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Vertical distribution of algal productivity in open pond raceways

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ABSTRACT

In this paper we report a method for experimental measurement of photosynthetic productivity as a function of simulated depth in open pond raceways for algae cultivation. Knowledge of the depth dependence of photosynthetic productivity aids in designing ponds with optimal depth with respect to biomass productivity and capital and operating costs. To simulate depth, we (i) measured irradiance attenuation coefficients of liquid algal cultures as a function of wavelength in the range of 400 to 700 nm, (ii) reproduced the magnitude and spectral content of the irradiance that would exist at various depths within open ponds using a programmable LED array, and (iii) measured photosynthetic rate as oxygen evolution under irradiances corresponding to various depths. We report the depth distribution of photosynthetic rate in simulated 20 cm deep ponds of the green alga *Chlorella vulgaris* and the cyanobacterium *Spirulina platensis* at a biomass concentration of 0.19 g dry biomass per liter (g/l). Under an incident irradiance corresponding to full sunlight, the compensation depth for *Chlorella* was 12 cm. Below this depth, net oxygen consumption due to respiration had a magnitude equal to 15% that of the total oxygen production above the compensation depth. For *Spirulina*, negative net oxygen production was not observed at any depth, but the top 13 cm of the pond accounted for 90% of its total oxygen production. These productivity cross-sections, in addition to knowledge of the dependence of capital and operating costs on pond depth, enable the design of open ponds for optimal depth for maximum return on investment.

1. Introduction

Open pond raceways provide a low cost platform for cultivating algae for nutritional supplements [1,2], agricultural and aquacultural feed [3–5], and biofuel feedstock [6–9]. Most open pond raceways consist of an oval shaped pond with a median separator such that fluid is circulated along the 'track' [10–13]. A paddle wheel is usually used to sustain this circulation. The resultant mixing prevents settling, diminishes vertical gradients of nutrients, carbon dioxide, and oxygen, and also moves cells into and out of the photic zone [14].

Typically, open ponds are about 20 to 30 cm deep [13], which represents compromise between areal productivity and hydraulic limitations [10,12]. Areal productivity is inversely related to pond depth because shallower ponds have a greater depth averaged irradiance [11,15]. Shallower ponds also reduce operating costs by reducing the total volumetric flow rate that needs to be sustained by the paddle wheels. Moreover, shallower ponds have been shown to increase the maximum achievable biomass density [15], which decreases harvesting costs [16]. On the other hand, the maximum achievable track length is proportional to pond depth because the amount of hydraulic head that the paddle wheel can provide is proportional to the height of the water column it can lift. Longer raceways decrease capital costs for hectare-scale algal farms by reducing the total number of raceways that need to be built to cover a prescribed total footprint area.

Optimizing pond depth with respect to these competing effects requires knowledge of the depth dependence of photosynthetic productivity. It is typical to report pond productivity in grams dry biomass per square meter of footprint area per day [6,13,17], but this metric neglects the non-uniformity of productivity with depth. Quantification of this non-uniformity enables calculation of overall productivity for different pond depths, which in turn enables quantitative optimization of pond depth for high productivity and low capital and operating costs.

Direct measurement of the vertical distribution of open pond productivity, or photosynthetic rate, is experimentally difficult. At the laboratory scale, photosynthetic rate is often measured as the time rate of change in dissolved oxygen concentration of a closed algal suspension after an illuminating light is switched on [18,19]. This technique cannot be applied to real ponds because diffusion and advective mixing make it very difficult to recover the local oxygen generation rate from the local oxygen concentration. Alternatively, a modeling approach can be taken for understanding the depth dependence of photosynthetic productivity in ponds [20]. However, it is difficult to accurately take into account the effect of a depth dependent irradiance spectrum on local productivity.

Abbreviations: OD, optical density; *P AR*, photosynthetically active range, 400 to 700 nm; *P U*, photosynthetically useful.

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Nomenclature

A	attenuation cross-section, $mm^{-1}/(g/l)$
a	action spectrum value
C	concentration, mol/l
d	pond depth, m
h_b	box thickness, m
$k_l a$	transfer coefficient, s^{-1}
G	irradiance, μmol photons/m^2
P	pressure, Pa
S	spectral matching parameter
t	time, s
v	velocity, m/s
$\dot{W}$	power consumption, W
w	width, m
X	biomass density, g/l
z	local depth, m

Greek symbols

α	irradiance attenuation coefficient, mm^{-1}
λ	wavelength, nm
π	production rate, mol $l^{-1}s^{-1}$

Subscripts

h	refers to headspace
i	refers to an initial value
O_2	refers to oxygen
p	refers to spectrum reconstruction

In this study, we present experimentally measured photosynthetic rates as a function of simulated depth within an open pond raceway. To simulate depth, we constructed irradiance spectra that cells would experience at different depths. First, we measured spectral irradiance attenuation coefficients in miniature ponds of the green alga *Chlorella vulgaris* and the cyanobacterium *Spirulina platensis*. Based on these attenuation coefficients, we used a programmable LED array to simulate the irradiance spectra that cells would experience at different depths in these ponds under full sunlight. For each simulated depth, we measured the photosynthetic rates of these species as rates of oxygen production. In this way, we were able to measure a productivity cross-section of a simulated open pond, taking into account variation in both the magnitude and the spectral content of irradiance with depth.

2. Materials and methods

2.1. Stock culture cultivation

The green alga *C. vulgaris* (UTEX 2714) and the cyanobacterium *S. platensis* (ATCC 29408) were used in this study. *C. vulgaris* is a spherical green alga approximately 10 μm in diameter [21]. It contains the pigments chlorophyll a, with absorption peaks at 440 nm and 680 nm, chlorophyll b, with peaks at 470 and 660 nm, and carotenoids, with a broad absorption band between 450 and 500 nm [22,23]. *C. vulgaris* is of interest in the biofuel market due to its high lipid productivity [24], as well as in the health food market due to its richness in protein, vitamins, polysaccharides, and polyunsaturated fatty acids [25,26]. Henceforth, *C. vulgaris* will be referred to simply as *Chlorella*.

S. platensis is a cylindrical cyanobacterium about 6 to 12 μm in diameter that forms spiral-shaped filaments tens to hundreds of cells in length [27]. It contains chlorophyll a and carotenoids. Additionally, light harvesting is accomplished by the phycobilisome, which consists of the phycobiliproteins phycocyanin and allophycocyanin [28], which absorb maximally at 620 nm and 650 nm, respectively [29,30]. *S. platensis* has been eaten by humans for centuries due to its high concentration of protein and vitamins [27,31]. For several decades, *S. platensis* has been cultivated at large scale in open ponds for sale as a nutritional supplement, with Earthrise® Nutritionals being the world's largest producer. Henceforth, *S. platensis* will be referred to simply as *Spirulina*. Although *Spirulina* is a cyanobacterium, henceforth both *Chlorella* and *Spirulina* will be referred to as 'algae,' as is common practice in the mass cultivation industry.

Stock cultures of *Chlorella* and *Spirulina* were grown in the freshwater medium BG11 (ATCC medium 616) and the *Spirulina* medium proposed by Schlösser [32], respectively. Aliquots containing 1 ml of culture were used to inoculate 100 ml of nutrient media in 250 ml Erlenmeyer flasks. The flasks were placed in an approximately 2 cm deep water bath which was maintained at 25 °C by a chiller (AquaEuroUSA, MC-1/2HP). The cultures were sparged with ambient air passed through a 0.2 μm filter at a rate of 30 ± 20 ml/min. The pH of the cultures was not controlled. Cultivation occurred in a greenhouse on the roof of the Ames Research Center (Moffett Field, CA) between August 29 and September 23, 2014. The greenhouse panels were made from acrylite OP4, which has a transmittance of about 92% throughout the ultraviolet, visible, and infrared range of the spectrum [33]. The cultures were not otherwise shaded.

2.2. Measurement of biomass density

In this study, biomass density X is reported in grams dry biomass per liter (g/l). Optical density at 750 nm (OD_{750}) was used as a proxy for biomass because relatively less time and culture volume are required to measure this parameter. Thus, calibration curves were generated between biomass density and OD_{750} for *Chlorella* and *Spirulina*. For this, the OD_{750} of 1×, 2×, 4×, and 8× dilutions of stock culture were measured in a plate reader spectrophotometer (Molecular Devices, SpectraMax M5). Then, a 50 ml volume of the same stock culture was centrifuged. The supernatant was discarded and the cells were rinsed with water and centrifuged again. The supernatant was again discarded, and the biomass was dried in an oven overnight at 80 °C. The dry biomass was then weighed, and the biomass density was calculated by dividing the biomass by the initial culture volume. It was assumed that the dilutions had biomass concentrations in accordance with their dilution ratios. A least squares regression line was then fitted to the data of biomass density versus OD_{750}. The biomass density of *Chlorella* could be expressed as $X_{Chl} = 0.539OD_{750}$, with an R^2 value of 0.998 for values of OD_{750} less than 0.7. The biomass density of *Spirulina* could be expressed as $X_{Spir} = 1.39OD_{750}$ with an R^2 value of 0.999 for values of OD_{750} less than 1.0. At higher optical densities, the relationship between OD_{750} and biomass density became nonlinear due to multiple scattering. Thus, when measuring the OD_{750} as a proxy for biomass in the following experiments, the culture was diluted until its OD_{750} was less than 0.7 and 1.0 for *Chlorella* and *Spirulina*, respectively.

2.3. Photosynthetic rate as a function of simulated depth

Photosynthetic rate was measured as a function of illumination by irradiance spectra that would occur at different depths within open ponds of *Chlorella* and *Spirulina*. The depth dependent irradiance spectra were determined by measuring the irradiance attenuation coefficient as a function of wavelength for each strain. We then calculated irradiance spectra for depths ranging from 0 to 20 cm within each culture given an incident solar irradiance provided by a standard reference [34]. We then reproduced these spectra using the programmable LED array with 16 different color LEDs (TeleLumen, Light Replicator) [33]. Finally, we measured the photosynthetic rate of small volumes of algal culture exposed to irradiance fields whose magnitude and spectral content corresponded to discrete depths. In their totality, these data comprise the "simulated pond."

Hereafter, the irradiance within a narrow spectral band (1.3 nm) will be referred to as the "spectral irradiance." Further, the irradiance as a