A HIGH-THROUGHPUT MICROFLUIDIC LIGHT CONTROLLING PLATFORM FOR BIOFUEL PRODUCING PHOTOSYNTHETIC MICROALGAE ANALYSIS

H.S. Kim[1], T.L. Weiss[2], T.P. Devarenne[2] and A. Han[1, 3*]

[1] Department of Electrical and Computer Engineering, [2] Department of Biochemistry and Biophysics, and [3] Department of Biomedical Engineering, Texas A&M University, College Station, TX, USA

ABSTRACT

We have developed a high-throughput microalgae screening platform capable of analyzing biomass production at different light conditions with single colony resolution. Combinations of different light intensities and day-night cycles resulting in 64 light conditions for each of 64 culture compartments were implemented on a single microfluidic chip by blocking the light from a single light source with different black color dye concentrations and by switching between water and black color dye, respectively. Five microalgae colony-trapping structures in each culture compartment allowed single-colony resolution growth and biofuel-production analysis. Oil-producing microalgae, *Botryococcus braunii*, were successfully cultured and characterized using this screening system.

KEYWORDS: Microalgae culture microsystem, Microfluidic light controlling platform, Biofuel, Photosynthetic microalgae, High-throughput analysis

INTRODUCTION

There have been a great interest in renewable and carbon-neutral fuels since petroleum-based fuels are not sustainable due to limited supplies and global warming resulting from the accumulation of carbon dioxide. Biofuels derived from oil crops such as corn and soybean can be potential alternatives for petroleum fuels, but their large-scale production is challenging and competition with food production is of concern [1]. Microalgae are promising renewable biofuel sources capable of satisfying the global need. Microalgae are photosynthetic microorganisms that convert sun light and atmospheric carbon dioxide to biomass. The relationship of biomass productivity versus light intensity and light cycle, however, remains largely unknown in most microalgae. Even in studies of a few well-known microalgae, the relationship is based on bulk analysis and can vary vastly depending on the culture systems used. Here, we present a novel high-throughput microalgae analysis platform for investigating biomass production in response to different light conditions (intensities and day-night cycles), capable of conducting 64 independent experiments in parallel with single colony resolution analysis.

EXPERIMENTAL

The microalgae culture platform is made of a poly(dimethylsiloxane) (PDMS) assembly consisting of 4 functional layers; a black PDMS layer to block scattered light, a microfluidic day-night cycle control layer, a microfluidic light inte-

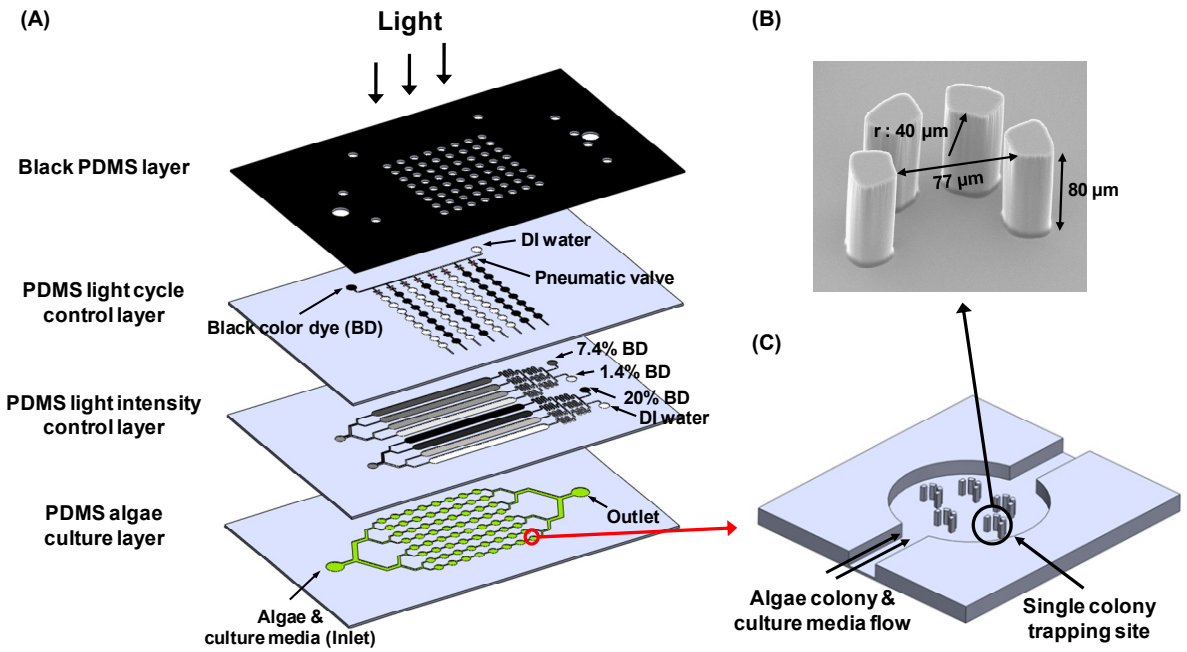

Figure 1: Illustration of the high-throughput microalgae analysis platform. (a) Four PDMS functional layers - a black PDMS layer, a microfluidic light cycle control layer, a microfluidic light intensity control layer, and a microalgae culture layer. (b) An SEM image of a single algae colony trapping site. (c) An enlarged view of a single culture compartment having 5 algae colony trapping sites enabling single-colony analysis.

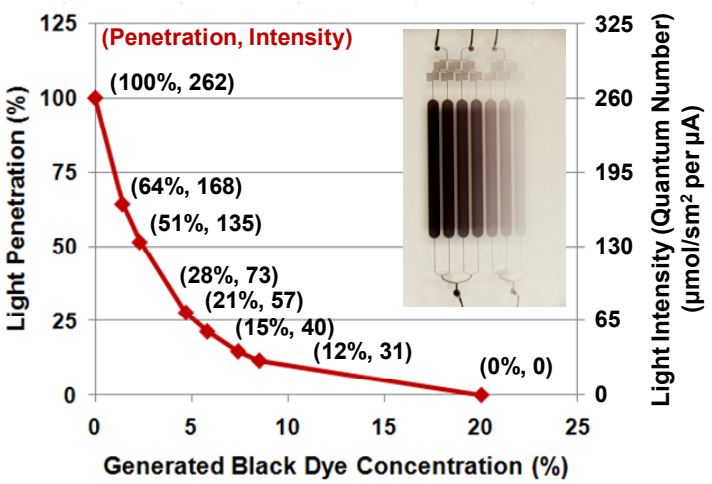

Figure 2: Light intensity and light penetrating through 8 different black color dye concentrations (0% (DI water), 1.4%, 2.3%, 4.7%, 5.8%, 7.4%, 8.5%, and 20%) generated from the microfluidic gradient generator. Each black color dye concentration has a different light penetration rate, resulting in 8 different light intensities within a single device. Inset shows a photograph of a fabricated PDMS light intensity control layer generating gradients of black color dye.

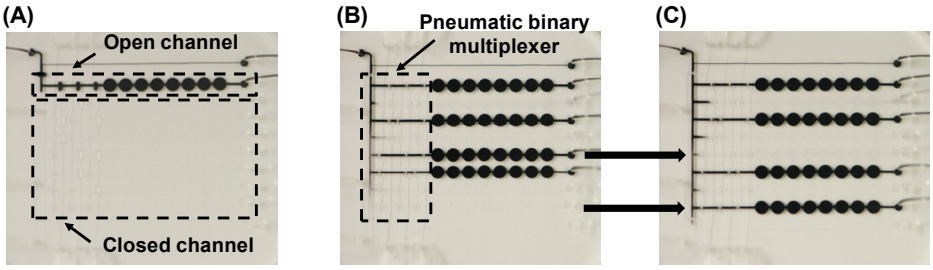

Figure 3: Operation of the light cycle layer controlled by a pneumatic binary multiplexer. By controlling pneumatic valves on top of the 8 microchannels, each channel can be independently filled or switched with either DI water or black color dye. (a) Only one channel is open while other channels are closed. (b) All channels are individually filled with either water or black color dye. (c)Water and black color dye in microchannels are switched by a binary multiplexer.

nsity control layer, and an algae culture layer containing 64 culture compartments (Figure 1A). To induce various light intensities within a single platform, a microfluidic gradient generator with 8 outputs was used. By flowing deionized (DI) water and black color dye - which have 100% and 0% light penetration, respectively - the gradient generator produced 8 different black color dye concentrations. When a single light source was placed on top of the gradient generator layer (Figure 1A), the underlying algae culture layer was exposed to 8 different intensities of light where different concentrations of black color dye resulted in different light penetration percentages (Figure 2). The day-night cycle layer, controlled by a pneumatic binary multiplexer, generated 8 different day-night cycles by filling the 8 microchannels placed 90^{o} against the light intensity control microchannels between DI water (100% light penetration, day) and 100% black color dye (0% light penetration, night) for different time periods (Figure 3). By combining these two layers, the 64 algae culture compartments could be exposed to 64 different light conditions (8 different light intensities x 8 different day-night cycles). The 5 single-colony trapping sites (opening: 77 µm) in each culture compartment enabled microalgae analysis with single colony resolution (Figure 1B & C). All functionalities of the PDMS assembly were automatically operated by a Labview^{TM} interface controlling syringe pumps and solenoid valves.

All four layers composing the device were fabricated in PDMS using soft lithography. Particularly, a commercial black PDMS kit (Sylgard 170, Dow Corning) was used in replicating the black PDMS layer from the acrylic master mold fabricated using a computer numerically controlled (CNC) milling machine. The PDMS assembly, where all layers were bonded with oxygen plasma treatment, was sealed with an acrylic chamber and 2.5% carbon dioxide (CO_2) enriched air was flowed into the chamber. CO_2 was thereby administered to the microalgae via PDMS gas diffusion.

Botryococcus braunii (*B. braunii*) is a green colonial and biopetroleum-producing microalga characterized by a significantly higher hydrocarbon content compared to other microalgae [2, 3]. *B. braunii* was cultured and analyzed by utilizing the microalgae screening platform.

RESULTS AND DISCUSSION

In the microalgae screening platform, a gradient generator with 4-inputs/8-outputs was used instead of the initial 2-inputs/8-outputs generator. The main performance criteria for this microfluidic gradient generator in this application is to generate a linear range of different light penetration, meaning a logarithmic scale black color dye concentration has to be generated (Figure 2). As large light penetration rate differences exist in the lower black color dye concentration range

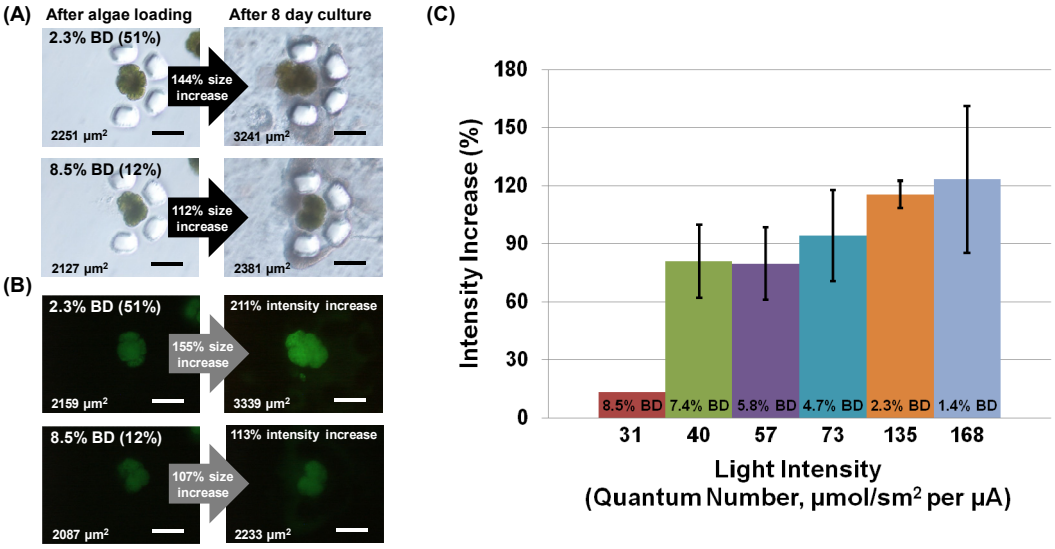

Figure 4: Botryococcus braunii growth analysis using the platform. Micrographs of a single B. braunii colony captured and cultured for 8 days under different light intensities. (a) Bright field (40×) and (b) Fluorescent field for chlorophyll detection (40×). (c) Biomass increase analyzed by chlorophyll detection based on the fluorescent emission light intensity from the B. braunii. (n = 6, scale bars: 50 μm).

(from 0 to 7.4% black color dye, the light penetration differs about 85%), outputs of the gradient generator need to cover this lower concentration range as much as possible. When 0% (DI water) and 20% black color dye - which have 100% and 0% light penetration respectively - are used as inputs for these two different gradient generators, the 4-inputs/8-outputs generator can produce 6 different concentrations in this lower concentration range (0 to 7.4%) while the 2-inputs/8-outputs generator can only generate 2 different concentrations within the same range. This indicates that the 4-inputs/8-outputs generator is more suitable for this platform. Using 0%, 1.4%, 7.4%, and 20% black color dyes as inputs, 8 outputs - 0%, 1.4%, 2.3%, 4.7%, 5.8%, 7.4%, 8.5%, and 20% black color dye concentrations - were generated. The light intensities through different black color dye concentrations were also measured using a quantum sensor (LI-250A Light Meter, LI-COR Bioscience) and different light penetrations were observed (Figure 2). This result demonstrates that 8 different light intensity conditions are achieved within a single platform by generating 8 different black color dye concentrations with the generator.

The 8 independent day-night cycles were implemented with 8 microchannels and a pneumatic binary multiplexer. As shown in Figure 3, 0% (DI water, day) and 100% black color dye (night) inside each channel were successfully switched by a pneumatic binary multiplexer without affecting other channels, and different cycles in each channel were implemented by switching both solutions over different time intervals. A normally closed valve scheme was used in the binary multiplexer design and no leakage was observed for flow rates up to 30 μl/min under 15 psi pressure. At the flow rate of 3 μl/min, switching time between black color dye and water inside the mirochannel was less than 2 minutes.

B. braunii was cultured for 8 days under light conditions shown in Figure 2 and a 12h-day/12h-night cycle condition. Biomass production was characterized through growth analysis using optical microscopy (Figure 4A) and chlorophyll detection (Figure 4B). The result shows that *B. braunii* produced more biomass under stronger light intensities within the same day-night cycle (Figure 4C).

CONCLUSION

A high-throughput microalgae analysis platform has been developed and implemented to investigate biomass production in response to different light conditions with single colony resolution. This platform is currently being used to analyze *B. braunii* biomass and oil production under combinations of different light intensities and day-night cycles at significantly higher throughput compared to conventional axenic flask culture. We expect that this system will serve as a powerful high-throughput microalgae analysis and screening tool to investigate optimum algae growth and oil-producing conditions at significantly lower costs and shorter times.

REFERENCES

[1] Y. Chisti, Biodiesel from microalgae. Biotechnology Advances, **25**, pp. 294-306, (2007).

[2] E. Casadevall, D. Dif, C. Largeau, C. Gudin, D. Chaumont, and O. Desanti, Studies on batch and continuous cultures of Botryococcus braunii: hydrocarbon production in relation to physiological state, cell ultrastructure, and phosphate nutrition. Biotechnology and Bioengineering, **27**, pp. 286-295, (1985).

[3] A. Banerjee, R. Sharma, Y. Chisti, and U. C. Banerjee, Botryococcus: A renewable source of hydrocarbons and other chemicals. Critical Reviews in Biotechnology, **22**, pp. 245-279, (2002).

CONTACT

*A. Han, tel: +1-979-8459686; arum.han@ece.tamu.edu