MONTANA STATE UNIVERSITY

Center for Biofilm Engineering

Investigating culture effects of light irradiance and initial biomass concentration in *Nannochloropsis Oceanica* sp. using response surface methodology

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INTRODUCTION

Microalgae have the capacity to produce precursors for biofuels, and are rapidly becoming a viable option for offsetting a significant portion of the world's fossil fuels demand. Triacylglycerol (TAG) is the most common lipid precursor for biofuels produced in microalgae, comprising over 50% wt./wt. in industrially relevant strains. Industrial production of microalgae is most practical in large outdoor photo-bioreactors or raceway ponds where light energy, an essential component of photosynthesis, is freely available from sunlight. Current limitations to industrial realization of algal biofuel production includes reduction in lipid potential from outdoor cultivation methods, particularly due to the unavoidable limiting factor of outdoor cultivation (*i.e.*, sun light). This project focused on optimizing advanced culturing techniques demonstrated with the industrial relevant strain *Nannochloropsis oceanica* sp. to evaluate light limitation and its impact on biomass and TAG production.

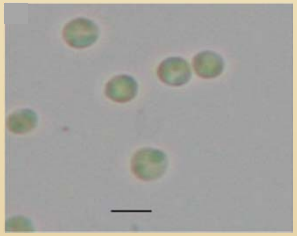

Figure 1. Transmitted light micrograph images of Nannochloropsis sp., industrially relevant strain with high TAG accumulation potential, *Scale bar = 5 μm*

METHODS

With traditional scientific research, it is commonplace to control one variable and evaluate the response. Here, a statistical approach is demonstrated in which two control variables were investigated and results were used to generate three dimensional surface response plots. The two control variables investigated were the initial concentration of biomass and the available photosynthetically active radiation (PAR). A two phase experiment was conducted to accomplish this task and is outlined below:

Phase I: Bulk Culture Growth

- *Nannochloropsis oceanica* sp. grown in pH controlled batch reactor
- ASP_II^{200} medium supplemented with 4x NO_3^- and 3x PO_4^{-3} and 5mM HCO_3^-
- Medium nutrients re-supplemented as NO_3^- was near depletion
- Culture grown until peak chlorophyll and biomass accumulation

Phase II: Lipid Accumulation

- Culture from bulk phase was centrifuged and re-suspended in fresh medium that was deplete of nitrogen and replete in all other nutrients
- 50 mM HCO_3^- supplemented to all conditions to as inorganic carbon source and lipid accumulation trigger
- Women's hosiery used to attenuate the light at medium and low levels

Table 1. Phase II experimental conditions, eight different experimental conditions were investigated, each representing a different combination of culture concentration and available PAR.

Reactor	PAR (μmol photons·m^{-2}·s^{-1})	Initial Cell Density (cell·mL^{-1})
1	900	1x10^8
2	900	2x10^8
3	900	3x10^8
4	600	1x10^8
5	600	3x10^8
6	300	1x10^8
7	300	2x10^8
8	300	3x10^8

RESULTS

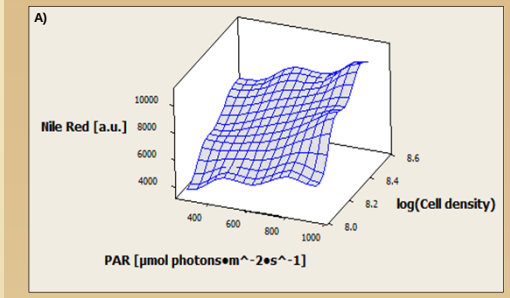

Figure 2. Response surface plots showing interaction between available PAR and biomass concentration with responses shown for Nile Red [a.u.] T=1 (A), Nile Red [a.u.] T=2 (B), and Chlorophyll A content [mg·L^{-1}] T=0 (C)

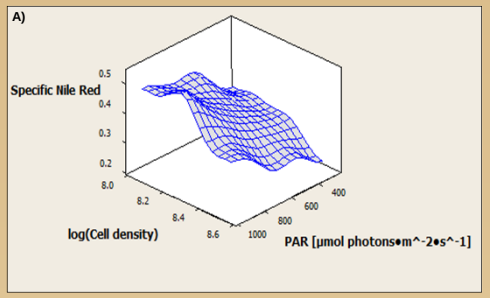

Figure 3. Response surface plots showing interaction between available PAR and biomass concentration with responses shown for Specific Nile Red [a.u.·10,000 cells^{-1}] T=1 (A), Specific Nile Red [a.u.·10,000 cells^{-1}] T=2 (B), and Specific Chlorophyll A content [mg·L^{-1}·10^{7}cells^{-1}] T=0 (C)

Table 2. Statistical models fitted to experimental data using a face-center cube design and quadratic model predicting responses in Nile Red, chlorophyll content, and specific Nile Red

Response	Model
Nile Red (T=2)	$NR = 213284 - 267.9 * PAR - (1.853 \cdot 10^{-2}) * PAR^2 - 46956 * log_{CD} + 2606.4 * log_{CD}{}^2 + 35.58 * PAR * log_{CD}$
Chlorophyll A (T=0)	$Chl_A = 117.91 + (3.32 \cdot 10^{-2}) * PAR + (4.331 \cdot 10^{-6}) * PAR^2 - 38.17 * log_{CD} + 2.945 * log_{CD}{}^2 - (4.58 \cdot 10^{-3}) * PAR * log_{CD}$
Specific Nile Red (T=1)	$NR_S = -85.503 + (2.15 \cdot 10^{-3}) * PAR - (2.545 \cdot 10^{-7}) * PAR^2 + 20.89 * log_{CD} - 1.272 * log_{CD}{}^2 - (1.961 \cdot 10^{-4}) * PAR * log_{CD}$

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DISCUSSION & CONCLUSION

During the course of this study a number of notable trends provided insight into further design considerations for repeating the experiment with decreased error. While the trends seen in the data are representative of quantitative interpretation it should be stated that further analytical work will be needed in future execution of this experiment.

- Nile Red intensity was seen highest in cultures with the most biomass and light intensity indicating high lipid potential
- Specific Nile Red intensity experienced a shift from its maximum at high light and medium biomass to medium light and low biomass indicating higher productivity per cell in these cultures
- Cultures exposed to high light intensity had rapid chlorophyll degradation after one day
- Cultures exposed to medium light intensity also underwent chlorophyll degradation but retained chlorophyll longer
- Low light cultures maintained a significant amount of chlorophyll throughout the study, indicating they retained more photosynthetic potential.

The fundamental principles of light intensity and culture density during bicarbonate-induced TAG accumulation were investigated and the results presented above. Overall trends of low, medium, and high cell densities were seen and maintained during the course of the experiment. Rapid chlorophyll degradation in all cultures

Figure 4. Experimental set-up showing each condition under LED light exposure with attenuating hosiery visible

INDUSTRIAL RELEVANCE

This experiment was initially designed such that the results could be a contribution in developing a strategy for outdoor cultivation methods. In an industrial plant, an ideal method for rapid lipid accumulation would be to seed with culture grown separately under optimal growth conditions. Biomass would then be primed for a lipid accumulation trigger, such as nitrogen depletion in concert with bicarbonate supplementation. The concerning factor is too dense of a seed culture will effectively limit light attenuation to cells further under the surface. Here, a method is used where these two variables are simultaneously investigated and their effect on culture responses is predicted.

FUTURE WORK

- Re-execution of experiment using identical conditions while monitoring chlorophyll and NO_3^- more frequently during bulk phase
- Gas chromatography analysis of dried biomass for lipid data
- Generate higher power models with more statistical significance

ACKNOWLEDGMENTS

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