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Biomass productivity of *Nannochloropsis* sp. grown in desalination brine culture medium

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ABSTRACT

Desalination technologies play a vital role in water production. Along with the production of clean drinking water, desalination plants produce waste in the form of hypersaline water or brine which may harm the surrounding environment. This paper proposes a method of brine management using microalgae. The alga species *Nannochloropsis* sp. was tested for its ability to grow in high salt stress conditions, up to 80 g L^{-1}, and in desalination brine with concentration of 70 g L^{-1}. Interestingly, four- and five-fold increases in biomass were observed in salt stress conditions of 60, 70, and 80 g L^{-1}. In the case of the desalination brine conditions, *Nannochloropsis* sp. growth resulted in comparable biomass values to the salt stress experiments, proving that algal growth was not inhibited due to the presence of brine. For cost efficiency, further research was conducted to optimize the concentration and components of the brine-nutrient medium. The best growth was obtained in the optimized brine-based F/2 medium (B_{UV}), which was scaled up and tested for its bio-desalination and biofuel capacities. Biomass productivity and lipid productivity were found to be 0.05 ± 0.016 g L^{-1} d^{-1} and 9.5% ± 2.1% w/w, respectively. This study presents a cost-effective sustainable method for brine management through which value is created.

Keywords: Desalination brine; *Nannochloropsis* sp.; Salt stress; Chlorophyll-a content; productivity; Lipid

1. Introduction

In an attempt to combat water scarcity, installations of desalination plants have steadily increased during the past years [1–4]. Over 150 countries currently practice desalination, having an average of 18,426 operational plants by the year 2015 and a capacity of about 86.5 million m^3 d^{-1}. The majority of desalination installations, 65%, use the membrane technology called reverse osmosis (RO), and most, 59%, are seawater based [5]. While desalination provides a feasible solution for alleviating water scarcity, the technology is under scrutiny due to its high energy demand and harmful waste [6–8].

The properties of desalination wastewater, also known as desalination-concentrate or brine, depends on the source of brine production. Brine produced from seawater reverse osmosis (SWRO) desalination stations, the most common desalination technology used globally, varies in concentration between 65 to 85 g L^{-1} and may contain traces of toxic and cleaning chemicals [9]. This led several studies to suggest that brine has a negative environmental impact on marine organisms [10–14]. Laboratory studies,

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conducted by Latorre [15] showed a correlation between exposure to highly saline waters, 40 g L^{-1}, and reduced mortality, 27%, of *Posidonia Oceanica*, the most common Mediterranean seagrass. Comparing a habitat of *P. Oceanica* seagrass that has been exposed to brine for 6 y to undisturbed sites, led [16] to report the absence of two marine species, sea cucumbers, and sea urchins, suggesting the change of salinity as a likely cause of their disappearance [16]. Other studies have reported no changes to the physical and chemical characteristics of certain marine plants [14]. The wide variety of conditions and species presents a daunting task for scholars to reach a consensus on the effects of brine on marine environments. However, management and treatment of brine is encouraged and currently practiced in some areas.

The three most common brine management technologies are based on improving disposal methods, volume minimization or the reuse, and extraction of useful chemicals [17,18]. Most of these methods utilize mechanical or chemical processes in brine management. Very few management techniques use biological processes as the foundation of the management technique.

Biological methods of brine management may be more sustainable as they can conform to the local biota and generate biodegradable waste. In some developing countries, where technology is limited but the land is abundant, the use of biological processes for brine management may prove to be more feasible than mechanical and chemical processes. An example of brine management using biological processes can be found in Brazil, the Agua Doce Program [19]. The program uses brine from an inland RO station and generates valuable goods through rearing several organisms including fish (Tilapia species), crops (Atriplex plant), and cattle [19]. However, as previously noted, the percentage of seawater desalination installations outnumber those of inland installations; hence, there is a more prominent need to test high saline brine. The biological management of SWRO brine is challenging as its high salinity creates an inhospitable habitat for most organisms. Nevertheless, some micro-organisms have the capacity to adapt to high saline conditions and several species have high economic values.

The marine microalgal species, *Nannochloropsis*, has been noted in the literature to survive under high salt stress conditions [20–22]. *Nannochloropsis* is a unicellular non-mobile microalga, which produces fatty acids and proteins making it a valuable food source [23]. Its ability to reproduce very rapidly places it as a favorable candidate for mass cultivation [24]. *Nannochloropsis* sp. has also gained a strong position in the biofuel research field. Several studies have experimented with the effect of salt stress on lipid production with different strains of *Nannochloropsis*. Findings record that lipid production is promoted under salt stress conditions [25–27].

This study aims to perform biological management of brine by testing the ability of *Nannochloropsis* sp. to grow under high saline conditions. This work is divided into three sections, *Nannochloropsis* sp. growth was first tested in nutrient-rich, artificially induced, salt stress media ranging from 30 g L^{-1}, similar to seawater, to 80 g L^{-1}, similar to brine salinity. The artificially induced salt stress tests were performed to determine biomass growth under different salinities and to use as a control in determining the sensitivity of algae to the possibly toxic chemicals within the brine medium. In the second section, biomass growth of *Nannochloropsis* sp. was tested in SWRO brine (70 g L^{-1}) under different nutrient concentrations. Once cultivation in brine was established, new tests were set to assess biomass growth supplemented with alternative and more cost-effective nutrients. Characterization was conducted on the alga produced to determine biomass production. The third section examines the capacity for biofuel utilization and bio-desalination on the best biomass performing test. Such analyses included lipid content, fatty acid profile, lipid production, and ion analysis.

2. Materials and methods

2.1. Experimental organism and cultivation conditions

The marine microalga, *Nannochloropsis* sp., was obtained from the Faculty of Science, Alexandria University, Egypt. The alga was grown and maintained in F/2 medium [28] with a 30 g L^{-1} sea salt base, mimicking the salinity of seawater (Fig. 1). Growth was achieved at room temperature (25°C–28°C) with an airflow of 4 L min^{-1} supplied by 5 W air compressors. Illumination was provided by 6 W LED bulbs providing an average light intensity of 5,000 lux under a diurnal cycle pattern of 18 h light/6 h darkness [29,30]. The composition of F/2 medium is as follows: sea salts, (concentration varied for each experiment), 75 mg L^{-1} of $NaNO_3$, 5 mg L^{-1} of $NaH_2PO_4{\cdot}H_2O$, 1 mL of trace element stock (3.15 mg L^{-1} of $FeCl_3{\cdot}6H_2O$, 4.36 mg L^{-1} of $Na_2EDTA{\cdot}2H_2O$, 1 mL of 9.8 g L^{-1} of $CuSO_4{\cdot}5H_2O$, 1 mL of 6.3 g L^{-1} of $Na_2MoO_4{\cdot}2H_2O$, 1 mL of 22 g L^{-1} of $ZnSO_4{\cdot}7H_2O$, 1 mL of 10 g L^{-1} of $CoCl_2{\cdot}6H_2O$, and 1 mL of 180 g L^{-1} of $MnCl_2{\cdot}4H_2O$) and 1 mL of vitamin stock (200 mg L^{-1} of Thiamine HCL (vit. B1), 1 mL of 1 g L^{-1} of Biotin (vit. H), and 1 mL of 1 g L^{-1} of Cyanocobalamin (vit. B12)).

Chlorophyll-a content (Chl-a) was monitored daily as a representative measurement of biomass growth. The measurements were conducted according to the methodology described by Chen et al. [31]. All tests were carried out in duplicates. The resulting biomass from each experiment was collected and used as an inoculum for the next experiment. This technique gradually adapted the microalga to higher levels of salt stress.

2.2. Desalination brine

The brine sample used in this study was obtained from Remalia SWRO desalination station in Marsa Matrouh, Egypt. Elemental analysis of the brine sample was performed at the Soil, Water, and Environment Research Institute (SWERI), Giza, Egypt (Table 1). The salinity was found to be 70 g L^{-1}. The electrical conductivity and pH were determined by electrical conductivity meter model WTW series Cond 720 and pH meter model WTW series pH 720. Methods of analysis for cations (Na^+, Ca^{++}, and Mg^{++}) and anions (Cl^-, SO_4^-, and HCO_3^-) were performed according to Estefan et al. [32]. Soluble potassium was determined by flame photometer method described in Estefan et al. [32].

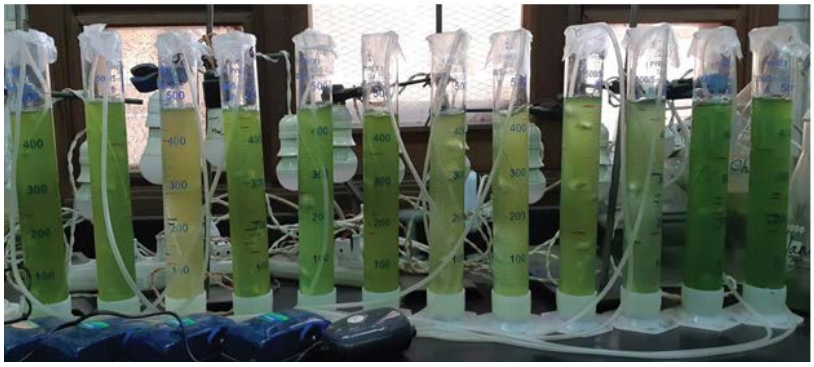

Fig. 1. Experimental setup depicting air compressors connected via 4 mm silicone tubes to 500 mL measuring cylinders which are illuminated with LED bulbs.

Table 1
Composition of sea water reverse osmosis desalination brine

Component[a]	Concentration (mg L^{-1})
Na^{+}	15,600
Ca^{++}	1,338
Mg^{++}	2,766
K^{+}	615
HCO_3^{-}	259
Cl^{-}	31,232
SO_4^{--}	4,470
NH_4^{+}	56
B	4.406
P	0.25
TN	2,227

[a]NO_3^{-} and Fe^{+3} less than 1 μg L^{-1}.
TN: total nitrogen.
TDS 70,160 mg L^{-1}, pH 8.3.

Soluble phosphorus and iron in water were acidified with conc. HNO_3 (1 mL nitric acid for 100 mL water) and filtered through filter paper, Whatman number 45 [33], and determined using inductively coupled plasma (ICP) spectrometry (model Ultima 2 JY Plasma) according to [34]. Soluble $N–NH_4^{+}$ and $N–NO_3$ in water were determined using micro-kjeldahl method according to AOAC [35]. Total nitrogen (TN) was determined by mixed acids; the ratio of sample to sulfuric acid of 1:10 and 1 g using Kjeldahl methods of determining total nitrogen in soil according to AOAC [35].

2.3. Cultivation of Nannochloropsis sp. under artificially induced salt stress

Several batches of F/2 medium, with sea salt base, were prepared to create concentrations of 30, 50, 60, 70, and 80 g L^{-1}, named S_{30}–S_{80}, respectively. S_{30} was conducted as a control test representing the optimum growth medium for the marine microalga *Nannochloropsis* sp. Each concentration was inoculated with 50 mL of a concentrated culture of *Nannochloropsis* sp. adjusted to contain 6–8 mg L^{-1} of Chl-a. The inoculum represented 10% of the corresponding medium in 500 mL glass measuring cylinders. Experiments were performed under the same aeration, temperature, and illumination conditions mentioned in section 2.1 (experimental organism and cultivation conditions). Chl-a content was monitored daily.

2.4. Cultivation of Nannochloropsis sp. in brine

Nannochloropsis sp. was tested for its ability to grow in the brine-based media, with the addition of F/2 nutrients at different concentrations (Table 2), 25%, 50%, and 100%, also referred to as B_{25}, B_{50}, B_{100}, respectively. A control test was set with no F/2 nutrients, B_0. The biomass obtained from cultivating *Nannochloropsis* sp. under artificial salt stress experiments (S_{30}–S_{80}) were collected and used as the starting inoculum for brine experiments. The experiments were performed under the same conditions as section 2.1 (experimental organism and cultivation conditions) and featured a microalgal inocula representing 10% of the total volume. All tests were carried out in duplicates. Chl-a content was monitored daily.

Previous studies have noted that the high cost of the culture medium offsets the value of the microalgae produced. Hence, three tests were conducted attempting to eliminate or substitute compounds within the F/2 medium with nutrients of lower economic value (Table 2). Experiment B_U tests the ability of *Nannochloropsis* sp. to grow in brine with F/2 nutrients substituting 75 mg L^{-1} of $NaNO_3$ with 0.5 M urea ($CO(NH_2)_2$), representing a cheaper nitrogen source. Experiment B_V tests the growth of algae in F/2 nutrient conditions excluding the vitamin stock solution. Experiment B_{UV} incorporates conditions of both B_U and B_V, substituting 75 mg L^{-1} of $NaNO_3$ with 0.5 M urea and eliminating the vitamin stock solution in the F/2 medium. Experimental conditions followed those described in section 2.1 (experimental organism and cultivation conditions). Biomass growth was observed and recorded as Chl-a content.

2.5. Characterization of Nannochloropsis sp. biomass produced in B_{UV} conditions

To determine the value of *Nannochloropsis* sp. cultivated in SWRO brine, its ability to perform bio-desalination and its ability to produce bio-products were tested. Water ion

Table 2
Variable compositions and concentrations of the nutrients applied in the brine-based F/2 culture medium experiments

Experiment[a]	Nutrient content				
	$NaNO_3$ (mg L^{-1})	NaH_2PO_4 (mg L^{-1})	Trace elements stock solution (mL)	Vitamin stock solution (mL)	Urea (mg L^{-1})
Concentration variation experiments					
B_{100}	75	5	1	0.5	na[a]
B_{50}	38	2.5	0.5	0.25	na
B_{25}	19	1.25	0.25	0.13	na
Component variation experiments					
B_U	na	5	1	0.5	30
B_V	75	5	1	na	na
B_{UV}	na	5	1	na	30

[a]Medium used in B_0 experiment was 100% brine.
na: not added.

analysis would determine the extent of individual nutrient uptake by the microalga and estimate the general change in total dissolved solids (TDS). To determine the potential of *Nannochloropsis* sp. as a source of biofuel, tests were conducted to determine biomass productivity (B_p), lipid content, lipid productivity, and fatty acid profile.

Test B_{UV} was scaled up, where *Nannochloropsis* sp. was cultivated in 5 L conical flasks. The brine-based medium is cost-effective as it contained 1 mM urea (60 mg L^{-1}) and no vitamins. Conditions of aeration and illumination followed the tests described in section 2.1 (experimental organism and cultivation conditions). The test was halted during the late exponential growth phase, after 10–12 d, and flasks were left for decantation.

2.5.1. Determining microalgal bio-desalination capacity

Samples of 100 mL were withdrawn at the start (day 0) and at the end of the experiment. The samples were centrifuged at 4,000 rpm and filtered through 0.45 μm syringe filters. The elemental analysis was performed at SWERI, following the same methods described for testing brine in section 2.2 (desalination brine).

2.5.2. Determining microalgal biofuel capacity

The biomass was collected through a process that included centrifugation, two washing cycles, the first with 0.5 M ammonium formate in order to remove the attached extracellular salts, the second with distilled water to remove any remains of ammonium formate, and drying in a circulating oven at 50°C [36]. Whenever necessary, the dried biomass was stored at 4°C until further analysis. The process was performed in duplicates and repeated until obtaining a satisfactory amount of biomass for further analysis.

2.5.2.1. Biomass productivity

Gravimetric determination of the harvested biomass productivity (B_p, g L^{-1} d^{-1}) was performed according to [36] using the following equation:

$$B_p = \frac{\left(X_t - X_0\right)}{t_x} \quad (1)$$

where, X_t is the biomass production (g L^{-1}) at the end of the exponential growth phase (t_x), X_0 is the initial biomass at the start of the experiment (g L^{-1}) at t_0 (d).

2.5.2.2. Lipid content and lipid productivity

In order to analyze the lipid content in the collected dried biomass, lipid extraction was performed using a mixture of *n*-hexane:isopropanol in a ratio of 3:2, followed by gravimetric determination of lipid fraction according to the method described by El-Sayed et al. [37]. The analysis was repeated twice.

Lipid productivity (mg L^{-1} d^{-1}) was determined as the product of B_p and the lipid content (w/w) in the biomass according to [38]:

$$\text{Lipid productivity} = \frac{\text{Biomass productivity}\left(B_p\right) \times \text{Lipid}}{\text{Biomass}\left(\text{w/w}\right)} \quad (2)$$

2.5.2.3. Fatty acid profile

The extracted dried lipid fraction was esterified, converted to fatty acids methyl esters (FAMEs), according to the method described by Ichihara and Fukubayashi [39] and followed by GC/EI-MS analysis. The GC/EI-MS analysis was performed at the Mass Spectrum Unit, Pharmacognosy Department, Faculty of Pharmacy, Ain Shams University, Egypt. Mass spectra were recorded using Shimadzu GCMS-QP2010 (Tokyo, Japan) equipped with a Rtx-5MS fused bonded column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) (Restek, USA) and a with a split-splitless injector. The initial column temperature was kept at 45°C for 2 min (isothermal) and programmed to 300°C at a rate of 5°C min^{-1} and kept constant at 300°C for 5 min (isothermal). Injector temperature was 250°C. Helium carrier gas flow rate was 1.41 mL min^{-1}. All the mass spectra were recorded applying

the following conditions: (equipment current) filament emission current, 60 mA; ionization voltage, 70 eV; ion source, 200°C. Diluted samples (1% v/v) were injected with split mode (split ratio 1:15).

2.6. Statistical analysis

Student *t*-test and one-way ANOVA ($p < 0.05$) were performed using GraphPad Prism 6.01 (GraphPad Software, Inc., San Diego). This was performed on tests with high biomass growth to determine statistically significant differences.

3. Results and discussion

3.1. Cultivation of Nannochloropsis sp. under artificially induced salt stress

The control test S_{30} showed a four-fold increase in Chl-a content within 3 days (Fig. 2). In experiment S_{50}, there was a lag phase of 1 d where growth was minimal. The lag phase is a result of the algal adaptation process brought by the increase in salt stress from 30 to 50 g L^{-1}. Nevertheless, by day 3, the alga was able to reach and exceed biomass growth observed in the control experiment (Fig. 2). These findings are superior to what was reported by Abu-Rezq et al. [40] and Gu et al. [41]. It was reported that *Nannochloropsis* grows best between the salinity range 20–40 g L^{-1} and in a culture time of 15 d [40]. Experiments S_{30} and S_{50} showed that the alga can grow in a salinity higher than 40 g L^{-1}, and in a shorter culture time, 3 d. Moreover, Gu et al. [41] examined the effect of salt stress on the growth of *Nannochloropsis oculata* in the range of 15 to 55 g L^{-1} with a culture time of 5 d, they reported a decrease in the specific growth rate and Chl-a content as the salinity increased from 45 to 55 g L^{-1}. Chl-a content in S_{50} did not decrease from that of S_{30} showing that under certain conditions the alga can adapt well to saline environments.

Experiments S_{60}–S_{80}, presented in Fig. 2, show the algal growth curve fluctuating in the lag and exponential phases. As the salinity increased the lag phase extended, depicting the algal adaptation process. Nevertheless, after a prolonged lag phase of 2 d in S_{70} and S_{80}, the algal growth rose rapidly, and biomass growth exceeded the levels found in the control test. The microalga grew best in S_{80} where the Chl-a content increased from 0.86 to 4.14 mg L^{-1} by day 8, portraying a five-fold increase. In both S_{60} and S_{70} Chl-a content increased by four-fold.

The growth of *Nannochloropsis* sp. under salt stresses higher than 55 g L^{-1} was previously tested by Bartley et al. [42]. They examined the effect of salinities up to 58 PSU (equivalent to 58 g L^{-1} as the authors equates 34 PSU to 34 g L^{-1}) on the growth of *Nannochloropsis salina*, and reported that higher salinities inhibit cell production. Another study investigated the response of *Nannochloropsis* sp. under salt stress of 13, 27, 54, and 81 g L^{-1} NaCl in a 4 d culture period. While the microalga was able to grow, they observed that biomass decreased as salinity increased [29]. On the other hand, the results of the present study proved that Chl-a, is not inhibited by increasing the salinity of the growth medium. These results show that *Nannochloropsis* sp. can be adapted in laboratory conditions to grow well in salinities up to 80 g L^{-1}, thus adding to the algal value worldwide.

3.2. Cultivation of Nannochloropsis sp. in brine

To determine the capability of the micro-algae to survive in desalination concentrate *Nannochloropsis* sp. was cultivated in brine, salinity 70 g L^{-1}, and supplemented with 100% of the F/2 nutrients, B_{100}. A four-fold increase in biomass was observed on cultivation day 8, which proves that algal growth was not inhibited by the chemical composition of brine (Fig. 3). This growth rate is comparable to S_{70} (non-statistically significant difference exists between

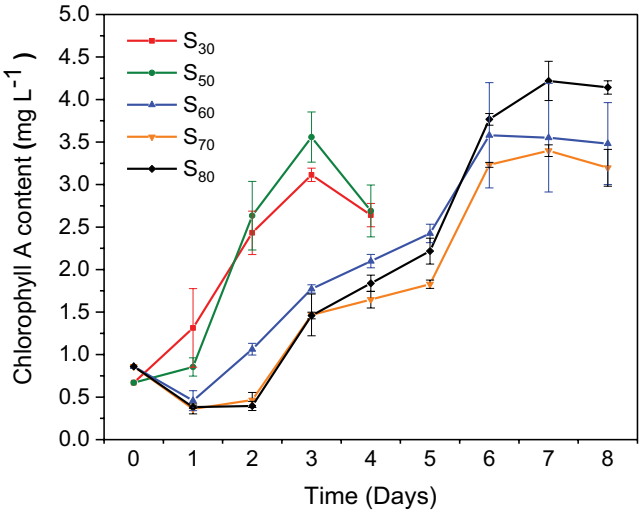

Fig. 2. Chlorophyll-a content of *Nannochloropsis* sp. observed during cultivation in sea salt-based F/2 culture medium at salt stress of 30–80 g L^{-1}.

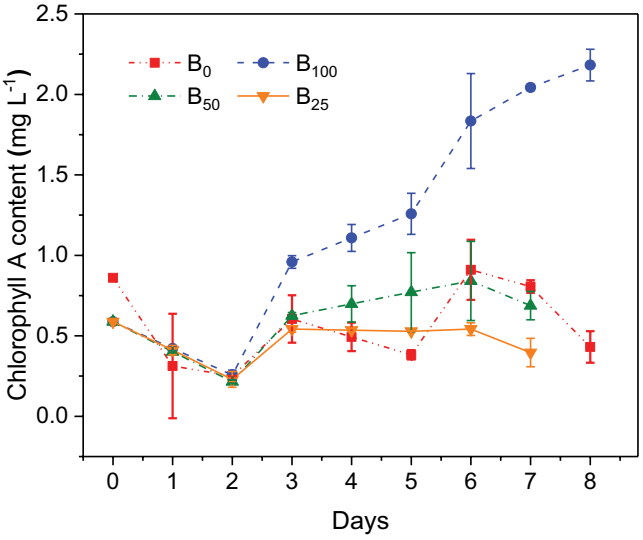

Fig. 3. Chlorophyll-a content of *Nannochloropsis* sp. observed during cultivation in brine-based F/2 culture medium at different nutrients' concentrations.

S_{70} and B_{100} Chl-a values, (*t*-test, $p < 0.05$)) which also portrayed a four-fold biomass increase in full F/2 nutrient conditions by day 8. The result of B_{100} indicates that desalination wastewater, brine, can be used as a base medium for the cultivation of *Nannochloropsis* sp. supplemented with full F/2 nutrient conditions.

It has been reported that the price of F/2 nutrients required is the most expensive aspect of microalgal growth [25,43]. In an attempt to decrease the cost of cultivation, experiments B_{50}, B_{25}, and B_0 were conducted to test the effect of F/2 nutrient concentrations, 50%, 25%, and 0%, respectively, on biomass growth (Fig. 3). In experiment B_{50}, the measured Chl-a content displayed an average increase of two-fold by day 6. The algal growth reached 46% of what was achieved in B_{100} experiment. In the case of experiments B_{25} and B_0 Chl-a declined by 33% and 50% as compared to the inoculation density. These results show that any decrease in the supplemented nutrient concentrations could have inhibitory effects on the microalgal growth. This result is also seen in Matos et al. [43] where *Nannochloropsis gaditana* cultivated in brine, salinity 4.5 g L^{-1}, with no supplementation of F/2 nutrients resulted in impaired algal growth.

The results of B_{50}, B_{25}, and B_0 showed that any decrease in nutrient concentrations inhibits *Nannochloropsis* growth; hence, a closer focus was placed on the breakdown of F/2 nutrients in a continued attempt to optimize the growth medium for lower cultivation costs. The components of F/2 medium include macronutrients and micronutrients. The macronutrient $NaNO_3$ is the main source of nitrogen, specified in the F/2 medium for active growth. This chemical is expensive and may not be economically feasible for scaling up [25]. Therefore, experiment B_U used urea, $CO(NH_2)_2$, as a substitute for $NaNO_3$ [44,45]. Urea is readily available in the global market and has a low price. The result of experiment B_U portrayed an increase of four-fold in Chl-a content by day 8 (Fig. 4). This finding surpassed the biomass growth obtained in B_{100} and this difference was statistically significant (*t*-test, $p < 0.05$), indicating that urea created more favorable conditions for algal growth in brine than the recommended F/2 nutrient $NaNO_3$.

The use of urea for optimizing *Nannochloropsis* growth was tested in previous studies. The effect of adding different nitrogen-containing compounds, as an extra nitrogen source, on the growth optimization of *N. gaditana* was examined by Rocha et al. [46]. The authors found that adding 10 mM of urea to F/2 medium leads to the best microalgal growth promotion. The result of experiment B_U correlates with the findings of Rocha et al. [46] and moves beyond this to suggest that in a growth medium of brine, urea can be used as a substitute nutrient for $NaNO_3$. Other studies have also found that the best population productivity, was obtained when urea was used as a nitrogen source for growing *N. salina* [45].

The work of Cheirsilp and Torpee [47] reported that urea is not only a source of nitrogen but can also be considered a carbon source. This would allow a microalga to grow in a mixotrophic pattern, eliminating the strict need of light that is required for photosynthesis and cell growth under photoautotrophic conditions; and therefore, enhancing biomass production. The result of B_U is in agreement with Cheirsilp and Torpee [47] who found that the growth of all tested microalgal strains, freshwater *Chlorella* sp., marine *Chlorella* sp. and *Nannochloropsis* sp., was better under mixotrophic conditions than under either photoautotrophic or heterotrophic cultures. The mixotrophic growth of *N. salina*, observed by Kumar and Saramma [48], in the presence of low concentrations of fructose and sucrose achieved higher growth rates than those observed under photoautotrophic conditions.

The micronutrients present in F/2 medium include trace elements and vitamin stock solutions, described in the section 2.1 (experimental organism and cultivation conditions). Expensive vitamins (thiamine HCL (vit. B1), biotin (vit. H), and cyanocobalamin (vit. B12)) are known to enrich and stimulate biomass growth [49]. Experiment B_V tested the effect of using F/2 nutrients without adding the expensive vitamin stock solutions. Microalgal growth reached a three-fold increase by day 7 (Fig. 4). In comparison with B_{100}, B_V was able to reach 75% of its potential growth in a shorter period. This result is in line with [20] who reported that cultivating *N. salina* in vitamin-free F/2 medium led to a decrease in the growth rate compared to cultivation in the original F/2 medium containing the vitamin stock solution.

Conditions of experiment B_{UV} were applied to observe the effect of a growth medium enriched with urea, with no vitamins, on *Nannochloropsis* sp. growth. B_{UV} conveyed an increase of Chl-a by about five-fold by day 8 (Fig. 4). This finding portrayed an increase from B_U, and it surpassed the biomass growth levels obtained in B_{100}, although these differences were non-statistically significant (*t*-test, $p < 0.05$), they indicate that vitamin micronutrients can be entirely omitted from the growth medium in the presence of urea as a substitute for $NaNO_3$ in F/2. Decreases in the expensive micro- and macro-nutrients tested will reduce the running cost of algae cultivation thus making it more sustainable for scaling up.

3.3. Characterization of *Nannochloropsis* sp. biomass produced in B_{UV} conditions

B_{UV} experimental conditions proved to be optimum for *Nannochloropsis* cultivation in SWRO brine. The medium is cost effective and generated the largest biomass growth compared to all other experiments performed. To determine the value that can be achieved from the growth of *Nannochloropsis* sp. in a brine medium, its capacity for utilization as a bio-desalination agent and as a biofuel were tested.

3.3.1. Capacity for bio-desalination

The main components of brine (Table 1) Na^+ and Cl^-, were found to have decreased in the growth medium by 8.6% and 8.4%, respectively (Fig. 5). Cations including Mg^{++} and K^+ have also shown slight decreases in their concentrations, with the highest percentage uptake found in Mg^{++}, 21%. Significant changes were observed in the levels of nutrient concentrations where 99% of the total nitrogen (TN) and 67% of phosphorus were absorbed by the algae (Fig. 5). Nevertheless, the overall TDS of the medium increased by 5% indicating that the uptake was offset by the presence of other compounds. One such compound is

HCO_3^- where the value increased in the growth medium by 26.6%. *Nannochloropsis* sp. proved to have a low capacity for bio-desalination of SWRO brine.

3.3.2. Capacity for biofuel production

Nannochloropsis has repeatedly been linked to biofuel production due to its ability to produce high lipid content under different stress conditions [27,29,43]. Characterization of *Nannochloropsis* sp. cultivated in B_{UV} growth medium showed biomass productivity to be 0.05 ± 0.016 g L^{-1} d^{-1}. Accumulated biomass at the end of the cultivation period, 10–13 d, was 0.567 ± 0.08 g L^{-1}. The result falls within the range reported by Matos et al. [43] who grew *N. gaditana* in seawater-based F/2 medium, with different concentrations of desalination concentrate (DC). For 25%, 50%, 75%, and 100% of DC, the respective biomass accumulated was 0.63, 0.81, 0.96, and 0.33 g L^{-1}. It is important to note that the DC origin for the abovementioned experiments was an inland source of much lower salinity of 2.2 g L^{-1}, as compared to the 70 g L^{-1} DC salinity used in this study. Nevertheless, biomass productivity results proved comparable to other studies and thus feasible for scaling up.

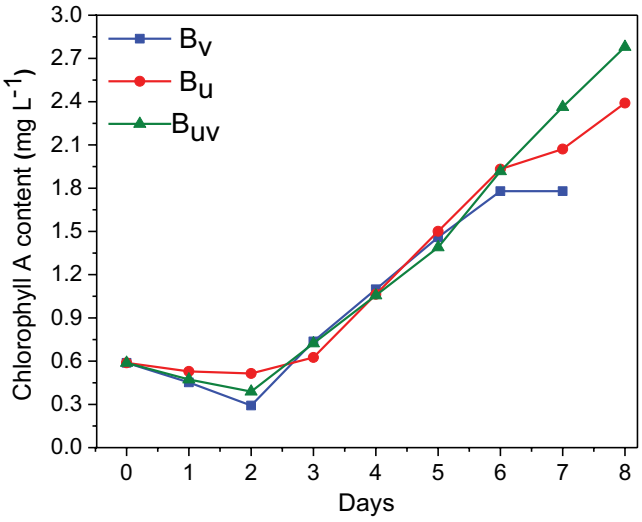

Fig. 4. Chlorophyll-a content of *Nannochloropsis* sp. observed in the brine-based F/2 culture medium at different nutrient compositions.

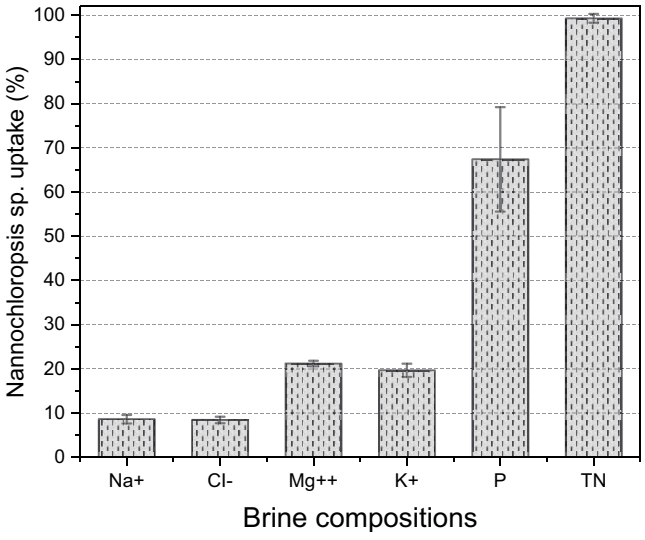

Fig. 5. Percentage of salt and nutrients uptake by *Nannochloropsis* sp. cultivated in the optimized brine-based culture medium (B_{UV}).

It has been previously noted that increases in salt stress induces the production of lipids in microalgae [29,43]. The stored lipids act as an energy reserve material until the conditions become favorable for growth [50]. In B_{UV} sample lipid productivity was found to be 4.8 ± 1 mg L^{-1} d^{-1} and the obtained lipid content of the accumulated biomass was 9.5% ± 2.1% w/w. Comparable results were reported by Martínez-Roldán et al. [29] where the lipid content of *Nannochloropsis* sp. cultivated in 81 g L^{-1} NaCl was 7.66%. Other studies also found the lipid content of *N. gaditana* grown in 100% DC to be 11.4% (w/w) [43]. Lipid accumulation found in experiment B_{UV} is comparable in literature thus promoting the use of brine as a culture medium.

Mass cultivation of *Nannochloropsis* is promising for utilization in biofuel production as it has a large capacity to accumulate high amounts of triacylglycerol (TAG) with high contents of saturated and monounsaturated fatty acids suitable for biofuel production [27,51,52]. TAGs are a good feedstock for biodiesel and their growth is mainly promoted in conditions of nutrient starvation. The cost effective, brine-based medium, B_{UV}, lipid content composition of *Nannochloropsis* sp. made up 9.5% ± 2.1% of the dry biomass. A similar conclusion, for utilizing cost-effective salt stress mediums for biofuel production from *Nannochloropsis* sp., was reached by Martínez-Roldán et al. [29]. Analysis of the fatty acid profile observed in B_{UV} is comparable to Matos et al. [43] (Table 3), cultivating *N. gaditana* in 100% DC conditions. The results portray great promise for *Nannochloropsis* sp. to be used in B_{UV} culture conditions as a source of biofuel.

4. Conclusion

Nannochloropsis sp. proved its ability to adapt to a brine-based culture medium with salinity 70 g L^{-1}. The algae

Table 3
Fatty acid profile of the harvested biomass grown in the optimized brine-based F/2 culture medium (B_{UV} culture conditions)

	Fatty acid	Percentage
Monounsaturated fatty acids	Palmitoleic acid	10.6
	Oleic acid	0.58
	9-Octadecenoic acid	20.27
Polyunsaturated fatty acid	Linoleic acid	6.6
Saturated fatty acids	Palmitic acid	44.9
	Stearic acid	2.99
	Pentadecyl hexanoic acid	0.34
Other volatile fragments		13.72

portrayed a five-fold growth increase in a cost-effective culture media based on desalination wastewater. While the microalga did not prove to be a good agent for bio-desalination, its lipid content, and fatty acid profile, confirmed its suitability as a valuable source of biofuel. The study's results validate the great potential of *Nannochloropsis* as a sustainable biological brine management tool where added value, in the form of biofuel, is created.

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