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Original article

Combined effect of salinity and pH on lipid content and fatty acid composition of *Tisochrysis lutea*

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ABSTRACT

The haptophyte microalga *Tisochrysis lutea* was heterotrophically grown in F2 medium with different combinations of pH and salinity. Growth, oil content and fatty acids (FAs) profile were determined under each set of conditions. The salinity was adjusted using NaCl at concentrations of 0.4, 0.6, 0.8, or 1.0 M, while pH was adjusted at 7, 8, or 9, and heterotrophic growth was performed using organic carbon in the form of sugar cane industry waste (CM). Fatty acid methyl esters (FAMEs) were identified by gas chromatography. The results showed that pH of 8.0 was the optimal for dry weight and oil production, regardless of the salinity level. At pH 8.0, growth at a salinity of 0.4 M NaCl was optimal for biomass accumulation (1.185 g L^{-1}). Under these conditions, the maximum growth rate was 0.055 g L^{-1} d^{-1}, with a doubling time of 17.5 h and a degree of multiplication of 2.198. Oil content was maximal (34.87%) when the salinity was 0.4 M and the pH was 9.0. The ratio of saturated to unsaturated FAs was affected by the pH value and salinity, in that unsaturated FAs increased to 58.09% of the total FAs, considerably greater than the value of 40.59% obtained for the control (0.4 M NaCl and pH 8.0).

1. Introduction

There is increasing worldwide interest in the mass production of microalgae to supply products such as proteins, oils, fuels, and drugs (Phang, 2010; Ashour et al., 2019). The continuing use of traditional fossil fuels and increasing the demand for energy worldwide have led to multiple environmental problems (Schenk et al., 2008; Abomohra et al., 2020a). Biofuels, which may help resolve the energy crisis, are liquid fuels yielded from both the biomass of agricultural and forest products and the biodegradable part of industrial wastes (Dufey, 2006). Among different biofuel feedstocks, microalgae have been widely discussed as a promising solution to the energy crisis because they are easy to grow with little care, inexpensive growth medium, can be grown rapidly without competitors, and the bioenergy product can be obtained in different forms such as biodiesel, bioalcohols, crude bio-oil, and biogas (El-Sayed et al., 2017; Wang et al., 2019; Almutairi, 2020; Xu et al., 2019).

Many previous studies have examined the microalgal lipids as a sustainable and environmentally friendly source for biodiesel (Griffiths and Harrison, 2009; Schnurr and Allen, 2015; Almarashi et al., 2020). However, to be economically feasible, microalgal cultivation must have high productivity of biomass and efficient production of lipids (Go et al., 2012; Touliabah et al., 2020). In order to enhance the productivity of biomass and lipids, researchers have applied different abiotic stresses to the microalgal cells (e.g., nutrient deprivation, low pH, high salinity, high light level, high temperature, and UV radiation) to stimulate the synthesis and accumulation of lipids (Gomaa et al., 2018; El-Sheekh et al., 2019; Abomohra et al., 2019; Chen et al., 2017; Paliwal et al., 2017). Microalgae use various mechanisms to adapt to these stresses, such as altering the morphology and growth due to changes in underlying physiological and biochemical processes (BenMoussa-Dahmen et al., 2016). Thus, different stressors are used commercially to enhance the productivity of certain algal components, mainly pigments and lipids.

Increasing of salinity alters the growth and biochemical composition of marine microalgae, even if they are tolerant to the high salinity (Khatoon et al., 2014; Abomohra et al., 2020b). In addition,

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accumulation of lipids increases when microalgal cells grow under high salinity, because they change from active cell division to the storage of energy as an adaptation to the stress environment (BenMoussa-Dahmen et al., 2016). At the cellular level, microalgae exposed to a saline environment undergo a survival response that includes restoration of turgor pressure, accumulation of osmo-protective molecules (glycerol), and induction of stress-related proteins. This stress then leads to increased production of lipids, an energy-rich substance that allows survival under extreme environmental conditions (Venkata Mohan and Devi, 2014; Ishika et al., 2019). On the other hand, the response to salinity stress differs among species, and growth of some species at high salt concentrations lowers the efficiency of photosynthesis and decreases the biomass accumulation (Chen et al., 2017).

The hydrogen ion concentration (pH) is another stress factor that can affect the growth and biochemical composition of microalgae. Microalgae exhibit optimal growth when the pH is within a certain range, and deviations from this range can inhibit growth, alter biochemical composition, or even cause cell death (Chen et al., 2017; Khatoon et al., 2014). Previous research reported that alkaline pH stress of *Chlorella* sp. stimulates the accumulation of triacylglycerols and decreases the membrane lipid content (LC) (Paliwal et al., 2017).

In general, recent studies have examined the effect of combined abiotic stressors on the growth and biochemical composition of microalgae. These studies found that the combined stress of two or more factors enhanced the growth and the lipid productivity of algae, and attributed this enhancement to the compensation between different stress factors (Cheng and He, 2014; Salama et al., 2018). However, effect of combined salinity and pH as a biotic stress on marine microalgae needs further investigation. In the present study, the marine microalga *Tisochrysis lutea* (previously known as *Tisochrysis* affinis *galbana*) was used. It was selected for this study because it is commonly used to feed mollusk larvae in aquaculture and the high lipid content makes it popular as a possible biodiesel fuel source (da Costa et al., 2017; Garnier et al., 2016). It belongs to haptophyte, prymnesiophyceae, isochrysidales, and family isochrysidaceae. The aim of the present study was to determine the effect of combining two stress factors (various levels of salinity and pH) on the growth, LC, and FA profile of *T. lutea.*

2. Materials and methods

2.1. Microalga and culture conditions

Tisochrysis lutea CCAP 927/14 was purchased from the Culture Collection of Algae and Protozoa (Oban, U.K.). A primary stock culture of the liquid *T. lutea* sample was prepared in a 100 mL flask. The marine F2 growth medium (Guillard and Ryther 1962) containing 33.6 g L^{-1} of artificial seawater salts was prepared, containing the mineral content shown in Table 1. All media used in the experiments were laboratory made and sterilized at 121 °C for 15 min.

Cultures were aerated by a gentle stream of dried air (free of oil and moisture), illuminated at 70 μmol m^{-2} s^{-1} from one side with a white LED light bank, and the medium was enriched with 0.7 g L^{-1} of sugar cane industry waste (CM) (Table 2). The temperature was maintained at 25 ± 1 °C.

Table 1
Mineral content of the artificial sea salt used for preparation of F2 growth medium.

N	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu
%						ppm			
0.80	traces	0.11	0.68	0.27	24.0	50	40	70	2

2.2. Growth measurement

Daily measurements of cellular dry weight were performed by passing a 10 mL of algal slurry through a membrane filter (0.46 μm). Filters were dried at 105 °C overnight, maintained over anhydrous $CaCl_2$ until they reached room temperature, and then reweighed. The weight difference represented the change in biomass during the sampling period (24 h). Growth analysis parameters including growth rate (μ), doubling time (DT), and degree of multiplication (DM) were calculated as described by Pirt (1975).

2.3. Lipid extraction and determination

Lipids were extracted by soaking cellulose extraction thimbles (33 × 94 mm) filled with the obtained dried biomass overnight in a solvent mixture comprised of 3:2 (*v*/*v*) n-hexane: isopropanol, followed by Soxhlet extraction (El-Sayed et al., 2017). Thimbles were then water-washed, dried at 105 °C for 60 min, and re-weighed. Weight differences were used to calculate the initial LC. FAMEs were identified and determined by gas chromatography (GC) using the GC Perkin Elmer Auto System XL. Volumetric lipid productivity (Lp; in mg/day) was calculated as P_{dwt} × Lc (Liu et al., 2011), where P_{dwt} is the sample dry weight at the end of the logarithmic growth phase and Lc is the total LC.

2.4. Statistical analysis

All experiments have been performed in triplicate and the results are reported as mean value ± standard deviation. The experimental results were analyzed by one-way analysis of variance (ANOVA) using Statistica package version 8.0 (StatSoft Inc., Tulsa, OK, USA) at probability level of ($P < 0.05$).

3. Results and discussion

3.1. Biomass and growth rate

Stress factors, including salinity, nutrient depletion, high light intensity and pH of the growth medium, markedly influence the growth and metabolic functions of microalgae. The high carbon content of algal cells, which can exceed 50% of the algal dry weight, confirms the importance of carbon sources for algal nutrition. Thus, a lack of bloom formation by algae in their natural habitat can be attributed to the carbon-deficient environment (El-Sheekh et al., 2018). In addition, access to sufficient amount of carbon, especially

Table 2
Chemical analysis of sugar cane industry waste used to enrich the F2 growth medium.

OC	TN	P	K	Ca	Mg	S	Mo	B	Mn	Fe	Zn	Cu
%							Ppm					
42.19	4.62	0.09	8.17	0.87	0.16	10.04	5.3	8.5	71.0	11.3	483.9	5.3

Tisochrysis lutea cultures were grown at various levels of salinity (0.4, 0.6, 0.8 and 1.0 M NaCl) and pH (7, 8, and 9). All experiments were conducted in 2 L flasks, with 3 replicates per treatment.

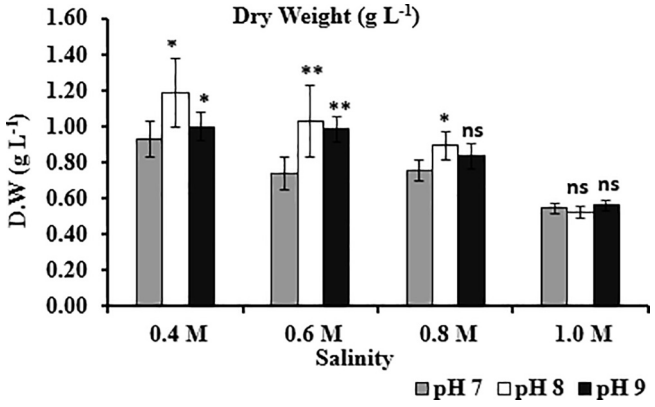

Fig. 1. Dry weight accumulation of *T. lutea* grown in media with different salinities and pH values. All tests were performed in triplicates (n = 3) and the standard deviations are shown as error bars. (*) indicate low significant difference compared to the control ($P < 0.05$), (**) indicate highly significant difference compared to the control ($P < 0.01$), (***) indicate very highly significant difference compared to the control ($P < 0.001$).

organic carbon, reduces the adverse effects of stressors such as pH and salinity on algae. Under ambient conditions, the organic carbon source used in the present work (CM-waste) was rich in both carbon and essential nutrients (N and K). Different salinities and pH showed that both of these factors affected the dry weight of *T. lutea* (Fig. 1). In particular, at pH 8.0, the highest change in the dry weight was obtained when algal cells were cultured at a low salinity level (0.4 M NaCl). This corresponds to the salinity level in the natural habitat of this marine microalga (23.3 g L^{-1} NaCl). Analysis of algal growth at 0.6 M NaCl and varying the pH showed a maximum biomass productivity at pH 8.0 (0.055 g L^{-1} d^{-1}), followed by pH 9.0 (0.049 g L^{-1} d^{-1}), then pH 7.0 (0.045 g L^{-1} d^{-1}).

At moderate salinity (0.6 to 0.8 M), algal growth was slightly inhibited and gave a smaller increase in the dry weight than was observed at 0.4 M NaCl, especially at pH 8.0. At a salinity of 0.6 M NaCl, the growth rate was 0.045 g L^{-1} d^{-1} at pH 7, 0.055 g L^{-1} d^{-1} at pH 8, and 0.049 g L^{-1} d^{-1} at pH 9, indicating that the microalgal growth was more affected by salinity than by pH of the growth medium. At 0.8 M salinity, the growth rate was 0.043 g L^{-1} d^{-1} at pH 7, 0.047 g L^{-1} d^{-1} at pH 8, and 0.47 g L^{-1} d^{-1} at pH 9. However, the poorest growth was observed for cultures grown at the highest level of salinity (1.0 M). Under this condition, the growth rate was 0.040 g L^{-1} d^{-1} at pH 7, 0.039 g L^{-1} d^{-1} at pH 8, and 0.035 g L^{-1} d^{-1} at pH 9. Taken together, these results suggest that a medium pH of 8 is optimal for maximizing biomass and growth rate.

Changing the salinity or pH of the medium in which microalgae grow leads to several changes in metabolites productivity and cell physiology, affecting levels of proteins, carbohydrates, lipids and pigments. In particular, Juneja et al. (2013) reported that environmental factors and nutrient availability strongly influence the amount of fixed carbon stored as lipids and carbohydrates (starch). Understanding the synergistic interactions among various environmental variables and nutritional factors is needed to improve sustainable high-productivity of the bio-algal systems to be suitable for use in commercial biofuel production. In addition to dry weight, two other growth parameters, namely doubling time (DT) and degree of multiplication (DM), were calculated for *T. lutea* grown under different combinations of salinity and pH (Fig. 2). DT and DM showed the same pattern of the dry weight in response to salinity and pH variation. These findings confirm that low salinity and slightly alkaline conditions are the most beneficial for the high growth of *T. lutea*. Many other studies have also reported a decline in growth (dry weight) of microalgae under unfavorable conditions, a response that can be attributed to a disruption of normal cell metabolism due to blocking of photosynthesis. The latter effect can be avoided by enriching the growth medium with organic carbon source. In that context, Masojidek et al. (2013) pointed out that under such conditions, photosynthesis and carbon dioxide fixation decline greatly, and dry weight increases due to the increase of accumulated sugars and oils.

Because sugars produced during photosynthesis affect the osmotic potential, cells store carbon in another form to avoid dysregulation of osmolarity. The most common storage products are lipids and starch, although the preferred storage product is species-specific (Rai, 1995; Rai et al., 1997; Abomohra et al., 2018). It is well known that microalgal cell composition and growth are very sensitive to nutritional status and multiple environmental factors. Thus, extreme growth conditions can often drastically affect the algal biomass production. However, the factors that affect dry weight accumulation, including nutritional and environmental factors, are well understood. In addition, growth patterns can also be changed due to changes in ambient conditions. Nitrogen content, salinity, carbon availability, light level, and pH are among the major factors affecting the growth patterns of microalgae. However, instead of considering growth pH as a factor that affects growth, pH could also be considered an indicator for the algal growth. This is because microalgal growth is accompanied by excretion of certain cell metabolites and uptake of acidic compounds, such as amino acids and nitrogenous compounds leading to increasing of alkalinity of the growth medium over time. Thus, a moderate increase of alkalinity is a good indicator for normal microalgal growth. An additional consideration is that microalgal growth is accompanied by processes affecting the pH, such as excretion of certain cell metabolites and absorption of acidic compounds (*e.g.*, amino acids and nitrogenous compounds). Thus, the growth medium tends to become more alkaline over time. Thus, pH is not simply a static factor that affects growth, but rather is also an indicator for healthy algal growth. Thus, the natural decline in acid reaction of the algal growth medium gives rise to a decline in algal growth, indicating a high death rate (Al-Mutairi and Toulibah, 2017). In the present study, it was found that the lowest growth occurred at the highest salt level (1.0 M) at all tested pH levels, and that increasing of salt concentration above 0.4 M reduced the dry weight accumulation.

3.2. Lipid content

Analysis of the LC of *T. lutea* grown under different pH and salinity values showed that both factors affected the LC (Table 3). In particular, at normal salinity (0.4 M), the LC (%) increased with increasing pH, from 8.97 at pH 7 to 13.78 at pH 8, and then to 34.9 at pH 9. This suggests that increasing the pH elicited a protective response. With increasing LC due to stress effects, attention should be given to the concomitant effects on dry weight accumulation. Specifically, a key problem during carotenogensis or lipid accumulation is dry weight failure due to the stress applied (mainly nitrogen deficiency and/or salinity).

T. lutea may be considered a novel source of lipid or oil feedstock. This alga accumulates more than 20% of its dry weight as lipid (Ohse et al., 2015; Renaud et al., 2002). By contrast, Silitonga et al. (2017) reported that the optimal lipid yield for *I. galbana* was 8.41% dry weight.

A completely opposite response occurred at a salinity of 0.6 M, in that the LC was 22.48% at pH 7, 16.06% at pH 8, and 5.85% at pH 9.0. This decline in LC with increasing the pH may be due to the combined lower nutrient availability at higher salinity. Therefore, a 0.4 M salinity level and a pH of 8 seems to be the most promising combination for high lipid production, because cultures accumulated 34.9% of their dry weight as lipid under these conditions. In

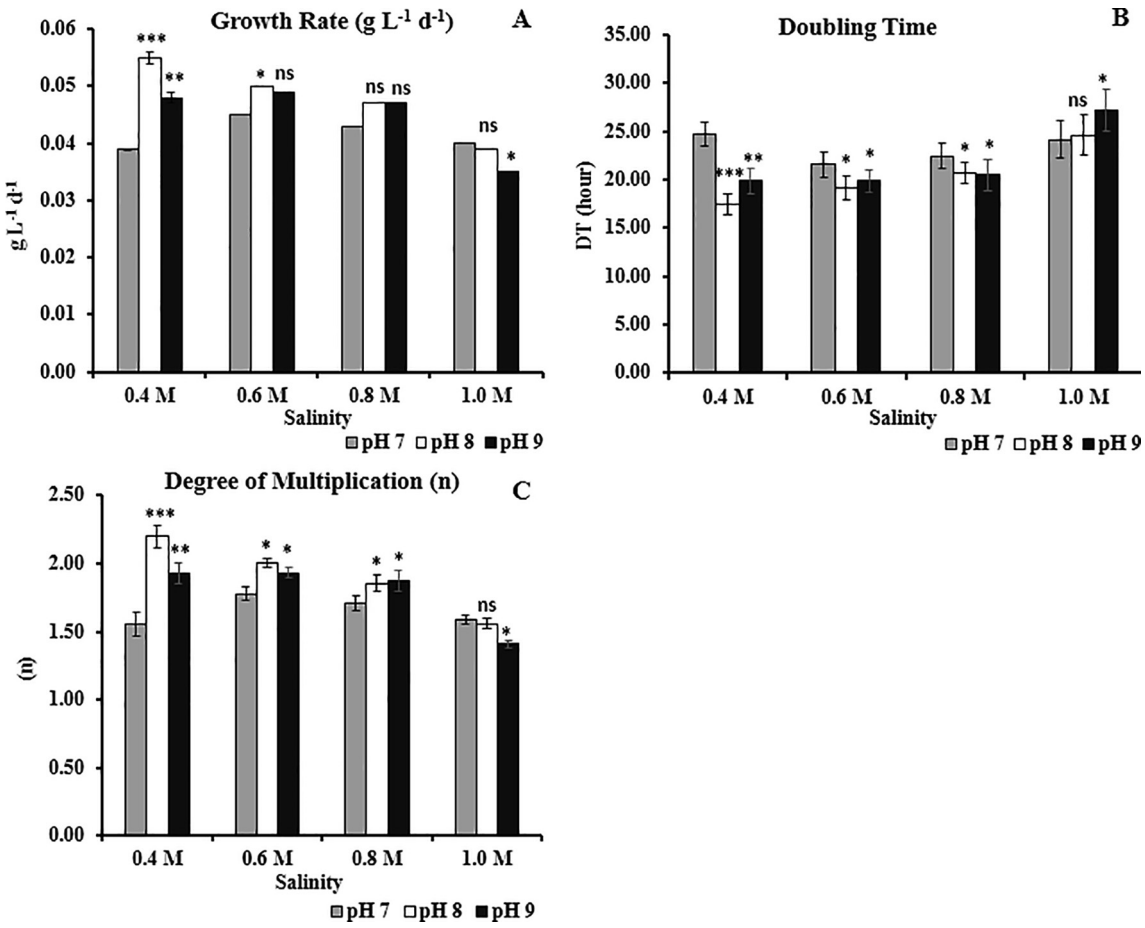

Fig. 2. A) Growth rate, B) doubling time and C) degree of multiplication of *T. lutea* grown in media with different salinities and pH values. All tests were performed in triplicates (n = 3) and the standard deviations are shown as error bars. (*) indicate low significant difference compared to the control ($P < 0.05$), (**) indicate highly significant difference compared to the control ($P < 0.01$), (***) indicate very highly significant difference compared to the control ($P < 0.001$).

Table 3
Dry weight (DW, g L^{-1}); lipid content (LC, %); and lipid productivity (LP, mg L^{-1} d^{-1}) of *T. lutea* grown at different salinities and pH values.

Salinity		pH 7			pH 8			pH 9		
0.4 M	DW	0.92	±	0.10	1.18 *	±	0.19	0.99 ns	±	0.08
	LC	8.97	±	1.10	13.78*	±	1.30	34.90***	±	2.90
	LP	8.32	±	0.90	16.33**	±	1.20	34.87***	±	2.30
0.6 M	DW	0.73	±	0.09	1.02**	±	0.20	0.98*	±	0.07
	LC	22.48	±	2.60	16.06*	±	1.50	5.86***	±	0.70
	LP	16.59	±	1.20	16.51 ns	±	1.20	5.77***	±	0.60
0.8 M	DW	0.75	±	0.06	0.89*	±	0.08	0.83*	±	0.07
	LC	8.64	±	1.00	32.40***	±	3.20	23.43***	±	2.60
	LP	6.51	±	0.80	28.97***	±	2.10	19.61***	±	1.30
1.0 M	DW	0.54	±	0.03	0.52 ns	±	0.03	0.56 ns	±	0.03
	LC	3.48	±	0.90	15.95***	±	1.70	14.49***	±	1.80
	LP	1.89	±	0.60	8.33***	±	0.90	8.13***	±	0.90

All tests were performed in triplicates (n = 3) and the standard deviation as an error bar. (*) indicate low significant difference compared to the control ($p < 0.05$), (**) indicate highly significant difference compared to the control ($p < 0.01$), (***) indicate very highly significant difference compared to the control ($p < 0.001$).

terms of dry weight accumulation, algae grown under conditions of 0.8 M and pH 8.0 yielded 0.894 g L^{-1} of cell dry weight. This corresponds to 0.05 g L^{-1} d^{-1}, which is a considerable productivity for mass production. However, the lowest LC (3.48%) was obtained for cultures grown in 1.0 M salinity and pH 7.0. Thus, the combined salinity and acidity effect is obviously shown by the moderate acidity habitat (pH 8.0), which simulates the natural habitat.

Previous studies have reported that carotenogensis levels of certain nutritional factors, lipids, sugars, and carotenoids during algal stress increase while protein and chlorophyll levels are markedly reduced (Tossavainen et al., 2019). As a result, photosynthesis becomes negligible and dry weight accumulation stalls, whereas oil and sugar contents increase. The lipid forms of carbon can be accumulated at much higher concentrations, hence improving the C/N ratio. Nitrogen deficiency often leads to decreased growth rate. In this case, large amounts of ATP and NADP, which are normally used for cell growth, are used for FAs biosynthesis and lipid accumulation (Harwood and Jones 1989; Thompson, 1996). In a

Table 4
Fatty acid profile of FAMEs produced from lipids of *T. lutea* grown at different salinities and pH values.

Fatty acid	Salinity 0.4 M			Salinity 0.6 M			Salinity 0.8 M			Salinity 1.0 M		
	pH 7	pH 8	pH 9	pH 7	pH 8	pH 9	pH 7	pH 8	pH 9	pH 7	pH 8	pH 9
Myristic 14:0	21.13 ± 1.60	19.09 ± 1.40	17.46 ± 1.50	22.04 ± 1.50	24.08 ± 1.60	22.09 ± 1.80	20.10 ± 1.90	19.42 ± 1.60	18.23 ± 1.20	19.52 ± 2.10	18.03 ± 1.30	16.51 ± 0.80
Myristoleic 14:1	3.05 ± 0.80	3.24 ± 0.35	3.52 ± 0.60	1.59 ± 0.20	2.17 ± 0.06	2.96 ± 0.10	3.08 ± 0.30	3.28 ± 0.20	3.16 ± 0.30	5.19 ± 0.80	3.86 ± 0.20	4.81 ± 0.32
Palmitoleic 16:1	3.12 ± 0.70	3.92 ± 0.85	4.15 ± 0.60	2.98 ± 0.30	3.05 ± 0.30	2.74 ± 0.20	3.87 ± 0.20	4.03 ± 0.30	3.98 ± 0.20	5.12 ± 0.32	3.94 ± 0.40	3.89 ± 0.20
Stearic 18:0	9.12 ± 0.80	8.07 ± 0.90	8.01 ± 0.40	10.08 ± 0.80	8.19 ± 0.80	8.24 ± 0.60	9.07 ± 0.30	7.08 ± 0.20	7.81 ± 0.30	4.71 ± 0.24	5.19 ± 0.18	8.08 ± 0.40
Oleic 18:1	12.08 ± 1.10	14.91 ± 1.10	14.99 ± 0.90	10.92 ± 0.90	10.35 ± 1.10	12.07 ± 0.50	10.02 ± 0.90	12.87 ± 0.10	12.08 ± 0.90	11.82 ± 0.90	13.06 ± 0.23	12.41 ± 0.6
Linoleic 18:2	0.74 ± 0.32	1.09 ± 0.20	1.24 ± 0.20	0.95 ± 0.90	1.16 ± 0.20	0.94 ± 0.10	2.14 ± 0.20	2.08 ± 0.10	2.65 ± 0.20	4.16 ± 0.76	1.98 ± 0.30	1.92 ± 0.20
α-Linolenic 18:3	4.19 ± 0.90	5.23 ± 0.85	6.02 ± 0.60	5.09 ± 0.60	4.88 ± 0.50	5.84 ± 0.20	6.48 ± 0.60	6.02 ± 0.60	6.18 ± 0.56	6.19 ± 0.36	5.92 ± 0.23	5.36 ± 0.36
γ-Linolenic 18:3	4.12 ± 0.85	4.01 ± 0.50	5.19 ± 0.56	3.74 ± 0.20	4.06 ± 0.60	4.92 ± 0.30	3.21 ± 0.20	4.72 ± 0.30	4.67 ± 0.35	3.58 ± 0.20	4.72 ± 0.30	4.83 ± 0.80
Arachidonic C20:0	8.02 ± 0.40	8.48 ± 0.65	7.62 ± 0.64	9.12 ± 0.90	7.63 ± 0.80	8.19 ± 0.90	8.02 ± 0.60	6.12 ± 0.40	6.24 ± 0.60	5.12 ± 0.15	8.22 ± 0.20	7.34 ± 0.36
Arachidonic (AA) 20:4	8.18 ± 0.50	8.61 ± 0.45	9.14 ± 0.90	9.16 ± 0.90	1.28 ± 0.20	9.14 ± 0.85	7.18 ± 0.50	9.27 ± 0.85	9.85 ± 0.80	10.02 ± 1.20	11.54 ± 1.20	9.82 ± 0.90
Eicosapentaenoic(EPA) 20:5	2.98 ± 0.30	2.05 ± 0.20	3.14 ± 0.48	3.22 ± 0.23	5.09 ± 0.50	1.98 ± 0.30	4.62 ± 0.70	4.69 ± 0.30	4.98 ± 0.30	5.45 ± 0.30	6.87 ± 0.30	5.13 ± 0.30
Docosahexaenoic(DHA)22:6	2.23 ± 0.20	2.12 ± 0.30	3.16 ± 0.26	3.19 ± 0.31	4.81 ± 0.50	4.15 ± 0.35	5.12 ± 0.90	4.88 ± 0.40	5.17 ± 0.40	4.66 ± 0.90	3.98 ± 0.20	6.07 ± 0.45
Total saturated	58.09 ± 5.60	53.8 ± 6.30	49.13 ± 5.60	58.86 ± 6.40	56.21 ± 6.30	55.61 ± 5.10	53.96 ± 6.20	47.64 ± 4.60	46.36 ± 4.30	41.44 ± 3.50	43.65 ± 4.50	44.85 ± 2.30
Total unsaturated	40.69 ± 4.80	45.18 ± 5.20	50.55 ± 5.80	40.84 ± 5.60	43.55 ± 4.50	44.74 ± 4.90	46.72 ± 4.60	51.84 ± 5.30	52.72 ± 4.90	58.19 ± 5.80	55.87 ± 5.30	54.24 ± 4.60

study of the putative relationship between salinity and lipid production, Ohse et al. (2015) documented that a raise in salinity leads to a decrease in LC. Further, the optimal salinity level for growth and lipid production seems to vary among algal species (Chaffin et al. 2012). Here, the maximum lipid productivity (Lp) of 34.87 mg L^{-1} d^{-1} was obtained when cultures were grown at a salinity of 0.4 M and a pH of 7.0 and, followed by the salinity of 0.8 M and a pH of 8.0 (28.97 mg L^{-1} d^{-1}). Accordingly, a closed or controlled system for oil production can maximize lipid accumulation by use of a salinity of 0.4 M and a pH of 7.0. On the other hand, an open system seems to operate best under the ambient levels of salinity and acidity (0.8 M and pH 8), although dry weight accumulation was the lowest under these conditions.

The present results confirmed that salinity and pH also affect the fatty acid methyl ester (FAMEs) profile of *T. lutea* (Table 4). These variations can also be interpreted as a protective response to the stress conditions. Under the standard growth conditions (0.4 M salinity and pH 8), short-chain FAs (C14-C16) accounted for more than 50% of the total FAs, medium-chain FAs (C18) accounted for more than 30%, while long-chain FAs (C20-24) accounted for about 20%. Overall, the most common FAs were C14:0 (20.19%), C16:0 (22.34%), and C18:1 (22.09%), and the least common FA was C18:2 (1.09%). These results differ from those of Napolitano et al. (1990), who reported that more than 70% of the total FAs in diatoms are short-chain FAs (C16), but<20% were recorded in *I. galbana*. In addition, more than 50% of the total FAs in *I. galbana* are medium-chain FAs (C18) and long-chain unsaturated FAs (22:6w3), compared to *Chaetoceros* spp. that are<10% of the total.

At a pH 7.0, there was 5% increase in the total saturated FAs comparing to that at pH 8.0. Increasing the pH to 8.0 also increased the unsaturated FAs content (about 50% of the total FAs). At a salinity of 0.4 M, the dominant saturated FAs were myristic acid (C14), palmitic acid (C16), stearic acid (C18), and arachidilic acid (C20). The dominant unsaturated FAs were oleic acid (18:1) and arachidonic acid (20:4).

Although salinity level affected the FAs profile of *T. lutea*, it had a little impact on the predominance of different FAs. Acidic reaction increases the saturated FAs, while alkaline reaction (pH 8.0) engaged the accumulation of unsaturated FAs as well as the long-chain FAs. Similarly, C16:0 and C18:1n-9 were the predominant FAs in the halophilic microalga *Tetraselmis elliptica* isolated from hypersaline environment (Abomohra et al., 2017). In addition, Almutairi and Toulibah (2017) reported that palmitic acid (C16:0) and stearic acid (C18:0) were the predominant FAs produced by *Tetraselmis* alga when grown in medium with 0.4 M NaCl. However, they detected several other FAs, including pentadecanoic acid (C15:0), *cis*-10-heptadecanoic acid (C17:1), elaidic acid (C18:1) and linolelaidic acid (C18:2), when the salinity was 0.6 M NaCl. Furthermore, these cells produced the same spectrum of FAs at salinity levels of 0.8 and 1.0 M NaCl.

4. Conclusion

Many stress factors affect the growth and LC of *T. lutea*. Regardless of nutritional status, *T. lutea* appears to be sensitive to the combination of salinity and pH of the growth medium. Growth under alkaline conditions increases the LC, as cells accumulate oils as a defense mechanism against stress conditions. However, stress conditions that result in lipid accumulation by algal cells should be avoided, due to simultaneous growth inhibition and reduction in biomass production. Therefore, using organic carbon as an essential additive to the algal growth medium might solve the issue by providing the essential carbon and providing a buffering effect, resulting in stimulation of both growth and lipid accumulation.

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