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Biosorption of Cadmium (II) Ion from Aqueous Solution Using Living Cell and Non-Living Cell Microalga *Scenedesmus Dimorphus*.

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ABSTRACT

The ability of microalga *Scenedesmusdimorphus,* living cells and non-living cells has been tested for bioremoval of a heavy metal, Cadmium (Cd^{+2}) in aqueous solution. Bioremoval capacity of living and non-living cells microalga *Scenedesmusdimorphus* was studied include the effects of pH solution, initial concentration of Cd^{+2} and contact time. For non-living cells microalga, the maximum adsorption was obtained at pH 6 with 2.465 mg_{Cd}/g of removal capacity. The highest extent of Cd^{+2} removal occurred at initial concentration 20 mg_{Cd}/L, and optimal contact time achieved when it exposed for 3 hours. For living cells microalga, the maximum adsorption was obtained at pH 7 with 2.765 mg_{Cd}/g of removal capacity. The highest extent of Cd^{+2} removal was also occurred at initial concentration 20 mg_{Cd}/L, and optimal contact time achieved when it exposed for 10 hours. Further the biosorbent was characterized by Fourier Transformer Infrared Spectroscopy (FT-IR) studies helped to identify the various functional groups contributing in the sorption process. From FTIR spectra analysis, hydroxyl was the major functional group contributed in biosorption process.The electron microscopy examination by Scanning Electron Microscopy (SEM) showed that there was a significant change in cell's surface structure after the uptake of Cd ions. There were tangled formations formed and change in pores size and shape. It was concluded that *S.dimorphus* biomass can be used potentially as biosorbent for the removal Cd in aqueous solution.

Keywords: Heavy metal, Microalga *Scenedesmusdimorphus*, living cell, non-living cell, Cadmium, biosorption.

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INTRODUCTION

The environmental pollution caused by the industrial waste has been increased due to the rapid growth of global industries. Most of the industrial waste contain heavy metals with certain concentration. So, the presence of heavy metals in water and wastewater is increasing due to the industrial development-disposal in the sewerage or in the water bodies [Zein *et al*, 2010].

This form of pollution has the particular disadvantage of not being susceptible to biodegradation, hence leading to bioaccumulation throughout the food chain even in low concentration, leading to serious problem on aquatic life as well as to animal, plant life and human health. Cadmium is one of heavy metals posing the greatest hazard to human health besides Mercury and Lead. It can displace essential metal, i.e Zn^{+2} and Ca^{+2} with specific biological function, so chronic exposure to high levels of Cd may result in kidney or liver damage, bone degeneration, and even cancer [Peralta-Videa *et al*, 2009]. This toxic metals is originated from metal plating, metallurgical alloying, mining, ceramics and other industrial operation [Nagy *et al*, 2013].

Many researches have been searching for alternative and better performing remediation strategies pertaining to toxic heavy metals, because conventional physico-chemical methods, such as electrochemical treatment, ion exchange, precipitation, reverse osmosis, evaporation, and sorption using waste streams are not fully effective at low level metal concentration and rather expensive[Nagy *et al*, 2013; Monteiro and Malcata, 2009].

The biosorption of metals by algae, bacteria, fungi and yeast has been extensively studied in the last two decades [Burgariu *et al,* 2010; Forster 2003]. Microalgae have become relatively popular as biosorbent of heavy metals due to the fact that microalgae, are a rich source in the ocean and other water bodies, relatively cheap to process and able to accumulate high metal content [Monteiro and Malcata, 2009]. Microalgae can possess molecular mechanism to discriminate non-essential metals ion for their growth [Parales *et al,* 2006]. Many microalgae species has been tested as potential heavy metal biosorbent such as*, Scenedesmus obliqus* [Monteiro and Malcata, 2009]*, Scenedesmus quadricauda. Phormidium ambiguum, Pseudochlorococcum typicum* [Shanab *et al,* 2012]*, Oudogonium urceolatum* [Yaqub et al, 2009]*, Dunaliella* [Imani *et al*, 2011]*, Spirulina* [Khan 2013]*, Chlorella,* and *Spirogyra* [Rezaee *et al*, 2006].

In this study, the ability of a microalga species, *Scenedesmus dimorphus,* both living cells and non-living cells for bioremoval of a heavy metal, Cadmium (Cd^{+2}) in aqueous solution under the effect of pH solution, initial concentration of Cd^{+2} and contact time has been tested.

EXPERIMENTAL

Microalga Biomass preparation

Microalga *Scenedesmus dimorphus* in this study is obtained from Microalgae Culture Collection of Biochemistry Laboratory, Andalas University. Microalga was cultivated in modified Bold's Basal Medium at room temperature and aerated with aquarium pump, under fluorescent light intensity of 3000 lux at 25 °C, photo period 12-12h and regularly subcultured until use. Cultures in the exponential growth phase were used in all experimental batch cultures. Cell growth was determined by measuring the optical density (OD) at 550 nm with UV-Vis Spectrophotometer (Genesys 20), and subsequently converting it to dry weight (DW) using calibration curve prepared in advance. For non-living cell, the biomass of *S. dimorphus* was harvested in the exponential phase by centrifugation at 4000 rpm for 15 min. Then, inactivated by heating at 100^{o}C for 24 h.

Reagents

Cadmium stock solution (1000 mg L^{-1}) was prepared by diluting $Cd(NO_3)_2$. $4H_2O$ in deionized water. The working solutions were prepared by serial dilution of stock solution. The initial pH was adjusted with NaOH 0.1 M and H_2SO_4 0.1 M.

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Biosorption Cd

The biosorption experiments were performed in batch condition under the effect of pH solution, initial concentration of Cd^{+2} and contact time. The effect of pH on the biosorption of Cadmium (II) by microalga *S. dimorphus* both living and non-living cell was determined at pH values 4,5,6,7 and 8. For batch experimental, 0.2 g microalga *S.dimorphus* biomass was added to 100 mL Cadmium (II) ion solution (1 mg L^{-1}) in erlenmeyer flask. The mixtures were shaken in orbital shaker at 150 rpm for 1 h. Then, the mixture was centrifuged at 4000 rpm for 15 min to separate the biomass. The residual concentration of Cadmium (II) ion in supernatant was determined by atomic absorption spectrophotometer (AAS, Varian AA240).

In order to investigate the effect of initial Cd^{+2} concentration, 0.2 g microalga biomass was exposed to 1, 5, 10, 20, and 30 mg L^{-1} of Cadmium (II) ion solution. pH was adjusted to the optimized pH experimented in previous method. The mixtures were shaken in orbital shaker at 150 rpm for 1 h. Following centrifugation, the supernatant was assayed for the remaining Cadmium (II) ion concentration.

For the determination of optimum contact time of biosorption Cadmium (II) ion, 0.2 g microalga biomass was exposed to an optimum initial Cd^{+2} concentration. pH was adjusted to the optimized pH experimented in previous method. The mixtures were shaken in orbital shaker at 150 rpm. The supernatant was analyzed for residual metal concentration after the contact period of 0, 30, 60, 120, 180, and 300 minutes.

In order to evaluate the amount of cadmium ions retained per unit mass of microalga biomass, the adsorption capacity (Q) was calculated using the following equation:

$$Q = \frac{V(C_o - C)}{m}$$

where Co and C were initial and final metal concentration in solutions (mg L^{-1}), respectively; V was volume of the solution (L); m was the amount of biomass (g).

FTIR Analysis

FT-IR spectroscopy was used to determine the vibration frequency groups in the biosorbent. For the IR studies, dried microalga biomass before and after biosorption were mixed with KBr and then grounded in an agate mortar (Merck,). The mixture was pressed to form pellets and used in the recording of spectra using Unican Mattson Mod 7000 FTIR Spectrometer.

SEM Analysis

Scanning electron microscopy is utilized for characterizing surface microstructures and porosity of biosorbents. The surface morphology of microalga *S. dimorphus* non-living cell before and after biosorption was determined using a scanning electron microscope. SEM examination result was used to compare the biosorbent before and after biosorption of Cd.

RESULTS AND DISCUSSIONS

Effect of pH

The pH is one of the most important controlling parameters in the adsorption process of heavy metal ions and algae tolerance. The pH can affect the solubility of the metal ion in aqueous solution and biosorbent ability to bind metal ions due to protonation state of functional group on the biosorbent cell wall [Nadeem 2013; Brinza *et al*, 2007]. Effect of pH on the absorption capacity of the metal ions Cd (II) is shown in Figure 1 in. It was observed that cadmium uptake increased with the rise in the initial pH solution.

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Figure 1: Effect of pH solution on the biosorption of Cadmium (II) ion

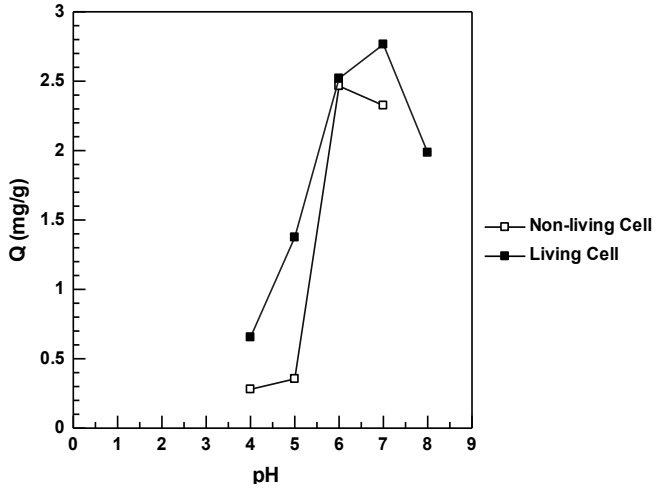
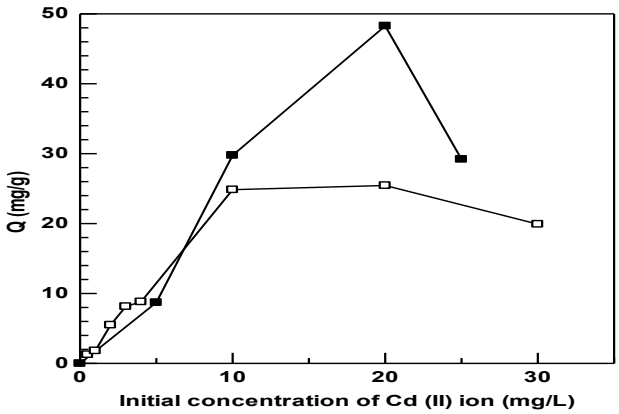


Figure 1 shows that ion uptake of Cd (II) by the biomass of non-living cells microalgae maximum at pH 6 while for living cells microalgae at pH 7. At low pH (acidic) absorption capacity metal by biosorbent tend to be low. At near neutral pH (pH 6 to 7) the absorption capacity significantly increase and at higher pH (alkaline) absorption capacity fell back. This is happened because at low pH, the surface charge of the cell wall is more positive, so it constrains binding of metal cations. As pH is raised, more ligands bearing negative charges become exposed on the surface of the cell wall, with subsequent attraction of metal ion and at higher pH, the precipitation is dominant [Monteiro and Malcata, 2009; Nadeem 2013]. Bayramoglu and Arica has shown the similar dependence of metal removal on pH [15]. Monteiro and Malcata also shown the similar result with the optimum pH for metal removal with microalga *Scenedesmus obliquus.*

Effect of Initial Concentration

The effect of variations of Cadmium (II) ion concentrations with absorption capacity by the both type of biomass can be seen in Figure 2. Cadmium absorption by the both type of biomass increased with increasing concentrations of metal ions. The highest absorption capacity occurred at Cadmium concentration 20 mg / L for both types of biomass. After reached the optimum concentration absorption capacity by both types biosorbent decreased.

Figure 2: Effect of initial concentration on the biosorption of Cadmium (II) ion



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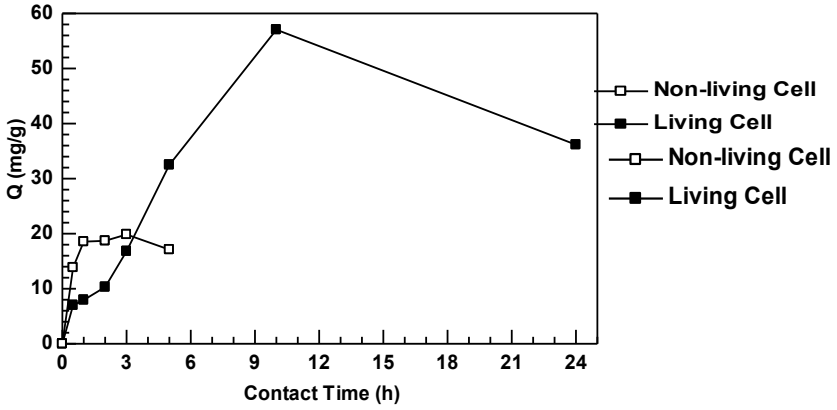
The concentration of metal ions is associated with the number of active sites on biosorbent, when the number of active side plenty the absorption capacity will be increased until the active sides in biosorbent is equal to the amount of metal ions in solution. Absorption capacity will decrease as the amount of metal exceeded the active side available on biosorben. Tichaona *et al* explain that the increase in the metal ions concentration increase absorption on the biosorbent surface and hence the increase on adsorption capacity [Tichaona *et al,* 2013]. The initial concentration of metal ions provides the driving force to overcome resistance of mass transfer between the two phases. It also encourages interaction between the adsorbent and sorbate [Rao 2013]. According to Nadeem *et al,* biosorben surface saturation depends on the concentration of metal ions. At low concentrations, the active sites on the biosorbent surface quickly bind the metal ion biosorbent available in solution. However, at higher concentrations, the metal ions need to diffuse to the surface of the biomass with intra-particles diffusion and usually ion will diffuse rather slow.

Although the optimum concentration for both biosorbent remain at the same conditions, their adsorption capacity is slightly different. Adsorption capacity of non-living cell biomass is 25.45 mg/g and 48.275 mg/g for living cells biomass. This occurs due to the mechanisms of metal uptake by living cells and non-living cells. Living microalga biomass has ability to remove such contaminants, either by adsorption onto the cell surface or by incorporation into the cells themselves. Two distinct biochemical paths can thus be followed: biosorption (or adsorption of metal ions onto the cell surface) and bioaccumulation (or absorption of metal into the cell to form complex with metallothioneins [Parales *et al,* 2006; Hassinen *et al,* 2011; Kaplan 2013] or phytochelatins [Chaidir *et al,* 2012] through the metabolism process. While in non-living microalgae cells, it is only adsorption on the cell surface.

Effect of Contact Time

The contact time is one of the fundamental parameters in batch biosorption mode. Determination of the contact time is necessary to know the minimum time needed to reach the maximum adsorption of heavy metal ions until saturation was reached where the absorption process stopped and desorption occurred. The results of the determination of the optimal contact time is presented in Figure 3.

Figure 3: Effect of contact time on the biosorption of Cadmium (II) ion



From data in Figure 3 can be seen an increase in the adsorption capacity of biosorbent along with the duration of metal exposure. The maximum absorption capacity is reached after 3 hours for non-living cell biomass and 10 hours for living cell biomass (19.85 mg/g and 57.05 mg/g). In biosorption by non-living cell biomass, adsorption capacity increased sharply in the early observations and after one hour exposure, biosorption tends to slow. It is characterized by a small increase in absorption capacity, even tend to stable. This situation occurs because the active site on the biosorbent surface has almost fully loaded [Shanab *et al*, 2012]. After 3 hours, absorption capacity decreased due to desorption process.

For living cell biomass, the optimal contact time is reached much longer than the non-living cell biomass. According to Shanab *et al,* biosorption of metal ions on the cell surface is a passive process and rapid introduction (achieved less than 30 minutes). While the process of accumulation of metal ions into the cell (which occurs in living cells) is an active process and tends to slow (can take place over one month) [Khan *et al*, 2013]. After 10 h, the adsorption capacity by living cell biomass decreased. This may occur because the equilibrium inside and outside the cell, the cells damage and break down bond between cell microalgae and ion metals by microorganisms, such as fungi and bacteria that growing during the experiment [Monteiro and Malcata, 2009].

FTIR Analysis

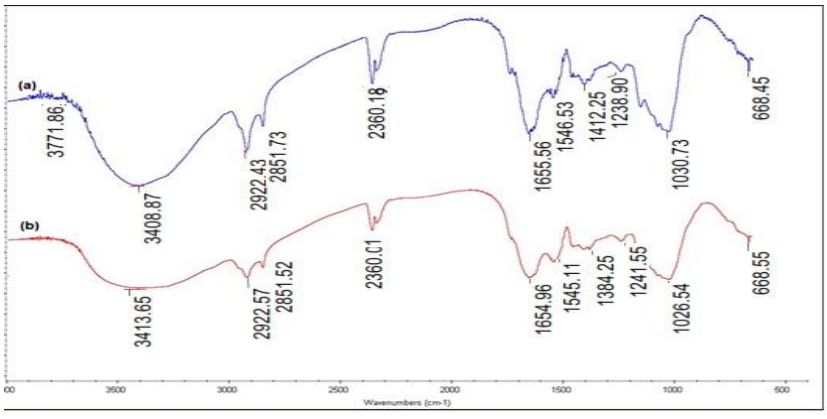
FT-IR analysis allows identification of some functional groups of organic compounds on biosorbent from characteristic peaks appear in the spectrum, including those involved in the biosorption process. To determine the functional groups involved in biosorption of Cd (II), it should be compared the FT-IR spectra before and after biosorption (Figure 4). Main identified peaks, their assignment and their shifted is presented in Table 1.

Peaks at wave numbers 3408.87 cm^{-1} indicated the presence of -OH groups from cellulose. This peak shifted to 3413.65 cm^{-1} after absorption. This is indicated the formation of a bond between cadmium ions and -OH groups. The peak at 2922 cm^{-1} indicating the presence of CH stretching and the shifted is not too obvious. The peak at 1655 cm^{-1} shifted to 1656 cm^{-1} where the peak indicates the presence of the C = O stretching vibration in carboxylic. At 1412 cm^{-1} shifted to 1384 cm-1 indicates -OH bending of carboxylate groups. From the wave number differences, the -OH group is of the most dominant functional group that role in Cd ion biosorption [Nagy *et al,* 2013; Khan *et al,* 2013].

Table 1: FTIR characteristic peaks of heat-inactifated Microalga *Scenedesmusdimorphus*before and after Cd (II) biosorption

***S. dimorphus* Biomass FTIR peaks (cm^{-1})**		**Differences**	**Functional group**
before	After		
3408.87	3413.65	-4.78	-OH stretching
2922.43	2922.57	-0.14	-CH stretching
1655.56	1654.96	0.6	C=O stretching
1412.25	1384.25	28	-OH bending
1030.73	1026.54	4.19	-CO stretching

Figure 4: FTIR spectra of heat-inactifated*S.dimorphus;* before (a) and after biosorbtion (b)

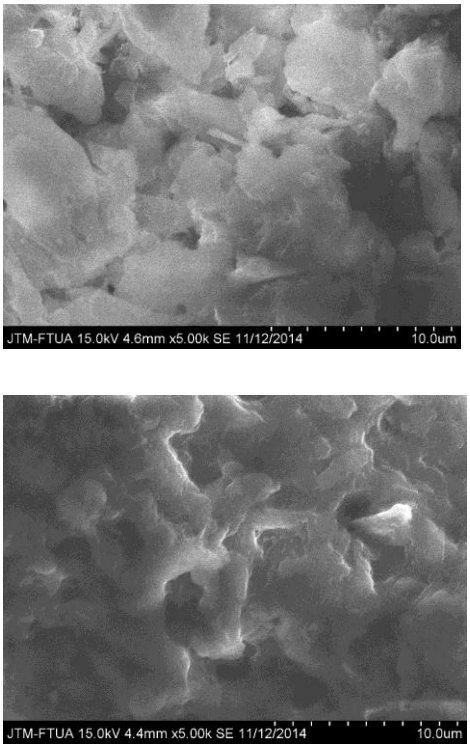


SEM Analysis

Figure 5 show the SEM micrographs of the microalga cell surface before and after Cd (II) biosorption. The SEM image in (fig.5a) 5000 x magnification shows that Microalga *S. dimorphus* biomass are highly porous, indicate the possibility of its good adsorption properties. The cell surface exhibited morphology change with the larger pore

after Cd (II) biosorbtion (fig 5b). The change in pore size and shape was probably caused by the release of some alkaline and earth alkaline ions which are bound to the biomass surface to bind the metal ions. The highly porous surface favors the diffusion of metal ions into the cell and leading to higher adsorption capacity [Dragana *et al,* 2011].

Figure 5: The 10.0 µm magnitude images of microalgae's cell structure:(a) Cell surface image before biosorption and (b) Cell surface image after biosorption



CONCLUSIONS

Microalga*S. dimorphus* both living and non-living cell is proved able to remove Cadmium (II) ions from aqueous solution, thus confirming its potential applicability in wastewater treatment.In batch mode studies, adsorption was dependent on pH, initial metal concentration and contact time.

FTIR analysis indicated the presence of the hydroxyl and carboxyl groups on the biomass surface which play an important role in the biosorption process, suggesting that the process takes place mainly by ionic exchange.

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