سومین همایش بین المللی و یازدهمین همایش ملی
بیوتکنولوژی جمهوری اسلامی ایران
۱۰ لغایت ۱۲ شهریور ماه ۱۳۹۸ - مرکز همایش های رازی

بسمه تعالی

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The effect of drying methods on simultaneous extraction of lipids, carbohydrates and proteins from *Chlorella vulgaris* microalgae

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Abstract
Microalgae have an extraordinary potential for rapid growth, synthesis and accumulation of lipids, proteins and carbohydrates. In this study, simultaneous assay for extraction of algal biochemicals was investigated. The effect of biomass drying methods on extraction yield was reported. For cell disruption two drying methods, oven drying and freeze drying followed by solvent extraction were adopted. Cell disruption acilitate the extraction process. Freeze drying proved to be better than oven drying for biochemicals extraction. Freeze drying improved extraction yield 68%, 66% and 30% for lipid, carbohydrate, and protein extractions; respectively. This result is a step forward towards obtaining value-added products from algal feedstock.

Keywords: Biochemical composition, Lipids, Carbohydrates, Proteins, *Chlorella Vulgaris*

Introduction

Biomass of microalgae is a renewable source for the production of multiple components like carbohydrates, proteins, and lipids. Carbohydrates can be used in the production of biochemicals, biopolymers and ethanol. Proteins of microalgae are low-volume but high-added value bioproducts that can find application in food and pharmaceuticals. Lipids can be used in biodiesel production cosmetics and nutraceuticals industries (1). The simultaneous extraction of valuable products from algal biomass may result in optimal value extraction and economically beneficial algal technology. A main consideration in the simultaneous extraction of products from algae is the effect of different treatments on the individual yields of such products (2). Disruption methods are generally used to increase yields of value products when combined with solvent extraction (4). Thus, many cell disruption methods were used to degrade cell wall of microalgae species (3). Ansari *et al.* (2) found microwave assisted extraction of oven dried samples resulted in maximum lipid yield while oven dried sample with ultrasonication and autoclave provided significantly higher protein and carbohydrate yeilds; respectively. Recently, combination of disruption methods resulted in higher yields for biochemicals extraction.
In this study the effect of two types of drying, freeze drying and oven drying as disruption methods combined with solvent extraction on the yield of simultaneous extraction of three bioproducts including lipids, proteins, and carbohydrates was investigated and the most effective cell wall break down and eventually the highest extraction efficiency was reported.

Experimental

Growth conditions and biomass production

Microalgae *Chlorella Vulgaris* was used as the algal species in this study. Cultivation was performed in a 50 L flat plate photobioreactor. At the seeding stage, 60 ml of algal solution was initially inoculated into a 500 ml of BG11 growth medium in an incubator shaker. After 48 h, 200 ml of algal broth was inoculated into a 4-liter BG11 culture. The lighting system was an array of white fluorescent lamps with the intensity of 4000 lux. The temperature was set at 27±1 ˚C. Finally, the seed culture was transferred to flat plate photobioreactor containing 48 L of BG11 growth medium. Flat plate operation performed under air flow rate of 1 vvm and contained 2% CO_2 with 16:8 light /dark cycle. The dual sparging system was used in which CO_2 and air supply were separated. The light intensity was 12000 lux and temperature was set at 27±1 ˚C. The flat plate cultivation took 7 days to raech maximum cell density. At the end of cultivation, pH of the culture was adjusted to 12 using sodium hydroxide and then upon sedimentaion the concentrated solution was collected.

drying of biomass

The concentrated algal broth was dried using two different methods: (a) oven drying, and (b) freeze drying. Oven drying was conducted at 45º C for 48 h (Wiseven, South Korea). Freeze drying was carried out at -50 ºC for 72 h (FD-10V , Pishtaz Engineering, Iran).

Lipid analysis

Lipid extraction was performed according to modified Bligh and Dyer (5) protocol with chloroform, methanol and water in the volume ratio of 5.7:3:1. Based on 1 gr of dried biomass, the final three-phase solution contained 13.6 ml top layer contaning carbohydrate and protein. Lipid mass in bottom layer was measured gravimetrically after evaporating chloroform. The lipid yield was calculated as the ratio of mass of extracted lipid (g) to mass of sample dried biomass (g).

Carbohydrates analysis

The carbohydrate concentration was quantified based on phenol-sulfuric acid method (6). Briefly, 0.5 ml of supernatant from previous step was reacted with 0.5 ml of phenol (5 %) and 2.5 ml of sulfuric acid (96%) to create a characteristic yellow-orange color. The mixture was incubated at room temperature. After 20 min, absorbance was measured at 490 nm (UV-Visible spectrophotometer, UNICO 2100, USA). Carbohydrate concentration was determined based on a standard curve. Glucose was used for standard curve preparation. Based on 13.6 ml of supernatant, carbohydrate yield was calculated as the ratio of total mass of carbohydrate (g) to mass of sample dried biomass (g).

Protein analysis

Modified Lowry (7) method was used to determine the protein content in algal biomass by following manufacturer's protocol. The blue color of the final solution was then measured at 650 nm (UV-Visible spectrophotometer, UNICO 2100, USA). Protein concentration was determined based on a standard curve. Bovine serum albumin (BSA) was used for standard curve preparation. Based on 13.6 ml of supernatant, protein yield was calculated as the ratio of total mass of protein (g) to mass of sample dried biomass (g).

Results and discussion

The effcet of drying method as a cell disruption technique on yield of simultaneous extraction of three bioproducts from the same algal biomass sample was examined. The disruption approach was combination of two methods including drying and chemical solvents. Table 1 indicates the extraction yields under freeze drying and oven drying techniques followed by solvent disruption. In general, extraction yield of freeze dried biomass was higher than oven dried biomass in all three bioproducts. Switching from oven drying to freeze drying increased lipid yield approximately 68%. This figure for carbohydrate and protein yields are 66% and 30%, respectively. Lower yields in oven dried biomass may be attributed to lower degree of cell disruption that was not maesured in this study. This, the results indicate that freeze drying is a more efective technique for cell disruption than oven drying.

The results indicate that carbohydrate and protein contents of the algal sample are remarkablly lower than lipid content. Although culture conditions and the type of algal species might affect the contents of different biochemicals, simultaneous extraction using different disruption protocols may affect the resulting contents as well in a positive or negative way. Ansari et al. (2,8) in two separate studies indicated that the extraction yields of carbohydrates and proteins changed from 20% and 58% to 25% and 33%, respectively, under two different disruption protocols. Lee et al. (9) reported that the carbohydrate and protein contents increased to 49.7% and 28.5% in the lipid-extracted residual biomass.

Also drying method may affect biochemicals contents so that some proteins may be degraded at oven temperature of 45 °C over the long period of 48 h. Furthermore, solvent application and cell disruption during lipid extraction may results in loss of some protein from the biomass reducing the total amount available for extraction (2).

Table 1: Extracted bioproducts under two drying methods

Drying methods	Lipid %w of DCW	Carbohydrate %w of DCW	Protein %w of DCW
Freeze dried	10.4	3.08	0.13
Oven dried	6.2	1.85	0.10

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