PROXIMATE AND BIOCHEMICAL ANALYSIS FOR MARINE AND FRESHWATER ALGAE

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ABSTRACT

Microalgae are well known as a great source of high level of lipids and starch accumulation. In this study, ten indigenous microalgae samples from freshwater and marine waters from Malaysia, cultured and analysed on proximate and biochemical analysis. The proximate and biochemical analysis consists of starch, carbohydrates, lipid, protein, ash and moisture contents. This study was more focused on screening of starch accumulation in marine and freshwater microalgae cultures. Based on screening, the results showed that Chlorella salina contents highest starch of 4.92±0.33%, followed by Spirulina sp. 2.58±1.18%, Isochrysis maritime 0.99±0.33%, and lastly for Nitzschia panduriformis and Navicula distans contents similar percentage of starch (0.44±0.10 and 0.40±0.07%, respectively). Besides starch analysis, proximate analysis have (ash, moisture, lipid, protein, and carbohydrates) have been conducted. The results obtained indicated that all the cultures contain more than 4.50% of carbohydrates in average, followed by lipid and protein <1%. The results demonstrate that further optimization and various harvesting stages (early of exponential phase, early of stationary phase and late stationary phase) may increase lipid, carbohydrates, starch, and protein accumulation. Based on the resulots obtained, Chlorella salina and Spirulina sp. have been selected further study on optimization of physical and chemical factors for high starch accumulation. In conclusion, this experiment focused more on preliminary screening for further application of starch uses in food and food packaging industries.

Keywords: Biochemical analysis, marine and freshwater microalgae, proximate analysis.

INTRODUCTION

The rising demand for natural products to be used in various applications has increased interest in algal biotechnology over the past two decades [1, 2]. Through algae biotechnology, various high-value compounds could be produced and isolated from numerous phototrophic and heterotrophic microalgae cultures [3]. Microalgae efficiently convert CO_2 to potential biofuels, feeds and high-value by products using a small foot print [2]. Microalgae can grow on non-arable land and use non-potable water without displacing food crops. This growth is considered environmental friendly as microalgal biofuels can take advantage of nutrients in wastewater and CO_2 from power plants; while crop plants cannot use these resources [2]. Other factors, which should be considered simultaneously for sustainable biofuel production include but are not limited to: lipid and high added value chemicals production (e.g. for pharmaceutical or cosmetic industry), extraction economics (solvents, ultrasound application, electromagnetic field use, etc.), incineration/pyrolysis/gasification of residual biomass, its anaerobic digestion for biogas production, etc. Microalgae are also excellent candidates for starch accumulation and production (feedstock for bioplastics production). These factors have been reviewed recently in Chisti [2], Schenk et al. [4] and Sostaric et al.[5].

Pulz and Gross [6] reported that successful algal biotechnology mainly depends on choosing the right alga with relevant properties for specific culture conditions and products. Large scale production of microalgal biomass therefore requires species, which also can tolerant of a wide range of conditions. Thus, microalgae can be harvested within a short span of time as compared to plants and crops and hence can meet the increasing demand of feedstock [7]. Cultivation and environmental conditions are very important factors in microalgae growth and intracellular substance accumulation [8, 9]. The aim of this study is to screen the proximate and biochemical analysis in marine and freshwater microalgae.

METHODS

Culture collection

Five marine and 5 freshwater microalgae cultures were obtained from Microalgal Culture Laboratory of School of Biological Sciences and School of Industrial Technology, Universiti Sains Malaysia. For freshwater microalgae cultures there are *Spirulina* sp., *Ankistrodesmus* sp., *Microcystis* sp., *Chlorococcum* sp., and *Chlorella vulgaris*. While for marine microalgae cultures there are *Chlorella salina*, *Tetraselmis* sp., *Isochrysis maritima*, *Nitzschia panduriformis* and *Navicula distans*.

Screening and growth profile of microalgae

The strains were sampled every day for determination of cell growth (biomass concentration) by spectrophotometer at wavelength 680 nm for 20 days. After 20 days of cultivation, algal biomass concentration was determined as total dry weight. The entire marine and freshwater microalgae cultures been harvested at the late exponential phase for further analysis.

Analysis

Moist and Ash determination

Moist and ash were determined using the method described in National Renewable Energy Laboratory [10].

Protein determination

The small-scale method developed for protein extraction of microalgae dry-weight (DW) was based on that used by Price [11] with extensive modifications. Protein quantification followed the method of Lowry et al. [12] as modified by Price [13].

Lipid determination

Lipid contains determined by using method by Bligh and Dyer [14].

Starch determination

Megazymes total starch analysis kit been used for starch determination. Amyloglucosidase/α-Amylase method (AOAC Official Method 996.11) [15] followed for the analysis.

Carbohydrates determination

For carbohydrates determination, Laurentin and Edwards [16] method was used.

Statistical Analysis

Statistical analyses were carried out using the Statistical Package for the Social Science (SPSS) for analyses of variance (ANOVA). Significance was defined at $P<0.05$. Three replications were performed for all experiments and analyses.

RESULTS AND DISCUSSION

Growth profile of microalgae

Growth profile been studied for all ten strains. The growth curve for all ten analysed strains were were shown in Figure 1a and Figure 1b. Almost all freshwater microalgae cultures reached at the late exponential on 9th day except for *Spirulina* sp. on 10th day. On the other hand, for marine microalgae cultures, *Chlorella salina*, *Isochrysis maritima* and *Tetraselmis* sp reached the late exponential phase on 7th day and followed by both diatoms cultures (*Nitzschia panduriformis* and *Navicula distans*) reaches late exponential on 9th day. Even though, the growth rate for marine microalgae is much faster than freshwater microalgae, but the biomass is still lower than freshwater cultures (high density growth). This can be seen when *Chlorella vulgaris* and *Microcystis* sp. possess similar highest dried cell weight of 0.23g/L followed by *Spirulina* sp. of 0.20g/L (Table 1). The dried cell weight obtained by the marine microalgae were below 0.12 g/L with the lowest was shown by *Isochrysis maritima* (0.06g/L). Overall lower biomass detected in this present study may due to the cultivation conditions at current stage (Screening) are not suitable. The growth characteristics and composition of microalgae are known to significantly depend on the cultivation conditions. There are four major types of cultivation conditions for microalgae:photoautotrophic, heterotrophic, mixotrophic and photoheterotrophic cultivation [17]. However, the fastest biomass growth can be enhanced at the optimum conditions.

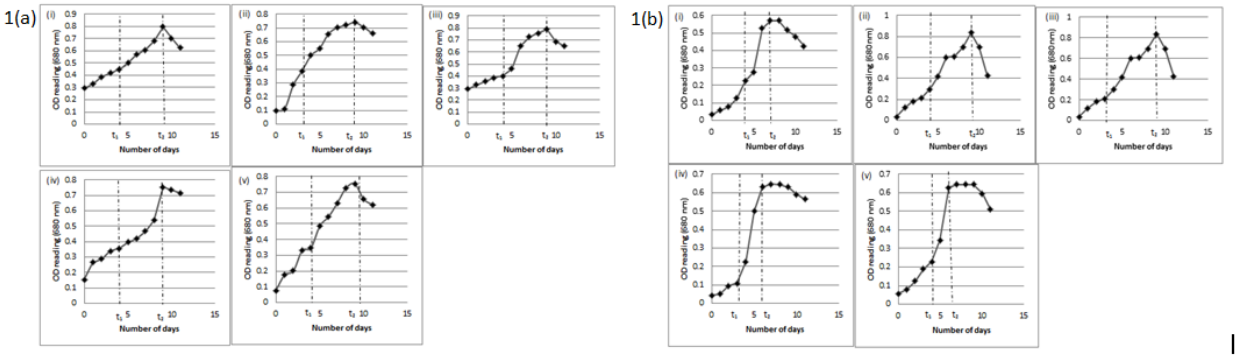

Figure 1. a Growth profile of five species of freshwater microalgae. (i) *Microcystis* sp. (ii*) Spirulina* sp. (iii) *Chlorella vulgaris* (iv) *Chlorococcum* sp. (v) *Ankistrodesmus* sp. **b.** Growth curves of five species of marine microalgae. (i) *Isochrysis maritima* (ii) *Navicula distans* (iii) *Nitzschia panduriformis* (iv) *Tetraselmis* sp. (v) *Chlorella salina*. . t_1 indicates at the early of exponential phase follow by t_2 is late exponential phase. Data was reported as means of triplicates.

Table 1. Summary of moist, ash and dry weight analysis.

Cultures	Microalgae cultures (F:Freshwater, M:Marine)	% Ash	Dry weight (g/L)
Spirulina sp.	F	30.82±1.71	0.20
Ankistrodesmus sp.	F	61.45±29.36	0.17
Microcystis sp.	F	85.33±0.35	0.23
Chlorella vulgaris	F	57.48±3.15	0.23
Chlorococcum sp.	F	42.30±9.87	0.14

Chlorella salina	M	41.03±3.45	0.08
Tetraselmis sp.	M	29.92±3.83	0.07
Isochrysis maritima	M	5.80±1.59	0.06
Nitzschia panduriformis	M	27.25±0.62	0.07
Navicula distans	M	25.31±0.85	0.11

*Data reported as means ± standard deviation of triplicates.

Moisture and ash determination

As shown in Table 1, freshwater green microalgae contain much higher ash than marine green, diatoms and brown microalgae. Almost all the microalgae cultures rich in ash contents (except for *Isochrysis maritima*), ranging from 5.80% to 85.33%. *Microcystis* sp. contained highest percentage of ash contents, and highest biomass. The higher contains of ash in freshwater green microalgae results in its high biomass. In recent years, many researchers have worked on discovering the characteristics of various microalgae. Most of their studies used green microalgae, and the results showed that about 20% DW of the microalgae biomass was left after slow pyrolysis and became char [18]. Oxidation of the incorporated minerals in microalgae into mineral oxides in the open-to-air furnace is the most likely contributor to this significant difference.

Protein determination

Testing for protein is necessary for understanding microalgae from another point of view. The results obtained indicated that all the cultures have <1% protein content (Figure 2). Nevertheless, statistical analysis indicated that the protein content obtained by *Chlorella vulgaris* (0.92%) was significantly higher (P<0.05) compared with all the tested microalgae strains. On the other hand, *Microcystis* sp. and *Chlorococcum* sp. showed no protein at all. This may due to the protein content was too low to be detected by the assay method. On top of that, it might due to; several substances may interfere with both the Lowry and Bradford method.

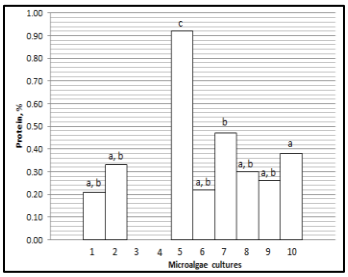

Figure 2. Protein contents (Percentage, %) of marine and freshwater microalgae culture. Data was reported as means of triplicates. Standard deviation has been removed for clearer bar chart. Means with the same letter indicated no significantly difference at 5% level of probability by Duncan Test. *1: *Spirulina* sp., 2: *Ankistrodesmus* sp., 3: *Microcystis* sp., 4: *Chlorococcum* sp., 5: *Chlorella vulgaris*, 6: *Isochrysis maritima*, 7: *Chlorella salina*, 8: *Tetraselmis* sp., 9: *Nitzschia panduriformis*, 10: *Navicula distans*

Lipid determination

Although the lipid content detected by *Chlorella vulgaris* and *Microcystis sp.* are significantly higher (P<0.05) compared with others tested microalgae strains. This is due to fast growing species of algae contain lower amounts of lipids, whereas cells accumulating lipids grow slower (Xiong et al., 2009). In the present study, all the strains contain less than 1% of lipid (Figure 3). This result was expected because most of the biochemical compounds, except for protein, increased when the culture aged, especially carbohydrate and lipid [19]. Furthermore, phytoplankton may physiologically acclimate in response to variation in temperature, changing their biochemical composition or adjusting their membrane lipid to increase their capacity to grow or survive [20]. Besides that, datas presented in the literature show that the lipid content in marine algae are less than 4% of the dry weight depending on the species [21].

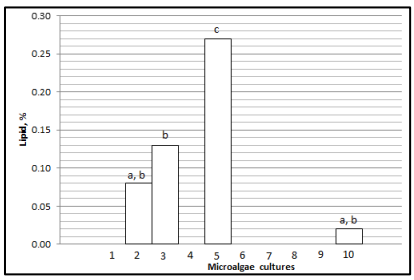

Figure 3. Lipid contents (Percentage, %) of marine and freshwater microalgae culture. Data was reported as means of triplicates. Standard deviation has been removed for clearer bar chart. Means with the same letter indicated no significantly difference at 5% level of probability by Duncan Test. *1: *Spirulina* sp., 2: *Ankistrodesmus* sp., 3: *Microcystis* sp., 4: *Chlorococcum* sp., 5: *Chlorella vulgaris*, 6: *Isochrysis maritima*, 7: *Chlorella salina*, 8: *Tetraselmis* sp., 9: *Nitzschia panduriformis*, 10: *Navicula distans*

Starch determination

Accumulation of starch can be induced by nitrogen depletion [22], sulfur depletion, high light intensity [23] or a high CO_2 concentration [24]. The marine *Chlorophyta* phylum which is *Chlorella salina* shows higher percentage (4.92%) and the productivity is 0.392 g/L of starch among all other tested strains ($P<0.05$), followed by freshwater *Cyanobacteria* microalgae *Spirulina* sp. (2.58%) and the productivity is 0.516 g/L (Figure 4). At this screening stage, none of the optimization tests have been conducted. These two cultures were selected for further studied on starch accumulation at the stress stage. *Chlorella salina* is significantly different from all other cultures as it does not appear in any subset together with any other cultures. Other 9 cultures are not differ significantly as it shown together in same subset. This shows that the mean percentage of starch in these cultures do not differ significantly. These cultures been selected for further optimization based on SPSS analysis data.

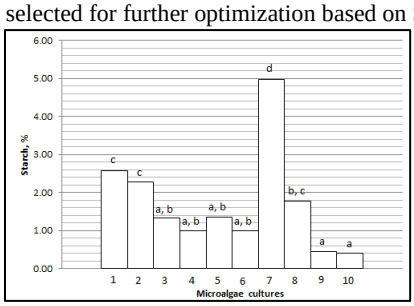

Figure 4. Starch contents (Percentage, %) of marine and freshwater microalgae cultures. Data was reported as means of triplicates. Standard deviation has been removed for clearer bar chart. Means with the same letter indicated no significantly difference at 5% level of probability by Duncan Test. *1: *Spirulina* sp., 2: *Ankistrodesmus* sp., 3: *Microcystis* sp., 4: *Chlorococcum* sp., 5: *Chlorella vulgaris*, 6: *Isochrysis maritima*, 7: *Chlorella salina*, 8: *Tetraselmis* sp., 9: *Nitzschia panduriformis*, 10: *Navicula distans*

Carbohydrates determination

In this experiment, carbohydrates content obtained by *Tetraselmis* sp. are significantly different ($P<0.05$) compared with all the tested microalgae cultures except of *Chlorella vulgaris* (Figure 5). It is interesting to be found that, the relationship between growth rate and high carbohydrates correlated. Based on growth profilling, *Tetraselmis* sp. shows fastest growth rate, and in terms of carbohydrates accumulation carries highest percentage of carbohydrates compare to other nine other cultures. In general, the carbohydrate content detected by freshwater and marine microalgae are not varied differently. *Tetraselmis* sp. (marine water culture) contains the highest carbohydrates (9.58%) followed by *Chlorella vulgaris* (8.20%) and the least is shown by *Microcystis* sp. (freshwater culture) with carbohydrate content of 2.27%. The values vary due to environmental conditions and usage of different media composition (location of isolates varies). Previous research shows that, accumulation of carbohydrates will be higher in microalgae is through depletion of nitrogen and phosphorus in the growth medium [22, 23].

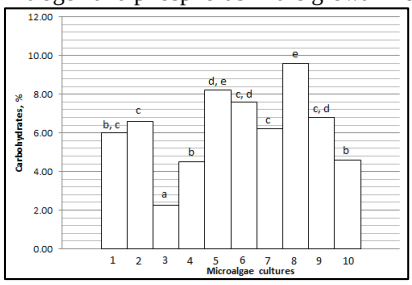

Figure 5. Carbohydrates contents (Percentage, %) of marine and freshwater microalgae cultures. Data was reported as means of triplicates. Standard deviation has been removed for clearer bar chart. Means with the same letter indicated no significantly difference at 5% level of probability by Duncan Test. *1: *Spirulina* sp., 2: *Ankistrodesmus* sp., 3: *Microcystis* sp., 4: *Chlorococcum* sp., 5: *Chlorella vulgaris*, 6: *Isochrysis maritima*, 7: *Chlorella salina*, 8: *Tetraselmis* sp., 9: *Nitzschia panduriformis*, 10: *Navicula distans*

CONCLUSIONS

The overall aims of this research work are to screen, determined and optimized the cultivation conditions of microalgae for high starch production and accumulation. Our data presented might low compate to literatures, however for ash, protein,

carbohydrates, lipid and starch contents may also be dependent of seasonal period, geographical location and environmental conditions. In present stage of study, *Spirulina platensis* and *Chlorella salina* been chose for further study in order to enhance starch production. Optimization will be done on physical factors and chemical factors (Nutrient availability). So that high starch content will be obtained and consequently the overall cost for starch production will be reduced. With that it can directly lower the cost for bioplastics production.

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