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Cocamidopropyl betaine-assisted foam separation of freshwater microalgae *Desmodesmus brasiliensis*

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HIGHLIGHTS

- The high collecting rate and extraction efficiency of the natural cocamidopropyl betaine can compete with chemical surfactants.
- The loading volume, air velocity and algae concentration of process were optimized, enhancing harvesting efficiency.
- Lutein production of microalgae *Desmodesmus brasiliensis* was improved by foam separation method than traditional processes.

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ABSTRACT

The downstream process of microalgae derived products is pinned by the harvesting/dewatering of microalgae biomass. Thus, a significant part in the industrial application of microalgae is to find a method to harvest microalgae on a large scale with a low energy cost and high financial efficiency. Foam separation technology is a simple, mild and efficient separating method based on bubbles as separating medium to separate substances of surfactivity. In this study, the cocamidopropyl betaine (CAPB), a natural surfactant, was used to harvest the freshwater microalgae *Desmodesmus brasiliensis* by foam separation. The paper shows when pH = 3, the recovery rate of cocamidopropyl betaine (CAPB) as surfactant (R(%) = 93.63 ± 1.75) can match the traditional surfactant CTAB, with a much larger enrichment ratio (E = 23.12 ± 0.028). Response Surface Methodology (RSM) was used to optimize the recovery rate R(%) after obtaining the optimum condition of harvesting the *Desmodesmus brasiliensis* and finally the optimum result of R (%) = 94.4 was reached. Compared with traditional centrifuge separation, the amount of algae lutein from *Desmodesmus brasiliensis* collected by foam separating was larger than that by centrifuge separation, suggesting this cheap and natural surfactant has the potential to be applied in commercial-scale microalgae biomass recovery and especially in boosting lutein yeild.

1. Introduction

The ingredients of microalgae are mainly protein, oil and carbohydrate, which have great resemblance to common food, and they also contain large amount of microelements with high nutritious value [1]. In countries such as the U.S, Germany and Japan, spiral seaweeds are utilized to supply daily nutrition and to prevent and cue diseases. Microalgae, which grows rapidly with high oil content, can be used in parallel for green environmental remediation such as wastewater treatment, fouling gases palliation, etc, [2–8]. Furthermore, microalgae contribute to the production of some products with high profit-leading goods like polyunsaturated fatty acids (PUFA) or carotenoid. Algaes like *Muriellopsis sp.* [9], *Chlorella zofingiensis* [10], *Chlorella sorokiniana* [11], *Scenedesmus almeriensis* [12] and *Scenedesmus obliquus* [13] contains lutein, a kind of product with high added-value, having good efficiencies in the prevention and treatment to atherosclerosis, diabetic retinopathy and other chronic diseases. However, present research has found only lutein extracted from natural plants has the bioactive function of antioxidant. Despite these advantages, microalgae-based products' industrialization is hindered by the high cost of harvesting and dewatering [14–17].

By far, methods applied to collect microalgae are mainly flocculation, centrifugation, filtration and foam separation methods. Flocculants must be separated from microalgae cell, otherwise it may pollute microalgae cells, increasing the collecting cost [18]. Centrifugation needs a huge investment on equipment and high mechanical

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strength with a quick wear of the equipment, leading to high energy consumptions. As for the filtration method, membranes adhered microalgae need cleanning repeatedly, adding extra microalgae collection cost [19]. Foam separation, known as foam fractionation, is a separation technology during which particles are separated within the foam phase [19]. This simple, mild, high efficient adsorption separating technology, which is conceived to be a better choice microalgae harvesting technology compared to conventional technologies, uses foam as separating medium to separate surface activity substance [20,21]. During foam separation, particles together with surfactant are fed into the separation vessel, then air will be pumped at the bottom of the vessel to produce bubbles that move upwardly [22]. The particles being separated attach themselves to the gas-liquid junctions of the generated bubbles, resulting in foam production [23]. However, algae and ingredients could be affected if using traditional chemical surfactant, therefore, choosing proper surfactant is of great importance.

This study aimed to find an efficient, cheap and low toxic surfactant for microalgae's foam separating process. After determining the optimal separating pH of eight surfactants, the hydrophobicity experiments under the optimum pH condition of each surfactant were conducted to figure out the best hydrophobic concentration, which also represents for the fittest surfactant for parting *Desmodesmus brasiliensis*. Next, Response Surface Methodology (RSM) was used to optimize the recovery percentage (R%) of foam separation on harvesting the algae, based on the optimal operating condition of the supreme surfactant obtained from single factor experiment. Finally, the qualities of collected samples and the feasibility of *Desmodesmus brasiliensis* separating by foam separation were tested by comparing the amount of lutein in the algae before and after separating process.

2. Materials and methods

2.1. Microalgal cultivation and lutein determination

The freshwater microalgae *Desmodesmus brasiliensis* (collection number FACHB-1495) used in this study was bought from the Freshwater Algae Culture Collection at the Institute of Hydrobiology, CAS, Wuhan, China. The strain was cultivated in Modified Bold 3 N medium, whose metal and vitamins were as depicted by Berges et al. [21]. Culture device is shown in Fig. 1 The microalgae cultivation was performed in a 1 L photobioreactor equipped with tungsten filament lamps (14 W TL5 tungsten filament lamps, Philips Co., China) to provide light, at the culture conditions of 30 °C for temperature, pH 7.5, 400 rpm for mixing rate, continuous light intensity of 600 μmol/m^2 s, continuous supply of 2.5% CO_2, and aeration rate of 0.2 vvm. The light intensity was measured by a Li-250 Light Meter fitted with Li-190SA pyranometer sensor (Li-COR lnc., Lincoln, Nebraska, USA). The alagal biomass was collected at the exponential growth phase.

The calibration curve (Eq. (1)) linking OD_{682nm} values to the dry cell weight (DCW) was used to estimate the biomass concentration. The OD_{682nm} was measured using the UV Spectrophotometer (UV- 1750, SHIMADZU, Japan) and the DCW was found by filtrating 50 mL of algae culture suspension by filtered membrane (pore size 0.45 μm, diameter 47 mm) and subsequently drying the filtered membrane in oven at 105 °C until constant weight of the filtration membrane is reached.

$$y = 0.39x - 0.06 \quad (1)$$

Where y is the DCW (g/L) and x is the OD_{682nm} value.

Chan's method [24] was used to extract carotenoid. In brief, the microalgal biomass was harvested by centrifugation in 2 min under 6000 rpm, pour out supernate, then clean algae twice with the same amount of deionized water and dry it by lyophilization. Put 10 mg of the lyophilized cells to 2 ml centrifuge tube, add 1 ml potassium hydroxide solution (60% w/w) and 0.5 g grind, use bead-beater to crush it for 7 min. Put the disposed broken sample under 40 °C for 40 min, then add 2 ml aliquots of ethyl ether to extract carotenoid. Repeat extraction process till the organic extract getting colorless. After extracting the carotenoid, HPLC, reported by Taylor [25], was used to measure the retention time of lutein and to compare its content. Chromatographic column was YMC30 RP-30(4.6 mm × 250 mm × 5 μm), the sample volume 20 μL; the detection wavelength 450 nm; 25 °C.

2.2. Foam separator set-up

Fig. 2 depicts the equipment used in this study. It consists of a bubble column (700 mm high and 35 mm inner diameter, made of polymethyl methacrylate tube), air supply system, rotameter to monitor the gas flow rate, a glass filter for gas distribution, and a collection beaker to collect the foam. The glass filter (10 mm height and 15 mm diameter, with pores of 1.0×10^{-6} m - 1.5×10^{-6} m mean diameter) used as the gas distributor was installed at the base of the separation vessel.

All experiments were performed at room temperature and in batch mode; the microalgae culture with surfactant solution was fed into

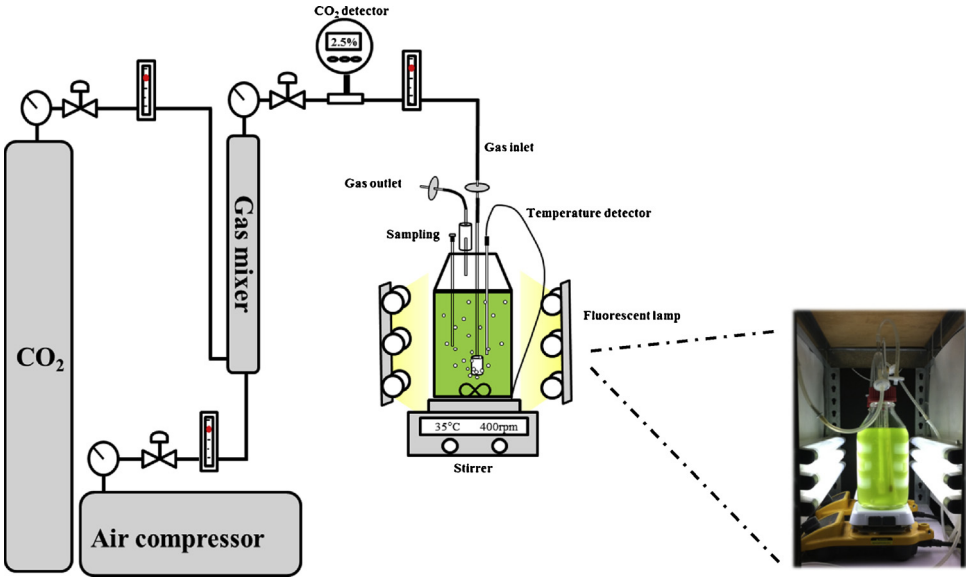

Fig. 1. Schematic description of 250 mL and 1 L photobioreactor.