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Simultaneous hydrolysis-esterification of wet microalgal lipid using acid

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HIGHLIGHTS

- Simultaneous hydrolysis-esterification could enable no uses of extraction and drying.
- Water content is the most significant factor in simultaneous process.
- Equimolar amounts of sulphuric acid and hydrochloric acid showed similar effects.

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ABSTRACT

This research demonstrated hydrolysis of wet microalgal lipid and esterification of free fatty acid (FFA) using acid in one-step process. The investigation of simultaneous hydrolysis-esterification (SHE) of wet microalgal lipid was conducted by using L_{27} orthogonal design and the effects of water content, volume of sulphuric acid, volume of methanol, temperature and time on SHE were examined. As a result, water content was found to be the most effective factor. The effects of various parameters on fatty acid methyl ester (FAME) content and equilibrium relation between FAME and FFA were also examined under water content 80%. Equimolar amounts of sulphuric acid and hydrochloric acid showed similar results. This method has great potential in terms of biodiesel production from microalgae since no organic solvents are used.

1. Introduction

Biodiesel is receiving much attention due to its potential as a viable alternative to fossil fuel. It can be produced from oils derived from plants, animals or microbes (Graboski and McCormick, 1998). Biodiesel is generated through various techniques such as direct/oil blends, microemulsion, pyrolysis and transesterification (Ma and Hanna, 1999). Especially, transesterification is the most common process for making biodiesel (Baroutian et al., 2008). Transesterification consists of a number of consecutive reversible reactions (Freedman et al., 1986). Triglyceride (TG) is converted stepwise to diglyceride, monoglyceride and finally glycerol. A mole of fatty acid methyl ester (FAME) is liberated at each step.

Microalgae are considered as advantageous materials to produce biodiesel over other sources. Microalgae have higher growth rates of biomass and oil productivities than conventional crops because of their simple cellular structure (Becker, 1994) and have been claimed to be up to 20 times more productive per unit area than palm oil (Chisti, 2008). In addition, microalgae do not compete for land used for food production, fodder and other products (Huang et al., 2010). Furthermore, microalgae can be grown in a number of environments that are unsuitable for growing other crops (Patil et al., 2008).

There are various methods which produce FAME from microalgal lipid such as oil extraction followed by transesterification, direct transesterification, supercritical method, and so on. Direct transesterification is typically the method which generates FAME from microbial biomass by using acid catalyst. It eliminates the need to extract and refine lipid before converting it to biodiesel, which could provide a reduction in the cost of biodiesel production (Haas and Wagner, 2011). In addition, direct transesterification can convert not only TG but also other lipids such as phospholipid into FAME (Vicente et al., 2009). Therefore, direct transesterification is a promising biodiesel production process from microalgae. However, it has a problem that water inhibits the reaction (Ehimen et al., 2010; Wahlen et al., 2011).

Free fatty acid (FFA) is more resistant to the FAME production inhibition caused by water existence than TG (Lepage and Roy, 1984). Hence, hydrolysis of lipid to FFA followed by esterification could be a valid method, which could reduce the inhibition and result in a decrease of the drying cost. There are some reports where hydrolysis followed by esterification of wet microalgal lipid was implemented under supercritical or subcritical condition. Two-step process from wet microalgal biomass was conducted by

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Table 1
Factors and levels for the L_{27} orthogonal experiment.

Factors	Level 1	Level 2	Level 3
A: Water content (%)	70	80	90
B: Volume of sulphuric acid (mL/kg-dry algae)	200	300	400
C: Volume of methanol (the ratio of methanol to wet biomass, vol./wt.)	1.33	2	2.67
D: Temperature (°C)	130	140	150
E: Time (h)	1	2	3

Levine et al. (2010). In their report, wet microalgal biomass (water content 80%) reacted in subcritical water to hydrolyze intracellular lipid in the first step and the wet fatty acid-rich solids underwent supercritical *in situ* transesterification with ethanol to produce FAME in the second step. Simultaneous hydrolysis-esterification (SHE) was also achieved under supercritical or subcritical condition (Patil et al., 2011; Tsigie et al., 2012). However, this method may be disadvantageous due to the adverse process economics as well as safety concerns (Marchetti and Errazu, 2008).

Only a few papers have dealt with hydrolysis followed by esterification using acid without application of pressure which is necessary for supercritical or subcritical condition. Wet lipid extraction from wet algal biomass (84%) via acid and base hydrolysis at 90 °C was performed by Sathish and Sims (2012). In our previous research, two-step hydrolysis followed by esterification was carried out (Takisawa et al., 2013). Acid hydrolysis provokes microalgal cell disruption and sugar extraction. When algal biomass was processed with physical methods (sonication, bead-beating, autoclaving and homogenization) and with physicochemical processes consisting of alkaline and acid hydrolysis (NaOH, HCl and H_2SO_4) in autoclave, acid process was the most effective hydrolysis method in all the processes (Miranda et al., 2012). In addition, when the effects of enzymatic hydrolysis and acid hydrolysis on ethanol fermentation were compared, acid hydrolysis showed higher ethanol yield than enzyme hydrolysis (Ho et al., 2013). Thus, acid hydrolysis can give a positive effect on bioethanol production as well as biodiesel production. In this study, the optimisation of SHE of wet microalgal biomass using acid was performed.

Table 2
L_{27} orthogonal experimental design.

No.	Water content (%)	Volume of sulphuric acid (mL/kg-dry algae)	Volume of methanol (the ratio of methanol to wet biomass, vol./wt.)	Temperature (°C)	Time (h)	FAME (%)
1	70	200	1.33	130	1	3.93
2	70	200	2	140	2	3.16
3	70	200	2.67	150	3	3.76
4	70	300	1.33	140	3	4.59
5	70	300	2	150	1	2.65
6	70	300	2.67	130	2	3.36
7	70	400	1.33	150	2	3.11
8	70	400	2	130	3	4.01
9	70	400	2.67	140	1	2.82
10	80	200	1.33	130	1	1.43
11	80	200	2	140	2	1.71
12	80	200	2.67	150	3	3.52
13	80	300	1.33	140	3	2.77
14	80	300	2	150	1	2.14
15	80	300	2.67	130	2	2.80
16	80	400	1.33	150	2	1.54
17	80	400	2	130	3	2.79
18	80	400	2.67	140	1	2.18
19	90	200	1.33	130	1	0.03
20	90	200	2	140	2	0.06
21	90	200	2.67	150	3	3.00
22	90	300	1.33	140	3	0.43
23	90	300	2	150	1	1.45
24	90	300	2.67	130	2	0.09
25	90	400	1.33	150	2	0.27
26	90	400	2	130	3	0.23
27	90	400	2.67	140	1	0.04

2. Methods

2.1. Materials

Hydrolysis is sensitive to microalgal cell wall. It is necessary to select the species with tough cell wall in order to hydrolyse any species cell. *Chlorella* is known to have rigid wall components embedded within a more plastic polymeric matrix (Gerken et al., 2013). This is why *Chlorella* was selected as a material in this study. Commercially available dried *Chlorella* powder was provided by Natural Health Inc., Japan. The powder was dried at 80 °C for 24 h and stored at −20 °C.

Distilled water was added into microalgal powder to reproduce harvested and concentrated microalgae where microalgal water content was assumed as 70–90%. Water content is shown on the basis of the weight of wet algal biomass (% (w/w)). Methanol (99.8%), sulphuric acid (95%) and hexane (96%) were purchased from Wako Chemical Industries Ltd. (Japan). As the internal standard of FAME and FFA, methyl pentadecanoate (>98%) and pentadecanoic acid (>98%) were bought from Tokyo Chemical Industry Co., Ltd., Japan.

2.2. Maximum FAME content

Maximum FAME content was determined by reference to the method of Wahlen et al. (2011). Dry microalgal powder (0.3 g) was mixed with 4 mL of methanol containing 2% sulphuric acid in a test tube. Each tube was sealed using PTFE lined screw cap and the microalgal lipid was transesterified by using dry block bath (MG-2200, EYELA, Japan) at 80 °C for 6 h. After the transesterification, 3 mL of hexane was added to the reaction mixture and vortexed. After the tube was centrifuged at 3000 rpm for 5 min, the hexane layer was removed. Further extractions with hexane were performed twice in the remaining water layer and FAME in the hexane was analysed.

2.3. SHE with orthogonal experiment

The experiment using L_{27} orthogonal array was implemented to inspect the effects of various parameters on SHE of microalgal lipid. Microalgal powder (0.3 g) was added with water, sulphuric acid and methanol in a test tube. Microalgal lipid simultaneously hydrolysed and esterified. After cooled at room temperature for 30 min, FAME extractions using hexane were performed as mentioned above and FAME was analysed.

Water content (Factor A), volume of sulphuric acid (Factor B), volume of methanol (Factor C), temperature (Factor D) and time (Factor E) were chosen as the conditional factors of the orthogonal experiment. Each factor was given at three levels as shown in Table 1. Water content was set at 70%, 80% and 90%, which are the possible values of centrifuged microalgae. Volume of sulphuric acid and temperature are set by considering the report by Takisawa et al. (2013). Volume of methanol was set based on the report of Tsigie et al. (2012), where the optimum condition of the ratio of methanol to wet biomass is 4 (vol./wt.). All the factors and levels were assigned to L_{27} orthogonal design as shown in Table 2. The interactions between water content and other parameters (A × B, A × C, A × D and A × E) were examined as well as main effects.