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Lipid accumulating microalgae cultivation in textile wastewater: Environmental parameters optimization

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ABSTRACT

The organic materials, nitrogen and phosphate nutrients in industrial textile wastewater could be utilized for algal growth. In order to increase algal wastewater treatment processing cost efficiency, microalgae collected in Taiwan could be grown using these wastewaters to produce biodiesel. The textile wastewater concentration, pH effects and phosphorus and nitrogen source effects on microalgae growth and lipid accumulation in *Chlorella* sp. G23 were investigated. The results showed the highest total fatty acid methyl ester content ($20 \pm 4\%$) when microalgae G23 was cultivated using textile wastewater, K_2HPO_4 (4 mg/L) and urea (1 g/L) at pH 10 with aeration (carbon dioxide sparging). The nitrogen source type had no effect on the overall NH_4^+-N and COD removal efficiency ($75 \pm 3\%$).

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1. Introduction

Microalgae may prove an effective alternative to terrestrial crops because they have higher photosynthetic efficiencies, higher yields and growth rates, fewer land cultivation requirements and they can be grown in saline waters and in arid and barren land areas [1]. Microalgae biomass can be converted into energy and fuels such as electricity, ethanol, hydrogen, methane and bio-oil through biochemical and thermochemical conversions because it has considerable advantages, such as environmental sustainability and lower greenhouse gas emissions benefits [2]. Biodiesel production from microalgae has been expected to replace petroleum-based energy sources owing to its high areal productivity and lipid contents due to the higher energy yield: 7–31 times higher than palm and several tens to several hundred times that of other oily plants such as corn, soybean, canola, jatropha, coconut and oil palm [3,4]. Using microalgae to produce bioenergy presents other advantages, such as (1) to low water consumption (per unit weight biomass and per energy unit) than that to grow other crops during cultivation [5]; (2) to avoid the competition with agriculture [5]; (3) to purify wastewater [6]; (4) to consume CO_2 in flue gas via photosynthesis [7]; (5) to reach commercial potential by optimizing the cultivation conditions to enlarge the lipid content in the microalgae biomass [8]; (6) to cultivate without herbicides or pesticides [9]; (7) to produce valuable byproducts like fatty acids, pigments, stable biochemical isotopes [10], animal feed, fertilizer, or to produce other biofuels (ethanol or methane) from the oil extraction residual biomass [9] and (8) to produce clean energy-hydrogen (H_2) [11]. Therefore, using microalgae to produce biodiesel for transportation fuel is a high potential renewable source.

Low-cost feedstock production plays an important role in biofuel production from algae commercialization. The addition of organic carbons, such as sugar alcohols, expand the algae growth medium and cultivation cost for the microalgae biomass. Recent attention has focused on the search for cheap microalgae culture media components [8]. Mixotrophic culture is a viable alternative strategy to improve the effective use of light or eliminate cell requirements as well as reducing the cost of microalgae biomass production. Mixotrophic growth can reduce the energy costs through relatively low light intensity [12]. Many types of wastewaters, including dairy wastewater [13], municipal wastewater [14], saline wastewater [15], brewery wastewater [16] and so on have been used to investigate the biofuel production potential using mixotrophic cultures because wastewater is a supplier of major nutrients such as nitrogen and phosphorus and also a source of organic carbon.

Textiles are an important traditional worldwide industry. The effluent from textile processes contains organic materials, dyes

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and other nutrients that contribute significantly to environmental pollution. These effluents are highly variable in composition with relatively low biochemical oxygen demand (BOD) and high chemical oxygen demand (COD) and are typically characterized by: (1) strong color due to residual dyes, (2) recalcitrance due to the presence of compounds such as dyes, surfactants and sizing agents, (3) high salinity (up to 100 g /m^3 NaCl) and (4) high temperature and highly variable pH [17]. These factors affect the water clarity and the gas solubility, which produces toxicity to aquatic plants and animals, leading to serious global environmental problems. The traditional textile wastewater treatment processes usually combine physical, chemical and sludge activity methods, producing high processing operating costs. Biological treatments are a relatively inexpensive way to remove dyes from wastewater. Several microorganisms, such as bacteria, fungi and microalgae, have been tested for their ability to accumulate heavy metals and dyes [18]. Therefore, algae cultivation is included as a unit in a wastewater treatment process. In order to increase the microalgae wastewater treatment process cost efficiency, microalgae could be grown using these wastewaters to produce biodiesel. Microalgae adopt various metabolic routes for their growth and survival using autotrophic, heterotrophic or mixotrophic modes. Some microalgae can grow heterotrophically utilizing only organic compounds as both carbon and energy sources and mixotrophically using both organic compounds and CO_2 as the energy source. Microalgae growth is also affected by many cultivation parameters, such as pH, temperature, nutrient sources and so on. This study developed a technology that simultaneously removes textile wastewater pollutants and accumulates the lipids in algae. The cultivation pH and aeration effects on lipid accumulation and wastewater removal are investigated.

2. Materials and methods

2.1. Microalgae collection and algal strain identification

Microalgae samples were collected from seawater around Taiwan, stored in sterile centrifugal tubes and sent to the laboratory within 3 days for algal cell isolation. Walne medium plates were prepared using full-strength seawater, containing 18 g/L of agar and 1 g/L of glucose. After inoculation, the plates were cultured at 30 °C for 2–7 days. Single colonies composed of spherical cells atypical of either yeast, fungi, or bacteria were extracted and carefully transferred to a new plate. Forty-nine species of microalgae were isolated and cultivated in sterilized textile wastewater as the medium to investigate the microalgae growth and lipid accumulation in textile wastewater.

2.2. Textile wastewater

Textile wastewater was collected from a buffer tank before the wastewater treatment process at a textile manufactory in Central Taiwan. This wastewater was used directly to cultivate 49 species of microalgae to investigate the wastewater inhibition on microalgae growth according to the wet biomass estimations using spectrophotometry in terms of density (D) at an OD of 650 nm. The raw textile wastewater is characterized as COD 2330 mg/L, pH 11.8, phosphate 23.2 mg/L, NH_4^+-N 27.84 mg/L and ADMI 900.

2.3. Experiment design

After 7 days cultivation the 37 species that could grow using textile wastewater (dilution rate of 0–80%) were selected to study the cultivation pH (7, 8, 9, 10, 11) effects with and without aeration for extra added carbon source. Moreover, the extra K_2HPO_4 (2–20 mg/L) and various nitrogen sources at 1 g/L concentration (peptone, urea, yeast extract, NH_4NO_3 and $(NH_4)_2SO_4$) were added to optimize the cultivation conditions. A glass tube with a working volume of 20 mL at 30 °C using an exponentially growing seed culture, and 4300 lx of light intensity was adopted. Aeration was performed by sparging air enriched with 10% CO_2 at 2 vvm. Both aeration and without aeration (static) were applied during environmental parameter optimization. During microalgae growth, liquid samples were collected from the serum bottle with respect to time to determine the microalgae biomass concentration, pH, residual sugar concentration, colorization, NH_4-N concentration and lipid content of the microalgae biomass. The detail cultivations in this study are shown in Table 1.

2.4. Analytical method

Biomass was determined by measuring the OD of each sample at 680 nm (OD680). The dry cell weights of the diluted samples were then detected and measured for plotting the standard curve. The Standard Methods [15] analytical procedures were used to determine pH, COD and colorization [19]. The detail information was presented in our previous study [12].

2.5. Total lipid extraction

The total lipid content (dry weight) was measured by employing a modified version of the method used by Bligh and Dyer [20]. After the cultivation was complete, the culture medium was centrifuged at 9000 rpm and 4 °C for 2 min; the cell pellets were then collected for freeze drying. The samples were pulverized after drying using a homogenizer and extracted using a chloroform–methanol mixture (1:2 v/v). Approximately 15 mL of solvent was used for 50 mg of dried sample in each extraction step. After the samples were mixed using a vortex mixer for 1 min they were ultrasonicated for 3 h and centrifuged at 3000 rpm for 10 min. The solid phases were separated carefully using Whatman No. 1 filter paper. The solids were then washed using 5 mL of chloroform. After this process 9 mL of sterilized water was added to the solvent phase and the solvent was then mixed using a vortex mixer. The solvent phase was centrifuged at 3000 rpm for 10 min. and the chloroform layer was collected. The lipids weight was measured after removing the solvent using a nitrogen blowing concentrator. The lipid content was then calculated.

2.6. Fatty methyl esters and fatty acid analysis

The samples were mixed with 2 mL of NaOH–methanol solution and disrupted using a sonicator, heated in a 100 °C water bath for 10 min and cooled to room temperature to observe the saponification/esterification reactions. The samples were then mixed with 2 mL of HCl in methanol and 1 mL of boron trifluoride solution (Sigma-Aldrich, St. Louis, MO, USA), heated again in a 90 °C water bath for 15 min and then cooled again. Three mL of a saturated NaCl aqueous solution and 4 mL of *n*-hexane were then added and the samples were mixed thoroughly. The upper liquid layer was transferred to a 4-mL amber glass vial, sealed using Parafilm®, and stored at −20 °C until analysis was performed.

The fatty methyl ester (FAME) samples were analyzed using a gas chromatography machine (YL6100 GC, Young Lin, Korea) equipped with a Carbowax column (fused silica, 30 m × 0.25 mm, Quadrex, USA) and a flame ionization detector according to the appropriate reference standards (Sigma, Louisiana, USA). The injector temperature was set at 220 °C, the column temperature was raised from 50 °C to 150 °C in increments of 15 °C/min, and then from 150 °C to 250 °C in increments of 5 °C/min. Methyl esters prepared from fatty acids, including C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, and C20:0, as well as a FAME standard mixture, were all purchased from Sigma-Aldrich and used as the standards for identifying fatty

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