Removal of Chromium (VI) by *Bacillus subtilis* Isolated from East Calcutta Wetlands, West Bengal, India

Srabanti Basu, Monikankana Dasgupta, and Bhaswati Chakraborty

***Abstract*—Chromium (VI), one of the major pollutants released from tanneries, dye and textile industries, is highly toxic and carcinogenic in nature. Chemical methods for bulk treatment of industrial effluents often fail to reduce the level to meet the environmental regulations. For end of the pipe treatment, bioremediation is considered a better alternative. East Calcutta Wetlands, the major sewage treatment site of Kolkata (previously known as Calcutta), has been reported to be contaminated with several heavy metals including chromium (VI). Therefore, there is a possibility that bacterial population of this region can tolerate chromium (VI) and would be useful for bioremediation of chromium (VI). A strain of *Bacillus subtilis* isolated from this region was grown in presence of chromium (VI) (2.5 μg/L–7.5 5 μg/L). There were 97% and 90% reduction of residual chromium concentration in growth media after 24 hours with initial concentrations of 2.5 μg/L and 5 μg/L respectively. Best removal was observed at 30°C. Growth of the *Bacillus* strain in presence of chromium (VI) was found to be best fit for Tessier model by non-linear regression analysis using MATLAB® 7.4. The *Bacillus* strain has the potential for the end of the pipe treatment removal of chromium (VI).**

***Index Terms*—Bioremediation, chromium, East Calcutta Wetlands, kinetic modeling.**

I. Introduction

Chromium (VI) is one of the most hazardous pollutants released from industries like textile dyeing, chemicals and pigment production, wood preservation, tanning and electroplating. [1]. Chromium exists in several oxidation states ranging from -2 to +6, among which chromium (IV) and chromium (III) are the most significant because of their persistence and stability. Chromium (VI) finds its place in the priority list prepared by the Agency of Toxic Substances and Diseases Registry (ATSDR). Chromium compounds can lead to mutation and cancer, and inhibit enzymes and nucleic acid synthesis. In contrast, chromium (III) is less toxic and less mobile [2]. Chemical methods are available for removal of chromium in bulk from industrial effluent but they often fail to meet the environmental regulations. Therefore polishing steps are or end of the pipe treatment is required.

Bioremediation is recommended as a better alternative to chemical treatment for this purpose as the chemical agents add to the environmental pollution. Studies have reported potential of certain species of bacteria like *Pseudomonas*, *Bacillus* and *Arthrobacter* for bioremediation of chromium [3]-[5].

In contaminated sites, chromium availability is influenced by processes like complex formation, oxidation-reduction, precipitation, which in turn depend on microbial activities. However, exposure to chromium for a long time can reduce microbial diversity, population and activity. Many bacterial species surviving in presence of chromium for years in contaminated sites are found to be highly resistant to chromium and are considered important for removal of chromium [6].

East Calcutta Wetlands, the major site for sewage treatment and resource recovery of the metropolitan city Kolkata (previously known as Calcutta), has been found to be contaminated with heavy metals like chromium, lead and mercury [7], [8]. The site receives domestic waste as well as industrial waste, particularly wastes generated by tanneries situated at the outskirts of the city. The Kolkata Municipality generates 600 million L of wastewater which flows through underground sewers to the pumping stations at the eastern fringes of the city and is then pumped into open channels. The wastewater is received by 286 sewage-fed fisheries and local farms for resource recovery. The wetland has been incorporated in the list maintained by the Ramsar Buraeu established under the Ramsar Convention and is recognized as a 'Wetland of International Importance' [9]-[11].

The present study attempts to check the potential of a strain of *Bacillus subtilis* isolated from East Calcutta Wetlands for bioremediation of chromium (VI). The bacterial strain, initially tested for biodegradation of phenolic compounds, was tested for its efficacy for removal of chromium.

II. Methods and Materials

A. Maintenance of the Bacteria in Laboratory

The strain of *Bacillus subtilis* isolated from the soil sample collected from East Calcutta Wetlands was maintained in laboratory in mineral salt agar containing 1% glucose as the sole carbon source. Composition of the media (for 1L) is KH_2PO_4: 0.68g, K_2HPO_4: 1.73g, $FeSO_4$: 0.03g, NH_4NO_3: 0.1g, $MgSO_4$: 0.10g, $CaCl_2$: 0.02g, $MnSO_4$: 0.03 g, pH maintained at 7.0. 24-hour old culture grown in mineral broth containing 1% glucose was used as inoculum for further studies.

B. Assay of Chromium and Determination of Biomass

Chromium concentration was determined by spectrophotometric analysis using diphenylcarbazide

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following standard protocol prescribed by APHA [12]. The reaction is specific for chromium (VI) and can be applied for estimation of chromium in the µg/L level. Bacterial biomass was quantified taking absorbance at 600 nm using spectrophotometer [13].

C. Preliminary Study for Removal of Chromium

The bacterial strain was grown in mineral salt broth having glucose as the sole carbon source in presence of chromium at 30°C. Residual chromium concentration was estimated after 24 hours.

D. Kinetic Study for Removal of Chromium and Bacterial Growth in Batch Mode

For kinetic study for removal of chromium, the isolate was grown in mineral salt broth having glucose as the sole carbon source in presence of chromium. Process parameters like volume of inoculum, initial concentration of chromium (VI) and pH were varied in a prescribed manner. Residual chromium (VI) was estimated at different time intervals. Bacterial growth was measured under the same experimental conditions.

E. Modeling the Kinetics of the Culture Growth in Presence of Chromium

The model equations for kinetic modeling were solved by non-linear regression method using MATLAB®7.4.

III. Results and Discussion

The bacterial strain removed 97%, 89% and 55% chromium (VI) from medium in 24 hours starting with the initial concentration of 2.5 mg/L, 5 mg/L and 7.5 mg/L respectively (Table 1). Percentage removal of chromium (VI) was similar to the observation made by Mahmood et al who reported 100% removal of chromium (VI) by *Pseudomonas putida* and *Serratia proteamaculans* starting with an initial concentration of 2 mg/L. Both the strains were isolated from tannery waste [14]. Results of the present study for chromium removal indicate that the isolate could tolerate chromium (VI) and further studies could be carried out with the isolate for removal of chromium (VI).

TABLE I: Reduction in Chromium (VI) Concentration in Media by the Isolate after 24 Hours (5% v/v Inoculum, 30°C, pH 7.0, Volume of Media 20 ml)

Initial concentration of chromium (VI) (mg / L)	Final concentration of chromium (VI) (mg / L)	Percent reduction
2.5	0.058	97.68
5	0.52	89.6
7.5	3.362	55

Less growth of bacteria was observed in 24 hours in presence of chromium (VI) using 5% (v/v) inoculum, at pH 7, and 30°C (Fig. 1). The lag phase was found to be considerably increased in presence of chromium (VI) since the cells were not initially acclimatized to chromium (VI). However, the change is similar for different concentrations of chromium (VI) ranging from 2.5–7.5 mg/L. Growth rate was also reduced with introduction of larger volume (10% and 15% v/v) of inoculum. Growth rate was also inhibited both at 20°C and 40°C (data not shown).

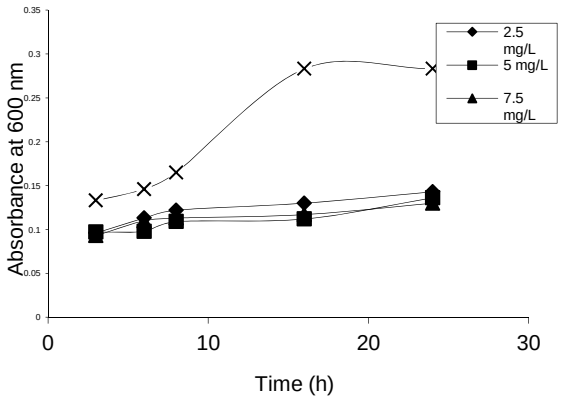

Fig. 1. Growth of bacteria in presence of different initial concentrations of chromium (VI) (5% v/v inoculum, temperature 30°C, pH 7.0, total volume 20 ml).

Percentage removal was decreased with increasing chromium concentration (Fig. 2). This is due to the fact that as the volume of inoculum was constant relatively less biomass was available for chromium (VI) removal from the media, in case of higher concentrations. The result is similar to the observation of Wang and Xiao [15] who reported lower chromium degradation with higher initial concentrations by a *Bacillus* sp. Isolated by them. DeLeo and Erlich have also reported higher reduction of chromium (VI) for lower initial concentrations by *Pseudomonas fluorescence* in batch culture. The organisms reduced 61%, 69% and 99.7% chromium (VI) respectively for initial concentrations of 314, 200 and 112.5 mg/L [17].

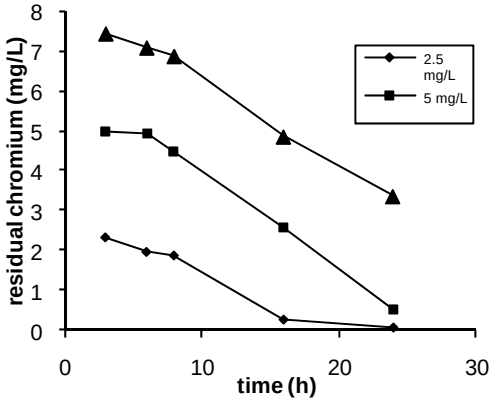

Fig. 2. Removal of chromium from growth media with different initial concentrations (5% v/v inoculum, temperature 30°C, pH 7.0, total volume 20 ml).

Introduction of larger volume of inoculum in media did not affect removal of chromium (VI) (Fig. 3). Moreover, amount of chromium (VI) removed from the medium after 24 hours is more with 5% v/v inoculum than with 10% and 15% inoculum. This may be due to the fact that higher number of bacterial cells reduces the probability of contact between bacteria cells and chromium. Similar observation was made by another group of researchers who reported higher biotransformation of chromium (VI) to chromium (II) at lower cell density [16].

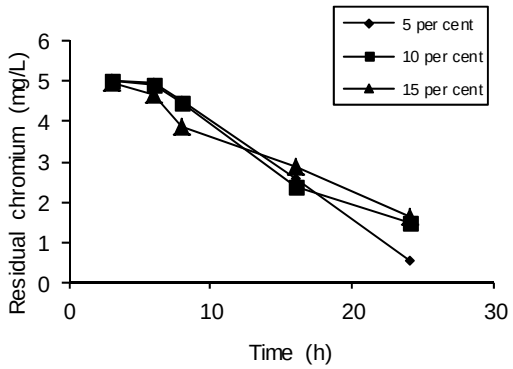

Fig. 3. Removal of chromium from growth media with different inoculum sizes (5 mg/L initial concentration, temperature 30°C, pH 7.0, total volume 20 ml).

Chromium (VI) removal by *Bacillus subtilis* was found to depend on temperature. There was negligible chromium removal at 20°C. Chromium (VI) removal was maximum at 30°C. When temperature was increased to 40°C, chromium (VI) removal was initially increased, but there was insignificant removal after 16 hours. From the results, it may be inferred that adsorption plays a role, at least partly, for removal of chromium. After 16 hours when the biomass is highly loaded with chromium (VI), desorption may take place at higher temperature.

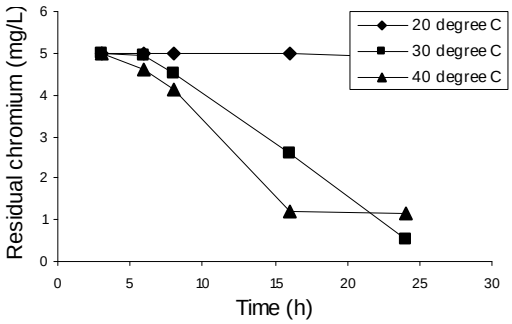

Fig. 4. Removal of chromium from growth media at different temperatures (5 mg/L initial concentration, 5% v/v inoculum size, pH 7.0, total volume 20 ml).

A. Modeling the Kinetics of the Bacterial Growth in Presence of Chromium (VI)

Different growth models used to describe the growth of microorganisms in absence of substrate inhibition are as follows. The earliest is the Monod model (equation 1) followed by Blackman model (equation 2), Tesssier model (equation 3) and Moser model (equation 4)

$$\mu = \mu_m S /(K_S + S) \quad (1)$$

$$\mu = \mu_m S/ 2Ks, \text{ if } S < 2Ks \quad (2)$$

$$\mu = \mu_m (1\text{-}e^{-KS}) \quad (3)$$

$$\mu = \mu_m S^n /(K_S + S^n) \quad (4)$$

The experimental values of specific growth rate μ were plotted against chromium (VI) concentration in the media. The equations were solved by nonlinear regression analysis was using MATLAB® 7.4. Fig. 5 showed the fit of these models to the experimental data. From the MATLAB® 7.4 analysis, it could be stated that the Tessier model best fitted the experimental data. It showed the least RMSE value of 0.0022574. The maximum growth rate (μ_m) values and saturation constant (*Ks*) values obtained from the kinetic modeling analysis given in Table II. The μ_m and *Ks* values calculated from the Tessier model were 0.026 h^{-1} and 2.6 mg/L respectively.

Activation energy (E) and Arrhenius constant (A) were calculated from Arrhenius equation. Arrhenius equation is given by:

$$\mu = Ae^{-E/RT} \quad (5)$$

The activation energy for the growth of cells in presence of chromium (VI) was 17467.39 J $kmole^{-1}$. Arrhenius constant for the growth reaction is 19.259.

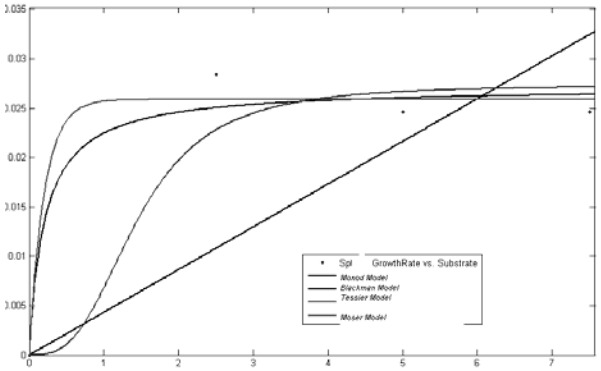

Fig. 4. Kinetic models of the bacterial growth in presence of chromium as solved by MATLAB® 7.4.

TABLE II: VALUE OF KINETIC PARAMETERS OF THE BACTERIAL GROWTH IN PRESENCE OF CHROMIUM (VI)

Model	μ_{max} (h^{-1})	K_2 (mg/L)	n	RMSE
Monod	0.02711	0.2055	…	0.00287
Blackman	0.0171	1.974	-	0.01379
Tessier	0.026	2.6	-	0.00222
Moser	0.02734	3.009	3	0.00647

IV. CONCLUSION

The strain of *Bacillus subtilis* isolated from East Calcutta Wetlands removed chromium from growth medium under laboratory condition. About 90% removal was observed in 24 hours with initial concentration of 5 mg/L. The strain, which was found to degrade phenolic compounds in previous studies, has a potential for end of the pipe treatment of industrial waste for removal of chromium.

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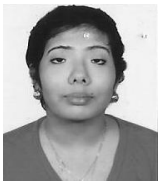

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