

Dual bioremediation of phenol and Cr(VI) by mixed microbial cultures in the presence of molasses

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ABSTRACT

Simultaneous phenol and Cr(VI) bioremoval by two different mixed cultures, from petroleum-contaminated soil (PS) and boron-contaminated wastewater (BW), was investigated in regard to different culture media, pH levels (6–8), initial phenol (25–100 mg/L) and Cr(VI) (15–50 mg/L) concentrations. The optimum medium was found to be mineral salt medium tested, which contained 1% (v/v) molasses (MSM). Optimum pH values were 6 for PS and 8 for BW. All of the phenol present in the samples was mineralized regardless of its concentrations tested, Cr(VI) bioremoval was enhanced by the increase in phenol concentrations, and molasses also exerted a positive effect on Cr(VI) removal, and the yields reached 100% for both pollutants, even at 13.1 mg/L Cr(VI) and 91.1 mg/L phenol concentration in PS samples. In MSM containing PS samples approximate efficiency was 100% for phenol removal; but Cr(VI) removal ratios were 64.9% and 41.7% at 25.8 mg/L and 41.3 mg/L concentrations, respectively. Finally it can be concluded that molasses stimulated Cr(VI) bioremoval at elevated phenolic conditions in the mixed microbial culture, and molasses might be of use for the bioremediation of phenol and Cr(VI) polluted wastewaters.

Key words | Cr(VI), bioremediation, molasses, mixed microbial culture, phenol

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INTRODUCTION

Although some of them are in the Priority Pollutants List, phenols are used widely in several industrial processes such as production of polycarbonate resins, paints, explosives, inks, perfumes, textiles, and antibacterial agents (Van Schie & Young 2000). Another priority pollutant, hexavalent chromium, can accompany phenol, naphthalene or trichloroethylene in wastewater effluents of several widely distributed industries like tannery, textile, dye and petrochemical (Aksu & Gönen 2006). Although both of chemical and biological procedures have long been used for treatment of these harmful effluents, chemical techniques are known to have some disadvantages such as the high cost, considerable sludge formation and generation of secondary pollution (Nawaz & Ahsan 2014). Biological treatment is considered as a less costly, easy to use alternative method, which is preferred especially for its ecofriendliness (Juwarkar *et al.* 2010). As is well known, organisms possessing sufficient bioremediation capability and high adaption ability to changing environmental conditions during the process are required for efficient biological treatment. Since phenol and Cr(VI) can often exist together in effluents,

their simultaneous bioremediation is therefore of great interest to investigate. In spite of this fact, there are not numerous studies on simultaneous bioremoval of microbially degraded phenol and Cr(VI), and the reports on the use of pure bacteria cultures to remove such pollutants are in particular very few (Wang & Chirwa 1998; Quintelas *et al.* 2006; Song *et al.* 2009; Wasi *et al.* 2011; Gunasundari & Muthukumar 2013; Zhou & Chen 2014). In a study done with *Pseudomonas aeruginosa* CCTCC AB91095, the concentration of Cr(VI) diminished from 20 mg/L to 3.36 mg/L, phenol declined from 100 g/L to 29.51 mg/L in experiments performed with 5% (v/v) inoculum (Song *et al.* 2009). According to another study investigating simultaneous bioremoval of phenol and Cr(VI) by *Stenotrophomonas* sp., isolated from tannery effluent contaminated soil, the highest Cr(VI) removal was 81.27% at 16.59 mg/L initial Cr(VI) concentration, while 200.05 mg/L phenols were entirely degraded (Gunasundari & Muthukumar 2013). Zhou & Chen (2014), on the other hand, showed that *Pseudomonas* sp. JF122 had the capability of degrading phenol and removing Cr(VI) simultaneously in a mineral medium with

2.4 mg/L Cr(VI) and 600 mg/L phenol. Liu *et al.* (2008) used mixed pure cultures of *Pseudomonas putida* Migula (CCTCC AB92019) and *Bacillus* sp. for binary bioremoval of phenol and Cr(VI) at 15 mg/L Cr(VI) and 100 mg/L phenol containing solution, and found 84.47% bioremoval of Cr(VI). Actually, these findings are in accordance with previously reported statement by Tziotziou *et al.* (2008), who claimed that mixed cultures could be more advantageous than pure cultures in removing such pollutants, due to their wider spectrum of metabolic activities.

The aim of this study is to present additional evidence showing simultaneous bioremoval of Cr(VI) and phenol by mixed microbial cultures effectively, economically and in an environmentally friendly way by using molasses, which are firstly tested here to increase the bioremoval rate of Cr(VI) in phenol containing media. In order to be able to test whether mixed microbial cultures could find a place in biological treatment systems for efficient dual bioremediation of phenol and Cr(VI), different conditions affecting pollutant bioremoval, such as media, pH, and initial pollutant concentration, were also investigated to determine the optimum removal capacity.

MATERIALS AND METHODS

Conditions employed in mixed microbial cultures

Two different mixed cultures were tested to see if they had binary phenol and Cr(VI) removal capacity, the one named as petroleum-contaminated soil (PS) was obtained in a previous study (Kiliç & Dönmez 2013). Boron-contaminated wastewater (BW) culture samples were taken from a BW effluent (Eti Mine General Directorate-Emet Boron Work Kütahya, Turkey). BW mixed cultures were enriched by periodic subculturing into the mineral salt (MS) medium or MS medium with 1% (v/v) molasses (MSM) at pH 7. The MS medium was composed of (in g/L) KH_2PO_4 , 1.7; $(\text{NH}_4)_2\text{SO}_4$, 2.69; MgSO_4 , 0.2; and CaCl_2 , 0.03 (Afzal *et al.* 2007). Beet molasses solution was approximately equivalent to 10 g/L sucrose. 1 mL of the samples were inoculated into 20 mL of the medium containing 50 mg/L phenol and 15 mg/L Cr(VI) in 50 mL Erlenmeyer flasks, which were kept 7 days at 30 °C on a rotary shaker at 100 rpm stirring rate.

Chromatographic grade phenol was used in the experiments (Riedel-de Haën, Germany); other chemicals used in the preparation of the microbial growth media were the products of Merck, Germany.

The effect of composition and pH of the media on bioremediation

To determine the effect of the composition of media and pH on the simultaneous bioremoval of phenol and Cr(VI) by PS and BW cultures, trials were performed at different pH values (6, 7, and 8) in MS media with 1 g/L glucose (MSG) and MS with 1% (v/v) molasses (MSM).

To find the bioremediation yields at different pH levels, the cultures were enriched at these pH values by inoculation of mixed microbial culture (1% v/v) into three each of different MSG and MSM media containing 50 mg/L phenol and 15 mg/L Cr(VI). The cultures were inoculated into 100 mL of media in 250 mL Erlenmeyer flasks and were incubated on a rotary shaker at 100 rpm stirring rate for 7 days at 30 °C. Further experiments were performed by using only the selected samples containing the media with a suitable pH level for the highest bioremoval capacity.

The effect of initial phenol concentration on Cr(VI) and phenol bioremediation

In these experiments, Cr(VI) concentrations were set as constant; bioremoval by PS mixed microbial culture was investigated in MSM with increasing phenol concentrations (pH 6). The initial phenol concentrations for PS were 28 mg/L, 52.4 mg/L, and 91.1 mg/L, and Cr(VI) concentrations were 15.1 mg/L, 14.7 mg/L, and 13.1 mg/L, respectively. Pollutant removal of BW was investigated in MSM at pH 8. The initial phenol concentrations for BW were 37.8 mg/L, 53.6 mg/L, 95.6 mg/L and Cr(VI) concentrations were 13.7 mg/L, 14.6 mg/L, and 13.2 mg/L, respectively. Mixed microbial cultures were inoculated 1% (v/v) into the media for these experiments.

The effect of initial Cr(VI) concentration on Cr(VI) and phenol bioremediation

To find the effect of initial Cr(VI) concentration on pollutant bioremediation, experiments were conducted in the series of samples with constant phenol concentration and increasing Cr(VI) concentrations. In the experiments done with PS in MSM (pH 6), initial Cr(VI) concentrations were increased from 13.1 mg/L to 25.8 mg/L, and to 41.3 mg/L; phenol concentrations were 91.1 mg/L in this media for the lowest Cr(VI) and 95.6 mg/L for other Cr(VI) concentrations tested. Trials done with BW in media at pH 8; ranging Cr(VI) concentrations from 13.2 mg/L, 34.6 mg/L, and to 62.6 mg/L, where phenol concentration was kept

constant at 96.6 mg/L. PS and BW mixed microbial cultures were inoculated as 1%(v/v) in these trials.

Analytical methods

For the determination of phenol and Cr(VI) bioremediation capacity of different mixed cultures, the samples were incubated for 7 days at 30 °C on a rotary shaker at 100 rpm. 4 mL samples were taken daily from each flask during the incubation period. The samples were centrifuged at $3,421 \times g$ for 10 min to remove the biomass. The concentration of phenol in the supernatant was determined by high performance liquid chromatography (Shimadzu, Japan), using a C-18 column (250 mm \times 4.6 mm inner diameter: 5 mm particle size). The mobile phase was acetonitrile:water (60:40 v/v), which was pumped at 1 mL/min rate; detection was performed with an UV detector at 275 nm.

The concentration of chromium in the supernatant was determined spectrophotometrically at 540 nm, using diphenyl carbazide reagent in acid solution as the complexing agent for Cr(VI) (Snell & Snell 1959). Absorbance measurements were done by using a Shimadzu UV 2001 model spectrophotometer. Microbial growth was determined by measuring the turbidity of the diluted samples at 600 nm, using a standard curve of absorbance against dry cell mass.

Phenol degradation and Cr(VI) removal by mixed cultures were investigated as a function of changes in initial pH, phenol and Cr(VI) concentrations. The percentage removal was calculated from Equation (1):

$$Y\% = \frac{(C_o - C_t)}{C_o} \times 100 \quad (1)$$

Pollutant removal capacity was calculated by measuring the concentration of pollutants left in the biomass, in terms of the mass balance principle (Equation (2)):

$$q_m = \frac{(C_o - C_t)}{X_m} \quad (2)$$

In these equations, q_m (the maximum specific pollutant removal) is the maximum amount of pollutants removed per unit dry weight of microbial cells (mg/g), X_m is maximum dried cell mass (g/L); C_o and C_t are the initial and final concentrations (mg/L), respectively. Control Erlenmeyer flasks contained phenol, Cr(VI) and media only; mixed cultures were omitted in order to determine and internalization of the effects of any direct reactions between media components and pollutants.

Statistical analysis

The experiments were set in a completely randomized design with three replicates up. The data were subjected to analysis of significant differences among treatment means, and were compared by standard deviations (\pm S.E.).

RESULTS AND DISCUSSION

In the study, biodegradation of phenol and Cr(VI) removal by two different mixed cultures (PS and BW) were determined in two different MS media, containing 1 g/L glucose or 1% (v/v) molasses instead. No reactions were observed between media ingredients and the pollutants in the control Erlenmeyer flasks containing only MSM media and pollutants. This observation proved that phenol and Cr(VI) were removed only by the mixed microbial cultures tested in the study.

Cr(VI) and phenol bioremediations in different media and some pH values tested

The effects of pH 6, 7 and 8 on phenol and Cr(VI) bioremediation rates were investigated in MSG and MSM containing 50 mg/L phenol and 15 mg/L Cr(VI) concentrations (Figure 1(a) and 1(b)). At pH 6, Cr(VI) removal was only 38.1% in MSG, but it went up to 95.5% in MSM; phenol degradation yield was 96.8% in MS media with glucose, and reached to 100% in the presence of molasses in PS mixed culture (Figure 1(a)). At pH 7, PS mixed culture removed 65.8% of Cr(VI) and degraded phenol at 99.4% and 100% levels in MSG and MSM, respectively. In MSG, Cr(VI) was removed with a yield of 21.6%, but the removal rate increased up to 78.8% in MSM at pH 8. Under the same condition, the applied phenol was completely degraded in MSG, instead of being mineralized 51.7% in MSM for PS samples.

Bioremediation of Cr(VI) and phenol by BW mixed culture are summarized in Figure 1(b), the results showed that BW mixed culture removed Cr(VI) with 23.2% in MSG and 99.2% yields in MSM at pH 6. At the same pH level, phenol degradation was 2.6% and 73.9% in MSG and MSM, respectively. In MSG at pH 7, 42.1% of the initial Cr(VI) was removed, but the removal rate nearly doubled, and the yield increased up to 86.0% in MSM. At pH 7, phenol bioremoval in MSG was only 21.8%, much lower than its level in MSM (89.0%). Although in pH 8 MSG media, 47.5% of the initially present Cr(VI) was removed, but in

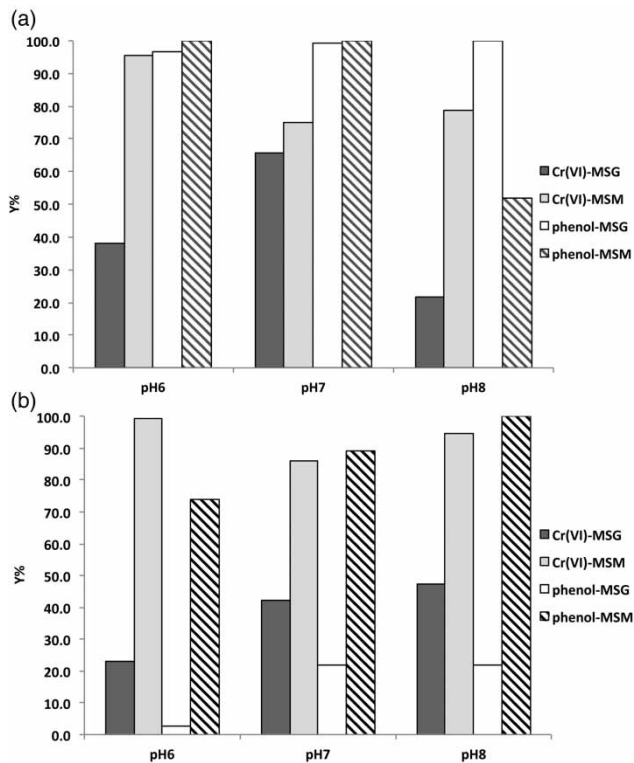


Figure 1 | Effect of pH and different media on phenol and Cr(VI) bioremoval by PS (a) and BW mixed culture (b) (C_0 : 50 mg/L phenol and 15 mg/L Cr(VI); T: 30 °C; incubation period: 7 d).

MSM with the same pH, only 5.4% of it remained. On the other hand, phenol removal at pH 8 was only 21.7% in MSG, but all of the phenol present was degraded by BW samples in MSM. In a previous study performed with mixed bacterial cultures, effective phenol and Cr(VI) bioremediation was carried out in a pilot-scale packed-bed reactor at alkaline pH ranging from 7.3 to 7.5 (Tziotziou *et al.* 2008). As in the current study with BW mixed culture at pH 8, it was shown by other researchers that pure cultures of bacterial strains also degraded phenol and removed Cr(VI) simultaneously at alkaline pH values (Liu *et al.* 2008; Song *et al.* 2009; Gunasundari & Muthukumar 2013). Zhou & Chen (2014), on the other hand, investigated phenol biodegradation and Cr(VI) reduction by the *Pseudomonas* sp. strain JF122 in mineral medium at pH 6.5, and showed that the strain was capable of bioremoving phenol and Cr(VI) simultaneously. In another study, Srivastava *et al.* (2007) selected potential Cr(VI) removing and pentachlorophenol degrading microbial strains in a minimal salt medium at pH 6; PS mixed culture also removed these pollutants with high yields at this pH in the present study.

The effect of different media on simultaneous phenol and Cr(VI) bioremediation was investigated in MSG and MSM with 50 mg/L phenol and 15 mg/L Cr(VI) initial concentrations. As shown in Figure 1(a) and 1(b), all the bioremoval yields of PS and BW mixed cultures in MSM were higher than the yields obtained in MSG, with only one exception, which was the phenol removal by PS mixed culture at pH 8 (Figure 1(a)).

In the current study, it was found relevant to test the effects of the addition of molasses, depending on its positive effect on microbial growth, as previously described elsewhere (Dönmez & Koçberber 2005; Kiliç & Dönmez 2013). As a matter of fact, molasses stimulated Cr(VI) bioremoval by mixed cultures significantly, and further trials were carried out in MS media with molasses (MSM). The optimum pH for the best bioremediation was 6 for PS and 8 for BW mixed cultures.

Dual bioremoval of Cr(VI) and phenol in media with increasing phenol concentrations

This series of experiments was performed by using PS and BW mixed cultures with 15 mg/L Cr(VI) and increasing phenol concentrations up to 100 mg/L. As seen in Figure 2, phenol degradation was quite high at all of the tested phenol concentrations. All of the initial phenol present in PS mixed culture samples was mineralized within 6 days of the incubation period, and Cr(VI) bioremoval increased by the increments in phenol concentration. In MSM samples containing 28 mg/L phenol and 15.1 mg/L Cr(VI), 56.0% of the ions were removed after incubation for 1 day and the removal rate reached 74.6% on the third day of the

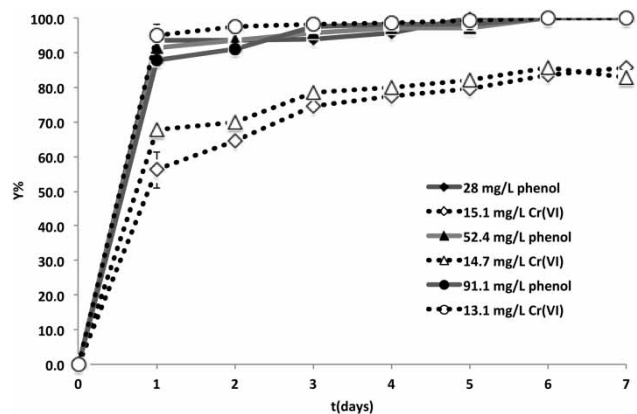


Figure 2 | Effect of initial phenol concentration on phenol and Cr(VI) removal by PS mixed culture in MSM with constant Cr(VI) concentration (T: 30 °C; incubation period: 7 d).

incubation. The highest Cr(VI) bioremoval rate was 85.8% in these trials. When the phenol concentration was increased to 52.4 mg/L, the removal yield reached 78.6% after 3 days of incubation; the maximum removal obtained was 85.6% at the end of 6 days. In media with the highest initial phenol concentration of 91.1 mg/L, PS removed 95.2% of Cr(VI) after incubation only for 1 day, and the removal was completed on the sixth day of the incubation; PS mixed culture degraded more than 90% of the initial phenol concentrations present within 2 days of the incubation. In addition, Cr(VI) bioremoval was elevated by the increases in initial phenol concentration; for example, with the increment from 28 mg/L to 91.1 mg/L, its removal was enhanced from 64.6% to 97.4%. This relation is in concordance with the previously reported findings by Liu *et al.* (2008) and Zhou & Chen (2014), which can be taken as an indication of the role of phenol degradation in enhancement of Cr(VI) bioremoval by providing electrons and energy for its reduction.

Figure 3 shows the changes in bioremoval capacity of BW mixed culture in MSM (pH 8) with 15 mg/L Cr(VI) and increasing phenol concentrations within the 37.8–95.6 mg/L range. BW mixed culture tolerated all of the present phenol concentrations, and the degradation yields were 100% for all of the tested concentrations; in the samples with 37.8 mg/L phenol and 13.7 mg/L Cr(VI), 52.0% Cr(VI) removal was found after incubation for 4 days. Under the same conditions, the highest Cr(VI) removal determined was 53.0% at the end of the incubation period. In MSM with 53.6 mg/L phenol and 14.6 mg/L Cr(VI), phenol bioremediation was completed and the Cr(VI) removal rate was 73.0% after incubation for 5 days, and it

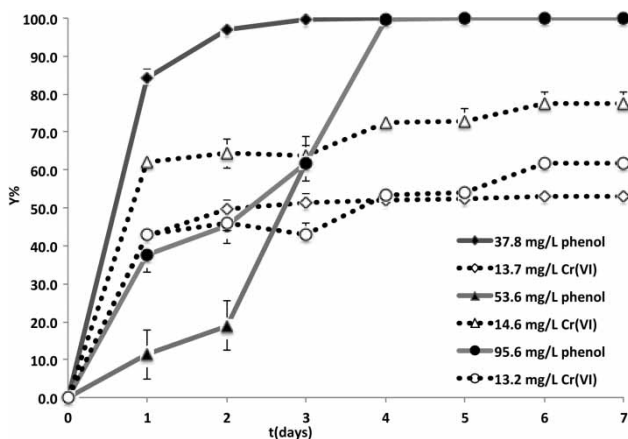


Figure 3 | Effect of initial phenol concentration on phenol and Cr(VI) removal by BW mixed culture in MSM with constant Cr(VI) concentration (T: 30 °C; incubation period: 7 d).

finally reached a 77.5% level at these conditions. Even in samples with the highest phenol concentration of 95.6 mg/L, its mineralization was completed, but 53.9% of the Cr(VI) present was removed after incubation for 5 days, and its final removal yield could not exceed the 61.7% level.

In the experiments performed with BW mixed culture, the incubation period required for complete phenol mineralization was longer than the corresponding periods in MSM at the higher phenol concentrations tested. In addition, Cr(VI) removal was enhanced by phenol up to concentrations near to 100 mg/L, with the exception of the slight decrease at 95.6 mg/L phenol and 13.2 mg/L Cr(VI), which may be taken as the result of the toxicological properties of phenol at its higher concentrations.

Dual bioremoval of Cr(VI) and phenol in media with increasing Cr(VI) loads

In these experiments, phenol concentrations were set to approximately 100 mg/L and Cr(VI) concentrations were increased up to 50 mg/L. In media with molasses, PS mixed culture had higher capability for binary bioremoval of phenol and Cr(VI), as shown in Figure 4. In the presence of 91.1 mg/L phenol and 13.1 mg/L Cr(VI), PS mixed culture removed both of the pollutants with the ratios of 87.9% and 95.2% respectively after 1 day incubation, and they were finally completely removed on the sixth day of incubation. The increments in Cr(VI) levels did not affect phenol bioremediation, but Cr(VI) bioremoval was affected negatively by them. In MSM with 95.6 mg/L phenol and 25.8 mg/L Cr(VI), phenol was completely degraded after incubation for 2 days, however

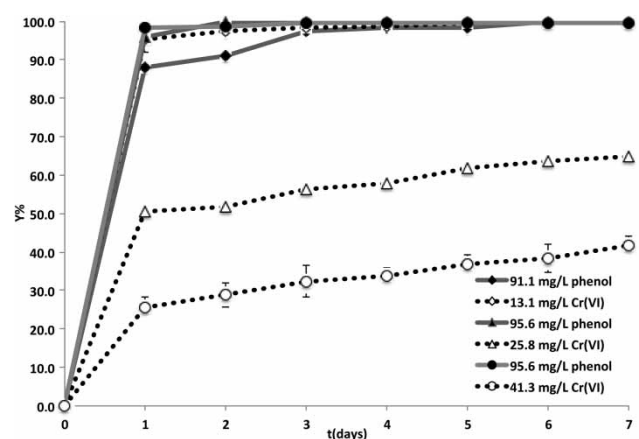


Figure 4 | Effect of initial Cr(VI) concentration on phenol and Cr(VI) removal by PS mixed culture in MSM with constant phenol concentration (T: 30 °C; incubation period: 7 d).

nearly half of the applied Cr(VI) (51.8%) was removed from the MSM by PS mixed culture. Under these conditions, the highest Cr(VI) bioremoval was 64.9% at the end of the incubation period. When Cr(VI) was increased to 41.3 mg/L, 99.5% of the initial phenol level was degraded and Cr(VI) removal was 32.4% at the third day of the incubation period. Extension of incubation to 7 days did not increase phenol degradation at all, but Cr(VI) removal increased to 41.7%.

In Figure 5, binary bioremoval of phenol and Cr(VI) in the set of culture samples with increasing Cr(VI) concentrations in BW mixed culture is summarized. In MSM containing 96.6 mg/L phenol and 13.2 mg/L Cr(VI), phenol mineralization was completed after incubation for 5 days, and the Cr(VI) removal was 53.9%. BW mixed culture removed Cr(VI) with the highest yield of 61.7% at the end of the incubation period. In MSM with 96.6 mg/L phenol and 34.6 mg/L Cr(VI), phenol was degraded completely and Cr(VI) removal reached its highest yield of 44.9%. A higher Cr(VI) level of 62.6 mg/L was also tested on BW mixed microbial culture, which showed the inability of the microorganisms to tolerate this concentration of the ion.

These data indicated that PS and BW mixed microbial cultures could mineralize all of the tested phenol concentrations, but they were affected negatively by certain Cr(VI) concentrations tried. In PS mixed culture samples, phenol degradation was not affected by increasing Cr(VI) levels, but BW mixed culture samples could not tolerate 50 mg/L Cr(VI) and phenol degradation could not be detected.

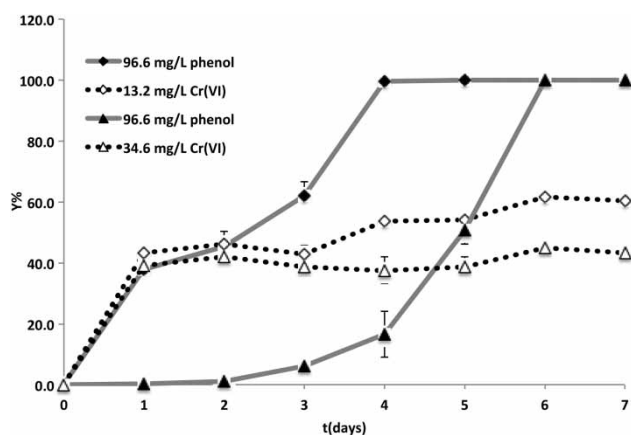


Figure 5 | Effect of initial Cr(VI) concentration on phenol and Cr(VI) removal by BW mixed culture in MSM with constant phenol concentration (T: 30 °C; incubation period: 7 d).

Table 1 | The maximum specific phenol and Cr(VI) bioremoval (q_m) of the PS mixed culture under nearly 100 mg/L phenol and increasing Cr(VI) concentrations (pH: 6; T: 30 °C; incubation period: 7 d)

C_o (mg/L)		q_m (mg/g)	
Phenol	Cr(VI)	Phenol	Cr(VI)
91.1	13.7	55.4 ± 0.3	8.0 ± 0.04
95.6	25.8	62.9 ± 0.3	11.1 ± 0.1
95.6	41.3	66.1 ± 0.2	12.0 ± 0.7

Table 2 | The maximum specific phenol and Cr(VI) bioremoval (q_m) of the BW mixed culture under nearly 100 mg/L phenol and increasing Cr(VI) concentrations (pH: 8; T: 30 °C; incubation period: 7 d)

C_o (mg/L)		q_m (mg/g)	
Phenol	Cr(VI)	Phenol	Cr(VI)
96.6	13.2	67.0 ± 0.3	5.6 ± 0.03
96.6	34.6	69.5 ± 0.6	11.2 ± 0.1

In Tables 1 and 2, the maximum specific phenol and Cr(VI) bioremediation (q_m) values of PS and BW mixed cultures in MSM with nearly 100 mg/L phenol and increasing Cr(VI) levels are presented for comparison. In samples with 91.1 mg/L phenol and 13.7 mg/L Cr(VI), q_m values were 55.4 mg/g and 8.0 mg/g, respectively for PS mixed culture (Table 1). In PS samples with 95.6 mg/L phenol and 25.8 mg/L Cr(VI), 62.9 mg phenol and 11.1 mg Cr(VI) were removed per 1 gram of biomass. In the experiments done with 95.6 mg/L phenol and 41.3 mg/L Cr(VI), the maximum specific phenol bioremediation found was 66.1 mg/g and the q_m value for Cr(VI) bioremoval by PS mixed culture was 12.0 mg/g; the value found for degraded phenol was 67.0 mg/g, and Cr(VI) removal was 5.6 mg/g for BW mixed culture in MSM with 96.6 mg/g phenol and 13.2 mg/L Cr(VI), as seen in Table 2. In samples with 96.6 mg/L phenol and 34.6 mg/L Cr(VI), q_m values were 69.5 mg/g and 11.2 mg/g, respectively.

This is the first study presenting experimental results evidencing the possibility of reaching the highest simultaneous removal efficiencies reported in the literature for both of the pollutants: 100% bioremoval in the presence of 100 mg/L phenol and 15 mg/L Cr(VI). Tziotzios *et al.* (2008) showed that only 5.5 mg/L Cr(VI) could be tolerated by the mixed microbial culture they tested, with an efficiency of 95.18% in the presence of both pollutants. Liu *et al.* (2008) also investigated dual bioremoval of

these pollutants, and found that the tested culture had higher removal capacity if the Cr(VI) concentration was lower than 15 mg/L. In that study, only 84.47% of 15 mg/L Cr(VI) was removed, whereas in the current study, PS mixed microbial culture had higher Cr(VI) removal capacity even in the media with higher initial phenol levels than the previous studies. Tziotzios *et al.* (2008) reported that their tested mixed microbial culture tolerated only 5.5 mg/L Cr(VI) in the media containing phenol, at 17 mg/L and even at 11 mg/L Cr(VI) concentrations the microorganisms they used could not remove any Cr(VI). In the current study, however, PS mixed culture had a very efficient Cr(VI) removal capacity in the presence of phenol, where microorganisms removed 13.1 mg/L, 25.8 mg/L, and 41.7% mg/L Cr(VI) concentrations with yields as 100%, 64.9% and 41.7%, respectively.

CONCLUSIONS

The simultaneous phenol mineralization and Cr(VI) bioremoval were carried out using two different mixed cultures, PS and BW. These cultures showed considerably high bioremediation capacities by removing both of the tested pollutants within 2 days in the media initially optimized for bioremoval efficiencies in 1% (v/v) molasses-MS media at the optimum pH values, which was 6 for PS, and 8 for BW. The mixed cultures tolerated 100 mg/L phenol concentration in the presence of Cr(VI) concentrations up to 25 mg/L; PS had higher bioremediation capacity than BW. The maximum specific phenol removal value was 66.5 mg/g, and q_m for Cr(VI) was 12.0 mg/g in MSM, which were the highest pollutant concentrations tested for PS samples. According to these results, it was concluded that the maximum specific pollutant removal values were increasing slightly as Cr(VI) concentrations were elevated as the result of the lower ratio of biomass to Cr(VI). On the other hand, PS mixed culture had higher tolerance to the pollutants in parallel with its higher biomass than BW mixed culture; thus, at nearly 25 mg/L Cr(VI) concentration and 100 mg/L phenol level, pollutant removal rates per 1 gram of PS biomass were lower than BW mixed culture samples. The presented results indicated that these mixed cultures can be useful in biological treatment processes with their satisfactory capacity of removing phenol and Cr(VI) simultaneously from effluents containing both pollutants.

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