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Microalgal growth with intracellular phosphorus for achieving high biomass growth rate and high lipid/triacylglycerol content simultaneously

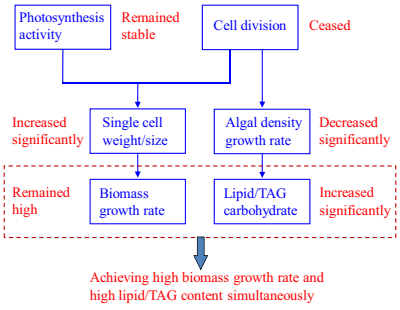
Wu Yin-Hu [a], Yu Yin [b], Hu Hong-Ying [a,*]

[a] Environmental Simulation and Pollution Control State Key Joint Laboratory and State Environmental Protection Key Laboratory of Microorganism Application and Risk Control (SMARC), School of Environment, Tsinghua University, Beijing 100084, PR China
[b] State Key Laboratory of Environmental Criteria and Risk Assessment, Research Center of Water Pollution Control Technology, Chinese Research Academy of Environmental Sciences, Beijing 100012, PR China

HIGHLIGHTS

- Photosynthesis activity remained stable during growth with intracellular phosphorus.
- After cell division ceased single cell weight and size showed sustained increase.
- At late stationary phase of algal density, biomass growth rate still remained high.
- At late stationary phase of algal density, lipid/TAG content increased significantly.
- After growth with intracellular P, biomass was rich in lipids and carbohydrates.

GRAPHICAL ABSTRACT



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ABSTRACT

Nutrient deprivation is a commonly-used trigger for microalgal lipid accumulation, but its adverse impact on microalgal growth seems to be inevitable. In this study, *Scenedesmus* sp. LX1 was found to show similar physiological and biochemical variation under oligotrophic and eutrophic conditions during growth with intracellular phosphorus. Under both conditions microalgal chlorophyll content and photosynthesis activity was stable during this growth process, leading to significant increase of single cell weight and size. Therefore, while algal density growth rate dropped significantly to below 1.0×10^5 cells mL^{-1} d^{-1} under oligotrophic condition, the biomass dry weight growth rate still maintained about 40 mg L^{-1} d^{-1}. Meanwhile, the lipid content in biomass and triacylglycerols (TAGs) content in lipids increased significantly to about 35% and 65%, respectively. Thus, high biomass growth rate and high lipid/TAG content were achieved simultaneously at the late growth phase with intracellular phosphorus. Besides, microalgal biomass produced was rich in carbohydrate with low protein content.

1. Introduction

Microalgae-based bioenergy lies at the intersection of some of the major challenges in the 21st century: global climate change, water body eutrophication and energy crisis. Microalgae are

* Corresponding author. Tel.: +86 10 62794005; fax: +86 10 62797265.
E-mail address: hyhu@tsinghua.edu.cn (H.-Y. Hu).

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photoautotrophic or mixotrophic microorganisms that can grow rapidly with great potential of carbon dioxide capture via photosynthesis (Razzak et al., 2013; Zhao and Su, 2014). Meanwhile, in the growth process, microalgae can achieve efficient nitrogen and phosphorus removal from water mainly by uptake into algal cells (Aslan and Kapdan, 2006). Furthermore, the lipids accumulated within microalgal cells, especially triacylglycerols (TAGs), can be used as feedstocks for biofuel production (Hu et al., 2008), which is considered as one of the most promising substitutes for fossil fuels (Chisti, 2007; Schenk et al., 2008).

Because of these superior advantages mentioned above, technologies related to microalgae attract worldwide attention recently. The coupled technology of wastewater treatment and microalgal biomass/bioenergy production is also proposed (Hu et al., 2011). Wastewater could provide most essential resources for large-scale microalgal cultivation, including water resource, organic matters and inorganic nutrients (Wu et al., 2014a). However, due to the massive demand of energy production, wastewater might not be able to provide sufficient resource for microalgal bioenergy production.

According to the Energy Independence and Security Act (EISA) of the US, the production of renewable fuels should be increased gradually to 36 billion gallons per year by 2022. Compared with bioethanol and biodiesel from oil crops, microalgal biofuels are a more promising choice (Chisti, 2008). As highlighted by Yang et al. (Yang et al., 2011), about 0.071 kg phosphorus was required to generate 1 kg microalgal biodiesel. The density of biodiesel is between 0.8 and 0.9 kg L^{-1} (Alptekin and Canakci, 2008). Assuming all the renewable fuels regulated in the EISA are provided by microalgal biofuels, the annual phosphorus consumption of biofuel production would be 7.7–8.7 billion kg. Typically, the total phosphorus concentration in domestic wastewater is about 5 mg L^{-1}. If all the phosphorus within domestic wastewater could be used for microalgal cultivation, 1540–1740 billion tons domestic wastewater would be needed for microalgal biofuel production, which would amount to over 40 times the total domestic wastewater amount of China in 2010. Therefore, phosphorus would become essential limiting resource for large-scale microalgal biofuel production.

In the previous study, the authors proposed a phosphorus-starvation cultivation mode to minimize the phosphorus consumption during microalgal cultivation (Wu et al., 2012b). This mode was meant to enhance microalgal growth with intracellular phosphorus, which was stored within microalgal cells, via optimizing cultivation conditions (Wu et al., 2014b), and thus to produce more microalgal biomass with the same consumption of external phosphorus.

During growth with intracellular phosphorus, microalgal cell is exposed to serious phosphorus deprivation, which might induce significant variation of the physiological and biochemical characteristics of microalgal cell. Nutrient deprivation is considered as an efficient approach to increase the lipid content in microalgal biomass, especially the deprivation of nitrogen (Guccione et al., 2014; Li et al., 2010a; Ren et al., 2013). It was found that when the initial nitrogen concentration in culture medium decreased from 25 to 2.5 mg L^{-1}, the lipid content in *Scenedesmus* sp. LX1 increased significantly to over 30% (Li et al., 2010a). However, with the deprivation of nitrogen as the trigger to enhance lipid accumulation, the total biomass production was always reduced, and thus high lipid content and high biomass production appeared to be in contradiction with each other.

In this study, the detailed variation of the physiological and biochemical characteristics of microalgal cell during growth with intracellular phosphorus was investigated, including the variation of photosynthesis activity, size/weight/shape of single cell, and biochemical composition of microalgal biomass. Furthermore, the relationship between lipid/TAG accumulation in microalgal biomass and microalgal growth rate was analyzed. Based on the research results, a promising approach was proposed to achieve high lipid/TAG content and high biomass growth rate simultaneously.

2. Methods

2.1. Microalgal strain

Scenedesmus sp. LX1 (patent No. CGMCC 3036 in China General Microbiological Culture Collection Center) used in this study was originally isolated by Li from tap water (Li et al., 2010a). This strain was maintained both on agar plate containing BG11 medium and in modified BG11 (mBG11) liquid medium. In mBG11 medium, $NaNO_3$ (15 mg N L^{-1}) and $K_2HPO_4{\cdot}3H_2O$ (1.5 mg P L^{-1}) were used as the nitrogen and phosphorus resource, respectively. A_5 solution was added the same as in BG11 and other components were added half as in BG11. Algal colonies picked from the agar plate were firstly cultured in 200 mL liquid mBG11 medium in an artificial climate chamber (HPG-280H) till logarithmic growth phase (cultivated for 5 days) and then used as inoculum for photobioreactors. According to the calculation based on biomass dry weight growth and external phosphorus uptake by microalgal cells, the initial phosphorus content of microalgal biomass was about 1.5%.

2.2. Experiment design

In this study, 4.8 L ($\varphi 70 \times 1250$ mm) column air-lift photobioreactors made of polymethyl methacrylate were used for algal batch cultivation. Filament lamps (Phillips, 36W) were used to provide light for the reactors. Light intensity was 200 ± 10 μmol photon m^{-2} s^{-1}, determined by an illuminometer (photoelectric instrument plant of Beijing Normal University, SI series). The light/dark ratio was 14:10. Mixing and aeration in the reactors were achieved by bubbling air through spargers located at the bottom of the reactors. The cultivation temperature was 25 ± 2 °C. Microalgal cultivation was conducted in triplicate (n = 3).

Scenedesmus sp. LX1 was cultivated in the photobioreactors under a bacteria-controlled condition. The reactors were filled with 0.05% copper sulfate solution for 12 h and then washed with sterilized water thoroughly before use. The medium components were sterilized (121 °C, 30 min) and then added into the reactors separately. The air was filtered through 0.22 μm membrane (PALL Corporation, USA) which was connected between the compressor and the air tube.

A 150 mL algal inoculum was centrifuged (10,000 rpm × 10 min at 4 °C), and the deposited algal cells were washed once with 15 mg L^{-1} $NaHCO_3$ solution. The inoculums were then re-suspended in 100 mL $NaHCO_3$ solution and inoculated into each photobioreactor. The initial microalgal density was about 1.5×10^5 cells mL^{-1}.

In order to reach the potential of growth with intracellular phosphorus, all the nutrients in the culture medium were provided sufficiently except total dissolved phosphorus (TDP).

Two different nutrient levels were set in this study. For the oligotrophic condition, the initial total dissolved nitrogen (TDN) and TDP was 30 and 0.2 mg L^{-1}, respectively; for the eutrophic condition, the initial TDN and TDP was 300 and 2 mg L^{-1}, respectively, to achieve a N/P ratio of 150:1.

Besides other components the same as BG11, 5 mL 5 g L^{-1} KCl solution was also added to each reactor to provide sufficient potassium element for algal growth.