Anaerobic biodecolorization of textile reactive anthraquinone and phthalocyanine dyebaths under hypersaline conditions

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Abstract The biological decolorization of two industrial, spent textile reactive dyebaths was investigated using a suspended-growth, halophilic mixed culture fed with glucose. Dyebath I contained mainly Reactive Blue 19 (RB19), an anthraquinone dye, whereas dyebath II contained mainly Reactive Blue 21 (RB21), a phthalocyanine dye. Batch assays under anaerobic conditions with the two neutralized dyebaths resulted in 87 and 37% extent of decolorization for dyebaths I and II, respectively. The rate of glucose utilization and the extent of acetate production were impacted in the presence of each dyebath as compared to the control culture. However, dyebath decolorization occurred despite moderate culture inhibition. Reuse of a biologically renovated RB19-containing dyebath in the dyeing process resulted in reproducible but not identical cotton fabric shades as compared to a standard dyeing (i.e., control) using fresh water. This difference is attributed to a variable degree of RB19 aggregation during the dyeing process and is not related to the efficiency of the biodecolorization process. Further improvement of the redyeing efficiency will lead to the development of an in-plant, closed-loop decolorization system resulting in significant water conservation and minimization of textile pollutants such as salt and dyes.

Keywords Anthraquinone dyes; biological decolorization; halophilic culture; phthalocyanine dyes; textiles; water reuse

Introduction Reactive dyes are the only textile dyes designed to covalently bond with cellulosic fibers (i.e., cotton). Cotton use has increased worldwide, resulting in a steady increase in reactive dye usage. Reactive dyeing is typically performed at 30–85 °C, with reactive dyebaths containing 25 to over 100 g/L salt (NaCl or Na2SO4), and 2–5 g/L Na2CO3 (or a combination of Na2CO3 and NaOH) (Aspland, 1997). Under typical reactive dyeing conditions, up to 50% of the initial dye remains in the dyebath in its hydrolyzed form, which has no affinity for the fabric and results in colored effluent (Zollinger, 1991; Aspland, 1997). In addition to the presence of dye, the high concentrations of salt, as well as high pH (11–13) further complicate the management of spent reactive dyebaths. Reactive dyes are highly water soluble, are non-degradable under the typical aerobic conditions found in conventional, biological treatment systems, and adsorb very poorly to biological solids, resulting in residual color in discharged effluents (Pierce, 1994; Vandevivere et al., 1998; Beydilli et al., 2000; Pearce et al., 2003).

Azo, anthraquinone, and phthalocyanine dyes are commercially important classes of reactive textile dyes. Although a substantial number of reports have documented the biological decolorization of reactive azo dyes under anaerobic conditions, relatively few reports exist on the biological decolorization of reactive anthraquinone and phthalocyanine dyes. In addition, very limited research dealing with the biological decolorization of commercial, full-strength, spent reactive dyebaths under hypersaline conditions has been
conducted. Although a high rate and extent of decolorization of a reactive anthraquinone dye bath was achieved, toxicity due to salt (30 g/L NaCl) and dyes prohibited the development of a sustainable biodecolorization process using mixed, methanogenic cultures (Fontenot et al., 2003; Lee and Pavlostathis, 2004a). Similarly, an unadapted activated sludge consortium maintained under anaerobic conditions was unable to decolorize a reactive azo dye at NaCl concentrations higher than 2 g/L (Bromley-Challenor et al., 2000).

Treatment of spent reactive dyebaths without dilution is necessary for the successful development of a closed-loop, in-plant dyebath renovation and reuse process, which is the primary goal of an ongoing research project. The objective of the work presented here was to assess the efficacy of using a mixed, halophilic enrichment culture for the decolorization of two industrial, spent reactive anthraquinone and phthalocyanine dyebaths under hypersaline conditions, as well as to assess the feasibility of reusing a biologically renovated dyebath in subsequent dyeing operations.

Materials and methods

Dyebaths

Two industrial, textile spent reactive dyebaths containing mainly anthraquinone and phthalocyanine dyes were collected at the Washington Manufacturing Co., textile plant in Washington, GA, USA. The dyebath recipes are shown in Table 1. The chemical structures of the two major dyes (RB19 and RB21) can be found in a previous report (Fontenot et al., 2003). Analysis of the two spent dyebaths included spectrophotometric scanning, pH, alkalinity, chemical oxygen demand (COD), dissolved organic carbon (DOC), total solids (TS), and volatile solids (VS).

Halophilic culture

A suspended-growth, halophilic mixed culture was enriched using a hypersaline lake (Mono Lake, CA, USA) sediment sample as inoculum. The enrichment culture was fed with glucose, yeast extract, and a medium containing mineral salts, trace metals, vitamins as well as NaCl at 100 g/L. The culture was maintained at 35 °C in a glass reactor open to the atmosphere with a 4-hour feeding cycle and a hydraulic (and solids) retention time of 10 d (Beydilli, 2001; Lee and Pavlostathis, 2004b). The 4-hour culture feeding cycle included four, 1-hour alternating anoxic and oxic phases. The glucose organic loading rate was 145 mg glucose/L-d and the steady-state biomass concentration was 1092 ± 42 mg VSS/L (mean ± standard deviation). The stock halophilic culture was maintained without any dye addition for three years prior to the initiation of the experiments described here.

Table 1 Components of the two industrial, reactive dyebaths

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dyebath I</th>
<th>Dyebath II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major dye 1, g/L</td>
<td>Remazol Blue R-W (RB19)¥, 4.9</td>
<td>Remazol Turquoise R-P (RB21)¥, 3.0</td>
</tr>
<tr>
<td>Major dye 2, g/L</td>
<td>Remazol Turquoise R-P (RB21)¥, 1.2</td>
<td>Remazol Blue R-W (RB19)¥, 2.2</td>
</tr>
<tr>
<td>Minor dye, g/L</td>
<td>Remazol Red RB (RR198)¥, 0.32</td>
<td>Everzol Yellow 4GL (RY160)¥, 0.0118</td>
</tr>
<tr>
<td>NaCl, g/L</td>
<td>113</td>
<td>–</td>
</tr>
<tr>
<td>Na₂SO₄, g/L</td>
<td>–</td>
<td>89.9</td>
</tr>
<tr>
<td>Na₂CO₃, g/L</td>
<td>3.0</td>
<td>3.5</td>
</tr>
<tr>
<td>NaOH, g/L</td>
<td>0.95</td>
<td>0.95</td>
</tr>
</tbody>
</table>

¥Initial dyebath composition (i.e., before dyeing); ²Anthraquinone dye; ³Phthalocyanine dye; ⁴Azo dye
Biodecolorization assays
Batch biodecolorization assays were performed using the halophilic, mixed culture which was pre-concentrated by centrifugation at 3000 rpm for 30 min in order to obtain a 15-fold concentrated biomass, thus allowing testing of the two dyebaths with only 10% dilution. Pre-concentrated culture aliquots were transferred to 250-mL serum bottles, which were either left open (aerobic assay) or previously sealed with rubber stoppers, covered with septa, aluminum crimped and flushed with helium for 10 min (anaerobic assay). Aliquots of 180 mL of each dyebath, which were previously neutralized to pH 7.0 with concentrated HCl, were added to each serum bottle along with a volume of concentrated culture media, yeast extract, glucose, NaCl, and NaHCO₃. The total liquid volume was 200 mL. The salt concentration was maintained at the same level as in each dyebath by the addition of NaCl. Previous experiments (data not shown) demonstrated that the culture performed similarly with either Na₂SO₄ or NaCl as long as the total Na⁺ concentration was the same. The initial biomass and glucose concentrations were 230 mg VSS/L and 250 mg/L, respectively. One culture prepared without any dyebath addition served as the control. In order to assess any possible reversibility of the reductive decolorization of the dyebath, at the end of the anaerobic decolorization assay, the dyebath decolorization products were aerated for 16 h. The effect of the initial spent dyebath concentration on the reductive decolorization and halophilic culture activity was assessed in another batch assay, which was performed at four different dyebath concentrations (100, 80, 50, and 20% v/v) under anaerobic conditions. All cultures were incubated statically in the dark at 35°C, and agitated daily by hand. During the incubation, the following analyses were performed: absorbance, pH, glucose, DOC, and acetate.

Decolorization and reuse of spent RB19 dyebath
The feasibility of dyebath reuse was examined using a RB19 (the major dye of dyebath I) solution. Low and high strength dyebath solutions were prepared with 625 and 4875 mg/L of RB19 dye (1.25 and 9.75% dye to fabric weight ratio, respectively). Dyeing of cotton fabric was first performed using RB19 solutions in tap water (standard dyeings). After dyeing, the remaining dye solutions were biologically decolorized using the halophilic culture, and then reused as process water in single redyeings (experimental dyeings) following previously reported procedures (Lee and Pavlostathis, 2004b).

Analytical methods
Alkalinity, pH, TS, VS, VSS, and COD analyses were carried out in accordance with Standard Methods (1998). Acetate was measured by gas chromatography (flame ionization detector). DOC, glucose, biomass, and spectrophotometric analyses were performed following previously reported procedures (Beydilli et al. 2001; Lee and Pavlostathis, 2004a, 2004b). Dyed cotton fabric samples were evaluated for color shade reproducibility and consistency following a standard procedure (AATCC, 1995). The X Y Z color tristimulus values were measured and used to calculate the ΔEcmc value, which is a single number defining the total color difference in CMC units of an experimental fabric sample from a standard fabric sample.

Results and discussion
Characterization of spent dyebaths
Characterization of the two spent dyebaths was performed and the results are shown in Table 2. The exact concentration of RB19 and RB21 in the two spent dyebaths could not be determined because the spectra of the two dyes overlap in the 550–700 nm range. Based on the dye bath recipe data and the measured equivalent dye concentration in the
spent dyebaths, the dye exhaustion in dyebaths I and II was approximately 79 and 83%, respectively.

Decolorization assay under aerobic conditions
Two halophilic, dyebath-amended cultures were incubated under aerobic conditions for 6 days. A 15–20% decrease of absorbance was observed immediately after the addition of each dyebath to the culture, which was attributed to dye aggregation and association with biomass. Further decrease of the absorbance was not observed for the remainder of the incubation period. These results demonstrate that effective decolorization of anthraquinone and phthalocyanine dyes/dyebaths is not feasible under aerobic conditions.

Decolorization assay under sequential anaerobic/aerobic conditions
A dyebath decolorization assay was performed under sequential anaerobic/aerobic conditions. The absorbance, glucose, and acetic acid profiles are shown in Figure 1. Although the initial decolorization rate was approximately the same for both dyebaths, the extent of decolorization was 87 and 37% for dyebaths I and II, respectively, which confirms the relatively higher resistance to biological decolorization of phthalocyanine dyes as compared to anthraquinone dyes. In a previous report, the extent of biological decolorization of RB19, the major dye in the spent dyebath I, was in the range 90–95% under methanogenic conditions without any salt amendment (Lee and Pavlostathis, 2004a). In addition, other researchers have reported relatively slow and partial bacterial decolorization of phthalocyanine dyes, which is due to their chemical stability and resistance to biotransformation (Nigam et al., 1996; Malpei et al., 1998; Beydilli et al., 2001; Fontenot et al., 2003). Therefore, the observed residual absorbance of both dyebath-amended cultures is likely due to RB21. At the end of the anaerobic dyebath decolorization phase (shown by a broken line in Figure 1), when the two dyebaths were aerated for 16 h, an absorbance increase of 17 and 6% was observed for dyebaths I and II, respectively. Spectral analysis of the decolorization products of dyebath I showed a decrease in the absorbance in the

<table>
<thead>
<tr>
<th>Parameter</th>
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<th>Dyebath II</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\lambda_{\text{max}}, \text{nm})</td>
<td>591</td>
<td>616/670</td>
</tr>
<tr>
<td>Dye concentration, mg/L</td>
<td>1032(^{a})</td>
<td>519(^{b})</td>
</tr>
<tr>
<td>pH</td>
<td>12.3</td>
<td>11.7</td>
</tr>
<tr>
<td>Alkalinity, mg CaCO(_3)/L</td>
<td>3173</td>
<td>4050</td>
</tr>
<tr>
<td>DOC, mg/L</td>
<td>1186</td>
<td>448</td>
</tr>
<tr>
<td>COD, mg/L</td>
<td>4670</td>
<td>1371</td>
</tr>
<tr>
<td>Total solids, g/L</td>
<td>108.4</td>
<td>86.0</td>
</tr>
<tr>
<td>Volatile solids, g/L</td>
<td>2.3</td>
<td>1.0</td>
</tr>
</tbody>
</table>

\(^{a}\)Based on absorbance measurement and expressed as RB19 equivalent (with NaCl)

\(^{b}\)Based on absorbance measurement and expressed as reacted RB21 equivalent (with Na\(_2\)SO\(_4\))

Figure 1 Relative absorbance, glucose, and acetate profiles in the halophilic cultures during the batch dyebath decolorization assay (broken line indicates switch from anaerobic to aerobic conditions)
420–460 nm region and a minor absorbance increase at 610 nm. Glucose consumption was delayed in the two dyebath-amended cultures and acetate production was suppressed as compared to the control culture (Figure 1). Acetate was completely removed in all three cultures within 16 h under aerobic conditions. During the anaerobic and aerobic incubation period, there was no significant change in DOC, other than that attributed to the glucose addition and acetate production and subsequent degradation. These data confirm that the observed absorbance decrease was due to biological decolorization and not to dye aggregation and/or association with biomass, and that biodegradation of the dye-bath dyes did not take place under anaerobic or aerobic conditions.

**Reuse of spent RB19 dyebath**

The remaining dye concentration after the first, standard dyeing in each spent dyebath was 71 ± 6 and 267 ± 7 mg/L, for the low and high strength dyebath, respectively. The dye concentration in the RB19 dyebaths after biological decolorization was 14 and 74 mg/L, for the low and high strength dyebath, respectively. A significant difference in initial and final dyebath pH values between standard and experimental dyeings using biologically renovated azo dyebaths was observed (Lee and Pavlostathis, 2004b). Similarly, for both the low and high strength RB19 dyebath, two sets of redyeings were carried out: one set with pH adjustment of the renovated dyebaths throughout the dyeing cycle by the addition of concentrated NaOH to raise the pH to the same level observed in the standard dyeing cycle, and another without pH adjustment. Fabric color and dye exhaustion data for each dyebath are shown in Table 3.

Figure 2 shows that the reflectance of fabric samples dyed in biologically decolorized dyebaths without pH adjustment was higher (lighter color shade) as compared to standards dyed in tap water, and that dyebath pH adjustment during the dyeing cycle resulted in a decrease of fabric reflectance (darker color shade). However, in spite of the improvement observed as a result of dyebath pH adjustment, reuse of the biologically decolorized RB19 dyebaths resulted in lower dye exhaustion, higher washing off, and a fabric color shade closer to, but not identical to that of the standard dyeing. For all dyebaths, ΔEcmc values were over 3 (acceptable limit is ΔEcmc ≤ 1; AATCC, 1995), which means that experimental dyeings resulted in fabric shades different than those of the standard. This difference is attributed to a variable degree of RB19 aggregation during the dyeing process and is not related to the efficiency of the biological decolorization process. However, all replicate experimental samples had identical color fabric shades (identical reflectance), showing that the experimental dyeings with RB19 renovated dyebaths were highly reproducible as was also the case with the reuse of biologically renovated industrial, azo dye-baths (Lee and Pavlostathis, 2004b).

| Table 3 RB19 dyebath exhaustion and color differences of fabrics dyed in biologically decolorized dyebaths with and without pH adjustment as compared to standards dyed in tap water |
|---|---|---|---|---|---|---|
| Dye level, %a | ΔL | ΔC | ΔH | ΔEcmc | Final pH | Exhaustion, %b |
| 1.25 (w/o pH adjustment) | 8.18 | −4.25 | −2.28 | 9.50 | 9.3 | 63.5 ± 1.4 (88.3 ± 1.0)c |
| 1.25 (w/ pH adjustment) | 3.43 | −1.40 | −1.10 | 3.87 | 11.3 | 71.2 ± 1.6 |
| 9.75 (w/o pH adjustment) | 5.82 | −1.74 | −1.17 | 6.19 | 9.2 | 66.9 ± 1.0 (94.2 ± 0.2)c |
| 9.75 (w/ pH adjustment) | −6.30 | 1.74 | 3.08 | 7.23 | 11.2 | 77.7 ± 0.4 |

aDye to cotton fabric weight ratio expressed as %
b(Abs. before dyeing − Abs. after dyeing) × 100/(Abs. before dyeing)
cValues in parenthesis are for standard dyeing in tap water

Abbreviations: ΔL, ΔC, ΔH, difference in lightness-darkness, brightness-dullness (or chroma), and hue, respectively; ΔEcmc, total difference in CMC units of a sample from a standard
Conclusions

Batch decolorization assays were conducted with a halophilic, mixed culture and two industrial, spent dyebaths under both aerobic and anaerobic conditions. Both the anthraquinone- and phthalocyanine-based dyebaths were not decolorized under aerobic conditions. In contrast, under anaerobic conditions, the anthraquinone-based dyebath was readily decolorized to over 87% within 2 days of incubation, whereas only partial decolorization of the phthalocyanine-based dyebath was observed. The extent of dyebath decolorization in all anthraquinone and phthalocyanine dyebath-amended cultures increased with decreasing initial dyebath concentration. Compared to a control culture, the two dyebaths and their decolorization products had a moderate inhibitory effect on the halophilic culture in terms of glucose utilization and conversion to acetic acid. In spite of the high extent of decolorization of the spent RB19 dyebath using the halophilic culture, reuse of the biologically decolorized RB19 dyebath at both 1.25 and 9.75% dye to fabric weight ratio was moderately successful possibly due to a variable degree of RB19 aggregation during the dyeing process. Further improvement of the redyeing efficiency will lead to the development of an in-plant, closed-loop decolorization system, resulting in significant water conservation and minimization of textile pollutants such as salt and dyes.

Acknowledgements

This work was supported by the National Science Foundation under grant no. BES-0114169. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

References


