Assessing the Nutritional Value of Algae Strains Grown in Biodiesel Wastewater as a Fish Food

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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 nitrate, potassium, and phosphorous[1].
- By studying the algae growth patterns in response to the amount of BWW a culture receives, students in the lab 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

Procedural Development

- Over the summer, First Year Research Experience (FYRE) students started researching methods for analyzing different macronutrients based on Association of Official Analytical Chemists guidelines[2].
- These methods were further developed and tested several times specifically for algae samples, and errors were worked through until a solution was found.
- After results were reproducible, samples were sent to an outside lab[3] to confirm the accuracy of our procedures.
- After receiving confirmation, further macronutrient analyses were performed on three strands of algae: *Chlorella protothecoides*, *Mougeotia transaui*, and *Scenedesmus dimorphus*.

Analyzing Algal Macronutrients

- Procedures to analyze ash, lipids, fiber, and protein were conducted; and carbohydrates and calories were then calculated from those results.
- Ash content was measured by burning the sample in a 600^{0}C furnace.
- Lipid content was measured using a Soxhlet Extraction Apparatus, in which anhydrous diethyl ether extracted the lipids from the sample (Image 1).
- Fiber content was measured by digesting the sample in sulfuric acid and then in sodium hydroxide (Image 2).
- Protein content was measured using a Flash 2000 Organic Elemental Analyzer (Image 3).
- Once we had data for all of the previously mentioned macronutrients, we were able to calculate carbohydrate content and calories using mathematical equations[4,5].

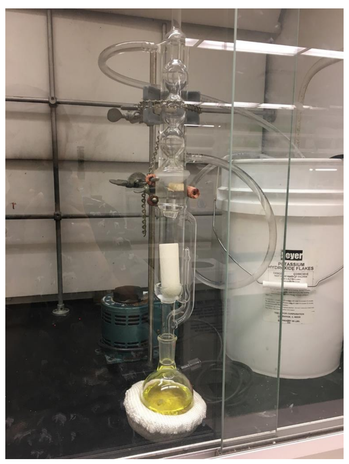

Image 1: Soxhlet extraction apparatus for lipid content analysis.

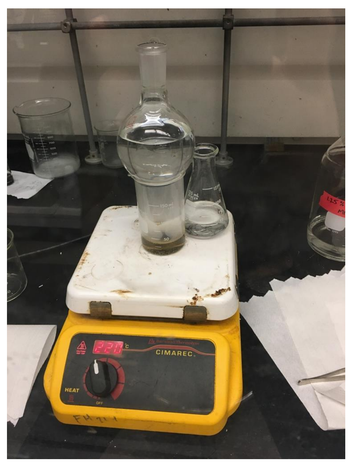

Image 2: Acid and base digestion for crude fiber content analysis.

Image 3: Flash 2000 Organic Elemental Analyzer for protein content analysis.

Results & Conclusions

Algal Nutrients

	Percent Dry Biomass						Calories
	Moisture	Ash	Lipids	Fiber	Protein	Total Carbs	(kcal/dry g)
Tilapia requirements[6,7,8,9]	N/A	N/A	10-15	8-10	30.00	40.00	9-11
Fish food	6.43	10.95	10.00	7.35	47.23	31.82	4.06
Fish food*	0.53	10.30	13.20	9.57	49.20	26.80	4.23
Chlorella**	N/A	6.50	1.00	32.15	46.74	45.76	3.79
Mougeotia**	N/A	15.33	11.00	26.60	32.71	40.96	3.94
Scenedesmus**	N/A	5.90	1.00	36.60	51.18	41.92	3.81
Red worms	87.31	8.29	7.20	7.51	69.56	14.95	4.03

Table 1: results on algal macronutrients compared to current fish food nutrients and tilapia nutritional requirements.
*Sample tested at Covance Commercial Labs **Number of replicates has been limited due to availability of algal samples.

Discussion

- After analyses were completed on all three algae strands, the same analyses were run on red worms to see if they could be used as an additive to our fish food so that we may achieve the correct nutrient levels for the tilapia.
- Now that we have a larger dataset of the macronutrient contents, we can begin looking into different ratios of algae and red worms to find a mix that will fulfill the nutritional requirements of the tilapia.
- We have found that *Chlorella* and *Scenedesmus* have very low lipid contents, but are high in protein and fiber.
- All of our samples have very high protein and carbohydrate content, especially *Scenedesmus*.

Potential Mixes for Final Product

	Percent Dry Biomass					Calories
	Ash	Lipids	Fiber	Protein	Total Carbs	(kcal/dry g)
Tilapia requirements	N/A	10-15	8-10	30.00	40.00	9-11
25% chlorella/75% red worms	7.84	5.65	13.67	63.86	22.65	3.97
50% chlorella/50% red worms	7.40	4.10	19.83	58.15	30.36	3.91
75% chlorella/25% red worms	6.95	2.55	25.99	52.45	38.06	3.85

Table 2: examples of possible mixes of algae and red worms and the macronutrient contents

Discussion

- We have decided to focus our attention on the strand of *Chlorella* because it is the strand that grew the best in our BWW conditions (Kelsey O'Malley, Provost Fellow).
- Table 2 shows three examples of different ratios of *Chlorella* and red worms and the resulting macronutrient contents of the mixtures.
- By looking into different ratios of algae and red worms, we can see what macronutrients are insufficient and which ones are excessive in order to find another additional ingredient to achieve the correct levels of macronutrients.

Future Research

- Since both the algae and the red worms have low lipid content, high protein content, and fiber content, we will begin looking into additional ingredients that are low in protein and fiber and high in lipids to create the correct balance of macronutrients for the tilapias' nutritional requirements.
- To find this additional ingredient, we will look into different plants and organisms that we have available in the Institute of Environmental Sustainability's greenhouse so that all of our ingredients are locally sourced.
- We will also conduct literary research to look at increasing the lipid content in our *Chlorella* strain to correct for the low lipid level in the fish food.
- We will then run any potential additional ingredients through the same analyses for macronutrient levels.
- Once we have found an ingredient that balances out the macronutrient levels of the algae and red worms, we will be able to create a more sustainable option for feeding the tilapia while also potentially improving their diet.

Citations: 1: Herrera, Daniella: Growing Algae in Biodiesel Wash Water for Waste Water Remediation. IES Climate Change Poster Session. Loyola University Chicago. March, 2017.
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Acknowledgements: Zach Waickman, MBA, Loyola University Chicago & Dr. Zhenwei Zhu, PhD, Loyola University Chicago