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Fractal microstructure characterization of wet microalgal cells disrupted with ultrasonic cavitation for lipid extraction

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HIGHLIGHTS

- Wet microalgal cytomembranes were destroyed to debris with ultrasonic treatment.
- Microalgal cells shrank due to dehydration but cell walls were not fragmented.
- Fractal dimension of cells increased with promoted power and time of ultrasonication.

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ABSTRACT

The effects of ultrasonic treatment on fractal microstructures of wet microalgal cells were investigated for lipid extraction. Fractal dimension of cells with distorted surfaces increased with power and ultrasonication time. Microalgal cells shrank owing to dehydration and cytomembranes were reduced to debris, but cell walls were not fragmented. When ultrasonication power increased from 0 to 500 W for 30 min, the fractal dimension of cells increased from 1.21 to 1.51, cell sizes decreased from 2.78 to 1.68 μm and cell wall thickness decreased from 0.08 to 0.05 μm. When ultrasonication time increased from 5 to 30 min with a power of 150 W, the fractal dimension of cells increased from 1.24 to 1.37, cell sizes decreased from 2.72 to 2.38 μm and cell wall thickness first increased to a peak of 0.22 μm and then decreased. Long-chain and unsaturated lipids were degraded into short-chain and saturated lipids with ultrasonic cavitation.

1. Introduction

With the global energy and environmental crisis, there is growing interest in research on renewable energy sources such as biomass (Gullberg et al., 2014; Ming et al., 2014). Biodiesel is an environmental friendly biofuel with characteristics similar to those of petroleum diesel (Wu et al., 2012; Banković-Ilić et al., 2012). Among all of the sources for biodiesel production, microalgae receive the most attention, owing to their high solar energy utilization efficiency, small size, abundant nutrients, high growth rate, strong environmental adopt ability and ease of cultivation (Perez-Garcia et al., 2011; Clarens et al., 2010; Demirbas, 2011; Huang et al., 2010).

Impressive advances have been achieved in biodiesel production from microalgae (Yeesang and Cheirsilp, 2011; Suali and Sarbatly, 2012). However, high cost is still a bottle-neck, in comparison with conventional petroleum diesel (Xu et al., 2011). Drying and milling are necessary but energy-intensive processes following the conventional harvest of wet algae from water. The energy consumed in these two steps is up to 80% of the total energy consumption in biodiesel production (Xu et al., 2011). Methods for directly utilizing wet algae without drying and milling are now a focus of research.

Fanny Adam et al. (2012) researched the solvent-free ultrasound-assisted extraction of lipids from fresh microalgal cells. They achieved the highest lipid recycling efficiency with 1000 W ultrasonication for 30 min. Although Scanning Electron Microscope (SEM) images showed the influence of ultrasound on the surface morphology of microalgal cells, the dynamic effects of ultrasonication power and time on the structures of the microalgal cells had not been studied. Ulker D. Keris-Sen et al. (2014) found that the highest release rate of intracellular substances was achieved when the energy intensity of ultrasonication was 0.4 kWh L^{-1}. The percentage of damaged cells decreased with higher energy intensity. The lipid extraction efficiency with solvents is 1–2 times of that without solvents. However, the microstructures of cells were not investigated, nor were changes in lipids mentioned. Meng Wang

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et al. (2014) studied the influences of ultrasound of high frequency (3.2 MHz) and low frequency (20 MHz) on microalgal cell disruption. They found that different species of algae cells with different cell wall structures affected the ultrasonic cell disruption efficiency. The fluorescence densities of lipids stained with Nile Red and chlorophyll a in two microalgae species (*Scenedesmus dimorphus* and *Nannochloropsis oculata*) both increased due to their cells disruption after ultrasonic treatment. The number of *S. dimorphus* cells (bean shape with a long axis of 10–20 μm) increased possibly because of the cells de-clumping, but the number of *N. oculata* cells (round shape with a diameter of 2–4 μm) decreased possibly because that cells were broken into smaller undetected particles. But the dynamic microstructural changes in cells were not investigated. Xiaoge Wu et al., 2012 investigated the different influences of different ultrasonication frequencies (20 kHz, 580 kHz and 1146 kHz) on cyanobacterial cell growth. They found that ultrasound at low frequency and high power inactivated the cells and that ultrasound at a frequency of 1146 kHz and a power of 0.0018 W cm^{-3} degraded the cells. The microstructural changes in cells were however not investigated.

The aforementioned studies clearly showed that ultrasound-assisted lipid extraction from microalgal cells is highly effective; however, how ultrasound affects the microstructures of cells during the treatment and what changes are produced by different ultrasonication conditions have not been thoroughly investigated. In the present study, the effects of different ultrasonication powers and times on lipid extraction from wet microalgal cells were investigated. The changes in cell fractal dimension during ultrasonication were revealed for the first time. The influence of ultrasonication on changes in lipid compositions and in cell walls and membranes was described.

2. Methods

2.1. Materials

Chlorella pyrenoidosa was purchased from the Aquatic Species Research Institute in Wuhan, China. The medium was the optimized Bristol's solution used by Cheng et al. (2013a). The microalgal cells were cultivated in six 1-L conical flasks for 6 days at 25 °C. Air was bubbled through tubes at the bottoms of the flasks at a flow rate of 500 mL min^{-1}. The illumination was 3640 lux under a 12 h/12 h light/dark cycle. The density was approximately 7.0×10^7 cells mL^{-1} when harvested. The cells were centrifuged (Avanti J-26 XP, Beckman Coulter, USA) at 5500 rpm for 5 min. The wet microalgal cells for experiments containing 0.101 g dry biomass per milliliter of solution were stored at 4 °C for subsequent experiments.

2.2. Experimental

The ultrasonication equipment was an ultrasonic processor (UH-500A, Auto-Science Instrument Co., Ltd., Tianjin, China). The equipment included an ultrasonic device, an amplitude-change pole with a frontal area of 132.73 mm^2 and a containment envelope. The maximum treatment power was 500 W and the ultrasonic frequency was 20 kHz (Fig. 1).

A 10-ml volume of wet algae was placed in twelve 25-ml beakers. The depth of the liquid in the beaker was 5–6 cm. The beakers were divided into two sets. The first set of six beakers was used for investigating dynamic changes of ultrasound-assisted lipid extraction with different treatment powers and the second set was used for investigating different ultrasonication times. In the first set, the first sample was the control sample. The beaker was placed in the oven for drying at 105 °C for 2 h and the dried cells were then

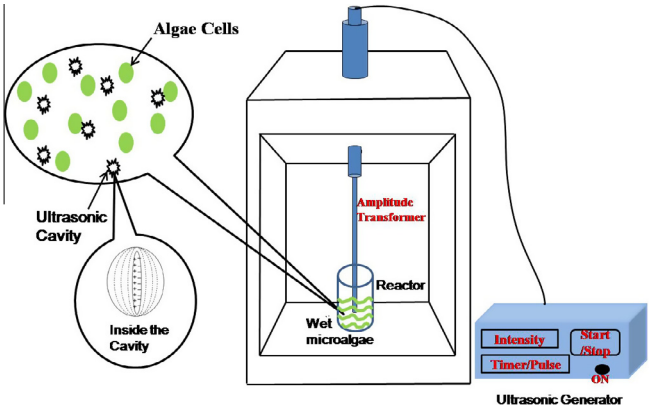

Fig. 1. Schematic of the ultrasonication equipment for disruption of wet microalgal cells.

ground for lipid extraction. The remaining five samples in this set were placed successively in the ultrasonication equipment. The amplitude-change pole was inserted into the wet algae and the end of the pole was immersed 2–3 cm deep in water. The ultrasonic treatments were of 50, 150, 250, 400 and 500 W ultrasonication for 30 min at 20 °C. The interval ratio was 3 s:3 s. In the second set too, the first sample was the control sample. The control sample was handled as in the first set. The remaining five samples in the second set were placed in the ultrasonication equipment as described for the first set. The samples were processed with 150 W ultrasonication for 5, 10, 15, 20 and 30 min at 20 °C.

Following ultrasonication, all the beakers were removed for lipid extraction. The lipids in the two control samples were extracted by the conventional Bligh–Dyer method, following Cheng et al. (2013b). The lipids in the samples with ultrasonication were extracted with isometric solvent containing chloroform and methanol (1:1, v/v). The microalgal cells and extraction solvent in the samples were thoroughly mixed with a mechanical stirrer bar for 2 h and the mixtures were centrifuged at 4000 rpm for 8 min. The solid phase remaining from centrifugation was separated from the liquid phase by filtering through two pieces of filter paper (Advantec filter paper, No. 1, Japan). The separation was performed twice to ensure that all the solid residue was completely removed. The liquid phase was a mixture of extraction solvents, lipids and other impurities. The liquid phase was then transferred to test tubes and heated in an oven at 80 °C for 24 h to evaporate the extraction solvents. The residue in the tubes was the crude lipids obtained from the samples. The weight of the crude lipids was determined with an electronic scale (ML104/02, Mettler Toledo, Switzerland). The experiments were performed three times and mean values were calculated to ensure the accuracy of the results.

Approximately 10-mg quantities of crude lipids extracted from wet microalgal cells by the different extraction methods described above were placed into capped test tubes. *n*-Hexane (2 mL) was first added into the tubes to dissolve the crude lipids, followed by 1 mL KOH–CH_3OH solution (the concentration of KOH was 2 mol L^{-1} CH_3OH) added for saponification. The tubes were shaken at 75 °C for 10 min and 1 mL of 5% HCl in methanol was added for methanolysis. The experimental transesterification process, which had been widely used in many literatures (Lee et al., 2010; Ke-Shun Liu, 1994), included two steps of saponification with base catalyst and methanolysis with acid catalyst. Compared with the one step transesterification with only acid catalyst, the two-step transesterification process with saponification and methanolysis markedly increased the production rate of transesterification reaction. On the other hand, compared with the one step transesterification with only base catalyst, the two-step transesterification process