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Variation of different solvent types for optimization of protein extraction from microalgae

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Abstract. Three microalgae isolates from Wakatobi waters collected by LIPI have been cultivated. i.e. LIPI 13-2-AL015, LIPI 13-2-AL025, LIPI 13-2-AL038. The three isolates were colony-shaped. Furthermore, the isolates were grown to make the growth curve, this was to determine the harvest time of biomass. Each isolate had different biomass harvest times: LIPI 13-2-AL015 isolates were harvested on day 10, LIPI 13-2-AL025 and LIPI 13-2-AL038 isolates were harvested on the 15th day. After harvesting, biomass was dried, and then the cell wall was destroyed by sonication. The next treatment was microalgae protein extraction. There were the solvent, namely: aquadest, buffer phosphate pH 7.4, and acetone 80%. Based on the three solvents, the most widely obtained protein consumption was phosphate buffer pH 7.4, followed by aquadest, and the last solvent was 80% acetone.

1. Introduction

Microalgae are one of the most promising groups of organisms for the sustainable, commercial production of bio-products such as oils, pigments, proteins and carbohydrates. They grow fast compared to terrestrial crops and can contain significant concentrations of lipids, proteins and other nutrients, depending of the strain and cultivation conditions.

Microalgae from Wakatobi Southeast Sulawesi has been explored by Indonesian Science Institute. Some of them have been investigated as anti-diabetic [1]. This time it will try to study the protein content of microalga of Wakatobi, and identify the amino acid composition. It is important to consider that each microalgae has a specific protein content.

The waters of Wakatobi National Park of Southeast Sulawesi covers an area of 13,900 km², which includes corals, islands, and communities therein [2]. Wakatobi National Park is located right in the Heart of Triangle Coral World, where 97% of waters and only 3% of land, so its biodiversity is very rich. The high biodiversity in this area is seen from the number of corals that reach 750 species, 942 species of fish, 9 species of seaweed from 12 species of Indonesian seaweed, 210 species of mollusks, and 85 species of seabirds [3]. With its diversity Wakatobi National Park of Southeast Sulawesi is believed to have a very high biodiversity due to the highly complex abiotic-biotic interactions and



biotic that occur in it. This of course affects the diverse species of microalgae and more specifically its "metabolite content".

The diversity of its species, the metabolite composition, and the genetics of microalgae are strongly influenced by climate and geographical location, changes in environmental parameters both spatially and temporally including light, temperature, nutrients, salinity, wave patterns, including biotic interactions [4]. Therefore, local microalgae species should be collected because they have a competitive advantage [5]. Hence, the purpose of this study was to find the best solvent for extracting microalgae proteins and to explore possible applications in food industry, and medical.

2. Material and Methods

2.1. Culture medium

The species microalgae was obtained from Bioenergy and Bioprocess Laboratory, Indonesian Institute of Sciences. These microalgae were isolate from Wakatobi Island South East Sulawesi, Indonesia. There were two mediums to growth microalgae, IMK and AF6. IMK Medium for LIPI 13-2-AI015, and LIPI 13-2-AI038, while AF6 for LIPI 13-2-AI025, IMK modified media made by weighing the IMK (Daigo, Japan) as much as 0.252 g, then added 1 L water that has been filtered. The media was sterilized for 15 minutes of temperature 121°C before use. Into sterile media added vitamin B12 0.015 mL, thiamine HCl 0.015 mL, biotin 0.015 mL and Vitamin B6 0.002 mL. The preparation of AF6 media was carried out by weighing NaNO_3 0.14 g, NH_4NO_3 0.002 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.03 g, KH_2PO_4 0.01 g, K_2HPO_4 0.005 g, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.01 g, CaCO_3 0.01 g, Fe Citrate 0.002 g, Citric Acid 0.002 g, enter 1 L of Iqu, PIV metal 1 mL, the media homogenized and pH adjusted to 6.6, then sterilized by autoclave. Subsequently sterile solutions were mixed with 0.01 mL of vitamin B6, thiamine HCl 0.01 mL, 0.012 mL vitamin B12, and biotin 0.02 mL.

2.2. Cultivation, harvesting, and protein extraction

The microalgae stock was prepared in 50 mL medium. Further microalgae were removed in 250 mL. The 250 mL microorganisms are stock to be cultivated with each two replications. Microalgae cultivation using a 500 mL culture bottle, was aerated at room temperature and given 24-hour illumination. The microalgae cell growth rate was observed daily using the Shimadzu UV-Vis spectrophotometer at a wavelength of 680 nm to reach the stationary phase. Growth curve is made by plotting time (day) with absorbance value. 125 mL cultures were harvested using centrifuge at 6000 rpm for 10 minutes. Then, it was washed with aquadest and centrifuged back to get the pellet. The obtained pellet was added solvent, and then the microalgae cell wall was solved by using a 30 Hz sonicator in 15 seconds for 20 times. This is done for each solvent (aquadest, phosphate buffer pH 7.4, and acetone 80%). After that it was centrifuged again and the supernatant was taken for analysis.

2.3. Determination of protein content and analysis of amino acid

Protein concentration was determined by the modified Bradford method [6]. Bovine serum albumin (BSA) in calibration curve was done in following concentrations: 20 ppm, 40 ppm, 60 ppm, 80 ppm, 100 ppm, 150 ppm, 200 ppm, 250 ppm, and 300 ppm. Every 100 μL BSA concentration mixed with 1 mL Bradford buffer. The BSA concentrations and the microalgae protein extracted with Bradford buffer were measured at optical density (OD) 595 nm. OD detection was done with spectrophotometer. Amino acid was analysed by UPLC (Ultra Pressure Liquid Chromatography), under taken in the PT Saraswanti Indo Genetech Laboratory, Bogor Indonesia

3. Results and Discussion

3.1. Sample characteristics

The three microalgae isolates were observed using the Leica microscope, to ensure that the isolates were isolates uncontaminated (figure 1).

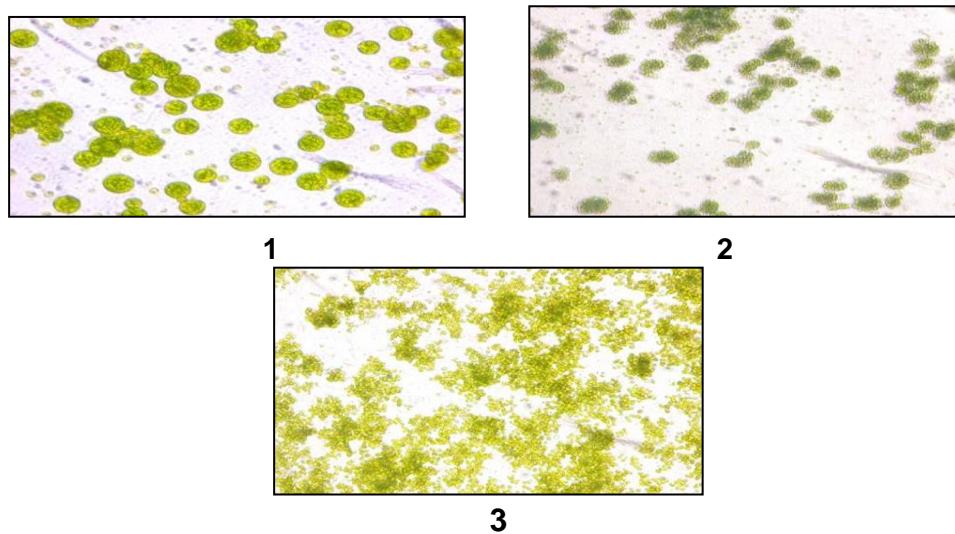


Figure 1. Microscopic image of cell microalgae (1) LIPI 13-2-AL015, (2) LIPI 13-2-AL025, (3) LIPI 13-2-AL038

Observation of morphology of cell microalga LIPI 13-2-AI015 obtained cell-shaped round with various sizes, light green, visible cells are grouped and not in groups. LIPI 13-2-AI025 obtained cell-shaped round, dark green, very small and group. LIPI 13-2-AI038 obtained cell-shaped round, light green, very small, looks clustered and looks solid.

3.2. Microalgae Growth

The first step, microalgae was growth for obtained growth curve, then each isolate is grown again and harvested in an exponential phase to obtain biomass. Cell density was measured at a wavelength of 680 nm.

Cultivation of microalgae is done in a bottle connected with aeration hose and 24 hour lighting. Microalgae growth was observed daily OD using the Shimadzu UV-Vis spectrophotometer. Measurements were made for each species 2 replicates, from day 0 to stationary phase. Based on the growth curve, LIPI 13-2-AL015 was harvested on day 10, LIPI 13-2-AL025 and LIPI 13-2-AL038 was harvested on the 15th day (figure 2). Isolate LIPI 13-2-AL015 growth is relatively fast compared with other microalgae. In terms of color this microalga looks light green. Isolate LIPI 13-2-AL025 looks more dark green than LIPI 13-2-AL038, which looks light green. The LIPI 13-2-AL025 microstructure that is precipitated is also more easily mixed by shaking the bottle than LIPI 13-2-AL038 where the precipitate must be piped in order to be mixed. After knowing the growth curve of microalga, then cultivation process done again by taking stock from volume 250 mL. Cultivation made each 2 replication with volume 500 mL.

After harvesting, visible LIPI 13-2-AI038 isolate has the highest dry biomass of 0.12 g/125 mL, then isolate LIPI-13-2-AI025 as much as 0.11 g/125 mL and the last is LIPI isolate 13-2 -AI015 of 0.08 g/125 mL (table 1). The difference in biomass weight correlate with the different cell division levels between one isolate and the other isolate.

Table 1. Production of microalgae dry biomass

Isolate	Biomass (g/125 mL)
LIPI 13-2-AI015	0.08 ± 0.01
LIPI 13-2-AI025	0.11 ± 0.00
LIPI 13-2-AI038	0.12 ± 0.02

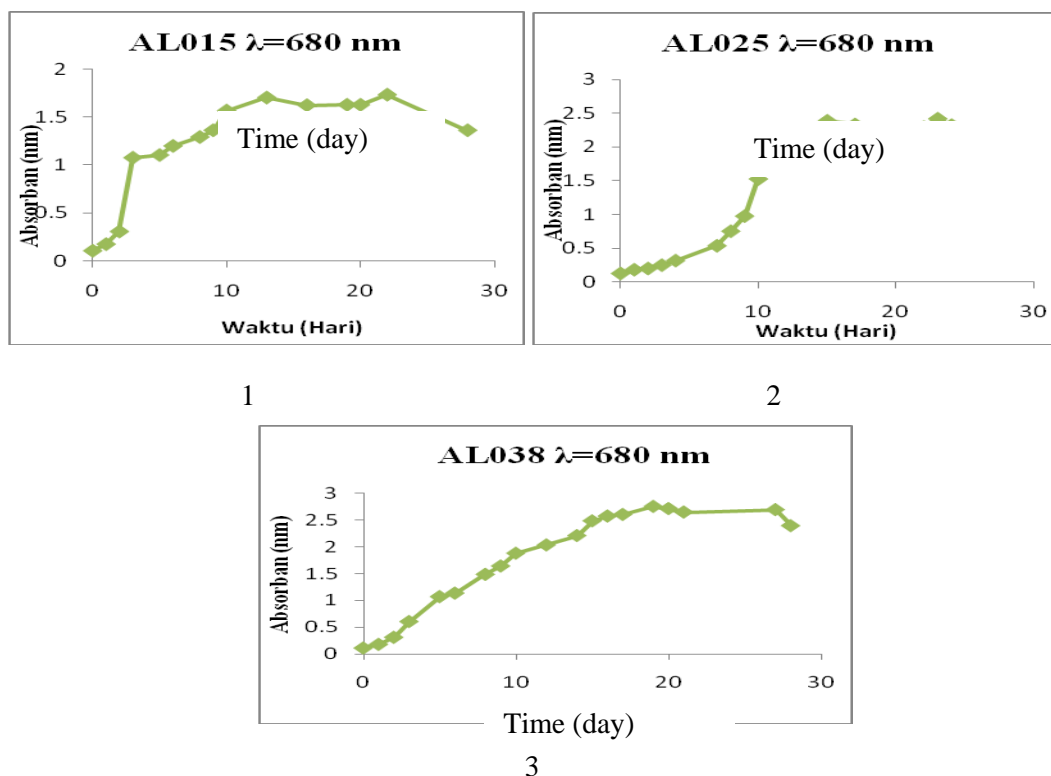


Figure 2. Growth curve of microalgae, (1) LIPI 13-2-AL015, (2) LIPI 13-2-AL025, and (3) LIPI 13-2-AL038

3.3. Crude protein quantification and amino acid composition

Bovine serum albumin (BSA) is generally used as a standard protein because it is available in pure form [7]. In this study using BSA as standard protein.

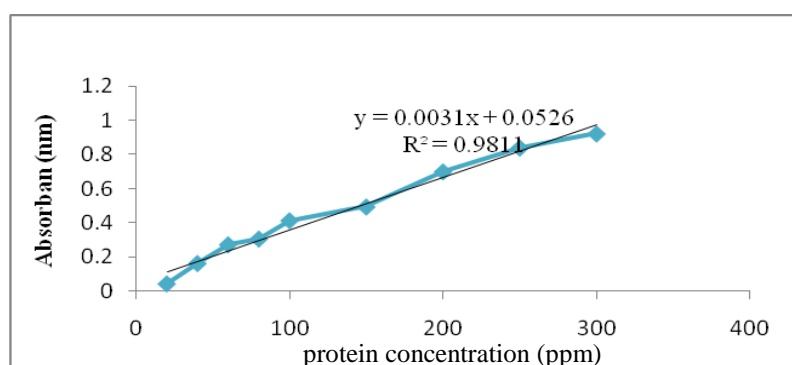


Figure 3. Standard curve using BSA as the standard.

From the figure 3 obtained equation used to measure protein content, $y = 0.0031x + 0.0526$, substitution y , absorbance value of sample, hence will get protein concentration (x). Each extract calculated the concentration of protein. The data show the highest protein concentration of extraction with buffer phosphate solution pH 7.4 from each isolate, followed by extraction with distilled water and the last is 80% acetone (table 2).

Table 2. Protein extraction yield of Aquadest, Phosphate buffer, and Acetone extracts from each isolate

Isolate	Protein concentration Extract (ppm)		
	Aquadest	Phosphate buffer pH 7,4	Acetone 80%
LIPI 13-2-015	280.94 ± 18.48	508.97 ± 26.00	74.97 ± 6.39
LIPI 13-2-025	252.81 ± 9.13	456.04 ± 36.49	188.29 ± 4.57
LIPI 13-2-038	260.94 ± 13.00	285.45 ± 4.79	66.58 ± 4.10

Proteins with varying polarization of the -R group of amino acid residues may be extracted by polar, or non-polar solvents. The results showed that phosphate buffer solvent was able to extract the most protein among aquadest, and 80% acetone. When referring to relative polarity, H₂O, 1 and acetone 0.3 [8]. The phosphate buffer solvent pH 7.4 is weak ionic. In other words, protein extraction from microalgae can be carried out optimally by solvents with the properties between polar and ionic.

Amino acid analysis was performed on the sample obtained from phosphate buffer extraction pH 7.4. This analysis is conducted by PT. Saraswanti Indo Genetech, Bogor.

The amino acid composition of microalgae protein extracted was characterized (table 3). Based on 15 parameters of amino acid investigated there are 7 of 9 essential amino acids in microalgae protein extract, LIPI A1038, namely: phenylalanine, valine, threonine, leucine, isoleucine, lysine, and histidine.

Table 3. Amino acid composition of microalgae protein extract

Parameter	Limit of Detection (ppm)	Result		
		A1015	A1025	A1038
L-Histidin	40.86	-	-	202.55
L-Threonin	71.88	-	-	< 239.36
L-Prolin	41.66	< 138.73	-	< 138.73
L-Tirosin	66.93	-	-	< 222.88
L-Leusin	67.81	-	-	< 225.81
L-Asam	119.93	-	-	< 399.37
Aspartat				
L-Lisin HCl	46.95	< 156.34	-	< 156.34
Glisin	120.72	-	-	< 402.00
L-Arginin	45.99	-	-	-
L-Alanin	101.7	< 338.66	< 338,66	< 338.66
L-Valin	46.74	-	-	< 155.64
L-Isoleusin	37.82	-	-	< 125.94
L-Fenilalanin	35.22	-	-	< 117.28
L-Asam	118.35	< 394.11	-	< 394.11
glutamate				
L-Serin	249.3	-	-	< 830.17

Note: - , not detected

4. Conclusion

Microalgae have attracted the attention of the food industry mainly because of the need for valuable, sustainable, unconventional, non-climate-dependent. In this study, it was shown that several of solvent were important in microalgae protein extraction. Solvent polarity factor is very influential in obtaining protein microalgae, especially on: LIPI 13-2-AL015, LIPI 13-2-AL025, LIPI 13-2-AL038. The phosphate buffer solvent extracts most of the microalgae proteins between water, and acetone 80%. Based on 15 parameters of amino acid investigated there are 7 of 9 essential amino acids in microalgae

protein extract, LIPI AL038, namely: phenylalanine, valine, threonine, leucine, isoleucine, lysine, and histidine.

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