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Factorial Design of Experiment for Biofuel Production by *Isochrysis Galbana*

Jen-Jeng Chen[1+], Yu-Ru Li[2], Meng-Zhi Xie[3], Chun-Yen Chiu[1], Shao-Wei Liao[4] and Wen-Liang Lai[1]

[1] Department of Environmental Resources Management, Tajen University, Pingtung 90741, Taiwan

[2] Graduate Institute of Bioresources, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan

[3] Department of Pharmacy and Graduate Institute of Pharmaceutical Technology, Tajen University, Pingtung 90741, Taiwan.

[4] Department of Tourism Management, Tajen University, Pingtung 90741, Taiwan

Abstract. The high biomass production of microalgae has greatly increased into attention as source of biofuel. Optimization of biofuel production, in terms of lipid content and calorific value, from microalgae was performed by varying four variables (temperature, light intensity, nitrogen content, and CO_2 addition) using a 2^4 full factorial design. A statistical analysis showing the influence of each variable and their interactions was conducted. The selected variables all influence biofuel production, the most significant variable is the CO_2 addition, light intensity, for cell growth rate and biofuel production, respectively. Interactive effects of light intensity with CO_2 addition for biofuel production were identified. 5 days was found to be the most favorable cultivation time for biomass under the investigated conditions.

Keywords: Biofuel, Factorial design, Microalgae.

1. Introduction

Microalgae, which has higher biomass production and faster growth than those of other energy crops, has attracted a lot of attention as a potential source of biofuel. There are several types of biofuel, including biomethane produced by the anaerobic digestion of an algal biomass, photobiologically produced biohydrogen, and biodiesel derived from microalgal oil [1]. Microalgae are photosynthetic microorganisms that convert sunlight, water, and carbon dioxide into algal biomass. Microalgae can reduce land use and do not require agricultural land.

All microalgae contain proteins, carbohydrates, lipids, and nucleic acids in varying proportions. Many microalgae are exceedingly rich in lipid content, which can be converted into biofuel [2]. The lipid content of microalgae varies in accordance with culture conditions. Several factors influence the lipid content of microalgae, including light (quality and quantity), temperature, nutrient concentration, O_2, CO_2, pH, salinity, and toxic chemicals. Few systematic studies have been conducted on the effects of these factors on the lipid content and growth rate of microalgae.

Light and temperature are major processing factors that affect the overall biomass production and biochemical composition of microalgae [3]. The effects of light and temperature are synergistic. Sandnes [4] observed that growth rates of *Nannochloropsis oceanica* increased with increasing light intensity at temperatures up to approximately 28 ºC. At low light intensity, the growth rate is less affected by temperature. For a number of microalgae, CO_2 is the only carbon compound which can support growth. Although the

[+] Corresponding author. Tel.: + 886-8-7624002 ext. 2613; fax: +886-8-7624002 ext. 5055.
E-mail address: jerry@mail.tajen.edu.tw

growth rate increased with increasing CO_2 concentration, relatively low CO_2 concentration favored lipid accumulation. Nitrogen limitation also improves the lipid accumulation of microalgae. Nitrogen limitation can result in a gradual change of lipid composition from free fatty acids to triacylglycerol.

Therefore, the optimum lipid content and growth rate of microalgae depends on the species. The relationship between the factors of cell conditions is complicated and difficult to identify from simple experiments. The present study was undertaken to examine the correlations among the factors (temperature, light intensity, nitrogen nutrient content, and carbon dioxide concentration) that affect the growth rate and lipid content of biomass using a full factorial design method to optimize biofuel production. The results predicted by the multiple regression analysis method are compared with experimental results.

2. Methods and Materials

2.1. Microalgal Culture

One of the dominant microalgal diatom, *Isochrysis galbana*, was cultured in a medium according to the method presented by Laing [5]. Axenic cultures of *Isochrysis galbana* were grown in batch mode in a 1-L modified serum bottle containing 800 mL of sterilized algal medium. The cultures were performed in an incubator according to the experimental design matrix. CO_2 was supplied to the cultures every day. Except for the growth phase stage test, cultures were harvested in the log growth phase after 7 days for experiments.

2.2. Biomass Concentration and Growth Rate

The optical density of microalgal cells was determined daily by measuring absorbance at 680 nm (OD_{680}) using an ultraviolet/visible spectrophotometer (Model U-2001, Hitachi, Japan). The dry weight of the microalgal biomass was determined gravimetrically. A known volume of microalgal culture was collected and dried at 90 °C for 3 hours. The growth rate (μ) was calculated according to the equation, $\mu = (\ln A1 - \ln A0) / (T1 - T0)$, where A1 and A0 are the dry weights of the microalgal biomass at times T1 and T0, respectively.

2.3. Experimental Design

A 2^4 full factorial design (FFD) was used to optimize the biofuel production [6]. The FFD was used to determine the joint effects of several factors on a response. It was also used to determine the individual and cumulative effects of these variables and the mutual interactions between them. The complete design consisted of 16 runs, which were performed in duplicate to optimize the levels of selected variables. Data processing and calculations were carried out using a commercial statistical package, STATISTICA 9.0, to estimate the coefficients of the regression equation.

2.4. Analytical Methods

A stock culture of *Isochrysis galbana* cells was collected by centrifugation at 5000 × g for 5 min. The precipitated algal cells were then washed and resuspended in deionic water in triplicate. Cells were collected by centrifugation again and then dried by a freeze dryer. The microalgal total lipids were extracted with n-hexane/methanol (2/1, v/v) in a Soxhlet extractor and quantified gravimetrically. The precipitated algal cells were dried by a freeze dryer at -80 °C at about 30 Pa. The calorific value was determined using an automatic adiabatic bomb calorimeter (C 2000 Basic, Germany). The sample was combusted in the bomb calorimeter. Filters without algae and a substance with a known calorific value were used to calibrate the calorimeter**.**

3. Results and Discussion

3.1. Growth of Microalgal Culture

Fig. 1 shows the effect of cultivation time of microalgae on the biomass concentration. The growth curve in optical density is in agreement with biomass concentration, which reaches a stationary phase as from 9 day of cultivation, the maximum cell densities being obtained in this period. However, the growth rate decreased from 0.235 d^{-1} during the first 5 days of cultivation to 0.061 d^{-1} at the end of cultivation. It was observed that a large

decrease in growth rate occurred after day 5 of cultivation. This result suggests that 5 days is the most favorable cultivation time for biomass production under the investigated conditions.

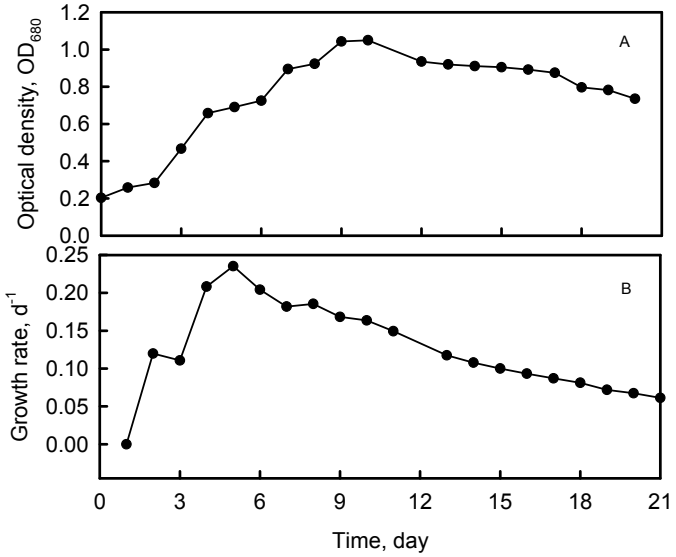

Fig.1: (A) Optical density and (B) growth rate of microalgae as functions of culture time.

3.2. Effects of Factors on Biofuel Production

The experimental results of growth rate, lipid content, and calorific value of the biomass in each case are presented in Table 1 along with the results predicted by the model with the help of STATISTICA® 9.0. The mathematical model representing the biofuel production as a function of the test variables in the experimental region is expressed by the following equations:

$$Y_{GR} = 0.2092 - 0.0007X1 + 0.0027X2 + 0.0021X3 - 0.0199X4 - 0.0002X1X2 - 0.0001X1X3 + 0.0007X1X4 - 0.0001X2X3 + 0.0002X2X4 - 0.0001X3X4 \quad (1)$$

$$Y_{LP} = 96.594 - 3.391X1 + 0.196X2 + 0.149X3 - 14.84X4 - 0.004X1X2 - 0.003X1X3 + 0.705X1X4 - 0.052X2X3 + 0.177X2X4 - 0.01X3X4 \quad (2)$$

where Y_{GR}, Y_L, and Y_C are the growth rate (d^{-1}), lipid content (%), and calorific value (Kcal Kg^{-1}), respectively. The X1, X2, X3, and X4 are the coded values of the main effects temperature, light intensity, nitrogen content, and CO_2 addition, respectively. Whereas the variables X1X2, X1X3, X1X4, X2X3, X2X4, and X3X4 represent the interaction effect of test variables. A positive term in the equations indicates a synergistic effect (i.e., increasing the variable increases the biofuel production), whereas a negative term indicates an antagonistic effect (i.e., increasing the variable decreases the biofuel production).

The results show individual effects of the combinations of the test variables, with significant variation between combinations. An analysis of these results shows that optimum growth rate, lipid content, and calorific value of the biomass were obtained at Run 16, R 10, and R 1, respectively.

Table 2 shows the effects of and interactions between the test variables. The three R values (0.97, 0.883, and 0.974 for growth rate, lipid content, and calorific value response, respectively) indicate a good fit between the model and the experimental data. Table 3 shows the statistical significance of test variables for biofuel production. The results indicate that the most significant variable is the CO_2 addition, light intensity, for cell growth rate and biofuel production, respectively. Growth rate is controlled by CO_2 addition, the interaction between temperature and light intensity, nitrogen content, and CO_2 addition, and that between nitrogen content and CO_2 addition. F-values with a very low probability ($p < 0.05$) indicate a very high significance for the corresponding coefficients. The significance of a coefficient increases with increasing magnitude of the F-value and decreasing P-value. The variable with the largest effect on growth rate was CO_2 addition. Growth rate increased by 0.032 d^{-1} on average when CO_2 addition was increased from 10 to 20 mL L^{-1} d^{-1}.

Lipid content is controlled by the interaction between light intensity and CO_2 addition ($p < 0.1$). Lipid content increased up to 3.93 % when light intensity was increased from 40 to 80 μmol photons m^{-2} s^{-1} accompanying with CO_2 addition was increased from 10 to 20 mL L^{-1} d^{-1}. For calorific value, only light

intensity, light intensity-CO_2 addition interaction, were significant ($p < 0.1$). Light intensity had the most important effect on calorific value.

Table 1: Factorial experimental matrix of observed and predicted biofuel yield for *Isochrysis galbana*.

Run number	Growth rate (d^{-1})		Lipid productivity (mg L^{-1} d^{-1})	
	Obs.	Pred.	Obs.	Pred.
1	0.155	0.154	26.76	24.18
2	0.149	0.150	20.74	23.33
3	0.150	0.151	19.94	22.53
4	0.092	0.091	8.84	6.25
5	0.136	0.137	18.36	20.95
6	0.150	0.148	21.60	19.02
7	0.131	0.130	14.12	11.54
8	0.124	0.125	12.48	15.07
9	0.131	0.132	15.09	17.67
10	0.181	0.180	45.40	42.81
11	0.169	0.168	42.76	40.17
12	0.121	0.122	12.63	15.22
13	0.133	0.132	16.44	13.86
14	0.209	0.211	25.52	28.10
15	0.186	0.187	48.57	51.16
16	0.213	0.212	38.79	36.20

Table 3: Significant influences for biofuel production.

Response	Significant influences
Growth rate	X_4; X_1X_2; X_1X_3; X_1X_4; X_3X_4
*Lipid content	X_2X_4
*Calorific value	X_2; X_2X_4

*Confidence level: 90 %

4. Conclusions

Four variables with two-level full factorial design were used to determine the effects of temperature, light intensity, nitrogen content, and CO_2 addition on biofuel production from microalgae. Results show that within the experimental range considered, the most important factor for biofuel production (in terms of lipid content and calorific value) is the CO_2 addition, light intensity, for cell growth rate and biofuel production, respectively. Temperature and nitrogen content are also significant for biofuel production.

The predictive model developed to describe the relation between the response and the variables allows the identification of statistically significant variables and the quantitative evaluation of the effect of each variable on biofuel production and the interactions between two variables. The interactive effects of light intensity with CO_2 addition for biofuel production were identified. In addition, it was found that 5 days is the most favorable cultivation time for lipid production under the investigated conditions.

5. Acknowledgement

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6. References

[1] Y. Chisti. Biodiesel from microalgae. Biotechnology Advances 2007, **25**: 294-306.

[2] J. Singh, S. Gu. Commercialization potential of microalgae for biofuels production. Renewable and Sustainable Energy Reviews 2010, **14**: 2596-2610.

[3] P. Carvalho, M. Cristina, F. Monteiro, X. Malcata. Simultaneous effect of irradiance and temperature on biochemical composition of the microalga *Pavlova lutheri*. J Appl Phycol 2009, **21**: 543-552.

[4] J. M. Sandnes, T. K¨allqvist, D. Wenner, & H. R. Gislerød. Combined influence of light and temperature on growth rates of *Nannochloropsis oceanica*: linking cellular responses to large-scale biomass production. Journal of Applied Phycology 2005, **17**: 515-525.

[5] Laing: Ministry of Agriculture, Fisheries and Food Directorate of Fisheries Research Laboratory, Lowestoft Vol. 67 1991, pp. 31.

[6] G.E.P.Box, W.G.Hunter, J.S.Hunter: Statistics for experiments: An introduction to design, data analysis, and model building (John Wiley & Sons, New York 1978), p. 374-1135.

Table 2: Estimated main effects and interactions for biofuel production.

	Term	Effect	Standard error	T-value	P-value
Growth rate	Mean	0.152	0.003	43.997	0.000
	X_1	0.006	0.007	0.863	0.428
	X_2	-0.007	0.007	-1.055	0.340
	X_3	0.017	0.007	2.406	0.061
	X_4	0.032	0.007	4.668	0.005
	X_1X_2	-0.028	0.007	-4.006	0.010
	X_1X_3	0.022	0.007	3.136	0.026
	X_1X_4	0.020	0.007	2.950	0.032
	X_2X_3	0.014	0.007	1.969	0.106
	X_2X_4	0.016	0.007	2.311	0.069
	X_3X_4	0.018	0.007	2.641	0.046
					R = 97.0 %
Lipid content	Mean	25.157	0.797	31.550	0.000
	X_1	-1.645	1.595	-1.031	0.350
	X_2	-2.927	1.595	-1.835	0.126
	X_3	-2.146	1.595	-1.346	0.236
	X_4	0.028	1.595	0.017	0.987
	X_1X_2	-3.206	1.595	-2.010	0.101
	X_1X_3	-0.920	1.595	-0.577	0.589
	X_1X_4	-1.087	1.595	-0.682	0.526
	X_2X_3	0.976	1.595	0.612	0.567
	X_2X_4	3.930	1.595	2.465	0.057
	X_3X_4	-0.641	1.595	-0.402	0.704
					R = 88.3 %
Calorific value	Mean	3183.301	125.163	25.433	0.000
	X_1	-419.160	250.326	-1.674	0.155
	X_2	-611.729	250.326	-2.444	0.058
	X_3	-370.853	250.326	-1.481	0.199
	X_4	-102.629	250.326	-0.410	0.699
	X_1X_2	-207.691	250.326	-0.830	0.445
	X_1X_3	-71.370	250.326	-0.285	0.787
	X_1X_4	-46.411	250.326	-0.185	0.860
	X_2X_3	126.633	250.326	0.506	0.634
	X_2X_4	541.568	250.326	2.163	0.083
	X_3X_4	-12.077	250.326	-0.048	0.963
					R = 97.4 %