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Effect of various carbon sources on biomass and lipid production of *Chlorella vulgaris* during nutrient sufficient and nitrogen starvation conditions

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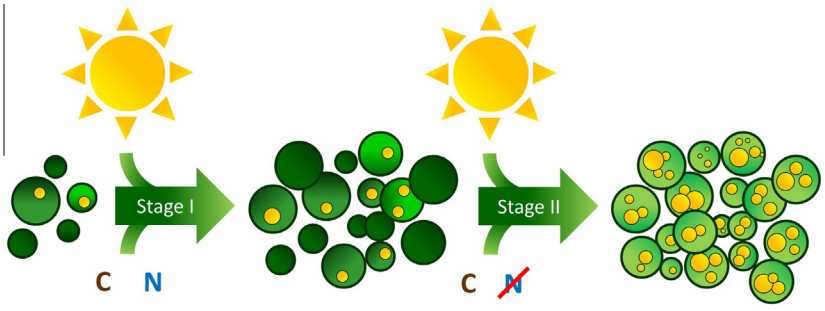
HIGHLIGHTS

- A two-stage process was applied to enhance lipid production in microalgae.
- In stage I the highest cell growth was achieved by using sodium bicarbonate.
- Sodium acetate resulted in highest lipid and fatty acid content during stage II.
- The highest lipid productivity belonged to sodium acetate in whole process.
- Molasses can be a suitable carbon source for lipid production in microalgae.

GRAPHICAL ABSTRACT



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ABSTRACT

In this research, a two-stage process consisting of cultivation in nutrient rich and nitrogen starvation conditions was employed to enhance lipid production in *Chlorella vulgaris* algal biomass. The effect of supplying different organic and inorganic carbon sources on cultivation behavior was investigated. During nutrient sufficient condition (stage I), the highest biomass productivity of 0.158 ± 0.011 g/L/d was achieved by using sodium bicarbonate followed by 0.130 ± 0.013, 0.111 ± 0.005 and 0.098 ± 0.003 g/L/d for sodium acetate, carbon dioxide and molasses, respectively. Cultivation under nitrogen starvation process (stage II) indicated that the lipid and fatty acid content increased continuously to a maximum value at day 2. Using carbon dioxide resulted in highest cell density, while using sodium acetate led to the highest fatty acid content. Molasses was not as effective as other carbon sources, but by taking into account its lower price, it can be considered as a suitable carbon source for algal lipid productivity.

1. Introduction

In recent decades, microalgae have gained attention as a promising resource for biodiesel production due to substantial amount of lipids (Mata et al., 2010), high growth rate (Rawat et al., 2013), and

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cultivation capability in saline water as well as nonagricultural land (Wijffels, 2008). However, various technological and economic constraints limit the industrial-scale production of microalgal biodiesel (Abedini Najafabadi et al., 2015). The major issues in cultivation system are high cell density culture with optimum lipid content and also cultivation in a suitable large-scale system (Huerlimann et al., 2010).

Many species of microalgae have the capability of accumulating large quantities of lipids as well as other valuable by-products (Subhadra and Edwards, 2010). Nevertheless, several growth conditions such as carbon supply, temperature, air flow rate and nutrient concentration should be adjusted to maximize production of a specific component. One way to increase the cell density as well as lipid content is to cultivate microalgae in heterotrophic or mixotrophic conditions where organic carbons, such as sugars and organic acids are used as carbon sources. Bhatnagar et al. (2011) have studied the mixotrophic cultivation of three different microalgal species and concluded that the mixotrophic growth of these microalgae resulted in production of biomass 3–10 times more than phototrophy. Heredia-Arroyo et al. (2011) investigated the growth rate and lipid content of *Chlorella vulgaris* under mixotrophic cultivation and showed that the acetate has the best performance between the studied carbon sources. However, even though the biomass and lipid productivities are significantly higher compared with those from autotrophic growth, the cost of the organic carbon sources is high when compared to all other added nutrients. This high cost issue can be solved by using cheap organic carbon sources. Molasses, which is a by-product of sugar factories, is an inexpensive material which is extensively used as a carbon source in cultivation of microorganisms (Lee and Kyun Kim, 2001; Lazaridou et al., 2002; Kongjan et al., 2011). Nevertheless, there are limited studies on investigating the use of molasses in microalgal cultivation (Andrade and Costa, 2007).

It has been shown that the lipid accumulation is enhanced during nitrogen-limited or deprived cultures of microalgae. Depletion of nitrogen along with availability of carbon source changes cellular carbon flux from protein synthesis to lipid synthesis (Sheehan et al., 1998). Beside this, neutral lipids in the form of triacylglycerols become the predominant components of lipids in nitrogen-depleted cells (Sheehan et al., 1998; Ho et al., 2012; Li et al., 2011). However, the enhanced lipid accumulation is concomitant with lower biomass productivity and consequently lower overall lipid (Klok et al., 2014). In order to reach high growth rate as well as high lipid content, two-stage process consisting of cultivation under nutrient sufficient followed by cultivation under nitrogen starvation condition could be applied (Mujtaba et al., 2012). Several researchers have studied this process, however they have mostly focused on CO_2 as carbon source (Sun et al., 2014; Breuer et al., 2012; Lucas-Salas et al., 2013). In contrast, the present study aimed at investigating the effect of various organic and inorganic sources on the cultivation of *C. vulgaris* microalgae during two-stage process. The studied carbon sources were carbon dioxide and sodium bicarbonate as inorganic sources, and sodium acetate and molasses as organic sources. Cell growth and fatty acid content are measured continuously at each stage and the best carbon source in terms of biomass growth and fatty acid productivity is identified.

2. Methods

2.1. Materials

The solvents used in this work included chloroform (99%, Merck) and methanol (99.5%, Merck). Sulfuric acid (95–98%, Merck) was used as the catalyst for transesterification reaction. All nutrients used for culture media were laboratory or American Chemical Society (ACS) grade. Methyl heptadecanoate (99%, Sigma–Aldrich) was used as an internal standard for fatty acid quantification.

2.2. Algal strain and culture media

The *C. vulgaris* CCAP (211/19) was obtained from the Culture Collection of Algae and Protozoa, Ambleside, Cumbria, UK. This species was at first pre-cultured in 250 ml erlenmeyer flasks containing 100 ml autoclaved Bold's Basal (BB) Medium (Stein, 1973) using a shaking incubator under continuous fluorescence illumination (100 μmol/m^2/s) at 25 °C. BB Medium contains $NaNO_3$ (250 mg/L), K_2HPO_4 (75 mg/L), KH_2PO_4 (175 mg/L), $CaCl_2{\cdot}2H_2O$ (25 mg/L), $MgSO_4{\cdot}7H_2O$ (75 mg/L), NaCl (25 mg/L), KOH (31 mg/L), Na_2EDTA (50 mg/L), $FeSO_4{\cdot}7H_2O$ (3.98 mg/L), H_3BO_3 (11.42 mg/L), $ZnSO_4{\cdot}7H_2O$ (8.82 mg/L), $MnCl_2{\cdot}4H_2O$ (1.44 mg/L), $CuSO_4{\cdot}5H_2O$ (1.57 mg/L), MoO_3 (0.71 mg/L) and $Co(NO_3)_2{\cdot}6H_2O$ (0.49 mg/L). Cells were transferred to a photobioreactor when they reached the constant phase.

2.3. Normal nutrient cultivation (stage I)

In the first stage, pre-cultured algal cells were cultivated in 5 L (ID, 16 cm; height, 25 cm) photobioreactor containing sterilized BB Medium. The culture was grown under 300–400 μmol/m^2/s of artificial light (continuous fluorescence illumination) and its temperature was adjusted to 25 °C. Rate of aeration was 5 L/min under an injection/non-injection cycle of 1/3 min. In order to provide carbon dioxide, it was continuously mixed with the air and injected to the photobioreactor. CO_2 concentration in the air was adjusted to 3% (v/v) with a flow meter. Other carbon sources including sodium acetate, sodium bicarbonate, and molasses was manually supplied to the system at the rate of 0.5 g/d. During growth period, samples were collected from the photobioreactor at different times to determine microalgal biomass concentration, residual nitrogen concentration, and lipid content.

2.4. Nitrogen depletion cultivation (stage II)

Cultivation in the first stage was stopped when the cells reached the constant phase. Algal Cells were recovered from the culture media through centrifugation at 4042*g* for 5 min. Subsequently, all flasks from stage I were pooled together to ensure an equal initial biomass composition for different experiments in stage II. After washing the cells three times with nitrogen-starved Bold's Basal (N-BB) Medium, the cells were transferred to the photobioreactor and re-suspended in the N-BB Medium. Cultivation condition (temperature, light intensity, aeration rate and carbon supply) was exactly the same as in stage I. The cultivation in the second stage was stopped after 7 days upon reaching the constant phase.

2.5. Determination of biomass concentration

The microalgal biomasses concentration was determined by measuring the optical density of the samples at 680 nm (OD_{680}) using a spectrophotometer (model 6715 UV–vis, Jenway, UK). When deemed necessary, the samples were diluted in such a way that their OD_{680} values fell between 0.2 and 0.9. Biomass concentration was then calculated by the following equation:

$$\text{Biomass concentration (g/L)} = OD_{680} \times 0.628 \quad (1)$$

Eq. (1) was established by plotting OD_{680} versus dry cell weight (DCW) of various samples with different biomass concentrations. DCW of the samples were determined gravimetrically after drying