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Botanica Marina
Vol. XXIV, pp. 57–61, 1981

Recycling the Residues from Anaerobic Digesters as a Nutrient Source for Seaweed Growth[1]

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(Received October 1, 1980)

Abstract

The rhodophyte *Gracilaria tikvahiae* is presently being cultivated in an aquaculture system to study its feasibility as a source of biomass that can be fermented to produce methane gas. Because nitrogen and other nutrients are conserved within the digester, the digester residues are a rich source of plant nutrients. Rather than being only waste products that require disposal, these residues can be recycled within the aquaculture system to produce additional seaweed biomass or, alternatively, might be used in agriculture to replace conventional fertilizers. For every 100 g of nitrogen added to the digester in the form of *Gracilaria*, 73 g of nitrogen were completely recycled from the digester back to cultures of *Gracilaria.*

Introduction

As fossil fuels become increasingly less available and more expensive, alternative sources of energy need to be developed. One possible renewable source, loosely referred to as "biomass," includes any type of organic substrate (e.g., wood, crop residues, animal wastes, aquatic weeds, garbage) that can be used either directly (i.e., combusted as is) or indirectly (i.e., fermented to fuels such as methane or alcohol). Most potential sources of biomass are derived from terrestrial plants, but their use as "energy crops" is probably seriously limited because 1) relatively little land is available for their cultivation, 2) terrestrial crops are more valuable for such products as food, fiber and lumber, and 3) the transformation of natural terrestrial plant communities into cultivated "energy plantations" would probably lead to serious ecological consequences.

The oceans of the world represent a vast area in which suitable biomass (e.g., seaweed) might be cultivated and harvested in the future (Wilcox 1975, Ryther 1979). Although a number of legal, policital and moral questions need to be resolved before any large-scale marine biomass system could be implemented, it is necessary that studies be made now to determine its feasibility for future implementation.

Accordingly, research has been conducted for several years at the Harbor Branch Foundation on the cultivation of seaweeds as a potential source of biomass for conversion to methane or other fuels (Ryther *et al.* 1978a, 1978b, 1979). Of over 40 species of seaweeds examined, the rhodophyte *Gracilaria tikvahiae* had the highest sustained yield and can be vegetatively propagated indefinitely in an aquaculture system. Its productivity can be as high as any terrestrial crop on earth (Lapointe and Ryther 1978). But to develop this species into an "energy crop," it is necessary not only to demonstrate its high biomass production, but also to digest this biomass to methane and recycle the digester residues as a source of nutrients. The latter is necessary in order to dispose of these waste products and to improve the economy of the whole system. This communication reports on the recycling of these digester residues to support additional growth of *Gracilaria.* Data on methane production during the digestion process has been reported elsewhere (Hanisak 1981).

Materials and Methods

Gracilaria tikvahiae was fermented in two digesters similar to those previously described and illustrated (Hanisak *et al.* 1980) for the digestion of the freshwater macrophyte *Eichhornia crassipes* (water hyacinth). These digesters, with overall dimensions of 45 cm × 45 cm × 80 cm (total volume = 162 ℓ), were constructed from 0.5 cm sheet plastic. Their lids were made from 0.3 cm thick sheet polyvinyl chloride (PVC) reinforced with 1.3 cm plywood and bolted to the digester after being sealed airtight with weatherstripping caulk. Loading and discharge ports were made of 10 cm PVC pipe with screwcap ends and two smaller (2.5 cm) PVC pipes with valves were provided for removal of liquid residue. Due to the location of the loading and discharge ports, the functio-

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0006-8055/81/0024-0057$02.00

nal volume (that which actually contained digesting material) was about 120 ℓ for each digester. One side of each digester contained a Plexiglass window for visual observation of the digester's contents. Gas lines led from the tops of the digester to inverted, submerged 208 ℓ steel drum manometers where the gas was collected and its volume measured.

The *Gracilaria* used in this study was cultivated in outdoor cultures (Hanisak 1979). The first attempts to digest *Gracilaria* were unsuccessful; these included using fermenting dairy manure or water hyacinths as an inoculum or using the *Gracilaria* alone. Digestion was eventually started by loading the following into the digester: 20 *l* of anaerobic sediments collected in the Indian River in an area of decaying seaweeds and seagrasses, 20 *l* of seawater, and 5–25 kg wet weight of *Gracilaria*. Gas production began within 5 days. The seaweed was not pretreated (e.g., shredded) in any way prior to being loaded into the digesters.

The digesters were loaded from October 1978 to June 1980, at approximately weekly intervals, usually with 5 kg wet weight; an equal volume of residue was removed. The contents of the digester were mixed manually at each loading; this was the only agitation employed. The digesters were kept at ambient temperature (30 C ± 5 C) during most of the year. During the cooler months of the first year (November–March), the digesters were kept partially submerged in a 3800 l circular water tank which was kept at approximately 30 C by an immersion heater.

To investigate the suitability of the digester residue as a nutrient source for growing *Gracilaria*, batch cultures, maintained at a density of 2.6 kg wet weight/m^2, were grown in aerated, 55 l chambers like those described by Lapointe and Ryther (1978). These cultures received either various amounts of liquid digester residue, solid digester residue, enriched seawater (see Tab. I), or unenriched seawater. The water was changed once a week, at which time the cultures were harvested, weighed and the incremental growth removed.

Levels of nitrate, nitrite, ammonium, and phosphate were monitored (APHA, 1971) in the tanks prior to and after the additions of digester residues. The nitrogen and carbon contents of plants or solid residue were determined with a Perkin-Elmer Model 240 Elemental Analyzer. The amount of organic matter (i.e., volatile solids) of plants or solid residue was determined after ashing dried samples (dried at 60 C for 48 hrs) for four hours at 550 C in a muffle furnace.

Results

A year-long study indicated that the liquid digester residue was an excellent source of nutrients for the growth of *Gracilaria* (Tab. II). There were no significant differences in growth between cultures that were

Tab. I. Composition of enrichment medium used for growing *Gracilaria tikvahiae.*

	mg/l
$NaNO_3$	127.3
KH_2PO_4	20.0
Trace metal mix[1]	
Fe	2.53
EDTA	1.52
Mn	0.45
Zn	0.45
Cu	0.06
Bo	0.012
Mo	0.006

[1] Prepared as a 2:1 mixture of Sunniland Nutri-Spray (Chase and Co., Sanford, FL) and Vigoro® Liquid Iron (Swift Agricultural Chemicals Corp., Winter Haven, FL).

Tab. II. Monthly mean productivity (± standard error) of *Gracilaria tikvahiae* in unenriched seawater, in inorganic enrichment, and in liquid digester residue, based on weekly harvests.

	Mean productivity (g dry weight m^{-2} day^{-1})			
Month (1979)	Unenriched	Inorganic Enrichment	Liquid Digester Residue[a]	
			A	B
January	4.56 ± 1.10	4.47 ± 1.14	5.70 ± 1.38	6.53 ± 1.41
February	4.33 ± 0.72	4.40 ± 0.90	6.47 ± 1.01	5.94 ± 0.78
March	3.46 ± 1.16	3.54 ± 0.95	7.62 ± 2.00	6.38 ± 1.89
April	1.67 ± 0.77	9.04 ± 1.42	13.79 ± 2.62	11.80 ± 2.16
May	–	12.83 ± 1.41	13.81 ± 1.77	13.92 ± 2.50
June	–	15.59 ± 1.41	15.32 ± 3.33	14.68 ± 0.88
July	–	19.06 ± 3.83	18.26 ± 2.87	23.72 ± 2.43
August	–	21.92 ± 3.01	26.88 ± 3.08	26.81 ± 2.96
September	–	8.35 ± 2.71	12.89 ± 2.20	15.45 ± 3.28
October	–	7.55 ± 4.17	14.85 ± 1.81	12.96 ± 1.25
November	4.97 ± 2.11	7.70 ± 2.37	13.31 ± 1.72	10.65 ± 3.03
December	3.64 ± 1.57	5.08 ± 1.44	7.79 ± 1.86	8.26 ± 2.20
Grand mean	3.81 ± 0.47	10.63 ± 0.90	13.36 ± 0.93	13.50 ± 0.96

[a] A and B received 0.5 and 1.0 liters respectively of liquid digester residue.

enriched with a defined chemical medium and those that received liquid digester residue. The unenriched controls grew slowly at the start of the study but ceased growing after their nutrient reserves were depleted. A second set of unenriched controls in the later part of the year also grew slowly.

The composition of *Gracilaria* grown on liquid digester residue is similar to those enriched with culture medium in terms of percentage ash, organic, and carbon (Tab. III). Enhanced nitrogen content and, consequently, a reduced C:N ratio of the plants grown in liquid residue were significantly different than those grown on the inorganic enrichment. There were no significant differences in composition resulting from the different concentrations of liquid residue used. The unriched controls had reduced ash and nitrogen levels and elevated C: N ratios relative to the enriched cultures.

A 12 week study employing a wider range of liquid digester levels (Tab. IV) indicated that at levels less than those used in the year-long study (Tab. II), productivity declined. As the amount of liquid residue added increased, the internal nitrogen of *Gracilaria* consistently increased while the carbon: nitrogen ratio consistently decreased.

The solid digester residue can also be used as a source of nutrients for the growth of *Gracilaria* (Tab. V). The productivity of *Gracilaria* was similar whether the inorganic enrichment, liquid residue, or solid residue was the source of nutrients. In addition, the ash (or organic) and carbon content was the same for all three nutrient sources. The internal nitrogen content was highest for plants grown on liquid residue and lowest for plants grown on solid residue. Because the internal carbon content was so similar for all treatments, carbon: nitrogen values were inversely related to the internal nitrogen content.

An approximate balance of the nitrogen recycled through the culture-digester-culture system was made. Over the course of the study, one digester was loaded with a total load of 536.0 kg wet weight of *Gracilaria* that contained 1.61 kg N. Of this N, 70.2% (1.13 kg) was recovered in the liquid residue and 23% (0.37 kg) was recovered in the solid residue. The liquid residue contained an average 1.69 g N/l, of which about 70% was in the form of NH_4-N and the remainder was organic nitrogen of an unknown identity. Addition of this liquid effluent over a 362 day period to cultures of *Gracilaria* (Tab. II) at the rate of 1 liter/week produced 1.1 kg dry weight which contained 39.3 g N; when added at the rate of 0.5 liter/week, 1.1 kg dry weight was produced containing 35.1 g N. This corresponds to a recycling efficiency of 46.6 and 83.2% respectively. Since there was no difference in biomass yields due to the different levels of residue addition, both cultures had sufficient nutrients for maximal growth. The higher enrichment provided excess nutrients that were not assimilated by *Gracilaria*, thus causing a reduction in recycling efficiency. At the present time, then, a recycling efficiency of 83.2% is the best estimate of the reassimilation of nitrogen by *Gracilaria* from the liquid residue.

Tab. III. Mean (± standard error) ash, organic, carbon, and nitrogen composition and carbon: nitrogen ratio of *Gracilaria tikvahiae* grown in unenriched seawater, in inorganic enrichment, and in liquid digester residue, January–December 1979.

	% of dry weight			
			Liquid Digester Residue[a]	
	Unenriched	Inorganic Enrichment	A	B
Ash	33.78 ± 0.45	36.78 ± 0.55	37.42 ± 0.53	37.90 ± 0.65
Organic	66.22 ± 0.45	63.22 ± 0.55	62.58 ± 0.53	62.10 ± 0.65
Carbon	29.25 ± 0.34	27.07 ± 0.31	27.23 ± 0.26	27.43 ± 0.28
Nitrogen	1.48 ± 0.09	2.59 ± 0.05	3.16 ± 0.09	3.50 ± 0.11
Carbon:Nitrogen	23.37 ± 1.50	10.70 ± 0.17	8.96 ± 0.20	8.33 ± 0.24

[a] A and B received 0.5 and 1.0 liters respectively of liquid digester residue.

Tab. IV. Mean (± standard error) values for primary productivity, nitrogen content and carbon:nitrogen ratio of *Gracilaria tikvahiae* grown on different amounts of liquid digester residue, November 1979–January 1980 (n = 12).

Volume of Residue Added (liters/week)	Mean Nitrogen Content (g N/week)	Productivity g dry weight.m^{-2}.day^{-1}	Nitrogen Content %	Carbon:Nitrogen
0.000	0.000	3.82 ± 0.79	0.97 ± 0.07	34.88 ± 2.46
0.125	0.135	6.29 ± 1.22	1.31 ± 0.03	23.67 ± 0.71
0.250	0.270	7.74 ± 1.71	1.53 ± 0.05	20.23 ± 0.67
0.500	0.540	9.51 ± 1.37	2.84 ± 0.07	9.54 ± 0.23
0.750	0.810	9.73 ± 1.34	3.54 ± 0.16	8.16 ± 0.24
1.000	1.080	9.45 ± 1.26	4.08 ± 0.24	7.60 ± 0.53

Tab. V. Mean (± standard error) values for primary productivity and selected chemical components of *Gracilaria tikvahiae* grown in inorganic enrichment, in liquid digester residue, and in solid digester residue, January–May 1980 (n = 32).

	Inorganic Enrichment	Liquid Digester Residue[a]		Solid Digester Residue[b]	
		A	B	C	D
Productivity ($g.m^{-2}.day^{-1}$)	12.83 ± 1.68	11.43 ± 1.24	10.42 ± 0.93	12.34 ± 1.26	15.71 ± 1.54
Ash (%)	37.77 ± 0.65	37.95 ± 0.65	37.56 ± 0.70	37.16 ± 0.83	37.35 ± 0.86
Organic (%)	62.23 ± 0.65	62.05 ± 0.65	62.44 ± 0.70	62.84 ± 0.83	62.65 ± 0.86
Carbon (%)	28.71 ± 0.28	28.87 ± 0.28	28.17 ± 0.26	29.64 ± 0.26	29.05 ± 0.28
Nitrogen (%)	2.84 ± 0.09	2.71 ± 0.07	3.75 ± 0.12	1.99 ± 0.11	2.95 ± 0.08
Carbon:Nitrogen	10.41 ± 0.32	10.90 ± 0.31	7.73 ± 0.26	16.26 ± 0.80	10.06 ± 0.24

[a] A and B received 0.5 and 1.0 liters respectively of liquid digester residue weekly.
[b] C and D received 250 and 500 g wet weight respectively of solid digester residue weekly.

The recycling efficiency for the solid digester residue was calculated based on the data in Table V. Addition of the solid residue over a 112 day period to cultures of *Gracilaria* at the rate of 500 g wet weight/week produced 405 g dry weight which contained 11.0 g N; when added at the rate of 250 g wet weight/week, 318 g dry weight was produced containing 9.4 g N. This corresponds to a recycling efficiency of 60.0 and 63.6% respectively.

Using these estimates, the culture-digester-culture system may be briefly summarized (Fig. 1). For every 100 g N added to the digester in the form of *Gracilaria*, 23.0 g N were recovered in the solid residue and 70.2 g N were recovered in the liquid residue. Of the 23.0 g N in the solid residue, 14.6 g (63.6%) could be reassimilated by *Gracilaria*; of the 70.2 g N in the liquid residue, 58.4 g N (83.2%) could be reassimilated by *Gracilaria*. Thus, of the original 100 g N, 73.0 g N would be completely recycled from the digester back to cultures, an overall recycling efficiency of 73%.

Discussion

This study demonstrates that the digester residues, both liquid and solid, resulting from the anaerobic digestion of *Gracilaria* can be readily recycled as a source of nutrients to produce additional biomass of *Gracilaria*. Utilization of the nutrients in these residues is a major development in the bioconversion of biomass to fuel. Rather than considering them only as a waste product of digestion and disposing of them, these residues may be considered a means of recovering a precious resource which can be recycled over and over again.

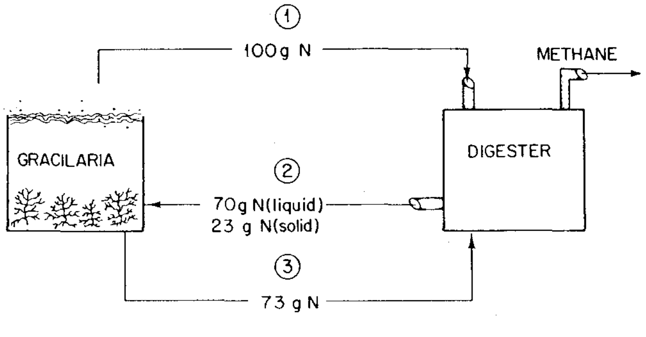

Fig. 1. Nitrogen balance in the recycling of digester residues from the anaerobic digestion of *Gracilaria tikvahiae*

Although other seaweeds besides *Gracilaria* are being considered as energy crops, there are no other studies on recycling their digester residues. The only comparison of nitrogen cycling in a bioconversion system that can be made is with the freshwater macrophyte *Eichhornia crassipes* (Hanisak *et al.* 1980). The overall recycling of nitrogen was higher for *Gracilaria* (73%) than for *Eichhornia* (65%) because the *Gracilaria* digesters had a greater amount of nitrogen in the liquid residue, which contained a higher percentage of NH_4-N that was more readily reassimilated. The nitrogen assimilation from the solid residues was remarkably similar for both species (64%).

The efficiency of recycling the digester residues could be enhanced by taking the seasonality of seaweed growth into consideration. *Gracilaria* requires less nutrients during the winter when growth is reduced. Less digester residue should be added to cultures then because relatively less of the nitrogen is absorbed by the plants and more is lost from the system due to such processes as denitrification and volatization to the atmosphere. The best way of determining the correct amount of digester residue to add is by monitoring the internal nitrogen concentration. This study (and other unpublished data) indicates that internal nitrogen values less than approximately 2% are limiting to growth. Plants should be fertilized when internal nitrogen levels drop to this critical internal concentration.

Nutrients are conserved during the fermentation process and were readily recycled in cultures of *Gracilaria*, with yields as high or higher than cultures receiving inorganic enrichment. This is important to the development of "energy farms" employing seaweeds because one of the major costs involved (in terms of both economics and energy) is that of fertilizer. Recycling of nutrients is probably a necessity for such an "energy farm" (Goldman and Ryther 1977, Oswald and Benemann 1977, Hanisak *et al.* 1980).

It is possible that digester effluent might be the only external source of nutrient enrichment needed to grow *Gracilaria* in a flowing seawater system. For example, *Gracilaria*, growing in the 929 m^2 pond being stocked at the Harbor Branch Foundation, filled to a depth of 1 m and receiving 2 exchanges of unenriched seawater per day (which has an average nitrogen concentration of 8 μmoles/l), would receive enough nitrogen to support 11.2 g dry weight.m^{-2}.day^{-1} (assuming an internal nitrogen concentration of 2.0%). If *Gracilaria* grew at an average of 20 g dry weight.m^{-2}.day^{-1} and was fermented to methane, the resulting residue could support an additional 14.6 g dry weight.m^{-2}.day^{-1}. Thus, the nutrients contained in only the unenriched seawater and the digester residue could provide more than enough nutrients to support the assumed 20 g dry weight.m^{-2}.day^{-1} without the need to use conventional fertilizers. The "energy farm" would then be operating like a natural ecosystem where energy flows through the system while nutrients cycle within the system. In this example, this could be accomplished even with a recycling efficiency of only 45%.

The chemical composition of the digester residues should be carefully characterized in the future. Besides serving as a nutrient source for additional seaweed growth, these residues should also be considered as potential fertilizers for agricultural crops, replacing more conventional sources that are becoming increasingly more expensive.

At the present time, it is technically feasible to cultivate *Gracilaria* in an aquaculture system, ferment it to produce methane, and to recycle the digester residues as a fertilizer, but it is uncertain as to when this might be economically feasible. Further improvements in such an "energy farm" might be achieved by combining the methane production and nutrient recycling with agar production from *Gracilaria* (Hanisak 1981). Agar is a commercially important and economically valuable natural product which might be extracted either before or after *Gracilaria* is loaded into the digesters. Such a combined system could lead to a major aquaculture crop being cultivated in a closed nutrient system, with energy requirements met, at least in part, by the methane generated within the system.

Acknowledgements

I thank Blake Faulkner, David Andrews, and Nat Corwin for performing various chemical analyses. This research was supported in part by Contract No. XR-9-8133-1 from the Solar Energy Research Institute.

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