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An integrative process for obtaining lipids and glucose from *Chlorella vulgaris* biomass with a single treatment of cell disruption

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ABSTRACT

To examine the possibility of better utilizing *Chlorella vulgaris* biomass including its carbohydrate as well as lipid contents, it was investigated whether cell disruption for lipid extraction could render the remaining microalgal residue (MR) suitable for enzymatic saccharification, possibly due to the disruption of cell wall structures. The *C. vulgaris* biomass was subjected to lipid extraction with different cell disruption methods (autoclaving, microwave irradiation, osmotic shock, and sonication), and recovered MRs were hydrolyzed using an enzyme produced from *Trichoderma koningiopsis* KUC21269 in this study. The enzyme was produced on-site with a highly simplified medium of barley straw, an agricultural byproduct. As a result, the saccharification rate of MR treated with microwave was more than twice that of the control group, and microwave irradiation appeared to be a promising method for both lipid extraction and subsequent saccharification. Our results suggested that both lipids and carbohydrates in *C. vulgaris* can be utilized by applying proper cell disruption method and a fungal enzyme produced on-site using an agricultural byproduct, respectively. This study revealed the high potential of *C. vulgaris* as an integrated bio-resource for both lipids and glucose, which can be converted to biodiesel and bioethanol, providing clues for overcoming hurdles in economically feasible biofuel production using microalgae.

1. Introduction

Because of the continuous decrease in oil prices due to shale gas production, renewable energy industries are having trouble achieving price competitiveness. Therefore, it has become even more important to reduce energy production costs [1]. In bioethanol production, the cost of raw material occupies a considerable proportion of the total production cost, and thus, many researchers have attempted to identify more economical raw materials.

Microalgae have been receiving increasing attention due to the potential of the third generation of biomass; however, a majority of studies on microalgal biomass have been biased toward biodiesel production without considering the high carbohydrate content in the biomass. Although there has been some effort to produce fermentable sugars or biogas from carbohydrate-rich microalgae, most previous studies have focused on chemical saccharification based on strong acids [2–5] or pretreatment with strong bases [6,7]. This approach is not desirable because the use of strong acids can cause environmental problems and additional costs for wastewater treatment, thus making bioethanol less environmentally friendly. Some studies were conducted with enzymatic saccharification using starch-rich microalgae [8,9], but this process also has a fundamental limit in that it may not be economically feasible to produce microalgal biomass for starch production alone. In this context, researchers alternatively focused on lipid-extracted microalgal residues (MRs), which would normally be considered as wastes and would thus require additional costs for disposal [10]. If the carbohydrates in those MRs can be utilized efficiently, the sum of both products will help the economic feasibility of microalgal biomass utilization. It was speculated that producing both bioethanol and biodiesel together from the microalgal biomass could achieve great benefit by broadening the extent of applications.

To the best of our knowledge, only few studies have attempted enzymatic saccharification of carbohydrates in MR for bioethanol production. However, they could not provide an appropriate process considering the issues on both economic feasibility and environmental friendliness. Lee et al. [11] investigated the saccharification of residual

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starch in MR, but starch is liable to be lost during lipid extraction, which limits the productivity. Mirsiaghi and Reardon [12] conducted saccharification of MR, but the MR was physically ground twice using a blender and a mortar with pestle causing the additional costs. Lee et al. [13] attempted to enhance the bioaccessibility of carbohydrates in MR by applying a chemical agent to lipid extraction and subsequent methylation. However, methylation efficiency of the agent is much lower than that of conventional agents [14], which inevitably reduces the biodiesel yield and thus causes fundamental problems in MR production. In addition, in all the studies, expensive commercial enzymes were used whose costs account for > 46% of the total bioethanol production cost [15]. It is evident that limited information is available for utilization of both lipids and carbohydrates from microalgal biomass without additional treatment or commercial enzymes.

The efficient utilization of both lipids and carbohydrates is entirely dependent upon high lipid productivity and high carbohydrate content in the microalgal biomass. According to previous studies, a green microalga, *Chlorella vulgaris*, exhibits high lipid content and also reserves high levels of cellulose in cell walls [16]; the cellulose constitutes up to 70–80% (w/w) of cell walls, and one *C. vulgaris* strain was reported to contain as much as 35% (w/w) cellulose in the total biomass [17,18]. For these reasons, *C. vulgaris* was thought to be a potential candidate for simultaneous application of both lipids and carbohydrates.

In order to fully utilize lipids and carbohydrates from *C. vulgaris* biomass, it is indispensable to develop treatment processes for efficient lipid extraction and saccharification. So far, a number of studies have suggested that efficient lipid extraction or saccharification is possible with the aid of various treatments. However, there has been no attempt to establish a single process for simultaneously improving both lipid extraction and saccharification efficiency.

It is likely that the cell disruption process itself serves to enhance both lipid extraction and saccharification owing to the fact that it leads to the destruction of the cell wall structures, thereby enhancing the bioaccessibility of the cellulose in *C. vulgaris*. Because cell disruption is essential for releasing lipid granules before extraction, we hypothesized that the same cell disruption process could be incorporated to simultaneously improve both lipid extraction and saccharification.

In this study, we attempted to establish the high efficiency of *C. vulgaris* biomass utilization. To do so, a single process of cell disruption was examined for enhancing both lipid extraction and enzymatic saccharification. Four approaches (autoclaving, microwave irradiation, sonication, and osmotic shock) were selected as the cell disruption processes in consideration of environmental impact avoiding the use of toxic chemicals and simplicity. We also tested the possibility of producing an enzyme from indigenous *Trichoderma koningiopsis* KUC21269 to save the cost of purchasing commercial enzymes. The fungus was cultured on barley straw, an agricultural byproduct, with a highly simplified medium, thereby reducing the enzyme production cost. Taken together, the aims of this study were to investigate the effects of different cell disruption methods on lipid extraction and the bioaccessibility of cellulose in MR and to reveal the potential of *C. vulgaris* as an integrated bio-resource for lipids and glucose which can be converted to biodiesel and bioethanol, respectively.

2. Materials and methods

2.1. Microalgae cultivation

A green microalga, *Chlorella vulgaris* UTEX 265, was obtained from the University of Texas Culture Collection (Austin, USA) and was cultured in a photobioreactor (light intensity: 60–90 μmol/m^2 s) for two weeks. The culture medium contained 1.25 g of KNO_3, 1.25 g of KH_2PO_4, 1.00 g of $MgSO_4{\cdot}7H_2O$, 83.5 mg of $CaCl_2$, 114.2 mg of H_3BO_3, 49.8 mg of $FeSO_4{\cdot}7H_2O$, 88.2 mg of $ZnSO_4{\cdot}7H_2O$, 14.4 mg of $MnCl_2{\cdot}4H_2O$, 7.1 mg of MoO_3, 15.7 mg of $CuSO_4{\cdot}5H_2O$, 4.9 mg of Co $(NO_3)_2{\cdot}6H_2O$, 500 mg of EDTA·2Na, and 350 mg of urea per liter of distilled water [16]. The microalgal biomass was harvested by centrifugation. The harvested biomass was freeze-dried at − 78 °C and stored in a deep-freezer at the same temperature.

2.2. Cell disruption of microalgae

Four grams of the dry cell biomass was blended with 800 mL of distilled water, and the mixture was placed in one of the four cell disruption methods as follows: autoclaving at 125 °C with 1.5 MPa for 5 min (from when the temperature reached 125 °C); microwave irradiation using a household microwave oven (Midea, China, MWO-2018, 2.45 GHz, 800 W) for 15 min; osmotic shock in a 10% (w/v) NaCl solution for 48 h; and sonication using a sonicator (Hwashin Instrument, Korea, POWERSONIC 620, 40 kHz, 700 W) for 15 min [19]. A biomass mixture without any treatment was established as a control.

2.3. Lipid extraction

The lipid was extracted by mixing chloroform and methanol with the samples in a proportion of 1:1:2 (chloroform:methanol:water (the biomass mixtures treated with/without cell disruption)) based on a modified version of Bligh and Dyer's method [20]. The mixture was shaken at room temperature for 5 min, and when it had separated into two layers, the chloroform layer (lipid fraction) was filtrated through a Whatman GF/C glass fiber filter with a vacuum pump. For the complete recovery of MR, the remaining MR on the filter paper was returned to its methanol-water layer using distilled water. After that, the methanol-water layer was centrifuged at 4000 rpm for 15 min and the supernatant was discarded. The MR was recovered by freeze-drying at − 70 °C [19].

2.4. Determination of the lipid extraction rate and the fatty acid composition

The chloroform layer was collected during the lipid extraction. Using a rotary evaporator, the solvent was completely removed and the weight of the extracted lipids was gravimetrically measured. The lipid extraction rate was calculated using the following equation:

$$\text{Lipid extraction rate (\%)} = 100 \times \frac{\text{Weight of the extracted lipids (g)}}{\text{Weight of the total TAGs in 4 g biomass (g)}}$$

It was assumed that majority of lipids in the sample were triacylglycerols (TAGs), and the total TAG content was regarded as total lipid content. It was reported that lipids in microalgae grown under the nitrogen-deficient condition mainly consist of TAGs [21]. The total TAG content in *C. vulgaris* was able to be obtained by a precise method using a gas chromatography–mass spectrometer (GC–MS; QP2010, Shimadzu, Japan) as described below. Thirty milligrams of dry *C. vulgaris* biomass was placed into capped test tubes, saponified with 2 mL of a NaOH-CH_3OH solution at 100 °C for 5 min, and then submitted to methanolysis with 4 mL of an HCl-CH_3OH solution at 80 °C for 10 min. Thereafter, the lipids were extracted with 2.5 mL of a C_6H_{14}-$(CH_3)_3COCH_3$ solution at room temperature for another 10 min. After the mixture was washed with 6 mL of a NaOH-D.W. solution for 5 min, the hexane layer that contained fatty acid methyl ester was separated. Pentadecanoic acid was used as an internal standard material, and the total TAG content of the sample was calculated. The fatty acid components were identified by comparing their retention times and fragmentation patterns with those for standards [42].

2.5. Estimation of biodiesel properties based on fatty acid composition

To evaluate the expected biodiesel properties of lipids in *C. vulgaris*, saponification value (SV), iodine value (IV), cetane number (CN),