Anaerobic degradation kinetics of reactive dye with different carbon sources

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Abstract: This study aims to investigate the anaerobic degradation kinetics of reactive dye, C.I. Reactive Red 141 (Evonic Red H-ETB) by partially granulated anaerobic mixed culture using three carbon sources, namely, modified starch (MS), polyvinyl alcohol (PVA) and acrylic size (AS) during batch incubation. There is a first-order kinetics reaction in the decolorization processes using MS and PVA as carbon sources, while a zero-order kinetics relationship describes the decolorization process for the AS carbon source. The k values and color removal rate of decolorization with MS carbon source was higher than those of PVA and AS carbon sources. This is because the MS carbon source was well degraded in comparison to AS and PVA, respectively. This study also found dye reduction could be enhanced through the addition of MS as a carbon source. The decolorization rates increased with decrease in dye concentrations of RR 141. In contrast, the decolorization rates increased with increase in COD concentration.

Key words: Anaerobic degradation, Biological treatment, Decolorization, Kinetics, Reactive dye

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Introduction

Large volumes of water pollution from dyestuffs are induced by the discharge of textile wastewater during manufacturing and processing. Subsequently, there has been a strong impact on the aquatic environment, such as the effects from bio-recalcitrant contaminants, which are a potential health hazard. The effluent from the dyeing and desizing processes contributes to the high colorant content and chemical oxygen demand (COD) of the total drainage. Reactive dyes are a very important class of textile dyes, whose losses through processing are particularly significant and difficult to treat. Many different methods have been used for the treatment of textile wastewater. In the case of biological treatment, the effluent from the conventional treatment system is typically low in COD, but high in color (Dong, 2005). The traditional aerobic wastewater treatment systems do not substantially decrease the color of wastewater (Shaw et al., 2002; Sani et al., 2006; Singh et al., 2007; Demir et al., 2007).

Some anaerobic processes can remove high COD and have nearly complete decolorization, such as upflow anaerobic sludge blanket (UASB) (Isik and Sponza, 2004; Bras et al., 2005), anaerobic baffled reactor (Bell and Buckley, 2003), upflow anaerobic fixed film (Rajaguru et al., 2000) and anaerobic fluidized bed reactor (Sen and Demirer, 2003). Anaerobic processes can enhance the ability of the microbial consortium to decolorize textile dye. Many studies have shown that adding carbon source to wastewater has an effect on biodegradation and biokinetics. In normal textile process, however, sizing agents such as MS, PVA and AS are used for the carbon sources. Therefore, the aim of this study is to investigate the rate of decolorization by granular sludge in the presence of these three carbon sources.

Materials and Methods

Medium: The basal medium used in these experiments contained the following contents (in g l-1): NaCl 0.15, (NH4)2SO4 0.28, NH4Cl 0.23, Na2PO4.12H2O 0.123 and Na2HPO4 0.38. The trace element solution (Prakash and Gupta, 2000) was prepared in one liter of distillation water. It contained the following components (in g l-1): MgSO4.7H2O 5, FeCl3.4H2O 6, CoCl2.4H2O 0.88, H3BO3 0.3, ZnSO4.7H2O 0.1, CuSO4.5H2O 0.05, NiSO4.6H2O 1, MnCl2.4H2O 5, (NH4)2MoO4.24H2O 0.64 and CaCl2.2H2O 5. One ml of this solution was added to 1 l of basal medium. The buffer solution contained 1.5 g l-1 NaHCO3 and the pH was adjusted to 7.00 ± 0.02.

Carbon sources preparation: Three carbon sources, namely, modified starch (MS), polyvinyl alcohol (PVA) and acrylic size (AS) were used in this study. MS is composed of starch ester, the PVA molecule contains acetate and acrylonitrile groups, and AS is mainly composed of polycrylate ester. Stock MS and PVA solutions (20 g l-1) were prepared by hydrolyzing the solution in 4% NaOH solution, and then heated for 2 hr at 80°C. Stock AS was prepared by diluting 40 ml of AS to 1000 ml with a 4% NaOH solution, and then heated for 2 hr at 80°C.

Dyestuff: Commercial diazo dye, C.I. Reactive red 141 (Fig. 1) was used in this study. The stock solution of dye has a concentration of 10 g l-1.
Microorganisms: The partially granulated anaerobic mixed culture from the UASB treatment plant of textile wastewater was used in this study.

Serum bottle batch assay: Experiments were performed in 120 ml serum bottles. The total working volume of 100 ml was used by mixing 70 ml of nutrient medium and 30 ml of granular sludge. The COD concentrations were between 400 and 1600 mg l⁻¹ for each of the three carbon sources used. The values of dye concentrations ranged from 20 to 60 mg l⁻¹. The concentration of MLVSS was 1.8 g l⁻¹. Serum bottles were flushed with nitrogen gas in the headspace and sealed with butyl rubber stoppers and aluminum crimp seals. Then the serum bottles were incubated at 37 ± 1°C for 324 hr. The samples were filtered through GF/C and the filtrates were used for analysis of chemical oxygen demand (COD) and color. The soluble and mixed liquor volatile suspended solids (MLVSS) were analyzed following standard methods (APHA, 2005), while the pH was measured by pH meter type CD500 WPA. The color was measured at the maximum wavelength of 520 nm by a DR/3000 HACH spectrophotometer. Samples were analyzed periodically until the end of the 324 hr incubation period.

Results and Discussion

Decolorization of RR 141 with respect to different carbon: The color of the dye comes from the azo bonds in the chromophore, therefore, the reduction of the azo bonds results in decolorization.

Various authors have emphasized the necessity of the involvement of azo reductase (cytoplasmic enzyme) in the decolorization of azo dyes, often assuming electron carriers, (coenzymes) flavin nucleotides [flavin mononucleotide (FMN); flavin adenine dinucleotide (FAD)] as cofactors. Gingell and Walker (1971), who regarded the soluble flavin as an electron shuttle between a dye and an NADH-dependent azo reductase. It was concluded that the dye acted as the final electron acceptor in the electron transport chain of the bacterial metabolism, and as a result, the azo bonds were reduced.

The percentage of color removal at different COD concentrations of MS, PVA and AS as carbon sources with RR 141 (40 mg l⁻¹) are shown in Fig. 2. The percentage of color removal for MS as the carbon source was higher than those of AS and PVA carbon sources, respectively. Color removal increased with increases in COD concentrations. The percentage of color removal slightly increased from 400 mg l⁻¹ to 800 mg l⁻¹ of COD concentration.

The maximum color removal rates achieved were 91.5%, 87% and 88.7% for MS, PVA and AS as carbon sources, respectively, at 1600 mg l⁻¹ of COD concentration.

Decolorization kinetics of RR 141: Zero-, first- and second-order kinetics were used to determine the colorization kinetics by plotting dye concentration (Cₜ) versus time (t), ln Cₜ/C₀ versus t, and 1/Cₜ versus t, respectively, where Cᵢ is the concentration of dye at time zero and Cₜ is the concentration of dye at time t.

Effects of increasing dye concentration: The rate constants for the decolorization kinetics in anaerobic batch tests containing different RR 141 concentrations of 20, 40 and 60 mg l⁻¹ with 1600 mg COD l⁻¹ in three different carbon sources are listed in Table 1. The decolorization kinetics with respect to dye concentrations for MS and PVA as carbon sources yielded high regression coefficients (R² > 0.972), which demonstrates that the color was removed according to first-order kinetics. On the other hand, zero-order kinetics described the data for AS as the carbon source, which had regression coefficients higher than 0.963. The decolorization rates were slightly decreased with increase in dye concentrations of RR 141 for MS and PVA as carbon sources. This may be caused by the accumulation of inter-metabolites through the simultaneous degradation and decolorization of dyes. Decolorization constants (kₗ) of 4.9x10⁻², 3.7x10⁻² and 3.4x10⁻² hr⁻¹, respectively, were achieved for MS as the carbon source at dye concentrations of 20, 40 and 60 mg l⁻¹, respectively, while rate constants (kₘ) of 1.5x10⁻², 1.2x10⁻² and 1.1x10⁻² hr⁻¹, respectively, were achieved for PVA as carbon source at the same dye concentrations, respectively. On the other hand, the decolorization rates for AS as the carbon source increased with increase in dye concentrations for RR 141. The decolorization constants (kₗ) of 0.138, 0.289 and 0.407 mg l⁻¹ hr⁻¹, were achieved, respectively, with the AS carbon source at dye concentrations of 20, 40 and 60 mg l⁻¹, respectively.

Effects of increasing COD concentration: Table 2 shows zero, first- and second order rate constants from decolorization kinetics in anaerobic batch tests containing RR141 (40 mg l⁻¹) with different carbon sources at the concentrations of 400, 800 and 1200 mg COD l⁻¹. The decolorization rate constants increased with increase in COD concentration. The decolorization rate constants in Table 2 follow first-order kinetics with respect to COD concentrations for the MS and PVA carbon sources (R² > 0.978), while zero-order kinetics with respect to COD concentrations describe the data from the AS carbon source (R² ed 0.980). The obtained kₗ values for

![Fig. 1: Chemical structure of C.I. Reactive red 141](image-url)
Anaerobic degradation kinetics of reactive dye

Fig. 2: Percentage of color removal at different COD concentrations of (MS) modified starch (a), (PVA) polyvinyl alcohol (b) and (AS) Acrylic size (c) with RR 141 (40 mg l⁻¹)

decolorization for the MS carbon source were 2.0x10⁻², 2.8x10⁻² and 3.8x10⁻² hr⁻¹, respectively, with COD concentrations of 400, 800 and 1200 mg l⁻¹, respectively, while the kₚ values for the PVA carbon source were 9.0x10⁻³, 8.7x10⁻³ and 1.4x10⁻² hr⁻¹, respectively at the same RR 141 concentrations, respectively. Meanwhile, the kₚ values for decolorization were 0.261, 0.270 and 0.275 mg l⁻¹ hr⁻¹, respectively, with COD concentrations of 400, 800 and 1200 mg l⁻¹, respectively, for AS as the carbon source.

Table 3 compares the first-order kinetics constant values and color removal in anaerobic batch tests containing dyes. The results of color removal from this research were shown higher than 85%. The first-order kinetic constants vary between 0.034-0.44 hr⁻¹. A probable explanation that the different carbon sources and dye structures effect to bacteria degradation of dye and bioactivity of wastewater bacteria. Therefore, the metabolic of anaerobic microbial community is a rate limiting factor in the decolorization of dye, requiring the carbon source to the system to maintain the rate of dye reduction.

From the experimental data, it can be proposed that the organic carbon transformed to volatile fatty acid (VFA), subsequently to methane production. In addition the azo bonds of RR 141 dye cleaved and transformed to aromatic amines. At higher dye concentrations, when aromatic amine concentrations (AA) and VFA accumulate, dyes are compelling for the active sites of azoreductase enzymes together with substrates (Isik and Sponza, 2004). Dyes and the intermediate substances may block the active sites of this enzyme, which inhibit cleavage of the azo bond. In other words, at high RR141 concentrations, the accumulation of both VFA and AA.
results in low substrate removal rate. This corresponds to the low rate of azo dye co-metabolism.

Many metabolic enzymes and co-factors are induced by utilization of growth substrate (carbon source) through decolorization of azo dyes. A non-growth substrate (dye) is biotransformed by the specific enzymes, but it cannot be utilized by the microorganisms to support their growth during color removal period. The dye was removed by the cleavage of azo bonds with electrons transferred from the carbon source under reductive environments. The concentration of auxiliary substrate (i.e. original electron donor) controls the rate of formation of the reduction equivalents or intermediate electron donors. Therefore, the decolorization rate should increase with increasing auxiliary substrate concentration (Carielli et al., 1995).

### Table 1: The rate constants from decolorization kinetics in anaerobic batch tests containing RR 141 (20, 40 and 60 mg l⁻¹) with three carbon sources (COD concentration 1600 mg l⁻¹)

<table>
<thead>
<tr>
<th>Carbon sources</th>
<th>Constants</th>
<th>20</th>
<th>40</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midified starch</td>
<td>( k_0 ) (mg l⁻¹ hr⁻¹)</td>
<td>0.325</td>
<td>0.691</td>
<td>0.724</td>
</tr>
<tr>
<td></td>
<td>( R^2 )</td>
<td>0.900</td>
<td>0.989</td>
<td>0.996</td>
</tr>
<tr>
<td>Polyvinyl alcohol</td>
<td>( k_0 ) (mg l⁻¹ hr⁻¹)</td>
<td>0.325</td>
<td>0.527</td>
<td>0.388</td>
</tr>
<tr>
<td></td>
<td>( R^2 )</td>
<td>0.915</td>
<td>0.906</td>
<td>0.935</td>
</tr>
<tr>
<td>Acrylic size</td>
<td>( k_0 ) (mg l⁻¹ hr⁻¹)</td>
<td>0.325</td>
<td>0.289</td>
<td>0.407</td>
</tr>
<tr>
<td></td>
<td>( R^2 )</td>
<td>0.963</td>
<td>0.967</td>
<td>0.993</td>
</tr>
</tbody>
</table>

### Table 2: Zero-, first- and second order rate constants from decolorization kinetics in anaerobic batch tests containing RR141 (40 mg l⁻¹) with different carbon sources (COD concentrations of 400, 800 and 1200 mg l⁻¹)

<table>
<thead>
<tr>
<th>Carbon sources</th>
<th>Constants</th>
<th>400</th>
<th>800</th>
<th>1200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midified starch</td>
<td>( k_0 ) (mg l⁻¹ hr⁻¹)</td>
<td>0.325</td>
<td>0.530</td>
<td>0.691</td>
</tr>
<tr>
<td></td>
<td>( R^2 )</td>
<td>0.868</td>
<td>0.831</td>
<td>0.933</td>
</tr>
<tr>
<td>Polyvinyl alcohol</td>
<td>( k_0 ) (mg l⁻¹ hr⁻¹)</td>
<td>0.249</td>
<td>0.241</td>
<td>0.291</td>
</tr>
<tr>
<td></td>
<td>( R^2 )</td>
<td>0.984</td>
<td>0.945</td>
<td>0.918</td>
</tr>
<tr>
<td>Acrylic size</td>
<td>( k_0 ) (mg l⁻¹ hr⁻¹)</td>
<td>0.261</td>
<td>0.270</td>
<td>0.275</td>
</tr>
<tr>
<td></td>
<td>( R^2 )</td>
<td>0.996</td>
<td>0.994</td>
<td>0.980</td>
</tr>
</tbody>
</table>
The first-order kinetics were present with respect to COD and dye concentrations for MS and PVA as carbon sources, while zero-order kinetics were shown with respect to COD concentrations for AS as the carbon source. These results are in agreement with first-order reactions for the degradation of dye with respect to different carbon sources, dye concentrations and COD concentrations reported by Carriell et al. (1995), Van der Zee et al. (2001), Isik and Sponza (2004), whereas others found zero-order kinetics (Brown, 1981). Meanwhile, Dos Santos et al. (2004) found zero-order and first-order kinetics based on the dye concentration depletion. A zero-order reaction, in which the rate is not proportional to the concentration of reacting substance, was found for the AS carbon source. A first-order reaction, in which the rate is proportional to the concentration of only one reacting substance, was obtained for the MS and PVA carbon sources.

Recalcitrant carbon removal: The removal rates of COD and color by anaerobic biodegradation with MS, PVA and AS at the COD concentration of 800 mg/l and color concentration of 20 mg/l are shown in Fig. 3. The COD removal rates at steady state for MS, PVA and AS were 71, 18 and 48%, respectively. Sacks and Buckley (1999) also reported an 85% reduction in COD by primary anaerobic digester with starch component. Use of PVA as carbon source shows poor degradation of COD. This is because the PVA molecule contains acetate and acylmonitrite groups, which prevent decay, and thus, it is reasonable to expect PVA to be resistant to biodegradation (Sacks and Buckley, 1999). Shaw et al. (2002) achieved an average COD removal of 8.9% degradation of PVA in an anaerobic stage of SBR, whereas there was 20-30% COD removal using a separate vessel. The AS carbon source was inert in inhibiting the anaerobic degradation of process, but it was better degraded than PVA. Therefore, it was concluded that the structure of the carbon source affects biodegradation.

Minke and Rott (1999) found good anaerobic biodegradability of MS, meanwhile PVA and AS (polyacrylate) were inert to the anaerobic biodegradation process. This may be attributed to the high acidogenic activity of acetogenic bacteria due to the degradation of the MS carbon source, while the degradation of the PVA and AS carbon sources was slow because of low acidogenic activity of acetogenic bacteria due to the inhibition of the dye concentrations.

Minke and Rott (1999) found good anaerobic biodegradability of MS, meanwhile PVA and AS (polyacrylate) were inert to the anaerobic biodegradation process. This may be attributed to the high acidogenic activity of acetogenic bacteria due to the degradation of the MS carbon source, while the degradation of the PVA and AS carbon sources was slow because of low acidogenic activity of acetogenic bacteria due to the inhibition of the dye concentrations.

Textile effluents also have a high organic load in the scouring and desizing processes. The discharge of desizing process has sizing agents, such as MS, PVA and AS. This study also found that MS has good anaerobic biodegradability. Meanwhile, the AS carbon source was largely inert to the anaerobic degradation process, but it was degraded more than PVA. At present, textile factories can recover PVA, thus it should decrease COD loading.

The pH values of the MS carbon source were slightly increased in the initial period (12 hr), after that, the pH dropped from 7.25 to 6.62 at 24 hr and then increased up to 7 (steady state) by the end of the incubation period. Meanwhile, the pH values for PVA as the carbon source increased in the initial period (12 hr), and after that, it was steady until the end of the 32 hr incubation period. The pH value of AS as carbon source showed a slight increase in the initial period (pH 7.62), then it dropped from 7.62 to 7.3 at 12-108 hr and achieved a steady pH value of 7.3 at the end of the incubation period. According to the above results, the MS carbon source had the highest acidogenic activity of acetogenic bacteria and degradation. It is inferred that as consumption of MS concentration increased, the rate of accumulation of organic acids in the medium was also increased (data not shown). This may be attributed to lower acidogenic activity of acetogenic bacteria due to the degradation of PVA and AS carbon sources. This demonstrates the importance of pH control for decolorization when various biodegradable carbon sources are present in dye wastewater.

The results of this study show that the anaerobic degradation kinetics of RR 141 with partially granulated anaerobic mixed culture can be concluded as follows:

- **First-order kinetics reactions are involved in decolorization for MS and PVA carbon sources, while a zero-order kinetics reaction was found for decolorization with the AS carbon source.**
- **The k values and color removal rates with MS as the carbon source were higher than those of the PVA and AS carbon sources.**
- **The decolorization rates increased with decreases in dye concentrations of RR 141.**
- **The decolorization rates increased with increases in COD concentrations for all three carbon sources.**

![Table - 3: First-order kinetics constants value and color removal in anaerobic batch tests containing dyes](image-url)
Acknowledgments

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References