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Culture modes and financial evaluation of two oleaginous microalgae for biodiesel production in desert area with open raceway pond

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HIGHLIGHTS

- Five microalgae were analyzed for their growth, lipid accumulation and FA profiles.
- *Chlorella* sp. L1 and *M. dybowskii* Y2 were selected for scale-up culture.
- Batch and semi-continuous mode were used in outdoors raceway pond.
- Lipid productivity of 13.91 and 14.45 ton ha^{-1} yr^{-1} in semi-continuous mode.
- 13.31 and 14.18 \$ gal^{-1} for crude biodiesel in two microalgae were obtained.

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ABSTRACT

Cultivation modes of autotrophic microalgae for biodiesel production utilizing open raceway pond were analyzed in this study. Five before screened good microalgae were tested their lipid productivity and biodiesel quality again in outdoor 1000 L ORP. Then, *Chlorella* sp. L1 and *Monoraphidium dybowskii* Y2 were selected due to their stronger environmental adaptability, higher lipid productivity and better biodiesel properties. Further scale up cultivation for two species with batch and semi-continuous culture was conducted. In 40,000 L ORP, higher lipid productivity (5.15 versus 4.06 g m^{-2} d^{-1} for *Chlorella* sp. L1, 5.35 versus 3.00 g m^{-2} d^{-1} for *M. dybowskii* Y2) was achieved in semi-continuous mode. Moreover, the financial costs of 14.18 \$ gal^{-1} and 13.31 \$ gal^{-1} for crude biodiesel in two microalgae with semi-continuous mode were more economically feasible for commercial production on large scale outdoors.

1. Introduction

Nowadays, heightened global concerns on energy consumption and serious environmental deterioration owing to emissions from CO_2 have driven the growing research on sustainable alternatives to energy production. Biodiesel production from microalgae has been considered as an important contributor to greenhouse gas (GHG) mitigation and environment-friendly renewable feedstock (Chisti, 2007). Microalgae possess high photosynthetic efficiency, fast growth rates, wide environmental adaptability and high lipid content which makes it a promising feedstock (Wijffels and Barbosa, 2010; Larkum et al., 2012).

The increasing demand for fossil fuels and technological advancements has motivated the exploration of commercial application on microalgae outdoors (Michels et al., 2016). There are two predominant cultivation systems employed in microalgal industry, i.e., open raceway ponds (ORPs) and closed photobioreactors (PBRs). Raceway reactors are commonly used in microalgal cultivation since the 1970s, their advantages in simplicity, low costs, convenient to operate and sustainable have led to the adoption in commercial application (Harun et al., 2010; Crowe et al., 2012; Ho et al., 2014). Under photoautotrophic conditions, the promotion of biomass productivity always along with an increased oxygen evolution rate through photosynthesis, thereby the paddlewheel is needed to reducing the oxygen concentration and sedimentation and enhancing gas and liquid mass transfer (Sato et al., 2014; Li et al., 2014). However, it will bring about a substantial level of shear stress which is harmful to the cell robustness (Michels et al., 2016). Moreover, poor light utilization, contamination by other microorganisms, cell adhesion and aggregation in outdoors cultivation all has negative effects on microalgae growth (Huo et al., 2012; Crowe et al., 2012; Sato et al., 2014; He et al., 2015; Gong et al., 2015). Therefore, only a few microalgae strains could survive in outdoor cultivation. And, it is of great importance to select the microalgae with high lipid production outdoors.

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Although microalgae have many advantages than other crops, but the high cost of cultivation systems hamper the commercialization for lipid production. It is thus necessary to develop more economically feasible culture mode to increase the lipid production and thereby reduce the cultivation costs (Oncel and Vardar-Sukan, 2009; Ho et al., 2014; Yoon et al., 2015). Conventional batch culture mode is common in microalgae production, but has the disadvantages such as high labor cost and easy pollution (Yoon et al., 2015). Moreover, the light may become significantly limited due to high cell density following prolonged cultivation course, thereby making biomass losses (Ho et al., 2014; He et al., 2015). Semi-continuous culture seems to be more practicable (Oncel and Vardar-Sukan, 2009; Ho et al., 2014; Yoon et al., 2015). Such mode can avoid both a low cell division rate in the early exponential stage and light limitation in the late stationary stage because it allows for maintaining the microalgal culture under exponential growth conditions, resulting in enhanced biodiesel production (Ho et al., 2014). By incorporating a semi-continuous regime, the possibility of the biodiesel production can be improved (Huang et al., 2010). However, it has rarely been utilized outdoors. Therefore, the feasibility of microalgae should be evaluated at outdoor conditions. Above all, to date, very few studies about the scalable cultivation and economically feasible process in microalgae-based biofuel production has been reported.

To know the commercial prospects of microalgae-based biodiesel, the financial feasible analysis for biodiesel production is necessary. Comprehensive feasibility evaluation of microalgae biodiesel cultivation systems that considers the resource consumption, emissions, and their impact across the entire life cycle is critical to assess the environmental sustainability (Richardson et al., 2014). However, relevant studies about financial analysis in microalgae biodiesel made mainly on *Nannochloropsis* sp., and optimistic productivities were assumed which did not accurately reflect the actual productivities and lipid content currently achievable in the existing ORPs (Stephenson et al., 2010; Davis et al., 2011; Crowe et al., 2012; Delrue et al., 2012; Richardson et al., 2014). Therefore, it is necessary to evaluate the actual financial costs of the selected microalgae cultivating outdoors. Besides, different culture mode has different cost components, thus, cultivation mode optimization could lead to more favorable economics by raising annual biomass yields and reducing capital and operating costs (Yoon et al., 2015).

It is of great importance to select superior algal strains, optimize culture mode, reduce total costs etc. to make microalgae biodiesel become commercial reality. Considering the superiority of the abundant light resources and lower land costs, the trials were conducted in the desert area. The environmental adaptability and lipid accumulation capability of five microalgae outdoors was determined by testing in 1000 L (5 m^2) ORP. The optimal microalgae were further selected for scale-up cultivation in 40,000 L (200 m^2) ORP. Moreover, to optimize the lipid production, not only batch mode but also semi-continuous mode was applied in two different ORPs. A comparison of growth, cellular lipid content and productivities was made in two modes. Furthermore, the economic analysis of biodiesel production from two alternative modes in 40,000 L (200 m^2) ORP was evaluated according to the methods of an algae farm used by Richardson et al. (2014). This work will provide insight into feasible outdoor mass cultivation for microalgae-derived biodiesel production.

2. Methods and materials

2.1. Microalgal strain and culture medium

Five microalgae (*Chlorella*. sp. L1, *Chlorella sorokiniana* H2, *Monoraphidium dybowskii* Y2, *M. dybowskii* XJ-151 and *Scenedesmus* sp. 99) selected from indoor experiments with good potential for lipid production were utilized in this experiment. Stock cultures for all the strains were grown in 500 mL flasks with 400 mL modified BG-11medium, in which the concentration of $NaNO_3$ was half of the normal BG-11. Other components remain unchanged. In outdoors scale-up cultivation, the $NaNO_3$ in BG-11 medium was replaced by 0. 25 g L^{-1} urea.

2.2. Outdoor cultivation

2.2.1. Open raceway pond geometry

The experiments were conducted in the Microalgae Experiment Station belonging to Institute of Hydrobiology, Chinese Academy of Sciences and situated in Dalate Banner of Inner Mongolia Autonomous Region, at the east edge of Hobq Desert (40°22′18.5″N, 10 9°50′37.1″E) for two years during the summer and autumn (May to October) from 2014 to 2015. Two scales of ORPs (5 m^2 and 200 m^2) were utilized. The length, width and maximum depth were 4.80 m, 1.05 m, 0.60 m and 34.50 m, 5.80 m, 0.60 m in 5 m^2 and 200 m^2 illuminated area of ORP, respectively. Moreover, considering the light availability, the culture depth set at 20 cm with 1000 L and 40,000 L culture volume, respectively. Each ORP was drove by paddlewheel to mix the cultures, but the velocity in 5 m^2 ORP (35 cm s^{-1}) was relatively faster than 200 m^2 ORP (25 cm s^{-1}). Due to the serious evaporative losses in open raceway system, corrected water was added to the system every day. In addition, more ventilating and shading were performed at the noon from 11:30 am to 14:30 pm in hot summer.

2.2.2. Experiment setup

For outdoor cultivation, a series of scale-up pre-cultivation was employed. First, the seed culture in 500 mL flasks was diluted in transparent barrel (20 L) with non-sterile medium, and incubated outdoors until exponential phase. Subsequently, another dilution was done in barrel (100 L). The culture was prepared for the ORP cultivation. After the pre-cultivation, the batch culture was conducted with five microalgae conducted in 1000 L ORP to select the optimal stains for lipid production. Then, the selected microalgae (*Chlorella* sp. L1 and *M. dybowskii* Y2) were cultivated in 40,000 L ORP. Further experiment was conducted with semi-continuous mode in two scales of ORP. In semi-continuous mode, half of the culture was harvested and the remaining culture used as the seed for next batches. In addition, three cycles and three days for each were performed after ten days rapid growth of the microalgae.

During the culture course, underground water from desertification area was used, which contained the concentration of Na^+ (89.39 ppm), SO_4^{2-} (62.92 ppm), and low levels of K^+ (1.69 ppm), Mg^{2+}(13.65 ppm), Ca^{2+} (12.66 ppm), Cl^- (24.12 ppm), $NO3^-$ (1.41 ppm). Parameters of incident light intensity were recorded right under the ORP with a Quantitherm light meter (Hansech, UK). Temperature, salinity and pH were measured daily utilizing respective sampling probes (YSI Instruments, Yellow Springs, OH, USA). All the parameters were determined everyday at7:00, 10:00, 13:00, 15:00 and 18:00 o'clock.

2.3. Analysis and calculation

2.3.1. Biomass analysis

Biomass of microalgae was determined gravimetrically, and the growth was expressed in terms of dry weight (DW). In brief, 20 mL of samples was harvested by centrifugation. Then, the cells was washed twice with distilled water, lyophilized, and weighed.