Biomass Nutrient Profiles of Marine Microalgae *Dunaliella salina*

Moh. Muhaemin[1)] dan Richardus F. Kaswadji[2)]
[1)]Aquaculture Department, University of Lampung, Lampung, Indonesia
[2)]Bogor Agriculture Institute, Bogor, Indonesia

Abstract: The unconventional micro algal sources for the production of feed, food, food additive, pharmaceutical, and fine chemical is growing in important. Researches in the field are expanding worldwide. Nutritional composition of marine micro algae *Dunaliella salina* data included proximate composition, nitrate, RNA, and pigments were analyzed under various conditions in semi cultured medium. On average, nutritional composition of biomass was highly influenced by external irradiance and residence time in bioreactor. The biomass collected for short residence times was richer in protein.

Keywords: biomass nutrient profiles, *dunaliella salina*

E-mail: m_muhaemin@unila.ac.id

September 2010

1 INTRODUCTION

Micro algae have been developed for feeding for fish and human consumption[1]. Micro algal biomass is used for food supplement in order to improve nutrient profile, and rich of some nutrient such as fatty acids (ω3 and ω6), and essential amino acid [1,2]. In addition, *Dunaliella* can accumulate very large amounts of -carotene. Those made the physiology and a biochemistry aspect of specific microalgae especially for biotechnological potential of *Dunaliella* as a potential source of nutrient was investigated already relatively.

Dunaliella salina is a type of halophile pink micro algae. *Dunaliella* cells lack a rigid cell wall, and the cell is enclosed solely by a thin elastic plasma membrane. As a result, the cells' morphology is strongly influenced by osmotic changes. *Dunaliella salina* is a kind of the unicellular green alga which is responsible for most of the primary production in hyper saline environments worldwide. In average, marine microalgae accumulate 6-18% of lipids, 50-60% of crude protein [3], and carbohydrates vary between 40-50%[4,1].

The research reports on the nutrient composition of the microalgae biomass of *Dunaliella salina*. The data provided on proximate composition, RNA, and pigments. Biomass was produced in semi outdoor culture at different conditions of solar irradiances. The research aim was to identify variations in nutrient profile of *Dunaliella salina* biomass.

2 MATERIALS AND METHODS

D. salina collected from Balai Besar Pengembangan Budidaya Laut (BBPBL, Mari-culture Development Station) Hanura pure isolate. *D. salina* grown in the photo bioreactor consisted of a airlift pump that dove the culture fluid through a horizontal tubular solar receiver. The total culture volume is 0.5 m^3. Air was continuously supplied at a flow rate 0.02 $mol.s^{-1}$. Biomass was collected directly from the photo bioreactor in a Pyrex glass container, and centrifuged at 4000 rpm for 4 min. the cultures obtained at different reactor residence times and external irradiance were analyzed. The growth medium was natural seawater enriched with f/2 at one sixth the normal trace metal concentration without Cu. The medium sterilized by filtration on 0.2 μm sterile membrane filters. The algae used were unicellular marine green algae *D. salina*. The research was conducted on October-December 2009.

Total N was determined in a semi-micro Kjeldahl apparatus. Total protein was calculated from the evaluated nitrogen, multiplying by 6.25, after allowing for N from nucleic acids and nitrate[4]. Lipids were determines as the extract obtained with chloroform: methanol (2:1) (v/v)[5]. Carbohydrates were estimated by the spectrophotometric method[6]. Total ash was determined by incineration of a representative 1.0 g sample in an oven at 450°C for 48h. Dietary fiber was determined by the natural detergent fiber method (Goering and Van Soest, 1970)[1]. RNA extraction was accomplished by the method of Shibko et al. (1967)[1]. Ribose was determined in

the supernatants by the orcinol spectrophotometric method (Ogur and Olsen, 1950) [1]. Total carotenoids were evaluated spectrophotometrically (Whyte, 1987) [1]. Chlorophylls were determined spectrophotometrically (Whyte, Jones and Gibbs, 1963) [1].

3 RESULTS AND DISCUSSION

For statistically analysis, in order to provide better global comprehension of the influence of the work variables on the value obtained for analysis nutrients, the data for residence time and external irradiance were grouped using codes, Table 1 and 2, respectively.

Table 1: Residence time groups

Codes	Days
1	1.0 - 1.25
2	1.5 - 1.75
3	2.0 - 2.25
4	2.5 - 2.75

Table 2: Irradiance groups

Codes	Eω (μE.m^{-2}.days^{-1})
A	$(05.25 - 10.21) \times 10^9$
B	$(10.21 - 15.32) \times 10^9$
C	$(15.32 - 20.45) \times 10^9$
D	$(20.45 - 25.53) \times 10^9$

As results of biomass analysis are expressed on a 100 g dry weight basis. Data referring to proximate composition, nitrate and RNA are providing in Table 4. In comparison with Table 3, available carbohydrates were lower. It probably occurred because the biomass was washed to eliminate the extra-cellular polysaccharide. The extra-cellular (capsular) polysaccharide functions are to protect cells from desiccation and enable the algae to grow in a marine environment [7,8]. Fibre amounts were very low and could be due to the fact that the exo-polysaccharide may replace the cell wall function. Low value of fibre may indicate that the alga was easily digestible biomass for human use. Protein content was high. Lipid contents were lower, possibly because the culture s was harvested with a short residence time. RNA amount was moderate. Nitrate values were also moderate. Ash contents were high.

Carotenoids are minor pigment in *D. salina*. Chlorophylls were analyzed because phaeophorbides can cause possible poisoning, cause inflammation in sensitive skins on exposure to sunlight. Phaeophorbides are lower than the recommended upper limit

Table 3: Approximate nutrient profiles of *D. salina* in g/100g dry weight biomass [3]

No	Variables	Value
1	Carbohydrates	40.21
2	Lipid	18.02
3	Fibre	2.10
4	Crude Protein	25.67
5	Nitrate	15.34
6	Ash	15.89
7	RNA	1.85
8	Pigments (Carotenoids)	42

(120 mg/ 100 g). Pigments contents were shown in Tabel 5.

PCA may describe the correlation among variables clearly in Figure 1. Figure 1 showed two different axis which correlated with their variance. The correlations among variables were high and generally significant, indicated that the variation could be due to a few related causes. In order to establish relationships between variables, a multi variables data analysis was performed for data obtained at various states. One of the powerful and useful methods is Principal Component Analysis (PCA), which may reduce the number of variables to a limited number of principal components [9]. PCA was initially applied to selected variables after the Pearson correlation analysis.

A plot for two first component weight (Figure 1) showed that there are grouping of variables. Residence time has a great influence on component 1; and may positively correlate with other variables such as Lipid, Fibre, and Chlorophyll-b. These variables with others form a group which increases jointly, for large residence time values. This explained by the fact that for high residence times, the energy is accumulated in reverse lipid form.

Ash is found in opposite form, which implies that a low residence time induces high salt accumulation in the cellular interior. It may occur in order to offset the high osmotic pressure from the culture medium. There are two variables (Chlorophyll-a, and Carotenoid) grouping with the ash variable in which located oppositely to the previous mentioned. Those opposed also to the three variables (available carbohydrates, C/N ratio, and biomass concentration). The phenomena indicated that low residence times (Eω) imply younger cells with increasing protein needs for the cell growth and cell reproduction [1].

External irradiance (Eω) was placed opposite to variables such as Chlorophyll, Lipid, and Biomass. It indicated that low irradiance may induce the cells to increase chlorophyll contents. Furthermore, low irradiance induces a longer cellular cycle and a greater lipid

Table 4: Proximate composition, RNA, and nitrate (g/100g dry weight biomass, mean±SD)

Steady	Variables						
State	AC	Lip	CP	Ash	Fib	RNA	Nit
1A	$34.08 \pm 0,23$	6.54 ± 0.32	42.79 ± 2.01	23.60 ± 2.0	0.34 ± 0.01	2.03 ± 0.15	0.15 ± 0.02
4A	37.19 ± 0.19	5.92 ± 0.39	40.69 ± 2.56	22.40 ± 2.0	0.24 ± 0.03	2.11 ± 0.21	0.16 ± 0.01
2Ba	26.90 ± 0.32	6.98 ± 0.12	41.67 ± 1.98	23.40 ± 1.8	0.42 ± 0.01	1.62 ± 0.15	0.16 ± 0.01
2Bb	25.70 ± 0.11	6.87 ± 0.15	38.99 ± 2.03	23.00 ± 1.4	0.46 ± 0.03	1.76 ± 0.21	0.13 ± 0.02
4B	24.22 ± 0.31	5.96 ± 0.21	39.09 ± 1.99	22.30 ± 1.5	0.54 ± 0.02	1.67 ± 0.18	0.15 ± 0.03
1C	32.09 ± 0.45	7.01 ± 0.24	39.90 ± 2.05	21.09 ± 2.1	0.39 ± 0.02	1.83 ± 0.15	1.14 ± 0.02
2C	37.29 ± 0.50	7.21 ± 0.32	42.29 ± 1.45	19.67 ± 1.3	0.43 ± 0.04	1.72 ± 0.14	0.12 ± 0.01
3Ca	35.97 ± 0.10	6.83 ± 0.20	43.35 ± 2.31	18.20 ± 1.5	0.33 ± 0.03	1.64 ± 0.15	0.09 ± 0.03
3Cb	36.18 ± 0.17	7.21 ± 0.41	42.78 ± 2.42	19.90 ± 1.4	0.26 ± 0.02	2.22 ± 0.19	0.22 ± 0.01
4C	35.66 ± 0.17	6.89 ± 0.11	30.98 ± 2.35	18.55 ± 1.3	0.56 ± 0.05	1.85 ± 0.11	0.19 ± 0.01
1Da	32.89 ± 0.12	7.25 ± 0.20	30.78 ± 2.30	16.92 ± 2.0	0.52 ± 0.04	1.87 ± 0.12	0.09 ± 0.01
1Db	31.76 ± 0.09	7.21 ± 0.30	29.93 ± 2.54	17.90 ± 1.9	0.62 ± 0.04	1.75 ± 0.14	0.09 ± 0.01
1E	35.77 ± 0.25	7.65 ± 0.12	30.44 ± 1.89	17.56 ± 1.8	0.66 ± 0.05	1.72 ± 0.21	0.12 ± 0.01

* AC, Available Carbohydrate; Lip, Lipid; CP, Crude Protein; Fib, Fiber; Nit, Nitrate

* State coincident for residence time and external irradiance code are distinguished by means of serial small letter

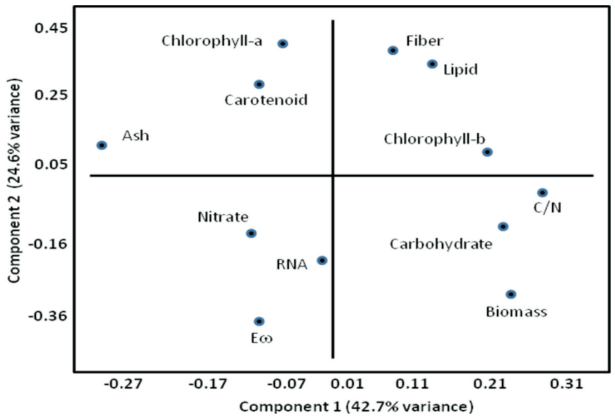

Figure 1: PCA profiles between variables

accumulation. In spite of that, external irradiance is placed next to RNA and nitrate variables, which confirms that high irradiance induces protein biosynthesis. RNA amount, as a function of nitrate concentration, have been cited elsewhere[10]. Cohen, Norman and Heimer[11] showed that when growth is slowed by any limiting factor, lipid and carbohydrate synthesis may be enhanced at the expense of protein synthesis.

Variables C/N, available carbohydrate and biomass concentration were located in the same area. It suggested that the cell concentration increase with the present of available carbohydrate as the main component. C/N ratio depends mainly on the available carbohydrate contents and biomass concentration of the micro algae.

4 CONCLUSION

D. salina nutrient profiles might vary due to research conditions and moderately have different results compared with Borowitzka and Borowitzka[3]. The nutrient profiles of biomass were highly influenced by external irradiance and residence time. Multivariable data analysis was suitable approach to find underlying structures in complicated biological systems. A PCA profiles showed that there were grouping in variables, thus residence time has a great influence.

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Table 5: Pigment contents in *D. salina* (g/100g dry weight biomass, mean±SD)

Steady	**Pigments**		
State	**Carotenoids**	**Chlorophyll**	
		a	**b**
1A	104 ± 5	165 ± 10	33 ± 4
4A	109 ± 7	192 ± 12	15 ± 4
2Ba	112 ± 10	325 ± 24	5 ± 1
2Bb	104 ± 8	228 ± 20	35 ± 5
4B	111 ± 9	230 ± 12	32 ± 4
1C	116 ± 6	269 ± 15	55 ± 5
2C	128 ± 7	455 ± 22	12 ± 2
3Ca	88 ± 6	320 ± 25	75 ± 5
3Cb	62 ± 4	112 ± 10	35 ± 7
4C	102 ± 5	67 ± 11	15 ± 3
1Da	98 ± 5	260 ± 12	135 ± 9
1Db	109 ± 6	212 ± 15	59 ± 4
1E	115 ± 4	330 ± 15	91 ± 8

* State coincident for residence time and external irradiance code are distinguished by means of serial small letter

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