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ORIGINAL ARTICLE

Effect of Sodium Chloride Concentrations on *Scenedesmus dimorphus* (Turpin) Kützing Growth and Fatty Acid Composition Under Mixotrophic Conditions

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ABSTRACT

The growing need for energy and the negative effects of using fossil fuels on the environment makes the search for sustainable and renewable energy sources imperative. Many studies are currently being conducted on the use of microalgae biofuels as a potential replacement for fossil fuels. This study examined how different concentrations of sodium chloride (NaCl) affected *Scenedesmus dimorphus*'s growth and essential fatty acid accumulation. The concentrations of NaCl used in cultivation of this strain in BG 11 medium under mixotrophic batch condition were 0.8g L^{-1}, and 0.16 gL^{-1} with a control (0.0g L^{-1} NaCl) . The control group, 0.8 gL^{-1}, and 1.6 gL^{-1} NaCl concentrations had specific growth rates of 0.93, 0.99, and 0.80 cells day^{-1}, respectively, suggesting that the culture with 0.8 g L^{-1} NaCl provided the best condition for cell proliferation. Conversely, the control (no NaCl) had the highest lipid productivity (16.94 $mgL^{-1}d^{-1}$), with increased total content of saturated and monounsaturated fatty acids. Addition of sodium chloride (1.6 gL^{-1}) elicited lower lipid productivity when compared with the control, but significantly increased the concentration of essential fatty acid (18:3) from 28.81 to 34.04 %, and arachidonic acid (20:4) from 1.00 to 2.50 %. These findings suggest that addition of NaCl (1.6 gL^{-1}) could induce high essential fatty acid synthesis in *Scenedesmus dimorphus*, necessary for biodiesel production.

Keywords: Biomass; Lipid production; Salinity; Fatty acids; *Scenedesmus dimorphus.*

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1.0 INTRODUCTION

Microbial oils, particularly triacylglycerols, or TAGs, produced by oleaginous microorganisms, have become a viable alternative to fossil fuels and plants due to the growing global demand for sustainable fuels and oleochemicals (Huo & Melis, 2018; Ratledge, 2013; Ikele & Ogbonna, 2022). Achieving a high biomass concentration and optimizing the biomass's intracellular lipid content are the two main parameters that directly affect this biotechnological process's economic viability (Panda & Gowthaman, 2020). Microbial physiology and metabolic pathways are significantly influenced by the physicochemical characteristics and content of the culture media. One important factor influencing cellular function is salinity, which is primarily regulated by the concentration of sodium chloride (NaCl). Sodium chloride (NaCl) has an effect on membrane permeability, enzyme activity, intracellular ion balance, and external osmotic pressure (Liang & Jiang, 2015). The integrity and functionality of the cell membrane, which is one of the basic cellular components affected by external stress, depend heavily on its lipid composition, particularly the fatty acid profile. Although basic cellular functions frequently require moderate salt concentrations, large quantities cause osmotic stress, which can impede growth (Oswald & Lurlin, 2018). A cellular adaptation response, such as preserving membrane fluidity under stress, is frequently indicated by changes in the ratios of Polyunsaturated (PUFA), monounsaturated (MUFA), and saturated (SFA) fatty acids. The rigidifying effects of stress are typically countered by a rise in unsaturation (increased MUFA and PUFA). Report shows that mild environmental stress, like nitrogen starvation or, in certain situations, osmotic stress caused by NaCl, is known to activate the organism's defense mechanism, redirecting carbon flux from growth-related synthesis (proteins, nucleic acids) to the build-up of storage compounds, mostly lipids (Xu et al., 2009). There is limited research on the impact of salt chloride concentrations on this strain's growth and fatty acid profile in the literature, thus it is necessary to look into how salinity stress affects these traits. There are few studies on how salinity affects *Scenedesmus* species' growth and fatty acid composition (Pushpakumari Kudahettige et al., 2018; Romanenko et al., 2017; Wiem et al., 2020).

This study aims to evaluate the effects of different NaCl concentrationson the kinetics and lipid synthesis ability of an oleaginous microalga, *Scenedesmus dimorphus*. By assessing the specific growth rate, oil content, biomass and lipid productivity, and fatty acid profiles, this study seeks to identify the optimal NaCl concentrations that will support both growth and lipid accumulation. In addition, influence of salt stress on the growth parameters and fatty acids production on microalgae is strain-specific and

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hence, the necessity to examine salinity effect on microalgae on strain basis.

2.0 MATERIALS AND METHODS

2.1 Preparation of the microalgae strain, inoculum, and medium

The oleaginous microalgae *Scenedesmus dimorphus*, strain ID NIES-93, was supplied by the National Institute of Environmental Studies (NIES) in Japan. The *Scenedesmus dimorphus* strain was subcultured in the lab using BG11 growth medium (Supriya et al., 2023). The inoculum was prepared by adding 10% stock culture with 200 mL of BG 11 media to a 500 mL Erlenmeyer flask. It was then subjected to continuous light illumination (40 µmol photons m^{-2} s^{-1}) using white fluorescent light (FHF32EX-N-HX-S, NEC Lighting Co., Ltd., Japan) provided by the Incubator-Biotron NK system (Nippon Medical & Chemical Instruments Co., Ltd., Japan) for ten days at 25ºC and 100 rpm in a rotating shaker. The following salts were used to prepare the BG 11 medium that was used for the cultivation of algal culture in this study - $NaNO_3$ (0.5 g), K_2HPO_4 (0.04 g), $MgSO_4.7H_2O$ (0.075 g), $CaCl_2.2H_2O$ (0.036 g), Citric acid (6.0 mg), Ferric ammonium citrate (6.0 mg), EDTA (1.0 mg), Na_2CO_3 (0.02 g), trace metal mixture (1.0 mL) (see below) and Distilled Water (1000 mL). The composition of trace metal mixture were H_3BO_3 (2.86 g), $MnCl_2.4H_2O$ (1.81 g), $ZnSO_4.7H_2O$ (0.222 g), $Na_2MoO_4.2H_2O$ (0.39 g), $CuSO_4.5H_2O$ (0.079 g), Co $(NO_3)_2.6H_2O$ (49.4 mg) and Distilled Water (1000 mL).

2.2 Cultivation methods

BG 11 media (200 mL) containing 0.0, 0.8, and 1.6 g L^{-1} NaCl and 5 g L^{-1} glucose as the carbon source was sterilized at 121ºC for 15 min in 500 mL Erlenmeyer flasks with foams sealed with rubber corks for ventilation. The algal cultures were exposed to 80 µmol m^{-2} s^{-1} of light during a 12 h light/dark photoperiod while being incubated at 25 ºC in a rotary shaker at 100 rpm. Three replications of the cultures seeded with 10% (v/v) inoculum were conducted. The cultures were kept at a temperature of 25 ºC using white fluorescent light (FHF32EX-N-HX-S, NEC Lighting Co., Ltd., Japan) provided by the Incubator-Biotron, NK system (Nippon Medical & Chemical Instruments Co., Ltd., Japan). A 2.0 mL was taken every day to measure cell growth during the strain's 10 days growth phase. The cell growth was measured using a spectrophotometer (U-2000A, Hitachi, Japan) at a wavelength of 680 nm and the optical density of the biomass factor was computed. . Growth rate, specific growth rate, biomass doubling time, and biomass productivity were all determined using the optical density technique. The following formulas were used to determine the biomass doubling time (*Td*) and specific growth rate (µ) (Krzeminskai et al., 2014; Yadavali et al., 2010).

$$\mu = \frac{\ln\left(\frac{N2}{N1}\right)}{T2-T1} \quad (1)$$

where N_1 is the initial and N_2 is the final optical density measured at 680 nm, T_1 is the initial and T_2 is the final cultivation period, and µ is the specific growth rate (day^{-1}).

$$T_d = \frac{ln2}{\mu} \quad (2)$$

Where T_d stands for biomass doubling time. The culture was centrifuged for five min at 5,000 rpm after cultivation. The cell pellet was washed twice with distilled water before being dried in an oven at 70ºC for 24 h. Both the concentration of biomass and the total oil content were measured. Dry cell weight was determined using the gravimetric method described by Rao et al. (Rao et al., 2007), and growth was reported in terms of dry cell concentration (g L^{-1}). The biomass productivity, P (mg L^{-1} day^{-1}), was calculated as the difference in biomass concentrations X (mg L^{-1}) over a culture time t (in days):

$$P = (X_1 - X_0) / (t_1 - t_0) \quad (3)$$

2.3 Biomass pretreatment and oil extraction

To ensure effective algal lipid extraction, the biomass was pretreated. Following lyophilisation in a freeze dryer (EYELA FDU-1200, TOKYO RIKAKIKAI CO., LTD., Japan), the harvested biomass was dried to constant mass at 60°C using a laboratory dryer. The freeze-dried biomass was ground into smaller particles using a pistol and motar. Following pulverization, it was sonicated at 500 w, 20 kHz, and 70% amplitude using a Sonics Vibra-Cell 500 sonicator. The samples were submerged in an ice bath to prevent overheating. A modified Bligh & Dyer (1959) technique using methanol and chloroform was used to extract lipid from microalgae biomass. Briefly, a 1.25 mL of chloroform and 2.5 mL of methanol (1:2 v/v) were added for each 100 mg of the dried and powdered biomass. Following that, a shaker was used to shake the samples for eighteen min. The samples were then placed on a vortex and 1.25 mL of chloroform was added. After adding 1.25 mL of water, the samples were vortexed once more. After centrifuging the samples for 10 min at 600 x g to form two separate layers, the lower layers were moved to round-bottom flasks. A vacuum evaporator (EYELA N-1110 with aspirator pump, Yamato Scientific co. Ltd., Japan) was used to extract the solvents. Gravimetric analysis was used to calculate the total yields following the triple lipid extractions.

2.4 Lipid analysis

Lipid analysis was carried out using the procedure outlined by Kotajima et al. (2014). To remove contamination, all glassware used for lipid analysis was heated to 450 °C for three hours. A 4 mL of a 0.1 M hydrochloric acid methanol solution (Wako Pure Chemicals, Osaka, Japan) were used to dissolve 0.1 mg of oil. Each oil sample was placed in a tube after 20 µL of the internal standard, 500 µg mL^{-1} heptadecanoic acid (17:0), was dissolved in hexane. In order to facilitate the saponification and methyl-esterification of free fatty acids and acyl groups in lipids for conversion into fatty-acid methyl esters (FAMEs), the tubes were firmly sealed and heated to 100°C for an hour in a water bath. Each tube was filled with four milliliters of n-hexane in order to extract fatty acids. The tubes were properly shaken before each top layer was moved to a fresh tube. This was carried out twice. A concentrating centrifuge (CC-105, Tomy Seiko, Tokyo, Japan) was used to evaporate the hexane phases. Before being moved to a new vial for GC analysis, each tube was vortexed after drying and 200 µL of hexane was added to recover FAME. To detect and measure FAMEs, a solution containing 1 µL of hexane was added to the gas chromatograph GC-2014, which has a flame ionization detector (Shimadzu, Kyoto, Japan). Helium was provided as a carrier gas in split-less mode at a steady flow rate of 1.25 mL min^{-1}. A CP-Sil5 CB column (Agilent Technologies, Santa Clara, CA) was used to raise the temperature from 60°C to 130°C for 1.5 min at a rate of 20 °C per minute. It was then raised to 300 °C at a rate of 4 °C per minute and maintained there for 40 min. Retention time was used to identify FAMEs, and commercial FAME standards (Nu-Chek Prep, Elysian, MN) were used to confirm the findings. By comparing the peak area of the total ion chromatogram with that of 17:0 methyl ester as an internal reference, the amount of each FAME (pg $cell^{-1}$) was calculated and standardized by cell number. Each fatty acid's identity was confirmed using a gas chromatograph (GC-2010) fitted with a mass spectrometer (QP-2010, Shimadzu). For the measurement of FAMEs, the operating conditions were similar to those described above. By examining their individual mass spectra, each FAME was identified.

2.5 Statistical analysis of data

All data underwent a one-way analysis of variance and were presented as mean ± standard deviation of triplicate determinations. Post-hoc multiple comparison tests and least square difference were used to distinguish mean differences. At $p < 0.05$, significant differences between means were determined.

3.0 RESULTS

3.1 Impact of varying NaCl concentrations on *S. dimorphus* growth at 80 µmol m^{-2} s^{-1} light intensity

The results obtained from different concentrations of NaCl (0.8 and 1.6 g L^{-1}) at 80 µmol $m^{-2}s^{-1}$ and 25 ºC showed that *S. dimorphus* were able to grow in culture media amended with NaCl. The growth curves of the culture showed no lag phase which means the algae started growing immediately after inoculation (Figure 1). The growth curves indicated that the control culture and culture supplemented with 0.8 g L^{-1} NaCl grew at the same rate and their growth rates were faster than culture supplemented with 1.6 g L^{-1} NaCl until the third day when the growth of culture with double concentration of NaCl picked up with other cultures. This slow growth was reflected in lower specific growth rate and higher doubling time of culture amended with 1.6 gL^{-1} NaCl when compared to other cultures. The slower growth of the culture supplemented with double concentration of NaCl within the first three days was due to its low adaptation to culture medium. Culture with 0.8 gL^{-1} NaCl adapted very fast to culture medium because of low concentration of NaCl. All the cultures entered stationary phase on day 5. When the culture was treated with 0.8

gL^{-1} NaCl, the specific growth rate significantly increased; however, when NaCl was doubled in the culture medium, the specific growth rate significantly decreased in comparison to the control (Table 1). Considering the two cultures amended with different salt concentrations in the present study, culture with lower NaCl concentration had higher specific growth rate with lesser cell doubling time than the culture containing higher levels of NaCl.

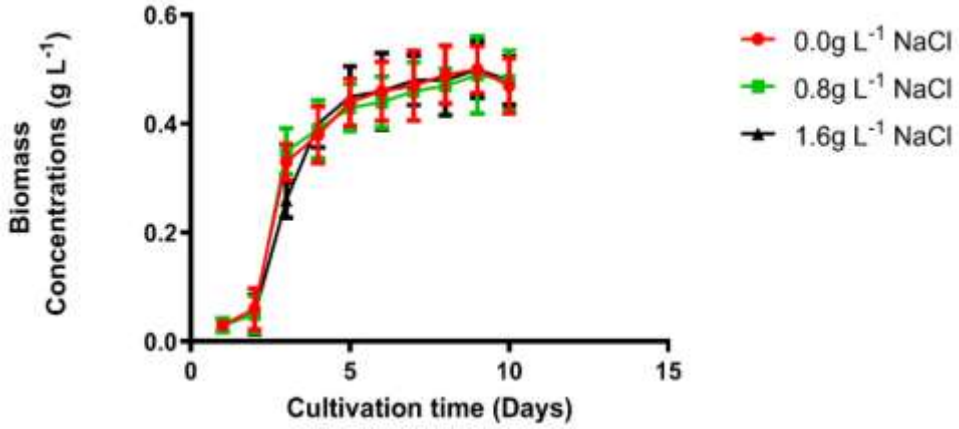


*Figure **1.** Effect of different concentrations of sodium chloride on growth curve of S. dimorphus mixotrophic culture at 80 µmol $m^{-2}s^{-1}$ and 25 ºC*

3.2 Impact of varying NaCl concentrations on *S. dimorphus*'s lipid contents and oil productivities

The effect of salt concentrations on the lipid contents and oil productivities of *S. dimorphus* was evaluated at 25ºC (Table 2). The control culture had oil contents of 24.2 ± 0.10% while cultures supplemented with 0.8 and 1.6 gL^{-1} NaCl had oil contents of 7.01 ± 0.12 and 8.0 ± 0.09% respectively. In the same manner, the oil productivity of control culture was 16.94 ± 0.004 $mgL^{-1}day^{-1}$ while the oil productivities of cultures consisting 0.8 and 1.6 gL^{-1} NaCl were 4.91 ± 0.02 and 5.60 ± 0.03 $mgL^{-1}day^{-1}$ respectively. When NaCl was added to the culture media, *S. dimorphus*'s lipid contents and productivities were considerably lower than in the control culture (Table 2). Compared to the culture fed with 0.8 gL^{-1} NaCl, the culture supplemented with 1.6 gL^{-1} NaCl had a significantly increased lipid content and oil productivity.

.Table **1**. Effect of different concentrations of NaCl on Specific growth rate (µ), doubling time (T_d) and biomass productivity of *S. dimorphus* at light intensity of 80 µmol and 25 ºC

Different concentrations of Sodium Chloride	Specific growth rate(day^{-1})	Doubling Time(day)	Biomass productivity ($mgL^{-1}day^{-1}$)
SD + 0.0 gL^{-1} NaCl + 5 gL^{-1} glc	0.93 ± 0.02^{a}	0.75 ± 0.05^{c}	70.00 ± 0.05^{e}
SD + 0.8 gL^{-1} NaCl + 5 gL^{-1} glc	0.99 ± 0.03^{a}	0.70 ± 0.05^{c}	70.00 ± 0.10^{e}
SD + 1.6 gL^{-1} NaCl + 5 gL^{-1} glc	0.80 ± 0.05^{b}	0.87 ± 0.05^{d}	70.00 ± 0.05^{e}

SD= Scenedesmus dimorphus; Glc= glucose. Significant differences at p < 0.05 are indicated by values with distinct letters.

Table **2**. Effect of different concentrations of NaCl on lipid content and lipid productivities of *S. dimorphus* mixotrophic cultures at light intensity of 80 µmol $m^{-2}s^{-1}$ and temperature of 25ºC

Different concentrations of Sodium Chloride	Biomass productivity ($mgL^{-1}day^{-1}$)	Oil content (%)	Oil productivity ($mgL^{-1}day^{-1}$)
SD + 0.0 gL^{-1} NaCl + 5 gL^{-1} glc	70.00 ± 0.05^{e}	24.20 ± 0.10^{f}	16.94 ± 0.004^{i}
SD + 0.8 gL^{-1} NaCl + 5 gL^{-1} glc	70.00 ± 0.10^{e}	7.01 ± 0.12^{g}	4.91 ± 0.02^{j}
SD + 1.6 gL^{-1} NaCl + 5 gL^{-1} glc	70.00 ± 0.05^{e}	8.00 ± 0.09^{h}	5.60 ± 0.03^{j}

SD= Scenedesmus dimorphus; Glc= glucose. Significant differences at p < 0.05 are indicated by values with distinct letters.

3.3 The effect of Sodium chloride on the fatty acids profile of *S. dimorphus*

Both the control culture and culture supplemented with 1.6 gL^{-1} NaCl of had fatty acid profiles ranging from C_{12} to C_{24} (Table 3). Individual fatty acid concentrations and numbers varied between the control and culture supplemented with 1.6 gL^{-1} NaCl. The control culture contained more long chain fatty acids than the cultures supplemeted with NaCl. With addition of NaCl into the culture, the expression of some fatty acids including $C_{19:1}$, $C_{20:2}$, $C_{22:0}$, $C_{24:0}$ and $C_{24:1}$ was lost. However, these fatty acids occurred in minute quantities in the control culture. The most significant increase in individual fatty acid occurred in $C_{18:3}$ followed by $C_{20:4}$ when *S. dimorphus* culture was supplemented with NaCl. The most abundant fatty acid expressed in both cultures was $C_{16:0}$ followed by $C_{18:3}$. $C_{18:3}$ fatty acid increased by 5.23 % in the presence of NaCl. The C_{16} and C_{18} formed the major part of the fatty acids accounting for 85.78 % and 90.97 % of the total fatty acids in control and culture supplemented with NaCl respectively. With supplementation of NaCl in the culture, PUFA increased by 4.09 % while SFA and MUFA decreased by 2.11 % and 1.98 % respectively. The increase in PUFA in culture amended with NaCl was due to rise in concentration of $C_{18:3}$. However, the relative concentration of SFA was higher compared to MUFA and PUFA in both cultures.

Table **3.** Effect of sodium chloride on fatty acids profile of *S. dimorphus* at light intensity of 80 µmol and temperature of 25ºC

Fatty acids	Control culture (%)	1.6 gL^{-1} NaCl (%)
12:0	1.30	1.37
14:0	1.02	0.71
15:0	1.07	0.74
16:0	41.74	41.19
18:0	2.26	2.64
20:0	0.92	0.83
22:0	0.61	ND
24:0	0.67	ND
16:1	5.94	6.44
18:1	5.46	5.81
19:1	1.52	ND
20:1	1.67	1.43
22:1	0.24	0.26
24:1	1.09	ND
18:2	1.57	0.86
18:3	28.81	34.04
20:2	2.22	ND
20:3	0.88	1.18

20:4	1.00	2.50
Total SFA	49.59	47.48
Total MUFA	15.92	13.94
Total PUFA	34.49	38.58

SFA= Saturated fatty acids; MUFA= Monounsaturated fatty acids and PUFA= polyunsaturated fatty acids. ND= Not detected

4.0 DISCUSSION

Analysis of growth parameter shows a clear ideal condition for cellular proliferation. At a moderate NaCl concentration of 0.8 g L^{-1}, the highest doubling time (0.70 d^{-1}) and maximum specific growth rate (0.99 d^{-1}) were observed. This probably implies that the 0.8 gL^{-1} NaCl condition offers the best osmotic balance for *Scenedesmus dimorphus*, enabling effective nutrient transport across the cell membrane and lowering the energetic cost of osmoregulation (Lian & Jiang, 2015). The specific growth rate (0.93 d^{-1}) was somewhat lower at 0.0 gL^{-1} NaCl (control), suggesting that the complete lack of added salt is not ideal for optimizing growth kinetics, either as a result of poor osmolality or inadequate necessary sodium or chloride ions. With the lowest specific growth rate (0.80 d^{-1}) and longest doubling time (0.87 d^{-1}), the 0.16 gL^{-1} NaCl concentration exhibited the lowest growth. This result is interesting because it implies that, unlike 0.8 gL^{-1}, 0.16 gL^{-1} is not stimulatory; rather, it may be just high enough to disrupt normal development processes without completely activating the advantageous stress response shown at other concentrations. There is negative correlation between oil concentration and growth rate. Even though the 0.8 gL^{-1} NaCl condition supported the fastest growth, it produced the lowest oil content (7.01%) and, as a result, the lowest lipid productivity (4.91 g L^{-1} d^{-1}). On the other hand, the condition with control culture (0.0 gL^{-1} NaCl) showed a notable rise in oil content (24.20%), which resulted in the greatest lipid productivity of 16.94 gL^{-1} d^{-1}. This event characterized a metabolic switch, in which *S. dimorphus* redirects the flow of carbon. The absence of additional NaCl under the 0.0 gL^{-1} condition could be explained as a modest environmental stressor (such as an osmotic or ionic imbalance) that causes the cell to enter a storage phase. Triacylglycerols (TAGs) build up in lipid bodies as a result of the activation of important lipogenesis-related enzymes and the downregulation of enzymes needed for rapid cell division (Xu et al., 2009; Chen et al., 2015). According to the values obtained, the biomass productivity was consistently 70.00 gL^{-1} d^{-1} under all circumstances. The variations in lipid productivity are closely correlated with the oil content because lipid productivity is equal to biomass productivity times oil content. The effects of varying NaCl concentrations on *S. dimorphus* generally showed that, as NaCl concentrations in culture media increased, specific growth rates decreased while lipid contents and productivities somewhat increased. This was similar with the work of Church et al. (2017) in which they found that the time required to reach maximum biomass increased with increase in concentration of NaCl. In the same vein, they reported that lipid production increased with increasing salt concentration in culture media. Similar report was obtained by Pushpakumari et al. (2018) who observed an increased lipid content in *S. dimorphus* when the culture was subjected to 5% NaCl concentration although they reported a significant decrease in biomass yield. Additionally, it was observed that *B. braunii*'s biomass concentration increased with increase in sodium chloride concentration, reaching its maximum at 0.017 M and 0.034 M of NaCl (Rao et al., 2007). The glucose concentration (5 $gL^{-1)}$ in the culture media may have contributed to the stability of the final dry weight of *S. dimorphus* in the current investigation. Our results are comparable with the work of El-Sayed (El-Sayed, 2010) who reported no significant increase in biomass concentration when 2 % NaCl was applied into the culture media during cultivation of *Scenedesmus sp*. However, Vazquez-Duhalt & Arredondo-Vega (1991) stated that there was no change in lipid accumulation and decreased biomass yield was noticed during haloadaptation of *B. braunii*. The reduction in biomass yield was due to low adaptability of microalgae under high salt concentrations ranging from 0.1 to 0.75 M. Furthermore, Yan & Jian (2005) found that there was no significant difference in the growth rate of *B. braunii* (UK 807-2, the UK strain) between the control and cultures supplemented with 0.15 M and 0.25 M NaCl, which is consistent with the findings of the current study. In their work, growth rate and lipid contents decreased when the concentration of NaCl reached 0.50 M NaCl. This goes to show that the effect of salinity on the growth and lipid contents of microalgae is dependent on the concentration of NaCl applied, other environmental conditions and the specie used. Addition of NaCl into the culture media decreased the lipid contents and lipid productivities of *S. dimorphus* in comparison to control culture (Table 2). It has been shown that growing microalgae under optimal conditions result in accumulation of large amounts of algal biomass with relative decrease in lipid contents while slowly growing algae produce high lipid (Sharma et al., 2012). Despite the stress induced by the addition of NaCl into the culture media in the present study, *S. dimorphus* was found growing almost at the same rate with control culture. The energy obtained from the glucose metabolism was channeled into cell division instead of lipid accumulation, and hence high biomass was obtained. This effect resulted in low lipid contents in comparison to the control. This could be attributed to the fact that the presence of glucose in the culture supplemented with NaCl countered the effect of salt stress towards lipid accumulation. Thus, *S. dimorphus* biomass production could not be negatively affected by salt-induced stress. In practice, in order to avoid counter effect of glucose on the salt stress, much lower quantity of glucose or organic carbon source should be added into the culture medium to stimulate high biomass at the early stage of cultivation. The effects of NaCl concentration on the fatty acid profile of *S. dimorphus* are shown in (Table 3). The most prevalent fatty acid expressed in both cultures was C16:0, followed by C18:3, according to the fatty acid profiles of the control culture and the culture treated with a double concentration of sodium chloride (which had the highest lipid content). In both cultures, the relative concentration of SFA at the end of culture cultivation was higher than either MUFA or PUFA, which is necessary for high-quality biodiesel since they guarantee the fuel's oxidative stability (Singh et al., 2014). The findings were comparable to that of Pushpakumari et al. (2018), who found that the relative percentage of SFA was higher than both MUFA and PUFA when *S. dimorphus* and *S. minutum* cultures were subjected to 5 % salt stress. It has been shown that *C. vulgaris* grown in wastewater with a high salinity produced more saturated and monounsaturated fatty acids than polyunsaturated fatty acids compared to that grown in wastewater with a lower salinity, producing high-quality lipids for biodiesel production (Church et al., 2017).

In the present study, an increase in PUFA in culture supplemented with NaCl was found mainly due to increase in concentration of $C_{18:3}$. Previous reports have shown increased concentrations of unsaturated fatty acids in some microalgae such as *T. suecica* and *N. oculata* CS 179 with increasing concentrations of NaCl in culture medium (Almutairi & Toulibah, 2026., Gu et al., 2012). Our results agree with the previous findings where salt stress induced increase in the concentrations of 18:1 and 18:3 fatty acids in two freshwater microalgae, *Scenedesmus obliquus and Chlamydomonas mexicana* (Salama et al., 2013). It has been shown that production of lipids in microalgae is classified into two namely non-polar lipids (storage lipid) and polar lipid (structural lipid) (Couee et al., 2006). Previous study shows that when the culture medium undergoes nutrient deficiency, neutral lipid accumulates in the stationary phase (Ratledge & Cohen, 2008). In the present study, lipids are extracted at the end of stationary phase. The fatty acids obtained from these lipids are stored in the form of TAG, an essential substrate for production of biodiesel (Miao & Hu, 2006). Previous study has shown that two genes including glycerol-3-phosphate dehydrogenase and glycerol-3-phosphate acyltransferase have been known to stimulate production of TAG in microalgae under salt stress (de Jaeger et al., 2018). Under this stress condition, 80 % of the total accumulated lipid consists of TAGs storage lipid which are used for biodiesel production (Tornabene et al., 1983; Kawata et al., 1998). The fatty acid contents of lipids obtained from *S. dimorphus* in the present study consist of mainly C_{16} and C_{18} comparable to those obtained from vegetable oils which are desirable for biodiesel production (Miao & Wu, 2006; Ozougwu et al., 2026). The dominant C_{16} and C_{18} fatty acids obtained from the present studies are similar to those obtained from freshwater *Scenedesmus obliquus* and *Chlamydomonas mexicana* (Salama et al., 2013). The remarkable effect of NaCl (1.6 gL^{-1}) in the current investigation was the increase in the concentration of linolenic acids (18:3) which can be used to produce biodiesel suitable for cold climates where cold-flow properties are important though it may affect the oxidative stability of biodiesel if subjected to long storage (Knothe, 2008).

Conclusion

According to the current study, *S. dimorphus* (Turpin) Kützing is a fast-growing, stress-tolerant strain that used low concentrations of NaCl and organic carbon sources to produce high concentrations of biomass and high-quality fatty acids in the culture media. The findings demonstrate that the effect of salt stress on lipid formation was countered by the presence of glucose in the culture media. Thus, *S. dimorphus* biomass production could not be negatively affected by salt-induced stress. In practice, in order to avoid counter effect of glucose on the salt stress, much lower quantity of glucose or organic carbon source should be added into the culture medium

to stimulate high biomass at the early stage of cultivation. Lipid content and productivity were higher in the culture supplemented with 0.16 g L^{-1} NaCl than in the culture supplied with 0.8 g L^{-1} NaCl. According to *S. dimorphus*'s fatty acid profile, the majority of its fatty acids are C16 and C18, which are suitable for making biodiesel.

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Competing interests
There are no pertinent financial or non-financial interests that the authors would want to declare.

Data availability
The data supporting the study's conclusions, according to the authors, are included in the report. If raw data files in a different format are required, the associated author can provide them upon reasonable request.

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