THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF CHEMICAL ENGINEERING
AND DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

DEVELOPMENT OF *RHODOBACTER* FOR THE PRODUCTION OF FUNCTIONAL MEMBRANE PROTEINS

BRANDON SCOTT CURTIS
Summer 2011

A thesis
submitted in partial fulfillment
of the requirements
for baccalaureate degrees
in Chemical Engineering and Biochemistry and Molecular Biology
with interdisciplinary honors
in Chemical Engineering and Biochemistry and Molecular Biology

Reviewed and approved* by the following:

Wayne Roger Curtis
Professor of Chemical Engineering
Thesis Supervisor

Darrell Velegol
Professor of Chemical Engineering
Honors Adviser

Philip Mohr
Professor of Biochemistry and Molecular Biology
Honors Adviser

Andrew Zydney
Professor of Chemical Engineering
Faculty Reader and Department Head

* Signatures are on file in the Schreyer Honors College.

ABSTRACT

Members of the genus *Rhodobacter* are examined as potential alternative bioprocessing platforms for products that span the value continuum, from small-scale, isotopically-labeled heterologous membrane proteins to large-scale biofuels. The salient metabolic and physiological features of *Rhodobacter* are discussed, in the context of the potential advantages they confer over traditional expression hosts such as *Escherichia coli* under certain processing conditions. The availability of molecular tools for delivering DNA and modifying the genome of *Rhodobacter* is surveyed. Genetic engineering, reactor design, and reactor operational strategies that may improve the viability of *Rhodobacter* as an expression host are also explored in the context of two potential applications: heterologous membrane protein expression and liquid biofuel production.

While significant work remains to be done to enhance the viability of *Rhodobacter* species as industrial production platforms, the analyses suggest that, in certain scenarios, the technical advantages of utilizing *Rhodobacter* as a production host may outweigh the technical challenges of using a non-traditional organism.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

FIGURES

TABLES

ACKNOWLEDGEMENTS

I thank my advisor, Dr. Wayne Curtis, for personal and technical mentorship that has extended far beyond my four years as an undergraduate researcher. I am indebted to many members of CurtisLab, past and present, whose passion for hard work and willingness to teach have inspired my own path. I also thank Dr. Ruth Mendum, for encouraging me to consider the ramifications of scientific research that extend far beyond the science itself.

CHAPTER I

INTRODUCTION AND BACKGROUND

Recently, much attention and federal funding has been focused on solving problems involving the production of very complex molecules, such as antibodies and membrane proteins used in biomedical research, and of very simple molecules, such as acids, alcohols, and hydrocarbons used for fuel and polymer production. Researchers have begun investigating nontraditional organisms or consortia of organisms to perform these conversions. It is hypothesized that the enhanced metabolic and physiological diversity of nontraditional organisms may provide a means to overcome technical and economic limitations of existing bioprocessing systems.

The traditional workhorse organisms of industrial bioprocessing are gram-negative bacteria such as *Escherichia coli* and yeasts such as *Saccharomyces cerevisiae* and *Pichia pastoris*. *E. coli* grows very rapidly, dividing up to three times per hour under ideal conditions, and high-intensity reactor systems have been developed that can support cell culture densities of over 100g/L (*1*). Production strains of *E. coli* have been engineered to reduce susceptibility to bacteriophages, disable potentially-deleterious homologous recombination, and remove native restriction enzymes that may interfere with foreign DNA delivery. Yeasts also divide rapidly, and have been used by man for thousands of years to produce fermented products such as beer and wine. *P. pastoris* has recently been engineered to impart human-like glycosylation patterns to heterologously produced proteins, improving their functionality and stability inside human patients (*2*).

Through a history of bioprocessing use, all of these organisms have acclimatized to the environmental conditions encountered in high-density culture systems. The tools and protocols for genetically manipulating these organisms have matured, and genes can be knocked out and introduced with relative ease using a wide variety of mobile genetic elements, robust selectable markers, and facile DNA delivery techniques.

These organisms are not without their metabolic and physiological limitations. They lack light-capture facilities, and are incapable of utilizing light for chemical energy production. They cannot fix atmospheric carbon dioxide nor dinitrogen into biologically-useful forms, and are instead dependent on reduced substrates, such as glucose, glycerol, and ammonia, for carbon and nitrogen (*3*). Because of this, they are obligate chemoheterotrophs. Robust, high-yield growth is further limited to aerobic conditions. Unless the desired molecule is the direct product of anaerobic fermentation, as in carbon dioxide in the biological leavening of bread and ethanol in alcoholic beverage production, then oxygen or another highly-electronegative electron acceptor is required for acceptable product yield.

This dependence on oxygen and sugar prevents processes that use these organisms from effectively competing with abiotic methods of monomer and fuel production. Sugar, and the high oxygen mass transfer coefficients necessary to support high-density aerobic growth, both cost significant energy to produce, undercutting the economics of these processes. During chemoheterotrophic growth, the substrate serves as the sole carbon, electron, and energy source for metabolism, ensuring that much of the carbon will be lost to oxidation to meet cellular energy requirements and limiting the substrate mass fraction available for incorporation into the desired product. High-

intensity bioreactors featuring these organisms, despite their usefulness for producing high-value products, are too expensive to run and too poorly scalable to be economical for high-volume monomer or fuel production.

The NIH has released several RFPs for the development of technologies targeted at relieving the heterologous production bottleneck to membrane protein structural determination ("Structural Biology of Membrane Proteins (R01), PA-06-119; "Membrane Protein Production for Structure Determination (R01) RFA-RM-08-012). These proteins are critically important in virtually every biological function, and have been estimated to account for over one-half of existing pharmaceutical targets (*4*). Knowledge of their structure and dynamics not only improves our basic understanding of fundamental biological mechanisms, but may eventually enable rational design of therapeutic molecules to effect a desired change in membrane protein functionality. Unfortunately, these proteins are difficult to heterologously express in a functional form using traditional expression hosts (*4*).

Occasionally, the unusual physiological characteristics possessed by a nontraditional expression host uniquely qualify it to produce a certain complex product; this is the case for heterologous membrane protein production. In *E. coli* and many other prokaryotes, the outer cellular membrane is the sole membrane in the cell, and its surface area is limited; attempts to overexpress membrane proteins in *E. coli* usually result in the formation of insoluble

Figure 1 - Rhodobacter grown under various environmental conditions

inclusion bodies of aggregated, misfolded protein (*5*). By developing *Rhodobacter sphaeroides* as the expression host and making use of its unique physiology, researchers at Argonne National Labs were able to achieve functional integration of heterologous membrane proteins for biochemical characterization (*6*).

Rhodobacter is a member of the purple non-sulfur bacteria (PNSB) in the prokaryotic class Alphaproteobacter (*7*). PNSBs are well-known among microbial ecologists for possessing an impressive array of metabolic capabilities that have allowed them to thrive in numerous environmental niches. It has been suggested by bacterial physiologists that *Rhodobacter capsulatus* is the most metabolically-diverse organism currently known; it has been confirmed to inhabit at least five different metabolic regimes, depending on substrate availability and the prevailing environmental conditions (*8*). Figure 1 provides a visual picture of this diversity under a variety of growth conditions (anaerobic and photoheterotrophic) and it is evident just from the changes in pigmentation that there are tremendous changes in cellular physiology for these different growth conditions. This metabolic flexibility goes significantly beyond that observed in traditional biotechnological production organisms such as *Escherichia coli*, *Saccharomyces cerevisiae*, and CHO cell lines, which are only capable of robust chemoheterotrophic growth under aerobic conditions.

This thesis seeks to examine the potential use of *Rhodobacter* species as platforms for industrial and environmental biotechnology, and investigates ways in which the increased metabolic complexity of these organisms may, in some instances, provide an advantage over traditional host organisms. Tools and techniques for growing and genetically engineering *Rhodobacter* are discussed. The applicability of *Rhodobacter* is

illustrated with experiments in membrane protein synthesis, peformed in collaboration with Argonne National Labs, and in liquid biofuel production, performed as part of the preliminary work supporting a Department of Energy Advanced Research Projects Agency - Energy (DOE ARPA-E) Electrofuels grant.

CHAPTER II

METABOLIC EVALUATION OF *RHODOBACTER*

Rhodobacter is a collection of freshwater aquatic microbial species. Communities are commonly found in oxygen-poor environments in the presence of decaying organic matter, which provide preferred organic acid substrates such as acetate, succinate, or fumarate. Free sugars are rarely encountered and poorly metabolized, but can be fermented anaerobically by a few species. Without light, organic acids and alcohols are chemoheterotrophically metabolizable in the presence of oxygen or another suitable electron acceptor such as dimethyl sulfoxide or trimethylamine-N-oxide (*7*). Light-induced charge separation under anaerobic conditions creates proton motive force for the production of ATP and NADH, and *Rhodobacter* growing phototrophically can reduce and assimilate carbon dioxide, organic acids, and alcohols. Chemolithotrophic growth with dihydrogen as the electron donor is also possible (*8*), and despite belonging to the purple non-sulfur bacteria, reduced sulfur compounds such as hydrogen sulfide and thiosulfate can also be used (*9*). While ammonia is the preferred nitrogen source, some species can also fix dinitrogen under anoxic conditions.

While *Rhodobacter* is capable of growing on many different substrates, biomass yields may vary significantly. For biotechnological applications such as protein expression for NMR structural analysis where extremely expensive radioisotopically-labeled substrates must be used, or biofuels production where feedstock costs are a significant contribution to the overall process economics, yield is of paramount importance.

The difference in yield of *Rhodobacter capsulatus* on ammonium succinate under chemoheterotrophic and photoheterotrophic conditions was qualitatively explored by examining the redox and stoichiometric considerations governing the conversion of substrate to biomass. Predictions made based on this analysis were then verified experimentally.

The redox states of the carbon in succinate and biomass were compared. *Rhodobacter capsulatus* biomass has been empirically determined to be $C_5H_{8.4}O_{2.35}N_{0.65}$ (*10*). Oxygen and hydrogen are expected to take their standard oxidation numbers of -2 and +1, respectively. Nitrogen in the cell is typically in the form of an amine or imine, which places N in the -3 oxidation state. Summing the contributions of H, O, and N, the five carbon atoms must therefore have an average oxidation state of +0.35 to balance the molecule. Performing the same calculation on succinate, each carbon must have an average oxidation state of -0.5. The nitrogen in ammonium is already in the -3 oxidation state and can be incorporated into biomass via glutamate synthase without any additional redox transfers. This suggests that the assimilation of succinate into biomass is an overall oxidative process, yielding excess reducing equivalents in the form of NAD(P)H. While biomass synthesis is overall an oxidation, some carbon dioxide is still evolved by decarboxylative metabolic processes:

$$\text{Succinate} + NH_4^+ \quad \rightarrow \quad \text{Biomass} + CO_2 + \text{NADH}$$

Previous work suggests that the redox balance of the cell is maintained by disposing of excess reducing power via RUBISCO, yielding reduced carbon species, via

a dihydrogenase, yielding dihydrogen, via a dinitrogenase, fixing dinitrogen to ammonia, or via a DMSO reductase acting on an exogenous species by a dissimilatory process (*11*):

$$NADH + CO_2 \rightarrow \text{Biomass}$$
$$NADH + H^+ \rightarrow H_2$$
$$NADH + N_2 \rightarrow NH_4^+$$
$$NADH + DMSO \rightarrow DMS$$

No suitable substrates for DMSO reductase are present in standard growth media, and the dinitrogenase is strongly repressed by the presence of ammonia. It was therefore hypothesized that the re-reduction of metabolic carbon dioxide could serve as the primary reducing equivalent sink, with the secondary sink via the evolution of hydrogen. The reductive pentose phosphate pathway, the primary carbon fixation pathway found in *Rhodobacter*, consumes two reducing equivalents and three molecules of ATP for each molecule of carbon dioxide fixed to glyceraldehyde-3-phosphate (*12*); ATP is produced at no direct cost to the cell from light energy capture, so it is likely that all of the carbon contained in the succinate molecule will be incorporated into biomass if excess reducing equivalent production sufficiently exceeds metabolic carbon dioxide production.

Under photoheterotrophic conditions, succinate serves as the carbon and electron source, while light provides the energy for ATP synthesis. No succinate must be oxidized to carbon dioxide to generate proton motive force for ATP synthesis, so the extra electrons are available as excess NAD(P)H which can be used to fix the small amount of carbon dioxide produced by other metabolic processes. The overall reaction scheme is therefore:

Light	→	ATP
Succinate + NH_4^+	→	Biomass + NADH + CO_2 (small quantities)
NADH + CO_2	→	Biomass
NADH + H^+	→	H_2 (if excess NADH is available)
Succinate + NH_4^+ + Light	→	Biomass + H_2

Under chemoheterotrophic conditions, succinate serves as the sole carbon, electron and energy source. Some of the succinate must be oxidized to carbon dioxide to generate proton motive force for ATP synthesis, and the remainder is available for biomass incorporation. The overall reaction scheme is therefore:

Succinate + O_2	→	ATP + CO_2
Succinate + NH_4^+	→	Biomass + NADH + CO_2 (small quantities)
NADH + CO_2	→	Biomass
Succinate + NH_4^+ + O_2	→	Biomass + CO_2

To test these predictions, *Rhodobacter* biomass yield on succinate and the complex components of YCC under chemoheterotrophic and photoheterotrophic conditions was compared. As predicted, biomass yield was nearly fourfold higher for photoheterotrophic conditions versus chemoheterotrophic conditions.

Cell yield on oxygen as the limiting substrate was also quantified. To adjust the concentration of available oxygen, the liquid culture volume in glass anaerobic culture tubes was varied prior to sealing (Figure 3). *Rhodobacter sphaeroides* cell yield on oxygen was calculated to be 1.2g dioxygen per gram of accumulated dry cell mass.

CHAPTER III

GROWTH KINETICS OF *RHODOBACTER*

The observed growth rate of a culture of cells is proportional to the number of cells in the culture and the rate at which those cells divide, i.e. the specific growth rate:

$$\dot{X} \equiv X * \mu_g, \mu_g\ [\equiv] time^{-1} \qquad \text{Eq (1)}$$

The specific growth rate is not a constant, but is a function of the strain and physiological state of the cells and of the environmental conditions:

$$\mu_g \equiv f(organism, T, S, [S], \dots) \qquad \text{Eq (2)}$$

The Monod growth kinetic approximation models a situation in which one substrate 'S' is the limiting factor for growth. This approximation partitions μ into an explicit functional dependence on substrate concentration and two fitting parameters, μ_{max} and K_S:

$$\mu_g \equiv \mu_{g:S,max} * \frac{[S]}{K_S + [S]} \qquad \text{Eq (3)}$$

For a given organism-substrate pair under defined environmental conditions, $\mu_{g:S,max}$ is equivalent to the maximum achievable specific growth rate, while K_S is equivalent to the substrate concentration at which μ_g is one-half the maximal value and accounts for the increased difficulty with which cells take up and metabolize the substrate as its concentration decreases.

The total substrate consumption rate of biomass can be subdivided into the fraction necessary to support the production of additional biomass and the fraction necessary to maintain existing biomass:

$$\dot{S} = \dot{S}_{growth} + \dot{S}_{maintenance} \quad \text{Eq (4)}$$

Substrate consumption rate for growth is related to cellular growth rate by $Y_{X,S}$, the theoretical yield of cells on substrate:

$$Y_{X,S}[\equiv] \frac{mass, cells\ produced}{mass, substrate\ consumed} \quad \text{Eq (5)}$$

$$\dot{S}_{growth} = \left(\frac{1}{Y_{X,S}}\right) * \frac{dX}{dt} \quad \text{Eq (6)}$$

$$\dot{S}_{growth} = \left(\frac{\mu_g}{Y_{X,S}}\right) * X \quad \text{Eq (7)}$$

Substrate consumption rate for maintenance is equivalent to the product of cell mass and a substrate- and organism-specific maintenance coefficient, $m_{X,S}$:

$$\dot{S}_{maintenance} = m_{X,S} * X \quad \text{Eq (8)}$$

$$m_{X,S}[\equiv] \frac{mass\ substrate\ /\ time}{mass\ cells} \quad \text{Eq (9)}$$

The substrate-specific maintenance coefficient can be generalized to a substrate-independent specific maintenance rate by division with a substrate-dependent maintenance yield:

$$m_{X,S} = \frac{\mu_m}{Y_{m:X,S}} \quad \text{Eq (10)}$$

$$\mu_m[\equiv] time^{-1} \quad \text{Eq (11)}$$

$$Y_{m:X,S}[\equiv] \frac{(mass, cells\ maintained)}{(mass, substrate\ consumed)} \quad \text{Eq (12)}$$

$$\dot{S}_{maintenance} = \left(\frac{\mu_m}{Y_{m:X,S}}\right) * X \quad \text{Eq (13)}$$

$$\dot{S} = \left(\frac{\mu_g}{Y_{X,S}}\right) * X + \left(\frac{\mu_m}{Y_{m:X,S}}\right) * X \quad \text{Eq (14)}$$

If it is assumed that the cellular processes that utilize substrate for maintaining and producing biomass proceed at a fixed efficiency ratio regardless of substrate, then $Y_{m:X,S} = Y_{X,S}$ and the expression simplifies:

$$\dot{S} = \left[\frac{\mu_g + \mu_m}{Y_{X,S}}\right] * X \quad \text{Eq (15)}$$

In this situation, the observed growth rate $\mu_{obs:g}$ and yield $Y_{obs:X,S}$ are less than the theoretical rate μ_g and yield $Y_{X,S}$ due to the biomass maintenance requirements:

$$\mu_{obs:g} = \mu_g - \mu_m \quad \text{Eq (16)}$$

$$Y_{obs:X,S} = Y_{X,S} * \frac{\mu_g - \mu_m}{\mu_g} \quad \text{Eq (17)}$$

For growth in a batch system, this series of approximations yields a sigmoidal growth curve. Cell growth is exponential when $[S] >> K_m$, eventually decreasing to zero as $[S] \rightarrow 0$.

When $\mu_g >> \mu_m$, the observed rate and yield are equivalent to the theoretical values; this is often a reasonable assumption for microorganisms that divide in minutes or

hours, in which substrate consumption is dominated by that required for biomass growth processes. $Y_{X,S}$ can be estimated from endpoint growth experiments at different starting substrate concentrations, and $\mu_{g:S,max}$ and K_m can be estimated from batch growth curves. If μ_m cannot be neglected, $Y_{X,S}$, $\mu_{g:S,max}$, and K_m must be determined by operating a chemostat and finding steady-state [X] and [S] at different dilution rates.

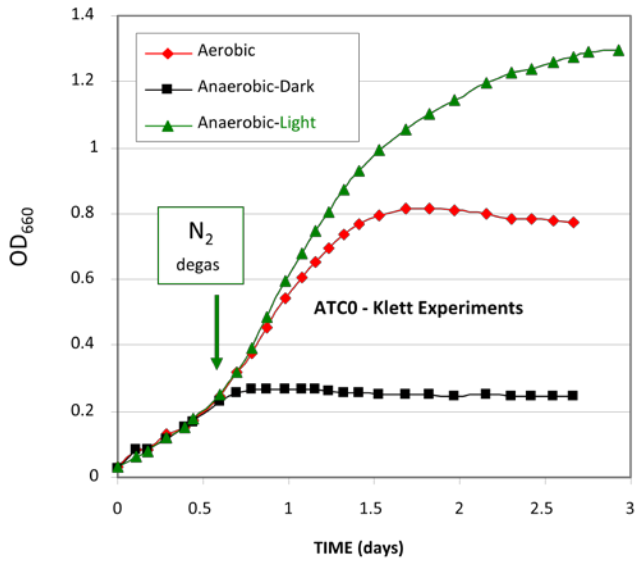

Figure 2 - Comparison of anaerobic and aerobic growth

A series of experiments were performed to evaluate *Rhodobacter* growth kinetics under chemoheterotrophic and photoheterotrophic conditions. One of the most informative growth comparisons is shown in Figure 2, which compares aerobic chemoheterotrophic, anaerobic chemoheterotrophic and anaerobic photoheterotrophic growth. Chemoheterotrophic growth in the dark was halted when the culture headspace was flushed with N_2. Although the cells do not replicate, they remained viable for several days. The anaerobic culture in the light grew to a significantly higher optical density than the aerobic culture, suggesting a yield enhancement in this growth regime. This difference can be explained in terms of the greater efficiency of photoheterotrophic growth on a carbon basis, due to the additional chemical energy harvested from light capture.

The multiplex growth apparatus (see Materials and Methods) enabled the aerobic/anaerobic culture transition to be investigated in detail. By filling the sealed culture tubes with different volumes of culture and thereby changing the headspace volume, the cell density and rate at which the transition occurs could be varied. This is depicted in the growth curves of Figure 3, where the headspace air volume was varied from nearly zero to 89% (the minimum liquid volume necessary to measure optical density).

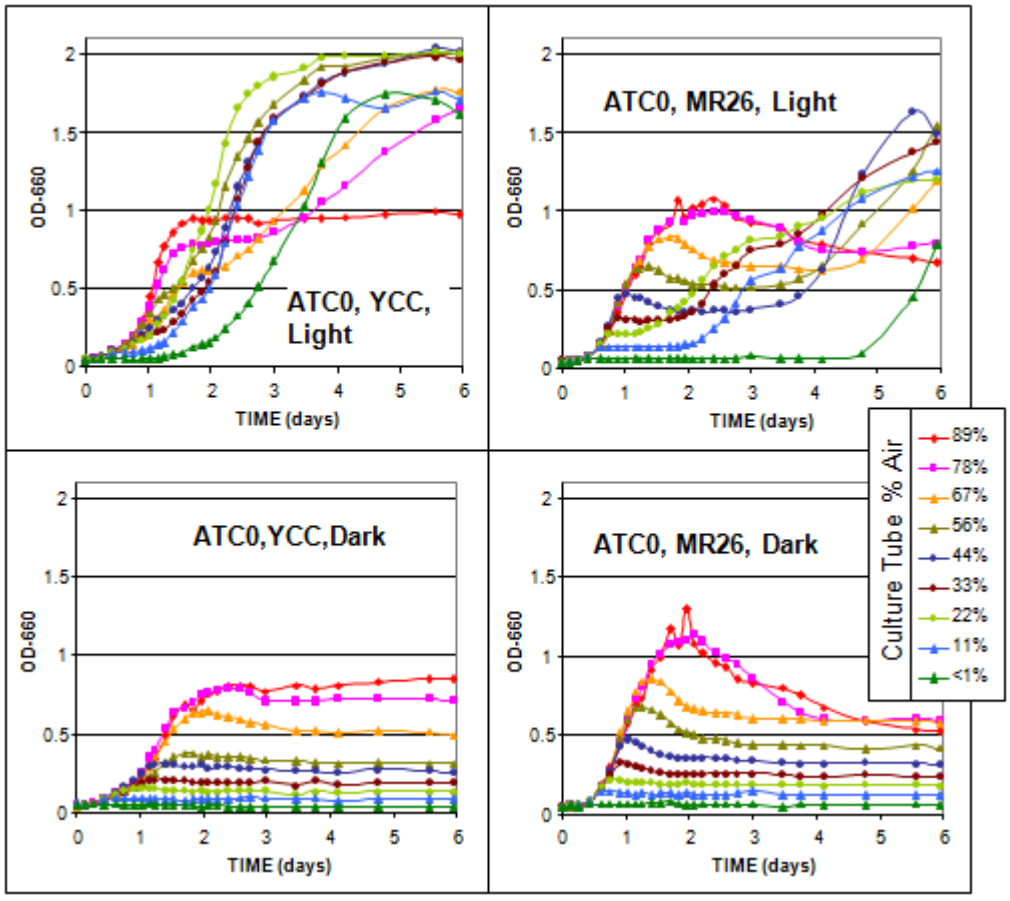

Figure 3 - Assessment of growth under aerobic/anaerobic, light/dark conditions

The results of this experiment confirm the previous observation of a dramatically improved growth yield for photoheterotrophic conditions. The difference in dry weight is more significant than the difference in optical density would suggest, due to saturation of the spectrophotometric measurement above the linear range. The upper left panel (ATC0 = wild type *R. sphaeroides* ATCC 2.4.1) illustrates interpretation: for large headspace volumes, the culture remains aerobic the entire culture period; this results in a substantially reduced aerobic heterotrophic yield. As the headspace volume decreases, overall yield increases substantially as a larger fraction of the substrate is consumed under photoheterotrophic conditions. In contrast, the growth curves of the lower left panel were performed in the dark; upon oxygen depletion, growth halted. In this situation, larger headspace volumes allowed the chemoheterotrophic consumption of a greater fraction of the available substrate, increasing observed culture growth.

CHAPTER IV

MOLECULAR METHODS IN *RHODOBACTER*

Molecular techniques for *Rhodobacter* species are much less well-developed than those available for traditional production organisms. Different strains of *Rhodobacter* produce a variety of restriction enzymes that make the introduction of DNA containing targeted sites impossible with standard techniques such as electroporation (*13*). Plasmids must instead be re-engineered to remove cut sequences or delivered by conjugation with a suitable donor bacterium.

Enzyme	**Sequence** (*14*)	**Strain** (*15*)
RshI (*16*)	(5’ - CGAT || CG)	2.4.1
RsrI (*17*, *18*)	(5’ - GAA || TTC)	RS630
RsaI (*19*)	(5’ - GT || AC - 3’)	RS28/5
RsrII (*20*)	(5’ - CG || G(A,T)CCG - 3’)	RS630, SE-12
RshII (*21*)	(5’ - CC(C,G)GG - 3’)	2.4.1

Table 1 - Restriction Enzymes in *Rhodobacter sphaeroides*

While conjugation is reasonably efficient, the process takes several days and methods must be developed for purifying the *Rhodobacter* transformants from the untransformed and donor cells. Most plasmids used in *Rhodobacter* are broad host range derivatives that exhibit very poor stability in the absence of selection pressure. The historical choice of selection pressure for these plasmids can also be problematic if the xenobiotic is not stable under the wide range of environmental growth conditions accessible by *Rhodobacter*.

The stability of the expression plasmid developed by Argonne National Labs for membrane protein production was evaluated and a method for conjugative transfer of plasmids into *Rhodobacter* was refined. *Rhodobacter sphaeroides* containing pRKPLHT1Dpuf and pRKPLHT7 were grown chemoheterotrophically or photoheterotrophically for five doublings, with or without 1μg/mL tetracycline. These cells were then plated on YCC with or without 1μg/mL tetracycline, and the number of colony-forming units per unit volume was compared to assess plasmid loss.

After five doublings, chemoheterotrophic cultures experienced over 80% plasmid loss, while phototrophic cultures were completely cured of plasmid. The difference may be attributable to the more rapid rate of growth under phototrophic conditions. This indicates that the vector's native degree of stability in *Rhodobacter* is extremely low, requiring selection pressure to prevent rapid plasmid loss.

Plasmids were electroporated into *E. coli* S-17 λ-pir and transferred into *Rhodobacter sphaeroides* and *Rhodobacter capsulatus* by conjugation. The mating mixture was then plated. The S-17 strain is a proline auxotroph, so initial attempts encouraged *Rhodobacter* growth whilst passively inhibiting *E. coli* by plating on MR26. Success was mixed - *Rhodobacter* exconjugates could take 5-7 days to appear, and had to be further purified by restreaking on YCC to avoid unintentionally carrying dormant *E. coli* into the cryopreserved glycerol stocks. A later technique made use of an MR26-based proline dropout medium to speed growth of *Rhodobacter*, but the medium was time-consuming and expensive to prepare, and exconjugates still had to be restreaked as above.

An alternative method, utilizing the host specificity of an *E. coli* T4r rapid-lysis bacteriophage and the native resistance of *Rhodobacter* to toxic heavy metal anions, was developed.

Rhodobacter was verified to be resistant to sodium tellurite at concentrations up to 200μg/mL, as mentioned in the literature (*22*). While the growth of *E. coli* was confirmed to be inhibited at sodium tellurite concentrations above 2μg/mL (*23*), preliminary testing revealed that *E. coli* mutants resistant to T4r or tellurite (up to 100μg/mL) could often arise when only one of these selection methods was used; combining them, however, proved to be rapidly and completely lethal to *E. coli* donor cells. 50μL of high-titer T4r phage was added to the mating mixture resuspension medium, and the resuspended mating mixture was streaked on YCC plates containing 10μg/mL of sodium tellurite.

With a plasmid selection pressure of 5ug/mL tetracycline on YCC plates, *Rhodobacter* exconjugates typically appeared within 2-4 days. Colonies were initially colorless and turned red as they grew, darkening as tellurite was intracellularly reduced to black tellurium. Overall transformation efficiency was not explicitly determined, but it was sufficiently high to yield dozens of exconjugates per attempt.

CHAPTER V

PHOTOTROPHY IN *RHODOBACTER*

Rhodobacter species are capable of capturing and converting light energy to chemical energy via a cyclic electron transfer mechanism that builds a proton motive force for ATP synthesis. This process is anoxygenic and does not involve the splitting of water, bearing some similarity to the cyclic electron transport mode of Photosystem I in green plants. Unlike green plant photosynthesis, *Rhodobacter* may only grow phototrophically under anaerobic conditions. Light capture, charge separation, and the initial electron transfer steps occur in the photosynthetic apparati, embedded within infoldings of the outer membrane formed when conditions are conducive to phototrophy.

The photosynthetic complexes in *Rhodobacter* consist of a reaction center (RC) and two light-harvesting complexes (LH1 and LH2, also known as B875 and B800-850 after their absorbance maxima). The core complex is composed of an RC dimer ringed by ~28 LH1; the ring is intercalated by the PufX protein, which ensures that the RC is not completely surrounded and allowing for quinone exchange (*24*). Two of the bacteriochlorophylls are in direct contact, forming the 'special pair' that carries out photochemistry. The X-ray crystal structure of the *R. sphaeroides* RC is available (*25*). A complete crystal structure of LH1 is not available, but an NMR solution structure is available for one of the polypeptide subunits (*26*), and LH1 has been studied in the core complex by cryoEM and AFM.

The RC, LH1, and LH2 are composed of the following elements:

RC	**LH1**	**LH2**
(3) polypeptides (L,M,H)	(2) polypeptides	(2) polypeptides
(4) bacteriochlorophyll a	(2) bacteriochlorophyll	(2) bacteriochlorophyll a
(2) bacteriopheophytin a	(2) carotenoids (SO)	(1) bacteriochlorophyll a
(2) ubiquinone		(1) carotenoid (SE)
(1) iron atom		
(1) carotenoid (SE)		

Table 2 - Photosystem composition

The LH2 protomers are composed of an alpha/beta polypeptide heterodimer that sandwiches a collection of bacteriochlorophylls and carotenoids. In *Rhodobacter sphaeroides*, the LH2 complex is a nonamer similar to that in *Rps. acidophila*. A crystal structure is available (*27*). While the RC:LH1 ratio is constant, LH2 organizes itself into clusters surrounding the core complex on an 'as-needed' basis to capture more light under low-light conditions (*28*). In the absence of LH1, LH2 is capable of directly transferring energy to the RC (*29*).

Carotenoids associated with the photosynthetic complex increase the spectral cross-section of the system by absorbing at shorter wavelengths that are poorly captured by bacteriochlorophylls, in addition to acting as antioxidants to protect from photoautooxidation. Carotenoid and bacteriochlorophyll biosynthesis are almost entirely inhibited under highly-oxygenated conditions (*30*), likely by the redox-sensitive RegA/RegB global regulators and the CrtJ transcriptional repressor (*31*).

Spheroidene (SE, yellow) and spheroidenone (SO, red) are the two major carotenoids in wild-type *Rhodobacter sphaeroides* and *R. capsulatus* (*32, 33*). Their interconversion depends on a monoxygenase and the presence of molecular oxygen, and is responsible for the switch from the dark red aerobic form to the yellow-brown

anaerobic photoheterotrophic form. The SE:SO ratio in LH1 is less than 1:1, giving it a red color; the SE:SO ratio is ~13:1 in LH2, giving it a yellow-brown color.

SE-SO interconversion has been shown to be regulated by light intensity and oxygen tension. Adding strong oxidizing agents such as tellurite to the growth medium also cause a shift toward SE, indicating that the conversion is redox state-controlled. Conversion also seems to be driven by a 'mass action' effect, where formation of LH1 or LH2 complexes encourages the synthesis of more of the appropriate carotenoid (*34*).

Significant work was performed in the 1950's, 60's, and 70's to characterize the physiology of *R. sphaeroides* carotenoid biosynthesis mutants as part of a larger effort to understand the mechanism of phototrophism (*30*, *35*). By analyzing the pigment content and growth habits of the mutants, details of the carotenoid biosynthesis pathways and the role of carotenoids in the light-capture process were elucidated (*35*).

In a production photoreactor, increasing cell density reduces light penetration into the culture, inducing *Rhodobacter* to increase the expression of LH2. This further increases the optical density of the culture and reduces light penetration, reducing the volumetric productivity of the system. Genetic engineering and reactor design strategies were explored for remedying this situation.

The cellular optical density / dry weight ratio (OD:DW) can be manipulated by knocking out accessory pigments, improving light penetration. Carotenoids cannot be knocked out due to their role in preventing photooxidation (*36*), but LH2 can be removed while retaining phototrophic competency. *Rhodobacter sphaeroides* PUC705-BA, a strain lacking LH2, was prepared for membrane protein expression by ANL (*37*).

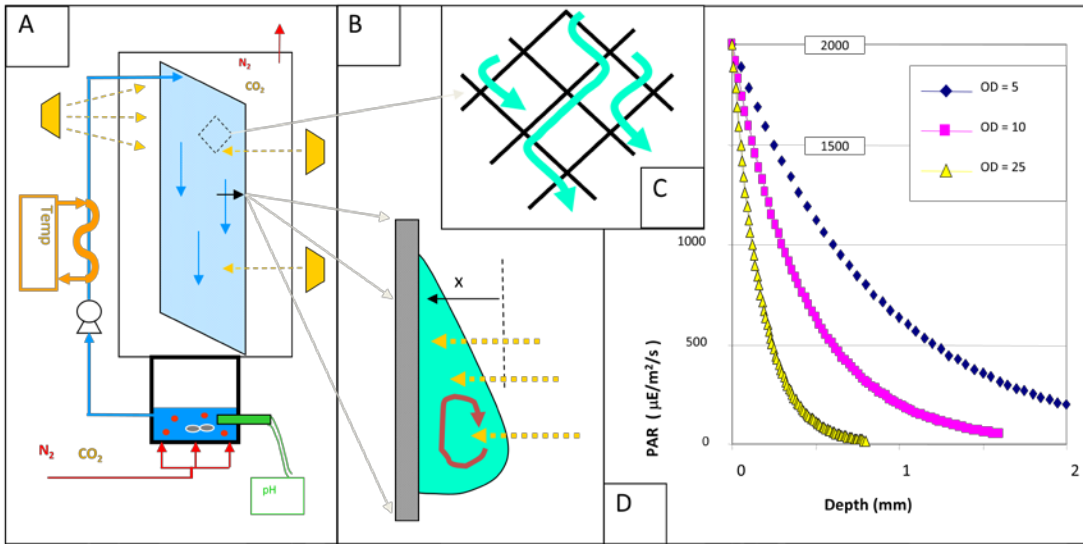

Figure 4 - Photobioreactor system used to examine high density growth

The bioreactor engineering strategy is depicted in Figure 4. For the purposes of photoheterotrophic growth, light can quickly become limiting at high density if the light path of the culture is too long. In panel A, the solution to obtaining a thin film is to run the culture down a screen. Panels B and C depict the flow behavior within a falling film. In addition to providing a thin light path, there is turbulent mixing of the fluid as it falls down the screen due to drag of the fluid as it flows (B) as well as changes in momentum as it traverses the screen (C). Using the Beer-Lambert law for estimation of light attenuation, the final panel (D) provides a quantification of the attenuation of light at several cell densities. An OD=5 (pink line) is moderate and corresponds to a dry cell concentration of less than 5 grams dry weight per liter (gDW/L), and densities approaching OD=25 (yellow line) are far more reasonable for a production system. At very high densities, light penetration of significant intensity is limited to less than 1mm.

This analysis frames the tremendous challenge of achieving high cell density culture in phototrophic systems.

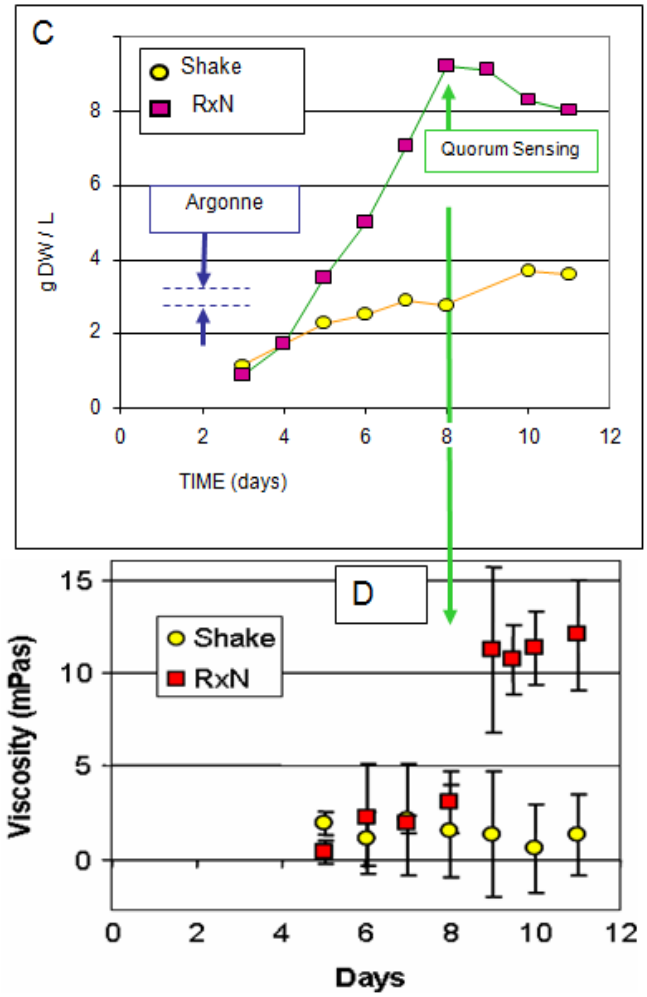

Figure 5 - Growth in Trickle Film Photobioreactor

Using the photoreactor described, *Rhodobacter sphaeroides* was grown to high density on pH-balanced batch feeds of succinate and ammonia. The results of the bioreactor run are shown in Figure 5 - the dry weight density reached 9.2 gDW/L (OD_{660}=19) on defined media containing succinate and ammonium This is nearly 20

times the batch growth reported by Argonne National labs on MR26 medium in simple flask culture.

Growth cessation occurred simultaneously with a seven-fold increase in culture viscosity that was not associated with a change in viable count, suggesting that polysaccharides or other polymers may have been secreted into the medium by the cells. The quorum sensing response described in *Rhodobacter* decreases cellular aggregation and extracellular polysaccharide production at higher densities, suggesting that the change occurred instead as a stress response.

An attempt was also made to grow *Rhodobacter sphaeroides* and *Rhodobacter capsulatus* photoheterotrophically on near-infrared radiation. It was hypothesized that a monochromatic light source operating near the photochemical excitation peak absorption band of the RC 'special pair' of bacteriochlorophyll molecules (870nm for *Rhodobacter sphaeroides* (*38*)) would most efficiently deliver light energy for phototrophic growth. 3-watt Infrared LEDs operating near 850nm were selected for this experiment. A single LED illuminating 10mL of culture in a sealed anaerobic tube was capable of maintaining growth rates similar to those observed under 400W high-intensity discharge lamps. This could allow the construction of small-scale photoreactors for research use that could be run on a small power supply with minimal requirements for heat dissipation. Delivery of IR light also avoids blue light photoinhibition of photosystem expression mediated by AppA (*39*).

CHAPTER VI

MEMBRANE PROTEIN EXPRESSION IN *RHODOBACTER*

The evaluation and optimization of heterologous membrane protein expression proceeded through a collaboration with Philip D. Laible and Deborah K. Hanson at Argonne National Laboratory (ANL), using pRK404-based expression plasmids developed there. These plasmids utilize the native *puf* and *puc* promoters, drivers of RC/LH1 and LH2 production that are controlled by light and oxygen levels. In addition to being among the most highly-expressed operons under phototrophic conditions, these promoters are also induced concurrently with the rapid intracellular membrane (ICM) expansion that occurs to make room for integration of the light-harvesting complexes. The availability of space in the membrane leads to improved yields of integrated, functionally-folded heterologous membrane proteins (*40*).

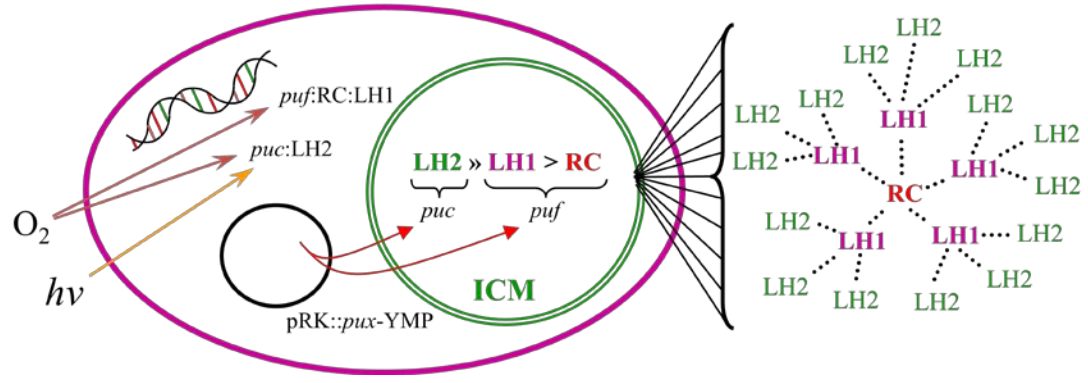

Figure 6 - Schematic representation of the genetic engineering strategy

To further improve yields, a strain of *Rhodobacter sphaeroides* 2.4.1 featuring a knockout of the LH2 operon was used, ostensibly with an even greater availability of space for heterologous membrane protein integration. In the schematic representation pRK::pux-YMP represents the pRK404-derived vector containing "Your Membrane Protein", with expression driven by either *puf* or *puc*.

The ANL production procedure relied on the induction of *puf* and *puc* under reduced oxygen tension in cultures grown chemoheterotrophically in the dark. The *puf* promoter was found to outperform the *puc* promoter, likely due to the lack of *puc* induction in the absence of light. It was hypothesized that the much stronger induction of the promoters under photoheterotrophic conditions could enable significantly higher expression levels.

Several constructs expressing a non-native cytochrome-c_y were grown in Klett flasks under anaerobic photoheterotrophic conditions; the Western blot quantification results provided by ANL are presented along with their associated growth curves in Figure 7.

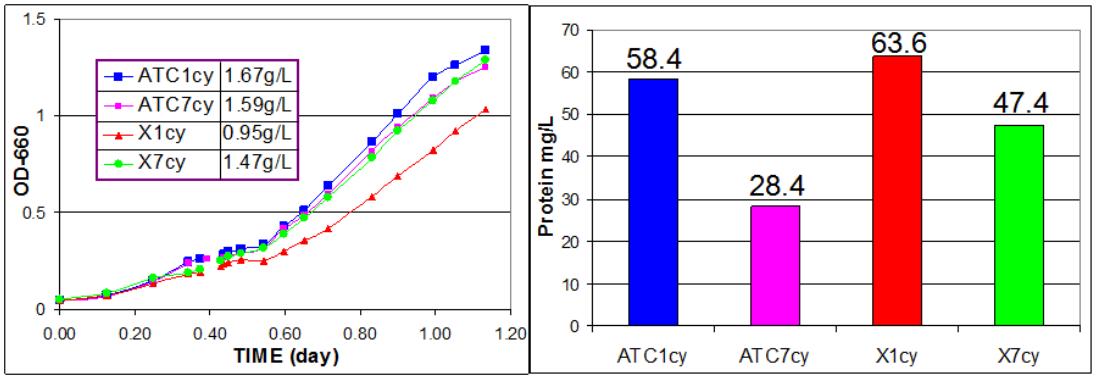

Figure 7 - Preliminary membrane protein production results

These results represent unoptimized productivities on the order of 60 mg/L/day in shake flasks. Noting that productivity of these shakes flasks is less than 1 gDW/L, this corresponds to roughly 70 mg protein/gDW, or on the order of 7% of the biomass consisting of the heterologous protein expressed. If these productivities are extrapolated to the demonstrated potential for nearly 10 gDW/L in the trickle-film photobioreactor (Figure 4), this suggests that ~500 mg/L of membrane protein can be expressed with this system.

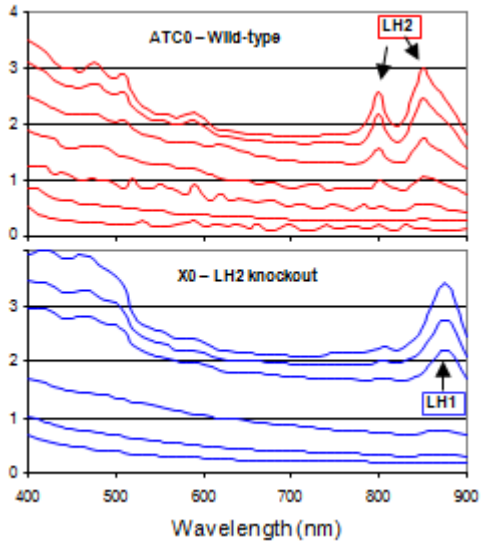

Figure 8 - Absorption spectra timecourses for phototrophic growth

Additional insight into promoter induction and photosystem regulation was gleaned from the absorption profile timecourses. In Figure 8, the time course of the absorption spectra was obtained during batch growth under anaerobic photoheterotrophic conditions for wild-type and LH2-knockout strains of *R. sphaeroides*. While the LH2 absorption peaks are clearly evident in this figure for the wild-type, there seems to be a compensating effect for the LH2 knockout in the form of increased LH1 expression levels. This hints at the complexity of the underlying photosystem regulatory mechanisms.

It was also noted that the optical density at 660nm was a function only of *Rhodobacter* cell concentration, uninfluenced by the extent of pigmentation. This suggests that the ratio of optical density at 660nm and 800/850/875nm may provide a convenient way to monitor the induction of the *puf* or *puc* operons. The plot of this ratio with time in Figure 9 forms a basis for quantifying the degree of heterologous induction, without requiring any physical manipulation of the growing culture.

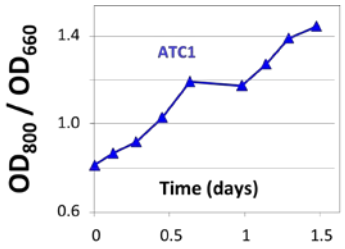

Figure 9 - Absorption ratio for bioreactor monitoring

CHAPTER VII

REDOX REGULATION IN *RHODOBACTER*

A cellular quinone pool acts as an electron transfer intermediate under both phototrophic and chemotrophic conditions. Ubiquinone can be reduced to ubiquinol directly by the RC in the presence of light, by hydrogenase in the presence of dihydrogen, or via the reoxidation of NADH produced from the oxidation of a reduced organic substrate. Ubiquinol shuttles its electrons to an electron transport chain that generates proton motive force and terminates in the reduction of oxygen or another suitable acceptor such as DMSO (chemotrophic) or return of the electron to the RC (phototrophic). Under environmental conditions in which oxygen or another oxidant is plentiful, the quinone pool becomes oxidized; when oxidant is scarce or reductant is plentiful (phototrophic growth on a reduced substrate; lithotrophic growth on hydrogen), the quinone pool becomes reduced. In this way, the redox state of the quinone pool reflects the redox state of the cell (*41*).

As illustrated by Dr. Samuel Kaplan, most the genes encoding the polypeptides and the chorophyll- and carotenoid-synthesizing enzymes responsible for construction of the phototrophic apparatus are contained in a single gene cluster (*42*). As illustrated by Carl Bauer, expression of this gene cluser is controlled primarily by two redox-sensing systems: a two-component global redox regulator (RegB/RegA in *R. capsulatus*, PrrB/PrrA in *R. sphaeroides*); and a redox-sensitive transcriptional repressor (CrtJ in *R. capsulatus*; PpsR in *R. sphaeroides*) (*43*).

RegB is a membrane-bound sensor kinase that regulates the expression of genes involved in phototrophy and other oxygen-sensitive processes by transducing the redox state of the quinone pool to the activation state of a transcriptional response regulator, RegA. RegB autophosphorylates and transfers the phosphate to RegA, activating it as a transcriptional regulator. RegA then upregulates genes involved in phototrophy, and downregulates genes involved in aerobic growth. This process is mediated by the interaction of RegB with ubiquinone and ubiquinol; ubiquinone binding inhibits RegB autophosphorylation, preventing the activation of RegA (*44*). This is the mechanism by which genes involved in phototrophism are repressed during aerobic chemoheterotrophic growth, and also explains the observation that photolithotrophic cultures grown on H_2 become pigmented due to light-harvesting complex production even in the presence of oxygen (*8*).

CrtJ is a transcriptional repressor that acts on genes involved in phototrophism, including those responsible for bacteriochlorophyll and carotenoid biosynthesis, preventing their transcription. It is also redox-sensitive, and its DNA binding affinity decreases when a disulfide bond is reduced. An additional light-sensitive antirepressor, AppA, is present in *R. sphaeroides* but not *R. capsulatus*. AppA catalyzes breakage of the disulfide bond, encouraging phototrophic gene expression, but is inactivated by blue light excitation of a flavin moiety. The result is that strong blue light actually represses phototrophic gene expression (*45*).

In a chemotrophic production system, expression of light-harvesting complexes is an unnecessary metabolic burden on the cell that diverts biosynthetic intermediates away from the desired product. Light-harvesting complex production may still occur in the

presence of oxygen if the cellular redox state tends toward oxidation, which may occur under oxygen mass transfer limitation of high-density aerobic cultures or under lithoautotrophic growth on dihydrogen. Under these conditions, product yields may be improved by reducing or eliminating light-harvesting complex expression.

Sistrom et al isolated a rare class of colorless *Rhodobacter* mutants that do not appear to synthesize bacteriochlorophyll or any carotenoids, even lacking the colorless carotenoid precursors found in most other mutants (*46*). While other carotenoid mutants were highly vulnerable to photoautooxidation in the simultaneous presence of light and oxygen, these mutants also lacked bacteriochlorophyll and therefore could not be photosensitized. These mutants exhibited a high degree of reversion, suggesting a single point mutation disrupted the biosynthesis of both classes of compounds. It is likely that the RegB-RegA redox-sensing system was defective in these mutants, preventing transcriptional upregulation of phototrophic genes. Knocking out this two-component system in a production organism would effectively disable the phototrophic machinery.

Beyond phototrophism, activated RegA has also been demonstrated to upregulate carbon fixation pathways in *R. sphaeroides*, and to indirectly activate nitrogenase synthesis and repress hydrogenase synthesis in *R. capsulatus* (*47*). PrrB knockouts of *Rhodobacter sphaeroides* are still capable of growing phototrophically at high light intensities, whereas PrrA knockouts are completely unable to grow phototrophically (*31*).

CHAPTER VIII

ONGOING AND FUTURE WORK

The full kinetics and thermodynamics of the numerous *Rhodobacter* growth regimes remain to be quantified. Detailed study of the pathways involved in the oxidation of organic substrates to carbon dioxide for ATP production, coupled with gas-phase analysis of actively growing cultures to quantify the rate of CO_2 or H_2 evolution under photoheterotrophic and chemoheterotrophic growth on more reduced (e.g. butyrate) or less reduced (e.g. malate) substrates, could provide quantitative insight into the cellular redox state under various conditions. Chemostat studies would enable the comparison of yields and maintenance rates of several *Rhodobacter* species grown on different substrates, allowing selection of the species and growth conditions most suitable for the efficient production of monomers and biofuels.

Plasmids native to *Rhodobacter* that do not require selection pressure for stable maintenance have been described. Recently, a vector derived from a highly-stable plasmid native to *Rhodobacter blasticus* has been created and used for heterologous expression in *Rhodobacter sphaeroides* (*48*). This vector makes use of kanamycin resistance for highly robust selection that is not photosensitive, and may therefore be ideal for phototrophic expression.

While conjugation can be used to successfully deliver plasmid DNA to *Rhodobacter* species, it is still significantly more complicated and time-consuming than electroporation. Other workers have reported limited success with electroporation using

plasmids that have been re-engineered to avoid native restriction enzyme cut sites (*13*), but this is a resource-intensive solution . A gene knockout technique for *Rhodobacter* species that uses the Cre-Lox recombination system has recently been described (*49*); it may be possible to use this system to knock out the native restriction enzymes in *Rhodobacter*, improving the viability of electroporation for delivering DNA.

Under chemoautotrophic growth conditions, product yields may be improved by reducing or eliminating light-harvesting complex expression. It was demonstrated (*50*) that RegA insertional mutants produce almost no detectable photopigments, exhibit RUBISCO activities similar to wild-type strains, and are fully capable of chemoautotrophic growth. RegA (PrrA) knockouts may exhibit improved yield under chemoautotrophic growth conditions, and the kinetics and thermodynamics of growth of this mutant should be explored.

The membrane protein expression strategy developed at Argonne National Labs and optimized in this lab is being explored for the heterologous production of human membrane proteins. A series of medically-relevant targets of increasing complexity (i.e. number of transmembrane regions) has been selected in collaboration with the research group of Dr. Fang Tian at Penn State Hershey Medical Center. The proteins will be analyzed structurally using Residual Dipolar Coupling Nuclear Magnetic Resonance spectroscopy. This system is also being evaluated for the mass production of aquaporins in collaboration with Dr. Manesh Kumar at Penn State University.

CHAPTER IX

CONCLUSION

Rhodobacter was evaluated as a potential alternative biotechnological production host. *Rhodobacter* species exhibit substantially more metabolic diversity than traditional expression hosts, enabling bioprocess designs utilizing a wide range of substrates and environmental conditions that are inaccessible with current methods. Physiological traits of *Rhodobacter*, such as an extensive, inducibly-expandable internal membrane, can provide unique biological functionality for the production of complex products such as membrane proteins. While the molecular tools for manipulating *Rhodobacter* are not as well-developed as those for traditional expression hosts, robust methods have been developed for the engineering of *Rhodobacter* species. While significant opportunity exists for the improvement of *Rhodobacter* as a production host, it offers unique advantages over traditional hosts that may be well-suited to specialized bioprocessing applications.

CHAPTER X

MATERIALS AND METHODS

Experimental procedures used in the above research are described.

Bacterial Strains

Rhodobacter sphaeroides used in this study is wild-type ATCC 17023 strain 2.4.1, provided by Philip Laible and Deborah Hanson at Argonne National Laboratory. The generation of the LH2 knockout strain PUC705BA is described elsewhere in detail (*40*). *Rhodobacter capsulatus* is wild-type strain SB1003, provided by Dr. Carl Bauer at Indiana University. *E. coli* DH5alpha and S17 lambda-pir were used for plasmid cloning and conjugation, respectively.

Growth Media

YCC complex medium was used for general maintenance of *Rhodobacter* and *E. coli* cultures; MR26+ ammonium succinate defined medium was used for quantitative *Rhodobacter* growth experiments. These media formulations are based on growth media used by Argonne National Laboratory (Deborah Hanson, personal communication):

Media Component	**[Stock]**	**Added to 1L**
Yeast Extract		5 g
Casamino Acids		6 g

YCC Base Concentrate		5 mL
Na2EDTA·2H2O	11.82 g/L	
CuSO4·5H20	0.04 g/L	
CoCl2·6H20	0.03 g/L	
(NH4)6Mo7O24·4H20	0.02 g/L	
MgSO4·7H2O	40.12 g/L	
FeSO4·7H2O	0.75 g/L	
H3BO3	0.0125 g/L	
Ca(NO3)2·4H2O	6.9 g/L	
The solution is brought to 1L with distilled water and pH-adjusted to 7. *The medium is autoclaved for 25 minutes at 121C, cooled, and stored aseptically.*		

Table 3 - YCC media formulation

Media Component	**[Stock]**	**Added to 1L**
Ammonium Succinate		2.36 g
MR26 Phosphates		20 mL
K2HPO4 (dibasic)	115 g/L	
KH2PO4 (monobasic)	44.9 g/L	
MR26 Micronutrients		1 mL
ZnSO4·7H20	10.9 g/L	
MnCl2·4H20	1.3 g/L	
CuSO4·5H20	0.392 g/L	
CoCl2·6H20	0.2 g/L	
(NH4)6Mo7O24·4H20	0.186 g/L	
H3BO3	0.114 g/L	
Fe-EDTA Solution		2.5 mL
Fe-EDTA·2H20	4 g/L	
Rhodobacter sphaeroides has a growth requirement for sodium, optimally 4mM. *Rhodobacter capsulatus does not have this requirement.*		
NaCl		0.234 g
The solution is brought to 1L with distilled water *The medium is autoclaved for 25 minutes at 121C and allowed to cool* *The following solutions are then added aseptically, reducing precipitation:*		
Magnesium Solution		1.205mL
MgSO4·7H20	493 g/L	
Calcium Solution		0.45mL
CaCl2·2H20	150 g/L	
Vitamin Stock		1mL
Nicotinic Acid	3 g/L	
Nicotinamide	3 g/L	
Thiamine-HCl	6 g/L	
Biotin	0.12 g/L	

Table 4 - MR26+ media formulation

Culture Storage and Seed Train

Cultures were stored long-term by mixing high-density late-log cultures 1:1 with a sterile 50% glycerol solution. The mixture was flash-frozen in liquid nitrogen and stored at -80C. Seed cultures for experiments were started by pressing the end of a sterile micropipette tip into the cryopreserved glycerol stock and rinsing gently by pipetting up and down in a 25mm glass culture tube containing 2mL of the growth medium of choice. The seed culture was incubated at 34C under agitation on a gyratory shaker in the dark, and in log phase used to inoculate a second seed culture incubated under the environmental conditions of the experiment.

Growth Apparatus

In the course of experimentation, growth studies were carried out in a variety of apparatus that ranged from simple shake flasks to a sophisticated trickle-film photobioreactor. A major constraint in executing growth experiments is control of the gas phase composition. For aerobic chemoheterotrophic growth, a shake flask is often assumed to provide sufficient aseptic gas exchange with the surrounding environment to allow for continuous oxygen addition and carbon dioxide removal. Growth under anaerobic conditions requires more explicit control. Cultures can be started anaerobically by gas-phase purging or liquid-phase sparging

Figure 10 - Klett Flasks used for growth study

with dinitrogen or argon, or may be allowed to transition due to their own metabolic activities in a sealed vessel. Sealed cultures may accumulate carbon dioxide or dihydrogen, depending on substrate availability and environmental conditions. Complete degassing was accomplished by purging the culture headspace with dinitrogen for 15 minutes while vigorously agitating the liquid phase.

Klett flasks, as illustrated in Figure 10, are particularly versatile. They can be incubated on a gyratory shaker and sealed with silicone stoppers, while also allowing non-invasive measurement of optical density to monitor cell growth via a hollow glass projection that can be placed directly into a 1cm pathlength spectrophotometric cuvette holder.

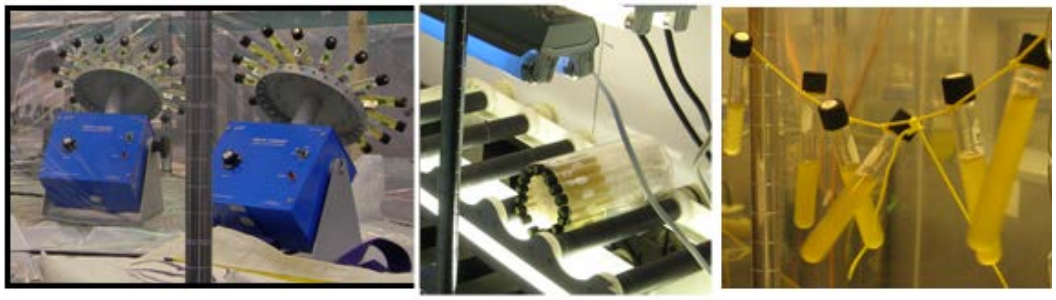

Figure 11 - Multiplexed growth apparatus

Figure 11 shows three additional configurations used to obtain multiple growth curves in sealed glass culture tubes. The rotator, left, rotates up to 16 culture tubes at up to 60 rpm; mixing and gas-liquid contacting is provided by the motion of the liquid in partially-filled tubes. Clamps were fabricated from aluminum channel and outfitted with hooks to allow simple attachment of culture tubes with rubber bands.

The roller bottle apparatus, center, provided for growth of tubes as the bottle revolved at ~10 rpm. By cutting apart a plastic roller bottle, large numbers of culture tubes could be placed within the periphery for light exposure.

Finally, the 'clothesline' configuration was developed to evaluate growth at very high light levels. Cultures were suspended a short distance from high-intensity discharge lamps, experiencing light intensities in excess of 1000 microE/m^2/s. The culture tubes were successively tied onto nylon masonry twine tethered to a gyratory shaker, causing mixing by a swinging motion.

Chemoheterotrophic Growth

Rhodobacter and *E. coli* cells were grown chemoheterotrophically under aerobic conditions. Mid-log seed cultures were centrifuged, rinsed with the desired growth medium, inoculated at 5% into a 25mm glass culture tube containing 2mL of growth medium, and the culture was incubated at 34^{o}C under agitation on a gyratory shaker. For growth under microaerobic conditions, 10mL of growth medium was used to reduce the surface area-to-volume ratio and increase oxygen demand rate without increasing supply rate. For chemoheterotrophic aerobic growth on solid media, agar was added at 8g/L prior to autoclaving.

Photoheterotrophic Growth

Rhodobacter cells were grown photoheterotrophically under anaerobic conditions. Mid-log seed cultures were centrifuged, rinsed with the desired growth medium, and inoculated at 5% into a 10mL anaerobic culture tube. Additional growth medium was

added to completely fill the culture tube, which was then sealed and incubated at 34C under illumination on a culture tube rotator.

Lighting Conditions

For photoheterotrophic growth, cultures were incubated under 4' 32W T-8 fluorescent lighting fixtures (~150W/m^2) or HID metal halide and sodium vapor lamps (~500W/m^2). For IR LED growth experiments, 3W 850nm infrared star LEDs were purchased from Shenzhen Xuancai Electronic Co. The LEDs were mounted to a section of sheet metal, and cultures were incubated in sealed anaerobic cultures tubes wrapped in a reflective aluminum foil tube and held upright directly over the diodes (~0.3W/m^3). The IR provided not only the light, but also mixing due to thermal gradients.

Plasmids

The vectors used in the membrane protein expression work are pRK404 derivatives, pRKPLHT1Dpuf and pRKPLHT7, provided by Philip Laible and Deborah Hanson at Argonne National Labs. pRK404 was derived from the naturally-occurring plasmid RK2, a conjugatable, broad host-range member of the P-1 incompatibility group (*51*). pRKPLHT1Dpuf features a promotor from the native puf operon, which encodes elements of the photosynthetic reaction center and light-harvesting complex 1; pRKPLHT7 features a promotor from the native puc operon, which encodes elements of light-harvesting complex 2 (*37*). Both plasmids also contain tetA, a tetracycline resistance gene encoding a proton antiporter of the Major Facilitator Superfamily that

utilizes proton motive force to pump tetracycline out of the cell. Cells containing these plasmids are maintained on 1-5μg/mL tetracycline.

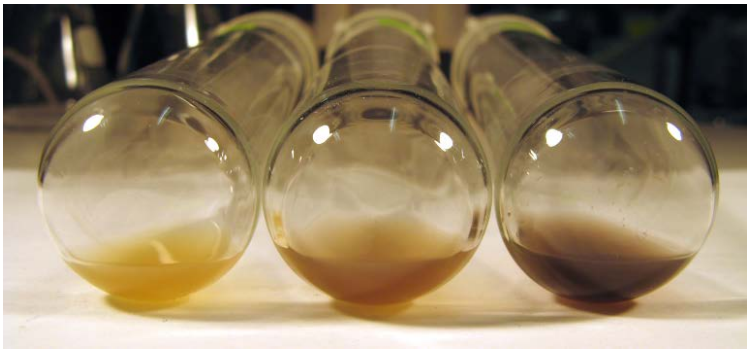

Figure 12 - Timecourse of biological tellurite reduction

Tetracycline

Tetracycline in powder form was purchased from Sigma-Aldrich. Stock solutions were prepared at 10mg/mL in 95% ethanol, agitated continuously at -20C for 24 hours to dissolve completely. Tetracycline stocks were stored long-term at -80C and short-term at -20C, and protected from light and oxygen whenever possible to prevent photooxidation to less-active forms that may also generate toxic reactive oxygen species (*52*).

Tellurite

Sodium tellurite was purchased from VWR Scientific. Stock solutions at 50mg/mL were prepared by filter sterilization and added aseptically to growth media prior to distribution. Autoclaving was avoided to avoid redox reactions with growth medium components, yielding insoluble black tellurium granules (Figure 12).

Bacteriophage

A high-titer stock (1x1011 plaque-forming units per mL) of the rapid-lysis phenotype of T4 coliphage, T4r, was purchased from Carolina Biological Supply Company. For the conjugation method utilizing T4r to selectively lyse *E. coli* donor cells, this stock was added 1:100 to mating mixture resuspensions and incubated for one hour at 30^{o}C prior to plating.

Cell Quantification Methods

Optical density was correlated to dry cell mass, and utilized as a rapid proxy for time-consuming direct determination by cell washing and lyophilization. *E. coli* was quantified by measurement at 600nm, while *Rhodobacter* was quantified by measurement at 660nm to avoid interference due to the light-harvesting pigments. Cell size (*3, 53*) and optical density to dry-weight ratios (OD:DW) (John Myers, personal communication) for *E. coli*, *Rhodobacter capsulatus*, and *Rhodobacter sphaeroides* are shown in Table 5.

Species	**Diam, Diam x Length, µm**	**OD:DW Ratio**
E. coli	0.5 x 1.0-3.0	3.0
R. capsulatus	0.5-1.2 x 2.0-2.5	1.8
R. sphaeroides	0.7-4.0	1.2
	2.0-2.5 x 2.5-3.5 µm (in sugar-containing media)	

Table 5 - Cell size and OD:DW ratio

Conjugation

The conjugation procedure was adapted from a method previously described (*13*). Mid-log cultures of plasmid donor and recipient cultures were centrifuged, washed, resuspended to a thick paste, mixed 100:1 (recipient:donor), and allowed to absorb into dried YCC agar plates (prepared by allowing plates to sit open in the laminar flow for half an hour after pouring). After incubation at 30C for 72 hours, the cells were recovered by overlaying the plates with 3mL of liquid medium of the same type used for the final plating, and vigorously agitating with a sterile stainless steel plate spreader. The resuspended mating mixture was then streaked with a sterile tungsten loop onto plates of the medium of choice.

Numerical Simulations

Numerical simulations of growth and substrate depletion were performed using Sage open source mathematics software (www.sagemath.org).

Thin-Film Photobioreactor

The implementation of this photobioreactor system is shown both as a photograph and a schematic in Figure 13. The basic reactor design strategy is to gravity flow the culture down a screen, illuminated and kept anaerobic within a transparent plastic bag.

The bag was composed of a very transparent plastic, most likely polyethylene copolymer, of dimensions 33.5" high and 29.5" wide and heat-sealed at the bottom. The bag was glued to a head plate with hot glue (Super Power Slow Setting, Arrow Fastener Company, Inc.; #B556-4), and a bead of silicone sealer (Chatham, Plumber's clear) was run along the top where the bag projected above the head-plate. Silicone sealer was also carefully placed in the 3/16" below the heat-sealed bottom and pressed smooth to provide an extra measure against leakage.

The liquid delivery to the trickle-screen was modeled after prior design for growth of cyanobacteria, but completely reconstructed to fit the 'head-plate' geometry required for this anaerobic application. The head-plate was snug-fit penetrated with 3/8" OD ridged white PVC pipe, sealed with silicone sealer and connected above and below with 90^{o} quick connect fittings (Watts Water Technologies, PL-3022). The liquid distributor was constructed as a tube-within-a-tube to even out flow; culture entering both ends was sprayed upward from the internal tube through small-diameter holes, to then roll down to an array of larger holes drilled in a 1/8" zig-zag array in the external tube to drain along the top of the screen. The head-plate was constructed from 0.25" thick, 8.25×22" Plexiglas acrylic (polymethyl methacrylate) to match the dimensions of the plastic bag. All drilling was done at 600-rpm using Aluminum cutting fluid to provide smooth bores (Relton, A-9). Corners were hung with stainless-steel hook-eyes (#8-32), bolts and

fender-washers. The bolt-nut-washer on the inside of the culture system was coated with a seal of silicone sealer to provide corrosion protection. Significant bowing of this design was noted during operation, and the headplate was reinforced in the next design. Gas inlets and outlets in head-plate and reservoir were fabricated from 3/16" OD thin-wall (1/64") stainless tubing to minimize the potential for airflow blockage by condensate.

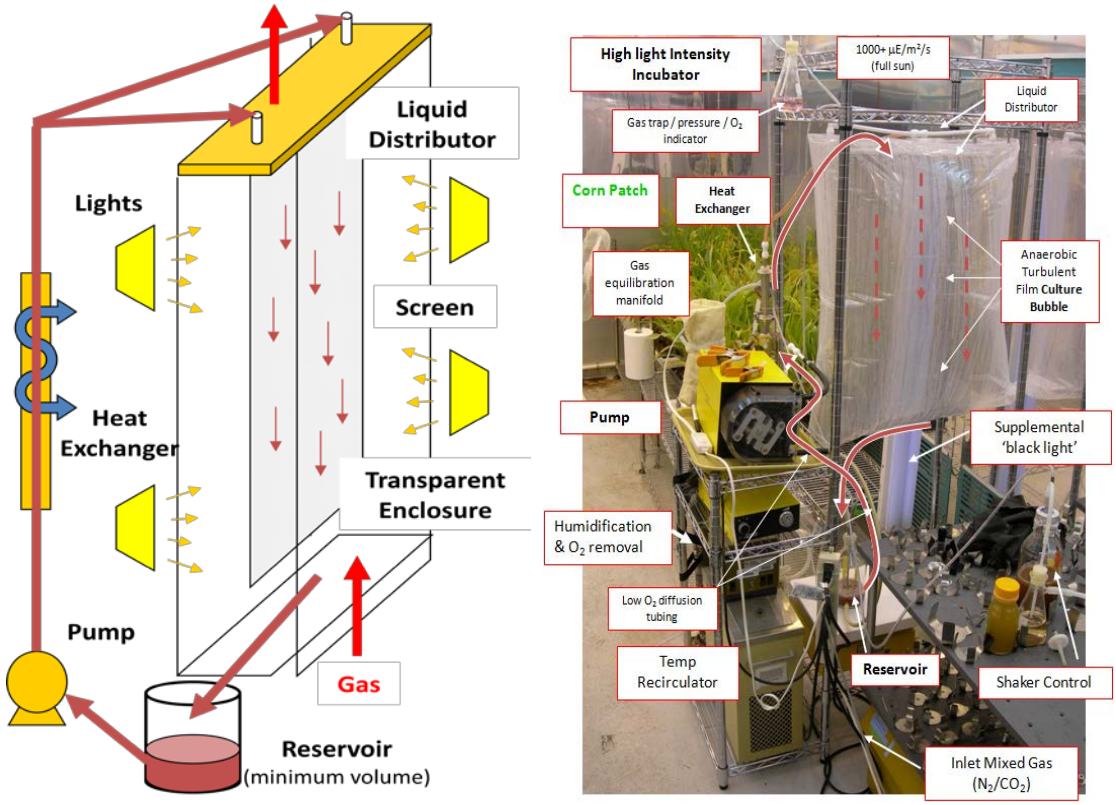

Figure 13 - Photograph and schematic of trickle-film photobioreactor

The trickle screen was constructed to be oriented 45^{o} from vertical to provide slow liquid down-flow, increase liquid holdup and induce liquid flow turbulence. The screen was cut from commercial window screen material (~17 threads / inch) using a cardboard template. It was discovered during operation that the screen was rendered

longer than necessary by the geometric effects of bag inflation. This did not alter flow on the screen, but increased holdup on the bottom outflow of the plastic bag.

A watertight liquid exit was created by pushing a 3/8”OD polyethylene tubing (Watts Water Technologies, ¼ ID, 048643-025592) through the bottom corner of the bag, into a 0.5” piece of silicone tubing. This created a very tight seal that which could be secured with cable ties. The poly-tubing on the inside of the bag was cut at ~60^{o} and glued in place with silicone sealer to provide an exit for flow with minimum dead volume. The outside end of the poly-tube was attached to a 90^{o} quick-connect fitting to direct flow downward to a culture reservoir.

The reservoir was a 1-L Aspirator Bottle with Bottom Sidearm (Corning, Corning PYREX® 1L Product #1220-1L). The reservoir rested on a large stir plate (Thermolyne Cimarec-3) that was used to agitate the culture during the simulated nighttime to prevent settling (setting #2). The reservoir top had a #6 silicone stopper, penetrated by two stainless steel gas ports (in/out as head-plate) and a fluid return (5/16” OD, 1/64” wall thickness) and a sample port (2mm OD, 1/16” ID, 12.5” in length with 0.45-mL dead volume) that reached the bottom of the reservoir. Sampling was facilitated with a piece of silicone tubing (3/16” OD, 1/6” ID) at the end of the sample tube that was clamped and capped with sterile aluminum foil between samplings. Culture was pumped from the reservoir through Neoprene tubing (Norton/Masterflex 6402-18) connected to Norprene® industrial grade tubing (3/8" ID x 9/16" OD x 3/32" wall), chosen to minimize oxygen diffusion into the system. A Watson-Marlow 601S peristaltic pump was used to minimize tubing damage and permit long-term operation with rollers adjusted to accommodate tubing wall thickness. Initially, flow was 0.7 L/min (setting 30%) which

was increased to 1-L/min during rapid growth (setting 40%) until the increase in viscosity required reduction back to 0.7-L/min to prevent excessive gas aspiration.

Cultures were grown in a Conviron incubator with 16-hr photoperiod. Room lighting provided 1000-2000 $\mu E/m^2/s$ during most of the day from one bank of high-pressure sodium vapor lamps and three banks of metal halide lamps. Lighting was ramped up and down over the course of one hour in a simulated sunrise and sunset. When the culture began to display rapid growth, supplemental near-UV light was provided by (2) 40-watt Actinic black lights (no dark glass filter; Phillips F40T12/BL) and subsequently with a 500-watt halogen work light (set at 3-ft distance) to provide additional far-red lighting.

The temperature in the room was 28^{o}C during 9am-11pm and fell to 25^{o}C at night. The temperature inside the reactor was monitored daily with a digital thermocouple placed in the offline sample as it was drawn. The bioreactor culture was passed through a heat exchanger to help manage heat and minimize evaporative water. The heat exchanger consisted of a 24" long piece of 3/8" OD thin wall stainless steel tubing that passed through a set of (3) 1.5" Tri-Clamp sanitary stainless steel Long-Tees (tube diameter ~1"). Culture flowed through the central pipe that was sealed to the sanitary fittings with silicone rubber stoppers and connected to the pump and headplate distributor with quick-connect fittings (Watts Water Technologies); cooling water flowed through the sanitary fittings through Tri-clamp hose barb caps on the top and bottom Tee. Fluid in the shell side was circulated through a refrigerated circulating bath (Fisher Isotemp, model #89100) that was adjusted between 28-29^{o}C, providing cooling to minimize evaporative water loss from the system.

An anaerobic environment was created inside the culture system by providing a 10% CO_2, 90% nitrogen gas supply that was scrubbed for oxygen removal. Gas was mixed using Brooks 1355 Sho-Rate "150" flow meters: CO_2 (R-215-AAA tube, 0.278-g tantalum float, low taper valve), N2 (R-2-15B, 0.133-g stainless steel float, medium taper). Vinyl tubing (1/4", Watts Water Technologies), inexpensive and exhibiting a low oxygen diffusion coefficient, was used to deliver gas to a 3"x28" humidification/scrubbing column (Corning flanged pilot plant glass) containing 304 stainless steel Pall ring tower packing (Norton 1"×1") to reduce gas bubble rise velocity in the column. Residual oxygen removal was facilitated by 3-L of 11.7-g sodium sulfite solution catalyzed by 2.3-mM Co^{2+} (1.7-mg $CoCl_2$-$6H_2O$); cobalt catalyst level was chosen to be twice the recommended level for measuring gas-liquid interface mass-transfer coefficients, to provide for interface-enhanced reaction rates for oxygen removal. An additional 17.6-g Na2SO3 was added to the humidification column on day 2 along with 250-mL 0.1% resazurin oxygen indicator to assure excess oxygen removal capacity. Gas then passed from a gas manifold to inlets on the reservoir and headplate. The headplate and reservoir gas outlets were connected to avoid flooding or bursting of the bag.

BIBLIOGRAPHY

1. Lee, S. Y. (1996) High cell-density culture of Escherichia coli, *Cell* 98-105.

2. Li, H., Sethuraman, N., Stadheim, T. a, Zha, D., Prinz, B., Ballew, N., Bobrowicz, P., Choi, B.-K., Cook, W. J., Cukan, M., Houston-Cummings, N. R., Davidson, R., Gong, B., Hamilton, S. R., Hoopes, J. P., Jiang, Y., Kim, N., Mansfield, R., Nett, J. H., Rios, S., Strawbridge, R., Wildt, S., and Gerngross, T. U. (2006) Optimization of humanized IgGs in glycoengineered Pichia pastoris., *Nature biotechnology 24*, 210-5.

3. Breed, R. S., and Murray, E. G. D. (1957) Bergey's Manual of Determinative Bacteriology (Breed, R. S., Murray, E. G. D., and Smith, N. R., Eds.), pp 335-340, The Williams & Wilkins Company, Baltimore, MD.

4. Sarramegna, V., Talmont, F., Demange, P., and Milon, a. (2003) Heterologous expression of G-protein-coupled receptors: comparison of expression systems from the standpoint of large-scale production and purification, *Cellular and Molecular Life Sciences (CMLS) 60*, 1529-1546.

5. Sarramegna, V., Muller, I., Milon, A., and Talmont, F. (2006) Recombinant G protein-coupled receptors from expression to renaturation: a challenge towards structure., *Cellular and molecular life sciences : CMLS 63*, 1149-64.

6. Laible, P. D., Scott, H. N., Henry, L., and Hanson, D. K. (2004) Towards higher-throughput membrane protein production for structural genomics initiatives., *Journal of structural and functional genomics 5*, 167-72.

7. Garrity, G. M., Bell, J. A., and Lilburn, T. (2005) Genus I. Rhodobacter. In *Bergey's Manual of Systematic Bacteriology* (Brenner, D. J., Krieg, N. R., Garrity, G. M., and Staley, J. T., Eds.) 2nd ed., pp 161-167, Bergey's Manual Trust.

8. Madigan, M. T., and Gest, H. (1979) Growth of the photosynthetic bacterium Rhodopseudomonas capsulata chemoautotrophically in darkness with H2 as the energy source., *Journal of bacteriology 137*, 524-30.

9. Hansen, T. a, and Gemerden, H. van. (1972) Sulfide utilization by purple nonsulfur bacteria., *Archiv für Mikrobiologie 86*, 49-56.

10. Hoekema, S., Douma, R. D., Janssen, M., and Tramper, J. (2006) Controlling Light-Use by Rhodobacter capsulatus Continuous Cultures in a Flat-Panel Photobioreactor, *Biotechnology*.

11. Tichi, M. A., and Tabita, F. R. (2001) Interactive Control of Rhodobacter capsulatus Redox-Balancing Systems during Phototrophic Metabolism, *Society 183*, 6344-6354.

12. Shively, J. M., Keulen, G. V., and Meijer, W. G. (1998) SOMETHING FROM ALMOST NOTHING : Carbon Dioxide Fixation in Chemoautotrophs Occurrence in Bacteria, *Biotechnology* 191-230.

13. Donohue, T. J., and Kaplan, S. (1991) Genetic Techniques in Rhodospirillaceae, *Methods in enzymology 204*, 459-485.

14. Roberts, R. J. (1980) Restriction and modification enzymes and their recognition sequences., *Nucleic Acids Research*, Oxford University Press *8*, r63.

15. Huo, J. (2011) Design of a BioBrick TM Compatible Gene Expression System for Rhodobacter sphaeroides.

16. Lynn, S. P., Cohen, L. K., Gardner, J. F., and Kaplan, S. (1979) Characterization of a site-specific restriction endonuclease from Rhodopseudomonas sphaeroides., *Journal of bacteriology 138*, 505-9.

17. Aiken, C., and Gumport, R. I. (1988) Restriction endonuclease RsrI from Rhodobacter sphaeroides, an isoschizomer of EcoRI: purification and properties., *Nucleic acids research 16*, 7901-16.

18. Kaszubska, W., Aiken, C., O'Connor, C. D., and Gumport, R. I. (1989) Purification, cloning and sequence analysis of RsrI DNA methyltransferase: lack of homology between two enzymes, RsrI and EcoRI, that methylate the same nucleotide in identical recognition sequences., *Nucleic acids research 17*, 10403-25.

19. Lynn, S. P., Cohen, L. K., Kaplan, S., and Gardner, J. F. (1980) RsaI: a new sequence-specific endonuclease activity from Rhodopseudomonas sphaeroides., *Journal of bacteriology 142*, 380-3.

20. O'Connor, C. D., Metcalf, E., Wrighton, C. J., Harris, T. J., and Saunders, J. R. (1984) RsrII--a novel restriction endonuclease with a heptanucleotide recognition site., *Nucleic acids research 12*, 6701-8.

21. Kramarov, V., Mazanov, A., Pachkunov, D., Smolyaninov, V., and Matvienko, N. (1984) Another site-specific endonuclease from Rhodopseudomonas sphaeroides, *BIOORGANICHESKAYA KHIMIYA 10*, 46-49.

22. Moore, M. D., and Kaplan, S. (1992) Identification of intrinsic high-level resistance to rare-earth oxides and oxyanions in members of the class Proteobacteria: characterization of tellurite, selenite, and rhodium sesquioxide reduction in Rhodobacter sphaeroides., *Journal of bacteriology 174*, 1505-14.

23. Turner, R. J., Weiner, J. H., and Taylor, D. E. (1999) Tellurite-mediated thiol oxidation in Escherichia coli., *Microbiology (Reading, England) 145 (Pt 9*, 2549-57.

24. Hunter, C. N., Daldal, F., and Thurnauer, M. C. (2009) Chapter 08 - Peripheral Complexes of Purple Bacteria, *Media* (Hunter, C. N., Daldal, F., Thurnauer, M. C., and Beatty, J. T., Eds.), pp 135-153, Springer Netherlands, Dordrecht.

25. Ermler, U., Fritzsch, G., Buchanan, S. K., and Michel, H. (1994) Structure of the photosynthetic reaction centre from Rhodobacter sphaeroides at 2.65 A resolution: cofactors and protein-cofactor interactions., *Structure (London, England : 1993) 2*, 925-36.

26. Conroy, M. J., Westerhuis, W. H., Parkes-Loach, P. S., Loach, P. a, Hunter, C. N., and Williamson, M. P. (2000) The solution structure of Rhodobacter sphaeroides LH1beta reveals two helical domains separated by a more flexible region: structural consequences for the LH1 complex., *Journal of molecular biology 298*, 83-94.

27. Cherezov, V., Clogston, J., Papiz, M. Z., and Caffrey, M. (2006) Room to move: crystallizing membrane proteins in swollen lipidic mesophases., *Journal of molecular biology 357*, 1605-18.

28. Hunter, C. N., Tucker, J. D., and Niederman, R. a. (2005) The assembly and organisation of photosynthetic membranes in Rhodobacter sphaeroides., *Photochemical & photobiological sciences : Official journal of the European Photochemistry Association and the European Society for Photobiology 4*, 1023-7.

29. Hess, S., Visscher, K., Ulander, J., Pullerits, T., Jones, M. R., Hunter, C. N., and Sundstroem, V. (1993) Direct energy transfer from the peripheral LH2 antenna to the reaction center in a mutant of Rhodobacter sphaeroides that lacks the core LH1 antenna, *Biochemistry 32*, 10314-10322.

30. Cohen-Bazire, G., Sistrom, W. R., and Stanier, R. Y. (1957) Kinetic studies of pigment synthesis by non-sulfur purple bacteria, *Journal of Cellular and Comparative Physiology 49*, 25-68.

31. Zeilstra-Ryalls, J., Gomelsky, M., Eraso, J. M., Yeliseev, A., O'Gara, J., and Kaplan, S. (1998) Control of photosystem formation in Rhodobacter sphaeroides., *Journal of bacteriology 180*, 2801-9.

32. Yoshida, K., Yoshioka, D., Inoue, K., Takaichi, S., and Maeda, I. (2007) Evaluation of colors in green mutants isolated from purple bacteria as a host for colorimetric whole-cell biosensors., *Applied microbiology and biotechnology 76*, 1043-50.

33. Scolnik, P. A., Walker, M. A., and Marrs, B. L. (1980) Biosynthesis of carotenoids derived from neurosporene in Rhodopseudomonas capsulata., *The Journal of biological chemistry 255*, 2427-32.

34. Yeliseev, a a, Eraso, J. M., and Kaplan, S. (1996) Differential carotenoid composition of the B875 and B800-850 photosynthetic antenna complexes in Rhodobacter sphaeroides 2.4.1: involvement of spheroidene and spheroidenone in adaptation to changes in light intensity and oxygen availability., *Journal of bacteriology 178*, 5877-83.

35. Griffiths, M., and Stanier, R. Y. (1956) Some mutational changes in the photosynthetic pigment system of Rhodopseudomonas spheroides., *Journal of general microbiology 14*, 698-715.

36. Sistrom, W. R., Griffiths, M., and Stanier, R. Y. (1956) The biology of photosynthetic bacterium which lacks colored carotenoids., *Journal of cellular physiology 48*, 473-515.

37. Laible, P. D., Mielke, D. L., and Hanson, D. K. (2009) Chapter 42 Foreign Gene Expression in Photosynthetic Bacteria, *Media* 839-860.

38. Lyle, P. a, Kolaczkowski, S. V., and Small, G. J. (1993) Photochemical hole-burned spectra of protonated and deuterated reaction centers of Rhodobacter sphaeroides, *The Journal of Physical Chemistry 97*, 6924-6933.

39. Masuda, S., and Bauer, C. E. (2002) AppA is a blue light photoreceptor that antirepresses photosynthesis gene expression in Rhodobacter sphaeroides., *Cell 110*, 613-23.

40. Laible, P. D., Mielke, D. L., and Hanson, D. K. (2009) Chapter 42 Foreign Gene Expression in Photosynthetic Bacteria, *Media* 839-860.

41. Kaplan, S. (2002) Photosynthesis genes and their expression in Rhodobacter sphaeroides 2.4.1: a tribute to my students and associates., *Photosynthesis research 73*, 95-108.

42. Oh, J. I., and Kaplan, S. (2001) Generalized approach to the regulation and integration of gene expression., *Molecular microbiology 39*, 1116-23.

43. Bauer, C. (2004) Regulation of Photosystem Synthesis in Rhodobacter capsulatus., *Photosynthesis Research 80*, 353-60.

44. Wu, J., and Bauer, C. E. (2010) RegB Kinase Activity Is Controlled in Part by Monitoring the Ratio of Oxidized to Reduced Ubiquinones in the Ubiquinone Pool *1*, 1-9.

45. Masuda, S., and Bauer, C. E. (2002) AppA is a blue light photoreceptor that antirepresses photosynthesis gene expression in Rhodobacter sphaeroides., *Cell 110*, 613-23.

46. Sistrom, W. R., Griffiths, M., and Stanier, R. Y. (1956) The biology of photosynthetic bacterium which lacks colored carotenoids., *Journal of cellular physiology 48*, 473-515.

47. Dubbs, J. M., and Robert Tabita, F. (2004) Regulators of nonsulfur purple phototrophic bacteria and the interactive control of CO2 assimilation, nitrogen fixation, hydrogen metabolism and energy generation, *FEMS Microbiology Reviews 28*, 353-376.

48. Inui, M., Nakata, K., Roh, J. H., Vertes, A. A., and Yukawa, H. (2003) Isolation and molecular characterization of pMG160, a mobilizable cryptic plasmid from Rhodobacter blasticus, *Applied and environmental microbiology*, Am Soc Microbiol *69*, 725.

49. Marx, C. J., and Lidstrom, M. E. (2002, November) Broad-host-range cre-lox system for antibiotic marker recycling in gram-negative bacteria., *BioTechniques*.

50. Gibson, J. L., Dubbs, J. M., and Tabita, F. R. (2002) Differential Expression of the CO 2 Fixation Operons of Rhodobacter sphaeroides by the Prr / Reg Two-Component System during Chemoautotrophic Growth, *Society 184*, 6654-6664.

51. Scott, H. (2003) Sequences of versatile broad-host-range vectors of the RK2 family, *Plasmid 50*, 74-79.

52. Halling-Sørensen, B., Sengeløv, G., and Tjørnelund, J. (2002) Toxicity of tetracyclines and tetracycline degradation products to environmentally relevant bacteria, including selected tetracycline-resistant bacteria., *Archives of environmental contamination and toxicology 42*, 263-71.

53. Garrity, G. M., Bell, J. A., and Lilburn, T. (2005) Genus I. Rhodobacter. In *Bergey's Manual of Systematic Bacteriology* (Brenner, D. J., Krieg, N. R., Garrity, G. M., and Staley, J. T., Eds.) 2nd ed., pp 161-167, Bergey's Manual Trust.

VITA

BRANDON SCOTT CURTIS
144 Creekside Drive
State College, PA 16801
brandon.curtis@curtislab.org

Education

The Pennsylvania State University (University Park, PA)
Schreyer Honors College - Graduation in August 2011
B.S. Chemical Engineering (Bioprocessing Option)
B.S. Biochemistry and Molecular Biology (Biochemistry Option)

Relevant Coursework

Bioprocessing, Biomolecular Engineering, Bioseparations, Analytical Biochemistry, Transport Phenomena, Mathematical Modeling, Microbiology, Business Basics, Intermediate German.
Graduate-Level: Biophysics, Enzymology, Thermodynamics, Kinetics, Molecular Genetics

Work Experience

Genentech, Inc — *S. San Francisco, CA*
Engineering Co-Op — **May – Dec 2007**
Engaged in sensor technology development within Process Development Engineering
Evaluated commercially-available pH technologies for use in Purification operations
Designed and executed experiments, analyzed data, and presented results to vendors
Recommended technologies and procedures to improve the quality of pH control
Supported bioreactor operation and sample analysis in mammalian cell culture

---

The Pennsylvania State University — *University Park, PA*
Laboratory Tech / Undergraduate Researcher — **1996 – Aug 2011**
Scaled up membrane protein expression using *Rhodobacter* in a novel photobioreactor system
Coordinated a team to characterize the metabolism and heterologous induction of *Rhodobacter*
Trained ~10 undergrads, grads, and international student interns in aseptic microbial culture
Developed protocols for genetic engineering, product purification, and seed train staging
Collaborated with researchers at Penn State, Hershey Medical Center, and Argonne National Lab

---

The Pennsylvania State University — *University Park, PA*
Data Management & Systems Administration — **Jun 2009 – Aug 2011**
Managed a 15-computer research network to explore and improve the use of IT in the lab
Deployed distributed backup and coordinated the archiving of 20 years of lab data
Modernized the group website and implemented online project collaboration tools
Configured and managed a 50-user Google Apps for Education domain
Implemented an improved CRM for the PSU Fellowship Office based on staff interviews
Provided information technology consulting for several Penn State student organizations
Performed day-to-day system maintenance, favoring free and open-source solutions

The Pennsylvania State University *University Park, PA*
Teaching Assistant / Teaching Intern **Aug 2008 – May 2011**
TA'ed courses in biomolecular (150 students)) and energy (20 students) engineering
Assisted with the first offering of the energy course by providing expertise in bioenergy systems
Redesigned biomolecular course outline, wrote homework and tests, and lectured independently
Held regular office hours and review sessions, in-person and electronically
Implemented a system to solicit anonymous, real-time student feedback on course content

---

PsuKnowHow Tutoring Service *State College, PA*
Private Instructor **Mar 2008 – Oct 2009**
Provided individual and group (100+) instruction in chemistry, biology, math and physics
Subject expert for General / Organic Chemistry, Molecular / Cellular Biology, Mechanics
Independently managed scheduling, material preparation, and professional interaction

Honors & Awards

2011 Recipient, National Science Foundation Graduate Research Fellowship
2010 National Finalist, Marshall Scholarship
2010 Nominee, Barry M. Goldwater Excellence in Education Program
2010 Recipient, Phi Beta Kappa Honors Thesis Research Scholarship
2009 Recipient, Excellence in Undergraduate Chemical Engineering Research
2008 - 2009 Recipient, Biomolecular Engineering Research Fellowship
2008 Recipient, College of Engineering Summer Research Grant
2006 – 2011 Recipient, Schreyer Academic Excellence Scholarship
2008 Merck Engineering & Technology Fellowship, declined to continue research

Activities & Organizations

President and Web Coordinator, Omega Chi Epsilon ChemE honors society
Web Developer and Member, Omicron Delta Kappa student leadership society
Executive Board and Web Designer, Center for Sustainability Community Garden
Member: AIChE (Penn State); ISPE (Delaware Valley); PSU Honors Mentor

Presentation & Publications

2009 First Place, AIChE National Student Paper Competition (*Rhodobacter* work)
2009 First Place, AIChE Mid-Atlantic Paper Competition (*Rhodobacter* work)
2008 Presenter, AIChE National Conference (*Rhodobacter* work)

Computer Skills

Computer Design, Construction, and Configuration, Data Management and Backup
Web Development (XHTML+CSS); Hosting and Site Maintenance (Google Apps, Wiki)
Proficient in Linux; Data Analysis, Statistics, and Numerical Methods in SAGE

Laboratory Skills

Plant Tissue: aseptic clonal propagation, cell and root culture initiation, Agro transforms
Microbial: culture of strict and facultative aerobes, microaerobes, anaerobes, lithotrophs
Microbial: cloning in *Rhodobacter* and *E. coli*; culture and analytical methods for algae
Protein purification, spectroscopy, AKTA chromatography; Enzymatic assays; biocatalysis
Basic organic chemistry; analytical methods (TLC, GC, NMR, IR, UV-Vis Polarimetery)
Design and operation of low-cost, high-density plant and microbial bioreactors