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Decolorization of azo dyes by *Rhodobacter sphaeroides*

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Abstract

Rhodobacter sphaeroides AS1.1737 decolorized more than 90% of several azo dyes (200 mg dyes l^{-1}) in 24 h. The optimal culture conditions were: anaerobic illumination (1990 lx), peptone as carbon source, temperature 35–40 °C and pH 7–8. Intracellular crude enzyme from this strain had azoreductase activity, optimized temperature as 45–50 °C, and decolorization kinetics which were consistent with a ping-pong mechanism.

Introduction

Azo dyes are the largest chemical class of dyes used regularly for textile dyeing and paper printing. These dyes are usually recalcitrant to conventional wastewater treatment (Chen 2002) but, because physical-chemical methods are generally expensive and produce large amounts of sludge, more studies are now focused on their biodegradation (Yang *et al.* 2003).

A prerequisite for the mineralization of many azo dyes is a combination of reductive and oxidative steps (Tan *et al.* 1999). The first step is decolorization of azo dyes by reduction or cleavage of azo bond by anaerobic digestion producing colorless compounds. The second step is the complete degradation of aromatic amines under aerobic conditions (Sponza & Isik 2002). Therefore integrated anaerobic/aerobic processes (Tan *et al.* 1999) are used to mineralize azo dyes.

The non-sulfur phototrophic bacterium, *Rhodobacter*, can grow under anaerobic or aerobic conditions, and degrade organics through diverse catabolic pathways which is then advantageous to biodegradation process for azo dyes. However, its ability to decolorize azo dyes has not yet been reported. In order to extend applications of *Rhodobacter sphaeroides*, the decolorization of azo dyes by this bacterium and the characters of its intracellular crude enzyme are now reported in this paper.

Materials and methods

Organism and growth

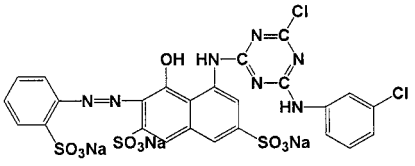
Rhodobacter sphaeroides AS1.1737 was grown anaerobically at 30 °C under illumination of 1990 lx in serum bottles, containing 100 ml medium (NH_4Cl 0.1 g, $NaHCO_3$ 0.1 g, K_2HPO_4 0.02 g, $MgSO_4 \cdot 7H_2O$ 0.02 g, NaCl 0.05∼0.2 g, peptone 4 g, distilled water 100 ml, pH 7).

Dyes and chemicals

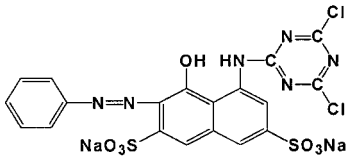
Reactive Brilliant Red K-2BP, Reactive Brilliant Red X-3B, Reactive Yellow X-6G and three Red Azo Dyes I, II, III (Figure 1) were from Dye Synthesize Laboratory, Dalian University of Technology.

Decolorization of azo dyes by growing cells

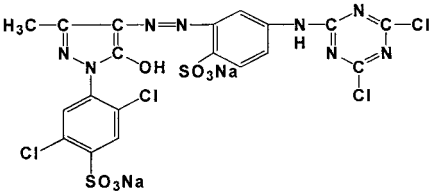
The decolorization experiments were performed in serum bottles. The bacterial strain was cultivated in the anaerobic medium containing different azo dyes. The decolorization rate was calculated at λ_{max} of medium supernatant after centrifugation at 8000 *g* for 10 min.



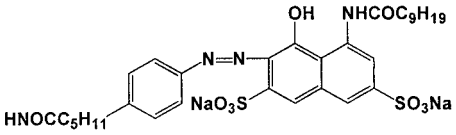
K-2BP



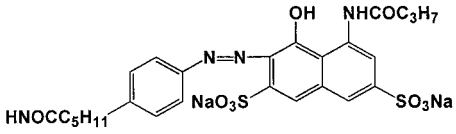
X-3B



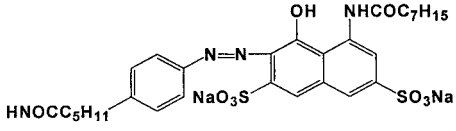
X-6G



Red Dye I



Red Dye II



Red Dye III

Fig. 1. The chemical structures of azo dyes.

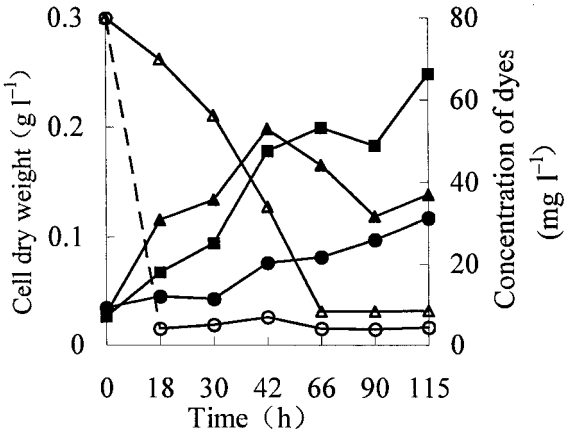


Fig. 2. Time-course of decolorization of Red Azo Dye I and II by growing cells (80 mg dye l^{-1}, ■: growth of culture without any dye; ♦: growth with Red Dye I; ▲: growth with Red Dye II; △: decolorization of Red Dye I; ○: decolorization of Red Dye II).

Table 1. Decolorization of six azo dyes by growing cells in 24 h (200 mg dye l^{-1} anaerobically at 30 °C).

Dyes	Decolorization (%)
X-3B	95
K-2BP	77
X-6G	67
Red Dye I	23
Red Dye II	95
Red Dye III	94

Cell concentration was measured at 660 nm and converted to the cell dry weight [OD_{660} of 0.505 = 1 g l^{-1} ($R^2 = 0.990$)].

Decolorization of Methyl Orange by azoreductase from Rhodobacter sphaeroides

The cells from 2000 ml culture were suspended in 40 ml 20 mM sodium-phosphate buffer (pH 7) and lysed twice by freezing and thawing followed by sonication (30 min, 225 W). After centrifugation at 8000 × *g* for 40 min, the supernatant was used as the intracellular enzyme for decolorization activity assays. The reaction mixture in a final volume of 2 ml consisted of various concentrations of Methyl Orange and crude enzyme in 20 mM sodium phosphate buffer, and adding NADH to start the reaction. The decolorization activity of crude enzyme was determined by measuring decrease at λ_{max} of Methyl Orange.

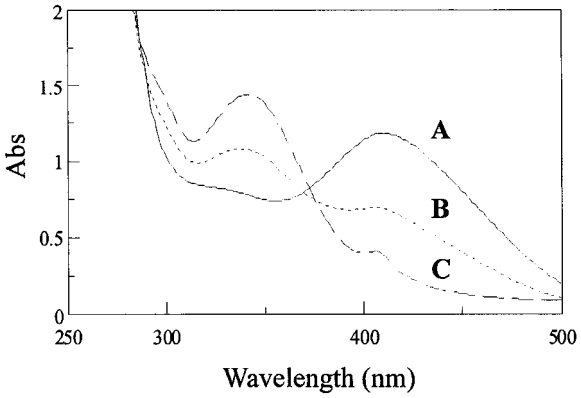


Fig. 3. Spectral scans (250–500 nm) of the culture supernatant with initial 100 mg X-6G l^{-1} at different time. Samples were centrifuged at 8000 rpm for 10 min (A: 0 h; B: 18 h; C: 24 h).

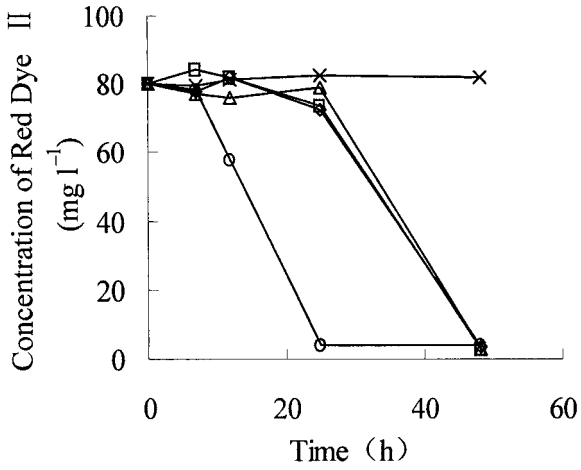


Fig. 4. Effect of carbon source on decolorization of Red Dye II (50 mg dye l^{-1}, 4 g carbon source l^{-1}, ○: peptone; □: glucose; △: sucrose; ◇: malic acid; ×: without carbon source).

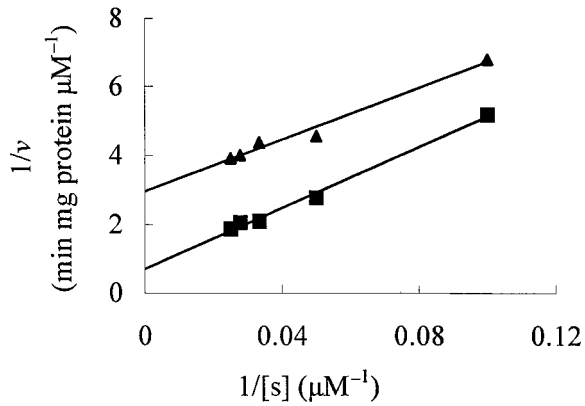


Fig. 5. Double-reciprocal plot of initial velocity (v) versus concentration of Methyl Orange [s] (10–40 μM). Methyl Orange decolorization activity was assayed at 40 °C with constant initial NADH concentration (▲: 500 μM, ■: 850 μM).

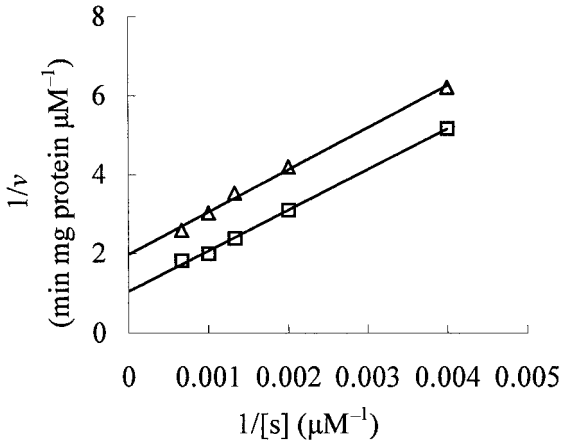


Fig. 6. Double-reciprocal plot of initial velocity (v) versus concentration of NADH [s] (250–1500 μM). Methyl Orange decolorization activity was assayed at 40 °C with constant initial Methyl Orange concentration (△: 20 μM, □: 40 μM).

Results and discussions

Effect of different dyes on cells growth and decolorization

Decolorizations of six different azo dyes by *Rhodobacter sphaeroides* AS1.1737 were shown in Table 1. X-3B and Red Dye II, III were decolorized by more than 90% in 24 h, and K-2BP, X-6G had about 70% decolorization. Although Red Dye I had only 23% decolorization rate in 24 h, it was almost completely decolorized at 66 h (Figure 2). Red Dye II was decolorized more rapidly, and the growth with it was better than with Red Dye I or without any dye in 42 h (Figure 2). An intermediate product with λ_{max} of 340 nm was formed after decolorization of X-6G (see Figure 3), which probably was an amino derivative (O'Neill *et al.* 2000).

Effect of additional carbon source

Addition of a carbon source accelerated decolorization of Red Dye II (Figure 4). The decolorization rate with peptone reached 95% in 24 h which was much faster than with the others.

Effect of temperature and pH

Decolorization of Red Dye II (200 mg dye l^{-1}) by growing cells was optimal at 35–40 °C and at pH 7–9 (data not shown).

Decolorization of Methyl Orange by crude enzyme from Rhodobacter sphaeroides

Methyl Orange was used as the experimental dye in enzyme assays. The highest decolorization rate of Methyl Orange by a crude enzyme preparation was at 45–50 °C. A double-reciprocal plot of initial velocity versus concentration of Methyl Orange resulted in parallel pattern (Figure 5), and the plot versus concentration of NADH also resulted in parallel pattern (Figure 6), which is consistent with a ping-pong mechanism (Nakanishi *et al.* 2001). According to the enzyme ping-pong kinetics, the initial velocity $v = (V_{\max}[\mathrm{A}][\mathrm{B}])/(K_{mA}[\mathrm{B}] + K_{mB}[\mathrm{A}] + [\mathrm{A}][\mathrm{B}])$, where [A] as concentration of Methyl Orange; [B] as concentration of NADH; K_{mA} and K_{mB} as the Michaelis constant for Methyl Orange and NADH, respectively. K_{mA} was calculated as 0.5 mM and K_{mB} as 12.6 mM.

Acknowledgement

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