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Original Research Article

Effect of Blue Light Color on Zn (II) and Cu (II) Metal Biosorption Using *Tetraselmis chuii* Microalgae

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Abstract

Direct release of inorganic compounds Cu (II) and Zn (II) into water bodies can disrupt ecosystems. Using microalgae biosorbent *Tetraselmis chuii* (*T. chuii*) is a promising approach for removing these metals from wastewater. This study investigated the effect of blue light on the absorption of Cu (II) and Zn (II) by analyzing the contact time and initial concentration. Statistical analysis (MANOVA) revealed differences in the biosorption process due to the contact time and Cu (II) concentration (P <0.05). The results showed that the most effective Cu (II) removal occurred with a 60-minute contact time at a concentration of 5 mg/L, achieving a 67.07% removal rate. Zn (II) removal was also efficient under blue light conditions with a 60-minute contact time at the same concentration, yielding a 56.23% removal rate. Additionally, this process led to a substantial reduction in microalgae *T. chuii* cell density, by 76% for Cu (II) and 89.2% for Zn(II). Characterization analyses using Fourier-transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) confirmed the presence of functional groups in T. chuii microalgae, which are crucial for the biosorption process. This study underscores the potential of microalgae as effective biosorbents for mitigating inorganic compound pollution in wastewater.

Keywords: Biosorption, *Tetraselmis chuii*, blue color, Cu (II), Zn (II)

1. Introduction

In developing countries, industrial wastewater is discharged directly into natural channels, sewer systems, internal septic tanks, or nearby fields. For example, in Bangladesh, a large amount of untreated industrial waste is discharged into open water bodies and adjacent lands. In addition, a considerable amount of enriched suspended solids is coming down from neighboring countries such as India through the Teesta and the Brahmaputra Rivers. According to Islam et al. (2015), the concentration of Cd (11 g/L in winter and 8 g/L in summer) at sites S6–S10 was much higher than that at the other sites because these sites are located downstream of the river and experience extensive discharge of urban waste, greatly exceeding the drinking water standard value (5 g/L). In recent years, urbanization and industrial activities have caused environmental damage in developing countries. The characteristics and amount of industrial wastewater discharged depend on the average daily product and water usage and vary from one industry to another (Ilyas et al., 2019; Islam et al., 2015).

Industrial wastewater streams containing inorganic compounds are generated from various industries, such as cadmium, zinc, lead, chromium, nickel, and copper are generated in the electroplating, electrolysis deposition, conversion plating, and anodization cleaning industries. All of these generators produce large amounts of wastewater, residue and sludge which can be categorized as hazardous waste requiring extensive waste processing (Gunatilake, 2015). In developing countries, inorganic compounds such as Cu, Ni, Cd, Zn, Cr, and Pb are often found in industrial wastewater in Southeast Asian countries

(Razman et al., 2023). A study conducted in Bintan Island, Indonesia, found that seawater, marine sediments, and *Caulerpa racemosa* contained high levels of zinc, zinc contained in sea waters were 32.59–45.79 mg/l (Raza'i et al., 2021).

Several methods have been used to reduce the inorganic compound content in wastewater; generally, the waste treatment that has been used is the treatment of physical and chemical waste, or a combination of both. However, such waste treatment has some disadvantages in the process of reducing inorganic compound content. Waste treatment by physical methods has disadvantages such as high operational costs, high capital investment, the need for engineering equipment, and the threat of exposure inorganic compounds to the surrounding environment. Likewise, processing waste using chemical methods also has serious disadvantages, such as high costs, difficult technical implementation, changes in water quality, damage to water flora and fauna, and other pollution problems. One of the current waste treatment potentials is the biological treatment method, which is the most efficient, simple, environmentally friendly, low-cost, technically applicable, and promising technique for removing inorganic compounds (Giripunje et al., 2015).

Microalgae-based bioremediation is a promising method for removing inorganic compounds from wastewater. Tetraselmis chuii, Pavlova lutheri, Nannochloropsis gaditana, and Chlorella muelleri are some of the microalgae species that have been used to remove inorganic compounds from wastewater (Goswami et al., 2022, Zabochnicka-Świątek & Krzywonos, 2014, Azizi et al., 2016, Dhokpande & Kaware, 2013). Microalgae can remove different types of contaminants through different methods, such as biosorption, bioaccumulation, and biodegradation. The efficiency of inorganic compound removal by microalgae depends on the microalgal species, the properties and concentration of the metal ions, and the culture period (Cameron et al., 2018).

Tetraselmis chuii was proven to be effective in the biosorption of inorganic compounds from wastewater. The inorganic compound removal efficiency of *Tetraselmis chuii* for inorganic compounds such as Cu2+, Fe+3, and Mn2+ was reported to be between 40-90%, 100%, and 20-50%, respectively, over a 72-hour period. for ion concentrations of 1.0 and 5.0 mg*L –1. (Cameron et al., 2018). In addition, another study showed that absorption and adsorption of inorganic compounds as well as lipid production were involved in the metal removal process by *Tetraselmis sp.* In addition, microscopic observations and FTIR analysis confirmed the inorganic compound removal mechanism of *the Tetraselmis sp.* FTIR analysis was conducted to determine the biochemical changes before and after metals 432 biosorption in *Tetraselmis sp.* cells by analyzing the position and the area of functional groups 433 infrared light absorptions (Dammak et al., 2022). This study aims to analyze the direct impact of the blue light on the absorption of Cu (II) and Zn (II) to the contact time and initial concentration.

2. Methods

2.1 Photobioreactor

The photobioreactor was made with length, width and height measurements of 100 cm, 40 cm and 30 cm respectively. The reactor was equipped with blue LEDs with wavelengths of 380–750 nm. Light acts as a source of energy for microorganisms (Sperling, 2007). The distance from the lamp to the bottom of the reactor is 15 cm, where 6 Erlenmeyer flasks are placed. The reactor walls are lined with aluminum foil as shown in **Figure 1.** During microalgae cultivation, coconut fertilizer and vitamin B612 are also used for microalgae growth and oxygen is pumped through an aerator (Zakir et al., 2022). Seawater was used as growth medium.

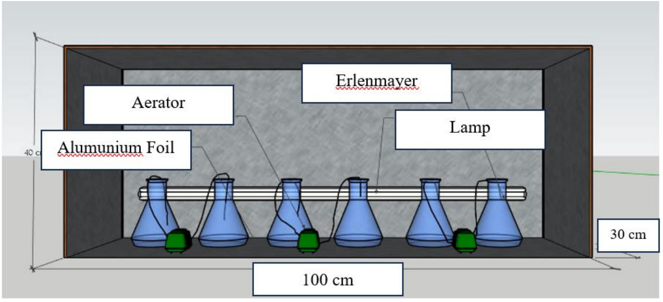


Figure 1. Photobioreactor design

2.2 Preparation of Mains Solution

The main solutions were $ZnCl_2$ and $CuSO_4.5H_2O$ with a concentration of 200 mg/L. Dissolved 0.417 gr $ZnCl_2$ into 1000 ml distilled water and 0.786 gr $CuSO_4.5H_2O$ was added to 1000 ml distilled water to obtain a concentration of Zn (II) and Cu (II) 1000 mg/L. $CuSO_4$ Merk SAP 99% and $ZnCl_2$ Merk SMART-LAB 99.8% were used to create a calibration curve with concentrations of 0 (blank), 2, 4, 6, 8, and 10 mg/L. The calibration curve represents the relationship between the concentration and absorbance values, which were analyzed using a UV-Vis spectrophotometer. In addition to curve calibration, the main solution was also used to prepare artificial Zn and Cu waste with concentrations of 5 mg/L and 15 mg/L, respectively. We took concentrations of 5 mg/L and 15 mg/L because based on research conducted by Yuda and Said in 2005, the electroplating industry produces wastewater discharges with a concentration of 15 mg/L but based on research conducted by Vania in 2016 the characteristics of Zn(II) waste in the electroplating industry are 5 mg/L so it can be concluded that the level of Zn(II) in electroplating industry wastewater is 5 mg/L - 15 mg/L.

2.3 Standard Curve Creation

Callibration curved can be accepted when linearity coefficient ≥ 0.95 according Indonesia National Standar 06-6989.7-2004.The concentrations used to generate the calibration curve were 0 (blank), 2, 4, 6, and 8 mg/L. The test was carried out using a standard solution with purity known for three repetitions with three different concentrations. The calibration curve is a graph related to the concentration of the solution and its absorption value. The linearity of the curve was considered satisfactory if the correlation coefficient (r) was close to 1. The value of r, which is close to 1, indicates that there is a linear relationship between the concentration of the analyte and the measured absorption (Chakti et al., 2019).

2.4 Acclimatization

Acclimatization is the adaptation of microorganisms to the conditions of waste water to be treated, including its food sources (Gunawan & Kahar, 2019). Acclimatization is carried out to help microalgal cells adapt to increased inorganic compound stress in photoautotrophic cultures (Kumar et al., 2020). The concentrations of Zn and Cu artificial waste used are 5 mg/L and 15 mg/L.

2.5 Biosorption

Two (duplo) biosorption samples with a working volume of 100 mL and a ratio of 7:3 for 70 mL of inorganic compounds Zn (II) and Cu (II) at concentrations of 5 mg/L and 15 mg/L, respectively. Biosorption was performed when the microalgae reached exponential and stationary phases. During biosorption, aeration is also carried out to meet the need for dissolved oxygen. Aeration was performed with a discharge of 1.5 L/min using an air pump aerator. Aeration Changes in exposure time for biosorption were 30, 45 and 60 minutes with blue LED light irradiation

2.6 Morphology Identification

2.6.1. SEM (Scanning Electron Microscopy)

SEM is a research method that uses a microscope that allows for clearer visualization of objects due to refractive magnification of up to tens of thousands of times (50x – 150000x) (Haty dkk, 2019). The working principle of SEM is that the electron source creates a beam of electron and is accelerated into a sample or specimen through a positive electrical potential. The electron beam is confined and focused using metal apertures and magnetic lenses into a thin, focused, monochromatic beam. The electrons in the beam interact with the atoms of the specimen, producing signals that contain information about their surface morphology (Subramanian et al., 2018). Microalgae were identified using a SEM-EDX HITACHI SU3500 and FEI type INSPECT S-50. Scanning electron microscopy (SEM) analysis aims to determine morphological changes from the effect of biosorption of *Tetraselmis chuii* microalgae on the inorganic compounds Zn (II) and Cu (II). SEM analysis was performed on *Tetraselmis chuii* microalgae before and after biosorption.

2.6.2. FTIR (Fourrier Transform Infrared Spectroscopy)

FTIR was used to identify the functional groups involved in the biosorption process. Infrared spectroscopy measures the absorption of IR radiation made by each bond in the molecule and, as a result, gives a spectrum that is commonly designated as % transmittance versus wavenumber (cm−1). A diverse range of materials containing covalent bonds can absorb electromagnetic radiation (Bankole et al., 2019). The FTIR apparatus specifications for identifying functional groups were FTIR Thermo Scientific Nicolet Is10 and SHIMADZU type IR-Prestuge 21.

2.7 Cell Density

Cell density analysis was carried out using a *Hemocytometer*. The *Hemocytometer* method is more often used to calculate cell density because it is easy to operate (Bahtiyar,2017). *The hemocytometer* contained four large boxes with a size of 1 mm on each side, using a *handcounter*. Cell density can be calculated using the following equation (1) (Zanuddin dkk., 2017).

$$k = \frac{N\ in\ 4\ blocks}{Number\ of\ blocks}\ x\ 10^4 \quad (1)$$

k = cell density of *Tetraselmis chuii* (sel/ml)
N = total number of *Tetraselmis chuii* in the 4 counting room boxes (sel)

2.8 Statistical Test

Two statistical testing methods will be performed: the normality test and the MANOVA test. The type of standardization test carried out is the Kolmogrov Smirnov method. The multivariate analysis of variance (MANOVA) test is a statistical technique used to calculate the significance of the mean differences between groups for two or more dependent variables (Sutrisno & Wulandari, 2018).

3. Result and Discussion

3.1 Cell Density

Figure 2 shows that if the contact time of microalgae with inorganic compounds increases, cell density decreases. Microalgae will decrease after reaching optimum conditions. This occurs because the amount of nutrients in the medium decreases with increasing microalgae density. Apart from that, the decrease in biomass is also caused by the accumulation of organic compounds such as $NH4^+$ (Kurniawan et al, 2014).

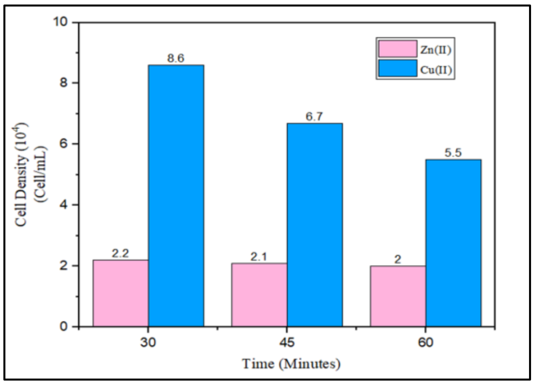


Figure 2. Cell density after biosorption in 5 mg/L concentration

Figure 2 shows the results of cell density after the biosorption process on Zn (II) metal. At a concentration of 5 mg/L with contact times of 30 min, 45 min, and 60 min, the cell density was respectively 2.2 x 10^4 cells/ml, 2.1 x 10^4 cells/ml, and 2 × 10^4 cells/ml, respectively. At a concentration of 15 mg/L with a contact time of 30 min, 45 min, and 60 min, the cell density was respectively 1.9 x 10^4 cells/ml, 0.8 x 10^4 cells/ml and 0.4 x 10^4 cells/ml.

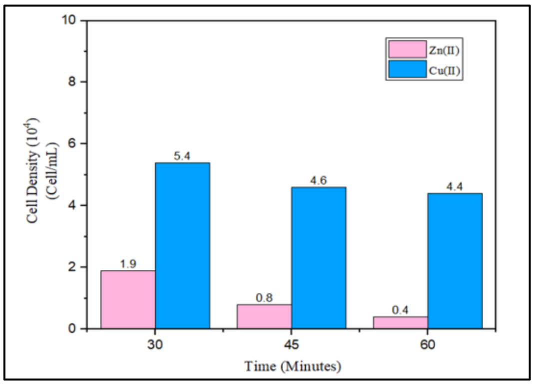


Figure 3. Cell density after biosorption in 15 mg/L concentration

Figure 3 shows the cell density after biosorption on Cu (II). At a concentration of 5 mg/L with contact times of 30 min, 45 min, and 60 min, the cell densities were 8.8 × 10^4 cells/ml, 6.7 x 10^4 cells/ml and 5.5 x 10^4 cells/ml, respectively. At a concentration of 15 mg/L with a contact time of 30 min, 45 min, and 60 min, the cell density was respectively 5.4 x 10^4 cells/ml, 4.6 x 10^4 cells/ml and 4.4 x 10^4 cells/ml, respectively.

3.2 FTIR and SEM Analysis Results

3.2.1. FTIR

Based on Figure 3, it can be compared between FTIR before and after biosorption of the inorganic compounds Cu(II) and Zn(II), showing that the absorption peak experienced a wave number shift. In the wave number range for the inorganic compounds Cu (II) before biosorption 3346.67, after biosorption 3342.80 cm^-1. Meanwhile, for the inorganic compounds Zn (II), it was 3339.29 cm^-1 before, while after it was 3338.52. 1640 - 3440 cm^-1 shows that the *T. chuii* microalgae has the -OH (hydroxyl) functional group from the absorbed water molecules (Mubarok et al., 2018). The occurrence of a wave number peak can be used to determine the number of water molecules (H2O) absorbed on the sample surface. The visible absorption at the wave number shows that the absorption of sharp stretching vibrations can result in changes in the length of a bond from a strong carboxyl group, namely O-H stretching vibrations. The bands resulting from the FTIR spectrum that have been read show that the functional groups contained in the *T. chuii* microalgae are hydroxyl (-OH), amine and amide groups. The results of FTIR analysis indicated that the functional group content in the microalgae cell walls changed in wave numbers before and after biosorption. In the cell wall, monovalent and divalent ions such as Na, Mg, and Ca are replaced by inorganic compounds, which are formed between the inorganic compound ions present in T. chuii microalgae functional groups such as carboxyl, hydroxyl, and amide. Figure 4 shows the mechanisms of Cu and Zn removal in this case. It was concluded that a shift occurs because functional groups play a role in the biosorption process.

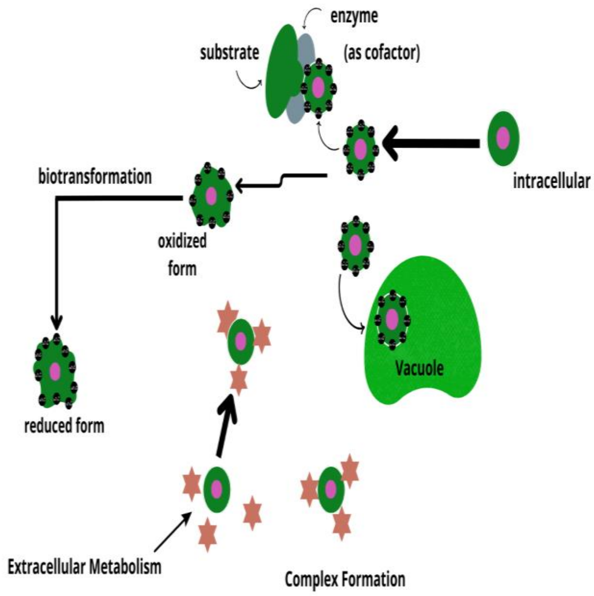
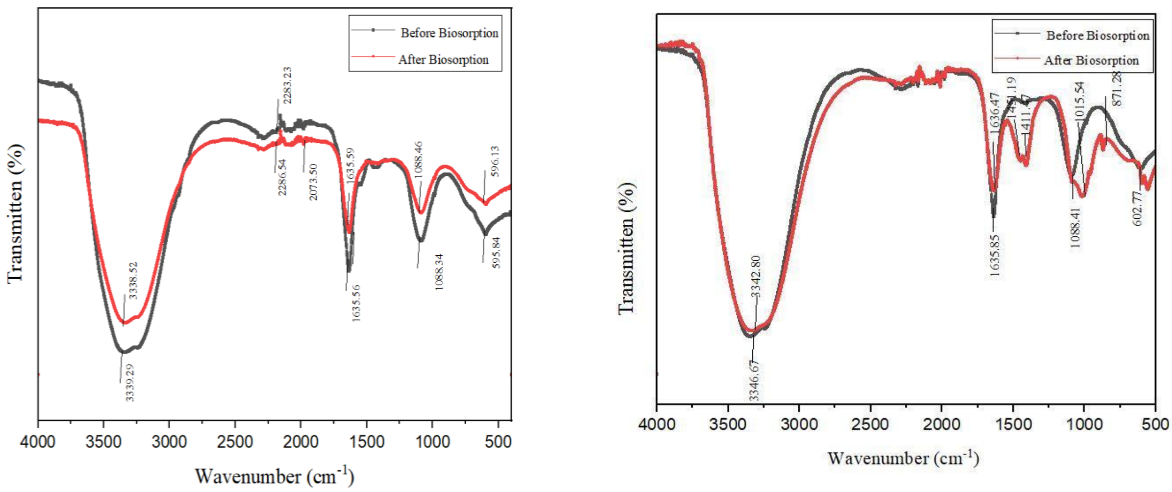


Figure 4. Mechanism of Cu (II) and Zn (II) removal

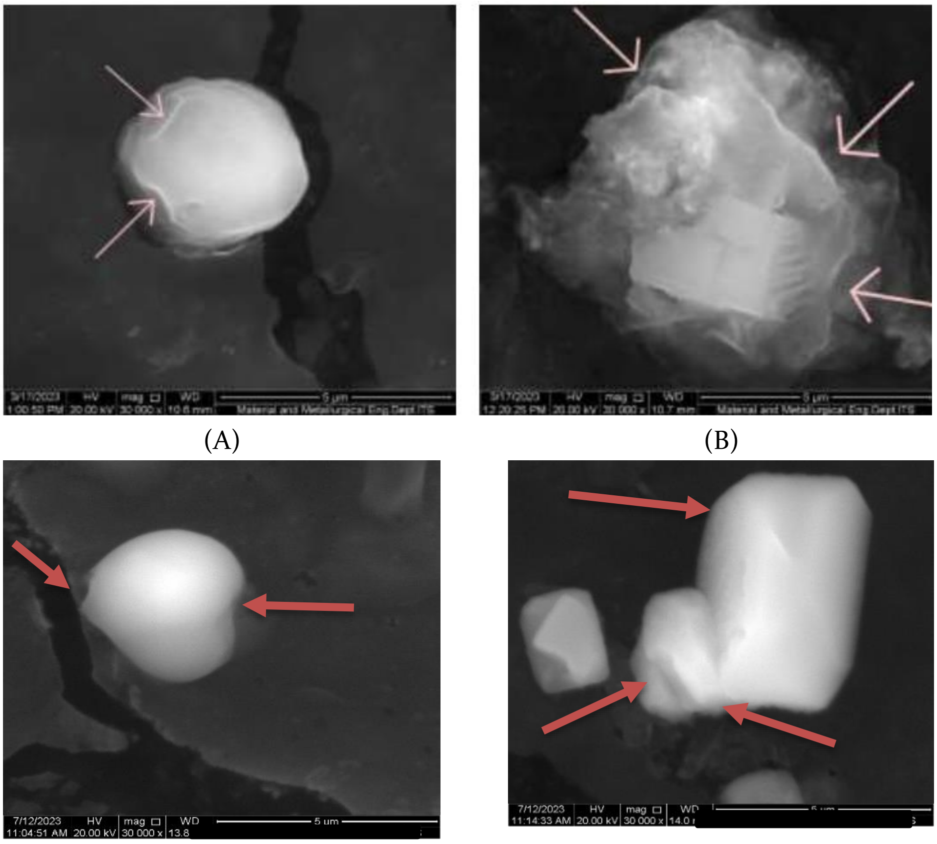


(A) (B)

Figure 5. Pre (A) and Post (B) Biosorption of *Tetraselmis chuii* inorganic compound Cu and Zn on FTIR spectra analysis

3.2.2. SEM

Morphological analysis of *Tetraselmis chuii* before and after biosorption of Cu (II) can be seen in **Figure 5.** (A) and (B) clearly visible changes between before biosorption and after biosorption. Before biosorption, healthy *Tetraselmis chuii* microalgae cells were round with a smooth surface, whereas after biosorption, *Tetraselmis chuii* microalgae cells experienced lysis and became irregularly shaped with a rough surface. There was also a slight difference in cell wall morphology during Zn(II) biosorption; before biosorption, the microalgae looked healthy and the cell texture was perfectly round, whereas after Zn(II) biosorption, the cell walls were enlarged and not as damaged as those of cells that adsorbed Cu(II). The difference in biomass surface being slightly rough and wavy texture on the surface after metal adsorption may be caused by the accumulation of different amounts of metal on the cell walls (Ajayan et al., 2015), Changes in microalgae cell walls (cell morphology) are caused by nodular deposits resulting in the attachment of metal to the surface of the microalgae cell walls (Tripathi et al., 2019). This indicates that the microalgae cell wall binds inorganic compound ions (Zn (II) and Cu (II)), which can result in microalgae cells in the environment and affect physiological properties and biochemical processes that can cause adverse effects on cell division, growth, photosynthesis, respiration, and the degeneration of major organelles (Bajguz, 2011). According to Wan Waznah et al. (2012), microalgae cells can experience lysis because the microalgae defense system, when in contact with metal ions, undergoes absorption in the cell wall and then enters the intracellular site.



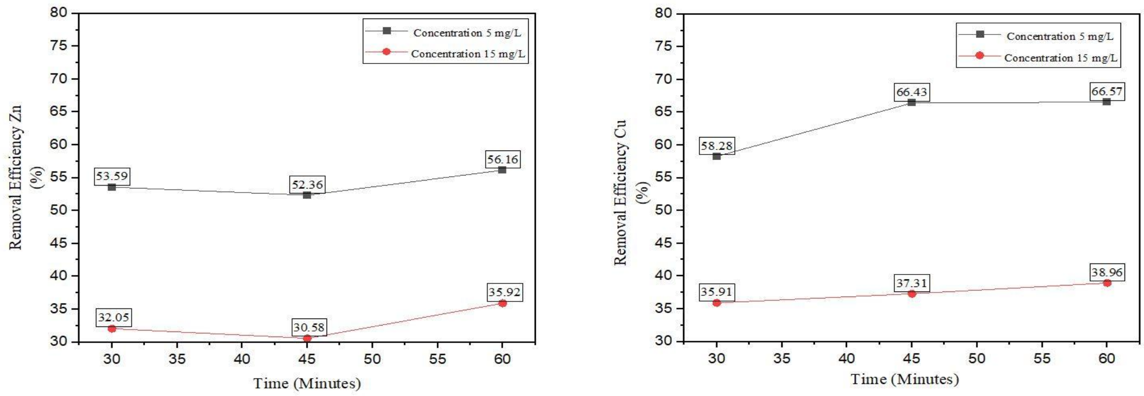
(A) (B)

(C) D

Figure 6. SEM test results of *Tetraselmis chuii* microalgae (A) before biosorption Cu (II), (B) after biosorption Cu (II), (C) before biosorption Zn (II) and (D) after biosorption Zn (II)

3.3 Relationship Analysis of Light Color, Concentration, and Contact Time on Zn (II) and Cu (II) Removal

Light is required 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 (Wanta et al., 2023). The results showed that blue light is effective in supporting the biosorption process; according to Ruyters (1984), blue light enhances enzyme activation and gene changes.



(A) (B)

Figure 6. Removal efficiency of Zn (II) and Cu (II) (A) Removal efficiency for Zn (II) (B) Removal efficiency for Cu (II)

An increase in the initial concentration of Cu (II) decreased the percentage of the resulting allowance. The concentration of inorganic compounds in excess Cu (II) can inhibit the growth of microalgae, and microalgae chlorophyll biosynthesis still influences the removal of Cu (II) metal as an inorganic compound from water media. The higher the concentration of metal ions, the greater the biosorption process decreases, so that metal ions increase on the surface of the adsorbent at high concentration levels, causing pores to be closed, preventing metal ions from entering the pore pores. Thus, process contact occurs on the surface of the microalgae cells. Internal functional groups, such as hydroxyl, amino, amide, carbonyl, and alkenyl group, can bond with the inorganic compound Cu (II) (Pinem,2016).

Based on the research, the longer contact time gives optimum result biosorption, in line with the research conducted by Haleem et al. (2010). A contact time of 60 min showed the optimum biorsorption process to remove Cu (II) and Zn (II). Optimal results can be determined based on the ability of microalgae to absorb inorganic compounds. The longer the contact time between microalgae and inorganic compounds, the effectiveness in absorbing them will decrease. This was because the microalgae entered the death phase (Hayati, 2022), and the percentage of Cu (II) removal at 67.07% was larger than that of Zn (II) at 56.229%.

3.4 Statistical Test Analysis

Statistical analysis was carried out to determine the effect of the variations used, namely concentration, contact time, and light color, on the biopsy of *Tetraselmis chuii* microalgae. The sample in this study consisted of 36 samples so that normality tests could be carried out using the results of Kolmogorov-Smirnov's interpretation referring to research (Pratama and Rita, 2021). Three-way MANOVA test analysis is used to determine the influence of the independent variable on the variable bound, which is interpreted based on the *P* value. with *P* value <0.05. The MANOVA test obtained *P* value <0.05. This confirm that changes in concentration, exposure time and light color had an effect on cell density and the ability to remove the inorganic compounds Zn (II) and Cu (II).

Table 1. MANOVA analysis results

No	Effect	Sig.	Information
1	Light Color	0.000	Influential
2	Concentration Variations	0.000	Influential
3	Contact Time	0.006	Influential
4	Light Color*Concentration Variations*Contact Time	0.000	Influential

4. Conclusions

Results of the research carried out show that the removal of Cu (II) and Zn (II) is influenced by the color of light, contact time and concentration. The highest percentage removal of Cu (II) was observed at a contact time of 60 min and concentration of 5 mg/L, with a yield of 67.07%. The highest percentage removal of Zn (II) was observed at a contact time of 60 min and a concentration of 5 mg/L, with a yield of 56.2299%. According to Hayati (2022), increasing contact time is not efficient for microalgae to absorb the inorganic compounds Cu (II) because the microalgae have entered the death phase, and morphological changes were observed in the microalgae *Tetraselmis chuii* before and after the biosorption process, as observed by SEM analysis, and there was also a change in functional groups as observed by FTIR analysis. The SEM analysis results show that Zn (II) biosorption in the cell walls is enlarged and not as damaged as those of cells that adsorb Cu (II).

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