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Biosorption of Cadmium, Lead and Nickel in their Aqueous Solution by *Nitzschia palea* and *Navicula incerta*

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Abstract: Powder of dried of *N. palea* and *N. incerta* were mixed together in equal ratio and used as different grams 0.2, 0.4, 0.6, 0.8 and 1 g were added to the selected heavy metals Ni, Cd and Pb in concentrations 0.5, 1, 2 ppm which recorded after 1, 4 and 6 h to determined the contact time of high percentage removal. The results showed the optimum removal percentage for cadmium was in pH 8 with room temperature 25±2°C. High decreased in cadmium ions with a range between 0.003-0.04 mg/L for the concentration 0.5 ppm with percentage removal reached 90.7-99.4%. Biosorption of lead indicated high removing in the concentrations 2 ppm with percentage removal 91.5-96.6%. As well as for nickel, the data showed the high decrease in the concentration 2 ppm with percentage removal reached 93-96.5%. FTIR technology was depended for detecting the active groups on cells surfaces for the two diatoms which responsible for biosorption of lead, nickel and cadmium.

Key words: Powder, dry diatoms, heavy metals, biosorption, immobilize calcium alginate, FTIR

INTRODUCTION

One of the most interesting pollution is polluted with heavy metals. The term heavy metal is widely used and refers to metals with an atomic density which reached >5 g/cm^3. Sometimes the term toxic heavy metals are used to indicate on the impact of these elements on the environment specifically on their effect on the biota. When heavy metals show toxic effects on living organisms, they are termed toxic heavy metals. Some of them such as nickel , copper and zinc are at very limiting concentrations, essential for life (also termed trace elements or microelements) because they act an important role in the metabolic processes which are taking place in living cells (Haferburg and Kothe, 2007). The search for new technologies to remove hazardous metals from wastewaters has focused attention on the metal binding abilities of different biological materials instead of the existing conventional physicochemical methods (Pavasant *et al*., 2006; Wenzel, 2009). Among the biological material, microalgae have proved to be advantageous because they present several advantages, i.e., economic regeneration, metal recovery potentiality, lesser volume of chemical and/or biological sludge to be disposed off, high efficiency in dilute effluents and large surface area to volume ratio (Echeveste *et al*., 2012; Dao and Beardall, 2016). Toxicity of heavy metals like Pb, Cd, Ni, Cu, Hg, Zn, etc. which enter the natural water bodies in various ways, to both freshwater and marine flora and fauna has long been recognized. Microalgae are very sensitive to different changes in their surroundings. Diatoms have high response to the trace levels of various organic and inorganic pollutants concentration including exposure to heavy metals in which changes that take place in their metabolism. One strategy to reduce heavy metal solution is to use microorganisms. Microalgae, due to their ubiquitous occurrence in nature have been studied extensively in this regard. They can sequester heavy metal ions by adsorption and absorption as do by other microorganisms. This ability may be induced in response to stress by toxic heavy metal exposure (Rai *et al*., 1981). The use of microalgae or diatoms for removing heavy metal has a greater performance at a lower cost than other conventional of water treatment. This is consistent with the recent trend for growing interest in biosorbent technology for removal of trace amounts of toxic metals from dilute aqueous waste by diatoms (Chong *et al*., 2000). Biosensors of microalgae havin various algal species as cyanobacteria, chlorophyta and diatoms and the advantages of these biosensors in comparison to the chemical and physical analyses are discussed by Brayner *et al*. (2011). The process of heavy metals biosorption by living immobilized eukaryotic microalgae cells and prokaryotic using various immobilizing compounds or material is an additional option for removal efficiency. Removing heavy metals by

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immobilized cells are more efficient in treatment processes in compared to the free living cells (Malik, 2004) that's could done by using immobilized cells of microalgae biomass is more efficient. The conditions which supporting microalgae in their processes and maintenance their growth was: light, temperature and pH. Removal of the metals from contaminated sites with high concentrations of metals can be achieved using dried (no active) biomass as biosorbents (Loutseti *et al*., 2009). It should be noted that, the microalgae biomass differ largely in their binding sites for various heavy metals (Micheletti *et al*., 2008; Mishra *et al*., 2011). The metal-binding sites of biosorbents (diatoms or algae) depend on the composition of the cell wall of these organisms. Calcium alginate as example in immobilized cells is nontoxic and let different type of microorganisms to grow inside. The transparency of small calcium-alginate beads is enough to permit the growth of immobilized microalgae. Additionally, it is an easy, cheap and feasible technique to be used in research laboratories (Papagregoriou, 1987).

MATERIALS AND METHODS

Metals: Lead A standard solution of 1000 parts per million of lead, nickel and cadmium were prepared by dissolving 1.342 g of $PbCl_2$ in one litter of de-ionized distilled water, 1.8 g of $CdCl_2.H_2O$ was dissolved in one litter of de-ionized distilled water. And for nickel which prepared by dissolving 5.4 g of Ni $(NO_3)_2$ in 1 L of de-ionized distilled water. All above were prepared according to the following equation:

$$C1V1 = C2\ V2$$

Measuring the concentration of heavy metals was by flame atomic absorption spectrophotometer in absorption wavelength 540 nm.

Powder of dried diatoms: The two species of diatoms were dried by Lyophilized methods were mention by Ratti (2008), each dried diatoms were tested in heavy metals and salinity removal in different grams. Using dried diatoms in lyophilized method to produce particles have very high surface area, minimized in contamination. The excellent shape of the product is maintained and quality of the rehydrated products are excellent (Ratti, 2008). The basic conditions used in lyophilized were temperature -40°C and pressure 0.12 millbar. Dry diatoms with particles size (50-45 μm) which measured by (FTIR) and kept in poly ethylene containers in refrigerator for used later (Vecchio, 2010).

Testing the ability of dry diatoms to remove heavy metals ions: The experiment was done in room temperature in 250 mL poly ethylene containers by added different grams of diatoms powder to the 100 mL of metal solution in different pH (5, 7 and 8.5). The containers were putted in vibrating incubator with speed 60 cycles/min for 3 h in T 250°C and then filtered to analyzing by atomic absorption. Calculating the percentage of Ni, Pb and Cd removal by equation of Vieira was mentioned by Uten (1978):

$$\text{Removal (\%)} = A\text{-}B/A*100$$

A = The initial concentration
B = The final concentration

Immobilizing diatoms: Sterile solutions of sodium alginate were prepared as follows: Na-alginate (1.5 g) was dissolved in distilled water (66 mL) by slow stirring for 4-6 h and a solution of NaCl (2 g) in distilled water (1 L) was added. The pH was adjusted to 7.5-8.0 by addition of 0.1 M NaOH. The 50 mM aqueous tris buffer at pH 7.5-8.0 was used instead of water for the dissolution of the alginate. The sterile solution was mixed thoroughly (gentle stirring) with drops of a very concentrated suspension of diatoms (dried). Forcing the mixture through a sterile Pastor pipette into a growth medium fortified with calcium (3/4 f-strength medium with 0.07 M $CaCl_2$) produced beads of calcium alginate with the entrapped algal cells. Bead diameter was regulated in the range of 0.5-2.0 mm. The beads remained in the calcium-enriched medium for at least 30 min. To secure gel hardening and were then transferred to the standard medium for growth. Diatoms were immobilized in calcium-alginate beads following Moreno-Garrido *et al*. (2005). Beads were kept in 250 mL spherical flasks containing 100 mL of f/2 medium with around 50 mL of beads. Samples were taken regularly till day 17 after the beginning of the test and fixed with formalin. Known volumes of beads were dissolved by soft sonication with known volumes of tri-sodium citrate (3% w/v) and cells number was counted (Moreno-Garrido *et al*., 2005).

Fourier Transformation Infrared (FTIR): This technique used for clearing the binding process of heavy metals ions into the active groups in the surfaces cells wall of adsorbents which important in biosorption and translate how the metals could binds by tested in spectrophotometer (Shimaozu FTIR-800). The 100 mL of Pb, Ni, Cd solutions with 20 mg/L were prepared according to the basic solution of each metals (1000 mg/L) and kept in 250 mL plastic containers, added 1 g of dry diatoms for each container of metals and kept in pH 8. The bottles or containers were sealed tightly and placed in vibrating incubator with speed 60 cycle/min for 3 h in 25°C. The samples were filtered by 0.45 micron filter papers finally, dried the diatomous suspended which binding the heavy metals and smash well and analysis by infrared device according to Naja *et al.* (2005).

RESULTS AND DISCUSSION

Powder of dried of *N. palea* and *N. incerta* were mixed together in equal ratio and used as different grams 0.2, 0.4, 0.6, 0.8 and 1 g were added to the selected heavy metals Ni, Cd and Pb in concentrations 0.5, 1, 2 ppm which recorded after 1, 4 and 6 h to determined the contact time of high percentage removal. The results showed the optimum removal percentage for cadmium was in pH 8 with room temperature 25±2°C, adsorption reactions are generally, exothermic and the extent of adsorption increases with decreasing temperature (Table 1). Haluk (2011) was recorded in his study the maximum biosorption capacity for Ni and Pb was obtained at 25°C and found to decrease as the temperature was increased to 40°C. The results showed high decreased in cadmium ions with a range between 0.003-0.04 mg/L for the concentration 0.5 ppm with percentage removal reached 90.7-99.4% in the concentration 1 ppm it's decreasing ranged reached to 0.1-0.06 mg/L with removal percentage reached 87-94% also, the percentage removal for 2 ppm was 90-97% with decreasing reached to 0.1-0.06 mg/L. So, the best contact time occurring in which highest percentage removal 100% was 4 h from (0.2 g) in 0.5 ppm because the biosorbent of heavy metals by dried diatoms would depended on the initial concentration for metal in liquids and removal ratio for sorption could increased by increasing the initial concentrations until saturated all active sites in the biosorbent (Mehta and Gaur, 2005).

When the pH value was increased from pH 3.0-8.0, there was an increase in Cd^{2+} sorption which could be attributed to the increase in electrostatic attraction between positively charged Cd^{2+} ions and negatively charged binding sites of functional groups present on the cell surface such as carboxylate, phosphate and amino groups (Adhiya *et al*., 2002). Solisio *et al.* (2008) was found the sorption efficiency of Cd^{2+} with *S. platensis* was at pH 8.0. However, there was a decrease in the uptake of Cd at alkaline pH 10 which could be attributed to the formation of a metal complex such as Cd-OH which might have competed with functional binding sites for metal ions and reduced the availability of Cd sorption (Rao *et al*., 2005; Kumar *et al*., 2006). Table 2 represents biosorption of lead by dried mix diatoms.

The results indicated to removing it in concentrations 0.5, 1 and 2 ppm with percentage removal 83.6-97, 88.6-91.5 and 91.5-96.6%, respectively and about the amount of residue of the concentrations 0.5, 1 and 2 was 0.08-0.01, 0.1- 0.05 and 0.1-0.06 mg/L, respectively. The best contact time occurring in which the highest percentage removal 100% was 4 h from (0.2 g) in 0.5 ppm. The present results were agreed with the study of Quintelas *et al*. (2007).

Table 1: Biosorbent of Cd ions by dried diatoms

2		**1**		**0.5**		**Cd**	
Percentage removal	Residue concentration (mg/L)	Percentage removal	Residue concentration (mg/L)	Percentage removal	Residue concentration (mg/L)	h	g
88.5	0.230	84	0.160	98.2	0.009	1H	**0.2**
96.5	0.070	92	0.080	100	0.000	4H	
97	0.060	100	0.000	100	0.000	6H	
94	**0.120**	**92**	**0.080**	**99. 4**	**0.003**		
91.5	0.170	85	0.150	90	0.050	1H	
94	0.120	97	0.030	99	0.004	4H	**0.4**
94.5	0.110	100	0.000	100	0.000	6H	
93. 3	**0.133**	**94**	**0.060**	**96. 3**	**0.018**		
94.5	0.110	86	0.140	**74**	0.130	1H	**0.6**
96.5	0.070	96	0.040	**98**	0.010	4H	
100	0.000	100	0.000	**100**	0.000	6H	
97	**0.060**	**94**	**0.060**	**90.7**	**0.046**		
92	0.160	87	0.130	88	0.060	1H	**0.8**
97.5	0.050	87	0.130	99.6	0.002	4H	
100	0.000	87	0.130	100	0.000	6H	
96.5	**0.070**	**87**	**0.130**	**95.9**	**0.020**		
86	0.280	77	0.230	84	0.080	1H	**1**
91.5	0.170	**98**	0.020	96	0.020	4H	
93.5	0.130	**100**	0.000	100	0.000	6H	
90.3	**0.193**	**91.67**	**0.083**	**93. 3**	**0.0333**		

Bold values are significance

Table 2: Biosorption of Pb ions by both dried *N. incerta* and *N. palea*

2		1		0.5		Lead	
Percentage removal	Residue concentration (mg/L)	Percentage removal	Residue concentration (mg/L)	Percentage removal	Residue concentration (mg/L)	h	g
94	0.12	90	0.1	92	0.04	1H	0.2
96	0.08	96	0.04	100	0.001	4H	
97	0.06	98	0.02	100	0	6H	
95.6667	0.08667	94.6667	0.05333	97.333	0.01367		
93	0.14	79	0.21	68	0.16	1H	
97	0.06	93	0.07	84	0.08	4H	0.4
100	0	99.9	0.001	99	0.004	6H	
96.6667	0.06667	90.6333	0.09367	83.6667	0.08133		
83.5	0.33	84	0.16	76	0.12	1H	0.6
95	0.1	91	0.09	82	0.09	4H	
96	0.08	99.7	0.003	99	0.005	6H	
91.5	0.17	91.5667	0.08433	85.6667	0.07167		
93.7	0.13	78	0.22	78	0.11	1H	0.8
95.5	0.09	94	0.06	90	0.05	4H	
100	0	94	0.06	100	0	6H	
96.4	0.07333	88.6667	0.113	89.3333	0.05333		
86	0.28	88	0.12	68	0.16	1H	1
96	0.08	91	0.09	88	0.06	4H	
98.5	0.03	93	0.07	98	0.01	6H	
93.5	0.13	90.6667	0.09333	84.6667	0.07667		

Table 3: Biosorption of Ni ions by both dried *N. incerta* and *N. palea*

2		1		0.5		Ni	
Percentage removal	Residue concentration (mg/L)	Percentage removal	Residue concentration (mg/L)	Percentage removal	Residue concentration (mg/L)	h	g
90	0.2	85.5	0.006	78	0.11	1H	0.2
94	0.12	100	0	94	0.03	4H	
95	0.1	100	0	94	0.03	6H	
93	0.14	95.1667	0.002	88.6667	0.056		
93	0.14	91	0.09	88	0.06	1H	
97	0.06	99.6	0.004	100	0	4H	0.4
98	0.04	100	0	100	0	6H	
96	0.08	96.8667	0.03133	96	0.02		
90.5	0.19	87	0.13	68	0.16	1H	0.6
99.5	0.01	96	0.04	94	0.03	4H	
99.5	0.01	99	0.01	100	0	6H	
96.5	0.07	94	0.06	87.3	0.063		
93.5	0.13	88	0.12	88	0.06	1H	0.8
96	0.08	98	0.02	90	0.05	4H	
97	0.06	99	0.01	90	0.05	6H	
95.5	0.09	95	0.05	89.3	0.05		
93	0.14	88	0.12	68	0.16	1H	1
97.5	0.05	96	0.04	82	0.09	4H	
98.5	0.03	97	0.03	86	0.07	6H	
96.3	0.073	93.66	0.063	78.66	0.106		

As well as for nickel, the data showed the decreasing for 0.5, 1 and 2 ppm were 0.1-0.02, 0.06-0.002 and 0.1-0.07 mg/L, respectively with percentage removal to the same concentrations was 78.6-96, 93.6- 96.8 and 93-96.5%, respectively (Table 3). The best percentage removal was 96.8 from (0.4 g) in 1 ppm. Our results indicated to the most metal removal was cadmium followed by and lead finally nickel (Cd>Pb>Ni). These results were agreed with Cherifi *et al.* (2016) who studied the biosorbent of Cd and Pb by diatoms (Navicula) and observed efficiency of biosorption metals high ratio. The lower sorption of nickel in comparison to lead and cadmium could be related to the fact that nickel has much lower selectivity coefficient for binds with amin, phosphoryl, than lead which features the highest one reflected in the highest values of binding.

Nickel belongs to the intermediate metals with high affinity not only to phosphoryl, -S032-, R-NH2 and Rz-NH but mainly to -COO groups which it likely shares with lead (Sari and Tuzen, 2007).

Immobilized the dry diatoms by calcium alginate: Powdery mixed of diatoms *N. incerta* and *N. palea* were added to calcium alginate and used as beads for

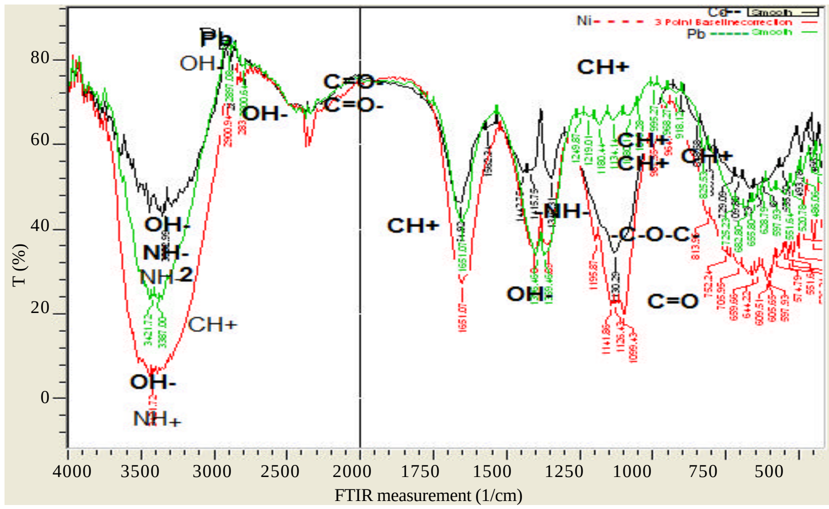

Fig. 1: The spectrum of adsorption heavy metals by the dried diatoms

Table 4: Immobilized dry diatoms to remove Cadmium ions in Temp 25, pH 8.5

	Cd ppm					
Metal/h	0.5	%	1	%	2	%
1	0.04	92	0.07	93	0.12	94.00
4	0.009	98	0.03	97	0.09	95.50
24	nil	100	0.03	97	0.06	97.00
	0.0245	97	0.04333	96	0.09	96.00

Table 5: Immobilized dry diatoms to remove Nickel ions in Temp 25, pH 8.5

	Ni ppm					
Metal/h	0.5	%	1	%	2	%
1	0.03	94	0.08	92	0.32	88.50
4	0.03	94	0.07	93	0.15	92.50
24	0.02	96	0.07	93	0.07	96.50
	0.02667	95	0.07333	93	0.18	92.50

Table 6: Immobilized dry diatoms to remove lead ions in Temp 25, pH 8.5

	pb					
Metal/h	0.5	%	1	%	2	%
1	0.004	99	0.08	92	0.07	96.50
4	nil	100	0.06	94	0.04	98.00
24	nil	100	nil	100	0.009	99.50
	0.004	100	0.07	95	0.03967	98.00

removing Ni, Pb, Cd in and chosen pH 8.5 for the beads maintenances. This was considered as biofilter for heavy metals removal. The result showed high removing rate in all concentrations and in all metals (Table 4-6).

Removal percentages for concentrations 0.5, 1 and 2 were Cd 97, 69 and 96%, respectively and lead were 100, 95 and 98%. Either for nickel the rates were 95, 93 and 92%. The most high percentage removal was in lead followed by cadmium and followed by nickel. Present results were agreed with Duygu who indicated that the sorption of Cd^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, Fe^{3+} and Cr^{6+} by a dried diatom *Nitzschia closterium* was investigated in a batch system. The experiments have been performed for the chosen temperature of 25°C and operational conditions such as constant agitation and pH 8 except for Cr^{6+} at pH 2.0. The sorption of all metal ions rapidly reached equilibrium within 100 min. The maximum sorption capacities of the various metal components on *N. closterium* biomass could be prioritized in order from high to low as: $Pb^{2+}>Cd^{2+}>Ni^{2+}>Zn^{2+}>Cr^{6+}>Fe^{3}$.

Karem *et al.* characterize the ability of the dried diatom *Planothidium lanceolatum* to biosorb Cadmium (Cd) from aqueous solutions he noticed the maximum uptake of metals was obtained at pH 8.0 for Cd. An increase in the biosorption of *P. lanceolatum* was observed with an increase in temperature from 15-250°C.

All the experiments for heavy metals removal indicated that the dry mass of diatoms either immobilized or as free without immobilizing by calcium alginate or other bindings were the most effective in removing metals during few 1-24 h that's could because the dry diatoms were not affected by toxic metals, not required culture media or purifications, it's could used for many times in removing process, also is stored for a long time without any corruptions and the most useful as excellent filters because the high surfaces.

Active groups for *N. incerta* and *N. palea*: FTIR technology was depended for detecting the active groups or active sites on cells surfaces for the two diatoms (Fig. 1). Detecting the specific groups which responsible for biosorption of lead, nickel and cadmium that represented by (amid I and II)-NH_2 and -SH (Sulfhydryl) functional groups in amino acides and glycoprotien of *N. palea* where's -$CONH_2$, -CONH-, alcohol, Carboxtlic and carbonyl groups were founds in liplic acids and polysaccharids in cell wall of *N. incerta*. Cadmium and lead uptake clearly binding with -NH_2 and -SH functional groups. It was similar to the results which get by Alejandro *et al.* and Zhang *et al.* This technological process had a quick detect on the active groups which needs very small amount of sample also, the machine not corrupt the sample (Kumar *et al.*, 2009) (Fig. 1).

CONCLUSION

Powder of dried *N. palea* and *N. incerta* was showed the highest percentage removal for Cd, Pb and Ni within few hour's efficiency. Immobilizing powdery mixed of dried diatoms *N. incerta* and *N. palea* by calcium alginate was used as beads (biofilter) to remove metals recorded the highest percentage removal than other method.

RECOMMENDATIONS

Both diatoms with good efficiency to remove heavy metals. So, we recommended to dependent on them in water treatment to remove different pollutants and there need to additional studies using other species of diatoms in biosorption of heavy metals. A study would suggest enhancing the scientific attention towards using powder of dry diatoms in the probable controlling other pollutants such as heavy metals

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