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Efficiency of the immobilized form of micro green alga *Chlorella vulgaris* for uranium uptake from aqueous solution

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The use of micro alga *Chlorella vulgaris* either free or immobilized in alginate or in spongy, composite to remove uranium from aqueous solution was examined under the effect of batch experiments. The experiments comprise different pH, concentrations of uranium, shaking and contact time. The maximum removal efficiency 82% was indicated when the alga coated with alginate at pH 4.5 on horizontal shaker for 25 min at 300 rpm. The adsorption of uranium was evaluated with Langmuir isotherm $R^2= 0.99$ with pseudo-second order where $R^2= 0.99$, $K_2= 0.00086$ and $q_{2nd} = 153.85$ mg/g. The lowest value of K_2 indicates that the great affinity of alga to adsorb uranium. FT-IR Infrared spectra confirmed that the alga donates different functional groups and the layer in algal biomass is heterogeneity due to the variable characteristics peaks of amino, carboxylic, hydroxyl, and carbonyl groups. For this reason, *C. vulgaris* in alginate form is more efficient to adsorb and uptake uranium ions from aqueous solution.

Keywords: Uranium- *Chlorella vulgaris*- Langmuir - Freundlich - FT-IR spectra

INTRODUCTION

Uranium as heavy metal is hazard due to its high toxicity and radioactivity. The activity of nuclear industry causes heavy load of uranium in different habitats (Laul, 1992). Immobilization of microalgae have wide varieties of biotechnological applications specially for heavy metals and nutrients removal (Lebeau and Robert, 2006). Such techniques allowed the alga to be live for long period and reused with high efficiency once again. The Immobilization is a process of common used natural polymers, alginate and carrageenan (Bashan, 1998 and Moreno-Garrido, 2008). Different studies on uranium biosorption showed that many types of biomass such as algae, yeast, and bacteria have ability to remove uranium from aqueous solution either living (Gavrilescu et al. 2009; Wang and Chen 2009) or non-living dead biomass (Tam et al. 2009). The process of

biosorption for heavy metal removal depends on many factors such as pH, metal ions, concentration, biomass dose, and temperature (Khani et al. 2008). The immobilization method has the priority in use due to the major advantages as it can be used several times and so easy to obtain during the filtration. Moreover, it is costless more than the other techniques (Moreno-Garrido, 2008).

The present investigation aims to evaluate the efficiency of *C. vulgaris* either free or immobilized to remove uranium from aqueous solutions.)

MATERIALS AND METHODS

2.1. Reagents and standards

All chemicals used for this study were analytical - grade and purchase from Sigma

Services Company and SDFCL fine - chem. Limited.

2.2. Isolation and growth of *Chlorella vulgaris*

The unicellular green non-motile *Chlorella vulgaris* was isolated from the Egyptian wheat soil farm at Zagazig city in solid modified BG11 medium according to (Stanier et al. 1971). Unistreakof purified alga was suspended in 5 ml sterile BG11 medium. Stock solution of *C. vulgaris* was cultivated in modified liquid BG11 medium. 3 liters flasks containing 2 l sterile BG11 medium. The flasks incubated under light intensity of (70 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$) at 25°C with continuous aeration for 20 days. The growth rate was determined every 48 hrs to assist the maximum growth rates of free alga (the greatest optical density). The total number of the cells was calculated using Hemocytometer then collected to ready for immobilization forms (alginate form and spongy, composite form).

2.3. Preparation of uranium solutions

Different uranium concentrations were prepared in Nuclear Materials Authority laboratories, New Cairo City, Egypt by diluting 1000 mg uranyl-acetate ($\text{C}_4\text{H}_8\text{O}_6\text{U}/\text{l}$) stock solution to the following concentrations (25,50,75,100, 125 and 250 mg/l). Dilution were carried out at room temperature with deionized water. The pH of the uranium solution was adjusted to the required pH values. The maximum rate of shaking was also adjusted to (300 rpm). The biosorption of uranium by the immobilized samples was performed in a batch experiment under the effect of pH, the contact time, substrate concentrations and shaking rate. The kinetics equilibrium and thermodynamics were taken in consideration.

2.4. Determination of uranium

Uranium was titrated and estimated according to (Davis & Gray, 1964). Through it 5 ml of uranium solution was taken and dropped and titrated against ammonium meta vanedate (NH_4VO_3) with titanium tri chloride (TiCl_3) as indicator.

2.5. Preparation of immobilized *Chlorella sp* by alginate and spongy/composite

The micro alga was harvested by centrifugation at 4000 rpm for 10 min. The cells were seeded in 100 ml of distilled water to form a concentrated algal suspension with a cell density of 10×10^6 cells /ml. 4% alginate solution was prepared, 1 ml of algal suspension was mixed with

1 ml alginate suspension to reach 2% alginate solution. The mixture was poured in to 50 ml burette and dropped into saturated calcium chloride solution. This method produced approximately 5650 uniform algal beads of approximately with diameter 2.35 mm and the volume of alginate beads was 6.7 mm^3 . For the formation of alga /spongy composite, a lot of clean white spongy pieces were mixed with algal suspension containing the same number in case of alginate then left to stand overnight and washed to remove the free cell. The concentration of the applicable beads and spongy were depend on the displacement volume of uranium solution alga suspensions.

2.6. Fourier Transform Infrared Spectroscopy Studies

Infrared Spectroscopy (FTIR) using a Shimadzo 1650 spectrophotometer (Tokyo, Japan) was employed. The spectra were taken within the range of 400: 4000 cm^{-1} of the different samples according to (Bayramoglu et al. 2006).

2.7. Effect of pH initial

was studied by performing a series of different pH ranging from (2.5 to 8.5). Solutions of 0.1 HNO_3 and 0.1 NaOH were used to adjust the different pH at room temperature. 3 Erlenmeyer flasks containing 125 ppm uranium mixed with equivalent number of beads to 0.035g and all the flasks were incubated at $32 \pm 2^\circ\text{C}$ in shaking incubator at 300 rpm. 5mls were taken and clarified by centrifuge for titration with ammonium meta vanedate (NH_4VO_3) using titanium tri chloride (TiCl_3) every 5min to the last contact time of 30 min for different shaking rate, the different samples were taken at each rate after constant time and optimum pH.

2.8. Effect of different concentrations of uranium

Under constant pH 4.5 and shaking rate at 300 rpm; an equivalent alginate bead and alga /spongy, composite were seeded to the different flasks containing different concentrations of uranium (25, 50,75,100, 125 and 250 mg/l). The flasks were shaken and different samples were taken after 25 min to compute the percentage of removal as previously mentioned.

Equilibrium models

At equilibrium Langmuir model (only monolayer adsorption of solute - L type) but Freundlich model (deals the development of

heterogeneous surfaces - F type). The Langmuir model expressed by Eq. (1)

$$Q_e = (q_{\max} \cdot b \cdot C_e) / (1 + b \cdot C_e) \quad (1)$$

Where, q_{\max} = maximum amount of metal ion adsorbed at C_e (equilibrium concentration of metal); and b = measure of the biosorption affinity or efficiency of the biomass. The parameter q_{\max} represents the maximum sorption capacity of the biosorbent when the surface is fully occupied by metal ions. It also represents the total number of binding sites (per unit weight) involved in metal sorption by the biosorbent where as Freundlich model expressed as :

$$Q_e = K_f C_e^{1/n} \quad (2)$$

where q_e = amount adsorbed (mg/g of sorbent) at equilibrium; C_e = equilibrium concentration (mg/l); and K_f and n = constants indicative of adsorption capacity and adsorption intensity, respectively.

2.9. Effect of shaking speed and contact time

Under optimum pH 4.5 and concentration of uranium (125 mg/l) as previously mention, a series of Erlenmeyer flasks containing 50 ml of sterile aqueous uranium solution were seeded with an equivalent wt of algal under investigation (0.035 g) were incubated on electric shaker. The flasks were classified in to two groups, one left under static conditions and the other were shaken from 100, 200, 300 and 400 rpm. For each speed, 5 ml was taken for estimating the concentration of residual uranium at the following time (5, 10, 15, 20, 25 and 30 min). The different data of different times and shaking rate were graphically represented in Figs (18, 19) and (17) respectively.

Kinetic studies

To analyze the rate of sorption of metal ions onto the biomass, two simple kinetic models namely, pseudo first-order may be represented as follows:

$$d_q / d_t = K_1 (q_e - q_t) \quad (3)$$

where k_1 = rate constant of pseudo first-order biosorption (min^{-1}) and q_e , q_t = amounts of biosorption (mg/g), at equilibrium and at time (t) respectively (Parab et al. 2005) and pseudo second-order model can be expressed as follows:

$$d_q / d_t = K_2 (q_e - q_t)^2 \quad (4)$$

where, k_2 (min^{-1}) = Rate constant of pseudo second-order biosorption (Bhat et al. 2008; Amini et al. 2009a).

2.10. Statistical analysis

The obtained data were analyzed statistically using one-way analysis of variance (ANOVA) and

either Fisher's LSD or Student's T-test was used to determine significant differences among the data.

RESULTS AND DISCUSSION

Characterization of immobilized forms of algae

The microstructures of alginate beads and algae / spongy, composite were clarified by Scanning Electron Microscopy in Fig (1 to 4) respectively. Fig (1) revealed that free *Chlorella* cells have highly similar abovate shape with multi area like cave between them.

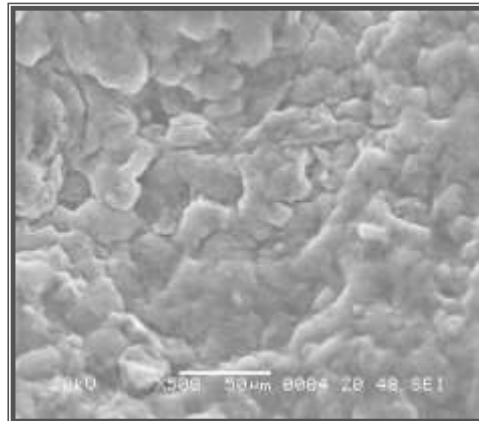


Fig (1) : SEM for Uranium free cells (Control)

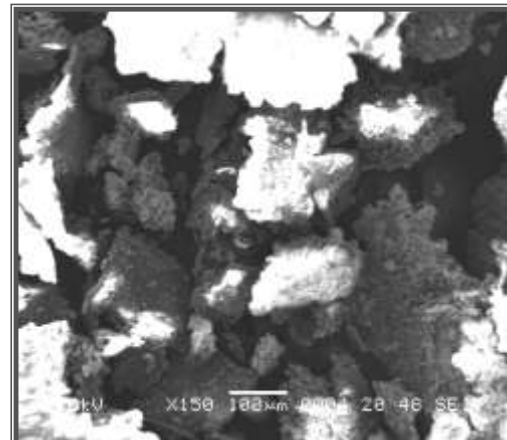


Fig (2) : SEM for Free *C. vulgaris* with U

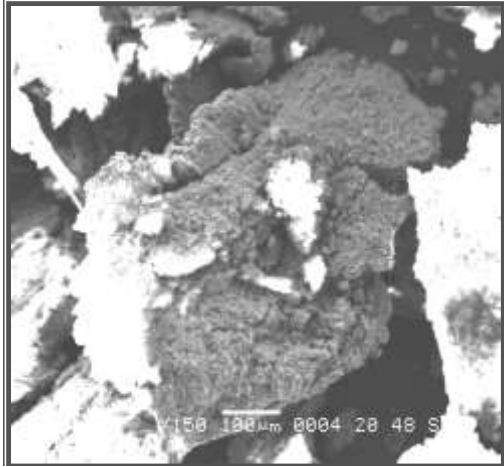


Fig (3) : SEM for Spongy *C. vulgaris* with U

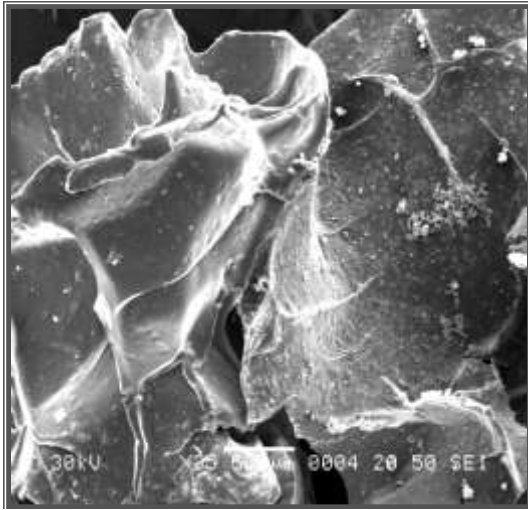


Fig (4) : SEM for Alginate *C. vulgaris* with U

Where as Fig (4) revealed that the different alginate beads have isodiametric size appeared with multi-minute pores that facilitate the passage of uranium ion to reach the active groups. The activity of alga/ spongy, composite is lower than alginate. This may be related to the capillarity index of pores in spongy is more sized than in alginate. X-Ray spectroscopy (XRF) spectrum in Fig(8) evaluated that U was adsorbed by alginate beads more than the other forms and shows 3 peaks at the range between 14 keV to 17 keV with maximum count reach $5.12 \cdot 10^3$. In case of spongy uranium appears at 14.7 keV uranium count reach $3.2 \cdot 10^3$. The results manifested that the alginate form is better than spongy form due to the appearance of active sites (-C-O-, -C-C-, and $-C \equiv C$) bonds, respectively. In case of free treated with U there is no peaks appear in XRF

spectrum.

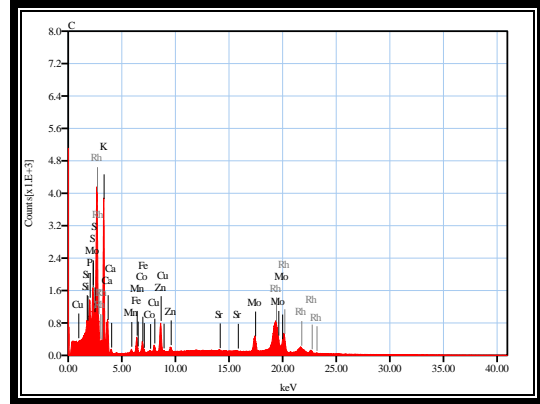


Fig (5) : XRF for Control *Chlorella vulgaris*

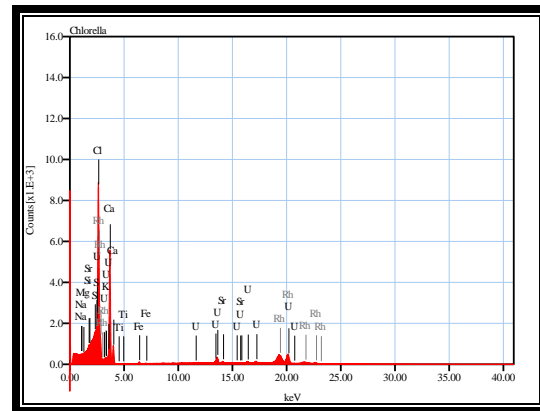


Fig (6) : XRF for free *C. vulgaris* with U

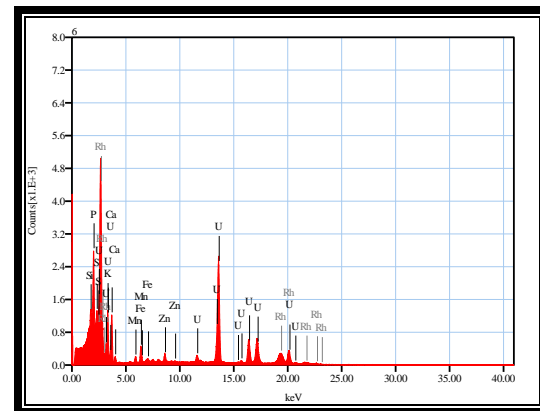


Fig (7) : XRF for spongy *C. vulgaris* with U

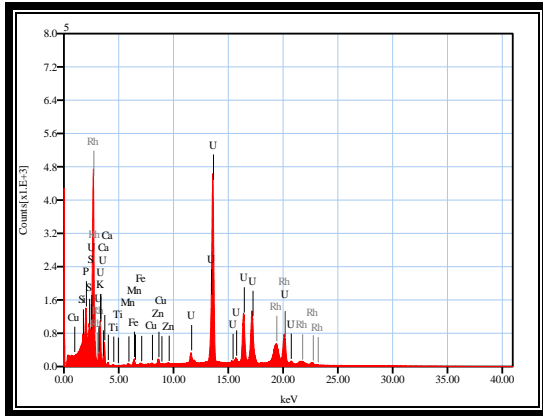


Fig (8): XRF for alginate *C. vulgaris* with U

Immobilization of microorganisms either in alginate or spongy exerts a significant stress which enhances the interaction between adsorbent and uranium moreover multi-minute channels in immobilization matrix forces the formation of different multi legend to combine with uranium (Bashan, 1986). The rate of intensive growth of immobilized form may be related to the reduction in competition process for nutrients (Faafeng et al. 1994). Photosynthesis and respiration process were also enhanced by the immobilized form rather than the free cells and the senescence was significantly delayed (Singh, 2003).

Infrared Spectroscopy for Sorption of uranium by *C. vulgaris*

The appearance of functional groups in the cell wall of the adsorbent may be realized a good answer for the ability of algal cells to the adsorption processes of uranium either by free living or immobilized cells. FT-IR spectra of *C. vulgaris* cells in Figs (9 to 12) show that different peaks which corresponding to the functional groups and confirm the properties of cell wall of *C. vulgaris*.

The combination between different groups on the algal biomass resulted in the heterogeneity characteristic peaks of the adsorption pattern. The different peaks of IR imply amino, carboxylic, hydroxyl, and carbonyl groups are characteristically present. In this regard, adsorbent forms (free and immobilized) have intense peaks at a frequency level of at 3347 cm^{-1} is due to O-H stretching vibration (Jing et al. 2016). The band at 2923 cm^{-1} is formed as a result of the combination of C-H asymmetric stretching vibration of methyl and methylene groups in cellulose (Kadous et al. 2009), whereas that at 2111 cm^{-1} is produced due to overtone or combination blend of amino acids in case of adsorption of uranium by free alga.

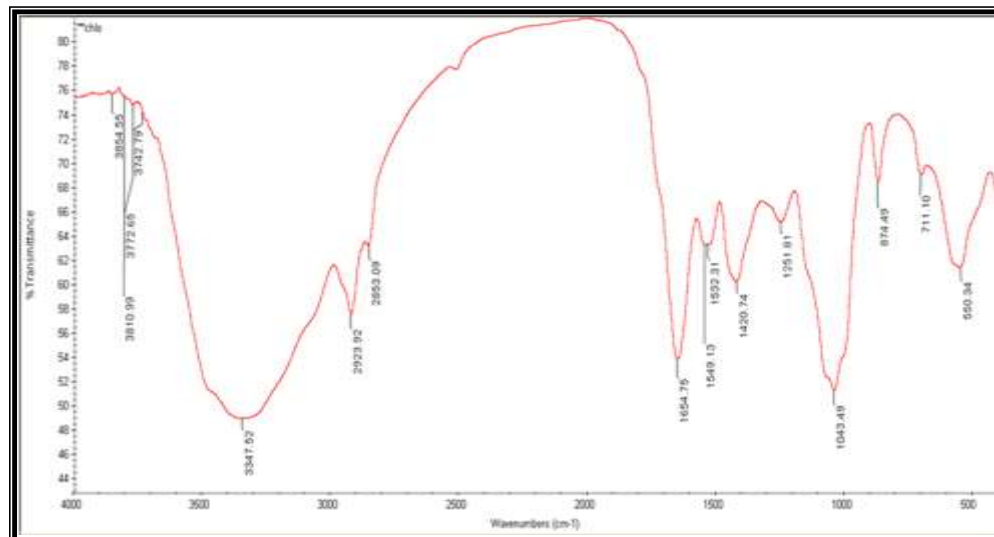


Fig (9) : The FT-IR spectra of *Chlorella vulgaris* (control)

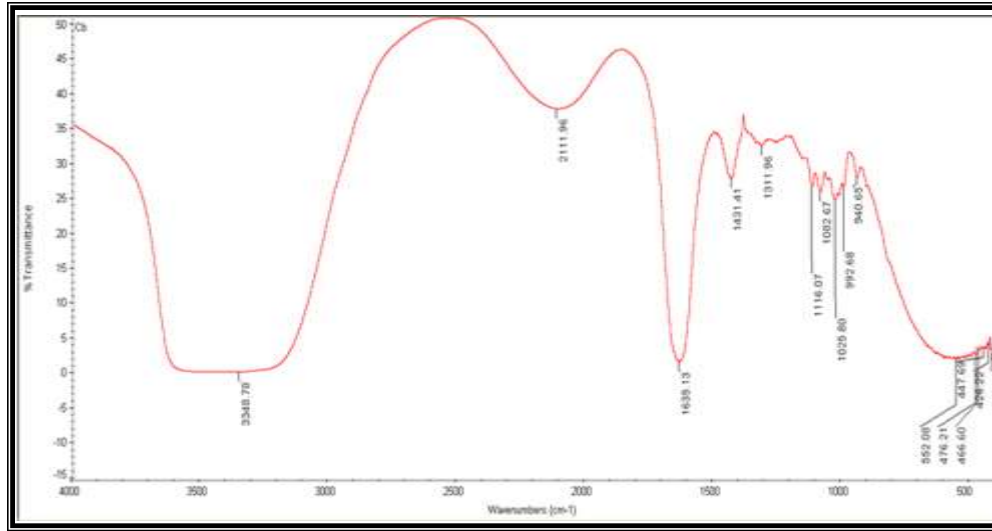


Fig (10): The FT-IR spectra of *C.vulgaris* (free) after U

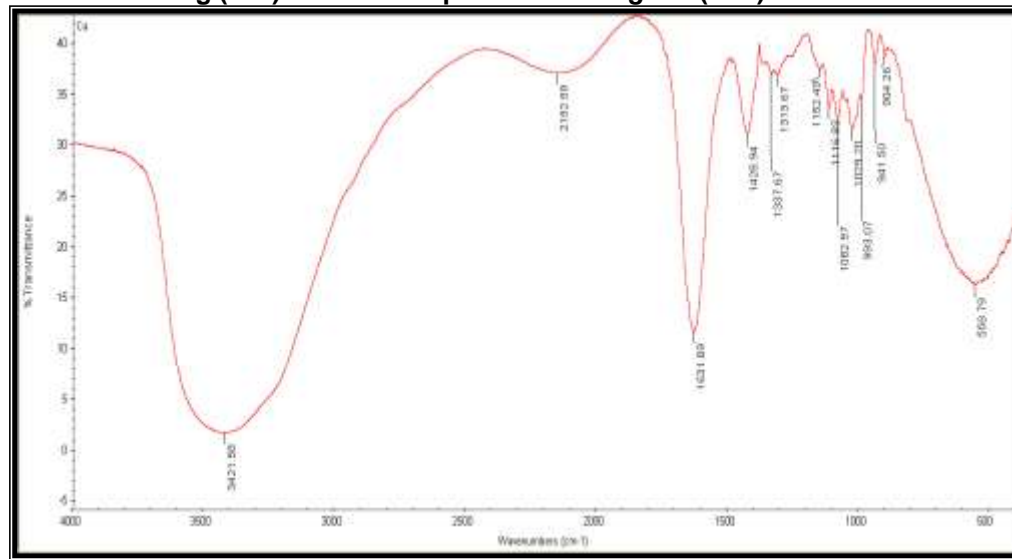


Fig (11): The FT-IR spectra of *C.vulgaris* (spongy) after U

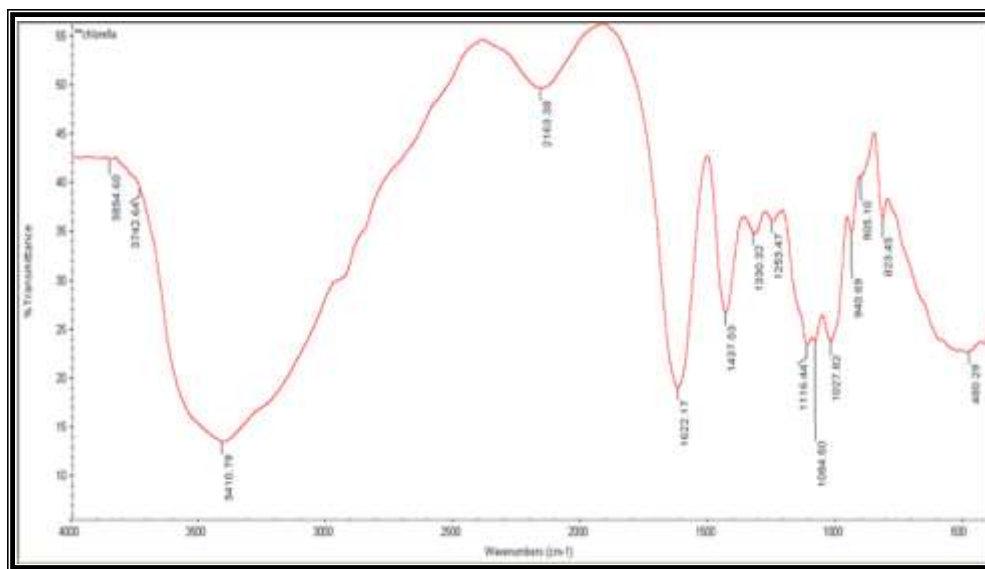


Fig (12): The FT-IR spectra of *C. vulgaris* (alginate) after U

Moreover, the peak located at 1635 cm^{-1} indicates the stretching vibration of carbonyl groups from carboxylates and ketones either the absorbent is free or immobilized (Belkhouche et al. 2006). The peak at 1433 cm^{-1} is due to C-H bending vibration but at 1026 cm^{-1} is attributed to C-O-C and asymmetric stretching vibrations of phosphate and silicate groups in case of all algal forms (Seanen, 2014). The peak of control is at 1043 cm^{-1} and represents C-O groups which were shifted to 1084 cm^{-1} in case of immobilized forms. The peak at 1240 cm^{-1} represents P-O groups; 1400 cm^{-1} represents O=C-O stretching of carboxylate groups; 1300 , 1430 and represents C-H groups; 1540 and 1650 cm^{-1} represents N-H stretches of amino groups in case of control and 3347 cm^{-1} representing O-H stretching of hydroxyl groups. In case of different forms of samples.

The strong peaks at approximately 3400 cm^{-1} are shifted and caused by the bending of N-H on the cell wall structure of immobilized forms of alga. The other peak at 2800 cm^{-1} , representing C-H stretching vibration, is caused by the several functional groups present on control algal cell wall. The peaks of O=C-O stretching vibrations at 1400 cm^{-1} and the peak at 1076 cm^{-1} representing C-O bending vibrations are observed in the algae species, too. After the uranium absorption, the following were detected from the spectrum changes of the functional groups on the biomass: (1) an increase of the peaks at the region 3400 cm^{-1} ; and (2) an increase of the hydroxyl groups

on the biomass (Lin et al. 2005). This could be attributable to hydroxylation on the cell wall of some polysaccharides to shorter saccharides, such as oligosaccharides, under acidic conditions (Deng and Ting 2005), decreasing the peak at 1400 cm^{-1} of a carboxylate functional group, and switching the peak at 1076 cm^{-1} because of the complication of the C-O bond of polysaccharides in U biosorption (Benning et al. 2004).

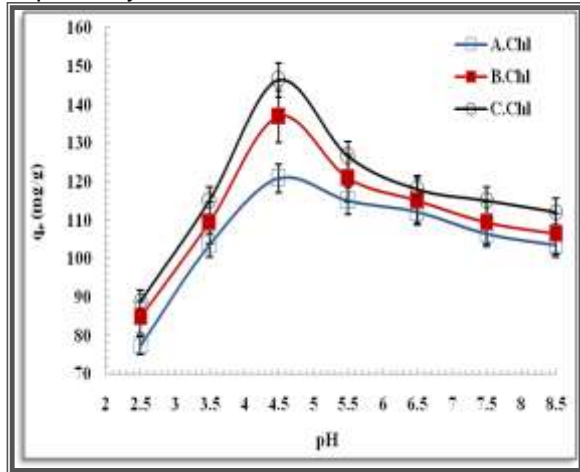
On the other hand, there is shifting of the peak 3343 in case of treated immobilized form to 3421 cm^{-1} and 3410 cm^{-1} indicating that the cell wall having multi-shifting C-H aldehydic group of some simple that sugar binding with uranium (Villaescusa et al. 2004). In a similar manner, the appearance of strong peak located at 2111 cm^{-1} to 2163 cm^{-1} indicated that C-H aldehydic groups are functionally to be a legend to unit with uranium and form the complex ion.

Effect of different pH values

Such experiment was conducted to obtain the optimum pH for absorption of uranium as well as the maximum biomass required for the maximum uptake.

Results in Fig (13) revealed that the immobilization process of micro-green alga *C. vulgaris* with (alginate) was found to be the best absorbent for uranium uptake. This is due to the rate of absorption by alginate (C) is greater than the other different processes under investigation spongy/ composite (B) and free (A). Also, it could be observed the maximum percent of removal of uranium was indicated in general at pH4.5 where

as the maximum removals attended as following : alginate 82 % > spongy 76.7% > free 67.68% respectively.



i.e. (Chl.) it means *Chlorella*

Fig (13) : Effect of pH on the biosorption of U by C. vulgaris at different forms

These results were dimensioned and decreased to the lower values at the alkaline pH. This meaning that the absorption process was mainly pH dependent either the algal cells in the form of immobilization or in the free living. The results explained that the pH plays a role in the nature of uranium through it able to adhere with the relatively static groups of the biosorbents and influences the speciation of uranium in aqueous solutions as well as the ionization of surface of functional groups as well as the binding sites on the biomass surface (Ghorbani et al. 2008; Gok and Aytas, 2009).

Effect of initial uranium Concentrations

Results in Fig (14) showed that the adsorption efficiencies of the different samples.

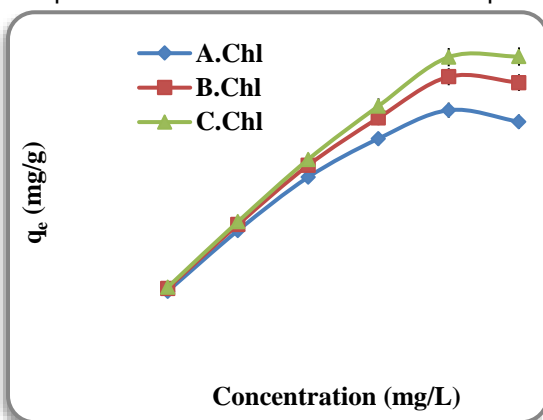


Fig (14) : Effect of concentrations for U adsorption by C. vulgaris forms

It showed an increase in their efficiencies as the concentrations increase up to reach the maximum at 125mg/l for all the samples. The suitable forms that induced the maximum adsorption capacity 146.43 mg/g with 82% removal efficiency was the alginate forms (C) that more than the alga in spongy/composite form (B) 76.7% and 67.68% free (A). The results explained that the biomass provides a greater number of active sites for exchange or sorption of metal ions on the mineral surface (Sharma and Tomar 2008). However, at higher biomass dose there is a partial aggregation of biomass, which results in a decrease in the effective surface area for the absorption. Therefore, 1 g/l was selected as the optimum biomass dose for further experiments. Fig (14) shows the effect of biomass dose on U sorption by C. vulgaris, obtained with pH 4.5, initial concentration of U. The previous results were parallel to (Horikoshi et al. 1981) and (Nakajima et al. 1979) confirmed the usefulness of algae as U adsorbents.

Adsorption isotherms were carried out with different initial concentrations of uranium varying from 25 to 250 mg/l. The experimental data were tested with Langmuir and Freundlich isotherm models.

Langmuir model

The adsorption isotherms obtained at 25°C were plotted according to Langmuir model and the obtained data were illustrated in Fig (15) and Table (1).

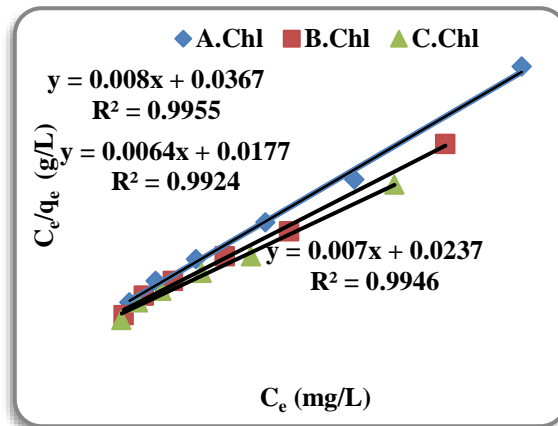


Fig (15): Langmuir isotherm model for U adsorption by C. vulgaris forms

Table (1): Adsorption isotherms for U adsorption by *C. vulgaris* forms

Isotherms model	Parameters	A.Chl	B.Chl	C.Chl	Temp.
Experimental	q_e (mg/g)	120.86	136.97	146.43	298 K
Langmuir	q_L (mg/g)	125	142.857	153.846	
	K_L (L/mg)	0.2162	0.2917	0.3611	
	R^2	0.99	0.99	0.992	
Freundlich	K_F (mg/g)	32.584	48.195	52.48	298 K
	N	2.9326	3.536	3.367	
	R^2	0.939	0.904	0.916	

These models can provide information regarding the metal uptake capacity, the removal efficiency, and the difference in metal uptake capacity under the various conditions. The results indicated that the adsorption of uranium ions onto *C. vulgaris* forms followed Langmuir isotherm $R^2 = 0.99$.

i.e. **A : free** , **B : spongy** and **C : alginate**

The energy for binding of different active sites were the same at all surface and does not depend on the degree of coverage according to (Ghorbani et al. 2008; Talip et al. 2009).

This indicates the applicability of the Langmuir isotherm to describe the adsorption process with the monolayer surface coverage system. Since, Langmuir isotherm means an interpretation hyperbolic adsorption. It is basically describe in a finite number of legends in a single layer, the sorbent has a fixed number of sites. All of the sites have equal binding enthalpies, independent of the extent of coverage. The maximum sorption is a monolayer on the substrate surface.

Freundlich model

The adsorption data of U ions at 25°C were tested according to the Freundlich model in Fig (16) and the values of Freundlich parameters were calculated in Table (1).

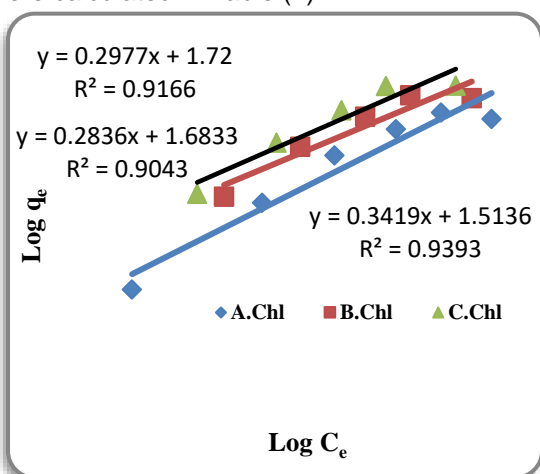


Fig (16) : Freundlich isotherm model for U

adsorption by *C. vulgaris* forms

The observed difference between theoretical adsorption capacities from Freundlich and the experimental one indicates the non-applicability of Freundlich of the studied adsorption process. It assumes that the frequency of sites associated with the free energy of adsorption decreases exponentially with increasing free energy. It suggests that sorption is not restricted to one specific class of the sites and assumes surface heterogeneity. It never predicts saturation of the surface of the sorbent by the sorbate, and the infinite surface coverage is predicted mathematically mode according to (Davis et al. 2003).

Effect of different shaking rates on uranium absorption

Results in Fig (17) revealed that the percentage of removals of uranium by the different samples was regularly increased as the speed of shaking increased. The maximum rate was indicated as the shaking speeds reached 300 rpm especially when the alga integrated with alginate (C) where the percentage of removal reached (82%). As the alga immobilized with spongy (B) (76.7 %) less than the previous one. On the other hand, it could be obvious that the least percentage of removal was reached as the algal sample seeded in the form of free (A) (67.68%) .Moreover the relative percentage of removal shows an obvious decrease as the shaking rate increase up .

The previous increment in case of alginate specially at 300 rpm may be explain on the basis that shaking enhances the collision between the ionic form of uranium and algal cell. Moreover, the collision speed resulted in an assist the protrusion

of many active side groups of algal wall to make complex ions with uranium (Mallick, 2002).

Effect of contact time

In order to understand the kinetics of uranium removal by *C. vulgaris* forms pseudo first-order fig (18) and pseudo second-order kinetic models fig

(19) are tested with the experimental data as in table (2). The goodness of the model may be tested for the value of regression coefficients and the consistency of the calculated value (q_{calc}) with the experimental one.

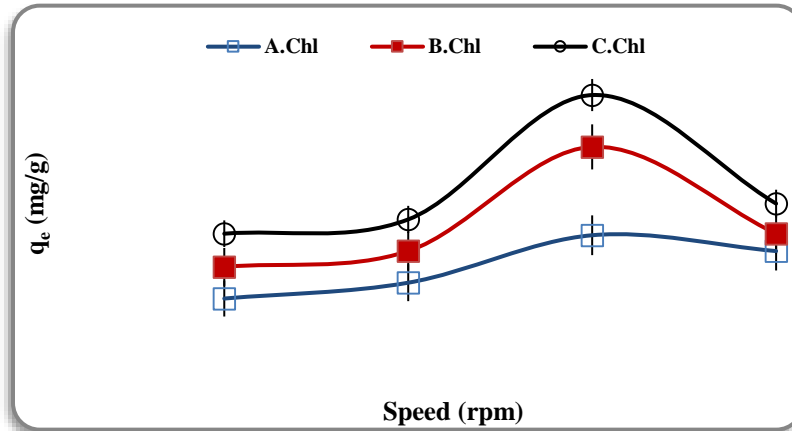


Fig (17) : Effect of Shaking on the biosorption of U adsorption by *C. vulgaris* forms

Table (2) : Kinetic data for U adsorption by *C. vulgaris* forms

Kinetic model	Parameters	A.Chl	B.Chl	C.Chl
Experimental	q_e (mg/g)	120.86	136.97	146.43
	q_{1st} (mg/g)	60.26	76.56	88.92
Pseudo first order	k_1 (min ⁻¹)	0.1497	0.1543	0.1681
	R ²	0.961	0.968	0.907
Pseudo second order	q_{2nd} (mg/g)	128.21	147.06	153.85
	K_2 (g/mg.min.)	0.003	0.00193	0.00086
	R ²	0.9998	0.9995	0.9994

The results indicated that the adsorption of uranium ions onto *C. vulgaris* forms followed the pseudo-second order rather than pseudo-first order kinetics. This implies that the rate of adsorption of uranium ions on the used adsorbents depends on the properties of the both alga and uranium.

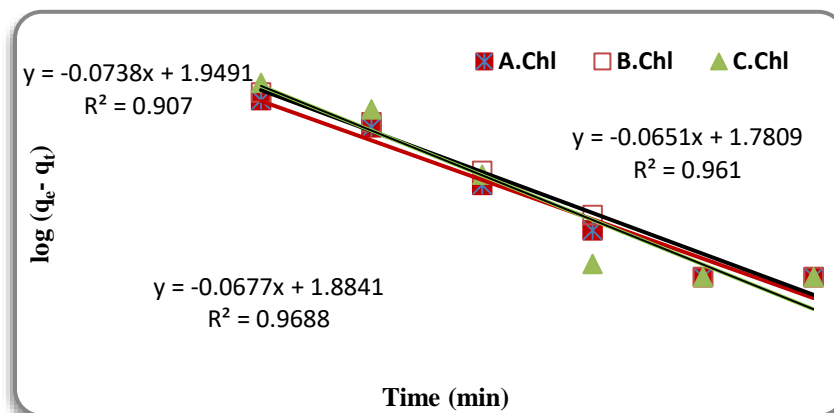


Fig (18) : Pseudo first-order model for U adsorption by *C. vulgaris* forms

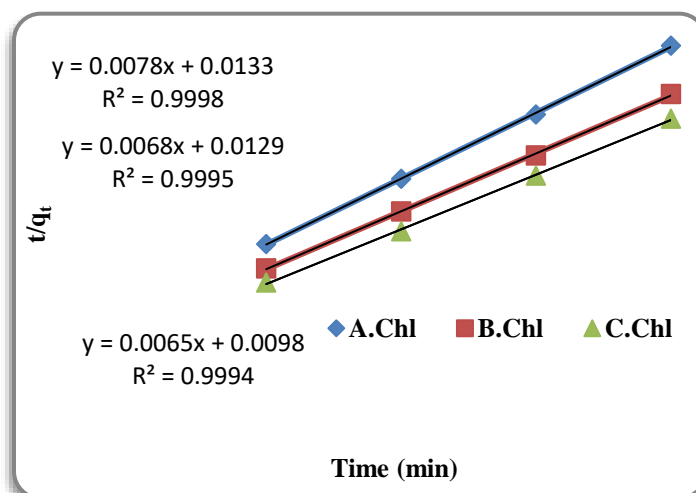


Fig (19) : Pseudo Second-order model for U adsorption by *C. vulgaris* forms

CONCLUSION

Results of this manuscript recommended that biological application for uranium uptake was promising technique and costless as compared with the other techniques. In this regard, *Chlorella vulgaris* is one of the most species that accumulate uranium under the acidic medium with in very short time.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEDGEMENT

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AUTHOR CONTRIBUTIONS

Yassin Mahmoud El Ayouty suggested the point of this manuscript and follow up the results of the different experiments. Ali Hassan Ali calculate the statistical analysis and draw the different figures. Mohamed Abdou Mahmoud Youssef and Zeinab Moussa Ahmed Moussa supply us uranium stock.

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REFERENCES

- Amini, M., Younesi, H., and Bahramifar, N. (2009a). "Biosorption of nickel(II) from aqueous solution by *Aspergillus niger*: Response surface methodology and isotherm study. " *Chemosphere*, 75(11), 1483- 1491.
- Bashan, Y. (1986). Alginate beads as synthetic inoculants carriers for the slow release of bacteria that affect plant growth. *Appl. Environ. Microbiol.* 51, 1089-1098.
- Bashan, Y. (1998). Inoculants of plant growth-promoting bacteria for use in agriculture. *Biotechnol. Adv.* 16, 729-770.
- Bayramoglu, G., Celik, G. K., and Arica, M. Y. (2006) "Studies on accumulation of uranium by fungus *Lentinussajor-caju*." *J. Hazard. Mater.*, 136(2),345-353.
- Belkhouche N-E, Didi MA, Villemin D 2006 *Solvent Extr Ion Exch* 23: 677.
- Benning, L. G., Phoenix, V. R., Yee, N., and Tobin, M. J. (2004) "Molecular

- characterization of cyanobacterial silicification using synchrotron infrared micro-spectroscopy." *Geochim. Cosmochim. Acta*, 68(4), 729-741.
- Bhat, S. V., Melo, J. S., Chaugule, B. B., and D'Souza, S. F. (2008) "Biosorption characteristics of uranium(VI) from aqueous medium onto *Catenellarepens*, a red alga." *J. Hazard. Mater.*, 158(2-3), 628-635.
- Davis, T.A., Volesky, B., and Mucci, A. (2003) "A review of the biochemistry of heavy metal biosorption by brown algae." *Water Res.*, 37(18), 4311-4330.
- Davis, W. and Gray, W. A. (1964) Rapid and specific titrimetric method for the precise determination of uranium using iron II sulphate as reductant. *Talanta*. 11: 1203-1211.
- Deng, S., and Ting, Y. P. (2005) "Characterization of PEI-modified biomass and biosorption of Cu(II), Pb(II) and Ni(II)." *Water Res.*, 39(10), 2167-2177.
- Faafeng, B.A., van Donk, E., Källqvist, S.T. (1994) In situ measurement of algal growth potential in aquatic ecosystems by immobilized algae. *J. Appl. Phycol.* 6, 301-308.
- Gavrilescu, M., Pavel, L. V., and Cretescu, I. (2009) "Characterization and remediation of soils contaminated with uranium." *J. Hazard. Mater.*, 163(2-3), 475-510.
- Ghorbani, F., Younesi, H., Ghasempouri, S. M., Zinatizadeh, A. A., Amini, M., and Daneshi, A. (2008) "Application of response surface methodology for optimization of cadmium biosorption in an aqueous solution by *Saccharomyces cerevisiae*." *Chem. Eng. J.*, 145(2), 267-275.
- Gok, C., and Aytas, S. (2009) "Biosorption of uranium(VI) from aqueous solution using calcium alginate beads." *J. hazard. Mater.*, 168(1), 369-375.
- Horikoshi, T., Nakajima, A., Sakoguchi, T. (1981) Accumulation of uranium by *Chlorella* cells grown under autotrophic heterotrophic and mixotrophic culture conditions. *Agricultural and Biological Chemistry* 45, 781-783.
- Jing Yu, Jianlong Wang and Yizhou Jiang. (2016) Removal of Uranium from Aqueous Solution by Alginate Beads. *Nuclear Engineering and Technology* 49, 534-540.
- Kadous A, Meddour- Boukhobza L, Didi MA, Villemin D (2009) *Sci Study Res* 10: 213.
- Khani, M. H., Keshtkar, A. R., Ghannadi. M., and Pahlavanzadeh. H. (2008) "Equilibrium, kinetic and thermodynamic study of the biosorption of uranium onto *Cystoseria indica* algae." *J. Hazard. Mater.*, 150(3), 612-618.
- Laul J C (1992) Natural radionuclides in ground water. *Journal of Radioanalytical and Nuclear chemistry* 156(1): 235-241.
- Lebeau, T., Robert, J.M. (2006) Biotechnology of immobilized micro-algae: a culture technique for the future? In : Rao, S. (Ed.), *Algal cultures, Analogues of Blooms and Applications*. Science publishers, Enfield, NH, PP. 801-837.
- Lin, Z., Wu, J., Xue, R., and Yang, Y. (2005) "Spectroscopic characterization of Au biosorption by waste biomass of *Saccharomyces cerevisiae*." *Spectrochim. Acta Pt. A: Mol. Biomol. Spectro.*, 61(4), 761-765.
- Mallick, N., Rai, L.C. (2002) Removal of inorganic ions from wastewaters by immobilized microalgae. *World J. Microb. Biotechnol.* 10, 439- 443.
- Moreno- Garrido, I., Blasco, J, Gonzalez – Del Valle, M., Lubian, L. M. (2008) Differences in copper accumulation by the marine microalgae *Nannochloropsis gaditana* submitted to two different thermal treatments. *Ecotox. Environ. Restor.* 1, 43-47.
- Nakajima, A., Horikoshi, T. (1979) Selective accumulation of heavy metals by microorganisms. *Applied Microbiology and Biotechnology* 24, 39- 64.
- Parab, H., Joshi, S., Shenoy, N., Verma, R., Lali, A., and Sudersanan, M. (2005) "Uranium removal from aqueous solution by coir pith: Equilibrium and kinetic studies." *Bioresour. Technol.*, 96(11), 1241-1248.
- Saenen E, Horemans N, Vandenhove H, Biermans G, Van Hees M, Wannijn J, Vangronsveld J, Cuypers A. (2014) The pH strongly influences the uranium-induced effects on the photosynthetic apparatus of *Arabidopsis thaliana* plants. *Plant Physiol Biochem.*
- Sharma, P., and Tomar, R. (2008) "Synthesis and application of an analogue of mesolite for the removal of uranium(VI), thorium(IV), and europium(III) from aqueous waste." *Microporous Mesoporous Mater.*, 116(1-3), 641-652.
- Singh, Y. (2003) Photosynthetic activity, lipid and hydrocarbon production by alginate-immobilized cells of *Botryococcus* in relation to growth phase. *J. Microbiol. Biotechnol.* 13, 687- 691.

- Stanier RY, Kunisawa R, Mandel M & Cohen-Bazire G (1971) Purification and properties of unicellular blue-green algae (Order Chroococcales). *Bacteriol. Rev.* 35 : 171-205.
- Talip, Z., Eral, M., and Hicsnmez, U. (2009) "Adsorption of thorium from aqueous solutions by perlite. "*J. Environ. Radioact.*, 100(2), 139-143.
- Tam, N. F. Y., Wong, Y. S., and Wong, M. H. (2009) " Novel technology in pollutant removal at source and bioremediation. " *Ocean Coast.Manage.*, 52(7), 368-373.
- Villaescusa, I., Fiol, Martinez, M., Miralles, N., Poch, J., and Serarols, J. (2004) "Removal of copper and nickel ions from aqueous solutions by grape stalks wastes. "*Water Res.*, 38(4), 992- 1002.
- Wang, J., and Chen, C. (2009) Biosorbents for heavy metals removal and their future.*Biotechnol. Adv.*, 27(2), 195-226.