Algae Thin-Film Immobilization

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Background

- To determine methods that effectively immobilize photosynthetic Cyanobacteria and microalgae that will allow for increased chemical and biofuel production from solar energy and CO_2
- The goal is to develop a thin film that contains the algae cells and allows for their growth
- The immobilization of the algae increases efficiency and increases the cultivation period of the cells

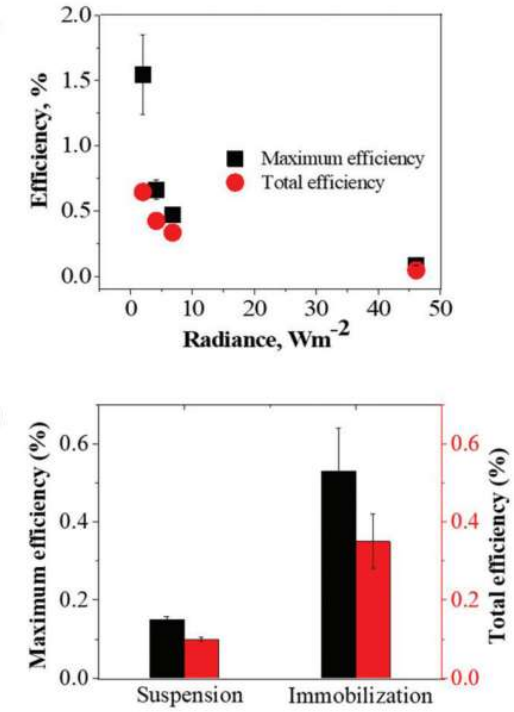

Figure 1. The light to ethylene conversion efficiencies for *Synechocystis efe* utilizing different methods.

Experimental Setup

Thin film immobilization- *Nannochloropsis oceanica*

1. Culture a fresh sample of cells, 50 mL in f/2 medium
   - Maintain at 25^{o}C and 150 rpm
2. Centrifuge the cells at 4000xg 10 mins, collect the cell pellet
3. Resuspend in 2 mL f/2 medium
4. Mix 0.5 mL of the cell slurry with 1.5 mL 4% alginate. (Total volume = 2 mL)
5. Spread on the plastic plate (size: 10x10 cm; area=100 cm^2; film thickness=200 µm)
6. Submerge it in 2% $CaCl_2$ immediately for at least 1 minute
7. Place the plate in f/2 medium, under light at 25 ^{o}C
8. Record pH, take photo, change f/2 medium daily.
9. Remove a sample the size of a 2 mL centrifuge cap and dissolve in 1 mL 5% sodium tripolyphosphate solution
10. Read the OD750 to measure growth

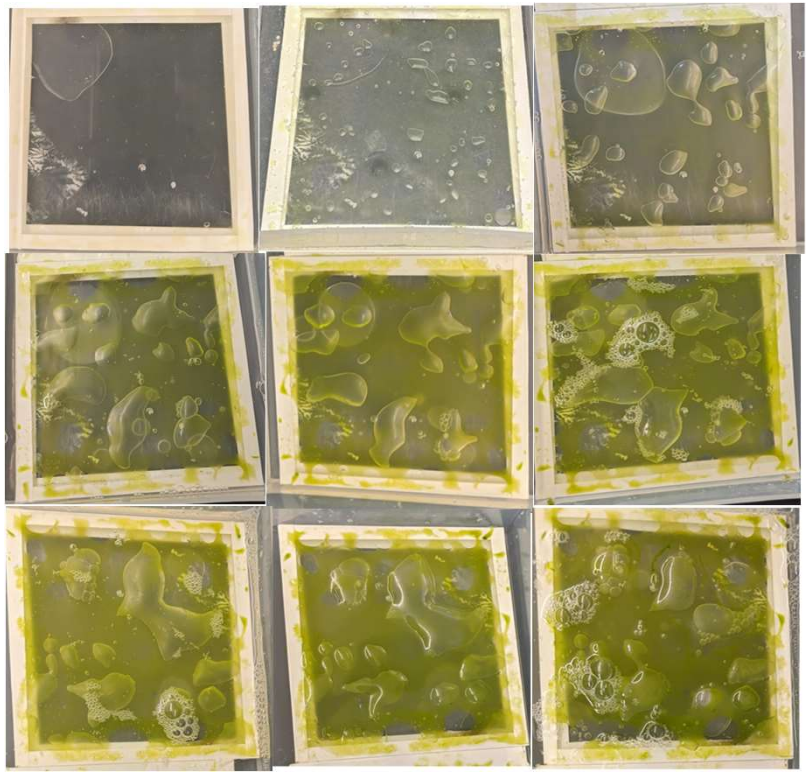

Figure 2. Algae Plates
Row 1: Day 0, Day 1, Day 2
Row 2: Day 3, Day 4, Day 5
Row 3: Day 6, Day 7, Day 9

Effect of $CaCl_2$

- The effect of $CaCl_2$ was analyzed by comparing the immobilization growth at 1 min and 10 mins in 2% $CaCl_2$ solution
- Cultivation and immobilization procedures remained unchanged
- One plate remained in 2% $CaCl_2$ for about 1 mins, the other one sat for 10 mins
- Both were placed in a box containing f/2 medium, under light at 25 ^oC

- **The cells grew well regardless of the time in** 2% $CaCl_2$
- **$CaCl_2$ had no significant effect on the cell growth.**

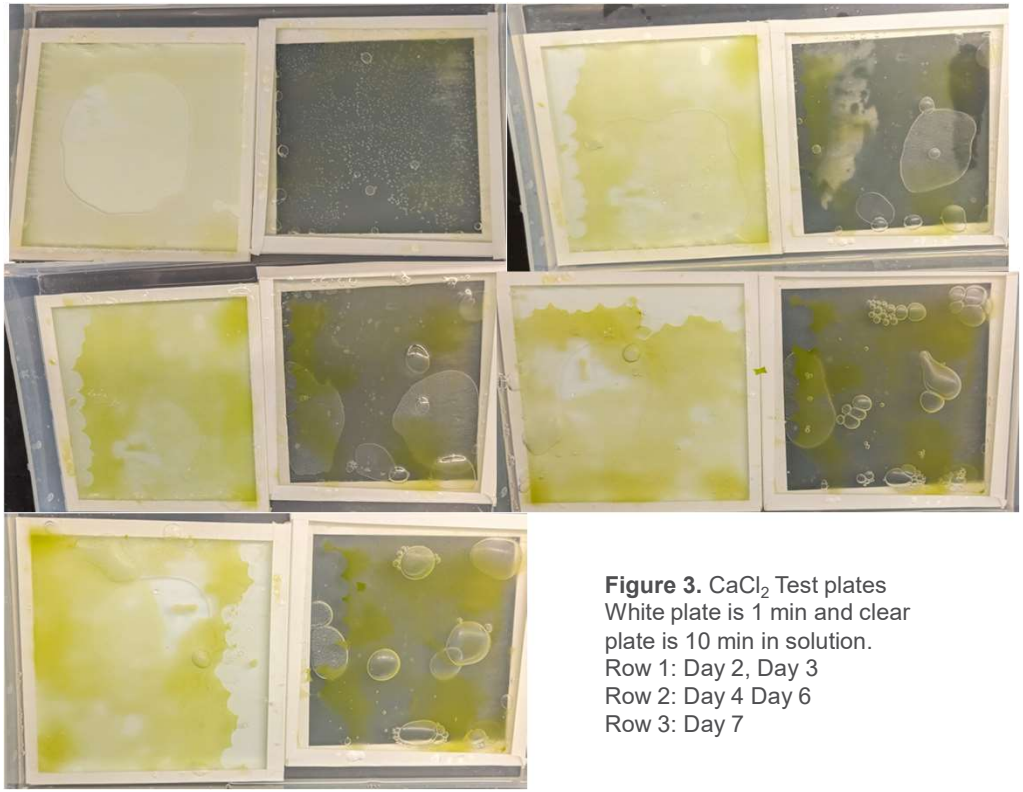

Figure 3. $CaCl_2$ Test plates
White plate is 1 min and clear plate is 10 min in solution.
Row 1: Day 2, Day 3
Row 2: Day 4 Day 6
Row 3: Day 7

Cultivation of *Nannochloropsis Oceanica*

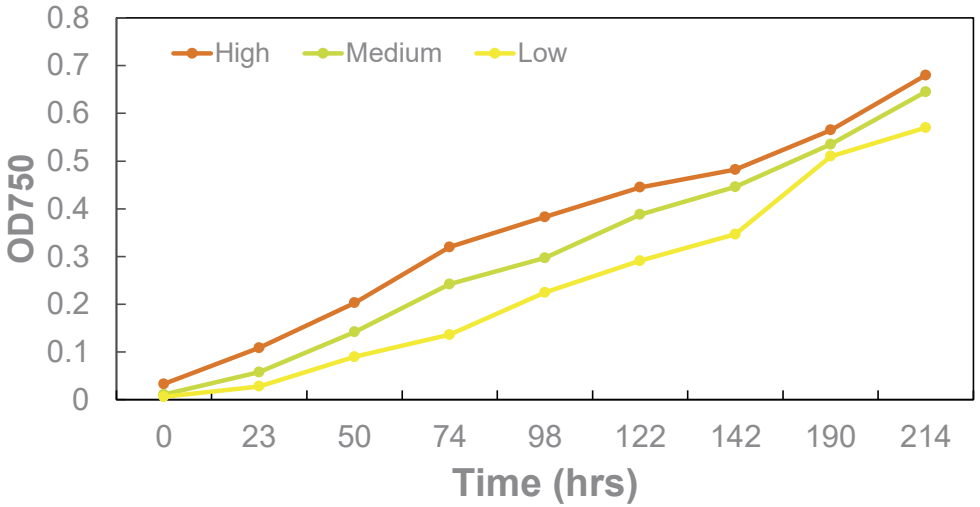

Figure 4. OD750 growth results of *Nannochloropsis Oceanica*

Fresh prepared *N. oceanica* (2-day old)

50 mL f/2 medium (fresh prepared) in 125 mL flasks. High (OD750=0.033), Medium (OD750=0.011), and Low (OD750=0.006) initial cell concentrations. 25 C, 150 rpm

The best time to cultivate the cells is after two days.

Results

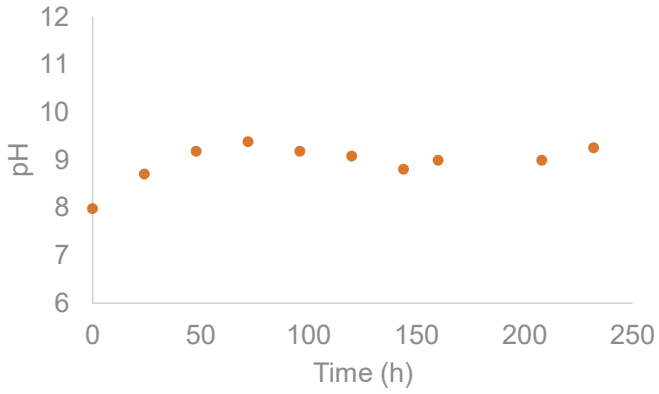

Figure 5. pH of the median of the algae plates.

The pH of the f/2 median increased slightly and then fluctuated slightly but remained between 8 and 9.

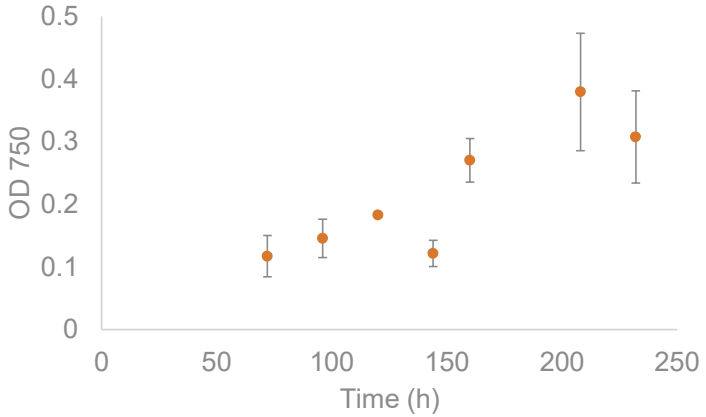

Figure 6. OD750 measurements from the immobilized plates.

The OD750 of the immobilized cells were measured daily.
Four samples were taken each time and then averaged.
The cell plates continuously grew until around the 10th day.
There were bubbles present on the plates, O_2 trapped under the film.

Discussion/Next Steps

- Develop a mass curve that relates the suspended volume of algae to the dry weight of the cells.
- Determine a method to remove the oxygen bubbles under the thin film.
  - A new plate that contains channels that run the f/2 media under the algae film.
- Obtain the Cyanobacteria and other genetically engineered strains to test how they function and their product efficiency

Conclusions

- *Nannochloropsis Oceanica* can successfully be immobilized in an Alginate-Ca2+ thin film and kept intact with f/2 median.
- As the *Nannochloropsis Oceanica* grew, the pH increased slightly while the OD750 continuously increased for approximately 10 days.
- The CaCl2 had no effect on the growth of the *Nannochloropsis Oceanica.*

What benefits did you get from you SURE experience?

- I was able to gain valuable experience in a lab setting that will benefit me in future courses and jobs.
- I learned more about natural applications within Chemical Engineering and one possible route I can go with the degree.
- I was able to connect and meet with other students and staff within my major and college.

References & Acknowledgements

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[1] Jamsa, M., Kosourov, S., et.al. (2018). Versatile Templates from Cellulose Nanofibrils for Photosynthetic Microbial Biofuel Production. *Journals of Material Chemistry A, 6(14), 5*825-5835. doi: 10.1039/c7ta11164a.

[2] Vajravel, S., Sirin, S., et. al. (2020). Towards Sustainable Ethylene Production with Cyanobacterial Artificial Biofilms. *Green Chemistry, 22(19)*, 6404-6414. doi: 10.1039/d0gc01830a.

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Thank you

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