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The new incorporation bio-treatment technology of bromoamine acid and azo dyes wastewaters under high-salt conditions

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Abstract The accelerating effect of quinones has been studied in the bio-decolorization processes, but there are no literatures about the incorporation bio-treatment technology of the bromoamine acid (BA) wastewater and azo dyes wastewaters under high-salt conditions (NaCl, 15%, w/w). Here we described the BA wastewater as a redox mediator in the bio-decolorization of azo dye wastewaters. Decolorization of azo dyes was carried out experimentally using the salt-tolerant bacteria under the BA wastewater and high-salt conditions. The BA wastewater used as a redox mediator was able to increase the decolorization rate of wastewater containing azo dyes. The effects of various operating conditions such as dissolved oxygen, temperature, and pH on microbial decolorization were investigated experimentally. At the same time, BA was tested to assess the effects on the change of the Oxidation–Reduction Potential (ORP) values during the decolorization processes. The experiments explored a great improvement of the redox mediator application and the new bio-treatment concept.

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Introduction

Synthetic dyes are extensively used in the textile, cosmetic, printing, drug, and food processing industries. Major classes of synthetic dyes including azo, anthraquinone, and triarylmethane dyes (Zollinger 1987). Azo dyes, aromatic moieties linked together by azo (–N = N–) chromophores, is the largest class of dyes used in textile-processing and other industries. These dye wastewaters are characterized by extreme fluctuations in many parameters such as Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), pH, color, and high salinity. Sequential or integrated anaerobic–aerobic treatment is the most logical strategy for the complete removal of azo dyes in biological systems. However, high salinity of the dye wastewaters usually causes plasmolysis and/or loss of activity of cells, and some traditional aerobic- and anaerobic-biological treatments in low-BOD removal performance (Peyton et al. 2002); at the same time, anaerobic azo dye reduction is a time-consuming process, reflected by the requirement of long reaction times. Hence, there is clearly a need for a salt-tolerant and efficient bio-treatment system for the removal of dye wastewaters.

As is known, large amounts of salts such as sodium nitrate, sodium sulfate, and sodium chloride are used in the dye manufacturing industries and in the dye-consuming industries (Carliell et al. 1994), as well as sodium hydroxide is widely applied to increase the pH to the alkaline range. It is estimated that the salt concentration is up to 15–20% (EPA 1997; Manu and Chauhari 2003). Hyper-salinity colored wastewater has been little investigated for the biological treatment systems, especially above 10% salt concentration (Woolard and Irvine 1995; Kargi and Uygur 1996; Panswad and Anan 1999; Karigi et al. 1998; Bromley-challenor et al. 2000; Lee and Pavlostathis 2004; Lee et al. 2005). At the same time, the decolorization rate of azo dyes is increased by using redox mediators, compounds that speed up the reaction rate by shuttling electrons from the biological oxidation of primary electron donors or from bulk electron donors to the electron-accepting azo dyes (Dos Santos et al. 2004; Van der Zee et al. 2003; Russ et al. 2000; Cervantes 2002; Keck et al. 1997). But continuous dosing of the redox mediators implies continuous expenses related to procurement of the chemical, as well as continuous discharge of this biologically recalcitrant compound. Therefore, incorporation of an alternative mediator and the salt-tolerant bacteria in the bio-treatment system of dye wastewaters would be a great improvement of the redox mediator application and the bio-treatment concept.

In this paper, the selected alternative mediator is Bromoamine acid (1-amino-4-bromoanthraquinone-2-sulfonic acid, BA), a major intermediate, which is widely used in synthesis of anthraquinone dyes and cause water bodies red resulting in serious environment pollution (Qu et al. 2005). And the experiments were conducted to explore the new incorporation bio-treatment technology of BA as the alternative mediator and the azo dyes by the salt-tolerant bacteria.

Methods and materials

Dyes and chemicals

The dyes used in this study were from Dye Synthesize Laboratory, Dalian University of Technology. The chemical structures of these dyes were shown in Fig. 1.

Organism and medium

The salt-tolerant bacteria, CAS, was obtained from The Gene Laboratory of Dalian University of Technology (Guo et al. 2005), the mixed bacterial culture was acclimatized in a salt-tolerant medium and incubated at 30°C, pH 7.0 on a rotary shaker at 150 r min^{-1}, which conditions was obtained by the experiment.

The salt-tolerant medium contained yeast extract 5 g L^{-1}, peptone 10 g L^{-1}, and NaCl 0–150 g L^{-1} (pH 7.0), which was abbreviated as STM.

Effects of NaCl on the decolorization by salt-tolerant cultures

The decolorization experiments were conducted in rubber-stoppered serum bottles, and acclimatization of dyes was not carried out. First, the salt-tolerant bacteria, CAS, were grown in STM with planned NaCl concentrations under the aerobic conditions until they reached the late exponential growth phase. Cells were harvested by centrifugation at 8,000 × *g* for 10 min and resuspended in STM with different salts and dye concentrations to optical density ($OD_{660\ nm}$ = 0.5). Then, these cell suspensions were transferred into rubber-stoppered serum bottles (100 mL), containing 100 mL STM with Reactive Brilliant Red K-2BP 100–500 mg L^{-1}. The serum bottles were transferred to an anaerobic incubation chamber at 30°C. The decolorization rate was calculated at λ_{max} (540 nm) of medium supernatant after centrifugation at 8,000 × *g* for 10 min. The cell concentration was measured by optical density at 660 nm.

Both cell-free and sterilized controls of CAS were conducted. To prevent possible contamination by oxygen during sampling, bottles were opened only once, and as many bottles were incubated as measurements were planed. The assays were performed in triplicate.

To avoid the affecting of dye, cells were harvested by centrifugation, which was washed with buffer solution (pH7.0), then resuspended in equal volume of buffer solution and measured at λ_{660} using an UV-Visible recording spectrophotometer (JASCO, V-560, UV/VS spectrophotometer).

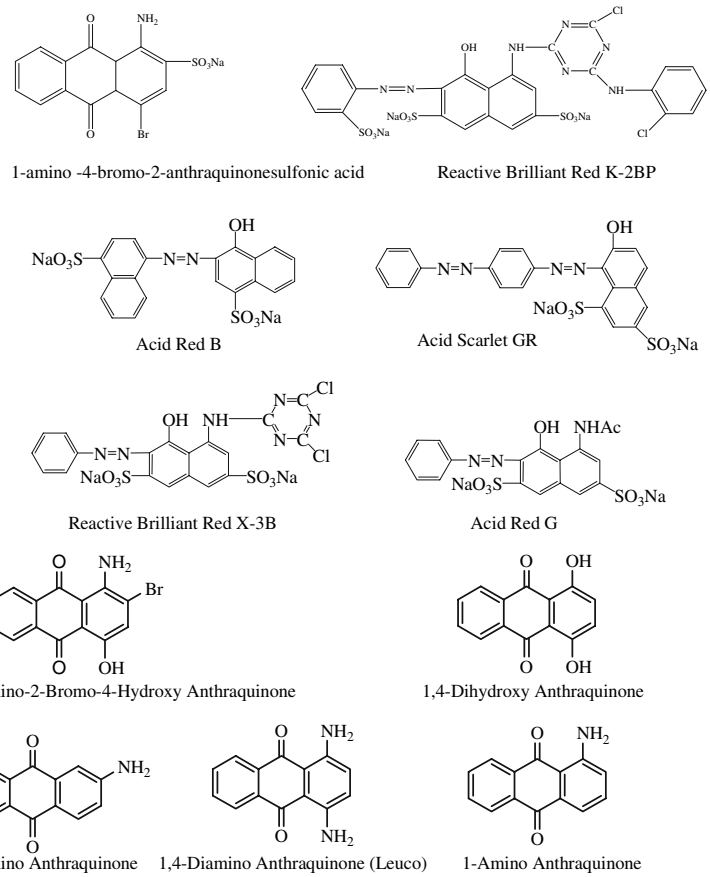

Fig. 1 The chemical structure of the dyes and the anthanquinone mediators

The effect of Oxidation–Reduction Potential (ORP) on decolorization

All oxidation–reduction potential (ORP) measurements were performed according the previous research (Guo et al. 2007).

The accelerating effect of BA on different dyes decolorization

The accelerating ability of BA on the bio-decolorization of different azo dyes was studied. The dyes included single-azo, diazo, and triazo. The experiment was conducted as the above-described procedure.

The accelerating effect of other anthanquinone mediator compounds on K-2BP decolorization

Various anthanquinone mediator compounds (for structural formulas see Fig. 1), such as 1-aminoanthraquinone (1-AAQ), 1-amino-2-bromo-4-hydroxy anthraquinone (ABHQ), 1,4-diamino anthraquinone (Leuco) (1,4-DAAQ), 2-amino anthraquinone (2-AAQ), and 1,4-dihydroxy anthraquinone (1,4-DHAQ), were selected to test the general applicability of the new incorporation bio-treatment technology of anthanquinone mediator compounds as the alternative mediator and the azo dyes by the salt-tolerant bacteria.

Analytical methods

Absorbance of the dye-containing solution was measured at its λ_{max} using an UV-Visible recording spectrophotometer (JASCO, V-560, UV/VS spectrophotometer), and absorbance was proportional to concentration over the range 0–75 mg L^{-1} for K-2BP and BA. The relationship between absorbance and concentration was not affected by pH in the range of 6–9 and NaCl concentration in the range of 5–150 g L^{-1}.

Results and discussion

Effect of pH, temperature, and dissolved oxygen (OD)

The optimum pH of the mixed culture was 6–9. Out of this range, the decolorization was much lower (data not shown). And 30–37°C was the optimum temperature for growth in the mixed cultures (data not shown). The dyes were decolorizated only under anaerobic conditions.

Effects of NaCl on the decolorization by salt-tolerant cultures

Figure 2 showed that if the concentration of K-2BP was kept constant with increasing the salt concentration, decolorization was more than 90%. With the elevated NaCl from 5 g L^{-1} to 150 g L^{-1}, it was obvious that the decolorization rate of those cultures decreased. The similar phenomenon occurred in other high-salt wastewater (Panswad and Anan 1999). The results showed the inhibition to microorganisms by high-salt concentration, which may cause plasmolysis and/or loss of activity of cells. At the same time, the effect of salt could be described as the first-order reaction kinetics (Karigi et al. 1998).

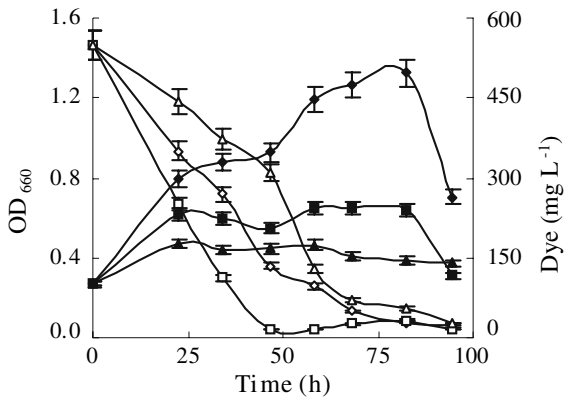

Fig. 2 Decolorization and growth of CAS at different NaCl concentrations and 550 mg L^{-1} K-2BP (*open diamond*: growth at 5 g L^{-1} NaCl; *filled diamond*: decolorization at 5 g L^{-1} NaCl; *open square*: growth at 80 g L^{-1} NaCl; *filled square*: decolorization at 80 g L^{-1} NaCl; *open triangle*: growth at 150 g L^{-1} NaCl; *filled triangle*: decolorization at 150 g L^{-1} NaCl)

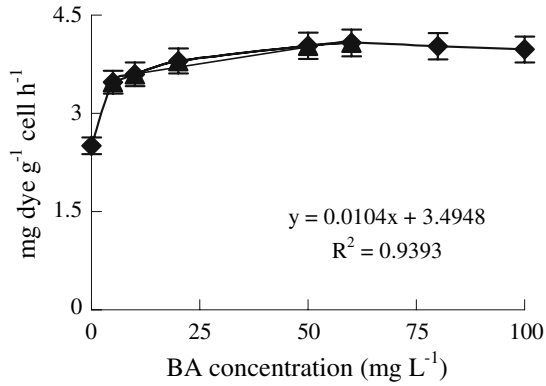

Fig. 3 Decolorization rate of K-2BP by the salt-tolerant bacteria at different concentrations of BA. The results are means of triplicate incubations (K-2BP, 100 mg L^{-1})

The effect of bromoamine acid on decolorization by salt-tolerant cultures

The BA was one of the redox mediators, which accelerated the electron transfer from a primary electron donor to a terminal electron acceptor and increased the reaction rates. Figure 3 showed that a linear correlation was found for BA concentrations from 5 to 60 mg L^{-1}. The decolorization rate increased 1.9-fold for the bottles with 60 mg L^{-1} BA compared to those lacking this mediator. However, the use of high concentrations of BA showed inhibition of the decolorization rate.

Figure 4 was the special analysis of the color during the decolorization, indicating that the dye structure was altered. It was to note that no new peaks in the visible region were observed.

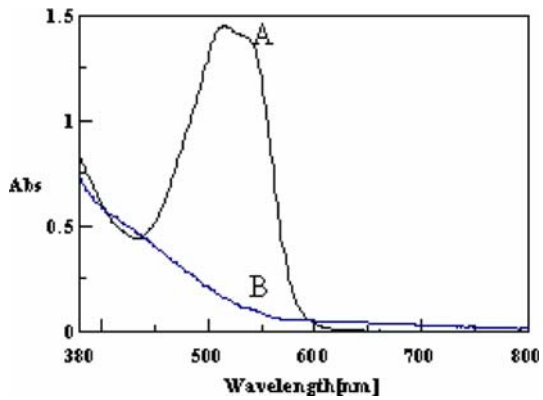

Fig. 4 Visible absorbance spectra of K-2BP (**a**) and the anaerobic products of K-2BP (**b**)

The effect of BA on the change of ORP during the decolorization processes

Redox mediators are compounds that accelerate the electron transfer from a primary electron donor to a terminal electron acceptor, which may increase the reaction rates by one to several orders of magnitude (Cervantes 2002). Reductive decolorization of azo dyes in the presence of redox mediators occurs in two distinct steps, the first step being a non-specific enzymatic mediator reduction, and the second step being a chemical re-oxidation of the mediator by the azo dyes (Keck et al. 1997). But very few studies were reported the effect of redox mediators on the change of ORP during the decolorization processes.

In the experiment, the ORP values stabilized around -275 to 280 mV after 6 h anoxic conditions, which was stimulated more negative values of -20 to 25 mV by BA (Fig. 5). The similar results were reported (Guo et al. 2007). The reason for the above-mentioned results was unknown, and needed to be studied further.

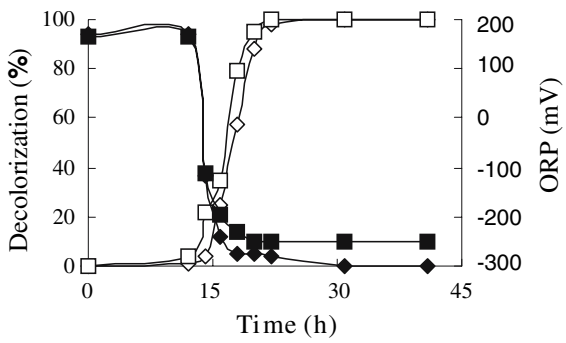

Fig. 5 Decolorization of K-2BP and ORP profiles in salt-tolerant culture under aerobic, anoxic, and anaerobic conditions

During the processes of decolorization experiment, a little amount of (<1%) decolorization occurred in cell-free controls in the presence of BA. However, controls conducted with autoclaved cell in BA-supplemented assays achieved about 7% of decolorization during the same period (results not shown), and a complete decolorization after prolonged periods (>30 days), which might be related with the contained quinines of autoclaved cell.

The accelerating effect of BA on different dye decolorization

The BA could significantly increase the reduction rate of K-2BP by the salt-tolerant bacteria. To demonstrate the general applicability of this system for the treatment of dye wastewater, various azo dyes (for structural formulas see Fig. 1) were incubated under anaerobic conditions with the salt-tolerant bacteria in the absence or presence of BA. Thus, it was demonstrated that the addition of BA increased the reduction rates of all azo dyes tested (Table 1).

The general applicability of other anthanquinone mediator compounds on K-2BP decolorization

To test the general applicability of the new incorporation bio-treatment technology of anthanquinone mediator compounds as the alternative mediator and the azo dyes by the salt-tolerant bacteria, various anthanquinone mediator compounds (for structural formulas see Fig. 1) were tested. Thus, it was demonstrated that the addition of the tested anthanquinone mediator increased the reduction rates of all azo dyes tested (data not shown). Because the solubility of these anthanquinone mediator compounds was very low, the detailed experiments were

Table 1 Effects of the BA on the decoloriazation of different azo dyes by the salt-tolerant bacteria

Dye (λ)	Reduction rates (mg dye/g cell/h)				
	1	2	3	4	5
Reactive brilliant red X-3B (538)	2.5	2.8	3.1	3.5	4.2
Acid black 10B (619)	2.6	2.9	3.5	4.1	4.7
Acid scarlet GR (518)	1.9	2.1	2.9	3.7	4.3
Acid red B (516)	4.0	4.2	4.5	4.9	5.2
Acid red G (531)	3.6	4.0	4.5	5.1	5.3

1: 0 mg L^{-1} BA; 2: 10 mg L^{-1} BA; 3: 20 mg L^{-1} BA; 4: 40 mg L^{-1} BA; 5: 60 mg L^{-1} BA

not conducted. But the results demonstrated that the new incorporation bio-treatment technology was a great improvement in the bio-treatment technology of dye wastewater.

Conclusion

The BA as well as other anthanquinone mediator compounds could play a very active role in the azo dye anaerobic decolorization by shuttling electrons. Therefore, the new incorporation bio-treatment technology of anthanquinone mediator compounds as the alternative mediator and the azo dyes by the salt-tolerant bacteria is a great improvement in the bio-treatment technology of dye wastewater.

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References

Bromley-challenor KCA, Knapp JS, Zhang Z, Gray NCC, Hetheridge MJ, Evans MR (2000) Decolorization of an azo dye by unacclimated activated sludge under anaerobic conditions. Water Res 34:4410–4418

Carliell CM, Barclay SJ, Naidoo N, Buckley CA, Mulholland DA, Senior E (1994) Anaerobic decolorisation of reactive dyes in conventional sewage treatment processes. Water SA 20:341–344

Cervantes FJ (2002) Quinones as electron acceptors and redox mediators for the anaerobic biotransformation of priority pollutants. Ph.D. Wageningen University, Wageningen, The Netherlands, p 166

Dos Santos AB, Bisschops IAE, Cervantes FJ, Van Lier JB (2004) Effect of different redox mediators during thermophilic azo dye reduction by anaerobic granular sludge and comparative study between mesophilic (30°C) and thermophilic (55°C) treatments for decolourisation of textile wastewaters. Chemosphere 55:1149–1157

EPA (1997) Profile of the textile industry. Environmental Protection Agency, Washington, USA

Guo JB, Zhou JT, Wang D, Wang J, Yu H (2005) Decolorization of dye wastewater with high salt concentration by the acclimatized salt-tolerant cultures. J Environ Sci 17(6):984–988

Guo JB, Zhou JT, Wang D, Tian CP, Wang P, Salah Uddin M, Yu H (2007) Biocalalyst effects of immobilized anthraquinone on the anaerobic reduction of azo dyes by the salt-tolerant bacteria. Water Res 41(2):426–432

Karigi F, Dinçer, Ali R (1998) Saline wastewater treatment by halophile-supplemented activated sludge culture in an aerated rotating biodisc contactor [J]. Enzyme Microb Technol 122:427–433

Kargi F, Uygur A (1996) Biological treatment of saline wastewater in an aerated percolator unit utilizing halophilic bacteria. Environ Technol 17:325–330

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

Lee YH, Pavlostathis SG (2004) Reuse of textile reactive azo dyebaths following biological decolorization. Water Environ Res 76(1):56–66

Lee YH, Matthews RD, Pavlostathis SG (2005) Anaerobic biodecolorization of textile reactive anthraquinone and phthalocyanine dyebaths under hypersaline conditions. Water Sci Technol 52(1–2):377–383

Manu B, Chauhari S (2003) Decolorization of indigo and azo dyes in semicontinuous reactors with long hydraulic retention time. Process Biochem 38(8):1213–1221

Panswad T, Anan C (1999) Impact of high chloride wastewater on an anaerobic/anoxic/aerobic process with and without inoculation of chloride acclimated seeds [J]. Water Res 33:1165–1172

Peyton BM, Wilson T, Yonge DR (2002) Kinetics of phenol biodegradation in high salt solutions. Water Res 36(19): 4811–4820

Qu YY, Zhou JT, Wang J, Fu X, Xing LL (2005) Microbial community dynamics in bioaugmented sequencing batch reactors for bromoamine acid removal. FEMS Microbiol Lett 246:143–149

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

Van der Zee FP, Bisschops IAE, Lettinga G, Field JA (2003) Activated carbon as an electron acceptor and redox mediator during the anaerobic biotransformation of azo dyes. Environ Sci Technol 37:402–408

Woolard CR, Irvine RL (1995) Treatment of hypersaline wastewater in the sequencing batch reactor. Water Res 29:1159–1168

Zollinger H (1987) Color chemistry—syntheses, properties and applications of organic dyes and pigments. VCH Publishers, New York, pp 92–102