The Effect of Ferrous Ammonium Sulfate Concentration on Marine *Synechococcus sp.* CO_2 Absorption and O_2 Production

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ABSTRACT

The purpose of this study was to determine the effects of iron concentration on marine *Synechococcus sp.* CO_2 absorption and O_2 production. *Synechococcus sp.* were cultured in varying concentrations of iron for 45 min., and O_2 and CO_2 concentrations were recorded. The lower the concentration of iron, the less CO_2 was absorbed by *Synechococcus sp*, but O_2 production was not affected as iron concentrations were deceased. Also, a concentration of iron was reached where CO_2 absorption and O_2 production reached maximum as iron concentrations were increased. A strong positive quadratic relationship was found between the iron concentration and the percent change in CO_2 absorption. There was no relationship between iron concentration and the O_2 production; indeed virtually all of the values were equal. This research showed that *Synechococcus sp.* used O_2 as an electron acceptor in iron-limited conditions. In the future, these results could be applied to an iron-limited marine environment.

KEYWORDS: *Synechococcus sp*., iron, ferrous ammonium sulfate, carbon dioxide, oxygen, photosynthesis
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1. INTRODUCTION

Cyanophyta are found in marine, freshwater and terrestrial environments. Cyanobacteria are widely distributed in ocean waters. There is currently great interest in the life cycle of the marine Cyanobacteria *Synechococcus* because it may play a crucial part of global primary productivity (Bailey *et. al*., 2008). Yin (1999) states that "Marine *Synechococcus* usually account for 5 to 50% of the total primary production in the oceans"; it dominates phytoplankton populations over large expanses of the world's oceans where

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bioavailable iron is scarce. In its unique adaptation to iron-limited conditions in oligotrophic areas of the ocean *Synechococcus* exhibits a significant alternative electron flow to oxygen in photosynthesis (Bailey *et. al*., 2008). According to *ScienceDaily* (2008) "this discovery impacts not only scientists' basic understanding of photosynthesis, but importantly, it may also impact how microorganisms in the oceans affect rising levels of CO_2". Further, Barsanti and Gaultieri (2006) state that "…phytoplankton [such as *Synechococcus,*] which form the base of the marine food chain … produce roughly 50% of the O_2 we inhale…" and marine cyanobacteria fix about 40% of the global total carbon fixed per year (Yin, 1999).

Synechococcus and other coccoid cyanobacteria are prevalent in subsurface waters (Whitton and Potts, 2000). Their frequency in these areas is attributed to their small size and efficient absorption of low light. However, since they require high levels of iron for survival, their existence in these high nutrient/low chlorophyll (HNLC) areas is unexpected since HNLC areas are characterized by a scarcity of iron (Yin, 1999). Cyanobacteria account for a significant amount of the biomass in the "deep chlorophyll maxima," or the lower 75-125m of the euphotic zone of the ocean, and they can survive near the compensation point. Additionally, they are common in nutrient poor ultraoligotrophic open ocean waters, as well as in other extreme conditions (Whitton and Potts, 2000).

Iron is the fourth most abundant element on earth (Bailey *et. al*., 2008,) making up almost 5% of the Earth's crust. Despite its abundance it can often be depleted in surface waters (euphotic zone) where *Synechococcus* is prevalent (Coale, 2001). Sources of iron in HNLC regions of the ocean are "the upward mixing of iron rich subsurface waters to the euphotic zone and atmospheric deposition of dust particles on the sea surface…" (Kraemer, *et. al*., 2005).

Cyanobacteria are especially vulnerable to iron deficiency, in part because of its role in functional photosynthesis (Bailey *et. al.,* 2008). This vulnerability arises because the photosynthetic apparatus is highly enriched with iron (contains 22-23 Fe atoms). Iron starvation can lead to changes in cellular structure, physiological activities, photosystem electron transport, respiratory system electron transport (Michel and Pistorius, 2004; Ivanov *et. al*., 2000)).

"The relationship between iron nutrition and physiological processes may be crucial in understanding global interactions among marine primary productivity, continental processes, and climate and in appreciating the potential for human disruptions of natural patterns in these processes" (Rueter and Unsworth, 1991). This regulation of biological processes by iron is common in aerobic alkaline marine ecosystems, where coccoid cyanobacteria, like *Synechococcus* are prevalent. Because of this iron limitation, these "ubiquitous" cyanobacteria's primary productivity is affected, proving that factors that influence these

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cyanobacteria regulate the entire marine ecosystem (Wilhelm and Trick, 1995). The marine ecosystem, in turn, acts as a reservoir for carbon dioxide, and the ocean reservoir contains about sixty times more inorganic carbon than the atmospheric reservoir. This means that the ocean carbon cycle regulates the atmospheric cycle through the "sea-air exchange," which is done through "physico-chemical forces," such as photosynthesis (Hanson, et. al., Eds., 2000). N.E. Tolbert and Jack Preiss put forth that regulation of CO_2 fixation is emphasized by scientists because it almost directly correlates with the atmospheric condition (1994). Tolbert and Preiss also give the perspective that "if one uses 250 ppm as the average preindustrial CO_2 concentration, the CO_2 level has now risen about 120 ppm or 48%, and in the next century it will have doubled to 500 ppm" (1994).

An understanding of Shaun Bailey and his colleagues' research is crucial to understanding *Synechococcus sp.*'s "alternative adaptation," so a brief description is as follows. The reaction center of PSI (P700) and cytochrome b6f complex are the most iron-enriched components of the photosynthetic system, thus low levels of PSI activity occur under low levels of iron. This is detrimental because this leads to excessive PSII excitation. Some types of cyanobacteria use isiA and Pcb as antenna proteins for PSI under low iron conditions, however, *Synechococcus* does not possess the isiA or Pcb genes, but has developed a unique alternative adaptation. *Synechococcus* extracts electrons from the intersystem electron transport chain prior to PSI, thus bypassing the most iron-enriched components of photosynthesis. Alternative electron sinks utilize O_2 as the terminal electron acceptor. The PTOX enzyme (an oxidase) is believed to catalyze this. Notably, this process may be common in oligotrophic oceans where *Synechococcus* is prevalent. Additionally, it is unclear how ATP and NADPH quotients are maintained during this alternative process though scientists know they are maintained.

The purpose of this study was to determine the effects of Fe^{2+} on the photosynthetic CO_2 absorption and O_2 production of the marine cyanobacterium, *Synechococcus sp*. This research is important because iron-limitation of *Synechococcus sp.*, which occurs in a significant part of the earth's oceans, could shift the electron acceptor in photosynthesis from CO_2 to O_2 leading to CO_2 production and thence to higher levels of atmospheric CO_2. It was hypothesized that as the concentration of ferrous ammonium sulfate was decreased *Synechococcus sp.* would absorb less CO_2 and produce less O_2. The hypothesis was tested by culturing *Synechococcus sp.* in varying concentrations of ferrous ammonium sulfate for a pre-determined amount of time. Measurements of O_2 and CO_2 concentrations were recorded over time.

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2. MATERIALS AND METHODS

A. Materials

Synechococcus sp. (UTEX- The Culture Collection of Algae/ University of Texas at Austin, TX; boat basin, University of Texas Marine Science Institute, Port Aransas, TX)

Vernier CO_2 gas sensor

Vernier O_2 gas sensor

Laptop with LoggerPro software

LabPro interface

Cool-white fluorescent lamps

Bench top (Clay Adams) centrifuge

Autoclave

Analytical balance

Electronic balance

Micropipetor

Pipettes

Boric acid (H_3BO_3)

Calcium chloride ($CaCl_2$)

Cobalt chloride ($CoCl_2.6H_2O$)

Copper sulfate ($CuSO_4.5H_2O$)

Cyanocobalamin (Vitamin B_{12})

Sodium dihydrogen phosphate ($NaH_2PO_4.H_2O$)

Ferrous ammonium sulfate ($Fe(NH_4)_2(SO_4)_2.6H_2O$)

Magnesium chloride ($MgCl_2.6H_2O$)

Na_2 EDTA ($C_{10}H_{14}O_8N_2Na_2.2H_2O$)

Potassium bromide (KBr)

Potassium chloride (KCl)

Sodium bicarbonate ($NaHCO_3$)

Sodium chloride (NaCl)

Sodium fluoride (NaF)

Sodium hydroxide (NaOH)

Sodium nitrate ($NaNO_3$)

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Sodium sulfate (Na_2SO_4)

Strontium chloride ($SrCl_2.6H_2O$)

Zinc sulfate ($ZnSO_4.7H_2O$)

B. Methods

Medium Preparation: Synthetic Ocean Water (SOW): Recorded masses of the anhydrous salts: 24.540g NaCl, 4.090g sodium sulfate Na_2SO_4, 0.700g KCl, 0.200g $NaHCO_3$, 0.100g KBr, 0.003g H_3BO_3, and 0.003g NaF. The recorded masses of the hydrous salts were as follow: 11.100g $MgCl_2.6H_2O$, 1.170g $CaCl_2$ [Annex "B,"] and 0.017g $SrCl_2.6H_2O$.

Each of the anhydrous salts were dissolved individually in 600mL distilled water in a sterile 900mL beaker by magnetically stirring for two minutes. Distilled water (300mL) was added to the mixture then, each of the hydrous salts was dissolved individually while stirring magnetically for two minutes. The major nutrient stock solutions were preparedNaH$_2$PO$_4$.H$_2$O and $NaNO_3$ for quantities (Annex "C.") to 250mL distilled water in a 600mL beaker while stirring magnetically for three minutes.

Metal/metalloid stock solution: a separate $CuSO_4.5H_2O$ stock solution was prepared [Annex "D."] as follows: 1.225g $CuSO_4.5H_2O$ was added to 250mL distilled H_2O in a sterile 400mL beaker and the solution was stirred magnetically for 3 min. The recorded mass of each metal/metalloid was: 29.200g $C_{10}H_{14}O_8N_2Na_2.2H_2O$, 0.230g $ZnSO_4.7H_2O$, and 0.012g $CoCl_2.6H_2O$. Na_2 EDTA was added to 900mL of distilled H_2O in a sterile 1L beaker and stirred for five minutes, then the $ZnSO_4.7H_2O$, $CoCl_2.6H_2O$, and 1mL of the $CuSO_4.5H_2O$ stock solution were added and the solution stirred again for five minutes. The volume was brought up to 1L with distilled H_2O and mixed vigorously by swirling.

Six crushed tablets [Annex "E."] were added to 100mL SOW stock solution in a sterile 150mL beaker and the solution stirred for five minutes, then allowed to settle for five minutes. Equal parts of the vitamin solution were measured into two sterile test tubes, and centrifuged for one minute. This process was repeated for sets of two test tubes until all of the Vitamin B_{12} solution had been used. The pink liquid containing the Vitamin B_{12} was poured into the SOW solution and mixed by stirring; the white pellet (filler) was discarded 1mL of the major nutrients stock solution and 1mL of the metal/metalloids stock solution were added and mixed by stirring for five minutes. The medium was covered tightly with aluminum foil and boiled. After reaching a boil, the medium was removed from the heat and 100mL of the solution was added to a sterile 150mL beaker. This was repeated four times to produce five 100mL solutions which were then

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chilled in a refrigerator to 20°C. In another sterile 1L beaker, 1L 39.2g ferrous ammonium sulfate ($Fe(NH_4)_2(SO_4)_2.6H_2O$) was added [Annex "F."] to 1L distilled water.

The pH of the five solutions (at 20°C) was brought to 8 using 0.1 M NaOH. Using a micropipetor, 50μmol (50μL) of the $Fe(NH_4)_2(SO_4)_2.6H_2O$ stock solution was added to the first 100mL of medium and the solution stirred for 2 min. This process was repeated for each concentration of $Fe(NH_4)_2(SO_4)_2.6H_2O$. The concentration of 100μmol was used as a control because this is the optimum concentration for *Synechococcussp* growth.

Growth of *Synechococcus sp.*: All experiments were carried out at room temperature (approximately 20^0C), Six mL of *Synechococcus sp* culture was pipetted into each $Fe(NH_4)_2(SO_4)_2.6H_2O$ solution, and the beaker covered with parafilm reinforced with duct tape around the side of the beaker to create a virtually gastight environment. Two holes were poked in the parafilm at the top of the beaker and the gas-sensing probes (O_2 and CO_2) were inserted and made gasticgh by taping with duct tape. The probes were connected a laptop computer through the LabPro interface. The apparatus was placed about 0.5 m below a single cool-white fluorescent lamp away from direct illumination by sunlight. After turning on the lamp the solution was stirred slowly to prevent oxygen dissolution. After 15 minutes stirring the O_2 and CO_2 gas levels were measured over a further 45 minutes. The experiment was repeated for each Fe^{2+} level.

3. RESULTS

Figure 1 shows that the oxygen gas concentration in a *Synechococcus sp*. Culture grown in an Fe^{2+}concentration of 50μmol (50μL,) increased almost linearly by 0.6% for 45 minutes. Over the same period the CO_2 concentration increased by 123ppm (Figure 2).

For an Fe^{2+} concentration of 75μmol (75μL,) there was a clear upward trend in the oxygen gas concentration (in percent,) as seen in Figure 1. The increase in concentration was roughly linear over 45 minutes. There are few places on the graph where the gas concentration dips temporarily, at about 0.70 and 0.90 hour, for example. The values ranged from 18.73% at 0.25 hour to 19.21% at one hour. The O_2 concentration increased by 0.48% over a period of 45 minutes.

For an Fe^{2+} concentration of 75μmol (75μL,) there was not much of a visible trend in the carbon dioxide gas concentration (in ppm.) However, in Figure 2, the graph's starting point is higher than its ending point, so there was a slight downward trend for carbon dioxide gas concentration (ppm.) It should be noted that on this graph, there are three points, at about 0.70, 0.85, and 0.90 hour, where the gas concentration

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spikes above 500ppm. The starting value was 454.29ppm at 0.25 hour, while the ending value was 375.80ppm at one hour. The CO_2 concentration decreased by 78.49 ppm over a period of 45 minutes.

For an Fe^{2+} concentration of 100μmol (100μL,) there was a clear upward trend in the oxygen gas concentration (in percent,) as seen in Figure 1. The increase in concentration was almost exactly linear over 45 minutes. The values ranged from 18.76% at 0.25 hour to 19.29% at one hour. The O_2 concentration increased by 0.53% over a period of 45 minutes.

For an Fe^{2+} concentration of 100μmol (100μL,) there was a clear downward trend in the carbon dioxide gas concentration (in ppm,) as seen in Figure 2. The decrease in concentration was roughly exponential over 45 minutes. The starting value was 996.61ppm at 0.25 hour, while the ending value was 476.80ppm at one hour. The CO_2 concentration decreased by 519.81 ppm over a period of 45 minutes.

For an Fe^{2+} concentration of 125μmol (125μL,) there was a clear upward trend in the oxygen gas concentration (in percent,) as seen in Figure 1. The increase in concentration was roughly linear, but the graph begins to look more exponential at about 0.60 hour. The values ranged from 19.10% at 0.25 hour to 20.00% at one hour. The O_2 concentration increased by 0.90% over a period of 45 minutes.

For an Fe^{2+} concentration of 125μmol (125μL,) there was a clear downward trend in the carbon dioxide gas concentration (in ppm,) as seen in Figure 2. The decrease in concentration was nearly exponential over 45 minutes. The starting value was 2726.18ppm at 0.25 hour, while the ending value was 1073.02ppm at one hour. The CO_2 concentration decreased by 1653.16 ppm over a period of 45 minutes.

For an Fe^{2+} concentration of 150μmol (150μL,) there was a clear upward trend in the oxygen gas concentration (in percent,) as seen in Figure 1. The increase in concentration was roughly linear, but the graph's slope begins to change at about 0.70 hour (during the 0.75 hour period.) The values ranged from 18.26% at 0.25 hour to 18.72% at 1.00 hour. The gas concentration increased by 0.46% over a period of 0.75 hour.

For an Fe^{2+} concentration of 150μmol (150μL,) there was a clear downward trend in the carbon dioxide gas concentration (in ppm,) as seen in Figure 2. The decrease in concentration is roughly exponential over 0.75 hour. The starting value was 412.14ppm at 0.25 hour, while the ending value is 219.80ppm at 1.00 hour. The gas concentration decreased by 192.34ppm over a period of 0.75 hour.

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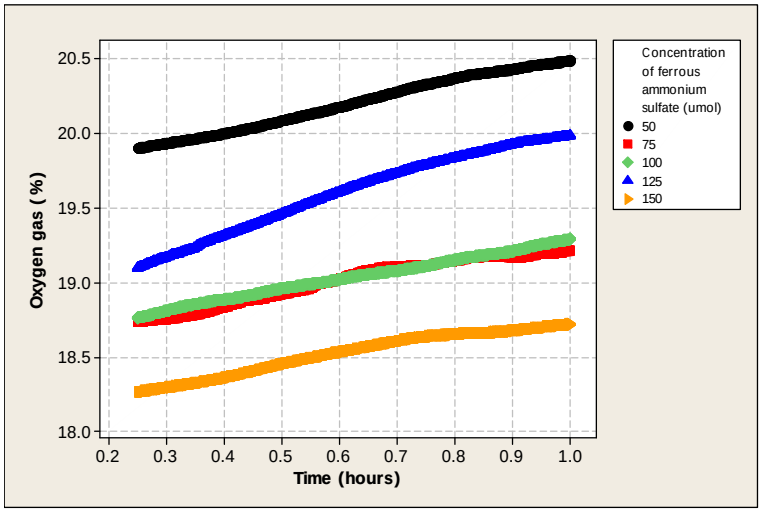

Figure 1. O_2 (%) during the growth of *Synechococcus sp*. in various concentrations of ferrous ammonium sulfate (µmol)

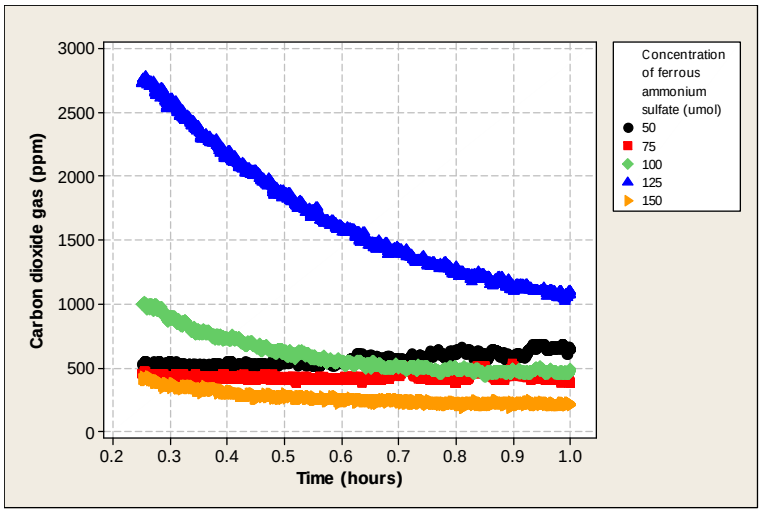

Figure 2. CO_2 (ppm) during the growth of *Synechococcus sp*. in various concentrations of ferrous ammonium sulfate (µmol)

Figures 3 and 4 show the relationships between the concentrations of ferrous ammonium sulfate added and the percent change in O_2 and CO_2.

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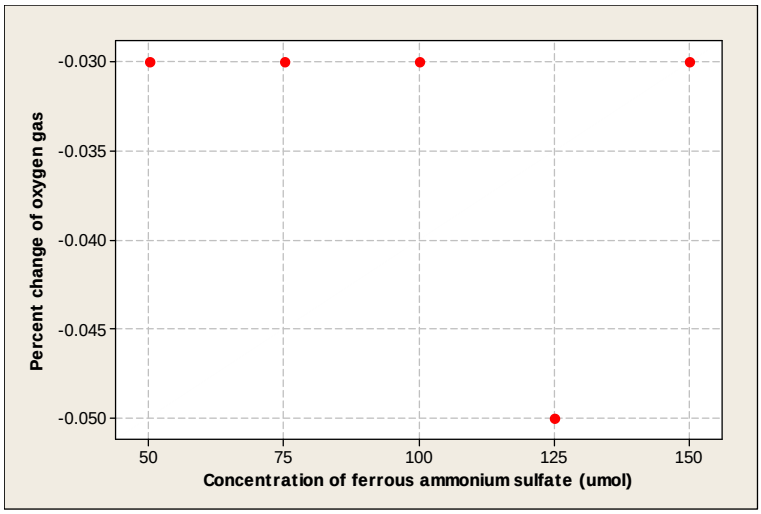

Figure 3. Concentration of ferrous ammonium sulfate (μmol) vs. the percent change of oxygen gas for each level in solutions of *Synechococcus sp.* during 0.75 of an hour growth period.

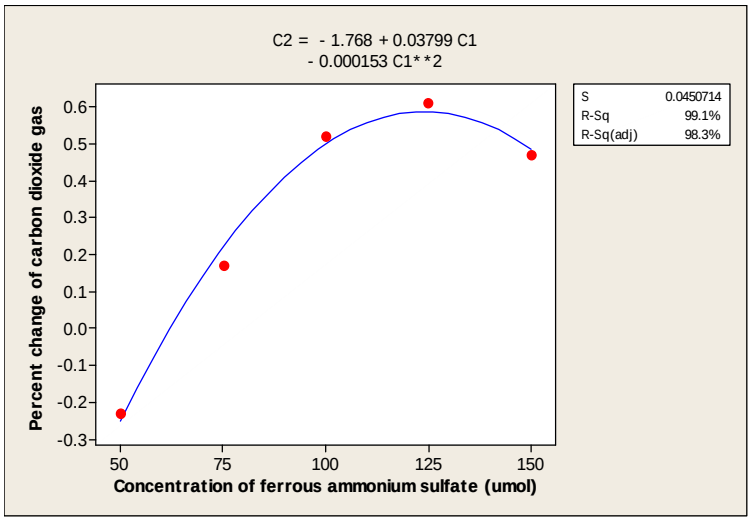

Figure 4. Concentration of ferrous ammonium sulfate (μmol) vs. the percent change of carbon dioxide gas for each level in solutions of *Synechococcus sp.* during 0.75 of an hour growth period.

4. DISCUSSION

This study evaluated the effects of iron on *Synechococcus sp.* CO_2 absorption and O_2 production. The research is important because iron-limited waters account for a significant part of the earth's oceans, so that an iron limitation in marine *Synechococcus* could lead to a shift from CO_2 to O_2 as the electron acceptor in photosynthesis. In turn, this could lead to higher levels of atmospheric CO_2. It was hypothesized that, at low ferrous ammonium sulfate concentrations, *Synechococcus sp.*, would less CO_2 and produce less O_2. At high ferrous ammonium sulfate concentrations the reverse was hypothesized. The hypothesis was only partially supported since low ferrous ammonium sulfate concentrations of were mostly associated with lower CO_2 (Figure 4). O_2 production was not affected by the ferrous ammonium sulfate level (Figure 3).

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The results of this study may have been affected by the following factors: (1) only one trial was performed and only 6mL of *Synechococcus sp.* culture was used at each ferrous ammonium sulfate concentration because of the limited amount of *Synechococcus sp.* culture available, and (2) the *Synechococcus sp.* was only exposed to each ferrous ammonium sulfate concentration for only 1hr because it was desired to carry out all experiments during the same day to prevent other more serious sources of error. A study such as this should be conducted in a natural iron-limited environment is which iron-fertilization program is being practiced.

6. ABBREVIATIONS AND ACRONYMS

CO_2 (carbon dioxide), O_2 (oxygen), HNLC (high nutrient/ low chlorophyll), m (meter), Gyr (Gigayear), NADPH (Nicotinamide adenine dinucleotide phosphate), ATP (adenosine triphosphate), PS (photosystem), ppm (parts per million), Na_2EDTA (sodium ethylene diamine tetra acetic acid), µmol (micromol), SOW (Synthetic Ocean Water), g (gram), C (Celsius), L (liter), mL (milliliter), µL (microliter), , minute (min.), hour (h).

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8. REFERENCES

Bailey, S., Melis, A., Mackey, K. R., Cardol, P., Finazzi, G., van Dijken, G., et al. (2008). Alternative photosynthetic electron flow to oxygen in marine *Synechococcus*. *Biochimica et Biophysica Acta, 1777*(3), (269-276). Retrieved July 8, 2008. doi:10.1016/j.bbabio.2008.01.002

Barsanti, L., & Gualtieri, P. (2006). *Algae: Anatomy, biochemistry, and biotechnology*. Boca Raton, Florida: CRC Press, Taylor & Francis Group.

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Carnegie Institution (2008, March 12). Startling Discovery About Photosynthesis: Many Marine Microorganism Skip Carbon Dioxide And Oxygen Step. *ScienceDaily*. Retrieved May 7, 2008, from http://www.sciencedaily.com /releases/2008/03/080311131851.htm

Coale, K. (2001, April). *Open ocean fertilization for scientific study and carbon sequestration*. Paper presented at American Society of Limnology and Oceanography (ASLO) Workshop. Retrieved February 1, 2009, from NETL Web site: http://www.netl.doe.gov/publications/proceedings/01/carbon_seq/6b1.pdf

Hanson, R. B., Ducklow, H. W., & Field, J. G. (Eds.). (2000). *The changing ocean carbon cycle: A midterm synthesis of the Joint Ocean Flux Study*. Cambridge, United Kingdom: Cambridge University Press.

Ivanov, A. G., Park, Y. I., Miskiewicz, E., Raven, J. A., Huner, N. P. A., & Oquist, G. (2000). Iron stress restricts photosynthetic intersystem electron transport in *Synechococcus* sp. PCC 7942. *FEBS Letters, 485*(2-3), (173-177). Retrieved July 8, 2008, from PubMed database. (11094162)

Kraemer, S. M., Butler, A., Borer, P., & Cervini-Silva, J. (2005, January). Siderophores and the dissolution of iron-bearing minerals in maine systems. *Reviews in Mineralogy and Geochemistry, 59*(1), 53-84. Retrieved February 1, 2009. doi:10.21318/rmg.2005.59.4

Michel, K.-P., & Pistorius, E. K. (2004). Adaptation of the photosynthetic electron transport chain in cyanobacteria to iron deficiency: The function of IdiA and IsiA. *Physiologia Plantarum, 120*(1), (36-50). Retrieved July 8, 2008.

Rueter, J. G., & Unsworth, N. L. (1991). Response of marine *Synechococcus* (Cyanophyceae) cultures to iron nutrition. *Journal of Phycology, 27*(2), (173-178).

Sandstrom, S., Ivanov, A. G., Park, Y.-I., Oquist, G., & Gustafsson, P. (2002). Iron stress responses in the cyanobacterium *Synechococcus* sp. PCC7942. *Physiologia Plantarum, 116*(2), (255-263). Retrieved July 8, 2008, from Wiley InterScience database. doi:10.1111/j.0031-9317.2004.0229.x

Tolbert, N. E., & Preiss, J. (Eds.). (1994). *Regulation of atmospheric carbon dioxide and oxygen by photosynthetic carbon metabolism* . New York: Oxford University Press.

Wilhelm, S. W., & Trick, C. G. (1995). Physiological profiles of *Synechococcus* (Cyanophyceae) in iron-limiting continuous cultures. *Journal of Phycology, 31*(1), (79-85).

Whitton, B. A., & Potts, M. (Eds.). (2000). *The ecology of cyanobacteria: Their diversity in time and space*. Dordrecht, The Netherlands: Kluwer Academic.

Xiong, J., & Bauer, C. E. (2002). Complex evolution of photosynthesis. *Annual Review of Plant Biology,*

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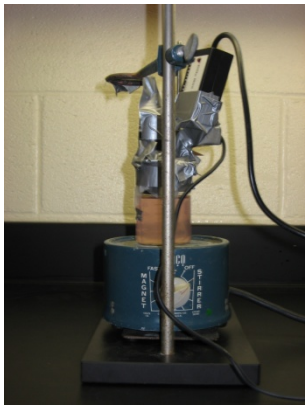
53(1), (503-521). Retrieved July 8, 2008. doi:10.1146/annurev. arplant.53.100301.13521

Yin, Y. (1999). Growth and photosynthesis in marine Synechococcus (cyanophyceae) under iron limitation. *Dissertation Abstracts International, 59* (8), (3888)B. (Publication number No. AAT 9901209) Retrieved July 8, 2008, from ProQuest database (732825851): http://proquest.umi .com/pqdweb ?did=732825851 &Fmt=2 &clientId=21321 &RQT=309 &VName=PQD

9. ANNEX



A. Apparatus- (solution on magnetic stirrer with probes assembled)

B. Note that anhydrous $CaCl_2$ was used instead of the hydrous form, which is $CaCl_2.2H_2O$. To obtain the correct concentration of $CaCl_2$ in the solution, the following calculation was performed.

1.54g * 110.99/ 146.99=
1.54g * 0.76=
1.17g $CaCl_2$

C. For a 1L stock solution of each, there should be 1.38g of $NaH_2PO_4.H_2O$ in a liter of dH_2O and 85.00g of $NaNO_3$ in a liter of dH_2O. The calculations are as follows.

$NaNO_3$:
1.380g/ 4.000=
0.345g $NaNO_3$ (in 250mL)
$NaH_2PO_4.H_2O$:
85.00g/ 4.00=21.25g $NaH_2PO_4.H_2O$ (in 250mL)

D. For a 1L stock solution of the $CuSO_4.5H_2O$, there should be 4.9g of $CuSO_4.5H_2O$ in a liter of dH_2O. To prepare a 250mL stock solution, the following calculation was performed.

4.900g/ 4.000=

1.225g $CuSO_4.5H_2O$ (in 250mL)

E. The number six was determined by the calculation below.

$\underline{6.11 * 10^{-3}g}$ * $\underline{1 \text{ tablet}}$ = $\underline{6.11 \text{ tablets}}$

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900mL 0.001g 900mL

F. This was determined by performing the following calculations.

$0.1\text{Mol} * \frac{392.16\text{g}}{\text{mol}} = 39.20\text{g Fe}(NH_4)_2(SO_4)_2.6H_2O$ (in 1L)

$(50 * 10^{-6}\text{M})(0.1\text{L}) = (0.1\text{M})(\text{XL})$ X= 50μmol (of the stock solution)

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