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Optimization of flocculation efficiency of lipid-rich marine *Chlorella* sp. biomass and evaluation of its composition in different cultivation modes

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HIGHLIGHTS

- Downstream process for lipid-rich marine *Chlorella* sp. biomass was optimized.
- Microalgal biomass in mixotrophic culture was easier harvested than other cultures.
- Cell debris after lipid extraction contained high protein and minerals.

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ABSTRACT

This study aimed to optimize flocculation efficiency of lipid-rich marine *Chlorella* sp. biomass and evaluate its composition in different cultivation modes. Among three flocculants including Al^{3+}, Mg^{2+} and Ca^{2+} tested, Al^{3+} was most effective for harvesting microalgal biomass. Four important parameters for flocculation were optimized through response surface methodology. The maximum flocculation efficiency in photoautotrophic culture was achieved at pH 10, flocculation time of 15 min, Al^{3+} concentration of 2.22 mM and microalgal cells of 0.47 g/L. The flocculation in mixotrophic culture required lower amount of Al^{3+} (0.74 mM) than that in photoautotrophic and heterotrophic cultures (2.22 mM). The biomass harvested from mixotrophic culture contained lipid at the highest content of 42.08 ± 0.58% followed by photoautotrophic (32.08 ± 3.88%) and heterotrophic (30.42 ± 1.13%) cultures. The lipid-extracted microalgal biomass residues (LMBRs) contained protein as high as 38–44% and several minerals showing their potential use as animal feed and their carbohydrate content were 16–29%.

1. Introduction

Energy security, rocketed oil price, depletive resources, and climate change have been worldwide problems for decades. Nowadays, biofuels and biorefineries are expected, at least to some extent, to mitigate those problems. Microalgae are well known as microorganisms with the capability to produce biofuel. This is because the microalgae have high biomass yields without requiring any arable land for growing (John et al., 2011). Moreover, some microalgal species can grow well in saline, brackish and wastewater environment and some can even accumulate lipid to a high content (>70%) (Mata et al., 2010). These characteristics make microalgae more promising as biodiesel feedstock than terrestrial crops which rely utterly on fresh water (Daroch et al., 2013). So far, most research on algal biofuels has been conducted in two areas i.e. biodiesel synthesis from algal lipids and fermentative ethanol production from algal feedstock (Daroch et al., 2013). However, many steps in the production of biodiesel from microalgae such as biomass harvesting, lipid extraction, and transesterification of microalgal lipid have jeopardized the massive interests of algal biomass due to these costly and energy-consuming processes (Yang et al., 2010; Zheng et al., 2012). Therefore, to be more sustainable in developing microalgal biodiesel industry, and in utilizing renewable energy, the effective downstream process for microalgal biomass should be investigated. Furthermore, the cell debris after lipid extraction should also be characterized and evaluated from the view point of biorefinery.

The low concentration and small size of microalgae make the harvesting process difficult. There are several methods that have been used for harvesting of microalgal biomass such as centrifugation, foam fractionation, filtration, flocculation, and gravity sedimentation. Most commercial systems choose centrifugation to harvest microalgae. Filtration could also be used in harvesting process, but membranes will be rapidly fouled by the extracellular

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organic matter if the medium is filtered directly (Babel and Takizawa, 2010). It has also been known that the use of filtration with pressure and vacuum are suitable methods to concentrate microalgal biomass that are large in size such as *Spirulina plantensis*. But for recovering of small sized algae strains such as *Chlorella* sp. and *Dunaliella* sp., the pressure or vacuum filtration methods are not suitable (Harun et al., 2010). Moreover based on life cycle analysis reported by Sander and Murthy (2010), the harvesting processes using centrifugation or filtration consume large amounts of energy and need intensive maintenance. On the other hand, the cost and energy demand for harvesting microalgae could be significantly reduced if the cultures are preconcentrated.

Flocculation is known as a typical preconcentration step that could rapidly reduce the large volumes of culture medium before further dewatering by centrifugation. Flocculation can be induced by pH increase (Wu et al., 2012), chemical flocculants such as inorganic metal salts (Eldridge et al., 2012; Rwehumbiza et al., 2012; Shen et al., 2013; Sanyano et al., 2013) and cationic polymers (Vandamme et al., 2010; Beach et al., 2012), and flocculating microorganisms (Papazi et al., 2009). Physical flocculation induced by ultrasound and electro-coagulation have also been reported (Vandamme et al., 2013). Among these flocculation methods, the flocculations by inorganic metal salts are widely used because of their low-cost and high efficiency. In addition, this process can be easily scaled up and applied for various species of microalgae (Uduman et al., 2010). Wu et al. (2012) investigated the flocculation of three freshwater microalgae and two marine microalgae by pH increase. They explained that Mg^{2+} in the growth medium might act as flocculant which coagulated microalgal cells at high pH. The pH, Mg^{2+} dosage and initial biomass concentration were set in the ranges of 8–12, 1.5–4.5 mg/L and around 1.7 g/L, respectively. The optimal pH for flocculation of freshwater microalgae (pH 10.5) was higher than that for marine microalgae (pH 9.2). Shen et al. (2013) optimized the flocculation of marine alga *Nannochloropsis oculata* with two cationic salts based on response surface methodology. The optimum flocculation conditions were predicted at microalgal cells of 1.7 g/L, pH 8.3, and flocculant dose of 383.5 μM for aluminum sulfate and at microalgal cells of 2.2 g/L, pH 7.9, and flocculant dose of 438.1 μM for ferric chloride. These results indicate that the optimal condition for flocculation is strongly dependent upon the type of flocculant used, and its dosage varied with different pH levels, microalgae strains and cell concentrations.

With the increasing microalgal biodiesel development, the lipid-extracted microalgal biomass residues (LMBRs) would be abundantly produced together with the microalgal lipid. LMBRs are rich in carbohydrates and proteins. Therefore, they can be not only used as a high-protein animal feed but also converted to some products such as fermentable sugars, hydrogen, methane, bioethanol, as well as nutrients for microalgae as a new crop (Harun et al., 2009; Zheng et al., 2012). The conversions of the LMBRs into valuable products not only provide an added bonus to offset the production costs of biodiesel but also lower the treatment or disposal costs of LMBRs. Moreover, this utilization would then give economic and environmental advantages for microalgal biodiesel production.

Because marine *Chlorella* sp. has been identified as a good source for production of lipid and its cultivation has been optimized (Cheirsilp and Torpee, 2012), the further optimization of its harvesting process and the characterization of its biomass as potential feedstocks for valuable products would contribute greatly to its industrialized production. In this study, inorganic metal salts were screened for their effectiveness in flocculation of marine *Chlorella* sp. biomass and the flocculation parameters were optimized through response surface methodology (RSM). As the microalgae can be cultivated in three different cultivation modes including photoautotrophic, heterotrophic and mixotrophic cultivation modes, these cultivation modes may influence the flocculation efficiency due to the differences in properties of microalgal cell surfaces, cell sizes, and various product formations (Vandamme et al., 2013). Therefore, the flocculation efficiencies of microalgal biomass in different cultivation modes were compared. The harvested microalgal biomass was then characterized for its lipid, carbohydrate, protein and mineral contents.

2. Methods

2.1. Microalgae strain and growth medium

Marine *Chlorella* sp. was obtained from the National Institute of Coastal Aquaculture, Thailand. The medium used in this study was BG-11 medium (Cheirsilp and Torpee, 2012). One liter of BG-11 medium contains 1.5 g $NaNO_3$, 0.04 g $K_2HPO_4{\cdot}3H_2O$, 0.2 g $H_2PO_4{\cdot}3H_2O$, 0.0005 g EDTA g, 0.005 g Fe ammonium citrate, 0.005 g citric acid, 0.02 g Na_2CO_3 and 1 mL of trace metal solution, pH 7.3. One liter of trace metal solution contains 2.85 g H_3BO_3, 1.8 g $MnCl_2{\cdot}4H_2O$, 0.02 g $ZnSO_4{\cdot}7H_2O$, 0.08 g $CuSO_4{\cdot}5H_2O$, 0.08 g $CoCl_2{\cdot}6H_2O$ and 0.05 g $Na_2MoO_4{\cdot}2H_2O$.

2.2. Cultivation of microalgae

Microalgae strain was pre-cultured in 400 mL of BG-11 medium in a 500 mL glass bottle. The pre-culture was incubated at 30 °C and air-aerated at a flow rate of 0.01 mL/min under a 3000 lux light intensity with a 16:8 h light and dark cycle for 3–5 days (Cheirsilp and Torpee, 2012). This was used as a seed culture. The batch cultivation of the microalgae was performed by inoculating 10% (v/v) seed culture into 3 L BG-11 medium in a 3.78 L (1 US gallon) glass bottle. The cultures were incubated at 30 °C, air-aerated at a flow rate of 0.01 mL/min, and illuminated with a 3000 lux light intensity with a 16:8 h light and dark cycle for 5 days. For mixotrophic and heterotrophic cultivation, glucose was used as a carbon source at a concentration of 2 g/L with and without light illumination, respectively. During microalgae cultivation the optical density at 660 nm (OD_{660}), pH, dry mass of microalgae, and lipid were determined.

2.3. Harvesting of the microalgal biomass

Microalgal biomass were harvested by flocculation method using several flocculants including $Al_2(SO_4)_3K_2SO_4{\cdot}24H_2O$, $MgSO_4{\cdot}7H_2O$, and $CaCl_2{\cdot}2H_2O$. The flocculant stock solution was added to the culture medium at the same molar ratio of 2.22 mM then the reaction tubes were vortexed for 5 s. The microalgal suspensions were left to settle for certain time without agitation. Subsequently, the optical density of the supernatant from half the height of the clarified layer and the sludge were measured at 660 nm. Agglomerates of microalgae were then washed twice with distilled water and dried at 60 °C in a hot air oven until a constant weight. The dried microalgae were then crushed and sieved using 120 mesh analytical sieve before used for lipid extraction. Concentration factor was calculated using Eq. (1) and flocculation efficiency was calculated using Eq. (2) (Wu et al., 2012):

$$\text{Concentration factor} = \text{Final cell concentration}/\text{Initial cell concentration} \quad (1)$$

$$\text{Flocculation efficiency } (\%) = (1 - A/B) \times 100 \quad (2)$$

A is the OD_{660} of supernatant from half the height of the clarified layer after flocculation and B is the initial OD_{660} of the microalgal culture suspension.