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Seawater with Added Monosodium Glutamate Residue (MSGR) Is a Promising Medium for the Cultivation of Two Commercial Marine Microalgae

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Abstract: *Phaeodactylum tricornutum* and *Nannochloropsis oceanica*, with their satisfactory performance in accumulating lipids and other high-value products, have been successfully used for commercial production in recent years. However, costly chemicals in culture media greatly increase the price of the resulting bioproducts. To control the cultivation cost, this paper assessed the potential of seawater supplemented with monosodium glutamate residue wastewater at a ratio of 1/500 (S-MSGR) to serve as a growing medium for these two marine species. Compared with the standard chemical culture medium, Erdschreiber's medium (EM), both the algal growth and metabolite accumulation of *P. tricornutum* and *N. oceanica* were greatly promoted in S-MSGR. The maximum biomass concentrations of *P. tricornutum* and *N. oceanica* reached 0.93 and 0.36 g/L, which were, respectively, 1.5 and 1.9 times higher than those in EM medium. For lipid accumulation, *P. tricornutum* exhibited an excellent lipid productivity of 22.9 mg/L/day in S-MSGR, a 64% increase compared to EM medium. Furthermore, the average yield coefficients indicated good performance of *P. tricornutum* and *N. oceanica* in transferring the nitrogen in S-MSGR to the biomass, at 74.8 and 174.8 mg/g of nitrogen. In addition, compared with EM, the costs of the medium for lipid production of *P. tricornutum* and *N. oceanica* cultured in S-MSGR were USD 2.3 and 5.8/(kg lipid), which saved 96.9% and 97.6%, respectively. Therefore, this paper demonstrates that S-MSGR is a suitable nutrient resource for *P. tricornutum* and *N. oceanica*, and it has a great potential to cut the cultivation cost during real commercial production.

Keywords: monosodium glutamate residue wastewater; biomass; lipid productivity; commercial marine microalga

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1. Introduction

Microalgal biological resources can be widely used in various sectors, including energy, food, animal breeding industry, medicine, cosmetics and environment, giving them great commercial potential and value [1,2]. There are many species of microalgae, which can adapt to living in a variety of environmental conditions and are easy to cultivate. Microalgae characteristically have a shorter growth cycle and less demand for agricultural land than general higher plants [3]. Moreover, compared with lignocellulosic biomass biofuels, the main disadvantages of lignocellulosic biomass biofuels utilization are biological conversion processes with low reaction rates and thermochemical conversion technologies, such as the quality of bio-oil products and low energy efficiency, and low product yield [4,5]. At present, several microalgal species have been used commercially due to their satisfactory performance in biomass and lipid accumulation, such as *Phaeodactylum tricornutum* and *Nannochloropsis oceanica*.

P. tricornutum is a widely studied marine diatom and has been explored to produce products on a pilot scale, including biodiesel, fatty acids of nutraceutical value (eicosapentaenoic acid, EPA and docosahexaenoic acid, DHA) and fucoxanthin [6]. *N. oceanica* is a species of miniature marine microalga with high nutritional value and lipid content, which has drawn attention as a potential feedstock for biofuels and aquaculture [7]. However, the main choice of culture medium for these two marine algae continues to be the use of chemical reagents as the nutrient sources, with the result that the high cost of nutrient supply is still a severe challenge for large-scale commercialization of microalgal biomass [8].

Although microalgae can survive and thrive in wastewater, and simultaneously synthesize lots of valuable metabolites through their ability to assimilate nitrogen (N), phosphorous (P) and other nutrients in wastewater [9], wastewater-based technologies of microalgal cultivation face some challenges, such as the capital consumed in the cultivation process and the selection of wastewater [10]. Recent studies have shown that some kinds of wastewater were successfully used for growing microalgae, including textile wastewater, municipal wastewater, swine wastewater, coal seam gas industrial wastewater and pharmaceutical wastewater [11], which effectively reduces the nutrient cost required for the cultivation of microalgae. For example, Dębowski et al. [12] used digestate from anaerobic digestion of dairy wastewater as culture medium to cultivate microalgae, which obtained 3 g/L biomass, and the lipid content also reached 20%. In addition, recent studies have shown that coke-oven wastewater containing high ammonia nitrogen [13] and monosodium glutamate residue (MSGR) wastewater were also very potential sources of nutrients. From the viewpoint of wastewater selection, it is better for it to contain no heavy metals or other toxic substances to meet the needs of microalgal culture and application after harvest.

MSGR wastewater is a nitrogen- and phosphorus-rich residual wastewater that is generated after glutamate extraction and sterilization in the process of monosodium glutamate production in the food industry [14]. MSGR does not contain hazardous or pathogenic matter [15], which seems to make it an ideal nutrient source for culturing microalgae. Previous studies in our group have proven that MSGR is suitable for limnetic microalgae, including *Chlorella vulgaris*, *Golenkinia* sp. and *Scenedesmus* sp., whether in freshwater or seawater [14,16]. However, basic data to grow marine microalgae in MSGR are still lacking. Inexhaustible natural seawater supplemented with nutrient-rich MSGR may be an ideal medium for cultivation of marine microalgae and would achieve the goal of saving nutrient costs [17]. Therefore, it is necessary to conduct trials to verify the feasibility of culturing marine microalgae in natural seawater supplemented with MSGR (S-MSGR), especially for the two commercial marine microalgae, *P. tricornutum* and *N. oceanica*.

Based on the foregoing considerations, in this study, two common commercial marine microalgae, *P. tricornutum* and *N. oceanica*, were grown in seawater combined with MSGR to provide an economic and effective strategy for microalgal cultivation to further improve the diversified application of microalgae in natural seawater. The main aims of this study were to: (1) investigate the growth characteristics and nutrient assimilation of the two microalgae under the optimal addition ratio of MSGR; (2) ascertain the main metabolites of these two microalgae in S-MSGR; and (3) analyze the potential application of this technology for industrial production of marine microalgae.

2. Materials and Methods

2.1. Microalgal Strain and Culture

During the experiments, two marine microalgae species were used, *Phaeodactylum tricornutum* FACHB-863 and *Nannochloropsis oceanica* FACHB-926, which were purchased from the Freshwater Algae Culture Collection of the Institute of Hydrobiology (FACHB). Before the experiments, *P. tricornutum* and *N. oceanica* were cultivated in Erdschreiber's medium (EM), consisting of 12 mL/L P-IV metallic stock solution, 200 mg/L $NaNO_3$, 18 mg/L $Na_2HPO_4{\cdot}7H_2O$, 0.135 mg/L cyanocobalamin and 50 mL/L soil extract solution in filtered seawater. One liter of P-IV metallic stock solution contained 750 mg $Na_2EDTA{\cdot}2H_2O$, 97 mg $FeCl_3{\cdot}6H_2O$, 41 mg $MnCl_2{\cdot}4H_2O$, 5 mg $ZnCl_2$, 4 mg $Na_2MoO_4{\cdot}2H_2O$ and 2 mg

$CoCl_2 \cdot 6H_2O$. To prepare the soil extract solution, 200 g of garden soil (without fertilizer) was put into a triangular bottle containing 1000 mL of distilled water. The mouth of the bottle was sealed with a porous plug, the bottle was heated by boiling water in a water bath for 3 h, and then, it was cooled and the contents allowed to precipitate for 24 h. This process was consecutively carried out 3 times. Then, the solution was filtered, and the supernatant was taken and sterilized in an autoclave. The microalgae were cultivated at 25 ± 1 °C under 45 μmol/m^2/s continuous illumination.

2.2. Optimization of Dilution Ratio of MSGR

MSGR used for the experiments was supplied by Liangshan Linghua Gourmet Powder (Jining, China). MSGR was filtered through 8 layers of gauze to remove larger particles before being used in this study, and the nutrient characteristics of MSGR were as follows: 85.0 ± 5.6 g/L total nitrogen (TN), 2.8 ± 0.4 g/L total phosphorus (TP), 78.3 ± 4.5 g/L ammonia nitrogen (NH_3-N), 108.3 ± 6.7 g/L COD_{Cr} and pH 3.2 ± 0.4.

In this study, based on the contents of nitrogen and phosphorus in the standard medium of these two species of algae, we designed three different dilution ratios (S-MSGR/seawater, 1/500, 1/1000, 1/2000, *v*/*v*). Under the same conditions (25 ± 1 °C and 45 μmol/m^2/s continuous illumination), three parallel experiments were carried out at the same time, and the growth status of microalgae was characterized by the concentration of total chlorophyll.

2.3. Experimental Design

The media were prepared by diluting MSGR with seawater at the optimum dilution ratio. The two species of microalgae were cultivated in 1 L conical flasks with 0.8 L working volumes of the respective aforementioned seawater media under the condition of 25 ± 1 °C temperature and 45 μmol/m^2/s continuous illumination. The initial inoculum concentration of microalgae in this experiment was about OD_{680} = 0.2. In addition, using standard medium (EM) as a control, three parallel experiments were carried out under the same conditions.

2.4. Algal Analysis

2.4.1. Determination of the Total Chlorophyll Content

The total chlorophyll (Total Chl) concentration of microalgae was measured by the method of methanol extraction [18]. Firstly, 1.5 mL of microalgal culture was transferred into a 2 mL centrifuge tube. After centrifugation, the supernatant was discarded, and the solids were resuspended to the same volume with methanol (99%). Next, the mixture was immersed at 45 °C for 24 h in the dark. After the dark treatment, the supernatant was collected after centrifugation, and the absorbances were measured at wavelengths of 652.4, 665.2 and 750 nm on a UV–visible spectrophotometer (UV-2450, Shimadzu, Japan). The total Chl concentration was then estimated by use of the following equation:

$$\text{Total Chl (mg/L)} = 1.44\ (A_{665.2} - A_{750}) + 24.93\ (A_{652.4} - A_{750}).$$

2.4.2. Measurement of Cell Biomass Concentration

The biomass concentration (X) was determined by weighing a certain volume of dry biomass [19]. In short, a 10 mL volume of microalgal solution was filtered through a 0.45 μm membrane, which was then dried to constant mass. The biomass concentration (g/L) was calculated as the dry mass per liter of algae culture solution. The OD_{680} (optical density at 680 nm) and corresponding biomass concentration of microalgae in different growth stages were measured, and the relation equations between OD_{680} and biomass concentration were fitted. Microalgal growth was monitored every day by measuring OD_{680}, and the relationships between biomass concentration and the OD_{680} of the two microalgae were calculated following the equations

$$X_{P.\ tricornutum} = 0.9773\ OD_{680} + 0.0313, \text{ with } R^2 = 0.9964,$$

and

$$X_{N.\ oceanica} = 0.2916\ OD_{680} + 0.0254, \text{ with } R^2 = 0.9951.$$

The biomass productivity, P_b (g/L/day), was calculated from the variation of the biomass concentration within a specific cultivation time according to the following equation:

$$P_b = \Delta X/\Delta T,$$

where ΔT (day) and ΔX (g/L) are the duration of the run and the variation of the biomass concentration, respectively.

The specific growth rate, μ (day^{-1}), of microalgae was calculated by the following equation:

$$\mu = [\ln(X_2) - \ln(X_1)]/(T_2 - T_1),$$

where X_1 and X_2 (g/L) represent the dry biomass concentrations at times T_1 and T_2, respectively.

2.4.3. Determination of Metabolites in Biomass

The carbohydrate content of algae was measured by the anthrone–sulfuric acid method [20]. Specifically, the algae were harvested by centrifugation, and the collected algal biomass was freeze dried to constant weight at −50 °C in a lyophilizer (EYELA FDU-1200, Tokyo Rikakikai, Japan) to obtain dried algal powder. After that, the dried algal sample, 10 mL hydrochloric acid and 15 mL water were mixed in a 50 mL conical flask and heated in boiling water for 20 min. The hot, mixed system was then filtered and diluted to 100 mL. Next a 1 mL sample with 4 mL of anthrone (2% H_2SO_4 by mass) was transferred into a clean glass vial with a screw cap, and the bottom of the glass vial was immersed in boiling water to boil for exactly 10 min, then cooled to room temperature with ice water. Finally, the extracted carbohydrate was measured by colorimetric assay on a 96-well plate at a wavelength of 620 nm in a microplate photometer (Multiskan FC, Thermo, Waltham, MA, USA).

The protein content of algae was measured by the Bradford method [21]. An amount of 10 mL of distilled water was added to the dried algal powder, which was accurately weighed, and then, the algal cells were broken by ultrasonic crusher under ice-bath conditions. The supernatant was collected by centrifugation. Next, a 1 mL extract with 5 mL of Coomassie brilliant blue G-250 (0.1 g/L) was transferred into a clean glass vial with a screw cap and oscillated. The absorbance of the supernatant was measured by UV–visible spectrophotometer at 595 nm. Bovine serum albumin was used as the standard curve to calculate the protein content.

The lipid content of algae was measured by the chloroform–methanol method [22]. Specifically, the dried algal sample and chloroform–methanol solution (2:1, *v*/*v*) were mixed, followed by ultrasound for 10 min. After that, the supernatant was collected in a long test tube by centrifugation. The entire extraction process was repeated twice. Sodium chloride solution (0.9%) of one-fifth the volume of the lipid extract was added to the system. The mixed liquid was vortex vibrated for 1 min, then left to stand for 15 min. All the liquid in the organic phase was transferred through a 0.22 μm organic membrane to a clean, short test tube of known mass. Finally, the contents of the tube were dried by blowing nitrogen at 60 °C to obtain the lipid mass.

2.5. Nutrient Measurements

The total nitrogen (TN) and total phosphorus (TP) of filtered samples were determined by the alkaline potassium persulfate digestion UV spectrophotometric method (HJ 636-2012) and the ammonium molybdate spectrophotometric method (GB/T 11893-1989) [23], respectively. The assimilation efficiency, *AE* (%), and the average yield coefficient, AYC (mg/g), were calculated by use of the following equations [24]:

$$AE = \Delta C/C_0,$$

and

$$AYC = \Delta C/\Delta X,$$

where C_0 is the initial concentration of nutrients (TN and TP), ΔC (mg/L) is the reduction in concentration due to consumption of nutrients during cultivation, and ΔX (g/L) is the increase in the biomass concentration.

2.6. Statistical Analysis

The results are presented in the mean values ± standard deviation (s.d.) from three independent experiments and analyzed using one-way analysis of variance in Duncan's test. A value of $p < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1. Effects of S-MSGR on Growth

According to the nutrients produced in standard medium by *P. tricornutum* and *N. oceanica*, three different dilutions of MSGR in natural seawater were specified to determine the optimal culture condition (Figure S1). The optimal dilution factor for *P. tricornutum* and *N. oceanica* was 500 times, which yielded total chlorophyll contents of 4.86 and 6.22 mg/L for the two respective algal species. Therefore, the condition of 1/500 was chosen in the following experiments.

Figure 1 presents the variation in growth characteristics of the two microalgae. The biomass concentrations of *P. tricornutum* and *N. oceanica* reached 0.93 and 0.36 g/L in S-MSGR, which were 1.5 and 1.9 times higher than the respective values in standard medium. During the first week of culture, the biomass productivity of *P. tricornutum* in S-MSGR fluctuated around 67 mg/L/day, and the maximum value of 72.27 mg/L/day, obtained on the sixth day, was evidently higher than the 50.04 mg/L/day obtained in EM medium. Subsequently, with the consumption of nutrients, the biomass productivity gradually decreased to 52.34 mg/L/day at the harvest time. For *N. oceanica*, the biomass productivity at the beginning of the experiment increased rapidly to 33.26 mg/L/day (on the fifth day). Similarly, the biomass productivity gradually decreased due to the reduction in nutrients in the medium and finally stabilized at 22.28 mg/L/day. Figure 2 shows the specific growth rates of the two microalgae under EM and S-MSGR culture conditions. The average specific growth rates of *P. tricornutum* and *N. oceanica* cultured in S-MSGR were clearly different from those in EM medium, exhibiting increases of about 33.3% and 58.6%, respectively. The results indicate that using S-MSGR as the medium for growth of *P. tricornutum* and *N. oceanica* could effectively improve their biomass productivity, and thus promote the rapid accumulation of biomass.

The nutrient assimilations of the two microalgae in the standard and S-MSGR media are shown in Figure 2 and Table S1. These two microalgae cultured with S-MSGR showed excellent capacities for TP assimilation, with *P. tricornutum* and *N. oceanica* achieving 97.8% and 66.9%, respectively. Compared with total phosphorus, the assimilation efficiency of total nitrogen (AE_N) was slightly lower. The maximum AE_N was only 38.9%, which was attained by *P. tricornutum*. This was mainly because the initial TN concentration in the MSGR medium was relatively high (179.4 mg/L), indicating that the growth of microalgae in this experiment was limited by the concentration of phosphorus. Therefore, this factor should be taken seriously in the following research. In this study, there was a high nutrient assimilation efficiency for *P. tricornutum* cultured in S-MSGR, which corresponded to its high biomass concentration, lipid, and protein productivity. Cultivating *P. tricornutum* in seawater with added MSGR not only enabled assimilation of N and P nutrients from MSGR, but also obtained higher lipid and protein productivity than in standard medium, indicating that S-MSGR, as the medium of growth for *P. tricornutum*, was fully in line with commercial demands. The average yield coefficient (AYC) was defined as the ratio of the amount of TN or TP assimilated by microalgae to the quantity of biomass produced; that is, the amount of nutrients used by the microalgae per gram of biomass produced [19].

N. oceanica manifested a maximum value of 174.8 mg/g for N-AYC (Figure 2), which showed an excellent ability to assimilate the nitrogen in MSGR.

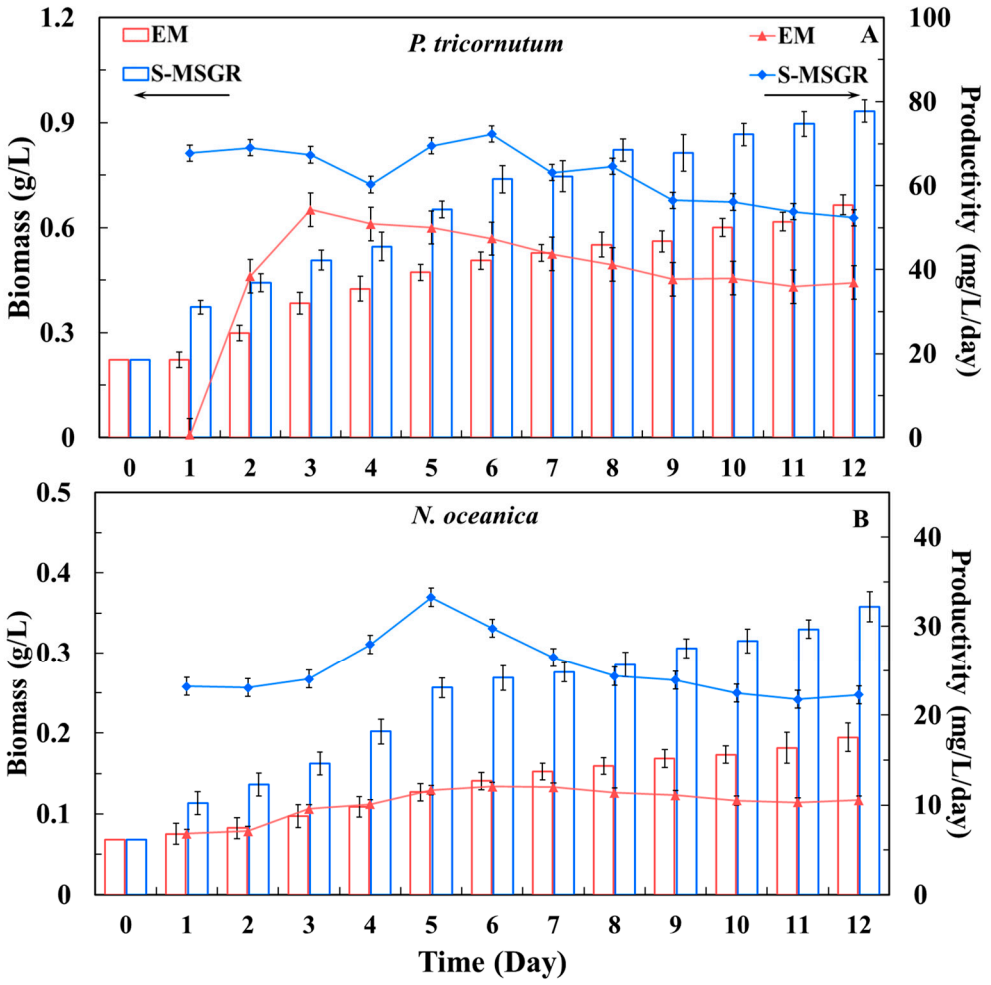

Figure 1. Changes in biomass concentration and the biomass productivity of the two microalgae, *P. tricornutum* (**A**) and *N. oceanica* (**B**), in EM and in S-MSGR. Values are expressed as means ± s.d. (n = 3).

3.2. Changes to Photosynthesis in S-MSGR

Chlorophyll is the main indicator in the photosynthetic system of algal cells, which can reflect the growth status of microalgae [25]. As shown in Figure 3, the total chlorophyll content of *P. tricornutum* and *N. oceanica* reached maximum values of 8.19 and 6.99 mg/L, respectively, within 7 days of being cultured in S-MSGR medium, while the total chlorophyll contents for EM medium were only 2.37 and 0.92 mg/L, respectively. The better chlorophyll synthesis of the two microalgae in S-MSGR demonstrated their higher photosynthetic efficiency in S-MSGR than in standard medium. It is generally considered that the ratio of Chl-a/Chl-b can reflect the ability to capture light energy related to the photochemical reaction rate of PS-II [26]. Great changes in Chl-a/Chl-b in the early stage of the two algae being cultured in EM or S-MSGR indicated the adaptation process in the new environment. On the fourth day, all the ratios tended to be stable, showing that the adaptation response of microalgae had finished. Finally, the Chl-a/Chl-b ratios for *P. tricornutum* stabilized at about 0.70 and 0.32 when cultured in EM and S-MSGR, respectively (Figure 3C). Similarly, the final ratio of *N. oceanica* cultured in S-MSGR was about 0.18, which was much lower than the 0.83 obtained with EM (Figure 3D). The results indicate that the activity of chloroplast photosynthetic phosphorylation increased in S-MSGR, thus improving the photosynthetic efficiency of microalgae.

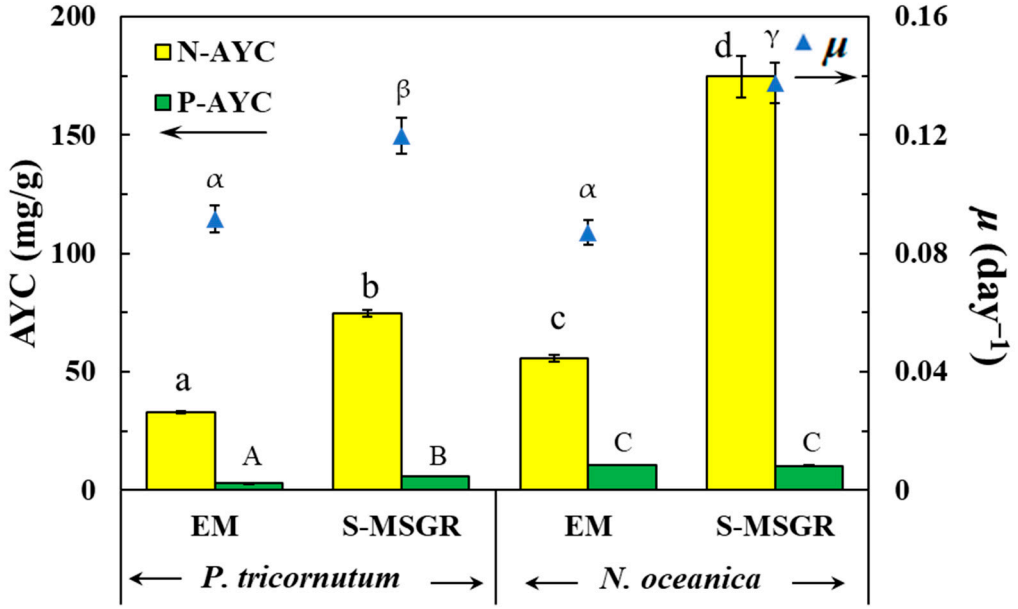

Figure 2. The nutritional assimilation and the average specific growth rate (μ, day^{-1}) of *P. tricornutum* and *N. oceanica* grown in standard medium (EM) and in S-MSGR. Values are expressed as means ± s.d. (n = 3). Values with the same letter (a, b, c, d; A, B, C or α, β, γ) show no significant difference of N-AYC, P-AYC or μ between groups, respectively ($p > 0.05$, Duncan's test).

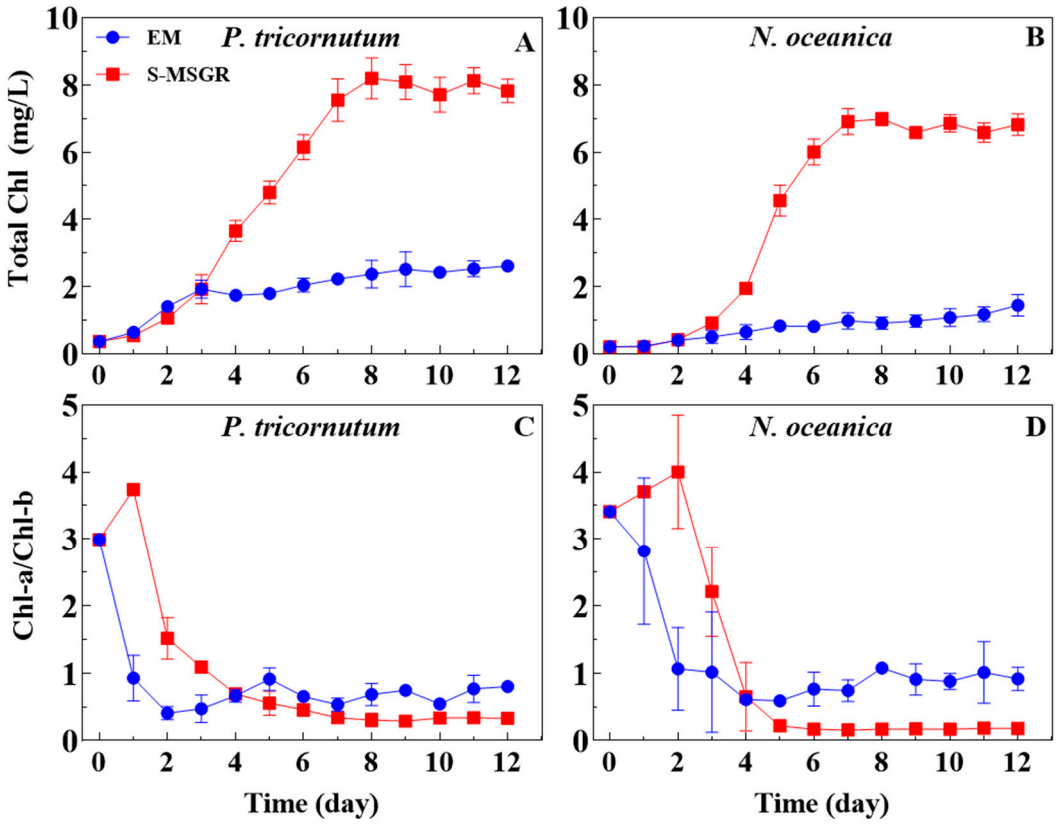

Figure 3. Changes in total chlorophyll content (**A**,**B**) and ratios of Chl-a/Chl-b (**C**,**D**) of the two marine microalgae, *P. tricornutum* and *N. oceanica*, grown in EM and in S-MSGR. Values are expressed as means ± s.d. (n = 3).

3.3. Metabolite Accumulation

Figure 4 shows the accumulation of metabolites (lipid, protein and carbohydrate) by the two microalgae in EM medium and S-MSGR. It was found that the lipid content was the highest among the three metabolites synthesized by these two microalgae, exceeding one-quarter of the dry mass. The high salinity in EM medium and S-MSGR was the main stimulating factor for lipid synthesis.

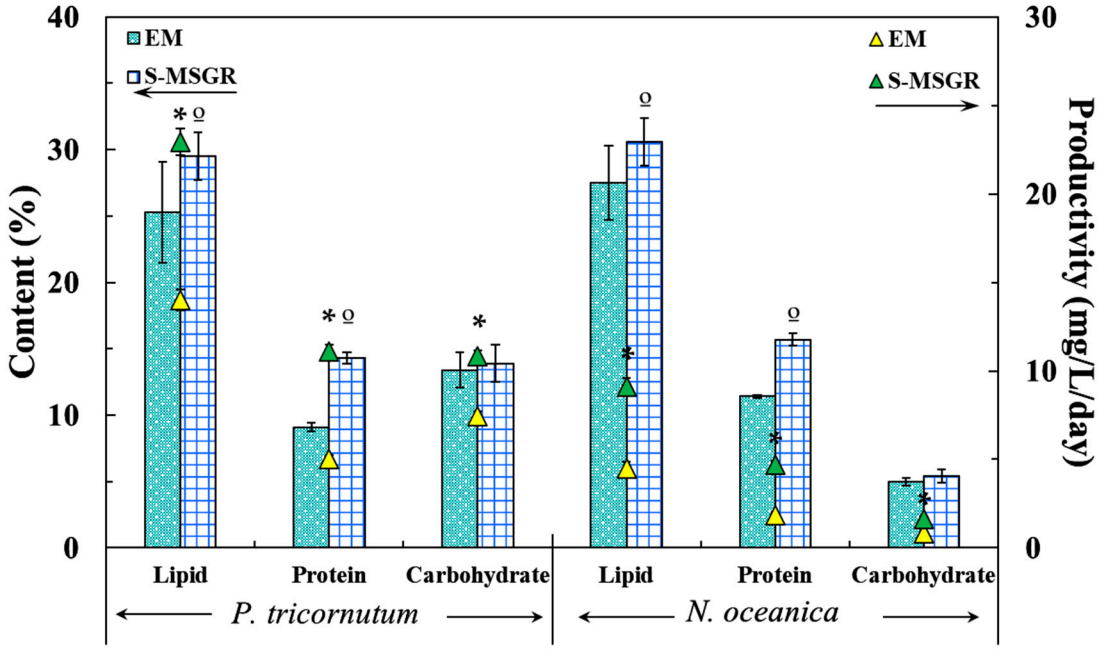

Figure 4. The content and productivity of lipids, proteins and carbohydrates for *P. tricornutum* and *N. oceanica*, grown in EM and in S-MSGR. Values are expressed as means ± s.d. (n = 3). The symbol * indicates the significant difference in the productivity groups ($p < 0.05$), The symbol º indicates the significant difference in the content groups ($p < 0.05$).

For S-MSGR-cultured *P. tricornutum*, the lipid content and productivity reached 29.5% and 22.9 mg/L/day, which were 1.2 and 1.6 times higher than the values in standard medium (25.3% and 14.0 mg/L/day). The results indicate that *P. tricornutum* cultured in S-MSGR could potentially be used as a raw material for biofuels production. Although the content and productivity of protein synthesized by *P. tricornutum* in S-MSGR were not as high as those of lipids, the protein productivity (11.1 mg/L/day) was still doubled compared with that in EM medium (5.0 mg/L/day). The carbohydrate content in both cultures did not show an obvious difference but, considering the high biomass productivity in S-MSGR, the carbohydrate productivity in S-MSGR (10.8 mg/L/day) was 1.46 times higher than the 7.4 mg/L/day in EM medium.

Likewise, the main metabolite in *N. oceanica* was also lipid, accounting for one-third of the total dry biomass, indicating that lipid accumulation is a common approach to withstand the stimulation of high salinity, whether for limnetic or marine microalgae. The protein content and productivity of *N. oceanica* in S-MSGR also showed increases of 37.7% and 153% compared to those in EM medium, but carbohydrate synthesis was not affected by S-MSGR.

The main reason causing such phenomena is that *P. tricornutum* and *N. oceanica* demonstrated the capacity for heterotrophy, and the ample organic carbon in MSGR provided extra carbon sources apart from atmospheric CO_2, which was conducive to promoting the accumulation of metabolites in microalgal cells [27]. Salinity was generally considered to be one of the triggers to changing the carbon partitioning from protein/carbohydrate synthesis to lipid synthesis, so that the lipid content was enhanced under exposure to high

salinity [28]. However, in this study, both lipid and protein content of the two microalgae in S-MSGR increased, yet carbohydrate synthesis did not show a difference, indicating that there may be other metabolites in the cells providing carbon to lipid synthesis.

3.4. Benefit Analysis of S-MSGR for Culture of Marine Microalgae

A benefit analysis of S-MSGR for culture of *P. tricornutum* and *N. oceanica* was conducted herein, based on a comparison of biomass and lipid production performances found in the current work with those reported in the literature.

Figure 5 shows the lipid content and productivity reported by different studies on *P. tricornutum* and *N. oceanica*. At present, the culture medium for *P. tricornutum* was commonly f/2 medium or modified f/2 medium (Table S2). Yodsuwan et al. [29] added different concentrations of $NaNO_3$ to f/2 medium for the cultivation of *P. tricornutum*, and the maximum lipid productivity was only 1.86 mg/L/day, which was far lower than that in S-MSGR. Notably, the lipid content in microalgal cells was low (2.8%) under the nitrogen-rich condition, and the corresponding biomass concentration was only 0.07 g/L. Similarly, Burch et al. [30] harvested the highest biomass concentration after culturing *P. tricornutum* in f/2 + 60% dairy manure wastewater, but the lipid content of algal cells was only 3.4%, which was far from meeting the demands of commercial application. Conversely, the total nitrogen content in S-MSGR was up to 179.4 mg/L, and the final lipid content of *P. tricornutum* increased to 22.9 mg/L/day, which suggested that S-MSGR as a nutrient source to cultivate *P. tricornutum* did not inhibit lipid synthesis in microalgae. Wang et al. [31] obtained the highest biomass concentration of *P. tricornutum* of 1.16 g/L with the help of high illumination and 5 g/L glucose, which increased the production cost. Simonazzi et al. [32] combined modified f/2 medium, effluent from anaerobic digestion and extra CO_2 supplementation to cultivate *P. tricornutum* and obtained lipid productivity of 22.4 mg/L/day, which was close to one in this study. However, extra power consumption for the aeration device (0.6 L(CO_2)/L/day) and high illumination (90–110 μmol/m^2/s) increased the cultivation costs. Wang et al. [33] obtained 5.5 mg/L/day for the maximum lipid productivity of *P. tricornutum* in municipal wastewater, indicating that the municipal wastewater was not an ideal nutrient source. Su et al. [34] cultured *P. tricornutum* in wastewater from ultrafiltered digestate (UF) supplied with glycerol, which achieved 3.25 g/L and 51 mg/L/day of biomass concentration and lipid productivity, respectively. However, the extra aeration device and high light intensity (312 μmol/m^2/s, versus 45 μmol/m^2/s in our study) greatly increased the cost of microalgal culture.

Table S3 lists some studies on the biomass and lipid of *N. oceanica*. It can be found that there were few studies on cultivating *N. oceanica* with wastewater, and the medium for cultivating *N. oceanica* was mainly one of the standard media, such as f/2, BG11 or Conway medium. Compared with the experimental conditions in other studies, high illumination intensity and stable aeration may improve the biomass and lipid productivity of *N. oceanica*. Although the biomass and lipid productivity obtained by these studies [35–37] were close to those in the current study, the medium they all used was prepared with standard chemical reagents. In contrast, the S-MSGR medium greatly reduced the cost of nutrition during microalgal cultivation. In the present study, we used MSGR as a nutrient source to cultivate *N. oceanica* and obtained biomass and lipid productivities of 30 and 9.1 mg/L/day, respectively. The results in this study were not the highest among all studies, but the low illumination intensity, absence of an aeration device and cost-efficient nutrient resource greatly saved the culture cost, which will promote the commercial production of *N. oceanica* biomass.

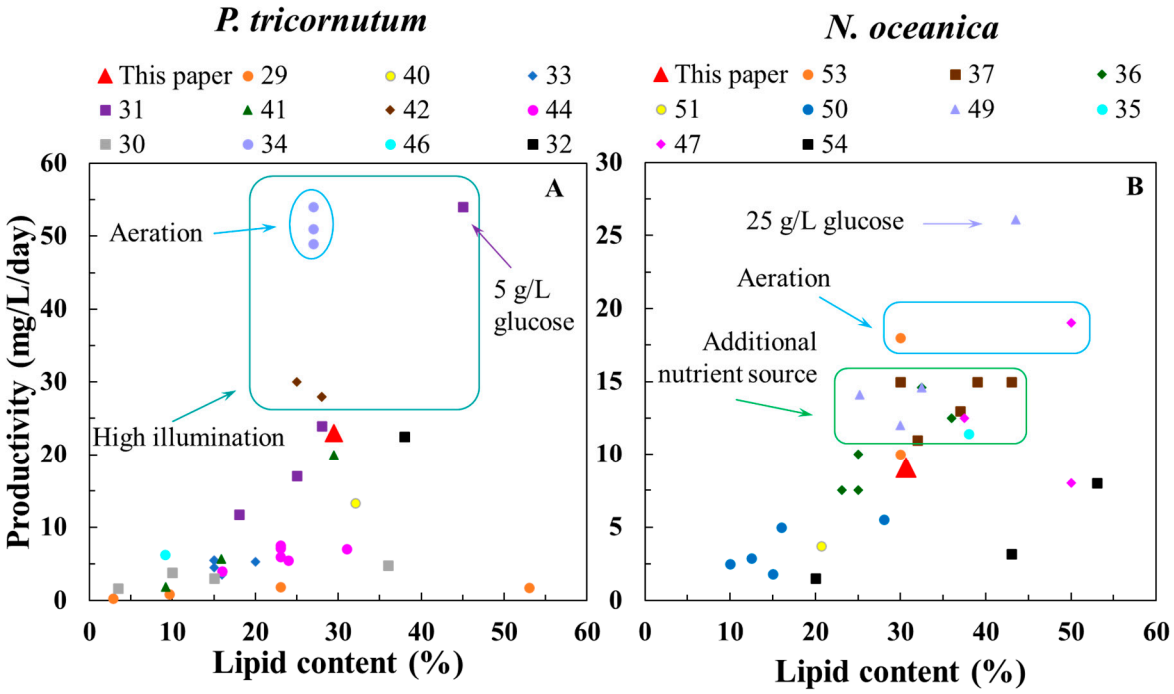

Figure 5. Comparison of the lipid content and lipid production performances of (**A**) *P. tricornutum* and (**B**) *N. oceanica* cultured in S-MSGR with other results from the literature. The legend indicates the references for the data.

Figure 6 shows the advantages of biomass and biofuel production by *P. tricornutum* and *N. oceanica* in S-MSGR. S-MSGR was able to promote the fast growth of *P. tricornutum* and *N. oceanica*, and the biomass concentration trebled within the first five days, while in the standard medium (EM), it only doubled. *P. tricornutum* and *N. oceanica* cultured in S-MSGR obtained higher lipid productivities, which were, respectively, 1.6 times and 2 times higher than those obtained in EM.

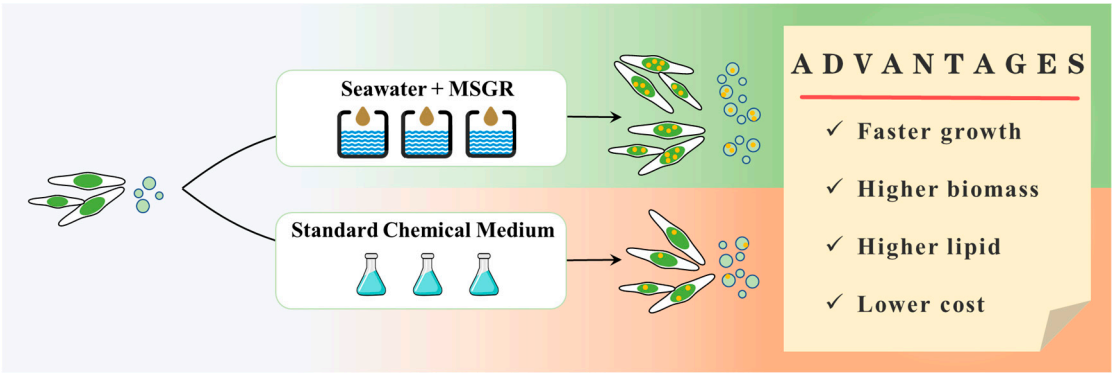

Figure 6. The benefits of using S-MSGR as a medium for biomass and biofuel production with *P. tricornutum* and *N. oceanica*.

To assess the economic benefits, the medium costs of biomass and lipid production of *P. tricornutum* and *N. oceanica* in EM and in S-MSGR were evaluated, without considering the cost of seawater needed for dilution of MSGR and preparation of EM. The cost of EM is mainly in chemical reagents, while the MSGR, as a waste nutrient source, has almost zero cost, so the main cost of S-MSGR medium was mainly from transportation. The medium costs of S-MSGR for biomass production of *P. tricornutum* and *N. oceanica* were USD 0.7 and 1.8/(kg biomass), which was merely 3.6% and 2.7% of the corresponding costs in EM.

Similarly, the medium costs of S-MSGR for lipid production of *P. tricornutum* and *N. oceanica* were USD 2.3 and 5.8/(kg lipid), respectively. Compared with EM, 96.9% and 97.6% of the medium costs were saved for lipid production by *P. tricornutum* and *N. oceanica* cultured in S-MSGR. Hence, the S-MSGR medium was more economical for algal cultivation.

4. Conclusions

P. tricornutum and *N. oceanica* survived well in the seawater-based medium supplemented with MSGR at a ratio of 1/500. The increase in total chlorophyll content and the decrease of Chl-a/Chl-b showed that S-MSGR promoted photosynthesis in the microalgae. S-MSGR was more suitable for lipid production, and the lipid productivities of *P. tricornutum* and *N. oceanica* reached 22.9 and 9.1 mg/L/day, which was 1.6 and 2.0 times higher than those in EM. Both of the microalgae cultured in S-MSGR for biomass and lipid production saved more than 96% of the cost of standard culture medium. Hence, this study indicates that S-MSGR has a great promise as a medium for cultivating *P. tricornutum* and *N. oceanica* and bright prospects for use in commercial production.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w14060975/s1, Figure S1: The changes in total chlorophyll of (A) *P. tricornutum* and (B) *N. oceanica*, cultured in seawater supplemented with different levels of MSGR. Values are expressed as means ± s.d. (n = 3); Table S1: The nutritional assimilation of the two microalgae, *P. tricornutum* and *N. oceanica*, grown in EM and in S-MSGR; Table S2: Comparison of the biomass and lipid production performances of *P. tricornutum* cultured in S-MSGR with other results reported in the literature; Table S3: Comparison of the biomass and lipid production performances of *N. oceanica* cultured in S-MSGR with other results from the literature. References [38–54] are cited in the supplementary materials.

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