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Investigation of The Bioethanol and Antioxidant Potential of *Borodinellopsis texensis* Grown in Wastewater under Various Copper Concentrations

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Antioxidant activity,
Wastewater

Abstract: Microalgae have the potential to grow at a rapid rate, which allows them to surpass other living organisms in terms of their prevalence in scientific research settings. Through their growth in wastewater, they are able to make a contribution to the treatment of wastewater. In addition, microalgae's metabolic components make them suitable for industrial applications. In this work, it is investigated how copper stress at various concentrations affected the biomass, carbohydrate, bioethanol, and antioxidant activities of *Borodinellopsis texensis* cultivated in 25% wastewater. The maximum biomass concentration was 0.79 ± 0.02 g/L at control group. In addition, carbohydrate content was the highest in a medium with 0.025 g/L copper, at 0.29 ± 0.02 g/L. The bioethanol productivities of *Borodinellopsis texensis* were 174.4 mg/g for 0.025 g/L Cu and 141.8 mg/ g for the control. DPPH, CAT, SOD and APX tests were performed to examine the antioxidant activities of *Borodinellopsis texensis*. The DPPH maximum scavenging, CAT, SOD, and APX activities of *Borodinellopsis texensis* were 83%, 17.4 µmol/mg, 8.87 µmol/mg, and 32.7 µmol/mg at 0.025 g/L of Cu, respectively. With the help of this study, research on how microalgae will respond when exposed to copper stress will be continued in larger reactors in the future.

Çeşitli Bakır Konsantrasyonları altında Atıksuda Büyütülen *Borodinellopsis texensis*'in Biyoetanol ve Antioksidan Potansiyelinin Araştırılması

Anahtar Kelimeler
Borodinellopsis texensis,
Cu stresi,
Biyoetanol,
Antioksidan aktivite,
Atıksu

Öz: Mikroalgler hızlı büyüyebilme özellikleri ile diğer canlıların önüne geçerek bilimsel çalışmalarda sıklıkla kullanılabilirler. Onlar atıksu içerisinde büyütülerek atıksuyunda arıtımına katkı sağlayabilirler. Ayrıca, mikroalgler içerisindeki metabolik bileşikler sayesinde sanayide kullanılabilir. Bu çalışmada %25 atıksu içerisinde büyütülen *Borodinellopsis texensis*'in çeşitli konsantrasyonlardaki bakır stresine karşı biyokütle, karbonhidrat, biyoetanol ve antioksidan aktivitelerine göstermiş olduğu değişim incelenmiştir. Maksimum biyokütle konsatrasyonu 0,79 ± 0,02 g/L ile kontrol grubunda bulundu. Buna ek olarak, en yüksek karbonhidrat içeriği 0,025 g/L bakır konsantrasyonunda 0,29 ± 0,02 g/L olduğu gözlemlendi. *Borodinellopsis texensis*'in biyoetanol verimi 0,025 g/L Cu konsatrasyonunda 174,4 mg/g iken, kontolün biyoetanol verimi 141,8 mg/g olarak bulundu. *Borodinellopsis texensis*'in antioksidan aktivitelerini incelemek için DPPH, CAT, SOD ve APX testleri yapıldı. *Borodinellopsis texensis*'in maksimum DPPH yakalama aktivitesi %83, CAT aktivitesi 17,4 µmol/mg, SOD aktivitesi 8,87 µmol/mg ve APX aktiviteside 32,7 µmol/mg olarak 0,025 g/L Cu konsantrasyonunda bulundu. Bu çalışmanın yardımıyla gelecekte daha büyük reaktörlerde mikroalglerin bakır stresine maruz kaldığında nasıl tepki vereceği konusundaki araştırmalara devam edilecektir.

1. Introduction

Microalgae are microorganisms with single or multicellular structures, as well as plant-like organ structures and functional functions. In this respect, just as microalgae can use carbon dioxide like plants and convert it into a sugar-derived organic substance such as glucose, some species can also use organic matter and convert it into catabolism products [1]. Microalgae can have green, brown, and red colors, and they can adapt to freshwater, marine, and terrestrial environments. Also, they can grow

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naturally or artificially for a variety of reasons. When grown in vitro, they need carbon, nitrogen, phosphorus, and trace elements [2]. The amounts of these elements and the substances they can use vary depending on the type of microalgal. Additionally, microalgae can grow rapidly in wastewater and freshwater and produce high amounts of metabolic products, which can be converted into biofuel [3].

Microalgae can grow in various wastewaters, such as industrial, municipal, domestic, medical, and agricultural wastewater. As a result, the expense of the growth medium's constituents is unnecessary. In addition to the elements we mentioned, external factors such as light, temperature, and pH may also be necessary for the growth of microalgae [4]. Like all living things, microalgae die when they cannot adapt to environmental conditions. Microalgae cultivated in a medium must be harvested before the nutrients deplete. So, harvesting microalgae is important, and one of the most expensive processes in the production process. The most popular harvesting methods are centrifugation, chemical and physical sedimentation, membrane application, and freezing [5]. The harvested microalgae need to be extracted to be transformed into a high-value-added substance [6]. Also, the extraction process varies depending on the desired product. While some products extract easily and quickly, others require meticulous attention to detail. In addition to all of these factors, the product's purification stage holds significant importance. Under certain conditions, the product might not be completely free of impurities. When it comes to fuel, for example, if it is going to be used in agricultural vehicles, it is possible to ignore it. Purification, on the other hand, is the most important phase in the process of obtaining a product, such as food, and there should be no impurities present in any way [7]. Microalgae's biochemistry and secondary metabolites make them valuable in a wide range of applications. Microalgae, for instance, have a wide range of applications in the food industry. The food industry has the potential to utilize the pigments produced from microalgae as colorants [1].

Environmental engineering uses microalgae grown in wastewater to remove nitrogen, phosphorus, and heavy metals, rendering the effluent harmless, and to produce goods used by other industries [8]. Fish aquaculture can use microalgae-made feed. The food supplement industry incorporates microalgae into their manufacturing processes. Also, microalgae can be used for biofuel production, and they can produce biodiesel, bioethanol, biobutanol, biomethane, and biohydrogen [9]. Each produces itself differently from the others, and the formation steps also vary within themselves. In order to obtain bioethanol from microalgae, carbohydrates must first be obtained. Compounds such as glucose, galactose, and fructose can combine to form sugars, and adding side groups can result in sugar derivatives. If the sugar in the solution contains additional side groups, it is more difficult to convert to fuel. The sugars obtained can be fermented and converted into bioethanol [10]. Every stage, from the harvesting of microalgae to product formation, can induce or increase bioethanol production. Furthermore, intervention at any of these stages may disrupt microalgae's antioxidant enzyme systems and alter metabolism. This system modification can be accomplished by interfering in the medium utilized by microalgae while they are growing, or it can be implemented after the microalgae have grown, i.e., when they have reached the stationary phase. Increasing the concentration of important components like nitrogen, carbon, and phosphorus in their surroundings can raise the biomass of microalgae [11]. In addition, changing the concentration of trace elements in the growing medium can affect the amount of biomass. In some cases, a change in trace elements may have a negative effect on the biomass, while it may have a positive effect on any component of the microalgae. Trace elements such as iron, copper, arsenic, molybdenum, zinc, chromium, and silicon can trigger these properties. The presence of an excessive amount of heavy metals in the environment or their total elimination from the environment might adversely impact biomass [12]. As a result of the changes that these elements make in the environment, antioxidant enzyme systems such as CAT, SOD, and APX can also come into play in the microalgae metabolism and show metabolism regulation. Their effects may differ depending on the concentration and nature of the elements that change in amount. Heavy metals increase the number of molecules called reactive oxygen species in microalgal systems, causing free radical formation. As a result, some changes occur in the microalgae protection system. These changes can be morphological and also affect the metabolic contents of microalgae. As a result, microalgal antioxidant enzyme systems respond to this event and change their amounts [13]. The type of reactors used to grow microalgae can also change the biomass and microalgae content. Column, flat, and loop photobioreactors are generally used for growing microalgae. Depending on the type of microalgae and the desired product, their effectiveness can vary [14]. If flat photobioreactors are thin, they can better absorb light, allowing a maximum number of microalgae to produce a maximum amount of biomass and product. Furthermore, controlling the parameters becomes more difficult as the reactor volume increases, but it also allows for more biomass production. Whether the bioreactor operates as a batch culture or continuously is a crucial factor in biomass production from microalgae [15]. Batch reactors provide easier control but produce biomass more difficult, while continuous systems produce more biomass while parameter control is difficult. *Borodinellopsis texensis* is a highly significant microalgae species. It is a member of the Chlorococcaceae family from the Borodinellopsis genus [16]. The aim of this study is to investigate the effects of microalgae grown in partial wastewater on

bioethanol and antioxidant systems when exposed to acute copper stress at different concentrations.

2. Material and Method

2.1. Condition of microalgae

Borodinellopsis texensis was purchased from the Culture Collection of Autotrophic Organisms in Czechia, and microalgae were propagated in Tris-Acetate-Phosphate (TAP) medium. Wastewater was prepared synthetically. In this study, 25% WW was used for control. 25% wastewater contains 25% wastewater and 75% TAP medium. Microalgae were subjected to Cu stress conditions for a period of forty-eight hours (48 hours) after different concentrations of $CuSO_45H_2O$ (0.01, 0.025, 0.05, and 0.1 g/L) were added to a stationary phase consisting of twenty-five percent wastewater. In the previous study [16], the preparation of wastewater and the growth of *Borodinellopsis texensis* were both described in detail.

2.2. Properties of photobioreactor (PBR)

During the course of this investigation, we employed a 1L flat photobioreactor (FPBR) to cultivate microalage until it reached a stationary phase. The air concentrations and light intensity were set at 0.3 $L.min^{-1}$ and 150 µmol m^{-2} s^{-1}, respectively. To eliminate the possibility of contamination, FPBR was exposed to 4 mM peroxyacetic acid for a period of thirty-five minutes before to the trials. Continuous mode was utilized for the study of FPBR.

2.3. Analitical experiments

Microalgae were centrifuged at 3500 g for 12 minutes, and the biomass was dried for 12 hours. Then, biomass was calculated gravimetrically, and carbohydrate concentration was determined using the anthrone technique spectrophotometrically. Various concentrations of microalgae extracts (100 µL) were added to the DPPH solution (1400µL), and DPPH procedure was applied. The samples were measured at 517 nm, and the detailed procedure was given in the previous study [17]. CAT and SOD activity were assessed using the nitroblue tetrazolium and thiobarbituric acid techniques, respectively, while APX was measured using the hydrogen peroxide method [16].

2.4. Hydrolysis of *Borodinellopsis texensis*

Microalgae samples were combined with 1M H_2SO_4 and autoclaved at 121 °C for 25 minutes. The residue was then brought to room temperature and centrifuged at 4500 g for 8 minutes. The supernatant was collected to determine carbohydrate concentration.

2.5. Bioethanol production and fermentation of microalgae

S. cerevisiae was cultivated on agar medium, and fermentation was observed at 25 °C in a shaking incubator at 140 rpm for 48 hours. Fermented samples were tested for bioethanol concentration and assessed spectrophotometrically in accordance with Rizza [18].

3. Results

3.1. Biomass content of microalgae under copper stress

In this study, *Borodinellopsis texensis* was grown in TAP medium. Because the growth rates of microalgae cultivated on TAP medium and 25% wastewater had the highest productivity in our previous study, 25% wastewater was used as a control in this one [16]. In addition, it was carried out as a continuous culturing system in 1 FPBR, and microalgae were grown for 8 days. The microalgae that had reached the stationary phase were then treated with varied Cu concentrations, and the samples were harvested 48 hours later to assess the results of the experiments. Then, the alterations were examined in biomass of *Borodinellopsis texensis* samples subjected to varying doses of Cu-induced stress. The biomass of the control group was 0.79 ± 0.02 g/L. The biomass concentration of the sample containing 0.01 g/L Cu was determined to be 0.71 ± 0.03 g/L. Furthermore, the biomass of the sample at 0.025 g/L Cu concentration was 0.62 ± 0.03 g/L. In the study, as the Cu concentration increased, the biomass concentration rate continued to decrease, and in the sample containing 0.05 g/L Cu, the biomass concentration of microalgae was observed to be 0.54 ± 0.03 g/L. Finally, it is investigated the biomass concentrations within 0.1 Cu, and the lowest biomass concentration emerged at 0.1 g/L Cu concentration. At this point, the concentration was 0.51 ± 0.03 g/L. Table 1 presents the biomass concentrations obtained by cultivating *Borodinellopsis texensis* under various Cu concentrations.

Table 1. Biomass and carbohydrate concentrations of *B. texensis* at the various concentrations of Cu

Cu Concentrations (g/L)	Biomass Concentrations (g/L)	Carbohydrate Concentrations (g/L)	Carbohydrate (dwt %)
Control	0.79 ± 0.02	0.30 ± 0.01	37.4 ± 0.9
0.01	0.71 ± 0.03	0.28 ± 0.01	39.9 ± 1.4
0.025	0.62 ± 0.03	0.29 ± 0.02	45.5 ± 0.8

0.05	0.54 ± 0.03	0.22 ± 0.02	39.3 ± 0.7
0.1	0.51 ± 0.03	0.19 ± 0.03	35.7 ± 0.7

With an increase in Cu content, there was a corresponding decrease in biomass concentration. It is designated the control group as the baseline, representing 100% in the experiments, and at a concentration of 0.01 g/L Cu, biomass decreased by 10%, calculated at 90%. The biomass exhibited a significant alteration when the concentration of Cu reached 0.025 g/L, resulting in an estimated value of 79%. At 0.05 g/L Cu concentration, biomass fell by 32% compared to the control, reaching 68%. Finally, at a Cu concentration of 0.1 g/L, biomass declined at a slower rate, reaching 64% with a 36% decrease. Figure 1 illustrates the biomass change percentages at various Cu concentrations.

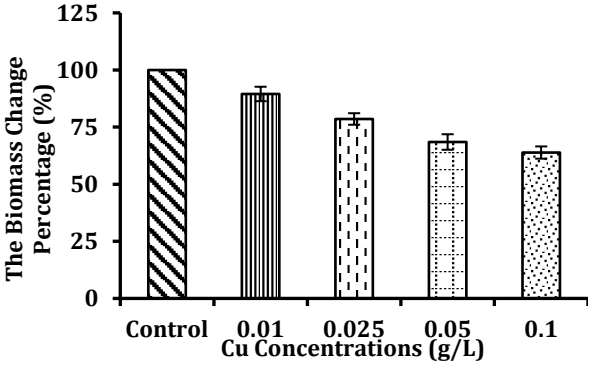

Figure 1. The biomass change percentages of microalgae at various Cu concentrations

3.2. Carbohydrate content of microalgae under copper stress

In addition, it is investigated how carbohydrate levels alter during Cu stress. The carbohydrate concentration of the control group was determined to be 0.30 ± 0.01 g/L. The maximum concentration of carbohydrates was seen in a medium containing 0.025 g/L of copper, with a value of 0.29 ± 0.02 g/L. The carbohydrate concentration in 0.01 g/L Cu was 0.28 ± 0.01 g/L. Furthermore, at 0.05 g/L Cu, the carbohydrate content was 0.22 ± 0.02 g/L. The lowest carbohydrate concentration was found to be 0.19 ± 0.03 g/L in 0.1 g/L Cu. In addition to the carbohydrate concentration in grams per liter, we computed it as a percentage due to variations in biomass levels. The carbohydrate concentration for the control group was 37.4 ± 0.9%, and the amount of carbohydrates was 39.9 ± 1.4% at a Cu concentration of 0.01 g/L. The highest carbohydrate concentration was observed at 0.025 g/L Cu, with 45.5 ± 0.8%. On the contrary, the lowest carbohydrate concentration was found to be 35.7 ± 0.7% in 0.1 g/L Cu. In this study, changes in carbohydrate quantities were also expressed as percentages, and the control group was considered to be %100. The greatest increase in carbohydrate content, up to 122%, was observed in a solution containing 0.025 g/L of Cu. The lowest carbohydrate change in quantity was determined to be 96% at 0.1 g/L Cu. Figure 2 demonstrates the percentage changes in the amount of sugar at various Cu concentrations.

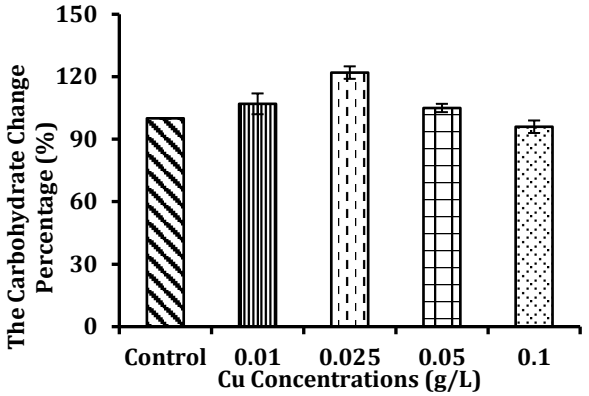

Figure 2. The percentage changes in the amount of sugar at various Cu concentrations

3.3. DPPH scavenging activity of microalgae under copper stress

In addition, the DPPH scavenging activity (SA) of *Borodinellopsis texensis* was carried out. The maximum scavenging activity of *Borodinellopsis texensis* was 83% at 0.025 g/L of Cu, whereas the SA of the control was 71%. SA increased as the Cu content increased, but it settled at 0.05 g/L Cu. At this point, the SA value at this point was 79%. The SA values were 77% and 78% at 0.01 and 0.1 g/L of Cu, respectively. Figure 3 illustrates the percentages of DPPH-scavenging activity in Cu at various concentrations.

3.4. The antioxidant enzyme activity of microalgae under copper stress

Moreover, the antioxidant enzyme activity of *Borodinellopsis texensis* was investigated under copper stress. Antioxidant enzyme activities changed at various concentrations of Cu. The maximum CAT activity was 17.4 µmol/mg for 0.025 g/L. On the other hand, the minimum CAT activity was 9.23 µmol/mg for control.

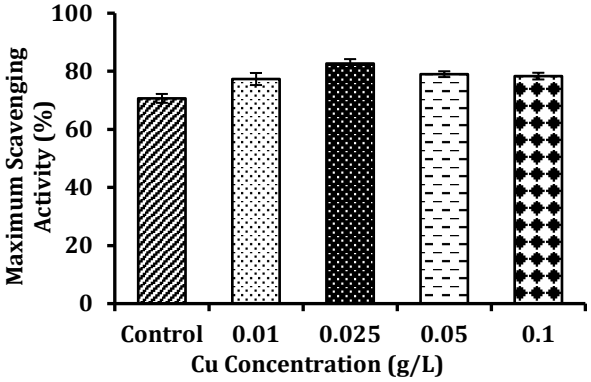

Figure 3. The percentages of DPPH-scavenging activity in Cu at various concentrations

CAT activities were similar at concentrations of 0.05 g/L and 0.1 g/L. CAT activities at 0.05 and 0.1 g/L Cu were 14.47 µmol/mg and 14.60 µmol/mg, respectively. Also, at 0.01 g/L Cu, CAT activity was 12.13 µmol/mg. CAT activities in Cu at different concentrations are shown in Figure 4.

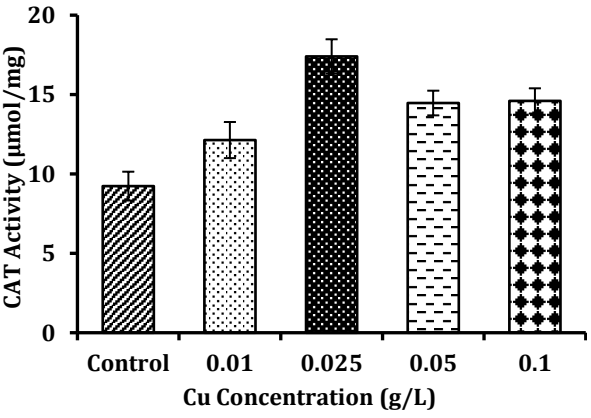

Figure 4. CAT activities in Cu at different concentrations

In addition, the SOD activities of microalgae was studied. The highest SOD activity was 8.87 µmol/mg at 0.025 g/L. However, SOD activities at 0.05 and 0.1 g/L were similar to these values. SOD activities were 8.73 µmol/mg and 8.60 µmol/mg, respectively. The SOD value of the control was 5.60 µmol/mg, and that of 0.01 g/L Cu was 6.80 µmol/mg. Figure 5 depicts the Superoxide Dismutase (SOD) activity in copper (Cu) at various concentrations.

Finally, the APX activities of some microalgal samples were investigated. The maximum APX activity was 32.7 µmol/mg for 0.025 g/L Cu. As Cu concentrations increased to this value, APX activity also increased. APX activity was 16.4 µmol/mg for control and 24.2 µmol/mg for 0.01 g/L Cu. APX

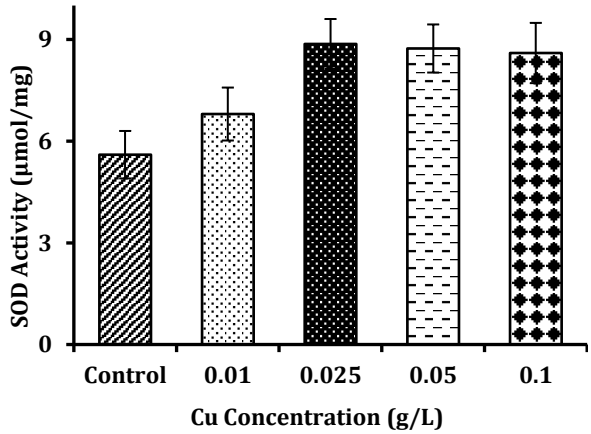

Figure 5. The SOD activities in Cu at various concentrations

activities were 27.7 µmol/mg and 28.2 µmol/mg for 0.05 g/L and 0.1 g/L, respectively. Figure 6 illustrates the APX alterations that occurred in the concentrations of microalgae with copper.

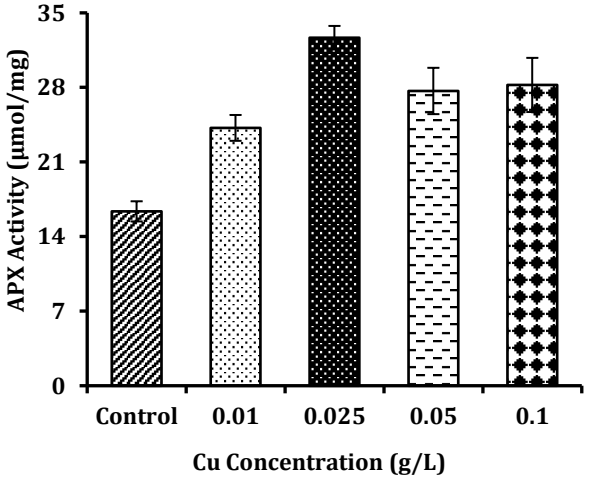

Figure 6. The APX activities in Cu at various concentrations

3.5. Bioethanol content of microalgae under copper stress

In addition, bioethanol from *Borodinellopsis texensis* was obtained, and microalgae samples are used, including the control and 0.025 g/L Cu.

Bioethanol concentration was 108.8 mg/L for 0.025 Cu, while that was 112.5 mg/L for control. However, when the findings were calculated based on the mass of microalgae per gram, there was a difference in the amount of bioethanol produced.

Bioethanol productivities were 174.4 mg g^{-1} and 141.8 mg g^{-1} for 0.025 g/L Cu and control, respectively. The bioethanol concentrations and productivities are presented in Table 2 for both the control and the 0.025 g/L Cu concentrations.

Table 2. Bioethanol concentration and productivity of *B. texensis* at the 0.025 g/L of Cu

Cu Concentration (g/L)	Bioethanol Concentration (mg/L)	Bioethanol Productivity (mg/g biomass)
Control	112.5 ± 3.8	141.8 ± 3.6
0.025	108.8 ± 7.5	174.4 ± 3.9

4. Discussion and Conclusion

The combined use of microalgae and copper appears in different forms in the literature. In general, investigations using microalgae and copper together stand out as those involving copper removal from wastewater. Yousef et al. studied the removal of copper and iron from medium-grown microalgae by the use of *Chlorella vulgaris* and *Scendesmus obliquus*. *Chlorella vulgaris* and *Scendesmus obliquus* were able to remove nearly 99% of the copper that was present in the medium in their various tests. Moreover, they demonstrated that copper has a high toxicity level for microalgae, and *Chlorella vulgaris* has a significant lack of tolerance to copper. Furthermore, they observed that the production of biofilm had an inverse relationship with the toxic to cells effect [19].

Also, copper can be utilized for harvesting of microalgae in nanotemplate investigations. Wong and his colleagues used 20 independent optimization strategies in their investigation, and they cultivated *Chlorella vulgaris* for their membrane study. The application of these membranes in conjunction with the copper-catalyzed template resulted in a 21% increase in flow rate. The effective application of copper led to an increase in the amount of biomass that was produced as well as an improvement in the effectiveness of membrane use [20]. In addition, Liu and coworkers investigated the removal of copper from synthetic piggery wastewater. *Desmodesmus sp.* was used as a microalga in their study, with a copper removal rate of about 89%. This rate demonstrates that copper in wastewater may be removed efficiently by these microalgae [21].

In addition, the toxicity of copper and its effect on microalgae may vary with temperature changes. Pascual and colleagues observed an increase in pH and a change in the permeability of microalgae when they increased the temperature of the water from 15 ^{0}C to 18 ^{0}C. In this case, there was a decrease in the toxicity of copper. On the contrary, when they raised the temperature from 21 to 30 degrees, they noticed a decrease in toxicity [22]. The study on the effect of copper on *Skeletonema costatum* found that as copper concentration grew, *Skeletonema costatum*'s growth rate reduced while its inhibition rate increased. After 96 hours, microalgae treated to a 2 mg/L nano-Cu concentration demonstrated an inhibitory rate of approximately 70%. However, after 24 hours of exposure to this dose, the value remained about 20% [23]. Similarly, Manikandan et al. investigated the impact that silver-copper nanocomposites had on *Tetraselmis suecica.* They found that the growth of these microalgae was inhibited in a manner that was dependent on both the duration of time and the amount of the nanocomposites. As a result of being subjected to a nanocomposite concentration of 1 mg/mL for a period of 72 hours, *Tetraselmis suecica* cells were decreased to 5.05 cells/mL [24]. Li et al. investigated the elimination of sulfonamides and copper with *Chlorella vulgaris*. Similarly, in our study, as the Cu concentration increased, the biomass concentration rate continued to decrease, and in the sample containing 0.05 g/L Cu, the biomass concentration of microalgae was observed to be 0.54 ± 0.03 g/L. The outcomes of these circumstances were comparable to our findings.

In addition, the effects of copper concentrations on protein and polysaccharide contents were examined in their study. The amount of protein and polysaccharide altered when Cu and sulfonamide concentrations increased. At a concentration of 200 ug/L Cu and 400 ug/L sulfonamides, the protein content was 92.71 mg/g, whereas the polysaccharide content was 556.52 mg/g. As a result, *Chlorella vulgaris* was found to be effective at removing copper and sulfonamide [25]. In another study, the effect of copper on the removal of estradiol was examined. If *Scenedesmus dimorphus* was exposed to 1 mg/L of copper, its growth increased, whereas exposure to 2 mg/L of copper slowed it down. In this investigation, 1 mg/L Cu and 0.5 mg/L estradiol were used, and approximately 90% of the estrogen and 77% of the copper were eliminated [26]. In another study, *Tetraselmis chuiif* was exposed to different concentrations of polyethylene plastic and copper, and their effect on the growth of the microalgae was examined. When growth rates were investigated at copper concentrations ranging from 0.02 to 0.64 mg/L, a decrease in growth was observed as the concentration increased. In contrast to the EC value of 0.139 mg/L that was obtained when copper was the only component utilized, the EC value of 0.145 mg/L was obtained when copper and microplastic were utilized together [27].

In order to determine the degree to which microalgae are able to absorb different elements, Saavedra et al. utilized a wide range of microalgal species. Chlorophyceae species played a significant role in determining the maximum removal rate for copper. The result was found to be 88 percent. During the course of this investigation, it was discovered that the sensitivity reached its peak point when the concentration of copper that was applied to Chlorophyceae was 4 mg/L [28]. According to the findings of these investigations, the metabolic components of microalgae may undergo changes when exposed to copper stress. In our study, the maximum concentration of carbohydrates was seen in a medium containing 0.025 g/L of copper, with a value of 0.29 ± 0.02 g/L, and the amount of carbohydrates and the percentages of microalgae both altered as a result of the change in the concentration of copper.

In the literature, the antioxidant properties of microalgae have been examined for various purposes. In the context of green synthesis, Rosyidah et al. synthesized gold from *Synechococcus moorigangae* and investigated the DPPH activity of the extracts that they produced. It was discovered that the antioxidant activities of the samples were dependent on the quantities of the samples. Maximum DPPH scavenging activity is nearly 60% in 100 µg/mL of the extract [29].

In another study, the antioxidant activities of *Chlorella sorokiniana* and *Scenedesmus acuminatus* under copper stress were examined. *Chlorella sorokiniana* showed a 4.6-fold increase in antioxidant activity, indicating that it was more sensitive to copper stress than the other species, although both species showed an increase in antioxidant activity [30]. Danouche et al. examined the effect of heavy metals on eight microalgae species. They found that low copper concentrations inhibited microalgae development. The EC_{50} value of copper was 5.8 ppm for *Scenedesmus obliquus*. According to the findings of this study, Scenedesmus is the most tolerant of heavy metals and the most resistant to lead [12]. In our study, the maximum scavenging activity of *Borodinellopsis texensis* was 83% at 0.025 g/L of Cu, whereas the SA of the control was 71%. SA increased as the Cu content increased. These findings demonstrated that, similar to the findings of the investigations mentioned previously, the DPPH activity of Borodinellopsis texensis was sensitive to the effects of copper stress.

Cylindrotheca closterium, *Phaeodactylum tricornutum*, and *Rhodomonas salina* were exposed to 5 and 10 µg/L copper concentrations, and their SOD, CAT, and APX activities were measured. The results of the control group showed that the CAT and SOD activities were higher, while the APX activity was lower, in the samples that were subjected to copper stress [31]. In a different study, the antioxidant capabilities of *Chlorella vulgaris* were investigated when copper stress was applied in the presence of sulphonamide. When copper content was 200 µg/L, SOD activity was at 161 U/mg protein. This result was 113 U/mg protein in the control group. The highest value for CAT activity was obtained at a copper concentration of 500 ug/L. Compared to the control, samples containing 500 ug/L copper showed an increase in CAT activity from 300 U/mg protein to 393 U/mg protein [25].

In another study, the antioxidant activity changes of *Scenedesmus dimorphus* were monitored under estradiol and copper concentrations. In the absence of estradiol, SOD activity increased as copper concentration increased, and the maximum SOD activity was found at 2 mg/L copper concentration. With an increase in copper concentration and the absence of estradiol, CAT activity also increased. The maximal residual activity was around 3 U/mg at a copper concentration of 2 mg/L [26]. Similarly, the SOD and CAT activities of *Chlorella sp.* in swine wastewater were investigated in the presence of different zinc and manganese levels. The highest SOD activity, approximately $1.1U/10^7$ cells, was obtained in a mixture of 1.85 mg/L ZN and 6 mg/L Mn. In addition, the highest CAT activity was found to be approximately $1.6\ U/10^7$ cells in 1 mg/L Mn [32]. SOD activity increased when *Chlorella vulgaris* was combined with copper in the presence of PVC. In the presence of copper, SOD activity was 1.05 U/mL, whereas the control had a SOD value of 0.97 U/mL [33].

Semi-inhibitory concentrations of Chlorella sp. and *Isochrysis zhangjiangensis* in the presence of heavy metals were calculated. When exposed to copper, *Isochrysis zhangjiangensis* had an EC_{50} value of 2.5 mg/L, whereas *Chlorella sp.* had an EC_{50} value of 2.78 mg/L. When two microalgae were combined, the EC_{50} value was 3.85 mg/L. The mixed culture exhibited the highest SOD value of approximately 550 $U.mg.protein^{-1}$ when subjected to 3 mg/L copper stress. The SOD activity values were approximately 300 $U.mg.protein^{-1}$ for Chlorella and nearly 500 $U.mg.protein^{-1}$ for *Isochrysis zhangjiangensis* [34].

The growth of *Graesiella sp.* MA1 in acid medium with copper and manganese led to an increase in the medium's pH from 3.5 to approximately 6, as well as a change in the antioxidant systems. The specific activity of APX varied between 0.04 and 0.06 with increasing copper stress. Similarly, our investigation revealed significant alterations in response to copper stress and the activity of APX [35]. In our study, the maximum CAT and SOD activities were 17.4 µmol/mg and 8.87 µmol/mg at 0.025 g/L, respectively. As was mentioned previously, the activities of CAT and SOD increased in response to copper concentrations, which is a

potential source of stress. Experiments using different types of microalgae, including *Tetradesmus dimorphus* and *Chlorella sp.,* revealed that the levels of APX increased by around 100% when subjected to various stressors such as nitrogen deprivation or the utilization of wastewater [36]. In our study, the maximum APX activity was 32.7 µmol/mg for 0.025 g/L Cu. As Cu concentrations increased to this value, APX activity also increased. These findings were consistent with the literature that was discussed earlier, which demonstrated that there was an increase in APX levels over time following the introduction of the stressor.

Different stressors can enhance the synthesis of bioethanol from microalgae. The researchers discovered that *Chlorella vulgaris* FSP-E produced a greater amount of bioethanol compared to the control group (0.22 g ethanol/g biomass) when exposed to an environment containing 25% swine wastewater and 2% carbon dioxide [2]. In a different study, *Chlorella sorokiniana* AK-1 and *Chlorella vulgaris* ESP-31 used things like 10% swine wastewater and changing the amount of light to make ethanol that was 4.2 g/L with an 84% fermentation rate [4]. Aside from microalgae, there are other ways to remove copper. One such method is to employ adsorbents. Using microbeads as adsorbents can help remove copper. One of them, [m-poly(DVB-VIM)], successfully removes copper from aqueous solutions [37]. Not only do beads remove copper from the environment, but they also successfully remove a wide range of other toxins. One of them is diethyl phthalate, which can be easily taken out of water with poly(EGDMA-MATrp) [38]. Antimicrobial pigments can also be adsorbed on beads. Cinnabarinic acid was adsorbed onto a bead [m-poly(EGDMA-MATrp)] [39]. Additionally, binding copper ions to m-poly(DVB–VIM) allows for the adsorption of alpha amylase. In their study, the highest amylase adsorption capacity was 10.84 mg/g [40]. In addition, adsorption can also be used to remove dyes from wastewater. Orange 16 dye in wastewater can be removed from wastewater using various polymer composites such as m-poly(EGDMA-VTA)-TiO_2 [41]. However, microalgae were used for the removal of Cu and biofuel production in this study.

In our study, bioethanol productivities were 174.4 mg g^{-1} and 141.8 mg g^{-1} for 0.025 g/L Cu and control, respectively. The results of this study demonstrated that microalgae that are subjected to copper stress can, up to a certain point, enhance the amount of bioethanol that they produce. With the help of this study, studies will continue on how microalgae exposed to copper stress will respond in larger reactors in the future.

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Declaration of Ethical Code

In this study, we undertake that all the rules required to be followed within the scope of the "Higher Education Institutions Scientific Research and Publication Ethics Directive" are complied with, and that none of the actions stated under the heading "Actions Against Scientific Research and Publication Ethics" are not carried out.

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