Fed-Batch Bioreactor Strategies for Microbial Decolorization of Azo Dye Using a Pseudomonas luteola Strain

J o-Shu Chang* and Yu-Chih Lin
Department of Chemical Engineering, Fengchia University, Taichung, Taiwan, Republic of China

A Pseudomonas luteola strain possessing azoreductase activity was utilized to decolorize a reactive azo dye (C.I. Reactive Red 22) with fed-batch processes consisting of an aerobic cell growth stage and an anaerobic fed-batch decolorization stage. The fed-batch decolorization was conducted with different agitation and aeration rates, initial culture volumes, dye loading strategies, and yeast extract to dye (Y/D) ratios, and the effect of those operation parameters on azo dye decolorization was evaluated. Dissolved oxygen strongly inhibited the azo reduction activity; thus aeration should be avoided during decolorization but slight agitation (around 50 rpm) was needed. With the periodical feeding strategy, the specific decolorization rate (v_dye) and overall decolorization efficiency (η_dye) tended to increase with increasing feeding concentrations of dye, whereas substrate inhibition seems to arise when the feeding concentration exceeded 600 mg dye L⁻¹. In the continuous feeding mode, higher initial culture volume resulted in better η_dye due to higher biomass loading, but lower v_dye due to lower dye concentration in the bioreactor. With a volumetric flow rate (F) of 25 mL/h, both v_dye and η_dye increased almost linearly with the increase in the loading rate of dye (F_dye) over the range of 50–200 mg/dye L⁻¹, while further increase in F_dye (400 mg/h) gave rise to a decline in v_dye and η_dye. As the F was doubled (50 mL/h), the v_dye and η_dye increased with F_dye only for F_dye < 80 mg/h. The best v_dye (113.7 mg dye g cell⁻¹ h⁻¹) and η_dye (86.3 mg dye L⁻¹ h⁻¹) were achieved at F_dye = 200 mg/h and F = 25 mL/h. The yield coefficient representing the relation between dye decolorized and yeast extract consumed was estimated as 0.8 g/g. With F_dye = 75 mg/h, the Y/D ratio should be higher than 0.5 to ensure sufficient supply of yeast extract for stable fed-batch operations. However, performance of the fed-batch decolorization process was not appreciably improved by raising the Y/D ratio from 0.5 to 1.875 but was more sensitive to the changes in the dye loading rate.

Introduction
Color removal from industrial effluents has been a major concern in wastewater treatment, especially for the wastewater that originates from dyestuff and textile plants which continuously discharge a great quantity of remaining dyes into the environment (1–3). The most commonly used synthetic dyes in Taiwan have been the azo dyes, which usually could not be removed effectively with conventional aerobic biological treatment units because they are recalcitrant to the majority of microbial genus under aerobic conditions (1, 2). As a consequence, traditional biological processes were often combined with physical and chemical treatments to achieve a better color removal efficiency (3). However, implementation of physical/chemical methods may be costly and often generates a significant amount of sludge or easily causes secondary pollution due to excessive chemical usage. Other emerging techniques, such as ozonation, Fenton’s reagent (3), electrochemical destruction (4, 5), and photocatalysis (6) may have the potential for decolorization, but they usually involve complicated procedures or are economically unfeasible. Apparently, there is still a demand for alternative dye decolorization approaches, such as microbial decolorization processes (1), for a more natural and complete cleanup of the pollutants in a more economical way.

Decolorization of azo dyes by bacterial strains was typically initiated by azoreductase-catalyzed anaerobic reduction or cleavage of azo bonds (7). The resulting metabolites could further be degraded under aerobic or anaerobic conditions (8, 9) by a mixed bacterial community (1, 10, 11). Therefore, an aerobic–anaerobic sequential environment was proposed for bacterial degradation or mineralization of azo dyes (12). With their ligninolytic degradation activity, wood-rotting fungi (e.g., Phanerochaete chrysosporium) have been found to effectively degrade a variety of azo dyes under aerobic conditions, primarily with the aid of fungal lignin peroxidase (13). A novel Penicillium isolate (ATCC 74414) was also found to aerobically decolorize polymeric dyes (14). Hence, the dye-degrading fungi have frequently been used in bioreactors for the decolorization and degradation of azo dyes (12, 15–17), despite the fact that decolorization by fungi is normally very time-consuming (12). Unlike the fungal decolorization system, bacteria-based color-removal processes have seldom been studied. Moreover, the existing bioreactor studies for bacterial decolorization have been performed exclusively with mixed bacterial consortia, such as activated sludge...
systems (12). The results obtained from the mixed-culture studies may be more comparable to practical situations, but they could only provide an averaged macroscopic view of what occurs in the culture and are poorly reproducible, making it very difficult to interpret the data. Therefore, the present work used a pure-culture system to ensure reproducibility of data, easier interpretation of the experimental observations, and more importantly, clear quantitative details of how the kinetics of azo-dye decolorization responds to changes of operation parameters in bioreactors.

The bacterial strain used in this study was a Pseudomonas luteola strain which was shown to remove the color of an azo dye from solutions via azoreductase-driven biotransformation (18, 19). A reactive azo dye (C. I. Reactive Red 22) that is currently used in a dyeing factory located in central Taiwan was chosen as the dye substrate for this study. The feasibility of utilizing the strain in practical decolorization processes was assessed with bioreactor studies. Since the oxygen requirements for cell growth and decolorization are completely opposite, chemostat operations that require simultaneous cell growth and decolorization seem less feasible. Instead, we applied a fed-batch process which enables batch growth of the cells under aerobic conditions, followed by a subsequent switch to anaerobic conditions for decolorization with appropriate feeding strategies of substrates. The dependence of decolorization efficiency on agitation/aeration rates, initial culture volumes, mercury loading strategies, and yeast extract supply strategies was investigated for the fed-batch bioprocesses. Experimental results obtained from this study were used to evaluate the feasibility of the microbial process for azo dye decolorization and to provide valuable information for further improvement of the bioprocess.

**Experimental Methods**

**Microorganism and Cultivation.** Pseudomonas luteola was isolated from an activated-sludge system utilized to treat dyed wastewater from a dyeing factory located in central Taiwan (18). The strain was found to express azoreductase activity and was able to decolorize a variety of azo dyes via a pathway initiated by enzymatic fission of azo bonds (18). The products resulting from the bacterial decolorization of azo dye were typically aromatic amines (18–20). The P. luteola strain was cultivated aerobically at 28 °C with a YG medium, typically containing 1.25 g/L of glucose (Difco) and 3 g/L of yeast extract (Difco). Normally, 200 mg/L of azo dye was incorporated with the YG medium to induce the azoreduction activity of the P. luteola strain (18).

**Analytical Methods.** The reactive azo dye used in this study was C. I. Reactive Red 22 (see Figure 1 for its chemical composition), which was obtained from Sumitomo, Inc. (Tokyo, J. Japan). The concentration of Reactive Red 22 in samples was determined by measuring the optical density (OD) of the supernatant of the sample at 510 nm with a UV–vis spectrophotometer (J ASCO model V-530). The dissolved oxygen levels in the culture were measured with an oxygen meter (WTW, model Oxi538).

**Decolorization at Different Agitation and Aeration Rates.** Pseudomonas luteola cells were cultivated aerobically with a 5-L fermentor (Eylea jar Fermentor MDF) equipped with devices that measure and control temperature, dissolved oxygen level (DO), pH, and agitation speed. The growth medium was YG medium amended with 200 mg/L Reactive Red 22. The cultivation temperature and agitation speed were controlled at 28 °C and 450 rpm, respectively. The air flow rate was set at 1 vvm (1 vol of air/vol of medium/min). To evaluate the effects of the agitation and aeration rates on the decolorization of Reactive Red 22, aerobic growth was terminated at early-stationary phase (approximately 1.0–1.5 g cell/L) and decolorization of the azo dye was performed with a range of agitation (0–200 rpm) and aeration (0–0.05 L/min) rates. The concentrations of cells, Reactive Red 22, and dissolved oxygen were monitored as a function of time. Each decolorization culture was supplemented with an appropriate amount of yeast extract to maintain the metabolic activity of P. luteola for the generation of the reduced electron carrier (NADH), which acts as a cofactor of azoreductase. Typically, 1 g of yeast extract was supplied for the addition of 0.8 g of Reactive Red 22.

**Fed-Batch Decolorization under Sequential Aerobic–Anaerobic Environments.** Aerobic Cell Growth and Pretreatment of the Seed Culture. The seed culture was prepared by cultivation of P. luteola in a 5-L jar fermenter at 28 °C with YG medium containing 200 mg/L Reactive Red 22. In general, the working volume of the seed culture was 2 L. The agitation and air aeration rates for the aerobic growth were 450 rpm and 1 vvm, respectively. As the seed culture reached the desirable cell density (approximately 1.0 g cell/L), the aeration and agitation rates were switched to those suitable for decolorization (aeration rate = 0, agitation rate = 50 rpm). Prior to being used for fed-batch operations, an appropriate amount of dye (typically 200 mg/L) was added into the seed culture to carry out decolorization of Reactive Red 22 in batch mode until the color in the reactor was completely removed. The main purpose of the pretreatment procedures was to stimulate the activity of azoreductase, which is reportedly inducible by the azo dyes (19). In addition, the nutrients in YG medium that were not completely consumed during aerobic cell growth could be utilized further during the adaptation procedures so that the subsequent fed-batch experiments would be started with a clear background without possible interference by the remaining nutrients from the growth medium.

**Fed-Batch Operations with a Periodical Dye Loading.** Previous studies (20) show that dissolved oxygen in the culture inhibits the decolorization of Reactive Red 22 in shake flasks. The fed-batch culture was thus operated without the supply of air, while a gentle agitation (50 rpm) was sustained to improve the mass transfer efficiency. Due to the above conditions, the typical DO level in the culture was around 0.1 mg/L. A fixed amount of filter-sterilized Reactive Red 22 (ranging from 200 to 600 mg/L) was added into the reactor containing the aforementioned 2-L seed culture at designated time intervals, until the total dye loading was 1.2 g. During the course of the operation, the yeast extract was supplied with a yeast extract to dye weight ratio (Y/D) of 1.25. The concentrations of cells and the azo dye in the reactor were measured as a function of time.
Fed-Batch Operations with a Continuous Dye Loading. The procedures were similar to those used for the periodical-loading experiments, except that the dye and yeast extract were continuously added into the reactor (initial culture volume = 2.0 L) at a constant rate of 25 or 50 mL/h, until a terminal volume of 4 L was reached. In the experiments with a fixed Y/D ratio of 1.25, the azo-dye loading rates ranging from 25 to 400 mg/h were examined. In the experiments with different Y/D ratios, the loading rate of dye was kept constant at 75 mg/h, while the loading rate of yeast extract was adjusted to achieve a Y/D ratio of 0, 0.5, 1.25, and 1.875. In the experiments with various initial culture volumes, the fed-batch operation was started with an initial culture volume of 1.5, 2.0, and 2.5 L with a constant dye feeding rate of 75 mg/h and a Y/D ratio of 1.25. For all experiments, concentrations of cells and the residual dye were monitored as a function of time.

Results and Discussion

Effects of Agitation and Aeration Rates on Decolorization of Reactive Red 22. It is known that azaeductase-driven bacterial decolorization of azo dyes is normally inhibited by the presence of oxygen primarily due to the competition in the oxidation of reduced electron carriers (e.g., NADH) with either oxygen or azo groups as the electron receptor (1). Consequently, in the bioreactor design, the aeration and agitation that provide oxygen for cell growth and stimulate the contact of the cells with medium substrates should be properly regulated to attain an optimal efficiency of decolorization. For this reason, the influence of agitation and aeration rates on the performance of the bioreactor was examined in batch mode. As no aeration was employed, the dissolved oxygen level in the culture was negligible for an agitation rate ranging from 0 to 100 rpm. Under these conditions, decolorization of the dye did occur, as indicated by the decrease in residual dye (regions A–C in Figure 2). The DO level rose slightly during the operation period of 40–50 h (agitation rate = 100 rpm), leading to a slower decolorization rate (region C in Figure 2). Further increasing of agitation rate to 200 rpm (region D in Figure 2) resulted in a visible increase in dissolved oxygen level from 0.2 to 1.5 mg/L; decolorization of Reactive Red 22 stopped correspondingly. As the aeration was provided at a rate of 0.05 L/min, the DO level increased abruptly from 1.5 to approximately 4.0 mg/L, and no decolorization of the azo dye was observed. The results in Figure 2 indicate that the bacterial decolorization was very sensitive to DO; thus for an efficient color removal, the agitation rate should be below 100 rpm, and aeration should be avoided.

Fed-Batch Operations with a Periodical Feeding of Reactive Red 22. In this study, the performance of decolorization of Reactive Red 22 with P. luteola was assessed with two parameters: specific decolorization rate (vdye) and overall decolorization efficiency (ηdye). The vdyec value is determined by dividing the fastest slope of the time profiles of residual dye by the total dry cell weight in the reactor; thus vdyec represents the ultimately achievable decolorization capability of P. luteola in the bioreactor on the cell-mass basis. On the other hand, ηdyec is an indication of the capacity of the bioreactor for decolorization of the azo dye, as it is defined as total amount of the dye decolorized per total volume treated per total operation time.

Decolorization results with pulse feeding of 210, 300, and 600 mg/L of Reactive Red 22 at designated time intervals are compared in Table 1. The identical amount of total dye loading (around 1.2 g) fed with 300 mg/L every 4 h exhibited a better specific decolorization rate (107.8 mg dye g cell\(^{-1}\) h\(^{-1}\)) and overall decolorization efficiency (75 mg dye L\(^{-1}\) h\(^{-1}\)) than those obtained from the other two pulse feeding strategies. Pulse feeding with 210 and 600 mg/mL resulted in similar vdyec values (49.4 and 45.8 mg dye g cell\(^{-1}\) h\(^{-1}\), respectively) and ηdyec values (40 and 37.5 mg dye L\(^{-1}\) h\(^{-1}\), respectively). Table 1 indicates the impact of the dye concentration on the decolorization performance of the bioreactor. Higher dye concentration in the culture seems to be the cause for the higher vdyec that resulted from a pulse feeding of 300 mg/L, in contrast to that obtained from a feeding of 210 mg/L. However, increasing the concentration of pulse feeding to 600 mg/L did not result in better vdyec, suggesting that a substrate-inhibition effect may arise from an excess loading of the dye. This is consistent with our previous observation with shake-flask decolorization experiments in which the rate of decolorization was optimal at an initial Reactive Red 22 concentration of approximately 400 mg/L, above which the rate declined considerably (data not shown).

Due to low DO levels (no aeration, low agitation rates) and limited carbon and nitrogen sources (only yeast extract was provided), there was essentially no cell growth during the course of fed-batch operation; thus total cell mass in the bioreactor was virtually unchanged throughout the experiments (data not shown). It is also noted from Figure 2 (regions A–C) that the residual dye profile resulting from periodical feeding decreased at a similar rate; thus the vdyec remained nearly constant during the majority of the operation period. As the cell concentration and specific decolorization rate remained nearly constant, higher vdyec gave rise to better ηdyec values, which is exactly what we observe from Table 1.


Figure 2. Decolorization of Reactive Red 22 by Pseudomonas luteola at different agitation and aeration rates: (A) no aeration, no agitation; (B) no aeration, agitation at 50 rpm; (C) no aeration, agitation at 100 rpm; (D) no aeration, agitation at 200 rpm; (E) aeration at 0.05 L/min, agitation at 100 rpm. •: Residual dye concentration. □: Dissolved oxygen level. All points in figures are averages from duplicate or triplicate experiments. Typical variations of the data were in a range of 2–6%.

Fed-Batch Operations with a Periodical Feeding of Reactive Red 22. In this study, the performance of decolorization of Reactive Red 22 with P. luteola was assessed with two parameters: specific decolorization rate (v_dye) and overall decolorization efficiency (eta_dye). The v_dye value is determined by dividing the fastest slope of the time profiles of residual dye by the total dry cell weight in the reactor; thus v_dye represents the ultimately achievable decolorization capability of P. luteola in the bioreactor on the cell-mass basis. On the other hand, eta_dye is an indication of the capacity of the bioreactor for decolorization of the azo dye, as it is defined as total amount of the dye decolorized per total volume treated per total operation time.

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et al. (2) found that the rate of azo reduction was sensitive to respiration substrates under anaerobic conditions, since decolorization of C. I. Reactive Red 141 as a sole carbon source gave a decolorization rate lower than the rate measured in the presence of a supplemental labile carbon source (such as glucose). They attributed this finding to the fact that catabolism of the respiration substrate is responsible for the production of reducing power required for azo reduction. Our recent work (19) showed that addition of yeast extract significantly enhanced the anaerobic decolorization of Reactive Red 22, whereas glucose, an efficient carbon source for the aerobic growth of P. luteola, strongly inhibited the microbial decolorization. The inhibitory effect of glucose most likely resulted from the catabolism repression provoked by intracellular glucose content (21). However, the glucose repression effect found in P. luteola is not universal, since Haug et al. (10) reported that addition of glucose into a bacterial consortium enhanced the decolorization of a group of sulfonated azo dyes. Due to its inhibitory effects, glucose was not supplemented to the decolorization culture. Alternatively, yeast extract was used as the sole supplemental respiration substrate to maintain the decolorization activity.

In an attempt to obtain quantitative correlation between consumption of yeast extract versus decolorization of Reactive Red 22, the dye was loaded at a constant rate of 75 mg/h into a 2-L culture (cell concentration was 0.9 g/L) containing approximately 900 mg/L of yeast extract. (a) time profiles of cumulative dye loading (●); (b) time profiles of the cumulative amount of dye loaded (□) and the cumulative amount of dye decolorized (●).

**Table 1. Fed-Batch Decolorization of Reactive Red 22 by Pseudomonas luteola with Periodical Feeding Strategies**

<table>
<thead>
<tr>
<th>dye feeding strategy</th>
<th>dye concn in feed (mg/L)</th>
<th>amt of dye added/reactor vol in each feeding (mg/L)</th>
<th>tot amt of dye loaded (mg)</th>
<th>specific decolor. rate; ( \eta_{dye} ) (mg g cell (^{-1} ) h(^{-1} ))</th>
<th>overall decolor. efficiency; ( \eta_D ) (mg L(^{-1} ) h(^{-1} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>add 14 mL every 5 h</td>
<td>30 000</td>
<td>210</td>
<td>1260</td>
<td>49.4</td>
<td>42.0</td>
</tr>
<tr>
<td>add 20 mL every 4 h</td>
<td>30 000</td>
<td>300</td>
<td>1200</td>
<td>107.8</td>
<td>75.0</td>
</tr>
<tr>
<td>add 40 mL at t = 0</td>
<td>30 000</td>
<td>600</td>
<td>1200</td>
<td>45.8</td>
<td>37.5</td>
</tr>
</tbody>
</table>

\( ^a \)Initial volume = 2.0 L; \( Y/D \) ratio = 1.25; initial cell concentration = 0.9–1.0 g/L.

**Table 2. Fed-batch Decolorization of Reactive Red 22 by Pseudomonas luteola with Continuous Feeding Strategies: Effect of Initial Culture Volume**

<table>
<thead>
<tr>
<th>init. culture vol (L)</th>
<th>term. culture vol (L)</th>
<th>dye loading rate (mg/h)</th>
<th>total amt of dye loaded (g)</th>
<th>dye concn at end of feeding (mg/L)</th>
<th>specific decolor. rate; ( \eta_{dye} ) (mg g cell (^{-1} ) h(^{-1} ))</th>
<th>overall decolor. efficiency; ( \eta_D ) (mg L(^{-1} ) h(^{-1} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>4.0</td>
<td>75</td>
<td>7.5</td>
<td>100</td>
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<td>28.4</td>
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<tr>
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<td>4.0</td>
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<td>6.0</td>
<td>75</td>
<td>43.2</td>
<td>35.6</td>
</tr>
<tr>
<td>2.5</td>
<td>4.0</td>
<td>75</td>
<td>4.5</td>
<td>63</td>
<td>35.5</td>
<td>47.2</td>
</tr>
</tbody>
</table>

\( ^a \)Initial cell concentration = 0.9–1.0 g/L; \( Y/D \) ratio = 1.25; volumetric flow rate = 25 mL/h.

**Figure 3.** Fed-batch decolorization with a constant-rate feeding (75 mg/h) of Reactive Red 22 into a 2-L initial culture containing 900 mg/L of yeast extract. (a) time profiles of cumulative dye loading (○) and residual dye concentration (●); (b) time profiles of the cumulative amount of dye loaded (□) and the cumulative amount of dye decolorized (●).

quantitative assessment of how the consumption of a respiration substrate is related to color removal of the azo dye.

**Effect of Initial Culture Volume.** Since essentially no cell growth occurs under decolorization conditions due to the low DO level, an increase in initial culture volume \( V_o \) indicates elevation of biomass loading for the decolorization process. Three initial culture volumes (1.5, 2.0, and 2.5 L) were examined for their performance on decolorization of Reactive Red 22 with a dye loading rate of 75 mg/L. The results are presented in Table 2, which shows that the specific decolorization rate \( \eta_{dye} \) decreased with an increase in \( V_o \) while an opposite trend was observed for the overall decolorization efficiency \( \eta_D \). It can be expected that \( \eta_{dye} \) value increased with increasing \( V_o \) since more biocatalyst (P. luteola cells) is present.
A volumetric flow rate was used, higher a lower decolorization rate. Likewise, when an identical dilution of the dye concentration in the culture, resulting feeding with a higher volumetric flow rate led to a faster enhancement on the decolorization rate and efficiency. Nevertheless, increasing the specific decolorization rate $V_{\text{dye}}$ and overall decolorization efficiency $\eta_{\text{dye}}$ on $F_{\text{dye}}$ and $F$ is illustrated in Figure 4. Inspection of Table 3 and Figure 4 shows that for $F = 25$ mL/h, the $V_{\text{dye}}$ and $\eta_{\text{dye}}$ values increased almost proportionally to the increase in dye loading rates, except for $F_{\text{dye}}$ higher than 200 mg/h. Increasing $F_{\text{dye}}$ to 400 mg/h caused a considerable drop in both $V_{\text{dye}}$ and $\eta_{\text{dye}}$ values, which may be attributed to the substrate inhibition, as the residual dye concentration rose to nearly 5900 mg/L (Table 3). In contrast, if the volumetric flow rate was doubled, the $V_{\text{dye}}$ and $\eta_{\text{dye}}$ values increased linearly with dye loading rates only for $F_{\text{dye}}$ lower than 80 mg/h, above which the increase in $V_{\text{dye}}$ and $\eta_{\text{dye}}$ values was limiting. Moreover, with the same dye loading rate, the two volumetric rates resulted in similar $V_{\text{dye}}$ and $\eta_{\text{dye}}$ values at low dye loading rates, whereas much better $V_{\text{dye}}$ and $\eta_{\text{dye}}$ Values resulted from a lower $F$ (25 mL/h) when $F_{\text{dye}}$ was larger than 80 mg/h (Figure 4).

Assessment of the effect of dye loading rate and volumetric flow rate on the performance of decolorization, as illustrated in Figure 4, shows that the key factor was the residual dye concentrations. For the same $F_{\text{dye}}$, feeding with a higher volumetric flow rate led to a faster dilution of the dye concentration in the culture, resulting in a lower residual dye concentration, and, consequently, a lower decolorization rate. Likewise, when an identical volumetric flow rate was used, higher $F_{\text{dye}}$ increased the dye concentration in the culture and thus led to the enhancement on the decolorization rate and efficiency. Nevertheless, increasing $F_{\text{dye}}$ did not ensure higher $V_{\text{dye}}$ and $\eta_{\text{dye}}$, since Table 3 shows that elevation of $F_{\text{dye}}$ to 400 mg/h led to overloading of the dye, resulting in decreases in $V_{\text{dye}}$ and $\eta_{\text{dye}}$ due to substrate inhibition. Besides, the final dye concentration ($C_{\text{dye}}$) in the culture (i.e., the dye concentration at the end of feeding) tended to increase with increasing dye loading rates (Table 3). For instance, $C_{\text{dye}}$ increased more than 100-fold if $F_{\text{dye}}$ was raised from 50 to 400 mg/h. Since the remaining dye concentration ($C_{\text{dye}}$) needed to be decolorized further with batch-mode operation to meet the government's requirements for the discharge of the treated water, a longer follow-up treatment would be required for fed-batch processes operated at higher dye loading rates.

### Table 3. Fed-Batch Decolorization of Reactive Red 22 by Pseudomonas luteola with Continuous Feeding Strategies: Effect of Volumetric Flow Rate and Dye Loading Rate

<table>
<thead>
<tr>
<th>vol. flow rate (mL/h)</th>
<th>dye loading rate (mg/h)</th>
<th>total amt of dye loaded (g)</th>
<th>dye concn at end of feeding (mg/L)</th>
<th>specific decolor. rate; $V_{\text{dye}}$ (mg g cell$^{-1}$ h$^{-1}$)</th>
<th>overall decolor. efficiency; $\eta_{\text{dye}}$ (mg L$^{-1}$ h$^{-1}$)</th>
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*Initial volume = 2.0 L; terminal volume = 4.0 L; initial cell concentration = 0.9–1.0 g/L; Y/D ratio = 1.25.

**Figure 4.** Dependence of specific decolorization rate ($V_{\text{dye}}$) and overall decolorization efficiency ($\eta_{\text{dye}}$) on the dye loading rate at a various volumetric flow rates: $\eta_{\text{dye}}$ at (●) 25 and (○) 50 mL/h, and $\eta_{\text{dye}}$ at (●) 25 and (○) 50 mL/h.

**Effect of Yeast Extract to Dye (Y/D) Ratios.** Recent studies (18–20) have shown that P. luteola was able to decolorize azo dyes, such as Red G, RP, and Reactive Red 22, under media containing exclusively yeast extract, and in the absence of any carbon and nitrogen sources. The metabolism of yeast extract is considered essential to the regeneration of NADH (2). It has also been found from shake-flask experiments that increasing yeast extract concentrations led to higher specific decolorization rates (20). Thus, the optimal strategy for the supply of yeast extract should be important in the bioreactor design. As mentioned earlier, a yield coefficient that correlates decolorization of Reactive Red 22 to the consumption of yeast extract was estimated as 0.8 g/g, equivalent to a Y/D ratio of 1.25. Therefore, we regulated the Y/D ratios in the feeding stream to reveal how yeast extract content affected the decolorization rates in fed-batch operations.

The residual dye profiles resulting from fed-batch bioreactors operated at different Y/D ratios are shown in Figure 5, and the kinetic characteristics of each fed-batch decolorization run is compared in Table 4. With Y/D = 0, decolorization occurred for the first 18 h, after which the residual dye concentration rose sharply, and 10 hours later, the residual dye and cumulative dye loading profiles increased at the same rate, indicating that the decolorization had stopped (Figure 5a), primarily due to the lack of yeast extract available to enable the azoreductase activity. Although yeast extract was ex-
enhanced the rate (\(v_{\text{ratio}}\) exceeded 0.5, further increase in extract at feeding (Figure 5a), suggesting that the supply of yeast decolorization operation for 50 h. No sharp increase in supply of yeast extract was critical to the stability of the increased to 1.25 and 1.875, indicating that adequate data indicated in Table 3 and Figure 4.

Overall efficiency of the bioprocess, as evidenced by the factor that controlled the decolorization rate and the increased to 1.25 and 1.875, indicating that adequate data indicated in Table 3 and Figure 4.

Factors that controlled the decolorization rate and the overall efficiency of the bioprocess, as evidenced by the data indicated in Table 3 and Figure 4.

The performance of the fed-batch bioreactor was closely affected by the initial biomass loading, the feeding strategy, the yeast extract-to-dye ratio (Y/D), but most importantly, the dye loading rate. The best specific decolorization rate and overall decolorization efficiency obtained in this study were 113.7 mg dye g cell\(^{-1}\) h\(^{-1}\) and 86.3 mg dye L\(^{-1}\) h\(^{-1}\), respectively, and both occurred when the dye was fed continuously into an initial culture volume of 2 L with a constant dye loading rate of 200 mg/L and a Y/D ratio of 1.25. To avoid an insufficient yeast extract supply that would depress the decolorization efficiency, the Y/D ratio should be maintained higher than 0.5. The decolorization efficiency can be enhanced significantly by increasing the dye loading rates but was insensitive to the elevation of Y/D ratios. However, dye loading rate should not be increased without limits since substrate inhibition may occur with overloading of the dye. Besides, higher loading rates would result in higher residual dye concentration at the end of feeding, and consequently, a longer follow-up batch decolorization treatment for complete color removal of the treated water.

### Conclusions

Fed-batch bioprocesses using Pseudomonas luteola were shown to effectively decolorize Reactive Red 22. Due to the inhibitory effect of oxygen on the bacterial decolorization, the bioreactor should be operated with gentle agitation and without aeration, but these conditions appeared to limit the cell growth during the course of decolorization. The performance of the fed-batch bioreactor was closely affected by the initial biomass loading, the feeding strategy, the yeast extract-to-dye ratio (Y/D), but most importantly, the dye loading rate. The best specific decolorization rate and overall decolorization efficiency obtained in this study were 113.7 mg dye g cell\(^{-1}\) h\(^{-1}\) and 86.3 mg dye L\(^{-1}\) h\(^{-1}\), respectively, and both occurred when the dye was fed continuously into an initial culture volume of 2 L with a constant dye loading rate of 200 mg/L and a Y/D ratio of 1.25. To avoid an insufficient yeast extract supply that would depress the decolorization efficiency, the Y/D ratio should be maintained higher than 0.5. The decolorization efficiency can be enhanced significantly by increasing the dye loading rates but was insensitive to the elevation of Y/D ratios. However, dye loading rate should not be increased without limits since substrate inhibition may occur with overloading of the dye. Besides, higher loading rates would result in higher residual dye concentration at the end of feeding, and consequently, a longer follow-up batch decolorization treatment for complete color removal of the treated water.

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### References and Notes


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