Colour Removal and the Effect of Reactive Dyes on Growth Substrate Utilization by an Unacclimated Ethanol-Enriched Anaerobic Culture

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The cultures from an ethanol-enriched anaerobic master culture reactor were dosed with 0, 50, 100, 200, 400, 800, and 1600 mg/L of a mixture of three Procion reactive dyes: Red MX-8B, Red MX-5B, and Orange MX-2R, in order to study the colour removal and the inhibitory effect of dyes on the methanogenic degradation of ethanol that was added as a co-substrate. The reduction in colour intensity, measured at 514 nm, was found to be similar to a trial using an inactivated culture. These results indicate that removal of colour by the unacclimated anaerobic population is due to adsorption of the dyes on the biomass. The dyes caused a competitive inhibition at doses above 200 mg/L on utilization of ethanol by the anaerobic bacteria.

Key words: adsorption, anaerobic, colour, inhibition, isotherm, kinetics

Introduction

Biological treatment of coloured wastewaters, like those originating from textile mills, has become popular over the last few decades (Bahorsky 1998; van der Zee and Villaverde 2003; Kaushik and Malik 2009). Unlike physico-chemical treatment of dyestuffs, which involves costly equipment and chemicals that can create secondary problems regarding disposal, biological treatment offers a cost-effective and environment friendly alternative (Azmi et al. 1998; Willetts et al. 2000; van der Zee and Villaverde 2005; Lee et al. 2006). Biological treatment of any wastewater can be aerobic or anaerobic, and depending on the characteristics of wastewater, one is favoured over the other. Conventional aerobic biological treatment is not very effective in the treatment of textile dyes because dyes are designed to be bio-resistant. Therefore, this method is generally unsuccessful in significantly removing dyestuff beyond the adsorbing capacity of the biomass (Bahorsky 1998). Anaerobic biological treatment of dyestuff is promising because the actual breakdown of chromophores (e.g. –N=N–) through reductive cleavage takes place under a reducing environment in the presence of a co-substrate which acts as an electron donor (Seshadri et al. 1994; Beydilli et al. 2000; Rai et al. 2005). However, complete mineralization of dyes under anaerobic environment may not be possible in every case. After the initial breakdown of the bonds, small-chain intermediate compounds may not be further degraded under anaerobic environment but can be mineralized completely by an aerobic biological polishing treatment. Hence, a sequencing anaerobic/aerobic treatment of dyestuffs is an area of research interest (Basibuyuk and Forster 1997; Luangdilok and Panswad 2000; O’Neill et al. 2000; Tan et al. 2000; Cruz and Buitrón 2001; Sponza and Işık 2002; Rai et al. 2005; Lourenço et al. 2006). While studies are being conducted for full-scale application of biological treatment of dyestuffs, researchers are also interested in kinetics and microbial aspects of dye degradation using batch experiments. So far, studies have focused on the kinetics of decolourization (van der Zee et al. 2001; Işık and Sponza 2004a, 2004b; Sponza and Işık 2004; Bafana et al. 2007; Sarioglu et al. 2007; Sarioglu and Bisgin 2007), the kinetics of co-substrate utilization (Işık and Sponza 2004a, 2005a; Işık and Sponza 2005b; Sarioglu et al. 2007; Sarioglu and Bisgin 2007), the decolourization and toxicity of dyes with acclimated and unacclimated anaerobic cultures (Beydilli and Pavlostathis 2005), the biotic and abiotic components of decolourization (Beydilli et al. 2000; Willetts and Ashbolt 2000; van der Zee et al. 2003; Wang et al. 2006), and the effect of oxidation states on decolourization of dyes (Lee et al. 2006).

Kinetic studies involving dyes have been done in the past using growth substrates like glucose (Gonçalves et al. 2001; Işık and Sponza 2004a, 2004b; Sponza and Işık 2004; Sarioglu and Bisgin 2007), starch (Işık and Sponza 2005b) and other complex substances like peptone/dextrin (Beydilli and Pavlostathis 2005). The degradation of the growth substrates, in most cases, involved all the important steps of anaerobic digestion – hydrolysis, acidogenesis, acetogenesis, and methanogenesis (van Haandel and Lettinga 1994) – brought about by different types of bacteria exhibiting complex symbiotic relationships. Among the different groups of bacteria that

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are present in any anaerobic biomass, those involved in methane formation are reported to be the most sensitive to inhibition (Yang and Speece 1985). However, in some cases, acetate forming bacteria or acetogens can be more sensitive (McCarty and Vath 1962). This research was designed to study the color removal and the inhibitory effect of dyes on growth substrate utilization by the most sensitive classes of anaerobic bacteria, the acetogens and the methanogens, using a batch experimental setup. An ethanol-enriched anaerobic culture was used to accomplish this goal. In an anaerobic microbial environment ethanol decomposes into methane and carbon dioxide via formation of acetic acid. Therefore, the anaerobic population in the culture consists primarily of the acetogens and the methanogens.

In this study, an ethanol-enriched microbial culture in batch reactors was dosed with a mixture of three Procion reactive dyes, Red MX-5B, Red MX-5B, and Orange MX-2R. These are the representatives of the class of reactive dyes, commonly used by textile industries. The color removal was studied using Freundlich’s adsorption isotherm (Allen et al. 1989; Metcalf and Eddy, Inc. 2003; Chan et al. 2008). The mathematical form of Freundlich’s isotherm is given by the following equation:

\[
\frac{x}{m} = K_f C_e^{\frac{1}{n}}
\]

The kinetics of growth substrate utilization was studied using Michaelis-Menten and Monod models. The inhibitory effect of the dyes on the microbial substrate utilization was related to the maximum specific substrate utilization rate and the half-velocity constant of the models (Han and Levenspiel 1988; Mulchandani and Luong 1989; Grady 1990; Kim et al. 1994; Bhattacharyya 2009) as given below:

\[
\frac{dS}{dt} = -\frac{kXS}{K_s + S}
\]

\[
X = X_o + Y(S_o - S)
\]

\[
k = k_o[k^*]
\]

\[
K_s = K_s[K^*_o]
\]

Competitive Inhibition: \[ k^* = 1.0 \quad K_s^* > 1.0 \]

Non-competitive Inhibition: \[ k^* < 1.0 \quad K_s^* = 1.0 \]

Uncompetitive Inhibition: \[ k^* < 1.0 \quad K_s^* < 1.0 \]

Mixed Inhibition: \[ k^* < 1.0 \quad K_s^* > 1.0 \]

The inhibitory effect of the dyes on single substrate (ethanol) utilization was also studied using the following equations (Işık and Sponza 2005a):

\[
\frac{dS}{dt} = -\frac{kXS}{K_s(1 + \frac{I_D}{K_{ID}}) + S}
\]

\[
\frac{dS}{dt} = -\frac{kXS}{(1 + \frac{K_s}{S})(1 + \frac{I_D}{K_{ID}})}
\]

\[
\frac{dS}{dt} = -\frac{kXS}{(\frac{K_s}{I_D / K_{ID}} + S)(1 + \frac{I_D}{K_{ID}})}
\]

**Batch Experiments and Experimental Procedures**

**Master Culture Reactor:** The seed culture used in the batch study was obtained from a 4-L capacity master culture reactor (MCR). The MCR, in turn, was inoculated with a sludge obtained from a full-scale anaerobic reactor (McCain Foods Limited, Grand Falls, N.B., Canada). Ethanol was used as a sole substrate in the MCR and the culture was not acclimated to any toxic or inhibitory compound. The nutrient, mineral (macro and micro elements), and buffer bases were added during the feed preparation following Young and Cowan (2004). Stock solutions of the above bases were made separately. Fresh feed solution (COD = 18,000 mg/L) was prepared every day by adding calculated amounts of 95% ethanol and the above stock solutions in distilled water. The reactor was operated on a draw-and-fill mode. Every day, 5% of the culture was removed from the reactor and replaced with the same volume of fresh feed solution. Thus, the reactor was operated at hydraulic and solids retention time of 20 days and a COD loading rate of 0.90 g/L-d.

Mixing was provided with a magnetic stirring device. The unit was maintained at a temperature of 35° C. The MCR was operated for a period of 60 days (3 SRTs) during which only biogas production was monitored. It was followed by a period of another 20 days (1 SRT) during which biogas production, methane composition, pH, and mixed liquor volatile suspended solids (MLVSS) were monitored. The average biogas production and methane content in the biogas, prior to the beginning of the batch experiment, were found to be 1865±66 mL and 80.73±0.35 percent, respectively. The MCR had a pH 7.1±0.2, and the MLVSS was 958±36 mg/L.

**Batch Test Reactors:** The tests were initiated by anaerobically transferring 100 mL cultures from the master culture reactor into 125 mL glass bottles which served as the test reactors. The test reactors were operated similar to the MCR for 3-4 days to allow the cultures to stabilize in the new setup. Daily methane production was measured using a Challenge AER-216 respirometer (Challenge Environmental Systems, Fayetteville, Ark,
USA). Test bottles producing expected amount of methane were selected for further study.

**Analytical procedures**

A mixture of equal proportion (by weight) of three commercial Procion reactive dyes, Red MX-8B, Red MX-5B, and Orange MX-2R, was used in this study. The test reactors were fed similar to the MCR and dosed with 0, 50, 100, 200, 400, 800, and 1600 mg/L of dye mixture. Mixed culture samples (7 mL) were removed from the test reactors at preset time intervals, inactivated with 2 drops of 16 g/L of mercuric chloride solution, and centrifuged at 3500 rpm for 10 minutes using a centrifuge (Allegra X-15, Beckman Coulter Inc., Fullerton, Calif., USA). Supernatant was then filtered through 0.45 μm membrane filter papers and analyzed for total organic carbon using a TOC analyzer (TOC-VCPH, Shimadzu Corporation, Kyoto, Japan) and for colour at 514 nm wavelength using a UV/Vis scanning spectrophotometer (Genesys 6, Thermo Electron Corporation, Waltham, Mass., USA). Total and volatile suspended solids were tested following methods 2540 D and E, described in Standard Methods (APHA et al. 1998).

**Adsorption study**

To find the colour removal by adsorption on to the biomass, a second set of experiments was run simultaneously using an inactive biomass. Here, the culture in the test reactors were first inactivated using 1 mL of mercuric chloride solution, and then gas production was monitored using a respirometer. No gas production was noticed in the test bottles indicating complete inactivation. The experiment was then conducted as described above.

**Results and Discussion**

**Decolourisation**

The test bottles were dosed with preset dye doses and the first set of samples were immediately drawn and analyzed so as to ascertain the adsorption of dyes by the biomass at time t=0. Figure 1(a-f) shows the colour removal by active and inactivated biomass at different doses of dyes. Concentrations of dye in the test bottles containing active biomass was found to be lower than those in the test bottles containing inactivated biomass at time t=0. A visible difference in the dye concentration between the active and inactivated cultures at all doses of dyes indicates that the inactivated biomass had likely undergone a change in its surface morphology during the process of inactivation with mercuric chloride. It is highly unlikely that the live and unacclimated culture decolourized a significant portion of dye instantaneously after doses are applied; therefore, that difference should not be attributed to the biotic component of decolourization. However, the trend of decolourisation for the rest of the experiment was found to be the same for both the active and the inactivated culture. The difference in concentration of dye in the solution between the inactive and the active cultures for every preset time interval and dose has been plotted in Fig. 1. The best-fit straight lines through these plots were found to be almost parallel to the time axis for all the dye doses, which indicates that the colour removal in the active culture did not increase significantly beyond the adsorbing capacity of the biomass. Hence, colour removal with the unacclimated culture in the present study was primarily abiotic.

The removal of colour in the present study has been expressed by adsorption isotherms. Figure 2 shows the Freundlich isotherms of adsorption by the active and the inactivated biomass. Freundlich isotherm, which was derived empirically, gave a good fit of the data. Table 1 shows the Freundlich capacity factor \((K_f)\) and intensity parameter \((n)\) for the active and the inactivated biomass. Literature suggests that for a favourable adsorption the value of \(n\) should fall within a range of 1 to 10 (Gulnaz et al. 2004; Vasanth Kumar et al. 2005; Mokhtar et al. 2006). The values of \(n\) for the active and the inactive biomass were found to be 1.26 and 0.98 respectively, which indicates that adsorption is not favourable for the inactive biomass.

Table 1 also compares the Freundlich capacity and intensity factors, obtained from the present study, with the literature values for a few bio-adsorbents treating reactive dyes. Freundlich parameters of the active anaerobic biomass used in the present study are comparable to some of the bio-adsorbents previously used. The active biomass showed favourable adsorption. Therefore, a significant amount of colour is likely to be removed by adsorption from a wastewater in a treatment plant during sludge wasting. Langmuir isotherm, which was derived from rational consideration, was found to be inappropriate for the present study, and hence, not shown.

**Kinetics of co-substrate (ethanol) utilization**

Figure 3 shows the plot of residual ethanol, expressed in mg/L as total organic carbon (TOC), against time. This plot was obtained by subtracting the TOC fraction of the residual dyes from the residual total soluble organic carbon in the samples. To find the organic carbon in the dyes, a TOC analysis was done on known standards of dye solution, and a relationship between the concentration of dye and the corresponding organic carbon was derived (Fig. 4). This relationship is given in the following equation:

\[
\text{TOC(mg/L)} = 0.22 \times \text{Dye(mg/L)}
\]  

(13)

The residual dye in the samples were converted into its TOC equivalent using equation 13 and subtracted from the TOC of the sample to obtain TOC fraction of the residual ethanol in the samples.

The decrease in TOC with time (Fig. 3) was attributed
Fig. 1. Decrease of residual dye in test bottles with time at different dye doses: (a) 50 mg/L, (b) 100 mg/L, (c) 200 mg/L, (d) 400 mg/L, (e) 800 mg/L, and (f) 1600 mg/L.

Fig. 2. Plot of Freundlich adsorption isotherm.

Fig. 3. Plot of residual ethanol in test bottle with time.

Fig. 4. Relationship between total organic carbon and dye
**TABLE 1. Freundlich’s constants for some bio-adsorbents treating reactive dyes from wastewaters**

<table>
<thead>
<tr>
<th>Adsorbate</th>
<th>Adsorbent</th>
<th>$K_f$</th>
<th>$n$</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Phanerochaete chrysosporium</em> (fungal biomass)</td>
<td>Reactive blue 4</td>
<td>1.12</td>
<td>1.31</td>
<td>Bayramoglu et al. (2006)</td>
</tr>
<tr>
<td><em>Posidonia oceanica</em> (marine biomass)</td>
<td>Cibacron red FNR</td>
<td>0.47</td>
<td>1.27</td>
<td>Ncibi et al. (2006)</td>
</tr>
<tr>
<td>Anaerobic sludge</td>
<td>Rhodamine B</td>
<td>1.95</td>
<td>1.43</td>
<td>Wang et al. (2006)</td>
</tr>
<tr>
<td><em>Lentimus sajor-caju</em> (fungal biomass)</td>
<td>Reactive red 120</td>
<td>0.39</td>
<td>1.12</td>
<td>Arica and Bayramoglu (2007)</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>Reactive blue 19</td>
<td>42.10</td>
<td>5.99</td>
<td>Çiçek et al. (2007)</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>Reactive red 195</td>
<td>31.40</td>
<td>4.24</td>
<td>Çiçek et al. (2007)</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>Reactive yellow 145</td>
<td>42.00</td>
<td>4.20</td>
<td>Çiçek et al. (2007)</td>
</tr>
<tr>
<td><em>Rhizopus nigricans</em> (fungal biomass)</td>
<td>Reactive black 8</td>
<td>0.48</td>
<td>1.23</td>
<td>Kumari and Abraham (2007)</td>
</tr>
<tr>
<td><em>Rhizopus nigricans</em> (fungal biomass)</td>
<td>Reactive brown 9</td>
<td>0.52</td>
<td>0.84</td>
<td>Kumari and Abraham (2007)</td>
</tr>
<tr>
<td><em>Rhizopus nigricans</em> (fungal biomass)</td>
<td>Reactive green 19</td>
<td>0.56</td>
<td>1.10</td>
<td>Kumari and Abraham (2007)</td>
</tr>
<tr>
<td><em>Rhizopus nigricans</em> (fungal biomass)</td>
<td>Reactive blue 38</td>
<td>0.79</td>
<td>1.12</td>
<td>Kumari and Abraham (2007)</td>
</tr>
<tr>
<td><em>Rhizopus nigricans</em> (fungal biomass)</td>
<td>Reactive blue 3</td>
<td>0.66</td>
<td>1.00</td>
<td>Kumari and Abraham (2007)</td>
</tr>
<tr>
<td><em>Corynebacterium glutamicum</em> (fermentation)</td>
<td>Reactive black 5</td>
<td>142</td>
<td>7.14</td>
<td>Vijayaraghavan and Yun (2007)</td>
</tr>
<tr>
<td>Chitosan</td>
<td>Remazol black 13</td>
<td>1.99</td>
<td>2.27</td>
<td>Annadurai et al. (2008)</td>
</tr>
<tr>
<td><em>Penicillium restrictum</em> (fungal biomass)</td>
<td>Reactive orange 122</td>
<td>22.7</td>
<td>2.80</td>
<td>Ilhan et al. (2008)</td>
</tr>
<tr>
<td>Brazilian pine fruit shell</td>
<td>Reactive red 194</td>
<td>3.41</td>
<td>3.03</td>
<td>Lima et al. (2008)</td>
</tr>
<tr>
<td>Active anaerobic culture</td>
<td>Procion reactive dyes mix - Red MX-8B, Red MX-5B, and Orange</td>
<td>1.76</td>
<td>1.26</td>
<td>Present study</td>
</tr>
<tr>
<td>Inactivated anaerobic culture</td>
<td>Procion reactive dyes mix - Red MX-8B, Red MX-5B, and Orange</td>
<td>0.26</td>
<td>0.98</td>
<td>Present study</td>
</tr>
</tbody>
</table>
solely to the decrease in ethanol since ethanol was the only source of carbon and energy for microbial growth and maintenance, and electrons, needed for the cleavage of azo bonds of dyes under reducing environment. Dyes, which are co-metabolized in anaerobic processes are considered non-growth substrates and cannot be utilized by anaerobic microorganisms as carbon and energy sources (Işık and Sponza 2004a; Işık and Sponza 2005a).

As shown in Fig. 3, the substrate-removal plots essentially overlap each other up to a dye-dosage of 200 mg/L, thereby indicating no or little inhibition. However, at higher doses, a noticeable drop in the rates of substrate-utilization was observed. The substrate utilization ceased after first 10 hours from the beginning for dye-doses for 0 mg/L, 50 mg/L, 100 mg/L, and 200 mg/L, and 12 hours and 14 hours for 400 mg/L and 800 mg/L of dye doses, respectively. For the 1600 mg/L dose, an increase in soluble organic carbon was noticed after 12 hours. This increase is possibly due to a release of colourless dye intermediates from the surface of the biomass into the liquid phase that increased the organic carbon concentration in the solution but was not detected by the spectrophotometer at 514 nm wavelength.

The ethanol-enriched master culture reactor produced an average biomass concentration of 958 mg VSS/L, which, at an organic loading rate of 0.90 g COD/L-d, a solids retention time of 20 days, and a decay coefficient of 0.024 d⁻¹ (Kim et al. 1994), gave a yield coefficient of 0.076 mg VSS/mg COD removed or 0.324 mg VSS/mg TOC removed. This value of the yield coefficient is obtained from a relationship proposed by Young (1981) and is given below:

$$X = YL_0 \frac{1 + 0.2k_d \theta_c}{1 + 1.2k_d \theta_c}$$  \hspace{1cm} (14)

The maximum specific rate of substrate utilization ($k$) and half-velocity coefficient ($K_s$) were estimated from the data using a spreadsheet method (Smith et al. 1998). Table 2 and Fig. 3 present the values of $k$ and $K_s$ at different doses of dyes, respectively. No noticeable trend of change in $k$ with dye-dosage was noticed within the tested range of dye doses, which implies that the inhibition term $k^*$ can be assumed to be equal to 1. However, $K_s$ increased at 200 mg/L and higher doses, which implies that the inhibition term $K_s^*$ is greater than 1 at dye doses of 200 mg/L and higher. The results of the inhibition terms $k^*$ and $K_s^*$ imply that the reactive dyes caused a competitive inhibition on ethanol utilization by the anaerobic biomass, which suggests that the dyes interfered with the binding of ethanol with enzymes, thereby decreasing the rate of ethanol utilization. This interference could have occurred either by the dyes competing with ethanol for the active sites on enzymes, or attaching to a secondary site causing a conformational change in the structure of the enzyme such that the growth substrate cannot bind. In competitive inhibition, the maximum speed of the reaction is unchanged, while the apparent affinity of the substrate to the binding site is decreased. Any given competitive inhibitor concentration can be overcome by increasing the substrate concentration in which case the substrate will displace or out-compete the inhibitor in binding to the enzyme (Rittmann and McCarty 2001; Marangoni 2003). Competitive inhibition of azo dyes on substrate utilization has also been reported in other studies (Hu 2001; Işık and Sponza 2004a; Işık and Sponza 2005a).

The inhibition coefficient $K_{ID}$, as shown in Table 2, was calculated by directly equating $K_s^*$ to the factor $(1+I/D/K_{ID})$ in equation 10 and substituting the values $K_s^*$ and $I_D$. Low $K_{ID}$ values in samples containing higher dye concentrations indicate that accumulation of the dye resulted in a significant inhibition to substrate utilization. $K_{ID}$ gives an idea of the sensitivity of the substrate utilization rate to a change in dosage of dyes. Low $K_{ID}$ increases the value of the factor $(1+I/D/K_{ID})$ causing a decrease in the affinity of the substrate to the binding sites of the enzyme.

![Fig. 5. Half-velocity coefficient at different dye doses](image)

<table>
<thead>
<tr>
<th>Constants</th>
<th>0 mg/L</th>
<th>50 mg/L</th>
<th>100 mg/L</th>
<th>200 mg/L</th>
<th>400 mg/L</th>
<th>800 mg/L</th>
<th>1600 mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k$</td>
<td>0.59</td>
<td>0.69</td>
<td>0.59</td>
<td>0.65</td>
<td>0.67</td>
<td>0.49</td>
<td>0.57</td>
</tr>
<tr>
<td>$K_{ID}$</td>
<td>---</td>
<td>No inhibition</td>
<td>242</td>
<td>93</td>
<td>249</td>
<td>127</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 2. Maximum specific substrate utilization rate ($k$) and constant of inhibition at different doses of dyes
Conclusions

The present study concludes that the removal of colour did not increase significantly beyond the adsorbing capacity of the biomass. Hence, colour removal with the unacclimated culture was primarily abiotic. A Freundlich adsorption isotherm produced a good fit to the data.

The dyes caused a competitive inhibition above a dose of 200 mg/L on ethanol utilization that acted as the sole growth substrate. The low KID values indicated that the affinity of the substrate to the binding sites of the enzyme was reduced at doses of dyes above 200 mg/L.

List of symbols

- \( C_s \): Equilibrium solute concentration (mg/L)
- \( \text{COD} \): Chemical oxygen demand (mg/L)
- \( I_d \): Dose of inhibitor (mg/L)
- \( K_f \): Freundlich capacity factor (mg adsorbate/g adsorbent)/(L water/mg adsorbate)\(^{1/n}\)
- \( K_{d} \): Half-velocity coefficient (mg/L)
- \( K_{w} \): Half-velocity coefficient without inhibitor (mg/L)
- \( k^* \): Inhibition term
- \( k_{id} \): Inhibition term (mg/L)
- \( k \): Maximum specific rate of substrate utilization (mg/mg-d)
- \( k_{a} \): Decay coefficient (d\(^{-1}\))
- \( k_{sa} \): Maximum specific rate of substrate utilization without inhibitor (mg/mg-d)
- \( L \): Organic loading rate (mg COD/L-d)
- \( \text{MCR} \): Master culture reactor
- \( n \): Freundlich intensity parameter
- \( S \): Substrate concentration (mg/L)
- \( S_0 \): Initial substrate concentration (mg/L)
- \( \text{SRT} \): Solids retention time (d)
- \( t \): Time (d)
- \( \text{TOC} \): Total organic carbon (mg/L)
- \( \text{VSS} \): Volatile suspended solids (mg/L)
- \( X \): Concentration of active cells (mg VSS/L)
- \( X_0 \): Initial concentration of active cells (mg VSS/L)
- \( x/m \): Mass of adsorbate adsorbed per unit mass of adsorbent (mg/g)
- \( Y \): Yield coefficient (mass of active cells produced per unit mass of substrate converted)
- \( \theta_c \): Solids retention time (d)

References


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