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Short Communication

CHANGES IN AGAR AND OTHER CHEMICAL CONSTITUENTS OF THE SEAWEED *GRACILARIA TIKVAHIAE* WHEN USED AS A SUBSTRATE IN METHANE DIGESTERS*

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INTRODUCTION

An alternative energy source currently under consideration is the production of ethanol or methane (biogas) from different kinds of biomass. Among potential sources are marine plants, some of which have been shown to be highly productive [1,2]. One particular seaweed under consideration as an "energy crop" is the agarophyte, *Gracilaria tikvahiae*. Previous research has demonstrated that this species can be successfully cultivated in a mariculture system [2] and fermented to produce methane gas [3]. Furthermore, in the interest of maximizing the economic benefits of producing fuels from biomass, the residues left in the digesters can be used as a source of recyclable fertilizer [4].

During these earlier experiments, it was noted that the liquid and solid phases of unagitated digesters tended to separate from each other. The solid residue contained portions of *Gracilaria* algal thalli which retained much of their original appearance. This observation suggested the possibility that the agar contained in the plants might be recoverable after digestion, as agar is considered to be relatively resistant to microbial breakdown. Agar is a commercially valuable phycocolloid widely used in the bacteriological and food industries. Accordingly, batch digestion experiments were initiated to determine the effects of methanogenesis on agar and other chemical constituents of the *Gracilaria* biosubstrate, and to determine if the agar could be recovered in a form of good commercial quality.

MATERIALS AND METHODS

The digester used in this study had a functional volume of 120 ℓ and was like those previously described [5]. Two batch experiments were performed,

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one from May 22 until July 31, 1980, and the other from September 23 to December 9, 1980. In each experiment, 60 kg of shredded wet *Gracilaria tikvahiae* were loaded into the digester with 60 ℓ of liquid taken from another active digester. The digester lid was removed weekly and the total bulk thoroughly mixed. The height of the slurry was noted for later determinations of the total volumes. A 400—600 mℓ sample was removed, the liquid filtered off, and the remaining material squeezed to remove additional liquid. The ratio of wet to dry weight was determined after drying the material at 70°C to a constant weight. A portion of dried material was ashed at 550°C for 4 h to obtain the percent volatile or organic solids. Due to the frequency of lid removal, gas production was not measured, but it was estimated from changes in volatile solids from the initiation of the experiments. Actual gas production data are discussed elsewhere for similar experiments [3].

Percent protein and percent soluble carbohydrate of the dried material were determined in triplicate following extraction and use of the Folin reagent and the phenol-sulfuric acid method, respectively [6]. Percent carbon and nitrogen were determined with a Perkin—Elmer elemental analyzer. Percent agar was determined after pretreatment of the samples in 1 *N* NaOH at 85°C for 1 h. After chilling and removal of the alkaline liquid, the samples were neutralized to a pH range of 6.5—8.0 in water, autoclaved for 2 h, and pressure filtered through Celite and a 10 μm filter. The filtrate was allowed to gel, and then frozen. Several freeze—thaw cycles were used to purify the agar, followed by drying the samples at 70°C. Yields were corrected for percent recovery efficiency, as determined by recovery of known gravimetric standards (Bacto—Agar). The percent and dry weight data were then used with total volume measurements and sampling aliquot measurements to calculate the total amounts of dry weight, ash free dry weight (volatile solids), agar, protein, and soluble carbohydrate in the digester. Melting and dynamic gelling temperatures and gel strength of the agars were determined on 1.5% gels. The gelometer had a plunger speed of 0.18 $cm \cdot s^{-1}$ and a plunger area of 1 cm^2. Techniques for agar analysis and characterization are described in detail elsewhere [7].

RESULTS

At the initiation of the experiments, the agar accounted for most of the soluble carbohydrate (90—100%). As the experiment progressed, a pattern appeared in which the percent soluble carbohydrate rose while the percent agar declined (Fig. 1). Percent soluble carbohydrate then fell approximately to the levels of percent agar, and the pattern repeated itself. The percent protein remained relatively constant in both experiments, declining only slightly with time (Fig. 1). In the first experiment, the C:N ratios ranged from 12.5 to 14.2 with a mean of 13.4 ± 6, and in the second experiment from 11.8 to 14.9, with a mean of 13.3 ± 0.9 (data not shown).

As indicated in Fig. 2, there was a decline in utilization of the total amounts of dried material, soluble carbohydrate, and protein throughout the

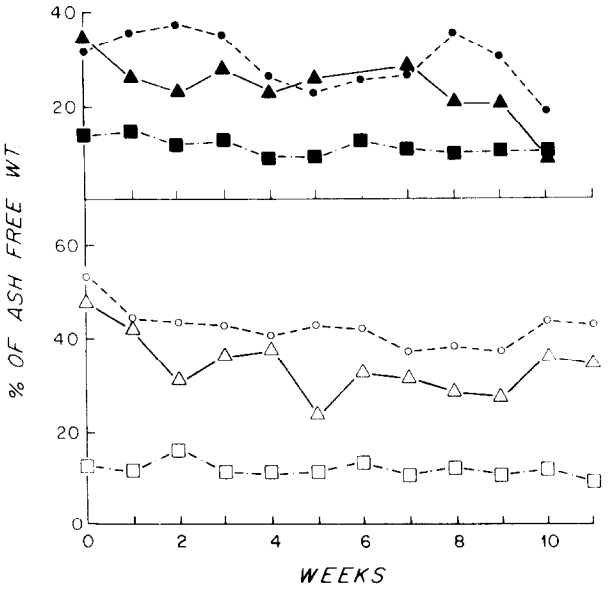

Fig. 1. Changes in the percent of agar, soluble carbohydrate, and protein of ash-free dry weight during the methanogenic digestion of *Gracilaria tikvahiae*. Experiment 1 was conducted in summer, experiment 2 in the fall-winter. Closed symbols and top portion of figure represent experiment 1, open symbols and bottom portion represent experiment 2. Circles represent soluble carbohydrate, triangles represent agar, and squares represent protein.

experiments. Throughout the course of the experiments, the amount of volatile solids (an indicator of gas production) decreased with time (Fig. 3).

All the parameters of commercial agar quality, i.e. melting and gelling temperature, and gel strength, decreased rapidly with time (Table 1). Gel strength was most strongly affected. Within one week, the gel strength was reduced to half of its initial strength in both experiments.

DISCUSSION

The patterns of percent agar decline followed by an increase in soluble carbohydrate suggest that soluble carbohydrate was produced from the degradation of agar by the microbial flora. While the percentages appear to decline and then rise again, it must be remembered that they are only relative measurements which can vary as other chemical constituents are used by the microbial flora. Although agar is generally considered to be relatively resistant to microbial breakdown, the data presented here indicate that agar can be rapidly broken under anaerobic conditions and serve as an important carbon source for the microbial flora in the digester. The rapid decrease in the total amounts of agar and soluble carbohydrate in both experiments and the strong effects of digestion on agar quality parameters further indicate that agar was meta-

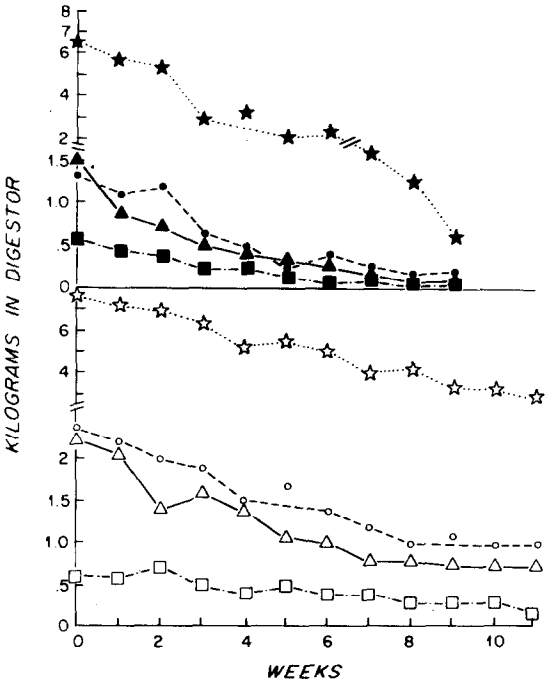

Fig. 2. Kilograms of dry weight, agar, soluble carbohydrate, and protein remaining in the digester solid phase during methanogenic digestion of *Gracilaria tikvahiae*. Experiment 1 was conducted in the summer, experiment 2 in the fall-winter. Closed symbols and top portion of figure represent experiment 1, open symbols and bottom portion represent experiment 2. Stars represent dry wt., remaining symbols same as for Figure 1.

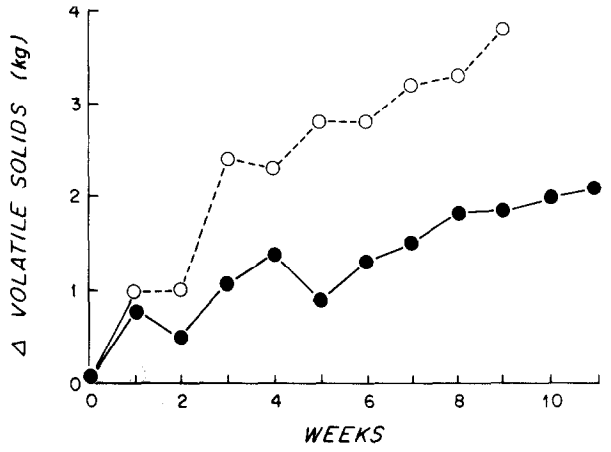

Fig. 3. Decrease in the kilograms of volatile solids in the solid phase during the methanogenic digestion of *Gracilaria tikvahiae*. Experiment 1 was conducted in summer, experiment 2 in fall-winter. Open circles represent experiment 1, closed circles, experiment 2.

TABLE 1

Changes in commercial agar quality parameters during methanogenic digestion of *Gracilaria tikvahiae*

Week	Melting temperature (°C)	Gelling temperature (°C)	Gel strength ($g \cdot cm^{-2}$)
Experiment 1			
0	86	47	306
1	76	44	130
2	76	44	20
3	74	41.5	20
4	71	38	10
5	73	42	—
6	—[a]	—	—
7	65	—	—
8	62	—	—
9	61.5	—	—
Experiment 2			
0	84	46.5	190
1	76	42	52.5
2	74	37	20
3	70	32	10
4	67.5	32	10
5	70	33.5	—
6	66	—	—
7	67	—	—
8	—	—	—
9	64	—	—
10	64	—	—
11	63	—	—

[a]— indicates that the condition of the gel was too poor to make accurate measurements.

bolized by the digester flora. Marine bacteria do have enzymes which are capable of hydrolyzing agar [8]. As melting temperatures of agar are indicators of polymer length [9], the decrease in melting temperatures and gel strength suggest that the agar polymers are hydrolyzed into successively smaller lengths. These patterns indicate that methanogenic fermentation of *Gracilaria tikvahiae* proceeds initially with a breakdown of the complex carbohydrate, agar, into simpler subunits. After this breakdown has occurred, the simpler oligosaccharides are utilized until exhausted, followed by further breakdown of the agar. This process could result from a mixed microbial flora in the digester, with each component having a specific role in the overall fermentation process. Observations on the methanogenic digestion of other substrates indicate that these types of synergistic effects of a mixed digester flora are important in the breakdown of the substrates and the operation of methane digesters [10]. No attempt was made to identify the microbial flora

of the seaweed digester; it would be of interest to isolate and identify these agents.

If algal biomass was to be cultivated for the purpose of conversion to biogas on a commercial scale, it would be economically more attractive if products other than fuel could also be obtained, e.g. fertilizer and/or phycocolloids. While the use of the residues for fertilizer appears to be practical [4,5], the recovery of phycocolloids does not. The "whole thalli" previously noted in the digester residues are probably remaining cell walls (cellulose) which form the thallus shape.

The possibility was also explored of extracting the agar first, and then using the remaining organic residues as a substrate for methanogenesis. This could provide a possible source of process energy as well as a waste treatment method for the agar industry. Preliminary attempts to do this were unsuccessful [11]; digesters loaded with agar-extracted residues quickly became too acidic (pH 4—5) for the digester flora. Both water extraction following alkaline pretreatment and the extraction techniques themselves remove most of the soluble carbohydrate, protein, and amino acids, leaving behind a residue which consists primarily of cellulose. As noted earlier, this appears to break down very slowly in the digesters. As the residues have been found to make a suitable fertilizer [4], this use will probably be of a more immediate and practical nature. Alternatively, the development of new technology may permit the conversion of the cellulosic material into ethanol [12,13]. Such a process could yield two energy crops, methane and ethanol, and a liquid residue fertilizer from a marine biomass farm.

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