OPTIMIZATION OF HEAVY METAL BIOSORPTION USING ATTENUATED CULTURES OF
Bacillus subtilis AND Pseudomonas aeruginosa

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ABSTRACT

Biosorption can be a part of to solve the water pollution problem caused due to toxic heavy metal contamination resulting from humans technological activities. Several gram positive and gram negative bacteria have the ability to adsorb the heavy metals and helps in effective cleaning of contaminated water and it has been documented that attenuated bacterial biomass have greater biosorption capability than viable cells. In the present study, biosorption of Chromium (Cr), using mixed culture of attenuated gram positive and gram negative, bacteria 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 comparative experiments have been carried out using individual and mixed bacterial culture and the biosorption parameters were optimized. Analytical results of the study revealed that 85% of biosorption of chromium was observed for mixed cultures of Bacillus subtilis and Pseudomonas aeruginosa and 60 % and 50% biosorption for individual cultures respectively in 30 min which was optimum. And also other parameters for Cr biosorption were established for mixed biomass cultures and found to be 32°C, 3 pH, 300 rpm agitation and 1gm/l of biomass concentration.

Key Words: Biosorption, Heavy metals, Attenuated cells, Optimization

INTRODUCTION

Heavy metals are widespread pollutants of great environmental concern as they are non-degradable and thus persistent ¹,². The presence of heavy metals in aquatic environments is known to cause severe damage to aquatic life. Conventional processes are expensive, ineffective and not eco-friendly especially when the heavy metal ions are in solutions containing in the order of 1-100 mg dissolved heavy metal ions/L.³ Biological methods such as biosorption / bioaccumulation for the removal of heavy metal ions may provide an attractive alternative to conventional methods.⁴ Bioaccumulation is defined as the phenomenon of living cells; where as, biosorption mechanisms are based on the use
of dead biomass. Biosorption can be defined as the passive uptake of toxicants by dead/inactive biological materials or by materials derived from biological sources. In addition, maintenance of healthy microbial population is difficult due to metal toxicity and other unsuitable environmental factors. Biosorption is a rapid phenomenon of passive metal sequestration by the non-growing biomass. Hence biosorption was regarded as an emergent technology with precise goals of identifying potential biomass. Metal-sequestering properties of non-viable biomass provide a basis for a new approach to remove heavy metals when they occur at low concentrations. Besides this, biosorption offers advantages of low operating cost, minimizes the volume of chemical and/or biological sludge to be disposed, is highly efficient in dilute effluents and has no nutrient requirements.

The bacterial cell wall is the first component that comes into contact with metal ions/dyes, where the solutes can be deposited on the surface or within the cell wall structure; since the mode of solute uptake by dead/inactive cells is extracellular, the chemical functional groups of the cell wall play vital roles in biosorption. Due to the nature of the cellular components, several functional groups are present on the bacterial cell wall, including carboxyl, phosphate, amine and hydroxyl groups. In the biosorption process bacterial cell wall plays the key role. It provides structural integrity to the cell, but differs from that of all other organisms due to the presence of peptidoglycan (poly-N-acetylglucosamine and N-acetylmuramic acid), which is located immediately outside of the cytoplasmic membrane. Peptidoglycan is responsible for the rigidity of the bacterial cell wall, and determines the cell shape. It is also relatively porous and considered as an impermeability barrier to small substrates. From Vijayaraghavan et al., the cell walls of all bacteria are not identical. Accordingly, Gram-positive bacteria are comprised of a thick peptidoglycan layer. Teichoic acids give the Gram-positive cell wall an overall negative charge, due to the presence of phosphodiester bonds between the teichoic acid monomers. In general, 90% of the Gram-positive cell wall is comprised of peptidoglycan. On the contrary, the cell wall of Gram-negative bacteria is much thinner, and composed of only 10–20% peptidoglycan. In addition, the cell wall contains an additional outer membrane composed of phospholipids and lipopolysaccharides. The highly charged nature of lipopolysaccharides confers an overall negative charge on the Gram-negative cell wall. This showed that the anionic functional groups present in the peptidoglycan, teichoic acids and teichuronic acids of Gram-positive bacteria, and the peptidoglycan, phospholipids, and lipopolysaccharides of Gram-negative bacteria were the components primarily responsible for the anionic character and metal-binding capability of the cell wall. Extra cellular polysaccharides are also capable of binding metals.

Our work includes study of biosorption by attenuated cultures of Bacillus and Pseudomonas species which are gram positive and gram negative bacteria, a comparative work also been carried out for individual and mixed cultures. Hence utilizing the advantages of both gram negative and gram positive bacteria biosorption studies of heavy metals were done. Attenuated cell sorption ability depends on various physical parameters. The parameters studied and optimized are pH, temperature, biomass concentration, agitation and time. Two adsorption models Freundlich and Langmuir isotherms applied and correlation found to the experimental data. The present study is directed towards effective use of mixed cultures for heavy
metal removal from the contaminated water bodies.

**Objective**

To utilize the advantages of both gram positive and gram negative cell walls in the studies of biosorption of heavy metals by attenuated cells. Optimization parameters like pH, temperature, biomass concentration and time with individual and mixed cultures.

**MATERIAL AND METHODS**

**Biomass preparation**

_Bacillus subtilis_ and _Pseudomonas aeruginosa_, showing good ability of sorption were obtained from NCIM pune. _Pseudomonas_ species was grown on cetrimide agar (Hi-media) and transferred to the nutrient media (Hi-media), _Bacillus_ strain is cultured on bromified media. The pH of the media was adjusted at the predetermined growth pH 7. The cultures are performed in 250 ml Erlenmeyer flasks containing 100ml of sterile media and incubated on rotary shaker at 200rpm at 30°C. Both the bacterial cells are harvested separately by centrifugation at 25ºC and 8000 rpm for 15 min and washed twice with distilled water. The cells are dried in hot air oven at 60°C for 24 hours to get the attenuated cell biomass.

**Metal solutions**

Different metal concentrations were prepared by dissolving K₂Cr₂O₇, salt in deionised water to have metal concentrations of (10,20,30,40,50) mg/L. All the glassware washed with 0.1 M HCl before and after each experiment to avoid binding of the metal to it.

**Biosorption process**

The metal solutions were prepared using the metal salts. Dried biomass of different concentrations was combined with 100ml of metal solution in 250ml Erlenmeyer flasks. The flasks were placed on a shaker with constant speed of 350 rpm. Metal uptake was determined by calculating the difference of the metal concentration in initial and final solutions at periodic intervals. Biosorption at different parameters like pH, temperature, time and biomass concentrations identified.

**Determination of metal concentration in the supernatant**

Heavy metal concentration was determined by the use of UV-Visible spectrophotometer, determination of chromium was done at the wave length of 540nm.

**Analytical estimation of Cr (VI)**

A 0.25% w/v solution of diphenylcarbazide was prepared in 50% acetone. 15 ml each of the sample solution, containing various concentrations of Cr (VI) was pipetted out into 25ml standard flasks. To this 2ml of 3M H₂SO₄ was added followed by 1ml of diphenylcarbazide and the total volume was made up to 25ml using double distilled water. The intensity of the color complex formed was measured using a UV–Vis. spectrophotometer (Jasco, Japan, V–530). The absorbance was measured against a reagent blank at 540-nm wave length maximum.

**Data evaluation**

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

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

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

**Biosorption models**

Langmuir and Freundlich isotherms were used to compare the experimental data.
The Langmuir model, \[ Q = Q_{\text{max}} \frac{b \cdot C}{1 + b \cdot C} \]

Where \( Q_{\text{max}} \) is the maximum metal uptake under the given conditions, \( b \) a constant related to the affinity between the biosorbent and sorbate.

Linearized Langmuir model \[ \frac{1}{Q} = \frac{1}{Q_{\text{max}}} \left( \frac{1}{b \cdot C} + 1 \right) \]

The Freundlich model, \[ Q = k \cdot C^{1/n} \]

Where \( k \) and \( n \) are Freundlich constants, which correlate to the maximum adsorption capacity and adsorption intensity, respectively.

Linearized Freundlich equation
\[ \log Q = \log k + \frac{1}{n} \log C \]

RESULTS AND DISCUSSION

Effect of pH

The result of the pH study given in Fig. 1 shows that maximum biosorption was obtained at pH 3 for mixed cultures and individual Bacillus subtilis and Pseudomonas aeruginosa. The percentage of biosorption is lower at higher pH values. This result support the earlier report that the biosorption is pH dependent. Surface adsorption is a physiochemical phenomenon. Results of this study indicate that the optimum pH value for chromium biosorption with mixed culture used was 3. It is well known that both the cell surface binding sites and the availability of metal in solution are related to pH; at low pH, the cell surface binding sites will be protonated at pH 3 and attracted negatively charged ions via electrostatic interaction. At higher solution pH, the solubility of metal complexes decreases sufficiently allowing precipitation, which may complicate the sorption process. Earlier studies revealed optimum pH of 2.5 for Bacillus species and pH 4 for Pseudomonas species. Our study with mixed culture of Bacillus and Pseudomonas reported the optimum pH was 3 where they can be applicable as individual strains are less potent at that pH.

Effect of temperature

The results of temperature study given in Fig. 2 shows that maximum biosorption was obtained at 32°C, where good biosorption results where obtained in the temperature of 32°C.

![Fig. 1: Effect of pH on biosorption of chromium (Cr\(^{6+}\))](image-url)
range of 24°C to 32°C for mixed and individual cultures. Temperature seems to affect biosorption only to a lesser extent within the range of 20°C - 35°C. With increase in temperature there is a decrease in biosorption percentage due to the physical damage to the biosorbent which can be expected at higher temperatures. It is said earlier that an increase in temperature is found to reduce the biosorption capacity of the biomass⁸.

**Effect of biomass concentration**

Previous studies reveal that with an increase in the biomass concentration generally increases biosorption percentage due to the increase in the surface area of the biosorbent, which in turn increases the number of binding sites¹⁰. The results of biomass concentration study given in Fig. 3 shows that there was an increase in the biosorption percentage with increase in biomass concentration up to a particular concentration of biomass (1mg/ml). Further increase in concentration made no difference in the biosorption percentage. This is expected due to complex interaction like interference between the binding sites. These studies report that biomass concentration strongly influences the extent of biosorption.

**Effect of contact time**

The biosorption process of chromium was completed rapidly. The result of effect of contact time on biosorption from aqueous solutions is shown in Fig. 4. It is observed that the adsorptive capacity of attenuated cells increased with increase in time. The biosorption was rapid for the first 30 min as a result of available binding sites on the biomass. In general the biosorption capacity and removal efficiency increases with increase in the contact time¹¹. The biosorption approached equilibrium within 60 min where after the first stage of biosorption (after 30 min) the process was very slow and remained almost constant. Hence optimization of contact time is necessary for economic reuse of biomass and metal desorption, so from our study it is reported that the optimum contact time is 30 min for the biosorption of chromium using attenuated mixed cultures of bacillus and pseudomonas species.
Effect of initial metal concentration

Biosorption equilibrium isotherms were plotted for the metal uptake Q against the residual metal concentrations in the solution. The Q verses $C_f$ sorption isotherm relationship was mathematically expressed by linearized Langmuir and Freundlich models. The higher the values of $k$ and $n$, lower the values of $b$, the higher the affinity of the biomass$^{12,13}$. The effect of initial metal concentrations on biosorption was carried out using a concentration range of 10-50 mg/L. 1mg/ml biomass concentration was added in each flask with 100ml metal solution at pH 3.
and 32°C. The result obtained was analyzed using Freundlich and Langmuir isotherms. These studies showed that the efficiency increased with increase in the initial metal concentration. However, the gradual increase in the efficiency of biomass shows nearness to saturation of the available binding site on it. The same experimental data was applied to both the Freundlich and Langmuir isotherm models as shown in Fig. 5.

![Fig. 5: Langmuir isotherm shown for mixed cultures by taking final metal concentration (Cf) vs metal uptake (Q).](image)

**CONCLUSION**

In the present study of the biosorption of heavy metals by mixed and individual cultures of pseudomonas and bacillus species, most of the metal ions were sequestered very fast from solutions within 30 minutes and almost no increase in the level of the bound metals have been occurred after this time interval and showed that their removals differed with different operating conditions and initial metal concentrations. Maximum Cr biosorption was found to be around 85% from the figures at pH 3, temperature 32°C, time 30 min and biomass concentration 1mg/ml. The comparison of the sorption performance of the different biosorbents i.e.; with mixed cultures and individual cultures of Bacillus subtilis and Pseudomonas aeruginosa was achieved under the fixed environmental conditions (i.e. pH, temperature, and biomass). The results of mixed culture biosorption are effective and interesting than individual cultures. More research is in this aspect encouraged as the process done with attenuated cultures, ecofriendly and more economical as it is minimizing maintenance cost.

**REFERENCES**