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Pyrolytic characteristics of biodiesel prepared from lipids accumulated in diatom cells with growth regulation

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Dynamic compositions of lipids accumulated in two diatoms *Chaetoceros gracilis* and *Nitzschia closterium* cultured with nitrogen and silicon deprivation were studied. It was found that short-chain fatty acids (C_{14}–C_{16}) content was much higher than long-chain fatty acids (C_{18}–C_{20}) content in lipids of two diatoms. The pyrolytic characteristics of biodiesel made from two diatoms and two plant seeds were compared by thermogravimetric analysis. The highest activation energy of 46.68 kJ mol^{-1} and the minimum solid residue of 25.18% were obtained in the pyrolysis of biodiesel made from *C. gracilis* cells, which were cultured with 0.5 mmol L^{-1} of nitrogen (no silicon) and accumulated the minimum polyunsaturated fatty acid ($C_{20:5}$). The pyrolysis residue percentage of *C. gracilis* biodiesel was lower than that of *N. closterium* biodiesel and higher than those of plant (*Cormus wilsoniana* and *Pistacia chinensis*) biodiesels.

[**Key words:** Biodiesel; Diatom; Fatty acids; Pyrolysis; Thermogravimetric analysis]

Microalgae is valued owing to its rapid growth rate, high lipid productivity, easy-to-cultivate characteristics, and adaptability to the environment (1–3). As a biological energy source, microalgae can absorb considerable CO_2 and NOx from power plant (4,5); this process can improve biomass productivity and clean the air, and it does not compete with land use for food production (6,7). A large quantity of mudflats and wetlands is available on the 18,000-km shorelines in China. These mudflats and wetlands are suitable for large-scale farming and cyclic utilization of cultivating microalgae, which are new types of oil sources for biodiesel production that can be developed in future (8,9).

At present, increasing number of studies on biodiesel production and analyses of fatty acid composition of green algae are being conducted. In the research of Stephenson et al. (10), *Chlorella* lipids were substantially enriched when the cells were cultured under nitrogen starvation. Futhermore, the concentration of $C_{18:3}$ was very high under various culture conditions. However, that study did not focus on how the composition of lipids changed with time during the cultivation. In the study conducted by Xu et al. (11), the heat value of biodiesel produced from green algae reached up to 41 MJ kg^{-1}, the density was 0.864 kg L^{-1}, and the viscosity was 5.2×10^{-4} Pa s (40°C). However, the difference between bio-oil extracted from microalgal cells cultured with standard medium and nitrogen-deprived medium was not compared. Although some reports are available on biodiesel production using diatoms, relatively few studies have focused on the characteristics of biodiesel prepared from diatoms (12–14). Breteler et al. (15) reported the lipid composition of the diatom *Thalassiosira weissflogii* under nitrogen and silicon deficiency, but did not consider biodiesel properties. Levitan et al. (16) summarized the lipid composition of most diatoms to account for their potential to replace fossil fuel. However, no detailed description had been provided about the pyrolytic characteristics.

Thermogravimetric analysis (TGA) is an effective technique, which can measure the weight changes with the increasing temperature in its physicochemical properties, to test the characteristics of the materials (17). Chand et al. (18) had proved TGA was a convenient, economical, and simple method for monitoring biodiesel production, which results were within ±1.5% relative to those of proton nuclear magnetic resonance method. Dantas et al. (19) reported the decomposition and thermal stability of biodiesel by using TGA-DTA curves analysis. The temperature programming, which started from 30°C to 600°C at a heating rate of 10°C min^{-1}, was conducted to obtain the mechanism, kinetic parameters and activation energy. However, it focused on the corn biodiesel instead of the algae biodiesel. Furthermore, TGA analysis was used to study the thermo oxidative behavior of *Jatropha curcas* oil extensively (20–22), but few of microalgae biodiesel.

The dynamic compositions of lipids accumulated in two diatoms *Chaetoceros gracilis* and *Nitzschia closterium* cultured with nitrogen and silicon deprivation were studied. The pyrolytic characteristics of biodiesel made from two diatoms and two plant seeds were compared by thermogravimetric analysis. The highest activation energy of 46.68 kJ mol^{-1} and the minimum solid residue of 25.18% were obtained in the pyrolysis of biodiesel made from *C. gracilis* cells with the minimum polyunsaturated fatty acid ($C_{20:5}$).

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MATERIALS AND METHODSMATERIALS-METHODS

Diatom strains and culture media The diatom *C. gracilis* and *N. closterium* were provided by the Institute of Oceanology, Chinese Academy of Sciences. Microalgal cells were cultured in f/2 medium (23), which contained no silicon. The cultivations were kept at 23°C ± 2°C under a 12 h: 12 h (light: dark) cycle illumination of 5000 lux in a BSG-250 incubator, BoXun, China. The initial nitrogen concentration was adjusted to 0.5, 2.0, 3.0, and 5.0 mmol L^{-1} to achieve a change in the rules of lipids in microalgal cells. The nitrogen and silicon concentrations in standard f/2 medium were 12.0 and 0.7 mmol L^{-1}, respectively. The initial pH values of the *C. gracilis* and *N. closterium* media were adjusted to 8.3 and 6.8 using 0.1 mol L^{-1} NaCl and 0.1 mol L^{-1} HCl, respectively. The lipid contents of the two stains cultured under different conditions are shown in Table 1.

Lipid extraction and biodiesel preparation The lipids were extracted from microalgal cells using the improved Bligh-Dyer method (24–26) after centrifugation (5000 ×*g*, 10 min, at 8°C) and drying (70°C, for 24 h). The lipids were converted into biodiesel using base catalysis interesterification, and then analyzed by gas chromatography (GC, GC160, FFAP 30 m × 0.25 mm × 0.32 μm, at the maximum temperature of 250°C) after being mixed with n-hexane. Temperature program was as follows: the initial oven temperature was 150°C, which was maintained for 5 min, heated up to 250°C at the heating rate of 4°C min^{-1}, and maintained for 5 min (27). The biodiesels prepared from lipids, which were extracted from ligneous plants (*Cormus wilsoniana* and *Pistacia chinensis*) by Soxhlet extraction (28,29), were used to compare with the diatom biodiesels.

Thermal analysis of biodiesel pyrolysis Thermal analysis is a type of technique, that uses thermal balance to measure the relationship between temperature and quality of materials under a certain temperature program. Differential thermogravimetry analysis (DTG) of biodiesel pyrolysis could be performed by considering the derivative of temperature time with respect to the TGA curves. TGA was performed under program control of constant heating rate with a high sensitivity using micro samples. Many experiments of biodiesel pyrolysis on TGA at different heating rates (20°C min^{-1}, 40°C min^{-1}, and 80°C min^{-1}) were conducted. It was found that experimental errors of biodiesel pyrolysis temperatures increased with an increased heating rate. This was because heat transfer between furnace wall and biodiesel samples was inadequate when furnace heating rate increased, which resulted in increased temperature gradient and pyrolysis temperatures of biodiesel. Therefore, the optimal experimental parameters were determined as follows: the heating rate was 20°C min^{-1}, the temperature ranged from 40°C to 600°C, the gas was nitrogen, and the gas flow rate was 50 mL min^{-1}.

RESULTS AND DISCUSSION

Dynamic compositions of lipids accumulated in diatom cells The moisture content in microalgae biomass after suspension centrifugation of *C. gracilis* and *N. closterium* were 81% and 83%, respectively. Then the microalgae biomass was dried in an oven at 105°C to a constant weight. The highest lipid contents in dried biomass of *C. gracilis* and *N. closterium* were 32.78% and 21.96%, respectively (Table 1). The ash contents in dried biomass of *C. gracilis* and *N. closterium* were 22% and 18%, respectively, which were weighed after microalgae biomass were burned in a furnace at 540°C for 4 h.

Figs. 1 and 2 show the influences of nitrogen concentration and culture time by considering the percentage of various lipid compositions of the biomass dry weight as investigation object. Based on the nitrogen consumption process in microalgal cultivation medium, three stages existed during the growth metabolic process: the nitrogen saturation stage, nitrogen deficiency-growth inhibition stage, and nitrogen deficiency-lipid enrichment stage. The metabolism of cells was on the rails in the first stage; thus the lipids exhibited a slow growth trend over time. As the nitrogen source was consumed, less and less nitrogen could be utilized by cells. Consequently, metabolic pathways of cells were restrained and lipid content displayed a downward trend. However, when the cells began to adapt to nitrogen starvation and protein synthesis sharply decreased, lipid synthesis was relatively enhanced. Consequently, the lipid components gradually increased. Nevertheless, the lipids could not be synthesized any longer because of slow metabolism and growth arrest, which seriously damaged the physical health of microalgal cells, when the cells experienced excessive nitrogen deficiency for a long time.

The nitrogen demand of *N. closterium* was much greater than that of *C. gracilis* (30). Nitrogen deficiency-growth inhibition period occurred on the 16th day, and the nitrogen deficiency-lipid enrichment period occurred on the 24th day. After the 32nd day, the growth of the *N. closterium* cells stopped, and the various lipid compositions decreased when the cells were cultured with 5.0 mmol L^{-1} nitrogen. In contrast, the nitrogen supply for *C. gracilis* cells was abundant until the 40th day under the same cultivation condition. The lipids were gradually increased with culture time. Lipid compositions of different algae species expressed different change trend to the nitrogen concentration. The content of short-chain saturated and partially saturated fatty acids decreased, whereas that of the short-chain and long-chain PUFAs increased with an increase in the nitrogen concentration in *C. gracilis* cells. It was coincided with algae *Dunaliella salina* and *Ankistrodesmus*, which were studied in the National Renewable Energy Laboratory's research (8). However, short-chain fatty acids increased, whereas the long-chain fatty acids decreased with an increase in the nitrogen concentration in *N. closterium* cells, which was similar to the algae *Botryococcus braunii* (8). Despite the different trends of the two diatom strains, the same point was obtained, i.e., the content of short-chain fatty acids was the highest, whereas that of long-chain fatty was the lowest, at the highest lipid content cultivation condition (nitrogen concentration: 0.5 mmol L^{-1} for *C. gracilis*, and 5.0 mmol L^{-1} for *N. closterium*).

Pistacia chinensis plays an important role in biodiesel industry because of its great advantages in terms of fatty acid components; mostly, the length of carbon chains of these fatty acid components is between C_{16} and C_{18} (31). Table 2 shows the comparison of lipid compositions between the two diatom strains and two ligneous plants. The saturation of the fatty acids in the *C. wilsoniana* and *P. chinensis* was higher than that in the diatoms. Mostly, the length of the carbon chains of fatty acids in two diatom strains was between C_{14} and C_{20}, including that of PUFA, such as $C_{20:5}$. PUFA and long-chain fatty acids may reduce the stability, liquidity, and low-temperature resistance of biodiesel. Understanding how to break them and modify and upgrade the processes will require further studies.

Pyrolytic characteristics of diatom biodiesel The heat values of biodiesel prepared from two diatoms *C. gracilis* and *N. closterium* were 40.6 MJ kg^{-1} and 40.1 MJ kg^{-1}, respectively. Fig. 3 shows the pyrolysis curves of the biodiesels of two diatom strains and two ligneous plants. The biodiesel pyrolysis procedure in N_2 atmosphere was divided into three stages: (i) low temperature stage: moisture was evaporated and small-molecule compositions were decomposed into volatile gas. (ii) Middle temperature stage:

TABLE 1. Lipid contents of diatoms cultured with different concentration of nitrogen and silicon in f/2 media for different time.

		C. gracilis	*C. gracilis*	*C. gracilis*	*C. gracilis*	*N. closterium*	*N. closterium*	*N. closterium*	*N. closterium*
Si concentration (mmol L^{-1})		200	0	0	0	200	0	0	0
N concentration (mmol L^{-1})		12	5	3	0.5	12	5	2	0.5
Lipid content (%)	8 days	13.25	13.49	17.92	20.19	12.23	17.38	15.07	17.12
	16 days	–	20.68	21.42	23.88	–	21.72	19.72	20.27
	24 days	–	20.9	22.4	24.27	–	21.96	21.11	20.98
	32 days	–	23.52	25.12	31.74	–	17.88	17.48	14.08
	40 days	–	24.59	24.75	32.78	–	14.79	15.33	15.72