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Short Communication

In situ transesterification of highly wet microalgae using hydrochloric acid

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HIGHLIGHTS

- *In situ* transesterification of highly wet microalgae using hydrochloric acid.
- FAME yield over 90 wt.% with less amount of solvent in HCl than in H_2SO_4 catalysts.
- HCl outperforms H_2SO_4 for *in situ* transesterification of highly wet microalgae.

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ABSTRACT

This study addresses *in situ* transesterification of highly wet microalgae with hydrochloric acid (HCl) as a catalyst. *In situ* transesterification was performed by heating the mixture of wet algal cells, HCl, methanol, and solvent in one pot, resulting in the fatty acid methyl ester (FAME) yield over 90% at 95 °C. The effects of reaction variables of temperature, amounts of catalyst, reactant, and solvent, and type of solvents on the yield were investigated. Compared with the catalytic effect of H_2SO_4, *in situ* transesterification using HCl has benefits of being less affected by moisture levels that are as high as or above 80%, and requiring less amounts of catalyst and solvent. For an equimolar amount of catalyst, HCl showed 15 wt.% higher FAME yield than H_2SO_4. This *in situ* transesterification using HCl as a catalyst would help to realize a feasible way to produce biodiesel from wet microalgae.

1. Introduction

Microalgae, a rising resource for biodiesel has been intensively investigated in a number of studies during past decades since they grow fast and store lots of lipids to be converted to biodiesel (Chisti, 2007; Halim et al., 2011). Despite many advantages of using microalgae as a potential resource for biodiesel, the cost of biodiesel production from microalgae hinders commercial uses due to the poor economic viability in the current production process involving cell harvest, oil extraction, transesterification, and purification. Among these processes, especially drying microalgae after harvesting them requires lots of energy to be consumed, which accounted for 20–30% of the total cost of biodiesel production (Mata et al., 2010). *In situ* transesterification is an efficient way to convert oil bearing biomass to biodiesel directly, hence, eliminating the extraction step which is required in the conventional method. Due to its simple process, various feedstock have been under the investigation using *in situ* transesterification including vegetable oil and microalgae. During *in situ* transesterification reaction, the importance of the presence of catalysts has been proved to be more critical for the improved yield of FAME (Atadahi et al., 2013). Therefore, studies on a wide variety of catalysts are actively ongoing including homogeneous catalysts (NaOH, KOH, H_2SO_4, HCl, etc.), enzymes, and solid catalysts (Zabeti et al., 2009). Due to the characteristic of algal cells that contain high percentage of free fatty acid whose presence causes saponification with alkaline catalysts, employing acid catalysts on biodiesel production from microalgae has been mainly studied. In addition, algal cells containing water also cause an adverse effect on FAME production when alkaline catalysts are used (Demirbas, 2009). Other acid catalysts such as BF_3 and H_3PO_4 were also studied (Bharathiraja et al., 2014). The FAME yield using aforementioned acid catalysts was able to be reached nearly 100 wt.%, however, none of them has been reported to be comparable with a cost-effectiveness of H_2SO_4. Nonetheless, using H_2SO_4 for transesterification requires additional purification step of products and wastewater treatment, thereby increasing the cost of producing biodiesel.

This work investigated the effectiveness of HCl as a catalyst since HCl has been rarely studied for *in situ* transesterification of

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wet microalgae to produce biodiesel. Then, the advantage of HCl as a catalyst for *in situ* transesterification of wet microalgae was addressed especially when the water contents of algal cells are high. Due to the high affinity of HCl with water, the performance of HCl may be unaffected by the moisture contents in algal cells (Su, 2013). As the conditional factors, the effect of HCl, co-solvent (methanol/chloroform and methanol/hexane), reaction temperature and moisture contents on the performance of *in situ* transesterification with highly saturated algal cells were elucidate. The effectiveness of HCl was demonstrated in achieving higher than 90% conversion of lipid to biodiesel even with smaller amounts of catalyst and solvent in comparison with the H_2SO_4 case.

2. Methods

2.1. Chemicals and strains

H_2SO_4 (98 wt.%), HCl solution (35 wt.%), and methanol are extra pure grade chemicals whereas chloroform and hexane are guaranteed reagent grade and all were provided from Junsei Chemical. For a standard reagent to quantify FAME in gas chromatography (GC) analyses, methyl heptadecanoate from Fluka was used. *N. gaditana* (*Nannochloropsis gaditana*) was acquired from AlgaSpring located in Almere, Netherlands. *N. gaditana* was selected due to its relatively high lipid productivity and growth rate under nearly any changeable growth conditions. Furthermore, above 84% of the total lipids in *N. gaditana* are the fatty acids containing C16 to C18, which classify themselves as a proper candidate to mimic petro-derived diesel.

2.2. In situ transesterification

Our previous *in situ* transesterification method (Im et al., 2014) was modified in this work. First, in a 14 ml Teflon-sealed tube (Daihan, South Korea), 0.15 g of dry microalgae was well saturated with DI water and water in HCl to have moisture contents of 80 wt.% in total. After an adequate saturation time of 0.5–24 h at room temperature, chloroform and methanol were added and evenly mixed. The tube cap was sealed tightly and then heated in a thermostat bath (WCH-8, Daihan, South Korea) set at a desired temperature for 2 h. After the reaction, the tube was cooled down to room temperature for 30 min. Then chloroform containing a known amount of standard agent and DI water were added for phase separation. To accelerate this phase separation, the tube was centrifuged at 3700 rpm for 10 min then the FAME phase containing chloroform on the bottom of the tube was collected for GC analyses. Temperature, amounts of catalyst, reactant and solvents, moisture content in algal cells and moisture saturation time were varied to find optimal reaction conditions and all experiments were duplicated or quadruplicated to secure the reproducibility of measurements.

2.3. Maximum FAME yield using two steps of lipid extraction and transesterification

To quantify the maximum amount of FAME that can be converted from lipids in *N. gaditana*, experiments were conducted based on Bligh and Dyer's method (Bligh and Dyer, 1959). Dry microalgae ranging from 10 to 20 mg in the tube was mixed with a 2 ml mixture of chloroform and methanol (2/1 v/v) and stirred for 5 min in order to allow lipids inside algal cells to be extracted. Then 1 ml of methanol and 0.3 ml of H_2SO_4 were added for transesterification reaction of lipids from lysed cells. The tightly sealed tube containing the mixture above was heated in a bath set at 100 °C for an hour followed by 30 min cooling at room temperature. In each tube, 1 ml chloroform containing 0.5 mg methyl heptadecanoate and 0.3 M of NaOH solution were added. The tube was centrifuged for 10 min at 3400 rpm for phase separation, then the organic phase was extracted for GC analyses.

2.4. FAME yield determined from GC analyses

To quantify the amount of FAME in each sample, Agilent 7890b equipped with HP-5 column (30.0 m × 0.32 mm × 0.25 μm) was used. The FID detector was set at 280 °C, with a column flow of 2.1 ml min^{-1} of helium as a carrier gas. The injected sample volume was 1 μl. The oven temperature began at 50 °C. It was increased to 175 °C at a rate of 25 °C min^{-1}, then to 240 °C at a rate of 4 °C min^{-1} and held constant for another 15 min.

The amount of FAME in algal cell was calculated by following equation:

$$\text{FAME contents (g)} = \frac{\text{Weight of standard agent in sample} \times \text{Sum of FAME area in GC}}{\text{Area of standard agent in GC}} \quad (1)$$

Based on the method described in Section 2.3, the maximum amount of FAME from lipids in *N. gaditana* was determined as 12.05 mg ± 0.59 FAME/100 mg dry cell. Thus, the FAME yield was calculated by using the following equation:

$$\text{FAME yield (wt.\%)} = \frac{\text{Weight of FAME obtained from each sample}}{\text{Weight of maximum FAME yield}} \times 100 \quad (2)$$

2.5. Control experiment using H_2SO_4

H_2SO_4 has been widely employed for *in situ* transesterification since H_2SO_4 along with primary alcohols are suitable to conduct both direct esterification and transesterification reaction simultaneously, make one step process possible (Marchetti and Errazu, 2008). Thus to compare the catalytic performance of HCl in *in situ* transesterification with that of H_2SO_4 on the FAME yield, experiments using an equimolar amount of H_2SO_4 corresponding to the HCl was used considering the difference in molecular weight. For example, 0.3 ml of 35 wt.% HCl contains 0.126 g of HCl, equimolar amount of H_2SO_4 then be calculated to be 0.334 g, which has 2.7 times greater than the one of HCl.

3. Results and discussion

3.1. Moisture contents on the FAME yield

As a term 'HCl solution' implies, HCl used as a catalyst in this work is fully dissociated in water. Therefore, the effect of moisture in HCl must be investigated first to understand whether it is a factor affecting the FAME yield. After HCl ranging from 0.1 to 1.5 ml added to dried microalgae and saturated for 0.5 h, 0.1 ml chloroform and 1.0 ml methanol were added and then the mixture sample in the tube was put in the thermostat bath for 2 h at 95 °C. With increasing HCl amounts added to the dried microalgae, the moisture contents move up and the corresponding FAME yield from *in situ* transesterification decreases as shown in Table 1. The result suggests that the water in the HCl solution negatively affects the FAME yield as the amount of HCl solution increases. Then, DI water along with water in the HCl solution was added to dried algal cells in order to reproduce harvested microalgae where the moisture content of microalgae is ranged as 70–85 wt.%. Takisawa et al. (2013) reported that during *in situ* transesterification employing H_2SO_4, the moisture contents has a profoundly adverse effect on the FAME yield from either dried or wet microalgae. With HCl,