Utilizing Algae to Treat a Combined Wastewater Stream and Harvesting Algae for Fish Food Production

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Introduction

- The Searle Biodiesel Lab produces 75-80 gallons of Biodiesel Wash Water (BWW) per batch of fuel (2,000 annually) during final fuel refining.
- BWW has been identified as a nutrient rich water that is a suitable media for algae based on their ability to absorb large quantities of nitrogen, potassium, and sodium (Herrera).
- By studying the algae growth patterns in response to the amount of BWW a culture receives, we can optimize the conditions for maximum nutrient redemption.
- Once the algae have cleansed the BWW so the water may be reused in biodiesel production, there is potential for the algae biomass to be harvested and converted into fish food for the tilapia that are grown on campus.
- Algae contain macronutrients that are similar to the macronutrients found in the food that is currently being fed to the fish.
- By studying the macronutrients in various algal strains as well as other ingredients available in our greenhouse, we aim to create a new sustainable fish food and eliminate the need for commercial fish food.

Methods

Optimization of Algae Growth in BWW

- 9 *Chlorella vulgaris* cultures- 3 controls received media, 3 cultures received the slow treatment, and 3 cultures received the fast treatment.
- Treatment periods lasted approximately 2 weeks while culture were under controlled light, temperature, and air flow.
- 5 mL samples were taken and filtered at the beginning and end of each treatment.
- Water samples were analyzed using Ion Chromatography for nutrient content.
- Algal samples were measured for biomass change over the two week treatment period.

Treatment Period	Fast Treatment		Slow Treatment	
	Media	TN-BWW	Media	TN-BWW
Pre-experiment	100%	0%	100%	0%
1st	88%	12%	94%	6%
2nd	76%	24%	88%	12%
3rd	64%	36%	82%	18%
4th	52%	48%	76%	24%
5th	40%	60%	70%	30%
6th	28%	72%	64%	36%
7th	16%	84%	58%	42%
8th	4%	96%	52%	48%

Table 1 (top): Percentage of Media and Treated, Neutralized-BWW (TN-BWW) each culture received during each treatment period.

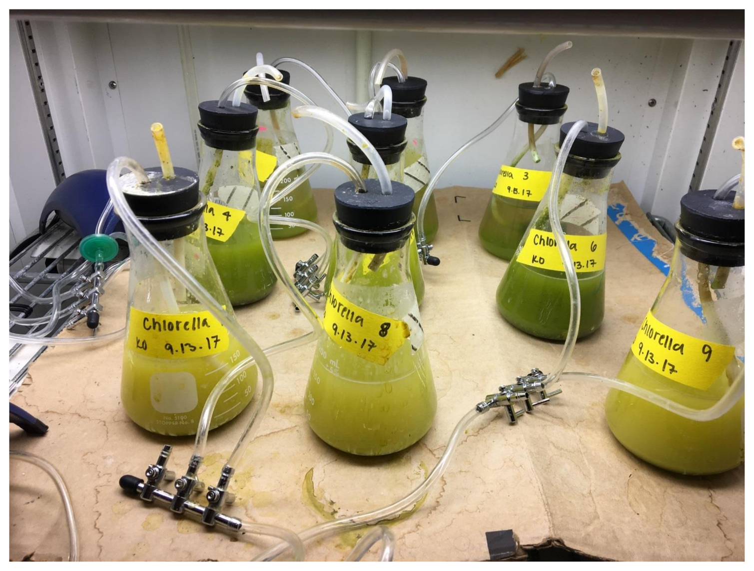


Image 1 (right): Algae culture experiment set up.

Analyzing Algal Macronutrients

- Over the summer, First Year Research Experience (FYRE) students started researching methods for analyzing different macronutrients based on Association of Official Analytical Chemists (AOAC) guidelines.
- These methods were further developed and tested several times specifically for algae samples, and errors were worked through in the procedures.
- After results were reproducible, samples were sent to an outside lab to confirm results.
- After receiving confirmation, further macronutrient analyses were performed on three strands of algae: *Chlorella vulgaris*, *Mougeotia transaui*, and *Scenedesmus dimorphus*.

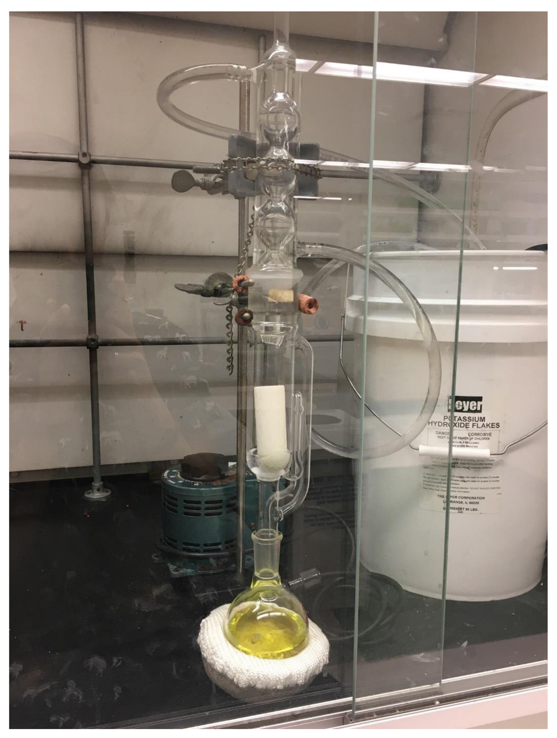


Image 2: Soxhlet extraction apparatus for lipid content analysis.

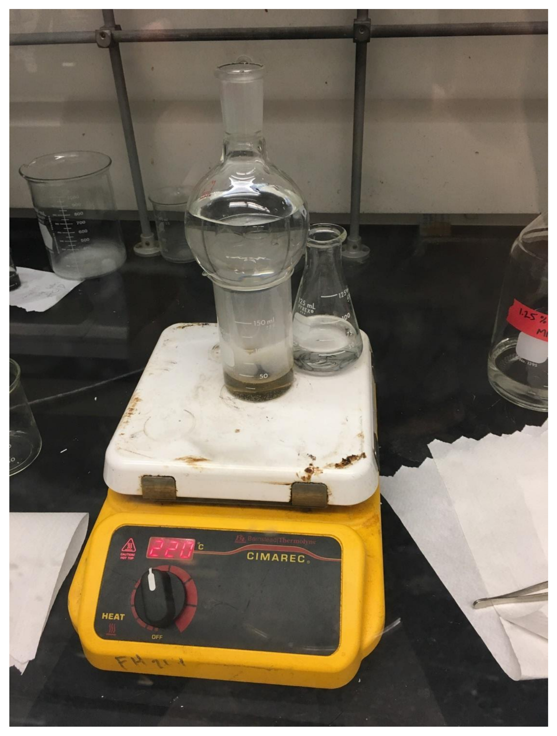
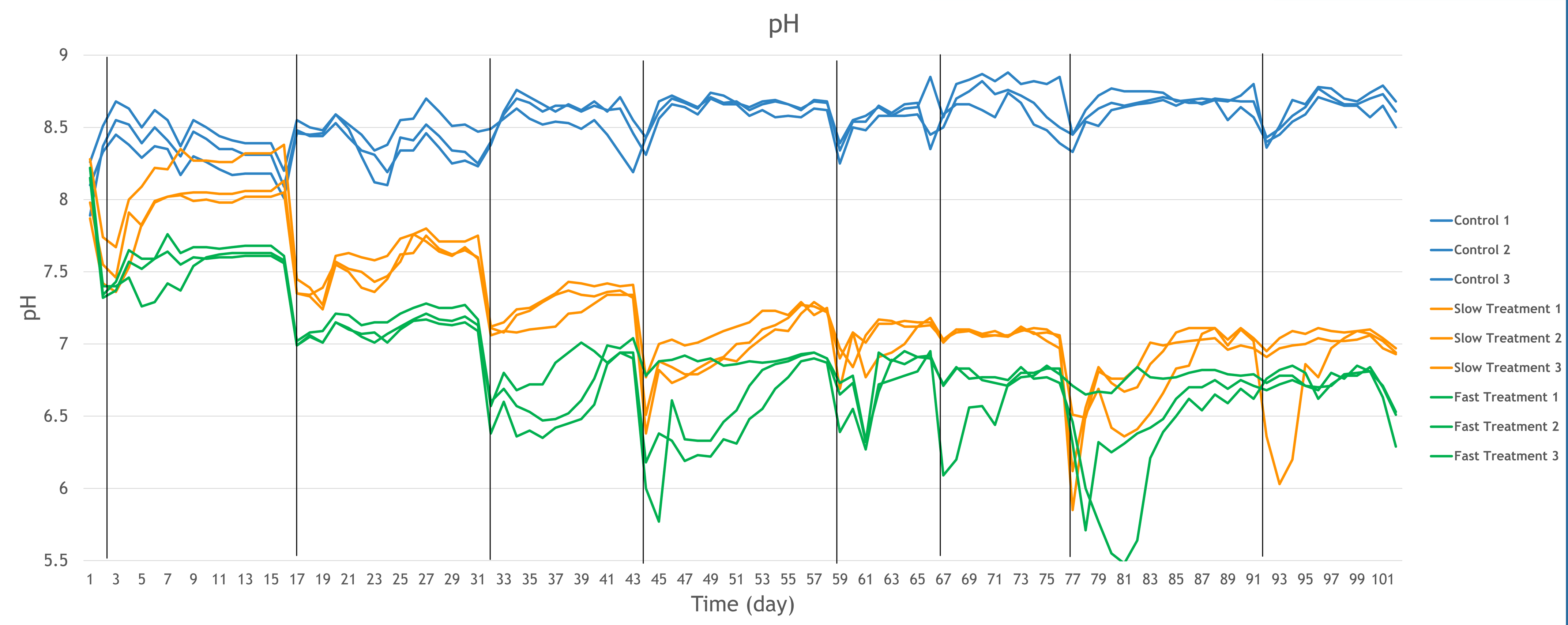


Image 3: Acid and base digestion for crude fiber content analysis.

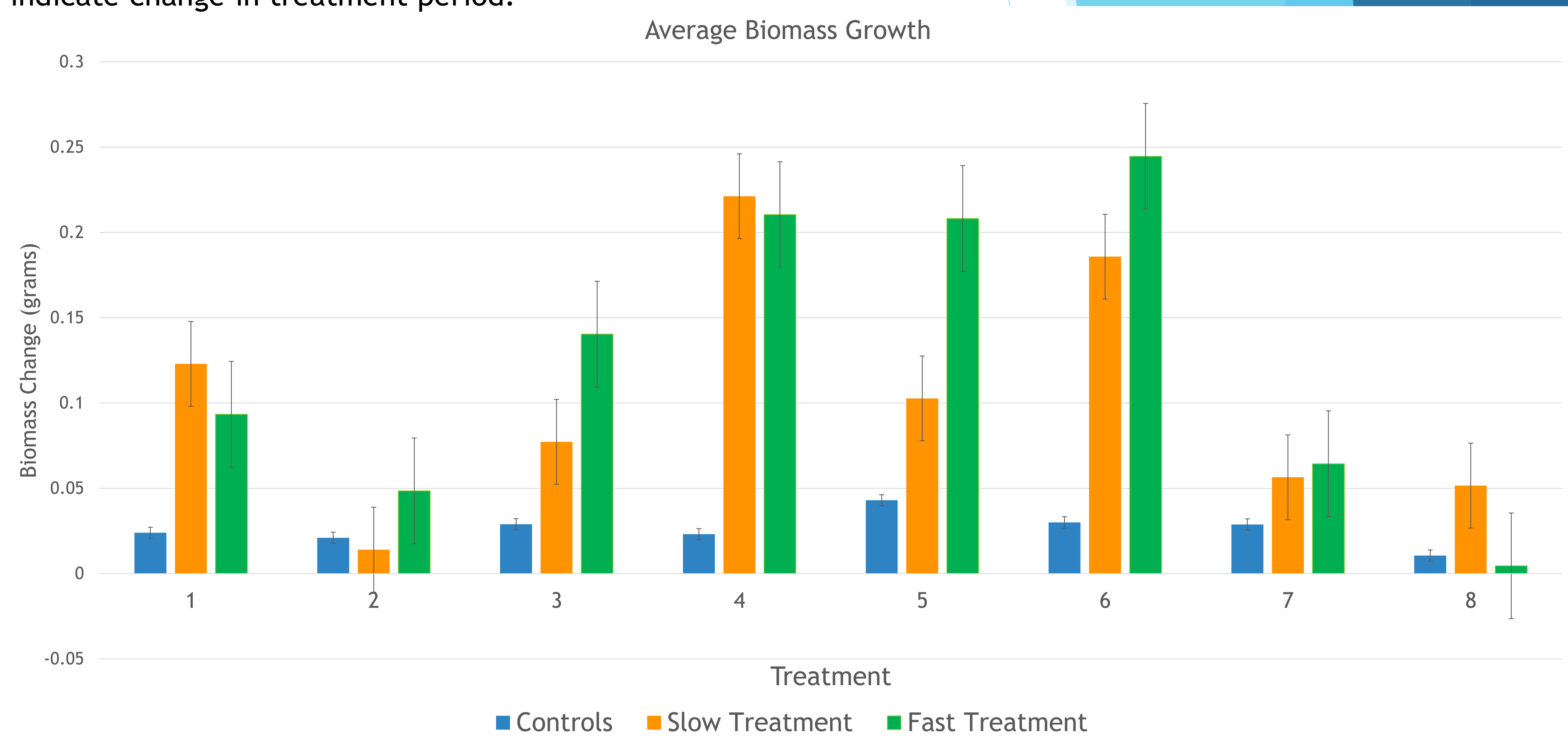
Image 4: Flash 2000 Organic Elemental Analyzer for protein content analysis.

Result & Conclusions

Optimization of Algae Growth



Graph 1: Daily pH measurements of 9 *Chlorella vulgaris* cultures throughout the experiment. Vertical lines indicate change in treatment period.



Graph 2: Average biomass growth of the 3 treatment replicate cultures during the treatment period.

Discussion

- After treatment 6, the fast treatment cultures rapidly declined. Also supported by observational data which showed algae lysing.
- Slow treatment cultures also experienced a decline after treatment 6- 36% TN-BWW. Due to no observations of algae death, this is predicted to be caused by limitations of culture conditions.
- Ion Chromatography analysis of water samples will give further information of nutrient absorption. It could indicate if nutrient concentration levels were inhibiting the growth of the fast cultures as they reached 84% TN-BWW and contributed to their decline.

Algal Nutrients

	Moisture	Ash	Lipids	Fiber	Protein	Carbs	Calories
Tilapia Needs			10-15%	8-10%	30%	40%	
Fish food	6.43%	10.95%	10.00%	7.35%	#	#	#
Chlorella*	N/A	6.50%	1.00%	32.15%	#	#	#
Mougeotia*	N/A	#	11.00%	26.60%	#	#	#
Scenedesmus*	N/A	#	#	#	#	#	#

Table 2: Current results on algal macronutrients compared to current fish food nutrients and tilapia needs.
* Number of replicates has been limited due to availability of algal samples.
# Testing in progress.

Discussion

- Once all of the analyses have been completed on the algae, the same analyses will be run on red worms that are grown in the greenhouse to see if they may be mixed with the algae to achieve the correct macronutrient content for fish food.
- Further ingredient testing and comparison with the nutritional requirements of the tilapia (FAO) will be needed to find a mixture that meets these requirements.
- By creating fish food from algae and red worms that are grown on campus, we will be able to create a more sustainable option for feeding the fish while also potentially improving their diet.

Citations: Association of Official Analytical Chemists (AOAC), Food and Agriculture Organization (FAO). Herrera, Daniella. Growing Algae in Biodiesel Wash Water for Waste Water Remediation. IES Climate Change Poster Session. Loyola University Chicago. March, 2017.
Acknowledgements: Zach Waickman, MBA, Loyola University Chicago & Dr. Zhenwei Zhu, PhD, Loyola University Chicago