

**Feasibility of Anaerobic Digestion of Algae Produced by an Algal Turf Scrubber at the
Port of Baltimore**

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1. Executive Summary

The Port of Baltimore is exploring Algal Turf Scrubber (ATS) technology as a method to remove nutrients from runoff that would otherwise pollute the Chesapeake Bay. The area of the current system at the Port is about 186 square meters; and it produces algae at a rate of 20 grams of dry algae per square meter per day or 26 kg of dry algae per week. The Port would like to scale up the ATS to a ½ acre system which would theoretically produce 282 kg of dry algae each week. Because the Port is trying to be more sustainable, they would like to investigate bioenergy production using the algae as a feedstock. The purpose of this study is to investigate the potential of Anaerobic Digestion (AD) of the algae as a fuel source to power the ATS pumps; thus creating a positive feedback loop in the system from what has been treated as waste. To assess the potential impact an AD system could have on the pumps' reliance on external energy inputs, a Bio-Methane Potential (BMP) test was conducted at a 28 day Hydraulic Retention Time (HRT) using a low-cost lab-scale bioreactor design. Two different (1:1 & 2:1) substrate to inoculum loading ratios (S:I) based on the Volatile Solid (VS) content of the inoculum (24.9gVS/g innoc) and dry algae (355.9gVS/kg algae) were tested in triplicate and compared to a control with a S:I of 0:1. Two of the three low-cost heating units failed, so only the data for a duplicate study at 23 degrees C could be analyzed. The best methane production was found to be 54.2 mL CH₄ per gram of algae. Assuming the algal production rate remains consistent the BMP can be expressed as a rate dependent on ATS area: 1.085 L CH₄/m²/d.

2. Introduction

Algal turf scrubbers (ATS) are used as wastewater treatment systems that biomimic natural processes. Algae and coral reefs have a symbiotic relationship in which turfs cover the hard surfaces of the reef crest where wave energy is highest. To simulate these processes, the ATS is designed as a shallow trough lined with a mesh screen over which water is passed with wave energy generated by a surge bucket (Kangas, 2004). The algae grow quickly and remove pollutants in the process. Their biomass is collected and the pollutants are permanently removed from the system.

Bacterial digestion reduces the algae and the inoculum in an anaerobic, or no oxygen, environment. The goals of anaerobic digesters are to destroy a significant portion of the volatile solids in the organic content (Gerardi, 2003). Digestion requires relatively long duration periods to allow for the slow bacterial processes of hydrolysis and dissolving of the solids. Once solubilized, the resulting complex organic compounds degrade to volatile acids and alcohols, methane, new bacterial cells, and a variety of simplistic inorganic compounds such as carbon dioxide and hydrogen gas (Gerardi, 2003).

Three stages occur during anaerobic digestion. The first stage is hydrolysis, which involves solubilization of organic compounds (polymers) to simpler compounds (monomers) such as volatile acids, fatty acids, and amino acids. The second stage is the process of acetogenesis that converts these compounds to formic, acetic, propionic, and butyric acids as well as hydrogen gas. The third stage, methanogenesis, involves the production of methane, carbon dioxide, ammonia, and hydrogen sulfide from the degradation of acetate (Gerardi, 2003). The product of digestion is biogas, innocuous digester sludge solids, and waste heat.

The algae biomass produced on the ATS primarily consists of *Melosira* and *Ulva*. Both species can be anaerobically digested in order to generate electricity to run the ATS at night. With solar power operating the pump during the day and algal biogas running the pump at night the entire system becomes self sufficient, creating a positive feedback loop.

3. Methods

3.a. *Bioreactor/Experimental Design*

To determine potential biomethane yields from the anaerobic digestion of algae, nine 1.5L bench-top bioreactors were constructed using plumbing materials available at Home Depot and online: 2' pieces of 3" PVC, with a 3" PVC toilet flange as a base, and a 3" test cap on top, fitted with a #4 rubber stopper, and ¼" plastic tubing and hose clamps so that the system was sealed airtight. The plastic tubing fed into 10L Tedlar gas bags where biogas was collected. Heating units for the reactors were fabricated by rewiring pipe defrosting heating tape through a HVAC temperature control unit, but the system did not work. Each triplicate of digesters cost \$151.

For our experimental design, we used digestate from the dairy manure digester at Beltsville Agricultural Research Center as inoculum. We mixed 1.5L of inoculum with dry algae at 3 predetermined ratios based on measured volatile solid contents and had 3 trials at each ratio. We took 250mL samples from each slurry for testing before loading the digesters. We wrapped one of each of our 3 heat tapes around one digester from each loading ratio. All digesters were purged with pure nitrogen and carbon dioxide gas after loading the digesters and before attaching gas bags, which were also purged.

3.b. Total Solids/Volatile Solids Testing

Total solids and volatile solids for dry algae, raw inoculum, and influent & effluent slurry samples were calculated using EPA Standard Method 1684. All samples were baked in pre-weighed crucibles at 105 degrees Celsius for 24 hours. Samples were then reweighed to determine the Total Solids (TS) and Moisture Content (MC) of the sample (Sample Calculation in Appendix). Samples were then baked for one hour at 550 degrees Celsius in a muffle furnace, massed and baked for an additional 15 minutes to be certain all Volatile Solids (VS) had been combusted. The TS of the sample is the mass of sample that remains after all water has been evaporated. The VS of a sample is a proxy measure of the organic content of the material; or in other words, an approximation of what percentage of the sample is available to bacteria and archaea involved in the AD process.

The VS ratio of inoculum to substrate has been proven to be a significant factor influencing the biogas yields in BMP studies (CITE). In this study we tested three different VS loading ratios (1:1, 2:1, 0:1) based on TS/VS results for dry algae from the ATS (Appendix 8.a.) and inoculum obtained from a dairy manure digester (Appendix 8.b.). Three trials were run at each loading ratio and example of the calculations to determine the loading ratios based on mass can be found in APPENDIX.

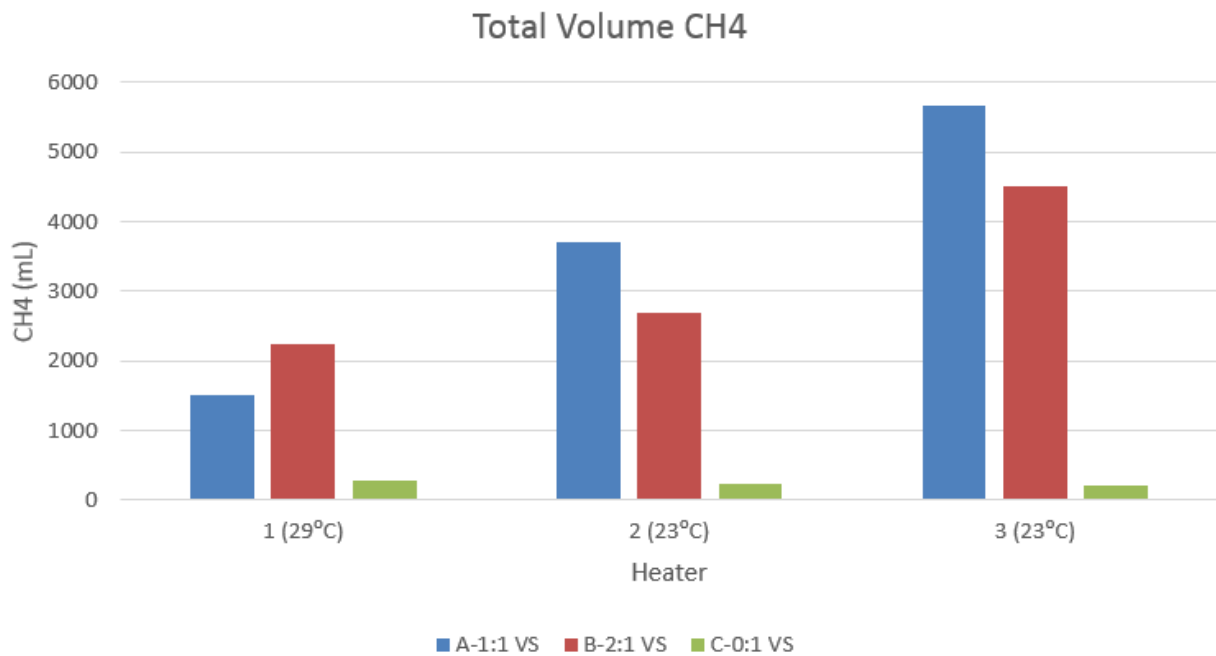
3.c. Biogas Monitoring

Biogas production was analyzed for volume and composition on days 7, 21, and 28. Volume was measured with a frictionless volumetric glass syringe. Triplicate samples of each trial were analyzed using a gas chromatograph. Methane content of the biogas was determined by comparing the peak areas of each run to a standard curve. The total volume of biogas production was multiplied by the methane percentage to determine the methane production of each trial.

4. Results & Discussion

For the 1:1 and 2:1 VS loading rates, the unheated digesters produced more biogas than the heated trials, as shown in the figure below. The only heater that was functioning at the end of the 28 day HRT was not supplying consistent heat, but fluctuating on and off throughout the day. Peces et al. observed an adverse effect on biogas production when mesophilic digesters were heated to thermophilic temperatures (55 degrees C) for 2 and 24 hour pulses (2013). The decrease in biogas production in this study was attributed to the sensitivity of methanogenic archaea to environmental instability. The same mechanism could explain the poor biogas production of the heated trials in this study. Because of this phenomena, any digester installed at the PoB needs to include a temperature regulation system.

The control treatments all had extremely low biogas production one order of magnitude lower than the reactors loaded with algae. This was expected because the methanogenic consortium of the controls were deprived of an available carbon source. In the two unheated runs biogas production was more efficient at the 1:1 loading rate (35.5 & 54.2 mL CH₄/g dry algae) than the 2:1 (12.9 & 21.6 mL CH₄/g dry algae). This was consistent with previous studies that investigated different VS loading rates for BMP experiments (.).



The best methane production was observed in trial A3 (54.2 mL CH₄/g dry algae) which was an unheated reactor at a 1:1 VS loading rate.

5. Conclusions

- A pilot-scale or full-scale digester at the Port would require a temperature control unit to protect the methanogenic community from temperature shocks, but heating to mesophilic temperatures is not essential to produce biogas.
- A digester maintained at 23 degrees C could theoretically produce 1.085 L CH₄/m² ATS or: 202 L CH₄/day from the algae produced at the current scale of the ATS and approximately 2200 L CH₄/day from the algae produced in the ½ acre scale-up.
- Biogas produced in the digestion of algae could be used to power pumps for approximately 2.7 hours if the mechanical energy of an engine could be used directly. This could be used to circulate water intermittently at night when algae is not being produced
- If a generator is absolutely necessary to convert mechanical energy to electrical energy digestion is not a feasible disposal method.

6. Work Cited

EPA Standard Method 1684 “Total, Fixed, and Volatile Solids in Water, Solids, and Biosolids”http://water.epa.gov/scitech/methods/cwa/bioindicators/upload/2008_11_25_methods_method_biological_1684-bio.pdf

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7. Appendices

7.a. TS/VS of Dried Algae

	Cruc. #	Crucible Mass (g)	Crucible+ Sample Mass (g)	Sample Mass (g)	Post 105 C	Post 550 C	TS (g/kg)	VS (g/kg)	%TS (of sample)	%VS (of TS)
1 (dry)	33	21.477	23.462	1.985	23.361	22.654	949.1	356.2	94.9%	37.5%
2 (dry)	14	21.532	23.466	1.934	23.365	22.678	947.8	355.2	94.8%	37.5%
3 (dry)	29	17.923	20.343	2.420	20.220	19.358	949.2	356.2	94.9%	37.5%
							948.7	355.9	94.9%	37.5%

8.b. TS/VS of Inoculum

	Crucible #	Crucible Mass (g)	Crucible+ Sample Mass (g)	Sample Mass (g)	Post 105 C	Post 550 C	TS (g/kg)	VS (g/kg)	%TS (of sample)	%VS (of TS)
Inoculum	3.2	18.624	28.902	10.278	18.955	18.702	32.2	24.6	3.2%	76.4%
Inoculum	22	16.720	26.862	10.142	17.048	16.795	32.3	24.9	3.2%	77.1%

Inoculum	5.1	17.810	27.210	9.400	18.111	17.874	32.0	25.2	3.2%	78.7%
							32.2	24.9	3.2%	77.4%

8.c. CH4 Volume per treatment per week

Treatment	Reactor	Volume CH4 (mL) Day 7	Volume CH4 (mL) Day 21	Volume CH4 (mL) Day 28
A	1	857	8	637
	2	1358	1865	488
	3	1941	2089	1633
B	1	570	1292	367
	2	455	2069	166
	3	808	2707	994
C	1	56	129	100
	2	28	104	111
	3	17	92	110

8.d. GC Analysis Week 1

3/30/2015	Trial 1	Trial 2	Trial 3	Average	Sam. Avg.	%CH4	Volume (mL)	Volume CH4 (mL)	Average
A1	3423.594 7	3216.385 5	3516.577 4	3385.5192		39.851 0	215 0	856.796 0	
A2	4141.742 2	4025.188 5	5212.644 5	4459.8584		52.635 6	258 0	1357.99 89	
A3	5054.789 1	5002.065 4	4929.553 2	4995.4692	4280. 2823	59.009 4	329 0	1941.40 87	1385.401 2
B1	1813.921 3	1779.554 9	1803.193 0	1798.8897		20.970 1	272 0	570.386 4	
B2	2124.022 2	2116.795 4	2235.004 6	2158.6074		25.250 7	180 0	454.513 1	

B3	2948.566 2	2573.109 9	2904.840 6	2808.8389	2255. 4453	32.988 5	245 0	808.217 8	611.0391
C1	1528.356 8	1498.226 6	1510.144 3	1512.2426		17.559 0	320	56.1888	
C2	1080.073 4	1042.477 4	1006.556 0	1043.0356		11.975 4	235	28.1422	
C3	884.4707	915.1802	881.7596	893.8035	1149. 6939	10.199 6	165	16.8293	33.7201

8.e. GC Analysis Week 2

4/13/2015	Trial 1	Trial 2	Trial 3	Average	Sam. Avg.	%CH4	Vol. (m L)	Vol. CH4 (mL)	Average
A1	163.8066	93.6729		128.739 7		1.5826	50 5	7.992 2	
A2	5904.818 9	5679.56 35	5250.97 41	5611.78 55		69.572 4	26 80	1864. 5401	
A3	5891.9229	5910.722 7		5901.322 8	5756. 5541	73.1627	285 5	2088.7 937	1320.442 0
B1	3449.709 2	3633.95 39	3382.64 60	3488.76 97		43.247 0	29 88	1292. 2202	
B2	4425.422 9	4547.54 15		4486.48 22		55.618 6	37 20	2069. 0130	
B3	5854.7559	5409.205 1	5680.995 6	5183.584 2	4386. 2787	64.2627	421 2	2706.7 447	2022.659 3
C1	2928.424 8	2943.72 93		2936.07 70		36.393 6	35 5	129.1 973	
C2	3261.312 5	3204.83 74		3233.07 50		40.076 4	26 0	104.1 986	
C3	2902.013 67	2877.03 125		2889.522 46	3019 .558 15	35.816 3	25 7	92.04 80	108.4813

8.f. GC Analysis Week 3

4/20/2015	Trial 1	Trial 2	Trial 3	Peak Avg.	%C H4	Vol.	mL CH4
A1	3393.6585	2870.9810	3275.1731	3179.937503	37.3	1710	637
A2	2377.8423	2077.6690	1873.7411	2109.750777	24.5	1990	488
A3	5808.1069	5516.4917	5568.7109	5631.10319	66.4	2460	1633
B1	5294.4287	3736.7581	5254.2275	5274.328125	62.1	590	367
B2	5709.6987	5315.3064		5512.502585	65	255	166
B3	6335.5635	5422.4434	6044.8784	5934.295087	70	1420	994
C1	3516.5120	3017.1541		3266.833005	38.3	260	100
C2	3437.7861	3726.7078		3582.246945	42	265	111
C3	2808.36621	3087.16846		2947.767335	34.5	320	110

8. g. Projections

Current		
Algal Prod. Rate	20	g(dry)/m ² /d
ATS Dimensions	93	m
	2	m
ATS Area	186	m ²
Algae Produced	3720	g/d
CH4 production best ()	1.94	mL CH4/g algae
Methane Prod. Rate	7205	mL CH4/d
Methane Prod. Rate	7.2	L CH4/d

Scale-up projection		
Algal Prod. Rate	20	g(dry)/m2/d
ATS Area	0.5	acres
	2023.4	
	3	m^2
Algae Produced	40468.	
	6	g/day
CH4 conv rate (A)	1.94	mL CH4/g algae
Methane Prod. Rate	78379	mL CH4/d
Methane Prod. Rate	78	L CH4/d