Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 20(2008) 1218–1225
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Biodecolorization and partial mineralization of Reactive Black 5 by a strain of *Rhodopseudomonas palustris*

WANG Xingzu, CHENG Xiang, SUN Dezhi*, Qi Hong

School of Municipal & Environmental Engineering, Harbin Institute of Technology, Harbin 150090, China. E-mail: wuxingzhizhong@yahoo.com.cn

Received 24 December 2007; revised 10 March 2008; accepted 1 April 2008

Abstract

A strain of photosynthetic bacterium, *Rhodopseudomonas palustris* W1, isolated from a lab-scale anaerobic moving bed biofilm reactor (MBBR) treating textile effluent was demonstrated to decolorize Reactive Black 5 (RB5) efficiently under anaerobic condition. By a series of batch tests, the suitable conditions for RB5 decolorization were obtained, namely, pH < 10, light presence, glutamine or lactate as carbon source with concentration more than 500 mg/L when lactate is selected, NH_4Cl as a nitrogen source with concentration more than 100 mg/L, NaCl concentration not exceeding 5%, and RB5 concentration less than 700 mg/L. In addition, ultraviolet-visible (UV-Vis) spectrum scan and High Performance Liquid Chromatography/Mass Spectrometry (HPLC-MS) were used to analyze the metabolites of RB5 decolorization of W1. The results showed that partial aromatic amines produced with RB5 reduction were further degraded during the extended period. Anaerobic partial mineralization of RB5 was suggested, and a possible degradation pathway was proposed.

Key words: decolorization; mineralization; Reactive Black 5 (RB5); photosynthetic bacterium; metabolite analysis

Introduction

Reactive azo dyes are widely used as textile colorants, typically for cotton dyeing, due to their variety of color shades, high wet fastness profiles, ease of application, brilliant colors, and minimal energy consumption. They approximately account for up to 70% of the total dyestuff produced (Carliell *et al*., 1995; Lee *et al*., 2004). However, as much as 20%–50% of the initial reactive dyes are washed off in the dyeing process resulting from their easy hydrolyzation and consequential low affinity for the fiber, therefore, they remain in the dyebath effluents (Sopa *et al*., 2000). Apart from the aesthetic concerns, the dye-containing effluents may cause serious problems since the cleavage of azo bonds produces aromatic amines, which are considered mutagenic and carcinogenic (Hu, 2001; O'Neill *et al*., 2000).

Many physicochemical methods have been employed to treat textile wastewater for decolorization and detoxification, which includes advanced oxidation processes (such as the use of Fenton reagent, hydrogen peroxide or ozone), coagulation-flocculation, activated carbon adsorption, membrane filtration, ion exchange, irradiation, and electrokinetic coagulation. However, almost none of these treatments are very feasibile, due to either high running cost or huge chemical sludge production (Sen and Demirer, 2003).

On the other hand, researchers found that azo dyes could be biologically transformed. White-rot fungus was reported to be a kind of microorganism capable of degrading a wide variety of recalcitrant compounds by their extracellular enzyme system such as, lignin peroxidase (LiP), laccase, and manganese peroxidase (MnP) (Fu and Viraraghavan, 2001). It was difficult, however, to keep them in functional form in activated sludge systems, because special nutrients and environmental conditions were generally required for fungal growth. Other publications recently showed that azo dyes could also be decolorized by azoreductase under anaerobic conditions to form aromatic amines, which were further biodegraded aerobically via hydroxylation and ring-opening (Albuquerquea *et al*., 2005; Brown and Hamburger, 1987; Frijtersa *et al*., 2006; Nuttapun *et al*., 2004; Sponza and Isik, 2002).

Biodecolorization and mineralization of textile wastewater is believed to be a promising technology, since it is cost-effective and environment-friendly. To obtain high efficiency of color removal from dyestuff wastewater, both microbial consortium and pure isolates were investigated by earlier studies. These related bacteria consist of *Bacillus*, *Sphaerotilus*, *Arthrobacter*, *Alcaligenes*, *Pseudomonas*, *Sphinogomonas*, *Proteus*, *Streptococcus*, *Aeromonas*, *Bacteroides*, *Shewanella*, *Clostridium*, *Hydrogenophaga*, *Kurthia*, *Desulfovibrio*, *Paenibacillus*, and so on (Pearcea *et al*., 2003).

Certain photosynthetic bacteria have recently been reported that arealso capable of biodegrading azo dyes,

* Corresponding author. E-mail: sdzlab@126.com.

although the mechanism is still unclear (Hong and Otaki, 2003; Hong *et al*., 2005; Song *et al*., 2003; Yan *et al*., 2004). In this study, a strain of photosynthetic bacteria, with high decolorization capability, was isolated from an anaerobic moving bed biofilm reactor (MBBR), which treats azo dye wastewater. By using 16S rRNA sequence determination and operation parameter optimization, the authors have obtained a basic knowledge of the bacterium and its potential application for azo dye wastewater treatment. Meanwhile, a probable metabolic pathway has been proposed using product analysis.

1 Materials and methods

1.1 Azo dye and chemical reagents

Reactive Black 5 (RB5), selected as a common azo dye, was obtained from Orichem International Ltd., Zhejiang, China (Fig.1). The commercial product was used without further purification. Stock solutions of the dyes were prepared at 5,000 mg/L, and diluted before use. Other chemicals and medium components were at least analytical grade reagents.

1.2 Screening of bacterial strain

Sludge sample as the microorganism source was from a lab-scale anaerobic MBBR reactor treating textile effluent, in which RB5 was the sole dye contaminant. The screening medium (SM) consisted of the following (per liter): 0.05 g RB5, 3.4 g sodium lactate (carbon source, with COD of 3,000 mg/L), 1 g NH_4Cl (nitrogen source), 1 g $NaHCO_3$, 0.2 g K_2HPO_4, 1 g NaCl, 0.2 g $MgSO_4{\cdot}7H_2O$, 0.1 g $FeSO_4{\cdot}7H_2O$, 0.05 g yeast extract and 10 ml trace solution. The trace solution contained the following components (per liter): 0.01 g $MnSO_4{\cdot}7H_2O$, 0.05 g $ZnSO_4{\cdot}7H_2O$, 0.01 g H_3BO_3, 0.01 g $CaCl_2$, 0.01 g Na_2MoO_4, 0.2 g $CoCl_2{\cdot}6H_2O$, and 0.01 g $AlK(SO_4)_2$. pH of the SM ($NaHCO_3$ and RB5 not included) was adjusted to 7.0 before it was autoclevaged at 121°C for 20 min. $NaHCO_3$ was prepared as 5% stock solution, and was filter-sterilized together with dye solution prior to its addition to the sterilized SM. The final concentrations of $NaHCO_3$ and RB5 were 1 g/L and 50 mg/L, respectively.

For screening high-performance bacterial decolorizer, Hungate roll-tube technique was adopted (Hungate, 1969). The isolates were cultured at 33 ± 1°C under 1,500 lx illumination for 2–3 d. The screening work was repeatedly conducted in 100-ml serum bottles until a pure culture was obtained.

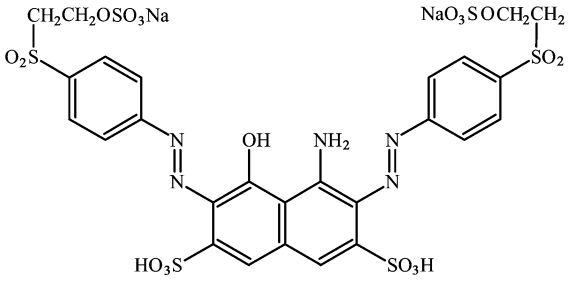

Fig. 1 Chemical structure of Reactive Black 5 (RB5) (λ_{max} = 597 nm).

1.3 Identification of dye-decolorizing bacteria

The bacterial strain obtained with the highest decolorizing activity was identified by biochemical and physiological investigation. To confirm that result, genomic DNA from that strain was extracted using the bacterial DNA mini kit (Watson Biotechnologies, China) according to the manufacturer's instructions. The 16S rRNA gene PCR amplifications were performed by using a pair of universal primers, 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1541R (5'-AAGGAGGTGATCCAGCC-3'). Sequencing was conducted by Bioasia Biological Technology Service, Shanghai, China. The obtained sequences were compared with those deposited at the GenBank database (National Center for Biotechnology Information, NCBI) and identified by using the Basic Local Alignment Search Tool (BLAST) also available in NCBI.

1.4 Decolorization tests

Decolorization tests were conducted in 100-ml serum bottles under different conditions. Temperature was kept at 33 ± 1°C by a thermostat. Media for all these tests were similar to the SM described in Section 1.2. In tests of carbon source, sodium lactate in the SM was replaced by formate, acetate, propionate, butyrate, oxalate, and glutamine, respectively, with the same COD concentration of 3,000 mg/L. Similarly in tests of nitrogen source, NH_4Cl was replaced by nitrite, nitrate, glutamine, and urea, respectively. To investigate the effects of the concentration of carbon and nitrogen source on RB5 decolorization, 0–3,000 mg/L of lactate and NH_4Cl were used. In the study on salinity, different amounts of NaCl were added and the salinities (0.5%–20%) were calculated by the mass concentration of NaCl (*m/V*). For tests on the dye tolerance of W1, different amounts of RB5 were dispensed in the medium with the concentration ranging from 50 to 1,000 mg/L. pH was adjusted to 7.2 ± 0.1 in all tests except in the study of the pH effect, in which pH 5–12 was achieved by using 1 mol/L of HCl or NaOH. The effect of light was evaluated by putting the control bottles in a dark environment. Bacterium concentration and color removal were monitored in all the tests.

1.5 Biomass measurement

Biomass concentration was determined by optical density (OD) at 660 nm using visible-light spectrophotometer (Precision Instrument Factory of Shanghai, China). Moreover, a calibration curve was established between cell dry weight (mg/L) and the corresponding OD value (Eq.(1), R^2 = 0.9989).

$$\text{Cell dry weight} = 413.1 \times \text{OD} - 2.4 \quad (1)$$

1.6 Measurement of color removal

Culture samples were first centrifuged at 8,000 × *g* for 10 min, and the supernatant was then examined at the maximum absorption wavelength of RB5 (λ_{max} = 597 nm)

to calculate the decolorization efficiency (F) (Eq.(2)).

$$F = \frac{(A_i - A_f)}{A_i} \times 100 \quad (2)$$

where, A_i is the initial absorbance, A_f is the absorbance of the decolorized sample.

Ultraviolet-visible (UV-Vis) spectrum scan was also performed to show color removal, using UV-2550 spectrophotometer (Shimadzu, Japan).

1.7 Measurement of decolorization metabolites

Samples for metabolite analysis were taken from decolorization tests, in which the bacteria were cultivated in SM at 33 ± 1°C under 1,500 lx illumination. Suspended particles were removed by the samples being filtrated through a 0.45-μm pore size membrane. The filtrate was then analyzed by the LCQ Deca XP MAX LC-MS system (Thermo, USA) for composition investigation. The sample volume injected was 25 μl. The chromatographic separation was done using a Spherisorb 3 ODS-2 column (100 × 2.0 mm i.d., Spherisorb, UK). The mobile phase was a mixture of methanol and deionized water (7:3, *V/V*) and was delivered at a flow rate of 0.2 ml/min. Mass spectra were obtained using an ion-trap mass spectrometer fitted with an electrospray (ESI, Thermo Finnigan LCQ-Duo, USA) interface operated in negative ionization mode, with a spray voltage of 5.0 kV, at a capillary temperature of 275°C, sheath gas at 35 AU (arbitrary unit), and auxiliary gas at 5 AU.

2 Results and discussion

2.1 Identification of the decolorizing strain

One of the total six isolates obtained, W1, was chosen for the next decolorization test, due to its highest decolorizing capability. W1 was identified as *Rhodopseudomonas palustris* according to biochemical and physiological investigation (Table 1). Atomic force microscopy (AFM) and transmission electron microscopy (TEM) investigation provided more information on the morphology (Fig.2). In addition, the result from 16S rRNA sequencing revealed that the strain W1 was most closely identical to *Rhodopseudomonas palustris* phylogenetically (99% sequence identity).

2.2 Effect of initial pH on the biodecolorization

High pH level of textile effluents is one of problems in their biological treatment. However, as shown in Fig.3, decolorization of RB5 by *R. palustris* W1 was not seriously inhibited at pH from 5 to 10, though the bacterial growth was obviously pH-sensitive, and the biomass concentration

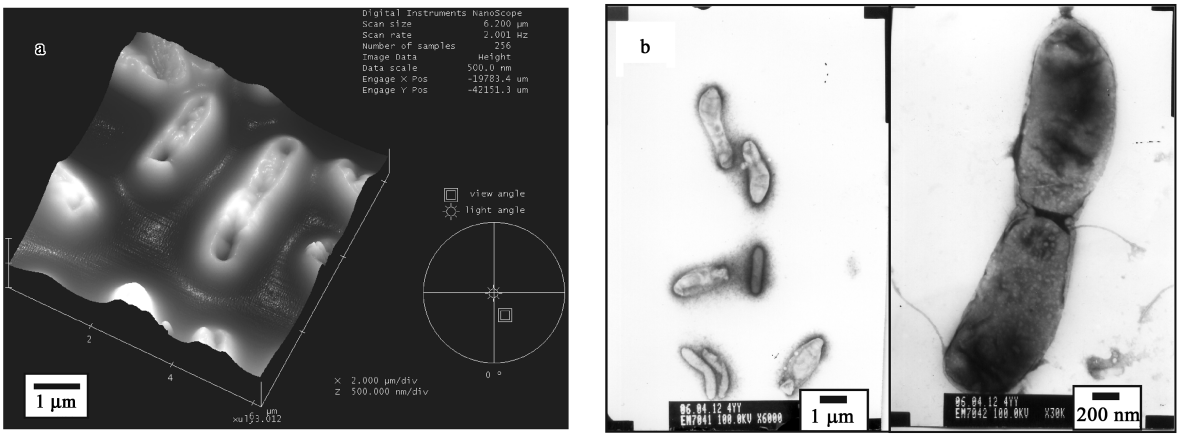

Fig. 2 AFM (a) and TEM (b) images of the isolated *Rhodopseudomonas palustris* W1.

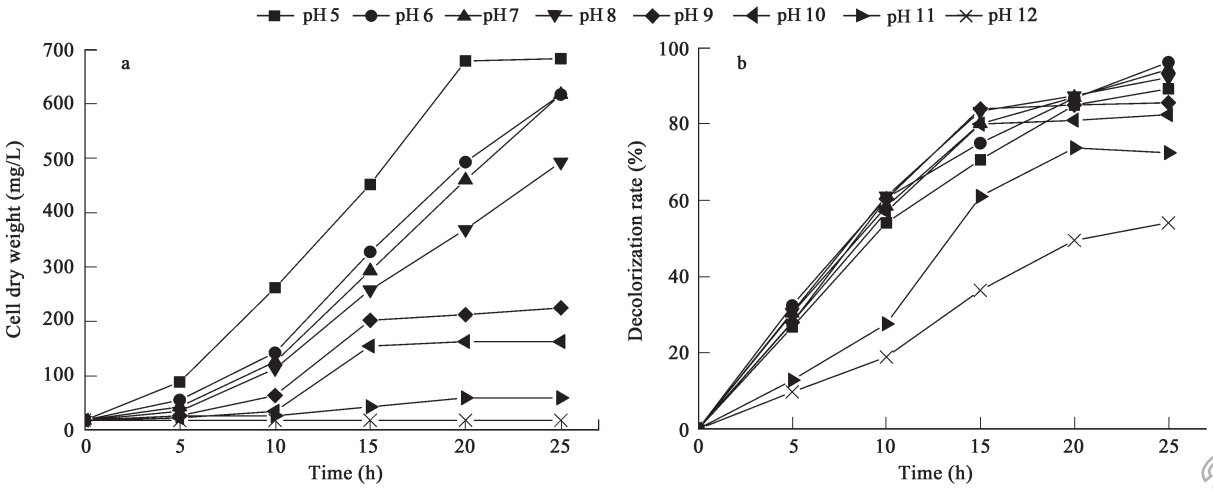

Fig. 3 Profiles of *R. palustris* W1 growth (a) and dye decolorization (b) at different initial pH levels.

Table 1 Biochemical and physiological profiles of the strain W1

Characteristics	Strain W1	Characteristics	Strain W1
Color of culture		Assimilation of	
Anaerobic-light	Red	Citrate	+
Aerobic-dark	White to pink	Ethanol	+
Cell shape	Short rod	Fumarate	+
Gram staining	–	Glucose	–
Motility	+	Succinate	+
Assimilation of		Mannitol	–
Lactate	+	Malonate	+
Nitrate	+	Thiosulfate	+
Formate	+	Sulfide	–
Acetate	+	Catalase	+
Benzoate	+	Sorbitol	–
Propionate	+	Indole	+
Butyrate	+		

after 25 h cultivation decreased gradually from 683.35 to 162.84 mg/L, with an increase in pH. At pH 6–8, over 90% of color could be removed. No strongly adverse effect on decolorization by *R. palustris* W1 was observed until the medium pH exceeded 10. Chen *et al*. (2003) found a similar result, where suitable pH ranged from 5.5 to 10.0 when a strain of *Aeromonas hydrophila* was employed to decolorize RED RBN. Besides growth inhibition, the dye molecule existed in an anionic form under a highly alkaline condition, which might account for the decolorization deterioration, due to its low affinity to the negative charged bacteria.

2.3 Effect of light presence on the biodecolorization

Growth rate of the photosynthetic bacterium, *R. palustris* W1, with light presence was almost four-fold higher than that in a dark environment, in 25 h (final biomass: light, 313.27 mg/L; dark, 88.48 mg/L). In terms of decolorization efficiency, a small, but still an obvious increase, was observed in the presence of light (Fig.4). Hence, light presence can be suggested to reach the optimal effect of decolorization by W1.

Up to now, few researches have covered decolorization of dye-contaminated effluent by photosynthetic bacteria. The correlation between light and color removal still needs further investigation.

2.4 Effect of carbon and nitrogen nutrients on biodecolorization

According to previous publications, azo bonds in the chromophores can be split by azoreductase-catalyzed reduction, under anaerobic conditions, which leads to biodecolorization of azo dye wastewater (Albuquerquea *et al*., 2005; Brown and Hamburger, 1987; Frijtersa *et al*., 2006; Nuttapun *et al*., 2004; Sponza and Isik, 2002). In this process, the azo bond acts as the terminal electron acceptor in the microbial electron transfer chain, thus, the presence of a labile carbon source is generally required (Carliell *et al*., 1995, 1996; Sen and Demirer, 2003). Fig.5 shows the relative ranking of the carbon cosubstrates in terms of RB5 decolorization efficiency, namely, glutamine > lactate > butyrate ⩾ propionate ⩾ acetate ⩾ oxalate > formate. During the first 10 h, *R. palustris* W1 exhibits a higher decolorization activity when glutamine or lactate is used as the carbon source. However, more color removal in the following stage is achieved when *R. palustris* W1 is fed with the other five carbon compounds. It can be explained by the fact that more dye remainder from the relatively poor decolorization in the beginning kept the reaction from slowing down sharply in the following stage.

In a further investigation, when lactate was the cosubstrate, the best decolorization of 98% at 20 h was obtained when 1000 mg/L lactate was added, but excessively high concentration did not further enhance the color removal efficiency (Fig.6).

The effect of different nitrogen nutrients on *R. palustris* W1 growth and its decolorization activity was also investigated in a batch test. Results indicated that ammonium chloride was the most suitable among all nitrogen sources, to promote W1 growth. High decolorization efficiencies of more than 83% were obtained under all nitrogen source conditions except the glutamine case, in which the corresponding efficiency was just some 40% (data not shown). This conclusion contradicted with Wuhrmann *et al*. (1980) and Carliell *et al*. (1996), who believed nitrate in the liquid was obviously a better electron acceptor than azo bond and prevented azo dye reduction.

In addition, no obvious difference in W1 growth was

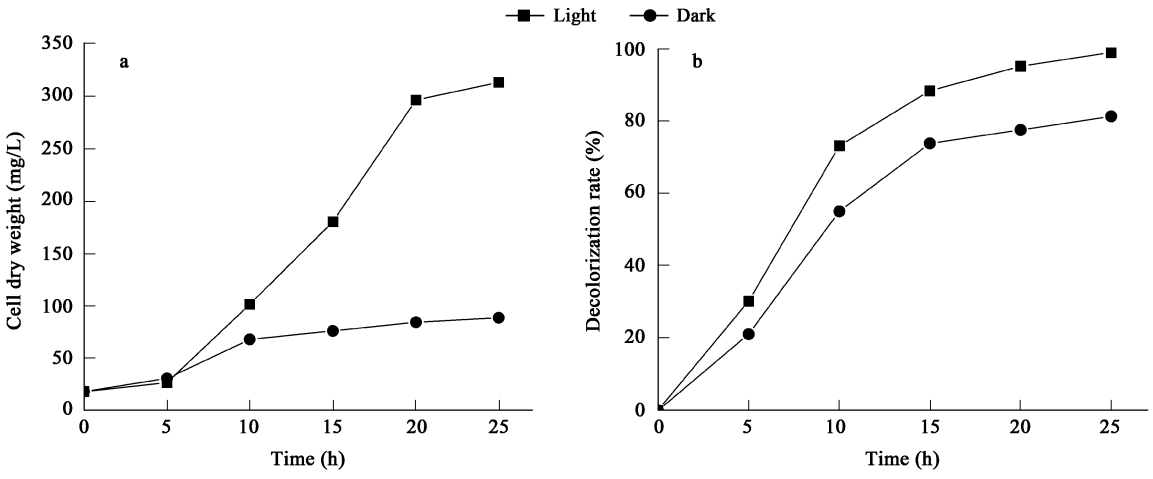

Fig. 4 Profiles of *R. palustris* W1 growth (a) and dye decolorization (b) under light and dark conditions.

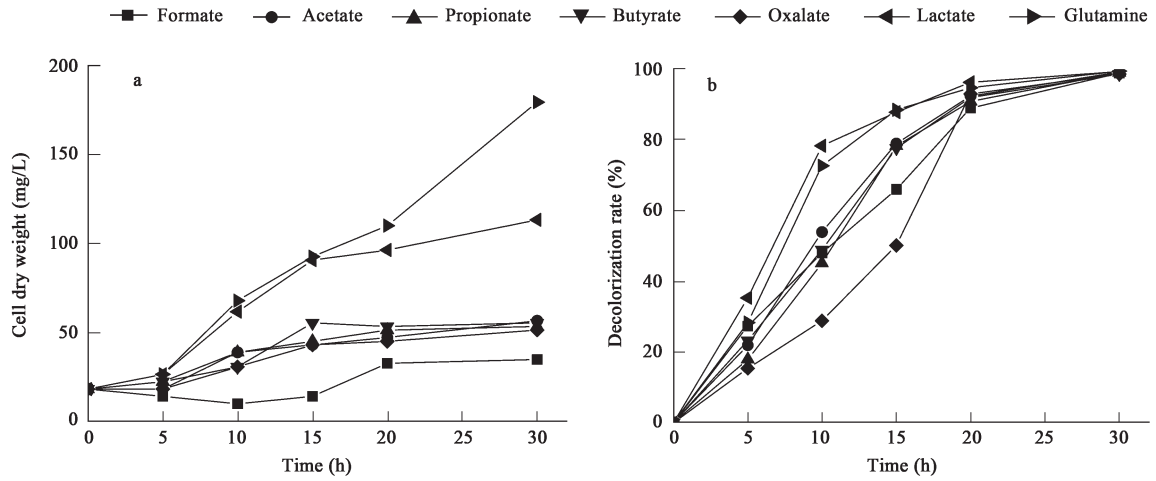

Fig. 5 Profiles of *R. palustris* W1 growth (a) and dye decolorization (b) under different carbon sources.

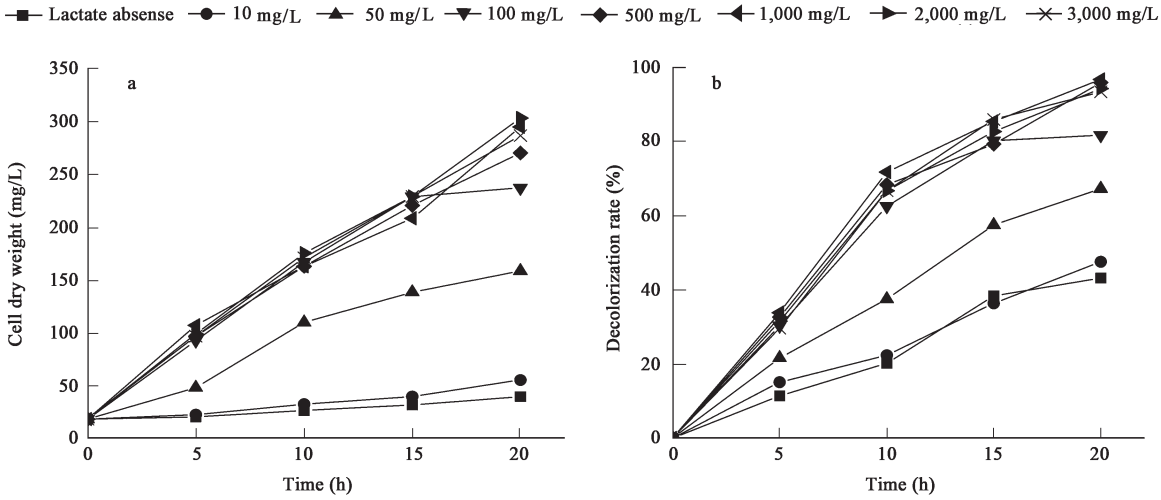

Fig. 6 Profiles of *R. palustris* W1 growth (a) and dye decolorization (b) under different lactate concentrations.

observed under ammonium chloride concentration from 0 to 3,000 mg/L, but color removal was seriously inhibited when the ammonium chloride fed was less than 100 mg/L (data not shown).

2.5 Effect of salinity on the biodecolorization

Reactive dye effluents generally contain a high concentration of salts (in the main form of NaCl), which are used in dyeing liquor, to lower the dye solubility and enhance bath dye exhaustion. High concentration of salts proved to have adverse effects on the dye biotreatment process (Delée *et al*., 1998; Manu and Chaudhari, 2003; Sen and Demirer, 2003). Two probable reasons for this inhibition are denaturing the functional azoreductase and reducing the substrate transfer rate. According to Fig.7, *R. palustris* W1 in this study seems to be strongly salt-resisting and the deterioration in the decolorization rate is not obvious until the NaCl concentration has exceeded 5%. It is undoubtedly important for the possible application of *R. palustris* W1 in real dye wastewater treatment.

2.6 Effect of dye concentration on biodecolorization

Microbial decolorization rate under anaerobic conditions was reported to decrease with increasing initial dye concentration (Carliell *et al*., 1995). Test results herein clearly indicate that the growth rate of *R. palustris* W1 was inversely related to the initial RB5 concentration in the medium. Zero order kinetic was demonstrated to describe W1 growth well ($R^2 > 0.96$), and the rate constant K decreased with an increase in the RB5 fed. Nevertheless, no obvious adverse effect of high dye concentration on decolorization was observed until over 700 mg/L, and even under these conditions just 40 h were required for complete decolorization (Fig.8). The decolorization efficiency herein was on level with or better than other related studies: 67% and 73% color removal in 10.5 and 79 h, respectively, 79% in 10 h and 66% in 24 h (Nuttapun *et al*., 2004). Results showed that first order kinetic was suitable to describe the decolorization process. Furthermore, the K value (rate constant of decolorization) gradually declined from 0.114 to 0.064 h^{-1} with dye concentration from 50 to 1,000 mg/L.

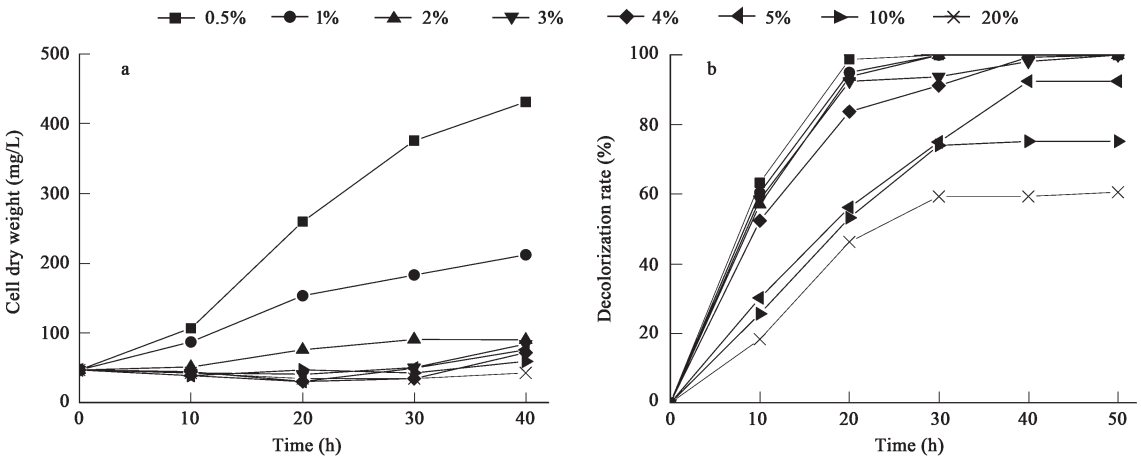

Fig. 7 Profiles of *R. palustris* W1 growth (a) and dye decolorization (b) at different NaCl concentrations.

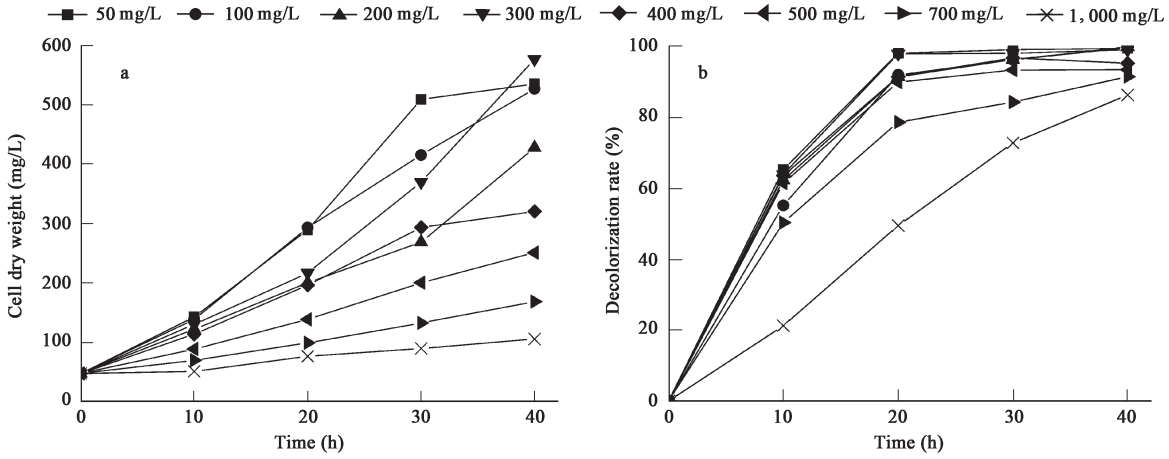

Fig. 8 Profiles of *R. palustris* W1 growth (a) and dye decolorization (b) at different RB5 concentrations.

2.7 Metabolites analysis of the biodecolorization

To make clear the metabolic products of RB5 biodecolorization, UV-Vis spectrum scan as well as HPLC-MS analysis was conducted. RB5 presented characteristic adsorption peaks at 597 and 310 nm, which could be ascribed to the presence of chromophoric azo bonds and aryl and naphthalene-like moieties (Lucas *et al*., 2006). As shown in Fig.9, RB5 was fast biodegraded in the first 15 h, accompanied by respectable intermediate production. These intermediates were generally suggested to be aromatic amines, due to the wavelength of the adsorption peak (254 nm) and the probable product interpretation of azo dye reduction. In more detail, HPLC-MS analysis of the culture sample at 15th hour demonstrated the production of compounds with molecular weight of 347, 348, 349, and 201, which could be interpreted as 7-amino-8-hydroxy-1,3-naphthoquinone-3,6-disulphonate-1,2-diimime ($TAHNDS_{DP1}$), dihydro-xynaphthoquinone-3,6-disulphonatediimine ($TAHNDS_{DP2}$), 1-2-7-triamino-8-hydroxy-3-6-naphthalinedisulphate (TAHNDS), and 2-(4-aminobenzenesulphonyl)ethanol (p-Base) (data not shown). After 60 h, p-Base were undetected. HPLC-MS analysis of the culture sample demonstrated the production of new compounds with molecular weights of 109 and 145, which could be interpreted as 4-aminophenol and 6-amino-6-oxohexanoic acid. The result indicated possible mineralization of partial aromatic amines during the extended period. It was of great importance since aromatic amines from azo dyes reduction were always supposed to be recalcitrant under anaerobic conditions. Previous studies, therefore, pointed out that anaerobic reduction of azo dyes was the first step of the whole textile wastewater

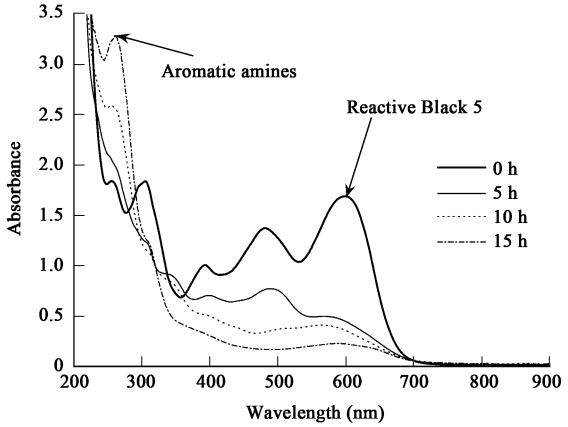

Fig. 9 Variation of UV-Vis spectra of RB5 solution after inoculation with *R. palustris* W1.

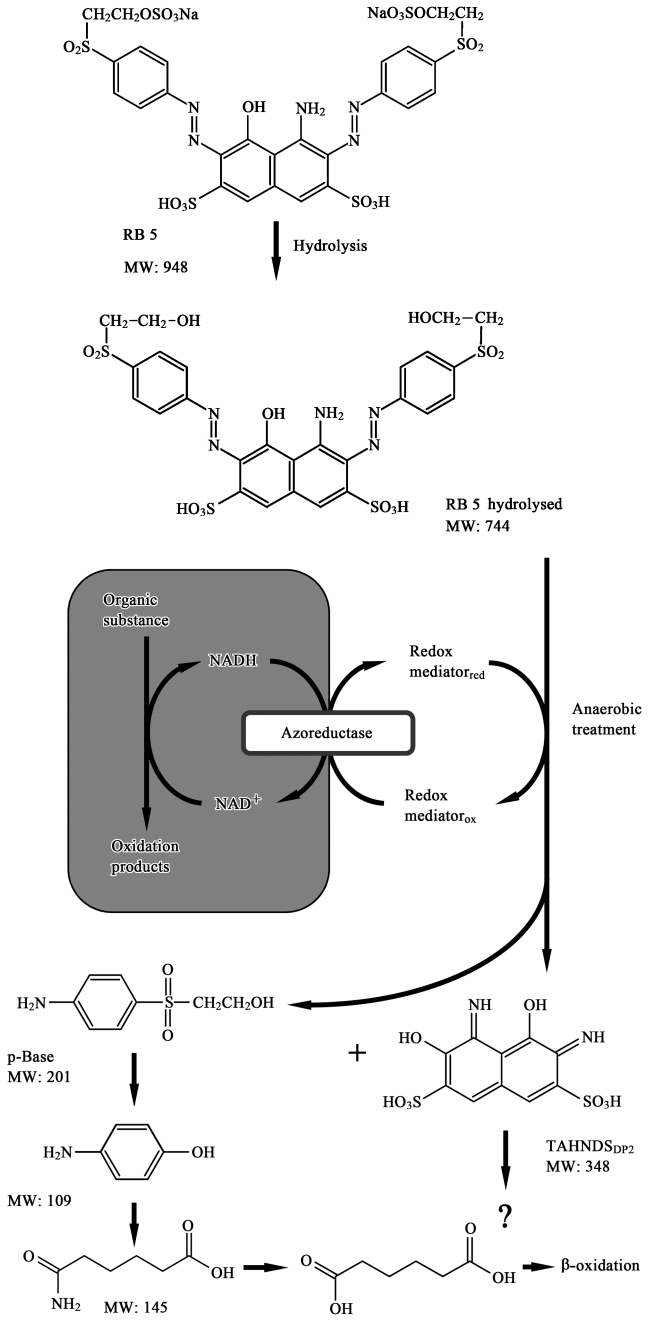

Fig. 10 Proposed metabolic pathway for the degradation of RB5 by *R. palustris* W1. MW: molecular weight.

treatment, which should be followed by aerobic units for complete mineralization of aromatic amines (Brown and Hamburger, 1987; Haug *et al*., 1991). Hence, the study herein gained a deeper insight about anaerobic partial mineralization of azo dyes by *R. palustris* W1. The possible degradation pathway was therefore proposed as shown in Fig.10.

3 Conclusions

An efficient bacterial decolorizer W1 was isolated from a lab-scale anaerobic MBBR reactor, treating azo dye-contaminated wastewater, and identified to be *Rhodopseudomonas palustris*. The results of decolorization batch tests suggested that to enhance W1 growth and its decolorization efficiency, suitable pH level ($\leqslant 10$), carbon source and its concentration (glutamine or lactate recommended, $\geqslant$ 500 mg/L when lactate is selected), nitrogen source and its concentration (NH_4Cl recommended $\geqslant$ 100 mg/L), salinity (NaCl concentration $\leqslant 5\%$), RB5 concentration (< 700 mg/L), and light presence were required. Under those optimal conditions, over 95% of color removal could be achieved in 20 h.

Metabolic product analysis revealed that partial aromatic amines produced in RB5 decolorization by *R. palustris* W1 were further degraded in extended periods. This should be of great use, because azo dye decolorization and mineralization were achieved in a single unit, whereas, the two-stage anaerobic/aerobic process was generally expected in previous publications, due to the recalcitrant characteristic of aromatic amines under anaerobic conditions. Therefore, further investigation and full understanding are quite necessary in future.

Acknowledgments

This work was supported by the Hi-Tech Research and Development Program (863) of China (No. 2007AA06Z300).

References

Albuquerquea M G E, Lopesb A T, Serralheirob M L, Novaisa J M, Pinheiroa H M, 2005. Biological sulphate reduction and redox mediator effects on azo dye decolorization in anaerobic-aerobic sequencing batch reactors. *Enzyme Microb Technol*, 36: 790–799.

Brown D, Hamburger B, 1987. The degradation of dyestuffs: Part III–investigations of their ultimative biodegradability. *Chemosphere*, 16: 1539–1553.

Carliell C M, Barclay S J, Buckley C A, 1996. Treatment of exhausted reactive dyebath effuent using anaerobic digestion: laboratory and full-scale trials. *Water SA*, 22: 225–233.

Carliell C M, Barclay S J, Naidoo N, Buckley C A, Mulholland D A, Senior E, 1995. Microbial decolourisation of a reactive azo dye under anaerobic conditions. *Water SA*, 21: 61–69.

Chen K C, Wu J Y, Liou D J, Hwang S C J, 2003. Decolorization of the textile dyes by newly isolated bacterial strains. *J Biotechnol*, 101: 57–68.

Delée W, O'Neill C, Hawkes F R, Pinheiro H M, 1998. Anaerobic treatment of textile effluents: a review. *J Chem Technol Biotechnol*, 73: 323–335.

Frijtersa C T M J, Vos R H , Schefferb G, Muldera R, 2006. Decolorizing and detoxifying textile wastewater, containing both soluble and insoluble dyes, in a full scale combined anaerobic/aerobic system. *Water Res*, 40: 1249–1257.

Fu Y Z, Viraraghavan T, 2001. Fungal decolorization of dye wastewaters: a review. *Biores Technol*, 79: 251–262.

Hu T L, 2001. Kinetics of azoreductase and assessment of toxicity of metabolic products from azo dye by *Pseudomonas luteola*. *Water Sci Technol*, 43: 261–269.

Haug W, Schmidt A, Nörtman B, Hempel D C, Stolz A, Knackmuss H J, 1991. Mineralization of the sulfonated azo dye Mordant Yellow 3 by a 6-aminonaphthalene-2-sulfonate-degrading bacterial consortium. *Appl Environ Microbiol*, 57: 3144–3149.

Hong J L, Emori H, Otaki M, 2005. Photodecolorization of azo

dyes by extracellular metabolites under fluorescent light and influence of operational parameters. *J Biosci Bioeng*, 100: 192–196.

Hong J L, Otaki M, 2003. Effects of photocatalysis on biological decolorization reactor and biological activity of isolated photosynthetic bacteria. *J Biosci Bioeng*, 96: 298–303.

Hungate R E, 1969. A roll tube method for cultivation of strict anaerobes. In: Methods in Microbiology (Norris J. R., Ribbons D. W., eds.). New York: Academic Press. Vol. 3B. 117–132.

Lee Y H, Spyros G, Pavlostathis, 2004. Decolorization and toxicity of reactive anthraquinone textile dyes under methanogenic conditions. *Water Res*, 38: 1838–1852.

Lucas M S, Amaral C, Sampaio A, Peres J A, Dias A A, 2006. Biodegradation of the diazo dye Reactive Black 5 by a wild isolate of *Candida oleophila*. *Enzyme Microb Technol*, 39: 51–55.

Manu B, Chaudhari S, 2003. Decolorization of indigo and azo dyes in semicontinuous reactors with long hydraulic retention time. *Proc Biochem*, 38: 1213–1221.

Nuttapun S, Kanchana J, Somsak D, Marie-Line D, Pierre S, 2004. Microbial decolorization of reactive azo dyes in a sequential anaerobic-aerobic system. *J Chem Eng*, 99: 169–176.

O'Neill C, Lopez A, Esteves S, Hawkes F R, Hawkes D L, Wilcox S, 2000. Azo-dye degradation in an anaerobic-aerobic treatment system operating on simulated textile effluent. *Appl Microbiol Biotechnol*, 53: 249–254.

Pearcea C I, Lloydb J R, Guthrie J T, 2003. The removal of colour from textile wastewater using whole bacterial cells: a review. *Dyes Pigm*, 58: 179–196.

Sen S, Demirer G N, 2003. Anaerobic treatment of real textile wastewater with a fluidized bed reactor. *Water Res*, 37: 1868–1878.

Song Z Y, Zhou J T, Wang J, Yan B, Du C H, 2003. Decolorization of azo dyes by *Rhodobacter sphaeroides*. *Biotechnol Lett*, 25: 1815–1818.

Sopa C, Munsin T, Thongchai P, 2000. Anaerobic decolorization of reactive dyebath effluents by a two-stage UASB system with tapioca as co-substrate. *Water Res*, 34: 2223–2232.

Sponza D T, Isik M, 2002. Decolorization and azo dye degradation by anaerobic/aerobic sequential process. *Enzyme Microb Technol*, 31: 102–110.

Wuhrmann K, Mechsner K, Kappeler T, 1980. Investigation on rate-determining factor in the microbial reduction of azo dyes. *Appl Microbiol Biotechnol*, 9: 325–338.

Yan B, Zhou J T, Wang J, Du C H, Hou H M, Song Z Y, Bao Y M, 2004. Expression and characteristics of the gene encoding azoreductase from *Rhodobacter sphaeroides* AS1.1737. *FEMS Microbiol Lett*, 236: 129–136.