Analysis of removal of cadmium by action of immobilized *Chlorella* sp. micro-algae in alginate beads [version 1; referees: 2 approved]

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Abstract
Cadmium (Cd) is a metal that can negatively interfere with the metabolic systems of living beings. The objective of this work was to evaluate the capacity for cadmium removal in aqueous solutions by immobilized *Chlorella* sp. in calcium alginate beads. Beads without *Chlorella* sp. were used as a control. All the treatments were established in triplicate for 80 min, at four concentrations of cadmium (0, 20, 100 and 200 ppm), taking samples of aqueous solution every 10 min, to be read using atomic absorption equipment. The study determined that the treatment of alginate beads with immobilized *Chlorella* sp. removed 59.67% of cadmium at an initial concentration of 20 ppm, this being the best removal result.

Keywords
*Chlorella* sp., cadmium removal, immobilization, alginate
Introduction
Pollution from the use of metals as a consequence of waste generated by industries is a constant concern, since they may end up being transferred to the environment. The need to develop technologies for the remediation of water contaminated with cadmium should be considered as a priority in Ecuador. The objective of this study was to evaluate the capacity for cadmium removal in aqueous solutions by immobilized Chlorella sp. in calcium alginate beads. This study used the micro-algae Chlorella sp., for the bio-removal of cadmium, since the use of cells of Chlorella sp. immobilized in beads of alginate has been successfully exploited and its potential use has been established in the bioremediation of contaminants such as metals, nutrients and other industrial pollutants.

Methods
Alginite beads
The assay was carried out with aqueous solutions of cadmium. This solution was placed together with the alginate beads in Florence flasks. Every 10 minutes a sample of the aqueous solution was taken (10 mL approximately) with a glass pipette, and each sample was placed in a test tube. The concentration of metal was measured by aspiration atomic absorption spectrophotometer.

To make the control beads, the procedure described in a previous work was followed. Briefly, 2 grams of sodium alginate (Loba Chemie, high density) were dissolved in 200 mL of ultra-pure water to obtain a homogeneous mixture, adjusting the pH between 7.8–8.0. pH was adjusted by addition of 0.1 molar sodium hydroxide or 0.1 molar hydrochloric acid, in sodium alginate paste (homogeneous mixture). One drop at a time was slowly poured into a 1% w/v calcium chloride solution with constant stirring.

For the immobilization of Chlorella sp. in alginate beads, liquid cultures were used. The volume of culture to be used was established to reach a concentration of 25 X 10^6 cells/mL per each mL of alginate. For the preparation of the beads, 2 g of sodium alginate were dissolved in microalgae culture, as above.

Removal of cadmium with alginate beads
Experimental liquids were placed in 12 Florence Flasks of 500 mL capacity, inside which were placed 50 g of alginate beads without immobilized Chlorella sp. and 200 mL of cadmium solution at different concentrations (0, 20, 100 and 200 ppm). Each concentration was performed in triplicate, essay was evaluated during 80 minutes, aqueous solution samples were taken at 10 min intervals. Each Florence flask was placed on a shelf in the laboratory, the location of each bead on this surface was performed randomly. The beads were subjected to constant aeration with air pressure of 0.012 MPa for 80 min. Every 10 minutes, an aqueous solution sample was taken to measure the concentration of metal present. These were the control treatments.

For the experimental samples, 50 g of alginate beads with immobilized Chlorella sp. were placed in each flask with the same concentrations of cadmium solution as the controls. These experiments were also performed in triplicate. These experimental samples were used to establish whether the addition of Chlorella sp. in the beads, increases or decreases the alginate removal activity.

The determination of metal concentration in the solutions was measured with a VARIAN direct aspiration atomic absorption spectrophotometer (VARIAN, Model: SpectrAA 55, Wavelength: 326.1 nm), using air-acetylene flame with cadmium lamp, and results are presented as % of removal of cadmium.

Viability of micro-algae exposed to cadmium
For viability tests, color change of the cultures exposed to cadmium was evaluated. For this purpose 100 mL of liquid Chlorella cultures were used (25 × 10^6 cells/mL) and they were subjected to different concentrations of Cd (0, 20, 100 and 200 ppm), in triplicate. The color change was monitored at the first hour of treatment, at 24 hours and at 7 days.

Results and discussion
Viability of micro-algae exposed to cadmium
Color changes were observed in the solutions (Figure 1, Dataset 1 and Dataset 2) of free micro-algae cells exposed to different concentrations of cadmium, which is a product of the toxicity of the metal, while the solutions without the presence of metal maintained a green color, characteristic of Chlorella sp.

It was determined that Chlorella sp. exposed to a concentration of 20 ppm cadmium at 60 minutes of contact does not react, and shows no noticeable changes in coloration. On the other hand, the micro-algae did not show resistance to concentrations 100 and 200 ppm of Cd, revealing that the cells did not remain viable due to the color variation and high toxicity of the metal used, going from green to a grayish brown in the first 60 minutes of the start of the trial. These results show to decrease algal growth and inhibit photosynthesis 100 ppm or higher.

Figure 1. Viability tests of Chlorella sp. subjected to different concentrations of Cd, at 60 minutes. (A) 0 ppm Cd, (B) 20 ppm Cd, (C) 100 ppm Cd, (D) 200 ppm Cd. Green color means that algae remain viable, brown color means that Chlorella loses viability.
Removal of cadmium with alginate beads

It is verified that the best treatment for Cd removal is the one corresponding to alginate beads with immobilized *Chlorella* sp. at the concentration of 20 ppm of Cd at 80 min. It was determined that at low metal concentrations the micro-algae enhances its removal capacity achieving a removal percentage of 59.67% with *Chlorella* sp., being significantly higher (p value <0.001) than the removal presented without *Chlorella* sp. (55.56%), explained by the viability process that showed that *Chlorella* sp. can withstand the toxicity of the metal for periods of at least 60 min at concentrations of 20 ppm (Figure 2 and Figure 3). As demonstrated by 9, micro-algae work better at lower concentrations of metal.

Chlorella* sp. immobilized in alginate beads could be used for bioremediation processes of cadmium at low concentrations of the metal, since the presence of viable biomass of the micro-algae potentiates the removal capacity of the alginate. Non-viable *Chlorella* sp., because of the high concentration of cadmium (100 and 200 ppm), decreases its removal capacity, yielding better results in beads treated without *Chlorella* sp. Thus, the alginate matrix without micro-algae could be used effectively in Cd removal processes at high metal concentrations.

**Data availability**

Dataset 1. Percentage of Cd in aqueous solution at 80 minutes. DOI, 10.5256/f1000research.13527.d190266

Dataset 2. Raw data for the percentage removal of cadmium with and without *Chlorella* alginate beads at all concentrations and triplicate experiments DOI, 10.5256/f1000research.13527.d190281

**Competing interests**

No competing interests were disclosed.

**Grant information**

The author(s) declared that no grants were involved in supporting this work.

**References**

5. de-Bashan LE, Moreno M, Hernandez JP, et al.: Removal of ammonium and


Open Peer Review

Current Referee Status:  

Bernadett Gálya  
Institute of Water and Environmental Management, University of Debrecen, Debrecen, Hungary

In my opinion, this article is well editable and easy to follow. The scope of each chapter is appropriate. In the Introduction, they briefly presented the problem behind the study. The study design is appropriate and the work is technically sound. They cited 11 references which is strongly connected to this topic. These are from 1998 to 2018, all in all, current literature was used. The method was described in detail, thus it is easy to replicate. They created three main parts in the methods. In my opinion is fine, but I suggest that the third subchapter should be the test used during the experiment. Otherwise, the materials, repetition and measurement which they are used are appropriate. The charts that are used are clear and easy to follow these in the text. In this article, the statistical analyses is applicable. May I suggest, that in the future they should use some other statistical test to compare different treatments (such as Tukey or Duncan-test). All source data are underlying the results available to ensure full reproducibility. The conclusions are drawn adequately supported by the results. Summary, this article is clear, easy to follow, used appropriate methods and scientifically based. Thus, it will be very useful to solve the examined problem.

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If applicable, is the statistical analysis and its interpretation appropriate?  
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Yes

Competing Interests: No competing interests were disclosed.
Referee Expertise: Environmental technologies

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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The problems of pollution with heavy metals is a serious environmental issue. The use of microalgae supported on different substrates is an eco-biotechnological variant of great importance in sustainable conditions. The work is clear, concise and develops a simple and applicable strategy in field and laboratory conditions. The utilization of alginate beds (Ca 2+) is a great technological solution.

Is the work clearly and accurately presented and does it cite the current literature?
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