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Bacterial Reduction of Hexavalent Chromium from Contaminated Overburden Soil

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Abstract This study was focused for the bioreduction of Cr(VI) from chromite mine overburden soil using a highly tolerant *Bacillus* sp. isolated from chromite mine soil. Under the optimized conditions of pH ~7.0, *Bacillus* sp. (4.05 x 10^7 cell mL^{-1}) reduced more than 98% of Cr(VI) from the soil sample in 16 h at 60% pulp density(PD). The exponential rate equation yielded rate constants in the range 4.923×10^{-1} h^{-1} – 2.141×10^{-1} h^{-1} for 20% and 60% PD respectively, which decreased with increase in Cr(VI) concentration. The bio-reduction was also carried out in absence of media at the higher pulp density (60%) as used in the chromite ore beneficiation plant. After bio-reduction the samples were characterized using FT-IR for the leaching of other valuable metals using bio-organic acids.

Keywords Chromite Mine Overburden, Bacillus sp, Cr(VI)

1. Introduction

India is the second largest producer of chromites in the world. Open cast chromite mining activity leads to various environmental problems due to the release of hexavalent chromium (VI). Contamination of soil and water in chromite mining areas is a widespread and serious problem. Orissa state accounts for 98% of the total chromite reserve of the country and the South Kaliapani chromite mine area of Orissa contributes about 97% of the total chromite reserve of the state[1-4]. As a result of growing open cast mining activities in the area, the environment is under threat[3]. The element chromium exists in two stable states, i.e., hexavalent chromium Cr(VI) and trivalent chromium Cr(III). Cr(VI) is the most toxic form of chromium[4] and is largely produced from anthropogenic sources, mining, and various industrial activities. In the hexavalent state, chromium exists as oxospecies such as CrO_3 and CrO_4^{2-} that are strongly oxidizing. Hexavalent chromium may exist in aquatic media as water soluble complex anions and may persist in water. Hexavalent chromium is a strong oxidizing agent and may react with organic matter or other reducing agents to form trivalent chromium[5]. Chromium toxicity results in the inhibition of plant growth and hazard to the living organisms.

The objectives of this study were mainly to investigate the extent of chromium contamination by mine overburden soil in the study area viz. Kaliapani chromite mine, India and bioremediation of hexavalent chromium using native microbial isolates.

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2. Materials and Methods

2.1. Collection and Characterization of Soil Samples

Overburden soil samples were collected in sterile glass bottles from Orissa Mining Corporation (OMC), South-Kaliapani chromie mines of Orissa, India with varying composition, storage time and microbial differences. The soil samples (15-20 cm depth) were collected and stored at 4℃., soil samples were dried and ground to powder for chemical analyses and phase identification. The chemical analyses of soils (before and after reduction) were carried out by conventional method while the mineral phases of chromite overburden were identified from the powder X-ray diffraction (PXRD) patterns (Siemens D500) using CuK_α radiation at a scanning speed of 2°(2θ)/min. The soil and treated samples were analysed for functional groups using FT-IR spectra.

2.2. Revitalization and Adaptation of isolated *Bacillus* Species

Previously isolated and hexavalent chromium reducing strain viz., CSB-4 identified as *Bacillus* sp.[5] was sub-cultured and adapted using real chromium(VI) contaminated overburden soil of OMC, South-Kaliapani chromite mines. Adaptation has been carried out using Luria broth media (containing g L^{-1}, 10 tryptone, 5 yeast extract, 10 NaCl) containing different pulp densities (5 - 60%) of contaminated soil. The flasks were incubated at pH 7.0 and 35℃ while

shaking at 100 rpm for five days. The pH was maintained to 7.0 on daily basis and cell count was monitored to check the growth of the bacteria.

2.3. Effect of pH, Nutrient, Temperature, and Pulp Density on Bacterial Reduction

The bacterial reduction of Cr(VI) was studied in the pH range 2.0 - 9.0 while inoculating 1mL of pre-grown adapted culture in different test flasks (adjusted to the desired pH) containing 10% pulp density (PD) under incubator at 35℃ with a shaking speed of 100 rpm. The effect of temperature was studied in the range 25 - 45℃. The composition of LB medium was also varied (tryptone, sucrose, beef extract and NaCl constituents) at different pulp densities (20 - 60%) to examine the Cr(VI) reduction from the contaminated soil. The data from duplicate sets of each experiment were collected with a variation within ±2% and were averaged to compute the extent of reduction.

2.4. Chemical Analysis

Hexavalent chromium in the overburden soil was leached rigorously with pure distilled water (solids to water mixture ratio 1:10) for 6h using horizontal shaking at rated speed of 100 rpm[5]. Leached water was separated by filtration using Whatman 42 filter paper and filtrate containing almost 99% of total dissolvable Cr(VI) was estimated by diphenylcarbazide method[6,7]. A certain volume of supernatant was taken with 2 mL of 6 N H_2SO_4 and 1 mL of 1,5-diphenyl carbazide (Hi-Media) (0.25%). After 10 min of incubation at room temperature, the absorbance of the resultant solution was measured at 540 nm by UV spectrophotometer against a known standard. All the experimental analysis was carried out using above method.

3. Results and Discussion

As stated above, the samples were collected and stored for their chemical composition and microbial isolation. The *Bacillus* sp. which was isolated from the Cr(VI) contaminated soil of 5-6 year old chromite mine overburden dump has a good potential for hexavalent chromium tolerance while growing in the same soil. The bacterial strain was repeatedly sub-cultured for several times and maintained in Luria broth inoculating with contaminated soil. Due to its high tolerance and growth, this strain was further chosen for chromium(VI) reduction from overburden soil[8-11].

The soil samples were characterized for their chemical composition and metal content by X-ray fluorescence (XRF make Phillips Axion). The XRD phase identification of one of the samples viz., OBS-4 (5-10 year old overburden dump) shows predominantly the presence of goethite phase along with minor quantity of hematite, chromite, and quartz. Small peaks of clay minerals possibly nontronite ($(Ca,Mg)_{0.5}Fe_2(Si,Al)_4O_{10}(OH)_2.xH_2O$) were also observed. The sample with these phases was consistent with its chemical analyses (Cr, 3.43; Fe, 58.624; Al, 4.42; Si, 2.69; Ni, 1.244; Mn, 0.404; Co, 0.177; Ti, 0.116; Mg, 0.154; wt% as the major and Ca, 0.018; Na 0.0659; K, 0.07; V, 0.015; Zn, 0.056; P, 0.012 wt% as the minor constituents). The overburden dump soil contains several metals including chromium as Cr(III) and Cr(VI).

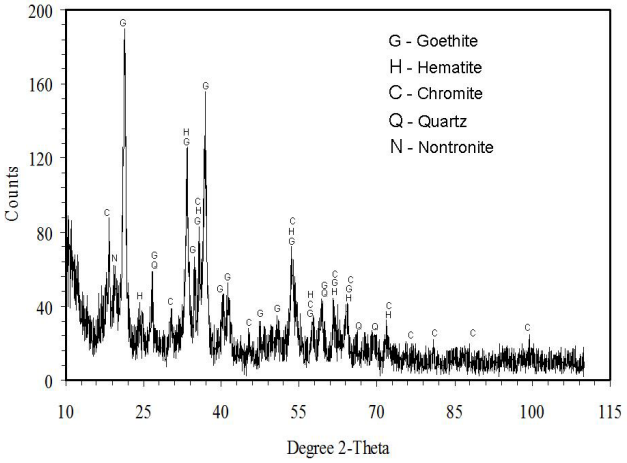

Figure 1. XRD of COS – 4 containing metallic phases

The leaching of hexavalent chromium from the soil depends on the particle size and leaching time (Fig not shown). The leaching of chromium (VI) increases from 390 mg kg^{-1} to 498 mg kg^{-1} with decrease in the particle size from >150 µm to < 100 µm in 6 h of leaching period at 100 rpm. In the overburden soil chromium (VI) is the main water soluble contaminant.

3.1. Effect of Working Parameters on Bioreduction of Cr(VI) of Soil Sample

3.1.1. Effect of pH

The reduction of Cr(VI) from the soil sample (OBS-4) was studied (Fig 2) in different pH range (2.0–9.0). It may be seen that the reduction increases with increase in pH up to 7.0 with 98% chromium(VI) reduction in 6 h, and then decreases on raising the pH[10,11]. The inhibitory effect below pH 5.0 is presumably due to the decrease in cell growth as the observed optimum pH value for cell growth is 6.0–7.0 in the same nutrient medium without Cr(VI). Consequently, further reduction experiments were carried out at pH 7.0.

3.1.2. Effect of Nutrient

As the process is aimed for pilot scale operation, the nutrient media is very important for economic point of view. The experiments were carried out with individual component of the nutrient of Luria broth, sucrose (10 g L^{-1}) and beef extract (5 g L^{-1}), along with the microbe during inoculation with the contaminated soil. The results showed that the most important nutrient was sucrose as 99% of Cr(VI) was reduced in 12h from 500 mg Cr(VI)/kg sample, which was the same as that of LB medium (Fig 3) at 60% pulp density. The lowest chromium(VI) reduction was found in only NaCl containing flask (~ 18%), due to the absence of carbon source to exhilarate the growth of the bacterial strain. It may

be mentioned that if cell growth is improper, the reduction of chromium(VI) is adversely affected[10,11].

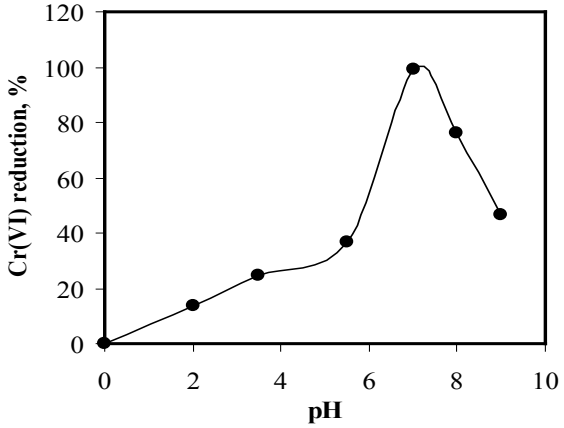

Figure 2. Effect of pH on Cr(VI) reduction from soil (35°C, 500mg Cr(VI)kg^{-1} soil, size <100 µm, 60%PD, time 12h)

3.1.3. Effect of Temperature

In the temperature range studied (25–45℃), the extent of Cr(VI) reduction increased with increase in temperature from 25 to 35℃ (Fig 4). About 93% Cr(VI) reduction was observed at 35℃(308 K) in comparison with only 14% reduction at 25℃(298 K) in 12 h. Okeke *et al*.[12] also reported a similar optimal temperature for growth and Cr(VI) reduction with *Bacillus* sp. isolated from soil enrichment cultures. At 45℃(318 K), 62% of Cr(VI) reduction was achieved in 12 h of incubation. At higher temperature the reduction was lower as compared to that of 35℃ which may be due to the improper cell growth at higher temperature. The growth temperature is thus optimized to be 35℃ for *Bacillus* sp. while growing in LB medium.

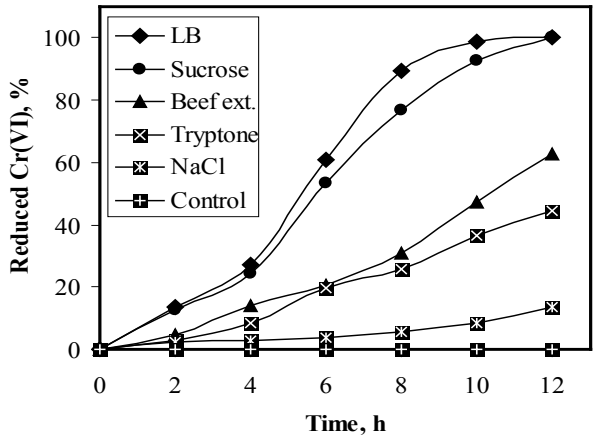

Figure 3. Effect of nutrient on Cr(VI) reduction from soil (35℃, 500mg Cr(VI)kg^{-1} soil, size <100 µm, 60%PD, time 12 h)

3.1.4. Effect of initial Cr(VI) concentration

The reduction of Cr(VI) from the overburden soil was further carried out with the optimized parameters established above at different pulp density and using different concentrations of Cr(VI) and results are presented in Fig 5.

As can be seen reduction was dependent on Cr(VI) concentration. Reduction of Cr(VI) was relatively high in case of 20 and 40% PD and complete reduction (≥ 98%) was observed in 12 and 14h, respectively. The reduction was very fast where the Cr(VI) content was low of 100 and 200 mg L^{-1} at the pulp density of 20 and 40% respectively[13]. But in the case of 60% PD, it consumed two hours extra as compared to that of 40% PD i.e. 16h for complete reduction due to high concentration of chromium (300 mg L^{-1}) and its relative toxicity on the bacterial cells.

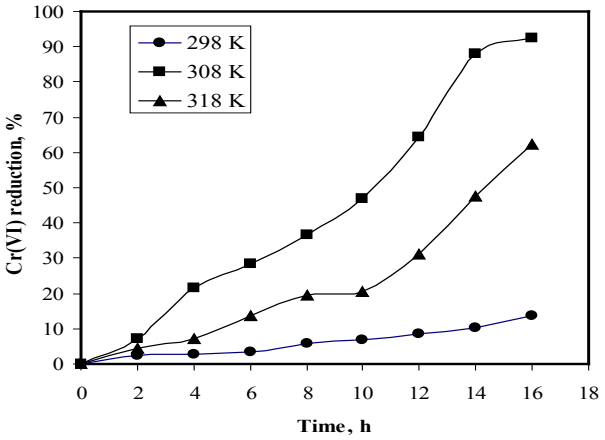

Figure 4. Effect of temperature on chromium (VI) reduction

The results of time course reduction of Cr(VI) at different pulp density (concentration) are calculated using exponential decay equation (1)[14].

$$y=a_e^{-kt} \quad (1)$$

$$C/C_0=a_e^{-kt} \quad (2)$$

Linearised from becomes:

$$ln\{C/C_0\}=lna-kt$$

Where, a is constant, y - C/C_0 - fraction of chromium (VI) reduction at time t, C - concentration of Cr(VI) at time t, C_0 - original Cr(VI) concentration, and k is the rate constant.

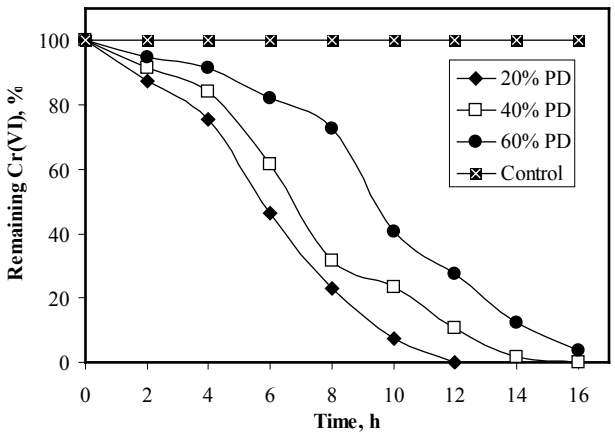

Figure 5. Effect of different pulp density on reduction of Cr(VI) (Temp 35℃, pH ~ 7)

The time course reduction data fitted well (Fig. 6) to linearised form of exponential rate equation ($R^2 \geq 0.98$) and showed highest rate of reduction (0.4923 h^{-1}) at lowest pulp density of 20% (~100 mg L^{-1} Cr(VI)) and the lowest rate

(2.141×10^{-1} h^{-1}) at the highest pulp density (60%) (~300 mg L^{-1} Cr(VI)). These data clearly show the possibility of reducing Cr(VI) present in the soil sample by using the native microbial species. The major achievement of the study is the possibility of chromium bio-reduction at 60% pulp density of, which is generally operated in the plant scale of chromite ore beneficiations plant using $FeSO_4$[14] and plant materials viz. Terminalia chebula[15]. Thisbio-reduction /bio-beneficiation process may be an alternative to the ongoing processes for the beneficiation plants without causing any pollution with waste water containing chromium entering to the natural streams.

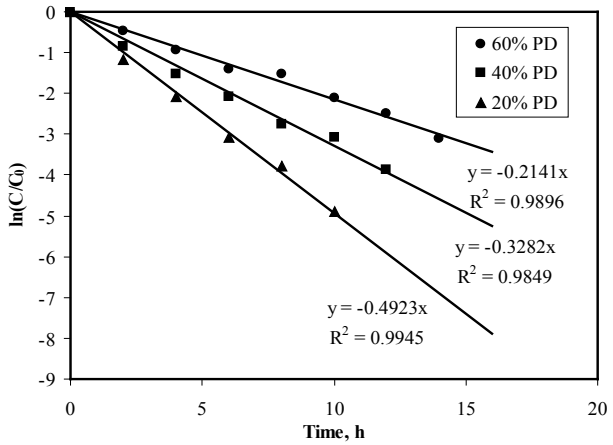

Figure 6. Rate of Cr(VI) reduction for different Cr(VI) levels at 35℃ & 7.0 pH

3.2. Characterization of bio-reduced Sample

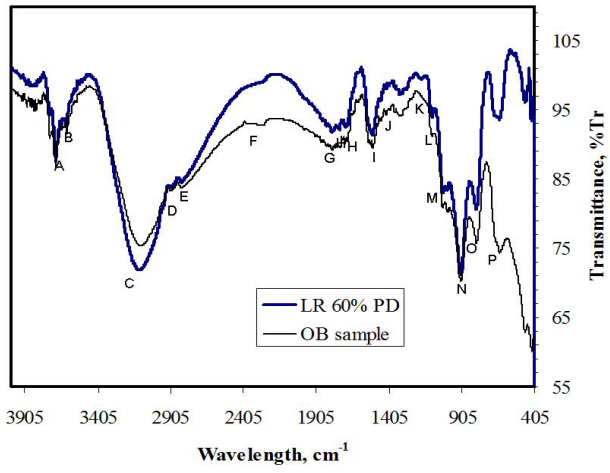

Figure 7. IR spectrum of COS – 4 before and after Cr(VI) reduction

The reduced chromite overburden soil was characterized by their chemical analysis and FT-IR. The FT-IR spectra of overburden soil samples before and after reduction are presented in Fig. 7, which indicates the functional groups attached or detached from the sample after the bacterial reduction of Cr(VI). Some characteristic peaks corresponding to the bond have been noticed due to the bacterial action on soil mineralogy and development of new functional groups on the soil surface. The broad absorptions peak around 3500 – 3650 cm^{-1} is indicative of the existence of the –OH bonded hydroxyl groups and the peak around 2100 – 2250 cm^{-1} represents the PO-H phosphonic acids on the reduced soil sample.

The peaks from 1700 – 1750 and 1750 – 1820 cm^{-1} represent the carboxylic acid group and their derivatives present on the reduced soil samples. The observed functional groups may be compared with the standard values and their respective assignments are presented in Table 1. From the comparison it is clearly emerged that the functional groups may either help in the leaching of valuable metals (Al, Ti, Ni, Zn and Co etc.) thereby creating the mobility from the overburden and or it has immobilize these metals. Further research needs to be carried out to understand the mobility of the Cr(VI) remediated soil to ensure for safe dumping.

Table 1. Functional groups on chromite overburden soil (COB) before and after Cr(VI) reduction

IR label names	Observed wavelength range (cm^{-1})	Standard wavelength range (cm^{-1})	Group assignments
A	3580 – 3630	3000 – 3650	O – H bonded hydroxyl groups (OH)
B	3590 – 3610		O – H group
C	3150 – 3140	3150 – 2800	C – H stretching
D	2930 – 2900	3000 – 2850	(O=) PO – H phosphonic acid
E	2860 – 2850		
F	2407 – 2288	2440 – 2280	C≡C group, P –H phosphine bending
G	1720 – 1705	1714 – 1710	C = O (H bonded) Carboxylic acid & derivatives
H	1820 – 1750	1809 – 1777	C = O & O – C (anhydride)
I	1650 – 1640	1670 – 1640	Primary & secondary amines & amides (N – H bending)
J	1380 – 1370	1375 – 1300	Sulfanide bonds (S = O)
K	1317.63	1350 – 1000	Amines (C – N stretching)
L	1260 – 1230	1300 – 1000	C – O stretching of COOH
M	1160 – 1150	1350 – 1000	Amines (C – N stretching)
	1040 – 1030		
N	900 – 850 & 785 – 750	990 – 690	C – H bendings
O	770 – 650		O – H bending (Out of the plane)
P	540 – 500		Weak S.S diluted sulfide

4. Conclusions

The *Bacillus* sp. isolated from the overburden soil sample is found to be fairly effective for reducing chromium(VI) from the chromium contaminated overburden soils. At optimal pH 7.0 and temperature 35℃, ~ 98% of[60%, PD; 500 mg Cr(VI) kg^{-1} of soil ~ 300 mg Cr(VI) L^{-1}] chromium (VI) is reduced in about 16 h of incubation. The rate of

reduction decreases with time, especially at the higher pulp density with higher initial Cr(VI) concentration due to Cr(VI) toxicity on the bacterial cell. Fitting of time course reduction data to the exponential rate equation ($C/C_0=a_e^{-kt}$) yields rate constants in the range 4.923×10^{-1} h^{-1} - 2.141×10^{-1} h^{-1} for 20% and 60% PD respectively. This strain can further be explored for reduction of Cr(VI) of chromite concentrates as COB plant operations are generally carried out at a 60% pulp density with a much lower chromium(VI) concentration. The adaptation of *Bacillus* sp. is going on for large scale experimental studies. The reduction of hexavalent chromium from the contaminated overburden soil has also been planned using different nutrient deficient condition for the large scale experiments. The carbon source, sucrose has same reducing ability as that of the supplied Luria broth. After chromium reduction the samples can be characterized for mobility of the metals which includes immobilization or leaching of metals with fungal species and thereby ensuring safe dumping.

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