Chad Weldy participated as an intern in the Science Undergraduate Laboratory Internship Program at Pacific Northwest National Lab, where he researched lipid production by microalgae for the purpose of biodiesel fuel. His paper is titled "Lipid Production by Dunaliella salina in Batch Culture: Effects of Nitrogen Limitation and Light Intensity." Mr. Weldy's undergraduate major was environmental sciences — environmental toxicology, at Western Washington University (Bellingham, Washington). He is a member of the Air and Waste Management Association, and the Society of Environmental Toxicology and Chemistry. His interests include competitive ultimate frisbee, backcountry hiking, climbing, and snowboarding, and his hometown is Bainbridge Island, Washington.

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LIPID PRODUCTION BY DUNALIELLA SALINA IN BATCH CULTURE: EFFECTS OF NITROGEN LIMITATION AND LIGHT INTENSITY

Chad Share Weldy and Michael Huesemann

ABSTRACT

Atmospheric carbon dioxide (CO_2) concentrations are increasing and may cause unknown deleterious environmental effects if left unchecked. The Intergovernmental Panel on Climate Change (IPCC) has predicted in its latest report a 2°C to 4°C increase in global temperatures even with the strictest CO_2 mitigation practices. Global warming can be attributed in large part to the burning of carbon-based fossil fuels, as the concentration of atmospheric CO_2 is directly related to the burning of fossil fuels. Biofuels which do not add CO_2 to the atmosphere are presently generated primarily from terrestrial plants, i.e., ethanol from corn grain and biodiesel from soybean oil. The production of biofuels from terrestrial plants is severely limited by the availability of fertile land. Lipid production from microalgae and its corresponding biodiesel production have been studied since the late 1970s but large scale production has remained economically infeasible due to the large costs of sterile growing conditions required for many algal species. This study focuses on the potential of the halophilic microalgae species *Dunaliella salina* as a source of lipids and subsequent biodiesel production. The lipid production rates under high light and low light as well as nitrogen sufficient and nitrogen deficient culture conditions were compared for *D. salina* cultured in replicate photobioreactors. The results show (a) cellular lipid content ranging from 16 to 44% (wt), (b) a maximum culture lipid concentration of 450mg lipid/L, and (c) a maximum integrated lipid production rate of 46mg lipid/L culture*day. The high amount of lipids produced suggests that *D. salina*, which can be mass-cultured in non-sterile outdoor ponds, has strong potential to be an economically valuable source for renewable oil and biodiesel production.

INTRODUCTION

An increase in atmospheric carbon dioxide (CO_2) concentration may be causing a dramatic change to our climate and deleterious effects to the environment [1, 2, 3]. The Intergovernmental Panel on Climate Change (IPCC) has stated in its latest assessment that atmospheric CO_2 concentrations between 550ppm and 1,000 ppm will be reached by 2,100. Even with the most aggressive CO_2 mitigation practices, these concentrations would result in 2°C and 4°C rises in temperature, respectively [2,3]. The increase in atmospheric CO_2 is in large part the result of fossil fuel combustion as the rise in fossil fuel combustion is directly proportional to the rise in atmospheric CO_2 [2, 3]. Although prices for fossil fuels are increasing due to more expensive extraction practices as oil becomes more scarce, demand for fuel has increased. It has been previously reported that energy demand for transport world wide will nearly double by 2020 [4]. Demand for energy and fuel will not disappear, but the optimal mitigation scenario is to completely shift from fossil fuel use to renewable fuels that do not add CO_2 to the atmosphere. If CO_2 emission rates are not reduced or eliminated, unknown environmental damage will result [5]. The potential worldwide effects from global warming, the observed increase in average atmospheric and oceanic temperatures, has led to an increase in efforts to develop reliable, economically feasible, and sustainable alternative energy sources to replace coal and petroleum [6, 7].

Studies that strategize the complete phase out of fossil fuel use by 2100 include the extensive use of biofuels as one source of energy [7]. The IPCC has concluded that biofuel production can offset fossil fuel emissions by 10–20% by 2050 [7]. Currently, biofuel production has focused on terrestrial plants and ethanol

production from starches. Terrestrial plants have been popular for biofuel production because of the low price for cultivation and production [7]. Terrestrial plants are severely limited by land availability which inherently limits the amount of fuel which can be produced and utilized by humans. If biomass were to supply all primary energy currently used, the production of biomass would have to increase by a factor of seven. Currently, 30-40% of worldwide primary terrestrial productivity is appropriated for food, fiber, and energy [5]. It is simply infeasible to dramatically increase biomass production of terrestrial plants without causing a detrimental impact on the environment.

The production of microalgal lipids that can be synthesized into biodiesel has been of interest to the U.S. Department of Energy (DOE) since the energy shortage in the mid-1970s. The possibility for renewable biofuels led the DOE to establish a program to develop microalgae technology in the late 1970s [8]. The major areas of interest in microalgae research were to determine which strains of algae are capable of producing high amounts of lipids, and what environmental conditions lead to highest lipid yields.

While many microalgae species were found to be capable of producing high amounts of lipids, it was also determined that higher lipid concentrations can be obtained in nitrogen limiting culture conditions [9]. For example *Monallantus salina* was reported to produce as much as 72% lipids in nitrogen-deficient conditions [10]. The relationship between nutrient availability and algal lipid concentration has been understood for more than fifty years. Spoehr and Milner [16] were the first to report that lipid concentration of algal cells can be increased when cultured in nitrogen-deficient medium.

Although biofuels produced by photosynthetic microbes received interest and funding for research, it is currently believed to be infeasible due to the inefficiency and large costs of fuel production [7]. The Aquatic Species Program (ASP) of the U.S. National Renewable Energy Laboratory (NREL) invested US $25 million into renewable fuels produced by photosynthetic microbes over a period of 20 years, only to terminate funding in the late 1990s due to concerns over economic feasibility [11].

Algal biodiesel production was determined to be economically infeasible because large scale sterile bioreactors are very expensive and difficult to develop [7]. Sterile bioreactors are required for many species that are known to produce high amounts of lipids (e.g., *Cyclotella cryptica*, *Tetraselmis suecica*, and *Monoraphidium minutum*). Relatively inexpensive, large outdoor algal ponds are critical to economically worthwhile production from photosynthetic species. Outdoor culture ponds have been studied over the last four decades and ponds which lead to maximum growth have been developed. Feasible, non-sterile open pond production has been found for only three taxa: *Spirulina platensis*, *Dunaliella salina*, and *Chlorella* [12]. This is due to the extreme environments that they live in, e.g. *S. platensis* can grow in highly alkaline medium with a pH up to 10, *D. salina* can grow in salinity levels greater than 200g NaCl L^{-1}. Because these species grow in extreme conditions, parasitic contamination is avoided and maximum productivity can be achieved [7].

Very little research has been done on the effects of nitrogen limitation and light intensity on lipid content of one of these taxa, *D. salina*, which can be industrially cultured in outdoor growing ponds. The lipid content of *Dunaliella salina* has been previously published to be between 45% and 55% of its total weight [13], but few tests have been done regarding N-limitation or light intensity. There has been some effort to look at photosynthetic hydrocarbon production by *D. salina* in Korea [6, 11, 12, 14, 15], but all papers published were poorly written and the data were inconsistent. The results reported range in lipid content from 0.22 to 52.12 to 350mg hydrocarbons L^{-1}.

Recently, the Pacific Northwest National Laboratory has submitted *Dunaliella salina* for genome sequencing at the Joint Genome Institute (JGI). This will open possibilities for metabolic regulation research and the potential to better understand metabolic regulation and lipid production with the goal to optimize certain products.

This study has three objectives: First, to determine if *Dunaliella salina* can produce high amounts of lipids, second, to determine if nitrogen limitation increases the cell lipid content, and third, to determine if a higher light intensity that is more representative of an outdoor culture pond increases lipid production. It is the hypothesis of this study that *D. salina* produces lipids at rates that could make large scale biodiesel production economically feasible, and that lipid production rates will increase with higher light intensity and with decreased nitrogen available in solution.

The results of this research can have great importance. If *D. salina* is capable of producing significant quantities of biodiesel, the microalgae biodiesel could become a large factor in reducing CO_2 emissions by replacing limited oil based fuel sources and thereby reducing the potential deleterious effects of global warming.

MATERIALS AND METHODS

The green algae, *Dunaliella salina*, strain *CCAP1918*, from the *Culture Collection of Algae and Protozoa* (CCAP), was cultured in four 1L Roux bottles. Two bottles were under a 24 hour high light exposure (800µmol/m^2*sec) and two bottles were under a 24 hour low light exposure (200µmol/m^2*sec). The medium of one bottle at each light intensity had a high starting nitrogen concentration (20mM NO_3) and the other bottle at each light intensity had a low starting nitrogen concentration (2mM NO_3), Table 1. Bottles were sparged with CO_2 enriched air (0.5% v/v CO_2) at 30mL/min for the duration of the experiment by a Pasteur pipette inserted into the culture medium. During the entire experiment, temperature and pH were maintained at 28°C and 8.1 respectively. Over-heating was avoided by the use of cooling bottles placed between the light source and the culture bottles. The culture medium within the Roux bottles was mixed constantly by shaker table at 60rpm. The experiment was continued for roughly 13 days and was conducted in replicate at the same time. The culture medium contained a NaCl

	Low Light	High Light
Low Nitrogen	2 mM NO_3, 200µmol/m^2*sec	2 mM NO_3, 800µmol/m^2*sec
High Nitrogen	20 mM NO_3, 200µmol/m^2*sec	20 mM NO_3, 800µmol/m^2*sec

Table 1. Table of experimental conditions.

concentration of 1M. The culture medium composition is described in detail in Table 2.

D. salina cell growth was measured by ash free dry weight (AFDW) and optical density at a wavelength of 590nm (OD590). AFDW was determined by vacuum filtering a selected volume (V) of culture through a Whatman, 55mm, GF/F glass microfibre filter. The difference in weight between the filter after drying overnight in a 105°C oven (M_1) and after combusting the cells on the filter in a 550°C furnace for 30 minutes (M_2) was then divided by the volume of culture that was vacuum filtered, i.e., AFDW= (M_1-M_2)/V. All AFDW measurements were conducted in replicate and expressed in mg/L. Filters were pre-vacuum rinsed with 25ml of de-ionized water and then pre-ashed in a 550°C furnace for 15 minutes. Two culture medium blanks were used to correct any blank error in AFDW determination. All weight measurements were made with a Mettler Toledo AG135 four place analytical balance. Optical density (OD590) was measured by using a UNICO 1100 Spectrophotometer. All samples were diluted with blank medium to the model's linear range (<0.5 A) if needed.

Nitrate concentration was measured by conductivity using an Orion Model 420A along with an Orion 93 series electrode body. The instrument was calibrated using the same culture medium as in the experiment with varying known nitrate concentrations. Due to a high salt concentration in the medium, the sensitivity of the instrument was low and the data are used as reference only since it is not entirely reliable.

Lipid measurements were made using a method adapted from Bligh and Dyer [16]. This method extracts the lipids from the algal cells by using a mixture of methanol, chloroform, and water. A culture sample is collected at three points during the experiments for lipid analysis. The culture sample is centrifuged at 3,500rpm for 10 minutes in a large (200ml) plastic centrifuge tube, the pelleted cells along with 35ml of supernatant are then transferred to a glass centrifuge tube (50ml) to be re-centrifuged again at 3,500rpm for 10 minutes. The supernatant is removed by pipette. The pellet is

RECIPE FOR THE GROWTH MEDIUM OF *DUNALIELLA SALINA*
(MODIFIED FROM THE ORIGINAL MEDIUM RECIPE FROM DR. POLLE, 6/26/06)

(A) CONCENTRATED NUTRIENT MIX	Per 1 L
Distilled H_2O	500 ml
2 M Tris-HCl (FW:=57.6 g/mol; 315.2 g/L) Adjust to pH 7.5	200 ml
1 M $MgSO_4$ (FW=120.4 g/mol; 60.2g/0.5L)	50 ml
60 mM $CaCl_2$ (FW=111.1 g/mol; 3.33g/0.5L)	50 ml
20 mM KH_2PO_4 (FW=136.1 g//mol; 2.72g/L)	100 ml
0.4 mM $FeCl_3$ (FW=162.3 g/mol; 0.065 g/L)	
in 4 mM EDTA (FW=292.2 g/mol; 1.17 g/L) Adjust to pH 7.5	100 ml
(B) MICRONUTRIENT MIX	Per 1 L
3M H_3BO_3 (FW of anhydrous form=61.83g/mol; 9.27g/50ml)	50 ml
200 mM $MnCl_2$ (FW of tetrahydrate form=197.9 g/mol; 1.98g/50ml)	50 ml
16 mM $ZnSO_4$ (FW of heptahydrate form=237.5 g/mol; 0.19g/50ml)	50 ml
6 mM $CuCl_2$ (FW of dihydrate form=170.5 g/mol; 0.051g/50ml)	50 ml
40 mM Na_2MoO_4 (FW=241.95 g/mol; 0.484g/50ml)	50 ml
40 mM $NaVO_3$ (FW=121.9 g/mol; 0.244g/50ml) ORDERED	50 ml
4 mM $CoCl_2$ (FW of hexahydrate form=237.9 g/mol; 0.048g/50ml)	50 ml
Distilled water	650ml
Dissolve each ingredient separately before mixing. Heat $NaVO_3$ and H_3BO_3 to dissolve. Add individual solution one by one (from the list above, top to bottom) slowly in distilled water (650 ml).	
(C) BICARBONATE SOLUTION (1.0 M)	
Dissolve 84 g of $NaHCO_3$ (sodium bicarbonate, FW=84 g/L) in about 900 ml of distilled water. Then titrate to pH of 7.4 and afterwards fill with distilled water up to 1,000 ml.	
(D) SODIUM CHLORIDE SOLUTION (4M)	
Dissolve 233.6 g of NaCl in 1 L of warm distilled water to obtain a 4 M solution.	
(E) POTASSIUM NITRATE (KNO_3) SOLUTION (0.4 M)	
Dissolve 40.48 g KNO_3 in 1 L distilled water to obtain a 0.4 M solution.	
GROWTH MEDIUM (1.0 M NaCl)	Per 1 L
H_2O	550 ml
SODIUM CHLORIDE SOLUTION (D)	250 ml
CONCENTRATED NUTRIENT MIX (A)	100 ml
MICRONUTRIENT MIX (B)	1 ml
NITRATE SOLUTION (E)	50 ml
1.0 M $NaHCO_3$ (C)	50 ml
STERILE FILTER MEDIUM, AUTOCLAVE ROUX BOTTLES	

Table 2. Growth Medium for *Dunaliella salina*.

then resuspended with 4ml of DIH_2O, then 10ml of methanol and 5ml of chloroform is added, resulting in a 10:5:4 ratio of methanol: chloroform:water. At this ratio, all solvents are miscible and form one layer. After overnight extraction on a shaker table, 5ml of water and 5ml of chloroform are added which results in a 10:10:9 ratio of methanol:chloroform:water. Tubes are centrifuged for 10 minutes at 3,500rpm. At this solvent ratio, two layers are formed, a water-methanol upper layer and a chloroform lower layer. The chloroform lower layer which contains the extracted lipids is then removed by Pasteur pipette and placed into a pre-weighed vial. After the first extraction, 10ml of additional chloroform is added to conduct a second extraction. The additional 10ml of chloroform again results is a 10:10:9 methanol:chloroform:water ratio and two layers are formed. The tube is centrifuged at 3,500rpm for 10 minutes, and the lower chloroform layer is removed by Pasteur pipette and placed into another pre-weighed vial. The chloroform is evaporated by heating in a 55°C water bath under a constant stream of nitrogen gas. After 1 hour in a 105°C oven, vials are weighed again. The weight difference represents weight of lipids extracted from the culture sample. Percentage of lipid content can be determined by measuring the AFDW of the culture sample at the same time as the lipid analysis. The mass of cells used for the lipid analysis can be determined by multiplying the AFDW by the volume of culture used for the lipid sample. The weight of lipids extracted can then be divided by the mass of cells extracted to determine the % lipid content.

Fifty milliliters of culture were collected daily for AFDW, OD590 and nitrate determination. Lipid analysis was performed for the inoculum culture to derive a lipid concentration value at time "zero", and at three points during growth, at the early exponential phase, the late exponential phase, and the late stationary phase (end of experiment). All data were analyzed using Microsoft Excel 2003.

RESULTS

Growth

Biomass growth in both high and low light 2mM NO_3 cultures slowed when an AFDW of 400 (mg/L) was reached (Figures 1 and 2). The 20mM NO_3 cultures continued to grow but the high light 20mM culture grew to a much higher AFDW than the low light 20mM culture (Figures 1 and 2). The highest AFDW was measured in Replicate 1 at over 1,500mg/L.

Nitrate

The results show that there is a general decline in N concentration and that there is a large difference in N concentration between the N-sufficient cultures and the N-deficient cultures (Figures 5 and 6). The results also indicate that the high N cultures did not reach N-limitation, while the low N-cultures did.

Lipid Content

The % lipid content for *D. salina* ranged from 16–44% when in early exponential phase to early stationary phase, respectively. (Figures 7 and 8). In Replicate 2, the maximum lipid content was measured at 44% for N-deficient under high light while only 38% for N-sufficient under high light. In Replicate 1, lipid content was

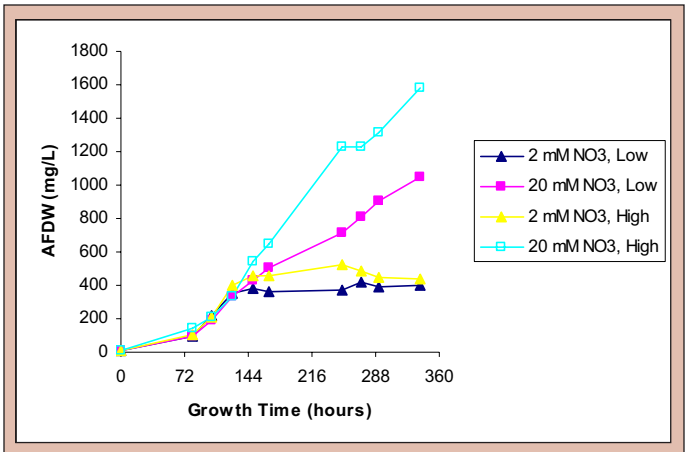

Figure 1. The growth of Replicate 1 expressed as AFDW (mg/L) over growth time (hours). The N-limited cultures halted growth at near 400mg/L. All values represent the average of two AFDW replicates.

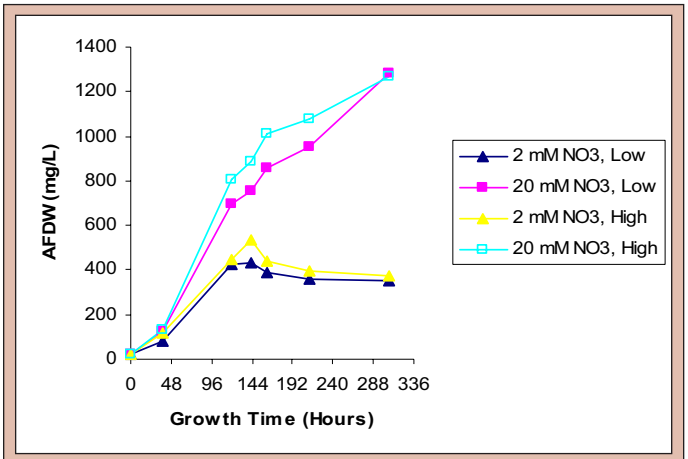

Figure 2. The growth of Replicate 2 expressed as AFDW (mg/L) over growth time (hours). All values represent the average of two replicates.

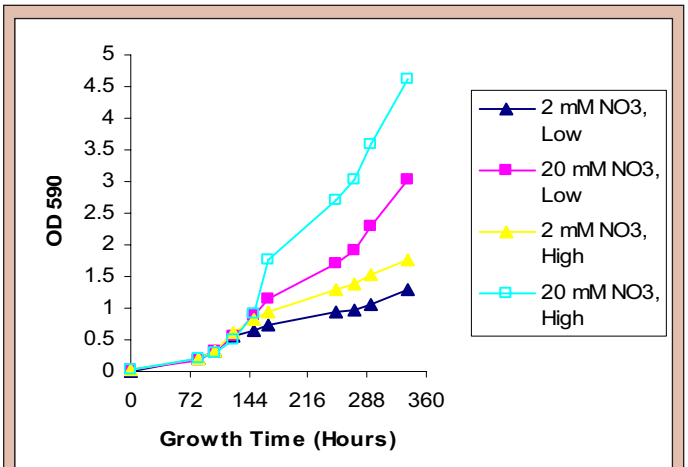

Figure 3. The optical density at wavelength 590nm for Replicate 1.

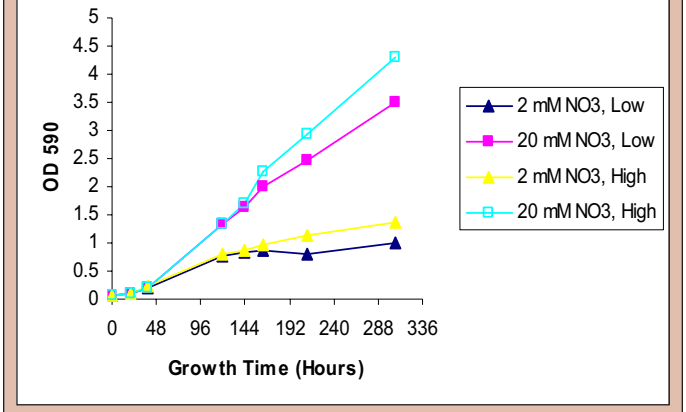

Figure 4. The optical density at wavelength 590nm for Replicate 2.

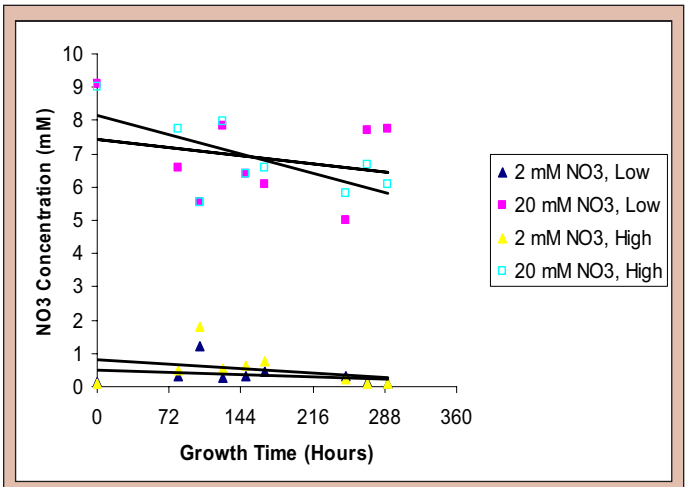

Figure 5. Nitrate concentration as a function of time for Replicate 1.

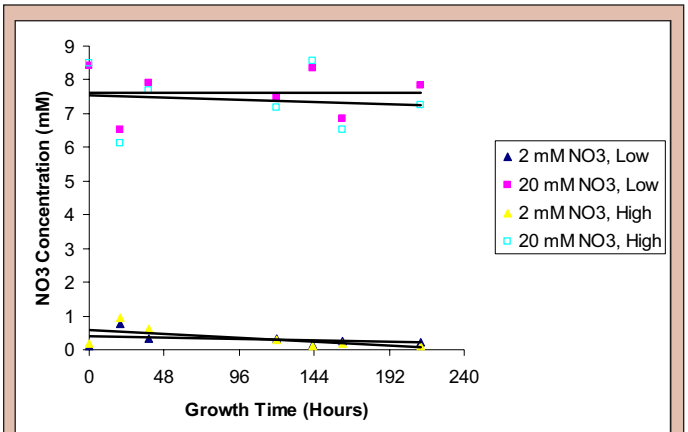

Figure 6. Nitrate concentration as a function of time for Replicate 2.

23% for the low light, N-sufficient culture while it was 30% for the high light, N-sufficient culture.

As shown in Figures 9 and 10, the highest lipid concentration is reached in N-sufficient medium under high light (Figures 9 and 10). Replicates 1 and 2 reached a maximum of 450 and 440mg lipid/liter culture respectively. This was reached in the high light 20mM NO_3 cultures. The highest lipid concentration for the low light cultures in N-deficient medium was recorded to be 110 and 135mg lipid/liter culture for Replicates 1 and 2 respectively (Figures 9 and 10).

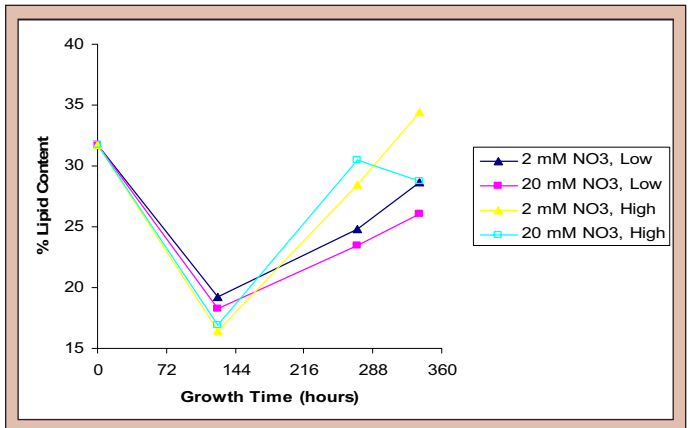

Figure 7. Percent lipid content as a function of growth time (hours) for Replicate 1. The minimum lipid content is 16% and the maximum is 34%.

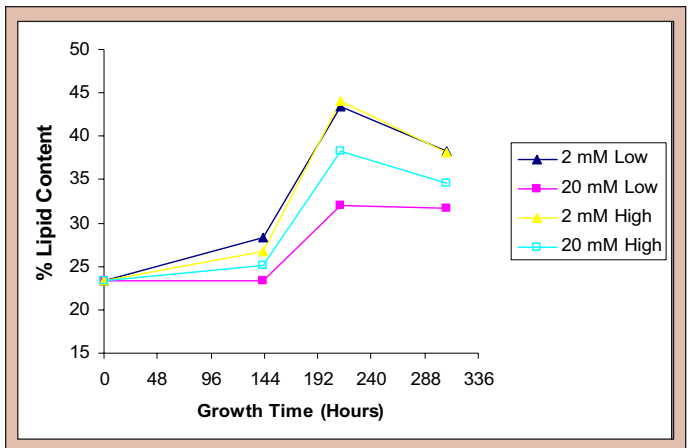

Figure 8. Percent lipid content as a function of growth time (hours) for Replicate 2. The minimum lipid content is 23% at inoculation and the maximum is 44%.

Lipid Production Rates

The rate of lipid production is expressed in mg lipid/ L*day. It is reported either as integrated lipid production rate over the number of total elapsed days (Figures 11 and 12) or lipid production rate between the (sampling) times of lipid analysis (Figures 13 and 14). The highest integrated rate of lipid production over total elapsed days was reached in Replicate 2 for the N-sufficient culture under high light. Lipid production was 46mg/L*day after about 9 days of growth. The highest lipid production rate found for Replicate 1 was

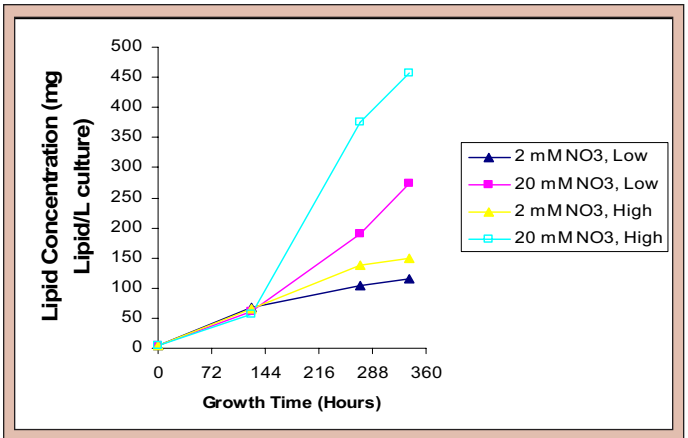

Figure 9. Lipid concentration in the culture medium over time (mg lipid/L culture) for Replicate 1. The highest lipid concentration was recorded for the N-sufficient, high light culture.

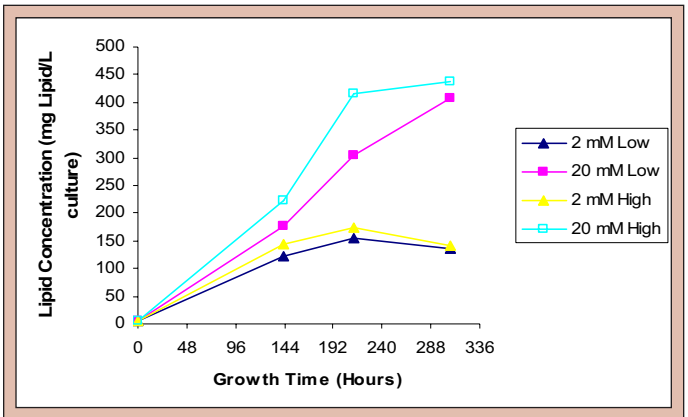

Figure 10. Lipid concentration in the culture medium over time for Replicate 2. Replicate 2 supports Replicate 1, i.e., the highest lipid concentration was recorded in high light and N-sufficient culture.

also in the N-sufficient culture under high light at 33mg lipid/L*day after 11 days (Figures 11 and 12).

Lipid production rates between the times of lipid analysis are also recorded in mg lipid/L culture*day. The rates are calculated for each time interval to determine when highest lipid production rates are achieved. Lipid production rates are greatest during high

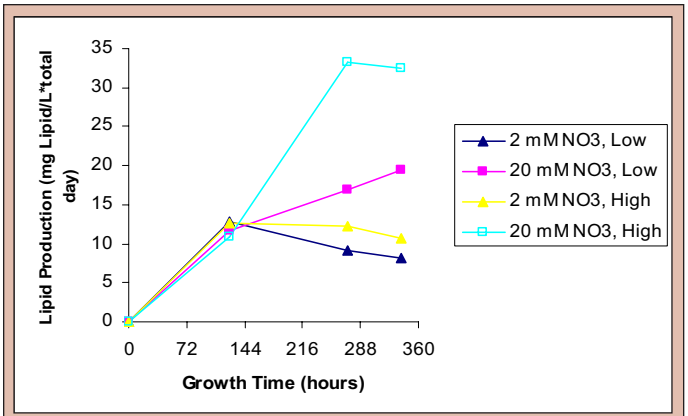

Figure 11. Integrated lipid production rate over total elapsed days for Replicate 1. The highest integrated production rate was recorded after 11 days for the N-sufficient, high light, culture at 36mg lipid/L*day.

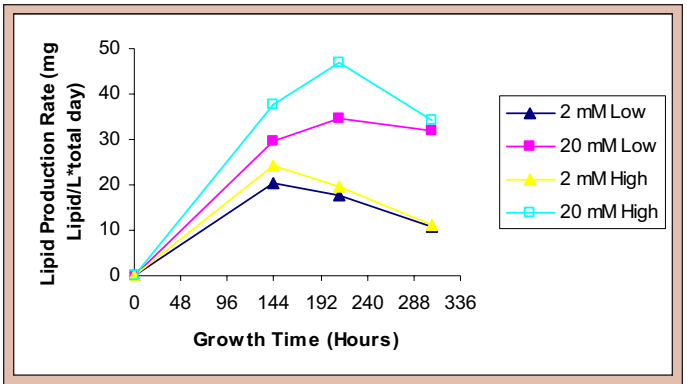

Figure 12. Integrated lipid production rate over total days for Replicate 2. The highest integrated lipid production rate was recorded after 9 days for the N-sufficient, high light, culture at 46mg lipid/L*day.

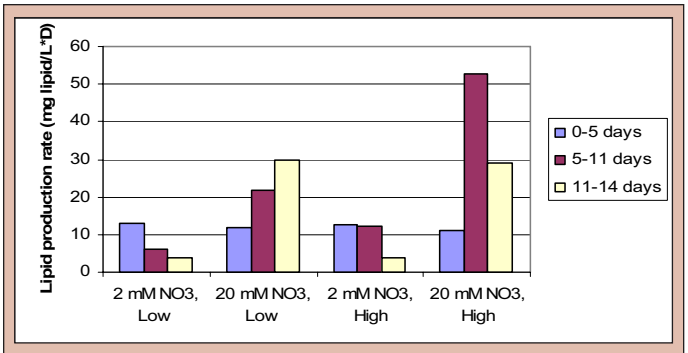

Figure 13. Lipid production rates between the times of lipid analysis for Replicate 1. The highest lipid production rate was achieved between days 5 and 11 at 52mg lipid/L*day.

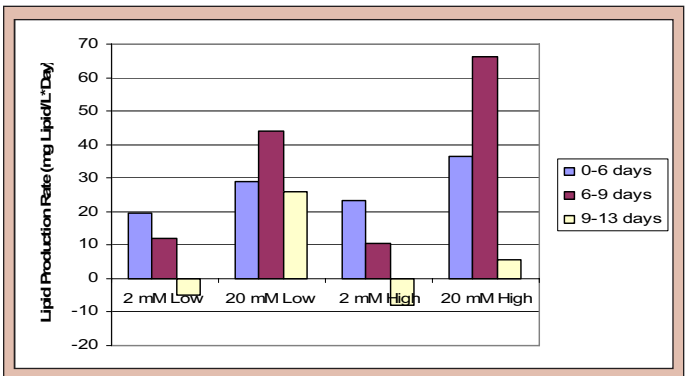

Figure 14. Lipid production rates between the times of lipid analysis for Replicate 2. The highest lipid production rate was for the N-sufficient, high light culture at 66mg lipid/L*day.

rates of biomass growth (Figures 13 and 14). The highest lipid production rate was recorded for Replicate 2 between day 6 and 9 of growth at 66mg lipid/Liter culture*day (Figure 14). As the algal culture reaches the late stationary phase and the death phase, the lipid production rate dramatically decreases (Figures 13 and 14). The highest lipid production for N-deficient cultures was recorded at 12mg lipid/L culture*day.

DISCUSSION

There are four conclusions that can be made from the results. First, the experimental results indicate that N-deficient cultures will develop higher % lipid content than N-sufficient cultures. Second, cultures under high light will develop higher % lipid content than cultures under low light. Third, culture lipid concentration and lipid production rates indicate that higher amounts of lipids are produced under N-sufficient conditions and high light due to higher biomass growth. Fourth, the % lipid content is less important than maximizing growth with respect to lipid production.

The results from this research suggest that actual lipid content of *D. salina* ranges from 15–45% depending on growing conditions and growth phase. This is less than the previously published 45-55% lipids by Tornabene et al. [13]. Lipid content data indicate that N-deficiency will increase lipid content for *D. salina*. There has been no published literature on this relationship with *D. salina*. Although the replicate tests differed in percent lipid content, they were very similar in general lipid trends and on lipid concentration of the culture, reaching a maximum of approximately 450mg lipid/liter culture for both replicates.

Currently, it is the general belief that large scale production of biodiesel from photosynthetic microalgae is economically infeasible due to the tremendous costs of sterile growing conditions [11]. But there has been little research on species which can grow in non-sterile conditions and their respective lipid production rates. Current microalgae biofuel research is focused on the species *Botryococcus braunii* due to its high lipid content [17], but little thought has been devoted to the fact that *B. braunii* requires sterile growing conditions and thus will not be economically feasible for large scale biofuel production. Not only does this species require sterile growing conditions, as this paper indicates, percent lipid content is not as important as actual biomass growth when considering total lipid production rates.

Future research for biodiesel production from microalgae must focus on species which can grow in non-sterile outdoor culture ponds. When considering the large scale production of biodiesel from microalgae, outdoor aquacultures are relatively inexpensive and efficient at maximizing the biomass growth of the species. Currently there are only three taxa which are known to be cultured in outdoor ponds, including *D. salina*. *D. salina* is currently mass cultured for beta-carotene production and results from this research suggest that *D. salina* can be cultured to produce high amounts of lipids (46mg lipid/L*day). Future research should examine the large scale production of lipids for biodiesel with *D. salina*, including an in depth economic analysis considering beta-carotene as a high value co-product.

Huntley and Redalje [7] mass-cultured *H. pluvialis* to determine lipid production rates and to perform an economic analysis on the large scale culturing of microalgae for biofuel production. Their results with *H. pluvialis* show lipid production rates equal to or less than those observed with *D. salina* in this study. Their average lipid production was 3.78g $m^{-2}d^{-1}$ grown in 12cm deep outdoor ponds, which is equivalent to 31.5mg lipid/L*day. Although Huntley and Redalje [7] cultured their species outside under natural diurnal (light/dark) lighting conditions, the light intensity outdoors can be twice as high as the high light intensity used in this study. The conclusion of the economic analysis of Huntley and Redalje [7] suggested that biodiesel mass production is economically feasible. They came to this conclusion by analyzing the lipid production rates and costs of culturing for their species with their method. They determined that the price of oil derived from microalgae would be $84/bbl. To mass culture *H. pluvialis*, Huntley and Redalje [7] used both sterile and non-sterile growing conditions, where they cultured the species under sterile conditions until inoculation of a large outdoor pond where the single species was being maintained.

Although *H. pluvialis* can potentially produce an economically feasible amount of biofuel, there are at least four reasons why *Dunaliella salina* would be better suited for large scale production of biofuel. First, *D. salina* produces higher amounts of lipids. The lipid production rate of *H. pluvialis* of 31.5mg lipid/L*day is nearly 30% lower than *D. salina* which was found to be near 45mg lipid/L*day. Not only does *H. pluvialis* have a lower lipid production rate, it has much lower percent lipid content than *D. salina* (25% compared to about 40%). Second, *D. salina* does not require sterile growing conditions. The cost of growing *H. pluvialis* under sterile conditions in the initial phase dramatically increases the cost of culturing compared to *D. salina* and its ability to be cultured in outdoor ponds. Third, *D. salina* is already currently mass produced in Australia, India, Israel, and USA for beta-carotene production. Finally, *D. salina* can synthesize beta-carotene as a high value co-product. The ability to increase sales by producing beta-carotene along with oil adds to the potential economic feasibility of mass culturing *D. salina*.

It is the opinion of the author that the halophilic marine microalgae *Dunaliella salina* has great potential to provide large quantities of hydrocarbons that can be converted into biodiesel. The need for renewable biofuels will continue to grow as fossil fuel reserves decline. Continued research leading to an economically viable process that produces biofuel and beta-carotene co-product from *Dunaliella salina* is suggested.

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