

## DIFFERENTIATION OF BIOMASS COMPOSITION BETWEEN ISOLATED AND COMMERCIAL STRAINS OF MICROALGAE

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### Abstract

Biomass produced from the cultivation of microalgae has many potential in producing high value product. Main objective of this research is to investigate the potential of biomass produced by algae from isolated strains and commercialize strains as well as to compare their potential in producing high quality of biomass. Isolated microalgaestrains used were *C. sorokiana* (UKM 3) and *Characium* sp. (UKM 1) extracted from Palm Oil Mill Effluent (POME) and commercial strains used were *C. vulgaris* and *Ankistrodesmus* sp.. Results shows that the commercial strain *Ankistrodesmus* sp has a highest biomass's weight which is 3.0 g/L, followed by another type of commercial strain, *C. vulgaris* which is at 2.72g/L and the other two types of isolated strains, *C.sorokiniana* and *Characium* sp. both at 2.52g/L and 2.27g/L respectively. For the biomass composition, the highest concentration of protein was found in locally isolated strain, *Characium* sp. with concentration of 0.52g/L, while for carbohydrates and lipid were found highest in commercial strain, *C. vulgaris* with concentration at 0.27g/L and 2.07g/L respectively.

Keywords: Isolated strain, Commercial strain, Biomass composition.

### 1. Introduction

The increasing population of worldwide had become a greatest concern for mankind. It leads to an increase in the demand of food and energy [1]. The production of biofuels and feedstock from microalgae represent one appealing alternative to overcome these challenges [2]. The uses of microalgae in producing high quality of biomass are getting much attention recently.

Many researcher concentrated their focus on the ability of microalgae to absorb CO<sub>2</sub> where from this cultivation method, not only it will helps reducing the amount of CO<sub>2</sub> in air but it also helps replacing our nature sources. Microalgae has ability to transform CO<sub>2</sub> gases from the air by using light energy through photosynthesis process to form various forms of chemical energies such as polysaccharides (types of carbohydrates), proteins, lipid and hydrocarbons [3]. The quality of microalgal biomass can be increased by considering the growth factors of microalgae such as CO<sub>2</sub>[4-8], macro and micro nutrients which can be obtain from medium[6-14] light intensity [6,8-11,15-18], aeration rates [4,14,19], pH and temperature [19] and the type of photobioreactor [20].

There are numerous commercial application of microalgae that has been studied and proved, for example it can be utilized to enhance the nutritional value of food and animal feed, plays a significant role in aquaculture, and can be incorporated into cosmetics [21-23] and medicines [24]. Its cultivation and harvesting for high value bio-products seems to be profitable for producers due to high quality and cost of the final products [25]. A large number of nutritional and toxicological evaluation demonstrated the viability of algal biomass as a valuable feed supplement or substitute for conventional protein sources such as soybean meal, fish meal, rice bran and etc. [21].

One of the many advantages of the microalgal biomass are the ability to represent a renewable and environmental-friendly feedstock [26]. Among the numerous potential sources of renewable energy, biofuels seems to be the most attractive bio-product and are anticipated to play a crucial role in the global energy infrastructure in the future [27].

The average lipid content from the cultivation from microalgae varies between 1-70% but can reach up to 90% of the dry weight under certain conditions [3]. Microalgal lipids are composed of glycerol, sugars or bases esterified to saturated or unsaturated fatty acids (12 to 22 carbon atoms). Microalgae have the potential in biofuel industry because of its capability to absorb CO<sub>2</sub>. This makes the content of C is high in microalgae where at least 80% w/w of oil is carbon [28]. The biofuels and biogas that produce from microalgal biomass doesn't content any sulphur that will lead to health hazard [29]. Microalgal lipids are the oils of future for sustainable biodiesel production [3] and it seems to be the only renewable biofuels that has the potential to completely replace the petroleum-derived transport fuels [3].

Although lipids are considered to be the most valuable component of microalgal biomass, other biomass components such as proteins and carbohydrates also make up a large fraction of the biomass [30]. In the early 1950's, the increase of world's population and predictions of an insufficient protein supply throughout the world has led to a search for a new alternative for protein sources [22]. Algal biomass appeared at that time as a good candidate for this purpose [31].

The high protein content of various types of microalgae species is one of the reasons to consider them as an unconventional source of protein [32]. Comprehensive analyses and nutritional studies have demonstrated that these microalgal proteins contain high quality and identical to conventional vegetable proteins [21]. By considering the available information on possible toxic properties or any other adverse effects of the different microalgae tested so far, it

can be stated that none of these microalgae showed any negative effect [21]. Many metabolic studies have been confirmed the capacity of microalgae as a novel source of protein and its quality is equal or even superior to that of other conventional high quality plant proteins [31].

Meanwhile, carbohydrates in microalgae can be found in the form of starch, glucose, sugars, and other polysaccharides [22]. The structure are complex and consist of a mixture of neutral sugars, amino sugars and uronic acids and these composition vary across species and growth condition [33]. The accumulation of carbohydrates from microalgal biomass also can be serve as a raw materials for bioethanol production [34].

The application of microalgae is very wide and yet to be studied thoroughly. In this study, our main focus is to determine the composition (lipid, protein, and carbohydrates) and comparing the quality of microalgal biomass between isolated and commercial strains.

## 2. Materials and Method

### 2.1. Seed culture

Two locally isolated strains, *C. sorokiniana* (UKM 3) and *Characium* sp. (UKM 1) were isolated from Palm Oil Mill Effluent (POME) while the other two commercial strains of microalgae used were *C. vulgaris*, obtained from the Algae Technology Malaysia and *Ankistrodesmus* sp. obtained from the Culture Collection of Sag German.

### 2.2. Experimental design

Microalgae were cultured in 1L Germany Conical Flask. The cultures were disbursed with CO<sub>2</sub> gas from air (0.04%) by using an aquarium pump through sterilized silicon tube with internal diameter 3 mm. The conical flasks were covered using silicon tube stoppers. Flow rate used was 1L/min per flask and was controlled by installing a flow meter (Cole Parmer, USA) on each flask.

Light was continuously supplied using a white fluorescent lamp at intensity of 400 $\mu\text{molm}^{-2}\text{s}^{-2}$ . The intensity was measured using lux meter (TES Digital Lux Meter, China) and was control by having an enclosed system that cover the whole flask where the light was installed inside the enclosed system to ensure that the light provided was focusing on the microalgae only. Temperature was controlled at 30°C. Medium used was Bold Basal Medium.

### 2.3. Determination of biomass and its content

The biomass was collected at the 10<sup>th</sup> day. Sample (50 mL) was taken and filtered using filter paper Whatman 1.2  $\mu\text{m}$  with diameter 47mm. The sample was dried overnight in an oven (105°C) (DHG-9146-A, China). Then, it was measured using an analytical balance (XB 220A, Switzerland) and the data was shown in Table 1. Another sample (50 mL) taken to identify the composition of lipids, proteins and carbohydrates. Method used to studied and differentiate the content of biomass are Folch 1957 (lipid), Dubois 1956 (carbohydrates) and Bradford 1976 (protein).

### 3. Results and Discussion

#### 3.1. Determination of biomass's weight

The biomass was collected and analysed. Table 1 shows the specific growth rates of each types of microalgae obtained from Matlab<sup>®</sup> using curve fit tool method. Growth rates equation was developed by [35]. Based on the biomass concentration shown in Table 1, *Ankistrodesmus* sp. shows a very high value of biomass concentration compare to other types of microalgae which is 3g/L. However, *Ankistrodesmus* sp. has a lower specific growth rates compare to *C. vulgaris* where *C. vulgaris* led *Ankistrodesmus* sp. by  $0.04d^{-1}$ . Both commercial strains seemed too superior as both produced large amount of biomass concentrations compared to the isolated strains as in *C.sorokiniana* only accumulated 2.52g/L and *Characium* sp. at 2.27g/L biomass.

**Table 1. Specific growth rates, growth rates equation and biomass concentration among four different types of microalgae. Biomass concentration was obtain from the weight of the biomass in g/L and the growth rates equation was developed using equation developed by [35].**

	Types of microalgae	Max Specific growth rate, $\mu$ ( $d^{-1}$ )	Growth rates equation	Biomass conc. (g/L)
Isolated strain	<i>C. sorokiniana</i> (UKM 3)	0.6128	$f(x) = \frac{0.026 \times e^{0.6128x}}{0.6105 + 0.04e^{0.6128x}}$	2.52
	<i>Characium</i> sp. (UKM 1)	0.5625	$f(x) = \frac{0.023 \times e^{0.5625x}}{0.542 + 0.04e^{0.5625x}}$	2.27
Commercial strain	<i>C. vulgaris</i>	0.753	$f(x) = \frac{0.009 \times e^{0.753x}}{0.738 + 0.012e^{0.753x}}$	2.72
	<i>Ankistrodesmus</i> sp.	0.624	$f(x) = \frac{0.007 \times e^{0.624x}}{0.604 + 0.012e^{0.624x}}$	3.00

#### 3.2. Determination of biomass composition

The analysis on the biomass was done at the 10<sup>th</sup> day where the culture was collected and prepared for analysis. Standard curves for each method were established by linear regression. For lipid standard, cholesterol was used, for protein standard Bovine Albumin Serum (BSA) was used and for carbohydrates standard, D+ glucose was used.

Table 2 shows the composition of lipid, protein and carbohydrates of *C.sorokiniana* (UKM 3), *Characium* sp. (UKM 1), *C. vulgaris* and *Ankistrodesmus* sp.. For protein concentrations, the maximum production shown by *Characium* sp. (UKM 1) with 0.52g/L concentration followed by *C. vulgaris* with 0.47g/L, *C.sorokiniana* 0.39 g/L and the least was *Ankistrodesmus* sp. which is 0.25g/L.

For carbohydrates concentration, *C. vulgaris* and *C.sorokiniana* (UKM 3) values were almost the same while the other two strain shows very little amount of carbohydrates. Both protein and carbohydrates concentrations were closely related to the media component content in BBM and the POME itself such as nitrate, phosphate and free copper ions [24].

For lipid composition, *C.vulgaris* shows a very significant value compared to another strains where its concentration amounted at 2.07g/L while the least concentration was *C.sorokiniana* (UKM 3), 0.38g/L. Lipid results shows that isolated strains have weak potential in producing lipidas proven in the isolated strains. A very significant difference can be seen from commercial strains and isolated strains in terms of lipid concentration. However, the quality and quantity of the biomass produced could be increased by increasing their growth factors such as light intensity, medium concentration, nutrient limitation [3].

**Table 2. Composition of biomass for *C. sorokiniana* (UKM 3), *Characium* sp. (UKM 1), *C. vulgaris*, *Ankistrodesmus* sp. based on lipid, protein and carbohydrates content.**

Type of microalgae	Lipid (g/L)	Percent of dry matter (%)	Protein (g/L)	Percent of dry matter (%)	Carbohydrates (g/L)	Percent of dry matter (%)
<i>C.sorokiniana</i> (UKM 3)	0.38	15.18	0.39	15.6	0.21	8.37
<i>Characium</i> sp. (UKM 1)	1.11	49.12	0.52	23	0.15	6.5
<i>C. vulgaris</i>	2.07	75.88	0.47	17.27	0.27	10
<i>Ankistrodesmus</i> sp.	0.72	24	0.25	8.29	0.08	2.7

Reported by Sakhivel et al. 2011, the percent of dry matter of liquid of *Chlorella* sp. cultivated in 250mL flask was 19.3% and can be increased up to 64% [27] at different culture condition, however for this study, the percent of dry matter of lipid for *C. Vulgaris* was 75.88% while for *C.sorokiniana* (UKM 3) the reported value was 19.2% [3] and 19.3% [27] while this experiment shows 15.18% of dry matter for lipid concentration.

Based on protein concentration, the percent of dry matter of *Chlorella* sp. has been reported by Becker 2007 was around 51- 58%. However, for this study, only 17.27% of protein had been extracted. While for carbohydrates, the percentage of *Chlorella* sp., was reported around 14-22% where as in this study only 10% of the carbohydrates was obtained. Overall, it has been proved by Becker 2007 that *C. vulgaris* has high content of lipid and carbohydrates compared to another strains and there is limited studies on the isolated strains of microalgae as yet.

Comparing the results with another study, it can be seen that the result was slightly different. These mainly due to the surrounding and climate condition of different places that will affect the microalgae growth has microalgae is reported very sensitive with pH changes, temperature, light and nutrient limitation [3].

Increasing the lipid accumulation will not causes the increase of lipid productivity as biomass productivity and lipid accumulation are not necessarily correlated [36]. Lipid accumulation refers to the increased of the concentration

of lipids within the microalgae cells without consideration of the overall biomass production [3]. This study shows that *Ankistrodesmus* sp. has produced higher biomass concentration however, for lipid concentration it is ranked third after *C.sorokiniana* (UKM 3) and *C. vulgaris*. Similar pattern were observed for protein and carbohydrates where it was not *Ankistrodesmus* sp. that has highest biomass composition.

While many microalgae strains naturally can produce high biomass content, it is possible to increase the concentration by optimizing the growth determining factors such as control the light provided, nutrient level [37], CO<sub>2</sub> concentration [5], harvesting procedure [37] and temperature [3]. Further study is on the pipeline on the optimization of growth conditions to obtain the highest composition of microalgal biomass.

#### 4. Conclusions

Overall, commercial strains of microalgae (both *C. vulgaris* and *Ankistrodesmus* sp.) gives a better performance compared to isolated strains in terms of their specific growth rates and biomass concentration. However, for the composition of carbohydrates, lipids and protein, only *C. vulgaris* shows a potential while *Ankistrodesmus* sp. recorded lowest composition of all parameters measured. Commercial strains have better performance mainly due to its strain purities' where isolated strains were extracted from POME which is not yet grown in enriched medium. From the results, the isolated local strains have a potential to growth well in synthetic media and can produce quality biomass.

#### References

1. Do Nascimento, M.; Dublan, M.D.L.A.; Ortiz-Marquez, J.C.F.; and Curatti, L. (2013). High lipid productivity of an *Ankistrodesmus*-*Rhizobium* artificial consortium. *Bioresour. Technol.*, 146, 400–407.
2. Brennan, L.; and Owende, P. (2010). Biofuels from microalgae-A review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and Sustainable Energy Reviews*, 14(2), 557–577.
3. Sakthivel, R.; Elumalai, S.; and Arif, M. (2011). Microalgae lipid research, past, present: a critical review for biodiesel production, in the future. *J. Exp. Sci.*, 2(10), 29–49.
4. Anjos, M.; Fernandes, B.D.; Vicente, A.a.; Teixeira, J.a.; and Dragone, G. (2013). Optimization of CO<sub>2</sub> bio-mitigation by *Chlorella vulgaris*. *Bioresour. Technol.*, 139, 149–154.
5. Chiu, S.Y.; Kao, C.Y.; Tsai, M.T.; Ong, S.C.; Chen, C.H.; and Lin, C.S. (2009). Lipid accumulation and CO<sub>2</sub> utilization of *Nannochloropsis oculata* in response to CO<sub>2</sub> aeration. *Bioresour. Technol.*, 100(2), 833–838.
6. Dragone, G.; Fernandes, B.; Vicente, A.; and Teixeira, J. (2010). Third generation biofuels from microalgae. *Curr. Res. Technol. Educ. Top. Appl. Microbiol. Microb. Biotechnol.*, 1355–1366.
7. Šoštarič, M.; Klinar, D.; Bricelj, M.; Golob, J.; Berovič, M.; and Likozar, B. (2012). Growth, lipid extraction and thermal degradation of the microalga

- Chlorella vulgaris*. *N. Biotechnol.*, 29(3), 325–331.
8. Yusuf, C. (2007). Biodiesel from microalgae. *Biotechnol. Adv.*, 25(3), 294–306.
  9. Blair, M.F.; Kokabian, B.; and Gude, V.G. (2014). Light and growth medium effect on *Chlorella vulgaris* biomass production. *J. Environ. Chem. Eng.*, 2, 665–674.
  10. Cheng, J.; Huang, Y.; Feng, J.; Sun, J.; Zhou, J.; and Cen, K. (2013). Improving CO<sub>2</sub> fixation efficiency by optimizing *Chlorella* PY-ZU1 culture conditions in sequential bioreactors. *Bioresour. Technol.*, 144, 321–327.
  11. Chisti, Y. (2008). Biodiesel from microalgae beats bioethanol. *Trends Biotechnol.*, 26, 126–131.
  12. Ho, S.H.; Chen, C.Y.; and Chang, J.S. (2012). Effect of light intensity and nitrogen starvation on CO<sub>2</sub> fixation and lipid/carbohydrate production of an indigenous microalga *Scenedesmus obliquus* CNW-N. *Bioresour. Technol.*, 113, 244–252.
  13. Jeon, Y.C.; Cho, C.W.; and Yun, Y.S. (2006). Combined effects of light intensity and acetate concentration on the growth of unicellular microalga *Haematococcus pluvialis*. *Enzyme Microb. Technol.*, 39, 490–495.
  14. Jiang, L.; Luo, S.; Fan, X.; Yang, Z.; and Guo, R. (2011). Biomass and lipid production of marine microalgae using municipal wastewater and high concentration of CO<sub>2</sub>. *Appl. Energy*, 88(10), 3336–3341.
  15. de Morais, M.G.; and Costa, J.a.V. (2007). Biofixation of carbon dioxide by *Spirulina* sp. and *Scenedesmus obliquus* cultivated in a three-stage serial tubular photobioreactor. *J. Biotechnol.*, 129, 439–445.
  16. Huang, G.; Chen, F.; Wei, D.; Zhang, X.; and Chen, G. (2010). Biodiesel production by microalgal biotechnology. *Appl. Energy*, 87(1), 38–46.
  17. Liu, Z.-Y.; Wang, G.-C.; and Zhou, B.-C. (2008). Effect of iron on growth and lipid accumulation in *Chlorella vulgaris*. *Bioresour. Technol.*, 99, 4717–4722.
  18. Xiong, W.; Gao, C.; Yan, D.; Wu, C.; and Wu, Q. (2010). Double CO<sub>2</sub> fixation in photosynthesis-fermentation model enhances algal lipid synthesis for biodiesel production. *Bioresour. Technol.*, 101(7), 2287–2293.
  19. Ho, S.H.; Chen, C.Y.; Lee, D.J.; and Chang, J.S. (2011). Perspectives on microalgal CO<sub>2</sub>-emission mitigation systems - A review. *Biotechnol. Adv.*, 29( 2), 189–198.
  20. Cheng, L.; Zhang, L.; Chen, H.; and Gao, C. (2006). Carbon dioxide removal from air by microalgae cultured in a membrane-photobioreactor. *Sep. Purif. Technol.*, 50, 324–329.
  21. Becker, E.W. (2007). Micro-algae as a source of protein. *Biotechnol. Adv.*, 25, 207–210.
  22. Spolaore, P.; Joannis-Cassan, C.; Duran, E.; and Isambert, A. (2006). Commercial applications of microalgae. *J. Biosci. Bioeng.*, 101(2), 87–96.
  23. Zeng, X.; Danquah, M.K.; Chen, X.D.; and Lu, Y. (2011). Microalgae bioengineering: From CO<sub>2</sub> fixation to biofuel production. *Renew. Sustain. Energy Rev.*, 15(6), 3252–3260.
  24. Chia, M.a.; Lombardi, A.T.; and Melão, M.D.G.G. (2013). Growth and biochemical composition of *Chlorella vulgaris* in different growth media.

- Anais da Academia Brasileira de Ciências. Retrieved January 10, 2016, from <http://www.ncbi.nlm.nih.gov/pubmed/24141409>.
25. Ferreira, a.F.; Ortigueira, J.; Alves, L.; Gouveia, L.; Moura, P.; and Silva, C. M. (2013). Energy requirement and CO<sub>2</sub> emissions of bioH<sub>2</sub> production from microalgal biomass. *Biomass and Bioenergy*, 49(1988), 249–259.
  26. Li, Y.; Horsman, M.; Wu, N.; Lan, C.Q.; and Dubois-calero, N. (2008). Articles : Biocatalysts and bioreactor design. *Biotechnol. Prog*, 24(1), 815–820.
  27. Chen, C.Y.; Yeh, K.L.; Aisyah, R.; Lee, D.J.; and Chang, J.S. (2011). Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: A critical review. *Bioresour. Technol.*, 102(1), 71–81.
  28. FAO, “Produced by: Agriculture and consumer protection title: Renewable biological systems for alternative sustainable energy production Retrieved February 1, 2012, from <http://www.fao.org/docrep/w7241e/w7241e0h.htm>.
  29. Johnson, M.B.; and Wen, Z. (2010). Development of an attached microalgal growth system for biofuel production. *Appl. Microbiol. Biotechnol.*, 85, 525–534.
  30. Laurens, L.M.L.; Dempster, T.A.; Jones, H.D.T.; Wolfrum, E.J.; Van Wychen, S.; McAllister, J.S.P.; Rencenberger, M.; Parchert, K.J.; and Gloe, L.M. (2012). Algal biomass constituent analysis: Method uncertainties and investigation of the underlying measuring chemistries. *Anal. Chem.*, 84(4), 1879–1887.
  31. Becker, W. (2003). *Microalgae in human and animal nutrition*, in handbook of microalgal culture: Biotechnology and applied phycology, Blackwell Publishing Ltd, Oxford, UK.
  32. Soletto, D.; Binaghi, L.; Lodi, A.; Carvalho, J.C.M.; and Converti, A. (2005). Batch and fed-batch cultivations of *Spirulina platensis* using ammonium sulphate and urea as nitrogen sources. *Aquaculture*, 243(1–4), 217–224.
  33. Pieper, S.; Unterieser, I.; Mann, F.; and Mischnick, P. (2012). A new arabinomannan from the cell wall of the chlorococcal algae *Chlorella vulgaris*. *Carbohydr. Res.*, 352, 166–176.
  34. John, R.P.; Anisha, G.S.; Nampoothiri, K.M.; and Pandey, A. (2011). Micro and macroalgal biomass: A renewable source for bioethanol. *Bioresour. Technol.*, 102(1), 186–193.
  35. Yang, J.; Rasa, E.; Tantayotai, P.; Scow, K.M.; Yuan, H.; and Hristova, K.R. (2011). Mathematical model of *Chlorella minutissima* UTEX2341 growth and lipid production under photoheterotrophic fermentation conditions. *Bioresour. Technol.*, 102(3), 3077–3082.
  36. Rodolfi, L.; Zittelli, G.C.; Bassi, N.; Padovani, G.; Biondi, N.; Bonini, G.; and Tredici, M.R. (2009). Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnol. Bioeng.*, 102(1), 100–112.
  37. Widjaja, A.; Chien, C.C.; and Ju, Y.H. (2009). Study of increasing lipid production from fresh water microalgae *Chlorella vulgaris*. *J. Taiwan Inst. Chem. Eng.*, 40(1), 13–20.