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Study of Increasing Lipid Production from Reused Medium for *Ankistrodesmus* sp. Culture

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Abstract. *Ankistrodesmus* sp. was cultivated in fresh medium and reused medium where the quality of algal biomass especially lipid content obtained from the various mediums was compared. Lipid content increased from 32.4 to 57.7% when the medium was reused straight after the first cultivation. Lipid in the 2nd reused medium was also greater than that in the fresh medium. The maximal biomass productivity and lipid productivity from the 2nd reused medium were 79.7 and 33.3 mg L^{-1} d^{-1}, respectively. The depletion of nitrogen source in reused mediums could be an important factor for the accumulation of lipid. Protein and carbohydrate accumulations were found to decrease when the medium was reused which could be as a result of depleted nitrogen source. Two major compositions of fatty acid in this algal biomass are palmitic and oleic acids. The highest oleic acid was 52.1% obtained from the use of the 1st reused medium.

Keywords: *Ankistrodesmus* sp., Lipid productivity, Reused medium

1. Introduction

Microalgae appear to be the promising alternative source for biodiesel production because they are easy to cultivate, can grow with little or no attention, can consume nutrients in water sources unsuitable for human consumption. In addition, mass production is not seasonally limited and they can be harvested daily. Past reports reveal that microalgae could grow in low major macronutrients such as nitrogen and phosphorous, and when cultivated under nitrogen deficient condition, microalgae tended to accumulate oils or polysaccharides [1]. Microalgae have an ability to produce many lipid classes, but triacylglycerols (TAGs) are considered more preferable as raw materials for the tranesterification reaction for biodiesel production.

Biomass productivity, lipid content and lipid productivity are the important parameters affecting the economic feasibility of microalgae for biodiesel production [2]. However, the current large scale cultivation microalga for fuel-oil production is economical infeasible. Major costs lie in the use of energy and the nutrient-medium preparation. Therefore one of the options to minimize production cost is to effectively utilize the nutrient through the reuse of spent medium. *Ankistrodesmus* sp. is an interesting fresh water microalga capable of producing oil due primarily to its high lipid content, about 28-40% of total cell dry weight [3] and [4]. This lipid content could be enhanced when cultivated in the proper growth condition such as light, temperature, CO_2 and nutrient media.

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The principal aim of the present study was to minimize the algal operating cost for the cultivation of green microalgae *Ankistrodesmus* sp. by medium recycling, and to investigate the influence of nutrient on the accumulation and the quality of lipids in *Ankistrodesmus* sp.

2. Materials and methods

2.1. Microalgal Strain and Medium

The strain of green microalgae used for this study, *Ankistrodesmus* sp., was obtained from Microbiological Resources Centre (MIRCEN) of Thailand Institute of Science and Technology Research (TISTR). *Ankistrodesmus* sp. was cultured with the BG11 medium which contains the following components (per liter): 1.5 g $NaNO_3$, 0.04 g $K_2HPO_4 \cdot 3H_2O$, 0.075 g $MgSO_4 \cdot 7H_2O$, 0.036 g $CaCl_2 \cdot 2H_2O$, 0.006 g Citric acid, 0.006 g Ammonium ferric citrate, 0.001 g $EDTANa_2$, 0.02 g $NaCO_3$. Microelement was prepared in the following composition (per liter of nutrient): 2.86 mg H_3BO_3, 1.81 mg $MnCl_2 \cdot 4H_2O$, 0.22 mg $ZnSO_4 \cdot 7H_2O$, 0.39 mg $Na_2MoO_4 \cdot 2H_2O$, 0.08 mg $CuSO_4 \cdot 5H_2O$ and 0.05 mg $Co(NO_3)_2 \cdot 6H_2O$.

2.2. Measurement of Microalgal Growth

The estimation of cell dry weight is one of the most direct ways to determine biomass production. Microalgal cells (25 mL) were collected and filtered through Whatman GF/C filter paper. The filtered biomass was dried at 80°C for 24 hours or until constant weight. The cell dry weight is calculated as follows:

$$\text{Algal dry weight (mg L}^{-1}) = \frac{\text{Wt}_\text{A} - \text{Wt}_\text{B}}{\text{V}} \times 1000 \quad (1)$$

where Wt_A is weight of filter paper and algae (mg), Wt_B is weight of filter paper (mg), and V is the culture volume (mL).

2.3. Lipid Extraction and Lipid Productivity Calculation

Microalgal cells were harvested at stationary growth phase and centrifuged at 2150×g for 5 min and freeze dried until completely dry. Lipids were extracted by adding 180 mL of chloroform/methanol (2:1) for each gram of dried microalga. A soxhlet extractor was operated for the separation of lipid from microalga. After extraction the solvents were removed in a rotary evaporator under 250 mbar and temperature of water bath at 45 °C. Lipid productivity is calculated as follows:

$$\text{Lipid productivity (gL}^{-1}\text{d}^{-1}) = \frac{\text{Lipid content (g)} \times \text{Biomass (gL}^{-1})}{100\text{g} \times \text{time of cultivation (d)}} \quad (2)$$

2.4. Experimental Setup

Ankistrodesmus sp. was cultivated in 3 L batch airlift photobioreactor (at least two replications), with superficial gas velocity (Usg) of 1.33 cm s^{-1}. The dimension of the airlift photobioreactor is as follows: wall thickness of outer column 3 mm, wall thickness of the inner tube 2 mm, inner height 40 cm, outer height 60 cm, inner column i.d. 5 cm and outer column i.d. 10 cm. The experiment was performed in 3 different mediums, i.e. fresh medium, 1st reused medium and 2nd reused medium. The fresh medium was prepared following the recipe provided in the BG11 standard medium [5]. After harvesting at 14 days of cultivation, the medium and microalga were separated by centrifugation at 2150×g for 5 minutes and the supernatant was reused in the next cultivation (1st reused medium). Similar procedure was applied for the 2nd reused medium. Note that our preliminary results suggest that the 3rd reused medium was no longer suitable for the cultivation of such alga.

3. Results

3.1. Growth of *Ankistrodesmus* sp. with different mediums

Figure 1 illustrates the biomass concentration of *Ankistrodesmus* sp. in different mediums (note that the initial biomass inoculum was 0.13 g L^{-1}). The growth profile in the fresh medium was lower than the 1st reused medium and the 2nd reused medium. At day 13, the growth of 2nd reused medium culture was increased rapidly and biomass reached 1.04 g L^{-1}. At the last day of cultivation, the dry weight for 2nd reused

medium was almost 1.5-fold greater than that obtained from the fresh medium. The concentration of nitrogen remaining in the different mediums is shown in Figure 2. A large quantity of nitrogen still remained after the first cultivation. The amounts of nitrogen being consumed in the fresh medium, 1st reused medium and 2nd reused medium (ΔN) were 118.61, 72.07 and 53.95 mg-N L^{-1}.

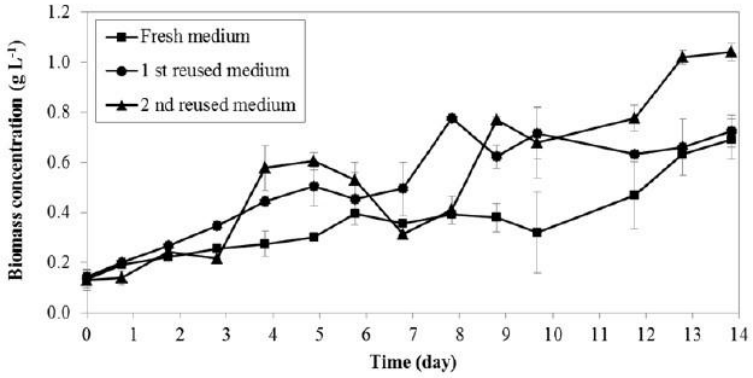

Fig. 1: Growth profile of *Ankistrodesmus* sp. in three different mediums

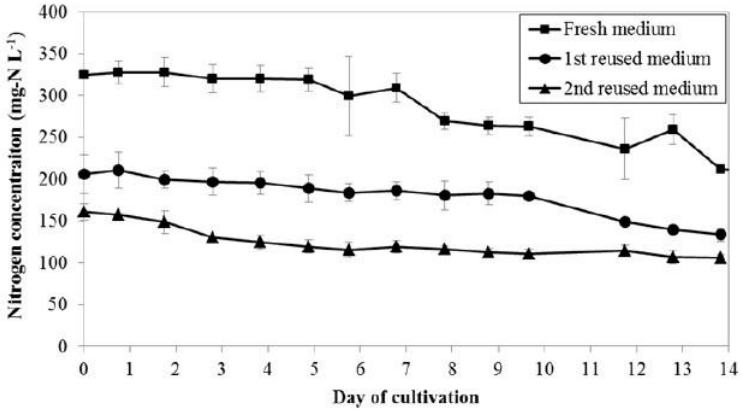

Fig. 2: Nitrogen concentration remaining in the three different mediums

3.2. Biochemical composition

Hexane was used as a solvent for extraction process. The lipid contents obtained from the growth in fresh medium, 1st reused medium and 2nd reused medium were 3.04, 14.41 and 5.35 %, respectively. Biomass productivity and lipid productivity were calculated at the last day of each batch. The lipid productivity was calculated by biomass concentration at last day of cultivation multiplied by lipid content extracted by chloroform and methanol as a solvent. The results as shown in Table 1 show that the maximum biomass productivity was 79.7 mg L^{-1} d^{-1} when cultivated in the 2nd reuse medium, which was about 2-fold higher than the biomass productivity from the fresh medium. Lipid productivity in the 1st reused was similar to that from the 2nd reused medium because both dry weight and lipid content were significant high.

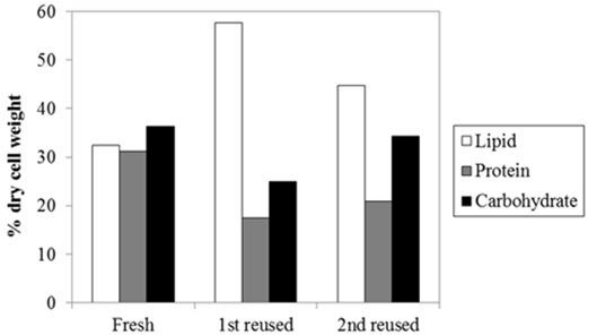

Fig. 3: Biochemical composition of *Ankistrodesmus* sp. cell from fresh medium, 1st reused and 2nd reused medium

The biochemical composition of *Ankistrodesmus* sp. growing in different mediums is shown in Figure 3. The highest lipid content occurred from the batch with the 1st reused medium (57.7%) but the protein content

was the lowest. Protein content declined from 31.2% in the fresh medium to 17.4% in the 1st reused medium because the reduction of nitrate impaired protein synthesis. Similarly, carbohydrate content decreased from 36.4 to 24.9%. Kilham reported that nitrogen limited (low N) cells had low protein content and the lipid content of low P cells was significantly high [6]. These results suggest that *Ankistrodesmus* sp. may accumulate lipids under nitrogen repletion.

Table 1 Performance of biomass productivity and lipid productivity of *Ankistrodesmus* sp. in different mediums

Medium	Max. dry weight ($g\ L^{-1}$)	Lipid content (% dry weight)	Biomass productivity ($mg\ L^{-1}\ d^{-1}$)	Lipid productivity ($mg\ L^{-1}\ d^{-1}$)
Fresh medium	0.69 ± 0.08	32.4	35.5 ± 7.4	16.0 ± 1.9
1st reused medium	0.73 ± 0.06	57.7	44.8 ± 6.3	29.9 ± 2.6
2nd reused medium	1.04 ± 0.03	44.8	79.7 ± 3.4	33.3 ± 0.1

3.3. Fatty acid

In addition to increasing the amount of lipid produced, it is important to obtain an appropriate composition of fatty acid profiles for the lipid produced from *Ankistrodesmus* sp. Table 2 showed the fatty acid profile of *Ankistrodesmus* sp., the C16 and C18 groups accounted for above 80% of the total fatty acids for microalgae cultivated in all mediums. However, about 5-14% of total fatty acids cannot be clearly identified (denoted as "unknown"). The two major compositions, C16:0 (Palmitic acid) and C18:1 (Oleic acid) were significantly high level, thus suitable for biodiesel production [7].

These results show that the maximum palmitic acid of 29.9% was obtained from the fresh medium culture. In 1st reused and 2nd reuse mediums, oleic acid was the majority of the accumulated fatty acids at 52.10 and 39.86%, respectively. In this study, the percentage of unsaturated fatty acid in 1st reused and 2nd reused medium was increased when compared to fresh medium.

4. Conclusion

The result showed that the cells of *Ankistrodesmus* sp. could be well cultivated with reused medium to reduce the cost of production. In terms of oil production, the reuse of nutrient does not seem to have negative effect as the lipid productivity became higher with lower nitrogen content in the reused nutrient. In other words, the biomass productivity and lipid productivity in 2nd reused medium were highest because cell density and lipid content were significant high. Further work is necessary to refine this result and make the whole process more economically viable.

Table 2 Fatty acid profile of *Ankistrodesmus* sp. cultivated in different mediums

Type of fatty acids	%, out of total fatty acid		
	Fresh medium	1st reused medium	2nd reused medium
Caprylic acid (C8:0)	0.1	0.1	0.1
Capric acid (C10:0)	0.1	-	-
Lauric acid (C12:0)	1.4	0.5	0.7
Myristic acid (C14:0)	0.8	0.4	0.4
Palmitic acid (C16:0)	29.9	24.4	23.7
Palmitoleic acid (C16:1)	0.8	0.3	0.4
Oleic acid (C18:1)	20.9	52.1	39.9
Linoleic acid (C18:2)	9.8	7.7	11.0

Gamma linolenic acid (C18:3 (ω6))	0.2	0.5	1.0
Alpha linolenic acid (C18:3 (ω3))	17.0	4.8	9.4
Stearidonic acid (C18:4 (ω3)	3.6	2.9	4.2
Erucic acid (C22:1 (n9))	-	0.1	-
Lignoceric acid (C24:0)	1.8	0.7	0.8
Unknown	13.6	5.5	8.4

5. Acknowledgement

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