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Short Communication

Nitrate repletion strategy for enhancing lipid production from marine microalga *Tetraselmis* sp.

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HIGHLIGHTS

- A two-stage process was applied to enhance the lipid productivity of *Tetraselmis* sp.
- Nitrate repletion enhanced both the cell growth and lipid content of *Tetraselmis* sp.
- Nitrate depletion resulted in the lowest lipid productivity from *Tetraselmis* sp.
- The fraction of polyunsaturated fatty acids (PUFAs) increased under N repletion.

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ABSTRACT

The cell growth rate and cellular lipid content of microalgae are affected by the nitrogen levels during cultivation. The growth rate and lipid content of marine microalga *Tetraselmis* sp. was found to increase under nitrate replete conditions, but not under deplete conditions. Thus, in order to enhance the lipid productivity of *Tetraselmis* sp., a two-stage culture process utilizing nitrate replete condition was applied. When the cells were cultivated in F/2 medium for five days in the first stage, the obtained lipid content and productivity were 22.4% and 26.7 mg L^{-1} d^{-1}, respectively. After second stage of cultivation for a further 36 h under nitrate replete conditions with 8.82 mM $NaNO_3$, increased biomass concentration of 1.32 g L^{-1} and lipid content of 30.5% were obtained, with an enhanced lipid productivity of 47.3 mg L^{-1} d^{-1}.

1. Introduction

Microalgae are regarded as an alternative feedstock for biodiesel production, owing to the fast growth rate and high lipid productivity. Microalgal culture requires less arable land than oil crops, does not compete with food production, and can also contribute to reducing the greenhouse gas emissions. However, there are still several obstacles that must be overcome for the commercial production, such as reducing costs and energy requirements, while maximizing lipid productivity (Wijffels and Barbosa, 2010). Particularly, in order to improve the lipid productivity of microalgae, it is important to select an appropriate strategy for inducing cellular lipid accumulation during cultivation, but not decreasing the biomass concentration. One of the most typical methods for increasing lipid content of microalgae is nitrogen limitation (Scott et al., 2010). However, the nitrogen limitation is accompanied by the reduction of cell growth rate or even a loss of biomass concentration (Huerlimann et al., 2010). Also, it is not a universal stress factor that stimulates lipid accumulation. For some microalgae, lipid synthesis does not respond to nitrogen limitation, and larger lipid production is observed at higher nitrogen levels (Xu et al., 2001; Feng et al., 2011).

The present study investigated the influence of nitrate concentrations on the biomass and lipid production of the marine green alga, *Tetraselmis* sp. The *Tetraselmis* species was reported to grow well under a wide salinity range up to 70 practical salinity units (PSU) and a low temperature range of 0–37 °C, with a lipid productivity of 18.6–48.9 mg L^{-1} d^{-1} depending on the culture conditions (Lee and Seong, 2015; Kim et al., 2015). In order to enhance the lipid productivity of *Tetraselmis* sp., a two-stage culture process was applied; a first stage for biomass production, followed by a second stage for stimulating lipid production through the nitrate repletion. The produced cell biomass was converted to fatty acid methyl esters (FAME) biodiesel and the composition of fatty acids at different nitrate levels were compared.

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2. Methods

2.1. Microalgal strain and culture conditions

Marine microalga *Tetraselmis* sp. KCTC 12236BP (Korean Collection for Type Cultures) was cultivated in artificial seawater F/2 medium without sodium silicate (Guillard, 1975). The artificial seawater had the following composition (g L^{-1}): NaCl, 24.72; KCl, 0.67; $CaCl_2{\cdot}2H_2O$, 1.36; $MgCl_2{\cdot}6H_2O$, 4.66; $MgSO_4{\cdot}7H_2O$, 6.29; $NaHCO_3$, 0.18; Tris, 0.606 (adjusted to pH 8.2). *Tetraselmis* cells were cultivated in aerated photobioreactors (PBRs) under the following conditions: aeration rate of 0.2 vvm with ambient air, continuous light supply with intensity of 110–120 μmol m^{-2} s^{-1}, temperature of 20–25 °C. All experiments were conducted in triplicate.

2.2. Analyses

Microalgal cell growth was monitored by measuring the dry cell weight (DCW). Nitrate concentrations were determined according to the Standard method of APHA. Carbohydrate content and protein content were determined using the phenol–sulfuric acid method and the Lowry method, respectively. Lipids were extracted following the method described by Bligh and Dyer. The cell biomass was converted to fatty acid methyl ester (FAME) through the acid-catalyzed transesterification proposed by the National Renewable Energy Laboratory (Wychen and Laurens, 2013). The method is based on a whole biomass transesterification procedure of lipids to FAME, which enables to convert all fatty acids including free fatty acids in the biomass. Fatty acids composition was analyzed using GC-FID (YL6500 GC, Younglin Instrument Co., Korea) equipped with an HP-INNOWAX capillary column (Agilent 19091N-213). GC analysis for each sample was conducted in triplicate.

3. Results and discussion

3.1. Effect of initial nitrate concentration on the cell growth and lipid production

As shown in Fig. 1a, the higher the initial nitrate concentration, the more cell biomass was produced. After 2 weeks of cultivation, both cultures supplemented with higher nitrate concentrations of 1.76 and 2.65 mM reached a cell concentration of 1.5 g L^{-1} without significant differences. The nitrate deplete culture had the lowest cell growth rate, however, it also showed continuous cell growth and achieved a cell concentration of 1.2 g L^{-1} on the final day. The results show that the *Tetraselmis* cells can survive for a relatively long time even under nitrogen deplete conditions, although the cell growth rate decreases due to nitrogen deficiency. Many

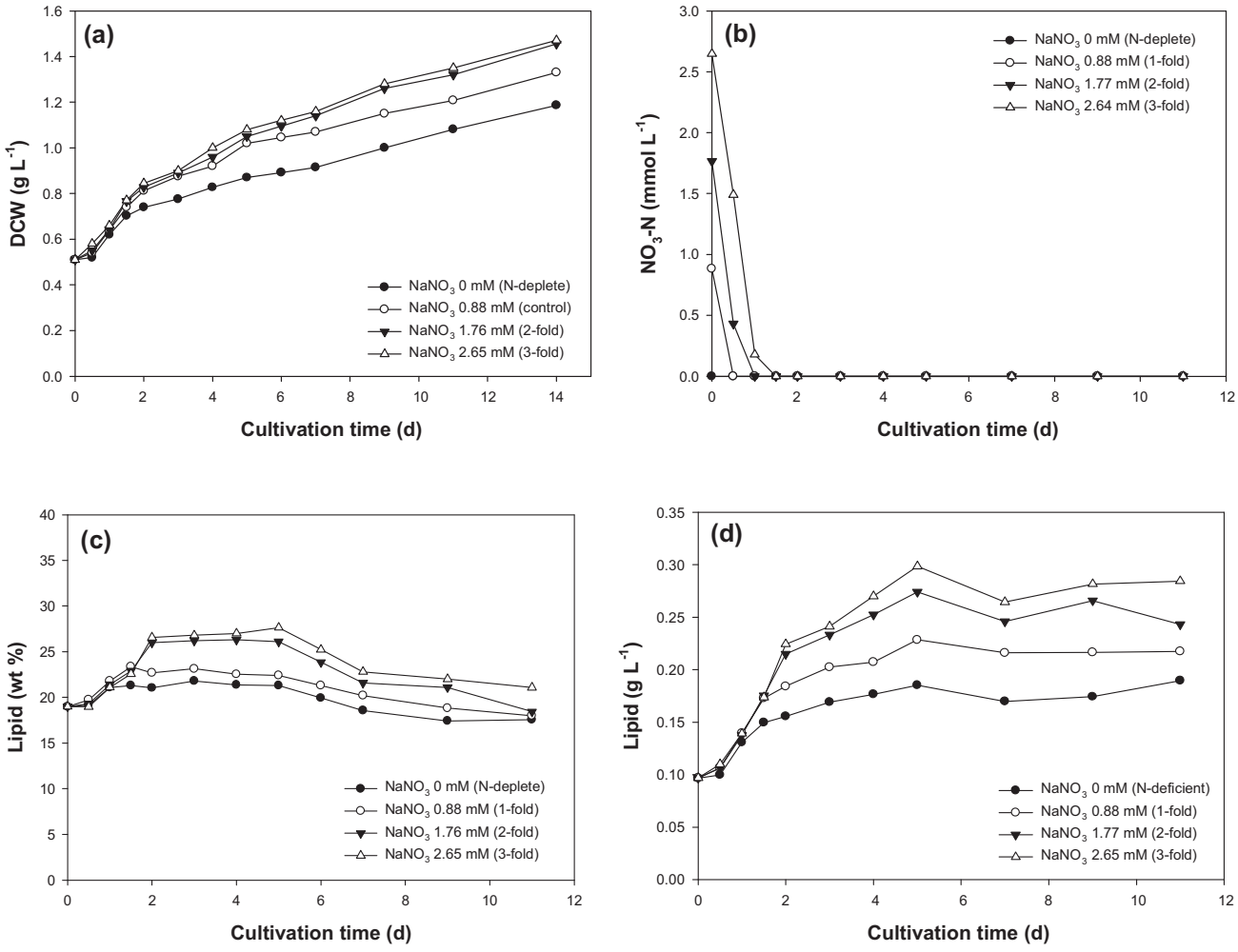

Fig. 1. The change of (a) cell concentration, (b) remaining nitrate concentration in medium, (c) cellular lipid content, and (d) lipid concentration per reactor volume at different initial nitrate levels in the first stage of cultivation.