Effective solvents for proteins recovery from microalgae

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Abstract. From an industrial perspective, the exploitation of microalgae as protein source is of great economical and commercial interest due to several attractive characteristics. However, the protein extraction efficiency is limited by the multiple layers of rigid and thick cell walls that are enriched with recalcitrant structure of cellulose. Therefore, an efficient method of cell disruption is necessary to disintegrate the cell wall and promote the release of protein contents. The conventional methods for downstream processing, e.g. disruption, isolation, extraction, concentration and purification, are energy-intensive and costly because they typically compose of several operational units. To reduce the overall process cost and establish an economical feasible process for the large-scale production of microalgae derived products, a more cost-effective and eco-friendly technique in downstream processing is in critical demand. One of the main challenges for protein extraction from microalgae cells is the recalcitrant structure of microalgae cell wall. This work aims to provide a guideline on the selection of the solvent to facilitate the proteins release during the cell disruption process. The influences of various solvent types (methanol, ethanol, 1-propanol and water) were evaluated and compared based on the protein yields. It was found that water solvent released the highest protein concentration from microalgae compared to the other tested solvents.

Keyword. Liquid biphasic system technologies, Microalgae, Protein, Solvents.

1 Introduction

The mass production of protein-derived products from microalgae cultures offers significant advantages as it is considered to be renewable biomass that fits to the concept of sustainable production [1]. Microalgal proteins obtained from aquaculture industry are one of the most promising and sustainable substitute sources to conventional proteins, for example fishmeal and soybean [2]. This is mainly due to the microalgal-enriched protein content, nutritional profile and quality of microalgae's proteins [3]. Microalgae cells have the ability to synthesize all types of essential amino acids, which are mostly equivalent or even better with that of other higher organisms, such as plants. Furthermore, their amino acid compositions are not significantly affected by changes or stresses in environmental conditions [4]. Additionally, microalgae possess high efficiency photosynthetic activity, high growth rate, short harvesting cycle, high disease resistance and high biomass density [5-7].

However, one of the main difficulties in the mass production of protein products in commercial and industrial process is the low efficiency in protein extraction and recovery because of the presence of multiple layers of thick and tough cell wall [8, 9]. The process development of simple and rapid cell disruption and cell lysis in large scale industry is necessary to facilitate the release of proteins [10]. One of the vital factor for promoting the protein release is to choose a suitable extraction solvent for the cell disruption process [11]. This study aims to evaluate the influence of extraction solvent and provides a guidance on the selection of the effective solvent to facilitate the proteins release during the cell disruption process of microalgae.

The relevancy of this research work also involves the green environmental initiative by creating high rates of photosynthesis in mitigation of carbon dioxide ($CO2$) adopted from the cultivation of *Chlamydomonas sp.* Tai-03 microalgae. In regards to reserve energy and sustainable solutions, the concept of using liquid biphasic system combines the pretreatment and extraction step into one-system, thereby creating an efficient and effective bioprocessing of protein extraction from microalgae cultures in aquaculture industries.

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2 Materials and methods

2.1 Chemicals

Food grade alcohols of ethanol, 1-propanol, 2-propanol (R&M Chemicals, Selangor, Malaysia) were purchased and used as the extraction solvents. Salts for the bottom phase during extraction that are utilized in this study were dipotassium hydrogen phosphate (K_2HPO_4), magnesium sulphate ($MgSO_4$), ammonium sulphate ($(NH_4)_2SO_4$) and potassium dihydrogen phosphate (KH_2PO_4) and all chemicals were purchased from R&M Chemicals (Selangor, Malaysia).

2.2. Setting up of liquid biphasic flotation system

Liquid biphasic flotation (LBF) unit of 50 mL volume capacity was used as the separation system, and it was provided by Donewell Resources (Puchong, Selangor, Malaysia). A 50 mL glass tube was connected from the bottom to a gas compressor. The bottom of the glass tube was drilled and fitted with a rubber tube to connect to the gas compressor. A sintered glass disk (Grade 4 porosity) was fitted at the bottom of the glass tube to allow the air bubbles that are generated during passing of the compressed air flow. The air flow rate that was supplied to the LBF unit is monitored and controlled by a flowmeter (model: RMA-26-SSV, Dwyer, Michigan, IN, USA) with a range of 50 to 200 cc/min.

2.3 Cultivation of *Chlamydomonas sp.* Tai-03 microalgae

The optimal culture conditions for the growth of *Chlamydomonas sp.* Tai-03 had been determined by our previous study [12]. Therefore, in this study, the culture conditions use BG-11 medium (containing 25% initial nitrate concentration (0.375 g/L $NaNO_3$). The microalgal culturing is conducted in the indoor 1 L photobioreactor. The light intensity is set up at 200 μmol/m^2.s. The initial concentration of cell inoculum is 0.12 g/L. The culture reactor is controlled in the mode of continuous stirring at 300 rpm, and is supplied by continuous sparging of 5% CO_2 gas at a flow rate of 0.1 vvm [12].

2.4. Protein Assay

The protein concentration is measured by using Bradford Reagent. The 2 mL of various dilutions of protein samples obtained from LBF unit at either top or bottom solutions are prepared with a serial dilution method (10x dilution) and mixed with 0.2 mL of Bradford Reagent, and the mixture was incubated for 10 min before the absorbance reading using UV-Vis spectrophotometer at a reading wavelength of 595 nm. The absorbance of the protein concentration was based on the calibration curve between Bovine serum albumin (BSA) concentrations and A595 values. BSA is commonly used as standard for measurement of protein concentration because of its commercial availability, high purity and low cost. The protein concentration of the microalgae samples can be calculated using the Equation (1) generated from the standard curve (Figure 1).

$$y = 0.0014x \tag{1}$$

Where y is the absorbance value at 595 nm and x is the protein concentration (μg/mL) equivalent to BSA

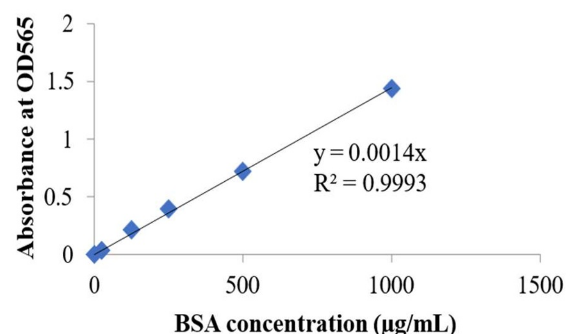

Figure 1. Standard curve for measurement of protein concentration using BSA as the standard based on the Bradford Reagent.

3. Results and discussion

In this study, four types of different solvents, including methanol, ethanol, propanol and water were used in extraction experiments (Figure 2). The protein concentrations (%) per dried weight of microalgae cells were measured by using Bradford protein assay. The experimental results showed that water showed the highest efficiency in protein extraction of *C. Tai-03* cultures because it provided the highest proteins concentration with about 3% of protein concentration per dry weight, and followed by 1-propanol (2.75%), ethanol (1.80%) and methanol (1.50%), respectively.

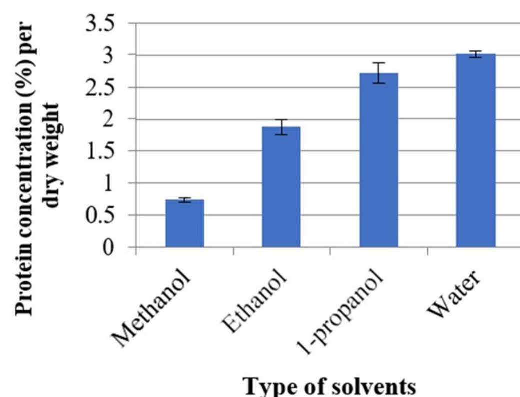

Figure 2. Total proteins contents of *Chlamydomonas Tai-03* obtained from extraction by LBF unit by using different types of solvents. Each bar is an average of three replicates.

In these recent years, liquid biphasic system (LBS) has become a proven tool for separation and purification process that fit to the concepts of circular economy and bioeconomy in microalgae industries. The application of controlling system based on Internet of Things (IoT) in LBSs in clarification, partitioning and partial purification of biomolecules and bioproducts, in this case, protein products, had showed the rapid development. This LBS technology helps to successfully achieve the high recovery yield and the high purity of targeted products in a single separation step. Additionally, the LBS shows the other interesting characteristics, such as high selectivity and easily to process scale up. Thus, LBS is proposed and proved to be an attractive technology that meets the requirements of the high throughput in commercial and industrial processes. Furthermore, it has good benefit in terms of economic and environmental protection.

Rapid progress and improvements in biorefining technology, new regulatory directives, product quality constraints, and the production efficiency have necessitated the development of more advanced and powerful downstream bioprocesses for various industries, such as biotechnology, biopharmaceuticals, as well as bioenergy. This situation motivates to intensely improvements in conventional biorefining processes, and drives the development of entirely new approaches, for example, numerous available extractive technology, including extractive fermentation, extractive bioconversion liquid biphasic system, liquid biphasic flotation system, and newly developed liquid biphasic flotation assisted with ultrasound and microwave, electricity. Combinations of the alcohol/salt LBF system and the ultrasonication have the ability of killing two birds with one stone, because it is not only able to execute the cell rupturing, it also capable to recover targeted bioproducts simultaneously and continuously.

Some other process parameters in LBS, such as crude feedstock concentration, flotation time, type of salt, concentration of salt, type of alcohol, concentration of alcohol, initial volumes of salt and alcohol were experimentally investigated. As demonstrated in this study, the small molecular weight aliphatic alcohols, methanol, ethanol, 1-propanol and 2-propanol can be combined with various types of salts, such as dipotassium hydrogen phosphate (K_2HPO_4), magnesium sulphate ($MgSO_4$), ammonium sulphate ($(NH_4)_2SO_4$) and potassium dihydrogen phosphate (KH_2PO_4). Under the optimal condition of LBF operation at 250 g/L of $(NH_4)_2SO_4$, 60% (v/v) of 2-propanol, initial VR of 1.0, 20 g/L of crude algal biomass load, 4 mm^3/min of air flowrate and 10 min of flotation time.

The recycling of phase components in LBF was also tested and evaluated to minimize the use of alcohol solvent and salt. It was demonstrated that top phase (containing alcohol) of recycling sample can achieve improved performance of extraction for at least three consecutive recycling runs. Under this optimized process conditions, the proportion of protein recovered in the top phase was 88.86% for the third recycling run in microalgae recovery studies.

4. Conclusions

In this work, comparing four types of solvents in LBF system, it was found that water could be an excellent solvent for microalgal cell disruption with the indication of higher proteins yield than the other solvents. Further investigation should be done on different strains of microalgae to evaluate the general effectiveness of water in improving the release of proteins yield from microalgae with different composition of cell wall.

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