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Extraction of alginate from *Azotobacter venillandii* A3 and its potential for copper and zinc ions adsorption

Husam Sabah Auhim and Shatha Salman Hassan

Department of Biology, College of Science, Baghdad University, Baghdad, Iraq

ABSTRACT

Azotobacter vinellandii A3 was isolated from corn field at College of Agriculture in Abu-Ghraib – Baghdad , alginate of A. vinellandii A3 was extracted and partial purified, Analysis of alginate by Fourier Transform Infrared spectrometry (FTIR) shows that the extracted alginate contain bending acetyl group in 1620.09 1/cm, stretching OH group in 3417.63 1/cm , and C-O-C bending group in 1072.35 and 1026.06 1/cm , the ability of alginate of A. vinellandii A3 to adsorb heavy metal ions (Cu^{+2} , Zn^{+2}) at different conditions was studied, the optimum conditions for adsorption process were determined, alginate has a high affinity and binding capacity for Cu^{+2} and Zn^{+2} ions, and the best adsorption level occurred after 10 hrs of treatment. Addition 150 ml of 1% alginate to 50 ml of solutions contain Cu^{+2} ions or Zn^{+2} ions, alkaline pH (9) was more suitable for Cu^{+2} and Zn^{+2} adsorption than neutral or acidic pH.

Keywords: Alginate, *Azotobacter venillandii*, Adsorption.

INTRODUCTION

Alginate, sometimes shortened to “algin”, is a main representative of a family of polysaccharides that neither show branching nor repeating blocks or unit pattern. Alginate is a negatively charged polymer, imparting material properties ranging from viscous solutions to gel [1].It forms gels with a large number of divalent cations under controlled conditions, alginate is a linear copolymer 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 [2].

Heavy metals can be released into the aqueous environment from a variety of sources such as effluents from the plastic, textile, microelectronic and wood preservative-producing industry, and even fertilizer and pesticide usage , the common pollutants are zinc and copper , wastewater that contains this ions are harmful for both irrigational and industrial applications [3]

Alginate has hydrophilicity, biocompatibility, nontoxicity, exceptional formability and it has a high affinity and binding capacity for metals ions and has already been widely used as heavy metals adsorbent in environmental protection [4].Adsorption of ions with alginate was calculated as ratio of ions removal, the ratio of removal measures the quantity in percentage of the heavy metals ions removal from the original solution [5].

MATERIALS AND METHODS

Microorganism

The microorganisms were isolated by using routine microbiological techniques from the soil of corn field at College of Agriculture in Abu- Ghraib – Baghdad, the isolated organisms were maintained on slant agar medium at 4 °C. The isolated bacteria was identified depending on morphological, biochemical test and microscopic examination [6].

Alginate production

A. vinellandii A3 was inoculated into a 250-ml flask containing 100 ml of Enrichment medium [7], contained (per liter) Sucrose, 20 gm; K_2HPO_4, 0. 3 gm; KH_2PO_4, 0. 7 gm; $MgSO_4.7H_2O$, 0. 2 gm; $CaCl_2$. $2H_2O$, 0. 1 gm; $FeSO_4$. $9H_2O$, 0.05 gm; Na_2MoO_4. $2H_2O$, 0.005 gm; Yeast extract,5gm. pH 7, the inoculated medium was incubated at 28 °C for 18 h . 5% of the culture was then transferred into another 250-flask containing 100 ml of fermentation medium [8] contained (per liter) Sucrose, 20 gm; K_2HPO_4, 3.2 gm; KH_2PO_4, 0. 8 gm; $MgSO_4.7H_2O$, 0. 4 gm; Nacl, 0.2 gm; $FeSO_4.9H_2O$, 0.02 gm; Na_2MoO_4. $2H_2O$, 0.03gm; $CaCO_3$, 0.05 gm.pH 7.2, the alginate produced by shaking the flask at 28°C for 5 days,

Extraction and partially purified of alginate

Capsular alginate was solubilized by adding 1 ml of 5.0M NaCl and 2ml 0.05 M disodium EDTA to 50 ml of the culture ,pH was adjusted to 7.0, and shaked for 5 min then 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 ,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, collected and dissolved in water at room temperature prior to assay by carbazole assay method [9] using commercial alginate (Sigma) as a standard, then extracted alginate was a analyses by Fourier transform infrared spectrometry (Shimadzu (8300) Japan).

Precipitation of alginate by different organic solvents.

Alginate in the culture supernatant fluid was precipitated by addition 1:3 volumes of different organic solvents included isopropanol, absolute methanol, and absolute ethanol then alginate was estimated by carbazole assay method.

Specific detection of alginate (gel formation).

Alginate (0.2 gm) was dissolved in 20 ml D.W. then 1 ml of calcium chloride (0.25M) was added to the solution above, immediately formation of gel indicates the presence of alginic acid [10]

Effect of time on adsorption of Cu^{+2} and Zn^{+2} ions by alginate

To determine the effect of treatment time on adsorption of Cu^{+2} by alginate, 50 ml of alginate solution (1%) was added to 100 ml of Standard solution of Cu^{+2} (500 µg / ml), mixed thoroughly and left for 4, 6,8,10 and 12 hrs. at room temperature, the solutions were filtered by Whatman No.1 filter paper, diluted 100-200 times and the concentration of Cu^{+2} was measured by atomic absorption spectrometer (Perkin-Elmer - USA). Adsorption of ions with alginate was calculated as ratio of ions removal %

$$R\ (\%) = (\ C_0 - C_1) / C_0 \times 100$$

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) [5] .Standard solution of Zn^{+2} ions (500 µg / ml) was treated with the same manner**.**

Effect of alginate solution volume on adsorption of Cu^{+2} and Zn^{+2} ions

To determine the relationship between alginate volume and efficiency of adsorption, different volumes (25, 50,100 and 150 ml) of 1% alginate solution were added to 100 ml of standard solution of Cu^{+2} (500 µg / ml), mixed thorough and left for 12 hrs. at room temperature, concentration of Cu^{+2} was measured by atomic absorption spectrometer and removal ratio was calculated. Standard solution of Zn^{+2} ions (500 µg / ml) was treated with the same manner**.**

Effect of pH on adsorption Cu^{+2} and Zn^{+2} ions by alginate

To determine the effect of pH on adsorption of Cu^{+2}, alginate solution 1% was prepared at different pH values (5,7 and 9), 100 ml of standard solution of Cu^{+2} (500 µg / ml)was added to 150 ml of 1% alginate solutions , mixed thoroughly and left for 12 hrs. at room temperature ,concentration of Cu^{+2} was measured by atomic absorption spectrometer and removal ratio was calculated. Standard solution of Zn^{+2} ions (500 µg / ml) was treated with the same manner**.**

RESULTS AND DISCUSSION

Extraction of alginate by different organic solvents.

Alginate was extracted from culture supernatant fluid by three different organic solvents (ethanol, methanol and isopropanol) at a ratio of 1:3 v/v. Results showed that alginate concentration was 3 gm / L in isopropanol extraction , 2.93 gm / L ethanol , and 2.6 gm / L methanol extraction (figure 1).

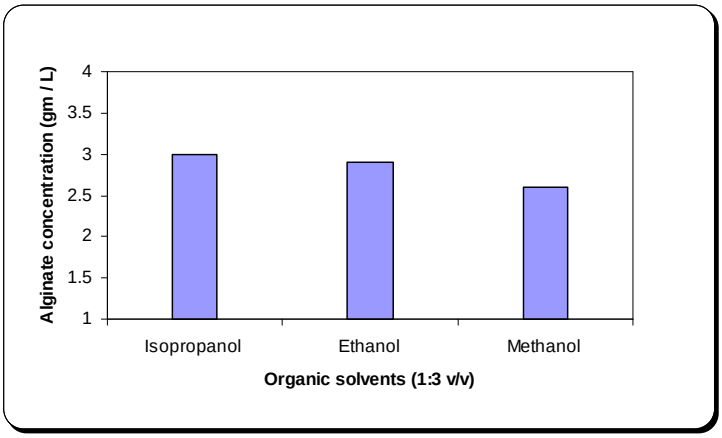

Figure 1: Alginate extracted by different organic solvents

Organic solvents permit separation of polysaccharide by lowering its solubility [11], isopropanol is more miscible with water than ethanol and methanol , miscibility is the property of liquids to mix in all proportions, forming a homogeneous solution**.**

Analysis of alginate by FTIR

Alginate from *A. vinellandii* A3 was analyzed by FTIR spectroscopy to detect the functional groups and confirm the chemical structure of alginate .FTIR technology is used for organic molecule diagnosis by detecting the active groups and bonds found in the molecule. This was done by exposing infrared energy on the sample and measuring the energy of bonds vibration connecting these groups, the group vibrations many forms like stretching, bending, twisting, rocking and wagging depending on the structure of molecules and configuration of the active groups within the molecule [12].

The results indicate that alginate sample contain bending acetyl group in 1620.09 1/cm, stretching OH group in 3417.63 1/cm , and C-O-C bending group in 1072.35 and 1026.06 1/cm (figure 2).

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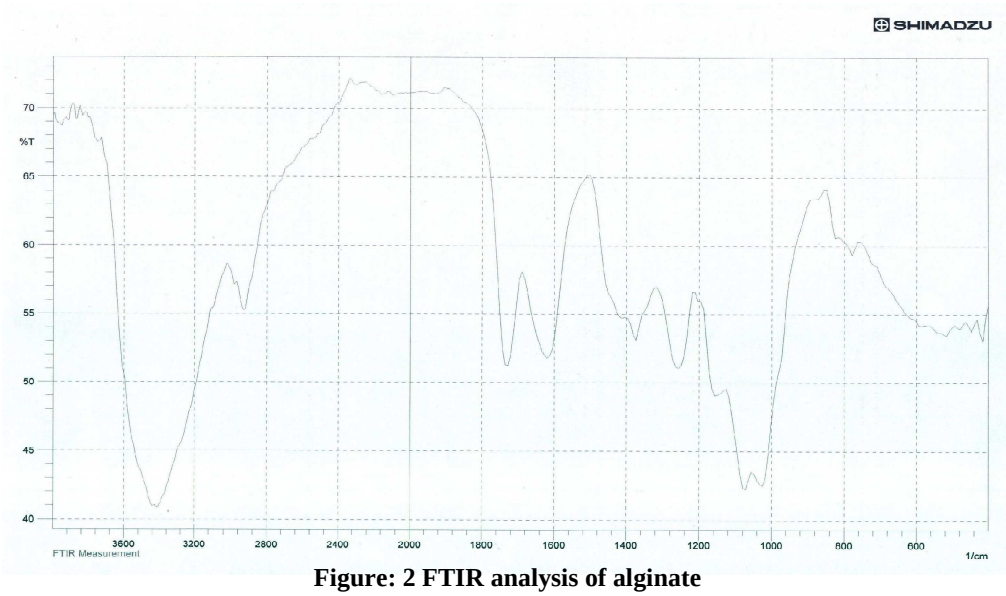

Figure: 2 FTIR analysis of alginate

Purified alginate sample of a strain of *pseudomonas aeruginosa* isolate from burns contain bending acetyl group in 1627.81 1/cm, stretching OH group in 3409.91 1/cm , and C-O-C (ether) bending group in 1288.38 and 1290 1/cm. [13].

Specific detection of alginate (gel formation)

The specific detection of alginate was done by addition of 1 ml calcium chloride (0.25M) to 20 ml of unknown sample, when this step was performed, gel was observed as highly viscous solution indicating the presence of alginate (figure 3). Alginate as a polymer, differ from other polymers such as levan and xanthan by its ability to bind with ions and form gel, a direct mixing of alginate and multivalent cations rarely produces homogeneous gels due to the very rapid and irreversible binding of such ions [14].

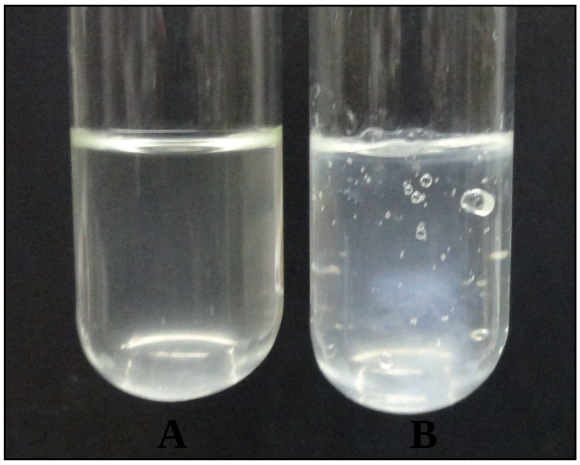

Figure 3 : Specific detection of alginate produced from *A. vinellandii* A3
A) Alginate solution, B) Alginate solution with calcium chloride

Effect of time on adsorption of Cu^{+2} and Zn^{+2} ions by alginate

Figure (4) shows that at the beginning of the treatment(adding the alginate producetion by *A. vinellandii* A3 to the solutions contain Cu^{+2} ions and Zn^{+2} ions) , there was a rapid rise of removal ratio, the process then reached equilibrium after 10 hrs**.**

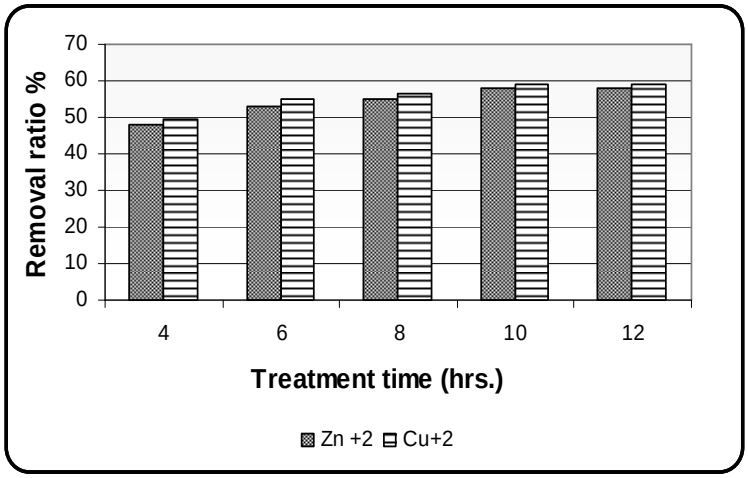

Figure 4: Effect of treatment time on adsorption of Cu^{+2} and Zn^{+2} ions, by *A. vinellandii* A3 alginate,pH7 and at room temperature

Results indicated that after 10 hrs of treatment, the removal ratio increased slightly from 58.9% to 59.1% for Cu^{+2} and 57.8% to 58 % for Zn^{+2}.

Effect of alginate solution volume on adsorption of Cu^{+2} and Zn^{+2} ions

When different volumes of 1% alginate produced by *A. vinellandii* A3 were incubated with 50 ml of Cu^{+2} and Zn^{+2} ions, it was observed that high adsorption occurred at volume 150 ,the removal ratio for both Cu^{+2} and Zn^{+2} were 73 % and 70.1 % respectively (figure 5).

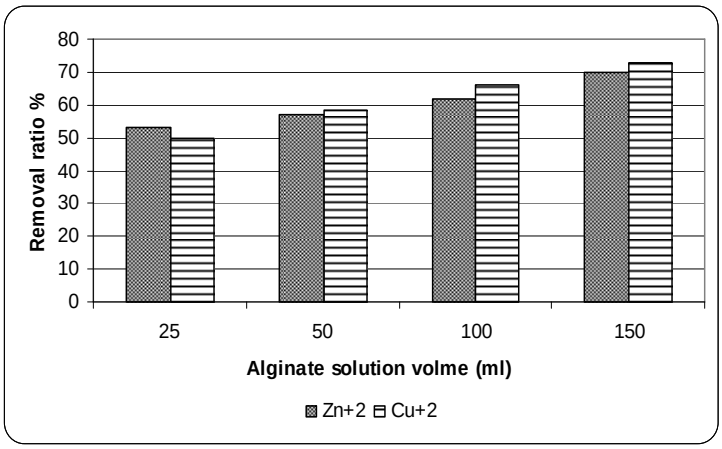

Figure 5 : Effect of *A. vinellandii* A3 alginate solution volume on adsorption of of Cu^{+2} and Zn^{+2} ions,pH7 and at room temperature

These results indicate that increasing of alginate solution volume leads to increaseing the functional groups (carboxylic groups) of the alginate, the binding sites and the removal ratio [5] .Qin et al. reported that the removal ratio was 80.7% when 50 ml of 1 % commercial alginate was added to 100 ml of solution containing Cu^{+2}(1000 µg / ml), and the Cu^{+2} ions in the original solution can be completely removed when 100 ml of commercial alginate is added.

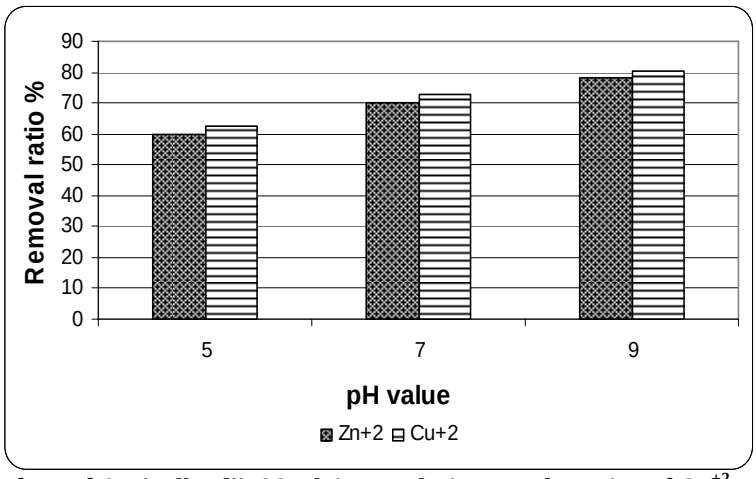

Figure 6 :Effect of pH of *A. vinellandii* A3 alginate solution on adsorption of Cu^{+2} and Zn^{+2} ions

Effect of pH on adsorption Cu^{+2} and Zn^{+2} ions by alginate

The pH is an important factor affecting the removal of cations from aqueous solutions. The dependence of metal biosorption on pH is related to both the metal chemistry in solution and the ionization state of the functional groups of the biosorbent which affects the availability of binding sites [3]. When alginate of *A. vinellandii* A3 was incubated with Cu^{+2} and Zn^{+2} ions at different pH levels , it was observed that the adsorption occurred at wide range of pH (5- 9),but it favored the alkaline pH (figure 6), removal ratio of Cu^{+2} ranged from 60.3% at pH 5 to 80.1% at pH 9 ,while removal ratio of Zn^{+2} was 59.6% at pH 5 and 78% at pH 9.

Acidic conditions are less favorable because most of the functional groups of the alginate are protonated leaving few available ionized groups. Competition between protons and metal species could thus explain the weak adsorption in acid medium, an increase of pH leads to an ionization of the functional sites inducing an increase of adsorption [3]. It can be recommended that in adsorption process, it is more suitable to prepare the alginate in neutral and alkaline environment.

CONCLUSION

The extracellular polysaccharide alginate has a high affinity and binding capacity for Cu^{+2} and Zn^{+2} ions and proved to be an effective adsorbent for removal of Cu^{+2} and Zn^{+2} ions from aqueous solutions, the removal ratio of Cu^{+2} and Zn^{+2} ions were 80.1% and 78% respectively after optimization of adsorption process. It can be concluded that the bacterial alginate is effective adsorbent for the removal of Cu^{+2} and Zn^{+2} ions from waste water.

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