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The feasibility of ultrasonic stimulation on microalgae for efficient lipid accumulation at the end of the logarithmic phase

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ABSTRACT

The microalgae *Scenedesmus quadricauda* were treated by ultrasound (200 W, 40 kHz) at the end of the logarithmic phase to investigate the effects on stimulating lipid accumulation. Firstly, the microalgae were treated by ultrasound for different times of 0, 5, 10, 15, 20 and 40 min, and then cultivated for 2 days. Although the biomass concentration did not increase, the maximum lipid content of 37.8% dry weight (DW), showing a significant improvement of 57.5%, was obtained after ultrasonic exposure for 20 min. Secondly, the cultivation of the microalgae at the end of the logarithmic phase was continued for a different number of days (1, 2, 3, 4, 5 and 6) after ultrasonic stimulation for 20 min to check the variation of biomass, lipid and other compositions accumulation. The results indicated that a maximum lipid content of 38.9% DW was obtained on day 2, showing an excellent performance. The improved fatty acid in microalgae cells was also analyzed after ultrasonic treatment. This study efficiently increased the accumulated lipid of microalgae by ultrasonic treatment at the end of the logarithmic growth phase without decreasing the biomass, which might be a useful method in practice.

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1. Introduction

The energy crisis is plaguing many countries as the natural fossil fuels like petroleum and natural gas are non-renewable and being rapidly consumed at an alarming rate [1,2]. Different biomaterials have been searched for renewable energy of bioethanol, biogas and biodiesel [3,4], which help alleviate the energy crisis and benefit the economy and society. The biomaterials of microalgae as the third-generation of biofuels have some distinguishing features [5–7] compared with the first- and second-generation [2], such as high growth rate (generally doubling the biomass in 24 h), high lipid content, environment adaptability and carbon neutrality [8].

The production of biodiesel from microalgae needs a large volume of biomass with high lipid content. However, microalgae can display two contradictory features: high biomass growth rate but low lipid content; high lipid content but low biomass concentration [9]. For example, the species of *Chlorella vulgaris* can grow rapidly, but the lipid content is relatively low [10]. The microalgae *Phaeodactylum tricornutum* with high lipid content have low biomass productivity [11]. Under the optimal environment conditions, microalgae can grow rapidly to accumulate biomass. The lipid content is often observed to be higher during stationary phase than that during growth phase in many algae species. Moreover, microalgae can productively accumulate lipid under stress environment induced by chemical or physical environmental stimuli [3]. Therefore, a two-step cultivation strategy seems to be a feasible way in solving the problem [12]. In the first step, the microalgae are cultured under optimal environment conditions to maximize the biomass. In the second step, the microalgae cells are treated in a harsh environment, suffering inhospitable or extreme conditions to increase lipid accumulation. Pancha et al. [13] improved the neutral lipid content (81.81%) of microalgae *Scenedesmus* sp. CCNM 1077 by nitrogen starvation for 3 days after the microalgae were first cultivated for 12 days in BG-11 medium. In the research of Venkata Subhash et al. [14], the microalgae were first cultured for 8 days in ambient temperature environment and then in the second step the algae were cultivated at the temperature of 35 °C for 8 days to induce lipid production, but this led to a significant reduction of biomass during the stress time. Phosphorus starvation has also been used to enhance the lipid accumulation in previous studies [15,16]. Although the methods mentioned above increased the lipid content, the biomass of microalgae might be decreased due to the long time of suffering the adverse environment. Furthermore, some practical obstacles exist in the implementation process of nutrients limitation or starvation [17,18] as in order to expose the microalgae to the adverse nutrients condition the microalgae cells have to be transferred to a new medium containing the decreased nutrients or the stress environment. In the two-step process, stress factors should be directly applied in situ in the same cultivation system, for instance, light, temperature and salt stress [17]. Therefore, an efficient treatment

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or stimulation should be explored by adopting practical and feasible stress factors, which involve less time without decreasing the biomass concentration.

It was reported that ultrasonic exposure has been studied in the related microalgae process, including lipid extraction and conversion [19–21], water-bloom or cyanobacteria control [22,23], and growth effects [22,24]. Some previous studies have also proved the biological effect of ultrasonic exposure on the plant cells and animal living tissues [25,26]. Ultrasonic treatment might be an external force or stress leading to the reduction, damage and collapse of microalgae cells, which could result in the decrease or cessation of the microalgae growth. The study of Joyce et al. [24] proved that the chlorophyll concentration of microalgae decreased due to the ultrasonic treatment, indicating an effect on the intracellular biochemical components. Ultrasonic treatment can induce the cavitation effect in the insonated liquid under sufficient intensity, which may lead to the change of the structure, function and permeability of membranes, growth rate, photosynthetic activity and chlorophyll in microalgae cells [22]. When the liquid is treated ultrasonically, it will lead to the generation of cavitation bubbles that can create implosive collapse along with producing extremely high local temperatures and pressure [27]. The environmental stress could trigger the synthesis of lipid or other compositions in microalgae cells. Therefore, the ultrasonic stimulation as a stress on growth conditions might have the potential ability to induce the lipid accumulation in microalgae for improving the biodiesel production.

However, the effects of ultrasonic exposure as a harsh environment for stimulating the lipid accumulation of microalgae have been rarely studied before. In the present study, the microalgae *Scenedesmus quadricauda* were chosen as the model species due to their widespread existence in nature, representatively common characteristics besides being an important biodiesel feedstock. This investigation adopted a process similar to the two-step cultivation method but focused on the ultrasound-induced stress stage instead of the regular culture stage. Different ultrasonic stimulation time and continuous cultivation time were tested on the algae at the end of the logarithmic growth phase with a fixed frequency and power. The first aim was to assess the feasibility of using ultrasonic exposure as a stress factor on microalgae to improve the lipid productivity and the second aim was to identify the optimal time of ultrasonic stimulation and continuous cultivation time for the efficient lipid production. The results might help set up a cultivation method for the biomass growth and rapid lipid accumulation.

2. Materials and methods

2.1. Microalgae strain

The microalgae used in the present study were *S. quadricauda* obtained from Shandong Provincial Engineering Centre on Environmental Science and Technology. The seed strains were cultured initially for 5–7 days in 1000-mL Erlenmeyer flasks for activation at the temperature of 25 ± 1 °C. The culture medium with activated microalgae was centrifuged to remove the liquid to form cell pellets which were resuspended and transferred to the photobioreactors for further inoculation. The microalgae in the photobioreactors were cultured at 25 ± 1 °C with the aeration flow 600 mL/min (HAILEA, 45 W, China) under the continuous illumination of 81 μmol photons/m^2/s with fluorescent lamps. When the microalgae culture reached the end of the logarithmic growth phase after about 15 days, these microalgae were used as the test microalgae (i.e. initial microalgae) in all the following ultrasonic stimulation investigation in the present study.

2.2. Culture medium

BG11 liquid culture medium was used in this investigation. The main compositions are as follows (g/L): $NaNO_3$, 1.5; K_2HPO_4, 0.04; $MgSO_4 \cdot 7H_2O$, 0.075; $CaCl_2 \cdot 2H_2O$, 0.036; citric acid, 0.006; ferric ammonium citrate, 0.006; EDTA·2Na, 0.001; Na_2CO_3, 0.02 and 1 mL/L A5 (trace mental solution) which contains (g/L): H_3BO_3, 2.86; $MnCl_2 \cdot 4H_2O$, 1.86; $ZnSO_4 \cdot 7H_2O$, 0.22; $Na_2MoO_4 \cdot 2H_2O$, 0.39; $CuSO_4 \cdot 5H_2O$, 0.08; $Co(NO_3)_2 \cdot 6H_2O$, 0.05, which were all prepared with distilled water. All the culture medium used in experiments was sterilized at 120 °C for 30 min. After sterilization, the pH values of BG11 medium were controlled at about 7.1 with appropriate volume of NaOH or HCl.

2.3. Experimental design

About 300 mL of the test microalgae solution (at the end of the logarithmic growth phase) was cultivated in an Erlenmeyer flask in the incubator after the ultrasonic stimulation treatment. The microalgae cultivated in the photobioreactor under optimal conditions without ultrasonic treatment were taken as the untreated group. All the experiments in the present investigation were done in triplicate. The data were expressed as the mean values of three independent experiments.

2.3.1. Effects of different ultrasonic exposure times

Different ultrasonic durations were investigated to study the effects on stimulating lipid accumulation at the ultrasonic power 200 W and frequency 40 kHz. The microalgae solution (at the end of the logarithmic phase) of 300 mL in Erlenmeyer flasks were treated in the ultrasonic bath (SCIENTZ, SB25-12DTD, China) for different times of 0, 5, 10, 15, 20 and 40 min. The microalgae solution was completely immersed in the water of the ultrasonic bath. After the ultrasonic stimulation, the microalgae were cultured statically for 2 days in an incubator at a temperature of 25 ± 1 °C and light intensity of 81 μmol photons/m^2/s. The microalgae cells were harvested through centrifugation (Luxiangyi, TDL-8M, China) at 4000 rpm and 4 °C. The biomass was measured to determine the cell dry weight and freeze-dried to powder for analysis of the lipid content and fatty acid methyl ester (FAME) compositions.

2.3.2. Lipid accumulation potential after ultrasonic stimulation

Following ultrasonic treatment, biomass dry weight, lipid content and FAME composition were measured over six days of static cultivation at 25 ± 1 °C and 81 μmol photons/m^2/s. The changes of biomass and lipid of microalgae cells were monitored to check the potential and ability of lipid accumulation and to determine the optimal harvest time for the maximum lipid production after the ultrasonic stimulation. As a result, the most efficient way of increasing lipid accumulation through ultrasonic stimulation could be revealed.

2.4. Measurement methods

2.4.1. Biomass dry weight

The biomass dry weight was determined by gravimetric method. A volume of 10 mL microalgae solution was centrifuged to form cell pellet which was washed twice with distilled water to remove the culture medium. And then the pellet was dried to constant weight in a thermostatic oven at 60 °C for 24 h. The biomass concentration was expressed as dry weight (DW) per liter.

2.4.2. Determination of lipid content and productivity

The lipid content was measured by solvent extraction and gravimetric method based on a reported method [28]. The total lipid was extracted from the freeze-dried biomass powder with the chloroform methanol solution (2:1, v/v) and ultrasonication (Ultrasonic Cell Crusher SCIENTZ-IID, China). The lipid content (LC) was calculated and expressed as a percentage of the biomass dry weight (% DW).

Lipid productivity (LP, g/L·d) was determined according to the following formula $LP = DW \times LC/T$ where DW (g/L) is the final biomass dry weight, LC (%) is the corresponding lipid content, T (day) is the total algae cultivation time (i.e. 15 days and the cultivation time after ultrasonic irradiation).

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