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Enhancement of Anaerobic Methanogenesis from Napiergrass by Addition of Micronutrients*

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ABSTRACT

*Mesophilic anaerobic digestion of Napiergrass (*Pennisetum purpureum *Schum.), supplemented with nitrogen and phosphorous, resulted in a low rate of methane production and high volatile fatty acid (VFA) concentrations. Daily addition of micronutrients — nickel, cobalt, molybdenum, selenium and sulfate (as a sulfur source) — increased methane production by approximately 40% and significantly decreased the VFA concentrations.*

Key words: Micronutrients, Napiergrass, *Pennisetum purpureum* Schum., energy crop, VFA metabolism, methanogenesis.

INTRODUCTION

Anaerobic digestion of complex organic matter to methane consists of a cascade of biochemical conversions catalysed by different physiological groups of interacting organisms. Conventional mixed digestion systems contain a mixture of organisms which can catalyse all phases of the cascade. The reaction rates normally obtained in different phases are markedly different with the net result that the overall process becomes rate restricted by the slowest phase.[1]

Considerations of the biochemical and physiological characteristics of bacteria may lead to useful application of factors affecting complex processes such as occur in digesters producing methane from biomass. One important factor is the mineral nutrition of the microorganisms involved. The low-level, trace element requirements of microorganisms

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are difficult to determine, since elements not detected by chemical assay may still be present in sufficient quantities for growth.[2] With plant biomass, these difficulties are compounded when the chemical analyses for these elements do not distinguish between total amounts present and those portions biologically available for cell metabolism.

Biomass yield is a major cost consideration in the production of crops for energy. Napiergrass (*Pennisetum purpureum* Schum.), water hyacinth (*Eichhornia crassipes* (Mart.) Solms.) and sugarcane (*Saccharum* Spp.) are among the most productive plant species. Biomass yields of Napiergrass and sugarcane have approached 50–75 Mg ha^{-1} in the south temperate to subtropical zones; sorghums (*Sorghum bicolor* (L.) Moench) yielded 20–30 Mg ha^{-1} in the north temperate zones; while water hyacinth yields in field tests have exceeded 50 Mg ha^{-1}.[3] Their high biomass yields make these plants attractive as potential feedstocks for the production of commercial quantities of methane gas, provided the plant species can be readily fermented. Fermentability is influenced by plant age, plant anatomy, plant variety, agronomic practices, environmental conditions, handling and storage methods.[4–7] Mature, hot air-dried Napiergrass was selected as feedstock for the present study since it was anticipated that it would have a poor fermentation profile. If this recalcitrant grass can be economically fermented, then other, more fermentable, plants could also be used.

This paper reports preliminary results from experiments on Napiergrass digestion in stirred digesters. The experimentation is part of an effort to enhance methane productivity by manipulating the biology of the fermentation.

MATERIALS AND METHODS

Digester design and operation

Six digesters were constructed from 4-liter aspirator bottles, sealed with No. 10 rubber stoppers containing three access ports, one for biogas output, one for feeding and one for a stirring mechanism. The access ports for feed introduction and biogas collection were made of glass tubing attached to amber latex tubing. The contents of the digesters were completely mixed intermittently (15 min h^{-1}) by a bent stainless steel rod rotated externally using a 60 rpm motor. The rod, enclosed in a sealed tygon tube, was inserted through the stopper. Digester temperature was maintained at 35°C in water baths. Effluent was removed daily through bottom aspirator spouts, which were attached by rubber tubing to ports in the lower side of the water baths. Daily biogas production was

measured by water displacement in a closed system consisting of a calibrated glass carboy and a polyethylene carboy reservoir, ensuring constant saturation of the gas collecting solution. Daily feed consisted of 5 g Napiergrass made up to 180 ml with tap water, giving a hydraulic retention time (HRT) of 20 days. The volatile solids (VS) loading rate was 1·23 g VS liter^{-1} day^{-1}. Samples for pH, VFA and gas analyses were taken while the digesters were being mixed.

Inoculum

The inoculum for the digesters was a 1:2 ratio of rumen fluid and mixed liquor from a packed-bed biological reactor treating Napiergrass leachate. The total culture volume was 3·6 liters. The rumen fluid was prepared from a sample of the rumen contents of a fistulated steer, maintained on a constant diet of Bermudagrass and protein supplement. In order to displace microorganisms from the particulate fraction, the rumen contents were blended for 30 s in a Waring Blendor under nitrogen gas, prior to decanting the fluid fraction. Sodium sulfide (0·05% final conc.) was subsequently added to the mixed inoculum to help poise a low oxidation-reduction potential.

Feedstock

The Napiergrass (United States Department of Agriculture introduction PI 300086) used was from a crop grown from early March until harvesting on 9 October, 1984. Top growth had been cut directly into a drying wagon and dried to constant weight at 49°C with a gas forced-air crop dryer. This material was then bagged and stored in a hay barn. Crop fertilization was 168-42-84 kg ha^{-1} of N-P_2O_5-K_2O once a season. One hundred kg of biomass was ground in a Wiley mill to a size sufficient to pass a 1 mm screen. The ground Napiergrass was stored in ventilated containers in the laboratory.

Analytical methods

Dry matter (DM) was determined by weight loss of samples dried at 105°C for 16 h and volatile solids were determined by difference after 3 h at 550°C.

Carbon (C), hydrogen (H), nitrogen (N) and sulphur (S) were determined using an elemental analyser. All other elemental analyses were carried out by atomic absorption spectrophotometry.

Volatile fatty acids were analysed by gas chromatography. Acids were separated in a glass column (1·8 m × 4 mm i.d.) packed with 8% SP-

1000, 2% SP-1200, and 1·5% phosphoric acid on 80/100 mesh Chromosorb W AW and measured with a flame ionization detector. The injector, oven and detector temperatures were 145, 130 and 175°C, respectively. Helium was the carrier gas at 30 ml min^{-1}. Samples were mixed with an equal volume of 4% *o-phosphoric* acid and centrifuged at 12 800 × g for 2 min. The supernatant fluid was analysed for VFA concentration. Standard VFA solutions were prepared by dilution of 0·1 M stock solutions which had been standardized by titration and were injected with each sample series.

Gases (N_2, CH_4, CO_2) were analysed by gas chromatography. They were separated at room temperature in a copper column (2·5 m × 2 mm i.d.) packed with 60/80 mesh Poropak Q and measured with a thermal conductivity detector. Helium was the carrier gas at 7·2 ml min^{-1}. Gas standards were injected with each sample series.

Hydrogen was measured using a Trace Analytical RGD2 Reduction Gas Detector attached to an RGA2 Gas Chromatograph Module (Trace Analytical, Menlo Park, Ca.). Compressed air was the carrier gas at 21 ml min^{-1}. Reference gas was 103 ppm (v/v) hydrogen in nitrogen.

EXPERIMENTS AND RESULTS

In general, feedstocks with a carbon/nitrogen (C/N) ratio exceeding 15 or a carbon/phosphorus (C/P) ratio exceeding 75 will be nutrient limited with respect to biological conversion.[8,9] Carbon, nitrogen and phosphorous analyses of Napiergrass showed it to have average C/N and C/P ratios of 69 and 346, respectively (Table 1). Consequently, the feed was supplemented to yield C/N and C/P ratios of 15 and 75, by addition of a nutrient solution containing NH_4Cl, K_2HPO_4 and KH_2PO_4. The pH of the reactors was maintained at 7·0 by addition of NaOH. After 6 HRTs (120 days) the digesters still maintained elevated concentrations of acetate, propionate and butyrate ranging from 20–40, 8–14 and 0·4–1 mM, respectively. Two of the six digesters were reinoculated to ensure the presence of an active acetate-utilizing microflora. However, after restabilization the VFA concentration was still elevated and the methane production rate was low. The high VFA concentrations observed, suggested that the metabolism of acetate, propionate and, to a lesser extent, butyrate was slow. The C/N and C/P ratios were further increased to 7·5 and 37·5, respectively, for 2 HRTs with no resulting alteration in the fermentation profile.

Elemental analyses were conducted to quantify levels of trace metals present in the feedstock. These analyses provided total rather than

TABLE 1
Elemental Analysis of PI 300086 Napiergrass ($g\ kg^{-1}$ DM)

Element	*Sample 1*	*Sample 2*
C	455	479
H	60·9	63·8
N	6·5	7·0
P	1·3	1·4
C/N ratio	70	68
C/P ratio	350	342
S	0·35	0·48
K	3·2	3·6
Ca	2·3	2·2
Mg	2·4	1·9
Na	0·12	0·09
Fe	0·09	0·15
Mn	0·078	0·091
Zn	0·029	0·028
Cu	0·005	0·005
Mo[a]	—	—
Ni[a]	—	—
Co[a]	—	—
Se[b]	—	—

— = not detectable.
[a] Detection limit = 10 $mg\ kg^{-1}$.
[b] Detection limit = 0·2 $mg\ kg^{-1}$.

TABLE 2
Daily Micronutrient Addition ($mg\ liter^{-1}$ of mixed liquor)

Nutrient	*Amount*
S	1·6
Ni	0·25
Co	0·19
Mo	0·30
Se	0·062

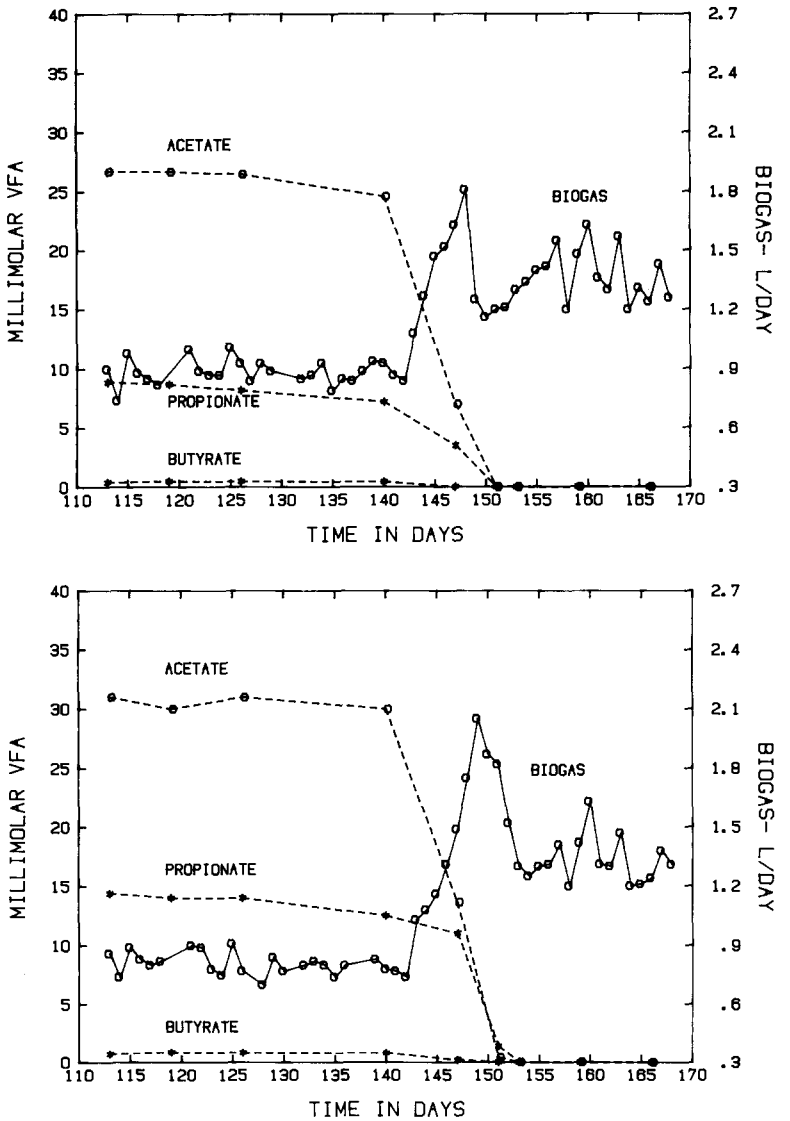

Fig. 1. VFA concentrations and biogas production in four Napiergrass-fed digesters, before and after micronutrient addition. Time axis is in days since start-up. Addition began on day 142. Last VFA measurements prior to addition were made on day 140.

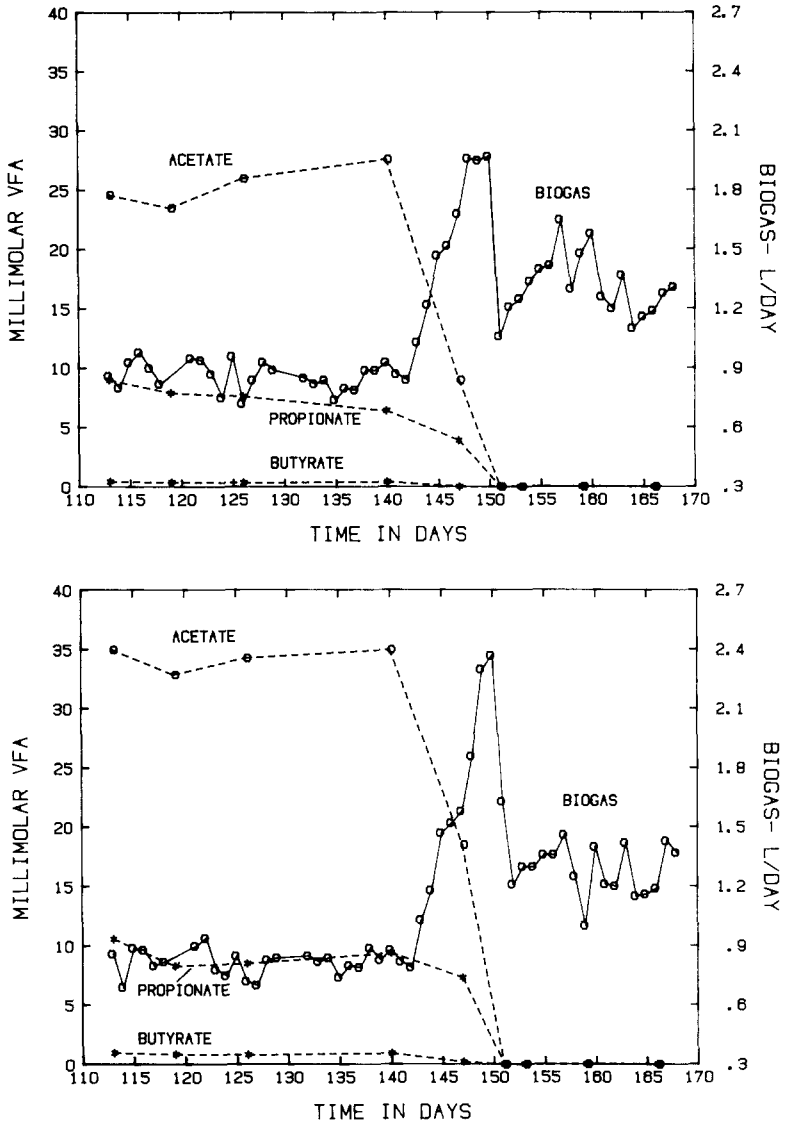
MILLIMOLAR VFA
BIOGAS- L/DAY
ACETATE
BIOGAS
PROPIONATE
BUTYRATE
TIME IN DAYS
MILLIMOLAR VFA
BIOGAS- L/DAY
ACETATE
BIOGAS
PROPIONATE
BUTYRATE
TIME IN DAYS

Fig. 1 — *contd.*

bioavailable element levels. Nickel (Ni), cobalt (Co), molybdenum (Mo) and selenium (Se) were not detected (Table 1). Consequently, a solution containing Ni, Co, Mo and Se was added daily with the feed (Table 2). This procedure was initiated on day 142 of the experiment. Although the feedstock contained 0·41 g S kg^{-1} DM, sulfate was also added since essential sulfides are lost in the product gas.

Biogas production increased immediately upon addition of the micronutrient solution, with a concurrent decrease in VFA concentration (Fig. 1). The decrease in acetate and butyrate was more marked than that of propionate with a substantial drop in acetate concentration during the first 5 days of micronutrient addition (Fig. 1). After 10 days of micronutrient amendment, VFA concentrations were below the detection limits of 0·1 mM for all three acids. Average methane production increased from 0·5 to 0·7 liter CH_4 day^{-1} (Table 3), expressed at standard temperature and pressure ($p < 0{\cdot}01$). No major changes in the concentration of evolved hydrogen were detected after micronutrient addition (Table 3).

DISCUSSION

Bacteria require a wide range of nutrients to maximize the probability of good growth. Various trace metal requirements exist for methanogens: growth is stimulated by iron and molybdenum;[10-12] growth of *Methanococcus vannielii* is markedly stimulated by selenium and tungsten[13] and growth of an acetate-fermenting strain of *Methanosarcina* is stimulated by the presence of cobalt in basal medium.[14] Growth of methanogens is also dependent on nickel.[15,16] Sulfur, required by most methanogens as sulfide, is an integral part of coenzyme M, which is involved in the methyl group transfer reactions in methanogenesis.[17,18] Conversely, one of the important inorganic elements which is toxic or inhibitory to methane fermentation is the sulfide ion. However, McCarty *et al.*[19] reported that equilibrium concentrations of soluble sulfide up to at least 200 mg $liter^{-1}$ exerted no significant toxic effect. In the present experiment, at 20 day HRT, the cumulative sulfide concentration added was 32 mg $liter^{-1}$ (in excess of that potentially available from the biomass).

Speece and Parkin[20] reported that iron supplementation of a municipal sludge digester, which had a chronically high volatile acids concentration of *c.* 4000 mg $liter^{-1}$, resulted in a reduction to 400 mg $liter^{-1}$ within 10 days. Nickel stimulation of specific acetate utilization rates from 2–4·6 to 10 g acetate g^{-1} VSS day^{-1} was reported by Speece *et al.*[21] Conversion of acetic acid to methane by a mixed methanogenic

TABLE 3
Average Methane Productivity and Average Evolved Biogas Composition from Four Reactors before and after Micronutrient Addition

Sample time (days):	*Before*					*After*				
	−29	*−23*	*−16*	*−9*	*Average*	*+12*	*+19*	*+22*	*+28*	*Average*
liter CH_4 d^{-1}:	0·44	0·52	0·52	0·51	0·50	0·70	0·68	0·71	0·69	0·70
% CH_4:	69·7	69·3	65·9	69·7	68·7	62·4	63·7	65·7	61·2	63·3
% CO_2:	27·0	28·6	32·8	25·5	28·5	36·6	36·4	34·8	38·9	36·7
ppm H_2:	24	24	23	19	22·5	24	21	18	17	20

population in an anaerobic fixed-film digester was stimulated by the addition of nickel cobalt and, especially, their combinations.[22]

The current results with Napiergrass as feedstock showed that addition of micronutrients stimulated acetate, propionate and butyrate metabolism (Fig. 1). Concentrations of hydrogen in the evolved biogas from the digesters never exceeded 24 ppm, even when VFA concentrations were elevated (Table 3). This observation suggests that the high VFA concentrations before nutrient addition were not a result of high hydrogen concentrations in the gas phase. In the digestion of domestic sewage sludge hydrogen concentrations as high as 8% did not inhibit acetate production from propionate.[23] The decrease in VFA produced as fermentation end-products suggests that micronutrient addition substantially affected the organisms which metabolize VFA. In contrast, the results suggest relatively little effect on the organisms which metabolize molecular hydrogen, since no corresponding change was observed in the amount of end-product hydrogen after micronutrient addition (Table 3).

In conclusion, the addition of micronutrients to four digesters operated at 20 day HRT, resulted in a shift of end-product formation from methane, carbon dioxide, acetate, propionate and butyrate to methane and carbon dioxide as the only detectable end-products. The 40% increase in average methane productivity (Table 3) represents an important step in efforts to enhance methanogenesis from Napiergrass. Alteration of a fermentation profile by manipulation of micronutrients may prove a useful tool in the regulation and control of methane formation from biomass or from any organic matter.

Further study is required to determine the specific micronutrient(s) responsible for these phenomena and to investigate the nature of the mechanisms involved. Comparatively little is known about the bioavailability of mineral elements from plants. The importance of micronutrients in anaerobic methanogenesis of energy crops may have been neglected due to the presence of seemingly adequate micronutrients and/or the practice of blending poor quality feedstocks with nutrient-rich animal manures or sewage. Aside from the economic consideration of increased methane production, reduction of effluent VFA levels is also desirable from an environmental standpoint.

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