การผลิตน้ำมันจากสาหร่ายสีเขียวขนาดเล็กภายใต้การเพาะเลี้ยงแบบเฮเทโรโทรฟิค

Microalgal Oil Production by Green Microalgae under Heterotrophic Cultivation

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บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อผลิตน้ำมันจากสาหร่ายสีเขียวขนาดเล็กภายใต้การเพาะเลี้ยงแบบเฮเทโรโทรฟิค เพื่อประโยชน์ในการใช้เป็นวัตถุดิบเพื่อผลิตไบโอดีเซล สภาวะที่เหมาะสมในการผลิตและสะสมลิปิดของสาหร่ายสีเขียวขนาดเล็กไอโซเลท KKU-S2 คือ อาหารเลี้ยงเชื้อที่มี $NaNO_3$ 0.5 กรัม/ลิตร กลูโคส 50 กรัม/ลิตร หรืออัตราส่วนคาร์บอนต่อไนโตรเจนที่ 280 ภายใต้สภาวะดังกล่าวสาหร่ายให้ปริมาณเซลล์ 6.3 กรัม/ลิตร อัตราการเจริญจำเพาะ 0.229 d^{-1} ปริมาณลิปิด 47.8 % โดยน้ำหนักเซลล์แห้ง ระยะเวลาเพาะเลี้ยง 8 วัน เมื่อวิเคราะห์องค์ประกอบของลิปิดที่สกัดได้จากเซลล์สาหร่ายสีเขียวขนาดเล็กด้วยเครื่องแก๊สโครมาโตกราฟฟีพบว่าเป็นกรดไขมันชนิดสายยาวที่มีคาร์บอน 16 และ 18 อะตอม โดยมีกรดสเตียริก กรดโอเลอิกและกรดปาล์มิติกเป็นองค์ประกอบหลัก เมื่อเปรียบเทียบกับน้ำมันพืชจะเห็นว่าลิปิดที่ผลิตจากสาหร่ายสีเขียวขนาดเล็กไอโซเลท KKU-S2 สามารถใช้เป็นวัตถุดิบที่มีศักยภาพในการผลิตไบโอดีเซล

Abstract

The objective of this research was to produce bio-oil from green microalgae under heterotrophic cultivation for used as feedstock for biodiesel production. The optimum conditions for lipid accumulation by the locally freshwater microalgae isolate KKU-S2 were obtained as follows: $NaNO_3$ as nitrogen source at 0.5 gL^{-1} and glucose as a carbon source at 50 gL^{-1} with C/N ratio at 280. Under the optimized conditions, a biomass of 6.3 gL^{-1}, specific growth rate at 0.229 d^{-1} and lipid content of 47.8 % on a cellular dry weight could be achieved after culture for 8 days. Gas chromatography analysis revealed that lipids or oils from the isolate KKU-S2 contained mainly long-chain fatty acids with 16 and 18 carbon atoms. Similar to vegetable oils, the lipid mainly contains palmitic acid, stearic acid, oleic acid and linoleic acid and the unsaturated fatty acids and saturated fatty acid amount to about 36.1% and 63.9% of total fatty acid, respectively. Based on these compositional data, microalgal oils from the freshwater microalgae isolate KKU-S2 are a potential feedstock for biodiesel production.

คำสำคัญ : น้ำมันสาหร่ายขนาดเล็ก ไบโอดีเซล สาหร่ายสีเขียวขนาดเล็ก ไขมันเซลล์เดียว

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Introduction

Because of the environmental problems and limited petroleum reserve on Earth, it is more and more difficult to exploit fossil fuel resources. Therefore, for solving the important issue above, a renewable alternative fuel should be developed. Biodiesel is defined as a fuel comprised of mono-alkyl esters of long-chain fatty acids from vegetable oils or animal fats, as a clean and renewable alternative to the traditional fossil fuel, has attracted more and more attention in recent years. However, the consumption of a large amount of vegetable oils for biodiesel production would result in a shortage in edible oils and soar of food price. Recently, there has been an increasing interest in looking for new oil sources for biodiesel production. Among them, microbial oils, namely single cell oils (SCOs), lipids produced by the oleaginous microorganisms involving yeasts, moulds, and microalgae are now considered as promising candidates because of their similar fatty acid composition to that of vegetable oils. Microalgae have received much attention as a renewable biofuel because of their advantages of higher photosynthetic efficiency, higher biomass production and faster growth compared to other energy crops (Minowa et al., 1995; Amin, 2009). In fact, microalgae have the highest oil or lipid yield among various plant oils, and the lipid content of some microalgae has up to 80% and they can also be used as potential feedstock for biodiesel production (Chisti, 2007). Heterotrophic cultivation of microalgae supplied with organic carbon source, results in high biomass and high cellular lipid contents comparing with photoautotrophic cultivation (Xu et al., 2006). To realize the industrialization of biodiesel from microalgal lipid, it was necessary to obtain a large amount of biomass and cellular lipid content. The freshwater microalgae isolate KKU-S2, is a microalgae that can be grown under photoautotrophic and heterotrophic cultivations. Therefore, in this paper, to improve the biomass and lipid yield, heterotrophic cultivation of the isolate KKU-S2 was investigated.

Materials and Methods

Green microalgae strain, pre-cultivation and optimization of culture condition

The freshwater green microalgae, isolate KKU-S2 used, were isolated from freshwater taken from pond in the area of Khon Kaen province. The pre-culture was performed on the BG-11 medium (20 gL^{-1} glucose) at 30°C and 130 rpm. BG-11 medium was consisted of (gL-1): $NaNO_3$ 1.5, $K_2HPO_4.3H_2O$ 0.04, $MgSO_4.7H_2O$ 0.075, $CaCl_2.2H_2O$ 0.036, citric acid 0.006, ferric ammonium citrate 0.006, Na_2EDTA 0.001, Na_2CO_3 0.02, pH 7.0. Then, 10% (v/v) seed culture was inoculated to BG-11 culture medium. Heterotrophic cultivation was carried out in a 1000 mL Erlenmeyer flask containing 400 mL medium under continuous mixing on magnetic stirrer in the dark. To investigate the influence of initial nitrogen source and glucose concentrations on the biomass and lipid production, different concentration of nitrogen source and glucose were added into the BG-11 medium.

Determination of glucose and biomass

The culture broth (5 mL) was centrifuged at 5,000 rpm for 5 min. The supernatants were analyzed for glucose concentration according to DNS method. Harvested biomass was washed twice with 5 mL of distilled water and then dried at 95 °C to constant weight. The biomass was determined gravimetrically.

Determination of lipid content

The lipid content was obtained by the ratio of total lipid concentration of biomass concentration. The total lipids were determined by the modified method of Know and Rhee (1989) with modifications. The fatty acid profile of the lipid was determined as fatty acid methyl esters (FAMEs) by the direct transesterification method with BF3-methanol at 100°C for 45 min, reported by Lepage and Roy (1984). FAMEs samples were analyzed by gas chromatography (Shimadzu) equipped with a flame ionization detector (FID). The condition of GC analysis was as follows: FID 350 °C, N_2 carrier gas 40 mL/min, injection port temperature 230 °C, oven temperature 190 °C.

Results

Time course of cell growth

The time course of cell growth, glucose utilization and lipid production of the isolate KKU-S2 were shown in Figure 1. It is apparent that glucose was used mainly for cell growth at the beginning of cultivation. Biomass, lipid content and utilized glucose gradually increased and lipid yield reached the maximum of 30.5 % of cellular dry weight. A slight decrease was found in biomass after day 8 while utilized glucose increased. The possible reason may be that nitrogen source was exhausted and a great deal of glucose consumption led to a decrease of pH, thus inhibiting cell growth. During the period between days 8 and 10, there showed an apparent decrease in biomass and lipid content was observed. The similar changes were also observed in lipid content of *Chlorella ptotothecoides*, after exhaustion of the carbon source in the growth environment (Xue et al., 2006).

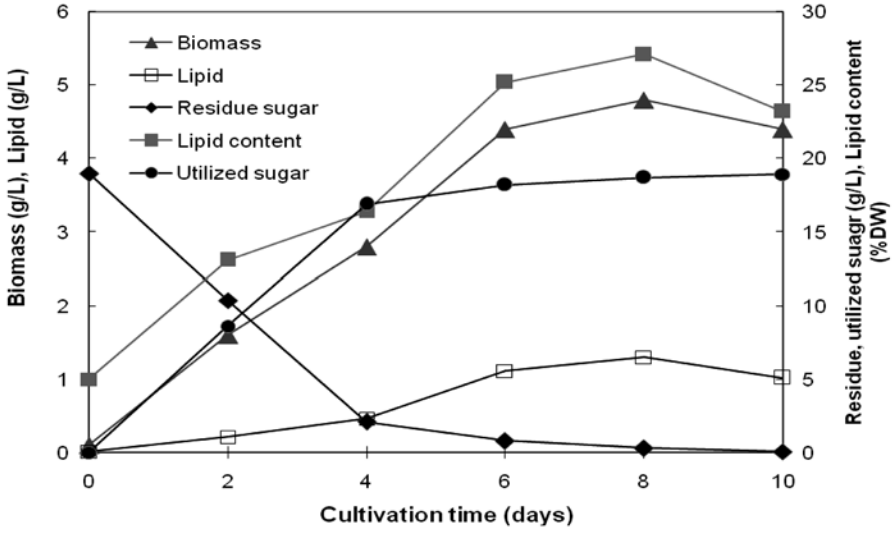

Figure 1. Time course of cell growth and lipid accumulation in the isolate KKU-S2. Culture was performed in the BG-11 medium supplement with 20 gL^{-1} glucose.

Effect of nitrogen source on the biomass and lipid content

Effects of inorganic (urea and $NaNO_3$) and organic (yeast extract) nitrogen sources on biomass and lipid production of microalgae isolate KKU-S2 was presented in Table 1. Among the nitrogen sources tested, $NaNO_3$ supported the maximum biomass of 5.22 gL^{-1} with lipid content of 30.3% of cellular dry weight. With respect to lipid content, $NaNO_3$ was the best and the lipid productivity of 0.198 $gL^{-1}d^{-1}$ was obtained.

Table 1. Effect of nitrogen source on biomass, specific growth rate, lipid content and lipid productivity of microalgae isolate KKU-S2 on BG-11 medium with 20gL^{-1} glucose as carbon source under heterotrophic cultivation.

Nitrogen source	Biomass (gL^{-1})	Specific growth rate (d^{-1})	Lipid content (%CDW*)	Lipid productivity ($gL^{-1}d^{-1}$)
Urea	5.08	0.203	27.9	0.176
Yeast extract	5.18	0.206	29.7	0.193
$NaNO_3$	5.22	0.207	30.3	0.198

*CDW represents the cellular dry weight

Effect of nitrogen concentration on the biomass and lipid content

The concentrations $NaNO_3$ of 0.5, 1.0, 1.5 and 2.0gL^{-1} were used as the initial nitrogen source to investigate the effects on cell growth and lipid content. After 8 days of cultivation, higher initial $NaNO_3$ concentrations of the nutrient medium led to an increase in biomass concentration, with the highest biomass of 5.61 gL^{-1} obtained by cultivation with an initial $NaNO_3$ of 2.0 gL^{-1}, as shown in Table 2. In the experimental data, an increase in the $NaNO_3$ concentration of the nutrient medium led to a decrease in lipid content of cells. The isolate KKU-S2 had the highest total lipid content of 33.8% of cellular dry weight by cultivation of an initial $NaNO_3$ at 0.5gL^{-1} as shown in Table 2. The lipid productivity decreased as $NaNO_3$ concentration increased from 0.5 to 2.0gL^{-1}. Consequently, initial concentration of $NaNO_3$ at 0.5gL^{-1}, was considered to be appropriated to achieve high lipid productivity. The growth rate and lipid accumulation of isolate KKU-S2 were strongly related to nitrogen concentration. The maximum volumetric lipid productivity obtained was 0.201 $gL^{-1}d^{-1}$ when initial $NaNO_3$ concentration was 0.5gL^{-1}.

Table 2. Effect of $NaNO_3$ concentration on biomass, specific growth rate, lipid content and lipid productivity of microalgae isolate KKU-S2 on BG-11 medium with 20gL^{-1} glucose as carbon source under heterotrophic cultivation.

$NaNO_3$ concentration (gL^{-1})	Biomass (gL^{-1})	Specific growth rate (d^{-1})	Lipid content (%CDW*)	Lipid productivity ($gL^{-1}d^{-1}$)
0.5	4.76	0.195	33.8	0.201
1.0	4.78	0.196	28.9	0.173
1.5	5.00	0.201	27.1	0.169
2.0	5.61	0.216	17.5	0.123

*CDW represents the cellular dry weight

Effect of glucose concentration on the biomass and lipid content

To study of glucose concentration or carbon to nitrogen molar ratio (C/N ratio) on cell growth and lipid accumulation, the concentration of glucose at 20, 30, 40, 50, 60, 70 and 80 gL^{-1} with $NaNO_3$ at 0.5 g L^{-1}, were investigated. As shown in Table 3, biomass increased gradually with the increase of C/N ratio and reached the maximum of 6.3gL^{-1} at C/N ratio of 280. Cellular lipid content was quite low at the C/N ratio of 112, then showed a sharp increase when C/N ratio increased from 112 to 280, and reached the maximum of 47.8% of cellular dry weight at 280. Further increase in C/N ratio beyond 280 resulted in a slight drop in lipid content and biomass, suggesting that a considerable glucose inhibitory effect had occurred.

Table 3. Effect glucose concentrations or C/N ratio on biomass, specific growth rate, lipid content and lipid productivity of the isolate KKU-S2.

Glucose concentration (gL^{-1})	C/N ratio	Biomass (gL^{-1})	Specific growth rate (d^{-1})	Lipid content (%CDW*)	Lipid productivity ($gL^{-1}d^{-1}$)
20	112	5.2	0.206	30.8	0.200
30	168	5.7	0.218	34.6	0.246
40	224	6.0	0.224	39.7	0.298
50	280	6.3	0.229	47.8	0.374
60	336	6.1	0.226	43.1	0.328
70	392	5.8	0.219	38.4	0.278
80	448	5.1	0.204	35.2	0.225

*CDW represents the cellular dry weight

GC analysis showed that the lipid extracted from microalgae isolate KKU-S2 mainly contained palmitic acid, stearic acid, oleic acid and linoleic acid, which is similar to that of vegetable oils, and the unsaturated fatty acids and saturated fatty acid amount to about 36.1% and 63.9% of total fatty acid, respectively (Figure 2).

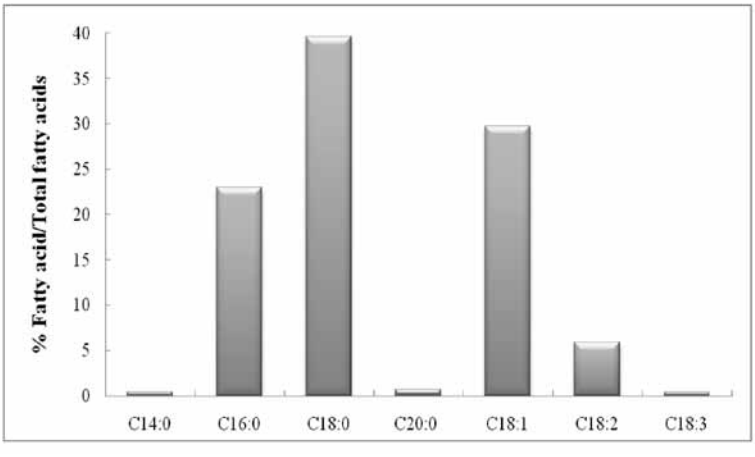

Figure 2. Fatty acid profile of the isolate KKU-S2 cultivated with 50 gL^{-1} glucose for 8 days under heterotrophic condition.

Discussion

Many factors including medium components, such as carbon and nitrogen concentration, C/N ratio as well as culture conditions have significant influences on the biomass and lipid accumulation of the oleaginous microorganism. The growth rate and total lipid content of isolate KKU-S2 varied with the level of $NaNO_3$ concentration in the cultivation. The cells had high lipid content but low biomass concentration with nitrogen limitation. The result was consistent with some reports, the total lipid content of *Neochloris oleoabundans* increase by limiting nitrogen concentrations (Li et al., 2008). According to literature reports, nitrogen limitation may increase the intracellular content of fatty acid acyl-CoA and activate diacylglycerol acyltransferase, which converts fatty acid acyl-CoA to triglyceride, it could be the causes of low nitrogen concentrations raising the total lipid content (Takagi et al., 2000). Additionally, the results imply that different nitrogen and glucose concentrations or C/N ratio influence cell growth and cellular lipid accumulation. The three major constituent fatty acids of the isolate KKU-S2, were stearic acid, oleic acid and palmitic acid that are comparable to vegetable oils. Based on these compositional data, microalgal lipids from the isolate KKU-S2 can be used as feedstock for biodiesel production. However, to realize the large-scale production of biodiesel from microalgal oils, it was necessary to obtain a large amount of biomass and lipid content as well as the low cost of cultivation process. At present, microalgal biomass production has been achieved by photoautotrophic cultivation by using solar energy and CO_2, and heterotrophic cultivation using organic carbon source. The microalgae isolate KKU-S2 accumulates much higher production of lipids and higher growth rate in heterotrophic cultivation, in heterotrophic cultures, production conditions can be easily controlled to realize high cell density with short period of cultivation. However, more economical carbon source should be employed to take the place of pure glucose as substrate. In this research work, using cassava starch hydrolysate and distiller slop from ethanol production plant (data not shown) as well as molasses to substitute glucose has been proved to technically feasible. In addition, potential and realistic progress in transforming of lignocelluloses to fermentable carbon sources might provide an optimal way to reduce the cost of microalgal oils production.

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