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The following collection of papers expands on some of the abstracts shown in the previous section. Some of the papers have been lightly refereed. We trust you will enjoy them.

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A BATCH TESTS FOR ASSESSING DECOLORIZATION AND KINETIC OF REACTIVE DYES BY GRANULATED ANAEROBIC MIXED CULTURE

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Abstract

Most of published studies have considered adding carbon sources to wastewater to affect the degradation and kinetics of dyes, but in the normally textile process has the sizing agents as the carbon source. Therefore, the aim of this study was to investigate the rate of decolorization and biological kinetics of dyes by partially granulated anaerobic mixed culture from an upflow anaerobic sludge blanket (UASB) reactor. The partial granulated anaerobic mixed culture (1.8 g MLVSS Γ^1) was used to investigate the treatability of two diazo dyes, reactive red 120 (RR 120) and reactive red 141(RR 141) in different carbon sources, dye concentrations and COD in batch test experiments with an incubation time of 324 hours. Three sizing agents, modified starch (MS), polyvinyl alcohol (PVA) and acrylic size (AS), were used as a carbon sources concentrations varying in the range of 400-1600 mg COD Γ^1 and color concentrations of 20,40 and 60 mg Γ^1 . The removal efficiencies and biological kinetics were evaluated. Results revealed that increasing dye concentration could inhibit the biodegradation, particularly when PVA is used as the carbon source. Additional experiments on the effect of dye concentration, using MS as the carbon source, revealed that degradation of RR 120 and RR 141 followed first-order kinetics. The decolorization rates increased with the decreasing dye concentrations of RR 120 and RR141. In contrast, the decolorization rates were increased with the increasing COD. The maximum decolorization of both RR 120 and RR141 was 96% when the 1600 mg COD Γ^1 and dye concentration of 60 mg Γ^1 were applied. In addition, the biological rates of RR120 and RR 141 were 2.61x 10^{-2} and 3.41x 10^{-2} h⁻¹, respectively. In conclusion, it is noted that anaerobic biodegradation of textile wastewater containing MS can be achieved, and that color removal of RR141 was faster than that of RR 120.

Keywords: Anaerobic biological treatment; Color removal; Kinetics; Reactive Dyes; Textile wastewater

1. Introduction

Azo dyes are the class of dyes most widely used industrially world market (Carliell et al., 1995). Approximately 10-15 % of production is released into the environment (Tan et al., 2000). This has been a strong impact on the aquatic environmental problem because some of azo dye or their breakdown products have a strong toxic, mutagenic and carcinogenic influence on the living organism.

Primary degradation and decolorization of dyes with azo-based chromophores can be achieved by the reduction of the azo bond (-N=N-). Azo bond are quite resistant to biodegradation aerobic condition (Supaka et al., 2004). On the other hand, azo bond are readily decolorized by anaerobic condition (Isik and Sponza, 2003). Although the phenomenon of anaerobic azo reduction is an animously

(Isik and Sponza, 2003). Although the phenomenon of anaerobic azo reduction is an animously accepted, some aspects of reaction mechanism remain to be clarified. Different observations have been reported on the involvement of enzymes and the local of the reaction and its kinetic order.

The aim of this present work to indicate color removal and kinetics of two reactive dyes namely RR 120 and RR 141, by three different carbon sources is namely MS, PVA and AS as a carbon sources concentrations varying in the range of 400-1600 mg COD I⁻¹ and color concentrations of 20, 40 and 60 mg I⁻¹ with partially granulated anaerobic mixed culture.

2. Materials and methods

2. I Medium

The basal nutrients of the medium and trace elements were used in all batch investigations following in Table 1. Sodium bicarbonate was used (1.5 g Γ^{-1}) to neutralize the medium and the pH was adjusted to 6.98 - 7.02.

Table 1 The nutrient medium and trace element compositions

Composition	Concentration (mg l ⁻¹)	
NaCl	150	
(NH ₄) ₂ SO ₄	280	
NH4CI	230	
Na ₂ HPO ₄	380	
Na ₃ HPO ₄ .12H ₂ O	123	
MgSO ₄ .7H₂O	5	
FeCl ₂ .4H ₂ O	16	
CoCl₂.6H₂O	0.88	
H ₃ BO ₃	0.1	
Zr SO ₄ .7H ₂ O	0.1	
CuSO _{4.} 5H ₂ O	0.05	
NiSO4,6H2O	1	
MhCl _{2.} 4H ₂ O	5	
(NH ₄) ₆ Mo ₇ O ₂₄ .4H ₂ O	0.64	
CaCl ₂ .2H ₂ O	5	

2.2 Carbon sources preparation

Three carbon sources namely MS, PVA and AS were used in this study. MS is composed of starch ester, the PVA molecule contains acetate and acrylonitrite groups and AS is mainly composed of polyacrylate ester. Stock MS and PVA solution (20 g l⁻¹) were prepared by hydrolyzing the solution in 4 % NaOH solution, then heated for 2 h at 80 °C. Stock AS was prepared by dilution a 40 ml of AS solution to 1000 ml by 4 % NaOH solution, then heated for 2 h at 80 °C.

2.3 Dyes

Commercially dyes were used in this investigation. Two dyes were C.I. Reactive Red 120 and C.I. Reactive Red 141. The characteristics of these dyes are summarized in Table 2. The structures of reactive dyes used in bath assays are give in Fig. 1.

Concentrated stock solutions of the dyes were prepared by 10 g l⁻¹ in distillation water. Both dyes were dissolved in the medium in a range of 20, 40 and 60 mg l⁻¹.

Table 2 Dyes and their properties

Name	Color Index	Molecular weight
Evercion red H-E3B	C.I. Reactive Red 120	1338
Evercion red H-E7B	C.I. Reactive Red 141	1774

C.I. Reactive Red 120

C.I. Reactive Red 141

Fig. 1. The structure of reactive dyes used in batch assays.

2.4 Microorganisms

The partially granulated anaerobic mixed cultures from the upflow anaerobic sludge blanket (UASB) treatment plant of textile wastewater were used in this study.

2.5 Batch assay

The anaerobic degradation kinetics of dyes decolorization assays were conducted in a 120 ml serum bottles (100 ml, total working volume) containing 70 ml of nutrient medium and 30 ml granular sludge. Three ranges of COD concentrations between 400 and 1600 mg I^{-1} with three carbon sources, and both dyes concentrations ranging from 20 to 60 mg I^{-1} were used. The concentration of MLVSS was 1.8 g/l of total working volume. Serum bottles were flushed with nitrogen gas in the headspace and then sealed with butyl rubber stoppers and aluminium crimp seals. The serum bottles were incubated at 37 \pm 1 °C without shaking. The samples were taken gradually during incubation until the end of 324 hours. Then they were filtered through GF/C and the filtrates were used for analysis of pH, chemical oxygen demand (COD). The mixed liquor volatile suspended solids (MLVSS) were also analyzed. All measurements are followed Standard Methods (APHA, AWWA and WPCF, 1995). The color were measured at the maximum absorbance in visible range of 520 nm for both dyes. Samples were analyzed periodically until the end of 324 hours of incubation time.

3. Results

3.1 Decolorization of RR 120 and RR 141 when present as different carbon sources

Decolorization of RR 120 and RR 141 with three different carbon sources at 800 mg COD I⁻¹ and color concentrations of 20, 40 and 60 mg I⁻¹ are shown in Fig. 2. Fig. 2 shows that there are two steps of decolorization of RR 120 with AS and PVA as carbon sources. The first step is high rate of decolorization while the second step was low rate of decolorization. It should be noted that it was probably due to the physical adsorption color concentrations of 20, 40 and 60 mg I⁻¹ are shown in Fig. 2.

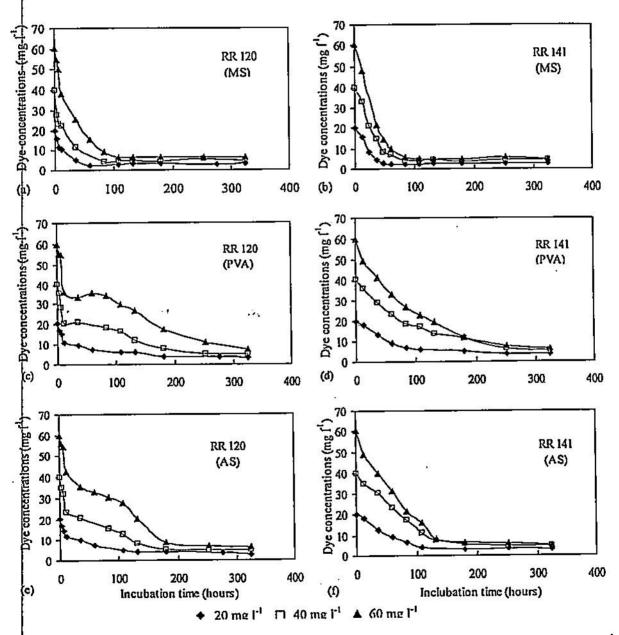


Fig.2. Decolorization of RR 120 and RR 141 with different carbon sources at 800 mg COD I⁻¹ and edlor concentrations of 20,40 and 60 mg I⁻¹.

Fig. 2 shows that there are two steps of decolorization of RR 120 with AS and PVA as carbon sources. The first step is high rate of decolorization while the second step was low rate of decolorization. It should be noted that it was probably due to the physical adsorption of dyes (RR 120) molecules by cell mass in the first step. On the other hand, the decolorization using three carbon sources of RR141 and MS as carbon source of RR120 that this reaction could be the exponential curve (one step) related to first-order kinetics.

Results revealed that the decolorization with MS was higher than AS and PVA as carbon sources. This is because, the MS carbon source was well degraded compared to AS and PVA. The maximum decolorization of RR120 with MS, PVA and AS as carbon sources were 88.6, 88 and 87.7 %, respectively when the dye concentration of 60 mg l⁻¹ were applied. Meanwhile, the maximum decolorization of RR 141 were 91.3, 88.5 and 90.1 %, respectively with MS, PVA and AS as carbon sources when used the dye concentration of 60 mg l⁻¹. It is noted that color removal of RR 141 was higher than that of RR 120.

3.2 Decolorization kinetis of RR 120 and RR 141

Zero-, first- and second-order were used to determine the colorization kinetic. Data were plotted in form of dye concentration (-C_t) versus time (t), $\ln C_t/C_0$ versus t, and $1/C_t$ versus t, respectively (where C_0 is the concentration of dye at time zero and C_t is the concentration of dye at time t).

Effect of color concentration

Azoreductase enzyme systems help bacteria to decolorize high concentrations of azo dyes with a cosubstrate under anaerobic conditions (Wuhrmann et al., 1980; Carliell et al., 1995; Yoo, 2002). Taking these characteristics into consideration, the effect of RR 120 and RR 141 dye concentrations on decolorization of dyes was determined.

Fig. 3(a), (b) and (c) show K_0 , K_1 and K_2 in anaerobic batch tests containing MS as carbon source (COD 1600 mg 1⁻¹) with RR 120 and RR141. The decolorization kinetics with respect to dye concentrations for MS carbon source was yield a high regression coefficients that the color was removed according to first-order kinetics. First-order kinetics with effect to dye concentrations have also been reported by Carliell et al. (1995) and Isik and Sponza (2004). The decolorization rate constants (K_1) of RR 141 are significantly higher than the rate constants of RR 120. The decolorization constants (K_1) of RR 120 were achieved 4.51×10^{-2} , 3.2×10^{-2} and 2.61×10^{-2} h⁻¹ a dye concentrations of 20, 40 and 60 mg 1⁻¹, respectively while the same rate constants were achieved 4.86×10^{-2} , 3.66×10^{-2} and 3.41×10^{-2} h⁻¹, respectively for RR 141 at the same dye concentrations, respectively. It is noted that decolorization rate constants of RR 141 was higher than that of RR 120. The decolorization rates increased with the decreasing dye concentrations of RR 120 and RR 141. This may because of the accumulating of inter-metabolites through simultaneous degradation and decolorization of dyes.

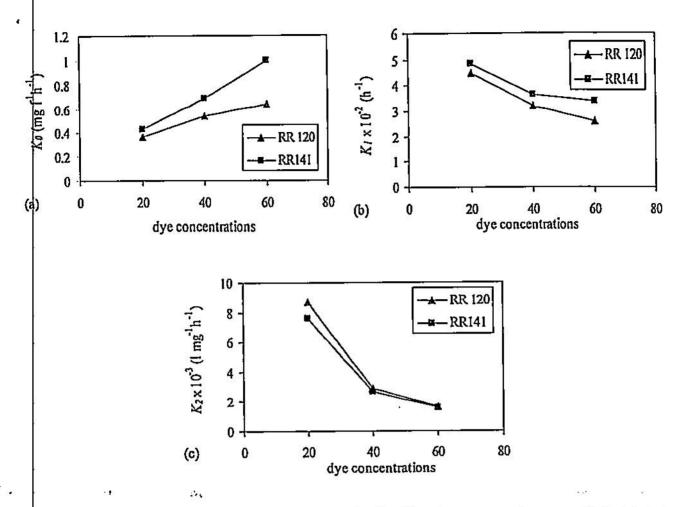


Fig. 3. Zero-, first- and second order from decolorization kinetic constants in anaerobic batch tests containing MS as carbon source (COD 1600 mg I⁻¹) with RR 120 and RR141.

Effect of COD concentration

Zero-, first- and second order from decolorization kinetic constants in anaerobic batch tests containing RR 141 (60 mg I^{-1}) with MS carbon sources at the concentrations in term of 400, 800 and 1600 mg COD I^{-1} , as show in Fig. 4(a), (b) and (c). The decolorization rate constants were first-order kinetic with effect to COD concentrations ($R^2 > 0.94$). The K_I values rate of decolorization of RR 120 were achieved 1.44×10^{-2} , 2.19×10^{-2} and 2.61×10^{-2} h⁻¹ with COD concentrations of 400, 800 and 1200 mg I^{-1} , respectively while the same rate constants were achieved 1.83×10^{-3} , 2.87×10^{-3} and 3.41×10^{-2} h⁻¹, respectively for RR 141 at the same COD concentrations. The maximum decolorization of both RR 120 and RR 141 was 96% when the 1600 mg COD I^{-1} and dye concentration of 60 mg I^{-1} were applied. The trend of decolorization rate constants increased with the increases in COD concentrations of RR 120 and RR 141. Enhancement of dyes reduction through addition of MS as carbon source.

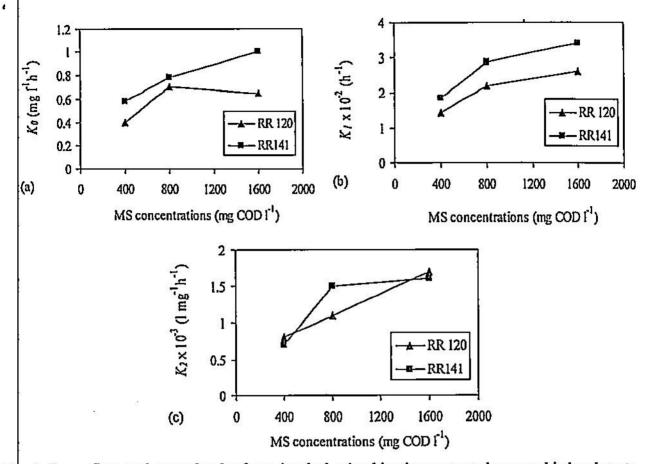


Fig. 4. Zero-, first- and second order from decolorization kinetic constants in anaerobic batch tests containing RR 120 and RR 141 (60 mg l⁻¹) during MS degradation.

3.3 Biological dyes reduction and COD removal

Carliell et al. (1994) reported that degradation of C.I Reactive Red 141 yielded 2-aminonaphthalene-1, 5-disulphonic acid (due to the cleavage of azo chromophore), 1,7-diamino- 8-naphtho-3,6disulphonic acid, p-diamino-benzene and cyanuric acid. The proposed degradation products from the decolorization of C.I. Reactive Red 120 were 2- aminonaphthalene-1-disulphonic acid, 1,7-diamino-8-naphtho-3,6-disulphonic acid, p-diamino-benzene and cyanuric acid.

The anaerobic biodegradations of dye and different carbon sources of MS, PVA and AS at 1600 mg COD I⁻¹ and dye concentration of 60 mg I⁻¹ were achieved 79, 28 and 56%, respectively for RR 120 while same condition were achieved 82, 27 and 66%, respectively for RR 141 (data not show). The anaerobic biodegradabilities of MS was higher than AS and PVA respectively. Sacks and Buckley (1999) achieved biodegradation of the starch component was efficient with 85% reduction in COD. Use PVA as a carbon source showed poorly degraded due to its polyacrylate structure. Sacks and Buckley (1999) found that the PVA molecule contains acetate and acrylonitrile groups which prevent decay, thus it was reasonable to expect PVA to be resistant to biodegradation. AS carbon source was inert to inhibiting the anaerobic degradation of process. Shaw et al. (2002) found that the AS concentrations less than 4 g I⁻¹, acrylic was not very efficient, with only a 45% reduction in COD.

The acrylic component could be treated anaerobically, in low concentrations, with acclimated biomass.

4. Discussion

The decolorization of azo dyes and COD removal occur in incubation that was link of anaerobic microorganisms. This implies that dye was degraded by microorganisms. Chung and Stevens (1993) reported that decolorization of azo dyes often assuming electron carriers and flavin nucleotides are cofactors. The dye acted as the final electron acceptor in the electron transport chain of the bacteria's metabolism; and as a result, the azo bonds were reduced. The color of the dye is due to the azo bonds in the chromophore, therefore, reduction of the azo bonds results in decolorization.

Decolorization of RR 120 and RR 141 under anaerobic condition with effect of different carbon sources revealed that the decolorization with MS was higher than AS and PVA as carbon sources. This is because, the MS carbon source was well degraded compared to AS and PVA. Meanwhile, PVA as carbon source was poorly degraded and then AS could be treated anaerobically, in low concentrations.

The color removal and decolorization kinetic rates of RR 141 was higher than that of RR 120. It it probable that the degradation potential of RR 141 metabolites in biological system is the higher sulphonated nature of these compounds than RR 121. Sulphonation tends to increase the regalcitrance of compounds by decreasing their ability to permeate through the microbial cell walls. It is possible that the compounds of RR 141 could pass through the microbial cell walls less than RR 120; therefore, permeation of the dyes through the microbial cell membranes and subsequent intercalation of the dyes between DNA base pairs is thought to be unlikely as a mechanism of inhibition.

The experimental data found in this study showed that decreasing rate of degradation of RR 120 and RR 141 with increasing dye concentration. It is probable that accumulation of metabolic products were also responsible for this phenomenon. As for high dye concentrations, the accumulated dyes are competed for the active sites of azoreductase enzyme. Dyes and the intermediate substances may block the active sites of this enzyme. Under reductive anaerobic conditions containing MS as carbon source, the kinetic rates of RR 141 was higher than the rate constants of RR 120. Degradation of reactive azo dyes are followed the first-order kinetic with increasing dye concentration.

The decolorization of reactive azo dyes by granulated anaerobic mixed culture are followed the first-order kinetic with increasing COD concentration. A first-order kinetic model gives the dependence of substrate degradation rate on initial concentration of dye which similar results was obtained by Carliell et al., 1995; Isik and Sponza, 2004. The trend of decolorization rate constants increased with the increases in COD concentrations of RR 120 and RR141. Enhancement of dyes reduction could be achieved through addition of MS as carbon source.

5. Conclusions

Microbial decolorization of RR 120 and RR 141 in an anaerobic condition occurs as a result of reduction and cleavage of the azo bonds. Decolorization of RR120 and RR141 containing MS as carbon source in the anaerobic system conform to a first-order relationship, with respect dye

concentration and COD concentration. However, k values decreased with increasing dye concentration in the anaerobic serum bottles, it is probable that accumulating metabolic products were also responsible for this phenomenon. On the other hand, the k values increased with the increasing COD concentration. Anaerobic biodegradation of textile wastewater containing MS can be achieved, and that color removal of RR141 was faster than that of RR 120.

Acknowledgments

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