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Bioremoval of Copper by Marine Blue Green Algae *Phormodium formosum* and *Oscillatoria simplicissima*

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Abstract

Objectives: Using bio-sorbents is regarded as one of the effective methods to remove heavy metals. Therefore, this study aimed to investigate Cu^{2+} adsorption and it's effecting on growth of *Phormodium formosum* and *Oscillatoria simplicissima*. **Methods/Statistical Analysis**: Two marine cyanobacteria isolates *P. formosum* and *O. simplicissima* were tested for tolerance and removal of copper supplemental individual as a single metal at different contact times (0, ½, 1½, 3 and 24h), different temperatures (20, 30, 35 and 40 °C), different pH (4, 5, 6, 7, 8) and different concentration (5, 10, 20, 30, 40 and 50 mg/L) under controlled laboratory conditions. **Findings:** The obtained results showed that lower concentration of Cu^{2+} (5, 10 and 20 mg/L) enhanced the algal growth (chlorophyll a), elevated concentration (30, 40 and 50 mg/L) were inhibitory to growth in case of two algal. The bioremoval of heavy metal ions (Cu^{2+}) by *P. formosum* and *O. simplicissima* from aqueous solution showed that the highest percentage of metal bioremoval occurred at 24 h of contact time recording 90% and 80% respectively. The maximum biosorption was observed at optimal conditions including 30°C, pH of 7 and 10 mg/L at 24h of contact time. **Application/Improvements:** The study findings revealed that *P. formosumalgae* can be effectively in order to adsorb Cu^{2+} due to its high efficiency of Cu^{2+} adsorption.

Keyword: Bioremoval, Copper, Marine Blue Green Algae, *Phormodium formosum*

1. Introduction

Microalgae are discovered to be a useful organism in many applications, like food, dietary supplements, medicine, fuel, and waste treatment. World population increase and manufacture will cause ever increasing evolution wastes. The earth population is projected to increase cardinal by 2050; from 7.3 billion in 2015 to 9.7 billion in 2050[1]. Serious metal pollution could be a problem of nice environmental concern. Serious metals are discharged from current municipal industrial, agricultural and domestic wastes; they modify the structure and productivity of aquatic ecosystem[2].

Some micro-organism strains are best-known that show very strong activity affinity for Hg, Au, Cu, Zn, Pb, and metal[3,4]. Some algae can take up serious metal ions and organic pollutants from the contaminated waters[5,6]. Output waste from fish farms usually contains high levels of waste, which can cause problems for the adjacent farms and various aquatic environments. Algae use most of this waste as a food provide so, it has been tried to grow these algae on the waste of fish farms[7]. Marine living thing algae with high metabolism rates fill the essential operate inside the first production and focus of great metals, that are pollutants with multiple consumption pathways to marine ecosystems. The hazard of great metal pollution is as a result of its capability to maneuver among marine associated near-shore ecosystems for an extended lasting length of it slow. By accumulating serious metals in their cells, algae encourage further accumulation inside the ordered elements of the natural phenomenon, likewise as in industrial aquatic organisms[8]. The responses to the incidence of deadly concentrations of great metals vary considerably in many species. Among the marine organisms, vegetation proof against serious metals is recommended as bio indicators for the assessment of marine pollution[9].

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The buildup of metals by algae, bacteria, fungi and yeast has been extensively studied inside the last twenty years of the organism studied, algae are gaining increasing attention, thanks to the particular incontrovertible fact that algae, considerably marine algae, arean upscale provide within the oceanic atmosphere, relatively low price to technique and able to accumulate high metal content[10]. The activity on the cell surface is that the dominant mechanism every surface activity and internal diffusion are involved inside the uptake of metals by algae[11]. Metal can cause to a lower place short exposure: abdominal pain, diarrhea, vomiting, and yellow skin, but to a lower place long- term exposure, its cause: internal organ injury, yellow eyes, aluminous vogue in mouth, weakness[12]. Serious metal process takes place in a very try of phases: 1) a speedy binding of ions to the skin of the cell wall, and 2) a slow transfer of metal particle into the algal cell. This may be chiefly thanks to the charge of the metal particle and conjointly the electrical charge of the ligands on the microorganism cell wall. Absorption is that the slow uptake of the metal ions into the inner microorganism cell. The metal particle diffuses across the cell wall and regularly depends on metabolic activity. The metal ions transport across the cell wall into the substance then usually accumulate inside the cavity. Uptake of metal ions into the inner cell entirely occurs in living algae, whereas activity can happen in living or non living algae[13]. Activity describes the first half, whereas absorption describes the second half. Activity is that the binding of molecules to a surface. Activity of metal ions to the algal cells generally takes place among minutes of being to bear[14]. This investigation was targeted on established of copper on growth, chlorophyll production by marine blue-green algae *Phormodium formosum* and *Oscillatoria simplicissima* for copper removal were administered to review the development of the activity of Cu^{2+} depends on such factors as pH, temperature, initial metal particle concentration, and make contact with time.

2. Materials and Methods

2.1 Algal Isolation

The algal species used in this study were isolated from Eastern harbor of Mediterranean Sea coast of Alexandria, Egypt. Samples were grown separately in 500 ml culture flasks containing F/2 medium[15,16]. The medium was then autoclaved at 120 °C for 30 minutes. The culture was incubated at temperature 30±1°C, pH of 7 and light intensity 3000 lux. The algae were kept under optimum conditions. The isolated strain was identified according to available literature[17–19].

2.2 Measurements of Algal Growth

Copper stock solutions were prepared from copper sulfate ($CuSO_4.5H_2O$). Algal tolerance to different copper concentrations (5, 10, 20, 30, 40 and 50 mg/L) was achieved by the determination of algal growth as a chlorophyll a[20]. Harvesting took place by centrifugation at 5000 rpm for 15 min. The pigment content in filtered extract was determined by the absorbance at 663 and 645 nm in a 1cm quartz cell against a blank of 80% aqueous acetone.

2.3 The Effective Parameters on Copper Removal

2.3.1 Contact Time

By using modified F/2 medium, the metal concentration used was 10 mg/ml and the exposure time was 0, ½, 1½, 3 and 24h. PH was adjusted to 7.0, 30°C and incubation was performed at the previous mentioned conditions. At the end of each exposure time, decantation was performed and the supernatant was used for the determination of heavy metal removal

2.3.2 Temperature

Different temperatures (20, 30, 35 and 40°C) by using modified F/2 medium Flasks were incubated at pH 7 and concentration of Cu^{2+} 10mg/L. At this stage the optimum contact time was 24 h.

2.3.3 pH

Different pH ranges from 4.0 to 8.0 by using modified F/2 medium. Flasks were incubated at 30 °C concentration of Cu^{2+} 10 mg/L. The optimum contact time was 24 h.

2.3.4 Copper Concentration

Different concentration (5, 10, 20, 30, 40 and 50ppm) by using modified F/2 medium and flasks were incubated at pH 7 and 30°C, taking into considerations a contact time at 24 h. Copper solution concentration was measured applying flame atomic absorption model (AA-240). The removal efficiency of copper was also investigated at optimum conditions calculated by the equation one[21].

2.4 The Equation (one)

% RE= $(C_0-C_t)\ C_0 \times 100$
RE: Removal efficiency
C0: Initial copper concentration (mg/l)
Ct: Copper concentration at the desired time (mg/l)

3. Statistical Analysis

In all measurements was carried out in triplicate. Statistical analyses were performed using one-way Analysis of Variance (ANOVA), and the significance of the difference between means was determined by Duncan's multiple range tests. Differences at $P<0.05$ were considered statistically significant. The results were presented as mean values (±SD, Standard Deviations).

4. Results and Discussion

4.1 Micro Algae Isolated

The algal strain was identified as *Phormidium formosum* and *Oscillatoria simplicissima*. The growth of *P. formosum* increased and reached to maximum value at stationary phase after 10 days under 30±2°C and pH 7, then, phase started to decrease but in case of *O. simplicissima*, the growth increased and reached to maximum value at stationary phase after 12 days (Figure 1). The most studies on the biochemical production of algal and their analysis were carried out in stationary phase Pf growth period[22].

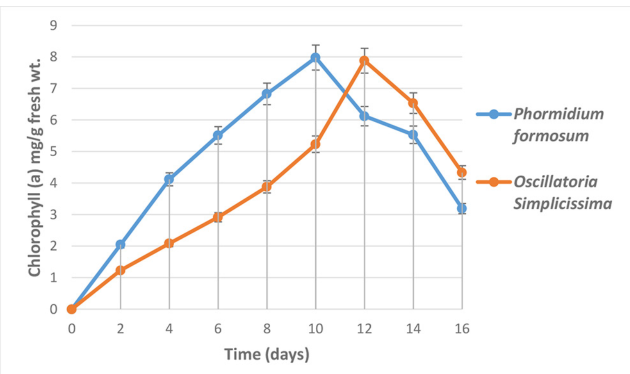

Figure 1. The growth of *P. formosum* and *O. Simplicissima* measured as chlorophyll (a) mg/g fresh wt.

4.2 Effect of Cu^{2+} on growth of *P. formosum* and *O. simplicissima*

Copper exhibited destructive effects at the 40 mg/L concentration and completely inhibited growth, copper is a known algicide[23]. One study the marine diatom *Phaeodactylum* found 0.1 mg L^{-1} Cu to reduce growth by 50% and 1 mg L^{-1} inhibited growth[24]. Another study determined Cu to kill microalgae at 0.300 mg L^{-1}[25]. Copper is known to react with glutathione which can inhibit cell division[26]. In addition, copper has shown to possess damaging effects on cell walls, leading to destruction[27]. Several inexperienced microorganism species are tolerant or proof against Cu2+, Cd2+, Pb2+ and Zn2+[28]. The two algae tolerated the toxicity of Cu^{2+} event at higher concentrations (30, 40 and 50 mg/L), moreover the lower concentration of Cu^{2+} (5-10 and 20 mg/L) induced a pronounced stimulation of chlorophyll(a) which was much more observed in two cyanobacteria (Figure 2, 3).

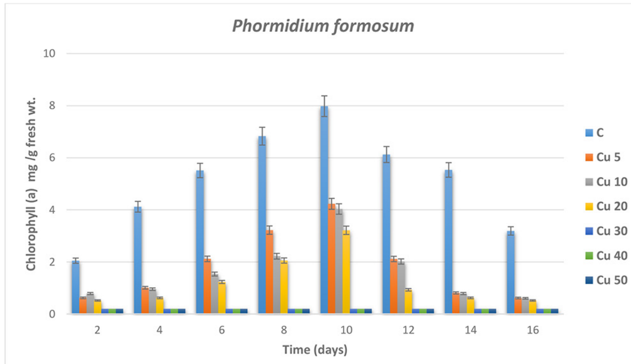

Figure 2. Effect of different concentration of copper on growth of *P. formosum* measured as chlorophyll (a) mg/g fresh wt. (C) represents algal treatment without Cu^{2+}.

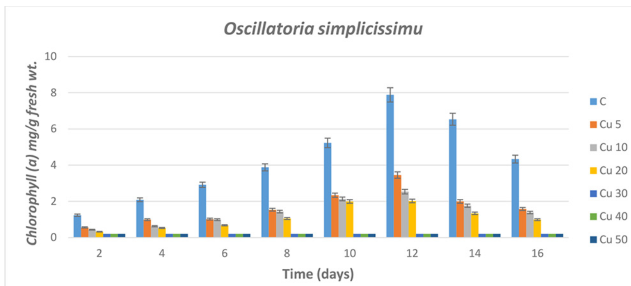

Figure 3. Effect of different concentration of copper on growth of *O. Simplicissima* measured as chlorophyll (a) mg/g fresh wt. (C) represents algal treatment without Cu^{2+}.

4.3 Effect of Contact Time on Cu^{2+} Removal

The results of the present study regarding the effect of contact time on copper removal by *P. formosum* and *O. simplicissima* are depicted in Figure 4. By increasing the exposure time (to 24h), the percentage of cu^{2+} removal decreases gradually till equilibrium establishment between the percentage of cu^{2+} removal by algal cells. After the equilibrium period, cu^{2+} sorbed by the algal biomass did not significantly changed with time[29].

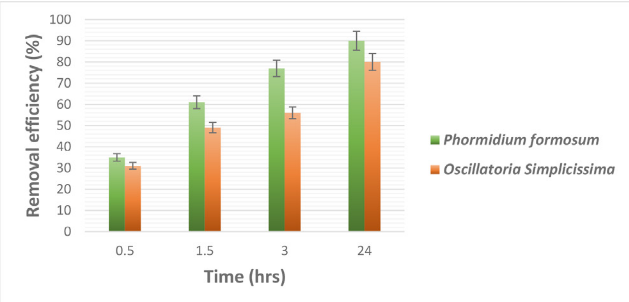

Figure 4. The effect of different times on removal efficiency (%) of Cu^{2+} by *P. formosum* and *O. Simplicissima* at pH 7, 30 °C and concentration 10 mg/L.

4.4 Effect of Temperature on Cu^{2+} Removal

Used algae vulgaris as a bio sorbent and settled that nickel (II) natural action steady hyperbolic from around 35 to cardinal mg/g over the vary of 15-45 °C[30]. The effect of temperature on Cu^{2+} removal by *P. formosum* and *O. simplicissima* is presented Figure 5. The most removal potency (90%) occurred at optimum temperature 30 °C by *P. formosum*. The removal percentage of cadmium (II) ions decreased from 61.0% to 34.9% when temperature was increased from 20 °C to 50 °C while using *C. vulgaris*[31]. The activity of great metals by *Ulothrixzonata* United Nations agency inarguable that the most effective activity was obtained at 30°C[32]. On the alternative hand, some studies even show metal natural action is not keen about medium temperature. Combined copper, zinc, iron, chromium, and lead using *C. vulgaris*[33]. Temperature had insignificant effects on lead and iron sorption, while having only slight effects on copper, zinc, and chromium. In most cases, there is an optimal range at which microalgae sorb metal ions; however, it is dependent on the microalgae and the metal ion. It is also dependent on whether living or non-living algae are being used. If biomass production is a desired by product, then the optimal sorption range will likely be influenced by the optimal growth temperature. Most microalgae have optimal growth between 20-30 °C[34,35].

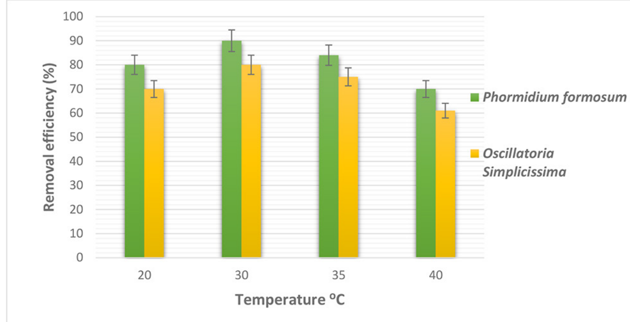

Figure 5. Effect of different temperature on cu^{2+} removal efficiency (%) by *P. formosum* and *O. Simplicissima* at 24h, pH 7 and concentration 10 mg/L.

4.5 Effect of pH on Cu^{2+} Removal

Metal ion adsorption is highly dependent on medium pH; pH is the measurement of H^+ or OH^- ions in a solution. Acidic solutions (pH < 7) contain more H+ ions than OH- ions, while the opposite is true for basic solutions (pH > 7). Typically, metal ions are cations, meaning they possess a positive charge. The algal cell wall carries a net negative charge. Therefore, in acidic solutions, H+ ions compete with metal cations to bind to the algal cell wall. For this reason, higher pH medium generally leads to higher metal ion sorption. However, heavy metals are more susceptible to precipitation at high pH levels[36,37]. The effect of pH on Cu^{2+} removal using *P. formosum* and *O. simplicissima* is demonstrated in Figure 6. The maximum Cu^{2+} removal by *P. formosum* was 90% at pH 7, at equilibrium time to 24h. The pH effect can be explained considering the surface charge on the adsorbent material. At low pH, due to high positive charge density caused by protons on the surface sites, adsorption of Cu(II) ions was intensively decreased. As a matter of fact, it is probably due to the competition of protons and Cu(II) ions towards a fixed number of adsorption sites[38]. Numerous studies are performed on the impact of proton concentration on metal natural action. For example, *Chlorella vulgaris* to sorb copper, zinc, cadmium, and nickel, on a personal basis. Proton concentration was varied for each metal system from concerning one .5 to 8.0, wishing on the metal[39]. They found that at proton concentration one. 5-2.0 metal natural action was nearly negligible for each metal. Natural action hyperbolic as proton concentration hyperbolic. At proton concentration values of 2.0, 4.0, 6.0, and 8.0 the natural action capability (in moll per g biomass) of number forty-eight was zero. 023, 0.254, 0.292, and 0.314. Natural action of copper, zinc, and nickel followed the identical trend as number forty-eight natural action.

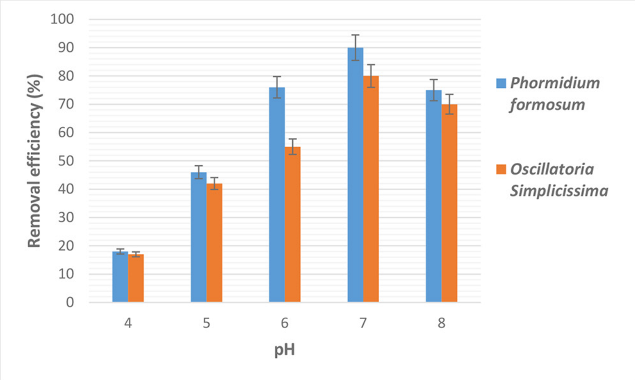

Figure 6. The effect of different pH on cu^{2+} removal efficiency (%) by *P. formosum* and *O. Simplicissima* at 24h, 30 °C and concentration 10 mg/L.

4.6 Effect of Initial Metal Ion Concentration Variation on Cu^{2+} Removal

Concentration of the adsorbed chemical plays an important role as a driving force to overcome the resistance caused by mass transfer between solid and liquid phase. The study results indicated that the maximum Cu^{2+} removal efficiency occurred at the concentration of 5, 10 and 20 mg/L for to cyanobacteria. In other words, Cu^{2+} removal efficiency decreased by increasing the initial concentrations of Cu^{2+} from 30 to 50 mg/L Figure 7. This might be associated with the increase in activity bands where the initial concentration of metals is lower. By increasing the surface charge of the adsorb able material on the adsorbent, the activity locations on the adsorbent are saturated and removal efficiency of the adsorbent decreases apace[40]. Increasing the concentration from 50 mg/L to 500 mg/L caused a discount in removal efficiency[41].

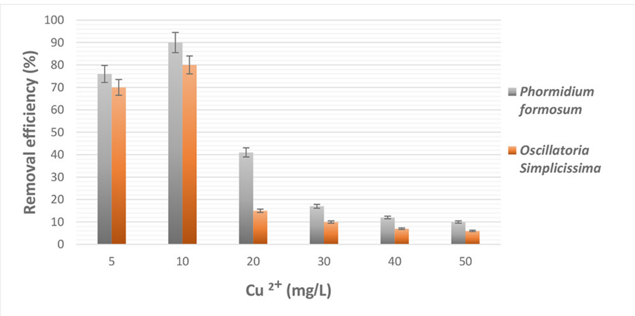

Figure 7. The effect of different Cu^{2+} concentration on removal efficiency (%) of Cu^{2+} by *P. formosum* and *O. Simplicissima* at 24h, pH 7 and 30 °C.

5. Conclusion

The present study provides clear evidence that the addition of copper in various concentrations brings about significant differences in the growth of microalgal species due to the differences in their resistances. Copper removal efficiency by *P. formosum* and *O. simplicissima* were 90% and 80%, respectively. The amount of adsorption depends on such factors as pH, temperature, initial metal ion concentration, and contact time. Therefore, applying the optimal condition is recommended in order to achieve higher removal efficiency.

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