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SUB-CRITICAL WATER TECHNOLOGY FOR ENHANCE EXTRACTION OF BIOACTIVE COMPOUND FROM MICROALGAE

AWALUDDIN S.A[1], SHAMSUL IZHAR[1], YOSHIDA HIROYUKI[1], MICHAEL K. DANQUAH[2], RAZIF HARUN[1*]

[1]Department of Chemical and Environmental Engineering, Universiti Putra Malaysia, 43400 Serdang, Malaysia
[2]Department of Chemical Engineering, Curtin University, 98009 Miri Sarawak, Malaysia
*Corresponding Author: mh_razif@upm.edu.my

Abstract

Current extraction technologies, including chemical, mechanical, and biological based methods, routinely used to extract biochemical compounds from microalgal biomass are disadvantaged with lengthy processing steps, energy intensive operations, high operational cost, lower product yields and environmentally unfriendly processes. Hence, the search for a sustainable low-cost technology for high throughput extraction of biochemicals from microalgal biomass is major research endeavour. Sub-critical water extraction (SWE) technology has been used for the extraction of active compounds from different biomass materials with low process cost, mild operating conditions, short process times, and environmental sustainability. With the limited application of the technology to microalgal biomass, this work investigates the factors that affect the production yield of bioactive compounds during SWE of microalgal biomass. The SWE process was investigated under different process conditions include temperature (180-374 ˚C), extraction time (1-20min) and biomass loading (5-40 wt%). The results showed that the highest carbohydrate and protein yields of 14.2 g/100g and 31.2 g/100g, respectively, were achieved at 277 ºC, 5 min with 5% of biomass loading. This productivity level which is in keeping or higher than that of current production systems endorses SWE as a promising technique for extracting bioactive compounds from microalgae.

Keywords: Sub-critical water extraction, Microalgae, Bioactive compounds, Biomass, Biochemical, Carbohydrate, Protein.

1. Introduction

The increasing of world population creates various environmental problems and demands on energy supply. It is important to develop and alternative and more sustainable energy sources based on renewable resources. Biofuels such as biodiesel and bioethanol are viable alternative energy sources to alleviate the existing dependence on petroleum-based fuel [1]. Biomass is one of the renewable energy sources that can be converted to produce biofuel. These include waste from agricultural, industrial, plant and residue obtained from bagasse, trees, rice bran and plants [2]. However, most of the biomass are not sustainable hence hinder the viability to produce biofuel.

In recent years, there have been an increasing interest to produce biofuel from microalgae due to high growth rate (double their size in 24 hours), easy to grow, efficient carbon dioxide fixation and does not required arable land to growth [3]. It contains high protein, lipids and carbohydrates compositions that can be further converted to produce variety bio-based products[4, 5]. For instance, the protein content can be used for animal feed, lipids for biodiesel and carbohydrate for fermentable sugar for ethanol production [3]. It is also has a potential as a source of natural resources of different functional compounds and used as alternative energy sources [6]. Some of the microalgal species is capable in treating wastewater and microalgae can use the organic and inorganic nutrients in wastewater as growth nutrients. This can make the process in producing bioproducts from microalgae becomes more cost-effective and sustainable.

Chemical composition of each microalgae strain is not similarly constant, but varies depending on environmental parameters such as temperature, illumination, pH value, and mineral content of the medium, CO_2 supply and mixing velocity [7]. According to Becker [7], variations in culture conditions, or changing of physical parameters such as radiation intensity, population density, light, or dark growth, producing different microalgal compositions. Bioactive compounds are also present in microalgal cells and synthesized as secondary metabolites via specific metabolic pathways. Microalgal bioactive metabolites are intracellularly produced and entrapped within the cells, thus an effective extraction technology is required to release those bioactive products [8].

One of the emerging extraction techniques is using sub-critical water extraction (SWE) technology. There has been an increasing interest in extraction of compounds from biomass materials using SWE [9]. SWE utilizes water at high temperature ranging between 100 °C (boiling point) to 374 °C (critical point) and pressure at high enough to keep it in the liquid state [10]. Figure 1 shows the water phase diagram of SWE. Two important characteristics during SWE are water ionization and dielectric potential. As the temperature of water elevated, its hydrogen bonding cleaves with decreasing dielectric constant and polarity [11]. This resulted in an increase in the concentration of hydrogen ion [12]. Using water as an extraction solvent, this offers lower production cost, safety, no net of pollution and non-toxic. Other than that, this technology is an attractive method due to shorter production period and milder operating conditions compared to the existing conventional methods.

As the technology is scarcely reported to extract products from microalgae, this work investigates different process conditions include temperature,

extraction time and biomass loading to extract bioactive compounds from microalgal biomass.

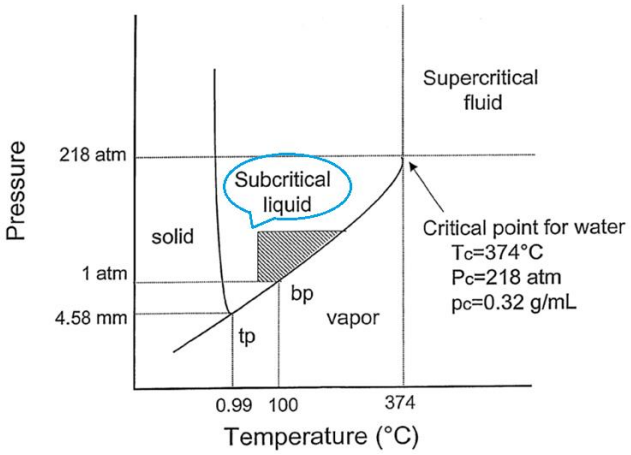

Fig. 1. water phase diagram [13].

2. Materials and methodology

2.1. Microalgal species and biomass development

Chlorella vulgaris ~~*(C. vulgaris)*~~(green microalgae) biomass was used for the extraction process. The microalgae specie was obtained from Pure Bulk Inc. (USA) and delivered in ~~a~~ green powdered form with an average particulate size of 100 µm. The powdered microalgal cells were stored in a desiccator until further used.

2.2. Characterization of microalgal biomass

2.2.1. Proximate analysis

The moisture, volatile matter, fixed carbon, and ash contents of *C. vulgaris* biomass were determined using a thermogravimetric analyser (TGA) (TGA/SDTA851, Mettler Toledo, USA). 20mg of fine biomass powder was placed in alumina crucible and heated inside a furnace. The sample was continuously heated under different conditions of temperature (0-1000 ℃), heating rate (5, 10, and 20 ℃/min), and at a constant gaseous nitrogen (N_2) / air atmosphere flowing at 25 ml/min.

2.2.2. Ultimate analysis

The elemental composition of *C. vulgaris* biomass was determined using CHNS analyser (model LECO True Spec CHNS628, USA). Approximately 1.0 mg of the biomass sample was weighed into a tin capsule and transferred to the auto sampler. The temperature was set at 1000 ℃, and oxygen, nitrogen, and helium were used as the carrier gases.

2.3. Sub-criticalwater extraction

Three process parameters were investigated at five different levels to understand the impacts on microalgal sub-critical water extraction. The three process parameters weretemperature (180 – 374 ℃), extraction time (1-20 min), and biomass loading (5-40 wt%). The schematic diagram of the sub-critical water extraction system is shown in Fig. 2. The dried microalgal biomass at a specific loading concentration was mixed with 6 ml of Milli-Q water and loaded into a stainless steel reaction tube (SUS316) having an inner diameter of 7.5 x 10^{-3} m and 1.5 x 10^{-1} m length. Argon gas was used to purge the reactor for 5 min to release trapped air from the reactor. The reactor tube was then closed tightly with Swagelok caps. The sample filled tube was immersed in a salt bath at a specific temperature for a specific time. After the reaction process, the reactor tube was quenched in a cooling water basin to terminate the reaction.

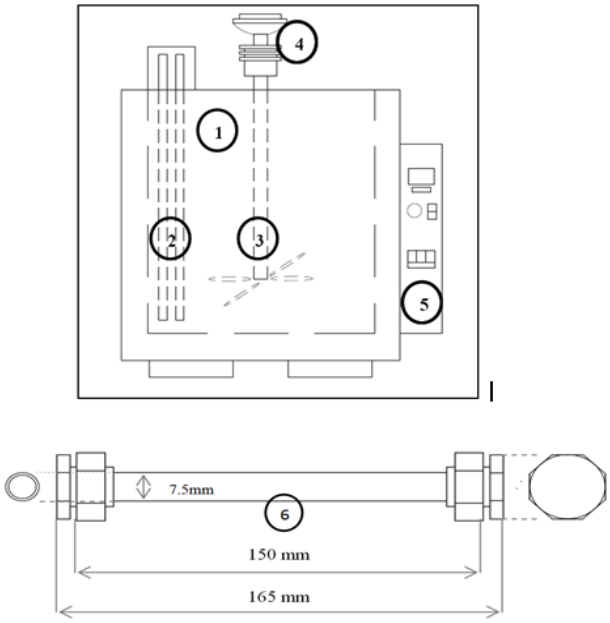

Fig. 2. Schematic diagram of the sub-critical water extraction experimental setup. The key components of the system are (1) inner salt bath, (2) heater, (3) stirrer, (4) stirring motor, (5) operation panel, (6) reactor.

2.4. SWE product analysis

2.4.1. Product separation

Product extracts from ~~the~~ SWE process were centrifuged (KUBOTA 2420, Tokyo, Japan) at 4000 rpm for 10 min. The centrifuged samples formed three different layers of oil, water, and solid residue as shown in Fig.3. About 1.5 ml of hexane (C_6H_{14}) (R&M Chemicals Ltd., MW 86.16, 99%) was added to the extracted samples for oil separation. The mixture was left for 10min before recovery by decantation. The process was repeated 8 times for complete oil

separation. The residual mixtures were further centrifuged at 4000 rpm for 10 min. The supernatant and solid residue were separated by filtration and stored at -20 °C until further analysis [14].

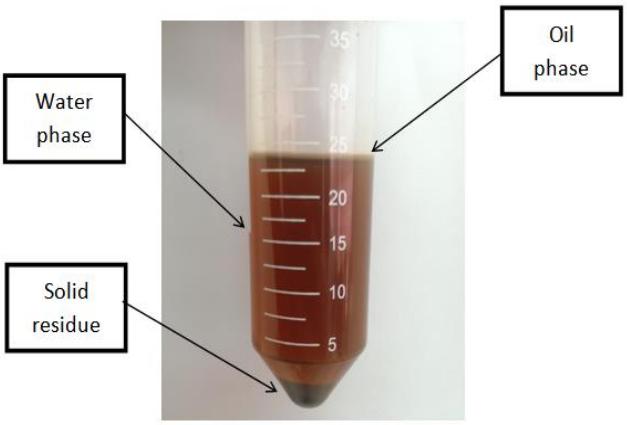

Fig. 3. Product fractions from sub-critical water extraction of *C. vulgaris* biomass.

2.4.2. Protein analysis

Protein concentration was determined using the Lowry method. A serial dilution of Bovine Serum Albumin (BSA) (R&M Chemicals Ltd) solution was prepared from its stock solution (1 mg/ml). 0.2 ml of BSA solutions with concentrations ranging from 0.05 to 1 mg/ml were pipetted into multiple test tubes. 2 ml of alkaline copper sulphate reagent was added to each aliquot and incubated for 10 min. The alkaline copper sulphate reagent consists of sodium carbonate (Na_2CO_3) (R&M Chemicals Ltd., MW 105.99, 99%), sodium hydroxide (NaOH) (Merck Pty Ltd., MW 40, 99%), copper sulphate ($CuSO_4.5H_2O$) (R&M Chemicals Ltd., MW 249.68, 99.6%), and sodium potassium tartarate ($C_4H_4KNaO_6.4H_2O$) (R&M Chemicals Ltd., MW 282.23, 99%). Also, 0.2 ml of Folin Ciocalteau (R&M Chemicals Ltd.) reagent was added to the solution, and the optical density was measured at 600 nm using UV spectrophotometer (UV-160A, SHIMADZU, Japan).

2.4.3. Carbohydrate analysis

Phenol-Sulphuric acid method was used to determine total carbohydrate content. A serial dilution of sugar standard (20 -100 mg/l) was prepared from glucose ($C_6H_{12}O_6$) (R&M Chemicals Ltd., MW 180.16, 99.5%). UV spectrophotometer was used to determine the optical density at 490 nm. 1 ml of sample was mixed with 1 ml of 5% phenol and 5 ml of sulphuric acid (H_2SO_4) (R&M Chemicals Ltd., MW 98.079, 99%). The sample was left at ambient conditions for 10 min, and the glass lid covered for 25 min incubation before UV spectrophotometric analysis.

3. Results and discussion

3.1. Biomass characterization

Proximate and ultimate analysis

Table 1 represents the composition of *C. vulgaris* biomass used in this study. The analysis in wt% indicated that the biomass contained 83.5% volatile matter, 6.3% moisture, 5.1% ash and 3.8% fixed carbon. The carbon content increase by 25% with SWE treatment compared to the intact cell, indicating that the extraction process resulted in some degree of biomass disruption and this enabled a higher amount of carbon to be available for combustion. The high carbon content of the biomass sample indicates that it contains substantial amounts of lipid, cellulose, and hemicelluloses which are good for biodiesel and bioethanol production.

Table 1. Proximate analysis and ultimate analysis of *C. vulgaris* biomass.

Proximate analysis	**wt%**	
Moisture	6.3	
Volatiles	83.5	
Fixed carbon	3.8	
Ash	5.1	
Ultimate analysis	**wt%**	
	Non-treated sample	**Treated sample**
Carbon (C)	47.11	58.88
Hydrogen (H)	7.47	8.55
Oxygen (O)	37.16	25.03
Nitrogen (N)	8.26	7.54
Sulphur (S)	-	-
Ash	-	-

3.2. SWE product analysis

3.2.1. Effect of time

Figure 4 illustrates the protein and carbohydrate concentrations under different extraction time. It was found that protein concentration decreased as the extraction time increased with the highest protein concentration of 25.5 g/100g at 1 min. The concentration was reduced to 12.5 g/100g when the extraction time was prolonged to 20 min, indicating protein was denatured as the extraction time increased. Protein denaturation happens when the heating time increases the molecular kinetic energy of the protein, causing it to vibrate rapidly and destroying the protein tertiary structure through the cleavage of its hydrogen bonds and non-polar hydrophobic interaction [15].

Furthermore, a similar declining trend with time was also observed while extracting carbohydrate composition from microalgal biomass. The carbohydrate inside the sample decomposed as the extraction time increase due to the long exposure period to heat. The highest concentration of carbohydrate of 4.1 g/100g was achieved at 1 min whereas the lowest carbohydrate of 2.0 g/100g was obtained at 20 min reaction time.

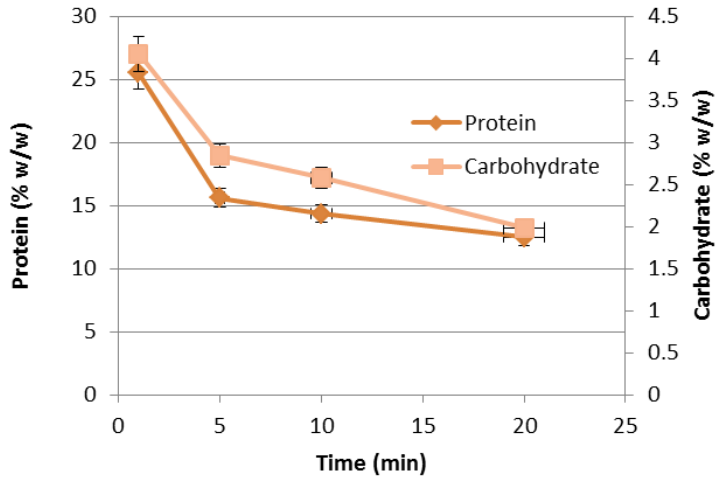

Fig. 4. The time course of protein and carbohydrate concentration. The results represent the average values of 3 replicates.

3.2.2. Effect of temperature

Figure 5 shows the effect of temperature on the production of protein and carbohydrate during SWE process. It was observed that protein concentration rapidly increased as the temperature increased (from 180-277 °C), but the decreasing pattern was observed after 277 °C. This is due to the protein denatured at high temperature [16]. The highest protein concentration of 15.6g/100g resulted at 277 °C while the lowest protein concentration of 7.8 g/100g was obtained at 374 °C.

However, the different trend was observed for carbohydrate production. As the temperature increased, carbohydrate concentration was decreased, producing the highest and lowest carbohydrate concentrations of 5.1 g/100g at 180 °C and 2.3 g/100g at 374 °C. This trend was consistent with the findings from other studies[17- 19].

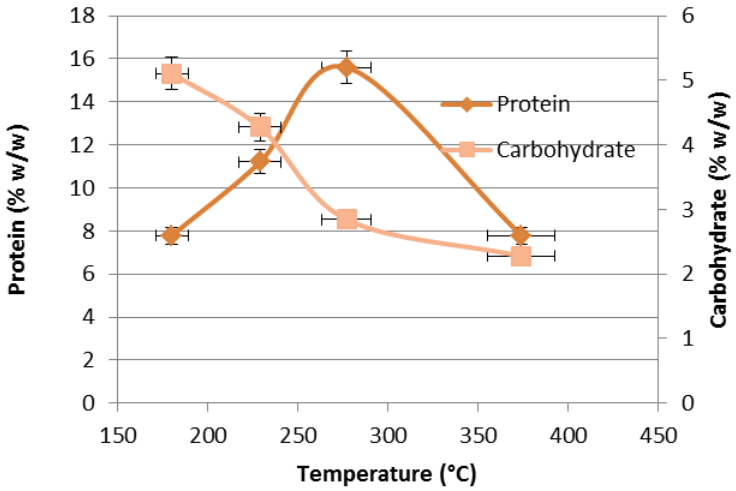

Fig. 5. Effect of different temperatures on protein and carbohydrate concentration. The results represent the average values of 3 replicates.

3.2.3. Effect of algal concentration

Figure 6 shows the effect of microalgal concentration on protein and carbohydrate concentrations. Both graphs illustrate a declining pattern on their productions while increasing the biomass loadings. The highest protein concentration was 31.2 g/100g with 5% loading while the lowest of 9.1 g/100g was achieved when loaded with 20% microalgal biomass. Meanwhile, the maximum carbohydrate concentration was found at14.2 g/100g with 5% concentration, and the lowest was 1.4 g/100g with 40% concentration. Biomass loading is one of the crucial parameters during SWE process due to the sample will not be completely extracted when the amount of sample inside the reactor is too concentrated. It is because microalgae has rigid cell walls which hard to break [20].

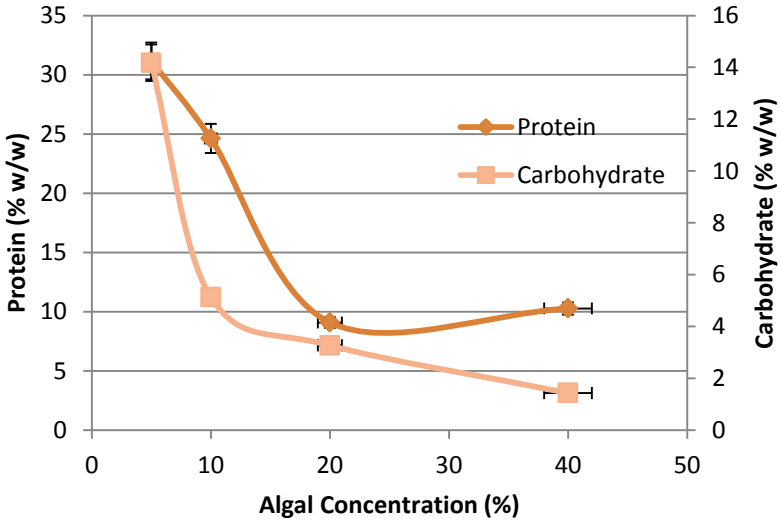

Fig. 6. Effect of algal concentration on protein and carbohydrate concentration. The results represent the average values of 3 replicates.

4. Conclusion

This work investigated the effect of different process parameters to extract biochemical compounds from microalgal biomass using sub-critical water extraction technology. The findings summarise that time, temperature and algal concentration provide a significant effect on protein and carbohydrate extraction using SWE. Protein concentration decreases when time and algal concentration increase. While carbohydrate concentration decreases as time, temperature and algal concentration increase. The obtained results demonstrate the potential of SWE of microalgal biomass for large-scale production biochemical compounds. From economic and environmental perspectives, sub-critical extraction has proven attractive in recovering valuable biochemical materials from a wide range of biomass feedstocks. The use of SWE for the generation of bioproducts from microalgal biomass is expected to herald future sustainable bio-extraction technologies, and this work contributes significantly to the validation of SWE technology for optimal biochemical products extraction.

Acknowledgement

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