

Development of the Photoheterotroph *Rhodobacter* for Functional Membrane Protein Expression

Brandon S. Curtis¹, Philip D. Laible², Deborah K. Hanson² and Wayne Curtis¹, (1) Chemical Engineering Department, Pennsylvania State University, Fenske Building, University Park, PA 16802, (2) Biosciences Division, Argonne National Laboratory, Argonne, IL 60439

Abstract:

This paper presents progress on the development of *Rhodobacter sphaeroides* as a platform for high-level expression of functional membrane proteins. The high capacity of *Rhodobacter* for membrane protein expression stems from the dense intracellular membranes synthesized by this organism under anaerobic photoheterotrophic conditions. Using plasmids with promoters from the light-harvesting pigments, inducible heterologous expression is accomplished simultaneously with membrane formation when the organism experiences reduced oxygen tension and low-light conditions. Unique growth characteristics of this organism are presented: although anaerobic growth ceases in the dark, anaerobic photoheterotrophic growth is as fast as aerobic growth and results in nearly 3-times greater biomass yield as a result of photosynthetic ATP production. Growth yield (on carbon basis) is also several-fold higher on defined media as compared to complex media – which is of particular interest for the application of isotopic labeling for membrane protein structural studies. Although transformed lines display dramatically reduced growth under low light conditions, this can be overcome with high light, even with ‘knockout’ strains where the dominant light harvesting protein (LH2) has been deleted to facilitate greater capacity for functional heterologous expression. Preliminary growth testing in a thin film photobioreactor combined with mass-balance rationalized fed-batch operation are shown to facilitate photoheterotrophic growth to nearly 10 grams DW/L (an order of magnitude higher than current practice in flask culture). Preliminary results demonstrate high-level membrane protein expression.

Background:

Rhodobacter is an extremely versatile organism, capable of growth under a myriad of unique culture conditions. Much of the preliminary work focused on characterizing growth behavior, since the expression system couples expression to promoters involved in the transition from aerobic heterotrophic growth to anaerobic photoheterotrophic growth. Despite extensive study of this versatility in growth behavior, there are very few reports of even simple growth kinetics; its inconvenient growth rate requiring sampling every few hours for 2-4 days, invariably contributed to the paucity of data. An important auxiliary objective was to achieve photoheterotrophic growth, and growth using a defined medium toward the objective of using this system for isotopic labeling of membrane proteins.

Results

Figure 1 shows growth curves executed in Klett flasks (shake flasks with side-arm for insitu optical density measurement) where OD @ 660nm were measured during aerobic, anaerobic and photoheterotrophic growth. In this experiment, nitrogen overlay was used to remove oxygen at an OD~0.2. This experiment displays three [important characteristics of](#)

Rhodobacter: (1) Anaerobic photo-heterotrophic growth results in growth as fast as aerobic growth, (2) Anaerobic photoheterotrophic growth provides much higher yield (as much as 3x greater yield based on dry weight measurements – data not shown); (3) *Rhodobacter* does not display significant anaerobic growth in the dark, though it does retain high viability as demonstrated by dilution plating (data not shown). Other useful characteristic of *Rhodobacter* include the distinct coloration patterns of colonies and growth rate, which allow for facile contamination testing.

While Klett flasks provide an excellent experimental system for volumes of material required for extensive protein analysis, they are cumbersome and time consuming to manipulate. A highly multiplexed means of growing *Rhodobacter* in anaerobic culture tubes will be presented that allows much more detailed examination of growth kinetics. A variety of *Rhodobacter* strains developed with and without plasmids (and blank expression vectors) have been characterized.

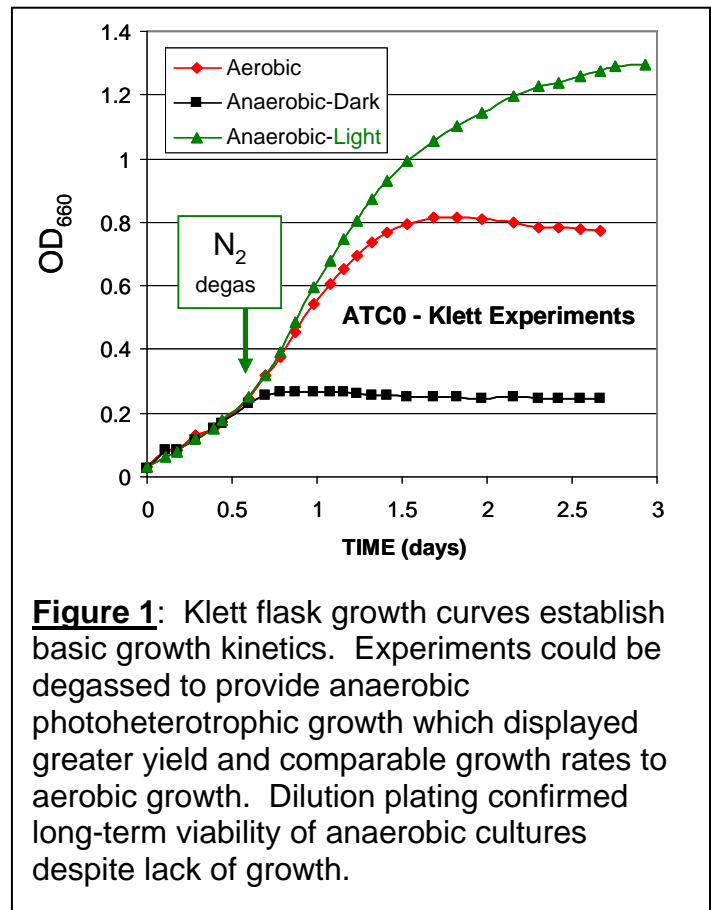


Figure 1: Klett flask growth curves establish basic growth kinetics. Experiments could be degassed to provide anaerobic photoheterotrophic growth which displayed greater yield and comparable growth rates to aerobic growth. Dilution plating confirmed long-term viability of anaerobic cultures despite lack of growth.

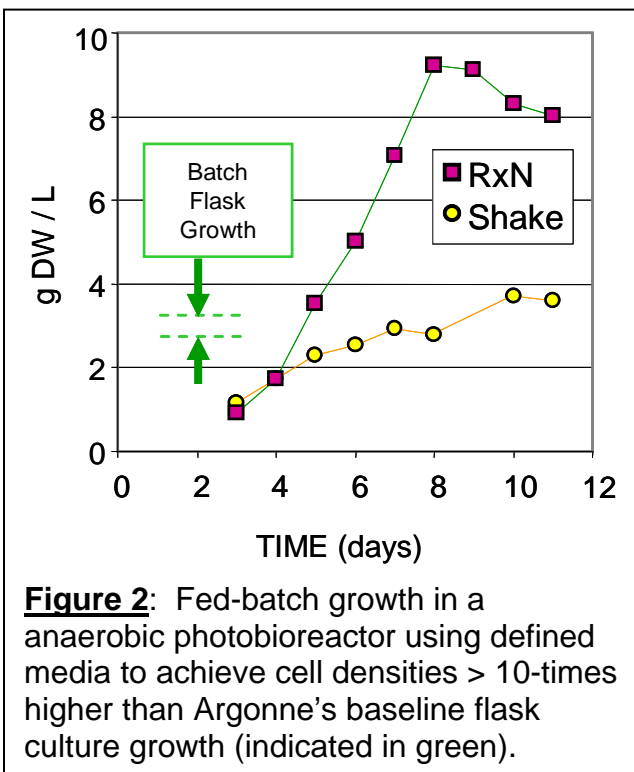


Figure 2: Fed-batch growth in a anaerobic photobioreactor using defined media to achieve cell densities > 10-times higher than Argonne's baseline flask culture growth (indicated in green).

An initial study of a novel bioreactor design was undertaken at a 1-L liquid volume scale as shown in **Figure 2**. This fed-batch strategy utilized mass balances and pH control to allow for accumulation of biomass to more than 10-times the standard batch flask culture.

References:

Laible PD, Scott HN, Henry L, Hanson DK. Towards higher-throughput membrane protein production for structural genomics initiatives. *J Struct Funct Genomics*. 2004;5(1-2):167-72.

Laible PD, Mielke DL, Hanson DK (2008). The Purple Phototrophic Bacteria. Series: Advances in Photosynthesis and Respiration , Vol. 28 Hunter, C.N.; Daldal, F.; Thurnauer, M.C.; Beatty, J.Th. (Eds.) ISBN: 978-1-4020-8814-8; In press, Available: November 3, 2008.