Biocatalysis and Agricultural Biotechnology 14 (2018) 10–17

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Effects of macro/micronutrients on green and brown microalgal cell growth and fatty acids in photobioreactor and open-tank systems

Syed Muhammad Usman Shah[a,b], Mohd Azmuddin Abdullah[c,*]

[a] Department of Chemical Engineering, Universiti Teknologi PETRONAS, 32610 Seri Iskandar, Perak, Malaysia
[b] Department of Biosciences, COMSATS Institute of Information Technology, Park Road, 44000 Islamabad, Pakistan
[c] Institute of Marine Biotechnology, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia

ARTICLE INFO

Keywords:
Microalgae
Lipid
Fatty-acids
Nutrient deficiency
Photobioreactor

ABSTRACT

This study described the optimization of growth parameters that affected locally-isolated green *Nannochloropsis oculata* and *Tetraselmis suecica* and brown *Isochrysis galbana* and *Pavlova lutheri* microalgae using Response Surface Methodology (RSM). Under deficiency conditions of 10–65 g L^{-1} KNO_3, 3–7.5 g L^{-1} Na_2HPO_4 and 2.5 g L^{-1} $FeCl_3$, the highest lipid accumulation of 37.3%, 23.6%, 28.3% and 37.2%, with slightly reduced cell growth of 0.64, 0.49, 0.54 and 0.38 g L^{-1} were achieved for *N. oculata*, *T. suecica*, *I. galbana* and *P. lutheri*, respectively. The macronutrients significantly influenced the biomass and lipid content positively. However, the interaction of phosphate-phosphate for *N. oculata*, and nitrate-nitrate for *I. galbana* may affect cell growth negatively. The highest biomass of 0.62–0.96 g L^{-1} and lipid content of 31.6–42.2% in 5 L PBR and the highest biomass of 0.45–0.72 g L^{-1} and lipid content of 24.4–38.5 in 300 L open tank were achieved for all the four species. The total saturated fatty acids (44.3–63.8% and 30.4–55.03%); monounsaturated fatty acids (6.1–37.0% and 4.2–13.1%); and polyunsaturated fatty acids (8.3–22.3% and 1.02–15.2%) were obtained, respectively, with pentadecanoic (C15:0), palmitic (C16:0), palmitoleic (C16:1), heptadecanoic (C17:0), oleic (C18:1), eicosanoic (C20:0), eicosapentaenoic (C20:5) and docosahexaenoic (C22:6) as predominant fatty acids.

1. Introduction

Algae represent the only means of current generation of renewable biofuels (Chisti, 2007; Schenk et al., 2008). Their naturally high-lipid content, semi-steady state production and suitability in variety of climates (Clarens et al., 2010) make microalgal biofuels having much lower impact on the environment and on the food supply than the conventional biofuel-producing crops. The low viscosity, high calorific value and low density also make algal biofuel a better option. If algal production could be scaled up to industrial capacity to meet the demand, less than 6 million hectares would be needed worldwide. This makes up less than 0.4% of arable land which is achievable from global agriculture perspective (Brennan and Owende, 2010). With more knowledge on algal biology and technological advances, the commercialization of algal biofuel production will be feasible in not too distant future.

Manipulation of temperature, salinity, light, pH, nutrients as well as culture duration, allows modulation of cell growth, biochemical and lipid composition for consequent optimization of overall yield and productivity (Carvalho et al., 2009; Abdullah et al., 2015, 2016a, 2017). The CO_2 concentration in natural air is low to aerate culture medium and sustain optimal growth and biomass productivity. For large scale cultivation, CO_2 supply from waste gas of industrial combustion process, diesel engine, cement plants or fermentation may be an option. Nitrogen is a vital component for composition, formation and functionality of protein and DNA and is normally supplied in the form of nitrate, nitrite, ammonia or urea. The average nitrogen requirement for most green algae is between 5% and 10% or 5–50 mM (Becker, 1994). Nitrogen starvation can either improve the biosynthesis and accumulation of lipids or reduce the process in some species. When lipid production is enhanced in nitrogen starved conditions, neutral lipids in the form of triacylglycerides (TAGs) accumulate in the depleted cells (Thompson, 1996).

Phosphates are essential for cellular metabolic process via formation of structural and functional constituent necessary for normal growth, biosynthesis of nucleic acid, and energy transfer (Becker, 1994; Goldman and Mann, 1980). Phosphorous concentration is often growth limiting in natural aqueous environment. Inorganic phosphate could occur in cells as polyphosphate where distinct polyphosphate granules accumulate in the cells when phosphorus is sufficient, but invisible

* Corresponding author.
E-mail addresses: azmuddin@umt.edu.my, joule1602@gmail.com (M.A. Abdullah).

https://doi.org/10.1016/j.bcab.2018.01.011
Received 21 November 2017; Received in revised form 16 January 2018; Accepted 22 January 2018

when phosphorous is deficient. The optimal absorption rates of phosphorous in most algal strains are 50 μg L^{-1} to 20 mg L^{-1} and any deficiency leads to similar nitrogen starved condition, with increased lipid and carbohydrate content, and a decrease in protein, nucleic acid and chlorophyll *a* content (Becker, 1994). Trace elements are required in small amounts of micro-, nano- or even pictogram per liter. The major trace elements in algae media are manganese, nickel, zinc, boron, vanadium, cobalt, copper, molybdenum and these cannot be replaced by other elements (Becker, 1994; Richmond, 2004).

Cultures lacking trace elements may show reversible signs of deficiency. Important metal ions include Ca, K, Na, Mg and Fe. Being a constituent of cytochromes, Fe is vital for metabolism, redox properties and effective in nitrogen assimilation and fixation, photosynthesis, respiration, DNA synthesis and for cell structure and constituent. As a functional part of ferredoxin, Fe affects the synthesis of phycocyanin and chlorophyll. Bleaching and yellowing of algae culture are often an indication of iron deficiency in the medium. The effect of iron source such as $FeCl_3$ or EDTA on lipid accumulation and biomass productivity has been investigated on marine *Chlorella* strain under laboratory conditions. The final cell density increases when chelated Fe^{3+} is added to the culture medium during late exponential growth phase. The total lipid content also increases when cells are re-inoculated into a new medium containing high level of iron (Behrenfield et al., 2006).

Most of the commercial, large-scale outdoor algal cultivation are artificial open ponds because they are cheap to build and easy to operate and scale up (Brennan and Owende, 2010). However, there are several disadvantages such as low productivity and biomass yield, high harvesting cost, water losses through evaporation, limited number of species which can be grown in ponds, vulnerability to contamination and lower efficiency of carbon dioxide use (Chisti, 2007; Schenk et al., 2008). Temperature fluctuations due to diurnal variations are difficult to control in open ponds (Chisti, 2007). Photobioreactors (PBRs), on the other hand, provide excellent reproducibility due to operational control, superior light and CO_2 utilization, minimal water losses, and lowered risk of contamination. The temperature, pH and salinity can be better controlled, while the higher surface-to-volume (S/V) ratio facilitate narrow light path and large illuminating area for higher volumetric productivities and cell concentrations (Abdullah et al., 2016a, 2017).

Response surface methodology (RSM) is an efficient and convenient method to screen the key factors rapidly from multiple factors and to optimize the culture conditions, avoiding the disadvantages often associated with a single-factor optimization (Qiu et al., 2013). The method has already been successfully utilized in the chemical industry, engineering and biology (Yücel, 2012). RSM optimization of growth parameters such as culture time, concentration of inoculum, and concentration of sodium bicarbonate has yielded maximal biomass production (0.0391 g L^{-1} day^{-1}) and lipid content (26.28% DCW) in *Scenedesmus abundans* (Chellamboli and Perumalsamy, 2014). Differences among species do exist and even among strains of the same species. Systematic studies on individual microalgal strain are needed to optimize the medium and processes to obtain maximal growth and lipid production (Yang et al., 2014; Abdullah et al., 2016a, 2017). The application of RSM in the optimization of autotrophic microalgal medium for lipid production is rather scarce. Plackett–Burman based statistical screening approach has exhibited an enhanced lipid production by a two-step strategy involving initial optimization of microalgal growth and the second optimization of lipid accumulation (Karemore et al., 2013).

The objectives of this study were to investigate the effects of macro/micronutrients using Response Surface Methodology (RSM) that could affect the productivity of locally-isolated *Nannochloropsis oculata*, *Tetraselmis suecica*, *Isochrysis galbana* and *Pavlova lutheri*. The kinetics of cell growth, lipid production and fatty acids profile in 5 L photobioreactor and 300 L open tanks cultivation were also established.

2. Materials and methods

2.1. Cultivation of microalgae

Four species of microalgae (*N. oculata*, *P. lutheri*, *I. galbana* and *T. suecica*) used in the present study, were collected from Dr. Mohd Fariduddin Othman, Fisheries Research Institute (FRI), Pulau Sayak, Sungai Petani, Kedah, Malaysia. All chemicals and solvents were obtained from Merck (Darmstadt, Germany).

Filtered seawater was sterilized by autoclaving for 20 min at 121 °C and 15 psi. After autoclaving, the media was left for 2–4 h to allow gases such as CO_2 to diffuse into the medium. To avoid precipitation during autoclaving, 1.44 mL of 1 N HCl and 0.12 g of $NaHCO_3$ per liter were added. These indirectly lower the pH, which help to reduce precipitation during autoclaving (MacLachlan, 1979). CO_2 may be added directly by bubbling the medium before autoclaving. The seawater was cooled quickly after autoclaving to room temperature, which also helped to prevent precipitation. Filtered seawater was stored in either glass or plastic bottles, often 5 L, for ease of handling. New containers were bleached for 24 h with diluted, 10% HCl, and then rinsed thoroughly before use. The seawater was kept in cold room and in the dark, before use.

The chemical composition of Conway (AQUACOP) and TMRL are as described before (Thompson et al., 1992; AQUACOP, 1984). For cultivation, 10% (v/v) inocula was added under sterile conditions using Conway medium (for small-scale 250 mL flasks and 1 L container) and TMRL Enrichment medium (for large-scale 5–300 L sizes). The culture media was prepared by mixing various nutrients in sea-water, filtered through 0.22–1 μm filter. All media constituents were added aseptically after sterilization (MacLachlan, 1979; Hamilton, 1979).

Cultures were subcultured on a fortnightly basis, and grown at pH 7–8, salinity 30 ppt,on an orbital shaker at 80 rpm, at 28 ± 2 °C, under 24 h illumination of 90 μmol photons m^2 s^{-1} intensity from white fluorescent tube (Philips).

2.2. Inoculum preparation

The comparative studies were carried out between 5 L working volume PBR and 300 L open-tank. The PBR system used was a 7 L BIOSYS STR 7LX6 PLUS (Biosys) and the open tank was an approximately 400 L tank. However, the detailed descriptions given below and the ensued discussion for both are based on the working volume of the reactor system.

The inoculum for 5 L PBR was firstly prepared from a 250 mL flask cultivation. Cultures of 8–11 days old were pooled and inoculated aseptically into PBR at 10% (v/v) ratio.

The inoculum for 300 L open tank was prepared first from a 1 L plastic container, and then transferred to a 5 L plastic container, before being inoculated into a 30 L tank, all at 10% (v/v) inoculum ratio. The 1 L and 5 L plastic containers were filled to two-third of their volume with microsterilized seawater. The plastic containers were disinfected by dilute solution of Chlorox (10 ppm) and dried in an oven at 60 °C for 30 mins. Aeration was provided non-aseptically and air was enriched with 5% CO_2. Plastic containers were covered with plastic caps pierced with two holes for the air inlet and outlet. Nutrients were added at the time of inoculation and the 1 L and 5 L plastic containers were inoculated with 8–11 days old culture from 250 mL or 1 L container, respectively. The plastic containers were kept at 28 ± 2 °C, under 24 h illumination of 90 μmol photons m^2 s^{-1} intensity from white fluorescent tube (Philips).

The 40 L fiber glass tanks were disinfected with Chlorox (20 ppm) and filled with 27 L microfiltered seawater. Media enrichment was made at the time of inoculation and tanks were inoculated at 10% (v/v) ratio with 8–11 days old stock cultures from 5 L containers. Polyvinyl chloride (PVC) distribution pipes were fixed with channels for condensing water to be purged. The distribution of air into the culture