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Biomass production of a *Scenedesmus* sp. under phosphorous-starvation cultivation condition

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ABSTRACT

Microalgae-based bioenergy has gained extensive attention, but the consumption of non-renewable resource such as phosphorous is inevitable in the production of its feedstock. In this work, the minimal phosphorous consumption for algal biomass production of *Scenedesmus* sp. LX1 was investigated by monitoring the growth and nutrient uptake under two different cultivation modes: phosphorous-starvation and luxury-nutrient. The results showed that continuous nitrogen and phosphorous feeding in luxury-nutrient mode had no stimulating effect on biomass productivity at the nutrient level in this study, TN: 245 mg L^{-1}, TP: 5.4 mg L^{-1}. However, the sustained growth of biomass after the exhaust of phosphate in phosphorous-starvation mode led to significant increase in the biomass yield of phosphorous up to 160 g biomass/g -P, which was nearly six times more than that with nutrient feeding. To minimize phosphorous resource consumption in production of algal biomass, a phosphorous-starvation cultivation mode is proposed.

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1. Introduction

The rapid development of human society is gradually exceeding the capacity of resource reserves on the earth, as exemplified by the energy crisis. Among fossil fuel substitutes, microalgae-based bioenergy is considered to be the most promising (Groom et al., 2008) because of its characteristics of high lipid content and rapid growth, which significantly result in real productivity higher than that of oilseed crops (Weyer et al., 2010). Compared with other energy forms, biomass-based energy has its own irreplaceable advantages, such as the possibility to be integrated with CO_2 capture (Teresa et al., 2010) and nutrient removal (Li et al., 2010a) during the production of its feedstock. Another advantage of algal biomass, other than a source of energy, is its capacity to be used as feedstock for many other industries, such as food and feeds (Spolaore et al., 2006), pharmaceuticals (Harun et al., 2010), fertilizers (DOE, 2009) and other specialty products, for example bioflocculants (Borowitzka, 1986), biopolymers, and biodegradable plastics (Wu et al., 2001; Philip et al., 2007). These valuable characteristics of microalgae have attracted growing worldwide attention.

Although microalgae-based energy is always considered renewable and sustainable (Schenk et al., 2008; Brennan and Owende, 2010), resource consumption (mainly referring to growth medium such as water and inorganic nutrients) in producing its feedstock is inevitable and accounts for over 70% total costs (Behzadi and Farid, 2007), which is the main bottleneck of commercial application. Among the resources necessary for biomass production, some are or will be limited, such as fresh water and phosphorous (Britton and Baur, 2010; Cramer, 2010; Van et al., 2010).

The lifecycle of water and nutrient consumption during the production of microalgae-based biofuel has been studied by Yang et al. (2011), and the necessity of recycling harvested water and using sea/wastewater as water source has been highlighted. However, phosphorous demand for algal biomass production has not been estimated from the aspect of resource consumption. Phosphorous is one of the necessary elements for algal growth. Typically, microalgae contain approximately 1% phosphorous by dry weight (DW) (Borchardt and Azad, 1968), but phosphorous consumption in the production of algal bioenergy may be far beyond this level. The uptake of phosphorous in the absence of growth without prior starvation can be defined as "luxury uptake" (Levin and Shapiro, 1965) and has been studied by other researchers (Borchardt and Azad, 1968; Powell et al., 2008). This phenomenon could induce significant increase in the phosphorous content of algal cell. Powell et al. (2009) reported phosphorous content as high as 3.85% in the algal mixture. In addition, according to a recent lifecycle analysis (Yang et al., 2011), 0.71 kg phosphorous is required to generate 1 kg biodiesel without harvested water recycling. The phosphorous reserve required to develop algal bioenergy could be roughly estimated using this resource consumption level as an example.

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The Energy Independence and Security Act (EISA) was passed in the US in 2007, which requires a gradual increase in the production of renewable fuels to reach 36 billion gallons (136.3 billion liters) per year by 2022. Generally, the density of biodiesel is between 0.8 and 0.9 kg L^{-1} (Alptekin and Canakci, 2008), which means that if biodiesel is composed of all renewable fuels required in EISA, 109–123 billion kg biodiesel is required per year by 2022. As 0.71 kg phosphorous is required for 1 kg biodiesel, 77–87 billion kg phosphorous is demanded per year to meet EISA, amounting to about 0.8% of the total phosphorous reserve in China. This ratio is expected to increase with the gradual depletion of fossil fuels.

Two possible strategies to reduce phosphorous demand for bioenergy production exist: cutting down biomass requirements by enhancing the energy substance content of biomass itself such as lipids and minimizing phosphorous consumption during biomass production. Several researchers have focused on the former, mainly using bio-chemical engineering, genetic engineering, and transcription factor engineering approaches (Courchesne et al., 2009). Some novel methods have been proposed recently, such as using trace amounts of natural organic matter to stimulate triacylglycerols accumulation in algal cells (Li et al., 2010b). However, minimizing direct phosphorous consumption during the production of commensurable biomass has been barely studied.

This paper aims to study the possibility of producing more algal biomass using less phosphorous, and thus directly decreasing the phosphorous consumption for biomass production. The work investigated algal growth, nutrient uptake, and chemical composition of algal cell with respect to time, under two different cultivation modes: phosphorous-starvation and "luxury-nutrient". While different cultivation modes to maximize algal biomass production have been studied, this work focused on biomass yield using unit mass of phosphorous. The results of the study allowed for the proposal possible methods to minimize phosphorous consumption in producing algal biomass through a proper cultivation mode.

2. Methods

2.1. Microalgae strain and growth medium

The *Scenedesmus* sp. LX1 (Patent No. CGMCC 3036 in China General Microbiological Culture Collection Center) used in this study was recently isolated from tap water (Li et al., 2010c). The previous studies showed that this strain could grow at a very low nutrient level and accumulate high lipid content (Li et al., 2010d), indicating that this microalga strain may become one of the most promising candidates in the large-scale microalgal biomass production. Therefore, it was used as a model strain in this study. This microalgae strain was maintained in 50% BG11 medium.

The 100% BG11 medium was used as the growth medium in this study, which contained 1.5 g L^{-1} $NaNO_3$ and 40 mg L^{-1} $K_2HPO_4{\cdot}3H_2O$ as the nitrogen and phosphorous sources, respectively. The growth medium also contained the following chemicals: 75 mg L^{-1} $MgSO_4{\cdot}7H_2O$, 36 mg L^{-1} $CaCl_2{\cdot}2H_2O$, 6 mg L^{-1} citric acid, 6 mg L^{-1} ferric ammonium citrate, 1 mg L^{-1} EDTA (dinatrium-salt), 20 mg L^{-1} Na_2CO_3, and 1.0 ml L^{-1} A_5 + Co solution. The A5 + Co solution contained 2.86 g L^{-1} H_3BO_3, 1.81 g L^{-1} $MnCl_2{\cdot}H_2O$, 222 mg L^{-1} $ZnSO_4{\cdot}7H_2O$, 79 mg L^{-1} $CuSO_4{\cdot}5H_2O$, 390 mg L^{-1} $Na_2MoO_4{\cdot}2H_2O$, and 49 mg L^{-1} Co $(NO_3)_2{\cdot}6H_2O$.

2.2. Column air-lift photobioreactor and inoculum

Four identical column air-lift photobioreactors with an effective volume of 80 L each were used. Each reactor was equipped with an external illumination system composed of four fluorescent lamps on the right and left sides. Mixing and aeration of the reactors were achieved by bubbling compressed air through spargers located at the bottom of the photobioreactors. Air flow increased with the growth of algal cells and the turbulence caused by air passing through the culture vessel was enough to mix the culture medium to keep the cells in constant suspension. Air was pumped through the spargers at a rate of 10–15 L min^{-1} of each column. The aeration supplied the cells with a continuous source of ambient levels of CO_2.

Prior to the main experiment, all reactors were inoculated with the *Scenedesmus* sp. LX1 strain, which was kept in the laboratory and fed with 20% BG11 for 10 days. At the end of pre-cultivation, aeration was stopped. After 24 h sedimentation, 5 L of culture medium discharged from the bottom of each reactor was kept and was used as inoculum for the subsequent experiment. The remaining medium of precultivation was drained and all photobioreactors were cleaned.

2.3. Cultivation modes of microalgae

Two cultivation modes were used in this study, referred to as phosphorous-starvation and luxury-nutrient modes (Fig. 1). In the phosphorous-starvation mode (a batch cultivation with the medium of a high initial nitrogen-to-phosphorous (N/P) ratio about 46:1 and no addition of nutrients during cultivation), the algal biomass exhausted the phosphorous in the earlier stage of growth. It was then exposed to phosphorous starvation for some time. Therefore, the potential to produce more algal biomass with a certain amount of phosphorous was investigated. Inversely, in the luxury-nutrient mode (a fed batch cultivation), microalgae were fed with nutrients according to the growth and nutrient uptake of algal biomass to reach maximum biomass production. The difference in biomass production in these two cultivation modes was expected to estimate the possibility of producing more biomass using less phosphorous.

$NaNO_3$ and $K_2HPO_4{\cdot}3H_2O$ were used in the experiments as the only nutrient feeding sources, and the amount of nutrient addition followed the differentials between the measured value of nitrogen and phosphorous concentrations in the medium and those at the beginning of cultivation.

The concentrations of dissolved total nitrogen (DTN) and dissolved total phosphorous (DTP) in the medium were measured every three days, and thus adding nutrients was based on the growth of microalgae cells at the day without the measured value of DTN and DTP. In the previous study, *Scenedesmus* sp. LX1 was cultivated under a nutrient level of secondary effluent (Li et al., 2010d), and the chemical composition of algae cells under such

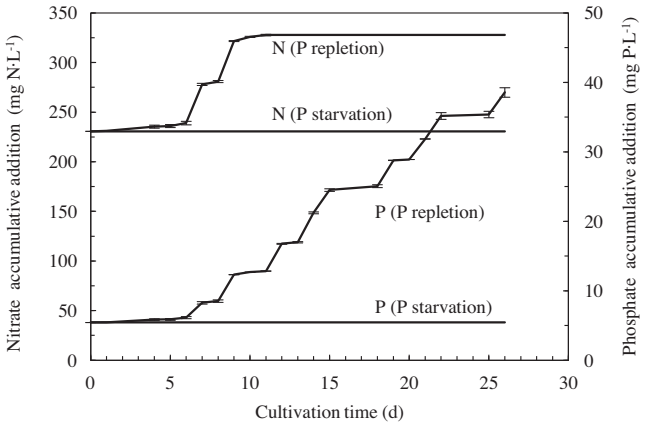

Fig. 1. Nitrogen and phosphorous addition under phosphorous-starvation and luxury-nutrient cultivation mode.

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