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RECYCLING THE NUTRIENTS IN RESIDUES FROM METHANE DIGESTERS OF AQUATIC MACROPHYTES FOR NEW BIOMASS PRODUCTION*

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ABSTRACT

The floating freshwater macrophyte *Eichhornia crassipes* (water hyacinth) was fermented anaerobically to produce 0.4 l of biogas/g volatile solids at 60% methane with a bioconversion efficiency of 47%. Both the liquid and solid digester residues were a rich source of nutrients that were recycled to produce additional biomass. An approximate balance of the nitrogen recycled through the culture—digester—culture system indicated that nitrogen was conserved within the digester. All of the nitrogen originally added to the digester in the form of shredded water hyacinths could be found in the liquid (48%) and solid (52%) residues; 64.5% of the nitrogen in these residues could be reassimilated by cultures of water hyacinths. This study indicated the potential of bioconversion of aquatic macrophytes to methane as a possible means of both producing and conserving energy.

INTRODUCTION

One of the most pressing problems of our industrialized society is the uncertainty of maintaining an adequate supply of energy. As fossil fuels continue to decline in availability, the search for alternative sources of energy intensifies. One alternative is the production of methane gas by the fermentation of various types of biomass.

Research has been conducted for the past three years at the Harbor Branch Foundation on the cultivation of seaweeds and freshwater macrophytes as potential biomass sources for conversion to methane or other fuels [1—4]. Primary emphasis has been devoted to determine maximum potential annual yields of organic matter by the aquatic plants under optimal growth conditions and to develop large scale culture methods that are both cost-and-energy-effective and that produce yields that approach the experimental optima. The most promising species studied to date, in terms of both

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biomass yield and the simplicity and reliability of their sustained cultivation, have been the red seaweed *Gracilaria tikvahiae* and the freshwater macrophyte *Eichhornia crassipes* (Water hyacinth), which have produced experimental annual yields equivalent to 127 and 88 (dry basis) Mg/hectare per year (Mg/h y) or 64 and 72 (ash-free dry) Mg/h y, respectively.

A potentially major cost (both in terms of economics and energy) of operating an "energy farm" is the cost of the nutrients (nitrogen, phosphorus, trace elements, etc.) required to grow the plant biomass. For example, a 100 km^2 energy plantation producing 30 (dry) Mg/h y of plant biomass would require of the order of 60,000 Mg of commercial fertilizer or its equivalent each year. However, methane production by anaerobic digestion of plant biomass neither consumes nor dissipates the plant nutrients which remain in the digester residue. Recycling of these nutrients would seem a logical procedure to provide nutrients to an energy farm. This communication reports on the anaerobic fermentation of water hyacinths and the recycling of nutrients in the digester residue to support further growth of this species.

MATERIALS AND METHODS

Several digesters (Fig. 1) 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 screw-cap 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 functional 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 water hyacinths that were digested had been grown in ponds containing nutrient-enriched water [3]. In order to facilitate handling of the plant material and loading of the digesters, the water hyacinths were shredded (Sears-Roebuck Electric Yard and Garden Shredder), producing a greenish-black slurry that had the consistency and texture of wet mud when wet and of peat moss when dry. The plants were chopped immediately after harvest and removal from the water. Even partial drying rendered the plant material tough, fibrous, and resistent to shredding; soaking in water did not reconstitute the plant material to a suitable form for shredding.

Digestion of water hyacinths could be initiated by two methods. In the first, fermenting dairy manure was used as an inoculum and was gradually replaced with water hyacinths. Shredded water hyacinths were added three

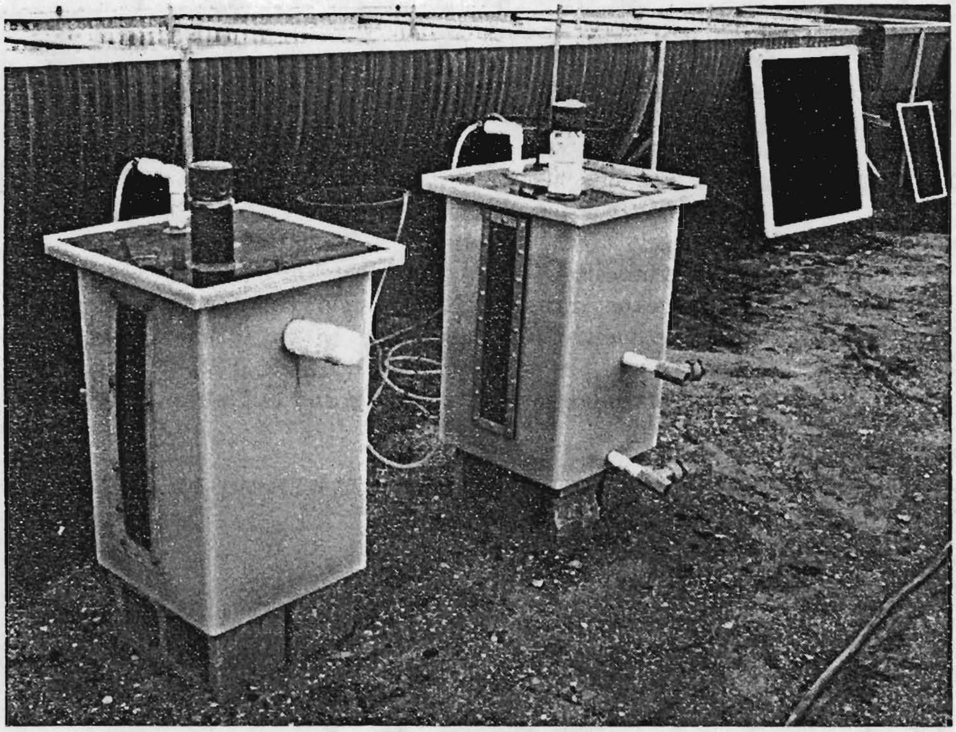

Fig. 1. Anaerobic digesters used for fermenting water hyacinths.

times a week at a loading rate equivalent to 1.10 to 1.38 g volatile solids/l digester volume per day (residence time of 30—38 days). Alternatively, water hyacinths could be digested in a "batch" mode in which 100 kg wet weight of water hyacinths were loaded into a digester. No pretreatment other than shredding was required for fermentation to occur. Gas production began in about 30 days. After the initiation of gas production, the digester could be loaded on a routine basis or continued in a batch mode without any further addition of biomass until gas production ceased (in 90—120 days). The start-up time for batch digesters could be decreased by adding 20% liquid effluent from another water hyacinth digester to the initial inoculum. The observations in this communication refer specifically to two digesters that were loaded on a regular basis for more than one year (April 1978—May 1979).

These two digesters were loaded 1—3 times a week with a loading rate equivalent to 0.74 g volatile solids/l day (15 kg wet weight of shredded water hyacinths) and a residence time of eight weeks. Gas production was not affected by changing the loading regimen from three smaller loads a week to one larger weekly load. Each time the digesters were loaded, an equivalent volume of liquid residue was removed from the digester. The contents of the digester were not stirred, and the solid fraction floated at the surface above the liquid fraction. To prevent the solids from plugging the discharge port, a screen was inserted below the discharge port in order to trap the solids.

The digesters were kept at ambient temperature (30°C ± 5°C) during most of the year. During the cooler months of the 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 water hyacinths, three batch cultures of this species, maintained at a density of 10 kg wet weight/m^2, were grown in Vexar plastic mesh cages (1.2 m^2) that had been placed in concrete tanks (2.20 × 0.80 × 0.45 m and 1.70 m^2 in water area) containing approximately 750 l of water (Fig. 2).

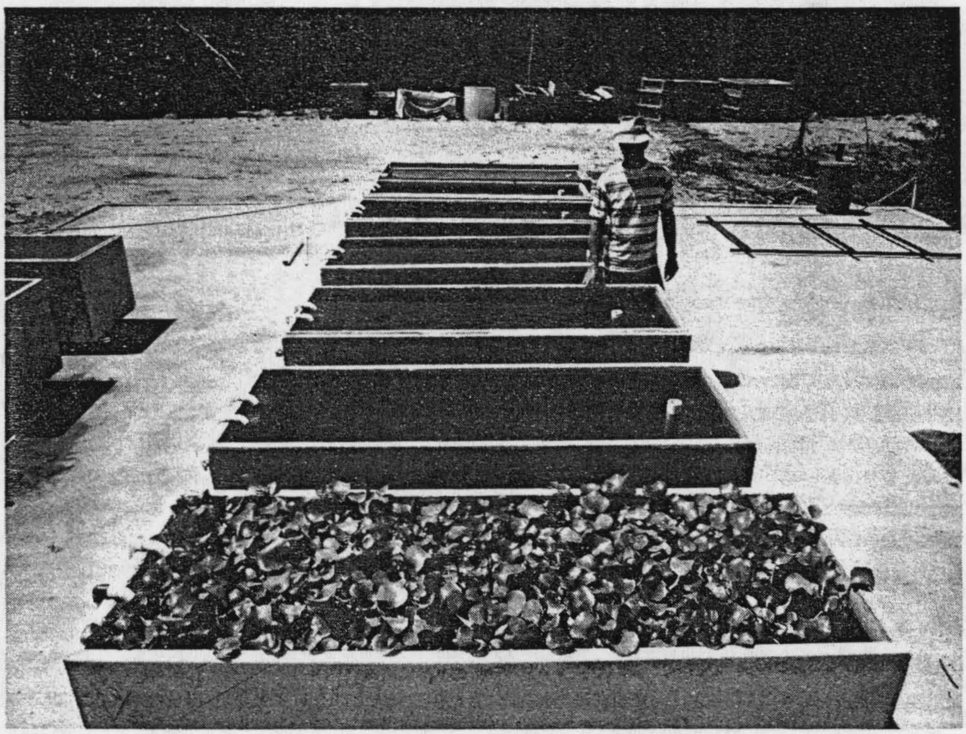

Fig. 2. Concrete vaults containing culture of water hyacinths in foreground.

Of these three cultures, the first was grown on unenriched well water, the second on water enriched with a nutrient medium normally used to grow water hyacinths (Table 1), and the third received liquid digester residue. Initially 5 l of this effluent was added three times a week, but this was

TABLE 1

Final composition of enrichment medium used for growing water hyacinths

	mg/l
$NaNO_3$	76.6
KNO_3	60.8
$CaCl_2 \cdot 2H_2O$	55.5
$Na_2HPO_4 \cdot H_2O$	24.0
$MgSO_4 \cdot 7H_2O$	23.8
Trace metal mix*	
Fe	2.0
S	1.5
Mn	0.75
Zn	0.75
Cu	0.10
B	0.02
Mo	0.01

*Sunniland Nutri-Spray (Chase and Co., Sanford, FL 32771)

changed to 10 l (during the winter when growth was reduced) or 15 l (after growth increased from its winter minimum) added weekly at the same time that the digesters were loaded. The recycling of the liquid residue began four months after the start-up of the digesters (a time period believed to be more than ample for steady state to have been achieved).

After a month, the artificially enriched culture became chlorotic and unhealthy in appearance and these plants were sprayed weekly with additional trace elements (Nutrispray, Table 1) which restored them to a normal appearance. It was subsequently found that this spraying was not necessary if chelated iron was added to the water.

The water in the first two cultures was changed weekly; the unenriched culture grew slowly for 15 weeks and was subsequently terminated. The water in the culture grown in liquid effluent was not exchanged nor did the plants receive the trace element spray or any other form of enrichment than the liquid digester residue.

Approximately every three months, the digesters were opened and the solid residue (i.e. sludge) that floated on the surface was removed and weighed. Studies were initiated to recycle the nutrients contained in this solid residue in a fashion similar to that described for the liquid residue, with the exception being that it was added only once every four weeks (12 kg sludge/four weeks).

Growth of the water hyacinths was determined by weekly lifting the Vexar baskets containing the cultures out of the tanks, draining them for four minutes, and weighing them with a spring scale. Incremental growth was removed from the cultures and the cultures were returned to their tanks. Productivity was expressed as mean daily yield of dry weight/m^2 for each weekly interval assuming a dry weight equivalent to 5% of the wet weight. These methods of measuring primary productivity are discussed more completely elsewhere [3].

Levels of nitrate, nitrite, ammonium, phosphate and total nitrogen were monitored [5] 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 h) for four hours at 550°C in a muffle furnace.

RESULTS

Stable, continuous anaerobic digestion of water hyacinths was maintained for longer than a year, with an average gas production of 0.4 l/g volatile solids, at 60% methane (Table 2). The heat of combustion of water hyacinths was determined by Boyd [6] to be approximately 3.8 kcal/g dry weight which would be equal to 4.6 kcal/g ash-free dry weight (since water hyacinths were on average 18% ash) or 19 kJ/g volatile solids. Since pure methane has an energy content of approximately 37 kJ/l, the above methane production represents an average bioconversion efficiency of about 47%.

TABLE 2

Characteristics of stable, continuous anaerobic digestion of water hyacinths

Temperature	30°C ± 5°C
Agitation	none
Load composition	chopped water hyacinths at 5% wt. total solids, of which 82% wt. is volatile solids
Mean loading rate	0.73 g volatile solids/l digester per day
Mean residence time	56 days
Normal pH range	6.8—7.3
Gas production	0.4 l/g volatile solids, at 60% methane

Both the liquid and the solid digester residues were a good source of nutrients for the growth of water hyacinths (Table 3). Cultures grown on these residues were consistently more productive than those which were chemically enriched, with an average productivity of 65% and 47% higher over the entire period for the liquid residue and the solid residue, respectively. The yields of the chemically enriched culture and that of the liquid residue were two and three times higher, respectively, than that of the unenriched control. All of these differences were determined to be statistically highly significant ($P < 0.01$) by the Student's *t* test using paired observations.

TABLE 3

Mean monthly yields (±standard error) of water hyacinths (*Eichhornia crassipes*) grown in unenriched water, in chemically-enriched medium, and in liquid and solid digester residues

Month	Mean monthly yield (g dry weight/m^2 day			
	Unenriched	Chemical medium	Liquid residue	Solid residue
August 1978	17.38±5.37	24.42±7.29	30.88±5.31	—
September 1978	8.09±1.56	14.69±3.46	24.16±2.15	—
October 1978	2.62±0.69	12.69±3.20	18.52±1.75	—
November 1978	1.94±1.42	12.50±1.86	17.50±1.80	—
December 1978	—	1.96±0.86	8.53±1.36	—
January 1979	—	2.58±1.73	5.02±1.97	—
February 1979	—	2.88±1.49	10.32±2.85	—
March 1979	—	13.13±2.19	16.74±2.01	13.98±2.75
April 1979	—	16.68±2.00	31.36±2.19	28.17±3.08
Time-weighted mean	6.85±1.88	11.01±1.35	18.20±1.60	23.44±3.19
n	15	39	39	9

The growth of water hyacinths grown on solid residue was 89% of those grown on liquid residues during the nine weeks these cultures were monitored concurrently. This difference was not statistically significant ($P < 0.05$).

Water hyacinths grown on digester residues have compositions similar to those grown on chemically enriched medium in terms of percentage ash, carbon, and nitrogen (Table 4). Cultures that did not receive nutrient enrichments had reduced levels of ash and nitrogen but enhanced carbon content and carbon:nitrogen ratios.

An approximate balance of the nitrogen recycled through the culture-digester-culture was made. Over the 39-week experimental period, one

TABLE 4

Mean (±standard error) ash, organic, carbon, and nitrogen composition and carbon-nitrogen ratio of water hyacinths (*Eichhornia crassipes*) grown in unenriched water, in chemically enriched medium, and in liquid and solid digester residues

	% of dry weight			
	Unenriched	Chemical medium	Liquid residue	Solid residue
Ash	15.46±0.66	21.21±0.29	20.70±0.23	19.89±0.43
Organic	84.54±0.66	78.79±0.29	78.30±0.23	80.11±0.43
Carbon	39.00±0.62	36.06±0.24	37.08±0.21	37.40±0.30
Nitrogen	1.04±0.04	3.04±0.07	2.65±0.07	2.75±0.20
Carbon:Nitrogen	38.36±1.55	12.19±0.35	14.58±0.44	14.16±1.03
n	15	39	39	9

digester was loaded with a total of 532 kg wet weight of water hyacinths. This biomass was 26.6 kg in dry weight and contained 577 g N. Of this N, 48% (276 g N) was recovered in the liquid residue and 52% (303 g N) was recovered in the solid residue. In all, 525 l of liquid effluent was removed containing an average of 526 mg N/l (a total of 276 g N), of which about 50% was in the form of NH_4^+—N and the remainder was organic N of an unknown identity. Addition of this liquid effluent to cultures of water hyacinths produced 5.9 kg dry weight which contained 179 g N over a 39 week period, a recycling efficiency of 65%. A total of 120.4 kg of solid residue was removed from the digester. This was equivalent to 6.7 kg wet weight and contained 303 g N. Recycling 24 kg wet weight (equivalent to 1.3 kg dry weight and containing 60 g N) of this material produced 1.4 kg dry weight containing 39 g N over an eight week period, a recycling efficiency of 64%.

In summary (Fig. 3), for every 100 g N loaded into the digester in the form of chopped water hyacinths, 48 g N were found in the liquid residue, half of which was in the form of NH_4^+—N. Of this 48 g N, 31.2 g N (65%) was reassimilated by water hyacinths. The solid residue removed from the digester contained 52 g N of the original 100 g N. Of this fraction, 33.3 g (64%) were reassimilated by water hyacinths. Thus, of the original 100 g N, 64.5 g N could be successfully recycled from the digesters back to cultures of water hyacinths (an overall recycling of 64.5%).

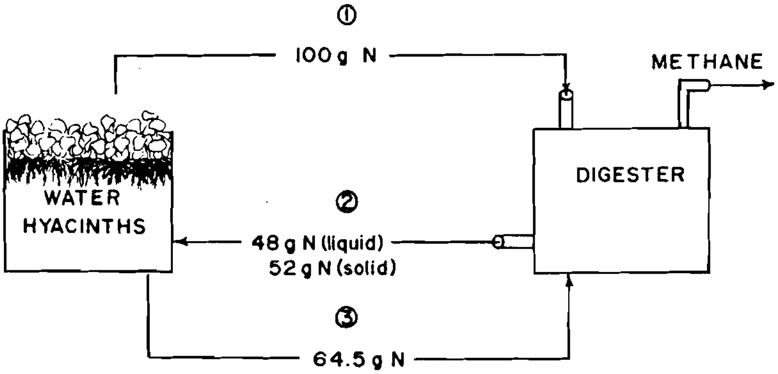

Fig. 3. Nitrogen balance in the recycling of digester residues from the anaerobic digestion of water hyacinths.

DISCUSSION

This study has demonstrated that water hyacinths can be easily fermented to produce methane gas and that the digester residues (both liquid and solid) are a rich source of nutrients that can be recycled to produce additional biomass of water hyacinths. Both the gas production (0.4 l/g volatile solids, at

60% methane) and the bioconversion efficiency (47%) compare favorably with those obtained with other substrates (e.g. [7—10]), although the loading rate employed in this study was low for a continuous digester. When the loading rate was increased, both pH and gas production decreased, an observation which suggests that the activity of the acidogenic bacteria inhibited the methanogenic flora at high loading rates.

Inasmuch as these digesters were not mixed in any way, a stratification of the solid phase may have prevented more complete digestion. Increased methane production might be expected to occur if the contents of the digester were mixed; however, the net energy yield might actually decrease with mixing as has been observed by Jewell [10] in the digestion of cow manure. Jewell has also observed similar effects of increasing the temperature on methane production, i.e. total methane production increases while net energy yield decreases. This work and that of Jewell indicate the potential favorable net energy yields of non-intensive digestion of biomass. The fact that water hyacinths can be successfully digested in a batch mode, with little or no inoculum, and at or near ambient temperature, suggests that this species could be readily digested on a large-scale using non-energy-intensive methods and involving low capital costs (e.g. covered plastic-lined ditches operated in a batch mode without mixing or heating).

The digester residues are an excellent source of nutrients for additional growth of water hyacinths. Both the liquid and the solid fractions can supply all the required nitrogen, phosphorus, and trace metals for maximal growth of water hyacinths. This study also indicates that a batch system of growing water hyacinths is as productive as a flow-through one daily requiring large amounts of water [3]. The ability to grow water hyacinths on as little water as possible increases the economic feasability of any large-scale cultivation of this species.

The enhanced yield of batch cultures grown on digester residues relative to those grown on chemically enriched ones is probably the result of better chelation of the trace elements required for growth. This conclusion is supported by the improved growth observed when the foliage was sprayed with a trace metal solution or when the trace metals were added in a chelated form (with ethylenediamine tetracetate).

The productivity of water hyacinths varies seasonally, as observed during this study and elsewhere [3]. The efficiency of recycling the digester residues could probably be enhanced by taking this seasonality of growth into consideration. Water hyacinths grow much less during the winter and therefore have much less nutrient requirements. Less digester residue should be added then because relatively less of the nitrogen is absorbed by the plants and more is lost from the system due to such processes as dentrification and volatilization to the atmosphere.

The nutritional value of the solid residue is as good as that of the liquid residue. It is important to try to recycle the solid fraction as well as the liquid fraction because more than half of the useable nutrients can be found there. Earlier studies with algae [11,12] also found a high percentage of the nutrients in the solid residue, but those studies suggested that these nutrients would not be readily assimilated by the algae until further oxidation of these solids by aerobic bacteria occurred. Presumably, this oxidation step occurs readily in the tanks of water hyacinths. The solids act as a complex fertilizer that breaks down slowly releasing required elements for plant growth.

Utilization of the nutrients in the digester residue 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, e.g. [13], they may be considered a means of recovering a precious resource which can be recycled and reused over and over again. The use of these nutrients is probably a necessity for any major bioconversion system using plant biomass [14,15].

Because of its high productivity and nutrient removal rates, water hyacinths could be used in advanced sewage treatment [2]. For example, a plant producing 3.8×10^7 l of secondary sewage per day containing 25 mg N/l could support an annual yield of water hyacinths of about 1.4×10^4 Mg/y dry weight. If this biomass was then fermented anaerobically, 100 TJ of energy in the form of methane would be produced as well as nutrient-rich fertilizer which would contain about 350 Mg of nitrogen. Use of this fertilizer obtained in the digestion process would help to conserve energy as it could be used to replace fertilizer produced in more conventional ways. For example, nitrogen fertilizer requires about 47—59 GJ/Mg of N [16] to manufacture conventionally. So the 350 Mg of N contained in the digester residue of the above sewage plant would conserve on average an additional 19 TJ of energy. Thus, the energy conserved by recycling just this one element would be significant, amounting to 19% of the energy produced in the form of methane.

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