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Ultrasound assisted extraction of carbohydrates from microalgae as feedstock for yeast fermentation

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HIGHLIGHTS

- Carbohydrates from microalgae can be a promising feedstock for yeast culture to produce biofuels.
- Pretreatment of microalgae and carbohydrates extraction from algal cell were performed.
- Four factors of ultrasound assisted extraction were examined by fractional factorial design.
- Refined model was confirmed as a good fitting model via analysis of variance (ANOVA).
- Yeast biomass of the group with glucose from microalgae was much higher than that of control group.

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ABSTRACT

Recently, carbohydrates biomass from microalgae is considered as a promising and inexpensive feedstock for biofeuls production by microorganism fermentation. The main obstacle of the process is microalgae pretreatment and carbohydrates extraction from algal cell. In this study, comparison of three pretreatment methods was performed and the results showed that ultrasonic assisted extraction (UAE) was very effective. The effects of four parameters (ultrasonic power, extraction time, flow rate and algal cell concentration, respectively) on extraction efficiency were also investigated. Additionally, in order to identify significant factors for glucose yield, combination of these four parameters was examined by using fractional factorial design (FFD) and the regression model was obtained. Meanwhile, the refined model was confirmed as a good fitting model via analysis of variance (ANOVA). After extraction, glucose obtained from microalgae was used as substrate for *Rhodosporidium toruloides* fermentation and yeast biomass was much higher than that of control culture.

1. Introduction

It is well known that energy has an essential influence on one country's economy and its continued economic growth and development. However, due to large consumption of fossil fuels and its non-renew ability, there exists serious energy crisis in the whole world and fossil fuels will be exhausted one day. Additional, global warming is increasingly severe and it is regarded as a consequence of large emissions of greenhouse gas such as carbon dioxide resulting from fossil fuels burning (Lam and Lee, 2012). Therefore, it is urgent to develop sustainable and clean energy from renewable resources. At present, biofuels produced from microorganisms are recognized as an attractive alternative of fossil fuels, such as biodiesel, bioethanol and biohydrogen (Chisti, 2008; Kito-Borsa et al., 1998; Wang and Wan, 2009; Levin et al., 2004).

Microalgae can serve as an excellent feedstock for the biofuels production. For example, many paper reported that microalgae can accumulate lipids in the range 20–50% of dry cell weight (DCW) for biodiesel production (Chisti, 2007; Zhao et al., 2012). Besides, according to the type of feedstock used, biofuels is classified into two generations that are produced from sugar/starch crops through conventional technology and lignocellulosic biomass, respectively (Nigam and Singh, 2011; Chiaramonti, 2007). Bioethanol production from microalgae is considered as the "third generation biofuels", which can overcome the major drawbacks of first and second generation biofuels to great extent (John et al., 2011). More important, some microalgae species like *Chlorella*, *Dunaliella*, *Chlamydomonas*, *Scenedesmus*, *Spirulina* have high carbohydrates (mainly starch) content (>50% of the DCW) (Ueda et al., 1996). The carbohydrates from microalgae can be hydrolyzed and converted into glucose which is a very significant substrate for heterotrophic microorganisms (like yeast, bacteria and fungus) to produce biofuels. For instance, in a study, *Chlamydomonas* could contain around 60% carbohydrates (44% of which was starch)

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which was hydrolyzed and converted into glucose. Then ethanol fermentation was conducted by *Saccharomyces cerevisiae* using hydrolyzate and 235 mg ethanol/g algae were obtained (Choi et al., 2010). A recent paper reported a novel process that was conversion carbohydrate in the algae into glucose prior to lipid extraction, which could increase up to 15% lipid accumulation by other oleaginous microorganism (Trzcinski et al., 2012). Apart from high lipid/carbohydrates content, microalgae can be considered as an excellent feedstock because of other advantages, such as fast growth, no competition with food crops for land surfaces and high CO_2 fixation efficiency. Thus, it will decrease the cost of biofuels production using glucose produced from microalgae via photosynthesis as the substrate for other microorganism's fermentation. In this study, *Chlorella* sp. was cultivated using CO_2 as carbon source for carbohydrates accumulation and glucose obtained was used to feed *Rhodosporidium toruloides* for lipid production.

Meanwhile, the main obstacle of carbohydrates/glucose production is that algae rigid cell walls are hard to break, so pretreatment or extraction is the most important step so that intercellular carbohydrates can be released (Choi et al., 2010; Libessart et al., 1995). Previous paper reported carbohydrates could be extracted using many pretreatment methods, such as enzymatic pretreatment (Choi et al., 2010; Correiaa and Beirão-da-Costa, 2012), chemical extraction method like alkaline pretreatment (Correiaa and Beirão-da-Costa, 2012; Ray and Lahaye, 1995) and physical method, such as hot-water treatment, microwave-assisted extraction and ultrasonic assisted extraction (Velmurugan and Muthukumar, 2012; Ying et al., 2011; Donot et al., 2012; Yoshida et al., 2010). There exist some selection criteria of extraction method that the pretreatment is effective for qualitatively and quantitatively, and the technology is simply to operate and economical for scale up. For instance, the composition of cell wall is complicated and unknown to some microalgae species, and some enzymes are very expensive. Thus, enzymatic pretreatment is not a feasible method. In order to obtain effective extraction method of carbohydrates, comparison of three methods was carried out and effects of several operation parameters on glucose yield for each method were studied in this paper. The results showed that ultrasonic assisted extraction was more effective than the other methods. Then significant factors of ultrasonic assisted extraction using FFD were found out in this work. Finally, carbohydrates extracted from microalgae were converted into glucose to feed *R. toruloides* culture for biofuels production.

2. Methods

2.1. Strains and culture conditions

The strain of *Chlorella* sp. (American Type Culture Collection, ATCC 14854) was provided by Dr. Raymond Lau and the algal cells were cultivated using modified R-medium. The modified medium increased the specific growth rate and biomass (Chen et al., 2011) and the lower sulfur and nitrogen adding could improve starch accumulation in microalgae cells (Brányiková et al., 2011). The composition of modified medium was: sodium citrate 0.5 g/L, sodium acetate 1.804 g/L, $MgSO_4{\cdot}7H_2O$ 0.1 g/L, NH_4NO_3 0.3 g/L, KH_2PO_4 0.2 g/L, $K_2HPO_4{\cdot}3H_2O$ 0.393 g/L, $FeCl_3{\cdot}6H_2O$ 0.01 g/L, $CaCl_2{\cdot}2H_2O$ 0.053 g/L, H_3BO_4 1.0 mg/L, $MnSO_4{\cdot}H_2O$ 0.303 mg/L, $ZnSO_4{\cdot}7H_2O$ 1.0 mg/L, $Na_2MoO_4{\cdot}2H_2O$ 0.2 mg/L, $CuSO_4{\cdot}5H_2O$ 0.0625 mg/L, $CoCl_2{\cdot}6H_2O$ 0.2 mg/L. The *Chlorella* sp. was cultured in 5 L air lift photo-bioreactor with mixture gas (air and CO_2) contained 2% (v/v) CO_2 under 25 °C and 100 μmol/m^2s. The total flow rate was set at 0.26 vvm.

The strain of *R. toruloides* (ATCC 10788) was obtained from American Type Culture Collection and stored using YM medium in the laboratory. The composition of the medium was as following: Glucose 10 g/L, Peptone 5 g/L, Yeast extract 3 g/L, and Malt extract 3 g/L, Agar 2%. In this study, the yeast cells were cultivated in the YM medium under different glucose concentration or in the YM medium with glucose obtained from microalgae. The yeast culture was carried out using sterilized 50 ml tube (with 15 ml medium) under 25 °C and 200 rpm. *R. toruloides* is oleaginous yeast and contains high lipid content up to 57% (Wu et al., 2011).

Table 1
Effects of three factors on carbohydrates extraction from *Chlorella* sp. using conventional solvent extraction.

Run	Time (min)	Temperature (°C)	Ratio (liquid/solid) (ml/g)	Glucose yield (g/100 g DCW)
1	30	100	30	6.67 ± 0.11
2	60	100	30	6.97 ± 0.17
3	90	100	30	8.34 ± 0.18
4	90	60	30	6.44 ± 0.05
5	90	80	30	7.50 ± 0.16
6	90	100	20	8.29 ± 0.15
7	90	100	40	9.06 ± 0.05

Table 2
Effects of three process parameters on carbohydrates extraction from *Chlorella* sp. using fluidized bed extraction.

Run	Time (min)	Air flow rate (L/min)	Cell concentration (g/L)	Glucose yield (g/100 g DCW)
1	90	6.12	1.0	3.53 ± 0.31
2	120	6.12	1.0	3.77 ± 0.28
3	150	6.12	1.0	3.93 ± 0.19
4	120	4.37	1.0	2.91 ± 0.20
5	120	7.65	1.0	4.56 ± 0.13
6	120	6.12	0.5	2.16 ± 0.21
7	120	6.12	1.5	4.81 ± 0.23

2.2. Conventional solvent extraction (CSE) method

Conventional solvent extraction was carried out in water bath so that the temperature could be controlled. Algal cells were harvested and lyophilized using freeze drier, then about 1.0 g dry microalgae was mixed with distilled water in 50 ml tube using vortex mixer. Extraction was performed total 7 runs according to different conditions, such as extraction time, extraction temperature and ratio of liquid to solid. Details were shown in Table 1.

2.3. Fluidized bed extraction (FBE) method

Fluidized bed extraction was carried out in a fluidized reactor with high flow rate of air. The *Chlorella* sp. culture was harvested and washed with distilled water, then diluted to certain cell concentration using distilled water. The obtained algal culture and about 10 ml glass beads were added into fluidized reactor with air aeration. Extraction was conducted referring to the conditions shown in Table 2.

2.4. Ultrasonic assisted extraction (UAE) method

An ultrasonic processor (UIP 1000hd ultrasonic mixer, Hiescher) was used to extract carbohydrates from microalgae in ultrasonic pretreatment. The algal cells were harvested and washed with distilled water, and diluted to certain biomass concentration. After that, 500 ml obtained algal culture was used for every run in extraction process. The microalgae culture could flow in a circle in ultrasound equipment but ultrasonic wave appeared only in