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Effect of immobilized biosorbents on the heavy metals (Cu^{2+}) biosorption with variations of temperature and initial concentration of waste

W P Siwi, A Rinanti, M D S Silalahi, R Hadisoebroto, M F Fachrul*

Environmental Engineering Department, Faculty of Landscape Architecture and Environmental Technology, Universitas Trisakti, Jakarta, Indonesia

*Corresponding Author: melati@trisakti.ac.id

Abstract. The aims of research is to studying the efficiency of copper removal by combining immobilized microalgae with optimizations of temperature and initial Copper concentration. The research was conducted in batch culture with temperature variations of 25°C, 30°C, and 35°C, as well as initial Cu^{2+} concentrations (mg/l) of 3, 5, 10, 15 and 20 using monoculture of *S. cerevisiae*, *Chlorella* sp., and mixed culture of them both as immobilized biosorbents. The optimum adsorption of 83.4% obtained in temperature of 30°C with an initial waste concentration of 17.62 mg/l, initial biomass concentration of 200 mg, pH of 4, and 120 minutes detention time by the immobilized mixed culture biosorbent. The cell morphology examined using Scanning Electron Microscope (SEM) has proved that the biosorbent surface was damaged after being in contact with copper (waste), implying that heavy metals (molecules) attach to different functional cell surfaces and change the biosorbent surface. The adsorption process of this research follows Langmuir Isotherm with the R^2 value close to 1. The immobilized mixed culture biosorbent is capable of optimally removing copper at temperature of 30°C and initial Cu^{2+} concentration of 17.62 mg/l.

Keywords: biosorbent, biosorption, heavy metals, immobilized microalgae, Langmuir Isotherm

1. Introduction

The development of industrial sectors such as mining, electroplating, agriculture, fisheries, and others increases heavy metal pollution in the environment. One of the heavy metal waste that pollutes the environment is copper (Cu). Cu is a heavy metal widely utilized in the power industry, also as fungicides and anti-pollutant paint. One of the alternative methods that can be applied to overcome the pollution problem is biosorption, a term to describe the removal of heavy metals by passive binding in biomass plants or microorganisms in a solution through the metabolic or chemical-physical steps including the removal of toxins from hazardous materials. The advantages of using biosorption method are economical, easily available, as well as environmentally friendly because it utilizes biological materials. The biosorption process is influenced by the initial concentration of metal-containing waste, temperature, pH, and biomass concentration in the solution [1]. This research was carried out by optimization of variations of temperature and initial concentration of the waste.

In general, biomass can be used as biosorbent in the form of microorganisms such as bacteria, microalgae, and fungi, whether living or dead. However, microalgae's weak structure and tiny size are not suitable for continuous operation of metal elimination. Immobilization of biomass is then



implemented to overcome this problem. Such immobilization is beneficial in improving the mechanical strength and resistance to chemicals, as well as facilitating the separation of biomass and generated effluent [2]. This Cu²⁺ metal biosorption study was conducted in a batch reactor using the immobilized consortium of *Chlorella* sp. and *S. Cerevisiae* with an optimization of temperature and initial concentration of waste to treat wastewater containing Cu²⁺.

2. Research Method

The optimal biosorption was achieved through three stages of testing, i.e., the determination of mobile and immobilized biosorbents with a concentration of 200 mg and 500 mg, followed by the evaluation of the effects of temperature using variations of 25°C, 30°C, and 35°C; and continued with the optimization of initial waste concentration using variations of 3 mg/l, 5 mg/l, 10 mg/l, 15 mg/l, and 20 mg/l.

2.1. Optimization Test of Temperature

The immobilized biosorbent was contacted with 100 ml of waste containing Cu²⁺ ions with a concentration of 10 mg/l at pH 4 and in 120 minutes detention time, by aeration and regulated with temperature variations 25°C, 30°C and 35°C. Afterwards, the solution was measured using AAS, and the final concentration was calculated to obtain the removal efficiency.

2.2. Optimization Test of Initial Waste Concentration

The immobilized biosorbent was contacted with 100 ml of waste containing Cu²⁺ ions with various concentrations of 3 mg/l, 5 mg/l, 10 mg/l, 15 mg/l, and 20 mg/l at the optimum temperature and pH 4, with a contact time of 120 minutes and aeration treatment. Afterwards, the solution was measured using AAS, and the final concentration was calculated to attain the removal efficiency.

2.3. Analysis of Removal Efficiency and Adsorption Capacity

The removal efficiency and metal adsorption capacity of Cu²⁺ can be calculated by the following formula [3]:

$$\text{Removal Efficiency (\%)} = \frac{C_0 - C_e}{C_0} \times 100\% \quad (1)$$

$$q \text{ (mg/g)} = \frac{(C_0 - C_e) \times V}{W} \quad (2)$$

3. Results and Discussion

The optimization of Cu²⁺ metal biosorption was carried out using a single culture of *S. cerevisiae*, monoculture of *Chlorella* sp., and a mixed culture consisting of *S. cerevisiae* and *Chlorella* sp. (1:1). The characterization results of electroplating industrial wastewater samples were used to acquire the initial concentration of waste by 53.674 mg/l. It was then diluted to get the intended variations of initial waste concentration for this research.

3.1. Determination of Mobile and Immobilized Biosorbents

The optimization test of Cu²⁺ metal biosorption was carried out using a single culture of *S. cerevisiae*, a monoculture of *Chlorella* sp., and mixed culture of *S. cerevisiae* and *Chlorella* sp. (1:1), treated with mobile and immobilized biosorbents. The experiment was conducted at pH 4, a contact time of 120 minutes, an initial concentration of Cu²⁺ wastewater of 2.99 mg/l, a temperature of 30°C, as well as initial biosorbent concentrations of 200 and 500 mg.

Based on the removal efficiency of Cu²⁺ as shown in Figure 1A, the biosorption using abiosorbent concentration of 200 mg indicated that mobile and immobilized biosorbents could adsorb Cu²⁺ ions, but the higher adsorption was delivered by immobilized biosorbent. It implies that both single culture and mixed culture are capable of performing adsorption, but higher adsorption is achieved by the mixed culture. The maximum adsorption of 71.91% was attained by the immobilized mixed culture biosorbent. Compared with the removal efficiency value shown in Figure 1B, the biosorption using a

biosorbent concentration of 500 mg showed its capability to absorb even higher, reaching 81.05% using the immobilized mixed culture. It reveals that the higher the initial biosorbent concentration, the greater the yielded removal efficiency. It is in line with [3] who reported that the biosorption of Uranium (VI) by immobilized biosorbent of *A. niger* with initial concentrations ranged from 0.1 to 1.2 g/l showed that the removal efficiency of Uranium (VI) increased from 55.2% to 93.4% for an increment of initial biosorbent concentration of 0.1–0.5 g/l.

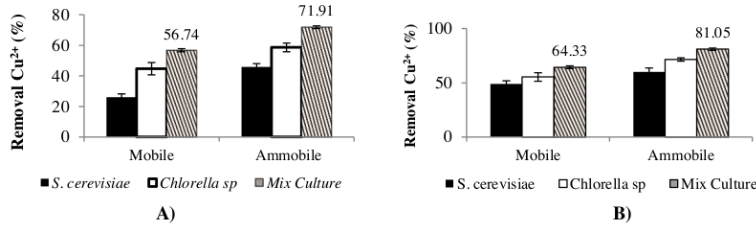


Figure 1. The relationship of Cu^{2+} removal efficiency with mobile and immobilized biosorbents at pH 4, 120 minutes contact time, initial Cu^{2+} concentration of 2.99 mg/l, as well as a biosorbent concentration of A) 200 mg and B) 500 mg

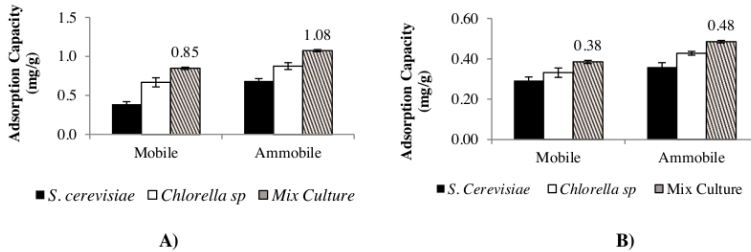


Figure 2. The relationship of Cu^{2+} adsorption capacity with mobile and immobilized biosorbents at pH 4, 120 minutes contact time, initial Cu^{2+} concentration of 2.99 mg/l, as well as initial biosorbent concentration of A) 200 mg and B) 500 mg

Adsorption capacity values in Figure 2A and Figure 2B are opposed to removal efficiencies in Figure 1 and Figure 2; the greater the initial concentration of biosorbent used, the smaller the adsorption capacities. The initial biosorbent concentration of 200 mg with mixed culture had a higher adsorption capacity compared with the initial concentration of 500 mg biosorbent. The maximum adsorption capacity was demonstrated by the immobilized mixed culture biosorbent with a concentration of 200 mg or equal to 1.08 mg/g.

The results obtained in this study are consistent with [4] which observed that the adsorption capacity of immobilized *Chlorella sp.* was greater than the mobile biomass of *Chlorella sp.*, reaching a removal capacity of Cu^{2+} ions of up to 24.91 mg/g. The study of [5] also reported using immobilized biosorbent of *Chlorella sp.* that had an adsorption capacity of Cu^{2+} ions of 28.5 mg/g.

Based on these matters, it can be concluded that immobilized biosorbents have a better ability to adsorb Cu^{2+} than the mobile ones. The initial concentration of 200 mg biosorbent has a higher adsorption capacity than the initial concentration of 500 mg biosorbent.

3.2. Temperature Optimization Test

At the temperature of 25°C, immobilized biosorbent could present a removal efficiency of 60.22–73.58%, while at the temperature of 30°C, it was capable of increasing the Cu²⁺ removal of up to 79.4%. At the temperature of 35°C, the adsorption efficiency gradually decreased of only up to 66.97%. It shows that the higher the temperature, the biosorbent becomes less capable of performing adsorption.

The results of this study correspond to [6] which researched the optimum adsorption temperature of heavy metals-Cr (VI), Cd, Cu²⁺, and Ni-using immobilized mixed culture biosorbent and temperature variations ranging from 10–50°C. It observed that the efficiency of metal uptake continued to increase at a temperature of 10–30°C, then the reduction of adsorption efficiency occurred along with the temperature increase. The optimum adsorption efficiency of the contained metal of 90% was achieved at the temperature of 30°C. According to [6], a decrease in the adsorption efficiency at 40–50°C was led by the increased absorption of heavy metals from the solid to liquid phase, the surface deactivation of biosorbent, as well as the damage to the active site on the surface of biosorbent due to the disruption or the weakening bonds of active site in binding heavy metals.

3.3. Optimization of Initial Concentration of Waste Containing Cu²⁺ Ions

The optimization test of initial concentration of waste containing Cu²⁺ by immobilized biosorbent was performed at pH 4 with concentration variations of 2 mg/l, 5 mg/l, 10 mg/l, 15 mg/l and 20 mg/l; using the optimum temperature obtained at the previous test of 30°C, 120 minutes contact time, and an initial biosorbent concentration of 200 mg.

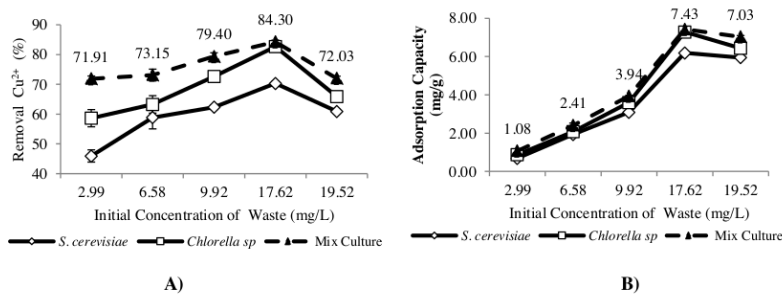


Figure 3. Effect of initial waste concentration optimization on A) the removal efficiency (%) and B) adsorption capacity (mg/g) at pH 4, 120 minutes contact time, 30°C temperature, and initial biosorbent concentration of 200 mg.

Figure 3A shows that the removal efficiency of Cu²⁺ by immobilized biosorbent was increasing and the highest removal efficiency of 84.30% was obtained at a concentration of 17.62 mg/l. Figure 3A and Figure 3B show that the immobilized mixed culture biosorbent could adsorb better than the single culture due to the active site discrepancy of the biosorbents. The mixed culture has more active sites used to carry Cu²⁺ adsorption than that owned by the monoculture.

The adsorption capacity of immobilized biosorbent had the range of 0.69–7.43 mg/g on the optimization of the initial concentration of Cu²⁺ waste with variations of 2.99 mg/l, 6.58 mg/l, 9.92 mg/l, 17.62 mg/l, and 19.52 mg/l. The highest adsorption capacity of 7.43 mg/g was generated by the immobilized mixed culture biosorbent with the initial Cu²⁺ waste concentration of 17.62 mg/l.

Researchers [7] stated that the higher the concentration of metal ions, the more the amount of the substance adsorbed to the achievement of the optimum concentration. Once the optimum concentration

is obtained, the biosorbent has surely been saturated. According to [8], the increase of the biosorption ability is directly proportional to the increase in concentration. It relates to the effect of stresses that occur, ergo increasing all ionic transfers and leading to a higher adsorption of metal ions.

3.4. Analysis of Fourier Transform Infra-Red (FTIR)

The analysis of Fourier Transform Infra-Red (FTIR) was conducted to determine changes in functional groups of biosorbent cells before and after being contacted with Cu^{2+} as they were estimated binding the Cu^{2+} ions. FTIR results of different wave lengths indicate a change of functional groups as listed in Table 1 which describes the wave lengths used, the functional groups, and the content changes of the functional groups of immobilized biosorbent cells.

Table 1. FTIR analysis of biosorbent before and after being in contact with Cu^{2+} .

Functional Groups	<i>S. cerevisiae</i>	<i>S. cerevisiae</i>	<i>Chlorella</i>	<i>Chlorella</i>	Mixed	Mixed
	Before (%)	After (%)	Before (%)	After (%)	Culture Before (%)	Culture After (%)
C-H (alkene)	97.71	95.86	97.60	94.45	96.03	94.45
C-O (carboxylic acid)	98.19	-	98.44	-	96.89	-
C-N (amine-amide)	98.19	96.73	98.44	95.84	96.63	95.84
C-H (alkene)	97.84	95.07	98.25	95.77	98.94	98.20
C=C (alkene, aromatic ring)	97.67	94.28	98.06	95.76	96.88	93.12
C=O (aldehyde, carboxylic acid)	97.62	-	98.48	98.43	98.45	95.83
O-H (phenol)	-	98.26	-	98.91	-	98.17
C≡C (alkalo)	-	98.68	-	98.79	-	98.79

Changes in functional groups that occurred to the immobilized biosorbents of *S. cerevisiae*, *Chlorella* sp., and mixed culture showed that the functional groups were involved in the adsorption process of Cu^{2+} ions. Changes of functional groups of biosorbents were present either in the form of losses of both formerly existed and non-existed functional groups or changes in the levels of biosorbent functional groups.

Previously, alterations of the biosorbent surface observed through SEM has explained that the occurred changes were due to the involvement of functional groups attached to the cell wall of the biosorbent during the biosorption process. It was reinforced by the FTIR results afterward that indicated a change of functional groups before and after the biosorbent being in contact with Cu^{2+} . The aspect affecting the loss or reduction of functional groups contained in the biosorbent as described by [9] is the chemical adsorption by bonding with a number of functional groups (hydroxyl, carboxyl, carbonyl, and amino groups) contained on the surface of the microalgal cell, ensuing exchanges of monovalent and divalent ions such as Na, Mg, and Ca in the cell walls to be replaced by heavy metal ions.

4. Conclusion

Both of mobile and immobilized biosorbents were capable of absorbing Cu^{2+} , but the higher adsorption of 71.91% was delivered by the immobilized biosorbent. Both single culture and mixed culture biosorbents were capable of performing adsorption as well, but the higher adsorption of 71.91% was achieved by the mixed culture. Temperature and initial waste concentration affect the adsorption of Cu^{2+} ions by single culture biosorbents of *S. cerevisiae* and *Chlorella* sp., as well as the immobilized mixed culture biosorbent. The optimum Cu^{2+} adsorption efficiency of 83.4% occurred at a temperature of 30°C, an initial waste concentration of 17.62 mg/l, an initial biosorbent concentration of 200 mg, pH 4, and 120 minutes contact time by the immobilized mixed culture biosorbent. FTIR test results indicated changes of functional groups of both formerly existed chains such as carboxylic

acids and formerly non-existed chains as phenols and alkynes, as well as reduced levels of functional groups after the immobilized biosorbent had been being in contact with Cu^{2+} ions.

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