

Evaluation of Different Solvent Types on the Extraction of Proteins from Microalgae

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The commercial production of microalgal proteins at low cost remains challenging. The release of proteins from microalgae is restricted by the presence of rigid thick cell wall that could hinder the extraction process and affect the proteins recovery yield. In view of this, this study aimed to provide some guidance on the selection of the efficient solvent in facilitating the proteins release during the cell disruption process. The effects of different solvent types such as methanol, ethanol, 1-propanol and water in facilitating the process of proteins release from microalgal cell wall were studied in the present study. Based on the results, it was found that water able to release the highest concentration of proteins from microalgae compared to the other solvents.

1. Introduction

Microalgae are a group of structurally simple microorganisms, with sizes ranging from 1µm to 1mm, except when they form natural proliferations which is known as algal blooms (Blackburn and Volkman, 2012). Microalgae do not possess structures that characterize land plants such as rhizoids, leaves, roots and stems (Cheah et al., 2014). Despite of lacking the complex tissues and organs, microalgae represent the largest and most robust microorganisms on earth (Chisti, 2007). They are ubiquitously distributed in a broad range of environmental conditions (Duong et al., 2015). These tiny microorganisms can even survive in harsh environments (Mata et al., 2010) such as saturated saline (Kumar et al., 2011), coastal seawater, brackish water, seawater as well as wastewater (Dragone et al., 2010) and in (Kumar et al., 2015).

Previous literature reported that the mass production of proteins source from microalgae has led to the increase of commercial interest due to several advantages possessed by microalgae over conventional crops (Solana et al., 2014) and other discussion in (Safi et al., 2014). In the recent decades, it was found that microalgal proteins emerge as the most promising and sustainable alternative to conventional protein sources (Safi et al., 2015) such as fishmeal and soybean in aquaculture industry (Roy and Pal, 2014). This is mainly owing to the high protein content (Blackburn and Volkman, 2012) and other discussion in (López Barreiro et al., 2013) and nutritional quality of microalgae (Norambuena et al., 2015). These extensively diverse and abundant group of microorganisms (Singh and Sharma, 2012) have the ability to synthesise all types of essential amino acids which are mostly equivalent or even better with that of other higher plant (Spolaore et al., 2006). It was found that their amino acid composition does not significantly affected by changes in environmental conditions (Blackburn and Volkman, 2012).

Apart from that, the farming of microalgae is cost-effective than conventional crops due to numerous attractive characteristics (Phong et al., 2016). It has been reported that although these photosynthetic microorganisms are tiny in size in comparison to the land-based crops, they have high photosynthetic efficiency (López

Barreiro et al., 2013), high growth rate, short harvesting cycle which is generally less than ten days (Cheah et al., 2014) and in (Chen et al., 2013) and further in (Taher et al., 2014), possess high disease resistance ability, high metabolites content and high biomass density (Roy and Pal, 2014). Compared to conventional farming, microalgal farming are characterized by a high productivity per unit area and therefore microalgal farming is amenable to mass culture (Chisti, 2007). Furthermore, microalgae can be cultured on non-arable land (Ferreira et al., 2013) with minimum land space requirement (Sambusiti et al., 2015). Microalgal cultivation are less seasonal and weather restrictions (Duong et al., 2015). Researchers also observed that microalgal farming does not compete with conventional agriculture for resources (Dragone et al., 2010). For example, the cultivation of microalgae require lesser fertilizer, simple nutrient input (Verma et al., 2010) and generally consumes less freshwater compared to the conventional agriculture. On top of that, microalgal farming able to consume nutrients contained in wastewater for growth (Tan et al., 2015). It has been proven experimentally that inorganic waste such as phosphates and nitrates can be effectively eliminated from wastewater (Solana et al., 2014). In addition, microalgae could also aid in combating the greenhouse effect and global warming attributed by their high rate of sequestration ability in fixing carbon dioxide (Li et al., 2008) and other discussion in (Yang et al., 2015). Due to this unique characteristic, the emissions of industrial exhaust gases such as carbon dioxide can be mitigated efficiently by these diverse and robust group of microorganisms (Ferreira et al., 2016) and other discussion in (Cheah et al., 2014).

However, one of the main difficulties faced in the mass production of proteins commercially is the low recovery of proteins due to the presence of multiple layers of rigid thick cell wall (González-Fernández et al., 2012) and also in (Günerken et al., 2015). Furthermore, the proteins are mostly trapped in globules or bound to complex membranes, making the extraction of proteins from microalgae a great challenge (Wang et al., 2014) and in (Günerken et al., 2015). The development of a simple, rapid and cost-effective cell disruption method is important to facilitate the release of proteins from microalgae (Safi et al., 2015) with minimum processing cost in microalgal industry especially at large scale application. This is because this process would have a significant impact on the efficiency of the subsequent steps in downstream processing (Phong et al., 2016). Therefore, it is imperative to choose a suitable and cost-effective solvent for use in the cell disruption and extraction process. In view of this, this study could provide some guidance on the selection of the efficient solvent to facilitate the proteins release during the downstream processing of microalgae.

2. Objectives

This research aimed to study the efficiency of different types of solvent on the proteins release from microalgae as well as to select the most suitable solvent for use in microalgal industry to facilitate the disruption and extraction process.

3. Methodology

Chlamydomonas sp. Tai-03 was chosen for evaluation in the present study. This microalgal strain belongs to the division of *Chlorophyta* and class of *Chlorophyceae*. *Chlamydomonas* sp. Tai-03 was cultivated in a *laboratory-scale* photobioreactor to obtain the maximum biomass production. After harvesting, the frozen paste of crude microalgal biomass was *freeze* dried using freeze dryer. Subsequently, the dried biomass was manually ground into fine powder using a mortar.

Four different types of solvents that were used for comparison and evaluation in this experiment were methanol, ethanol, 1-propanol and water. Microalgal biomass weighed 0.1g was dispersed in 10 mL of each solvent. After centrifugation, the pellet was discarded while the supernatant was collected for proteins analysis. The amount of proteins released from the microalgal cells was estimated by BCA protein assay. The absorbance was measured at 562 nm using microplate absorbance reader. The overall flow of the experimental process was summarized in Figure 1.

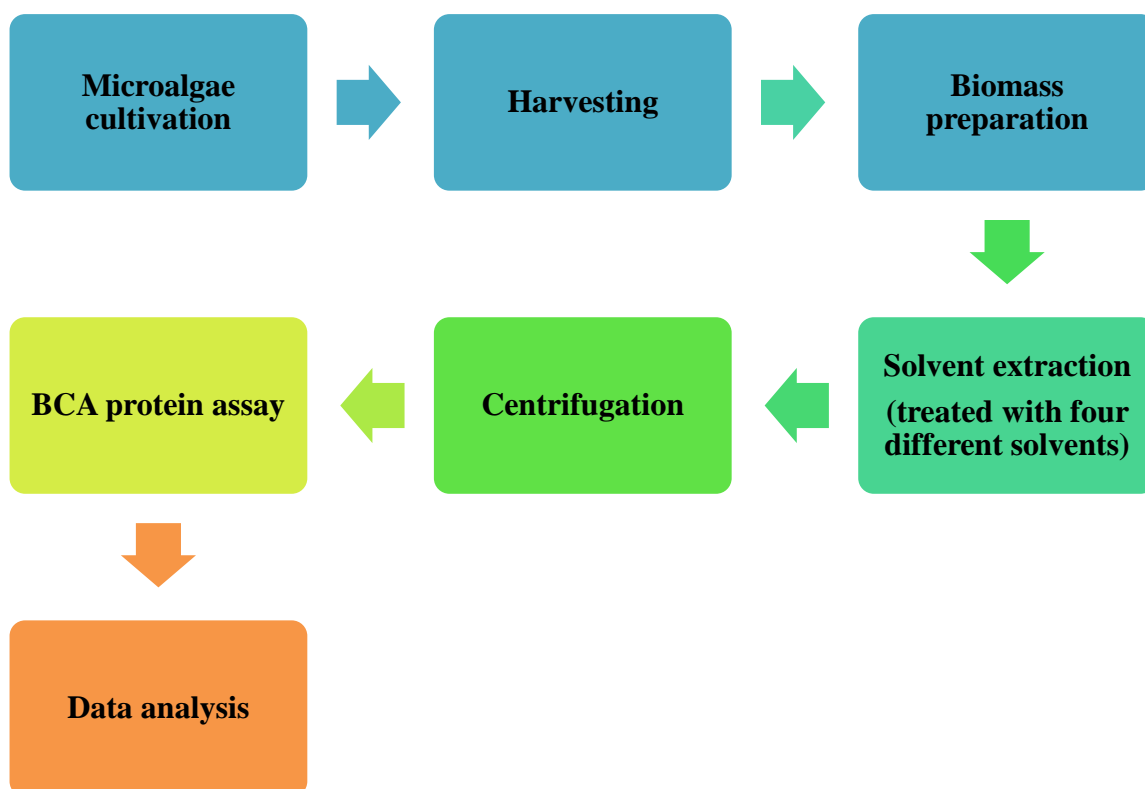


Figure 1: Flow diagram of experimental process.

4. Results and Discussions

A protein standard curve was constructed to estimate the concentration of proteins in a sample. In this experiment, bovine serum albumin (BSA) was used as a standard for the construction of a standard curve. BSA is the most common used protein standard owing to its high purity and low cost (Sattayasai, 2012). The protein concentration of the microalgae can be measured using the equation generated from the standard curve, namely $y = 0.0014x$ as shown in Figure 2.

In order to obtain the maximum recovery of cell contents from microalgae, selecting a suitable solvent to facilitate the release of the cell contents during the cell disruption and extraction process is undoubtedly one of the most crucial initial steps in downstream processing. It is important to enhance the assimilation and bioavailability of the intracellular compounds in the extraction solvent (Safi et al., 2015) so as to ensure the liberation of the internal components into the liquid medium, making them readily available for the subsequent extraction and separation process. Based on the results of the present study, it was found that among all the solvents tested, water was found to be able to extract the highest proteins concentration from *C. Tai-03* with about 3% of protein concentration per dry weight, followed by 1-propanol, ethanol and methanol (Figure 3). As illustrated in Figure 3, the percentage of proteins release in water as the solvent was approximately four-fold higher than that of using methanol as the solvent.

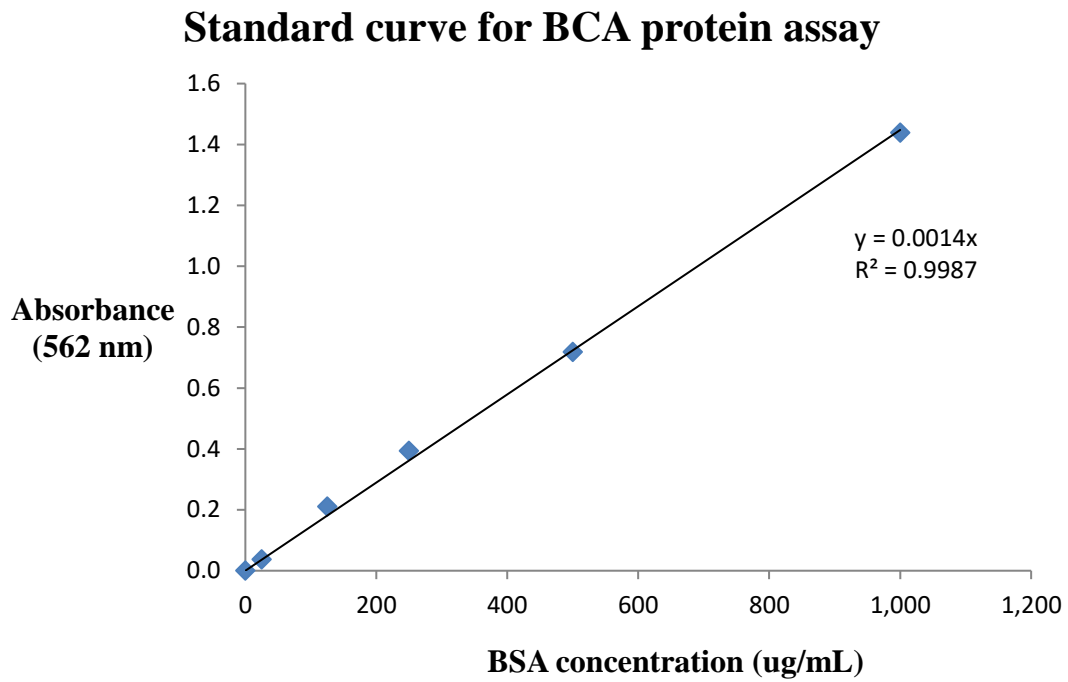


Figure 2: Standard curve for BCA protein assay using BSA as the standard.

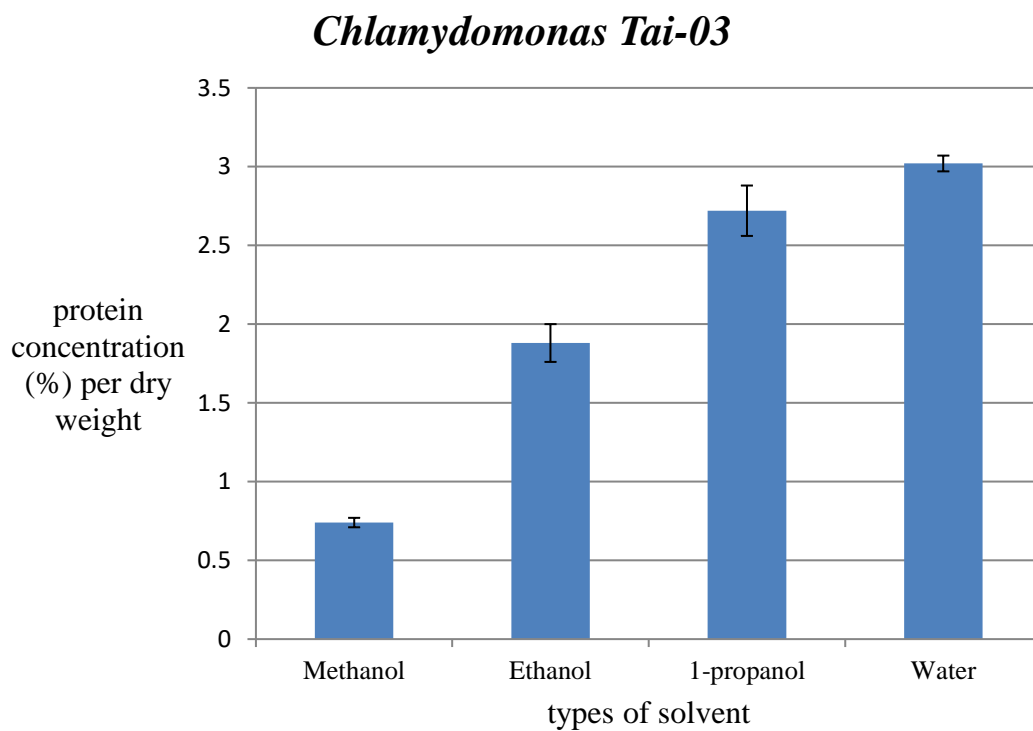


Figure 3: Total proteins content of *Chlamydomonas Tai-03* subjected to different types of solvent. Each bar is an average of three replicates.

This study provided a preliminary investigation into the effect of solvent types on the proteins liberation from microalgae. Apart from evaluating the effectiveness of a solvent, other factors such as cost, safety and the ease of scalability should also be considered when determining the suitability of a solvent for use at large scale. This is important to ensure the long-term environmental and economical sustainability in the aquaculture industry. Besides from being the most effective extraction solvent, water has several advantages over the other solvents such as low-cost, ubiquitous availability, safety and ease of production scaling. As such, it can be suggested that water is an excellent choice of solvent in aiding the proteins release from microalgae at large scale downstream processing. As the process of releasing protein from microalgae using water as the solvent is cost-effective, the mechanism behind the chemical interaction between the water and microalgal proteins could be an exciting subject for further investigation.

5. Conclusion

Overall, it was found from the study that water could be an excellent solvent for microalgal downstream processing with the indication of higher proteins yield compared to the other solvents. Further investigation using water as the choice of solvent should be tested on different strains of microalgae so as to evaluate its general effectiveness in facilitating the liberation of proteins from various types of microalgae with varying composition of cell wall.

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