Decolorization and biogas production by an anaerobic consortium: effect of different azo dyes and quinoid redox mediators


ABSTRACT

The inhibitory effect of azo dyes and quinoid compounds on an anaerobic consortium was evaluated during a decolorization process and biogas production. In addition, the impact of quinoid compounds such as lawsone (LAW) and anthraquinone-2,6-disulfonate (AQDS) on the rate of decolorization of Direct Blue 71 (DB71) was assessed. The anaerobic consortium was not completely inhibited under all tested dye concentrations (0.1–2 mmol l\(^{-1}\)), evidenced by an active decolorization process and biogas production. The presence of quinoid compounds at different concentrations (4, 8, and 12 mmol l\(^{-1}\)) also inhibited biogas production compared to the control incubated without the quinoid compounds. In summary, the anaerobic consortium was affected to a greater extent by increasing the quantity of azo dyes or quinoid compounds. Nevertheless, at a lower concentration (1 mmol l\(^{-1}\)) of quinoid compounds, the anaerobic consortium effectively decolorized 2 mmol l\(^{-1}\) of DB71, increasing up to 5.2- and 20.4-fold the rate of decolorization with AQDS and LAW, respectively, compared to the control lacking quinoid compounds.

Key words | anaerobic consortium, azo dyes, biogas, decolorization, redox mediators

INTRODUCTION

Among all dyes employed by the textile industry, azo compounds represent the most common class of dyes used by this industrial sector, representing up to 60–70% of the worldwide market. Azo dyes can be grouped as mono-, di-, or tri-azo, according to the number of azo bonds (\(-\text{N} = \text{N}–\)) in their structure. The release of this class of colored compounds is undesirable because of the color and chemical oxygen demand that confer to water bodies, but also for their adverse impact on the photosynthesis of aquatic plants and their toxicity, mutagenicity, and carcinogenicity (van der Zee 2002).

Azo compounds remain unaffected during conventional aerobic wastewater treatment systems because the azo bond is an electron-accepting structure. However, under anaerobic conditions, these pollutants are susceptible to redox biotransformations (Field et al. 1995) using anaerobic bioreactors, such as up-flow anaerobic sludge bed (UASB) and expanded granular sludge bed systems (Donlon et al. 1996; Christiansen et al. 1997; Razo-Flores et al. 1999). Nevertheless, the biotransformation of many recalcitrant compounds proceeds very slowly due to electron transfer limitations and toxicity effects, leading to poor performance or even the collapse of anaerobic bioreactors (Rodgers & Bunce 2001; van der Zee et al. 2001b). The toxicity, resistance to reduction, and recalcitrance of azo dyes can be directly associated with the chemical structure (Carantino et al. 2001), the redox potential (Zille et al. 2004), and the number of azo bonds (Encinas-Yocupicio et al. 2006). Moreover, the adverse impacts of azo dyes are not similar for all microbial communities present in the anaerobic consortium. For example, the presence of Reactive Blue 19 resulted in a higher degree of inhibition of both acidogens and methanogens in relation to the impact observed by Reactive Blue 4 (Lee & Pavlostathis 2001). Because of the large diversity of dyes used by the textile and other industries, the color removal from wastewaters generated by these sectors is a complex task.

Employment of redox mediators (RMs) has been used as a suitable strategy to overcome limitations during a
biodecolorization process. As described by Van der Zee & Cervantes (2009), the use of humic substances and different quinoid compounds, acting as RM, increased the rate of decolorization by several orders of magnitude due to an increment in the electron transfer capacity between the oxidation of organic substrates and the reduction of azo dyes by an anaerobic consortium. In some cases, the presence of RM is an essential prerequisite for reaction to take place. In spite of the advantages of using RM during biodecolorization processes, it is important to emphasize that this class of compounds also have an inhibitory effect on anaerobic microorganisms. In a previous study, the addition of anthraquinone-2,6-disulfonate (AQDS) proved to be toxic for methanogens, but the use of this quinoid compound as a terminal electron acceptor was the preferred pathway during the respiratory process (Cervantes et al. 2000). The objective of the present study is to evaluate the inhibitory effects of different azo dyes and quinoid compounds on an anaerobic consortium. The catalytic effect of quinoid compounds during a decolorization process is also evaluated.

**MATERIALS AND METHODS**

**Reagents**

Three azo dyes were selected as model compounds to assess the inhibitory effects on an anaerobic consortium: Reactive Red 2 (RR2, 40% of purity), Congo Red (CR, 85% of purity), and Direct Blue 71 (DB71, 50% of purity), which are mono-, di-, and tri-azo compounds, respectively. Lawsone (LAW) and AQDS were selected as model RM. The chemical structures of the azo dyes and RM are illustrated in Figure 1.

**Inoculum and basal medium**

Anaerobic granular sludge was collected from a full scale up-flow anaerobic reactor (UASB reactor) installed in a brewery located in Cd. Obregón, México. The sludge, with 9.6% volatile suspended solids (VSS), was never acclimatized to any condition in the laboratory, including exposure to azo dyes and RM. Prior to inoculating batch assays, the

![Figure 1](https://iwaponline.com/wst/article-pdf/72/5/794/466776/wst072050794.pdf)
granular sludge (2–3 mm) was washed with distilled water and disintegrated with a sieve of 0.4 mm. Then, a portion of the consortium was added to vials at a concentration of 0.1 g VSS l\(^{-1}\). The bottles for incubation were supplied with basal medium (pH 7.0) with the following composition (mg l\(^{-1}\)): NaHCO\(_3\) (5,000), NH\(_4\)Cl (300), K\(_2\)HPO\(_4\) (200), MgCl\(_2\)-6H\(_2\)O (50), CaCl\(_2\) (10), and 1 ml l\(^{-1}\) of trace element solution, whose composition was as follows (mg l\(^{-1}\)): FeCl\(_3\)-4H\(_2\)O (2000), H\(_3\)BO\(_3\) (50), ZnCl\(_2\) (50), CuCl\(_2\)-2H\(_2\)O (38), MnCl\(_2\)-4H\(_2\)O (500), (NH\(_4\))\(_6\)Mo\(_7\)O\(_24\)-4H\(_2\)O (50), AlCl\(_3\)-6H\(_2\)O (90), CoCl\(_2\)-6H\(_2\)O (2000), NiCl\(_2\)-6H\(_2\)O (92), Na\(_2\)SeO\(_3\)-5H\(_2\)O (162), EDTA (1000), and 1 ml l\(^{-1}\) of HCl (36%).

**Effects of azo dyes and quinoid RMs on the anaerobic consortium**

Batch incubations were conducted in 120 ml glass serum bottles. A first assay set was conducted to evaluate the inhibitory effects of azo dyes on the anaerobic consortium by evaluating biogas production and decolorization of dyes. The microbial consortium was exposed to different concentrations (0.1, 0.5, 1, and 2 mmol l\(^{-1}\)) of CR, RR2, and DB71. The second set of assays consisted of evaluating the adverse effect of LAW and AQDS on biogas production by the anaerobic consortium. The tested concentrations of LAW and AQDS were 4, 8, and 12 mmol l\(^{-1}\). In these two assays, the working volume was 80% of the total volume of the serum bottle, and glucose was provided as an energy source at 2 g chemical oxygen demand (COD) l\(^{-1}\). Finally, a third set of assays was conducted to assess the catalytic effect of LAW and AQDS (1 mmol l\(^{-1}\)), acting as RM, on microbial decolorization of DB71. Before the addition of DB71, the bottles were pre-incubated for 24 h to promote an initial reduction of quinoid compounds, and then 2 mmol l\(^{-1}\) of the azo dye was added to each bottle. Moreover, the cultures received a pulse of glucose of 1 g COD l\(^{-1}\) in both the pre-incubation and incubation period. In all cases, anaerobic conditions were established by flushing the headspace of the bottles with nitrogen (N\(_2\)), after sealing them with rubber stoppers and aluminum crimps. The bottles were placed in an orbital shaker at 30 °C and 150 rpm.

**Analysis**

Decolorization of azo dyes was spectrophotometrically measured at their maximum wavelength of visible absorbance at 495, 540, and 585 nm for CR, RR2, and DB71, respectively. Samples (0.5 ml) were withdrawn, centrifuged and diluted up to an absorbance of less than 1 in a phosphate buffer (10.86 g l\(^{-1}\) Na\(_2\)HPO\(_4\)-2H\(_2\)O; 5.38 g l\(^{-1}\) Na\(_2\)HPO\(_4\)-H\(_2\)O). The biogas volume was determined by liquid displacement using a 2% NaOH solution to absorb the CO\(_2\) produced. The VSS content of the anaerobic consortium was determined according to *Standard Methods* (APHA 1985).

**RESULTS AND DISCUSSION**

**Effect of azo dyes: decolorization and biogas production**

The capacity of the anaerobic consortium to decolorize was evaluated under different concentrations of azo dyes and during a period of ~170 h. The decolorization process followed a first-order kinetic and the rate constants were calculated at the maximum slope observed as follows:

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

where \( C_t \) is the concentration of the azo dye in a given time; \( C_0 \) is the initial concentration of azo dye; \( k_d \) is the first-order rate constant of decolorization (h\(^{-1}\)); and \( t \) is the elapsed time (h). Figure 2 shows the typical decolorization profiles (top panel) and biogas production (bottom panel) in the absence of RM by anaerobic consortium under different concentrations of the tested dyes. In general, a better decolorization process occurred at the lowest concentration of dye tested. For instance, after the incubation period (~170 h), the decolorization efficiencies for RR2 and CR at 0.1 mmol l\(^{-1}\) were 76% and 74% and decreased to 58% and 41% when 2 mmol l\(^{-1}\) of dyes was provided, respectively. Conversely, the highest decolorization efficiencies were obtained with DB71, with values of 99% and 66% for the incubations at 0.1 and 2 mmol l\(^{-1}\), respectively. Table 1 shows the rates of decolorization achieved under all tested conditions. In general, lower rates of decolorization were achieved by increasing the concentration of dyes. The rates of decolorization (h\(^{-1}\)) obtained with RR2 were 0.0142 and 0.0043 for a concentration of 0.1 and 2 mmol l\(^{-1}\), respectively. In addition, the rates of decolorization proceeded as follows DB71 > CR > RR2, for all concentrations, except for the incubation at 2 mmol l\(^{-1}\) (CR > RR2 > DB71). The production of biogas (Figure 2, bottom panel) was not completely inhibited during the first hours of incubation and under all tested conditions, indicating that the anaerobic consortium remained physiologically active, even in the presence of any type and
concentration of dye. The highest values of cumulative biogas production were observed at the lowest concentration (0.1 mmol l⁻¹) tested for each azo dye. Both RR2 and CR incubations showed similar cumulative biogas production at 0.5–2 mmol l⁻¹ of azo dye, with values of ∼15 and ∼18.5 ml, respectively. The biogas production in incubations with DB71 was negatively affected according to the increment of its concentration. For instance, the volumes (ml) of biogas obtained were 14.9, 16.3, 18.3, and 22.1 for the concentrations (mmol l⁻¹) of 2, 1, 0.5, and 0.1, respectively. In general, the inhibition of biogas production by the addition of DB71 occurred after 48 h of incubation, where different profiles were observed (Figure 2, bottom panel). This result indicates that methanogenic microorganisms present in the consortium are initially not affected by the presence of azo dyes. Conversely, there is not a direct relationship between the affectation of the decolorization process (rates of decolorization observed) and the biogas produced by the anaerobic consortium. In a previous study, Lee & Pavlostathis (2004) found similar results during the decolorization of Reactive Blue 19 (RB19). Certainly, the extent of decolorization of RB19 for initial concentrations of 50–300 mg l⁻¹ was >90%, with a severe inhibition of methane production, achieving a maximum ≤8% of the control lacking the dye. Moreover, this behavior was not observed for incubations with Reactive Blue 4. These authors suggested that

Table 1 | First-order rate constants (kₐ) of decolorization by anaerobic consortium using different concentrations of azo dyes

<table>
<thead>
<tr>
<th>Azo dye concentration (mmol l⁻¹)</th>
<th>RR2 (mono-azo)</th>
<th>CR (di-azo)</th>
<th>DB71 (tri-azo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.0142 ± 1.0 × 10⁻³</td>
<td>0.0183 ± 1.5 × 10⁻³</td>
<td>0.0445 ± 3.6 × 10⁻³</td>
</tr>
<tr>
<td>0.5</td>
<td>0.0058 ± 2.7 × 10⁻⁴</td>
<td>0.0078 ± 6.6 × 10⁻⁴</td>
<td>0.0117 ± 7.7 × 10⁻⁴</td>
</tr>
<tr>
<td>1</td>
<td>0.0052 ± 1.4 × 10⁻⁴</td>
<td>0.0072 ± 1.1 × 10⁻⁴</td>
<td>0.0097 ± 5.8 × 10⁻⁴</td>
</tr>
<tr>
<td>2</td>
<td>0.0043 ± 2.6 × 10⁻⁴</td>
<td>0.0061 ± 2.5 × 10⁻⁴</td>
<td>0.0021 ± 1.2 × 10⁻⁴</td>
</tr>
<tr>
<td>2 + AQDS</td>
<td>ND</td>
<td>ND</td>
<td>0.0422 ± 2.7 × 10⁻³</td>
</tr>
<tr>
<td>2 + LAW</td>
<td>ND</td>
<td>ND</td>
<td>0.1639 ± 1.3 × 10⁻²</td>
</tr>
</tbody>
</table>

ND, not determined.

Figure 2 | Decolorization profiles (top panel) and cumulative biogas production (bottom panel) by anaerobic consortium. Symbols represent azo dye concentration (mmol l⁻¹): (diamonds) 0.1, (squares) 0.5, (triangles) 1, and (circles) 2. In all cases standard deviation was ≤10%.
methanogenic microorganisms were inhibited by the presence of RB19, which was evidenced by the accumulation of volatile fatty acids. Then, the high extent of reduction of the dyes could be attributable to the reduced conditions created by the consortium.

The increment of azo dye concentration significantly affected the decolorization process. Certainly, the rates of decolorization obtained with azo dyes at 2 mmol l\(^{-1}\) were 3.3-, 3.0-, and 21.1-fold, which were lower than those values achieved in the incubations at 0.1 mmol l\(^{-1}\) for RR2, CR, and DB71, respectively. Previously, Hu (2000) indicated that the recalcitrance of azo dyes increases with the presence of di-azo and tri-azo structures; nevertheless, Table 1 shows that the rate of decolorization (\(k_d\)) observed in the present study follows DB71 > CR > RR2, indicating a direct relationship between the number of azo bonds and the rate of decolorization. This relationship was also observed during the decolorization of DB71 (tri-azo), Direct Blue 53 (di-azo), and Reactive Orange 14 (mono-azo; Encinas-Yocupicio et al. 2006), confirming that the number of azo bonds is not associated with the toxicity effects or recalcitrance of azo dyes on the anaerobic consortium. Nevertheless, some functional groups of the azo dyes have been reported as being responsible for the toxicity or resistance being reduced. Van der Zee et al. (2006a, b) reported that reactive dyes with a triazine reactive group are slowly reduced among other types of dyes tested. This agrees with the lower decolorization rates obtained in the present study for RR2 containing the above-mentioned nitrogen-heterocyclic group. In addition, incubations with RR2 produced the lowest volume of biogas, presumably also caused by toxicity effects from the triazine group of the azo compound. The presence of sulfonate groups in the structure of azo dyes also greatly impacts the rate of reduction. For instance, if dye reduction takes place intracellularly, the presence of sulfonate groups will hamper the transport of the azo compound through the cell membrane. Therefore, the rate of dye reduction will decrease as the number of sulfonate groups increases. However, if the dye reduction occurs outside the microbial cell, the presence of sulfonate groups will have little effect on the rate of decolorization (Sandhya 2010). In this regard, the high extent of decolorization observed in this study for DB71 (with four sulfonate groups) can be caused by an extracellular mechanism. Certainly, the high rates of decolorization shown in Table 1 for DB71 in relation to those obtained with CR (two sulfonate groups), can be attributable to the higher electronegativity of sulfonate groups, which makes the azo bonds more accessible to receiving electrons (Beydilli et al. 2000) and confirms that the reaction is extracellular. In addition, azo dyes with linear structures are more susceptible to being reduced compared to those with non-linear structures, because steric hindrance effects are minimized. This could also be responsible for the results here obtained with respect to decolorization assays (DB71 > CR > RR2, see Figure 1 and Table 1). Another reason for the higher rate of decolorization achieved with DB71 compared to RR2, could be an auto-catalytic input provided by the aromatic amines produced from the reductive decolorization (Encinas-Yocupicio et al. 2006). In general, azo dyes with simple structures are more susceptible to being reduced, conversely it is more difficult to establish an efficient decolorization process with highly substituted dyes (Sani & Banerjee 1999).

**Effects of RMs: decolorization and biogas production**

The inhibitory effects of AQDS and LAW on the anaerobic consortium during biogas production were assessed in the presence of 4, 8, and 12 mmol l\(^{-1}\) of these compounds. Figure 3 shows the cumulative biogas production after ~170 h of incubation, which indicates that the control lacking quinoid compounds achieved the highest production of biogas with 19.8 ml. Considering all tested concentrations of quinoid compounds, assays with LAW affected biogas production to a greater extent than AQDS assays (as
indicated in Figure 3). The incubations reflected a decrement in biogas production up to 37, 53, and 58% at 4, 8, and 12 mmol l\(^{-1}\) of LAW, whereas AQDS affected biogas production up to 5.1, 19, and 24% at the same RM concentrations, both compared to biogas produced by the control lacking quinoid compounds.

The catalytic effect of RM during the decolorization of DB71 was evaluated at the highest concentration (2 mmol l\(^{-1}\)) previously tested, which also reflected a higher inhibitory effect than RR2 and CR on the anaerobic consortium, evidenced by the lowest rate of decolorization observed (0.0021 h\(^{-1}\)). In addition, AQDS and LAW were added at 1 mmol l\(^{-1}\), which is significantly lower than those concentrations applied to the incubations to assess the effect of these compounds during biogas production. The endogenous and sterile controls showed negligible decolorization efficiency, ranging between 8.7 and 13%, for both AQDS and LAW incubations. Figure 4 shows a better performance of LAW acting as RM for the decolorization of DB71 compared to AQDS after 12 h of incubation. The efficiencies obtained for decolorization were 58% and 87%, for AQDS and LAW, respectively. Table 1 shows the rates of decolorization of DB71 using AQDS and LAW, with values of 0.0422 and 0.1639 h\(^{-1}\), respectively, which are significantly higher than the incubations without RM.

The presence of LAW and AQDS inhibited the production of biogas when the concentration of these quinoid compounds was increased. The volume of biogas produced after ~170 h of incubation was 1.5, 1.7, and 1.8 times higher in AQDS supplemented cultures in comparison with LAW-added cultures for the concentrations of 4, 8, and 12 mmol l\(^{-1}\), respectively. These results clearly indicate that the presence of LAW affected the anaerobic consortium to a greater extent than AQDS, but biogas production was not completely inhibited under the different concentrations tested. The inhibitory effects of quinoid compounds can be explained by the redox potential of the culture solution. Previously, Cervantes et al. (2000) showed that a solution of 20 mmol l\(^{-1}\) of AQDS increased the redox potential to +130 mV, but this value decreased gradually to about −175 to −250 mV, according to the extent of AQDS reduction in the cultures. The decrement of redox potential promoted recovery of the capacity of the anaerobic consortium to produce methane. Certainly, the initial redox potential affected the biochemical process of methanogenesis because methane production is only possible if redox potential in the medium is lower than −200 to −400 mV (Fetzer & Conrad 1993).

The catalytic capacity of quinoid compounds to act as RM was evidenced by the high extent of decolorization achieved (Table 1 and Figure 4). The rates of decolorization in the cultures at 2 mmol l\(^{-1}\) of DB71 were 5.2 and 20.4 times higher with AQDS and LAW, respectively, compared to the control experiment without RM (according to the data obtained in the experiment presented in Figure 4). Moreover, the addition of LAW to the incubation at 2 mmol l\(^{-1}\) of DB71 accelerated the reaction of decolorization (3.6 times higher) even above the value observed at 0.1 mmol l\(^{-1}\), but in absence of the quinoid compound. In the case of AQDS, the assay at 2 mmol l\(^{-1}\) of DB71 increased the decolorization rates up to similar values observed at 0.1 mmol l\(^{-1}\) but in the absence of RM (Table 1). The higher capacity of LAW to decolorize DB71 faster than AQDS is due to its redox potential, whose values are −137 and −184 mV, respectively. A mediator's redox potential should not be much lower (ideally higher or less negative) than that of the biological reducing system, typically −320 mV for NAD(P)H, otherwise it will not be reduced sufficiently (Van der Zee & Cervantes 2009). Conversely, in spite of the concentration of RM (1 mmol l\(^{-1}\)) tested in the present study, it is too high compared to those used in similar studies; the molar ratio RM/azo dye established here is 0.5, which is lower than those reported during decolorization assays by anaerobic sludge (Kudlich et al. 1997; Van der Zee et al. 2000a; Rau et al. 2002; Dos Santos et al. 2004). In the study developed by Van der Zee et al. (2000a, b), a high concentration of AQDS (1.18 mmol l\(^{-1}\)) with a molar ratio RM/RR2 of 5.7
(a ratio 7.3 times higher than the selected in this study) was also used, achieving an increment up to 16-fold higher than the control without RM, but using an anaerobic consortium at 2 g VSS l⁻¹ (20 times higher than that applied in this study).

The application of RM for the treatment of textile wastewaters under anaerobic conditions could represent a suitable technology. Nevertheless, some aspects need to be considered before applying RM in anaerobic bioreactors at full scale. For instance, the use of humic substances instead of quinoid compounds represents a better cost-effective alternative, taking into account that these compounds are very abundant in nature, are inert, and have a high content of quinone groups in their structure (Steven-

son 1994). In addition, RM does not necessarily have to be added abundantly; in fact, adding a small concentration of AQDS (19 μM) to a continuous reactor influent caused an important biological activity, evidenced by the increase of dye decolorization and COD removal (Van der Zee et al. 2001a).

CONCLUSIONS

The results of this study indicate that the inhibitory effects of azo dyes on the anaerobic consortium mainly depend on their chemical structure and concentration, and there is no relationship with the molecular weight and/or the number of azo bonds. Both the rate of decolorization and biogas production were affected by increasing the azo dye concentration. The presence of quinoid compounds, namely LAW and AQDS, also inhibited biogas production by the anaerobic consortium by up to 58% and 24%, respectively, in comparison with the control experiment in the absence of quinoid compounds. Furthermore, the findings of the present study also indicate that the addition of LAW and AQDS to incubations contributed significantly to decolorizing DB71, evidenced by the high decolorization rates achieved compared to the control without redox mediators.

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