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Assessment of Phytoremediation Efficiency and Valuable Biomass-Yielding of Different Microalgal Strains Grown on Aquaculture Wastewater

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ABSTRACT

This work aimed to study the potential of aquaculture wastewater (AWW) as a nutrient source for *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, and *Haematococcus lacustris* cultivation. Investigating nutrient removal for these microalgae was evaluated. The best phytoremediation was observed by *C. reinhardtii* with removal efficiencies of 82 and 62.3% for nitrate and chemical oxygen demand (COD), respectively, while all strains were able to remove 100% of total phosphorus (TP). Although the aquaculture wastewater (AWW) was a suitable medium for microalgae, the 3 microalgae grew faster with more biomass on BG11 than on AWW. In AWW, nutrients were lower than in BG11, which may contribute to microalgae stress; this stress enhanced lipid and carbohydrate production compared to BG11. *C. reinhardtii* exhibited the highest biomass of 1.00±0.07mg/L in AWW and the highest Diphenylpicrylhydrazyl (DPPH) scavenging activity of 63.08±2.09%. These findings demonstrate the potential of microalgae to remove and utilize nutrients in AWW while achieving valuable biomass productivity.

INTRODUCTION

Aquaculture is one of the fastest-growing sectors in the food industry. It demands vast amounts of water for fish farming, hatcheries, and processing **(Teoh *et al.*, 2023)**. Globally, especially in developing countries, water scarcity and the depletion of natural waters are the major challenges in seafood security today **(Rifna *et al.*, 2024)**. Moreover, aquaculture effluents are typically high in nitrate, nitrite, ammonia, and phosphorus, thus they can cause contamination and have adverse effects on the surrounding water bodies **(Abdo *et al.*, 2019; Doma *et al.*, 2021; Hala *et al.*, 2021; Ahmad *et al.*, 2022)**. As a result, researchers are working on producing low-cost, environmentally friendly technologies for treating aquaculture effluent, reducing environmental impact, and maintaining natural water resources. The conventional AWW treatment solutions, viz. aerobic filters or constructed wetlands, require high energy demands, cost, and large areas **(Tom *et al.*, 2021)**. Bioremediation treats discharged water by using naturally occurring microorganisms. Bioremediation is less expensive than other technologies for the hazardous waste cleanup **(McMartin, 2014)**.

Table 1. A comparison between microalgae-based wastewater treatment methods and traditional methods

	Microalgae-based wastewater treatment	**Traditional wastewater treatment**	**Ref.**
Treatment Efficiency	Removing the pollutant in only one step	Requires multiple steps, such as primary and secondary treatment, to achieve the same level of nutrient removal	**(Molinuevo-Salces *et al.*, 2019)**
Cost	• No chemicals are required for treatment. • Able to simultaneously treat and generate value-added products. • The harvested microalgae biomass can be used for various applications, such as biofuels, animal feed, or fertilizer. • Using • Using pollutants as nutrients for microalgae growth reduces about 60% of the cost required for algae biomass production.	Requires significant energy inputs. Adding chemicals may be required to complete the treatment process.	**(Andrade *et al.*, 2018; Cheng *et al.*, 2021)**
Environmental impact	• Can effectively remove nitrogen and phosphorus from wastewater, preventing their discharge into bodies of water, where they can cause eutrophication and harm aquatic ecosystems. • Has the potential to sequester carbon dioxide during their growth, mitigating greenhouse gas emissions.	• May generate sludge as a by-product, which requires further treatment and disposal. •	**(Al-Tohamy *et al.*, 2022; Devi *et al.*, 2023)**

Phytoremediation is a type of bioremediation, exploiting algae to improve the quality of the water. It has been discovered that microalgae can efficiently use the phosphorus and nitrogen in wastewater to support cell growth. These nutrients can be absorbed by microalgae, which then convert them into valuable biomass **(El-Baz *et al*., 2016a, b; Nasir *et al*., 2023)**. Hence, Microalgae-based technologies for wastewater treatment have several advantages over traditional methods in terms of efficiency, cost, and environmental impact. A comparison between microalgae-based wastewater treatment methods and traditional methods is summarized in Table (1).

In recent years, microalgae biomass has emerged as a highly promising feedstock to produce sustainable biofuels, including biodiesel, bioethanol, and biogas **(Khan *et al.*, 2018; Hossain *et al.*, 2020)**. In addition, the potential of microalgae to sequester carbon dioxide during photosynthesis and prevent its release during metabolism enhances the carbon footprint and carbon credit significantly for industrial processes and provides a promising tool against climate change **(Choi *et al.*, 2019)**. The cultivation of microalgae for biomass production typically requires significant amounts of water, mainly for growing, harvesting, and processing. Depending on the water source and the efficiency of water usage, there may be concerns about the depletion of local water resources or increased pressure on freshwater ecosystems. Moreover, expensive chemicals such as Conway or Walne fertilizers are used to recover nutrients in growing media **(Santos *et al.*, 2019; Hossain *et al.*, 2020)**. A suitable concentration and composition of nutrients will significantly affect microalgae biomass production and its biocomponents **(Darwesh *et al.*, 2022)**. Therefore, macronutrients and micronutrients must be added to the culture media to meet the microalgae's nutritional needs. Using wastewater instead of culture media can be an effective alternative. The use of nutrient-rich aquaculture wastewater as a medium for microalgae growth might reduce the need for water and expensive chemicals **(Mahmoud *et al.*, 2022)**.

To our knowledge, it is the first time for *Chlamydomonas reinhardtii*, *Chlorella vulgaris,* and *Haematococcus lacustris* isolated from the River Nile to be applied for phytoremediation of the aquaculture wastewater. It is worth noting that, this study is the first of its kind addressing the effect of aquaculture wastewater on the content of phenolic compounds, total flavonoid content, and radical scavenging capacity RSC/DPPH in the resulting biomass.

In the present study, three green microalgal strains: *Chlamydomonas reinhardtii*, *Haematococcus lacustris*, and *Chlorella vulgaris* were evaluated to treat aquaculture wastewater from a fish tank. The produced microalgae biomasses were harvested and value-added compounds (Carbohydrates, fats, proteins, and total phenolics) were evaluated.

MATERIALS AND METHODS

1. Aquaculture wastewater collection and preparation

The aquaculture wastewater used in this study was collected from a 6-month operated fish tank established in our previous study **(Mahmoud *et al.*, 2024)** and filtered to remove suspended impurities using a membrane filter with pores, having an average diameter of 0.45µm. Because aquaculture wastewater contains residual nutrients, it can help microalgae grow and remove pollutants.

2. Candidate microalgal isolates

Pure cultivation of freshwater green microalgae local strains, *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, and *Haematococcus lacustris*, isolated from the River Nile (Egypt) were obtained from the culture collection of the Hydrobiology Lab, Water Pollution Research Department, National Research Center (NRC), Egypt. Microalgae isolates were chosen to carry out this study due to their easy availability and high potential for wastewater treatment **(Almutairi, 2023; Kumar *et al.*, 2023; Nasir *et al.*, 2023)**. Before being used, the microalgae isolates were grown in Erlenmeyer flasks filled with 50ml of BG11 medium under 2500 Lux illumination and 25°C temperature until they reached an exponential phase. Centrifuging the medium for five minutes at 2000rpm yielded the initial microalgae culture, from which the supernatant was then retrieved.

3. Phytoremediation process

To evaluate the potential of the recommended microalgal strains for aquaculture wastewater treatment, the harvested microalgal pellet after centrifuging at 2000 rpm for 5min was inoculated in 500mL Erlenmeyer flasks containing aquaculture wastewater (250mL per each). Batch culture with initial optical density of 0.3OD_{680} for each microalgal isolate was incubated at 25 ± 2°C under continuous illumination of 2500 Lux, provided by white fluorescent lamps for 16 days. The three algal strains were grown in BG11 media under the same previous cultivation conditions as control. Three replications were performed in all experiments. The microalgae cultures were regularly agitated to ensure proper mixing and exposure of the microalgae to light and nutrients, enhance gas exchange and prevent stagnant conditions. At the end of the experiments, the produced microalgae biomass for the three microalgal strains was harvested by centrifugation (2000rpm for 5min). Since the three microalgal strains are motile, centrifugation is the most suitable method for harvesting.

4. Analytical methods

4.1. Screening microalgae growth

To monitor the microalgae growth on the aquaculture wastewater, optical density at 680 nm was monitored every 2 days.

4.2. Aquaculture wastewater analysis

The levels of total phosphorus (TP) and nitrate (NO_3^-), ammonia (NH_3), and COD in the aquaculture wastewater were analyzed using standard methods **(Federation *et al*., 2005)**.

The removal efficiencies (RE) of nutrients (TP, nitrate, and COD) were calculated using the following equation:

$$RE\% = \frac{(C_B - C_A)}{C_B} \times 100 \qquad (1)$$

Where, C_B is the concentration of nutrients (TP, nitrate, or COD) in AWW before microalgae cultivation, and C_A is the concentration of nutrients (TP, nitrate, or COD) in AWW after microalgae cultivation.

4.3. Analysis of microalgae biomass components

A. Chemical composition

The proximate evaluation was performed using the techniques of **AOAC (1995)** for the following parameters: moisture, ash, crude fat, protein, and total carbohydrate content.

B. Phenolic content

The phenolic content was measured according to **Yu *et al.* (2002)**. Sodium carbonate (1.5mL), Folin Ciocalteu reagent (500µL), and extracts (100µL) made up the reaction mixture. DI was added to reach the final amount of 10mL, and the mixture was then vortexed. The absorbance was measured at 765nm after the reaction had been going on for two hours, and the results were represented as Gallic acid equivalent.

C. Total flavonoid content

Rutin served as a reference compound for the technique of estimating the flavonoid concentration **(Chang *et al*., 2002)**. Ethanol (1.5 ml), aluminum chloride (0.1 ml), 1 M potassium acetate (0.1 ml), and DI (2.8 ml) were combined with the extracts (0.5 ml). Using a UNICO UV/VIS-2100A spectrophotometer (Dayton, USA), the absorbance of the reaction mixture was measured at 415nm following a 30-minute incubation period at room temperature. Using a standard calibration of rutin solution, the flavonoid content was determined and represented as micrograms of rutin equivalent (RE) per gram of material.

D. Radical scavenging capacity

Based on the study of **Huang *et al*. (2005)**, the sample solution (0.1ml) was combined with 3.9ml of DPPH solution. For precisely thirty minutes, the absorbance of the mixtures was measured at 515nm.

RESULTS

1. Phytoremediation of aquaculture wastewater (AWW)

The three microalgal strains chosen for this study have a significant potential to remove nutrients when grown on AWW. The physicochemical characteristics of the AWW before and after the phytoremediation process are shown in Table (2). The suspended solids (SS) of the raw AWW was 75±5.1mg/ L. It is worth mentioning that, before starting the cultivation process, the AWW was filtered to remove suspended impurities (using a membrane filter with pores with an average diameter of 0.45µm) that may reduce light penetration during microalgae cultivation, and thus there was no information about the suspended solids (SS) after AWW treatment.

Table 2. Physicochemical characterization of aquaculture wastewater before and after phytoremediation by the 3 recommended algal strains

Parameter	Raw AWW	AWW after *C. reinhardtii* cultivation	AWW after *C. vulgaris* cultivation	AWW after *H. lacustris* cultivation
pH	8.5±0.3	10.3±0.2	9.9±1.1	10.8±0.76
EC (µS/cm^{-2})	1250±20	634±12	756±23	725±15
TP (mg/L)	12.1±0.5	0.0	0.0	0.0
Suspended solids (SS) (mg/L)	75±5.1	-	-	-
Ammonia NH_3 (mg/L)	0.12±0.01	0.0	0.0	0.0
Nitrate (mg/L)	50.3±2	9±2.2	10±1.23	10.8±0.98
COD (mg/L)	127±5	47.9±6.7	65.9±10	52.1±7.6

As shown in Table (2), the electrical conductivity (EC) of raw AWW was decreased from 1250±20 to 634±12, 756±23, and 725±15µS/ cm^{-2} by *C. reinhardtii*, *C. vulgaris*, and *H. lacustris*, respectively. This reduction in EC value indicated the consumption of nutrients in AWW by microalgae. In addition, it was observed that raw AWW contained a high nitrate concentration of 50mg/ L, while the ammonia concentration was very low (0.12±0.01mg/ L).

In terms of nutrient removal efficiency, different microalgal strains showed slightly different efficacies. The nutrient removal efficiency by *C. reinhardtii* was found to be 82% for nitrates. *C. vulgaris* cultivation in AWW removed 80% of nitrates while *H. lacustris* removed 78.4% of nitrates (Fig. 1B).

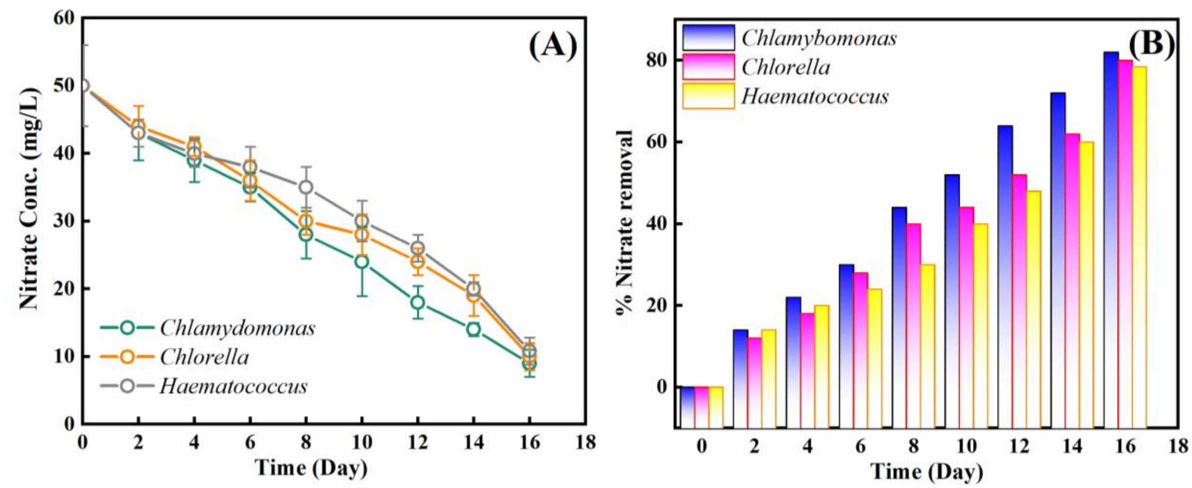

Fig. 1. (A) Variation in concentration of nitrate, B) % of removal of nitrate during cultivation of *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, and *Haematococcus lacustris* on aquaculture wastewater

Notably, *C. reinhardtii*, *C. vulgaris,* and *H. lacustris* showed approximately 100% removal efficiency for total phosphates (Fig. 2B).

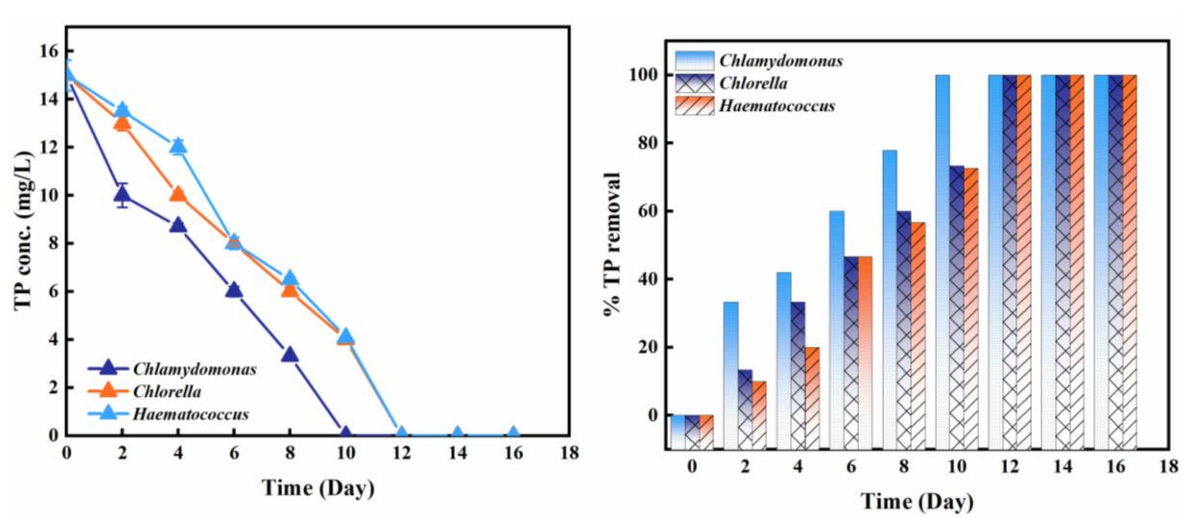

Fig. 2. (A) Variation in concentration of total phosphorus, (B) % of removal of total phosphorus (TP) during cultivation of *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, and *Haematococcus lacustris* on aquaculture wastewater

Likewise, in a previous study, *Chlorella sorokiniana* and *Scenedesmus obliquus* demonstrated high (> 70%) removal of phosphates in 14 days of cultivation when grown on wastewater from aquaculture **(Ansari *et al*., 2017; Bhatti *et al*., 2023)**. With respect to COD removal, *C. reinhardtii* exhibited the highest removal efficiency of 62.3%, followed by 58.97% and 48.1% for *H. lacustris* and *C. vulgaris,* respectively (Fig. 3).

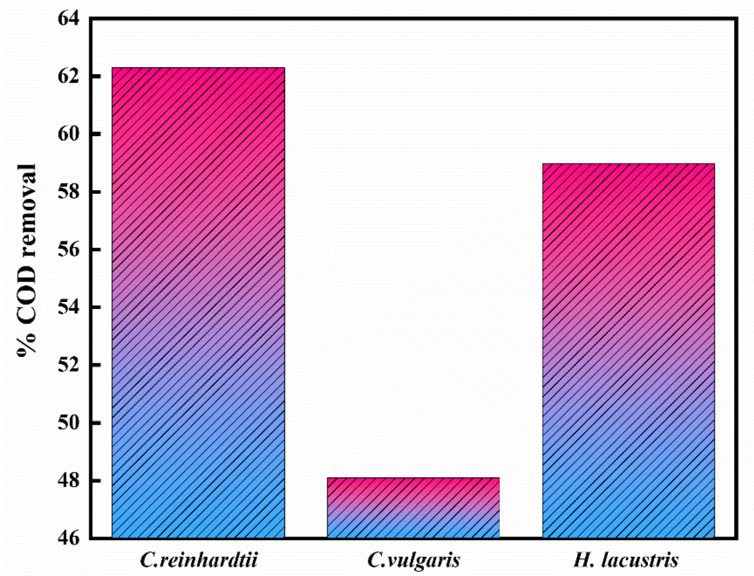

Fig. 3. % of removal of COD during cultivation of *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, and *Haematococcus lacustris* on aquaculture wastewater

2. Microalgal biomass production using aquaculture wastewater as a nutrient source

The growth of microalgal biomass was tracked and evaluated in terms of biomass concentration (g/L). In Fig. (4), it is noticed that the aquaculture wastewater nutrients content was a suitable medium for the growth of the 3 microalgal strains since the rise in biomass concentration was observed without the addition of additional nutrients. The cultivation of *C. reinhardtii*, *C. vulgaris*, and *H. lacustris* exhibited biomass yields of 1.00±0.07, 0.64±0.03, and 0.71±0.023g/ L, respectively. However, the BG11 medium's biomass outputs were 1.5±0.021, 0.93± 0.014, and 1.1± 0.012g/ L. It is worth mentioning that, microalgae growth increases the pH of AWW from 8.5±0.3 in raw AWW to 10.3±0.2, 9.9±1.1, and 10.8±0.76 for C. *reinhardtii, C. vulgaris, and H. lacustris,* respectively (Table 2). This increase in pH was caused by a combination of factors, including microalgae's high photosynthetic efficiency and their absorption of CO_2 in the aquatic environment, as well as their releasing of alkaline metabolites **(Wu *et al.*, 2022)**.

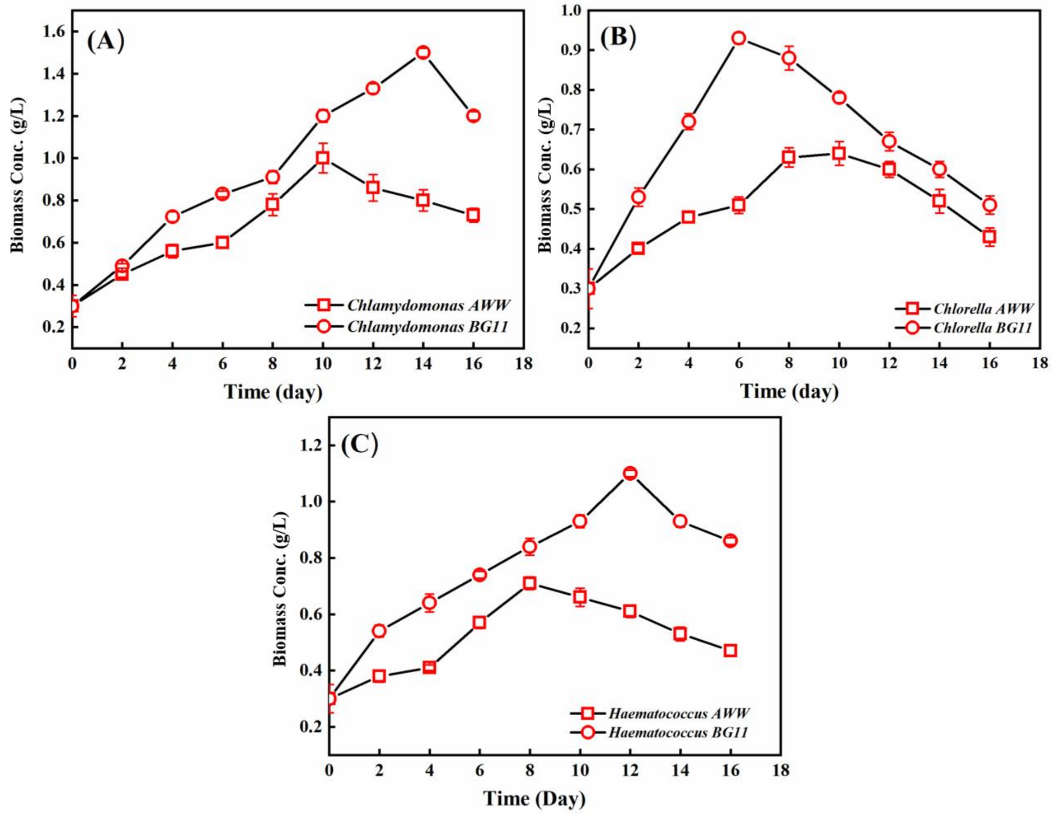

Fig. 4. Biomass concentration (g/L) of microalgae strains grown in AWW compared to BG11 synthetic media as control (A) *Chlamydomonas reinhardtii*, (B) *Chlorella vulgaris*, and (C) *Haematococcus lacustris*

The composition of microalgal biomass
Chemical composition

The ash, moisture, crude fibers, crude fat, carbohydrates, and proteins of harvested microalgae biomass from selected microalgal strains grown with AWW were determined and compared to the BG11 media. It was observed that the ash and moisture percentage in the three microalgae strains grown in BG11 were higher than the AWW (Fig. 5). Crude fat for *C. reinhardtii*, *C. vulgaris*, and *H. lacustris* were 5.3±0.1, 3.67±0.2, and 4.1±0.21% dry cell weight (DCW), respectively when cultivated using BG11 media. While the increased values in crude fat of 6.956±0.135, 4.323±0.274, and 4.543±0.132 were respectively detected when grown on AWW (Fig. 6A). Similarly, total carbohydrates for *C. reinhardtii*, *C. vulgaris*, and *H. lacustris* were 40.6±2, 25±2, and 15.9±1.3 % dry cell weight (DCW) for control cultures (grown in BG11 medium), which is lower than 45.083±1.165, 25.905±1.04, and 20.9255±1.13 % dry cell weight (DCW), respectively, for AWW cultures (Fig. 6B). Under stress or mixotrophic conditions, microalgae tend to accumulate more carbohydrates and fats **(Sarat Chandra *et al*., 2016; Singh *et al*., 2016)**. In AWW, nitrogen and phosphorus contents were lower than in synthetic BG11 medium, which may contribute to stress the microalgae. Given that AWW contains fewer nutrients than synthetic medium, microalgae growth is also slower, resulting in lower protein levels **(Ansari *et al.*, 2017)**. The protein content of *C. reinhardtii*, *C. vulgaris*, and *H. lacustris* cultivated on BG11 medium were 75.112±0.380, 48.190±1.263, and 36.540±1.643%, respectively. On the other hand, the protein levels were 50.6±3, 36.7±3, and 27.8±1.7 % in AWW nutrients.

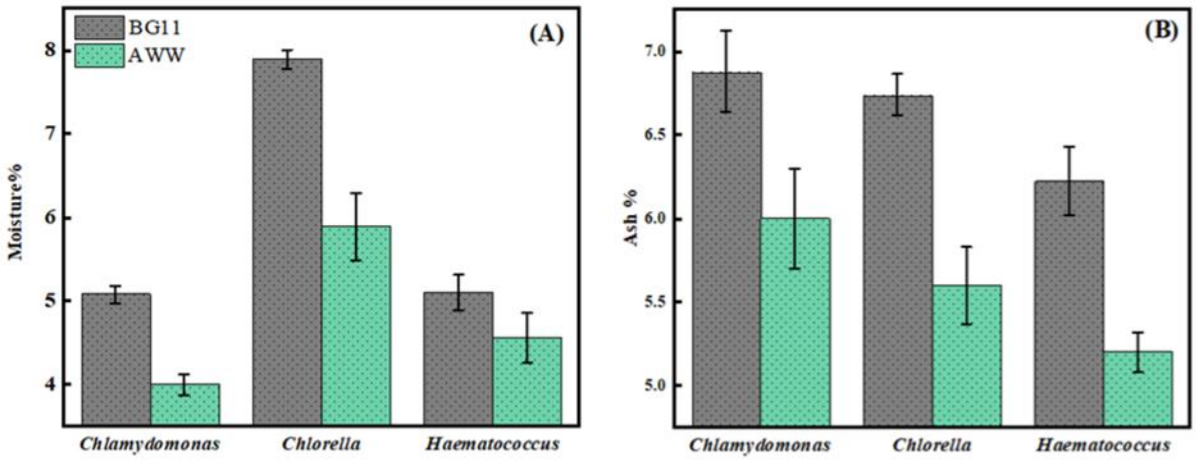

Fig. 5. (A) Moisture %, (B) Ash % of *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, and *Haematococcus lacustris* biomasses grown in aquaculture wastewater compared to BG11 synthetic media as control

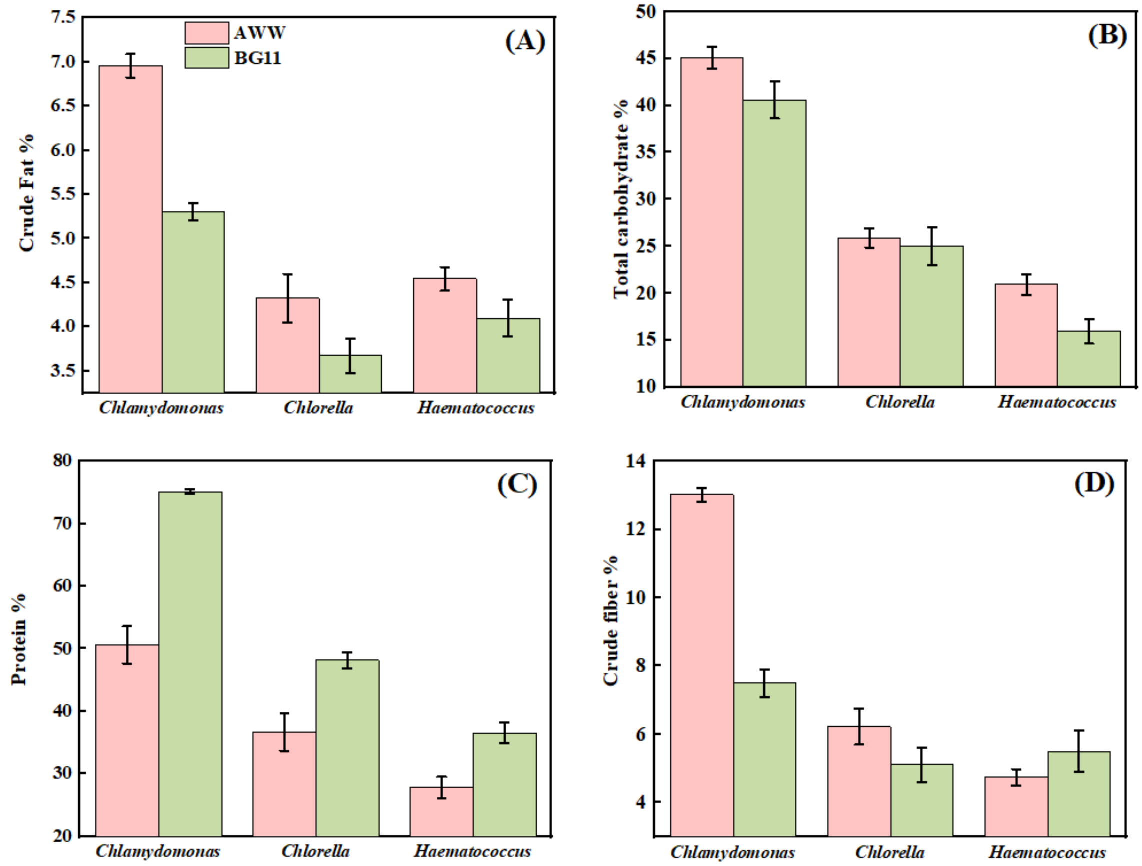

Fig. 6. (A) crude fat, (B)Total carbohydrates, (C) Protein content, and (D) Crude fiber of *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, and *Haematococcus lacustris* biomasses grown in aquaculture wastewater compared to BG11 synthetic media as control, in aquaculture wastewater compared to BG11 synthetic media as control

Total phenolic content

The phenolic content in the analyzed for *C. reinhardtii*, *C. vulgaris*, and *H. lacustris* cultivated on AWW were 99.303±0.907, 66.529±0.4 and 76.779±0.12 mg GAE/g, respectively (Fig.7A). These values were higher than that of 78.9±3, 60.9±2.3, and 69±3 mg GAE/g, respectively in the biomasses grown in synthetic BG11 media.

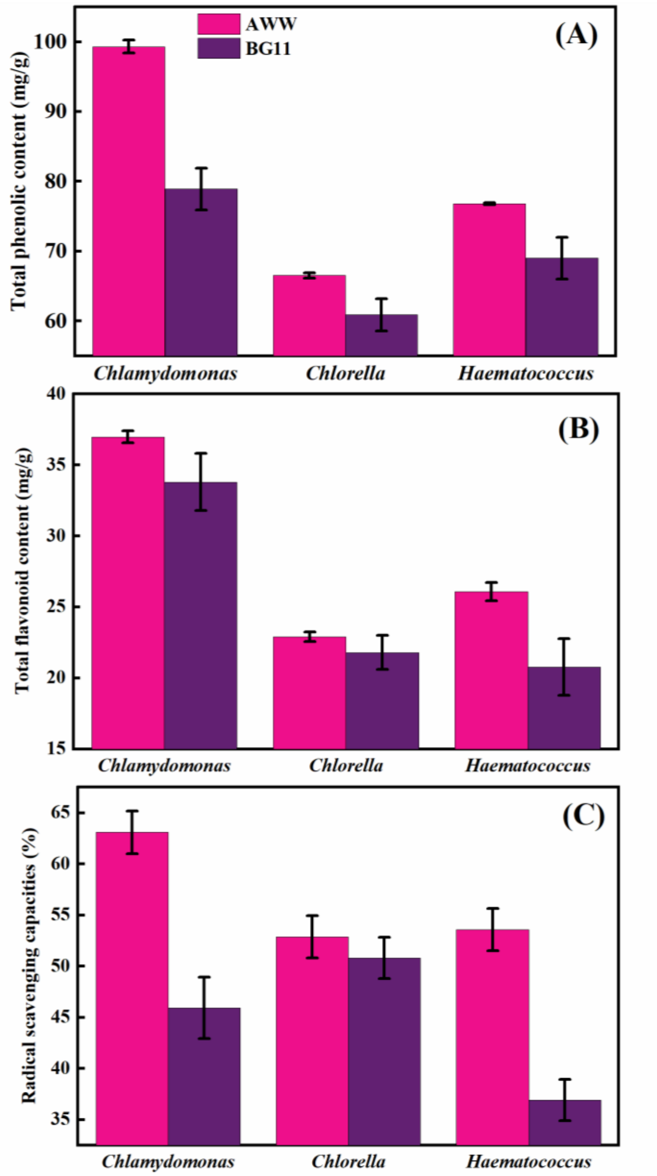

Fig. 7. (A) Total phenolic content, (B) Total flavonoid content, and (C) Radical scavenging capacities of fiber of *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, and *Haematococcus lacustris* biomasses grown in aquaculture wastewater compared to BG11 synthetic media as control, in aquaculture wastewater compared to BG11 synthetic media as control

Total flavonoid content

C. reinhardtii, *C. vulgaris*, and *H. lacustris* cultivated on cultured in AWW displayed significantly higher flavonoid content of 36.978±0.42, 22.908±0.33, and 26.078±0.65mg/ g RE, respectively, than that observed in BG11, recording values of 33.8±2, 21.8±1.2, and 20.78±2mg/ g RE, respectively (Fig. 7B).

Free radical scavenging capacity using DPPH assay

All tested microalgae were capable of scavenging DPPH at varying levels. *C. reinhardtii*, *C. vulgaris*, and *H. lacustris* cultivated on cultured in AWW had higher scavenging activities of 63.088±2.09, 52.854±2.06, and 53.578±2.06 %, respectively, than those recorded in BG11 with 45.9±3, 50.8±2, and 36.9±2% (Fig. 7C). Total phenolic content is positively correlated with antioxidant activity, based on phenolic content data. Accordingly, microalgae biomass grown in AWW has a higher level of scavenging activity when compared with biomass collected from BG11 for it contains more phenolic compounds. Our results are lower than those of the crude extract of the red alga *Polysiphonia urceolata* (96.8 ± 0.07 %) in which ascorbic acid was the solvent fraction (*Duan et al.*, 2006), and those recorded in the study of **Custódio *et al.* (2012)** who discovered that, *Nannochloropsis oculata* hexane extracts had the highest radical scavenging ability at 70.3 ± 0.6%. This variation may result from variations in the chemical structure of the substances supportinng the antioxidant responses in these species' cellular structures **(Ahmed *et al.*, 2014)**.

DISCUSSION

Through the phytoremediation process, microalgae demonstrated the ability to remove nutrients from wastewater effectively **(Caporgno *et al.*, 2015)**. The cultivation of microalgae in large-scale systems might lead to changes in local ecosystems. The introduction of non-native species or genetically modified strains could impact native biodiversity. However, if the cultivation is carried out in closed systems, the risk of environmental contamination or invasiveness can be minimized. Furthermore, species ought to be localized with a wide geographical distribution **(Barrington *et al*., 2009; Neori *et al*., 2004; Paul & de Nys, 2008)**. Hence, *C. reinhardtii*, *C. vulgaris*, and *H. lacustris* isolated from the River Nile were recommended for this study, as they are widely present, especially in the Egyptian water streams.

Generally, microalgae remove nutrients from wastewater through phosphorylation and nitrogen assimilation **(Barsanti *et al.*, 2022)**. During nitrogen assimilation, inorganic nitrogen (e.g., ammonia, ammonium, nitrate, and nitrite) is converted into the organic building block of proteins, peptides, enzymes, and energy transfer molecules (e.g., adenosine triphosphate (ATP), and adenosine diphosphate (ADP)). In the nitrogen assimilation process, nitrite and nitrate are reduced to ammonium with the aid of nitrite and nitrate reductase, respectively **(Gonçalves *et al*., 2017)**. After that, the ammonium is incorporated into intracellular amino acid glutamine, which is facilitated by glutamate (Glu) and ATP **(Cai *et al.*, 2013)**. In addition to nitrogen, inorganic phosphorous is incorporated into intracellular organic compounds, such as proteins, lipids, and nucleic acids through phosphorylation process. To take up inorganic P for cellular phosphorous transformation, this mechanism involves several phosphate transporters at the microalgae plasma membrane. The production of ATP from ADP and the synthesis of polyphosphate (such as acid-soluble and acid-insoluble polyphosphate) by polyphosphate kinase are two aspects of the transformation occurring under light conditions, or photosynthesis **(Mohsenpour *et al.,* 2021; Nguyen *et al.,* 2022)**. It was noted that AWW contains macronutrients that are essential for microalgae growth, especially nitrates, and phosphates. The high concentration of nitrate in this type of AWW may be traced back to the presence of some species of nitrifying bacteria that convert ammonia into nitrate during the nitrification process **(Kuhn *et al.*, 2010)**, the low concentration of ammonia in the AWW confirms this phenomenon. The physicochemical characteristics of the treated AWW after the 3 microalgae cultivation indicated the efficiency of the phytoremediation process, and the potential of the treated water to be safely discharged into the environment without harmful effects. Microalgae use the nutrients in wastewater for metabolic processes that result in biomass which is high in proteins, lipids, and carbohydrates. The ability of microalgae to remove nutrients is evident in their capacity to generate biomass.

The increased nutrient contents in BG11 compared to AWW are thought to be the cause of the higher biomass yields in this medium. *C. reinhardtii* exhibited the highest biomass production. It is known that when organic carbon is available, *C. reinhardtii* adopts a mixotrophic mode of nutrition **(Moon *et al*., 2013)**. These findings demonstrate that the microalgae growth needs for nutrient intake and adaptation to the wastewater substrate vary by strain. Based on these findings, *C. reinhardtii*, *C. vulgaris*, and *H. lacustris* were chosen for AWW supplementation studies to increase biomass productivity. The quantity of fats, carbohydrates, and proteins in microalgae biomass is affected by the medium's composition and growth conditions **(Ansari *et al.*, 2017)**.

Among the most potent natural antioxidants found in microalgae are the phenolic compounds **(Hajimahmoodi *et al.,* 2010)**. Numerous factors affect the total phenolic content of microalgae, including algae species, environment, cultivation area, and seasonal in addition to the physiological variations **(Marinho-Soriano *et al*., 2006)**. The findings showed that the growth media had a major impact on the total phenolic content of the three microalgal strains.

The phenolic contents evaluated in the current study were higher than the levels reported by previous studies. It was found that *Chlorella protothecoides* had a phenolic content of about 15mg GAE/g and *Chlorella pyrenoidosa* (14 mg GAE/g) where n-hexane was used for fractionation **(Li *et al.,* 2007)**. Furthermore, the water extract of *Chlorella* exhibited a very low phenolic content of about 1.5mg tannic acid equivalent/g **(Wu *et al.,* 2005)**. The current outcomes are similar to those reported for *Chlorella* methanolic extracts (65.89–258.83 mg GAE/g) **(Tirado *et al.,* 2017)**. In their previous study, **Maisuthisakul *et al*. (2007)** found that the native Thai plants had flavonoid contents ranging from about 1.5 to 50mg RE/ g dry weight, which is less than the current findings. This demonstrates that microalgae are a suitable natural antioxidant source.

Antioxidants protect cell tissues from free radical damage, which protects against infections and degenerative diseases among which is cancer. The antioxidant activity of microalgae indicates the presence of molecules capable of interacting with free radicals and donating electrons **(Tirado *et al.*, 2017)**. The common compound DPPH has been widely applied as a free radical to assess natural compounds' capacity to scavenge radicals **(Zhong *et al.*, 2010)**. With a significant absorption band centered at approximately 517 nm, DPPH is a stable free radical whose strength is reduced when antioxidants are present and can donate electrons or hydrogen to reduce it to its hydrazine form.

CONCLUSION

When grown in AWW, novel Egyptian microalga freshwater microalga *C. reinhardtii* showed high removal efficiencies for nitrate (> 82%), COD (62.3%), and TP removal efficiency was 100% with all 3 strains. In addition, *C. reinhardtii* had the highest biomass productivity of the evaluated strains. It is worth mentioning that, the free radical scavenging capacity was evaluated for the first time in microalgae grown in AWW. Furthermore, microalgae biomass grown in aquaculture wastewater has high crude fat and carbohydrate productivities that can be utilized for a variety of feed and bioenergy applications. As a result, microalgal cultivation was shown to be superior to other treatments for aquaculture wastewater. For future studies, it is crucial to adopt sustainable practices such as using renewable energy sources, optimizing water usage, and minimizing the introduction of non-native species to mitigate potential negative environmental consequences.

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