Desalination and Water Treatment
www.deswater.com

doi: 10.1080/19443994.2013.838521

53 (2015) 221–229
January

Taylor & Francis
Taylor & Francis Group

Anthraquinone 2-sulfonic acid-mediated reduction of Cr(VI) by *Bacillus* sp. BT1

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Received 1 April 2013; Accepted 14 August 2013

ABSTRACT

Bacillus sp. BT1 exhibited enhanced efficiency as well as rate of Cr(VI) reduction in the presence of anthraquinone-2-sulfonic acid (AQS), a quinone compound analogous to humic acid. The culture exhibited 5.67-fold higher Cr(VI) reduction rate in Luria broth containing 100 mg Cr(VI)/L augmented with 1 mM AQS. Furthermore, in the absence of AQS, *Bacillus* sp. BT1 reduced only 100 mg Cr(VI)/L, whereas in the presence of AQS, it reduced a total of 400 mg Cr(VI)/L, added in form of repeated spikes of 100 mg Cr(VI)/L in each cycle. The activation energy of mediated Cr(VI) reduction was found to be 2.1-fold lower in comparison to nonmediated Cr(VI) reduction. The Cr(VI) and AQS, both induced intracellular quinone and chromate reductases in *Bacillus* sp. BT1. The results demonstrate the potential of AQS-mediated Cr(VI) reduction by *Bacillus* sp. BT1 in bioremediation of Cr(VI) containing wastewaters.

Keywords: Anthraquinone 2-sulfonic acid; Redox mediators; Cr(VI) reduction; Bacillus

1. Introduction

Hexavalent chromium [Cr(VI)] and trivalent chromium [Cr(III)] are the most prevalent forms of chromium in the natural environment. Hexavalent chromium, a wide spread industrial waste, is a strong oxidant and toxic to life forms [1–3]. The reduction of highly toxic and mobile Cr(VI) to less toxic; less mobile Cr(III) is considered to be a feasible remedy for Cr(VI) contaminated soils and waters. Both chemical and biological methods can be employed for the reduction of Cr(VI) to Cr(III) [4–6]. However, chemical reduction requires high energy input and leads to generation of large quantity of chemical sludge posing subsequent disposal problems. On the other hand, biological methods involving microbial reduction of Cr(VI) are economical, effective, and eco-friendly in nature. Bacteria facilitate Cr(VI) reduction enzymatically involving soluble cytosolic chromate reductase/s under aerobic condition or a membrane bound chromate reductase during anaerobic respiration [7,8]. The enzymatic reduction of multivalent Cr affects its solubility and mobility in the environment. Such changes in solubility make microbial Cr(VI) reduction an attractive process for removing Cr(VI) from contaminated waters and soils. The studies reported in the literature indicate that the primary function of most of the chromate reductases is not chromate reduction; for instance, many oxidoreductases such as quinone reductases, iron reductases, nitroreductases, and flavin reductases have been

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demonstrated to exhibit chromate reductase activity [9–12]. The Cr(VI) reductases from *Pseudomonas putida* and *Escherichia coli* have been purified and characterized completely [13]. The *P. putida* chromate reductase (chrR) and *E. coli* chrR are annotated as a flavin-bound quinone reductase and flavin reductase, respectively. Several organisms like *Enterobacter cloacae* HO1 [14] and *Shewanella putrefaciens* [7] have been proposed to utilize Cr(VI) as terminal electron acceptor under anaerobic conditions. The formate-dependent chromate reductase activity in *S. putrefaciens* was inhibited by *p*-chloromercuriphenylsulphonate, azide, 2-heptyl, 4-hydroxyquinolone-N-oxide, and antimycin A, suggesting the involvement of a multicomponent electron transport chain including cytochromes and quinones.

The lower rate of Cr(VI) reduction by various micro-organisms has been attributed to electron transfer limitation and toxic effects of Cr(VI) to reducing micro-organisms [15,16]. Since last two decades, extensive research has been carried out to explore the catalytic effect of different electron shuttles on the redox biotransformation processes [17]. The electron shuttles also referred as redox mediators are organic molecules that undergo reversible oxidation and reduction, thereby conferring the capacity to serve as an electron carrier in multiple redox reactions. The most commonly studied redox mediators in reference to reductive biotransformation of various contaminants include humic acid and their quinoid analogs [18–21]. The presence of humic acid or anthraquinone-2-sulfonic acid (AQS) in cultures of metal-reducing bacteria has been shown to enhance the rate and extent of reduction of metals like U(VI), Fe(III), Tc (VII), Cr(VI), and Se(VI) [20,22–25].This effect has been attributed to an electron shuttle mechanism, whereby the bacteria reduce soluble redox mediators which in turn reduce metal ions, thereby regenerating redox mediators for next cycle of reduction.

The present study describes AQS-mediated Cr(VI) reduction by *Bacillus* sp. BT1 and its association with intracellular quinone and chromate reductase activities.

2. Materials and methods

2.1. Chemicals

Luria–Bertani (LB) broth (dehydrated powder) and redox mediator (Anthraquinone sulphonate) were purchased from Hi-Media Chemicals, India. Diphenyl carbazide and potassium dichromate ($K_2Cr_2O_7$) were procured from Qualigens, India. All stock solutions and media were prepared using deionized water.

2.2. Bacterial strain and growth medium

The Cr(VI)-tolerant bacterial strain BT1 identified as *Bacillus* sp. was isolated from a long-term chromium-polluted soil collected from Tannery industry, Tamilnadu, India by enrichment culture technique. The bacterial strain was grown on LB agar plates (containing; Tryptone; 10 g/L, Yeast Extract; 5 g/L, NaCl; 10 g/L, Agar–Agar: 20 g/L) amended with 100 mg Cr(VI)/L. $K_2Cr_2O_7$ was used as source of Cr(VI) in all experiments.

2.3. AQS-mediated Cr(VI) reduction by Bacillus sp. BT1 in batch mode

Bacillus sp. BT1 was inoculated in 150 mL Cr(VI) (100–300 mg/L)-amended LB broth augmented with AQS as redox mediator (1 mM). Cr(VI) reduction was monitored at regular time intervals in 1 mL sample withdrawn aseptically. Control experiments were performed in the same manner, except that no AQS was added to LB broth. Uninoculated media served as abiotic controls for the corresponding experiment.

The Cr(VI) reduction by *Bacillus* sp. BT1 in the presence of different concentrations of AQS was studied, wherein Cr(VI) (100 mg/L)-amended LB broth was supplemented with AQS in the concentration range of 0–5 mM. Cr(VI) reduction was monitored from samples withdrawn at different time intervals.

2.4. Repeated cycles of AQS-mediated Cr(VI) reduction in fed batch mode

LB broth (200 mL) supplemented with or without AQS (1 mM) was spiked with 100 mg Cr(VI)/L. The Cr(VI) reduction was monitored immediately after inoculation of *Bacillus* sp. BT1. The samples were withdrawn and Cr(VI) was estimated. On complete reduction of Cr(VI), the medium was spiked with another aliquot of Cr(VI) and its reduction was monitored. The cycles of Cr(VI) spiking and reduction were repeated till Cr(VI) reduction ceased.

2.5. Effect of temperature on AQS-mediated Cr(VI) reduction

The AQS-mediated and nonmediated Cr(VI) reduction was determined at various incubation temperatures (25–40˚C). The Cr(VI)-amended LB broth supplemented either with or without 1 mM AQS was inoculated with overnight grown bacterial culture of *Bacillus* sp. BT1 to an initial cell density of 0.05 absorbance (660 nm). The inoculated growth medium was incubated at various temperatures (25–40˚C).

Samples were withdrawn at regular time intervals and Cr(VI) was estimated.

Cr(VI) reduction rate was calculated using pseudo-first-order reaction and the first-order rate constant k (h^{-1}) was calculated using following equation:

$$A_t = A_0 e^{-kt} \quad (1)$$

where A_t is Cr(VI) concentration at time t (h), A_0 is initial Cr(VI) concentration at time t = 0, and k is the first-order rate constant (h^{-1}). k (h^{-1}) was calculated from slope of $\ln \frac{A_t}{A_0}$ vs. t (h).

The activation energy of the AQS-mediated and nonmediated Cr(VI) reduction was calculated by employing Arrhenius equation as follows:

$$\ln k = -E_a/RT + \ln A_0 \quad (2)$$

where k is the first-order rate constant (h^{-1}) and E_a is activation energy. The activation energy (E_a) can be determined from the Slope ($-E_a$/R) of ln k vs. $1/T$ plot [26].

2.6. Characterization of quinone and chromate reductase

The effect of pH on the chromate reductase and quinone reductase activity in the cell-free extract of *Bacillus* sp. BT1 was determined by incubating the reaction mixture at various pH values ranging from 5.0 to 8.5 using the following buffers (50 mM) under standard assay condition: sodium acetate buffer (pH: 5–5.5), potassium phosphate buffer (pH: 6.0–7.5), and Tris-HCl buffer (pH: 7.5–8.5).

To evaluate the effect of temperature on the chromate reductase and quinone reductase activity in the cell-free extract of *Bacillus* sp. BT1, the enzyme assay was conducted at various temperatures ranging 25–40 °C using temperature-controlled spectrophotometer.

The kinetic parameters K_m was determined from Lineweaver–Burk plots of quinone and chromate reductase employing Lawsone and $K_2Cr_2O_7$ as substrates (0.1 mM).

3. Analytical methods

3.1. Preparation of cell lysate

To prepare the crude cell-free extract, the isolate *Bacillus* sp. BT1 was grown in 200 mL LB broth for 24 h at 30 °C. The cells were harvested by centrifugation at 8603 × g for 15 min. and were resuspended in 3 mL of 100 mM potassium phosphate buffer (pH: 6.0). The cells were disrupted by sonication for 15 min (Sonics & Materials, Inc., USA) in ice bath. The resultant homogenate was centrifuged at 8603 xg for 30 min at 4 °C to remove cell debris and intact cells. The clear supernatant thus obtained was used as cell-free extract for all enzyme assays.

3.2. Enzyme assays

The quinone and chromate reductases were assayed as described previously by [27]. Briefly, quinone and chromate reductase activity was assayed spectrophotometrically at constant temperature of 30 °C by monitoring the oxidation of NADH at 340 nm (Molar extinction coefficient of NADH at 340 nm = 6.22 mM^{-1} cm^{-1}). The reaction was initiated by adding 0.1 mL of appropriately diluting cell-free extract to 1 cm path length cuvette containing 2.9 mL reaction mixture. The reaction mixture consisted of 0.1 mM lawsone (for quinone reductase) or 0.1 mM $K_2Cr_2O_7$ (for chromate reductase) and 0.2 mM NADH in 50 mM phosphate buffer (pH: 6.0).

One unit of enzyme activity was defined as the amount of enzyme required for the oxidation of 1 μmole of NADH per min under specified assay conditions [27].

3.3. Estimation of Cr(VI)

The Cr(VI) concentration in the cell-free supernatant was measured spectrophotometrically at 540 nm using the Diphenylcarbazide (DPC) reagent [28]. Briefly, the hexavalent chromium containing samples (in the range of 1–10 μg) were acidified by adding 330 μL of 6 N sulfuric acid. To this acidified solution of the hexavalent chromium, DPC was added at concentration of 0.25% and final volume was made up to 10 mL. The mixture was incubated for 10 min at room temperature and the concentration of DPC: Cr(VI) was assessed by measuring the absorbance at 540 nm.

3.4. Protein estimation

The protein concentrations of the cell-free extract (CFE) were estimated using Folin-phenol reagent by reading absorbance at 720 nm, following the principle of Lowry et al. [29].

4. Results and discussion

4.1. AQS-mediated reduction of Cr(VI) by Bacillus sp. BT1

AQS at 1 mM concentration has been reported to act as a potential redox mediator in the biotransformation of recalcitrant pollutants like sulfonated azo dye, iron, 2,4 dichlorophenoxy acetic acid, and carbon tetra

chloride [18,30–32]. The initial Cr(VI) reduction rates in batch cultures of *Bacillus* sp. BT1 were found to be 0.74, 0.61, and 0.041 mg Cr(VI)/L/h corresponding to the initial Cr(VI) concentration of 100, 200, and 300 mg/L, respectively. However, in batch cultures augmented with 1 mM AQS, initial Cr(VI) reduction rates were found to be approximately 4.03, 1.53, and 1.64 Cr(VI)/L/h corresponding to initial Cr(VI) concentration of 100, 200, and 300 mg/L, respectively (Fig. 1). The extent of Cr(VI) reduction was also enhanced in cultures augmented with 1 mM AQS. For instance, 72 and 55.3% of initial 200 and 300 mg Cr (VI)/L was reduced by *Bacillus* sp. BT1 in LB augmented with 1 mM AQS, respectively. However, in LB without AQS, *Bacillus* sp. BT1 reduced only 36% of initial 200 mg Cr(VI)/L, which was 2.25-fold lower in comparison with AQS augmented cultures. In the absence of AQS, *Bacillus* sp. BT1 failed to grow in LB containing 300 mg Cr(VI)/L.

Our results are in agreement with observations of Liu et al. (2010) who reported enhanced Cr(VI) reduction by resting cells of *E. coli* in the presence of quinone redox mediators like lawsone, menadione, AQS, and anthra quinone di sulfonate (AQDS) [23]. Hong et al. also reported significant enhancement in Cr(VI) reduction efficiency in liquid cultures of *Bacillus* sp. $3C_3$ upon augmentation with AQS and AQDS [25].

The pure cultures of many bacterial species are capable of utilizing quinone as an electron shuttle to increase the biotransformation rate or extent of various organic/inorganic pollutants; for example, the role of quinone-based electron shuttles in acceleration of azo dye reduction has been well documented [23,30,33]. Ling et al. [23] demonstrated a very high reduction rate of sulfonated azo dye (0.2 mM) by *Sphingomonas xenophaga* in the presence of 0.05 mM AQS (70.96 μmol/g h) in comparison with the AQS deficient assays (36.11 μmol/g h). Quinones are known for their potential role as redox centers in humic acid and, therefore, the higher Cr(VI) reduction rate by *Bacillus* sp. BT1 in the presence of AQS is presumably a result of facilitated flux of electrons towards Cr(VI) [25]. Furthermore, the transfer of electrons from AQS to Cr(VI) is a thermodynamically feasible phenomenon as the standard redox potential (E°) of AQS is −0.218 V, which is significantly lower than the redox potential of CrO_4^{2-} (1.28 V). Similar possibilities were investigated by Fredrickson et al. [22] who demonstrated that *Deinococcus radiodurans* could couple the reduction of AQDS to the reduction of Fe oxides like hydrous ferric oxide, ferric pyrophosphate, ferric citrate, and goethite. *D. radiodurans* exhibited higher rates of Cr(VI) reduction in the presence of AQDS using lactate as electron donor under anaerobic conditions. It also exhibited reduction of U(VI) and Tc(VII) to insoluble U(IV) and Tc(IV) only in the presence of AQDS. One additional property of AQS which supports its ability to act as redox mediator is its permeability across bacterial cell membrane [17].

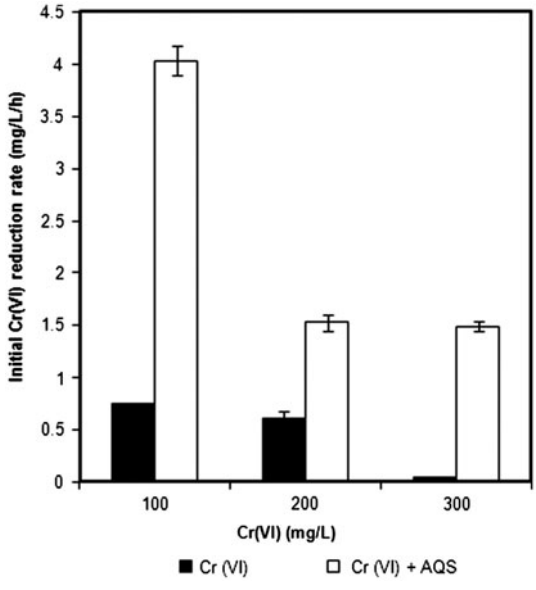

Fig. 1. Cr(VI) reduction by *Bacillus* sp. BT1 in the presence or absence of AQS. The cells were cultured in LB broth amended with 100, 200, 300 mg Cr(VI) L^{-1} supplemented with or without AQS (1 mM).

4.2. Effect of AQS concentration on Cr(VI) reduction

Zee and Cerventes [17] stated that redox mediator concentration used in the batch experiment may be a limiting factor for mediated biotransformation of any organic/inorganic pollutant as the biotransformation rate increased with increasing mediator concentration. Therefore, the effect of increasing AQS concentration (0–5 mM) on efficiency and the rate of bacterial Cr(VI) reduction was tested (Fig. 2). The augmentation of medium with AQS was found to have a positive effect on both efficiency and rate of Cr(VI) reduction (100 mg/L). *Bacillus* sp. BT1, in the presence of 1 mM AQS, was found to reduce 100% of Cr(VI) in 24 h with a specific Cr(VI) reduction rate of 3.89 mg Cr(VI)/L/h. This was found to be 5.67-fold higher in comparison with Cr(VI) reduction rate in the absence of AQS. The impact was disconcerting beyond 1 mM AQS as at higher AQS concentrations (2 and 5 mM), only 15.32 and 7.97% of Cr(VI) was reduced with initial reduction rate of 0.299 and 0.15 mg Cr(VI)/L/h, respectively. This may be attributed either to the AQS

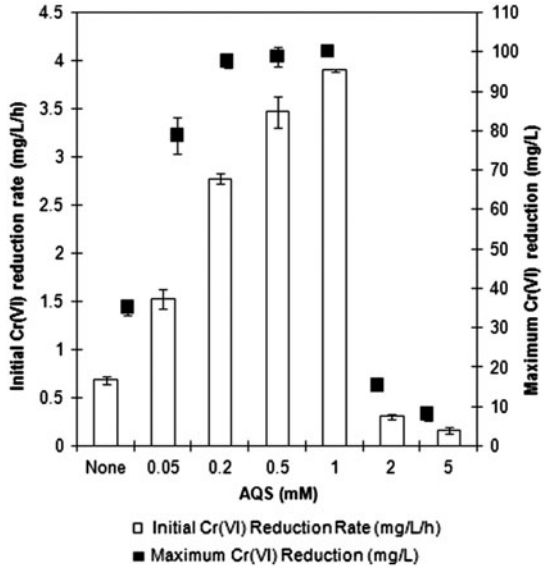

Fig. 2. Effect of AQS concentration on Cr(VI) reduction by *Bacillus* sp. BT1. The cells were cultured in Cr(VI) amended (100 mg Cr(VI) L^{-1}) LB broth supplemented with 0, 0.05, 0.2, 0.5, 1.0, 2.0, and 5.0 mM AQS.

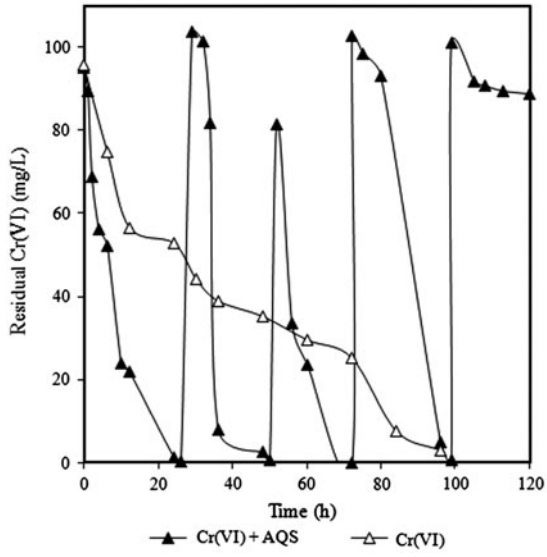

Fig. 3. Repeated cycles of Cr(VI) reduction by *Bacillus* sp. BT1 in the presence and absence of 1.0 mM AQS. The Cr (VI) (100 mg/L) was spiked every time after previously added Cr(VI) was reduced completely.

toxicity at higher concentration or to the limiting concentrations of electron donor [34].

4.3. Reduction of repeated spikes of Cr(VI) in batch cultures

Bacillus sp. BT1 reduced 100 mg Cr(VI)/L in 120 h with initial reduction rate of 0.83 mg Cr(VI)/L/h in the absence of AQS. However, in presence of AQS, *Bacillus* sp. BT1 reduced a total of 400 mg Cr(VI)/L (during 100 h of incubation) in spiking of 100 mg Cr (VI)/L four times, each with initial reduction rate of 4 mg Cr(VI)/L/h (Fig. 3). The failure of Cr(VI) reduction upon further spiking of Cr(VI) may be attributed to the exhaustion of primary electron donor. Liu et al. [23] reported four rounds of 100 mg Cr(VI)/L reduction by resting cells of *E. coli* in the presence of lawsone, indicating the stability and persistence of mediated Cr(VI) reduction.

4.4. Effect of temperature on AQS-mediated Cr(VI) reduction

Santos et al. [27] observed that at higher incubation temperature, the rate of AQDS-mediated azo dye reduction increased significantly in comparison with nonmediated azo dye reduction and suggested that AQDS stimulated azo dye reduction by lowering the activation energy. Hence, the effect of temperature on mediated and nonmediated Cr(VI) reduction was followed. The nonmediated Cr(VI) reduction rate (k, h^{-1}) of *Bacillus* sp. BT1 increased from 0.017 to 0.034 h^{-1} with the increase in temperature from 25 to 35°C. The mediated Cr(VI) reduction rate increased from 0.038 to 0.164 h^{-1}, with increase in temperature from 25 to 35°C (Table 1). The AQS-mediated Cr(VI) reduction rate of *Bacillus* sp. BT1 were found to be 2.2-, 3.7-, and 4.8-fold higher than nonmediated Cr(VI) reduction at 25, 30, and 35°C, respectively (Table 1).

Activation energy, E_a was calculated using Arrhenius plot of ln k (h^{-1}) against $1/T$ (kelvin). The calculated E_a value of AQS-mediated and nonmediated Cr(VI) reduction were found to be 52.7 and 111.4 kJ/mol, respectively (Fig. 4). The 2.1-fold lower activation energy of AQS mediated in comparison with nonmediated Cr(VI) reduction reflected the difference in the mechanism of Cr(VI) reduction by *Bacillus* sp. BT1 in the presence and absence of AQS. Santos et al. [33] reported 1.2-fold lower

Table 1
First-order rate constant k (h^{-1}) of nonmediated and AQS-mediated Cr(VI) reduction

Temperature (°C)	First-order rate constant, k (h^{-1})	
	Nonmediated	AQS-mediated
25	0.017	0.038
30	0.023	0.087
35	0.034	0.164

activation energy of AQS-mediated azo dye reduction in comparison with nonmediated azo dye reduction by anaerobic sludge.

4.5. Effect of AQS on quinone reduction and Cr(VI) reduction activity in the CFE of *Bacillus* sp. BT1.

Chromate reductases from *P. putida*, *E. coli*, and *Paracoccus denitrificans* have been demonstrated to exhibit quinone reductases activity as well [11,13,35]. Liu et al. [36] demonstrated that over expression of quinone reductase in *E. coli* JM109 accelerated quinone-dependent azo dye AR27 reduction, which otherwise does not reduce azo dye AR27 in the absence of quinone. Since Cr(VI) reduction activity of *Bacillus* sp. BT1 was found to be enhanced in the presence of quinone, both chromate and quinone reductase activities were assayed in cells cultured in the presence/absence of AQS and/Cr(VI).

The chromate and quinone reductases (U/mg protein) in CFE of *Bacillus* sp. BT1 grown in LB containing Cr(VI) (100 mg/L) and varying AQS concentration (0–1 mM) displayed high, nonlinear, positive correlation with AQS concentration ($\gamma > +90$).

The specific chromate and quinone reductase activities in *Bacillus* sp. BT1 grown in LB amended with 100 mg Cr(VI)/L were 0.033 and 0.044 U/mg protein, respectively. Both these enzymes activities were found to increase in cells grown in LB augmented with increasing concentration of AQS while keeping initial Cr(VI) (100 mg/L) concentration constant. The intracellular levels of quinone reductase were found to increase by 4.8-, 22.2-, and 62.6-fold in *Bacillus* sp. BT1 grown in the presence of 0.1, 0.5, and 1.0 AQS in LB amended with 100 mg Cr(VI)/L, respectively, in comparison with control (Fig. 5). It was interesting to observe that along with quinone reductase, even chromate reductase levels in cells grown in presence of 0.1, 0.5, and 1.0 AQS in LB amended with 100 mg Cr (VI)/L were found to increase by 2.16-, 7.0-, and 22.8-fold, respectively, in comparison with control (Fig. 5).

Alternately, when *Bacillus* sp. BT1 was grown in the presence of 50 and 100 mg Cr(VI)/L in LB augmented with 1 mM AQS, the intracellular levels of quinone reductase increased by 2.37- and 7.06-fold, while chromate reductase increased by 1.83- and 12.93-fold, respectively (Fig. 6).

It can be inferred that while both AQS as well as Cr(VI) caused increase in intracellular levels of quinone and chromate reductase, AQS induced levels of both enzymes to the similar extent, whereas Cr(VI) seems to be a better inducer of chromate reductase than for quinone reductase in *Bacillus* sp. BT1.

The role of redox mediators such as AQS, AQDS, and lawsone in anaerobic bacterial azo dye reduction has been widely investigated [30,36]. Rau et al. [37] proposed a two-step mechanism for redox mediator dependent reduction of azo dyes, which involves enzymatic reduction of quinones (redox mediators) to the corresponding hydroquinone followed by a nonenzymatic cleavage of azo dyes mediated by hydroquinones. Ling et al. [30] suggested enzymatic

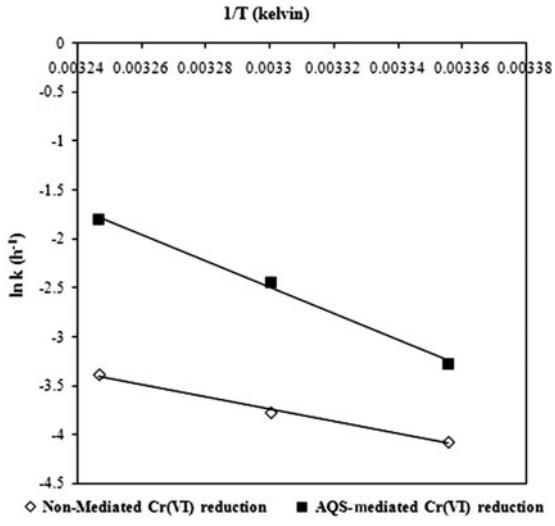

Fig. 4. Arrhenius plot of AQS-mediated and nonmediated Cr(VI) reduction monitored at incubation temperatures from 25 to 35˚C.

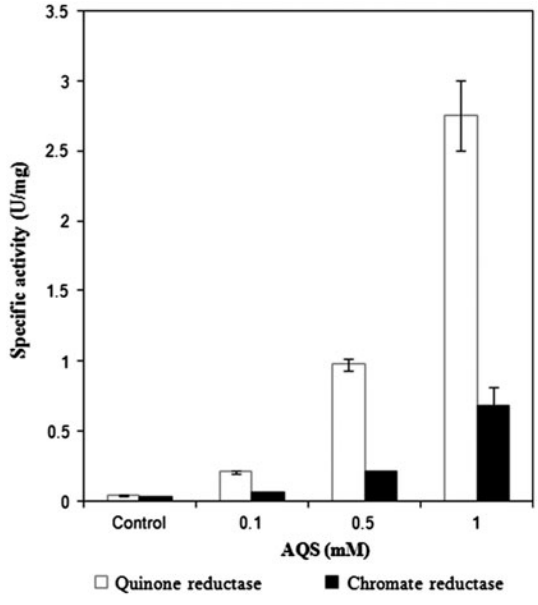

Fig. 5. Effect of AQS concentration on intracellular levels of quinone and chromate reductases of *Bacillus* sp. BT1 grown in LB containing 100 mg Cr(VI)/L.

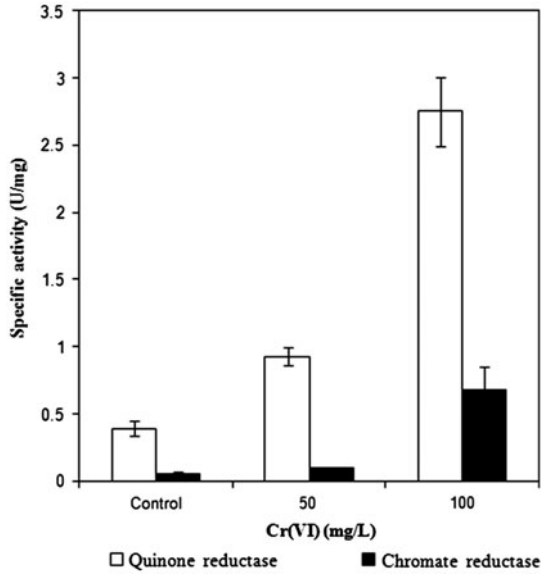

Fig. 6. Effect of Cr(VI) concentration on intracellular levels of quinone and chromate reductases of *Bacillus* sp. BT1 grown in LB augmented with 1 mM AQS.

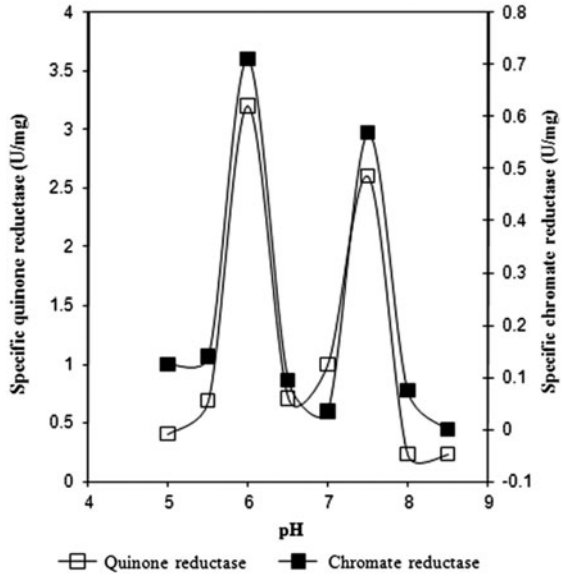

Fig. 7. Effect of pH on chromate and quinone reductase activities of *Bacillus* sp. BT1.

reduction of redox mediators as a rate limiting step in mediated degradation of azo dyes. The mediated azo dye reduction by *S. xenophaga* was shown to be associated with the membrane-bound respiratory chain enzyme, NADH: ubiquinone oxidoreductase [30]; whereas in *E. coli*, two cytosolic quinone reductases were identified as mediator-dependent azo reductases [38].

Both quinone and chromate reductases in CFE of *Bacillus* sp. BT1 were NADH-dependent (data not shown) with maximum activity at pH 6.0 and temperature 30˚C (Figs. 7 and 8). However, the CFE of *Bacillus* sp. BT1 retained approximately 80% of its maximum quinone and chromate reductase activity at pH 7.5 and 40˚C (Figs. 7 and 8). Both the enzyme activities were flavin independent (data not shown). The results are consistent with the pH and temperature optima of Cr(VI) reduction activity from CFE of *Exiguobacterium* sp. and *Bacillus* sp. [39].

The steady state kinetic parameter, K_m of quinone and chromate reduction activity from CFE of *Bacillus* sp. BT1 was found to be 0.0186 mM (using lawsone as substrate) and 0.222 mM (using chromate as substrate), respectively. The K_m of chromate reductase was found to be similar to the K_m value of chromate reductase (ChrR) (0.190 mM) from *P. putida* reported by Gonzalez et al. [10]. The primary biological role of several chromate reductases has been demonstrated in quinone reduction rather than in reduction of Cr(VI) [9–11]. Gonzalez et al. [11] demonstrated low K_m value of *P. putida* ChrR for quinone substrates compared to chromate substrate.

In conclusion, the efficiency and rate of Cr(VI) reduction was enhanced in the presence of AQS in *Bacillus* sp. BT1. The higher intracellular levels of quinone and chromate reductase in the presence of AQS may be attributed to increased efficiency of Cr(VI) reduction. It seems that Cr(VI) reduction in *Bacillus* sp. BT1 occurs by two different pathways; (1) Direct reduction of Cr(VI) by chromate reductase, (2) Indirect reduction involving quinone reductase mediated

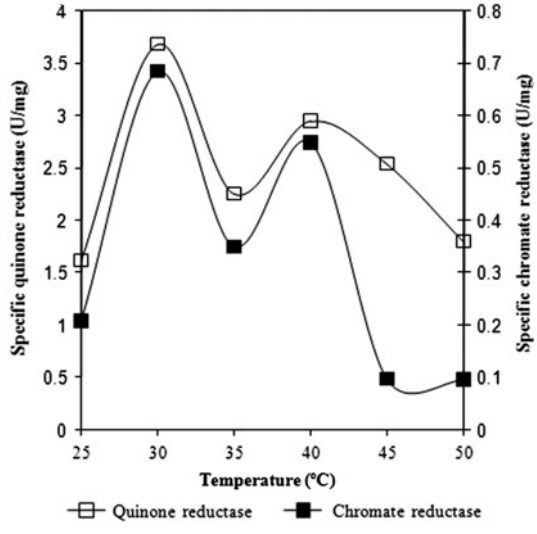

Fig. 8. Effect of temperature on chromate and quinone reductase activities of *Bacillus* sp. BT1.

reduction of AQS to $AQSH_2$ followed by chemical reduction of Cr(VI) by $AQSH_2$ regenerating AQS. Further in-depth understanding of biochemical and molecular mechanism of mediated Cr(VI) reduction will be valuable in the development of an efficient Cr (VI) bioremediation process.

Acknowledgment

Authors are grateful to Department of Science and Technology (DST) and University Grant Commission (UGC), New Delhi, India for providing financial assistance.

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