Appl Biochem Biotechnol (2010) 160:2000–2013
DOI 10.1007/s12010-009-8716-7

Sonoassisted Microbial Reduction of Chromium

Mathur Nadarajan Kathiravan · Ramalingam Karthick · Naggapan Muthu · Karuppan Muthukumar · Manickam Velan

Received: 21 February 2009 / Accepted: 12 July 2009 /
Published online: 29 July 2009

Abstract This study presents sonoassisted microbial reduction of hexavalent chromium (Cr(VI)) using *Bacillus* sp. isolated from tannery effluent contaminated site. The experiments were carried out with free cells in the presence and absence of ultrasound. The optimum pH and temperature for the reduction of Cr(VI) by *Bacillus* sp. were found to be 7.0 and 37 °C, respectively. The Cr(VI) reduction was significantly influenced by the electron donors and among the various electron donors studied, glucose offered maximum reduction. The ultrasound-irradiated reduction of Cr(VI) with *Bacillus* sp. showed efficient Cr(VI) reduction. The percent reduction was found to increase with an increase in biomass concentration and decrease with an increase in initial concentration. The changes in the functional groups of *Bacillus* sp., before and after chromium reduction were observed with FTIR spectra. Microbial growth was described with Monod and Andrews model and best fit was observed with Andrews model.

Keywords *Bacillus* sp. · Sonolysis · Chromium reduction · Electron donors · Growth kinetics

Nomenclature

C_0	initial chromium concentration (mg/l)
K_S	half saturation constant (mg/l)
K_I	inhibition constant (mg/l)
$[S]$	substrate concentration (mg/l)
S	speed (rpm)
t	time (min)
T	temperature (°C)
μ	specific growth rate (h^{-1})

M. N. Kathiravan · R. Karthick · K. Muthukumar (✉) · M. Velan
Department of Chemical Engineering, A.C. College of Technology, Anna University, Chennai 600 025, India
e-mail: muthukumar@annauniv.edu

N. Muthu
Department of Biotechnology, Holy Cross College, Trichy 620002, India

Introduction

Extensive use of chromium in industries such as leather tanning, metallurgical, electroplating etc., resulted in industrial wastes containing hexavalent chromium (Cr(VI)). Toxic Cr(VI) ions cause physical discomfort and sometimes life-threatening illness including irreversible damage to vital body system [1]. Compared to Cr(VI), Cr(III) is nontoxic and, due to its lower environmental mobility, exhibits limited environmental impact. For this reason, the reduction of Cr(VI) to Cr(III) remains as a primary method for the treatment of chromium containing wastes. The traditional chemical and electrochemical methods used for the reduction are expensive and generate large volume of sludge. Microbial techniques developed to treat chromium-contaminated wastewater were found to be economic and was first demonstrated by Romanenko and Korenkov [2], following that a wide diversity of chromium reducing bacteria (CRB) has been isolated. Prime Cr(VI) reducing microorganisms include *Escherichia*, *Pseudomonas*, *Pantoea*, *Cellulomonas*, *Micrococcus*, *Staphylococcus, Achromobacter* sp. strain Ch1, *Ochrobactrum intermedium* SDCr-5 , *P. agglomerans* SP1, *Sporosarcina ureae*, *Shewanella putrefaciens*, *Leucobacter* sp., and *Exiguobacterium* sp. [3–10]. Conventional methods for reduction of Cr(VI) from industrial wastewater include chemical reduction, ion exchange, electrocoagulation [11], electrochemical reduction [12], photo-reduction [13], bulk liquid membranes process [14], and reduction using iron particles [15]. To improve the chromium reduction efficiency, an improved method, which combines ultrasound and microbial reduction of Cr(VI) is presented. The integration of ultrasonic with biological reactions demonstrated that the sonication increases the mass transfer in aqueous solution [16, 17], which in turn enhances the bioavailability [18, 19]. Gli et al. [20] analyzed the effect of ultrasonic irradiation on the reduction of Hg(II) and achieved a maximum reduction of 94%. The scope of the present investigation was to study the microbial reduction of Cr(VI) with free cells in the presence and absence of ultrasound and to optimize the parameters which influence the Cr(VI) reduction.

Materials and Methods

Microorganism

The microorganism used in this study was isolated from tannery effluent contaminated site in Chennai, India. The soil sample (10% *w*/*v*) was inoculated with nutrient broth containing 100 mg/l of Cr(VI) and incubated at 37°C under controlled conditions. After incubation, 10 ml of the culture was serially diluted (10^{-3} to 10^{-8}). Samples (0.1 ml) were withdrawn from 10^{-5} dilution (in which single colonies were observed) and were then transferred to nutrient agar plates containing Cr(VI). After 2 days of incubation at 37°C, the colonies were screened for their ability to survive in the chromium-amended agar plates. Potential isolates were inoculated with fresh nutrient broth and purified by streak plate technique. The isolate was identified by colony morphology, cell morphology, and biochemical tests and isolated bacterium, identified as *Bacillus* sp., reduced Cr(VI) effectively.

Minimal Inhibitory Concentration

The minimal inhibitory concentration (MIC) of Cr(VI) was determined by inoculating overnight-grown culture of bacterial isolate into freshly prepared agar plates containing different concentrations of Cr(VI) (50 to 600 mg/l) at pH 7.0 and 37°C

and incubating for 48 h. The minimal concentration of metal inhibiting the growth completely was taken as MIC.

Batch Reduction of Cr(VI) with Free Cells

The isolated *Bacillus* sp. was used for Cr(VI) reduction in minimal salt medium(MSM) containing (g/l) glucose—1.0; $Na_2HPO_4{\cdot}2H_2O$—6.0; KH_2PO_4—3.0; NaCl—0.5; $CaCl_2$—0.01; $MgSO_4$—0.246 and various initial concentrations of Cr(VI) ranging from 100 to 500 mg/l. The MSM was inoculated with overnight grown bacterial culture and incubated at optimum conditions. The samples were withdrawn at every 6 h intervals and subjected to Cr(VI) estimation using spectrophotometeric method [21]. The MSM containing appropriate concentration of Cr(VI) without *Bacillus* sp. was used as control.

Effect of Temperature

To study the influence of temperature on Cr(VI) reduction, experiments were carried out with free cells without ultrasound at 30, 32, 35, 37, and 40°C. Minimal salt medium containing 500 mg/l Cr(VI) was inoculated with overnight grown bacterial culture and incubated at desired temperature. Samples were withdrawn at regular time intervals and analyzed for Cr(VI) reduction and the optimum temperature was arrived based on the maximum reduction of Cr(VI) achieved.

Effect of pH

The influence of initial pH on Cr(VI) reduction was studied by varying pH as 5.0, 6.0, 7.0, 8.0, and 9.0 and pH was adjusted using 1 N NaOH (or) 1 N HCl . Overnight grown bacterial culture was inoculated with MSM containing Cr(VI) and incubated at desired pH. The optimum pH was arrived based on the maximum Cr(VI) reduction.

Effect of Electron Donor

The effect of various electron donors/carbon sources on Cr(VI) reduction was estimated by adding glucose, fructose, sucrose, lactose, and sodium acetate with MSM containing Cr(VI) . The influence of electron donors was analyzed based on maximum Cr(VI) reduction.

Effect of Ultrasonic Irradiation on Cell Viability

The effect of ultrasonic irradiation on *Bacillus* sp. viability was determined using pour plate method, heterotrophic (pour) plate method and colony counts [22]. Samples (1 ml) were withdrawn at every 3 min intervals from sonicator and were serially diluted with sterile saline solution. One milliliter from each dilution was then inoculated in agar plates and incubated at 37°C. Colonies were counted after 24 h incubation and the bacterial count was reported as colony-forming units per milliliter of sample. The results are presented in Table 1.

Studies on Ultrasound Mediated Microbial Reduction of Cr(VI)

The pre-cultured overnight-grown *Bacillus* sp. nutrient-rich broth of desired volume having approximately 50 g/l biomass concentration was disrupted in an ultrasonic reactor for 15 min (30 kHz, Sai Sonics, India). Then, 500 ml of solution containing desired

Table 1 Effect of sonolysis time on viability of *Bacillus* sp.

Time	CFU/ml of ultrasound-irradiated *Bacillus* sp.
0	2.6×10^{9}
3	1.6×10^{6}
6	0.92×10^{5}
9	0.65×10^{3}
12	0.21×10^{2}
15	2.10×10^{1}

concentration of Cr(VI) was added into the sonolyzer. The heat-killed *Bacillus* sp. crude was used as control. Reduction efficiency was evaluated with various initial concentrations of Cr(VI) (10–500 mg/l), initial pH (5–9), and volume of broth (10–60 ml). Samples were collected at regular time intervals, centrifuged at 6,000 rpm for 10 min and the supernatant was subjected to Cr(VI) concentration estimation.

Analytical Methods

The samples obtained during the batch reduction with free cells were centrifuged at 6,000 rpm for 20 min at room temperature and the cell pellet obtained was washed with distilled water. The pellet was subsequently dried at 85 °C, and was reported as dry cell weight. The supernatant obtained after centrifugation was subjected to Cr(VI) concentration analysis. The decrease in concentration of Cr(VI) with time was determined through spectrophotometric method using specific colorimetric reagent 1, 5-diphenylcarbazide (DPC) [21]. In a 10-ml test tube, 1 ml of the supernatant sample was mixed with 9 ml of 0.2 M H_2SO_4. Then 0.2 ml of freshly prepared 0.25% (*w*/*v*) DPC in acetone was added. The absorbance was measured by using UV–visible spectrometer at 540 nm against a reagent blank.

FTIR Analysis

The changes in the functional groups of the *Bacillus* sp. before and after Cr(VI) reduction were analyzed and interpreted by FTIR spectroscopy. The spectra were recorded using Fourier transform infrared spectrometer (Tensomax Bruker, Tensor-27) in the range of 400–4,000 cm^{-1} with samples prepared as KBr disks.

Modeling

In case of single-substrate-limited mechanism, Monod model [23] can be used to model the microbial growth and is given as

$$\mu = \frac{1}{X}\frac{\mathrm{d}X}{\mathrm{d}t} = \frac{\mu_{\max} S}{K_S + S} \tag{1}$$

where μ is the specific growth rate (h^{-1}), μ_{max} is the maximum specific growth rate (h^{-1}), S is the limiting substrate concentration (mg/l), K_s is the half saturation constant (mg/l), and X is the biomass concentration (g/l). At higher substrate concentrations, substrate inhibition

occurs and hence, Monod model might not hold well under such conditions. To overcome this problem, the Andrews model [23] is normally used to represent microbial growth.

$$\mu = \frac{\mu_{\max} S}{K_S + S + \frac{S^2}{K_I}} \tag{2}$$

where K_I is the inhibition constant (mg/l).

Results and Discussion

Identification of Microorganism

The colony morphology, cell morphology and biochemical test results of the isolated species are presented in Table 2 and from the results, isolated species was identified as *Bacillus* sp. The isolate showed a minimum inhibitory concentration of 500 mg/l but the values reported in the literature for *Arthobactor* sp. and *Bacillus* sp. isolated from chromium-contaminated sites were around 100–150 mg/l [24, 25].

Cr(VI) Reduction without Ultrasound

Effect of pH

Microbial growth rate and the activity of enzymes were significantly affected by pH. The variation in pH of the medium causes changes in the ionic form of active site and affects the activity of chromium reductase enzyme. Therefore, the effect of initial pH on the reduction of

Table 2 Cell morphology and biochemical test results for the isolate.

Analysis	Results
Gram staining	+
Endospore staining	+
Motility	+
Morphology	Rod
Anaerobic growth	−
Catalase test	+
Indole test	−
Lactose fermentation test	−
Urease	+
Lactose	−
Amylase test	+
Nitrate reduction test	+
Voges–Proskquer	+
Citrate test	+
Growth in 10% NaOH	+
MR/VP test	+/−

+ positive; − negative

Cr(VI) using *Bacillus* sp. was studied and the results are shown in Fig. 1. In the pH range studied, pH 7.0 was found to offer 91.78% of reduction at an initial concentration of 500 mg/l after 78 h of incubation. The percent reduction observed for 5.0, 6.0, 8.0, and 9.0 were 74.87%, 87.18%, 87.9%, and 60.27% respectively for the same conditions. For most of the cases, the optimum pH value reported was in the range of results obtained in the present investigation. Urvasi and Datta [26] reported that the *Ochrobactrum* sp. reduced Cr(VI) in the pH range from 6.0 to 8.0, whereas the maximum growth as well as reduction was reported at pH 7. Wang et al. [27, 28] also reported that the Cr(VI) reduction by *Enterobacter* strain occurred between pH 6.5–8.5 but pH 5 and 9 were found to inhibit very strongly. Slobodkina et al. [25] reported a pH range of 6.0-7.0 for *B. thermoamylovoras SKC1* for the optimum growth and reduction.

Effect of Temperature

The influence of temperature on Cr(VI) reduction ability of *Bacillus* sp. was analyzed by varying temperatures from 30 to 40°C and the results are shown in Fig. 2. The reduction of 91.86% was obtained at 37°C with 500 mg/l of Cr(VI) after 78 h. In the temperature range between 35 and 40°C, the percent reduction was nearly the same but the reduction observed for 30 and 32°C was less and this may be due to the structural changes of chromium reductase enzymes at these temperatures. The optimum temperature observed in the present investigation was in the range reported for bacterial chromium reductase in the literature [29, 30]. Liu et al. [31] reported complete reduction of Cr(VI) at 37°C with 40 mg/l of Cr(VI) after 72 h of incubation. The cell growth is also significantly affected beyond the optimum temperature because the variation in temperature affects the viability of the cells and also lethal. At low temperatures, the fluidity of the membrane decreases sufficiently which prevent the functioning of the transport systems, so the substrates cannot enter into cell rapidly to support even low rate of growth. Increase in temperature affects proteins by causing thermal denaturation, which is usually

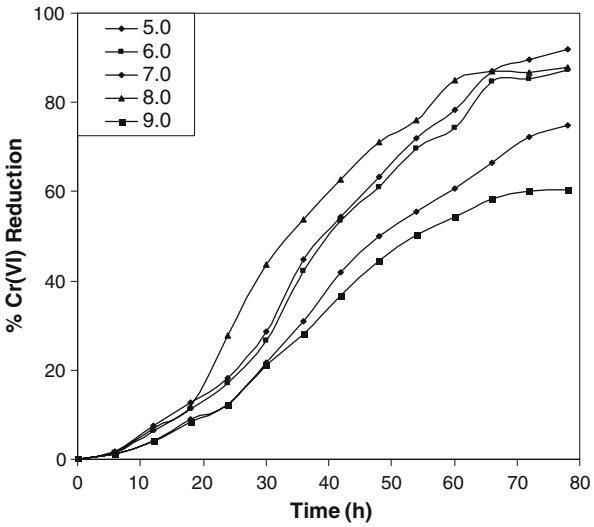

Fig. 1 Effect of pH on % Cr(VI) reduction [conditions, temperature 37°C, C_0=500 mg/l and S=150 rpm]

irreversible. Thermal denaturation of proteins can cause loss of an essential enzyme function, alteration of membrane structure, or inactivation of the protein synthesizing mechanism due to alteration of ribosome conformation. The optimum temperature for Cr (VI) reduction depends mainly on the nature of the species. For example, Sarangi and Krishnan [10] reported that *Exiguobacterium* sp. were optimally active at 35°C but inactive at 45°C. Slobodkina et al [24] reported that the optimum temperature for the reduction of Cr(VI) by *B. thermoamylovoras SKC1* as 50°C.

Effect of Initial Concentration

The effect of initial concentration on Cr(VI) reduction by *Bacillus* sp. was studied over an initial concentration range of 100 to 500 mg/l and the results are shown in Fig. 3. The percent reduction was increased with increase in incubation time and decreased with increase in concentration. Maximum Cr(VI) reduction obtained for 100, 200, 300, 400, and 500 mg/l of initial concentration at 78 h of incubation were 95.2%, 94.1%, 93.2% 92.8%, and 87.8% respectively. The trend observed in the present study was similar to that of reported in the literature [32, 33]. The results of the control experiments showed negligible Cr(VI) reduction.

The influence of initial concentration on biomass concentration was studied and the results are shown in Fig. 4. Higher amount of biomass concentration was observed at an initial concentration of 100 mg/l and increase in initial concentration of Cr(VI) decreased the biomass concentration. The rate of *Bacillus* sp. growth and Cr(VI) reduction were faster during the log phase, because large quantity of active chromium reductase enzymes was produced during that phase.

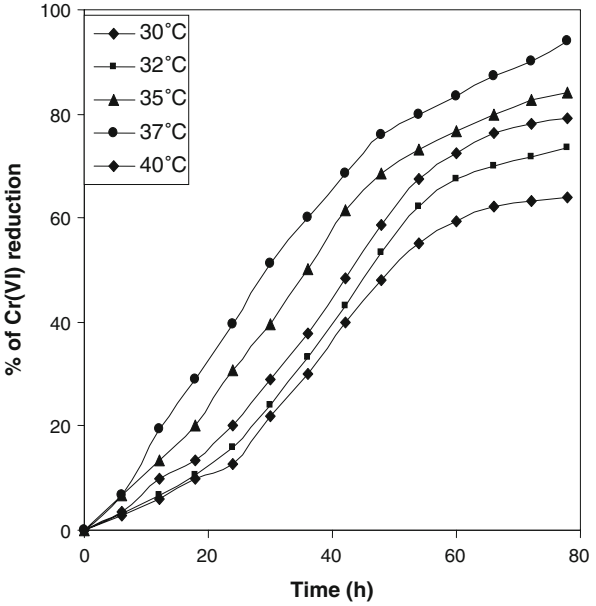

Fig. 2 Effect of temperature on % Cr(VI) reduction [conditions, pH=7, C_0=500 mg/l, and S=150 rpm]

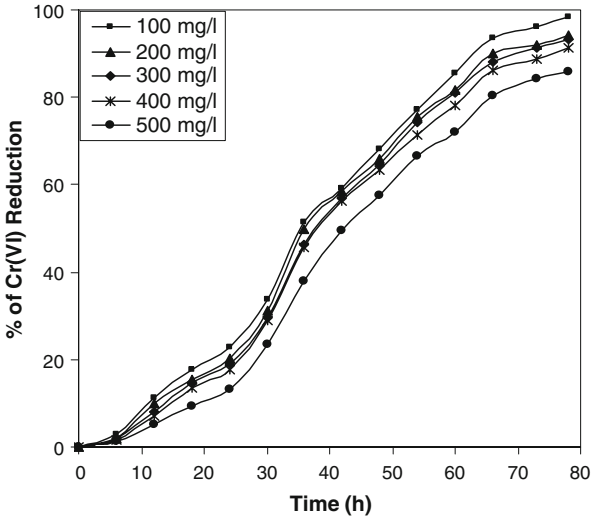

Fig. 3 Effect of initial concentration on % Cr(VI) reduction [conditions, T=37°C, pH=7, and S=150 rpm]

Effect of Electron Donor

Microbial reduction of toxic Cr(VI) to nontoxic Cr(III) is an electron requiring process and hence influence of electron donors such as lactose, glucose, fructose, sucrose, and sodium acetate on chromium reduction was studied. The percent reduction of Cr(VI) was significantly influenced by electron donors/carbon sources and the results obtained are shown in Fig. 5. Among the various electron donors studied, 94% reduction was obtained with glucose. The other electron donors such as

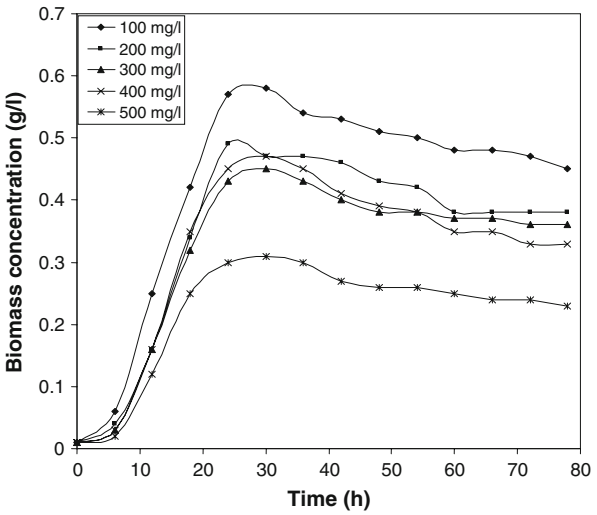

Fig. 4 Effect of initial concentration on bacterial growth [conditions, pH=7, T=37°C, and S=150 rpm]

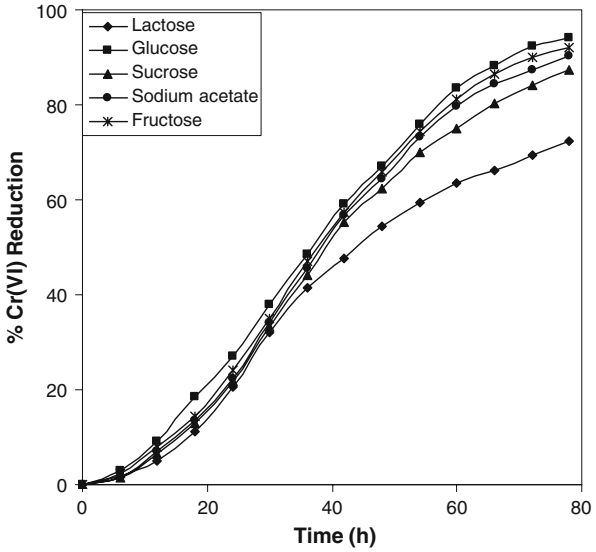

Fig. 5 Effect of electron donor on % Cr(VI) reduction [conditions T=37°C, C_0=500 mg/l, pH=7, and S= 150 rpm]

lactose, sucrose, sodium citrate, and fructose offered 72.4%, 87.3%, 90.4%, and 92% reduction, respectively. Glucose showed maximum reduction because it is catabolized to pyruvate through glycolysis process, then entered the TCA cycle, whereas the other electron donors such as sucrose and lactose are disaccharide molecules which ought to be converted into monosaccharides and are further catabolized. Similar results were observed by Philip et al. [34] for *B. coagulans.* Even though glucose showed maximum efficiency in the present investigation, actually the electron donor/carbon sources utilization is species dependent, and *Bacillus* sp. utilized glucose molecules easily. Mclean and Beveridge [35], reported that *Pseudomonas* sp. reduced Cr(VI) significantly in the presence of lactate and Fulladose et al. [36] reported that the Cr(VI) reduction capacity was increased when media supplemented with glycerol. The complexity of metabolic pathways and the involvement of various enzymes led to lesser reduction in presence of other electron donors except glucose.

Cr(VI) Reduction with Ultrasound

Effect of pH

To study the influence of pH on Cr(VI) reduction with ultrasound, the pH was varied as 5, 6, 7, 8, and 9 and the results shown in Fig. 6. The maximum Cr(VI) reduction was obtained at pH 7.0.

Effect of Broth Volume

The biomass content is an important factor which determines the efficiency of the Cr(VI) reduction by crude chromium reductase in sonolyzer. Approximately 64%, 81%, 96%, and 100% of Cr(VI) reduction were observed at 5, 10, 20, 30, and 40 ml of broth/500 ml of feed

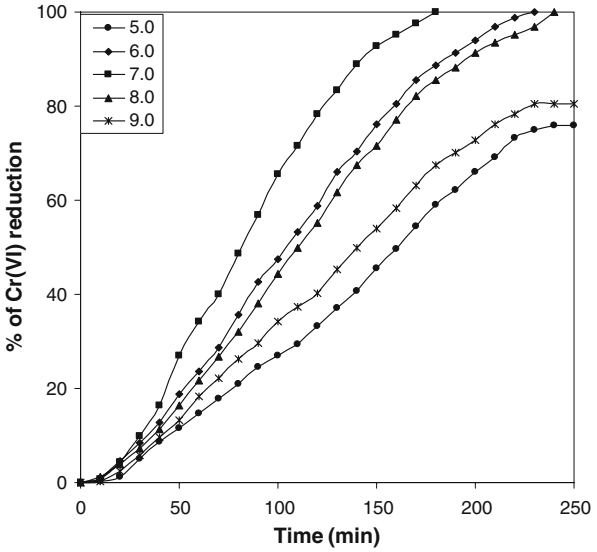

Fig. 6 Effect of pH on ultrasound mediated microbial reduction of Cr(VI) [conditions, 50 ml *Bacillus* sp. culture broth crude, C_0=200 mg/l, T=37°C, frequency=30 kHz, volume=500 ml of Cr(VI)]

respectively. The 100% reduction was obtained for 40, 50, 60 ml at 230, 180,170 min, respectively, and the results are shown in Fig. 7. The highest Cr(VI) reduction was observed with 50 ml of broth/500 ml of 200 mg/l Cr(VI) and further increase did not affect the reduction significantly. Hence, further studies were carried out with 50 ml.

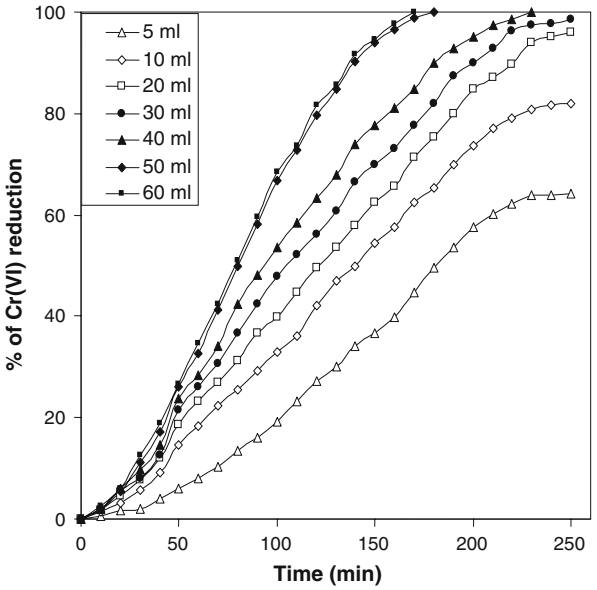

Fig. 7 Effect of volume of broth on ultrasound mediated microbial reduction of Cr(VI) [conditions, C_0= 200 mg/l, T=37°C, frequency=30 kHz, volume=500 ml of Cr(VI)]

Effect of Initial Concentration

The effect of initial concentration on Cr(VI) reduction with ultrasound in the presence of *Bacillus* sp. was studied and the results are shown in Fig. 8. The conditions such as temperature, pH and biomass concentration maintained were 37±2°C, 7.0 and 50 ml/500 ml of Cr(VI) solution, respectively. Complete Cr(VI) reduction was obtained within 180 min in the initial concentration range 10–200 mg/l but 300 mg/l required 260 min. For initial concentrations of 400 and 500 mg/l, the percent reduction obtained were 87.02% and 70.6%, respectively. The decrease in time required for the reduction compared to reduction with free cells in the absence of ultrasound was due to the disruption of *Bacillus* sp. by ultrasonication to release the chromium reductase enzyme, which is responsible for the conversion of Cr(VI) to Cr (III). Since the reductase enzyme had direct contact with the substrate, there are no mass-transfer limitations like in the case of free cells. No Cr(VI) reduction was observed when the heat-killed biomass was used in the sonicator and this confirmed that the reduction was due to the enzymatic reaction.

FTIR Spectral Analysis

The FTIR spectra of the *Bacillus* sp. before and after Cr(VI) reduction without ultrasound were given in Fig. 9. The spectra were obtained in the wavelength range between 400 and 4000 cm^{-1} and compared with each other to find out the functional groups present in the *Bacillus* sp. The peaks appeared at 3,772 cm^{-1}, 3,783 cm^{-1} (strong), and 3,695 cm^{-1} indicated the presence of O–H stretching of carboxyl groups and N–H stretching of secondary amides for 78 h chromium treated *Bacillus* sp. The peaks for non treated *Bacillus* sp. were observed at 3,294 cm^{-1} (bonded hydroxyl group), 1,624 cm^{-1} (C–N stretching and N–H deformation), 1,603 cm^{-1} and 1,624 cm^{-1} (COO– anions and C=C aromatic

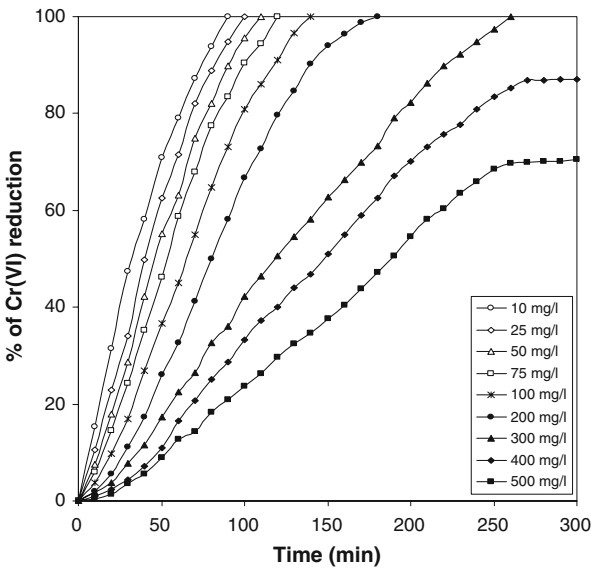

Fig. 8 Effect of initial concentration on ultrasound mediated microbial reduction of Cr(VI) [conditions, 50 ml *Bacillus* sp. culture broth crude, pH=7.0, *T*=37°C, frequency=30 kHz, volume=500 ml of Cr(VI)]

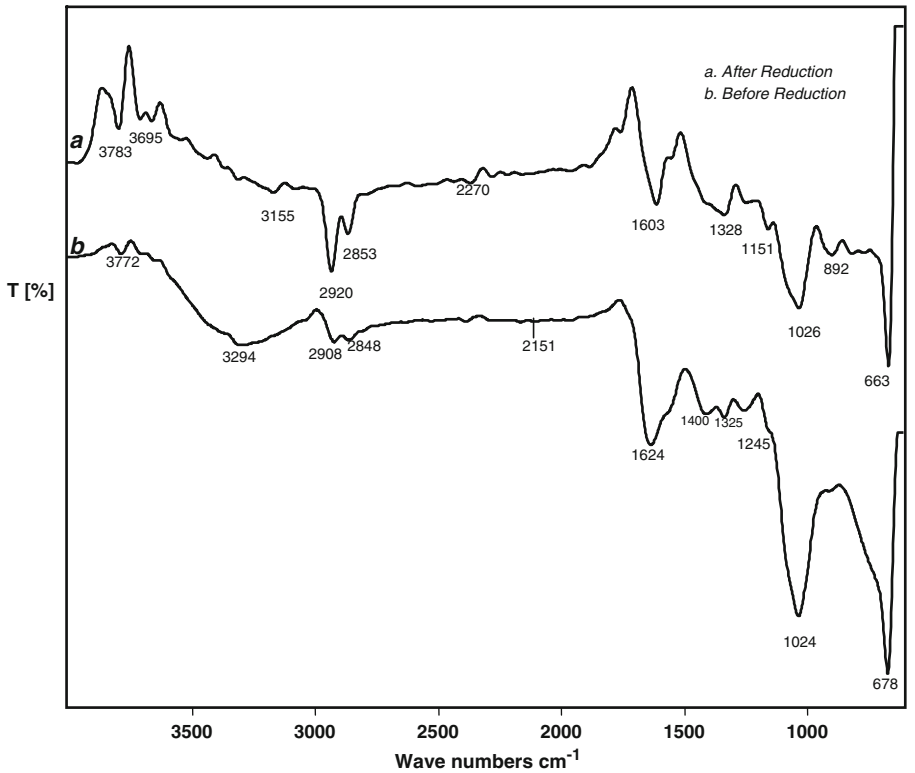

Fig. 9 Functional group comparison of the *Bacillus* sp. before and after 78 h chromium reduction

conjugated), 1,400−1,240 cm^{-1} (C–H stretching vibrations, N–H bending, $–CH_3$ wagging and C–OH stretching vibrations), whereas the sharp peaks appeared at 1,026 cm^{-1}, 1,024 cm^{-1}, and at 662 cm^{-1} represent the C–O stretching and aromatic –CH deformation, C═O bending vibrations, respectively. The FTIR analysis showed slight modification in the functional groups of *Bacillus* sp. during the 78 h of Cr(VI) reduction.

Growth Kinetics

The kinetic parameters of Monod and Andrews models were estimated using the specific growth data obtained at various initial Cr(VI) concentrations. The experimental and theoretically predicted specific growth rates are shown in Fig. 10. It was observed that the Andrews model was found to fit the data well than Monod model. This clearly indicates that the bioconversion of Cr(VI) by *Bacillus* sp. follows substrate inhibition kinetics. The fitted equations for Monod and Andrews models are given below along with their correlation coefficients.

$$\mu = \frac{0.038S}{116.20 + S} \qquad R^2 = 0.92 \tag{3}$$

$$\mu = \frac{0.102S}{399.4 + S + \frac{S^2}{259.9}} \qquad R^2 = 0.97 \tag{4}$$

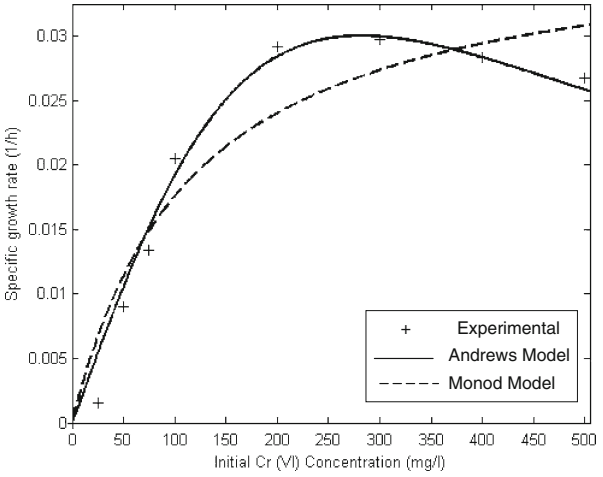

Fig. 10 Monod and Andrews models fitted to the results of batch growth experimental data

Andrews model gave a better correlation coefficient compared to Monod model. Since, the Monod model is a non-inhibitory model and the kinetic constants obtained will not consider the effect of the inhibition. Therefore, the Monod equation fits well with lower concentration whereas at higher concentrations best fit was not observed.

Conclusions

In the present study, the microbial reduction of Cr(VI) using *Bacillus* sp. isolated from chromium-contaminated environment was investigated with and without ultrasound. The ultrasound mediated reduction was found to offer higher efficiency. The effects of operating parameters such as pH, temperature, initial concentration, and carbon sources were studied and the optimum conditions were arrived based on the maximum reduction for free cells and ultrasound mediated reduction. The percent reduction of Cr(VI) decreased with an increase in initial concentration. The optimum pH and temperature for the maximum reduction were found to be 7 and 37 °C, respectively, for free cells without ultrasound. The reduction increased with biomass content and decreased with increase in initial concentration in sonoassisted Cr(VI) reduction. The pH of 7 was found to offer maximum reduction in this case also. The microbial growth kinetics was analyzed with the Monod and Andrews models. The growth data were found to fit well with the Andrews model and therefore, it can be concluded that the bioconversion of Cr(VI) follows substrate inhibition kinetics. The changes in the functional groups of *Bacillus* sp. after chromium reduction were identified using FTIR and the analysis showed slight modification in the functional groups of *Bacillus* sp. after 78 h.

References

1. Malik, A. (2004). *Environment International, 30*, 261–278.
2. Romanenko, V. I., & Korenkov, V. N. (1977). *Mikrobiolgiya, 46*, 414–448.

3. Viamajala, S., Smith, W. A., Sani, R. K., Apel, W. A., Petersen, J. N., Neal, A. L. (2007). *Bioresource Technology, 98*, 612–622.
4. Badar, U., Ahmed, N., Beswick, A. J., Pattanapitpais, P., & Macaskie, L. E. (2000). *Biotechnology Letters, 22*, 829–836.
5. Saxena, D., Levin, R., & Firer, M. A. (2000). *Water Science Technology, 42*, 93–98.
6. Zhu, L., Li, W., & Dong, X. (2003). *International Journal of Systems and Evolution Microbiology, 53*, 1619–1623.
7. Sultan, S., & Hasnain, S. (2007). *Bioresource Technology, 98*, 340–344.
8. Chris, A. F., Anna, Y. O., & Bradley, M. T. (2000). *Applied and Environmental Microbiology, 66*(2), 543–548.
9. Fein, J. B., Fowle, D. A., Cahill, J., Kemner, K., Boyanov, M., & Bunker, B. (2002). *Geomicrobiology Journal, 19*, 369–382.
10. Sarangi, A., & Krishnan, C. (2007). *Bioresource Technology,* . doi:10.1016/j.biortech. 2007.08.059.
11. Heidmann, I., & Calmano, W. (2008). *Separation and Purification Technology, 61*, 15–21.
12. Lakshmipathiraj, P., Bhasar Raju, G., Basariya, R., Parvathy, S., & Prabhakar, S. (2008). *Separation and Purification Technology, 60*, 96–102.
13. Kleber, R. J., & Helr, G. R. (1992). *Environmental Science Technology, 26*, 307–312.
14. Ahmet, O., Safi, S. A., & Sirit, A. (2006). *Journal of Membrane Science, 283*, 448–455.
15. Shao-Feng, N., Yong, L., Xin-hua, X., & Zhang-hua, L. (2005). *Journal of Zhejiang University - Science B, 6*(10), 1022–1027.
16. Thomas, J. M., Yordy, J. R., Amador, J. A., & Alexander, M. (1986). *Applied Environmental Microbiology, 52*, 290–296.
17. Harms, H., & Zehnder, A. J. B. (1995). *Applied Environmental Microbiology, 61*, 27–33.
18. Reynolds, C., & Wills, E. D. (1974). *International Journal Radiation Biology, 25*(2), 113–120.
19. Reid. I. M., & Sikov. M.R., Interaction of Ultrasound and Biological Tissues, Proceedings of Workshop, Washington, 1972
20. Gil, S., Lavilla, I., & Bendicho, C. (2008). *Ultrasonics Sonochemistry, 15*(3), 212–216.
21. Pattanapipitpaisal, P., Brown, N. L., & Macaskie, L. E. (2001). *Applied Microbiology and Biotechnology, 57*, 257–261.
22. APHA, 2005. Standard methods for the examination of water and wastewater. In: Eaton, A.D., Clesceri, L.S., Rice, E.W., Greenberg, A.E., & Franson, M.A.H. (Eds.), 21st ed. American Public Health Association, American Water Works Association, Water Environment Federation, USA (centennial edition)
23. Shuler, M. L., & Kargi, F. (2005). *Bioprocess engineering—basic concepts* (2nd ed., pp. 175–180). New Delhi: Prentice-Hall.
24. Megharaj, M., Avudainayagam, S., & Naidu, R. (2003). *Current Microbiology, 47*, 51–54.
25. Slobodkina, G. B., Bonch-Osmolovskaya, E. A., & Slobodkin, A. I. (2007). *Microbiology, 76*(5), 530–534.
26. Urvasi, T., & Datta, M. (2005). *World Journal of Microbiology Biotechnology, 21*, 891–899.
27. Wang, P. C., Mori, T., Toda, K., & Ohtake, H. (1990). *Journal of Bacteriology, 172*, 1670–1672.
28. Wang, Y. T., & Shen, H. (1995). *Journal of Industrial Microbiology, 14*, 159–163.
29. Camargo, F. A. O., Bento, F. M., Okeke, B. C., & Frankenberger, W. T. (2003). *Journal of Environmental Quality, 32*, 1228–1233.
30. Bae, W. C., Lee, H. K., Choe, Y. C., Jahng, D. J., Lee, S. H., Kim, S. J., et al. (2005). *Journal of Microbiology, 43*, 21–27.
31. Liu, Y., Xu, W., Zeng, G., Li, X., & Gao, H. (2006). *Process Biochemistry, 41*, 1981–1986.
32. Lakshman, R. S., & More, S. (2002). *Minerals Engineering, 15*, 831–837.
33. Deleo, P. C., & Ehrlich, H. L. (1994). *Applied Microbiology Biotechnology, 40*, 756–759.
34. Philip, L., Iyengar, L., & Venkobachar, C. (1998). *Journal of Environmental Engineering ASCE, 124*, 1165–1170.
35. Mclean, J. S., Beveridge, T. J., & Phipps, D. (2000). *Environmental Microbiology, 2*, 611–619.
36. Fulladose, E., Dejardin, V., Murat, J. C., Gourdon, R., & Villaescusa, I. (2006). *Chemosphere, 65*, 644–650.