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Efficient enzyme-catalysed transesterification of microalgal biomass from *Chlamydomonas* sp.

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ABSTRACT

Facing the global issues of dwindling oil reserves and global warming, the search for alternative green energy source has become a priority. Microalgal biofuels has been regarded as a potential sustainable energy source, due to the high oil yield per area of land, ease of culturing microalgae, zero net carbon emission and reduced competition for arable land. In this paper, five different lipid extraction methods were studied using dry biomass of the microalga *Chlamydomonas* sp. Folch et al. method gave the highest oil yield of 26.27 wt%. The extracted microalgal oil underwent transesterification process using immobilised lipases. The highest conversion achieved was 72.09% in the following optimized conditions: 0.100 g of immobilised enzymes and solvent to methanol volume ratio of 1:1 with tert-butanol as the organic solvent.

1. Introduction

Two of the most pressing issues of our time are the dwindling world crude oil reserves and the greenhouse emissions associated with burning fossil fuels. We are heavily dependent on conventional fossil fuels to support our civilisation, but this energy source is unsustainable [5]. In view of the growing concerns, many research initiatives have focused on biodiesel production, in hopes of developing a sustainable and renewable energy source [7].

The search for lipid-rich biological materials for biodiesel production has attracted major interest in order to resolve the world's energy shortage crisis [17]. Biodiesel derived from oil crops can potentially become a renewable and carbon neutral alternative to petroleum fuel. However, biodiesel derived from oil crops, waste cooking oil and animal fats may not meet the huge global demand for transport fuel. Microalgae-derived biodiesel is thus proposed as a good candidate to completely displace petroleum-derived transport fuels without compromising any food production and other cash crops. Oil palm, being the most productive oil crop today, yields only a fraction of oil compared to microalgae per dry weight of feedstock [7]. Bioethanol yield from sugarcane is also less than microalgal biodiesel per dry weight of feedstock [8].

Extraction of microalgal oil for large-scale biofuel production is relatively new in terms of technology, when compared to biofuel generation from oil crops and lignocellulosic biomass [9]. Due to the inherent hardiness of microalgal cells, it is very difficult to extract its lipids without the use of cell disruption methods. Cell

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disruption methods (such as bead-beating, French press, ultrasonication) are commonly used to break the microalgal cell walls, and achieve a more complete lipid extraction [19,22].

The lipid extraction process is followed by transesterification, where alcohols are reacted with long-chain lipids to form short-chain fatty acid methyl esters (FAMEs). Short-chain alcohols are commonly used, such as methanol, ethanol, propanol, butanol and amyl alcohol [14]. Alkaline-catalysed transesterification are relatively less corrosive and faster compared to acid-catalysed transesterification [25]. Therefore, base catalysts are widely used in industrial scale. Chemical transesterification has many disadvantages although it is known that the yield of chemical transesterification is higher. The disadvantages include complex and expensive downstream processes such as the recovery of glycerol, removal of inorganic salt and water from the products, and the treatment of alkaline wastewater [2,12]. In view of that, enzymatic transesterification is proving to be an alternative method for large-scale microalgal biodiesel production. Enzymes enable transesterification of feedstock with high free fatty acid (FFA) content, lower reaction temperature (30–50 °C), production of glycerol with higher quality and generate less wastewater [14].

Therefore, this paper optimized the transesterification of microalgal oils to FAMEs via enzyme catalysis. Five different lipid extraction techniques were performed on dry biomass of *Chlamydomonas* sp. The microalgal oils extracted were transesterified by reacting with methanol and organic solvent in the presence of immobilised lipases. The experimental parameters optimized included lipase concentration, methanol concentration and the choice of organic solvent used.

2. Materials and methods

2.1. Microalgae source and lipid extraction methods

The dry microalgal biomass of *Chlamydomonas* sp. chosen for this study was obtained from the National Cheng Kung University (NCKU), Taiwan. The biomass had been previously lyophilized and was in surplus.

Five methods of lipid extractions were studied: Bligh and Dyer [4], Chen et al. [6], Folch et al. [13], Hara and Radin [16] and the heating block method. The biomass was pretreated by ultrasonication before applying the first four methods. The heating block method did not include ultrasonication and was used as a reference to compare the results of the other four methods.

0.25 g of dried microalgae biomass was used in this study. Ultrasonication was conducted in an ultrasonic bath operating continuously at 37 kHz, 30% power and 25 °C (Elmasonic P 30 H, ELMA, Germany). The duration of ultrasonication for Bligh and Dyer method was 20 min, Chen et al. and Folch et al. methods were 27 min, and Hara and Radin method was 56 min. The ultrasonication acted as a cell disruption treatment which assisted in the release of lipids within the microalga cells. All experiments were carried out in triplicate.

2.2. The heating block method

The biomass was mixed with 10 ml of chloroform, 5 ml of methanol and 1 ml of distilled water in a test tube. The sample was vortexed until a homogenised mixture was obtained. The mouth of the test tube was sealed with PTFE. The sample was heated at 60 °C for an hour using a heating block (Wise Therm HB-48, Witeg, Germany). After the sample had cooled down, the bottom phase (chloroform) was collected into a glass vial using a pipette. The sample was allowed to dry at room temperature and the remaining lipid in the glass vial was weighed.

2.3. Lipase-catalysed transesterification of microalgal oil

The extracted microalgal oil was dissolved in 3 ml tert-butanol and mixed with 3 ml methanol. 0.100 g of immobilised enzymes (lipase acrylic resin from *Candida Antarctica*, Novozyme 435, Sigma, U.S.A.) were then added to start the transesterification reaction. The reaction was carried out in a tightly-sealed 30 ml McCartney bottle to prevent evaporation. The immobilised enzymes acted as biocatalysts to convert the lipids into biodiesel, a process called transesterification . The sample was shaken at 150 rpm and incubated at 50 °C for 24 h [18]. After incubation, the immobilised enzymes were removed using filter paper. The top oil layer in the remaining liquid fraction was pipetted and diluted with 1 ml of *n*-heptane for gas chromatography-mass spectrometry (GC-MS) analysis. Tert-butanol was chosen for this study because it is the most commonly used solvent for biodiesel production [27].

2.4. Determination of oil content from microalgal biomass

After transesterification, the mixture was filtered to remove the solids using filter paper and filter funnel. The remaining liquid fraction was mixed with 15 ml of potassium chloride (KCl) solution (0.88% w/v) and then allowed to settle down for 24 h in a separating funnel. The bottom phase was collected in round-bottomed conical flask and transferred to a rotary evaporator (Rotary Evaporator RE300, Yamato, Japan) at 45 °C and vacuum condition was applied. The liquid fraction was allowed to dry out until all solvent had evaporated. The remaining oil phase was collected by using dichloromethane and pipetted into a glass vial where its weight was measured. The samples were allowed to dry out at the room temperature and the dried microalgae oil remaining in the glass vial was weighed.

2.5. Gas chromatography-mass spectrometry (GC-MS) analysis

The crude FAME samples were analysed by GC-MS (Agilent 7200 Quadrupole TOF GC-MS, Agilent Technologies, U.S.A.) equipped with a DB-5 column and flame ionisation detector (FID). The GC-MS was used in a splitless mode and helium gas was used as the carrier gas which flowed at 1.2 ml/min. The inlet temperature was maintained at 250 °C and the temperature of the column was programmed in the following sequence: The temperature was initially maintained at 50 °C for 1 min. The temperature was increased at a rate of 25 °C/min to 200 °C and maintained for 10 min. The temperature was further increased to 300 °C at a rate of 10 °C/min and kept for 10 min. The total FAME yield percentage was summed up based on the area percentage given by the chromatogram [1].

3. Results and discussions

3.1. Comparison of five different lipid extraction methods

Based on the results tabulated in Table 1, it can be seen that the lipid yields obtained from all the solvent and ultrasonication-assisted methods (Bligh and Dyer, Chen et al., Folch et al., as well as Hara and Radin) were higher than the solvent-assisted only heating block method. The five methods being studied were highly reproducible because the standard errors calculated from the five methods ranged from 0.8 to 1.8%. Out of the fi-ve methods being studied, Folch et al. method achieved the highest lipid yield of 26.27 ± 1.80 wt% of biomass. The other lipid extraction yields were arranged in the following descending order: Chen et al. method (23.73 ± 0.61 wt%), Bligh and Dyer method (17.87 ± 1.40 wt%), Hara and Radin method (11.73 ± 1.80 wt%) and heating block method (8.00 ± 0.80 wt%). The combination of ultrasonication and solvent

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