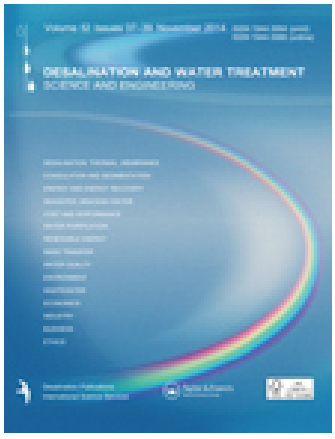

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Reduction of Cr(VI) to Cr(III) by thermal Bacillus licheniformis B22 under different temperatures using binary and ternary combinations of organic acids

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ABSTRACT

This study presents optimization of process variables for hexavalent chromium reduction using thermotolerant *Bacillus licheniformis* B22 isolated from Pamukkale (Denizli, Turkey). We examined the effects of binary and ternary combinations of different electron-donating substrates (galacturonic acid, glucuronic acid, and humic acid) at 45 and 50°C. The influence of different pH values (6.0, 7.0, 8.0, 9.0, and 10.0) and initial inoculation rates (2, 4, 6, and 8%) were also enumerated. The strongest stimulatory effect on Cr(VI) reduction was obtained with ternary combination of galacturonic acid, glucuronic acid, and humic acid. At 45 and 50°C, 8% inoculation rate, the reduction in ternary combination is relatively fast, completely reducing 100 mg/l Cr(VI) in 6 h compared with 2% inoculation rate (12 h). This bacterium exhibited a rapid Cr(VI) reduction ability under optimized conditions. Cr(VI) reduction of *B. licheniformis* B22 increased with an increase in initial inoculation rate, and the optimum initial pH for Cr(VI) reduction was 7.0. In addition, the results suggested that the reduced Cr(III) was not precipitated in the form of $Cr(OH)_3$, and that organic acids significantly enhanced microbial Cr(VI) reduction rates by forming less toxic and highly soluble organo-Cr(III) complexes despite Cr(III) having very low solubility.

Keywords: Chromate reduction; Hexavalent chromium; Thermal Bacillus; Organic acids

1. Introduction

Hexavalent chromium is an important pollutant present in the environment at elevated concentrations due to its extensive use in various industries including leather tanning, textiles, and electroplating [1–4]. Uncontrolled release of industrial wastes has caused severe contamination of soil–water systems and subsequent chromium toxicosis because of its carcinogenic, mutagenic, and teratogenic potential [5]. Chromium generally exists in two stable oxidation states: trivalent chromium and hexavalent chromium. Hexavalent chromium is more toxic and soluble than trivalent chromium, which is mobile [6]. Batch sorption studies, primarily performed with pure mineral phases, suggest that Cr(III) is highly reactive and may strongly sorb to the mineral phases [7]. Cr(VI) exhibits weak to medium binding affinity for metal oxides such as Fe- and Al-oxides [8–10] depending on the environmental conditions (e.g. pH and organic matter content). One of the most important factors affecting chromium

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mobility in underground systems is natural organic substances that are abundant in soil and water. These organic substances act as electron donors and convert Cr(VI) compounds to Cr(III) compounds. A number of studies have demonstrated that organic ligands, clay, dissolved metal ions, and Fe(II; III)-bearing minerals may function as a catalysts in the reduction of Cr(VI) to less soluble Cr(III) [11–13]. Organic ligands may also compete against metal ions for sorption sites on mineral surfaces thereby reducing the extent of metal ion sorption to mineral surfaces [14,15]. Recently, organic ligands capable of reducing Cr(VI) have emerged as a reasonable means of enhancing or reducing Cr mobility [16]. Several researchers have, for example, identified the potential role of microbial exudates in Cr oxide solubilization as a central challenge in the development of remediation strategies and accurate assessment of environmental risks [16,17]. Exopolymeric substances (EPS) are produced by micro-organisms for a variety of purposes in response to environmental stresses. Several studies showed that the quantity and composition of EPS vary according to bacterial strain and metal exposure [18,19]. For example, in a study with the hydrogen-producing photosynthetic bacteria strain *Rhodopseudomonas acidophila*, Sheng et al. [20] found that toxic substances such as Cr(VI) and Cd(II) stimulated the production of microbial EPS.

Some uronic acids, such as alginic, galacturonic, and glucuronic acids identified as a main constituent of microbial EPS, were used as model organic ligands with known chemical structures [21]. Uronic acids are continuously produced by some soil bacteria in subsurface systems. For example, bacterially produced alginate is commonly present in subsurface systems due to its production by N_2-fixing bacteria of the genus *Azotobacter* and *P. aeruginosa* [22].

Removal of Cr(VI), either by reduction or by biosorption, can significantly reduce the risks to human health [23]. Conventional technologies for remediation of chromium-contaminated wastewater, including ion exchange, precipitation, and adsorption on alum or kaolinite, cannot be applied on a large scale due to high cost and subsequent secondary environmental pollution. Alternatively, bacterial bioremediation sites contaminated by toxic metals are gaining increasing attention due to its efficiency, affordability, and environmentally friendly advantages [24]. It is obvious that thermal cultures with such a capability are particularly useful in industrial applications, where most discharged effluents have elevated temperatures requiring the use of cooling or holding tanks. The availability of thermotolerant and/or thermophilic micro-organisms may therefore significantly reduce treatment costs.

A wide variety of micro-organisms such as bacteria, yeast, algae, protozoa, and fungi are found in water, and these micro-organisms have developed various mechanisms to protect themselves from heavy metal toxicity, such as adsorption, uptake, methylation, oxidation, and reduction [23]. Micro-organisms present in water and soil develop resistance to chromium, and can play an important role in the detoxification and removal of hexavalent chromium from polluted sites. It has been reported that hexavalent chromium can be reduced under aerobic and anaerobic conditions by many bacteria belonging to genera such as *Bacillus* [6,25], *Lysinibacillus* [24], *Pseudomonas* [26–28], *Escherichia* [29], *Enterobacter* [30], *Providencia* [31], and *Achromobacter* [32]. However, there is relatively little information in the literature on the use of thermophilic and/or thermotolerant strains for chromium bioremoval. Thermophilic micro-organisms are amongst the most studied extremophiles, and are gaining wide industrial and biotechnological interest because they are well adapted to harsh industrial processes. Thermal springs and hot geothermal outflows have been screened worldwide to find the appropriate metabolite for every application [33–36]. Therefore, the present study examined the Cr reduction potential of *Bacillus licheniformis* B22 bacteria, which are the isolates of Pamukkale Geothermal region of Turkey [37].

The main objectives of this study are: (i) to investigate the effects of various environmental conditions such as different pH values and initial inoculation rates, (ii) to determine the effect of binary and ternary combinations of different electron-donating substrates under different temperatures, (iii) to optimize the process variables for chromium reduction and to evaluate total Cr under these conditions, (iv) and to provide a useful reference for further development of effective chromium reduction bioprocesses utilizing thermal isolate.

2. Materials and methods

2.1. Chemicals

Unless otherwise stated, all chemicals used in the experiments were reagent grade or better. Water for all experiments was supplied from a Human Power-Pure water system (Zeener Power, Korea). Cr(VI) stock solution was prepared by dissolving 2.829 g $K_2Cr_2O_7$ (294.19 g/mol) (Merck) in 1 l UV-water, which was autoclaved separately and added to the media before experiments. D(+)-glucuronic acid sodium salt monohydrate ($C_6H_9NaO_7{\cdot}H_2O$) (Merck), D(+) galacturonic acid monohydrate ($C_6H_{10}O_7{\cdot}H_2O$) (Sigma-Aldrich), and humic acid (Sigma-Aldrich) were used in the experiments as organic acids (ligands). In addition,

diphenylcarbazide (Merck) reagent was prepared in acetone. All stock solutions were stored in amber glass bottles in darkness at 4 °C.

2.2. Preparation of media and growth conditions

B. licheniformis B22 was isolated from thermal resources, as previously reported [37]. The bacterial culture was inoculated in growth media tryptic soy broth (TSB) consisting of peptone from casein (17.0 g/l), peptone from soymeal (3.0 g/l), glucose (2.5 g/l), NaCl (5.0 g/l), and dipotassium hydrogen phosphate (2.5 g/l). The culture was aerobically incubated at 40, 45, or 50 °C with constant shaking at 125 rpm; culture growth was monitored by measuring optical density (OD) at 600 nm. The culture suspension was prepared and adjusted by comparing against 0.5 McFarland turbidity standard tubes (1.5×10^8 cfu/ml) for all tests.

2.3. Cr (VI) reduction experiments

Microbial Cr(VI) reduction experiments were performed using *B. licheniformis* B22 in the absence and presence of organic acids. First, 250 ml flasks containing 100 ml TSB with 100 mg/l Cr(VI) and 1 g/l organic acid concentration were inoculated with the culture at logarithmic phase. The initial pH of the media was buffered to 7.0 (± 0.2) using an appropriate amount of $NaHCO_3$ (0.088 mM) [2]. All media were autoclaved at 121 °C for 15 min before use in microbial Cr(VI) reduction experiments. The culture was then aerobically incubated at 40, 45, or 50 °C with constant shaking at 125 rpm. Immediately after inoculation with bacteria, samples were taken at regular time intervals (every 6 or 12 h) and centrifuged at 6,000 rpm for 20 min. The concentration of Cr(VI) in the supernatant was determined colorimetrically at 540 nm by UV spectrophotometer (Hach Lange, DR 5000, Germany) using diphenylcarbazide reagent [38]. Total chromium content of samples was determined using ICP-MS (Agilent 7500 ce, USA) with a detection limit of 4×10^{-10} M. The Cr(III) content was the difference between total chromium and Cr(VI). Each experiment was carried out in duplicate.

2.4. Effect of combinations of organic acids under different temperature and inoculation rates

In order to determine the synergistic effect of organic acids under different temperatures (45 and 50 °C) and inoculation rates (2 and 8%), galactronic acid, glucuronic acid, and humic acid were tested in binary and ternary combinations. Organic acids were added in the growth media at 1.0 g/l. The initial pH of the media was 7.0 (± 0.2).

2.5. Effect of pH

To determine the effect of pH values on chromium reduction, Cr(VI) reduction experiments were carried out in media containing galactronic acid or glucuronic acid at 40 °C and at pH 6.0, 7.0, 8.0, 9.0, and 10.0. The pH of the medium was set with 6 N HCl and 6 N NaOH; $NaHCO_3$ buffers for pH 6.0, 7.0, and 8.0, and NH_3–NH_4Cl buffers for pH 9.0 and 10.0 were added to the medium in order to stabilize the pH.

2.6. Effect of initial cell inoculation rates

To determine the effect of initial cell density on Cr(VI) reduction, we tested 2, 4, 6, and 8% inoculation rates at 40 °C and pH 7.0 for all organic acids (galactronic acid, glucuronic acid, and humic acid) that were used in the previous study as organic ligands [37].

3. Results

3.1. Effect of combinations of organic acids under different temperature and inoculation rates on bacterial chromium reduction

Binary and ternary combinations of organic acids were studied at 45 and 50 °C, and at 2 and 8% inoculation rates. The reduction time for 100 mg/l Cr(VI) was the same for all binary combinations. Using galactronic acid+glucuronic acid, galactronic acid+humic acid, and glucuronic acid+humic acid combinations, Cr(VI) reduction was completed in 24 h. The highest synergic effect was observed in ternary combinations of organic acids. With the ternary combinations of galactronic acid, glucuronic acid, and humic acid, 100 mg/l Cr(VI) reduction by *B. licheniformis* B22 bacterium was completed in 12 h (Fig. 1).

3.2. Effect of different pH levels on bacterial chromium reduction

In order to determine the effect of pH on chromium reduction, pre-activated *B. licheniformis* B22 strain was inoculated 2% by volume. TSB media with pH set to 6.0, 7.0, 8.0, 9.0, and 10.0. The experiments at different pH values were carried out in TSB media including galactronic acid and glucuronic acid, which were the most effective of all the organic acids used in previous work [37]. In both media, chromium reduction at pH 6.0, 7.0, and 8.0 was very similar to each other; at pH 6.0 and 7.0, the 100 mg/l Cr(VI) was completely reduced at 48 h incubation. At pH 9.0, reduction slowed slightly and was completed at the

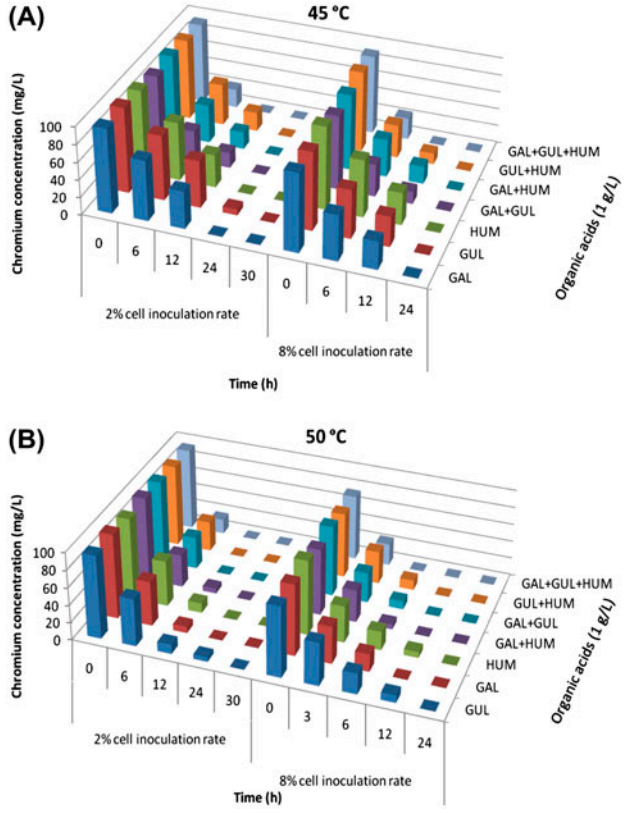

Fig. 1. Effect of binary and ternary combinations of organic acids on Cr(VI) reduction by *B. licheniformis* B22 at 45˚C (A) and 50˚C (B) (pH 7.0). GAL: galactronic acid (1 g/l), GUL: glucuronic acid (1 g/l), and HUM: humic acid (1 g/l).

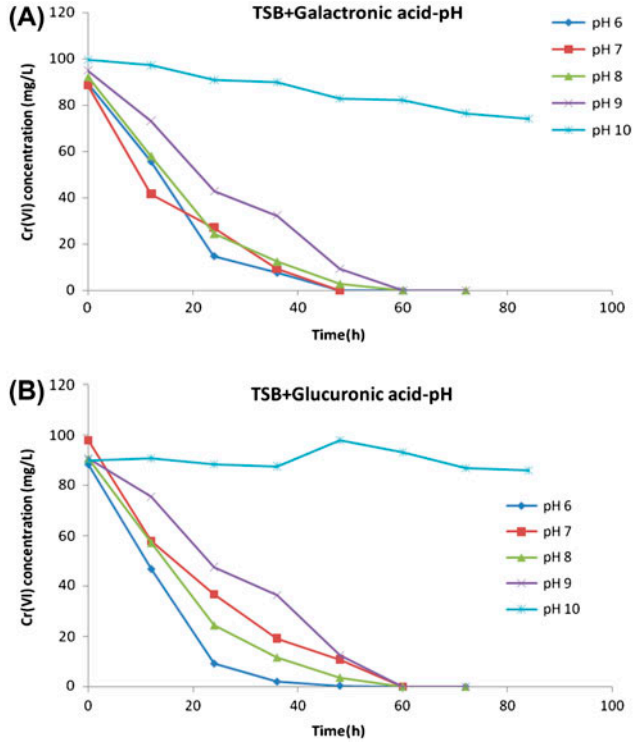

Fig. 2. Effect of different pH levels on Cr(VI) reduction by *B. licheniformis* B22 in TSB medium supplied with galactronic acid (A) and glucuronic acid (B).

60th hour. At pH 10.0, there was very limited Cr(VI) reduction. Fig. 2 shows effect of pH on chromium reduction in media containing galactronic acid and glucuronic acid.

3.3. Effect of initial cell inoculation rates on bacterial chromium reduction

Inoculation rates of 2, 4, 6, and 8% were tested for each organic compound. As shown in Fig. 3, Cr(VI) reduction by *B. licheniformis* B22 increased with greater initial cell concentration from 2% (120 h completion) to 8% (60 h completion).

3.4. Chromium reduction at optimal conditions and determination of the reduction of Cr(VI) to Cr(III) by *B. licheniformis* B22

The optimal conditions at which *B. licheniformis* B22 bacterium reduced 100 mg/l Cr(VI) were established as 50˚C, pH 7.0, and 8% initial inoculation rate. Under these conditions, reduction was most effective in the medium containing the ternary combination of organic acids (Fig. 4). ICP-MS analysis of the reduction medium with and without galactronic acid revealed that the reduction of Cr(VI) by *B. licheniformis* B22 led to the production of soluble Cr(III) rather than the precipitation of Cr(III) as $Cr(OH)_3$ (Fig. 5). Cr $(OH)_3$ precipitate was not measured in our spectroscopic studies; however, mass balance calculations indicated that the mass of total Cr(Cr(VI)+Cr(III)) was equal to the mass of Cr(VI) added to the flasks. Thus, we conclude that the reduced Cr(III) remained in the liquid phase after Cr(VI) was transformed to Cr(III)—in other words, there was no precipitate. This may be due to the organic content of the medium. Therefore, future studies will seek to establish the effect of other organic types of matter on $Cr(OH)_3$ precipitate.

4. Discussion

Chromium is of great economic importance in industrial processes, but also a major metal pollutant of the environment [39]. The ability of some bacteria to reduce Cr(VI) has raised the possibility of using these micro-organisms as a biotechnological tool for

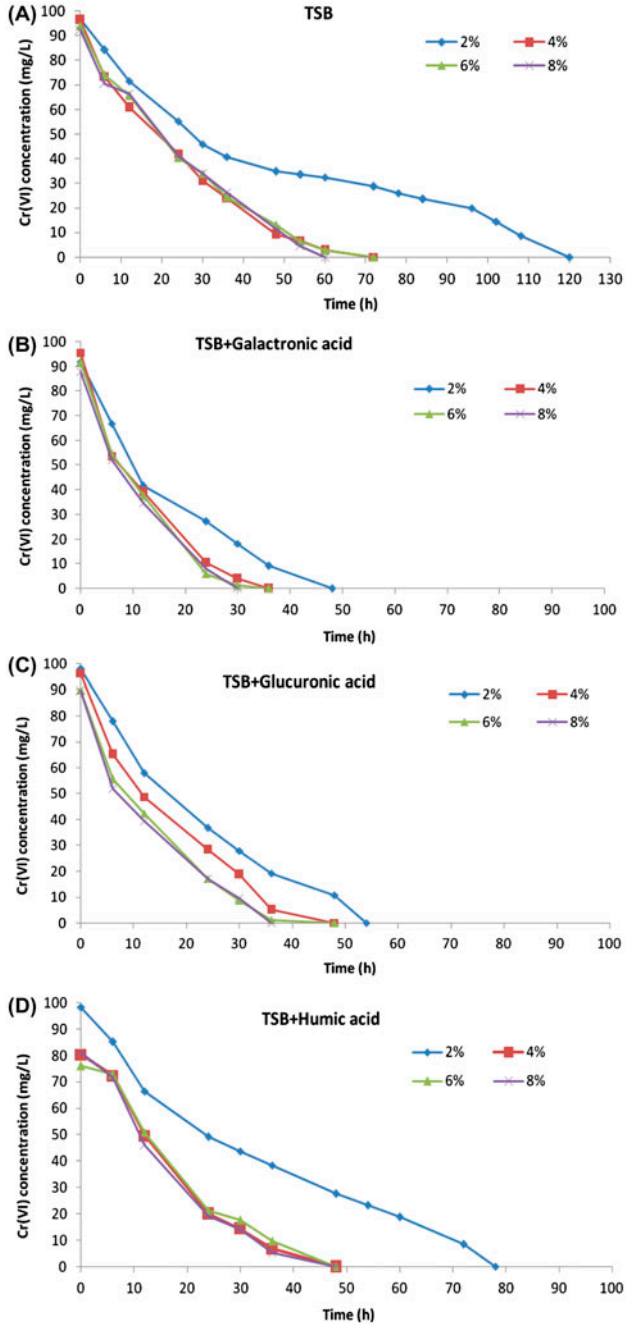

Fig. 3. Effect of initial inoculation rates on chromate reduction in TSB medium (control) and TSB medium supplied with organic acids by *B. licheniformis* B22 at 40°C (pH 7.0). (A) TSB medium, (B) TSB medium with galactronic acid, (C) TSB medium with glucuronic acid, and (D) TSB medium with humic acid.

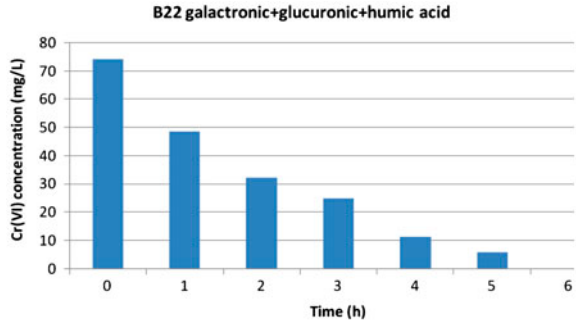

Fig. 4. Cr(VI) reduction of *B. licheniformis* B22 in optimization conditions (50°C, pH 7.0, 8% inoculation rate).

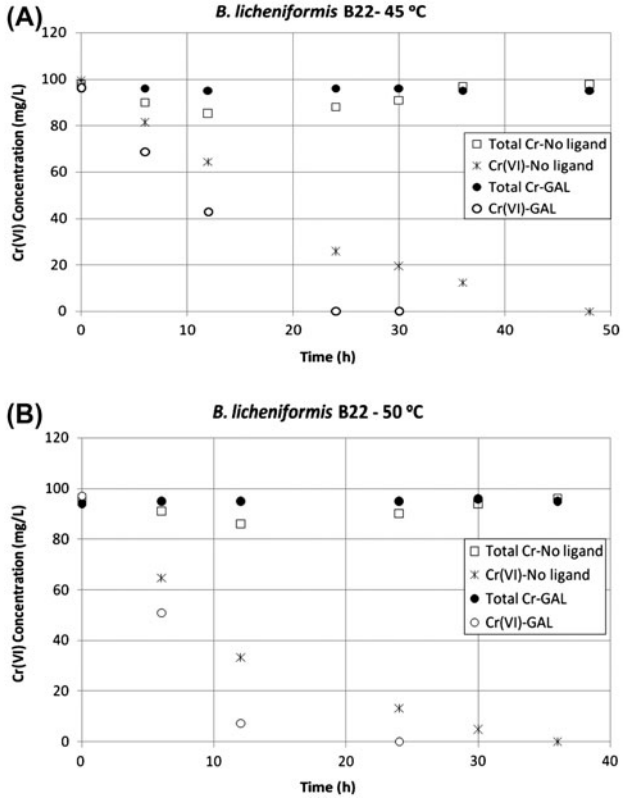

Fig. 5. Whole cell reduction of Cr(VI) and total chromium (total Cr) analysis in the absence or presence of 1 g/l galactronic acid (GAL) at 45°C (A) and 50°C (B) by *B.licheniformis* B22: Cr(VI) = 100 mg/L. All solutions prepared TSB nutrientmedia were buffered to pH 7 with 0.088 mM $NaHCO_3$.

bioremediation of chromium-polluted zones [40,41]. The main advantages of using bacterial Cr(VI) reduction are: it does not require high energy input or toxic chemical reagents, and the possibility of using native, non-hazardous strains. It is known that bacterial chromium reduction is related to multiple factors such as pH, chromium concentration, carbon sources, organic acids, temperature, and cell inoculation level.

The effects of organic acids such as galacturonic acid, glucuronic acid, humic acid, alginic acid, and citric acid on Cr (VI) reduction by *B. licheniformis* B22 were reported in our previous paper; we found bacterial Cr(VI) reduction was enhanced significantly by adding organic acids [37]. Also, in the previous experiment, we reported that the binary and ternary combinations of galactronic acid, glucuronic acid, and humic acid had a synergic effect on the reduction of chromium by B22 bacterium at 40˚C [37]. In the present study, we examined whether there is also a synergic effect between organic acids at different temperatures (45 and 50˚C) and inoculation rates (2 and 8%). In addition, via ICP-MS analysis, we demonstrated that *B. licheniformis* B22 reduced Cr(VI)–Cr(III).

We are not aware of any previous study within the literature on combinations of organic acids. In natural media such as soil and water, these kinds of compounds undoubtedly exist together and interact with each other. In addition, we know that microbial EPS is present in natural media; that its production increases under conditions of environmental stress; and that the main constituents of EPS are organic acids such as alginic, galacturonic, and glucuronic acids. The present study therefore used the binary and ternary combinations of natural organic acids, and determined the relationship between these combinations and Cr(VI) reduction.

According to the results of synergistic action, studies were conducted through the inoculation of B22 bacteria between 2 and 8% volumes at 45 and 50˚C; At 45˚C and 2% inoculation rate, the reduction in ternary combination is relatively fast, completely reducing 100 mg/l Cr(VI) in 12 h.

At 8% initial inoculation, chromium reduction accelerated and was completed in 12 h (Fig. 1(A)). At 50˚C the reduction speed was also fastest in ternary combinations, reducing 100 mg/l Cr(VI) in 12 h at 2% inoculation, and in 6 h at 8% (Fig. 1(B)). In our previous study of the same bacteria, for ternary combinations of organic acids at 40˚C and with 2 and 8% inoculation rates, reduction times were 24 and 12 h, respectively. These results are in good agreement with those of previous studies [16,17,27,42]. For example, Mabbett et al. [42] reported on the reduction of Cr(VI) by *Desulfovibrio vulgaris* ATCC 29579 in anaerobic resting cell suspensions. Their results indicate that bioreduction occurred only in the presence of low molecular chelating agents (e.g. EDTA, citrate). Similarly, Puzon et al. [16] found that the biotic Cr(VI) reduction by *E. coli* (ATCC 11105) in the presence of cellular organic metabolites (e.g. citrate) formed both soluble and insoluble organo-Cr(III) end-products. Xu et al. [43] reported enhanced chromate reduction activity by *Pannonibacter phragmitetus* LSSE-09 in the presence of electron donors such as acetate, lactate, and pyruvate.

The initial pH of the culture plays a crucial role in chromium reduction. Many studies have investigated the optimum pH values for bacterial reduction of chromium. Cheng and Li [44] reported that the optimum pH range for reduction of chromium by *Bacillus* sp. MDS05 strain is 7.0–9.0, maximum chromium reduction occurs at pH 8.0 and at extreme pH values (5.0, 6.0 and 10.0), bacterial reduction of chromium is restricted. In *Bacillus* sp. and *Pseudomonas fluorescens*, optimum pH is 7.0, and chromium reduction is inhibited at pH 6.0 [45]. In *Bacillus* sp. XW4 and one Gram-positive isolate, the optimum initial pH was 9.0 [6]. The variation observed in optimal pH indicates that it is important to individually determine the optimum pH value in different cultures and to modify the pH in order to achieve maximum Cr(VI) reduction of chromium detoxification. We therefore studied the effect of pH variation on Cr(VI) at pH levels of 6.0, 7.0, 8.0, 9.0, and 10.0. The pH of the medium was stabilized using buffers. In our study, the lowest reduction occurred at pH 8.0, 9.0, and 10.0. However, chromium reduction also decreased at increased alkalinity. The pH values of 6.0 and 7.0 had a positive effect on reduction due to good cell growth. The general trend with reference to the influence of pH on reduction was pH 6.0 > pH 7.0 > pH 8.0 > pH 9.0 > pH 10.0 (Fig. 2). The results show that *B. licheniformis* B22 bacterium completed 100 mg/l Cr(VI) reduction at the 48th hour of incubation in TSB medium supplied with galactronic acid at pH 6.0 and 7.0 (Fig. 2(A)). At pH 8.0 and 9.0, reduction was completed at the 60th hour, and at pH 10.0 no notable reduction occurred. Although the greatest reduction by B22 bacterium occurred at pH 6.0 and 7.0, reduction experiments were conducted at pH 7.0 because of good cell growth (Table 1).

As seen in Fig. 3, for all the organic acids examined, chromium reduction time decreased as the inoculation percentage increased. Fig. 3 shows that initial inoculation concentration affects Cr(VI) reduction as the number of initial cells increases, reduction occurs more rapidly. The slowest reduction was observed at 2% inoculation rate and the fastest at 8%. The reduction times are very similar at 4, 6, and 8% inoculation rates. Generally, an increase in the amount of inoculated cells resulted in shorter reduction time. It is concluded that a greater amount of inoculum promotes more efficient reduction. In a similar study, it was observed that as the initial cell concentration of *Lysinibacillus fusiformis* ZC1 increased, the reduction time decreased. Reduction was completed in 13 h

Table 1
Effect of different pH levels on cell growth of *B. licheniformis* B22 in 100 mg/L Cr(VI) containing medium supplied with galactronic acid and glucuronic acid (OD: 600 nm)

Time (h)	TSB + galactronic acid					TSB + glucuronic acid				
	pH 6.00	pH 7.00	pH 8.00	pH 9.00	pH 10.00	pH 6.00	pH 7.00	pH 8.00	pH 9.00	pH 10.00
12	0.04 ± 0.04	0.82 ± 0.05	0.38 ± 0.01	0.19 ± 0.01	0.13 ± 0.05	0.46 ± 0.01	0.74 ± 0.03	0.72 ± 0.01	0.41 ± 0.03	0.12 ± 0.02
24	0.47 ± 0.00	0.78 ± 0.11	0.53 ± 0.02	0.34 ± 0.04	0.11 ± 0.08	0.57 ± 0.00	0.78 ± 0.01	0.48 ± 0.00	0.58 ± 0.00	0.11 ± 0.12
36	0.65 ± 0.03	0.68 ± 0.06	1.08 ± 0.03	0.26 ± 0.02	0.20 ± 0.03	0.51 ± 0.05	0.65 ± 0.09	0.63 ± 0.04	0.49 ± 0.07	0.20 ± 0.03
48	0.97 ± 0.01	0.83 ± 0.02	0.87 ± 0.10	0.47 ± 0.01	0.47 ± 0.04	0.45 ± 0.02	0.72 ± 0.04	0.83 ± 0.06	0.63 ± 0.01	0.37 ± 0.09
60	1.05 ± 0.07	1.15 ± 0.01	1.01 ± 0.02	1.07 ± 0.01	0.44 ± 0.05	0.73 ± 0.03	0.9 ± 0.03	0.79 ± 0.08	0.64 ± 0.04	0.36 ± 0.02

when the initial cell concentration was 4.86×10^7 cell/ml, and in 10 h at 1.26×10^9 cell/ml [24]. When these findings are taken into account, it is concluded that bacterial Cr(VI) reduction is directly correlated with cell concentration, and that reduction is accelerated by adding organic acids to the medium.

At optimal conditions for *B. licheniformis* B22 bacterium, complete reduction of 100 mg/l occurred at 6 h: ternary combination of organic acids, initial pH 7.0, temperature 50˚C, and 8% inoculation rate (Fig. 4). The addition of heat-killed bacterial cells as the control only led to 5% Cr(VI) reduction after 84 h incubation, which might be attributed to the adsorption by dead bacterial cells [37]. These results provide strong evidence that reduction by *B. licheniformis* B22 occurred mainly via reduction rather than biosorption. More importantly, *B. licheniformis* B22 showed capacity for rapid Cr(VI) reduction compared to other micro-organisms, which therefore makes it a suitable candidate for bioremediation. For example, Liu et al. [6] found that *Bacillus* sp. (XW-4) bacterium did not completely reduce 100 mg/l Cr(VI) at 72 h. According to Zahoor and Rehman [23], *Bacillus* sp. JDM-2-1 could reduce 85% of 100 μg/ml Cr(VI) at 96 h. Cheng and Li [44] reported that *Bacillus* sp. MDS05 reduced 10 mg/l Cr(VI) at 24 h. Under optimal conditions, halophilic *Vigribacillus* sp. reduced 90.2 and 99.2% of 100 mg/l Cr(VI) within 70 h in the absence and presence of 6 wt.% NaCl, respectively [46]. Briefly, B22 bacterium is a more economical candidate for future Cr(VI) detoxification applications.

Cr(III) reduced by bacteria is precipitated out at neutral pH and is eventually removed from the medium in the form of chromium hydroxide ($Cr(OH)_3$) [47–49]. In our study, the end-products were analyzed at neutral pH. Mass balance analysis revealed that the reduction of Cr(VI) by *B. licheniformis* B22 led to the production of soluble Cr(III) end-products rather than the precipitation of Cr(III) as $Cr(OH)_3$. In addition, the presence of complexing ligands increased Cr solubility, thus Cr(III) was retained in the solution and prevented any precipitation. For *B. licheniformis* B22, the amount of total Cr in solution decreased slightly during the early stages of Cr(VI) reduction ($t < 12$ h), then started to increase after 12 h, indicating the formation of soluble Cr species in solution (Fig. 5). In experiments involving galactronic acid, all of the chromium added remained in solution in soluble form (Fig. 5) with no precipitation occurring at any stages of microbial reduction. Similarly, Dogan et al. [2] stated that microbial Cr(VI) reduction with *P. putida*

produced soluble end-products, especially in the presence of complexing ligands such as alginic acid.

5. Conclusions

The use of thermophilic bacteria for chromium reduction has received relatively little attention and there are few studies of this issue within the literature [34,37]. We optimized inoculation rate, combinations of organic acid, pH, and temperature to achieve maximum reduction by the selected bacterial isolate. We also studied reduction of chromium with optimum conditions. Newly isolated *B. licheniformis* B22 was found to reduce Cr(VI); 92.4 and 100% reduction were observed after 5 and 6 h, respectively, under the optimum conditions: ternary combination of organic acids, initial pH 7.0, temperature 50˚C, and 8% inoculation rate. We also evaluated total Cr: ICP-MS results indicate that bacterial Cr(VI) reduction by *B. licheniformis* produced soluble end-products, especially in the presence of complexing ligands such as galacturonic acid.

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