Characterizing and Optimizing Lipid Production in Microalgae Using Matrixed Photobioreactors

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Introduction:

There are many sources of plant lipids that have been used to create biodiesel, including soybean and rapeseed, but these often compete with food crops for desirable farmland and other resources, driving up the price for both the food and biofuel. In addition, the growth cycle for many plant crops is relatively long, usually lasting several months.

Certain strains of microalgae can contain lipids of up to 70% or more, greatly exceeding the oil productivity of the best-producing terrestrial plants. Additionally, microalgae grows much faster than other plant sources of lipids, producing high-volume, high-lipid-density material; with many harvests (crops) per year per acre (doubling time can be as fast as 3.5 hours).

However, not all strains of microalgae produce high levels of lipids. First, it is necessary to identify strains that can potentially be most productive. Second, it is then necessary to optimize culture conditions to ensure the highest possible yield (balancing biomass and by-product production). Further, this second step must be conducted under local climate conditions (in the case of ponds or raceways). Therefore, the bench-top system must be capable of faithfully mimicking production conditions (whether outdoor raceways or closed system reactors).

It is extremely time-consuming and expensive to try to systematically optimize strains and growing conditions on a large scale (e.g. raceway or production-scale reactor). Instead, the use of a bench-top photobioreactor that mimics production conditions has been demonstrated as a cost effective tool to facilitate the discovery, characterization and optimization of high lipid producing microalgae purpose. If multiple reactors can be “matrixed”, or networked or linked by the control software such that multiple experiments or variations of the same experiment can be performed simultaneously, this will dramatically reduce time and resources. By extension, we examine the use of a system of matrixed Phenometrics PBR101 photobioreactors for rapid optimization of strain characterization and optimization of growth of high-lipid producing microalgae.

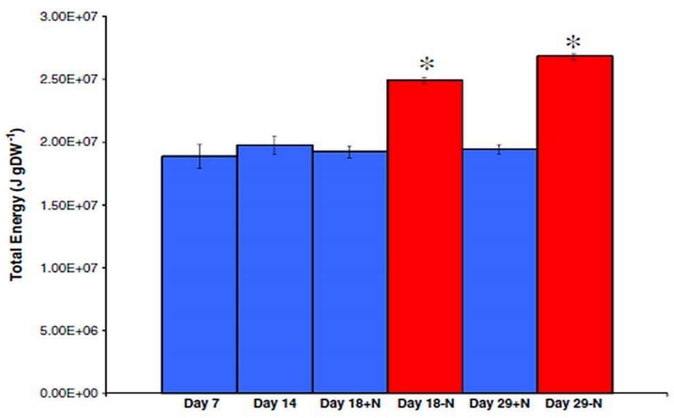
The Use of Small Photobioreactors for Large-Scale Reactor Optimization:

The advantages of a properly-designed and equipped bench-top (<1L) photobioreactor are that it can perform several functions: 1) it can accurately mimic production conditions, making results scalable (which is not the case for most systems; 2) Researchers can work on one strain or several simultaneously (with a matrixed system); 3) Studies can be performed on a small-scale prior to up-scaling in raceways or closed system reactors, providing design guidance; 4) the PBR101 bench-top reactor can be matrixed with up to 256 units operating simultaneously, all controlled from one computer. Each PBR101 can be programmed and controlled to run a unique experiment. It is this matrix capability that dramatically reduces cost, research time, and ultimately improves the value of the data produced. Herein, we will discuss the benefits of matrixed photobioreactors and demonstrate their value towards algal experimentation.

The Value of Matrixed Photobioreactors – Optimizing Lipid Production Via N-Depletion:

The PBR101 has become an extremely valuable experimental tool for many types of algal research and production. A recent paper[1] cites the use of matrixed PBR101 systems for the investigation to determine if a particular algal strain could provide increased biomass yield coupled with the induction of oil accumulation under conditions of nitrogen limitation or starvation. Normally, withholding nitrogen increases the induction of oil accumulation in algae. However, this usually comes at a cost of lower total biomass yield. What is desired is to retain both: high or elevated biomass yield coupled with increased oil accumulation. The ultimate goal was of course, maximum induction of lipid accumulation; in other words, how much oil (i.e. convertible energy) can be produced? What is the optimum balance between biomass and product yield? The PBR101 excels as an experimental tool in answering these questions.

It has been shown that a particular strain of microalgae, *Chlorella sorokiniana*, has a high lipid content under a wide range of environmental growth conditions. In these experiments, a total of 18 PBR101 systems were matrixed. N-replete and N-deplete media were studied, and triacylglycerol (TAG) accumulation (i.e. lipid) as well as total biomass were measured. On day 7 (7 days after inoculation), day 14, day 19, and day 29, the total contents of the individual PBR101 vessels were harvested. The matrixed PBR101s allowed for a wide variety of measurements under varied conditions. In addition, all of the data was collected on a single computer, allowing for rapid and efficient examination and comparison of the results.

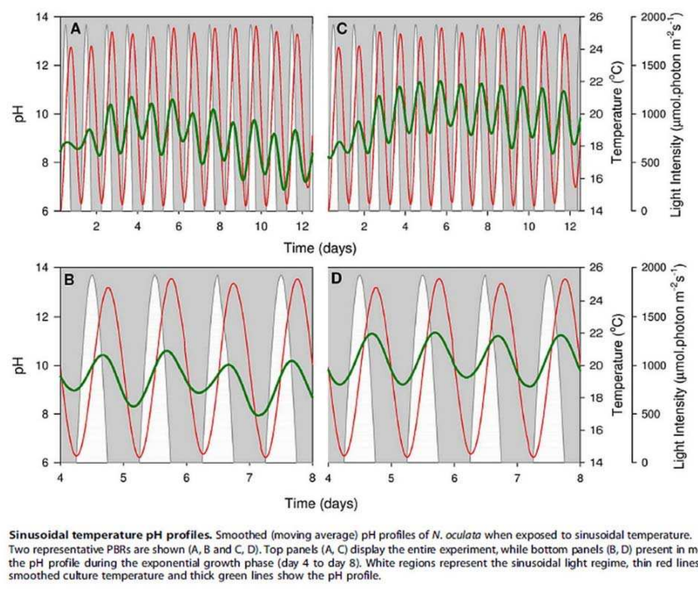


It was found that for *Chlorella sorokiniana*, TAG content increased 20-fold, and energy density increased by 64% over the 1 month time course of the experiment for cells grown under N-deplete conditions relative to cultures grown in the presence of N. At the same time N depletion had less impact on total cell numbers or biomass yield. Regardless, the increases in energy density coupled with the differences in biomass yield between N-deplete and N-replete cultures resulted in a total energy yield of 23.6×10^6 J for the N-deplete and 14.4×10^6 J for the N-replete culture or a 1.6-fold greater total energy yield for the N-deplete culture.

The Value of Matrixed Photobioreactors – Optimizing a Particular Strain of Algae for Lipid Production:

Another example of using a matrix of PBR101 photobioreactors studied the effect of diel temperature and light cycles on the growth of *Nannochloropsis oculata*.[2] There are several strains of *Nannochloropsis* that are known to provide relatively high lipid content usable for the production of biofuels. *N. oculata* was selected for this study based on its ability to grow in ponds of saline, brackish and hypersaline water, ensuring that its potential use for biofuel production will not compete with food crops for freshwater or arable land.

In these experiments, the growth of *N. oculata* in PBR101s was studied (i) at constant temperature, and (ii) with variable temperature following a diel pattern, where both treatments were held under varying light intensity that also follows the diel cycle. The objective was to understand the combined effects of light and temperature on algal physiology, and to determine the importance of temperature in controlling large-scale biofuel production. Chlorophyll *a* content was measured to determine algal growth rates, and dissolved oxygen concentrations and pH were monitored continuously *in situ*.



Sinusoidal temperature pH profiles. Smoothed (moving average) pH profiles of *N. oculata* when exposed to sinusoidal temperature. Two representative PBRs are shown (A, B and C, D). Top panels (A, C) display the entire experiment, while bottom panels (B, D) present in more detail the pH profile during the exponential growth phase (day 4 to day 8). White regions represent the sinusoidal light regime, thin red lines give the smoothed culture temperature and thick green lines show the pH profile.

In these experiments, a varying number of PBR101s (up to 16 here) were matrixed. Matrixed photobioreactors provided an ideal methodology for this work, as a) multiple parameters could be examined simultaneously; b) multiple changes to a single parameter could be made simultaneously; and c) all of the data was collected and examined from a single computer. Further, the environmental control features and high-resolution monitoring of the algal growth and physiology made possible by the PBR101 demonstrated its versatility in studies of this kind.

Accordingly, it was through this matrixed experimental setup that the combination of changes in light intensity, temperature, pH, and dissolved oxygen both a) individually and b) combined allowed for the study of the growth and physiology of the biofuel candidate microalgal species *N. oculata*. Further, what was not explored in these experiments, but could also have been easily done with a PBR101 matrix, was to determine which specific strain of *Nannochloropsis* could have been best optimized for lipid content production.

About the PBR101:

The PBR101 has been proven time-and-again to provide direct scale-up (100%, + or - 10%) from bench-top to much larger reactors. Accordingly, it is an ideal system for companies with large-scale reactors to optimize their production conditions on a small scale much more quickly and at greatly reduced. For a researcher working on one strain, or exploring many different ones, the system is also ideal, due to its technical capabilities and flexibility, small size, affordability, and speed of experimental setup and acquiring results.

The PBR 101 has the following Standard Features (controlled using the PBR101 and Algal Command):

- 3000 µ-Einstein LED specially-designed lighting system with conical vessel that accurately mimics BOTH raceway or production tube bioreactors (controllable from 0-3000 µ-Einsteins
- Fully programmable Diurnal Cycles; you set the length of sunlight day and also the hour of peak sunlight
- Fully programmable active heating and cooling from 5 - 50° C, linkable to the diurnal cycle; you can independently set the hour of peak heating (if different from peak sunlight)
- cooling and heating,
- Easy to use Algal Command Software permits complete, simultaneous, integrated control of up to 256 bioreactors from a single computer. All the reactors are linked together via Ethernet Hub.
- CO_2 programmable and controlled from the software
- Active magnetic stirring
- Active sparging of up to two gasses (the first is included; second is optional)
- Temperature measurement
- True Turbidostatic measurement and control (with the optional turbidostatic pump)
- Field-configurable, fully autoclavable, low cost durable polycarbonate reactor vessel
- The reactor vessel can have as many inlets and outlets as you require
- 100% (+/- 10%) directly-proportional (by volume) up-scale to pond, raceway, or indoor reactor

- pH probe (Optional) for control and measurement of pH
- Turbidostatic pump; fully programmable and computer-controlled (Optional)

Conclusion:

Lipids are an important by-product of algal culturing. They represent a valuable and easily-renewable energy source. However, it is important to properly optimize their production. The PBR101 is an important tool in the research of algae culture, physiology, and by-products such as lipids.

In addition, to its ability to control and manage experiments, it is the matrix capability of the PBR101 that adds a unique extra dimension to the system, and to the experimental capabilities of the researcher. Up to 256 separate reactors can be controlled by one computer, with all of their data stored together. Thus, multiple matrixed PBR101 systems have been shown to dramatically reduce time, cost, and resources.

References: 1) A. Barry et al., “Impact of Nitrogen Limitation on Biomass, Photosynthesis, and Lipid Accumulation in Chlorella Sorokiniana “, J Appl. Phycol., DOI 10.1007/s10811-015-0652-z; 2) B. Tamburic et al., “The Effect of Diel Temperature and Light Cycles on the Growth of Nannochloropsis oculata in a Photobioreactor Matrix”, PLoS ONE 9(1): e86047. doi:10.1371/journal.pone.0086047