Blue Biotechnology

RESEARCH

Open Access

Potential of culturing microalgae *Chlorella vulgaris* and *Nannochloropsis oculata* with aquaculture wastewater for simultaneous aquafeed production and wastewater remediation

Wong Ryan Lieng Song[1], Yeap Swee Keong[1], Fatimah Md. Yusoff[2], Tan Jian Ping[3] and Norazira Abdu Rahman[1*]

Abstract

Aquaculture expansion has resulted in nutrient pollution in aquatic ecosystems, primarily due to nitrogen-rich effluents, leading to eutrophication and degraded water quality. Although conventional wastewater treatment methods are effective, they are often costly and environmentally risky. Microalgae offer a promising alternative, enabling both wastewater remediation and the production of nutrient-rich biomass. However, most research has mainly focused on nutrient removal efficiencies, with relatively little attention given to the quality of the microalgal biomass and its suitability for simultaneous aquafeed production. This study evaluates the growth, nutritional content, and nutrient removal efficiencies of *Chlorella vulgaris* (*C. vulgaris*) and *Nannochloropsis oculata* (*N. oculata*) in synthetic aquaculture wastewater (AW). The findings reveal that both species showed significant growth in AW and F/2 media, with *N. oculata* reaching the highest cell density (17.6×10^6 cells/mL) in AW. After seven days, *C. vulgaris* removed $83.7 \pm 0.42\%$ of nutrients in AW and $78.0 \pm 4.35\%$ in F/2, while *N. oculata* achieved $71.3 \pm 1.50\%$ and $72.3 \pm 10.0\%$, respectively. Biomass from both species was also rich in protein (35.9–57.4%) and carbohydrates (12.7–40.9%). Particularly, *N. oculata* produced 46% dw protein and 40.9% dw carbohydrates in aquaculture wastewater, with protein levels higher than most previously reported values in such conditions. Additionally, with over 71% nutrient removal in only seven days, a longer culture duration and higher initial biomass inoculum could further enhance the efficiency. These findings highlight the potential of *N. oculata* and *C. vulgaris* for sustainable aquaculture, effectively treating aquaculture wastewater and producing high-quality aquafeed biomass, thereby supporting environmentally friendly and cost-effective practices.

Keywords Microalgae, Aquaculture, Wastewater, Aquafeed, Protein, Carbohydrate

*Correspondence:
Norazira Abdu Rahman
norazira.abdurahman@xmu.edu.my
[1] China-ASEAN College of Marine Sciences, Xiamen University Malaysia, Selangor, Malaysia
[2] International Institute of Aquaculture and Aquatic Sciences, Universiti Putra Malaysia, Selangor, Malaysia
[3] School of Energy and Chemical Engineering, Xiamen University Malaysia, Selangor, Malaysia

Introduction

Water pollution, driven by the uncontrolled discharge of human and industrial waste, remains a critical environmental challenge, particularly in urbanized regions where nutrient-rich effluents are increasingly released into aquatic ecosystems [1]. The aquaculture industry, while crucial in addressing the global demand for protein, is a significant contributor to this problem when

wastewater management practices are inadequate [2]. As the depletion of wild fish stocks accelerates, aquaculture has expanded rapidly, emerging as a key food production system worldwide. However, this expansion has also led to environmental concerns, particularly the generation of nutrient-dense wastewater, which poses substantial risks to aquatic environments if not properly treated. Particularly, the intensive farming practices prevalent in modern aquaculture often result in excessive nitrogen and phosphorus compounds in wastewater, primarily derived from fish waste and uneaten feed.

Excessive nutrients, especially nitrogen in the form of ammonium (NH_4^+), can cause eutrophication and other ecological disruptions when discharged into natural water bodies [3]. Ammonium pollution especially, can lead to pH shifts, increased toxicity, and reductions in dissolved oxygen, further endangering aquatic life and ecosystem stability [4]. Consequently, there is an urgent need for effective and sustainable nitrogen removal strategies in aquaculture. Conventional wastewater treatment methods, though effective, are often expensive and require extensive maintenance, making them less feasible for widespread application in the aquaculture industry [5]. As a result, there is growing interest in alternative, sustainable methods that not only mitigate environmental impacts but also add value to the aquaculture process. Microalgae have emerged as a promising solution, offering a natural and cost-effective means of nutrient removal from wastewater [6]. Nitrogen, a critical nutrient for microalgal growth, plays a key role not only in cell proliferation but also in shaping the biochemical composition of the resulting biomass [7].

Microalgae, such as *Chlorella vulgaris* and *Nannochloropsis oculata*, are particularly well-suited for wastewater remediation due to their rapid growth rates, high photosynthetic efficiency, and adaptability to various environmental conditions [8]. These microalgae not only remove nitrogen and phosphorus from wastewater but also produce biomass rich in valuable nutritional components. Both *Chlorella* sp. and *Nannochloropsis* sp. are known for their high protein, lipid, carbohydrate, essential fatty acids, vitamins, and antioxidants, making them highly beneficial for use as aquaculture feed. The unique nutritional profile of these microalgae offers significant advantages over conventional feed ingredients, contributing to enhanced growth, immunity, and overall health of aquaculture species. The high protein content in microalgae is particularly important, as it provides a sustainable alternative to traditional fishmeal, reducing the reliance on wild-caught fish stocks [9]. Additionally, microalgae are also rich in omega-3 and omega-6 fatty acids, which are crucial for the development and health of aquatic organisms. These fatty acids, along with other bioactive compounds such as carotenoids and vitamins, improve the nutritional quality of aquaculture products, making them more appealing to consumers [10]. Furthermore, the presence of antioxidants in microalgae can also enhance the immune response of farmed species, reducing the disease related loss and improving overall survival rates and production.

However, the form of nitrogen—whether nitrate, nitrite, ammonium, or organic nitrogen—can significantly affect the quality and nutritional value of the microalgal biomass produced [9]. In addition, different microalgae species and strain may exhibit varying preferences and responses to specific nitrogen forms, which subsequently influence their metabolic pathways and nutrient uptake efficiency [11]. This presents both a challenge and an opportunity: by selecting and optimizing the right microalgal species for specific wastewater compositions, it is possible to enhance both nutrient removal and the production of high-value biomass. Several studies have reported the effectiveness of microalgae in nutrient removal from wastewater. This includes *Isochrysis zhanjiangensis* which has demonstrated the ability to remove between 60 and 85% of nitrogenous compounds, including ammonia, from aquaculture wastewater [12]. Similarly, Nasir et al. (2023) [13] also found that *Chlorella sp.* achieved ammonia, nitrite, and phosphate removal efficiencies ranging from 75.96% to 96.77%, depending on inoculum dosage. Other species, such as *Haematococcus* sp., *Neochloris* sp., *Monoraphidium* sp., were also able to assimilate > 70% of the total nitrogen in brackish aquaculture wastewater [14]. However, many studies have focused primarily on nutrient removal efficiencies, with limited attention to the nutritional composition of the resulting biomass, particularly its suitability as aquafeed. Additionally, cultivating microalgae in aquaculture wastewater often results in low yields of proteins, carbohydrates, or lipids in the biomass, limiting its practicality for simultaneous aquafeed production and wastewater remediation. For instance, *Chlorella sorokiniana* cultivated in Nile tilapia (*Oreochromis niloticus*) wastewater achieved a 75.6% reduction in ammonia, 96.4% in nitrite, and 84.5% in nitrate, with a biomass composition of 39.1% lipids and 36.1% carbohydrates [15]. In contrast, He et al. (2023) [16] reported ammonia removal of up to 86.42% by *Chlorella sorokiniana*, but with a lower protein content of only 21.5%. Similarly, *Chlorella vulgaris* cultivated in trout farm wastewater produced low protein content (17.93%), lipids (15.82%), and carbohydrates (48.64%), despite nutrient removal efficiencies exceeding 90% [17]. Given the species- and strain-specific variations in nutrient uptake and metabolic responses, more research is needed to fully explore the potential of

microalgae for simultaneous nutrient removal and aquafeed production.

Hence, this study investigates the nitrogen removal efficiency of *Chlorella vulgaris* and *Nannochloropsis oculata* when cultivated in synthetic aquaculture wastewater, with a focus on its effect on their growth performance, nutritional content, and potential for simultaneous aquafeed production. Although both species can effectively absorb nitrogenous compounds, their efficiency and biochemical profiles will differ due to their distinct metabolic pathways. By identifying the potential of microalgae strain for aquaculture wastewater remediation and assessing its biomass quality, this study also aims to contributes to the development of more resilient and sustainable aquaculture production and practices.

Materials and methods

Algae strain and culture conditions

The pure stock of microalga *Chlorella vulgaris* and *Nannochloropsis oculata* was obtained from Universiti Putra Malaysia. The cultures were grown in F/2 medium [18] using filtered and autoclaved seawater at 24 °C, 60 µmol/m2/s light intensity and 12 h light:12 dark photoperiod. Freshwater microalgae, *Chlorella vulgaris* and marine microalgae, *Nannochloropsis oculata* were grown in 0 ppt and 30 ppt salinity, respectively. Sub-culturing was done every two weeks to maintain pure and healthy stock culture.

Preparation of culture media

Synthetic aquaculture wastewater was formulated by adding ammonium, nitrite, and phosphate to sterilized seawater and distilled water to create appropriate media for marine and freshwater algal cultures, respectively. The final concentrations in the synthetic wastewater were set at 3 mg/L ammonium, 2 mg/L nitrite, and 2 mg/L phosphate. These nutrient levels were achieved by dissolving ammonium sulfate, sodium nitrite, and potassium dihydrogen phosphate, which provided the necessary sources of nitrogen and phosphorus. These concentrations were selected based on reported nutrient profiles of aquaculture wastewater, especially in Malaysia [3, 19]. Meanwhile, the F/2 nutrient media was also prepared and used as control (Table 1).

Experimental design

Marine microalgae *Nannochloropsis* sp. (30 ppt salinity) and freshwater microalgae *Chlorella* sp. (0 ppt salinity) was cultured using the prepared F/2 medium and synthetic aquaculture wastewater. Each culture was inoculated into experimental flasks (triplicates) at an initial cell density of 1×10^6 cells/mL. The cultures were then incubated in a controlled environment at a temperature of 23°C and light intensity of 60 µmol/m2/s with 12-h light:dark photoperiod. On Day 5 of culture (exponential phase), cells were harvested by centrifugation at 5000 rpm and then freeze dried. The harvested cells were subsequently stored at -20°C until further analysis.

Table 1 Chemical composition of F/2 culture media

	Component	Final concentration (mg/L)
Nitrogen (Nitrate)	$NaNO_3$	75
Phosphate	$NaH_2PO_4{\cdot}H_2O$	5
	Na_2CO_3	30
Trace metal	$FeCl_3{\cdot}6H_2O$	3.15
	$Na_2EDTA{\cdot}2H_2O$	4.36
	$CuSO_4{\cdot}5H_2O$	9.8
	$Na_2MoO_4{\cdot}2H_2O$	6.3
	$ZnSO_4{\cdot}7H_2O$	22
	$CoCl_2{\cdot}6H_2O$	10
	$MnCl_2{\cdot}4H_2O$	180
Vitamin	Thiamine HCl (Vitamin B1)	0.1
	Biotin (Vitamin H)	0.05
	Cyanocobalamin (Vitamin B12)	0.5

Growth parameter analysis

Microalgae growth was measured in terms of cell density and optical density. Cells were sampled and counted every alternate day using a haemacytometer (Hawksley AC1000, UK). Meanwhile, the optical density for all the cultures were determined daily using a spectrophotometer (Shimadzu UV-1601, Japan) where the medium was used as blank at 750 nm wavelength.

Determination of total ammonia nitrogen (TAN) and nutrient removal efficiency

Total ammonia nitrogen, which is the main form of available nitrogen found in aquaculture wastewater was measured at the start and end of experiments on Day 0 and Day 7 following the method by [20]. Water samples (5 mL) were filtered and then mixed with 0.2 mL each of phenol solution and sodium nitroprusside, followed by 0.5 mL of oxidizing solution. After incubation at room temperature for 1 h, absorbance was then measured at 640 nm and used for determination of nutrient removal efficiency.

Nutritional analysis

Protein

Protein content was assessed using the Lowry method [21]. Five milligrams of freeze dried microalgal sample were dissolved in 25 mL distilled water, with 0.5 mL

used for analysis in triplicate. The mixed reagent was made by combining 1 mL of 1% potassium sodium tartrate with 50 mL of 2 g sodium carbonate in 100 mL of 0.1 M NaOH. The sample, with 0.5 mL of 1 M sodium hydroxide, was incubated at 100 °C for 5 min, cooled for 10 min, then mixed with 2.5 mL of the mixed reagent and 0.5 mL of Folin reagent. After a 30-min dark incubation, absorbance was measured at 750 nm using a Shimadzu UV-1601 spectrophotometer.

Carbohydrates

The sample solution was prepared by dissolving 5–6 mg of the sample in 25 mL of distilled water [22]. Subsequently, 1.0 mL of a 5% phenolic solution and 5.0 mL of sulfuric acid were added to the mixture. The absorbance was then measured at 488 nm using a Shimadzu UV-1601 spectrophotometer (Japan).

Lipids

Lipid analysis was performed using the method described by [23]. Carbonization was carried out with tripalmitin as a standard following lipid extraction based on [24]. To extract lipids, 4.5 mL of chloroform (1:2) was added to the sample and centrifuged at 10,000 rpm for 10 min. The supernatant was collected in a clean tube. The biomass was re-extracted by adding 1.5 mL of chloroform and 1.5 mL of distilled water, followed by a second centrifugation. The combined supernatants were evaporated under vacuum at 35 °C after removing the polar phase. After the residue was completely dry, 2 mL of concentrated sulfuric acid was added, and the mixture was cooled to 0 °C. Absorbance was then measured at 375 nm after adding 3.0 mL of distilled water.

Statistical analysis

The experiments were carried out in triplicates, and all results are expressed as mean ± standard error. Data were then analysed using two-way variance analysis (ANOVA), followed by Tukey's post hoc comparison test to measure differences between data. Statistical significance was set at $p < 0.05$. Statistical analysis was carried out using the statistical software SPPS, version 23 (SPSS Inc., USA).

Results

Effect of synthetic aquaculture wastewater and F/2 media on growth of *Chlorella vulgaris* and *Nannochloropsis oculata*

Figures 1 and 2 illustrate the growth patterns of *Chlorella vulgaris* (*C. vulgaris*) and *Nannochloropsis oculata* (*N. oculata*) cultured in synthetic aquaculture wastewater (AW) and F/2 media (F2), as measured by cell density and optical density (OD_{750}) over a 7-day period.

Both *C. vulgaris* and *N. oculata* showed an increase in optical density throughout the culture period, with the highest OD_{750} values observed on Day 7 (Fig. 1). For *C. vulgaris*, the initial OD_{750} on Day 0 was 0.113 A for both AW and F2 media. By Day 7, the OD_{750} had increased to 0.246 ± 0.003 A in the AW and 0.323 ± 0.008 A in the F/2 media. *C. vulgaris* demonstrated significantly higher optical density when cultured in F/2 media compared to synthetic aquaculture wastewater ($p < 0.05$). In contrast, *N. oculata* exhibited a smaller increase in OD_{750},

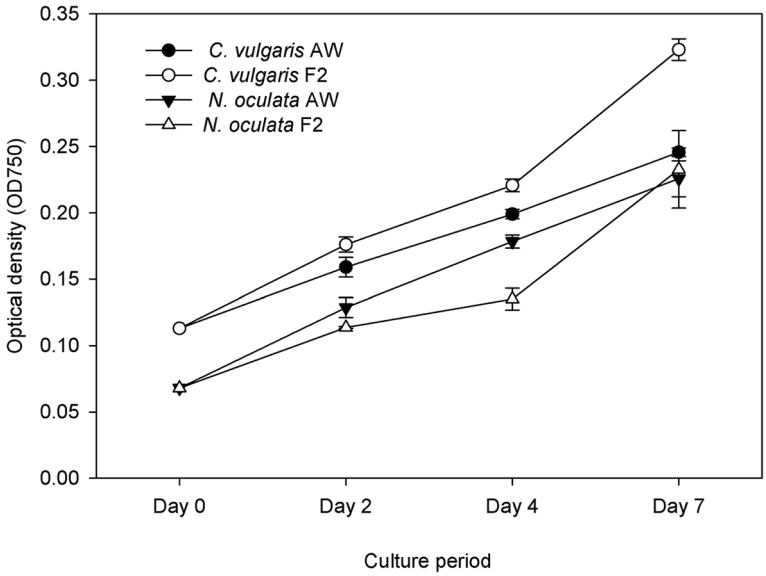

Fig. 1 Optical density (OD_{750}) of *Chlorella vulgaris* (*C. vulgaris*) and *Nannochloropsis oculata* (*N. oculata*) in synthetic aquaculture wastewater (AW) and F/2 media (F2). Data are presented as means ± standard errors

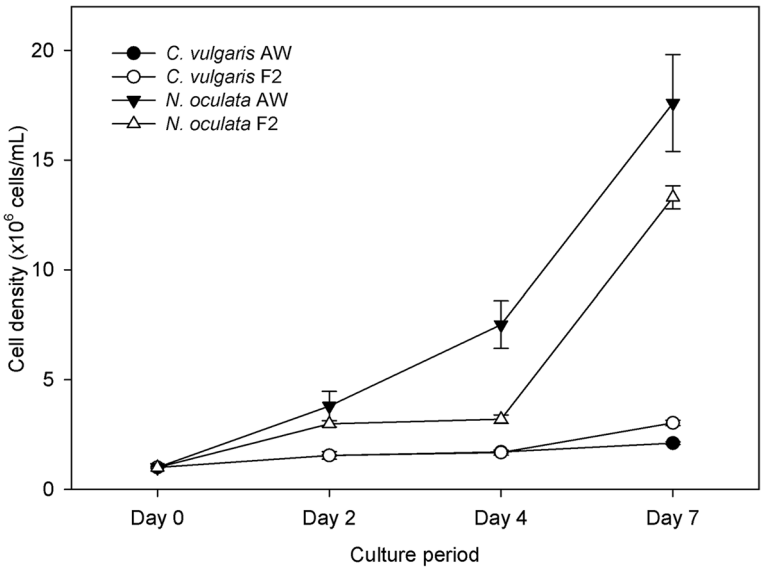

Fig. 2 Cell density (1×10^6 cells/mL) of *Chlorella vulgaris* (*C. vulgaris*) and *Nannochloropsis oculata* (*N. oculata*) in synthetic aquaculture wastewater (AW) and F/2 media (F2). Data are presented as means ± standard errors

starting at 0.068 A on Day 0 and reaching 0.226 ± 0.014 A in AW and 0.233 ± 0.029 A in F2 by Day 7. The difference in growth between the two media for *N. oculata* was not significantly different ($p > 0.05$), although a slightly higher OD_{750} was observed in the F/2 media. When comparing the two microalgal species, *C. vulgaris* generally achieved higher optical density than *N. oculata* under both culture conditions. On Day 7, the OD_{750} for *C. vulgaris* in F/2 media (0.323 ± 0.008 A) was significantly higher ($p < 0.05$) than that of *N. oculata* in the same medium (0.233 ± 0.029 A). Similarly, in synthetic aquaculture wastewater, *C. vulgaris* also outperformed *N. oculata*, with OD_{750} values of 0.246 ± 0.003 A compared to 0.226 ± 0.014 A, respectively.

Cell density observed throughout the culture period supported the trends observed in optical density (Fig. 2). Initially, all cultures had a cell density of 1×10^6 cells/mL. By Day 7, *N. oculata* cultured in AW showed significantly higher ($p < 0.05$) cell density at $(17.6 \pm 2.21) \times 10^6$ cells/mL, followed by *N. oculata* in F2 at $(13.3 \pm 0.52) \times 10^6$ cells/mL. In comparison, *C. vulgaris* cultured in F2 reached a cell density of $(3.03 \pm 0.12) \times 10^6$ cells/mL, while those in AW at $(2.11 \pm 0.05) \times 10^6$ cells/mL by Day 7. On Day 2, *C. vulgaris* cultures in both AW and F2 had similar cell densities, recorded at $(1.55 \pm 0.17) \times 10^6$ cells/mL and $(1.55 \pm 0.03) \times 10^6$ cells/mL, respectively. However, *N. oculata* consistently showed a significantly higher cell count ($p < 0.05$) than *C. vulgaris* from Day 2 onward, with AW maintaining the highest cell count throughout the experiment.

Overall, *N. oculata* demonstrated better growth in terms of cell density and optical density compared to *C. vulgaris* in both AW and F2 media. Despite *C. vulgaris* achieving higher optical density, *N. oculata* maintained a higher cell count, particularly in AW, hence indicating that AW can also be an effective medium for sustaining growth of both microalgae species.

Effect of aquaculture wastewater and F/2 media on specific growth rate of *Chlorella vulgaris* and *Nannochloropsis oculata*

Figure 3 presents the specific growth rates (μ) of *C. vulgaris* and *N. oculata* cultured in synthetic aquaculture wastewater (AW) and F/2 media over a 7-day period. All cultures showed positive specific growth rates throughout the experiment. Overall, the specific growth rates for both microalgae species ranged from 0.107 ± 0.004 day^{-1} to 0.409 ± 0.018 day^{-1}. Particularly, *N. oculata* in AW obtained the highest ($p < 0.05$) specific growth rate of 0.409 ± 0.018 day^{-1}, compared to *Chlorella vulgaris* in AW which showed the lowest ($p < 0.05$) specific growth rate of 0.107 ± 0.004 day^{-1}. In addition, with F/2 medium, the specific growth rate of *C. vulgaris* was at 0.159 ± 0.006 day^{-1}, significantly higher compared to 0.107 ± 0.004 day^{-1} when cultured in AW. This indicates that *C. vulgaris* grows more effectively in F/2 media. In contrast, *N. oculata* exhibited a significantly higher ($p < 0.05$) specific growth rate in AW (0.409 ± 0.018 day^{-1}) compared to F/2 media (0.370 ± 0.006 day^{-1}). These results highlight the differential growth responses of *Chlorella vulgaris* and *Nannochloropsis oculata* to the two culture media.

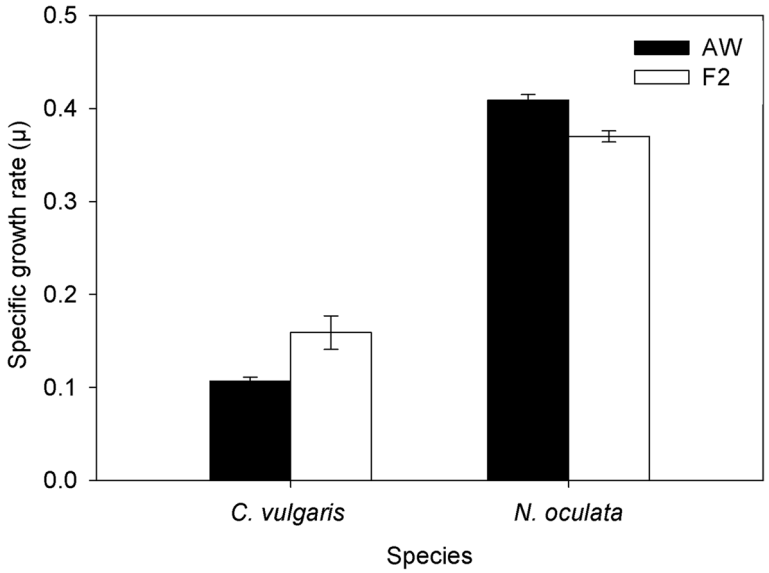

Fig. 3 Specific growth rate (μ) of *Chlorella vulgaris* (*C. vulgaris*) and *Nannochloropsis oculata* (*N. oculata*) in synthetic aquaculture wastewater (AW) and F/2 media (F2). Data are presented as means ± standard errors

Nutrient removal efficiency

The ammonium nitrogen removal efficiency of *C. vulgaris* and *N. oculata* varied across the different media (Fig. 4). *C. vulgaris* in AW demonstrated the highest ammonium (TAN) removal efficiency at 83.7 ± 0.42%, while in F2 media, the removal efficiency was slightly lower at 78.0 ± 4.35%. For *N. oculata*, TAN removal efficiency in F2 media was 72.3 ± 10.0%, and in AW, it was 71.3 ± 1.50%, with no significant difference between the two media ($p > 0.05$). Overall, although both species exhibited good TAN removal efficiency, *C. vulgaris* performed slightly better in terms of nutrient removal in AW.

Protein content of *Chlorella vulgaris* and *Nannochloropsis oculata* cultured in aquaculture wastewater and F/2 media

The protein content of *C. vulgaris* and *N. oculata* cultured in AW and F2 exhibited significant variations across the

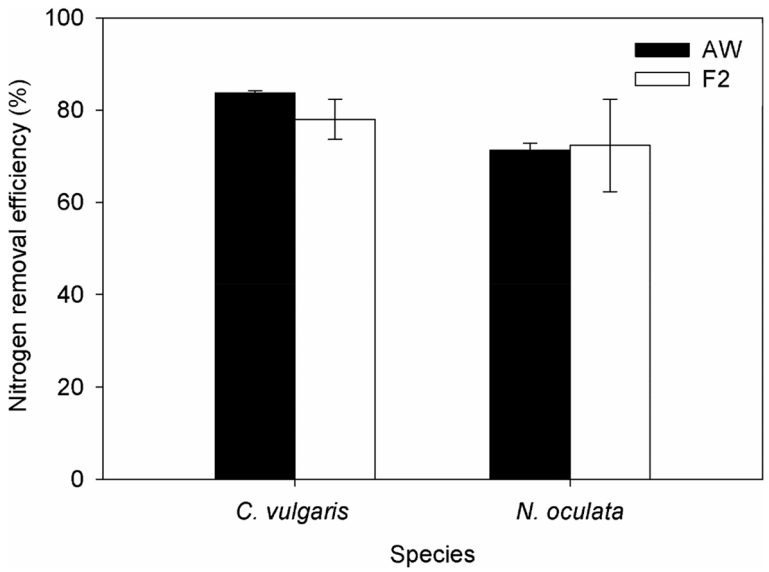

Fig. 4 Total ammonia nitrogen removal efficiency of *Chlorella vulgaris* (*C. vulgaris*) and *Nannochloropsis oculata* (*N. oculata*) in synthetic aquaculture wastewater (AW) and F/2 media (F2). Data are presented as means ± standard errors

different culture conditions (Fig. 5a). For *C. vulgaris*, the protein content was significantly lower when cultured in AW, reaching 35.9% dw (dry weight). In comparison, the protein content in F2 media was substantially higher at 53.0% dw, reflecting a 17.1% increase. Meanwhile, *N. oculata* showed a generally higher protein content across both media types. When cultured in AW, *N. oculata* recorded a protein content of 46.3% dw, which was significantly higher ($p < 0.05$) than *C. vulgaris* (35.9% dw) in the same medium. In F2 media, *N. oculata* obtained the highest protein content ($p < 0.05$) observed among all treatments, with 57.4% dw (11.1% increase in protein content than F2). Overall, these results indicate that F2 media significantly enhances protein content in both *C. vulgaris* and *N. oculata*, with *N. oculata* showing a better total protein content.

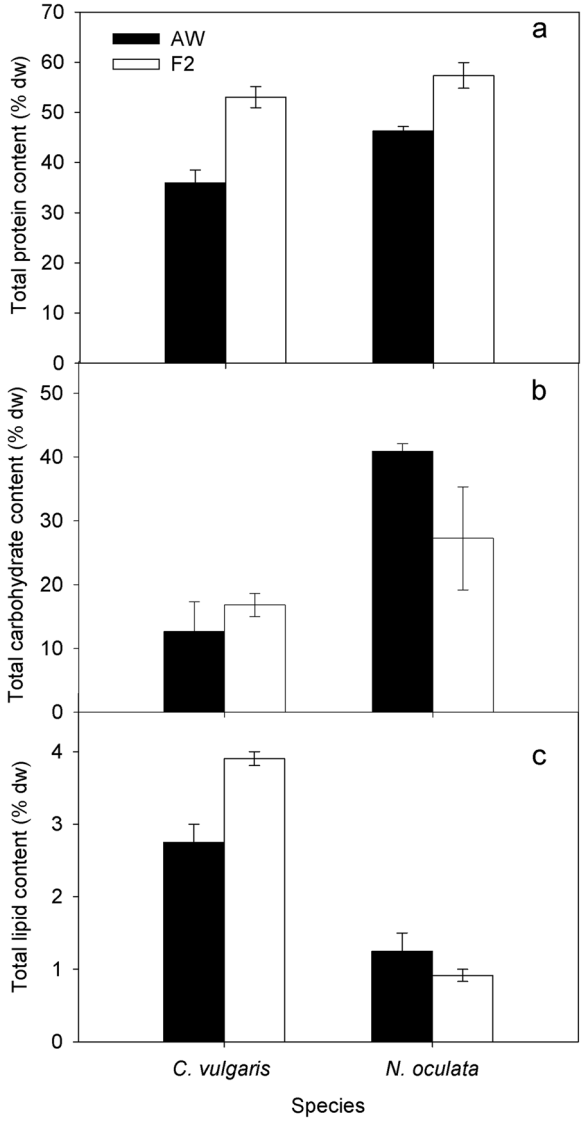

Fig. 5 Total content of (**a**) protein, (**b**) carbohydrate and (**c**) lipid (% dw) in *Chlorella vulgaris* (*C. vulgaris*) and *Nannochloropsis oculata* (*N. oculata*) cultured in synthetic aquaculture wastewater (AW) and F/2 media (F2). Data are presented as means ± standard errors

Carbohydrate content of *Chlorella vulgaris* and *Nannochloropsis oculata* cultured in aquaculture wastewater and F/2 media

The carbohydrate content of *Chlorella vulgaris* (C. vulgaris) and *Nannochloropsis oculata* (N. oculata) cultured in synthetic aquaculture wastewater (AW) and F/2 media (F2) are shown in Fig. 5b. Among the treatments, *N. oculata*cultured in AW exhibited the highest carbohydrate content at 40.9 ± 1.82% dw, followed by *N. oculata* in F2 media, which had a carbohydrate content of 27.3 ± 8.06% dw. In contrast, *C. vulgaris* showed significantly lower ($p < 0.05$) carbohydrate levels than *N. oculata*, with 16.8 ± 5.03% dw in F2 media and 12.7 ± 4.62% dw in AW. Overall, *N. oculata* had a significantly higher carbohydrate content ($p < 0.05$) compared to *C. vulgaris* under both culture media. However, for each species, there was no significant difference ($p > 0.05$) in carbohydrate content between the aquaculture wastewater (AW) and F/2 treatments.

Lipid content of *Chlorella vulgaris* and *Nannochloropsis oculata* cultured in aquaculture wastewater and F/2 media

The total lipid content of *C. vulgaris* and *N. oculata* cultured in synthetic aquaculture wastewater (AW) and F/2 media (F2) is as shown in Fig. 5c. Both *C. vulgaris* and *N. oculata* accumulated lipids, with *C. vulgaris* obtained the higher ($p < 0.05$) lipid levels compared to *N. oculata*. Specifically, *C. vulgaris* cultured in F2 media (C-F2) achieved the highest lipid content at 3.90 ± 0.10% dw, followed by *C. vulgaris* in AW (C-AW) with a lipid content of 2.75 ± 0.25% dw. In contrast, *N. oculata* exhibited significantly lower lipid accumulation, with 1.25 ± 0.25% dw in AW (N-AW) and 0.92 ± 0.08% dw in F2 media (N-F2). Overall, *C. vulgaris* had significantly higher ($p < 0.05$) lipid content compared to *N. oculata* in both AW and F2 media.

Discussion

Effect of synthetic aquaculture wastewater and F/2 media on growth of *Chlorella vulgaris* and *Nannochloropsis oculata*

The present study evaluated the growth dynamics of *Chlorella vulgaris* (*C. vulgaris*) and *Nannochloropsis oculata* (*N. oculata*) in synthetic aquaculture wastewater (AW), using F/2 media (F2) as a control. Growth parameters, including optical density (OD_{750}) and cell density, were monitored, showing an overall increasing trend in both OD_{750} and cell counts across all treatments.

These finding confirm the successful proliferation of both microalgal species in both AW and F2 media, highlighting their adaptability to diverse nutrient environments.

During the initial phase (Days 0 to 4), both species exhibited a lag phase characterized by minimal changes in OD_{750} and cell count, a common occurrence as cells acclimatize to new environments. Following this period, both species entered an exponential growth phase from Days 4 onwards. Notably, *C. vulgaris* cultured in F2 media achieved the highest optical density, suggesting better biomass accumulation in this medium. This is likely due to the nutrient composition of F2 media, as detailed in Table 1, which has been optimized to support robust microalgal growth by providing ideal levels of nitrogen sources, trace metals, minerals, and vitamins. In contrast, *N. oculata* cultured in AW recorded the highest cell density, despite lower optical density readings. This discrepancy suggests that optical density may not fully capture the growth dynamics of *N. oculata* in AW especially, potentially due to factors such as cell size, morphology, and the presence of extracellular materials that can easily affect light scattering and absorption. These results indicate that while optical density is a useful proxy for biomass estimation, it should be complemented with cell density or other methods, such as determination of dry weight or chlorophyll content, for more accurate growth assessments, particularly in wastewater media [25].

Meanwhile, the specific growth rate (SGR) analysis also revealed obvious differences in growth responses of both microalgae species in AW and F2 media. *N. oculata* exhibited the highest SGR in AW (0.409 ± 0.018 day^{-1}), indicating that the nutrient composition of AW may be particularly suited to the metabolic requirements of this species. This observation is consistent with previous studies, such as [26], where *Nannochloropsis* species showed enhanced growth in wastewater environments. In addition, the presence of ammonium as the main nitrogen source in AW could provide *N. oculata* with a competitive advantage, as this species has been found to prefer ammonium with faster uptake rate and growth than nitrate, even when both compounds were available to the microalgae [27]. In contrast, while *C. vulgaris* can adapt to and utilize nutrients in AW, it performs better with optimal nutrient media, as indicated by the higher SGR observed in F2 culture. Therefore, the differential growth responses observed in this study have important implications for the application of these microalgae in aquaculture wastewater remediation and bioresource production. Particularly, *N. oculata* robust growth in AW highlights its potential for bioremediation in aquaculture systems, where it can effectively utilize nutrients from wastewater for growth.

Nutrient removal efficiency

To effectively utilize microalgae for nutrient removal in aquaculture, a thorough assessment of their growth and nitrogen removal efficiency is crucial, as responses can be species-specific. In aquaculture environments especially, the forms of nitrogen present—primarily ammonia and nitrate—play critical roles in water quality management. Ammonia, even at relatively low concentrations (> 0.5 mg/L), is toxic to most aquatic organisms and can lead to fish mortality if not adequately controlled, while nitrate, though less harmful (if less than 10 mg/L), still requires careful management [28]. Generally, removal efficiencies of more than 50% indicate effective nutrient removal, regardless of the algal species involved [29].

The present study found significant variations in Total Ammonia Nitrogen (TAN) concentration and removal efficiency between *C. vulgaris* and *N. oculata* in AW and F2. In F2 media, *C. vulgaris* consistently showed higher TAN removal efficiency compared to *N. oculata*. Specifically, in AW treatments, *C. vulgaris* achieved a high removal efficiency of $83.7 \pm 0.42\%$. These suggest that *C. vulgaris* has better TAN removal capabilities, potentially due to its greater tolerance to ammonium nitrogen and its efficient uptake of ammonium as a primary nitrogen source for growth. For many microalgae, ammonium is often the preferred nitrogen form because it requires less energy for assimilation compared to nitrate. This is because it enters microalgal cells through specific transporters and is immediately incorporated into amino acids via the glutamine synthetase-glutamate synthase (GS-GOGAT) pathway, making it a highly efficient nitrogen source for growth [30]. Meanwhile, despite *N. oculata* achieving higher growth, its TAN removal efficiency ($71.3 \pm 1.50\%$) was still slightly lower than that of *C. vulgaris*. This may be due to the differences in cell size and nutrient uptake mechanisms. Larger cells, like those of *C. vulgaris*, have a greater surface area-to-volume ratio, which may enhance their nutrient uptake capacity [31]. As the uptake of nutrients in microalgae cells is usually facilitated through active transport mechanisms across the cell membrane, involving specific transporters or channels [32].

In addition, another critical factor influencing the nutrient removal efficiency of microalgae is the nitrogen-to-phosphorus (N/P) ratio in the culture medium. Different microalgal species and strains usually prefer varying optimal N/P ratios, which can significantly affect their ability to simultaneously assimilate nitrogen and phosphorus for biomass growth. Thus, when the N/P ratio is not optimal, nutrient limitation or excess can occur, leading to reduced growth rates and nutrient uptake efficiency [32, 33]. High ammonium concentrations can also inhibit microalgal physiological activity

by causing metabolic stress, which highlights the importance of maintaining balanced nutrient levels for optimal nutrient uptake and growth. For instance, Choi and Lee [34] reported that *C. vulgaris* could achieve ammonia-nitrogen removal efficiencies which varies from 3.59% to 99.61%, depending on the ammonium concentration. In the current study, *C. vulgaris* achieved a high removal efficiency of 83.7% in AW and 78.0% in F2. Meanwhile, *N. oculata* also demonstrated a higher ammonia nitrogen removal efficiency (71.3% to 72.3%) compared to the findings by [27], where the same species achieved 50% ammonia removal and 33.24% nitrate removal efficiency from F2 media. This also further emphasis on the preference of these microalgae in utilizing ammonia over nitrate as a nitrogen source. The ammonium preference over nitrate for most microalgae can be attributed to the lower energy cost of assimilation, as nitrate reduction to ammonium within the cell requires energy-intensive enzymatic reactions (e.g., nitrate reductase and nitrite reductase [35]. The present study further highlights the species- and strain-specific nature of nutrient removal in microalgae, emphasizing the importance of selecting microalgal species that are tailored to the specific nutrient composition of wastewater to optimize both nutrient removal efficiency and biomass production [36].

Furthermore, the nitrogen removal efficiencies from aquaculture wastewater achieved in this study, with 83.7% for *C. vulgaris* and 71.3% for *N. oculata* after just 7 days of culture, are comparable to values reported in the literature. For instance, *Chlorella sorokiniana*, *Scenedesmus obliquus*, and *Ankistrodesmus falcatus* have been shown to achieve ammonia removal efficiencies ranging from 86.45–98.21% after 14 days of culture [37]. Meanwhile, Esteves et al. (2022) [17] found that *C. vulgaris* required a minimum culture period of 11 days to exceed 90% nitrogen removal efficiency, with only around 50% of nitrogen removal by day 5. Thus, prolonging the culture duration for both *C. vulgaris* and *N. oculata* is likely to result in higher nitrogen removal efficiencies. Moreover, increasing the initial inoculum density of microalgal biomass can also enhance nutrient removal efficiencies in wastewater treatment [13].

Effect of synthetic aquaculture wastewater and F/2 media on nutritional content of *Chlorella vulgaris* and *Nannochloropsis oculata*

The nutrient removal capabilities of microalgae, coupled with their ability to synthesize key nutritional and bioactive compounds such as proteins, carbohydrates, and lipids, provide a promising approach for sustainable aquaculture wastewater treatment and the production of value-added products including food, feed, and biofuels [38]. The present study investigated the effect on protein, carbohydrate, and lipid content of *C. vulgaris* and *N. oculata* when cultivated in synthetic aquaculture wastewater (AW) compared to F/2 media. The findings indicate significant variations in nutritional content between both microalgae species, which have important implications for their application in aquaculture, particularly in sustainable aquafeed production. Despite these differences, both species demonstrated nutritional content that are adequate for their potential use in aquaculture, even when cultured in aquaculture wastewater.

In terms of protein content, *N. oculata* exhibited higher protein content compared to *C. vulgaris*, indicating its potential as a more suitable microalgal species for use in aquafeeds. This is because a high protein content in aquafeeds is usually desirable, as it enhances the nutritional value of aquaculture organisms across various growth stages [39]. Specifically, *C. vulgaris* demonstrated a protein content of 53% dw in F/2 media, consistent with the reported range of 51% to 58% for this species [40]. In contrast, *C. vulgaris* in AW had a reduced protein content of 35.9% dw. Similarly, Viegas et al., (2021) [41] also reported protein content or 31% for *C. vulgaris* and 35% for *Scenedesmus obliquus* cultured in brown crab aquaculture wastewater. Although this represents a decrease, the protein content remains substantial and can still be considered adequate for aquaculture use. For aquafeed, including live feed, formulated feed, or feed additives, protein content of microalgae is generally targeted to be above 30% to ensures optimal nutritional value for aquatic species [42]. The nitrogen limitation in AW culture likely impairs the protein synthesis because it is crucial for amino acid production and overall cellular function in microalgae [43]. Typically, when nitrogen is scarce, microalgae adapt by redirecting resources toward carbohydrate production, often resulting in a decrease in protein content [44]. Furthermore, *N. oculata* also demonstrated a higher protein content of 57.4% dw in F2, indicating optimal nutrient conditions. Although its protein content decreased slightly to 46.3% dw in synthetic aquaculture wastewater (AW), it remains within the desirable range for aquafeed, making *N. oculata* a viable option for aquaculture use. The protein content of *N. oculata* in this study is also higher than most previously reported value in previous research using aquaculture wastewater. For instance, Bhatti et al. (2023) [45] assessed 37 different wastewaters as culture media for *Chlorella sorokiniana* and *Scenedesmus* sp., reporting protein levels of only 41.0% to 42.1%. Additionally, Ding et al. (2024) [46] reported that co-cultivated *Chlorella* sp. and *Phaeodactylum tricornutum* in aquaculture wastewater resulted in biomass containing only 37.11% protein. In another study, *Chlorella vulgaris* cultivated in trout farm wastewater exhibited a reduced protein content of only

17.93% [17]. Ansari et al. (2016) [37] also reported protein levels ranging from only 19% to 36% for *Scenedesmus obliquus*, *Chlorella sorokiniana*, and *Ankistrodesmus falcatus* grown in Nile tilapia aquaculture wastewater. Overall, the variation of nutritional content in response of *C. vulgaris* and *N. oculata* to AW and F2 media in the current study highlights the adaptability of both microalgae to different nutrient environments, demonstrating their capacity to produce adequate protein levels even when cultivated in aquaculture wastewater. The high protein content observed in *N. oculata* also indicates its suitability for aquafeeds, particularly in applications where high protein content is desired [39].

In general, the nutritional composition in microalgae can vary widely, ranging from 4 to 64% depending on the species. Specifically, for *Chlorella* sp., the innate carbohydrate content typically ranges from 12 to 17% [44]. In this study, *C. vulgaris* cultured in AW had a carbohydrate content of 12.7% dw, while those in F2 had a content of 16.8% dw, both within the expected range for the species. Meanwhile, *N. oculata* cultured in AW produced high carbohydrate content of 40.9% dw, which was the highest recorded among all treatments. This is particularly notable as carbohydrate content in microalgae intended for aquaculture use often targets more than 10% to ensure adequate energy provision for aquatic species [47]. This is comparable to other related studies on various microalgae using aquaculture wastewater which reported carbohydrate content ranging from 19 – 70%, depending on species [15, 17, 37, 41, 46, 48]. In contrast, the carbohydrate content of *N. oculata* grown in F/2 media was lower than *C. vulgaris* but still substantial at 27.3% dw. The obvious difference in carbohydrate levels between the media types suggests that *N. oculata* may respond to nutrient stress differently than *C. vulgaris*, by significantly increasing carbohydrate accumulation under nitrogen-limited conditions (AW). In general, nitrogen is a critical element in protein synthesis and overall cellular function, and its limitation often leads microalgae to reallocate resources away from protein and lipid synthesis towards carbohydrate storage. This shift occurs because excess carbon and electrons are channelled into carbohydrate production when nitrogen is insufficient for protein and polar lipid synthesis [11]. These stress responses indicate how microalgae adjust their metabolic pathways to adapt to different environmental conditions, though this may vary between species.

In addition to other nutritional components, lipid content can also vary significantly between species and strains, with lipid accumulation potential also influenced by culture conditions. In this study, *Chlorella* sp. had a lipid content of 2.72% dw in AW and with slightly higher lipid accumulation observed in the nutrient-rich F2 (3.90% dw). Meanwhile, for *N. oculata* the lipid content differences between AW (1.25% dw) and F2 (0.92% dw) were minimal. Typically, lipid accumulation in microalgae is known to increase under nutrient stress, such as nitrogen or phosphorus limitation, as a survival strategy where excess carbon is stored in the form of lipids [49]. However, in this study, the observed trend was the opposite especially for *C. vulgaris*, indicating that factors beyond nutrient stress, such as strain-specific characteristics, may have influenced the lipid production. This further emphasis the complexity of lipid metabolism in microalgae and further research is still needed to fully understand the interplay between strain properties and wastewater media in lipid accumulation. Nevertheless, the ability of *N. oculata* and *C. vulgaris* to grow well and accumulate high levels of protein and carbohydrates, even when cultured in aquaculture wastewater, highlights its potential for wastewater remediation for production of aquafeed, biofuels, and other applications.

Conclusions

This present finding highlights the potential of microalgae, particularly *Chlorella vulgaris* and *Nannochloropsis oculata*, for aquaculture wastewater remediation and nutrient-rich aquafeed production. Both microalgae species demonstrated significant adaptability to the synthetic aquaculture wastewater, with *N. oculata* achieving high growth, nutrient removal efficiency, protein, and carbohydrate content. This robustness indicates the suitability of *N. oculata* for utilizing aquaculture wastewater as a culture medium to produce aquafeeds where high protein and carbohydrate content is essential. Although *C. vulgaris* exhibited lower protein and carbohydrate content in aquaculture wastewater, it still fell within acceptable ranges for aquafeed. Overall, our findings suggest that both *C. vulgaris* and *N. oculata* are viable for potential integration into aquaculture systems for a cost-effective wastewater remediation and aquafeed production. While further research is needed to assess the integration into practical aquaculture systems, the current findings provide valuable insights for advancing towards a more sustainable aquaculture industry.

Authors' contributions

Ryan Wong Lieng Song and Norazira Abdu Rahman conceived and designed the experiments, collected and analyzed the data, prepared the manuscript, and participated in its assembly and editing. Yeap Swee Keong, Fatimah Md. Yusoff contributed to technical support, data interpretation, and manuscript assembly. Tan Jian Ping provided technical support and assisted with manuscript preparation.

Funding

This study was funded by the Malaysia Higher Education Ministry's Fundamental Research Grant Scheme (FRGS) (Project number: FRGS/1/2023/

WAB04/XMU/03/1), and the Xiamen University Malaysia Research Fund (XMUMRF/2022-C10/ICAM/0010).

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All the authors agree to the submission.

Competing interests
The authors declare no competing interests.

Received: 26 August 2024 Accepted: 10 October 2024
Published online: 09 December 2024

References

1. Abdel-Raouf N, Al-Homaidan AA, Ibraheem IBM. Microalgae and wastewater treatment. Saudi J Biol Sci. 2012;19(3):257–75. https://doi.org/10.1016/j.sjbs.2012.04.005.
2. Khatoon H, PenzPenz K, Banerjee S, Redwanur Rahman M, Mahmud Minhaz T, Islam Z, Ara Mukta F, Nayma Z, Sultana R, Islam Amira K. Immobilized Tetraselmis sp. for reducing nitrogenous and phosphorous compounds from aquaculture wastewater. Bioresour Technol. 2021;338:125529. https://doi.org/10.1016/j.biortech.2021.125529.
3. Kawasaki N, Kushairi MRM, Nagao N, Yusoff F, Imai A, Kohzu A. Release of nitrogen and phosphorus from aquaculture farms to Selangor River, Malaysia. Int J Environ Sci Dev. 2016;7(2):120–5.
4. Ashour M, Alprol AE, Heneash AMM, Saleh H, Abualnaja KM, Alhashmi-alameer D, Mansour AT. Ammonia bioremediation from aquaculture wastewater effluents using *Arthrospira platensis* niof17/003: Impact of biodiesel residue and potential of ammonia-loaded biomass as rotifer feed. Materials. 2021;14(18):5460. https://doi.org/10.3390/ma14185460.
5. Tom AP, Jayakumar JS, Biju M, Somarajan J, Ibrahim MA. Aquaculture wastewater treatment technologies and their sustainability: a review. Energy Nexus. 2021;4:100022. https://doi.org/10.1016/j.nexus.2021.100022.
6. Ramírez Mérida LG, Rodríguez Padrón RA. Application of microalgae in wastewater: opportunity for sustainable development. Front Environ Sci. 2023;11. https://doi.org/10.3389/fenvs.2023.1238640.
7. Kim G, Mujtaba G, Lee K. Effects of nitrogen sources on cell growth and biochemical composition of marine chlorophyte Tetraselmis sp. for lipid production. Algae. 2016;31(3):257–66. https://doi.org/10.4490/algae.2016.31.8.18.
8. Mojiri A, Baharlooeian M, Zahed MA. The potential of *Chaetoceros muelleri* in bioremediation of antibiotics: performance and optimization. Int J Environ Res Public Health. 2021;18(3):1–13. https://doi.org/10.3390/ijerph18030977.
9. Salbitani G, Carfagna S. Ammonium utilization in microalgae: a sustainable method for wastewater treatment. Sustainability. 2021;13:956. https://doi.org/10.3390/su13020956.
10. Kube M, Mohseni A, Fan L, Roddick F. Energy and nutrient recovery by treating wastewater with fluidised-beds of immobilised algae. J Water Process Eng. 2020;38:101585. https://doi.org/10.1016/j.jwpe.2020.101585.
11. Morales-Plasencia E, Ibarra-Castro L, Martínez-Brown JM, Nieves-Soto M, Bermúdez-Lizárraga JF, Rojo-Cebreros AH. The effect of nitrogen limitation on carbohydrates and β-glucan accumulation in *Nannochloropsis oculata*. Algal Res. 2023;72:103125. https://doi.org/10.1016/j.algal.2023.103125.
12. Zheng J, Hao J, Wang B, Shui C. Bioremediation of aquaculture wastewater by microalgae *Isochrysis zhanjiangensis* and production of the biomass material. Key Eng Mater. 2011;460–461:491–5. https://doi.org/10.4028/www.scientific.net/KEM.460-461.491.
13. Nasir NM, Jusoh A, Harun R, Ibrahim NNLN, Rasit N, Ghani WAWAK, Kurniawan SB. Nutrient consumption of green microalgae, Chlorella sp, during the bioremediation of shrimp aquaculture wastewater. Algal Res. 2023;72:103110. https://doi.org/10.1016/j.algal.2023.103110.
14. Kashem AH, Das P, AbdulQuadir M, Khan S, Thaher MI, Alghasal G, Hawari AH, Al-Jabri H. Microalgal bioremediation of brackish aquaculture wastewater. Sci Total Environ. 2023;873:162384. https://doi.org/10.1016/j.scitotenv.2023.162384.
15. Guldhe A, Ansari FA, Singh P, et al. Heterotrophic cultivation of microalgae using aquaculture wastewater: a biorefinery concept for biomass production and nutrient remediation. Ecol Eng. 2017;99:47–53. https://doi.org/10.1016/j.ecoleng.2016.11.013.
16. He Y, Lian J, Wang L, Tan L, Khan F, Li Y, Wang H, Rebours C, Han D, Hu Q. Recovery of nutrients from aquaculture wastewater: effects of light quality on the growth, biochemical composition, and nutrient removal of Chlorella sorokiniana. Algal Res. 2023;69:102965. https://doi.org/10.1016/j.algal.2022.102965.
17. Esteves AF, Soares SM, Salgado EM, Boaventura RAR, Pires JCM. Microalgal growth in aquaculture effluent: coupling biomass valorisation with nutrients removal. Appl Sci. 2022;12:12608. https://doi.org/10.3390/app122412608.
18. Guillard RRL. Culture of phytoplankton for feeding marine invertebrates. In: Smith WL, Chanley MH, editors. Culture of Marine Invertebrate Animals. Plenum Press; 1975. p. 26-60. https://doi.org/10.1007/978-1-4615-8714-9_3.
19. Hambali N, Mohd Yusof AA, Sabria NH, Abdol Jani WNF, Abdullah M. An in-depth review of the critical water analysis parameters and water quality management technology in cage aquaculture within Malaysian coastal regions. J Kejuruteraan. 2024;36(3):861–75. https://doi.org/10.17576/jkukm-2024-36(3)-03.
20. Parsons TR, Maita Y, Lalli CM. A manual of chemical and biological methods for seawater analysis. Oxford: Pergamon Press; 1984. p. 173. https://doi.org/10.25607/OBP-1830.
21. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193:265–75.
22. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Anal Chem. 1956;28:350–6.
23. Marsh JB, Weinstein DB. Simple charring method for determination of lipids. J Lipid Res. 1966;7(4):574–6.
24. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. J Biochem Physiol. 1959;37:911–7.
25. Thiviyanathan VA, Ker PJ, Amin EPP, Tang SGH, Yee W, Jamaludin MZ. Quantifying microalgae growth by the optical detection of glucose in the NIR waveband. Molecules. 2023;28(3):1318. https://doi.org/10.3390/molecules28031318.
26. Shaari AL, Sa SNC, Surif M, Zolkarnain N, Ghazali R. Growth of marine microalgae in landfill leachate and their ability as pollutants removal. Trop Life Sci Res. 2021;32(2):133–46. https://doi.org/10.21315/tlsr2021.32.2.9.
27. Hii YS, Soo CL, Chuah TS, Ambak M, Abol-Munafi A. Interactive effect of ammonia and nitrate on the nitrogen uptake by *Nannochloropsis sp*. J Sustain Sci Manage. 2011;6:60–8.
28. Boyd CE, Tucker CS. Handbook for aquaculture water quality. Auburn: C.E. Boyd & Associates, Inc.; 2015. p. 439.
29. Borg-Stoveland S, Draganovic V, Spilling K, Gabrielsen TM. Successful growth of coastal marine microalgae in wastewater from a salmon recirculating aquaculture system. J Appl Phycol. 2024. https://doi.org/10.1007/s10811-024-03310-1.
30. Wang J, Zhou W, Chen H, Zhan J, He C, Wang Q. Ammonium nitrogen tolerant *Chlorella* strain screening and its damaging effects on photosynthesis. Front Microbiol. 2019;10:3250. https://doi.org/10.3389/fmicb.2018.03250.
31. Marañón E. Phytoplankton size structure. In: Encyclopedia of Ocean Sciences. Elsevier; 2019. p. 599-605. https://doi.org/10.1016/B978-0-12-409548-9.11405-8.
32. Kumar A, Bera S. Revisiting nitrogen utilization in algae: a review on the process of regulation and assimilation. Bioresour Technol Rep. 2020;12:100584. https://doi.org/10.1016/j.biteb.2020.100584.
33. Nguyen LN, Aditya L, Vu HP, Johir AH, Bennar L, Ralph P, Hoang NB, Zdarta J, Nghiem LD. Nutrient removal by algae-based wastewater

treatment. Curr Pollut Rep. 2022;8(4):369–83. https://doi.org/10.1007/s40726-022-00230-x.
34. Choi HJ, Lee SM. Performance of *Chlorella vulgaris* for the removal of ammonia-nitrogen from wastewater. Environ Eng Res. 2013;18(4):235–9. https://doi.org/10.4491/eer.2013.18.4.235.
35. Lachmann SC, Mettler-Altmann T, Wacker A, Spijkerman E. Nitrate or ammonium: Influences of nitrogen source on the physiology of a green alga. Ecol Evol. 2019;9(3):1070–82. https://doi.org/10.1002/ece3.4790.
36. Lv J, Wang X, Feng J, Liu Q, Nan F, Jiao X, Xie S. Comparison of growth characteristics and nitrogen removal capacity of five species of green algae. J Appl Phycol. 2019;31(1):409–21. https://doi.org/10.1007/s10811-018-1542-y.
37. Ansari FA, Singh P, Guldhe A, Bux F. Microalgal cultivation using aquaculture wastewater: Integrated biomass generation and nutrient remediation. Algal Res. 2017;21:169–77. https://doi.org/10.1016/j.algal.2016.11.015.
38. Yirgu Z, Leta S, Hussen A, Khan MM. Nutrient removal and carbohydrate production potential of indigenous Scenedesmus sp. grown in anaerobically digested brewery wastewater. Environ Syst Res. 2020;9:40. https://doi.org/10.1186/s40068-020-00201-5.
39. Hulatt CJ, Wijffels RH, Bolla S, Kiron V. Production of fatty acids and protein by *Nannochloropsis* in flat-plate photobioreactors. PLoS One. 2017;12(1). https://doi.org/10.1371/journal.pone.0170440.
40. Wang Y, Tibbetts SM, McGinn PJ. Microalgae as sources of high-quality protein for human food and protein supplements. Foods. 2021;10:3002. https://doi.org/10.3390/foods10123002.
41. Viegas C, Gouveia L, Gonçalves M. Aquaculture wastewater treatment through microalgae: biomass potential applications on animal feed, agriculture, and energy. J Environ Manag. 2021;286:112187. https://doi.org/10.1016/j.jenvman.2021.112187.
42. Guedes A, Malcata FX. Nutritional value and uses of microalgae in aquaculture. In: Aquaculture. InTech; 2012. p. 1516. https://doi.org/10.5772/30576.
43. Michelon W, Da Silva MLB, Mezzari MP, Pirolli M, Prandini JM, Soares HM. Effects of nitrogen and phosphorus on biochemical composition of microalgae polyculture harvested from phycoremediation of piggery wastewater digestate. Appl Biochem Biotechnol. 2016;178:1407–19. https://doi.org/10.1007/s12010-015-1955-x.
44. Merz CR, Arora N, Welch M, Lo E, Philippidis GP. Microalgal cultivation characteristics using commercially available air-cushion packaging material as a photobioreactor. Sci Rep. 2023;13(1):3792. https://doi.org/10.1038/s41598-023-30080-6.
45. Bhatti S, Richards R, Wall CL, MacPherson MJ, Stemmler K, Lalonde CG, Patelakis SJJ, Tibbetts SM, McGinn PJ. Phycoremediation and simultaneous production of protein-rich algal biomass from aquaculture and agriculture wastewaters. J Chem Technol Biotechnol. 2023. https://doi.org/10.1002/jctb.7409.
46. Ding W, Zhou X, He M, Jin W, Chen Y, Sun J. Pollutant removal and resource recovery of co-cultivated microalgae Chlorella sp. and Phaeodactylum tricornutum for marine aquaculture wastewater. J Water Process Eng. 2024;67:106182. https://doi.org/10.1016/j.jwpe.2024.106182.
47. Ahmad A, Hassan SW, Banat F. An overview of microalgae biomass as a sustainable aquaculture feed ingredient: food security and circular economy. Bioengineered. 2022;13(4):9521–47. https://doi.org/10.1080/21655979.2022.2061148.
48. Cardoso LG, Duarte JH, Andrade BB, Lemos PVF, Costa JAV, Druzian JI, Chinalia FA. Spirulina sp. LEB 18 cultivation in outdoor pilot scale using aquaculture wastewater: High biomass, carotenoid, lipid, and carbohydrate production. Aquaculture. 2020;525:735272. https://doi.org/10.1016/j.aquaculture.2020.735272.
49. Maltsev Y, Kulikovskiy M, Maltseva S. Nitrogen and phosphorus stress as a tool to induce lipid production in microalgae. Microb Cell Fact. 2023;22:239. https://doi.org/10.1186/s12934-023-02244-6.