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Changes in total lipid contents of marine diatom *Nitzschia frustulum* at various temperatures under Si deficiency

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Abstract−The photosynthetic marine diatom *Nitzschia frustulum* was grown autotrophically in a photobioreactor in two stages. In the first stage, cells were grown for 7 days to Si deficiency at the optimally adapted temperature of 22 °C. In the second stage, they were grown for 2 more days at a new temperature ranging from 12 °C to 37 °C; then the contents of total lipids in the harvested diatom samples were measured. The total lipid content of the final diatom cells grown at the altered temperatures between 17 and 32 °C changed little from 15.1% (wt) of the dried cells harvested before the temperature changes. However, it increased to 21% (wt) for cells grown at a low temperature of 12 °C and decreased drastically to 7% (wt) at a high temperature of 37 °C.

Key words: Biodiesel, Diatom, Silicon Deficiency, Temperature Effects, Total Lipids

INTRODUCTION

Biodiesels are the alkyl esters of fatty acids that originate primarily from biomass-derived triacylglycerols which consist of three fatty acids bound to a glycerol through ester links. Biodiesels have drawn great interest as promising environmentally friendly alternative fuels to replace the currently available fossil fuels [1,2]. Biomass-derived biodiesels from oilseeds such as soybean and rapeseed, which constitute most of the currently produced biodiesels, are, however, known to suffer from several drawbacks. First, oilseed crops have become very expensive due to the competitive markets of foods and feeds from the same feedstock. This is an especially serious obstacle for the economic production of biodiesels because the feedstock price is known to comprise more than 70% of the total production cost of biodiesels [3]. Second, the net effect of carbon dioxide reduction is doubted due to the destruction of forests to cultivate crops as feedstock for biodiesels.

Algae and microalgae are considered as alternative feedstocks for biodiesel production due to several potential advantages over oilseed crops [3,4]. They can be cultivated on non-arable areas without destructing forests, thereby enhancing carbon dioxide reduction efficiency. Furthermore, they can grow fast with short doubling times of normally around 1 day, enabling mass production in a small area. Lastly, they do not compete with the markets for foods and feeds.

Among microalgae, diatoms were reported to have high lipid contents [5]. In addition, the lipid production by these diatoms is stimulated when they are stressed by nutrient deficiency for elements such as phosphorous, silicon, and nitrogen [6,7]. Cultivation temperature is also known to affect the composition and content of lipids in diatoms. However, most of the previous studies about the temperature effects on the lipid production from diatoms focused on the production of a particular bioactive lipid component such as eicosapentaenoic acid instead of the total lipids for biodiesel production [8-10].

The aim of this study was to elucidate the composite effect of temperature and Si deficiency on the total lipid content of a marine diatom, *Nitzschia frustulum*. In our study, we employed a two-stage cultivation method to grow the cells: In the first stage, cell growth can be performed for maximum cell-mass production, while in the second stage for maximum lipid production. Previous studies about the changes in total lipids at different cultivation temperatures were performed in one stage process, thus sacrificing cell mass production at temperatures that might be different from the optimal temperatures for the growth of cells [11-13].

EXPERIMENTAL

1. Growth Conditions of Diatom

Pure cultures of *Nitzschia frustulum* were obtained from the UTEX culture collection of algae (UTEX #2042, Austin, TX). The Harrison's artificial seawater medium supplemented with f/2 nutrients was used for both maintenance cultures and two-stage cultures [14]. The maintenance cultures of the diatom were performed without agitation in 500 mL flasks (100 mL per flask) at 22 °C as previously described [15]. *Nitzschia frustulum* for lipid production was grown in the two stages in a 1 L agitated vessel (780 mL initial medium with 25 mL of four week-grown inoculum). The bioreactor was illuminated from both sides with an average incident light intensity of 120 mmol photons/(m^2 s) on a 14 h light/10 h dark photoperiod and aerated with 0.13 L air/(L culture min). Soluble Si, the limiting nutrient for growth, was added to the medium as $Na_2SiO_3 \cdot 5H_2O$ with an initial concentration of 0.3 mM, which is less than half of the initial Si concentration previously verified for Si deficiency for *Nitzschia frustulum* [16]. In the first stage, cells were grown for 7 days to Si starvation at the optimally adapted temperature of 22 °C.

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The growth temperatures were then changed between 12 °C to 37 °C spanning an approximate range of outdoor cultivation temperatures, and the cells were grown for 2 more days at the newly-set temperatures. Culture samples of 200 mL were harvested for lipid analysis at 0 h, 24 h and 48 h after the temperature change. The spectrophotometric assay of the Si concentration in the filtrates of the harvested samples was performed as previously described [17]. Cell numbers were counted by using a hemocytometer (Hausser Scientific).

2. Extraction and Analysis of Total Lipids

A sample of harvested cells was centrifuged at 1,000 rpm (170 g) for 15 min. The supernatant was removed, and the cells were suspended with distilled deionized water. The cell suspension was centrifuged and the supernatant was removed. And the final cell pellets were freeze-dried, then stored in the freezer for lipid extraction. Total lipids were extracted from the freeze-dried cells by a chloroform-methanol (2 : 1, v/v) solution according to the procedures [18]. The total lipid content in the final extract was determined using previously published methods as follows [19]. Lipid extract samples of 0.2 mL were placed into glass vials and then the solvents inside the vials were completely evaporated with N_2 gas. Into the glass vials, 2 ml dichromate solution in concentrated H_2SO_4 (2.5 g/L) was added. The vials were closed with teflon lined caps and then boiled for at least 45 min with frequent shaking. After the vials were cooled to room temperature, 1 mL dichromate solutions were taken from the vials and diluted with 9 ml distilled water in 20 mL glass scintillation vials. The mixtures were cooled to room temperature and their absorbance values at 350 nm were measured against water as a blank solution on a spectrophotometer. Palmitic acid was used as a standard for the calibration of total lipids.

RESULTS AND DISCUSSION

1. Characteristics of Cell Growth

As shown in Fig. 1, the cell number density increased from the initial value of 0.67×10^6/mL to 5.9×10^6/mL, while the Si concentration decreased to 0.016 mM from its initial value of 0.301 mM over the first 7 days of the culture at 22 °C. After the Si concentration in the culture solution decreased close to its minimum value in 4 days of culture, the rate of cell growth decreased considerably and became almost constant for a longer period of culture. The doubling time measured during the initial exponential growth period between days 0 to 4 was 35.2 h. No significant initial lag period for the growth of *Nitzschia frustulum* was observed. The cell mass density after 7 days of growth was 0.15 (±0.017) g/L. The growth temperature was changed to one of five temperatures ranging from 12 °C to 37 °C at day 7, and the cells were grown for 2 more days. As shown in Fig. 2, cell growth changed little in 48 h after the temperature changes, indicating that the cells were under Si deficiency. At 37 °C, however, cell density decreased dramatically to approximately one-third of the value at day 7 at 22 °C, implying that the diatom becomes very vulnerable to high temperatures.

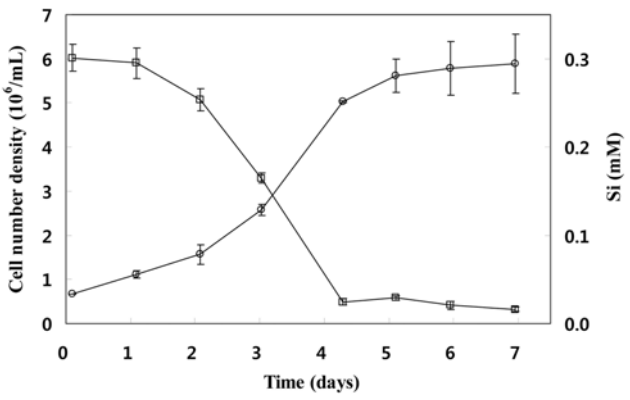

Fig. 1. Changes in the cell number density (○) and Si concentration (□) over time for the growth of *Nitzschia frustulum* in an artificial sea water medium at 22 °C. The culture solution pH varied between 8.5 and 9.5.

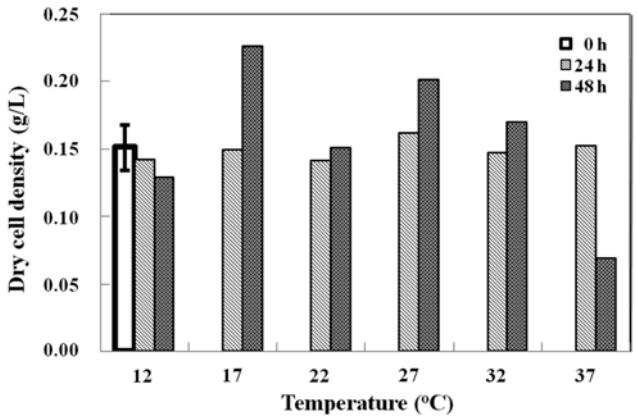

Fig. 2. Effects of temperature changes of the second-stage culture on the dry cell density of *Nitzschia frustulum*. In the first stage, cells were grown in an artificial sea water medium for 7 days at 22 °C. The growth temperature was then changed to a new temperature between 12 and 37 °C and the cells were further cultured for 48 h in the second stage. Cells were harvested at 0, 24, and 48 h after the temperature change. The average dry cell mass density of the samples harvested at 0 h in the second stage was 15.1±3.0% by weight.

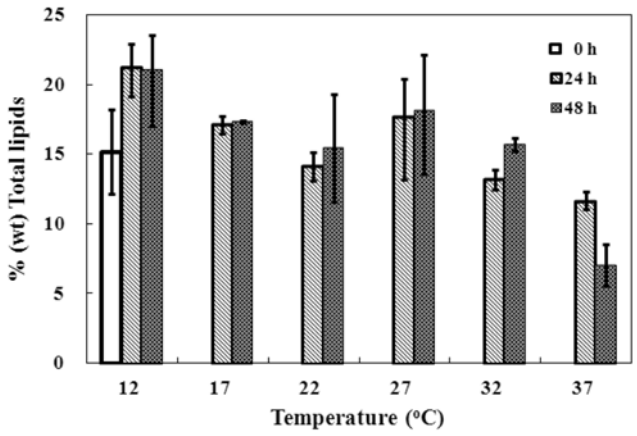

Fig. 3. Effects of temperature changes of the second-stage culture on the total lipid content of *Nitzschia frustulum*. The average content of total lipids of cells harvested at 0 h in the second stage was 15.1±3.0% by weight. The two-stage cultures were performed as described in Fig. 2.

2. Temperature Effects on the Contents of Total Lipids

The primary goal when using algae for biodiesel production is to find organisms and their growth conditions that produce high amounts of total lipids. Limiting nutrients and varying temperatures are two growth conditions that can be adjusted easily to improve lipid production. Optimizing these two growth conditions together may promote the production of lipids by the diatoms to a greater extent than when they are adjusted separately. Fig. 3 shows that, in general, more total lipids were produced at lower temperatures, which is consistent with the previous reports for other diatoms [11,12]. The total lipid content of the dried cells was 15.1 (±3) after 7 days of growth at 22 °C and before temperature changes were introduced. This value is slightly higher than the total lipid contents of other diatoms reported by others [20]. The total lipid content of the cells changed when the cells were grown further at different temperature under Si deficiency: The maximum content of total lipid was 21.2% (wt) of the dried cells when the diatom was grown further for 48 h at 12 °C under Si deficiency. The total lipid content did not change significantly until the temperature was increased to the highest temperature of 37 °C, at which point the total lipid content decreased significantly to 7.1% (wt) of the dried cells 48 h after the temperature change.

Consequently, our results indicated that diatoms can be a promising feedstock for biodiesel production having the total lipid content over 20% (wt) of its dry weight. However, a special consideration should be used in culturing these organisms, as the total lipids and the volumetric productivity of the diatoms can decrease significantly as the second-stage culture temperature increased above 30 °C. A two-stage culture of diatoms, where the cells grow under the optimal conditions for high cell density in the first stage followed by a short period of second stage culture for maximum lipid production, can be adopted for cultivation of diatoms as feedstock for biodiesel production.

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