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Integration process of biodiesel production from filamentous oleaginous microalgae *Tribonema minus*

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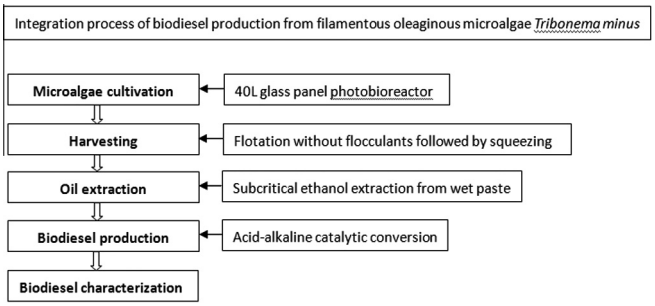
HIGHLIGHTS

- Biodiesel production from filamentous microalgae *Tribonema* sp. for the first time.
- Microalgal cells were successfully harvested by flotation without any flocculants.
- Lipids were extracted from wet pastes based on subcritical ethanol extraction.
- Acid-alkaline catalytic conversion was applied to produce microalgal biodiesel.

GRAPHICAL ABSTRACT



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ABSTRACT

Biodiesel production from microalgae has been receiving considerable attention. Past studies mainly relied on tiny sized single-cell oleaginous microalgal species, the biodiesel based on filamentous oleaginous microalgae was rarely reported. Thus, integrated process of biodiesel production from filamentous oleaginous microalgal strain *Tribonema minus* was studied in this work. The filamentous microalgae was cultivated for 21 days in 40 L glass panel, microalgae cells was harvested by DAF without any flocculants after the lipid content was 50.23%. After that, total lipid was extracted by subcritical ethanol from wet algal paste and 44.55% of crude lipid was triacylglycerols. Two-step catalytic conversion of pre-esterification and transesterification was adopted to convert the crude algal oil to biodiesel. The conversion rate of triacylglycerols reached 96.52% under the methanol to oil molar ratio of 12:1 during catalysis with 2% potassium hydroxide at 65 °C for 30 min. The biodiesel product from *T. minus* conformed to Chinese National Standards.

1. Introduction

With growing concerns surrounding the continued use of fossil fuels and the change of environment, biofuels have received attentions because of their sustainability and less eco-toxic (Hulatt and Thomas, 2011; Williams and Laurens, 2010). Biodiesel is composed of fatty acid alkyl esters produced from triacylglycerols (TAG), diacylglycerols (DAG), free fatty acids (FFA) and phospholipids (PL) (Vyas et al., 2010). Among three generations of biofuel feedstocks, the economically significant production of carbon-neutral biodiesel from microalgae has been hailed as one of promising alternative to deplete resources of petro-diesel due to its high cellular concentration of lipid, efficiency of CO_2 fixation, resources and economic sustainability and overall potential advantages over other sources of biofuels (Chisti, 2007).

Many oleaginous microalgae species have been screened for microalgae biodiesel production, but most of the oleaginous microalgae species were focused on single-cell oleaginous microalgae (cell dimension <30 μm) such as *Scenedesmus* sp. and *Chlorella* sp. (Abou-Shanab et al., 2011; Phukan et al., 2011). Though such algal

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species have higher lipid oil content and have been cultivated successfully in open pond and variety of photobioreactors, some obstacles derived of its tiny size deteriorate the technical and economic viability. The oleaginous algal cells with tiny size are usually palatable for grazers to promote the massive proliferation during cultivation, and then cause the crash of mass cultivation (Wang et al., 2013). Another is the difficulty of microalgae harvesting. Harvesting and then dewatering of single-cell microalgae from water remains a major hurdle to industrial scale processing and it was estimated to account for 20–30% of the total costs of microalgal biomass production (Mata et al., 2010). Flocculant additives are usually required for filtration, gravitational sedimentation or dissolved air flotation to ensure the effective harvesting recovery of such tiny single-cell algae cells. However the flocculant residuals in both algal biomass and harvested water are not only negative for later processing but also disadvantages for culture medium recycling. Adversely, filamentous algal species with bigger size like *Spirulina platensis* is easier to harvest (Zhang and Hu, 2012).

In addition, lots of literature researches on microalgae biodiesel production are available, but most of them are focused on separated single steps of algal strains screening (Reda et al., 2011; Sydney et al., 2011), cultivation (Angel et al., 2013; Sara et al., 2011), cell harvesting (Bosma et al., 2003; Zhang and Hu, 2012), oil extraction (Fajardo et al., 2007; Pourmortazavi and Hajimirsadeghi, 2007) and biodiesel conversion (Johnson and Wen, 2009; Chen et al., 2012). Meanwhile, lots of literatures are available on the single steps but only a few reports are available on the integrated process for microalgae-to-biodiesel approach.

A special filamentous microalgae species *Tribonema minus* from the Culture Collection of Algae of Gottingen University, was proved as a potent candidate for biodiesel production in preliminary evaluation. Hence, here an integrated process approach of microalgae biodiesel production with this strain for further study was carried out. The process includes cultivating in 40 L glass panel, harvesting by dissolved air flotation without flocculants, oil extracting with subcritical ethanol and followed biodiesel producing upon two-step catalytic conversion. It is shown that *T. minus* is a potential candidate oleaginous microalgae species for biodiesel production.

2. Methods

2.1. Microalgae and cultivation media

Microalgae *T. minus*, a genus of filamentous, freshwater yellow–green algae was provided by the Culture Collection of Algae of Gottingen University. Even though the single cell of *T. minus* shows column shape only in length of 7–9 μm long and width of 3–5 μm, *T. minus* is usually shaped bunchy in unbranched filaments to be relatively large, with sizes of 0.5–3 mm in length, which makes some individual cells visible to the naked eye. In this research, the culture medium used for cultivation was BG11 solution, the composition of which was described in Table 1.

Table 1
Composition of BG11 solution.

BG11		A5 Solution	
$NaNO_3$	1.5g L^{-1}		
K_2HPO_4	0.04 g L^{-1}	H_3BO_3	2.86 g L^{-1}
$MgSO_4{\cdot}7H_2O$	0.075 g L^{-1}	$MnCl_2{\cdot}4H_2O$	1.81 g L^{-1}
$CaCl_2{\cdot}2H_2O$	0.036 g L^{-1}	$ZnSO_4$	0.222 g L^{-1}
Na_2CO_3	0.02 g L^{-1}	Na_2MoO_4	0.39 g L^{-1}
Citric acid	0.006 g L^{-1}	$CuSO_4{\cdot}5H_2O$	0.079 g L^{-1}
Ferric ammonium	0.006 g L^{-1}	$Co(NO_3){\cdot}6H_2O$	49.4 g L^{-1}
EDTA	0.0001 g L^{-1}		
A5 solution	1 mL		

2.2. Microalgae cultivation in panel photobioreactor

The inoculation culture of *T. minus* was cultivated in 700 mL bubble column containing 500 mL culture bubbled with compressed air (1% CO_2) under 100 μmol photons m^{-2} s^{-1} of artificial light for 7 days. And then, algae paste was collected by silk screen and inoculated into each 40 L glass panel with 30 L BG11 medium. Artificial lights with 100 μmol photons m^{-2} s^{-1} were set in both sides of panels. Cultures were grown at 25 ± 2 °C and bubbled with compressed air (1.5% CO_2).

Microalgae dry weight (DW) was measured according to a method previously reported (Liu et al., 2013). A certain volume of microalgae culture was filtered to pre-weighted 0.45 μm GF/C filter membrane (Whatman, DW_0). The membrane was oven dried at 105 °C overnight and then weighted (DW_1). The *DW* was calculated as:

$$DW = \frac{DW_1 - DW_0}{v} \quad (1)$$

The total lipid content from algal sample was measured with gravimetric method (Bligh and Dyer, 1959). The lipid was extracted with methanol–chloroform (2:1, v/v) and then separated into chloroform and aqueous methanol layers by the addition of chloroform and 1% sodium chloride solution to give a final solvent ratio of methanol:chloroform:1% NaCl of 1:1:0.9. The chloroform layer was evaporated to dryness. Thereafter, the weight of the total lipid obtained from *T. minus* was measured gravimetrically.

Nile red staining was conducted to detect intracellular lipid droplets (Greenspan et al., 1985). After 21-day-cultivation, microalgal culture (0.5 mL) were collected by centrifugation for 10 min and washed with physiological saline solution three times. After the collected cells were re-suspended in the same solution (0.5 mL), the Nile red solution (0.1 g L^{-1} in acetone) was added to cell suspensions (1:10 v/v) and incubated for 10 min. After washing once, stained microalgal cells were observed by fluorescent microscopy.

2.3. Harvesting of algae paste

Flotation was applied to harvest microalgal cells from culture after 21-day-cultivation. After the lipid content of *T. minus* in two panels reached 50% at stationary phase, the culture grown in two panels was firstly harvested by dissolved air flotation (DAF) method without any coagulant. The flow rate of air dissolved water and water-feed volume ratio were 60 L h^{-1} and 2:1. Microalgal cells started to flotation after microalgal suspension was placed in the flotation column with 50 cm height and 50 mm diameter and the gas dissolved water was draw. The recovery efficiency was calculated as the ratio of biomass recovered to the total biomass and the concentration factor was the ratio of the final product concentration to the initial concentration (Bosma et al., 2003). The recovery efficiency and concentration factor were calculated as:

$$\text{Recovery effciency } (\%) = \frac{C_0V_0 - C_1V_1}{C_0V_0} \times 100 \quad (2)$$

$$\text{Concentration factor} = \frac{C_2}{C_0} \quad (3)$$

where C_1 is concentration of the supernatant from half the height of the clarified layer after flotation, C_2 is concentration of the sludge after flotation, and C_0 is concentration of the microalgal suspension before flotation. V_0 is the volume of microalgal suspension before flotation, and V_1 is the volume of microalgal supernatant after flotation.

The wet algae cake containing 75.56% of moisture was obtained after microalgal paste was squeezed in a net with 300 mesh for dewatering followed by flotation.

2.4. Extraction of oil from wet algae paste and analysis of lipid composition

Method of oil extraction from wet algae paste was used as described by Chen et al. (2011a). Briefly, algae paste was thawed at room temperature, and mixed with ethanol before it was loaded into the chamber of a high pressure extractor. Nitrogen gas was driven into the chamber to maintain a pressure of 1.5 MPa. The temperature of the extractor was maintained at 105 °C for 100 min. Samples were cooled to room temperature before the pressure was decreased. The extraction mixture was centrifuged to separate the oil solution and residual algae, the solvent was then evaporated using a rotary evaporator to recover algae oil. The recovery of the total lipids can be calculated by the following equation:

$$\text{Recovery rate of the total lipids} = \frac{\text{the lipid extracted in SEE}}{\text{total lipid content by B \& D method}} \quad (4)$$

Total lipid composition of *T. minus* was analysed using a thin-layer chromatography (TLC) system (TLC-FID, MK-6, Iatron Laboratories, Inc., Japan) (Fedosov et al., 2011). The individual lipid component was identified by co-chromatography with pure standards (SE&HC; FAME; FFA; TAG; DAG; MAG&PL, purchased from Sigma, St. Louis, MO, USA).

2.5. Biodiesel production from algae oil

Biodiesel production from algae oil was followed by two-step catalytic conversion described by Chen et al. (2012). A 5 g sample was mixed with 2 ml methanol containing 3.3% sulfuric acid. The mixtures were stirred at 65 °C for 120–180 min. After that, the treated oils were mixed with 2 ml of methanol containing potassium hydroxide (KOH) at 65 °C for 30 min. After the reaction, the mixture was cooled at room temperature and solvent was evaporated.

2.6. Fatty acid analysis of algae biodiesel

A 0.5 mg sample of purified biodiesel was dissolved in 1 mL heptane containing 50 μg heptadecanoic acid methyl ester ($C_{18}H_{37}COOCH_3$) as internal standard for FAME analysis on a Varian 450GC (Varian Inc., USA) equipped with a flame ionisation detector (FID) and Agilent HP-5 GC Capillary Column (30 m × 0.25 mm × 0.25 μm). Nitrogen was used as carrier gas. The injector temperature was set at 280 °C with an injection volume of 2 μL under split mode (10:1). The detector temperature was set at 280 °C. The individual FAMEs were identified by comparing their retention time against those of authentic standards.

2.7. Characterization of biodiesel

Density, kinematic viscosity, moisture content and distillation temperature (atmospheric equivalent temperature, 90% recovered) were tested to according to the procedures recommended by Chinese National Standard GB/T 20828-2007. Gross heating value was determined using an IKA Calorimeters C2000 (Germany) according to Chinese National Standard GB/T 384-81. To determine the acid value, samples were dissolved in a mixture of anhydrous ethanol and diethyl ether (1:1), and titrated with 0.1 mol L^{-1} KOH, according to Chinese National Standard (GB/T 264-1983).

2.8. Statistical analysis

Statistical analyses were conducted using the software SPSS 11.0 software (SPSS Inc., Chicago, USA). One-way analysis of variance (ANOVA) was used to evaluate the differences of specific growth rate, biomass concentration, FAME productivity among the treatments. If ANOVA effects were significant, comparisons between the different means were made using post hoc least significant differences (LSD) with a confidence level of 0.05.

3. Results and discussion

3.1. Biomass and lipid accumulation of T. minus in bench scale cultivation

The biomass and total lipid content of *T. minus* grown with 1.5% CO_2 under 100 μmol photons m^{-2} s^{-1} at 25 ± 2 °C in two 40 L glass panels are presented in Fig. 1. From an initial biomass of 0.13 g L^{-1}, the biomass increased rapidly and reached 2.88 g L^{-1} after 15-day-cultivation, and the biomass productivity was 0.17 g $L^{-1}d^{-1}$, which was similar to those of *Chlorella* sp., *Scenedesmus* sp., *Nanonochloropsis* sp. and *Isochrysis* sp. in similar culture scale reported by others (Ahmad et al., 2011; Angel et al.,2013). After that, the relatively slow growth of *T. minus* was shown, and finally a culture of 30 L with 3.14 g L^{-1} was obtained after 21 days.

The microalgae oil was extracted using methanol–chloroform method as described above. During the whole cultivation, the total lipid of *T. minus* increased slowly firstly and then rapidly rose after 9 days (Fig. 1). Total extracted oil content, expressed as dry weight percentage of microalgae, was 50.23% after 21-day-cultivation without any nutrient limitation. Though lots of reports have shown ca. 50% of the dry biomass weight (Reda et al., 2011; Spolaore et al., 2006), most of them were obtained in small culture device of ca. 150 mL^{-1} L. It's known that the total lipid content in large scale cultivation is usually much lower than that in small size of culturing device because of the differences of varieties of environmental factors, thus it's valuable to compare the lipid content of *T. minus* with those of other oleaginous microalgae species cultured in similar scale. According to the previous literatures (Chen et al., 2011b; Takagi et al., 2000), lipid contents of 19.3–34%, 12.7–19.6% and 29.9–40.3% of dry weight were obtained from *Chlorella* sp., *Scenedesmus* sp. and *Nannochloris* sp. respectively, in 10–60 L medium, all of which were much lower than that of *T. minus* in this study. Based on the biomass productivity and lipid content, the filamentous microalgal strain could be considered as a potential raw material for biodiesel production.

Triacylglycerols are main feedstock for transesterified in large scale processing. Therefore, quantities and fatty acid compositions

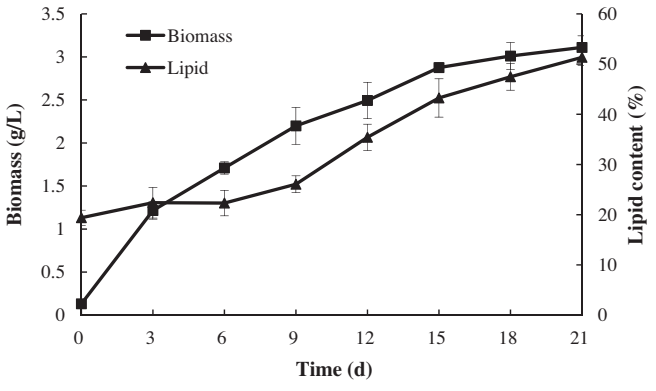

Fig. 1. Biomass and total lipid content of *T. minus* with 30 L culture.

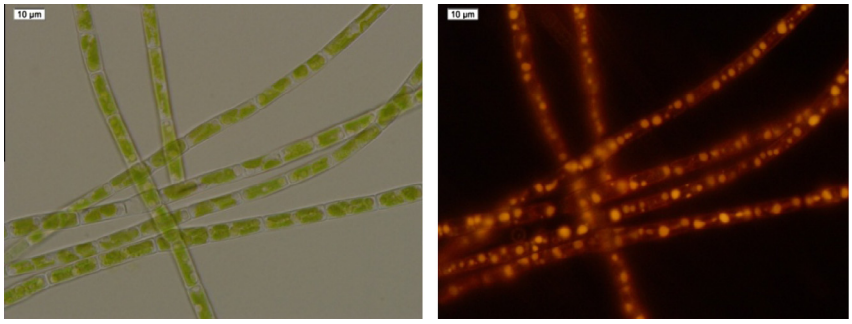

Fig. 2. Nile red staining of cells of *T. minus*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

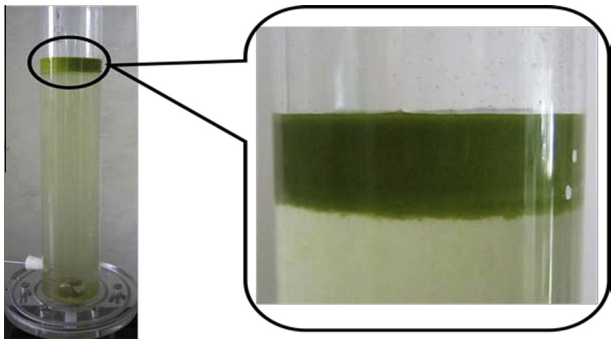

Fig. 3. Flotation harvesting of *s* without any flocculants.

of triacylglycerols in *T. minus* are important factors to evaluate whether they can serve as feedstocks for biodiesel production. Oil bodies in *T. minus* cells were visualised by Nile red staining as shown in Fig. 2. *T. minus* has unbranched filaments composed of a single row of elongated cells, the cytoplasm contains remarkable lipid droplets (neutral lipid), which presents a high neutral lipid content.

3.2. Recovery efficiency of T. minus with flotation without any flocculant

Flotation and finally dewatering by centrifugation or filtration appears to be the most promising option in microalgae harvesting. However, the addition of chemical flocculant, such as aluminum chloride ($AlCl_3$) and ferrus chloride ($FeCl_3$) is not only costly, but also deteriorates any later downstream processes (Angel et al., 2013). In previous study, Kim et al. reported that the flotation activity of *S. platensis* without flocculant showed a maximum value of 80% after 3 h of settling (Kim et al., 2005). That is, flotation without coagulation maybe suitable for filamentous microalgae with their natural flotation activity. Thus in this study, microalgae cells which were cultivated for 21 days in glass panel were harvested by dissolved air flotation (DAF) without any flocculants in laboratory.

Flocs appeared and an interphase was clearly observed(Fig. 3). The recovery efficiency was calculated as the ratio between the biomass in the flocs zone to the biomass of the entire volume at the beginning of the experiment (g/g), and the concentration factor was calculated as the ratio of the final product concentration to the initial concentration. From this formula, the recovery efficiency (95.57%) and the concentrate factor (11.3) indicated a good flotation activity of filamentous microalgae *Tribonema* sp. In our latest experiments of the cultivation of *T. minus* in 24 m^2 raceway pond, 98.69% recovery efficiency was obtained by air dissolved flotation without any flocculants in 3000 L/h and the harvested water was pumped into the ponds for a next batch cultivation, which had no negative effect on the growth of this microalgae.

Table 2
Chemical composition of lipid extracted from tested microalgae strain.

Chemical composition	Crude oil	Biodiesel
SE&HC	2.82 ± 0.01	2.78 ± 0.00
FAME	0 ± 0.00	95.47 ± 0.02
FFA	8.22 ± 0.01	0 ± 0.00
TAG	44.55 ± 0.02	1.54 ± 0.05
DAG	12.26 ± 0.01	0 ± 0.00
MAG&PL	40.36 ± 0.02	0.21 ± 0.00

3.3. The recovery rate of total lipid and chemical composition of algae oil and biodiesel

The use of solvents is a quicker and more efficient method for lipid extraction from microalgal cells than mechanical compression because of the tiny sizes of microalgal cells. While chloroform–methanol is effective in lipid extraction, large scale lipid extraction using chloroform is precluded by environmental and health risks.

Subcritical extraction used elevated pressures to keep the solvent as liquid state when the temperature reached above the boiling point, which greatly improved extraction efficiency. In previous study by Chen et al. (2012), subcritical ethanol and subcritical ethanol–hexane were used for oil extraction from microalgae paste. Considering ethanol is much cheaper than hexane, subcritical ethanol extraction was performed in total lipid extraction from microalgal paste. The advantage of this technology is that no freeze-drying process is needed, and thus could avoid the energy cost. The recovery rate of the total lipid was 72.82% when the solvent (ethanol (95%, v/v) to microalgae (dry weight) ratio (v/w) was 20:1 at 105 °C for 100 min. Therefore, the solvent to microalgae phase ratio, extraction temperature, pressure and time will be necessary to discussed in future to improve the recovery rate of total lipid.

The changes in chemical composition of lipid from wet algae pastes and biodiesel produced from lipid were listed in Table 1. The *T. minus* oil was transesterificated in presence of methanol and potassium hydroxide. As presented in Table 2, FFA (8.22%) in wet algae pastes was totally transformed into FAME. The TAG, DAG, MAG and PL contents (% of oil weight) decreased significantly from 44.55%, 12.26% and 40.36% to 1.91%, 0% and 1.85%, respectively. In contrast, the content of FAME was 94.47% on the basis of biodiesel weight while no FAME was detected in total lipid from wet algal paste. The conversion rate of triacylglycerols reached 96.52% under this experiment, indicating the conversion conditions could be explored in future study.

Table 3
Fatty acid analysis of algae biodiesel.

Fatty acid composition		Content
Saturated fatty acids (SFA)	C14:0	6.85 ± 0.7
	C15:0	0.56 ± 0.3
	C16:0	28.35 ± 1.5
	C18:0	1.02 ± 0.5
Monounsaturated fatty acids (MUFA)	C16:1	50.65 ± 3.5
	C18:1	2.96 ± 0.4
Polyunsaturated fatty acids (PUFA)	C16:2	1.55 ± 0.2
	C18:2	0.71 ± 0.0
	C20:3	1.20 ± 0.1
	C20:4	3.02 ± 0.1
	C20:5	3.14 ± 0.2

Table 4
Fatty acid analysis of algae biodiesel.

Item	Biodiesel	Limitation	Test methods
Density at 15 °C (kg/L)	0.83	0.82–0.90	GB/T 2540
Acid value (mg KOH/g oil)	0.64	0.80	GB/T 264
Kinematic viscosity at 40 °C (mm^2/s)	4.25	1.9–6.0	GB/T 265
Moisture content (%)	0.06	0.05	SH/T 0246
Sulfur content (%)	0.04	<0.05	SH/T 0689
Methyl ester content (%)	98.25	>96.5	EN 14103
Distillation temperature (°C)	314	<360	GB/T6536
Gross heating value (MJ/kg)	39.63	>35	GB/T 384-81

3.4. Fatty acid analysis of algae biodiesel

Fatty acid profile of microalgae biodiesel is also an important characteristic as it ultimately affects the quality of the biodiesel product (Knothe, 2005; Ramos et al., 2009), since the carbon chain length of saturated and unsaturated fatty acids affects biodiesel properties such as cetane number, oxidative stability and cold-flow properties (Smith et al., 2010). FAME composition of microalgae biodiesel which was determined by gas chromatography is shown in Table 3. *T. minus* biodiesel contains of saturated and unsaturated carbon chain lengths from C14 to C20. Special attention should be taken to the polyunsaturated fatty acids with four or more double bonds (C20:4, C20:5), which are susceptible to oxidation during storage and this reduces the acceptability as biodiesel. However, fatty acids with more than four double bonds can be reduced easily using an additional treatment such a partial catalytic hydrogenation of the oil (Dijkstra, 2006), which would not be a significant limitation for production of biodiesel. Moreover, the major components of *T. minus* biodiesel are saturated fatty acids (C14:0, 6.85% and C16:0, 28.35%) and palmitoleic acid (C16:1, 50.65%), which was the dominant monounsaturated fatty acid. Monounsaturated fatty acid methyl esters was considered to be better than polyunsaturated fatty methyl esters such as methyl linolenate and methyl linolenate for improving oxidative stability without any concomitant adverse effect on the cold properties of the diesel.

3.5. Characterization of biodiesel

Density, acid value, kinematic viscosity, sulfur content satisfied the criteria set by Chinese National Standards (Table 4). The methyl ester content of biodiesel from *T. minus* was 98.25%, which is higher than the required value of 96.5%. Distillation temperature (314 °C) was lower than Limitation (360 °C), meanwhile, the heating value of biodiesel sample was 39.63 MJ kg^{-1}, which is comparable to that of fossil oil of 42 MJ kg^{-1}. An exception was the moisture content (0.06%) of biodiesel from *T. minus*, which was slightly higher than the limit of 0.05%.

4. Conclusions

To explore the potential and integrated process of filamentous microalgae for biodiesel production, a filamentous microalgal strain *T. minus* was tested in this study. The biomass and lipid content of *T. minus* were 3.14g L^{-1} and 50.2% of dry weight after 21-day-cultivation in 40 L glass panel. Microalgal cells were successfully harvested by flotation without any flocculants followed by squeezing. Two-step catalytic conversion of was adopted to produce biodiesel after subcritical ethanol extraction of lipid. The properties of *T. minus* biodiesel satisfied the criteria set by Chinese National Standards. This study indicates that *T. minus* is a valuable candidate for use in biodiesel production.

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References

Abou-Shanab, R.A.I., Matter, I.A., Kim, S.N., Oh, Y.K., Choi, J., Jeon, B.H., 2011. Characterization and identification of lipid-producing microalgae species isolated from a freshwater lake. Biomass Bioenergy 35, 3079–3085.
Ahmad, A.L., MatYasin, N.H., Derek, C.J.C., Lim, J.K., 2011. Microalgae as a sustainable energy source for biodiesel production: a review. Renew. Sust. Energy Rev. 15, 584–593.
Angel, S., Rocio, M., Angeles, C., Alfonso, P., 2013. Culture aspects of *Isochrysis galbana* for biodiesel production. Appl. Energy 101, 192–197.
Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.
Bosma, R., Sporonsen, W., Tramper, J., Wiffels, R., 2003. Ultrasound, a new separation technique to harvest microalgae. J. Appl. Phycol. 215, 143–153.
Chen, C.Y., Yeh, K.L., Aisyah, R., Lee, D.J., Chang, J.S., 2011b. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. Bioresour. Technol. 102, 71–81.
Chen, M., Chen, X.L., Liu, T.Z., Zhang, W., 2011a. Subcritical ethanol extraction of lipid from wet microalgae paste of *nanochloropsis* sp.. J. Biobased Mater. Bioenergy 5, 1–5.
Chen, L., Liu, T.Z., 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.
Dijkstra, A.J., 2006. Revisiting the formation of transisomers during partial hydrogenation of triacylglycerol oils. Eur. J. Lipid Sci. Technol. 108, 249–264.
Fajardo, A., Cerdan, L.E., Medina, A.R., Fernandez, F.G.A., Moreno, P.A.G., Grima, E.M., 2007. Lipid extraction from the microalga *Phaedactylum tricornutum*. Eur. J. Lipid Sci. Technol. 109, 120–126.
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.
Greenspan, P., Mayer, E.P., Fowler, S.D., 1985. Nile red: a selective fluorescent stain for intracellular lipid droplets. J. Cell Biol. 100, 965–973.
Hulatt, C.J., Thomas, D.N., 2011. Productivity, carbon dioxide uptake and net energy return of microalgal bubble column photobioreactors. Bioresour. Technol. 102, 5775–5787.
Johnson, M.B., Wen, Z.Y., 2009. Production of biodiesel fuel from the microalga *Schizochytrium limacinum* by direct transesterification of algal biomass. Energy Fuels 23, 5179–5183.
Kim, S.G., Choi, A., Ahn, C.Y., Park, C.S., Park, Y.H., Oh, H.M., 2005. Harvesting of *Spirulina platensis* by cellular flotation and growth stage determination. Lett. Appl. Microbiol. 40, 190–194.
Knothe, G., 2005. Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. Fuel Process. Technol. 86, 1059–1070.
Liu, T.Z., Wang, J.F., Cheng, P.F., Ji, B., Liu, J.L., Chen, Y., Zhang, W., Chen, X.L., Chen, L., Gao, L.L., Ji, C.L., Wang, H., 2013. Attached cultivation technology of microalgae for efficient biomass feedstock production. Bioresour. Technol. 127, 216–222.
Mata, T.M., Martins, A.A., Caetano, N.S., 2010. Microalgae for biodiesel production and other applications: a review. Renew. Sust. Energy Rev. 14, 217–232.
Phukan, M.M., Chutia, R.S., Konwar, B.K., Kataki, R., 2011. Microalgae *Chlorella* as a potential bio-energy feedstock. Appl. Energy 88, 3307–3312.

Pourmortazavi, S.M., Hajimirsadeghi, S.S., 2007. Supercritical fluid extraction in plant essential and volatile oil analysis–review. J. Chromatogr. A 1163, 2–24.

Ramos, M.J., Fernandez, C., Casas, A., Rodriguez, L., Perez, A., 2009. Influence of fatty acid composition of raw materials on biodiesel properties. Bioresour. Technol. 100, 261–268.

Reda, A.I., Abou-Shanab, I., Ivrahim, A.M., Su-Nam, K., You-Kwan, O., Jaeyoung, C., Byong-Hun, J., 2011. Characterization and identification of lipid-producing microalgae species isolated from a freshwater lake. Biomass Bioenergy 35, 3079–3085.

Sara, R.A., Nima, M.N., Mohamad, A.M., Samira, H.A., Younes, G., 2011. *Chlorella* sp.: a new strain with highly saturated fatty acids for biodiesel production in bubble-column photobioreactor. Appl. Energy 88, 3354–3356.

Smith, P.C., Ngothai, Y., Nguyen, Q.D., O'Neill, B.K., 2010. Improving the low-temperature properties of biodiesel: methods and consequences. Renewable Energy 35, 1145–1151.

Spolaore, P., Joannis-Cassan, C., Duran, E., Isambert, A., 2006. Commercial applications of microalgae. J. Biosci. Bioeng. 101, 87–96.

Takagi, M., Watanabe, K., Yamaberi, K., Yoshida, T., 2000. Limited feeding of potassium nitrate for intracellular lipid and triglyceride accumulation of *Nannochloris* sp. UTEX LB1999. Appl. Microbiol. Biotechnol. 54, 112–117.

Vyas, A.P., Verma, J.L., Subrahmanyam, N., 2010. A review on FAME production processes. Fuel 89, 1–9.

Wang, H., Zhang, W., Chen, L., Wang, J.F., Liu, T.Z., 2013. The contamination and control of biological pollutants in mass cultivation of microalgae. Bioresour. Technol. 128, 745–750.

Williams, P.J.I.B., Laurens, L.M.L., 2010. Microalgae as biodiesel & biomass feedstocks: review & analysis of the biochemistry, energetic &economics. Energy Environ. Sci. 3, 554–590.

Zhang, J.G., Hu, B., 2012. A novel method to harvest microalgae via co-culture of filamentous fungi to form cell pellets. Bioresour. Technol. 114, 529–535.