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EFFECT OF NITROGEN SUPPLY IN CULTURE MEDIA AND LIGHT INTENSITY ON PHOTOSYNTHESIS OF *Chlamydomonas reinhardtii*

Pengaruh Suplai Nitrogen pada Media Kultur dan Intensitas Cahaya Terhadap Proses Fotosintesis *Chlamydomonas reinhardtii*

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Abstract
Organisms use nitrogen to produce, among others, amino acids, proteins, and nucleic acids. In this study, the effects of various concentrations of ammonium in culture media on the photosynthetic performance of Chlamydomonas reinhardtii *were done under two light conditions: low and high intensity. The microbes were grown at low (75% NH_4Cl dosage), normal (100% NH_4Cl dosage, which was 2 M NH_4Cl), and high (125% NH_4Cl dosage) nitrogen content. Cells density and chlorophyll content were quantitatively determined. Immunoblotting technique was used to separate proteins based on molecular mass. In both low and high light intensity, cells grown in 75% NH_4Cl dosage culture medium showed lower cell density and chlorophyll concentration than those grown in 100% and 125% NH_4Cl dosage media. The later two media produced almost the same amount of cell density and chlorophyll concentration. In conclusion, 75% NH_4Cl dosage was insufficient for* C. reinhardtii *cells to grow well. The results also showed that accumulation of photosystem I (PsaA and PsaD/F) and light harvesting complex II (LHCII) were higher in low light than in high light intensity.*

Keywords: Chlamydomonas reinhardtii*, chlorophyll, light intensity, nitrogen, photosynthesis*

Abstrak
Organisme menggunakan nitrogen diantaranya untuk memproduksi asam amino, protein, dan asam nukleat. Dalam percobaan ini pengaruh berbagai konsentrasi amonium dalam media pada fotosintesis *Chlamydomonas reinhardtii* dilakukan di bawah dua kondisi cahaya: intensitas rendah dan tinggi. *C. reinhardtii* ditumbuhkan dalam medium dengan dosis nitrogen (N) rendah (75% dosis NH_4Cl), normal (100% dosis NH_4Cl, yakni NH_4Cl 2 M), dan tinggi (125% dosis NH_4Cl). Parameter yang diukur adalah kepadatan sel dan konsentrasi klorofil. Analisis protein dilakukan dengan imunobloting untuk memisahkan protein berdasarkan massa molekul. Pada intensitas cahaya rendah dan tinggi, sel-sel pada medium dengan 75% NH_4Cl menunjukkan kepadatan sel dan konsentrasi klorofil lebih rendah dibandingkan dengan 100% NH_4Cl dan 125% NH_4Cl, di mana kedua media ini menghasilkan kepadatan sel maupun konsentrasi klorofil yang hampir sama. Dengan demikian dapat disimpulkan bahwa 75% NH_4Cl tidak cukup bagi *C. reinhardtii* untuk tumbuh dengan baik. Selain itu, akumulasi fotosistem I (PsaA dan PsaD/F) dan kompleks pemanenan cahaya II (LHCII) lebih tinggi pada sistem fotosintesis dengan intensitas cahaya rendah dibandingkan cahaya tinggi.

Kata kunci: *Chlamydomonas reinhardtii*, fotosintesis, intensitas cahaya, klorofil, nitrogen

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INTRODUCTION

Chlamydomonas is commonly used as a model organism in the efforts to understand the basic principles of biological processes such as chloroplast inheritance, photosynthesis and biology, carbon and nitrogen metabolism as well as nutrient deprivation. A number of factors have been known to influence cellular physiology and fat metabolism in algae. One of them is nitrogen availability (Chen et al. 2011; Forde, 2002; Ikaran et al. 2015; Juergens et al. 2015; Lardon et al. 2009). Altering the ratio of carbon to nitrogen changes the control of nitrogen assimilation, photosynthetic capacity, starch and sugar accumulation, and also activities of metabolic enzymes (Ball et al. 1990; Li et al. 2011). At the same time, algae slow down their growth and turn their metabolic state towards the cellular structure protection by synthesizing more lipids (Wang et al. 2009).

Extreme modifications in the availability of macronutrients triggered large alterations in gene expression as well as in biochemistry in the photosynthetic alga. Metabolism in *Chlamydomonas* readily gives response to total medium nitrogen availability with temporal rises in short-chain free fatty acids and turnover of internal proteins, long before nitrogen resources are depleted (Lee et al. 2012). In different circumstances, nitrogen-starved *D. tertiolecta* was shown to produce only 1% of dry cell weight (DCW) in neutral lipids, whereas starch was quickly accumulated up to 46% DCW. The rise in starch content occurred in parallel with a coordinated overexpression of genes switching carbon towards starch synthesis, a response not seen in the oleaginous microalgae *Nannochloropsis oceanica*, *Chlamydomonas reinhardtii* or *Chlorella vulgaris* (Tan et al. 2016).

Flexibility in the metabolism of microalgae has been known that a comprehensive description of the interaction between the various metabolic pathways is a complicated undertaking. But, there are restrictions that administer central carbon metabolism in *C. reinhardtii* that are demonstrated by the compartmentalization and regulation of the pathways and their relation to important cellular processes like cell motility and division, carbon uptake and partitioning, internal and external rhythms, as well as nutrient stress (Johnson and Alric, 2013). The aim of this experiment was to find out the effect of nitrogen source (ammonium chloride supply) and light intensity on the photosynthetic activities of *C. reinhardtii*.

MATERIALS AND METHODS

Cell culture and harvest

The *C. reinhardtii* wild type (WT) strain was used in this study. The strain was cultivated in tris acetate phosphate (TAP) medium (Harris 1989) at 25°C under constant illumination of cool-white fluorescent bulbs at an irradiance of 400 µmol m^{-2} s^{-1} and with continuous shaking at 100 rpm. Strain stock (WT) was used to inoculate a starter culture, which was harvested at late log-phase and used to inoculate a new culture at a starting density of 6.0 x 10^5 cells mL^{-1}. All cell numbers were counted by using a Hemacytometer.

These experiments were done at two treatments of light intensity, namely low (40 µmol m^{-2} s^{-1}) and high (300 µmol m^{-2} s^{-1}) (Figure 1) light intensity. The *C. reinhardtii* strains were grown at 75% (=1.5 M NH_4Cl), 100% (=2 M NH_4Cl) and 125% (=2.5 M NH_4Cl) of the normal NH_4Cl dosage.

One millilitre of cell culture was harvested at different points of growth phase (24, 33, 48, 72, and 96 h), transferred into microtubes, spun down and the pellets were stored at -80°C for further analysis. A half millilitre of cell culture was taken and quantified to plot growth curves based on cell density (cells·mL^{-1}) and chlorophyll content (µg·mL^{-1}). Chlorophyll concentration was measured by spectrophotometer using the following equation:

Chlorophyll concentration = Chlorophyll (a + b)

Protein analyses

Chlorophyll-protein complexes separation was carried out according to the method of Ozawa et al. (2010). Proteins were analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) method and were electrophoretically blotted onto nitrocellulose filters, probed with antibodies, and visualized by enhanced chemiluminescence (ECL). Signals on nitrocellulose membranes were detected

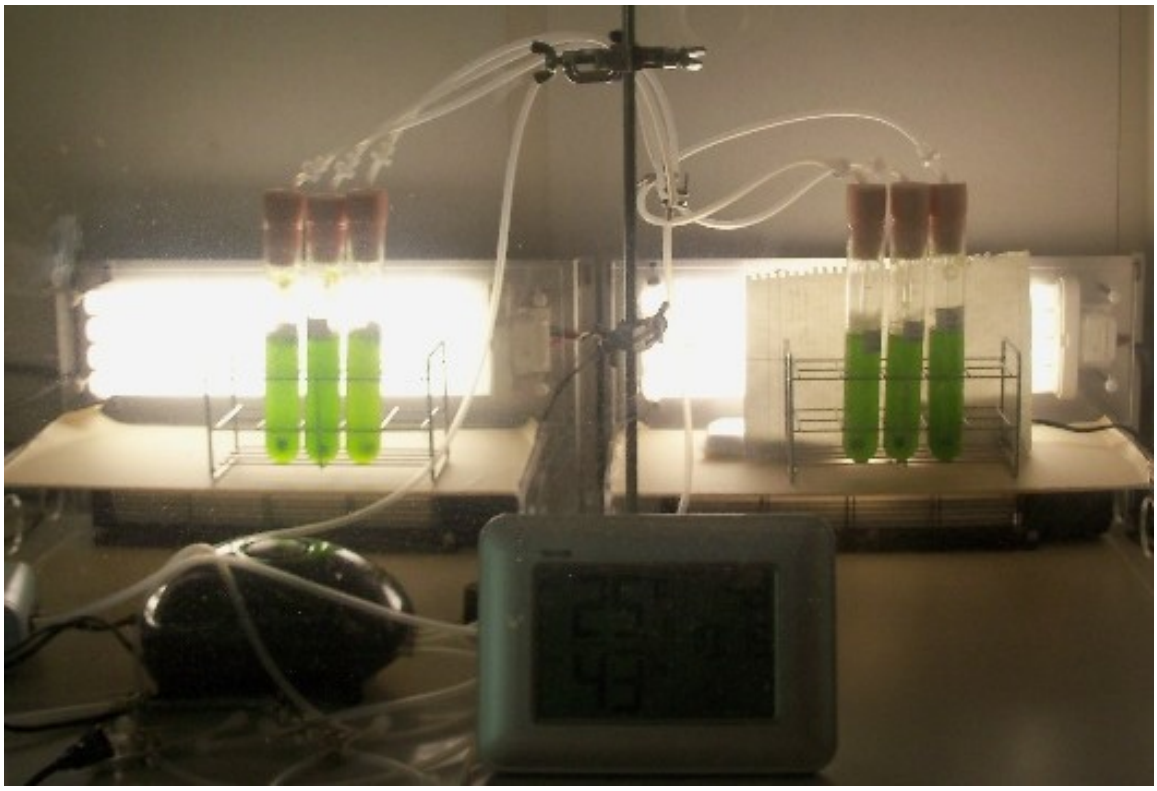

Figure 1. *C. reinhardtii* cultures grown in two light conditions: low and high light intensity

using a LAS-4000 mini luminescent image analyzer (Fujifilm, Tokyo) (Ozawa et al. 2010).

RESULTS AND DISCUSSION

In this study the effects of various concentrations of ammonium chloride in the culture media of *C. reinhardtii* on the performance of photosynthesis were measured. These microbes were grown at low (75% of NH_4Cl normal dosage), normal (100% of NH_4Cl normal dosage) and high (125% of NH_4Cl normal dosage) concentrations. Measurements were made on the cell density and chlorophyll content. In addition, analysis using immunoblotting technique to separate photosystem proteins based on their molecular mass was carried out.

Cell density for all cases showed positive correlation with time (Figure 2). For the cultures grown in the media containing 100% and 125% NH_4Cl dosage, the cells density increased steadily until 72 h, then became roughly stable, or slightly declined, until 96 h. An exception was noted for low light growth with 125% NH_4Cl, where the cell density kept increasing until 96 h. At 96 h, all the growth curves for high light condition had higher peaks than their counterparts for low light, indicating high light promoted higher biomass formation. In addition, steeper slopes were observed for high light than that for low light during 48-72 h growth period, showing faster growth in high light setting.

There was a similarity between the two light treatments, however, in terms of the effect of the nitrogen source. Higher NH_4Cl concentration cell density tended to promote higher cell density throughout growth period, with the exception of 75% NH_4Cl dosage supplementation. This least nitrogen concentration caused a lag phase during the first 24 h of cell growth, with more severe effect seen in high light condition. This suggests that during early culture period, just after inoculation was done until about 24 h, the use of low light intensity might support better growth and avoid higher cell mortality.

Based on the curves relating chlorophyll content to growth period (Figure 3), it can be seen that in low light condition chlorophyll concentration increased considerably from 33 to 96 h, but with continuously decreasing gradients. On the contrary, during the same growth period but high light intensity, chlorophyll concentration rose steadily with approximately constant

slope. At the end of 96 h, low light condition gave higher chlorophyll concentration than the high light did. It is important, however, to note that the *C. reinhardtii* cells grown in 75% NH_4Cl dosage-containing medium with high light intensity lost their chlorophyll content after being cultured for 72 h.

In low light intensity, antenna (LCHII) could harvest more light than in the high light intensity, so the concentration of chlorophyll became higher and the culture became greener. High light antenna photosystem could not be better in harvesting light, so the culture became less green. In both low and high light conditions, cells growing in 75% NH_4Cl culture medium showed lower cell density and chlorophyll concentration compared to those grown in 100% NH_4Cl and 125% NH_4Cl culture medium. Both 100% NH_4Cl and 125% NH_4Cl culture media produced almost the same cell density and chlorophyll concentration.

According to Gargouri et al. (2017), nutrient deprivation leads to significant stress to the *C. reinhardtii* which reacts by significantly modifying its metabolic program. After N deficiency, the starch and triacylglycerols (TAGs) accumulation is

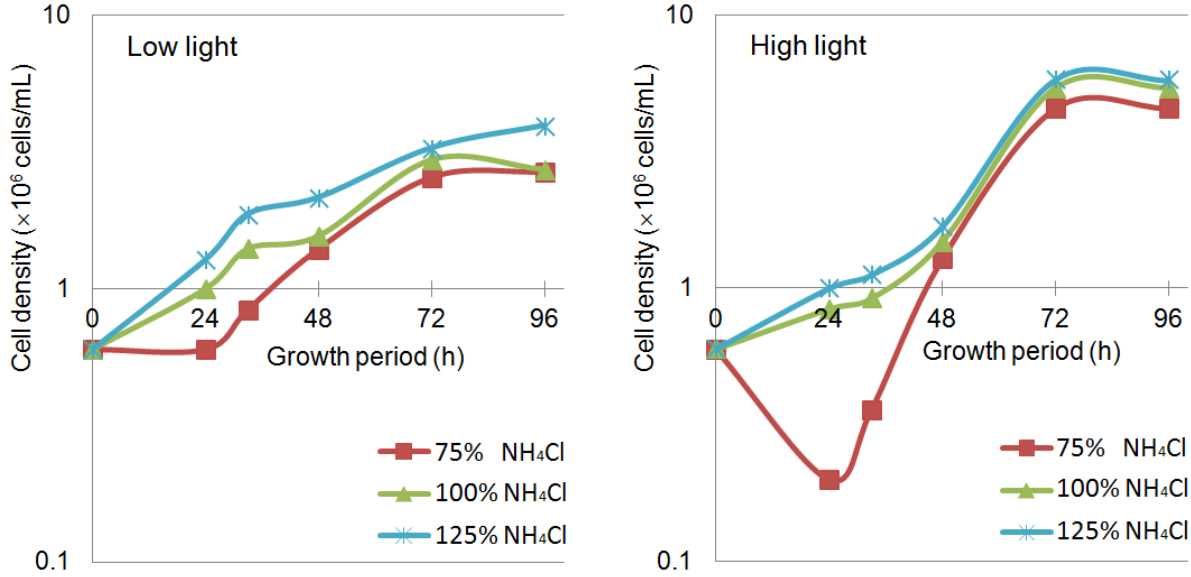

Figure 2. Growth profile in relation to cell density

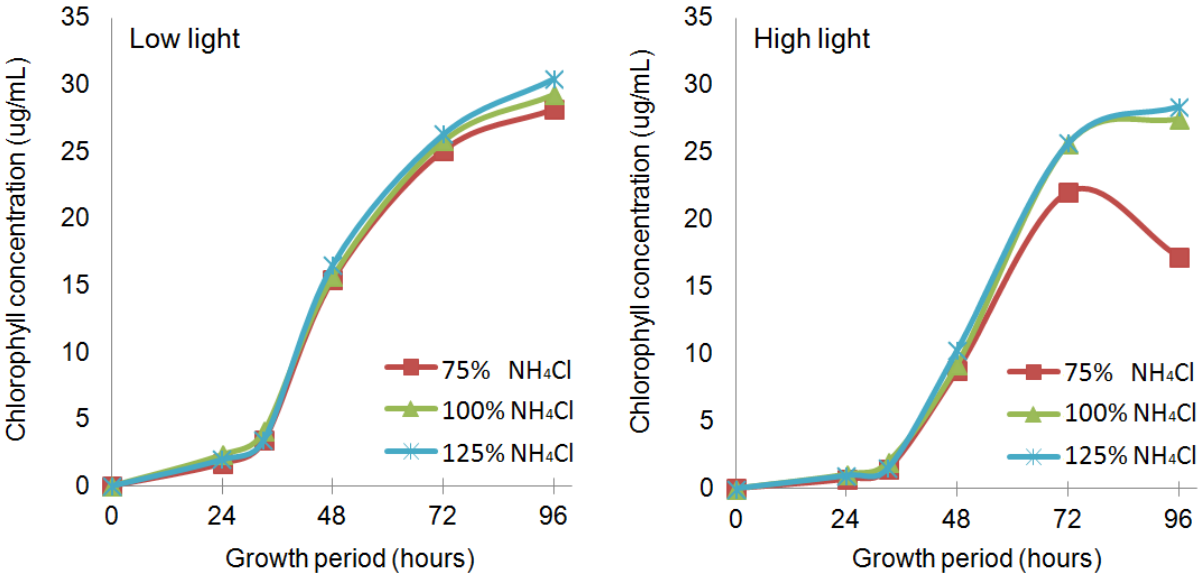

Figure 3. Growth profile in relation to chlorophyll content

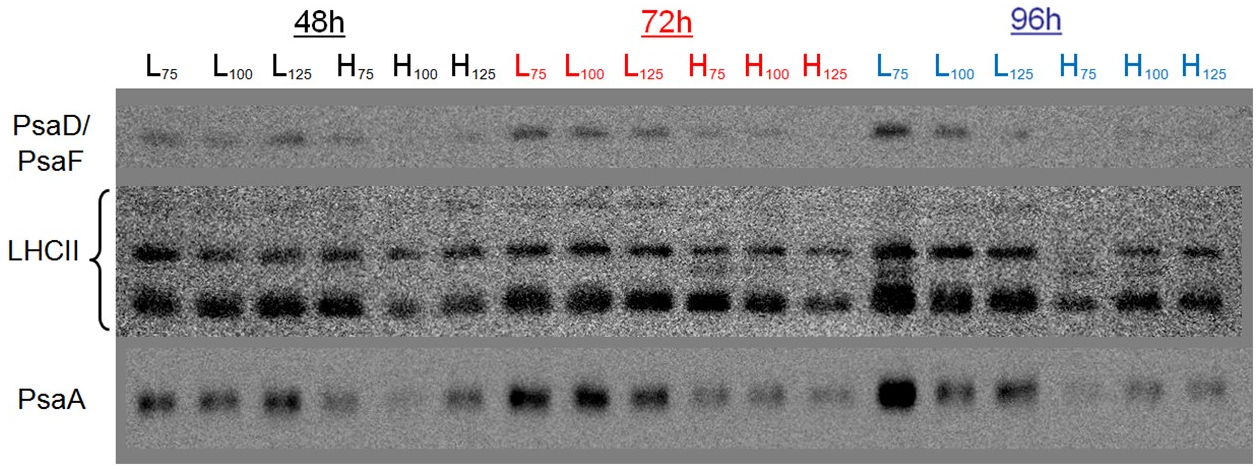

Figure 4. Protein separation based on cell biomass by using immunoblotting technique

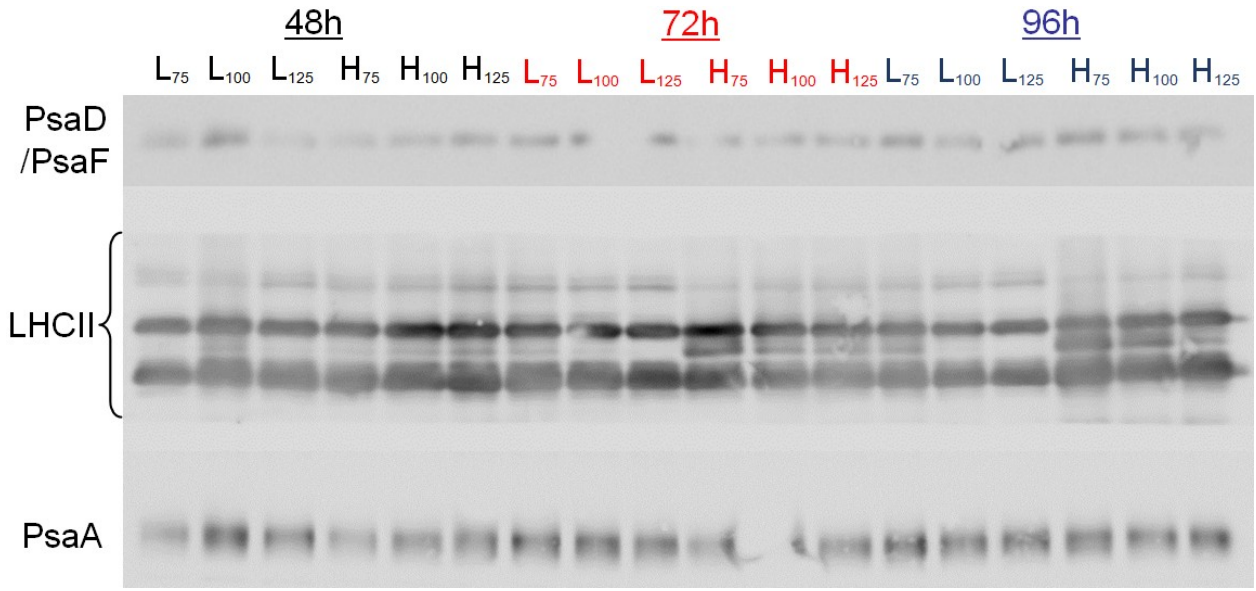

Figure 5. Protein separation based on chlorophyll content by using immunoblotting technique

significantly changed following considerable reprogramming of metabolism at cellular level.

By using immunoblotting technique based on the cell density, low light intensity resulted in accumulation of Photosystem I (PsaA, PsaD/F) and LHCII protein complexes (Figure. 4) which were of higher quantity than in high light intensity. On the other hand, based on the chlorophyll content, the results showed that in both of low light and high light intensity, accumulation of PSI (PsaA, PsaD/F) and LHCII protein complexes were almost similar or slightly different (Figure 5).

A relatively large part of reduced N in both of plant or *Chlamydomonas* is associated with enzymes that are required for energy metabolism (photosynthesis, respiration). According to de Groot et al. (2003), under N stress photosynthesis was reduced by a decreased light absorption and by the decreased utilization of assimilates. Under N stress foliar starch levels increased and the oxygen sensitivity of CO_2 fixation decreased. The relationship between starch accumulation and oxygen sensitivity (increased starch accumulation correlated with the decreased oxygen sensitivity) was always the same across the nutrient treatments. These results are consistent with N deprivation producing an increasing limitation of photosynthesis.

CONCLUSION

Under N limitation (75% NH_4Cl) photosynthetic ability was reduced, whereas in both 100% NH_4Cl (normal dosage) and

125% NH_4Cl dosage media the effect was similar. In these concentrations of NH_4Cl, under low light intensity, photosynthesis worked better than in high light condition.

REFERENCES

Ball SG, Dirich L, Decq A, Marhat JC, Matagne R (1990) Psysiology of starch storage in the monocellular alga *Chlamydomonas reinhardtii*. Plant Sci 66:1-9

Chen M, Tang H, Ma H, Holland TC, Ng K, Sailey SO (2011) Effect of nutrients on growth and lipid accumulation in the green algae *Dunaiella tertiolecta*. Bioresour Technol 102:1649-1655

De Groot CC, Boogaard RVD, Marcelis LFM, Harbinson J, Lambers H (2003) Contrasting effects of N and P deprivation on the regulation of photosynthesis in tomato plants in relation to feedback limitation. J Exp Bot 54:1957-1967

Forde BG (2002) Local and long-range signaling pathway's regulating plant responses to nitrate. Annu Rev Plant Biol 53:203-224

Gargouri M, Bates PD, Park JJ, Kirchhoff H, Gang DR (2017) Functional photosystem I maintains proper energy balance during nitrogen depletion in *Chlamydomonas reinhardtii*, promoting triacylglycerol accumulation. Biotechnol Biofuels 10:89

Harris EH (1989) The *Chlamydomonas Sourcebook:* a Comprehensive Guide to Biology and Laboratory Use. Academic Press, San Diego

Johnson X, Alric J (2013) Central carbon metabolism and electron transport in *Chlamydomonas reinhardtii*: metabolic constraints for carbon partitioning between oil and starch. Eukaryot Cell 12:776-793

Juergens MT, Deshpande RR, Lucker BF, Park JJ, Wang H, Gargouri M, Kramer DM (2015) The regulation of photosynthetic structure and function during nitrogen deprivation in *Chlamydomonas reinhardtii*. Plant Physiol 167:558-573

Ikaran Z, Suárez-Alvarez S, Urreta I, Castañón S (2015) The effect of nitrogen limitation on the physiology and metabolism of *Chlorella vulgaris* var L3. Algal Res 10:134-144

Lardon L, Helias A, Sialve B, Steyer JP, Bernard O (2009) Life-cycle assessment of biodiesel production from microalgae. Environ Sci Technol 43: 6475-6481

Lee DY, Park JJ, Barupal DK, Fiehn O (2012) System response of metabolic networks in *Chlamydomonas reinhardtii* to total available ammonium. Mol Cell Proteomics 11:973-988

Li Y, Han D, Sommerfeld M, Hu Q (2011) Photosynthetic carbon partitioning and lipid production in the oleaginous microalga *Pseudochlorococcum* sp. (*Chlorophyceae*) under nitrogen-limited conditions. Bioresour Technol 102:123-129

Ozawa S, Onishi T, Takahashi Y (2010) Identification and characterization of an assembly intermediate subcomplex of photosystem I in the green alga *Chlamydomonas reinhardtii*. J Biol Chem 285:20072–20079

Tan KWM, Lin H, Shen H, Lee YK (2016) Nitrogen-induced metabolic changes and molecular determinants of carbon allocation in *Dunaliella tertiolecta*. Scientific Reports, *6*

Park JJ, Wang H, Gargouri M, Deshpande RR, Skepper JN, Holguin FO, Gang DR (2015) The response of *Chlamydomonas reinhardtii* to nitrogen deprivation: a systems biology analysis. Plant J 81:611-624

Wang ZT, Ulrich N, Joo S, Waffenschmidt S, Goodenough U (2009) Algal lipid bodies: stress induction, purification and biochemical characterization in wild type and starchless *Chlamydomonas reinhardtii*. Eukaryot Cell 8: 1856-1868