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Sustainable Production of *Monoraphidium* Microalgae Biomass as a Source of Bioenergy

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Abstract: Microalgae are a renewable source of unconventional biomass with potential application in the production of various biofuels. The production of carbon-neutral fuels is necessary for protecting the environment. This work determined the possibility of producing biomass of microalgae belonging to *Monoraphidium* genus using saline wastewater resulting from proecological salmon farming in the recirculating aquaculture system. The tests were carried out in tubular photobioreactors using LED light. As a part of the analyses, the growth and productivity of microalgal biomass, cell density in culture, and lipid concentration and ash content in biomass were determined. In addition, the concentration of selected phosphorus and nitrogen forms present in wastewater corresponding to the degree of their use by microalgae as a nutrient substrate was determined. The biomass concentration estimated in the tests was 3.79 g·L^{-1}, while the maximum biomass productivity was 0.46 g·L^{-1}·d^{-1}. The cells' optical density in culture measured at 680 nm was 0.648. The lipid content in biomass was 18.53% (dry basis), and the ash content was 32.34%. It was found that microalgae of the genus *Monoraphidium* effectively used the nitrogen as well as phosphorus forms present in the wastewater for their growth. The total nitrogen content in the sewage decreased by 82.62%, and total phosphorus content by over 99%. The analysis of the individual forms of nitrogen showed that N-NO_3 was reduced by 85.37% and N-NO_2 by 78.43%, while orthophosphate (V) dissolved in water was reduced by 99%. However, the content of N-NH_4 in wastewater from the beginning till the end of the experiment remained <0.05 mg·L^{-1}.

Keywords: microalgae; bioenergy; biomass; lipids; aquaculture wastewater

1. Introduction

The rapid increase in population has resulted in a growing energy need, which is estimated to increase further by ≥50% by the year 2030 [1]. As conventional energy resources are shrinking, intensive research is underway not only exploring the technologies for using renewable energy resources but also analyzing the possibilities of reducing the costs of their use. In addition, the use of renewable energy can enable reducing air pollution and carbon dioxide emissions worldwide, thus maintaining the principles of sustainable development [2]. Although a large part of the world's energy demand is covered by conventional energy sources, including coal, oil, and natural gas, in recent years, competition for them has widely increased, and therefore, sources including wind, sun, water, and geothermal energy are being studied [3]. It is also expected that in the coming decades energy from biomass—bioenergy—will play an increasingly important role [4]. Bioenergy increases energy independence and reduces greenhouse gas (GHG) emissions [5]. The implementation of modern technologies will significantly increase its share in the energy mix to a level of 10^8 exajoules (EJ)

globally by 2030. Thus, bioenergy can contribute to 20% of the total supply of primary energy with 60% of final consumption of renewable energy. The global biomass potential is sufficient to fulfill the growing energy need, but the different biomass resources seem to be unevenly distributed [6]. Currently, only agricultural waste, forest products and waste, and energy plants [7] are used primarily to generate heat. Bioenergy potential is limited due to the fact that land is required first of all for producing food and feed, as well as for protecting the environment and climate [8]. Unlike traditional biomass, the use of modern biomass is definitely more beneficial. It is obtained in an environmentally, socially, and economically sustainable way [9] and enables the production of not only heat but also electricity and transport fuels [10]. Biofuels include those products obtained from biomasses as well as their residues. The properties of these fuels may allow their use in conventional engines as such, or after mixing with fossil fuels. Both first-generation and second-generation biofuels can be acquired from different feedstocks, which include food crops (e.g., sugar beet, sorghum, sugar cane), energy crops (e.g., lignocellulosic masses), and wastes (e.g., organic fraction derived from municipal solid waste, landfill leachate) [11].

Algae can be an alternative, modern, and sustainable source of biomass. They are used as food, feed, nutraceuticals, pharmaceuticals, and substrates for producing advanced third-generation fuels [12], including bioethanol [13], biomethane [14], and biohydrogen [15]. Due to the rapid growth and the higher lipid content in cells than oilseeds, microalgal biomass is considered as a potential substrate for producing biodiesel [16]. An important problem associated with using microalgae for biofuel production on an industrial scale is, however, the cost of their cultivation as well as the collection and dehydration of biomass [17,18]. Even though the yield of microalgae is higher, while the GHG footprint is lower compared to previous generations, microalgae require enormous nutrients and water for their production [19]. Freshwater and nutrients account for 50% of total production costs [20]. Different types of wastes generated by food production, industries, or municipal activities contain significant amounts of elements that can be used as raw materials for producing energy based on renewable energy sources [21], including municipal sewage sludge [22], sewage from industrial production [23], and sewage from animal production [24]. Municipal and industrial wastewaters are rich in nitrogen, phosphorus, and macro- and microelements. They are also a source of biogenic compounds for biomass, the development of which can contribute to wastewater treatment and thus have a positive impact on aquatic ecosystems [25–27]. Waste nutrients obtained from local industries are another valuable option that can act as an affordable and constant source of nutrients, and their use is associated with an added advantage of wastewater management. Furthermore, biomass production costs can be significantly reduced by connecting the biomass-producing system to industries that generate waste nutrients [28], energy, and water [29] which can support biomass growth. Additionally, the residues of microalgal biomass used for energy purposes have a high amount of proteins and carbohydrates, and hence can act, for example, as substrates for food production [12].

In the present study, we investigated whether (1) *Monoraphidium* can be grown using the saline aquaculture-derived wastewater and (2) how the uptake of nutrients from such wastewater can decrease the costs of fertilizer. In addition, we analyzed the algal biomass to determine its lipid accumulation efficiency for its potential application in biodiesel production and estimated its ash content.

2. Materials and Methods

2.1. Microalgae and Preculture Conditions

Microalgae were purchased from the Culture Collection of Baltic Algae (CCBA). *Monoraphidium* sp. (Figure 1) were precultured in the F/2 medium containing $NaNO_3$ (0.075 g), $NaH_2PO_4{\cdot}2H_2O$ (0.00565 g), trace element stock solution (1 $mL{\cdot}L^{-1}$; made of Na_2EDTA (4.16 g), $FeCl_3{\cdot}6H_2O$ (3.15 g), $CuSO_4{\cdot}5H_2O$ (0.01 g), $ZnSO_4{\cdot}7H_2O$ (0.022 g), $CoCl_2{\cdot}6H_2O$ (0.01 g), $MnCl_2{\cdot}4H_2O$ (0.18 g), and $NaMoO_4{\cdot}2H_2O$ (0.18 g)), and vitamin mix stock solution (1 $mL{\cdot}L^{-1}$; made of cyanocobalamin (vitamin B12, 0.0005 g), thiamine HCl (vitamin B1, 0.1 g), and biotin (0.0005 g)) [30]. After preparation, the medium's pH was

adjusted to 8.0 with 0.1N NaOH. Precultivation was performed for 7 days in batch cycle (in 1 L volume photobioreactor), at room temperature (23 ± 1 °C) under illumination with a light-emitting diode (LED; intensity = 8.4 W/m^2). The duration of illumination was controlled with a timer to provide a 16 h light/8 h dark cycle. The culture was aerated by bubbling with ambient air (flow rate = 7.2 L·min^{-1}). Air filtration was done using a sterile filter (0.22 µm, polytetrafluoroethylene filter—PTFE).

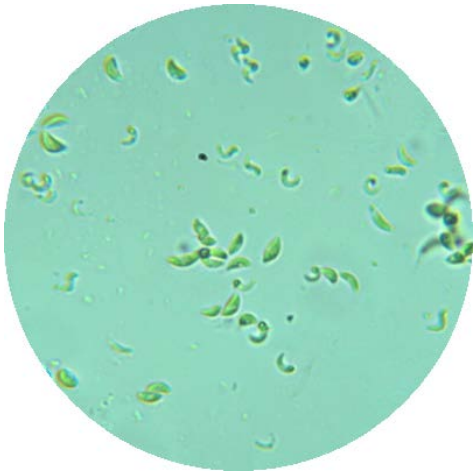

Figure 1. Light microscopy image of *Monoraphidium* sp. obtained during cultivation.

2.2. Aquaculture Wastewater

Aquaculture wastewater (AWW) was procured from a Jurassic Salmon company (Karnice, Poland), which is the first complete ecological farm in the world. It carries out fish production under a low temperature using saline (10‰) geothermal water obtained from over 1220 m depth below the sea level. For this study, AWW was taken from a storage tank. The collected water was stored at 4 °C in a cold room for further utilization.

2.3. Experimental Setup

The experiment was done in two vertical column photobioreactors (volume 14 L, with dimensions 22 × 56 cm, Figure 2). The cultures of pure *Monoraphidium* sp. (1.2 L) were placed in the photobioreactors with 10.8 L of F/2 medium or wastewater after sterilization with UV-C light (waterproof 13 W lamp, 15 min). Before that, AWW was filtered twice by pressure membrane filtration for removing the suspended dirt and subsequently through filters with pores of 1.2 µm average diameter. These photobioreactors were held at 23 ± 1 °C with an LED light.

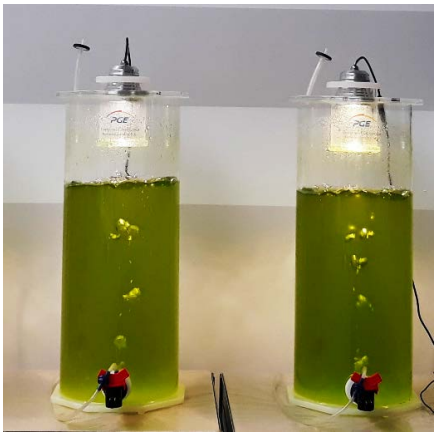

Figure 2. Experimental setup applied for research of *Monoraphidium* microalgae.

Cultures were allowed to grow under a 16 h light/8h dark cycle. Aseptic air and CO_2 were provided through a membrane pump (flow rate = 14 L·min^{-1}). Experimentation was carried out over a period of 10 days.

2.4. Analytical Methods

2.4.1. Determination of Microalgal Growth and Biomass Production

During batch cultivation, the growth of microalgae was determined by biomass concentration as well as by optical density measured at 680 nm. The biomass concentration of *Monoraphidium* sp. (g·L^{-1}) was calculated using the gravimetric method. Briefly, the broth biomass sample (40 mL) was centrifuged (Eppendorf Centrifuge 5702) for 15 min at 4000 rpm and then allowed to dry at 105 °C till a constant weight was obtained. Optical density at 680 nm was determined with a spectrophotometer (SEMCO S91E). For this, 2 mL of microalgal suspension was added to a cuvette and optical density was measured. The measurement of biomass concentration and optical density was done once every 2 days. From the yield, biomass productivity (g·L^{-1}·d^{-1}) was calculated as follows:

$$\text{Biomass productivity (BP)} = (B_f - B_0)/d, \quad (1)$$

where B_f is the final biomass amount (g), B_0 is the initial biomass amount (g), and d is the cultivation time (day).

2.4.2. Determination of Lipid and Ash Content

Lipid extraction from microalgal biomass was performed using Soxhlet extraction method with hexane. For this, the biomass was first dried at a temperature of 80 °C. Then, 0.3 g of samples was weighed and transferred to a cellulose casing. Extraction was conducted for 6 h at 20 cycles·h^{-1}. After solvent removal, the quantity of lipids was measured gravimetrically. The lipid content in dry weight percentage was calculated using the below equation:

$$\text{Lipid content (LC)} = (m_L/m_{DAB}) \times 100, \quad (2)$$

where m_L is the mass of lipids (g) and m_{DAB} is the mass of dry algal biomass (g).

The content of ash in biomass was measured gravimetrically. First, the biomass was dried at a temperature of 105 °C. Then, 0.3 g samples of dry biomass were incinerated for 6 h at 650 °C in a muffle furnace. The incinerated material was allowed to cool to the ambient temperature in a desiccator.

2.4.3. Determination of Nutrient Concentration

For performing chemical analysis, samples were collected from photobioreactors on the day they were set up and after 2, 4, 6, 8, and 10 days. The samples were analyzed for pH, total content of nitrogen (TN), content of nitrite-nitrogen (N-NO_2), content of nitrate-nitrogen (N-NO_3), content of ammonium nitrogen (N-NH_4^+), total content of phosphorus (TP), and concentration of orthophosphate ions. The contents of orthophosphate (PN EN 1189:2000) [31], nitrate-nitrogen (PN-C-04576-08) [32], nitrite-nitrogen (PN-C-04576-06) [33], and ammonium nitrogen (PN ISO7150-1:2002) [34] were estimated using the spectrophotometric method described in the Polish Standards. The concentration of mineral nitrogen forms was calculated as a sum of the concentration of all measured nitrogen compounds (N_{min} = N-NO_3^- + N-NH_4^+ + N-NO_2^-). The pH was determined potentiometrically in the tested water with a CI-316 microcomputer pH-meter.

2.4.4. Removal Efficiency and Ratio of Consumed Nutrients

Removal efficiency was calculated in percentage as follows:

$$\text{Percentage removal of nutrient (\%)} = (\text{initial concentration} - \text{final concentration})/\text{initial concentration} \quad (3)$$

Consumed nutrients were determined using the following equations:

$$\text{Consumed phosphorus after 2 days during experiment} = (\text{content P-}PO_4^{3-})_t - (\text{content P-}PO_4^{3-})_{t-2}, \quad (4)$$

$$\text{Consumed } N_{min} \text{ after 2 days during experiment} = (\text{content } N_{min})_t - (\text{content } N_{min})_{t-2}, \quad (5)$$

for t = 2, 4, 6, 10, and 12 (days).

2.4.5. Statistical Analysis

All the determinations were performed in triplicate. The measured values were expressed as the mean ± SD. The AWW algae-related parameters were evaluated by the analysis of variance (ANOVA). Tukey's honestly significant difference test was used to determine significant differences at $\alpha = 0.05$. Pearson's correlation coefficient (r) was calculated for the association between biomass content and optical density ($\alpha = 0.001$). All analyses were conducted in the statistical software package for Windows (Dell Statistica (data analysis software system) version 13.3 (2016); Dell Inc., Tulsa, OK, USA).

3. Results and Discussion

3.1. Microalgal Growth and Biomass Production

Algal growth and accumulation of lipids are influenced by various factors such as the concentration and availability of macro- and micronutrients, CO_2, pH, temperature, and intensity of light as well as photoperiod. In this study, nutrients in AWW positively impacted microalgal growth (Figure 3).

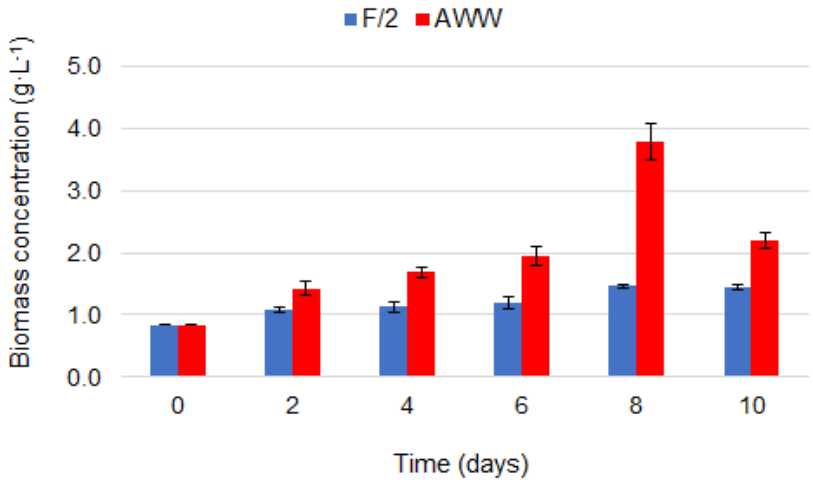

Figure 3. Changes observed in the biomass concentration during the cultivation of *Monoraphidium* sp. (mean ± SD; F/2—synthetic medium, AWW—aquaculture wastewater).

No lag phase was observed during the experiment which indicated that *Monoraphidium* is well adapted to grow in AWW. Similar results were reported by Liu et al. [35] for *Chlorococcum* sp. GD, *Chlorella vulgaris*, *Parachlorella kessleri* TY, *Scenedesmus obliquus*, and *Scenedesmus quadricauda* cultivated in real AWW from a fishery. In the present study, the concentration of biomass was higher in AWW than in the F/2 synthetic medium, and biomass concentration profiles were found to change with time. The maximum concentration of *Monoraphidium* biomass was achieved after culturing for 8 days. The biomass concentration was observed to increase to 3.79 and 1.47 $g \cdot L^{-1}$ in wastewater and in the F/2 medium, respectively. Biomass concentration in AWW correlated with that reported by Guiza-Franco et al. [36] for *C. vulgaris*. However, this concentration is much higher than that observed by Halfhide et al. [37] for *Scenedesmus* (0.41 $g \cdot L^{-1}$) and by Nogueira et al. [38] for *Spirulina platensis* (0.22 $g \cdot L^{-1}$) on the tilapia culture effluent. The microalgae *Monoraphidium* used biogens from AWW which indicated the elimination of the need for freshwater as well as chemical nutrients for algal

cultivation [39]. After culturing *Monoraphidium* sp. in AWW for 10 days, we noted a significant decline in the concentration of biomass. According to Tossavainen et al. [40], this indicates the limitation of bioavailable nutrients, which has also been confirmed by our previous results [26].

The productivity of *Monoraphidium* sp. biomass was 0.46 $g \cdot L^{-1} \cdot d^{-1}$. Kuo et al. [41] reported that after cultivating *Chlorella* sp. for seven days, the productivity of biomass equaled 0.31 $g\ L^{-1} \cdot d^{-1}$. Ansari et al. [39] cultivated microalgae using AWW and observed the maximum biomass productivity amounting to 160.79 $mg \cdot L^{-1} \cdot d^{-1}$ for *Ankistrodesmus falcatus* and lower biomass productivity amounting to 107.86 and 89.61 $mg \cdot L^{-1} \cdot d^{-1}$ for *Chlorella sorokiniana* and *S. obliquus*, respectively. The utilization of microalgae for treating wastewater and producing biomass can allow achieving a reduction in the energy cost, decreasing GHG emission, and cutting down the costs of nutrients as well as freshwater resources needed for biofuel generation [42].

A linear regression coefficient (r) of 0.95 was determined for the correlation between the dry weight biomass and optical density. Figure 4 shows the changes in OD_{680}, which is also commonly used for determining microalgal abundance. These results confirmed that *Monoraphidium* sp. grew well without a lag on the fish farm. Algal cell density in AWW increased almost 5 times of the initial level, suggesting that the components of AWW were assimilated into biomass. In F/2 medium, it was observed that OD_{680} reached a maximum value of 0.22 after eight days of incubation. Similar differences were noted while cultivating microalgae in AWW, where the optical density reached the highest value after eight days (0.65). Microalgal cultivation in AWW can effectively protect the environment against excess nutrients while enabling biomass accumulation with the possibility of further wide utilization as a source of bioenergy. Moreover, the production of microalgal biomass on AWW does not compete with food, and the cultivation requires much less time compared with agriculture crops for energy or biofuel [43].

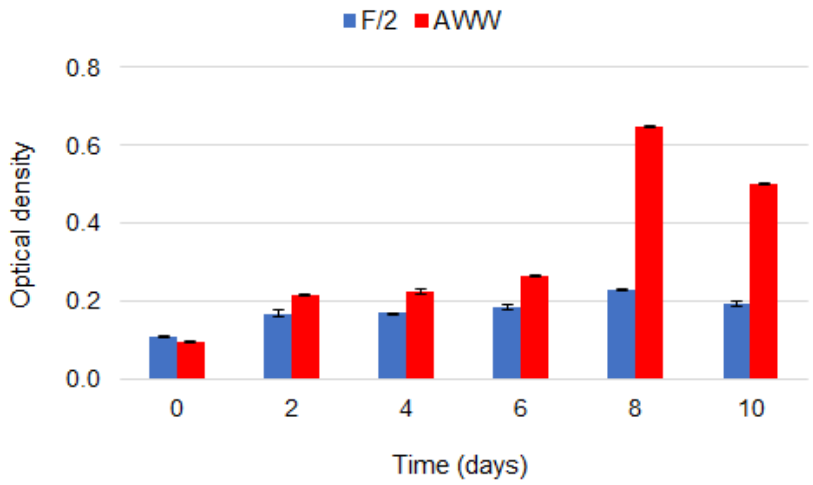

Figure 4. Changes observed in the optical density during the cultivation of *Monoraphidium* sp. (mean ± SD; F/2—synthetic medium, AWW—aquaculture wastewater).

3.2. Lipid Production and Ash Content

Microalgal biomass can be an excellent feedstock for the production of biodiesel, due to the fact that microalgae are capable of accumulating high concentrations of lipids in the cell [44]. The content of lipids in *Monoraphidium* biomass is estimated to be 19–35% in relation to the dry weight [45]. The results obtained in our study (18.53%) are similar to this data (Figure 4). The average content of lipids in microalgae can widely vary from 1% to 70% [46], depending on the type of organisms [47], environmental conditions [48], and extraction method [45]. Microalgae form lipids as an energy reserve mainly in the stationary phase [49], but this growth phase was relatively short during cultivation in AWW. Microalgae cultivated in AWW showed a different lipid content than synthetic medium. This can be related to the low concentration of phosphorus [50]. For biomass grown in AWW, it was

about 10% lower than the F/2 medium. However, the maximum biomass concentration was higher by about 150%. It should be noted that biodiesel production requires a considerable concentration of biomass, appropriate amount of lipids, and proper composition of fatty acids [45].

Bi and He [51] showed that green microalgae possess a lower amount of ash (from 13.8% to 18.8% wt dry basis) compared to brown microalgae (from 28.1% to 43.4% wt dry basis), which is favorable for biofuel processing. High amount of ash would very likely affect the purification procedure and the quality of a product. Metsoviti et al. [52] reported that ash content in microalgal biomass ranged from 10.9% (*Botryococcus braunii*) to 12.9% (*Euglena gracilis*). In the present study, the ash content in biomass from AWW was 32.34% and F/2 medium was 27.15% (Table 1). Differences in the microalgal ash content can be related to different ashing conditions, including temperatures. Ash content decreases when a high temperature is applied during ashing. This is due to, among others, increased vaporization of some ash components [53]. The ash content in biomass may be significantly affected by microalgae growing conditions. In the study of Roostaei et al. [54], in mixotrophic conditions, microalgal biofilms had a lower ash content of 40–60% compared to those grown in autotrophic conditions. Thus, considering the high ash content, the preferred conversion process would be pyrolysis and oil production from microalgal biomass [55].

Table 1. Lipid and ash content of microalgal biomass (mean ± SD; F/2—synthetic medium, AWW—aquaculture wastewater).

Content %	Objects	
	F/2	AWW
Lipid	20.80 ± 1.25	18.53 ± 0.83
Ash	27.15 ± 1.66	32.34 ± 2.25

3.3. Nutrient Removal

Eutrophication is considered a global problem affecting aquatic ecosystems, mainly caused by human-induced enrichment with phosphorus and nitrogen [56]. Biological methods are often used for cleaning the wastewaters originating from fish farming. The use of these methods in the form of a hydrophyte lagoon or open-air "raceway" algae ponds aims at using the biogenic compounds or metals remaining in the wastewater for the development of aquatic plant biomass [25] or algae [26]. The biomass thus obtained can be utilized in various ways. The advantage of this technological solution is the reduction in the charge of phosphorus and nitrogen to almost zero, which can counteract the eutrophication of surface waters [57].

The phosphorus and nitrogen contents in wastewater can differ significantly depending on the type and stage of treatment [58]. Wastewater resulting from Recirculating Aquaculture System (RAS) has comparatively low total nitrogen and phosphorus, at a concentration of approximately 23–33 and 1–4 $mg \cdot L^{-1}$, respectively (Table 2). Because the ratio of carbon, phosphorous, and nitrogen required by algae greatly varies, the Redfield ratio ($C_{106}H_{181}O_{45}N_{16}P$) has been recognized as a standard for predicting growth [59].

Table 2. Initial nutrient concentration in algal cultivation.

Total N $mg \cdot L^{-1}$	Total P $mg \cdot L^{-1}$	N/P	Reference
24.10–23.70	3.50–3.60	6.64	Tossavainen et al. [40]
31.53–32.13	1.08–1.12	28.94	Present study

The total nitrogen as well as total phosphorus content estimated in the wastewater is presented in Table 3. The initial total nitrogen content was 31.83 $mg \cdot L^{-1}$, but after 10-day treatment with *Monoraphidium* sp., the concentration reduced by 82.62%. The initial total phosphorus content was 1.1 $mg \cdot L^{-1}$, which decreased by 99.06% following microalgal treatment. Gao et al. [60] showed that

the average decrease in the total phosphorus and nitrogen concentrations in AWW achieved with *C. vulgaris* was 82.7% and 86.1%, respectively. In another study, Van Den Hende et al. [61] analyzed a mixed microalgal and bacterial culture and observed a reduction in phosphorus and nitrogen contents by 88.6% and 57.9%, respectively. On the other hand, Jiang et al. [62] reported that *Monoraphidium* sp. use ammonia first in the presence of various sources of nitrogen, and then uptake high concentrations of phosphorus from wastewater. This resulted in the removal of ammonia and phosphorus by almost 100%, thus fulfilling the pollutant discharge standard required to be met by municipal wastewater treatment systems.

Table 3. Nutrient removal efficiency of *Monoraphidium* sp.

	TN Concentration	**TP Concentration**
Nutrient removal efficiency (%)	82.62	99.06
Initial mg·L^{-1}	31.83 ± 0.31	1.10 ± 0.02
Final mg·L^{-1}	5.53 ± 0.06	0.01 ± 0.00

Nitrogen is the second major nutrient required for algal growth next to carbon [63]. The nitrogen forms that are best assimilated are nitrate (NO_3^-) and ammonium nitrogen (NH_4^+) [64]. Prior to treatment with *Monoraphidium*, N-NO_3 concentration in wastewater was 27.67 mg·L^{-1}, and that of N-NO_2 was 1.53 mg·L^{-1}, N-NH_4 was 0.23 mg·L^{-1}, and PO_4 was 1.29 mg·L^{-1} (Table 4). A remarkable decrease in all their concentrations was observed after just 2 days of culture, but the reduction course differed.

Table 4. Changes in nutrient concentration observed during the cultivation of *Monoraphidium* sp. in wastewater.

Term (Days)	**Nutrient Concentration, Mean ± SD (mg·L^{-1})**			
	N-NO_3^-	**N-NH_4^+**	**N-NO_2^-**	**PO_4^{3-}**
0	27.67 ± 0.69 ^{a,*}	0.23 ± 0.02	1.53 ± 0.02 ^{a}	1.29 ± 0.09 ^{a}
2	24.50 ± 1.04 ^{b}	0.00	0.55 ± 0.01 ^{d}	0.52 ± 0.07 ^{b}
4	19.14 ± 1.58 ^{c}	0.00	0.63 ± 0.00 ^{b}	0.07 ± 0.01 ^{c}
6	13.46 ± 1.68 ^{d}	0.00	0.59 ± 0.01 ^{c}	0.00
8	12.04 ± 0.20 ^{d}	0.00	0.38 ± 0.01 ^{e}	0.00
10	4.05 ± 0.15 ^{e}	0.00	0.33 ± 0.01 ^{f}	0.00

PO_4^{3-}: $F = 1054$; N-NO_2^-: $F = 13{,}788$; N-NO_3^-: $F = 591$; N-NH_4^+: $F = 1515$; all at $p < 0.05$; * ^{a,b,c,d,e,f}-homogeneous groups, mean not marked with the same letter is significantly different at $p < 0.001$.

Of all the nutrients, the analyzed microalgal species utilized ammonium nitrogen and orthophosphates at a higher rate (Table 4), and after 2 and 6 days, ammonium nitrogen and orthophosphates decreased to <0.05 mg·L^{-1}. These results confirm that of Jiang et al. [62]. It is worth mentioning that, in the study of Beuckels et al. [65], microalgal utilization of phosphorus was found to be associated with nitrogen content in wastewater.

In this study, low orthophosphate content seemed to influence the biological removal rate of oxidized nitrogen forms from the sixth day of culture (Figure 5). As a result, a considerably lower availability was observed in the case of nitrates till the end (Table 3). Nitrite reduction calculated during the study was 78%, nitrate reduction was 85%, and orthophosphate reduction was 100%, while after 10 days the content of these compounds in wastewater was 0.33, 4.05, and <0.05 mg·L^{-1}, respectively. Liu et al. [35] studied the ability of five microalgal species (*C. vulgaris*, *Chlorococcum* sp. GD, *P. kessleri* TY, *S. quadricauda*, and *S. obliquus*) to treat AWW and observed that the amount of nitrites, nitrates, phosphorus, and total ammonium was reduced by 94.3–99.8%, 85.7–97.1%, 90.2–98.9%, and 97.9–98.9%, respectively, after five days. Ansari et al. [39] stated that ammonium ions were reduced by

C. sorokiniana, *S. obliquus*, and *A. falcatus* by 86.45–98.21%, nitrates by 75.76–80.85%, and phosphates by 98.52–100%.

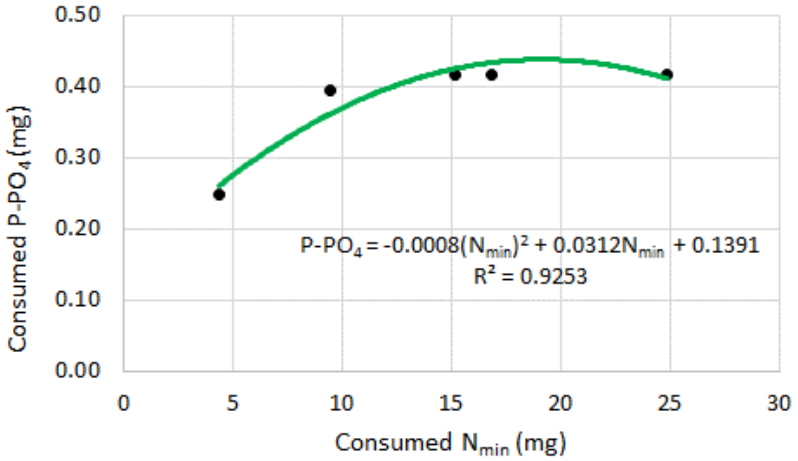

Figure 5. Changes in consumed $P\text{-}PO_4^{3-}$ in relation to changes in consumed N_{min} during the cultivation of *Monoraphidium* sp. in AWW ($R^2 = 0.9653$ at $p < 0.05$).

The ratio of $P\text{-}PO_4$ consumed and N_{min} differed during the microalgal cultivation. The studied algae showed the fastest phosphorus consumption in the initial two days. Rapid P uptake was confirmed by Qu et al. [66]. According to Wu et al. [67] microalgae absorb phosphorus from wastewater and store it in a cells. Under P deficiency, they can utilize this intracellular nutrient to biomass growth.

Literature suggests pH as a significant environmental factor determining the content of algal biomass, lipids, and fatty acids [68]. In the present study, the growth environment of the tested *Monoraphidium* algae had a slightly alkaline pH of 7.98–8.76 (Figure 6). The changes in pH may be caused by CO_2 utilization from medium or nitrate absorption by the analyzed microalgae [69].

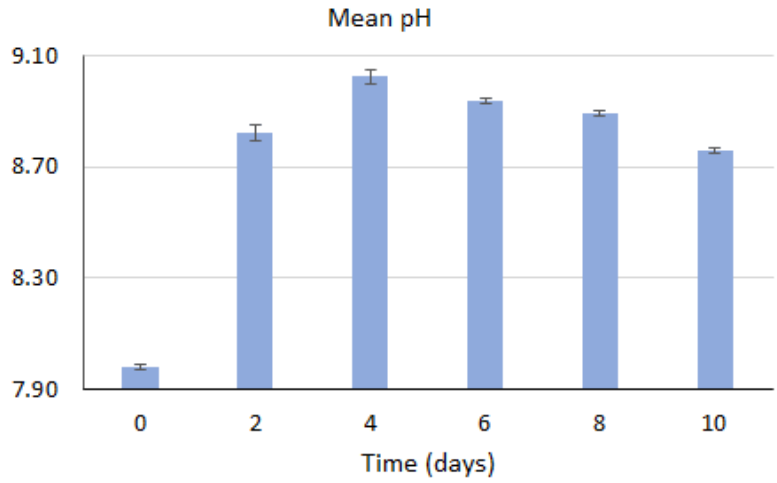

Figure 6. Reaction pH of AWW with algae (mean ± SD; ANOVA: $F = 3993$, $p < 0.05$).

4. Conclusions

This study investigated the value of AWW as a nutrient source for producing microalgal biomass. To our knowledge, this study is the first in which *Monoraphidium* sp. were tested in such conditions. *Monoraphidium* sp. exhibit high efficiencies of decreasing total phosphorus and nitrogen (over 99% and by 82.6%, respectively), nitrate (85%), nitrite (78%), and orthophosphate (100%). Biomass produced using aquaculture did not have high lipid content (18.53%), but its productivity in AWW (3.79 $g \cdot L^{-1}$) was significantly higher compared to the synthetic medium (1.47 $g \cdot L^{-1}$). Phosphorus deficiency

limited biomass productivity and lipid content. Its source could be the sewage sludge obtained at the initial stage of AWW treatment in the RAS system. Microalgal biomass production using AWW can be economically feasible and environmentally sustainable. The use of AWW for cultivating microalgae is promising for modern bioenergy production which integrates biomass production and nutrient removal.

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