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Growth and lipid accumulation of indigenous algal strains under photoautotrophic and mixotrophic modes at low temperature

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ABSTRACT

In the present work, 10 strains of algae indigenous to Quebec Province of Canada were used to investigate the performances of lipid accumulation and biomass growth of algae cells in autotrophic and mixotrophic modes at a low cultivation temperature of 10 °C. It is found that mixotrophic cultivation of algae cells brought more steady growth than photoautotrophic growth at low temperature, but had only a weak influence on growth phases, and that the high C/N ratio of the mixtotrophic culture medium played a positive role in algal lipid accumulation. Furthermore, compared with photoautotrophic cultivation, mixotrophic cultivation enhanced lipid accumulation at a cultivation temperature of 10 °C. Most of the experimented algal strains consumed little phosphate in the Bold's Basal Medium used under two trophic modes. The results indicate that it is necessary to further optimize the culture medium used in order to enhance algal growth during mixotrophic cultivation, and that the metabolic mechanisms of these algae in the mixotrophic mode need to be further investigated.

1. Introduction

As common photosynthetic organisms, algae can be technologically developed as an excellent cell factory that can capture solar energy and transform atmospheric carbon dioxide to useful organic compounds such as lipids [1]. Lipids from algal biomass has been proposed as one of the promising feed stocks for biodiesel production due to the algae's intrinsic characteristics of fast growth rate, high lipid content and productivity, lack of a requirement for arable land and environmentally friendly production process without conflict with food production [2–5].

Microalgae can adopt various routes of metabolism for their growth and lipid production. For instance, microalgae can grow photoautotrophically using light as a sole energy source that can be converted to chemical energy through photosynthetic reactions, or heterotrophically utilizing only organic compounds as both carbon and energy sources, or mixotrophically using both organic compounds and CO_2 to support growth of algal biomass under conditions of illumination [6,7]. Photoautotrophic cultivation may lead to a low density of microalgal biomass and low lipid productivity due to mutual shading of cells and limited light penetration [8,9]. Mixotrophic growth regime of microalgae is a variant of the heterotrophic growth regime, where CO_2 and organic carbon are simultaneously assimilated and both respiratory and photosynthetic metabolism operate concurrently [10]. Liang et al. [11] found that autotrophic growth of *C. vulgaris* gave a higher cellular lipid content (38%), while lipid productivity was highest in the mixotrophic mode using glucose as substrate. Liu et al. [12] reported that when compared with autotrophic cultivation, *C. zofingiensis* showed higher lipid productivity and better lipid quality under heterotrophic conditions. Kim et al. [13] indicated that heterotrophic cultivation resulted in a good potential in microalgae biomass growth and nitrogen and phosphorus removal rates of *C. sorokiniana* in waste water treatment system. Chojnacka and Noworyta [14] observed high specific growth rate for *Spirulina* sp. in a high light intensity of 33 W/m^2 in mixotrophic cultivation and low specific growth rate in a low light intensity of 17 W/m^2 in autotrophic cultivation. Hallenbeck et al. found that the investigated algae strains demonstrated good growth at low culture temperatures and lipid production was favored by mixotrophic growth at either temperature [15]. In summary, numerous investigations have shown that mixotrophic conditions can improve both the growth of the microalgae and the accumulation of triacylglycerols (TAGs) [16].

Most microalgae accumulate lipids under specific environmental stress conditions such as nitrogen or phosphate limitation or low temperature, at the cost of a decreased growth rate of biomass [17]. Culture temperature is one of the important environmental parameters which impacts algal cellular physiology by changing the rate of chemical reactions and the stability of cellular components. To maintain structural integrity, some algal strains regulate lipid composition to achieve membrane fluidity at a low temperature, resulting in an increased cellular level of unsaturated fatty acids [18]. Temperatures ranging between 15 and 25 °C are usually considered optimal for algal growth [18], while lipid content decreased with increasing temperature, temperature

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control, therefore, may be a possible approach to enhance the lipid productivity [19,20]. Thompson et al. [21] and Lynch et al. [22] found a considerable increase in unsaturated fatty acids of algae cells at low temperatures of 10 and 12 °C. Roleda et al. [23] found that some algae species grown under low temperature (10 °C) and nutrient-replete conditions were able to acclimate to the culture temperature condition and significantly increased lipid content to 57–113% in autotrophy with 16:8 h light:dark photoperiod. However, little research on mixotrophic culture at low temperature has been reported, especially on effects of different trophic modes coupled with low temperature stress on the characteristics of lipid biosynthesis.

In the present work, 10 strains of algae indigenous to Quebec Province of Canada were used to investigate the performances of lipid accumulation and biomass growth of algal cells in autotrophic and mixotrophic modes at a low cultivation temperature, and a comparison of lipid accumulation and biomass growth between photoautotrophic and mixotrophic modes with low culture temperature was conducted. The aim of this study was to reveal effects of autotrophic and mixotrophic modes with low cultivation temperature on accumulation of lipids and growth of algae cells.

2. Methods and materials

2.1. Cultivation of algal strains

In this experiment, 10 indigenous algal strains (Labelled as MA2H1, MA2H4, HA1A1*, HA1B1*, PCH03, PCH04, PCH10, PCH19, PCH22 and PCH37) used to investigate biomass growth and lipid accumulation in mixotrophic and autotrophic cultivations were isolated from the five different locations in Canada (Lac Croche (45°59′24.37″N 74 °0′21.01″ W), Lac Pilon (46 ° 0′14.02″N 74 ° 1′7.09″W) and University of Montreal biological station (45°59′17.11″N 74 ° 0′20.55″W)) [15,18]. The purity of the cultures was ensured by repeated plating and by regular observation under microscope. The isolated microalgal strains were previously identified as *Chlorella* sp. (MA2H1, MA2H4, PCH03, PCH04, PCH10, PCH37), *Chroococcus* sp. (HA1A1*), *Scenedesmus* sp. (HA1B1*), *Desmodesmus* sp. (PCH19) or *Botrydiopsis* sp. (PCH22) [15]. During the experiment, 10 algal strains were respectively incubated in two 12-well microplates for autotrophic and mixtrophic growth, and the samples located in top-left and bottom-right wells of the microplates were made as controls without inoculation. One microplate was used for autotrophic cultivation with Bold's Basic Medium (BBM) only and another one for mixotrophic cultivation with 25 mM glycerol in BBM as substrate. Prior to inoculation, the culture medium was made sterile by filtration through a 0.45 μm Millipore filter. After placing 3.5 mL of sterile culture medium into the wells of the two microplates, the wells were individually inoculated with the algal strains to be studied. The inoculated samples covered by a transparent lid to decrease water evaporation were then put in an incubator for cultivation. The environmental temperature for incubation was set at 10 °C in the incubator (Controlled Environment Incubator Shaker, New Brunswick Scientific, America). The CO_2 concentration in the incubator was set at about 2% using a CO_2 controller (Thermo Scientific, Electron Corporation, America) according to the results of preliminary experiments, and the average illumination intensity was adjusted to about 14 W/m^2 (approximately 64.34 μmol/m^2/s) from fluorescent light lamps. All of the experiments were carried out in duplicate.

2.2. Analytical methods

During the cultivation, microalgal growth was monitored via measurement of absorbance at 630 nm every 24 h using a universal plate reader (EL800, Biotek Instrument, America). Prior to measurement, the cultures were mixed sterilely and then measured at least three times to obtain steady absorbance values. At end of the experiment, the neutral lipid concentration of the cultures in every well was detected using Nile Red staining [18,24]. After 23 days of incubation, 50 μL of the diluted algal culture sample was stained with Nile Red at 15 μg/mL final concentration, using dimethylsulfoxide (DMSO) as carrier. The assay was brought to a final volume of 200 μL and was incubated for 15 min with agitation using a microtiter plate shaker (DSG Titertek/4 Flow Laboratories, Meckenheim, Germany). The fluorescence was then read in a Synergy NEO HTS Microplate Reader (America) with excitation set to 520 nm and emission captured at 570 nm. The fluorescence output was compared to a standard curve made using extra virgin olive oil. The average value of the analytical readings of each biological replicate was calculated.

To obtain calibration curve of biomass weight versus OD_{630nm}, the algae pre-cultures with different OD_{630nm} values were centrifuged at 7370 × g (D37520, Sigma, Germany). The obtained biomasses were transferred to a microporous filter and weighed after being dried at 40 °C for 48 h in an oven. The filter had been previously dried at 40 °C for about 24 h till a constant weight was obtained.

At end of this cultivation, the cultures in every well were centrifuged at 7370 × g. The suspension was used for determination of residual nitrate and phosphate concentrations. Phosphate concentrations in the culture medium were determined colorimetrically at 630 nm using ammonium heptamolybdate and malachite green [25]. Nitrate concentration was detected colorimetrically using diphenylamine [26]. The 96-well microplates were used to read absorbance at 630 nm for phosphate and nitrate determinations. Detection of residual glycerol included three parts: 1) transformation of the glycerol into formaldehyde, 2) detection of the formaldehyde using Hantzsch's reaction, 3) detection of absorbance of the reaction solution at 410 nm (Shimadzu UV-2101PC, Japan) [27].

Biomass productivity was calculated from the following equation: BP (g/L/d) = $(B_1 - B_0)/t$, where B_1 (g/L) was the biomass concentration at end of the cultivation, B_0 (g/L) was that at the beginning, and t was the duration of cultivation (23 days).

The utilization efficiencies of nitrate, phosphate and glycerol were calculated according to the equation: utilization efficiency = $(C_0 - C_1)/C_0 \times 100$, where C_1 was the residual concentration of nitrate or phosphate or glycerol in culture medium (g/L), C_0 the initial concentration (g/L).

The cellular content of total neutral lipid was calculated using the equations: Lipid content (%) = $(W_L/W_B \times 100)$, where W_L (g) was the weight of obtained lipids and W_B (g) the dry weight of obtained algal biomass.

2.3. Statistical analysis

All cultivations were carried out in duplicate. All the determinations were carried out in at least duplicate and the final values were expressed as mean ± standard deviation. Analysis of variation (ANOVA) of single factor was conducted to analyze the variance of these results with significance declared at $P < 0.05$ using Origin Pro 8.0.

3. Experimental results

3.1. Algal biomass growth

The biomass concentrations of the 10 algal strains cultured in BBM and BBM plus glycerol were measured over the experimental period. It can be seen that the trophic mode had different effects on biomass growth at the low cultivation temperature (10 °C) depending upon the strain (Fig.1). As shown in Fig 1 A, PCH03 in BBM grew faster in the photoautotrophic mode than in BBM plus glycerol (mixotrophic mode), while the growth of PCH10 was faster under mixotrophic conditions than under photautotrophic conditions. Unlike these two algal strains, PCH19 grew slowly under both trophic modes at the low temperature of cultivation used. At end of cultivation, the biomass