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Some Metals Removal by Biomass of *Bacillus* spp. and *Pseudomonas* spp. from Wastewater of Second Campus of Mosul University

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ABSTRACT

This paper highlighted a study on the use of bacteria as biological material for heavy metals removal from wastewater, using biomass of *Bacillus* spp. and *Pseudomonas* spp. in two time intervals (4, 24 hrs.) of biosorption process.The maximum capacity was found to be the highest for zinc by the two types of bacteria and two time intervals.While the minimum capacity was found for lead by *pseudomonas* spp.. The other metals have differed in the percentage uptake by two types of bacteria and two time intervals.

The study has found that there is an increase in uptake after 24 hrs. of biosorption process except the lead which its percentage of uptake has decreased after 24 hrs. by *Bacillus* spp. while the percentage of zinc for two types of bacteria has remained constant after 24 hrs. of biosorption.

As well as the research has studied the effect of addition of glucose on biosorption as an additional carbon and energy source and the results revealed that there is variation in bacterial ability for metal adsorption within the two intervals 4 and 24 hrs.

Keywords: *Bacillus* spp., *Pseudomonas* spp., Wastewater, Metal uptake, biosorption.

إزالة بعض المعادن باستخدام الكتلة الحيوية لجرثومتي *Bacillus* **spp.** و *Pseudomonas* **spp.** من مياه فضلات المجمع الثاني لجامعة الموصل

الملخص

ألقى هذا البحث الضوء على دراسة استخدام الجراثيم بوصفها مواداً حيوية لإزالة المعادن الثقيلة من مياه الفضلات، مستخدما الكتلة الحيوية لجرثومتي *Bacillus* spp. و *Pseudomonas* spp. وبفترتين زمنيتين 4 و 24 ساعة لعملية الامتصاص الحيوي.

وجد أن أعلى قدرة امتصاص كانت للزنك من الجرثومتين وبالفترتين الزمنيتين، وأقل قدرة امتصاص كانت للرصاص من جرثومة *Pseudomonas* spp..

أما المعادن الأخرى فقد تباينت في قدرتها على الامتصاص من الجرثومتين وللفترتين الزمنيتين. إذ توصلت الدراسة إلى أن هناك زيادة في الامتصاص بعد فترة 24 ساعة من عملية الامتصاص الحيوي ما عدا في حالة الرصاص التي كانت النسبة المئوية للامتصاص قد تناقصت بعد 24 ساعة من جرثومة *Bacillus* spp. ، فيما بقيت نسبة الامتصاص المئوية لمعدن الزنك ثابتة لكلا الجرثومتين بعد 24 ساعة من الامتصاص الحيوي.

استهدف البحث التحري عن قدرة جرثومتي *Bacillus* spp. و*Pseudomonas* spp على إزالة بعض المعادن الثقيلة من مياه الفضلات ودراسة تأثير إضافة سكر الكلوكوز على هذه العملية بوصفه مصدرا إضافيا للكربون والطاقة وأظهرت النتائج تباينا في قدرة الجرثومتين على إزالة المعادن وفي كلا الفترتين 4 و24 ساعة.

INTRODUCTION

Contamination of the aquatic environment by heavy metals is a worldwide environmental problem. The pollutants of serious concern include lead, chromium, mercury, uranium, selenium, zinc, arsenic, cadmium, gold, silver, copper and nickel, due to their carcinogenic and mutagenic nature (Leung *et al.*, 2000).

Heavy metals, in particular are a group of pollutants (mostly from domestic, agricultural and industrial activities) of major concern in the aquatic environment due to their toxicity.(Opeolu *et al*., 2010)

Bioremediation can be defined as any process that uses microorganisms or their enzymes to return the environment altered by contaminants to its original condition, Biosorption can be defined as the selective sequestering of metal soluble species that result in the immobilization of the metals by microbial cells, The speedy development and increasing sophistication of various industries in the past century has remarkably increased the amount and complexity of toxic waste effluents, which may be bioremediated by appropriate plants and microbes, either natural occurring or tailor-made for the specific purpose (Kumar *et al*., 2010).

An increased use of metals and chemicals in the industries has resulted in the generation of large quantities of aqueous effluents that contain high levels of heavy metals, thereby creating serious environmental disposal problems (Antunes *et al*., 2003). Also exponential growth of the world’s population has resulted in environmental build up of waste products, of which heavy metals are of particular concern (Appel and Ma, 2002).

The major advantages of biosorption over conventional treatment methods include: low cost; high efficiency; Minimization of chemical and biological sludge; no additional nutrient requirement; regeneration of biosorbent; and possibility of metal recovery (Kratochvil and Volesky, 1998).

In the biosorption mechanisms, the complex structure of microorganisms implies that there are many ways for the metal to be taken up by the microbial cell. The biosorption mechanisms are various, they may be classified according to various criteria. According to the dependence on the cell's metabolism, biosorption mechanisms can be divided into: Metabolism dependent; and Non -metabolism dependent. According to the location where

the metal removed from solution is found, biosorption can be classified as: (1) Extra cellular accumulation/ precipitation; (2) Cell surface sorption/ precipitation; and (3) Intracellular accumulation (Narasimhulu and Rao, 2009).

With the increasing need for the safe removal of heavy metals and the continued public interest of environmental problems, this research has aimed to use the microbial treatment as an eco-friendly alternative method which considered as important way in the future of waste management, as well as, the research has aimed to enhance biosorption by addition of glucose as additional carbon and energy source.

MATERIALS AND METHODS

Sampling

Sewage water samples were collected from the second campus of Mosul university which located in Al-Shurta neighborhood in the left side of Mosul city, from equalization tank for the sewage water treatment plant, then they were put in sterile container and were brought to laboratory directly to achieve the tests.

To make the samples ready for metal determinations, sewage samples were digested according to (APHA, 1985) in following steps:

Five hundreds ml of the sample was taken from sewagewater, and was divided to five beakers with 100ml volumes, then 5 ml conc. HNO_3were added for each beaker. Slow boil was brought and evaporated on a hot plate to the lowest volume possible (about 15 ml) before precipitation or salting-out occurs. Then 5 ml conc. HNO_3 were added, and covered with a watch glass, and heated to obtain a gentle refluxing action. Heating was continued and conc. HNO_3 was added as necessary until digestion was complete as shown by a light-colored, clear solution, without let sample to dry during digestion. 1 to 2 ml conc. HNO_3 were added and were warmed slightly to dissolve any remaining residue. The beaker walls and watch glass were washed down with water.

Volume was completed to 100 ml deionized distilled water for each beaker, 200 ml for *Pseudomonas*, 200 ml for *Bacillus*, 100 for control.

Bacterial Isolates

Bacillus spp. which is gram positive bacteria and *pseudomonas* spp. which is gram negative bacteria were used in this research obtained from Biology Department / College of Science /Mosul University each of them were isolated from environmental samples.

Then the biochemical tests were done to assure its identification and diagnosis according to (Forbes *et al*., 2007; Winn *et al*., 2008).

Biomass Preparation

To obtain as more as possible heavy growth, bacterial culture was inoculated in 100 ml nutrient broth, then incubated in a rotary shaker at 250 rpm at 35°C for 48 hrs.

Biomass was harvested from the medium by centrifugation at 5000 rpm for 20 min., the supernatant was discarded and the cells were re-suspended in deionized water for washing and again centrifuged as above to make sure that no media remain on the cell surface.

The biomass killed by heating in a conventional hot air oven at 60°C for 24 hrs. it was weighted and maintained until used in biosorption process (Tarangini, 2009).

Preparation of Metal solution

To obtain of a stock solution of 1000 mg/L (ppm), this solution was prepared by dissolving 1 g of cobalt metal in 50 ml 5M HCl, and dissolving of 1 g of zinc metal in 30 ml of 5M HCl. Then they were diluted to 1 liter in a volumetric flask with deionized water.

Dissolving of 1.598g of lead nitrate $pb(NO_3)_2$ in 100 ml of deionized water, 2.1032g of cadmium nitrate $Cd(NO_3)_2$ in 250 ml of deionized water, 4.9530g of nickel nitrate $Ni(NO_3)_2.6H_2H$ in 100 ml of deionized water.

Then each of metal salt was diluted to 1 liter in volumetric flask with deionized water, to obtain a stock solution of 1000 mg/L, then the other concentrations were obtained from it as a standard concentration, (Whiteside, 1976).

Acid was added to used glassware and subsequently rinsed with deionized water to remove any possible interference by other. All glassware used for experimental purposes was washed in 60% nitric metal, (Quintelas *et al*., 2008).

Biosorption process

Four hundreds ml of digested sewagewater samples were distributed into two main portions :

The first one of them (200 ml) was for *Bacillus* spp. which was subdivided into two parts, (100 ml) with addition of glucose sugar and the other (100 ml) was without any addition, and according to (Kumar *et al*.,2010) the pH was adjusted to 6.2 .

The second was for *Pseudomonas* spp. which was also subdivided into two parts and were performed as above and according to (Kumar *et al*., 2010) the pH was adjusted to 6.5, the temperature also was adjusted at 35°C for both bacteria according to (Tarangini, 2009).

All the four flasks were placed in a shaker (Controlled Environmental Incubator Shaker. New Brunswick scientific. Edison, N.J. U.S.A.) with a constant speed at 300 rpm for 24 hrs.

Samples were collected at defined time intervals (4 hrs, 24 hrs) to knowing whether the attachment between the cell surface and heavy metal is constant (versible) or reversible, then were centrifuged at 3000 for 10 minutes .

The amount of metal in the supernatant was determined.

Analytical estimation of metals

The concentrations of heavy metals were measured by atomic absorption spectrophotometer (analytic jana. novAA_{350}. Germany). Determination of cobalt, lead, cadmium, nickel and zinc were done by using the specific lamp for each metal and at a specific wavelength.

The amount of metal bound by the biosorbents was calculated as follows:

$Q = v (Ci – Cf) / m$

Where Q is the metal uptake (mg metal per g biosorbent), v is the liquid sample volume (ml) , Ci is the initial concentration of the metal in the solution (mg/l) , Cf is the final (equilibrium) concentration of the metal in the supernatant (mg/l) and m is the amount of the added biosorbent on the dry basis (mg).

RESULTS

The present study demonstrates the differences in the ability of *Bacillus* and *Pseudomonas* spp. in removal of heavy metals from sewage water although biosorption was performed under the same conditions (pH, temperature, agitation and speed). On the contrary, these bacteria have displayed similarity in zinc uptake as they have gave the highest uptake percentage among other heavy metals. The results showed that the uptake percentage of zinc by *Bacillus* spp. after 4 and 24 hrs. of biosorption process were the same percentage which was 85% while *Pseudomonas* spp. gave 82% removal at the same time intervals (Fig. 1 , 2).

Nickel uptake by the bacteria *Bacillus* spp. after 4 hrs. of biosorption experiment was shown to be 4% and has increased to be 20% after 24 hrs. of biosorption experiment. On the other hand, a slight increase was exhibited by *Pseudomonas* spp. from 20% at 4 hrs. to 24% after 24 hrs. from the biosorption process.

Another metal was cadmium and the uptake of it was shown to be very low (1%) by *Bacillus* spp. and (4%) by *Pseudomonas* spp. after 4 hrs. from the process of biosorption.

Differences in behavior was observed toward lead uptake from sewage water by these two bacteria, the results have revealed that *Bacillus* spp. was able to remove 12% after 4 hrs of mixing the bacteria with the sample, while *Pseudomonas* spp. was unable to do so at all. Nevertheless, after 24 hrs. the percentage of biosorption by *Bacillus* spp. decreased from 12% to 4% and has reached to 4% by *pseudomonas* spp.

Four percent of cobalt was removed by *Bacillus* after 4 hrs. and was fixed at 12% after 24 hrs. of the process. On the contrary, *Pseudomonas* spp. uptake value was shown to be 44% after 4 hrs. of biosorption process and 52% after 24 hrs.

Effect of glucose on heavy metal removal by bacteria :

The effect of glucose on biosorption was examined by adding 1% glucose to a flask containing wastewater sample, two types of bacteria as an attempt to test the sugar ability to the enhancing or decreasing bacterial removing of the heavy metals. Results as shown in fig. 1 and fig. 2 demonstrated both activation as well as inactivation of biosorption.

In the case of zinc metal, both bacteria and both time interval (4, 24 hrs) have revealed a slight decrease in removing this metal and this slight zinc uptake might not be significant. The uptake percentage for *Bacillus* was 77%, and 72% at 4hr and 24 hrs. respectively, and for *Pseudomonas* was 76% and 72% respectively.

Results of Nickel uptake also indicated that glucose added has activated nickel uptake which showed 24%, and 32% removal after 4 and 24 hrs. respectively of biosorption process by *Bacillus* spp. However *Pseudomonas* exhibited a slow absorption after the first 4 hrs (16%), followed by enhanced uptake at 24 hrs. interval (36%).

Cadmium showed an enhanced uptake exhibited by both bacteria after 4 hrs. giving 14% and 16% for *Bacillus* and *Pseudomonas* respectively. However, after 24 hrs., inactivation was noted by *Bacillus* were uptake decreased from 12% to 8%. On the other hand *Pseudomonas* persisted the percentage on 12% removal.

Glucose greatly enhanced lead biosorption by *Bacillus* spp., percentage has register 48% after 4 hrs. in a percentage equal to 8% has accomplished after 24%. On the contrary, *pseudomonas* spp. has given 4% and 8% after 4 and 24 hrs. respectively.

Biosorption of cobalt was not affected throughout the experiment by *Bacillus* spp. after 4 and 24 hrs. with glucose as indicated by 12% removal (which is the same value for biosorption after 24 hrs. without glucose), this situation reveals that the sugar has affected just after the first 4 hrs mildly, while after 24 hrs. there was no effect of glucose. However, cobalt uptake by *Pseudomonas* spp. was greatly influenced by the addition of glucose, 4% removal was demonstrated after 4, and 24 hrs. Which indicates an inhibitory effect of glucose on cobalt uptake by this bacteria.

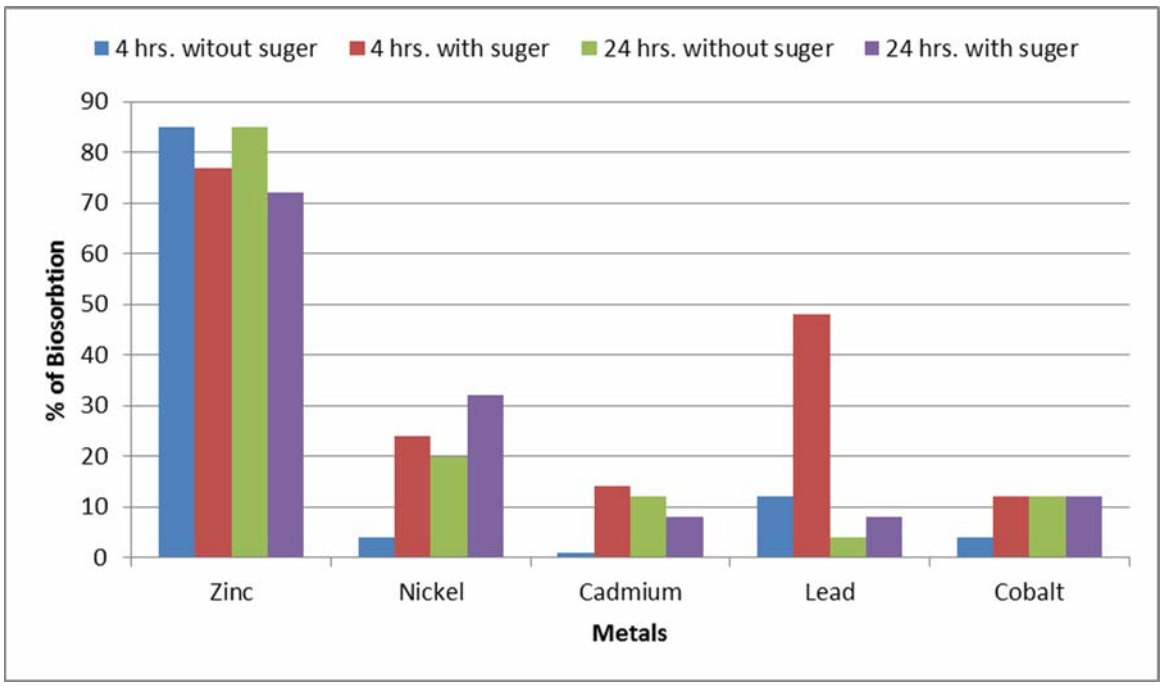

Fig. 1: percentages uptake of metals by *Bacillus* spp.

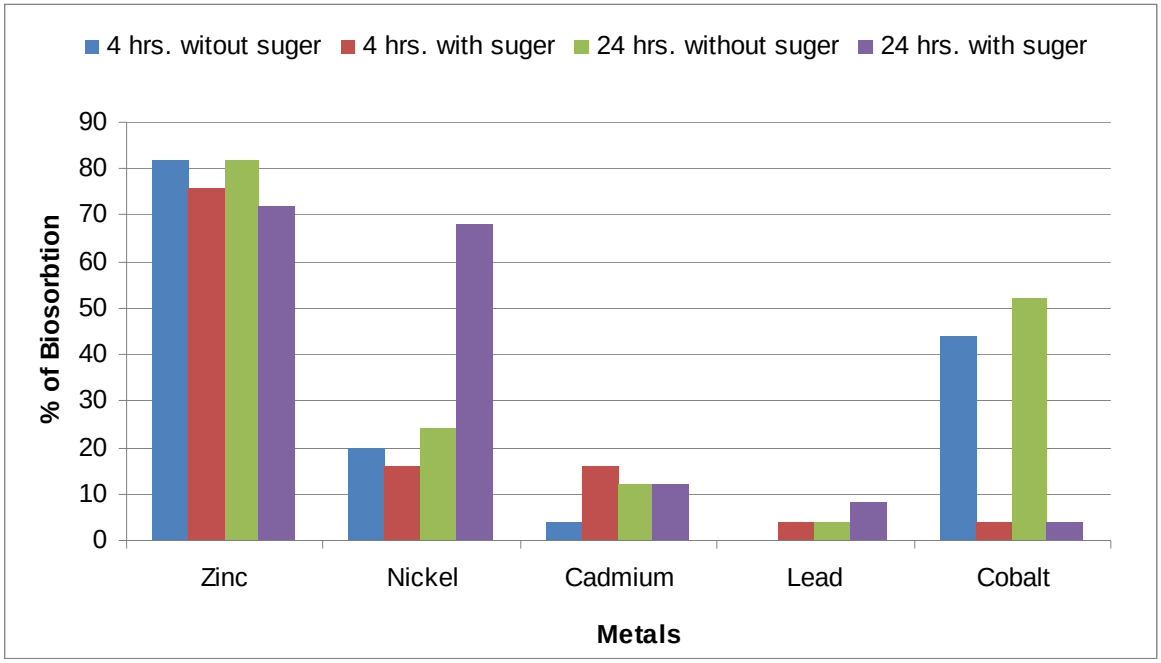

Fig. 2: percentages uptake of metals by *Pseudomonas* spp.

DISCUSSION

Pollution is a serious environmental concern and interest in bacterial resistance to heavy metals of practical significance. The strong biosorbent behavior of certain types of microbial biomass toward metallic ions is a function of the chemical makeup of microbial cells (Ramaiah *et al*., 2008).

In fact, the biomass is dead and all cells are metabolically inactive (Voelsky and Holan 1995). There is also much confusion in the methodology used and in the evaluation and expression of experimental data which shows an inadequate understanding of sorption principles (Hussein *et al*., 2004). According to (Beverdige,1989), bacteria make excellent biosorbent because of their high surface-to-volume ratio and a high content of potentially active chemisorptions site such as on teichoic acid in their cell wall .

Our results were approach with the results of (Bafubiandi *et al*., 2009) who found that the percentage of cobalt removal ranged between 40-65% by *Pseudomonas* spp. at PH 6 and temperature 37ºC within 24 hrs. while the results of (Dlamini *et al*., 2010) did not agree with our results, by using *Bacillus* spp. which obtain 83% after 48 hrs. at pH 5.5. On the other hand, (Mamba *et al*., 2009) who used a mixture of many microorganisms to remove this metal, the uptake percentage were between 23-70% at pH 2 and temperature 37ºC after 24 hrs.

Our results showed that *Bacillus* spp. was more active than *Pseudomonas* spp. in removal of lead metal after 4 hrs. of biosorption. In comparison with (Khanafari *et al*., 2008) who used *Bacillus circulans* to remove lead from sewage water, the uptake after 72 hrs. 65% in pH 7, while (Kumar *et al*., 2010) found that *Staphylococcus* spp. was the best in removal of lead than *Bacillus* spp. and *Pseudomonas* spp. and the rate was 93%. The results showed that *Staphylococcus* spp. Uptake the lead in very significant amount. In Turkish study introduced by (Ilhanet *et al*., 2004) showed that 100% yield was determined for lead ions.

Lead poisoning in humans may cause severe damage to the kidneys, nervous system and reproductive systems, Alzheimer's-Disease, liver and brain severe exposure to lead is also associated with sterility, abortion, still birth and neonatal deaths (Leung *et al*, 2000) .

The uptake of cadmium metal in our results by using *Bacillus* spp. and *Pseudomonas* spp. were low in comparison with (Jaysanker *et al*., 2008) who used *Bacillus* to remove Cadmium metal after 24 hrs. which reached 70%. As well as (Narasimhu and Rao, 2009) used *Pseudomonas* to removing cadmium the rate were 90%.

Ions of lead and cadmium are of serious concern as they are non-bio degradable, highly toxic and are present in a variety of waste streams that contaminate the environment (Cameron, 1992).

Our results about removing nickel metal did not agree with Kumar and his workers at 2010 who obtained 48% for *Bacillus* and 65% for *Pseudomonas.* As well as (Dlamini *et al*., 2010) found that this metal uptake from waste water by *Pseudomonas* increased to 90%.

Concerning to cadmium and nickel similar removal ratios were obtained, since it was ranged between 35 to 88%. and their maximum removal were obtained in the case of individual cadmium and nickel (Dlamini *et al*., 2010).

Our results about zinc agree with (Wierzba, 2010) who obtain 86.5% at pH 5 to removal of zinc. While (Kumar *et al*., 2010) used fungi *Aspergillus niger* to remediate zinc and the rate which obtained 58% at PH 8 and tem. 35ºC.

This ratio is less than the ration of our result by using bacteria to remov this metal; using of *Bacillus* to examine further the bacterium interaction with cadmium, lead. The results quantified not only the deprotonation constants for the important organic functional groups on the bacterial cell wall, but also the stability constants for absorption of environmentally important metal species onto the individual sites. Bacterial cell walls are negatively charged under acidic condition and the cell wall chemically functional groups display a high affinity for metal ions in solution (Fein *et al*.,1997).

Bacterial mobile genetic elements, such as plasmids or transposones, can carry multiple genes encoding metal and antibiotic resistance, thus exposure to one agent select for microbial resistant to toxicants, such organisms may be important in performing biological processes in contaminated habitats (Ramaiah *et al* ., 2008).

Cellulosic materials and their derivatives have shown quite good metal ion adsorptive capacity. Although, the efficiency of activated carbon in adsorbing heavy metal ions from wastewater is high enough, because of significant costs involved in preparation of activated carbon and its regeneration, it is only used as a tertiary step in the treatment of wastewater.

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