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The effects of nitrogen sources and temperature on cell growth and lipid accumulation of microalgae

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ABSTRACT

Microalgal lipids are potential sustainable biodiesel feedstocks in the future. In this study, effects of temperature, nitrogen sources and potassium ion on cell growth and lipid accumulation of *Monoraphidium* sp. SB2 isolated from Taiwan ponds and the cultivation in industrial wastewater were studied. The results indicated that the biomass obtained with potassium nitrate as nitrogen source was higher than with other nitrogen sources (ammonium chloride and ammonium nitrate). Potassium ion stimulated the uptake of ammonium in the artificial medium. *Monoraphidium* sp. SB2 could grow well under 25–35 °C but decay at 40 °C. The highest microalgal lipid content (32.9%) was obtained at 25 °C, but the highest biomass concentration (650 mg l^{-1}) and lipid productivity (29.2 mg l^{-1} d^{-1}) was achieved at 30 °C. Cultured in industrial wastewater, *Monoraphidium* sp. SB2 can also produce 34.7% of lipid content. *Monoraphidium* sp. SB2 is a promising microalgal strain for biodiesel production because of its ability to grow in industrial wastewater.

1. Introduction

Fossil fuels are non-renewable resources because of their limited supplies and not environmental friendly due to their combustion being regarded as the major source of greenhouse gases responsible for global warming (Verma et al., 2010). Biodiesel, one of the alternative energy resources, has received much attention recently as a result of its environmental benefits and renewable feedstocks. Biodiesel is the monoalkyl ester of long-chain fatty acids derived from the chemical reaction of renewable feedstocks (Meher et al., 2006). Microalgal lipids are regarded as the future feedstock for sustainable biodiesel production because microalgae have a much higher growth rate and photosynthetic efficiencies than conventional crops (Chisti, 2007). Microalgae can accumulate large quantities of triacylglycerols, grow at high rates, fix CO_2 from atmosphere, adapt to wide area including extreme environment and utilize nutrients from wastewater (Hu et al., 2008). If oil producing microalgae are cultivated in wastewater, they can remove nutrients in the wastewater and produce oil at the same time. The apparent benefits of combining microalgal biodiesel production and wastewater treatment are minimizing the use of freshwater, reducing the cost of nutrient addition for microalgal cultivation and removing nitrogen and phosphorus from effluents (Li et al., 2008; Pittman et al., 2011).

Depending on species and culture conditions such as temperature, nutrient and light intensity, microalgal oil content and composition are varied (Solovchenco et al., 2008; Converti et al., 2009; Li et al., 2009, 2010). Temperature is one of the most important factors in culture conditions and affects the growth and the types of fatty acids produced by microalgae (Renaud et al., 2002; Converti et al., 2009; Taoka et al., 2009). Many microalgal species increased unsaturated fatty acids with decreasing growth temperature and increased saturated fatty acids with increasing growth temperature. (Renaud et al., 2002). Biomass productivity, lipid content and lipid productivity are some of the key parameters affecting the economic feasibility of algal for biodiesel production (Griffiths and Harrison, 2009). Different nitrogen sources would affect the growth and lipid content of microalgae (Li et al., 2009). Some species of microalgae can be induced to significantly increase in lipid content under nitrogen starvation or other stress factors (Illman et al., 2000; Khozin-Goldberg and Cohen, 2006; Liu et al., 2007). Oil content of some microalgae such as *Scenedesmus* sp., *Chlorella* sp., *Neochloris oleoabundans* can achieve from 20% to 50% of total cell dry weight (Gouveia and Oliveira, 2009), revealing the significant potential of biodiesel production.

The objective of this study was to investigate the effect of nitrogen sources and temperature on cell growth as well as lipid accumulation by *Monoraphidium* sp. SB2 isolated from Taiwan

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freshwater. Besides, the possibility of microalgae cultivated in industrial wastewater for cell growth and lipid production was estimated.

2. Materials and methods

2.1. Microalgal strain and cultivation conditions

Monoraphidium sp. SB2 is a strain of freshwater microalga isolated from a pond in Taichung city of Taiwan. The microalga was cultivated in a artificial medium (pH 6.8) (Becker, 1994), containing the following components (per liter) 1.01 g KNO_3, 0.246 g $MgSO_4 \cdot 7H_2O$, 0.003 g $CaCl_2 \cdot 7H_2O$, 0.621 g $NaH_2PO_4 \cdot H_2O$, 0.134 g $Na_2HPO_4 \cdot 7H_2O$, 0.0139 g $FeSO_4 \cdot 7H_2O$, 0.0186 g EDTA, 0.062 mg H_3BO_3, 0.169 mg $MnSO_4$, 0.287 mg $ZnSO_4$, 0.0025 mg $CuSO_4$, 0.0129 mg $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$. This microalgal strain was cultivated in 250 mL Erlenmeyer flask containing 100 mL of sterile cultural medium. The flasks were incubated at room temperature with intermittent illumination (14:10 h light–dark cycle) of 25 μmol photons m^{-2} day^{-1} and shaken at 120 rpm on an orbital shaker.

Untreated industrial wastewaters used in this study were collected from a wastewater treatment factory in Taiwan. The wastewater consists of 13.9 mg l^{-1} O_2 COD, 63.3 mg l^{-1} $NH_4^+ - N$, 6.8 mg l^{-1} $NO_3^- - N$, 43.6 mg l^{-1} $PO_4^{3-} - P$, 984.3 mg l^{-1} Na^+ and 82.9 mg l^{-1} K^+. Wastewater samples were collected in 20-L plastic containers and stored in a cold room at 4 °C. Wastewater samples using in the experiments were filtered through a 0.22 μm membrane in advance.

2.2. Evaluation of microalgal growth and lipid accumulation

A Photobioreactor used in this study was a 400 mL glass tube attached to a glass pipe from the bottom (Fig.1). Batch experiments were carried out in a photobioreactor holding 300 mL of microalgal suspension, illuminated with 120 μmol photons m^{-2} day^{-1} and aerated with air at a constant flow rate (50 mL min^{-1}). Samples were taken everyday during a 7-day period to measure the optical density at the wavelength of 680 nm ($O.D._{680}$), pH and nitrate/ammonium concentration. After 7-day cultivation, the microalgal biomass were harvested to extract the amount of lipid and analysis fatty acid composition.

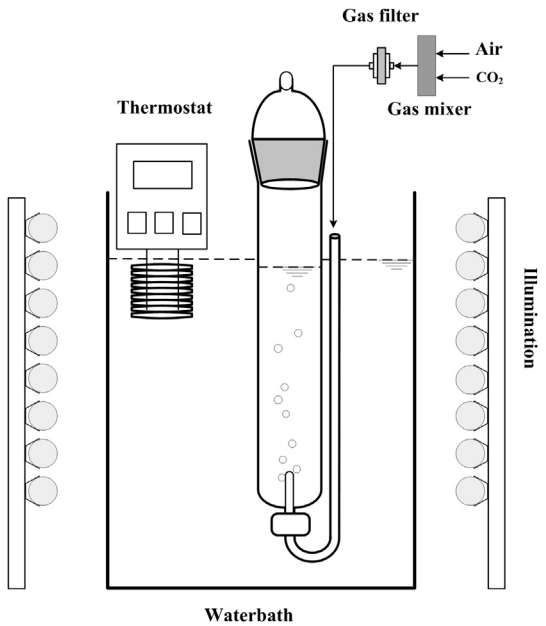

Fig. 1. The diagram of a photobioreactor.

Temperature and nitrogen sources in artificial medium were selected as the independent variables. Effect of temperature on growth and lipid accumulation of microalgal cells were evaluated at 25–40 °C. Effect of nitrogen sources on growth and lipid accumulation of microalgal cells were studied on potassium nitrate, ammonium chloride and ammonium nitrate. The initial nitrogen concentration in the artificial medium was altered to 50 mg l^{-1} N. Besides, the effect of industrial wastewater as a culture medium on growth and lipid accumulation of microalgal cells were evaluated.

2.3. Analysis of lipid content and fatty acid composition

The microalgal cells were harvested from the cultural mixture by centrifugation at 8000 rpm for 10 min (Himac CR22GII, Hitachi, Japan) and the cell pellet was frozen at −20 °C. Total lipids were extracted from lyophilized biomass in $CHCl_3$-MeOH (2:1, v/v) by a modified method of Folch (Christie, 2003). Freeze-dried biomass was suspended in a 3 ml $CHCl_3$–MeOH (2:1, v/v) solution, extracted by sonication for 90 min, and then collected for extractant by centrifugation at 2000 rpm for 10 min. The pellet was re-extracted in a 3 ml $CHCl_3$–MeOH solution twice. The collected extractant was evaporated, and subsequently weighed. Lipid content of microalgae was calculated by dividing the residue weight by the freeze-dried cell weight.

Fatty acids were transmethylated with 5% H_2SO_4 in dry methanol mixture at 80 °C for 4 h (Christie, 2003; Krienitz and Wirth, 2006), using heptadecanoic acid as an internal standard. Gas chromatography (HP 6890, USA) equipped with 30 m DB-WAXETR (J&W, Agilent) capillary column was used for qualitative and quantitative determination of fatty acid composition. The oven temperature program started at 190 °C, and increased at 4 °C min^{-1} until 220 °C. Carrier gas, N_2, was kept at a constant rate of 15 mL min^{-1}. Injector and detector (flame ionization) temperature were kept at 220 °C. The individual fatty acid compositions were identified by comparison between the retention time and the authentic standards (Sigma), and were quantified by comparing their peak area with that of the internal standard. Fatty acid composition was calculated as percentage of the total fatty acids present in the sample.

2.4. Analytical methods

The microalgal concentration was determined by measuring the optical density of the algal culture at 680 nm by spectrophotometer (Hitachi, Japan), and biomass concentration was related to optical density by the equation $y = 3.8904\ x + 0.0422$ ($R^2 = 0.995$) for *Monoraphidium* sp. SB2, where y is the biomass concentration measured in mg per liter, and x is the OD_{680}.

The nitrate concentration in the medium was determined using ion chromatography. Separated ions were detected by the conductivity detector. The column (Shodex, IC NI-424) temperature was controlled at 40 °C. The mobile phase was composed of 18 mM 4-hydroxybenzoic acid, 2.8 mM Bis-tris, 2 mM phenylboronic acid, and 5.0 μM trans-1, 2-diaminocyclohexane-N,N,N′,N′-tetraacetic acid and its flow rate was 1.0 mL/min. The injection volume of samples was 25 μl. The concentration of nitrate ion in the sample was identified by comparing the integral area with that of the standard curve of the potassium nitrate.

Ammonium concentrations were determined colorimetrically (APHA-AWA-WEF, 2005). All the analyses were carried out with culture supernatants obtained after filtration through a 0.22 μm membrane.

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