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MODELLING OF MICROALGAE GROWTH AND OIL PRODUCTION BY USING SEWAGE AND CARBON DIOXIDE FOR BIODIESEL

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ABSTRACT

With the depletion of the world fossil oil reserves, microalgae biodiesel might become one of the economic, renewable and carbon-neutral sources of transportation biofuels. This study investigates marine microalgae growth and biodiesel production by cultivating highly productive oleaginous microalgae at large scale using sewage as nutrient supply and waster CO_2 as carbon source. Five strains of marine microalgae will be screened in the laboratory for their biomass productivity and lipid content. The best strain will be optimised for the cultivation parameter, including initial pH value, dissolved carbon dioxide concentration, proportion of sewage mixture and illumination intensity. The result showed Chlorella and Nannochloropsis Local have the better biomass production.

Keywords： Microalgae, Sewage, Carbon Dioxide, Biodiesel

INTRODUCTION

The enormity of energy crisis has multifolded dramatically over the past decade (Behera, 2003), as well as the environmental consequences of exhaust gases from fossil diesel, modern bioenergy has gained increased attention(Ma and Hanna, 1999). Biomass is one of the better sources of energy (Kulkarni and Dalai, 2006). Large-scale introduction of biomass energy could contribute to sustainable development on several fronts, environmentally, socially and economic(Turkenburg, 2000). Biodiesel (monoalkyl esters) is one of such alternative fuel, which is obtained by the transesterification of triglyceride oil with monohydric alcohols. It has been well-reported that biodiesel obtained from canola and soybean, palm, sunflower oil, algal oil as a diesel fuel substitute(Lang et al., 2002; Spolaore, 2006). Unfortunately, biodiesel derived from oil crops, waste cooking oil, and animal fat can realistically satisfy only a small fraction of the existing demand for transport fuels(Chisti, 2007).

In view of Table1(Chisti, 2007), take USA for example, oil crops cannot significantly contribute to replacing petroleum derived liquid fuels in the foreseeable future[7], microalgae appear to be the only source of biodiesel that has the potential to completely displace fossil diesel. Unlike other oil crops, microalgae grow extremely rapidly and many are exceedingly rich in oil. Microalgae commonly double their biomass within 24 h. Biomass doubling times during exponential growth are commonly as short as 3.5 h. Oil content in microalgae can exceed 80% by weight of dry biomass[7].

Table 1: Comparison of some of biodiesel

Crop	Oil yield (L/ha)	Land area needed (M ha)[a]	Percent of existing US cropping area[a]
Com	172	1540	846
Soybean	446	594	326
Canola	1190	223	122
Jatropha	1892	140	77
Coconut	2689	99	54
Oil palm	5950	45	24
Microalgae[b]	136,900	2	1.1
Microalgae[c]	58,700	4.5	2.5

[a] For meeting 50% of all transport fuel needs of the United States.
[b] 70% oil (by wt) in biomass.
[c] 30% oil (by wt) in biomass.

So far, microalgae oil is not a mature technology. The main question still remaining is how to bridge the gap between the high current cost of algae biomass production, about US$5kg-1 being the lowest possible, and the very low required for biofuel production(about US$0.25kg^{-1} prior to conversion to biofuel (Rodolfi, 2009). So the most important issue should be decreasing the cost of the biomass production. At the same time, poor sewage treatment has been blamed as being one of the causes of corals slowly dying in the sea off the coast of Malaysia, as algae was found to have smothered some reefs, indicating nutrient (Li, 2008). The power sector is polluting too, with Malaysia ranked 24th among the top 50 countries with the highest CO_2 emitting power sectors, emitting 61,100,000 tons of CO_2 per year (CARMA, 2009).In the USA, process integration was set up in the open pond, including CO_2 capture, water treatment and utilization of multiple compounds accumulated by cell in addition to the lipids. The similar system of the biomass production for biodiesel is well worth investigating in Malaysia.

EXPERIMENTAL SET UP

Material

Microalage

Five strains of 20 ml marine microalgae were collected from Jabatan Perikanan Malaysia. They are Tilapia, Nannochloropsis Taiwan, Nannochloropsis Local, Chlorella, Isochrysis Tahiti.

Medium

F/2 medium was used in culture maintaining the five microalgae, as shown in the Table.2. Seawater was collected from Teluk Cempedak Beach, Kuantan, Malaysia. Reconstituted model of sewage was made by mixture up the dairy pollution discharge pond, secondary treatment plant.

Table 2: Composition and preparation of Guillard's F/2 medium (modified from Smith *et al.*, 1993a).

Nutrients	**Final concentration (mg.l^{-1} seawater)[a]**
$NaNO_3$	75
$NaH_2PO_4.H_2O$	5
$Na_2SiO_3.9H_2O$	30
$Na_2C_{10}H_{14}O_8N_2.H_2O$ (Na_2EDTA)	4.36
$CoCl_2.6H_2O$	0.01
$CuSO_4.5H_2O$	0.01
$FeCl_3.6H_2O$	3.15
$MnCl_2.4H_2O$	0.18
$Na_2MoO_4.2H_2O$	0.006
$ZnSO_4.7H_2O$	0.022
Thiamin HCl	0.1
Biotin	0.0005
B_{12}	0.0005

METHOD

Microalgae Cultivation in the Flask

F/2 medium are prepared and filtered through 0.2µm filter. All the culture materials are autoclaved under 121℃ for 20min, including the flask, air filter and silicon rubber tubing. 20ml of microalgae is poured into a flask containing 250ml of fresh culture medium. All operation should be conducted in the biohazard safety cabinet in case of contamination. Five strains of the microalgae was aerated with filtered air(70 l/min) and cultivated with the same condition of temperature 22±2℃, 2000 lx illumination intensity 12:12 hr photoperiods for 7days, as shown in the Fig.1. The growth of microalgae culture is

measured by both the cell density using hemocytometer and by the cell dry weight.

Figure 1: Microalgae Culture: Small Scale in the Flask

Cell Density Measurement

Cell Density was measured by using the Neubaur-Improved hemocytometer. 1ml of the sample was transferred into the test tube, and diluted with 9ml of seawater. After mixing, 1ml of the mixture was further diluted with 9 ml of seawater, which made up a total dilution of 100 times.10µl of this mixture was transferred to the hemocytometer for cell counting.

Cell Dry Weight Determination

Cells are harvested by centrifugation at 10,000rpm, washed twice with distilled water, and dried using the freeze dryer at minus 65 for 36h. The dry weight of five algae biomass is determined gravimetrically and growth is expressed in terms of dry weight.

FUTURE WORK

Future work included three sections, measurement of the lipid content, optimisation of the microalgae growth parameters and open pond.

Measurement of the Lipid Content

Lipid measurements will be used a method adapted from Bligh and Dyer (Bligh and Dyer, 1959). This method extracts the lipids from the algal cells by using a mixture of methanol,

chloroform, and water. The culture sample is centrifuged at 8,000 rpm for 10 minutes in a large (200 ml) plastic centrifuge tube. The pelleted cells along with 35ml of supernatant will then transfer to a centrifuge tube (50 ml) to be re-centrifuged at 8,000 rpm for 10 minutes. The supernatant will removed by pipette. The pellet will be dried by freeze dryer to get the weigh. then sample will be resuspended with 4ml of distilled water, then 10ml of methanol and 5 ml of chloroform will be added, resulting in a 10:5:4 ratio of methanol: chloroform: water. At this ratio, all solvents will be miscible and form one layer, homogenizer will be used to break the cell wall for 2 h. Then after extraction on a shaker table for 2 h, 5 ml of water and 5ml of chloroform are added which results in a 10:10:9 ratio of methanol: chloroform: water. Tubes will be centrifuged for 10 minutes at 8,000 rpm. At this solvent ratio, two layers will be formed, a water methanol upper layer and a chloroform lower layer. The chloroform lower layer which contains the extracted lipids will be then removed by Pasteur pipette and placed into a pre-weighed vial. After the first extraction, 10ml of additional chloroform will be added to conduct a second extraction. The additional 10ml of chloroform again results in a 10:10:9 methanol: chloroform:water ratio and two layers are formed. The tube will be centrifuged at 8,000 rpm for 10 minutes, and the lower chloroform layer will be removed by Pasteur pipette and placed into the same pre-weighed vial. Repeat this extraction step again and then 30ml of chloroform dissolved with lipid can be collected. The chloroform will be evaporated by heating in a 55°C water bath under a constant stream of nitrogen gas. After 1 hour in a 105°C oven, vials will be weighed again. The weight difference represents weight of lipids extracted from the culture sample. Percentage of lipid content can be determined by measuring the dry weight of the culture sample at the same time as the lipid analysis.

Optimisation of Microalgae Growth Parameters

The optimisation of microalgae growth parameter will be done in 2 parts:

Optimise the Proportion of the Mixture with Sewage

Fix the initial pH value 7.0, illumination intensity1750lx, 12:12 hr photoperiods, room temperature, cultivate the microalgae in the fermentor with the proportion of mixture F/2 medium and sewage at 0:10, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1. Freeze dry the microalgae after cultivation for 7days. The best strain will be selected based on the highest lipid content obtained.

Optimise the Condition of Illumination

Intensity(X_1), initial pH Value(X_2) and CO_2 flow rate(X_3), dissolved CO_2 concentration will be measured on the CO_2 system. CO_2 will be mixed with the air. Y represents the lipid content of the biomass. Experiments are designed based on 2^3 Factorial, as shown in the Table 2, with the values of variable as in Table 3.

Table 2 : 2^3 Factorial Design

	X_1	X_2	X_3	Y
1	–1	–1	–1	
2	+1	–1	–1	
3	–1	+1	–1	
4	+1	+1	–1	
5	–1	–1	+1	
6	+1	–1	+1	
7	–1	+1	+1	
8	+1	+1	+1	

Table 3: Range of Variable Study

	α=–1	α=0	α=+1
X_1	1000 lx	1500 lx	2000 lx
X_2	pH6	pH7	pH8
X_3	1 L/min	2L/min	3L/min

Open Pond Culture

Figure 2 shows a detail of the Algae Open Breeding Pond. In the middle of the pond there is a dike to be able to circulate the water in the pond by means of a paddle wheel. The plastic liner will be installed on the bottom of pond, over the dike in the middle of the pond and over and on top pf the surrounding dikes. The gas pipeline is installed at the bottom of the sink, carbon dioxide will be mixed with air tubing into the gas pipeline. The depth of the pond is designed as 20 cm.

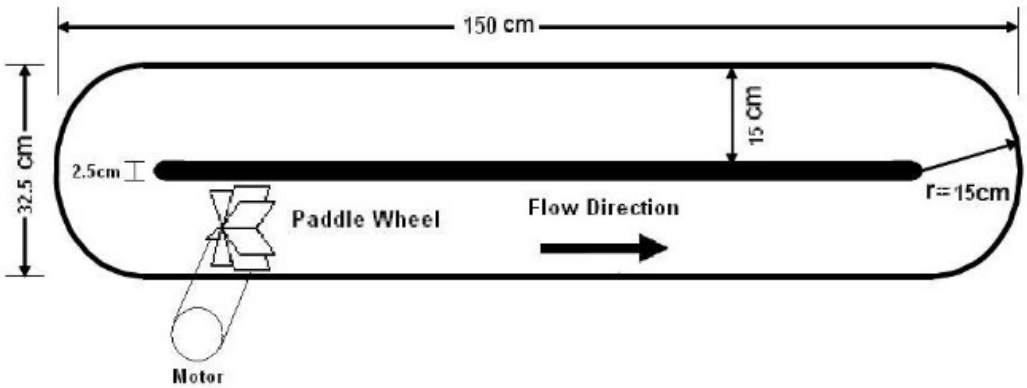

Figure 2: Design of the Open Pond Culture

RESULTS AND DISCUSSION

In the small scale, five strains of the microalgae dry powder have been harvested, the weight are shown in Table 4, the Chlorella have the largest mass of biomass. Lipid content

will be later to measure to determine which strain of them has the highest oil productivity. The growth curve was shown in Figure 3. Both Table 4 and Figure 3 clearly showed that Chlorella and Nannochloropsis Local have the better biomass production.

Table 4: Weight of Five Strains

Microalgae	Weight(mg)
Tilapia	30.0
Nannochloropsis Taiwan	30.5
Nannochloropsis Local	35.0
Chlorella	37.0
Isochrysis Tahiti	25.5

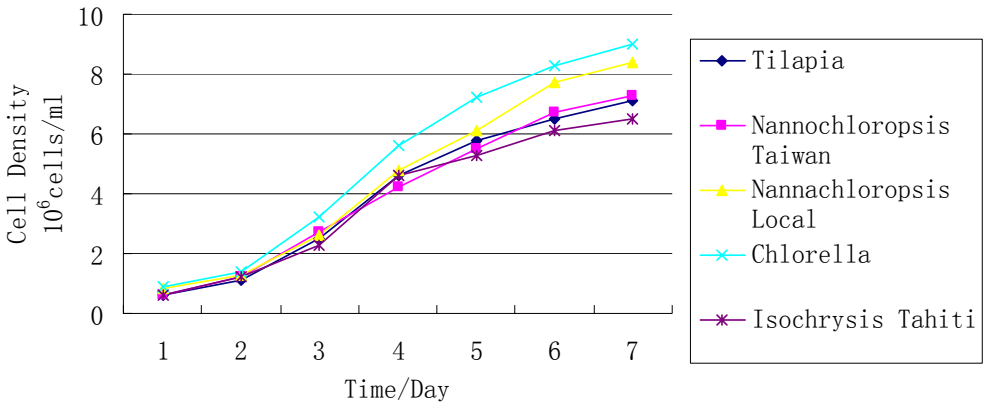

Figure 3: Growth of the five microalgae

In the optimisation section, the relation between the three factors mentioned above will be investigated to identify the optimisation of the micioalgae cultivation. The carbon dioxide system will be used to measure the dissolved CO_2 concentration in the medium to investigate the relation between pH value and dissolved CO_2 concentration. From optimisation in the fermentor to open pond culture, it should be checked regularly to ensure that the microalgae are not contaminated by bacteria.

CONCLUSION

Compared with the results of other strains, Chlorella and Nannochloropsis Local have the largest amount of biomass. They are chosen for the optimisation of oil productivity.

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