Photosynthetic Growth of Synechocystis sp. PCC 6803 with Different Levels of CO_2 Availability

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Abstract: In response to global warming becoming a popular and complex issue in today's society, scientists began to take a closer look into what specific systems could slow and stop the process of increasing CO2 concentrations, and exactly how those systems function. In Dr. Burnap's lab, we are focusing on cyanobacteria, a photosynthetic bacteria rich with biochemical diversity. It serves as a model system for the photosynthetic process. However, the main unknown of the photosynthetic process is how specific genes are involved, and the role each gene plays in the process. With the constant burning of fuel, coal, and natural gases, large amounts of CO2 are being emitted into the environment. This is causing many detrimental effects on the world since these are 'greenhouse gases', which traps heat that would otherwise be lost as infrared radiation into space. If, by doing our research, we are able to exactly measure the photosynthetic process under controlled conditions, we can determine how to produce biomass in the form of cyanobacterial metabolites to create chemicals and energy. This could affect how biofuels, plastics, and industrial products are made substituting renewable chemical inputs that come from cyanobacterial photosynthetic products instead using chemical inputs derived from fossil fuels. This would shave the effect of reducing carbon dioxide levels within our environment. Research indicates that the increase in the speed of cyanobacterial photosynthetic production to produce, as well as production of other photosynthetic organisms would allow for faster and increased in carbon intake, which could overall serve to alter the progression of global warming by decreasing levels of carbon dioxide and pollutants.

Keywords: *Synechocystis* sp. PCC 6803, Growth Rates, Cell Physiology, CO_2 Limitation

Introduction

Research indicates that CO2 intake system are possible to engineer and carbon dioxide exchanges can be measured with a device called, FLUXNET. This device would allow researchers to measure the speed and effectiveness of engineered CO2 intake systems. These engineered devices would overall serve to alter the progression of global warming by effectively decreasing levels of carbon dioxide and pollutants within the environment (Baldocchi et al. 2001). In order to achieve this overall goal, our lab aims to answer the question of how do cyanobacteria actively acquire CO_2 to carbonate their cytoplasm, and what specific genes control the carbonation? However, quantitative growth and physiology of CO_2 metabolizing cells is lacking in literature. Without this basic knowledge, we are unable to solve a larger and worldwide problem, more efficient and effective uses of energy. In Dr. Burnap's lab, we are focusing on how cell's CO_2 metabolism affects physiology and growth rates of cells. We used wild type *Synechocystis*, a naturally occurring bacterial strain of Cyanobacteria widely used as an experimental model to study cyanobacteria. *Synechocystis* is an excellent

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model organism for a range of biological studies because of its robust environmental stress responses and because it can be genetically manipulated very easily (Simon et al. 2002). Despite its wide use in many experimental studies, we discovered that it has still not been characterized with respect to basic growth analysis. Since out subsequent studies of specific genes may require a detailed knowledge of growth, my objective was fill in this gap in our knowledge. Accordingly, I developed three techniques for measuring growth of *Synechocystis*, and tested each under four different conditions. We tested growth rate, cell size, and cellular chlorophyll within pH 7 low carbon, pH 7 high carbon, pH 8 low carbon, and pH 8 high carbon conditions. It was important to control the pH of the growth media because the amount of available CO_2 depends upon the acidity of the aqueous media as discussed below.

Table 1 - Cell densities (cells/mL) observed at 24 hour intervals under different pH and carbon conditions.

Time(h)	pH 7 Low Carbon	pH 7 High Carbon	pH 8 Low Carbon	pH 8 High Carbon
24	$6.89x10^6 \pm 1.6x105$	$6.71x10^6 \pm 1.0x105$	$6.55x10^6 \pm 2.3x105$	$6.56x10^6 \pm 3.8x105$
48	$8.70x10^6 \pm 3.1x10^5$	$8.98x10^6 \pm 2.9x105$	$8.28x10^6 \pm 5.2x104$	$8.54x10^6 \pm 1.9x105$
72	$1.81x10^7 \pm 4.1x105$	$1.78x10^7 \pm 6.4x105$	$1.73x10^7 \pm 1.7x105$	$1.73x10^7 \pm 3.5x105$
96	$4.40x10^7 \pm 2.1x106$	$4.55x10^7 \pm 1.4x106$	$3.63x10^7 \pm 9.1x105$	$4.69x10^7 \pm 5.0x106$

Methods

To create our weekly cyanobacterial cultures, we inoculated liquid cultures with cells obtained as scrapings of *Synechocystis* colonies from agar growth plates. Cells were then collected by centrifugation and re-suspended in liquid BG-11 media . The cells were then inoculated by pipetting a small amount of cells into a large amount of BG-11 media to OD_{750} 0.1. The resuspended cells were mixed and separated into six different test tubes using in the Multi Cultivator growth apparatus. All six test tubes were placed into a PSI Multi-Cultivator OD_{730} (Photon Systems Instruments, Czech Republic) and bubbled with 3% CO_2. At hour 40, 3 test tubes were switched to air bubbling (.04% CO_2). Growth was measured by OD_{730} using the PSI instrument and plotting the log of each OD at a specific time (Table 3). We took the log of the OD to see an exponential curve which is easier to analyze. Each week we tested the cells under a specific pH condition, either pH 7 or pH 8. The first technique used to analyze growth rate was cell counts at 24 hour intervals. Small samples were taken daily from each test tube and placed into a Nexcelom Auto X4 cell counting machine (Nexcelom Bioscience, Massachusetts, USA). Data was recorded and averaged by taking 4 counts throughout the course of the growth experiment, and 4

Table 2 - Chlorophyll per cell under different carbon conditions. Measured in femtograms per cell

Time(h)	pH 7 Low Carbon	pH 7 High Carbon	pH 8 Low Carbon	pH 8 High Carbon
48	71.86±3.7	80.29±7.1	88.83±4.6	94.59±1.7
72	88.41±3.6	90.51±6.7	97.83±4.4	108.98±3.6
96	68.87±2.1	111.30±.35	93.69±2.3	120.59±1.1

Table 3 - Chlorophyll per milliliter (µg/mL) under different inorganic carbon conditions.

Time(h)	pH 7 Low Carbon	pH 7 High Carbon	pH 8 Low Carbon	pH 8 High Carbon
48	.628±.03	.647±.06	.705±.02	.695±.01
72	1.72±.02	1.74±.10	1.69±.05	1.67±.12
96	3.17±.48	5.75±.27	3.55±.06	5.42±.57

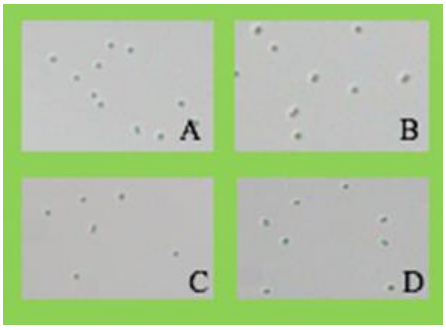

Figure 1 - Pictured cells under different carbon conditions: A: pH 7 LC, B: pH 7 HC, C: pH 8 LC, D: pH 8 HC

counts for each tube were averaged and the standard deviation calculated. Another technique we used was cell size. Every 24 hours, samples were taken from each test tube and analyzed and pictured using a microscopic camera at zoom 200x. Then, an image analysis application, called ImageJ, was used to determine height and width of cells pictured. Ten cells in each photo were analysed, and width and heights were averaged (Figure 1). The last technique we used to characterize growth rate was chlorophyll concentration per cell, and per milliliter of solution. Samples of all six test tubes were taken at 24 hour intervals and placed into a spectrophotometer along with a baseline water sample. The equation used to obtain this number was (14.96*(OD_{678}-OD_{750}))-(0.607*(OD_{620}-OD_{750}))*Dilution Factor. This number gave us chlorophyll per milliliter of solution. We then divided this number by the number of cells obtained in solution to get the amount of chlorophyll found in each cell. This allowed us to evaluate the photosynthetic capacity for individual cell under the different growth conditions.

Figure 2 - Growth of cultures in multi-cultivator after switching to air bubbling

Results

When growing the cells, we saw a significant visual difference with the coloration of cells air bubbled and CO_2 bubbled cells. The left three cultures (Figure 3) bubbled with CO_2 show healthy, thick, and dense growth. While the right three cultures, bubbling with air, show a more pale color and, apparently, sparse growth. This visual difference can also be shown quantitatively (Table 2 and Table 3). These two tables are showing chlorophyll per cell and chlorophyll per milliliter respectively. Between hours 72 and 96 and carbon becomes limited, the cultures fail to exhibit large increase in chlorophyll concentration per milliliter of culture as compared to cells maintained under high CO_2 conditions (Table 3). Note the large increase in concentration with the high carbon cells. We characterized the growth rates of each pH under different carbon limitations (Figure 3.) Up until the low carbon cultures were switched to air bubbling, hour 40, the cultures have very similar growth curves. Growth rates drastically decrease in both pH 8 and pH 7 low carbon conditions at hour 40. However, for pH 8 it takes longer for growth curve to deflect then it does for pH 7. We hypothesize that within a higher pH condition more CO_2 is present. Therefore, when switched to air bubbling, pH 8 takes longer to deplete carbon simply because it

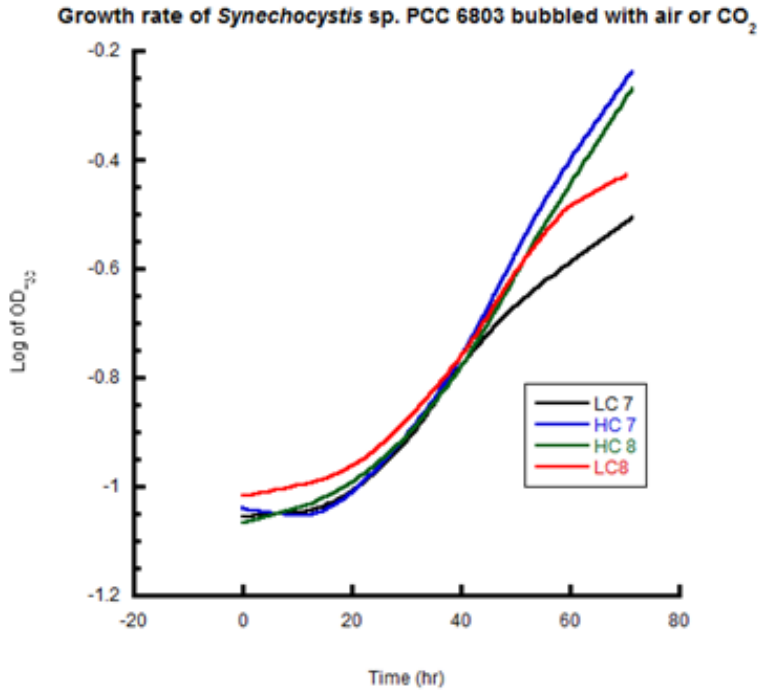

Figure 3 - Growth rate curve for cultures under different pH and carbon conditions. Measured by OD_{730}

has more dissolved carbon than pH 7. Unfortunately when looking at photos taken to analyze cell size, we had many technical difficulties and results on cell size were not conclusive.

Discussion

Growth rates are drastically altered when cultures are switched to air bubbling. When switched to air bubbling, the cells go from 3% to .04% CO_2. This causes cells to become inorganic carbon limited. We see that when cells become carbon limited they begin to decrease chlorophyll concentration. Accumulation of chlorophyll is much higher in high carbon cells than low carbon cells, as shown in Table 2. Chlorophyll plays a primary role in converting CO_2 and water into sugar and oxygen. Sugar and oxygen are necessary for cells to have an optimum growth rate. With less CO_2, cells have less of these necessary substances and the cell's growth rate is drastically decreased (Figure 2 and Figure 3). Hypothesized differences in cell size as a function of growth condition were not reliably obtained because of technical difficulties.When adequate and sufficient CO_2 is provided, pH does not have a sufficient effect on growth rates (Table 1 and Figure 3). Low carbon cells grown in pH 7 deplete much faster than low carbon cells grown in pH 8. We believe that this is because there is a higher availability of dissolved carbon in pH 8 than pH 7, meaning pH 8 has more carbon left in its system when switched to air bubbling than at pH 7 . This allows the growth rate to sustain longer before the growth rate begins to diminish.When determining cell physiology in carbon limiting conditions, pH 7 will be the most useful to use because is depletes fastest. This will allow us to obtain accurate results within a shorter amount of time.

Future Research

In 1996, sequencing the entire *Synechocystis* genome was completed (Kaneko et al. 1996). This sequence determined the presence of multiple copies of these genes. Further analysis showed that these genes would only be present in cyanobacteria, however their function was still unknown (Shibata et al. 2001). Further research has been done to conclude that the specific genes encode components specifically involved in the induced CO_2 uptake systems (Shibata et al. 2002). We will use this data to work with mutants deficient in CO_2 uptake by reprogramming

the genome to alter specific genes hypothesized to affect CO_2 uptake. After similar physiological and growth rate data is taken with the mutant, we will compare results within mutant and non-mutant. This will allow us to understand what is happening within the mutant by how far the two results deviate. This will allow us to understand the role of specific genes involved with CO_2 uptake.

Acknowledgements

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