The effect of nitrogen fertilization on chlorophyll content and composition in the green algae *Neochloris oleoabundans*

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Plant Nutrition

Abstract

Chlorophyll concentration was monitored under different levels of nitrogen stress. Chlorophyll levels dropped to less than half the concentration found in replete nitrogen treatments. The drops in chlorophyll level was associated with decreased growth rates, but not decreased final densities. Carotenoid levels decreased in a similar manner to chlorophyll levels. The chlorophyll a: ratio also decreased in as nitrogen levels decreased.

Introduction

Commercial algae production has the potential to produce a number of beneficial products, such as fuel and food (protein and oils), as well as the capability for phytoremediation of wastewater (Mata et al 2010). The advantages of algae come from the fact that they are fast growing (not needing roots or vascular tissues), do not require soil (can be grown anywhere), and can be grown in any shape of container. Some algal species can reach lipid levels of over 50% of biomass by weight.

In order to fully exploit this potential, the limiting factors of these microbes must be understood. Algae, along with all other photosynthetic organisms, have two major limiting factors, inorganic nutrients, especially

nitrogen, and light. Consequentially, these two factors are related in a number of ways, one of which is that nitrogen content affects chlorophyll levels. This relationship is understood in higher plants, as higher nitrogen levels produce higher levels of chlorophyll in leaves, and nitrogen stress induces chlorosis.

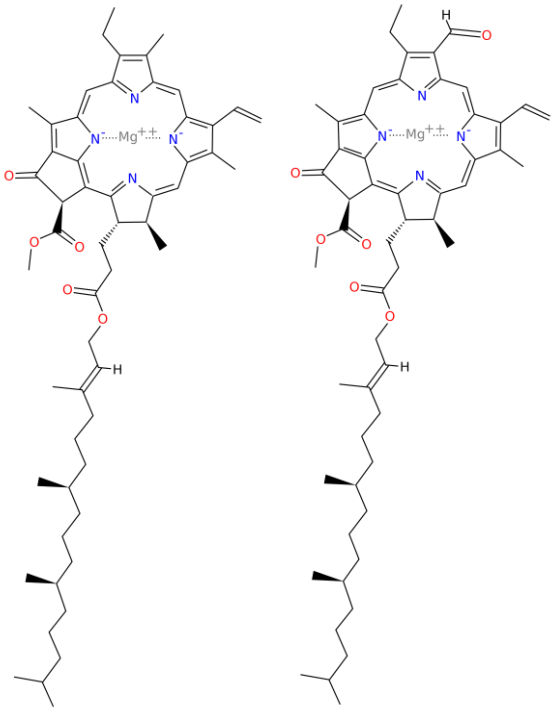

Illustration 1: Molecular diagrams of chlorophylls a (left) and b (right)

As seen in Illustration 1, the difference between chlorophyll a and b is the addition of a hydroxyl group in chlorophyll b. Chlorophyll a is actually the precursor to b, being only one enzymatic transformation away. As reviewed extensively in Green & Durnford (1996) chlorophyll a is the pigment common to all photosynthetic organisms, and is associated with Photosystem II. In contrast chlorophyll b is common to only a few organisms most notably green algae and all plants, this pigment is associated with the light harvesting complexes (LHC II has a chlorophyll a:b ratio of 1.3-1.4) (Green & Durnford 1996). Carotenoids, which include compounds such as β-carotene and lutein's, are generally associated with protection of the photosynthetic complexes from excess light, along with some light harvesting, and photosynthetic complex structure(Green & Durnford 1996).

The chlorophyll a:b ratio gives an indication of the PSII to LHC II ratio, and therefore the amount of water splitting capabilities relative to light harvest. In higher plants, there are multiple models for optimal partitioning of nitrogen in respect to photosynthesis and growth (Anten et al. (1995) and Hikosaka &

Terashima (1995) for example). Chlorophyll a:b ratio strategies have not been extensively explored in algae.

This study focuses on the effect of nitrogen fertilization on chlorophyll content and composition. As nitrogen stress increases, chlorophyll content, as well as associated photosynthetic pigments such as carotenoids, are expected to decrease. The chlorophyll a:b ratio will be monitored in an effort to understand the nitrogen allocation strategies of algae under stress conditions.

Methods and Materials

One liter glass tube bioreactors were inoculated with 30 ml of *Neochloris oleoabundans* culture. The USU Research Greenhouse Freshwater media was used, with KNO_3 added to give four nitrogen treatments: 3, 6, 9, and 12 mM N. The experiment had two replications, giving 8 total bioreactors. Each bioreactor was mixed by aeration, with a 1% CO_2 enrichment. Nitrogen levels were measure at the end of the study using a nitrate test strip.

Density was determined by measuring optical density at 750 nm and converted to g/l using a correlation developed by Curtis Adams (unpublished data). To extract chlorophyll, 3 ml of algal solution containing between 0.3 to .6 mg of biomass was filtered onto glass microfiber filter paper. The filter paper was then placed in 10 ml of DMSO and heated to 60° C for 20 minutes. chlorophylls a and b, and total carotenoid concentrations were quantified using optical density as described by Wellburn (1994).

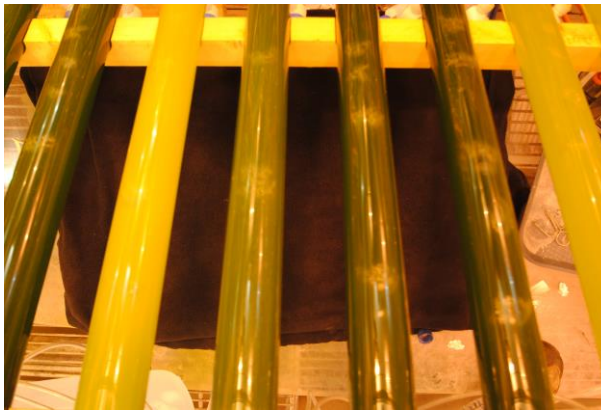

Illustration 2: A photo of the 1 liter bioreactors. Changes in color are due to changes in chlorophyll concentration.

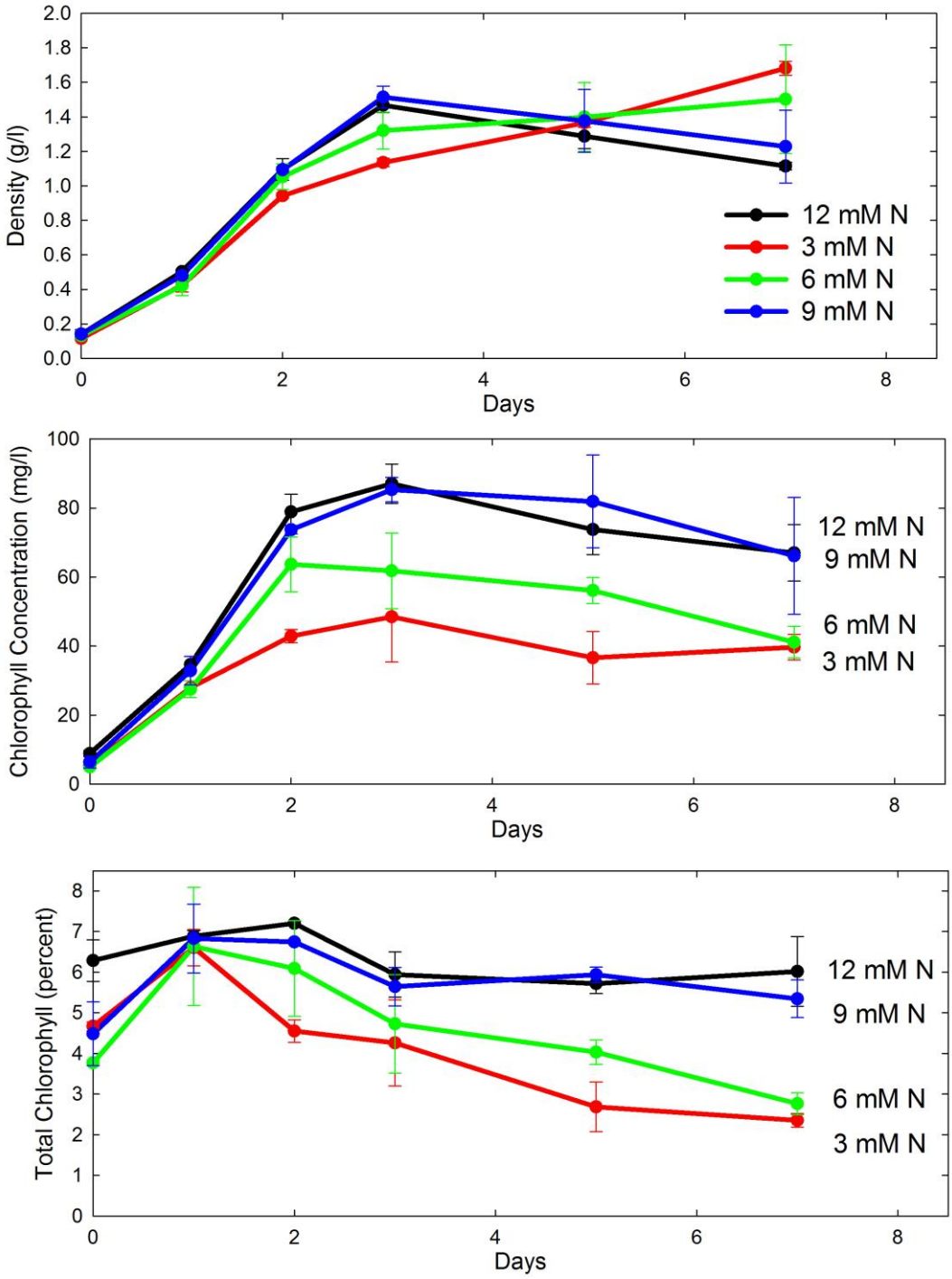

Figure 1: Density and chlorophyll over time
Top: Biomass density in grams per liter
Mid: Chlorophyll concentration in mg total chlorophyll per liter
Bottom: Total chlorophyll as a percent of biomass

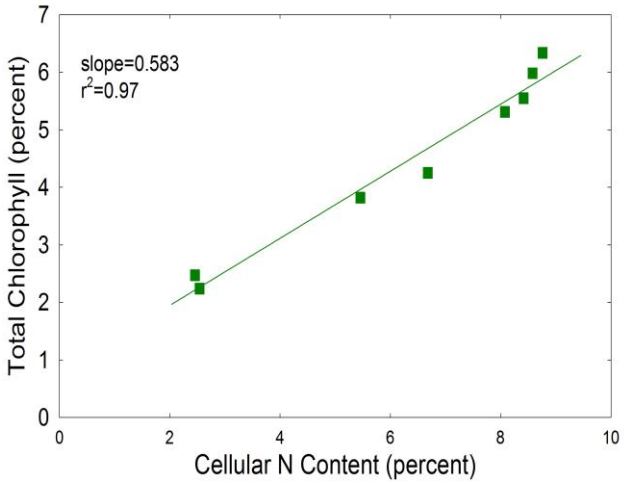

Figure 2: Total chlorophyll as a response to cellular nitrogen content, both as a percentage of biomass, at peak density

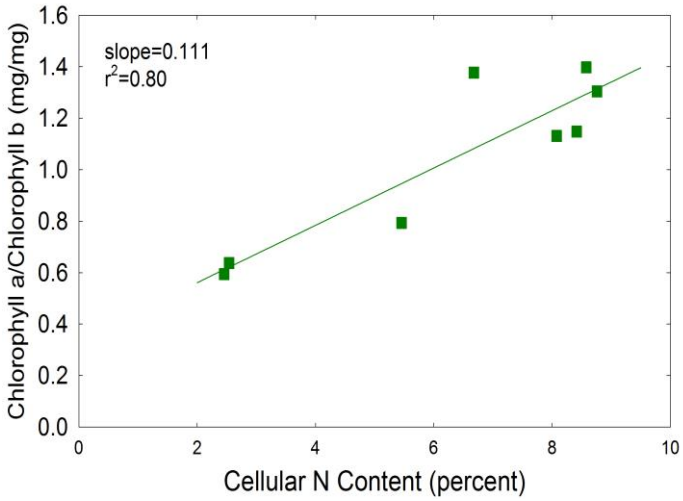

Figure 3: The ratio of chlorophyll a to chlorophyll b as a response to cellular nitrogen, as a percent of biomass, at peak density

Results

As seen in Figure 1 (top), all cultures reached similar final densities. All nitrogen treatments depleted the solution nitrogen by the end of the study, except the 12 mM treatment. Due to differences in growth rate, the data selected for Figures 2-5 were at final densities. All chlorophyll and carotenoid levels increased linearly with cellular nitrogen content. The chlorophyll a to b ration also increased linearly with cellular nitrogen content. The slopes of chlorophyll a and b in Figure 4 were 0.40 and 0.18 respectively.

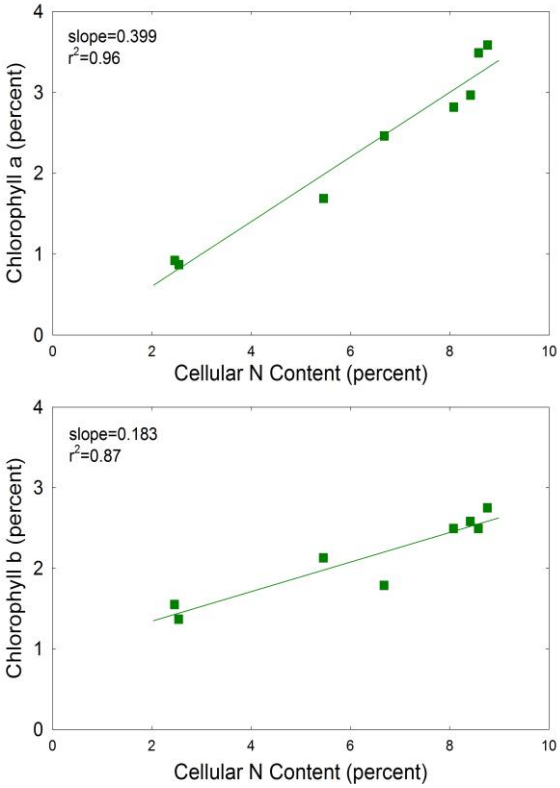

Figure 2: Above: chlorophyll a as a response to cellular nitrogen, both as percent of biomass, at peak density Below: chlorophyll b as a response to cellular nitrogen, both as percent of biomass, at peak density

Discussion

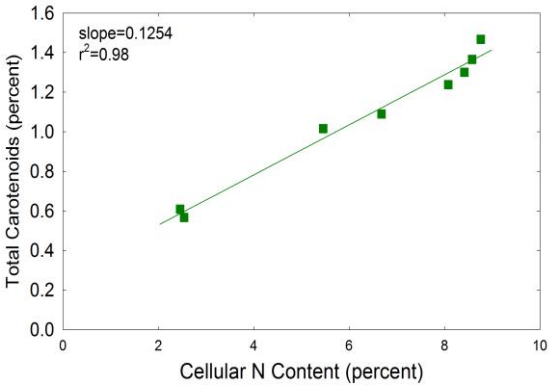

Figure 3: Total carotenoids as a response to cellular nitrogen, both as percent of biomass, at peak density.

Figure 1 (top) shows that the peak densities were similar indicates that nitrogen limitations were not so severe that growth potential was limited, although it should be noted that the replete nitrogen treatments (9 and 12 mM N) reached peak density in about half the time, resulting in higher biomass productivity. The lower N treatments (3 and 6 mM N) had longer sustained growth, whereas the replete N treatments saw declines in the latter half of the study. These declines were due to settling of algae, thought the reason for settling was not examined.

Chlorophyll levels reached a peak, followed by a slow decline (Figure 1 mid). This can be attributed to either a decline in cellular N content as cultures continue to grow, or in the case of the replete nitrogen treatments a decline in biomass due to settling. All cultures showed approximately the same percentage of chlorophyll (Figure 1 bottom) by day one (which could be attributed to the baseline replete chlorophyll content), followed by a slow decline in the 3 and 6 mM N treatments. This decline is due to greater N limitation per cell as the culture grows and runs out of nitrogen.

The percentage of chlorophyll in relation to biomass increased linearly as a function of cellular N content (Figure 2), this is as expected based on the relationship in higher plants. The 12 mM N treatment never ran out of nitrogen, so the increase in nitrogen fertilization did not increase chlorophyll concentration, indicating the saturation point in our system was 6% N. This

could be a useful benchmark in other studies, though different systems may see different results.

Chlorophyll a to b ratios increased linearly with increasing nitrogen content. The shift in this ratio was due to an increase in chlorophyll a, as the rate of increase of chlorophyll a was more than double that of chlorophyll b. This would suggest that as the nitrogen content of the cell increases, more resources are allocated to the conversion of light energy to chemical energy, relative to the resources allocated to light harvest. It's hard to say what advantage this strategy has for the algae.

Carotenoids decreased with increase nitrogen stress, which was expected. As chlorophyll levels drop, their associated compound should drop as well.

Future research should be conducted to verify that these patterns are consistent in other species of algae. The effect of varying light levels could also give further understanding as to the nitrogen/chlorophyll allocation strategies. In high light conditions, would the decrease in chlorophyll be as pronounced. The effect light level has on chlorophyll a/b ratios would also help in understanding what advantages varying chlorophyll a/b ratios have. The effect of chlorophyll on growth rate could also be further studied, with a better characterization of biomass productivity and chlorophyll levels.

Literature Cited

Mata, T. M., Martins, A. A. & Caetano, N. S. (2010) Microalgae for biodiesel production and other applications: A review. *Renewable and Sustainable Energy Reviews* **14**, 217–232

Green, B. R. & Durnford, D. G. (1996) The Chlorophyll-Carotenoid Proteins of Oxygenic Photosynthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47**, 685–714

Anten, N. P. R., Schieving, F. and Werger M. J. A. (1995) Patterns of Light and Nitrogen Distribution in Relation to Whole Canopy Carbon Gain in C_3 and C_4 Mono- and Dicotyledonous Species. *Oecologia*, Vol. 101, No. 4 (1995), pp. 504-513

Hikosaka K, Terashima I (1995) A model of the acclimation of photo-synthesis in the leaves of C3plants to sun and shade with respect to nitrogen use. Plant Cell Environ 18:605–61

Wellburn, A. R (1994) The Spectral Determination of Chlorophylls a and b, as well as Total Carotenoids, Using Various Solvents with Spectrophotometers of Different Resolution. J Plant Physiol 144: 307-313