Int Aquat Res
https://doi.org/10.22034/IAR.2025.2009393.1866

ORIGINAL RESEARCH

Microalgal growth, bioremediation, and nutritional composition in aquaculture wastewater

Lily Anne G. Piñosa . Greta G. Gabinete . Mary Jane S. Apines-Amar . Eleodoro L. Alicante . Helen G. Genandoy . Joel A. Araquil. Jr . Jerry A. Ceballos . Fiona L. Pedroso

Received: 10 March 2025 / Accepted: 17 September 2025 / Published online: 02 October 2025

Abstract Microalgae have been utilized as eco-friendly bioremediation agents in aquaculture wastewater with concomitant biomass production. However, growth, bioremediation efficiency, and the nutritional components of the resulting biomass still need assessment to evaluate their potential as bioremediation agents, growth performance, and fertilizer sources. This study evaluated five microalgae, namely, *Tetraselmis tetrathele*, *Nannochlorum* sp., *Chaetoceros calcitrans, Isochrysis galbana,* and *Thalassiosira* sp., grown using milkfish wastewater. Specific growth rate (SGR), biomass productivity and concentration, nutrient removal efficiency, and the nutritional components of the biomass were analyzed as bases for determination. Results showed that *Nannochlorum* sp. and *Isochrysis galbana* had the highest SGR of 0.263 and 0.255 μ day^{-1}, respectively. However, due to their larger size, *T. tetrathele* and *Thalassiosira* sp. had the highest biomass productivity and concentrations of 0.075 and 0.065 g L^{-1} day^{-1} and of 0.933 and 0.879 g L^{-1}, respectively. *Tetraselmis tetrathele* is best in removing N and P, achieving removal efficiency of 98.24% and 98.87% for NH_3-N and NO_2-N, respectively, while 72.50% for P. *T. tetrathele* and *Thalassiosira* sp. had significantly higher N, with no significant difference among microalgae for P. At the same time, *Thalassiosira* sp. was significantly higher in K. There was no significant difference among algae in terms of Cu, while *T. tetrathele* and *Thalassiosira* sp. were significantly higher in Zn, *T. tetrathele, Nannochlorum* sp., and *I. galbana* for Mn, and *Thalassiosira* sp. for Fe. Results indicate that *T. tetrathele* emerged as the most efficient species for bioremediating aquaculture wastewater compared to the other microalgae. While *T. tetrathele* and *Thalassiosira* sp. exhibited high biomass productivity and potential as fertilizer sources, they outperformed the other microalgae.

Keywords Biomass productivity . Specific growth rate . Macronutrients . Micronutrients

Introduction

Aquaculture pollution is an unfortunate result of the rapidly growing industry. Shrimp farming in the Philippines, for instance, was a significant success in the 1980s until its decline in the mid-1990s (Tendencia and de la Peña 2001), with an estimated loss of 40,080 mt in 1997 and 51,000 mt in 2014 (Macusi et al. 2022) due to disease outbreaks caused by water pollution when culture intensified (Iber and Kasan 2021). Accordingly, only 20 to 40% of nitrogen and phosphorus applied to ponds from feed is recovered in harvested fish,

Lily Anne G. Piñosa (✉) . Mary Jane A. Amar . Fiona L. Pedroso
Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao, Iloilo, Philippines
e-mail: lgpinosa@up.edu.ph

Greta G. Gabinete . Eleodoro L. Alicante . Helen G. Genandoy . Joel A. Araquil, Jr . Jerry A. Ceballos
College of Agriculture and Forestry, West Visayas State University, Lambunao, Iloilo, Philippines

Fiona L. Pedroso
School of Marine Fisheries and Technology, Mindanao State University at Naawan, Misamis Oriental, Philippines

and less than 30% of nitrogen and 10% of phosphorus applied to ponds exit in the effluent (Facey 2024). The high levels of these nutrients may cause pathogen proliferation (Niu et al. 2025).

One way to mitigate the adverse effects of nutrient-rich aquaculture effluents is through bioremediation (Skriptsova and Miroshnikova 2011). In aquaculture, this means utilizing living organisms, such as algae or bacteria, to break down waste generated by operations. Algal bioremediation, on the other hand, uses live algae to absorb excess nutrients from the wastewater (Vijayaram et al. 2024). Algae are known to remove up to 90% of the ammonium produced by marine animals (Skriptsova and Miroshnikova 2011) and over 90% of pathogenic bacteria (Abdel-Raouf et al. 2012). However, further research is needed to determine which microalgal species are most effective at sequestration. Liu et al. (2018) suggested that screening microalgal species is a promising strategy to improve the removal efficiency of aquaculture pollutants.

Aside from removing pollutants and nutrients from wastewater, microalgae also produce biomass. This is a promising approach that combines biomass generation with nutrient removal (Huang et al. 2022). This method also addresses three main goals: remediation of aquaculture wastewater for cleaner water and sustainable aquaculture, provision of a low-cost medium for microalgae cultivation through wastewater nutrients utilization, and generation of microalgal biomass for product development.

While microalgae can grow in most wastewaters, their growth rates, however, vary with species and culture medium, making it essential to assess the growth rate for each type to select high-yielding species and enhance production output. For instance, in a study performed by Ansari et al. (2017), *Chlorella sorokiniana* produced lower biomass yields, ranging only from 1.25 g L^{-1} to 2.20 g L^{-1}, compared to *Ankistrodesmus falcatus,* which ranged from 2.25 g L^{-1} to 2.86 g L^{-1}, even though both were grown in the same aquaculture wastewater. In terms of microalgal responses to culture media, Zhang et al. (2023) observed two growth patterns: the non-influencing growth, where microalgae hardly accumulate biomass and cell division in a nitrogen-rich medium, and the inhibitory growth, where they can accumulate biomass and divide more readily under nitrogen-limited conditions.

On the other hand, microalgal biomass holds potential for various product applications. However, most research has focused on its use in biofuels, nutraceuticals, and pharmaceuticals, with relatively little attention given to its role as a fertilizer source. This may be because organic fertilizers are devalued compared to the products mentioned above. In agriculture, however, the value of organic fertilization is indispensable, as this prevents soil from degradation, reduces chemical residues, promotes a balanced ecosystem, and ultimately contributes to long-term agricultural sustainability. Among the microalgal studies that explored their use as fertilizers were: microalgal extracts and biomass on seed germination, hydroponic systems, and soil-based crop cultivation (Zhang et al. 2024), as biofertilizer (Castro et al. 2024), and as biomass from wastewater to enhance the shoot, root, and grain biomass of barley (Suleiman et al. 2020). However, these studies only incorporated microalgae into the growing medium and evaluated their impact on plant growth, whereas studies that focus on the characterization of microalgal nutritional components for fertilizer production are scarce. Though Alvarez-González et al. (2022) were able to characterize the nutritional components of microalgal biomass grown in wastewater as a potential replacement for inorganic fertilizer, their study utilized mixed algae and domestic wastewater. Niccolai et al. (2019) noted that the biochemical composition of microalgae can vary depending on the culture conditions and the microalgal strains used.

In addition, a wide range of factors must be considered in the bioremediation process, and accordingly, selecting an appropriate target species is one of the critical steps for the removal of aquaculture waste. The algal characteristics in the selections are: high growth rates, endemic to the area, and with broad distribution (Lawton et al. 2013). The five microalgae in this study, however, were selected due to their availability and local abundance, making them practical candidates for testing.

Based on the previously identified research gaps, there is a need to assess the growth and productivity of microalgae for selecting high-yielding species, assess their nutrient removal efficiency for effective bioremediation, and understand their nutritional composition to identify those best suited as organic fertilizer. Thus, this study aimed to evaluate the growth, bioremediation efficiency, and nutritional composition of five microalgal species: *T. tetrathele, Nannochlorum* sp., *C. calcitrans, I. galbana*, and *Thalassiosira* sp., grown in aquaculture wastewater.

Materials and methods

This study is a single-factor experiment in which the single variable factor is the different species of microalgae. The experimental set-up was laid out in a Randomized Complete Block Design (RCBD) where the experimental runs acted as treatment replicates or blocks to control potential sources of variability. Time was used as the blocking factor because environmental conditions could vary across different runs. By treating each run as a block, the design controls for this variability, ensuring that temporal changes do not confound any differences among treatments. A total of three (3) experimental runs were performed. The experiment was done at the Multispecies Hatchery of the Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao, Iloilo, Philippines.

Preparation of microalgae and wastewater source

The microalgae used in this study, namely, *T. tetrathele, Nannochlorum* sp*., C. calcitrans, I. galbana,* and *Thalassiosira* sp*.,* were obtained from the Southeast Asian Fisheries Development Center/Aquaculture Department (SEAFDEC-AQD) in Tigbauan, Iloilo, Philippines. Upon arrival at the hatchery, the microalgae were examined under a microscope for possible contamination, cultured in 1-L-capacity glass bottles, and subsequently scaled up to 10-L-capacity carboys using chlorinated water. The microalgal propagation used Conway media (Piñosa and Apines-Amar 2024), with aeration, and illuminated horizontally using 40 W fluorescent lamps until growth stabilized and the required volume of microalgae for the experiment was obtained.

The aquaculture wastewater came from the milkfish culture tank. The wastewater inorganic nitrogen (N) (ammonia-N (NH_3-N) and nitrite-N (NO_2-N)) and phosphorus (P) concentrations were checked, as these nutrients are toxic to fish at higher concentrations, but are needed for microalgal growth. The analyses were performed using a UV-Vis Spectrophotometer (Shimadzu UV-1280, Germany) following the procedures described in Strickland and Parsons (1972) to ensure that it contained sufficient nutrients to support the growth of algae. When the desired nutrient concentrations were reached, the wastewater was filtered through a 12-inch-wide and 23-inch-long, 5 µm mesh filter bag (BNH Marketing, Philippines) to remove debris and other impurities from the water column, and subsequently distributed in five 500-L-capacity fiberglass tanks. Initial concentrations of water NH_3-N, NO_2-N, and P were determined using a UV-Vis Spectrophotometer, while water salinity and pH were determined using a refractometer (Atago S/Mill-E, Japan) and a digital pH meter (Suntex TS-1, China), respectively. The characteristics of aquaculture wastewater were as follows: NH_3-N - 0.934±0.1 - 1.23±0.2 mg L^{-1}, NO_2-N - 0.857±0.1 - 1.01±0.1 mg L^{-1}; P - 2.11±0.2 - 2.52±0.2 mg L^{-1}, pH - 7.16 - 7.58, and salinity - 29 - 32 ppt.

Experimental setup and sampling

The experiment used five 500-L-capacity fiberglass containers filled with 450 L filtered aquaculture wastewater. The microalgal inoculants were stocked at a density of 0.2 optical density. Stocking was performed by diluting the concentrated algal inoculants with the wastewater until the density of 0.2 for each alga was achieved at a wavelength of 680 nm using a UV-Vis spectrophotometer. All cultures were aerated and placed in an open structure with transparent roofing, allowing sunlight to penetrate, which was needed for microalgal growth. The use of transparent roofing allowed the sunlight to penetrate the culture area, which can enhance algal growth but may also introduce variability in light intensity, potentially affecting microalgal reproducibility. However, this set-up also served to protect the experiment from rain, which could negatively impact the cultured algae. Sampling for the microalgae's specific growth rate was performed daily for five days, at the onset and the end of the experiment, for biomass productivity and bioremediation efficiency, while samples were taken at the end of the experiment for biomass concentration before all microalgae were harvested. After five days of culture, microalgae were harvested through electro-flocculation. Floated and settled algae in the tanks were collected, filtered, and dewatered using a fabricated microalgal collector, a plastic container with a 30 µm mesh size net at the bottom. The five-day culture duration was adopted from other studies, where microalgae exponential growth was spotted during the first three days (Wang et al. 2014; Piñosa and Apines-Amar 2024). Harvested algae were air-dried, homogenized, and sam-

pled for their macronutrient and micronutrient content, ash, organic matter (OM), organic carbon (OC), and moisture. Specific analysis and its procedures were outlined as follows:

Specific growth rate

The specific growth rate of the five microalgae was evaluated through cell enumeration using a Neubauer improved hemocytometer (Germany) and a compound microscope (Motic BA410, China) following the methods of Piñosa (2018) for algal count and Martinez et al. (1975) for calculations. The specific growth rate was calculated based on Gani et al. (2016) formula:

$$\text{Specific growth rate } (\mu/\text{day}) = \frac{\ln (Nf/Ni)}{Tf - Ti}$$

Where Nf and Ni were defined as the cell concentration (cell mL^{-1}) at time Tf and Ti, respectively.

Biomass productivity and concentration

The biomass productivity and concentration were determined by quantifying the dry weight of the algae through filtration using a glass microfiber filter (GF/C Whatman, USA), washed with distilled water to remove the salt, and dried at 100 °C until a constant weight was obtained. The dry weight was determined by obtaining the difference between the filter weights before and after filtration. The dry weight is the biomass concentration, while the biomass productivity calculation follows the formula of Kumar et al. (2019) as shown below:

Biomass productivity (g $L^{-1}d^{-1}$)

$$P = \frac{C1 - C0}{T1 - T0}$$

Where C1 and C0 are the microalgae biomass concentrations at the end and at the beginning of the culture at time T1 and T0, respectively.

Nutrient removal efficiency

The water samples for nutrient removal efficiency were analyzed immediately after sampling by obtaining a clear supernatant through centrifugation with a tabletop centrifuge at 3000 rpm. The concentrations of NH_3-N, NO_2-N, and reactive P in the water were analyzed using the methods described by Strickland and Parsons (1972) with modifications, using a Shimadzu UV-Vis Spectrophotometer. The phenol hypochlorite method was used for the inorganic NH_3-N analysis. Briefly, 25 mL of the supernatant water sample was transferred into a 125 mL Erlenmeyer flask. Successive additions of 1 mL phenol solution, 1 mL sodium nitroprusside solution, and 2.5 mL of oxidizing reagent were made, with thorough mixing after each addition. The samples were allowed to stand for 1 hour, and the absorbance was recorded against distilled water using a UV-VIS spectrophotometer at a wavelength of 640 nm. For the analysis of NO_2-N, the diazotization process was used. Briefly, 25 mL of the supernatant water sample was measured into a 125 mL Erlenmeyer flask. A sulfanilamide solution of 0.5 mL was added, mixed, and allowed to react for 2 and 8 minutes, then the naphthyl-ethylenediamine solution of 0.5 mL was also added and mixed. The absorbance of the solution was read against distilled water between 10 minutes and 2 hours using a UV-VIS spectrophotometer at a wavelength of 543 nm. For the analysis of reactive P, the orthophosphate molybdate method was used. Briefly, 25 mL of the supernatant water was transferred into a 125 mL Erlenmeyer flask and allowed to react with a 2.5 mL composite reagent containing ammonium molybdate solution, sulfuric acid solution, ascorbic acid solution, and potassium antimonyl solution. After 5 minutes or preferably 2-3 hours, the extinction of the solution was measured against distilled water in a UV-VIS spectrophotometer at a wavelength of 885 nm. The calculation for nutrient removal efficiency of these three nutrients followed the formula of Aníbal et al. (2014):

$$\text{Nutrient Removal Efficiency} = 100 - \left(100 \text{ x } \frac{\text{Nutrient concentration at the beginning}}{\text{Nutrient concentration at the sampling moment}}\right)$$

Microalgal nutritional compositions

Macronutrient and micronutrient

Homogenized air-dried algal samples were sent to two analytical laboratories, the Department of Agriculture, Regional Organic Soils Laboratory, in Iloilo City, Philippines, and the Department of Agriculture – Sugar Regulatory Administration, Bacolod City, Philippines for the analyses of macronutrients N, P, and Potassium (K) and micronutrients Iron (Fe), Manganese (Mn), Zinc (Zn), and Copper (Cu) to determine their potential as source of organic fertilizers. For the macronutrient content, total N was analyzed using the Kjeldahl method described in AOAC (2005). The volumetric method was used in the analyses of total P and total K. The phosphoric acid method was used to determine total P, while sodium tetraphenyl borate (STPB) was used to determine K; both methods followed the procedure described in AOAC (1975). For the micronutrients Cu, Mn, and Fe, samples were prepared into pressed powdered pellets and analyzed using the elemental analyzer X-ray fluorescence spectroscopy (Malvern Panalytical Epsilon 4 XRF Analyzer, The Netherlands) following Karathanasis and Hajel (1996), while diethylenetriaminepentaacetic acid (DTPA) micronutrient extraction method was performed for Zn and was analyzed using the Atomic Absorption Spectroscopy (Agilent Technologies, 200 Series AA, USA), following the procedure described in AOAC (2005).

Microalgal ash, organic carbon (OC), organic matter (OM), and moisture

The microalgal ash, OC, and moisture contents were determined gravimetrically by oven drying at 105 °C to a constant weight, and incineration in a muffle furnace at 550 °C, respectively. For the OM, values were derived by multiplying the percent OC by 1.72. This conversion factor assumes that OM contains 58% OC.

Statistical analysis

All data were analyzed statistically for their significance using the Statistical Package for the Social Sciences (SPSS version 16.0). Levene's test was performed to evaluate whether the data were distributed normally and to determine the homogeneity of variances. The differences among microalgae in terms of specific growth rate, biomass production, and concentrations, macronutrient and micronutrient contents, were evaluated using a one-way analysis of variance (ANOVA). The Duncan's Multiple Range Test (DMRT) post hoc analysis was performed at a 5% level of significance to evaluate the comparisons in the differences in means.

Results

Specific growth rate

Measurement of specific growth rates was performed daily to identify the optimal growing days for each alga, aiding future culture for improved production. Results showed a high specific growth rate in four microalgae on the first day of culture. The rate declined thereafter, except for *T. tetrathele* where an almost equal increase in growth was observed for the entire culture period at a range of 0.210-0.274 μ day^{-1} (Figure 1). *Thalassiosira* sp. had the highest specific growth rate on day 1 of 0.525 μ day^{-1} but was statistically comparable with other algae, except for *T. tetrathele,* which was the lowest. From day 2 to day 3, the specific growth of all the microalgae was significantly the same, except again for *T. tetrathele,* which was low on day 2 at 0.274 μ day^{-1}. On day 4, *Nannochlorum* sp. had the highest specific growth rate of 0.295 μ day^{-1} but was not significantly different from other algae except *C. calcitrans,* which had the lowest specific growth rate of 0.256 μ day^{-1}. On day 5, *Nannochlorum* sp. and *Isochrysis galbana* had the highest specific growth rate among microalgae of 0.263 μ day^{-1} and 0.255 μ day^{-1}, respectively. After five days of culture, the specific growth rate of the five microalgae grown in aquaculture wastewater ranged from 0.214 μ day^{-1} to 0.263 μ day^{-1}.

Microalgal biomass productivity and concentration

The biomass productivity of microalgae grown in aquaculture wastewater is presented in Figure 2. *Tetraselmis tetrathele* had significantly high biomass productivity among the algae (0.075 g L^{-1} day^{-1}) but was statistically comparable with *Thalassiosira* sp. (0.065 g L^{-1} day^{-1}). *Nannochlorum* sp. and *I. galbana* followed, with *C. calcitrans* being the lowest. The range of biomass productivity among the algae in this study was 0.032 - 0.075 g L^{-1} day^{-1}. Likewise, the biomass concentration of the five microalgae after 5 days is presented in Figure 3. *T. tetrathele* and *Thalassiosira* sp. had the highest microalgal biomass concentrations of 0.933 g L^{-1} and 0.879 g L^{-1}, respectively.

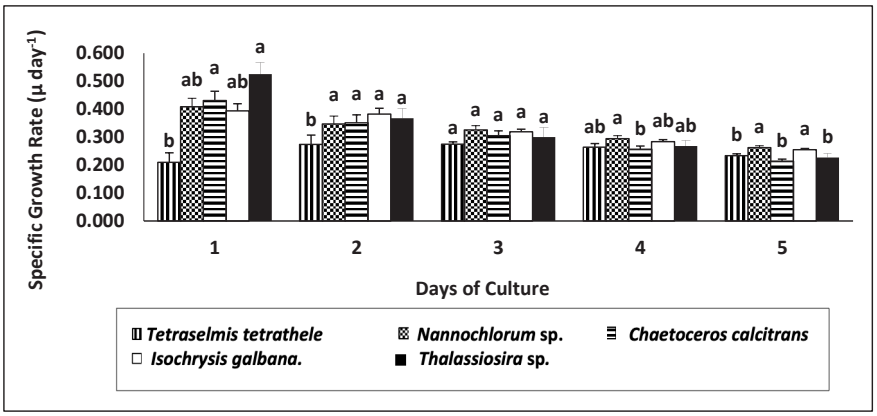

Fig. 1 Daily specific growth rate of microalgae over five days of cultivation using aquaculture wastewater. Bars represent means ± SD, n=3. Different letters above the bars denote significant differences ($p < 0.05$)

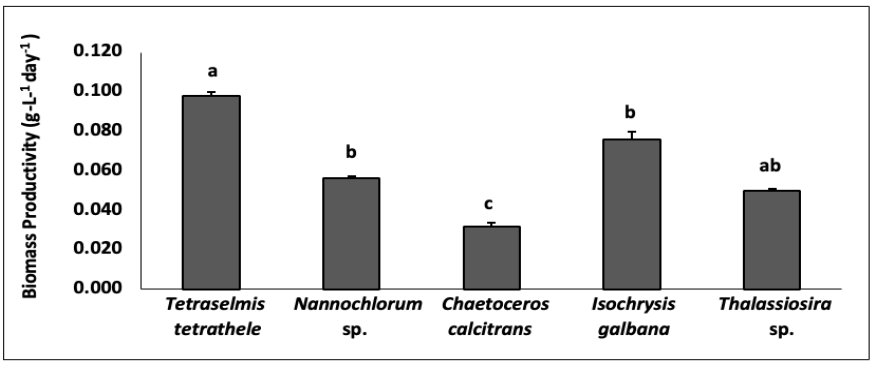

Fig. 2 Biomass productivity of microalgae after five days of cultivation using aquaculture wastewater. Bars represent means ± SD, n=3. Different letters above the bars denote significant differences ($p < 0.05$)

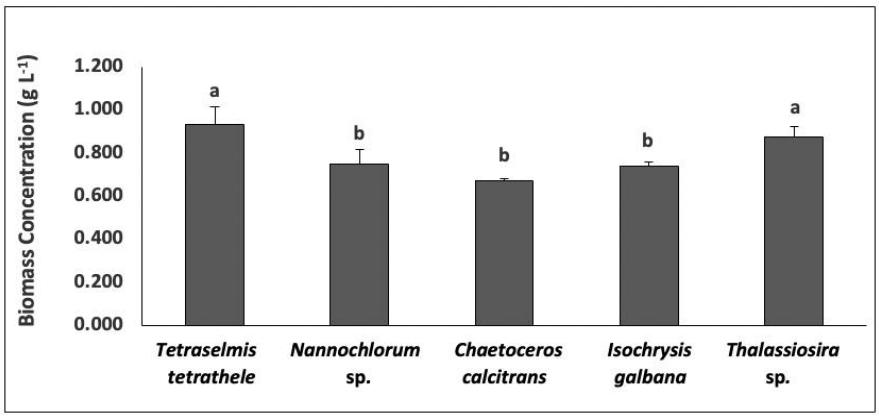

Fig. 3 Microalgal biomass concentrations after five days of cultivation using aquaculture wastewater. Bars represent means ± SD, n=3. Different letters above the bars denote significant differences ($p < 0.05$)

Microalgae nutrient removal efficiency

The nutrient removal efficiency of N and P by five microalgae was evaluated after five days of culture. For NH_3-N removal, all microalgae achieved a removal efficiency of over 93% (Figure 4a). *T. tetrathele* exhibited the highest removal (98.24%), which was not significantly different from *I. galbana* (98.00%). *Thalassiosira* sp. followed (94.64%), which was significantly comparable to *Nannochlorum sp.* (95.14%) *and C. calcitrans* (93.16%), both of which had relatively lower NH_3-N removal efficiencies.

For NO_2-N removal, *T. tetrathele* achieved the highest removal efficiency (98.87%), which was significantly comparable to that of *I. galbana* (97.42%) and *Thalassiosira* sp. (96.58%). *Nannochlorum* sp. (95.05%) and *C. calcitrans* (95.54%) displayed lower removal efficiencies, but were significantly comparable to *Isochrysis galbana* and *Thalassiosira* sp. (Figure 4b).

For P removal, all microalgae had low removal efficiencies of less than 72% (Figure 4c). *T. tetrathele* (72.50%) and *C. calcitrans* (62.00%) showed high phosphorus removal efficiencies, followed by *Nannochlorum* sp. (55.23%) and *Thalassiosira* sp. (54.40%). *I. galbana* demonstrated the lowest removal, achieving only 42.90%.

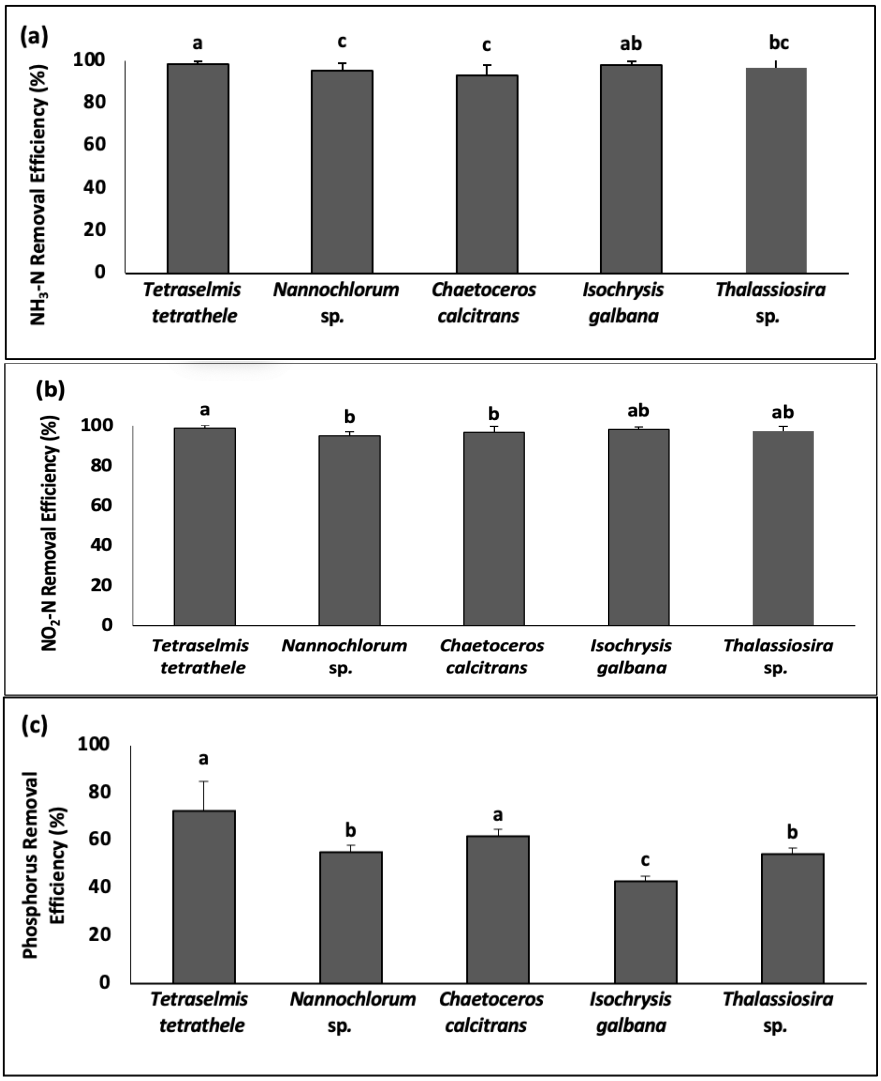

Fig. 4 Nutrient removal efficiency of five microalgae (a) NH_3-N, (b) NO_2-N, (c) Phosphorus grown using aquaculture wastewater. Bars represent means ± SD, n=3. Different letters above the bars denote significant differences ($p < 0.05$)

Microalgae macronutrient content

The macronutrients N, P, and K of the five microalgae grown using aquaculture wastewater are presented in Table 1. After five days of culture, the total N contents among the algae ranged from 0.87%-2.01% with *T. tetrathele* as the highest, followed by *Thalassiosira* sp., while *C. calcitrans*, *Nannochlorum* sp., and *I. galbana* were significantly low. The total P content of the microalgae was found to be high, ranging from 1.43% to 1.84%. Although *T. tetrathele* had the highest P content among the algae, the value was not significantly different from that of the other algae. On the other hand, of the five microalgae, *Thalassiosira* sp. had the highest total K content, while the rest of the microalgae were not significantly different from each other. The five microalgae had K values ranging from 1.34% to 1.84

Microalgal micronutrient content

Results of the micronutrient analysis of the five microalgae are shown in Table 2. The Cu contents found in five microalgae ranged from 41.33 to 48.41 mg kg^{-1} dry-weight samples. Although *T. tetrathele* and *I. galbana* had the highest Cu values, these values did not differ significantly from those of the other microalgae. In terms of Zn content, *Thalassiosira* sp. and *T. tetrathele* had significantly higher Zn, compared to *C. calcitrans*, *I. galbana,* and *Nannochlorum* sp. The amount of Zn found in five microalgae ranged from 3.76 to 7.44 mg kg^{-1}. Results on Mn contents showed that *T. tetrathele, Nannochlorum* sp., and *Isochrysis galbana* had significantly higher content, compared to *C. calcitrans* and *Thalassiosira* sp. The Mn contents among algae ranged from 336.67 to 482.67 mg kg^{-1}. On the other hand, the Fe content of the five microalgae ranged from 1484 to 2140 mg kg^{-1}. *Thalassiosira* sp. had the highest Fe content of 2140 mg kg^{-1}, followed by *T. tetrathele* (1880 mg kg^{-1}), which was significantly higher than *Nannochlorum* sp., *C. calcitrans,* and *I. galbana*.

Microalgal ash, OC, OM, and moisture content

Table 3 presents the Ash, OC, OM, moisture, and pH contents of the five microalgae cultured in aquaculture wastewater. Results showed that the five microalgae had ash and OC contents ranging from 54.45% to 59.88% and from 40.11% to 45.55% respectively. OM values ranged from 68.99% to 78.35%, and moisture from 19.18% to 23.52%. *Thalassiosira* sp. had higher ash while *Tetraselmis tetrathele* had higher OM and

Table 1 The macronutrient contents of air-dried microalgal biomass cultured in aquaculture wastewater (n = 3, mean ± SD)

	Macronutrients		
	Total N (%)	Total P (%)	Total K (% K_2O)
Tetraselmis tetrathele	2.01±0.11^{a}	1.85±0.42^{a}	1.653±0.41^{b}
Nannochlorum sp.	1.11±0.13^{c}	1.76±0.10^{a}	1.449±0.21^{b}
Chaetoceros calcitrans	0.88±0.09^{c}	1.53±0.48^{a}	1.730±0.31^{b}
Isochrysis galbana	0.87±0.15^{c}	1.43±0.24^{a}	1.338±0.29^{b}
Thalassiosira sp.	1.45±0.14^{b}	1.66±0.01^{a}	1.835±0.50^{a}
F-values	41.252	0.885	0.959
P-values	0.000	0.507	0.041

Means within a column having different letter superscripts indicate significant differences

Table 2 The micronutrient contents of air-dried microalgal biomass cultured in aquaculture wastewater (n = 3, mean ± SD)

	Micronutrients			
	Cu (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Fe (mg kg^{-1})
Tetraselmis tetrathele	48.04±2.94^{a}	6.59±0.78^{a}	482.67±18^{a}	1880±105^{b}
Nannochlorum sp.	44.35±3.54^{a}	3.60±0.59^{b}	441.00±30^{a}	1500±50^{c}
Chaetoceros calcitrans	41.33±3.51^{a}	4.15±0.78^{b}	340.00±15^{b}	1484±145^{c}
Isochrysis galbana	48.41±4.13^{a}	3.76±0.89^{b}	400.00±20^{a}	1640±80^{c}
Thalassiosira sp.	41.00±4.00^{a}	7.44±1.43^{a}	336.67±19^{b}	2140±90^{a}
F-values	2.820	1.188	288.038	23.802
P-values	0.084	0.001	0.000	0.000

Means within a column having different letter superscripts indicate significant differences

OC contents, but values were not significantly different from the rest of the algae. For moisture content, *Nannochlorum* sp. had the highest, followed by *C. calcitrans* and *I. galbana,* then *T. tetrathele*, while *Thalassiosira* sp. showed a significantly lower value.

Discussion

Microalgae have been utilized to bioremediate effluents through their autotrophic metabolism, absorbing N and P for growth. Generally, growth corresponds to the amount and kind of nutrients being removed, and the rate may vary depending on the environmental conditions and the species of microalgae. Using synthetic wastewater, for instance, with the algae *Chlorella vulgaris*, Ferro (2019) observed removal of 58.8% and 100% for N and P, respectively, under heterotrophic and mixotrophic conditions with specific growth rates of 0.02 h^{-1} and 0.114 h^{-1}, respectively. Even though nutrient removal efficiency was the same in both conditions, growth was higher in the mixotrophic condition. In contrast, the present study found greater N removal (NH_3-N from 93.16% to 98.24%; NO_2-N from 95.05% to 98.87%) than P (42.90% to 72.50%), with a specific growth rate of 0.210 to 0.525 μ day^{-1}, comparable to the heterotrophic condition in Ferro (2019). The results of this study were understandably lower than the growth observed under mixotrophic conditions in the above-cited study, because the heterotrophic bacterium *Rhizobium* aided algal bioremediation. The current study relies solely on the nutrients present in the aquaculture wastewater. However, despite nutrient limitations, the specific growth rate of microalgae in this study was within the range reported by Kawaroe et al. (2015) for *Nannochloropsis* sp. cultured in a photobioreactor and an open raceway pond.

On the other hand, when comparing microalgal growth in other studies using aquaculture wastewater as growth media, the five microalgae in this study exhibited a slightly higher growth rate than that reported for *Chlorella vulgaris,* which had a growth rate of 0.260 d^{-1} (Esteves et al. 2022). While the specific growth rates of mixed algal cultures of *Euglena gracilis* with *Selenastrum* grown in aquaculture wastewater ranged from 0.27 day^{-1} to 0.38 day^{-1} (Tossavainen et al. 2019), supporting the findings of this study

On the other hand, *Nannochlorum* sp. and *I. galbana* had higher specific growth rates, which almost surpassed the other three algae on day 4 and exceeded them all on day 5. The higher growth of *Nannochlorum* sp. was observed in the current experiment and confirmed in another study (Kawaroe et al. 2015). On the other hand, despite the potential of *T. tetrathele* for removing N and P (NH_3-N - 98.24%, NO_2-N - 98.87% and P - 72.50%), the microalgae-specific growth rate was observed to be low. It could be speculated that, due to its larger size, the growth of *T. tetrathele* was slightly affected, resulting in a lower specific growth rate compared to the rapid proliferation observed in smaller algae such as *Nannochlorum* sp. and *I. galbana*. The same observation was also seen in the larger algae *Thalassiosira* sp., confirming the above hypothesis. These findings were also confirmed by the report of Piñosa and Amar (2024) on two microalgal species, *T. tetrathele* and *Nannochlorum* sp., grown using the same cell density. The larger microalga, *T. tetrathele,* exhibited a significantly higher specific growth rate at lower densities, but its growth rate declined at higher densities. Conversely, the smaller microalga, *Nannochlorum* sp. maintained a consistent specific growth rate across all tested densities. The measured mean length and width of *T. tetrathele* and *Thalassiosira* sp. ranged from 12.90 to 14.04 μm and 8.41 to 11.84 μm, respectively, while for *I. galbana* and *Nannochlorum* sp., they only ranged from 2.29 to 4.13 μm and 2.13 to 3.66 μm, respectively.

The five microalgae exhibited a high specific growth rate after only a day grown in aquaculture wastewater, indicating their good adaptability to the culture medium, and supporting its effectiveness for microal-

Table 3 Ash, OC, OM, and moisture of air-dried microalgal biomass cultured in aquaculture wastewater (n = 3, mean ± SD)

	Ash (%)	OC (%)	OM (%)	Moisture(%)
Tetraselmis tetrathele	54.45±0.6 ^{a}	45.55±0.6 ^{a}	78.35±0.4 ^{a}	20.31±0 ^{c}
Nannochlorum sp.	57.12±0.4 ^{a}	42.88±0.9 ^{a}	73.76±0.5 ^{a}	23.52±3 ^{a}
Chaetoceros calcitrans	57.14±0.4 ^{a}	42.86±0.4 ^{a}	73.72±0.2 ^{a}	22.34±6 ^{b}
Isochrysis galbana	59.44±0.9 ^{a}	40.14±1.0 ^{a}	69.76±0.6 ^{a}	21.47±5 ^{b}
Thalassiosira sp.	59.88±0.6 ^{a}	40.11±0.4 ^{a}	68.99±0.2 ^{a}	19.18±3 ^{d}
F-values	2.875	2.875	2.875	28.435
P-values	0.080	0.080	0.080	0.000

Means within a column having different letter superscripts indicate significant differences

gal production. Exponential growth was further observed during the first 1-3 days. Although growth was still observed in the succeeding days, production was already low, and most microalgae tended to collapse at any time if not added with nutrients or re-cultured. The same observation was also reported by Wang et al. (2014), who noted that the cell density of *I. zhangjiangensis* increased only up to three days under depleted N conditions.

For biomass productivity, findings showed that *T. tetrathele* and *Thalassiosira* sp. had higher values than the other three microalgae, making both microalgae candidates for production. The microalgae's high biomass productivity and concentration could be attributed to the organism's efficiency in absorbing nutrients and converting them to biomass, or to their size, where the larger the microalgae, the higher their biomass. In this study, *T. tetrathele* and *Thalassiosira* sp. were both bigger (12.01 - 14.04 µm in length) than the rest of the algae (2.13 - 3.66 µm in length). Likewise, both had high N and P wastewater absorption, with more than 90% removal efficiencies for N and 72.50% and 54.40% removal efficiencies for P in *T. tetrathele* and *Thalassiosira* sp., respectively. Although *Nannochlorum* sp. and *I. galbana* had higher specific growth rates than the rest of the algae, *T. tetrathele* displayed the lowest growth, and *Thalassiosira* sp. had low growth only in the latter days; however, due to the large size of their cells, both had higher biomass production at the end of the experiment. The biomass productivity in this study was lower than that reported by Piñosa and Apines-Amar (2024), despite using the same aquaculture wastewater and microalgal species. Their study utilized smaller containers of 10 L carboys, and the culture was performed under controlled room temperature (20-22 °C) and with continuous lighting support. In contrast, the present experiment utilized larger fiberglass containers of 500 L, conducted in an open structure with a transparent roof, allowing sunlight as the sole source for algal growth. According to Razzak et al. (2024), culture conditions such as light and solar irradiation affect the growth of microalgae. However, compared to some studies, biomass productivity values obtained in the current experiment were comparable to those of Viegas et al. (2021), which ranged from 20.9 to 146.4 mg L^{-1} day^{-1} using *Nannochlorum* sp. and *Chlorella vulgaris* cultured in brown crab effluent under laboratory conditions. This comparison suggests that aquaculture wastewater is a promising culture medium for microalgae, as they can still grow abundantly despite environmental and nutrient limitations.

On the other hand, studies have shown that microalgae possess remarkable nutrient bioremediation potential. Mohamed and Abdallah (2024) reported a 93% reduction in NH_4-N from sewage plant effluent by mixed cultures of *Chlorella vulgaris* and *Micrococcus luteus*. In this study, the five microalgae tested also exhibited high NH_3-N removal efficiency after five days of culture, ranging from 93.16% to 98.24%. Among them, *T. tetrathele* exhibited the highest NH_3-N removal efficiency, making it particularly promising for wastewater treatment.

Similar to NH_3-N, the removal of NO_2-N in this study was also high, ranging from 95.05% to 98.87% after five days of culture. These results fall within the range (73.83% to 99.73%) reported by Ansari et al. (2017) in a study using *Scenedesmus obliquus*, *Chlorella sorokiniana*, and *Ankistrodesmus facultatus* to treat aquaculture wastewater. Among the five microalgae tested in this study, *T. tetrathele* again demonstrated the highest NO_2-N removal efficiency, highlighting its potential as an effective agent for remediating NO_2-N contaminated ponds.

Although most microalgae have demonstrated high P removal efficiency (Lananan et al. 2014), this study observed relatively low removal rates ranging from 42.90% to 72.50%. These findings, however, are supported by a study using water hyacinth in an aquatic treatment system, which reported that P removal rates rarely exceed 60% -70% (Abdel-Raouf et al. 2012). In addition, general observations on the nutrient removal efficiency by the five microalgae showed that N removal was higher than P removal. This may be because microalgae preferentially take up N over P (Collos and Harrison 2014). Several studies have reported lower P removal compared to N (Choi and Lee 2012), which supports the current findings. Among the five microalgae tested in this study, *T. tetrathele* exhibited significantly higher removal efficiency, with *C. calcitrans* being similarly effective in removing P.

For the macronutrients of the microalgal biomass grown in aquaculture wastewater, the N content among the microalgae was lower (0.87% to 2.01%) than that reported for freshwater and marine microalgae (3.74% to 11.67%) (Tibbetts et al. 2015). This lower N content may be attributed to the low N concentrations in the aquaculture wastewater, particularly NH_3-N (0.934 - 1.23 mg L^{-1}), which was controlled, as elevated levels would be harmful to fish.

In contrast, the five microalgae in this study offered the advantage of providing higher P levels (1.43% to 1.85%) compared to most commonly used animal-based manures (Sager 2007). Like N, the higher P levels in microalgae may be attributed to the higher P concentrations in the aquaculture wastewater (2.1 to 2.5 mg L^{-1}). The microalgae likely absorbed and stored this nutrient in their tissues, as many species are capable of taking up and storing large amounts of P whenever available (Solovchenko et al. 2019).

On the other hand, although the K content of the microalgae in this study was lower than that grown in synthetic effluent (Silva et al. 2015), the aquaculture wastewater appeared comparable with commercial media. The K content of the microalgae in this study was similar to that reported by Tibbetts et al. (2015), which ranged from 0.67% to 2.39% grown using f/2, f/2+Si, or Bold's media.

For the micronutrients, the Cu contents of microalgae in this study were comparable to those grown using commercial F/2 medium (Di Lena et al. 2020), suggesting that aquaculture wastewater could serve as an alternative nutrient source. On the other hand, Zn levels were lower than in most algae grown with growth media (Silva et al. 2015; Tibbetts et al. 2015), likely due to the limited Zn availability in aquaculture wastewater, with the only source coming from uneaten milkfish feed. Compared to most animal-based manures, microalgae had lower Cu and Zn levels (Sager 2007). The Cu and Zn in animal-based manures are logically high because they were used as feed additives for poultry and livestock. However, lower Cu and Zn levels are preferred for use as fertilizers, as these minerals, when in excess, lead to soil pollution. In China, the use of animal-based manure has already contributed to soil Cu pollution (Xiong et al. 2010), and in the Philippines, allowable limits of only 300 mg kg^{-1} for Cu and 5 mg kg^{-1} for Zn in organic fertilizers have already been established. Other requirements, including acceptable levels of pathogens, heavy metals, and additional criteria for classifying organic fertilizers, have also been standardized (PNS/BAFS 2013).

In terms of Mn, the microalgae in this study had Mn contents ranging from 340 to 482.62 mg kg^{-1}. Consistent with values from microalgae grown in photobioreactors (Tibbetts et al. 2015). For suitability as a fertilizer source, these values were slightly lower than in poultry manure (Sheppard and Sanipelli 2012), but higher than in cattle manure and pig and poultry dung (Sager 2007). While the Fe contents of microalgae in this study ranged from 1,484 mg kg^{-1} - 2,140 mg kg^{-1}, comparable to some microalgae (Tuzen et al. 2009), and within the range of some known animal-based manures (Sager 2007). Although some variability was observed in the data, this is expected since the replicates were conducted in separate runs.

While microalgae hold great potential for bioremediating aquaculture wastewaters and generating valuable biomass, challenges remain that can affect microalgal growth. Among these, fluctuations in wastewater quality are one such factor. In this experiment, nutrient levels in the wastewaters varied, affecting microalgal growth. However, to minimize variations among runs, nutrient levels were checked before use. Likewise, light intensity influences the growth performance of microalgae. Generally, as *light intensity* increases, microalgal *growth* also increases up to a photoinhibitory threshold (Razzak et al. 2024*)*. Light limitation in the current experiment may have contributed to the lower specific growth compared to microalgae cultivated using wastewater but provided with sufficient light (Borg-Stoveland et al. 2024).

Conclusion

In conclusion, our study highlights the potential of microalgae as effective bioremediation agents for aquaculture wastewater, with *T. tetrathele* demonstrating superior performance compared to four other microalgae. The study likewise suggested that aquaculture wastewater is a cost-effective medium for microalgae cultivation, as evidenced by the rapid proliferation of all microalgae, even at the onset of wastewater exposure. Moreover, microalgae cultured in aquaculture wastewater are a possible source of macronutrients with minimal concentrations of micronutrients, making them a potential source of organic fertilizer. Among the microalgae studied, *T. tetrathele and Thalassiosira* sp. showed significant potential as organic fertilizer sources due to their high macronutrient and micronutrient contents, as well as their biomass productivity and concentration. While aquaculture wastewater enhances the nutrient content of microalgal cells, further research on other types of wastewater, such as fish processing waste, which may have lower heavy metal contamination than industrial wastewater. This could help optimize nutrient levels in microalgal biomass, enhancing its potential as a source of organic fertilizer and other valuable products for utilization.

Competing interests The authors declare no competing interests.

Authors' contributions LAGP, Conceptualization, experimental work, data collection and analysis, and writing of manuscript; GGG, conceptualization, scheduling of activities and parameters to be taken, collection of data, review and editing the manuscript; MJAA, conceptualization, monitoring of experiment, review and editing the manuscript; ELA, conceptualization, statistical analysis, review and editing the manuscript; HGG, JAA Jr., JAB, FLP, conceptualization, data collection, review and editing the manuscript.

Acknowledgments The authors wish to acknowledge the University of the Philippines Visayas, Office of the Vice Chancellor for Research and Extension, In-house Research Grant, for the financial support. Professor Roman C. Sanares for the statistical advice, and the Institute of Aquaculture of the University of the Philippines Visayas, College of Fisheries and Ocean Sciences, for the assistance and support.

References

Abdel-Raouf N, Al-Homaidan AA, Ibraheem IBM (2012) Microalgae and wastewater treatment. Saudi J Biol Sci 19:257-275. https://doi.org/ 10.1016/j.sjbs.2012.04.005

Alvarez-González A, Uggetti E, Serrano L, Gorchs G, Ferrer I, Díez-Montero R (2022) Can microalgae grown in wastewater reduce the use of inorganic fertilizers? J Environ Manage 323:116224. https://doi.org/10.1016/j.jenvman.2022.116224

Aníbal J, Madeira HT, Carvalho LF, Esteves E, Veiga-Pires C, Rocha C (2014) Macroalgae mitigation potential for fish aquaculture effluents: an approach coupling nitrogen uptake and metabolic pathways using *Ulva rigida* and *Enteromorpha clathrata*. Environ Sci Pollut Res Int 21:13324–13334. https://doi.org/10.1007/s11356-013-2427-x

Ansari FA, Singh P, Guldhe A, Bux F (2017) Microalgal cultivation using aquaculture wastewater: integrated biomass generation and nutrient remediation. Algal Res 21:169-177. https://doi.org/10.1016/j.algal.2016.11.015

AOAC (1975) Official methods of analysis, 12th edn. Association of Official Analytical Chemists, Washington, DC

AOAC (2005) Official methods of analysis, 18th edn. Association of Official Analytical Chemists, Washington, DC

Borg-Stoveland S, Draganovic V, Spilling K, Gabrielsen TM (2024) Successful growth of coastal marine microalgae in wastewater from a salmon recirculating aquaculture system. J Appl Phycol 36:2851–2861. https://doi.org/10.1007/s10811-024-03310-1

Castro IMP, Rosa A, Borges A, Cunha F, Passos F (2024) The effects of microalgae use as a biofertilizer on soil and plant before and after its anaerobic (co-)digestion with food waste. Sci Total Environ 934:17330. https://doi.org/10.1016/j.scitotenv.2024.173301

Choi HJ, Lee SM (2012) Effects of microalgae on the removal of nutrients from wastewater: Various concentrations of *Chlorella vulgaris.* Environ Eng Res 17(S1): S3-S8. http://dx.doi.org/10.4491/eer. 2012.17. S1. S3

Collos Y, Harrison PJ (2014) Acclimation and toxicity of high ammonium concentrations to unicellular algae. Mar Pollut Bull 80:8–23

Di Lena G, Casini I, Lucarini M, Sanchez del Pulgar J, Aguzzi A, Caproni R, Gabrielli P, Lombardi-Boccia G (2020) Chemical characterization and nutritional evaluation of microalgal biomass from large-scale production: a comparative study of five species. Eur Food Res Technol 246:323-332. https://doi.org/10.1007/s00217-019-03346-5

Esteves AF, Soares SM, Salgado EM, Boaventura RAR, Pires JCM (2022) Microalgal growth in aquaculture effluent: coupling biomass valorisation with nutrients removal. Appli Sci. https://doi.org/10.3390/app122412608

Facey KK (2024) Evaluation of aquaculture effluents and management practices: a case study for Jamaica. Final project. GRÓ Fisheries Training Programme under the auspices of UNESCO, Iceland. https://www.grocentre.is/static/gro/publication/1819/document/Facey23prf.pdf. Accessed 12 Dec 2024

Ferro L, Colombo M, Posadas E, Funk C, Muñoz R (2019) Elucidating the symbiotic interactions between a locally isolated microalga *Chlorella vulgaris* and its co-occurring bacterium *Rhizobium* sp. in synthetic municipal wastewater. J Appl Phycol 31:2299-2310. https://doi.org/10.1007/s10811-019-1741-1

Gani P, Sunar NM, Matias-Peralta H, Abdul Latiff AA, Abdul Razak AR (2016) Influence of initial cell concentrations on the growth rate and biomass productivity of microalgae in domestic wastewater. Appl Ecol Environ Res 14:399-409. https://doi.org/10.15666/aeer/1402_399409

Huang H, Zhong S, Wen S, Luo C, Long T (2022) Improving the efficiency of wastewater treatment and microalgae production for biofuels. Resour Conserv Recycl 178:106094. https://doi.org/10.1016/j.resconrec.2021.106094

Iber BT, Kasan NA (2021) Recent advances in shrimp aquaculture wastewater management. Heliyon 7:e08283. https://doi.org/10.1016/j.heliyon.2021.e08283

Karathanasis AD, Hajel BF (1996) Elemental analysis by X-Ray fluorescence spectroscopy. In: Sparks DL, Page AL, Helmke PA, Loeppert RH, Soltanpour PN, Tabatabai MA, Johnston CT, Sumner ME (eds) Methods of soil analysis, Part 3: chemical methods. Soil Sci Soc Am, Madison, pp 161-223. https://doi.org/10.2136/sssabookser5.3.c7

Kawaroe M, Hwangbo J, Augustine D, Putr HA (2015) Comparison of density, specific growth rate, biomass weight and doubling time of microalgae *Nannochloropsis* sp. cultivated in open raceway pond and photobioreactor. Aquac Aquar Conserv Legis Int J Bioflux Soc 8:740-750

Kumar KP, Krishna SV, Naidua SS, Verma K, Bhagawan D, Himabindu V (2019) Biomass production from microalgae *Chlorella* grown in sewage, kitchen wastewater using industrial CO_2 emissions: comparative study. Carbon Resour Convers 2:126-133. https://doi.org/10.1016/j.crcon.2019.06.002

Lananan F, Hamid SHA, Din WNS, Ali N, Khatoon H, Jusoh A, Endut A (2014) Symbiotic bioremediation of aquaculture wastewater in reducing ammonia and phosphorus utilizing effective microorganism (EM-1) and microalgae (*Chlorella* sp.). Int Biodeterior Biodegr 95:127-134. https://doi.org/10.1016/j.ibiod.2014.06.013

Lawton RJ, Mata L, de Nys R, Paul NA (2013) Algal bioremediation of wastewaters from land-based aquaculture using *Ulva*: selecting target species and strains. PloS One 8(10):e77344. https://doi.org/10.1371/journal.pone.0077344

Liu Y, Lv J, Feng J, Liu Q, Nan F, Xie S (2018) Treatment of real aquaculture wastewater from a fishery utilizing phytoremediation with microalgae. J Chem Technol Biotechnol 94:900-910. https://doi.org/10.1002/jctb.5837

Macusi ED, Estor DEP, Borazon EQ, Clapano MB, Santos MD (2022) Environmental and socioeconomic impacts of shrimp farming in the Philippines: a critical analysis using PRISMA. Sustainability 14(5):2977. https://doi.org/10.3390/su14052977

Martinez MR, Chakroff CL, Pantastico JF (1975) Direct phytoplankton counting techniques using the haemacytometer. Philipp Agric Sci 55:43-50

Mohamed A, Abdallah AM (2024) The bioremediation effect of microalgae on wastewater: a green technology approach. J Eng Res 8(3):31. https://digitalcommons .aaru.edu.jo/erjeng/vol8/iss3/31

Niccolai A, Zittelli GC, Rodolfi L, Biondi N, Tredici MR (2019) Microalgae of interest as food source: biochemical composition and digestibility. Algal Res 42:101617. https://doi.org/10.1016/j.algal.2019.101617

Niu S, Li C, Xie J, Li Z, Zhang K, Wang G, Xia Y, Tian J, Li H, Xie W, Gong W (2025) Influence of aquaculture practices on microbiota composition and pathogen abundance in pond ecosystems in South China. Water Res X 27:100302. https://doi.org/10.1016/j.wroa.2025.100302

Philippine National Standard/Bureau of Agriculture and Fisheries Product Standards (2013) PNS/BAFPS 40:2013 - Organic fertilizer. Department of Agriculture, Bureau of Agriculture and Fisheries Product Standards, Philippines. Available from ResearchGate: Philippine National Standard PNS/BAFPS 40:2013 – Organic fertilizer. Access 12 Dec 2023

Piñosa LAG (2018) Influence of colonization time on phytoperiphyton growth during wet and dry seasons in brackish water pond. J Appl Phycol 30:3633–3641. https://doi.org/10.1007/s10811-018-1497-z

Piñosa LAG, Apines-Amar MJS (2024) Optimization of stocking density for *Isochrysis galbana, Nannochlorum* sp., and *Tetraselmis tetrahele* in the bioremediation of aquaculture wastewater. Aquac Int 32:3597-3616. https://doi.org/10.1007/s10499-023-01340-z

Razzak SA, Bahar K, Islam KMO, Haniffa AK, Faruque MO, Hossain SMZ, Hossain MM (2024) Microalgae cultivation in photobioreactors: sustainable solutions for a greener future. Green Chem Eng 5(4):418-439. https://doi.org/10.1016/j.gce.2023.10.004

Sager M (2007) Trace and nutrient elements in manure, dung and compost samples in Austria. Soil Biol Biochem 39(6):1383-1390. https://doi.org/10.1016/j.soilbio.2006.12.015

Sheppard SC, Sanipelli B (2012) Trace elements in feed, manure, and manured soils. J Environ Qual 41(6):1846-1856. https://doi.org/10.2134/jeq2012.0133

Silva NFP, Gonçalves AL, Moreira FC, Silva TFCV, Martins FG, Alvim-Ferraz MCM, Boaventura RAR, Vilar VJP, Pires JCM (2015) Towards sustainable microalgal biomass production by phycoremediation of a synthetic wastewater: a kinetic study. Algal Res 11:350–358. https://doi.org/10.1016/j.algal.2015.07.014

Skriptsova AV, Miroshnikova NV (2011) Laboratory experiment to determine the potential of two macroalgae from the Russian Far-East as biofilters for integrated multi-trophic aquaculture (IMTA). Bioresour Technol 102:3149–3154. https://doi.org/10.1016/j.biortech.2010.10.093

Solovchenko AE, Ismagulova TT, Lukyanov AA, Vasilieva SG, Konyukhov IV, Pogosyan SI, Lobakova ES, Gorelova OA (2019) Luxury phosphorus uptake in microalgae. J Appl Phycol 31:2755–2770. https://doi.org/10.1007/s10811-019-01831-8

Strickland JDH, Parsons TR (1972) A practical handbook of seawater analysis, 2nd edn. Fish Res Board Can Bull 167:1-130. https://doi.org/10.25607/OBP-1791

Suleiman AKA, Lourenço KS, Clark C, Luz RL, da Silva GHR, Vet LEM, Cantarella H, Fernandes TV, Kuramae EE (2020) From toilet to agriculture: fertilization with microalgal biomass from wastewater impacts the soil and rhizosphere active microbiomes, greenhouse gas emissions and plant growth. Resour Conserv Recyl 161:104924. https://doi.org/10.1016/j.resconrec.2020.104924

Tendencia EA, de la Peña LD (2001) Antibiotic resistance of bacteria from shrimp ponds. Aquaculture 195:193-204. https://doi.org/10.1016/S0044-8486(00)00570-6

Tibbetts SM, Milley JE, Lall SP (2015) Chemical composition and nutritional properties of freshwater and marine microalgal biomass cultured in photobioreactors. J Appl Phycol 27:1109-1119. https://doi.org/10.1007/s10811-014-0428-x

Tossavainen M, Lahti K, Edelmann M, Escola R, Lampi AM, Piironen V, Korvonen P, Ojala A, Romantschuk M (2019). Integrated utilization of microalgae cultured in aquaculture wastewater: wastewater treatment and production of valuable fatty acids and tocopherols. J Appl Phycol 31:1753-1763

Tuzen M, Verep B, Ogretmen AO, Soylak M (2009) Trace element content in marine algae species from the Black Sea, Turkey. Environ Monit Assess 151:363-368. https://doi.org/10.1007/s10661-008-0277-7

Viegas C, Gouveia L, Goncalves M (2021) Aquaculture wastewater treatment through microalgal. Biomass potential applications on animal feed, agriculture, and energy. J Environ Manage 286:112187. https://doi.org/10.1016/j.jenvman.2021.112187

Vijayaram S, Ringø E, Ghafarifarsani H, Hoseinifar SH, Ahani S, Chou CC (2024) Use of algae in aquaculture: a review. Fishes 9(2):63. https://doi.org/10.3390/fishes9020063

Wang HT, Yao CH, Ai JN, Cao XP, Xue S, Wang WL (2014) Identification of carbohydrates as the major carbon sink of the marine microalga *Isochrysis zhangjiangensis* (Haptophyta) and optimization of its productivity by nitrogen manipulation. Bioresour Technol 171:298-304. https://doi.org/10.1016/j.biortech.2014.08.090

Xiong X, Li Y, Liao W, Li C, Huang W, Yang M (2010) Copper content in animal manures and potential risk of soil copper pollution with animal manure use in agriculture. Resour Conserv Recycl 54:985-990. https://doi.org/10.1016/j.resconrec.2010.02.005

Zhang Y, Wang Q, Liu X, Zheng H, Li A (2023) Two types of growth pattern of five microalgal species under different nitrogen supplies. Biomass Bioenergy 173:106720. https://doi.org/10.1016/j.biombioe.2023.106720

Zhang Z, Xu M, Fan Y, Zhang L, Wang H (2024) Using microalgae to reduce the use of conventional fertilizers in hydroponics and soil-based cultivation. Sci Total Environ 912:169424. https://doi.org/10.1016/j.scitotenv.2023.169424

Publisher's Note