DECOLORIZATION OF AZO DYES BY PURPLE NON-SULFUR BACTERIA

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ABSTRACT
Purple non-sulfur bacterial isolates from different water resources have shown decolorizing activity on different azo dyes. Five isolates of different purple nonsulfur bacterial species, i.e. *Rhodobacter adriaticus, R. blasticus, R. capsulatus, Rhodovulum strictum, Rhodopseudomonas palustris*) could decolorize up to 96% of tested dyes after two days of illuminated and anaerobic incubation. The decolorizing activity varied by the phototrophic isolates and by the dye substrates. Although phototrophic bacteria have been known to be able to metabolize N-heterocyclic compounds, this is the first report on the metabolizing activity of these bacteria on azo dyes.

KEYWORDS
Azo dye, Decolorization, Purple non-sulfur bacteria, Textile dyestuff

INTRODUCTION

Azo dyes are widely used in industrial, printing as well as textile dyeing process because of their diversity of chemical structure, ease of synthesis and chemical stability. Recently, azo dyes became of concern in wastewater treatments because 10-15% of the dyes used in dyeing processing are discharged in effluent (Vaidya, 1982), particularly because of their bio-recalcitrance and potential toxicity to animals and humans (Levine, 1991; Chung, 1992). Thus the wastewater with azo dyes must be decolorized and furthermore mineralized in appropriate systems. Biological treatment of azo dyes-polluted wastewater has the advantage of cost effectiveness. Although, aerobic decolorization of azo dyes by white rot fungi has been reported (Ollikka et al., 1993; Swamy and Ramsay 1999a, 1999b), azo dyes have rarely been decolorized by bacteria under aerobic conditions (Zimmermann et al., 1982; Idaka et al., 1978, 1987). The anaerobic treatment of textile wastewater can decolorize a variety of dyes (Brown and Laboureur, 1983). Treatment schemes for colored wastewater based on the anaerobic bacterial reduction of the azo bond are being used by the textile industry, as well as being studied by many groups (Walker, 1970; Whurmann et al. 1980; Chung and Stevens, 1993). Microbial reductive decolorization has been suggested to involve an azo reductase (Zimmermann et al., 1982; Chung and Stevens, 1993), an intracellular reduction by reduced flavin nucleotides ($FADH_2$) (Whurmann et al. 1980) or secreted extracellular redox mediators (H_2S) (Yoo E.S., 2000). Various obligate and facultative anaerobes have been reported to be able to decolorize azo dyes. However, the ability has not shown yet for the diverse group of phototrophic bacteria. Phototrophic bacteria are Gram-negative cells predominating in most of natural habitats and having a very broad phenotypic range. Purple non-sulfur bacteria have been known for their diverse energy metabolisms such as chemolithotroph, chemoheterotroph, S-, Fe-oxidizers, S- reducers, etc. They can also carry out anoxygenic photosynthesis with H_2S or H_2 as electron donor for NADPH synthesis in CO_2 fixation (Madigan M.T. et al., 2002). These bacteria have been shown to degrade varied heterocyclic xenobiotics (Blasco R. and Castillo F., 1992; Harwood C.S. and Gibson J., 1988; Kamal V.S. and Wyndham R.C., 1990; Khanna P. Rajkumar B.S. and Jothikumar N., 1992; Montgomery L. and Vogel T.M., 1992). Recently, we have isolated and identified of purple non-sulfur bacterial isolates from different water-bodies in Vietnam and many of theses isolates showed high capacity of metabolizing N-heterocyclic xenobiotics (Ai et al., 2002). We have also found that some of these isolates could also decolorize azo dyes. Experimental results to demonstrate their decolorizing activity were reported in this communication.

MATERIALS AND METHODS

Microorganism and media

Purple non-sulfur bacteria used in this study had been isolated and identified by Ai et al., 2002. Strains were maintained on 2% agar SA (1g/l sodium succinate, 0.5g/l KH_2PO_4, 0.6g/l K_2HPO_4, 1g/l $(NH_4)_2SO_4$, 0.2g/l $MgSO_4.7H_2O$, 0.2g/l NaCl, 0.05g/l $CaCl_2.2H_2O$, 0.1g/l $Na_2S_2O_3.5H_2O$, 0.1g/l yeast extract, 1ml/l vitamin solution, 1ml/l mineral solution, pH 6.8 - 7.0) or, alternatively for salt-tolerant strains, on 2% agar SSW (1g/l sodium succinate, 0.2g/l KH_2PO_4, 0.9g/l K_2HPO_4, 1g/l $(NH_4)_2SO_4$, 0.2g/l $MgSO_4.7H_2O$, 20g/l NaCl, 0.05g/l $CaCl_2.2H_2O$, 0.1g/l $Na_2S_2O_3.5H_2O$, 2g/l $MgCl_2$, 0.1g/l yeast extract, 1ml/l vitamin solution, 1ml/l mineral solution, pH 6.8 - 7.0) media; vitamin solution (0.5g/l thiamin hydrochloride, 0.5g/l niacin, 0.3g/l p-aminobenzoic acid, 0.1g/l pyridoxal hydrochloride, 0.05g/l biotin, 0.05g/l B_{12}), mineral solution (1g/l EDTA-2Na, 2g/l $FeCl_3$, 0.1g/l $ZnCl_2$, 0.1g/l $MnCl_2$, 0.1g/l H_3BO_3, 0.1g/l $CoCl_2.6H_2O$, 0.02g/l $Na_2MO_4.2H_2O$, 0.01g/l $CuCl_2.2H_2O$, 0.01g/l $NiCl_2.6H_2O$, 0.005g/l Na_2SeO_3) (Hoshino, 1984; Kawasaki, 1993).

Azo dyes

The azo dyes used in this study (Table 1) include Orange II, Orange 16 and some commercially significant dyes in Vietnam.

Solid-plate decolorization studies

Solid SA or SSW medium containing azo dye was prepared by adding agar powder and a dye solution to a final concentration of 2% and 10ppm, respectively. Each plates containing one of the dyes and a control plate with no dye added were inoculated with one purple non-sulfur strain and incubated under anaerobic and illuminated (2000lux provided by a lamp) condition at 30°C for 7 days. Decolorization was assessed by appearance of halo clear zone surrounding the bacterial colonies on the plates. Uninoculated plates served as controls for abiotic decolorization.

Aqueous batch decolorization studies

Each strain of *Rhodobacter blasticus* PN5, *R. capsulatus* PN16, and *Rhodopseudomonas palustris* PN15 was inoculated into different 250ml-Erlenmeyer flasks containing 100ml SA medium. Alternatively, each strain of *Rhodobacter adriaticus* PN21, and *Rhodovulum strictum* PN31, was inoculated into different 250ml-Erlenmeyer flasks containing 100ml SSW medium. Each flask was added an aliquot of dye solution to a final concentration of 50ppm, and incubated under illuminated anaerobic condition at 30°C for 3 days to be used as seeding cultures.

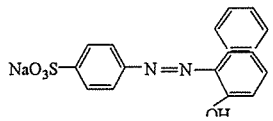
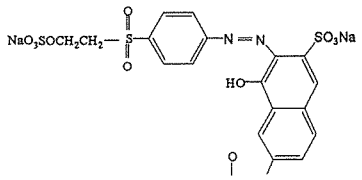
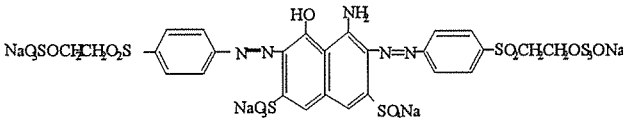
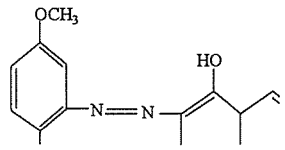
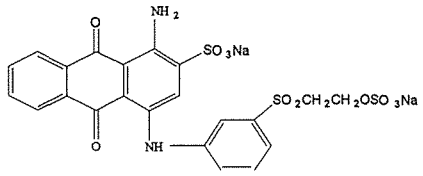
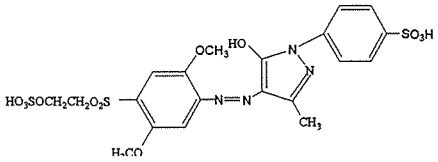
Ten ml of each seeding culture was correspondingly transferred into flask containing 90ml of the same medium with corresponding dye as the seeding culture. The seeded flasks were incubated in the same condition as for seed cultivation. After 24 and 48 hours of incubation, sample was taken. Bacterial cells were eliminated by centrifuged at 10.000rpm for 5 min and the supernatant was used for spectrophotometric measurement. Residual concentration of a certain tested dye was determined basing on the corresponding calibration curve of the absorbance at maximal wavelength of the dye versus a range of 1 - 5ppm of the dye. Relative decolorizing activity was expressed as the residual percentage of the dye after incubation taking the initial concentration of dye in the medium as 100.

RESULTS AND DISCUSSION

Screening of potential purple non-sulfur bacterial isolates being capable of decolorizing azo dye

Azo dye-decolorizing activity was screened on our collection of 17 strains belonging to 8 species of identified purple non-sulfur phototrophic bacteria, which have been isolated from different water bodies in Vietnam. This initial evaluation of dye decolorizing was done using solid

Table 1. Structure and maximal visible wavelength of azo dyes

Dye	λ_{max} (nm)	Structure
Orange II	485	
Orange 16	492	
Reactive Black 5	597	
Remazol Red 3BS	546	
Remazol Blue G133	675	
Remazol Yellow 3RS	499	

medium with the two analytical grade azo dyes Orange II and Orange 16. After 7 days of illuminated anaerobic incubation, decolorization activity was evaluated basing on the diameter of clear zone around the colonies (Table 2). Among the screened phototrophic bacterial strains, only the 5 strains of *Rhodobacter blasticus* PN5, *Rhodobacter adriaticus* PN21, *Rhodobacter capsulatus* PN16, *Rhodovulum strictum* PN31 and *Rhodopseudomonas palustris* PN15 showed strong decolorizing activity on both Orange II and Orang 16. These strains were selected and their

dye decolorizing activity was quantified by aqueous batch culture in later experiments. Other strains showed no activity or very weak activity on these two dyes.

Decolorization of azo dyes by purple non-sulfur bacterial strains in liquid culture

Rhodobacter blasticus PN5 could decolorize 40 and 44% of Orange II after 24 and 48h incubation, respectively (Table 3). Decolorizing activity on Orange 16 was limited to 16% after 48h incubation. As for Reactive Black 5, the activity was limited 20% after 24h but could be extended to 40% after 48h. This strain showed almost no activity on the tested Remazol dyes

Table 2. Screening of purple non-sulfur isolates for dye decolorizing activity

Strain	Decolorizing activity*		Strain	Decolorizing activity*	
	Orange II	Orange 16		Orange II	Orange 16
Rhodobacter adriaticus			*Rhodobacter sphaeroides*		
PN17	+	-	PN11	-	+
PN30	-	+	PN26	+	-
PN21	++	+++	*Rubrivivax gelatinosa*		
Rhodobacter blasticus			PN2	+	-
PN5	+++	+++	PN3	+	-
Rhodobacter capsulatus			PN10	-	+
PN6	+	-	*Rhodopseudomonas acidophilus*		
PN9	-	+	PN24	-	+
PN13	-	+	*Rhodopseudomonas palustris*		
PN16	+++	+++	PN15	++	+++
Rhodovulum strictum			PN20	+	-
PN31	++	+++			

*, Decolorizing activity basing on the diameter of the halo ring surrounding the bacterial colony.

Table 3. Relative decolorizing activity of purple non-sulfur bacterial strains in liquid culture

Dye	Relative decolorization activity (%)*				
	Rhodobacter blasticus PN5**	*Rhodobacter capsulatus* PN16**	*Rhodopseudo-monas palustris* PN15**	*Rhodobacter adriaticus* PN21***	*Rhodovulum strictum* PN31***
24h incubation					
Orange II	40.0	32.0	0	22.0	0
Orange 16	16.0	30.0	70.0	16.0	0
Re. Black 5	20.0	30.0	0	6.0	0
Rm. Red 3BS	0	0	46.0	22.0	58.0
Rm. Blue G133	0	0	0	26.0	0
Rm. Yellow 3RS	0	0	10.0	22.0	0
48h incubation					
Orange II	44.0	32.0	0	22.0	0
Orange 16	16.0	94.0	96.0	70.0	80.0
Re. Black 5	40.0	36.0	0	20.0	0
Rm. Red 3BS	12.0	44.0	76.0	44.0	58.0
Rm. Blue G133	0	18.0	70.0	16.0	0
Rm. Yellow 3RS	0	0	10.0	26.0	0

*, Percentage of residual dye compared to the initial amount before incubation.
, SA medium. * SSW medium. Re., reactive; Rm., Remazol.

after 24h but could decolorizing 12% of Remazol Red 3BS after 48h. *Rhodobacter capsulatus* PN16 could decolorize a wide range of dyes. After 24h of incubation, the strain could decolorize 30-32% of Orange II, Orange 16 and Reactive Black 5, while showing no activity on Remazol groups. When the incubation was extended to 48h, except for Remazol Yellow 3RS, which could not be decolorized at all, other tested dyes were decolorized from 18-94%. *Rhodopseudomonas palustris* PN15 could not decolorize Orange II and Reactive Black 5 within 48h. On the other hand, the strain showed high activity on Remazol group, especially on Orange 16, 70% and 96% of the dye was decolorized after 24h and 48h, respectively. Red 3BS and Blue G133 were decolorized up to 76% after 48h, while Yellow 3RS was limited at 10%. Among the two salt- tolerant strains

(*Rhodobacter adriaticus* PN21 and *Rhodovulum strictum* PN31), *R. adriaticus* PN21 strain showed decolorizing activity on all the tested dyes. The activity was at 44 and 70% of Remazol Red 3BS and Orange 16, respectively, and 16 - 26% of the other dyes after 48h incubation. *Rhodovulum strictum* PN31 had more substrate selectivity. After 24h incubation, the strain could decolorize only Remazol Red 3BS but with fairly high 58% relative activity. After 48h, it could also decolorize Red 3BS and Orange 16 at 58% and 80%, respectively. The strain could not decolorize Orange II, reactive Black 5, Remazol Blue G133 and Yellow 3RS after 48h incubation. Thus, studied purple non-sulfur strains exhibited different decolorizing capacity on different azo dyes. *R. capsulatans* PN16 could decolorize 32-94% of 5 among the 6 tested dyes. *R. adiaticus* PN21 showed activity on all 6 studied dyes with relative activity varied from 16 up to 70%. *R. palustris* PN15 and *R. blasticus* PN5 showed 8-96% relative activity on 4 of the 6 tested dyes. *R. strictum* PN31 could showed 20-80% relative activity on 2 among the tested dyes. On the other hand, Orange 16 and Red 3BS were widely sensitive to all 5 purple non-sulfur strains. Remazol Blue G133 was decolorized by 3 among 5 studied strains, and Orange II, Reactive Black 5 were sensitive to 3 of tested bacterial strains within 2 days. Remazol Yellow 3RS was most recalcitrant and could be decolorized slightly by only 2 among studied bacterial strains.

CONCLUSIONS

Purple non-sulfur bacteria have been known for their diverse energy metabolisms and various application properties. These bacteria have interesting properties such as protein rich cell mass to produce single cell protein, H_2-producing capacity for renewable energy strategy, high content of biological active substances such as vitamins, ubiquinon, good capacity of degradation of xenobiotics, especially heterocyclic comounds. However, the ability of decolorizing and metabolizing azo dyes was not reported for purple phototrophic bacteria in general and purple non-sulfur phototrophic bacteria, in particularly. We have recently isolated and identified of purple non-sulfur bacterial isolates from different water-bodies in Vietnam. Many isolates showed high capacity of metabolize N-heterocyclic xenobiotics (Ai et al., 2002). In this report, we successfully demonstrated that some of these isolates could also decolorize different azo dyes, including commercially significant monoazo and disazo dyes used in Vietnam. This is the first report on decolorization of azo dyes by phototrophic bacteria. Our experimental results showed that the decolorizing activity varied by the phototrophic isolates and by the dye substrates. A mixture of strains rather a pure strain, therefore, would be the choice for dye-containing wastewater treatment purpose. On the other hand, none of studied strains could decolorize a 100% of the dyes after 48h incubation. Further studies on the optimizing of treatment condition in order to improve decolorizing activity are needed.

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