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Methane Production from the Anaerobic Digestion of some Marine Macrophytes

by

Clifford Habig and John H. Ryther

School of Forest Resources and Conservation
University of Florida
Gainesville, FL

INTRODUCTION:

Recently, considerable interest has developed concerning the use of biomass as an alternative fuel source. Among the possible substrates, marine plant biomass has frequently been mentioned, primarily due to the fact that such plants do not have competing, more valuable uses for food or fiber and their cultivation does not compete for valuable agricultural lands (1, 2). Also, recent research has demonstrated that at least one potential marine energy crop, the red alga Gracilaria tikvahiae, is capable of extremely high production rates that equal or exceed those of terrestrial plants, and are rivaled only by the productivity of another possible aquatic energy crop, the water hyacinth (3, 4, 5).

To date, seaweed energy research has emphasized cultivation, while a marked paucity of information exists regarding the comparative performance of these algae as a methanogenic substrate. Only two species, the giant kelp, Macrocystis pyrifera and Gracilaria tikvahiae, have been tested in fermentation trials (6, 7, 8). The relative merits of a red, a green, and a brown algae, run vis à vis at four different loading rates, will be discussed in this report. In addition, two loading procedures were utilized to access what , if any, effect they might have on digester performance.

MATERIALS AND METHODS

a) Set-up:

The digesters consisted of sealed 2 liter Nalgene(R) bottles, placed in boxes filled with poured-foam insulation. A gas line made from Nalgene tubing ran from each digester to an inverted one-liter graduated cylinder. The cylinders were filled with a 0.05N sulfuric acid solution, and were placed in a tub containing approximately 4 cm of the acid solution. Gas production was monitored by displacement of the acid solution. The digesters were housed in an insulated building whose temperature was maintained at $28^{0}C \pm 3^{0}$ by the use of heat lamps.

Three species of seaweeds were examined for their digestion properties. These included the Rhodophyte, Gracilaria tikvahiae, the Chlorophyte, Ulva sp., and the floating pelagic Phaeophyte, Sargassum fluitans. Gracilaria and Ulva were grown at the Harbor Branch aquaculture facility, while the Sargassum was collected from the Ft. Pierce, FL, beaches. The Gracilaria digester consisted of 1 kg wet weight of chopped (2-3 cm lengths) seaweed, 0.8 liters of seawater, and 0.2 liters of inoculum. For the Sargassum digesters, one kg wet weight of shredded Sargassum (about .5 cm pieces) was used with 0.5 liters of seawater, and 0.2 liters of inoculum. The Ulva digesters initially contained one kg wet weight of chopped seaweed (pieces 2-3 cm on a side), .7 liters of seawater, and .2 liters of inoculum. However, due to acid difficulties during start-up, all but one Ulva digester was re-started, with .5 kg of seaweed, 1.2 liters of seawater, and the same dosage of inoculum. A previously described 120 liter digester (9), re-started with 20 kg of anaerobic sediments and 10 kg of Gracilaria, provided the inoculum source in each case.

b) Loading:

Gracilaria and Sargassum digesters were loaded at rates corresponding to 20, 30, 40, or 50 day retention times, while the Ulva digesters were operated at 30, 40 or 50 day retention times. Prior to the experiment, the seaweed of each species was pre-packaged in 100 gram wet weight packets and frozen to minimize possible effects due to using different batches of seaweed. Unfortunately, not enough Sargassum could be collected at once, so two batches of this seaweed were necessary. The digesters were loaded according to one of two schedules by adding either 10% or 20% of the wet weight of seaweed in the digester at each loading. The equal amount of digester residue removed during loading contained both solid and liquid residue phases. The digesters were agitated only during loading. At this time, a digester's contents received a 10-15 second stirring. Since the 10% exchange digesters were loaded twice as often as their 20% counterparts, the latter set of digesters were swirled for the same amount of time when the 10% digesters were loaded. The data reported here represent a ten week period commencing four to six weeks after initiating differential loading.

The experimental protocol for digester loading and operation is shown in Table 1.

c) Sampling:

Gas samples and digester residue samples were taken either at each loading, or on alternate loadings, depending upon a digester's retention time and loading schedule. The digester residue samples were separated into solid and liquid phases by filtering through a .5 mm mesh. Determination of the volitile solids content of plants or solid digester residues consisted of drying samples for four days at 70^{O}C, followed by measurement of weight loss after combustion at 550^{O}C in a muffle furnace for four hours. Biogas production was measured daily, with gas samples being analyzed for methane content using an MSA total hydrocarbon analyzer standardized against known amounts of methane. All gas production numbers have been corrected to standard conditions of temperature and pressure.

RESULTS:

Pertinent information about each species of seaweed tested is presented in Table 2. Most importantly, Sargassum contains the greatest amount of volitile solids per unit of wet weight, followed by Ulva, then Gracilaria. Energy content per unit wet weight is also highest in Sargassum, lower by 15% in both Gracilaria and Ulva.

Initially, within two days following inoculation, all of the digesters displayed a significant drop in pH. Ulva reacted the most drastically, dropping from 7.2-7.3 at inoculation to 5.8-5.9. Gracilaria and Sargassum reacted similarily, with the pH of Gracilaria digesters falling to 6.1-6.2, while Sargassum digesters dropped to 6.2-6.3. These pH changes were countered by titrating the digesters back to the nuetral range with sodium hydroxide. The digesters required daily titration for a period of eight to ten days before the pH stabilized at 6.7-6.8. Titration with alkali proved to be a more effective method of pH control than the addition of carbonate buffers in these salt water digesters.

A) Effects of 10% vs. 20% loading rates

Initially, there was concern that exchanging as much as 20% of the digester volume would shock or disrupt digester performance, either by disturbing the organisms or causing harmful pH fluctuations. However, those digesters showed similar characteristics to the ones in which 10% of the biomass was exchanged at each loading. There were, however, two noticeable trends. The 20%- loaded digesters characteristically combined a slightly higher methane content with a somewhat lower biogas production per gram of volitile solids added. However, T-tests between matched digesters indicated no difference at the α- .05 level. Therefore, the data presented in Table 3 represent the combined 10% and 20% exchange results for each retention time. These results permit seaweed digesters to be run with only half the maintenance necessary if loading exchanges of 10% or less were required.

B) Methanogenisis:

All three species of seaweed showed increased methanogenisis with increased retention time, but the methane output patterns were somewhat different for each species (Table 3). *Sargassum* maximized its methane production at the 30 to 40 day retention times. *Gracilaria*, on the other hand, did not attain high methane production until the 40 and 50 day retention times. *Ulva* displayed good methanogenisis for all three retention times that were tested. The greatly increased gas production of *Ulva* per gram of volitile solids added is particularly noticeable, especially at higher loading rates. The gas output of *Sargassum* slightly exceeded that of *Gracilaria* at higher loading rates, while *Gracilaria*'s production eventually surpassed *Sargassum*'s at longer retention times. Also note that yields of 60% or more of methane could be obtained from each species. With high gas production coupled to high methane content, *Ulva* displayed the best bioconversion efficiency.

Figure 1 portrays the trends in daily biogas and methane production at different retention times. For each species, the maximum daily biogas output occurs at the lowest retention time. However, under those conditions, methane content is quite low for *Gracilaria*, but is near 50% for both *Sargassum* and *Ulva*. These changes in methane content with retention time result in shifting optimization for daily methane production to longer retention times for *Gracilaria*. The daily methane output of Sargassum, on the other hand, is similar for 20 to 40 day retention times, due to faily equal compensation between biogas production and methane content, but it drops off significantly at the 50 day retention time.

DISCUSSION

Of the three marine macrophytes tested, Ulva appears to be more readily digestable than Gracilaria or Sargassum. This is indicated by (a) the large initial pH drop of Ulva digesters, suggesting a rapid release of volitile organic acids; and (b) the gas produced per unit of volitile solids reduced. The latter reflects a higher amount of volitile solids destruction per unit of gas produced, most notably at shorter retention times. The relatively poor performance of Gracilaria at low retention times most probably results from initial resistance of agar to microbial attack, as was also concluded by Hanisak (7). However, Bird et al (10) also observed that, with time, agar becomes a suitable substrate for methanogenic fermentation. Those observations correlate well with the present performance of Gracilaria at longer retention times. The present results suggest that once initially resistant polysaccharides, such as agar, are partially degraded, they provide a good methanogenic substrate. The agar content of Gracilaria may be very important in determining optimum retention time.

Sargassum presents a different picture. This brown seaweed appears to contain some biochemical constituants that provide a relatively digestable methanogenic substrate, which accounts for the good performance at low retention times. However, at the longest retention time (50 days), Sargassum's performance falls off considerably, suggesting that a fairly resistant, poor substrate component, possibly a fiber fraction, must also be utilized as a methanogenic substrate. Sargassum does contain a larger fiber fraction and a smaller soluble carbohydrate fraction than either Gracilaria or Ulva (unpublished data). There is also the possibility that the Sargassum digesters became nutrient limited, since the C/N ratio of the feed exceeded 20. Fannin et al (8) reported that a C/N ratio greater than 15 represented nutrient limitation in Macrocystis digesters.

Seaweeds represent a biochemically complex and diverse group (11). Therefore, considering the variety of components, particularly polysaccharides, available as substrates, interspecific variations in biogas output and methane content are not surprising. Indeed, there are several indications that intraspecific variation between batches of seaweed can affect gas yields and alter optimal retention times (8). Clearly, more information concerning the relationships between environmental growth conditions, biochemical composition, and digestability of seaweeds would be desirable.

The methods used in this study have emphasized low-cost, non-intensive anaerobic fermentation. Energy inputs to the digesters were minimized, with temperature maintenance above 25°C via heat lamps being the only significant input. The digesters were only briefly stirred during loading, and the pretreatment of raw seaweed (coarse chopping) represents minimal substrate preparation. Even with such low energy inputs, the methane production of these digesters compares well with that of other manure and plant biomass digesters (6, 7, 8, 12, 13, 14). In fact, the only significant difference between this study and the previous one using Gracilaria (7) is the high methane production at 30 days retention time reported in the latter study. Equally good methanogenisis did not occur until 40 days retention time in the present experiment. This difference can be attributed to either the slightly lower temperature of this experiment, variability of seaweed batches or a combination of the two factors.

The net energy production of these simplified digesters most probably would equal or exceed that of other digesters, since all previous digesters were maintained at slightly higher (30-35°C) to much higher (55-58°C) temperatures. Many also involved a considerable amount of mixing. Considering Jewell's (13) observations on cow manure digesters, these increased inputs, along with increased construction costs of elaborate digesters, tend to shift the net energy and economic balance toward lower temperature, simplified digesters. However, 27°C may represent the lowest practical temperature for operating these seaweed digesters, since temperatures below this appeared to greatly reduce gas output.

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Table 1. Experimental protocol for digester operation.

Organism	Digester Load (kg wet wt.)	Retention (days)	Loading Freq (days)	Loading Rate[1/] %	kg/addition
<u>Sargassum</u> <u>and</u> <u>Gracilaria</u>	1.0	20	2	10	0.1
	1.0	30	3	10	0.1
	1.0	40	4	10	0.1
	1.0	50	5	10	0.1
	1.0	20	4	20	0.2
	1.0	30	6	20	0.2
	1.0	40	8	20	0.2
	1.0	50	10	20	0.2
<u>Ulva</u>	0.5	30	3	10	0.05
	0.5	40	4	10	0.05
	0.5	50	5	10	0.05
	0.5	30	6	20	0.1
	0.5	40	8	20	0.1
	0.5	50	10	20	0.1

[1/] Loading rate includes both liquid and solid fraction of the feed and is equivalent to volume exchange of the digesters, as referred to in text.

Table 2. Composition and energy content of seaweeds.

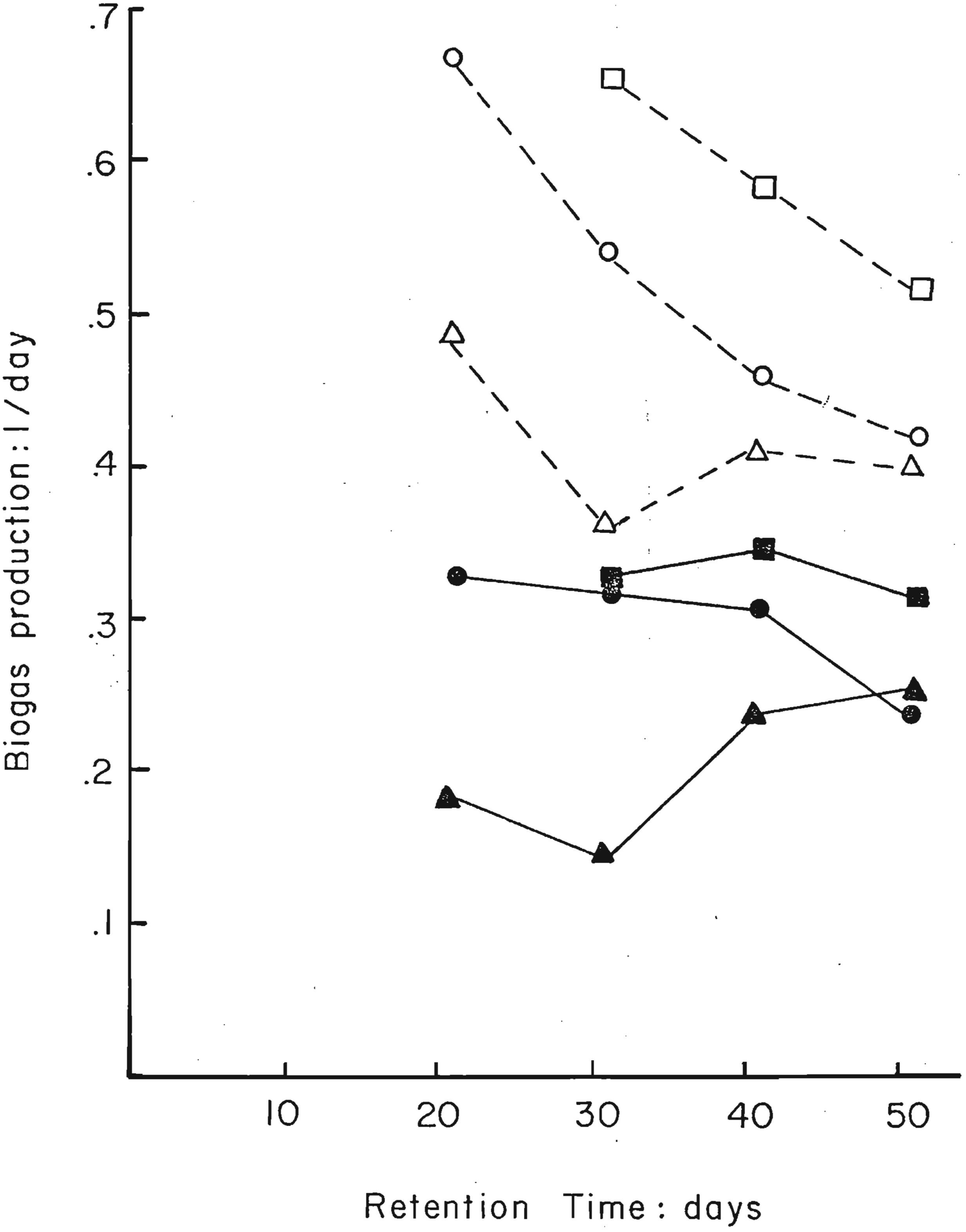
Organism	Dry wt. (% wet wt.)	Volatile Solids % wet wt.	 % dry wt.	Nitrogen % dry wt.	Energy C/g VS
Sargassum	13.4	8.4	62.8	1.04	4.2
Gracilaria	10.8	6.6	60.9	2.07	4.5
Ulva	11.6	6.9	59.7	2.35	4.3

Table 3. Characteristics and efficiency of seaweed methanogenesis

Organism	Retention (days)	pH	Biogas Produced (1/g VS added)	Methane Produced (% Biogas)	Methane Produced (1/g VS added)	Methane Produced (1/g VS reduced)	VS Reduced (%)	Energy Recovered (% VS as methane)
<u>Sargassum</u>	20	6.9	0.16	49.5	0.08	0.39	20.2	17.0
	30	7.0	0.19	58.5	0.11	0.42	27.4	24.5
	40	7.1	0.22	67.5	0.15	0.44	33.3	31.5
	50	7.2	0.25	57.4	0.14	0.34	40.4	30.6
<u>Gracilaria</u>	20	6.8	0.14	36.8	0.05	0.29	18.2	10.7
	30	6.9	0.16	39.5	0.06	0.30	21.2	12.8
	40	7.1	0.25	58.4	0.14	0.45	31.8	28.9
	50	7.2	0.30	62.7	0.19	0.48	39.4	38.3
<u>Ulva</u>	30	6.9	0.29	50.3	0.14	0.34	41.3	30.4
	40	7.0	0.34	59.0	0.20	0.39	50.4	41.7
	50	7.2	0.38	60.6	0.23	0.41	56.1	48.1

Figure Legend

Figure 1. Daily gas production vs. retention time from anaerobic digestion of the seaweeds *Sargassum* (circles), *Gracilaria* (triangles) and *Ulva* (squares). Open symbols and dashed lines, total biogas. Closed symbols and solid lines, methane.


.7
.6
.5
.4
.3
.2
.1
Biogas production: l/day
10
20
30
40
50
Retention Time: days

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