# The influence of culture medium and light cycle on the productivity of the green algae *Neochloris oleoabundans*

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## Introduction

Biofuels from algae are a promising source of alternative energy. One algae species, *Neochloris oleoabundans,* shows potential for successful biodiesel production, where biodiesel is produced from the neutral lipid content of the algae. Algal neutral lipid content may be influenced and increased by changes in the surrounding environment. One possible way to influence lipid synthesis is through the growth medium. Two published growth mediums were compared using photobioreactors: 1) modified soil extract (SE+)[1] and 2) modified bold basal (MBBM)[2].
Two experimental runs were performed with each medium: 1) continuous light and 2) 12 hour on, 12 hour off light cycle. In addition to medium composition, the effect of light cycle on kinetics and energy content was investigated.

## Materials and Methods

Two complete experiment runs were completed in two photobioreactors (Fig. 1) each containing three liters of media. Light was provided by fluorescent plant and aquarium lights. The average light intensity during the light period was 150 µmol $s^{-1}$ $m^{-2}$. One run was completed on continuous light, and the other on a twelve hours on and twelve hours off light cycle. The average temperature was 25 degrees Celsius. Filtered air was continuously sparged at a rate of 1.5 L/min. Media was prepared as specified in literature (Table 1) with slight modifications of micronutrients. Carbon dioxide was added when pH levels reached a set point of 7.5 for MBBM and 7.0 for SE+ as specified in published articles.

**Figure 1: Algal photobioreactor**

**Table 1: Major constituents of the two media recipes**

| Constituent | MBBM [gm/L] | SE + [gm/L] |
|---|---|---|
| Magnesium Sulfate Heptahydrate | 0.22 | 0.15 |
| Sodium Nitrate | 0.75 | 0.85 |
| Potassium Phosphate Monobasic | 0.06 | 0.35 |
| Potassium Phosphate Dibasic | 0.22 | 0.15 |
| Sodium Chloride | 0.03 | 0.05 |
| Calcium Chloride Dihydrate | 0.03 | 0.05 |

Optical density measurements were recorded daily on a Shimadzu UV-1800 spectrophotometer at a wavelength of 750 nm. Dry weight measurements were taken at the end of a run. Bomb calorimetry measurements were taken with a Parr 1241 Oxygen Bomb Calorimeter, and gas chromatography was performed on a Shimadzu GA -2010.

## Results and Discussion

With continuous light, SE+ medium resulted in a higher final culture density than MBBM. The MBBM initially had a steeper growth curve, but it tapered off in the end (Fig. 2). The final culture density measurements for each media were substantially different. In the 12/12 light cycle run, both media obtained approximately the same culture density.

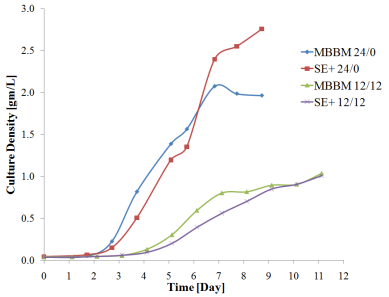


**Figure 2: Culture density [gm/L] of all experimental runs**

A comparison of growth rates and energy content is provided (Table 2). In both instances, MBBM showed increased FAME (fatty acid methyl ester) content, though it was not significant. Energy content was higher in MBBM then in SE+ media in both instances; however, the difference was slight.

**Table 2: Comparison of growth rates and energy content**

| Information Collected | Continuous | | 12/12 | |
|---|---|---|---|---|
| | MBBM | SE+ | MBBM | SE+ |
| Max OD 750 nm | 10.48 | 14.8 | 5.44 | 5.28 |
| Dry Weight [gm/L] | 1.48 | 1.76 | 0.96 | 0.84 |
| Max Specific Growth Rate [$days^{-1}$] | 2.59 | 1.44 | 2.00 | 2.54 |
| Max Doubling Time [hours] | 6.42 | 11.55 | 8.32 | 6.55 |
| FAME Content | 16.4% | 15.1% | 14.9% | 14.0% |
| Energy Content [Cal/gm] | 5209 | 4938 | 5601 | 5425 |
| Energy Content [kJ/gm] | 21.8 | 20.7 | 23.5 | 22.7 |

When dissolved oxygen (DO) concentration was compared in the MBBM (Fig. 4), continuous light showed an increasing DO concentration, while 12/12 showed a decrease in DO concentration during the dark cycle.

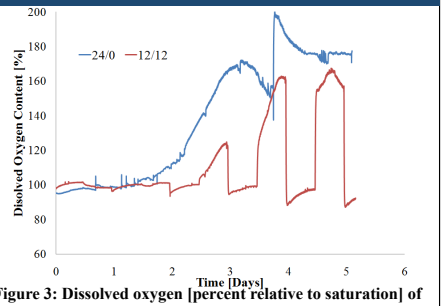


**Figure 3: Dissolved oxygen [percent relative to saturation] of continuous and 12/12 light cycle**

Biomass production and lipid fraction was compared between our current study and published data. Biomass production values of the MBBM were similar to those of the published data (Fig.4). SE+ media biomass production values were greater than published data. Because data was only published on continuous light cycles, there are no comparisons with the 12/12 light cycle. Lipid mass fraction was lower in the current study than in published data (Fig. 5). However, the "lipid" mass fraction measured in this study represents the portion convertible to FAME, while in the published literature it is all neutral lipids.

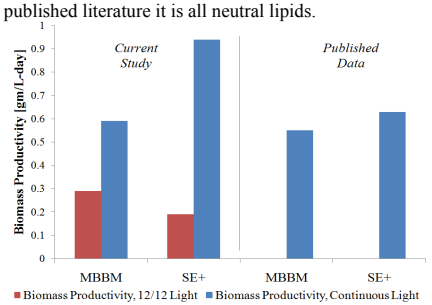


**Figure 4: Biomass productivity of current study and published data**

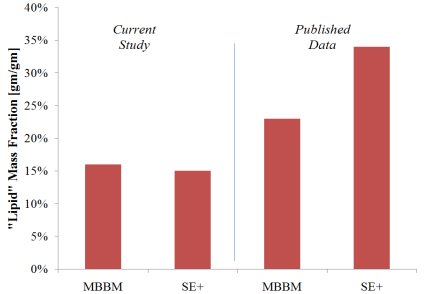


**Figure 5: Lipid mass fraction of current study and published data**

## Conclusion

**Table 3: Comparison of biomass production and lipid content between current study and published data**

| Information Collected | Current Study | | Published Data | |
|---|---|---|---|---|
| | MBBM | SE+ | MBBM | SE+ |
| Biomass Production [gm/L-day] Continuous | 0.59 | 0.94 | 0.55 | 0.63 |
| Biomass Production [gm/Lday] 12/12 Light | 0.29 | 0.19 | - | - |
| Total Lipid Content, Continuous | 16% | 15% | 23% | 34% |

Overall, only slight differences were seen between the two media (Table 3). The most significant difference occurred between the light cycle. Biomass productivity decreased significantly during a 12/12 light cycle compared to continuous light. There is no published data available for 12/12 light cycle. Lipid content between the two light cycles do not show significant differences. The decrease in biomass productivity is due to respiration during the dark cycle, where the cells are consuming stored biomass and using oxygen. During the dark cycle, cells are not performing photosynthesis and must use stored energy to stay alive. The published data is from experiments which were performed with continuous light. Although continuous light results in higher biomass productivity, continuous light is not realistic for commercial application. When algae is being considered for commercial production it is necessary to use a light cycle, since commercial production would occur outdoors. This study shows that it is critical to use a diurnal light cycle when studying kinetic and productivity data.

## Literature Cited

[1] Pruvost, J., Van Vooren, G., Cogne, G., Legrand, J., 2009. Investigation of biomass and lipids production with *Neochloris oleoabundans* in phtobioreactor. Bioresource Technology 100, 5988-5995.

[2] Li, Y., Horsman, M., Wang, B., Wu, N., Lan, C., 2008. Effects of nitrogen sources on cell growth and lipid accumulation of green alga *Neochloris oleoabundans.* Applied Microbiology and Biotechnology 81, 629-636.

## Acknowledgments

Thank you to USTAR and USU Biofuels Center for funding. Thank you to Nathan Phillips for bomb calorimetry, and Brad Wahlen and Lynsey Talbot for GC analysis, and to Mikey Morgan for reactor construction.

USU Biofuels Center

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