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Simultaneous Cr(VI) reduction and phenol degradation in pure cultures of *Pseudomonas aeruginosa* CCTCC AB91095

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ABSTRACT

Simultaneous Cr(VI) reduction and phenol degradation were investigated in a reactor containing *Pseudomonas aeruginosa* CCTCC AB91095. Phenol was used as carbon source. *P. aeruginosa* utilized metabolites formed during phenol degradation as energy source for Cr(VI) reduction. Cr(VI) inhibited both Cr(VI) reduction and phenol degradation when Cr(VI) concentration exceeded the optimum value (20 mg/L), whereas phenol enhanced both Cr(VI) reduction and phenol degradation below the optimum initial concentration of 100 mg/L. Cr(III) was the predominant product of Cr(VI) reduction in cultures after incubation for 24 h. Both Cr(VI) reduction and phenol degradation were influenced by the amount of inocula. The concentration of Cr(VI) and phenol declined quickly from 20, 100 to 3.36, 29.51 mg/L in cultures containing of 5% (v/v) inoculum after incubation for 12 h, respectively. The whole study showed that *P. aeruginosa* is promising for the reduction of toxic Cr(VI) and degradation of organic pollutants simultaneously in the mineral liquid medium.

1. Introduction

With the development of industrialization, more and more industrial waste water containing chromium is discharged from industrial processes such as plating, leather tanning, and wood preservation (Nkhalambayausi-Chirwa and Wang, 2000). Chromium is an essential micronutrient required for the growth of many organisms; however, at high concentration it is toxic, carcinogenic, and teratogenic. Chromium has been designated as a priority pollutant by US EPA. Though chromium exists in nine valence states ranging from −2 to +6, trivalent chromium (Cr(III)) and hexavalent chromium (Cr(VI)) are of major environmental significance because of their stability in the natural environment (Thacker et al., 2006; Mishra and Doble, 2008; Gupta and Rastogi, 2008; Kılıç and Dönmez, 2008). Cr(VI) is approximately 100 times more toxic (Yao et al., 2008), and 1000 times more mutagenic than Cr(III) (Barrera et al., 2008).

The conventional remediation processes for water contaminated with Cr(VI) involve physical and chemical removal technologies. Physicochemical treatment strategies are, however, expensive and produce secondary waste streams that require remediation (Viamajala et al., 2002). Bioremediation using microorganisms offers an attractive treatment option because the technology is cost-effective and environmentally compatible. Thus, there is a growing interest in the use of microorganisms for reduction of Cr(VI) to Cr(III) (Camargo et al., 2005). It has been reported that Cr(VI) is reduced to Cr(III) by a number of bacterial species such as *Pseudomonas fluorescens* LB300, *Bacillus* sp., *Enterobacter cloacae* HO1, *Enterobacter aerogenes* (Thacker et al., 2006), *Escherichia coli* (Ackerley et al., 2004), *Shewanella* spp. (Viamajala et al., 2003), and *Pseudomonas aeruginosa* (Xu and Liu, 2005). In those studies, glucose or organic acids were used as carbon and energy source.

In fact, a wide range of organic pollutants including phenol, naphthalene, and trichloroethylene (TCE) has been found at high concentrations in water containing Cr(VI) (Wang and Nkhalambayausi-Chirwa, 2001). Cr(VI) and its organic copollutants often originate from industrial sources such as leather tanning, photographic-film making, wood preservation, car manufacturing, petroleum refining, and agricultural activity (Aksu and Gönen, 2006). It is reported that aromatic compounds can be used as carbon and energy source for Cr(VI) reduction in cultures of some microorganisms, which show different behavior in terms of their ability to utilize metabolites formed during aromatic compounds degradation as carbon source for Cr(VI) reduction (Kumar et al., 2005). Cr(VI)-reducing bacteria may utilize a variety of organic compounds as electron donors for Cr(VI) reduction, though the organic compounds are generally limited to natural aliphatics, mainly low-molecular-weight carbohydrates, amino acids, and fatty acids (Wang and Xiao, 1995).

Although chromate reduction ($CrO_4^{2-} \rightarrow Cr^{3+}$) is thermodynamically favorable, Cr(VI) reduction to Cr(III) may be limited by reaction kinetics under physiological conditions (Glaze, 1990). Generally, Cr(VI) reduction increased in the presence of organic com-

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pounds, a possible reason for that is the fact that the kinetics of Cr(VI) reduction may be improved by coupling Cr(VI) reduction to other energy yielding reactions such as phenol degradation (Quintelas et al., 2006). Several researchers have investigated that the suitable consortium of bacteria can reduce Cr(VI) and degrade phenol simultaneously (Nkhalambayausi-Chirwa and Wang, 2000; Wang and Nkhalambayausi-Chirwa, 2001; Aksu and Gönen, 2006; Quintelas et al., 2006; Liu et al., 2008). In most of these studies, phenol was used as carbon source and Cr(VI)-reducing bacteria utilized organic acid byproducts of phenol degradation for growth and Cr(VI) reduction. However, few studies have reported on simultaneous Cr(VI) reduction and aromatic compounds degradation by using pure cultures of bacteria—*P. aeruginosa*, which is an obligate aerobe capable of degrading phenol via meta-pathways (Chen et al., 2003) and reduces Cr(VI) under aerobic condition as described in earlier studies by Xu and Liu (2005). In this study, phenol was chosen as a model aromatic copollutant of Cr(VI) due to its relatively wide occurrence in Cr(VI) contaminated sites. This paper demonstrates the feasibility of simultaneous Cr(VI) reduction and phenol degradation using pure cultures of bacteria—*P. aeruginosa*.

2. Methods

2.1. Bacterial strains and cultivation conditions

The bacterium *P. aeruginosa* (CCTCC AB91095) was purchased from China Center for Type Culture Collection (CCTCC) in lyophilized form. Bacterial strain was first grown on agar Petri dish containing agar medium, which consisted of beef extract (3.0 g), peptone (5.0 g), agar (20.0 g), NaCl (5.0 g) in 1 L distilled water, and the pH was adjusted to 7.0 ± 0.2 with 10% (w/v) NaOH and 10% (w/v) HCl. After the incubation of cultures at 37 °C for 24 h in agar plates, the bacteria were inoculated from the plates onto the agar slants and stored at 4 °C until needed for further experiments.

Before the beginning of each experiment, strains were enriched by transferring one loop of cells from the agar slants to 100 mL of previously sterilized liquid nutrient medium in 250 mL flasks and incubated at 37 °C for 24 h by shaking at 150 rpm in an orbital incubator (LRH-250-Z, Guangdong, China). The liquid medium contained the same components described above in agar medium except agar, and the pH value was also adjusted to 7.0 ± 0.2 in the same way mentioned above. The cells grown in liquid nutrient medium were centrifuged at 10,000 × *g* for 15 min at 4 °C. The supernatant was discarded and the cell pellets were washed three times and suspended in phosphate buffer (1/15 mol/L NaH_2PO_4, 1/15 mol/L Na_2HPO_4, pH 7) before used in experiments.

The components of the mineral liquid medium were KH_2PO_4 0.5 g/L, K_2HPO_4 0.5 g/L, $MgSO_4{\cdot}7H_2O$ 0.2 g/L, $CaCl_2$ 0.1 g/L, NaCl 0.2 g/L, $MnSO_4{\cdot}H_2O$ 0.01 g/L, NH_4NO_3 1.0 g/L, and phenol was added to the required concentration.

2.2. Preparation of solutions

All the chemicals including potassium dichromate and phenol were AR grade with more than 99% purity. Stock solutions of phenol and Cr(VI) were obtained by dissolving the exact quantities of 99% purity of phenol and potassium dichromate, in 1 L of double-distilled water, respectively. Stock solution of phenol was stored in a brown glass bottle to avoid photo-oxidation. The test solutions containing separate phenol or Cr(VI) were prepared by diluting 1.0 g/L of stock solutions of phenol and Cr(VI) to the desired concentrations. The test solutions containing desired combinations of phenol and Cr(VI) were prepared by diluting 1.0 g/L of stock solutions of phenol and Cr(VI) and mixing with test medium.

2.3. Analytical methods

The concentration of Cr(VI) in the supernatant was determined by colorimetric method using a UV–vis spectrophotometer (UV 754N shanghai, China) at 540 nm by reaction with diphenylcarbazide (Camargo et al., 2005). The absorbance of the purple colored solution was read after 10 min. The concentration of phenol in the solution was also determined spectrophotometrically using 4-amino antipyrine as a color reagent (λ_{max} = 500 nm) (Annaduraia et al., 2002). The absorbance was recorded after 10 min and the concentration was determined from the calibration curve. Samples for total chromium analysis were first digested with sulphuric–nitric acid (V:V = 1:1) and oxidized with 4% (w/v) potassium permanganate before reacting with diphenylcarbazide and determined colorimetrically (Liu et al., 2006). Cr(III) were measured at a wavelength of 520 nm using an ion chromatograph equipped with a CS5 column after reaction with pyridine-2,6-dicarboxilic-acid (PDCA) (Nkhalambayausi-Chirwa and Wang, 2000; Wang and Nkhalambayausi-Chirwa, 2001). Total organic carbon (TOC) was determined by using a Carbon Analyzer (OI-1010, America). Total organic acids were determined by titrating the eluent from a column packed with 200 mesh silicic acid to the phenolphthalein end point with 0.02 N NaOH (Nkhalambayausi-Chirwa and Wang, 2000). Cell density of the liquid cultures was determined by measuring the absorbance of l-cm cuvette at 610 nm with a spectrophotometer (Wang and Xiao, 1995).

2.4. Cr(VI) reduction and phenol degradation experiment

Cr(VI) reduction experiments were carried out in batch reactors using 250 mL conical flasks. The 250 mL conical flasks containing 100 mL mineral liquid medium autoclaved at 120 °C for 20 min were filled with 5 mg/L Cr(VI), 100 mg/L phenol, and 2% (v/v) cells suspension. The flasks were incubated at 37 °C by shaking at 150 rpm in the orbital shaker. Chromium and phenol were measured at an interval of 0, 12, 24, and 72 h. Because of the possibility of volatilization of phenol during the experiments and adsorption of Cr(VI) and phenol to the microorganism, and to confirm the activity of microorganism, control experiments were carried out at the same operating conditions of the biodegradation experiments. Five controls were prepared by: (1) autoclaving cells for 20 min at 120 °C and adding phenol in the culture; (2) using active cells and adding no phenol in the culture; (3) autoclaving cells in the same way mentioned above and adding no phenol in the culture; (4) inoculating no cells and adding phenol in the culture; and (5) autoclaving cell-free growth medium as described above and adding no phenol in the culture. These five controls were carried out for 72 h in 5 mg/L Cr(VI) and 100 mg/L phenol. All samples were subjected to centrifugation at 10,000 rpm for 10 min prior to analysis to remove the biomass from the liquid phase.

In order to understand the role of phenol on Cr(VI) reduction, further experiments were carried out using a wide range of Cr(VI) concentrations, phenol concentrations, and amount of inocula.

Effect of initial Cr(VI) concentration on the rates of Cr(VI) reduction and phenol degradation were conducted using conical flasks. A series of conical flasks containing 100 mL mineral liquid medium were prepared with 2, 5, 10, 15, 20, 30, and 40 mg/L Cr(VI), respectively, and phenol concentration in all of flasks was 50 mg/L. The flasks were inoculated with 2 mL cells suspension and then incubated by shaking as described above. Samples were taken from the flasks at intervals and analyzed for the residual Cr(VI) and phenol concentration.

The initial phenol concentration was adjusted to the desired values including 10, 25, 50, 100, 150, and 200 mg/L to evaluate the effect of initial phenol concentration on Cr(VI) reduction and phenol degradation. A series of flasks were autoclaved at 120 °C

for 20 min and filled with 100 mL mineral liquid medium autoclaved under the same condition. Some flasks containing 100 mL liquid medium and 2 mg/L Cr(VI) were inoculated with autoclaved cells suspension, other flasks were filled with 2 mg/L Cr(VI) and 10, 25, 50, 100, 150, and 200 mg/L phenol, respectively, then inoculated with 2 mL cells suspension. Then, the flasks were incubated by shaking as described above. Samples were taken from the mixture at intervals and analyzed for the residual Cr(VI) and phenol concentration.

The various amounts of inocula in experiments were inoculated with desired values including 0.5%, 1%, 2%, 3%, and 5% (v/v) to observe its influence on Cr(VI) reduction and phenol degradation. A series of autoclaved flasks with 100 mL mineral liquid medium were filled with 20 mg/L Cr(VI), 100 mg/L phenol, and 0.5%, 1%, 2%, 3%, and 5% (v/v) cells suspension, respectively. *P. aeruginosa* were contacted with Cr(VI) and phenol at 37 °C in the shaker. Samples were taken at intervals and analyzed for the residual Cr(VI) and phenol concentration.

2.5. Statistical analysis

All the experiments carried out in triplicate. At least three separate flasks were usually prepared for one treatment. Each time three readings were taken, their means, and standard deviations were determined by the computer software package (SPSS 7.5).

3. Results and discussion

3.1. Cr(VI) reduction and phenol degradation

Cr(VI) reduction was first investigated using phenol as the carbon source. Results showed that Cr(VI) reduction occurred in the culture medium with concomitant phenol degradation (Table 1). Reduction of about 5.00 mg/L Cr(VI) was nearly complete after 72 h of incubation with 100 mg/L phenol as the carbon source. The rate of reduction was faster during the initial stages (12 h) of incubation, which slowed down gradually with time, as it has been reported before (Pal and Paul, 2004). Cr(VI) reduction was not observed in autoclaved controls (Controls 1, 3, and 5), indicating Cr(VI) reduction was mainly accomplished through biological activity. Results obtained with the cell-free growth medium (Control 4) indicated that almost no abiotic Cr(VI) reduction occurred in the culture medium, some phenol degradation in the absence of cells may be attributed to volatilization of phenol during the experiments. The higher extents of Cr(VI) reduction in cultures with added phenol than in cultures without added phenol (Control 2) demonstrated that phenol degradation enhanced Cr(VI) reduction.

Cr(VI) reduction with concomitant Cr(III) formation was observed in the reactor. The data in Fig. 1 show that Cr(VI) was nearly reduced to Cr(III) after incubation for 24 h, Cr(III) was the predominant product of Cr(VI) reduction in cultures.

3.2. Effect of Cr(VI) concentration

The effect of initial Cr(VI) concentration on the rates of Cr(VI) reduction and phenol degradation was examined under an initial phenol concentration of 50 mg/L over a Cr(VI) concentration range of about 2–40 mg/L (Fig. 2A and B). The results in Fig. 2A show that Cr(VI) reduction in *P. aeruginosa* occurred even under the highest Cr(VI) concentration, but complete Cr(VI) reduction was not observed for initial concentration higher than 10 mg/L in 72 h. The percent of Cr(VI) reduction exceeded 90% under low initial Cr(VI) concentrations (2–10 mg/L) after 72 h incubation. However, the rate of Cr(VI) reduction was relatively higher at higher initial Cr(VI) concentration range. The above observed phenomenon is consistent with the rate of Cr(VI) reduction observed earlier by Wang and Xiao (1995). The average rates of Cr(VI) reduction were 0.12, 0.16, 0.17, 0.20, 0.24, 0.19, and 0.18 mg/L/h at initial Cr(VI) concentrations of 2, 5, 10, 15, 20, 30, and 40 mg/L, respectively. Thus, the average rate of Cr(VI) reduction increased with increasing initial Cr(VI) concentration until an optimum Cr(VI) concentration of 20 mg/L was reached. The average rate began to decrease at Cr(VI) concentrations higher than 20 mg/L. These data clearly revealed the existence of a finite Cr(VI) reduction capacity possibly due to Cr(VI) toxicity toward biological activity. The concept of Cr(VI) reduction capacity of cells has been illustrated earlier using *E. coli* cultures (Nkhalambayausi-Chirwa and Wang, 2000). An earlier work showed that Cr(VI) was deoxidized predominantly by the soluble enzyme of the *P. aeruginosa* (Xu and Liu, 2005). The toxicity and high oxidative potential of chromate (CrO_4^{2-}) may reduce activity of the soluble enzyme.

The effect of Cr(VI) concentration on phenol degradation was shown in Fig. 2B. Analysis of the data in Fig. 2B reveals that the average phenol degradation rate decreased with increasing Cr(VI) concentration (1.84, 1.69, 1.50, 1.27, 0.82, 0.21, and 0.13 mg/L/h at initial Cr(VI) concentrations of 2, 5, 10, 15, 20, 30, and 40 mg/L, respectively). The average rate of phenol degradation decreased slowly from 1.84 to 0.82 mg/L/h within 2–20 mg/L Cr(VI) but decreased rapidly when the initial Cr(VI) concentration was increased from 20 to 40 mg/L. The concentration of phenol was declined quickly only under low initial Cr(VI) concentration indicating

Table 1
Cr(VI) reduction and phenol degradation using *P. aeruginosa* at initial Cr(VI) concentration 5 mg/L and initial phenol concentration 100 mg/L. Five control experiments were carried out at the same operating conditions. Control 4 did not contain cells of the *P. aeruginosa*; autoclaved controls proceeded with heat killed *P. aeruginosa* by autoclaving the cultures.

Incubation time (h)	Concentration* (mg/L)								
	Test 1		Control 1[a]		Control 2[b]	Control 3[c]	Control 4[d]		Control 5[e]
	Cr(VI)	Phenol	Cr(VI)	Phenol	Cr(VI)	Cr(VI)	Cr(VI)	Phenol	Cr(VI)
0	5.00 ± 0.21	100 ± 16	5.00 ± 0.19	100 ± 23	5.00 ± 0.18	5.00 ± 0.16	5.00 ± 0.09	100 ± 11	5.00 ± 0.12
12	2.15 ± 0.17	68 ± 14	4.98 ± 1.21	99 ± 21	4.86 ± 0.09	4.99 ± 0.13	4.96 ± 0.11	98 ± 15	5.00 ± 0.16
24	1.09 ± 0.12	35 ± 18	4.97 ± 0.13	98 ± 18	4.90 ± 0.06	4.97 ± 0.16	4.98 ± 0.16	99 ± 12	4.98 ± 0.18
36	0.76 ± 0.06	19 ± 6	4.98 ± 0.07	99 ± 20	4.81 ± 0.13	4.98 ± 0.06	4.97 ± 0.13	96 ± 21	4.99 ± 0.07
48	0.26 ± 0.13	10 ± 3	4.82 ± 0.12	97 ± 11	4.82 ± 0.15	4.94 ± 0.07	4.95 ± 0.03	94 ± 18	5.00 ± 0.05
60	0.07 ± 0.02	5 ± 3	4.67 ± 0.04	97 ± 9	4.79 ± 0.07	4.93 ± 0.00	4.87 ± 0.01	98 ± 9	4.97 ± 0.20
72	0.02 ± 0.00	2 ± 1	4.59 ± 0.11	95 ± 16	4.84 ± 0.23	4.91 ± 0.18	4.85 ± 0.07	94 ± 14	4.99 ± 0.13

[a] Autoclaved cells with phenol added.
[b] Active cells without phenol.
[c] Autoclaved cells without phenol.
[d] Cell-free growth medium with phenol added.
[e] Autoclaved cell-free growth medium without phenol.
* Mean ± SD.

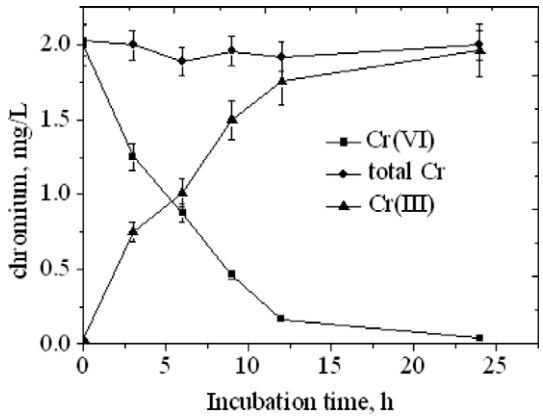

Fig. 1. Cr(VI) reduction and Cr(III) formation. Initial Cr(VI) concentration was 2 mg/L and initial phenol concentration was 100 mg/L.

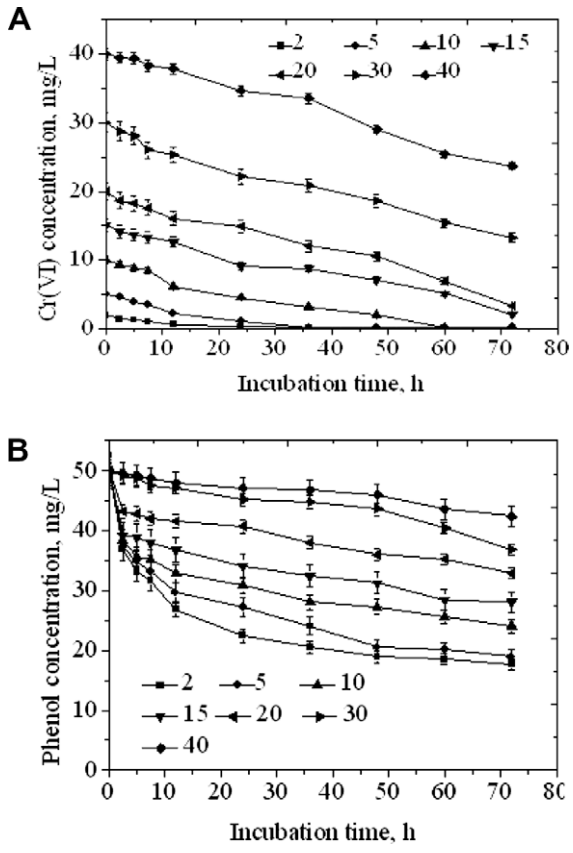

Fig. 2. Effect of initial Cr(VI) concentration on (A) Cr(VI) reduction, and (B) phenol degradation using *P. aeruginosa* at initial phenol concentration 50 mg/L, initial Cr(VI) concentration in the medium was adjusted to 2, 5, 10, 15, 20, 30, and 40 mg/L, respectively.

Cr(VI) inhibition on phenol degradation, which is in agreement with that of an earlier study by Wang and Nkhalambayausi-Chirwa (2001).

3.3. Effect of phenol concentration

In order to minimize Cr(VI) toxicity toward biological activity, a low initial Cr(VI) concentration (2.0 mg/L) was used in the experiments determining the effect of initial phenol concentration on the rate of Cr(VI) reduction over a phenol concentration range of about 10–200 mg/L. The data in Fig. 3A show that Cr(VI) reduction occurred in all active cell cultures, but no significant reduction was detected with the autoclaved control. The percentages of Cr(VI) reduction were (as %) 89.14, 90.86, 92.58, 97.76, 88.45, and 78.8 at initial phenol concentration of 10, 25, 50, 100, 150, and 200 mg/L, respectively. As shown in Fig. 3A, an increase in initial phenol concentration enhanced Cr(VI) reduction until an optimum level of 100 mg/L was reached. Meanwhile, the average phenol degradation rate increased from 0.836 to 17.448 mg/L/h when initial phenol concentration increased from 10 to 100 mg/L. Further increase in phenol concentration from 100 to 200 mg/L led to the decrease in average phenol degradation rate from 17.448 to 6.62 mg/L/h. The data in Fig. 3A and B show that phenol played an important role during Cr(VI) reduction. The observed average 2.5-h Cr(VI) reduction rates were (in mg/L/h): 0.272, 0.299, 0.327, 0.410, 0.382, and 0.134 at phenol concentration of 10, 25, 50, 100, 150, and 200 mg/L, respectively. Optimum Cr(VI) reduction rate was thus observed at the initial phenol concentration of 100 mg/L. Increasing the concentration of phenol from 100 to 200 mg/L resulted in a decrease in Cr(VI) reduction rate from 0.410 to 0.134 mg/L/h. These results show that Cr(VI) reduction and phenol degradation became less efficient with increasing the concentration of phenol as initial phenol concentration was higher than the optimum phenol concentration (100 mg/L). An earlier work also showed that phenol will inhibit Cr(VI) reduction and phenol degradation when its concentration was above the optimum value (Nkhalambayausi-Chirwa and Wang, 2000).

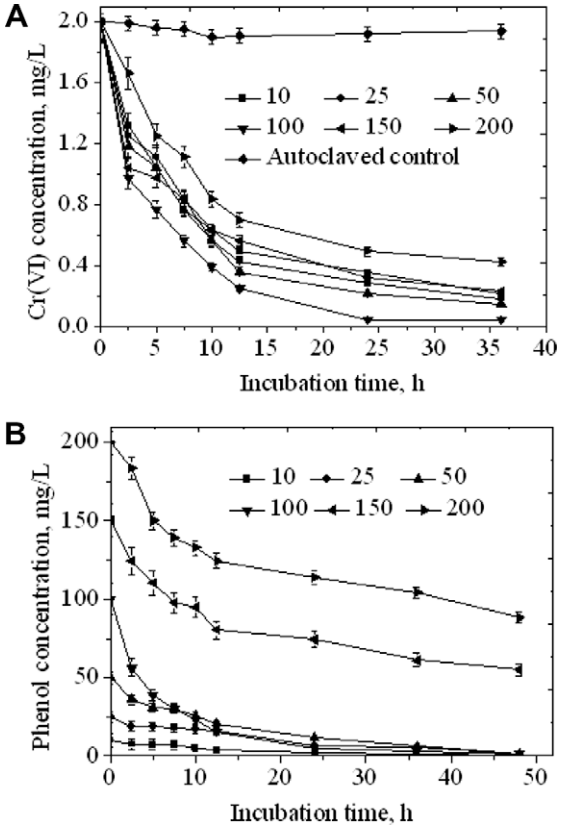

Fig. 3. Effect of initial phenol concentration on (A) Cr(VI) reduction, and (B) phenol degradation by *P. aeruginosa*. Initial Cr(VI) concentration was 2 mg/L and initial phenol concentration in the medium was adjusted to 10, 25, 50, 100, 150, and 200 mg/L, respectively.

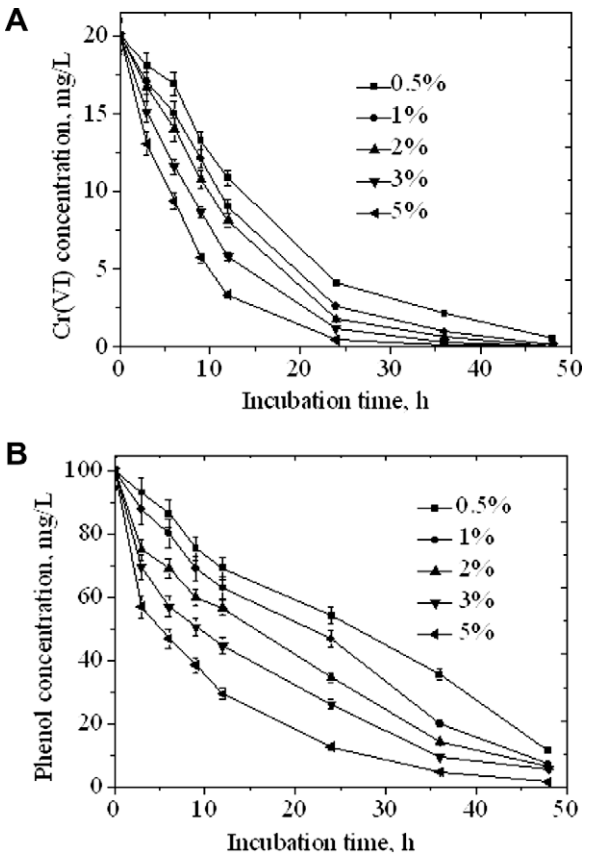

Fig. 4. Effect of amount of inocula on (A) Cr(VI) reduction, and (B) phenol degradation using *P. aeruginosa* at initial Cr(VI) concentration 20 mg/L and initial phenol concentration 100 mg/L, with amount of inocula 0.5%, 1%, 2%, 3%, and 5%, respectively.

3.4. Effect of amount of inocula

Effect of various amounts of inocula (0.5, 1, 2, 3, and 5% (v/v)) was studied on Cr(VI) reduction and phenol degradation with a level of phenol concentration (100 mg/L) at initial Cr(VI) concentration of 20 mg/L. (Fig. 4A and B). Both Cr(VI) reduction and phenol degradation were influenced by the amount of inocula. The data in Fig. 4A show that Cr(VI) was almost completely reduced after incubation for 48 h in the presence of different concentrations of inocula in the medium. Whereas the concentration of Cr(VI) declined quickly when more cells were inoculated. After 12 h, the concentrations of Cr(VI) were 10.89, 9.07, 8.15, 5.81, and 3.36 mg/L in cultures containing of 0.5%, 1%, 2%, 3%, and 5% inoculum, respectively. A high cell density has been recommended for significant Cr(VI) reduction to occur, as also reported earlier (Wang and Xiao, 1995; Pal and Paul, 2004). Similar results were obtained for phenol degradation (Fig. 4B), in which significant phenol degradation was observed. But Ohtake et al. (1990) previously observed that Cr(VI) reduction rate did not increase with increasing the cell density when the optimum cells amount was exceeded. Analysis of the data in Fig. 4A reveals that Cr(VI) reduction rate generally decreased with time regardless of the initial cell concentration. This observation suggested that biological reduction of Cr(VI) may be inhibited by Cr(VI) toxicity, which is in agreement with previous findings of Wang and Xiao (1995).

3.5. Bioenergetics and mass balance

In this study, both Cr(VI) reduction and phenol degradation data were analyzed in order to determine the relationship between Cr(VI) reduction and phenol degradation.

Analysis of the data in Table 2 reveals that a good correlation between cumulative Cr(VI) reduced and cumulative phenol degraded with correlation coefficients ranging between 0.939 and 0.976. The data in Table 2 also indicate that the amount of Cr(VI) reduced per unit weight of phenol degraded increased with increasing initial Cr(VI) concentration. This result is in agreement with that of an earlier study by Nkhalambayausi-Chirwa and Wang (2000). It is assumed that phenol was completely degraded to HCO_3^- and H_2O.

$$C_6H_6O + 9\frac{1}{3}CrO_4^{2-} + 40\frac{2}{3}H^+ \rightarrow 9\frac{1}{3}Cr^{3+} + 6HCO_3^- + 20\frac{1}{3}H_2O$$

According to the above equation, it is determined that the efficiency of Cr(VI) reduction per unit weight of phenol degraded was 5.14 mg Cr(VI)/mg phenol. However, the highest observed efficiency of Cr(VI) reduction per unit weight of phenol degradation (1.33 mg Cr(VI)/mg phenol) was far less than the theoretically required. Therefore, the amount of phenol consumed was much more than the stoichiometrically required for Cr(VI) reduction because phenol was not being used fully.

In fact, during the course of the experiment, organic acid was detected. This result indicates that phenol was degraded step by step—organic acid may be one of the metabolites. The above analysis indicated that *P. aeruginosa* possibly utilized organic acid rather than phenol as carbon source for Cr(VI) reduction.

3.6. Analysis of metabolites

Total organic carbon, total organic acids, and phenol were monitored throughout the incubation with a level of phenol concentration (100 mg/L) at initial Cr(VI) concentration of 2.0 mg/L. The data in Fig. 5 show that 100 mg/L of phenol was almost completely degraded with concomitant production of total organic acids within 72 h of incubation. Total organic acids had been increasing with

Table 2
Relationship between cumulative Cr(VI) reduced and cumulative phenol degraded. Initial phenol concentration was 50 mg/L, initial Cr(VI) concentration in the medium was adjusted to 5, 10, 20, and 40 mg/l, respectively.

Cr(VI) concentration (mg/L)		Cumulative reduced concentration (mg/L)									Correlation coefficient (r^2)
		2.5 h	5 h	7.5 h	12 h	24 h	36 h	48 h	60 h	72 h	
5	Phenol	12.75	15.14	16.73	20.32	22.71	25.9	29.48	29.88	31.08	0.939
	Cr(VI)	0.37	1.06	1.48	2.77	3.93	4.85	4.86	4.86	4.86	
10	Phenol	11.55	14.34	14.74	17.13	19.12	21.91	22.71	24.3	25.9	0.975
	Cr(VI)	0.69	1.17	1.51	3.92	5.48	6.85	7.99	9.83	9.84	
20	Phenol	6.77	7.17	7.97	8.36	9.16	11.95	13.94	14.74	17.13	0.976
	Cr(VI)	1.13	1.8	2.42	3.96	5.11	7.99	9.48	13.11	16.69	
40	Phenol	0.4	0.8	1.2	1.99	2.79	3.18	3.98	6.37	7.57	0.967
	Cr(VI)	0.51	0.65	1.68	2.16	5.29	6.44	10.96	14.58	16.31	

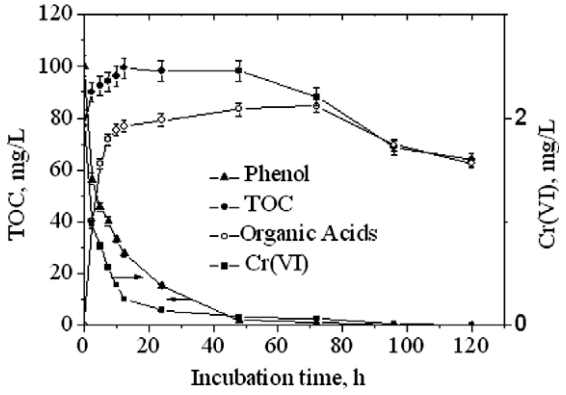

Fig. 5. TOC and organic acid accumulation in the medium at initial Cr(VI) concentration 2 mg/L, initial phenol concentration 100 mg/L.

phenol degradation until about 72 h into the incubation and then began to decrease; this may be attributed to depletion of phenol such that the organisms start consuming organic acids at that point. The representative data shown in Fig. 5 indicate that total organic acids accounted for approximately 100% total measured TOC after phenol was almost completely degraded. Organic acids accumulation in the solution demonstrated that metabolites formed from phenol degradation were produced at a rate faster than the rate of being utilized. It was reported that the biological degradation of phenol was accomplished through aromatic ring cleavage using the enzyme present in the microorganism (Kumar et al., 2005). While Ampe and Leonard (1998) previously observed that organic acids at a certain high concentration inhibited ring cleavage during biological aromatic compound degradation. Thus, *P. aeruginosa* utilized some organic acid intermediates to reduce Cr(VI), which may enhance the rate of phenol degradation thereby increasing biological activity in cultures.

4. Conclusions

This paper demonstrates that phenol can be used as a carbon source for Cr(VI) reduction under aerobic conditions. There is a good correlation between cumulative Cr(VI) reduced and cumulative phenol degraded. *P. aeruginosa* utilized phenol metabolites for growth and Cr(VI) reduction. Optimum Cr(VI) reduction and phenol degradation were observed at a Cr(VI) concentration of 20 mg/L and a phenol concentration of 100 mg/L. More cells inoculum amount resulted in higher rates of Cr(VI) reduction and phenol degradation. This study indicates the ability of a single organism, *P. aeruginosa* to carry out both of Cr(VI) reduction and phenol degradation formerly performed by mixed cultures.

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