

AN INNOVATIVE APPROACH FOR SCREENING MARINE MICROALGAE FOR MAXIMUM FLUE GAS CO₂ BIOFIXATION POTENTIAL

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ABSTRACT

A series of growth experiments were carried out with six strains of marine microalgae in batch and semi-continuous culture to measure maximum growth rates and biomass productivities, respectively. Experimental results confirmed the hypothesis being tested that there is no correlation between maximum biomass productivity (i.e., CO₂ biofixation potential) and the maximum specific growth rate. Consequently, it is not possible to select microalgal species exhibiting maximum biomass productivities for CO₂ biofixation of power plant flue gases based on maximum specific growth rates as measured in laboratory batch culture experiments. A mathematical model of algal CO₂ biofixation was developed to better understand which algal parameters other than maximum specific growth rate influence biomass productivities. Future research will focus on using this newly developed model in conjunction with laboratory measurements of key algal parameters to predict CO₂ biofixation by marine microalgae in outdoor mass culture for biofixation of CO₂ and greenhouse gas abatement.

INTRODUCTION

Microalgae are currently being investigated by scientists in the U.S. and abroad as agents for the fixation of CO₂ from power plants. The U.S. Department of Energy (through the National Energy Technology Laboratory) is supporting several projects in this area. Recently an "International Network for Biofixation of CO₂ and Greenhouse Gas Abatement with Microalgae" was formed through the International Energy Agency Greenhouse Gas R&D Programme, with support from the U.S. DOE, to coordinate and channel R&D activities in this field (Pedroni et al., 2002). Presently the Network has the following members: Arizona Public Services, ENEL Produzione Ricerca, EniTecnologie, Electric Power Research Institute, ExxonMobil, Gas Technology Institute, Rio Tinto, and the U.S. DOE-NETL with additional participants expected to join in the months ahead.

The advantages of microalgae in greenhouse gas mitigation are that they can utilize CO₂ from power plant and other flue gases directly, that they can produce high value liquid and gaseous fuels (biodiesel, hydrocarbons, ethanol, methane, hydrogen), that they could potentially exhibit very high biomass productivities approaching the theoretical limits of photosynthesis (about 10% solar conversion efficiency), and that they are able to use waste water and nutrients, allowing for integration of such processes with waste treatment. However, these advantages must still be realized, with many practical problems and issues remaining to be overcome through focused R&D.

There are presently no established or proven technical protocols for the selection of microalgal species that would prove effective in outdoor algal mass cultures, with the goal of maximizing flue gas carbon dioxide sequestration. It is generally assumed that those species that exhibit the highest maximal specific growth rate will also have the highest biomass productivity, i.e., the best CO₂ biofixation potential (Eppley and Dyer, 1965). As a result, "fast growth rate" and "high productivity" are often used synonymously in the literature, particularly in research related to algal mass culture for CO₂ mitigation. However, a positive correlation between these parameters has not been established, either experimentally or theoretically, in the literature. Indeed, based on analogy with other biological systems, physio-genetic

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characteristics that would result in fastest growth may not be linked with highest efficiency in light energy conversion, the underlying determinant of biomass productivity.

Before discussing the experimental and modeling results, it is instructive to briefly define the terms “growth rate”, “biomass productivity”, and “CO₂ biofixation potential” since they will be used frequently throughout this paper. Growth rate [r , mg/L·day] refers to the rate of increase in algal cell concentration [B] with time as measured in batch culture, i.e., $r = dB/dt$. The specific growth rate [μ , day⁻¹] is the growth rate normalized by the algal biomass concentration [B], i.e., $\mu = r/B = (dB/dt) \cdot 1/B$. The specific growth rate is also related to the biomass doubling time [t_d] as follows: $\mu = \ln 2/t_d$. Thus, the specific growth rate [μ] in batch culture can easily be determined by measuring the time it takes for biomass concentrations to double. The shorter the doubling time, the greater the specific growth rate. The maximum specific growth rate [μ_{max}] is the value of the specific growth rate that is measured during the exponential growth phase in a batch culture system where all nutrients (CO₂, minerals, etc.) and light are present in excess, i.e., growth is limited only by the inherent physiology of the algal species.

Biomass productivity [P , mg/L·day] is the production of algal biomass concentration per unit time in a continuous or semi-continuous culture system. Since continuous algal cultures are generally light-limited because of shading by dense cell suspensions, biomass productivity is not only a function of the specific maximum growth rate of the selected algal species (as measured in non light-limited batch culture, see above) but also the operating conditions of the continuous bioreactor system (i.e., dilution rate, light intensity) and other genetic physiological parameters (i.e., half saturation constant for light, biomass absorption coefficient), as indicated in the model below. Finally, since according to the stoichiometry of photosynthesis one mole of biomass carbon is produced for each mole of CO₂ fixed, the CO₂ biofixation potential is directly related to biomass productivity.

It was the objective of this research to determine whether maximal biomass productivities or maximal specific growth rates are the better selection criterion for algal species suitable for mass culture and CO₂ fixation. The hypothesis is that maximal biomass productivities are not correlated with maximum specific growth rates. In other words, strains with the fastest growth rates do not necessarily have the highest biomass productivities. We have tested the validity of this hypothesis by growing a number of different microalgal species (see list below) in both batch and semi-continuous cultures, and measuring the maximum specific growth rates (in batch cultures) and maximal biomass productivities (at optimal dilution rates in semi-continuous cultures), respectively.

MATERIAL AND METHODS

All culture experiments were carried out in sterile 1 L (flat) Roux bottles filled with 750 mL enriched seawater medium containing 3.15 g/L sea salts, 500 mg/L urea, 100 mg/L NaH₂PO₄·H₂O, 20 mg/L Fe-sequester, 50 µg/L CuSO₄·5H₂O, 125 µg/L ZnSO₄·7H₂O, 50 µg/L CoCl₂·6H₂O, 900 µg/L MnCl₂·4H₂O, 32.5 µg/L Na₂MoO₄·2H₂O, 500 µg/L thiamine HCl, 25 µg/L biotine, and 25 µg/L vitamin B₁₂. For culturing diatoms, the medium also contained 300 mg/L NaSiO₃·9H₂O (neutralized with HCl). The Roux bottles were placed in a water bath with temperature controlled at 28 °C, sparged with CO₂ enriched air (1.4 % v/v CO₂) at 100 mL/min to maintain a pH of 7.5, and illuminated continuously (i.e., 24 hrs/day) from both sides with a set of four 48 inch 40 Watt fluorescent lights (Vita Lite) at a light intensity of 120 µM/m²·sec. The culture medium within the Roux bottles was mixed continuously with the help of magnetic stirrers.

The maximum specific growth rate for a given microalgal species was measured in batch cultures as follows: A temperature equilibrated Roux bottle filled with the enriched seawater medium was inoculated with the selected microalgal species that had been grown previously in a separate shake flask culture to an optical density (OD) of about 1. A small culture sample was periodically removed aseptically from the continuously sparged, mixed, and illuminated Roux bottle and the algal biomass concentration was measured both photospectrometrically as optical density (OD @ 570 nm) and gravimetrically as ash free dry weight (AFDW, mg/L). The maximum specific growth rate (μ_{max}) was determined from the biomass doubling time (t_d) during the exponential growth phase, where $\mu_{max} = \ln 2/t_d$. The maximum specific

growth rate (μ_{\max}) measured in batch culture should also be similar to the dilution rate (D_w) at which biomass washout is observed in semi-continuous culture.

We cultured 6 different marine microalgal species from two different culture collections (UTEX = University of Texas Culture Collection, UHAW = University of Hawaii Culture Collection): Unicellular green algae *Chlorella salina* (UTEX), *Chlorella sp. S/CHLOR-3* (UHAW), *Chlorococcum sp. CHLOCO3* (UHAW), *Nannochloris eucaryotum* (UTEX), *Nannochloris sp. NANNO02* (UHAW), and the unicellular diatom *Phaeodactylum tricorutum* (courtesy of Dr. M. Tredici, University of Florence, Italy).

After successful completion of the batch culture experiments, a set of at least four Roux bottles were inoculated with the particular microalgal species and grown under conditions similar to those used in batch experiments, with the exception that a fraction of the culture suspension was removed and replaced with fresh medium on a daily basis. The ratio of the rate of new medium addition (F) to the total culture volume (V) is defined as the dilution rate D ($D = F/V$). For example, if 250 mL of suspension are removed from the 750 mL culture and replaced with 250 mL fresh medium every day, the dilution rate is 0.33 d^{-1} . In general, these semi-continuous cultures were carried out at four different dilution rates, such as 0.35 d^{-1} , 0.5 d^{-1} , 0.65 d^{-1} , and 0.8 d^{-1} . In some cases, additional dilution rates were tested to determine the rate (D_w) at which biomass washout occurs.

The reproducibility of steady-state conditions was tested by performing triplicate semi-continuous culture experiments for *Nannochloris sp. NANNO02* at two dilutions rates (i.e., $D=0.5 \text{ d}^{-1}$ and $D=0.8 \text{ d}^{-1}$). The variability of steady-state biomass concentrations (AFDW) was 5.7% and 1.8% relative standard deviation for cultures operated at $D=0.5 \text{ d}^{-1}$ and $D=0.8 \text{ d}^{-1}$, respectively, indicating good reproducibility of the experiments.

EXPERIMENTAL RESULTS

Figure 1 shows a typical batch growth curve (i.e., both (a) arithmetic and (b) logarithmic presentation of the data) for *Nannochloris eucaryotum*, demonstrating the maximal growth rate (linear region in b) under these conditions. Figure 2 shows typical biomass concentrations (OD_{570}) for semicontinuous cultures of *Nannochloris eucaryotum* as a function of time for four different dilution rates. Approximately 2 weeks after inoculation from a batch culture, the biomass concentrations diverge in the four cultures in response to the imposed dilution rates and different steady-state biomass concentrations are reached. The biomass productivities (P) at each steady-state can then be calculated as the product of dilution rate (D) and biomass concentration (B), i.e., $P = D \cdot B$ (see also equation 1 below).

Figure 3 shows steady-state biomass productivities (P) as a function of dilution rate (D) for the six different microalgal species (i.e., *Chlorella salina*, *Chlorella sp.*, *Chlorococcum*, *Nannochloris eucaryotum*, *Nannochloris sp.*, and *Phaeodactylum tricorutum*) that were grown successfully in the semi-continuous culture experiments. While all species exhibit characteristic P-D curves resembling inverted parabolas as predicted by theory (see equation 7 and Figure 5), there are significant differences in maximum biomass productivities among the various tested algal species. For example, the maximum biomass productivity for *Nannochloris eucaryotum* was approximately 1,350 mg/L-d while it was only around 830 mg/L-d for *Phaeodactylum tricorutum*. It is also interesting to note that the P-D relationship is not fixed for a given species, but appears to be strongly affected by strain characteristics. For instance, there is very little similarity in the P-D curves for the two *Chlorella* or the two *Nannochloris* strains.

As shown in Figure 4, the maximum biomass productivities (P_{\max}) for each species were plotted as a function of the respective maximum specific growth rates (μ_{\max}) that were independently measured in batch culture experiments. Based on this limited data set of 6 different marine microalgal strains, it is clear that there is no strong correlation ($R^2 = 0.23$) between P_{\max} and μ_{\max} . For example, while *Chlorella salina* and *Nannochloris sp.* exhibit almost exactly the same maximum productivity in semi-continuous culture (i.e., ca. 1000 mg/L d), their respective maximum specific growth rates differ by almost a factor of three (i.e., $\mu_{\max} = 0.78 \text{ d}^{-1}$ versus 2.27 d^{-1} , respectively). In addition, the greatest P_{\max} was observed for *Nannochloris eucaryotum* which had a smaller μ_{\max} value than *Nannochloris sp.* In summary, based on the data shown in Figure 4, there appears to be no clear relationship between P_{\max} and μ_{\max} and it is therefore

impossible to predict maximum biomass productivities in semi-continuous cultures (both laboratory and outdoor) from measurements of maximum specific growth rates in laboratory batch cultures.

ALGAL CO₂ BIOFIXATION MODEL DEVELOPMENT

We also developed a simple conceptual model for a light-limited continuous “flat” bioreactor (i.e., Roux bottle). We briefly review this “Algal CO₂ Biofixation Model” here to show the essential steps that are involved and to demonstrate how the model can be used to provide first-order estimates of biomass productivities if key biological and physical input parameters are known. For example, as will be shown below (see discussion of equation 7), biomass productivity in flat photobioreactors is a complex function of biological parameters such as the maximum specific growth rate (μ_{max}), the maintenance energy requirement (m), the biomass absorption coefficient (K_a), and the half-saturation constant for light (K_I). If these fundamental parameters are measured for a wide range of potential algal species, it will be possible to use the model to rank the different species in terms of their predicted biomass productivities and to select the one with the highest CO₂ biofixation potential.

The modeling of light-limited chemostats is complicated because light is attenuated via absorption and scattering as it penetrates into the culture which contains both medium and biomass (Cornet et al., 1992a,b; 1995; 1998; Cornet and Albiol, 2000; Fernandez et al., 1998; Frohlich et al. 1983; Grima et al., 1994; 1996). Under steady state conditions, the biomass productivity (P) in a chemostat is given as:

$$(1) \quad P = B \cdot D = r$$

where r is the biomass production rate within the entire bioreactor volume, B is the steady-state biomass concentration, and D is the dilution rate. Since the light-intensity changes as a function of depth (z) inside the bioreactor, it follows that the “localized” biomass production rate is also changing as a function of depth (z). Consequently, the total biomass production rate within the entire bioreactor volume can be obtained by integrating $r(z)$ over the entire illuminated bioreactor thickness L (Cornet and Albiol, 2000):

$$(2) \quad r = \frac{1}{V} \int_V r(z) dV = \frac{1}{V} \int_0^L r(z) A \cdot dz = \frac{1}{L} \int_0^L r(z) dz$$

The localized biomass production rate $r(z)$ is related to the localized specific growth rate $\mu(z)$ as follows (Evers, 1991; Grima et al., 1994):

$$(3) \quad r(z) = (\mu(z) - m) \cdot B$$

where m is the maintenance energy requirement. Under light-limited conditions, the localized specific growth rate $\mu(z)$ has been hypothesized to be a function of the localized light intensity $I(z)$ according to a relationship that is similar to the Monod model for nutrient-limited cultures (Cornet and Albiol, 2000):

$$(4) \quad \mu(z) = \mu_{max} \cdot \frac{I(z)}{I(z) + K_I}$$

where μ_{max} is the maximum specific growth rate which is observed when light is not limiting and K_I is the half-saturation constant, i.e., the light intensity where $\mu = 1/2 \mu_{max}$.

Light absorption by algal biomass according to Lambert-Beer’s Law results in an exponential decrease in light intensity, $I(z)$, as a function of culture depth z :

$$(5) \quad I(z) = I_0 \cdot e^{-K_a \cdot B \cdot z}$$

where I_o is the incident light intensity entering at the bioreactor surface, and K_a is the biomass absorption coefficient. Although equation (5) does not account for light scattering, it is a reasonable first approximation to the light regime in a dense algal culture.

By combining equations (2), (3), (4), and (5), it is then possible to obtain an expression for the biomass production rate within the entire illuminated photobioreactor volume:

$$(6) \quad r = \frac{1}{L} \int_0^L r(z) dz = \frac{1}{L} \int_0^L (\mu(z) - m) \cdot B \cdot dz = \frac{B}{L} \int_0^L \mu(z) dz - mB = \frac{B\mu_{\max}}{L} \int_0^L \frac{I_o \cdot e^{-K_a Bz}}{I_o \cdot e^{-K_a Bz} + K_I} dz - mB$$

This integral has the following analytical solution:

$$(7) \quad P = r = -\frac{\mu_{\max}}{K_a L} \cdot \ln \left(\frac{K_I/I_o + e^{-K_a B L}}{K_I/I_o + 1} \right) - mB$$

Equations (1) and (7) may be combined to calculate steady-state biomass concentrations (B) as a function of dilution rate (D), and then biomass productivity ($P = D \cdot B$) can be computed and plotted as a function of D. Figure 5 shows the results of model simulations for biomass productivity (P) as a function of dilution rate (D) for three hypothetical algae species which differ only with respect to their maximum specific growth rate (μ_{\max}). The observation that the calculated parabolic biomass productivity profiles closely resemble in overall appearance the measured curves shown in Figure 3 indicates that the mathematical model appears to be conceptually correct.

According to equation (7), biomass productivity (P) in a light-limited photobioreactor with external illumination (i.e., a flat Roux bottle) is a complex function of both biological and physical parameters. The biological parameters of importance are the maximum specific growth rate (μ_{\max}), the maintenance energy requirement (m), the biomass absorption coefficient (K_a), and the half-saturation constant for light (K_I). The significant physical parameters are the thickness of the flat photobioreactor (L), the incident light intensity (I_o), and the dilution rate (D), which determines the steady-state biomass concentration (B). For a given photobioreactor, the physical parameters L and I_o are fixed, and the maximum biomass productivity (P_{\max}) is only a function of the biological parameters. If these biological parameters can be independently measured in simple experiments and the resulting numerical values used as inputs to the above equation (7), it should be possible to mathematically estimate which microalgae species are most likely to have the greatest CO₂ biofixation potential in this particular bioreactor system. In addition, if models are developed for other photobioreactor types (such as tubular or tank photobioreactors, with light impinging from more than one direction), it will be possible to use the measured biological parameters as numerical inputs to these predictive models. Future research will focus on the experimental validation of the algal CO₂ biofixation model and its application to screening marine microalgae for maximum biomass productivities in outdoor pond cultures.

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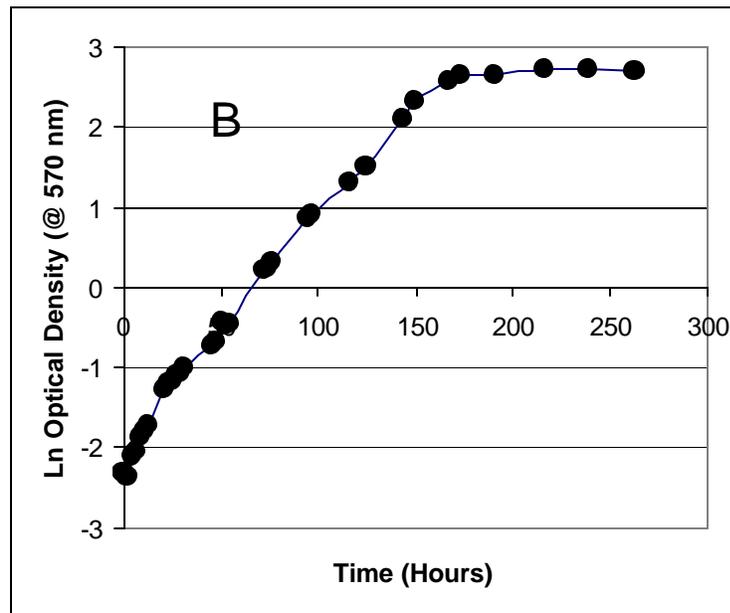
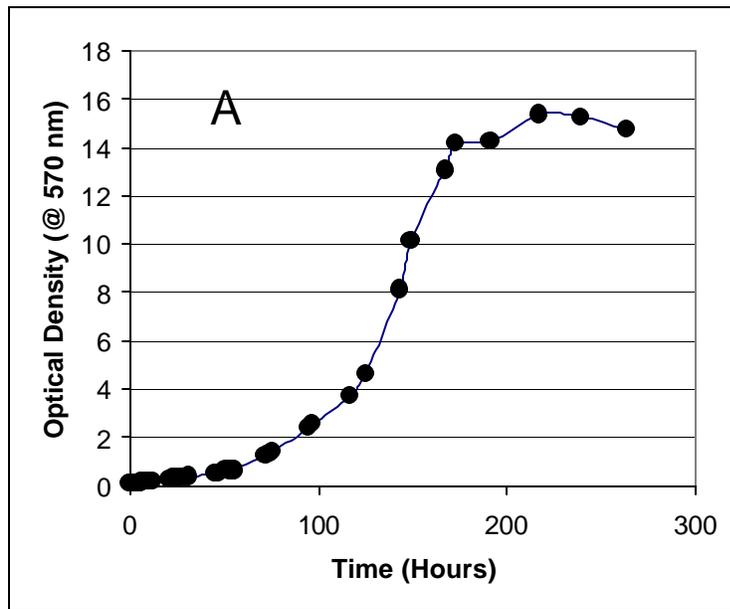


Figure 1 Increase of Biomass Concentration as Measured by (A) Optical Density (OD) at 570 nm or (B) Ln(OD) as a Function of Incubation Time in Batch Culture of *Nannochloris eucaryotum*.

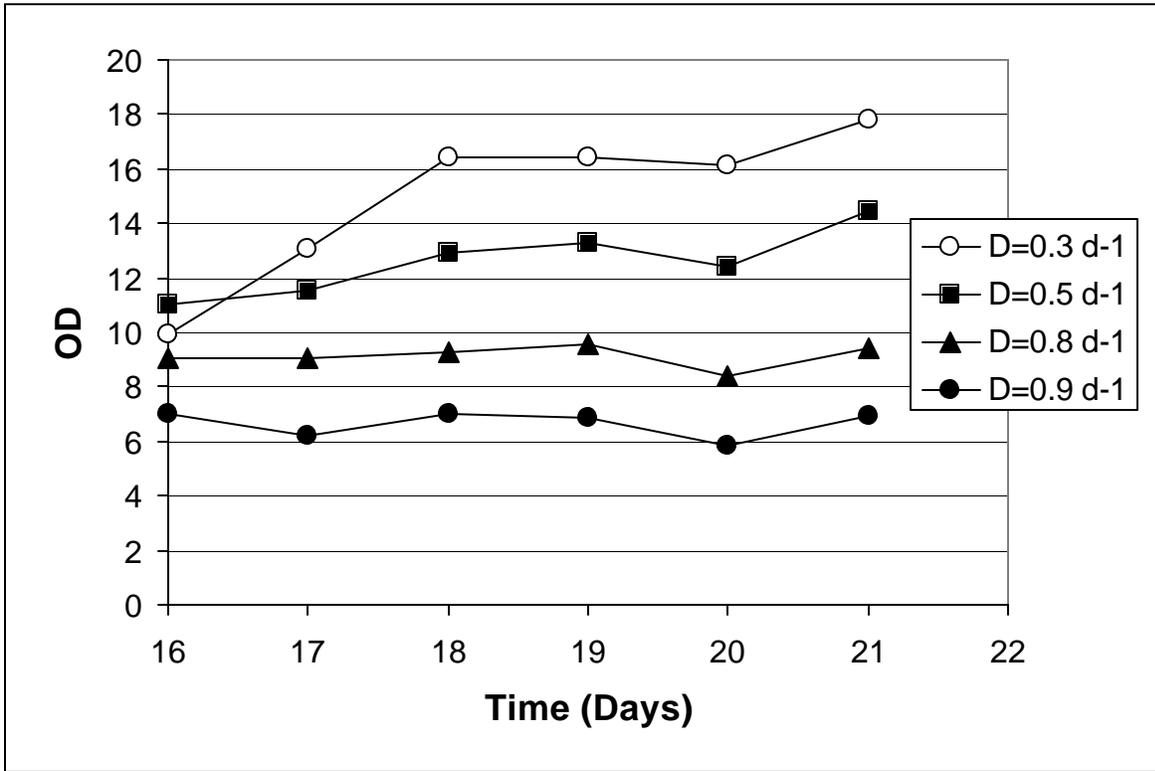


Figure 2 Biomass Concentrations as Measured by OD as a Function of Time in Semi-continuous Cultures of *Nannochloris eucaryotum* at Four Different Dilution Rates.

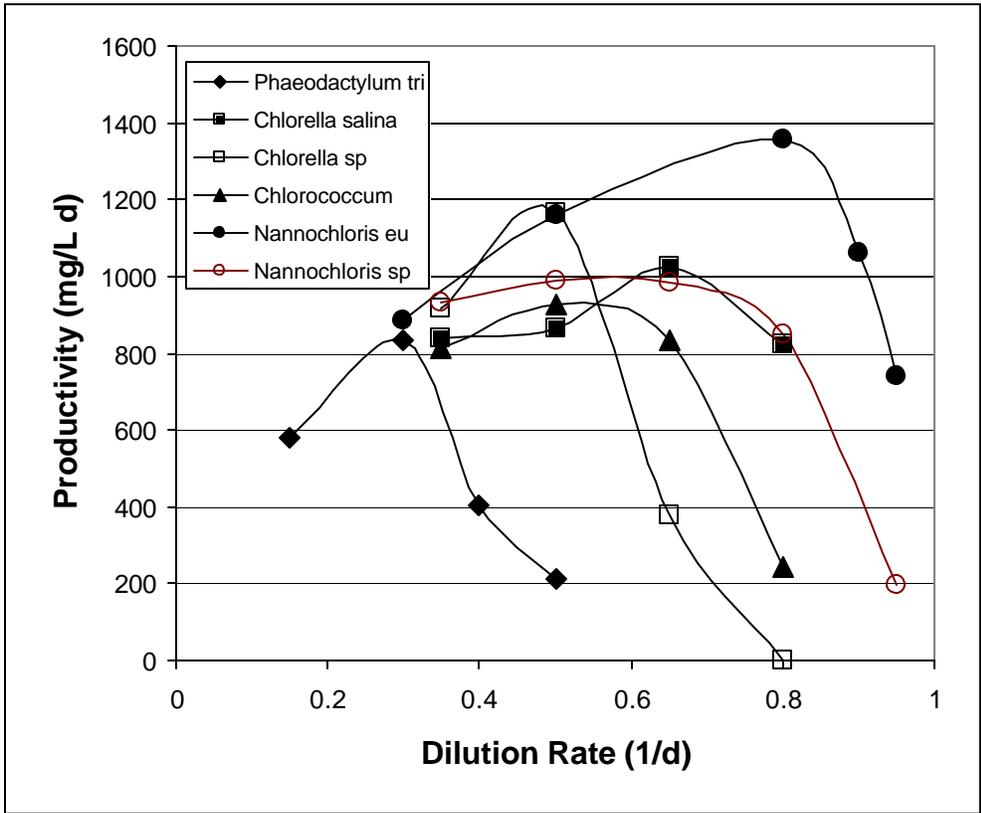


Figure 3 Steady-State Biomass Productivity as a Function of Dilution Rate in Semi-Continuous Cultures of Six Marine Microalgal Strains.

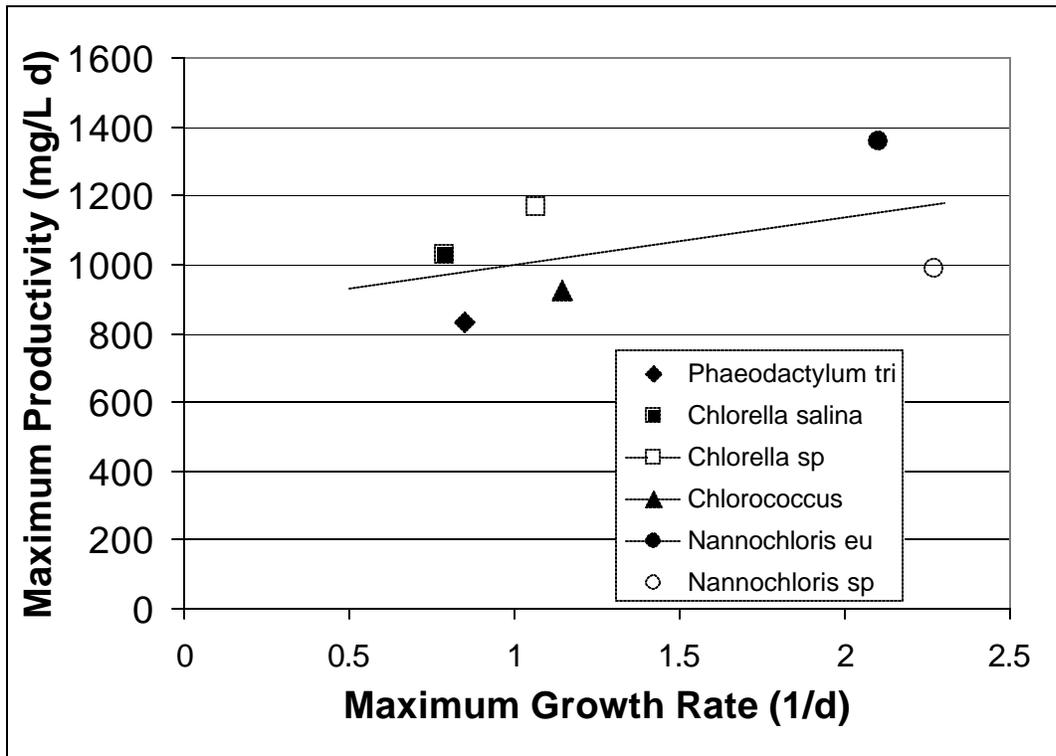


Figure 4 Maximum Steady-State Biomass Productivity (P_{max}) in Semi-continuous Culture as a Function of Maximum Specific Growth Rate (m_{max}) in Batch Culture for Six Marine Microalgal Strains. The Linear Regression Line has an $R^2 = 0.23$, Indicating Poor Correlation Between the two Measured Parameters.

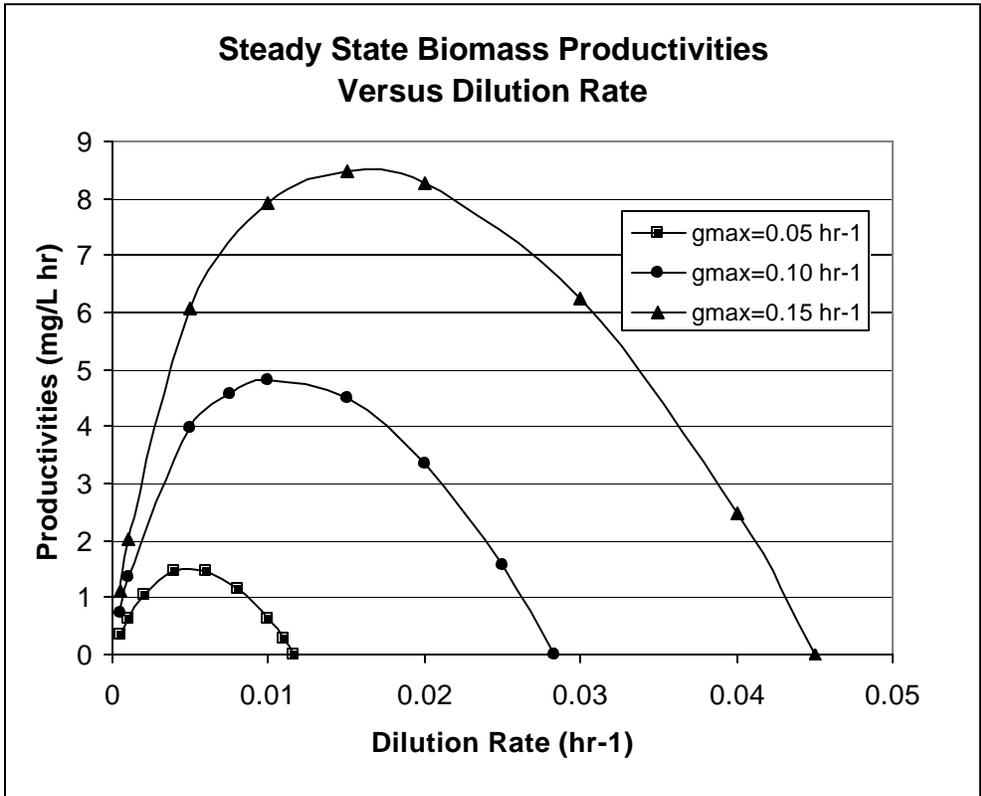


Figure 5 Model Predicted Steady-State Biomass Productivities as a Function of Dilution Rate in Continuous Cultures of Three Hypothetical Microalgae Species Characterized with Maximum Specific Growth Rates of 0.05 hr⁻¹, 0.10 hr⁻¹, and 0.15 hr⁻¹. The Selected Physical and Biological Modeling Parameters (see also Equation 7) were as Follows: $L = 0.1$ m, $I_0 = 10$ W/m², $K_a = 0.5$ cm²/mg, $K_i = 20$ W/m², and $m = 0.005$ hr⁻¹.