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Effects of cytoplasm and reactant polarities on acid-catalyzed lipid transesterification in wet microalgal cells subjected to microwave irradiation

Rui Huang, Jun Cheng *, Yi Qiu, Tao Li, Junhu Zhou, Kefa Cen

State Key Laboratory of Clean Energy Utilization, Zhejiang University, Hangzhou 310027, China

HIGHLIGHTS

- Cytoplasm and microwave change polarity of reactant and affect transesterification.
- Cytoplasm organic decrease contact angle of reactants by 13.92° and promote reaction.
- Hydrophilic algae biomass make apolar lipid more accessible to hydrophilic alcohol.
- Cytoplasm water increase contact angle of reactants and inhibit transesterification.
- Microwave decrease dielectric constant of alcohol and promote transesterification.

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ABSTRACT

The polarities of the cytoplasm and reactants were measured through dielectric spectroscopy, contact angle test, NMR, and FTIR to investigate the mechanisms underlying acid-catalyzed lipid transesterification in wet microalgal cells subjected to microwave irradiation. Organics with apolar functional groups in the cytoplasm decreased the contact angle of methanol against triglyceride by 13.92°, which subsequently increased transesterification efficiency by 2.4 times. The microalgal biomass, given its higher hydrophilicity index of 1.96 than lipids, was more accessible to hydrophilic alcohols, which subsequently promoted transesterification. Water in the cytoplasm promoted the dielectric constant of methanol and increased the contact angle of methanol against triglyceride by 20.51°, which subsequently decreased transesterification efficiency by 72.6%. The inhibitory effect of water on transesterification weakened with the prolonged carbon lengths of the alcohols because of decreased polarity. Microwave decreased the electric constants of alcohols and reduced the polarity difference between alcohols and lipids, thereby improving transesterification efficiency.

1. Introduction

In consideration of the growing fossil fuel shortage and environmental concerns related to massive carbon dioxide emissions, biodiesel produced from microalgae is currently regarded as a promising alternative fuel for diesel engines (Hidalgo et al., 2015a; Ma et al., 2015; Song et al., 2015; Teo and Idris, 2014). Using microalgae as a biodiesel source presents several significant advantages, such as high growth ratios, high cellular lipid concentration, and no competition for land with crops (Callejon et al., 2014; Jin et al., 2014; Tran et al., 2013). Despite the numerous advantages of using microalgae for biodiesel production, various technical and economic challenges, including the development of an efficient lipid extraction procedure, must be addressed to commercialize the industrial-scale production of microalgal biodiesel (Hidalgo et al., 2015b; Im et al., 2014, 2015). Traditional biodiesel production involves not only high energy consumption related to microalgae drying but also toxic chloroform utilization for oil extraction, which hinder the efficient scale-up of biodiesel production (Sitthithanaboon et al., 2015). Direct lipid transesterification in wet microalgal cells have attracted increasing attention in recent years because the process eliminates microalgae drying and chloroform utilization for oil extraction (Park et al., 2015).

Alcohol molecules diffuse into wet microalgal cells and react with lipids in cells; thus, lipid transesterification is significantly influenced by the cytoplasm in microalgal cells. Lipid transesterification is inhibited by the water contained in the cytoplasm

* Corresponding author. Tel.: +86 571 87952889; fax: +86 571 87951616.
E-mail address: juncheng@zju.edu.cn (J. Cheng).

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(Sathish and Sims, 2012). The inhibitory effect of water may be attributed to the reversible reaction of water with the resulting biodiesel, the deactivated acid catalyst by water, and the shield effect of water on lipids (Sathish et al., 2014). However, Takisawa et al. (2013) reported that the inhibitory effect of water is significantly weaker on the esterification of free fatty acids than on the transesterification of triglycerides. However, the different inhibitory effects of water on the esterification and transesterification in microalgal cells remain unexplained.

Considering the high water and free fatty acid contents of microalgae, previous studies used acids to catalyze the direct lipid transesterification in wet microalgal cells (Hidalgo et al., 2014; Kim et al., 2015a; Macias-Sanchez et al., 2015). However, acid-catalyzed transesterification shows a low efficiency. A co-solvent is required to promote the transesterification of lipids extracted from microalgae. Tetrahydrofuran reportedly yields the highest biodiesel among different solvents (Lam and Lee, 2013). However, a co-solvent is not needed to achieve a high biodiesel yield when acid-catalyzed transesterification is performed in microalgal cells (Cheng et al., 2014; Kim et al., 2015b). This situation indicates that the cytoplasm significantly influences acid-catalyzed transesterification, possibly acting as a co-solvent during the reaction. However, the effect of the cytoplasm on acid-catalyzed transesterification remains to be clarified.

Microwave heating is highly advantageous for chemical applications and has become a widely accepted nonconventional energy source to promote transesterification (Hsiao et al., 2011; Martinez-Guerra et al., 2014). The biodiesel production rate and yield of acid-catalyzed transesterification are simultaneously increased with microwave irradiation (Cheng et al., 2013b) possibly because of the high temperature generated during microwave heating. However, the increased reactivity may also be caused by a specific radiation effect (de la Hoz et al., 2005). The mechanism by which microwave irradiation promotes acid-catalyzed transesterification has not been experimentally investigated.

The polarity of reactant mixtures significantly influences lipase-catalyzed transesterification (Bezbradica et al., 2007; Sun et al., 2013). However, the effect of reactant polarity on acid-catalyzed lipid transesterification has not yet been investigated. In the present study, the polarities of the cytoplasm and reactants were measured through dielectric spectroscopy, contact angle test, nuclear magnetic resonance (NMR), and Fourier-transform infrared (FTIR) spectroscopy to investigate the mechanisms underlying acid-catalyzed lipid transesterification in wet microalgal cells subjected to microwave irradiation. Methanol, ethanol, and isopropanol were employed for acid-catalyzed lipid transesterification under different conditions and the polarities of the reactant mixtures were measured and compared to reveal the mechanism by which the cytoplasm and microwave influence acid-catalyzed lipid transesterification in wet microalgal cells. The cytoplasm of wet microalgal cells was characterized through FTIR, C^{13}NMR, and H^{1}NMR to reveal how it affects reactant polarity.

2. Methods

2.1. Materials

Bristol's medium-cultivated *Chlorella pyrenoidosa* was used to investigate the effects of the cytoplasm on acid-catalyzed lipid transesterification in wet microalgal cells (Cheng et al., 2013a). A 2 L bioreactor was pumped with air to cultivate *C. pyrenoidosa* for 15 days with illumination (2500 lx, dark/light cycle: 12/12 h). The cultivated *C. pyrenoidosa* was centrifuged in individual centrifuge tubes to obtain a microalgal paste (~1 g) with 77 wt.% water. The paste was then stored at −20 °C until processing. All reagents, including hexane, methanol, ethanol, isopropanol, sulfuric acid, ester, fatty acid, and triglyceride, were purchased from Sinopharm Chemical Reagent (Shanghai, China).

2.2. Microwave-assisted lipid transesterification with cytoplasm-added alcohols

To reveal the effects of cytoplasm organics on microwave-assisted lipid transesterification, the cytoplasm was extracted from wet microalgal cells with alcohols in accordance with the following steps. The stored wet *C. pyrenoidosa* biomass (1 g) was thawed and then transferred into the 60 mL digestion reactor of the microwave digestion system. Alcohols (6 mL) were subsequently added into the reactor to extract the cytoplasm in the WX-4000 microwave digestion system (2.45 GHz; Shanghai Yiyao Microwave Chemistry Company, Shanghai, China) (Cheng et al., 2014). After the reactor was sealed, the mixture in the digestion reactor was heated to 90 °C via microwave irradiation with a power output of 600 W. The power output of microwave irradiation was set at 500 W to maintain the mixture at 90 °C for 30 min.

After the treatment, the mixture was cooled in air to reach room temperature. The cooled mixture was then poured into a centrifuge tube. After the removal of the microalgal residue through centrifugation, the cytoplasm-added alcohols were obtained and added into the 60 mL digestion reactor of the microwave digestion system. Triglyceride (100 mg) and concentrated sulfuric acid (0.24 mL) were added into the digestion reactor. After the reactor was sealed, the mixture in the digestion reactor was heated to 90 °C via microwave irradiation with a power output of 600 W. The power output of microwave irradiation was set at 500 W to maintain the mixture at 90 °C for 30 min.

After microwave-assisted lipid transesterification, the mixture was cooled in air to reach room temperature. The cooled mixture was then poured into a centrifuge tube. Hexane (8 mL) and water (8 mL) were added into the centrifuge tube and mixed with the cooled reactant through 5 min of vigorous shaking to extract the resulting biodiesel after microwave-assisted transesterification. After centrifugation of the mixture, the resulting upper hexane layer was transferred into a glass vial and heated in a baking oven at 65 °C to regenerate hexane and subsequently produce crude biodiesel. The crude biodiesel was resolved in 1 mL of hexane with an internal standard (C19:0) for gas chromatography (GC) to determine the weight of the resulting fatty acid alkyl ester (FAAE).

Considering that the lipids in the microalgal cells may also be extracted using the alcohols, 0.24 mL of concentrated sulfuric acid were added into the cytoplasm-added alcohols to conduct microwave-assisted lipid transesterification without triglyceride addition. The weight of FAAE (named as cytoplasm-derived FAAE) converted from the lipids in the cytoplasm-added alcohol was then determined through GC analysis.

The transesterification efficiency of microwave-assisted lipid transesterification with the cytoplasm-added alcohols was calculated as follows:

$$\text{Transesterification efficiency} = \frac{\text{weight of total FAAE} - \text{weight of cytoplasm} - \text{derived FAAE}}{\text{weight of used triglyceride}}. \quad (1)$$

2.3. Microwave-assisted lipid transesterification and esterification with water-added alcohols

Microwave-assisted lipid transesterification with water-added alcohols was conducted in the same WX-4000 microwave digestion system. Triglyceride (or fatty acid; 100 mg), alcohols (methanol, ethanol, or isopropanol; 6 mL), and concentrated sulfuric acid