Modeling of the Acid Orange 7 anaerobic biodegradation

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Abstract It was found that 1-amino-2-naphthol, an intermediate generated during the anaerobic degradation of Acid Orange 7, is a redox mediator which plays a significant role in the transport of electrons to the dye, thus giving to the whole process an autocatalytic nature. Evidences of the autocatalytic behaviour were observed in experimental data previously obtained under batch and fed-batch conditions. In this paper, a kinetic model considering all these factors is proposed and validated. In batch assays, this model agrees satisfactorily with the experimental data. In the case of fed-batch assays, the autocatalytic model only can be applied satisfactorily after the first feeding, since the degradation of Acid Orange 7 after the second and third feedings followed a first-order kinetic. This fact can be explained due to the presence of the redox mediator previously generated during the reactions that took place after the first feeding.

Keywords Acid Orange 7; anaerobic reduction; autocatalysis; azo dye; redox mediator

Introduction

Among the different colorants used in the textile industry, azo dyes constitute nowadays the most common compounds used for this purpose, being subsequently present in significant amounts in the liquid effluents generated. These compounds have an azo bond (N=N), which is responsible for the colour. The anaerobic treatment of these compounds causes, in most cases, the azo bond to break down, producing its decolorisation and the generation of aromatic amines.

It has been postulated that anaerobic reduction of azo dyes is a chemical reaction between reduced enzymatic cofactors (reduced flavins) and the dye (Gingell and Walker, 1971). The presence of external carbon sources is favourable for the rate of the process (Carliell et al., 1995), because the oxidation of these compounds yields electrons used for the formation of reduced cofactors (FAD, FMN, NADH). In this sense, glucose has been reported to be a good cosubstrate to get a high removal rate of azo dyes (Wuhrmann et al., 1980; Haug et al., 1991; Carliell et al., 1995). If an additional carbon source is not present, the endogenous lysis of the sludge would yield the reducing equivalents (Van der Zee et al., 2001a).

Furthermore, the external addition of cofactors such as FAD or FMN (Chung et al., 1978; Haug et al., 1991), or artificial redox mediators, such as benzyl viologen (Chung et al., 1978; Brown, 1981; Kudlich et al., 1997; Bragger et al., 1997) or quinones (Kudlich et al., 1997; Van der Zee et al., 2000 and 2001a) causes a high increase in the reduction rate of azo dyes.

Biological azo dye reduction kinetics depends on the concentration of dye and reducing equivalents. In the case of the presence of an external carbon source which provides a high excess of reducing equivalents, it is expected that the kinetics will depend only on the dye concentration (Yoo, 2000). In this sense, several researchers found that the anaerobic degradation of azo dyes follows a first-order kinetic model (Wuhrmann et al., 1980; Mecshner and Wuhrmann, 1982; Carliell et al., 1995; Willetts and Ashbolt, 2000; Van der Zee et al., 2001b), although zero-order kinetics were also found (Brown, 1981; Dubin and Wright, 1975). This different kinetic behaviour was explained in terms of the different...
experimental conditions, such as the use of pure cultures or anaerobic sludge environments (Van der Zee et al., 2001b).

In previous works different batch and fed-batch experiments were carried out to study the anaerobic biological degradation of AO7 (Méndez-Paz et al., 2003a and 2003b). The experimental data obtained indicate a different evolution of the AO7 removal rates, some of them with maximum values at time zero and in other cases after an initial period. The latter evidence points to a certain autocatalytic behaviour, as reported by Van der Zee et al. (2000) in abiotic assays.

The aim of this work is focused on the determination of the kinetics of the reduction of the azo dye Acid Orange 7 (AO7) based on those experimental data.

Materials and methods
Experimental assays
The experimental assays were carried out under batch and fed-batch conditions. Both experimental settings can give complementary information for a better understanding of the process. The main difference between both assays, apart from the initial concentration of the dye, is that under fed-batch conditions it is possible to determine the behaviour of the system when there are intermediates present. Besides, fed-batch operation allows us to avoid the drop in the removal rates produced when the substrate concentration is low, a typical drawback of batch assays.

The preparation of the assays and the analytical methods used to follow the operation has been previously reported (Méndez-Paz et al., 2003a and 2003b). Non-adapted granular biomass was used in both cases. All assays were carried out in 500 ml serum bottles. The initial AO7 concentrations used were between 0.3 and 0.9 mmol l\(^{-1}\) (100–300 mg·l\(^{-1}\)). Glucose was added as external electron donor.

The experimental design of batch and fed-batch assays is shown in Table 1.

Mathematical models
First order and a autocatalytic kinetic model A will be used.

Batch assays. From the first-order differential Eq. (1), Eq. (2) is obtained after integration, \(C_0\) being the AO7 concentration at the beginning of the assay (\(t = 0\)).

\[
\frac{dC}{dt} = k_1C
\]

\[
C = C_0 e^{-kt}
\]

Eq. (3) corresponds to the autocatalytic model proposed, which has a first-order kinetic term \((k_1)\) corrected with an autocatalytic term \((k_2)\). With the factor \(a\) equal to 1, this

| Table 1 Experimental design of batch (A–D) and fed-batch (E) assays |
|----------------|----------------|----------------|
| Assay | Feeding | AO7 (mmol·l\(^{-1}\)) | Glucose (g·l\(^{-1}\)) |
| A | 1st | 0.31 | 1.8 |
| B | 1st | 0.56 | 1.8 |
| C | 1st | 0.78 | 2.5 |
| D | 1st | 0.90 | 2.6 |
| E | 1st | 0.31 | 2.4 |
| | 2nd | 0.34 | _ |
| | 3rd | 0.32 | _ |
equation was previously used by Van der Zee et al. (2001) to study the autocatalytic role in the chemical reduction of AO7.

\[ r = -\frac{dC}{dt} = k_1 C + k_2 C(C_0 - C)^a \]  

(3)

Using \( a = 1 \), Eq. (4) is obtained after integration.

\[ C = C_0 \frac{(k_1 + k_2 C_0) e^{-(k_1 + k_2 C_0)r}}{k_1 + k_2 C_0 e^{-(k_1 + k_2 C_0)r}} \]  

(4)

Thus, the maximum removal rate can be calculated by derivation of Eq. (3) (for \( a = 1 \)) and equalled to zero, which is shown in Eq. (5). The concentration of AO7 corresponding to these conditions (\( C_{max} \)) is given by the Eq. (6). Finally, the expression for the maximum removal rate is shown by the Eq. (7).

\[ \frac{dr}{dC} = \frac{d}{dC} \left[ k_1 C + k_2 C(C_0 - C) \right] = 0 \]  

(5)

\[ C_{max} = \frac{k_1 + k_2 C_0}{2k_2} \]  

(6)

\[ r_{max} = k_1 \frac{k_1 + k_2 C_0}{2k_2} + k_1 \frac{k_1 + k_2 C_0}{2} \left( C_0 - k_1 + k_2 C_0 \right) \]  

(7)

**Fed-batch assays.** In the case of fed-batch assays the equation corresponding to an autocatalytic model must be generalized for all feedings. Considering the volume changes due to the different azo dye additions negligible, the model is represented by the Eq. (8).

\[ -\frac{dC}{dt} = k_1 C + k_2 C \left( \sum_{i=1}^{n} C_{0i} - C \right)^a \]  

(8)

\( n \) being the number of feedings added and \( C_{0i} \) the initial AO7 concentration corresponding to the feeding \( i \). After the integration of Eq. (8) for \( a = 1 \), Eq. (9) is obtained.

\[ C = C_0 n \frac{\left[ k_1 + k_2 \sum_{i=1}^{n} C_{0i} \right] e^{-(k_1 + k_2 \sum_{i=1}^{n} C_{0i})t}}{k_1 + \sum_{i=1}^{n-1} k_2 C_{0i} + k_2 C_{0n} e^{-(k_1 + k_2 \sum_{i=1}^{n} C_{0i})t}} \]  

(9)

The concentration of AO7 in which the removal rate is maximum is obtained by Eq. (10).

\[ C_{max} = \frac{k_1 + k_2 \sum_{i=1}^{n} C_{0i}}{2k_2} \]  

(10)

*Table Curve* for Windows (version 1.0) was used to adjust the experimental data to these equations.

**Results and discussion**

**Batch assays**

When assays with different AO7 concentrations were carried out, it was observed that the removal rates had a maximum value between the first and second day of operation (Méndez-Paz et al., 2003a).
Due to the high concentration of glucose, it can be assumed that there should be an excess of reducing equivalents in the medium, since the oxidation of each mole of glucose to pyruvate yields two moles of NADH, so the kinetics would depend only on the AO7 concentration. This observation was previously reported by Yoo (2000). From the trend of the experimental data it can be observed that there is an initial period (the first day of operation) in which the removal rates are lower than afterwards. However, for the following days the first-order kinetic model can be applied for all points satisfactorily (Figure 1), with similar values obtained for the kinetic constant (0.9–1.1 d⁻¹), as shown in Table 2.

The other possibility is to explain the different removal velocities between the first days on the basis of an autocatalytic process. Thus, Eq. (4) was used to fit the experimental data, which was completely successful (Figure 2). With the exception of assay A, the values of the parameters obtained were very similar between the different assays (Table 3). The maximum removal rate calculated with Eq. (7) is higher when the initial dye concentration is

Figure 1 AO7 concentrations for assays A–D. Experimental data (■) and first-order model (—)

Table 2 First-order kinetic constant and correlation factor obtained for assays A–D (Eq. (2))

<table>
<thead>
<tr>
<th>Assay</th>
<th>$k$ (d⁻¹)</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.95</td>
<td>0.992</td>
</tr>
<tr>
<td>B</td>
<td>0.72</td>
<td>0.996</td>
</tr>
<tr>
<td>C</td>
<td>0.89</td>
<td>0.995</td>
</tr>
<tr>
<td>D</td>
<td>1.11</td>
<td>0.997</td>
</tr>
</tbody>
</table>

Table 3 Kinetic constants, correlation factor and maximum removal rates calculated with the autocatalytic kinetic model for the assays A–D (Eqs (4) and (7))

<table>
<thead>
<tr>
<th>Assay</th>
<th>$k_1$ (d⁻¹)</th>
<th>$k_2$ (l mmol⁻¹·d⁻¹)</th>
<th>$r^2$</th>
<th>$r_{max}$ (mmol·l⁻¹·d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.22</td>
<td>3.84</td>
<td>0.999</td>
<td>0.53</td>
</tr>
<tr>
<td>B</td>
<td>0.09</td>
<td>2.55</td>
<td>0.999</td>
<td>0.70</td>
</tr>
<tr>
<td>C</td>
<td>0.11</td>
<td>2.05</td>
<td>0.998</td>
<td>0.79</td>
</tr>
<tr>
<td>D</td>
<td>0.09</td>
<td>2.30</td>
<td>1.000</td>
<td>1.03</td>
</tr>
</tbody>
</table>
increased. Van der Zee et al. (2000) also observed an autocatalytic process during the study of the chemical reduction of AO7 with sulphide. They found that 1-amino-2-naphthol (1A2N), an aromatic amine generated after AO7 reduction, catalyses AO7 removal, acting as redox mediator.

**Fed-batch assays**

A fed-batch assay (E) with three AO7 feedings was carried out to study the AO7 removal rate under these conditions (Méndez-Paz et al., 2003b). As shown in Table 1, glucose was only added during the first feeding and the initial AO7 concentration ranged between 0.31 and 0.34 mmol l\(^{-1}\). The evolution of the medium after the first feeding was quite similar to the one observed in assay A, with the maximum AO7 removal rate after the first day of operation. On the other hand, after the second and third feedings, removal rates were continuously decreasing (Figure 3A).

The autocatalytic model represented by Eq. (9) was applied for the three trends obtained. In all cases this model reproduces the experimental data very well. However, for the second and third feedings the values obtained for \(C_{\text{max}}\) have no physical sense, since they are higher than each respective initial AO7 concentration, \(C_{0i}\), which invalidates this model under these conditions.

On the other hand, the first-order kinetic model was successfully used to reproduce the data corresponding to the second and third feedings (Figure 3A). Table 4 shows the parameters obtained for the two kinetic models (autocatalytic for the first feeding and first-order for the second and third ones) and the maximum removal rates. The evolution of the removal rates for the different feedings calculated by the models is shown in Figure 3B. It is clear that the behaviour in the first feeding is different with respect to the other feedings. Thus, the maximum removal rate during the second feeding was achieved at the beginning and was higher than the one achieved in the first feeding.

These differences between the first and the other feedings can be explained on the basis of the generation of the redox mediator, 1A2N. During the first feeding, its generation gives to the system its autocatalytic nature. However, in the following ones this compound was already present, which caused that AO7 removal rate will depend only on its concentration.

Since glucose was added only in the first feeding, the time needed to achieve complete
AO7 removal was longer in the other feedings. Considering that glucose is a better cosubstrate than volatile fatty acids for azo dye reduction (Donlon et al., 1997) and propionic acid is the predominant carbon source present during the second and third feedings, this evolution was predictable.

Conclusions

An autocatalytic model was successfully used to reproduce the experimental data obtained during the anaerobic degradation of AO7 in batch and fed-batch assays. The autocatalytic nature is related to the generation of 1A2N, an intermediate produced after the anaerobic breakdown of the dye, which acts as a redox mediator favouring the reduction of the dye. However, the data obtained after the second and third feedings of the fed-batch assays cannot be reproduced by this model, whereas a simple first-order kinetic model was successfully used. This indicates that the presence of 1A2N in the medium, already generated after the first feeding, means that AO7 removal will be only dependent on its concentration.

Acknowledgements

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References


