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Growth and lipid productivity of a promising candidate *Micractinium reisseri* (JN169781) under changes in salinity and some carbon sources

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ABSTRACT

The objective of the current research was to cultivate *Micractinium reisseri* (JN169781), a promising microalga for high biomass and lipid accumulation. The algal culture was exposed to 0.71 and 0.94 g L^{-1} of sodium chloride (NaCl) for 20 days, resulting in 3.66 and 6.71 % increases in the growth, and biomass productivity (3.82 and 12.56 %), respectively at the two NaCl concentrations over the control. The growth and biomass productivity of *M. reisseri* gradually decreased with increasing seawater ratios (25, 50, 75, and 100%), however the highest lipid content and lipid productivity at 25 % SW concentration. *M. reisseri* was observed the highest biomass at 1 g L^{-1} of glucose. The greatest levels of protein, lipid content, and lipid productivity were obtained at 5 g L^{-1}. With 1 g L^{-1} Na-acetate, *M. reisseri* showed a high increase in growth and biomass productivity, while the greatest growth and biomass productivity were observed at 2 g L^{-1} of $NaHCO_3$. In comparison to the control, the maximum protein content (32.37%) was found at 5 g L-1 of $NaHCO_3$, while the highest lipid productivity was found at 2 g L^{-1} of $NaHCO_3$. The maximum growth, biomass, protein, lipid, and lipid productivity of *M. reisseri* were achieved at 0.1 g L^{-1} glycerol. Cultivation of *M. reisseri* at 2 g L^{-1} Na-acetate obtained the maximum protein, lipid, and lipid productivity. The results clarified that the culture of *M. reisseri* grown with enriched sodium salt and few carbon sources produced great biomass and lipid productivity.

INTRODUCTION

The pressure on available energy resources and efforts to develop sustainable energy from microalgae has intensified due to the development in the world's population. **(Abomohra *et al.*, 2020; El-Sayed *et al.*, 2020; Almutairi *et al.*, 2021; Goswami, *et al.*, 2022),** they can also lower production costs and increase biomass yields **(Lo *et al.*, 2010; Huang *et al.*, 2017; Cheng *et al.*, 2021).** The selection of excellent species with high biomass production, lipid content, and productivity is crucial for the success of making biodiesel from microalgae **(Gong and Jiang, 2011).** In order to produce microalgal biodiesel at a cheaper cost, it is crucial to investigate the optimal culture conditions for

microalgae in addition to eliminating those species that have a high growth rate and lipid content **(Pruvost *et al.*, 2011).**

Many microalgae can change their lipid biosynthesis routes from membrane lipid synthesis to the storage of neutral lipids, typically in the form of triacylglycerols, under unfavourable environmental or stress circumstances (TAGs). Since it was added at the outset of cultivation, stress conditions like sodium chloride (NaCl) severely impacted cellular proliferation, which subsequently had a considerable detrimental impact on lipid accumulation **(Almutairi *et al.*, 2021). Kaewkannetra *et al.* (2012) and Zhao *et al.* (2012)** have previously looked at how changes in salinity and other carbon sources affect the fatty acid. These variables influence the oleaginous microalgae's growth rate and lipid production, increasing the lipid contents **(Hu *et al.*, 2008; Mohammed *et al.*, 2013).** According to **Wan *et al.* (2011; 2012),** the lipid productivity of *Nannochloropsis oculata* and *Chlorella sorokiniana* rises when the concentration of glucose rises.

Different carbon sources can be used to develop microalgae in photoautotrophic, heterotrophic, and mixotrophic modes (supplementation of organic and inorganic carbon sources in the presence of light conditions) demonstrated a high biomass output. Using CO_2 as a carbon source in the presence of light, phototrophic development of microalgae is a natural growth mode; however it has low biomass (**Goswami *et al.*, 2020**). Additionally, *Picochlorum* sp. was grown in a phototrophic manner by Vega *et al.* (2011), who noted that little biomass was, produced (1.8 g L^{-1}). Compared to photoautotrophic and heterotrophic cultivation modes, mixotrophic cultivation, the addition of organic and inorganic carbon sources showed a high biomass production **(Gupta and Pawar, 2018).** However, different microalgal strains have varied optimal sodium acetate concentrations, necessitating careful testing; **Huang *et al.* (2017)** employed glucose and acetate as well when growing *Chlorella sorokiniana* in a mixotrophic environment.

It was stated that the major strategies for increasing the productivity of biomass and biomolecules involve the optimization of medium components. In order to attain the highest levels of biomass and lipid production under mixotrophic farming conditions, carbon sources are crucial. Carbon dioxide (CO_2) and HCO_3 are examples of inorganic carbon sources that have good properties that can help to reduce the possibility of medium contamination by undesirable bacteria **(Goswami *et al.*, 2022).** *Scenedesmus obliquus* and *M. reisseri*, two potential microalgae for the production of biodiesel, were examined for growth, lipid content, and lipid productivity of these algae (**Abomohra *et al.*, 2016)**. They came to the conclusion that *M. reisseri*, when grown on KC medium, produced substantial levels of lipids, showing promise as a potential source of biodiesel. Therefore, in this work, we will make certain adjustments to the *M. reisseri* (JN169781) growth medium in order to increase growth, biomass, and lipid productivity in order to be used for the manufacture of biodiesel.

MATERIALS AND METHODS

Algal strain

Micractinium reisseri was acquired from Tanta University's Phycology Laboratory in the Faculty of Science. According to **Abou-Shanab *et al.* (2014),** this species was previously isolated from agricultural drainage mixed with urban wastewater at El-Gharbya Governorate, Egypt, and genetically identified with strain number (JN169781) (Photo 1).

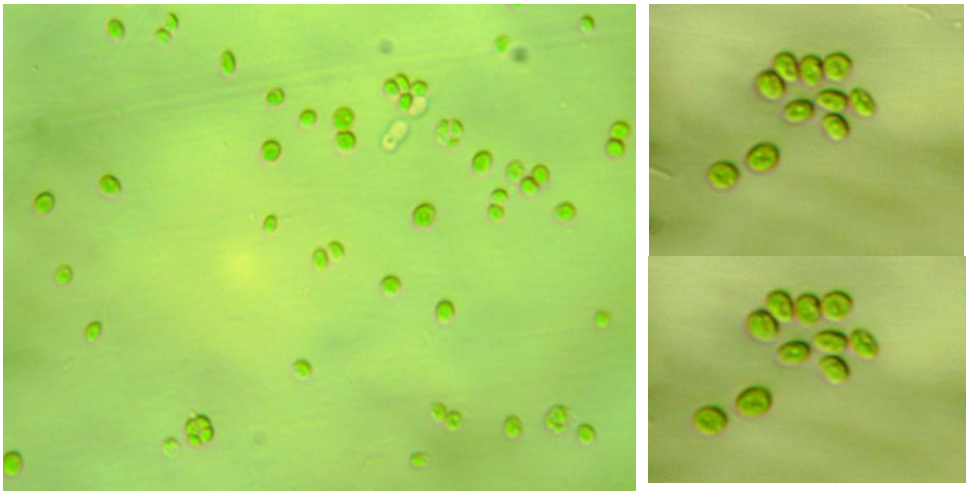

Photo 1. Freshwater microalga *Micractinium reisseri* (JN169781) (400 x)

Growth condition of algal culture

In batch cultures, *Micractinium reisseri* was grown in 1000 ml Erlenmeyer flasks with 700 ml of KC medium **(Kessler and Czygan, 1970)** as follows: 10 ml L^{-1} of Macronutrient (81 gm L^{-1} KNO_3, 47 gm L^{-1} NaCl, 47 gm L^{-1} $NaH_2PO_4.H_2O$, 36 gm L^{-1} $Na_2HPO_4.H_2O$, 25 gm L^{-1} $MgSO_4.7H_2O$), and 1 ml L^{-1} Micronutrients (20 mg/100 ml NH_4, $6Mo_7O_2.4H_2O$, 500 mg/100 ml $CaCl_2$ $.2H_2O$, 20 mg/100 ml $ZnSO_4$ $.7H_2O$, 50 mg/100 ml $H3BO_3$, 50 mg/100 ml $MnCl_2.4H_2O$, 600 mg/100 ml $FeSO_4$ $.7H_2O$, 800 mg/100 ml EDTA). Prior to autoclaving, the growth medium's pH value was adjusted to 6.5. The cultures were incubated at 25±2 °C under constant illumination from tubular fluorescent lamps (FL 40 T9D/38) with a light intensity of 45 mole $m^{-2}s^{-1}$.

Experimental design

KC medium has been modified by utilizing various NaCl concentrations, various ratios of seawater, and various carbon sources (such as glucose, sodium acetate, sodium bicarbonate, and glycerol) as shown in Table (1). A certain volume of *M. reisseri* cells at exponential growth phase (day 20) was inoculated in the medium, and the initial optical density was 0.01 at 680 nm. Continuous aeration was supplied to the culture to provide necessary CO_2 at a flow rate of 1 L min^{-1} by bubbling of filter-sterilized air.

Table 1. Different concentrations of salinity, seawater and different carbon sources used

Treatements		Concentrations
Salinity	NaCl	(0.47) control, 0.71, 0.94,0.24 and 0 g L^{-1}
	Seawater ratios	0 (control), 25, 50, 75 and 100 %
Carbon sources	Glucose	0 (control), 0.5, 1, 2 and 5 g L^{-1}
	Sodium acetate	
	Sodium bicarbonate	
	Glycerol	0 (control), 0.01, 0.03,0.05 and 0.1 g L^{-1}

Growth measurement

The growth of *M. reisseri* was determined by measuring the optical density (OD) of the culture at 680 nm (OD_{680}) **a**s reported in **Markle *et al.* (2000),** at 2 days intervals, using UNICO UV/Visible spectrophotometer, model 2000 UV, power source, AC220V/50HZ. The respective growth curves were developed by plotting the optical density against the incubation time, and the algal cellular dry weight (CDW) was estimated and expressed as grams per liter (g L^{-1}).. The biomass productivity (BP) was calculated according to **Andrade and Costa (2007)** (Equ. 1), and modified by **Abomohra *et al.* (2013).**

$$BP\ (g\ L^{-1}\ d^{-1}) = (CDW_L - CDW_E).\ (T_L - T_E)^{-1} \qquad \text{(Equ. 1)}$$

Where CDW_E illustrating the CDW (g L^{-1}) at days of early exponential phase (T_E)

Where CDW_L illustrating the CDW (g L^{-1}) at days of late exponential phase (T_L)

Estimation of total soluble protein

Total protein content was estimated according to Bradford method **(Bradford, 1976).** The absorbance was determined by the spectrophotometer at 595 nm wavelength, using bovine serum albumin (BSA) as standard, the protein content was determined as mg/g CDW.

Estimation of total soluble carbohydrate

Total carbohydrate content was estimated as described by **Payne and Stewart (1988)** using glucose as standard. The absorbance was measured at 490 nm.

Lipid extraction

Total lipid was measured after 20 days of incubation. Extraction process of total lipid was conducted by modified Folch method **(Folch *et al.*, 1957).** The lipid content was calculated as mg g^{-1} CDW. Lipid productivity was calculated according to **Andrade and Costa (2007)** (Equ. 2) and modified by **Abomohra *et al.* (2013).**

Lipid productivity (mg L^{-1} d^{-1}) = (TL_L – TL_E). (t_L-t_E)$^{-1}$ (Equ. 2)
Where TL_L representing the total lipid (mg L^{-1}) at days of Late exponential phase (t_L)
Where TL_E representing the total lipid (mg L^{-1}) at days of early exponential phase (t_E)

Statistical analysis

Results were represented as the mean of three replicates ± standard deviation (SD). The statistical analyses were carried out using SPSS (IBM, Version 22). Data obtained were analyzed statistically to determine the degree of significance using one way analysis of variance (ANOVA) at $P \leq 0.05$.

RESULTS

Effect of different salinity and seawater concentrations on growth, protein, carbohydrate and lipid productivity of *M. reisseri*

The obtained results revealed that the growth of *M. reisseri* increased with increasing salinity concentration in culture medium. The cultures treated with 0.71 and 0.94 g L^{-1} of NaCl, resulted in 3.66 and 6.71 % increasing in the growth, respectively, over the control (Fig. 1). Biomass productivity showed the similar tendency, attained 3.82 and 12.56 %, respectively at the same NaCl concentration (Table 2), $P \leq 0.05$.

The findings clarified that the cultures exposed to 0, 0.24, 0.71 and 0.94 g L^{-1} NaCl showed in significant decreases in protein content by 18.48, 11.44, 30.38, and 21.38 %, respectively (Fig. 2) as compared with control. Using 0.24 and 0 g L^{-1} of NaCl significantly increased carbohydrate content with 18.33 % and 32.95 %, respectively ($P \leq$ 0.05), where reaching its maximum (29.2 % of CDW) at 0 g L^{-1} NaCl as compare with control (Fig. 2). After 20 days of incubation, *M. reisseri* lipid content that was significantly enhanced by 5.92 and 46.93 %, at 0.24 and 0 g L^{-1} NaCl, respectively (Table 2) as compared with control. The highest lipid content (34.7 % of CDW) was obtained at 0 g L^{-1} NaCl. As compared with control (13.07 mg $L^{-1}d^{-1}$), the maximum lipid productivity (16.05 mg $L^{-1}d^{-1}$) was observed at the highest NaCl concentration (Table 2).

As a result of the findings NaCl increased *M. reisseri* biomass productivity, seawater (SW) was chosen to replace NaCl in the culture. Figure (3) depicts the impact of various seawater ratios on *M. reisseri* growth over the course of 20 days of incubation. The findings showed that, with increasing SW ratios (25, 50, 75, and 100%), *M. reisseri* growth (Fig. 2) and biomass productivity (Table 3) rapidly declined ($P \leq 0.05$).

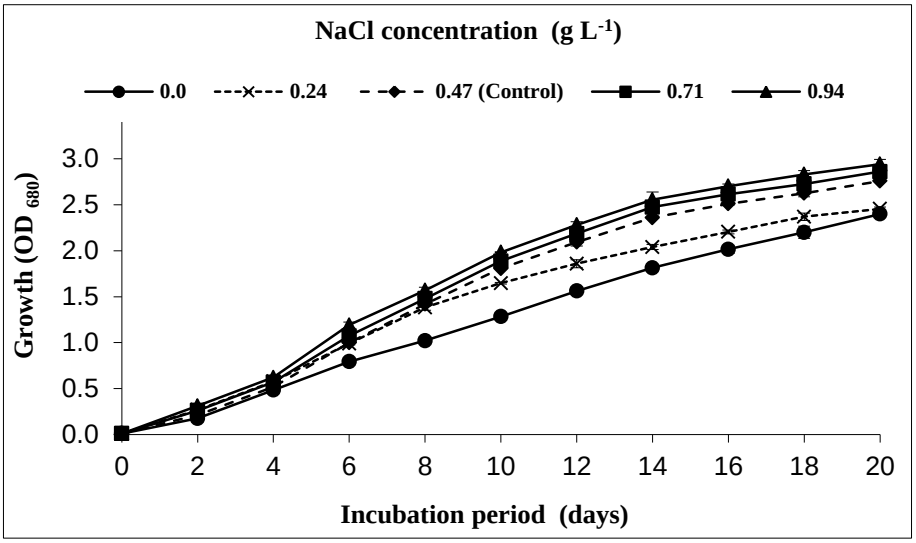

Fig. 1. Effect of different concentrations of NaCl (g L^{-1}) on growth of *M. reisseri*.

Table 2. Effect of different concentrations of NaCl on biomass, lipid content, and lipid productivity of *M. reisseri*.

NaCl concentration (g L^{-1})	Biomass (g L^{-1} d^{-1})	Lipid content (mg g^{-1}dw)	Lipid productivity (mg $L^{-1}d^{-1}$)
0.0	0.0376±0.0007^{e}	347.72±10.54^{c}	13.07±0.15^{d}
0.24	0.0462±0.0012^{d}	250.66±9.19^{b}	11.58±0.13^{c}
0.47 (Control)	0.0629±0.0012^{a}	236.65±6.83^{a}	14.88±0.21^{a}
0.71	0.0653±0.0005^{b}	226.96±1.20^{a}	14.81±0.06^{a}
0.94	0.0708±0.0016^{c}	226.84±5.81^{a}	16.05±0.06^{b}

Significant of differences for each studied parameter was denoted by different letters (at $P \leq 0.05$).

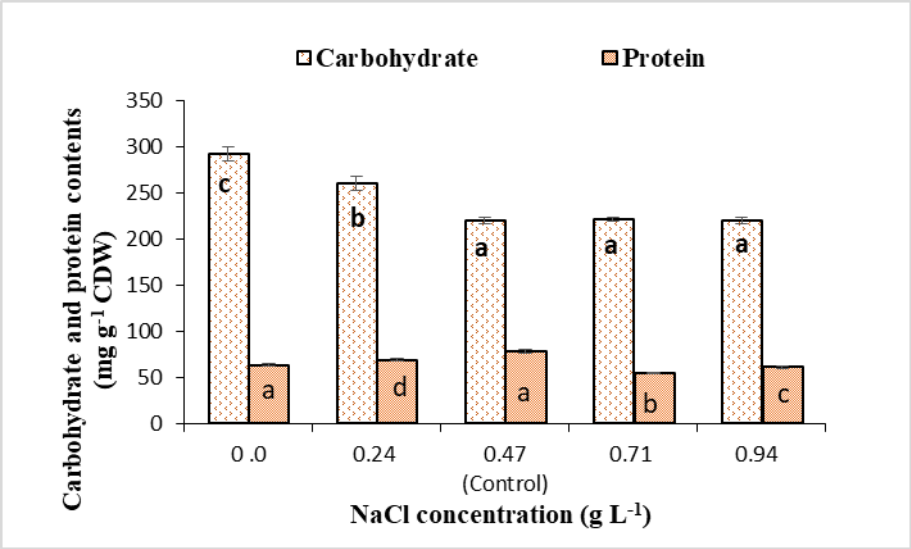

Fig. 2. Carbohydrate and protein contents of *M. reisseri*, at different NaCl concentrations.

Error bars show the SD for three measurements; significant of differences was denoted by different letters (at $P \leq 0.05$).

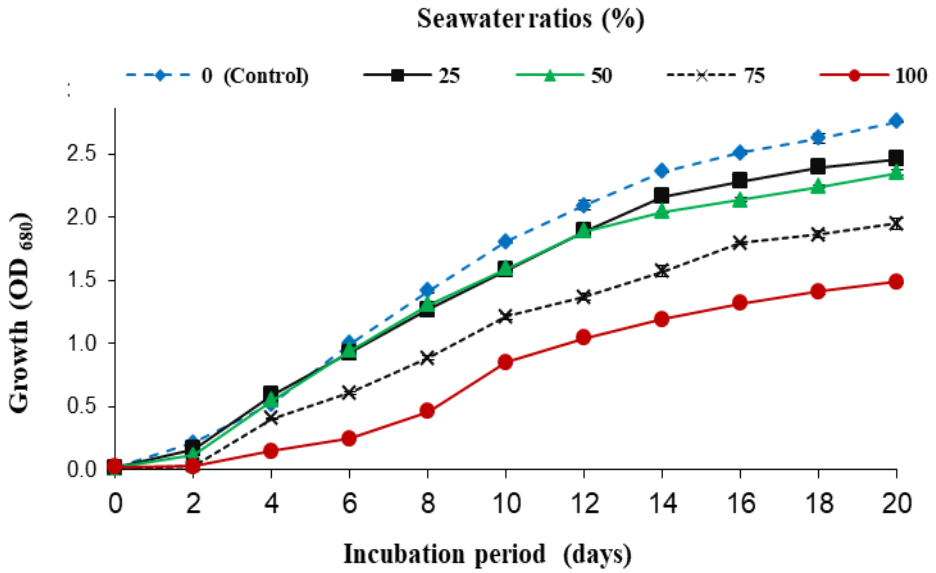

Fig. 3. Effect of different seawater ratios (%) on the growth of *M. reisseri*.

Concerning the effect of seawater ratios on the protein content of *M. reisseri*, Fig. (4) showed drastically decrease by 16.22, 42.31, 53.02 and 87.21% below the control, with increasing seawater ratios by 25, 50, 75, and 100 %, respectively. On the other hand, using 25, 50 and 75 % seawater led to significant increases in carbohydrates by 63.64,

58.17 and 55.99 %, respectively, as compared with control (Fig. 4). The results showed significant increases in lipid content and lipid productivity, and attained their maximum (364.78 and 16,54 mg $L^{-1}d^{-1}$, respectively) at 25 % SW concentration (Table 3).

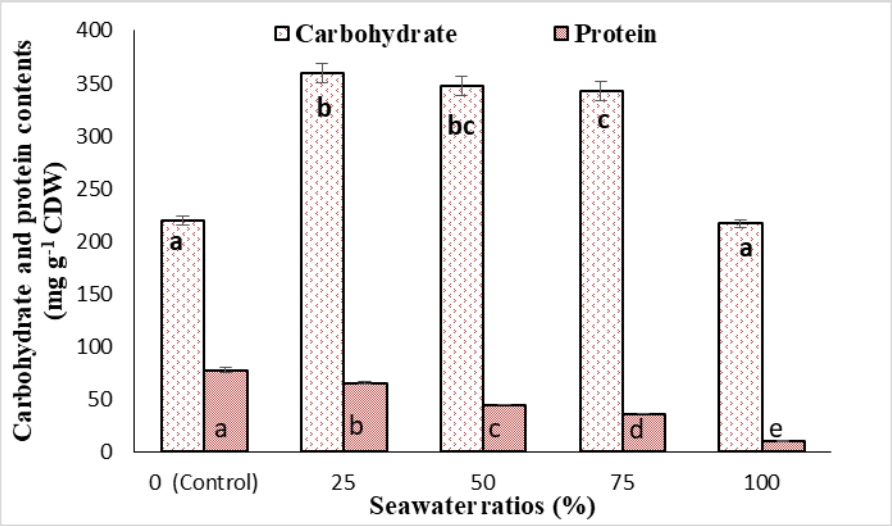

Fig. 4. Carbohydrate and protein contents of *Micractinium reisseri* at different ratios of seawater.

Error bars show the SD for three measurements; significant of differences was denoted by different letters (at $P \leq 0.05$).

Table 3. Biomass, lipid content and lipid productivity of *M. reisseri* at different ratios of seawater.

Seawater (%)	Biomass (g L^{-1} d^{-1})	Lipid content (mg g^{-1} dw)	Lipid productivity (mg L^{-1} d^{-1})
0 (Control)	0.0629±0.0012^{a}	236.65±6.83^{a}	14.88±0.21^{a}
25	0.0454±0.0012^{b}	364.78±10.13^{b}	16.54±0.08^{b}
50	0.0438±0.0008^{b}	338.46±7.01^{c}	14.82±0.11^{a}
75	0.0364±0.0009^{c}	341.59±10.75^{c}	12.44±0.16^{c}
100	0.0337±0.0008^{d}	338.42±3.66^{c}	11.39±0.17^{d}

Significant of differences of each studied parameter was denoted by different letters (at $P \leq 0.05$).

Effect of different carbon sources on growth and lipid productivity of *M. reisseri*

The obtained result showed that *M. reisseri* have higher growth (OD_{680}) when supplemented with 1 g L^{-1} glucose as compared with control (Fig. 5). The same trend was obtained for biomass productivity (Table 4). Figure (6) clarified the enhancement of

protein content by 39.88, 47.42 and 88.66 % with the different concentrations of glucose (0.5. 1.0, 2.0, and 5.0 g L^{-1}), respectively. While, the maximum carbohydrate content (25.2 % of CDW) was found at 0.5 g L^{-1} of glucose. Lipid content of *M. reisseri* was significantly enhanced by 30.61 % when the culture supplemented with 5 g L^{-1} of glucose (Table 4). On the other hand, using 1 and 5 g L^{-1} glucose significantly increased lipid productivity by 7.46 and 32.06 %, respectively compared with the control (Table 4).

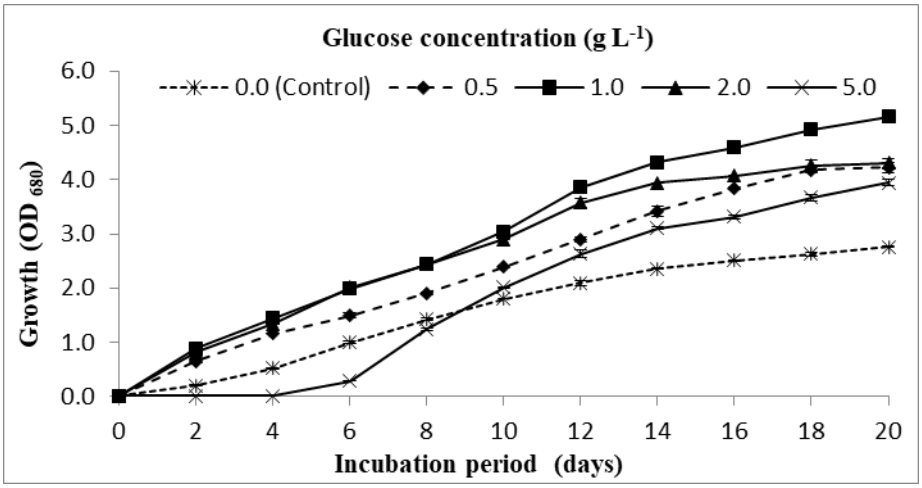

Fig. 5. Effect of glucose concentrations (g L^{-1}) on the growth of *M. reisseri*.

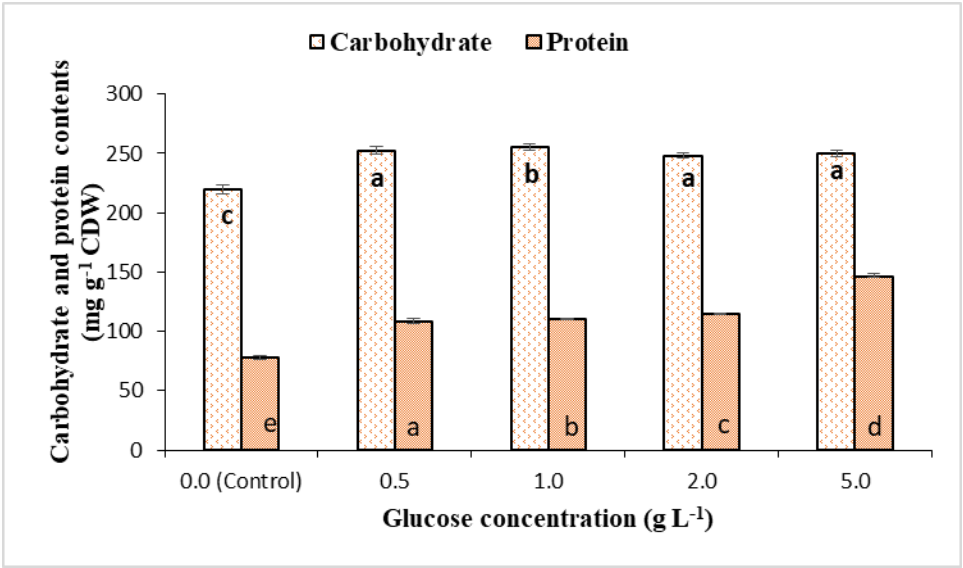

Fig. 6. Carbohydrate and protein contents of *M. reisseri* grown with different concentrations of glucose.

Error bars show the SD for three measurements; significant of differences was denoted by different letters (at $P \leq 0.05$).

Table 4. Effect of different concentrations of glucose on biomass, lipid content, and lipid productivity of *M. reisseri*.

Concentration ($g\ L^{-1}$)	Biomass ($g\ L^{-1}\ d^{-1}$)	Lipid content ($mg\ g^{-1}$ dw)	Lipid productivity ($mg\ L^{-1}\ d^{-1}$)
Control	0.0629 ± 0.0012^{b}	236.65 ± 6.83^{a}	14.88 ± 0.21^{a}
0.5	0.0649 ± 0.0009^{a}	230.67 ± 3.75^{a}	14.98 ± 0.14^{a}
1.0	0.0871 ± 0.0006^{c}	183.64 ± 0.35^{b}	15.99 ± 0.09^{b}
2.0	0.0683 ± 0.0005^{d}	216.24 ± 2.63^{c}	14.77 ± 0.13^{a}
5.0	0.0646 ± 0.0007^{ab}	309.08 ± 5.55^{d}	19.65 ± 0.14^{c}

Significant of differences of each studied parameter was denoted by different letters (at $P \leq 0.05$).

As regarded to using Na-acetate as carbon source for the growth of *M. reisseri*, the results showed that 1 g L^{-1} Na-acetate clearly stimulated the growth and biomass productivity by 89.27 % and 41.49, respectively as compared with the corresponding control (Fig. 7).
The results showed significant stimulation of protein content of *M. reisseri* at 2 g L^{-1} sodium acetate by 27.90 % over the control (Fig. 8). The maximum carbohydrate content was observed at 0.5 g L^{-1} of Na-acetate (24.1 % of CDW) as shown in Figure (8). Lipid content of *M. reisseri* was significant enhanced by 51.65 and 18.28 % in cultures containing 2 and 5 g L^{-1} of Na-acetate, respectively (Table 5). Application of 2 g L^{-1} of Na-acetate in the medium resulted in the highest lipid productivity by 82.39 % as compared with control (Table 5). However, 26.34 % significant drop was found in the culture treated with 0.5 g L^{-1} of Na-acetate (Table 5).

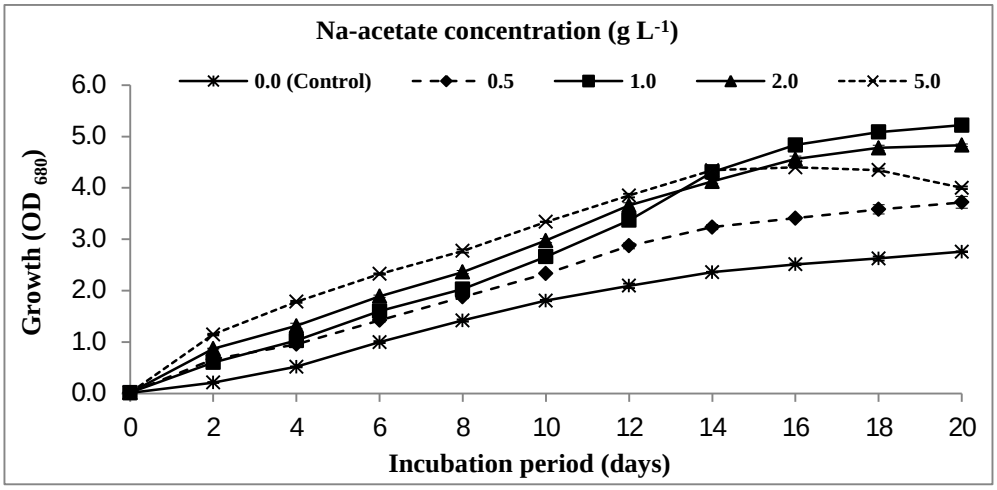

Fig. 7. Effect of Na-acetate concentrations (g L^{-1}) on the growth of *M. reisseri*.

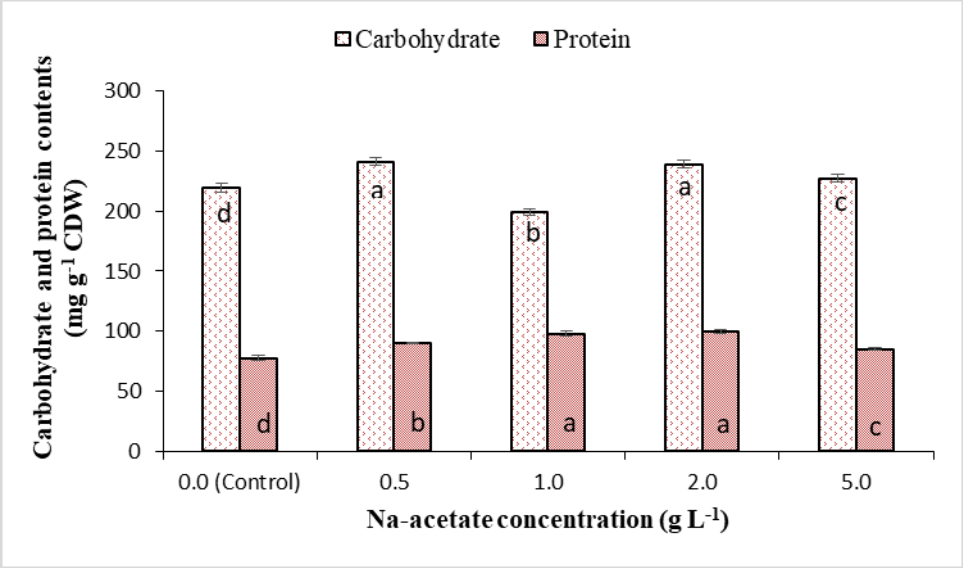

Fig. 8. Carbohydrate and protein contents of *M. reisseri*, grown with different concentrations of Na-acetate.

Error bars show the SD for three measurements; significant of differences was denoted by different letters (at $P \leq 0.05$).

Table 5. Effect of different concentrations of Na-acetate on biomass, lipid content, and lipid productivity of *M. reisseri*.

Concentration	Biomass	Lipids content	Lipid productivity
(g L^{-1})	(g L^{-1} d^{-1})	(mg g^{-1} dw)	(mg L^{-1} d^{-1})
0.0 (Control)	0.0629±0.0012[a]	236.65±6.83[e]	14.88±0.21d
0.5	0.0633±0.0007[a]	173.31±3.18[a]	10.96±0.09b
1.0	0.089±0.0009[b]	200.29±3.1[b]	17.82±0.15 [a]
2.0	0.0756±0.0015[c]	358.89±6.4[c]	27.14±0.09[c]
5.0	0.0635±0.0007[a]	279.91±1.89[d]	17.77±0.09a

Significant of differences of each studied parameter was denoted by different letters (at $P \leq 0.05$).

The highest growth of *M. reisseri* was observed at 2 g L^{-1} of $NaHCO_3$ (Fig. 9). While using 2 g L^{-1} of $NaHCO_3$ caused significant increases in the biomass by 117.97 % over the control (Table 6). Significant increases in protein content of *M. reisseri* (32.37 %) were obtained when the cultures treated with 5 g L^{-1} of $NaHCO_3$ as compared with control (Fig. 10). However, using 0.5 and 5 g L^{-1} of $NaHCO_3$ led to insignificant changes in carbohydrate content.

The obtained results confirmed that cultures treated with all used concentration of $NaHCO_3$ resulted in significant reductions in lipid content of *M. reisseri* below the control (Table 6). While, significant increase in the lipid productivity by 24.46 % was observed with 2 g L^{-1} of $NaHCO_3$ as compared with control. The highest lipid productivity (18.52 mg L^{-1} d^{-1}) was recorded at 2 g L^{-1} of $NaHCO_3$ (Table 6).

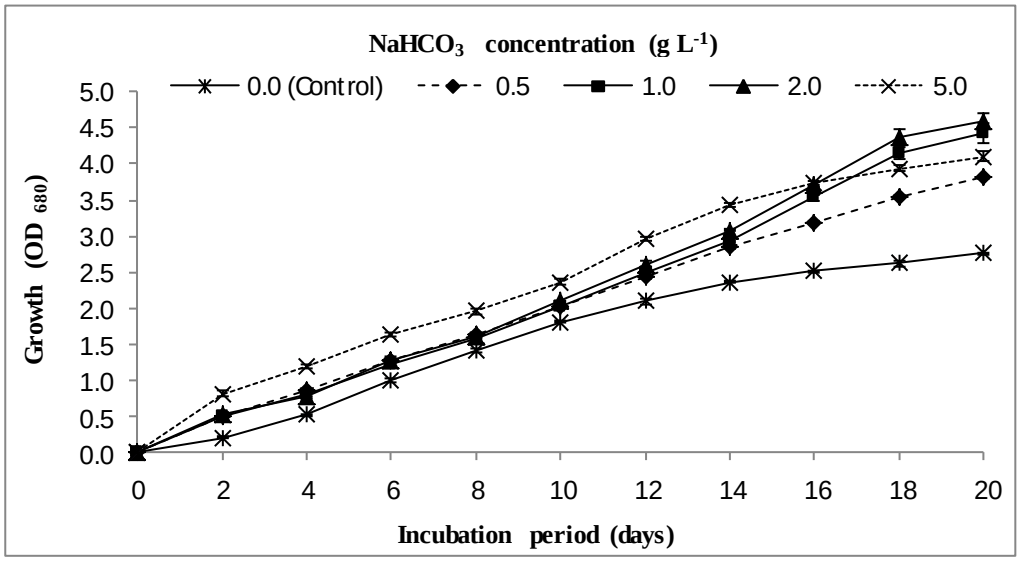

Fig. 9. Effect of $NaHCO_3$ concentrations (g L^{-1}) on the growth of *M. reisseri*.

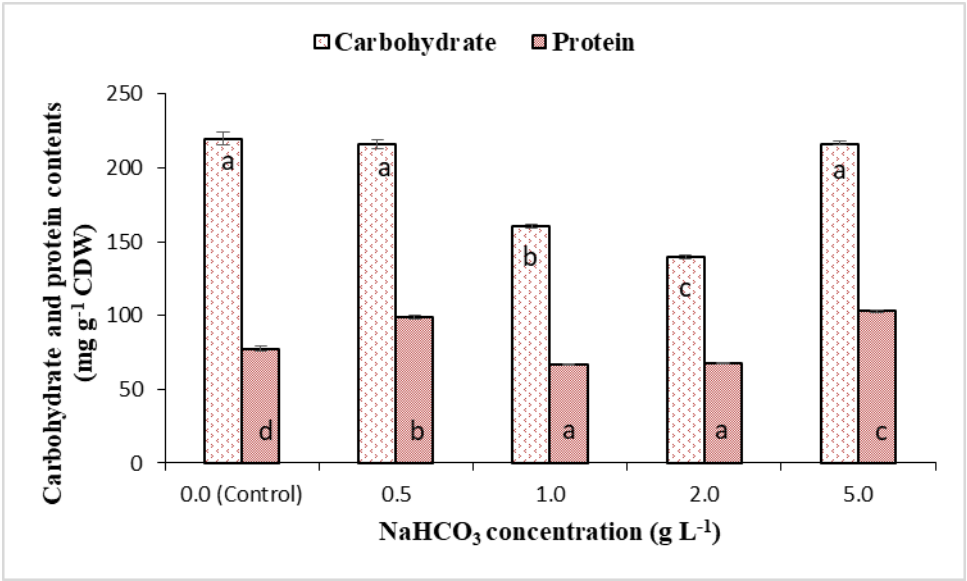

Fig. 10. Carbohydrate and protein contents of *M. reisseri* grown with different concentrations of $NaHCO_3$.

Error bars show the SD for three measurements; significant of differences was denoted by different letters (at $P \leq 0.05$).

Table 6. Effect of different concentrations of $NaHCO_3$ on biomass, lipid content, and lipid productivity of *M. reisseri*.

Concentration	Biomass	Lipids content	Lipid productivity
(g L^{-1})	**(g L^{-1} d^{-1})**	**(mg g^{-1} dw)**	**(mg L^{-1} d^{-1})**
0.0 (Control)	0.0629±0.0012^{e}	236.65±6.83^{e}	14.88±0.21^{e}
0.5	0.0657±0.0009^{a}	126.87±1.78^{a}	8.33±0.10^{a}
1.0	0.1148±0.0007^{b}	146.65±1.26^{b}	16.84±0.15^{b}
2.0	0.1371±0.0014^{c}	135.10±1.34^{c}	18.52±0.10^{c}
5.0	0.0760±0.0006^{d}	214.56±1.56^{d}	16.30±0.09^{d}

Significant of differences of each studied parameter was denoted by different letters (at $P \leq 0.05$).

The results in Figure (11) showed the maximum growth of *M. reisseri* occurred with 0.1 g L^{-1} of glycerol by 43.22 %, over the control. While no change in growth at 0.01 g L^{-1} glycerol. The same results were obtained with the biomass productivity. Application of 0.01, 0.03 and 0.05 g L^{-1} glycerol resulted in insignificant increase in the biomass productivity. However, significant enhancement in the biomass productivity (11.29 %) was observed with culture treated with 0.1 g L^{-1} of glycerol, compared with control (Table 7).

Figure (12) showed increase of glycerol concentration in the medium resulted in significant increases in protein content in *M. reisseri*, where the maximum protein content (8.9 % of CDW) was observed at 0.1 g L^{-1} glycerol. On the other hand with 0.03 g L^{-1} glycerol led to significant increases in carbohydrate content by 5.72 % compared with the control after 20 days of incubation (Fig. 12).

Lipid content of *M. reisseri* was stimulated significantly by 9.20 % when the culture provided with 0.1 g L^{-1} glycerol, as compared with control. However, the culture treated with 0.03 g L^{-1} glycerol resulted in significant reduction in lipid content by 4.58 %, below the control (Table 7). Application of 0.05 and 0.1 g L^{-1} glycerol concentrations in medium caused significant enhance in the lipid productivity by 3.16 and 21.51 %, respectively, as compared with control (Table 7).

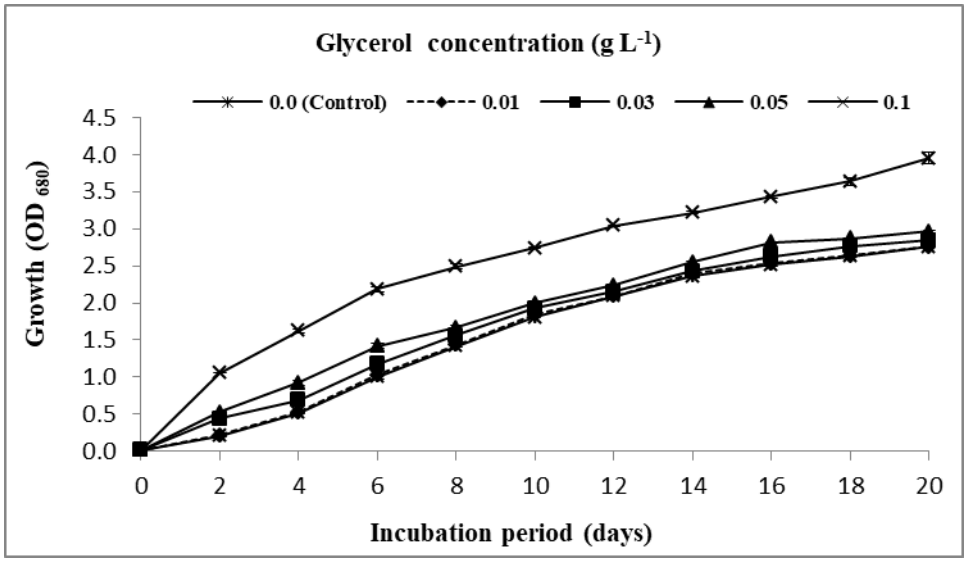

Fig. 11. Effect of glycerol concentrations (g L^{-1}) on the growth of *M. reisseri*.

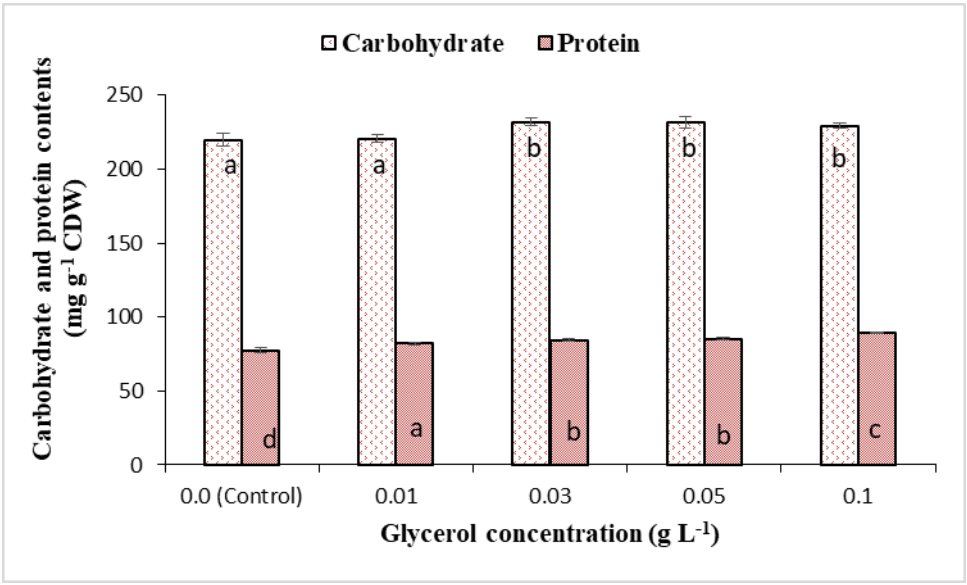

Fig. 12. Carbohydrate and protein contents of *M. reisseri*, grown with different concentrations of glycerol.

Error bars show the SD for three measurements; significant of differences was denoted by different letters (at $P \leq 0.05$).

Table 7. Effect of different concentrations of glycerol on biomass, lipid content, and lipid productivity of *M. reisseri*.

Concentration ($g\ L^{-1}$)	Biomass ($g\ L^{-1}\ d^{-1}$)	Lipids content ($mg\ g^{-1}$ dw)	Lipid productivity ($mg\ L^{-1}\ d^{-1}$)
Control	0.0629 ± 0.0012^{a}	236.65 ± 6.83^{b}	14.88 ± 0.21^{d}
0.01	0.0630 ± 0.0007^{a}	232.53 ± 1.1^{ab}	14.64 ± 0.12^{ad}
0.03	0.0639 ± 0.0008^{a}	225.81 ± 4.04^{a}	14.44 ± 0.15^{a}
0.05	0.0644 ± 0.0011^{a}	238.41 ± 4.28^{b}	15.35 ± 0.09^{b}
0.10	0.0700 ± 0.0011^{b}	258.43 ± 2.23^{c}	18.09 ± 0.16^{c}

Significant of differences of each studied parameter was denoted by different letters (at $P \leq 0.05$).

DISCUSSION

A crucial factor impacting the viability of oil for the production of biodiesel is the lipid content and biomass of the microalgae used to produce biofuel. In contrast, because of their ability to store lipids and relatively higher biomass production compared to other oil plants, microalgae are a prospective source of biofuel. Additionally, utilizing wastewater or seawater, algae can be grown in desert areas **(Wang *et al.*, 2022).**

The inorganic components that make up an algal cell should be supplied by the growth medium. Cells growth and lipid accumulation are significantly affected by salt stress (**Farghl *et al.*, 2015)**. The acquired data showed that, in comparison to the control, *M. reisseri* grew more quickly at higher NaCl concentrations 0.94 g L^{-1}. This is consistent with the findings of **Pandit *et al.* (2017)**, who came to the conclusion that the initial rise in NaCl accelerated the growth of *Chlorella vulgaris* and *Acutodesmus obliquus.* **Gu *et al.* (2012)** analysis of the initial 10-day cultivation *Nannochloropsis oculata* had the largest dry biomass at a salinity of 25 ‰ among the treatments ($P < 0.05$). Additionally, these algae had the maximum lipid productivity at 35 ‰ (64.71mg $L^{-1}d^{-1}$; P<0.001), which contributed to their capacity to promote growth **(El-Sayed and Abdel-Maguid, 2010). Rai *et al.* (2015)** reported that *Chlorella* sp. showed highest growth of 1.021 g L^{-1} under 0.2 M NaCl. However, the maximum lipid production of 0.18 g L^{-1} was estimated by growing the cells in Fogg's medium including 0.5 M NaCl with slight compromise in cell growth (0.86 g L^{-1}). **Talukdar *et al.* (2012)** observed that increasing salinity up to 160 mM NaCl resulted in improved growth and total lipid levels.

The results showed that as the seawater ratio increased in the medium, *M. reisseri* growth gradually dropped but its lipid content significantly increased. This result was consistent with a previous study by **Battah *et al.* (2014)** who found that increasing salinity inhibited algal development and increased the total lipid content of *Chlorella vulgaris*. Salt stress,

which may impact photosynthetic efficiency, rate of respiration, membrane permeability, and buildup of reactive oxygen species (ROS), is responsible for decreasing *M. reisseri* growth with increasing seawater ratios **(Kalita *et al.*, 2011).**

Additionally, the accumulation of lipid as a secondary metabolite and energy storage material f may be due to adaptive responses of algae and protection under salt conditions **(Zhang *et al.*, 2010).**

Carbon is necessary for photosynthesis, formation of lipids, and growth of microalgae **(Hsueh *et al.*, 2007).** The results showed that *M. reisseri* can grow mixotrophically and that they were able to produce their maximum biomass using every carbon source that was examined. Our findings demonstrated that all tested glucose doses from 0.5 to 5.0 g L^{-1} enhanced the development of *M. reisseri.* This result supported mixotrophic conditions for *Scenedesmus* sp. by **Dittamart *et al.* (2014).** According to **Liu *et al.* (2021)** the addition of glucose, maltose, and sodium acetate at 2 and 4 g L^{-1} could considerably increase the production of biomass, lipid content, and productivity.

Many microalgal organisms prefer glucose over other organic carbon sources because it can be quickly absorbed and creates energy-rich molecules such neutral storage lipids **Marudhupandi *et al.* (2016)**. The fact that simple sugar is easily assimilated, broken down by various enzymes, and converted into glucose-6-phosphate, a crucial intermittent product involved in both glycolysis and the pentose-phosphate cycle **(Stewart, 1974)**, may be the cause of glucose's stimulated effect on *M. reisseri* growth in mixotrophic culture**.** In addition, glucose contains more energy than other substrates **(Boyle and Morgan, 2009).**

Regarding the impact of various glucose concentrations on the lipid content of *M. reisseri*, it was discovered that high glucose concentrations of 5 g L^{-1} greatly increased the lipid content. According to their findings, **Kong *et al.* (2011)** concluded that *Chlorella vulgaris* accumulated lipids in response to high glucose concentrations. On the other hand, *M. reisseri* lipid content is dramatically reduced by low glucose concentrations of 1 and 2 g L^{-1}. The decrease in lipid content at low glucose concentrations may be caused by the fact that glucose is a byproduct of photosynthesis and, as a result, *M. reisseri* uses glucose directly as an energy source for stimulating cell division and growth rather than storing it as lipid.

In comparison to the control, adding Na-acetate to *M. reisseri* cultures clearly enhanced growth at all investigated doses, ranging from 0.5 to 5.0 g L^{-1}. In line with our findings, **Wang *et al.* (2012)** discovered that *Phaeodactylum tricornutum* growth rate in mixotrophic batch cultures was greatly increased by Na-acetate. Acetyl CoA can be utilized to convert acetate into pyruvate, which can then be further oxidized in the metabolic process **(Dittamart *et al.*, 2014).**

The lipid accumulation in *M. reisseri* was boosted by the addition of Na-acetate as a carbon source. Similar findings from recient studies indicate that increasing the amount

of Na-acetate may increase the lipid content of microalgae **(Lu *et al.*, 2021; Ghosh *et al.*, 2021).** These findings, however, were in contrast to those of earlier research by **Dittamart *et al.* (2014),** who discovered that the biomass and lipid content of *Scenedesmus* sp. in the presence of Na-acetate supplementation were not substantially different from those under the photoautotrophic condition. Furthermore, **Rai *et al.* (2013)** reported that the addition of 10 g m^2 Na-acetate promoted a 13.5-fold higher lipid compared to photoautotrophic conditions of *Chlorella Pyrenoidosa*. Similarly, **Ghosh *et al.* (2021)** reported that 3 g L^{-1} of Na-acetate showed the highest lipid productivity of 176.80 ± 68.80 µg mg^{-1}. Na-acetate can boost the metabolic process within algal cells, where acetyl-CoA catalyses the creation of acetyl-CoA from acetate in algal cells and participates in the citric acid cycle metabolism for lipid synthesis. Additionally, it enhances the intracellular citric acid cycle's carbon metabolic flux, supporting growth and biomass yield.

The current findings suggest that the growth enhancement of *M. reisseri* under the influence of $NaHCO_3$ may be due to the ability of some microalgal species to actively transport carbonate across the plasma membrane into the cytosol where it can be used for cell growth by extracellular carbonic anhydrase (CA) activities. That is what causes carbonate to turn into free CO_2 to speed up CO_2 assimilation **(Young *et al.*, 2001).** On the other hand, all examined $NaHCO_3$ concentrations reduced the lipid content of *M. reisseri.* The lipid productivity of *M. reisseri* peaked at 2 g L^{-1} $NaHCO_3$, and the only factor that increased it was an increase in biomass. These findings conflict with those of **Devgoswami *et al.* (2011),** who discovered that strains of *Chlorella, Haematococcus*, and *Scenedesmus* cultivated in medium supplemented with bicarbonate salt had higher lipid contents.

By increasing glycerol concentrations from 0.01 to 0.1 g L^{-1}, *M. reisseri* grew faster than the control and began to produce its greatest amounts of biomass and lipids. The lipid productivity of *M. reisseri* in the current study peaked at 0.1 g L^{-1} glycerol, and its improvement was brought on by an increase in both total lipid content and biomass at the same time. This finding is consistent with earlier findings made by **Kong *et al.* (2013),** who noted that in mixotrophic circumstances, *C. vulgaris* biomass, lipid production, and lipid content rose with an increase in glycerol concentration. **Marey *et al.* (2022)** concluded that *Tetraselmis elliptica* lipid productivity was significantly improved by 0.01 g L^{-1} glycerol.

Although mixotrophic growth of microalgae has better biomass and lipid productivities than photoautotrophic growth, the high cost of organic carbon substrate, may make mixotrophic cultivation of microalgae economically untenable. Finding inexpensive organic substrates that provide the dietary requirements is thus required.

Micractinium reisseri (JN169781) is a freshwater green microalgae, and its biomass contains high lipid productivity, which increases its valuation. As we tested the different salinity and some carbon sources, among them wide variation was obtained in their lipid

production. Among all studied factors, 0.94 g L^{-1} of sodium chloride (NaCl), 25 % SW concentration, 5 g L^{-1} of glucose, 2 g L^{-1} Na-acetate, and 0.1 g L^{-1} of glycerol significantly enhanced the lipid productivity of *M. reisseri*.

CONCLUSION

Micractinium reisseri (JN169781) is a freshwater green microalgae, and its biomass contains high lipid productivity, which increases its valuation. As we tested the different salinity and some carbon sources, among them wide variation was obtained in their lipid production. Among all studied factors, 0.94 g L^{-1} of sodium chloride (NaCl), 25 % SW concentration, 5 g L^{-1} of glucose, 2 g L^{-1} Na-acetate, and 0.1 g L^{-1} of glycerol significantly enhanced the lipid productivity of *M. reisseri*.

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