

Heavy Metal Adsorption Characteristics of Extracellular Polysaccharide Produced by *Zoogloea ramigera* Grown on Various Carbon Sources

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Abstract *Zoogloea ramigera* produces an extracellular polysaccharide called zooglan, which adsorbs heavy metals. In the current study, *Zoogloea ramigera* was cultured in media containing various carbon sources. When different carbon sources were included in the cultivation medium, there was a change in the composition of zooglan that is mainly composed of glucose, galactose, and pyruvic acid. The various zooglan compositions were analyzed by HPLC, and changes in the heavy metal (lead (II) and cadmium) adsorption characteristics relative to a change in the composition were examined using an atomic absorption spectrophotometer. A high adsorption capacity was observed at a pH higher than 3.0. The adsorption of metal ions was the highest at 35°C, and a higher adsorption was obtained with a lower flow rate. Changes in the zooglan composition did result in changes in the heavy metal adsorption characteristics. Furthermore, it was also found that the pyruvic acid content was more important than the glucose or galactose content for heavy metal adsorption.

Key words: Heavy metals, polysaccharides, adsorption, *Zoogloea ramigera*, immobilization

Microbial polysaccharides are widely used as gelling agents, emulsifiers, stabilizers, binders, and lubricants in the food, pharmaceutical, and chemical industries. Dextran produced by *Leuconostoc mesenteroides* and hyaluronic acid produced by *Streptococcus* sp. are used in the pharmaceutical industry, while xanthan gum produced from *Xanthomonas campestris* is used in the food, chemical, and oil industries [14]. Microbial polysaccharides are also known to play important roles in biosorption of heavy metals [5, 8, 15].

Zoogloea ramigera is a Gram-negative, floc-forming bacterium that produces an exopolysaccharide from various

carbon and nitrogen sources [17]. One of the potential applications of *Zoogloea ramigera* and zooglan is the removal of organic matter from a solution as a part of wastewater treatment processes [1]. Purified zooglan, a polysaccharide produced by *Zoogloea ramigera*, consists of glucose, galactose, and pyruvic acid [2]. The composition of zooglan is known to vary with the growth conditions. Zooglan has a high viscosity and high molecular weight and is very resistant to pH, heat, and mechanical shear stress [14]. It is also known to have the properties of a polyelectrolyte, i.e., it binds to a high concentration of metal ions [2]. Although zooglan has already been studied as an effective adsorbing agent for heavy metal ions [16, 20], the influence of the composition of zooglan on its characteristics of heavy metal adsorption has not yet been investigated.

Accordingly, the current study cultivated *Zoogloea ramigera* with various carbon sources and examined the effect of the resulting compositional change of zooglan on the adsorption of heavy metals (lead (II) and cadmium) ions. The effects of pH, temperature, and flow rate on the heavy metal adsorption were also investigated using calcium alginate beads.

MATERIALS AND METHODS

Microorganism and Culture Conditions

Zoogloea ramigera (KCTC 2582) was used in the current study. The seed culture medium was a tryptic soy broth (30 g/l), while the main culture medium was a mineral salts medium (MSM) that contained 25 g/l glucose, 2 g/l K₂HPO₄, 1 g/l KH₂PO₄, 1 g/l NH₄Cl, 0.2 g/l MgSO₄·7H₂O, and 0.01 g/l yeast extract (Difco Lab., Detroit, U.S.A.). The glucose and MgSO₄·7H₂O were autoclaved separately [18].

The *Zoogloea ramigera* was cultivated in 250 ml Erlenmeyer flasks containing 50 ml of MSM using a rotary shaker (Vision Scientific Co., Korea). The rotation speed was 200 rpm and the temperature was 26°C [18].

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Immobilization

Zooglan was separated from the culture broth by centrifugation at 10,000 rpm for 30 min and resuspended in a 3% sodium alginate solution. The ratio of sodium alginate to polysaccharide was 3 (w/w). This mixture of polysaccharide and sodium alginate was then dropped into a 0.2 M CaCl₂ solution using a peristaltic pump (Cole Parmer, U.S.A.) to produce gel beads [23]. The diameter of the gel beads was 3.0±0.5 mm. Finally, the gel beads composed of calcium alginate and zooglan were stored in a CaCl₂ solution at 4°C for 2 h for hardening.

Effect of Carbon Sources

To investigate the effect of different carbon sources on the composition of zooglan and the resulting changes in its heavy metal adsorption characteristics, *Zoogloea ramigera* was cultivated in a medium containing various carbon sources, i.e., galactose, lactose, maltose, fructose, and sucrose, respectively [9, 10]. After cultivation, the polysaccharides were recovered from the culture broth, and immobilized beads were prepared using the same method described above.

Effect of Glucose Analogs

Seed cultures were prepared in MSM at 30°C and 180 rpm for 3 days. A 3% (v/v) inoculum was applied to 150 ml of MSM (1% glucose and 2% glucose analog (g/l): glucose, 10; glucose analog, 20; K₂HPO₄, 2; KH₂PO₄, 1; NH₄Cl, 1; MgSO₄·7H₂O, 0.2; yeast extract, 0.01) in 500-ml Erlenmeyer flasks. The main culture was carried out for 5 days under the same conditions used for the seed culture preparation. Glucosamine (2-amino-2-deoxy-D-glucose, Sigma Chemical Co., U.S.A.) and N-acetylglucosamine (2-acetamido-2-deoxy-D-glucose, Sigma Chemical Co., U.S.A.) were used as the glucose analogs [11]. After cultivation, the culture broth was separated, and immobilized beads were prepared using the same method described above.

Isolation of Zooglan and Analysis of Its Composition

After the cultivation of *Zoogloea ramigera*, the culture broth was recovered by centrifugation, then subjected to ultrasonication twice using a sonicator (Sonic U200S, IKA, Germany) with a 40 sec interval. The centrifugation was carried out at 10,000 rpm for 30 min to remove the cells and cellular debris (Centrifuge 5403, Eppendorf, Germany). One percent (w/v) KCl and 2 vol of propanol were added to the supernatant, and the solution was maintained at 4°C for 24 h to precipitate the polysaccharide. The precipitate was then dried at 60°C for 24 to 48 h, and the dried zooglan was ground and screened through a US sieve No. 200 (75 µm).

Next, the dried zooglan powder was dissolved in 5 ml of 0.1 M trifluoroacetic acid (TFA) and hydrolyzed at 120°C for 6 h [24]. The hydrolysate was then filtered using 0.2 µm

cellulose acetate filters (Satorius, Germany) and analyzed by high performance liquid chromatography (HPLC). The sample analysis was performed at 45°C using an HPLC 10A system (Shimadzu, Japan) equipped with both an RI detector LC-10AD (Shimadzu, Japan) for a sugar analysis and UV detector SPD-10A (Shimadzu, Japan) for an organic acid analysis (220 nm). The HPLC column was an Aminex HP87H (Bio-Rad Laboratories, U.S.A.), and 0.008 N sulfuric acid at a flow rate of 0.5 ml/min was used for the mobile phase.

Adsorption Studies

Lead (II) and cadmium solutions were prepared by diluting a 1.0 g/l stock solution of each heavy metal. The stock solutions of lead (II) and cadmium were prepared by dissolving N₂O₆Pb and CdN₂O₆·4H₂O (Fluka, U.S.A.) in deionized water. Ten polysaccharide beads were prepared and mixed with 10 ml of the desired metal solution in an Erlenmeyer flask. The flasks were agitated in a shaker at 35°C and 250 rpm for metal adsorption. After reaching equilibrium, the supernatant was diluted with 1% HNO₃ solution and the metal ion concentrations were analyzed [22, 23]. The amounts of unadsorbed lead (II) and cadmium ions in the supernatant were determined using an AAS (Atomic Absorption Spectrophotometer) 5EA (Analytik Jena, Germany).

After the metal adsorption, the removal efficiency of the regenerated immobilized zooglan for heavy metal ions was investigated using 0.1 N HNO₃ solution. Ten polysaccharide beads were prepared and mixed with 10 ml of the desorbent solution in an Erlenmeyer flask. The flasks were shaken at 35°C and 250 rpm for metal desorption. Thereafter, the supernatant was diluted with 1% HNO₃ solution and the metal ion concentrations were analyzed using an AAS [22, 23]. All the experiments were performed in duplicate, and mean values were used in the data analysis.

Effect of pH

pH is one of the most important parameters that affect adsorption capacity. As such, the adsorption experiments were performed at various pH values to investigate any change in the adsorption characteristics of zooglan relative to the pH. The heavy metal solutions were prepared at a concentration of 100 mg/l, and HNO₃ and NaOH were used for the pH adjustment [3]. The pH of the metal solutions was adjusted between 1.0 and 7.0, based on 1.0 unit increments.

Packed Bead Column Reactor

A packed bead column, 2.6 cm in diameter and 60 cm in height with a total volume of 25 ml, was used in the adsorption studies. The column was filled with 500 immobilized beads, then the heavy metal solution was passed through the column and the effluent was analyzed

for its metal ion concentrations. After the metal adsorption experiment, the heavy metals were desorbed by 0.1 N HNO_3 [22].

Effect of Temperature and Flow Rate

The adsorption experiments were also carried out at various temperatures and flow rates. The temperature was maintained at 25°C, 35°C, and 45°C using a low-temperature water bath (VS-1205 CW, Vision Scientific Co., Korea) [23], while the flow rate of the heavy metal solutions was regulated with a peristaltic pump [4, 22]. The flow rates used in the current study were 1.0 ml/min, 3.0 ml/min, 6.0 ml/min, and 10.0 ml/min.

RESULTS AND DISCUSSION

Adsorption Studies

The adsorption of lead (II) and cadmium by the zooglan beads reached an equilibrium approximately 12 h after the adsorption started. Therefore, in the batch experiments, the metal adsorption was carried out for 24 h to ensure sufficient time to achieve an adsorption equilibrium.

Since the biosorbent used in metal adsorption should not cause secondary pollution, the adsorbed heavy metal should be recoverable through desorption after adsorption [22]. The desorption of cadmium by the regenerated zooglan beads was higher than that of lead (II) (60–70% for lead (II) and 80–90% for cadmium).

Effect of pH

In the adsorption process, it is already known that the adsorption capacity is very sensitive to a change in the pH of the aqueous solution, while the optimum pH for heavy metal adsorption is affected by the type of biosorbent [6]. A variation in the pH can change the speciation and availability of the metal ions in the solution, along with the state of the chemical functional groups responsible for metal binding in the polysaccharides. In the current study, adsorption experiments were carried out at various pHs between pH 1 and pH 7. The precipitation of heavy metals was observed at pH 8, which was probably due to excess hydroxide ions (OH^-) in the metal solution [20].

The adsorption and desorption of lead (II) and cadmium by the alginate beads and zooglan beads containing alginate and zooglan are shown in Figs. 1 and 2, respectively. When the alginate beads were used alone, the heavy metal adsorption and recovery were less than 50% for lead (II) and cadmium for the pH range examined (Fig. 1). However, the heavy metal adsorption and recovery were much higher for both metals when the beads were mixed with the polysaccharide produced from the glucose-containing medium (Fig. 2). A low adsorption of cadmium and lead (II) ions (less than 50%) was observed at pH 1 and 2, while

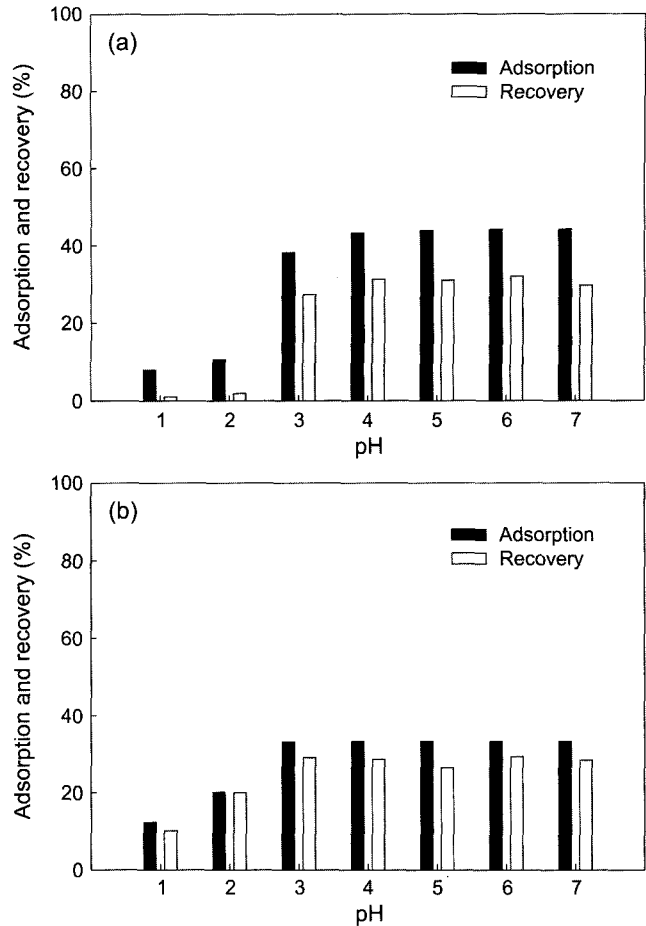


Fig. 1. Effect of change in pH on metal adsorption and recovery with immobilized alginate beads. (a) Lead (II), (b) Cadmium.

a high adsorption was observed at a pH higher than 3.0. Adsorption and desorption experiments using beads that contained alginate and the polysaccharides produced by *Z. ramigera* which were grown on media containing different carbon sources, also showed similar results (data not shown). The low metal adsorption at a low pH (pH 1 and 2) was attributed to competition between the heavy metal ions and the hydrogen ions for available adsorption sites. As such, at a very low pH, the hydrogen ions competed strongly with the metal cations, thereby decreasing the percentage removal of metals from the solution [3, 7].

Effect of Temperature

The adsorption of heavy metals is a physicochemical process and thus likely to be affected by temperature. The effect of temperature on the adsorption capacity of lead (II) and cadmium by the zooglan beads was investigated in a continuous packed bed column (Figs. 3 and 4). The concentration of the feed heavy metal solution was 1.0 g/l. The polysaccharide produced from the glucose-containing medium was used together with alginate to prepare the

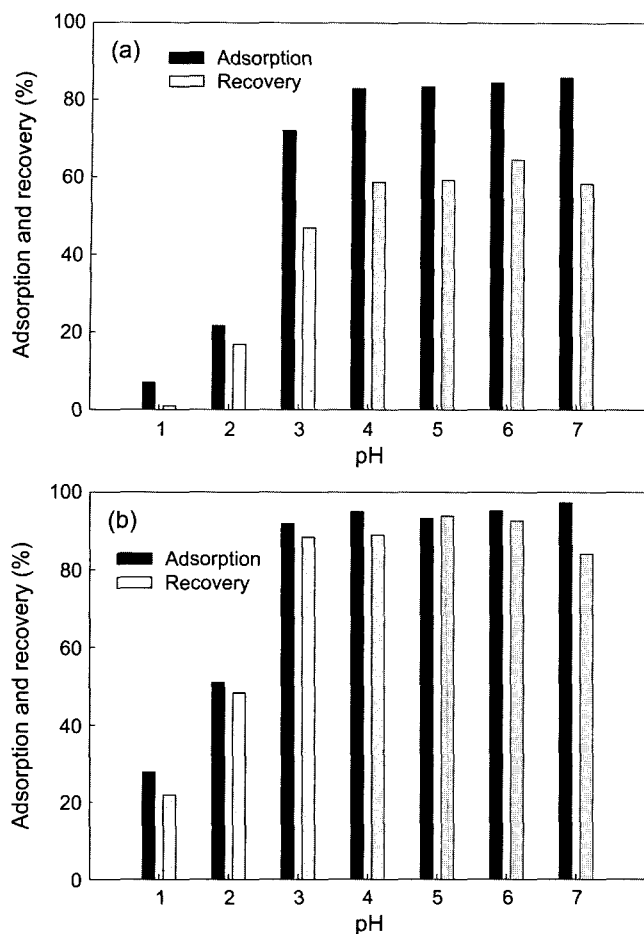


Fig. 2. Effect of change in pH on metal adsorption and recovery with immobilized zooglan beads. (a) Lead (II), (b) Cadmium.

beads. The adsorption experiments were carried out at 3 different column temperatures: 25°C, 35°C, and 45°C. The adsorption and desorption were the highest at 35°C (Figs. 3 and 4). A physical adsorption reaction is exothermic and reversible, and is dominant in heavy metal adsorption at a lower temperature. However, chemical adsorption, which can be either reversible or irreversible, generally occurs at higher temperature [23]. Consequently, the efficiency of the heavy metal recovery process can be improved by selecting the proper temperature for the adsorption and desorption processes. The optimal temperature for the recovery of cadmium and lead (II) by the zooglan beads was 35°C.

Effect of Flow Rate

To investigate the effect of the flow rate on the adsorption of lead (II) and cadmium by the zooglan beads, the influent metal concentration was held constant at 1.0 g/l, while the flow rate was changed from 1.0 to 10.0 ml/min. The maximum adsorption of lead (II) and cadmium at a flow rate of 1.0 ml/min was 408.04 mg/l (81.61%) and 474.35 mg/l (94.87%), respectively. As shown in Fig. 3, the

adsorbed metal ion concentrations decreased with flow rate, regardless of the temperature. The metal recovery of the continuous system was much lower than that of the batch system. At higher flow rates, the residence time of the solute metal ions was probably not long enough to reach an adsorption equilibrium. Consequently, a lower flow rate was required for a higher metal recovery in the continuous system.

For the effective recovery of the heavy metals from the beads, the metal ions should be adsorbed on the surface of the beads or inside the pores. The porous structure of calcium alginate causes difficulties in the mass transfer of metal ions into the beads [22] by requiring longer residence time for the metal ions to diffuse into the pores. Thus, at a higher flow rate, the residence time of heavy metal ions is too short for either surface adsorption or pore diffusion [20]. Consequently, the longer the contact time between the heavy metal ions and the immobilized zooglan beads, the greater the amount of heavy metal adsorbed. Similar results were also obtained when a biomass was used for metal adsorption instead of polysaccharides [21].

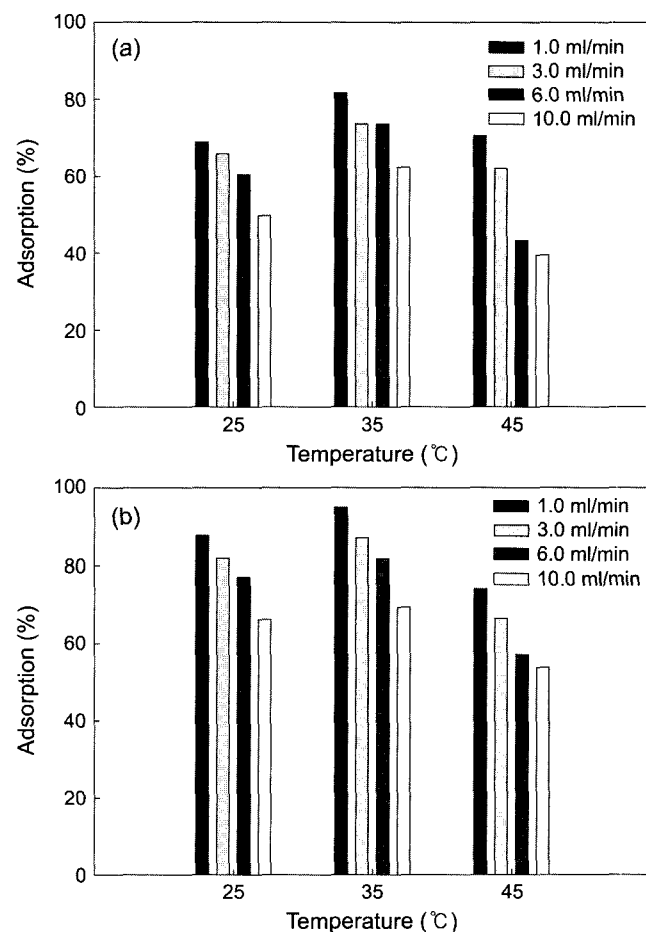


Fig. 3. Effect of change in flow rate and temperature on metal adsorption in continuous system. (a) Lead (II), (b) Cadmium.

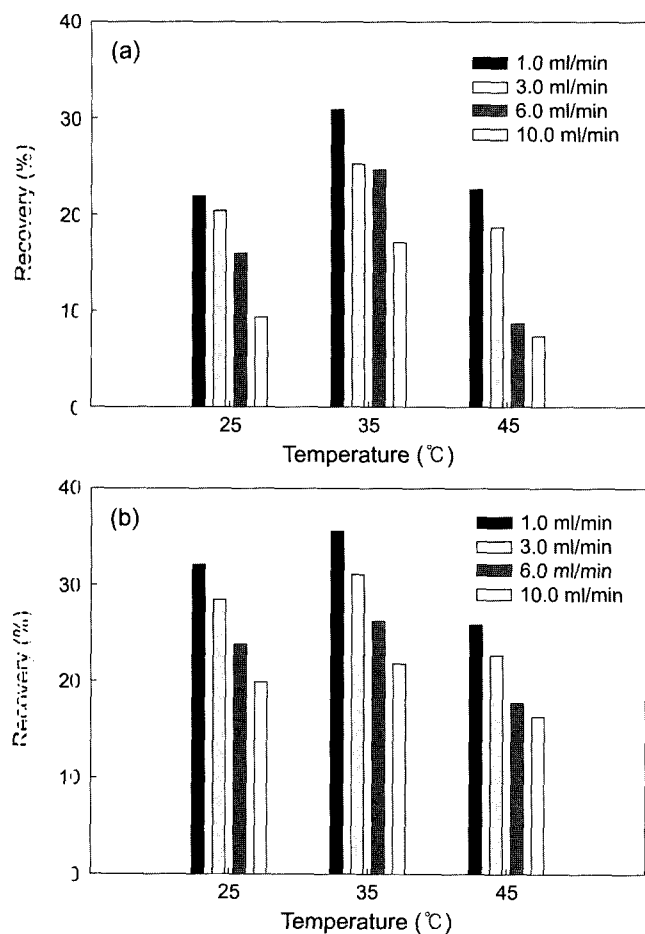


Fig. 4. Effect of change in flow rate and temperature on metal recovery in continuous system. (a) Lead (II), (b) Cadmium.

Effect of Carbon Source on Composition of Zooglan and Heavy Metal Adsorption Characteristics in Packed Bead Column System

Adsorption experiments using immobilized zooglan beads prepared using different carbon sources were carried out in

a packed bead column reactor at 35°C and 1.0 ml/min (Table 1). Immobilized beads made of just alginate were used as the control experiment, and the maximum adsorption percentage of lead (II) and cadmium with these beads was 37% and 19%, respectively. However, when alginate beads were prepared with zooglan produced from a glucose-containing medium, the maximum adsorption of lead (II) was 81% and that of cadmium was 94%.

The various zooglan compositions were determined by a HPLC analysis after acid hydrolysis. The polysaccharides were produced by *Z. ramigera* in media containing different carbon sources, such as glucose, galactose, lactose, maltose, fructose, sucrose, or glucose analogs. There was a significant difference in the zooglan composition, when glucose analogs (glucosamine, *N*-acetylglucosamine) were added to the medium. Since glucose analogs alone do not support cell growth [11], they were added to the culture medium along with glucose.

The composition of the polysaccharide produced by *Z. ramigera* from the glucose-containing medium was mainly glucose, galactose, and pyruvic acid with a molar ratio of approximately 12.7:3.4:1.5. When *Z. ramigera* was cultivated with different carbon sources, changes were found in the molar composition and metal adsorption characteristics of the resulting zooglan. When glucose, lactose, maltose, fructose, or sucrose was used as the carbon source, there was not much change in the zooglan composition (ratio of glucose, galactose, and pyruvic acid). However, the fraction of pyruvic acid was lower when galactose was used as the sole carbon source (Table 1), and the adsorption percentages of lead (II) and cadmium were also lower in comparison with those of the polysaccharides prepared using the other carbon sources.

When glucosamine and *N*-acetylglucosamine were added to the glucose-containing medium, the glucose content in the resulting polysaccharide was much lower than that in the other polysaccharides produced from the other carbon sources. However, the adsorption percentages of lead (II)

Table 1. Effect of various carbon sources on composition and metal adsorption and recovery of polysaccharides produced by *Z. ramigera*.

Control		Pb adsorption (%)	Pb recovery (%)	Cd adsorption (%)	Cd recovery (%)
Alginate		37.39	15.50	18.83	17.41
Carbon sources	Glu ^a :Gal ^b :Pyr ^c	Pb adsorption (%)	Pb recovery (%)	Cd adsorption (%)	Cd recovery (%)
Glucose	0.72:0.20:0.08	81.60	61.79	94.87	71.09
Galactose	0.76:0.21:0.03	60.63	43.13	69.18	51.77
Lactose	0.74:0.19:0.07	82.19	63.05	93.66	68.29
Maltose	0.71:0.23:0.06	83.77	63.39	95.43	70.75
Fructose	0.74:0.21:0.05	77.05	56.53	85.94	62.70
Sucrose	0.74:0.20:0.06	83.92	63.46	95.57	69.94
Glucose+glucosamine	0.27:0.68:0.05	92.25	69.18	95.67	71.62
Glucose+ <i>N</i> -acetylglucosamine	0.25:0.70:0.05	81.99	61.09	93.48	68.73

^aGlucose. ^bGalactose. ^cPyruvic acid.

and cadmium were similar to the adsorption percentages obtained, when using the other carbon sources. This indicates that glucose and galactose did not play an important role in the lead (II) and cadmium adsorption, making the carboxyl group of pyruvic acid more significant for the adsorption of lead (II) and cadmium by zooglan [12, 13, 19].

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