

## Batch Studies on Biosorption of Chromium from Waste Water using Bacterial Cultures

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**Abstract**—Biosorption can be an effective technique for the treatment of heavy metal bearing waste water resulting from humans and industrial activities. Several gram positive and gram negative bacteria have the ability to remove the heavy metals and there by making water contaminant free. It has been reported that attenuated bacterial biomass have greater biosorption capability than viable cells. In the present study, the biosorption of heavy metals using individual and mixed culture of attenuated bacteria (gram positive and gram negative) like *Bacillus subtilis* and *Pseudomonas aeruginosa* and parameters affecting the biosorption of heavy metals; such as time, pH, biomass concentration and initial metal concentration have been investigated. The batch experiments have been carried out using individual and mixed bacterial culture and the biosorption parameters were optimized using univariate procedures. The biosorption of Chromium (VI) showed that 77.6% for mixed cultures, 60.5 and 81.3 for *Pseudomonas aeruginosa* and *Bacillus subtilis* respectively. The optimum biomass concentration was found to be 1.5, 1.5 and 2 for mixed cultures (*Pseudomonas aeruginosa* and *Bacillus subtilis*) at 32°C and 3 pH. Maximum sorption was achieved with *Bacillus subtilis* compared to *Pseudomonas* and mixed cultures.

**Keywords**— Biosorption; *Bacillus subtilis*; *Pseudomonas aeruginosa*; heavy metals; wastewater.

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### I. INTRODUCTION

In recent years, biosorption has been widely studied for the removal of metal ions, especially at the concentrations ranging from 1-100 mg L<sup>-1</sup>, due to its lower cost and higher effectiveness than conventional methods such as chemical precipitation and ion exchange [1]. A better understanding of the biosorption efficiency and mechanism is important to the design and optimization of the biosorption process [2]. Biosorption is a metabolic-independent process and the sequestration of metals by cells can take place through adsorption, ion exchange, coordination, complexation, etc. by the presence of functional groups on the biomass such as carboxyl, hydroxyl, amine and phosphate, which exhibit affinity to metal ions [3]. According to the Indian standards [4], the permissible limit of Cr (VI) is 0.05 and 0.1 mg/L for potable and industrial discharge water respectively. Chromium and its compounds are pollutants commonly found in the environment and occur mainly in the oxidation states from 0 to VI in nature. Among all these oxidation states, Cr(II) can be easily oxidized to Cr(III) by air, while Cr(IV) and Cr(V) are unstable intermediates formed during Cr bearing reactions. Cr(VI) and Cr(III) are the two stable ionic forms, and because of their different oxidation states, they have different physicochemical properties as well as chemical and biochemical reactivity. Cr(VI) forms several species and the relative proportions of each species depends on both pH and total Cr(VI) concentration [5]. The growing, resting and non-living cells of microorganisms are reported to remove Cr(VI) from aqueous solutions [6],[7],[8]. However, most of the works to remove Cr(VI) have been carried out using non-living cells and a very little information is available on use of growing and resting cells.

### II. MATERIALS AND METHODS

#### 2.1 Materials

##### 2.1.1 Chemicals

The metal salts used in this work are K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> as a source of metal ions Chromium. Nutrient medium for subculturing, Nutrient Agar for slant culture, Hydrochloric acid (HCl) and sodium hydroxide (NaOH) for adjusting pH. The Bromfield medium for culturing composed per litre (pH-7) each of KH<sub>2</sub>PO<sub>4</sub> - 0.50g, MgSO<sub>4</sub>.7H<sub>2</sub>O- 0.20g used for chromium analysis by UV-Vis. spectrophotometer at an absorbance of 540 nm.

##### 2.1.2 Microorganisms

Gram negative and gram positive microorganisms *Pseudomonas aeruginosa*, *Bacillus subtilis* for the study were isolated from NIT Warangal wastewater treatment plant. Each strain maintained in the nutrient medium and appropriate proportions used for the experiment. Standard sterile techniques were used for inoculation of cultures. Medium used for the microorganism and all the glassware were properly sterilized autoclaved at 15 lb/in<sup>2</sup> pressure and 121°C for 30 minutes.

#### 2.2 Methods

##### 2.2.1 Preparation of Metal solutions and Biosorbent preparation

Chromium metal solutions were prepared by dissolving K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> in double distilled water to get metal concentrations of 5, 10, 15, 20, 30 mg/L. A stock solution of 1000 mg/L was prepared and all other concentrations were obtained from it. Nutrient medium (1000 ml) was prepared with standard composition in a conical flask. The pH for the medium was adjusted accordingly and then the media was sterilized at 15 lb/in<sup>2</sup> pressure and 121°C for 30 minutes. Nutrient agar medium was

prepared, autoclaved and allowed to cool. Loop full of bacterial culture was taken and streaked on the agar plate to obtain more colonies. They were later transferred to nutrient broth and grown on specific media (Bromfield medium-*Bacillus subtilis*; Cetrinide medium-*Pseudomonas aeruginosa*) for subculture. 100 ml of sterilized culture media was transferred to 250 ml Erlenmeyer flask. The media was allowed to cool and then the 100µl microbial solution was inoculated into the medium in laminar air flow chamber. The inoculated flasks were incubated in an orbital shaker at 250 rpm at 32°C for 2 days to obtain the biomass. Mixed cultures were prepared by adding equal amounts (1:1) of individual cultures. Biomass was harvested from the medium by centrifugation at 9000 rpm for 10 min. The supernatant was discarded and the cells were re-suspended in purified water for washing and again centrifuged as above to make sure that no media remain on the cell surface. The biomass was heated in a conventional hot air oven at 60°C for 24 hrs. This biomass was used for the biosorption experiments. Both the biomasses were added in equal amounts for biosorption experiments with mixed culture.

#### **2.2.2 Biosorption experiment and Analytical estimation of Chromium (VI)**

Different concentrations of biomass (pure/mixed cultures) were combined with 100 ml of metal solution in 250 ml Erlenmeyer flask. The flasks were placed on a shaker with a constant speed of 300 rpm and left to equilibrate. Samples were collected at predefined time intervals, centrifuged as above and the amount of metal in the supernatant was determined. A 0.25% w/v solution of diphenyl carbazide was prepared in 50% acetone. 15 ml each of the sample solutions, containing various concentrations of Cr (VI) were pipetted out into 25ml standard flasks [9]. To this 2 ml of 3M H<sub>2</sub>SO<sub>4</sub> was added followed by 1 ml of diphenyl carbazide and the total volume was made upto 25 ml using deionised, double distilled water such that the final concentrations were in the range of 0.15 to 0.3 ppm. Chromium concentration estimated by the intensity of the colour complex formed was measured using a UV-visible spectrophotometer. The absorbance was measured against a reagent blank at 540-nm wavelength maximum. A linear plot was obtained indicating adherence to the Beer Lambert's law in the concentration range studied.

$$\% \text{Biosorption} = \left[ \frac{\text{Initial metal concentration} - \text{Final metal concentration}}{\text{Initial metal concentration}} \right] 100$$

#### **2.2.3 Batch biosorption studies**

Biomass was harvested from the medium by centrifugation at 9000 rpm for 10 min. The supernatant was discarded and the cells were re-suspended in purified water for washing and again centrifuged as above to make sure that no media remain on the cell surface. The biomass was heat killed in a conventional hot air oven at 60°C for 24 hrs. This biomass was used for the sorption experiments. Biosorption studies were done using biomass as a function of various parameters such as

- a) pH
- b) Biomass concentration
- c) Temperature
- d) Time
- e) Initial metal concentration

#### **2.2.4 Effect of pH**

The metal sorption monitored for pH range 1 to 7. NaOH and HCL were used as pH regulators. 1 mg/ml biomass was dispersed in 100 ml of the solution containing 10mg/L of each metal concentration. All flasks were maintained at different pH values ranging from 1 to 7 for about 12 hours. Solutions were centrifuged as above and the supernatant was analysed for the residual concentrations of the metal ions. The final pH values have been plotted. Many other researchers reported the optimum pH value for bacterial Cr (VI) reduction but not the optimum initial pH value. It is reported that the optimum pH was 9 for Cr (VI) reduction by gram-negative bacterium [10], but it was found that the optimum pH was 7 in case of *Pseudomonas aeruginosa* and *Bacillus subtilis* [11], [12], [13].

#### **2.2.5 Effect of biomass concentration**

Biomass was centrifuged at 9000 rpm and different weights of the biomass ranging from 0.5 to 3 mg/mL were dispersed in solutions containing the 10 mg/L metal concentration. The solutions were adjusted to the optimum pH in which maximum biosorption of the metal ion occurred. Flasks were left for equilibration. The solutions were later centrifuged at 9000 rpm and the metal ion concentrations were determined using the procedures described earlier.

#### **2.2.6 Effect of temperature**

Optimum biomass concentration with optimum pH was used to monitor the temperature effect on biosorption. Experiments were carried out at different temperatures from 10-50°C for each culture and kept on rotary shaker at 240 rpm. The samples were allowed to attain equilibrium. The sample collected at regular intervals as above and analysed for metal concentration.

#### **2.2.7 Effect of contact time**

The cell pellet dispersed in metal solution of 10 mg/L concentration with a working volume of 100 ml. the experiment was carried out at the optimum pH system. Flasks were allowed to attain equilibrium on rotary shaker at 240 rpm and samples were collected at regular time intervals. Centrifugation at 9000 rpm was done and the supernatant was analysed for the residual metal content.

#### **2.2.8 Effect of initial metal concentration**

Biosorption experiments were conducted by taking different initial metal concentrations by fixing all the parameters such as biomass concentration, pH, temperature and time. Metal solutions were prepared as stated above. With increase in metal concentration (5 to 30 mg/L) percentage biosorption was observed.

#### **2.2.9 Adsorption isotherms**

The optimum biomass of each culture was dispersed in a desired concentration ranging from 5 mg/L to 30 mg/L for each metal. In all these cases the initial pH was adjusted to that of the optimum value, namely 3, 5, 6 for Chromium. The flasks were incubated for 50 minutes period of time at the end of which the residual concentrations were determined.

The amount of metal bound by the biosorbents was calculated as follows:

$$Q = \frac{v(C_i - C_f)}{m}$$

Where Q is the metal uptake (mg metal per g biosorbent), v is the liquid sample volume (ml), C<sub>i</sub> is the initial concentration of the metal in the solution (mg/L), C<sub>f</sub> is the final (equilibrium) concentration of the metal in the supernatant (mg/L) and m is the amount of the added biosorbent on the dry basis (mg).

The Langmuir model

$$Q = \frac{Q_{max} \cdot b \cdot C_f}{1 + b \cdot C_f}$$

Where Q<sub>max</sub> is the maximum metal uptake under the given conditions, b a constant related to the affinity between the biosorbent and sorbate.

Linearized Langmuir model

$$\frac{1}{Q} = \frac{1}{Q_{max}} \left( \frac{1}{b} \cdot C_f + 1 \right)$$

The Freundlich Model,

$$Q = K \cdot C_f^{\left(\frac{1}{n}\right)}$$

Where k and n are Freundlich constants, which correlated to the maximum adsorption capacity and adsorption intensity, respectively.

Linearized Freundlich equation

$$\text{Log } Q = \text{Log } K + \frac{1}{n} \text{Log } C_f$$

### 2.2.10 Rate kinetics

As aforementioned, a lumped analysis of adsorption rate is sufficient to practical operation from a system design point of view. The commonly employed lumped kinetic models, namely a) the pseudo-first-order equation b) the pseudo-second-order equation are presented below [14]. The pseudo first-order and pseudo-second-order kinetic models assume that adsorption is a pseudo-chemical reaction process and the adsorption rate can be determined respectively by the first-order and second-order reaction rate equations.

$$\begin{aligned} \frac{dq_t}{dt} &= k_1 (q_e - q_t) \\ \frac{dq_t}{dt} &= k_2 (q_e - q_t)^2 \end{aligned}$$

Where q<sub>e</sub> (mg/g) is the solid phase concentration at equilibrium, q<sub>t</sub> (mg/g) is the average solid phase concentration at time t (min) . k<sub>1</sub> (min<sup>-1</sup>) and k<sub>2</sub> (g mg<sup>-1</sup> min<sup>-1</sup>) are the pseudo-first- order and pseudo-second-order rate constants respectively. The above equations represent initial value problems and have analytical solutions when combined with the initial condition t=0, q<sub>t</sub>=0.

The solutions for the above equations are as follows:

$$\begin{aligned} \ln(q_e - q_t) &= \ln(q_e) - k_1 \cdot t \\ \frac{t}{q_t} &= \frac{1}{k_2 q_e^2} + t/q_e \end{aligned}$$

If the adsorption follows the pseudo-first order rate equation, a plot of ln (q<sub>e</sub> - q<sub>t</sub>) against time t should be a straight line. Similarly, t/q<sub>t</sub> should change linearly with time t if the adsorption process obeys the pseudo-second order rate equation. Available studies have shown that the pseudo-second order rate equation is reasonably good fit of data over the entire fractional approach to equilibrium and therefore has been employed extensively in the study of adsorption kinetics [15]. However, it is not uncommon to observe multi linearity on the ln (q<sub>e</sub>-q<sub>t</sub>) - t plot or t/q<sub>t</sub> - t plot. The trend is usually such that the rate constant decreases with time or more specially decreases with increase in solid phase concentration.

## III. RESULTS AND DISCUSSION

### 3.1 Batch biosorption studies using attenuated cells of *Pseudomonas aeruginosa*

In the investigation carried out so far, the attenuated cells of *Pseudomonas aeruginosa* were used for the biosorption of Chromium. The parameters affecting the biosorption of chromium using this single culture of *Pseudomonas aeruginosa* were studied. The effect of these parameters was discussed below.

#### 3.1.1 Effect of pH

The pH value of the solution is an important factor that controls the sorption of Chromium. Figure 1 shows that the highest biosorption efficiency of chromium is at pH 3 and the influence of pH on the percentage sorption of Chromium is depicted in the Figure 1. The percentage sorption increased from 56% at pH 2 to 68% at 3 and significantly decreased with increase in pH and at pH of 6 it was around 18%. From this study it was concluded that at pH of 3 *Pseudomonas aeruginosa* showed maximum percent of biosorption.

#### 3.1.2 Effect of biomass concentration

Batch experiments were conducted to investigate the influence of biomass concentration on the percentage biosorption of Chromium is depicted in Figure 2. To achieve the maximum biosorption capacity of the biosorbent for Chromium, the biomass concentration was varied from 0.5 to 3 mg/ml and it was found that a concentration of 1.5 mg/ml was sufficient for

maximum percentage of Chromium biosorption. These findings are shown in Figure 2. It can also be seen from this Figure that a further increase in biomass does not affect the sorption percentage greatly. This may be due to the unavailability of binding sites to the metal and also due to the blockage of binding sites with excess biomass.

### **3.1.3 Effect of temperature**

Effect of temperature on Chromium biosorption is presented in Figure 3. It was observed that the temperature 32<sup>o</sup>C is favourable than that of the lower or higher temperatures. Good sorption percentage around 64% was observed at 32<sup>o</sup>C. In these experiments there was an increase in sorption percentage with increase in the temperature but there was a gradual decrease with further increase in temperature. This is because of the shrinkage of cells in the higher and lower temperatures which reduces the surface area of contact.

### **3.1.4 Effect of contact time**

The adsorption experiments of Chromium were carried out for different contact times with a fixed adsorbent dose of 1.5 mg/ml concentration at pH 3 and at 32<sup>o</sup>C. The results were plotted in Figure 4. The sorption percentage of metal increased with increase in contact time. The equilibrium time was 30 min for Chromium at which percent of biosorption was 64%.

## **3.2 Batch biosorption studies using *Bacillus subtilis***

In the studies carried out so far, the attenuated cells of *Bacillus subtilis* were used for the biosorption of Chromium. The parameters affecting the Biosorption of Chromium using single culture of *Bacillus subtilis* were studied. Effects of these parameters were discussed below:

### **3.2.1 Effect of pH**

The pH of the system exerts profound influence on the sorption of adsorbate molecule due to its influence on the surface properties of the adsorbent and ionization/dissociation of the adsorbate molecule. Figure 1 depicts that the highest biosorption efficiency is at pH 3 and the percentage sorption increased from 72% at pH 2 to 84% at 3 and significantly decreased with increase of pH. From this study it was concluded that at pH 3, *Bacillus subtilis* showed maximum percent of biosorption of chromium.

### **3.2.2 Effect of biomass concentration**

Biosorption of Chromium was observed at various biomass concentrations of *Bacillus subtilis*. To achieve the maximum biosorption capacity of the biosorbent for Chromium, the biomass concentration was varied from 0.5 to 3 mg/ml. The variation in sorption from Figure 2 was observed and the optimum biomass concentration noted at 2 mg/ml and beyond this it was constant. This could be due to the unavailability of binding sites to the metal and also due to the blockage of binding sites with excess biomass.

### **3.2.3 Effect of temperature**

Effect of temperature on Chromium biosorption is presented in Figure 3. Sorption experiments were conducted from 10<sup>o</sup>C to 50<sup>o</sup>C. Good sorption percentages around 78% were observed in the temperature range of 24<sup>o</sup>C to 40<sup>o</sup>C. Maximum biosorption percentage of 82% was noted at 32<sup>o</sup>C. In these experiments there was an increase in sorption percentage with increase in the temperature. A gradual decrease with further increase in temperature was noted. This is because of the shrinkage of cells in the higher and lower temperatures which will reduce the surface area of contact.

### **3.2.4 Effect of contact time**

The sorption experiments of Chromium were carried out for different contact times with a fixed adsorbent dose of 2 mg/ml concentration at pH 3 at 32<sup>o</sup>C. The results were plotted in Figure 4. The sorption percentage of metal increased with increase in contact time. The equilibrium time was 25 min for Chromium at which the percent of biosorption was 82%.

## **3.3 Biosorption studies on Chromium using mixed cultures of *Pseudomonas aeruginosa* and *Bacillus subtilis*.**

Present investigation deals with the utilization of mixed cultures of gram positive and gram negative bacteria as biosorbent for the sorption of Chromium. Considering the advantage of both bacterial surfaces in biosorption, studies on Chromium sorption using this mixed biomass were done. Batch studies were done to address various experimental parameters like pH, contact time, adsorbent dose for the sorption of Chromium. Greater percent of sorption was observed at lower concentrations of metal. Adsorption isotherms and kinetic studies were done. The effect of various parameters on the biosorption of Chromium using mixed cultures of *Pseudomonas aeruginosa* and *Bacillus subtilis* (1:1) was discussed below.

### **3.3.1 Effect of pH**

The sorption treatment of metals in water is pH dependent. However, pH is also known to affect the sorption process as magnitude of electrostatic charges imparted by ionized metal molecules is controlled by the pH of the medium. The percent of metal sorption vary with pH of the medium. The experimental results of Chromium sorption using mixed cultures of *Pseudomonas aeruginosa* and *Bacillus subtilis* (1:1) at varying pH ranges was shown in the Figure 1. Effect of pH on biosorption has been studied over a range of 1 to 7. Mixed cultures showed the highest percent sorption of chromium at around 74% at pH 3. Figure 1 shows a decrease in sorption percentage with further increase of pH.

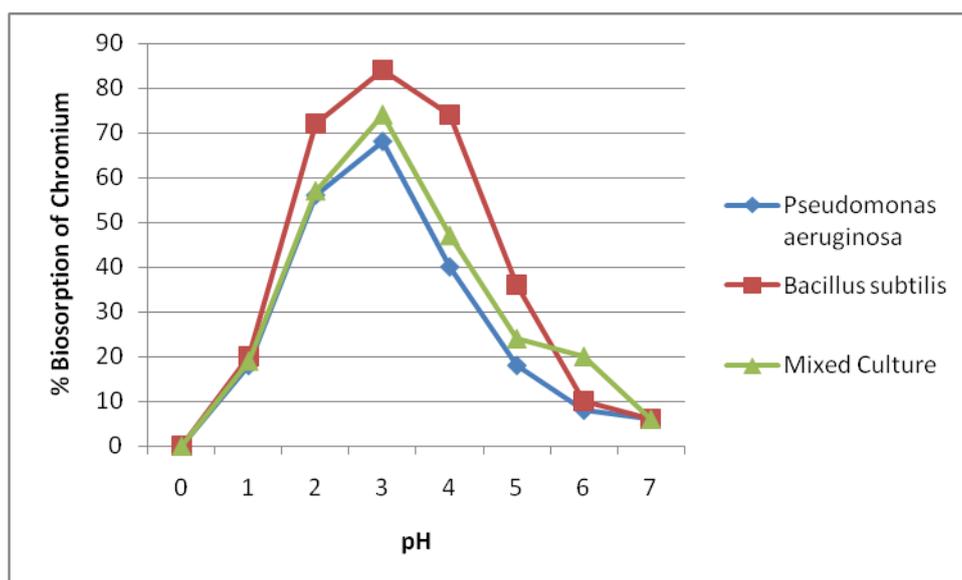


Figure.1 Effect of pH on Biosorption of Chromium at 32<sup>0</sup>C and 10mg/L initial metal concentration of *Pseudomonas aeruginosa*, *Bacillus subtilis* and mixed culture (1:1)

### 3.3.2 Effect of biomass concentration

The effect of biomass concentration on sorption of Chromium was studied using various amounts ranging from 0.5 mg/ml to 3 mg/ml. These studies were done at a fixed of pH 5. The Figure 2 depicts the effect of biomass concentration on biosorption percentage of Chromium. It was observed that the biosorption percentage increased with increase in biomass. Significantly high biosorption of around 78% was achieved at 1.5 mg/ml and this biomass concentration was chosen for all further studies. It was observed that there was no further increase in the percentage sorption of Chromium with increase of biomass beyond 1.5 mg/mL. It was concluded that 1.5 mg/ml concentration of biomass gave optimum biosorption for Chromium with mixed cultures of *Pseudomonas aeruginosa* and *Bacillus subtilis* (1:1).

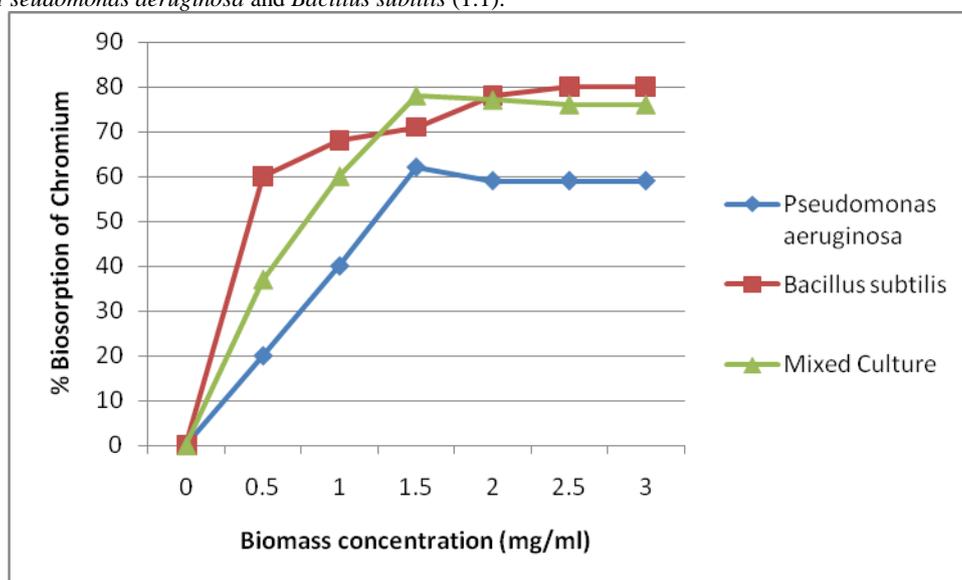


Figure.2 Effect of biomass concentration on percent biosorption of chromium at 32<sup>0</sup>C temperature, pH 3 and initial metal concentration 10 mg/L for *Pseudomonas aeruginosa*, *Bacillus subtilis* and mixed culture (1:1)

### 3.3.3 Effect of temperature

Biosorption studies of Chromium using mixed cultures were carried out at different temperatures ranging from 10<sup>0</sup>C to 50<sup>0</sup>C. The effect of temperature on metal sorption is presented in Figure 3. The percentage of metal sorption was increased from 10<sup>0</sup>C to 32<sup>0</sup>C and then showed decrease in sorption percentage with further increase in temperature. Figure 3 shows a maximum percent of sorption of around 77% was achieved at 32<sup>0</sup>C at fixed pH and biomass concentrations.

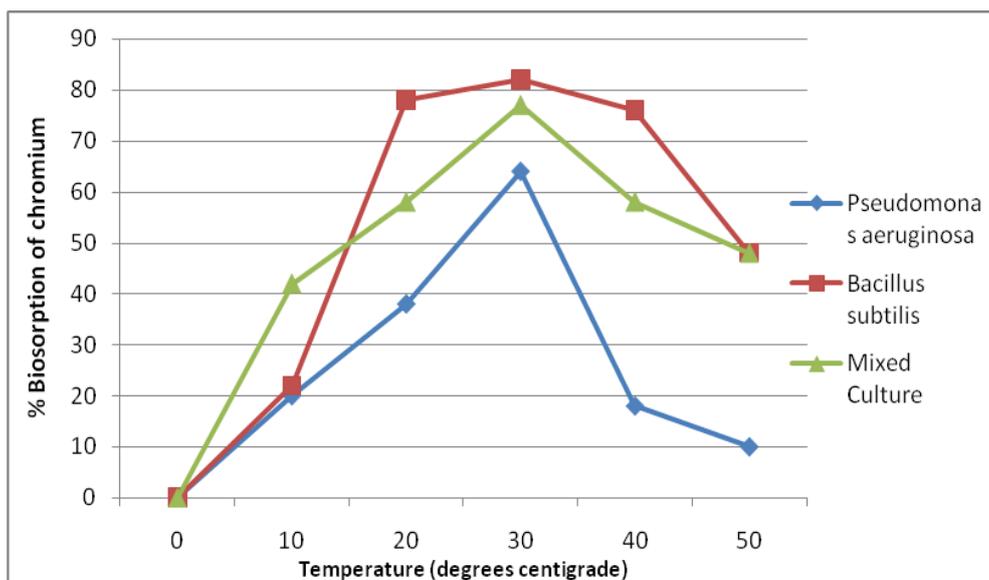


Figure.3 Effect of temperature on percent biosorption of chromium at pH 3 and initial metal concentration 10 mg/L for *Pseudomonas aeruginosa*, *Bacillus subtilis* and mixed culture(1:1).

### 3.3.4 Effect of contact time

Biosorption experiments of Chromium using mixed cultures were carried out for different contact times at fixed pH, biomass concentration and temperature. The figure 4 depicts the percent sorption with time. The sorption percentage of the metal increased with time and a sorption of 78% was reached 25 min, the sorption of metal was rapid in the initial stages of contact time and gradually decreased with lapse of time until saturation. Maximum sorption was achieved with *Bacillus subtilis* compared to *Pseudomonas* and mixed cultures.

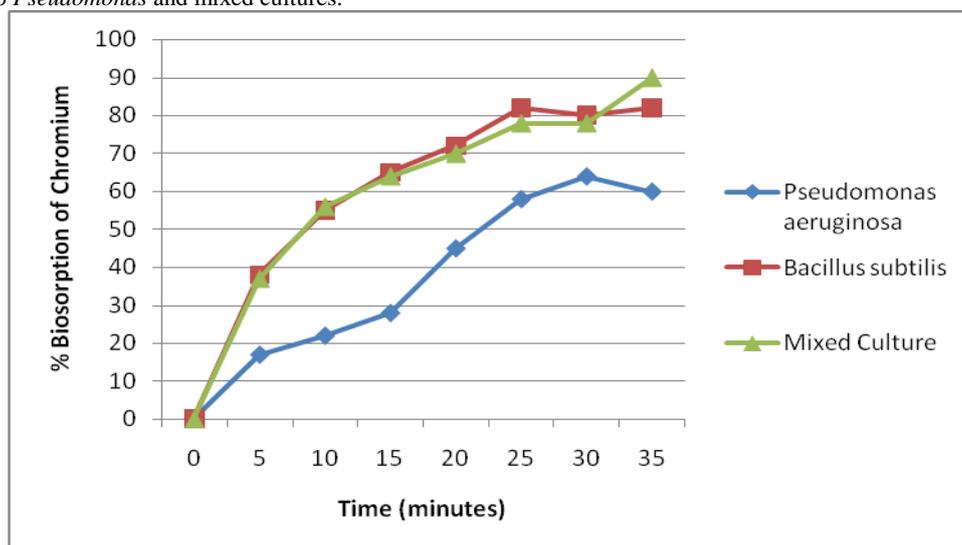


Figure.4 Effect of contact time on percent biosorption of chromium at 32°C temperature, pH 3 and initial metal concentration 10 mg/L for *Pseudomonas aeruginosa*, *Bacillus subtilis* and mixed culture (1:1)

### 3.3.5 Rate kinetics

Biosorption of metal onto biomass was monitored specific time intervals of 5min. the metal uptake was calculated from the data obtained from the metal uptake and plotted against time to determine a suitable kinetic model. The adsorption data was fitted into first order and second order kinetics. The first order equation was plotted for  $\ln(q_e - q_t)$  against  $t$ . the values of  $\ln(q_e - q_t)$  were calculated from the kinetic data of Figures 6 and 7. The  $k_1$  values were calculated from the slope of this plot. The value of  $k_1$  was shown in Table 2. The second order equation was plotted for  $t/q_t$  against  $t$ . The values of  $q_e$  and  $k_2$  are calculated from the slope and intercept of this plot. The values of  $q_e$  and  $k_2$  are shown in Table 2. The correlation coefficient  $R^2 = 0.992$  for pseudo-first order and  $R^2 = 0.995$  for pseudo-second order kinetic equation states that both values were very near and well suited. But pseudo-second order best fitted with experimental values as it was close to 1. By maintaining all the parameters at optimum levels, the initial metal concentrations were varied (10, 15, 20, 25, 30 mg/L). The percentage sorption was decreased constantly with increase in initial metal concentration. The decline in the percentage biosorption was depicted in Figure 5.

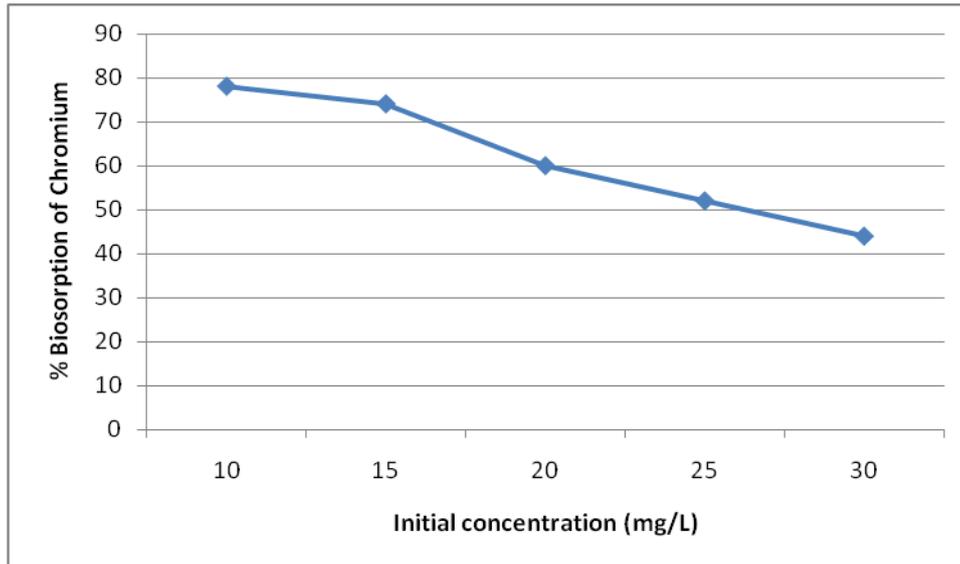


Figure.5 Effect of initial metal concentration on percent biosorption of chromium at 1.5 mg/ml biomass, 32<sup>0</sup>C temperature, pH 3 and 30 minutes of contact time.

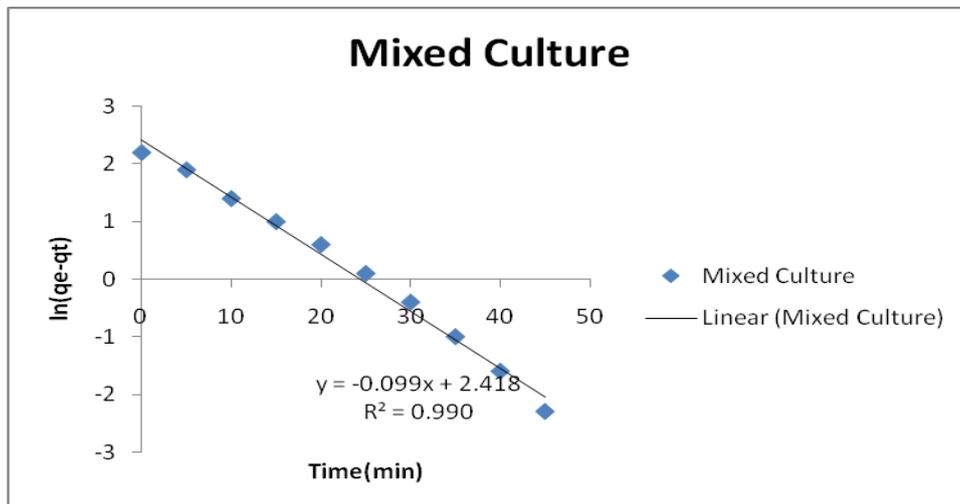


Figure.6 First order kinetics for Chromium by *Pseudomonas aeruginosa* and *Bacillus subtilis* (1:1) mixed culture at 1.5 mg/ml biomass concentration, 10 mg/L metal concentration, pH.3, 32<sup>0</sup>C temperature.

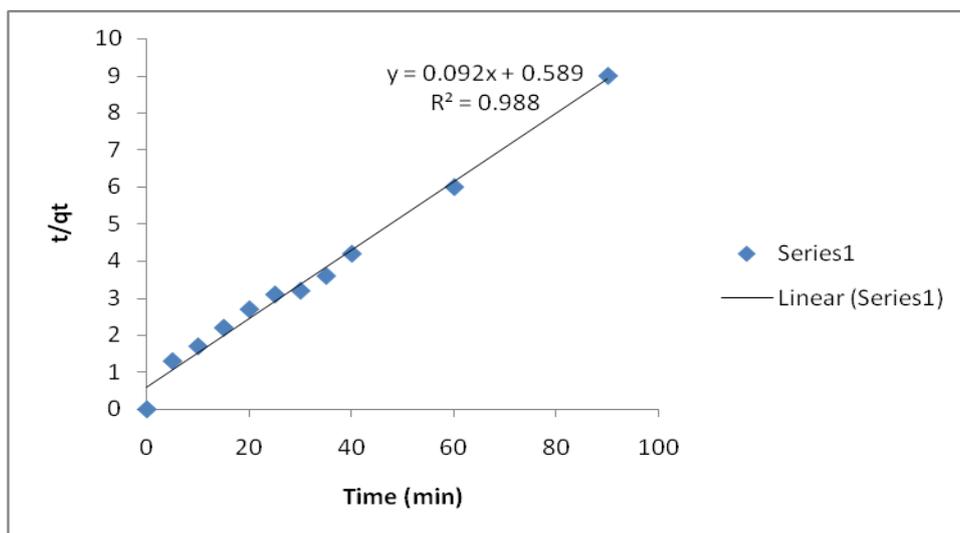


Figure.7 Second order kinetics for Chromium by *Pseudomonas aeruginosa* and *Bacillus subtilis* (1:1) mixed culture at 1.5 mg/ml biomass concentration, 10 mg/L metal concentration, pH.3, 32<sup>0</sup>C temperature.

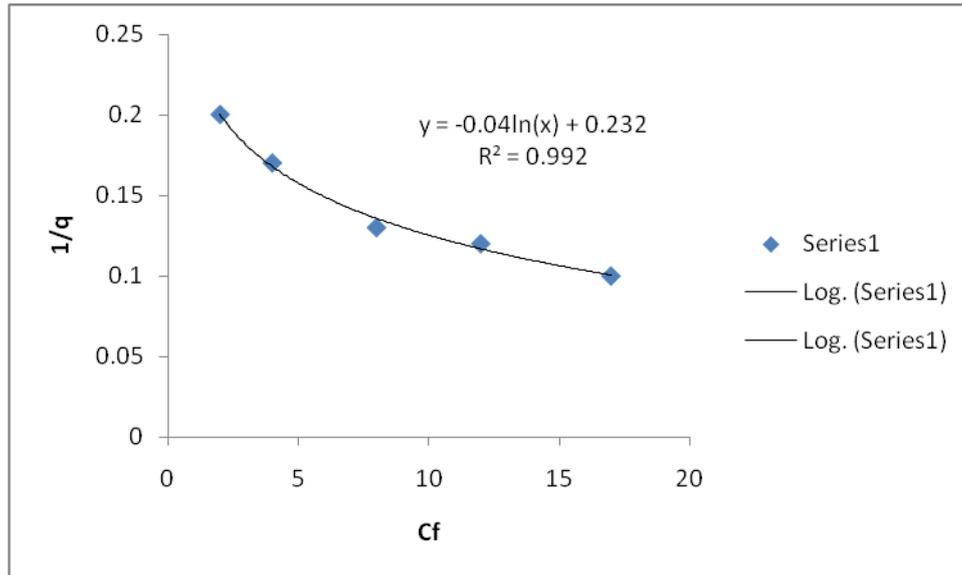


Figure 8: Adsorption isotherm (Langmiur) for Chromium by *Pseudomonas aeruginosa* and *Bacillus subtilis* (1:1) mixed culture at pH 3, 1.5 mg/ml biomass, 32°C temperature, and 30 min of contact time.

### 3.3.6 Adsorption isotherms

The equilibrium experimental results of Chromium ions have been fitted in the Langmuir and Freundlich models. For biosorption of Chromium using mixed cultures of *Pseudomonas aeruginosa* and *Bacillus subtilis* (1:1) the coefficient of determination ( $R^2$ ) of both models was mostly greater than 0.95 and close to 1 (Figure 8 and figure 9). This indicates that both models adequately describe the experimental data of the biosorption of Chromium. Data evaluation was done in the same manner as described. In the biosorption of Chromium by mixed cultures of *Pseudomonas aeruginosa* and *Bacillus subtilis*, most of the metal ions were sequestered very fast from the solutions in the first phase of contact time 30 minutes and almost no increase in the level of bound metal have been occurred after this time interval as shown in Figure 4. The sorption performance of the mixed biosorbent was studied under fixed environmental conditions. Biosorption equilibrium isotherms were plotted for metal uptake  $q$  against the residual metal concentration in the solution. The  $q$  versus  $C_f$  sorption isotherm relationship was mathematically expressed by Langmuir and Freundlich models. The higher the values of  $k$  and  $n$ ; lower the value of  $b$ , the higher the affinity of the biomass. Table 1 describes summaries of linear regression data for Langmuir and Freundlich isotherms for Chromium biosorption using attenuate mixed biomass. Langmuir and Freundlich constant  $k$  were obtained from the linear equations of both models. As indicated in the Table 1, the coefficients of determination ( $R^2$ ) of both models are 0.99 close to 1. In the Table 2, the values of  $K_f$ ,  $1/n$ ,  $Q_{max}$  and  $b$  were given.

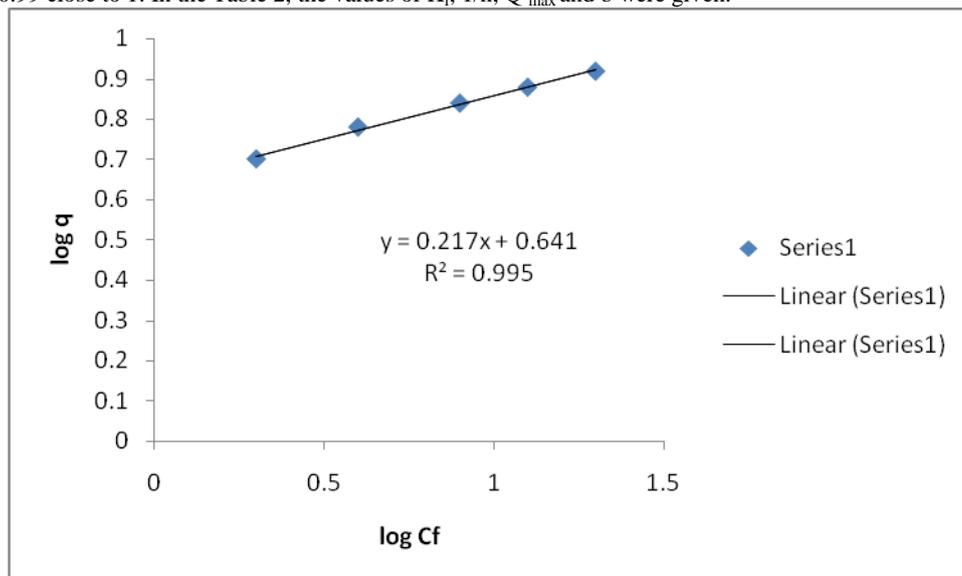


Figure 9: Adsorption isotherm (Freundlich) for Chromium by *Pseudomonas aeruginosa* and *Bacillus subtilis* (1:1) mixed culture at pH 3, 1.5 mg/ml biomass, 32°C temperature, and 30 min of contact time.

Metal	Pseudo first order			Pseudo second order		
	$K_1$	$q_e$	$R^2$	$K_2$	$q_e$	$R^2$
Chromium	0.0867	10.69953	0.990	0.1584	10.32	0.9880

**Table.1 Kinetic data of *Pseudomonas aeruginosa* and *Bacillus subtilis* (1:1) mixed culture of chromium.**

Metal	Pseudo first order			Pseudo second order		
	$K_f$	$1/n$	$R^2$	$q_m$	$b$	$R^2$
Chromium	4.0355	0.3088	0.992	4.422	-5.06	0.995

**Table.2 Parameters of isotherm models for heavy metal chromium**

#### IV. CONCLUSION

Biosorption of heavy metals is one of the most promising technologies involved in the removal of toxic materials from the industrial wastewater and natural waters. The biosorption process depends significantly on the pH of the solution and is favoured at around pH value of 3.0. The optimum biomass concentration noted at 2 mg/ml and beyond this it was constant for *Bacillus subtilis*. This could be due to the unavailability of binding sites to the metal and also due to the blockage of binding sites with excess biomass. The maximum uptake capacity of biomass for Cr(VI) increased with the increase in initial metal ion concentration and decreased with increase in biomass concentration. Biosorption obeys the pseudo first order kinetics, which implies that the rate of biosorption process is independent of initial concentration. The adsorption is well described by Langmuir isotherms that expresses that monolayer adsorption exist under the experimental conditions. The adsorption-desorption experiments were successfully carried out three times. Percent biosorption of chromium by both bacterial strains were compared and *Bacillus subtilis* was found to be efficient. Hence, *Bacillus subtilis* biomass, a fermentation by-product can be used as an effective, inexpensive and alternative biosorbent for the removal of Cr(VI) from the industrial wastewaters.

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