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# BIOSORPTION OF CU (II) BY *SCENEDESMUS OBLIQUUS*: OPTIMIZATION IN PHOVASOLI HAEMOTOCOCCUS MEDIUM

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**ABSTRACT:** Biosorption of copper ($Cu^{2+}$) in electroplating wastewater by biosorbent using *Scenedesmus obliquus* green algae has been investigated in the artificial medium of Phovasoli Haemotococcus Medium (PHM). The study aims to find an alternative for the treatment of wastewater containing copper ($Cu^{2+}$) using *S.obliquus* as a biosorbent, uses a batch test with various pH 4, 5, 6, 7 and 8 as well as the various contact time of 60, 120, 180, 240 and 300 minutes. Some ratios of wastes versus nutrients ($Cu^{2+}$ : nutrient) in the treatment were A (1: 3), B (1: 1), C (3: 1) and D (100% of waste) with the initial concentration respectively, A (5.05 mg/L), B (16.54 mg/L), C (21.85 mg/L) and D (30.28 mg/L) at 28°C ± 2. The results of the study show that the biosorption rate and the abilities of the sorbent are influenced by pH and contact time (adsorption rate). The maximum uptake rate for $Cu^{2+}$ was obtained at pH 6 in 240 minutes. In the ratio of waste: nutrient = 1: 3, the uptake rate was 65.54% ± 8.04 while when 100% waste, the uptake rate was 41.99% ± 1.01. The adsorption of copper ($Cu^{2+}$) is due to the destruction of the cell wall. Analysis of Fourier Transform Infrared (FTIR) shows the adsorption band of functional groups such as carboxyl, amine, and alkene. It indicates the interaction between *S.obliquus* and the copper ion ($Cu^{2+}$). The adsorption process of this study follows the Freundlich isotherm equation.

***Keywords:*** *Biosorption; Copper ($Cu^{2+}$); Electroplating wastewater; Isotherms Adsorption; Kinetics Adsorption, S.obliquus*

## 1. INTRODUCTION

The increasing of technological advances and the rise of industrial activities has an impact on the increase of the waste amount generated in the form of liquid waste, solid waste and gas waste. These wastes may cause environmental pollution which will affect the environment and human health. One of the most hazardous wastes is the heavy metal such as copper ($Cu^{2+}$).

The existence of $Cu^{2+}$ metal is generated from electroplating industrial activities into water environment, that usually in form of a complex salt or still remain in its elemental form. The concentration of $Cu^{2+}$ metal in electroplating industrial wastewater is regulated in Decree of Ministry of Environment Republic of Indonesia No. 5 of 2014, about Wastewater Quality Standard, with a maximum of 0.5 mg/L. $Cu^{2+}$ metal ion concentration exceeds the Quality Standard judged to be harmful directly to habitat and water organisms life, and indirectly to human health like poisonous, headache and diarrhea [1]

Several sorption methods to separate the heavy metal are chemical precipitation, reverse osmosis, ion exchange, and bioreduction. These processes have several weaknesses, such as requiring high costs and less effective when applied at low waste concentrations for small concentration scale between 0-100 ppm [2, 3].

Biosorption is a popular technique to separate and regain the metals from liquid waste by utilizing biomass that can bind heavy metal ions from a solution [4, 5, 6]. This method is competitive, relatively cheap and effective as well [4]. The high efficiency of biosorption in a dilute solution can minimize the formation of sludge and ease the regeneration process [3, 7]. From the regeneration process, the sorbent can be used repeatedly and the metal ions can be regained/recovered from the solution (5, 8).

One of the biomasses that can be used in the biosorption process is microalgae such as bacteria, fungi, and algae. The microalgae have an ability to accumulate the heavy metals from waste. Microalgae have the greatest ability to absorb heavy metals [8, 9]. *Scenedesmus obliquus* is one of the types of microalgae that can be used for the adsorption of heavy metals. Based on the previous research [10,11], *S.obliquus* has a resistance property towards heavy metal, thus, it can adsorb copper ($Cu^{2+}$) in a sufficiently high amount.

The purpose of this study is to find an alternative for the treatment of heavy metal waste especially copper ($Cu^{2+}$) using *S.obliquus* as a biosorbent. In this study, the growth response of microalgae in the electroplating of industrial waste containing heavy metals of copper ($Cu^{2+}$) will be determined. This research is a preliminary study, that pH variation as an early indicator of biochemical mechanisms in heavy metal removal by microalgae, while contact time is needed for kinetics study. So, the adsorption optimum condition regarding the acidity and the contact time will be studied.

## 2. RESEARCH METHODOLOGY

### 2.1. Characterization and Preparation of *S.obliquus* as Biosorbent

The isolated *S.obliquus* obtained from Bojongsoang WWTP, Bandung is grown in *Haemotococcus Phavosoli Media* (PHM) as a nutrient [10,12]. The waste characterization aims to determine the initial concentration of copper waste ($Cu^{2+}$) and to make sure that the original waste consists only copper ($Cu^{2+}$). The solutions containing only waste and the waste with the addition of in the ratio of 1:1 were studied in order to investigate the mechanism of biosorption of $Cu^{2+}$ on *S.obliquus*.

### 2.2. Optimization of acidity value (pH)

This research was conducted by preparing ratio of (A) waste : nutrient (PHM growth media as a nutrient) = 1: 3, (B) waste: nutrient = 1:1, (C) waste: nutrient = 3:1 and (D) 100% waste. The acidity (pH) was adjusted by adding a buffer solution to obtain a pH of 4, 5, 6, 7 and 8. In each treatment, the aeration was supplied by a water compressor pump with a flow rate of 0.8 mL/minutes.

### 2.3. Optimization of contact time variation (Td)

The optimum pH obtained in this study was 6. The study of contact time variation was conducted at this optimum pH. The contact time variations are 60, 120, 180, 240 and 300 minutes. The experiments of pH and contact time influences were performed under the condition where the ambient light intensity was 4000-5000 lux sourced from artificial lighting and at the room temperature of 28°C ± 2.

The concentration of copper ions ($Cu^{2+}$) remained in the waste after the adsorption process was measured using an *Atomic Absorption Spectrophotometer* (AAS). Analysis using AAS was done to know the concentration of metal ion $Cu^{2+}$ contained on wastewater before and after biosorption process in the batch column. AAS could measure concentration inside the samples until µg/L. This quantitative analysis technic is using light wave length absorbed by metal element.

The *Fourier Transform Infra-Red* (FTIR) was used to study the functional groups of the biosorbent. The *Scanning Electron Microscopy* (SEM) analysis was performed in order to find the morphological surface of the biosorbent. SEM is an electronic microscope using electron bundles which reflected in high energy to describe surface form material analyzed. Biosorbent sample was analyzed its morphologic form before and after contacted with Cu2+ waste on electroplating wastewater. After dried, the samples (0,71 hingga 1 mm) layered with a thin gold layer (10 nm) using sputter coater and observed using *Scanning Electron Microscope* (20 kV) in vacuum condition of 1,33 x $10^{-6}$ mBar. SEM analysis is a qualitative analysis instead of quantitative.

The determination of dry weight was conducted by filtrating the solution from the treatment using Whatman no. 42 filter paper with a pore size of 2.5 µm. After filtration, the filter paper was dried in the oven at 105°C for 2 hours and then weighed using the analytical balance.

## 3. RESULTS AND DISCUSSION

### 3.1 Determination of Optimum (pH)

It is seen from Fig. 1 that the addition of more nutrients than waste (ratio A) causes the metabolism of *S.obliquus* to be better when compared to the treatment without the addition of the nutrients. Thus, the *S.obliquus* will be able to remove the heavy metals when the nutrients are added. PHM is chosen as microalgae growth media based on research by Rinanti et al. [10]. The research result shows that PHM media contains macro and micronutrient that fitted for S. obiquus growth and proved to gives the highest biomass with fast growth rate compared to other growth media like Zarrouk and Schlosser. Rinanti et al. [10] report that microalgae could grow in those three media, but PHM proved to be best growth media, with Optical Density (OD) and cell density (cell/mL) 0.25 and 34.49, respectively, and a total of chlorophyll content (mg/L) of 0.43

In cultures with the acidity value (pH) that is highly acidic such as 4 and 5, the growth of *S. obliquus* is suspected to be inhibited. It was shown by the small removal of heavy metal in treatments A and D. The pH value of 6 and 7 is fairly good for the growth of *S.obliquus*. By giving an adequate amount of the nutrients at pH 6, *S.obliquus* is capable of removing copper ions ($Cu^{2+}$) for treatment A and B, respectively by 48.84% ± 3.48 and 43.17% ± 0.97. At ratio (C), *S.obliquus* is also capable of removing copper ions ($Cu^{2+}$) with the removal percentage of 34.25% ± 0.92. This result is similar to that of the ratio (D) with the removal percentage of 36.04% ± 0.69.

Cu accumulation increases ion H+ concentration. Due to equilibrium reaction, increasing in pH makes reaction moves to ion H+ production that means more Cu being complexed. This metal ion accumulation process tends to remain in the cells due to rate constant value of metal releases that lower than its absorption. Absorption process and toxic material accumulation inside the cell will break down and excreted, stored or metabolized by microalgae, depends on concentration and chemical potential of the material. Hydrophilic chemical material like Cu usually easier to excreted than hypoxic metal.

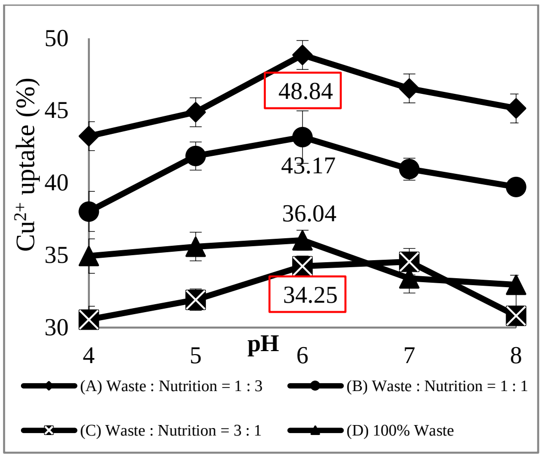


Fig. 1 The effect of variation of acidity value (pH) against the removal (%) at a temperature (28 °C ± 2) and contact time (Td) 60 minutes

Although Cu is hydrophilic metal, some researchers report that Cu could bound and accumulated inside cells. In pH base, metal ion spontaneously reacted with hydroxide ion forming hydroxide metal bond while in pH acid will be a competition between metal ion with H+ ion to binding with microalgae cells wall. [2], [4], [14]. Future research will study in various temperature and concentration solid/liquid ratio.

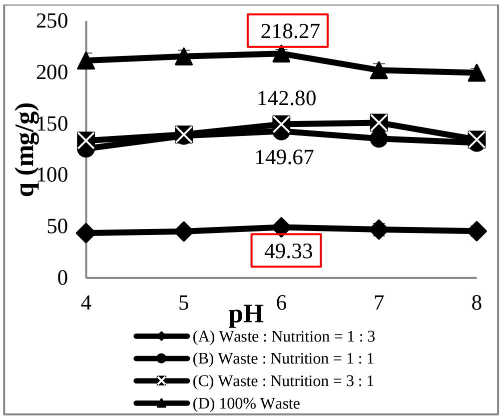


Fig. 2 The effect of variation of adsorption capacity (q) against the removal (%) at a temperature (28 °C ± 2) and contact time (Td) 60 minutes

Fig. 2 shows that the adsorption capacity has the same pattern as the removal efficiency, or in other words, the adsorption capacity is directly proportional to the removal efficiency. At the medium acidity value (pH) of 6 and 7, *S.obliquus* shows the adsorption capacity (q) for both ratio (A) and ratio (D), respectively providing q value of 49,33 mg/g and 218,27 ± 3,51 mg/g ± 4,16. According to the previous research that has been conducted [10,11, 13, 14], *S.obliquus* can grow well at the optimum pH of 6.

### 3.2 Determination of Contact Time

Fig. 3 shows that the removal efficiency of copper ions ($Cu^{2+}$) at a ratio (A) tends to be constant from the contact time (Td) 60 minutes to 240 minutes, and this efficiency then declines at the contact time of 300 minutes. The best removal efficiency was 65.54% ± 8.04, obtained at the contact time of 240 minutes. The similar results on the removal efficiency of copper ions ($Cu^{2+}$) are shown in the ratio (D) although its removal efficiency is less than that of the ratio (A). At ratio (D), *S. obliquus* is still able to adsorb copper ions and shows similar removal efficiency patterns as a ratio (A). The highest removal efficiency on the ratio (D) also occurs on contact time (Td) 240 minutes with a removal efficiency of 41.99% ± 1.01.

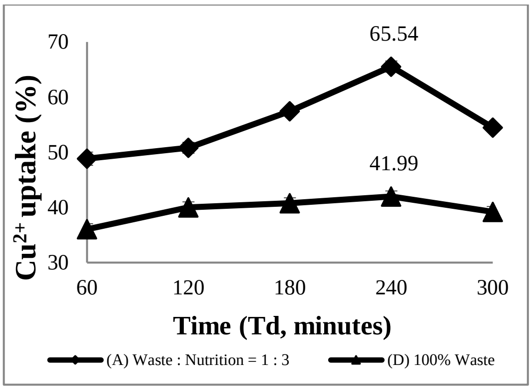


Fig. 3 The effect of variation of contact time (Td, minutes) against the removal (%) in ratio A and D, pH 6 (optimum) and temperature 28°C ± 2

The decrease in the removal efficiency which occurs at the contact time (Td) 300 minutes might be because the cell of *S. obliquus* has reached the saturation point in adsorbing the copper ions ($Cu^{2+}$). The adsorption of heavy metal ions by microorganisms occurs quickly and is reversible. In the reversible reaction, the biosorbent will be saturated at a certain point. After reaching the saturation point, the sorbent will release back the heavy metal ions which have been adsorbed into the solution. When the condition is not saturated, the sorbent will re-adsorb the heavy metals contained in the solution. The reaction will take place continuously [15,16].

Fig. 4 shows that the adsorption capacity (q) is proportional to the removal efficiency for either ratio (A) or ratio (D). The higher the removal efficiency, the higher the adsorption capacity. The highest adsorption capacity (q) was achieved in treatment A and D on the contact time (Td) 240 minutes. The values of the adsorption capacities are 59.58 mg/g ± 7.31 and 228.84 mg/g ± 5.50 for treatment A and D, respectively. From the calculation of the removal efficiency and the adsorption capacity (q) values, the optimum contact time is at 240 minutes.

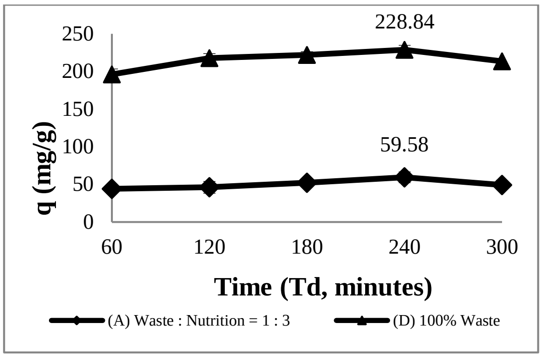


Fig. 4 The effect of variation of contact time (Td, minutes) to the capacity of adsorption (q) against the removal (%) in ratio A and D, pH 6 (optimum) and temperature 28°C ± 2

Either living or dead biomass could be used in biosorption study, but dead biomass gives more benefit, which is: the effect of poisonous of dissolved heavy metal could not affect the biomass sorption capacity; continuously supply nutrient is unnecessary; could be regenerated and reused in several times of cycles [17,18].

Usage of living microalgae biomass as the absorbent is an interesting option, with economically and effectively advantages of microalgae than other biomass [19]. Those advantages are the low of nutrient demand, high biomass production due to its autotroph, and unlike another biomass such as bacteria and fungi, microalgae usually not produce harmful compound [9]. Based on biosorption statistical data studied by Brinza [20], algae had used as biosorbent 15,3% more than other biomass and 84,6% more than fungi and bacteria.

The mechanisms involved in biosorption hardly identified, except in simple laboratory system. Biological materials are very complex. Besides that, bounding mechanisms of sorbate by biosorbent in biosorption is a complicated process so various kind of different mechanisms could occur at the same time at different rates [21].

Based on its dependence to cell metabolisms, biosorption mechanisms divided into two, dependent metabolisms and independent metabolisms [8,9]. Heavy metal absorption by microorganisms happens at two-stage, passive uptake and active uptake. Related to its dependence to cell metabolisms, passive uptake is metabolisms independent, since its physical-chemical absorption process at the cell surface and not involving cell metabolisms. Active uptake is metabolisms dependent, since its absorption process involving cell metabolisms [8, 22].

Active uptake is a slow process, including transport between cell membrane, sedimentation, and precipitation. Passive uptake is a fast process, including precipitation, physical-chemical adsorption, ion exchange and complexation [8, 22, 23].

When heavy metal ion dispersed around the cell, metal ion will be bounded in the element at cell walls based on its ability of cells chemical affinity power [24]. Before metal ion reaches cells membrane and cells cytoplasms, it must through the microalgae cell walls containing various polysaccharide and protein that has some active sites capable to bond with metal ion [23].

At that moment, there is an exchange of monovalent and bivalent, e.g. Na, Mg, Ca in the cell wall, replaced by heavy metal ion and then built complex formations between heavy metal ions and functional groups such as carbonyl, amino, thiol, hydroxy, phosphate and hydroxy-carboxyl.

This biosorption process is occurred in short time, reversible, and in dead or living cells. The process effectively occurred in certain pH and present another ion so heavy metal could become sedimented undissolved salt. Therefore, cell walls commonly state as the most important part of cell defense mechanisms, since cell walls are the first barrier against accumulation of toxic heavy metal.

After the biosorption process occurs (passive uptake), the next mechanism is active uptake where *S. obliquus* moves metal ion bounded into cell walls to deeper cell organ (bioaccumulation/absorption). This mechanism happen as metal ion consumptions occurred for cell growth and accumulation of metal ion

Usage of living *S.obliquus* in water remediation process needs to improve due to still many weakness on its process. This free cell using fitted to laboratory necessity, but not applicable in the field. The relatively small cell, low mechanisms power, excess hydrostatic pressure, will reduce cell capability to remediate. In future research, this system needs to improve by immobilized *S.obliquus* cells. Immobilized cells could load more biomass, minimize clogging, more resiliently pressure, maintenance and nutrition unneeded could be using many times, even possible to remove more concentration of heavy metal. In this research, metal ion Cu 2 valence will replace divalent or monovalent ion, existed at cell walls of *S. obliquus* so metal ion outside the cell will be reduced.

FTIR analysis aims to detect the changes in the variation of functional groups of *S.obliquus*. This analysis provides three major advantages as an analytical technique: a fast, non-destructive and requires a very little amount of sample [16]. Fig. 5

shows a spectrum of *Fourier Transform Infra-Red* (FTIR) which indicates the functional groups contained in *S.obliquus*. Before the adsorption process, the absorbance band was observed on 3338.80 $cm^{-1}$. This band shifted to 3339.52 $cm^{-1}$ in the FTIR spectra of the treatment A and to 3337 $cm^{-1}$ in the treatment D.

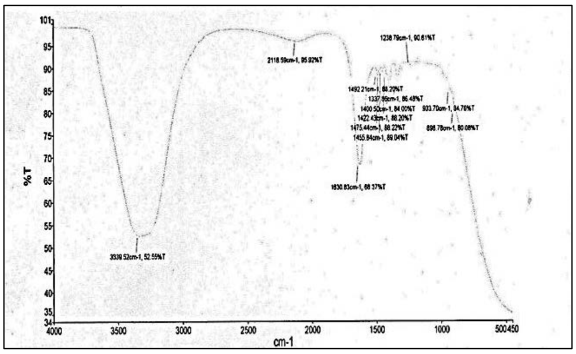


Fig. 5 *Fourier Transform Infra-Red* (FTIR) which indicates a functional group of *S.obliquus* after contacted with copper ($Cu^{2+}$)

The ratio A shows the presence of peak for C-C in the wavenumber of 1455.84 $cm^{-1}$. This means that the alkene bond has been broken and turned into a group. In addition, there is also a shifting for the absorbance of the amine functional group which is originally showed at the wavenumber value of 1340.70 $cm^{-1}$. It shifted to 1238.70 $cm^{-1}$ for ratio A and to 1230.20 $cm^{-1}$ for ratio D. The results of FTIR analysis shows that there has been a change in the functional groups of *S. obliquus* due to the interactions with copper ions ($Cu^{2+}$).

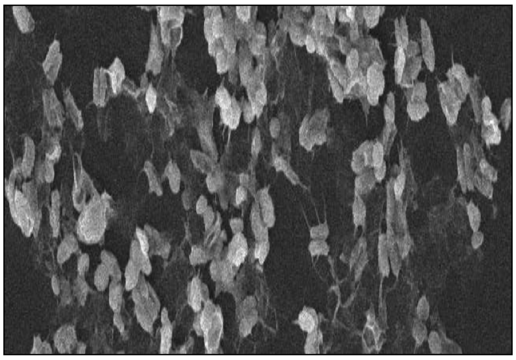

Fig. 6 *S.obliquus* prior to treatment with a magnification of 1000 times with *Scanning Electron Microscopy* (SEM)

The *Scanning Electron Microscopy* (SEM) analysis made it possible to evaluate the morphological changes in the surface of the cells. However, SEM analysis only provides a qualitative analysis and a surface structure estimations. Fig. 6 shows the surface morphology of *S.obliquus* before the adsorption process. The *S.obliquus* cells have a whip shape, flat, thin, and arranged in a parallel arrangement. The type of colony formed is called *coenobium* [25, 26].

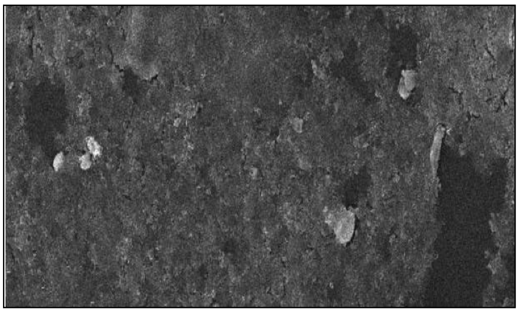

Fig. 7 *S.obliquus* after treatment with waste ratio : nutrient (1:3) to initial concentration 5.05 mg/L, at temperature (28°C ± 2), pH 6 (optimum) and contact time (Td) 240 minutes (optimum), the magnification of 1000 times seen with *Scanning Electron Microscopy* (SEM)

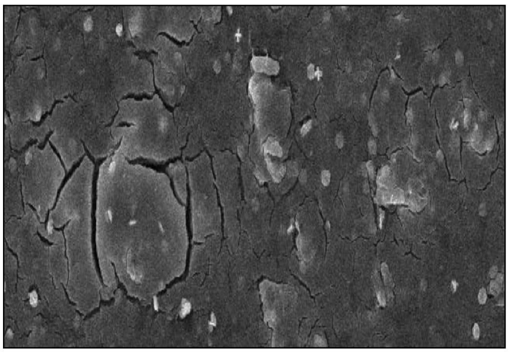

Fig.8 *S.obliquus* after treatment with 100% of waste to initial concentration of early 30.28 mg/L, at a temperature (28°C ± 2), pH 6 (optimum) and contact time (Td) 240 minutes (optimum), the magnification of 1000 times seem with *Scanning Electron Microscopy* (SEM)

Fig. 7 and Fig. 8 show the occurrence of damages of *S.obliquus* cells after the adsorption of copper ions ($Cu^{2+}$). The cells are shrinking, the cell shape is not flattened and the cell wall matrix is broken. The changes in the morphology of the surface are normally associated with the cross interference between metal ions and the negatively charged functional groups such as carboxyl groups in the cell wall polymers [25, 27, 28].

Fig. 9 shows the dry weight obtained at the optimum pH of 6. The dry weight for ratio A and ratio D are 1.07 x $10^{-1}$ and 8.69x$10^{-2}$, respectively. The dry weight at pH 6 is higher than the dry weight at pH 5 and 7, which are 1.07 x $10^{-1}$ mg/mL and 8.69x$10^{-2}$ mg/mL, respectively. Fig. 10 shows that the *S.obliquus* is able to perform an optimum adsorption of copper ions at pH 6 and the contact time of 240 minutes. The dry weight of the biomass is 3.70 x $10^{-1}$ mg/mL for ratio A and 2.37 x $10^{-2}$ mg/mL for ratio D.

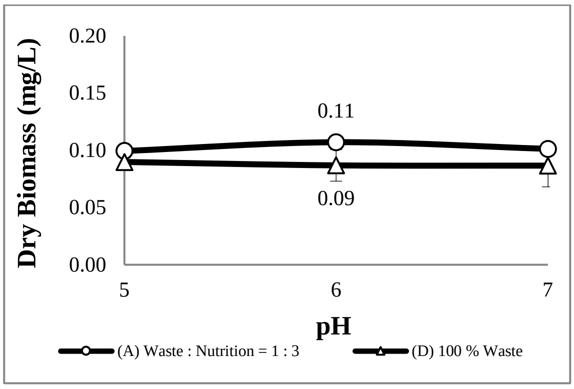


Fig. 9 Dry weight of *S. obliquus* after treatment with acidity value variations (pH) at a temperature (28°C ± 2) and contact time (Td) 60 minutes

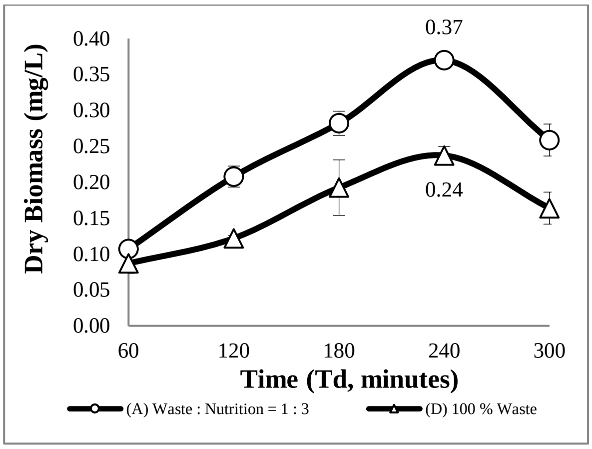


Fig. 10 The dry weight of *S. obliquus* after treatment with the variation of contact time (Td) at a temperature (28°C ± 2) and pH 6 (optimum)

The adsorption processes for ratio A and D fitted the Freundlich and Langmuir isotherm model both. The isotherm is based on the variation of contact time (Td) (in this research: 60, 120, 180, 240 and 300 minutes) at the acidity (pH) value of 6 (optimum), the initial concentration of copper ions ($Cu^{2+}$) for the ratio A: 5.05 mg/L and for ratio D: 30.28 mg/L. The data fitted the linear curve of Freundlich isotherm with the $R^2$ value of 0.9961 and 0.9985 for ratio A and D, respectively. Otherwise, $R^2$ value of Langmuir isotherm is lower, which are 0.9844 and 0.9939 for ratio A and D, respectively.

The Freundlich isotherm is used to describe a non-ideal adsorption on the heterogeneous surfaces. Heterogeneity can be caused by the differences in functional groups on the surface of the adsorbent. Freundlich adsorption isotherms assumed that the adsorption occurs at different layers (multilayer) of the adsorbent [15, 28].

The adsorption processes of A and D fitted the Freundlich isotherms with the n value less than ten. It can be concluded that the adsorption is reversible [20]. The Freundlich isotherm is based on assumptions that adsorbent consists of different classes of adsorption sites. In another side, The Langmuir isotherm assumes that the adsorbent surface adsorption has a number of specific points that are comparable with the adsorbent surface area and the adsorption occurs only in monolayer. According to the theoretical models of adsorption, it is not possible for the adsorption process to proceed on the surface which has been occupied by an adsorbate. If the adsorption point is saturated and the concentration of the adsorbate is increased, the amount of heavy metal ion adsorbed is either increased or decreased [15, 25, 27, 30].

### 3.3 Adsorption Kinetics.

In this study, the approach in the calculation of the kinetic parameter was using one order model since the biological process occurred mainly in one order. To draw the graph, data at 300 minutes contact is deleted, since it is a decreasing in adsorbate concentration value.

Based on the results of kinetic calculations for treatment A and D, it is obtained that the $R^2$ value of 0.8103 for treatment A (Figure 11) and 0.8791 for treatment D (Fig. 12). The adsorption kinetics study aims to know the reaction order of the adsorption process. The prediction from the flow of biosorption gives an important information in generating a biosorption system of batch scale [15].

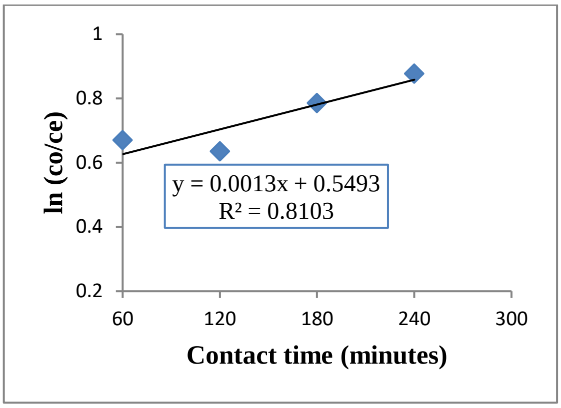


Fig. 11 The kinetics graph-one order of ratio A (waste : nutrient = 1: 3) with initial concentration 5.05 mg/L, temperature (28°C ± 2), pH 6 (optimum) and contact time (Td) 240 minutes (optimum)

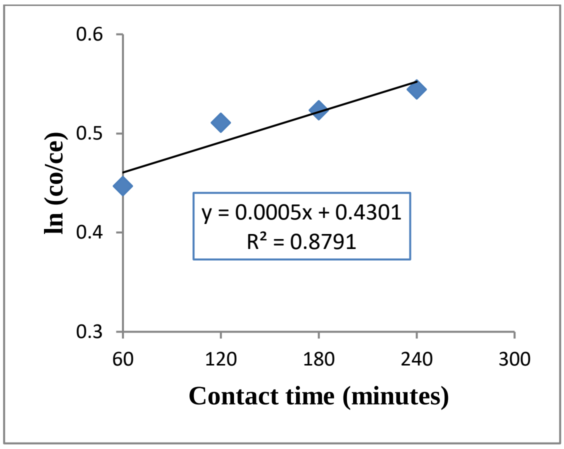


Fig. 12 The kinetics graph of order one in ratio D (100% waste) with initial concentration 30.28 mg/L, temperature (28°C ± 2), pH 6 (optimum) and contact time (Td) 240 minutes (optimum).

## 4. CONCLUSION

*S.obliquus* able to grow in a wastewater containing copper heavy metals ($Cu^{2+}$) either with or without the nutrient addition. In conditions of optimum pH 6 and optimum contact time (Td) of 240 minutes, the removal efficiency of heavy metal ions of copper ($Cu^{2+}$) reached 65.54% ± 8.04 in the solution containing waste and nutrients in the ratio of 1: 3.

In the solution containing only waste, the removal efficiency was 41.99% ± 1.01. In this study, the effect of heavy metals ($Cu^{2+}$) on *S.obliquus* cells is shown by the breaking of (the destruction) of carboxyl and amine functional groups, as well as-as the alkene group. The morphological analysis conducted using SEM shows that there is a damage to the cells of *S.obliquus*. The results of this study fitted the Freundlich isotherm models with $R^2$ value nearly reaching 1, a little higher than R2 value of the Langmuir isotherm model. The adsorption kinetics was studied in the order one kinetics with $R^2$ value of 0.2486 for the solution containing waste and nutrients in the ratio of 1:3 and 0.336 for the solution containing only wastewater.

## 5. ACKNOWLEDGMENTS

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