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Cultivation of four microalgae for biomass and oil production using a two-stage culture strategy with salt stress

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ABSTRACT

A two-stage culture strategy was used for maximum biomass production under nutrient-sufficient conditions, followed by cultivation under low-salt stress, to cause the accumulation of oil in the biomass. Controlled conditions of nitrate, salt concentration, and time to exposure to stress were optimized for oil production with four species of microalgae, *Isochrysis galbana*, *Nannochloropsis oculata*, *Dunaliella salina*, and *Dunaliella tertiolecta*. Using conditions with addition of nitrate to 24.0 mg/L, *I. galbana* and *N. oculata* showed higher biomass productions than *D. salina* and *D. tertiolecta*. The oil contents of the microalgae increased from 24.0% to 47.0% in *I. galbana* with 10 psu for 2 days, from 17.0% to 29.0% in *N. oculata* with 0 psu for 3 days, from 22.0% to 43.0% of *D. salina* with 10 psu for 1 day, and from 23.0% to 40.0% (w/w) in *D. tertiolecta* with 0 psu for 2 days as the second stage culture with low-salt stress. Thus, *I. galbana* could be a suitable candidate microalga for oil production.

1. Introduction

Microalgae have been attracted as potential resources for renewable biofuels [1]. The economic feasibility of biofuel production depends on the productivities of biomass and oil from microalgae culture [2].

A few microalgae species, including some *Chaetoceros calcitrans* [3], *Isochrysis* species [4,5], *Dunaliella* species [6,7], *Nannochloropsis* species [8], *Chlorella* species [9,10] and *Botryococcus braunii* [11,12], have been focused on growth-associated oil production during culture. Among these species, *Isochrysis galbana*, *Nannochloropsis oculata*, *Dunaliella salina*, and *Dunaliella tertiolecta* were used in this study because they can use inorganic nutrients present in seawater along with sunlight to produce biomass using CO_2 as the carbon source. They are species with high tolerances for salt, temperature, and light. They are also relatively easy to culture and have high cell growth and oil production rates.

Nitrogen sources are the essential inorganic salts for cell growth and metabolism. Nitrate, ammonia, and urea can be used as nitrogen sources for growing microalgae [13]. The use of nitrate as the nitrogen source enhanced cell growth and resulted in higher accumulated oil content than with urea [14]. Also, the cells grew poorly in medium with ammonium as the nitrogen source [15]. Thus, nitrate was used as the nitrogen source in this study. Microbial growth kinetics has been dominated by an empirical model proposed by Monod [16]. The Monod model fits to a wide range of data for cell growth. The model is commonly applied to unstructured and nonsegregated model of microbial cell growth. The Monod model describes substrate-limited growth when growth is slow and population density is low. Under these circumstances, culture conditions could be related simply to substrate. The growth kinetics of four microalgae in this study was investigated in the prediction of algae cell growth and nitrogen concentration as substrate.

The oil content in some microalgae can be increased by varying the cultivation conditions, such as nitrogen depletion [9], light intensity [17], temperature [18], salt concentration [6], and iron concentration [19]. Thus, a two-stage culture strategy can be used to enhance the oil productivity as well as cell growth. In the first stage, the microalgae are grown under nutrient-sufficient conditions to obtain maximum biomass production. Then, in the second stage, for example, salt stress is imposed to trigger increased accumulation of oils. Some microalgae species can accumulate oil to 40–70% of the dry cell weight, however their cell growth is

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generally slow. The well-known *Botryococcus braunii* can accumulate oil up to 70% of the dry cell weight; however the doubling time of the cell is in the range of 5–7 days [11,12]. Indeed, the high oil contents of microalgae under environmental stress are associated with low biomass productivity.

This study was carried out to examine biomass production in the first stage and oil production with salt stress in the second stage using a two-stage process with the four species of microalgae, *I. galbana*, *N. oculata*, *D. salina*, and *D. tertiolecta*. Changes in oil content were monitored and the effects of limitations on nitrate, salt concentration, oil, and time were evaluated in a 5-L circular cylindrical photobioreactor.

2. Materials and methods

2.1. Strains and preculture conditions

Four species of microalgae, *I. galbana*, *N. oculata*, *D. salina*, and *D. tertiolecta*, were obtained from NLP Corp. (Busan, Korea). After preculture in a 1-L flask, the microalgae were cultivated at 20 ± 1 °C at a light intensity of 108.9 μmol photon/m^2/s in a 5-L circular cylindrical photobioreactor with a working volume of 3 L using *f*/2 medium, as described by Guillard and Ryther [20]. Aeration with filtered air was provided through an air stone at a rate of 2.5 L/min. All cultures were carried out with a 12/12-h light/dark cycle.

2.2. Photobioreactor and cultivation conditions

Cells were cultured photoautotrophically with 3 L of *f*/2 medium in a 5-L circular cylindrical photobioreactor (radius, 8.2 cm; height, 27 cm) at 20 ± 1 °C (first stage). Filtered air was supplied to the reactor through a 0.2-μm PTFE membrane at a rate of 2.5 L/min. Illumination for the 12/12-h light/dark cycle was provided at 108.9 μmol photon/m^2/s light intensity using white fluorescent lights. The influences of nitrogen content on biomass and oil production were evaluated with and without N source. Cultures were carried out in the reactor containing *f*/2 medium with nitrate concentrations of 6, 12, 18, and 24 mg/L with various N sources. Biomass productivity was calculated as

$$P_{\text{Biomass}}(\text{mg/L/d}) = \frac{\text{DCW(mg/L)} - \text{DCW}_0\text{(mg/L)}}{T(\text{d})}$$

where P_{Biomass} is biomass productivity (mg/L/d), DCW is the final dry cell weight of the microalgae, DCW_0 is the initial dry cell weight of the microalgae, and T is the culture time in days.

The Monod model introduced the concept of growth limiting substrate. The relationship of specific growth rate to substrate concentration often assumes the form of saturation kinetics. The Monod model is shown in the equation (1), where μ is specific growth rate (h^{-1}), $\mu_{\max}$ is maximum specific growth rate, S is substrate concentration as nitrogen (mg/L), K_s is substrate saturation constant (i.e. substrate concentration (mg/L) at half $\mu_{\max}$). And μ can be calculated from equation (2),

$$\mu = \frac{\mu_{\max}[S]}{K_s + [S]} \tag{1}$$

$$\mu = \frac{\text{DCW}_{(t)} - \text{DCW}_{(t-1)}}{\text{DCW}_{(t-1)} \times \left(T_{(t)} - T_{(t-1)}\right)} \tag{2}$$

The specific growth rate is dependent on the concentration of a growth-limiting substrate through the parameters of $\mu_{\max}$ and K_s. To obtain these parameters, it is necessary to draw double reciprocal plot of equation (1) in growth phase in the first stage in the experiment. A double reciprocal plot of the Monod equation (3) can be written as;

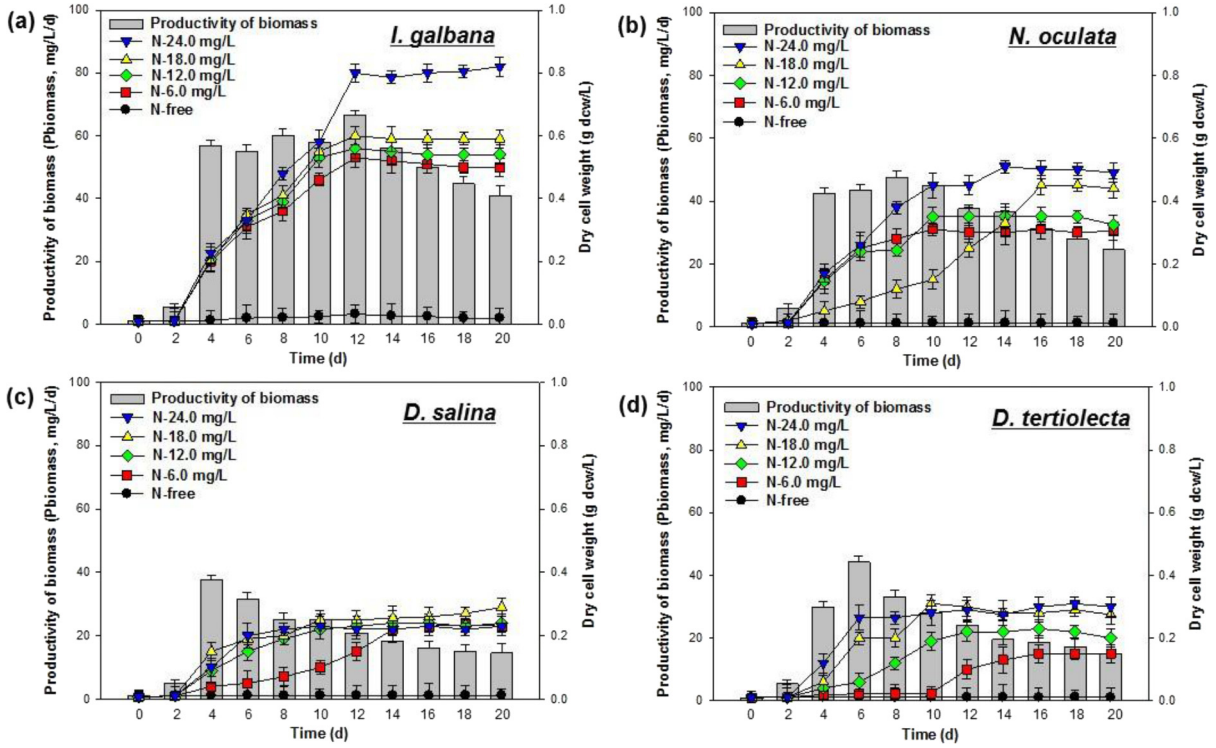

Fig. 1. Time courses of biomass production and productivity of (a) *I. galbana*, (b) *N. oculata*, (c) *D. salina*, and (d) *D. tertiolecta* at different initial nitrate concentrations. Cultivation with initial nitrate concentrations of N-free, N-6.0, N-12.0, N-18.0, and N-24.0 mg/L as $NaNO_3$.