Heavy Metal uptake Potentials of *Pseudomonas aeruginosa* and *Micrococcus luteus*

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Abstract

Uptake of heavy metals, silver and cadmium by Pseudomonas aeruginosa (a Gram negative bacterium) and Micrococcus luteus (a Gram positive bacterium) was investigated in Cadmium and Silver stock solution using ion selective electrodes. Silver and cadmium uptake by the two organisms was described by Langmuir isotherms. Binding at one site type is indicated. It was observed that both organisms showed rapid uptake of metal mostly in the first minute. Uptake was complete by ten minutes. Micrococcus luteus concentrated more metal than Pseudomonas aeruginosa. M. luteus took up 200% more silver and 100% more cadmium than P. aeruginosa. We conclude that the two organisms, particularly M. Luteus have very high bioremediation potentials in heavy metals polluted environment.

Keywords: Heavy metals, Pseudomonas, Langmuir isotherms, Silver, Cadmium

Introduction

Man's activities generate heavy metal wastes. For instance, the processes of industrial manufacturing (Ajmal and Khan, 1985; Gazsó, 2001) and metal mining (Denny and Welsh, 1979,; Johnson and Eaton, 1980,; Beyer *et al.,* 1985) release large quantities of metals into the environment. Discharge of cadmium into natural waters may be through electroplating activities (Higgins and Desher, 1986), nickel-cadmium battery manufacture and/or smelter operations (Butterworth, *et al*., 1972). Silver is a waste generated by photographic and radiographic applications.

Industrial wastes that contain these metals find their way into sewage treatment works (Cheng *et al.,* 1975; Tyagi *et al.,* 1988). In many cases the sludge from these works contains high concentrations of metal, which is deposited on agricultural land (Schauer *et al*., 1980; Dressler *et al*., 1986).

There are three main areas of interest in heavy metal uptake by microorganisms (a) these heavy metals find their way into the environment resulting in bioconcentration and biomagnification (Broda, 1972; Martin and Coughtrey, 1975; Gipps and Biro, 1978; Eja *et al*., 2003). (b) Another area is in the economics of alternative methods of recovery of metals in low concentrations. Microbial extraction causes less pollution and energy use, and may be amenable to unique biological improvement techniques such as genetic engineering. (c) As more stringent effluent limitation standards have resulted in a need to remove or reduce heavy metal contaminants before they find their way into the environment, more sophisticated and costly treatment methods will be required, making biological treatment most attractive.

Therefore the aim of this study is to examine the metal uptake potentials of *P. aeruginosa* and *M. Luteus*, for their possible application in the bioremediation of heavy metal polluted environment.

Materials and Methods

Cultures: *Pseudomonas aeruginosa* (NCIB 950) and *Micrococcus luteus* (NCIB 8553) were obtained from The National Collections of Industrial and Marine Bacteria ltd. 23 Machar Drive, Aberdeen, Scotland.

Metal solutions: All solutions were made using deionised water. Sterilization was achieved by autoclaving at 121^{o}C for 15 minutes.

Cadmium and silver stock solution: Cadmium stock solutions were made using cadmium nitrate (ANALAR BDH Chemicals Ltd Poole England) while Silver stock solutions were prepared using silver nitrate (ANALAR, Hopkin and Williams, Chadwell Heath Essex). To avoid photoreduction, solutions were stored in brown bottles.

Ion selective electrodes

Silver and cadmium: Silver was measured continuously using a silver/sulfide Electrode Model 94-16 ORION (Orion Research Incorporated Products Group), while cadmium was measured continuously using a cadmium Electrode Model 94-4489 Russell. Electrodes were calibrated before use.

Silver and cadmium uptake over time: Investigations were carried out using 20 ml of metal solution (20 mg/l) in 50 ml beakers that had previously been rinsed in distilled water. A suspension of the test organism (2.6mg dry weight in 2mls sterile distilled water) was added to the metal solution and decrease in free ion concentration was measured using an ion selective electrode. To ensure that any decrease in metal concentration was due to the test organism, controls were used which consisted of metal solution minus the test organism. The controls were continuously monitored using the same ion selective electrode. To allow for the slight increase in volume caused by introducing the test organism,

the controls had an identical amount of distilled water added to them. Adjustments were made where necessary based on the controls. The investigations were repeated three times but results were not pooled. The binding/uptake of silver and cadmium by the two organisms was described by Langmuir plots. The Langmuir isotherm was expressed as $[M_F] / [M_B] = 1/KB + [M_F]/B$ (Hughes & Poole, 1989), where a plot of $[M_F] / [M_B]$ against $[M_F]$ should be linear. $[M_F]$ = Free metal ion concentration, $[M_B]$= Bound metal, K= Binding affinity, B = binding capacity.

Results

Of the total silver taken up over a ten minute period *Micrococcus* accumulated 97% of its total uptake in the first minute, while *Pseudomonas* took up 87%. *Micrococcus* took up 200% more silver than *Pseudomonas* over the ten minute period (Fig. 1).

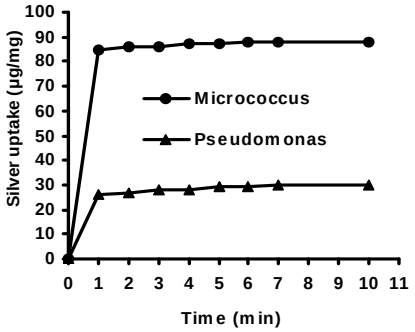

Fig. 1: Uptake of Silver by *Micrococcus* and *Pseudomonas*

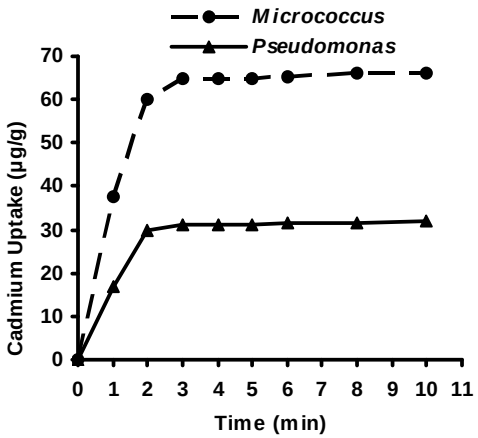

Fig. 2: Uptake of cadmium by *Micrococcus* and *Pseudomonas*

Of the actual silver taken up from solution during the first minute, *Micrococcus* took up 21.3% of silver present, and *Pseudomonas* took up 6.5% of silver present. In the first minute 9.2% of total cadmium present was taken up by *Micrococcus* while *Pseudomonas* took up 4.3%. Of the total uptake over 10 minutes both organisms took up just over 50% in the first minute. *Micrococcus* took up over 100% more than *Pseudomonas* over the 10 minutes (Fig. 2). Cadmium and silver uptake by the two organisms can be described by Langmuir isotherms (Figs 3 - 6) which were straight line plots.

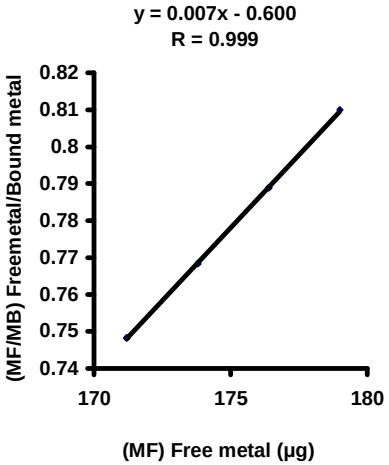

Fig. 3: Langmuir Isotherm for silver uptake by *Micrococcus luteus*

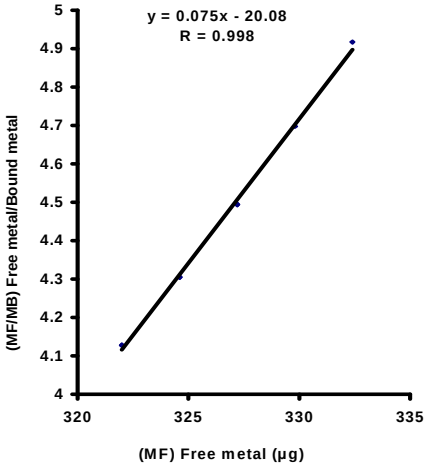

Fig. 4: Langmuir Isotherm for silver uptake by Pseudomonas aeruginosa

Discussion

In this study uptake of silver and cadmium by bacterial species *P. aeruginosa* and *M. luteus*, was compared. Metal removal by both the organisms studied here, showed the same general profile. That is, a rapid initial uptake, usually in the first one or two minutes, where the largest proportion of the removal takes place followed by a decrease in uptake, and ceasing or becoming negligible by about 10 minutes. Similar observations with microbial heavy metal uptake have been reported elsewhere (Khummongol *et al.,* 1982; Costa and Leite, 1991; Arikpo and Eja, 2003; Gőksungur *et al*., 2003).

Of the two organisms studied *Micrococcus* took up a greater quantity of both silver and cadmium. The Gram positive *Micrococcus* took up far more metal than the Gram negative *Pseudomonas*.

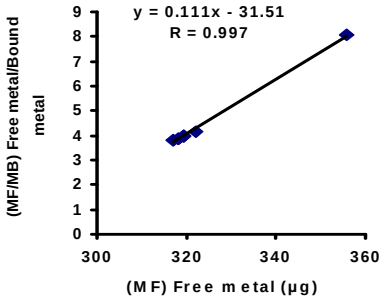

Figure 5: Langmuir isotherm for cadmium uptake by *Pseudomonas aeruginosa*

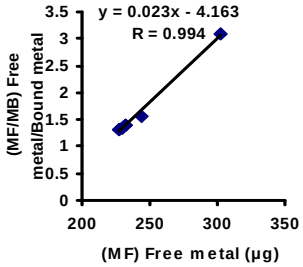

Fig. 6: Langmuir isotherm of cadmium uptake by *Micrococcus luteus*

It has been mentioned that Gram-negative bacteria bind less metal than Gram-positive bacteria, sometimes up to ten times less (Hughes & Poole 1989). It may be suggested that Gram-positive bacteria have more porous walls, allowing metal to build up due to nucleation and precipitation of insoluble salts (Beveridge, 1988; 1989).

Cadmium and silver uptake by the two organisms is described by Langmuir isotherms which indicate binding at one site. On the other hand a biphasic curve may indicate the availability of more than one site type, while a sigmoidal curve, indicates that initial binding of the metal has brought about structural changes and an increase in the availability of binding sites (Hughes and Poole, 1989).

It was observed that the rates of uptake of silver and cadmium by the two organisms were measurable, with *M. luteus* taking up 200% more silver and 100% more cadmium that *P. aeruginosa.* We conclude that the two organisms, and particularly *M. luteus*, have very high metal uptake and bioaccumulation factor which underline their potential application in bioremediation of heavy metal-polluted environment.

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