

Biosorption of uranium from aqueous solutions by nonliving biomass of marine algae *Cystoseira indica*

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Biosorption of heavy metals can be an effective process for the removal and recovery of heavy metal ions from aqueous solutions. In this paper uranium biosorption by protonated, Ca-pretreated and non-pretreated *Cystoseira indica* algae biomass was investigated in a batch system. The results of the kinetic studies showed that the sorption of uranium on protonated and non-pretreated biomass followed pseudo-second order kinetics. The effect of pH on the equilibrium uranium sorption capacity of *Cystoseira indica* exhibited that highest uptake occurred at pH 4 at a solution with 350 mg/l uranium concentration. At various initial uranium

concentrations from 50 to 1000 mg/l, batch sorption equilibrium at 30°C was reached within 3 hrs and the sorption isotherms were interpreted in terms of the Langmuir and Freundlich models. Equilibrium data fitted very well to Langmuir model for all studied forms of *Cystoseira indica* algae. The Freundlich isotherm cannot fit as well as the Langmuir model the equilibrium data of protonated and non-pretreated *Cystoseira indica* algae. The maximum uranium adsorption capacity on the Ca-pretreated, protonated and non-pretreated *Cystoseira indica* algae predicted by Langmuir isotherm at pH 4 and 30°C was 454.5, 322.58

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and 224.67 mg/g respectively.

Toxic heavy metal contamination of industrial water is a significant universal problem. They accumulate in living tissues throughout the food chain which has humans at its top. These toxic metals can cause accumulative poisoning, cancer and brain damage when found above the tolerance levels. Uranium is one of the most seriously threatening heavy metals because of its high toxicity and some radioactivity. Excessive amounts of uranium have found their ways into the environment through the activities associated with the nuclear industry. Uranium contamination poses a threat in some surface and groundwaters (Laul, 1992). There is a need for controlling the heavy metal especially uranium emissions into the environment. Conventional methods for removing heavy metals from industrial effluents (e.g. precipitation and sludge separation, chemical oxidation or reduction, ion exchange, reverse osmosis, membrane separation, electrochemical treatment and evaporation) are often ineffective and costly when applied to dilute and very dilute effluents (Aksu, 1998). Recently, biological removal processes has been attracting considerable attention for removing heavy metals from aqueous wastes and screening for microorganisms having higher potential for removing heavy metals from wastes has been promising so far (Volesky and Holan, 1995; Kratochvil et al. 1997; Kratochvil and Volesky, 1998; Pagnanelli et al. 2001; Singh et al. 2001; Kaewsarn, 2002; Nuhoglu et al. 2002; Pessoa de Franca et al. 2002; Tien, 2002; Axtell et al. 2003; Padmavathy et al. 2003). Microbial removal of heavy metals offers the advantages of low operating cost, minimizing secondary problems with metal-bearing sludge and high efficiency in detoxifying very dilute effluents. Metal uptake by microorganisms can occur actively (bioaccumulation) and/or passively (biosorption) (Ehrlich, 1997; Nies, 1999). Feasibility studies for large-scale applications demonstrated that the biosorptive processes are more applicable than the bio-accumulative processes because living systems often require the addition of nutrients and hence increase biological oxygen demand (BOD) or chemical oxygen demand (COD) in the effluent, maintenance of a healthy microbial population is difficult due to metal toxicity and other unsuitable environmental factors and mathematical modelling of an undefined system is difficult (Sternberg and Dorn, 2002; Chandra Sekhar et al. 2003).

Table 1. Kinetics parameters for the sorption of uranium by *Cystoseira indica* algae.

Sorbent	$q_{eq}(mg/g)$	$K(g/mg \times min)$	R^2	$q_{eq-exp}(mg/g)$
Protonated algae	161.29	2.4×10^{-3}	0.9999	167.86
Non-pretreated algae	151.51	1.7×10^{-3}	0.9998	150.3

Although the fact that marine algae are capable of biosorbing radionuclides such as radium, thorium and uranium has been known for a long time (Edgington et al. 1970), the biosorption of uranium by *Cystoseira indica* algae, a brown algae biomass which found vastly in Iran, has not been investigated. The aim of this paper is to determine the effect of the basic parameters such as pH, contact time and initial metal concentration on the uranium biosorption by *Cystoseira indica* algae and to compare between sorption on protonated, Ca-pretreated and non-pretreated algae biomass in a batch system.

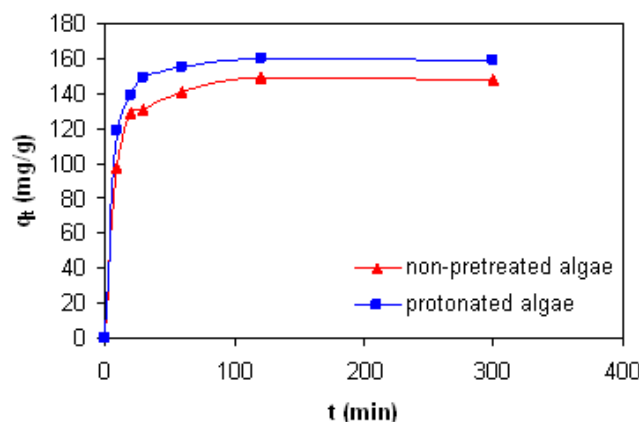


Figure 1. Biosorption rate of uranium ions by *Cystoseira indica* algae at 350 mg/l initial uranium concentration, pH 4 and 30°C.

MATERIALS AND METHODS

Preparation of algae and uranium solutions

Cystoseira indica, a brown alga, obtained from Persian gulf on the coast of Qeshm, Iran was extensively washed with distilled water and sun-dried on the beach and in an oven at 50°C overnight. Dried biomass was ground in a laboratory blender and sorted by sieving using the standard test sieves. The batch of biomass with particle size 1.0-1.25 mm was selected for subsequent pre-treatment. Pre-treatment of the biomass was carried out as follows: A sample of 10 g of biomass was treated with 0.1 M CaCl₂ solution (1000 ml) for 12 hrs under slow stirring. The solution pH was kept constant at pH 5.0 by using 0.01 M HCl or 0.01 M NaOH solution if deviations were observed. The calcium-treated biomass was washed several times with deionised water to remove excess calcium from the biomass. Also protonation of the biomass was carried out by using 0.1 N HCl (10 g biomass/L). After 12 hrs of contacting with acid, the biomass was rinsed with deionised water in the same volume many times until a stable wash solution pH 5.0 was reached. The Ca-pretreated and protonated biomasses were then dried in an oven at 50°C overnight. The prepared biomasses was stored for later uses.

Uranium solutions were prepared by diluting 1000 mg uranium/stock solution which was obtained by dissolving a

weighed quantity of uranium in nitrate salt form, $UO_2(NO_3)_2 \cdot 6H_2O$, analytical grade. Diluted solutions were prepared at room temperature in distilled and deionised water.

Analysis of uranium concentration

Dissolved uranium concentrations in solution were assessed by an inductively coupled plasma atomic emission spectrophotometer (ICP-AES, Thermo Jarrel Ash, Model TraceScan). The ICP analyses were conducted at wavelength of 409.014 nm.

Batch biosorption studies

In order to determine the contact time required for the sorption equilibrium experiments, the sorption dynamics experiments were conducted first. On a shaker incubator 0.2 gram of biomass was mixed with 100 mL of 350 mgU/l $UO_2(NO_3)_2$ solution at 150 rpm and 30°C. A series of 0.5 mL samples of solution were removed from the vessel at predefined time intervals. After appropriate dilution, the samples were analyzed by the ICP-AES for metal concentrations. After the equilibrium time was determined, a series of uranium nitrate solution with different concentrations (100 mL) were mixed with 0.2 g biomass and the sorption equilibrium experiments were conducted in the same way. The suspensions were brought to the desired pH by adding 0.05 N HCl or NaOH during the sorption process and the volume of HCl or NaOH added was recorded. After 3 hrs of contact (according to the preliminary sorption dynamics tests), equilibrium was reached and the solution was filtered. The supernatant was diluted for uranium concentration analyses by the ICP-AES.

The amount of uranium ions adsorbed per unit of empty sorbent was obtained by using the following expression:

$$q = (C_i - C_e) \times V/M \quad [1]$$

where q is the amount of uranium adsorbed onto the unit mass of the adsorbent (mg/g), C_i and C_e are the concentrations of the metal ions before and after biosorption (mg/l), V is the volume of the aqueous phase (l), and M is the amount of the adsorbent (g).

All sorption experiments were carried out in triplicate. Values used in calculations were the arithmetic averages of the experimental data.

RESULTS AND DISCUSSION

Biosorption rate of uranium ions and kinetic modelling

Generally speaking, the uptake of heavy metal ions by microorganisms has often been observed to occur in two stages; an initial rapid uptake due to surface adsorption on the cell walls and a subsequent slow uptake due to membrane transport of the metal ions on the cytoplasm of the cells. Uranium biosorption by *Cystoseira indica* algae exhibited the same behaviour (Figure 1). As can be seen from Figure 1, the uranium ions adsorption rate by *Cystoseira indica* algae is high at the beginning but plateau values are reached in about 3 hrs. This result is typical for biosorption of metals involving no energy-mediated reactions, where metal removed from solution is due to purely physico/chemical interactions between the biomass and metal in solution. The fast dynamic behaviour of uranium uptake suggests a minimal residence time (or contact time) for a process based on these preparations. Figure 1 also shows that a larger amount of uranium removed by protonated biomass in the first 30 min of contact time so that the initial solution rate for protonated and non-pretreated biomass is 11.88 and 9.76 mg/g x min, respectively. The rapid kinetics has significant practical importance as it will facilitate smaller reactor volumes ensuring efficiency and economy.

In order to modelling the sorption rate of uranium on *Cystoseira indica* algae suspension in a well-agitated batch system, pseudo-second order rate equation was applied (Ho and McKay, 1999). Assuming the sorption capacity of uranium on the biomass is proportional to the number of active sites occupied on the sorbent, then the pseudo-second order equation is given by:

$$\frac{dq_t}{dt} = k(q_{eq} - q_t)^2 \quad t = 0, \quad q_t = 0 \quad [2]$$

Where k is the equilibrium rate constant of pseudo-second order sorption kinetics (g/mg x min), q_t the amount of sorbate on sorbent at time t (mg/g), and q_{eq} the equilibrium uptake (mg/g). Equation [2] can be integrated and rearranged in to:

$$\frac{t}{q_t} = \frac{t}{q_{eq}} + \frac{1}{q_{eq}^2 k} \quad [3]$$

Table 2. The Langmuir and Freundlich adsorption parameters for the biosorption of uranium on the *Cystoseira indica* algae.

Sorbent	Langmuir model			Freundlich model		
	Q^0 (mg/g)	b (l/mg)	R^2	k_F (mg/g)(mg/l) ⁿ	n	R^2
Ca-pretreated algae	454.5	0.041	0.9887	30.729	3	0.9576
Protonated	322.58	0.0852	0.9914	49.0944	2.82	0.8785
Non-pretreated algae	224.67	0.0194	0.9922	15.547	2.02	0.9341

Figure 2 shows a plot of t/q_t versus t , for the sorption of uranium on the biomass at 350 mg/l initial concentration and 30°C. The values of the parameters, q_{eq} and k are calculated from the slopes and the intercepts of the straight lines shown in Figure 2 and tabulated in Table 1. The values of the theoretical q_{eq} for both sorbents are in good agreement with those found experimentally. Also the correlation coefficients for both sorbents are greater than 0.999. These results indicate that the sorption of uranium on the sorbents investigated in this study follows a pseudo-second order kinetics.

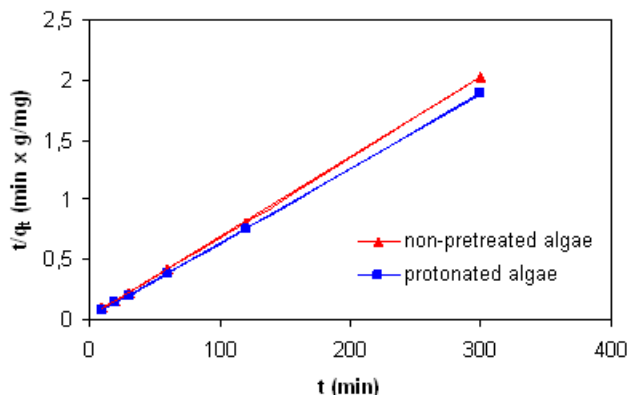


Figure 2. Kinetics of sorption of uranium by *Cystoseira indica* algae at 350 mg/l initial uranium concentration, pH 4 and 30°C.

Effect of pH on uranium biosorption

Earlier studies on heavy metal biosorption have shows that pH is an important parameter effecting the biosorption process. The effect of initial pH on uranium ion biosorption capacity of *Cystoseira indica* algae was studied at 350 mg/l initial uranium concentration and at 30°C. As seen from Figure 3 the biosorption of uranium for both protonated and non-pretreated algae increased with pH up to 4 and then declined with further increase in pH. The maximum equilibrium uptake value was found at pH 4.

The metal ion binding in biosorption could be attributed to several mechanisms such as ion exchange, complexation, electrostatic attraction and micro precipitation. For algae biomass, ion exchange has been considered as a main mechanism responsible for metal sequestering (Crist et al. 1988). The ion exchange mechanism for uranyl ions binding to the biomass is complicated by the fact that the uranium cation UO_2^{2+} is hydrolyzed in aqueous solutions within the range of the sorption system pH. Portioning of the hydrolysed uranium species depends on the solution pH and on the total uranium concentration in the solution. In the range of acidic to near neutral pH values, four major hydrolysed complex ions, UO_2^{2+} , $(UO_2)_2(OH)^{2+}$, UO_2OH^+ , $(UO_2)_3(OH)^{5+}$ and a dissolved solid schoepite ($4UO_3 \cdot H_2O$), a hydrous uranium oxide, exist in the solution (Baes and Mesmer, 1976). The hydrolysis equilibrium constants are $pK = 5.8$ for UO_2OH^+ , $pK = 5.62$ for $(UO_2)_2(OH)^{2+}$ and pK

$= 15.63$ for $(UO_2)_3(OH)^{5+}$ (Baes and Mesmer, 1976). At pH 4 and uranium concentration 350 mg/l all hydrolyzed ions exist in the solution.

According to Collins and Stotzky (Collins and Stotzky, 1992), the hydrolyzed species can obviously be sorbed better than the free hydrated ions. Particularly the monovalent, compared with the divalent hydrolyzed ions, have even higher affinity to the biomass in ion exchange with protons because they could replace single protons on separate binding sites in the biomass.

The percentage of UO_2^{2+} in the solution increases with decreasing the pH of system. The lower pH suppresses the enhancement of uranium biosorption occurring normally because of the hydrolyzed ions. When the pH becomes low enough, for example at pH 2.6, the divalent free UO_2^{2+} becomes the dominant ion form in the solution for a wide uranium concentration range from 0.3 to 1000 mg/l. In addition, since the UO_2^{2+} is divalent, it can only replace two protons on the adjacent binding sites of the biomass but cannot react with those sites which are farther apart from each other. In other words, at low pH some binding sites are not available to the divalent UO_2^{2+} . On the other hand, the non-ion dissolved solid schoepite starts appearing in the solution when the pH is too high. The uranium sorption may be hindered by the decrease in ion concentration in this situation.

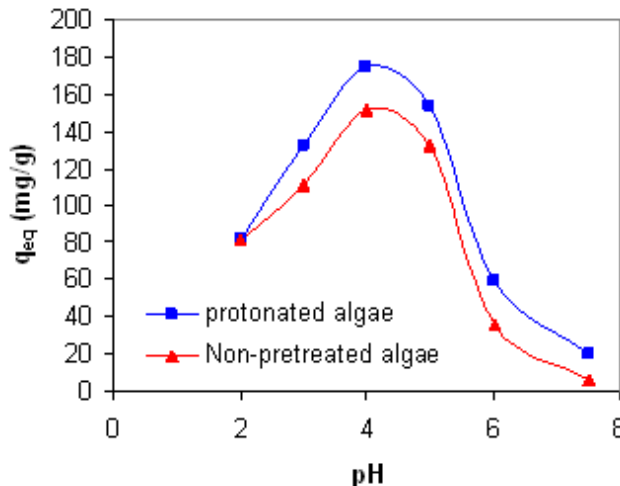


Figure 3. The effect of pH on the equilibrium sorption capacity of *Cystoseira indica* algae at 350 mg/l initial uranium concentration and 30°C.

Effect of initial uranium concentration

The initial concentration provides an important driving force to overcome all mass transfer resistance of uranium between the aqueous and solid phases. Hence a higher initial concentration of uranium will enhance the adsorption process. Such an effect is clearly demonstrated in Figure 4 where the experimental data of q_{eq} versus initial

concentration of uranium have been illustrated for protonated, non-pretreated and Ca-pretreated algae biomass at pH 4 and 30°C. As can be seen from Figure 4, at low initial uranium concentration, the equilibrium sorption capacity of the all sorbents is directly proportional to the initial uranium concentration in the solution and in the high initial uranium concentration range, the equilibrium sorption capacity of the all forms of the algae biomass go to a constant value. Also the results show that the pre-treatment of the algae biomass considerably influences the equilibrium sorption capacity of the sorbent. Figure 4 shows that the Ca-pretreated *Cystoseira indica* algae is better than protonated and non-pretreated *Cystoseira indica* algae and the protonated algae is better than non-pretreated algae in the all range of initial uranium concentration. The pre-treatment process enhances biomass surface ions. Since the biosorption of uranium is largely ion exchange process, the processed samples of the biomass have higher sorption capacity in comparison with raw. Also the Ca-treatment is more effective than the protonation, because the Ca²⁺ ions are divalent and can replace with two monovalent uranium ions. Proton, nevertheless, can replace with only one. In this paper, the characteristics of the surface groups on *Cystoseira indica* algae were not determined. Acidic sites, carboxylic and phosphatic types or amino-type sites are more likely to take part in ion exchange. It is supposed to be determined by potentiometric titration.

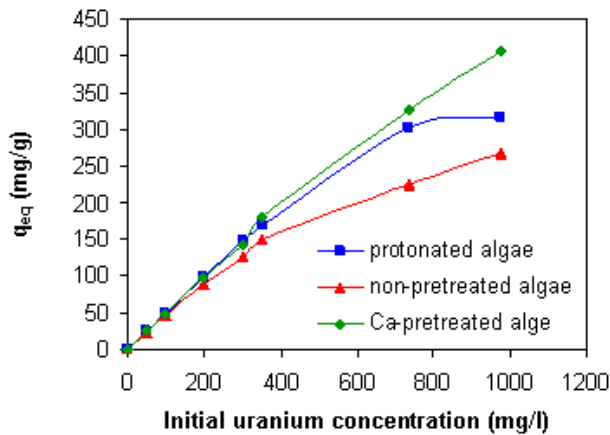


Figure 4. Equilibrium sorption capacity of *Cystoseira indica* algae for uranium removal from aqueous solution at pH 4 and 30°C.

Modelling of adsorption isotherms

The equilibrium established between adsorbed metal ion on the biosorbent and unadsorbed metal ion in solution can be represented by adsorption isotherms. Two isotherm equations have been tested in the present study, namely, the Langmuir, Freundlich (Aksu, 1998; Gin et al. 2002; Nuhoglu et al. 2002; Donat et al. 2005; Kumar Meena et al. 2005; Xiangliang et al. 2005).

The Langmuir equation which is valid for monolayer

sorption onto a surface with a finite number of identical sites which are homogeneously distributed over the sorbent surface is given by Equation [4].

$$q_{eq} = \frac{Q^0 b C_{eq}}{1 + b C_{eq}} \quad [4]$$

where q_{eq} is the amount of metal ion bound to per gram of dried biomass at equilibrium and C_{eq} is the residual (equilibrium) metal ion concentration left in solution after binding, respectively. Q^0 is the maximum amount of metal ion per unit weight of sorbent to form a complete monolayer on the surface bound at high C_{eq} , and b is a constant related to the affinity of the binding sites. Q^0 and b can be determined from C_{eq}/q_{eq} versus C_{eq} plot which gives a straight line of slope $1/Q^0$ and intercept $1/bQ^0$.

The empirical Freundlich equation based on sorption on a heterogeneous surface is given by Equation [5].

$$q_{eq} = k_F C_{eq}^{1/n} \quad [5]$$

where k_F and n are the Freundlich constants related to the sorption capacity and sorption intensity of the sorbent, respectively. Equation [5] can be linearized in logarithmic form and Freundlich constants can be determined. Both models were developed for a single-layer adsorption, but the Freundlich isotherm provides no information on the monolayer adsorption capacity, in contrast to the Langmuir model.

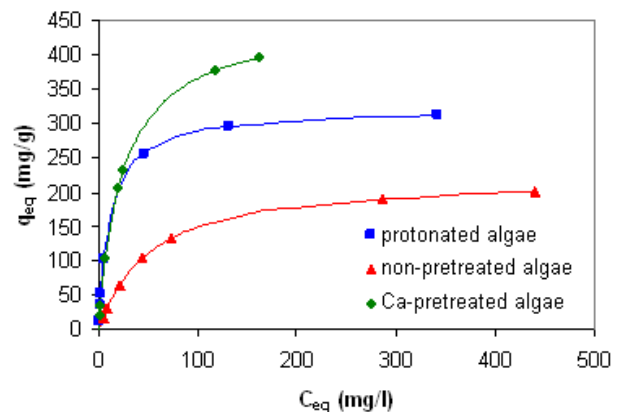


Figure 5. Langmuir isotherms of uranium ions sorbed on different sorbents at pH, 4 and 30°C. The points represents experimental data and the lines are model isotherms that fitted for them.

The Langmuir and Freundlich adsorption parameters with the correlation coefficients (R^2) for the biosorption of uranium on the biosorbent are listed in Table 2. High regression correlation coefficients (>0.9887) for the Langmuir model show that the adsorption process of

uranium by *Cystoseira indica* algae can be well defined by this model. As can be seen from Table 2, the Freundlich isotherm is only suitable for describing the biosorption equilibrium of uranium by Ca-pretreated and non-pretreated algae and it can not fit reasonably the biosorption equilibrium of uranium by protonated algae. While the Freundlich model does not describe the saturation behaviour of the biosorbent, Q^0 , the Langmuir constant represents the monolayer saturation of equilibrium. The maximum capacity, Q^0 determined from the Langmuir isotherm defines the total capacity of the biosorbent for uranium. From Table 2 the maximum adsorption capacity for uranium on the Ca-pretreated, protonated and non-pretreated *Cystoseira indica* algae is 454.5, 322.58 and 224.67 mg/g, respectively. The Langmuir isotherm with the experimental data of q_{eq} versus C_{eq} are shown in Figure 5.

CONCLUDING REMARKS

The study indicated that the biomass of the *Cystoseira indica* algae particularly its Ca-pretreated form could be used as an efficient biosorbent material for the treatment of uranium ions-bearing waste water streams. The kinetics of adsorption by this biomass was rapid with 90% of the total adsorption occurring within first 30 min. Also the uranium biosorption on the biomass was found to follow pseudo-second order kinetics. The adsorption capacities were solution pH dependent and maximum adsorption capacities of Ca-pretreated, protonated and non-pretreated *Cystoseira indica* algae was determined to be 454.5, 322.58, 224.67 mg/g respectively at a solution pH of 4. The Langmuir and Freundlich adsorption models were used for the mathematical description of the biosorption equilibrium of uranium ions to *Cystoseira indica* algae and the obtained results showed that the adsorption equilibrium data fitted very well to the Langmuir model in the studied concentration range for all investigated biomass forms. Assuming the batch biosorption as a single-staged equilibrium operation, the separation process can be mathematically defined using these isotherm constants to estimate the residual concentration of metal ions or amount of biosorbent for desired purification.

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