The kinetics of Fe$^{2+}$ heavy metal adsorption by microalgae Desmodesmus sp. beads

D A Widyaningrum$^1$, A Rinanti$^2$, R Hadisoebroto$^{3*}$

$^{1,2,3}$Environmental Engineering Study Program, Faculty of Landscape Architecture and Environmental Technology, Universitas Trisakti, Jakarta, Indonesia

rositayanti@trisakti.ac.id

Abstract. Industrial waste that contains high concentration of iron heavy metal (Fe$^{2+}$) needs to be reduced into a safer limit for water ecosystem using an environmentally-friendly, sustainable and low cost technology. This literature study was aimed to gather information on Fe$^{2+}$ metal ion removal with biosorption method by mobilized beads-shaped Desmodesmus sp. green microalgae. Biosorbent beads are made of Desmodesmus sp. green microalgae powder binded with Na-alginate polymer. Desmodesmus sp. microalgae were cultivated in an Erlenmeyer flask with wastewater contains Fe$^{2+}$ and Beijerinck growth media controlled in batch system. Analysis was conducted to study the influence of pH, size, beads, beads concentration, and Na-alginate concentration towards Fe$^{2+}$ heavy metal removal efficiency on biosorption process. Research results revealed that Fe$^{2+}$ heavy metal was effectively adsorbed by 1 mm beads up until 46% at maximum capacity on pH level of 6. These beads contain 8% gr/L of microalgae powder and 9% gr/L of Na-alginate. The involved function groups in Fe$^{2+}$ sorption and the beads biosorbent morphology changes were analyzed by using FTIR and SEM. This biosorption research was following by analysis of Freundlich isotherm and reaction kinetics order two with linear approach. This was to shows the beads possess adsorption kinetics with optimum Fe$^{2+}$ absorption capacity. Desmodesmus sp. beads are highly potential to be used as biosorbent since the heavy metal removal process was selectively conducted on wide pH level and temperature ranges, fast adsorption kinetics, and low operational cost. The smaller the beads size the wider the surface area, which leads to higher removal efficiency rate.

1. Introduction
The toxic and persistent heavy metal nature and its tendency to be accumulated in the environment has become the main cause of declined ecosystem quality due to heavy metal pollution. To reduce heavy metal ion concentration, further processing stage should be implemented before removing it to the environment. Currently, there are three popular waste water processing technology namely physical, chemical and biological waste water processing. Physic-chemical methods that commonly used to remove Fe$^{2+}$ from the environment are adsorption process, reverse osmosis, oxidation process, membrane removal, ion changes, and chemical precipitation [1, 2, 3]. The advantage of this method is that the dose of the absorbent can be arranged to effectively and efficiently remove the metal compound at high/low pollutant concentration. However, this method has weaknesses due to its high energy consumption, high cost, low metal removal efficiency, not environmentally friendly, and can produce toxic side product [3, 4].
Another method that can be utilized to reduce polluting heavy metal compound is biotechnology method through biosorption, which is a method to remove heavy metal compounds by living or dead microorganism assistance through ion change process, complex formation development, and heavy metal absorbance. The best microorganism to play this role is microalgae, because its cellular wall contains active function groups that play important role in metal ion binding process [5]. Factors that influence biosorption process are acidity level (pH), waste concentration, temperature, biomass concentration in the solution, and contact time [3, 7, 8]. On a passive manner, heavy metal compound will be absorbed by biosorbent through physic-chemical binding on the surface of cellular walls, but in an actively manner it will occur through cellular metabolism [9].

Microalgae as autotroph microorganism has the potential to be utilized as biological material (biosorbent) because of its capacity to absorb non-organic compounds in biosorption process, which makes heavy metal solution that contains high level of affinity can be easily bound by it [3, 4, 10, 12]. Complex formulation of cation heavy metal will interact with anion active group at cellular [12], which place microalgae as the most potential compound to absorb and accumulate heavy metal in waste water [13, 14]. Researcher [4] discovered that Ca-alginate mobilized microalgae are effectively potential to absorb Cu\(^{2+}\) heavy metal ion from a solution in a batch system.

2. Methodology

2.1. Desmodesmus sp. biomass and growth media preparations

Secondary data in this research refer to literature [3]. Desmodesmus sp. green microalgae were isolated from Parkview Lake freshwater in Johannesburg, South Africa. 1 liter of Beijerinck growth media that contains stock 1 solution (1.5 gr of NH\(_4\)NO\(_3\), 0.2 gr of KH\(_2\)PO\(_4\), 0.2 gr of MgSO\(_4\).7H\(_2\)O, and 0.2 gr of CaCl\(_2\).2H\(_2\)O), stock 2 solution (9.07 gr KH\(_2\)PO\(_4\)), stock 3 solution (11.61 gr of KH\(_2\)PO\(_4\)), and micronutrients (10 gr of H\(_3\)BO\(_3\), 5 gr of MnCl\(_2\).5H\(_2\)O, 50 gr of EDTA, 1.5 gr of CuSO\(_4\).5H\(_2\)O, 22 gr of ZnSO\(_4\).H\(_2\)O, 1.5 gr of CoCl\(_2\).6H\(_2\)O, 5 gr of FeSO\(_4\).7H\(_2\)O, and 1 gr of (NH\(_4\)).6Mo.7O\(_{24}\).4H\(_2\)O). Incubated Desmodesmus sp. in an Erlenmeyer flask with a batch system containing modified Beijerinck medium, streamed with CO\(_2\) on a 50 mL/minutes rate, placed on room temperature of 25±2\(^{\circ}\)C, completed with artificial lighting from TL lamp. Mixing process was conducted by utilizing Boeco, MS 300 (German) magnetic stirrer. Desmodesmus sp. cultivation was observed each day for 25 days by utilizing UV-VIS 4802 spectrophotometer with 680 nm wavelength.

2.2. Immobilized biosorbent with Na-alginate polymer

Desmodesmus sp. biomass was harvested on the exponential phase at the 18\(^{th}\) day. 50 mL of Desmodesmus sp. biomass was separated from the solution through centrifugation on 4,000 rpm speed for 30 minutes. Growth media was poured and biomass was filtered to remove water excess. To create beads or immobilized biosorbent, 3% of Na-alginate powder was dissolved into aquades and stirred by using magnetic stirrer for one hour. After that, filtered 1.5 gr of biomass powder was mixed into 50 mL of Na-alginate, while stirring it on a magnetic stirrer to accelerate mixing process. A mixture of biomass and Na-alginate was inserted into a syringe and added with a drop technique into 0.2M 500 mL CaCl\(_2\) solution, homogenized on magnetic stirrer to avoid beads pasting. The beads formed in the CaCl\(_2\) solution were soaked for 24 hours so that the formation of the beads took place completely. The beads were picked up with a filter, and rinsed for 3 times to remove remaining CaCl\(_2\). The beads were stored in a duran schott glass bottle and covered by wet cotton on the lid to create humid environment.

2.3. Fe\(^{2+}\) heavy metal ion biosorption

Biosorption process of Fe\(^{2+}\) using beads was conducted in batch system on pH variations of 4, 5, 6; contact time of 15, 60, and 120; size of beads at 1, 2, 3, and 4 mm. Five grams of Desmodesmus sp. Beads were poured into 50 mL of Fe\(^{2+}\) solution with initial concentration of 120 mg/L on optimum level of pH. All research stages were conducted in 1L Erlenmeyer flask at room temperature. The Fe\(^{2+}\) concentration analysis prior and post sorption was measured on model PG-990 (Leicestershire, UK)
Atomic Absorption Spectrophotometry (AAS). Function groups analysis on biosorbent beads was conducting by utilizing Fourier Transform Infrared (FTIR, Bruker, Tensor 27, Germany) spectroscopy. Biosorbent beads surface morphology analysis was measured on a Scanning Electron Microscope (SEM, Hitachi S-4160).

2.4. Biosorption modeling
The maximum Fe$^{2+}$ absorption by biosorbent beads was determined by adjusting adsorption data with Langmuir isotherm and Freundlich isotherm. Langmuir isotherm (equation 1) utilization is meant to produce linear regression coefficient value ($R^2$) by plotting $C_e/q_e$ value on $C_e$ value [8] and implemented it on research data as shown below:

$$\frac{C_e}{q_e} = \frac{1}{q_m \cdot L} + \frac{C_e}{K_L}$$  \hspace{1cm} (1)

$C_e$: metal ion concentration at balanced state (mg/L); $q_e$: number of metal ion absorb at balanced state (mg/g); $q_m$: maximum absorption capacity (mg/g); $K_L$: Langmuir empirical constant

Freundlich isotherm (equation 2) [8] applied on research data is as below:

$$q_e = K_f C_e^{1/n}$$  \hspace{1cm} (2)

$q_e$: absorbed metal ion on biomass surface at balanced state (mg/g); $C_e$: metal ion concentration at balanced state (mg/L); $K_f$: Freundlich adsorption constant

The next stage is to determine Freundlich isotherm constant based on a graphic plotting between $q_e$ and $C_e$, and logarithmized it into:

$$\log q_e = \log K_f + \frac{1}{n} \log C_e$$  \hspace{1cm} (3)

Difference in absorption time was implemented to determine the correct required time by biomass to bind metal ion [9]. Adsorption kinetics refer to reaction order one (equation 4) and reaction order two (equation 5) [9, 10, 11].

$$\ln (q_e - q_t) = \ln q_e - K_1 \cdot t$$  \hspace{1cm} (4)

$$\frac{1}{q_t} = \frac{1}{K_2} \cdot q_e^2 + \frac{1}{q_e}$$  \hspace{1cm} (5)

$q_e$: number of absorbed metal ion at balanced state (mg/g); $q_t$: number of absorbed ion during t time (mg/g); $K_1$, $K_2$: reaction order one and two equation reaction constant rate; $t$: time (minutes).

3. Result and discussion
Microalgae growth pattern cultivated in a photobioreactor batch is influenced by nutrition, type of microalgae, initial cell population in culture media, and other environmental factors [6]. *Desmodesmus* sp. microalgae cultivation was conducted on 1L Erlenmeyer flask by temperature setting, CO$_2$ gas stream with air flow of 50 mL/minute, and artificial lighting made from TL lamp and magnetic stirrer utilization as mixing tool (Figure 1). Microalgae growth pattern was started from adaptation phase happened on day 1 (Figure 2). During this phase, microalgae are still adapting to their new environment which lead to insignificant cell divisions. Exponential phase occurred on the 10$^{th}$ to the 20$^{th}$ day, marked by rapid cell division, on a constant metabolism activity and balanced growth between microalgae cell increase and nutrition supply. Biomass permanent was conducted with centrifugation method conducted on the 18$^{th}$ day where significant biomass increase happened between exponential phase ranges. Fe$^{2+}$ metal biosorption process is influenced by pH variable, contact time, and size of biosorbent beads (Figure 3 and Figure 4).
Figure 1. *Desmodesmus* sp. Microalgae Cultivation on 1L Erlenmeyer Flask [3].

Figure 2. *Desmodesmus* sp. microalgae growth pattern on modified Beijerinck medium [3].

Figure 3 shows that on pH level of 2 and 6 Fe$^{2+}$ removal efficiencies on each level reached 43% and 46%, before finally declined at pH level of 7 into 38%. On lower pH level, there can be a rivalry between hydrogen (H) and metal ion to attach themselves on biosorbent active side, due to function group protonation and positive charged biomass surface. However, as pH level increases, proton concentration will decrease and deprotonated that would lead to a more negative biomass [5]. The increased pH level happened along with metal ion binding on biosorbent surface [5, 15].

Figure 4 shows that Fe$^{2+}$ metal ion absorption reached 48% by 1 mm beads in just 30 minutes, followed by 2 mm beads with 34% of Fe$^{2+}$ metal absorption in 120 minutes. Researchers [14, 16, 17] explained that the smaller the size of the biosorbent, the larger the surface contact between biosorbent and heavy metal ion will be. Besides that, the surface area is also proportional to the number of pores contained on a biosorbent unit, which create higher level of heavy metal ion removal [15].

Figure 3. Fe$^{2+}$ removal under variation of pH levels on biosorbent beads [3].

Figure 4. Fe$^{2+}$ removal with variation of size of biosorbent beads [3].

FTIR analysis shows that the involved active function groups in adsorbing Fe$^{2+}$ metal ion are carboxylate acid, hydroxyl, sulphonate acid, and alcohol. Biosorbent morphology structure change after biosorption process were analyzed on SEM, which marked by the more visible pores and amorphous structure [14, 17]. Biosorption process has rapid reversible nature between heavy metal ion binding on cellular wall, both on living and dead cells. The basic principle of biosorption process with microalgae
utilization is by substituting function group with heavy metal, so one of compounds contained in function group can be replaced by heavy metal ion [12, 17].

Based on the secondary data, we can create biosorption models based on Langmuir and Freundlich adsorption isotherm modelling and adsorption kinetics (reaction order one and two).

**Figure 5.** Langmuir isotherm curve. **Figure 6.** Freundlich isotherm curve.

Langmuir isotherm curve produce an equation of \( y = 8.0229x - 434.05 \), with linear coefficient value \((R^2)\) of 0.9891 (Figure 5). Based on the equation, we were able to obtain the adsorption capacity value \((q_m)\) at 0.1246 and Langmuir empirical constant value \((K_L)\) at -0.0185. On the other hand, Figure 6 shows Freundlich Isotherm curve with an equation of \( y = -2.0631x + 3.52 \), and linear regression coefficient value \((R^2)\) of 0.9947. Based on the equation, we were able to obtain Freundlich constant factor value \((n)\) at -0.4847 and Freundlich \((K_f)\) Constant value of 3,311.31. Based on the findings, \(Fe^{2+}\) metal biosorption process by mobilized *Desmodesmus* sp. followed Freundlich Isotherm model, because the obtained \(R^2\) value is closer to 1. This means that Freundlich Isotherm model is able to decently interpret the data, because the sorption mechanism occurred physically with multilayer formation and heterogeneous. Biosorption occurs in an effective way because microalgae possess active function groups supported by broad surface area, which enable \(Fe^{2+}\) metal ion to be optimally adsorbed [16, 17]. Adsorption kinetics is defined as heavy metal removal rate caused by heavy metal contact with microalgae biosorbent. This research was conducted with reaction order one and two which were plotted on a curve to produce linear regression coefficient value \((R^2)\) (Table 1).

**Table 1.** Adsorption kinetics recapitulation.

| Order | pH | \(R^2\) | \(q_e\) (mg/g) | Reaction Rate Constant |
|-------|----|--------|---------------|-----------------------|
| One   | 4  | 0.0101 | 0.1912        | 0.0250 /minutes       |
|       | 5  | 0.2157 | 0.4850        | 0.0130 / minutes      |
|       | 6  | 0.0064 | 0.2246        | 0.0018 / minutes      |
| Two   | 4  | 0.9486 | 0.1926        | 0.0861 /g.mg/ minutes |
|       | 5  | 0.9580 | 0.2720        | 0.1294 /g.mg/ minutes |
|       | 6  | 0.9880 | 0.4547        | 0.7924 /g.mg/ minutes |

Based on Table 1, we can see the linear regression coefficient value \((R^2)\) comparison results. \(Fe^{2+}\) metal concentration was decently adsorbed on reaction order two at pH level of 6, 120 minutes of contact time, with \(q_e\) value of 0.4547 mg/g and reaction rate constant value of 0.7924/g.mg/minutes. This finding shows that adsorption kinetics reaction order two can interpret decent \(Fe^{2+}\) biosorption kinetics rate by biosorbent beads.
The implementation of biosorption process on pilot scale to process 75L of waste that contain Fe\textsuperscript{2+} metal by *Desmodesmus* sp., beads is shown on Table 2. The calculation is also completed with reaction design on Figure 7.

| Description                          | Value | Units |
|--------------------------------------|-------|-------|
| Fe\textsuperscript{2+} Volume        | 75    | L     |
| Biosorbent beads required            | 7,500 | gram  |
| Biomass powder required              | 121   | gram  |
| Microalgae harvest required          | 605   | L     |
| Reactor volume required              | 1,500 | L     |
| Reactor height                       | 2     | m     |
| Reactor diameter                     | 1     | m     |
| Reactor design volume                | 1,571 | L     |
| Detention time                       | 69.88 | minutes |

Figure 7. Reactor design.

Based on calculation results by utilizing adsorption capacity obtained from Freundlich isotherm value and reaction order two adsorption kinetics on pH level 6 condition, we can calculate the required biosorbent beads to process 75 L of Fe\textsuperscript{2+} metal ion with initial metal concentration of 50 mg/L into final concentration of 7 mg/L, which is at 7,500 grams for 69.88 minutes.

4. Conclusion
Na-alginate mobilized *Desmodesmus* sp. microalgae were able to remove Fe\textsuperscript{2+} heavy metal by utilizing 1mm biosorbent beads in controlled environmental condition, with initial metal concentration at 120 mg/L, on 60 minutes of contact time. Biosorption process was marked by heavy metal binding into microalgae function group located on cellular wall surface. Based on the findings, biosorption process of Fe\textsuperscript{2+} metal by mobilized *Desmodesmus* sp. followed Freundlich Isotherm model and adsorption kinetics reaction order two can interpret decent Fe\textsuperscript{2+} biosorption kinetics rate by biosorbent beads. The implementation of biosorption process on pilot scale to process 75 L of waste that contains Fe\textsuperscript{2+} heavy metal design to be conducted in a 1,500 L reactor, requires 7,500 gram of biosorbent beads that for 69.88 minutes to remove similar level of Fe\textsuperscript{2+} heavy metal content at the same removal efficiency level.

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