TITLE: BIOLOGICAL RENOVATION AND REUSE OF SPENT REACTIVE DYEBATHS

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Project Goal: To evaluate the feasibility of using anaerobic/anoxic biological processes for color removal and reuse of spent reactive dyebaths and demonstrate the effectiveness of a fixed-film bioreactor system as a low-cost dyebath decolorization and reuse technology.

ABSTRACT

The objective of this project was to biologically renovate reactive dyebaths and reuse the high-salt containing mixture in the dyeing process. This approach accomplishes two goals: (a) elimination of the color from the plant effluent; and (b) reuse of the renovated process water and salts, thus leading to final wastewater volume reduction, and water, as well as salt conservation. Nine commercial reactive dyes (Black 5; Blue 4, 7, and 19; Red 2 and 120; Yellow 3, 15 and 17) were used in this study. Decolorization assays of simulated reactive dyebaths were conducted with an anaerobic, methanogenic culture at low salt concentrations as well as with a halotolerant culture maintained under microaerophilic conditions and at elevated salt concentrations. In addition, decolorization of a spent dyebath obtained from a package-dyeing plant was tested using the suspended growth, methanogenic culture. Overall, fast decolorization kinetics were observed, especially with the reactive azo dyes. A fixed-film bioreactor was developed with the halotolerant culture and used for the decolorization of Red 2 simulated dyebath solutions under microaerophilic conditions. Repetitive dyeing tests were conducted with the same Red 2 solution which was decolorized by the halotolerant culture under anoxic conditions and then the decolorized liquor was reused in a new dyeing test. The results of dyeing tests conducted with decolorized and recycled dye liquor when compared with those of standard reactive dyeings (i.e., dyeings conducted with fresh dye) resulted in very small and acceptable color differences. In conclusion, biological decolorization of spent reactive dyebaths is feasible under anoxic/microaerophilic conditions and reuse of the renovated process water in subsequent dyeings results in an acceptable fabric color.

INTRODUCTION

The key question addressed by this project was whether an effective (both technically and economically) means can be found to reuse spent reactive dyebaths. In addition to the dye, these streams have very high salt and heavy metal concentrations. Existing physical/chemical technologies for color removal are very expensive and commercially unattractive. Although it is extremely difficult to oxidize reactive dyes, their reduction can be achieved relatively easily. Microbial anoxic and anaerobic processes are capable of producing and maintaining low oxidation-reduction potential conditions. Under such conditions, reductive cleavage (e.g., azo bond cleavage) or other reductive transformation (e.g., reduction of anthraquinone to dihydroxyanthracene) results in dye decolorization.
The following information was included in the two previous annual reports: characterization of the nine commercial dyes, description of a methanogenic stock culture, and results of a series of batch assays (effect of culture activity on color removal, comparison of decolorization kinetics between reacted and unreacted dyes, effect of repetitive dye addition to culture activity and color removal, toxicity and color removal as a function of dye concentration). These assays were conducted with a methanogenic, suspended-growth culture in the absence of elevated salt concentrations. In the third annual report, the results of additional assays conducted with the methanogenic culture as well as with a halotolerant culture maintained under microaerophilic conditions were presented. Decolorization of a spent dyebath obtained from a package-dyeing plant was tested using the methanogenic culture. Red 2 solution decolorized by the halotolerant culture under anoxic conditions was used as dye liquor and compared to a standard reactive dyeing. In the present report, the results of batch decolorization assays of Blue 7 – a Cu-phthalocyanine, monochlorotriazinyl reactive dye – using the suspended-growth and halotolerant culture, as well as the decolorization of Red 2 – a monoazo, dichlorotriazinyl reactive dye – using the continuous-flow, fixed-film reactor at an elevated salt concentration are presented. In addition, repetitive dyeing tests were conducted with the same Red 2 solution which was decolorized by the halotolerant culture under anoxic conditions and then the decolorized liquor was reused in new dyeing tests five times.

MATERIALS AND METHODS

Suspended-Growth, Halotolerant Mixed Culture

An anaerobic, halotolerant mixed culture was enriched using inoculum from a salt lake sediment (Mono Lake, California). This culture was fed with a mixture of organic compounds and salts as follows (in g/L): glucose, 10; yeast extract, 0.2; K$_2$HPO$_4$, 0.9; KH$_2$PO$_4$, 0.5; NH$_4$Cl, 0.5; CaCl$_2$·2H$_2$O, 0.10; MgCl$_2$·6H$_2$O, 0.20; FeCl$_2$·4H$_2$O, 0.10; MgSO$_4$·7H$_2$O, 0.4; Na$_2$CO$_3$, 1.6; NaCl, 100; trace metal and vitamin stock solutions. The anaerobic culture was maintained at 35°C and was batch-fed. After 2.5 months of operation under anaerobic conditions, a subculture was set up and maintained in a 4-L reactor (2 L liquid volume) open to the atmosphere. Oxygen was supplied by vortex mixing of the culture over a magnetic stir plate. This culture was batch-fed once every 2 days with the above mentioned mixture of organic compounds and salts, with a hydraulic (and solids) retention time of 10 days. Under these conditions, and especially immediately after feeding, anoxic/microaerophilic conditions prevailed. Finally, this culture was maintained open to the atmosphere with a 4-hour feeding cycle using an electronic timer as follows: feed was added via a feeding pump for 2 min while aeration was stopped; then, after 1 hour, aeration was switched on at a predetermined flow rate and lasted for 1 hour; then the aeration was switched off for another hour and then again was switched on for another hour. Parameter variation during a typical feeding cycle of this culture is shown in Figure 1. This culture has been maintained under this feeding protocol for over 5 months.
Continuous-Flow, Fixed-Film Reactor

The continuous-flow, fixed-film reactor was constructed with a 5.0-cm internal diameter glass tube (1.3 L total working volume). Porous glass beads were used as the support material and the reactor was operated with a high enough recirculation flow rate to achieve complete bed fluidization. A separate recirculation line was used to pass a fraction of the support-free reactor contents through an small oxygenation tube where oxygen was delivered via a peristaltic pump and sparged through a fine-pore diffuser. The reactor was started with mixed liquor from the above described suspended-growth, halotolerant culture and was fed with the same media containing glucose/yeast extract, inorganic salts, trace metals and vitamins. The feed for this reactor also included 100 g/L NaCl. The feed dissolved organic carbon (DOC) concentration was 200 mg/L. The reactor was maintained at 35°C with water recirculation through a water jacket surrounding the reactor. The reactor was operated with a hydraulic retention time of 2.25 h for 4 months before the assessment of dye decolorization. The dissolved oxygen (DO), pH and dissolved organic carbon (DOC) in the bulk of the reactor contents were monitored. After 3 months of operation, the steady-state values of these three parameters were as follows: DO, 0.5 – 0.8 mg/L; pH, 6.8 – 7.0; and DOC, 10 – 12 mg/L.

Dyeing Procedure

Reactive dyeing of prepared 100% cotton twill fabric was carried out using an AHIBA Texomat dyeing machine to ensure constant temperature and precise time control. Dyeings using both fresh dye solution and biologically decolorized Procion Red MX-5B (C. I. Reactive Red 2) solution (referred to as standard and experimental dyeing, respectively) were performed. Standard dyebaths were made up of a 20:1 liquor ratio containing 1.25% of Reactive Red 2, 5 g of fabric, 100 g/L sodium chloride, and 10 g/L of sodium carbonate. All dyeings were carried out by the modified "all in method" in which sodium chloride was added at the beginning of dyeing. Dyebaths were set to calculated volumes of deionized water, dye, salt, and sodium carbonate. The dyebaths were brought to a constant temperature of 28°C before insertion of the fabric and then the prepared fabric.
was added to the dyebaths. The temperature of the dyebath was raised to 42°C over a 10-minute period. The dyebath was then held at 42°C for 15 min for exhaustion. Sodium carbonate was then added to the dyebath. The fixation cycle was run for 60 min at 42°C. At the end of the fixation cycle, the fabric was placed in a soaping bath containing 1 g/L of I.C.I. Synthrapol SP at 100°C for 10 min. The fabric was then rinsed with warm tap water to remove the soap. After drying, fabric samples were evaluated for color. An Ultrascan XE Colormeter and Hunter Universal Software (Ver. 3.0) was used to compare standard dyed swatches versus experimental swatches (i.e., swatches dyed with biologically decolorized and recycled dyebaths). Standard and experimental swatches were evaluated for color consistency using the Test Method 173-1992 (AATCC, 1995). Residual dyebaths were evaluated for exhaustion using a spectrophotometer.

Repetitive Dyeings with a Biologically Decolorized Dyebath

In order to demonstrate the efficacy of reusing biologically renovated spent reactive dye bath, repetitive dyeing tests were conducted with the same Red 2 solution which was decolorized by the halotolerant culture under anoxic/aerobic conditions. The spent dyebath obtained after standard dyeing was neutralized using concentrated HCl to pH 7.2±0.1. Mixed liquor obtained from the halotolerant stock culture with a volume equal to that of the spent dyebath was centrifuged at 5,000 rpm for 30 min in order to obtain concentrated biomass for the decolorization experiment. To the neutralized spent dyebath, concentrated biomass from the halotolerant stock culture and the concentrated mixture of organic compounds and inorganic salts were added. The final concentrations of the organic compounds and salts were as follows (in g/L): glucose, 0.250; yeast extract, 0.005; K2HPO4, 1.9; KH2PO4, 1.0; NH4Cl, 1.0; CaCl2·2H2O, 0.2; MgCl2·6H2O, 0.4; FeCl2·4H2O, 0.2; MgSO4·7H2O, 0.8; Na2CO3, 8.0; NaCl, 100; trace metal and vitamin stock solutions. The culture was maintained under anoxic conditions for the first 24 h and then was aerated for the next 24 h, with a total incubation period of 2 d. Each subsequent decolorization was performed by reusing the same spent dye bath and fresh biomass from the halotolerant stock culture. Due to biomass, organic compounds and inorganic salt additions during culture set-up for each decolorization cycle, the spent dyebath after each dyeing cycle was diluted by about 6.5%. Therefore, at the end of five decolorization/dyeing cycles the initial spent dyebath volume was diluted by 37%.

The decolorized Red 2 solution was further prepared for a new dyeing by the removal of suspended particulate matter and neutralization as follows. Solids were removed by centrifugation of the dyebath at 8000 rpm and then filtration using a 1.2 µm, 0.80 µm, 0.45 µm, and 0.22 µm filters in order to remove any left over suspended particles. The dyebath was then neutralized to a pH of approximately 7.15 using 4 N HCL and aerated for 15 min to raise the oxidation-reduction potential (ORP) of the solution in order to prevent any reduction of the new dye. New salt was not added to the renovated dyebath for the repetitive experimental dyeings, since salt was conserved. Only fresh dye and sodium carbonate were added to the renovated dyebaths just before each dyeing at the same levels as in the above described standard dyeings. Five repetitive dyebath renovations (decolorizations) and dyeings were performed according to the above described procedure using the same Red 2 dyebath.

Dye Analyses
Reacted dyes were prepared by simulating the dyebath conditions as follows. Aliquots of 5.0 g dye and 25.0 g sodium carbonate were dissolved in deionized water, heated to 80°C for 1 h and then diluted to 1 L. All spectrophotometric analyses were carried out using a UV/VIS Hewlett Packard 8453 spectrophotometer equipped with a diode array detector. Samples were first centrifuged at 14,000 rpm in a microcentrifuge in 1.5-ml polypropylene microcentrifuge tubes. Supernatants were used directly for spectrophotometric analyses. Red 2 was also measured using a high performance liquid chromatography (HPLC) Hewlett Packard Series 1100 unit equipped with a diode array detector and a Waters Spherisorb C-18 (250 mm x 4.6 mm I.D.) column. The ion-pairing chromatography technique was used and the mobile phase consisted of 50/50% (v/v) methanol/water and 0.005 M tetrabutylammonium hydroxide as the ion-pairing reagent. Isocratic elution with a flow rate of 0.8 ml/min was used.

RESULTS AND DISCUSSION

Decolorization of Reactive Blue 7

Commercial quality Reactive Blue 7 – a Cu-phthalocyanine, monochlorotriazinyl dye – was used in this test after it was hydrolyzed. The batch decolorization assay was performed using inoculum from the microaerophilic and halotolerant culture amended with 250 mg/L Blue 7, 1,000 mg/L glucose and 20 mg/L yeast extract, 100 g/L NaCl and incubated under anoxic conditions. Figure 2A shows that the halotolerant culture achieved 65.2% color removal in 19 d of incubation at an initial decolorization rate of 33 mg/L-d. In order to assess the effect of culture activity and maintenance of anoxic conditions on further decolorization of Blue 7, the culture was fed with only the glucose/yeast extract mixture and incubated for a total of 39 d. During the extended incubation period, slow decolorization of Blue 7 was observed and reached an extent of decolorization equal to 69.2% after a total 39 d of incubation (Figure 2A). Similar results were obtained with the anaerobic, methanogenic culture where the decolorization of Blue 7 was tested in the absence of salt (Figure 2B). Under methanogenic conditions, the initial decolorization rate was 14 mg/L-d and after 41 d of incubation, the extent of color removal reached 59.7%. Addition of organic feed to the methanogenic culture and extension of the incubation period for another 42 d did not result in further decolorization of Blue 7 (data not shown). The pH and oxidation-reduction potential (ORP) in the halotolerant and the methanogenic cultures were 8.2/-494 mV and 7.4/-375 mV, respectively. Therefore, the halotolerant culture performed better than the methanogenic culture, in terms of both the rate and extent of decolorization of Blue 7, perhaps as a result of a lower redox potential. The absorbance ratio values (A664/A620) for both assays are also shown in Figure 2. The change in the absorbance with increasing incubation time indicates that the observed color removal was due to dye transformation and not to sorption onto biomass, in which case the absorbance ratio values would have remained unchanged.

In the decolorization assays conducted with both the methanogenic and the halotolerant culture, significant concentrations of residual color remained. Chemical decolorization assays of Blue 7 using sodium dithionite as the reducing agent confirmed that the observed residual color was the result of partial oxidation of a reduced intermediate upon exposure to air, indicating that the reduction and decolorization of Blue 7 are partially reversible. To overcome the partial decolorization of phthalocyanine dyes, other means should be investigated in conjunction with the biological decolorization method developed in the present research project. In contrast to these results, decolorization of the monoazo, dichlorotriazinyl reactive dye Red 2 at an initial rate of 224 mg/L-d and to an extent greater than 95% at a salt concentration of 100 g/L was achieved with the
same halotolerant culture, indicating the relative stability of phthalocyanine dyes as compared to the azo dyes.

Figure 2. Color profile and absorbance ratio during the Blue 7 batch decolorization assays with the halotolerant culture under anoxic conditions (A) and the methanogenic culture (B).

Performance of the Continuous-Flow, Fixed-Film Reactor

After 4 months of operation without any dye addition, the reactive Red 2 azo dye was added to the reactor feed at a concentration of 200 mg/L. First, the reactor was operated at a hydraulic retention time of 2.25 h. The steady-state DO concentration in the bulk of the reactor contents was maintained at 0.1±0.05 mg/L. Under these conditions, the steady-state effluent color concentration was 25±5 mg/L. In an effort to assess the effect of bulk reactor DO concentration on the decolorization of Red 2, the DO concentration was raised to 4.5 mg/L. As seen in Figure 3A, with the increase of the DO level, the effluent color concentration increased quickly and approached that of the feed solution (200 mg/L). A subsequent decrease and then increase of the bulk DO level resulted in lower and then higher effluent color concentration, respectively (Figure 3A). These results demonstrate that the degree of decolorization achieved by the fixed-film reactor was primarily controlled by the bulk DO concentration. The kinetics of Red 2 decolorization by the fixed-film reactor were also assessed by stopping the continuous feeding of this reactor and operating it as a batch reactor for a short time with a bulk DO concentration below 0.05 mg/L (Figure 3B). Under these conditions, the initial rate of Red 2 decolorization was 1,858 mg/L-d. This is the fastest decolorization rate achieved in this study.
Figure 3. Red 2 profiles attained by the continuous-flow, fixed-film reactor after deliberate changes of the dissolved oxygen (DO) level (A) and Red 2 profile during the batch testing of the same reactor (B).

For comparison purposes, decolorization of Red 2 at an initial rate of 224 mg/L·d was achieved with the suspended-growth, halotolerant culture. The fast decolorization kinetics achieved by the fixed-film reactor are attributed to localized, low redox conditions within the biofilm. Operation of the fixed-film reactor at a hydraulic retention time of 4.5 h and a bulk DO concentration of 0.05 mg/L resulted in a steady-state effluent color concentration of 25 mg/L. These results show that due to the fast dye decolorization kinetics, a high degree of decolorization was achieved by the fixed-film reactor at very low hydraulic retention times as long as the reactor bulk DO concentration was maintained below 0.1 mg/L. Operation at low hydraulic retention times allows relatively high flow rates, thus a significant reactor throughput can be achieved with a relatively small reactor volume.

Repetitive Dyeing Tests with a Biologically Decolorized Dyebath

During the repetitive decolorization of the spent Red 2 solutions, fast decolorization kinetics with very low levels of residual color were achieved. A detailed characterization of the first decolorization cycle was performed and results are presented in Figure 4A. Dissolved organic carbon removal was mainly achieved during the aerobic incubation period (last 27 h). The initial and final pH in the culture were 7.9/8.9. The ORP value during the anoxic incubation period (first 41 h) was -520 mV. In the first 14 h of the anoxic incubation period about 90% of the initial Red 2 was removed. During the subsequent aerobic incubation period further color removal was observed resulting in a total color removal of 96% as determined by absorbance measurements. Further analysis of the decolorized solution revealed that all the residual color was due to the decolorization products, not Red 2. Therefore, the halotolerant culture achieved 100% transformation of Red 2. A total of five decolorization/dyeing cycles were conducted. It is noteworthy, that the residual color after each decolorization cycle remained relatively constant with no build up (see Figure 4B). On the other hand, dissolved organic carbon increased after each decolorization cycle, as a result of a build up of Red 2 decolorization products.
Figure 4. Profiles of Red 2, dissolved organic carbon (DOC) and acetate during the decolorization of the first-cycle spent dyebath (A) (anoxic period: 41 h; aerobic period: 27 h) and initial Red 2, final Red 2 and DOC levels as a function of decolorization cycle (B).

Table 1 shows the color data and dye exhaustion values for each experimental dyeing with the renovated dyebath. The coefficient of variation of the XYZ tristimulus values were less than 5% showing that there was not much statistical difference between the standard dyeings and the experimental dyeings. Statistical analysis using a t-test showed that there was no significant difference at the 1% and 5% value between the standard and experimental XYZ values. The XYZ tristimulus values were used to calculate the ΔEcmc value for standard and experimental dyeings (the ΔEcmc value is a single number defining the total color difference in CMC units of a sample from a standard). The ΔEcmc data for each dyeing fall within the acceptable limit of less than one ΔEcmc units according to the AATCC Test Method 173-1992 (see Table 1).

Experimental swatches were identified as being too light or too dark when compared to the standard swatch. A negative ΔLCmc value indicates a lighter shade and a positive ΔLCmc value indicates a darker shade. The Y tristimulus value also indicates lightness-darkness. A Y value greater than the standard Y value of 19.80 gives a lighter shade swatch; whereas, a Y value less than 19.80 gives a darker shade swatch. In all shades (except for cycle four), the experimental dyeings produced a higher Y value than the standard dyeings. Thus, the shades for the experimental dyeings were slightly lighter. This could be due to overall differences in pH. The initial and final pH values for standard dyeings were 5.97 and 10.71, respectively. The initial and final pH values for the experimental dyeings were 7.76 and 9.01, respectively. Therefore, the lower experimental pH values may have resulted in lower dye fixation, which in turn may be responsible for the observed higher Y values (lighter shades). Differences in chroma (ΔC) and hue (ΔH) values show that fabric samples from the first and second experimental dyeing to be duller than the standard and samples from the third, fourth and fifth experimental dyeings to be brighter than the standard. Therefore, the observed color differences are not systematic and related to the reuse of the same spent dyebath, but they are rather due to experimental differences.

Table 1. Data of fabric color after dyeing with the standard and a renovated/reused Red 2 spent dyebath.
Figure 5 shows the reflectance values for the standard and experimental samples. These data show that the experimental dyeings are very close to the standard reflectance curve. All samples reflect mostly red light or light of longer wavelengths than the yellow or blue region. The less light they absorb or the higher the reflectance factor, the lighter they look. It can be seen that all samples lie above the standard curve giving a lighter shade, except sample no. 4 which lies below the standard curve thus giving a darker shade.

The exhaustion values for each dyeing are not significantly different with the exception of dyeing 5. Experimental dyeings one through four resulted in an overall exhaustion of approximately 79%, whereas dyeing 5 resulted in a slightly lower exhaustion of 75%. This difference in exhaustion can be seen in the higher ∆Ecmc value for sample 5 (∆Ecmc = 0.93; see Table 1). While this value is less than the acceptable ∆Ecmc limit of one, it is higher than the ∆Ecmc values obtained with the other four experimental dyeings. Therefore, the difference in dye exhaustion resulted in a lighter shade of fabric color. Overall, these results demonstrate that the biological renovation and reuse of the same dyebath in five repetitive dyeings did not adversely affect the dyeing process and resulted in practically identical color of cotton twill fabric to that obtained with standard dyeings.

CONCLUSIONS
Color removal of reactive dyebaths prepared in the laboratory by simulating typical dyebath conditions was achieved under both methanogenic and non-methanogenic conditions. A halotolerant
culture maintained under microaerophilic conditions and at a salt concentration of 100 g/L achieved fast decolorization kinetics under anoxic conditions when it was first exposed to Red 2. Decolorization of Red 2 with a continuous-flow, fixed-film reactor maintained under microaerophilic conditions and with an influent salt concentration of 100 g/L achieved over 95% decolorization of Red 2 when operated at relatively short hydraulic retention times (2.25 and 4.5 h), thus demonstrating the potential of this bioreactor system for fast decolorization of spent reactive dyebaths. A plant spent dyebath composed of three commercial dyes was successfully decolorized by the methanogenic culture, in spite the severe inhibition of methane production. Blue 7 – a Cu-phthalocyanine dye – was biologically decolorized using both a methanogenic and a microaerophilic and halotolerant culture, but due to a partial reversibility of the decolorization upon exposure to air, the extent of decolorization of this dye was less than 70%. Dyeing tests conducted with Red 2 standard solutions and the same spent Red 2 dyebath biologically decolorized and reused resulted in a practically identical color of cotton twill fabric. Overall, the results of this study have demonstrated that biological decolorization of spent reactive dyebaths is feasible under a wide range of redox conditions and reuse of the renovated process water in subsequent dyeings results in an acceptable fabric color.

**DISSEMINATION OF PROJECT RESULTS**


