Effects of marine algal proximate composition on methane yields

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Abstract

The suitability of different *Gracilaria* spp. and two *Sargassum* species for bioconversion to methane was determined through bioassays of methane yield. *Gracilaria* species and strains were excellent feedstocks for high methane yields, ranging from 0.28 to 0.40 m^3 kg^{-1} volatile solids added. These yields ranged from 58 to 95% of theoretical stoichiometric yields. Methane yields were highly correlated with acid soluble carbohydrate components of the *Gracilaria* spp. Both *Sargassum fluitans* and *S. pteropleuron* were poor feedstocks, with methane yields ranging from 0.12 to 0.19 m^3 kg^{-1} volatile solids added, 27 to 46% of theoretical stoichiometric yields, respectively. The various tissue types of these *Sargassum* species were also poor feedstocks for anaerobic digestion to methane. While there is no clear explanation for the low methane yields, the two *Sargassum* spp. appear to contain a high proportion of an insoluble, non-extractable component which may not be available as a substrate for bioconversion to methane.

Introduction

Production of methane by anaerobic digestion of biomass is strongly dependent on several factors such as bioreactor design, its operation and the chemical composition of the feedstock. Compositional effects are so significant that they affect the choice of reactor design (Fannin *et al.*, 1983). Even within a specific biomass resource such as *Sorghum bicolor*, variability in compositional fractions such as cellulose, fiber and starch can significantly affect methane yield (Jerger & Chynoweth, 1987).

Marine biomass has been found to be an excellent feedstock for anaerobic digestion to methane. Methane yields on the order of 0.43 m^3 kg^{-1} volatile solids (V.S.) added have been achieved at low loading rates for the kelp, *Macrocystis pyrifera*. Comparisons of methane yields from different lots of kelp indicated that yields, ranging from 0.17 to 0.43 m^3 kg^{-1} V.S. added, were strongly dependent on the amounts of mannitol and algin. Methane yield has been modelled as a function of the mannitol concentration (Chynoweth *et al.*, 1987); but the relationship between methane yield and substrate composition has been poorly defined for other marine biomass species. A significant correlation was found between yield and acid soluble carbohydrate content of four macroalgae, *Ulva, Gracilaria*,

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Hypnea and *Sargassum* (Habig & Ryther, 1984).

Gracilaria spp. are an excellent biomass candidate because of their high productivity. *Gracilaria* can be grown in shallow water areas (<1.5 m depth) in simple enclosures of nets supported by pilings (Bird, 1987; Bird & Ryther, in press). Such areas are extensive in tropical latitudes such as Caribbean, with up to 180 000 hectares in northwest Florida alone. A large number of *Gracilaria* strains have now been isolated. Their differences in composition and chemistry of a major carbohydrate, agar, make them ideal for examining the relationship of methane yield to differences in carbohydrate composition.

Sargassum is a morphologically complex plant, with bladders, blades, and stipes. The gas filled bladders provide buoyancy, making it possible to cultivate this plant as a floating crop similar to water hyacinth. Little is known about the compositional chemistry of *Sargassum* species. There are reports that different plant tissues vary considerably in compositional chemistry, and that variability also occurs seasonally (Prince & Daly, 1981; Daly & Prince, 1981). Methane yields may vary, not only as a function of composition as affected by environmental conditions, but also due to morphology. This paper examines the composition of two *Sargassum* species, *fluitans* and *pteropleuron*, as a function of morphological differences and relates these differences to methane yield.

Materials and methods

Growth conditions

The following strains of *Gracilaria* represent either clones or single collections of the cultured *Gracilaria* species: Strain G-1, *G. tikvahiae* olive green pigmented phenotype; Strain G-3, *G. verrucosa*; Strain G-4, *G. tikvahiae*, brown pigmented phenotype; Strain G-5, *G. tikvahiae*, green pigmented phenotype; Strain G-8, *G. tikvahiae*; Strain G-9, *G. tikvahiae*; Strain G-16, *G. verrucosa*. The collection history of the different strains

is summarized in Cote and Hanisak (1986). The *Gracilaria* strains were grown in outdoor continuous suspension culture in 1.8 m^2 vaults receiving 3–4 water exchanges d^{-1}. The cultivation system is described in Bird (1989).

All strains were grown in nitrogen replete conditions by pulse fertilizing weekly in 0.6 mM NH_4NO_3 for 24 h. Reported yields were determined weekly over a 4 week period prior to harvest. Harvested material was frozen and shipped for methane yield bioassays via overnight carrier. A portion was retained and dried at 60 °C for compositional analyses.

Sargassum morphological comparisons

Large collections of *Sargassum fluitans* and *S. pteropleuron* were made in the Florida Keys. Ten fronds of each species were quantitatively dissected into bladders, blades and stipes. These samples were individually weighed to determine their percent contribution to total frond weight. The remainder of the collections was dissected until there was sufficient biomass (ca. 500 g fresh weight) of each tissue for compositional and bioassay analyses. The samples were frozen and handled as described earlier.

Compositional analyses

Dried material was used for the compositional analyses, all of which were based on triplicate biomass samples. Organic content was determined gravimetrically after ashing samples for 4 h at 450 °C. Total carbon and nitrogen contents were determined using an elemental analyzer. Percent protein and acid soluble carbohydrate were determined as previously described (Bird *et al.*, 1981a). For *Gracilaria* analyses, agar extractions of native agars and the characterization of 3% agar gels are also described in Bird *et al.* (1981a). For *Sargassum* analyses, mannitol was determined by oxidation with periodic acid (Larsen, 1978). Alginate and fiber were determined by extracting dry tissue with 0.16 *M* HCl

for 24 h on a rotary shaker, followed by centrifugation and extraction of the pellet in 0.1 M NaOH for 24 h. This supernatant was saved and the pellet reextracted for 24 h, with the subsequent supernatants combined with the initial supernatants. The pellet was dried at 60 °C, weighed and corrected for ash content, and the gravimetric difference considered to be fiber. Lipid levels of *Sargassum* range up to 3% of dry weight (Dawes *et al.*, 1988), hence contribute little to any plant component. Alginate was determined in the alkaline fraction using the differences in absorption of comparative samples treated with H_2SO_4 and H_2SO_4/borate reagents (Cheshire & Hallam, 1985). Alginate from *Macrocystis pyrifera* (Sigma) was used as a standard.

Biochemical methane potential (BMP) assay

Frozen samples were weighed and quantitatively transferred to 250 ml (282 ml actual volume) Wheaton serum bottles (No. 223950) prepared in triplicate. Approximately 180–220 mg feed V.S. were added per reactor which provided a measurable amount of methane (10–100 ml) during the incubation period. The bottles were outgassed with 30:70 volume ratio of CO_2/N_2, which passed through a heated, reduced copper column for oxygen removal. Simultaneously, a defined nutrient medium was purged with 30:70 volume ratio of CO_2/N_2 (Owen *et al.*, 1979).

The seed inoculum was obtained from a sludge digester operated at a 15-day retention time and a loading of 1.6 g V.S. $l^{-1} d^{-1}$. The inoculum was anaerobically transferred to the outgassed nutrient medium, resulting in a tenfold inoculum dilution. While the diluted inoculum was being purged and mixed, a 100 ml aliquot was anaerobically transferred to the serum bottle containing the feedstock, resulting in a feed/inoculum volatile solids ratio of approximately one. The reactor was purged for an additional 10 minutes, rubber-stoppered, crimp-sealed, and incubated at 35 °C in an inverted position to minimize gas leaks.

Gas production and gas composition measurements were typically performed at 3, 7, 10, 20, 30 and 60 days. Gas production measurements were performed with a glass syringe equipped with a 20-gauge needle. The assay bottles were equilibrated to room temperature before measuring gas production. The sample syringe was lubricated with deionized water and flushed with a CO_2/N_2 mixture prior to gas reading. Gas volume determinations were made by allowing the syringe plunger to equilibrate between the bottle and atmospheric pressure. At the termination of the test period, the quantity of methane within the assay volume was added to that removed during incubation and sampling, and the methane yield was determined.

Gas composition analysis for CO_2, N_2, and CH_4 was performed by gas chromatography. At the termination of the 60-day incubation period, the reactor contents were analyzed for pH, vola-

Table 1. Productivity, percent ash free composition, agar characteristics and methane yields (experimental and theoretical) from different *Gracilaria* strains.*

Strain	Productivity	Composition (%)								Agar characteristics			Methane yield	
		Ash	C	N	H	Pro	Carb	Agar	Agar of carb	Gel St	Gel T	Melt T	Exper.	Theor.
G-1	15	39	43	6.1	7.5	22.3	51.4	48.4	94	545	42	77	0.40	0.46
G-3	12	29	48	6.6	6.1	11.9	75.5	45.0	60	25	34	83	0.28	0.48
G-4	16	33	42	6.0	6.7	27.7	42.5	44.6	100	172	42	73	0.40	0.42
G-5	14	35	44	5.7	6.9	13.3	54.1	44.9	83	272	45	83	0.36	0.42
G-8	15	33	46	6.1	6.8	36.2	45.8	46.7	100	208	39	78	0.40	0.45
G-9	15	42	42	7.9	7.7	13.4	54.7	53.7	98	414	46	83	0.35	0.48
G-16	13	25	45	4.5	6.2	12.3	64.0	39.0	61	822	44	92	0.35	0.46

* Productivity as gafdw $m^{-2} d^{-1}$; methane yield as $m^3 kg^{-1}$ V.S. added.

tile fatty acids, and total and volatile solids concentrations. An inoculum control and positive control (Avicel cellulose) were run concurrently with all samples. Theoretical stoichiometric yields were calculated as described in Chynoweth *et al.* (1981).

Results

The seven *Gracilaria* strains showed marked differences in both overall composition and in characteristics of their major carbohydrate, agar. The high thallus nitrogen contents indicate that the biomass samples were not nitrogen limited. Ash content varied widely between strains, and ranged from 25% (Strain G-16) to 39% (Strain G-1) of dry weight. Acid soluble carbohydrate of the V.S. ranged from 44–76%, and was highest in Strain G-3. Protein ranged from 12–36% of the V.S., and was highest in Strain G-8. Percent ash, protein and carbohydrate combine to comprise 101–115% of the sample weight ($\pm 12\%$) indicating that these constituents comprise the major components of biomass. Agar ranged from 39–53% of the ash free weight, and comprised 60–100% of the acid soluble carbohydrate. Gelling temperature of the agars from the different strains ranged from 39–46 °C, melting temperatures from 73–92 °C, and gel strengths from 25–822 g cm^{-2}. Theoretical stoichiometric methane yields ranged from 0.42 to 0.48 m³ kg^{-1} V.S. added. Experimental methane yields ranged from 0.28 to 0.40 standard m³ kg^{-1} V.S. added. It was lowest in Strain G-3 and highest in Strains G-1, G-4, and G-8 (Table 1). Regression analyses were used to determine statistically significant relationships between composition and methane yield. Significant linear relationships were found for methane yield with percent carbohydrate and agar melting temperature. These slopes were negatively correlated with increases in the independent variables. There was also a significant linear regression with a positively correlated slope for methane yield with percent agar of acid soluble carbohydrate (Fig. 1). Other compositional characteristics
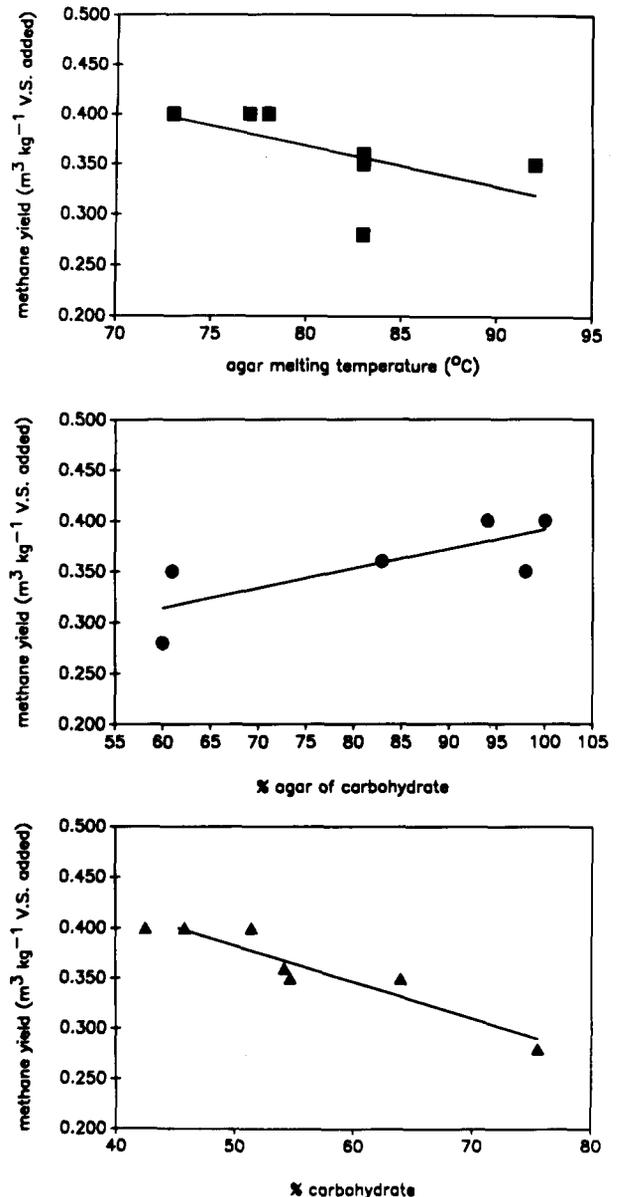

Fig. 1. Methane yield as a function of agar melting temperature (top), % agar of carbohydrate (middle), and % carbohydrate (bottom). The regression coefficients are r = 0.57, 0.79 and 0.94, respectively.

yielded no statistically significant correlation with methane yield.

The two *Sargassum* species were different in regard to contributions of different plant tissues to total thallus biomass. For *Sargassum fluitans*, the blades contributed 60% of the total V.S. in the thallus, and only a small portion in the stipe

(11%). For *S. pteropleuron*, the blades contributed ca. 50% of the V.S. of the thallus biomass, with the remaining biomass evenly distributed between bladders and stipe. There were minimal differences in most of the proximate analyses for the different tissue types. Mannitol content of the V.S. ranged from 3.1–4.5%, and was an average of 45% ($\pm$ 7%) of the total acid soluble carbohydrate pool. There were some apparent differences in alginate, with the stipe of *S. fluitans* containing almost twice as much alginate as any other tissue type. *S. pteropleuron* contained slightly less alginate in its stipe than in other tissues. The largest component of the V.S. is an insoluble component, left after acid and alkaline extraction, which usually represented more than 30% of the V.S. (Table 2). Methane yields from anaerobic digestion of the different *Sargassum* tissues were low, ranging from 0.15 to 0.19 standard $m^3 kg^{-1}$ volatile solids added. Theoretical methane yields ranged from 0.41 to 0.47 $m^3 kg^{-1}$ V.S. added. There was no clear correlation of methane yields from *Sargassum* with any compositional component. A toxicity assay did not indicate that the *Sargassum* biomass itself was toxic to the methane fermentation of volatile acids at concentrations of *Sargassum* used in these studies.

Discussion

The *Gracilaria* strains used in these experiments reflected the kind of chemical composition characteristic of N enriched cultures. Percent N was higher than 2% of dry weight for all these strains (G-16 was the lowest with 3.4% N of dry weight). A comparison of alkaline extracted protein to thallus N also suggests that these strain have significant N pools in other metabolic forms (Bird et al., 1982). Several of the *Gracilaria* strains served as excellent substrates for bioconversion to methane, with yields on the order of the best lots of *Macrocystis* or low fiber *Sorghum* (Chynoweth et al., 1987). The theoretical methane yields of the *Gracilaria* strains were slightly lower than those reported for *Macrocystis* (0.42–0.48 vs. 0.44–0.52 $m^3 kg^{-1}$ V.S. added). However, actual methane yields fell within the same range as those reported for *Macrocystis*.

A negative correlation of methane yield with percent acid soluble carbohydrate in these *Gracilaria* strains contradicts the general literature of biomass compositional effects on biodegradability. Typically, if the process is not nutrient limited (as in bioassays), an increase in soluble carbohydrate content in accompanied by increased methane yields (Habig & Ryther, 1984).

Table 2. Comparison of weight, percent ash free chemical composition, and methane yields (experimental and theoretical) from different tissue types of *Sargassum fluitans* and *S. pteropleuron.**

	% of tissue		% of whole plant			Composition %								Methane yield	
	Dry	Ash	Wet	Dry	V.S.	C	N	H	Protein	Carb	Mannitol	Alginate	Fiber	Exper.	Theor.
Fluitans															
Bladders	9.6	32.9	38	28	29	46.9	1.2	6.1	8.9	7.9	3.4	13.4	34.6	0.18	0.45
Blades	16.0	34.5	43	52	60	46.6	2.0	6.1	7.6	7.0	3.7	22.7	31.7	0.15	0.45
Stipe	27.9	36.8	19	20	11	46.2	1.5	6.9	6.4	7.8	3.2	45.2	32.4	0.20	0.47
Whole plant	14.0	39.6	–	–	–	46.9	1.9	6.1	8.6	8.3	4.5	28.3	36.5	0.18	0.45
Pteropleuron															
Bladders	14.3	27.0	33	23	24	44.5	1.3	5.8	6.0	8.6	3.7	27.4	18.7	0.19	0.41
Blades	20.0	28.9	44	44	49	44.3	1.7	5.8	6.2	6.5	3.4	32.8	33.0	0.15	0.41
Stipe	28.3	21.8	23	33	27	48.2	1.4	5.6	8.3	8.3	3.1	21.0	34.7	0.12	0.45
Whole plant	24.9	23.5	–	–	–	45.5	0.9	5.9	5.1	9.2	3.5	24.5	40.6	0.15	0.42

* V.S. is volatile solids (organics); carb is acid soluble carbohydrate; methane yield is $m^3 kg^{-1}$ V.S. added.

Methane yields were lower in strains where agar was a lower percentage of acid soluble carbohydrate, suggesting that some unknown acid soluble fraction in *Gracilaria* spp. may be recalcitrant to anaerobic digestion. This unknown fraction may be some form of an acid soluble fiber component which hydrolyzes during hot acid extraction to react as carbohydrate. The significant positive relationship between methane yield and percent agar of carbohydrate suggests agar is easily degraded and is the primary substrate during anaerobic digestion. Batch anaerobic digestion studies of *Gracilaria tikvahiae* Strain G-1 have indicated that agar is readily broken down into smaller molecular weight components (Bird *et al.*, 1981b).

Agar melting temperature displayed a significant and negative correlation with methane yield. Melting temperature of agar reflects molecular size, with larger agar polysaccharides correlated with an increase in agar melting temperature (Selby & Wynne, 1973). In these experiments, the negative correlation suggests that more complex agars are more difficult to degrade, resulting in lower yields.

There appeared to be some differences in compositional patterns between different tissue types of *Sargassum* spp. which is in agreement with the analyses by others (Prince & Daly, 1981; Daly & Prince, 1981). The two *Sargassum* species proved to be poor feedstocks for methane production. The theoretical methane yields were in the same range as those reported for *Macrocystis*. The experimental methane yields were less than half those of the theoretical methane yields. The mannitol yields of the two species were also less than half of those reported for poor lots of *Macrocystis* which have 8% mannitol of V.S. (Chynoweth *et al.*, 1987). In addition, there is a high percentage (> 30%) of an acid and alkaline insoluble component (considered herein as fiber) in the V.S. of these two *Sargassum* spp. The composition of this fiber is uncertain. Both this fiber component and the low mannitol content may have contributed to the low methane yields found in the *Sargassum* biomass samples.

Marine algae are excellent substrates for bioconversion to methane, as indicated by high methane yields obtained with some lots of *Macrocystis* (Chynoweth *et al.*, 1987) and several of the *Gracilaria* strains. However, the often cited virtue of a lack of lignin may well be an oversimplification. While little is known about the fiber components of algae, both acid detergent and neutral detergent fiber have been reported (Tompkins, 1982; Habig & Ryther, 1984; Larsen, 1978). This study suggests that there exists several types of recalcitrant material in macroalgae which reduce biodegradability. Identification of such components may provide suggestions for modifying composition to increase methane yields from marine biomass. Careful strain selection can also lead to algal biomass candidates with a higher content of biodegradable components.

Acknowledgements

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