

Biodesalination of brackish water coupled with lipid production using native *Scenedesmus* sp. isolated from a saline lake in Ethiopia, Lake Basaka

Muluwork Maru^{a,*}, Feleke Zewge^b, Demeke Kifle^c, Endalkachew Sahle-Demissie^d

^aAfrica Centre of Excellence for Water Management, Addis Ababa University, Addis Ababa, Ethiopia, ORCID No: 0000-0001-8060-9542, email: muluworkmaru24@yahoo.com/muluwork.maru@aau.edu.et

^bAddis Ababa University, College of Natural and Computational Science, Addis Ababa, Ethiopia, email: feleke.zewge@aau.edu.et
^cAquatic Science, Fisheries and Aquaculture Stream, Department of Zoological Sciences, Addis Ababa University, Addis Ababa, Ethiopia, email: tsegakif@yahoo.com

^dCenter for Environmental Solution and Emergency Response, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH 45268, USA, email: sahle-demissie.endalkachew@epa.gov

Received 21 January 2022; Accepted 3 June 2022

ABSTRACT

Microalgae-based water desalination is an emerging method that uses microalgae to remove salt from brackish and seawater through a natural photosynthetic process. Because of its low energy consumption, this technology is expected to provide low-cost desalted water. Furthermore, algal lipid, which is an excellent feedstock for biodiesel production, could be extracted from the recovered algal biomass. Thus, combining algae-based desalination with lipid production could be a solution to the world's water and energy crises. This study used native *Scenedesmus* sp. isolated from a brackish Rift Valley lake for simultaneous brackish water desalination and lipid production. *Scenedesmus* sp. was cultured in Bold's basal medium containing 0–15 g/L NaCl for 20 d at 30°C and a light level of 65 $\mu\text{mol}/\text{m}^2 \text{ s}$ in a 12 h:12 h light:dark cycle. Maximum and minimum desalination efficiency of 23% and 15% were observed in cultures treated with 2.5 and 15 g/L NaCl, respectively. The highest lipid content of 26% was observed in the culture treated with 10 g/L NaCl, which is 27% higher than the control. The sample treated with 5 g/L NaCl appears to be suitable for the integrated desalination and lipid production system, achieving a desalination efficiency of 17% with lipid production of 109 mg/L. According to the findings of this study, microalgal species from a saline habitat could be used for brackish water biodesalination and lipid production simultaneously.

Keywords: Desalination; Microalgae; Algal lipid

1. Introduction

Water scarcity has become one of the most serious problems that many parts of the world are dealing with as a result of rapid population and economic growth [1]. Water shortage have an impact on public health, food security, economic development, and international relations in the utilization of transboundary water. Currently, more than 40% of the world's population suffers from acute water scarcity,

which is expected to rise to 60% by 2025 [2]. Despite this fact, 97% of the world's water is contained in the seas and oceans that are too saline to use. As a result, desalination technologies have emerged as a viable option for sustainable water supply by exploiting saline water resources like the sea and brackish waters. Desalination technologies, such as thermal and membrane-based, have been widely used in water-stressed areas, producing roughly 95 million m^3/d of desalinated water [2]. However, the high energy

* Corresponding author.

demand, the environmental impact of brine discharge, and greenhouse gas (GHG) emissions from the current desalination methods have become significant concerns [1]. As a result, there is a demand for eco-friendly and low-cost desalination technology [3].

Biodesalination is a relatively new desalination method that employs microorganisms to remove salt from saline water. Previous research has shown that microalgae can remove salt from brackish and seawater via bioaccumulation and biosorption [4]. Microalgae are photosynthetic organisms that use solar radiation to generate energy from CO₂ and water. As a result, the algae-based desalination method uses less energy to remove the salt than thermal and membrane technologies. It also helps to reduce greenhouse gas emissions by sequestering CO₂ in the atmosphere [5]. Microalgae can be mass cultured in algal ponds, which are relatively simple infrastructures to manage and maintain. Hence, highly skilled workers may not be required to run the system in remote areas [4]. Another advantage of the algae-based desalination method is the possibility of reusing the biomass for biofuel production such as biodiesel and biogas. Microalgae grown in saline water have been found to have a higher lipid content and are therefore suitable for biodiesel production [6–8]. Thus, coupling desalination with biomass re-use for biodiesel production will reduce the operational cost and environmental impact. The total expenditure (TOTEX) and the water production cost were estimated to be decreased by 26.8% when biomass was re-used compared to the system without biomass re-use [9]. However, very few studies have been done on such an integrated system [10] and additional research is needed to determine the viability of this coupled process.

Several factors influence the effectiveness of algal desalination, including the species/strain used, the initial salt concentration of the saline water, ambient temperature, light intensity, pH, algal dose, and so on [5]. The first and crucial step in designing an algae-based desalination system is to choose the appropriate algal species. The salt tolerance and salt removal capabilities of algal species are the two most important selection criteria for desalination [11]. The utilization of native microalgal species for any biotechnological application is advantageous since they adapted to the local environmental conditions such as salt stress, temperature, and light [12]. As a result, microalgae from saline and hypersaline environments are particularly appealing for biodesalination applications because they have already adapted to salt stress.

Lake Basaka is one of the brackish lakes in the Ethiopian Rift Valley. Previous studies reported an electrical conductivity of the lake ranging from 1.4 to 10.7 mS/cm [13,14]. *Scenedesmus* sp. is one of the most abundant microalgal species in this lake [15]. Thus, in the present study, the potential of native *Scenedesmus* sp. for simultaneous biodesalination and lipid production was investigated at different salt levels, for the first time.

2. Methods and materials

2.1. Growth media preparation

Bold's basal medium (BBM), a common green microalgae medium without vitamins, was used in this study [16].

The composition of BBM is shown in Table 1. The pH of the medium was adjusted to 7.5 using concentrated NaOH. To prepare the agar medium, 1.5% (w/v) agar was added to the liquid medium, transferred to Petri plates, and then allowed to solidify. All glassware and nutrient medium was sterilized in an autoclave at 121°C for 15 min.

2.2. *Scenedesmus* sp. isolation

The water sample was randomly collected from different locations of Lake Basaka and mixed. The electrical conductivity (EC) of the lake during sample collection ranged from 4.71 to 4.76 mS/cm. A 100 mL of the composite sample was transferred into a volumetric flask containing 100 mL of BBM. Then, the enriched sample was allowed to grow in a growth chamber at a room temperature of 20°C ± 2°C and a light level of 40 μmol/m² s in a 12 h:12 h light: dark cycle for two weeks. *Scenedesmus* sp. was isolated from the algal population by repeated serial dilution and agar plating methods [17]. Then a unialgal *Scenedesmus* sp. was sub-cultured into a fresh BBM for further investigation. The purity of the stock culture was ensured by a regular microscopic observation.

2.3. Experimental design

Different concentrations of NaCl (2.5, 5.0, 7.5, 10, and 15 g/L NaCl) were added to BBM to study the effect of salinity on algal growth, biodesalination ability, and lipid content of *Scenedesmus* sp. A sample without NaCl was used as a control culture. A single stock culture was used as an

Table 1
Bold's basal medium (BBM) composition

Component	Stock solution concentration (g/L)	Amount used (mL)
Macronutrients		
NaNO ₃	25	10
MgSO ₄ ·7H ₂ O	7.5	10
NaCl	2.5	10
K ₂ HPO ₄	7.5	10
KH ₂ PO ₄	17.5	10
CaCl ₂ ·2H ₂ O	2.5	10
EDTA-KOH solution		1
EDTA (with 31 g KOH)	50	
Ferric solution		1
FeSO ₄ ·7H ₂ O (with 1 mL H ₂ SO ₄)	4.98	
Boron solution		1
H ₃ BO ₃	11.42	
Trace-element solution		1
ZnSO ₄ ·7H ₂ O	8.82	
MnCl ₂ ·4H ₂ O	1.44	
MoO ₃	0.71	
CuSO ₄ ·5H ₂ O	1.57	
Co(NO ₃) ₂ ·6H ₂ O	0.49	

inoculum for all sample cultures. 30 mL of the stock culture in the logarithmic phase (5 d old) was centrifuged at a speed of 4,000 rpm (g force = 1,790 g) for 5 min using a laboratory centrifuge (Model 800B). The pellet was then washed twice with distilled water and inoculated into 500 mL cotton stoppered Erlenmeyer flasks containing 300 mL BBM treated with different levels of NaCl. The initial algal cell density in all samples was around 1.18×10^5 cell/mL.

All the batch cultures were incubated in a growth chamber for 20 d at a controlled temperature of 30°C and a light level of 65 $\mu\text{mol}/\text{m}^2 \text{ s}$ on a 12 h:12 h light: dark cycle. These growth conditions (temperature and light level) had previously been identified as the best temperature and light level for the growth of this species (data not shown here). Using small fans mounted on the growth chamber, the air was continuously circulated to provide CO_2 . The contents of the flasks were agitated by hand three times daily. For each salinity, six replicates were prepared, and the same three replicates were used to study algal growth and desalination efficiency. The remaining biomass from the batch culture was then used for lipid extraction.

2.4. Algal growth and pH monitoring

To monitor algal growth, the algal cell density was determined every other day using a hemocytometer (Improved Neubauer Haemocytometer, 0.2 mm deep chamber) under a light microscope (Hund H600, Germany, with 100 \times to 1,000 \times magnification). The mean cell number of three replicates was calculated and the number of cells per milliliter was expressed using Eq. (1) [18]. The growth rate during the exponential growth phase was calculated using Eq. (2) [19].

$$\text{Cell density} \left(\frac{\text{No. of cells}}{\text{mL}} \right) = \left(\frac{\text{total cells counted}}{\text{squares counted}} \right) \times \text{DF} \times 10^4 \quad (1)$$

where DF is the dilution factor and 10^4 is the volume conversion factor for the hemocytometer.

$$\text{Specific growth rate} (\mu) = \frac{\ln N_{t_2} - \ln N_{t_1}}{(t_2 - t_1)} \quad (2)$$

where N_{t_1} and N_{t_2} are algal cell densities at time t_1 and t_2 , respectively.

The pH of the culture was also monitored every other day using a benchtop pH meter (pH-016) at 25°C.

2.5. Biomass as dry weight

To measure the biomass as cellular dry weight (CDW), a 50 mL culture suspension at the end of the batch culture was filtered through a pre-dried and weighted membrane filter with a 0.45 μm pore size. The pellet was rinsed twice with deionized water and oven-dried at 60°C until it reached a constant weight. The dry biomass weight produced per liter was calculated using Eq. (3) [20].

$$\text{Dry biomass weight (g/L)} = \left(\frac{m}{50} \right) \times 1000 \quad (3)$$

where m is the algal cell dry weight (g) in 50 mL of growth media.

2.6. Desalination efficiency

To evaluate the algal desalination efficiency, a change in electrical conductivity of the culture medium (EC at 25°C) was monitored using a conductivity meter (Bante-520). Before algal inoculation, the initial EC of the medium was measured and recorded as EC_i . 30 mL of culture were taken every fourth day, centrifuged at a speed of 4,000 rpm (g force = 1,790 g) for 10 min, and filtered through a 0.45 μm membrane filter. Then the EC of the supernatant was measured and recorded as EC_f . The desalination efficiency was calculated according to Eq. (4) [10].

$$\text{Desalination efficiency (\%)} = \left(1 - \frac{\text{EC}_f}{\text{EC}_i} \right) \times 100 \quad (4)$$

2.7. Lipid extraction and determination

The remaining culture treated with different NaCl levels was centrifuged separately at a speed of 4,000 rpm for 10 min. The pellets were washed twice with deionized water and oven-dried at 60°C for 24 h. The pellet was then ground to a fine powder and algal lipid was extracted using modified Bligh and Dyer's method [21]. 100 mg of dried biomass was mixed with 10 mL of chloroform-methanol (2:1) mixture and left to stand overnight. The mixture was vortexed for 2 min, and a 2.5 mL chloroform-methanol (2:1) mixture was added and vortexed again for 1 min. Then, it was centrifuged at a speed of 4,000 rpm for 10 min, and filtered through a filter paper (Whatman # 41). 10 mL of 5% NaCl solution were added and mixed completely. The liquid in the lower phase was separated and transferred into a pre-weighed test tube (W_i) and dried at 60°C until constant weight (W_f) was obtained. The lipid content and lipid production were estimated according to the Eqs. (5) and (6) [10].

$$\text{Lipid content (\%)} = \frac{(W_f - W_i)}{W_a} \times 100 \quad (5)$$

$$\text{Lipid production (mg/L)} = \text{Lipid content (\%)} \times \text{Dry weight (mg/L)} \quad (6)$$

where W_a is the weight of dry algae used.

2.8. Data analysis

All experiments were carried out in triplicate and data were presented as mean \pm standard deviation. Data analysis was performed using SPSS version 21.0. One-way analysis of variance (ANOVA) was used to test the differences among treatments. Tukey's test was applied to compare means among treatments when necessary. Differences were considered to be significant at a 5% confidence level ($p < 0.05$).

3. Results and discussion

3.1. Effect of salinity on algal cell density and biomass

Fig. 1 shows the growth curves for the batch cultures of *Scenedesmus* sp. grown in the presence of different NaCl concentrations. At all salt levels, the algal cell density increased with cultivation time till the 18th culture day. The cell density of the culture containing 5 g/L NaCl remained constant after the 18th day, whereas that of the culture containing 10 g/L NaCl decreased. Moreover, the effect of salt on algal cell density depended on the age of the culture. Until the 6th culture day, the cell density of the control culture was found to be higher than those of the salt-treated cultures. However, the effect of salt was less afterward, with the cell density of cultures with 2.5 and 5.0 g/L NaCl exceeding that of the control after the 8th and 10th culture days, respectively. The slow algal growth at the early stage in the salt-treated cultures could be due to sudden salt stress, which improved with time after the acclimatization period [22].

As can be seen from Fig. 1, the highest cell density of 8.41×10^6 was observed for the culture treated with 2.5 g/L NaCl, which was followed by 7.31×10^6 and 6.38×10^6 for the culture with 5.0 g/L NaCl and the control culture, respectively. On the other hand, cultures treated with more than 5 g/L NaCl had lower cell densities than the control, with the lowest algal density of 1.83×10^6 recorded in cultures treated with 15 g/L NaCl.

The naked eye could also observe the algal growth difference between cultures treated with various NaCl concentrations due to the difference in the intensity of the green color of the cultures. Cultures containing moderate NaCl concentration (0–5 g/L NaCl) had intense green color compared to cultures containing higher salt concentrations (7.5–15 g/L NaCl), probably due to differences in chlorophyll concentrations.

Fig. 2 shows the specific growth rate and biomass as dry weight of the batch cultures of *Scenedesmus* sp. cultured in BBM treated with different NaCl concentrations. Significant differences in the specific growth rate ($p < 0.05$) were observed among algal cultures treated with different NaCl levels. As shown in the figure, the specific growth rate of cultures with 2.5 g/L (2.31 d^{-1}) and 5.0 g/L (0.229 d^{-1}) NaCl was higher than that of the control culture (0.213 d^{-1}), although the differences were not significant ($p > 0.05$). On the other hand, the specific growth rates of cultures containing 7.5 g/L

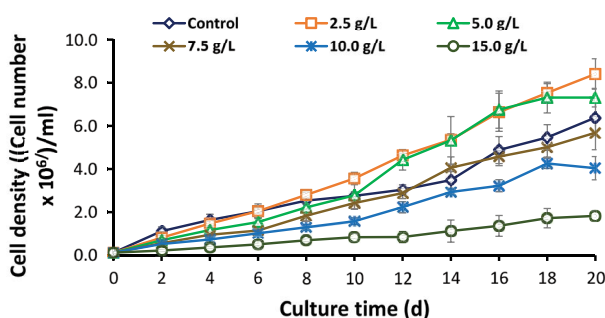


Fig. 1. Growth curves for the batch cultures of *Scenedesmus* sp. in BBM media with different levels of NaCl.

NaCl (0.208 d^{-1}) and 10 g/L NaCl (0.199 d^{-1}) were significantly lower ($p < 0.05$) than those of the cultures treated with 2.5 and 5.0 g/L NaCl. The culture treated with 15 g/L NaCl had the lowest specific growth rate of 0.149 d^{-1} , which was significantly lower ($p < 0.001$) than of all other cultures.

Similarly, a significant difference ($p < 0.05$) in the dry biomass weight of *Scenedesmus* sp. was observed among cultures treated with different NaCl concentrations. In the control culture (BBM without NaCl), dry cell weight and biomass productivity of 442 mg/L and 22.1 mg/L d, respectively, were obtained. These are comparable with the results of [23], who reported dry cell weight and biomass productivity of 421.85 mg/L and 22.72 mg/L d for *Scenedesmus* sp. CCNM 1077 culture grown in BG-11 medium. The biomass as dry weight of *Scenedesmus* sp. increased significantly ($p < 0.001$) with the addition of 2.5 and 5.0 g/L NaCl. In both cultures treated with 2.5 and 5.0 g/L NaCl, the maximum cell dry weight and biomass productivity were 450 mg/L and 22.5 mg/L d, respectively. On the other hand, high concentrations of NaCl (7.5–15 g/L NaCl) significantly reduced ($p < 0.05$) the dry biomass weight as compared to that of the control.

The above-results indicated that native *Scenedesmus* sp. isolated from Lake Basaka grew well in the presence of 0–15 g/L NaCl in the culture medium. Besides, cultures with 2.5 and 5.0 g/L NaCl exhibited enhanced growth compared to the control culture. However, a pronounced reduction in the algal growth was observed in the culture treated with 15 g/L NaCl. Elevated salt concentration negatively affects algal growth and physiological metabolism due to the osmotic and ionic stress it exerts [24]. Reduced algal growth rate and cell dry weight were reported for different strains of *Scenedesmus* sp. at higher salt levels [19,25]. Similar to our study, different *Scenedesmus* strains have previously shown increased growth in the presence of moderate salt levels [26–28]. In contrast, other studies reported a reduced algal growth with salinity increment irrespective of the salt concentration [6,10,23,29]. The enhanced growth of *Scenedesmus* sp. under moderate salt levels observed in this study could be due to the brackish nature of the lake from which the alga originated. Previous studies have also reported enhanced growth of algae in media with salt levels close to those of their habitats. Park

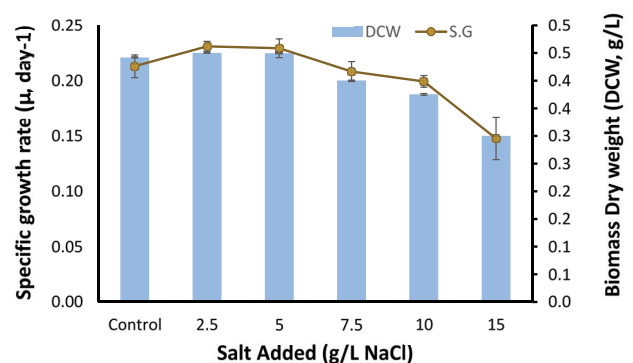


Fig. 2. Specific growth rate and biomass as dry weight of *Scenedesmus* sp. cultured under different salt levels. Values are means of three independent measurements ($n = 3$) and bars indicate standard deviation.

et al. [25] studied the growth of *Scenedesmus quadricauda* isolated from a brackish water reservoir (with an average salinity of 3.7 g/L) at different salt levels and reported enhanced algal growth at moderate salt levels (3 and 6 g/L), which were similar to those of field conditions [24].

3.2. pH of the culture

The variations in the average pH values of the culture during the cultivation period are illustrated in Fig. 3. For all samples, the pH of the culture was found to increase with culture age. The variations in pH of the culture medium treated with relatively lower NaCl levels (0 to 5.0 g/L) were found to be higher than those of cultures treated with salt levels above 5 g/L NaCl mainly due to the enhanced photosynthetic activity at the lower salt levels. The pH levels of the control and cultures treated with 2.5 and 5 g/L NaCl increased by four units, ranging from 7.38 to 11.52. The pH of the culture medium treated with 15 g/L, however, ranged from 7.25 to 9.78. At a higher photosynthetic rate, the amount of CO₂ utilized becomes higher than the supply, which leads to a rise in culture pH [30]. In the actual conditions where seawater was used, pH variation is expected to be less than the experimental values due to the better carbonate buffering capacity of seawater as compared to that of freshwater [30].

3.3. Desalination (Conductivity reduction)

In the present study, the elimination of salt from the culture medium was monitored using measurements of electrical conductivity. Before alga inoculation, the electrical conductivity (EC) of the autoclaved culture media containing various levels of NaCl was determined. Then the EC of the filtered cultures was checked at 4 d interval. The changes in EC of the culture media of the batch cultures of *Scenedesmus* sp. grown in BBM containing different levels of NaCl (0 to 15 g/L NaCl) are shown in Fig. 4.

In the present investigation, the conductivity of the cultures declined significantly ($p < 0.05$) from the beginning to the end of the cultivation period of the batch cultures of both control and treatment cultures. For all cultures, the conductivity of the culture medium decreased steadily from the start of the cultivation to the 12th day, during which more than 70% of the total salt removed was observed. On the 16th

culture day, the conductivity of the culture started to rise again for cultures containing 5.0, 7.5, and 10.0 g/L. Except for the control culture, which showed a rise in conductivity after the 16th day, the conductivity of all cultures began to drop again until the end of the culture period. The maximum salinity reduction occurred on the 20th culture day in samples treated with 2.5, 7.5, 10, and 15 g/L NaCl. Whereas in the control and culture with 5 g/L NaCl, the maximum salinity reduction was observed on the 16th and 12th days, respectively.

The unexpected increase in culture conductivity could be attributed to salt release from the dead algal cells. An abrupt salt release from a dead algal cell was also reported to have caused an increase in electrical conductivity in batch cultures of *Scenedesmus* sp. and *Chlorella vulgaris* on the 18th culture day [29]. This indicated that pronounced salt removal is expected to occur when the number of cells generated is higher than the number of cells lost. As a result, algal biomass must be harvested during this period to prevent salt from being released from the dead cells [31]. The other possible explanation for the unexpected rise in the conductivity of the culture is an active salt extrusion from the algal cells. Under saline and hyper conditions, salt-tolerant microalgae use different salt export mechanisms to maintain the ionic balance inside the cell [32]. Active Na⁺ export from living cells consumes the cell's ATP. Thus depleting internal ATP stores once the culture has achieved high cell density protects salt extrusion from the algal cell. This can be achieved through environmental manipulations such as changing the light intensity, depleting nutrients and essential metals, changing pH, and so on [31].

Microalgae remove salts both through bioaccumulation (salt uptake) and biosorption (surface adsorption) [4,29]. Adsorption was discovered to be the primary mechanism responsible for approximately 75% of salt removal [4]. Though adsorption is expected to be the main salt removal mechanism, the contribution of bioaccumulation cannot be overlooked. Yao et al. [33] reported a significant increment in cellular Na⁺ (12.0%–275.7%) for *S. obliquus* cultivated at different salinities (5–25). The amount of salt removed

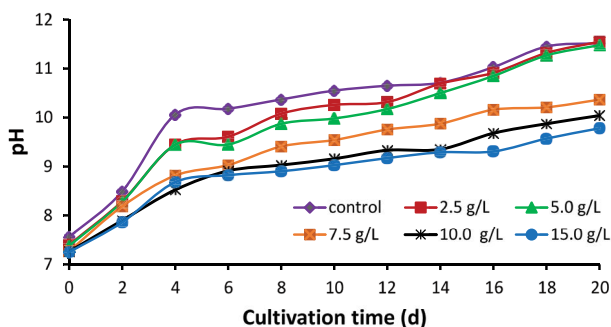


Fig. 3. Average pH values of culture media with the cultivation period.

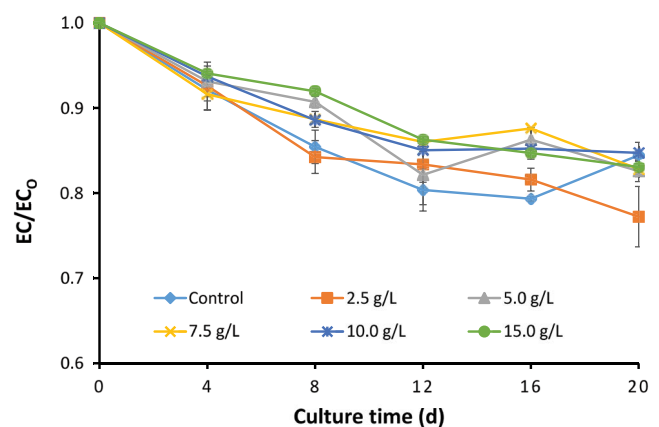


Fig. 4. Conductivity reduction (EC/EC₀) in *Scenedesmus* sp. grown in BBM dosed with different NaCl concentrations. Values are means of measurements in three independent samples ($n = 3$). The error bar indicates the standard deviation.

either by adsorption or salt uptake is expected to increase as the number of algal cells in the culture increases. Sahle-Demessie et al. [29] observed a steeper decline in electrical conductivity during the growth phase. Similarly, Sergany et al. [34] observed a minor reduction in the total dissolved solids (TDS) of saline water after the 16th day of the batch culture of *Scenedesmus* sp. probably due to a reduced algal growth after this culture period. As a result, adjusting favorable algal growth conditions for biomass production, such as temperature, light, and nutrients, is expected to increase the efficiency of desalination

3.4. Effect of salt concentration on desalination

Fig. 5 shows the desalination efficiency (%) of *Scenedesmus* sp. cultured in BBM with various NaCl concentrations at the end of the batch culture (20th day). Results of one-way ANOVA indicated a significant difference ($p = 0.002$) in the desalination efficiency (%) among cultures treated with different NaCl levels. As can be seen from Fig. 5, the sample treated with 2.5 g/L NaCl had a maximum desalination efficiency of 23%, which was substantially greater ($p < 0.05$) than all the other cultures. Whereas, the minimum desalination efficiency of 15% was recorded in the culture treated with 10 g/L NaCl. The desalination efficiency observed in this study is within the range of values recorded [11], which reported a conductivity reduction of 3% to 26% for batch cultures of *S. obliquus* and *Scenedesmus obutus* grown in BBM treated with 0.5 to 10 g/L NaCl. A higher desalination efficiency (30%) was reported for different *Scenedesmus* sp. cultivated at different initial salt levels and culture conditions [10,29].

In the present study, no direct relationship between NaCl concentration and desalination efficiency was observed. As the amount of NaCl rose from 0 to 2.5 g/L, the desalination efficiency increased from 15.6% to 22.8%. Then it decreased to 17.4% (for culture treated with 5.0 and 7.5 g/L NaCl) and 15.3% for culture with 10 g/L NaCl. However, the desalination efficiency raised to 16.9% as the amount of salt added increased to 15.0 g/L NaCl. Similar to our study, no direct relationship between salt level and salinity reduction was observed for *S. obliquus* and *S. obutus* [11]. The correlation between initial salinity and desalination efficiency is

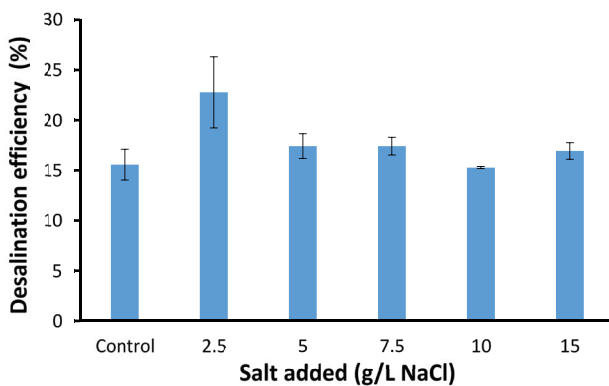


Fig. 5. Desalination efficiency (%) of batch cultures of *Scenedesmus* sp. in BB medium dosed with different NaCl concentrations. Values are the means of three replicate samples. The error bar indicates the standard deviation.

not consistent between studies. Gan et al. [10] observed a consistent increase in desalination efficiency with salinity increments from 2.2 to 8.8 g/L. In contrast, an inverse relationship between conductivity reduction and initial conductivity levels (24–59.8 mS/cm) was observed for indigenous microalgal consortium isolated from evaporation pond concentrates [35].

The algal desalination potential observed in this study could be improved further by altering various factors. Operational conditions such as algal dose, temperature, light intensity, pH, and CO₂ concentration have been reported to influence algal salt removal effectiveness [4,34,36,37]. In this study, an initial algal dose of 1.18×10^5 cell/mL was used for the batch culture study. Increasing the initial algal cell density is expected to improve the salt removal efficiency. Sergany et al. [34] reported a direct relationship between algal dose and TDS removal efficiency for *Scenedesmus* sp. cultured in a pilot plant consisting of three consecutive basins. Accordingly, the TDS removal increased from 0% of algal levels of 150 mL/path to 62.7% and 87.7% of 300 and 400 mL/path, respectively [34]. In addition to algal dose, growth conditions were also found to affect the algal desalination capacity. Sergany et al. [36] reported a 0.5% increment in TDS removal efficiency for each 2°C temperature increment. Another study Azmi et al. [37] also reported a change in desalination potential due to the interactive effect of temperature, light, and pH for the cyanobacterium *Synechococcus* sp. PCC 7002. In the present study, the culture was grown at experimentally determined optimal temperature and light levels. However, the air was used as a CO₂ source, which contains only roughly 0.04% of CO₂. Due to the low CO₂ input, the pH of the culture gradually increased throughout the cultivation time. A previous study has shown that an extra source of CO₂ is required for improved algal growth, biochemical composition as well as management of pH variations in the culture medium [38]. As a result, the provision of more CO₂ may boost algal growth and the entire desalination process. Nonetheless, further research is needed to fully comprehend the impact of each element of the culture condition on algal biodesalination ability.

3.5. Effect of salt on lipid content and production

Microalgae are becoming increasingly attractive as a source of high-lipid material for biofuel generation for different reasons. Unlike first-generation biofuel feedstocks, microalgae do not compete with food crops and can be grown on non-arable land [39]. Microalgae could be cultivated in brackish and seawater, which reduces the cost of water and also the risk of contamination by other species. Furthermore, the algal lipid content can be induced under stressed conditions such as high salinity [40]. The lipid content and lipid production of *Scenedesmus* sp. at various salt concentrations are shown in Fig. 6. A significant change in lipid content ($p < 0.05$) was observed among cultures treated with various NaCl levels. The lipid content of *Scenedesmus* sp. grown in the control culture (BBM without additional NaCl) was 20.2% of the dry cell weight. This value is consistent with a previous study [22], which reported a lipid content of 20.8% for the same local species grown in BBM medium. Meanwhile, lower lipid contents of 12.8% [6] and

18% [28] were reported for *S. obliquus* XJ002 and *S. obliquus* HM103382 grown in BG-11 and BB media, respectively.

Stressful growth circumstances, such as high salinity, are reported to induce the algal lipid content. An increase in algal lipid content under salt stress has been documented for different *Scenedesmus* strains [6,10,23]. Under salt stress, lipid biosynthetic pathways shift from membrane lipid synthesis towards neutral lipid accumulation, primarily triacylglycerol, and glycerol, which are ideal feedstocks for biodiesel [41]. The lipid content of *Scenedesmus* sp. increased from 20.2% to 25.7% as the concentration of NaCl added to the medium grew from 0 to 10 g/L, respectively. However, it decreased again to 24% as the concentration of NaCl further increased to 15 g/L. Yet, the amount of lipid in the cultures treated with NaCl was considerably higher ($p < 0.05$) than in the control culture, except in the culture treated with 2.5 g/L NaCl. Similarly, an increase in lipid content from 18% to 34% was observed in *S. obliquus* as the quantity of NaCl added to BBM grew from 0 to 25 mM (approximately 1.5 g/L NaCl). However, the lipid content dropped to 21% as the salt concentration increased further to 100 mM NaCl (about 5.8 g/L) [28]. The highest lipid content of about 26% was observed in the culture with 10 g/L NaCl, which is about 27% higher than in the control. The maximum lipid content obtained in this study is high compared to *S. obliquus*, which exhibits 21% when grown at a salinity of 8.8 g/L NaCl [10]. Others report a lipid content higher than 30% [6,28] for *Scenedesmus* sp. treated with different salt levels.

Because of the reduced algal growth under salt stress, most investigators found an inverse correlation between lipid content and lipid production rate [10]. In our study, the lipid production of *Scenedesmus* sp. increased as the level of NaCl in the culture medium increased from 0 to 5 g/L NaCl. This is due to the increased algal growth and biomass at moderate salt levels of 2.5 and 5.0 g/L NaCl. For cultures with 0, 2.5, and 5.0 g/L NaCl, lipid production was found to be 89, 94.6, and 108.7 mg/L, respectively. Cultures treated with 7.5, 10, and 15 g/L NaCl, on the other hand, produced 98, 96, and 72 mg/L of lipids, respectively. Algal cultures with greater lipid productivities but intermediate lipid accumulation (20%–50%) are favored for mass cultivation over those with higher lipid content but lower lipid production

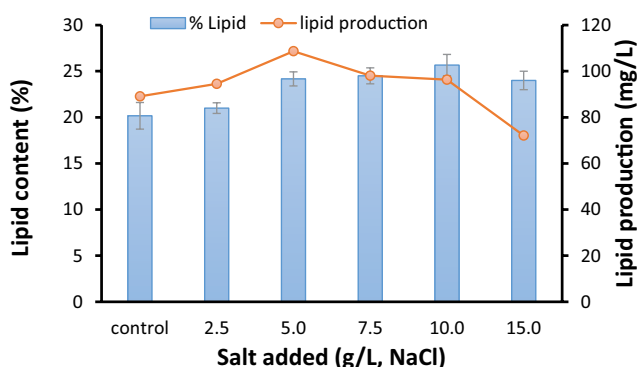


Fig. 6. Lipid content (%) of dry cell weight and lipid production rate (mg/L) of *Scenedesmus* sp. biomass at a different salt concentration.

[42,43]. As a result, a culture medium with 5 g/L NaCl can be considered to have the optimum salinity level for lipid production.

3.6. Integrating biodesalination with lipid production

The above-results indicated that *Scenedesmus* sp. isolated from Lake Basaka could be employed for the simultaneous use of brackish water desalination and lipid production. Table 1 summarizes the overall results for the batch culture of *Scenedesmus* sp. grown in BBM treated with 0–15 g/L NaCl at the end of the batch culture. The salt level at which maximum desalination efficiency and lipid production occurred at different salt levels, as shown in the table. Maximum desalination efficiency of 23% was observed in the culture treated with 2.5 g/L NaCl. However, if we consider the total amount of salt removed from the given culture, 17% EC reduction in the culture treated with 15 g/L NaCl was by far better than the 23% reduction in the culture treated with 2.5 g/L NaCl. Maximum lipid production (109 mg/L), on the other hand, was recorded in a culture containing 5 g/L NaCl. As a result, the best salt level for simultaneous desalination and lipid production was found to be for the culture treated with 5 g/L NaCl. A similar study by Gan et al. [44] examined the potential of *Scenedesmus obliquus* for simultaneous use of desalination and lipid production using a Blue-Green medium (BG-11) having salinity between 1.2 to 8.8 g/L NaCl. According to this study, a salinity of 4.8 g/L NaCl was found as an acceptable salt level for this coupled system. At this salt level, a salt removal of 1 g/L (around 20.8% desalination efficiency) and lipid production of 100 mg/L was achieved. The desalination efficiency reported in the study [44] was slightly higher than our result, while the lipid production was lower. This could be due to the difference in culture growth conditions such as nutrient media used. In Gan et al. [44], study BG-11 media, having relatively higher nitrate concentration (1.5 g/L of NaNO_3) [45] compared to BBM (0.25 g/L NaNO_3), was used. This high nitrate concentration could increase the algal growth and biomass produced, which in turn improve the salt removal process. Similarly, the high lipid production observed in our study could be due to the lower nitrate concentration in BBM, which facilitates lipid accumulation. The increase in lipid accumulation under a nutrient limitation is well documented in previous studies [46,47]. However, additional investigations on the effect of nutrient concentration on the performance of this coupled system are required.

According to the present and prior studies, total desalination cannot be accomplished in a single batch cycle. Hence, a multi-stage desalination process is required for the practical implementation of algal-based desalination to produce the necessary water quality for the intended use (Fig. 7). The number of desalination stages required is determined by the desired water quality, the water's initial salinity, and the algal desalination capability in a single batch cycle [48].

In a multi-stage desalination process, the saline water desalted in the first stage of the process will be transferred to the second-stage and desalted with new microalgae culture, and so on. Thus, the overall salt removal capacity will then be boosted as a result of this operation. The biomass collected at each stage will be collected and used for lipid

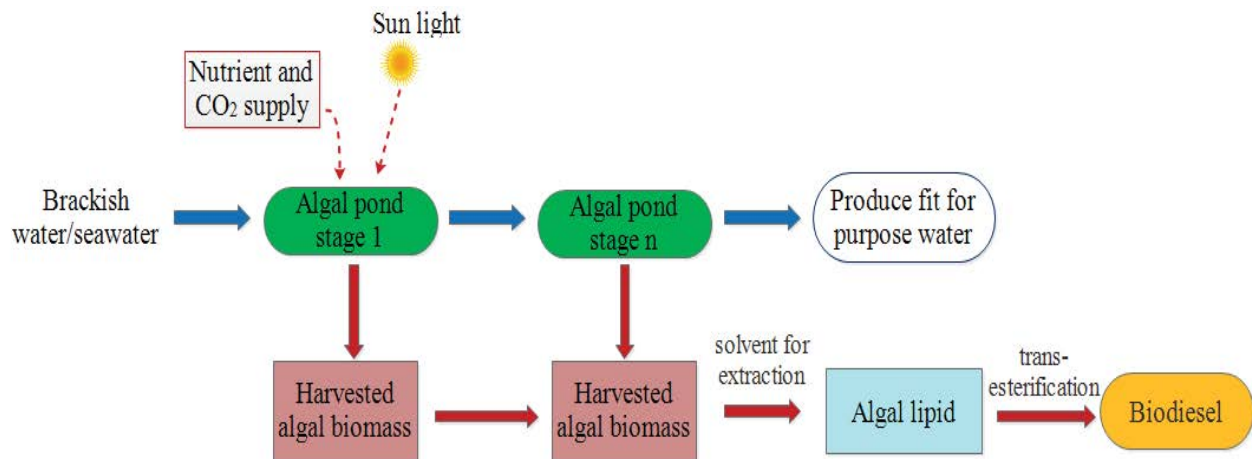


Fig. 7. Multi-stage microalgae-based desalination integrated with biodiesel production.

Table 2

Summary of the desalination efficiency, lipid content, and lipid production of *Scenedesmus* sp. grown in BBM containing various levels of NaCl

Culture (g/L NaCl added)	Desalination efficiency (%)	Lipid content (%)	Lipid production (mg/L)
Control	15.6 ± 1.5	20.2 ± 1.4	89.1
2.5	22.8 ± 3.5	21.0 ± 0.6	94.6
5.0	17.4 ± 1.2	24.2 ± 0.8	108.7
7.5	17.4 ± 0.9	24.5 ± 0.9	98.1
10.0	15.3 ± 0.1	25.7 ± 1.2	96.3
15.0	16.9 ± 0.8	24.0 ± 1.0	72.1

extraction, which could be converted into biodiesel through the transesterification process. A group of researchers from Egypt has built an outdoor continuous algal pond containing three consecutive basins to study the desalination capacity of *Scenedesmus* sp. under natural conditions. They reported a TDS removal above 95% from the three basins with initial TDS of the water ranging from 2,000 to 40,000 ppm [49].

3.7. Biomass harvest and downstream processing

Algal harvesting or dewatering is the biggest downside connected with algal downstream processing, accounting for 20%–30% of the overall production cost. The small size and density of most algal cells with negatively charged cell surfaces make biomass harvesting challenging [50]. The most common algal harvesting techniques include centrifugation, filtration, and gravity sedimentation. The choice of biomass recovery depends on the biomass slurry's density and the value of the end product [51]. The use of attached cultivation can also be considered as an alternative for the suspended culture due to the ease of harvesting [52].

The presence of salt in the algal biomass is another major obstacle to utilizing salt-water-grown microalgae to produce valuable biochemicals. For marine microalgae cultivated in saline water, the cell dry weight of non-washed algal biomass was found to be 1.2 times higher than that of biomass

cleaned with distilled water [53]. Furthermore, the presence of salt on the algal cell wall was visible in a scanning electron microscopy (SEM) image of salt-grown alga [54], which could impair downstream processing. Knoshaug et al. [55] reported a difference in lipid extraction yield from the fermentation broth between the salt-water and fresh-water grown algal biomass. Washing solvents such as distilled water, HCl, and ammonium bicarbonate have been suggested to remove salt from the algal biomass to produce fatty acids and other valuable products from marine microalgae. The washing solvent was found to affect the composition of the biochemicals produced from the biomass. Significant differences in the relative abundance of saturated fatty acids were observed between algal biomass cleaned with different washing treatments and reported that the untreated biomass resulted in a better fatty acid profile than distilled water-washed biomass for biodiesel production [54]. However, further studies are required to understand the effect of different washing treatments on algal lipid yield and biodiesel quality.

4. Conclusions

The present study shows that native *Scenedesmus* sp. isolated from a saline lake, Lake Basaka, can be used for simultaneous brackish water desalination and lipid production. The maximum desalination efficiency of 23% was achieved in the sample treated with 2.5 g/L NaCl. Whereas, the minimum desalination efficiency of 15% was observed in the sample treated with 10 g/L NaCl. The algal lipid content increased from 20% to 26% as the amount of NaCl in the medium increased from 0 to 10 g/L. Due to the enhanced algal growth at moderate salt levels, a maximum lipid production of 108 mg/L was observed in the culture treated with 5 g/L NaCl. Thus, the sample treated with 5 g/L NaCl seems suitable for the simultaneous desalination and lipid production system. At this salinity level, a desalination efficiency and lipid production of 17% and 109 mg/L, respectively, were achieved. This study shows that native algal species originating from a saline habitat could be utilized for simultaneous desalination and lipid production systems.

Acknowledgments

The authors are grateful to the Africa Centre of Excellence for Water Management (ACEWM), Addis Ababa University, Ethiopia, for financial support of this work. This study was funded by ACEWM, Addis Ababa University, Ethiopia. The views expressed in this manuscript are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

Conflict of interest

The authors declare that they have no conflict of interest.

References

- [1] M. Elimelech, W.A. Phillip, The future of seawater desalination: energy, technology, and the environment, *Science*, 333 (2011) 712–717.
- [2] E. Jones, M. Qadir, M.T.H. van Vliet, V. Smakhtin, S.-m. Kang, The state of desalination and brine production: a global outlook, *Sci. Total Environ.*, 657 (2019) 1343–1356.
- [3] K. Minas, E. Karunakaran, T. Bond, C. Gandy, A. Honsbein, M. Madsen, J. Amezaga, A. Amtmann, M.R. Templeton, C.A. Biggs, L. Lawton, Biodesalination: an emerging technology for targeted removal of Na⁺ and Cl⁻ from seawater by cyanobacteria, *Desal. Water Treat.*, 55 (2015) 2647–2668.
- [4] J. Wei, L. Gao, G. Shen, X. Yang, M. Li, The role of adsorption in microalgae biological desalination: salt removal from brackish water using *Scenedesmus obliquus*, *Desalination*, 493 (2020) 1–7, doi: 10.1016/j.desal.2020.114616.
- [5] L. Gao, X. Zhang, L. Fan, S. Gray, M. Li, Algae-based approach for desalination: an emerging energy-passive and environmentally friendly desalination technology, *ACS Sustainable Chem. Eng.*, 9 (2021) 8663–8678.
- [6] X. Ji, J. Cheng, D. Gong, X. Zhao, Y. Qi, Y. Su, W. Ma, The effect of NaCl stress on photosynthetic efficiency and lipid production in freshwater microalga—*Scenedesmus obliquus* XJ002, *Sci. Total Environ.*, 633 (2018) 593–599.
- [7] L. Xia, J. Rong, H. Yang, Q. He, D. Zhang, C. Hu, NaCl as an effective inducer for lipid accumulation in freshwater microalgae *Desmodesmus abundans*, *Bioresour. Technol.*, 161 (2014) 402–409.
- [8] R. Kakarla, J.W. Choi, J.H. Yun, B.H. Kim, J. Heo, S. Lee, D.H. Cho, R. Ramanan, H.S. Kim, Application of high-salinity stress for enhancing the lipid productivity of *Chlorella sorokiniana* HS1 in a two-phase process, *J. Microbiol.*, 56 (2018) 56–64.
- [9] L. Gao, G. Liu, A. Zamyadi, Q. Wang, M. Li, Life-cycle cost analysis of a hybrid algae-based biological desalination – low pressure reverse osmosis system, *Water Res.*, 195 (2021) 1–15, doi: 10.1016/j.watres.2021.116957.
- [10] X. Gan, G. Shen, B. Xin, M. Li, Simultaneous biological desalination and lipid production by *Scenedesmus obliquus* cultured with brackish water, *Desalination*, 400 (2016) 1–6.
- [11] A. Figler, V. B-Béres, D. Dobronoki, K. Márton, S.A. Nagy, I. Bácsi, Salt tolerance and desalination abilities of nine common green microalgae isolates, *Water*, 11 (2019) 2527, doi: 10.3390/w11122527.
- [12] Indrayani, Haslianti, Asriyana, Isolation and screening of marine microalgae from kendari waters, southeast Sulawesi, Indonesia suitable for outdoor mass cultivation in hypersaline media, *AAFL Bioflux*, 11 (2018) 1445–1455.
- [13] M. Olumana, W. Loiskandl, J. Fürst, Effect of Lake Basaka Expansion on the Sustainability of Matahara SE in the Awash River Basin, Ethiopia, *Water, Sanitation and Hygiene: Sustainable Development and Multisectoral Approaches*, 34th WEDC International Conference, Addis Ababa, Ethiopia, 2009, pp. 1–9.
- [14] M.O. Dinka, Analysing the temporal water quality dynamics of Lake Basaka, Central Rift Valley of Ethiopia, *IOP Conf. Ser.: Earth Environ. Sci.*, 52 (2017) 1–7, doi: 1755-1315/52/1/012057.
- [15] R. Nega, Microalgal diversity study of Lake Basaka, Metehara, Ethiopia, *J. Ecol. Nat. Resour.*, 5 (2021) 1–4, doi: 10.23880/jenr-16000256.
- [16] H. Bischoff, H. Bold, Some soil algae from enchanted rock and related algal species, *Phycol. Stud.*, 44 (1963) 1–95.
- [17] R.A. Andersen, *Algal Culturing Techniques*, Elsevier Academic Press, Amsterdam, 2005, pp. 83–98.
- [18] A.L. Abubakar, Effect of salinity on the growth parameters of halotolerant microalgae, *Dunaliella* spp., *Niger. J. Basic Appl. Sci.*, 24 (2017) 85–91.
- [19] R.S. Gour, V.K. Garlapati, A. Kant, Effect of salinity stress on lipid accumulation in *Scenedesmus* sp. and *Chlorella* sp.: feasibility of stepwise culturing, *Curr. Microbiol.*, 77 (2020) 779–785.
- [20] R. Devasya, A. Bassi, Investigation of phyco-remediation of road salt run-off with marine microalgae *Nannochloropsis gaditana*, *Environ. Technol. (United Kingdom)*, 40 (2019) 553–563.
- [21] E.G. Bligh, W.J. Dyer, A rapid method of total lipid extraction and purification, *Can. J. Biochem. Physiol.*, 37 (1959) 911–917.
- [22] T.T. Mamo, Y.S. Mekonnen, Microwave-assisted biodiesel production from microalgae, *Scenedesmus* species, using goat bone-made nano-catalyst, *Appl. Biochem. Biotechnol.*, 190 (2020) 1147–1162.
- [23] I. Pancha, K. Chokshi, R. Maurya, K. Trivedi, S.K. Patidar, A. Ghosh, S. Mishra, Salinity induced oxidative stress enhanced biofuel production potential of microalgae *Scenedesmus* sp. CCNM 1077, *Bioresour. Technol.*, 189 (2015) 341–348.
- [24] P. Shetty, M.M. Gitau, G. Maróti, Salinity stress responses and adaptation mechanisms in eukaryotic green microalgae, *Cells*, 8 (2019) 1–16, doi: 10.3390/cells8121657.
- [25] M.-H. Park, C.-H. Park, Y.B. Sim, S.-J. Hwang, Response of *Scenedesmus quadricauda* (Chlorophyceae) to salt stress considering nutrient enrichment and intracellular proline accumulation, *Int. J. Environ. Res. Public Health*, 17 (2020) 1–12, doi: 10.3390/ijerph17103624.
- [26] P. Kaewkannetra, P. Enmak, T. Chiu, The effect of CO₂ and salinity on the cultivation of *Scenedesmus obliquus* for biodiesel production, *Bioprocess Eng.*, 17 (2012) 591–597.
- [27] J.F. Sánchez, J.M. Fernández, F.G. Acien, A. Rueda, J. Pérez-Parra, E. Molina, Influence of culture conditions on the productivity and lutein content of the new strain *Scenedesmus almeriensis*, *Process Biochem.*, 43 (2008) 398–405.
- [28] E.S. Salama, H.C. Kim, R.A.I. Abou-Shanab, M.K. Ji, Y.K. Oh, S.H. Kim, B.H. Jeon, Biomass, lipid content, and fatty acid composition of freshwater *Chlamydomonas mexicana* and *Scenedesmus obliquus* grown under salt stress, *Bioprocess Biosyst. Eng.*, 36 (2013) 827–833.
- [29] E. Sahle-Demessie, A. Aly Hassan, A. El Badawy, Biodesalination of brackish and seawater using halophytic algae, *Desalination*, 465 (2019) 104–113.
- [30] C.Y. Chen, E.G. Durbin, Effects of pH on the growth and carbon uptake of marine phytoplankton, *Mar. Ecol. Prog. Ser.*, 109 (1994) 83–94.
- [31] J.M. Amezaga, A. Amtmann, C.A. Biggs, T. Bond, C.J. Gandy, A. Honsbein, E. Karunakaran, L. Lawton, M.A. Madsen, K. Minas, M.R. Templeton, Biodesalination: a case study for applications of photosynthetic bacteria in water treatment, *Plant Physiol.*, 164 (2014) 1661–1676.
- [32] H. Gimmler, Primary sodium plasma membrane ATPases in salt-tolerant algae: facts and fictions, *J. Exp. Bot.*, 51 (2000) 1171–1178.
- [33] Z. Yao, C. Ying, J. Lu, Q. Lai, K. Zhou, H. Wang, L. Chen, Removal of K⁺, Na⁺, Ca²⁺, and Mg²⁺ from saline-alkaline water using the microalga *Scenedesmus obliquus*, *Chin. J. Oceanol. Limnol.*, 31 (2013) 1248–1256.
- [34] E. Sergany, F.A. Gh. E. Hosseiny, E. Nadi, The optimum algae dose in water desalination by algae ponds, *Int. Res. J. Adv. Eng. Sci.*, 4 (2019) 152–154.
- [35] M.T. Myint, W. Hussein, A. Ghassemi, Microalgal process for treatment of high conductivity concentrates from inland desalination, *Desal. Water Treat.*, 57 (2016) 4313–4321.
- [36] E. Sergany, A. Fadhli, E. Nadi, Temperature effect on desalination by algae, *Aust. J. Basic Appl. Sci.*, 8 (2014) 277–281.

- [37] A.S. Azmi, F.S. Sani, F. Ali, M. Mel, P.A. Baru, Interactive effect of temperature, pH and light intensity on biodesalination of seawater by *Synechococcus* sp. PCC 7002 and on the cyanobacteria growth, *J. Adv. Res. Fluid Mech. Therm. Sci.*, 52 (2018) 85–93.
- [38] S.P. Singh, P. Singh, Effect of CO₂ concentration on algal growth: a review, *Renewable Sustainable Energy Rev.*, 38 (2014) 172–179.
- [39] Y. Chisti, Biodiesel from microalgae beats bioethanol, *Trends Biotechnol.*, 26 (2008) 126–131.
- [40] J.P. Cañavate, C. Fernández-Díaz, An appraisal of the variable response of microalgal lipids to culture salinity, *Rev. Aquacult.*, 14 (2022) 192–212.
- [41] H.Y. El-Kassas, Growth and fatty acid profile of the marine microalga *Picochlorum* sp. grown under nutrient stress conditions, *Egypt. J. Aquat. Res.*, 39 (2013) 233–239.
- [42] T.M. Mata, A.A. Martins, N.S. Caetano, Microalgae for biodiesel production and other applications: a review, *Renewable Sustainable Energy Rev.*, 14 (2010) 217–232.
- [43] H.M. Amaro, A.C. Guedes, F.X. Malcata, Advances and perspectives in using microalgae to produce biodiesel, *Appl. Energy*, 88 (2011) 3402–3410.
- [44] X. Gan, G. Shen, B. Xin, M. Li, Simultaneous biological desalination and lipid production by *Scenedesmus obliquus* cultured with brackish water, *Desalination*, 400 (2016) 1–6.
- [45] R. Rippka, J. Deruelles, J.B. Waterbury, Generic assignments, strain histories and properties of pure cultures of cyanobacteria, *J. Gen. Microbiol.*, 111 (1979) 1–61.
- [46] Y. Bulut Mutlu, O. Işık, L. Uslu, K. Koç, Y. Durmaz, The effects of nitrogen and phosphorus deficiencies and nitrite addition on the lipid content of *Chlorella vulgaris* (Chlorophyceae), *Afr. J. Biotechnol.*, 10 (2011) 453–456.
- [47] M. Cobos, J.D. Paredes, J.D. Maddox, G. Vargas-Arana, L. Flores, C.P. Aguilar, J.L. Marapara, J.C. Castro, Isolation and characterization of native microalgae from the Peruvian Amazon with potential for biodiesel production, *Energies*, 10 (2017) 1–16.
- [48] L. Gao, J. Zhang, G. Liu, Life cycle assessment for algae-based desalination system, *Desalination*, 512 (2021) 1–9.
- [49] M.H.A. El Nadi, F.A.G.H. El Sergany, O.M. El Hosseiny, Desalination using algae ponds under nature Egyptian conditions, *J. Water Resour. Ocean Sci.*, 3 (2014) 69–73.
- [50] E.M. Grima, F.G. Acie, A.R. Medina, Y. Chisti, Recovery of microalgal biomass and metabolites: process options and economics, *Biotechnol. Adv.*, 20 (2003) 491–515.
- [51] I. Rawat, R.R. Kumar, T. Mutanda, F. Bux, Biodiesel from microalgae: a critical evaluation from laboratory to large scale production, *Appl. Energy*, 103 (2013) 444–467.
- [52] J. Wang, W. Liu, T. Liu, Biofilm based attached cultivation technology for microalgal biorefineries — a review, *Bioresour. Technol.*, 244 (2017) 1245–1253.
- [53] C.J. Zhu, Y.K. Lee, Determination of biomass dry weight of marine microalgae, *J. Appl. Phycol.*, 9 (1997) 189–194.
- [54] J.T. Mary Leema, T. Persia Jothy, D. Magesh Peter, T.S. Kumar, G. Dharani, A critical look into different salt removal treatments for the production of high value pigments and fatty acids from marine microalgae *Chlorella vulgaris* (NIOT-74), *Biotechnol. Rep.*, 30 (2021) e00627, doi: 10.1016/j.btre.2021.e00627.
- [55] E.P. Knoshaug, T. Dong, R. Spiller, N. Nagle, P.T. Pienkos, Pretreatment and fermentation of salt-water grown algal biomass as a feedstock for biofuels and high-value biochemicals, *Algal Res.*, 36 (2018) 239–248.