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On-line modeling intracellular carbon and energy metabolism of *Nannochloropsis* sp. in nitrogen-repletion and nitrogen-limitation cultures

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HIGHLIGHTS

- On-line monitoring and calculation of carbon and energy metabolism of microalgae.
- Only 30–60% of carbon fixed in Calvin cycle was used for biomass material.
- Protein, lipids, carbohydrates, and nucleic acids contents were calculated on-line.
- The energy for biomass formation and maintenance were quantitatively analyzed.
- Light respiration rate decreased with the intracellular nitrogen reduction.

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ABSTRACT

In this study, a photobioreactor cultivation system and a calculation method for on-line monitoring of carbon and energy metabolism of microalgae were developed using *Nannochloropsis* sp. in nitrogen-repletion and nitrogen-limitation cultures. Only 30–60% of carbon fixed in Calvin cycle was used for biomass and the rest was lost in light respiration. The net fixed carbon was assumed to be incorporated into protein, lipids, carbohydrates, and nucleic acids, whose contents calculated on-line fitted well with the experimental measurements. Intracellular ATPs were quantitatively divided for biomass production and cell maintenance, and the result is in accordance with known reports. Due to light limitation induced by high cell concentration in batch cultures, the proportion of CO_2 loss in light respiration and the proportion of energy for maintenance rapidly increased in culturing process. Nitrogen starvation reduced the light respiration, thus decreasing CO_2 loss and maintenance energy, but no effect on ATP requirement for cell growth.

1. Introduction

Microalgae have recently received specific attention as the most potential biofuel feedstock due to their unique characteristics such as rapid growth rate, high lipid content and no requirement of high quality agriculture land (Chisti, 2007; Scott et al., 2010). In addition, microalgae can utilize CO_2 in flue gases as carbon source, which can mitigate the increasing CO_2 emission and help reduce the green house effect (Wang et al., 2008).

The growth rate and composition of microalgae vary greatly under different environmental conditions. There have been known reports on the effects of cultural conditions on cell growth rate and lipid productivity (Breuer et al., 2013; Lv et al., 2010), whereas little information is available on the dynamic change of intracellular carbon and energy flux of algae according to growth conditions. Photoautotrophic organisms utilize CO_2 and light energy to synthesize biomolecules. In eukaryotic microalgal cells, photosynthesis and respiration are two important processes that coexist in light. CO_2 is fixed through photosynthesis, but a fraction of carbon is lost by respiration in the form of CO_2 evolution. The respiration should not only be regarded as carbon loss because the precursor and the energy generated during respiration are necessary for the formation of high-value biomass components such as proteins and lipids (Wilhelm and Jakob, 2011). The biomass composition usually varies with environmental conditions, and light respiration rate may change according to types of components synthesized. Therefore,

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light respiration rate should be an important factor in carbon metabolism. However, it is usually considered to have a negative impact on growth, and is assumed to remain constant in classical macroscopic modeling approaches (Takache et al., 2012). The conventional measurements of cell composition require the disruption of the cell and can only be carried out off-line, thus being invasive and time-consuming (Carvalho et al., 2009; Kliphuis et al., 2012). Although several models have been developed for microalgal growth and lipid accumulation (Mairet et al., 2011; Packer et al., 2011), the methods for calculating biomass concentration and contents of main components on-line are not reported. On energy metabolism, some theoretical estimations of the energy in the form of ATP required for biomass production are solely based on the main biomass composition. This method gives a value lower than that experimental measurements. Additional energy is required for the assembly of biopolymers into growing biomass defined as "growth-associated maintenance" and cell maintenance called "non-growth-associated maintenance" (Baart et al., 2008). Kliphuis et al. (2012) quantified the energy parameters for biomass formation and maintenance of *Chlamydomonas reinhardtii* by performing chemostat experiments at different growth rates. Monitoring and analysis of intracellular carbon and energy flux could provide further insight on intracellular metabolic processes. To the best of our knowledge, there has been no known report on on-line calculations of carbon and energy allocation in culture of microalgae.

In this study, a photobioreactor cultivation system and a calculation method were established to monitor process parameters and acquire information on carbon and energy flux on-line. For oleaginous microalgae, light intensity and nitrogen stress are the most widely studied approaches to regulate cell growth rate and lipid production. (Breuer et al., 2012; Liu et al., 2012; Pal et al., 2011). In this study, *Nannochloropsis* sp., a promising marine alga for biodiesel production (Quinn et al., 2012), was used as a model strain to investigate the change of carbon and energy flux in nitrogen-repletion (NR) and nitrogen-limitation (NL) batch cultures in a self-built photobioreactor culture system.

2. Methods

2.1. Microorganism and culture medium

The *Nannochloropsis* sp. used in this study was obtained from Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences. The algae were cultivated in sterilized modified artificial f/2-Si medium (Guillard and Ryther, 1962). $NaNO_3$ was used as the sole nitrogen source. In NR culture, $NaNO_3$ was added to the medium according to the biomass production every day, with 0.8 g/L of initial concentration. In NL culture, initial $NaNO_3$ concentration was set at 0.375 g/L.

2.2. Analytical methods

The biomass concentration was determined by gravimetric method. An aliquot of culture was sampled and centrifuged (5000 rpm, 8 min), washed twice with distilled water to remove adhering inorganic salts, lyophilized for more than 48 h, and weighed. Each experimental measurement was performed in triplicate and averaged.

Carbohydrates content was measured according to the phenol/concentrated sulfuric acid method, and glucose was used as a standard (Dubois et al., 1956). Total protein content was assayed by the Lowry method, and bovine serum albumin was used as a standard (Lowry et al., 1951). The relative amino acid composition was determined by HPLC using amino acid analysis column (Eclipse AAA 4.6 × 150 mm; Agilent, USA). After extraction of lyophilized algal powder with modified Bligh and Dyer method, lipid content was determined gravimetrically, and the relative fatty acid composition was determined by GC analysis using capillary column (VF-5ht 30 m × 0.25 mm; Agilent, USA) and flame ionization detection (Liang et al., 2013). Nucleic acids were not measured, DNA and RNA contents were set at 0.35% and 2.20%, respectively, according to the literature (Rebolloso-Fuentes et al., 2001).

Nitrate content in the medium was measured with an ion chromatograph (Metrohm, 761 Compact IC, CH), and intracellular nitrogen quota was determined with an elemental analyzer (Vario EL III CHN, GER).

2.3. Photobioreactor culture system

Batch cultures were carried out in a cylindrical air-lift glass photobioreactor (Φ0.165 m × 1.0 m, working volume of 18 L). Continuous illumination was provided by 16 fluorescent lamps (tubes) arrayed symmetrically around the bioreactor, providing the incident light intensity of 120 μE/(m^2 s). The pH, dissolved oxygen (DO), and dissolved carbon dioxide (DCO_2) of the medium were monitored by autoclavable pH electrode, DO electrode, and DCO_2 electrode, respectively (Mettler-Toledo, CH). The pressure in the photobioreactor was measured by a pressure transducer and maintained at 10 ± 1 kPa above ambient pressure. Aeration was supplied with air filtrated through a 0.22-μm gas filter at the flow rate of 1.0 L/min, monitored by an air mass flow controller (Brooks, USA). CO_2 was intermittently injected into the bioreactor to control pH at 7.8 ± 0.2, monitored by a CO_2 mass flow controller (Brooks, USA). The off-gas was channeled to the CO_2/O_2 analysis unit, comprising a dehumidifier, a pressure regulator, and a gas mass spectrometry (Sunny Hengping, CHN). The CO_2/O_2 analysis unit was designed with multiple channels. Part of the air in the inlet was channeled to the CO_2/O_2 analysis unit for cross-calibration. The software developed by our lab was used to collect and store the data on-line in a local computer.

2.4. Light respiration rate measurement

The light respiration rate was determined by measuring the rate of post-illumination O_2 uptake (Kliphuis et al., 2011). The photosynthesis and respiration rates were measured by a Clark-type electrode (Hansatech Oxylab, UK), referring to the method described by Langner et al. (2009). A computer controlled the program of increasing light intensity from 50 to 500 μE/m^2 s (each light irradiance with 3-min duration) alternating with subsequent dark phase of 3 min. Both oxygen increase rate and decrease rate were recorded, representing net oxygen evolution rates (P_N) and respiration oxygen consumption rate, respectively. This respiration rate represents the oxygen consumption during the light period, denoted as R_L. Gross photosynthetic oxygen production (P_G) was derived from P_N corrected by corresponding R_L.

2.5. PQ monitoring on-line and carbon flux analysis

For photoautotrophic organisms, CO_2 and inorganic nitrogen NO_3^-, NO_2^- and NH_4^+ are used as carbon source and nitrogen source, respectively. PQ (Photosynthetic Quotient) was determined from the ratio between O_2 evolution rate and CO_2 uptake rate (Eriksen et al., 2007; Kroon and Thoms, 2006).

Bubbled CO_2 into the phtotobioreactor was utilized by cells for biomass (C_X) or dissolved in medium (C_{aq}), or escaped with off-gas (C_O). The carbon balance in the photobioreactor could be described as follows:

$$C_{in} = C_X + C_{aq} + C_O \quad (1)$$