

CHAPTER 4

GROWTH COMPARISON OF THE PROMISING STRAIN IN JAWORSKI'S MEDIUM WITH ALGAL MEDIUM AND OPTIMIZATION OF NUTRIENT COMPONENTS FOR ENHANCING LIPID ACCUMULATION

4.1 Introduction

In the previous experiment, *Carteria* sp. AARL G045 was found to be the best strain with fast growth rate and high lipid content. However, its lipid productivity is still unsatisfied and the medium (JM) used in the cultivation is expensive. Thus the development of optimal conditions is required for enhancing the algal biomass with high lipid content.

Algal medium (AM) is the basic medium for algal isolation and cultivation which has been widely used. AM composes of N source, P source and some trace elements which are necessary for microalgae. Moreover, this medium is interesting due to its cheaper cost than JM hence it was also used in this study.

The appropriate culture condition is quite important for enhancing the productivity of algal lipid content. Generally, medium is essential for microalgal growth and some nutrient components in the medium such as phosphorus, nitrogen, inorganic salt and some of trace elements also effected lipid accumulation in microalgal cells (Li *et al.*, 2008). In addition, decreasing and starvation of some nutrients were examined for encouraging microalgal lipid accumulation. Normally,

the effects of medium components could be evaluated through using conventional screening for maximizing the product.

Thus in this experiment, *Carteria* sp. AARL G045 was cultivated in JM and compared with AM. Furthermore, the nutritional requirement was evaluated for lipid accumulation in the cells by initial screening using PBD and the suitable significant factors were optimized using BBD.

4.2 Materials and methods

4.2.1 Comparison of the growth and lipid productivity of *Carteria* sp. AARL G045 in JM with AM

The best native strain, *Carteria* sp. AARL G045 from the previous experiment was selected for cultivation in two media. The stock culture of *Carteria* sp. AARL G045 was washed with sterile distilled water before being transferred to 500 mL of JM and AM to obtain the initial optical density at 665 nm (OD_{665}) of approximately 0.05. The culture was cultivated at 25 °C under continuous shaking at 120 rpm with continuous illumination of $10.8 \mu\text{mol.m}^{-2}.\text{s}^{-1}$ by florescent light for 16 days.

The growth rate of *Carteria* sp. AARL G045 was determined by spectrophotometrically measured at OD_{665} nm and cell numbers were counted under compound microscope with haemocytometer counting chamber every two days. When the culture reached the stationary phase on Day 16, cells were harvested by centrifugation and dried at 60 °C for 48 hr, Lipid extraction was prepared by pulverization of dried cells in mortar and extracted with hexane soxhlet extraction

(Wangchai, 2009). The growth and lipid accumulation of *Carteria* sp. AARL G045 cultivated in JM was compared with those in AM.

4.2.2 Optimization of nutrient components for enhancing lipid accumulation in *Carteria* sp. AARL G045 by response surface methodology (RSM).

Stock culture of *Carteria* sp. AARL G045 was maintained in AM. The seed culture was prepared on a shaker at 25°C under continuous shaking at 120 rpm and 10.8 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ illumination by florescent lamp.

4.2.2.1 Culture condition

Before transferring into the new flask experiments, the initial cells were washed 2-3 times with sterile distilled water to remove the old medium. The washed cells were inoculated to 500 mL of fresh AM according to the statistical experimental design, response surface methodology (RSM) to obtain an initial OD_{665 nm} of 0.05. The flask was kept at the condition following as the stock culture. The cells were harvested by centrifugation and cellular lipid content was determined by extraction with 30 mL of CHCl₃: methanol (2:1 v/v) per 0.5 g of dried cells according to the method of Bligh and Dyer (1959).

4.2.2.2 Experiment designs

Plackett - Burman design (PBD)

PBD was used to screen multifactor that most significantly influence lipid accumulation. Each variable was represented at two levels (Plackett and Burman, 1946), -1 for low level (fixed at 0 g.L⁻¹) and +1 for high level, which was as follows: NH₄Cl 0.1 g.L⁻¹, FeCl₃ 0.006 g.L⁻¹, CaCl₂ 0.116 g.L⁻¹, NaNO₃ 2 g.L⁻¹, K₂HPO₄ 0.4

g.L^{-1} and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1.03 g.L^{-1} . Plackett - Burman design with 2 levels of concentrations, high and low levels, for 6 different nutrient components are shown in Table 5 and the six variables in 12 experimental run are represented in Table 6, based on the first – order model:

$$Y = \beta_0 + \sum \beta_i x_i \quad (1)$$

Where Y is the predicted response (lipid productivity), β_0 and β_i are the constant coefficients and x_i represents the code independent factor.

Table 5 Plackett - Burman design for screening 6 different nutrient components with 2 levels of concentrations

Factor (g.L^{-1})	Variables	Low levels (-1)	High levels (+1)
NH_4Cl	A	0	0.1
CaCl_2	B	0	0.116
K_2HPO_4	C	0	0.5
FeCl_3	D	0	0.006
NaNO_3	E	0	2
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	F	0	1.026

Table 6 PBD variables for optimization of nutrient compositions

Runs	Variables					
	A	B	C	D	E	F
1	+1	+1	-1	+1	+1	+1
2	+1	-1	+1	+1	+1	-1
3	-1	+1	+1	+1	-1	-1
4	+1	+1	+1	-1	-1	-1
5	+1	+1	-1	-1	-1	+1
6	+1	-1	-1	-1	+1	-1
7	-1	-1	-1	+1	-1	+1
8	-1	-1	+1	-1	+1	+1
9	-1	+1	-1	+1	+1	-1
10	+1	-1	+1	+1	-1	+1
11	-1	+1	+1	-1	+1	+1
12	-1	-1	-1	-1	-1	-1

Box - Behnken Design (BBD)

The BBD is a tool for the multivariable optimization, which are rotatable second – order designs with three levels, coded -1 for low level, 0 for middle level and +1 for high level (Box and Behnken, 1960). Four different factors were chosen as main variables and designated as NaNO_3 , K_2HPO_4 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and NH_4Cl . The low level (fixed at 0 g.L^{-1}), normal level was followed as: NaNO_3 (1 g.L^{-1}), K_2HPO_4 (0.25 g.L^{-1}), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.513 g.L^{-1}) and NH_4Cl (0.05 g.L^{-1}) and high levels: NaNO_3 (2 g.L^{-1}), K_2HPO_4 (0.5 g.L^{-1}), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (1.026 g.L^{-1}) and NH_4Cl (0.1 g.L^{-1}) of each variable were investigated. The four variables in 29 experimental runs are represented in Table 7.

Table 7 Four significant variables in 29 experimental runs by using BBD

Runs	Factor 1	Factor 2	Factor 3	Factor 4
	A: NH_4Cl (g.L^{-1})	B: K_2HPO_4 (g.L^{-1})	C: NaNO_3 (g.L^{-1})	D: $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (g.L^{-1})
1	0.05	0	1	0
2	0.05	0.5	1	1.026
3	0.1	0.25	2	0.513
4	0.1	0.25	1	0
5	0.05	0	2	0.513
6	0.05	0.25	1	0.513
7	0	0.25	0	0.513
8	0.05	0	1	1.026
9	0	0.5	1	0.513
10	0.05	0.25	2	1.026
11	0.1	0.25	0	0.513
12	0.05	0.25	2	0
13	0	0.25	2	0.513
14	0	0	1	0.513
15	0	0.25	1	0
16	0.1	0.25	1	1.026
17	0.05	0.25	0	1.026
18	0.05	0.5	2	0.513
19	0.05	0.5	0	0.513
20	0.1	0.5	1	0.513
21	0.05	0.25	0	0
22	0.05	0.5	1	0
23	0.05	0.25	1	0.513
24	0.05	0.25	1	0.513
25	0	0.25	1	1.026
26	0.05	0.25	1	0.513
27	0.05	0	0	0.513
28	0.05	0.25	1	0.513
29	0.1	0	1	0.513

The data obtained from the relationship and interrelationship of the variables which were analyzed by the second order polynomial equation:

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \beta_{ij} x_i x_j$$

Where Y is the predicted response surface variables, β_0 is the offset term, β_i is linear effect, β_{ii} is the square effect, β_{ij} is interaction effect and X is the coded levels of independent variables; x_i and x_j ($i \neq j$) represent the independent variables. The regression equation above was optimized for optimal value also using Design Expert Software version 6.0.2.

In addition, these optimized values of each nutrient composition were verified in triplicate sets of flasks and the batch experiment was performed in an open glass tank containing 5 L of optimized AM. The biomass, percentage of lipid and the total lipid were compared with the predicted value.

4.2.2.3 Lipid extraction methods

Lipid content was determined using a modified procedure according to Bligh and Dyer method (1959). One gram of dried sample was added to 30 mL of the chloroform: methanol (2:1v/v) in a glass bottle with cover. Then, it was left for 24 hr at room temperature. The extracted lipids were separated from the cell debris by centrifugation at 6000 rpm for 20 min. The lower organic phase was transferred to a new pre-weighed centrifuge tube and dried at 36 – 40 °C to determine the dry lipid weight.

4.3 Results and discussion

4.3.1 Comparison of the growth and lipid productivity of *Carteria* sp.

AARL G045 in JM with AM

Cultivation of *Carteria* sp. AARL G045 in JM and AM showed a similar pattern both in terms of the number of cells and the absorbance at 665 nm. The microalga reached stationary phase on Day 16 with cell concentration at 1.06×10^6 cell.mL⁻¹ and on Day 14 with cell concentration at 1.03×10^6 cell.mL⁻¹ when cultivated in AM and JM respectively (Figure 13).

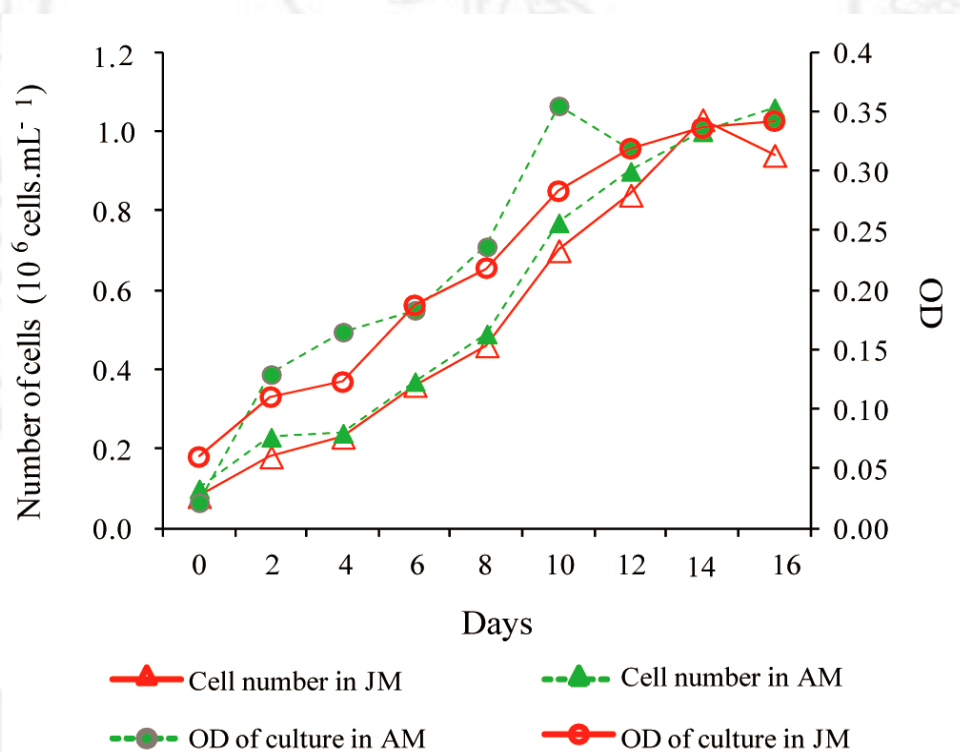


Figure 13 Growth and cell number of *Carteria* sp. AARL G045, in JM and AM

The dry biomass samples obtained from AM and JM were not significantly different ($p>0.05$) i.e. AM 0.42 g.L^{-1} and JM 0.41 g.L^{-1} (Figure 14). Although, the percentage of lipid content from cultivation in the two media was similar, the total lipid accumulation in AM (7.10 % lipid content of dry weight, 29.59 mg.L^{-1}) was significantly higher than that in JM (6.6 % lipid content of dry weight, 27.18 mg.L^{-1}) ($p<0.05$) (Figure 15). This may due to the combined effect of having slightly higher biomass and lipid content.

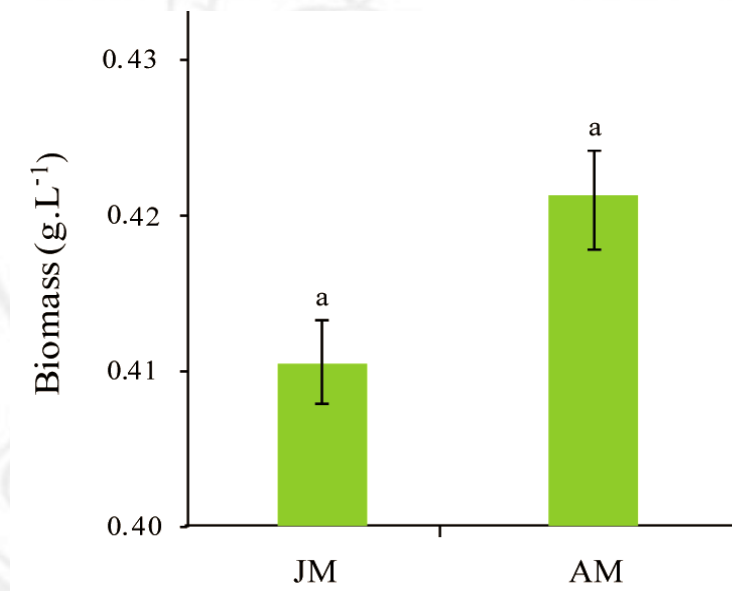


Figure 14 Biomass of *Carteria* sp. AARL G045 (g.L^{-1}) cultivated in JM and AM.

Letters on the top are statistical comparison among groups using analysis of variance (ANOVA) and post-hoc least-significant difference (LSD) test,

$p < 0.05$

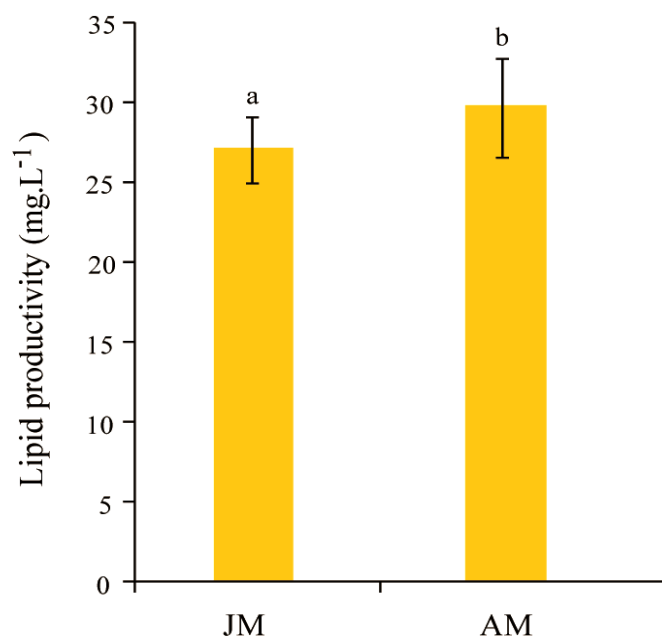


Figure 15 Lipid productivity of *Carteria* sp. AARL G045 (mg.L⁻¹) cultivated in JM and AM. Letters on the top are statistical comparison among groups using analysis of variance (ANOVA) and post-hoc least-significant difference (LSD) test, $p < 0.05$

Interestingly, the cost of AM is lower than JM by about 11% (Table 8). Higher lipid productivity of *Carteria* sp. AARL G045 which was cultivated in AM, related with cheaper cost of media resulting in lower crude lipid cost (Table 9). AM does not contain any carbon source and trace elements but it did not affect the algal growth or lipid production. Thus, it should be of interest as a supply of a cheap carbon source and trace elements in AM which can enhance the growth of *Carteria* sp. AARL G045 at low cost.

Table 8 Comparison of cultivation cost of JM and AM

Jaworski's medium (JM)				Algal medium (AM)			
Chemicals	Prices (฿.g ⁻¹)	Conc. (g.L ⁻¹)	Cost (฿.L ⁻¹)	Chemicals	Prices (฿.g ⁻¹)	Conc. (g.L ⁻¹)	Cost (฿.L ⁻¹)
Ca(NO ₃).4H ₂ O	0.72	0.02	0.0144	NaNO ₃	0.70	1	0.6960
K ₂ HPO ₄	0.67	0.0124	0.0083	NHCl ₄	0.54	0.05	0.0270
MgSO ₄ .7H ₂ O	0.54	0.05	0.0270	MgSO ₄ .7H ₂ O	0.54	0.513	0.2770
NaHCO ₃	0.46	0.0159	0.0072	CaCl ₂	0.38	0.058	0.0223
EDTA Na ₂	1.13	0.00225	0.0025	FeCl ₃	1.08	0.003	0.0032
H ₃ BO ₃	0.53	0.00248	0.0013	K ₂ HPO ₄	0.67	0.25	0.1680
MnCl ₂ .4H ₂ O	1.56	0.00139	0.0022				
Vitamin B12	25,000	0.00004	1.0000				
Vitamin B1	33.6	0.00004	0.0013				
Biotin	5,130	0.00004	0.2052				
NaNO ₃	0.70	0.08	0.0557				
Na ₂ HPO ₄	0.91	0.01428	0.0130				
Total			1.34	Total			1.19

Table 9 Comparison of lipid cost in *Carteria* sp. AARL G045 cultivated in JM and

AM

Media	Lipid yield (mg.L ⁻¹)	Cost of media (฿.L ⁻¹)	Cost of lipid (฿.kg ⁻¹)
JM	27.18	1.34	49,300.96
AM	29.59	1.19	40,216.29

Although AM is cheaper than JM, the long-term cultivation for seed or stock culture, trace elements are necessary. The lack of micro nutrients in AM may not be enough to sustain long term cultivation as was evident in *Spirulina* cultivation. Even though, *Spirulina* grew well in simple media, stock cultures still need some supplemental nutrients in Zarrouk medium (Peerapornpisal, 2003). However, stock culture for seeding should be strong and healthy, it should be maintained in the complete medium such as JM.

4.3.2 Optimization of nutrient components for enhancing lipid accumulation in *Carteria* sp. AARL G045 by response surface methodology (RSM).

Nutrient components in AM were screened for enhancing lipid productivity by PBD. Table 10 demonstrates the effect of 6 variables on cells biomass, percentage of lipid content and lipid productivity, which were decided with the values by using ANOVA analysis. Table 11 shows the factors significantly affected productivity, the plus represents the positive and the minus refers to the negative effects. It was found that NaNO_3 , K_2HPO_4 and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ were the significant factors positively influencing biomass with confidence levels above 80%. However, only NH_4Cl was found to be significantly positive factor affecting the percentage of lipid content and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ was the only significantly positive factor for lipid productivity. Therefore, the optimal quantities of four positive factors were predicted by using BBD. Conversely, the negative and the non-significant factors were fixed at normal values.

Table 10 Experimental results of biomass, percentage of lipid content and lipid productivity from Plackett-Burman design

Runs	Variables						Experimental results		
							Biomass	Lipid content	Lipid productivity
	A	B	C	D	E	F	(g.L ⁻¹)	(%)	(mg.L ⁻¹)
1	+1	+1	-1	+1	+1	+1	0.0292	66.18	19.32
2	+1	-1	+1	+1	+1	-1	0.0250	25	6.25
3	-1	+1	+1	+1	-1	-1	0.2011	12.98	26.1
4	+1	+1	+1	-1	-1	-1	0.0329	28.76	9.46
5	+1	+1	-1	-1	-1	+1	0.0422	59.69	25.19
6	+1	-1	-1	-1	+1	-1	0.0152	71.01	10.79
7	-1	-1	-1	+1	-1	+1	0.1381	20.72	28.61
8	-1	-1	+1	-1	+1	+1	0.3133	10.01	31.36
9	-1	+1	-1	+1	+1	-1	0.1236	12.78	15.8
10	+1	-1	+1	+1	-1	+1	0.0308	38.46	11.85
11	-1	+1	+1	-1	+1	+1	0.2707	11.04	29.89
12	-1	-1	-1	-1	-1	-1	0.0189	32.95	6.23

-1= low level; +1= high level

Table 11 Linear regression analysis of PBD to screen for the factors that significantly affected the products, cells biomass, percentage of lipid content and lipid productivity

Source	Biomass		% Lipid content		Lipid productivity	
	Coefficient	P value	Coefficient	P value	Coefficient	P value
Model		0.0144***		0.0333***		0.1331*
A-NH ₄ Cl	-0.0742	0.0025***	15.718	0.0049***	-4.5942	0.0630**
B-CaCl ₂	0.0132	0.3631	-0.560	0.8710	2.5558	0.2424
C-K ₂ HPO ₄	0.0422	0.0240***	-11.423	0.0175***	0.7475	0.7143
D-FeCl ₃	-0.0121	0.4006	-3.112	0.3858	-0.4158	0.8378
E-NaNO ₃	0.0261	0.1050*	0.205	0.9525	0.4975	0.8067
F-MgSO ₄	0.0340	0.0498***	1.885	0.5899	5.9658	0.0271***
	R ²	= 0.9156	R ²	= 0.8794	R ²	= 0.7703

* Significant at level, $p < 0.2$; ** Significant at level, $p < 0.1$; *** Significant at level, $p < 0.05$

The statistical model describing the correlation between 6 variables, including NH₄Cl (A), CaCl₂ (B), K₂HPO₄ (C), FeCl₃ (D), NaNO₃ (E) and MgSO₄ (F) and where Y are the cells biomass, percentage of lipid content and lipid productivity presented by equations 3 – 5, respectively:

$$Y = 0.10 - 0.074 A + 0.013B + 0.042 C - 0.012D + 0.026E + 0.03F \quad (3)$$

$$Y = 32.47 + 15.72 A - 0.56 B - 11.42 C - 3.11D + 0.20 E + 1.8F \quad (4)$$

$$Y = 18.40 - 4.59A + 2.56 B + 0.75 C - 0.42 D + 0.50 E + 5.97F \quad (5)$$

4. 3.2.1 Interaction between medium components

The experimental results were analyzed by standard ANOVA. Regression analysis showed the biomass and lipid productivity related among the levels of 4 factors, NH_4Cl (A), K_2HPO_4 (B), NaNO_3 (C) and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (D). The ANOVA of linear model was used to consider on biomass production. It demonstrates that the model is significant ($p < 0.05$). Each coefficient is shown in Table 12 on p value, listed.

The 3D contour plots (Figure 16) demonstrates that K_2HPO_4 affected positively on biomass in Figure 16A. In contrast, NH_4Cl was significant factor that affected negatively on biomass in Figure 16B ($p < 0.05$). The actual biomass increased under an increase in K_2HPO_4 but it decreased at higher amount of NH_4Cl .

Table 12 Model coefficient estimated by linear regression

Factor	Sum of Squares	DF	Mean Square	F Value	Prob > F
Model	0.049	4	0.012		0.0127****
A- NH_4Cl	0.018	1	0.018	1.93190	0.0216****
B- K_2HPO_4	0.016	1	0.016	1.93190	0.0309****
C- NaNO_3	2.024	1	2.024	1.93190	0.4226
D- MgSO_4	0.012	1	0.012	1.93190	0.0567

* Significant at level, $p < 0.05$

DESIGN-EXPERT Plot

One Factor Plot

DW

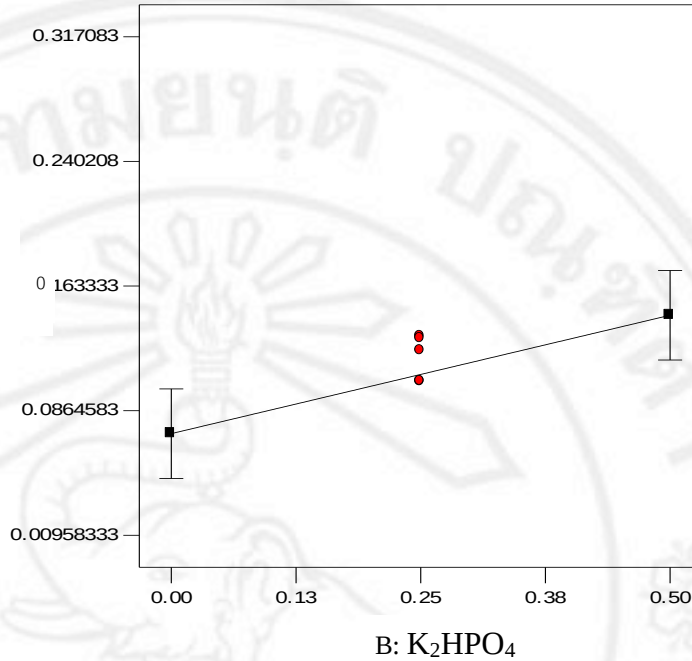
X = B: K₂HPO₄

● Design Points

Actual Factors

A: NH₄Cl = 0.05C: NaNO₃ = 1.00D: MgSO₄ = 0.51

Biomass



DESIGN-EXPERT Plot

One Factor Plot

DW

X = A: NH₄Cl

● Design Points

Actual Factors

B: K₂HPO₄ = 0.25C: NaNO₃ = 1.00D: MgSO₄ = 0.51

Biomass

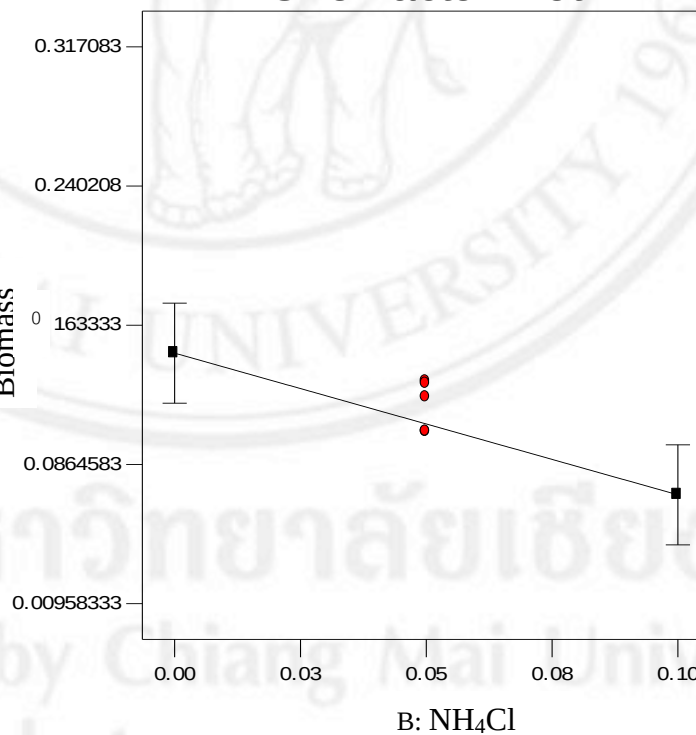


Figure 16 Effect of factors on biomass:

(A) Effect of K₂HPO₄

(B) Effect of NH₄Cl

On other hand, the effect of 4 factors on the lipid productivity were analyzed from the ANOVA of quadratic regression model that is significant [$(p > F) < 0.2$]. Each coefficient is shown in Table 13 on p value, listed.

Table 13 Model coefficient estimated by quadratic regression

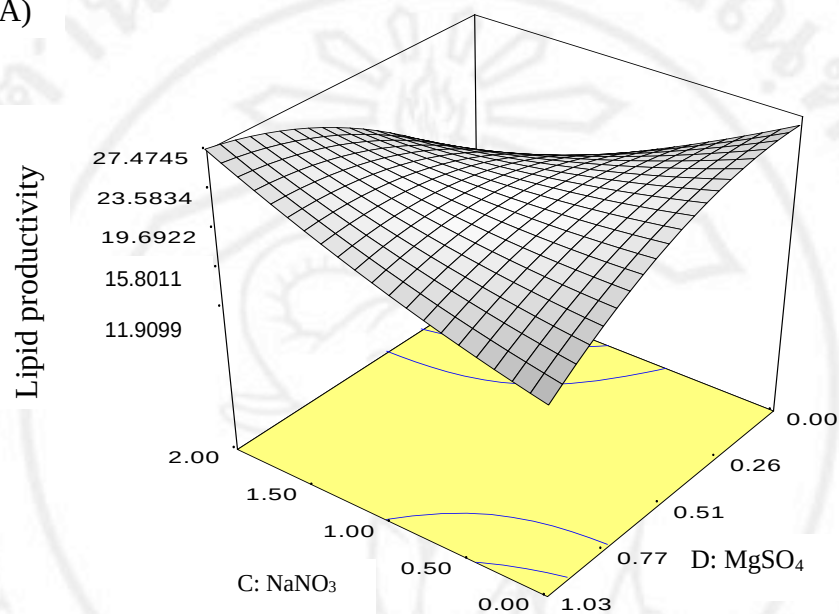
Factor	Sum of Squares	Coefficient Estimate	Standard Error	Prob > F
Model	1263.8809			0.1010*
A-NH ₄ Cl	2.6133	0.4667	1.93190	0.8126
B-K ₂ HPO ₄	343.4729	5.3500	1.93190	0.0151***
C-NaNO ₃	7.683	-0.8002	1.93190	0.6850
D-MgSO ₄	19.7696	1.2835	1.93190	0.5172
A ²	29.3416	-2.1269	2.62766	0.4318
B ²	287.0052	-6.6518	2.62766	0.0240***
C ²	5.53399	0.9237	2.62766	0.7304
D ²	49.1004	-2.7513	2.62766	0.3128
AB	24.01	2.4500	3.34614	0.4761
AC	198.81	-7.0500	3.34614	0.0536**
AD	60.84	3.9000	3.34614	0.2633
BC	9.61	1.5500	3.34614	0.6503
BD	79.2075	4.4499	3.34614	0.2048
CD	168.9355	6.4988	3.34614	0.0725**

* Significant at level, $p < 0.2$; ** Significant at level, $p < 0.1$; *** Significant at level, $p < 0.05$

The 3D contour plots (Figure 17) demonstrates the interactions between each factor. Positive coefficient was found in an interaction between NaNO₃ and MgSO₄ (CD) in Figure 17A ($p < 0.1$). In contrast, an interaction between NaNO₃ and NH₄Cl

(Figure 17B) affected negatively on lipid productivity. It seems likely that K_2HPO_4 was a significant factor for lipid productivity. The actual lipid productivity in the cells increased, while K_2HPO_4 was added at higher amount than normal.

(A)



(B)

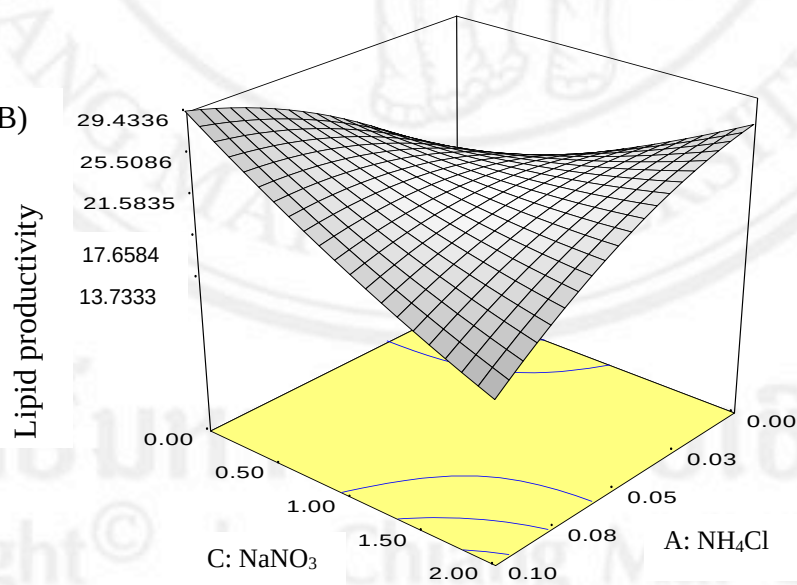


Figure 17 Interaction of factors for lipid productivity:

(A) Effect of $NaNO_3$ and $MgSO_4 \cdot 7H_2O$

(B) Effect of $NaNO_3$ and NH_4Cl

The nutrient components in the media such as organic and inorganic carbon sources, phosphorus, nitrogen, and some macro- and micro-nutrients like magnesium and iron influenced the growth rate and involved in lipid accumulation (Li *et al.*, 2008; Grobbelaar, 2004). From previous study, the algae grown under various N:P ratios showed different growth rates, lipid contents and cell sizes (Verma and Acharya, 2004). Many studies reported that the increase in lipid accumulation in algae was due to the nutritional stress (Li *et al.*, 2008). Thus, the nutrient concentrations which were suitable for algal growth might not enhance the lipid production.

Some nutrients, especially, nitrogen is the most important parameter on biomass production. Besides, nitrogen depletion leads to a decrease in chlorophylls of the cells but increase in the carotinoids and accumulation of organic carbon compounds such as polysaccharides and oils in the form of polyunsaturated fatty acids (Becker, 1994). Lipid content normally increases under N limited condition while the growth is suppressed (Hu *et al.*, 2008).

Phosphorus is another factor which is essential for growth and some metabolism in the cell such as electron transfer, biosynthesis of nucleic acids and DNA (Grobbelaar, 2004). An *et al.* (2003) reported a noticeable increase in the amount of hydrocarbon production in *Botryococcus braunii* under the presence of excess phosphate. Moreover, in this study, MgSO_4 was another factor needed to be optimized. Mg is also very important in biological system. It is required as co-factor in many enzymes and especially, for chlorophyll synthesis (Maeschner, 1995). The increase in MgSO_4 concentration supported algal biomass production, as could be noticed from PBD. However, excess MgSO_4 could reduce the lipid productivity.

Conversely, NH_4Cl has the negative effect on algal growth but positive on the percentage of lipid content. This is because an increase in NH_4Cl concentration could be toxic and a stress for algal cells (Ip *et al.*, 1982). Therefore this results in biomass production and may induce an increase in lipid accumulation. Therefore, the balance between nutrient concentration such as K_2HPO_4 , NaNO_3 , MgSO_4 which increase biomass production and enhanced lipid accumulation such as NH_4Cl are required.

However, in this study it was aimed to enhance the lipid productivity. The highest lipid productivity was predicted by the Design Expert program as 32.04 mg.L^{-1} . The maximum productivity could be obtained in AM containing NH_4Cl 0.03 g.L^{-1} , NaNO_3 2 g.L^{-1} , K_2HPO_4 0.4 g.L^{-1} and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1.03 g.L^{-1} (Table 14)

Table 14 Prediction of productivity by Design Expert program

Product	Prediction	S E Mean	95%CI low	95%CI high	95% PI low	95% PI high
Dry weight (g.L^{-1})	0.19	0.044	0.088	0.30	0.044	0.35
Lipid content (%)	13.19	11.16	-10.75	37.13	-21.76	48.14
Lipid yield (mg.L^{-1})	32.02	6.29	18.53	45.51	12.32	51.72

4.3.2.2 Validation of the optimized condition

The optimized values of each nutrient composition from Design Expert program were verified in triplicate sets of experiments and it was shown that the mean of maximum lipid productivity obtained was 35.04 mg.L^{-1} while the biomass was 0.17 g.L^{-1} which agreed well with the predicted value. *Carteria* sp. AARL G045 which was cultivated with optimized AM represented the amount of lipid droplets inside

their cells (Figure 18A) compared with normal cells grown in normal AM (Figure 18B).

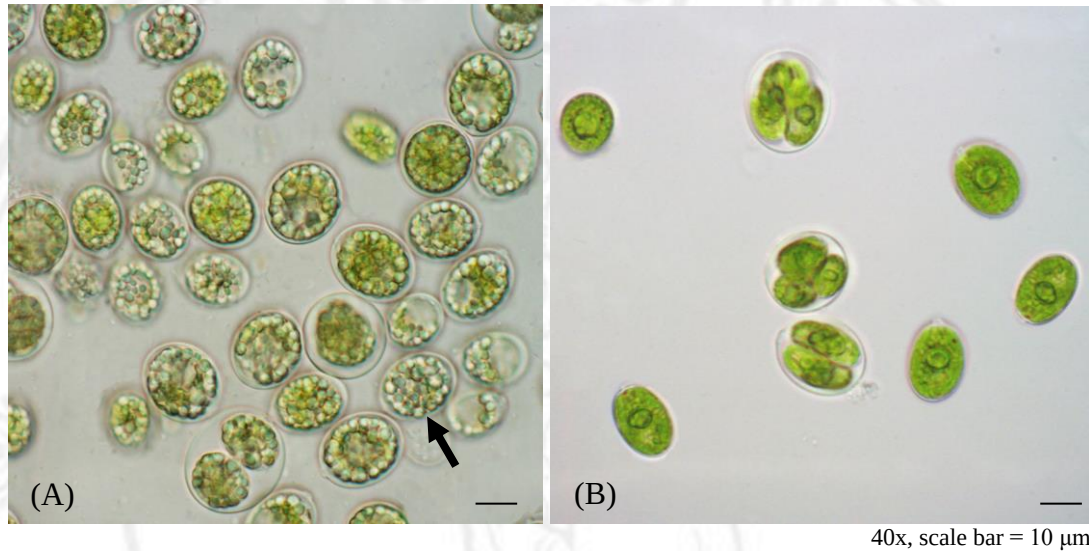
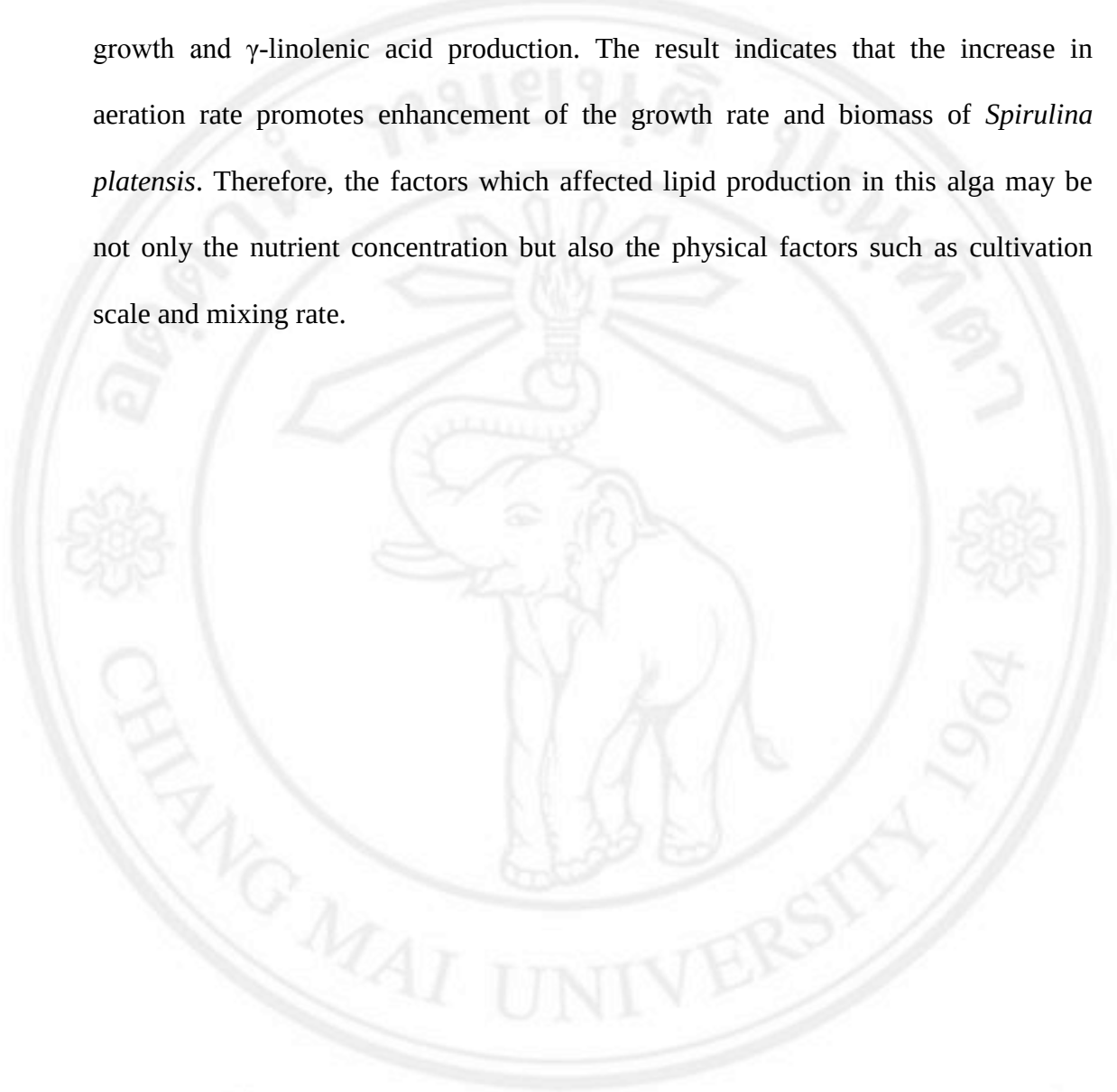


Figure 18 Lipid droplets in *Carteria* sp. AARL G045 cultivated with optimized AM (A) compared with normal cells grown in normal AM (B)

In addition, the batch experiment was performed in 5 L open tank under the optimized conditions with continuous aeration by air bubble and illumination. However, the biomass and lipid productivity obtained were 0.28 g.L^{-1} and 28.48 mg.L^{-1} , respectively. Lipid productivity was less than that cultured in the flask because the aeration rate in the tank (air pump) differed from that in the flask (shaking). Hence, better mixing in the tank would encourage better growth of microalgae and the high growth reduced lipid accumulation in the cells. Mixing is essential to prevent sedimentation of algal cells, encourage a stimulation of the nutrient uptake (Schumacher and Whitford, 1965) and increase effective utilization of light (Gates and Borchardt, 1964).

Ronda *et al.* (2012) studied in the effect of aeration on *Spirulina platensis* growth and γ -linolenic acid production. The result indicates that the increase in aeration rate promotes enhancement of the growth rate and biomass of *Spirulina platensis*. Therefore, the factors which affected lipid production in this alga may be not only the nutrient concentration but also the physical factors such as cultivation scale and mixing rate.



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

Copyright© by Chiang Mai University
All rights reserved