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Dynamic microstructures and fractal characterization of cell wall disruption for microwave irradiation-assisted lipid extraction from wet microalgae

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HIGHLIGHTS

- Fractal dimension of algae cells increased with enhanced disruption of cell walls.
- Increased microwave treatment temperature led to increased cell fractal dimension.
- Cell wall thickness and pore diameter increased with prolonged microwave time.
- Cell walls treated by microwave started to disrupt at the maximum curvature.
- Long-chain and unsaturated lipids decreased due to microwave electromagnetic effect.

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ABSTRACT

To extract lipids from wet microalgae through cell disruption, the effects of microwave treatment on the dynamic cell wall microstructures were investigated. The fractal dimension of raw, untreated microalgal cells was 1.46. The disruption level of microalgal cell walls was enhanced when microwave treatment temperature increased from 80 to 120 °C, resulting in an increase in microalgal cell fractal dimension from 1.61 to 1.91. The cell wall thickness and pore diameters in cell walls increased from 0.11 to 0.59 μm and from 0.005 to 0.18 μm, respectively, when microwave treatment time increased from 0 to 20 min. The outer pectin layers of cell walls gradually detached and the porosity of inner cellulose layers increased when microwave treatment time increased to 26 min. The initial point of disruption appeared at the maximum curvature (approximately 1.01×10^7 m^{-1}) of cell walls. Numbers of short-chain and saturated lipids increased because of microwave electromagnetic effect.

1. Introduction

Microalgae have high potential as raw materials for biodiesel production because of their high photosynthetic efficiency, short cycling time and high lipid content. These microorganisms are considered high-potential renewable biomass resources that can replace traditional fossil fuels (Chisti, 2007). The use of microalgae in biodiesel production has other additional advantages, such as the capture of carbon dioxide as a carbon source from flue gas via photosynthesis (Demirbas, 2011; Fulke et al., 2010), wastewater purification by absorption of nutrient salts in water for nutriments (Wu et al., 2012) and residue fermentation for methane or alcohol production after lipid-extraction (Kirrolia et al., 2013).

However, high production costs severely limit the industrial application of microalgae in biodiesel production. In the traditional method for biodiesel production, microalgae are dried by evaporation of the total moisture in the cells after harvest. The dried microalgal cells are then ground for cell disruption. Both drying and grinding require large amounts of energy. Therefore, the development of low-cost, high-efficiency microalgal cell disruption methods has become a research hotspot (Gonçalves et al., 2013).

Lee et al. (2012) compared the energy consumed by lipid extraction from microalgae with those required by other methods. The energy consumption of mechanical disruption was higher than those of nonmechanical disruption methods, including physical, chemical and enzymolysis techniques. These methods had different advantages and disadvantages. Microwave irradiation features short processing times, high disruption efficiency and low energy consumption. Although solvent addition (e.g. hexane, ethanol) could reduce the energy consumption, the disadvantage was that solvents for lipid extraction might react with algae cells, which could affect the disruption of algae cell walls. The changes of algae cell walls in TEM images would attribute to the combined influence of solvents and microwave irradiation. It was hard to investigate the influence of only microwave irradiation on disruption of wet algae cell walls with solvents addition. Lee et al. (2010)

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compared the performances of five disruption methods (i.e., high-pressure steaming, grinding, microwave irradiation, ultrasonication and osmotic-pressure techniques) during lipid extraction from freeze-dried microalgal cells. A maximum extraction efficiency exceeding 80% was achieved with microwave irradiation treatment at 100 °C for 5 min. However, neither the effects of microwave irradiation on the microstructure of the microalgal cell walls not the effects of different microwave treatment conditions on the lipid extraction efficiency and lipid compositions were discussed. Balasubramanian et al. (2011) investigated lipid extraction from freeze-dried *Scenedesmus* (at a concentration of 5×10^6 cells/mL of aluminium sulphate solution) using successive microwave irradiation treatment. A maximum lipid extraction efficiency of 77% was achieved with microwave irradiation at 95 °C for 30 min. However, the effects of microwave irradiation on microalgal cell walls were not determined, and the energy consumed by the method was not calculated. Pasquet et al. (2011) compared the effects of microwave (microwave-assisted extraction and vacuum microwave-assisted extraction) and conventional methods on pigment extraction from freeze-dried algae. Both microwave and ultrasound methods showed shortened treatment times and enhanced extraction efficiencies. Whilst the effects of microwave irradiation on algae cell shapes were investigated in this work, the microwave effects on cell wall structures were not discussed. McMillan et al. (2013) studied the effects of solid–liquid shear, thermolysis and microwave and laser treatments on the disruption of wet *Nannochloropsis oculata*. Samples were observed every 5 min under microwave treatment at 90 °C for 20 min. The cell disruption percentage increased with increasing treatment time, and a maximum cell disruption percentage of 94.92% was found. However, the effects of different microwave treatment temperatures on cell disruption were not elucidated, and the effects of different treatment times on the cell wall microstructure and lipid composition were not discussed.

All of the aforementioned studies indicate that microwave irradiation is a highly effective as a microalgal lipid extraction method. Several questions, however, have yet to be answered. Why, for example, do the electromagnetic and heat effects of microwave irradiation lead to cell disruption? How does microwave irradiation affect the microalgal cell wall microstructure as well as the composition of the extracted lipid? This paper investigates the effects of different microwave treatment conditions on wet microalgal cell disruption during lipid extraction, and the dynamic microstructures of wet microalgal cell walls are reported for the first time. The fractal dimensional changes of wet microalgal cells during microwave treatment are analysed extensively.

2. Methods

2.1. Materials

Chlorella PY-ZU1 (Cheng et al., 2013) that had been mutated by nuclear irradiation and domesticated under high-concentration CO_2 were cultivated in the laboratory. Brostol's solution (also known as the soil extract, SE) containing 0.25 g of $NaNO_3$, 0.075 g of $K_2HPO_4{\cdot}3H_2O$, 0.075 g of $MgSO_4{\cdot}7H_2O$, 0.025 g of $CaCl_2{\cdot}2H_2O$, 0.175 g of KH_2PO_4, 0.025 g of NaCl, 40 mL SE, 0.005 g of $FeCl_3{\cdot}6H_2O$, 1 mL of Fe-EDTA and 1 mL of A_5 solution in 958 mL of distilled water was used as the cultivation medium. SE was obtained from the supernate of a boiled soil solution. The Fe-EDTA solution used in this study contained 10 g L^{-1} Na_2EDTA, 0.81 g L^{-1} $FeCl_3{\cdot}6H_2O$ and 500 mL of 0.1 M HCl. The A_5 solution contained 286 mg of H_3BO_3, 181 mg of $MnCl_2{\cdot}4H_2O$, 22 mg of $ZnSO_4{\cdot}7H_2O$, 7.9 mg of $CuSO_4{\cdot}5H_2O$ and 3.9 mg of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ in 100 mL of distilled water. Microalgae were cultivated in six one-liter flasks for approximately 7 d at 27 °C under a 12 h/12 h light/dark cycle with illumination of 3000 lux. Cells were harvested when they had accumulated to approximately 7.5×10^7 cells/mL of medium. The microalgal cells were centrifuged (Avanti J-26 XP, Beckman Coulter, USA) at 5000 rpm for 3 min, and wet microalgae, which contained 0.105 g dry biomass per milliliter of solution, were stored at 4 °C for subsequent experiments.

2.2. Experimental

The equipment used in this experiment was a microwave synthesis/extraction reaction system (MAS-II, Sineo Microwave Chemistry Technology (Shanghai) Co., Ltd., China). The pressure of the extraction vessel during the microwave irradiation was atmospheric pressure. The microwave power was automatically controlled by a frequency-converting technology based on temperature changes. Noncontact infrared temperature measurements were adopted. The actual inside temperature was approximately 2 °C above sensed infrared temperature, which was calibrated by the microwave system manufacturer. The system could monitor and control the temperature in time and had two stirring methods, namely, electromagnetic and mechanical.

Approximately 10 mL of the wet microalgae was placed in 25 mL three-necked flasks. The first sample was the control sample (without microwave irradiation). The cells in the flask were first dried in an oven at 105 °C for 2 h and then pulverized using a mortar for lipid extraction. Subsequent samples were preprocessed in the MAS-II microwave synthesis/extraction reaction system. The microwave power output and duty cycle were automatically modulated to provide a stable treatment temperature for the samples. The samples were placed in the microwave treatment system under different conditions. In one set of experiments, the treatment time was maintained for 5 min and treatment temperatures were set to 80, 90, 100 and 120 °C. In another set of experiments, the treatment temperature was maintained at 80 °C and the treatment times were set to 5 and 10 min. 40 mL of distilled water were used to flush the whole reactor system to ensure no algae residue after each experiment. So, the total volume of each wet algae sample after microwave for lipid extraction is 50 mL. To minimize microalgal growth-induced changes in the cell structures during long-term storage, the experiments were all performed within 1 week after microalgal harvesting.

The lipids in the control sample were extracted by the improved Bligh–Dyer method using a chloroform:methanol (1:1, v/v) mixture. Approximately 50 mL of solvent was used for every gram of dried sample in each extraction step (Widjaja et al., 2009). The sample was stirred with a magnetic stirrer bar for 2 h and then centrifuged at 3000 rpm for 10 min. The solid phase was carefully separated twice using two pieces of filter paper (Advantec filter paper, No. 1, Japan) to provide complete separation. The procedure was repeated three times until all lipids were extracted from the solid phase to the solvent phase. The solvent phase was evaporated in an oven at 80 °C for 24 h. The lipids in the microwave-treated samples were extracted using the modified method reported by Lee et al. (2010), which involved mixing chloroform:methanol (1:1, v/v) with the samples at a ratio of 1:1. The samples were stirred using a magnetic stirrer bar for 10 min and then centrifuged at 3000 rpm for 10 min. The solid phase was carefully separated using filter paper, as previously described. The solvent phase was evaporated in an oven at 80 °C for 24 h. The weight of the crude lipid obtained from each sample was determined using an electronic scale.

The crude lipid extracted from algae cells was directly used for transesterification without any separation and purification. 50 mg of crude lipid extracted from three replicates for each experiment was placed into capped test tubes, saponified with 1 mL of a