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Not all culture is created equal: A comparative study in search of the most productive cultivation methodology

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ABSTRACT

Considerable investment through public/private consortia has been towards increasing the productivity from mass algal cultivation for making the industries involved more economically viable. Although high-tech solutions have been popular in recent years, I questioned whether low-tech answers through the method that algae are cultivated could shed light on increasing productivity in mass cultivation systems. Shade-limited growth was studied for *Thalassiosira pseudonana* (marine diatom) cultures exposed to 85 Ein m^{-2} d^{-1} under continuous light, and mixed through a 20 cm water column (a standard depth for open pond cultivation), to compare ash-free dry weight (AFDW) and chlorophyll-a (Chl-a) concentrations, net primary productivity (P) and specific growth rates (μ) obtained by batch culture, continuous culture, and semi-continuous culture. Under shade-limiting conditions, both AFDW and Chl-a concentrations varied inversely with μ for the three cultivation methods. During the linear growth phase of batch culture, P did not vary in relation to μ or biomass (B), therefore $\mu = P/B$, and growth rate varied as a power for biomass. For continuous culture methods, B is a function of μ (as dilution rate), therefore $B = P\mu^m$, and $m = -1$ if linear growth is modeled. Net primary production did vary in relation to μ for both continuous and semi-continuous cultures, and $m > -1$. Therefore continuous and semi-continuous culture methods did not reproduce a linear growth phase as found for shade-limited growth. For growth rate > 0.5 doubling d^{-1}, semi-continuous culture had highest, and continuous culture had lowest P for the three methods compared. These results provide evidence that the method of cultivation introduces large variability to net primary productivity under shade-limiting conditions, and needs consideration in the design of cultivation systems and microbiological experiments.

1. Introduction

Following the exponential growth phase for algae grown in a nutrient-replete, batch culture environment is a phase of linear growth due to self-shading, and incurs declining growth rates while biomass increases arithmetically [9]. For batch culture, maximum productivity is achieved, and does not vary during the linear growth phase that can last for days. Under continuous culture conditions, and whereby self-shading controls the light regime and growth rate, net primary productivity has been described to have a pronounced maximum in relation to biomass and growth rate ([4,14,25,26]). This peak for the bell-shaped productivity response has been modeled ([6]; p. 133) as occurring when "the light dissipation efficiency factor [self-shading]… is a maximum … and cell decay is still relatively unimportant." Goldman [6] described "cell decay" as attributed to several variables, importantly respiration, to provide curvature in the growth dynamic between exponential and stationary phases as described using shade-limiting continuous culture. However, this productivity rate maximum has also been described as a broad plateau in relation to biomass or growth rate for *Spirulina* continuous culture [30], or completely absent for large (2000 l), outdoor fed-batch *Chlorella* culture [3], and of *Nannochloropsis oceanica* grown outdoors in biofences using semi-continuous culture methods [27]. Laws et al. [17,18] obtained conflicting results as to whether a maximum in productivity occurred for outdoor, shade-limited semi-continuous cultures grown in shallow flumes. Increasing the productivity for mass algal cultivation systems has been a major concern of research efforts in applied phycology. Whether or not productivity has some maximum between exponential and stationary growth phases, or whether such productivity varies as a function of cultivation methodology, are important questions for an algal industry whose economic survival depends on production.

There has been a long period of contention within the history of microbiology that continuous culture technique is the only true manner to observe growth dynamics for substrate-limiting growth, and that batch culture technique results with artifact (see [23]). Continuous culture technique allowed the phase of declining growth to be resolved by forcing culture into sustaining sub-maximum growth rates in order that resulting phenomena could be observed (i.e., [22]); this was not possible using batch culture as events evolved too rapidly that hindered

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their study. This attitude has been somewhat relaxed regarding physiological responses for light-limited algal growth, whereby results are often considered as mirrored independently of how culture is grown (see [21]), and is because sub-maximal growth rates are obtained as a function of irradiance intensity (i.e., light-limiting growth) while culture is growing exponentially [1].

Yet, what are the growth dynamics when self-shading becomes important in regulating the light regime that a culture is exposed? Hewes [9] described net productivity (P; as weight / volume / time, or weight / area / time) for shade-limited batch culture as a function of both biomass (B; as weight / volume, or weight / area) and specific growth rate (μ; as 1 / time), and because $P = B\,\mu$, that therefore, $\mu = P / B$. During linear (i.e., shade-limiting) growth in batch culture under a variety of light-limiting conditions, μ is a dependent variable as a power function of B. However, if net productivity does have a pronounced maximum during shade-limited growth in relation to biomass and growth rate, the growth dynamic could not have a linear phase. Could photophysiological responses to irradiances be different for algae culture in exponential vs. linear growth phases [9]? Could such difference influence shade-limiting growth dynamics leading to productivity differences by the methods that algae are cultivated?

A retrospective analysis was done of shade-limited algal growth kinetics comparing batch, continuous, and semi-continuous culture techniques. *Thalassiosira pseudonana* (marine Bacillariophyceae) cultures were grown under identical nutrient replete, quantum defined conditions, and were mixed through a photic zone contained within a 20 cm water column (a standard depth for outdoor cultivation ponds; [28]), to examine and compare their net photosynthetic efficiencies under a range of sub-maximum growth rates. The hypothesis that net photosynthesis varies as a function of growth rate or biomass during shade-limited growth is tested, as well as is the hypothesis that net photosynthesis during shade-limitation is not influenced by cultivation method. This study furthermore establishes a benchmark method that can be applied to outdoor cultivation for comparing net photosynthesis among species/strains and cultivation technique as functions of biomass and growth rate.

2. Methods

A laboratory apparatus [10] was used for quantitative studies on the variability of net photosynthesis as functions of depth and quantum flux using traditional batch, continuous, and semi-continuous cultivation methods. Cultures of *T. pseudonana* were grown under continuous light, irradiated only from the bottom by light emitting diode (daylight) with a daily *in situ* irradiance of 85 Ein m^{-2} d^{-1}, being through a 20 cm water column (172 ml), and at 22 ± 1 °C. This is an *in situ* irradiance of ~980 μEin m^{-2} s^{-1}, being an intensity to saturate the photosystems, but not as high as to cause photoinhibition (see [21] regarding photoinhibition under nutrient-replete conditions). Culture vessels were borosilicate glass, sealed that allowed bubbling by humidified air (4 ml s^{-1}) for mixing from bottom of the culture vessel providing a uniform water column, and for continuous culture, to provide pressure that was vented to allow excess gasses and culture to escape through an exit tube. Cultures were grown on 2–4 times strength Guillard's F-media (ProLine® F/2 Algae Feed, Aquatic Eco-Systems, Inc.) to ensure that nutrients were not limiting algal growth.

From a separate experiment, pH was ~8.1 at the beginning, and 8.4 at the end of the phase of linear growth. Most of this variation in pH was due to the compositional change of the media itself (nutrient draw down, organic excretions), causing pH of filtered media (and bubbled 30 min with air) to range 7.8–8.3 during the linear growth phase. The difference between culture and filtered media at beginning and end of the linear growth phase had a pH difference of ~0.2, being evidence that net productivity (*via* CO_2 consumption) was the same at the beginning and end of linear growth (4 day period), and pH did not influence net production for batch culture. This range of pH was well within bounds described by Chen and Durbin [2] for *T. pseudonana* where pH does not influence growth rate *via* carbon limitation with pH 8.0–8.7.

Chl-a concentration was measured by adapting standard fluorometric methods [12,13] used in oceanography (*e.g.*, [11]); 50 μl culture was injected into 10 ml absolute methanol (Sigma), allowed to extract (minimum of 15 minutes) in the dark. Fluorescence, before and after acidification with 1 N HCl, was measured by a calibrated Chl-a fluorometer (Turner Designs, TD-700). To measure biomass, samples (5–10 ml depending upon cell concentration) were filtered onto pre-weighed, oven-dried (100 °C, overnight) 2.4 cm Whatman GF/F glass fiber filters, the filtered material was then dried overnight at 100 °C, and afterwards weighed (Mettler B5 analytical balance). Subsequently, the filters were combusted by natural gas flame and weighed again to obtain weight of remaining ash and salt. The difference between weights of the oven dried (whole biomass) and the combusted (ash) filters provided raw ash free dry weight, and an averaged combusted filter blank (~1 mg of combustible materials) was subtracted from this to obtain ash free dry weight (AFDW; see [9]).

Samples were obtained daily at approximately the same time of day for all culture experiments, and prior to sampling, culture volumes were measured and adjusted with distilled water to offset losses due to evaporation (~1 ml d^{-1}). For semi-continuous culture, a measured volume of the culture (V') was harvested, with the remaining culture replenished with fresh culture medium to the total culture volume (V), and the procedure was repeated after elapsed time (t) of ~24 h. Growth rate was determined as $\mu = (\ln(V) - \ln(V - V')) / t$. For continuous culture, either Beckman or Gilman Scientific Instruments peristaltic pumps supplied culture medium at constant rate. Samples were obtained directly from the culture, and volume (<6% total culture volume) replenished from the collected overflow. Specific growth rate was the dilution rate, i.e., $\mu = V' / V / t$. For both continuous and semi-continuous cultures, samples for analyses were obtained over 4–8 day periods after the cultures had stabilized (taking 1–6 weeks depending on growth rate).

Batch cultures were grown intermittently throughout the experimental period that allowed a test to assure that the culturing apparatus had no unexpected changes in experimentally controlled variables. For batch cultures, only measured values obtained during the linear (shade-limited) growth phase were used in the analyses presented here; this phase lasted ~4 days. Sixteen runs of batch culture were made during the course of the experiment. Equal volumes from a surrogate culture grown under similar conditions were used to replace those volumes removed for sampling (<6% total culture volume). The measure of net productivity (P) for batch culture was calculated for each run as the slope (linear regression) for increase of AFDW from beginning to end of the linear growth phase. Growth rate during each run was estimated from the daily AFDW measured as $\mu = P$ / AFDW.

3. Theoretical considerations

Growth kinetics for batch culture differs considerably between light- vs. substrate-(nutrient or carbon) limitation. After exponential growth, substrate-limitation causes a decline in growth rate often modeled as a hyperbola after the work of Monod [22]:

$$\mu = \mu_{max} s / (s + K_s)$$

where μ is the specific growth rate, μ_{max} is the maximum rate, s is substrate concentration, and K_s is the half-saturation equilibrium constant for growth by the substrate. This leads to a non-linear increase of biomass in time with declining μ between the exponential and stationary growth phases as a function of declining substrate concentration.

The dynamics of light-limited growth in batch culture differs from that of substrate-limitation with two distinctive phases. Exponential growth will occur at sub-maximum rates as a function of light intensity