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Research Article

Investigating the Suitability of Remineralized Aquaponics Sludge for Microalgae Culture: Biomass Production and Nutritional Composition

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ABSTRACT

Microalgae are promising resources for valuable products, and cultivating them requires a suitable culture medium to optimize growth and desired biochemical content. Aquaponic sludge, a byproduct of aquaponics systems, offers a sustainable and cost-effective alternative to conventional media by recycling waste and reducing environmental impact. This study aimed to compare the performance of standard BG-11 (Blue-Green 11) medium with remineralized sludge-water (RSW) and RSW supplemented with micronutrient solution (RSW+Mn) for cultivating *Chlorella minutissima, Botryococcus braunii,* and *Haematococcus pluvialis*. The highest specific growth rate (μ) of 0.097±0.011 was observed for *C. minutissima* in BG-11 medium, nearly 28% higher than in RSW medium. However, the highest dry biomass productivity (P_b) of 0.012±0.011 was achieved by *H. pluvialis* in RSW+Mn medium, significantly 94% higher than in RSW medium. Additionally, the volumetric productivity of biomass (Q_x) for *H. pluvialis* in RSW medium was 0.045±0.017, nearly 50% higher than in BG-11 medium. The best doubling time (td) of 8.83±0.93 days was observed for *H. pluvialis* in RSW medium. Notably, *C. minutissima* cultured in RSW medium yielded the highest crude protein (55.77±1.81%) and total lipid (4.69±0.88%) contents. These results demonstrate that RSW medium can be tailored to achieve desired outcomes, such as optimizing growth rate or lipid content. This study highlights the potential of remineralized aquaponic sludge as a sustainable culture medium for microalgae, contributing to waste recycling and resource efficiency in aquaponics systems. Future studies should focus on optimizing RSW medium for large-scale cultivation of target microalgae species with specific biochemical profiles.

Keywords: Microalgae, aquaculture, wastewater, recirculating aquaculture system, sustainability, waste recycling

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INTRODUCTION

The integration of aquaponics and microalgae production using wastewater represents a significant advancement in sustainable aquaculture. Medium-scale integrations offer valuable data for aquapreneurs, enabling them to develop new initiatives.

In aquaponics, fish and plants are grown together in a closed recirculating system (Goddek and Keesman, 2020). The system is managed by mimicking a natural aquatic environment with fish, plants, and nitrification bacteria consortia in a limited area (Tunçelli and Memiş, 2024). The recirculation characteristics of aquaponics make it a valuable system that contributes to reducing natural freshwater consumption by up to 90% (Danish et al., 2021). As compared to conventional irrigation systems, recirculating aquaculture systems like aquaponic systems use less irrigation water for agricultural activities and prevent soil salinization (Colt et al., 2022). However, recirculating aquaculture systems produce sludge that contains a variety of nutrients, including nitrogenous compounds,

phosphorus, and other dissolved organic carbons that could negatively affect the environment when their concentration is higher than usual (Jasmin et al., 2020). Furthermore, the water in aquaponics is typically warm, which is ideal for microalgae growth. Due to these kinds of opportunities, aquaponics byproduct, sludge-water, has the potential to be a sustainable alternative as it can be collected easily and remineralized for microalgae cultivation (Boedijn et al., 2021; Addy et al., 2017).

Three primary methods are commonly used for integrated vegetable and fish production in aquaponics systems. Nutrient film technique (NFT), media bed, and deep water culture methods are frequently used methods for establishing aquaponics (Li et al., 2019). NFT aquaponics utilizes thin water surfaces and air simultaneously to provide optimal conditions for plant growth (Su et al., 2020). With its wide air surface area, NFT aquaponics is considered one of the most advantageous aquaponic systems for converting nitrogenous ions to nitrates through aerobic oxidation bacteria (Thakur et al., 2023). Moreover, aquaponic systems can be integrated with microalgae cultivation to enhance ecosystem-friendly aquaculture (Ansari et al., 2021). However, this sludge-water can be treated through remineralization and repurposed as a nutrient-rich medium for microalgae cultivation.

Studies have shown that microalgae grown in different wastewaters have similar growth rates and biomass yields as microalgae grown in traditional culture mediums. Studies have shown that microalgae can effectively utilize various wastewaters for growth, including urban wastewater (Robles et al., 2020), textile wastewater (Wu et al., 2020), pig farm wastewater (Nagarajan et al., 2019), brewery effluent (Ferreira et al., 2019), shrimp wastewater (Krasaesueb et al., 2019), and even palm oil wastewater (Ahmad et al., 2019). Because microalgae culture medium is one of the most expensive entries of the microalgae production after labor, illumination conditions, agitation, and photosynthetic efficiency of the system (Mtaki et al., 2023). As a result, using cheaper raw materials like aquaponics sludge-water, optimizing nutrient composition in culture medium, recycling wastewaters, cost-effective production techniques, and exploring alternative microalgae culture mediums can be done to reduce the cost of the culture medium. Microalgae have the ability to produce highly valuable bioactive compounds such as vitamins, minerals, essential amino acids, fatty acids, carotenoids, and enzymes (Zhou et al., 2022). Therefore microalgae that are cultured in aquaponics sludge-water are very good candidates to get biomass for bioactive compounds. Nitrification bioreactors can recover nitrogen from sludge-water via remineralization (Wongkiew et al., 2021), and the resulting culture medium can be used for microalgae cultivation.

Ammonium-oxidizing bacteria like *Nitrosomonas* sp., *Nitrosococcus* sp., and *Nitrosospira* sp. are the leading microorganisms of the nitrification process (Al-Ajeel et al., 2022). These bacteria are commonly used in wastewater treatment facilities. Aerobic-activated sludge application is one of the widely used processes for municipal and industrial wastewaters to get rid of their hazardous compounds such as ammonium, nitrite, and nitrate ions (Singh and Dey, 2024). Because these pollutants can cause eutrophication with precipitation in narrow bays, closed-water basins or lakes (Zhang et al., 2020). Eutrophication is usually characterized by algal blooms, low water quality, and mass fish mortality (Kapsalis and Kalavrouziotis, 2021). Compounds like ammonium and nitrite in sludge-water that pose a danger to aquatic and terrestrial animals make it possible for microalgae to grow effectively (Chamoli et al., 2024).

First studies of using sludge extracts to produce microalgae date back to the 1970s. Using sludge extracts, Wong et al. (1977) attempted to increase the production of *Chlorella pyrenoidosa* in the Kuhl Medium. In a different study, sludge extracts were discovered to be more beneficial than other common microalgae cultivation mediums (Wong, 1977). Aquaponics sludge-water is a waste product that would otherwise need to be disposed of, so using it as a culture medium can help to reduce the amount of waste produced by the aquaponics system and potentially reduce the cost of microalgae cultivation. Additionally, using aquaponics sludge water can help to improve the efficiency of the aquaponics system by recycling the nutrients that are present in the water.

However, depending on the species of fish and plants in the aquaponics system, the sludge-water might be contaminated with some pathogens, heavy metals, or other pollutants, which might impact negatively on microalgae growth. There are some important physicochemical parameters such as temperature (Elisabeth et al., 2021), pH (Fernandes et al., 2022), culture medium (de Medeiros et al., 2020), carbon dioxide (Li et al., 2023), and conductivity (Barahoei et al., 2021) that have effects on the concentration and quality of lipids and proteins in microalgae. Therefore, it's important to evaluate the quality of the sludge water before using it as a culture medium and to consider other factors such as pH, temperature, and light conditions to optimize the growth of the microalgae. Plants and protists require nitrogenous nutrient salts such as ammonia, nitrite, and nitrate as well as algae to take advantage of these compounds, as well (Ribeiro et al., 2020; Kyriacou et al., 2019).

Chlorella sp. is one of the most common microorganisms used in the production of biomass from industrial, municipal, and even aquaponics wastewater (Fimbres-Acedo et al., 2020; Chen et al., 2019; Wang et al., 2019; Addy et al. 2017; Fang et al., 2017). On the other hand, *B. braunii* and *H. pluvialis* are other microalgae species that have a growing trend of experimental and commercial cultivation. *B. braunii* is known for its rich lipid content and hydrocarbon production ability (Nazloo et al., 2024), *H. pluvialis* is known for its natural astaxanthin production capability (Mularczyk et al., 2020). Bioethanol wastewater streams (Nishshanka et al., 2022), primary treated wastewater (Pan et al., 2021), synthetic brewery wastewater (Yap et al., 2022), and domestic secondary effluent (Sirotiya et al., 2023) were used as culture mediums for the production of *H. pluvialis* biomass. *B. braunii* were used as a wastewater treatment organism for piggery wastewater (Mkpuma et al., 2023), sewage wastewater rich in ammonium nitrogen (Miura et al., 2022), aerated swine lagoon wastewater (Li et al., 2022). According to these studies, a sustainable ecosystem approach to biomass production was made by using the help of different microalgae.

This study's hypothesis focuses on a novel and potentially sustainable approach to microalgae culture medium using aqua-

ponic byproducts. Traditionally, microalgae are cultivated using well-defined but expensive medium like BG-11. This study investigates a potentially lower-cost option by exploring remineralized sludgewater from aquaponics as a culture medium. There's a growing interest in using wastewater as a culture medium, and this study specifically focuses on remineralized sludgewater due to its potentially enhanced nutrient profile, making it even more suitable for microalgae growth.

The purpose of the study was to determine the performance of microalgae biomass production by utilizing sludge-water obtained from aquaponics. A comparison was made among the performance of microalgae grown in RSW medium, RSW medium including micronutrient solution (RSW+Mn), and commonly used BG-11 medium. For the cultivation of microalgae species, the BG-11 medium is one of the most commonly used nutrient mediums, and it can be compared with other microalgae culture mediums easily. Microalgae cell numbers, doubling times, biomass productivities, specific growth rates, and biochemical contents were determined and evaluated in the RSW and RSW+Mn culture mediums.

MATERIALS AND METHODS

Aquaponic system setup

This study was conducted at the Mediterranean Fisheries Research Production and Training Institute's (MEDFRI) Nutrient Film Technique (NFT) aquaponics research facility in Antalya Province for 42 days, from September to October. The recirculating aquaculture system (RAS) consisted of a 2.5 m^3 fiber aquaculture tank, two 80-liter radial flow separators, two 150-liter biofilter tanks (each containing 20 liters of media), 12 PVC rafts, and a 150-liter sump/pump tank. A 0.25 kW submersible water pump (Pedrollo, Tamworth UK) was installed in the sump/pump tank to circulate water. Daily, 10 liters of sludge-water were collected from the aquaculture tank discharge pipe and radial flow separators. This untreated discharge poses a risk of eutrophication in nearby inland waters. The collected sludge-water underwent a one-day remineralization process in 10-liter tanks. This process employed an air pump (2.5 L/min) to convert hazardous nutrients into bioavailable forms. A 0.45-micron cellulose ester membrane filter removed solid waste. To prevent bacterial interference, the remineralized sludge-water (RSW) was autoclaved daily at 121 °C and 1 atm for 25 minutes. The combined RSW was used as a microalgae culture medium and compared to the BG-11 medium. Dead fish were promptly removed from the aquaculture tank to minimize bacterial contamination. Figure 1 illustrates the catfish-lettuce aquaponics system utilized in this study.

Fish

A total of 348 African catfish (*Clarias gariepinus*) were used with an average weight of 28.83±11.68 g were used in the study. The total biomass of the fish was 10 kg (4 kg/m^3 initial density). The fish were fed twice daily (09:00 and 16:00) at a rate of 2% of their total biomass per feeding (4% total daily ration) for six weeks using commercial carp feed. The diet contained 50% raw protein, 8% total fat, 3.7% cellulose, 9.7% ash, 1.5% calcium, 0.94% phosphorus, 0.42% sodium, 8000 $IUkg^{-1}$ vitamin A, 3000 $IUkg^{-1}$ vitamin D3, 350 $mgkg^{-1}$ vitamin E, 30 $mgkg^{-1}$ manganese oxide, 60 $mgkg^{-1}$ zinc oxide, 20 $mgkg^{-1}$ iron chelate of glycine hydrate, 2 $mgkg^{-1}$ calcium iodide, 6 $mgkg^{-1}$ copper sulfate pentahydrate, 0.2 $mgkg^{-1}$ sodium selenite. The following fish performance parameters were assessed during the study: feed conversion ratio (FCR), specific growth rate (SGR) (μmax), survival rate (SR), relative growth rate (RGR), and weight gain (WG). Calculation methods for each parameter are described as follows:

FCR=Dry feed weight (g) / Weight gain (g)

SGR= ((ln Last Weight – ln Initial Weight) / Duration of experiment) × 100

SR= (Last number of fish in fish tank / Initial number of fish in fish tank) × 100

RGR= ((Last weight of fish – Initial weight of fish) / Initial weight of fish) × 100

WG= (Last weight – Initial weight)

Figure 1. Nutrient film technique aquaponics 1: Fish tank, 2: Separation tanks, 3: Bio-filter, 4: Hydroponic unit, 5: Sump/Pump, 6: Sludge-water discharge pipe

Plant

Three-week-old lettuce (*Lactuca sativa*) seedlings, with an average initial weight of 1.69±0.42 g, were planted in a hydroponics unit at a density of 12 × 12 cm, using fiber as a substrate. The total of 120 lettuce plants were used in the experiment to ensure statistical reliability. The total wet weights of all plants were meticulously measured after separating them from the fiber substrates. The lettuce shoots were separated from the roots by cutting them just above the root collar (the point where the stem meets the roots). The following parameters were calculated:

Total harvested plant weight (g): Measured for each plant.

Leaf number: Counted for each plant.

Yield (gm^{-2}): Calculated as Biomass/Area, where the area of the hydroponics unit was 2.88 m^2.

Survival rate (SR): Determined as (Final number of plants/Initial number of plants) × 100.

Shoot/root ratio (s/r): Calculated as plant shoot length divided by plant root length.

Microalgae strains and culture system setup

Chlorella minutissima, *Haematococcus pluvialis*, and *Botryococcus braunii* were selected for cultivation using standard BG-11, RSW, and RSW+Mn mediums. Firstly, all stock microalgae species used in the present study were pre-cultured in standard BG-11 medium ($NaNO_3$ (1500 mgL^{-1}), K_2HPO_4 $3H_2O$ (40 mgL^{-1}), $MgSO_4$ $7H_2O$ (75 mgL^{-1}), $CaCl_2$ $2H_2O$ (36 mgL^{-1}), $C_6H_8O_7$ (6 mgL^{-1}), $C_6H_8O_7$.xFeH_3N (6 mgL^{-1}), Na-EDTA (1 mgL^{-1}), Na_2CO_3 (20 mgL^{-1}) and micronutrient solution: H_3BO_3 (2.86 μgL^{-1}), $Co(NO_3)_2$ $6H_2O$ (0.494 μgL^{-1}), $MnCl_2$ $4H_2O$ (1.81 μgL^{-1}), $ZnSO_4$ $7H_2O$ (2.22 μgL^{-1}), Na_2MoO_4 $2H_2O$ (3.9 μgL^{-1}), $CuSO_4$ $5H_2O$ (0.79 μgL^{-1})) in 1 L erlenmeyer flasks. The microalgae were then cultivated in three different media: standard BG-11, RSW, and RSW+Mn in the study. All microalgae species were cultivated in 10 L low-density polyethylene (PE) plastic bags. Each species was cultured in triplicate 10-liter low-density polyethylene (PE) plastic bags within a microalgae culture platform (Figure 2). A 16:8 light/dark photoperiod was used as one of the most important parameters for biomass production (Ratomski et al., 2021). The cultures were illuminated with 36-watt cool-white fluorescent lights. It was attempted to maintain the temperature at 25 °C by using the central heating system of the Aquarium Unit at MEDFRI. The light adjustment at the surface of the microalgae culture plastic bags was realized by using a light meter, TES 1332A (TES Electrical Electronic Corp., Taipei, China). Microalgae cultures were continuously aerated using filtered air at a constant flow rate of 2.5 $Lmin^{-1}$ for each plastic bag. Microalgae were grown in the live feed laboratory of the Mediterranean Fisheries Research Production and Training Institute for 27 days. Microalgae cell numbers were monitored every day using a light microscope (Leica Microsystems, Wetzlar, Germany) and a haemocytometer (depth 0.1mm; five replicates were averaged). At the end of the cultivation process, batch cultures were harvested and then freeze-dried by a high-vacuum freeze dryer (Telstar Cryodos-50, Terrassa, Spain) for 2 days at 0.023 mBar and -55±2 °C. All biochemical analyses were conducted on freeze-dried samples obtained from batchwise cultures, and they were stored at -18 °C.

Physicochemical parameters and ion concentration of microalgae culture mediums

Evaluating the physicochemical parameters and nutrient salts of the microalgae culture medium is an important step in optimizing microalgae growth. This influences the biochemical composition of the obtained microalgae, such as crude protein, total carbohydrates, and total lipids. By doing this, microalgae researchers can minimize culture medium costs while maintaining optimal growth conditions using alternative techniques and resources.

BG-11 medium is commonly used for green microalgae and cyanobacteria species (Rippka et al., 1979; Ozturk et al., 2019). The RSW culture medium, obtained daily from the aquaponics system, underwent a 42 day oxidation and sterilization process before being pooled (or "combined"/"formulated") in PE plastic bags. After remineralization, the ion concentration of RSW was analyzed using a Dionex ICS 3000 ion chromatography system consisting of a dual gradient pump unit (Dionex, Sunnyvale, CA, USA). Ion concentrations of BG-11, RSW, and RSW+Mn culture mediums are shown in Table 4.

Figure 2. Microalgae batch culture platform 1: Polyethylene plastic bags with microalgae, 2: 36 Watt fluorescent lamps, 3: 2.5 L/min aeration

A handheld multiparameter unit, the Yellow Spring Instrument Pro-DSS (YSI, Xylem Inc., Yellow Springs, OH, USA), was used to measure physicochemical parameters of microalgae culture medium, including temperature (°C), conductivity (μScm^{-1}), salinity (ppt), and pH every day. At the end of the remineralization process, the daily collected and remineralized sludge-waters were combined and analyzed by ion chromatography. Daily collected RSW culture medium samples (3 mL) were filtered using 0.22 μm pore size polytetrafluoroethylene (PTFE) filters before analysis with two Dionex ICS 3000 ion chromatographs (Thermo Scientific, Waltham, Massachusetts, USA) equipped with IonPac AS9-HC and IonPac AS11-HC columns according to the manufacturer instructions.

Microalgae cell number and biomass

Maintaining optimal nutrient levels and growth conditions for microalgae leads to a higher biomass yield. By understanding how culture medium and conditions affect cell production, they can manipulate the conditions to favor the desired product. By doing this, it can be formulated cost-effective and optimized culture medium for large-scale production.

Three different mediums (BG-11, RSW, and RSW+Mn) were compared to determine microalgae growth (cell numbers). Microalgae cultures were counted daily using a binocular microscope (Leica DM2000, Leica Microsystems Canada, Richmond Hill, Ontario). The microalgae cells were counted daily after homogenization using a Neubauer haemocytometer and results were expressed as cellsmL^{-1}. For determining dry biomass, 10 mL algal suspension was filtered daily using glass microfiber filters (Whatman GF/F, 47 mm, nominal pore size 1.6 μm) and all microalgae dried at 105 °C for 4 hours. Pre-weighed glass microfiber filters were used for algae culture filtration. After filtration, the filters containing the algae were dried at 105 °C for 4 hours. The filters were then cooled down in a vacuum desiccator and weighed again. The difference in weight represents the dry biomass of the algae culture (mgL^{-1}) (Simonazzi et al., 2021; APHA, 1997). Vacuum pressure during filtration was maintained at 45 mm Hg. All microalgae groups were filtered in triplicate.

According to Vonshak (1986), the below formulation was used to calculate specific growth rate (μ).

$\mu = \ln(X2 - X1) / t$

$X1$= Initial biomass concentration

$X2$= Final biomass concentration

t= Time

Productivity (Q*x*) was calculated using the formula below (Liu ve ark., 2013).

Qx (gLday^{-1})= $(X2-X1)/t$

$X1$= Initial biomass concentration

$X2$= Final biomass concentration

t= Time

Doubling time (t*d*) was determined using the following formula (Yoshimura ve ark., 2013).

t*d* (day)= $\ln 2/\mu = 0.693/\mu$

t*d*: Doubling time or regeneration time

Dry biomass productivity calculated was applied using the following formula (Liu et al., 2013).

Biomass productivity (P*b*) (gm^{-2} day^{-1})= $(DWL_n \times DWL_0)/n$

DWL_n= Dry weight of day n

DWL_0= Dry weight of day 0

n= cultivation days

Determination of biochemical content of microalgae

Determining the biochemical content of obtained microalgae biomass is a key aspect for evaluating target products like crude protein, total carbohydrates, and total lipids. Crude protein in the biomass is suitable primarily for human consumption and animal feed. Total carbohydrates from the algae can be used for bioethanol and other biofuel applications. Total lipids are valuable for mainly supplements and biodiesel production.

Triplicate analyses were conducted on lyophilized microalgae to determine their total lipids, crude protein, raw ash, carbohydrates, and moisture content. The total lipid concentrations were determined gravimetrically after extraction using the modified Bligh and Dyer (1959) method by Kates (1972). Using the Dumas combustion method, the crude protein content of microalgae was determined (Chiacchierini et al., 2003). In order to determine the moisture content in freeze-dried microalgae biomass, approximately 1 g of the sample was oven dried at 105°C for 1 hour (Lee et al., 2013). Microalgae raw ash content was determined using the protocol of the AOAC method 942.05 (Helrich, K., 1990). Total carbohydrates (including crude fiber) were calculated as 100% minus the sum of the moisture, protein, fat, and ash contents obtained using proximate analysis as previously explained (Eyeson & Ankrah, 1975).

Statistical analysis

The statistical analyses were performed using JMP 13 software (SAS Institute Inc., Cary, N. C.). Following a Shapiro-Wilk homogeneity test, analysis of variance (One-way and two-way ANOVA) was conducted to compare results for culture mediums (BG-11, RSW, and RSW+Mn) within each algal species. Tukey's HSD tests were used to determine significant differences (P <0.05) among all culture mediums and algal species. The effects of temperature, pH, conductivity, and salinity were assessed using a repeated measures ANOVA. Data are presented as means±standard deviations in the tables.

RESULTS AND DISCUSSION

Growth performance of fish

The growth performance of African catfish was evaluated using the following criteria: fish feed conversion ratio (FCR), specific growth rate (SGR), relative growth rate (RGR), and survival ratio

(SR). At the conclusion of the 42 day of the aquaponics study, 45.14 kg of fish were harvested. However, accumulation of organic matter in the fish and separation tank, leading to declining dissolved oxygen (DO) levels throughout the study period and the cannibalistic nature of African catfish resulted in the mortality of 47 out of 348 fish. The final average weight of the fish was 149.97±70.38 g. FCR, SGR, RGR, and SR were calculated to be 0.98, 3.59% day-*1*, 351.1%, and 86.49%, respectively. The overall health of the African catfish was assessed as good. The absence of supplemental aeration in the fish tank is a potential factor in the 13.5% fish mortality observed by the end of the study. FCR of African catfish were found as 1.18-1.33 in spinach and mustard green integrated aquaponics (Endut et al., 2016), 1.02-1.09 in floating raft hydroponics integrated with cucumber (Baßmann et al., 2017), and 1.03-1.14 in co-cultivated basil integrated aquaponics (Baßmann et al., 2018). African catfish fed with fish diets containing different proportions of potassium diformate and potassium chloride exhibited specific growth rates (SGR) ranging from 1.25±0.09% day^{-1} to 1.52±0.12% day^{-1} (Siqwepu et al., 2020) which is lower than this study's results. In another study, African catfish grown in an aquaponic system with basil (*Ocimum basilicum*) at moderate and high densities had lower SGR values (0.71% day^{-1} and 0.80% day^{-1}, respectively) compared to this study (Baßmann et al., 2018). Hagar et al. (2019) reported an RGR value of 97.28±0.03 for African catfish grown in recirculating aquaculture systems (RAS). In comparison, studies have shown higher survival rates for African catfish integrated with aquaponics (e.g., 94.25±2.12% with pumpkin cultivation) compared to recirculating systems (80.60±1.20%) and static systems (59.24±1.91%) (Oladimeji et al., 2020). African catfish are found to be an appropriate candidate for aquaponics systems. Studies have shown promising results, with survival rates reaching 100% when integrated with lettuce cultivation using microwave pyrolysis biochar (Su et al., 2020). Additionally, research by Suhl et al. (2018) reported total weight gain ranging from 10.0 kg $tank^{-1}$ to 267.5 kg $tank^{-1}$ in a tomato-African catfish aquaponics system with an innovative suction filter that reduces nitrogen loss. However, cannibalism can be a major cause of mortality in African catfish.

Growth performance of plant

Lettuce was harvested from the hydroponics unit after 42 days of the experiment. Average plant weight, average leaf number, average leaf area, average stem diameter, fresh weight (yield), survival rate, and average shoot/root ratio were calculated as 91.85±35.36 g, 36.79±7.33, 48.26±32.20 cm^2, 2.16±0.39 cm, 164.06±54.09 gm^{-2}, 100%, and 4.83±1.21, respectively. Tunçelli and Memiş (2024) Palm et al. (2014), Calone et al. (2019), and Byrd et al. (2022) reported similar results for hydroponic lettuce production in aquaponics. However, the fresh weight was lower compared to the findings of Maucieri et al. (2019). Badrey et al. (2024) found that lettuce (*Lactuca sativa* L.) grown in a polyculture aquaponic system using polyculture effluent (ASTAF-Pro) achieved a significantly higher average weight (450±70 g) compared to those grown in monoculture (360±45 g). Matysiak et al. (2023) reported a romaine lettuce yield of 86 g per plant within 21 days in a vertical aquaponic farm. This translates to a yield of 3.4 kg m^{-2} at a planting density of 40 plants m^{-2}. In a study of lettuce (*Lactuca sativa*) production in northern latitudes using aquaponics, Abbey and Anderson (2019) observed significant differences in fresh weight based on fish species. Lettuce grown in a deep water culture (DWC) system with perch had the lowest mean fresh weight (65.6 g), while those grown with tilapia achieved the highest (172.3 g).

Leafy vegetables like lettuce are the most preferred plant species in aquaponics due to their easy integration. However, in this study, the lettuce showed a relatively lower growth performance compared to other studies. This might be attributed to the specific ion concentration in the effluent (circulating water) of the aquaponic system, which may not have been optimal for lettuce growth.

Ion concentrations of the microalgae culture mediums

As expected, the BG-11 medium had the highest NO_3-N concentration at 247.06 mgL^{-1}. In contrast, RSW and RSW+Mn media had higher concentrations of NH_4-N, NO_2-N, SO_4, K, Mg, Ca, and Cl compared to standard BG-11 (Table 1). Statistically significant differences ($p<0.05$) were found in nutrient concentrations between RSW and RSW+Mn media compared to BG-11, suggesting they might be more suitable for *Chlorella* sp. growth. Based on the combined daily aquaponic sludge-water, ammonia levels were relatively low (1.06±1.18 mgL^{-1}) compared to nitrite (3.97±6.63 mgL^{-1}) and nitrate (4.32±7.9 mgL-*1*) levels. Phosphate levels were also moderate (1.52±1.65 mgL^{-1}). Green microalgae like *Chlorella* sp. require adequate nitrogen (particularly NO_3-N) and phosphorus (PO_4-P) for maximum biomass production (Chakraborty et al., 2016). These values are all considered relatively low compared to other studies (e.g., Gao et al., 2016; Tanikawa et al., 2018). In another study, for an axenic cultivation, nitrate and phosphate concentrations of aquaculture wastewater were found as 17.6 mgL^{-1} and 16.9 mgL^{-1}, respectively. Indigenous microalgae consortia consisting of *Chlorella* sp. (95.2%), *Chlamydomonas* sp. (3.1%), *Stichococcus* sp. (1.1%), *Chlorella* sp., and *Scenedesmus quadricauda* were used to produce microalgal biomass with aquaculture wastewater, successfully (Halfhide et al., 2014).

Physicochemical parameters of *Chlorella minutissima*culture medium

The trends in physicochemical parameters (e.g., temperature, pH, salinity, EC) for *H. pluvialis* cultured in BG-11, RSW, and RSW+Mn media are shown in Figure 3. Average values of these parameters for *C. minutissima* culture are presented in Table 2. The maximum temperature (26.8±0.10 °C) was observed in the BG-11 medium on day 7, while the minimum temperature (22.53±0.05 °C) was measured in the RSW+Mn medium on day 21. Average temperatures were calculated as 24.59±1.23 °C in BG-11, 24.32±1.26 °C in RSW, and 24.42±1.26 °C in RSW+Mn media. While temperature showed no significant differences among groups ($F(2,81) = 0.3420$, $P>0.05$), pH ($F(2,81) = 9.711$), salinity ($F(2,81) = 109.3$), and EC ($F(2,81) = 140.5$) exhibited statistically significant differences ($P < 0.05$). The highest EC was measured in the BG-11 medium on the 27th day (1599.33±57.41 μScm^{-1}), while the lowest conductivity was found in the same medium on day 1 (700.33±1.70 μScm^{-1}). The average EC was calculated as 1499±227.69 μScm^{-1} in BG-11, 992.35±31.86 μScm^{-1} in RSW, and 976.45±15.99 μScm^{-1} in RSW+Mn mediums. BG-11 ex-

Table 1. The ion concentrations of BG-11, RSW, and RSW+Mn mediums

Descriptions	BG-11	RSW	RSW+Mn
NO_2-N (mg/L)	-	3.97±6.83	3.97±6.83
NO_3-N (mg/L)	247.06	4.32±7.9	4.32±7.9
PO_4-P (mg/L)	9.25	1.52±1.65	1.52±1.65
SO_4 (mg/L)	29.33	109.42±43.41	109.42±43.41
NH_4-N (mg/L)	0.32	1.06±1.18	1.06±1.18
K (mg/L)	5.67	8.68±5.15	8.68±5.15
Mg (mg/L)	7.40	33.36±7.88	33.36±7.88
Ca (mg/L)	9.81	155.5±29.36	155.5±29.36
Na (mg/L)	410.29	55.65±58.14	55.65±58.14
Cl (mg/L)	9.00	89.87±132.74	89.87±132.74
Co (mg/L)	0.0091	-	0.0091
Mo (mg/L)	0.1546	-	0.1546
Mn (mg/L)	0.5025	-	0.5025
Zn (mg/L)	0.0500	-	0.0500
Cu (mg/L)	0.0204	-	0.0204

BG-11: Blue-green microalgae culture medium, RSW: Remineralized sludge-water, RSW+Tr: Remineralized sludge-water + BG-11 microalgae culture medium trace element solution

hibited a statistically significant difference (P <0.05) in conductivity compared to the other culture media. No significant differences were found in salinity among all mediums (P >0.05). Figure 3 illustrates the observed trends in pH, with a maximum value of 8.78±0.02 on day 16 and a minimum of 7.42±0.01 at the beginning of the experiment. Despite the difference in nutrient salt concentration between BG-11 and RSW, the RSW medium yielded better results in terms of biomass production compared to BG-11. The average physicochemical parameters of *Chlorella minutissima* culture are presented in Table 2 below. Temperature of the culture mediums of *C. minutissima* showed no significant changes. However, RSW and RSW+Mn mediums differed from BG-11 in terms of pH, salinity and conductivity, potentially indicating a difference in nutrient composition.

Temperature of the culture mediums of *C. minutissima* showed no significant changes. However, RSW and RSW+Mn mediums differed from BG-11 in terms of pH, salinity and conductivity, potentially indicating a difference in nutrient composition.

For comparison, a study by Ribeiro et al. (2020) found an optimal temperature of 28°C for *Chlorella sorokiniana* production using a combination nitrogen medium (urea, ammonia, and nitrate). Microalgae growth also depends on pH response and reaction (Berge et al., 2012). Chiu (2015) reported that agricultural and livestock breeding wastewater offered a good potential for *Chlorella* sp. cultivation due to higher nutrient concentrations. These

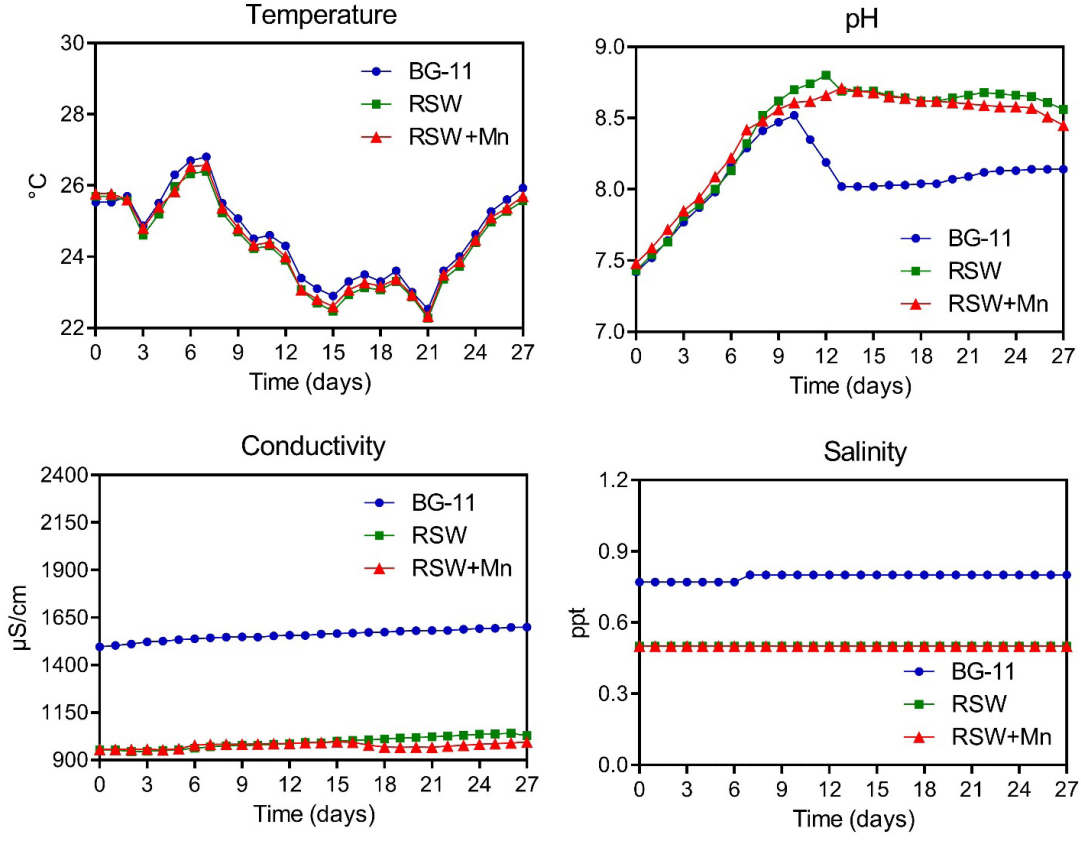

Figure 3. Time-dependent change of physicochemical parameters of BG-11, RSW, and RSW+Mn for *Chlorella minutissima*

Table 2. *C. minutissima* culture mediums average physicochemical parameters

Parameter/ Culture medium	BG-11	RSW	RSW+Mn
Temperature (°C)	24.59±1.23^{A}	24.32±1.26^{A}	24.42±1.26^{A}
pH	8.06±0.25^{A}	8.42±0.41^{B}	8.41±0.36^{B}
Salinity (ppt)	0.76±0.13^{A}	0.50±0.00^{B}	0.50±0.00^{B}
Conductivity (µScm^{-1})	1499.01±227.69^{A}	992.35±31.86^{B}	976.45±15.99^{B}

Letters A-B indicates significant differences between samples (P <0.05)

Table 3. Growth parameters of *C. minutissima*

Abbreviations	BG-11	RSW	RSW+Mn
µ	0.097±0.011^{A}	0.070±0.010^{A}	0.068±0.016^{A}
Q_x	0.0046±0.0006^{A}	0.0034±0.0001^{A}	0.0041±0.0005^{A}
t_d	7.05±0.98^{A}	10.18±1.67^{A}	10.96±3.04^{A}
P_b	0.000054±0.000024^{A}	0.000064±0.000021^{A}	0.000117±0.000047^{A}

µ= specific growth rate, Q_x= productivity, t_d= doubling time, P_b= biomass productivity

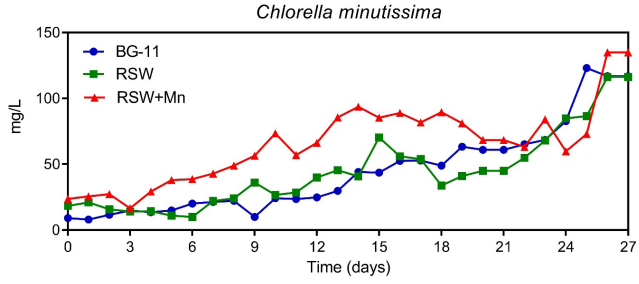

Figure 4. Time-dependent change of *C. minutissima* dry biomass

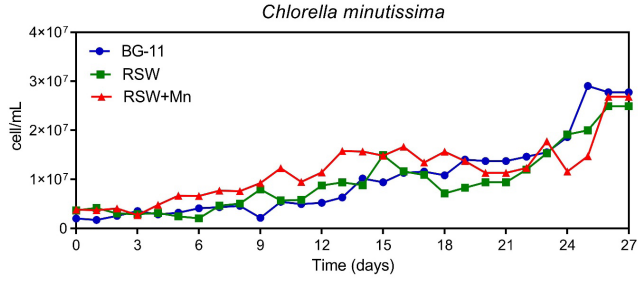

Figure 5. Time-dependent change of C. minutissima cell number

wastewaters contained total nitrogen ranging from 185-3213 mgL^{-1} and total phosphorus around 30-987 mgL^{-1}. In contrast, domestic secondary effluent had a relatively low concentration of both total nitrogen (15-90 mgL^{-1}) and total phosphorus (5-20 mgL^{-1}). *Chlorella* sp. demonstrates adaptability to various wastewater sources. For instance, Wang et al. (2010) cultivated *Chlorella* sp. in municipal wastewater, reporting effluent from an aeration tank to contain nitrite (0.074±0.003 mgL^{-1}) and nitrate (16.95±0.07 mgL^{-1}). In contrast, Yu et al. (2019) used anaerobic digestion effluent containing high ammonium (40 mgL^{-1}) for *Chlorella vulgaris* and *Chlorella protothecoides*. A pH of 5.7 to 6.5 was sufficient for optimal growth of *Chlorella pyrenoidosa* grown in anaerobically digested activated sludge. However, when pH levels increased above 9.1 to 9.6 it was unable to grow in the wastewater (Tan et al., 2016). *Chlorella vulgaris* was used for removal of toxic chemicals from tannery wastewater, as well (Das et al., 2017). Temperature, pH, electrical conductivity, ammonium-nitrogen, and phosphate of the diluted tannery wastewater were found to be 15-20 °C, 7.78±0.20, 2.19±0.16 mScm^{-1}, 8.12±0.60 mgL^{-1} and 10.68±1.63 mgL^{-1}, respectively (Subashini and Rajiv, 2018).

Growth trend of *Chlorella minutissima*

As shown in Figures 4 and 5, *C. minutissima* exhibited higher growth rates or cell densities in RSW and RSW+Mn media compared to BG-11. A distinct separation in algal dry biomass concentrations between cultures was observed from day 3 to day 22, when comparing all media used for *C. minutissima* cultivation. Figure 5 illustrates a time-dependent increase in cell number of *C. minutissima*, with all culture media exhibiting either exponential or linear growth patterns. The maximum cell concentration of *C. minutissima* was observed as (2.70±1.17)×10^{7} cellsmL^{-1} in the RSW+Mn culture medium on the 27th day of the study. No statistically significant differences (P >0.05) were found in *C. minutissima* cell concentrations among all culture media. The average biomass of all culture mediums was measured 51.62±38.40 mgL^{-1} in BG-11, 42.06±27.53 mgL-*1* in RSW, and 65.61±29.49 mgL^{-1} in RSW+Mn.

Dry weight of biomass is one of the most important parameters for assessing biomass yield in microalgae culture (Chioccioli et al., 2014). Interestingly, Mutanda et al. (2011) reported no statistically significant difference in growth parameters between *Chlorella* spp. cultured in BG-11 and post-chlorinated wastewater, despite observing a higher biomass yield (116.3 mgL^{-1}) in the post-chlorinated medium compared to BG-11 (69.9 mgL^{-1}). *Chlorella* sp. was used as a phytoremediation species and it can produce biomass in highly concentrated municipal wastewater. *Chlorella* sp. exhibited a high biomass concentration of 86 mgL^{-1} (Li et al., 2011). In another study, Cabanales et al. (2013) investigated the use of five distinct stages of domestic wastewater depuration for eliminating nutrient salts while producing *Chlorella vulgaris* biomass. They reported biomass yields ranging from 39 to 195 mgL^{-1} dry weight per day (mgL^{-1} dW day^{-1}), similar to the values observed in our study. A study reported biomass yields of 0.1 gL^{-1} dW day^{-1} for *C. vulgaris*, 0.4 gL^{-1} dW day^{-1} for *Scenedesmus obliquus*, and 0.9 gL^{-1} dW day^{-1} for a consortium of Chlorella, Chaetophora, Scenedesmus, and Navicula when cultivated using urban wastewater in a photobioreactor (Gouveia et al., 2016). *C. minutissima* has gained attention for its ability to serve

two purposes: remediating wastewater by removing nutrient salts and producing microalgae biomass, even when cultivated in saline aquaculture water. A study reported that the cell density of microalgae increased almost fivefold during wastewater treatment, reaching a peak five times higher than the initial concentration after 10 days (Hawrot-Paw et al., 2020). In another study, *Scenedesmus* sp., *C. variabilis*, and *C. sorokiniana* were applied to tannery wastewater to produce biomass, which could then be used for biofuel production. *Scenedesmus* sp., *C. variabilis*, and *C. sorokiniana* cultivated in different tannery wastewater concentrations exhibited substantial growth, as evidenced by increased cell density, chlorophyll content, and sugar content, compared to the control group. *C. sorokiniana* displayed impressive growth in a short period, achieving a threefold increase in biomass compared to the BG-11 control group within just 16 days (Nagi et al., 2020). There were no statistically significant differences among the groups when it comes to microalgal growth parameters as seen in Table 3 [F(2,9) = 0.1272], (*P*=0.8821). Researchers found that the specific growth rate ranged from 0.289 to 0.408 day^{-1} after adhering to photoheterotrophic fermentation and adding glycerin to the culture medium (Yang et al., 2011). BG-11 medium supported faster growth, higher productivity, and potentially greater overall biomass production compared to RSW and RSW+Mn mediums. Doubling time is inversely related to growth rate, the higher μ value in BG-11 suggests a potentially shorter doubling time compared to RSW and RSW+Mn mediums.

Physicochemical parameters of *Botryococcus braunii* culture medium

The average physicochemical parameters of the *B. braunii* cultures in BG-11, RSW, and RSW+Mn media are presented in Table 2, while Figure 6 illustrates the trends observed in these parameters throughout the experiment. Temperature remained consistent across all culture media (BG-11, RSW, and RSW+Mn) throughout the experiment, with no statistically significant differences observed [F=(2, 81)= 0,2701] (*P* >0.05). For conductivity and salinity, BG-11 was found statistically important compared to other culture mediums [F(2, 81)= 96.79], (*P* <0.05). When it comes to pH, among all groups were found statistically significant differences (*P* <0.05). The maximum pH level was determined as 8.87±0.07 at RSW medium on the last day of experiment while minimum pH level was measured as 7.66±0.04 on the 5th day of experiment. A statistically significant difference was found between BG-11 and other mediums in terms of pH [F(2.81)= 2795] (*P* <0.05). Figure 6 shows physicochemical parameters of *B. braunii* in different culture mediums over time. The average physicochemical parameters of *B. braunii* culture can be seen in Table 4 below.

Repeated measures ANOVA revealed a statistically significant effect of treatment on temperature [F(1.049, 28.33)= 23.25], (*P* <0.0001), pH [F(2.142, 57.83)= 59.31], (*P* <0.0001), conductivity [F(1.148, 31.00)= 831.4], (*P* <0.0001), and salinity [1.086, 29.32)= 32.47], (*P* <0.0001) over time.

All three mediums had similar average temperatures with some small variations. BG-11 medium had a slightly lower pH compared to RSW and RSW+Mn. While *B. braunii* tolerates a wide pH range, it generally prefers slightly acidic conditions (pH 6-6.5) to produce hydrocarbons (Nugroho et al., 2020). BG-11 had slightly higher salinity (0.66 ppt) compared to RSW (0.59 ppt) and RSW+Mn (0.58 ppt), but the differences are minor. BG-11 had the highest conductivity (around 1325 μScm^{-1}), followed by RSW (around 1180 μScm^{-1}) and RSW+Mn (around 1153 μScm^{-1}). This difference in nutrient composition might influence *B. braunii* growth and other parameters. A culture medium temperature of 23 °C was determined to be the optimum temperature for growing *B. braunii* (Qin and Li, 2006). Yoshimura et al. (2013) found the growing temperature of *B. braunii* strain SHOWA between 5 to 35 °C and optimum growth temperature was determined as 30 °C. Tarhan et al. (2021) tried to grow *C. minutissima* and *B. braunii* using different dilutions (50x, 100x 200x, and 400x) of orange and olive pomace aqueous phases. At low dilution rates they found shorter generation times and higher growth rates for microalgae. In another study, *B. braunii* strain CHN 357 was cultured at different temperatures among 20 to 30 °C and the optimum temperature was found as 23 °C (Qin and Li, 2006). The highest EC was measured as 1385.33±46.58 μScm^{-1} at the end of the study in BG-11 medium. Similarly, Órpez et al. (2009) found the EC as 978 μScm^{-1} in secondarily treated sewage wastewater in the study of production performance of *B. braunii*. The growth performance of *B. braunii* strain BOT-22 was also evaluated in soybean curd wastewater (SCW). SCW is diluted as 1%, 2%, 5%, and 10% and compared to control AF-6 medium's microalgae biomass performance. SCW medium ion concentration was reported as 3 mgL^{-1} ammonium, 100 mgL^{-1} phosphate, 92 mgL^{-1} sulfate, 35 mgL^{-1} magnesium, 1280 mgL^{-1} potassium, 366 mgL^{-1} calcium, and 41 mgL^{-1} sodium. Compared to AF6 microalgae culture medium like the one used in that study, SCW yielded better biomass results with its nutrient variables (Yonezawa et al., 2012). In another study, secondarily treated sewage (STS) was used as *B. braunii* culture medium in the batch culture system. STS derivatives were found to have better concentrations of nitrite, ammonium, conductivity, and total phosphorus compared to CHU 13 microalgae culture medium (Sawayama et al., 1992). Aerated swine lagoon wastewater without sterilization and pH adjustment was also tested as an alternative *B. braunii* culture medium for open microalgae production systems. When aerated swine lagoon wastewater (ASLW) is compared with swine lagoon wastewater (SLW), it was observed that there are significant differences in terms of pH, conductivity, dissolved oxygen, nitrate-nitrogen, ammonium-nitrogen, total nitrogen, and total phosphorus. Similarly to the present study, *B. braunii* cultivation was made at 25 °C temperature and light intensity of 120 μmol photons $m^{-2}s^{-1}$ (Liu et al.,

Table 4. *B. braunii* culture mediums average physicochemical parameters

Parameter/ Culture medium	BG-11	RSW	RSW+Mn
Temperature (°C)	26.75±1.68^{A}	26.52±1.56^{A}	26.81±1.55^{A}
pH	8.03±0.34^{A}	8.64±0.19^{B}	8.51±0.15^{B}
Salinity (ppt)	0.66±0.05^{A}	0.59±0.05^{B}	0.58±0.04^{B}
Conductivity (μScm^{-1})	1325.16±44.00^{A}	1180.00±60.91^{B}	1153.75±43.43^{B}

Letters A-B indicates significant differences between samples (*P* <0.05)

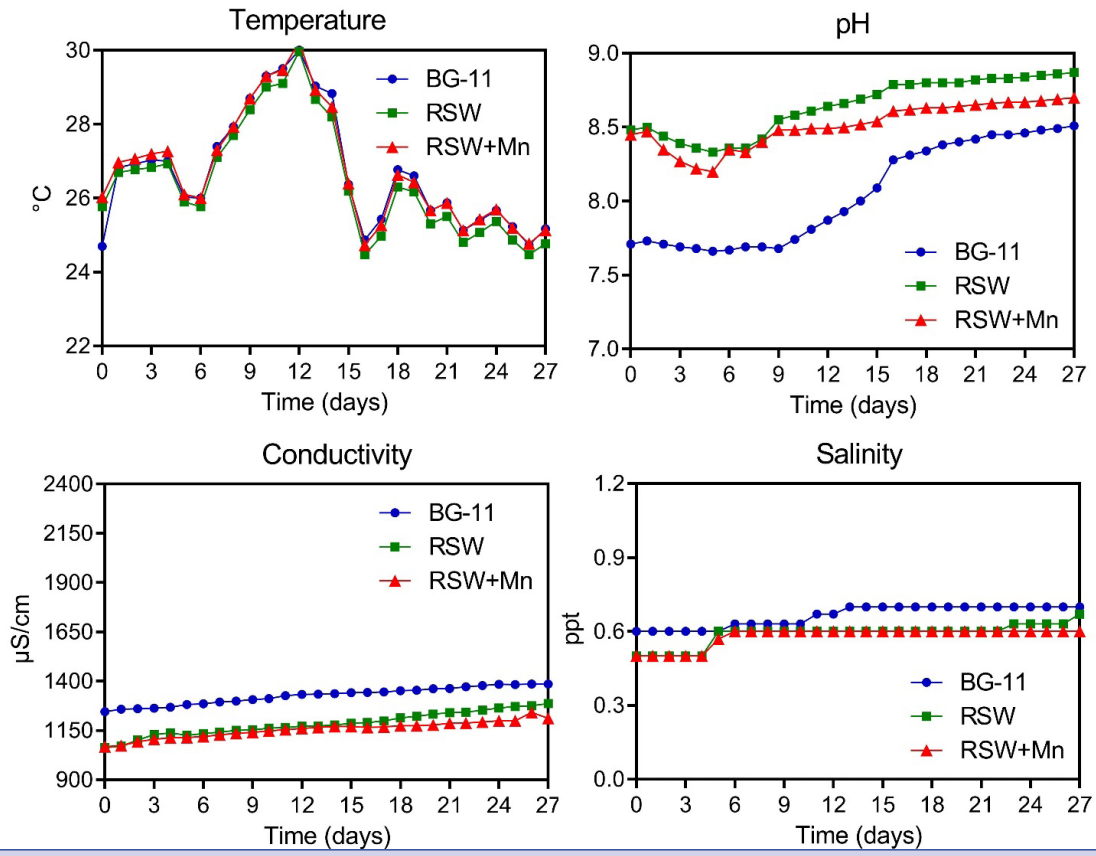

Figure 6. Time-dependent change of physicochemical parameters of BG-11, RSW, and RSW+Mn for *Botryococcus braunii*

2013). A pretreated or untreated wastewater resource can be an effective microalgae medium, since it can be used for microalgae cultivation at a low cost.

Growth trend of *Botryococcus braunii*

Dry biomass and cell concentration changes of *B. braunii* grown in different culture mediums are given in Figure 7 and Figure 8, respectively. Mean dry biomass of *B. braunii* obtained from BG-11, RSW, and RSW+Mn were calculated as 379.40±200.12 mgL^{-1}, 385.19±305.01 mgL^{-1}, 130.20±138.63 mgL^{-1}, respectively. As can be seen in Figure 7, *B. braunii* grew better in the RSW medium compared to others. The 21st day of the experiment revealed the highest cell concentration of *B. braunii* in RSW+Mn medium at 3.6×105±5.3×104 $cellsmL^{-1}$. 21 days of *B. braunii* production in the RSW culture medium can be considered sufficient.

After the 18th day of the experiment, the biomass yield of the *B. braunii* cultured in RSW medium, skyrocketed. There were no statistically significant differences among the groups when it comes to microalgal growth parameters as seen in Table 5 [$F(2,9) = 0.5292$], ($P = 0.6063$).

RSW medium had the highest specific growth rate compared to BG-11 and RSW+Mn. This suggests *B. braunii* grew faster in RSW. Similar to specific growth rate, RSW medium had the highest productivity compared to BG-11 and RSW+Mn mediums. RSW medium (8.04±0.47) has the shortest doubling time, followed by BG-11 (11.78±2.59) and RSW+Mn (13.31±0.80). This aligns with the trend observed in specific growth rate. Biomass productivity of the BG-11 medium was found as the highest when compared to RSW and RSW+Mn.

In a study investigating the biomass production performance of secondarily treated piggery wastewater, the removal of nitrogen-phosphorus and produce *B. braunii* biomass was found advantageous and sustainable. In that study, biomass production was found as between 1 gL^{-1} and 7.5 gL^{-1} in different nitrogen concentrations (102 $mgNL^{-1}$, 204 $mgNL^{-1}$, 510 $mgNL^{-1}$, +1020 $mgNL^{-1}$) in batch culture (An et al. 2003). Nitrogen concentrations of secondarily treated wastewaters were found much higher compared to this study and low values of biomass may be caused by the lack of nitrogenous compounds in the culture mediums. Órpez et al. (2009) grew *B. braunii* in secondarily treated sewage wastewater at

Table 5. Growth parameters of *B. braunii*

Abbreviations	BG-11	RSW	RSW+Mn
µ	0.061±0.012^{A}	0.086±0.0052^{A}	0.052±0.0031^{A}
Q_x	0.033±0.023^{A}	0.044±0.0062^{A}	0.013±0.0015^{A}
t_d	11.78±2.59^{A}	8.04±0.47^{A}	13.31±0.80^{A}
P_b	0.0086±0.009^{A}	0.0062±0.0008^{A}	0.0021±0.00019^{A}

µ= specific growth rate, Q_x= productivity, t_d= doubling time, P_b= biomass productivity

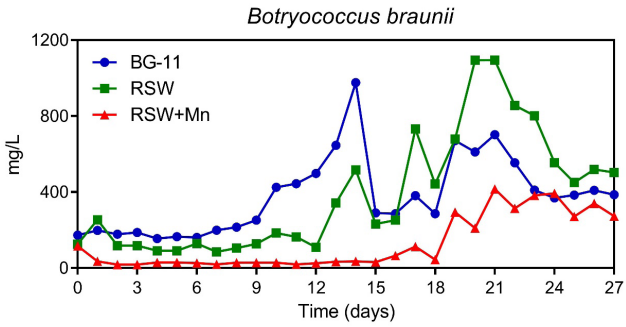

Figure 7. Time-dependent change of *B. braunii* dry biomass

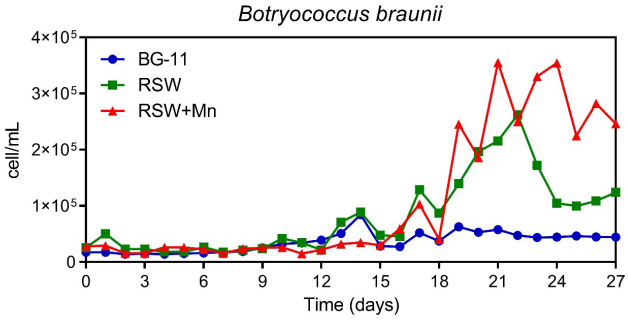

Figure 8. Time-dependent change of *B. braunii* cell number

pH 8 and maximum specific growth rate was found as 0.21 gL^{-1} day^{-1}. Secondarily treated sewage wastewater showed similar findings to this study with its nutrient concentrations like ammonium (15 mgL^{-1}), nitrates (0.9 mgL^{-1}), nitrites (0.14 mgL^{-1}), phosphates (11.5 mgL^{-1}). In a research that aims carotenoid production from *B. braunii* at different light intensities (100 and 500 µmol photons $m^{-2}s^{-1}$), and in various culture mediums (modified CHU 13 medium, modified CHU 13 medium without nitrogen, and modified CHU 13 without N+2Fe), the highest biomass yield was determined as 0.6 gL^{-1} on day 16 at the highest light intensity (Indrayani et al., 2022). When compared to this study, similar results were found with the biomass of *B. braunii* cultured in BG-11 medium. Qin and Li (2006) reported specific growth rates between 0.061±0.003 - 0.095±0.003 which was very similar to this study.

Physicochemical parameters of *H. pluvialis* culture medium

The trend differences among physicochemical parameters of BG-11, RSW, and RSW+Mn culture mediums for culturing *H. pluvialis* were shown in Figure 9. The average physicochemical parameters of the study were shown in Table 3. While the maximum temperature was determined as 26.83±0.05 °C at RSW+Mn culture medium on the 3rd day, the minimum temperature was observed as 22.13±0.05 °C at BG-11 medium on the 17th day of the experiment. In this study, temperature was held at appropriate levels. The maximum pH level was determined as 8.57±0.07 at RSW medium on the 8th day, and the minimum pH level was measured as 7.41±0.03 at RSW+Mn in the beginning of the experiment. In RSW+Mn medium, the highest conductivity was measured as 2316.33±30.07 µScm^{-1} at the end of the study, while the lowest conductivity was measured as 1070.33±3.40 µScm^{-1} on the first day of the experiment. The average physicochemical parameters of *H. pluvialis* culture can be seen in Table 6 below.

Table 6. *Haematococcus pluvialis* culture mediums physicochemical parameters

Parameter/ Culture medium	BG-11	RSW	RSW+Mn
Temperature (°C)	24.26±1.27^{A}	24.35±1.25^{A}	24.54±1.26^{A}
pH	7.83±0.15^{A}	8.27±0.35^{B}	8.22±0.32^{B}
Salinity (ppt)	0.79±0.05^{A}	0.58±0.04^{B}	1.24±0.70^{C}
Conductivity (µS/cm)	1597.91±70.93^{A}	1146.29±35.63^{B}	2207.35±54.02^{C}

Letters A-C indicates significant differences between samples (P <0.05)

BG-11, RSW, and RSW+Mn had very similar average temperatures with some variations. Temperature wasn't a differentiating factor for *H. pluvialis* growth in the experiment. BG-11 medium had a slightly lower pH compared to RSW and RSW+Mn. *H. pluvialis* can tolerate a wide range of pH (6-8.5) (Do et al., 2021). The statistically significant differences suggest that the pH levels in the BG-11 medium are different from both RSW and RSW+Mn. RSW+Mn had a significantly higher salinity (1.24 ppt) compared to both BG-11 and RSW mediums. The statistically significant differences suggest variations in the amount and type of dissolved nutrients between the mediums. BG-11 and RSW+Mn likely have higher concentrations of dissolved salts compared to RSW medium.

Optimum growth temperature of *H. pluvialis* was found between 25-28 °C in a study that aims to find optimal temperature and irradiance for *H. pluvialis* (Fan et al., 1994). 35 °C had detrimental effects on *H. pluvialis* cells according to Borowitzka et al. (1991). The beginning pH level of the culture medium for *H. pluvialis* was determined as 7.5, according to Choi et al. (2017).

Growth trend of *H. pluvialis*

The maximum *H. pluvialis* cell concentration was elicited as 8.9×10^{4}±3.2×10^{4} in RSW+Mn medium on the 20th day of the study. The dry weight of biomass obtained from BG-11, RSW, and RSW+Mn mediums were calculated as 70.62±20.29 mgL^{-1}, 169.42±84.21 mgL^{-1}, 631.52±336.90 mgL^{-1}, respectively (Figure 10 and Figure 11). As can be seen in Figure 10, the highest dry biomass increase was found at the RSW+Mn medium at the 20th day of the experiment for *H. pluvialis*. Some green cells transitioned to aplanospore stage but more than 90% of the cells never transitioned into the red (astaxanthin production) stage. There were no statistically significant differences among the groups when it comes to microalgal growth parameters as seen in Table 7 [$F(2,9) = 0.6714$], (P =0.5348).

There was no statistically significant difference between RSW (0.079±0.0081) and RSW+Mn (0.080±0.015) medium, which have the higher specific growth rates. This suggests *H. pluvialis* grew slower in BG-11 medium. Similar to growth rate, RSW+Mn medium (0.045±0.017) has the highest productivity, followed by RSW (0.010±0.0057) and BG-11 (0.0030±0.0001). This indicates that RSW+Mn supported the highest rate of biomass production. A lower doubling time signifies faster

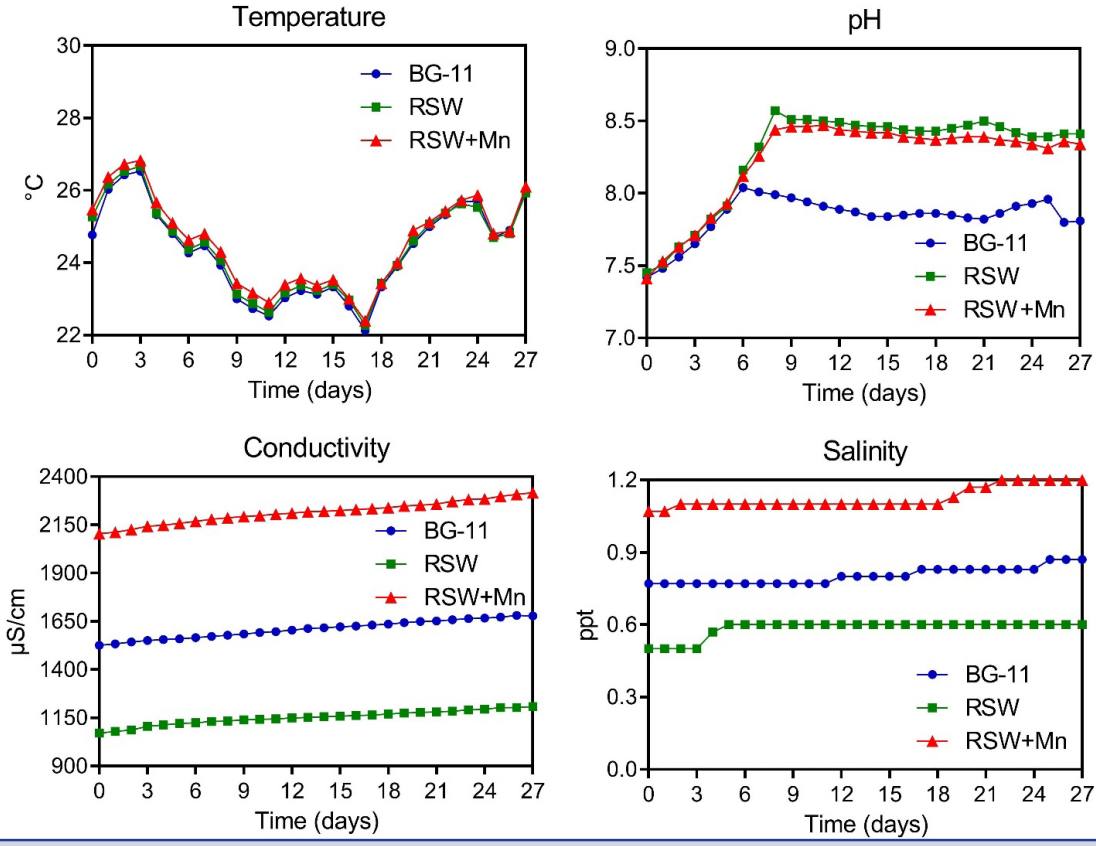

Figure 9. Time-dependent change of physicochemical parameters of BG-11, RSW, and RSW+Mn for *H. pluvialis*

Table 7. Growth parameters of *H. pluvialis*

Abbreviations	BG-11	RSW	RSW+Mn
μ	0.044±0.012^{A}	0.079±0.0081^{A}	0.080±0.015^{A}
Q_x	0.0030±0.0001^{A}	0.010±0.0057^{A}	0.045±0.017^{A}
t_d	17.40±5.69^{A}	8.83±0.93^{A}	8.92±1.44^{A}
P_b	0.00020±0.00013^{A}	0.00074±0.00083^{A}	0.012±0.011^{A}

μ= specific growth rate, Q_x= productivity, t_d= doubling time, P_b= biomass productivity

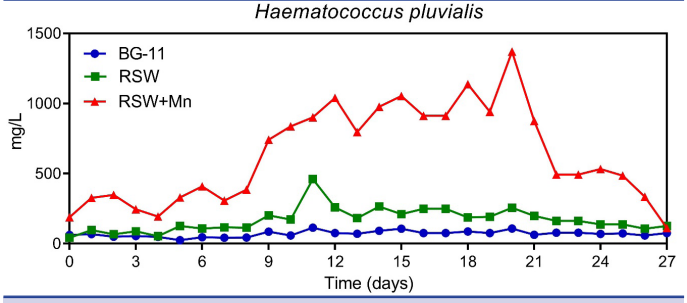

Figure 10. Time-dependent change of *H. pluvialis* dry biomass.

population growth. RSW medium (8.83±0.93) has the shortest doubling time, followed by RSW+Mn (8.92±1.44) and BG-11 (17.40±5.69). This aligns with the trend observed in specific growth rate. RSW+Mn medium showed the highest biomass productivity (0.012±0.011).

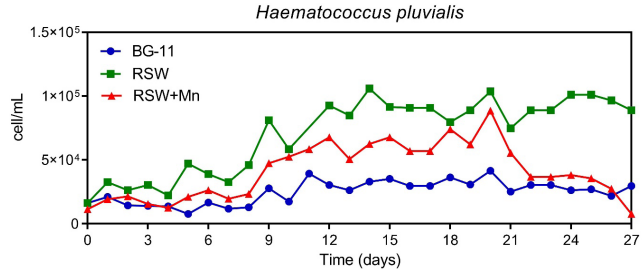

Figure 11. Time-dependent change of *H. pluvialis* cell number.

In general two-step production policy followed for commercial cultivation. In the first stage of production, the vegetative process is maximized. At the second stage, stress conditions are tried to be provided for astaxanthin production (Shah et al., 2019). Only vegetative processes were studied in this study. The results of a study which was related to 32 days of cultivation of *H. pluvialis* using standard laboratory prepared wastewater under different light spectrums has a slightly better performance in terms of cell concentration (51×10^4 cellsmL^{-1}) than this study (Mourya et al., 2023). The maximum biomass concentration was found as 27.8 mgL^{-1} day^{-1} cultured in a domestic secondary treated effluent for *H. pluvialis* (Wu et al. 2013). In another study, cas-

sava wastewater obtained from three different cassava producers diluted to different concentrations (2.5%, 5%, and 10%) to produce microalgae. The highest biomass was obtained from 2.5% diluted CW medium as 9.31 cells mL^{-1} (Rodrigues et al., 2021).

Statistical analyzes revealed significant differences in cell densities among the tested microalgae species (*C. minutissima*, *B. braunii*, and *H. pluvialis*) across varying culture media (BG-11, RSW, and RSW+Mn) $F(27, 504)= 17.09$, ($P <0.0001$). As expected, *C. minutissima* exhibited higher cell densities compared to both *B. braunii* and *H. pluvialis*. Furthermore, the RSW+Mn medium negatively impacted *C. minutissima* growth, resulting in significantly reduced cell densities compared to BG-11 and RSW media $F(8, 504)= 299.3$, ($P <0.0001$). This effect was specific to *C. minutissima*, as the other species did not exhibit the same sensitivity to the RSW+Mn medium.

Two-way ANOVA and Tukey's HSD post-hoc test revealed significant effects of all microalgae species (*C. minutissima, B. braunii,* and *H. pluvialis*) and culture mediums (BG-11, RSW, and RSW+Mn) on biomass productivity $F(27, 504)= 5.182$, ($P <0.0001$). *C. minutissima* exhibited significantly greater biomass productivity in BG-11 compared to *B. braunii* ($P <0.0001$). *H. pluvialis*, however, achieved maximal biomass productivity in RSW+Mn, significantly outperforming all other species-medium combinations $F(8, 504)= 68.84$, ($P <0.0001$). The significant interaction effect between species and medium ($P <0.0001$) highlights the differential responses of these microalgae to varying culture conditions.

Nutritional value of microalgae in aquaponics remineralized sludge-water

Nutritional value of *C. minutissima, B. braunii* and *H. pluvialis* were determined and discussed below following headings. In Table 8, 9, and 10 are shown comparison of the biochemical contents of *C. minutissima*, *B. braunii*, and *H. pluvialis*, respectively. Microalgae's biochemical composition can be affected by multiple factors, such as nutrient concentration, composition, light intensity, and temperature.

Crude protein

C. minutissima crude protein concentrations were determined for BG-11, RSW, and RSW+Mn groups as 51.88±0.32%, 55.77±1.81%, and 21.53±0.70%, respectively. *C. minutissima* cultured in RSW+Mn showed a statistically significant difference from other cultures in terms of crude protein levels. RSW medium was found to have the best protein concentration compared to other groups for *C. minutissima*. *B. braunii* crude protein contents were determined for BG-11, RSW, and RSW+Mn groups as 49.77±0.62%, 38.50±0.78%, and 37.39±0.61%, respectively. Comparatively, *B. braunii* cultured in BG-11 medium exhibited statistically significant difference ($P <0.05$). *H. pluvialis* crude protein contents in BG-11, RSW, and RSW+Mn were calculated as 40.61±4.06%, 33.86±2.75%, and 30.25±0.84%, respectively. BG-11 culture medium had statistically significant difference compared to other RSW culture mediums for *H. pluvialis* ($P <0.05$).

The lowest protein contents in this study were obtained from *H. pluvialis*. Nitrogen to protein conversion factor (ki) have determined as 4.78 by Lourenço et al. (2004) and the factor mostly used in microalgae studies. In this study, results were also calculated using a 4.78 nitrogen to protein conversion factor. The presence of trace elements in the culture medium can result in a high ash content in the biomass and a reduction in protein concentration (Schüler et al., 2020). And low nitrogen concentration in the culture medium causes low crude protein in the biomass (Ördög et al., 2012). Freeze-dried Chlorella 71105 strain grown in standard culture medium was found to contain 55.5% crude protein (Lubitz, 1963). This percentage of protein concentration is similar to this study's conclusion. Protein concentrations of different culture groups of *B. braunii* were shown to be similar and relatively high results when compared to other studies (Cabanelas et al., 2015; Ashokkumar and Rengasamy, 2012). Sydney et al., (2011) investigated *C. vulgaris* and *B. braunii* microalgae species biodiesel production potential using domestic wastewater and they found the maximum protein content as 40.4% of biomass. It is possible that macronutrients and micronutrients obtained from RSW can influence the nutritional value of microalgae. All microalgae species cultured in an RSW medium can be evaluated as having the potential to be a raw material in different sectors with their high protein concentrations.

Total lipids

C. minutissima cultured in BG-11, RSW, and RSW+Mn the total lipid contents were determined as 4.18±0.33%, 4.69±0.88%, and 1.35±0.48%, respectively. Comparing RSW+Mn with other groups, a statistically significant difference was found ($P <0.05$). *B. braunii* is known for the production of hydrocarbons and high levels of lipids. However, cultured *B. braunii* in BG-11, RSW, and RSW+Mn mediums the total lipid contents were found as 0.62±0.11%, 0.48±0.11%, and 0.53±0.31%, respectively. Finally, *H. pluvialis* BG-11, RSW, and RSW+Mn culture groups' total lipid contents were found as 0.57±0.30%, 0.70±0.07%, and 0.11±0.02%, respectively. No statistically significant difference was found among all culture mediums regarding total lipids for *H. pluvialis* and *B. braunii* ($P >0.05$).

Lipid contents of the all microalgae species were found relatively low when compared to other studies (Sonkar et al., 2023; Liang et al., 2015; Jackson et al., 2020). Microalgae cultures under controlled conditions that promote biomass multiplication resulted in a low total lipid concentration (Mularczyk et al., 2020). Different Chlorella strains which have 40% crude protein, 20-25% carbohydrates, and 20-26% lipids were reported grown at seawater based F2 medium in outdoor cultivation (Guccione et al. 2014). *C. vulgaris* which is grown at modified Fitzgerald medium (Hughes et al. 1958) showed 40-55% lipid composition with nitrogen depletion in medium (Widjaja, 2009). In another study, *C. vulgaris* cultured in artificial wastewater medium had 42% average lipid content and the lipid productivity was 147 $mgL^{-1}day^{-1}$ (Feng et al., 2011). Giraldo et al. (2021) reported that high bicarbonate dosages increased biomass and lipid productivity in *B. braunii*. In another study, total lipid concentration of *B. braunii* was found higher than 40% (Cheng et al., 2013). Damiani et al. (2010) investigated the impact of continuous high light intensity with nitrogen-sufficient medium and high light intensity with nitrogen-deprivation medium on the total lipid content (dry weight) and they found 34.85% and 32.99%, respectively. Similar to other studies, the high light intensity and nitrogen deprivation have the ability to change the lipid production (46.71%-

56.92%) of *H. pluvialis*, dramatically (Liang et al., 2015). All microalgae species used in this study had low total lipid concentrations compared to above studies.

Total carbohydrates

Total carbohydrate concentrations of *C. minutissima* were determined in BG-11, RSW, and RSW+Mn as 18.66±3.68%, 16.90±0.99%, and 20.20±5.47%, respectively. *B. braunii* total carbohydrate contents were calculated in BG-11, RSW, and RSW+Mn as 23.67±1.89%, 39.14±2.43%, and 31.32±2.35%, respectively. *H. pluvialis* total carbohydrate content was found in BG-11, RSW, and RSW+Mn as 34.94±5.04%, 27.94±6.75%, and 28.52±0.80%, respectively. In comparing all culture mediums for *C. minutissima* and *B. braunii*, there was no statistically significant difference (P >0.05). However, BG-11 exhibited a statistically significant difference for *H. pluvialis* (P <0.05).

The dominant energy storage products in Chlorophytic microalgae are carbohydrates and oils (Subramanian et al., 2013). The low lipid content in dry microalgal biomass can be explained by the high carbohydrate produced within the microalgae cells. It has been suggested by Freitas et al. (2017) that arabinose and xylose can be used as carbon sources for microalgal cultures to increase the amount of carbohydrates in biomass (53.8%). In another study, Andreeva et al. (2021) reached 47.9% carbohydrate content in *Chlorella vulgaris* biomass using carbohydrate additives (a mixture of glucose, fructose, sucrose, and maltose). The total carbohydrate concentration was determined between 20 to 76% in 16 different *B. braunii* strains (Gouveia et al., 2017). The maximum sugar content of *B. braunii* was found to be 28.96% in treated domestic sewage wastewater, which was lower than the carbohydrate content of this study (Sydney et al., 2011). It has been understood that the content of microalgae culture medium directly affects the carbohydrate content of microalgae.

Moisture

C. minutissima, cultured in BG-11, RSW, and RSW+Mn culture mediums, moisture content was determined as 11.83±0.18%, 11.09±1.01%, respectively. *B. braunii* moisture content was calculated in BG-11, RSW, and RSW+Mn as 16.76±2.67%, 15.43±0.17%, and 21.59±0.51%, respectively. *H. pluvialis* moisture content was found in BG-11, RSW, and RSW+Mn as 10.76±1.04%, 17.22±0.90%, and 30.10±1.61%, respectively. No statistically significant difference among all culture mediums for *C. minutissima* and *B. braunii* (P >0.05), but BG-11 showed statistically significant difference for *H. pluvialis* (P <0.05).

Fresh algal cells constitutes around 70-95% water after centrifugation (Da Silva et al., 2008). Hosseinizand et al. (2017) stated that the moisture content of Chlorella should be decreased from 35-75% to 10% due to preservation of the biochemical properties. Chlorella species moisture content were decreased using hot air drying from 70.38±2.90% to 0.88±0.05% and using freeze drying from 70.38±2.90% to 3.58±0.19% (Stramarkou et al., 2017).

Ash content

As shown in these tables below, *C. minutissima* ash contents in BG-11, RSW, and RSW+Mn were determined as 11.84±0.19%, 11.09±1.01%, and 55.94±4.57%, respectively. *B. braunii* ash content was calculated in BG-11, RSW, and RSW+Mn groups as 16.76±2.67%, 15.43±0.17%, and 21.59±0.51%, respectively. *H. pluvialis* ash content was found in BG-11, RSW, and RSW+Mn as 10.76±1.04%, 17.22±0.90%, and 30.10±1.61%, respectively. There was no statistically significant difference among all culture mediums for *C. minutissima* and *H. pluvialis* (P >0.05). However, it was determined that there was a statistically significant difference in the RSW and RSW+Mn groups used in the production of *B. braunii* species when compared to BG-11 (P <0.05).

The highest ash content was found in RSW+Mn medium similar to another study which used landfill leachate based mediums (dos Santos et al., 2022). With this study, it is understood that high conductivity and salinity concentrations in the culture mediums caused an increase of the ash content of harvested spe-

Table 8. *C. minutissima* nutrition facts in different culture mediums

Descriptions	BG-11 (%)	RSW (%)	RSW+Mn (%)
Crude protein	51.88±0.32^{A}	55.77±1.81^{A}	21.53±0.70^{B}
Total lipid	4.18±0.33^{A}	4.69±0.88^{A}	1.35±0.48^{B}
Total carbohydrate	18.66±3.68^{A}	16.90±0.99^{A}	20.20±5.47^{A}
Moisture	10.60±0.33^{A}	10.84±0.88^{A}	10.90±0.48^{A}
Ash	11.84±0.19^{A}	11.09±1.01^{A}	55.94±4.57^{A}

Letters A-B indicates significant differences between samples (P <0.05)

Table 9. *B. braunii* nutrition facts in different culture mediums

Descriptions	BG-11 (%)	RSW (%)	RSW+Mn (%)
Crude protein	49.77±0.62^{A}	38.50±0.78^{A}	37.39±0.61^{A}
Total lipids	0.62±0.11^{A}	0.48±0.11^{A}	0.53±0.31^{A}
Total carbohydrates	23.67±1.89^{A}	39.14±2.43^{A}	31.32±2.35^{A}
Moisture	11.41±0.11^{A}	7.20±0.11^{A}	11.49±0.31^{A}
Ash	16.76±2.67^{AB}	15.43±0.17^{A}	21.59±0.05^{B}

Letters A-B indicates significant differences between samples (P <0.05)

Table 10. *H. pluvialis* nutrition facts in different culture mediums

Descriptions	BG-11 (%)	RSW (%)	RSW+Mn (%)
Crude protein	40.61±4.06^{A}	33.86±2.75^{B}	30.25±0.84^{B}
Total lipid	0.57±0.30^{A}	0.70±0.07^{A}	0.11±0.02^{A}
Total carbohydrate	34.94±5.04^{A}	27.94±6.75^{AB}	28.52±0.80^{B}
Moisture	13.11±0.30^{A}	10.28±0.07^{B}	11.03±0.02^{AB}
Ash	10.76±1.04^{A}	17.22±0.90^{A}	30.10±1.60^{A}

Letters A-B indicates significant differences between samples (P <0.05)

cies. It may be possible to use leachate and effluent-based wastes as microalgae culture mediums, however the biomass would have a higher ash content than commercially produced microalgae.

CONCLUSION

The comparative study of using BG-11, RSW and RSW+Mn mediums to culture *C. minutissima*, *B. braunii*, and *H. pluvialis* has shown sufficient concentrations and value-added compounds. Removal of nitrogen and phosphorus from remineralized sludge-water of aquaponics to microalgal biomass was successfully achieved. RSW obtained from aquaponics had an advantage to BG-11 microalgae culture medium due to its low cost, easy for obtaining and more environmentally friendliness. Protein composition of *C. minutissima* in RSW had the highest scores compared to other culture mediums (BG-11 and RSW+Mn). While RSW medium was found as an advantageous for *C. minutissima* cultivation due to its high protein content, but *B. braunii* and *H. pluvialis* were characterized by low lipid contents. Because of this reason RSW medium is not recommended for cultivation of *B. braunii* and *H. pluvialis*. *B. braunii* culture was found to have the highest carbohydrate content in the RSW medium. *B. braunii* is known for its low growth rate and long regeneration time. The RSW medium, however, can be an advantageous sustainable resource with its fertile properties for the production of biomass. Crude protein levels of the microalgae species were suitable when compared to other culture mediums in the literature. However, total lipid contents of the microalgae species were found very low, between 0.11±0.02-4.69±0.88%, due to the nitrogen rich culture mediums. RSW culture medium might be suggested as an alternative culture medium for green microalgae production for batchwise systems. When it comes to culture period, it may be suggested that *B. braunii* and *H. pluvialis* be cultured for 20 days using RSW based microalgae mediums, however, *C. minutissima* may require more than 27 days for cultivation batchwise. Additional research is required to find the optimum physico-chemical conditions, remineralization process of RSW and techno-economic analysis of *C. minutissima*, *B. braunii*, and *H. pluvialis* cultured in RSW.

This study partially fulfills the expected objective. *C. minutissima, B. braunii*, and *H. pluvialis* grew well in remineralized sludge water (RSW) medium obtained from nutrient film technique (NFT) aquaponics. All microalgae species effectively removed nutrients form RSW, achieving bioremediation. *C. minutissima* exhibited the highest crude protein content in RSW medium compared to the other cultures. RSW proved to be a low-cost, readily available, and environmentally friendly alternative to the standard BG-11 for *C. minutissima* cultivation. However, all microalgae species had very low lipid content due to the nitrogen-rich RSW medium. Consequently, RSW was not suitable for maximizing lipid production in *B. braunii* and *H. pluvialis*.

Given its characteristically high total oil content and hydrocarbon production, *B. braunii* is a promising candidate for further studies on maximizing oil yield. Future research can investigate the effects of manipulating nutrients, light, carbon source, and stress factors (including high salinity and various trace elements) on oil production for this species in remineralized aquaponics sludge water. *H. pluvialis*, valued for its high concentration of important antioxidant substances like astaxanthin, canthaxanthin, and lutein, is a promising candidate to investigate the effects of remineralized aquaponics wastewater on production of these antioxidants.

In conclusion, microalgae species exhibited differential growth responses and biomass productivities depending on the culture medium. While *C. minutissima* thrived in BG-11 and RSW, *H. pluvialis* achieved superior biomass productivity in the RSW+Mn medium, underscoring the importance of optimizing culture conditions for each species to maximize yields. Finally, the effects of different concentrations of aquaponic wastewater and stress factors on the biomass and biochemical composition of *C. minutissima* can be studied, as well.

Ethics committee approval: This study was ethically reviewed by the Local Ethics Committee for Animal Experiments of the General Directorate of Agricultural Research and Policies, Mediterranean Fisheries Research, Production, and Training Institute. It was approved in compliance with the principles of the Local Ethics Committee Directive for Animal Experiments on January 28, 2019, under decision number 2019/01.

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