Biodegradation (2008) 19:643–650
DOI 10.1007/s10532-007-9169-8

ORIGINAL PAPER

Decolorization of anthraquinone dye intermediate and its accelerating effect on reduction of azo acid dyes by *Sphingomonas xenophaga* in anaerobic–aerobic process

Hong Lu · Jiti Zhou · Jing Wang · Haixin Ai · Chunli Zheng · Yusuo Yang

Received: 24 September 2007 / Accepted: 27 November 2007 / Published online: 11 December 2007

Abstract Decolorization of 1-aminoanthraquinone-2-sulfonic acid (ASA-2) and its accelerating effect on the reduction of azo acid dyes by *Sphingomonas xenophaga* QYY were investigated. The study showed that ASA-2 could be efficiently decolorized by strain QYY under aerobic conditions according to the analysis of total organic carbon removal and UV–VIS spectra changes. Moreover, strain QYY was able to reduce azo acid dyes under anaerobic conditions. The effects of various operating conditions such as carbon sources, temperature, and pH on the reduction rate were studied. It was demonstrated that ASA-2 used as a redox mediator could accelerate the reduction process. Consequently the reduction of azo acid dyes mediated by ASA-2 and the decolorization of ASA-2 with strain QYY could be achieved in an anaerobic–aerobic process.

Keywords Azo acid dyes · Anthraquinone dye intermediate · *Sphingomonas xenophaga* QYY · Reduction · Redox mediator · Decolorization

H. Lu (✉) · J. Zhou · J. Wang · H. Ai · C. Zheng · Y. Yang
School of Environmental and Biological Science and Technology, Dalian University of Technology, 116023 Dalian, P.R. China
e-mail: lvhonghj@163.com

Abbreviations

AQDS	Anthraquinone-2,6-disulfonic acid
AQS	Anthraquinone-2-sulfonic acid
ASA-2	1-Aminoanthraquinone-2-sulfonic acid
BAA	1-Amino-4-bromoanthraquinone-2-sulfonic acid
DBSM	Defined basal salts medium
LB medium	Luria–Bertani medium
SM	Synthetic medium

Introduction

With the development of modern industries, the output of acid dyes has been increasing. It is reported that output of Chinese acid dyes is the largest in the world (Lin and Xi 2006). They are widely used in textiles and printing industries. In acid dyes, azo group is the most important chromophore (Baughman and Weber 1994). Azo acid dyes containing sulfonic groups usually resist biodegradation in conventional sewage treatment plants (Rau et al. 2002). Thus they could be found in industrial effluents. But the release of these colored effluents into the environment is undesirable, not only because of their color, but also because azo acid dyes and their breakdown products are toxic and mutagenic to life (Dos Santos et al. 2007). Compared with chemical and physical treatment processes, biological methods have received more attention owing to their cost effectiveness,

environmental friendliness, and less sludge production (McMullan et al. 2001). Therefore, the development of a practical bioprocess for treating dye-containing wastewater is of great significance.

Integrated anaerobic–aerobic treatment is the most logical strategy for the complete removal of azo acid dyes in biological systems (Stolz 2001). But the anaerobic reduction of these compounds in these systems usually proceeds rather slowly (Rau et al. 2002). It has been demonstrated that the addition of quinones, such as AQS, AQDS, and BAA etc., results in significantly increased reduction rates of azo acid dyes under anaerobic conditions (Guo et al. 2007; Dos Santos et al. 2004; Rau et al. 2002; van der Zee et al. 2001). However, additional redox mediators imply additional expenses for using and treating the recalcitrant compounds. Therefore, incorporation treatment of an alternative redox mediator and azo acid dyes would be a great improvement to the application of the redox mediator.

It was reported that *Sphingomonas xenophaga* QYY isolated from sludge samples could decolorize BAA under aerobic conditions (Qu et al. 2005). In addition, AQS could accelerate the reduction of azo acid dyes by *S. xenophaga* BN6 under anaerobic conditions (Rau et al. 2002). ASA-2, an important anthraquinone dye intermediate, has similar chemical structure with AQS and BAA. In this paper, the decolorization of ASA-2 under aerobic conditions and the reduction of azo acid dyes mediated by ASA-2 under anaerobic conditions with *S. xenophaga* QYY were investigated in detail. To our knowledge, this is the first report of ASA-2 accelerating effect on the reduction of azo acid dyes and the decolorization of ASA-2 by strain QYY in an anaerobic–aerobic process.

Materials and methods

Dyes and chemicals

Acid Red B (C.I.14720), Acid Red GR (C.I.27290), ASA-2, and BAA (Fig. 1) were obtained from Dye Synthesis Laboratory of Dalian University of Technology. They were purified before use. Acid Red 3R (C.I.16255), Amaranth (C.I.16185) (Fig. 1), methanol, acetic acid, and dibutylammonium were purchased from Sigma. All other reagents used were of analytical grade.

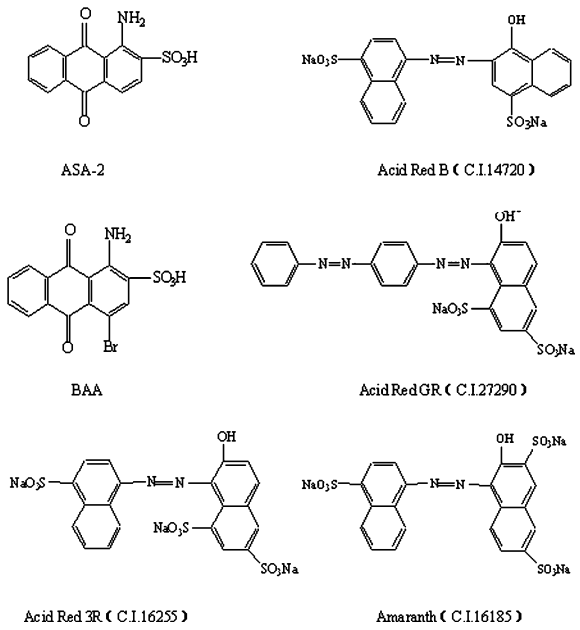

Fig. 1 Chemical structures of azo acid dyes and anthraquinone dye intermediates used in the study ASA-2: 1-aminoanthraquinone-2-sulfonic acid, BAA: 1-amino-4-bromoanthraquinone-2-sulfonic acid

Strains and media

Sphingomonas xenophaga QYY isolated from sludge samples by our laboratory (Qu et al. 2005) was used in this study. *E. coli* JM109 was purchased from Sino-American Biotechnology Company. DBSM and LB medium were used under aerobic conditions (Qu et al. 2005). SM used under anaerobic conditions contained: NH_4Cl: 1 g l^{-1}, K_2HPO_4: 0.2 g l^{-1}, $MgSO_4 \cdot 7H_2O$: 0.2 g l^{-1}, NaCl: 1 g l^{-1}, and glucose: 4 g l^{-1} (pH 8.0).

Reduction of Acid Red 3R by strain QYY

Strain QYY was grown aerobically in 50 ml LB medium containing 0.66 mM ASA-2 at 30°C with shaking at 150 rpm until they reached the late exponential growth phase. Cells were harvested by centrifugation at 8,000*g* for 10 min, and resuspended in SM containing certain concentration of Acid Red 3R to an optical density (OD_{660}) of about 0.4–0.45. Then these cell suspensions were transferred into rubber-stoppered serum bottles (20 ml). The serum bottles were transferred to an anaerobic incubation

chamber at 30°C. The effects of various factors, such as additional carbon sources (sugar, sodium acetate, starch, etc.), pH (6–9) and temperature (20–40°C), on bacterial reduction of 0.1 mM Acid Red 3R were investigated. The effects of the concentrations of ASA-2 and BAA (0–0.4 mM, each) on the reduction of 0.2 mM Acid Red 3R were also studied.

Analysis of the reduction products

Samples were taken before and after the reduction of 0.5 mM Acid Red 3R, and the cells in the samples were removed by centrifugation at 8,000*g* for 10 min. The supernatants were immediately filtrated through a 0.22 μm filter. Then they were analyzed using HPLC-MS.

Cyclic voltammetric experiments

0.15 mM ASA-2 or BAA was added into SM. Cyclic voltammetric experiments were performed using a Voltalab Potentiostai/Galvanostat Model 263A (Advanced Measurement Technology, USA). The method used was described previously (Guo et al. 2006).

Effect of ASA-2 on reduction of different azo acid dyes by different strains and activated sludge

The effect of 0.05 mM ASA-2 on the reduction of azo acid dyes (Acid Red B, Amaranth, Acid Red GR) by strain QYY, *E. coli* JM109 and activated sludge was investigated. The initial concentration of azo acid dyes was 0.2 mM. The experiment with strain QYY was conducted as mentioned above. *E. coli* JM109 was grown aerobically in LB medium at 30°C with shaking at 150 rpm until they reached the late exponential growth phase. The remaining experiments were the same as described for the reduction of Acid Red 3R by strain QYY mentioned above. The activated sludges were obtained from the aerobic parts of Chunliu sewage treatment plant in Dalian City. Particulate material was collected by centrifugation (8,000*g*, 10 min) and resuspended in SM. The remaining experiments were the same as described for the reduction of Acid Red 3R by strain QYY mentioned above.

Degradation of ASA-2 under aerobic conditions

Strain QYY was grown aerobically in 50 ml DBSM containing 0.66 mM ASA-2 at 30°C and 150 rpm. The effects of additional carbon sources (glucose, sodium acetate, starch, etc.) on ASA-2 degradation were investigated. The initial concentration of additional carbon sources was 0.2 g l^{-1}. The degradation of ASA-2 aerobically by strain QYY in SM was also studied after ASA-2 (0.2 mM) mediated reduction of 0.2 mM Acid Red 3R was performed.

Analytical methods

The concentrations of azo acid dyes, BAA and ASA-2 in medium supernatant were determined using HPLC. The samples were monitored at λ_{240} with HP1100 Liquid chromatography fitted with Shim-pack VP-ODS column (4.6 × 200 mm). The mobile phase was composed of methanol and water containing 5.0 mM dibutylammonium acetate. The elution program began with 30% (v/v) methanol, then linearly increased to 45% over 15 min, then linearly increased to 80% over 15 min and maintained for 25 min. The flow rate was 1.0 ml min^{-1}. HPLC-MS (Shimadzu HPLC-2010A system) with Shim-pack VP-ODS column (2.0 × 200 mm) was used. The mobile phase mentioned above was used at a flow rate of 0.2 ml min^{-1}. MS was performed under the following conditions: electrospray ionization source (ESI-), CDL temperature 250°C, block temperature 200°C, detector pressure 1.6 V, scan mode.

TOC was measured using Total Organic Carbon Analyzer (5050A, Shimadzu, Japan). UV–VIS spectra of the samples taken at intervals were recorded using UV–VIS spectrophotometer (V-560, JASCO, Japan). The glucose concentration was measured by the 3,5-dinitrosalicylic acid reducing sugar (DNS) assay (Ghose 1987).

The cell concentration was measured from optical density of the cultures at 660 nm. The relationships between the bacterial cell concentration and OD_{660} were 1.0 OD = 1.11 g dry cell weight l^{-1} for *S. xenophaga* QYY, 1.0 OD = 2.117 g dry cell weight l^{-1} for *E. coli* JM109.

To prevent possible contamination by oxygen during sampling, bottles were opened only once, thus many bottles were incubated according to the

requirement of measurements. The assays were performed in triplicate and the mean values of the data were presented.

Results and discussion

Decolorization of ASA-2 by strain QYY

It was previously reported that strain QYY was able to grow with bromoamine acid as the sole carbon. The optimal conditions of the degradation were pH 6.0–7.0, temperature 30°C, and shaking speed 150 rpm (Qu et al. 2005). As ASA-2 has similar structure with BAA, the decolorization of ASA-2 by strain QYY was investigated. As shown in Fig. 2, the specific wavelengths of ASA-2 were 226, 247, and 475 nm. The maximum absorbance peak at 475 nm gradually decreased and finally disappeared completely after 48 h cultivation. Meanwhile, the peaks at 226 and 247 nm also quickly decreased. During the above process, TOC of ASA-2 decreased gradually, and then remained stable. The data in Fig. 3 showed that more than 50% TOC of ASA-2 was removed. Combined the results of analysis of UV–VIS spectra and TOC removal, it was concluded that the structure of ASA-2 changed and anthraquinone ring might be destroyed.

Effects of various factors on reduction rate of Acid Red 3R

The effects of various factors on the reduction of Acid Red 3R were investigated (Fig. 4). Among additional carbon sources, glucose was shown to be the best. Sodium acetate took second place. And no decolorization was observed when Acid Red 3R was used as the sole carbon source. These findings suggested that the reduction by strain QYY was a co-metabolism process. Thus glucose was used as additional carbon source in the following studies and its optimal concentration was 4 g l^{-1} in SM. The optimal pH and temperature for the reduction of Acid Red 3R were 8.0 and 30°C, respectively.

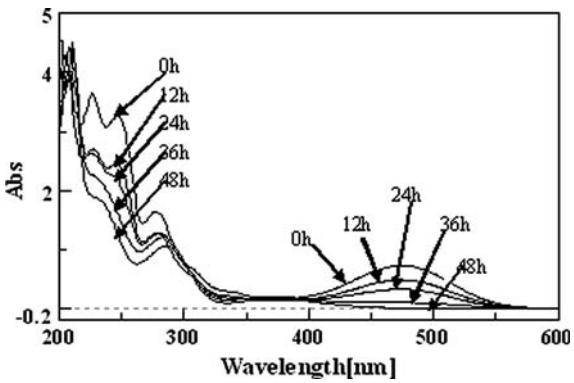

Fig. 2 UV–VIS spectra of ASA-2 degradation by *S. xenophaga* QYY under aerobic conditions. Aerobic conditions: 0.66 mM ASA-2, 30°C and 150 rpm. The supernatants were diluted to 1/4 with water before measuring

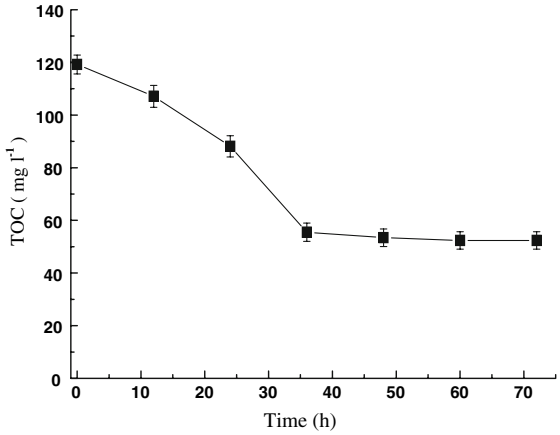

Fig. 3 Time course of TOC during ASA-2 biodegradation by *S. xenophaga* QYY under aerobic conditions. Aerobic conditions: 0.66 mM ASA-2, 30°C and 150 rpm. Error bars indicate the standard deviation obtained in triplicate experiments

Identification of reduction products

As shown in Fig. 5, one reduction product (RT 6.552 min) of Acid Red 3R showed *m/z* 222 in mass spectra. Another product (RT 12.033 min) showed *m/z* 318 in mass spectra. This indicated that azo bond was cleaved and Acid Red 3R was reduced to 4-amino-1-naphthalenesulfonic acid and 7-hydroxy-8-amino-1,3-disulfonatenaphthalene.

Effect of the concentration of ASA-2 on the reduction of Acid Red 3R

ASA-2 mediated reduction of Acid Red 3R by strain QYY was investigated and compared with

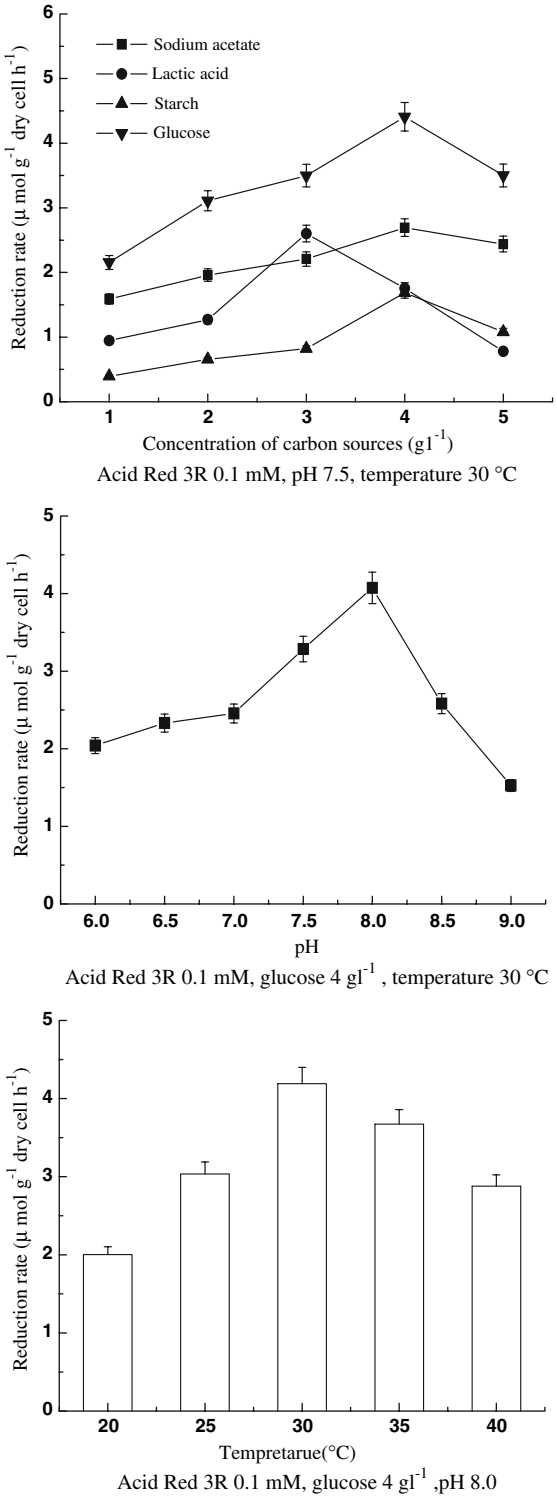

Fig. 4 Effects of carbon sources, pH, and temperature on the reduction rate of Acid Red 3R by *S. xenophaga* QYY under anaerobic conditions. Error bars indicate the standard deviation obtained in triplicate experiments

BAA. The data presented in Fig. 6 showed that the reduction rates increased with the increase of the concentrations of ASA-2 and BAA. Moreover, ASA-2 mediated reduction rate of Acid Red 3R was faster than that mediated by BAA. When the concentration of ASA-2 reached 0.4 mM, the reduction rate increased 5-fold compared to those lacking this compound. The results showed that ASA-2 was a more effective redox mediator than BAA. Their electrochemical characteristics in the cyclic voltammograms showed that the reduction potential of ASA-2 (−0.71 V) was larger than that of BAA (−0.78 V). This indicated that ASA-2 was more easily reduced than BAA. Rau et al. reported that enzymatic reduction of the quinones was the rate-limiting step for the redox mediator dependent reduction of azo dyes by *S. xenophaga* BN6. The proposed mechanism included two independent reactions: first, the quinones are enzymatically reduced to the corresponding hydroquinones, and second, the hydroquinones cleave the azo dyes in a purely chemical reaction (Rau et al. 2002). Based on this, the accelerating effect of ASA-2 on the reduction of azo acid dyes was greater than that of BAA.

Effect of ASA-2 on reduction of different azo acid dyes

The addition of ASA-2 resulted in significantly increased reduction rate of Acid Red 3R by strain QYY. To demonstrate the general applicability of this system for the treatment of textile wastewaters, the reduction of three azo acid dyes was investigated with different strains and activated sludge in the absence or presence of ASA-2 (Table 1). The results showed that ASA-2 could accelerate the reduction of azo acid dyes tested with different strains, and ASA-2 was also effective in combination with nonadapted activated sludge. Furthermore, it became evident that ASA-2 was a more effective redox mediator with strain QYY than with *E. coli* JM109. Previous reports (Rau et al. 2002) showed that lawsone was more effective than AQS with *E. coli*. Thus the results suggested that different enzymatic systems might be responsible for the enzymatic reduction of the relevant quinones.

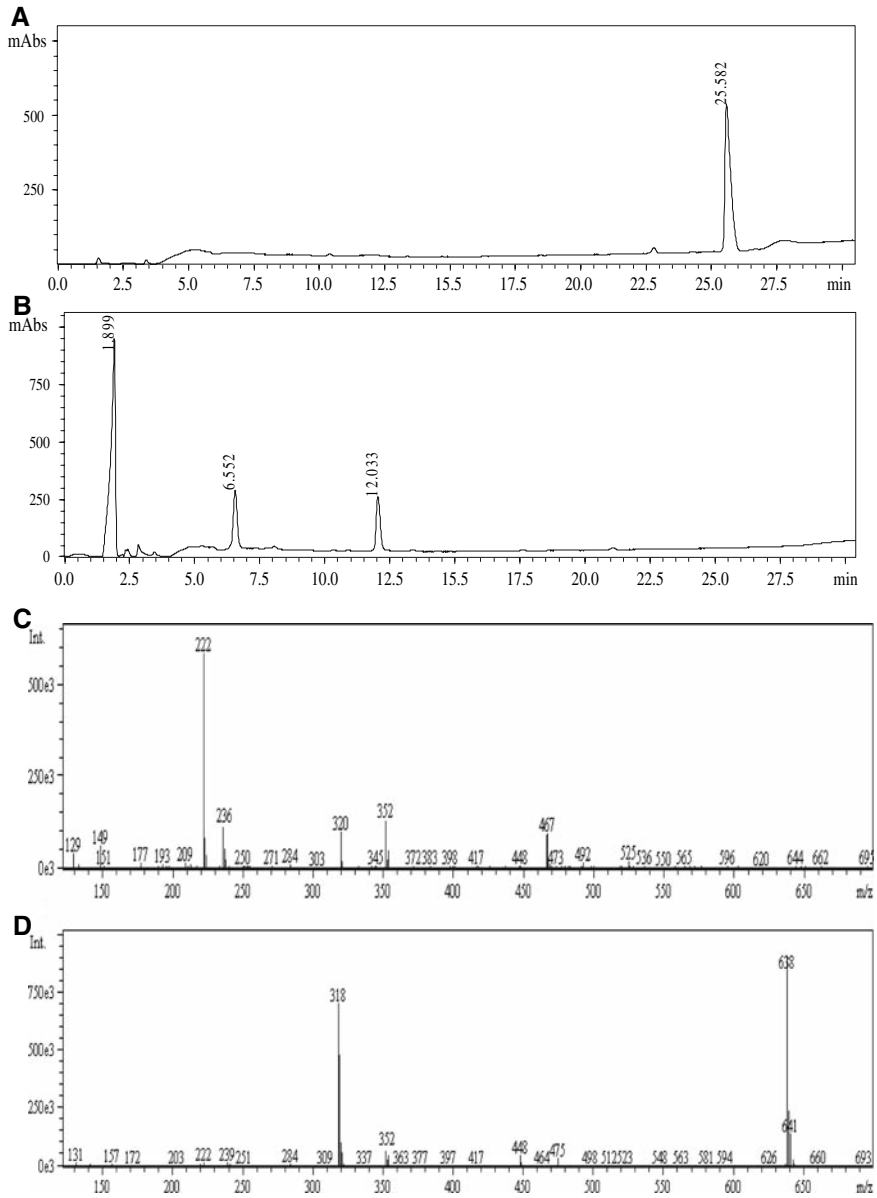

Fig. 5 HPLC-MS of Acid Red 3R and its reduction products. (**a**) HPLC of Acid Red 3R; (**b**) HPLC of the reduction products of Acid Red 3R; (**c**) mass spectrum of the peak (RT 6.552 min) in (**b**); (**d**) mass spectrum of the peak (RT 12.033 min) in (**b**). Samples were taken before and after the reduction of 0.5 mM Acid Red 3R by *S. xenophaga* QYY under anaerobic conditions. Anaerobic conditions: glucose 4 g l^{-1}, 30°C, pH 8.0

ASA-2 degradation and ASA-2 mediated reduction of Acid Red 3R

As shown in Fig. 7, 0.2 mM Acid Red 3R could be completely reduced in 8 h under anaerobic conditions when ASA-2 as a redox mediator was added into SM with an initial concentration of 0.2 mM. In the process, pH of the culture solution was changed from initial 8.0 to 7.0 and the concentration of residual glucose was 3.82 g l^{-1}.

For efficiently degrading ASA-2 in SM, the effect of additional carbon sources on the degradation of ASA-2 under aerobic conditions was investigated (Fig. 8). Compared with control experiment (no additional carbon source), only lactic acid and sodium acetate supplement could improve the degradation rate of ASA-2. Glucose obviously inhibited the degradation of ASA-2, although strain QYY displayed good growth in DBSM containing glucose. Thus sodium acetate was added into SM with an

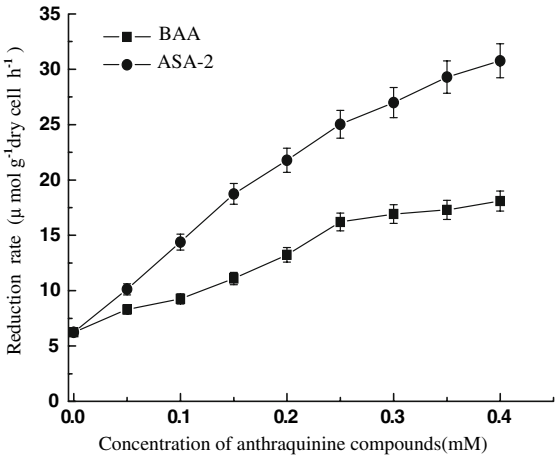

Fig. 6 Effects of the concentrations of ASA-2 and BAA on the reduction rate of Acid Red 3R by *S. xenophaga* QYY under anaerobic conditions. Anaerobic conditions: 0.2 mM Acid Red 3R, glucose 4 g l^{-1}, 30°C, pH 8.0. Error bars indicate the standard deviation obtained in triplicate experiments

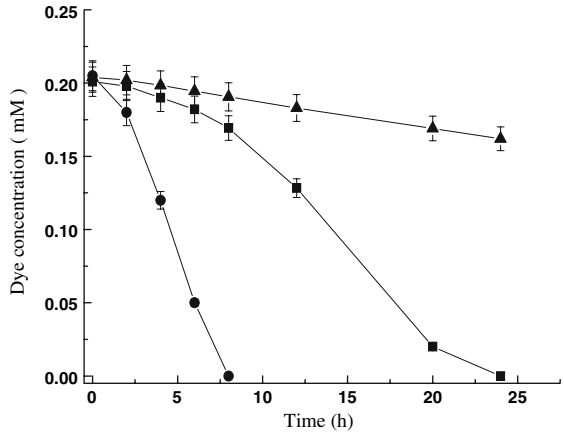

Fig. 7 The reduction of Acid Red 3R mediated by ASA-2 and the degradation of ASA-2 with *S. xenophaga* QYY under anaerobic–aerobic conditions. The reduction of Acid Red 3R in the presence of ASA-2 (●) and in the absence of ASA-2 (▲) under anaerobic conditions. ASA-2 degradation under aerobic conditions (■) after ASA-2 mediated reduction of Acid Red 3R. Error bars indicate the standard deviation obtained in triplicate experiments. The cells were grown aerobically in LB containing 0.66 mM ASA-2, washed, suspended in SM (Acid Red 3R 0.2 mM ASA-2 0.2 mM) to an optical density ($OD_{660\ mM}$) of about 0.4–0.45, and the cells were transferred to an anaerobic chamber. After the reduction of Acid Red 3R, the cells in SM were removed by centrifugation and sodium acetate was added to the supernatant with an initial concentration of 8 g l^{-1}. Strain QYY was inoculated into the supernatant and cultured aerobically at 30 °C and 150 rpm. The concentrations of Acid Red 3R and ASA-2 in medium supernatant were determined using HPLC

initial concentration of 4 g l^{-1} to eliminate the inhibition effect of residual glucose.

However, the studies showed that strain QYY could not efficiently degrade ASA-2 in SM under aerobic conditions in 48 h. The reason for this might be that toxic reduction products of Acid Red 3R might inhibit the normal growth and metabolism of strain QYY. Thus the cells in the cultures were removed by centrifugation after ASA-2 mediated reduction of Acid Red 3R was performed. Then sodium acetate was added into the culture supernatants with an initial concentration of 8 g l^{-1}. The results showed that ASA-2 could be efficiently degraded in 24 h when strain QYY was inoculated into the modified supernatants.

Conclusions

In conclusion, this study demonstrated that strain QYY could degrade 0.66 mM ASA-2 in 48 h with anthraquinone ring cleavage. Moreover, ASA-2 could accelerate the reduction of azo acid dyes by strain

Table 1 Reduction rate of different azo acid dyes by different strains and activated sludge under anaerobic conditions

Azo acid dyes	Reduction rate (μmol g^{-1} dry cell h^{-1})				Reduction rate (μmol g^{-1} h^{-1})	
	S. xenophaga QYY		*E. coli* JM109		Activated sludge	
	Control	ASA-2	Control	ASA-2	Control	ASA-2
Acid Red B	36.11	52.08	5.87	8.30	1.44	2.14
Amaranth	2.73	4.55	2.21	2.81	1.03	1.21
Acid red GR	13.90	31.65	4.02	4.73	1.22	1.48

Anaerobic conditions: 0.05 mM ASA-2, 0.2 mM azo acid dyes, glucose 4 g l^{-1}, 30°C, pH 8.0

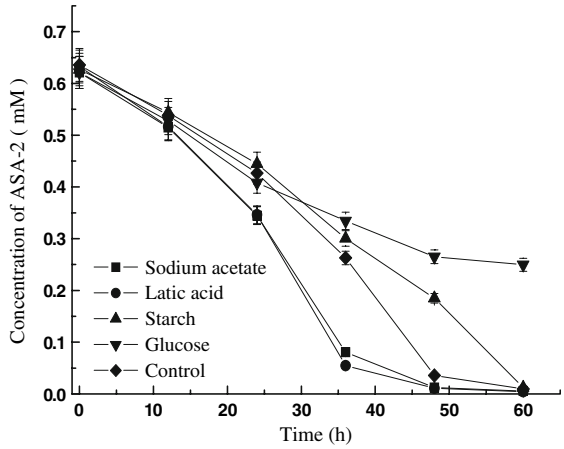

Fig. 8 Effects of additional carbon sources on ASA-2 degradation by *S. xenophaga* QYY under aerobic conditions. Aerobic conditions: 0.2 g l^{-1} additional carbon sources, 30°C, and 150 rpm. Control experiment (no additional carbon source) was conducted. Error bars indicate the standard deviation obtained in triplicate experiments

QYY under anaerobic conditions. On this basis, the studies showed the general applicability of ASA-2 as a redox mediator. Furthermore, in an anaerobic–aerobic process ASA-2 mediated reduction of azo acid dyes and ASA-2 degradation by strain QYY could be achieved under the optimal conditions. These findings provide possibility of the incorporation treatment of anthraquinone dye intermediate as the redox mediator and azo acid dyes by strain QYY in an anaerobic–aerobic process.

References

Baughman GL, Weber EJ (1994) Transformation of dyes and related compounds in anoxic sediment: kinetics and products. Environ Sci Technol 28:267–276

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 decolorisation of textile wasterwaters. Chemophere 55:1149–1157

Dos Santos AB, Cervantes FJ, van Lier JB (2007) Review paper on current technologies for decolorisation of textile wastewaters: perspectives for anaerobic biotechnology. Bioresour Technol 98:2369–2385

Ghose TK (1987) Measurement of cellulase activities. Pure Appl Chem 59:257–268

Guo JB, Zhou JT, Wang D, Xiang XM, Yu H, Tian CP, Song ZY (2006) Correlation of anaerobic biodegradability and electrochemical characteristic of azo dyes. Biodegradation 17:341–346

Guo JB, Zhou JT, Wang D, Yang JL, Li ZX (2007) The new incorporation bio-treatment technology of bromoamine acid and azo dyes wastewaters under high-salt conditions. Biodegradation (in press). doi: 10.1007/s10532-007-9118-6

Lin Y, Xi D (2006) Study on aerobic biodegradability of acid azo dyestuffs. Ind Water Treat (Chinese) 26:25–27

McMullan G, Meehan C, Conneely A, Kirby N, Robinson T, Nigam P, Banat IM, Marchant R, Smyth WF (2001) Microbial decolorisation and degradation of textile dyes. Appl Microbiol Biotechnol 56:81–87

Qu YY, Zhou JT, Wang J, Xing LL (2005) Decolorization of bromoamine acid by a newly isolated strain of *Sphingomonas xenophaga* QYY and its resting cells. Biochem Eng J 27:104–109

Rau J, Knackmuss HJ, Stolz A (2002) Effects of different quinoid redox mediators on the anaerobic reduction of azo dyes by bacteria. Environ Sci Technol 36:1497–1504

Stolz A (2001) Basic and applied aspects in the microbial degradation of azo dyes. Appl Microbiol Biotechnol 56:69–80

Van der Zee FP, Bouwman RHM, Strik DPBTB, Letting G, Field JA (2001) Application of redox mediators to accelerate the transformation of reactive azo dyes in anaerobic bioreactors. Biotechnol Bioeng 75:691–701