Mechanical Extraction of Protein Solution from Microalgae by Ultrasonication

A Kuhavichanan[1,2], P Kusolkumbot[2], S Sirisattha[2] and *C Areeprasert[1]

1 Department of Mechanical Engineering, Faculty of Engineering, Kasetsart University.
50 Ngam Wong Wan Road, Lat Yao, Chatuchak, Bangkok Thailand 10900.
2 Biodiversity Research Centre, Thailand Institute of Scientific and Technological Research.
35 Mu 3 Tambon Khlong Ha, Amphoe Khlong Luang, Pathum Thani 12120 Thailand
E-mail: achinatun@gmail.com and apichay.ku@gmail.com

Abstract. This study investigated the condition on microalgae cell disruption method by the combination of ultrasonication and buffer solution. Various buffer solutions have been utilized and the duration of the sonication was also tested. The quantity in term of protein concentration and the quality of the protein product were evaluated. Results showed that Mixed buffer III and Triton x lysis buffer showed the high concentration of protein but low quality. SDS lysis buffer showed high quality protein product but low the concentration of protein. For the study on the effect of sonication duration, the concentration of protein significantly increased when longer duration of sonication was applied and this result was observed during continuous sonication test as well. The concentration of protein was not only the important aspect, but the quality of the protein needs to be satisfied. Thus, it could be suggested that the optimum condition of protein extraction from microalgae needs the combination of the use of buffer solution to ensure high quality product and an appropriate duration of ultrasonication to maximize the quantity of protein.

1. Introduction

Many researchers have investigated the sustainable and renewable fuels including solar, wind, biomass, geothermal, hydropower, etc. Microalgae have been identified as a potential bioenergy source for fuel production. Advantages of microalgae over terrestrial biodiesel feedstock include easy to cultivate, higher growth rates and productivity than other terrestrial biomass sources, capable of storing solar energy into energy-rich compounds such as lipids and no direct competition for agricultural land [1]. Microalgae contain water around 80-90% [2], so traditional thermochemical processes like pyrolysis and gasification are not economically feasible. However, an innovative thermochemical process, i.e. Hydrothermal Liquefaction (HTL) that uses hot compressed water as a medium, appears to be more suitable for wet microalgae feedstock. The HTL utilizes high pressure and temperature steam at the condition of 250 to 350 ℃, 10-20 MPa for conversion of wet biomass. For this result, the high-value intracellular component, such as protein, in microalgae was destroyed by the HTL processes. Protein was denatured easily under high temperature condition; therefore, the high value product must be extracted from the microalgae by other cell disruption methods before the HTL process.

Currently, many literature studies focused on evaluation of cell disruption methods. Cell disruption is important for the extraction of various cell inclusions [3], [4]. Cell disruption methods can be divided

into mechanical, such as ultrasonication [5], and non-mechanical, such as chemical. Ultrasonication is one of the most effective microalgae cell breaking methods. This method used sonic wave with frequencies around 25 kHz for cell disruption. Evaluation on the effectiveness of high-frequency focused ultrasound (HFFU) in microalgae cell disruption was performed [6]. However, only sonication mean was not enough for cell disruption of microalgae; thus, addition of buffer for cooperation on cell breaking was necessary. Buffer solution was able to protect the released protein from disrupt microalgae giving better protein quality. Therefore, this paper aims to optimize the condition on microalgae cell disruption method by the combination of ultrasonication and buffer solution.

2. Material and methods

2.1. Microalgae

Coelastrum sp. microalgae was used in this study. It was cultured with open pond system and was harvested by gravity sedimentation method [7]. The microalgae raw material was supported by the Biodiversity Research Centre (BRC)**,** Thailand Institute of Scientific and Technological Research (TISTR), Thailand**.** Concentrated microalgae were separated and prepared for pretreatment. For pretreatment method, freeze-thaw was employed as the slurry microalgae were frozen at -40 ℃ prior to the experiment. Original microalgae (no pretreatment) was also prepared by storing at 4 ℃.

(a) (b) (c) (d)

Figure 1. (a): suspension microalgae; (b): slurry microalgae; (c): dewatering by vacuum pump; (d): concentrated microalgae.

2.2. Preparation of microalgae suspension

The freeze microalgae suspensions was prepared at the concentrate (slurry) microalgae (g) and water (ml) ratio of 1:4 and kept at 4 ℃. (Figure 1) Similarly, the fresh microalgae suspensions (Figure1a) was prepared at the concentrate (slurry) microalgae (Figure1b) then dewatering by vacuum pump (Figure1c). Finally, The concentrated microalgae (Figure1d) (g) were mixed with other solvents (ml) ratio of 1:4 and kept at 4 ℃. Solvents used in this study can be named as follows: Water; Mixed buffer I (pH 7, 100 mM Tris, 10 mM EDTA (Ethylenediaminetetraacetic Acid) and 100 mM NaCl); Mixed buffer II (150 mM NaCl, 1% Triton X (Triton^{TM} X-100) and 50 mM Tris pH 8), Mixed buffer III (150 mM NaCl, 1% Triton X, 50 mM Tris pH 8 and 0.1% SDS (Sodium Dodecyl Sulfate)), SDS lysis buffer (pH7, 100 mM Tris, 10 mM EDTA and 100 mM NaCl added SDS buffer), and Triton-x lysis buffer (pH7, 100 mM Tris, 10 mM EDTA and 100 mM NaCl added Triton x buffer). In this experiment, an evaluation on the effect of the concentration of SDS and Triton x lysis buffers and duration of cell disruption by ultrasonication was performed. Comparison of the concentration of protein and the quality of protein after extracted from the different solvents during ultrasonication was also performed.

2.3. Ultrasonication

Cells disruption method was done by ultrasonication device VCX 750 by Sonics & Materials Inc. The ultrasonic processors was operated at 750 Watt 20 kHz. The ultrasonic probes are fabricated from high-grade titanium alloy Ti-6Al-4V with 13 mm diameter and 159 mm length. The pulse generator was operated for 20 s and stop for 5 s and worked at 60 % amplitude. In the experiments, the ultrasonic heat, which occurred when probe is working, was absorbed by ice.

2.4. Quality and quantity of the product

After the microalgae suspensions were disrupted by ultrasonication, the product was centrifuged at 10,000 rpm 4 ℃ for 30 min (Sorvall™ Stratos™ Centrifuge by Thermo Fisher Scientific) for separation liquid and solid phase. A liquid phase (Figure 2) was measured concentration of protein by Bradford assay and quality of protein was determined by Sodium Dodecyl Sulfate polyacrylamide gel electrophoresis (SDS-PAGE). In this work, the test was triplicated and reported in mean value ± SD.

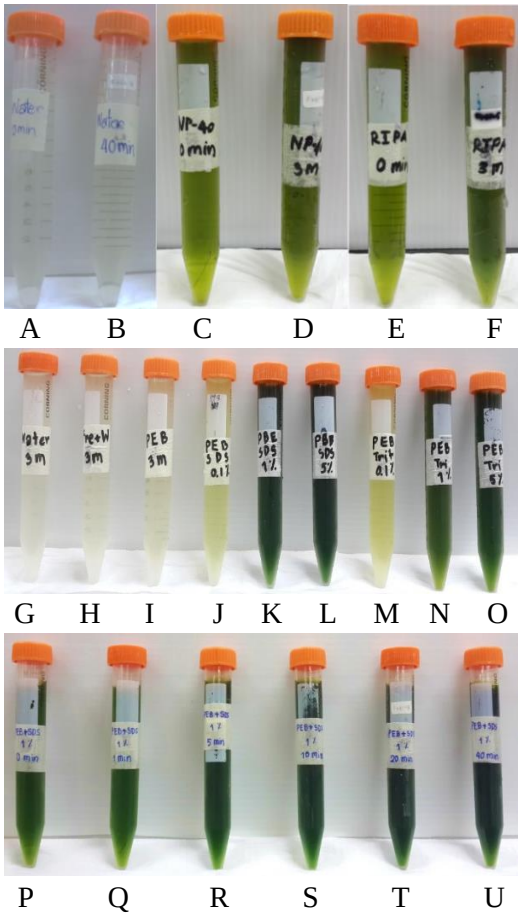

Figure 2. Supernatant solvents at various condition.

In Bradford assay[8], the absorbance was measured at 595 nm by spectrophotometer and the concentration of protein was determined by Monochromator (Infinite M200 PRO, Tecan Trading AG, Switzerland). Bovine Serum Albumin (BSA) was used to a calibration curve. In SDS-PAGE method separated molecules based on the difference of their molecular weight. Gels was stained with Coomassie brillianl blue (CBB). Figure 2 shows supernatant from various condition and solvent. A, G, and B are supernatant from water co-extraction with sonicate at 0, 3, and 40 min. H is supernatant from freeze-thaw co-extraction with sonicate at 3 min. I is supernatant from Mixed buffer I co-extraction with sonicate at 3 min. C and D are supernatant of Mixed buffer II co-extraction with sonicate at 0 and 3 min. E and F are supernatant of mix buffer III co-extraction with sonicate at 0 and 3 min. J, K and L are supernatant solvents of SDS lysis buffer concentrate 0.1, 1 and 5% co-extraction

with sonicate at 3 min. M, N and O are supernatant from Triton x lysis buffer concentrate 0.1, 1 and 5% co-extraction with sonicate at 3 min. P, Q, R, S, T and U are supernatant from SDS lysis buffer 1% co-extraction with sonicate at 0, 1, 5, 10, 20 and 40 min.

2.5. Continuous cell disruption method by ultrasonication

The operation of the continuous ultrasonication cell disruption was performed at three liter/s. The slurry was recirculating until cell disruption was completed. The duration of the test was from 0 to 90 min. Figure 3 shows the schematic diagram of the continuous ultrasonication cell disruption system. The freeze microalgae was used as feedstock for this test. The feedstock was stored and sucked up by centrifugal pump then entering the disruption region from the bottom of the chamber. Sonication was done at 60% amplitude and frequency of 20 kHz. Then, the overflow was guided to the feedstock storage waiting for recirculation and re-disruption. The continuous system was used for cells disruption of microalgae by the combination of buffer solution and ultrasonication.

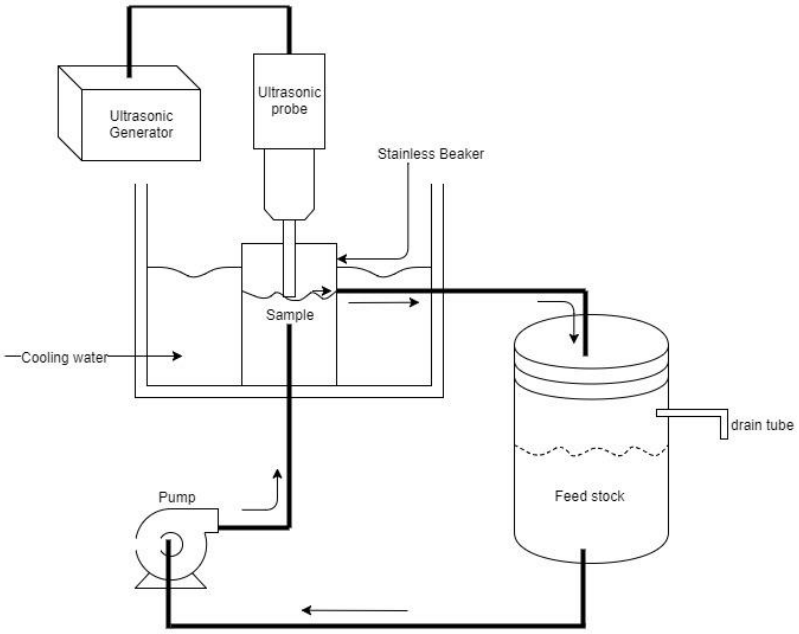

Figure 3. Schematic diagram showing the arrangement for continuous cell disruption method by sonication of the suspension microalgae.

3. Result and discussion

The concentration of protein is shown in Table 1. This method requires comparing the spectrophotometric absorbance of the unknown suspension at wavelength of 595 nm to a calibration curve prepared by standard solution of a protein (BSA). Table 1 presents the comparison of concentration of protein, which was measured from the suspension microalgae after cell disruption by sonication methods. In this Table 1, the highest of concentration of protein was 3.6540 mg/ml by Triton x lysis buffer 5 % 3 min. From the results of the water sonication at 0 min (0.0242 mg/ml), 3 min (0.0303 and 0.0418 mg/ml) and 40 min (0.1324 mg/ml), it revealed that the long duration increased the efficiency of cell disruption giving higher protein concentration. When comparison water, Mixed buffer I, Mixed buffer II and Mixed buffer III, the highest concentration of protein were measured in the case of Mix buffer III at 1.4975 mg/ml. Comparing the water, Mixed buffer I, various concentration of SDS lysis buffer as 0.1%, 1%, and 5%, various concentration of Triton x lysis buffer as 0.1%, 1%, and 5%) at 3 min sonication, result showed that Triton x lysis buffer gave the highest concentration of protein at 3.654 mg/ml. The final test was a comparison of SDS lysis buffer 1% at various sonication duration of 0 to 40 min. It was found that sonication with SDS lysis buffer 1% at 40 min gave the maximum protein concentration value at 0.3505 mg/ml.

In all cases, the Mixed buffer III and Triton x lysis buffer gave the high concentration of protein; however, the quality of protein determined by the SDS-PAGE shown in Figure 4 was no observed band. It can be suggested that the solvents distorted molecule of protein from long to short chain. This is represented by the poor quality band color shown in Figure 4a and 4b. SDS lysis buffer gave the concentration of protein not higher than that of the other solvents but the quality of protein was the best in all cases (Figure 4b, 4c). Figure 4b shows a comparison of the quality of protein SDS lysis buffer to Triton x with various solvent concentration of 0.1%, 1% and 5%. The H, I, and J were from Triton x lysis buffer 0.1%, 1% and 5%, respectively while the E, F, and G were from SDS lysis buffer 0.1%, 1%, and 5%, respectively. The H, I, and J were intent band but the E, F, and G were no observed band. Therefore, the amount of obtained protein was not only the importance, but also the quality of the protein product need to be consider for increasing the value of the final product.

For the evaluation of sonication duration, the data of the concentration of protein using SDS lysis buffer with various sonication duration as 0, 1, 5, 10, 20 and 40 min are summarized in Table 1. The concentration of protein increased when longer duration of sonication was applied, for example, at 0 min the yield was 0.1730 mg/ml and it was increased to 0.2963 mg/ml at 20 min and 0.3505 mg/ml at 40 min, accounting 71% and 102%, respectively. This was clear that the duration of sonication affected the yield of the protein.

Table 1. Quantification of protein in suspension by Bradford methods.

Solvent	Time (m)	Concentration protein (mg/ml)	SD
Water (1)	3	0.0303	0.0080
Mixed buffer I (1)	3	0.1025	0.0115
Mixed buffer II	0	1.0690	0.0100
Mixed buffer II	3	1.0950	0.0147
Mixed buffer III	0	1.4975	0.0125
Mixed buffer III	3	1.3457	0.0266
Water (2)	3	0.0418	0.0055
Pretreated-water	3	0.1139	0.0141
Mixed buffer I (2)	3	0.1010	0.0103
SDS lysis buffer 0.1 %	3	0.1606	0.0039
SDS lysis buffer 1 %	3	0.3159	0.0080
SDS lysis buffer 5 %	3	0.3346	0.0087
Triton x lysis buffer 0.1 %	3	0.1573	0.0092
Triton x lysis buffer 1 %	3	0.6176	0.0173
Triton x lysis buffer 5 %	3	3.6540	0.0112
Water (3) 0 min	0	0.0242	0.0088
Water 40 min	40	0.1324	0.0257
SDS lysis buffer 1 %	0	0.1730	0.0009
SDS lysis buffer 1 %	1	0.1737	0.0132
SDS lysis buffer 1 %	5	0.1948	0.0151
SDS lysis buffer 1 %	10	0.2270	0.0227
SDS lysis buffer 1 %	20	0.2963	0.0306
SDS lysis buffer 1 %	40	0.3505	0.0458

For continuous cell disruption by sonication, the first test used pretreated freeze-thaw microalgae as feedstock. The test used water to solvents and various duration of sonication. Results are presented in Table. 2. The concentration of protein at 40 min (longest duration in this study) was higher than that of at 20 min (shortest duration) for 12.3%. Additionally, the quality of protein obtained from this process is shown in Figure 4d was not satisfied due to denaturation of obtained protein. Therefore, the solvents need to be consider to improve the quality of the protein. Base on the previous experiment, the protection of extracted protein that can enhance the quality can be the SDS lysis buffer.

Table 2. Quantification of protein in suspension by Bradford methods.

Concentrate of protein from freeze microalgae		
Time(min)	Total protein (mg/ml)	SD
20	0.4232	0.0096
25	0.4300	0.0272
30	0.4488	0.0405
35	0.4584	0.0109
40	0.4754	0.0224

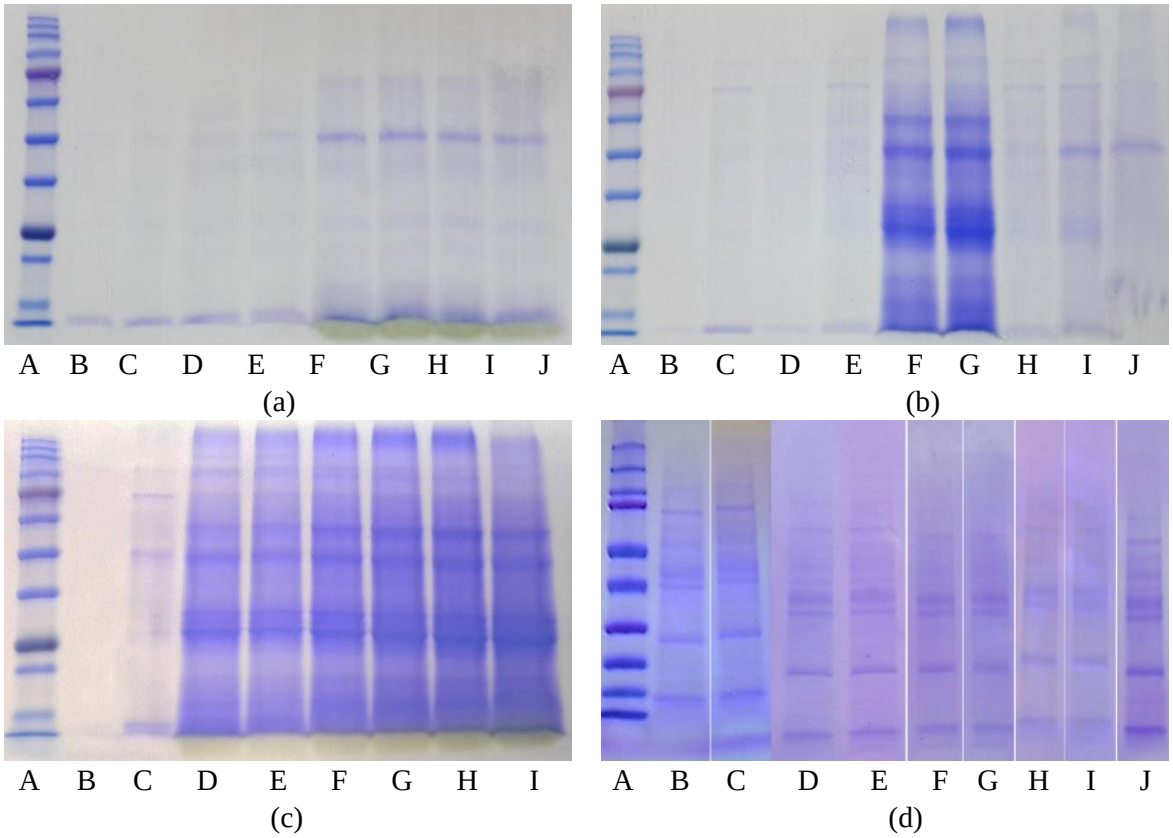

Figure 4. SDS-PAGE gel stained by Coomassie blue (a): A(Maker) B(Water at 0 min) C(Water at 3 min) D(Mixed buffer I at 0 min) E(Mixed buffer I at 3 min) F(Mixed buffer II at 0 min) G(Mixed buffer II at 3 min) H(Mixed buffer III at 0 min) I(Mixed buffer III at 3 min); (b): A(Maker) B(Water) C(Freeze microalgae ,water) D(Mixed buffer I) E(SDS lysis buffer 0.1 %) F(SDS lysis buffer 1 %) G(SDS lysis buffer 5 %) H(Triton x lysis buffer 0.1 %) I(Triton x lysis buffer 1 %) J(Triton x lysis buffer 5 %) at 3 min; (c): A(Maker) B(Water at 0 min) C(Water at 40 min), SDS lysis buffer 1 % at D(0 min) E(1 min) F(5 min) G(10 min) H(20 min) I(40 min); (d): A(Maker), continuous sonication at B(20 min) C(25 min) D(30 min) E(35 min) F(40 min) G(45 min) H(50 min) I(55 min) J(60 min).

Figure 4 shows the quality of total protein concentration which determined by SDS PAGE. A in all gels is protein marker. In Figure 4a as B, C, D and E which had total protein concentration was 0.6821 mg/ml (B and C) and 2.3068 mg/ml (D and E) , in Figure 4b as B, C, D and E which had total protein concentration was 0.9402, 2.5624, 2.2714, and 3.6133 mg/ml, respectively. Figure 4c as B and C,

which had total protein concentration was 0.5455 and 2.9795 mg/ml, respectively, show unintent band because the total were low. On the other hand, Figure 4a presents F, G, H and I which had total protein concentration was 24.0516, 24.6383, 33.6934 and 30.2792 mg/ml, respectively. Figure 4b as H, I and J which had total protein concentration was 3.5403, 13.8961, and 82.2149 mg/ml had high concentration of protein. They were not intent band and no observed band. Figure 5 shows a comparison of three processes, water sonication, SDS lysis 1% sonication, and water continuous sonication. The SDS lysis buffer gave the concentration of protein more than water sonication as well as better quality of protein. The highest concentration of protein from this comparison was the continuous sonication. From all curves, it can be summarized that duration of sonication contributed to cells disruption.

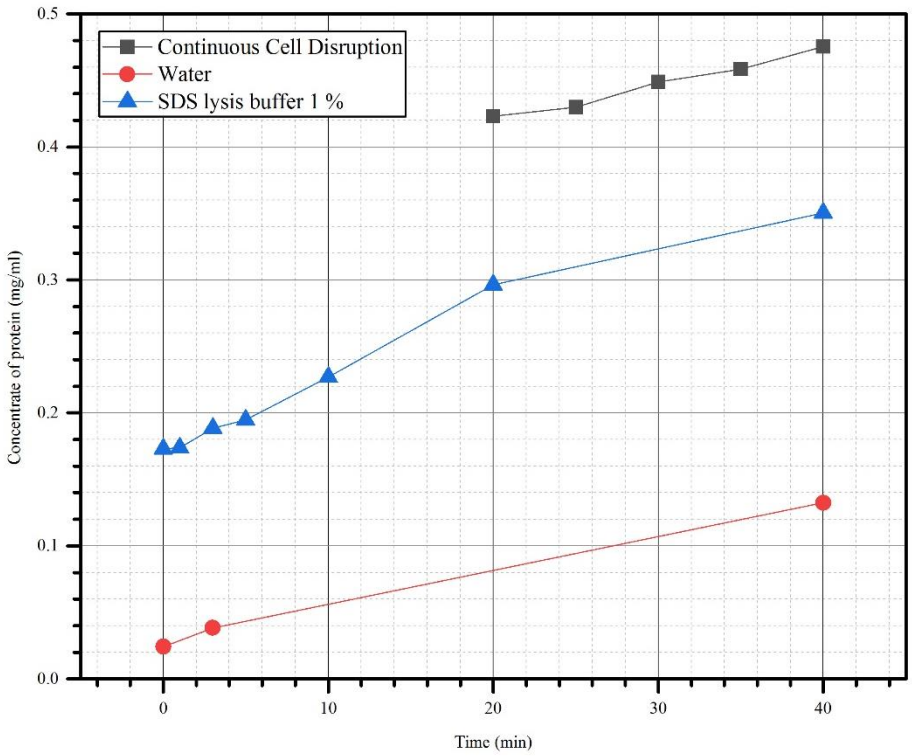

Figure 5. Soluble protein concentration released in each solvents after cell disruption

4. Conclusion

This paper examines the condition on microalgae cell disruption method by the combination of ultrasonication and buffer solution. It can be summarized as follows: (1) Mixed buffer III and Triton x lysis buffer showed the high concentration of protein; however, the quality of protein was not satisfied; (2) SDS lysis buffer showed high quality protein product but low the concentration of protein; (3) When using SDS lysis buffer, the concentration of protein significantly increased when longer duration of sonication was applied and this result was observed during continuous sonication test as well; thus, the duration of sonication affected the yield of the protein; (4) The amount of obtained protein was not only the importance, but also the quality of the protein product need to be consider for increasing the value of the final product; therefore, the optimum condition of protein extraction from microalgae needs the combination of the use of buffer solution to ensure high quality product and an appropriate duration of ultrasonication to maximize the quantity of protein.

6. References

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