Biodegradation of Hexavalent Chromium by Acclimatized *Pseudomonas Putida*: Optimization and Kinetic Study

Ravi Kumar Sonwani*, Prarabdh Jain, Balendu Shekhar Giri, Ram Sharan Singh, Birendra Nath Rai

Department of Chemical Engineering & Technology, Indian Institute of Technology (BHU), Varanasi-221005, Uttar Pradesh, India

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ABSTRACT

Hexavalent Chromium (Cr (VI)) is considered as priority pollutants to human health and aquatic life. The USEPA has set the maximum permissible limit of 0.05 mg/L of Cr (VI) in domestic uses of water. In the present work, the acclimatized culture of *Pseudomonas putida* (MTCC 1072) was used for the degradation Cr (VI) in wastewater. The process variables such as pH, temperature, and process time were optimized and found to be 7.0 ± 0.2 , 35 ± 2 °C, and 8.0 days, respectively in the batch reactor. The optimized process condition was used to scale-up the batch process at laboratory scale. The maximum elimination of 86.4% was obtained at the optimum condition. The kinetics of biodegradation were studied using Monod and Andrew-Haldane model.

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1. Introduction

The industrial effluents contain Cr (VI) which is one of the most toxic heavy metal. It is carcinogenic, mutagenic, and concern to the environmentalist [Pandey et al., 2016]. It has an adverse effect on human health and aquatic life. Industrial processes like leather tanning, electroplating, steel production, textile industries and chromate preparation discharge effluent that contains chromium ions [Sultan and Hasnain 2017; Sukla et al., 2012]. Chromium is the major industrial pollutant found in the wastewater and it exists in nature in their valence states from -2 to +6 in solution [Shukla et al., 2012]. According to World Health Organization and Environmental Protection Agency of the USA, the maximum permissible limit of Cr (VI) for domestic use of water is 0.05 mg/L [Ge et al., 2015; Kathiravan et al., 2010]. Based on the literature survey, various chemical and physical methods have been used to the remediation of Cr (VI) from wastewater. The method namely: chemical reduction, ion exchange, reverse osmosis, adsorption, and electro dialysis are efficient methods for the removal of Cr (VI) [Qin et al., 2005; Gheju and Balcu 2011; Kongsricharoern et al., 1996; Mukherjee et al., 2016; Thacker et al., 2006]. But these methods generate secondary pollutant and require high operating cost [Zahoor and Rehman 2005]. To overcome these demerits, bioremediation can be used for removal of pollutant, as it is cost-effective and an environment-friendly technique [Yadav et al., 2014; Srivastva et al., 2016; Chung et al., 2003]. Nandi et al. [2015] and Balamurugan et al. [2015] studied the degradation of hexavalent chromium in wastewater using *Pseudomonas putida* and *Bacillus subtilis* as microbial

species. Similarly, Ge et al. [2015] used two isolated strains of bacteria from chromium contaminated soil and observed that species were able to remove Cr (VI) in wastewater.

To the best of our knowledge, very few works are reported on the kinetic study of Cr (VI) removal. Keeping this fact in mind, the aim of present work was to optimize the process parameter along with kinetic study using Monod and Andrew-Haldane model. In the present study, the acclimatized culture of *Pseudomonas putida* was used for the biodegradation of Cr (VI) in wastewater.

2. Materials and Methods

2.1 Chemical and culture medium

An analytical grade (>99% purity) potassium dichromate was acquired from Sigma-Aldrich for the preparation of Cr (VI) stock solution. The bacterial strain (*Pseudomonas putida* (MTCC 1072)) was procured from the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India. The acclimatization of bacterial culture was performed in mineral salt medium (MSM) which was added with 25 mg/L of Cr (VI). This process was repeated thrice by gradually increasing the Cr (VI) concentration. The composition of MSM (g/L) was; KH_2PO_4 : 1.5 (g/L), Na_2HPO_4 : 1.5 (g/L), NaCl : 2 (g/L), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$: 0.2 (g/L), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$: 0.3 (g/L), and yeast extract: 2.0 (g/L). Initially, the stock solution was filtered through a 0.22 μm filtration unit to sterilize the solution.

* Corresponding Author: raviks.rs.che16@itbhu.ac.in

2.2 Batch Study

The batch experiment was performed in Erlenmeyer flasks containing sterilized MSM, $K_2Cr_2O_7$, and inoculated with 2.0 mL *Pseudomonas putida* culture. The flasks were kept at 37 °C and 150 rpm in a rotary incubator (NSW 256) for ten days. The scale-up process was performed in a cylindrical borosilicate reactor of 1424 mL (Fig. 1). The treated samples were taken at regular interval for the analysis of residual Cr (VI). The samples were centrifuged at 8000 rpm for 15 min and the supernatant was used to measure the Cr (VI) concentration using 1, 5-diphenylcarbazide (DPC) method at 540 nm [APHA, 2005].

Fig. 1 Schematic diagram of the experimental set up for biodegradation of hexavalent chromium

The pellets of centrifuged samples were re-suspended in the equal amount of double distilled water (DW) and the absorbance of bacterial samples was analyzed by UV-Vis spectrophotometer (ELICO SL 210) at 600 nm [Apte et al., 2005]. The removal (%) of hexavalent chromium was measured by the equation given below:

$$\% \text{ Removal} = \left(\frac{C_i - C_f}{C_i} \right) \times 100 \quad (1)$$

where C_i is the initial concentration of Cr (VI) solution (mg/L) and C_f is the final concentration of Cr (VI) solution (mg/L).

2.3 Kinetic study

The kinetics of Cr (VI) degradation is related to the growth rate of microorganisms. Monod model was used for the calculation of the specific growth rate. The experimental data of log phase was used to analyze the biokinetic parameters by eq.(2)

$$\mu = \frac{1}{X} \frac{dX}{dt} = \frac{\mu_{\max} S}{K_s + S} \quad (2)$$

where, X_0 is the initial concentration of biomass (mg/L); X is the concentration of biomass (mg/L) at time t (day); μ is the specific growth rate (day^{-1}); $\mu_{\max}$ is the maximum specific growth rate (day^{-1}); S is the concentration of substrate (mg/L).

When the concentration of the substrate was increased, the Monod model was irrelevant due to substrate inhibition. The substrate inhibition was well studied by Andrew-Haldane Model using eq.(3)

$$\mu = \frac{\mu_{\max} S}{K_s + S + \frac{S^2}{K_i}} \quad (3)$$

where K_i is the substrate inhibition constant (mg/L).

3. Results and Discussion

The effective degradation of the substrate during the biological process depends on the process variables such as pH, temperature, substrate concentration, and process time.

3.1 Effect of process time

The effect of process time on Cr (VI) removal has been represented in Fig. 2. The removal (%) of Cr (VI) was perceived in two phases. In the initial phase, the removal was slow due to the lag phase while the second the phase of fast degradation. The low rate of degradation may be due to the saturation of binding sites [Singh et al., 2013]. As shown in Fig. 2, by increasing the process time, the removal increased up to 16.2% in two days. Further increased in time, the removal of Cr (VI) was increased up to 8th days. The maximum removal efficiency was observed to be 76.8% on the 8th day of the process and above this, no increment was found.

Fig. 2 Effect of process time on the biodegradation of Cr (VI).

3.2 Effect of pH

The pH of the solution was varied from 5 to 10. It can be seen in Fig. 3, by increasing the pH at constant time and temperature, the removal increased up to 7.0 pH and on further increasing pH up to 10, the removal was decreased. The maximum Cr (VI) removal was obtained to be 76.7% at 7.0 pH. Poornima et al. [2010] reported the optimum reduction of Cr (VI) was obtained at the optimum pH of 7.0. The minimum removal was observed to be 58.7% and 59.4% at pH 5.0 and 7.0, respectively. The soluble NADH dependent hexavalent chromium reducing enzyme have optimum activity at pH 7.0 (Thacker et al., 2006). These results agree with the previous work done by the various researcher [Batool et al., 2012].

Fig. 3 Effect of pH on the biodegradation of Cr (VI).

3.3 Effect of Temperature

The temperature of the solution is one of the most important variable that affects the biological activity. The temperature of the process was varied between 25-45 °C. It can be seen in Fig. 4, by increasing the temperature, the response was increased up to 35 °C and the further increase in the temperature, the removal of Cr (VI) decreased. The maximum removal efficiency was observed to be 81.3% at 35 °C (Fig. 4). The obtained result was similar to Polti et al. [2014], which reported that the maximum removal was obtained at 35 °C. Thus, the growth of the bacterial species was inhibit due to loss of metabolic activity [Thacker et al., 2006; Batool et al 2012].

Fig. 4 Effect of temperature on the biodegradation of Cr (VI)

3.4 Batch study in bioreactor

The optimized process conditions (pH 7.0, temperature 35.0°C, and process time 8.0 days) was used to scale-up the batch process. For this, a cylindrical borosilicate reactor of volume 1424 mL (1000 mL of working volume) was fabricated and operated at the optimized condition. Fig. 5, represents the biodegradation of Cr (VI) in scale-up bioreactor at the optimized condition. Initially, the rate of Cr (VI) removal (%) was slow which further increased with process time. It can be seen in Fig. 5, the amount of Cr (VI) was decreased with increasing process time. The equilibrium of Cr (VI) removal was observed in 8.0 days. The maximum removal efficiency was observed to be 86.4%. The obtained results are comparable with the outcomes of previous researches [Polti et al., 2014].

Fig. 5 Biodegradation study of Cr (VI) in scale-up bioreactor at optimized condition (pH 7.0, temperature 35.0 °C, and process time 8.0 days)

3.5 Kinetic Study

The cell growth rate (in terms of OD) with respect to time was evaluated at varying concentration of hexavalent chromium from 10 to 100 mg/L. Initially, the lag phase were observed which followed by exponential and stationary phase. The exponential phase data was used to evaluate the kinetics study. The growth kinetics of *Pseudomonas putida* during Cr (VI) reduction was described by Monod model. The model was used to find out the kinetic parameters for free cell between 10- 50 mg/L concentration. The details of kinetic parameters have been presented in Table 1. The value of specific growth rate (μ_{max}) and half saturation rate constant (K_s) was observed to be 0.154 (day^{-1}) and 22.53 (mg/L), respectively (Fig. 6A). Further, as the concentration of substrate increased, the Model model was not well fitted. This may be due to substrate inhibition observed at higher concentration. Under substrate inhibition condition, Andrew-Haldane model was used to predict the kinetic parameters (Geed et al., 2017). The value of specific growth rate (μ_{max}) half saturation rate constant (K_s), and inhibition constant (K_i) were found to be 0.047 (day^{-1}), 23.51 (mg/L), and 42.31 (mg/L), respectively (Fig. 6B). The value of the coefficient of determination (R^2) was observed to be 0.98 and 0.96 for Monod and Andrew-Haldane model, respectively which is a good agreement for model fitting (Kureel et al., 2017). The small value of K_s and the large value of μ_{max} are preferable during the biodegradation process. it is desirable to use ratio (μ_{max}/K_s) as a convenient index to express the biodegradation potential of microbes for particular substrate [Sonwani et al., 2018; Geed et al., 2017; Kureel et al., 2017]. In this study, the values of μ_{max}/K_s were found to be 0.0068 and 0.0105 L/mg.day for Monod and Andrew-Haldane model, respectively. The obtained results reveal that acclimatized *Pseudomonas putida* is effective for the removal of Cr (VI) from wastewater. The kinetic parameter can't be compared with previous works due to unavailability of kinetic data.

Table 1: Kinetic parameters evaluated by Monod growth kinetics and Andrews-Haldane inhibition model

Substrate concentration (mg/L)	μ_{max} (day^{-1})	K_s (mg/L)	K_i (mg/L)	μ_{max}/K_s	R^2
10-50 (Monod)	0.154	22.53	-	0.0068	0.98
10-100 (Andrews-Haldane)	0.247	23.51	42.34	0.0105	0.96

Fig. 6 Experimental data fitted with (A) Monod model and (B) Andrew Haldane model.

4. Conclusion

In the present study, biodegradation of Cr (VI) has been studied by *Pseudomonas putida*. The process parameters such as pH, temperature, and process time were optimized and found to be 7.0 ± 0.2 , $35 \pm 2^\circ\text{C}$, and 8.0 days, respectively in the batch reactor. The maximum removal of Cr (VI) was found to be 86.4% at the optimized condition in the bioreactor. The biokinetics parameters were evaluated by Monod and Andrew-Haldane model. These biokinetic parameters were observed to be $\mu_{\max}$: 0.154 day^{-1} ; K_s : 22.35 mg/L by Monod while $\mu_{\max}$: 0.247 day^{-1} ; K_s : 23.51 mg/L ; 42.34 mg/L by Andrew – Haldane. This study would be helpful in the designing of the bioreactor and practical application of *Pseudomonas putida* culture for the removal of Cr (VI) in the wastewater.

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