A Review of Recent Advancements of Microalgae Technology and Production for Commercial Applications Using the Bench-Top Photobioreactor

T.J. Alavosus, Ph.D. and M. G. Chaparian, Ph.D.*
Phenometrics, Inc., 2727 Alliance Drive, Lansing, MI 48910

Introduction:

There are tens of thousands of known species of microalgae, some of which have been shown to be excellent sources of biofuels, foods, feedstock, nutraceuticals, health foods, industrial chemicals, pharmaceuticals, and other major commercial products. These often benefit from their ability to grow across a wide-range of environmental conditions with smaller acreage requirement and much shorter harvesting times when compared to equivalent yields of traditional crops such as corn, sugar cane, and others.

Some strains of microalgae are better than others for different commercial applications. For example, high-lipid production is desirable for biofuel development, while biomass co-products (mostly protein and carbohydrate) may be processed into many other products such as: foods, chemicals, medicines, vaccines, minerals, animal feed, fertilizers, pigments, salad dressings, ice cream, puddings, laxatives and skin creams.

In order to even further optimize microalgae production, strains are often genetically engineered. These modified strains typically require unique culture conditions and containment; therefore, each potential production strain must be optimized for both co-product yield and culture growth prior to production scale implementation. Thus, if optimization at bench scale can accurately predict production yield, then time, resources and CAPEX can be significantly reduced and ROI maximized.

This poster examines the use of the Phenometrics PBR101 photobioreactor to optimize cultivation conditions at bench scale that are predictive of large-scale yield in raceways or enclosed photobioreactors for several commercially relevant applications of microalgae. The papers cited herein represent many different commercial applications for which the PBR101 can be an enabling tool for enhancing algae and co-product production.

Bench-Scale Prediction of Production Yields:

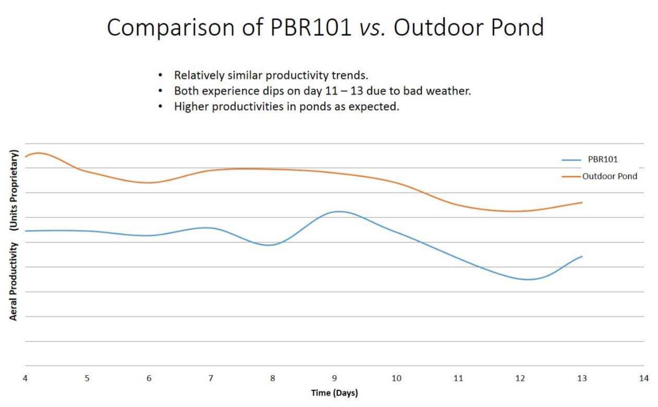
The Phenometrics PBR101 is a bench-top photobioreactor (approximately 600-650 ml working volume) that has been demonstrated to accurately predict the required growing conditions of algae in much larger systems, such as raceways and industrial-scale bioreactors, often tens of thousands of liters or larger.

Many experiments have been performed by commercial companies to directly compare the productivity of a real-world large scale bioreactor (a raceway pond) to that predicted from preliminary bench-scale yields using the Phenometrics PBR101 photobioreactor. In this example[1], an experiment was performed to establish the correlation between biomass productivity in PBR101 systems and AA outdoor 1-acre growth raceway ponds.

Environmental data, including temperature and Photosynthetically Active Radiation (PAR), recorded at a raceway pilot production facility, was programmed into an array of PBR101s and used to compare yields in the two systems. The average daily aerial productivity was calculated.

Conditions that were duplicated in the PBR101 included Temperature, PAR, pH, and Carbon delivery. Conditions that were not duplicated in the PBR101 included Humidity, Evaporation rate, Wind, Solar irradiance (spectrum/light quality), interaction with foreign microorganisms such as grazers, algae, etc., and rainfall.

The PBR101 was proven to accurately predict raceway production to within 10%. Similar accuracy in up-scale yield predictions have been demonstrated by other PBR101 users.



Animal Feed:

Corn is a popular animal feed, and is a good representative of the feedstock commercial market. Algae has a much shorter yield-cycle than corn, and thus significantly reduces total growth time (from planting to harvest) and also greatly improves total annual yield. That is, with algae, you get multiple harvests in a single year rather than just one with corn, while at the same time also reducing costs.

Nitrogen and Phosphorous are important components of animal feed. Accordingly, a study was performed to determine if algae could convert N and P into animal feed in smaller land acreage than crops such as corn[2]. At the same time, this study sought to optimize the nutritional value of algae produced for animal feed.

An array of PBR101s was programmed to simulate solar radiation and day length. Pond cultures were isolated and pH was controlled by the addition of CO_2. As a result, a variety of pure strains were isolated in the lab from the pond cultures and determined to be appropriate for pond inoculation based on several requirements, including maximization of the nutritional value of algae for animal feed, optimization of pathogen inactivation methods, and quantification and control of any toxic cyanobacteria.

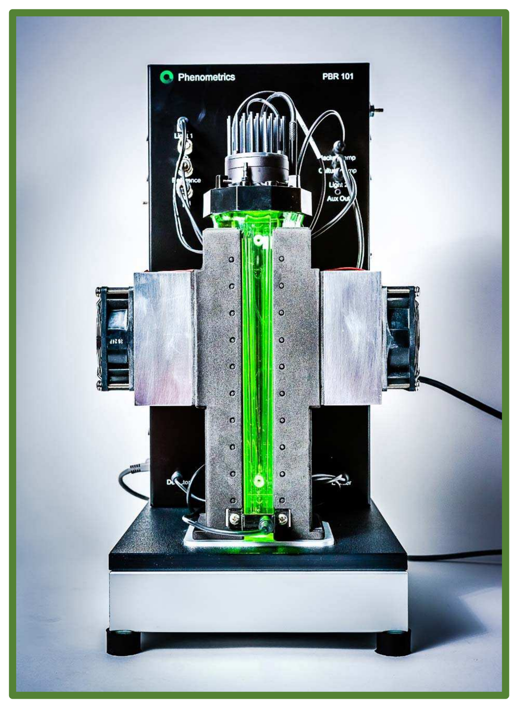
Biofuel:

The promise of one day using algae as a low-cost source for biofuels is coming closer and closer to fruition. Existing algal strains are already producing high lipid content, which can be extracted and converted to fuels. New strains and genetically-modified strains are also continually being researched, as well as variations in many different culture conditions in order to increase lipid and desirable co-product production.

A recent study[3] examined the effect of light and temperature variations on the growth and physiology of the biofuel candidate marine microalgal species *Nannochloropsis oculata*.

An array of interconnected PBR101 photobioreactors integrated with metabolic sensors was used to vary light and temperature conditions, varying them according to sinusoidal day/night light/dark and heating/cooling cycles. The specific experiments were performed with algal cultures maintained at a constant 20°C versus a 15°C to 25°C diel temperature cycle, where light intensity also followed a diel cycle. While no differences in algal growth were found, it was determined that the changes in environmental conditions had a great effect on the metabolic processes. The combination of strong light and high temperature in the second set of experiments caused greater damage to this second photosystem. In addition, overnight metabolism also was found to perform differently; this was thought to be due to the effect of temperature on respiration.

These experiments demonstrated the prediction of the effectiveness of deploying *Nannochloropsis oculata* in similar field conditions for commercial biofuel production. In addition, this study showed that the PBR101, with high-level environmental control features combined with high-resolution monitoring of algal growth and physiology, can be used to answer many unresolved questions in algal biofuel production.


The PBR101

A related study[4] provided the first in-depth analysis of CO_2 limitation on the biomass productivity of *Nannochloropsis oculata* using PBR101 photobioreactors. Net photosynthesis decreased by 60% from 125 to 50 µmol $O_2 L^{-1} h^{-1}$ over a 12 h light cycle as a direct result of carbon limitation. Continuous dissolved O_2 and pH measurements were used to develop a detailed diurnal mechanism for the interaction between photosynthesis, gas exchange and carbonate chemistry in the PBR101 photobioreactor. Gas exchange determined the degree of carbon limitation experienced by the algae. Carbon limitation was confirmed by delivering more CO_2, which increased net photosynthesis back to its steady-state maximum.

Further research examined the induction of oil accumulation in algae for biofuel production[5]. This effect is often achieved by nitrogen starvation. However, withholding nitrogen also often reduces total biomass yield, which reduces crop yield. In this report, it was demonstrated using the PBR101 photobioreactor that *Chlorella sorokiniana* will not only accumulate substantial quantities of neutral lipids when grown in the absence of nitrogen, but will also exhibit unimpeded growth rates for up to 2 weeks, a finding with significant commercial ramifications.

Industrial Wastewater Cleanup:

Industrial wastewaters are often produced on extremely large volumes. Many studies have shown that these wastewaters can potentially serve as a source for both carbon (essential nutrient) and water for microalgal production. The effluent of an anaerobic internal circulation (IC) reactor, used to treat the waste of a biotechnology production facility, was chosen as the cultivation medium for *Chlorella sorokiniana* in batch and continuous cultures using the PBR101 photobioreactor[6]. The aim was to determine the relationship between the rate of waste (algae nutrients) removal and biomass production at varying dilution rates.

Batch culture using undiluted wastewater showed biomass productivity of 1.33 $g\ L^{-1} day^{-1}$, while removing over 99% of the ammonia and phosphate from the wastewater. Deceleration-stat (D-stat) experiments performed at both high and low light intensities of 2100 and 200 (µmol photon $m^{-2} s^{-1}$) established the optimal dilution rates to reach volumetric productivity of 5.87 and 1.67 $g\ L^{-1} day^{-1}$ respectively. The corresponding removal rates of nitrogen were 238 and 93 $mg\ L^{-1} day^{-1}$ and 40 and 19 $mg\ L^{-1} day^{-1}$ for phosphorous. The yield at low light intensity was as high as had been observed in any previous report indicating that the waste stream allowed the algae to grow at its full potential.

Nutraceuticals and Health Foods:

Nutraceuticals – dietary nutrients and supplements – are a growing industry worldwide. The search for new and more diverse nutritional materials at a lower cost has led many researchers to discover that algae is an very viable source for a wide variety of nutraceutical ingredients.

As an example, it is now common to find Spirulina, as well as other algae strains, in nutritional drinks and foods (e.g. Odwalla *Green Machine*). A recent study[7] found that microalgae lipids could serve as a source of eicosapentaenoic acid (EPA) or omega-3 as an alternative to traditional fish oils. This and other research of its kind is important for the increasingly large population of consumers who are strict vegetarians and will not accept fish as a source of nutrients or supplements. However, the yield of EPA and lipid extracted from microalgae vary with different growth conditions. Therefore, for commercial feasibility, the importance of optimizing EPA production is critical. The effects of temperature, light intensity, and nitrate level on cell growth, lipid productivity, and omega-6 (arachidonic acid (ARA))/omega-3 (EPA) ratio of *Porphyridium cruentum*, one of the most promising oil-rich species of microalgae, were investigated.

This study demonstrated that temperature, illumination, nitrogen level, and CO_2 levels (studied using the PBR101 photobioreactor) significantly impacted cell growth, lipid production, and fatty acid compositions of *P. cruentum*. The results demonstrated that decreased temperature and enhanced light intensity resulted in higher lipid content and lipid production, but also reduced biomass. Therefore, maximizing biomass production does not lead to optimizing lipid production. In addition, higher lipid production and lower biomass were observed when 5% CO_2 in air was used as CO_2 supplementation as compared to pure CO_2. Further, optimal growth conditions were also experimentally determined to produce the lowest ARA/EPA ratio, which is most desirable for maximum nutritional value.

Biofuel, Fish + Animal Feeds, Fertilizer:

In 2013, the National Association for Advanced Biofuels and Bioproducts (NAABB) had benchmarked five of their strains in outdoor facilities[8]. These were put into cultivation in an outdoor environment, studied for their productivity over time, and produced an understanding of how they develop in those environments as compared to the laboratory. Those strains were taken through the entire, fully-integrated process including cultivation, harvesting, extraction, conversion of the lipids into fuels (both diesel and other hydrocarbons), and the lipid-extracted algae processed into additional fuel components and/or animal feed products.

José Olivares, founder of the original NAABB 3-year DOE-backed consortium, said that the Phenometrics PBR101 photobioreactor "was valuable in our cultivation research. It allowed us to mimic the environment of an algal pond throughout different temperatures, different nutrient conditions, different sunlight conditions – within a small system that can be multiplexed in the laboratory so that we didn't have to have, say, 30 ponds in a very large field to do a particular set of experiments. In fact, Los Alamos (National Laboratories) has acquired 24 of these photobioreactors and is utilizing them quite heavily."

Further, he added, "From our harvesting process we did a separation primarily through hexane and wet extraction with one of our partners and the lipid-extracted algae (LEA) then went into a number of development activities. One of them was to take that LEA and understand its value as feed for a number of different types of animals, including ruminants, pigs, fish and shrimp. What we found was that the value of LEA as a feed supplement for ruminants could add up to $160 of value per ton to the process. And this is in direct comparison to soybean meal, which is the normal protein supplement added to cattle feed. In aquaculture that value seems to be much higher, up another $100 a ton added into the process."

Molecular Biology of a Model Organism for Biofuel, Industrial chemicals, and Pharmaceuticals Production:

Cyanobacteria are an excellent model for the study of photosynthesis in the laboratory. However, only a small percentage of cyanobacterial genes and intergenic regions have been experimentally evaluated for their impact on fitness and survival. A recent investigation determined the complete set of essential genomic regions necessary for survival in a cyanobacterium[9].

Synechococcus elongatus PCC 7942, a model organism for studying photosynthesis and the circadian clock, is also being developed for the production of fuel, industrial chemicals, and pharmaceuticals. It would be thus beneficial to identify a comprehensive set of genes and intergenic regions that impacts fitness in *S. elongatus.* A pooled library of ~250,000 transposon mutants was created and sequencing was used to identify the insertion locations. For testing the library, growth under standard laboratory conditions was accomplished by cultures grown in several conditions, including in a Phenometrics PBR101 Photobioreactor. By analyzing the distribution and survival of these mutants, 718 of the organism's 2,723 genes were identified as essential for survival under laboratory conditions. In addition to improving our fundamental understanding of Cyanobacteria, from a commercial perspective this research more broadly defines the essential genes and intergenic regions that must be maintained in any genetically engineered strains designed for optimized commercial production.

References: 1. T.J. Alavosus and M. Chaparian, (2014) *Algae Biomass Summit*; 2. Shelton Murinda, Marcia Murray, *et al.*, (2015) *115th General Meeting, American Society for Microbiologists;* 3. Bojan Tamburic, Peter J. Ralph, *et al.*, (2014) *PLoS ONE* 9(1): e86047; 4. Bojan Tamburic, Peter J. Ralph, *et al.*, (2016) *ChemSusChem*, in press; 5. Sangeeta Negi, Amanda N. Barry, Richard Sayre *et al.*, (2015) *J Appl Phycol*, published on-line 08 July; 6. Jon Van Wagenen, Mathias Leon Pape, Irini Angelidaki, (2015) *Water Research* 75 301-311; 7. Maryam Asgharpour, Brigitte Rodgers and Jamie A. Hestekin, (2015) *Energies* 8, 10487-10503; 8. . David Schwartz, (2013) *Algae Industry Magazine*, published on-line 21 July; 9. Golden, SS *et al.*, (2015) *Proceedings of the National Academy of Sciences of the United States of America*, 112(48).