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Immobilize Algae To Removal Copper And Lead From Aquatic Ecosystem

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Abstract

Laboratory experiments were performed to study Cu^{+2} and Pb^{+2} uptake by the unicellular green microalga Chlorella vulgaris immobilized in calcium alginate beads. was carried out by using sodium alginate and calcium chloride bound with calcium alginate to form the beads of the organism. In all immobilization samples, regular solutions of lead Immobilization and copper at concentrations of (10, 20, 30, 40 and 50) ppm were used. The heavy metal concentrations were measured using the atomic absorption device during the analysis. Also, Scanning electron microscope (SEM) was used to examine immobilization of algae on calcium alginate. In order to remove trace elements from the aqueous solution, the immobilization of algae will typically lead to an increase in biomass collection of trace elements and eventually increase the removal efficiency of Chlorella vulgaris.

Key words: calcium alginate beads ; algae ; Chlorella vulgaris; immobilized; Cu^{+2} and Pb^{+2} ; SEM .

Introduction

In various works, the microalgae Chlorella vulgaris has been used to generate full cell biosensors to track toxic contaminants in aquatic media.(Pandard P et al. 1993;Durrieu C and Tran-Minh C , 2002). Because of its major ecological benefits, this single-cell green algae was selected (it is ubiquist in all dulcicol environments and is able to accumulate large quantities of pollutants). Pandard et al. (1993) created the first biosensor using whole algal cells.. Toxic effects and accumulation tendencies throughout the food chain, heavy metals represent a serious problem to human health (Maneand Bhosle 2011). The immobilization of microalgae is thus becoming a global trend used for a wide range of biotechnological applications. Ca-alginate microalgae immobilization is used in wastewater treatment by removing nutrients, phosphorus, phosphate, organic carbon, pharmaceutical compounds, hazardous textile pigment compounds and heavy metals. (Wang et al. 2016; EL-Sheekh et al. 2016; Moreno Garrido 2008; Salam et al. 2017). Immobilization of microorganisms is one of the general applications of bacteria and algae in the treatment of dangerous pollutants. (Munoz and Guieysse 2006). The resilience of immobilized cells is distinguished by the fact that the cells are contained in the matrix and are shielded from harmful compounds in waste water; thus, they are more effective in extracting nutrients. (Hernandez et al. 2006; EL-Sheekh et al. 2016), Dyeing (Revathi et al. 2017), and heavy metals (EL-Sheekh and Mahmoud 2017) Compared to free cells. In the bioremediation of contaminants in waste water, immobilized cells have many advantages: (1) immobilized cells provide stability for the operational stability of the performance of microorganisms; (2) a limited amount of high population density can be trapped; (3) they provide protection for microorganisms against the toxicity of pollutants in waste water; (4) minimization by diffusion constraints of inhibition and toxicity to microbial cells; (5) immobilized cells can be reused for successive applications and this decreases costs because they can remain viable for long periods of time; (6) they can be processed for long periods without loss of their bioremediation activities. (Quek et al. 2006; Bikram et al. 2014).

Materials and Methods

Algal strain

Chlorella vulgaris (green algae) was collected from the Center for Environmental Research and Analysis, University of Babylon, Iraq. Microscopic observation has established Chlorella vulgaris (Maulood et al., 2013) Incubated under controlled conditions of 286 LE/m2/s light strength, 16:8 hours light/dark duration and 25+2°C temperature (Chia et al. , 2013).

Algae Immobilization

Algae Cultivation

Chlorella vulgaris was taken from the Department of Ecology Laboratory /Biology /College of Sciences/Babylon University and cultivated in the culture medium of CHU 10 (Chu, 1942) and updated by Kassim (1998). The medium was prepared by Stock Solutions. After that, 2.5 ml was taken and the size of 1 liter was completed and pH 7 was changed, either by adding 0.0IN hydrochloric acid or sodium hydroxide to the same normality. Then sterilized by autoclave and left and used for the next day for cultivation. Algae isolates were cultivated in a 100 ml culture media as a batch culture method and 10 ml of pure cultures (Fig. 1) were incubated at 25 ± 2 in Plant Cabinet and 50 um / m2 / s with light intensity and 16: 8 hours light: dark for 7 days and finally completed from culture media to 1000 ml(Fig. 2). Algae growth was tracked by measuring the optical density at a wavelength of 540 nm using a spectrophotometer.

Beads formation

In the stationary process, 3000 ml were taken and condensed from the studied organism culture by filtering Millipore filter paper 0.45um (Fig.3), then the concentrated species were combined with an equal volume of 2% soluble sodium alginate solution and shaken well to homogenize these ingredients and placed the mixture in a medical syringe. At this time, In a separate beaker, calcium chloride solution (3%) was prepared and the contents of the syringe were gradually lowered into the calcium chloride solution. A drop of solution is solidified and immobilized in the form of beads in the beaker (organisms and sodium alginate) and left for 5-10min (each of 4 mm in diameter) Singh et al. (2012).The tea strainer then removes the beads (immobilized organisms) from the calcium chloride solution and gently washes them with tap water and thoroughly rinses them with distilled water (Fig.4) (Adlercreutz and Mattiasson, 1982).

Heavy Metals Treatment

Measurement by Atomic Absorption:

In order to assess the removal efficiency of immobilized organisms with sodium alginate in their treatment, lead and copper were selected. For use in an experiment with 5 beads, five normal concentrations of each metal (10,20,30,40 and 50) mg/l and 10 ml of each concentration. The sample of heavy metals was inserted in the tube and left for 15 minutes for each metal concentration. The sample was then collected and measured by atomic absorption. Removal Efficiency (R.E.) was calculated as below:

$$R.E\% = \frac{C1-C2}{C1} * 100$$

Were:

R.E%: Removal efficiency, C l : Heavy metal concentration before treatment,C2: Heavy metal concentration after treatment.

Fig. 1 100ml culture media and 10 ml

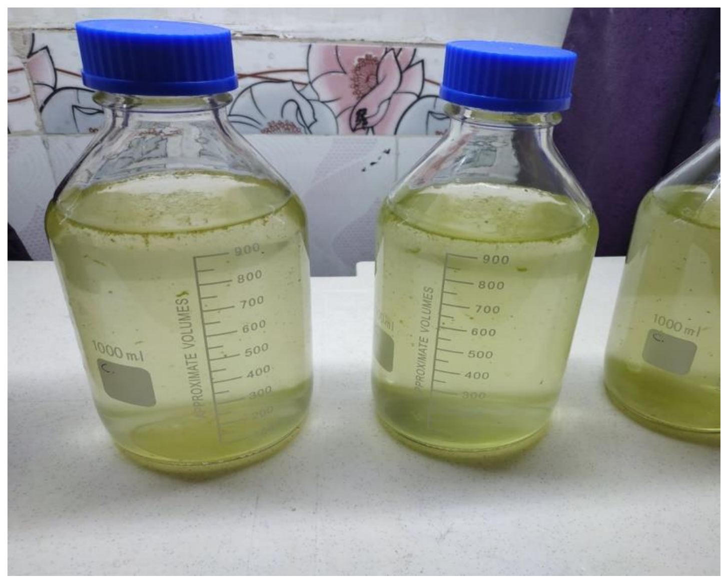


Fig. 2 1000ml from culture media of pure cultures

Fig.3 Millipore filter paper 0.45um

Fig.4 Beads formation

Scanning Electron Microscopy

Scanning electron microscope (SEM) was used to examine immobilization of algae on calcium alginate (Hawser and Douglas 1994). Surface binding between immobilized algae and heavy metals was examined by SEM that carried out at the University of Technology- Iraq.

Results and Discussion

Chlorella vulgaris is commonly used to test trace element adsorption. (Aksu and Dönmez, 2006; Ruangsomboon and Wongrat, 2006). For the most part, it is accounted (Ting et al., 1989) It is possible to divide the take-up of element particles into 2 phases: rapid and moderate phase. Particles of elements are adsorbed on the outside of the microorganism in the rapid process. While the element particles pass over the microorganisms into the interior cells in the moderate phase.

Through the results of the present study, immobilized algae (C.vulgaris) were used to show their ability to treat copper and lead concentrations by using Standards concentrations (10, 20, 30,40 & 30) ppm. The copper concentrations after treatment with these immobilized algae were (9.2, 18.2, 16.39, 31.39 and 45.8) ppm. respectively , with a removing ratio (8, 9, 45.3, 21.5 and 8.4)% for each concentration, respectively. As well as lead concentrations after treatment with these immobilized algae were (8, 15.25, 28.1, 38 and 48.6) ppm. respectively, with a removing ratio (20, 23.75, 6.3, 5, and 2.8)% for each concentration, respectively. These results mean the immobilization of algae will in general lead to increment trace elements gathering by biomass and finally increase the removal efficiency of Chlorella vulgaris to remove the trace elements from aqueous solution this results agree with (Darnall et al., 1986; Aksu et al., 1998). Also immobilization increases the rate of biodegradation of pollutants through increasing cell loading and this also improve the catalytic stability as well as the tolerance against toxic pollutants (Baskaran and Nemati 2006; Wang et al. 2007). The degradation rates by immobilized cells on Ca-alginate and chitosan were higher than those of free cells (Wu and Wan 2009).

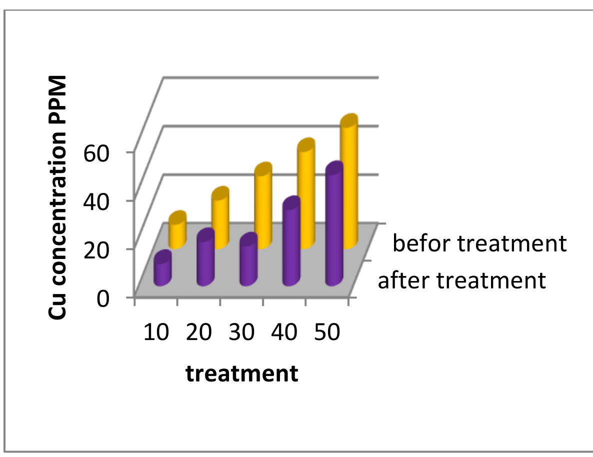
Scanning electron microscope (SEM) was applied in this study to show the immobilization of investigated microbial cells on Ca-alginate matrix. The results showed heavy aggregations and affinity of algal cells to be immobilized on Ca-alginate. Also immobilized matrices (Ca-alginate) acts as cell protector against toxic sewage wastewater by reducing the number of attack sites and by decreasing the flow rate toward the entrapped cells, so the biodegrading capacity using immobilized microorganism was stable than using free microorganism. These results agree with Ettayebi et al. (2003). The Scanning Electron Microscope Fig.9 showed the before and after effects of treatment procedure on immobilized algal cells. So as to check whether the layer was as yet adsorbed of copper and lead on the surface of beads and concentrations were penetrate inside beads. The time used is 15 minutes for each concentration, Before examination, the beads should be dried by hot air

Table (1). Concentration of Cu^{++} before & after treatment and removal efficiency of Chlorella vulgaris

Treatment Cu^{++}					
Before	10 ppm	20 ppm	30 ppm	40 ppm	50 ppm
After	9.2 ppm	18.2 ppm	16.39 ppm	31.39 ppm	45.8 ppm
Removal Efficiency%	8	9	45.3	21.5	8.4

Table (2). Concentration of Pb^{++} before & after treatment and removal efficiency of Chlorella vulgaris

Treatment Pb^{++}					
Before	10 ppm	20 ppm	30 ppm	40 ppm	50 ppm
After	8 ppm	15.25 ppm	28.1 ppm	38 ppm	48.6 ppm
Removal Efficiency%	20	23.75	6.3	5	2.8



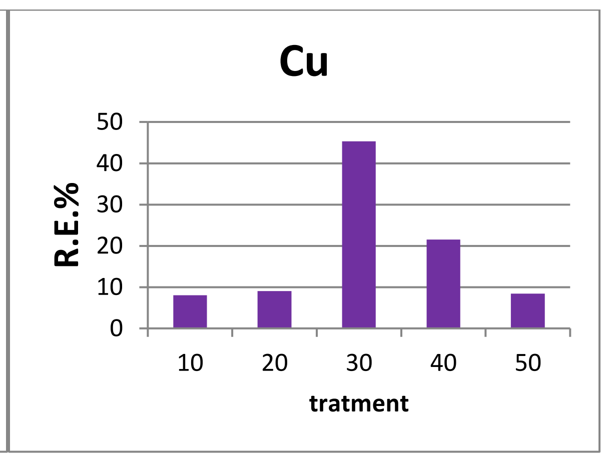
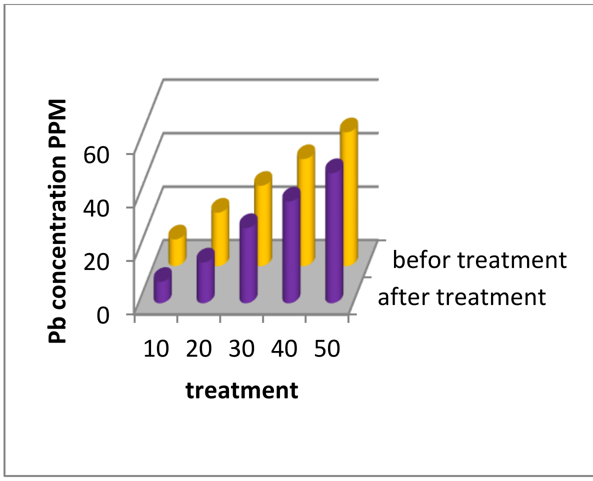


Fig.5 Cu^{++} concentration before and after treatment by C. vulgaris

Fig.6 Removal Efficiency of immobilized C. vulgaris for copper



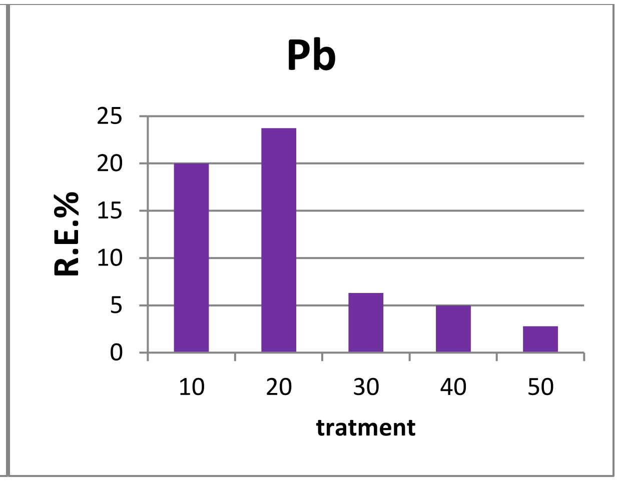
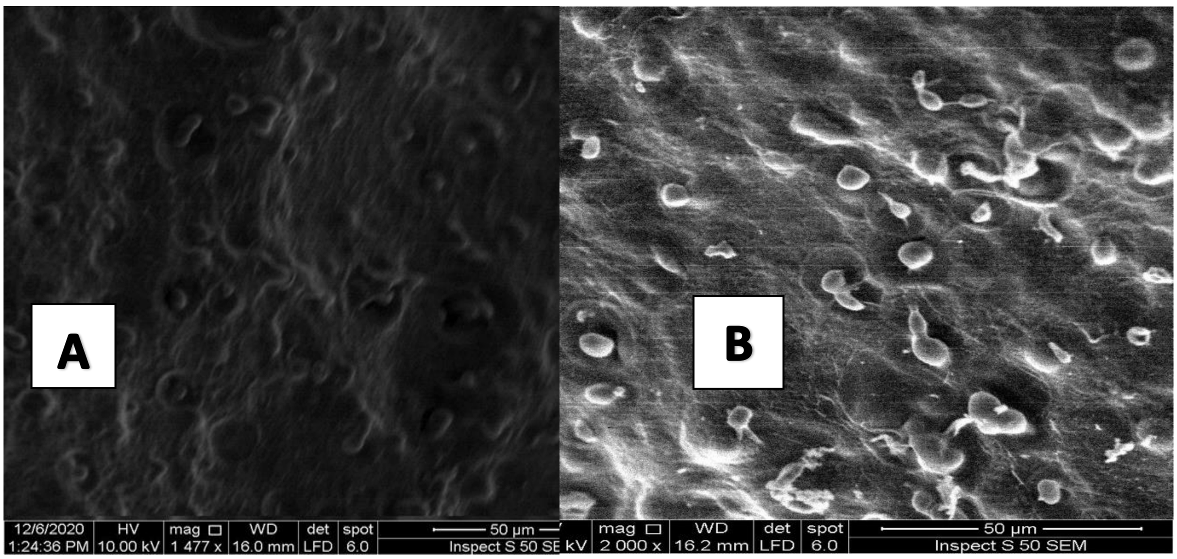
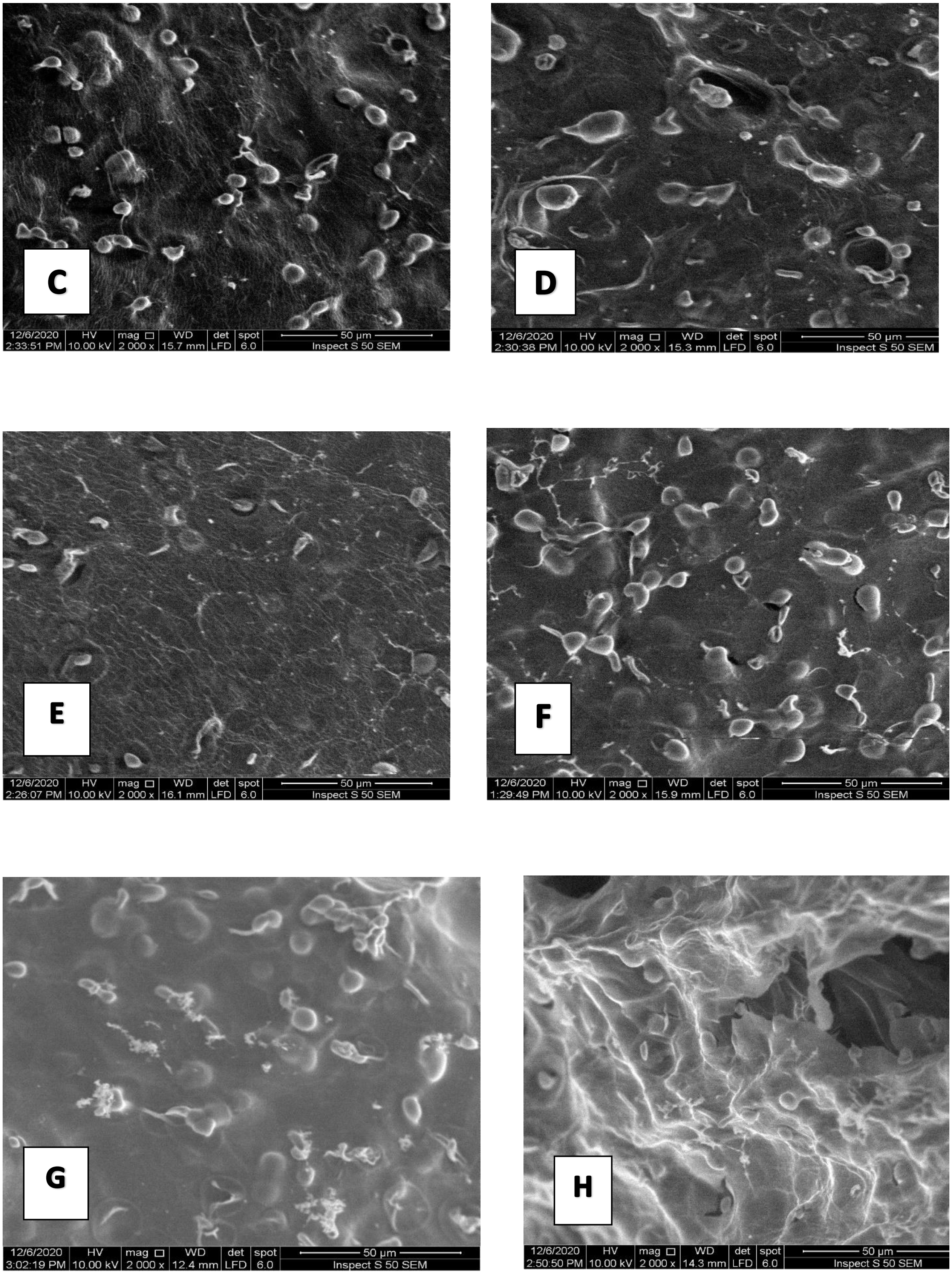


Fig.7 Pb^{++} concentration before and after treatment C. vulgaris

Fig.8 Removal Efficiency of immobilized C. vulgaris for lead by

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12/6/2020 3:02:19 PM | HV 10.00 kV | mag 2 000 x | WD 12.4 mm | det LFD | spot 6.0 | 50 µm | Inspect S 50 SEM
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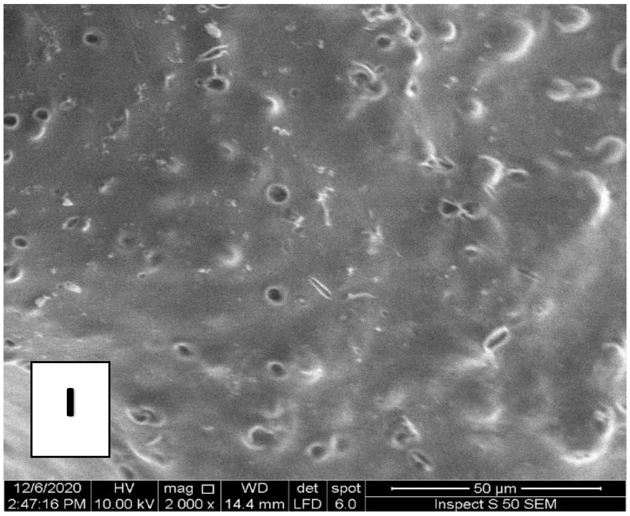
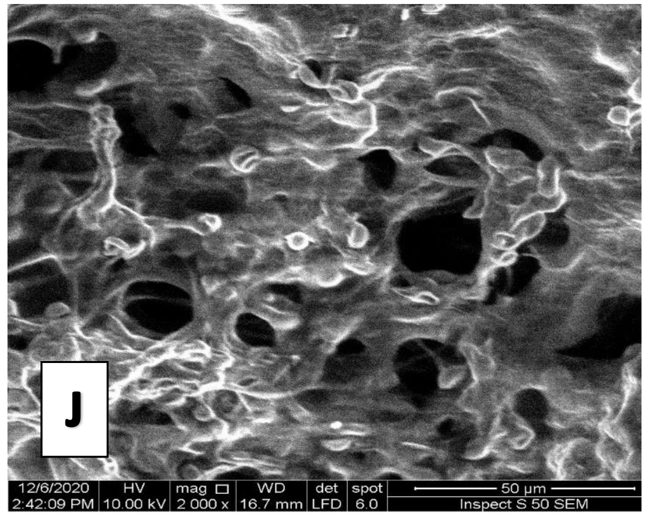
12/6/2020 2:50:50 PM | HV 10.00 kV | mag 2 000 x | WD 14.3 mm | det LFD | spot 6.0 | 50 µm | Inspect S 50 SEM





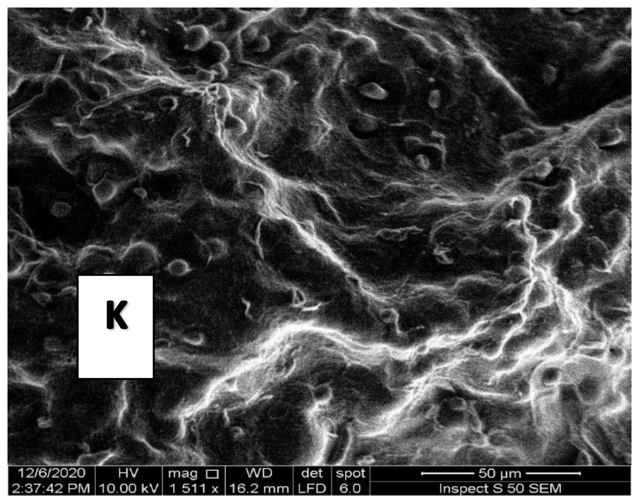


Fig.9 Scanning electron microscope of immobilized Chlorella vulgaris cells on Ca-alginate without treatment (A), Cu10ppm(B),Cu20ppm(C), Cu30ppm(D), Cu40ppm(E) ,Cu50ppm(F).

Pb10ppm(G), Pb20ppm(H),Pb30ppm(I),Pb40ppm(J) ,Pb50ppm(K).

Fig.9 (A) which showed the SEM without any treatment for algal beads. This photo shows the raw material of Calcium Alginate without adsorbed copper & lead and shows the porosity of beads that imply the ability of beads to adsorb and penetrate copper & lead into inside beads to enhance the efficiency of removal. Algal biomass has a fundamental role in the purification of waste water and thus in the service of water supplies. (Knauer et al . , 1998; Corder and Reeves , 1994) . The sterilization features of impure aqueous systems can be improved by immobilized algae. (Tam et al ., 1998) .

Results of this study show that the concentrations of copper after treatment by immobilized C. vulgaris were 9.2ppm, 18.2ppm, 16.39ppm, 31.39ppm and finally 45.8ppm when the initial concentrations before treatments were (10, 20, 30, 40&50) ppm respectively. Removal Efficiency was 8%,9%,45.3%,21.5%and8.4%for respectively(table1). Also, the concentrations of lead after treatment by immobilized C. vulgaris were 8ppm, 15.25ppm, 28.1ppm, 38ppm and finally 48.6ppm when the initial concentrations before treatments were (10,20, 30, 40&50) ppm respectively. Removal Efficiency was 20%, 23.75%,6.3%, 5%and 2.8% for respectively(table 2). As a result, Chlorella vulgaris has been able to adsorb and remediate the copper & lead components and reduce its concentration in aqueous solution containing copper & lead because Chlorella vulgaris has bound trace elements in particular and non-specific positions. (Mehta and Gaur, 2001), In this way, it should be sensibly known that the immobilized algae have a larger number of negatively charged locations/identity species for bioadsorption elements (Knauer et al., 1997). Bioadsorption includes ionic charge and covalent bonds and the wall of cell components is also considered to have a vital role in bioadsorption (Mahan et al., 1989). These findings are the same as those of Hamdy (2000) and Chong et al., (2000). There are several possible binding sites in the cell membrane and alginate types of entire species of algae that have found significant quantities of trace elements such as copper & lead for amassing. (Saitoh et al., 2001).

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