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The effect of temperature on growth and lipid and fatty acid composition on marine microalgae used for biodiesel production

Panjaphol Chaisutyakorn[1,2] · **Jantana Praiboon**[1,2] · **Chatcharee Kaewsuralikhit**[1,2]

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Abstract Cultivation temperature is one of the major factors affecting the growth and lipid accumulation of microalgae. In this study, the effects of temperature on the growth, lipid content, fatty acid composition and biodiesel properties of the marine microalgae *Chaetoceros* sp. FIKU035, *Tetraselmis suecica* FIKU032 and *Nannochloropsis* sp. FIKU036 were investigated. These species were cultured at different temperatures (25, 30, 35 and 40 °C). The results showed that the specific growth rate, biomass and lipid content of all microalgae decreased with increasing temperature. With regards to fatty acids, the presence of saturated fatty acids (SFAs) in *T. suecica* FIKU032 and *Nannochloropsis* sp. FIKU036 decreased with increasing temperature, in contrast with polyunsaturated fatty acids (PUFAs). Moreover, *Chaetoceros* sp. FIKU035 was the only species that could grow at 40 °C. The highest lipid productivity was observed in *Chaetoceros* sp. FIKU035 when cultivated at 25 °C (66.73 ± 1.34 mg L^{-1} day^{-1}) and 30 °C (61.35 ± 2.89 mg L^{-1} day^{-1}). Moreover, the biodiesel properties (cetane number, cold filter plugging point, kinematic viscosity and density) of the lipids obtained from this species were in accordance with biodiesel standards. This study indicated that *Chaetoceros* sp. FIKU035 can be considered as a suitable species for biodiesel production in outdoor cultivation.

✉ Jantana Praiboon
ffisjtn@ku.ac.th

[1] Department of Fishery Biology, Faculty of Fisheries, Kasetsart University, Bangkok 10900, Thailand

[2] Center for Advanced Studies for Agriculture and Food, Kasetsart University Institute for Advanced Studies, Kasetsart University, Bangkok 10900, Thailand

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Introduction

Currently, the majority of biodiesel production comes from oil extracted from oleaginous seed plants, such as rapeseed, soybean, sunflower and palm. However, a potentially better alternative to these crops is photosynthetic microalgae, which could provide at least 10 to 20 times higher oil yields per unit land area and have the added benefit of little competition for agricultural land (Chisti 2008). Moreover, microalgae can potentially use part of carbon dioxide produced by combustion of fossil fuels; from this point of view, the microalgae can also be seen as simple CO_2 sequestrants to use for greenhouse gas emission control, thereby reducing greenhouse gas emissions (Converti et al. 2009). The demand for biofuel is extremely strong and currently rising; therefore, the production of algae for biofuel and/or CO_2 capture requires extremely large-scale culturing (Borowitzka and Moheimani 2013). With this in mind, the outdoor cultivation systems seem to be the most suitable and commercialized, as they would require lower initial capital costs (Brennan and Owende 2010; Lee 2011; Acién et al. 2012; Slade and Bauen 2013). However, large-scale outdoor culture systems are subject to wide fluctuations in diurnal and seasonal temperature (Borowitzka 2016). Thus, the selection of microalgae strains adapted to a wide range of temperatures would be beneficial for microalgae cultivation in outdoor systems at different time of the year.

Temperature influences all metabolic processes, and the optimum temperature for a particular algae strain will have a pronounced effect on the achievable productivity of a culture (Borowitzka 2016). The different culture environmental conditions, such as seasonal fluctuation, result in low and high

temperatures and can cause variable growth rates and lipid accumulation of microalgae (Oliveira et al. 1999; Renaud et al. 2002; Sheng et al. 2011; Markou et al. 2012; Wei et al. 2015; Ippoliti et al. 2016). The photosynthesis rate, respiration and growth of microalgae all decline when optimal temperatures are exceeded due to imbalances between adenosine triphosphate (ATP) production and energy demand, inactivation or denaturation of necessary proteins for photosynthesis (Raven and Geider 1988; Ras et al. 2013) or stress on photosystem II activity (Sheng et al. 2011). Temperature also has the effect of changing fatty acid composition. It has been reported that total unsaturated fatty acids decreased with high temperature (Montensen et al. 1988; Renaud et al. 1995; Jiang and Chen 2000; Wei et al. 2015). In microalgae, unsaturated fatty acids are necessary for the maintenance of membrane fluidity, and the degree of membrane fluidity depends on the length of fatty acid chains (Hochachka and Somero 1984; Harwood 1988; Sargent et al. 1989). Moreover, the properties of biodiesel are determined by the fatty acid profile of the microalgae. Thus, fatty acid composition of algal lipids is very important for biodiesel production. Saturated fatty acids provide a more favourable cetane number, oxidative stability, heat of combustion and viscosity (Ramos et al. 2009). A higher content of saturated fatty acids improves oxidative stability and combustion while worsening cold flow, while a higher content of unsaturated fatty acid, especially polyunsaturated fatty acid, improves cold flow but leads to poorer oxidative stability and combustion (Knothe 2013). An optimal proportion of saturated to unsaturated fatty acids in the fatty acid methyl ester (FAME) yields the best quality of biodiesel (Islam et al. 2013).

Therefore, the objectives of this study were to investigate effects of temperature (25, 30, 35 and 40 °C) on the specific growth rate, biomass and lipid productivity of marine microalgae. Their fatty acid profiles were analysed and biodiesel properties were estimated based on their fatty acid methyl esters.

Materials and methods

Strains and culture conditions

Chaetoceros sp. FIKU035, *Tetraselmis suecica* FIKU032 and *Nannochloropsis* sp. FIKU036 were obtained from the Chantaburi Coastal Fisheries Research and Development Centre, Chantaburi Province, Thailand. All species were cultured in 1 L of sterilized F/2 Medium (Guillard and Ryther 1962) with a salinity of 27 ± 1 ppt. Cultures were maintained at room temperature (25 ± 1 °C) under 50 μmol photons m^{-2} s^{-1} light intensity on a 12:12-h light/dark cycle and were aerated continuously.

The analyses of different growth temperatures were carried out by cultivation of 10^4 cells mL^{-1} of *Chaetoceros* sp. FIKU035 and *Tetraselmis* sp. FIKU032 and 10^6 cells $mL^{-1}$of *Nannochloropsis* sp. FIKU036, in 250-mL cylinder tubes (OD 40 mm × 350 mm) containing 200 mL of sterilized F/2 medium with salinity of 27 ± 1 ppt. Experiments were performed at a constant temperature of 25, 30, 35 or 40 °C under 100 μmol photons m^{-2} s^{-1} light intensity with 12:12-h L/D cycle in a growth chamber (LGS-5201, Labtech, Korea). The growth rate of each species was characterized based on cell counts using a haemocytometer or via optical density measurements at 680 nm (UV-1700 Pharma Spec, Shimadzu, Japan). All experiments were carried out in triplicate.

The specific growth rate (μ) was calculated from the slope of the linear regression of time (days) and cell density (cell mL^{-1}) according to eq. (1) (Wood et al. 2005):

$$\mu = (\ln_{Nt} - \ln_{N0}) \Big/ \Delta t \quad (1)$$

where μ (day^{-1}) is the specific growth rate in log phase, N_0 is the cell density at the beginning of log phase and N_t is the cell density at late log phase.

Biomass and biomass productivity

Microalgae at late log phase were harvested by centrifugation at 1520×*g* for 10 min. The pellet was dried at 60 °C for dry weight measurement. The biomass productivity was calculated according to eq. (2) (Song et al. 2013). In addition, algal biomass was stored at −20 °C until chemical analysis.

$$\text{Biomass productivity } \left(\text{mg L}^{-1}\ \text{day}^{-1}\right) = \mu \times \text{biomass} \quad (2)$$

Total lipid and fatty acid analysis

Lipids of marine microalgae were extracted following Bligh and Dyer (1959). Briefly, dried microalgae were extracted with chloroform/methanol/water (1:2:0.8) for 3 h and then filtered through Whatman No. 2 filter paper and rinsed with the same solvent mixture. This process was repeated a second time. The filtrates were pooled in a separation funnel, and then, chloroform and water were added to produce a 1:1:0.9 solvent ratio. The separation funnel was shaken and left to stand overnight, and then, the lower chloroform lipid layer was collected. The solvent was removed under a vacuum at 40 ± 1 °C using a rotary evaporator. Total lipid in the form of a viscous green residue was weighed, redissolved in methanol and stored at −20 °C until further analysis. Total lipids and lipid productivity were calculated according to eqs. (3–4) (Song et al. 2013).

$$\text{Total lipid content} = \left(\text{total lipids} \Big/ \text{biomass}\right) \times 100 \quad (3)$$

$$\text{Lipid productivity} = \text{lipid content} \times \text{biomass productivity} \quad (4)$$

Transesterification and fatty acid analysis

FAMEs of algal lipids were prepared following the method of Prevot and Mordret (1976). Briefly, an aliquot of lipid (~9–12 mg) was added to 2 mL of 0.5 M CH_3ONa and was incubated at 55 °C for 30 min. Then, 2 mL of distilled water and n-hexane were added and gently mixed to recover the upper n-hexane layer containing FAMEs, which was neutralized by washing with distilled water many times and was finally purified by silica gel column chromatography using a 5% mixture of diethyl ether in hexane. The resulting FAMEs were analysed by GC-FID (7890 A, Agilent Technologies, USA) equipped with a DB-Wax column (127-2012, Agilent Technologies, USA). The oven temperature was set at and held at 170 °C for 2 min, then was raised 5 °C min^{-1} to 240 °C where it was held for 14 min. The injector and detector temperatures were set at 250 °C. FAME components were identified by comparisons with retention times of FAME standards (37 FAME standards C4-C24, Supelco, USA).

Estimation of biodiesel properties

The essential biodiesel parameters were estimated with a mathematical model based on FAME molecular structure, carbon chain size and the number and/or position of double bonds, as follows:

The cetane number (CN) of fatty acid methyl esters was calculated using eq. (5) (Krisnangkura 1986):

$$\text{CN} = 46.3 + \left(5458 \Big/ \text{SV}\right) - (0.225 \times \text{IV}) \quad (5)$$

The saponification value (SV) in mg KOH g^{-1} and the iodine value (IV) in g I_2 $(100\ g)^{-1}$ of fatty acid were estimated by eqs. (6–7) (Kalayasiri et al. 1996):

$$\text{SV} = \sum_i \left((560 \times n_i) \Big/ \text{MW}\right) \quad (6)$$

$$\text{IV} = \sum_i \left((254 \times d_i \times n_i) \Big/ \text{MW}\right) \quad (7)$$

where N_i is the percentage of each FAME and D_i is the number of double bonds of each FAME.

The degree of unsaturation (DU) was calculated based on the total of MUFA and PUFA using eq. (8) (Ramos et al. 2009):

$$\text{DU} = \sum \text{MUFA} + (2 \times \text{PUFA}) \quad (8)$$

The long chain saturation factor (LCSF) and the cold filter plugging point (CFPP) were calculated according to eqs. (9–10) (Ramos et al. 2009):

$$\text{LCSF} = (0.1 \times \text{C16} : 0) + (0.5 \times \text{C18} : 0) + (1 \times \text{C20} : 0) + (2 \times \text{C24} : 0) \quad (9)$$

$$\text{CFPP} = (3.1417 \times \text{LCSF}) - 16.477 \quad (10)$$

The kinematic viscosity (υ, $mm^2\ s^{-1}$) at 40 °C, density (ρ, $g\ cm^{-3}$) at 20 °C and higher heating value (HHV) of biodiesel were calculated by eqs. (11, 12, 13) (Ramírez-Verduzco et al. 2012):

$$\ln(\upsilon) = \sum_{ni} \left(-12.503 + \left(2.496 \Big/ \ln(M_{wi})\right) - (0.178 \times D_i)\right) \quad (11)$$

$$\rho = \sum_{ni} \left(0.8463 + \left(4.9 \Big/ M_{wi}\right) + (0.0118 \times D_i)\right) \quad (12)$$

$$\text{HHV} = \sum_{ni} \left(46.19 - \left(1794 \Big/ M_{wi}\right) - (0.21 \times D_i)\right) \quad (13)$$

where Mw_i is the molecular weight of a fatty acid, N_i is its percentage of the fatty acid composition and D_i is the number of double bonds in the fatty acid.

Statistical analysis

The results were expressed as the mean values ± standard deviation. Comparisons of the means were conducted by one-way analysis of variance (ANOVA), followed by a Duncan's multiple range test to determine significance. In all cases, comparisons that showed a p value <0.05 were considered significant.

Results

Effect of temperature on growth and biomass

The growth rate of microalgae is highly variable under different temperature, and the results of our studies of this phenomenon are given in Table 1. In *Chaetoceros* sp. FIKU035, the highest specific growth rate was observed in culture at 25 °C (0.537 ± 0.027 day^{-1}). However, there were no significant change ($p > 0.05$) in culture growth rates at 25, 30 and 35 °C. The growth rate of *Chaetoceros* sp. FIKU035 was decreased at 40 °C. This result shows that this species is adaptable to growth at a wide temperature range (25–40 °C). Growth temperatures above 30 °C were lethal for *T. suecica* FIKU032, with the highest specific growth rate of 0.378 ± 0.012 day^{-1} observed at 25 °C. In addition, *Nannochloropsis* sp. FIKU036 grew at 25° and up to 35 °C, the specific growth rate of this microalga decreased with

Table 1 Effect of temperature on specific growth rate (day^{-1})

Strains	Temperature (°C)			
	25	30	35	40
Chaetoceros sp. FIKU035	0.537 ± 0.027	0.500 ± 0.007	0.484 ± 0.017	0.425 ± 0.037
T. suecica FIKU032	0.353 ± 0.018	0.378 ± 0.013	–	–
Nannochloropsis sp. FIKU036	0.331 ± 0.006	0.298 ± 0.002	0.289 ± 0.003	–

"–" cannot grow

increasing temperature and the optimal temperature was 25 °C with specific growth rate of 0.331 ± 0.006 day^{-1} at this temperature. Among the three species investigated, *Chaetoceros* sp. FIKU035 was the most eurythermal species, exhibiting a significantly higher specific growth rate than the other two species.

Biomass and biomass productivity are shown in Fig. 1. The highest biomass and biomass productivity of *Chaetoceros* sp. FIKU035 (777.93 ± 58.33 mg L^{-1}, 388.97 ± 49.13 mg L^{-1} day^{-1}) and *T. suecica* FIKU032 (978.43 ± 25.58 mg L^{-1}, 369.84 ± 9.67 mg L^{-1} day^{-1}) was found at 30 °C. The highest biomass and biomass productivity of *Nannochloropsis* sp. FIKU036 (885.35 ± 64.78 mg L^{-1}, 293.05 ± 21.44 mg L^{-1} day^{-1}) were found at 25 °C and decreased with increasing temperature. Among the three species, the highest biomass was observed for *T. suecica* FIKU032, while the highest biomass productivity was observed for *Chaetoceros* sp. FIKU035.

Effect of temperature on lipid content and fatty acid composition

Total lipid content and productivity at different temperatures are shown in Fig. 2. At its optimum growth temperature of 25 °C, the lipid content of *Chaetoceros* sp. FIKU035 was 20.42 ± 0.41%. However, the lipid content of this microalga decreased about two-fold at 35 °C (10.82 ± 0.68%) and two and a half fold at 40 °C (8.03 ± 0.01%). Similarly, the lipid productivity of *Chaetoceros* sp. FIKU035 decreased about two-fold from 25 to 35 °C (66.73 ± 1.34 to 29.98 ± 1.82 mg L^{-1} day^{-1}) and by four-fold at 40 °C (15.92 ± 1.09 mg L^{-1} day^{-1}). Moreover, *Chaetoceros* sp. FIKU035 had significantly higher lipid content and lipid productivity than the other assayed species.

The effect of temperature on fatty acid composition is shown in Fig. 3. The content of saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) for *T. suecica* FIKU032 and *Nannochloropsis* sp. FIKU036 decreased with increasing temperature. Conversely, polyunsaturated fatty acids (PUFAs) of these two species increased with increasing temperature. The content of SFAs and PUFAs in *Chaetoceros* sp. FIKU035 did not change significantly with temperature. Moreover, the MUFA content of *Chaetoceros* sp. FIKU035 seemed to decrease with increasing temperature.

Table 2 shows the change of the fatty acid profile of microalgae under different growth temperatures. The major fatty acids of *Chaetoceros* sp. FIKU035 were C14:0, C16:0 and C16:1, while *T. suecica* FIKU032 and *Nannochloropsis* sp. FIKU036 mainly contained C16:0 and C18:1n9. The

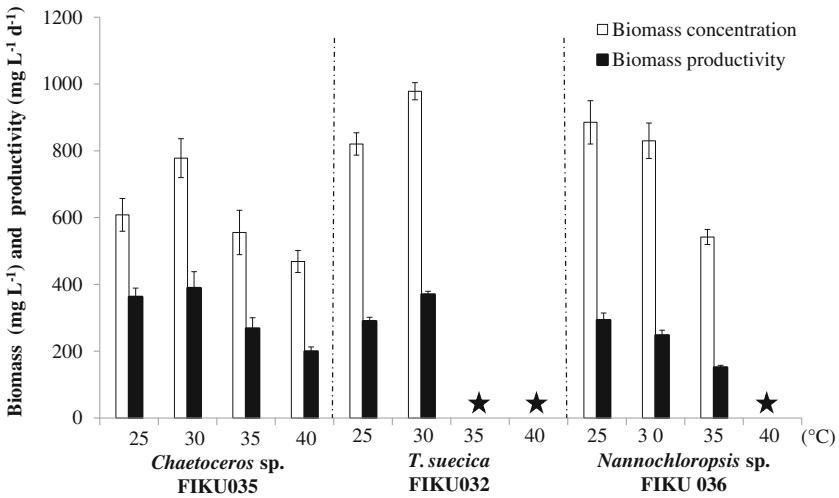

Fig. 1 Effect of temperature on biomass and biomass productivity; *white star* cannot grow; *error bars* indicate standard deviation from the mean ($n = 3$)

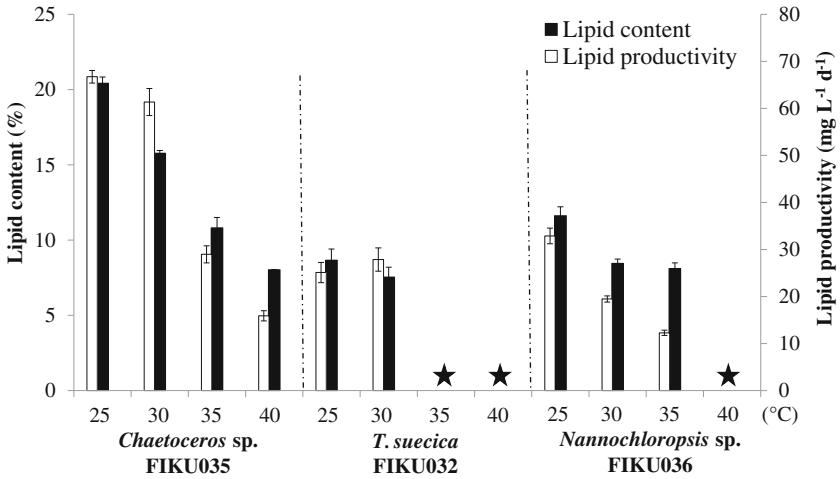

Fig. 2 Effect of temperature on lipid content and lipid productivity; *white star* cannot grow; *error bars* indicate standard deviation from the mean ($n = 3$)

contents of SFAs and MUFAs were highest in *Chaetoceros* sp. FIKU035 cultured at 25–30 °C, which represented approximately 80% of total FAMEs. Moreover, the content of C16:0, C18:0 and C18:1n9 decreased with increasing temperature for all species. *Chaetoceros* sp. FIKU035 showed higher C16:1 and lower C18:2n6 and C18:3n3 contents than the other two species assayed. The content of C18:3n3 in *Nannochloropsis* sp. FIKU036 increased with increasing temperature.

Effects of temperature on biodiesel properties

The essential biodiesel properties of microalgae cultured at different temperatures are shown in Table 3. The results showed that FAMEs of all species had biodiesel properties, including iodine value, cetane number, kinematic viscosity and density in accordance with European (EN14214) and American (ASTM D6751) standards. However, the cold plugging point of *Chaetoceros* sp. FIKU035 at 40 °C and of *Nannochloropsis* sp. FIKU036 at 30 and 35 °C were not in accordance with the European standard.

Discussion

Many researchers have reported on the importance of temperature on microalgae cultivation for biodiesel production (Wigmosta et al. 2011; Ras et al. 2013; Slade and Bauen 2013). Cultivation temperature is a very important factor, especially in a tropical country such as Thailand. In Thailand, the average temperature is approximately 30 °C, while the highest temperature is nearly 40 °C in summer (Meteorological Department 2015). In selecting a suitable microalgae strain for biodiesel production, factors such as lipid content, fatty acid composition and biomass production of the particular strain under different growth temperatures must be considered. Using strains adapted to wide range of temperature would be beneficial for microalgae cultivation at different time of the year.

In the present study, *Chaetoceros* sp. FIKU035 was found to be able to grow in a wide range of temperatures, from 25 to 40 °C (Eurythermal species) similar to findings of a previous study by Ras et al. (2013). Growth of *T. suecica* FIKU032 and *Nannochloropsis* sp. FIKU036 did not occur above 35

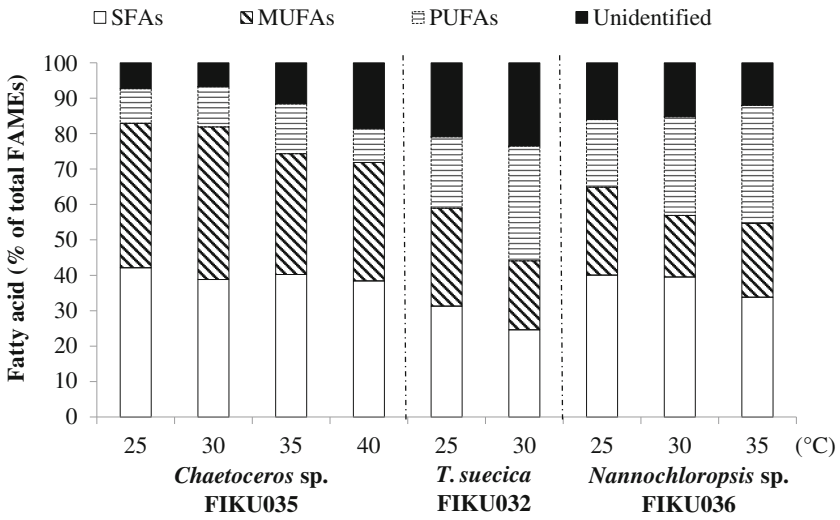

Fig. 3 Effect of temperature on fatty acid composition; *SFAs* saturated fatty acids, *MUFAs* monounsaturated fatty acids, *PUFAs* polyunsaturated fatty acids

Table 2 Effect of temperature on fatty acid profile ($n = 3$)

Fatty acid (%)	Temperature (°C)								
	Chaetoceros sp. FIKU035				*T. suecica* FIKU032		*Nannochloropsis* sp. FIKU036		
	25	30	35	40	25	30	25	30	35
Saturated fatty acid, SFA									
C14:0	10.77 ± 0.25	16.84 ± 0.84	20.26 ± 1.21	16.37 ± 0.83	0.91 ± 0.02	0.75 ± 0.00	0.79 ± 0.03	0.66 ± 0.11	0.63 ± 0.10
C15:0	0.61 ± 0.04	0.56 ± 0.03	0.65 ± 0.05	–	0.19 ± 0.00	–	0.18 ± 0.04	0.25 ± 0.00	0.29 ± 0.07
C16:0	24.27 ± 3.97	15.98 ± 1.45	11.16 ± 1.39	11.14 ± 1.72	25.92 ± 0.19	20.09 ± 0.42	32.10 ± 2.24	24.32 ± 3.45	25.20 ± 2.04
C17:0	2.22 ± 0.47	2.92 ± 0.19	4.27 ± 0.27	1.67 ± 0.31	0.73 ± 0.01	2.53 ± 0.12	0.16 ± 0.10	0.33 ± 0.01	0.16 ± 0.01
C18:0	3.55 ± 0.87	1.99 ± 0.53	1.55 ± 0.32	1.18 ± 0.10	3.57 ± 0.07	1.29 ± 0.01	6.62 ± 1.28	1.75 ± 0.95	2.59 ± 1.84
C20:0	0.72 ± 0.46	0.13 ± 0.10	2.15 ± 1.44	8.06 ± 4.20	–	–	0.23 ± 0.07	12.23 ± 4.46	4.59 ± 0.10
C22:0	–	–	0.23 ± 0.01	–	–	–	–	–	–
C24:0	–	0.43 ± 0.01	–	–	–	–	–	–	–
Monounsaturated fatty acid, MUFA									
C14:1	0.18 ± 0.08	0.14 ± 0.01	0.29 ± 0.06	–	0.13 ± 0.01	0.23 ± 0.00	0.18 ± 0.14	–	0.18 ± 0.01
C15:1	–	0.09 ± 0.01	–	–	–	–	–	–	–
C16:1	28.07 ± 0.76	32.84 ± 1.55	22.47 ± 1.41	23.94 ± 0.68	2.71 ± 0.08	0.57 ± 0.06	1.06 ± 0.13	1.10 ± 0.05	1.21 ± 0.24
C17:1	3.43 ± 1.07	2.98 ± 0.25	5.74 ± 0.44	3.89 ± 0.21	2.01 ± 0.05	1.54 ± 0.07	2.86 ± 1.03	4.60 ± 0.70	5.61 ± 1.47
C18:1n9	9.10 ± 0.24	6.80 ± 1.42	5.57 ± 1.52	5.61 ± 1.71	21.77 ± 0.42	16.19 ± 0.19	20.68 ± 3.80	11.70 ± 6.10	13.84 ± 1.20
C20:1n9	–	–	–	–	1.01 ± 0.03	0.92 ± 0.02	–	–	–
C22:1n9	–	0.22 ± 0.01	–	–	–	–	–	–	–
Polyunsaturated fatty acid, PUFA									
C18:2n6	1.87 ± 0.16	1.34 ± 0.19	1.65 ± 0.42	1.82 ± 0.44	3.67 ± 0.13	11.19 ± 0.29	9.65 ± 1.48	13.52 ± 0.60	16.66 ± 1.69
C18:3n3	–	0.39 ± 0.03	0.48 ± 0.14	–	10.66 ± 0.12	14.43 ± 0.47	7.44 ± 2.27	12.93 ± 1.93	16.69 ± 4.40
C20:2	–	–	0.81 ± 0.01	–	–	–	–	–	–
C20:3n6	0.28 ± 0.06	0.32 ± 0.02	0.30 ± 0.13	–	–	–	–	–	–
C20:3n3	1.65 ± 0.47	3.69 ± 0.25	4.15 ± 0.33	1.91 ± 0.12	0.57 ± 0.01	1.25 ± 0.03	–	–	–
C20:4n6	–	–	–	–	0.19 ± 1.22	–	–	–	–
C20:5n3	5.96 ± 1.82	4.79 ± 0.36	5.61 ± 0.40	5.80 ± 0.30	4.41 ± 0.08	3.56 ± 0.10	–	–	–
C22:6n3	–	0.82 ± 0.10	1.08 ± 0.01	–	0.67 ± 0.01	1.98 ± 0.07	2.03 ± 0.31	1.40 ± 0.09	–
Identified	92.68	93.27	88.42	81.39	79.13	76.52	83.98	84.79	87.79
Unidentified	7.32	6.73	11.58	18.61	20.87	23.48	16.02	15.21	12.21

"–" not detected

and 30 °C, respectively. These results were in accordance with previous reports of *Nannochloropsis salina*, which cannot grow at temperatures above 35 °C (Wagenen et al. 2012), *Tetraselmis chui*, which can grow between 15 and 30 °C (Chen et al. 2012), and *Tetraselmis subcordiformis* and *Nannochloropsis oculata*, which can grow between the ranges of 15–30 and 20–35 °C, respectively (Wei et al. 2015). In addition, temperature was observed to have effects on specific growth rate and biomass. The specific growth rate and biomass of *Chaetoceros* sp. FIKU035 and *Nannochloropsis* sp. FIKU036 both decreased with increasing temperature. The biomass productivity of *T. suecica* FIKU032 was lower than *Chaetoceros* sp. FIKU035 at 30 °C. The best biomass productivity was found for *Chaetoceros* sp. FIKU035 cultured at 25–30 °C. However, these results were not significantly different from *T. suecica* FIKU032 cultured at 30 °C. Temperature affects microalgae growth due to changes of cell metabolic activities and activity of important enzymes, such as ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), an essential enzyme in photosynthesis (Feller et al. 1998; Leggat et al. 2004; Wei et al. 2015). Fogg and Thake (1987) reported that the specific growth rate of microalgae declined when cultured at high temperature, due to increasing respiration.

It has been reported that lipid content and productivity of microalgae are dependent on growth conditions such as growth phase, medium composition, irradiance and temperature. In this study, the lipid content of *Chaetoceros* sp. FIKU035 and *Nannochloropsis* sp. FIKU036 significantly decreased with increasing temperature. This was in agreement with previous reports that lipid content of microalgae

Table 3 Effects of temperature on biodiesel properties ($n = 3$)

Strains	Biodiesel parameters							
	SV	I_2	CN	LCF	CFPP	υ	ρ	HHV
Chaetoceros sp. FIKU035								
25 °C	200.02 ± 1.76	67.70 ± 9.45	58.36 ± 2.36	5.20 ± 0.10	−0.14 ± 0.32	4.75 ± 0.01	0.85 ± 0.00	36.29 ± 0.49
30 °C	201.31 ± 1.69	79.34 ± 2.74	55.56 ± 0.49	2.92 ± 0.52	−6.35 ± 0.18	4.72 ± 0.01	0.88 ± 0.00	36.19 ± 0.26
35 °C	187.03 ± 9.13	74.01 ± 0.69	58.88 ± 1.56	4.09 ± 1.04	−3.62 ± 3.28	4.65 ± 0.05	0.88 ± 0.00	33.66 ± 1.54
40 °C	173.72 ± 11.38	67.11 ± 3.31	63.17 ± 2.87	11.70 ± 1.73	*20.27 ± 5.42*[a]	4.62 ± 0.08	0.88 ± 0.00	31.72 ± 2.17
T. suecica FIKU032								
25 °C	162.11 ± 2.75	82.87 ± 2.57	60.61 ± 1.48	4.38 ± 0.05	−2.73 ± 0.17	4.63 ± 0.02	0.88 ± 0.00	31.25 ± 0.58
30 °C	155.90 ± 3.46	104.58 ± 2.85	57.79 ± 1.42	2.66 ± 0.03	−8.13 ± 0.11	4.56 ± 0.02	0.88 ± 0.00	30.11 ± 0.67
Nannochloropsis sp. FIKU0036								
25 °C	179.79 ± 5.86	65.99 ± 1.80	60.39 ± 0.64	6.14 ± 0.13	2.82 ± 0.41	4.70 ± 0.08	0.88 ± 0.00	32.99 ± 2.29
30 °C	171.60 ± 8.91	82.34 ± 0.16	59.62 ± 1.69	15.53 ± 3.64	*32.33 ± 11.44*[a]	4.73 ± 0.04	0.88 ± 0.00	33.40 ± 1.45
35 °C	181.80 ± 0.78	94.88 ± 6.63	55.26 ± 1.71	6.85 ± 2.65	*17.02 ± 8.59*[a]	4.73 ± 0.02	0.88 ± 0.00	34.56 ± 0.45
Biodiesel standard								
European standard (EN 14214)	–	<120	>51	–	+5- (−20)	3.5–5.0	0.87–0.90	–
American standard (ASTM D6751)	–	–	>47	–	–	1.9–6.0	-	-

[a] Indicates that biodiesel parameter is inconsistent with European standard (EN14214)

decreased when cultured at extremely low or high temperatures (Aaroson 1973; Opute 1974; Renaud et al. 1995). There have also been reports that the lipid contents of *Chaetoceros* sp., *C. calcitrans* and *C. simplex* decreased with increasing temperature (Renaud et al. 2002). In contrast, Wei et al. (2015) reported that the lipid content of *N. oculata* and *T. subcordiformis* increased with increasing temperature. However, beyond the optimal temperature, lipids of microalgae are known to decrease due to stress on photosystem II activity (Sheng et al. 2011). Thus, it can be said that the effect of temperature on growth rate, lipid content and lipid productivity of microalgae is species-specific.

Temperature also has an effect on fatty acid composition and biodiesel properties (Converti et al. 2009; Ramos et al. 2009; Islam et al. 2013; Knothe 2013). In this study, the PUFA content of *T. suecica* FIKU032 and *Nannochloropsis* sp. FIKU036 increased with increasing temperature. These results contrast those of a previous study that reported that some microalgae respond to a decrease in temperature by increasing the ratio of unsaturated fatty acids. Enhanced stability and fluidity of cellular membranes, particularly thylakoid membranes (through increased levels of unsaturated fatty acid in membrane lipids), protect the photosynthetic machinery from photoinhibition at low temperatures. Conversely, the microalgae cultured at a high temperature contained a lower content of unsaturated fatty acid (Renaud et al. 2002; Hu 2003). In addition, there was no significant difference in PUFA content in *Chaetoceros* sp. FIKU035 over the experimental temperature range.

In an assessment of general biodiesel properties, the fatty acid composition of microalgae is an important parameter that has to be considered. Commonly, palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3) are strong candidates for suitable biodiesel production (Knothe 2008). In this study, *Chaetoceros* sp. FIKU035 cultured at 25 and 30 °C showed fatty acid profiles that are suitable for biodiesel production due to high content SFAs and MUFAs (more than 80% of FAMEs). MUFAs (i.e., C16:1 and C18:1) are advantageous over SFAs and PUFAs for their desirable oxidative stability, cold flow and combustion properties (Knothe 2009). A certain amount of PUFA can have positive impact on the biodiesel flow properties, especially during cold weather, but can have adverse effects on oxidative stability (Knothe 2008). However, *Chaetoceros* sp. FIKU035 exhibited a low content of PUFAs (~10–14% of total FAMEs). The fatty acid composition of *Chaetoceros* sp. FIKU035 was in accordance with a previous report by Wang et al. (2014) in that the dominant fatty acid of *Chaetoceros muelleri* were C14:0, C16:0 and C16:1, while the PUFA content was only 10–16% of total FAMEs. This predominance of short chain fatty acid (C14-C18) and low percentage of PUFAs are very significant for the potential of *Chaetoceros* sp. FIKU035 for the biodiesel production.

These findings support previous reports that short chain fatty acids, which contain high SFAs and MUFAs, are suitable for biodiesel production by having increased energy yield, oxidative and thermal stability and higher cetane number

(Monyem et al. 2000; Knothe 2007, 2008, 2009). However, lipids dominated by these fatty acids are prone to solidify at low temperature. Lipids with high PUFA content provide a poor cetane number and oxidative stability (Ramos et al. 2009; Hoekman et al. 2012). A low cetane number causes longer ignition delay time (Gopinath et al. 2009). In addition, poor oxidative stability increases viscosity, as well as gum formation and sediment in biodiesel (Knothe 2005). In this study, the cold filter plugging points of *Chaetoceros* sp. FIKU035 at 40 °C and of *Nannochloropsis* sp. FIKU036 at 30 and 35 °C were not in accordance with accepted standards due to accumulated long chain saturated fatty acids. Too high of a cold filter plugging point in biodiesel leads to wax formation and engine starving due to reduced fuel flow (Hoekman et al. 2012).

This study revealed that the specific growth rate, biomass and lipid productivity, fatty acid composition and biodiesel properties of *Chaetoceros* sp. FIKU035, *T. suecica* FIKU032 and *Nannochloropsis* sp. FIKU036 varied with the temperature of cultivation. The highest specific growth rate, biomass and lipid productivity values were found for *Chaetoceros* sp. FIKU035. Moreover, the fatty acid profiles produced by this strain were suitable for biodiesel production. In addition, the biodiesel parameters were in accordance with European (EN14214) and American (ASTM D6751) standards. These finding suggest that *Chaetoceros* sp. FIKU035 can be considered as suitable species for outdoor cultivation for biodiesel production, as it can grow in wide range temperatures, up to 40 °C. Thus, it is suitable for the environment of Thailand and other tropical countries.

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