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Growth Profile and Fatty Acid Accumulation of Four *Chaetoceros* Taxa Isolated from Coastal Water of Pahang, Malaysia

(Profil Tumbesaran dan Pengumpulan Asid Lemak oleh Empat Takson *Chaetoceros* diasingkan dari Pesisiran Pantai Pahang, Malaysia)

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ABSTRACT

This indoor study was aimed to analyze the production of fatty acids with their growth profile from few marine algae under the genus Chaetoceros *isolated from coastal water of Pahang, Malaysia. The algae were established into culture using standard marine media (f/2 media) and the variation of fatty acid for each species was determined using GCMS. Statistical analysis of one-way ANOVA was performed to evaluate the significant and homogeneity data on the growth of each alga and total fatty acid percentage obtained. The results showed that four taxa were successfully cultivated and identified as* Chaetoceros baculites, Chaetoceros anastomosans, Chaetoceros affinis *var.* willei and Chaetoceros affinis *var.* affinis. *Out of four* Chaetoceros, C. baculites *showed the highest growth rate (0.75 cell.day^{-1}) and division's value (1.08) while* C. anastomosans *showed the highest doubling time value (8.66). Statistical analysis showed that all species have significantly different growth rate (*p<*0.05). Myristic acid was the main component for fatty acid storage for* C. baculites, C. anastomosans *and* C. affinis *var*. willei *whereas palmitic acid for* C. affinis *var.* affinis. *All species contained about 35 to 75% of total percentage fatty acids throughout the growth day. Based on total percentage, both affinis varieties had high fatty acid percentage compared with the other two species with the total percentage of more than 70%. As a conclusion, all four taxa are suitable to be used in lipid industry in Malaysia with* C. affinis *var.* affinis *is the best candidate for bio-fuel industry and* C. anastomosans *for pharmaceutical industry.*

Keywords: Chaetoceros*; fatty acids; growth; indoor; industry*

ABSTRAK

Kajian secara dalam persekitaran dijalankan untuk menganalisis pengeluaran asid lemak dengan profil tumbesaran daripada beberapa spesies alga marin di bawah genus Chaeotoceros *yang diasingkan dari pesisiran pantai Pahang, Malaysia. Kesemua alga dimantapkan dalam bentuk kultur dengan menggunakan media marin yang dipiawai (f/2 media) dan variasi asid lemak untuk setiap spesies ditentukan dengan menggunakan GCMS. Analisis statistik ANOVA satu-hala digunakan untuk menilai kesignifikan dan kehomogenan data ke atas tumbesaran setiap alga serta jumlah peratusan asid lemak yang diterima. Hasil menunjukkan empat takson berjaya dikulturkan dan dikenal pasti sebagai* Chaetoceros baculites, Chaetoceros anastomosans, Chaetoceros affinis *var*. willei *dan* Chaetoceros affinis *var*. affinis. *Daripada empat spesies tersebut,* C. baculites *menunjukkan kadar pertumbuhan yang tinggi (0.75 cell.day^{-1}) dan nilai pendua yang tinggi (1.08) manakala* C. anastomosans *menunjukkan kadar masa pendua yang tinggi (8.66). Analisis statistik menunjukkan kesemua spesies mempunyai kadar pertumbuhan yang bererti (*p<*0.05). Asid miristik merupakan komponen simpanan asid lemak bagi* C. baculites, C. anastomosans *dan* C. affinis *var*. willei *manakala asid palmitik bagi* C. affinis *var.* affinis. *Kesemua spesies mengandungi lebih kurang 35 hingga 75% jumlah peratusan asid lemak mengikut hari tumbesaran. Berdasarkan jumlah peratusan, kedua-dua variasi affinis mempunyai jumlah peratusan asid lemak yang tinggi berbanding dua spesies yang lain dengan jumlah peratusan melebihi 70%. Kesimpulannya, kesemua empat takson adalah sesuai digunakan di dalam industri lipid di Malaysia dengan* C. affinis *var.* affinis *merupakan calon yang sesuai bagi industri bio-bahan api dan* C. anastomosans *bagi industri farmaseutikal.*

Kata kunci: Asid lemak; Chaetoceros*; dalam persekitaran; industri; tumbesaran*

INTRODUCTION

Currently, microalgae are known as a promising new source of feedstock for the production of various fatty acid compounds. During photosynthesis process, microalgae convert sunlight, water and CO_2 to sugars and macromolecules such as lipids and triacylglycerols (TAGs) (Singh & Gu 2010). Since the cells grow in aqueous body they are more efficient in accessing the water, CO_2 and other nutrients from the water body. In addition, numerous algal strains have been shown to have the ability to produce more than 50-80% of lipids (Metting 1996) and 20-50% of oil levels. For these

reasons, microalgae are capable of producing much higher amount of oil per unit area of land, compared with many terrestrial oil seed crops, such as soybean, coconut and palm (Mata et al. 2010).

The Genus *Chaetoceros* is one of the largest cosmopolitan marine phytoplankton genera (Be´rard-Therriault et al. 1999) and most important genus in marine planktonic diatoms. This genus has been listed as major contributor to primary production in near-shore upwelling regions and coastal areas (Rines & Theriot 2003), where it contributes about 20-25% of the total marine primary production. For the past decade, the genus has widely been used in aquaculture such as for larvae fish feedings. The usage of the genus is mainly due to high qualities of nutrients that was being stored in the cells such as PUFA. Moreover, the genus is widely used in marine hatcheries as food sources as well as to maintain water quality (Khatoon et al. 2007). In addition, initiatives has been taken to improvise and multiple the usage into different disciplines. Some of the research proposed the genus can be used in pharmaceutical mainly in antimicrobial compound (Mendiola et al. 2007). The genus can also be used in lipid production for industrial purpose. Some industries are focusing on producing high quality of fatty acid such as PUFA extracted from *Chaetoceros* spp that is beneficial for human. It has been proven that other species under the same genus such as *C. gracilis* (Rika Partiwi et al. 2009) is capable of producing high quality of fatty acids for lipid industry.

Due to the promising results of using algae as lipid producer, many studies have been conducted to study the potential of algae as the source of lipid. However, all the research conducted is mainly based on freshwater algae (Mata et al. 2010) and the study on marine microalgae is still limited. Marine environment contain a very diverse species compared to freshwater and explorations of the marine species are beneficial. Due to that, the purpose of this study was to determine the growth pattern of four *Chaetoceros* taxa namely *C. affinis* var. *affinis*, *C. affinis* var. *willei*, *C. anastomosans* and *C. baculites*. The production of fatty acids by each species will also be discussed. The results obtained from this study will be used in proposing the best potential candidates for the production of high concentration of fatty acids.

Materials and Methods

Sample Collection

The samples of microalgae were collected using 20 µm plankton net meshes along the coastal water. The samplings were randomly selected which the locations covered from Pantai Cherating to Tanjung Gemok from August 2011 until August 2012. The samples were then kept in polyethylene bottle, covered with newspaper and kept in ice chest. The samples were then transported to the laboratory for isolation.

Establishment of Pure Culture

Single cell of the microalgae was isolated using one cell isolation technique (Mohammad-Noor 2012) under compound light microscope, Model: Leica DME. The isolation was done by using micropipette or glass pipette and was done in a laminar flow. The isolated cell was put into 24 well plates containing 1 mL of F/2 media (Harrison & Berges 2004). The pH of the medium was adjusted from 7.2 to 8.2, salinity of 28±1 ppt, pressure was within 35 PSV (normal atmosphere) and temperature of 24±1°C. For light intensity, cold lights were used with the intensity of 4000-5000 Lux (Rika Partiwi et al. 2009). During cultivation, the air flow was given for 24 h using air aerator and the light: Dark cycle was 12: 12 h. For up scaling, 250 mL conical flaks were used to cultivate for stock culture and proceed by scale phase until mass cultivation using 15 L of sterile bottles.

Identification Process

Both light microscope (LM) and scanning electron microscope (SEM) were used for species identification up to the species level. For LM, identification was done under compound light microscope, Model: Leica DME with a magnification of 40, 100, 400 and 600. For detailed view of the morphology, SEM was used. The method used followed Mohammad-Noor (2012).

Cell Counting

For the growth analysis, all strains were cultured in 5 L of media with the proportion of 1:1 (stock culture: fresh media). The starting density of algae was about 5000 to 6000 cells/mL. The counting was done thrice using a Sedgwick rafter cell at total magnification of 100. The data obtained were used to analyze the growth pattern and growth rate of each strain. For growth curve, population growth rate (r), divisions per day (k) and population doubling time (T_2), followed a formula by Andersen (2005):

$$\text{Growth rate (r)} = \ln(Nt/No)/\Delta T, \quad (1)$$

where Nt is the population size at the end of the time interval; No is the population size at the beginning of the time interval; and ΔT is the length of the time interval:

$$\text{Divisons per day (k)} = r/0.6931 \quad (2)$$

$$= \text{Log2}\,(Nt/No)/\Delta T. \quad (3)$$

$$\text{Population doubling time } (T_2) = 0.6931/r. \quad (4)$$

Lipid Analysis

To determine the fatty acid profile of each strain, l L of cultures were harvested every two days. The samples were then centrifuged to collect the pellet and the pellet were freeze dried. The preparation of FAME followed

the Ministry of Palm Oil Board (MPOB) test method in 2004 (Ainie et al. 2005) and the method that being used was MPOB p3.4 – Part 1: 2004 (FAME – BF_3 method) ISO 5509:2000. The end solution contains about 100 mg/mL of methyl ester. The analysis was performed using GCMS Model 6890N Network GC system with the detector Model 5973 Network Mass Selective Detector and injected automatically into the GCMS using automatic injector Model 7683 Series Injector. Five µL of samples were injected into GCMS within the column (30 long and 0.25 mm), the flow calibrates to 1.2, the temperature was set programming between 50 and 250°C and the pressure of the gas was 64.5 Kpa (Rika Partiwi et al. 2009).

STATISTICAL ANALYSIS

The data obtained from this study were subjected to the statistical analysis using SPSS 15.0. Most data were evaluated using one - way ANOVA to assess significant differences of the algal growth and lipid percentages between strains.

RESULTS AND DISCUSSION

GROWTH RATE

From the result obtained, *C. baculites* showed the highest growth rate which is 0.75 cell.day^{-1} followed by *C. affinis* var. *willei* (0.51 cell.day^{-1}), *C. affinis* var. *affinis* (0.15 cell.day^{-1}) and *C. anastomosans* (0.08 cell.day^{-1}). Based on cell count, *C. baculites* recorded the highest cell number which is 5.3×10^5 cells/mL, followed by *C. affinis* var. *willei* (2.8×10^5), *C. affinis* var. *affinis* (9.3×10^4 cells/mL) and *C. anastomosans* (7.1×10^4 cells/mL). The two varieties, *C. affinis* var. *willei* and *C. affinis* var. *affinis* have different growth rate which are 0.51 and 0.15 cell.day^{-1}, respectively. The difference between these two strains could be influenced by the cell thickness where var. *affinis* has very thick cell compared to var. *willei* (Jensen & Moestrup 1998). Thus, with this features, var. *affinis* is less efficient in absorbing nutrient from the environment and this lead to slower growth rate (Fogg & Thake 1987). All the growth estimation for each species can be referred to Tables 1 to 4.

As for division per day (K), *C. baculites* has the higher value (1.08 Div.day^{-1}) followed by *C. affinis* var. *willei* (0.74 Div.day^{-1}), *C. affinis* var. *affinis* (0.22 Div.day^{-1}) and *C. anastomosans* (0.12 Div.day^{-1}). We can conclude that the higher the growth rate and cell numbers per day, the higher the division value obtained. Doubling times referred to the capability of the algae to produce another cell within one day (Andersen 2005). If the values are smaller, we can conclude that the algae have higher growth rate and cell number during the cultivation. Due to high growth rate, the time for population to double from initial cells for *C. baculites* (0.92) are lesser compared with *C. anastomosans* (8.66) which has low growth rate.

For the statistical analysis, the homogeneity test showed that the data has not violated the assumption of the homogeneity (Sig>0.05). For one-way ANOVA analysis, there is a significant difference between the species growth data (p<0.05). By referring to the results obtained, this explanation ensures that the data are genuine and has difference in terms of growth peaks and cell numbers.

TABLE 1. Growth estimation for *Chaetoceros baculites*

Time Interval (Days)	0-1	1-2	2-3	3-4	4-5	5-6	0-3	3-6
Nt/N0	2.24	2.84	1.49	0.63	0.83	0.77	9.50	0.40
ln(Nt/N0)	0.81	1.04	0.40	-0.46	-0.19	-0.26	2.25	-0.92
Log 2 (Nt/N0)	1.16	1.51	0.58	-0.67	-0.27	-0.38	3.25	-1.32
ΔT=(T2-T1)	1	1	1	1	1	1	3	3
r (Eq.4) a	0.81	1.04	0.40	-0.46	-0.19	-0.26	0.75	-0.31
K (Eq.6)	1.17	1.50	0.58	-0.66	-0.26	-0.38	1.08	-0.45
K (Eq. 7)	1.16	1.51	0.58	-0.67	-0.27	-0.38	1.08	-0.44
T2 (Eq.8)	0.86	0.67	1.73	-1.51	-3.85	-2.67	0.92	-2.24

a Assuming exponential growth and zero mortality, r=µ, the intrinsic growth rate of the population rate of the population. Eq : Equation of the text

TABLE 2. Growth estimation for *Chaetoceros anastomosans*

Time Interval (Days)	0-1	1-2	2-3	3-4	4-5	5-6	0-4	4-6
Nt/N0	1.15	1.03	1.05	1.13	0.93	0.73	1.39	0.68
ln(Nt/N0)	0.14	0.03	0.05	0.12	-0.07	-0.31	0.33	-0.39
Log 2 (Nt/N0)	0.20	0.04	0.07	0.18	-0.10	-0.45	0.48	-0.56
ΔT=(T2-T1)	1	1	1	1	1	1	4	2
r (Eq.4) a	0.14	0.03	0.05	0.12	-0.07	-0.31	0.08	-0.20
K (Eq.6)	0.20	0.04	0.07	0.17	-0.10	-0.45	0.12	-0.29
K (Eq. 7)	0.20	0.04	0.07	0.18	-0.10	-0.45	0.12	-0.28
T2 (Eq.8)	4.95	23.10	13.86	5.78	-9.90	-2.24	8.66	-3.47

a Assuming exponential growth and zero mortality, r=µ, the intrinsic growth rate of the population rate of the population. Eq : Equation of the text

TABLE 3. Growth estimation for *Chaetoceros affinis* var. *affinis*

Time Interval (Days)	0-1	1-2	2-3	3-4	4-5	5-6	0-4	4-6
Nt/N0	1.23	1.08	0.97	1.40	0.79	0.80	1.81	0.63
ln(Nt/N0)	0.21	0.08	-0.03	0.34	-0.24	-0.22	0.59	-0.46
Log 2 (Nt/N0)	0.30	0.11	-0.04	0.49	-0.34	-0.32	0.86	-0.67
ΔT=(T2-T1)	1	1	1	1	1	1	4	2
r (Eq.4) a	0.21	0.08	-0.03	0.34	-0.24	-0.22	0.15	-0.23
K (Eq.6)	0.30	0.12	-0.04	0.49	-0.35	-0.32	0.22	-0.33
K (Eq. 7)	0.30	0.11	-0.04	0.49	-0.34	-0.32	0.22	-0.34
T2 (Eq.8)	3.30	8.66	-17.33	2.04	-2.89	-3.15	4.62	-3.01

a Assuming exponential growth and zero mortality, r=μ, the intrinsic growth rate of the population rate of the population . Eq : Equation of the text

TABLE 4. Growth estimation for *Chaetoceros affinis* var. *willei*

Time Interval (Days)	0-1	1-2	2-3	3-4	4-5	5-6	0-3	3-6
Nt/N0	2.51	1.69	1.10	0.97	0.67	0.25	4.67	0.16
ln(Nt/N0)	0.92	0.52	0.10	-0.03	-0.40	-1.39	1.54	-1.83
Log 2 (Nt/N0)	1.33	0.76	0.14	-0.04	-0.58	-2.00	2.22	-2.64
ΔT=(T2-T1)	1	1	1	1	1	1	3	3
r (Eq.4) a	0.92	0.52	0.10	-0.03	-0.40	-1.39	0.51	-0.61
K (Eq.6)	1.33	0.75	0.14	-0.04	-0.58	-2.00	0.74	-0.88
K (Eq. 7)	1.33	0.76	0.14	-0.04	-0.58	-2.00	0.74	-0.88
T2 (Eq.8)	0.75	1.33	6.93	-17.33	-1.73	-0.50	1.36	-1.14

a Assuming exponential growth and zero mortality, r=μ, the intrinsic growth rate of the population rate of the population. Eq : Equation of the text

GROWTH PATTERN

All four taxa of *Chaetoceros* have different growth rates and patterns during the cultivation process with a life span about 6 days starting from the cultivation day. Based on Figure 1, only *C. affinis* var. *willei* showed bell shape growth rate. *C. baculites* showed rapid growth pattern but starting Day 3, the growth decrease with death phase occurred after Day 4. Both *C. anastomosans* and *C. affinis* var. *affinis* showed slow growth pattern where at Day 5 the algae started to undergo death phase.

C. baculites and *C. affinis* var. *willei* have similarity on the rate where both species take only three days for optimum growth before decrease whereas *C. anastomosans* and *C. affinis* var. *affinis* take about four days for optimum growth before started undergo death phase. This indicates that the growth pattern is species specific (Thompson et al. 1990) whereby each species has its own preferable condition for optimum growth. There are several factors been considered to influenced the algae growth such as the intensity of light source (Go et al. 2012), CO_2 levels from aerators (Tsuzuki et al. 1990), salinity (Asulabh et al. 2012), pH (Chen & Durbin 1994), temperature (Guschina & Harwood 2009; Harwood 1998; Thompson 1994) and available nutrients. For this study all the parameters are constant with the nutrient not added throughout the cultivation. Therefore, all the growth obtained were based on the ability of algal species to survive with the limitation appeared during cultivation (Fogg & Thake 1987). Based on laboratory condition, *C. baculites* and *C. affinis* var. *willei* shown the best growth profile than other species

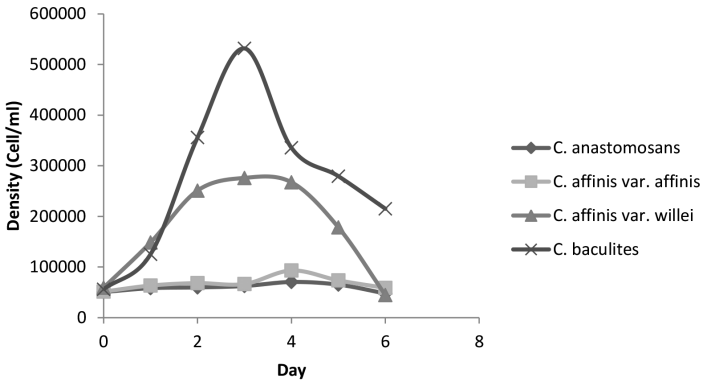

FIGURE 1. The graph show growth pattern of the *Chaetoceros* species

based on the results. All the data were recorded and will be used for future studies.

FATTY ACID ANALYSIS

Eight fatty acids were detected from all four strains. The percentage of the fatty acids and their profile are shown in Table 5. From the total eight fatty acids recorded, one hazardous fatty acid has been detected in *C. affinis* var. *willei* at Day 4 (2.9±1.57%) and Day 6 (3.68±1.59%). Pelargonic acid is one of the compounds that are used to control weed and can affect organism tissue by binding to the fats and oil (Lakeridge & Olympia 2000). The compound produces toxic effect the tissue through bioaccumulation process.

The common fatty acids such as myristic and palmitic were determined in all taxa at Day 2 and 4. Both varieties of *C. affinis* have palmitic acid and myristic acid in all their growth day. However, during Day 6, myristic acid was not detected for *C. anastomosans* and *C. baculites* and palmitic acid was not recorded for *C. baculites*. Palmitic acid is recognized as primary fatty acids for lipid storage in marine diatom. This compound is considered as one of signature compound for Bacillariophycea, which is highly found in the cell (Mansour et al. 2003). However, for this study, myristic acid detected was higher in all taxa except for *C. affinis* var. *affinis*. Therefore, we can conclude that myristic acid is the main component for SFA storage for *C. baculites, C. anastomosans* and *C. affinis* var. *willei*. During Day 2, *C. baculites* recorded higher percentage of myristic acid which is 39.24±16.33% followed by *C. affinis* var. *willei* (23.13±3.82%), *C. anastomosans* (18.56±9.36%) and *C. affinis* var. *affinis* (14.81±1.88%). However, for palmitic acid, *C. affinis* var. *affinis* recorded the higher percentage which is 23.57±5.97% followed by *C. affinis* var. *willei* (19.33±2.73%), *C. baculites* (14.43±8.70%) and *C. anastomosans* (11.17±2.19%). For Day 4, both myristic and palmitic acids still accumulate within 20-40% within the cells. During this period, all four strains reached their optimum growth where all the cell activities are in their highest active level. Based on Fogg and Thake (1987), during stationary phase all activities in the cell are in high level within their growth generation. Therefore, the accumulation of metabolic and storage compound is in the highest percentage.

For Day 6, both varieties of *C. affinis* contain both myristic and palmitic acids whereas var. *willei* still maintain the highest percentage for both compounds compared to var. *affinis*. Not only that, *C. anastomosans* contain palmitic acid about 13.47±2.26% which was much higher than *C. affinis* var. *affinis*. However, for *C. baculites*, both fatty acids already depleted. The content of myristic and palmitic acid in the cells depends on the species cell membrane, energy storage and metabolic processes (Orhan et al. 2003). Therefore, different percentages of these acids were recorded. Based on statistical analysis, the homogeneity test shows both fatty acid did not violated the homogeneity of the data obtain (Sig.>0.05). For one-way ANOVA analysis, the results showed that both fatty acids showed no significant difference between the species (p>0.05).

Stearic acid was detected in both varieties of *C. affinis* where the highest recorded in Day 4 for var. *affinis* (6.05±1.71%). Previous research reported that stearic acid was recorded from *Chaetoceros muelleri* (Mendiola et al. 2007) with low percentage. From the statistical analysis, the homogeneity test showed that the data obtained did not violate the data homogeneity (Sig>0.05). One-way ANOVA analysis appeared that there are significant difference (p<0.05) between both species.

Valuable fatty acids such as palmitoleic acid and oleic acid were also detected. For palmitoleic acid, all studied species have this fatty acid in all their growth phases except for *C. anastomosans* (Day 6) and *C. baculites* (Day 2 and 4). The highest percentage of palmitoleic acid was recorded in *C. anastomosans* during Day 4 (20.58±8.44%). Palmitoleic acid is a form based on biosynthesize process from palmitic acid with the help of the action enzyme delta-9 desaturase during all the growth day. This compound is beneficial because it can help to increase insulin sensitivity by suppressing inflammation, as well as inhibit the destruction of insulin-secreting pancreatic beta cells (Yang et al. 2011). Oleic acid was detected in *C. affinis* var. *affinis* in all growth day. However, the highest percentage of oleic acid was recorded at Day 6 for *C. anastomosans* (26.78±12.67%). Oleic acid is important because it is used in curing breast cancer (Tin Win 2005), enhances cancer drug effectiveness (Mendez et al. 2005) and improving the immune systems (Carillo et al. 2012).

Due to limited data on the biochemistry of the Genus *Chaetoceros*, all the results obtained from this study will be kept in view for further research. All the parameters and the media used are of standard procedure/information that currently being used to cultivate algae in the laboratory. Several factors have been listed to influence the biochemistry of the algae, such as the intensity of light source (Go et al. 2012), CO_2 levels (Tsuzuki et al. 1990), salinity (Asulabh et al. 2012), pH (Chen & Durbin 1994), temperature (Guschina & Harwood 2009; Harwood 1998; Thompson 1994) and nutrients (nitrogen, phosphorus, silica).

These factors are considered to greatly influence the biochemical composition of microalgae (Fábregas et al. 2004; Gordillo et al. 1998; Kalacheva et al. 2002) in affecting the photosynthesis process, which altering carbon fixation inside the cells and the allocation of carbon receive into different types of macromolecules. As a result, the cell's macromolecular composition varies throughout the growth process. As mentioned earlier, all the parameters are constant with no added nutrient during cultivation process. All the changes inside the lipid contents were based on biochemical activities appeared inside the cells (Orhan et al. 2003). Therefore, the data obtained in this study were recorded and added for biochemistry data and be used for future studies.

TABLE 5. Fatty acids detected within all strains in every growth phase. CAA: *Chaetoceros affinis* var. *affinis*, CAW: *Chaetoceros affinis* var. *willei*, CBA: *Chaetoceros baculites*, CAN: *Chaetoceros anastomosans*

Fatty Acids	Fatty acid percentage of total fatty acid											
	Day 2				Day 4				Day 6			
	CAA	CAW	CAN	CBA	CAA	CAW	CAN	CBA	CAA	CAW	CAN	CBA
	Saturated fatty acid (SFA)											
9(0): Pelargonic Acid	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.91 ± 1.57	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	3.68 ± 1.59	0.00 ± 0.00	0.00 ± 0.00
14(0): Myristic Acid	14.81 ± 1.88	23.13 ± 3.82	18.56 ± 9.36	39.24 ± 16.33	16.33 ± 3.46	19.22 ± 3.99	21.44 ± 9.50	26.43 ± 14.92	8.51 ± 3.32	24.70 ± 3.85	0.00 ± 0.00	0.00 ± 0.00
16(0): Palmitic Acid	23.57 ± 5.97	19.33 ± 2.73	11.17 ± 2.19	14.43 ± 8.70	24.42 ± 6.25	14.22 ± 2.87	16.79 ± 2.31	21.41 ± 8.91	10.76 ± 6.10	20.97 ± 2.97	13.47 ± 2.26	0.00 ± 0.00
18(0): Stearic Acid	4.04 ± 1.64	3.79 ± 0.50	0.00 ± 0.00	0.00 ± 0.00	6.05 ± 1.71	3.76 ± 0.57	0.00 ± 0.00	0.00 ± 0.00	1.87 ± 1.57	5.08 ± 0.62	0.00 ± 0.00	0.00 ± 0.00
Total SFA (%)	42.42 ± 9.49	46.25 ± 7.05	29.73 ± 11.55	53.67 ± 25.03	46.80 ± 11.42	40.11 ± 9.00	38.23 ± 11.81	47.84 ± 23.83	21.14 ± 10.99	54.43 ± 9.03	13.47 ± 2.26	0.00 ± 0.00
	Monounsaturated fatty acid (MUFA)											
16 (1:9): Palmitoleic Acid	10.87 ± 3.25	3.31 ± 3.54	8.67 ± 7.02	0.00 ± 0.00	14.97 ± 3.29	13.01 ± 4.36	20.58 ± 8.44	7.30 ± 3.44	6.91 ± 3.15	16.22 ± 5.49	0.00 ± 0.00	0.00 ± 0.00
18 (1:9): Oleic Acid	4.18 ± 1.53	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	8.93 ± 1.95	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	6.13 ± 1.81	0.00 ± 0.00	26.87 ± 12.67	0.00 ± 0.00
18 (1:9:Trans): Elaidic Acid	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	3.09 ± 1.46	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
18 (1:11): Vaccenic Acid	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	4.27 ± 2.01	0.00 ± 0.00	0.00 ± 0.00
Total MUFA (%)	15.05 ± 4.78	3.31 ± 3.54	8.67 ± 7.02	0.00 ± 0.00	23.90 ± 5.24	16.10 ± 5.82	20.58 ± 8.44	7.30 ± 3.44	13.04 ± 4.96	20.49 ± 7.50	26.87 ± 12.67	0.00 ± 0.00
Others (Tetradecane, Nonacosane, Eicosane)	42.53 ± 12.27	50.44 ± 10.74	61.60 ± 9.20	46.33 ± 12.83	29.30 ± 7.55	43.79 ± 9.32	41.19 ± 8.49	44.86 ± 12.73	65.82 ± 15.10	25.08 ± 5.37	59.66 ± 8.92	100 ± 25.65
Total Percentage (%)	57.47 ± 14.27	49.56 ± 10.59	38.40 ± 18.57	53.67 ± 25.03	70.70 ± 16.66	56.21 ± 14.82	58.81 ± 20.25	55.14 ± 27.27	34.18 ± 15.95	74.92 ± 16.53	40.34 ± 14.93	0.00 ± 0.00

Based on the total percentage, the highest lipid percentage was recorded in *C. affinis* var. *willei* in Day 6 (74.92±16.53%), followed by *C. affinis* var *affinis* in Day 4 (70.70±16.66%). *C. affinis* var. *willei* increases of the fatty acids percentage within the growth day. This is most likely that the species tends to store the energy in the form of energy storage which is lipid storage due to lipid accumulation from previous culture age, lack of nutrient and downfall condition from the environment (De Castro et al. 2005). Pernet et al. (2003) reported that lipid increases in *C. muelleri* is due to silicon-depletion whereas for *C. wighamii*, lipid accumulation is triggered by nutrient deficiency (De Castro et al. 2005). However, for *C. affinis* var. *affinis*, lipid increases during Day 4 but decrease during Day 6. This may due to fatty acids accumulation is species specific (Thompson et al. 1990) and biochemistry of the cell (Orhan et al. 2003).

Previous studies on 35 microalgae including *Spirullina* sp. and *Chlorella* sp. have reported that fatty acid contents are between 5 and 67% (Mata et al. 2010). Moreover, a potential biofuel candidate, *Ankistrodesmus falcatus* (Corda) Ralfs (Talukdar et al. 2012) recorded 45% of lipid throughout all the growth day. Comparing with these studies, Genus *Chaetoceros* has the ability to produce within or higher percentage. From the result, all species contain saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) group. The absence of polyunsaturated fatty acids (PUFA) indicates that the species are good candidate for biodiesel production as suggested by Yanqun et al. (2008). This is because the bonds inside the PUFA group need extra process to turn the compound into biodiesel. On the other hand, if the algae contain more saturated fatty acids (SAFA), it is ideal to be used in biodiesel production (Tornabene et al. 1983). Moreover, few species such as *C. anastomosans* could be used in pharmaceutical industry for producing good quality fatty acids.

Conclusion

C. baculites has the highest growth rate(r) and divisions value (K) while *C. anastomosans* has highest doubling time value (T_2). Myristic acid is the main component for fatty acid storage for *C. baculites, C. anastomosans* and *C. affinis* var. *willei* whereas for *C. affinis* var. *affinis* is palmitic acid. *C. affinis* var. *willei* has pelargonic acid, hazardous fatty acid whereas *C. affinis* var. *affinis* has valuable fatty acid, oleic acid. Both *C. affinis* varieties recorded highest percentages of fatty acids where var. *affinis* was highest during Day 4 and var. *willei* during Day 6. All the taxa can be listed as potential candidates in lipid industry with *C. affinis* var. *affinis* concluded as the best candidates for bio-fuel industry due to var. *willei* contain pelargonic acid and can produce high lipid production and *C. anastomosans* for pharmaceutical industry.

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