Bioresource Technology 193 (2015) 386–392

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

# Concurrent production of biodiesel and chemicals through wet in situ transesterification of microalgae

Hanjin Im, Bora Kim, Jae W. Lee *

*Department of Chemical and Biomolecular Engineering, KAIST, 291 Daehak-ro, Yuseong-gu, Daejeon 305-701, Republic of Korea*

## HIGHLIGHTS

- Coproduction of biodiesel and chemicals from wet in situ transesterification.
- Elucidation of mechanism for coproducing biodiesel, EL, EF, and DEE.
- EL and EF are originated from acid hydrolysis of algal cells.
- DEE is produced from acid dehydration of ethanol.
- FAEE yield exceeding 94% with EL, EF, and DEE yields of 23%, 10%, and 52%.

## ARTICLE INFO

*Article history:*
Received 18 May 2015
Received in revised form 23 June 2015
Accepted 24 June 2015
Available online 29 June 2015

*Keywords:*
Biodiesel
Wet in situ transesterification
Microalgae
Cell hydrolysis
Chemicals

## ABSTRACT

This work addresses an unprecedented way of co-producing biodiesel (FAEE) and valuable chemicals of ethyl levulinate (EL), ethyl formate (EF) and diethyl ether (DEE) from wet in situ transesterification of microalgae. EL, EF, and DEE were significantly produced up to 23.1%, 10.3%, and 52.1% of the maximum FAEE mass with the FAEE yield higher than 90% at 125 °C. Experiments to elucidate a detailed route of EL and EF synthesis were fulfilled and it was found that its main route to the production of EL and EF was the acid hydrolysis of algal cells and esterification with ethanol. To investigate the effect of reaction variables on the products yields, comprehensive experiments were carried out with varying temperatures, solvent and alcohol volumes, moisture contents and catalyst amounts. Coproduction of DEE, EL, EF and FAEE can contribute to elevating the economic feasibility of microalgae-based biodiesel supply chain.



## 1. Introduction

Over the years, the growing concern of global warming and the decline of petroleum-derived energy source have resulted in a shift of interest to alternative resources for the production of fuels and valued commodities. Microalgae are one of promising biofuel sources because they can uptake carbon dioxide from the air or industrial flue gas and grow faster than terrestrial plants. In addition, higher algal cell concentrations can be obtained from a wide variety of cultivation media even in extreme environments like contaminated water (Jiang et al., 2011). Despite all the advantages, however, research on microalgae-derived biofuels still faces hurdles to commercialization due to the cost of production. In the entire biodiesel production process, drying algal cells is needed to improve the efficiency of lipid extraction. Even if the drying process could accelerate the yield in extraction and conversion processes, it is a highly energy-intensive step and it elevates production costs of biodiesel (Razon and Tan, 2011). Thus, eliminating the drying step in biodiesel production is crucial in reducing the overall production cost. However, obtaining the biodiesel from wet microalgae without the drying process still remains challenging.

To this end, the alternative 'in situ' approach which integrates lipid extraction and conversion processes has been designed to reduce processing costs in producing biodiesel (Leung et al., 2010). This approach can accelerate the conversion of triacylglyceride (TAG) in microalgae into biodiesel by combining each extraction and transesterification step into a single operation process. Many previous works have successfully investigated in situ transesterification using conventional methods of microwave, supercritical fluid, and solvent extraction.(Im et al., 2014; Johnson and Wen, 2009; Koberg et al., 2011; Patil et al., 2013). These in situ experiments except for the supercritical method were normally done at

* Corresponding author. Tel.: +82 42 350 3940; fax: +82 42 350 3910.
*E-mail address:* jaewlee@kaist.ac.kr (J.W. Lee).

http://dx.doi.org/10.1016/j.biortech.2015.06.122
0960-8524/

ambient pressure with a temperature range of 60–100 °C and only focused on the production of biodiesel.

At elevated temperatures higher than 100 °C, however, this study newly emphasized the production of new chemicals of ethyl levulinate (EL), diethyl ether (DEE), and ethyl formate (EF) in addition to biodiesel when in situ transesterification of wet microalgae was carried out. These valuable chemical compounds were produced when in situ transesterification of wet algal cells occurs in one pot with ethanol as a reactant, chloroform as a solvent, and sulfuric acid as a catalyst. They have a wide range of applications. EL that is an esterification adduct of levulinic acid (LA) can be used as a flavoring agent (Leonard, 1956). Furthermore, it was reported that EL has similar properties to biodiesel and addition of EL to biodiesel fuels can improve the low temperature properties of biodiesel (Demirbas, 2011; Joshi et al., 2011). Another co-product EF has widely used as a fumigant for storing grain and fruit (Ren and Mahon, 2006). DEE can also be blended with diesel for better fuel property because DEE has a high cetane number (more than 125) and miscibility in diesel fuel without any solvent (Bailey et al., 1997; Miller Jothi et al., 2007). Furthermore, one previous study observed that DEE blended diesel showed simultaneous reduction in $NO_x$ and smoke emissions (Mahalakshmi and Anand, 2007).

Therefore, this work will demonstrate the high yield of both FAEE and valuable secondary chemical compounds of EL, EF and DEE using one-pot in situ transesterification of wet microalgae. We will elucidate the formation mechanism of EL and EF by decoupling the production of LA and formic acid through algal cell hydrolysis, and its esterification to EL and EF. We will also investigate the effect of moisture contents of microalgae, reaction temperature, ethanol volume, chloroform volume, sulfuric acid catalyst amount, and produced glycerol amount on the FAEE and the other chemical compound yields. Experimental outcomes will show significantly high yields of FAEE and the other chemical compounds at a reaction temperature of 125 °C. Co-producing FAEE with EL, DEE, and EF can further decrease the cost of biodiesel production and improve the biodiesel value chain, which improves the competitiveness of the microalgal biodiesel production system.

## 2. Methods

### 2.1. Chemicals and reagents

The *Nannochloropsis gaditana* powder was purchased from AlgaSpring (Netherlands) and stored in the dark at −10 °C. Extra pure grade (95%) sulfuric acid and reagent grade (99%) chloroform were purchased from JUNSEI (Japan). Extra pure grade (99.5%) ethanol was purchased from Daejung (Korea). Analytical grade (99%) heptadecanoic acid ethyl ester (C17 ethyl ester), reagent grade (97%) ethyl formate (EF) and diethyl ether (DEE, 99.7%) were purchased from Sigma–Aldrich (USA) and used as a reference component for gas chromatography (GC) analysis. Ethyl levulinate (EL) with a purity of 98% were acquired from TCI (Japan).

### 2.2. GC analysis

For the quantification of each sample, the liquid organic phase including FAEE and other chemicals was injected to GC (Agilent 7890b, USA) equipped with HP-5 column (30.0 m × 0.32 mm × 0.25 μm). 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 injected. The yield of FAEE was calculated based on the ratio between the peak area of standard C17 ethyl ester and the peak area of produced FAEE from microalgae. The yields of the other chemicals (EL, EF, and DEE) were measured in GC in a similar way to the measurement of FAEE yield. However, the correction factor was used to calculate the yield of each chemical because there is a large difference in molecular weights between each chemical and C17 ethyl ester.

### 2.3. Maximum FAEE yield

To investigate the maximum quantity of FAEE in *N. gaditana* cells, experiments based on Folch's method were conducted (Folch et al., 1957). In a Teflon-sealed screw-capped tube, 10 mg of lyophilized cells were mixed with 2 ml of chloroform and ethanol mixture (2/1 v/v) and vortexed for 10 min for lipid extraction. 1 ml of ethanol and 0.3 ml of sulfuric acid were then added to the sample tube and heated to 100 °C for 30 min. After the transesterification reaction, the tube was cooled to room temperature and a standard solution of 1 ml chloroform containing 0.5 mg heptadecanoic acid ethyl ester was added to the tube. In each sample, the phase separation was done by centrifugation at 3500 rpm for 10 min and the organic phase was extracted for further GC analysis.

### 2.4. Procedure of wet in situ transesterification

The dry microalgal powder was mixed with deionized water and settled for 30 min for saturation and then wet in situ transesterification was conducted in a Teflon-sealed screw-capped tube. The wet algal cell, chloroform, ethanol, and sulfuric acid were mixed in the tube and the sample tube was heated to the temperature range of 95–140 °C in a water bath (WCH-8, Germany) and was maintained at a desired temperature for 2 h. To analyze the effect of each reaction parameter on the yields of FAEE and other chemicals, reaction conditions were varied in the following range: temperature (95–140 °C), moisture content (0–90 wt.%), chloroform (0–3 ml), ethanol to dry biomass ratio (0.15–9 ml/g), and sulfuric acid (0.03–0.7 ml/ml ethanol).

Once the experiments were done, 1 ml chloroform containing 0.5 mg heptadecanoic acid ethyl ester was added as a standard reagent for GC analysis and sodium hydroxide solution was added to neutralize the liquid sample. The yields of FAEE and other chemicals were calculated by the following equations.

$$\text{FAEE yield} = \frac{\text{Weight of obtained FAEE}}{\text{Weight of maximum FAEE}} \times 100\ (\%) \quad (1)$$

$$\text{Chemical compound yield} = \frac{\text{Weight of obtained chemical}}{\text{Weight of maximum FAEE}} \times 100\ (\%) \quad (2)$$

The yields of EL, EF, and DEE were calculated based on the weight of the maximum FAEE yield to compare with the FAEE yield. All the experiments were repeated more than three times in order to guarantee the reproducibility of data.

### 2.5. Procedure for verifying the generation of EL, EF, and DEE

The cellulosic part in the microalgal cell could be converted to LA and formic acid by acidic hydrolysis (Weingarten et al., 2012). Because LA and formic acid are esterification precursors of EL and EF, the presence of ethanol in in situ transesterification naturally converts LA and formic acid into EL and EF. Therefore, the production level of LA and formic acid through the cell hydrolysis significantly affects the EL and EF yield during in situ transesterification. Fig. 1a illustrates the experimental procedure to confirm the generation of EL and EF that are co-products from the single-step in situ transesterification of wet microalgal cells (Fig. 1b). To verify the generation of EL and EF, the confirmation