GLOBAL JOURNAL OF PURE AND APPLIED SCIENCES VOL. 10, NO. 2, MARCH 2004: 257 - 262
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CADMIUM UPTAKE BY THE GREEN ALGA *CHLORELLA EMERSONII.*

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(Received 20 July 2003; Revision accepted 23 July 2003)

ABSTRACT

Investigations were carried out on the uptake of the heavy metal cadmium (Cd) by the green alga *Chlorella emersonii* with the aid of an ion selective electrode. Cadmium uptake by *Chlorella* was very rapid with 70% of total uptake occurring during the first 10 seconds. Uptake of cadmium by *Chlorella* showed a direct relationship to the amount of metal present, suggesting 'equilibrium conditions' being responsible for the amount of metal removed from solution rather than just number of binding sites available.. Absence of metabolic involvement in the uptake process was observed as there was no difference on final uptake of cadmium whether cells were alive or dead.. It is concluded that living or dead *Chlorella* biomass has a high biosorption potential for cadmium, and its rapid uptake makes it an attractive candidate in the current proposed application of biosorption technology for industrial waste treatment.

KEY WORDS: Cadmium uptake, alga *Chlorella*, industrial waste treatment, biosorption technology, cell biomass.

INTRODUCTION

Heavy metals are generated in many activities of man. The process of mining (Johnson and Eaton, 1980, Denny and Welsh, 1979, Beyer et al., 1985) and manufacturing (Ajmal and Khan, 1985) release large quantities of metals into the environment. Also discharge of cadmium into natural waters may be a consequence of electroplating activities (Higgins and Desher, 1986), nickel-cadmium battery manufacture and/or smelter operations (Butterworth, *et al.*,1972). Much of the wastes from industry containing these metals finds its way into sewage treatment works (Cheng *et al.*, 1975, Tyagi *et al.*, 1988). In many cases the sludge from these works containing high concentrations of metal is deposited on agricultural land (Schauer *et al.*, 1980, Dressler *et al.*, 1986).

The interest in microbial heavy metal uptake stems from three main areas:
(a) These heavy metals find their way into the environment resulting in, bioconcentration and successive accumulation in the trophic levels (Broda, 1972, Martin and Coughtry, 1975, Gipps and Biro, 1978). The oceans are frequently the final reservoir of heavy metal pollutants and since algae have a major role in marine primary production, there is a need to understand the microbial heavy metal interactions.

(b) The economics of alternative methods of recovery of metals in low concentrations. Microbial extraction uses less energy, causes less pollution and may be amenable to unique biological improvement techniques such as genetic engineering.

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.

Rorrer (1999) gives three principal advantages of bioprocess technologies for heavy metal ion removal;

(1) Biologically based processes can be carried out in situ at the heavy metal ion contaminated site.

(2) Bioprocess technologies are usually environmentally benign.

(3) Bioprocess technologies are potentially low cost.

In this study the aim was to examine the ability of *Chlorella emersonii*, a green alga, to take up cadmium. The approach was to measure Cadmium uptake directly.. This was possible with the aid of an ion selective electrode which was found to be reliable, rapid, cheap, stable and measured metal in its ionic form only. This was a departure from the way metal is measured by most workers, (Hughes and Poole (1989), which

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Table 1: Effect of Other metals on cadmium removal by *Chlorella*.

	Cadmium uptake (µg/mg)	Percentage uptake (%)
Control (cadmium only)	9.02	22.5
Cadmium + Lead	10.56	26.4
Cadmium + Copper	11.11	27.8
Cadmium + Lead + Copper	10.45	26.1

involves using atomic spectrophotometric (AA), and stripping voltammetry methods which do not discriminate between metal in its ionic and non ionic form.

Materials and methods

A stock culture of *Chlorella emersonii* (CCAP 211/11N) was obtained from Culture Collection of Algae and Protozoa, Freshwater Biological Association, The Ferry House, Ambleside, Cumbria LA22 OLP U.K.

JM (Jaworski's medium) Algal growth medium described by Thompson *et al.* (1988) and JM agar which was prepared from JM medium solidified by the addition of 15g/l of Agar No. 3 (Oxoid) were used for the maintenance of algal cultures.

Maintenance of Cultures

Cultures were stored in JM algal growth medium and on JM agar slopes at 5°C in a refrigerator. Fresh cultures were prepared by inoculating fresh media with a loopful of culture and by streaking a fresh agar slope every two months. These were then incubated, in natural daylight for two weeks at room temperature. Checks were made for purity using a light microscope. These were then stored in a refrigerator and used as required. Workng growing cultures were grown in 20l (working volume 15l) glass vessels at room temperature by a window.

Solutions

All solutions were made using glass distilled water. Sterilization was achieved by autoclaving at 121°C for 15 minutes.

Metal solutions.

All metal solutions were made using deionised water. Stock solutions (1000 mg l^{-1}) were made up every two months. Working solutions were made fresh from stock solution as required.

Cadmium

Cadmium stock solutions were made up using cadmium nitrate. (ANALAR BDH Chemicals Ltd Poole England).

Copper

Copper stock solution was prepared using cupric sulphate {copper (II) sulphate pentahydrate} ANALAR BDH Chemicals Ltd. Poole England.

Lead

Lead stock solution was prepared using lead nitrate A.R. grade Fisons Scientific Apparatus, Loughborough, Leics. England.

Analysis

Atomic absorption spectrophotometry

Concentrations of copper and lead were determined using a Varian AA-1275 Series Atomic Absorption Spectrophotometer. Methods used

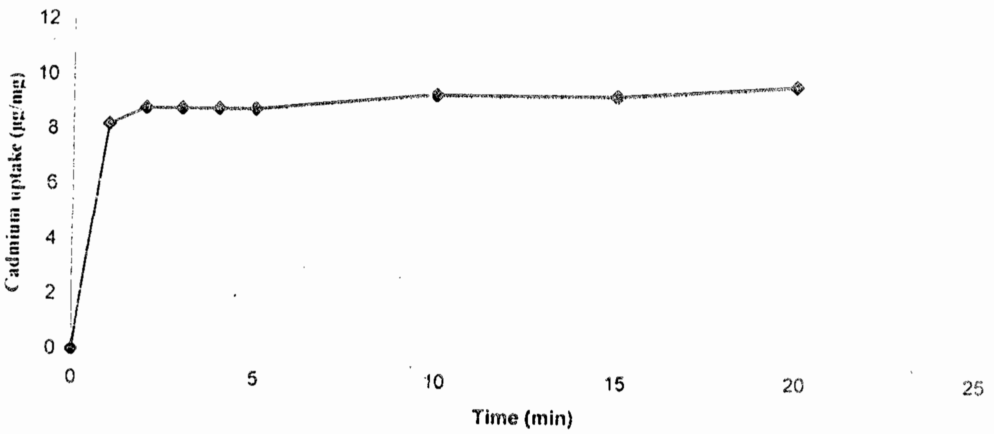

Figure 1. Cadmium uptake by *Chlorella*.

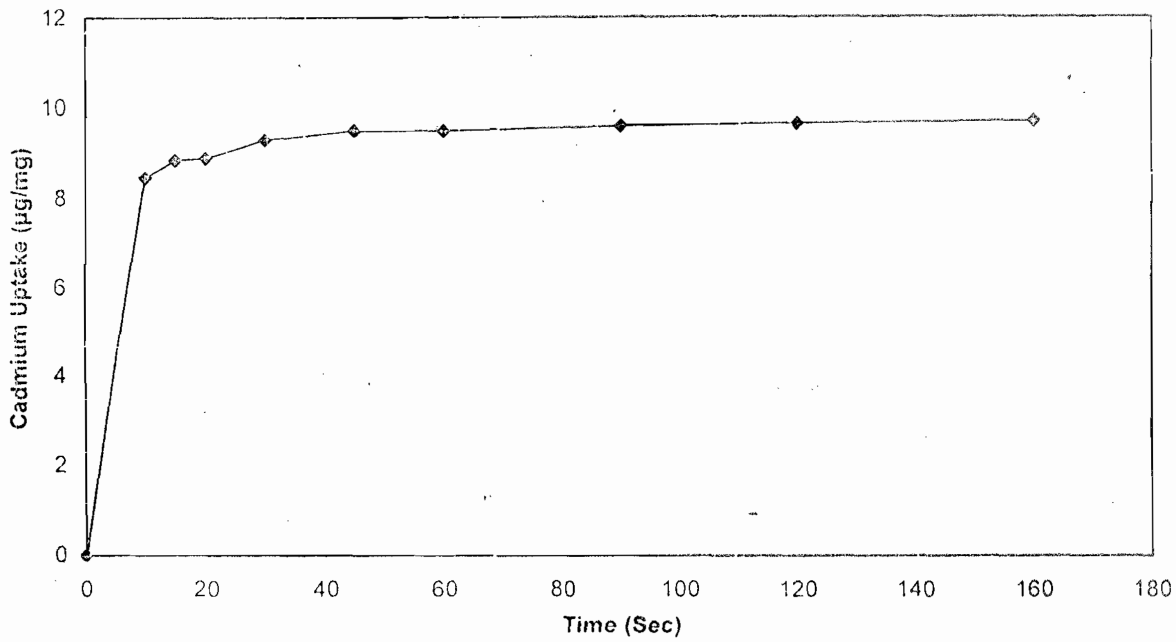

Figure 2. Cadmium uptake by *Chlorella.*

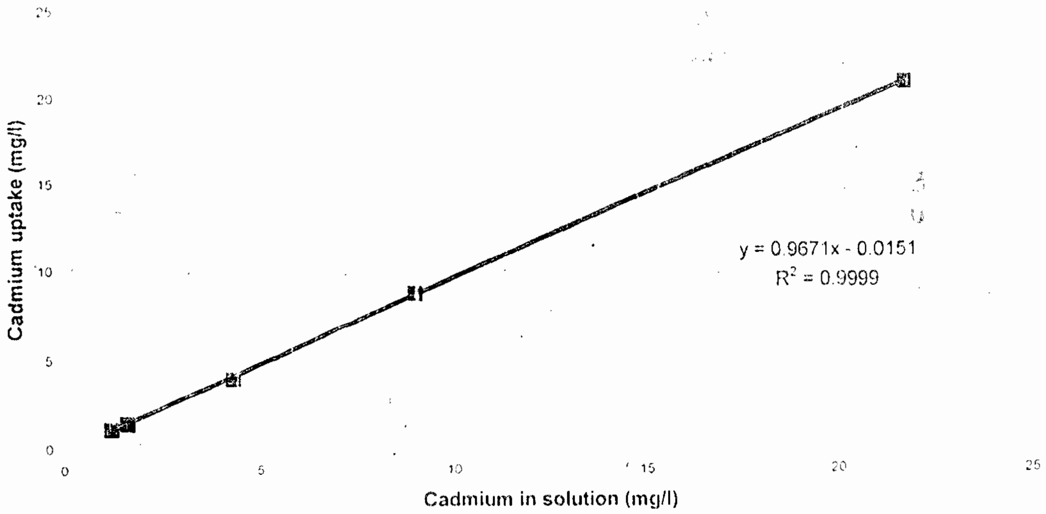

Figure 3. Effect of cadmium concentration on its uptake.

were as described in "analytical methods for flame spectroscopy" (Varian 1979). Metal standards were made from stock solutions as described above.

Cadmium

Cadmium was measured continuously using a cadmium Electrode Model 94-4489 Russell.

The electrode was calibrated daily before use.

Biomass Determination

Biomass levels were obtained by dry weight analysis. Cells were centrifuged, resuspended in distilled water, and the procedure duplicated to avoid any carry over of salts that might affect the dry weight. Resuspended cells (10 mls) were added to a dry pre-weighed aluminium dish. This was dried at 55°C until constant weight was achieved on two consecutive weighings.

Metal uptake investigations

Cadmium uptake over time

Investigations were carried out using 20 ml of metal solution (19mg/l) in 50 ml beakers that had previously been rinsed in distilled water. A suspension of *Chlorella* cells (5mg dry weight in 2 ml) 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 an ion selective electrode. To allow for the slight increase

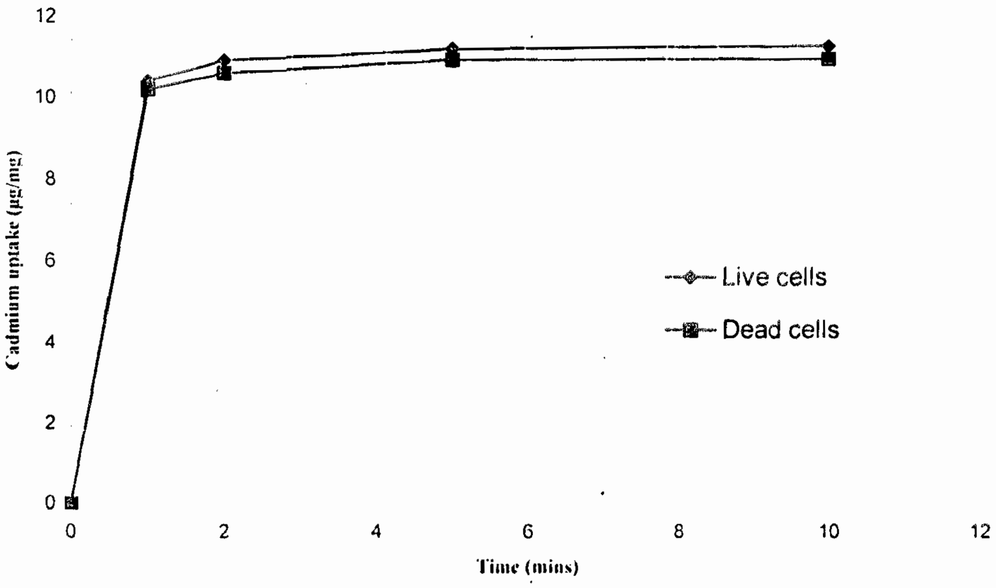

Figure 4. Cadmium uptake by live and heat killed *Chlorella* cells.

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.

Effect of cadmium concentration on its uptake by *Chlorella*

Studies on effect of cadmium concentration on its uptake by *Chlorella* was carried out using 20 ml of metal solution (1.2mg/l, 1.6mg/l, 4.3mg/l, 9mg/l, 21.7mg/l respectively) in 50 ml beakers that had previously been rinsed in distilled water. A suspension of *Chlorella* cells (35mg dry weight in 2 ml) was added to the metal solution and decrease in free ion concentration was measured using a cadmium ion selective electrode. Controls were used as earlier mentioned.

Cadmium uptake by dead and live *Chlorella* cells

Dead *Chlorella* cells were prepared by using 20 mls of cell suspension from a growing culture. This was placed in a 100 ml beaker and boiled for 2 minutes in a microwave oven. Cells were centrifuged, resuspended in 2 mls distilled water. Live cells were treated in the same way except for boiling. A suspension of the living and dead cells (5 mg dry weight in 2 ml respectively) was added to the metal solution and decrease in free ion concentration was measured using an ion selective electrode.

Effect of other metals (copper and lead) on uptake of cadmium by *Chlorella*

Investigations were carried out using 20 ml of Cadmium (10 mg/l), cadmium and lead (10mg/l respectively), cadmium and copper (10mg/l respectively) and cadmium, lead and copper (10mg/l respectively) in 50 ml beakers that had previously been rinsed in distilled water. A suspension of the test organism (5mg dry weight in 2 ml) was added to the metal solution and decrease in free ion concentration was measured using an ion selective electrode. Controls were used as previously mentioned.

The binding of cadmium by *Chlorella* was described by a Langmuir plot . 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 AND DISCUSSION

On monitoring cadmium uptake by *Chlorella* over a twenty minute period it was found that about 84% of total uptake occurred in the first minute (fig1). It was then decided to monitor the uptake over shorter time periods (fig2) where it was found that over 70% of total uptake occurred in the first 10 seconds. The rapid uptake was followed by a slower uptake with no further increase after about 3 minutes, and this indicates that *Chlorella* is a potential bisorbent for cadmium, especially as it allows for very fast throughput of waste streams. Other workers have made similar observations with other metals and organisms. (Khummongkol, *et al*.. 1982, Costa and Leite, 1991)..

Uptake of cadmium by *Chlorella* showed a direct relationship to the amount of metal present, suggesting 'equilibrium conditions' being

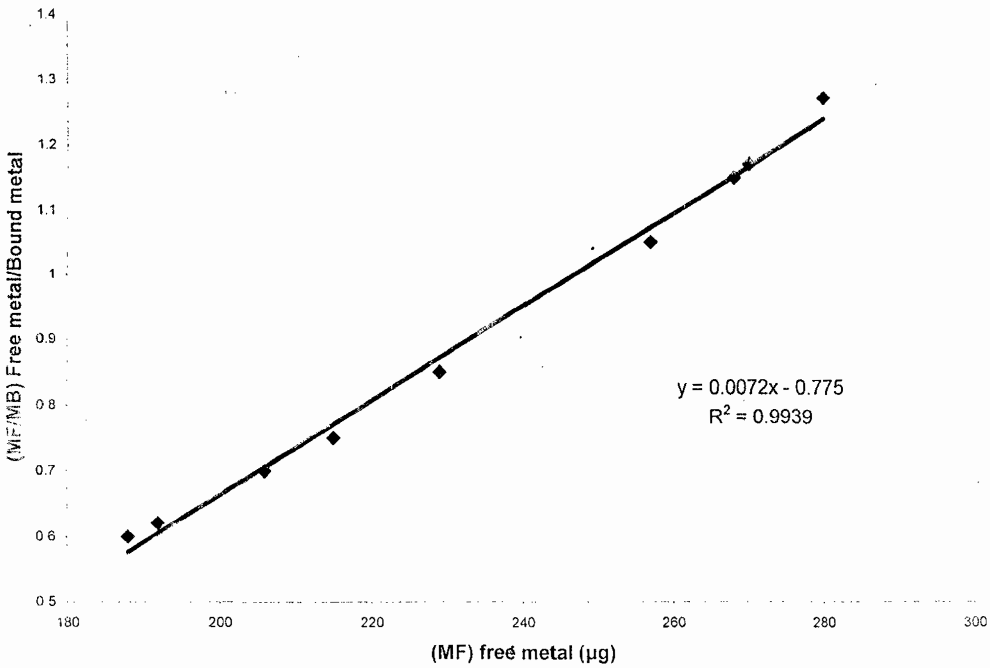

Figure 5. Langmuir Isotherm for Cadmium Removal by *Chlorella.*

responsible for the amount of metal removed from solution rather than just the number of binding sites available (fig. 3)

A comparison of cadmium uptake by living and dead (heat-killed) *Chlorella* cells (fig 4) showed no pronounced variation in uptake of cadmium at the end of 10 minutes. This has been observed by other workers using other organisms and/or metal (Ratcliffe and Beeby, 1980, Tsezos and Volesky, 1982, Göksungur *et al.*, 2003, Arikpo and Eja, 2003). This implication makes *Chlorella* biomass an attractive bisorbent , as cell death caused by the effluent would be unlikely to reduce the efficiency of metal uptake.

It was found that the presence of other metals (Copper and Lead) did not cause any reduction in the uptake of cadmium by *Chlorella* (Table 1). This is an indication that cadmium may have specific binding sites on the *Chlorella* cell wall. It also indicates that waste streams containing a number of dissolved metals can be treated without a decrease in efficiency of uptake of a particular metal.

Cadmium uptake by *Chlorella* can be described by a Langmuir isotherm (fig 5) showing a straight line plot. This being an indication of binding at one site type, a biphasic curves may indicate the availability of more than one site type, while a sigmoidal curve when a plot of bound metal against free metal is undertaken 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)

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