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Effects of light intensity and carbon dioxide on lipids and fatty acids produced by *Synechocystis* sp. PCC6803 during continuous flow

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ABSTRACT

We studied the effects of light intensity (LI) and CO_2 supply on pH and total lipid production and fatty acids by *Synechocystis* sp. PCC6803 during continuous-flow operation of a photobioreactor having continuous nutrient supply. The temperature was fixed at 30 °C, and the LI pattern mimicked a day/night light cycle from 0 to 1920 μmol/m^2 s. The CO_2 supply varied from 1 to 5% v/v of total air. The total lipid content increased proportionally to LI, reaching a high content of 14% of dry weight (DW) at the highest LI at 3% CO_2. In contrast, LI had no significant influence on the total fatty acid content, which was 3.4% ± 0.5% DW, measured as fatty acid methyl esters (FAMEs). Palmitic acid (C16:0) was the main fatty acid (52% of FAMEs), but γ-linolenic acid (C18:3^{n6}) and linoleic acid (C18:2) were significant at 20% and 14% of total FAMEs, respectively. Also, α-linolenic acid (C18:3n3), oleic acid (C18:1), and palmitoleic acid (C16:1) represented 5%, 4%, and 4% of the total FAMEs, respectively. In case of C16:0, its highest content was achieved at LI of 400 to 1500 μmol/m^2 s and pH media values from 7.2 to 8.8 (3% CO_2). The highest formation of C16:1 and C18:1 (desirable for biodiesel production) occurred with LI up to 600 μmol/m^2 s at pH 9 (3% CO_2). Stearic acid (C18:0) and linoleic acid (C18:2) contents did not vary with LI or pH, but α-linolenic acid (C18:3^{n3}) formation occurred with patterns opposite to C18:3^{n6}, C16:0, and C16:1. LI of 400 to 1600 μmol/m^2 s and pH range from 7.7 to 8.7 led to the highest values of C18:3^{n6} (0.8% DW), but C18:3^{n3} was suppressed by these conditions, supporting a desaturation pathway in *Synechocystis*. These results point to strategies to optimize LI, CO_2, and pH, to enhance the fatty acid production profile for biofuel production.

1. Introduction

Depletion of fossil fuels, increases in oil prices, and the buildup of greenhouse gases have forced countries to investigate renewable energy alternatives to fossil sources. Biofuel production offers opportunities to develop long-term replacements for fossil fuels while also promoting the economies of rural areas [1–3]. Biological CO_2 fixation can be achieved through the photosynthesis of terrestrial plants and microorganisms [4]. Because their CO_2-fixation efficiency is 10–100 times higher than for plants, eukaryotic algae and cyanobacteria can produce renewable fuel feedstock with much less land, and they do not require arable land [2, 4–6]. Therefore, the mass cultivation of microalgae and cyanobacteria as biomass feedstock for liquid biofuels is being assessed worldwide [7,8].

According to Quintana et al. [9], cyanobacteria have promise for bioenergy generation because they possess higher photosynthesis and growth rates compared to algae. They grow well when provided only basic nutritional requirements, such as water, CO_2, mineral salts (especially phosphorus and nitrogen), and light as the only energy source. Moreover, cyanobacteria are amenable to genetic manipulation through the introduction or deletion of genes that allow them to produce more or higher-value products [9–12].

Biodiesel can be produced by transesterification of microalgal lipids, reaction of a simple alcohol (usually methanol) with tri- or di-acylglycerides to produce fatty acid methyl esters (FAMEs) [13]. FAMEs composition determines a biodiesel's oxidative stability and performance properties. Polyunsaturated fatty acids (PUFAs) are susceptible to oxidation, and fully saturated lipids increase the cloud point and viscosity of biodiesel [14]. Therefore, the most desirable lipids are monounsaturated fatty acids (MUFAs) [8]. Microalgae lipids also can be input to a refinery *in lieu* of petroleum feedstock; the fatty acid profile controls fuel quality in a similar way as for biodiesel.

Environmental growth conditions determine the fatty acid profile of lipid in microalgae and cyanobacteria [8,15–20]. Quintana et al. [9] reported that growth conditions affected the fatty acid composition of different cyanobacteria species, Liu et al. [21] noted that the degree of

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unsaturation in fatty acids increased at lower temperatures, Walsh et al. [22] found that the amount of polyunsaturated fatty acids (PUFAs) decreased in favor of monounsaturated fatty acids at high light intensities, and Gombos et al. [23] found that temperature had a strong impact on the degree of unsaturation of fatty acid groups.

Synechocystis sp. PCC6803 was the first photosynthetic organism to have its genome sequenced and is one of the best characterized cyanobacteria in general [11]. It also has been studied for large-scale biomass production based on its growth capability for wide ranges of environmental conditions, such as salt concentration, pH, temperature, and carbon dioxide (CO_2) level [24–26]. The intracellular lipids in *Synechocystis* are mostly diacylglycerols located in the thylakoid membranes [24,27]. Sheng et al. [16] studied the temperature effect on lipids and fatty acids production in *Synechocystis* sp. PCC6803 and found that the highest fatty acid content in the cells occurred at 30–33 °C. In contrast, 22 °C showed a 20% suppression of lipid synthesis, and 35–40% suppression occurred at 18 °C and 44 °C. Moreover, low temperature (18 and 22 °C) led to higher amounts of PUFA linolenic acid (α-C18:3 and γ-C18:3).

Cyanobacteria optimize membrane-barrier functions, permeability properties, activities of membrane-bound enzymes, and signaling mechanisms in response to temperature changes [16,28]. Acyl-lipid desaturases introduce double bonds into fatty acids that have been esterified to glycerolipids and are bound to the thylakoid membrane in cyanobacterial cells [29]. *Synechosystis* sp. PCC6803 has been well studied, and its metabolic fatty acid desaturation follows the pathway shown in Fig. 1. Desaturases introduce double bonds at the Δ6, Δ9, Δ12, and Δ15 positions of the fatty acids [30].

In this work, we study how lipid and fatty acid production patterns are controlled by diurnal variations in light intensity, carbon dioxide, and pH for *Synechocystis* sp. PCC6803. These are factors that can vary over wide ranges during the normal day–night conditions of bioenergy generation, and they can be manipulated by engineering measures.

2. Materials and methods

2.1. Experimental set-up

All experiments were conducted in a vertical, flat-plate photobioreactor (PBR) that consisted of a rectangular body made of polymethyl methacrylate plastic, light panels for irradiation along two sides, and sampling ports with liquid discharging valve along the other two sides. Details of the PBR system are provided by Kim et al. [24,31]. The liquid volume inside the PBR was 14 L. Two mass-flow controllers, connected to a compressed-air tank and a pure-CO_2 tank, regulated the ratio of CO_2 in the air supplied to a gas diffuser installed in the bottom of PBR to achieve good mixing of the reactor contents. Also, an outer-layer water jacket contained water circulating through a water bath (Thermo Scientific Digital Plus) for temperature control. Day–night light intensity (LI) cycling for both sides of the PBR was achieved by using a computer program that created a stepwise pattern imitating the solar irradiation average of a typical day near the autumn equinox in Phoenix, AZ [24,31].

2.2. PBR start-up and operating conditions

The PBR was disinfected by washing it once with deionized water contained 0.04% NaOCl. After two rinses with sterile deionized water, the PBR was loaded with 12 L of BG-11 5P medium, which is the standard BG-11 medium with five-fold increased phosphate, or ~28.4 mg P/L [24]. Then, the PBR was inoculated with 2 L of *Synechocystis* sp. PCC6803 culture that had been grown in a glass bottle connected to a filtered-air supply, surrounded by fluorescent lamps that illuminated the vessel continuously, and maintaining at a temperature of ~30 °C. Standard BG-11 medium [32] was used for culture growth. All media were autoclaved before use.

To avoid nutrient depletion and to ensure enough light penetration in the system, the reactor was operated with continuous flow. During

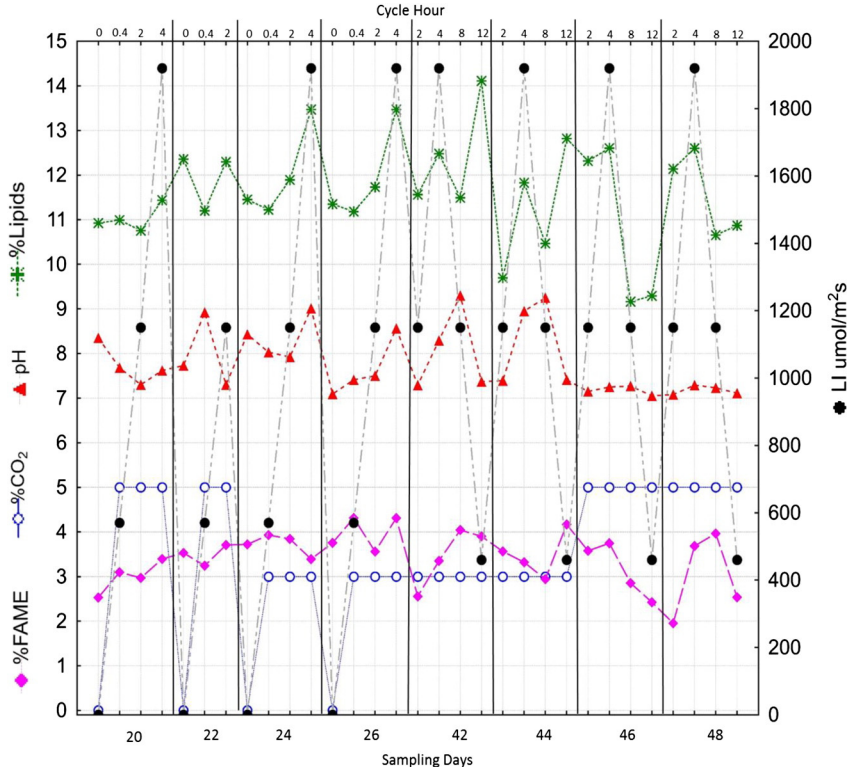

Fig. 1. *Synechocystis sp.* PCC6803 pathway of fatty acid desaturation by acyl-lipid desaturases [29,30]. %Lipids, %FAME, and pH values throughout the set of experiments with different LI and %CO_2 inputs for *Synechocystis* sp. PCC6803 in continuous flow. %Lipids and %FAME are percentages of dry weight (DW).