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BIOSORPTION OF COPPER USING AN EXOPOLISACCHARIDE PRODUCED BY *PAENIBACILLUS POLYMYXA*

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Welcome to the XIII International Conference on Heavy Metals in the Environment ICHMET (2005), completing its Thirtiest Anniversary in marvelous Rio de Janeiro, Brazil.

The Technical Advisory Team approved 480 abstracts from investigators representing the following 40 countries: Argentina, Australia, Bangladesh, Belgium, Bolivia, Brazil, Bulgaria, Canada, Chile, China, Colombia, Croatia, Cuba, Czech Republic, Denmark, Egypt, England, Finland, France, Germany, Guatemala, Hungary, India, Indonesia, Iran, Ireland, Italy, Kosova, Malaysia, Moroccos, Mexico, Nigeria, Norway, Pakistan, Portugal, Philippines, Poland, Romania, Russia, Slovenia, South Africa, Spain, Switzerland, Tunisia, Ukraine, United Arab Emirate, United Kingdom, United States, Uruguay, Venezuela.

We wish to thank the Steering Committee, the Technical Advisory Team, the Invited Speakers, and all the participants who helped make this Event come true. We want also to mention and thank CETEM's staff for contributing in various manners for the success of the Conference.

Have a good Conference!

The Executive Committee

SESSION ONE
Analytical Chemistry

SESSION TWO
Air Atmospheric Processes: emissions, exchange modeling

SESSION THREE
Biological Processes: biomonitoring, bioaccumulation, physiologica response

SESSION FOUR
Contaminated Sites

SESSION FIVE
Geochemistry: soils, sediments, water, historical trends

SESSION SIX
Human Health: exposure, epidemiology, toxicology

SESSION SEVEN
Remediation: bioremediation, phytoremediation, physico chemical processes

SESSION EIGHT
Sustainable Processes: Recycling, recovery, clean technology

SESSION NINE
Risk Assessment and Life Cycle

BIOSORPTION OF COPPER USING AN EXOPOLISACCHARIDE-PRODUCED BY *PAENIBACILLUS POLYMYXA*

ABSTRACT

Human activities, such as mining operations and the discharge of industrial wastes, have resulted in the accumulation of metals in the environment, which poses serious health threats as these heavy metals tend to persist indefinitely. In recent years, there has been a significant effort to search for new mechanisms of heavy metal removal from contaminated sites. This search has focused on the utilization of Gram positive non-pathogenic and non-toxicogenic species Paenibacillus polymyxa P13 and its purified exopolysaccharide (EPS) to act as biosorbents of copper.

After extraction from the fermentation medium, EPS was dialyzed against water and dried. Sorption assays carried out using 100 mg L^{-1} initial metal concentration and 0.1 g biosorbent L^{-1} showed that EPS has higher uptake capacity when compared to whole-cells of P13. Experiments with different EPS concentrations showed higher copper uptake (900 mg g^{-1}) with 0.05g EPS L^{-1}. The process of Cu removal was inhibited in the presence of other ions (Zn^{2+}, Ca^{2+}, K^{+}, Na^{+}, Mg^{2+}) using 100 mg L^{-1} initial metal concentration and 0.5 g biosorbent L^{-1}. Particularly, Ca^{2+} was the greater inhibitor although high concentrations were required to observe the complete displacement of Cu^{2+}. Ca^{2+} and Zn^{2+} produced an important inhibition of Cu^{2+}, probably due to the very close characteristics, the same charge and practically the same hydrated ionic radii (HIR), aprox. 600 pm. K^{+} (HIR: 300 pm) presents a ratio radius:charge similar to Cu^{2+}, suggesting that the charge density plays an important role in the adsorption process. Mg^{2+} has a bigger radius (800 pm) and Na^{+} (450 pm) a smaller charge density.

Binding of heavy metals by EPS of P13 is thought to be an important mechanism in the natural detoxification of heavy metal contaminated sites and in heavy metal bioremediation.

INTRODUCTION

Aqueous waste released by a number of industries contains heavy metals that pollute the environment. This contamination of the environment poses serious health threats to humans and animals, as these heavy metals tend to persist in the environment indefinitely (Volesky & Holan 1995). In recent years, there has been a significant effort to search for new methods of heavy metal removal from contaminated sites. Biological methods to remove metals from liquid effluents present many potential advantages. Heavy metal accumulation processes by biological cells are grouped together under the general term “biosorption”. The mechanisms of biosorption may involve intracellular uptake and storage via active cationic transport systems, surface binding or some undefined mechanisms. The biological and chemical characteristics of these uptake processes are important not only as an aid in the understanding of the role of metallic ions in basic cellular functions but also for use in detoxification of metal-polluted industrial effluents by application of biomass. Emphasis has been placed on the use of microorganisms as biosorbents because they are propagated inexpensively (Volesky & Holan 1995; Salehizadeh & Shojaosadati 2003; Ince Yilmaz 2003). The use of bacteria and fungal biomass as biosorbents should be of special interest to industries in undeveloped countries where pollution generators cannot afford to install costly high-performance treatment facilities. In heavy metal pollution, bacterial exopolymers have become an alternative of interest as metal-binding agents in detoxification of contaminated waters (Gutnick & Bach 2000).

EXPERIMENTAL

Exopolysaccharide purification

In order to purify the exopolysaccharide (EPS) produced by *P. polymyxa*, an entire culture (cells plus broth) was boiled for 10 min to remove attached EPS and centrifuged at 20,000*g* for 20 min to remove cells. Trichloroacetic acid (TCA) was added to 10% final concentration to precipitate proteins and peptides. After centrifugation, supernatant was filtered through 0.45 µm filter membrane. One volume of cold ethanol (4°C) was added to the supernatant and the crude EPS precipitated. After centrifugation, 1/10 original volume sterile distilled water was used to re-suspend it, and was dialysed overnight against sterile distilled water in cold room. The dialysed EPS was lyophilised overnight to remove the water, weighed and stored at 8°C. For biosorption experiments, it was dissolved in sterile distilled water to give a stock solution with final concentration of 10 mg ml^{-1}, adjusting the pH to 6 with NaOH.

Copper biosorption experiments

Purified EPS was evaluated for biosorption capacity and was added to metal solutions at pH 6 at 25°C. After biosorption had reached equilibrium EPS was removed by adding one volume of cold ethanol to the solution and centrifuged at 20,000*g* for 20 min to remove insoluble EPS-Metal complex. Residual, unsorbed metal in the supernatant was determined by the BCA method (Brenner & Harris 1995). In order to account the effect of the ethanol precipitation on the metal solubility, a separate set of control experiments was run under the same conditions without adding EPS. All experiments were run in duplicate. The influence of other metal ions at different concentrations (25, 250 and 500 mg l^{-1}) was also evaluated on copper (50 mg l^{-1}) biosorption by EPS (0.25 mg ml^{-1}).

RESULTS

Metallic ions in nature are rarely represented by a single kind of metal. Na, Ca, Mg and K are elements frequently found in nature and of great solubility. The competitive effect of different metal ions on Cu^{2+} adsorption to EPS was studied by pairing Cu^{2+} with each of the other ions at three different concentrations (50, 250 and 500 mg l^{-1}). The process of Cu^{2+} removal was inhibited in the presence of other ions (Figure 1).

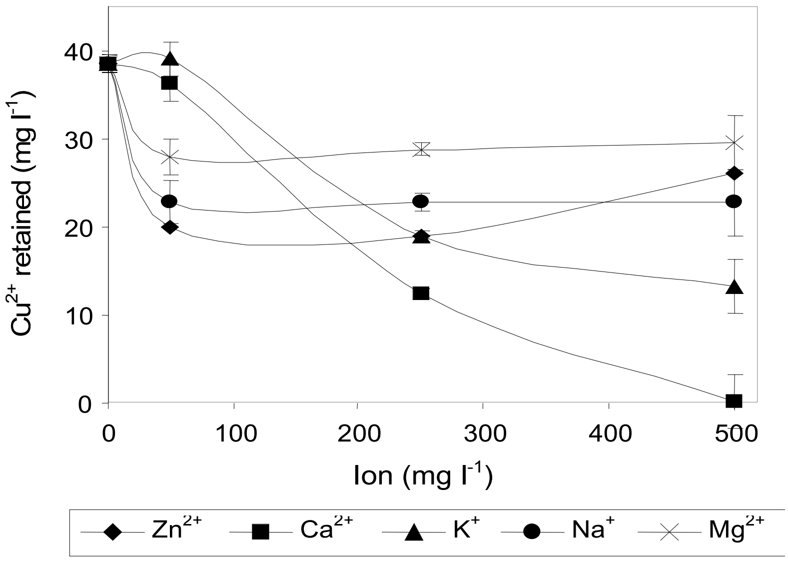

Figure 1 – Effect of other ions (50 mg l^{-1} initial Cu^{2+} concentration; 0.25 mg EPS ml^{-1})

Particularly, Ca^{2+} was the greater inhibitor although high concentrations are required to observe the complete displacement of Cu^{2+} by Ca^{2+} (Prado Acosta *et al.* 2005). It can also be seen that Ca^{2+} and Zn^{2+} produced an important inhibition of Cu^{2+} uptake.

DICUSSION

The use of the inhibition assay was the approach used to determine that EPS was able to adsorb other ions. Other heavy metals, like zinc, interacted with EPS inhibiting copper biosorption. This is probably due to the very close characteristics of Ca^{2+} and Zn^{2+} ions to Cu^{2+}, the same charge and practically the same hydrated ionic radii, approx. 600 pm (Kielland 1937). K^+ (hydrated ionic radius: 300 pm), presents a ratio radius:charge, similar to Cu^{2+}, suggesting that the charge density plays an important role in the adsorption process. Mg^{2+} has a bigger radius (800 pm) and Na^+ (450 pm) a smaller charge density. The four elements tested belong to the same period of the periodic table, Cu and Zn are neighbours and generally presented in polluted wastes.

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CONCLUSIONS

The process of copper removal using the EPS from P13 was inhibited in the presence of other ions. A study on the combined effect of two or more metals will be conducted to evaluate the potential of this EPS for use in biodetoxification of metal-polluted waters.

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