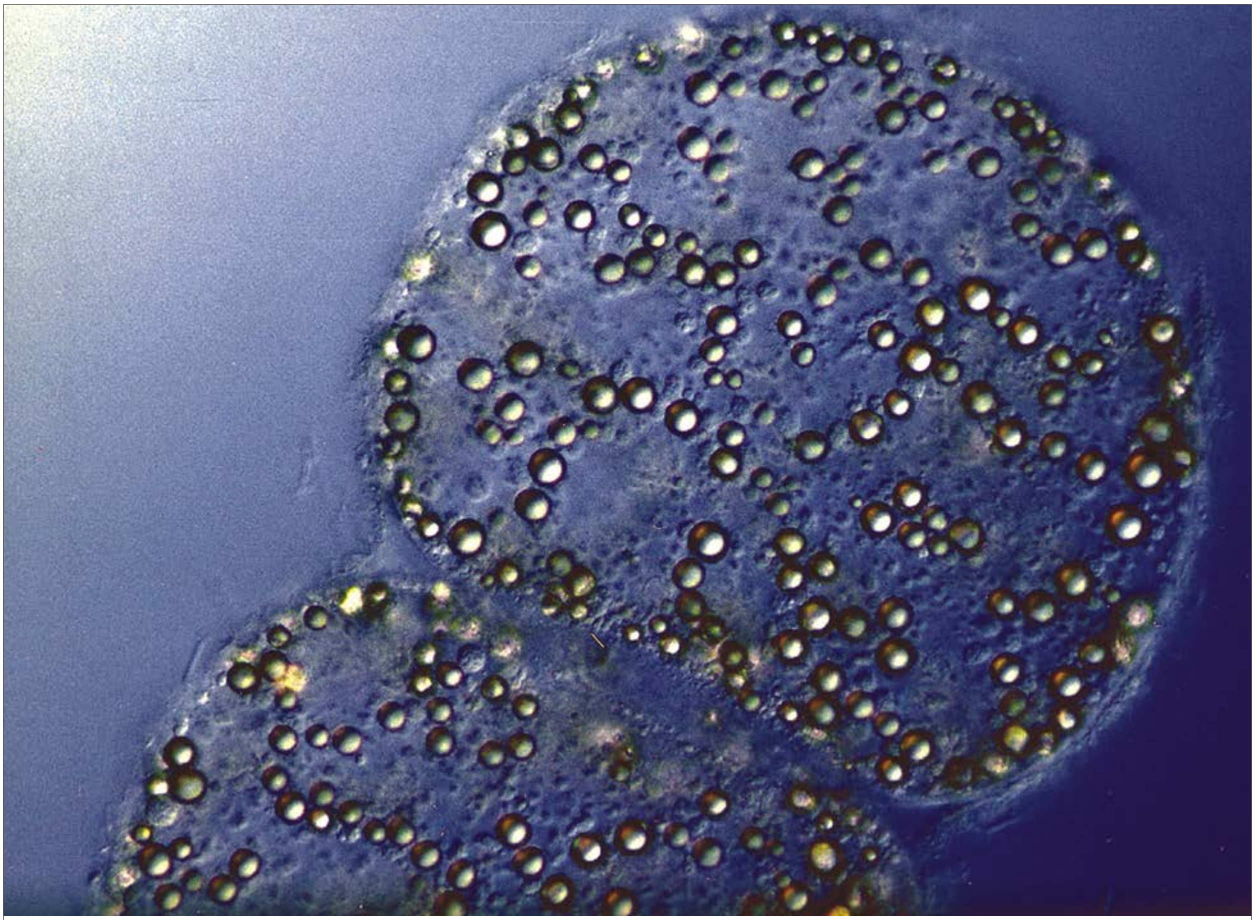

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Study of Various Parameters Influencing the Biosorption of Aluminum by Actinomycetes

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ABSTRACT

Streptomyces albus and *Streptomyces diastaticus* drying cells were used for biosorption of aluminum ions from aqueous solutions. The maximum adsorption values 98.70 and 99.60% were recorded by *S. albus* and *S. diastaticus*, respectively at the hydrogen ion concentration (pH) 7 and 7 days old for either organism. The highest adsorption value of aluminum by *S. albus* was 95.50% when glucose was utilized and reached its maximum value of 96.20% by *S. diastaticus* when fructose was utilized. The biosorption of aluminum by *S. albus* reached its maximum values of 89.90 and 99.00% when lower biomass weights 0.10 and 0.60 g, respectively were using. Considering *S. diastaticus*, the biosorption ratio reached its maximum value of 96.00% when 0.10 g biomass was used. The presence of Zinc in the same solution increased the biosorption value by *S. albus* or *S. diastaticus* and it decreased by increasing zinc concentration. Sodium or calcium had not a significant effect on the biosorption by two organisms. The total values of aluminum biosorption by dried cell of *S. albus* or *S. diastaticus* columns during three stages and for different period sranged from 10 to 120 min were 98.52 and 95.74%, respectively. The application of 1 mM sulfuric acid led to recovery of the adsorbed metals from the surfaces of the dried cell columns. The total values of the recovered aluminum from the cell surfaces of either *S. albus* or *S. diastaticus* during these stages were 96.55 and 94.25%, respectively.

Key words: *Streptomyces albus*, *Streptomyces diastaticus*, aluminum biosorption, drying cells, adsorption values

INTRODUCTION

The metal-uptake ability of microorganisms has known for a long time. It has been suggested that biomass could be used to decontaminate metal-bearing waste waters and to concentrate metals. Biological surfaces consist of different functional groups where coordination complexes with metal ions can form. Among these functional groups are carboxyl (-COOH), amide ($-NH_2$), thiol (-SH), phosphate (PO_{3-4}) and hydroxide (-OH). When a metal ion in solution interacts with a solid surface it can adsorbed by physical adsorption, associated with the weak forces of physical attraction such as van der Waals' forces or by chemical adsorption, associated with the exchange of electrons and the formation of a chemical bond between the adsorbent and the solid surface. The other possibility is the ion exchange, which may take place between the incoming cation and hydrogen ions of functional groups at the surface (Yalcinkaya *et al*., 2002).

Most recently actinomycetes have been used to remove heavy metals from industrial effluent successful example is of: Cr (VI) bioaccumulation by Streptomyces strains that has been reported by Amoroso *et al.* (2001). Laxman and More (2002) determinate Cr (VI) reduction by *Streptomyces griseus*, Polti *et al.* (2007) showed the potential capacity of actinomycetes as tools for Cr (VI) bioremediation. Sineriz *et al.* (2009) present the potential capacity of *Streptomyces* sp. F4 for Cd (2+) bioremediation. Current investigation was designed to explore the metal Al (II) removal capacity of *Streptomyces albus*, *Streptomyces diastaticus* from aqueous solution. Parameters such as the influence of initial pH, contact time, temperature, mixed-metal, carbohydrate sources and initial metal ion concentration on biosorption evaluated.

MATERIALS AND METHODS

Microorganisms and culture medium: *S. albus* and *S. diastaticus* (local species) were previously isolated from soil collected from Second Industrial City, Riyadh, Saudi Arabia and identified by Al-Rokban (2009). Actinomycetes species were grown on Albumin medium, which contained (per liter) 0.25 g of egg albumin, 1.0 g of glucose, 0.5 g of potassium dihydrogen phosphate, 0.2 g of magnesium sulphate, 1.0 g of iron sulphate. Cultures were maintained in agar slants (liquied medium plus 15 g agar).

Preparation of metals solutions: Aluminum solutions were prepared from $Al(NO_3)_3.9H_2O$ (Laboratory, Rasayan), while sodium, calcium and zinc solutions were prepared from $NaCl_2$, $CaCl_2$ (Winlap, UK) and $ZnCl_2$ (Merck, Germany). The pH of the working solution was adjusted to 7.0. Fresh dilutions were using for each biosorption study.

Determination of aluminum ions concentrations in the solutions: The concentrations of aluminum ions in the biomass and in the supernatant determined by atomic absorption spectrophotometer. Also, the metal quantity added to the media determined by atomic absorption spectrophotometer.

The pH effects on aluminum biosorption: It well known that the pH of the medium affects the solubility of metal ions and the concentration of the counter ions on the functional groups of the biomass cell walls, so pH is an important parameter on biosorption of metal ions from aqueous solution (Antunes *et al.*, 2003). The levels of pH used were those expected to found in the field. Metal solution with 0. 4 mM of aluminum salts were adjusted to pH 5, 7, 9 or 11 with NaOH prior addition 0.1 g of *S. albus* or *S. diastaticus*. The pH of residual solution were also measured.

The effect of temperature on aluminum biosorption: The effect of temperature on heavy metals uptake by *S. albus* or *S. diastaticus* were generated by adding 0.1 g of biomass with rotary shaker set on 160 rpm and pH 7 using 250 mL flask containing 25 mL aqueous solution comprised of 0.4 mM of aluminum. The temperature chosen in experiments were 25, 28, 30 and 35°C.

The effect of initial cell biomass: The effect of initial cell biomass of *S. albus* and *S. diastaticus* on aluminum ions uptake was tested using 0.1 g of biomass from 7 day old culture. The biomass was separately added into 250 mL flask containing 25 mL aqueous solution comprised of 0.4 mM of aluminum.

Biosorption in mixed-metal system: For the determination of synergistic/antagonistic effect of cations present in the effluent metal solution, the biosorption of metal ions by *S. albus* and

S. diastaticus in mixed-metal system was investigating. To test the binding of each metal ion in the presence of others, metal solutions containing 0.4 mM aluminum ions alone or mixed with one other (4 mM of aluminum ions and 0.4 mM of sodium, calcium or zinc ions) or (4 mM of aluminum ions and 8 mM of sodium, calcium or zinc ions) were tested.

The effect of time on aluminum uptake by *S. albus* and *S. diastaticus*: In this experiment the effect of time exposure on aluminum ions uptake by *S. albus* and *S. diastaticus* was studied using 0.1 g of *S. albus* or *S. diastaticus*. The biomass was separately added into 250 mL flask containing 25 mL aqueous solution comprised of 0.4 mM of aluminum. The pH was measured and a sample was taken from each flasks at different exposure times: 3, 5 and 7 days.

Effect of carbohydrate sources on aluminum biosorption by actinomycetes: To study the effect of carbohydrate source on aluminum biosorption by *S. albus* and *S. diastaticus*. The organisms culture in the albumin media contain either glucose, fructose or starch as a carbon source. After harvesting, biomass was drying in oven at 80°C and was utilized in biosorption studies.

Biosorption studies: Biosorption of aluminum ions was investigated by oven dried biomass of *S. albus* and *S. diastaticus* in batch biosorption experiments. Batch experiments were performed by adding 0.1 g of biomass using 250 mL flask containing 25 mL aqueous solution comprised of 0.4 mM of aluminum. There were 3 replicates per isolate.

Un added flask (no actinomycetes) were used as controls. All flasks were incubated at 28°C on a rotary shaker set on 160 rpm. After 2 h of incubation, the samples were withdrawn from the flask and filtered through a 0.2-µm syringe filter and residual aluminum concentration was measured by an Atomic Absorption Spectrophotometer (AAS).

Removal of heavy metals in column experiments: Since the *S. albus* and *S. diastaticus* biomass was able to effectively bind heavy metals, the next step was to examine heavy metal uptake in the column experiments. The solution containing 0.4 mM aluminum was contact with (0.1 g dry wt. basis) dried cells packed in columns for 120 min at room temperature. This system permits to overcome the inter ionic competition and to improve the uptake.

Recovery of the adsorbed Al from the surface of the dried *S. albus* and *S. diastaticus*: For the recovery of the adsorbed Al from the surface of the dried *S. albus* and *S. diastaticus*, microbial cells with accumulated aluminum were washed three times with 10 mL of $H_2SO_4$1 mM solution.

Reproducibility and data analysis: Data presented are the mean values from three separate experiments. Statistical analysis of the data was carried out using the statical package for the social sciences (SPSS) version 13. The concentration of metal ions adsorbed per unit actinomycetes biomass (mg metal g^{-1} dry biosorbent) was determined using the following expression:

$$q = V(Ci-Ceq) /M$$

where, q is the metal uptake (mg metal ions g^{-1} dry weight of fungal biomass) V is the volume of metal solution (mL), C_i is initial concentration of metal ions in the solution(mg L^{-1}), C_{eq} is the final concentration of metal ions in the solution and M is the dry weight of fungal biomass.

RESULTS

Effect of temperature on Al biosorption by *S. albus* and *S. diastaticus*: The results in Table 1 demonstrated, that the temperature range of 25-35°C apparently exhibited no significant influence on biosorption potential of test actinomycetes species that the value of aluminum biosorption by *S. albus* and *S. diastaticus* was range of 97.3-99.3 and 97.28-99.28%, respectively.

Effect of pH: The influence of pH on the biosorption capacity for aluminum is shown in Table 2. It is clear from the results that the optimum metal ion uptake is 931 and 989.65 (µg g^{-1}) for *S. albus* and *S. diastaticus*, respectively at pH value 7 and decrease with increasing pH. Thus, the accumulation of aluminum by actinomycetes cells is markedly affected by the pH of the solution.

Effect of age on Al biosorption by *S. albus* and *S. diastaticus*: It is evident from Table 3 that the highest amounts of aluminum accumulated by *S. albus* and *S. diastaticus* dried cells were recorded 7 days old for either organism. The highest values of aluminum adsorption by the two organisms were 98.70 and 99.60%, respectively.

Effect of carbon sources on Al biosorption by *S. albus* and *S. diastaticus*: Table 4 showed effect of carbon sources on Al biosorption by *S. albus* and *S. diastaticus* after 2 h incubation at 28°C on a rotary shaker (160 rpm). Considering effect of carbohydrate sources on the adsorption process by actinomycetes, the results demonstrated that the highest adsorption value of aluminum by *S. albus* was 95.50% when glucose was utilized and reached its maximum value of 96.20% by *S. diastaticus* when fructose was utilized.

Table 1: Effect of temperature on Al biosorption by *S. albus* and *S. diastaticus* after 2 h incubation at 25, 28, 30 and 35°C on a rotary shaker (160 rpm)

Temperature (°C)	Al biosorption by *S. diastaticus* (µg g^{-1})	Al biosorption by *S. albus* (µg g^{-1})
25	97.28±0.00	98.9±0.00
28	99.28±0.01	98.7±0.10
30	98.23±0.01	99.3±0.05
35	99.05±0.02	97.3±0.15

Sig = 0.000, values are in Mean±SD%

Table 2: Effect of pH on Al biosorption by *S. albus* and *S. diastaticus* after 2 h incubation at 28°C on a rotary shaker (160 rpm)

pH	Al biosorption by *S. diastaticus* (µg g^{-1})	Al biosorption by *S. albus* (µg g^{-1})
5	986.4±0.110	915.8±0.950
7	989.65±0.01	931.0±0.030
9	956.89±0.02	870.68±0.51
11	599.42±0.01	628.04±0.56

Sig = 0.000, values are in Mean±SD

Table 3: Effect of age on Al biosorption by *S. albus* and *S. diastaticus* after 5, 7 and 9 d incubation at 28°C on a rotary shaker (160 rpm)

Days	Al biosorption by *S. diastaticus* (µg g^{-1})	Al biosorption by *S. albus* (µg g^{-1})
5	98.9±0.01	91.3±0.01
7	99.6±0.02	98.7±0.00
9	92.4±0.03	94.5±0.05

Sig = 0.000, values are in Mean±SD%

Table 4: Effect of carbon sources on Al biosorption by *S. albus* and *S. diastaticus* after 2 h incubation at 28°C on a rotary shaker (160 rpm)

Carbon source	Al biosorption by *S. diastaticus* (μg g^{-1})	Al biosorption by *S. albus* (μg g^{-1})
Glucose	95.3±0.050	95.5±0.020
Fructose	96.2±0.010	94.3±0.020
Starch	95.09±0.01	86.11±0.02

Sig = 0.000, values are in Mean±SD%

Table 5: Effect of biomass on Al biosorption by *S. albus* and *S. diastaticus* after 2 h incubation at 28°C on a rotary shaker (160 rpm)

Biomass (G)	Al biosorption by *S. albus* (μg g^{-1})	Al biosorption by *S. diastaticus* (μg g^{-1})
0.1	96±0.00	98.9±0.01
0.6	93±0.05	99.0±0.01
1.2	90±0.00	89.0±0.01

Sig = 0.000, values are in Mean±SD%

Table 6: Effect of Mixed-metal on Al biosorption by *S. albus* and *S. diastaticus* after 2 h incubation at 28°C on a rotary shaker (160 rpm)

Metal concentration (mM)		Al biosorption by *S. diastaticus* (μg g 1)	Al biosorption by *S. albus* (μg g^{-1})
ZnCl$_2$	0.4	99.4±0.01	99.8±0.010
	0.8	96.5±0.01	98.8±0.020
NaCl	0.4	97±0.01	94.48±0.01
	0.8	95.52±0.00	97.0±0.030
CaCl$_2$	0.4	98.2±0.01	96.9±0.030
	0.8	96.4 ±0.01	96.3 ±0.05
Control		98.23±0.00	99.3±0.020

Sig = 0.000, values are in Mean±SD%

Effect of biomass on Al biosorption by *S. albus* and *S. diastaticus*: The effect of biomass on Al biosorption is shown in Table 5. It is clearly seeing that the removal efficiency increases as the biomass increases from 0.1 to 0.6. The rate of increase gradually decreases with increasing adsorbent mass.

Effect of mixed-metal on Al biosorption by *S. albus* and *S. diastaticus*: The effect of other heavy or light metals present in the same aqueous solutions on the process of Aluminum biosorption had been studied. The gained data showed that the presence of zinc in the same aqueous solution increased the biosorption value of either aluminum by *S. albus* or *S. diastaticus*. Such increase was found to be decreased by increasing zinc concentration. The results showed that neither sodium nor calcium had a significant effect on the biosorption of aluminum by either *S. albus* or *S. diastaticus* (Table 6).

Removal of heavy metals in column experiments: The efficiency of heavy metal removal (mg heavy metal removed mg^{-1} heavy metal added) in a column system is given in Table 7. The results of absorption columns made from drying cells experiment showed that the two organisms could absorb heavy metals after solutions were contacting with the organism's surfaces during three stages and for different periods ranged from 10 to 120 min. The total values of aluminum biosorption by columns of *S. albus* or *S. diastaticus* during these stages were 98.52 and 95.74%, respectively.

Table 7: Biosorption of Al by *S. albus* and *S. diastaticus* using column

Time (min)	Al biosorption by *S. diastaticus* (μg g^{-1})	Al biosorption by *S. albus* (μg g^{-1})
10	19.31±0.00	70.7±0.010
30	27.85±0.01	18.59±0.01
120	48.58±0.01	72.86±0.00

Sig = 0.000, values are in Mean±SD%

Table 8: Recovery of the adsorbed Al from the surface of the dried *S. albus* and *S. diastaticus* columns

Time (min)	Al biosorption by *S. diastaticus* (μg g^{-1})	Al biosorption by *S. albus* (μg g^{-1})
10	13.21±0.00	90.52±0.01
30	30.62±0.01	14.59±0.02
120	50.42±0.01	72.08±0.02

Sig = 0.000, values are in Mean±SD%

Recovery of the adsorbed Al from the surface of the dried *S. albus*: The gained results showed that application of 1 mM sulfuric acid led to recovery of the adsorbed metals from the surfaces of the dried cell columns after three stages and for different periods ranged from 10 to 120 min. The total values of the recovered aluminum from the cell surfaces of either *S. albus* or *S. diastaticus* during these stages were 96.55 and 94.25%, respectively (Table 8).

DISCUSSION

The gained results demonstrated that the temperature range of 25-35°C apparently exhibited no significant influence on biosorption potential of test Actinomycetes species. This result agreed with that reported by Saglam *et al*. (2002) and Javaid *et al*. (2010) which demonstrated that the temperature range of 20-45°C apparently exhibited no significant influence on biosorption potential of test fungal specie, probably due to exothermic. The maximum adsorption values were recorded by *S. albus* or *S. diastaticus* at the hydrogen ion concentration 7 followed by the concentrations values of 5, 9, 11 for *S. albus* and 5, 11 and 9 for *S. diastaticus*, this revealed that absorption of aluminum depends greatly on the hydrogen ion concentration of the solution. Also, Tsuruta (2007) reported that the amounts of uranium accumulated by *Arthrobacter* sp., US-10 are highest at around pH 5 and decrease with increasing acidity below pH 4. Regarding the effect of utilized organism's age on the process of adsorption, the recorded results showed that the highest adsorption values for aluminum 98.70 and 99.60% by *S. albus* or *S. diastaticus*, respectively were recorded at 7 days old for either organism. Considering effect of carbohydrate sources on the adsorption process by actinomycetes, the results demonstrated that the maximum adsorption value of aluminum by *S. albus* was 95.50% when glucose was utilized and reached its maximum value of 96.20% by *S. diastaticus* when fructose was utilized. Goyal *et al*. (2003) demonstrated that *S. cerevisiae* grown in glucose or sucrose generally had a greater uptake than those fed with fructose at higher concentration of Cr (VI). Regarding the effect of biomass on the biosorption rate of heavy metals the obtained results revealed that the biosorption of aluminum by *S. albus* reached its maximum values of 89.90 and 99.00% when lower biomass weights 0.10 and 0.60 g were used, respectively. The results showed that the biosorption rate of this metal decreased by increasing the biomass of this organism. Considering *S. diastaticus*, the results demonstrated that the biosorption ratio for this metal reached its maximum value of 96.00% when 0.10 g biomass was used and a decrease in the biosorption rate was noticed when larger biomass was used. As the biomass increases the number of binding sites for the ions also increases (Al-Qodah, 2006). After some point, sorption capacity was steady or decreased with biomass concentration due to a screen effect between cells, this produced a block of the cell active sites by an increase of biomass in the system (Hammaini *et al.*, 2007).

The effect of other heavy or light metals present in the same aqueous solutions on the process of aluminum biosorption had been studied. The gained data showed that the presence of zinc in the same aqueous solution increased the biosorption value of either aluminum by *S. albus* or *S. diastaticus*. Such increase was found to be decreased by increasing zinc concentration results showed that neither sodium nor calcium had a significant effect on the biosorption of aluminum by either *S. albus* or *S. diastaticus*. The results of absorption columns made from drying cells experiment showed that the two organisms could absorb heavy metals after solutions contacted with the organism's surfaces during three stages and for different periods ranged from 10 to 120 min. The total values of aluminum biosorption by columns of *S. albus* or *S. diastaticus* during these stages were 98.52 and 95.74%, respectively. Tsekova and Petrov (2002) showed that cobalt and iron were removed more than 98% by immobilized mycelia of *Rhizopus delemar* in first column.

The gained results showed that application of 1 mM sulfuric acid led to recovery of the adsorbed metals from the surfaces of the dried cell columns after three stages and for different periods ranged from 10 to 120 min. The total values of the recovered aluminum from the cell surfaces of either *S. albus* or *S. diastaticus* during these stages were 96.55 and 94.25%, respectively.

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