# Adsorptive Removal of Heavy Metals by Microalgae

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## Abstract

In the present study, the optimization of immobilization structures along with *Calothrix* sp. and the determination of heavy metals absorption rate have been investigated. Polymers in combination with salts were performed in alginate in order to study the stability of formed structures in cross linking agents. Beads with stable structure in synthesized heavy metal solution inoculated and the adsorption percentage has been recorded. Results showed that the most stable beads were formed in 2 and 3% alginate along with $CaCO_3$ application beside $BaCl_2$ as solidified solution. Reduction of heavy metal in these structures had the most percentage and it was observed that if the incubation time of beads in solidified solution decreased, the percentage of heavy metal reduction would increase to 86%. Although alginate beads showed the highest absorption rate of heavy metal, the presence of *Calothrix* sp. in all treatments occasionally increased heavy metal absorption up to 15%.

**Keywords**: Alginate, Heavy metal Removal, Microalgae, Stability.

## Introduction

Environmental pollutions especially heavy metal pollutions, which are caused from industrialization, have become one of the fundamental problems of the present century due to their accumulation in living organisms. Among heavy metal, Nickel (Ni) toxicity has been considered in recent years due to its harmful effects on the human and environment. Nickel is one of many trace metals widely distribute in the environment. It is present in the soil, water and air in different form. Nickel is essential element for plant in low concentration but high concentration is toxic. It is also toxic for human health. Nickel occurs predominantly as the ion $Ni (H_2O)_6^{2+}$ in natural waters at pH 5-9 (Rator et al., 2014). Cr is known to be as mobile and highly toxic element in nature and easily penetrates into the cell wall and causes different forms of cancer. Different forms are found in wastewater from the leather, paint, wood industries and plating from tens to hundreds $mgl^{-1}$.

Conventional methods for elimination of heavy metal are included chemical precipitation, membrane separation, ion exchange and evaporation. In addition, these methods do not

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have enough efficiency at <100 mgl$^{-1}$ concentrations, they have no economical aspect and cannot be used in large-scale. Therefore, researchers have focused on surface absorption in two recent decades and more researches have been used biosorbent to eliminate heavy metal (Han et al., 2007; Yilleng et al., 2013). Studies on alternative and innovative treatment techniques using biological methods due to their high efficiency and low cost have led to biosorbent investigation in recent years. Among different biosorbent green algae, due to their special nature in adsorption and high ratio of their surface to volume has been considered (Han et al., 2007; Gupta and Rastogi, 2009).

In detoxification or recycling where concentration of metal pollutant is very low, the use of metabolically active immobilized microalgae is an especially attractive option in applications; a large amounts of the metal are bound to living or dead algae surfaces, and most of these connections will be discharged and recycle by acid treatment. These connections are based on light, temperature, metal competition and metal surface during incubation time (Gupta and Rastogi 2008). Selection of stable matrix for microalgae immobilization is a key factor in stability and absorption process. Many natural polymers such as sodium alginate, carrageenan, glutaraldehyde, agarose, cellulose, chitosan, acetate derivations have been known as absorbent of heavy metal (Pandey et al., 2007). Among them, the most commonly used polymers are alginate and carrageenan. Although the sustainability of these natural polymers in wastewater is less than artificial ones, alginates have a high level of human health, widely available and inexpensive. Physiologically, the greatest advantage of alginate is gel permeability and lack of intense stress for immobilized cells (De-Bashan and Bashan, 2010).

Due to the necessity of eliminating heavy metal from industrial effluents, application of "green technology" which cause less secondary damages to the environment, is in priority. Therefore, in this research, the stability of immobilized microalgae structures has been investigated.

## Materials and Methods

### Preparation of algae biomass

Microalga *Calothrix* sp. was developed from algal collection of the Research Institute of Applied Science of Academic Center for Education, Culture and Research (ACECR) and cultivated in BG11 medium at $25 \pm 1°C$, 16/8 photoperiod, and light intensity of 135 $\mu Em^{-2}s^{-1}$ in aerated condition. Cells in exponential phase were harvested by centrifugation at 10,000 rpm for 15 min. *Calothrix* sp. cells were resuspended in distilled water to use at immobilized matrix. To prepare dry biomass, samples were placed an overnight at 70°C.

### Preparation of immobilized matrix

### Beads preparation

To prepare sodium alginate suspension, the mixture was placed on the stirrer for an hour to achieve uniform suspension of 1, 2 and 3% sodium alginate in room temperature. The cells were added in last step to suspension and the mixture was transferred to a 50 ml burette and beads were formed when "titrated" into cross

linking agent on the Na-alginate containing 2% $CaCl_2$ or $BaCl_2$ solution. The beads were kept for hardening in last solutions for an overnight at 25 ±2°C then rinsed with 0.85% NaCl and subsequently washed with distilled water and kept in room temperature for further investigations. Also, a concentration of 2: 100 beads: wastewater (v/v) included 0.1 g microalga had been considered for wastewater treatment.

*Usage of secondary polymers in internal and external structure of beads*

For internal usage of co-polymers, chitosan 0.1% (Dabbagh et al., 2008), hydroxyl methyl cellulose 1% (Li and Buschle-Diller, 2017; Cruz et al., 2013), and PVP 1% (Cruz et al., 2013) were added after the formation of alginate suspension and before adding algae. For external application of last co-polymers, beads were coated with chitosan 1%, hydroxyl methyl cellulose 2.5% and PVP 20% an overnight then were kept in distilled water. It should be noted that chitosan solution was prepared in 1% acetic acid solution.

*Using of salt in beads structure or cells treatment*

NaCl (Gao et al., 2011) at 1% and $CaCO_3$ (Sobecka and Bartkowiak, 2009) at 1, 5 and 10% in powder form were used in alginate suspension before adding algae. To investigate the effect of saline treatment on heavy metal removal, harvested cells resuspended and incubated at 0.85% NaCl for 72 h.

*Investigation of beads deformation and their resistance*

Presence of anionic ions such as phosphate in a medium containing calcium alginate, produce calcium phosphate that destruct beads

structures (Gaserød et al., 1999). Different concentrations of phosphate buffer were used for 4 days to study the strength of the structures. For this purpose, 3 beads in 3 replicates were placed in micro-tubes containing 0.1, 0.5 and 1 M buffer and destruction of beads structures were recorded at least 4 days. Therefore, beads with resistant structure in high concentration of phosphate during days were selected as stable patterns. In order to investigate the effect of acidity in the presence of phosphate ions (0.5 M), pH range were considered from 3 to 9.

*Statistical analysis*

All results are expressed as mean ± SE. A one-way analysis of variance (ANOVA) followed by multiple comparisons with the Tukey post hoc test was used to compare different parameters between the samples. P value <0.05 was considered significant.

**Results**

*Preparation of beads*

*Stability in presence of phosphate ions*

Findings indicated that beads stability increased along with alginate concentration to 3% in presence of phosphate ions. Between cross linking agents, $BaCl_2$ resulted in pale green color in algal beads immediately after dropping. Ba-alginate beads showed higher stabilization in compare to Ca-alginate beads. The results of secondary polymers showed that internal application of chitosan in beads composition increased their resistance and external application or coating beads with chitosan has no effect on their resistance structure. The death of algae observed in both internal and

external application of chitosan. Moreover, other polymers showed no effect on beads structures. Among salts, sodium chloride in beads structures reduced their stability against phosphate ions but calcium carbonate strongly resisted their structures to phosphate buffer. Increasing the percentage of calcium carbonate up to 10% especially in barium beads resulted in good resistance structures.

*Resistance in various pH*

Incubation of resistance structures of barium beads in combination with calcium carbonate in various pH showed that more resistance was observed at pH 3.0 in presence with phosphate ions than pH 9.0 or higher. Also coating beads especially with PVP and hydroxyl methyl cellulose has improved the stability especially in alkaline pH. Ba-Beads containing $CaCO_3$, showed higher resistance at pH 3.0 than pH 9.0.

*Heavy metal absorption from artificial wastewater*

*Heavy metal reduction*

Algae treatment within 72 hours with different structures showed that maximum absorption of Cr was performed in the first 24 hours somehow in barium beads containing calcium carbonate, more than 90% of final Cr absorption was done in the first 24 hours. It was also observed that $BaCl_2$ as cross-linking agent on the alginate, can cause increasing of Cr absorption content in all treatments. In first step, Ba-beads containing $CaCO_3$ showed maximum reduction (58.6%). The presence of algae in immobilized form has increased absorption capacity by 15% in the first 24 hours while free algae have little capacity in

Cr absorption. Free algae cells at 80 $mgl^{-1}$ Cr, were dead rapidly and released absorbed Cr into the medium After 24 h. Absorption in dry biomass was equal to wet biomass inoculated into the medium around 6.5%, by functional groups localized on algal cell wall. It was also observed that internal application of chitosan has no desired results and also prevent algal absorption (Fig. 1).

*Heavy metal absorption rate*

Since the maximum absorption occurred in Ba-beads containing $CaCO_3$, reduction rate had been investigated. Results showed 66% of total reduction occurred in first 6 hours. Higher amount Cr absorption by algal beads in compare with blank beads determined the role of immobilized algae on it. Quick absorption of immobilized algae (9.5%) in early hours indicated passive transport that applied by functional group localized on cell. This process especially for Ba-beads containing $CaCO_3$ is more obvious (Fig. 2).

*Effect of co-polymers on heavy metal removal*

Additional treatment in 0.85% NaCl for 72 hours in free and immobilized algae had no important effect on Cr removal. Cr absorption in fresh and dry biomass and beads with chitosan coverage observed less than 5%. Although co-polymers application into bead structure and its coat showed a negative effect on Cr absorption, algae presence in all structures had a positive effect especially in first 24 hours. Same rate of absorption were observed at first 2 hours with an exception cellulose coated bead that reduce Cr content in double than other biosorbent.

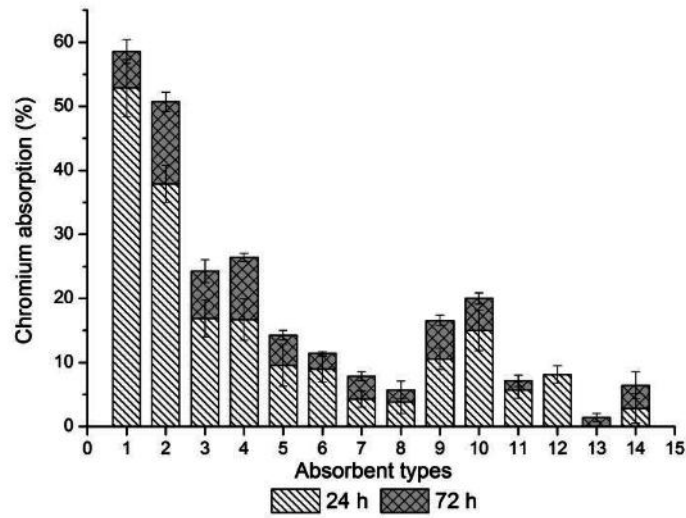All beads are titrated in $BaCl_2$ and contain 2%

**Fig. 1.** The absorption rate of Cr by different adsorbent species in 24 and 72 hours. Algae in immobilized form have increased alginate absorption capacity while it has been decrease by chitosan application.
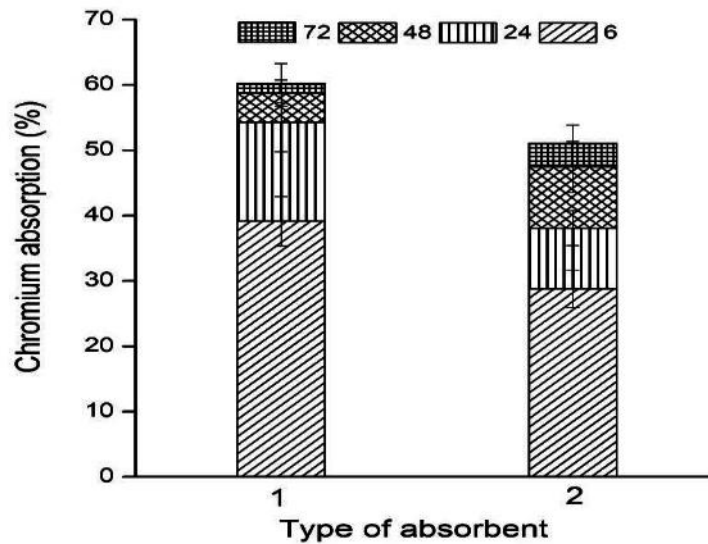


**Fig. 2.** The process of chromium absorption over a period of 72 hours by various adsorbents showed the highest percentage of absorption in the early hours. Columns with odd numbers represent algal beads and even numbers represent beads without algae.
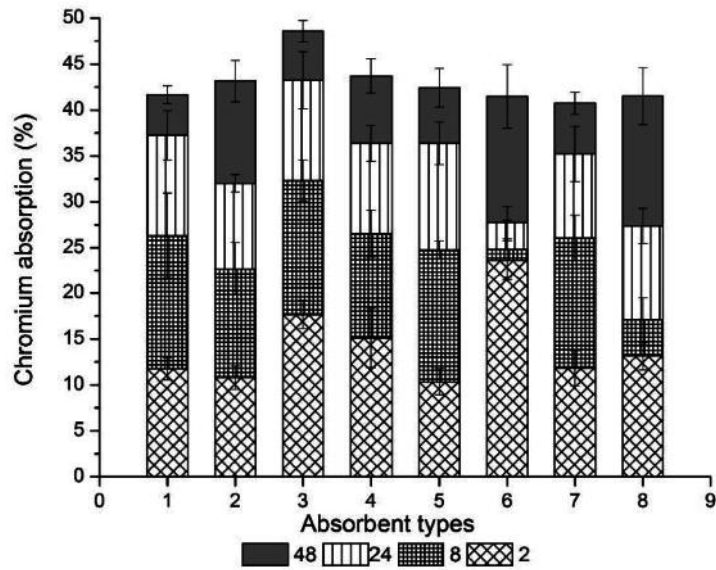
**Fig. 3**. The process of chromium absorption by coated beads show the highest absorption rate in the first 8 hours.
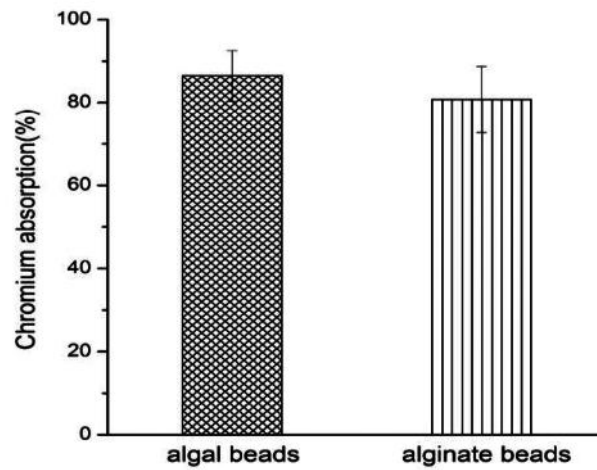


**Fig. 4.** The percentage of chromium adsorption by 4% volumes of bead after 24 hours, 86% chromium adsorption was observed by the algal bead structure.

alginate and 1% calcium carbonate. Columns with odd numbers represent algal beads and even numbers represent beads without algae. According to last results, direct application of 4.0 gl$^{-1}$ *S. obliques* in alginate and solidification in BaCl$_2$ solution in 4 hours with 4% v/v beads: wastewater ratio presented 2.7 times absorption over 24 hours compared to first observation (from 31.7% to 86.3%).

*Effects of primary pH on heavy metal absorption*

Alginate bead presence in medium had increased final pH from 3.0-6.0 to 7.5 but when the initial pH was 2.0, final pH had reached to 4.0. Maximum Cr absorption was in pH $\geq 3$ and by increasing pH to neutral parts the amount of Cr elimination increased significantly in blank beads while it has no significant effect in algal beads.

## Discussion

Calcium alginate is extremely unstable when exposed to chelators such as phosphate, lactate, citrate or cations like sodium or magnesium ion. The exchange of these factors with calcium or cation in the alginate structure could disrupt the structure of the beads (Gaserød et al., 1999). Cruz et al. (2013) reported that 90% of phosphate was absorbed by alginate and algae in non-sterile wastewater. Bivalent ions such as calcium and barium play an important role as cross linking agent on the Na-alginate.

Cruz et al. (2013) also reported that calcium chloride showed better stability to alginate beads than strontium chloride. Higher stability of alginate beads in barium chloride solution

was reported by Mørch et al. (2006). The results of this study like the other reports authenticated the presence of barium in stability of alginate structure and algal beads relative to calcium. However, Ibanez and Umetsu (2002) reported that morphology of the calcium and barium alginate beads had the same reaction and behavior in the environment but a slight decrease was observed in the size of barium beads, which was also described by Mørch et al. (2006).

Using two natural hydrophilic natural polymers (Sodium Alginate and Chitosan) in BSA release revealed that 3% alginate and 0.25% chitosan concentration had a good effect on preserving Bovine serum albumin protein (Dabbagh, 2008). Ibanez and Umetsu (2002) reported that the stability of alginate-chitosan capsules depends on the amount of chitosan conjugate to the capsules (Gaserød et al., 1999). However, our results also show that, beads coated with chitosan were weaker than the ones that chitosan entered into their structure but presence of calcium carbonate played more significant role in stability improvement. Cruz et al. (2013) also reported that presence of calcium carbonate was very effective in improvement of alginate beads stability's but presence of polymers containing PVP and carboxymethyl cellulose had no effect in increasing stability of alginate beads. Other reports also showed the positive effect of calcium carbonate in increasing alginate beads stabilities (Sobecka and Bartkowiak, 2009; Mahmood et al., 2015). In alkaline pH, connection bond in alginate beads began to break and treatment with strong acidic solutions caused to loss of

stabilized biomass (Gaserød et al., 1999). Our results also show that in acidic pH, resistance of these structures was better and presence of beads in phosphate solution with alkaline pH, caused to break them down and presence of PVP or cellulose coating were effective in increasing beads resistance.

The elimination of anionic heavy metal is a complex process which reduction, forming of surface complex and ion exchange are involved. Heavy metal is able to implicate surface complexes with functional proton groups in bio-sorbent like -COOH, -NH$_2$, -SO$_3$H (Yang and Chen, 2008). In brown algae, bio absorption has been considered due to presence of alginate in their cell wall which have functional groups such as carboxylic acids (gluronic and manoronic acids) as metal binding site through electrostatic force and complexity (Blanes at al., 2011).

Al-Rub et al. (2004) reported that although beads without algae or blank beads have also shown high percentage of absorption but immobilization of *Chlorella* dry biomass could increase nickel absorption (to 10 % in pH 5.0). In addition, Rangsayatorn et al. (2004) reported that free cells of *Spirulina platensis* and immobilized ones in alginate and silica gel were able to absorb cadmium 98, 71 and 37 mg/g respectively. They proved that alteration in cell wall structure during immobilization might be one of the reasons of preventing in absorption. It is also possible that some parts of wall surface became unavailable by gel materials. It had been shown that alginate beads have higher tendency for Cr compared to Pb and Cu. Pandey et al. (2007) have suggested

that this system can be used as filtering metal streams. Our results also indicated that alginate had very high tendency to absorb heavy metal and algae could improve this absorption. This action had a higher rate in the early hours. However, its application with other polymers, especially chitosan, has decreased absorption quantity. Udaybhaskar et al. (1990) reported an absorption capacity of 273 mg of Cr per gram of chitosan at pH 4.0. Chitosan had usually cross linked with chemical agent such as diisocyanate, epoxy compounds, carbodiimides, glutaraldeides. Modified chitosan without these cross linked had 30 mg g$^{-1}$ more absorption. The excellent capacity of chitosan is due to its hydrophilic properties that related for presence of a large number of hydroxyl functional groups, primary amino groups with high activity and a chitosan polymer chains flexible structure. The involvement of the above couplings reduces its capacity in absorbing pollutant due to increasing competition in connecting to sites. Pandey et al. (2007) showed that in the range of 20-200 mgl$^{-1}$ Cr, the percentage of adsorption (86%) showed no difference but the increase in Cr concentration up to 1000 mgl$^{-1}$ showed a significant decrease in Cr absorption (Mahmoud and Mohamed, 2017). In high concentrations of Cr (1000 mgl$^{-1}$), it seems that the roles of algae is more important and enhance the absorption capacity of bead algae structure. As indicated by Mahmoud and Mohammad (2017), in Cr enriched yeast in Alginate, with an increase in Cr concentration from 200 to 1000 mgl$^{-1}$, absorption was significantly reduced.

It is suggested as the best option to use algae,

since at 1000 mgl$^{-1}$ algae has shown the ability to absorb it after initiating saturation of alginate up to 102.2 mgl$^{-1}$. In line with our results, Pandey et al. (2007) reported that the inoculation of 1-4% (w/v) beads in synthetic effluent to absorb Cr, Pb and Cu did not significantly differ in the percentage of metal adsorption. They said that increasing the amount of adsorbent in the environment can be related to the difference in porosity of the beads, and thus the amount of active and ready positions with metals would be decreased.

Although the early acidity was adjusted by HCl and NaOH but the final acidity in almost all media reached pH 6-7 within 24 hours. Cr absorption by algal beads was almost constant at pH 6.0 while decreased only at pH 2.0. Mahmoud and Mohamed (2017) reported that in the pH range of 1.5 to pH 7.5, the maximum adsorption was at pH 3.5. Pandey et al. (2007) also indicated that chromium absorption (86%) was within the range of 3.0 to 4.0. Phycoremediation using microalgae like *Calothrix* sp. for wastewater provides an effective and environmentally adequate option for wastewater remediation, which recycles heavy metal and also improves water quality. The use of calcium carbonate in the alginate structure and BaCl$_2$ as cross linking agent, when was incubated in 70 degree for 1 hour creates highly stable structures of the beads, which would remain stable even against phosphate ions. The presence of fresh cells. in this structure, not only resulted in stabilization of the structure but also has led to an increase in absorption, which indicates the involvement of the functional groups in the cell wall. The heavy metal absorption in the algae beads structure during its early hours has passed and the occupancy of remaining other sites has continued during next 72 hours. It could be concluded that application of 2% alginate in combination with CaCO$_3$ and algae when titrated in BaCl$_2$ would cause to design biofilters for the purpose of absorbing heavy metal contaminants.

## Acknowledgment

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