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Bioremediation growth kinetics of Cr(VI) contaminated aqueous solution using *Pseudomonas sp.*

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Abstract. Bioremediation of metal wastes using enriched mixed consortium of microbial species is a very promising and viable alternative in environmental biotechnology. Present study deals with the bioremediation of Cr(VI) contaminated aqueous solution using indigenous microorganisms Pseudomonas sp. (GenBank Accession Number: JF911384.1), isolated from activated sludge. The kinetic studies were carried out for initial Cr(VI) concentrations ranging from 10-90 mg L-1. The maximum consumption of Cr(VI) was achieved after 40 hrs at the solution pH 7.0. Various growth kinetic models were fitted with the obtained experimental data. The obtained results for different growth kinetic models indicate that the growth kinetics of Pseudomonas sp. for bioremediation of Cr(VI) can be better understood by Luong model (R2 = 0.913).

Keywords: Pseudomonas sp., Activated sludge, Bioremediation, Luong model.

1. Introduction

Heavy metal contaminants exist in aqueous waste streams of many industries, such as mining, metal processing, tanneries, pharmaceuticals, pesticides and organic chemicals. Usually chromium, cadmium, mercury, lead and arsenic are always in focus and considered as hazardous to human and other living creatures. Most of these heavy metals which are relatively abundant in the Earth's crust and frequently used in industrial processes or agriculture are toxic. These can make significant alterations to the biochemical cycles of living things. During the last two decades a lot of importance has been given to manage industrial pollution and regulate the usage of toxic heavy metals [1]. Among these heavy metal ions, chromium is considered to be most toxic and carcinogenic. It is listed as class A human carcinogen by the US Environmental Protection Agency (U.S. EPA) [2]. Chromium exists in the environment in various forms such as trivalent Cr (III) and hexavalent Cr(VI), of which Cr(VI) is a potential soil, surface water and ground water contaminant [3]. High Cr(VI) concentration causes health problems to human beings because of its high toxicity and carcinogenicity [4]. According to the U.S. EPA, the allowable concentration of Cr(VI) in drinking water is 0.05 mg L-1 [2].

For the last few decades, various physico-chemical methods (adsorption, chemical precipitation, ion-exchange, chemical reduction etc.) have been used for removing Cr(VI) [2, 5, 6, 7]. However, large-scale applications of these methods are highly energy exhausting and utilize huge amounts of reagents in addition of their high cost. As a result, present research is more focused towards the cost effective technologies such as biological based separation. Bioremediation of Cr(VI),is gathering attention as it is more efficient, economical and environmentally friendly. In bioremediation, living microorganisms are used to break down

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toxic and hazardous compounds to simple and less toxic compounds. Various studies have been carried out using microorganisms including bacteria, algae, fungi and yeasts to remove Cr(VI) from synthetic and industrial waste water [8, 9, 10]. Pure and acclimated mixed culture of microorganisms are used to reduce Cr(VI) to Cr(III) in contaminated water. Recent studies shows that the strains isolated from contaminated soil water have excellent capability of removing Cr(VI) from both aqueous solution and waste-water. Enterobacter cloacae and Klebsiella spp. isolated from contaminated soil have shown resistance to high concentrations of Cr(VI) in the growth media and could remove approximately 85% Cr(VI) during growth [11]. The present study deals with the removal of Cr(VI) from synthetic aqueous solution using an indigenous strain Pseudomonas sp., isolated from activated sludge. The kinetic studies were conducted for initial Cr(VI) concentrations ranging from 10-90 mg L-1. Various growth kinetic models were fitted with the obtained experimental data.

2. Materials and methods

2.1. Preparation of reagents and media

Analytical grade reagents and media were used in the present study. A 1000 mg L-1 aqueous stock solution of Cr(VI) was prepared by dissolving specific amount of anhydrous K2Cr2O7 salt in distilled water, and the solution volume was made up to 1000 mL. This solution was used to prepare different concentrations of Cr(VI) (such as 10, 30, 50, 70 and 90 mg L-1) for the present study. The minimal salt media (MSM) was prepared by dissolving six different mineral salts in distilled water. The composition of MSM was (in g L-1): K2HPO4-0.8, KH2PO4-0.2, CaSO4 ·2H2O-0.05, MgSO4 ·7H2O-0.5, (NH4)2SO4-1.0 and FeSO4-0.01. The media were sterilized by autoclaving at 15 kPa at 120oC for 30 min. Stock glucose solution of 10,000 mg L-1 was prepared by dissolving 10 g of D-glucose in 100 mL distilled water. The nutrient agar media used for microbial growth was prepared by dissolving 5 g of peptone, 1.5 g of beef extract, 1.5 g of yeast extract, 15 g of agar and 5 g of sodium chloride in 1 L of distilled water [16]. The pH of MSM and nutrient agar media were maintained at 7 ± 0.2 by using 1 M HCl or 1 M NaOH.

2.2. Microorganism culture condition

An aerobic mixed microbial culture in the form of sludge was obtained from the Municipal Sewage Treatment Plant of Birla Institute of Technology & Science (BITS), Pilani. The sludge was then mixed thoroughly with distilled water. It was allowed to settle for 3 h in order to separate the dissolved impurities present in the supernatant. The sludge was retained as it contains the microbial culture. Ten grams of sludge was taken and again thoroughly mixed with 100 mL of distilled water in a beaker. The shaking was carried out gently, and then the sludge was allowed to settle for a short time (1 min) in order to screen out the settled impurities. Then 2 mL of the supernatant was taken and placed in a centrifuge tube. The centrifugation was carried out for 2 min at 10,000 rpm at 4 ℃ in a centrifuge (Remi Cooling Centrifuge, India). A clear pellet obtained after centrifugation was taken out with a loop. It was used for the enrichment study. A control, keeping all conditions same without the addition of microbial culture, was employed to quantify the abiotic reduction of Cr(VI) in all the experiments.

2.3. Enrichment procedure

A loop full of sludge obtained after centrifugation was added to 100 mL of autoclaved MSM. Enrichment of the culture was carried out over a period of 6 days by decreasing the amount of glucose from 1000 to 0 mg L-1 with a decrement of 200 mg L-1 each day and increasing the Cr(VI) concentration from 2 mg L-1 to 20 mg L-1. Each day, the solution was kept in a Biological Oxygen Demand (BOD) incubator and shaker (Macro Scientific Works, India) at 37oC and at 100 rpm for 24 h. The final acclimated culture was obtained with only 20 mg L-1 of Cr(VI) which was then used for the isolation study.

2.4. Isolation and identification of bacterial strain

For isolation of Cr(VI) resistant bacteria, One milliliter of enriched culture was spread on PYE medium (peptone and yeast extract) agar plates containing 100 μg of Cr(VI) mL-1 supplemented to the medium. PYE medium agar plates were prepared in a 250 mL flasks by dissolving 1 g NaCl, 1 g peptone and 0.5 g yeast extract in 100 mL distilled water. pH of the solution was adjusted at 7. The solution is then mixed with 1.5 g

of agar and autoclaved at 15 kPa at 120oC for 30 min. The growth of the microorganisms was observed after 24 h of incubation at 37oC. Single isolated colonies were picked up with sterilized wire loop and then streaked on PYE medium agar plate. The plates were incubated at 37oC for 24 h. This process is repeated with higher concentrations of Cr(VI). Colonies that grew in Cr(VI) rich environment were considered as Cr(VI) resistant and selected for further study. The identification of isolated strain was carried out at Genei, Merck Millipore Laboratory, India.

2.5. Bioremediation of hexavalent chromium

Batch experiments were carried out to determine the effect of time on the bioremediation of Cr(VI) at different initial concentrations of Cr(VI) ranging from 10 - 90 mg L-1 in 250 mL flask. In these experiments, 100 mL of MSM was autoclaved and added with 2 mL of isolated bacterial strain obtained from the enrichment procedure. A known amount of stock Cr(VI) solution was also added to maintain the required concentrations. The samples were kept in a BOD incubator and shaker which was maintained at 37oC and at 100 rpm. The samples were collected at regular intervals of time based on visual observation (turbidity). In the above study, final samples were analyzed to find out biomass concentration and the amount of Cr(VI) consumed from the solution.

2.6. Analytical techniques

Residual hexavalent chromium in the solution was measured spectrophotometrically at 540 nm by reaction with diphenyl carbazide in acidic conditions [12]. The results were also confirmed using Atomic Absorption Spectrophotometer (Shimadzu, Japan). The optical densities of the microbial cultures were determined by using UV-Vis spectrophotometer (Thermo Scientific, India).

2.7. Determination of kinetic parameters

The kinetics of Cr(VI) remediation is related to the specific growth rate of microorganisms. The growth rate of the microorganisms at a given time in the log phase is proportional to the number of microorganisms present at that time. The experimental data of log phase obtained for biomass concentration during kinetic studies were used to estimate the specific growth rate (μ) by Eq. 1:

$$\mu = \frac{1}{x}\frac{dx}{dt} \tag{1}$$

where x is the biomass concentration (g L^{-1}) at time t (h), and dt is the change in time (h). Integrating Eq. 1 results in Eq. 2:

$$\ln x = \ln x_o + \mu t \tag{2}$$

where x_o is the biomass concentration (g L^{-1}) at t = 0. A plot of ln x vs t gives a straight line with $ln\ x_o$ as its intercept and μ as the slope. The obtained values of specific growth rates in batch bioremediation study were used to determine the model parameters in various growth kinetic models (Monod model, Haldane model and Luong model).

2.8. Growth kinetic modeling

The log phase experimental data of kinetic studies were used to determine the specific growth rate (μ) values of isolated strain at different initial concentrations of Cr(VI). The values of μ for different initial concentrations of Cr(VI) were determined by plotting the ln$x$ versus time (t) data. Various growth kinetic models used in the present study are given in Eqs. 3-5

Monod model: $$\mu = \mu_m \frac{S}{K_s + S} \tag{3}$$

Haldane model: $$\mu = \frac{\mu_m S}{K_s + S + \left(\frac{S^2}{K_I}\right)} \tag{4}$$

Luong model: $$\mu = \frac{\mu_m S}{K_S + S}\left(1 - \frac{S}{S_m}\right)^n \quad (5)$$

where μ_m is the maximum specific growth rate (h^{-1}), S is the substrate concentration (mg L^{-1}), K_s is the substrate affinity constant (mg L^{-1}), K_I is the substrate inhibition constant (mg L^{-1}), n is a positive constant in the Luong model.

3. Results and discussions

3.1 Identification of bacterial strain

Based on nucleotide homology and phylogenetic analysis, the isolated strain was identified as Pseudomonas sp. (GenBank Accession Number: JF911384.1).

3.2 Effect of time on initial concentration of Cr(VI)

The consumption of Cr(VI) by the isolated bacterial strain for various initial concentrations of Cr(VI) is shown in Fig. 1 against the time. The time required for the consumption of Cr(VI) was found as 20, 22, 28, 36.5, and 40 h for 10, 30, 50, 70 and 90 mg L-1 of initial concentration of Cr(VI) respectively. Fig. 1 indicates that the consumption of Cr(VI) was highest (80.2 mg L-) for 90 mg L-1 initial concentration of Cr(VI).

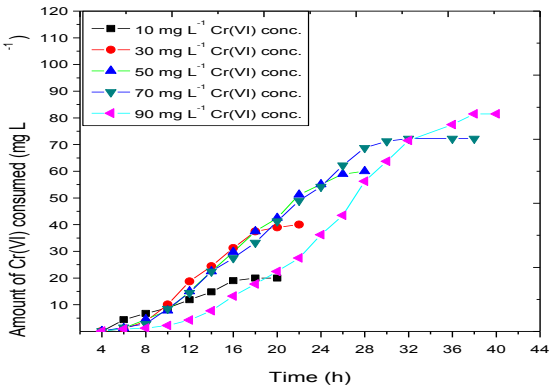

Fig. 1: Amount of Cr(VI) consumed at different time for various initial Cr(VI) concentrations.

Fig. 2 shows the biomass concentration (g L-1) profile of the isolated strain against time for different values of initial Cr(VI) concentrations. The maximum biomass concentrations were obtained as 0.178, 0.215, 0.223, 0.252 and 0.253 g L-1 for initial Cr(VI) concentrations of 10, 30, 50, 70 and 90 mg L-1 respectively. The growth curve for Cr(VI) was categorized in phases such as lag, log, stationary and death phase. Initially, significant increment in the biomass concentration was not observed with time which results in lag phase. This might be due to the fact that microbes take some time for adaptation to a new environment. In the next phase, namely the log phase, the biomass concentration was increased exponentially. In this phase, most of the substrate, Cr(VI), was utilized by the microbes for their growth. It was also observed that there was no increase in biomass concentration, which gives the stationary phase of the growth curve. After the stationary phase, the biomass concentration was ceased which finally leads to the death phase.

3.3 Growth modeling

The values of μ for different initial concentrations of Cr(VI) are determined using the log phase experimental data of kinetic studies. Various biodegradation growth kinetic models such as the Monod model, Haldane model, and Luong model were fitted with the obtained bioremediation experimental data using Origin 8.0 software package (Fig. 3). Values of various growth kinetic parameters are determined. Monod model does not fit the obtained experimental data adequately (Monod model: R2 = 0.839) as self-

inhibition effects during the bioremediation process at higher initial concentrations of Cr(VI) are not considered in this model. The specific growth rate is decreased at high initial Cr(VI) concentration which is an indication of self-inhibition of Cr(VI). The obtained smaller values of Ks (52.201) using the Monod model also indicates the self-inhibition of Cr(VI). Haldane and Luong models consider inhibition effects of the substrate during the growth of microorganisms. Higher value of coefficient of determination (R2 = 0.913) shows that the Luong model fits the experimental growth data better than the Monod model, and Haldane model.

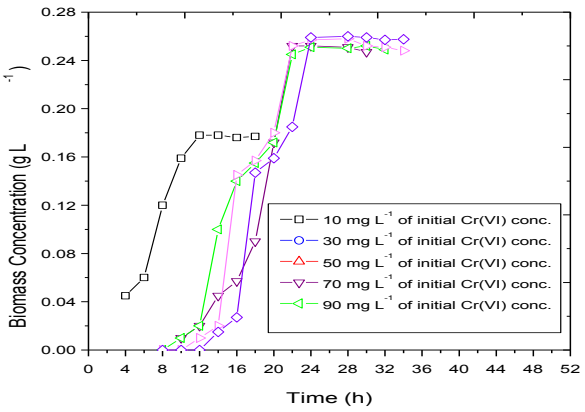

Fig. 2: Change in biomass concentration with respect to time for initial concentrations of Cr(VI).

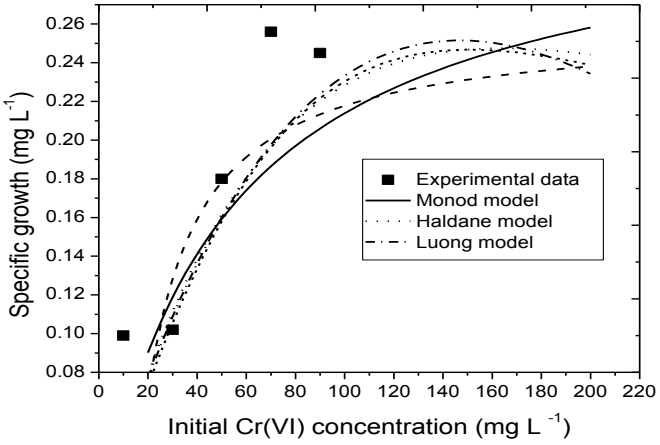

Fig. 3: Experimental and predicted specific growth rate values for different growth kinetics models at different initial Cr(VI) concentrations.

4. Conclusions

An indigenous bacterial strain capable of remediating Cr(VI) was isolated from activated sludge and identified as Pseudomonas sp. It was used for bioremediation of Cr(VI) from aqueous solution. Maximum Cr(VI) consumption was found to be 80.2 mg L-1 for an initial Cr(VI) concentration of 90 mg L-1 at a solution pH of 7.0 after 40 h. The obtained bioremediation experimental results were better understood by the Luong growth kinetic model (R2 = 0.913).

5. Acknowledgements

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