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Evaluation of the potential of 9 *Nannochloropsis* strains for biodiesel production

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HIGHLIGHTS

- The potential of 9 *Nannochloropsis* species for biodiesel production was evaluated.
- Growth rate, biomass accumulation and lipid productivity were investigated.
- Further lipid composition, fatty acid profile and biodiesel property were examined.
- The best strain was *Nannochloropsis oceanica* IMET1 with highest lipid productivity.

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ABSTRACT

Nannochloropsis have attracted sustained interest from algal biodiesel researchers due to their high biomass accumulation rate and high lipid content. There are six recognized species in the *Nannochloropsis* genus that are phylogenetically divided into *Nannochloropsis gaditana*, *Nannochloropsis salina*, *Nannochloropsis granulata*, *Nannochloropsis limnetica*, *Nannochloropsis oceanica* and *Nannochloropsis oculata*. In this study, the potential of 9 *Nannochloropsis* species from the 6 genus for biodiesel production was evaluated by determining their growth rate, biomass accumulation, lipid productivity, lipid composition, fatty acid profiles and biodiesel properties. The results showed that the best strain was *N. oceanica* IMET1, with lipid productivity of 158.76 ± 13.83 mg L^{-1} day^{-1}, TAG production of 1.67 ± 0.20 g/L, favorable fatty acid profiles of C16–C18 (56.62 ± 1.96%) as well as suitable biodiesel properties of higher cetane number (54.61 ± 0.25), lower iodine number (104.85 ± 2.80 gI_2/100 g) and relative low cloud point (3.45 ± 0.50 °C). *N. oceanica* IMET1 could be consider as valuable feedstock for microalgal biodiesel production.

1. Introduction

With the need to reduce carbon emissions, and the dwindling reserves of easily exploitable fossil fuel, biofuels especially biodiesel have attracted much more attention worldwide in recent years. Microalgae are among the most promising feedstock for biodiesel production due to their ability to produce substantial amount of triacylglycerides (TAG), high growth rate and no compete for land with crops used for food production (Ahmad et al., 2011; Chisti, 2007). However, several challenges need to be tackled to allow commercial production of biodiesel from microalgae. Choice of suitable algal strain is the key consideration in microalgal biodiesel production pipeline (Scott et al., 2010).

In screening microalgae strain for biodiesel production, Griffiths and Harrison (2009) suggested that the key criterion for choosing oleaginous algae is lipid productivity. Higher lipid productivity could result in higher biodiesel production in microalgae outdoor large-scale cultivation, and vice versa. Suitable microalgae candidates for biodiesel production require not only high lipid productivity, but also suitable fatty acid composition. Fatty acid composition could significantly influence biodiesel fuel properties including kinematic viscosity, specific gravity, cetane number, cloud point and iodine value (Tan and Lin, 2011; Knothe, 2011; Hoekman et al., 2012). In addition, TAG content in total lipid could significantly influence efficiency of microalgal biodiesel production. Although almost all types of microalgal lipids can be extracted, only TAGs are easily transesterified into biodiesel by traditional methods (Gong and Jiang, 2011). Other types of microalgal lipids such as polar lipid can result in loss of biodiesel production

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due to precipitation and saponification (Balasubramanian and Obbard, 2011; Chen et al., 2012). Besides lipid productivity and fatty acid composition, TAG content is another important consideration in microalgae selection for biodiesel production.

At present, several species of *Nannochloropsis* have attracted sustained interest from algal biodiesel researchers due to their high biomass accumulation rate, high lipid content (Rodolfi et al., 2009; Doan et al., 2011; Chen et al., 2012; Ma et al., 2013), and their successful cultivation at large scale using natural sunlight by companies such as Solix Biofuels, Aurora Algae, Seambiotic, Hairong Electric Company/Seambiotic and Proviron (Radakovits et al., 2012). There are six recognized species in the *Nannochloropsis* genus that are phylogenetically divided into *Nannochloropsis gaditana*, *Nannochloropsis salina*, *Nannochloropsis granulata*, *Nannochloropsis limnetica*, *Nannochloropsis oceanica* and *Nannochloropsis oculata* (Vieler et al., 2012; Jinkerson et al., 2013). To our knowledge, no research has been conducted to assess which one is the best feedstock for biodiesel production.

In this study, 9 *Nannochloropsis* from six recognized species, including *N. gaditana*, *N. salina*, *N. granulata*, *N. limnetica*, *N. oceanica* and *N. oculata*, was selected to evaluate their potential for biodiesel production by assessing their biomass accumulation and lipid production. To investigate the reason of high biomass accumulation, the photosynthesis data (Fv/Fm) of the 9 *Nannochloropsis* was determined. Fv/Fm, indicating the potential maximum quantum efficiency, can directly reflect the photosynthesis activity of PS II (Li et al., 2010). Furthermore, lipid composition and fatty acid profile as well as biodiesel properties estimated by fatty acid profile such as kinematic viscosity, specific gravity, cetane number, cold flow and oxidative stability were also analyzed. The aim of this study was to identify the best feedstock for microalgal biodiesel production from the 9 species of *Nannochloropsis*.

2. Methods

2.1. Algal strains and cultivation conditions

N. oceanica IMET1 was kindly provided by Dr. Feng Chen from The University of Maryland Center for Environmental Science. *N. oceanica* 805 from local water system was kindly provided by Dr. Tianzhong Liu from Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences. *N. limnetica* CCMP505, *N. granulata* CCMP525, *N. gaditana* CCMP527, *N. oculata* CCMP529, *N. oceanica* CCMP531, *N. salina* CCMP537 and *N. salina* CCMP1176 were kindly provided by Dr. Jian Xu from Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences. The strain was cultured in seawater except *N. limnetica* CCMP505 in freshwater, supplemented with BG-11 medium under 24 h cool white fluorescent lights at 80–100 μmol photons m^{-2} s^{-1} irradiance.

2.2. Microalgal growth property

The 9 *Nannochloropsis* strains were inoculated to 400 mL bubble column bioreactor with sterile gas composed of air supplemented with 2% (V/V) CO_2. Aeration was carried out at 80 mL/min. The microalgae were cultured at 25 °C under continuous illumination at 100 μmol photons m^{-2} s^{-1}. After cultured for 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22 days, the growth of microalgae cells was estimated by measuring the dry cell weight (DCW). A 10-mL sample was filtered through pre-weighed 5 μm microporous filter paper, and washed twice with 10 mL distilled water to remove adhering inorganic salts. The filter paper was oven-dried overnight at 105 °C. The difference between the final weight and the weight of the paper before filtration was taken as the DCW.

The specific growth rate of each strain was calculated from the slope of the linear regression of time and nature log dry weight in exponential growth phase:

$$k = (\ln N - \ln N_0)/(t - t_0)$$

where k (d^{-1}) is the specific growth rate in exponential growth phase, N_0 is dry weight at the beginning of the exponential phase (t_0) and N represents the dry weight at time (t) of the exponential phase.

Doubling time can be calculated based on the specific growth rate:

$$T = \mathrm{Ln}2/k$$

2.3. In vivo monitoring of Fv/Fm

Fv/Fm (potential maximum quantum efficiency) was determined after 15 min of dark adaptation using an imaging pulse amplitude-modulated fluorometer (Imaging PAM; Heinz Walz, Effeltrich, Germany) (Maxwell and Johnson, 2000).

2.4. Determination of lipid content

The microalgae cells were harvested after 22 days of cultivation by centrifugation at 4722 g for 10 min. Cell pellets were lyophilized using a freeze drier (Alpha1-2LD Plus; Martin Christ GmBH, Osterode, Germany). The total lipids contained in the algal cells were extracted with a modified chloroform–methanol–water solvent system (Ma et al., 2013).

2.5. Lipid composition and fatty acid composition analysis

Lipid components were analyzed using a thin-layer chromatography (TLC) system (TLC-FID, MK-6, Iatron Laboratories, Inc., Tokyo, Japan) (Fedosov et al., 2011; Chen et al., 2012). Samples were dissolved in chloroform to a concentration of 10 mg/mL, and 2 μL of solutions containing lipids was spotted onto Chromarod S-III silica coated quartz rods held in a frame. The rods were developed in a solvent system of benzene:chloroform:acetic acid (150:60:2, v/v/v) for the first migration to 7.5 cm, followed by a solution of benzene:hexane (50:50, v/v) for the second migration to 10 cm. The rods were dried at 70 °C for 3 min before they were scanned in the Iatroscan analyzer, which was operated at a flow rate of 0.16 L/min for hydrogen and 2 L/min for air. The scan speed was 30 s per rod. The recorded profiles were analyzed by SIC-480 II program. The individual lipid components were identified by co-chromatography with pure standards (sterol ester, SE; fatty acid methyl ester, FAME; triacylglycerol, TAG; diacylglycerol, DAG; phospholipids, PL; purchased from Sigma, St. Louis, MO, USA). The quantities of individual components were estimated from the peak areas of pure standards (Chen et al., 2012).

Methyl esters were generated from the microalgae lipids by heating them in a 2% H_2SO_4-methanol solution at 85 °C for 2.5 h. Fatty acid methyl esters (FAME) were analyzed by GC using a Varian (Walnut Creek, CA) 450 GC equipped with an FID detector and a Varian capillary column CP-Wax 58 (FFAP) CB (25 m × 0.25 mm × 0.20 μm). Carrier gas was nitrogen at 1 mL/min and the split ratio was 1:30. The oven temperature was initially held at 100 °C for 2 min, followed by an increase to 250 °C at 10 °C/min, which was then held for 8 min. The detection system was equipped with a flame ionization detector (FID) operating at 280 °C. FAME peak identification was carried out by GC–MS (NIST, 2.0) operating in the same conditions as the GC-FID. The relative percentage of the fatty acid was calculated on the basis of the peak area of a fatty acid species to the total peak area of all the fatty acids in the oil sample.

2.6. Estimation of biodiesel fuel properties based on FAME profiles

Biodiesel is a renewable transportation fuel consisting of fatty acid methyl esters (FAME). FAME profiles, especially size distribution and the degree of unsaturation, could significantly influence the physical and chemical properties of biodiesel (Hoekman et al., 2012). At present, many equations based on FAME profiles have been built to predict biodiesel properties (Francisco et al., 2010; Hoekman et al., 2012; Nascimento et al., 2013). Especially, the equations of Hoekman et al. (2012) was widely accepted due to the calculated values by the equation were more close to the measured values (Song et al., 2013). In this study, the equations of Hoekman et al. (2012) were selected to predict the biodiesel properties and detailed calculations are as follows:

Average degree of unsaturation was determined as previous described (Song et al., 2013). The relationships between average degree of unsaturation and biodiesel properties including kinematic viscosity, specific gravity, cloud point, cetane number, and iodine value are as shown in Eqs. (1–5) (Hoekman et al., 2012).

$$Y = -0.6316X + 5.2065 \quad (1)$$

$$Y = 0.0055X + 0.8726 \quad (2)$$

$$Y = -13.356X + 19.994 \quad (3)$$

$$Y = -6.6684X + 62.876 \quad (4)$$

$$Y = 74.373X + 12.71 \quad (5)$$

2.7. Statistical analysis

Data were presented as the mean ± standard deviation of the mean of triplicate samples. Significant differences between means from the 9 species of *Nannochloropsis* were determined using one-way analysis of variance followed by Duncan's multiple-range tests, using the SPSS statistical package (version 13.0; SPSS Inc., Chicago, IL, USA) at a significance level of $p < 0.05$.

3. Results and discussion

3.1. Growth and lipid accumulation properties

Lipid productivity is a key criterion for choosing oleaginous algae (Griffiths and Harrison, 2009), which could be calculated by biomass productivity and lipid content. Therefore, biomass and lipid accumulation characteristics of the 9 *Nannochloropsis* were investigated. The results showed that *N. oceanica* IMET1 exhibited the fastest specific growth rate ($0.21 \pm 0.0072\ d^{-1}$), followed by *N. oculata* CCMP529 ($0.20 \pm 0.0042\ d^{-1}$), *N. salina* CCMP1176 ($0.19 \pm 0.0087\ d^{-1}$), *N. salina* CCMP537 ($0.19 \pm 0.0069\ d^{-1}$), *N. gaditana* CCMP527 ($0.18 \pm 0.0019\ d^{-1}$), *N. oceanica* 805 ($0.17 \pm 0.0042\ d^{-1}$), *N. granulata* CCMP525 ($0.13 \pm 0.0045\ d^{-1}$) and *N. oceanica* CCMP531 ($0.11 \pm 0.0046\ d^{-1}$), and finally *N. limnetica* CCMP505 showed a relatively low specific growth rate ($0.07 \pm 0.0064\ d^{-1}$) (Table 1). The double time showed the similar tendency with the specific growth rate (Table 1).

Biomass productivity was calculated by the cultivation time and final biomass at the end of cultivation. The results showed that *N. salina* CCMP1176 had the highest biomass productivity at $363.10 \pm 9.77\ mg\ L^{-1}\ day^{-1}$, while *N. salina* CCMP537 ($316.18 \pm 4.50\ mg\ L^{-1}\ day^{-1}$), *N. oceanica* IMET1 ($300.55 \pm 20.25\ mg\ L^{-1}\ day^{-1}$), *N. gaditana* CCMP527 ($296.41 \pm 6.62\ mg\ L^{-1}\ day^{-1}$), *N. oceanica* 805 ($291.91 \pm 19.93\ mg\ L^{-1}\ day^{-1}$), *N. oculata* CCMP529 ($263.64 \pm 9.09\ mg\ L^{-1}\ day^{-1}$), *N. granulata* CCMP525 ($216.00 \pm 1.93\ mg\ L^{-1}\ day^{-1}$) and *N. oceanica* CCMP531 ($186.36 \pm 2.73\ mg\ L^{-1}\ day^{-1}$) with lower biomass productivity, and finally *N. limnetica* CCMP505 with the lowest biomass productivity ($181.14 \pm 2.38\ mg\ L^{-1}\ day^{-1}$) (Table 1). The biomass productivity of the 9 *Nannochloropsis* was not consistent with the specific growth rate and the double time. Although *N. salina* CCMP1176 showed not highest specific growth rate, its biomass accumulation was constantly increased in 22-day cultivation which may result in its highest biomass productivity (Fig. 1).

As indicated in Fig. 2, Fv/Fm showed the similar tendency with the biomass production except *N. limnetica* CCMP505. For example, similar with the biomass production, the Fv/Fm of *N. salina* CCMP1176 and *N. salina* CCMP537 showed higher value at the end of cultivation, while *N. granulata* CCMP525 and *N. oceanica* CCMP531 with lower value. In the 9 *Nannochloropsis*, only *N. limnetica* CCMP505 was cultured in fresh water which may result in its strange phenomenon in Fv/Fm and biomass production. Microalgae biomass production is directly proportional to the efficiency with which the algal cells assimilate carbon from the atmosphere through photosynthesis (Williams and Laurens, 2010). Photosynthesis efficiency is the deciding factor in microalgae biomass production. In this study, our results were consistent with the previous report.

Lipid productivity was not only in connection with biomass productivity, but also lipid content. Furthermore, lipid content of the 9 *Nannochloropsis* at the end of 22-day cultivation was analyzed. The results showed *N. granulata* CCMP525 attained the highest lipid content at 60.35 ± 1.20%, while *N. salina* CCMP1176 showed the lowest at 36.95 ± 0.91% (Table 1). Compared to *N. salina* CCMP1176, *N. oceanica* CCMP531, *N. oceanica* IMET1, *N. oculata* CCMP529, *N. oceanica* 805, *N. gaditana* CCMP527, *N. limnetica* CCMP505 and *N. salina* CCMP537 could achieve greater lipid content of 53.20 ± 0.55%, 52.92 ± 1.04%, 52.10 ± 0.30%, 48.96 ± 2.25%, 44.89 ± 1.58%, 41.17 ± 0.35% and 38.75 ± 1.67%, respectively (Table 1). Compared to the biomass productivity, we found the top biomass producers in the present study did not correspond to the top lipid producers. For example, *N. salina* CCMP1176 and *N. salina* CCMP537 showed the highest biomass productivity, while their lipid contents were lowest. The phenomenon was consistent with the previous report

Table 1
Growth kinetics, lipid content and lipid productivity of microalgae strains. Data are given as means ± S.D., $n = 3$. Different superscripts indicate significant difference among the 9 *Nannochloropsis* species (ANOVA, Duncan's test; $p < 0.05$).

Strains	Specific growth rate (day^{-1})	Double time	Biomass productivity ($mg\ L^{-1}\ day^{-1}$)	Lipid content (%)	Lipid productivity ($mg\ L^{-1}\ day^{-1}$)
Nannochloropsis oceanica IMET1	0.21 ± 0.0072^{a}	3.39 ± 0.12^{a}	300.55 ± 20.25^{b}	52.92 ± 1.04^{b}	158.76 ± 13.83^{a}
Nannochloropsis oceanica 805	0.17 ± 0.0042^{d}	4.06 ± 0.10^{a}	291.91 ± 19.93^{b}	48.96 ± 2.25^{c}	$142.92 \pm 16.32^{a,b}$
Nannochloropsis limnetica CCMP505	0.073 ± 0.0064^{g}	9.54 ± 0.84^{d}	181.14 ± 2.38^{e}	41.17 ± 0.35^{e}	74.58 ± 1.61^{e}
Nannochloropsis granulata CCMP525	0.13 ± 0.0045^{e}	5.21 ± 0.18^{b}	216.00 ± 1.93^{d}	60.35 ± 1.20^{a}	$130.36 \pm 3.76^{b,c}$
Nannochloropsis gaditana CCMP527	$0.18 \pm 0.0019^{c,d}$	3.81 ± 0.040^{a}	296.41 ± 6.62^{b}	44.89 ± 1.58^{d}	$133.06 \pm 1.72^{b,c}$
Nannochloropsis oculata CCMP529	$0.20 \pm 0.0042^{a,b}$	3.49 ± 0.074^{a}	263.64 ± 9.09^{c}	52.10 ± 0.30^{b}	$137.36 \pm 5.53^{b,c}$
Nannochloropsis oceanica CCMP531	0.11 ± 0.0046^{f}	6.25 ± 0.26^{c}	186.36 ± 2.73^{e}	53.20 ± 0.55^{b}	100.21 ± 0.43^{d}
Nannochloropsis salina CCMP537	$0.19 \pm 0.0069^{b,c}$	3.71 ± 0.14^{a}	316.18 ± 4.50^{b}	$38.75 \pm 1.67^{e,f}$	122.52 ± 3.53^{c}
Nannochloropsis salina CCMP1176	$0.19 \pm 0.0087^{b,c}$	3.74 ± 0.18^{a}	363.10 ± 9.77^{a}	36.95 ± 0.91^{f}	$134.17 \pm 6.91^{b,c}$

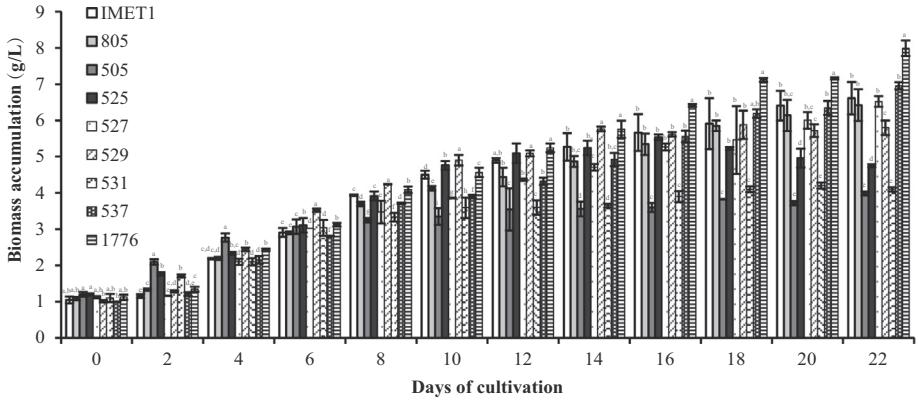

Fig. 1. Biomass accumulation of 9 *Nannochloropsis* species during cultivation. Data are means of three repeated experiments and error bars indicate standard deviations. Different superscripts indicate significant difference among the 9 *Nannochloropsis* species (ANOVA, Duncan's test; $p < 0.05$).

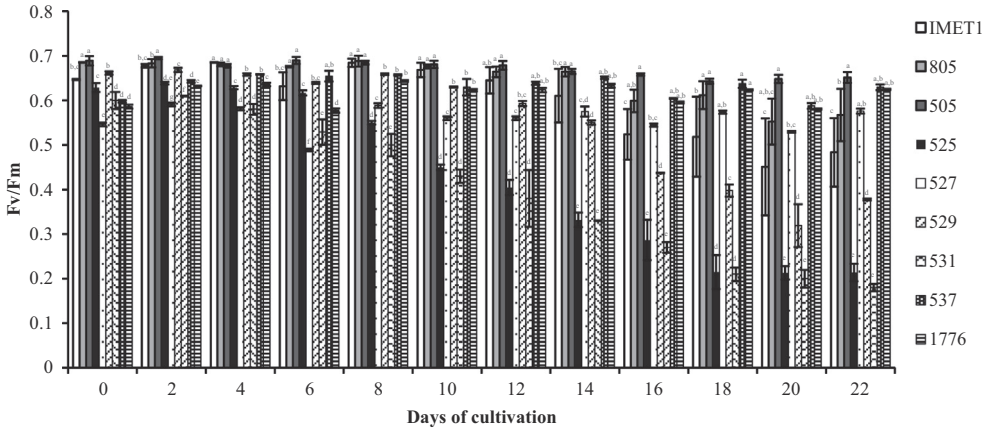

Fig. 2. Fv/Fm of 9 *Nannochloropsis* species during cultivation. Data are means of three repeated experiments and error bars indicate standard deviations. Different superscripts indicate significant difference among the 9 *Nannochloropsis* species (ANOVA, Duncan's test; $p < 0.05$).

(Nascimento et al., 2013). Naturally, a strain which has high biomass productivity may manifest in a relative low lipid content and vice versa (Rodolfi et al., 2009).

Combined with the biomass productivity, the lipid productivity of the 9 *Nannochloropsis* was analyzed. The results showed that lipid productivity of *N. oceanica* IMET1 was the highest (158.76 ± 13.83 mg L^{-1} day^{-1}), followed by *N. oceanica* 805 (142.92 ± 16.32 mg L^{-1} day^{-1}), *N. oculata* CCMP529 (137.36 ± 5.53 mg L^{-1} day^{-1}), *N. salina* CCMP1176 (134.17 ± 6.91 mg L^{-1} day^{-1}), *N. gaditana* CCMP527 (133.06 ± 1.72 mg L^{-1} day^{-1}), *N. granulata* CCMP525 (130.36 ± 3.76 mg L^{-1} day^{-1}), *N. oceanica* CCMP537 (122.52 ± 3.53 mg L^{-1} day^{-1}), and *N. oceanica* CCMP531 (100.21 ± 0.43 mg L^{-1} day^{-1}), while *N. limnetica* CCMP505 (74.58 ± 1.61 mg L^{-1} day^{-1}) was the lowest (Table 1). Based on the lipid productivity, *N. oceanica* IMET1 could be consider as the best feedstock for microalgal biodiesel production in 9 *Nannochloropsis*.

3.2. Lipid composition properties

Besides lipid productivity, lipid composition is another important factor in screening microalgae strain for biodiesel production. To further analyze the lipid characteristics for biodiesel production,

Table 2
Lipid composition analyses of 9 *Nannochloropsis* species. Data are given as means ± S.D., $n = 3$. Different superscripts indicate significant difference among the 9 *Nannochloropsis* species (ANOVA, Duncan's test; $p < 0.05$).

Lipid composition	IMET1	805	505	525	527	529	531	537	1776
SE[A]	$5.43 \pm 3.72^{a,b}$	$3.53 \pm 1.46^{a,b}$	1.71 ± 0.1^{b}	1.99 ± 0.15^{b}	$3.33 \pm 0.18^{a,b}$	7.18 ± 0.15^{a}	1.60 ± 0.07^{b}	1.76 ± 0.24^{b}	$4.79 \pm 2.78^{a,b}$
FAME[B]	$6.08 \pm 0.15^{a,b}$	$4.62 \pm 4.59^{a,b}$	$4.97 \pm 0.25^{a,b}$	9.56 ± 1.02^{a}	1.54 ± 0.054^{b}	1.79 ± 0.10^{b}	2.39 ± 0.20^{b}	$6.89 \pm 0.99^{a,b}$	$5.38 \pm 5.15^{a,b}$
TAC[C]	$47.56 \pm 1.64^{b,c}$	$44.17 \pm 3.90^{c,d}$	30.85 ± 1.52^{f}	$36.10 \pm 2.00^{e,f}$	$38.24 \pm 3.96^{d,e}$	$54.05 \pm 1.75^{a,b}$	58.43 ± 2.00^{a}	31.38 ± 2.40^{f}	23.79 ± 4.63^{g}
FS[D]&DAG[E]	$4.53 \pm 2.56^{c,d,e}$	$2.64 \pm 1.54^{d,e}$	12.49 ± 0.91^{a}	$2.63 \pm 0.058^{d,e}$	9.28 ± 1.34^{b}	$5.20 \pm 0.56^{c,d}$	1.50 ± 0.15^{f}	$7.05 \pm 1.49^{b,c}$	8.62 ± 0.65^{b}
PL[F]	36.40 ± 2.65^{c}	45.05 ± 5.49^{b}	$49.98 \pm 3.52^{a,b}$	$49.72 \pm 3.52^{a,b}$	47.60 ± 5.44^{b}	31.78 ± 2.66^{c}	36.10 ± 2.51^{c}	$52.92 \pm 2.61^{a,b}$	57.42 ± 2.91^{a}

[A] SE: percentage of sterol ester (% of total lipid).
[B] FAME: percentage of fatty acid methyl ester (% of total lipid).
[C] TAG: percentage of triacylglycerol (% of total lipid).
[D] FS: percentage of free sterol (% of total lipid).
[E] DAG: percentage of diacylglycerol (% of total lipid).
[F] PL: percentage of phospholipids (% of total lipid).

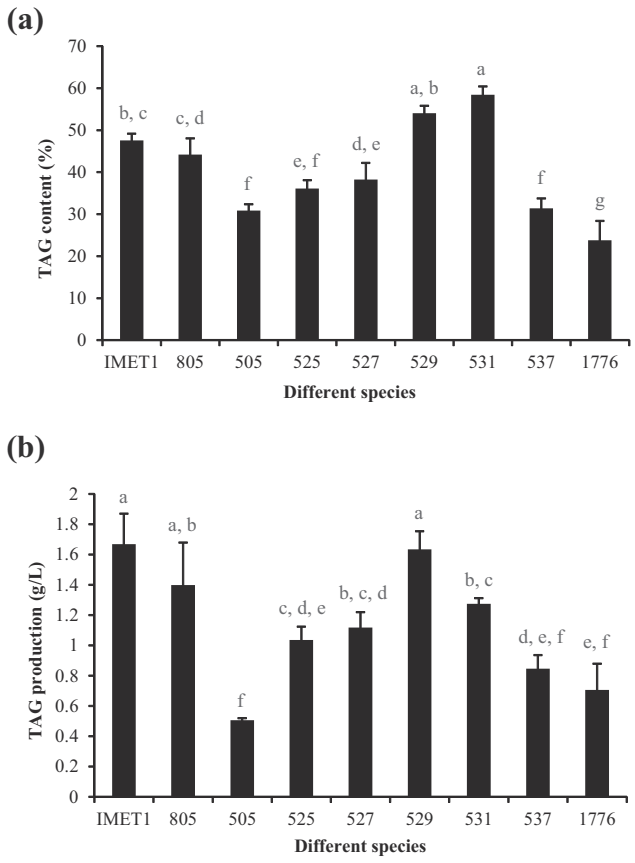

Fig. 3. TAG content (A) and TAG production (B) of 9 *Nannochloropsis* species. Data are means of three repeated experiments and error bars indicate standard deviations. Different superscripts indicate significant difference among the 9 *Nannochloropsis* species (ANOVA, Duncan's test; $p < 0.05$).

the lipid composition of the 9 *Nannochloropsis* was analyzed. The results showed that polar lipid and TAG were main composition in the total lipid of the 9 *Nannochloropsis*, while other component contents, including sterol esters (SE), fatty acid methyl ester (FAME), free sterol (FS) and diacylglycerol (DAG) were lower than both TAG and polar lipid content (Table 2). In the all lipid component contents, only TAGs are easily transesterified into biodiesel by traditional methods (Gong and Jiang, 2011). Therefore, TAG content in the total lipid could significantly influence production efficiency of microalgal biodiesel. In this study, TAG content and production of the 9 *Nannochloropsis* were highlighted. The results showed that the TAG content of *N. oceanica* CCMP531 (58.43 ± 2.00%) and *N. oculata* CCMP529 (54.05 ± 1.75%) was the highest, followed by *N. oceanica* IMET1 (47.56 ± 1.64%), *N. oceanica* 805 (44.17 ± 3.90%), *N. gaditana* CCMP527 (38.24 ± 3.96%), *N. granulate* CCMP525 (36.10 ± 2.00%), *N. oceanica* CCMP537 (31.38 ± 2.40%) and *N. limnetica* CCMP505 (30.85 ± 1.52%), while *N. oceanica* CCMP1776 (23.79 ± 4.63%) was the lowest (Fig. 3a). Combined with the lipid production, the TAG production of *N. oceanica* IMET1 was the highest, followed by *N. oculata* CCMP529 and *N. oceanica* 805, while *N. oceanica* CCMP531, *N. gaditana* CCMP527, *N. granulata* CCMP525, *N. oceanica* CCMP537, *N. oceanica* CCMP1176 and *N. limnetica* CCMP505 were relatively lower (Fig. 3b). Based on TAG production, *N. oceanica* IMET1 showed the highest biodiesel production efficiency in 9 *Nannochloropsis*.

3.3. Fatty acid profiles properties

Ideal microalgae candidates for biodiesel production require not only high lipid and TAG production, but also suitable fatty acid composition. Fatty acid composition of 9 *Nannochloropsis* was analyzed. The results showed that C16:0 (palmitic acid), C16:1 (palmitoleic acid), C18:1 (oleic acid), C20:4 (eicosatetraenoic acid) and C20:5 (eicosapentaenoic acid) were major fatty acids in 9 *Nannochloropsis* except *N. limnetica* CCMP505 (Table 3). Compared with other species, *N. limnetica* CCMP505 showed the different fatty acid profile, which may due to its inconsistent cultivation condition in freshwater. When cultivated in seawater, *N. limnetica* CCMP505 showed the similar fatty acid profile with the other *Nannochloropsis* (Unpublished results). Further analysis showed saturated fatty acids (SFA) ranged from 21.23 ± 0.27% to 50.87 ± 1.73%, monounsaturated fatty acids (MUFA) ranged from 34.37 ± 2.08% to 52.56 ± 1.24%, while polyunsaturated fatty acids (PUFA) ranged from 6.66 ± 0.64% to 41.35 ± 2.46% in 9 *Nannochloropsis* species. Previous study revealed that the most favorable biodiesel would have rather low levels of polyunsaturated fatty acids and low levels of saturated fatty acids to decrease oxidative stability and cold flow problems (Knothe, 2009; Hoekman et al., 2012). Therefore, monounsaturated fatty acids were capable of giving the finest compromise between oxidative stability and cold flow (Knothe, 2009;

Table 3
Fatty acid profiles of 9 *Nannochloropsis* species. Data are given as means ± S.D., $n = 3$. Different superscripts indicate significant difference among the 9 *Nannochloropsis* species (ANOVA, Duncan's test; $p < 0.05$).

Fatty acids	IMET1	805	505	525	527	529	531	537	1776
14:0	2.80 ± 0.26^{c}	$2.86 \pm 0.02^{b,c}$	–	$2.40 \pm 0.03^{c,d}$	2.74 ± 0.31^{c}	2.07 ± 0.12^{d}	4.47 ± 0.26^{a}	3.34 ± 0.19^{b}	2.08 ± 0.31^{d}
16:0	27.62 ± 0.92^{e}	32.95 ± 2.36^{c}	16.64 ± 0.19^{f}	26.21 ± 0.92^{e}	39.19 ± 1.61^{b}	$29.13 \pm 0.95^{d,e}$	45.85 ± 0.92^{a}	32.23 ± 0.67^{c}	$32.04 \pm 1.80^{c,d}$
16:1	$26.41 \pm 0.27^{a,b,c,d}$	$29.36 \pm 4.58^{a,b}$	2.92 ± 0.33^{e}	24.04 ± 0.64^{d}	$24.14 \pm 1.45^{c,d}$	$28.29 \pm 1.20^{a,b}$	22.70 ± 0.27^{d}	$25.41 \pm 0.82^{b,c,d}$	29.94 ± 0.85^{a}
16:2	–	–	3.05 ± 0.24	–	–	–	–	2.98 ± 0.95	–
18:0	$2.71 \pm 0.05^{b,c}$	1.90 ± 0.62^{c}	4.59 ± 0.08^{a}	3.25 ± 0.30^{b}	3.07 ± 0.78^{b}	1.85 ± 0.15^{c}	0.55 ± 0.05^{d}	$2.47 \pm 0.70^{b,c}$	3.19 ± 0.18^{b}
18:1	20.82 ± 0.18^{b}	22.35 ± 3.78^{b}	31.45 ± 1.75^{a}	28.52 ± 0.60^{a}	14.17 ± 0.45^{c}	22.84 ± 0.85^{b}	22.15 ± 0.18^{b}	15.46 ± 0.71^{c}	9.37 ± 0.15^{d}
18:2	$3.80 \pm 0.69^{b,c}$	$2.18 \pm 0.14^{c,d}$	23.84 ± 2.12^{a}	4.71 ± 0.83^{b}	$2.29 \pm 0.11^{c,d}$	$2.64 \pm 0.12^{c,d}$	0.72 ± 0.19^{d}	$2.89 \pm 0.29^{b,c}$	$2.58 \pm 0.40^{c,d}$
18:3	1.67 ± 0.12^{b}	$0.91 \pm 0.32^{d,e}$	17.51 ± 0.34^{a}	1.60 ± 0.10^{b}	0.96 ± 0.06^{c}	1.61 ± 0.13^{b}	0.52 ± 0.12^{e}	$0.65 \pm 0.04^{d,e}$	$0.90 \pm 0.06^{d,e}$
20:4	$6.70 \pm 0.08^{a,b}$	$6.11 \pm 0.80^{a,b}$	–	4.52 ± 0.13^{c}	5.81 ± 0.76^{b}	$6.15 \pm 0.55^{a,b}$	2.52 ± 0.08^{d}	$3.64 \pm 0.07^{c,d}$	7.16 ± 0.75^{a}
20:5	$7.45 \pm 0.25^{b,c}$	8.35 ± 1.45^{b}	–	$4.74 \pm 0.17^{d,e}$	$7.63 \pm 1.20^{b,c}$	$5.41 \pm 0.32^{c,d}$	2.90 ± 0.25^{e}	10.93 ± 0.14^{a}	12.74 ± 1.84^{a}
SFA[A]	$33.13 \pm 1.23^{d,e}$	37.71 ± 3.00^{c}	21.23 ± 0.27^{f}	31.86 ± 1.25^{e}	45.00 ± 2.70^{b}	$33.05 \pm 1.22^{d,e}$	50.87 ± 1.73^{a}	38.04 ± 1.81^{c}	$37.31 \pm 2.29^{c,d}$
MUFA[B]	$47.23 \pm 0.45^{a,b,c}$	$51.71 \pm 8.36^{a,b}$	34.37 ± 2.08^{e}	52.56 ± 1.24^{a}	$38.31 \pm 1.90^{d,e}$	$51.13 \pm 2.05^{a,b}$	$44.85 \pm 0.45^{b,c,d}$	$40.87 \pm 1.53^{c,d,e}$	$39.31 \pm 1.00^{d,e}$
PUFA[C]	$19.62 \pm 1.14^{b,c}$	17.55 ± 2.71^{c}	41.35 ± 2.46^{a}	15.57 ± 1.23^{c}	16.69 ± 2.13^{c}	15.81 ± 1.12^{c}	6.66 ± 0.64^{d}	18.11 ± 0.54^{c}	23.38 ± 3.05^{b}
UFA[D]	$66.85 \pm 1.59^{a,b}$	$69.26 \pm 11.07^{a,b}$	75.72 ± 4.54^{a}	$68.13 \pm 2.47^{a,b}$	55.00 ± 4.03^{c}	$66.94 \pm 3.17^{a,b}$	51.51 ± 1.09^{c}	$58.98 \pm 2.07^{b,c}$	$62.69 \pm 4.05^{b,c}$

[A] SFA: percentage of saturated fatty acids (% of total fatty acids).
[B] MUFA: percentage of monounsaturated fatty acids (% of total fatty acids).
[C] PUFA: percentage of polyunsaturated fatty acids (% of total fatty acids).
[D] UFA: percentage of unsaturated fatty acids (% of total fatty acids).

Table 4
Comparison of five properties of biodiesel from microalgae oil, biodiesel and ASTM biodiesel, EN biodiesel standard. Data are given as means ± S.D., $n = 3$. Different superscripts indicate significant difference among the 9 *Nannochloropsis* species (ANOVA, Duncan's test; $p < 0.05$).

Property	IMET1	805	505	525	527	529	531	537	1776	Biodiesel	US (ASTMD6751-08)	Europe (EN14214)
Kinematic viscosity 40 °C ($mm^2 s^{-1}$)	4.42 ± 0.024^{b,c}	4.42 ± 0.13^{b,c}	4.32 ± 0.074^{d}	4.52 ± 0.029^{b}	4.53 ± 0.072^{b}	4.49 ± 0.041^{b}	4.75 ± 0.017^{a}	4.41 ± 0.047^{b,c}	4.33 ± 0.090^{d}	4–5	1.9–6.0	3.5–5.0
Specific gravity ($kg L^{-1}$)	0.879 ± 0.00^{a,b}	0.879 ± 0.00^{a,b}	0.880 ± 0.00^{a}	0.879 ± 0.00^{b}	0.878 ± 0.00^{b}	0.879 ± 0.00^{b}	0.877 ± 0.00^{c}	0.879 ± 0.00^{a,b}	0.880 ± 0.00^{a}	0.87 −0.89	0.85–0.90	
Cloud point (°C)	3.45 ± 0.50^{b,c}	3.30 ± 2.68^{b,c}	0.84 ± 1.56^{c}	5.49 ± 0.61^{b}	5.68 ± 1.52^{b}	4.92 ± 0.87^{b}	10.32 ± 0.37^{a}	3.24 ± 0.99^{b,c}	1.36 ± 1.89^{c}	–		
Cetane number	54.61 ± 0.25^{b,c}	54.54 ± 1.34^{b,c}	53.31 ± 0.78^{c}	55.64 ± 0.30^{b}	55.73 ± 0.76^{b}	55.35 ± 0.43^{b}	58.05 ± 0.18^{a}	54.51 ± 0.50^{b,c}	53.57 ± 0.95^{c}	44–55	Report min47	
Iodine number ($gI_2/100$ g)	104.85 ± 2.80^{a,b}	105.67 ± 14.92^{a,b}	119.35 ± 8.67^{a}	93.45 ± 3.40^{b}	92.41 ± 8.44^{b}	96.67 ± 4.82^{b}	66.58 ± 2.05^{c}	105.98 ± 5.52^{a,b}	116.47 ± 10.55^{a}			Min51 Max120
Avg. unsaturation	1.24 ± 0.038^{a,b}	1.25 ± 0.20^{a,b}	1.43 ± 0.12^{a}	1.09 ± 0.046^{b}	1.07 ± 0.11^{b}	1.13 ± 0.065^{b}	0.72 ± 0.028^{c}	1.25 ± 0.074^{a,b}	1.40 ± 0.14^{a}	0.6–1.6		

Hoekman et al., 2012). In this study, it was interesting to find that the monounsaturated fatty acids in *N. oceanica* 805, *N. granulata* CCMP525 and *N. oculata* CCMP529 presented a major percentage of approximately 50%, and in other species it had small differences ranging from 34.37 ± 2.08% to 47.23 ± 0.45%.

The consensus view is that the most common feedstocks suitable for biodiesel production were enriched in the five most common C16-C18 fatty acids, including C16:0 (palmitic acid), C18:0 (stearic acid), C18:1 (oleic acid), C18:2 (linoleic acid), and C18:3 (linolenic acid) (Knothe, 2009). The results showed that the 9 *Nannochloropsis* species possessed considerable amounts of C16 and C18 species, ranging from 53.70 ± 2.41% to 94.03 ± 4.48%, except for *N. salina* CCMP1176 which contained a substantially low value at 48.08 ± 2.59%. In addition, polyunsaturated fatty acids appear to be more commonly occurring in the fatty acids of 9 *Nannochloropsis* species. Several species rich in C20:5 (eicosapentaenoic acid), a functional ingredient showing great benefits to human health (Chen et al., 2013), were *N. salina* CCMP1176 (12.74 ± 1.84%), *N. oceanica* CCMP537 (10.93 ± 0.14%) and *N. oceanica* 805 (8.35 ± 1.45%). However, it was not suitable for biodiesel production as it would likely result in low oxidative stability. Fatty acid composition could significantly influence biodiesel fuel properties, moreover, due to the diversity and conflicting impacts of fatty acid profiles on biodiesel properties, it is difficult to describe clearly the suitability of fatty acid profiles. Hence, a comprehensive analysis of fatty acid profiles is urgently needed to evaluate biodiesel fuel properties.

3.4. Estimation of biodiesel's fuel properties of microalgae oils

As shown in Table 4, five important biodiesel properties besides average degree of unsaturation of the 9 *Nannochloropsis* species were analyzed. Average degree of unsaturation was proved to have high correlation with several other properties, such as higher average unsaturation leads to lower cetane number and poorer oxidation stability, but improved low temperature performance (Hoekman et al., 2012). Five important biodiesel properties including kinematic viscosity, fuel density, cloud point cetane number and iodine number were estimated based on the relationships built across a range of realistic biodiesel types (Hoekman et al., 2012; Song et al., 2013).

Kinematic viscosity is a measure of resistance to flow of biodiesel (Knothe, 2005). In general, higher viscosity leads to overall poorer combustion, higher emissions, and increased oil dilution. The kinematic viscosity of FAME increases with increasing saturation (Hoekman et al., 2012). The results showed that the estimated kinematic viscosity for microalgal biodiesels was varied from 4.32 ± 0.074 $mm^2 s^{-1}$ to 4.75 ± 0.017 $mm^2 s^{-1}$ among the 9 *Nannochloropsis* species. *N. oceanica* CCMP531 showed the highest kinematic viscosity may due to its higher saturated fatty acids content.

Fuel density is a key characteristic that influences engine performance (Hoekman et al., 2012). As fuel injection pumps meter fuel by volume, not by mass, a greater or lesser mass of fuel is injected due to its density. The biodiesel density is affected by average unsaturation (Hoekman et al., 2012). In this study, specific gravity varied within a narrow range of 0.877–0.880 kg L^{-1} for microalgal biodiesels from 9 *Nannochloropsis* species.

Cloud point is the temperature at which the least soluble biodiesel component crystallizes from solution. Low temperature performance is one of the most important properties for biodiesel. Poor low temperature performance may result in filter plugging due to wax formation, and engine starving due to reduced fuel flow. In general, poor cold flow properties result from the presence of long chain, saturated FAME present in biodiesel (Hoekman et al., 2012). In this study, the results showed that the estimated cloud point for microalgal biodiesels varied among the 9 *Nannochloropsis*

species from 1.36 ± 1.89 °C to 10.32 ± 0.37 °C. *N. oceanica* CCMP531 showed the highest cloud point may due to its higher saturated fatty acids content.

The iodine number is a measure of unsaturation involving the weighted sum of the masses of MUFA and PUFA, important for the biodiesel oxidative stability (Nascimento et al., 2013). High unsaturation levels may result in polymerization of glycerides and formation of deposits (Francisco et al., 2010). The results indicated that *N. oceanica* CCMP531 showed the lowest iodine number 66.58 ± 2.05 gI_2/100 g, while *N. limnetica* CCMP505 with the highest iodine number at 119.35 ± 8.67 gI_2/100 g.

The cetane number is a dimensionless descriptor related to the ignition quality of a fuel in a diesel engine. Generally, the higher the cetane number, the better the ignition quality of the fuel and vice versa (Knothe, 2009). The cetane number of FAME increases with increasing saturation. In this study, the cetane number varied within a narrow range of 53.31 ± 0.78 – 58.05 ± 0.18 for microalgal biodiesels from 9 *Nannochloropsis* species. *N. oceanica* CCMP531 showed the highest cetane number may due to its higher saturated fatty acids content.

Taken together, according to the two most common quality standards for biodiesel, ASTM D6751 in the US and EN 14214 in Europe, and the ranges of qualities occurring in common biodiesel feedstocks (Hoekman et al., 2012), the values of kinematic viscosity, specific gravity, cetane number and iodine number of the 9 candidates completely satisfied the specifications. There are no definite specifications of cloud point, due to the different climate condition in the United States and Europe (Knothe, 2011). *N. oceanica* CCMP531 exhibited high cloud point around 10 °C, indicating poor cold flow properties, but they achieved a high cetane number ideal for diesel fuels in terms of combustion (Knothe, 2009). *N. oceanica* IMET1, the best feedstock for microalgal biodiesel production in 9 *Nannochloropsis* based on lipid and TAG productivity, also showed favorable biodiesel qualities within the prescribed limits.

4. Conclusions

Nannochloropsis has been considered as promising feedstock for microalgal biodiesel production. In this study, 9 *Nannochloropsis* from six recognized species including *N. gaditana*, *N. salina*, *N. granulata*, *N. limnetica*, *N. oceanica* and *N. oculata* was selected to evaluate their potential for biodiesel production. The best strain was *N. oceanica* IMET1, with lipid productivity of 158.76 ± 13.83 mg L^{-1} day^{-1}, TAG production of 1.67 ± 0.20 g/L, the favorable FA profiles of C16–C18 (56.62 ± 1.96%) as well as suitable biodiesel properties of higher cetane number (54.61 ± 0.25), lower iodine number (104.85 ± 2.80 gI_2/100 g) and relative low cloud point (3.45 ± 0.50 °C).

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References

Ahmad, A.L., Mat Yasin, N.H., Derek, C.J.C., Lim, J.K., 2011. Microalgae as a sustainable energy source for biodiesel production: a review. Renew. Sustainable Energy Rev. 15, 584–593.
Balasubramanian, R.K., Obbard, J.P., 2011. Heterogeneous catalytic transesterification of phosphatidylcholine. Bioresour. Technol. 102, 1942–1946.
Chen, C.Y., Chen, Y.C., Huang, H.C., Huang, C.C., Lee, W.L., Chang, J.S., 2013. Engineering strategies for enhancing the production of eicosapentaenoic acid (EPA) from an isolated microalga *Nannochloropsis oceanica* CY2. Bioresour. Technol. 147, 160–167.
Chen, L., Liu, T., Zhang, W., Chen, X.L., Wang, J.F., 2012. Biodiesel production from algae oil high in free fatty acids by two-step catalytic conversion. Bioresour. Technol. 111, 208–214.
Chisti, Y., 2007. Biodiesel from microalgae. Biotechnol. Adv. 25, 294–306.
Doan, T.T.Y., Sivaloganathan, B., Obbard, J.P., 2011. Screening of marine microalgae for biodiesel feedstock. Biomass Bioenergy 35, 2534–2544.
Fedosov, S.N., Brask, J., Xu, X., 2011. Analysis of biodiesel conversion using thin layer chromatography and nonlinear calibration curves. J. Chromatogr. A 1218, 2785–2792.
Francisco, E.C., Neves, D.B., Jacob-Lopes, E., Franco, T.T., 2010. Microalgae feedstock for biodiesel production: carbon dioxide sequestration, lipid production and biofuel quality. J. Chem. Technol. Biotechnol. 85, 395–403.
Gong, Y.M., Jiang, M., 2011. Biodiesel production with microalgae as feedstock: from strains to biodiesel. Biotechnol. Lett. 33, 1269–1284.
Griffiths, M.J., Harrison, S.T.L., 2009. Lipid productivity as a key characteristics for choosing algal species for biodiesel production. J. Appl. Phycol. 21, 493–507.
Hoekman, S.K., Broch, A., Robbins, C., Ceniceros, E., Natarajan, M., 2012. Review of biodiesel composition, properties, and specifications. Renew. Sustainable Energy Rev. 16, 143–169.
Jinkerson, R.E., Radakovits, R., Posewitz, M.C., 2013. Genomic insights from the oleaginous model alga *Nannochloropsis gaditana*. Bioengineered 4 (1), 37–43.
Knothe, G., 2005. Viscosity of biodiesel. In: Knothe, G., Van, G.J., Krahl, J. (Eds.), The Biodiesel Handbook. AOCS Press, Urbana, pp. 81–83.
Knothe, G., 2009. Improving biodiesel fuel properties by modifying fatty ester composition. Energy Environ. Sci. 2, 759–766.
Knothe, G., 2011. A technical evaluation of biodiesel from vegetable oils vs. algae. Will algae-derived biodiesel perform? Green Chem. 13, 3048–3065.
Li, Y.T., Han, D.X., Hu, G.R., Sommerfeld, M., Hu, Q., 2010. Inhibition of starch synthesis results in overproduction of lipids in *Chlamydomonas reinhardtii*. Biotechnol. Bioeng. 107 (2), 258–268.
Ma, Y.B., Wang, Z.Y., Zhu, M., Yu, C.J., Cao, Y.P., Zhang, D.Y., Zhou, G.K., 2013. Increased lipid productivity and TAG content in *Nannochloropsis* by heavy-ion irradiation mutagenesis. Bioresour. Technol. 136, 360–367.
Maxwell, K., Johnson, G.N., 2000. Chlorophyll fluorescence – a practical guide. J. Exp. Bot. 51 (345), 659–668.
Nascimento, I.A., Marques, S.S.I., Cabanelas, I.T.D., Pereira, S.A., Druzian, J.I., Souza, C.O.D., Vich, D.V., Carvalho, G.C.D., Nascimento, M.A., 2013. Screening microalgae strains for biodiesel production: lipid productivity and estimation of fuel quality based on fatty acids profiles as selective criteria. Bioenerg. Res. 6, 1–13.
Radakovits, R., Jinkerson, R.E., Fuerstenberg, S.I., Tae, H., Settlage, R.E., Boore, J.L., Posewitz, M.C., 2012. Draft genome sequence and genetic transformation of the oleaginous alga *Nannochloropsis gaditana*. Nat. Commun. 3, 686.
Rodolfi, L., Zittelli, G.C., Bassi, N., Padovani, G., Biondi, N., Bonini, G., Tredici, M.R., 2009. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnol. Bioeng. 102 (1), 100–112.
Scott, S.A., Davey, M.P., Dennis, J.S., Horst, I., Howe, C.J., Lea-Smith, D.J., Smith, A.G., 2010. Biodiesel from algae: challenges and prospects. Curr. Opin. Biotechnol. 21, 227–286.
Song, M.M., Pei, H.Y., Hu, W.R., Ma, G.X., 2013. Evaluation of the potential of 10 microalgal strains for biodiesel production. Bioresour. Technol. 141, 245–251.
Tan, Y.X., Lin, J., 2011. Biomass production and fatty acid profile of a *Scenedesmus rubescens*-like microalga. Bioresour. Technol. 102, 10131–10135.
Vieler, A., Wu, G.X., Tsai, C.H., Bullard, B., Cornish, A.J., Harvey, C., Reca, I.B., Shiu, S.H., Benning, C., 2012. Genome, functional gene annotation, and nuclear transformation of the heterokont oleaginous alga *Nannochloropsis oceanica* CCMP1779. PLoS Genet. 8 (11), e1003064.
Williams, P.J.B., Laurens, L.M.L., 2010. Microalgae as biodiesel & biomass feedstocks: review & analysis of the biochemistry, energetics & economics. Energy Environ. Sci. 3, 554–590.