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Experimental Study on the Factors Affecting Hexavalent Chromium Bioreduction by *Bacillus cereus*

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A B S T R A C T

In this work, batch studies were conducted to evaluate the effect of environmental factors on the rate of Cr(VI) reduction from synthetic wastewater of metal plating industry by *Bacillus cereus*. The effect of different inoculum volumes (5, 10, 15 and 20 mL), pH (5, 7 and 9), temperatures (20, 30 and 40 °C) and initial concentrations of Cr(VI) (10, 50, 100 and 200 mg/L) for the best performance of chromium removal were investigated during 72 h of cultivation by *Bacillus cereus*. Complete reduction of Cr(VI) by *Bacillus cereus* was achieved after 48 h of incubation under optimized conditions of pH 7, inoculum volume of 5 mL, initial chromium concentration of 50 mg/L, and temperature of 40 °C. The results showed the highest rate of reduction at the lowest Cr(VI) concentration (0.104 mg/L.h). Atomic absorption spectroscopy analyses under optimized conditions showed the concentration of Cr(III) in the culture supernatant was 49 mg/L after 48 h. The presence of almost all the reduced Cr(III) in the supernatant revealed Cr(VI)-reductase in *Bacillus cereus* is mainly associated with the soluble fraction of the enzyme. High Cr(VI) concentration resistance and high Cr(VI) reducing ability of *Bacillus cereus* make it a suitable candidate for bioremediation.

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1. INTRODUCTION

Water contamination by heavy metals, such as mercury, cadmium and chromium has attracted considerable attentions as it poses a serious threat to human health and the environment [1, 2]. They are easily bio-accumulated through the food chain and beyond permissible quantities causes various chronic disorders [3]. Chromium has many industrial application, such as textile dyeing, chemicals and pigments production, wood preservation, tanning activity and electroplating for surface treatment [4]. Chromium commonly found in the environment in two valence states: trivalent Cr(III) and hexavalent Cr(VI). The Cr(III) is less toxic and mobile, while Cr(VI) is easily soluble and more toxic than Cr(III). The Cr(VI), usually present in form of chromate (CrO_4^-) and dichromate ($Cr_2O_7^-$) possesses significant higher levels of toxicity than other valence states. Chromate (CrO_4^-) is a strong oxidizing agent and causes mutagenic and carcinogenic effect on biological systems [5].

The maximum acceptable limit for the total chromium in drinking water recommended by World Health Organization (WHO) is 0.05 mg/L [6]. Vossoughi et al. determined the concentration of the most important heavy metals contaminants (cadmium, nickel and chrome) in the sediments of the Persian Gulf coast and found that the concentration of heavy metals in all areas were high and the maximum of Cr was 39.6 mg/kg sediment [7].

Various techniques, including chemical precipitation, biological treatment, membrane technology, electrolytic reduction, ion exchange and adsorption, have been developed to tackle this problem [1, 8]. During recent years, many bacteria have been reported to reduce Cr(VI) to Cr(III). A wide

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variety species of microorganisms, including strains of *Achromobacter* sp. [9], *Brucella* sp. [10], *Pseudomonas* [11, 12], *Escherichia* [13, 14], *Enterobacter* [15, 16], *Bacillus* [17-20] and *Shewanella* [21, 22] have been found to be able to reduce Cr(VI) to Cr(III). The mechanisms of Cr(VI) reduction by these microorganisms are variable and species dependent. Some species use Cr (VI) as the final electron acceptor in the respiratory chain whiles in some other strains certain soluble enzymes are responsible for reduction of Cr (VI) to Cr (III) [5, 14].

Microbial chromate reduction becomes a bit complicate as a result of the effects of environmental conditions under which microbial Cr(VI) reduction proceeds. Thus, determining the optimum conditions is also quite important for the maximum conversion of chromium (VI) [5]. Mishra et al. isolated a moderately halophilic Cr(VI) tolerant bacterial that was identified as *Vigribacillus* sp. and could tolerate up to 1000 mg/L Cr(VI) concentration and reduced 90.2 and 99.2% of 100 mg/L Cr(VI) under optimized condition within 70 h in absence and presence of 6 wt. % NaCl [23]. Chatterjee et al. investigated Cr(VI) reduction by *Pseudomonas aeruginosa* from synthetic solution and tannery effluents. They found that the maximum absorption was at 30 mg/L of Cr(VI) at pH 8 [24]. Dhal et al. reported 99% reduction of Cr(VI) in the chromite concentrate (25.2 mg Cr(VI)/L) after 12 min of incubation under the optimized conditions of pH 7, temperature 35 °C and 60% pulp density with the *Bacillus* sp. (3.55×10^7 cells/mL) [25]. Pal and Paul showed that *Bacillus sphaericus* was tolerant to 800 mg/L Cr(VI) and reduced >80% Cr(VI) during growth. Optimum pH and temperature for reduction were 6.0 and 25 °C, respectively [26]. Das et al. isolated *Bacillus amyloliquefaciens* with relatively high tolerance to Cr(VI) (≤900 mg/L) and fast reduction rate of Cr(VI) under optimized conditions of 100 mg/L Cr(VI), pH 7 and temperature 35 °C within 45 h [27]. Chaturvedi was isolated *Bacillus circulans* strain from tannery effluent and reported that optimum conditions for Cr(VI) removal was pH 5.6 and temperature of 30 °C and cells growth was heavily influenced when initial Cr(VI) concentration was increased between 1110 mg/L and 4500 mg/L while Cr(VI) at 500 mg/L to 1110 mg/L did not suppressed the cells growth [5].

In the literatures, to the knowledge of the authors there has been few researches reported on the Cr(VI) reduction potential of *Bacillus cereus* from synthetic wastewater of metal plating industry. Niak et al. [28] isolated *Bacillus cereus* IST105 from electroplating effluent and found that absorption of chromate on the bacterial cell wall takes place through surface functional groups like carboxyl, amide, phosphoryl and hydroxyl. The aim of this study was to evaluate the ability of a strain of *Bacillus cereus* in removal of Cr(VI) from synthetic wastewater of metal plating industry in different Cr(VI) concentrations, pH, temperatures and concentrations of bacteria. Another aspect of this study was to find the mechanism of Cr(VI) reduction and removal by *Bacillus cereus*. It should be noted that the effect of the bacteria to remove chromium has not been studied previously in Iran.

2. MATERIALS AND METHODS

2. 1. Microorganisms' Source *Bacillus cereus* 1665 was procured from the Persian Type Culture Collection (PTCC) of Iranian Research Organization for Science and Technology (IROST), IR Iran.

2. 2. Synthetic Wastewater of Metal Plating Industry The synthetic metal plating wastewater was prepared by adding appropriate concentration of Cr(VI) (10, 50, 100 and 200 mg/L), as the only pollutant source, to the deionized water. The pH of the synthetic metal plating wastewater was adjusted to the desired level using 1 N NaOH or HCl.

2. 3. Optimization of Parameters for Cr (VI) Reduction (Inoculum volume, pH, Temperature and Initial Cr(VI) concentration) To optimize Cr (VI) reduction efficiency of the selected strain of *Bacillus cereus*, the effects of temperature (20, 30, 40 °C), pH (5, 7, 9), initial Cr(VI) concentration (10, 50, 100 and 200 mg/L) and inoculum volume (5, 10, 15 and 20 mL) were investigated during 72 h. The experiments were performed in 100 mL autoclaved Luria–Bertani (LB) broth (g/L: tryptone 10, yeast extract 5, NaCl 5) taken in 250 mL culture flasks using pre grown cells of *Bacillus cereus* (5 mL of cells from the log phase of bacterial culture (cell density at 660 nm was about 1 and concentration of biomass was 1.9 g/L) were inoculated in these flasks except otherwise mentioned.). Autoclaved LB medium was supplemented with Cr(VI) and cells were inoculated in these and incubated at the appropriate temperature and pH with shaking (160 rpm). Samples were drawn out at regular time intervals and analyzed for disappearance of Cr(VI). The percentage of Cr(VI) removal from aqueous solution was calculated by the Equation (1):

$$\text{Cr (VI) removal (\%)} = ((C_0 - C) / C_0) \times 100 \quad (1)$$

where, C_0 and C are the concentrations of Cr(VI) at time 0 and time t, respectively. The optimum pH and temperature were arrived based on the maximum Cr(VI) reduction. The mechanism Cr(VI) reduction was studied under the optimized conditions.

2. 4. Hexavalent and Total Chromium Analysis All Sample from each experimental flask(s) was collected after 0, 4, 8, 24, 48 and 72 h of bacterial inoculation. Samples were subjected to centrifugation at

4000 rpm for 15 min prior to analysis. Hexavalent chromium in the culture supernatant was estimated spectrophometrically by diphenylcarbazide (DPC) method [29], in which 1 mL of supernatant was taken with 1 mL of phosphoric acid and 400 µL of 0.25% (w/v) 1,5-diphenylcarbazide (prepared in acetone). After incubation for 15 min at room temperature, the color intensity of Cr(VI)–diphenylcarbazide complex (purple color) was measured at 540 nm using UV–Visible spectrophotometer (T80+ UV/VIS Spectrometer PG Instruments Ltd., England). Calibration chart at different concentrations of chromium (from 0 to 1 mg/L) was drawn. The Cr(IV) determination analysis involved dilution of the initial Cr(IV) concentrations to the level sensitive enough to be determined by employing colorimetric method. Final Cr (IV) values were obtained by incorporating dilution factor into the calculations. The total chromium concentration was measured by atomic absorption spectroscopy (AAS) (GBC Scientific Equipment Ltd. - 932 Plus), after adding HNO_3 to the sample supernatant. The Cr(III) concentration in the sample was calculated by subtracting the Cr(VI) content from the total chromium content taken by AAS [29].

2. 5. Statistical Analysis All experiments including controls were performed in triplicates. The statistical calculation was done according to the standard method [30], and results are presented as mean ± SD values.

3. RESULTS AND DISCUSSION

3. 1. Effect of pH The pH affects the availability of metal ions in solution (speciation), as well as the metal binding sites onto cell surface [31]. In the present study, the effect of incubation time on Cr(VI) reduction at different pH (5, 7 and 9) was studied (operation condition was: 50 mg/L initial Cr(VI) concentration, temperature 37 °C and inoculum volume of 5 mL). According to the data presented in Figures 1 and 2 with increasing pH from 5 to 7 an increasing in Cr (VI) reduction was observed and at pH 7 the bacterium *Bacillus cereus* showed highest removal efficiency of Cr (VI) (100%) after 72 h and hence, further studies were carried out with pH 7. however, with further increase in pH from 7 to 9, Cr (VI) reduction was declined and reached to 89.6±0.2% after 72 h. The initial pH of medium culture was shown to be an effective factor for bacterial growth and Cr(VI) reduction and deviation from the optimum pH may lead to the formation of number of dissociated and undissociated substances, which may penetrate into the cell membrane and control the entire metabolic and inhibitory activity.

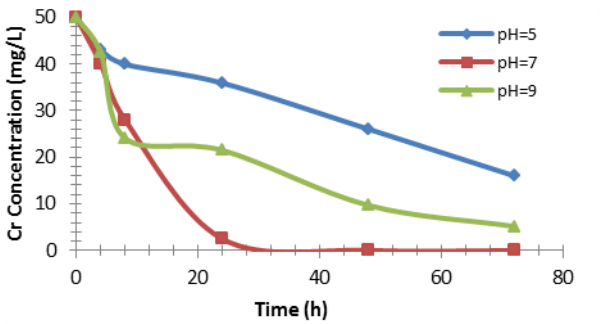

Figure 1. Effect of incubation time on Cr(VI) reduction at pH 5, 7 and 9 [conditions: inoculum volume of 5 mL, T 37 °C, initial Cr(VI) concentration of 50 mg/L].

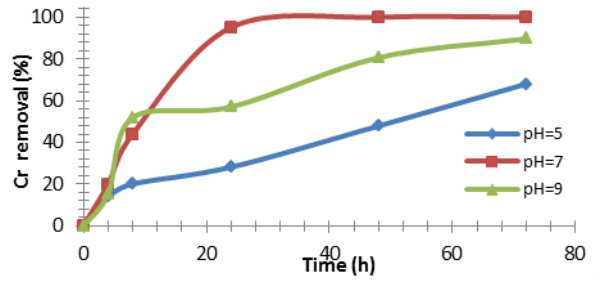

Figure 2. Effect of incubation time on % Cr(VI) reduction at pH 5, 7 and 9 [conditions: inoculum volume of 5 mL, T 37 °C, initial Cr(VI) concentration of 50 mg/L].

The modification in structure and activity of chromium reductase also occur when pH was deviated from the optimum pH [32]. The similar trend was reported for *Bacillus* sp. and *Bacillus amyloliquefaciens* and at pH 7 the bacterium showed highest Cr(VI) reduction [27, 32]. Report of chromium reduction by *Nesterenkonia* sp. strain in range of pH 5-10 showed that the maximum Cr(VI) reduction was exhibited at pH 8 [33]. However, in two other another study a higher optimum pH of 9 for Cr(VI) reduction by *Bacillus* sp. was reported [19, 31]. The difference in optimum pH value suggests that pH modification is important for different cultures to achieve the maximum Cr(VI) reduction in the bioremediation of chromate.

3. 2. Effect of Temperature Temperature is an important factor that has effect on microbial Cr(VI) reduction. In the present study, the effect of incubation time on Cr(VI) reduction by the strain *Bacillus cereus* at different temperatures (20, 30 and 40 °C) was studied (operation condition was: 50 mg/L initial Cr(VI) concentration, pH 7 and inoculum volume of 5 mL). According to the data presented in Figures 3 and 4, with increasing temperature from 20 to 40 °C, Cr(VI) reduction was increased and at 40 °C complete reduction of Cr (VI) was achieved after 72 h. The deviation from the optimum temperature decreased the

Cr(VI) reduction and this may be due to the change in cell membrane structure, metabolism or denaturation of chromium reductase enzyme [32]. At low temperatures, with decreasing the fluidity of the membrane, the substrates cannot enter into the cell rapidly [27]. The temperature of 37 °C has been previously obtained as the optimum temperature for Cr(VI) reduction by *Bacillus* sp [32, 34]. In other two studies the temperature of 35 °C has been obtained as the optimum temperature for Cr (VI) reduction by *Bacillus amyloliquefaciens* and *Nesterenkonia* sp [27, 33]. Also in another study, optimum temperature for Cr(VI) reduction by *Bacillus sphaericus* was reported 25 °C [26].

3. 3. Effect of Initial Cr(VI) Concentration In the present study, the effect of incubation time on Cr(VI) reduction at different initial Cr(VI) concentrations (10, 50, 100 and 200 mg/L) was investigated (operation condition was: pH 7, temperature 37 °C and inoculum volume of 5 mL). As shown in Figures 5 and 6, Cr(VI) reduction occurred even at the highest concentration of 200 mg/L, but with increasing initial Cr(VI) concentration from 10 to 200 mg/L an decreasing in percentage of Cr (VI) reduction was observed. A complete reduction of Cr(VI) was observed at lower Cr(VI) initial concentrations of 10 and 50 mg/L after 48 and 72 h, respectively. However, at higher initial concentrations of 100 and 200 mg/L, Cr(VI) reduction of 98.9±0.2 and 61.9±0.2 % were recorded, respectively after 72 h. This is in agreement with earlier reports that Cr(VI) reducing cells were irreversibly inactivated in batch cultures when the initial concentration exceeded a certain limiting concentration [27]. Similar trends were observed by *Brucella* sp. that reduced Cr(VI) to 100, 94.1, 93.2, 66.9 and 41.6% at concentrations of 50, 100, 150, 200 and 300 mg/L, respectively at pH 7 and temperature 37 °C [10]. Complete Cr(VI) reduction by *Nesterenkonia* sp. was achieved for initial Cr(VI) concentration of 0.1 and 0.4 mM after 12 and 72 h, respectively [32].

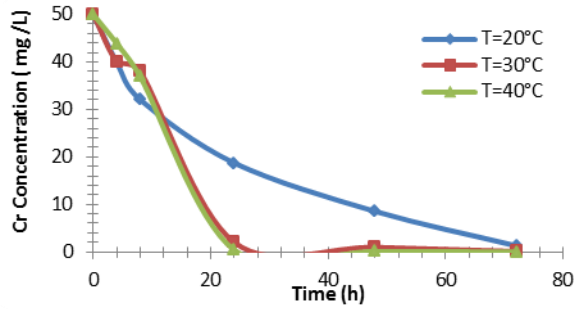

Figure 3. Effect of incubation time on Cr(VI) reduction at temperatures of 20, 30 and 40 °C [conditions: inoculum volume of 5 mL, pH 7, initial Cr(VI) concentration of 50 mg/L]

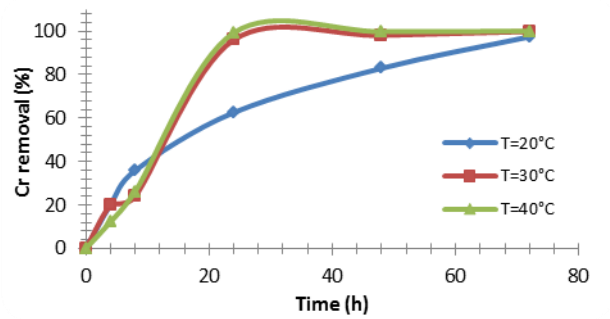

Figure 4. Effect of incubation time on % Cr(VI) reduction at temperatures of 20, 30 and 40 °C [conditions: inoculum volume of 5 mL, pH 7, initial Cr(VI) concentration of 50 mg/L].

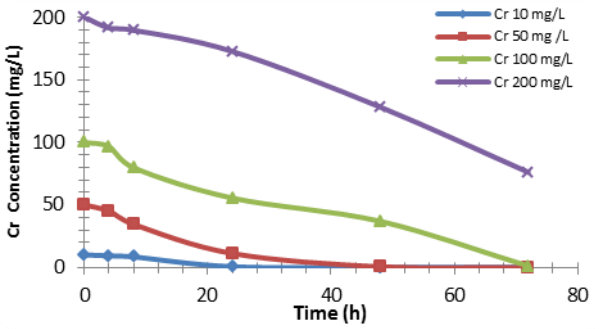

Figure 5. Effect of incubation time on Cr(VI) reduction at initial Cr(VI) concentrations of 10, 50, 100 and 200 mg/L [conditions: inoculum volume of 5 ml, pH 7, T 37 °C].

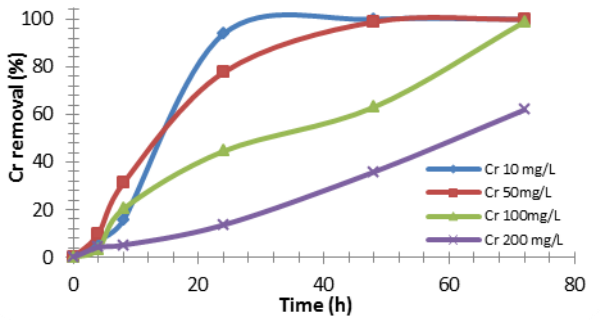

Figure 6. Effect of incubation time on % Cr(VI) reduction at initial Cr(VI) concentrations of 10, 50, 100 and 200 mg/L [conditions: inoculum volume of 5 mL, pH 7, T 37 °C]

3. 4. Effect of Inoculum Volume In the present study, the effect of incubation time on Cr(VI) reduction by the strain *Bacillus cereus* at different inoculum volumes of bacteria (5, 10, 15 and 20 mL) was studied (operation condition was: 50 mg/L initial Cr(VI) concentration, pH 7 and temperature 37 °C). According to the data presented in Figures 7 and 8, by increasing the concentration of bacteria from 5 mL to 20 mL, the rate of hexavalent chromium removal has been increased. Complete reduction of Cr(VI) was observed

at inoculum volume of 5, 10, 15 and 20 mL after 72, 48 and 24 h, respectively. The results showed that in the concentration of 20 mL bacteria, the complete removal of chromium (100%) has been obtained only after 24 h. By reducing the concentration of bacteria to 15 mL the concentration of remaining chromium in the wastewater after 24 h slightly increased into 0.02 mg/L and removal percentage of 99.9±0.1 has been reached. Also, at the inoculum volume of 10 ml and 5 mL of the bacteria, the final concentration and removal percentage of chromium have been reached to 4.2±0.1 mg/L and 8.3±0.3 mg/L, and to 91.6±0.2 and 83.4±0.6% after 24 h. Kathiravan et al. [32] also observed that by increasing the concentration of *Bacillus* sp. from 5 mL to 20 mL, the Cr(VI) reduction rate has been increased and the removal percentage of chromium has increased into 18.3%. Pal and Paul [26] observed that chromate reduction by *Bacillus sphaericus* increased with increasing cell density. Another study also showed that mercury removal rates increased with increasing *Sargassum bevanom* algae dosage at 50 mg/L concentration of mercury because the surface area increased with increase of the adsorbent [34].

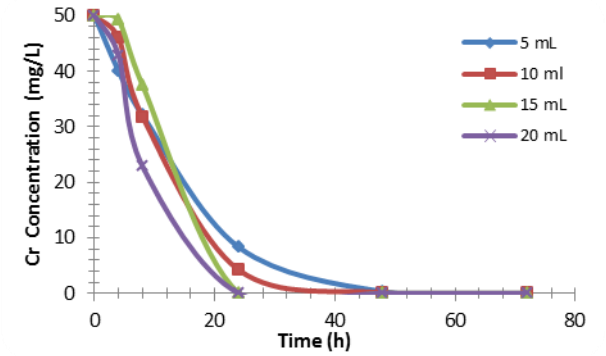

Figure 7. Effect of incubation time on Cr(VI) reduction at inoculum volumes of 5, 10, 15 and 20 mL [conditions: initial Cr(VI) concentration of 50 mg/L, pH 7, T 37 °C].

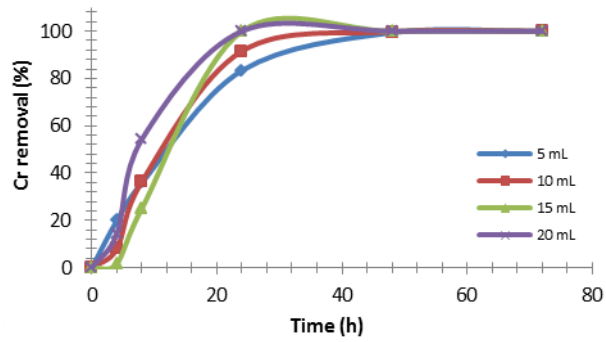

Figure 8. Effect of incubation time on % Cr(VI) reduction at inoculum volumes of 5, 10, 15 and 20 mL [conditions: initial Cr(VI) concentration of 50 mg/L, pH 7, T 37 °C]

3. 5. Kinetics of Cr(VI) Reduction Using linearized form of exponential decay Equation (2) as reported earlier [25], the results of time course reduction of Cr(VI) at different initial chromium concentrations can be analyzed.

$$y = a.e^{-kt} \quad (2)$$

$$\frac{C}{C_0} = a.e^{-kt} \quad (3)$$

$$\ln\frac{C}{C_0} = \ln a - kt \quad (4)$$

where, a is a constant, C_0 and C are the concentrations of Cr(VI) at time 0 and time t, respectively, and k is the rate constant. Thus, k can be calculated from the slope of the plot between ln (C/ C_0) versus time. The data presented in Figure 9 showed the highest rate of reduction at the lowest Cr(VI) concentration (0.104 mg/Lh) and the lowest at the highest Cr(VI) concentration (1.17×10^{-2} mg/Lh).

However, within the tested range of 10–200 mg/L, the change of Cr(VI) concentration indicates that in the same incubation time (72 h), more amount of Cr(VI) was reduced at higher initial Cr(VI) concentration. Similar trend was observed by Das et al. when used *Bacillus amyloliquefaciens* [27]. Pal and Paul [26] observed that the rate of reduction increased with increase in initial Cr(VI) concentration from 10 to 100 mg/L and it was highest (1.42 mg Cr(VI)/L.h) over the initial 24 h of incubation. In another study, the rate of Cr(VI) reduction by *Bacillus* sp. increased with initial Cr(VI) concentrations ranging from 20 to 70 mg/L and decreased from 70 to 100 mg/L, but the rate of Cr(VI) reduction by *Pseudomonas fluorescens* steadily increased with the initial Cr(VI) concentrations over the entire test range of 20-100 mg/L Cr(VI) .

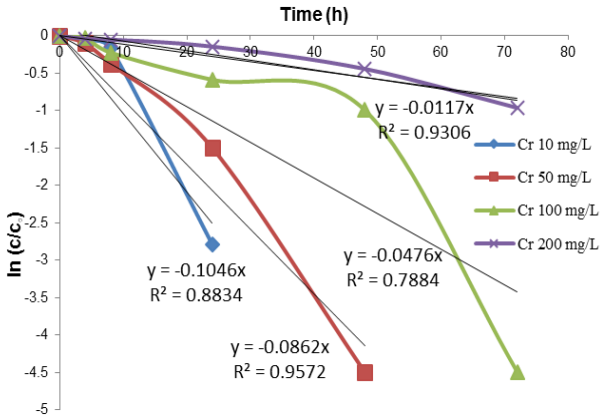

Figure 9. Kinetics of Cr(VI) reduction at different initial Cr(VI) concentrations by *Bacillus cereus*

However, in another study, it was observed that initial Cr(VI) concentration did not affect the chromate reduction rate and the reduction rates were almost equivalent [33].

3. 6. Cr (VI) Reduction under Optimized Conditions A confirmation experiment was conducted using optimized parameters (pH 7, T 40°C, inoculum volume of 5 mL and initial Cr(VI) concentration of 50 mg/L). The results showed (not presented here) that complete reduction of Cr(VI) to Cr(III) by *Bacillus cereus* occurred in 48 h. Das et al. [27] also observed that complete reduction of Cr(VI) (100 mg/L) by *Bacillus amyloliquefaciens* under optimized conditions occurred in 45 h.

The reduction rate of Cr(VI) by *Bacillus cereus* at optimized conditions was found to be 1.04 Cr(VI) mg/L.h, while for *Serratia* sp. the reduction rate was investigated to be 0.28 Cr(VI) mg/L.h [35]. Thus, it is evident that the *Bacillus cereus* is an efficient Cr(VI) reducer.

3. 7. Mechanism of Cr(VI) Reduction *Bacillus cereus* reduced Cr(VI) to Cr(III) completely under optimized conditions (pH 7, T 40 °C, inoculum volume of 5 mL and initial Cr(VI) concentration of 50 mg/L) after 48 h.

Chromium reduction can be attributed to one of the chromate tolerance mechanisms in bacteria such as reduction, methylation, precipitation at the cell surface, blocking cellular uptake by altering the uptake pathway and removal from the cytoplasm by efflux pumps. In general, the mechanisms adopted by different microorganisms for Cr(VI) reduction are variable and are species dependent. In one mechanism, some species use Cr(VI) as the final electron acceptor in the respiratory chain, while some other strains use certain secreted soluble enzymes for reduction of Cr(VI) to Cr(III) [36].

The presence of reduced Cr(III) in the culture supernatant was found to be 49 mg/L after 48 h, as measured by AAS. This finding revealed the presence of almost all the reduced Cr(III) in the supernatant as soluble Cr(III) end product. Evidently, Cr(VI)-reductase in these bacteria is mainly associated with the soluble fraction of the enzyme [37]. As a result, the presence of almost all the reduced Cr(III) in the supernatant revealed Cr(VI)-reductase in *Bacillus cereus* is mainly associated with the soluble fraction of the enzyme.

The above values are very similar with those reported earlier for Cr(VI) reduction by *Arthrobacte*r sp. and *Bacillus* sp [37]. In another research by *Bacillus amyloliquefaciens* a majority of the reduced Cr(III) was immobilized by the bacteria [27]. Niak et al. [28] reported that absorption chromate on the *Bacillus cereus* cell wall takes place through surface functional groups like carboxyl, amide, phosphoryl and hydroxyl.

4. CONCLUSIONS

The present study highlights the application of bacterial strain, *Bacillus cereus*, for detoxification Cr (VI) from wastewater of metal plating industry. It is interesting to see the tolerance capacity of strain against heavy metal toxicity and its ability to detoxify variety of toxic heavy metals like Cr(VI). Various factors such as inoculum volume, initial Cr(VI) concentration, pH and temperature were found to have a profound effect on Cr(VI) reduction and complete reduction of Cr(VI) by *Bacillus cereus* was achieved after 48 h of incubation under optimized conditions of pH 7, inoculum volume of 5 mL, initial chromium concentration of 50 mg/L and temperature of 40 °C. The presence of almost all the reduced Cr(III) in the supernatants revealed Cr(VI)-reductase in *Bacillus cereus* is mainly associated with the soluble fraction of the enzyme.

High Cr(VI) concentration resistance and high Cr(VI) reducing ability of *Bacillus* can be further exploited for the possible utilization of the selected native strain in bioremediation of Cr(VI) in a contaminated environment and synthesis of chromium nanoparticle of industrial and pharmaceutical importance.

5. ACKNOWLEDGEMENT

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Experimental Study on the Factors Affecting Hexavalent Chromium Bioreduction by *Bacillus cereus*

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چکیده

در این کار، مطالعات ناپیوسته به منظور بررسی اثر عوامل محیطی بر میزان نرخ کاهش کروم (VI) از پساب سنتزی صنعت آبکاری فلز توسط باسیلوس سرئوس انجام شد. اثر حجم های مختلف مایه تلقیح (۵، ۱۰، ۱۵ و ۲۰ میلی لیتر)، pH (۵، ۷ و ۹)، درجه حرارت (۲۰، ۳۰ و ٤٠ درجه سانتی گراد) و غلظت های اولیه کروم (VI) (۱۰، ۵۰ ، ۱۰۰ و ۲۰۰ میلی گرم بر لیتر) برای بهترین عملکرد حذف کروم در طول ۷۲ ساعت مورد بررسی قرار گرفت. کاهش کامل کروم (VI) توسط باسیلوس سرئوس بعد از ٤٨ ساعت انکوباسیون تحت شرایط بهینه pH ۹، حجم مایه تلقیح ۵ میلی گرم، غلظت اولیه کروم ۵۰ میلی گرم بر لیتر و درجه حرارت ٤٠ درجه سانتی گراد به دست آمد. نتایج نشان داد که بیشترین نرخ کاهش در کمترین غلظت کروم (VI) (۰/۱۰٤ mg/Lh) بود. آنالیز جذب اتمی در شرایط بهینه نشان داد که غلظت کروم (III) در مایع رویی کشت بعد از ٤٨ ساعت ٤٩ میلی گرم بر لیتر بود. حضور تقریبا تمام کروم (III) کاهش یافته در مایع رویی نشان داد که کروم (VI)–ردوکتاز در باسیلوس سرئوس به طور عمده با بخش محلول آنزیم همراه است. مقاومت در برابر غلظت بالای کروم (VI) و توانایی کاهش بالای کروم (VI) باسیلوس سرئوس، آن را یک گزینه مناسب برای تصفیه زیستی می سازد.

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