Array Microhabitat Platform for Microalgae Growth

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Abstract:

The occurrence of harmful algal blooms (HABs) is increasing at an alarming rate worldwide, threatening water resources and aquatic ecosystems. Nutrients are known to trigger the onset of HABs and systematic investigation at cellular level is lacking. To study the combination effects of multiple nutrients on microalgae growth in a high throughput way, we built a dual-gradient microhabitat device and a micro-scale light gradient generation platform. Using these platforms, the effect of chemical and physical microenvironment on the growth of model microalgal *Chlamydomonas reinhardtii* was revealed.

Summary of Research:

Harmful algal blooms, or HABs, are serious environmental problems, where a sudden growth of algae or cyanobacteria poses threat to freshwater and marine ecosystems. HABs deteriorate drinking water quality and have huge environmental and economic costs. Nutrient enrichment is believed to be the fundamental cause of HABs, and climate change may further intensity the problem [1]. However, there lacks a quantitative/mechanistic understanding of the roles of environmental factors in the onset of HABs at cellular level. The goal of this project is to investigate the synergistic roles of multiple environmental factors in the growth of cyanobacteria.

Environmental conditions known to affect algae growth include nutrients, mainly nitrogen (N) and phosphorous (P), light intensity and temperature. These conditions are hard to control in nature, and also cannot be quantified in a high throughput way in flasks and chemostats. To address the need for quantitative, systematic, and high-throughput screening of environmental factors, we first developed a high throughput array microhabitat platform, capable of generating stable dual nutrient gradients, and used it for monitoring growth of photosynthetic microbes [2]. The device consists of 64 microhabitats in the form of an 8 × 8 array and each habitat is 100 µm × 100 µm × 100 µm. The microhabitat array is surrounded by two sets of side channels each with the width of 400 µm and height of 200 µm. The device design is shown in Figure 1.

The silicon master was fabricated using two-layer SU-8 negative resist photolithography, and pattern was transferred to a thin agarose membrane later used for cell seeding via soft lithography. Applying this platform, we discovered that nutrients N and P synergistically promoted the growth of *Chlamydomonas reinhardtii* (*C. reinhardtii*) (see Figure 2).

In addition to controlling the chemical environment in the microfluidic device, we developed microscope-based light gradient generation platform, compatible with the array microhabitats, for the investigation of the impact of light intensity on algal growth [3]. The controlled light gradient was generated by modifying the transmitted light path of a commercially available inverted microscope (see Figure 3). A piece of 45 mm half-moon mask, made of optical resin, was fabricated using the CO_2 laser cutter, and customized for a half-dark, half-bright gradient. Light gradient was characterized by grayscale values of bright field images and photosynthetically active radiation (PAR) meter measurements. Growth of algal cells under the controlled light gradient was monitored by fluorescence imaging for five days, and the growth curves and growth rates were obtained (see Figure 4). Results showed that the growth of the microalgae was significantly regulated by the light intensity and a Monod kinetics model fit revealed the half saturation constant of light to be 1.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for *C. reinhardtii*.

Our results provided the enabling capability of creating multiple controlled environmental parameters, nutrients and light intensity within one platform, which is suitable for growth studies of all photosynthetic micro-organisms. Future study will move a step further to combine the chemical and physical cues and actively working towards incorporating microbial communities in the HAB-on-a-chip platform.

References:

[1] Paerl, Hans W., et al. Environmental Science and Technology (2018): 5519-5529.

[2] Liu, Fangchen, et al. Lab on a Chip 20.4 (2020): 798-805.

[3] Liu, Fangchen and Larissa Gaul, et al. Lab on a Chip (2022) Advance Article.

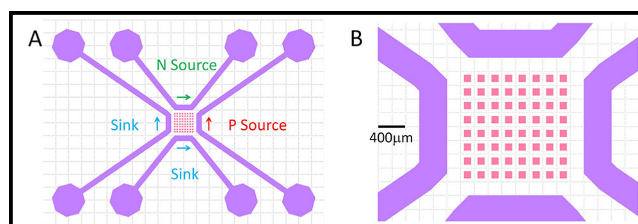

Figure 1: Dual-gradient microfluidic platform design. A. Top view of a device. B. A zoomed-in view of microhabitats and channel. The 8 × 8 array of 100 µm cubic habitats are separated by 100 µm from each other. These habitats are surrounded by four channels with width of 400 µm and height of 200 µm. N source and P source runs through the top and right channel respectively, and the other channels are sink channels. A gradient is generated for each chemical species in the microhabitat array region through molecular diffusion.

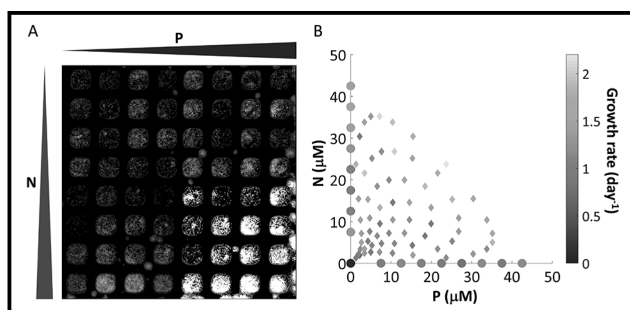

Figure 2: Growth of C. reinhardtii *under nutrients (N, P) gradient. A. Fluorescence images of nutrients co-limited cells growing under N and P dual gradients at day 4. B. The growth rate of* C. reinhardtii *under: control condition, no N or P (dot at the origin), single P gradient (dots on x axis), single N gradient (dots on y axis), and dual-gradient (all the diamonds). Shade is coded for the value of the growth rate.*

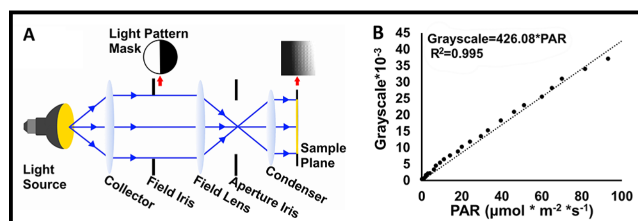

Figure 3: Experimental setup for micrometer-scale light intensity gradient generation and characterization. A. Modifying the light path of a microscope for light gradient generation. Light comes from a halogen lamp. A half-moon light pattern mask was placed directly below the field iris to create the light gradient. Both field and aperture irises were fully open throughout all experiments for optimal, reproducible light gradient generation. (B) Calibration curve of grayscale value from the CCD camera as a function of the light intensity measured by the PAR (photosynthetic active radiation) meter. Dots are the adjusted grayscale values, and the line is a fit to a linear function.

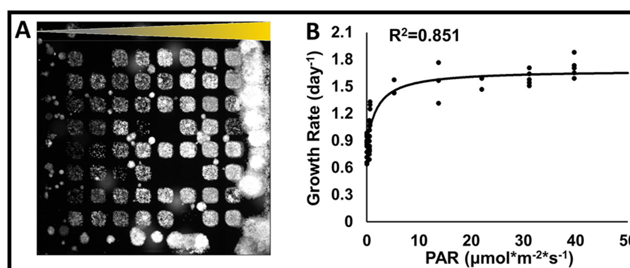

Figure 4: Growth response of C. reinhardtii *to light intensity gradient in an array microhabitat. A. Fluorescence images of cells growing in the array microhabitats under a light intensity gradient at day 5, with approximately 0 PAR on the left side and about 47.7 PAR on the right. B. Growth rate as a function of PAR. Dots are experimental values and line is a fit to Monod model. The fitted coefficients with 95% confidence bounds are μ_0 = 0.823±0.07 day^{-1} µmax' = 0.860±0.099 day^{-1}, and K_S = 1.9±1.18 µmol/(m^2*s), with an R-squared value of 0.851. This data is collected from one of three replicates.*