



## BIOSORPTION OF $\text{Ag}^+$ , $\text{Cu}^{2+}$ AND $\text{Fe}^{3+}$ BY GELLAN GUM

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**ABSTRACT:** The biosorption behaviors and mechanisms for  $\text{Ag}^+$ ,  $\text{Cu}^{2+}$  and  $\text{Fe}^{3+}$  by a novel exopolysaccharide (gellan) from *Sphingomonas paucimobilis* have been studied in this paper. The influences of gellan concentration, solution pH, desorption study and ionic strength on adsorption property were investigated. The gellan showed strong biosorption capability, up to 169.15 mg/g of  $\text{Ag}^+$ , 216.09 mg/g of  $\text{Cu}^{2+}$  and 84.47 mg/g of  $\text{Fe}^{3+}$ , respectively. Infrared spectrometry analysis demonstrated that the groups of O—H, C=O, C—O—C and  $\text{CH}_2$  of the gellan which involved in metal biosorption process were the main functional groups for binding metal ions. Solvent of 0.1N Sulphuric acid showed higher desorption of  $\text{Ag}^+$  (94%),  $\text{Cu}^{2+}$  (90%),  $\text{Fe}^{3+}$  (86%) and adsorption-desorption process can be continued till seven cycles efficiently.

**Key words:** Gellan, exopolysaccharide, biosorption, metal ions.

## INTRODUCTION

Heavy metals in the environment are difficult to remove by chemical or biological means. Today, many heavy metals constitute a global environmental hazard (Mejare and Bülow, 2001). Heavy metals usually can be toxic, carcinogenic or mutagenic even at trace levels. It has been established that all metals or elements with metallic characteristics can form compounds that are toxic to human (Goyer, 1991).

Eco-toxicity on living organism has become a prime concern from the last few decades. Massive urbanization are continuously releasing waste and waste water to the ecosystem and, causing pollution to environment and eventually toxicity to living being. The industrial effluents which contain different derivatives of heavy metals such as Cd, Pb, Ni, Cr, As, Cu, Fe *etc.*, are continuously discharging to the ecosystem and producing a significant toxic impact on aquatic environment. Among the heavy metals, copper is the major available type of heavy metal in the aquatic environment. Copper in the blood system may generate reactive free oxygen

species and damage the protein, lipids and DNA (Brewer, 2010). The excess copper compound in the body may also affects on aging, schizophrenia, mental illness, Indian childhood cirrhosis, Wilson's and Alzheimer's diseases (Brewer, 2007; Faller, 2009; Haureau and Faller, 2009). Copper has damaged the marine ecosystem and damaged the gills, liver, kidneys, the nervous system and changing sexual life of fish (Flemming and Trevors 1989; Van Genderen *et al.*, 2005). Recent awareness of toxicity originated from water and waste water are attending the interest for environmental scientist and researcher.

The extracellular polysaccharide, commercially known as gellan gum or heteropolysaccharide, was produced by *Sphingomonas paucimobilis* (formely *Pseudomonas elodea*) (Kang and Veeder, 1982 ; Martin *et al.*, 1996). The gellan gum consists of linear repeating tetrasaccharide ( $\rightarrow 3$ )- $\beta$ -D-Glc-(1  $\rightarrow$  4)-  $\beta$ -D-GluA-(1  $\rightarrow$  4)- $\beta$ -D-Glc-(1  $\rightarrow$  4)- $\alpha$ -L-Rha-(1 $\rightarrow$ ) composed of d-glucose (Glc), d-glucuronic acid (GlcA), and l-rhamnose residues (Rha) (Jansson *et al.*, 1983). The gellan gum exhibits good stability over a wide pH range (3.5–8.0). Acid stability of gellan

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gum is a distinct advantage in fruit-based products. According to its property to produce a thermoreversible gel, gellan gum can be substituted for agar (Sylvain and Lacroix, 1990). Due to diversity of its structure and properties, gellan gum has a wide range of applications in the food, pharmaceutical, and other industries as texturizing, stabilizing, thickening, emulsifying, and gelling agents (Francois *et al.*, 1986; Hans and Olav, 1987). The total gelatin market in the Western Europe alone is 60,000 ton per year, of which 80% is in foods. One of the possible gelatin alternatives for the food industry is gellan gum (Morrison *et al.*, 1999).

The main aim of this research is to determine the potentiality and adsorption capacity of gellan as bioadsorbent.

## MATERIALS AND METHODS

### Materials

Gellan was purchased from sigma Inc.  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{AgNO}_3$  and  $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$  were supplied by Gmhourai chemical reagent Co., All reagents were of chemical grade.

### Preparation of Metal Solution

The stock solutions of heavy metals at 1g/l concentrations were prepared by dissolving a required quantity of copper, silver and ferric (1000 mg/l) was prepared of copper nitrate ( $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$  silver nitrate ( $\text{AgNO}_3$ ) and ferric chloride ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ) and used for all experiments with required dilution. The pH of the solution was adjusted to required value by adding either 1 M  $\text{HNO}_3$  or 1 M  $\text{NaOH}$ . The resulting stock solutions were stored in air tight bottle.

### Methods

#### Scanning Electron Microscopy (SEM)

The Scanning Electron Microscopy (SEM), Model: Hitachi SU-1500 was used for the study of gellan gum structure. The micrographs obtained before and after the adsorption of metal ions were compared to study the adsorption efficiency. Data size was 1280 x 960, accelerating voltage 15000 V, deceleration voltage was 0V and emission current was 86000 nA. SEM analysis was used for the study of

gellan gum structures using high magnification, the micrographs obtained before and after the adsorption of metal ions were compared to study the adsorption efficiency.

#### Study of adsorbent doses

The effect of adsorbent doses on the equilibrium adsorption of copper was investigated with gellan of 0.05, 0.1, 0.2, 0.5, 1, and 2 g in five set of 100 ml water which contained 1, 2.5, 5, 10 and 15 mg/l of copper, silver and ferric concentration each. The Erlenmeyers were shaken for 24 hours with 120 rpm at room temperature. The water samples were then filtered and analyzed in terms of heavy metals by AAS.

#### Study of pH

The effect of pH for copper adsorption onto gellan was investigated with 0.5g of gellan in 100 ml water containing 10mg/l of heavy metals and each sample were adjusted to pH between 2.0 to 13.0 using either 1N  $\text{HNO}_3$  or  $\text{NaOH}$  solution. The Erlenmeyers were shaken for 24 hours with 120 rpm at room temperature.

#### Study of contact time and shaking speed

The effect of temperature, contact time and shaking speed (rpm) on adsorption of heavy metals were examined with different temperature (30-70°C), time (3 hr.) and speed (30-200 rpm) for 10 mg/l copper concentration with 0.5 g gellan in 100 ml water.

#### Infrared analysis

The infrared (IR) spectrum of gellan gum was determined using a Fourier transform infrared (FTIR) spectrophotometer (Spectrum One-B, Perkin Elmer, US) for detection of various functional groups. The purified gellan gum was ground with KBr powder and pressed into pellets for FTIR measurement in the frequency range of 4000–400  $\text{cm}^{-1}$  (Zhbakov *et al.*, 1997).

#### Desorption study

Desorption of gellan was studied using 7 types of solvent medium including tap water, distilled water, 0.1N  $\text{H}_2\text{SO}_4$ , 0.1N  $\text{HCl}$ , 0.1N  $\text{HNO}_3$ , 0.1N  $\text{NaOH}$  and 0.1 N  $\text{CH}_3\text{COOH}$ . For this purpose, 0.5 g of saturated (with heavy metals) gellan was taken in 100 ml of above mentioned medium and shaken at 120 rpm for 24 hr.

## Analysis

The collected water samples from different experiments were filtered with filter paper (Whatmann 5) and prepared for AAS analysis. The samples were analyzed in term of heavy metals by Atomic Absorption Spectrometer (AAS 932 plus, GBC, Australia). The pH was measured by Hanna HI 9025 (HANNA Instrument, Romania) pH meter. Extracted gellan and heavy metal loaded gellan were characterized by FT -IR spectroscopy (IRPrestige-21, Shimadzu, Japan).

### Adsorption experiments

Batch adsorption experiments were carried out in which aliquots of 25 ml of Cu (II), Ag or Fe (III) solutions of known concentration were poured into flask (100 ml) containing accurately weighed amounts of the adsorbents. The gellan gum weight ranged from 0.1 to 2.0 g per 100 ml of solutions. The flasks were shaken at 120 rpm using an electric shaker for a prescribed length of time to attain equilibrium. After filtration thorough the filter paper, copper and ferric ions remaining in the solutions were determined by AAS. The amount of metal ion adsorbed was calculated as

$$\text{Adsorption (\%)} = \frac{C_0 - C_e}{C_0} \times 100$$

Where:

$C_0$  and  $C_e$  are the initial and equilibrium concentrations of the adsorbate, respectively.

### Adsorption model

The Langmuir equation (Duddridge and Wainwright, 1981) is in the linear form,

$$\frac{C_e}{x/m} = \frac{1}{KX_m} + \left(\frac{1}{X_m}\right) C_e$$

Where:

$x/m$  represents the amount of metal ions adsorbed at equilibrium,  $C_e$  are the equilibrium concentrations,  $X_m$  and  $K$  are Langmuir constants related to maximum adsorption capacity (monolayer capacity) and energy of adsorption, respectively. Langmuir isotherm was obtained from the experiments.

## RESULTS AND DISCUSSION

### Scanning Electronic Microscopy (SEM)

The microporous structure of gellan with particle sizes of 150-212  $\mu\text{m}$  was observed at a resolution of 500x (Fig. 1). The micrograph of biosorbent shows some cavities in the surface structure capable of uptaking heavy metal ions as well as an irregular and porous microstructure of the biosorbent. It is clearly seen that there is a considerable modification on morphology of biosorbent before and after heavy metal ions binding.

The SEM images obtained for the raw adsorbent before any adsorption with the metal ions reveal the surface texture and morphology of the adsorbent, the microporous structure of potato peel with particle sizes of 150-212  $\mu\text{m}$  was observed at a resolution of 1500x (Fig. 1). The micrograph of biosorbent shows some cavities in the surface structure capable of uptaking heavy metal ions as well as an irregular and porous microstructure of the biosorbent. It is clearly seen that there is a considerable modification on morphology of biosorbent before and after heavy metal ions binding. Clear through SEM is no difference between the gellan gum before absorption Fig. 1(A) and after absorption (B), (C) and (D) with both copper, silver and iron, respectively.

### Effect of Initial Gellan Concentration

The influence of gellan concentration in equilibrium adsorption was shown in Fig. 2. Results indicated that, with the dosage of the gellan increasing from 0.4 to 1.5 g/l, the biosorption amounts for all heavy metal ions increased significantly, which increased from 81.05 to 139.75 mgAg<sup>+</sup>/g gellan, from 79.05 to 163.5 mg Cu<sup>2+</sup>/g gellan and from 31.57 to 70.49 mg Fe<sup>3+</sup>/g gellan, respectively. It could be attributed to more available binding sites for metal ions biosorption presented by the increased gellan at low concentration. Although adsorption sites increased at excessive gellan, but the bound metal ions may be unstable and the metal ions surrounded by unit mass of gellan decreased (Zhou *et al.*, 2009).

The biosorption capacity of gellan is selective. It depends on structure and functional

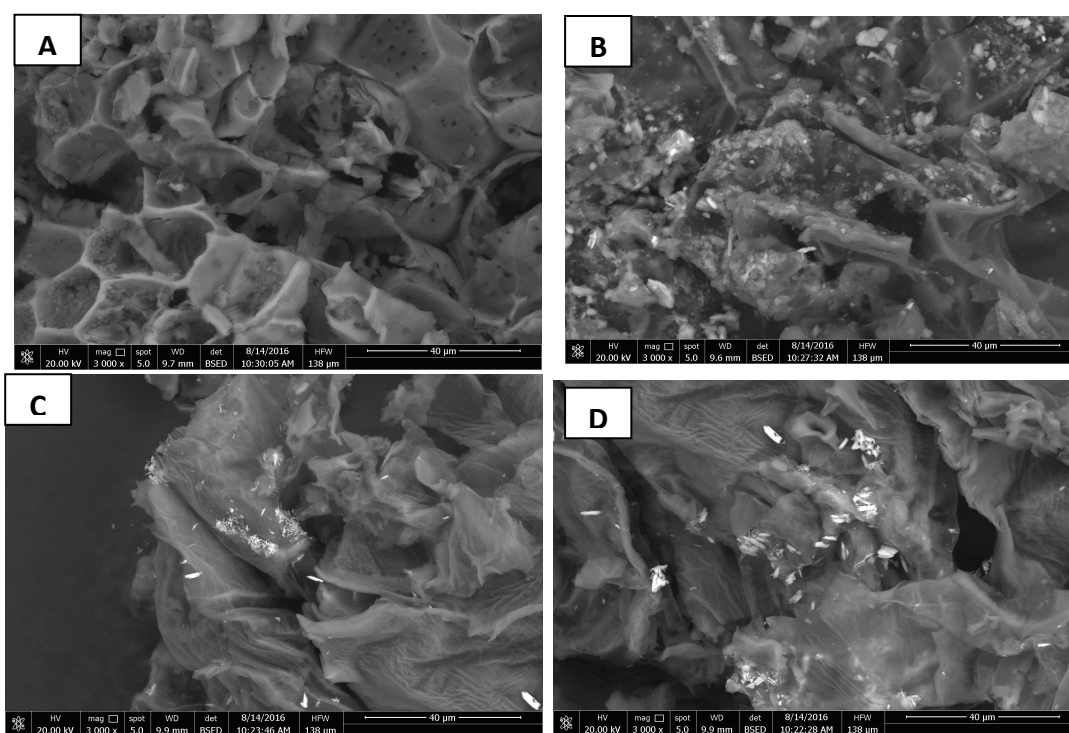


Fig. 1. SEM micrograph of (A), gellan before biosorption of heavy metal ions. (B), after biosorption of ferric ions. (C), after biosorption of copper ions (D) after biosorption of silver ions

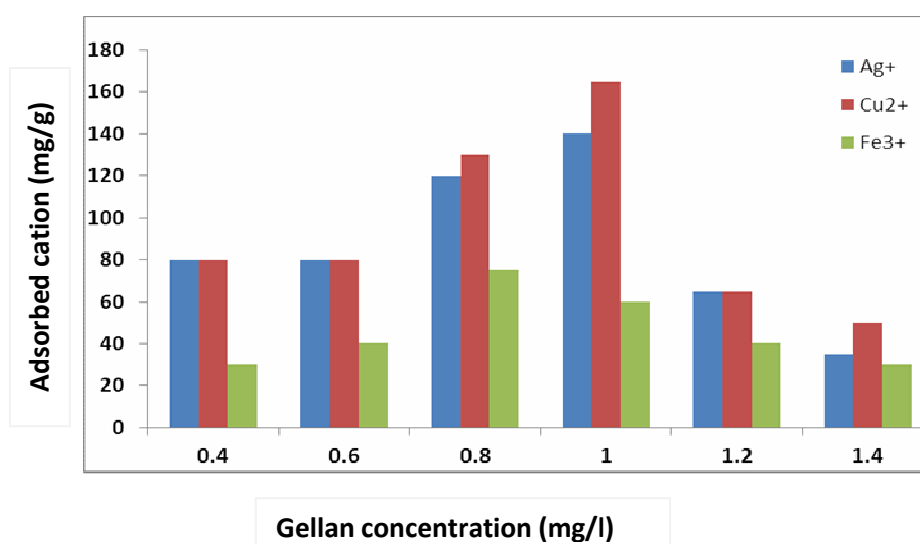


Fig. 2. Influence of gellan concentration on adsorption of heavy metal ions

groups of adsorbent and state, size and bond energy of metal ions. However, binding sites for metal ions biosorption increased and metal ions surrounded by unit mass gellan decreased when gellan concentration was too high (Ye *et al.*, 2001). But metal ions may be unable combined and could be divorced from gellan. The biosorption capacities of gellan for three kinds of metal ions followed the order of  $\text{Ag}^+ > \text{Cu}^{2+} > \text{Fe}^{3+}$ . The main reason for this phenomenon could result from atomic mass, molecular size and deformability of metal ions (Ye *et al.*, 2001).

### Effect of pH on Metals Adsorption

The effect of solution pH on metals adsorption was shown in Fig. 3. The biosorption capacity of the gellan for the metals increased as pH increased, and then it decreased at high pH. The maximum uptakes were 169.15 mg/g for  $\text{Ag}^+$ , 216.09 mg/g for  $\text{Cu}^{2+}$  and 84.47 mg/g for  $\text{Fe}^{3+}$  at pH 5.0, 5.5 and 6.0, respectively.

The solution pH, which affects the biosorption performance of biosorbent, is an important controlling parameter in the process (Han *et al.*, 2006). The effect of pH on the metal biosorption can be achieved through the competition between  $\text{H}_3\text{O}^+$  ions and metal ions for the biosorptive sites. At low pH, little biosorption occurs, as the large amount of  $\text{H}_3\text{O}^+$  exists in solution and the competition biosorption takes place (Cruz *et al.*, 2004). Moreover, the protonation state of ligands on the surface will endorse reaction with metal ions (Krishnani *et al.*, 2008). As pH increased, these negatively charged functional groups would be exposed in cations to accumulate in the double layer surrounding the polyelectrolyte (Lamelas *et al.*, 2006). Hence the biosorption on the gellan surface was increased, which was corresponding to the maximum biosorption capacity obtained at pH 5.0 for  $\text{Ag}^+$ , 5.5 for  $\text{Cu}^{2+}$  and 6.0 for  $\text{Fe}^{3+}$ , respectively. At high pH, the precipitation of metals occurs by the formation of metal hydroxides, so it is impossible for the biosorption (Veglio and Beolchini, 1997).

### Infrared analysis

The IR analysis of the gellan was shown in Table 1. The peaks assignments of the gellan

were as follows:  $3400\text{--}3200\text{ cm}^{-1}$  was related to the stretch vibration of O—H or hydrogen bond existing in all polymers. The signals at  $3000\text{--}2800\text{ cm}^{-1}$  were related to the stretch vibration of  $\text{CH}_2$  bond, and the signals at  $1850\text{--}1600\text{ cm}^{-1}$  were due to the stretch vibration of C = O bond. The signals at  $1400\text{--}1370\text{ cm}^{-1}$  were attributed to the vibration of C—O bond and the signals at  $900\text{--}1150\text{ cm}^{-1}$  for C—O—C bond (Sun *et al.*, 2009). There were shifts in wave numbers of peaks at  $3399.72\text{ cm}^{-1}$ ,  $2936.10\text{ cm}^{-1}$ ,  $1640.02\text{ cm}^{-1}$ ,  $1418.17\text{ cm}^{-1}$  and  $1063.64\text{ cm}^{-1}$  after loading  $\text{Ag}^+$ ,  $\text{Cu}^{2+}$  and  $\text{Fe}^{3+}$  (Table 1).

The functional group is one of the keys to analyze the mechanism of metal binding onto the gellan. Peaks appearing the FTIR transmission spectra of the gellan were related to various groups and bonds in accordance with respective wave numbers ( $\text{cm}^{-1}$ ). The FTIR spectra revealed the presence of many functional groups such as hydroxyl, carboxyl and glycosidic bond on the gellan surface. In comparing between the gellan and the metal-gellan, it can be observed that there were some shifts in wave number of dominant peaks associated with the metal-laden gellan. These shifts in wave number were corresponding to the metal binding process taking place on the surface of biosorbent (Pavasant *et al.*, 2006). The O—H stretching group and the C = O stretching group were involved with metal biosorption. The wave numbers of the other groups such as  $\text{CH}_2$  group, C—O—C group and C = O group were also changed (Table 1). This phenomenon showed that there existed a metal binding process on the surface of gellan. This is because O of the polysaccharide structure complexes with  $\text{Ag}^+$ ,  $\text{Cu}^{2+}$  and  $\text{Fe}^{3+}$  in adsorption process to reduce electron cloud density of functional groups containing oxygen and to change their vibration frequency and vibration intensity (Pal and Paul, 2008; Sun *et al.*, 2006). The wave numbers of several functional groups by  $\text{Ag}^+$  and  $\text{Cu}^{2+}$ -laden gellan were close than those by  $\text{Fe}^{3+}$ -gellan, which may be related to the number of electric charges of metal ions. The IR analysis results in this study demonstrated that O—H, C = O, C—O—C and  $\text{CH}_2$  played an important role in the binding  $\text{Ag}^+$ ,  $\text{Cu}^{2+}$  and  $\text{Fe}^{3+}$  by the gellan.

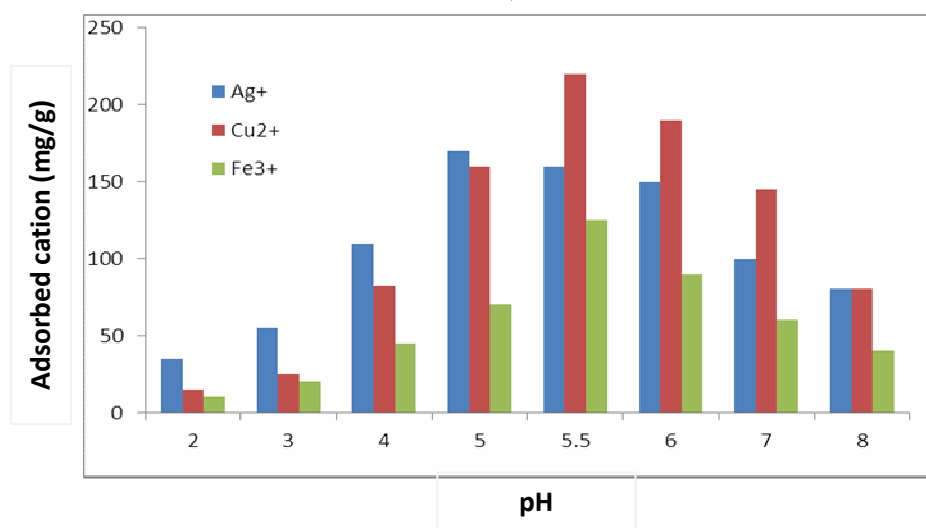


Fig. 3. Influence of gellan concentration on adsorption of heavy metal ions

Table 1. Wave number ( $\text{cm}^{-1}$ ) of dominant peaks obtained from FTIR transmission spectra of gellan +  $\text{Ag}^+$ , gellan +  $\text{Cu}^{2+}$ , gellan +  $\text{Fe}^{3+}$

Sample	Functional group				
	O—H	CH <sub>2</sub>	C=O	C—O	C—O—C
Gellan	3399.72	2936.10	1640.02	1418.17	1063.64
Gellan + $\text{Ag}^+$	3420.99	2941.17	1636.47	1384.74	1060.13
Gellan + $\text{Cu}^{2+}$	3421.92	2943.01	1636.84	1384.78	1060.02
Gellan + $\text{Fe}^{3+}$	3419.23	2927.34	1635.18	1381.56	1131.28

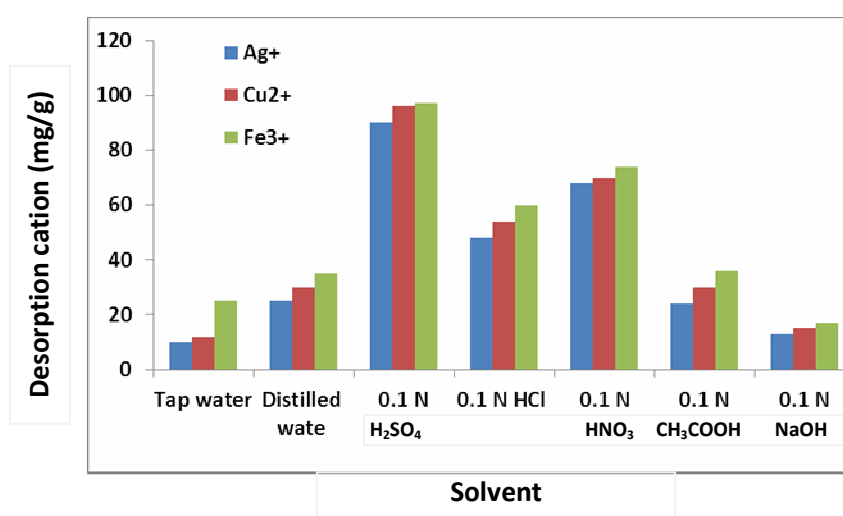


Fig. 4. Desorption of heavy metals  $\text{Ag}^+$ ,  $\text{Cu}^{2+}$  and  $\text{Fe}^{3+}$  (from gellan (Co: 10 mg/l; desorption time: 24hr.; rpm: 120; T: 20°C)

### Desorption performance

Desorption of metals or regeneration of adsorbents from used-adsorbents are crucial to repeat the use of adsorbents, recover of precious metals and reduce the cost of operation in any water treatment system. The adsorbed metals on adsorbents can not be completely reversible as reported by several observations on literature (Farrah and Pickering 1978; Brummer *et al.*, 1988; Ainsworth *et al.*, 1994). Eight solvent were used for desorption/regeneration experiment and the results are presented in Fig. 4. Among the solvents the tap water, distilled water, CH<sub>3</sub>COOH and NaOH were resulted a limited amount of metals ion recovery (<30%). The highest (94%) recovery was found with the use of 0.1N H<sub>2</sub>SO<sub>4</sub>. The other two acids 0.1 N HCl and 0.1 N HNO<sub>3</sub> were shown lower efficiencies (54 and 72%). In the desorption system H<sup>+</sup> released from the acids which replaced metal cation (heavy metals : Ag<sup>+</sup>, Cu<sup>2+</sup> and Fe<sup>3+</sup>) on the surface of the gellan (Karthikeyan *et al.*, 2007). The recovery and reuse processes for gellan gum can be continued upto 10 times with minor deviation in removal efficiency.

### Adsorption kinetics

It is important to perform kinetic analysis for any kind of biosorption process as it helps in determining the applicability of biosorption process and provides useful design related information. It not only describes the mechanism of binding of metals on adsorbents but also quantifies the rate constant which control the required contact time of metal at solid-liquid interface. The kinetics of Cu (II) adsorption was investigated using various models such as pseudo first order and pseudo

second order kinetics. The best fit model has been selected based on the value of linear regression correlation coefficient, R<sup>2</sup> Langmuir constant and correlation coefficients calculated from the adsorption data are given in Table 2. The high correlation coefficients showed that both models are suitable for describing the adsorption equilibrium of copper ions. But lead adsorption fitted well with the Langmuir more than the Freundlich model. The adsorption capacity (X<sub>m</sub>) of potato peel for the uptakes of Ag(I), Cu (II) and Fe (III) are 7.56, 6.645 and 13.05 mg/g, respectively. The adsorption capacity of various sorbents for Ag(I), Cu (II) and Fe (III) ions taken from literature are presented in Table 2.

### Conclusions

The gellan biosorption capacity on heavy metals was tested and the results indicated that the biosorption capacity of Ag<sup>+</sup>, Cu<sup>2+</sup> and Fe<sup>3+</sup> were obviously affected by gellan concentration, pH and ionic strength. The optimum biosorption capacity on Ag<sup>+</sup> were 216.09 mg Ag/g gellan gum, 169.15 mg Cu<sup>2+</sup> /g gellan gum and 84.47 mg Fe<sup>3+</sup>/g gellan gum

Interactions between metal ions and functional groups of the gellan were determined by FTIR. The results demonstrated that the groups of O—H, C=O, C—O—C and CH<sub>2</sub> of the gellan involved in metal biosorption process were the main functional groups for binding metal ions. The overall results suggest that the gellan obtained from *Sphingomonas paucimobilis* has potentials as a novel biosorbent in waste water treatment and the future work will be focused on the removal of trace metal ions.

**Table 2. Values of Langmuir constant for of Ag(I), Cu(II) and Fe(III)**

Adsorbate	Adsorption capacity X <sub>m</sub> (mg g <sup>-1</sup> )	K (L mg <sup>-1</sup> )	r <sup>2</sup> (corr coeff.)
Ag (I)	7.562	0.168	0.9782
Cu (II)	6.645	0.179	0.9874
Fe (III)	13.05	0.255	0.9933

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## ادمصاص كاتيونات الفضة والنحاسيك والحديدك بواسطة صمغ الجيلان

سارة السيد عثمان - سيد سليمان السعدني - صلاح الدين لبيب - حفناوي طه حفناوي

قسم الكيمياء الحيوية - كلية الزراعة - جامعة الزقازيق - مصر

في هذا البحث تم دراسة آليات ادمصاص لكاتيونات الفضة والنحاسيك والحديدك بواسطة سكر الجيلان والذي يتم انتاجه من بكتيريا *Sphingomonas paucimobilis*، تم دراسة تأثير تركيز صمغ الجيلان، ودرجة حموضة المحلول واعادة ادمصاص، والقوة الايونية علي خصائص ادمصاص، أظهر سكر الجيلان قدرة قوية علي ادمصاص تصل إلى 216,09 ملجم أيون فضة/جم صمغ الجيلان و 169,15 ملجم أيون نحاسيك/جم صمغ الجيلان، 84,47 ملجم أيون حديدك/جم صمغ الجيلان علي التوالي، أظهر التحليل الطيفي بالاشعة تحت الحمراء FT-IR أن مجموعات الهيدروكسيل والكيتون والايثير لسكر الجيلان تشارك في عملية ادمصاص المعادن والتي هي المجموعات الوظيفية الرئيسية لعمل روابط مع المعادن، أظهر حمض الكبريتيك 0,1 عياري درجة عالية من القدرة على فك الارتباط بين السكر والمعدن عن المذيبات الأخرى كالماء حيث كانت نسبة الاسترجاع (94-90-86%) لأيونات (الفضة والنحاسيك والحديدك على التوالي ويمكن إعادة استخدام السكر في عملية ادمصاص سبع مرات بكفاءة عالية.

المحكمون:

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