Journal of Korean Society on Water Environment, Vol. 32, No. 2, pp. 197-204 (March, 2016)
pISSN 2289-0971 eISSN 2289-098X http://dx.doi.org/10.15681/KSWE.2016.32.2.197

Application of Saccharified Acorn-starch for Biomass and Lipid Accumulation of Microalgae

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당화된 도토리의 전분이 미세조류 바이오매스 증식과 바이오오일 함량에 미치는 영향

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(Received 4 January 2016, Revised 9 March 2016, Accepted 10 March 2016)

Abstract

The growth of the algae strain *Chlorella vulgaris* under mixotrophic conditions in the presence of saccharified acorn-starch (acorn-glucose) was evaluated with the objective of increasing biomass growth and triacylglycerols (TAGs) content. The results indicated that 81.3% of starch was converted to glucose in acorns. *C.vulgaris* algal strains grown with acorn-glucose produced higher biomass and TAGs content than with autotrophic growth. The highest biomass production and TAGs content with 3 g/L acorn-glucose were 12.44 g/L and 32.9%, respectively. Biomass production with 3 g/L acorn-glucose was 16.4 fold higher than under autotrophic growth condition. These findings suggested that 3 g/L acorn-glucose is economic and efficient for biomass production/productivity and TAGs content of microalgae. This study provides a feasible way to reduce the cost of bioenergy production from microalgae.

Key words : Acorn-starch, Biomass, Glucose, Microalgae, Mixotrophic, Oil content

1. Introduction

Algae strains that are robust and highly productive are selected for the conversion of biomass into energy (Spolaore et al., 2006), and strains with relatively high lipid contents are very attractive for biodiesel fuel production (Choi, 2015b; Rudolfi et al., 2009). Microalgae have received considerable interest as a source of renewable energy; however, further optimization of the mass culture conditions is necessary to make microalgal biofuel production economically viable and sustainable (Choi, 2014; Pittman et al., 2011).

Many algal organisms can use either autotrophic, heterotrophic or mixotrophic metabolic processes for growth. The growth rate and biomass production for some algae in mixo- or heterotrophic conditions can be several times higher than in photoautotrophic-only conditions (Qiao et al., 2009; Yang et al., 2000; Zhang et al., 2011). Moreover, the synthesis of metabolic products, such as lipids and pigments, is influenced by the quality and quantity of organic carbon. The use of organic carbon in mixotrophic cultures reduces the need for carbon dioxide in the culture and facilitates the growth of algal species that are sensitive to agitation (Andrade and Costa, 2007; Chojnacka and Noworyta, 2004). Bouarab et al. (2004) reported that *Micractinium pusillum* grew in the presence of organic substrates, such as glucose and acetate, under both mixotrophic and heterotrophic conditions. It can be concluded from the above that mixotrophism is an ideal nutritional mode for high density cultivation of microalgae for the production of biofuels and functional components. However, even though the biomass and lipid productivities are significantly higher compared with those from autotrophic growth, the cost of organic carbon sources (usually in the form of glucose or acetate) is high when compared to all other added nutrients. To overcome this high carbon cost, a cheap resource must be found.

Acorns are capable of providing such a supply. Acorns are much cheaper than other organic carbon sources and are found everywhere in the world. The acorn is the nut from oaks and their close relatives (genera *Quercus* and *Litho-*

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carpus, in the family Fagaceae). It usually contains a single seed (rarely two seeds), enclosed in a tough, leathery shell and borne in a cup-shaped cupule. Acorns vary from 1-6 cm in length and 0.8-4 cm in width. Acorns are also rich in nutrients. Percentages vary from species to species, but all acorns contain large amounts of water (10.6%), protein (0.8%), carbohydrates (87.3%) and fats (1.2%), as well as various minerals (e.g., calcium, phosphorus, magnesium, potassium and the vitamin niacin) and sugar (e.g., rhamnose, galactose, arainose, mannose, fucose and xylose) (Choi, 2015a). Glucose is especially rich in the acorn, representing 97.9% of the sugar composition. Glucose is an important carbohydrate in biology, indicated by the fact that cells use it as a secondary source of energy and a metabolic intermediate. Glucose is one of the main products of photosynthesis and fuels for cellular respiration (Pyle et al., 2008). In mixotrophic growth, there are two distinctive processes: photosynthesis and aerobic respiration. The former is influenced by light intensity, and the latter is related to the organic substrate (glucose) concentration (Andruleviciute et al., 2014; Heredia-Arroyo et al., 2011). The high cell density in mixotrophic cultures has demonstrated that the growth stimulating effects of light and CO2 in mixotrophic cultures are as strong as the effects of glucose (Kong et al., 2013).

The above findings suggest that acorn-glucose is a potential substrate for the mixotrophic cultivation of microalgae that may reduce the production cost of microalgal biodiesel. However, there are few reports on the use of acorn-glucose in biomass production and algal cell components under mixotrophic conditions. Therefore, the effects of various concentrations of saccharified acorn-starch (acorn-glucose) on the biomass growth and triacylglycerols (TAGs) content of *Chlorella vulgaris* (*C. vulgaris*) were evaluated under mixotrophic conditions in this study.

2. Materials and Methods

2.1. Microalgae Cultures and Medium

The investigated microalgae were isolated from KMMCC (Korea Marine Microalgae Culture Center). The *C. vulgaris* (FC-16, KMMCC-135) cells were round in shape and 3-8 μm in diameter. *C. vulgaris* cells were cultivated in Jaworski's medium (JM), which was prepared using deionised water under LED lamps at ambient temperature. Jaworski's medium is comprised of 4.0 g $Ca(NO_3)_2H_2O$, 2.48 g KH_2PO_4, 10.0 g $MgSO_4 \cdot 7H_2O$, 3.18 g $NaHCO_3$, 0.45 g EDTAFeNa, 0.45 g $EDTANa_2$, 0.496 g H_3BO_3, 0.278 g $MnCl_2 \cdot 4H_2O$, 0.20 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.008 g cyanocobalamin, 0.008 g thiamine HCl, 0.008 g biotin, 16.0 g $NaNO_3$ and 7.2 g $Na_2HPO_4 \cdot 12H_2O$ in 200 ml of deionised water. The initial concentration of *C. vulgaris* was 0.357 g/L, and the pH was adjusted to 7.3±0.3. Each culture was grown at room temperature (23±2°C) under 200~250 μmol photons/m 2s illumination using white LEDs (light emitting diodes) for dark and light cycles of 12 h each. Light intensity was measured with a data logger (model LI-1400) using an LI-190SA quantum sensor. For batch cultivation in laboratory conditions, Erlenmeyer glass flasks with a working volume of 5 L were used, and a 15-day cultivation period was chosen. The cultivation period of 15 days was not chosen randomly; the highest biomass concentration is reached during the stationary growth phase, and the lipid content increases with increasing cultivation time.

Mixotrophic conditions for microalgae cultivation were achieved with acorn-glucose. This acorn contained considerable amounts of starch and glucose. The corresponding amount of acorn-glucose was added to the JM growth medium to achieve the desired mixotrophic medium. The cultures were shaken by hand several times a day to avoid sticking.

2.2. Pretreatment of Acorn

Acorns were collected in Ganuneung City in Korea. To remove adherent and interference materials, such as organics and salts, the acorns were rinsed several times with deionised water. After cleaning, the hard shell of the acorn was removed, and the nuts were dried in an oven at 105°C for 24 h then crushed into a fine powder using a mortar and pestle. Tannins were removed by soaking chopped acorns in several changes of water until the water no longer turned brown. The acorn powder was extracted using ultrasonic cycle extraction equipment (JYD-US01, Shenzhen Jiayuanda Technol. Co., Ltd, Guangdong, China) with 20-kHz ultrasonic frequency in an 80% ethanol solution for 120 min with a liquid-to-solid ratio of 15:1 (Pan et al., 2014).

2.3. Saccharification of Acorn-starch

Acorn powder was added to deionized water at a ratio of 1:5 and mixed with calcium chloride and α-amylase (30 U/g dry acorn) for 2h at 90°C. The liquefied mixture was saccharified with glucoamylase (150 U/g dry acorn) for 4h at pH 4.0 and 60°C (Tang et al., 2011). Acorn powder was treated as above for the liquefaction step; however, the saccharification step was allowed to continue for 12 h to completely convert all the starch to glucose. The glucose was measured to determine the total starch in the powder.

2.4. Analytical Methods

The experiment was carried out five times, and the mean values and standard deviations were calculated. Saccharifica-

tion of starch represents a normal glucose equivalent (Dextrose Equivalent, DE).

DE = (Glucose form saccharified starch/ total solid) × 100

2.4.1. Proximate Compositions

The moisture content of the acorns was determined by drying the various parts in an oven at 105°C until a constant weight was obtained. The crude protein content was calculated by converting the nitrogen content, which was determined using the method proposed by Kjeldahl (6.25 × N). The fat content was determined with the acid saccharification Soxhlet system using the method described by the AOAC (2005). The ash content was determined by dry ashing in a furnace oven at 600°C for 10 h. The carbohydrate content was estimated by subtracting the sum of the weights of the protein, fibre and ash from the total dry matter. All determinations were performed in triplicate.

2.4.2. Measurement of Minerals

The samples used for mineral determination were first digested in HNO_3/HCl. The elements K, Ca, Fe, Na and Mg were measured with atomic absorption spectrophotometry (AAS) using a Varian Spectra atomic absorption spectrophotometer, Buck Scientific 210 GVP model. All determinations were performed in triplicate, and we also added spike samples to verify the accuracy of the procedure. P was analysed according to the Mo-blue method using a UV/Vis Spectrophotometer DU800 (Beckman Coulter, USA).

2.4.3. Determination of Monosaccharides for Acorn

The monosaccharide content was determined by a method of separation described by Blakeney et al. (1983). The 10-mg sample was placed into a Teflon-lined screw cap tube and mixed with 125 µL of 73% (w/w) H_2SO_4. After 45 minutes, the solution was saccharified with 1.35 mL of distilled water at 100°C for 3 hour, and then it was neutralised by adding 320 µL of 15M NH_4OH. After neutralization, 1 mL of 2% $NaBH_4$ in DMSO was added to the mixture to react for 90 minutes at 40°C. Next, 100 µL of 18 M glacial acetic acid, 200 µL of 1-methylimidazole and 2.0 mL of acetic anhydride were added to the reaction mixture and allowed to stand at room temperature for 10 minutes. After decomposing, the excess acetic anhydride was separated into a microcentrifuge tube for analysis by GLC. The GLC analysis conditions are shown in Table 1.

2.4.4. Measurement of Cell Weight and Specific Growth Rate

Acorn-glucose at various concentrations (0, 1, 2, 3 and 5 g/L) was added during the initial growth phase and the growth of the algae biomass as well as the lipid accumulation was evaluated. To determine the biomass concentration, a sample of microalgae in growth medium was centrifuged for 10 min at 628 g, washed with distilled water and dried in an oven at 105ºC for 24 h to constant weight. The biomass productivity P (g/(L·day)) was calculated from the variation in biomass concentration X (g/L) within a cultivation time t (in days), according to the following equation:

$$P = (X_1\text{-}X_0) \ / \ (t_1\text{-}t_0) \quad (1)$$

The specific growth rate µ (in days) was calculated using equation (2):

$$\mu = \ln (X_1/X_0) \ / \ (t_1\text{-}t_0) \quad (2)$$

where X_1 and X_0 are the biomass concentration (g/L) on days t_1 and t_0, respectively.

Table 1. Instrument and operation conditions for monosaccharide analysis by GLC

Instrument		Agilent 6890N gas chromatograph
Column		ZB-Wax capillary column (30 m × 0.25 mm id × 0.25 µm df)
Column Temperature	Initial Temperature	140°C
	Initial Time	3 min
	Program rate	8°C / min
	Final Temperature	250°C
	Final Time	20 min
Injector Temperature		250°C
Detector (FID) Temperature		260°C
Carrier gas flow rate		0.8 mL/min (N_2)
Hydrogen flow rate		40 mL/min
Air flow rate		450 mL/min
Split ratio		5:1

2.4.5. Extraction of Lipids

The algal biomass for lipid extraction was prepared by centrifugation and drying. After oven drying, the algae were pulverised and subjected to Soxhlet extraction. All Soxhlet extractions were performed for 72 h using 500 mL of solvent for 1 g of pulverised dry algae with a cycle time of 10-15 min. The Soxhlet extraction with hexane was selected because the Bligh and Dyer (1959) extraction method is suitable for the extraction of all lipids, including triglycerides, phospholipids and other pigments (Sobczuk and Chisti 2010). The lipid content does not reflect the exact amount of triacylglycerols (TAGs) because only triglycerides are used in the synthesis of biodiesel, and other components are undesirable. The excess hexane was evaporated by rotary evaporation until the total volume reached 30-40 mL. The solutions were diluted to 50 mL and used to determine the TAG

content. The amount of TAGs was determined using a Fourier transform infrared (FTIR) spectrometer Spectrum RX 1 (Perkin Elmer) according to the carbonyl stretching absorption at 1740/cm (Stehfest et al., 2005). The amount of TAGs in the extract solutions was determined using a standard graph, and the amount of TAGs was calculated in the dry algae (%, w/w). The experiments were performed five times, and the mean values and standard deviations were calculated.

3. Results and Discussion

3.1. Saccharification of Acorn Powder

The composition of raw acorn and saccharified acorn is represented in Table 2. The proximate compositions such as moisture, crude ash, crude protein, crude fat and carbohydrate decreased from 70.2-78.8% to 14.7-20.5%. In contrast, sugar composition (Glucose, Rhamnose, Galactose, Arabinose, Mannose, Fructose and Xylose) increased after sacchrification of the acorns, from 23.2-28.6% to 75.3-91.4%. These results indicated that most of the starch in the acorns was converted to glucose through saccahrification. Mineral contents (K, P, Ca, Na, Mg and Fe etc.) and fatty acid composition decreased slightly from 4.3-5.2% to 3.8-4.9% and 5.6-6.2% to 5.1-5.4%, respectively, with saccharification.

Fig. 1 illustrates glucose release during the saccharification of acorn starch from the pretreated materials. Starch concentration decreased to 14.50 g/L from 123.50 g/L. While starch concentration decreased by 88.3%, glucose increased 9.8 fold in 5 hours after saccharification. The saccharification rate reached 81.3% within 5 hours but the starch could not be converted completely. After 5 hours of saccharification, the glucose and starch concentrations did not change further. α-amylase and glucoamylase, saccharified enzymes, convert the α-1,4- bond polymer in starch to form low molecular weight dextrins such as glucose, maltose, oligosaccharides. The activities of α-amylase and glucoamylase are inhibited by tannins (Pan et al., 2014), which comprise about 6.2% of acorns. After the saccharification enzymes saccharified amylose to dextrins (6 to 8 form glucose), the dextrin is decomposed into oligosaccharides such as maltoteratose, maltopentaose and maltotriose by the enzyme. Thereafter, oligosaccharides are slowly broken down to glucose and maltose by the enzyme and the concentration becomes

Table 2. The acorn composition

Composition [%]	Acorn powder	Saccharified acorns
Proximate compositions	70.2-78.8	14.7-20.5
Mineral contents	4.3-5.2	3.8-4.9
Sugar composition	23.2-28.6	75.2-81.1
Fatty acid composition	5.6-6.2	5.1-5.4

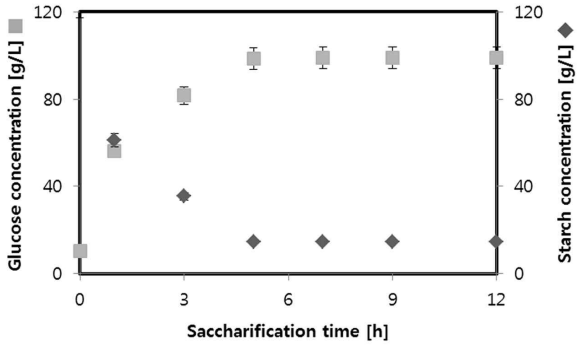

Fig. 1. Process of glucose and starch concentration during saccharification of acorn starch.

diluted (Charef et al., 2008). However, if the concentration of oligosaccharides is high, isomaltose and panose can be regenerated using saccharified energy from maltose and glucose. In particular, since the concentration of glucose and maltose increase rapidly in accordance with the progress of sacchrification, the regeneration of isomaltose and panose also occurs rapidly (Tang et al., 2011). Thus, obtaining a large amount of glucose from starch saccharification is difficult. Glucose is a very important carbon source for the growth of microalgae under mixotrophic conditions and was the main sugar composition in the acorns evaluated. Chaudhary et al. (2012) reported that glucose is a very important factor for microalgae growth and that E. coli growth with glucose is 3 times faster than with glycerol. The results of the current study indicated that the acorns contained a considerable amount of carbonate and glucose, which positively affected the growth of microalgae.

3.2. Effect of Acorn-glucose Dosage on the Growth of Algal Species

Figure 2 shows the effect of different acorn-glucose concentrations on the growth of *C. vulgaris* compared to growth in the autotrophic condition. On the first day, the microalgae grew at the same rate in all concentrations of acorn-glucose. After 2 days, microalgae growth in 5 g/L acorn-glucose was significantly higher than the other samples. The biomass increased for the first 6 days in the sample containing 5 g/L acorn-glucose. Thereafter, the rate of biomass production was similar to the 3 g/L acorn-glucose sample, indicating that high glucose concentrations inhibit the growth of microalgae. Maximum biomass productions were 0.76±0.03, 3.49±0.05, 8.06±0.35, 12.44±1.34 and 10.62±0.75 g/L in the media containing 0, 1, 2, 3 and 5 g/L acorn-glucose, respectively. This was 4.6, 10.6, 16.4 and 14.0 fold higher than the concentration achieved with *C. vulgaris* in autotrophic medium, respectively. The highest biomass production in the mixotrophic condition was obtained with 3 g/L acorn-glucose. All media with acorn-glucose yielded a higher biomass than the autotrophic condition.

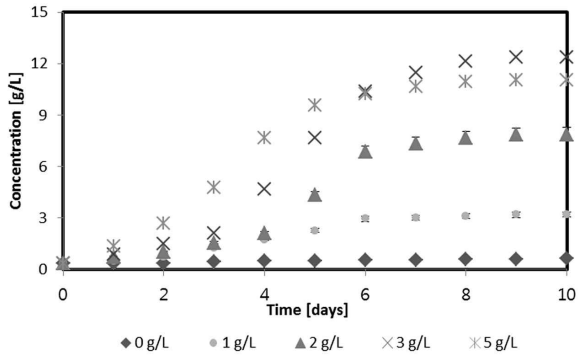

Fig. 2. Growth of *C. vulgaris* in various acorn-glucose concentrations.

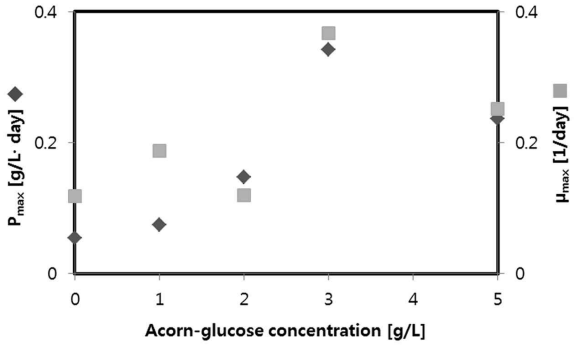

Fig. 3. P_{max} and μ_{max} with various acorn-glucose concentrations.

The oxidation of glucose in microalgae contributes to a series of complex biochemical reactions that provide the energy needed by cells (Choi and Yu, 2015; Liang et al., 2009). The first step in the breakdown of glucose in all cells is glycolysis to produce pyruvate, which is the starting point for all other processes in cellular respiration. In cells where oxygen is present (aerobic respiration), these processes are modelled in the tricarboxylic acid cycle (TCA) or the Krebs cycle. The majority of the energy generated from glucose oxidation is used in the conversion of adenosine diphosphate (ADP) to adenosine triphosphate (ATP), with the energy-rich molecule ATP used subsequently as the energy currency of the cell (Mitra et al., 2012; Perez-Garcia et al., 2010). Bouarab et al. (2004) reported that *Micractinium pusillum* grew in the presence of organic substrates, i.e., glucose and acetate, under mixotrophic conditions, as well as they did under heterotrophic conditions. The growth of *M. pusillum* was much more eugenic in the light than in the dark and was higher in the presence of glucose than acetate. It can be concluded from the above that mixotrophism is an ideal nutritional model for the production of biofuels and functional components. In the present study, the acorn-glucose concentration influenced the biomass production of *C. vulgaris*. The results of this study suggest that the investigated algae species may be excellent biofuel producers because organic materials stimulate the growth rate of these strains.

Growth of *C. vulgaris* in various acorn-glucose concentrations depicted in Figure 3. *C. vulgaris* achieved a maximum biomass productivity of 0.342±0.015 g/L·day and a maximum specific grow rate of 0.367±0.021 g/L·day with 3 g/L acorn-glucose. The maximum biomass productivity and maximum specific growth rate in samples with acorn-glucose were higher than the authotrophic condition.

An early report indicated that mixotrophic growth had the potential to greatly increase the microalgal cell concentration and volumetric productivity in a batch system (Yamane et al., 2001). The report established that the adenosine triphosphate formed in photochemical reactions accelerated glucose anabolism in the mixotrophic culture of *Euglena gracilis* and was the reason why growth in the mixotrophic culture increased. The results of our study suggest that *C. vulgaris* has the potential to be an excellent biofuel producer because the growth rate of the strains can be stimulated by organic materials.

3.3. Total TAGs (triacylglycerols) Content in Algae Species

The total TAGs content with different acorn-glucose concentrations are represented in Table 3. The highest TAGs content was 32.9% for *C. vulgaris* with 3 g/L acorn-glucose. Furthermore, 3 g/L acorn-glucose resulted in 29.2% more TAGs content in *C. vulgaris* compared with the autotrophic condition. The TAGs content increased approximately 2 fold with each 1 g/L acorn-glycerol increment, up to 3 g/L acorn-glucose. However, slight inhibition was observed during the initial cultivation when the acorn-glucose concentration reached 5 g/L. Therefore, to obtain high TAGs content, the recommended mixotrophic condition for the microalgae species is 3 g/L acorn-glucose.

Liang et al. (2010) observed an increase in lipid content with increasing concentrations of glucose. The lipid content increased from 22% with 1 g/L glucose to 32% with 2 g/L glucose; however, the highest amount (10 g/L) of glucose had an inhibitory effect on the growth of algae and on TAGs content. Another study demonstrated that *Chlorella protothecoides* was slightly inhibited by glucose and could still grow even when the salinity of the culture medium

Table 3. Total TAGs content in dry biomass for different acorn-glucose concentrations

Acorn-glucose concentration [g/L]	Total TAGs content in dry biomass* (%)
0	3.71 ± 0.51
1	8.73 ± 1.12
2	18.21 ± 1.83
3	32.91 ± 2.82
5	18.35 ± 2.27

* The data from the 15-day cell growth was used for the determination.
TAGs: Triacylglycerols

reached 35 g/L (Chaudhary et al., 2012). *Chlorella vulgaris*, however, had a much lower tolerance of glucose and its growth was inhibited when the glucose concentration reached 15 g/L. In this study, slight inhibition was observed during the initial cultivation when the acorn-glucose concentration reached 5 g/L. The TAGs content and biomass production with acorn-glucose increased with increasing acorn-glucose concentration. The results showed that using acorn-glucose as a carbon source for the mixotrophic cultivation of *C. vulgaris* is a feasible way to solve the problem of low algal cell density when the acorn-glucose is the sole carbon source, which stimulates additional utilization of the acorn-glucose. Furthermore, this study demonstrated the feasibility of acorn-glucose as an alternative carbon substrate to glucose for microalgae cultivation, and cost reduction of the carbon substrate feed for microalgal lipid production is expected. The TAGs content and efficiency of microalgae growth are important for biodiesel production (Ramos et al., 2009). Improved lipid accumulation with slower microalgae growth may result in lower oil yields compared to faster growing microalgae with less lipid accumulation.

Various carbon sources, such as sodium acetate (Qiao et al., 2009), fructose (Gao et al., 2009), glucose (Yeh and Chang, 2012), glycerol (Heredia-Arroyo et al., 2010), sucrose (Gao et al., 2009), and acetate (Heredia-Arroyo et al., 2010), have been successfully applied to increase the rate of growth and lipid content of microalgae. However, these methods are cost-intensive (Borowitzka and Moheimani, 2013; Lin and Wu, 2015; Vidotti et al., 2014). The carbon source used in this study is simple and cost effective. The prices of glucose (obtained from starch produced from plants that are cultivated under phototrophic conditions, e.g. corn), glycerol and acetate are in the range of 0.5-0.8, 0.6-0.7 and 0.9-0.94 US dollars per kg, respectively. While the use of carbon dioxide from flue gases has an additional bonus due to the reduction of emissions to the atmosphere (Gouveia and Oliveira, 2009), additional cleanup steps are likely to be required for the flue gas. In contrast, acorns are inexpensive, costing approximately $0.15-0.3 USD per kilogram (Leon-Camacho et al., 2004; Shim et al., 2005). Acorn-glucose does not contaminate the growth medium, which can be recycled to reduce not only the cost and the demand for water but also the extra operational costs for reusing growth medium. This cost effective carbon source will help reduce the production cost of using algae for biodiesel.

4. Conclusions

The growth of the algae strain *C. vulgaris* under mixotrophic conditions in the presence of saccharified acorn-starch (acorn-glucose) was investigated with the objective of increasing the biomass growth and TAGs content. Acorn-starch concentration decreased by 88.26% and glucose increased 9.8 fold within 5 hours after saccharification. The saccharification reached 81.3% in 5 hours. Biomass production from 0.357 g/L of *C. vulgaris* was determined to be 0.76, 3.49, 8.06, 12.44 and 10.62 g/L with 0, 1, 2, 3 and 5 g/L acorn-glucose, respectively. Biomass production with 3 g/L acorn-glucose was 16.4 fold higher than that of the autotrophic growth condition. In addition, the amount of TAGs in the algal strains was 3.7, 8.7, 18.2, 32.9 and 18.4% for 0, 1, 2, 3 and 5 g/L acorn-glucose, respectively. The 3 g/L acorn-glucose concentration under the mixotrophic conditions was most effective for maximum increases in biomass production/productivity and TAGs content. The acorn-glucose enhanced the investigated microalgae growth, biomass productivity and TAGs content.

국문요약

본 연구는 당화된 도토리의 전분이 미세조류(*Chlorella vulgaris*) 바이오매스 증식과 바이오오일 함량에 미치는 영향을 알아보고자 하였다. 실험결과 도토리의 전분은 당화 후에 81.3%가 글루코스로 전환되었다. 도토리-글루코스를 이용한 복합영양 상태에서 미세조류는 독립영양 상태보다 높은 바이오매스 증식률과 TAGs (Triacyglycerols)의 함유량을 나타내었는데, 최대 바이오매스 생산량과 TAGs의 함유량은 3 g/L의 도토리-글루코스의 농도에서 각각 12.44 g/L와 32.9%를 나타내었다. 이는 3 g/L의 도토리-글루코스 농도에서 독립영양 상태의 바이오매스 생산량과 비교하면 16.4배의 많은 양의 바이오매스를 생산하였음을 알 수 있었다. 따라서 경제성과 바이오매스의 생산량/생산률 그리고 TAGs의 함유량을 고려한다면 3 g/L의 도토리-글루코스 농도가 미세조류의 증식에 가장 효과적이였다. 본 연구의 결과는 미세조류 유래 바이오에너지의 생산 비용을 절감하는 데 도움이 될 수 있을 것으로 생각된다.

Acknowledgments

This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2013006899).

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