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Biosorption of Cu(II) Ions Using Living Microalgae Chlorella sp.: Effects of Microalgae Concentration, Salinity, and Light Color

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Abstract. Chemical industry wastewater containing metals must be treated so as not to threaten the environment or human life. One of the wastewater treatments is the biosorption process using living microalgae. Although living microalgae can provide better results as a biosorbent, the mechanism of this biosorption process is complex because it involves two steps of the process, active and passive uptake, which run simultaneously. In addition, several process parameters need to be adjusted for the biosorption process to operate optimally. This study aims to investigate the effect of several parameters such as microalgae concentration, salinity, and light color. Synthetic $CuSO_4$ solution at a concentration of 40 mg/L and pH 5 is used as artificial waste, while microalgae *Chlorella sp.* is used as biosorbent. The biosorption process was operated in a batch system at room temperature for 6 days. The experimental results show that 96.83% of the Cu(II) ions could be removed when the microalgae concentration, salinity, and light color were conditioned at 1.5 x 10^6 cells/mL, 3,000 mg/L, and red light, respectively.

Keywords: Biosorption; *Chlorella sp.*; Copper removal; Living biosorbent; Wastewater treatment

1. Introduction

Nowadays, various chemical industries are emerging and developing rapidly in order to fulfill human needs. It has had a positive impact on various sectors, including the economy. However, on the other hand, it also creates new challenges and problems, particularly in terms of industrial waste treatment. Improper industrial waste processing will negatively impact the environment, especially hazardous and toxic waste. Metal wastewater is one of the hazardous wastes requiring proper treatment. This waste can originate from a variety of chemical industries, such as the mining, electroplating, and metallurgical industry (Kim *et al.,* 2018; Sun *et al.,* 2013). Metal wastewater is classified as hazardous waste because some metal elements have toxic and even carcinogenic properties (Yang *et al.,* 2019; Gunatilake, 2015). It has a significant potential to endanger human life and public health. Therefore, reducing and eliminating this hazardous potential must be carried out effectively and efficiently.

Physical and/or chemical wastewater treatment, e.g., adsorption, membrane filtration, ion exchange, chemical precipitation, coagulation and flocculation, oxidation/reduction, and electrochemical treatment is an industrially applicable method for metal wastewater

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wastewater treatment (Ullah *et al.,* 2020; Gunatilake, 2015; Barakat, 2011). This method involves microorganisms to reduce metal content in the wastewater.

One method that can be used to treat metal wastewater is the biosorption process. The definition of biosorption process is a process that utilizes the ability of biological materials (living and/or non–living) to reduce metal content in the wastewater (Kusrini *et al.,* 2021; Volesky, 2007). This process will adsorb and/or consume the metal ions physicochemical and metabolically (Volesky, 2007). Biosorption is an option that should be considered because it has many advantages, which include low capital and operating costs, using fewer chemicals, producing less sludge, being effective at low metal concentration, and high efficiency (Anuar *et al.,* 2019; Abdi and Kazemi, 2015; Abbas *et al.,* 2014; Fomina and Gadd, 2014). Bacteria, fungi, microalgae, and yeast are the biological materials used as biosorbents (Wang and Chen, 2009). As a biosorbent, microalgae has some advantages, including the ability to remove metals from wastewater with high efficiency, the ability to be regenerated, does not produce toxic sludge, a high growth rate, can be applied in batch and continuous systems, being safe, inexpensive, and can live in open water (fresh or marine water) (Daneshvar *et al.,* 2018; Brinza, Dring, and Gavrilescu, 2007; Borowitzka, 1999).

Most studies related to the biosorption process of metal wastewater use non–living microalgae (biomass) as biosorbents (Chu and Phang, 2019; Kücüker, Nadal, and Kuchta, 2016; Utomo *et al.,* 2016). However, another interesting thing to be applied is the use of living microalgae as biosorbent and this is still not much to be explored further. Theoretically, living microalgae provide better removal results because of their mechanism which involves two steps. The first step is called passive uptake, and this step will bind (or adsorb) the metal ions to the cell surface. The second step is active uptake which the metal ions will be accumulated in the cell across the cell membrane (Das, Vimala, and Karthika 2008). Generally, the passive uptake has a faster rate process than the active uptake (Hawari and Mulligan, 2006). Living microalgae as a biosorbent has an advantage where passive and active uptake will run simultaneously during the biosorption process. On the other hand, when the biosorption process uses non–living microalgae, active uptake will not occur. Thus, living microalgae have a big potential to develop further to remove the metal ions from wastewater.

In simple terms, the biosorption process's main principle using living microalgae is (1) to ensure microalgae grow and reproduce as much as possible, and (2) an adsorption step through the cell surface of microalgae will occur. However, this process is categorized as complex. This process's complexity will be significantly influenced by several factors, such as solution pH, temperature, biosorbent dosage, ionic strength, initial solute concentrate, agitation rate, time, nutrients, light intensity, and salinity (Shihab, Dhahir, and Mohammed, 2020; Luangpipat and Chisti, 2017; Lee, Jalalizadeh, and Zhang, 2015; Das, 2010). These process parameters can either support or conflict with the performance of the biosorption process. For example, Wanta *et al.* (2020) studied the effect of the initial concentration of Cu(II) ions in the solution. The results of their study showed that there was a certain concentration that provides optimum biosorption results. A higher concentration of Cu(II) ions would kill the microalgae and decrease biosorption performance. It was characterized by a very low removal percentage when higher concentrations were used (Wanta *et al.,* 2020). Thus, it is necessary to investigate the effect of other parameters on the biosorption process using living microalgae. When the process is implemented on an industrial scale, it can operate optimally.

In this study, the microalgae concentration, salinity, and light color were studied for the biosorption process with living microalgae. The number of microalgae used as a biosorbent affects the reducing Cu(II) ions in the waste solution. However, it should also be noted that a large number of living microalgae may be detrimental to the performance of the biosorption process due to nutritional competition. It can cause rapid destruction of microalgae, and the active uptake step does not run optimally. In addition, the growth of microalgae is also influenced by the salinity level and light color. Therefore, the purpose of this study is to investigate the effect of these three parameters on the biosorption process, particularly its mechanism.

2. Methods

2.1. Materials

The microalgae *Chlorella sp.* which was used as a biosorbent in this study was harvested at the Center for Study of Water Technology and Waste Management, Parahyangan Catholic University. Walne's medium is a nutrient-rich growth medium for microalgae. The synthetic wastewater solutions were prepared using copper sulfate pentahydrate ($CuSO_4.5H_2O$) (Merck), while NaOH and HCl solutions were used as pH regulators. Sodium chloride (NaCl) (Merck) was also used to regulate the synthetic waste solution's salinity level. When the Cu^{2+} ion content in the liquid phase was analyzed, ammonia (NH_3) solution (Merck) was also used as a complexing agent. The entire biosorption process was carried out using RO water as a solvent.

2.2. Cultivation of Microalgae Chlorella sp.

For the microalgae cultivation process, the first step was to prepare a nutrient solution. This solution was made by mixing 1 mL of Walne's medium with 1 L of RO water. Then, this solution was then sterilized using an autoclave at 121°C for 15 minutes. After that, 500 mL of this solution was mixed with 1,500 mL of microalgae; then the solution is adjusted to a pH of 5. The equipment consists of a closed box, a 40-watt LED lamp, a glass bottle as a bioreactor, an air pump as an aerator, and also functions as a mixer. The cell density of microalgae *Chlorella sp.* was calculated daily using a hemocytometer for 6 days during the cultivation process.

2.3. Biosorption of Copper(II) Ions Using Microalgae Chlorella sp.

The biosorption process was carried out using a $CuSO_4$ solution where the Cu^{2+} ions concentration was 40 mg/L, and the pH of the solution was 5 (Wanta *et al.,* 2020). 950 mL of the solution was mixed with 50 mL of microalgae with various concentrations of 1.5–4.5 x 10^6 cells/mL. In addition, the solution's salinity level was also adjusted by adding 0, 3,000, and 6,000 mg/L of NaCl solution into the bioreactor. The lights used in this study were also varied in light color (white, red, and blue). After adjusting the settings for the light intensity, the lights were programmed to be on for 12 hours and off for 12 hours automatically.

The liquid phase was taken periodically for 6 days. That sample was centrifuged at 10,000 rpm for 5 minutes. The supernatant was analyzed for the remaining Cu^{2+} ion content using a UV–vis spectrophotometer (Mapada UV–6100 PC) at a wavelength of 610 nm. Before being analyzed, the supernatant was first mixed with 1 mL of NH_3 (as a complexing agent). In addition to testing the remaining Cu^{2+} ions content, the supernatant was also analyzed for cell density using a hemocytometer.

2.4. Data Analysis

2.4.1. Cell density analysis

The cell density of microalgae indicates the number of cells in a solution. The following equation was used to calculate cell density:

$$Number\ of\ cells = \frac{x}{80\ x\ 25\ x\ 10^{-8}} \quad (1)$$

where x is the number of living microalgae cells counted with a hemocytometer.

2.4.2. Removal percentage analysis

The removal percentage indicates the number of metal ions successfully adsorbed by the microalgae. The following equation was used to calculate the removal percentage:

$$\%\ R = \frac{Co - Ct}{Co}\ x\ 100\% \quad (2)$$

where %R is the percentage removal of Cu^{2+} ions, C_o is the concentration of Cu^{2+} when t = 0 (mg/L), and C_t is the concentration of Cu^{2+} ions at t (mg/L).

3. Results and Discussion

3.1. Effect of Microalgae Concentration on the Copper(II) Ions Biosorption Process

As a biosorbent, the quantity of microalgae in the biosorption system greatly influences the ability of microalgae to adsorb metal ions both actively and passively uptake. In this study, the microalgae concentration was varied between 1.5 x 10^6, 3.0 x 10^6, and 4.5 x 10^6 cells/mL, while the salinity level of the solution was 0 mg/L.

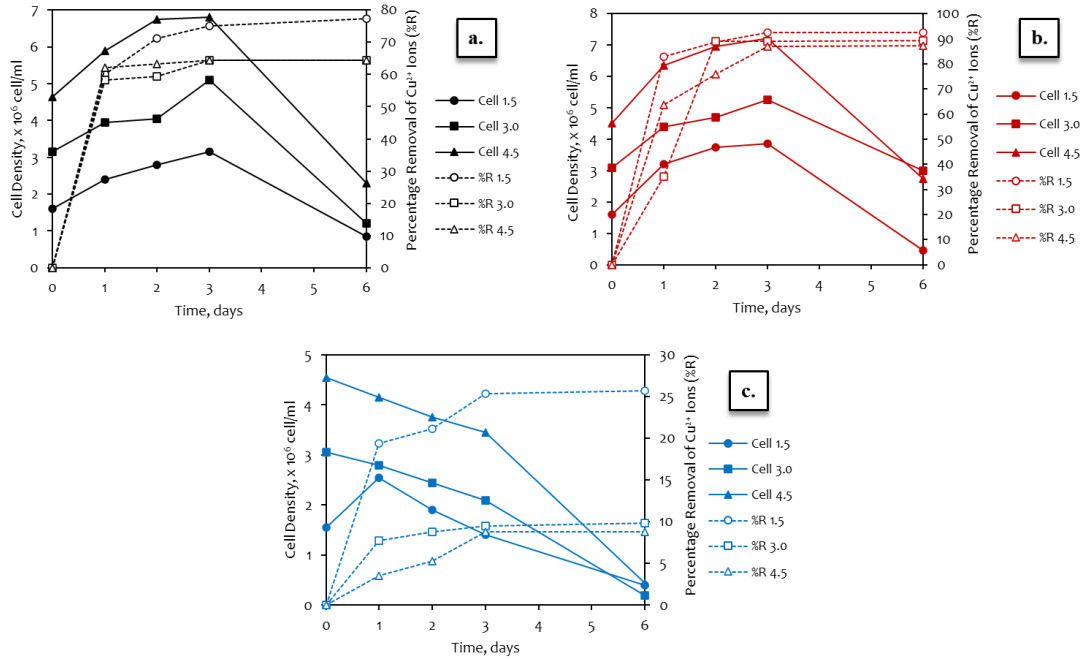

Figure 1 Experimental data on biosorption process by varying microalgae concentration with (a) white, (b) red, (c) blue lights and without regulating salinity levels

Figure 1 shows that the best Cu(II) ions removal percentage profile was achieved for the three types of light colors used when the lowest microalgae concentration was applied, namely 1.5 x 10^6 cells/mL. Increasing the concentration of microalgae inhibits the biosorption of Cu(II) ions. There are conflicting conditions between the number of microalgae cells and the performance of the biosorption process. The more microalgae cells in the solution cause the microalgae cells to be aggregated (Magdalena and Rygał, 2017;

Dönmez, Öztürk, and Kutsal, 1999). This study also reveals intercellular microalgae aggregation, as demonstrated in Figure 2. This phenomenon causes the surface area of contact between the microalgae cells and Cu^{2+} ions to decrease. The binding site (active site) of the microalgae used as an adsorbent medium will be blocked. Consequently, the metal uptake per unit of microalgae cells will decrease, as will biosorption performance.

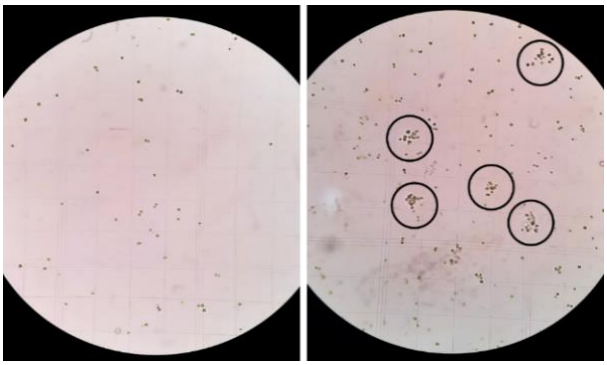

Figure 2 The appearance of microalgae growth after the biosorption process at a concentration of 1.5 x 10^6 cells/mL (left) and 4.5 x 10^6 cells/mL (right)

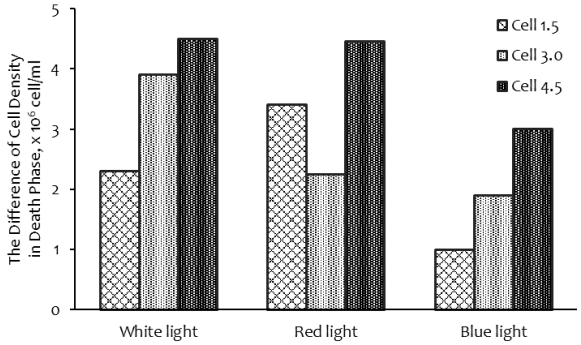

Figure 3 The decrease of cell density during the biosorption process

In addition, the large number of microalgae causes competition for nutrients between microalgae. Since the number of nutrients added to each variation of the experiment at t = 0 was the same, many microalgae did not get the optimal nutrition they needed. As a result, there will be a significant increase in the death phase of many microalgae. Based on the experimental data presented in Figure 3, the decrease in microalgae cell density in the highest death phase was achieved at a variation of the concentration of microalgae 4.5 x 10^6 cells/mL, where the difference in microalgae density during the process from the 3^{rd} to 6^{th} day ranged from 3.0–4.5 x10^6 cells/mL.

The kinetics of the Cu^{2+} ion biosorption process by *Chlorella sp*. are also depicted in Figure 1. In general, the biosorption process takes place rapidly, within 24 hours. This can be seen from the tendency of the removal percentage profile to increase sharply. The fast biosorption rate occurs due to the microalgae *Chlorella sp.* being able to adsorb Cu^{2+} ions both actively and passively. Cu^{2+} ion is one of the nutrients that contribute to microalgae cell metabolism. Microalgae will absorb as many Cu^{2+} ions as necessary for metabolic functions at the beginning of the biosorption process. This phenomenon is known as "active uptake." On the other hand, the surface of microalgae cells is the active side of microalgae as biosorbents. In this part, the solution's Cu^{2+} ions will be absorbed and reduce the Cu^{2+} ion levels in the solution. This is known as the passive-active mechanism.

After a day of the biosorption process, the removal percentage of Cu^{2+} ions still increased, although not as significantly as on the first day. This indicates that the microalgae are still carrying out the Cu^{2+} ion adsorption process. This insignificance trend indicates that the two mechanisms are not working optimally. This phenomenon is possible considering that the microalgae cell's active site has begun to be fully charged as a result of the rapid adsorption of Cu^{2+} ions on the first day of the biosorption process. The condition in which the biosorbent has begun to saturate will affect the adsorption rate, decreasing it.

The active uptake mechanism will dominate the increase in the removal percentage that still occurs. It can be proved that the cell density continued to increase during this period. In other words, the reduction of Cu^{2+} ions in the system occurs because the newly growing microalgae take up Cu^{2+} ions to meet their nutritional needs and accumulate in the microalgae cell.

After the third day of the biosorption process, the removal percentage of Cu^{2+} ions was already stagnant. These conditions suggest that the microalgae are unable to adsorb Cu^{2+} ions. Passive uptake occurs when the biosorbent has experienced saturation, where all the active sites of the biosorbent are filled with Cu^{2+} ions and are no longer able to adsorb these ions. In addition, by active uptake, microalgae cells have not grown or are already in the death phase. The absence of a change in the concentration of Cu^{2+} ions in this solution is called an equilibrium condition.

3.2. Effect of Salinity on the Copper(II) Ions Biosorption Process

As living organisms, microalgae require specific environmental conditions for survival. One of the factors that influence the growth of microalgae is the level of salinity. In this study, the salinity level was conditioned by the addition of NaCl salts at concentrations of 0; 3,000 and 6,000 mg/L. At the same time, the microalgae concentration was kept at 1.5 x 10^6 cells/mL because, in the previous section, these conditions gave the maximum biosorption results.

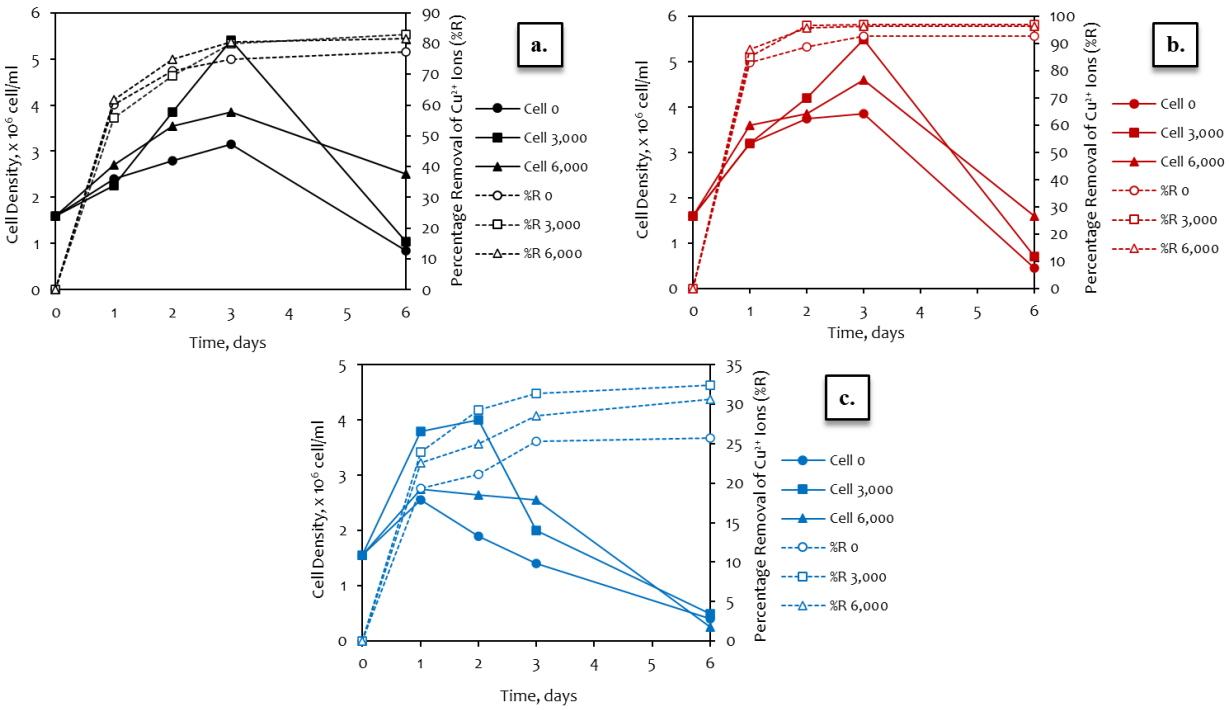

Figure 4 Experimental data on biosorption process by varying salinity with (a) white, (b) red, and (c) blue lights and microalgae concentration at 1.5 x 10^6 cells/mL

Figure 4 shows that the addition of 3,000 and 6,000 mg/L of NaCl salt has a positive effect on the growth of *Chlorella sp*. microalgae, as evidenced by the increase in microalgae cell density. However, this parameter's effect is not directly proportional because there are optimal conditions for adding the salinity level of the solution. When 6,000 mg/L of NaCl salt is added, it inhibits the growth of microalgae cells (compared to the addition of 3,000 mg/L of NaCl salt). This optimum condition occurs due to a decrease in microalgae's ability to absorb water for growth and cell division needs. This phenomenon is known as the salinity-water deficit effect.

In addition, the changes from low to high salinity environments will experience obstacles in the photosynthesis process. The addition of NaCl salt increases the concentration of Cl^- ions. The Cl^- ions have an essential physiological function in photosynthesis, especially the light phase. In the light-phase photosynthesis process, there is a transfer of electrons in the chlorophyll to form ATP, which will form carbohydrates in the dark phase. If no ATP is formed, the formation of carbohydrates in the dark phase will also be inhibited. Moreover, if Cl^- ions are unavailable, the growth and development of plants will also be hampered. This theory is reinforced by research conducted by Rai, Gautam, and Sharma (2015) where it was stated that the formation of ATP requires the presence of chloride ions during the process of photosynthesis. In his research, the microalgae *Chlorella sp.* proved to only live normally in environmental conditions with low salinity. When these microalgae are exposed to environmental conditions with high salinity, *Chlorella sp.* will experience chlorophyll degradation and cause microalgae cell death. Therefore, the increase in salinity levels helps microalgae cell growth, but only to a certain limit.

The relationship between cell density and the removal percentage of Cu^{2+} ions is illustrated in Figure 4. The results showed that although the differences in salinity levels impacted the growth and density of microalgae cells, this condition did not significantly impact the performance of the biosorption process, particularly the use of white and red light colors. The trend in the biosorption process's performance for the three variations of salinity levels tends to be the same for the white and red lights. In addition, the results in Figure 4 reinforce the information in Figure 1 regarding the kinetics of the Cu^{2+} ion biosorption process with the microalgae *Chlorella sp.* During the logarithmic growth phase of microalgae, the Cu^{2+} ion adsorption rate is incredibly fast.

3.3. Effect of Light Color on the Copper(II) Ions Biosorption Process

The system's light color is a significant factor in the microalgae growth process. Light is needed in the microalgae cultivation process as a source of energy in the photosynthesis process to form organic compounds that influence the growth and number of microalgae cells. In this study, the light source came from a lamp where the lamp's color was varied by using white, red, and blue light. Figures 1, 4, and 5 illustrate the entire cultivation performance of microalgae.

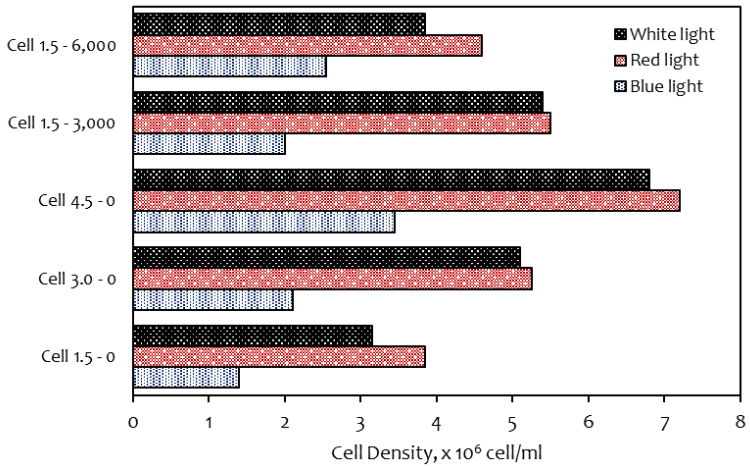

Figure 5 The cell density of microalgae in optimum condition (day 3)

The growth of the microalgae *Chlorella sp*. is maximized by red light, followed by white and blue light, as seen in Figures 1, 4, and 5. The results of this study support the results of research conducted by (Kendirlioglu and Cetin, 2017; Sudibyo *et al.,* 2017). Determination of whether or not microalgae growth can be seen in the system's value of cell density. Figure 5 depicts that the density of microalgae cells in red light is always higher than the other colors. Microalgae absorb red light efficiently for photosynthesis because their spectrum range is 625–740 nm (Kendirlioglu and Cetin, 2017; Das *et al.,* 2011). These wavelengths optimize the performance of the photosynthesis process. There are two photosystem mechanisms in the photosynthetic process: (I) the photosystem I process can only absorb light with a 700 nm wavelength, and (II) the photosystem II process can only absorb light with a 680 nm wavelength (Pettai *et al.,* 2005). In other words, these two photosystem processes run optimally when the system receives red light. In addition, red light is also known to increase the synthesis of organic material and the amount of chlorophyll which can accelerate microalgae growth (Wang, Fu and Liu, 2007).

The blue light spectrum plays no significant role in the growth process of *Chlorella sp*. microalgae, specifically in the photosynthetic process of the microalgae, as depicted in Figure 5. The density value of microalgae cells in white and red light is significantly higher than in blue light. On average, the cell density of microalgae under white and red light was 2.17 and 2.38 times higher than the density of microalgae cells in blue light, respectively. The poor performance of blue light in this cultivation process is due to blue light's characteristic wavelength. Blue light has a wavelength of 455–492 nm; therefore, this light cannot be absorbed properly in the photosystem step and affects the photosynthesis process.

On the other hand, white light is better than blue light. White light has a diverse spectrum of colors, including red light and blue light. This mixed color spectrum causes the photosystem to absorb red light from the white light spectrum even though that the quantity of red light absorbed is less than the full use of red light. According to Figure 5, the cell density in white light is less than in red light. This condition also indicates that the amount and intensity of light play an important role in light absorption in microalgae. The large amount and intensity of light have a positive tendency toward the photosynthesis process. However, this also depends on the cultivation volume and the microalgae density. The required light intensity increases proportionally to the microalgae density.

However, it should be noted that the light factor is not the only factor that affects microalgae's growth process. Figures 1, 4, and 5 demonstrate and explain that microalgae

growth is complex, and all factors will influence one another and can have other impacts, including nutritional competition and others.

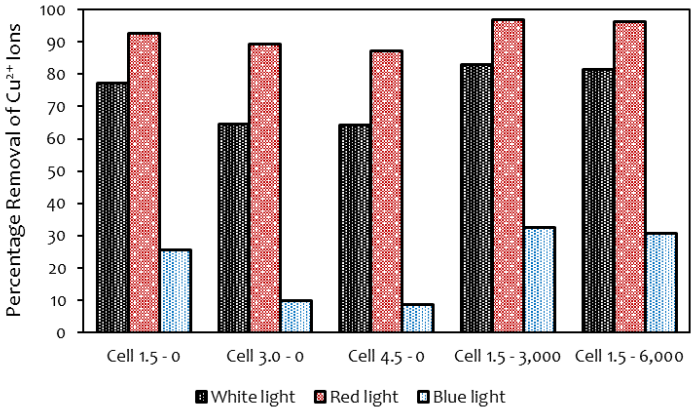

Figure 6 The percentage removal of Cu^{2+} ions during the biosorption process

The effect of light on the growth of microalgae also impacts the performance of the Cu^{2+} ion biosorption process. Figure 6 shows the percentage loss of Cu^{2+} ions in the liquid phase. The highest removal percentage of Cu^{2+} ions was 96.83%. These results were achieved when the biosorption process conditions were set at a microalgae concentration of 1.5 x 10^6 cells/mL, a salinity concentration of 3,000 mg NaCl/L, and red light. In this study, the percentage of Cu(II) ion removal is slightly higher than in the previous one (Wanta *et al.,* 2020). Based on these experimental results, this study has proved that each parameter influences the biosorption process significantly.

4. Conclusions

The biosorption process of metal wastewater using living microalgae *Chlorella sp.* is complex because many process parameters influence it. The experimental results of this study indicate that the effect of each parameter is not always linear. For instance, the larger the number of microalgae in a system, the less efficiently it can remove the Cu^{2+} ion concentration from the solution, as the microalgae cell density increases, thereby increasing competition for nutrients. This also applies to salinity levels where optimum conditions must be adjusted for optimized biosorption results. Moreover, the biosorption process is also related to the photosynthesis process that occurs in these microalgae. It causes the influence of the light color given to the system and will affect the microalgae's photosynthesis rate. Based on the experimental results, the process conditions that gave the maximum removal of Cu^{2+} ions were when the microalgae concentration, salinity, and light color were conditioned at 1.5 x 10^6 cells/mL, 3,000 mg/L, and red light, respectively. In this condition, 96.83% of Cu^{2+} ions were successfully removed from the wastewater. These results revealed that this biosorption process has promising potential and needs further development on a larger scale (continuous system or industrial scale).

Acknowledgments

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