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Effect of permeate recycling and light intensity on growth kinetics of *Synechocystis* sp. PCC 6803

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ABSTRACT

Permeate reuse for cultivating *Synechocystis* sp. PCC 6803 was studied in batch photosynthetic-growth experiments having a wide range of the ratio of membrane permeate to BG-11 medium. The 50% dilution of permeate gave the greatest biomass concentration and specific growth rate for *Synechocystis*, but up to 100% permeate had minimal impact on biomass growth; thus, a high ratio of permeate supported good growth of *Synechocystis*. With initial values of the spatially averaged light intensity (LI_{SA}) ranging from 49 to 479 μE m^{-2} s^{-1}, *Synechocystis* exhibited typical photo-inhibition growth kinetics in 50% permeate. The half-maximum-rate light irradiance (K_L) was 39 μE m^{-2} s^{-1}, and the half-inhibition-rate light irradiance (K_I) was 281 μE m^{-2} s^{-1}. The K_I value is larger than seen previously for *Synechocystis* in BG-11 medium, which suggests that culturing in permeate may have made *Synechocystis* less sensitive to photo-inhibition. The exponential growth rate of *Synechocystis* sp. PCC 6803 was not inhibited by recycled permeate through multiple rounds of growth in 50% permeate. These results demonstrate that permeate recycling is a feasible strategy for reusing nutrients and minimizing water loss.

1. Introduction

Biofuels produced from microalgae provide an opportunity to replace fossil fuels and diversify sustainable energy sources [1–3]. Improvements in the design of photobioreactors (PBRs) are needed to boost productivity and to minimize the use of water and nutrients [4–6].

Three important features of PBR design and operation are the nutrient demand, which contributes to operating costs [7]; the water demand [8], which is especially important in sunny, but arid regions; and the concentration of the harvested biomass [9], which is advantageous if it is considerably greater than inside the PBR. Dynamic control of water recycling would enable increased algal biomass productivity and decreased nutrient demand, while also reducing water demand, an especially important goal for arid and semi-arid regions [10]. Having a higher solids concentration in the harvested biomass reduces the volume to be handled in downstream processing, which usually lowers the costs of downstream processing.

Microfiltration separators have drawn attention due to their ability to concentrate algal biomass without cell disruption [11,12], their significantly lower energy costs as compared to centrifuging, and the convenience of their integration with PBRs [13–15]. Furthermore, the permeate from a microfiltration unit can be recycled to the PBR, which minimizes water loss and the need to supply exogenous nutrients. For example, previous studies of permeate recycling demonstrated nutrient accumulation to levels not limiting biomass growth [16] and substantial reduction (77%) in water demand [17]. Life-cycle assessments of microalgae cultivation show that continuous recycling of the culture medium could substantially reduce production costs by minimizing the water and nutrient footprints [18]. Because the majority of the water footprint comes from the cultivation system, it is necessary to recover and recycle the maximum amount of medium possible by recycling permeate. Without recycling, unused nutrients are lost through the discharge of the permeate (or any process effluent). By increasing the utilization of water and nutrients, water recycling after biomass harvesting will reduce the demand for each and enhance the long-term sustainability of microalgal production [19].

While recycling microfiltration permeate offers important benefits, the benefits may be accompanied by risks to process performance. Possible risks include the build-up of salts, non-available forms of nutrients, and soluble organic materials that could lead to inhibition or light attenuation [20–22]. For instance, microalgae using recycled permeate may experience salt stress or decreased PSII activity [23]. Of particular interest is light intensity, which ideally should be the main growth-limiting factor. A kinetic model that captures the impacts of light intensity on photosynthetic growth is essential for efficient design

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and control of PBRs [24].

Using a series of experiments with the cyanobacterium *Synechocystis* sp. PCC 6803, we systematically tested permeate dilution ratios and spatially averaged light intensities (LI_{SA}), using values typical for studies on C_i-based and light-based growth kinetics. We were able to separate the effect of C_i (from permeate recycling) and light intensity on photosynthetic-growth kinetics. To interpret the results of these experiments, we used Monod [25] and Aiba [26] models to quantify the maximum specific growth rate (μ_{max}), the half-maximum-rate concentration for C_i, the half-maximum-rate light irradiance (K_L), and the half-inhibition-rate light irradiance (K_I).

2. Material and methods

2.1. Inoculum and culture medium

Wild-type *Synechocystis* sp. PCC 6803 was obtained from the laboratory of Dr. Willem Vermaas in the School of Life Sciences at Arizona State University (Tempe, AZ, USA). A 10-mL suspension of *Synechocystis* taken from a mother culture was inoculated into 100 mL of modified BG-11 medium [27] that had been autoclaved (121 °C and 15 min in a Tabletop Sterilizer, SterileMax, Thermo Scientific, IA, USA) in 250-mL flasks. Then, filtered air (1-μm pore-size hydrophobic glass laminate filter, Pall Corporation, USA) was supplied by sparging through a tube inserted into the bottle, and continuous illumination was provided with fluorescent lamps (93 $\mu E\, m^{-2}\, s^{-1}$) for 3 weeks, during which time the temperature was maintained at room temperature (~26 °C). *Synechocystis* doubled its biomass within ~24 h during the exponential growth phase. The modified BG-11 medium contained 1.5 g $NaNO_3$, 200 mg $K_2HPO_4{\cdot}3H_2O$, 75 mg $MgSO_4{\cdot}7H_2O$, 36 mg $CaCl_2{\cdot}2H_2O$, 6 mg citric acid, 6 mg ferric ammonium citrate, 1 mg EDTA disodium salt, 20 mg Na_2CO_3, and 1 mL mixed trace-metal solution. The trace-metal mix contained (per liter): 2.86 g H_3BO_3, 1.81 g $MnCl_2{\cdot}4H_2O$, 222 mg $Zn_2SO_4{\cdot}7H_2O$, 390 mg $Na_2MoO_4{\cdot}2H_2O$, 79 mg $CuSO_4{\cdot}5H_2O$, and 49.4 mg $Co(NO_3)_2{\cdot}6H_2O$.

2.2. Batch setup for different permeate rations and light intensity

We obtained permeate from a bench-top photobioreactor (PBR) [28] by filtering *Synechocystis*-culture medium through a 0.45-μm pore-size, hydrophilic polyvinylidene fluoride (PVDF) membrane preceded by a coarse screen (C screen) contained within a microfiltration system (MFS) (Pellicon 2 Durapore cassette filter module, Millipore, USA). The bench-top PBR was fed with BG-11 medium [28] that had been augmented with 5-times the normal P concentration, since BG-11 is relatively P deficient. With the added influent P, the membrane-filtered effluent contained a residual P concentration of at least 7.5 mg/L. The chemical characteristics of the permeate were (mg/L, except pH and alkalinity): pH, 9.6 ± 0.3; alkalinity, 4.0 ± 0.1 meq L^{-1}; soluble chemical oxygen demand (SCOD), 58 ± 2; dissolved organic carbon (DOC), 18 ± 2; inorganic carbon (C_i), 163 ± 2; SO_4^{2-}, 4.3 ± 0.2; NO_3^--N 85 ± 2; NO_2^--N, 5.9 ± 0.5; and PO_4^{3-}-P, 23.9 ± 0.2. We diluted the permeate from 0% to 100% (v/v) in sterilized BG-11 to evaluate the effects of permeate on growth of *Synechocystis*. Table S1 lists the characteristics of the dilutions.

Batch growth experiments were performed in duplicate using 500-mL Erlenmeyer flasks (VWR® Laboratory Bottles, USA) having a working volume of 300 mL; Fig. 1 has a schematic of the experimental system. Each series of batch experiments was initiated by adding 300 mL of appropriately diluted permeate and setting the light intensity. The temperature was room temperature (~26 °C), and mixing and CO_2 were supplied by sparging filtered air. In addition, any off gas was vented using a disposable syringe needle to relieve pressure build up. Before each experiment, we added 0.1 M Tris-HCl buffer to give the desired pH 8.5. During the experiment, we adjusted the pH to 8.5 using a Tris-HCl buffer solution titrated into the medium after pH measurement every 5 h.

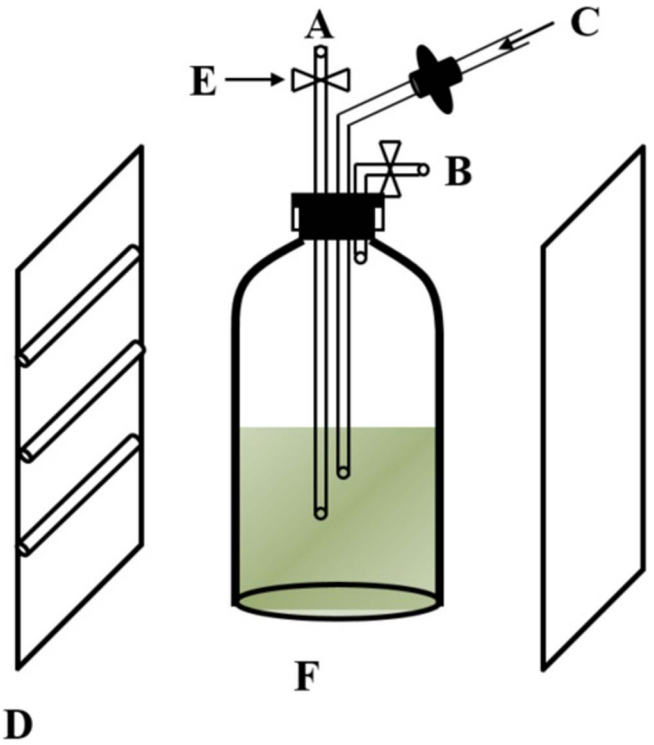

Fig. 1. Schematic of the batch-experiment setup (A: Sample port, B: Disposal gas port, C: Air, D: Light, E: Stop cock, F: Permeate + *Synechocystis* sp.).

The light panel illuminating the flasks had three fluorescent lamps (Prolume F24 T5/841/HO, Halco Lighting Technologies, Norcross, GA) that supplied photosynthetically active radiation (PAR) [29,30] with a controllable incident light intensity (LI_0) in the range of 60 to 590 $\mu E\, m^{-2}\, s^{-1}$ in fixed 50% permeate in order to test the relationship between biomass concentration and light availability during PBR operation. The light intensity to an individual PBR was controlled by adjusting the distance of the light panel from the PBR. The incident light intensity was measured by a U1252 microammeter (Agilent, USA) equipped with a LI-190 PAR sensor (LI-COR, USA). The PAR sensor was placed on the inside PBR surface to measure the average internal initial LI (LI_0) actually entering the PBR (without biomass), and values are listed in Table 1.

The spatially-averaged LI inside a flask (LI_{SA}, $\mu E\, m^{-2}\, s^{-1}$) was computed with the Beer-Lambert law (Eq. (1)), which describes how LI decreases exponentially depending on the depth from the light source and the biomass concentration [31]:

$$LI_{SA} = \frac{LI_o \int_0^d e^{-\varepsilon X w} dw}{d} \quad (1)$$

where ε = the Beer-Lambert constant ($m^3\, g^{-1}\, m^{-1}$), X = biomass concentration ($g\, m^{-3}$), w = depth of light path from the light-entry surface (m), and dw is a differential distance of the light path. We used $\varepsilon = 0.255\, m^3\, g^{-1}\, m^{-1}$, which Kim et al. [31] determined for *Synechococcus* sp. PCC 6803. The initial LI_{SA} was about ~17% attenuated from LI_0 with the initial biomass concentrations of ~34 mg/L for all experiments.

2.3. Data analysis

The specific growth rate (μ, day^{-1}) was computed based on biomass concentrations in samples taken at the beginning and end of a time interval:

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