



Re-use of aquaculture wastewater in cultivating microalgae as live feed for aquaculture organisms

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ABSTRACT

Culturing microalgae using commercial media is expensive. Proliferation of aquaculture is generating high amount of wastewater containing nitrogen and phosphorus and this could be a source of nutrient for cultivating microalgae thereby reducing the production costs. This study compared the growth, productivity, and proximate composition of *Chaetoceros calcitrans*, *Nannochloris maculate*, and *Tetraselmis chunii* cultured in aquaculture wastewater and Conway medium. Results indicated that selected microalgae cultivated in wastewater and Conway medium did not show any significant differences ($p > 0.05$) in terms of cell density, optical density, and biomass. Further, volumetric and areal productivity showed similar trend for all the three species. But lipid productivity (LP) in *N. maculate* was significantly higher ($p < 0.05$) when cultured in wastewater medium compared to the Conway medium. There were no significant differences ($p > 0.05$) for *C. calcitrans* and *T. chunii* in terms of LP when cultivated in the two media. *N. maculate* and *T. chunii* had significantly higher ($p < 0.05$) protein and lipid content compared to *C. calcitrans* when cultured in wastewater medium. The three species did not show any significant differences ($p > 0.05$) in terms of carbohydrate content when cultured either in wastewater or Conway medium. The results indicate that aquaculture wastewater can be re-used as a possible source of low-cost nutrient for culturing selected microalgae for live feed utilization in aquaculture.

Keywords: Wastewater; Microalgae; Proximate composition, productivity; Live feed

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1. Introduction

Aquaculture provides feed for 47% (51 million tons) of the global human fish consumption and its production is growing. Aquaculture output is set to increase by 60–100% over the next 20–30 years to keep up with the increasing per capita fish consumption and the population [1]. According to Timmons and Losordo [2], the production of 1.0 kg of penaeid shrimp requires about 20,000 L of water, which is ultimately released into the coastal environment. Effluent from production systems is typically high in waste nutrients such as nitrogen and phosphorus, total suspended solids, volatile suspended solids, biochemical oxygen demand, and chemical oxygen demand [3,4].

Nitrogen and phosphorus are considered as waste components of fish farming, causing serious environmental problems such as eutrophication [5]. Traditional wastewater treatment methods are mostly physical, chemical, and biological. Several researchers have sought to develop processes that remove nutrients, primarily nitrogen and phosphorus from wastewater by growing different types of microalgae. Bio-treatment with microalgae is particularly attractive because of their photosynthetic capabilities, converting solar energy into useful biomass, using the eutrophication causing nutrients such as nitrogen and phosphorus [6]. The nutrients, instead of being wasted, become feed for the microalgae, which in turn become either a feed or a fuel source. The resulting wastewater will be of quality and suitable for many industrial applications.

Microalgae are the primary food source for a large number of aquatic organisms and play a key role in aquaculture development. Products made from microalgae have excellent potential for commercialization since pure biomass has high market demand and value. Biomass has been used as live feed for larviculture, premix for feed supplement, pharmaceutical, nutraceutical, cosmeceutical, production of high health organisms, and enhancement of animal color.

Chaetoceros sp., *Nannochloropsis* sp. and *Tetraselmis* sp. are typical marine microalgae widely used as live feed in aquaculture industry due to their high nutritional quality and suitable for the growth and development of larvae and juveniles [7,8]. In addition, *Tetraselmis* sp. has ability to produce lipid that can be converted to biodiesel [9]. *Chaetoceros* sp. has also been used to feed *Acartia* sp. [10].

One of the major challenges of using wastewater for microalgae cultivation is the low or imbalanced nutrient profile of wastewater that leads to low biomass yield and poor quality [11]. In this study, tropical marine microalgae *Chaetoceros calcitrans*,

Nannochloris maculate and *Tetraselmis chuii* isolated from South China Sea were grown using aquaculture wastewater and compared with Conway medium to evaluate their growth and productivity. In addition, harvested microalgae were further analyzed for their nutritional quality.

2. Materials and methods

2.1. Wastewater collection

Wastewater was collected from aquaculture shrimp ponds located at Setiu Wetland, Terengganu, Malaysia. Prior to use, wastewater was filtered using a filter bag to eliminate macro particles, followed by glass microfiber filter (GFC, Whatman, pore size 1.2 μm). The wastewater was then treated with submerged UV for 48 h for eliminating the contamination by bacteria, protozoa, or other species. Contamination has been an issue with monospecific cultures of microalgae. The wastewater was then stored in a refrigerator ($4 \pm 2^\circ\text{C}$) for future use as a medium for growing *C. calcitrans*, *N. maculate* and *T. chuii*. The pH was measured using a pH meter (Orion, USA) and the nutrients in wastewater were analyzed by ion chromatography (882 Compact IC Plus, Metrohm, Switzerland).

2.2. Stock culture of microalgae

Tropical marine *C. calcitrans* (UMTAQT01), *N. maculate* (UMTAQT02) and *T. chuii* (UMTAQT03) were collected from the Institute of Tropical Aquaculture, Universiti Malaysia Terengganu. All the three species were isolated from the South China Sea. The cultures were grown under controlled conditions using artificial light ($50 \mu\text{mol}/\text{m}^2/\text{s}$) at 25°C in Conway medium [12] (Table 1). Cultures were later inoculated in flask

Table 1
Chemical composition of Conway medium

Conway Medium (Tompkins et al. 1995)	
Nitrate	KNO_3 (100 g m^{-3})
Phosphate	Na_3PO_4 (20 g m^{-3})
Trace metal	$\text{Na}_2\text{H}_2\text{EDTA}\cdot 2\text{H}_2\text{O}$ (45 g m^{-3})
	$\text{FeCl}_3\cdot 6\text{H}_2\text{O}$ (1.3 g m^{-3})
	ZnCl_2 (4.2 g m^{-3})
	$\text{MnCl}_2\cdot 4\text{H}_2\text{O}$ (0.36 g m^{-3})
	$\text{CoCl}_2\cdot 6\text{H}_2\text{O}$ (4.0 g m^{-3})
	$\text{CuSO}_4\cdot 5\text{H}_2\text{O}$ (4.0 g m^{-3})
	$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ (1.8 g m^{-3})
	H_3BO_3 (33.4 g m^{-3})
Vitamin	Thiamin HCl (200 mg m^{-3})
	Cyanocobalamin (10 mg m^{-3})

containing filtered and autoclaved wastewater and maintained at 25°C at a light intensity of 50 $\mu\text{mol}/\text{m}^2/\text{s}$. The *C. calcitrans*, *N. maculate*, and *Tetraselmis chuii* cultured using wastewater were used as the stock cultures for the experiments.

2.3. Experimental design

C. calcitrans, *N. maculate* and *T. chuii* were grown under laboratory conditions in 15 L closed photobioreactors using Conway medium and wastewater. Four replicates were prepared for all samples and the initial cell density was maintained at 1×10^3 cells/mL. Cultures were maintained at 25°C and with a light intensity of 50 $\mu\text{mol}/\text{m}^2/\text{s}$ using cool fluorescent lamps for a photoperiod of 24 h. Filtered air was provided to all the flasks throughout the experimental period to keep the microalgal cells in suspension. The cultures were then harvested using a continuous harvester (Hanil, Korea) when they reached the stationary phase for protein, lipid, and carbohydrate analyses (Figs. 1 and 2).

2.4. Analyses of physical and growth parameters

During the experimental period, physical parameters such as temperature and pH were measured every day both morning and evening. Microalgal growth was measured in triplicates to estimate the cell density, optical density (OD), and biomass. The cell density was determined by placing an aliquot of well-mixed culture suspension on a Neubauer haemocytometer (Assistant, Germany) under microscope.

The OD for all the cultures was determined daily using a spectrophotometer (UV-vis 1601, Shimadzu, Japan). The wavelengths used were 750 nm for *C. calcitrans*, 540 nm for *N. maculate* and 530 nm for *T. chuii* [13,14]. Biomass was estimated using 1 mL microalgal samples filtered through pre-combusted (100°C, 4 h) and pre-weighed glass fiber filters (Whatman, UK). After filtration, samples were rinsed with 1 ml of 0.5 M ammonium formate. The filters were dried at 100°C for 4 h, cooled in a desiccator and then weighed. The dry biomass concentration in the culture was calculated by dividing the difference between the weights of the dried filter paper (after and before filtration) by the filtered volume [14].

2.5. Proximate composition

Protein and carbohydrate were analyzed according to the methods of Lowry et al. [15] and Dubois et al.

[16], respectively, using 6 mg freeze-dried microalgal culture. Lipid was analyzed using 1 mL of fresh microalgal culture following the carbonization method of Marsh and Weinstein [17]. Tripalmitin was used as the standard.

2.6. Productivity

Volumetric productivity (VP) was calculated by $VP (\text{g}/\text{L}/\text{d}) = (X_n - X_0)/N$, where X_n = final biomass; X_0 = initial biomass and N = culture days. Areal productivity (AP) was calculated by $AP (\text{g}/\text{m}^2/\text{d}) = (VP \times V)/A$, where VP = volumetric productivity; V = total volume of the culture and A = surface area occupied on ground. Lipid productivity (LP) was calculated by $LP (\text{mg}/\text{L}/\text{d}) = VP \times (\% \text{ lipid}/100)$, where VP = volumetric productivity of the microalgae cultured and % lipid is lipid content.

2.7. Statistical analysis

The collected data were analyzed using one-way analysis of variance. All the data which were expressed in percentages were arcsine-transformed to satisfy the condition of homogeneity of variance [18]. Statistical analyses were accomplished using the Statistical Analysis System (SAS 9.2) computer software [19].

3. Results

3.1. Physicochemical analysis of wastewater

The physicochemical characteristic of the wastewater from the shrimp pond used in the present experiment as a medium to grow three different marine microalgae is shown in Table 2. It contained sufficient amount of nitrate, nitrite, ammonium, phosphate, potassium, sodium, and sulfate for culture of microalgae. Physical parameters such as pH, temperature, dissolved oxygen, and salinity were in the recommended range suitable for microalgae culture as shown in Table 2.

3.2. Growth

There were no significant differences ($p > 0.05$) in terms of cell density, OD, and biomass in *C. calcitrans*, *N. maculate*, and *T. chuii* when cultured either in Conway or wastewater media. Highest growth in terms of biomass was found on day 8 for the three species cultured in both media as shown in Figs. 3–5.

Table 2
Physicochemical characteristics of the shrimp pond wastewater. Values are means \pm standard errors ($n = 3$)

Properties	Value
<i>Physical Properties</i>	
pH	8.05 \pm 0.01
Temperature ($^{\circ}$ C)	24.01 \pm 0.02
Dissolved oxygen (mg/l)	5.20 \pm 0.02
Salinity (ppt)	24.17 \pm 0.03
<i>Chemical Properties (mg/l)</i>	
Calcium	5.40 \pm 0.12
Phosphate	7.20 \pm 0.03
Nitrate	3.47 \pm 0.02
Nitrite	3.80 \pm 0.03
Ammonium	6.12 \pm 0.01
Potassium	5.45 \pm 0.01
Sodium	14.25 \pm 0.01
Sulfate	5.05 \pm 0.02

3.3. Productivity

Referring to Fig. 6(a) and (b) and comparing the growth in both the media, it is seen that volumetric and AP were not significantly different ($p > 0.05$) for *C. calcitrans*, *N. maculate* and *T. chuii*. However, for *N. maculate*, LP was significantly higher ($p < 0.05$) when cultured in wastewater medium compared to the Conway medium. There were no significant differences ($p > 0.05$) between the two different media for *C. calcitrans* and *T. chuii* in terms of LP as shown in Fig. 6(c).

3.4. Proximate composition

Protein and lipid content in *N. maculate* and *T. chuii* were significantly higher ($p < 0.05$) when cultured in wastewater medium compared to Conway medium. For *C. calcitrans*, protein and lipid content did not show any significant difference ($p > 0.5$) as seen from Fig. 7(a) and (b). Also no significant differences ($p > 0.05$) were found in the carbohydrate content for all the three species cultured either in wastewater or Conway as seen in Fig. 7(c).

4. Discussion

Microalgae have become one of the main live foods for rotifers, cladocerans and other zooplanktons as well as larvae of fish and shrimp [20]. In addition, microalgae also play an important role in supplying energy nutrients, such as protein, lipid, carotene, polyunsaturated fatty acids, vitamins, amino acids, and minerals for the development of aquatic



Fig. 1. Microalgae cultured in photobioreactors.

organisms [21]. Growing microalgae using inorganic and commercial media is cost-intensive [22].

Microalgae have demonstrated capability to grow in industrial, municipal, agricultural, and aquaculture wastewaters [23,24]. Microalgae cultivated in wastewater can be economically viable and environmentally friendly. Sustainable production levels can be maintained and nutrients required for microalgae growth could be saved resulting in lesser operational costs. Microalgae utilize nutrients such as nitrogen and phosphorous derived from wastewater thus providing bioremediation and production of by-products and biofuels in a sustainable way [25,26]. In addition, Raja et al. [27] reported that microalgae can be used to treat industrial effluents to reduce the pollutants. With wastewater usage for microalgae, other advantages over traditional cultivation methods may be envisaged.

Indigenous microalgae have been selected for this study since they are already adapted with the local environment, and are not likely to become invasive in nature [28]. *C. calcitrans*, *N. maculate*, and *T. chuii* which are naturally found in South China Sea were selected for the same reason. In an earlier study by Khatoon et al. [29] it was shown that tropical algae such as *N. maculate* and *T. chuii* isolated from South China Sea can grow easily under laboratory and natural conditions. These species are widely used in aquaculture due to their rapid growth rate, resistance to adverse conditions, and high nutritional quality.

Chaetoceros sp. is considered as the most popular strain used in hatcheries, especially for shrimp larvae.



Fig. 2. Continuous harvester.

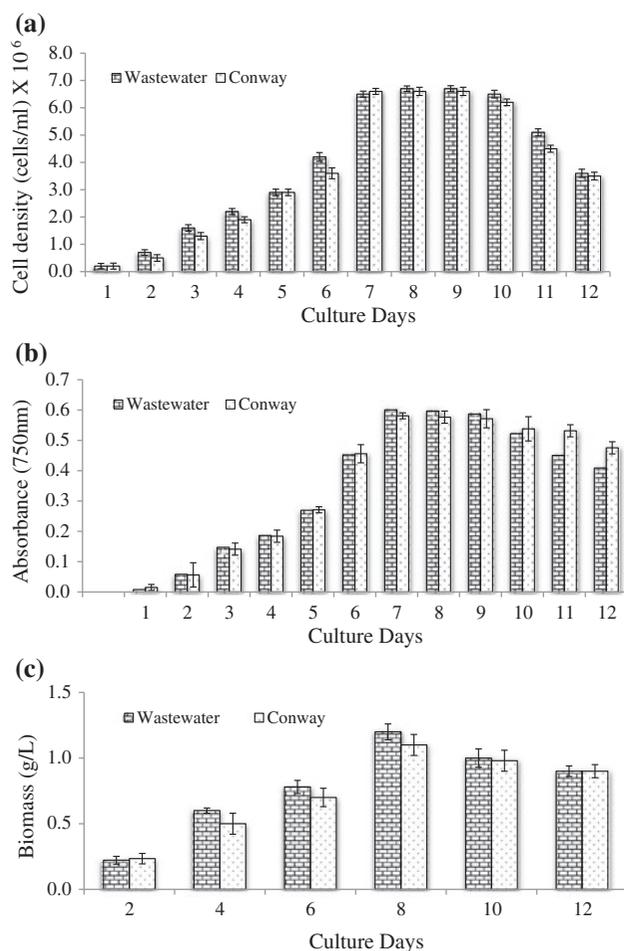


Fig. 3. Cell density (a), optical density (b), and biomass (c) of *C. calcitrans* cultured in different media. Values are mean \pm standard error ($n = 4$).

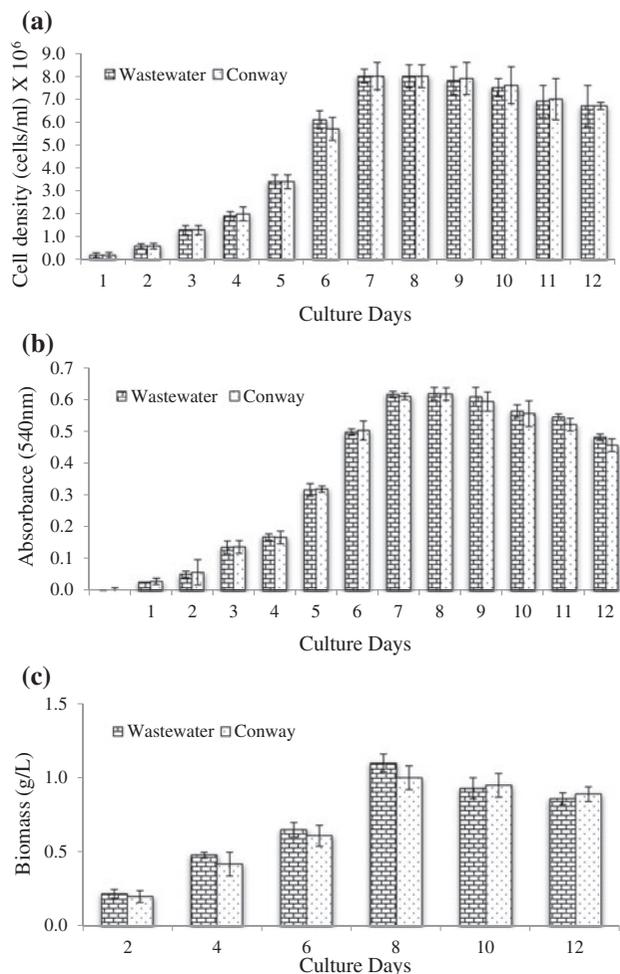


Fig. 4. Cell density (a), optical density (b), and biomass (c) of *N. maculate* cultured in different media. Values are mean \pm standard error ($n = 4$).

It has been shown to be an efficient diet for the protozoa of many panaeids [30] and is widely used in many hatcheries [8]. These species give vital energy and organic nutrients for the growth and development of larvae and juveniles [7]. Furthermore, most of the native green microalgae have been selected as good candidates for biodiesel production as well as live feed for zooplankton and crustacean [31].

The results clearly indicate that growth and production of *C. calcitrans*, *N. maculate*, and *T. chuii* in aquaculture wastewater was at par with Conway medium. This assures the availability of sufficient nutrients in aquaculture wastewater to support microalgal growth. In another study by Chopin et al. [32], similar growth trends were reported while culturing selected microalgae either in commercial medium or wastewater from fish and shrimp farm. Further, Sacristán de Alva et al. [33] had reported

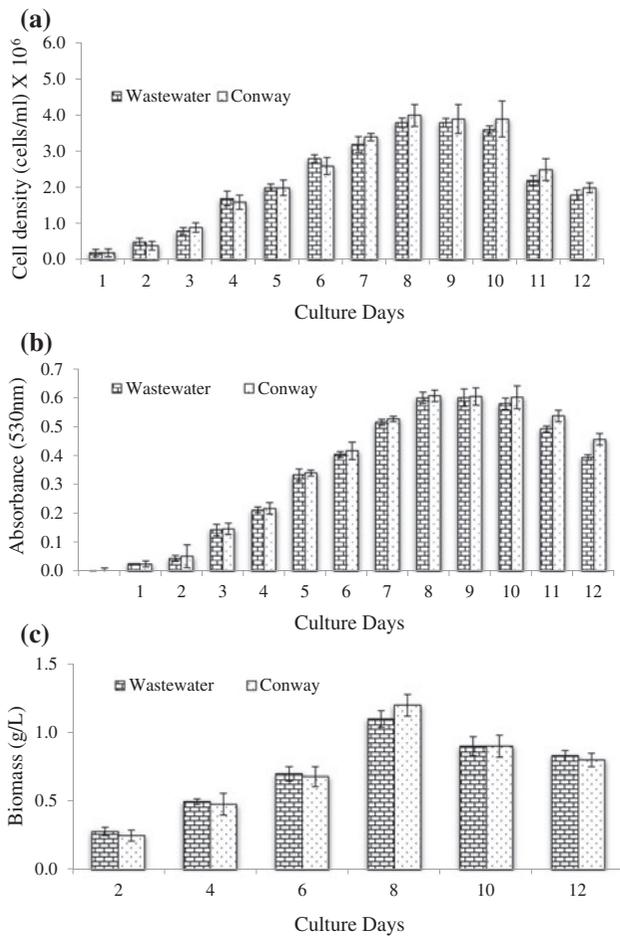


Fig. 5. Cell density (a), optical density (b), and biomass (c) of *T. chuii* cultured in different media. Values are mean \pm standard error ($n = 4$).

better biomass productivity in *Scenedesmus acutus* cultures using pre-treated wastewater instead of enriched medium. This again indicates the availability of adequate amount of nutrients and organic compounds. Wastewater employment can then have the same effects as a media similar to the commercial medium [34]. It may be concluded that wastewater can be a major source of nutrient for the culture of *C. calcitrans*, *N. maculate*, and *T. chuii*.

Major nutrients required to grow microalgae are nitrogen and phosphorous. Hillebrand and Sommer [35] recommended N and P are the major nutrients for algal growth. Singh et al. [36] found that composite wastewater from dairy, sago, and textile mills could be treated by acclimatized microalgal cultures such as *C. pyrenoidosa* and *C. vulgaris*. Aquaculture wastewater contain sufficient amount of nitrogen and phosphorous which can be used as a growth media to grow microalgae. This would facilitate the removal of

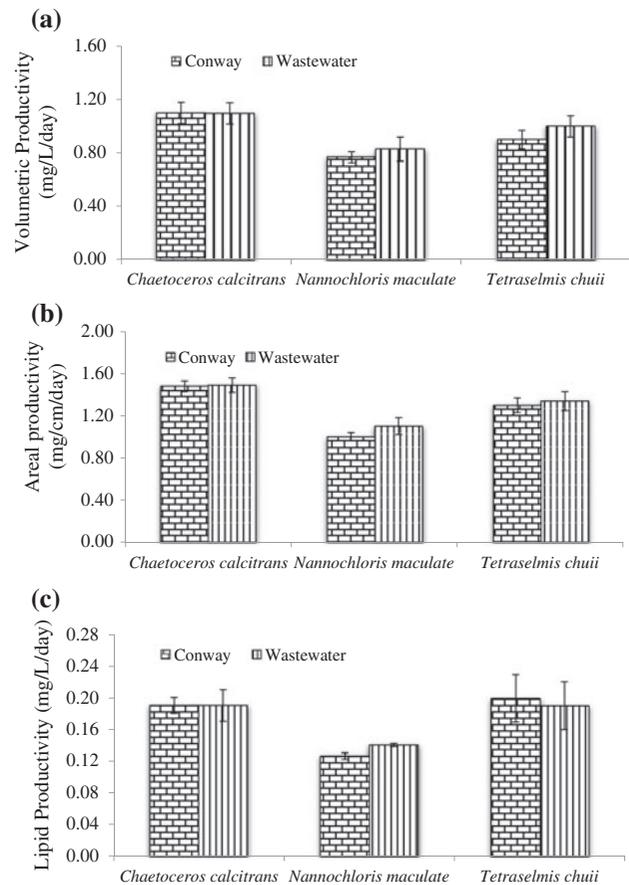


Fig. 6. Volumetric productivity (a), areal productivity (b), and lipid productivity (c) of *C. calcitrans*, *N. maculate*, and *T. chuii* cultured in different media. Values are mean \pm standard error ($n = 4$).

nutrients from wastewater and production of microalgal biomass concurrently [37].

Other parameters such as pH, temperature, dissolved oxygen, and salinity of wastewater for present study were found within the recommended range [14]. The total amount of nitrogen and phosphorus in the collected wastewater samples were sufficient and therefore supported good growth of *C. calcitrans*, *N. maculate*, and *T. chuii*. Nitrogen is an important element in the general metabolism of the species and the assimilation of nitrogen source is directly connected with photosynthesis [38]. Nitrogen was supplied as nitrates, nitrites, and ammonium salts by the wastewater medium and they were readily available to microalgae when present in the inorganic form. Besides that, phosphorus is another key element required for normal growth of microalgae. Phosphorus source was found naturally in the form of inorganic phosphate in the wastewater. In addition to nitrogen and phosphorus, other major ions such as, calcium,

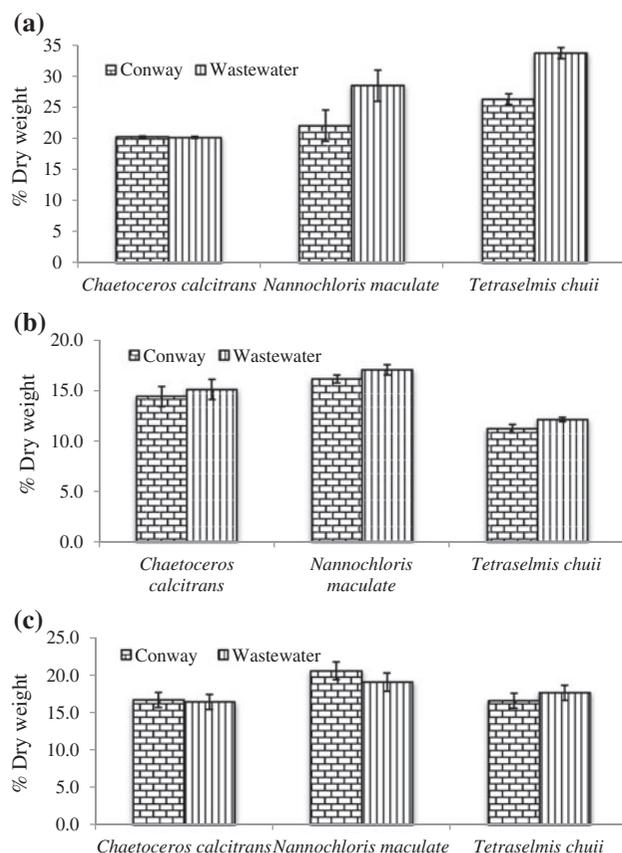


Fig. 7. Protein (a), lipid (b), and carbohydrate (c) content of *C. calcitrans*, *N. maculate* and *T. chuii* cultured in different media. Values are mean \pm standard error ($n = 4$).

sodium, and potassium were also present in the wastewater and these were found to be essential as an important constituent of living matter and act as cofactors for enzymatic reactions in microalgae [38].

Microalgae typically contain 30–40% protein, 10–20% lipid, and 5–15% carbohydrate [39,40]. The results indicated the ranges to be within this recommended range in the wastewater profiles [14]. These nutritional profiles for wastewater grown microalgae make them well suited as live feed for aquaculture organisms. Sacristán de Alva et al. [33] had reported better accumulation of lipids in *S. acutus* cultures using pre-treated wastewater instead of enriched medium.

5. Conclusion

The biomass cultivated from wastewater promises a wide range of applications. The results showed that the aquaculture wastewater could be used as an alternative source to cultivate microalgae (like *C. calcitrans*,

N. maculate, and *T. chuii*) under laboratory conditions. In this way, nutrients in the wastewater are utilized by algae otherwise, it would have been harmful to the environment and by growing algae in wastewater it reduces the medium costs and also saves the environment. Hence, more detailed study may improve or give a fruitful solution to utilize the aquaculture wastewater for mass production of microalgae.

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