International Journal of Environmental Science and Technology

Cadmium removal by Anabaena sp. ATCC 33047 immobilized in polyurethane foam

--Manuscript Draft--

Manuscript Number:	JEST-D-14-00654R1
Full Title:	Cadmium removal by Anabaena sp. ATCC 33047 immobilized in polyurethane foam
Short Title:	Cadmium removal by immobilized Anabaena
Article Type:	Short Communication
Keywords:	Anabaena; algal immobilization; Cadmium; heavy metals; polyurethane
Corresponding Author:	Mercedes Garcia-Gonzalez, PhD University of Seville-CSIC Sevilla, SPAIN
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	University of Seville-CSIC
Corresponding Author's Secondary Institution:	
First Author:	Marta E Clares
First Author Secondary Information:	
Order of Authors:	Marta E Clares
	Miguel G Guerrero
	Mercedes Garcia-Gonzalez, PhD
Order of Authors Secondary Information:	
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Cadmium removal by *Anabaena* sp. ATCC 33047 immobilized in polyurethane foam

Cadmium removal by immobilized Anabaena

Clares, Marta E; Guerrero, Miguel G and García-González, Mercedes

Instituto de Bioquímica Vegetal y Fotosíntesis, Universidad de Sevilla-Consejo Superior de Investigaciones Científicas, Centro de Investigaciones Científicas Isla de la Cartuja, Avda. Américo Vespucio, 49, 41092-Sevilla, Spain.

Author for correspondence

Prof. Mercedes García-González
Instituto de Bioquímica Vegetal y Fotosíntesis
Centro de Investigaciones Científicas Isla de la Cartuja
Avda. Américo Vespucio, 49, 41092-Sevilla, Spain
Phone: + 34 954489508
Fax: +34 954460165
Email: mggonza@us.es

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ABSTRACT

The nitrogen fixing cyanobacterium *Anabaena* sp. ATCC 33047, which generates substantial amounts of exopolysaccharide, was immobilized by entrapment within the reticulate network of polyurethane foam discs. The immobilized *Anabaena* sp. system has been investigated as a potential biosorbent for the removal of cadmium from aqueous solutions. The results showed that it was a highly fast process, with 80% of the adsorption taking place in the first 10 min, reaching full equilibrium in about 50 min. Data analysis indicated that the behaviour of the system accurately fit to a monolayer adsorption model (Langmuir isotherm). The maximal biosorption capacity determined for the immobilized *Anabaena* sp. system was as high as 162 mg Cd (II) per gram dry biomass. The outstanding properties established for immobilized *Anabaena* sp. in polyurethane foam underline the relevance of such a system as an alternative to current treatments of variety effluents or wastewater contaminated with cadmium.

INTRODUCTION

Recalcitrant heavy metals accumulate in the food chain, representing a major environmental risk (Doshi et al 2007). Cadmium is one of the most toxic heavy metals, since it generates kidney disorders, respiratory failure, bone lesions, hypertension and cancer in humans. The mining and metallurgy of cadmium, the manufacture of batteries, ceramics and pigments industries generate effluents that contain cadmium, which is also present in many wastewaters. These effluents are usually treated by physico-chemical methods such as precipitation, evaporation, adsorption and ion exchange, among others (Gupta and Rastogi 2008). All these methods entail significant economic investment, with high operation costs and even generation of secondary waste, difficult to remove (Chen et al 2014).

Removal of heavy metals from effluents and wastewater is a very interesting issue of the biotechnology of photosynthetic microorganisms (Muñoz et al 2006). These biological agents possess outstanding surface properties, such as the presence of polysaccharides, peptidoglycan layer and proteins with numerous functional groups, potential metal binding sites (Chojnacka et al. 2005). However, the use of free cells for the treatment of industrial effluents presents serious operational constraints, since the algal biomass is made up of small particles of low density, low mechanical stability and low stiffness. The most important drawback is the separation of the cells after use (Bashan and Bashan 2010). To circumvent this problem, the immobilization of the cells in systems with appropriate characteristics of size, strength and porosity is recommended (Abdel Hameed and Hammouda 2007; Liu et al 2012), although some authors invoke a higher metal sorbing efficiency of free cells as compared to immobilized ones (Wong and Pak 1992). A variety of methods are available for the immobilization of cells and enzymes, entrapment being by far the most frequently used in the case of algae. Several natural (e.g. collagen, agar, agarose, cellulose, alginate, carragenate) and synthetic (e.g. acrylamide, polyurethane, polyvinyl) polymers are used for this purpose. Carragenate and alginate gels have been widely used in immobilization, although these systems have major disadvantages as limited diffusion, low mechanical resistance of the gels or lack of space for cell growth, together with a prohibitive cost (Moreno-Garrido 2013). Physical entrapment in inert media, both natural (plant fibre) or synthetic (polyurethane), is an alternative immobilization technique that does not share the above limitations. Comparative studies of immobilization of *Chlorella vulgaris* and *Scenedesmus quadricauda* in polyurethane and carragenate or alginate gels have highlighted the stability of polyurethane for wastewater treatment (Travieso et al. 1996). Polyurethane foams (PUF) present advantages over gels, such as greater resistance to the majority of effluents, low cost, resistance to microbial agents, possibility of reuse and flexibility for scaling up. Polyurethane has proven to be a versatile support, being therefore used in a number of applications based on immobilised algal systems, such as the production of hydrogen by *Gleocapsa olpicola* and *Synechocystis* sp. PCC 6803 (Antal and Lindblad 2005) and of exopolysaccharides by *Anabaena* sp. ATCC 33047 (Clares et al. 2012), as well as the adsorption of nitrate, phosphate or various heavy metals by *C. vulgaris* and species of the genus *Scenedesmus* (Chen et al 2014). The lack of toxicity of PUF makes it especially interesting as sorbent for immobilization of photosynthetic microorganisms (Urrutia et al. 1995).

The presence of a large number of negative charges on the external cell layers in exopolysacharide (EPS)-producing cyanobacteria has led to consider these organisms very promising as chelating agents for the removal of positively charged heavy metal ions from aqueous solutions (Comte et al 2008; De Phillipis and Micheletti 2009). Nitrogen-fixing *Anabaena* sp. ATCC 33047 exhibits properties that make this strain attractive for its use in bioremediation of heavy metals. Among them are the ability to remain upheld in polyurethane housings, and the presence of uronic acids in the EPS that enhances the anionic nature of the cell surface and its capacity to retain metal cations (Moreno et al. 2000). Based on the above criteria, polyurethane foam has been selected as the support for the retention of *Anabaena* sp. filaments.

The aim of this work was to assess the ability for cadmium removal of the EPS-producing cyanobacterium *Anabaena* sp. ATCC 33047 immobilized in polyurethane foam.

MATERIALS AND METHODS

Organism and culture medium

Anabaena sp. ATCC 33047 from the American Type Culture Collection, Rockville (USA), was grown on the medium described by Moreno et al. (1995). The cells were grown aseptically to exponential phase, in 120-mL glass cylindrical containers, bubbling through the medium air supplemented with 1% (v/v) CO_2 as the source of carbon and nitrogen, at 30ºC, under continuous illumination to 200 μmol photon m^{-2} s^{-1}.

Microalgal immobilization

Immobilization was performed on polyurethane foam Filtrey TM23190 of 1.6-2.2 mm pore size. This material exhibited the best biomass holding capacity as compared with other PUF types assayed, such as TM23133 and TM23450. The foam was cut into discs of approximately 15 mm diameter, 10 mm thick, soaked in boiling water for 30 min, thoroughly washed under running tap water, and oven dried at 70ºC till constant weight (24 h). For immobilization of *Anabaena* sp. cells, PUF discs contributing a surface of 250 cm^2 were transferred to 200 mL of culture medium and the cylindrical containers with the mixture were sterilized by steam pressure for 20 min at 120ºC. Each container was inoculated with 10 mL of a cyanobacterial culture in exponential growth phase (about 0.2 g dry weight biomass L^{-1}) and maintained for 8 days under regular growth conditions. The PUF discs with entrapped biomass were removed from the culture and they were used in the studies of cadmium sorption, after being washed with fresh culture medium to remove free cells. The amount of cyanobacterial biomass immobilized on and within the foam was measured after extracting the cells from the matrix using a Potter homogenizer and determining their dry weigh as previously described (Fontes et al. 1987). Under the defined conditions, the *Anabaena* sp. load in the PUF disks was 0.7-0.8 mg (dry weight) per cm^2 of outer surface of PUF disks.

Cadmium biosorption assays with Anabaena sp. immobilized in PUF discs

Biosorption experiments were carried out by suspending an amount of PUF discs contributing 125 cm^2 outer surface and holding about 90-100 mg (dry weight) *Anabaena* sp. biomass in 200 mL of a $CdCl_2$ fresh solution, prepared with ultrapure water, containing 100 mg Cd(II) L^{-1} (100 ppm) in 500 mL-capacity Erlenmeyer flasks. The mixture was incubated in an orbital shaker at 100 rpm for up to 300 min at room temperature.

Cadmium determination

After the time established, the PUF discs were removed and the residual concentration of Cd (II) in the metal supernatant solution was determined spectrophotometrically according to Lee and Choy 2001. An aliquot of 200 μL was diluted with pure water to 1 mL and then 0.5 mL of 0.1% (w/v) ammonium pyrrolidine dithiocarbamate was added and the mixture shaken. One mL of 1% (w/v) Tween 80 solution was then added and the volume was completed to 10 mL with 1 M phosphate buffer, pH 7.0,

and the mixture shaken thereafter. After 30 min, absorbance at 323 nm was measured. The amount of cadmium in the sample was calculated from a calibration curve.

Data analysis

All experiments were performed in triplicate and statistical analysis of the data was performed by using software SPSS. The concentration of metal ions adsorbed per unit of immobilized microalgal mass (mg metal g^{-1} biosorbent) was determined using the following expression (Akhtar et al. 2004):

$$q_e = \frac{(C_0 - C_e)}{C_b} \qquad \text{eq. 1}$$

where q_e is the metal uptake (mg metal ions g^{-1} dry weight of algal biomass entrapped within the PUF discs), C_0 and C_e are the initial and final concentration, respectively, of metal ion in the solution (mg L^{-1}), and C_b is the dry weight of *Anabaena* biomass.

The Langmuir equilibrium model was used for the evaluation of the biosorption data. This model assumes monolayer adsorption, which is expressed as:

$$q_e = \frac{q_{max} bC_e}{(1 + bC_e)} \qquad \text{eq. 2}$$

where q_{max} is the maximum uptake capacity (mg g^{-1} biosorbent), C_e is the equilibrium concentration (mg L^{-1} solution) and b is the Langmuir equilibrium constant concerning the energy of adsorption-deadsorption.

RESULTS AND DISCUSSION

The filamentous nature of the N_2-fixing cyanobacterium *Anabaena* sp. ATCC 33047 facilitates its immobilization and permanence in porous carriers, as is the case of polyurethane foam. The invasive adsorption technique, consisting essentially in maintaining discs of the PUF matrix suspended in a cyanobacterial liquid culture under regular growing conditions has been used. After four days, the polyurethane foam was totally covered by *Anabaena* (Fig 1A), with the filaments occupying not only the surface but also the inner spaces of the material (Fig 1B). This distribution of the biological material is an important feature for achieving maximal biosorption of the heavy metal ions (Akhtar et al. 2004).

About 1 mg dry biomass of *Anabaena* sp. ATCC 33047 was effectively immobilized per cm^2 of outer surface of the matrix. This corresponds to a load of about 120 mg biomass per g of the PUF discs.

Availability of Cd (II) to biological systems in a solution can be limited by the pH value or by the concurrence of other salts and chelating agents that, under certain conditions, can lead to Cd precipitation, withdrawing part of the metal from the solution (Elmaci et al. 2013). Due to its composition, the medium used for Anabaena culture could interfere with Cd (II) solubility. Therefore all cadmium absorption assays have been performed in aqueous solution, to avoid the above mentioned interference.

In order to properly assess the potential of immobilized *Anabaena* sp. on PUF discs to retain cadmium, occurrence of nonspecific adsorption of the metal by the support must be excluded. To this end, the kinetics of Cd (II) withdrawal from a solution containing 100 ppm Cd (II) was simultaneously determined for PUF discs containing immobilized *Anabaena* sp. filaments and for the same amount of disks that did not contain the biological material (Figure 2). The PUF discs without *Anabaena*, embedded in either water or in culture medium did not retain any cadmium, thus allowing excluding nonspecific adsorption by the matrix. The uptake of cadmium by PUF-immobilized *Anabaena* sp. was very fast at the beginning, with 65% of total Cd (II) being removed from the solution within the first 5 min of incubation, followed by a slower phase. The biosorption equilibrium was achieved in about 50 min. By that time, 97.5% of initial Cd (II) had been retained by the immobilized system. Similar absorption profiles, with a rapid drop in the dissolved free metal in the first 10-20 min, followed by a slower descend during the next 30-40 min of treatment have been previously described (Ozturk et al. 2009). These observations indicate that this *Anabaena* strain has a high affinity for cadmium. The anionic nature of the EPS covering the outer surface of this cyanobacterium probably contributes to an efficient metal adsorption. Faster equilibria for cadmium biosorption have been reported for other photosynthetic microorganisms, with removal of 93.5% of initial cadmium in solution for *Chlorella sorokiniana* (Akhtar et al. 2003) and 94.3% for *Synechococcus sp* (Saeed and Iqbal 2013) in 5 min, using loofa sponges as matrix and cadmium solutions of only 10 ppm. Gadd (1988) proposed that a first rapid phase of biosorption involved bulk transport of cadmium, resulting in the binding of cations to the negatively charged reactive groups on the surface of cells, followed by a slower second phase of cellular uptake.

To further analyse the Cd removal capacity of *Anabaena* sp., different amounts of immobilized biomass were added to an aqueous solution containing 100 ppm Cd (II), and the cadmium remaining in the aqueous solution at equilibrium (C_e, cadmium remaining in the medium at the end of the withdrawal

process) was determined. The results presented in Figure 3 clearly show that the amount of removed cadmium was a function of that of immobilized *Anabaena* biomass present in the assay. Thus, with 0.445 g L^{-1} biomass, virtually all of the initial cadmium was removed after 300 min.

The cadmium removing capacity of the immobilized system was determined with a fixed amount of PUF-immobilized *Anabaena* sp. and different initial concentrations of Cd (II) (Figure 4). According to the results, about 0.2 g Cd (II) were retained per g (dry weight) *Anabaena* sp. biomass, regardless the initial concentration of the ion.

Based on the experimental data obtained, values for biosorption of Cd (II) per biomass unit (q_e) at the equilibrium by PUF-immobilized *Anabaena* sp. were calculated (equation1, see Materials and Methods), and plotted against the corresponding metal concentration at equilibrium, C_e (Figure 5). Values for q_e increased with increasing C_e, to reach a maximum of about 160 mg Cd (II) per g dry biomass for C_e values above 100 ppm. The relationship between cadmium removal capacity (q_e) and Cd (II) concentration at equilibrium (C_e) was further examined using equation 2 (see Material and Methods). The Langmuir linear regression approach was used to analyse the experimental data, yielding a linear relationship between C_e/q_e and C_e (Figure 6) that showed a good fit (r^2 = 0.998). The value for q_{max} calculated from the linearized Langmuir plot for PUF-immobilized *Anabaena* sp. was 161.8 mg Cd (II) g^{-1} dry biomass. The estimated value for the Langmuir constant (b) was 0.41 L mg^{-1}, indicative of a strong binding of Cd (II) to the immobilized biomass. The q_{max} value found for Cd (II) sorption by PUF-immobilized *Anabaena* sp. was higher than the corresponding values determined for other systems with photosynthetic microorganisms (Iqbal and Saeed 2011; Katircioglu et al. 2008; Saeed and Iqbal 2006), indicating the high potential of the PUF-immobilized *Anabaena* sp. system for Cd (II) removal in comparison to others based on either free or immobilized biomass of different nature.

The Langmuir isotherm describes a reversible monolayer adsorption phenomenon, by which metal ions adhere to a certain number of binding sites, each one chemically equivalent, holding a single ion, and the particles attached to the surface not interacting between them, so that the amount of adsorbed cadmium does not affect the rate of cadmium retention (Senthilkumar et al 2007). The system used in this study thus would behave according to these bases. Furthermore, the relationship between cadmium removal and the amount of *Anabaena* sp. biomass is indicative of a passive adsorption process. The fact that the immobilized system exhibits the same Cd (II) retention capacity in either light or darkness (data not shown) does also support the contention that Cd (II) sorption by the PUF-immobilized *Anabaena* sp. biomass is a passive process.

CONCLUSION

The retention of cadmium by PUF-immobilized biomass of *Anabaena* sp. ATCC 33047 is a passive process, taking place at a high speed and behaving accordingly to a monolayer adsorption model (Langmuir isotherm).

Anabaena sp. ATCC 33047 biomass immobilized on PUF matrix exhibits outstanding capacity for Cd (II) biosorption, being able to retain 162 mg Cd (II) per g dry biomass, the highest value so far reported for a biological system to the best of our knowledge. A vast potential can be envisaged for the application of this system in wastewater treatment.

ACKNOWLEDGEMENTS

This research was supported by Consejería de Innovación, Ciencia y Empresas de la Junta de Andalucía nº CVI 422 (Proyecto de Excelencia), and Plan Andaluz de Investigación (group CVI 131).

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FIGURE CAPTIONS

Graph 1 PUF disks containg immobilized *Anabaena* sp. ATCC 33047 (A). Micrograph showing *Anabaena* sp. filaments embedded in the matrix of the PUF disks (B)

Graph 2 Time course of the evolution of cadmium concentration in 200 mL of a 100 mg Cd (II) L^{-1} solution following the addition of PUF disks (125 cm^2 outer surface) soaked in water (open circles), culture medium (black circles), or containing 90 mg (dry weight) immobilized *Anabaena* sp. (squares)

Graph 3 Effect of the concentration of immobilized *Anabaena* sp. biomass on cadmium removal from a 100 mg Cd (II) L^{-1} solution. C_e = equilibrium concentration (after 300 min)

Graph 4 Effect of Cd (II) initial concentration (C_o) on concentration in the equilibrium (C_e) in presence of 100 mg (dry weight) of *Anabaena* sp. biomass, immobilized on PUF disks (125 cm^2 outer surface). Assay volume: 200 mL

Graph 5 Equilibrium isotherms for Cd (II) binding to PUF-immobilized *Anabaena* sp. biomass (0.5 g L^{-1}). q_e stands for the quantity of biosorbed Cd (II) per biomass unit

Graph 6 Langmuir linear regression plot for Cd (II) binding by the PUF-immobilized *Anabaena* sp. system

Figure

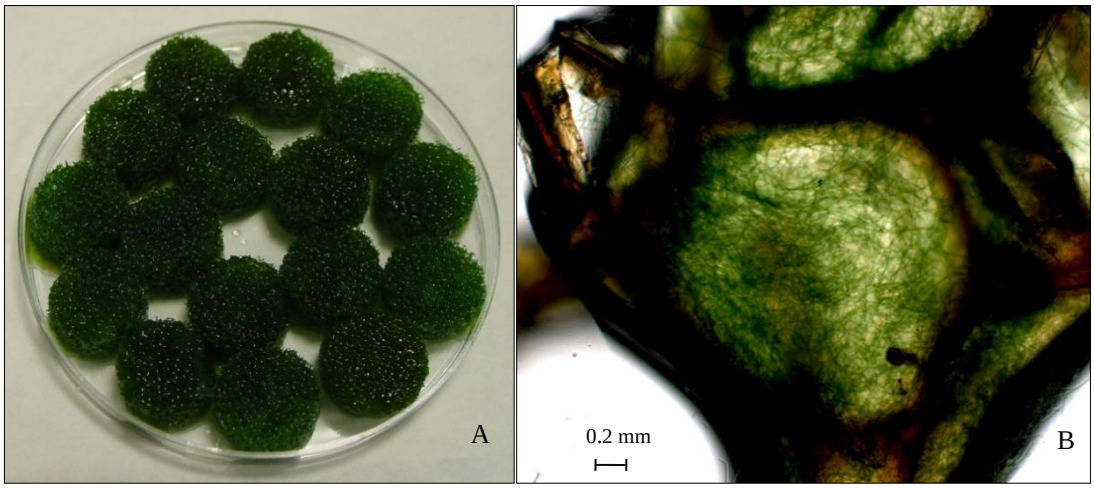

Figure 1

Cadmium removal by *Anabaena* sp. ATCC 33047 immobilized in polyurethane foam

Graphs

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