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Short Communication

Two-stage heterotrophic and phototrophic culture strategy for algal biomass and lipid production

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ABSTRACT

A two-stage heterotrophic and phototrophic culture strategy for algal biomass and lipid production was studied, wherein high density heterotrophic cultures of *Chlorella sorokiniana* serve as seed for subsequent phototrophic growth. The data showed growth rate, cell density and productivity of heterotrophic *C. sorokiniana* were 3.0, 3.3 and 7.4 times higher than phototrophic counterpart, respectively. Hetero- and phototrophic algal seeds had similar biomass/lipid production and fatty acid profile when inoculated into phototrophic culture system. To expand the application, food waste and wastewater were tested as feedstock for heterotrophic growth, and supported cell growth successfully. These results demonstrated the advantages of using heterotrophic algae cells as seeds for open algae culture system. Additionally, high inoculation rate of heterotrophic algal seed can be utilized as an effective method for contamination control. This two-stage heterotrophic phototrophic process is promising to provide a more efficient way for large scale production of algal biomass and biofuels.

1. Introduction

Algal lipid is considered as an ideal feedstock for transportation fuels (Pienkos and Darzins, 2009). However, prior to industrial scale application, a series of key challenges have to be resolved. For example, in northern climates, phototrophic biomass production is limited in the winter because of the cold temperature and lack of available sunlight. Even in summer months, open algae cultures have relatively low growth rates and biomass productivity (Chisti, 2007). Usually, light limitation is the major limiting factor, since light penetration is inversely proportional to the cell concentration (Chen, 1996). Although a relatively higher biomass productivity can be achieved in photobioreactors (PBRs), its high cost in facility and operation leads to a lower economical viability than open pond (Chisti, 2007). Additionally, open systems are continuously threatened by invading species, such as undesired algae and bacteria. Due to these problems, phototrophic algae are only commercially used to produce high value products (Spolaore et al., 2006). Large scale culture of phototrophic algae for biofuel production still has too high production costs, compared to the produced value (Pienkos and Darzins, 2009).

Compared to phototrophic growth, heterotrophic algae culture takes advantage of fast growth, high production rate, and convenient harvesting. A series of heterotrophic microalgae species were successfully used in industry-scale polyunsaturated fatty acids production (Chi et al., 2009). Recently, heterotrophic microalgae culture to produce biodiesel was reported and showed its promise, however, a high cost of organic carbon is one of limiting factors for this process (Chi et al., 2011).

To develop an efficient phototrophic process and overcome potential contamination issues, we have investigated a process that takes advantages of both heterotrophic culture's high efficiency and phototrophic culture's low cost. In this two-stage heterotrophic and phototrophic culture process, heterotrophic culture provided an efficient way for seed cells production, which can be used as inoculums in the subsequent phototrophic open pond cultivation for algal biomass and lipid production.

2. Methods

2.1. Organism and medium

Chlorella sorokiniana (UTEX 1602) was obtained from the Culture Collection of Alga at the University of Texas (Austin, TX, USA). Kuhl medium was used for phototrophic culture (Kuhl and Lorenzen, 1964). Heterotrophic culture was supplemented with different concentrations of glucose, as indicated in individual experiments.

2.2. Culture conditions

Flask cultures were conducted in 0.25-L Erlenmeyer flasks. Phototrophic cultures contained 0.2 L Kuhl medium and were

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bubbled with air supplemented with 0.9% CO_2 at a rate of 0.08 L min^{-1}. Heterotrophic cultures contained 50 mL Kuhl medium with glucose respectively, and incubated at a rotary rate of 200 rpm. For the experiments with bioreactors, phototrophic cultures were carried out with 1-L and 5-L closed PBR containing 1.0 L and 3.0 L Kuhl medium, and bubbled with 0.9% CO_2 in air at the rate of 0.32 and 0.96 L min^{-1}, respectively. Heterotrophic cultures were performed in 5-L NBS Bioflo 110 fermentors (New Brunswick Scientific) with 3.0 L Kuhl medium (20 g L^{-1} glucose). The dissolved oxygen (DO) concentration was maintained at 50% with cascading DO control to agitation speed, and the aeration rate was 3.0 L min^{-1}.

2.3. Heterotrophic algae culture with food waste and wastewater

Food waste was collected from a cafeteria at Washington State University, Pullman, WA. The preparation for Food Waste Hydrolyzed Broth (FWHB) was described by Chi et al. (2011). The primary wastewater was collected from Pullman Wastewater Treatment Plant at Pullman, WA. Heterotrophic cultures were conducted in 5-L fermentors containing 3.0 L mixture of FWHB and primary wastewater (1:1, v/v).

2.4. Algal seeds comparison

After heterotrophic culture with the mixture of FWHB and wastewater, the produced algal seeds were harvested as the hetero-seed. The phototrophic cultured algal seeds in the 5-L PBR were used as the photo-seed. For closed system comparison, the hetero- and photo-seeds were inoculated into the 1-L PBR for phototrophic culture. For open system comparison, the hetero- and photo-seeds were inoculated into open tanks containing 40 L Kuhl medium with the depth of 0.15 m. The open tank was placed in a greenhouse and the temperature was kept around 23 °C. The cultures were mixed with air bubbling at the rate of 4.0 L min^{-1}, and pure CO_2 was injected at the rate of 0.2 L min^{-1}. The average photon flux density was 400 μmol m^{-2} s^{-1} during the daytime with sunlight, and 200 μmol m^{-2} s^{-1} in the night with artificial light.

2.5. Contamination test

C. sorokiniana was used as the desired alga species, while a native green alga *Chlamydomonas* sp. (with a higher growth rate of 0.75 d^{-1} compared with *C. sorokiniana*) isolated from the northwest pacific area in US and *Escherichia coli* (Top 10, Invitrogen) were used as the contaminants. Cultures were performed in 0.25-L flasks phototrophically. The experimental groups contained both *C. sorokiniana* and *Chlamydomonas* sp., or *C. sorokiniana* and *E. coli*, while the control group only contained *C. sorokiniana*.

2.6. Analytical procedure

The analytical methods of cell density, dry cell weight (DCW), fatty acids, sugars and chemical oxygen demand (COD) were described by Chi et al. (2011).

3. Results and discussion

3.1. Heterotrophic and phototrophic culture of C. sorokiniana for seed cells production

Flasks cultures were conducted to investigate the effect of initial glucose concentrations on the alga *C. sorokiniana* growth. As shown in Table 1, the final algal cell density increased when glucose went up from 5 to 20 g L^{-1}, but the high concentration glucose 40 g L^{-1} showed inhibitory effects. At 20 g L^{-1} glucose, *C. sorokiniana* reached the highest growth rate, cell density and productivity of 1.48 d^{-1}, 397×10^6 cells mL^{-1} and 182×10^6 cells mL^{-1} d^{-1}, respectively. Compared with heterotrophic growth, phototrophic culture of *C. sorokiniana* showed a shorter lag time of 24 h. However, the growth rate, final cell density and average productivity were only 0.73 d^{-1}, 261×10^6 cells mL^{-1} and 62×10^6 cells mL^{-1} d^{-1}, respectively. These results showed that the heterotrophic culture had higher growth rate and cell productivity for the green alga *C. sorokiniana*.

For dual-trophic algae, such as many *Chlorella* species, the uptake of glucose is based on an inducible hexose/H^+ symport system (Tanner, 2000). In the presence of inducer glucose, algae can change the trophic mode in a short time. Additionally, when algae switch from phototrophy to heterotrophy, the transporter activity can increase more than 200-fold. Under heterotrophic conditions, growth rate, DCW, ATP generated from the supplied energy and the yields of biomass on ATP are higher than those of phototrophic cultures (Liang et al., 2009; Liu et al., 2010; Yang et al., 2000). Many other algal species outside of the *Chlorella* genus also exhibit higher growth rates under heterotrophy (Azma et al., 2011; Chen and Johns, 1995; Ogbonna et al., 1999), and can be potentially applied for this two-stage heterotrophic and phototrophic system.

To study algal productivity at a larger scale, *C. sorokiniana* was cultured in larger size bioreactors. For heterotrophic culture, a much higher final cell density of 542×10^6 cells mL^{-1} was achieved in the fermentor than that in the 0.25-L flask (Table 1), which was attributed to the well controlled DO and mixing. However, for phototrophic culture, the scaling up had negative effects on algae growth. The growth rate, final cell density and average productivity in 5-L PBR were only 0.48 d^{-1}, 165×10^6 cells mL^{-1} and 24×10^6 cells mL^{-1} d^{-1}, respectively, which were much lower than those in 0.25-L flask (Table 1). In small-size flasks (0.25-L), the growth rate, final cell density and average productivity of heterotrophic culture (20 g L^{-1}glucose) were 2.0, 1.5 and 2.9 times higher than those of phototrophic culture, respectively. In larger scale bioreactors (5-L), the advantage of heterotrophic growth was more obvious, which gave 3.0-fold growth rate, 3.3-fold final cell density and 7.4-fold average productivity compared with phototrophic culture.

Traditionally, seed cells for algae culture are generated in PBRs and/or in open ponds. For these phototrophic culture processes, light is a significant limiting factor. It is suggested that the light is hardly disperse efficiently and evenly inside the PBR at operational volumes of 50–100 L or higher, which leads to a low productivity in large scale production (Perez-Garcia et al., 2011). Heterotrophic culture is more easily to be scaled up and does not compete with illuminated surface area of the dense open cultures, e.g. the heterotrophic cultivation of microalgae has been successfully scaled-up to 50,000 L (Chen, 1996). In addition, the heterotrophic culture can use the existing infrastructures, which results in a significant reduction in costs (Perez-Garcia et al., 2011). It is clearly demonstrated that heterotrophic culture has significant advantages over phototrophic culture in large scale application.

3.2. Heterotrophic algae seed culture with food waste and wastewater

The mixture of FWHB and wastewater was used for the heterotrophic culture of *C. sorokiniana* in 5-L fermentor. The results showed that *C. sorokiniana* was able to reach a high cell density of 463×10^6 cells mL^{-1}, which was 2.8-fold higher than that of phototrophic culture in 5-L PBR. Generally the cost of substrates is considered as one of major limitations of heterotrophic fermentation (Chen et al., 2010; Perez-Garcia et al., 2011), but it can be reduced if the low value waste materials is utilized. Our results