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Biodegradation of phenol with chromium (VI) reduction by the *Pseudomonas* sp. strain JF122

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ABSTRACT

A bacterium capable of degrading phenol coupled with reducing chromium (VI) was isolated and was identified as being *Pseudomonas* sp. JF122 using 16S rRNA sequence analysis. The bacterium was able to simultaneously remove 600 mg/L phenol and 2.0 mg/L Cr(VI) within 72 h. Results from a study of the phenol biodegradation coupled with chromium (VI) reduction suggested that the electron donors involved in the reduction of Cr(VI) were intermediate phenol degradation products. Phenol concentrations higher than 800 mg/L inhibited both phenol degradation and Cr(VI) reduction. However, at a phenol concentration of 200 mg/L, not enough electron donors were produced to fully reduce the initial Cr(VI) concentration (1.2 mg/L). Cr(VI) concentrations below 2.0 mg/L enhanced both phenol degradation and Cr(VI) reduction. Cr(VI) was reduced to soluble Cr(III), which remained in the supernatant solution. This bacterium seems to have potential for the efficient and simultaneous treatment of organic pollutants and heavy metals in complex wastewaters.

Keywords: Phenol; Biodegradation; Chromium (VI) reduction; *Pseudomonas* sp. JF122; Bioremediation; Chromium

1. Introduction

Chromium is present in the environment mostly as hexavalent chromium, Cr(VI), and trivalent chromium, Cr(III). Cr(VI) is highly toxic and soluble, and it poses a serious health threat to humans because it is a potential carcinogen [1], whereas Cr(III) is less soluble and less toxic than Cr(VI). Cr(VI) is widely used in industrial processes, including manufacturing degradable pyrotechnics, electronics, chromate, dyes, pigments, and corrosion inhibitors [2,3], and is therefore released into the environment from effluents from these industries. Reducing Cr(VI) to Cr(III) is a key part of treating wastewater contaminated with Cr(VI). The bioremediation of Cr(VI) can be effectively achieved using microbes that reduce it to the less toxic Cr(III) [4,5]. A variety of organic compounds have recently been tested as alternative carbon and energy sources for the microbes in bioremediation processes.

Phenol and phenolic compounds are major pollutants that are present in many kinds of industrial effluents, such as wastewaters from coal conversion processes, oil refineries, petrochemical plants, steel industries, pharmaceutical industries, textile production, paper mills, and resin, dye, and paint production [6,7]. Phenol and phenolic compounds are toxic when ingested or inhaled and through contact, even at

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low concentrations [8]. Phenolic compounds have traditionally been removed from industrial effluents using physicochemical or biological methods [9,10]. Compared with physicochemical methods, biological treatment is cost-effective and is less likely to cause secondary effluent problems. Biological treatments that have been studied in depth have used various phenol-degrading micro-organisms, including bacteria [11–14], yeasts [15–18], algae [19], and filamentous fungi [20–22].

Phenol and chromium (VI) are often present together in industrial wastewater [23], and approaches in which chromium (VI) and phenol can be simultaneously removed are of great interest for treating such wastewater. The simultaneous biodegradation of phenol and reduction of chromium (VI) using micro-organisms have therefore received much attention recently [24–28]. Coupled phenol degradation and chromium (VI) reduction has been extensively studied using co-culture of bacteria, and the two main bacterial species responsible are a phenol-degrading strain *Pseudomonas putida* DMP-1 and a Cr(VI)-reducing organism *Escherichia coli* ATCC33456 [29,30]. However, phenol biodegradation coupled with chromium (VI) reduction using an isolated species of bacterium has not been reported. The aim of the work reported here was to demonstrate the feasibility of using an isolated *Pseudomonas* strain to simultaneously bioremediate phenol and chromium (VI) in complex wastewater.

2. Methodology

2.1. Materials

Phenol and potassium dichromate were purchased from Sinopharm Chemical Reagent Co. Ltd., China. All other chemicals were of analytical grade from Xilong Chemical Co. Ltd., China. The stock solution of phenol and Cr(VI) was prepared by dissolving a known amount of phenol and potassium dichromate in 1 L of distilled water in a brown glass bottle and stored in the dark at 4˚C, respectively. The experimental work of phenol biodegradation coupled with chromium (VI) reduction by strain JF122 was carried out in mineral medium (MM). The isolate was incubated aerobically at 30˚C in MM, which contained 0.5 g/L NaH_2PO_4, 0.5 g/L K_2HPO_4, 1.0 g/L $(NH_4)_2SO_4$, 0.2 g/L $MgSO_4{\cdot}7H_2O$, 0.2 g/L NaCl, 0.01 g/L $Fe_2(SO_4)_3{\cdot}7H_2O$, and 0.2 g/L $CaCl_2$. The medium had a pH of 6.5 ± 0.1. The medium was autoclaved for 25 min at 121˚C before use, and different concentrations of phenol (the sole carbon source) and Cr(VI) were added to the sterilized MM for the experiments.

2.2. Micro-organisms

The bacterial strain JF122 was isolated from the soil samples that were collected from a polluted river bank near a steel plant in Anhui Province, China. This bacterium was found to be Gram-negative, to have an estimated size of 0.6 × 1.4 μm, to be motile, to use citrate, to reduce nitrate, oxidase, and catalase positive, and to produce acid from glucose. Strain JF122 grew aerobically at 20–41˚C and was negative in the methyl red and Voges–Proskaue tests. Analysis of the 16S rRNA gene sequence (1,498 bp) showed that strain JF122 (GenBank number HM016864) was closely related to *Pseudomonas fluorescens* (GenBank number DQ207731.2), with 99% identical sequence identities.

2.3. Phenol biodegradation coupled with Cr(VI) reduction

To investigate the role of strain JF122 in biodegrading phenol and reducing Cr(VI), four control tests were prepared as follows. Strain JF122 was inoculated into 200 mL MM supplemented with phenol (300 mg/L) and incubated aerobically for 24 h at 30˚C and shaken at 150 rpm to produce a strain JF122 culture. Test 1: 1.0 mL of the strain JF122 culture was added to 150 mL MM containing 600 mg/L phenol and 2.0 mg/L Cr(VI). Test 2: Cell-free growth MM (150 mL) was prepared containing 600 mg/L phenol and 2.0 mg/L Cr(VI). Test 3: 1.0 mL of the strain JF122 culture was added to 150 mL MM containing 600 mg/L phenol and no Cr(VI). Test 4: 1.0 mL of the strain JF122 culture was added to 150 mL MM containing 2.0 mg/L Cr(VI) but no phenol. The control test samples were incubated in 250-mL Erlenmeyer flasks on a rotary shaker (operated at 150 rpm and kept at 30˚C) for 72 h. Samples were withdrawn from each flask at specific intervals during the incubation period and centrifuged at 4,472 × g for 10 min. The residual phenol and Cr(VI) concentrations in the supernatants were analyzed.

To determine the growth curves of strain JF122 on MM that contained phenol and Cr(VI), the various amounts of strain JF122 culture (0.25, 0.5, 1, and 1.5 mL) were added to 150 mL MM aliquots that contained 600 mg/L phenol and 2 mg/L Cr(VI) in a series of 250-mL Erlenmeyer flasks, and the flasks were incubated on a rotary shaker (operated at 150 rpm and kept at 30˚C) for 72 h. Control flasks without strain JF122 Cell were similarly prepared in parallel. Samples were withdrawn from the flasks at specific intervals, and cell density in the culture was measured at 600 nm.

To assess the effects of the initial Cr(VI) concentration on phenol biodegradation coupled with Cr(VI) reduction by strain JF122, 1.0-mL cultures of strain JF122 were added to 150 mL MM aliquots that

contained 600 mg/L phenol and Cr(VI) concentrations between 1.2 and 2.4 mg/L in a series of 250-mL Erlenmeyer flasks, and the flasks were incubated on a rotary shaker (operated at 150 rpm and kept at 30˚C) for 72 h. Control flasks without strain JF122 Cell were incubated in parallel under the same conditions. Samples were withdrawn from the flasks at specific intervals, and the residual phenol and Cr(VI) concentrations were determined.

To assess the effects of the initial phenol concentration on phenol biodegradation coupled with Cr(VI) reduction by strain JF122, 1.0-mL cultures of strain JF122 were added to 150 mL MM aliquots that contained 1.2 mg/L Cr(VI) and phenol concentrations between 200 and 800 mg/L in a series of 250-mL Erlenmeyer flasks, and the flasks were incubated on a rotary shaker (operated at 150 rpm and kept at 30˚C) for 72 h. Control flasks without strain JF122 Cell were incubated in parallel under the same conditions. Samples were withdrawn from the flasks at specific intervals, and the residual phenol and Cr(VI) concentrations were determined.

2.4. Analytical methods

All of the tests were carried out in triplicate, and the results presented are the averages of the triplicate results. Cr(VI) concentrations were measured using a colorimetric method using a spectrophotometer (UV 754N; APL, Shanghai, China). The measured wavelength was 540 nm, and the Cr(VI) was complexed with diphenylcarbazide for 10 min in an acid solution before the colorimetric measurements [31]. The total chromium concentration was measured using an atomic absorption spectrometer (WFX-130; Rayleigh, Beijing, China). The chromium (III) concentration was calculated as the difference between the total chromium and chromium (VI) concentrations [32]. The formation of elemental chromium from the reduction of Cr(VI) was determined using solid-state X-ray photoelectron spectroscopy (XPS). Phenol was determined using a 4-aminoantipyrine colorimetric test [9]. Cell growth of strain JF122 was monitored by measuring cell density (OD) at 600 nm. Organic acids were determined by gas chromatography (GC2010; Shimadzu, Japan).The standard curve adopted for Cr(VI) and phenol estimation was prepared by standard methods [33].

3. Results and discussion

3.1. Phenol biodegradation coupled with Cr(VI) reduction

Phenol degradation and Cr(VI) reduction were investigated using four control tests (Tests 1–4). As is shown in Table 1, phenol biodegradation coupled with chromium reduction occurred in the medium containing strain JF122. Strain JF122 was able to simultaneously remove 600 mg/L phenol and 2.0 mg/L Cr (VI) within 72 h (Test 1). Neither phenol degradation nor Cr(VI) reduction was observed in the experiment in which strain JF122 was not present (Test 2). This means that the removal of phenol and Cr(VI) was caused by the growth of strain JF122 [34]. Strain JF122 was able to degrade 600 mg/L phenol in the culture medium when Cr(VI) was not present (Test 3), but Cr (VI) was not reduced in the experiment in which phenol was not present (Test 4). The reason for this may be that the phenol was the only carbon source in the medium and that the electron donors necessary to reduce Cr(VI) were intermediate phenol degradation products.

3.2. Growth of strain JF122

Fig. 1 shows the results of the growth of strain JF122 on 150 mL MM that contained 600 mg/L phenol and 2 mg/L Cr(VI) with different inocula, it can be observed that cell growth (OD_{600}) of JF122 promoted prominently with the increase of inocula amount (in the 0.25–1 mL range), and the maximal OD_{600} value was increasing up to 0.79. However, there was no significant difference in curve patterns when inocula ranged from 1 to 1.5 mL, the lag period of growth was 24 h, and growth of JF122 reached the stationary phase in 60 h, which indicated that the growth of JF122 did not increase with increasing of inocula amount when the optimum amount was exceeded. Similar findings were reported by Ohtake et al. [35].

3.3. Effect of the initial Cr(VI) concentration

The effect of the initial Cr(VI) concentration (1.2–2.4 mg/L) on the capacity of strain JF122 to degrade phenol and reduce Cr(VI) was tested at a phenol concentration of 600 mg/L (Fig. 2(a) and (b)). Fig. 2(a) shows that strain JF122 could completely reduce Cr(VI) in 72 h when the initial Cr(VI) concentration was in the range of 1.2–2.0 mg/L, but the complete reduction of Cr(VI) was not observed within 72 h at a Cr(VI) concentration of 2.4 mg/L. The average Cr(VI) reduction rates were 0.02, 0.026, 0.028, and 0.022 mg/(L h) when the initial Cr(VI) concentrations were 1.2, 1.6, 2.0, and 2.4 mg/L, respectively. Therefore, it appears that the reduction of Cr(VI) by strain JF122 was not inhibited at an initial Cr(VI) concentration of up to 2.0 mg/L, but that the average rate began to decrease as the Cr(VI) concentration increased above 2.0 mg/L. The average

Table 1
Results of tests of the ability of *Pseudomonas* sp. JF122 to biodegrade phenol and reduce Cr(VI)

	Concentration (mg/L)					
	Test 1		Test 2		Test 3	Test 4
Time (h)	Phenol	Cr(VI)	Phenol	Cr(VI)	Phenol	Cr(VI)
0	600 ± 0	2 ± 0	600 ± 0	2 ± 0	600 ± 0	2 ± 0
12	579.5 ± 8.1	1.96 ± 0.02	589.1 ± 8.9	1.96 ± 0.02	541.9 ± 20.6	1.96 ± 0.01
24	560 ± 4.9	1.89 ± 0.01	581.4 ± 11.5	1.96 ± 0.01	369.5 ± 17.3	1.94 ± 0.01
36	440.8 ± 19.9	1.41 ± 0.03	572.2 ± 18.5	1.86 ± 0.08	225.9 ± 33.6	1.9 ± 0.08
48	60 ± 11.3	0.7 ± 0.04	566.3 ± 20.8	1.81 ± 0.13	122.4 ± 46.6	1.9 ± 0.08
60	28.7 ± 4.5	0.3 ± 0.04	565.5 ± 22.8	1.71 ± 0.13	45.8 ± 21.9	1.92 ± 0.05
72	1.2 ± 0.2	0 ± 0	552.9 ± 19.2	1.77 ± 0.01	4.3 ± 2.5	1.87 ± 0.08

Notes: Test 1: Strain JF122 cultivated in mineral medium (MM) containing phenol and Cr(VI); Test 2: Cell-free MM containing phenol and Cr(VI); Test 3: Strain JF122 cultivated in MM containing phenol but no Cr(VI); Test 4: Strain JF122 cultivated in MM containing Cr(VI) but no phenol.

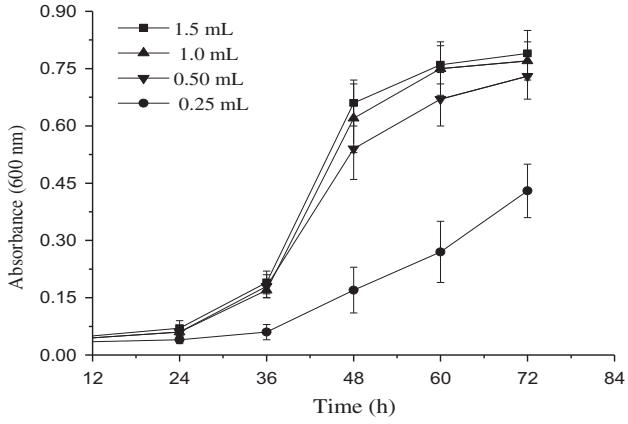

Fig. 1. Cell growth of strain JF122 on MM that contained phenol and Cr(VI) with different inocula.

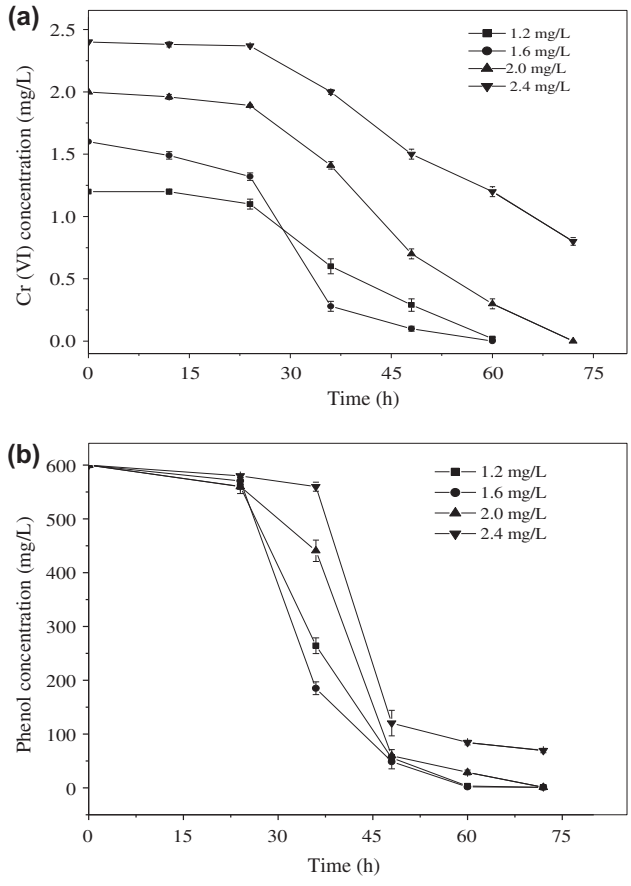

Fig. 2. Reduction of Cr(VI) (a) and degradation of phenol (b) by strain JF122 at different Cr(VI) concentrations.

Cr(VI) reduction rate was higher at 2.4 mg/L than at 1.2 mg/L, and a possible explanation for this is that the electron donors produced during phenol biodegradation were used at a faster rate than they were produced at a Cr(VI) concentration of 1.2 mg/L but were not enough to reduce Cr(VI) concentrations of 2.4 mg/L. The accumulation of Cr(VI) in the medium may therefore decrease the biological activity of strain JF122, so the complete reduction of Cr(VI) was not observed within 72 h at a Cr(VI) concentration of 2.4 mg/L. These results show that there was a maximum capacity for Cr(VI) reduction by strain JF122 at a phenol concentration of 600 mg/L and that when Cr(VI) was present above a certain concentration, the reduction capacity was inhibited by metal toxicity. Similar behavior was found by Song et al. [36].

The capacity of strain JF122 to degrade phenol at different Cr(VI) concentrations (1.2, 1.6, 2.0, and 2.4 mg/L) during the incubation period is shown in Fig. 2(b). It is evident from Fig. 2(b) that there were no clear differences in the phenol biodegradation yields

when the initial Cr(VI) concentration was in the range of 1.2–2.0 mg/L. Strain JF122 showed a fast phenol degradation rate after an initial lag phase, and phenol was then rapidly removed, reaching complete removal within 60 h. However, there was a longer phenol degradation lag phase when the initial Cr(VI) concentration was around 2.0–2.4 mg/L, but after that lag phase, the highest degradation rate was at a Cr(VI) concentration of 2.4 mg/L. The reason for this may be that the electron donors involved in Cr(VI) reduction were derived from phenol degradation, and the removal of these intermediates may occur at a faster rate at higher initial Cr(VI) concentrations, increasing the phenol degradation rate. The incomplete phenol degradation at a Cr(VI) concentration of 2.4 mg/L was caused by there being insufficient electron donors produced for Cr(VI) reduction. Cr(VI) accumulation in the medium will inhibit phenol degradation, and similar results were found in an earlier study by Chirwa and Wang [34].

3.4. Effect of the initial phenol concentration

The effect of the initial phenol concentration (200–800 mg/L) on the capacity of strain JF122 to degrade phenol and reduce Cr(VI) was examined at an initial Cr(VI) concentration of 1.2 mg/L (Fig. 3(a) and (b)). The results in Fig. 3(a) show that the capacity of strain JF122 to reduce Cr(VI) was affected by the initial phenol concentration. When the initial phenol concentration was around 400–600 mg/L, the reduction of Cr(VI) by strain JF122 was enhanced, and the complete reduction of Cr(VI) was found within 60 h. We observed clear inhibition when the phenol concentration was 800 mg/L; the reduction of Cr(VI) was appreciable. However, there was no inhibition at a phenol concentration of 200 mg/L, the final phenol concentration being similar to that reached at other initial phenol concentrations, but the complete reduction of Cr(VI) was not found within 60 h. According to the explanation given by Nkhalambayausi-Chirwa and Wang [26], it is possible that the initial phenol concentration (200 mg/L) was not high enough to cause the initial Cr(VI) concentration (1.2 mg/L) to decrease completely, because the electron donors involved in the reduction of Cr(VI) were intermediate phenol degradation products.

Variation in the capacity of strain JF122 to degrade phenol with the initial phenol concentration (200–800 mg/L) is shown in Fig. 3(b). We found that an increase in the initial phenol concentration (in the 200–600 mg/L range) resulted in the phenol degradation rate increasing to a maximum of 10 mg/(L h).

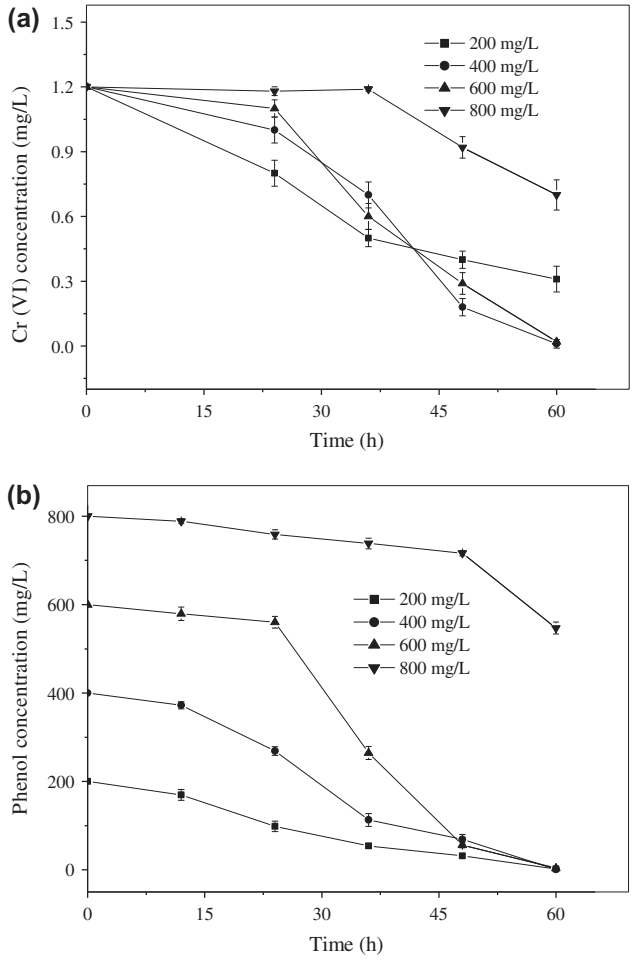

Fig. 3. Reduction of Cr(VI) (a) and degradation of phenol (b) by strain JF122 at different phenol concentrations.

However, when the initial phenol concentration was increased from 600 to 800 mg/L, a considerable decrease in the phenol degradation rate was found, and phenol was not removed completely, which was probably caused by the biological activity of strain JF122 being inhibited by the phenol toxicity at these concentrations. Phenol is toxic to organisms at high concentrations, as has been reported by Zaitsev et al. [37].

3.5. The chromium forms produced by the reduction of Cr(VI)

The Cr(VI) and total chromium concentrations were determined in experiments using 600 mg/L phenol and 2.0 mg/L Cr(VI) to determine the formation of chromium in the cultures when Cr(VI) was reduced by strain JF122. As is shown in Fig. 4, the Cr(VI) concentrations decreased over the incubation time, and

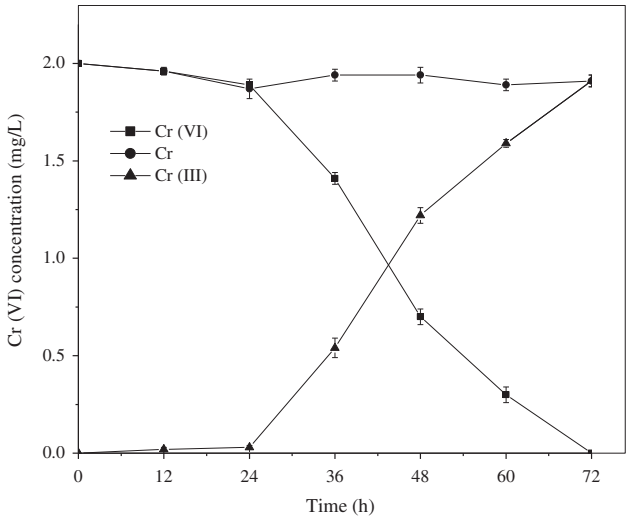

Fig. 4. Formation of Cr(III) and the reduction of Cr(VI) by strain JF122 over time.

the complete removal of Cr(VI) was observed by 72 h. However, the total chromium concentration in the solutions remained relatively constant over the incubation period. These results demonstrate that Cr(VI) was reduced rather than biosorbed, and a similar observation was reported by Juvera-Espinosa et al. [38].

It is reasonable to predict that strain JF122 transformed phenol into water and bicarbonate under aerobic conditions. However, the data shown in Table 2 show that organic acids accumulated in the medium after the removal of 600 mg/L phenol and 2.0 mg/L Cr(VI). Organic acid accumulation may inhibit the biodegradation of aromatic compounds [39]. The complete degradation of phenol, determined by the biological activity of strain JF122, was not inhibited by the accumulation of organic acids, and the reason for this may be that the microbial degradation of phenol was affected by the composition of the medium. The degradation of phenol in the presence of Cr(VI) was enhanced because phenol degradation intermediates were used as electron donors in the reduction of Cr(VI).

Table 2
Organic acids accumulated in the medium at the end of the phenol biodegradation coupled with Cr(VI) reduction tests

Substrate	Chemical formula	Concentration (mg/L)
Phenol	C_6H_6O	0 ± 0
Acetic acid	CH_3COOH	30.3 ± 17.9
Ethanol	C_2H_5OH	19.7 ± 14.5

The XPS analysis of the solids formed in the experiments did not show any sign of elemental chromium being produced (data not shown), probably because the accumulation of organic acids impeded the precipitation of Cr(III), so Cr(VI) was reduced to soluble Cr(III) (in the supernatant).

4. Discussion

A phenol-degrading and chromate-reducing bacterial strain was isolated and identified as being a *Pseudomonas* sp., from its biochemical characteristics and 16S rRNA sequence analysis. The strain (called JF122) was able to simultaneously remove 600 mg/L phenol and 2.0 mg/L Cr(VI) from an aqueous solution within 72 h. Phenol concentrations higher than 800 mg/L inhibited both phenol degradation and Cr(VI) reduction, whereas Cr(VI) concentrations lower than 2.0 mg /L enhanced both phenol degradation and Cr(VI) reduction. These results found in the current research are similar to previous work by Chirwa and Wang, where the optimum Cr(VI) reduction in an anaerobic consortium was observed at a Cr(VI) concentration of 2.0 mg/L and a phenol concentration of 200 mg/L [34]. Moreover, Tziotzios et al. studied the biological phenol removal with simultaneous Cr(VI) reduction by a single pilot-scale bioreactor; the results indicated that highest Cr(VI) reduction rate was observed at the initial phenol concentration of 350 mg/L under a Cr(VI) feed of 5.5 mg/L, while the optimum phenol removal rate was observed at the initial phenol concentration of 500 mg/L [28].

The amount of Cr(VI) reduced was far lower than the amount of phenol degraded, which supports the hypothesis that the electron donors involved in reducing the Cr(VI) by the isolated bacteria were derived from phenol degradation metabolites in the aerobic culture. A similar observation was made in an earlier study by Nkhalambayausi-Chirwa and Wang [26], who found that the highest Cr(VI) reduction efficiency per unit of phenol degradation was two orders of magnitude lower than the 1,106 mg Cr(VI)/g phenol theoretically required by the following equation:

$$C_6H_6O + 4.5O_2 + 2CrO_4^{2} + 8H^{+} + \frac{1}{2}C_6H_6O_5 \rightarrow 3CO_2 + 2HCO_3^{-} + 2Cr^{3+} + \frac{9}{2}H_2O + HCOO \cdot (CH_2)_2 \cdot COOH$$

Less toxic, insoluble Cr(III) from the reduction of Cr(VI) was expected to be formed, but our results ruled out the formation of Cr(III) precipitate, and XPS

analysis of the solids produced did not show any paramagnetic signal to indicate the presence of elemental chromium (data not shown). There were no changes in the total Cr concentrations in the aqueous portions of the samples (Fig. 4). Therefore, we concluded that the reduction of Cr(VI) led to the formation of soluble Cr(III) in the supernatant, which is in agreement with some earlier studies [40,41]. The reduction of Cr(VI) by aerobic bacteria is based on a reductase that is mainly associated with soluble enzyme activity [42]. The enzymatic reduction of Cr(VI) resulting in the formation of soluble organo-Cr(III) complexes is well known [43–45]. Further research into the mechanisms by which strain JF122 degrades phenol and reduces Cr(VI) is underway.

5. Conclusions

A phenol-degrading and chromate-reducing bacterial strain, *Pseudomonas* sp. JF122, was isolated from soil samples collected from a river bank near a steel plant. It was evident that the electron donors involved in reducing Cr(VI) by the isolated bacteria were intermediate phenol degradation products. This work clearly demonstrates the feasibility of using a bacterial isolate, JF122, for the biodegradation of phenol coupled with the reduction of chromium (VI), which has previously been performed using consortia of bacteria. These results show that strain JF122 and other microorganisms could be useful for simultaneously removing organic pollutants and heavy metals from complex wastewaters.

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