Characterization of Algal Cell Culture in Photobioreactors for Biodiesel Production

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Abstract

Microalgal biofuels are a viable alternative for fossil fuels to reduce the amount of greenhouse gases released into the atmosphere from fuel consumption. However, microalgal biofuel production requires large volumes of algae and costs approximately 50% more than traditional fuel production. Therefore, there is a need to improve cell and lipid productivities in the photobioreactor process. This project focuses on determining cell productivity in the shaker flask and the traditional mechanically-stirred bioreactor, the quantification of lipids by spectroscopy, and the design of a novel space-saving bioreactor. The strains studied are: *Scenedesmus bijuga*, *Chlorella sorokiniana*, and *Chlorella vulgaris*.

The multiple cell strains grown in the shaker flask were kept at varying conditions to determine the optimum environment for cell growth. The cells did not grow when initially placed under fluorescent lights in a standard shaker. As an alternative, a specifically-designed shaker for algal culture with LED lights fixed on the interior ceiling was used. Cell growth was monitored by measuring spectroscopic absorbance at wavelengths of 680 nm and 750 nm.

The traditional stirred tank bioreactor (STR) was operated with 2 L of algae and was kept running for 10 days. The doubling time was observed to be approximately forty-eight hours.

The novel space saving reactor consists of growing the algae on a substrate surface rather than suspended in medium. Medium is sprayed over the algae while it is attached to a supporting surface. This method significantly increases the amount of algae produced per unit volume, since most of the volume in the traditional reactor is water, and not biomass.

Algae biofuel and biomass production are limited by a lack of real-time knowledge of viable cell growth, and cellular lipid content. Thus, developing a real-time monitoring system for algae cultures is of great advantage to effectively increase their productivity. Visible/near-infrared spectroscopy was used to establish a low-cost sensor for lipids. In addition to measuring the daily algal cell count with a hemocytometer, the cells were stained with fluorescent Nile red dye in DMSO to observe the intracellular lipid droplets from which biofuels are derived. The stained cultures were then analyzed via fluorescent microscopy (reference method) and spectroscopy, daily. Afterwards, these two methods were correlated to ultimately identify a strong correlation in which the implementation of spectroscopy is an effective real-time cellular lipid monitoring system for microalgae.

Experimental Procedure

Shaker Platform:

Cells were transferred from culture vials (50 mL) into individual 250-mL flasks under aseptic conditions, then 50 mL of BG-11 medium added to it. (flasks to maintain ratio of 100 mL liquid in 250-mL flask). Flasks of cells were incubated at 25°C with shaker speed 120 rpm with 60% humidity, LED light (Cool white, 6500K) at a diurnal schedule of 12 hours. CO_2 content was varied from ambient (0.03%) to 2.5% v/v.

Novel Space Saving Reactor:

Different porous media were used was solid substates. Finally filter paper (Size: 11 cm). 8 mL of algal cell culture were filtered through a glass filter paper, using a Büchner funnel under moderate pressure. The run off filtrate was continuously re-filtered till all the cells were seeded to the filter medium.

Stirred Tank Bioreactor:

200 mL of cell culture with an optical density of 11 was aseptically inoculated in a Sartorius Biotstat® Aplus bioreactor. It was then adjusted to 2 L with BG-11 culture medium.

Optimization of a Cost-Effective Lipid Monitoring System for Green Microalgae with Real-Time Applications:

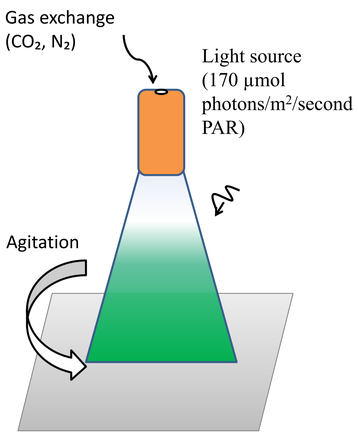
Fluorescence microscopy was selected to quantify algal lipid concentrations; microscopy with *Nile Red* lipophilic stain with DMSO as solvent is an accepted reference method for algal lipid detection.

A design for low-cost, real-time lipid sensor utilizing visible fluorescence micro-spectroscopy monitoring system is being proposed for about $4,000 (current systems cost upwards of $25,000).To successfully establish it; the real-time and time-point approaches were correlated using statistical significance tests ("*t*-test") to evaluate the effectiveness of the spectroscopic monitoring system.

This would help establish a low-cost option for feedback control to optimize algal biofuel production

Shaker Experimental Setup

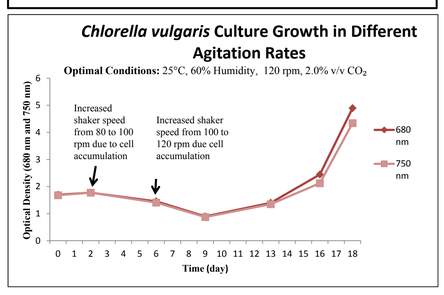
Specifically designed, climate controlled shaker for algal culture incubation (Kuhner Climo-Shaker ISF1-X),



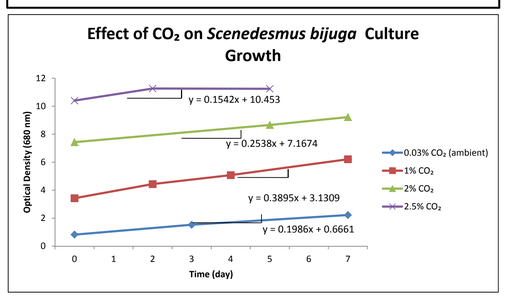
Autotrophic algal cell culture under photosynthesis

Results And Observations

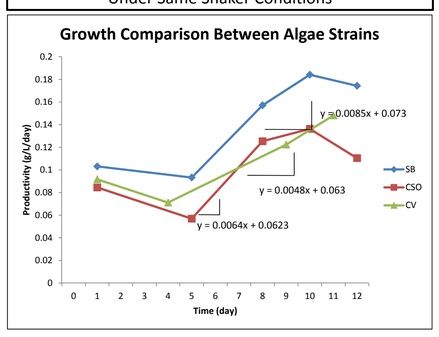
Determination of Optimal Conditions of Growth



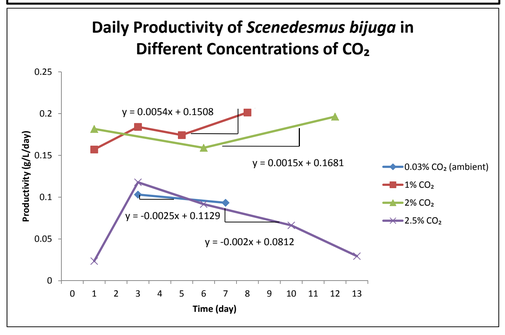
Effect of CO_2 on Growth Kinetics



Growth Comparison of Different Strains of Algae Under Same Shaker Conditions



Daily Productivity

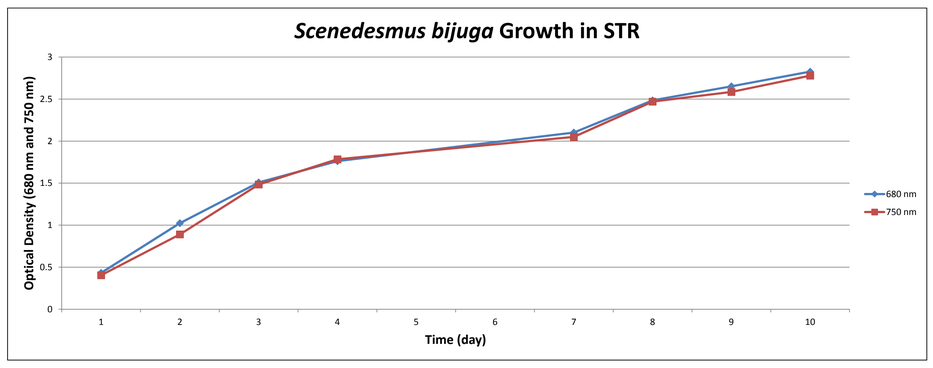


STR Setup

Conditions:

- Temperature = 20°C
- pH of 7.1 with phosphate buffer.
- Aeration rate of 80 cm^3/min of 2.5% CO_2 (97.5% N_2)
- Agitation rate = 350 rpm

Result For STR



It was observed that the culture in the STR was sustainable with a doubling time of about two days.

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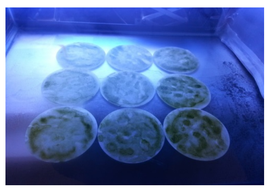
Novel Space Saving Reactor

Algal cell culture in medium leads to poor optimization of the cells and lipid production.

Our Approach: Cell culture on a solid substrate and optimal medium transfer through nebulization of medium.

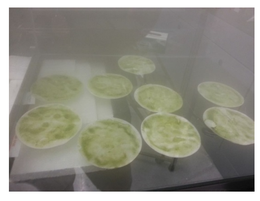
Challenges:

- Layered growth affects uniform spread of nutrients, gas and light.
- Finding optimum growth rate and harvest time



Immediately after inoculation

Conditions: 2.5 % CO_2, 12 hour diurnal cycle, fluorescent lighting, periodic transfer of nebulized medium. Transfer of media was optimized through the use of a humidistat.



Four days after inoculation

Design And Optimization of A Cost-Effective Lipid Monitoring System For Green Microalgae With Real-time Applications

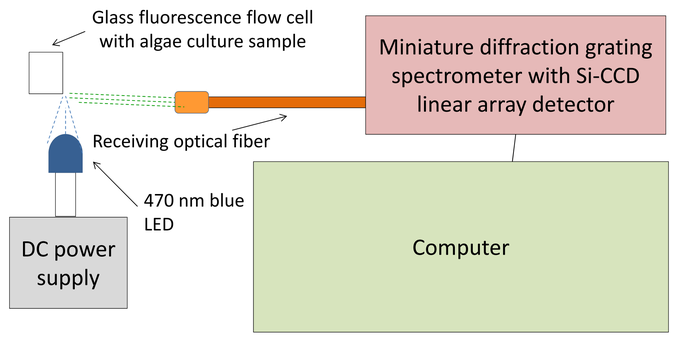
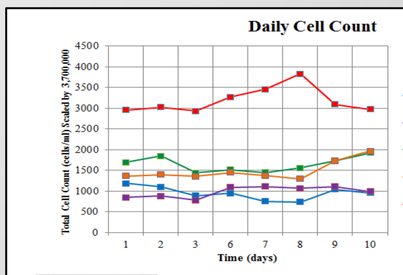
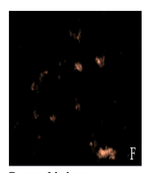


Diagram of the fluorescence spectroscopy monitoring system used to measure fluorescence emission spectra of algae cell culture samples stained with fluorescent dyes

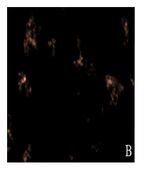
Results From Cell Count and Fluorescence Microscopy Experiments



C. vulgaris Reference staining - Successful

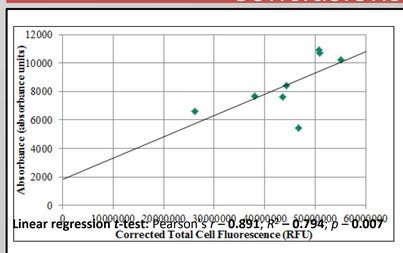


C. sorokiniana Reference staining - Successful



S. bijuga Reference staining - Successful

Spectroscopy Results – Data Correlation – Conclusions



Experimental Conclusions

- Spectroscopic monitoring system works for particular strains of microalgae
- Possibly an effect of the high optical densities of the *S. bijuga* and *C. sorokiniana* strains
- Thus, visible/near-infrared spectroscopy may be utilized in future real-time lipid sensors for experimentally-determined species of microalgae

Nile Red Spectroscopic Absorption Spectrum for Freshwater Green Microalgae

Algae Culture	Absorbance (absorbance units) - Normalized to Cell Count						
	0 hours	5 hours	27 hours	47 hours	70 hours	118 hours	123 hours
S. bijuga Master Culture	0.000	0.000	0.000	1.385	0.088	0.339	0.000
C. sorokiniana Master Culture	0.004	0.040	0.000	0.720	0.381	0.211	0.239
S. bijuga Sub-Culture	0.000	0.000	0.000	0.436	0.000	0.000	0.000
C. sorokiniana Sub-Culture	0.000	0.000	0.000	0.092	0.000	0.029	0.000
C. vulgaris Sub-Culture	2.234	2.589	2.546	3.733	2.859	2.576	2.382

Algae Culture	Absorbance (absorbance units) - Normalized to Cell Count						
	143 hours	147 hours	166 hours	171 hours	191 hours	195 hours	215 hours
S. bijuga Master Culture	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C. sorokiniana Master Culture	0.000	0.017	0.000	0.000	0.000	0.000	0.000
S. bijuga Sub-Culture	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C. sorokiniana Sub-Culture	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C. vulgaris Sub-Culture	2.214	1.626	2.802	1.583	3.304	1.793	1.824

Future Work

Shaker Platform:

- Continue to experiment with different conditions to identify optimal conditions for all three strains of algae being studied.

Novel Space Saving Reactor:

- Continue experimenting with different substrates to find one that most favors algae cell attachment
- Identify optimum time to harvest cells

Stirred Tank Bioreactor (STR):

- Additional runs will be done in order to identify optimal CO_2 levels and quantity of cell volume to use for inoculation.

Optimization of a Cost-Effective Lipid Monitoring System for Green Microalgae:

- Optimize the Nile red signal
- Use hexane-based solvent extraction to characterize the lipids in the stained cells
- Expand the sensor to include cellular monitoring capabilities

Acknowledgements

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References

1. S.A. Khan, Rashmi, M.Z. Hussain, S. Prasad, U.C. Banerjee. Prospects of biodiesel production from microalgae in India. Renewable and Sustainable Energy Reviews 13 (2009), Vol.9, 2361-2372.
2. T. McConville. Fat content determination methods. Unpublished Manuscript,1999.
3. L. Rodol, G.C. Zittelli, N. Bassi, G. Padovani, N. Biondi, G. Bonini, M.R.Tredici. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnology and Bioengineering 102 (2008), Vol.1, 100-112.
4. R. Sakthivel, S. Elumalai, and M.M. Arif. Microalgae lipid research, past, present: A critical review for biodiesel production, in the future. Journal of Experimental Sciences 2 (2011), Vol.2, 29-49.
5. M. Sato, Y. Murata, M. Mizusawa, H. Iwahashi, S. Oka. A simple and rapid dual-uorescence viability assay for microalgae. Microbiology and Culture Collections 20 (2004), Vol. 2, 53-59.
6. L. Xin, H. Hong-ying, Z. Yu-ping. Growth and lipid accumulation properties of a freshwater microalga Scenedesmus sp. under different cultivation temperature. Bioresource Technology 102 (2011), 3098-3102.
7. Sobczuk, et al. Effects of agitation on the microalgae Phaeodactylum tricornutum and Porphyridium cruentum. Bioprocess Biosyst Eng (2006) 28: 243–250.
8. Suh, et al. Photobioreactor Engineering: Design and Performance. Bioprocess Biosyst Eng (2003) 8: 313-321.
9. Li, Ma et al. Effects of Filtration Seeding on Cell Density, Spatial Distribution,and Proliferation in Nonwoven Fibrous Matrices. Biotechnology Progress 2001, 17, 935-944