-5 azo dye. **A**, Zero; **B**, first and **C**, second order

Table 1. The kinetic rate constants and correlation coefficients for azo dye decolourization (concentration of **150 mg/l** R.B.-5).

Reaction order		Constants	
	0.	K_0 (mg/l.h)	3.26
		R^2	0.69
	1.	K_1 (1/h)	0.175
		R^2	0.98
	2.	K_2 (l /mg.h)	0.032
		R^2	0.70

Conclusion

In this study, with partially granulated mixed anaerobic cultures, glucose as co-substrate and RB 5 azo dye was decolorized throughout the experimental period of 24 h. Results obtained from this work show that the mixed bacterial culture possesses high decolourisation efficiency. The anaerobic batch reactors achieved complete decolourisation and up to 99% removal of dye load of synthetic dye wastewater containing 150 mg/L of C.I. Reactive Black 5 dye. Batch anaerobic decolourisation experiment showed that RB 5 dye was decolorized according to the first order reaction kinetic. Kinetic rate constant of the dye decolourisation was 0.1751 h^{-1}.

Textile industries wastewaters in Turkey are generally treated via mixing activated sludge systems, but toxic colour resulted from azo colours cannot be removed due to its toxic effects on

aerobic microorganisms, treatment efficiency was negatively affected. The result of the study showed that textile industry wastewater will be treated in anaerobic system in front of aerobic treatment, which colour removal efficiency will be increased after two treatment reactors.

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