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Evaluation of lipid extractability after flash hydrolysis of algae

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ABSTRACT

Microalgae is identified as a promising feedstock for producing renewable liquid transportation fuels; however, lipids extraction from microalgae for downstream processing to biofuels is one of the important challenges for algal based biorefineries. This work aims at evaluating the potential of applying flash hydrolysis (FH) as a chemical-free technique to increase the lipids extractability of algal biomass as well as its integration with the hydrothermal liquefaction (HTL) of microalgae to enhance the biocrude yields and characteristics for fuel production. To this aim, the FH process was performed on three different algal species (*Scenedesmus* sp., *Nannochloropsis* sp., and *Chlorella vulgaris*) at 280 °C and 10 s of residence time. Following FH, in addition to the nutrients rich hydrolysate, approximately, 40 wt% of solids containing almost all (> 90 wt%) the lipids termed as biofuels intermediates (BI), were recovered. Kinetics study on lipids extractability from the BI and their lipid profile analyses were conducted for each algal species. The results showed that the FH process had significantly enhanced the lipids extractability. For all three algae species, lipid yields from BI were higher than that of the raw algae. Lipid yields of *Chlorella vulgaris* in the first 15 min were more than five times higher (52.3 ± 0.8 vs. 10.7 ± 0.9 wt%) than that of raw algae during n-hexane based solvent extraction. The kinetics of lipids extractability followed a zero-order reaction rate for all wet raw microalgae and the BI of *Scenedesmus* sp., while the BI recovered from the other two algal species were determined as a second-order reaction. Comparison of fatty acids profiles indicated the contribution of the FH process in saturating fatty acids. Subsequent to lipids extraction, a conventional hydrothermal liquefaction was performed at 350 °C and 1 h to compare the biocrude yields from raw versus BI of *Chlorella vulgaris* microalgae. The results showed that the biocrude yields from the BI and its quality was significantly enhanced post FH than that of raw algae. The FH process was proven to be a viable option for lipid extraction by increasing the extent of recovery and decreasing the extraction time. Its integration with HTL notably impact the biocrude yields and characteristics for fuel production.

1. Introduction

Efforts to reduce fossil fuel consumption have been attempted around the world with the aims of mitigating negative environmental harms such as air and water pollution, establishing energy independence, and inspiring innovation in alternative fuels development. The dependence on fossil fuels is heavily ingrained into society, while alternative energy only accounts for less than 10% of the global energy supply according to the United States Department of Energy [1]. Outstanding biological photosynthetic carbon assimilation potential is one of the main reasons that algal biomass is being considered as a clean fuel and bioproducts source [2]. There are over thousands of species of algae, but their basic composition mainly consist of proteins, lipids, and carbohydrates [3]. In particular, microalgae can accumulate lipids up to 20–50 wt%, which have high interest for a variety of bioproducts in food, cosmetics, and pharmaceutical industries [4] in addition to its biofuels potential. The algae to biofuels production process has had much success from pilot to large scale operation, but equally as many obstacles that prevent it from becoming competitive with conventional fossil fuels. One of the scientific challenges of algal biofuels commercialization includes lipid extractability from algae cells [5]. Lipid extraction methods are key to the biofuels/bioproducts quality and yield from algae. The conventional oil extraction steps include breaking the algae cell walls, freeing the oil, and separating the oil out of the oil cake [6,7]. There are multiple technologies for lipid extraction from microalgae that are categorized under solvent extractions (Folch, Bligh and Dyer method), mechanical approaches (expeller press, bead beating, ultrasonic-assisted, microwave), and solvent-free methods (osmotic pressure, isotonic, enzyme-assisted) [8]. Choice of oil extraction typically depends on moisture content, quantity to be treated, quality of end-product, extraction efficiency, safety aspects, and cost economics [7]. Three methods including expeller, supercritical CO_2 fluid

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extraction, and hexane extraction seem to be the most viable for industrial scale [5,7]. Among the three, hexane has been used in most applications of oil extraction [9]. It has high stability, low greasy residual effects, and low corrosiveness [9,10]. It has less toxicity compared to chloroform and methanol [6]. In addition, it is apolar (water immiscible) with low latent heat of boiling that makes it possible to be separated through low energy separation recovery methods [6,11]; however, it has a poor extractability efficiency compared to chlorinated solvents (i.e. chloroform) [12]. Techno-economic analyses (TEA) has shown that costs involved for lipid extraction with hexane is the second largest operational cost [13]; therefore, there is persistently strong demands for novel pretreatment methods of feedstock resulting in the overall improvement of the hexane extraction process. Alternative to the lipid extraction approach, hydrothermal liquefaction (HTL) of microalgae feedstock can directly convert the lipids into a biocrude oil, which is then subjected to further upgradation to fuel [14]. Many efforts have been made to optimize the process in terms of enhancing the biocrude yields [15–17]; however, high nitrogen content in HTL biocrude causes catalyst poisoning during downstream processing for liquid hydrocarbons/transportation fuels [18]. Production of high amounts of NO_x emissions in the downstream processing originating from nitrogenous compounds in proteins and chlorophyll content of microalgae is another serious challenge that this process needs to overcome [14,18].

Flash hydrolysis is a chemical-free subcritical water-based continuous process that fractionates microalgae components in a short residence time of 10 s. Our previous studies have shown multiple advantages of using the FH process for microalgae in terms of nutrient management either in forms of recycling or bioproducts formation [19–27] while protecting the lipids in solids. It was reported that 24–52 wt% (depending upon algal species) of the solid residue, known as biofuels intermediates (BI) are recovered after FH with diminished ash and nitrogen content [19]. We have also demonstrated that more than 90 wt% of total lipids available in the raw microalgae has been retained in the biofuels intermediates (BI) after the FH process [19,21]. The previous SEM images [19] of BI have shown its globular condensed appearance after the FH treatment. It has indicated that the process affected the physical dimensions of the particles to a smaller size; however, it is not clear if the FH process adversely affected the lipids extractability from the BI due to reduced solvent accessibility or entrapping oil after the recondensation process [6].

The current study investigates the lipid extraction efficiency of these BIs recovered after the FH process. In addition, a novel integrated FH-HTL process has been proposed to improve the biocrude yield and characteristics for fuel conversion. The main objectives of this study are to (i) conduct a kinetics study on the lipids extractability of the raw and BI from three common algal species (*Scenedesmus* sp., *Nannochloropsis* sp., and *Chlorella vulgaris*), (ii) analyze the fatty acids profile of lipids and compare it with that of lipids from untreated algae, (iii) produce biocrude from the BI of *Chlorella vulgaris* using HTL and compare the biocrude yield and quality with biocrude produced via direct HTL (no FH) of the same microalga. Fig. 1 shows the overall process including objectives and the products analyses among this study.

2. Materials and methods

2.1. Microalgae characterization

Three microalgae species including *Scenedesmus* sp., *Nannochloropsis* sp., and *Chlorella vulgaris* (*Chlorella v.*) was selected for this study. These species are known as the most promising candidates for biofuels and bioproducts production due to their lipid productivity and growth rate [2,6]. *Chlorella v.* was purchased from Arizona Center for Algae Technology and Innovation (AzCATI), *Nannochloropsis* sp. microalgae was received from Sandia National Laboratory (SNL), and *Scenedesmus* sp. was cultivated in a raceway open pond near Spring Grove, Virginia [23]. All samples were freeze dried (FD) and stored at −20 °C until application. In order to collect an adequate amount of BI for subsequent lipids extraction and HTL experiments, 10 FH tests were performed on each microalgae species at 280 °C and 10 s of residence time using the method explained in our previous studies [21,25]. Briefly, solids (0.9–1.2 dry wt%) are loaded in the reactor at specified conditions; based on the reactor set up, deionized water is pumped until the desired temperature is reached, and the second pump delivers the algae slurry into the reactor. Followed by FH, products were centrifuged (Fisher Scientific accuSpinTM 400) and vacuum filtered (1.5 μm, Whatman 47 mm glass microfiber filters) to separate the solids (i.e. lipid rich BI) from the hydrolysate. The recovered BI from each algal species was freeze dried and stored at −20 °C until application. All microalgae samples and their respective BIs were subjected to ash analysis using the dry oxidation method at 575 ± 25 °C for 24 ± 6 h as described by the National Renewable Energy Laboratory (NREL) analytical procedure [28] followed by elemental analysis. Thermo Finnigan Flash EA 1112 elemental analyzer (ThermoFisher Scientific, Waltham, MA) with 2,5-Bis(5-tert-butylbenzoxazol-2-yl) thiophene (BBOT) standard (certified no. 202147-10/03/2015, ThermoFisher Scientific, Cambridge, UK) were used to characterize the elemental composition of algal biomass [19].

2.2. Experimental setup and procedure

2.2.1. Total lipid yields and FAME composition

To evaluate the lipid extraction performance of the microalgae feedstock, two critical factors including lipid yields and fatty acid methyl ester (FAME) profile needed to be considered [29]. First, the total extractable lipid was quantified from the respective BIs to evaluate if the FH process had positive impact on improving lipids yield. For quantifying the total extractable lipids, 0.35 g of dry biomass (BI or untreated algae) was fed into a glass tube and 4 ml of deionized (DI) water was added to the tube to fully soak the dry biomass at 4 °C overnight. To assist the oil extraction, specifically free fatty acids (FFA) [6,30], 0.5 wt% of sulfuric acid was added to reduce the pH. A magnetic stir bar was added to stir the biomass on a multi-position magnetic plate and 3 ml of hexane was added to the tube. The extraction was carried out for 2 h on the magnetic plate. Tubes were vortexed for 30 s, every 30 min to improve the extraction. After the extraction, the tubes were centrifuged at 2000*g* for 10 min for phase separation. Then, the upper phase was moved to a preweighted glass tube. The solvent was evaporated in a vacuum oven at 40 °C overnight [31]. The experiment was carried out in triplicate. The extractable lipid yields were calculated using the following Eq. (1) on moisture-free basis:

$$\text{Total lipid yields(wt\%)} = \text{extracted lipids(g)/starting biomass(g)} \times 100\% \quad (1)$$

Fatty acid content in the biomass was measured as total FAME content after an in situ transesterification procedure [32]. A total of 7 to 10 mg of lyophilized biomass was transesterified with 0.3 ml of HCl/methanol (5%, v/v) at the presence of 0.2 ml of chloroform/methanol (2:1, v/v) for 1 h at 85 °C with a known amount of tridecanoic acid (C13) methyl ester as an internal standard. FAMEs were extracted with hexane (1 ml) at room temperature for 1 h and analyzed by gas chromatography–flame ionization detection (GC–FID) on an Agilent 7890 N; DBWax-MS column (30 m × 0.25 mm i.d. × 0.25 μm film thickness). The individual FAME concentrations were normalized against the internal standard tridecanoic acid methyl ester.

2.2.2. Lipid extraction kinetics

To better understand the lipid extraction process, a lipid kinetic study was conducted. Lipid yields represent extraction efficiency and was measured through the Eq. (1). To perform the lipid extraction experiments, 1 g of raw algae or BI were added to 20 ml of Milli-Q water (EMD Millipore, Milli-Q Direct 16 water purification system) to make a