Bioresource Technology 229 (2017) 119–125

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Pyrolysis characteristics and pathways of protein, lipid and carbohydrate isolated from microalgae *Nannochloropsis* sp.

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HIGHLIGHTS

- Protein, lipid and carbohydrate were isolated for microalgal component pyrolysis.
- Relationship between DTG curves of microalgae and their isolated components.
- Experimental *N* was 0.6776, 0.3861 and 0.2856 for lipid, protein and carbohydrate.
- Recommended pyrolysis pathways by biocrude composition of isolated algal components.
- Steroids and lipopolysaccharides increased hydrocarbon content in bio-crude.

ARTICLE INFO

Article history:
Received 11 November 2016
Received in revised form 7 January 2017
Accepted 9 January 2017
Available online 12 January 2017

Keywords:
Microalgae
Component isolation
Pyrolysis mechanism
Protein
Lipid
Carbohydrate

ABSTRACT

Microalgal components were isolated gradually to get lipid-rich, protein-rich and carbohydrate-rich components. The aim of this work was to study pyrolysis mechanism of microalgae by real isolated real algae components. Thermogrametric analysis (DTG) curve of microalgae was fitted by single pyrolysis curves of protein, lipid and carbohydrate except special zones, which likely affected by cell disruption and hydrolysis mass loss. Experimental microalgae liquefaction without water index *N* was 0.6776, 0.3861 and 0.2856 for isolated lipid, protein and carbohydrate. Pyrolysis pathways of lipid are decarboxylation, decarbonylation, fragmentation of glycerin moieties and steroid to form hydrocarbons, carboxylic acids and esters. Pyrolysis pathways of protein are decarboxylation, deamination, hydrocarbon residue fragmentation, dimerization and fragmentation of peptide bonds to form amide/amines/nitriles, esters, hydrocarbons and N-heterocyclic compounds, especially diketopiperazines (DKPs). Pyrolysis pathways of carbohydrate are dehydrated reactions and further fragmentation to form ketones and aldehyde, decomposition of lignin to form phenols, and fragmentation of lipopolysaccharides.

1. Introduction

As the third generation biofuels microalgae have drawn much attention. They have high lipid content, fast growth rate and less land competition with agricultural crops. Microalgae can be feedstock source in many liquid biofuel production processes, such as bio-diesel (Miao and Wu, 2006), hydrotreated renewable jet fuels (Zhou and Lawal, 2015) and Fischer-Tropsch fuels (Kim et al., 2013). Microalgae pyrolysis is also one of important pathways to produce alternative liquid fuels (Rizzo et al., 2013). It is a thermal decomposition process at an oxygen-free condition with the main products of biocrude, char and gas. Many researches have investigated pyrolysis temperature/holding time (Peng et al., 2000), reactors type (Hu et al., 2012; Wang et al., 2013), and biocrude composition and so on. The main components of microalgae are lipid, protein and carbohydrate. The indexes *N* of the apparent liquefaction ability, containing biocrude and water, were 0.7, 0.6 and 0.3 for microalgae lipid, carbohydrate and protein respectively (Yang et al., 2014). Defatted microalgae pyrolysis pathway with lipid extraction has the higher total apparent oil yield than direct pyrolysis (Wang et al., 2015a). These microalgal components have different chemical structures and their pyrolysis pathways should be researched for the aim of alternative biofuel production process optimization and microalgae-based biorefineries.

As one of important components in microalgae (Eboibi et al., 2015), the monomers of protein are amino acids, which are connected by peptide bond. During pyrolysis process, cyclic reactions and dimerization reactions proceed among these amino acids (Choi and Ko, 2010, 2011). Pyrolysis of twenty amino acids in the presence of catalyst tetramethylammonium hydroxide (TMAH) by

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http://dx.doi.org/10.1016/j.biortech.2017.01.018
0960-8524/

pyrolysis-gas chromatography–mass spectrometry (Py-GC–MS) provided diagnostic signals of amino acid profile for natural samples containing protein (Gallois et al., 2007). Catalytic pyrolysis of aliphatic amino acids and cyclic amino acids also showed that chemical structures of amino acids had significant effects on product distribution (Liu et al., 2016). For extracted microalgal protein, the main pyrolysis temperature range was 300–500 °C based on thermogrametric curves (Kebelmann et al., 2013).

Lipid content in microalgae depends on microalgae species. Its content can be up to 40% for some engineered microalgae (Feng et al., 2011). The main compositions of lipid are neutral lipids, phosphoglycerides, sphingolipids, glycolipids, peptidolipids and peptidoglycolipids. For triglycerides, the main structures are glycerin with three long chain ester moieties, which can form fatty acids during pyrolysis. Pyrolysis of oleic acid (fatty acid model compound) in inert and light hydrocarbon pyrolysis environment indicated that light hydrocarbon pyrolysis environment significantly increased liquid yield (Asomaning et al., 2014). Gasoline production from fatty acids by catalytic cracking was evaluated and gasoline yield of 44 wt% was obtained from oleic acid at 550 °C (Bielansky et al., 2012). The unsaturation degree of fatty acids had significant effect on the formation of volatile compounds in pyrolysis as well as pyrolysis temperature and reaction time (Lappi and Alen, 2009).

Carbohydrate pyrolysis characteristics have also been studied. Four kinds of carbohydrates of brown macroalgae have been pyrolyzed by Py-GC–MS and the relationship between their biochemical composition and thermogrametric analysis (TGA) data was established (Anastasakis et al., 2011). The thermal conversion of glucose to aromatic hydrocarbons via pressurized secondary pyrolysis indicated that secondary cracking of primary pyrolysis products of biomass oxygenates undergo gas-phase homogenous molecular restructuring (Gunawardena and Fernando, 2011). Fast pyrolysis of several mono-, di- and polysaccharides was studied with the help of micro-pyrolyzer, which indicated that levoglucosan and the low molecular weight compounds were formed through competitive pyrolysis reactions rather than sequential pyrolysis reactions (Patwardhan et al., 2009). The research about chain length (or end-group) effect indicated that levoglucosan increased continuously with degree of polymerization (Mettler et al., 2012).

Besides, interactions can occur among these components of microalgae. One of interactions occurs between protein and carbohydrate, named Maillard reaction (Zhang et al., 2016), which forms a large number of products such as aroma compounds, ultra-violet absorbing intermediates, and dark-brown polymeric compounds (Wijewickreme et al., 1997). Synergetic deoxy reforming of cellulose and fatty acid esters can form liquid hydrocarbon-rich oils (Wang et al., 2015b). Production of surfactants and detergents was also based on their interactions, such as protein-fatty acid condensates (Peng et al., 2016) and alkyl polyglycosides (El-Sukkary et al., 2008).

However, most of these researches focused on pyrolysis parameter optimization of raw microalgae or defatted microalgae (Watanabe et al., 2014). Researches about protein, lipid and carbohydrate pyrolysis were also conducted by model components or their monomers only and further researches are needed to figure out the detailed pyrolysis reaction mechanisms or pathways by real microalgal components. In this study, pyrolysis characteristics and pathways of protein, lipid and carbohydrate isolated from microalgae *Nannochloropsis* sp. were conducted. *Nannochloropsis* sp. has the potential for industrial application by large-scale cultivation with a coal based power plant (Zhao et al., 2016), which is also one of potential microalgae feedstocks for biofuel production (Beacham et al., 2014). Microalgae component pyrolysis mechanisms were inferred by thermogrametric analysis and biocrude composition tested by Gas Chromatography–Mass Spectrometry method (GC–MS). Accordingly, novel and detailed microalgae components pyrolysis pathways are proposed for microalgae pyrolysis reactor design and process optimization and aimed product quality upgrading in biomass refineries.

2. Materials and methods

2.1. Microalgae sample

Microalgae *Nannochloropsis* sp. powder was purchased from Yantai Hairong Microalgae Breeding Co., Ltd. (Shandong Province of P.R. China). The element and composition analysis and their test methods were shown in Table 1. The parameter test methods including moisture, ash, biochemical composition and element analysis can also refer to previous researches (Tang et al., 2016). Duplicate or triplicate experiments were conducted for each test. The fatty acid profile of microalgae (Table 2) was measured by direct transesterification and Gas Chromatography–Mass Spectrometry method (Agilent GC–MS 7890A/5975C). The detailed transesterification procedure can refer to Laboratory Analytical Procedure of National Renewable Energy Laboratory (American) for determination of Total Lipids as Fatty Acid Methyl Esters (FAME) by *in situ* transesterification (Van Wychen and Laurens, 2013).

2.2. Protein, lipid and carbohydrate isolation

Isolation of protein, lipid and carbohydrate from microalgae were conducted. Firstly, microalgal lipid was extracted by co-solvent (dichloromethane and methanol, 2:1, v/v) and the amount of co-solvent was 250 mL per 10 g dry microalgae. The operation temperature was controlled at 30–35 °C and the stirring time was 5 h. The extraction process was conducted twice and for the second extraction the stirring time was 2 h. After each extraction, the mixture of microalgae and co-solvent was filtered and the co-solvent was evaporated by a rotaevaporator (atmospheric pressure and 80 °C). When all the solvent had evaporated, lipids were determined gravimetrically. Microalgae after solvent extraction were named defatted microalgae.

Secondly, microalgal protein was isolated by hydrolysis (2 M NaOH) and precipitation method. Before the protein isolation, the isolation parameters (hydrolysis temperature and pH in precipitation) were optimized by measuring soluble protein content in hydrolysis process with Branford method (Coomassie® Brilliant Blue G-250 dye). Because the maximum absorbance of Coomassie® Brilliant Blue G-250 dye shifts from 465 nm to 595 nm when binding to protein. The optimal isolation parameters were 80 °C and

Table 1
Composition and element analysis of microalgae *Nannochloropsis* sp.

	Percentage (w/w, ar)	Test methods or standards
Moisture (%)	3.14 ± 0.06	Drying (105 °C, 24 h)
Ash (%)	8.9 ± 0.32	Heating (575 °C, 3 h)
Protein (%)	36.4 ± 4.6	Bicinchoninic Acid (BCA) method
Carbohydrate (%)	12.4 ± 0.9	Sulfuric acid-anthrone method
Fatty acids (%)	19.05 ± 0.13	*In situ* Transesterification
Lipid (%)	27.8 ± 0.9	Chloroform-methanol extraction
C (%)	49.27 ± 0.93	GB/T 476-2001
H (%)	7.27 ± 0.12	GB/T 476-2001
O (%)	32.39 ± 0.50	GB/T 476-2001
N (%)	6.29 ± 0.09	GB/T 476-2001
S (%)	0.83 ± 0.01	GB/T 214-1996
High heat value (MJ/kg)	20.5	Calculated according to Dulong's function