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Short Communication

Acid-catalyzed hot-water extraction of lipids from *Chlorella vulgaris*

Ji-Yeon Park *, You-Kwan Oh, Jin-Suk Lee, Kyubock Lee, Min-Ji Jeong, Sun-A Choi

Department of Clean Fuel, Korea Institute of Energy Research, Daejeon 305-343, Republic of Korea

HIGHLIGHTS

- Acid-catalyzed hot-water treatment for efficient lipid extraction from wet microalgae.
- Conversion of extracted microalgal lipids to biodiesel by esterification.
- Improvement of extraction yield through cell disruption and demulsification by acid.

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ABSTRACT

Acid-catalyzed hot-water treatment for efficient extraction of lipids from a wet microalga, *Chlorella vulgaris*, was investigated. For an initial fatty acids content of 381.6 mg/g cell, the extracted-lipid yield with no heating and no catalyst was 83.2 mg/g cell. Under a 1% H_2SO_4 concentration heated at 120 °C for 60 min, however, the lipid-extraction yield was 337.4 mg/g cell. The fatty acids content, meanwhile, was 935 mg fatty acid/g lipid. According to the severity index formula, 337.5 mg/g cell of yield under the 1% H_2SO_4 concentration heated at 150 °C for 8 min, and 334.2 mg/g cell of yield under the 0.5% H_2SO_4 concentration heated at 150 °C for 16 min, were obtained. The lipids extracted by acid-catalyzed hot-water treatment were converted to biodiesel. The biodiesel's fatty acid methyl ester (FAME) content after esterification of the microalgal lipids was increased to 79.2% by the addition of excess methanol and sulfuric acid.

1. Introduction

Microalgae are photosynthetic microorganisms capable of converting, under light conditions, carbon dioxide and water into macromolecules such as lipids, polysaccharides, and proteins (Fu et al., 2010). Some microalgae boast high oil productivity compared with plant oils, and offer the additional advantage of not competing with food crops. For enhanced economic and environmental feasibilities, moreover, waste water or sea water can be used in place of fresh water in the microalgae production process (Li et al., 2008; Schenk et al., 2008). Conversion of microalgae to biodiesel typically includes the following four steps: microalgae cultivation, cell harvesting, lipid extraction, and biodiesel conversion (Li et al., 2008). Lipid extraction from microalgae can be achieved via a number of methods including solvent extraction, enzymatic hydrolysis, fractionation as well as microwave- and ultrasound-based modalities (Lee et al., 2013; Shin et al., 2011). Polysaccharides in post-extraction cell debris, additionally, can be utilized as a source of sugar for bioethanol production (Sugiyama et al., 1991; Sun and Cheng, 2002).

Recovery of microalgal lipids from dried microalgae has been achieved by many researchers (Demirbas, 2009; Xu et al., 2006); however, given the high costs incurred in the dewatering process, wet extraction has emerged an attractive alternative approach. Several methods of wet lipid extraction have been proposed, though the yields remain unsatisfactory (Cho et al., 2013; Lee et al., 2013).

Hot-water treatment facilitates the pressure maintenance necessary to keep water in the liquid state at elevated temperatures. This method has been typically applied to cellulosic biomass pretreatment in order to weaken or disrupt the crystalline structure of cellulose (Mosier et al., 2005; Yu et al., 2013). To improve the pretreatment efficiency, catalysts such as sulfuric acid or sodium hydroxide were used. Given that microalgal cell walls contain cellulose, it is expected that hot-water treatment will become a proven alternative approach to the extraction of lipids from microalgae.

In this study, the efficiency of sulfuric-acid-catalyzed hot-water extraction of lipids from wet microalgae was investigated. The relationship between the reaction conditions and the lipid-extraction yield was determined using the severity index formula.

* Corresponding author. Tel.: +82 42 860 3041; fax: +82 42 860 3495.
E-mail address: yearn@kier.re.kr (J.-Y. Park).

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2. Experimental methods

2.1. Lipid extraction from microalgae

Chlorella vulgaris (hereafter: *C. vulgaris*), a freshwater microalga, was isolated locally and cultured in a nutrient media (constituents: KNO_3, 3 mM; KH_2PO_4, 5.44 mM; Na_2HPO_4, 1.83 mM; $MgSO_4{\cdot}7H_2O$, 0.20 mM; $CaCl_2$, 0.12 mM; FeNaEDTA, 0.03 mM; $ZnSO_4{\cdot}7H_2O$, 0.01 mM; $MnCl_2{\cdot}4H_2O$, 0.07 mM; $CuSO_4$, 0.07 mM; $Al_2(SO_4)_3{\cdot}18H_2O$, 0.01 mM) adjusted to a pH of 6.5. The *C. vulgaris* was then cultivated at 30 °C in a Pyrex bubble-column reactor (working volume: 6 L) equipped with 12 fluorescent lamps (light intensity: 80 μmol/m^2/s) and maintained in a constant-temperature room. The reactor was supplied with 10% (v/v) CO_2 in air at a rate of 0.75 L/min. For the purposes of lipid-extraction experiments, cells were harvested by centrifugation (4000 rpm and 10 min) until the cell concentration was 20 g/L. Prior to analyses of fatty acids and polysaccharide contents, cells were harvested by centrifugation, washed with deionized water, and freeze-dried (FD5512, IlShin BioBase Co., Korea) for 4 days.

As a control, lipids were extracted from lyophilized *C. vulgaris* using three organic solvents: hexane (96%, Junsei, Japan), hexane:methanol (99.6%, Junsei, Japan) = 7:3 (v/v), and chloroform (99%, Junsei, Japan): methanol = 2:1 (v/v). The *C. vulgaris* loading was 5% (w/w). The mixture was stirred at 1000 rpm for 6 h at room temperature, and then separated into the organic-solvent and cell-debris layers by 4000 rpm centrifugation for 10 min. Finally, the solvent of lipids-containing organic-solvent layer was removed using a vacuum evaporator (EZ2 PLUS, Genevac, UK), and the lipids were recovered. The lipid-extraction yield was determined by reference to the weight of the recovered lipids. All of the experiments were performed in duplicate.

2.2. Acid-catalyzed hot-water treatment of microalgae

Hot-water treatments were carried out in a 100 mL autoclave reactor. To enhance the lipid-extraction efficiency, sulfuric acid (H_2SO_4; 95%, Junsei, Japan) solution was injected into the harvested cells before heating. The H_2SO_4 concentration of the culture solution was adjusted to 0%, 0.25%, 0.5%, 0.75%, 1%, 2%, and 3% (w/w), respectively. Various reaction temperatures, namely 120, 130, 140, 150, and 160 °C, were applied. After the reactor was cooled, organic-solvent hexane was mixed with the treatment solution at 1000 rpm for 2 h at room temperature, after which the hexane layer was separated from the cell-debris layer by centrifugation. Finally, the hexane of lipids-containing hexane layer was removed by vacuum evaporation, and the lipids were recovered. The lipid-extraction yield was determined by reference to the weight of the recovered lipid. All of the experiments were performed in duplicate.

2.3. Microalgal lipids conversion to biodiesel

The extracted microalgal lipids were converted to biodiesel by esterification. Specifically, the lipids were reacted with methanol under H_2SO_4 catalysis (concentrations: 1%, 5%, and 10% (w/w) of lipids) at 100 °C for 1 h in a Pyrex-glass tube with a Teflon-sealed screw-cap. The excess methanol (lipids:methanol = 1:1, w/w) was then used to enhance the biodiesel conversion efficiency. Finally, after washing with distilled water and 10,000 rpm centrifugation for 5 min, the fatty acid methyl ester (FAME, biodiesel) content was analyzed. All of the experiments were performed in duplicate.

2.4. Analyses

The fatty acids content of the microalgae was analyzed using the modified direct esterification method (Lepage and Roy, 1984). Specifically, cells totaling approximately 10 mg were put in a vial. Two milliliter of chloroform–methanol mixture (2:1, v/v) was added to the cells, which solution was then vigorously agitated for 10 min. One milliliter of chloroform solution containing heptadecanoic acid (Sigma, USA) as an internal standard (500 μg/L), 1 mL of methanol, and 300 μL of H_2SO_4 were sequentially added to the vial and vortex-mixed for 5 min. The vial was then reacted in a 100 °C water bath for 10 min, after which it was cooled to room temperature, supplemented with 1 mL of distilled water, and intensely mixed for 5 min. After centrifugation, the lower layer (organic phase) was injected into a gas chromatograph. The FAME was analyzed using a gas chromatograph equipped with an automatic injector (Agilent 7890, USA). Mix RM3, Mix RM5, GLC50, and GLC70 (Supelco, USA) were utilized as the standards. The other reagents used were of analytical grade.

The fatty acids contents of the microalgal lipids were determined following the modified direct esterification method noted above (Lepage and Roy, 1984). Initially, extracted microalgal lipids (approx. 10 mg per vial) were used instead of cells for esterification. The subsequent procedure was the same as detailed above.

The biodiesel's FAME content was analyzed using a gas chromatograph equipped with an auto-injector (Agilent 6890A, USA). In steps, approximately 250 mg of sample was put in a 10 mL vial. The sample was then mixed with 5 mL of heptane solution containing methyl heptadecanoate (Fluka, Switzerland) as an internal standard (10 mg/mL), and the resultant solution was injected into a gas chromatograph (Park et al., 2008). The cells were hydrolyzed by H_2SO_4 according to NREL's Chemical Analysis and Testing Standard Procedures Nos. 001–004 (NREL, 2004); on this basis, the initial polysaccharide content of the *C. vulgaris* was measured. A UV–VIS Spectrophotometer (Optizen 2120UV, MECASYS, Korea) was used to measure the culture solution's optical density (600 nm), according to which the cell concentration was then calculated.

2.5. Severity index

The severity index is a useful tool for experimental design (Chum et al., 1990). Using this formula, the reaction conditions (reaction time, temperature, and H_2SO_4 concentration) of H_2SO_4-catalyzed hot-water-based lipid extraction from microalgae can be easily determined.

The severity index can be employed to evaluate hot-water treatment as a function of reaction time (t, min) and temperature (T, °C):

$$\text{Severity Index (SI)} = \log R_0 = \log(t\ \exp((T-100)/14.75)) \quad (1)$$

When treatment is performed under acidic conditions, the effect of pH can be taken into consideration by means of a combined severity index:

$$\text{Combined Severity Index (CS)} = \log R_1 = \log R_0 - \text{pH} = \log R_0 + \log[H^+] \quad (2)$$

The severity and combined severity indices, given their assumption that a first-order reaction takes place, can be considered to be proximate methods (Chum et al., 1990; Fernandez-Bolanos et al., 2001; Park et al., 2012).

3. Results and discussion

3.1. Fatty acids and polysaccharide contents of microalgae

The *C. vulgaris* fatty acids contents and compositions are summarized in Table 1. The total fatty acids content was 381.6 mg/g cell; the palmitic acid content was 100.5 mg/g cell (26.3% of total