

Decolorization of sulfonated azo dyes with two photosynthetic bacterial strains and a genetically engineered *Escherichia coli* strain

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Abstract Sulfonated azo dyes were decolorized by two wild type photosynthetic bacterial (PSB) strains (*Rhodobacter sphaeroides* AS1.1737 and *Rhodopseudomonas palustris* AS1.2352) and a recombinant strain (*Escherichia coli* YB). The effects of environmental factors (dissolved oxygen, pH and temperature) on decolorization were investigated. All the strains could decolorize azo dye up to 900 mg l⁻¹, and the correlations between the specific decolorization rate and dye concentration could be described by Michaelis–Menten kinetics. Repeated batch operations were performed to study the persistence and stability of bacterial decolorization. Mixed azo dyes were also decolorized by the two PSB strains. Azoreductase was overexpressed in *E. coli* YB; however, the two PSB strains were better decolorizers for sulfonated azo dyes.

Keywords Azo dye · Azoreductase · Decolorization · Photosynthetic bacteria · *Rhodobacter sphaeroides* · *Rhodopseudomonas palustris*

Introduction

Dyes are widely found in the textile, printing, food, pharmaceutical, leather, cosmetics and many other

industries (Raffi et al. 1997). Almost 10⁶ tonnes of dyes are produced annually around the world, of which azo dyes, characterized by one or more azo groups ($R_1-N = N-R_2$) linking substituted aromatic structures, represent about 70% by weight (Hao et al. 2000). The release of these compounds into the environment is undesirable, not only because of their color, but also because many azo dyes and their breakdown products are toxic and/or mutagenic to life (Shore 1996; Chung et al. 1978). Effective and economic treatment of a diversity of effluents containing azo dyes has become a problem. Currently, much research has been focused on chemically and physically degrading azo dyes in wastewaters. However, many of these technologies are cost-prohibitive and therefore are not viable options for treating large waste streams (Chang et al. 2001; Yang et al. 2003).

Compared with chemical/physical methods, biological processes have received much interest because of their cost effectiveness, lower sludge production and environmental friendliness (Banat et al. 1996; Stolz 2001). Various wood-rotting fungi were shown to decolorize azo dyes using peroxidases or laccases with high oxidative capacities, which oxidize the azo dyes to form compounds of lower molecular weights (Cripps et al. 1990). But fungal treatment of effluents was usually time-consuming (Banat et al. 1996). Aerobic treatment of azo dyes with bacteria usually achieved low efficiencies because oxygen is a more efficient electron acceptor than azo dyes. Under static or anaerobic conditions, bacterial decolorization generally demonstrated good color removal effects. Anaerobic processes are usually unspecific with regard to the organisms involved and the dyes converted (Stolz 2001).

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Azoreductase is reported to be the key enzyme expressed in azo-dye-degrading bacteria and catalyses the reductive cleavage of the azo bond. Azoreductase activity has been identified in several species of bacteria recently, such as *Caulobacter subvibrioides* strain C7-D, *Xenophilus azovorans* KF46F, *Pigmentiphaga kullae* K24, *Enterobacter agglomerans*, *Enterococcus faecalis*, and *Staphylococcus aureus* (Mazumder et al. 1999; Blümel et al. 2002; Blümel and Stolz 2003; Moutaouakkil et al. 2003; Chen et al. 2004, 2005). These azoreductases all demonstrated better abilities at degrading azo dyes than whole cells, and possessed new potentials for the enzymatic degradation of azo dyes. However, the importance of intracellular azoreductase in anaerobic decolorization of azo dyes was doubted recently (Keck et al. 1997; Kudlich et al. 1997). Previous studies with facultative anaerobic bacteria suggested that the unspecific reduction of azo dyes was due to the reduced flavins generated by cytosolic flavin-dependent reductases (Roxon et al. 1967; Walker 1970). Some reports have suggested the involvement of membrane-bound enzyme systems and redox mediators in the bacterial decolorization process (Keck et al. 1997; Kudlich et al. 1997).

For the overexpression and characterization of azoreductase, different gene-engineered strains (usually as *Escherichia coli* strains) were constructed. It has been reported that a recombinant *E. coli* strain carrying the azoreductase gene (*azo B*) from *X. azovorans* KF46F, expressed almost 50 times higher azoreductase activity in its cell extracts than that observed in *X. azovorans* KF46F. However, the resting recombinant cell suspension showed no detectable azoreductase activity (Blümel et al. 2002). The gene of a flavin reductase (*fre*) functioned as azoreductase in vitro was transferred to *Sphingomonas* sp. strain BN6 and resulted in 30-fold increase in azoreductase activity in the wild-type strain. But the whole cells of the recombinant *Sphingomonas* sp. BN6 showed only approximately threefold increase in the reduction rate for azo dyes Amaranth and Mordant Yellow three compared to the wild type BN6 strain (Russ et al. 2000).

Reports on the comparative study of azo dye decolorization by different species of bacteria are scarce. To our knowledge, this is the first comparative study on azo dye decolorization with photosynthetic bacterial (PSB) strains and a genetically engineered one harboring gene encoding azoreductase from PSB strain. It provides fundamental information for the development of a bacterial bioprocess for azo dye wastewater treatment and demonstrates that intracel-

lular azoreductase is not the dominant factor in the bacterial decolorization of sulfonated azo dyes.

Materials and methods

Chemicals

Dyes used in this study were kindly provided by Dye Synthesis Laboratory, Dalian University of Technology (Table 1). Reduced nicotinamide adenine dinucleotide (NADH) was purchased from Sigma Chemicals, USA. BSA and isopropyl- β -D-thiogalactopyranoside (IPTG) were purchased from TaKaRa Dalian. All other reagents used were of analytical grade. All the reagents were used as purchased without purification.

Microorganism and cultivation method

The two PSB strains, *Rhodobacter sphaeroides* AS1.1737 and *Rhodospseudomonas palustris* AS1.2352 were identified by the Institute of Microbiology, Chinese Academy of Science and studied for azo dye decolorization and CO₂ fixation in our laboratory (Yan et al. 2004; Du et al. 2003).

The gene encoding azoreductase from *R. sphaeroides* AS1.1737 (*azr*, 537 bp) was cloned and inserted into plasmid pGEX 4T-1 under the control of the *lac* promoter. The recombinant plasmid designated as pGEX4T-AZR, which directs the high expression of azoreductase (Yan et al. 2004), was transformed into CaCl₂-treated competent cell *E. coli* JM 109. The recombinant strain was named *E. coli* YB.

All the strains were cultivated aerobically at 30°C with Luria–Bertani broth (supplemented with 1 mM ampicillin for *E. coli* YB) until reaching their early stationary phases (ESP), respectively. In addition, IPTG was added into *E. coli* YB culture and cultivated for 4 h to induce the expression of decolorizing genes

Table 1 Characteristics of azo dyes used in this work

Dye	Color index name (C.I. No.)	Molecular weight	Maximum absorption wavelength (λ_{\max} , nm)
Acid Red B	Acid Red 14 (14720)	502.43	513
Reactive Brilliant Red K-2BP	Reactive Red 24 (18208)	788.07	535
Reactive Brilliant Red X-3B	Reactive Red 2 (18200)	615.34	544

(Yan et al. 2004). After that, azo dyes of specific concentrations were added to ESP cultures and color removal investigations were conducted under static conditions (i.e., neither aeration nor agitation was employed), except for those designed to investigate the effect of oxygen on decolorization.

Measurement of dye concentration and cell concentration

The dye and cell concentrations were monitored with a UV/VIS spectrophotometer (Jasco V-560, Tokyo, Japan) at intervals during the decolorization process.

The concentration of azo dye was detected spectrophotometrically by reading the culture supernatant at its specific λ_{\max} after centrifugation (8,000 rev min⁻¹, 10 min). The efficiency of color removal was expressed as color removal (%) = $C_i - C_r / C_i \times 100\%$, where C_i and C_r were initial and residual concentrations, respectively.

The cell concentration was determined from optical density (OD) of the cultures at 660 nm (OD₆₆₀). The relationships between the bacterial cell concentration and OD₆₆₀ were 1.0 OD₆₆₀ = 1.980 g l⁻¹ dry cell weight for *R. sphaeroides* AS1.1737, 1.0 OD₆₆₀ = 0.594 g l⁻¹ dry cell weight for *R. palustris* AS1.2352 and 1.0 OD₆₆₀ = 2.326 g l⁻¹ dry cell weight for *E. coli* YB.

Batch decolorization operations

In order to investigate the effects of dissolved oxygen on decolorization, the decolorization of Acid Red B (ARB) was carried out under agitation (180 rev min⁻¹) and static conditions, separately. Effects of pH, temperature and initial dye concentration were studied under static conditions. Parameters of batch operations are summarized in Table 2.

Repeated batch operations

Under static conditions, around 90 mg of ARB l⁻¹ was decolorized by the strains. As more than 80% of the color was removed, the culture was again amended with ARB to 90–110 mg l⁻¹ for the next batch. These

procedures were repeated six times to investigate the stability and persistence of decolorization by these strains.

Decolorization of mixed azo dyes

Decolorization of a mixture of three sulfonated azo dyes, Reactive Brilliant Red X-3B (RBR X-3B), Reactive Brilliant Red K-2BP (RBR K-2BP), and ARB, was investigated. Each of them was added with a final concentration of 25–30 mg l⁻¹, respectively. Decolorization processes were monitored according to the method reported by Harazono and Nakamura (2005) with little modification. Dye decolorization was measured photometrically at visible wavelengths (400, 450, 500, 550, and 600 nm). \sum OD of mixed azo dyes was calculated as the sum of absorbances at each wavelength and color removal (%) was calculated as the extent of decrease from the initial value of \sum OD. The reaction was also monitored at the absorbance maximum of each dye.

Preparation of cell extracts

Cell extracts of the three strains were obtained as follows. Cells of 1,000 ml culture were harvested by centrifugation (8,000 rev min⁻¹, at 4°C for 20 min), and the pellet was washed twice with sodium phosphate buffer (20 mM, pH 7.0). Then the washed pellet was resuspended in 20 ml of the same buffer and lysed by freezing and thawing followed by sonication (225 W, at 4°C for 30 min). Cell debris was removed by centrifugation (22,000 rev min⁻¹, at 4°C for 30 min). The supernatant was stored at -20°C and used as cell extracts in the following decolorization studies.

Protein concentration was measured according to the Bradford procedure, using BSA as a standard.

Decolorization of azo dye by cell extracts

The total volume of the reaction mixture was 2.0 ml, which contained 1.3 ml 40 mg l⁻¹ RBR X-3B in phosphate buffer (20 mM, pH 7.0), 0.5 ml cell extracts mentioned above and 0.2 ml 10 mM NADH. The

Table 2 Summary of parameters of batch operations

Factors investigated	Dissolved oxygen (mg l ⁻¹)	pH	Temperature (°C)	Initial dye concentration (mg l ⁻¹)
Dissolved oxygen	Agitation (6.0–6.5) Static (0.1–0.4)	7.0	30	90
pH	0.1–0.4	5.0–9.0	30	185
Temperature	0.1–0.4	7.0	25–40	185
Dye concentration	0.1–0.4	7.0	30	90–900

decolorization experiments were performed under static conditions. The reaction was started by the addition of NADH, and was assayed spectrophotometrically at 544 nm with the corresponding buffer as control. The crude enzyme activity was defined as the amount of reduced dye by per mg protein per minute.

Statistical analysis

All the experiments were conducted in triplicate. The Tukey's studentized range test was used to compare the means of separate replicates. Unless otherwise stated, conclusions are based on differences between means significant at $p < 0.05$.

Results and discussion

Effects of dissolved oxygen

Under static conditions, the two PSB strains demonstrated significantly higher decolorization rate than the recombinant strain. Cultures of *R. sphaeroides* AS1.1737 and *R. palustris* AS1.2352 decolorized 85% ARB within 1 and 4 h, respectively. However, *E. coli* YB culture reached an 88% color removal in 11.5 h (Fig. 1).

Under agitated conditions, all the cultures still demonstrated decolorization effects, though poor compared with their performances under static conditions. In the 14.5 h investigated, *R. sphaeroides* AS1.1737, *R. palustris* AS1.2352, and *E. coli* YB decolorized 84.2, 33.1, and 41.4% ARB, respectively.

When azo dyes were added to the cultures at the beginning of the inoculation, anaerobic or static conditions were necessary for bacterial decolorization and nearly no decolorization effects were observed under

aerobic or agitated conditions (Song et al. 2003; Liu et al. 2006). It was speculated that under agitated conditions, the aerobic respiration of the strain might dominate the utilization of NADH and inhibit the azoreductase from obtaining electron from NADH to decolorize azo dyes (Stolz 2001). In this study, ARB was added into the culture when the strains reached their ESP. Under static conditions, ARB was decolorized by PSB strains shortly after its addition. Decolorization with *E. coli* YB also preferred static conditions. However, ARB decolorization was still carried out under agitated conditions by all the ESP cultures. Oxygen in the ESP cultures was greatly consumed due to growth and metabolism (data not shown), thus little oxygen-inhibition effect was observed.

Effects of pH and temperature

Decolorization of ARB at various pH values by *R. sphaeroides* AS1.1737, *R. palustris* AS1.2352, and *E. coli* YB was compared. It was demonstrated that the decolorization rates of the two PSB strains were significantly higher than that of the engineered one at all the pH values investigated (data not shown). The most favorable pH for *R. sphaeroides* AS1.1737 and *R. palustris* AS1.2352 was around 6.0, with decolorization rates of 322.05 and 288.06 mg dye g cell⁻¹ h⁻¹, respectively, indicating that neutral and slightly acidic pH values (6.0–7.0) were favorable for ARB decolorization by ESP cultures of PSB strains. However, the optimal pH for *E. coli* YB was around 8.0, with a specific decolorization rate of 44.12 mg dye g cell⁻¹ h⁻¹. A slightly basic condition was preferable for ARB decolorization by the engineered strain. It has been suggested that the difference in optimal pH values may be attributed to the difference in genetic determinants responsible for decolorization or in bacterial physiology (Chang and Lin 2001).

Decolorization of ARB by the three strains was investigated over a range of 25–40°C. Different most favorable temperatures, 299.77 mg dye g cell⁻¹ h⁻¹ at 35°C for *R. sphaeroides* AS1.1737, 247.13 mg dye g cell⁻¹ h⁻¹ at 30°C for *R. palustris* AS1.2352 and 46.44 mg dye g cell⁻¹ h⁻¹ at 40°C for *E. coli* YB, were observed.

Effects of dye concentration

Figure 2 shows dependences of the specific decolorization rate on substrate concentration. The correlations between decolorization rate and dye concentration could be described by Michaelis–Menten kinetics ($v_{\text{dye}} = v_{\text{dye, max}} [\text{Dye}] / (K_m + [\text{Dye}])$); where $v_{\text{dye, max}}$

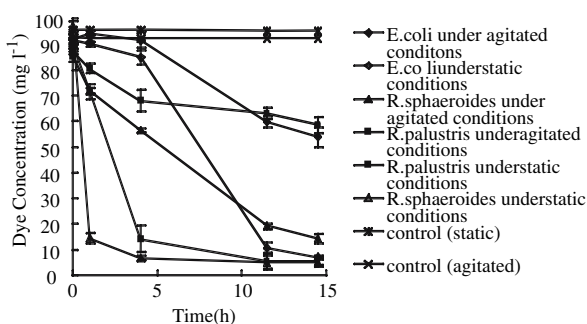


Fig. 1 Effects of oxygen on the decolorization of Acid Red B by the three strains. No strain was inoculated in the control studies. Three replicates performed and confidence limits calculated at 95% confidence level

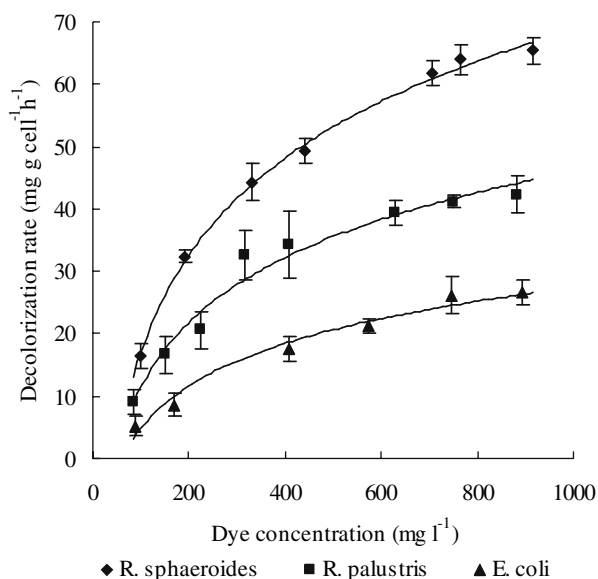


Fig. 2 The effects of dye concentration on the specific decolorization rate of Acid Red B by the three strains. Three replicates performed and confidence limits calculated at 95% confidence level

and K_m denoted maximum decolorization rate and Michaelis constant, respectively, and [Dye] represented the concentration of ARB. The predicted $v_{\text{dye, max}}$ and K_m values were shown in Table 3.

The $v_{\text{dye, max}}/K_m$ ratio may be used as an indicator for the performance of decolorization ability (Yeh and Chang 2004). The ratio was 0.2, 0.15, and 0.06 l g cell⁻¹ h⁻¹ for *R. sphaeroides* AS1.1737, *R. palustris* AS1.2352, and *E. coli* YB, respectively, suggesting that the PSB strains were more effective decolorizers for ARB.

Effects of repeated uses

All the three strains kept their normal growth during the batch operations and decolorized ARB repeatedly in five runs (Fig. 3). *R. sphaeroides* AS1.1737 finished five runs of decolorization in 6 h, much faster than the performances of *R. palustris* AS1.2352 and *E. coli* YB. Compared to *R. sphaeroides* AS1.1737, *R. palustris* AS1.2352, and *E. coli* YB demonstrated long start-up periods in the first run (5 and 10 h, respectively). After that, their decolorization rates increased dramatically

Table 3 Kinetic parameters of the three strains

Strains	v_{max} of dyes (mg g cell ⁻¹ h ⁻¹)	K_m (mg l ⁻¹)
<i>R. sphaeroides</i> AS1.1737	116.28	578.69
<i>R. palustris</i> AS1.2352	101.01	837.15
<i>E. coli</i> YB	49.75	831.59

for runs 2–5. This might be attributed to the adaptation effect for their repeated exposure to ARB. It was notable that *E. coli* YB finished the remaining four runs of decolorization with only 4 h. A great amount of dead cells were found settled at the bottom of the flasks in the sixth operation of all the three strains, which was attributed to nutrition/O₂ limitation and the accumulation of toxic products in the culture. The hostile and stressful environment inhibits the normal growth and metabolism of the bacteria. As a result, no further repeated decolorization operation was carried out.

All these three strains held good persistence and stabilities in repetitive operations. *R. sphaeroides* AS1.1737 showed the most stable decolorization rate in five runs, whereas the adapted *E. coli* YB decolorized the last four runs efficiently.

Decolorization of mixed azo dyes

As shown in Fig. 4, both of the two PSB stains could decolorize the mixed azo dyes. All the three dyes were decolorized simultaneously. Culture of *R. palustris* AS1.2352 demonstrated the best decolorization effects, which reached 50% color removal in 4 h. *R. sphaeroides* AS1.1737 decolorized 43% mixed dyes in 9.5 h. However, the *E. coli* YB culture reached only a 7% color removal.

Decolorization of azo dye by cell extracts

Decolorization of RBR X-3B by cell extracts of the three strains were conducted respectively. As expected, *E. coli* YB demonstrated significantly more intracellular azoreductase activity than the other two PSB strains, which was in line with our previous report (Yan et al. 2004). The decolorization rates of cell extracts from *R. sphaeroides* AS1.1737, *R. palustris* AS1.2352, and *E. coli* YB were 1.13, 0.86, and 9.92 $\mu\text{mol mg protein}^{-1} \text{min}^{-1}$, respectively.

In the study of bacterial decolorization of azo dyes, the two PSB strains demonstrated better performances than the engineered one who expressed more azoreductase in vivo. On the other hand, azoreductase was overexpressed in *E. coli* YB (Yan et al. 2004) and decolorized azo dyes efficiently in vitro. It seems that the intracellular azoreductase is of little importance for the reduction of sulfonated azo dyes, whose highly polar characteristics inhibit their permeability through cell membranes (Russ et al. 2000).

It was suggested that bacterial decolorization of azo dyes intracellularly will require not only the presence of azoreductase but also a transport system(s) which allows the uptake of the sulfonated dyes into the cells

Fig. 3 Effects of repeated uses on cells growth and decolorization of the three strains, (a) *R. sphaeroides* AS1.1737, (b) *R. palustris* AS1.2352, and c *E. coli* YB. Three replicates performed and confidence limits calculated at 95% confidence level

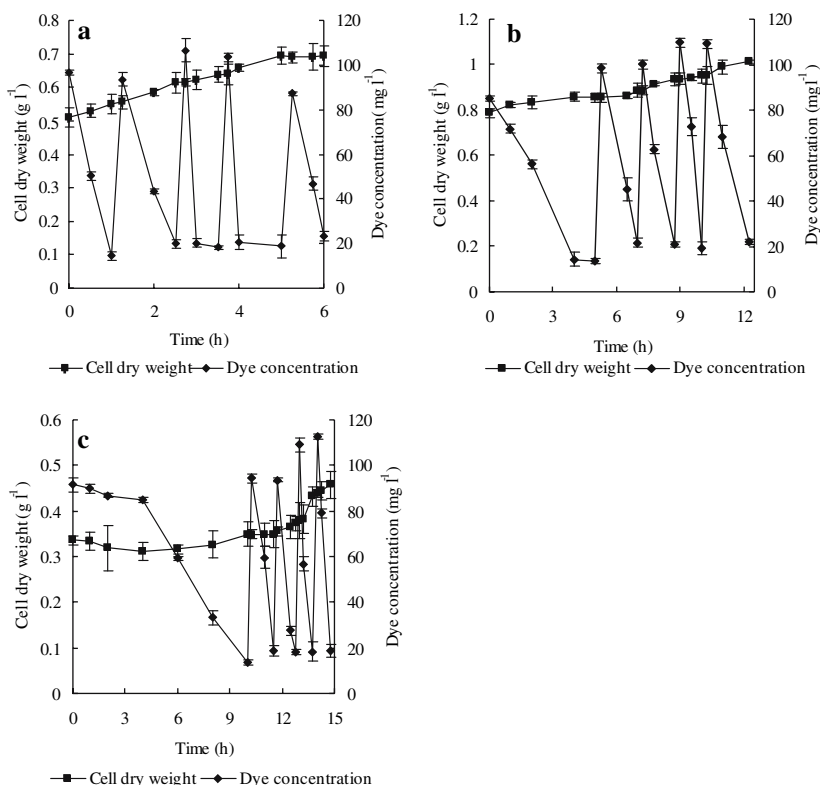
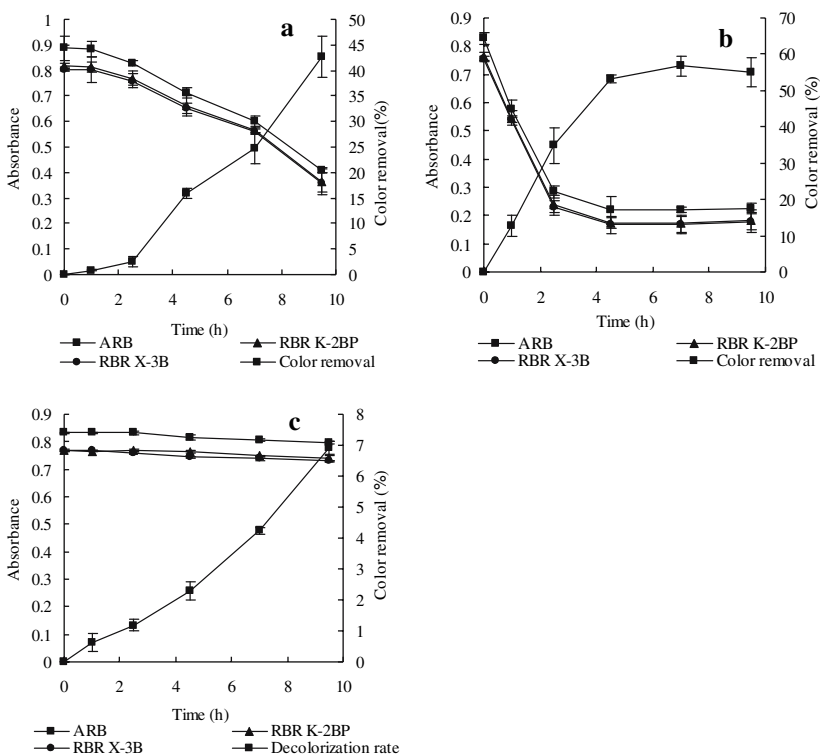


Fig. 4 Decolorization of mixed sulfonated azo dyes Acid Red B, Reactive Brilliant Red X-3B and Reactive Brilliant Red K-2BP by the three strains, (a) *R. sphaeroides* AS1.1737, (b) *R. palustris* AS1.2352, and (c) *E. coli* YB. Three replicates performed and confidence limits calculated at 95% confidence level



(Blümel et al. 2002). It was speculated that the poor performance of the recombinant *E. coli* strain was due to its lack of an efficient transport system of sulfonated azo dyes. There might be a particular transport sys-

tem(s) of sulfonated azo dyes in PSB strains. Although there is no information available about the transport system of sulfonated azo dyes, system involved in the transport of other kinds of sulfonated substrates in

bacteria have been reported (Eichhorn et al. 2000; Kahnert et al. 2000; Locher et al. 1993). The transfer of the *azr* gene on a shuttle plasmid to a PSB strain has been conducted. As expected, the recombinant PSB strain demonstrated better performance than the wild type PSB strains and this will be reported later.

Conclusions

Sulfonated azo dyes were decolorized by PSB strains *R. sphaeroides* AS1.1737, *R. palustris* AS1.2352, and the gene-engineered *E. coli* YB harboring azoreductase gene from *R. sphaeroides* AS1.1737. The *E. coli* YB expressed almost ten times higher azoreductase activity in the cell extracts than PSB strains, however, no better decolorization abilities were demonstrated under batch operation investigations. The cytosolic azoreductase was not the dominant factor in bacterial decolorization of sulfonated azo dyes.

References

- Banat IM, Nigam P, Singh D, Marchant R (1996) Microbial decolorization of textile-dye containing effluents: a review. *Bioresour Technol* 58:217–227
- Blümel S, Knackmuss HJ, Stolz A (2002) Molecular cloning and characterization of the gene coding for the aerobic azoreductase from *Xenophilus azovorans* KF46F. *Appl Environ Microbiol* 68:3948–3955
- Blümel S, Stolz A (2003) Cloning and characterization of the gene coding for the aerobic azoreductase from *Pigmentiphaga kullae* K24. *Appl Microbiol Biotechnol* 62:186–190
- Chang JS, Chou C, Lin YC, Lin PJ, Ho JY, Hu TL (2001) Kinetic characteristics of bacterial azo-dye decolorization by *Pseudomonas luteola*. *Wat Res* 35:2841–2850
- Chang JS, Lin CY (2001) Decolorization kinetics of a recombinant *Escherichia coli* strain harboring azo-dye-decolorizing determinants from *Rhodococcus* sp. *Biotechnol Lett* 23:631–636
- Chen HZ, Wang RF, Cerniglia CE (2004) Molecular cloning, overexpression, purification, and characterization of an aerobic FMN-dependent azoreductase from *Enterococcus faecalis*. *Protein Expr Purif* 34:302–310
- Chen HZ, Hopper SL, Cerniglia CE (2005) Biochemical and molecular characterization of an azoreductase from *Staphylococcus aureus*, a tetrameric NADPH-dependent flavoprotein. *Microbiology* 151:1433–1441
- Chung KT, Fulk GE, Egan M (1978) Reduction of azo dyes by intestinal anaerobes. *Appl Environ Microbiol* 35:558–562
- Cripps C, Bumpus JA, Aust SD (1990) Biodegradation of azo and heterocyclic dyes by *Phanerochaete chrysosporium*. *Appl Environ Microbiol* 56:1114–1118
- Du CH, Zhou JT, Wang J, Yan B, Lu H, Hou HM (2003) Construction of a genetically engineered microorganism for CO₂ fixation using a *Rhodospseudomonas/Escherichia coli* shuttle vector. *FEMS Microbiol Lett* 225:69–73
- Eichhorn E, Vanderploeg JR, Leisinger T (2000) Deletion analysis of the *Escherichia coli* taurine and alkanesulfonate transport systems. *J Bacteriol* 182:2687–2695
- Hao OJ, Kim H, Chiang PC (2000) Decolorization of wastewater. *Critical Rev Environ Sci Technol* 30:449–505
- Harazono K, Nakamura K (2005) Decolorization of mixtures of different reactive textile dyes by the white-rot basidiomycete *Phanerochaete sordida* and inhibitory effect of polyvinyl alcohol. *Chemosphere* 59:63–68
- Kahnert A, Vermeij P, Wietek C, James P, Leisinger T, Kertesz MA (2000) The *ssu* locus plays a key role in organosulfur metabolism in *Pseudomonas putida* S-313. *J Bacteriol* 182:2869–2878
- Keck A, Klein J, Kudlich M, Stolz A, Knackmuss HJ, Mattes R (1997) Reduction of azo dyes by redox mediators originating in the naphthalenesulfonic acid degradation pathway of *Sphingomonas* sp. strain BN6. *Appl Environ Microbiol* 63:3684–3690
- Kudlich M, Keck A, Klein J, Stolz A (1997) Localization of the enzyme system involved in anaerobic reduction of azo dyes by *Sphingomonas* sp. strain BN6 and effect of artificial redox mediators on the rate of azo dye reduction. *Appl Environ Microbiol* 63:3691–3694
- Liu GF, Zhou JT, Wang J, Song ZY, Qv YY (2006) Bacterial decolorization of azo dyes by *Rhodospseudomonas palustris*. *World J Microbiol Biotechnol* 22:1069–1074
- Locher HH, Poolman B, Cook AM, Konings WN (1993) Uptake of 4-toluenesulfonate by *Comamonas testosteroni* T-2. *J Bacteriol* 175:1075–1080
- Mazumder R, Logan JR, Mikell AT Jr, Hooper SW (1999) Characteristics and purification of an oxygen insensitive azoreductase from *Caulobacter subvibrioides* strain C7-D. *J Indust Microbiol Biotechnol* 23:476–483
- Moutaouakkil A, Zeroual Y, Dzayri FZ, Talbi M, Lee K, Blaghen M (2003) Purification and partial characterization of azoreductase from *Enterobacter agglomerans*. *Arch Biochem Biophys* 413:139–146
- Raffi F, Hall JD, Cerniglia CE (1997) Mutagenicity of azo dyes used in foods, drugs and cosmetics before and after reduction by *Clostridium* species from the human intestinal tract. *Food Chem Toxicol* 35:897–901
- Roxon JJ, Ryan AJ, Wright SE (1967) Enzymatic reduction of tartrazine by *Proteus vulgaris* from rats. *Food Chem Toxicol* 5:645–656
- Russ R, Rau J, Stolz A (2000) The function of cytoplasmic flavin reductases in the reduction of azo dyes by bacteria. *Appl Environ Microbiol* 66:1429–1434
- Shore J (1996) Advances in direct dyes. *Ind J Fib Text Res* 21:1–29
- Song ZY, Zhou JT, Wang J, Yan B, Du CH (2003) Decolorization of azo dyes by *Rhodobacter sphaeroides*. *Biotechnol Lett* 25:1815–1818
- Stolz A (2001) Basic and applied aspects in the microbial degradation of azo dyes. *Appl Microbiol Biotechnol* 56:69–80
- Walker JR (1970) The metabolism of azo compounds: a review of the literature. *Food Cosmet Toxicol* 8:659–676
- Yan B, Zou JT, Wang J, Du CH, Hou HM, Song ZY, Bao YM (2004) Expression and characteristics of the gene encoding azoreductase from *Rhodobacter sphaeroides* AS1.1737. *FEMS Microbiol Lett* 236:129–136
- Yang QX, Yang M, Pritsch K, Yediler A, Hagn A, Schloter M, Kettrup A (2003) Decolorization of synthetic dyes and production of manganese-dependent peroxidase by new fungal isolates. *Biotechnol Lett* 25:709–713
- Yeh MS, Chang JS (2004) Bacterial decolorization of an azo dye with a natural isolate of *Pseudomonas luteola* and genetically modified *Escherichia coli*. *J Chem Technol Biotechnol* 79:1354–1360