Integration of semi-batch cultivation and extraction for maximal lipid production in Chlamydomonas sp. Tai-03

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Abstract. Rapid improvements in bioseparation technology, new regulatory directives, product quality constraints, and the production efficiency have necessitated the development of more advanced and powerful downstream bioprocesses for biotechnology and biopharmaceuticals industrial. This has transformed in dramatically improvements in traditional bioseparation processes as well as the development of entirely new approaches. In this paper, we highlight some of these recent advances of integration of semi-batch cultivation and extraction for maximal lipid production in Chlamydomonas sp. Tai-03. This includes extractive cultivation, extractive bioconversion aqueous two-phase system, aqueous two-phase flotation, and newly developed liquid biphasic flotation. Alcohol/salt liquid biphasic flotation (LBF) with aid of ultrasonication which have the ability of killing two birds with one stone, it not only capable in cell rupturing, it also able to recover bioproducts simultaneously and continuously. The effect of varying crude feedstock concentration, flotation time, type of salt, concentration of salt, type of alcohol, concentration of alcohol, initial volumes of salt and alcohol were investigated. Microalgal biofuels or generation three biofuels have been widely recognized as potential replacements of fossil fuels. One of the most attractive option is the partial or full replacement of diesel fuel with microalgal biodiesel. Here, Chlamydomonas sp. Tai-03 was cultured using semi-batch cultivation to enhance its lipid production. Upon lowering the culture replacement fraction to 25%, the greatest biomass and lipid productivities were obtained at 1.23 ± 0.02 g/L/d and 239.6 ± 24.8 mg/L/d. After transesterification, palmitic acid (C16:0), oleic acid (C18:1), and linoleic acid (C18:2) were the main fatty acid methyl esters (FAMEs) present. These short-chain FAMEs and high productivities of Chlamydomonas sp. Tai-03 are suitable for biodiesel output.

1. Introduction

Compared with generation one and two biofuels, microalgal biofuels are becoming more attractive options. This is due to the advantages of culturing microalgae, which includes fast growth rate, large accumulation of lipids, able to flourish using wastewater and brackish water, does not contest against food crops and cash crops, and does not require arable land. The culture conditions of microalgae can also be altered to increase the production of several types of biofuels, such as biodiesel, biohydrogen, bioethanol, and others [1]. Of these biofuels, microalgal biodiesel is a potential substitution for petrodiesel [2]. Biodiesel is different from petrodiesel, where biodiesel is not comprised of sulphur or aromatic compounds and combusting biodiesel gives off lesser unburned hydrocarbons, particulate matter, and carbon monoxide [3]. Biodiesel can also be blended with gasoline to improve fuel
performances such as better thermal efficiency and lower ignition delay [4]. Therefore, in this study, we aim to optimize the growth rate of Chlamydomonas sp. Tai-03 using semi-batch cultivation.

2. Methodology

2.1. Microalga strain, medium composition, and culture conditions

The microalgal species used in this study is *Chlamydomonas* sp. Tai-03. This species was isolated in Taiwan and obtained from National Cheng Kung University (NCKU). The medium chosen to culture the microalgae was BG-11 medium, which has a chemical composition (in g/L) of: NaNO₃, 1.5; K₂HPO₄, 0.03; MgSO₄.7H₂O, 0.075; Citric acid anhydrous, 0.006; Na₂CO₃, 0.02; CaCl₂.2H₂O, 0.036; Ammonium iron(III) citrate, 0.006; EDTA.2Na, 0.011; H₃BO₃, 0.00286; Na₂CO₃, 0.02; CaCl₂.2H₂O, 0.036; Ammonium iron(III) citrate, 0.006; EDTA.2Na, 0.011; H₃BO₃, 0.00286; MnCl₂.4H₂O, 0.00181; ZnSO₄.7H₂O, 0.000222; Na₂MoO₄.2H₂O, 0.00039; CuSO₄.5H₂O, 0.000079; Co(NO₃)₂.6H₂O, 0.000049.

The optimal culture conditions for maximal lipid production in *Chlamydomonas* sp. Tai-03 had been determined by our previous study, and the conditions were: BG-11 medium with 25% initial nitrate concentration (0.375 g/L NaNO₃), 200 µmol/m²s light intensity, 0.12 g/L initial cell concentration, 300 rpm continuous stirring, and continuous sparging of 5% CO₂ gas at a flow rate of 0.1 vvm. The operation of the indoor 1 L photobioreactor used in this study was identical to PS [5].

2.2. Determination of microalgal cell concentration

The microalgal cell concentration in the photobioreactor was determined daily via optical density measurement at a wavelength of 680 nm (OD₆₈₀) using a spectrophotometer (UV-1800, Shimadzu, Japan) after appropriate dilution with distilled water. The dry cell weight (DCW) of the microalgal biomass was obtained by drying 5 ml of the culture in a weighing moisture analyser (ML-50, A&D, Japan). The dry weight of the biomass was determined using an electronic balance and converted to biomass concentration via calibration between OD₆₈₀ and DCW.

2.3. Determination of nitrate concentration

The nitrate concentration of the culture was determined daily via optical density measurement at a wavelength of 220 nm (OD₂₂₀) using the spectrophotometer. Before measurement, the samples were centrifuged at 6000 rpm for 5 min and appropriately diluted with distilled water.

2.4. Determination of oil/lipid content

After appropriate cell growth, the microalgal cells were harvested from the culture broth by centrifugation (9000 rpm for 10 min). The cells were washed twice with deionized water, lyophilized, and weighed. The lipid composition was determined as fatty acid methyl esters (FAMEs) through the direct transesterification method described by Lepage and Roy [6]. The sample was analysed by gas chromatography (GC-2014, Shimadzu, Kyoto, Japan) equipped with a flame ionization detector (FID). Samples were injected into a 30 m long capillary column (Type no. 260M143P, Thermo Fisher Scientific, Waltham, MA, USA) with an internal diameter of 0.32 mm. Helium was used as the carrier gas, with a flow rate of 1.3 ml/min. The temperatures of the injector and detector were set at 250 and 280°C, respectively. The oven temperature was initially set at 110°C, increased from 150 to 180°C at a rate of 10°C/min, 180 to 220°C at a rate of 1.5°C/min, 220 to 260°C at a rate of 30°C/min, and held at 260°C for 5 min.

2.5. Operation of semi-batch culture with medium replacement

Upon reaching the intended day of harvest, the photobioreactors and fresh media were put into a horizontal laminar flow cabinet equipped with ultraviolet (UV) light (AHC-5A1, Esco Micro Pte. Ltd., Singapore), and a medium replacement was carried out. A portion (in this case 25%, 50%, or 75%) of
the cultures were harvested, and the remaining cultures were topped up to the starting volume (1 L) with fresh media.

3. Results and Discussion

Three culture replacement fractions (CRFs) were chosen in this study (25%, 50%, and 75%). In Table 1, upon culturing *Chlamydomonas* sp. Tai-03 under 25% CRF, the biomass concentration bounced back to its highest value after 1 day. Meanwhile, when using 50% and 75% CRF, the biomass concentration bounced back to their highest values after 5 days (Table 1). The peak biomass concentration in two replacement steps of 25%, 50%, and 75% CRF were 4.15 ± 0.12, 4.18 ± 0.08, and 4.27 ± 0.14 g/L. Within both replacement steps in all three CRFs, the nitrate contents were reduced back to 10% in 1 day after medium replacement. This shows that the microalgal cells were rapidly absorbing the added nitrates. This was evident from the high biomass productivities in 25%, 50%, and 75% CRF of 1.23 ± 0.02, 0.96 ± 0.03, and 1.21 ± 0.08 g/L/d.

From Table 1, the greatest lipid productivity of 239.6 ± 24.8 mg/L/d was achieved by 25% CRF. Unlike 25% CRF, the lipid productivities of 50% and 75% CRF were comparatively lesser. The greater lipid productivity value of 25% CRF were brought upon by the fast growth rate of *Chlamydomonas* sp. Tai-03 which allowed harvesting after 1 day. As 75% of the active culture remained after medium replacement, 25% CRF had a larger initial biomass after culture replacement, and this helped to speed up the microalgal growth. Although the biomass concentrations were able to return to peak value in all MRRs, the lipid content were not observed to increase to the initial levels (28 – 31%). This may be due to the nutrient limitation during each replacement step. The scarce nutrients were primarily consumed to accumulate biomass, and less nutrients were diverted to synthesis of lipids. Hence, 25% CRF was selected to be the optimum replacement fraction for *Chlamydomonas* sp. Tai-03.

| CRF (%) | Culture Timea (d) | Biomass | Lipid |
|---------|-------------------|---------|-------|
|         | Growth (g/L) | Pr.a (g/L/d) | Content (%) | Pr. (mg/L/d) |
| 25      | 8     | 4.21 ± 0.10 | 0.53 ± 0.01 | 27.6 ± 2.1 | 145.2 ± 14.4 |
|         | (2 Repl) 1 | 4.15 ± 0.11 | 1.23 ± 0.15 | 19.4 ± 2.0 | 239.6 ± 24.8 |
| 50      | 8     | 4.13 ± 0.11 | 0.52 ± 0.01 | 31.1 ± 2.3 | 160.5 ± 7.7 |
|         | (2 Repl) 5 | 4.18 ± 0.08 | 0.44 ± 0.02 | 21.9 ± 2.4 | 95.6 ± 9.9 |
| 75      | 8     | 4.26 ± 0.08 | 0.53 ± 0.01 | 28.3 ± 2.5 | 150.9 ± 16.0 |
|         | (2 Repl) 5 | 4.27 ± 0.14 | 0.65 ± 0.02 | 13.4 ± 2.7 | 87.5 ± 15.6 |

a Productivity
b Culture time of replacement steps are in the form of (X Repl) Y, where X = quantity of replacement steps, and Y = the culture days after replacement

4. Conclusions

The biomass and lipid productivities of *Chlamydomonas* sp. Tai-03 were enhanced when cultured under 25% MRR. The rapid growth pattern implied that harvesting and medium replacement could be done every 24 h, which might become a useful mode of microalgal culture during large scale cultivation.
this study, the microalgal growth was stable for two replacement cycles, but for future works, the culture time could be extended to study the stability of the culture and consistency of lipid accumulation.

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