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Research Article

Determination of changes in biodiesel production by *Gloeocystis vesiculosa* cultured in wastewater with the artificial sweetener aspartame and its metabolites

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ABSTRACT

Microalgae can grow and form biomass in wastewater to produce various end products. In this study, the biomass, lipid content, and lipid yield changes of *Gloeocystis vesiculosa* cultured in wastewater containing different concentrations of the artificial sweetener APM and its metabolites DKP and PHE were evaluated. The highest biomass amount was 1080 ± 20 mg/L at 250 mg/L of DKP. Conversely, the highest lipid percentage was 31 ± 2% at 250 mg/L APM. There was no significant difference in lipid yield due to the lower biomass content relative to the higher lipid percentages. APM acts as a stress trigger. In addition, the antioxidant enzyme activities of *Gloeocystis vesiculosa* in wastewater with artificial sweeteners were determined. At 250 mg/L APM, the highest levels of SOD, CAT, APX, and MDA activity were 91 ± 4 U/mg protein, 78 ± 4 U/mg protein, 18 ± 2 U/mg protein, and 3.9 ± 0.2 nmol/mg protein. Future studies using a large-scale photobioreactor will investigate the effects of *Gloeocystis vesiculosa* on biodiesel production and wastewater treatment in wastewater contaminated with APM, DKP, and PHE.

Keywords: Biodiesel, *Gloeocystis vesiculosa*, Aspartame, Wastewater, Antioxidant activity

Introduction

Microalgae are the subject of research in the scientific community because of their rapid growth and the ease with which they may be manipulated to create the product of interest. In a medium known as a nutrient medium, which contains a variety of elements, microalgae can thrive (Jeffers et al., 2025). Alternatively, they can grow in municipal wastewater, which can supply them with all of the elements or minerals they require. This media typically contains a source of carbon, either organic or inorganic, that microalgae are able to harvest and absorb. If the microalgae are autotrophic, the carbon source that they use is typically carbon dioxide, which they then convert into an organic compound such as sugar (Duan et al., 2020). However, if the microalgae are heterotrophic, they are able to consume an organic molecule like glucose or a derivative in an anabolic cycle to make use of the carbon (Morales-Sánchez et al., 2013). In addition, if the microalgae are facultative, they are able to utilise any carbon source that they recognise, depending on the conditions of the surrounding environment (Abiusi et al., 2021). Microalgae not only require carbon for growth but also utilise high amounts of nitrogen and phosphorus (Salgado et al., 2023). Generally, increasing nitrogen levels accelerates microalgae growth and increases biomass. This process is a common manipulation method used in studies to increase microalgae biomass. However, increasing nitrogen levels and biomass also result in a decrease in microalgae metabolic products such as carbohydrates, lipids, or proteins (Maltsev et al., 2023). One strategy for increasing lipid content and biodiesel yield is to use a nitrogen-deficient medium. This allows for manipulation of lipid levels and changes in FAME content (Oğuz et al., 2024). The growth of microalgae and the production of lipids can be enhanced by adjusting the concentrations of key components in the culture media, including nitrogen, carbon, and phosphorus. Microalgae cultivated in various media, such as BG-11, TAP medium, and BBM, exhibit fluctuations in both their growth rates and lipid content based on the different sources and concentrations of these components (Vishwakarma et al., 2019). If the process negatively impacts the synthesis of products used as precursor molecules, it is undesirable for industrial production. For this purpose, increasing the desired precursor molecule and applying appropriate manipulation methods to increase the amount of the final product are more suitable for industrial studies. Another important issue in microalgae growth is the high cost of chemicals in artificially prepared media, which creates additional costs on an industrial scale. At this point, growing microalgae in wastewater may be considered (Dias et al., 2025). Wastewater provides a large portion of the nitrogen, phosphorus, and often carbon required by microalgae. This process allows for both wastewater treatment and microalgae growth, enabling the desired product to be obtained from the resulting biomass (Nur & Buma, 2019). If the wastewater is municipal or industrial, using proper microalgae can clean it while also producing a larger volume of product, as previously stated. The effluent from municipal facilities may contain significant quantities of artificial sweeteners (Shen et al., 2023). The metabolites of aspartame, which are known as (2S,5S)-3,6-Dioxo-5-(phenylmethyl)-2-piperazineacetic acid (DKP) and phenylalanine (PHE), can be discovered in wastewater when products that contain aspartame (APM) are released directly into wastewater without being used or mixed with wastewater after being metabolised. APM is commonly used as a sweetener in the pharmaceutical and food industries. Because of its expiration date or disposal in the bath and kitchen water, it directly enters municipal wastewater. Furthermore, when humans consume aspartame, the body converts it into PHE, which is expelled in urine. When exposed to harsh conditions such as high temperatures, acidic or neutral pH, and long-term storage, APM undergoes chemical reactions that result in DKP formation. Thus, DKP and PHE can be found in wastewater. DKP and PHE are referred to as APM metabolites since they are formed in the human body as a result of APM metabolism (Wawryk et al., 2023). It is possible for the artificial sweetener APM, as well as its metabolites DKP and PHE, to simultaneously promote the growth of microalgae that are suited for wastewater and to eliminate them from it. This investigation made use of the microalgae *Gloeocystis vesiculosa*, which had been cultured in the past on a variety of different media. This particular microalgae is classified as a member of the phylum Chlorophyta and the genus Gloeocystis (Capek et al., 2023). Any industrial product can be produced by microalgae that have been cultured in any medium or effluent. Microalgae have emerged as a highly influential actor in the energy industry. Microalgae have the potential to be recycled into biofuels. In this category, biodiesel, bioethanol, biogas, and biohydrogen are considered the most significant (Sharma et al., 2025). To manufacture biodiesel, the first step is to cultivate them for the generation of biomass, the acquisition of lipids, and finally the transesterification of the lipids (Geng et al., 2025). In addition, manipulation of any environmental component in microalgae may increase or decrease the metabolic product produced by stress factors. The status of antioxidant enzyme systems formed as a result of stress factors in microalgae can be examined. The most important antioxidant activity systems are superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and malondialdehyde (MDA). Their levels can vary depending on the microalgal stress level (Fal et al., 2022).

In this study, the microalgae *Gloeocystis vesiculosa* were cultivated in wastewater containing varying concentrations of artificial sweeteners (APM) and their metabolites (DKP and PHE). The purpose of this research was to investigate the biomass, lipid, and biodiesel yields of *Gloeocystis vesiculosa* grown in wastewater with APM, DKP, and PHE. Additionally, the study aimed to analyse the concentrations of aspartame and its metabolites present in wastewater, which microalgae can utilise. The study also aimed to investigate whether the stress generated by these sweeteners affected the levels of metabolic products in *Gloeocystis vesiculosa*.

Materials and Methods

Growth of Microalgae

The microalga *Gloeocystis vesiculosa* was obtained from the Culture Collection of Autotrophic Organisms (CCALA), Czechia. The microalgae were first cultivated in a medium containing 50% (v/v) Tris-Acetate-Phosphate (TAP) and 50% (v/v) Blue-Green-11 (BG-11). 80% (v/v) of a 1:1 TAP and BG-11 mixture was then combined with 20% (v/v) municipal wastewater for use in the studies. The final mixture consisted of 40% TAP, 40% BG-11, and 20% municipal wastewater (v/v). The wastewater used in this study had a COD value of 715 ± 23, a TN value of 68 ± 5, and a TP value of 21 ± 2. The final pH was adjusted to 7.2. A prior study provided detailed information on prepared wastewater (Onay, 2020). Microalgae were cultured at a speed of 60 rpm, a temperature of 24 ±1°C, and a light intensity of 90 µmol m^{-2} s^{-1}. Then, experiments were carried out on a 1 L flat photobioreactor. Experimental setups were carried out in three parallel samples by adding different concentrations (25 mg/L, 75 mg/L, 125 mg/L, and 250 mg/L) of the artificial sweetener aspartame (APM) and its metabolites (2S,5S)-3,6-Dioxo-5-(phenylmethyl)-2-piperazineacetic acid (DKP) and phenylalanine (PHE) to the medium. Microalgae were grown for 12 days until they reached the stationary phase at OD 680, after which their dry weights were measured gravimetrically. For harvesting, the samples were then centrifuged at 3000g for 10 minutes. After harvesting, the samples were washed three times with distilled water. Then, they were filtered through filter paper (1.2 µm GF/C; 47 mm). The samples were lyophilised. Then, they were placed in a desiccator to remove moisture, and they were weighed gravimetrically for biomass analysis. The samples were stored at -20 °C for further analysis.

Determination of Lipid Percentage

The Folch method was used to determine the lipid content of *Gloeocystis vesiculosa* samples (Folch et al., 1957). 1g of microalgae samples was used for lipid extraction, and the samples were prepared by combining chloroform and methanol in a 2:1 ratio, evaporating the chloroform, and measuring the residual oil gravimetrically.

Determination of Antioxidant Enzyme Activities

Superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and malondialdehyde (MDA) activities were determined spectroscopically, and the procedures were detailed in a previous study (Onay, 2020).

Biodiesel Production

Methanol, together with 0.1 M potassium hydroxide, was used to transesterify the lipids of microalgae extracts to produce biodiesel. A period of 4 h was spent incubating the mixture at 60 °C. Thereafter, it was stored at room temperature for the entire night. The glycerol and biodiesel phases were separated with the help of a flask separator, and the biodiesel was rinsed three times with distilled water to make sure that any undesirable residues were removed. Biodiesel content was determined gravimetrically (Onay & Ayas, 2024).

Statistical Analyses

The experimental groups contained three parallel samples. All statistical analyses were performed by one-way analysis of variance (ANOVA) and Tukey's test. The confidence level was higher than 95%. In this report, the results are expressed as the mean ± standard deviation (SD). When $P < 0.05$, * shows significant, and ** shows very significant.

Results and Discussion

This study focused on how *Gloeocystis vesiculosa* microalgae, grown in wastewater with aspartame (APM), (2S,5S)-3,6-Dioxo-5-(phenylmethyl)-2-piperazineacetic acid (DKP), and phenylalanine (PHE), affected their metabolic content and contribution to biodiesel production by examining changes in biomass concentrations. The control group comprised microalgae cultivated in wastewater that did not include APM, DKP, or PHE. The biomass of the control was 827 ± 15 mg/L. The lowest biomass content was 663 ±15 mg/L at 250 mg/L of APM, while the maximum was 1080 ± 20 at 250 DKP. The study demonstrated that when the APM concentration grew, the biomass concentration fell. The biomass concentration at 25 mg/L APM was 830 ± 15 mg/L, but at 75 mg/L APM, it was 753 ± 6 mg/L. Furthermore, as the APM concentration grew, the biomass decreased. The biomass concentration at 125 mg/L APM was 720 ± 10 mg/L, with the lowest concentration at 250 mg/L. Unlike APM, DKP showed a different growth pattern. As DKP concentration increased, biomass concentration also increased. The increase in biomass concentration was slow at 25 mg/L (837 ± 6) and 75 mg/L (880 ± 10 mg/L), but it became rapid at 125 mg/L DKP (963 ± 15 mg/L), with the maximum biomass (1080 ± 20 mg/L) reached at 250 mg/L DKP. PHE showed a different effect than APM and DKP. At low PHE concentrations, the biomass amount was close to the control. Accordingly, at 25 mg/L and 75 mg/L PHE, biomass amounts were 827 ± 6 mg/L and 853 ± 15, respectively. Similarly, at 125 mg/L and 250 mg/L PHE concentrations, the biomass concentrations were 900 ± 10 mg/L and 920 ± 10, respectively. As a result, APM and its metabolite products, DKP and PHE, which are structurally similar, had distinct effects on microalgae development in wastewater. When the results are evaluated statistically, biomass concentrations of 125 and 250 mg/L for APM, DKP, and PHE are highly significant. In contrast, biomass at 75 mg/L for DKP and PHE is considered significant, while APM also shows high significance.

Although studies on *Gloeocystis vesiculosa* are scarce in the literature, a few publications exist, mostly about carbohydrate and sugar production. Exopolysaccharide from *Gloeocystis vesiculosa* was found to contain α-D-Glcp residues, the majority of which were found to be 1,4-linked. To a lesser extent, they were found to be the terminal sugar. It can be inferred from this that β-D-xylo-α-D-mannan was slightly contaminated with amylose, around 10 weight per cent (Capek et al., 2023). In another study, the microalgae *Gloeocystis vesiculosa* was grown in BBM medium, and its biomass yield was examined under different light sources. The highest biomass was obtained under red light (0.62 g/L), while under white light, it was found to be 0.59 g/L (Çeliktaş, 2020). The impacts of altering the amounts of carbon, nitrogen, sulfate, sodium, and calcium in wastewater were explored for *Gloeocystis vesiculosa* in another study. That study focused on the concentration of biomass. It was discovered that the highest concentration of biomass was 0.63 g/L (Al-Badri, 2021). The findings of these studies were comparable to ours, although they were distinct from our own. Differences in biomass concentrations were observed because of the utilisation of various light wavelengths, as well as various media and wastewater ingredients. Figure 1 depicts the fluctuation in the biomass amount of *Gloeocystis vesiculosa* growing in wastewater containing APM, DKP, and PHE.

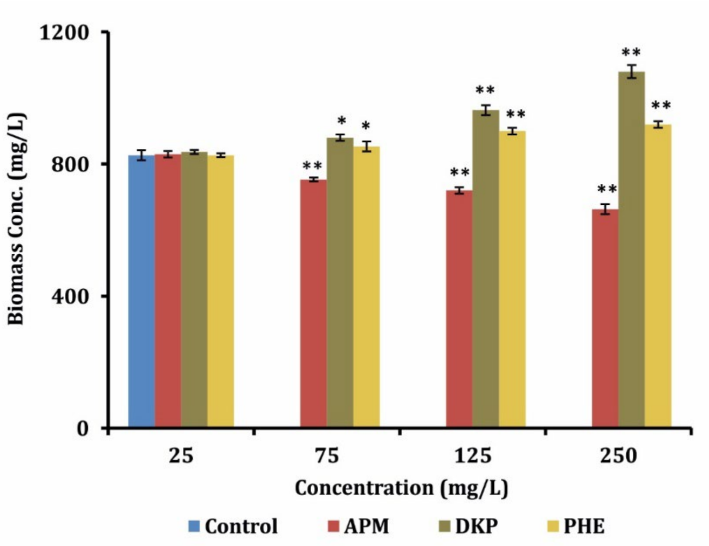

Figure 1. The changes in the biomass amount of *Gloeocystis vesiculosa* growing in wastewater containing APM, DKP, and PHE

Then, lipid content changes were investigated after biomass change in *Gloeocystis vesiculosa* cultured in wastewater containing APM, DKP, and PHE. The control had a lipid content of 25% ± 1%. Furthermore, the maximum lipid percentage was 31 ± 2% at 250 mg/L APM, and the lowest was 20 ± 1% with 250 mg/L DKP. According to the results, as the APM increased, the amount of lipids also increased. At 25 mg/L APM, the lipid percentage was 26 ± 1%, while at a 75 mg/L APM concentration, the lipid percentage (27 ± 1%) was close to this value. The increase in lipid percentage accelerated after this value. At a 125 mg/L APM concentration, the lipid percentage was 28 ± 1%, and at a 250 mg/L APM concentration, it reached a maximum value of 31 ± 2%, as mentioned above. DKP, on the other hand, exhibited the opposite behaviour to APM. As the amount of DKP increased, the amount of lipid decreased. At low concentrations such as 25 mg/L DKP, the lipid percentage was the same as the control (25 ± 1%). At 75 mg/L DKP, the lipid percentage was 24 ± 2%, and at 125 mg/L DKP, it was 22 ± 1%. However, at high DKP concentrations such as 250 mg/L, the lipid percentage was 20 ± 1%. PHE showed similar behaviour to DKP. As PHE concentration increased, the lipid percentage decreased slightly but steadily. At a 25 mg/L PHE concentration, the lipid percentage was the same as the control (25 ± 1%). At 75 mg/L PHE, it was 24 ± 1%. The decrease continued as the concentration increased. At a 125 mg/L PHE concentration, it was 23 ± 1%, and at a 250 mg/L PHE concentration, it was 21 ± 1%. This indicates that while higher levels of APM lead to greater lipid accumulation, the presence of DKP and PHE influences the lipid composition, causing variability in lipid percentages. Consequently, the interplay between these compounds affects the overall lipid profile differently. The statistical interpretation of the results indicated that the differences in lipid percentages were most pronounced at a concentration of 250 mg/L. At this concentration, there were very significant differences observed in APM and DKP, while PHE was noted to be significant as well.

There are articles in the literature showing changes in lipid amounts by manipulating the environmental properties of microalgae. In a study with *Chlamydomonas reinhardtii*, fatty acid yield increased by 150% when nitrogen and phosphorus were depleted from the medium, and 4 g/L sodium acetate was utilised as the carbon source (Yang et al., 2018). In another study, *Chlamydomonas reinhardtii* microalgae were cultivated with *Azotobacter chroococcum* in nitrogen-deficient medium, and the lipid content increased from 29% to 66% when compared to the control (Xu et al., 2018). This showed that, in addition to removing nitrogen from the environment, the use of biological material could increase the amount of lipid. Additionally, *Chlorella sp.* have demonstrated the ability to increase lipid levels. Under zinc stress, *Chlorella sp.* cells created stress conditions, increasing lipid levels to 23% (Mihiraj et al., 2022). These results showed that any stress factor or change in the concentration of the chemical can cause changes in the lipid content of microalgae. Figure 2 shows how the lipid percentage of *Gloeocystis vesiculosa* changes when it grows in wastewater with APM, DKP, and PHE.

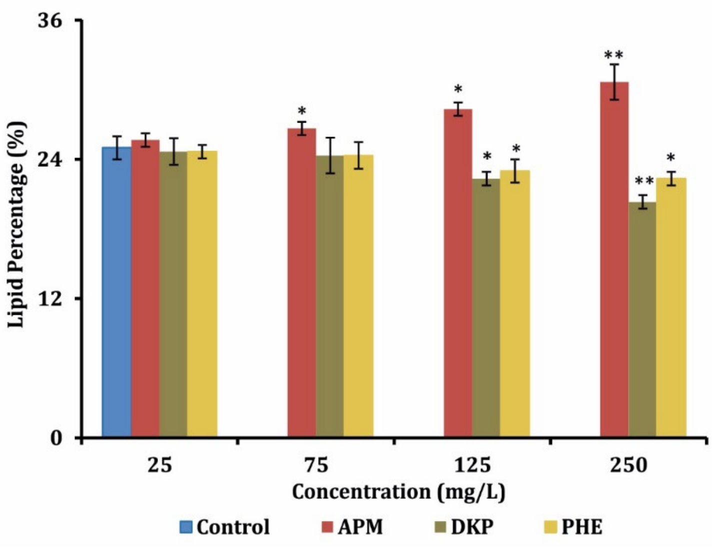

Figure 2. The lipid percentage of *Gloeocystis vesiculosa* grown in wastewater with APM, DKP, and PHE

In addition to changes in biomass and lipid content, the antioxidant capabilities of *Gloeocystis vesiculosa* cultured at various doses of APM and its metabolites were assessed. Changes in biomass and lipid levels can influence antioxidant activity; hence, a stress state can be described by these changes. For this purpose, SOD activities were first examined. The highest SOD activity was 91 ± 4 U/mg protein at 250 APM. The lowest SOD activity was 32 ± 2 U/mg protein at 25 DKP. The SOD value of the control group was 35 ± 3 U/mg protein. When we evaluated the results in terms of APM, SOD activity also increased as the APM amount increased. At a 25 mg/L APM concentration, SOD activity was 36 ± 2 U/mg protein, while at a 75 mg/L APM concentration, it was 44 ± 4 U/mg protein. When the APM was increased to 125 mg/L, SOD activity increased to 69 ± 5 U/mg protein. Although the results for DKP seemed similar, the increases were more limited. Probably, the decreases in lipid levels corresponded with a lesser effect on SOD activity. As mentioned above, SOD activity at a 25 mg/L DKP concentration was 32 ± 5 U/mg protein, while at a 75 mg/L DKP concentration it was around 36 ± 2 U/mg protein. At a 125 mg/L DKP concentration, it was 48 ± 4 U/mg protein, and at a 250 mg/L DKP concentration, it was 46 ± 4 U/mg protein. This condition showed that SOD activity remained constant at high concentrations of DKP. PHE's SOD activities were shown to be opposing those of DKP. SOD activity was unaltered at low PHE amounts but increased at high PHE concentrations. SOD activity was 34 ± 1 U/mg protein at PHE concentrations of 25 mg/L and 35 ± 3 U/mg protein at 75 mg/L. Then, at 125 mg/L and 250 PHE concentrations, SOD activity was 45 ± 2 U/mg protein and 49 ± 4, respectively. The SOD activities of the microalgae samples were statistically analysed. At a concentration of 250 mg/L, the SOD activities of the APM and PHE samples were found to be highly significant compared to the control, while the DKP samples were noted as significant. At a concentration of 125 mg/L, APM was highly significant, whereas DKP and PHE were deemed significant.

There are literature articles showing changes in SOD activity in microalgae exposed to varying heavy metal concentrations. In one of these, *Chlorella sorokiniana* and *Scenedesmus acuminatus* were exposed to 1 mM and 0.6 mM zinc concentrations. Due to its antioxidant properties, *Chlorella sorokiniana* was less exposed to stress, while SOD activity increased 2.2-fold compared to the control (Hamed et al., 2017). An additional investigation was conducted in which *Coelastrella sp.* was grown in swine wastewater and subjected to different doses of zinc ranging from 0 to 8 mg/L. The metabolic contents of the organism were then evaluated. It was discovered that a zinc concentration of 8 mg/L (63 U/mg protein) produced the maximum SOD activity measured. As the zinc concentration grew, so did the activity of SOD, which is evidence of the harmful effects of zinc (Li et al., 2020). The application of physical elements is another method by which microalgae can be subjected to stress. The degrees of stress experienced by *Scenedesmus obliquus* and *Nannochloropsis gaditana* were evaluated in one of these experiments, which involved the animals being subjected to a magnetic field. SOD activity of *Scenedesmus obliquus* increased by 60% when it was subjected to a magnetic field, but the SOD activity of *Nannochloropsis gaditana* increased by 115% when it was subjected to stress (Serrano et al., 2021). Microalgae may experience an increase in SOD activity and changes in metabolic content when they are subjected to any stress, as may be deduced from these investigations, which are conducted in parallel with our research. Figure 3 demonstrates the changes in *Gloeocystis vesiculosa* SOD enzyme activity as it grows in wastewater containing APM, DKP, and PHE.

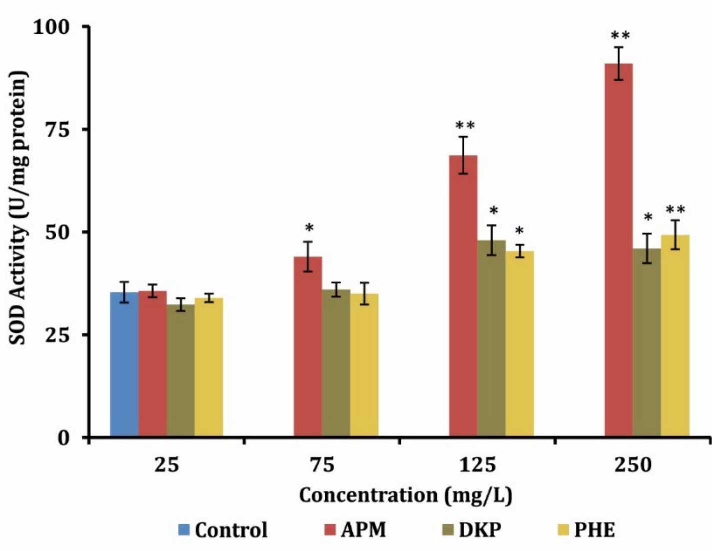

Figure 3. The changes in SOD enzyme activity for *Gloeocystis vesiculosa* grown in wastewater containing APM, DKP, and PHE

CAT activity was also investigated, in addition to the assessment of SOD activity. Comparing the results of the CAT activity to those of the SOD activity revealed comparable findings. The variation is most likely the result of the interrelated nature of the two enzymes and their relatively minor impact on the various enzyme systems. The system likely produced oxygen radicals and hydrogen peroxide in proportionate amounts. The highest CAT activity was observed at 250 mg/L APM, with a protein concentration of 78 ± 4 U/mg. On the contrary, the lowest CAT activity was observed at 25 mg/L DKP, with 26 ± 3 U/mg. In the control group, the CAT activity was 29 ± 3 U/mg protein. CAT activities were evaluated at low amounts of 25 mg/L and 75 mg/L, with 31 ± 3 U/mg protein and 44 ± 3, respectively, at the relevant doses. At concentrations of 125 mg/L APM, the increase continued, and the CAT activity was determined to be 62 ± 6 U/mg protein at 250 mg/L APM. The CAT activity was also measured to be 78 ± 4 U/mg protein at these levels. At low concentrations in the medium with DKP, CAT activity did not rise noticeably. CAT activity was 26 ± 3 U/mg protein when DKP was 25 mg/L. However, when DKP was 75 mg/L, the CAT activity was 28 ± 2 U/mg protein. Additionally, aggregation took place at 125 mg/L and 250 DKP per volume. Although the CAT activity was 38 ± 4 U/mg protein when the DKP was 125 mg/L, it was 41 ± 3 U/mg protein when the DKP was 250 mg/L. The CAT activity of PHE exhibited behaviours that were comparable to those of the SOD activity. CAT activities were measured at 25 mg/L and 75 mg/L PHE, with the former exhibiting 26 ± 3 U/mg protein and the latter exhibiting 28 ± 3 U/mg protein. According to the findings of this investigation, the activity of CAT was comparable to that of the control at low values of PHE. However, when PHE was 125 mg/L, the CAT was 40 ± 4 U/mg protein. However, when it was 250 mg/L, the CAT activity was 39 ± 3. After 125 mg/L of PHE was added, the results demonstrated that there was no change in CAT. CAT activity may change with the emergence of stress factors. When the CAT activity levels were evaluated in the microalgae statistically, we observed results similar to those for SOD activity. The samples with concentrations of 125 and 250 mg/L showed more significance. While APM samples at these concentrations were very significant, DKP and PHE samples could be considered significant.

In one study, *Scenedesmus sp.* was exposed to aluminium stress, and CAT activity was observed to decrease at the highest concentration of 100 μM. It has been explained that this is due to the decrease in protein expression (Ameri et al., 2020). In another study, *Pseudochlorella pringsheimii* was exposed to salinity and iron stress. CAT activity increased under both stress factors, reaching a two-fold increase compared to the control (Ismaiel & Piercey-Normore, 2023). Similarly, in another study, CAT activities were investigated when *Phormidium ambiguum* and *Microcystis aeruginosa* were grown at different light intensities, and the maximum CAT activity for *Phormidium ambiguum* increased 12-fold at 600 μmol·m^{-2}·s^{-1}after 15 hours. However, CAT activity for *Microcystis aeruginosa* increased 5-fold at 600 μmol·m^{-2}·s^{-1}after 15 hours. This suggests that both microalgae species are exposed to stress at high light intensities, causing an increase in CAT activity (Muhetaer et al., 2020).

These results were parallel to our results. Figure 4 demonstrates the variations in CAT activity of *Gloeocystis vesiculosa* in wastewater containing APM, DKP, and PHE.

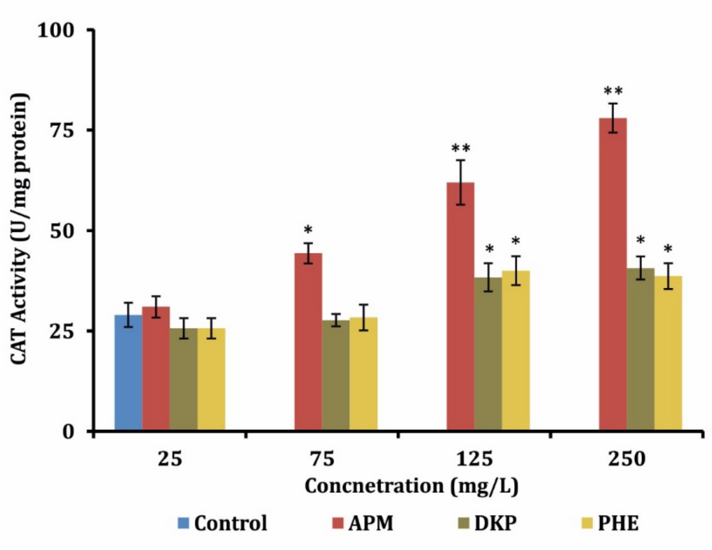

Figure 4. The variations in CAT activity of *Gloeocystis vesiculosa* in wastewater containing APM, DKP, and PHE

Another antioxidant enzyme studied was APX in this study. The changes observed in APX activity were somewhat more pronounced. The APX value in the control group, which did not contain APM, DKP, or PHE, was 6 ± 1 U/mg protein. The highest APX value was 18 ± 2 U/mg protein at 250 mg/L APM, three times the control level. The lowest APX activity value was 5 ± 1 U/mg protein at 25 mg/L DKP, very close to the control level. The lower DKP concentration proved to have no effect on APX activity. The situation was similar at low APM concentrations. The APX activity value at 25 mg/L APM was 6 ± 2 U/mg protein, while the APX activity at 75 mg/L APM was 9 ± 2 U/mg protein. As the amount of APM increased, the APX activity value continued to increase. The APX activity at 125 mg/L APM was 13 ± 2 U/mg protein, while the APX activity value at 250 mg/L APM was 18 ± 2 U/mg protein, as mentioned above. The levels of APX activity at various concentrations of DKP were quite comparable to one another. When DKP was 25 mg/L, the activity of APX was measured to be 5 ± 1 U/mg protein. However, when DKP was 75 mg/L, the APX activity was 7 ± 1 U/mg protein. Without taking into account the other findings, the APX activity was 8 ± 1 U/mg protein when the DKP was 125 mg/L. At these three concentrations, the APX activities were highly comparable to each other. When DKP was increased to 250 mg/L, the activity of APX was found to be 11 ± 1 U/mg protein following this increase. There was a close relationship between the APX activity levels of DKP and the APX activity values at various concentrations of PHE. The APX value was found to be 6 ± 1 U/mg protein when PHE was 25 mg/L. However, when PHE was 75 mg/L, the APX value was 7 ± 1 U/mg protein. After increasing PHE to 125 mg/L, the APX activity value was 8 ± 1 U/mg protein following the increase. When PHE was significantly raised to 250 mg/L, the APX activity value was found to be 10 ± 1 U/mg protein. The APX activities of microalgae samples were statistically distinct at concentrations of 125 and 250 mg/L. While 125 mg/L APM, DKP, and PHE samples showed statistically significant results, 250 mg/L APM, DKP, and PHE samples were considered highly significant.

Studies on APX in the literature were consistent with our results. In one of these, the APX activity of the microalgae *Chlamydomonas reinhardtii* grown at a high light intensity of $1400\ \mu E \cdot m^{-2} \cdot s^{-1}$ increased, reaching a maximum of around 0.12 mmol/mg protein, and this increase was found to be associated with an increase in transcript levels (Kuo et al., 2020). Another study investigated how APX activity changes during various growth phases of *Chlorella vulgaris*, and it revealed that APX activity was induced in the lag phase and was 37 U/mg protein (Yusuf et al., 2022). *Chlorella sorokiniana* and *Scenedesmus acuminatus* were subjected to zinc concentrations of 1 mM and 0.6 mM, respectively. *Chlorella sorokiniana* had considerably higher APX activity (about 0.7 U/mg protein) than *Scenedesmus acuminatus* (0.25 U/mg protein); however, APX activity increased in both microalgae (Hamed et al., 2017). As a result, the APX activities of microalgae exposed to stress factors increased, as in this study. Figure 5 shows the differences in APX activity of *Gloeocystis vesiculosa* in wastewater containing APM, DKP, and PHE.

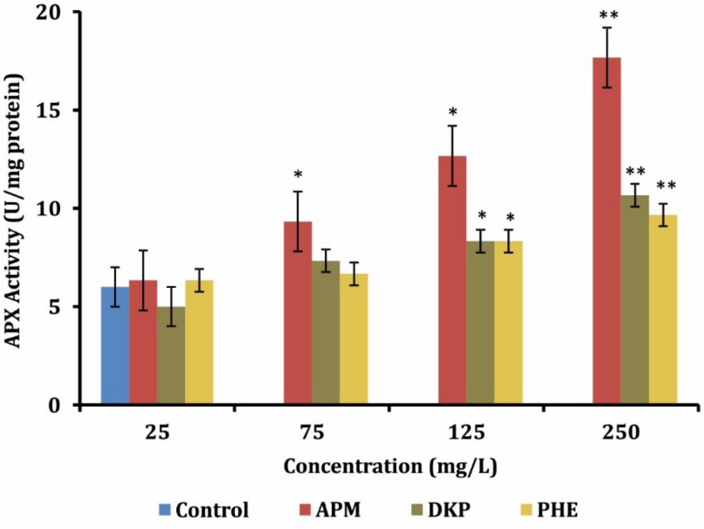

Figure 5. The differences in APX activity of *Gloeocystis vesiculosa* in wastewater containing APM, DKP, and PHE

Finally, MDA activity was examined to understand the response of *Gloeocystis vesiculosa* to stress factors (APM, DKP, and PHE) in wastewater. MDA activity gave comparable results to APX activity and was consistent with other enzyme activities. The MDA activity of the control was 1.3 ± 0.1 nmol/mg protein. The highest MDA activity was found to be 3.9 ± 0.2 nmol/mg protein at 250 mg/L APM concentration. In contrast, the lowest MDA activity was 1.3 ± 0.1 at 25 and 75 DKP. In addition, the MDA value at low APM concentration was very close to the control value. MDA values at 25 and 75 mg/L APM were 1.4 ± 0.1 nmol/mg protein and 1.5 ± 0.1 nmol/mg protein, respectively. As the amount of APM increased, MDA activity increased. At an APM concentration of 125 mg/L, MDA activity was 2.8 ± 0.2 nmol/mg protein. At an APM concentration of 250 mg/L, it reached its maximum value, as mentioned above, and became 3.9 ± 0.2 nmol/mg protein. Changes in DKP concentrations also had a minor impact on MDA activity. At DKP dosages of 25 mg/L and 75 mg/L, MDA activity was 1.3 ± 0.1 nmol/mg protein, comparable to the control group. In other words, lower concentrations of DKP did not influence MDA activity, but as the concentration grew, it increased slightly. MDA activity was 1.7 ± 0.1 nmol/mg protein at 125 mg/L DKP but rose to 2 ± 0.1 nmol/mg protein at a 250 mg/L DKP concentration. PHE, in contrast to APM and DKP, did not react to a significant amount of MDA activity. When PHE was utilised, the MDA activity reached its peak at 250 mg/L, with a value of 1.3 ± 0.1 nmol/mg. At PHE concentrations of 25 and 75 mg/L, the protein concentration was found to be 1.4 ± 0.1 nmol/mg protein and 1.5 ± 0.1 nmol/mg protein, respectively, during the experiment. In addition, at 125 mg/L PHE, the MDA activity value was 1.6 ± 0.1 nmol/mg protein. MDA activity can vary depending on the nature of the microalgae and the stress conditions applied. When the MDA activities of microalgae were statistically evaluated, samples of 250 mg/L APM, DKP, and PHE were found to be highly significant. In contrast, samples of 125 mg/L DKP and PHE were noted as significant. Additionally, samples of 125 mg/L APM were also considered highly significant.

There is a wealth of information in the literature regarding varying MDA activity. One study examined the MDA changes in Spirulina platensis grown at different concentrations of lead, copper, and zinc. The highest MDA activity increased by almost 100% at the 0.2 mg/L copper concentration, while an almost 90% increase was observed at 0.2 mg/L zinc and lead concentrations. This demonstrates that MDA activity increased significantly at high amounts of heavy metals (Choudhary et al., 2007). In another study, *Scenedesmus vacuolatus* and *Chlorella kessleri* were exposed to copper (between 6.2 mM and 414 µM), and their MDA activities were examined. While the samples at 414 µM copper concentrations of *Scenedesmus vacuolatus* had maximum activity with approximately 10 nmol 10^6 cells^{-1} MDA activity, no significant change was observed in the MDA of *Chlorella kessleri* (Sabatini et al., 2009). *Chlorococcum sp.* was also subjected to different levels of copper and cadmium, and microalgae grew. The maximum concentration of both heavy metals was 200 mg/L, and the highest MDA activity was found at 200 mg/L copper, with a value of approximately 35 mmol/g ww. In contrast, the MDA value at 200 mg/L cadmium was approximately 29 mmol/g ww (Qiu et al., 2022). These instances demonstrate that, similar to ours, MDA activity increased in response to stress. Figure 6 illustrates the variations in MDA activities of *Gloeocystis vesiculosa* cultivated in wastewater containing APM, DKP, and PHE.

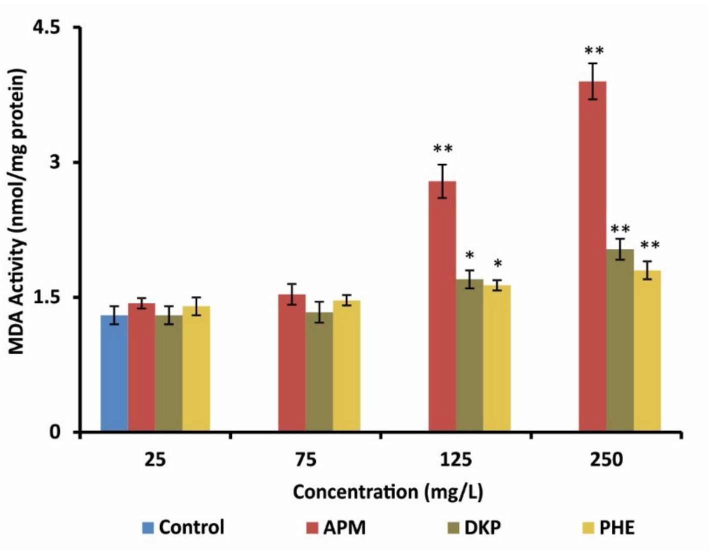

Figure 6. The variations in MDA activities of *Gloeocystis vesiculosa* cultivated in wastewater containing APM, DKP, and PHE

Finally, this study looked into the effect of *Gloeocystis vesiculosa* microalgae cultivated at various concentrations of APM, DKP, and PHE on lipid yields. Microalgae samples cultivated at a DKP concentration of 250 mg/L produced the maximum lipid yield (220 ± 3 mg/L). Microalgae samples with 75 mg/L APM had the lowest lipid yield (201 ± 5 mg/L). In fact, when the concentration of APM increased, the lipid yield decreased, but this decline was not substantial. The lipid yield of samples with 25 mg/L APM was 213 ± 7 mg/L, whereas the highest APM content yielded 203 ± 10 mg/L. In fact, APM significantly increased cellular lipid content while decreasing biomass concentration. The metabolic shift was caused by stress rather than an overall increase in lipid productivity. APM seems to shift carbon flow away from processes that help growth and toward lipid storage as a way to survive. However, when looked at from the point of view of biofuel production, this rise in lipid percentage did not lead to a higher total lipid yield because biomass was lost at the same time. The trade-off between biomass suppression and lipid accumulation greatly limits the practical benefit of APM treatment because total lipid yield is the most important factor in determining whether biodiesel is possible. Consequently, APM ought to be regarded as a modulator of lipid metabolism at the cellular level, rather than a method to enhance overall lipid productivity, unless integrated with process optimisation techniques that can reduce biomass loss. Increases in SOD, CAT, APX, and MDA concentrations support this conclusion, strengthening the concept that it functions as a stress trigger. In samples grown in DKP, an increase in lipid yield was observed as the DKP concentration increased. This increase was gradual but small in amount. At a DKP concentration of 25 mg/L, the yield was 206 ± 8 mg/L, while at a DKP concentration of 250 mg/L, as mentioned above, the maximum value was 220 ± 3 mg/L. The yields at 75 mg/L and 125 mg/L DKP concentrations were quite close to each other. The yields at 75 mg/L and 125 mg/L DKP concentrations were 214 ± 11 mg/L and 215 ± 4 mg/L, respectively. Although PHE exhibited similar behaviour to APM and DKP, the lipid yields here were closer to each other, and increases in PHE concentration did not significantly affect lipid yield. The lowest yield was 204 ± 6 mg/L at a PHE concentration of 25 mg/L, while the highest yield was 208 ± 7 mg/L at a PHE concentration of 75 mg/L.

Most studies on biodiesel synthesis from microalgae involve changes in ambient pollutants. In one of these studies, *Chlorella ellipsoidea* was exposed to salt stress, and lipid levels were boosted to increase biodiesel yield. The maximum lipid content was 46% at an NaCl concentration of 5 g/L, resulting in a higher biodiesel output (Satpati et al., 2016). According to the findings of another study, the lipid content of *Skeletonema costatum* increased when it was grown in a medium that was limited in both nitrogen and silica. Microalgae grown in a medium with 6.8 µmol/L of nitrogen and 0.36 µmol/L of silicon, under N-Si restriction, demonstrated a lipid content that increased by 114% (Gao et al., 2019). In another study, the lipid content of the thermo-resistant microalga *Micractinium sp.* was examined to determine how it altered as temperature increased. The experiment indicated that the highest lipid content was 23% at a temperature of 25°C (Onay et al., 2014). Table 1 shows the amounts of biomass and lipid yield produced by *Gloeocystis vesiculosa* grown in different amounts of APM, DKP, and PHE.

Finally, *Gloeocystis vesiculosa*, when grown in various concentrations of artificial sweetener and its metabolites (APM, DKP, and PHE), responded by altering its metabolic contents, which led to changes in biomass and lipid. However, it did not cause significant changes in lipid yield. This situation is observed as a decrease in the biomass amount and an increase in the lipid amount, and it can be said that APM, in particular, increases the lipid amount due to stress. It is a stress trigger. Conversely, in DKP, lipid percentage decreased with concentration. Variations in SOD, CAT, APX, and MDA activity levels also explained these findings. In addition, the concentrations of aspartame and its breakdown products in this study (25–250 mg/L) are higher than those in wastewaters (µg/L–ng/L range). This study was designed to test how well the treatment system works under heavy loads. Additionally, point-source or industrial wastewaters, particularly those from the food, drink, or drug industries, may contain these high levels. Accordingly, the results should be considered a high-load toxicity test that shows how well the system can handle stress, what effects it might have on performance, and what its limits are in the worst-case scenario.

Table 1. The yield of biomass and lipid of *Gloeocystis vesiculosa* cultivated at various APM, DKP, and PHE concentrations

Biomass (mg/L)	APM	DKP	PHE
25 mg/L	830 ± 10	837 ± 6	827 ± 6
75 mg/L	753 ± 6**	880 ± 10*	853 ± 15*
125 mg/L	720 ± 10**	963 ± 15**	900 ± 10**
250 mg/L	663 ± 15**	1080 ± 20**	920 ± 10**
Lipid Yield (mg/L)	**APM**	**DKP**	**PHE**
25 mg/L	213 ± 7	206 ± 8	204 ± 6
75 mg/L	201 ± 5	214 ± 11	208 ± 7
125 mg/L	204 ± 2	215 ± 4	207 ± 10
250 mg/L	203 ± 10	220 ± 3	205 ± 4

Conclusion

When grown in wastewater containing artificial sweeteners such as APM and its metabolites, such as DKP and PHE, Gloeocystis vesiculosa metabolises some of these substances, altering biomass and lipid contents, and affecting antioxidant activities such as SOD, CAT, APX, and MDA. In addition, no significant change was observed in lipid yield. High levels of APM increase lipid percentage, while high levels of DKP increase microalgal biomass. APM functions as a stress trigger and induces severe stress. Increases in the antioxidant enzymes SOD, CAT, APX, and MDA provide evidence of this. In both cases, these chemicals are metabolised, benefiting wastewater treatment and leading to product formation. In light of these results, future studies using a large-scale photobioreactor will investigate the effects of *Gloeocystis vesiculosa* on biodiesel production and wastewater treatment in wastewater contaminated with APM, DKP, and PHE.

Compliance with Ethical Standards

Conflict of interest: The author(s) declare no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: The authors declare that this study does not involve experiments with human or animal subjects, and therefore, ethics committee approval is not required.

Data availability: The data will be made available upon request from the author.

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Disclosure: -

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