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Adsorption of Lead (II) Ions from Aqueous Solutions by Bacterial Alginate

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Abstract: Bacterial strain H 8, which produces high amount of extracellular polysaccharide alginate, was isolated from soil, and identified as strain of *Azotobacter chrococcum* by its biochemical /physiological characteristics, alginate was extracted, partially purified and used as adsorbent. Analysis of alginate by Fourier transform infrared spectrometry (FTIR) show that the -OH groups present in bacterial alginate are clearly seen at 3433.06 cm-1, the peaks attributed to the -CH_3 groups present at 2916.17 cm-1,and some distinct peaks such as carboxyl group showed strong absorption bands at 1604.66 cm-1, 1411.80 cm-1 and 1303.79 cm-1. Adsorption of lead (II) ions onto bacterial alginate was investigated with the variation in the parameters of contact time, temperature, pH and the amounts of adsorbent. Alginate has a high affinity and binding capacity for lead (II) ions, best adsorption level occurred after 30 min of treatment. Addition 50 mL of 1% alginate to 50 mL of solution containing lead (II) ions at 35°C, alkaline pH (10) was more suitable for lead (II) ions adsorption than neutral or acidic pH, and the lead (II) ions in the original solution can be removed completely by bacterial alginate, the removal ratio for lead (II) ions was 100 %.

Keywords: Aginate, *Azotobacter chrococcum*, adsorption, lead (II) ions.

INTRODUCTION

Heavy metal pollution has become a serious health concern in recent years. Continuous exposure to low levels of heavy metals may result in bioaccumulation and resulting health consequences in humans[1]. Lead (II) ion is one of the most poisonous heavy metal ions that accumulates in muscles,

bones, and kidney and brain tissues and has the potential to cause various disorders, and is regarded as the priority controlled pollutant in many countries[2]. Lead contamination is mainly due to industries related to lead batteries, phosphate fertilizer, electronic, wood production, paint, oil, metal, and also combustion of fossil fuel, automobile emissions, mining activity, forest fires, and sewage wastewater etc[3]. The methods used for the removal of trace metals from water include precipitation[4], ion exchange[5], solvent extraction[6] and adsorption[7].

Among these methods, adsorption has been proved to be an efficient and economical technique. Activated carbon and silica gel are the two most popular adsorbents[8], in trace element analysis. But they are relatively expensive materials since the higher the quality, the greater their cost. Looking for alternative adsorbents has intensified in recent years. At present, the focus is on alginate, alginate is a negatively charged polymer composed of two monomeric units, β - D- mannuronic acid and C5-epimer-α-L-guluronic acids, its unique and random structural pattern has attracted a lot of scientific and commercial interest over the past decade[9].

Alginate has hydrophilicity, biocompatibility, nontoxicity, exceptional formability and it has a high affinity and binding capacity for metals ions[10]. In the present work, we have used the bacterial alginate as adsorbent for lead (II) ions. A series of experiments were then performed to optimization the adsorption process.

MATERIALS AND METHODS

Microorganism: The organisms were isolated by using routine microbiological techniques from the soil; the isolated organisms were maintained on slant agar medium at 4 °C.

Alginate production: Highest alginate producer isolate was inoculated into a 250-mL flask containing 100 mL of enrichment medium contained (per liter) 20 g sucrose, 0.3g K_2HPO_4, 0.7g KH_2PO_4, 0.2 g $MgSO_4.7H_2O$, 0.1 g $CaCl_2.2H_2O$, 0.05 g $FeSO_4.9H_2O$, 0.005 g $Na_2MoO_4.2H_2O$,5 g yeast extract, pH 7 and cultivated at 28 ˚C for 18 h . 5% of the culture was then transferred into another 250-flask containing 100 mL of fermentation medium[11], as modified by Husam[12] , containing (per Liter) 10 g sucrose, 3.2 g K_2HPO_4, 0.8 g KH_2PO_4, 0.4 g $MgSO_4.7H_2O$, 0.2 g NaCl, 0.02 g $FeSO_4.9H_2O$, 0.03 g $Na_2MoO_4.2H_2O$, 0.05 g $CaCO_3$, pH 6.5. The alginate was produced under optimum conditions by incubation at 30˚C for 5 days in shaking incubator at 150 rpm.[12]

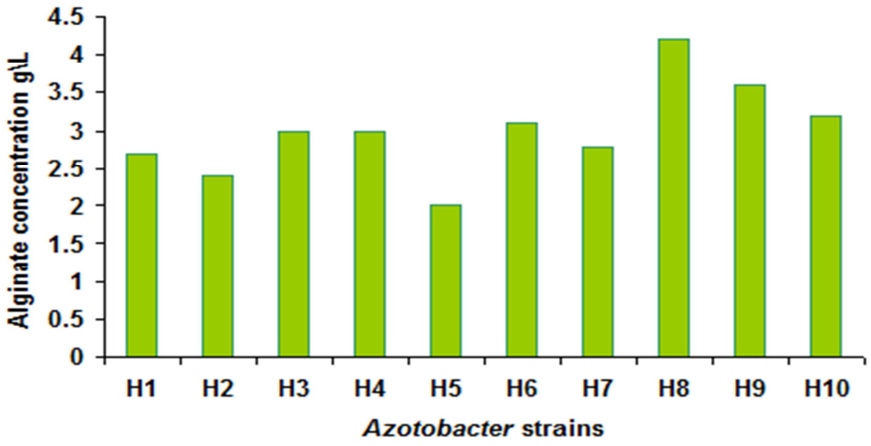
Extraction and partially purified of alginate: Capsular alginate was solubilized by adding 2 mL of 5.0M NaCl and 4 mL of 0.05 M disodium EDTA into a 250-mL flask containing 100 mL of the culture; pH was adjusted to 7.0, and the flask was shaked for 5 min then the content was centrifuged at 18000 rpm at 15°C for 30 min to precipitate the cells. Alginate in the culture supernatant fluid was precipitated by addition of 3-volumes of ice cold isopropanol, and the precipitated alginate was collected on a Whatman filter paper No.1 and dissolved in water and precipitated again by addition of 3-volumes of ice cold isopropanol[13], collected and dissolved in water at room temperature prior to assay by carbazole assay method[14]. Then extracted alginate was an analyzed by Fourier transforms infrared spectrometry (Shimadzu (8300) Japan).

Adsorption experiments: The adsorption experiment was carried out by the contact method. In brief 25 mL of 1% alginate was added to a 50 mL solution containing (1000 µg / mL) of lead (II) ions which prepared by dissolving 1.83 g of $(CH_3COO)_2Pb.3H_2O$ in 1 Liter of D.W. , mixed thoroughly and left at room temperature (20 ± 0.5°C) for 24 h. The solution was filtered by Whatman No.1 filter paper, diluted 100-200 times and the concentration of lead (II) ions was measured by atomic absorption spectrometer (Perkin-Elmer - USA). Adsorption of ions with alginate was calculated as ratio of ions removal %:

R (%) = ($C_0 - C_1$) / C_0 x 100 where R= Removal Ratio (%); C_0 = concentration of heavy metals ions in the original solution (µg / mL) and C_1 = concentration of heavy metals ions in the treated solution (µg / mL) [15]. Four series of experiments were performed to evaluate the influence of the following variables: contact time, temperature, pH value of the initial alginate solution (adsorbent) and dosage of alginate.

RESULTS AND DISCUSSION

Isolation and identification of the alginate producing strain: In this study, a total of 10 bacterial strains have been isolated from the soil. All those isolates revealed mucoid colonies when grown on enrichment agar medium. These bacterial isolates were screened for their ability to produce alginate, strain H 8 showed the highest alginate production which was 4.2 g / L. Thus it was selected for the further steps of this study **(Figures 1)**. Strain H8 was identified as strain of *Azotobacter chrococcum* by its biochemical and physiological characteristics[16], as shown in **Table 1**.



Figures 1: Screening of alginate producing isolates

Table-1: Taxonomic characteristics of isolated strain H8

Characteristics	Result	Characteristics	Result
Morphology		**Acid from**	
Gram stain	-	Maltose	+
Shape	Rods to more coccoid forms	Rhamnose	-
Cysts produced	+	Fructose	+
Motile	+	Mannitol	+
Physiological characteristics		Sucrose	+
Catalase test	+	**Culture characteristics**	
Oxidase test	+	Anaerobic growth	-
Starch hydrolysis	+	Growth in 1%NaCl	-
Gelatin liquefaction	+	Growth in 0.1%phenol	-
Indole formation	+	Growth in1%sodiumbenzoate	-
Acid from		Growth in 2%glycerol	-
Glucose	+	Yellow - Green Pigment	-
Lactose	+	Brown - Black Pigment	+

Species of *Pseudomonas* and *Azotobacter* are the only prokaryotic sources for extracellular polysaccharide alginate. *Pseudomonas aeruginosa* (a human pathogen causing chronic respiratory infections of cystic fibrosis patients) was first reported to produce this polysaccharide being important for the virulence of this strain and its survival in the lung. Also several species of the genus *Pseudomonas* (*Pseudomonas mendocina* and *Pseudomonas syringae*) have the ability to produce alginate under several conditions[17].

Many strains of *Azotobacter* (a nitrogen fixing soil bacterium) were also found to produce this polymer in complex and synthetic media, considering pathogenicity associated with species of *Pseudomonas* and in view of its potential exploitation as food and pharmaceutical additives, *Azotobacter* appears to be more suitable for a commercial alginate production[13].

Analysis of alginate by FTIR: FTIR spectroscopy is used for organic molecule diagnosis by detecting the active groups and bonds found in the molecule. The results indicate that the -OH groups present in bacterial alginate are clearly seen at 3433.06 cm^{-1}, and the peaks attributed to the $-CH_3$ groups are present at 2916.17 cm^{-1}. In bacterial alginate some distinct peaks such as for carboxyl groups showed strong absorption bands at 1604.66 cm^{-1}, 1411.80 cm^{-1} and 1303.79 cm^{-1}**(Figure 2)**. Containing carboxylic groups in their structure, alginates are defined by the adsorption capacity for metals.

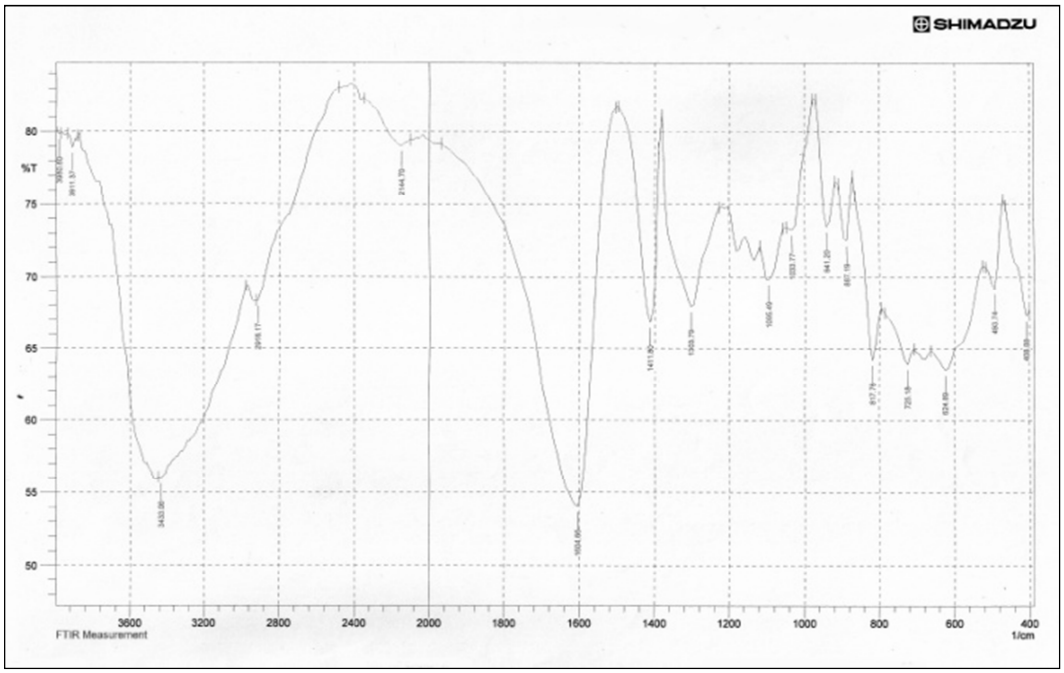
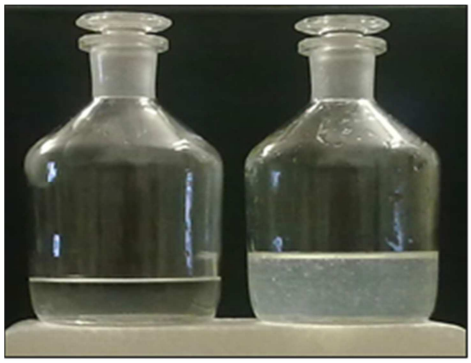


Figure 2: FTIR analysis of alginate

ADSORPTION EXPERIMENTS

Effect of contact time: When 25 mL of bacterial alginate 1% (pH 7) was added to 50 mL of solution containing (1000 µg / mL) of lead (II) ions and left at room temperature (20 ± 0.5°C) for different period of time (10, 20, 30, and 40 min) was used to study the effect of contact time on adsorption process. The lead (II) ions were bound immediately with alginate molecules to form visible gel **(Figure 3)**. The result showed that the removal ratio increased slightly during 10, 20 and 30 min, and then the equilibrium was reached after 40 min. which was 63%, 64%, 65% and 65%, respectively **(Figure 4)** this result indicate that the adsorption process was not dependent on time.



A B

Figure 3: (A) Lead (II) ions solution without alginate. (B) Lead (II) ions solution with alginate.

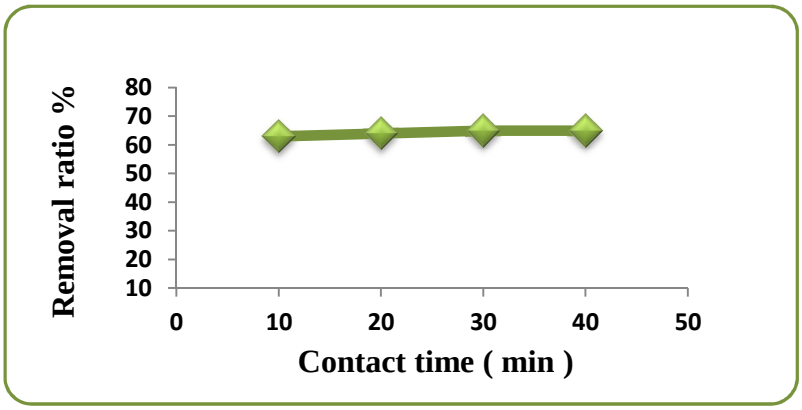


Figure 4: Effect of contact time on adsorption process.

Effect of temperature: The adsorption of lead (II) ions on alginate molecules was studied at temperatures in the range of 20, 25, 30, 35 and 40 °C. The results showed that the removal ratio increased with increase in temperature which was 65, 67, 69.2, 71% respectively, then reached equilibrium at 40 ° C 71 % **(Figure 5)**.

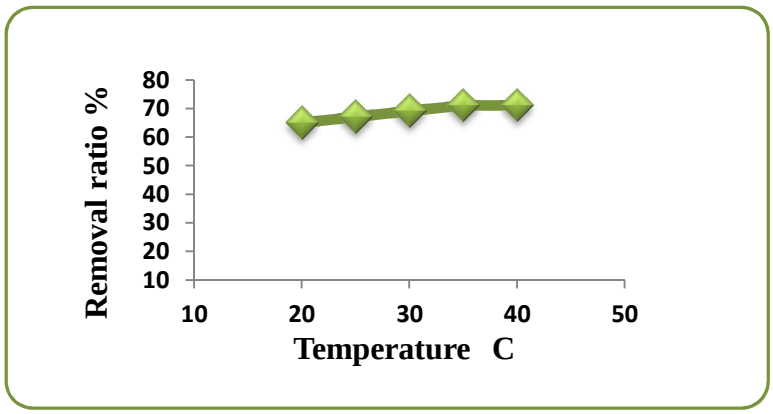


Figure 5: Effect of temperature on adsorption process

The results may be explained by the fact that with the increase in temperature, a greater number of active sites may be generated on the bio polymeric because of the enhanced rate of protonation/ deprotonation of functional groups present on the bio polymeric ,which will clearly bring about an

increase in the adsorption of lead (II) ions. The increased adsorption at higher temperature also suggests the possibility of formation of some co-ordinate type of bonds between the lead and electron rich donor atoms of the adsorbent[18], increased adsorption at increasing temperature is also ascribed to enhanced mobility of metal ions from bulk solution[19]. Removal ratio reached equilibrium at 40 °C due to all the functional groups occupied by lead (II) ions.

Effect of initial pH of alginate solution: The pH is an important factor affecting the removal of cations from aqueous solutions. The dependence of metal bio sorption on pH is related to both the metal chemistry in solution and the ionization state of the functional groups of the bio sorbent which affects the availability of binding sites[20]. When 25 mL of bacterial alginate 1% was added to 50 mL of solution containing (1000 µg / mL) of lead (II) ions and left at 35°C for 30 min. at different pH levels, it was observed that the adsorption occurred at wide range of pH (4 - 10), but it favored the alkaline pH **(Figure 6)**, removal ratio of lead (II) ions ranged from 40% at pH 4 to 80 % at pH 10.

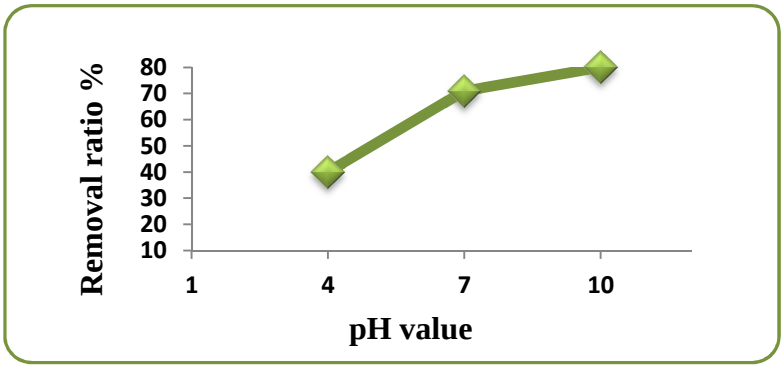


Figure 6: Effect of initial pH of alginate solution on adsorption process

Acidic conditions are less favorable because most of the functional groups of the alginate are prorogated leaving few available ionized groups. Competition between protons and metal species could thus explain the weak adsorption in acidic condition, an increase of pH leads to an ionization of the functional sites inducing an increase of adsorption[20].

Effect of alginate solution volume: When different volumes (25, 50, and 75 mL) of bacterial alginate 1% (pH 10) were incubated with 50 mL of solution containing (1000 µg / mL) of lead (II) ions and left at 35°C for 30 min. It was observed that lead (II) ions in the original solution can be completely removed when 50 mL of alginate was added; the removal ratio was 100% **(Figure 7)**. These results indicate that increasing of alginate solution volume leads to increases the functional groups (carboxylic groups) of the alginate, the binding sites and removal ratio[15].

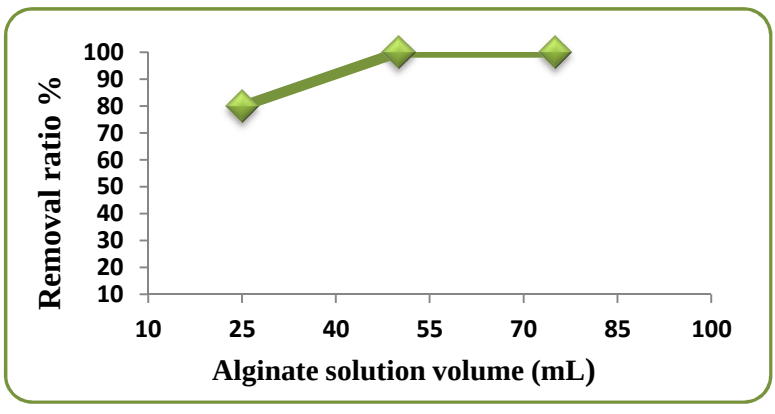


Figure 7: Effect of alginate solution volume on adsorption process

CONCLUSION

The extracellular polysaccharide alginate has a high affinity and binding capacity for lead (II) ions and proved to be an effective adsorbent for removal of lead (II) ions from aqueous solutions, lead (II) ions in the original solution can be completely removed, the removal ratio was 100% after optimization of adsorption process. It can be concluded that the bacterial alginate is effective adsorbent for the removal of lead (II) ions from waste water.

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