BIOSORPTION EFFICIENCY OF CADMIUM IONS BY GREEN ALGAE (*CHLOROPHYTA*) IN AQUEOUS SOLUTIONS

HYNSTOVA VERONIKA, KLEJDUS BORIVOJ, HEDBAVNY JOSEF
Department of Chemistry and Biochemistry
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

veronika.hynstova@mendelu.cz

Abstract: The aim of this study is to explore the biosorption process of Cd^{2+} ions by the dry algal biomass (*Coccomyxa subellipsoidea, Chlorella vulgaris, Parachlorella kessleri, Scenedesmus quadricauda, Trebouxia erici*) and to investigate the biosorption process by the plant biosorbent *Tillandsia usneoides* from aqueous solutions. In this study, we tested the effect of biosorbent dosage and contact time of Cd^{2+} ions with different dried algal biomass or plant on biosorption efficiency (%). The initial concentration of Cd^{2+} ions was 10 mg/L. The solutions were filtered in four time intervals. Final concentration of Cd^{2+} ions in the filtrates was determined by the atomic absorption spectrometry on the CONTRAA 700 (Analytik Jena) at the wavelength 228.8 nm. The biosorption efficiency was found to be biomass dosage dependent. When the biomass dose was increased ten times, from 0.2 g/L on 2 g/L, the maximum biosorption efficiency of Cd^{2+} increased 75.05% from 39.01. The alga *Parachlorella* was demonstrated as the most effective biosorbent of ions Cd^{2+} with maximum percentage biosorption 73.14% (corresponds to 3.657 mg Cd^{2+}/g DW), in comparison to *Tillandsia usneoides* which only reached 37.66% (corresponds to 1.916 mg Cd^{2+}/g DW).

Key Words: biosorption, heavy metal ions, cadmium, algae, *Chlorophyta*, waste water

INTRODUCTION

Environmental pollution by heavy metals such as cadmium, chromium, lead and copper due to industrial development is one of the most important worldwide problems today. Heavy metals are considered persistent environmental contaminants since they cannot be degraded or destroyed, thus they pose an important problem due to their toxic effect and accumulation throughout the food chain leading to serious ecological and health problems. Methods proposed for removal of Cd^{2+} ions from wastewaters are similar to those employed for most heavy metals, which include chemical precipitation, chemical oxidation or reduction, evaporation, adsorption and ion exchange. These processes are either ineffective or extremely expensive (Vilar et al. 2006).

In recent years, the use of innovative biosorption technology by living organisms and/or non-living biosorbents for the removal and recovery of heavy metals has been considered. The extensive research has focused on the use of biosorbents such as bacteria, fungi, micro/macro algae for water and wastewater treatments (Mirghaffari et al. 2015). The advantages of these methods include the low operating cost, minimization of the volume of chemical and/or biological sludge to be disposed of and high efficiency in detoxifying very dilute effluents and no nutrient requirements. The removal process is rapid; it takes only a few minutes and it takes place under normal pressure and normal temperature conditions (Kadukova, Vircikova 2005).

The term biosorption indicates a metabolism-independent binding of heavy metals by dead (inactive) biological materials. The mechanisms of cell surface sorption are based on physico-chemical interactions between metal and functional groups of the cell wall. The microorganism's cell wall consists mainly of polysaccharides, proteins and lipids, which have many binding possibilities for metals. The binding of metals is very quick (up to 1 min) and mostly reversible (Kadukova, Vircikova 2005). Biosorption includes a combination of several mechanisms including electrostatic attraction, complexation, ion-exchange, covalent binding, Van der Waals' attraction, adsorption and microprecipitation (Montazer-Rahmati et al. 2011).

Cadmium is one of the most toxic metals affecting the environment and it causes renal disturbances, lung insufficiency, bone lesions, cancer and hypertension in humans. The permissible limits of cadmium discharge in wastewater and drinking water are 0.1 and 0.05 mg/L, respectively (Gupta, Rastogi 2008, Vilar et al. 2006).

This study examines the use of the dried biomass of different species of green algae (Chlorophyta), namely *Coccomyxa subellipsoidea, Chlorella vulgaris, Parachlorella kessleri, Scenedesmus quadricauda, Trebouxia erici*, for biosorption processes responsible for the removal of heavy metal Cd^{2+} from aqueous systems. The plant *Tillandsia usneoides* was used for comparison with algae. The biosorption efficiency was monitored using the effect of biomass dose and the effect of contact time of Cd^{2+} ions.

MATERIAL AND METHODS

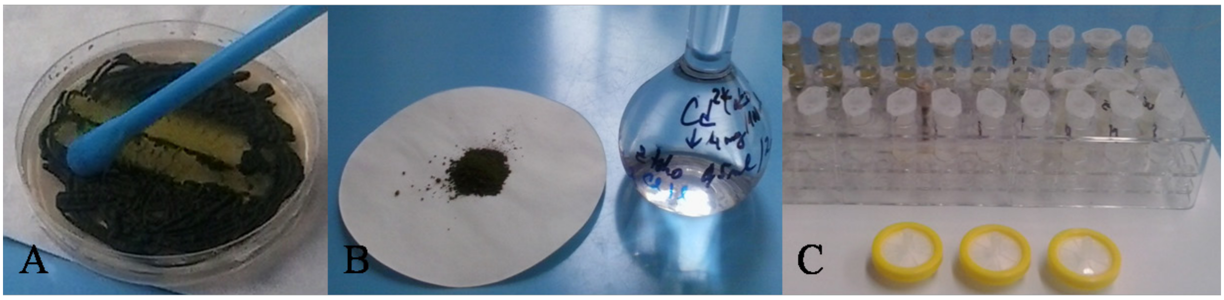
Chemicals

Stock solution of Cd^{2+} (10 mg/L) was prepared by dissolving analytically pure $CdCl_2$ (6.52 mg) in deionized water in 100 mL volumetric flask (Figure 1B).

Cultivation and drying of biomass of green algae

Green algae *Coccomyxa subellipsoidea, Chlorella vulgaris, Parachlorella kessleri, Scenedesmus quadricauda, Trebouxia erici* (Chlorophyta), were cultured under sterile conditions on Petri dishes in cultivation room with controlled temperature (25/20°C day/night) in „Milieu Bristol medium“ containing inorganic salts (in mg/L: 750 $NaNO_3$, 175 KH_2PO_4, 75 K_2HPO_4, 75 $MgSO_4$ g · L^{-1} $7H_2O$, 25 $CaCl_2 \cdot 7H_2O$, 20 Fe-EDTA, 20 NaCl, 2.86 H_3BO_3, 1.81 $MnCl \cdot 4H_2O$, 0.22 $ZnSO_4 \cdot 7H_2O$, 0.1 $CoCl_2 \cdot 6H_2O$, 0.08 $CuSO_4 \cdot 5H_2O$ and 0.052 $Na_2MoO_4 \cdot 2H_2O$, pH corrected to 6.5), 2% glucose, 1% casein hydrolysate and solidified with 1% agar. Algae were collected from the surface of cultivation medium 4–5 week after inoculation (Figure 1A), when colonies achieved enough biomass (Kovacik et al. 2015). Then samples were dried at 70°C for 12 hours (Figure 1B) (Kovacik et al. 2011).

Figure 1 A – cultivated biomass of Scenedesmus quadricauda, B – dried biomass of Scenedesmus quadricauda, stock solution of Cd^{2+} C - batch biosorption experiment, filtration



Batch biosorption experiments

Effect of biomass dose on biosorption efficiency of Cd^{2+}:

This experiment was performed by using dry biomass *Scenedesmus quadricauda* and Cd^{2+} solution (initial concentration c_i = 10 mg/L). Two aqueous suspensions with different concentrations of dried biomass *Scenedesmus quadricauda* (0.2 g/L and 2.0 g/L) were prepared by weighing of required amount of biomass into plastic tubes (volume 50 mL), and adding deionized water (pH 5.5) to final volume 48 ml. Dispersion of algal biomass in the water was done by placing the tubes in an ultrasonic bath for 15 minutes. Thereafter, 1.5 mL algal suspensions were pipetted into the Eppendorf plastic tubes (in series 2x12) and 0.5 mL of Cd^{2+} solution was added to each Eppendorf plastic tube. Two triplets of solutions were filtered through filter (Nylon Membrane Syringe filter, 0.22 µm pore size, Sigma-Aldrich) at intervals 10, 30, 60 and 180 minutes to clean Eppendorf tubes (Figure 1C). The concentration of non-adsorbed Cd^{2+} ions in the filtrates (final concentration c_f) was determined by atomic absorption spectrometry (AAS) using the CONTRAA 700 (Analytik, Jena). Cadmium was measured at the wavelength 228.8 nm.

Effect of contact time on the biosorption efficiency of Cd^{2+}:

Batch biosorption experiments were conducted to study the effect of contact time on the biosorption of Cd^{2+}. The test was carried out by application of Cd^{2+} heavy metal solution (initial concentration c_i = 10 mg/L) to the suspensions of different species of dried algal biomass (*Coccomyxa subellipsoidea, Chlorella vulgaris, Parachlorella kessleri, Scenedesmus quadricauda, Trebouxia erici* and the plant *Tillandsia usneoides.* The biomass dosage was 2 g/L aqueous solution. The aqueous biomass suspensions were prepared by weighing the required amount of biomass into plastic tubes (volume 50 mL), then adding deionized water (pH 5.5) to final volume 48 mL and dispersing by placing the tubes in an ultrasonic bath for 15 minutes. An aliquot of 1.5 mL of algal suspensions and 0.5 ml of Cd^{2+}metal solution were pipetted into 2 mL Eppendorf plastic tubes. Each of the triplet of suspensions were filtered through filter (Nylon Membrane Syringe filter, 0.22 µm pore size, Sigma-Aldrich) at intervals 5, 10, 30 and 60 minutes to clean Eppendorf tubes. The concentration of non-adsorbed Cd^{2+} ions in the filtrates (final concentration c_f) was determined by atomic absorption spectrometry (AAS) using the CONTRAA 700 (Analytik, Jena). Cadmium was measured at the wavelength 228.8 nm.

Calculations and outputs of biosorption experiments

The amount of metals adsorbed q (mg/g) by dried algal biomass was calculated using the following equation: $q\ (mg/g) = \frac{v\,.(c_i - c_f)}{m}$, where c_i and c_f (mg/L) are the initial and final metal ion concentrations in the solution, respectively. v (L) is the solution volume and m (g) is the mass of the biosorbent (Montazer-Rahmati et al. 2011). The percentage biosorption of metal ions was calculated as follows: $\text{biosorption}\ (\%) = \frac{(c_i - c_f)}{c_i} \times 100$, where c_i and c_f (mg/L) are the initial and final metal ion concentrations, respectively (Tamilselvan et al. 2012).

Statistical analysis

All the experiments were performed in triplicate and the obtained data were expressed as mean ± standard deviation. The statistical analysis (F-test and ANOVA) was done using software Statistica Version 12 and the graphs were done in Excel 2010.

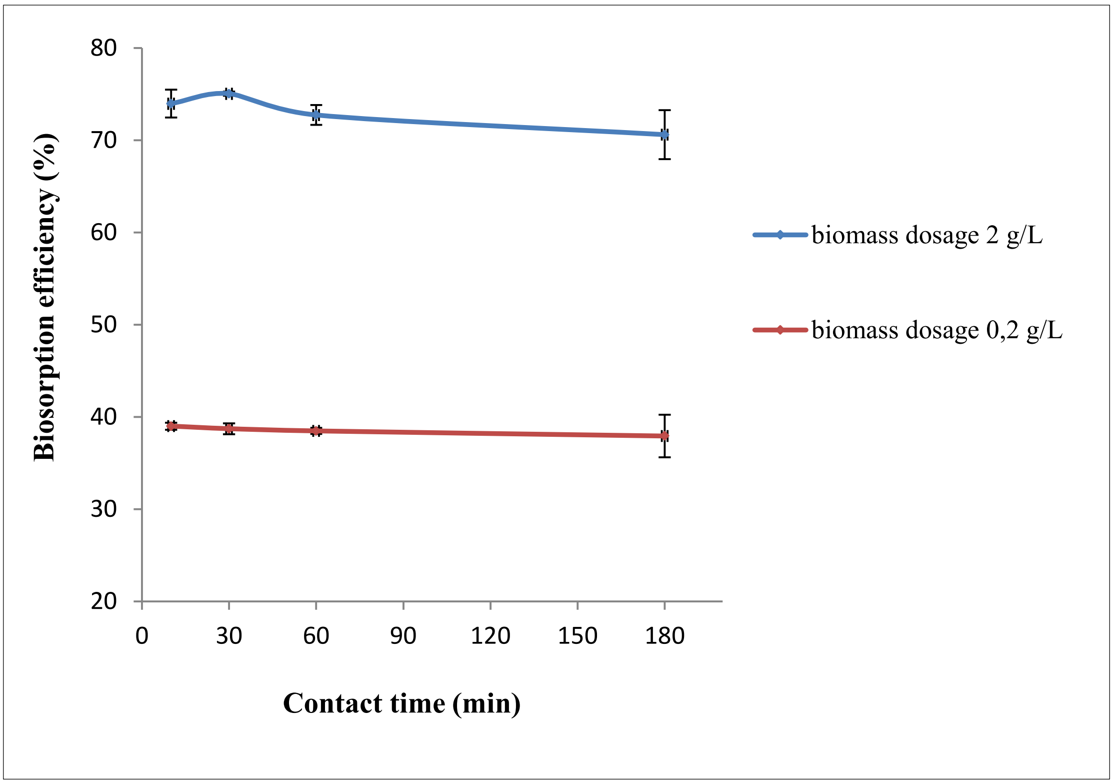
RESULTS AND DISCUSSION

Effect of biomass dosage on biosorption efficiency of Cd^{2+}

Q values were determined in the range of 18.97 ± 1.16 – 19.51 ± 0.19 mg/g at biomass dosage 0.2 g/L and in the range of 3.54 ± 0.13 – 3.75 ± 0.01 mg/g at biomass dosage 2 g/L. Biosorption efficiency (%) was at biosorption dose 0.2 g/L calculated in the values of 37.94 ± 2.31 – 39.01 ± 0.38 % and at dose 2 g/L in the values of 70.61 ± 2.67 – 75.05 ± 0.22 % (Figure 2). The biosorption efficiency was found to be biomass concentration dependent. When the applied biosorbent dose was increased ten times, the maximum biosorption efficiency increased from 39,01 to 75.05%. This phenomenon can be explained by increasing the surface area of biosorbent in aqueous solution and thereby increasing the number of functional groups of cell walls of algal biomass responsible for binding of metal ions. The performed F-test and ANOVA test showed values for dosage 2 g/L: F = 3.903, p = 0.0548 and for 0.2 g/L: F = 0.419, p = 0.744.

It was previously reported that the biosorption was increased from 18.3 to 64% for Cd as the algal adsorbent dose was increased from 0.05 to 0.7 g/L. Correspondingly, the biosorption amount was reduced from 198.0 to 49.4 mg/g for Cd (Mirghaffari et al. 2015). Also in another work, researchers reported that maximum biosorption (90%) of Cd^{2+}metal ions by *Caulerpa racemosa* (*Chlorophyta*) was observed at 40 g/L, at pH 5 and concentration of metal ions 100 mg/L. *C. racemosa* biomass showed maximum uptake of Cd^{2+} with 10.4 mg/g (Tamilselvan et al. 2012).

Figure 2 Effect of apllied biomass dose Scenedesmus quadricauda on biosorption efficiency (%) after various times of Cd^{2+}ions exposure (c_i = 10 mg/L)



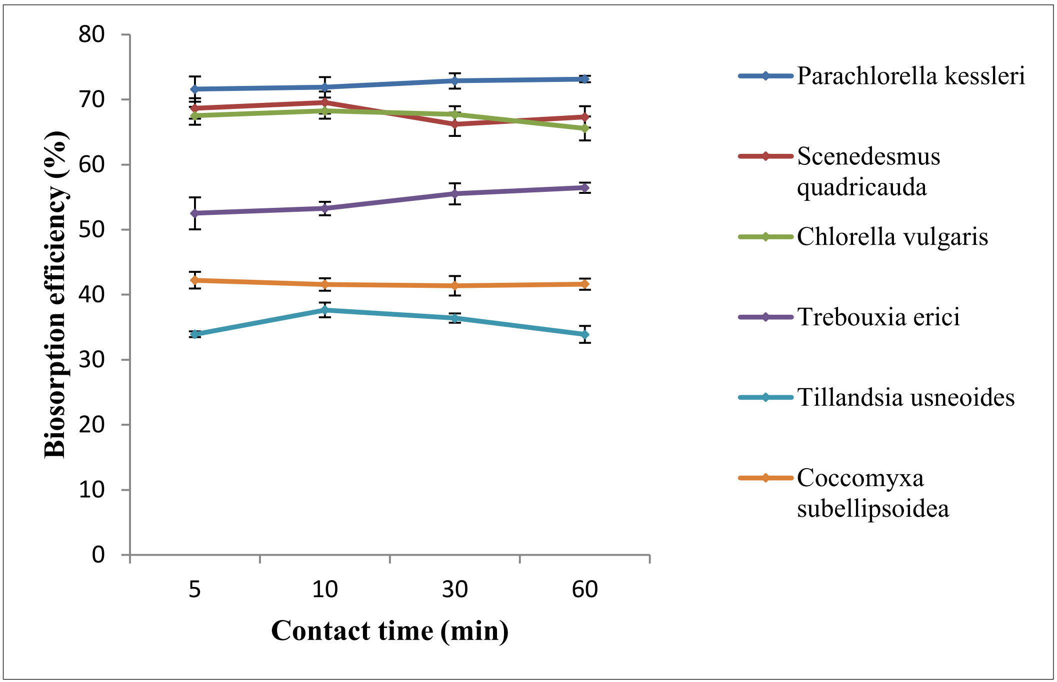
Legend: (%) – biosorption efficiency with aplicated biomass dosage of Scenedesmus quadricauda 2 or 0.2 g/L

Effect of exposure algal biomass to heavy metal ions Cd^{2+}

Figure 3 shows the biosorption of Cd^{2+} ions from aqueous solutions by dry biomass of 5 different green algae of division Chlorophyta: *Coccomyxa subellipsoidea, Chlorella vulgaris, Parachlorella kessleri, Scenedesmus quadricauda, Trebouxia erici* and by plant biosorbent *Tillandsia usneoides* as a function of contact time. The metal initial concentration was 10 mg/L and biosorbent amount was 2 g/L. The metal removal was quick – state of saturation of functional groups of cell walls by ions Cd^{2+} was achieved within 30 minutes. As the most effective biosorbent of Cd^{2+} ions was shown by the alga *Parachlorella kessleri* with maximum percentage biosorption 73.14 ± 0.49% (q = 3.66 ± 0.02 mg/g), followed by *Scenedesmus quadricauda* with 69.56 ± 1.69% (q = 3.48 ± 0.09 mg/g), *Chlorella vulgaris* with 68.24 ± 1.18% (q = 3.41 ± 0.06 mg/g), *Trebouxia erici* 56.44 ± 0.77% (q = 2.76 ± 0.11 mg/g) and the least effective alga in biosorption of Cd^{2+}ions was demonstrated by *Coccomyxa subellipsoidea* with 42.22 ± 1.27% (q = 2.11 ± 0.06 mg/g). For comparison with the algae, the plant biosorbent *Tillandsia usneoides* reached only 37.66 ± 1.12% of Cd^{2+} biosorption (q = 1.92 ± 0.24 mg/g). The performed F-test and ANOVA test showed values for *Parachlorella*: F = 0.855, p = 0.502, for *Scenedesmus*: F = 2.324, p = 0.151, for *Chlorella*: F = 1.955, p = 0.199, for *Trebouxia*: F = 0.986, p = 0.447, for *Coccomyxa*: F = 0.2867, p = 0.834 and for *Tillandsia*: F = 0.669, p = 0.595.

The biosorption of Cd^{2+} ions from the synthetic solutions by the *S. quadricauda* dry biomass is a function of contact time (1 to 240 min). The optimum contact time was determined at the metal concentration of 10 mg/L, pH 5, and biomass dosage of 0.2 g/L. The metal removal was relatively rapid, and the equilibrium times for metals were between 30 and 60 min (Mirghaffari et al. 2015). Maximum percentage removals of Cd^{2+} ions at saturation were found to be 87% (ci = 11.0 mg/L; q = 4.7 mg/g) for *Gelidium* (Rhodophyta) and 79% (c_i = 6.4 mg/L; q = 1.67 mg/g for algal waste, an amount of weighted biomass was 0.2 g of *Gelidium* or 0.3 g of algal waste. The pH was initially adjusted to 5.3 before adding the biomass (Vilar et al. 2006).

Figure 3 Effect of contact time on the biosorption efficiency of Cd^{2+} (c_i = 10 mg/L) by different dry biomass (biomass amount = 2 g/L)



Legend: (%) – biosorption efficiency, PCH – Parachlorella, SQ – Scenedesmus quadricauda, CH – Chlorella, TRE – Trebouxia erici, TIU – Tillandsia usneoides, COC – Coccomyxa.

CONCLUSION

In this study we tested the effect of biosorbent dosage and contact time of Cd^{2+} ions with different dried algal biomass on biosorption efficiency (%).The biosorption efficiency was found to be biomass concentration dependent. When the applied biosorbent dose increased ten times (0.2 g/L and 2 g/L), the maximum biosorption efficiency increased from 39.01 to 75.05%. The most effective biosorbent of ions Cd^{2+} was shown by the alga *Parachlorella kessleri* with maximum percentage biosorption 73.14%, followed by *Scenedesmus quadricauda* with 69.56%, *Chlorella vulgaris* with 68.24%, *Trebouxia erici* 56.44%, whereas the least effective alga in biosorption of Cd^{2+} ions was demonstrated by *Coccomyxa subellipsoidea* with 42.22%. In comparison to algae, the plant biosorbent *Tillandsia usneoides* reached only 37.66%. The results of this study suggest that green algae biomass can be used for biosorption of Cd^{2+} ions from waste water. Development and improvement of biosorption methods are very important in terms of protecting the environment by removing toxic metals or reducing their levels in environmentally acceptable limits from industrial waste water.

ACKNOWLEDGEMENT

The research was financially supported by the Internal Grant Agency of Faculty of Agronomy, Mendel University (project no. 2101 SPP: SP 2150431) and the Grant Agency of Czech Republic (project no. GA14- 28933S). The author is thankful to doc. RNDr. Jozef Kováčik, PhD. for cooperation in the realization of this experiment.

REFERENCES

Gupta V. K., Rastogi A. 2009. Biosorption of hexavalent chromium by raw and acid-treated green alga *Oedogonium hatei* from aqueous solutions. *Journal of Hazardous Materials,* 163: 396–402.

Gupta V. K., Rastogi A. 2008. Equlibrium and kinetic modelling of cadmium (II) biosorption by non-living algal biomass *Oedogonium* sp. from aqueous phase. *Journal of Hazardous Materials,* 153: 759–766.

Gupta V. K., Shrivastava A. K., Jain N. 2001. Biosorption of chromium (VI) from aqueous solutions by green algae *Spirogyra* species. *Water Research,* 35 (17): 4079–4085.

Kadukova J., Vircikova E. 2005. Comparison of differences between copper bioaccumulation and biosorption. *Environment International,* 31: 22–232.

Kovacik J., Babula P., Hedbavny J., Krystofova O., Provaznik I. 2015. Physiology and methodology of chromium toxicity using alga Scenedesmus quadricauda as model object. *Chemosphere,* 120: 23–30.

Kovacik J., Klejdus B., Stork F., Hedbavny J., Backor M. 2011. Comparison of methyl jasmonate and cadmium effect on selected physiological parameters in *Scenedesmus quadricauda* (Chlorophyta, Chlorphyceae). *Journal of Phycology,* 47: 1044–1049.

Mirghaffari N., Moeini E., Farhadian O. 2015. Biosorption of Cd and Pb ions from aqueous solutions by biomass of the green microalga, Scenedesmus quadricauda. *J Appl Phycol,* 27: 311–320.

Montazer-Rahmati M. M., Rabbani P., Abdolali A., Keshtkar A. R., 2011. Kinetics and equilibrium studies on biosorption of cadmium, lead, and nickel ions from aqueous solutions by intact and chemically modified brown algae. *Journal of Hazardous Materials,* 185: 401–407.

Tamilselvan N., Saurav K., Kannabiran K. 2012. Biosorption of Cr (VI), Cr (III), Pb (II) and Cd (II) from Aqueous Solutions by *Sargassum wightii* and *Caulerpa racemosa* Algal Biomass. *Oceanic and Coastal Sea Research,* 11 (1): 52–58.

Vilar V. J. P., Botelho C. M. S., Boaventura R. A. R. 2006. Equlibrium and kinetic modelling of Cd (II) biosorption by algae *Gelidium* and agar extraction algal waste. *Water Research,* 40: 291–302.