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Temperature effects on lipid properties of microalgae *Tetraselmis subcordiformis* and *Nannochloropsis oculata* as biofuel resources*

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Abstract Microalgae *Tetraselmis subcordiformis* and *Nannochloropsis oculata* were cultured at 15, 20, 25, 30, and 35°C and their properties as potential biofuel resources were examined. The results indicate that *T. subcordiformis* and *N. oculata* grew best at 20°C and 25°C and yielded the highest total lipids at 20°C and 30°C, respectively. With increased temperature, neutral lipid and polyunsaturated fatty acids (FAs) decreased while saturated FAs increased, accompanied by increased monounsaturated FAs (MUFAs) in *T. subcordiformis* and decreased MUFAs in *N. oculata*; meanwhile, the predicted cetane number of FA methyl esters increased from 45.3 to 47.6 in *T. subcordiformis* and from 52.3 to 60.3 in *N. oculata*. Therefore, optimizing culture temperatures is important for improving microalgal biodiesel production.

Keyword: fatty acids (FAs); lipid class; *Nannochloropsis oculata*; temperature; *Tetraselmis subcordiformis*; total lipid

1 INTRODUCTION

Microalgae are recognized as a promising source of raw materials for biofuel production because of their superiority over higher plants and other organisms by having fast growth, rich lipid content, cost-effective photosynthetic mechanisms, less competition for agricultural land, useful by-products, and being environment friendly (Scott et al., 2010). The potential of many microalgae species or strains, such as *Nannochloropsis oculata* (Converti et al., 2009; Huang et al., 2012, 2013; Wei et al., 2013) and *Tetraselmis subcordiformis* (Ahmad et al., 2011; Xu et al., 2013), has been demonstrated for possible biodiesel production. Biodiesel from microalgae essentially comprises the production of a series of monoalkyl esters of long-chain fatty acids (FAs) and, for now, is obtained principally from acylglycerols. FA composition affects the quantity and quality of synthesized biodiesel (Bruton et al., 2009; Ramos et al., 2009). The longer and more saturated the FA carbon chains, the higher the cetane number (CN) related to the ignition delay time and combustion quality (Ramos et al., 2009). Therefore, FA profiles as well as the growth rate, total lipid (TL) content, and neutral lipid (NL)/TL ratio are common parameters used to evaluate the qualities of microalgae as biodiesel production devices.

Temperature is an important factor that affects an organism's distribution, growth, biomass composition, and metabolism. The adaptability of microalgae to a temperature regime is species dependent. *T. subcordiformis* and *N. oculata* can grow in wide temperature ranges of 7–30°C and 10–35°C, respectively (Cheng et al., 2005). As microalgal

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growth performance and secondary metabolism vary with temperature (Yao et al., 2012; James et al., 2013; Roleda et al., 2013), better understanding of their biochemical response to temperature might help in the design of optimal systems for biofuel production. Converti et al. (2009) have reported that microalgal lipid content is strongly influenced by temperature variation; increasing temperature from 20°C to 25°C almost doubled the lipid content of *N. oculata*, from 7.90% to 14.92%. The present study is different from that described by Converti et al. (2009), because a wider temperature range was chosen and the resulting lipid classes and detailed FA profiles examined. To date, alterations in the properties of *T. subcordiformis* lipids in response to growth temperature have not been reported. Therefore, the growth performance and lipid properties of two microalgal strains of *T. subcordiformis* and *N. oculata* cultured at different temperatures were investigated for their potential for biodiesel production.

2 MATERIAL AND METHOD

2.1 Culture of microalgae

Strains of *T. subcordiformis* and *N. oculata*, obtained from the Culture Collection of Microalgae at Shanghai Ocean University in China, were cultured axenically in 60-L photobioreactors containing f/2 medium (Guillard, 1975). The cultures were grown at 20 salinity and aerated with continuous 0.2 vvm sterile air (Cheng et al., 2005). The separate *T. subcordiformis* and *N. oculata* cultures were incubated at 20±1°C under continuous irradiance (full spectrum fluorescent light, Phillips TWG128) of 100 μE/(m^2·s) and at 25±1°C under 150 μE/(m^2·s), respectively.

For trials at different temperatures, the initial cultures were harvested by centrifugation at 11 590×*g* for 15 min after a 10-d cultivation. Cell pellets were resupended in fresh f/2 medium, transferred to 3-L flasks, and then incubated in illumination incubators under the same light intensity, salinity, and air flow rate as above for each strain. The initial inoculation densities for *T. subcordiformis* and *N. oculata* were 1.0×10^6 and 1.0×10^7 cells/mL, respectively. Considering their growth temperature ranges, *T. subcordiformis* was cultured at 15, 20, 25, and 30°C and *N. oculata* at 20, 25, 30, and 35°C. The cells of each strain were further cultivated for 10 d and then collected for determination of final cell densities and lipid properties.

2.2 Assay of microalgae growth performance

Microalgae cell densities (cells/mL) were measured by hemocytometer. The specific growth rates (μ) were calculated using the following equation: $\mu=(\ln N_t-\ln N_0)/t$, where N_0 is initial cell density and N_t the cell density at time t (d) (Cheng et al., 2005).

2.3 Assays of lipid and FA characteristics

Cells were harvested using centrifugation at 11 590×*g* for 15 min and dried to a powder in a freeze-drier at -46°C and stored for later determination of TL content, lipid classes, and FA profiles (Huang et al., 2012).

In brief, TL of a microalgal powder sample were extracted with chloroform-methanol (2/1, v/v) at 4°C for 24 h and with two 30 min ultrasonic homogenizations (JY92-IID, Ningbo Scientz Biotechnology Co. Inc., Ningbo, China). After filtration of the mixture, the filtrate was transferred into a 50-mL preweighed flask and washed with 0.88% KCl solution. Then, the lower liquid phase was collected and volatilized to dryness at 40°C under vacuum to yield the crude algal lipids. Gravimetric analysis of the total TL content was determined by the equation: Y(% dry wt)=WL/WDA, where WL and WDA are the dry weights of the extracted lipids and algae biomass, respectively.

The dried TL were dissolved in n-hexane and divided into two portions. For lipid class analysis, one portion was fractionated by liquid-phase extraction into NL and polar lipids (PL) using a solvent mixture of 95% methanol-water and petroleum ether (1/1, v/v): mixing the two solvents via oscillation to form the liquid-phase layering. NL was dissolved in petroleum ether, and PL in 95% methanol-water, followed by volatilization of the isolated organic phase to obtain two lipid fractions. The other portion was saponified and esterified to fatty acid methyl esters (FAMEs) using 0.4 mol/L KOH in methanol with gentle swirling and let stand at room temperature for 20 min and the FAMEs extracted into benzene-petroleum ether mixture (1/1, v/v) after the addition of water. For FAME detection, an Agilent HP6890A gas chromatograph (Agilent Technologies, Santa Clara, Ca, USA) equipped with a FID detector and a Omegawax 320 column (30 m×0.32 mm I.D., 0.25 μm; Cat. No. 24152, Sigma-Aldrich Inc., St. Louis, MO, USA) was employed and samples analysed using a temperature program described by Wei et al. (2013). Peak identification was accomplished

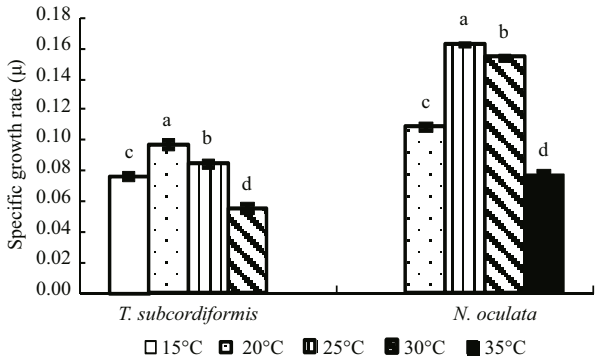

Fig.1 Effects of temperature on specific growth rates of *T. subcordiformis* and *N. oculata*

Different letters indicate significant differences among treatments of the same microalgae (P<0.05).

by comparison of retention times with those of standard mixtures (Sigma-Aldrich Inc.) and the FA proportions (% of total FAs) calculated via a normalization method.

2.4 Statistics

All experiments were performed in triplicate and results expressed as means±standard deviation. Because of the importance of the correlationship between CN and FAME composition, the predicted theoretical CNs were estimated according to the method of Piloto-Rodríguez et al. (2013). Comparisons of mean values were conducted by one-way analysis of variance (ANOVA) obtained from SPSS statistical software, followed by Duncan's new multiple-range test for statistical significance. In all cases, comparisons that represented P<0.05 were considered significant.

3 RESULT AND DISCUSSION

3.1 Effects of temperature on the growth of *T. subcordiformis* and *N. oculata*

The specific growth rates of the two microalgae first increased and then decreased in correlation with increased culture temperatures (P<0.05, Fig.1). The maximal growth rate for *T. subcordiformis* appeared at 20°C, while its growth was significantly inhibited when the temperature exceeded 25°C. In contrast, *N. oculata* cultured at 25°C displayed significantly higher growth rate than those at 20, 30, and 35°C.

Temperature is the most important environmental factor influencing microalgal growth. Temperature change is generally considered to mainly influence the photosynthetic process. For a particular microalgal strain, there is an optimal temperature range in which it grows best. The growth performance of microalgae is impaired when culture temperatures are outside the optimal temperature range. Further, temperature affects nutrient uptake, cell membrane fluidity, and influences the oxygen evolving activity of photosystem II (Vonshak, 2002). In addition, temperature also affects microalgae growth by altering the activities of important enzymes vital for assimilation. For example, it has been demonstrated that temperature influences the concentration of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), as a key enzyme involved in carbon assimilation in photosynthetic organisms. Despite a low correlation between Rubisco activity and temperature, Rubisco activity declines with increasing temperature (Feller et al., 1998; Leggat et al., 2004); Rubisco activity largely determines photosynthetic efficiency (Doubnerová and Ryšlavá, 2011). Jiang (2002) and Converti et al. (2009) have demonstrated that 20°C is the best temperature for *N. oculata* growth. However, Cheng et al. (2005) have reported that *N. oculata* grows best at 25–30°C. In the present study, *N. oculata* displayed maximal specific growth rate at 25°C and *T. subcordiformis* at 20°C, showing growth rates significantly better than at other temperatures (Fig.1). These results imply that different species, even different strains of the same microalgae species, can possess different adaptabilities to temperature conditions.

3.2 Effects of temperature on TL content in *T. subcordiformis* and *N. oculata*

Temperature is an environmental factor that requires the most in-depth study, particularly as it has significant impact on the lipid composition of photosynthetic organisms. The TL content of these two microalgae varied significantly at different culture temperatures (Fig.2). Winth increasing temperature, the TL content of *T. subcordiformis* and *N. oculata* first increased then decreased. *T. subcordiformis* displayed the highest content (22.25%) at 20°C, which was consistent with its best growth temperature. In contrast, the highest content (24.44%) in *N. oculata* appeared at 30°C, which was higher than its optimal growth temperature (25°C).

A number of different descriptions of temperature effects on lipid accumulation in microalgae have been reported. Increased lipid content with increasing temperature has been demonstrated in other microalgae species, such as *Spirulina* spp., *Isochrysis*

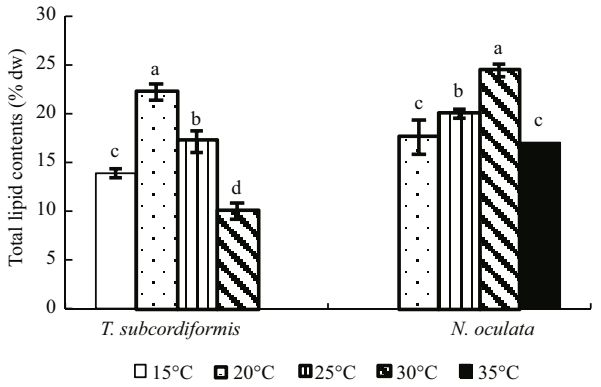

Fig.2 Effects of temperature on TL content (% dry wt) of *T. subcordiformis* and *N. oculata*

Different letters, significant differences among treatments of the same microalgae (P<0.05).

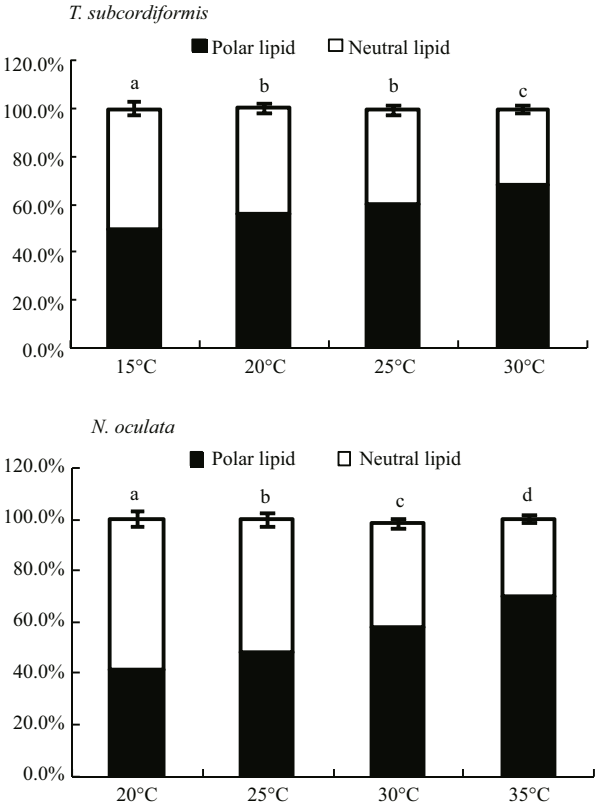

Fig.3 Effects of temperature on lipid classes of *T. subcordiformis* (a) and *N. oculata* (b)

Different letters, significant differences of NL/TL ratio among treatments (P<0.05).

galbana, and *Ettlia oleoabundans* (Sayegh and Montagnes, 2011; Yang et al., 2013). However, two cryptomonads (*Rhodomonas* sp., and the unidentified Prymnesiophyte NT19) and four diatoms (*Chaetoceros* sp., *C. calcitrans*, *C. simplex*, and *Nitzschia* spp.) exhibit decreased lipid content when grown at higher temperatures (Renaud et al., 2002). Converti et al. (2009) have reported that *N. oculata*, at its optimal 20°C for maximal specific growth rate, displays lower lipid content than at 15 and 25°C. Similarly, *Chlorella vulgaris* at 30 and 35°C display lower lipid content than at 25 and 38°C. Li et al. (2011) have also reported that 20°C is the optimal temperature for biomass and lipid content for the freshwater microalga *Scenedesmus* sp. LX1. Compared with the optimal growth temperature of 30–33°C for *Synechocystis* sp. PCC6803, lower temperatures of 18 and 22°C and a higher temperature of 44°C have been found to seriously inhibit the lipid production rate (up to an 80% decrease) and specific growth rate (up to a 66% decrease; Sheng et al., 2011). These seemingly contradictory data imply that temperature effects on microalgal lipid content do not suggest a uniform or single principle, possibly because of the different temperature ranges adopted in different studies. The temperature effects on lipid accumulation of microalgae is also species specific, although the details of this mechanism remain unclear. However, three proposals regarding temperature effects on lipid accumulation have been suggested to explain the different observed lipid changes. Although different microalgae species respond differently to environmental stress conditions, there apppears to be a negative correlation between microalgal lipid content and growth rate. Roleda et al. (2013) have suggested that the default pathway for micoalgae under stressful conditions is to synthesize and deposit triacylglycerols (TAG) into cytosolic lipid bodies and divert internal resources that would have gone into growth. Sayegh and Montagnes (2011) have suggested that the increased lipid accumulation in microalgae at high temperatures is due to the important role of TL or the greater use of lipids as a storage product. In contrast, Sheng et al. (2011) have speculated that the lower specific growth rate and lipid content of microalgae beyond the optimal growth temperature results from stress on photosystem II activity. In the present study, it is speculated that there are different adaptation strategies among microalgae for altering lipid properties in response to temperature changes. More detail and relevant evidence should be seen in the future following more extensive morphological and biochemical measurements.

When grown at different temperatures, the lipid classes of these microalgae also varied (Fig.3a, b). At the lowest experimental temperature, both microalgae showed the maximal NL/TL, at 49.56% and 40.93% for *T. subcordiformis* and *N. oculata*, respectively.

Table 1 Effect of temperature on the FA profile (%) of *T. subcordiformis*

Fatty acids	Treatment temperature (°C)			
	15	20	25	30
C16:0	15.43±1.01^{b}	14.93±0.04^{b}	18.49±0.63^{a}	18.27±0.31^{a}
C20:0	9.04±0.35	9.62±0.76	9.49±0.31	10.09±0.49
∑SFAs	25.30±0.65^{b}	25.45±0.62^{b}	29.14±0.95^{a}	29.45±0.27^{a}
C14:1	2.52±0.87	2.67±1.70	4.57±1.84	3.32±0.16
C18:1	9.19±0.19^{b}	9.68±0.33^{b}	14.30±0.96^{a}	16.02±1.13^{a}
C20:1	1.62±0.12^{a}	1.52±0.01^{a}	1.18±0.13^{b}	1.29±0.03^{b}
∑MUFAs	14.23±0.55^{b}	14.77±1.73^{b}	21.48±0.63^{a}	22.40±0.57^{a}
C16:2	2.12±0.68	2.84±0.73	3.33±0.44	2.99±0.14
C16:3	13.09±0.80^{a}	13.06±0.25^{a}	11.02±0.65^{b}	8.41±0.37^{c}
C18:2n6	3.81±0.53^{c}	3.58±0.30^{c}	5.49±0.24^{b}	8.53±0.30^{a}
C18:3n3	23.65±1.28^{a}	23.61±1.29^{a}	17.74±1.34^{b}	15.99±0.59^{b}
C18:4n3	5.39±0.06^{a}	5.44±0.21^{a}	2.99±0.15^{b}	2.32±0.30^{c}
C20:4n6	1.59±0.21^{b}	1.79±0.16^{ab}	1.54±0.18^{b}	2.17±0.11^{a}
C20:5n3	6.49±0.46^{a}	6.29±0.11^{a}	3.74±0.44^{b}	3.45±0.60^{b}
∑PUFAs	58.71±1.13^{a}	59.21±1.24^{a}	47.48±0.81^{b}	45.63±0.27^{b}
∑16C	31.55±2.63	31.74±1.20	34.28±2.51	31.43±0.88
∑18C	42.84±2.26	43.20±2.44	41.36±3.05	44.17±1.41
∑20C	20.49±1.20^{a}	20.92±1.15^{a}	16.74±1.15^{b}	17.47±0.20^{b}

Data expressed as mean±standard deviation of three independent measurements; values within same row marked with different letters, significantly different (P<0.05), and other FAs, such as C13:0, C14:0, C16:1, C18:3n6, and C20:4n3, with FAs with content <1% of total FAs not listed.

The algae lipid composition included NL, comprising TAG and wax esters, and PL, which included phospholipids, glycolipids, and betaine lipids. TAG were the dominant NL component and also the principle energy storage form in these cells, while glycolipids were the major component of the PL (Huang et al., 2012). PL are important structural components of cell membranes and perform specific membrane functions. The NL/TL ratio in both microalgae were found to decrease significantly with increasing culture temperature (P<0.05), which was similar observations by Zhu et al. (1997). In their study, culturing *Isochrysis galbana* at 30°C showed a slight decrease, relative to growth at 15°C, in the NL proportion along with a higher TL accumulation. However, other studies have described different changes. A temperature lower than that for best growth in the diatom *Nitzschia laevis* favored PL formation and specifically showed increased phosphatidylglycerol, the major phospholipid in *N. laevis* extraplastidic membranes (Chen et al., 2008). Yao et al. (2012) also have reported that one type of *Chlamydomonas reinhardtii* mutant showed a shift in NL content instead of polar membrane lipids after a temperature increase from 22 to 34°C (the latter being a restrictive temperature).

3.3 Effects of temperature on FA profiles of *T. subcordiformis* and *N. oculata*

The main FAs in *T. subcordiformis* were C16:0, C16:3n3, C18:3n3, and C20:0 (14.93%–18.49%, 6.77%–12.30%, 15.99%–23.65%, and 9.04%–10.09%, respectively), with the sum of the 16 and 18-carbon FAs accounting for ~80% of the total FAs (Table 1). Culture temperature affected the FA composition of *T. subcordiformis* significantly (P<0.05). With increasing temperature, the proportions of C16:0, C18:1, saturated FAs (SFAs), and monounsaturated FAs (MUFAs) increased significantly, while the proportions of C16:3n3, C18:3n3, C20:5n3, and polyunsaturated FAs (PUFAs) decreased significantly (P<0.05).

In *N. oculata*, C14:0, C16:1, and C20:5n3 were the preponderant FAs (6.05%–11.08%, 20.46%–24.81%, and 3.84%–15.24%, respectively), with the sum of the 16 and 18-carbon FAs accounting for 70.89%–77.70% of the total FAs. With increased culture temperature, the content of C14:0, C16:0, SFAs, and 16-carbon FAs increased significantly and the content of C14:1, C16:1, C20:0, C20:5n3, 20-carbon FAs, MUFAs, and PUFAs decreased significantly (P<0.05, Table 2).

In sharp contrast to the observed temperature effects on lipid content, temperature effects on the FA profiles of microalgae appear to be highly similar among microalgae and have been well elucidated. The physical properties of membrane bilayers are influenced by lipid compositional changes that help maintain normal metabolic functions under different temperature regimes such as photosynthesis, ion permeability, and respiratory processes. Many studies have demonstrated that algae generally increase their relative amount of unsaturated FAs when subjected to lower environmental temperatures (Jiang, 2002; Sheng et al., 2011). Rousch et al. (2003) have demonstrated that *Chaetoceros muelleri* exhibits a clear increase in MUFAs and reduction in PUFAs when the culture temperature increases from 24 to 40°C. Similarly, Li et al. (2011) have illustrated that the PUFA content in *Scenedesmus* sp. LX1 decreases

Table 2 Effects of temperature on the FA profile (%) of *N. oculata*

Fatty acids	Treatment temperature (°C)			
	20	25	30	35
C14:0	7.10±0.12^{c}	8.99±0.67^{b}	6.05±0.70^{c}	11.08±0.04^{a}
C16:0	13.64±0.08^{d}	26.53±1.90^{c}	33.89±1.15^{b}	41.96±1.23^{a}
C20:0	9.39±0.27^{a}	6.69±0.66^{b}	4.81±0.24^{c}	2.66±0.44^{c}
∑SFAs	32.88±2.24^{c}	44.34±3.55^{b}	47.33±2.60^{b}	57.04±1.91^{a}
C14:1	9.35±0.49^{a}	8.75±1.76^{ab}	7.52±0.46^{ab}	6.30±0.14^{b}
C16:1	23.72±0.24^{a}	22.64±1.81^{ab}	24.81±0.58^{a}	20.46±1.00^{b}
C18:1	4.96±0.40^{a}	2.79±0.35^{b}	3.41±0.72^{b}	5.64±1.09^{a}
∑MUFAs	38.79±1.04^{a}	34.48±4.06^{ab}	36.01±1.82^{ab}	32.58±2.33^{b}
C16:2	2.15±1.26^{ab}	3.05±0.39^{a}	2.56±0.04^{b}	1.99±0.03^{c}
C18:2n6	4.34±0.05^{a}	2.22±0.31^{b}	2.02±0.21^{b}	1.36±0.07^{a}
C20:4n6	5.47±0.08^{a}	4.49±0.58^{b}	2.93±0.07^{c}	2.62±0.20^{d}
C20:5n3	15.24±0.17^{a}	10.56±1.06^{b}	8.29±0.75^{c}	3.84±0.51^{d}
∑PUFAs	27.57±1.88^{a}	20.75±2.46^{b}	16.21±1.14^{c}	9.92±0.85^{d}
∑16C	40.79±1.52^{c}	52.66±4.23^{b}	61.67±1.86^{a}	64.52±2.36^{a}
∑18C	9.83±0.46^{a}	5.92±0.89^{b}	6.57±1.26^{b}	7.47±1.27^{b}
∑20C	30.10±0.51^{a}	21.74±2.3^{b}	16.03±1.06^{c}	9.12±1.16^{d}

Data expressed as means±standard deviation of three independent measurements; values within same row marked with different letters, significantly different (P<0.05), and other FAs, such as C12:0, C15:0, C16:3, C17:0, C17:1, and C18:0, with FAs with content <1% of total FAs not listed.

Table 3 Theoretical CN of the FAMEs from *T. subcordiformis* and *N. oculata* cultured at different temperatures

Microalgae	Treatment temperature (°C)				
	15	20	25	30	35
T. subcordiformis	45.3	45.2	47.6	47.6	/
N. oculata	/	52.3	56.0	57.5	60.3

as the cultivation temperature increases. The present results generally concur with the findings of previous studies. The reduction of environmental temperature resulted in an increase in the total FA degree of unsaturation. The SFAs proportion increased and PUFAs decreased with increasing culture temperature, accompanied by increased MUFAs in *T. subcordiformis* (Table 1) and decreased MUFAs in *N. oculata* (Table 2). This interesting phenomenon has been widely observed in many other organisms because lower temperatures can stimulate cells to synthesize unsaturated FAs to maintain membrane fluidity and functions (Jiang and Chen, 2000). Decreased temperature generally causes increased unsaturated FA content, which increases cell membrane fluidity as a protection against low temperatures (Los and Murata, 2004). Conversely, increased SFA contents with higher culture temperatures are regarded as a method for preserving algae cell membrane integrity (Jiang and Chen, 2000; Chen et al., 2008).

In addition, growth temperatures above optimal have resulted in increased C18:1 (oleic acid) content in *Chlorella vulgaris* (Converti et al., 2009). In the present study, oleic acid in *T. Subcordiformis* was observed to increase significantly at above optimal temperatures (Table 1). In *N. oculata*, such oleic acid variation with increased temperature was small because of its low proportion in the FAs (Table 2).

FA composition influences both the quantity and quality of biodiesel. Excess unsaturated FAs can result in the formation of tar, produced by FA chain crosslinking, and low CNs. Further, the oxidation stability of biodiesel decreases as polyunsaturated methyl esters increases (Ramos et al., 2009). The CN has been regarded as an essential part of the biodiesel quality specifications, with a minimum CN requirement of 51 in some European countries (e.g., European Standard EN 14214:2003) and a minimum of 47 prescribed for neat biodiesel or B100, according to the American Society for Testing and Materials D6751-07a standards (Wadumesthrige et al., 2008). The predicted theoretical CN from the present results are shown in Table 3. CN values of FAMEs from *T. subcordiformis* appeared to increase from 45.3 to 47.6 when the culture temperature increased from 15 to 30°C. In *N. oculata*, FAME CN values increased from 52.3 to 60.3 when the culture temperature increased from 20 to 35°C. Thus, *N. oculata* appeared more suitable for biodiesel production in terms of CN values, with the chemical structure of its FAMEs contributing significantly to the CN. Fewer double bonds, via inceased saturated FAMEs, or longer FA chains both lead to increased CN values. Using artificial neural networks and multiple linear regressions, according to Piloto-Rodríguez et al. (2013), the CN values of FAMEs from both microalgae improved with increased culture temperature.

4 CONCLUSION

Temperature significantly influenced the growth and oil-yielding properties of these two microalgae, *T. subcordiformis* and *N. oculata*, and its effects were species specific. The temperatures for optimal growth and high lipid content were the same in *T.*

subcordiformis. But in *N. oculata*, the temperature for high lipid content was higher than that for optimal growth. With increasing culture temperature, SFAs increased while PUFAs decreased in both species, accompanied by increased MUFAs in *T. subcordiformis* and decreased MUFAs in *N. oculata*. These changes resulted in an improvement of the predicted theoretical CN values of FAMEs from both microalgae. Thus, optimization of culture temperature was concluded here to be a key condition for improving the prospects of efficient microalgal biodiesel production.

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