In situ Transesterification of Wet Microalgae under Acid Catalysts

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ABSTRACT

One of energy intensive processes of biodiesel production is concentrating and drying wet algal cells to facilitate the subsequent lipid extraction. The cost for this process occupies 20-30% of the total cost of biodiesel production. To this end, this work addresses a reliable *in situ* transesterification process which integrates lipid extraction from wet microalgae, and its conversion to biodiesel without having a drying process. The process enables one-step production of biodiesel by heating the mixture of wet *Nannochloropsis gaditana* with co-solvent, alcohol, and acid catalyst in one pot. Effects of reaction parameters (temperature and co-solvent volume) on the biodiesel yield will be investigated under various conditions. It will be shown that the fatty acid ethyl ester yield of 90-100% is achieved.

Keywords: biodiesel, *in-situ* transesterification, lipid extraction, wet microalgae

1 INTRODUCTION

Microalgae can take carbon dioxide from the air and grow faster than terrestrial plants. Using microalgae, high-concentration culture is possible in large quantities and even in extreme environments [1]. Producing biodiesel from microalgal lipids is feasible since microalgae can accumulate lipid levels that are higher than 50% of their dry cell weight [2]. Usually microalgae are photoautotrophic microbes, but in addition to using free sunlight as an energy source, microalgae can be cultivated using the nitrogen, phosphorus and hydrocarbons in industrial wastewater [3, 4].

After extraction of the lipids from cultivated microalgae, the extracted oils are usually converted to fatty acid ethyl ester (FAEE) using ethanol and acid catalyst in a wet transesterification reaction. Such FAEE can be used as a biodiesel after purification [5]. Because microalgae are grown in liquid media, extraction is usually classified as wet extraction or dry extraction depending on whether or not the culture broth was dried before the lipid extraction step. Most of the current extraction processes are carried out after drying the wet microalgae to increase the amount of lipid extracted from the biomass [6, 7]. However, this drying process is responsible for 59% of the total energy consumed during biodiesel production [8].

To reduce the impact of the energy consumed for the current drying process, improvement of the efficiency of wet extraction by adding other operations has recently emerged as a very important issue [9-11]. These operations include mechanical pressing, as well as ultrasonic, microwave, supercritical fluid, and solvent extraction methods, all of which have been used for lipid extraction. Mechanical pressing is easy to design, but it has the drawbacks that it consumes lots of energy and time. The ultrasonic and microwave methods need shorter extraction time than other methods, and allow improved penetration of solvent with less solvent used, but are difficult to scale up [6, 12]. When it comes to extraction using a supercritical fluid, there is the benefit of not needing to use toxic solvents. However, because of intensive energy consumption and large capital costs, it still faces hurdles for scaling up [11, 13]. Solvent extraction, except for toxicity issues, provides reasonable performance and has the advantage of low costs for scaleup [14].

The oils (triacylglycerides) recovered from microalgae can be converted into biodiesel by a transesterification reaction. Usually extraction and transesterification proceed separately, but there is a process called 'in-situ transesterification' which integrates the two processes into a single-step process as an economically feasible alternative. The in-situ process allows direct extraction of the lipid from microalgae, and its transformation to biodiesel, while reducing the processing cost [15-17]. This in-situ process delivers verified success in the dry state, but with moisture present, low yields are typical without an additional energy input such as microwave [6, 18].

In contrast, our new process presents extraordinarily high yields of FAEE using wet microalgae by combining the extraction and transesterification processes into a single step. Heating wet microalgae in a mixed solution of ethanol, solvent, and acid catalyst leads to production of FAEE with its yield of around 90 wt.%. This is a significant improvement over *in situ* transesterification of wet microalgae; without additional energy inputs. This work focuses on the effects of reaction parameters (e.g., amounts of solvent, ethanol and temperature) on conversion yields for one-pot extraction and transesterification.

2 METHOD

2.1 Chemicals and reagents

Extra pure grade sulfuric acid and reagent grade chloroform were obtained from JUNSEI Chemical (Japan).

Extra pure grade ethanol (99.5%) was obtained from Samchun (Korea). Heptadecanoic acid ethyl ester as a standard material for GC analysis from Sigma Aldrich (> 99%) was also used. The *Nannochloropsis gaditana* powder was purchased from AlgaSpring (Netherlands) and stored at a dark room.

2.2 *In situ* transesterification procedure

Numerous experiments for optimizing the reaction parameters used for biodiesel production were performed with wet *Nannochloropsis gaditana*. The algal paste used in this study was confirmed to contain 65 wt.% moisture. In-situ transesterification was started by mixing ethanol, solvent and sulfuric acid with the wet microalgae in a teflon-seal, screw-capped tube (14 ml). Ethanol and sulfuric acid were used as transesterification reactant and catalyst. Simultaneous extraction and reaction were conducted by immersing the tube in a waterbath. To minimize leakage of ethanol and chloroform from the vapor head, the tube was capped tightly. After the reaction, sodium hydroxide was added to prevent further reaction and the mixture was centrifuged at 3700 rpm for 10 min to achieve phase separation. Then, ethanol containing sulfuric acid and water formed an upper layer and chloroform containing the converted FAEE and unconverted TAG formed a lower layer. Reaction parameters (temperature and choroform solvent) were varied to find optimal conditions for maximizing the FAEE yield.

2.3 FAEE analysis and yield calculation

For the quantification of each sample, the liquid organic phase including FAEE and was injected to GC (Agilent 7890b, USA) equipped with HP-5 column. The GC analysis was performed with a flame ionization detector (FID) and helium was used as a makeup gas. At each analysis, 1 μl of the liquid sample was used. The yield of FAEE was calculated based on the ratio between the peak area of standard C17 ethyl ester and the peak area of the corresponding chemical compound. To determine maximum yield of transesterifiable lipids in *N. gaditana* cells, experiments based on Folch's method were conducted [19]. 10 mg dried cells and 2 ml 2/1 v/v mixture of chloroform and ethanol were added to the teflon-sealed, screw-capped tube and lipids were extracted by vortexing for 10 min. One ml ethanol and 300 μl sulfuric acid were added and the tubes were heated to 100˚C for 20 min. After the transesterification reaction, the tube was cooled down to room temperature and left to settle about one hour for phase separation. 1 ml chloroform containing 0.5 mg heptadecanoic ethyl ester (C17:0) was added to the tube for GC analysis to determine the maximum FAEE yield. Then the FAEE yield is calculated as follows: the maximum amount of FAEE that could be derived from the lipids was determined as 12.05 mg FAEE / 100 mg dry cell. Thus, the FAEE yield could be obtained by dividing an experimentally determined FAEE amount by the maximum FAEE amount

3 RESULT AND DISCUSSION

3.1 Reaction temperature effect on the FAEE yield

The results from previous studies of *in situ* transesterification showed higher yields of FAEE when higher reaction temperatures were used [18]. In this study, the reaction temperature was varied from 65 ℃ to 140 ℃ To 0.3 g dry biomass, 0.3 ml sulfuric acid and a 2 ml, 2/1 v/v mixture of chloroform and ethanol were added. For wet condition, moisture content was maintained to 65%. Then the reaction was allowed to continue in a water bath for two hours at a specified temperature.

It is clear that the FAEE yield increases with rising temperature (Fig.1). At the dry condition, the FAEE yield was already 88 wt.% at 95 ℃ and reached almost 100 wt.% with flattening out after 110 ℃. Less amount of FAEE was produced at the wet condition and FAEE yield more than 90% was obtained between 125 - 140 ℃. Although other studies obtained an optimal reaction temperature near 80°C higher temperatures in this study helped chloroform, ethanol and sulfuric acid disrupt the cell walls as well as accelerate the transesterification reaction [6, 18]. To confirm FAEE production, the distribution of FAEE was illustrated in Fig.2. Other FAEE components were not shown because they account for only 7.3 % of the total FAEE. Each FAEE component shows a similar trend in which the yield increases along with the temperature rise. From the result, *N. gaditana*, is suitable to be selected as a biodiesel source considering that the ester products of C16-C18 has been a proper candidate for biodiesel [20].

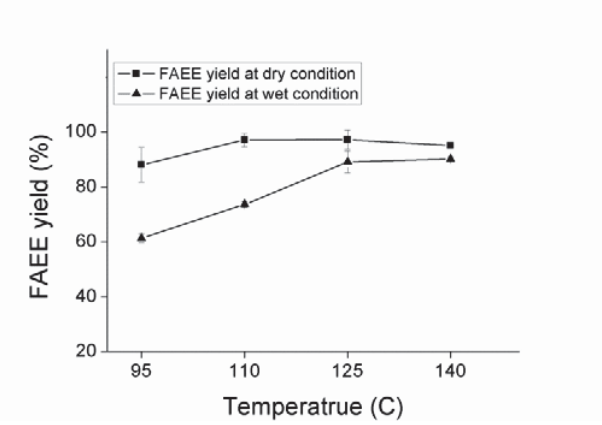

Fig. 1 FAEE yields subject to various reaction temperatures.

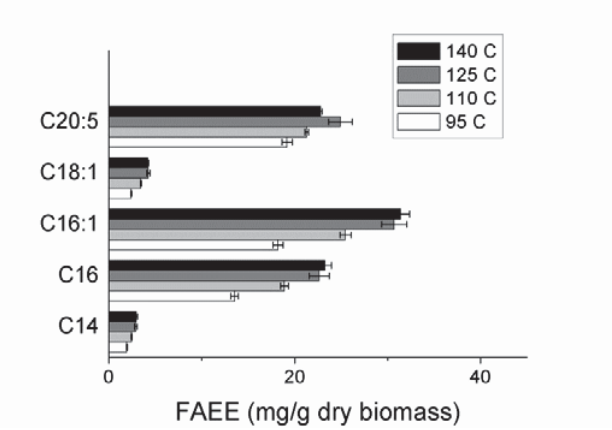

Fig. 2 FAEE product distribution at the wet condition.

3.2 Chloroform effect on the FAEE yield

Originally, chloroform was employed in order to improve efficiency of extraction and conversion of microalgal lipid as a solvent [16]. To confirm the effectiveness of chloroform for the production of FAEE, the solvent volume varied from 0 to 3 ml/ethanol in *in situ* transesterification at 125 °C while other experimental conditions remained the same as in Section 3.1.

Without chloroform, the FAEE yields was 66 wt. %, respectively, as shown in Fig. 3 . However, addition of 0.5 ml chloroform made a dramatic rise of the yields – 86 % of FAEE yield. In the reaction tube containing wet microalgae, ethanol, sulfuric acid and chloroform, ethanol and sulfuric acid with wet microalgae generate the aqueous phase and chloroform forms the organic phase. According to the molecular structure, FAEE is easily miscible with the organic phase and ethanol prefers staying in the aqueous phase. Thus, in the sample tube, the reactant (ethanol) and the product (FAEE) were stabilized in the separate phases with the enhancement of the forward direction of transesterification reaction.

Once lipids from *N. gaditana* were released in the aqueous phase, it reacts with ethanol via transesterification from which FAEE and glycerol were produced. Because FAEE has a low solubility in the aqueous phase, it remains in the organic phase. With regard to the aqueous phase, the remaining product in transesterification was only glycerol. The depletion of product FAEE from the aqueous phase was effective enough to make more FAEE by the reaction equilibrium shift.

4 CONCLUSION

The wet, in-situ transesterification process in this work has been demonstrated to achieve conversion yields as high as 90% from wet *N. gaditana* (65% moisture) using chloroform, ethanol, and sulfuric acid in the one pot. This significant improvement of FAEE yield is possible without using an energy-intensive drying process before extracting the lipid. The reaction parameters for producing biodiesel via this process have been successfully analyzed. This *in situ* approach can be used to produce biodiesel directly from harvested algae and provides a useful step forward for the next generation of renewable biodiesel.

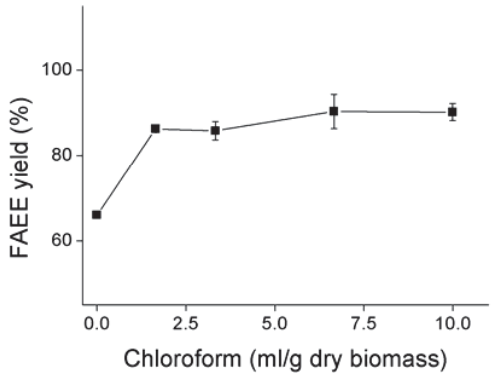

Fig. 3 FAEE yield changes depending on the amount of chloroform.

5 ACKNOWLEDGEMENT

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