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Decolorization of azo dyes with high salt concentration by salt-tolerant mixed cultures under anaerobic conditions

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Abstract: Because the lack of detailed study of biological decolorization in high salt dye wastewater, it is still difficult to evaluate the biological treatment on high-salinity dye wastewater. The experiments were carried out to study the salt-tolerant bacteria, which is useful in the treatment of high-salinity colored wastewater. Simulated wastewater containing 5—150 g/L salt (NaCl) and 50—2000 mg/L Reactive Brilliant Red K-2BP was treated with three salt-tolerant mixed cultures (CAS, TAS, DSAS), which were under a gradually acclimated procedure. With the increase of concentrations of salt and dye, the decolorization became low. The abilities of decolorization of dyes wastewater by three mixed cultures (CAS, TAS, DSAS) were studied, CAS and DSAS mixed cultures showed more active for the treatment of high-salinity colored wastewater than TAS mixed cultures. The results suggested that there might be a simple process for the high salt wastewater treatment, which could be incorporated into conventional activated sludge plants.

Keywords: anaerobic decolorization; hyper-salinity; salt-tolerant mixed cultures

Introduction

Hyper-salinity wastewaters are defined herein as brines, which contain organic compounds and at least 3.5% w/v total dissolved solids (TDS) (Woolard, 1995a; 1995b). These waters are generated by several industrials. In arid and coastal areas, take Hong Kong for an example, using seawater to flush toilet has been practiced in which this wastewater was produced. Some industries, such as tanning, textile dyeing, pickling, chemical/petrochemical manufacturing, etc., also discharge salty wastewater from manufacturing processes. In addition, waste minimization practices promise to generate additional brines in the future. Sodium concentration above 3 g/L can cause moderate inhibition of bacteria activities (De Baere, 1984). Hyper-salinity wastewater usually causes plasmolysis and/or loss of activity of cells, therefore, some traditional aerobic- and anaerobic-biological treatments result in low BOD removal performance.

Hyper-salinity coloured wastewater is a consequence product of batch processes both in the dye manufacturing industries and in the dye-consuming industries, and the salt concentration is up to 15%—20%. Textile wastewaters are complex waste products containing dyes, sizing agents and dyeing aids that are characterized by their deep color and high concentration of salt (Manu, 2003). Various physicochemical, advanced oxidation processes, biological processes are applied to treat them to meet regulatory discharged limits. In contrast, biological treatment investment are five to twenty times less than chemical ones such as ozone or hydrogen peroxide and the running costs are three to ten times less than chemical processes (Mario, 1997).

However, there are a limited studies about the effects of salt on biological treatment systems, especially above 10% salt concentration (Woolard, 1995a; 1995b; Panswad, 1999). And industrialization of salt-tolerant bacteria could not be conducted because there are many questions on the application and theory of salt-tolerant bacteria in hyper-salinity wastewater treatment. In this paper, the experiments were conducted to explore the method of acclimatization of salt-tolerant bacteria and investigate decolorization of different concentrations of azo dye by salt-tolerant bacteria under anaerobic conditions and different concentrations of NaCl.

1 Materials and methods

1.1 Dyes and chemicals

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

1.2 Organism and medium

The mixed bacterial cultures were obtained from the aerobic part of the Sewage Treatment Plant of Chunliu (Dalian, China), from the aerobic part of the wastewater treatment plant of a food industry (Tianjin, China) and from the bay of Dalian (China), which were abbreviated as CAS, TAS and DSAS, respectively. These mixed bacterial cultures were acclimatized in a salt-tolerant medium and incubated at 30℃ on a rotary shaker at 150 r/min.

The salt-tolerant medium contained yeast extract 5 g/L, peptone 10 g/L and 0—250 g/L NaCl (pH 7.0), which was abbreviated as STM.

1.3 Decolorization of azo dye (K-2BP) by salt-tolerant cultures

The decolorization experiments were conducted in rubber-stoppered serum bottles, and acclimatization of dyes were not carried out. First, salt-tolerant CAS, TAS and DSAS were grown in STM with 150 g/L NaCl. Cells were harvested by centrifugation at 8000 × g for 10 min and resuspended in STM with different salt and dye concentration to optical density 0.26—0.30 at 660 nm (OD_{660} = 0.26—0.30). Then, these cell suspensions were transferred into rubber-stoppered serum bottles (100 ml), containing 100 ml STM with Reactive Brilliant Red K-2BP 100—1000 mg/L. The culture was cultivated in anaerobic medium containing different concentrations of dye at 30℃. The decolorization rate was calculated at λ_{max} (540 nm) of medium supernatant after centrifugation at 8000 × g for 10 min. The cell concentration was measured by optical density at 660 nm.

In order to study the effect of NaCl and dyes concentration on decolorization by these cultures, two kinds of experiments were run. In the first set of experiments, the

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initial dye concentration was kept constant (350, 550, 750 mg/L, respectively) and NaCl was fed at a range of concentrations from 5 g/L to 150 g/L. In the second set of experiments, the NaCl concentration was kept constant and dye was supplemented with concentrations ranging from 350 mg/L to 750 mg/L. Both cell-free and sterilized controls of those mixed cultures 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 duplicate.

1.4 Effect of salt-tolerant bacteria on different dye decolorization

The ability of these mixed cultures degrading various dyes was studied. The dyes included azo, anthraquinone, triphenylmethane and metal complex dyes. The experiment was conducted as the above-described procedure. The NaCl concentration and the dye concentration of the culture were 150 g/L and 50 mg/L, respectively.

1.5 Analytical methods

Absorbance of the dye-containing solution was measured at their respective λ_{max} values 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 for K-2BP. The relationship between absorbance and concentration was unaffected by pH in the range of 6—9 and NaCl concentration in the range of 0.5—150 g/L.

2 Results and discussions

2.1 Comparison of the acclimatizing ability of three mixed cultures under high salt concentration

In a comparative study among CAS, TAS and DSAS, the effect of gradual increasing concentration of NaCl(5, 50, 100, 150, 200 and 250 g/L) was examined using STM medium, initial pH 7.0, 30℃, and shaking speed 120—130 r/min. The mixed culture of DSAS was the most salt-tolerant one among the three mixed cultures and the worst one was that of TAS(Fig.1, 2; data of other NaCl concentrations not shown). When incubation was conducted under 150 g/L NaCl, the difference between the three mixed cultures was slight. At the same time, the length of the lag phase increased from 20—30 h to 60—80 h at the NaCl concentration from 150 g/L to 250 g/L. This suggested that the high salt concentration inhibited the growth of various bacteria. How might this effect be explained? For thriving under conditions of elevated osmolarity, it is absolutely essential for the cells to measure external osmolarity and to adjust their metabolism accordingly by inducing the biosynthesis or uptaking of osmolytes, such as proline and glycine betaine, that may aid in osmotic adjustment(Markus, 2003). Therefore, adjusting and acclimating of the organisms cost time. While the lag phase could disappear after conducting these acclimating experiments many times.

2.2 Effects of NaCl and dye concentrations on decolorization by salt-tolerant cultures

Fig.3, 4 and 5 show that if the concentration of K-2BP was kept constant with increasing the salt concentration, decolorization was more than 90%. At the concentration of 5, 80 and 150 g/L NaCl, CAS and TAS showed slight differ-

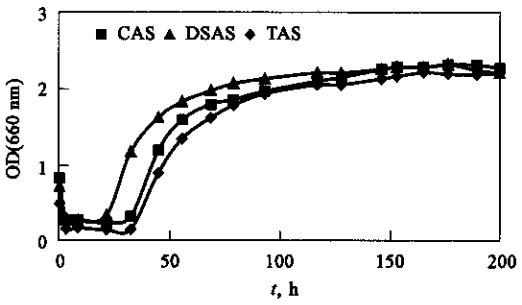


Fig.1 Growth of the three mixed cultures(150 g/L NaCl)

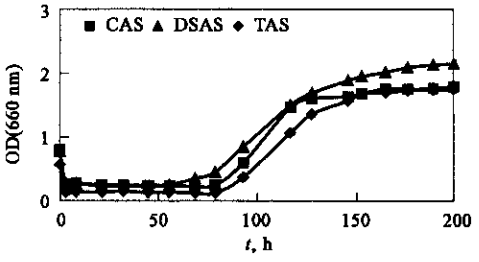


Fig.2 Growth of the three mixed cultures(250 g/L NaCl)

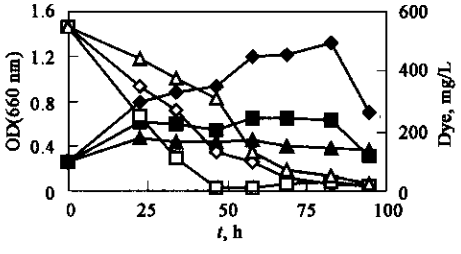


Fig.3 Decolorization and growth of CAS at different NaCl concentrations and 550 mg/L K-2BP
◇. growth at 5 g/L NaCl; ◆. decolorization at 5 g/L NaCl; □. growth at 80 g/L NaCl; ■. decolorization at 80 g/L NaCl; △. growth at 150 g/L NaCl; ▲. decolorization at 150 g/L NaCl

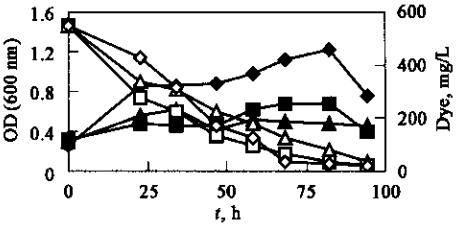


Fig.4 Decolorization and growth of SAS at different NaCl concentrations and 550 mg/L K-2BP
◇. growth at 5 g/L NaCl; ◆. decolorization at 5 g/L NaCl; □. growth at 80 g/L NaCl; ■. decolorization at 80 g/L NaCl; △. growth at 150 g/L NaCl; ▲. decolorization at 150 g/L NaCl

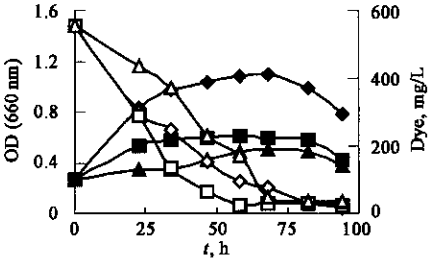


Fig.5 Decolorization and growth of TAS at different NaCl concentrations and 550 mg/L K-2BP
◇. growth at 5 g/L NaCl; ◆. decolorization at 5 g/L NaCl; □. growth at 80 g/L NaCl; ■. decolorization at 80 g/L NaCl; △. growth at 150 g/L NaCl; ▲. decolorization at 150 g/L NaCl

ence in the decolorization rate, DSAS showed the higher decolorization activity. In contrast, with the elevated NaCl from 5 g/L NaCl to 150 g/L, it was obvious that the decolorization rate of those cultures decreased. The similar phenomenon occurred in other high salt wastewater(Panswad, 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, 1998).

When NaCl concentration was constant, the decolorization rate decreased with the increase of concentration of azo dye. The decolorization rate varied from mixed cultures to mixed cultures(Fig.6, 7 and 8), and the greatest rates were obtained with the mixed cultures of DSAS, followed by the mixed cultures of CAS, and the mixed culture of TAS gave the lowest rates. There was a linear correlation between the concentration of dye and the decolorization rate in the range of 350—750 mg/L. The decolorization reaction could be described as first order kinetics, which has also been observed previously(Wuhrmann, 1980). As the initial dye concentration increased above 750 mg/L, the decolorization rate showed a decreasing tendency. This decrease in decolorization may be due to an inhibition effect introduced by the dye metabolites(i.e. aromatic amines) accumulated in the medium, which are formed by cleavage of azo-bonds, to inhibit intracellular azoreductase activity. In addition, the lower activity under high salt conditions limited cells to generate sufficient NADH, which is a required electron donor for microbial decolorization. Controls with sterilized-cell and with cell-free gave no significant reduction. There was no visual adsorption of dyes by the mixed cultures.

Finally, after four continually transfers, at the NaCl concentration of 150 g/L, 2000 mg/L K-2BP, the complete decolorization was observed in 36 h.

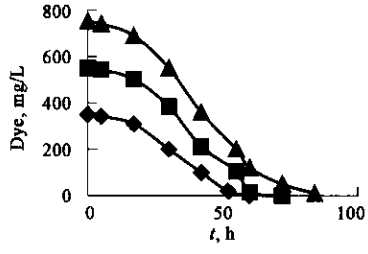


Fig.6 Decolorization of CAS at different K-2BP concentrations and 100 mg/L NaCl
◆. 350 mg/L K-2BP; ■. 550 mg/L K-2BP; ▲. 750 mg/L K-2BP

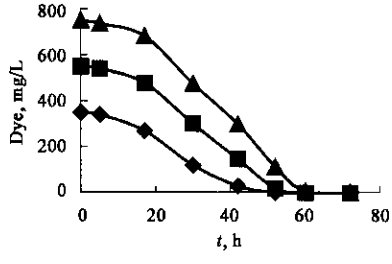


Fig.7 Decolorization of DSAS at different K-2BP concentrations and 100 mg/L NaCl
◆. 350 mg/L K-2BP; ■. 550 mg/L K-2BP; ▲. 750 mg/L K-2BP

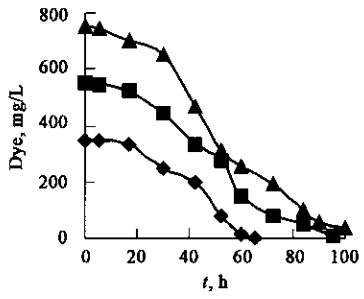


Fig.8 Decolorization of TAS at different K-2BP concentrations and 100 mg/L NaCl
◆. 350 mg/L K-2BP; ■. 550 mg/L K-2BP; ▲. 750 mg/L K-2BP

2.3 Effect of pH

Because the ability of decolorization is correlated with the activity of bacteria, which is lower and even loses completely out of the range of 6—10, the optimum pH of three mixed cultures was almost the same range of 6—10 (Fig.9).

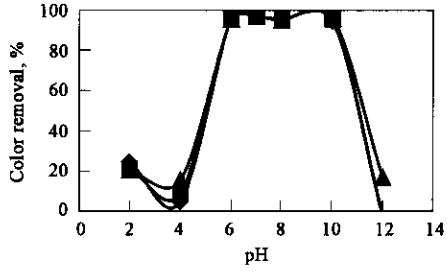


Fig.9 The effect of decolorization by pH(100 g/L K-2BP)

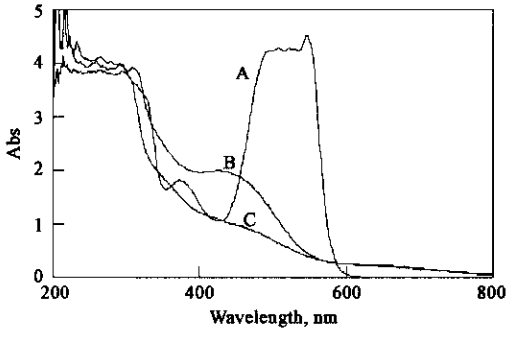


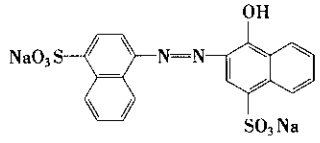
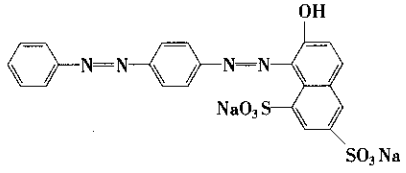
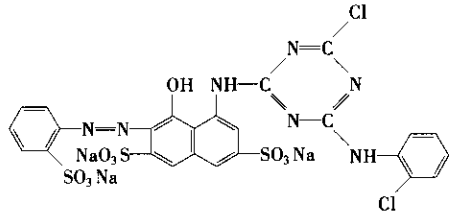
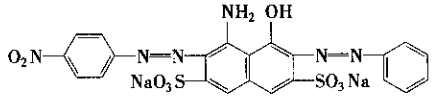
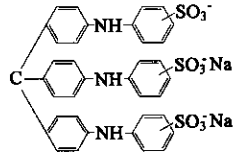
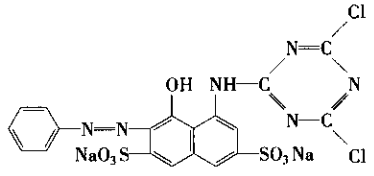
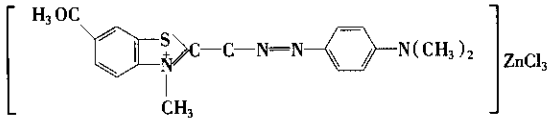
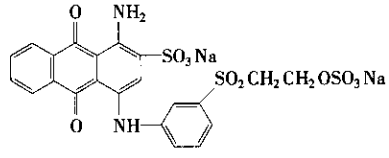
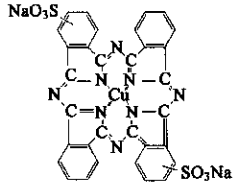
Fig.10 UV-visible absorbance spectra of K-2BP(A), the oxidation products of the anaerobic products of K-2BP(B) and the anaerobic products of K-2BP (C)

2.4 Effect of re-aeration

At high concentration of dye, the contents would be almost colorless after the cultivation, but, color would gradually reappear if exposed to air. The absorbance increased in the range of 400—500 nm due to the oxidation products of the anaerobic products of K-2BP, which resulted in the darken color of the reduced dye solution(Fig.10). Partial oxidation of reduced compounds with reappearance of color of reduced dye molecules have also been observed in other research(Beydili, 2000). The organic dye molecules was reported to be modified by biomass to such an extent that they produce other colored chromospheres and also the partially broken amines could self-polymerize to form colored and biological recalcitrant compounds(Hao, 2000). The phenomena were also explained in detail that *o*-amino-

hydroxybenzenes and *o*-aminohydoxybaphthalenes, which were commonly occurring products of the anaerobic reduction of azo dyes by microorganisms, were oxygen-sensitive and decomposed under aerobic conditions (Kudlich, 1999). Oxidation of the hydroxyl groups and the amino groups were converted to quinones and quinone imines (Pearce, 2003). These compounds were capable of dimerization or polymerization, and such reactions could lead to the development of new chromophores. However, when the reappeared solution was in the sealed bottles, the darken color could disappear. And the darken color matters inhibited the rate of cleavage of the azo bond under anaerobic conditions. This point may imply that the toxicity of the darken color matters to cells is greater than K-2BP, as constant cell density was maintained during K-2BP decolorization.

Table 1 Dye structures and decolorization efficiency

Dyes	Dye structures	Color removal, % *	λ_{max}, nm	The mixed cultures	Structure type
Acid Red B		100(3 d)	515	CAS, TAS, DSAS	Azo
Acid Scarlet GR		100(3 d)	509	CAS, TAS, DSAS	Azo
Reactive Brilliant Red K-2BP		100(3 d)	540	CAS, TAS, DSAS	Azo
Acid Black 10B		100(7 d) 50(7 d)	619	CAS, SAS TAS	Azo
Acid Ink Blue G		100(1 d)	582	CAS, DSAS, TAS	Triphenyl methane
Reactive Brilliant Red X-3B		100(7 d)	538	CAS, DSAS, TAS	Azo
Cationic Brilliant Blue 2RL		100(7 d)	598	CAS, DSAS, TAS	Complex metal
Reactive Brilliant Red K-NR		100(7 d)	597	CAS, DSAS, TAS	Anthraquinone
Direct Fast Turquoise GL		70(7 d)	616	CAS, DSAS, TAS	Complex metal

Notes: * 150 g/L NaCl and 50 mg/L dye

2.5 Effect of salt-tolerant bacteria on different dye decolorization

All azo dyes could be decolorizated by the unadapted cultures in 7 d, and most azo dyes were reduced completely by three mixed cultures in 1—3 d (Table 1). Unadapted mixed cultures decolorized these structurally dissimilar azo dyes, which suggested that decolorization of azo dyes under anaerobic conditions was not a specific process and probably widespread (Seshadri, 1994; Bromley-challenor, 2000; Brown, 1993). The decolorization rates of azo dyes were affected by their molecular weights, substitution group of the dye molecules, and the intramolecular hydrogen bond between the azo and hydroxyl groups. It is considered that the low decolorization of Direct Deep Blue L-3RB is attributed to those factors (Supaka, 2004). The color of anthraquinone dyes could also be decolorized by those salt-tolerant mixed cultures and the color of metal complex dyes were not easy to be removed, such as Direct Fast Turquoise GL. In terms of decolorization rate, the mixed culture of CAS was the best, and the worst was TAS.

3 Conclusions

Among the three mixed cultures, in the light of the acclimation of salt and the decolorization effect of different azo dyes and other dyes (anthraquinone types and metal complex dyes), CAS was the best cultures applied in high salt concentration of dye wastewater, the next culture was DSAS, and the final one was TAS. Therefore, in terms of theory, activated sludge could be used to treat high salt wastewater by a gradually acclimated procedure. This was demonstrated in many studies (Benedict, 2002; Hamoda, 1995; Panswad, 1999). There exists a potential to produce a simple process for the high salt wastewater, which could be incorporated into conventional activated sludge plants. While, in practice, there still were many questions to be explored, such as the addition of co-metabolism substrates.

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