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

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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. 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/h, 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

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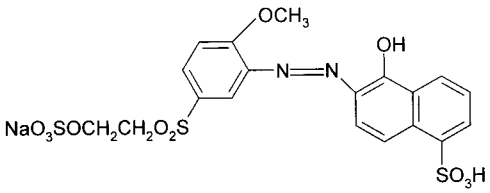

Figure 1. Schematic description of the composition of C. I. Reactive Red 22.

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

Microorgansim 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, 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 (JASCO 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 (Eyela 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.

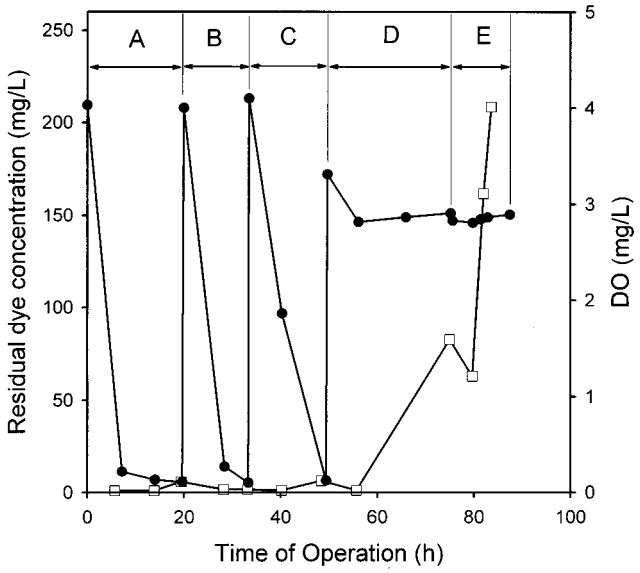

Figure 2. Decolorization of Reactive Red 22 by *Pseudomonas luteola* at different agitation and aeration rates: (A) no aeration, no agitation; (B) no aeration, agitation at 50 rpm; (C) no aeration, agitation at 100 rpm; (D) no aeration, agitation at 200 rpm; (E) aeration at 0.05 L/min, agitation at 100 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 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 azoreductase-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 (v_{dye}) and overall decolorization efficiency (η_{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, η_{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.

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/L resulted in similar v_{dye} values (49.4 and 45.8 mg dye g cell^{-1}h^{-1}, respectively) and η_{dye} 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 v_{dye} 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 v_{dye}, 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 v_{dye} remained nearly constant during the majority of the operation period. As the cell concentration and specific decolorization rate remained nearly constant, higher v_{dye} gave rise to better η_{dye} values, which is exactly what we observe from Table 1.

Fed-Batch Operations with a Continuous Feeding of Reactive Red 22. ***Estimation of Yeast Extract Consumption Versus Azo Dye Decolorization.*** Carliell

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

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

[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[a]

init. culture vol (L)	term. culture vol (L)	dye loading rate (mg/h)	total amt of dye loaded (g)	dye concn at end of feeding (mg/L)	specific decolor. rate; v_{dye} (mg g cell^{-1} h^{-1})	overall decolor. efficiency; η_{dye} (mg L^{-1} h^{-1})
1.5	4.0	75	7.5	100	49.3	28.4
2.0	4.0	75	6.0	75	43.2	35.6
2.5	4.0	75	4.5	63	35.5	47.2

[a] Initial cell concentration = 0.9−1.0 g/L; *Y*/*D* ratio = 1.25; volumetric flow rate = 25 mL/h.

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 substrates 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 initially. No external feeding of yeast extract was provided. The resulting residual dye profiles are shown in Figure 3. For the first 15 h, the residual dye concentration increased at a rate much slower than that of the cumulative dye loading, which signals that decolorization of the dye occurred. The residual dye rose sharply after 15 h of operation, and the rate of its increase became similar to the rate of dye loading (Figure 3a), resulting in the termination of decolorization as the cumulative amount of dye decolorization leveled off after 15 h (Figure 3b). The retardation of decolorization of Reactive Red 22 above 15 h may be ascribed to the depletion of the key components in yeast extract that are essential to the decolorization activity of the *P. luteola* strain after continuous feeding of the dye at 75 mg/h for 15 h. Accordingly, we may obtain a yield coefficient relating yeast extract and dye by calculating the ratio of the amount of azo dye decolorized within the first 15 h and the amount of yeast extract present. The yield coefficient was estimated to be approximately 0.8 g dye/g yeast extract. The information obtained here provides a clear

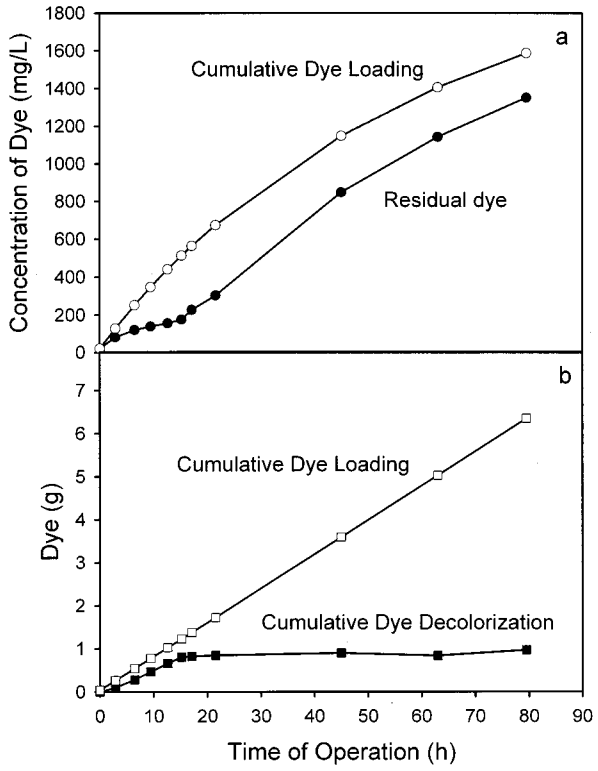

Figure 3. Fed-batch decolorization with a constant-rate feeding (75 mg/h) of Reactive Red 22 into a 2-L initial culture containing 900 mg/L of yeast extract. (a) time profiles of cumulative dye loading (○) and residual dye concentration (●); (b) time profiles of the cumulative amount of dye loaded (□) 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 (v_{dye}) decreased with an increase in V_o, while an opposite trend was observed for the overall decolorization efficiency (η_{dye}). It can be expected that η_{dye} value increased with increasing V_o since more biocatalyst (*P. luteola* cells) is present

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

vol. flow rate (mL/h)	dye loading rate (mg/h)	total amt of dye loaded (g)	dye concn at end of feeding (mg/L)	specific decolor. rate; v_{dye} (mg g cell^{-1} h^{-1})	overall decolor. efficiency; η_{dye} (mg L^{-1}h^{-1})
50	25	1.0	4	15.3	12.3
	50	2.0	42	27.0	22.9
	75	3.0	163	44.9	29.4
	125	5.0	492	49.1	37.9
	200	8.0	976	55.5	51.2
25	50	4.0	48	28.3	23.8
	75	6.0	75	43.2	35.6
	125	10.0	124	67.2	59.4
	200	16.0	548	113.7	86.3
	400	32.0	5868	71.0	53.3

[a] Initial volume = 2.0 L; terminal volume = 4.0 L; initial cell concentration = 0.9−1.0 g/L; *Y/D* ratio = 1.25.

with a larger V_o. On the other hand, larger initial volumes also dilute the added dye to give a lower concentration, which is unfavorable for decolorization according to reaction kinetics, and thus results in lower v_{dye} values.

Effect of Dye Loading Rate (F_{dye}) and Volumetric Flow Rate (F). Table 3 summarizes the operation conditions and the color removal performance of fed-batch bioreactors with dye loading rates (F_{dye}) in the range of 25−400 mg/h and with volumetric flow rates (F) of 25 and 50 mL/h. The dependence of the specific decolorization rate (v_{dye}) and overall decolorization efficiency (η_{dye}) on F_{dye} and F is illustrated in Figure 4. Inspection of Table 3 and Figure 4 shows that for F = 25 mL/h, the v_{dye} and η_{dye} values increased almost proportionally to the increase in dye loading rates, except for F_{dye} higher than 200 mg/h. Increasing F_{dye} to 400 mg/h caused a considerable drop in both v_{dye} and η_{dye} values, which may be attributed to the substrate inhibition, as the final residual dye concentration rose to nearly 5900 mg/L (Table 3). In contrast, if the volumetric flow rate was doubled, the v_{dye} and η_{dye} values increased linearly with dye loading rates only for F_{dye} lower than 80 mg/h, above which the increase in v_{dye} and η_{dye} values was limiting. Moreover, with the same dye loading rate, the two volumetric rates resulted in similar v_{dye} and η_{dye} values at low dye loading rates, whereas much better v_{dye} and η_{dye} values resulted from a lower F (25 mL/h) when F_{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_{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_{dye} increased the dye concentration in the culture and thus led to the enhancement on the decolorization rate and efficiency. Nevertheless, increasing F_{dye} did not ensure higher v_{dye} and η_{dye}, since Table 3 shows that elevation of F_{dye} to 400 mg/h led to overloading of the dye, resulting in decreases in v_{dye} and η_{dye} due to substrate inhibition. Besides, the final dye concentration ($C_{dye,f}$) 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, $C_{dye,f}$ increased more than 100-fold if F_{dye} was raised from 50 to 400 mg/h. Since the remaining dye concentration ($C_{dye,f}$) 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.

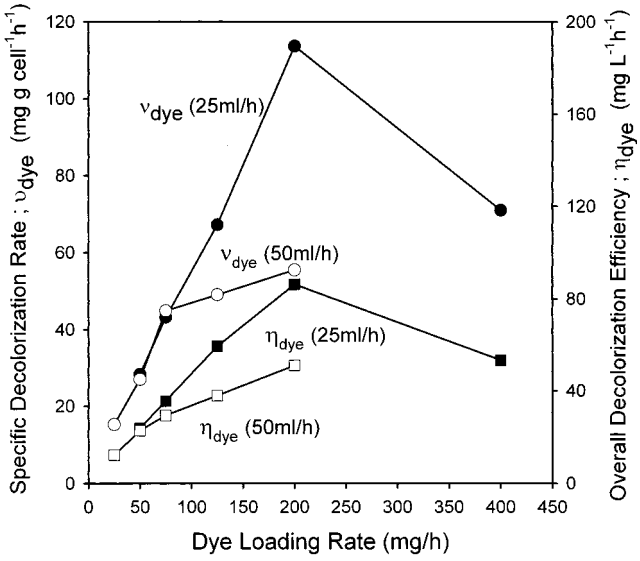

Figure 4. Dependence of specific decolorization rate (v_{dye}) and overall decolorization efficiency (η_{dye}) on the dye loading rate at a various volumetric flow rates: v_{dye} at (●) 25 and (○) 50 mL/h, and η_{dye} at (■) 25 and (□) 50 mL/h.

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_2B, 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-

Table 4. Fed-Batch Decolorization of Reactive Red 22 by *Pseudomonas luteola* with Continuous Feeding Strategies: Effect of *Y/D* Ratio[a]

Y/D ratio	dye loading rate (mg/h)	total amt of dye loaded (g)	dye concn at end of feeding (mg/L)	specific decolor rate; v_{dye} (mg g cell^{-1} h^{-1})	overall decolor. efficiency; η_{dye} (mg L^{-1} h^{-1})
0	75	6.0	1,180	37.0	8.0
0.5	75	6.0	348	44.2	28.8
1.25	75	6.0	75	43.2	35.6
1.875	75	6.0	52	44.6	36.2

[a] Initial volume = 2.0 L; terminal volume = 4.0 L; initial cell concentration = 0.9−1.0 g/L; volumetric flow rate = 25 mL/h.

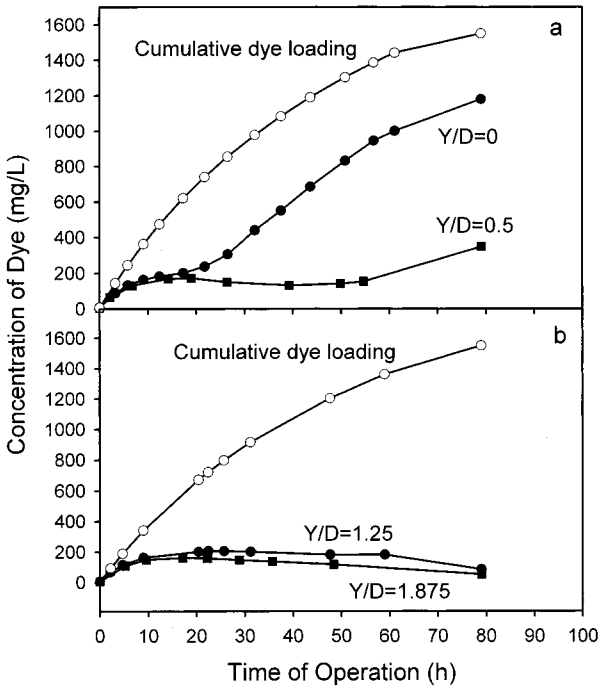

Figure 5. Residual dye profiles resulted from fed-batch decolorization with continuous-feeding strategies under different *Y/D* ratios: residual dye profiles for (a) *Y/D* = 0 (●) and 0.5 (■), and (b) *Y/D* = 1.25 (●) and 1.875 (■). Initial volume = 2.0 L; initial cell concentration = 0.9−1.0 g/L; volumetric flow rate = 25 mL/h; dye loading rate = 75 mg/h.

cluded from the feeding stream, the fed-batch culture still had decolorization activity for a short period of time, which was most likely contributable by the residual nutrients in the seed culture as well as the remaining intracellular metabolic energy and cofactors. However, since the decolorization failed within 20 h because of the shortage of yeast extract supply, the v_{dye} and η_{dye} values were much lower than those obtained from higher *Y/D* ratios (Table 4). As the *Y/D* ratio was increased to 0.5, the sharp rise in residual dye similar to that observed for *Y/D* = 0 still existed, but it took place after 50 h of feeding (Figure 5a), suggesting that the supply of yeast extract at *Y/D* = 0.5 could only maintain a stable decolorization operation for 50 h. No sharp increase in residual dye was observed for nearly 80 h as *Y/D* was increased to 1.25 and 1.875, indicating that adequate supply of yeast extract was critical to the stability of the fed-batch decolorization operations. However, as the *Y/D* ratio exceeded 0.5, further increase in *Y/D* did not enhance the rate (v_{dye}) and overall efficiency (η_{dye}) of decolorization significantly (Table 4). This seems to imply that the concentration of yeast extract was not the rate-limiting factor during the fed-batch operations adapted in this study. Instead, the dye loading rate was the key factor that controlled the decolorization rate and the overall efficiency of the bioprocess, as evidenced by the data indicated in Table 3 and Figure 4.

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/h 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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