0273-2289/88/1700-0245/$03.40

Effects of Addition of Soluble Oxidants on the Thermophilic Anaerobic Digestion of Biomass to Methane

CHRISTOPHER J. RIVARD,*† FRANCIS BORDEAUX,**
J. MICHAEL HENSON,‡ AND PAUL H. SMITH

Department of Microbiology and Cell Science, University of Florida, G069 McCarty Hall, Gainesville, FL

ABSTRACT

Bioconversion of polymeric substrates in anaerobic digesters is slow. Exploratory research was conducted on the effects of the addition of soluble oxidants to a thermophilic, anaerobic, semicontinuous stirred tank reactor (CSTR) fed a biomass feedstock. After adaptation, added nitrate was quantitatively reduced to ammonia and isotope-labeling experiments confirmed that denitrification reactions did not occur. Addition of sulfate to a continuous nitrate amended digester resulted in sulfate accumulation, whereas sulfate addition to nonnitrate-amended digesters resulted in sulfate reduction. These results support the hypothesis that nitrate is preferentially reduced in the presence of sulfate and nitrate.

Index Entries: Oxidants; nitrate; sulfate; methane digester; anaerobic digestion.

INTRODUCTION

Anaerobic digestion is a slow, naturally occurring process that was first observed (because of the flammable nature of gas evolving from marsh sediments) by Alessandro Volta (*1*) in 1776. Since that time, re-

*Author to whom all correspondence and reprint requests should be addressed.

**NDRC Laboratory, 6284 Brookhill Dr., Houston, TX

†Present Address: Biotechnology Research Branch, Solar Energy Research Institute, Golden, CO

‡Robert S. Kerr Environmental Research Laboratory, US Environmental Protection Agency, Ada, OK

search has been conducted to elucidate the pathway(s) (2–7) by which organic matter is converted to methane. Quantitative characterization of reaction pathways may suggest procedures to improve the efficiency of the process either for destruction of undesirable organic materials or to improve the yield of methane in applications for fuel production.

The anaerobic digestion of organic matter to methane and carbon dioxide results in an easily purified gaseous product. The conversion process is also energy efficient because it results in a small amount of microbial growth and 90% or more of the original substrate energy is retained in the methane produced (*8,9*). The process occurs slowly, requiring in many cases a 60–90 d retention time for nearly complete digestion of lignocellulosic material such as biomass in standard stirred digesters.

Various parameters have been addressed in attempts to increase the digestion rate, among these, modifications of higher temperature and decreased retention time (*10,11*).

Anaerobic digestion of biomass to methane gas is a complex microbial process requiring a consortium of microorganisms that can be divided basically into three microbial groups (*12*). Fermentative microbes hydrolyze polymeric substrates such as cellulose and proteins to simple, soluble compounds that are further metabolized to organic acids, hydrogen, and carbon dioxide. The second group, collectively named the obligate proton-reducing acetogenic bacteria, produce acetate, hydrogen, and carbon dioxide from organic acids such as propionate and butyrate. Finally, methanogenic bacteria utilize the hydrogen, carbon dioxide, and acetate to produce the endproducts methane and carbon dioxide.

Hydrogen serves an important role as an electron sink for many microorganisms in the anaerobic digestion consortium, and its effects on the overall process have only recently been elucidated (*12–22*). Rates of polymer hydrolysis as well as acid utilization are affected by the concentration of hydrogen present in the digesting material. The major mechanism for the removal of electrons (as hydrogen) in the anaerobic digestion system is the reduction of carbon dioxide by methanogenic bacteria. Research on the addition of sulfate to various freshwater and marine anaerobic systems has established that sulfate is reduced preferentially compared to carbon dioxide (*23–27*) and lowers the partial pressures of hydrogen in the process. This successful competition for electrons may be predicted from the calculated free energy of reactions (*16*), as shown below:

Reaction	$G^{o\prime}$ (free energy)
$HCO_3^- + 4H_2 + H^+ \rightarrow CH_4 + 3H_2O$	−32.4
$SO_4^{-2} + 4H_2 + 2H^+ \rightarrow H_2S + 4H_2O$	−36.3
$NO_3^- + 4H_2 + 2H^+ \rightarrow NH_4^+ + 3H_2O$	−143.3

Sulfate reduction has been used to maintain lower hydrogen partial pressures than that attainable by carbon dioxide reduction in coculture work with microorganisms that require low hydrogen levels for growth (*28–30*).

The effects of nitrate as a possible oxidant are also of interest. Nitrate reduction in the anaerobic digestion process may allow for greater electron removal and serve to lower hydrogen levels, and thus allow increased energetics and metabolic rates of various microbial groups that produce hydrogen as a means of electron disposal.

As opposed to sulfate, the information on nitrate effects on anaerobic digesters is scarce. Nitrate has been found to inhibit methanogenesis in salt marsh sediments (*31*) as well as inhibiting sulfate reduction to sulfides in stream sediments (*32*). In anaerobic systems, nitrate is known to undergo reduction via two different pathways (*33,34*). In denitrification, nitrate is reduced to nitrite, nitric oxide, nitrous oxide, and finally, nitrogen gas. In dissimilatory nitrate reduction, nitrate is reduced to nitrite and finally to ammonia. In anaerobic sediments, silage, and one study using digested sludge, added nitrate was reduced via both pathways resulting in nitrogen gas and ammonia (*35–37*). Studies on the fate of nitrate in the rumen of animals have focused on evaluation of the toxic nature of nitrates in feedstocks (*38–43*). In the rumen system most of the added nitrate is reduced by the dissimilatory pathway to ammonia (*44*).

In the present study, the fate and effects of added nitrate and sulfate on the thermophilic anaerobic digestion of biomass is examined.

MATERIALS AND METHODS

Digester Design and Operation

Digesters were constructed from 4-L aspirator bottles with a 3.5 L working volume. The bottles were sealed with a stopper having ports for stirring, gas collection, and feeding. The digesters were stirred for 15 out of each 30 min, and maintained at 55°C in a water bath. The water baths were constructed with side ports which were connected to the aspirator spouts on the digesters for sludge removal. Gas was collected and measured using calibrated water displacement vessels.

The digesters were initially seeded with sludge from a thermophilic digester treating cattle waste. Digesters were batch fed 16 g of feed in 200 mL of tap water daily, yielding a hydraulic retention time of 20 d. The feedstock was a mixture of 75% bermuda grass and 25% universal cattle feed (Seminole brand). The feedstock was reduced in size using a food processor (Sunbeam), followed by pebble milling (24 h, 4°C), and then sifted through a 1.5 mm sieve.

Oxidant additions to digesters were made with a multiplace syringe pump (Harvard Apparatus).

Serum Bottle Experiments

Experiments were conducted with sludge external to the digesters in 150 mL serum bottles (Wheaton). Sludge was removed from the digesters 2 h after feeding and vigorously mixed with a magnetic stirrer while flushing with oxygen-free N_2/CO_2 (80/20% mixture, Matheson) gas. While stirring, either 25 or 100 mL aliquots were pipeted into the serum bottles while the bottles were being flushed with the same gas mixture. The bottles were sealed with black rubber stoppers and crimp caps. Additions to the sludge were made through the stoppers using 20-gage needles and 1 or 5 mL plastic syringes.The bottles were shaken at 55°C using a New Brunswick Model G-76 shaking water bath. Gas production was measured using a pressure transducer (Setra Systems). Samples of the headspace were removed for gas composition analysis using a 250 μ-L gas-tight syringe (Hamilton) with a 25-gage needle. Sludge was removed using 20 gage needles and 1, 5, or 10 mL syringes.

Gas Analysis

Methane, hydrogen, carbon dioxide, and nitrous oxide were measured using a Hewlett Packard Model 5880 gas chromatograph equipped with a thermal conductivity detector and a Porapack Q column (80/100 mesh, 6 ft. × 1/8 in. OD Alltech Assoc.). Detector and oven temperatures were maintained at 145 and 120°C, respectively. Helium served as the carrier gas.

Nitrogen was measured with a Loenco Model 15A gas chromatograph equipped with a thermal conductivity detector and integrator. A Molecular Sieve 5A column (80/100 mesh, 6 ft. × 1/8 in. OD, Alltech Assoc.) was used with helium as carrier gas. Detector and oven were kept at room temperature. Chromatographs were calibrated with ultrahigh purity standards (Matheson).

pH, Volatile Fatty Acid, and Ion Analysis

An Orion Research Model (701A) Digital Ionalyzer and pencil-type, gel-filled probe (Corning) were used for pH measurements. Sludge samples were analyzed within 10 min of removal from the digester.

Volatile fatty acids (VFA) were measured using a Hewlett Packard gas chromatograph (model 5880) equipped with a flame ionization detector, autosampler, and integrator. The column used for VFA separation was packed with 8% SP1000, 2% SP1200, and 1.5% phosphoric acid (6 ft. × 1/8 in. ID, Supelco). Injection port and column temperature were maintained at 145 and 120°C, respectively, Helium served as carrier gas.

Digester sludge was prepared for analysis by mixing 1:1 (v/v) with 4% *o*-phosphoric acid solution in water. The acidified samples were loaded into microcentrifuge tubes and centrifuged (Eppendorf Microcentrifuge) for 15 min. The supernatant was loaded into autosampler vials for gas chromatography.

Nitrate and sulfate were determined with a Hewlett Packard liquid chromatograph (Model 1084B) equipped with an autosampler, conductivity detector (Wescan), and integrator. Ions were separated on an Anion Exclusion Column (Wescan) using 0.004*M* potassium hydrogen phthalate, pH 3.9, as eluent. Eluent, detector, and oven temperatures were maintained at 45°C. Samples for ion analysis were diluted 1:1 with acetonitrile to stop further metabolic activity, diluted 1:10 (final concentration) with distilled water, centrifuged, and the supernatant loaded into autosampler vials. Nitrite was determined by a standard colorimetric method (*45*) at 540 nm using a Perkin-Elmer (Model 35) spectrophotometer.

Ammonium ion plus ammonia was determined with an ammonia electrode (Corning) and an Orion Research Digital Ionalyzer (Model 701A). Digester sludge was diluted (2 mL into 88 mL of distilled water) and 10 mL of 1.0*M* NaOH added for electrode detection. The resulting millivolt reading was taken after 1.5 min of mixing using a magnetic stir bar.

Stable Isotope (^{15}N-Nitrate) Experiments

For ^{15}N-label analysis, sludge samples (4 mL) were acidified to a pH of approximately 4.0 by the addition of 0.3 mL of 10% sulfuric acid to convert ammonia to the stable ammonium ion form. The samples were frozen until analysis. Ammonium ion and/or nitrate (first reduced to ammonium ion) were distilled as described (*46*). The distillates were concentrated to approximately 3 mL by evaporation on a hot plate while flushing with air. Samples were transferred to small vials and analyzed for ^{15}N-ammonium ion ratio as described (*47*). Percent ^{15}N-label calculations were based on ^{15}N recovered in ammonium ion distillation from sludge removed during the experiment versus ^{15}N recovered from both ammonium ion plus nitrate distillation at the time of introduction of the label (zero time).

Digester Sludge Composition Analysis

Sludge samples were analyzed for dry matter and volatile solids. Sludge (200 mL) was removed from each digester (while stirring) into 250 mL flatbottom boiling flasks. The sludge was vigorously mixed on a magnetic stirrer while samples (25 mL) were removed with 25-mL wide-tip pipets and placed into crucibles (30 mL, Coors) that had been previously

ashed overnight at 550°C, cooled to room temperature in a desiccator, and weighed. Sludge samples were evaporated overnight at 45°C, dried at 110°C for 4 h, cooled to room temperature in a desiccator, and weighed. The samples were then ashed at 550°C in a muffle furnace for 2 h and again cooled and weighed.

Digester sludge was analyzed for acid detergent fiber (ADF), lignin, and cellulose, as described (*48*).

Enumeration of Digester Microflora

Anaerobic digester sludge was analyzed for viable cell numbers of several important groups of microorganisms. Digester effluent (100 mL) was removed 24 h after feeding and blended in a semimicro blender (Eberbach, Waring) for 30 s to break up microbial flocks and dislodge microorganisms attached to particulates in the sludge. The sludge was flushed with oxygen-free N_2/CO_2 (80/20%) gas during blending. The blended sludge was subjected to 10-fold dilutions in 9 mL anaerobic dilution blanks. Anaerobic dilution blanks consisted of basal medium under an oxygen-free N_2/CO_2 gas phase. Anaerobic basal medium was prepared in serum tubes by the methods of Hungate (*49*), as described by Balch et al. (*50*). Basal medium composition was as follows: trace vitamin solution (*51*) 10 mL; trace mineral solution (*51*) 10 mL; NH_4Cl 1.0 g; KH_2PO_4 2.0 g; $NaHCO_3$ 5.0 g; resazurin 0.001 g; cysteine HCl 0.5 g; clarified digester sludge dilution was injected into 25 mL of tempered agar medium that consisted of basal medium, specific additions if any, and 2% Noble agar (Difco), contained in 150 mL serum bottles. Bottles were rolled in a level pan containing ice water until the agar solidified. The roll bottles were incubated at 55°C, inverted, with either a N_2/CO_2 or H_2/CO_2 gas phase (both 80/20%). Colonies were examined and counted with an Olympus stereo microscope after 1, 2, and 3 wk of incubation.

Cellulose degraders were enumerated in basal medium plus cellulose (1% final concentration). Cellulose fibers used in the medium preparation were Whatman #1 filter paper blended in a standard Waring blender and extensively washed with distilled water. Colonies that degraded the opaque cellulose fibers, producing clear zones, were counted.

Hydrogen-metabolizing methanogenic bacteria were cultivated in basal medium under a H_2/CO_2 gas phase. These methanogens were enumerated by counting colonies which autofluoresced when illuminated with 420 nm light (*52*).

Acetate-metabolizing methanogenic bacteria were enumerated by counting colonies which developed in basal medium containing 4 g/L sodium acetate under a N_2/CO_2 gas phase and which autofluoresced when illuminated with 420 nm light.

Sulfate-reducing bacteria oxidizing hydrogen were enumerated. The basal medium was supplemented with sodium sulfate (1.0 g/L), and prior

to inoculation 0.25 mL of a sterile $FeSO_4 \cdot 7H_20$ solution (0.5 g/L final concentration) was added. Colonies were counted which produced a black FeS precipitate, resulting from the sulfide produced by sulfate reduction.

Nitrate reducers were enumerated by the incorporation of sodium nitrate (100 μmol/mL), glucose (3.0 g/L final), and yeast extract (3.0 g/L, difco) to the basal medium. The medium surrounding colonies was tested for the presence of nitrate by the addition of a few drops of a mixture of 33% diphenylamine and 66% concentrated sulfuric acid as described (*50*). The production of a dark blue-black color indicates nitrate presence. Colonies counted were those producing clear zones upon treatment.

RESULTS

Six 4-L anaerobic digesters, operated at 55°C with a 20-d retention time and batch fed a synthetic "biomass" feedstock, were utilized in the study. Three digesters served as controls and also provided sludge for serum bottle studies. The effect of added calcium nitrate was studied by continuous syringe pump addition (day 0–130) and resulted in dramatic reductions in gas produced followed by long recovery periods after cessation of nitrate addition. Upon subsequent nitrate addition, greater total amounts of nitrate were required to attain the same reduction in gas production, and recovery of gas production rates occurred sooner, indicating an adaptation to nitrate addition.

Continuous nitrate addition at the level of 0.5 μmol/mL sludge/h resulted in digester failure. Addition at the rate of 0.4 μmol nitrate/mL sludge/h was sustained without digester failure, and the long-term effects on gas production and acetic acid and ammonia pools are shown in Fig. 1. In the nitrate acclimated digester, ammonia pools (ammonia plus ammonium ion) increased and corresponded to theoretical values obtained if all the added nitrate was reduced to ammonia (Fig. 2), which indicates a dissimilatory pathway of nitrate reduction. Analysis of gas produced in the control and nitrate acclimated digesters were similar in nitrogen content with detected levels below 1%, indicating that little if any denitrification of the added nitrate occurred. This minor amount of nitrogen was attributed to introduction of air during the daily feeding.

To further investigate the ability for denitrification in the nitrate acclimated sludge, nitrous oxide reduction was examined (Fig. 3). Although nitrous oxide was reduced in the nitrate amended sludge, it occurred only after a 4-d lag period, indicating the reduction of nitrous oxide was induced by its presence, representing an alternative pathway.

The fate of added nitrate was rigorously established by adding ^{15}N-labeled nitrate and determining the percentage of added label occurring in ammonia pools produced (Fig. 4). Of the ^{15}N-label added as

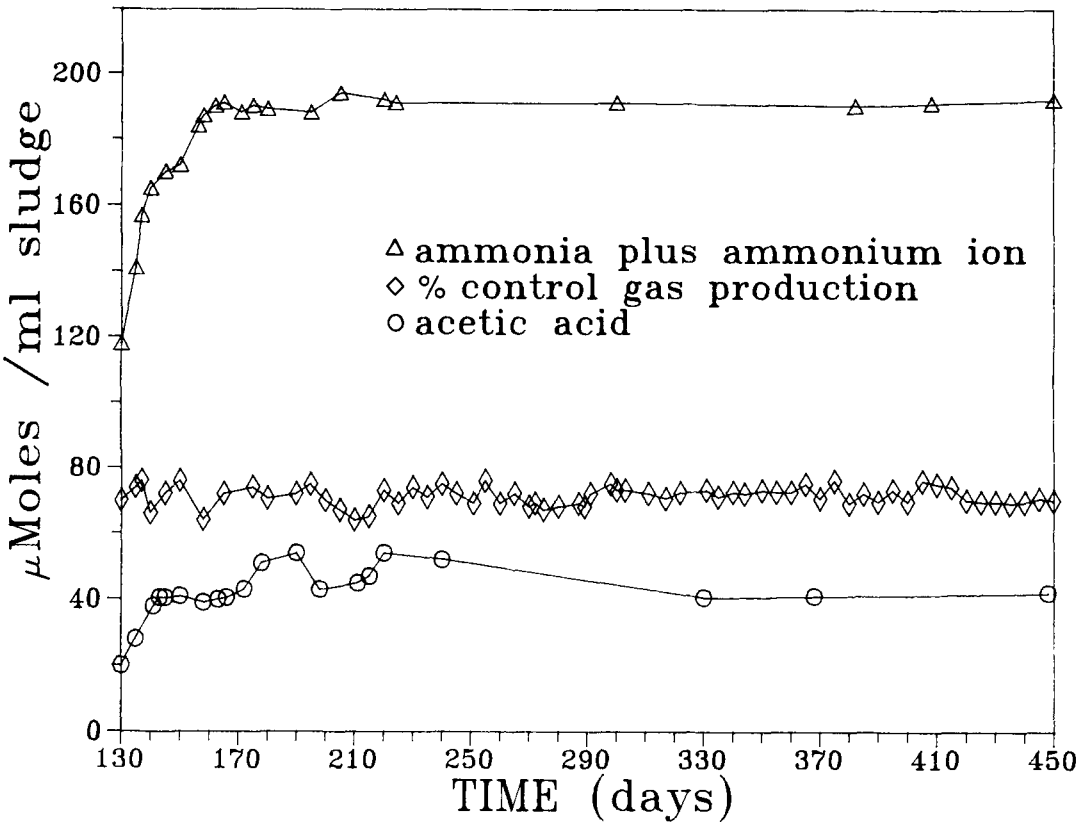

Fig. 1. Digester gas production, and acetate and ammonia pools during acclimation to continuous nitrate addition (0.4 µmol NO_3/mL sludge/h). Acclimation to nitrate "spikes" occurred from d 0–130.

nitrate, as much as 97% accumulated in the ammonia pools (28 h). In nitrate amended digester sludge, added nitrate is reduced to the intermediate nitrite before final reduction to ammonia.

To assess the effect of nitrate addition on digester gas production, nitrate, nitrite, and ammonia were added at various concentrations (Fig. 5). It is evident that low levels of added ammonium chloride can stimulate gas production in control digester sludge, and at levels as great as 200 µmol/mL sludge, only a minor inhibition occurred. Nitrite was the most toxic and inhibited 94% of the gas production with addition of as little as 5 µmol/mL sludge. Inhibition by nitrate depended on the concentration in the sludge with a maximum inhibition of approximately 80% occurring at levels of 100–200 µmol/mL sludge.

Larger pools of acetate were observed in the nitrate amended digester (sodium nitrate) and the effects of the sodium ion and nitrate were investigated by acclimation of separate digesters to added calcium nitrate and ammonium chloride (Table 1). The calcium salt of nitrate resulted in less reduction in gas production than the sodium salt, as evidenced by

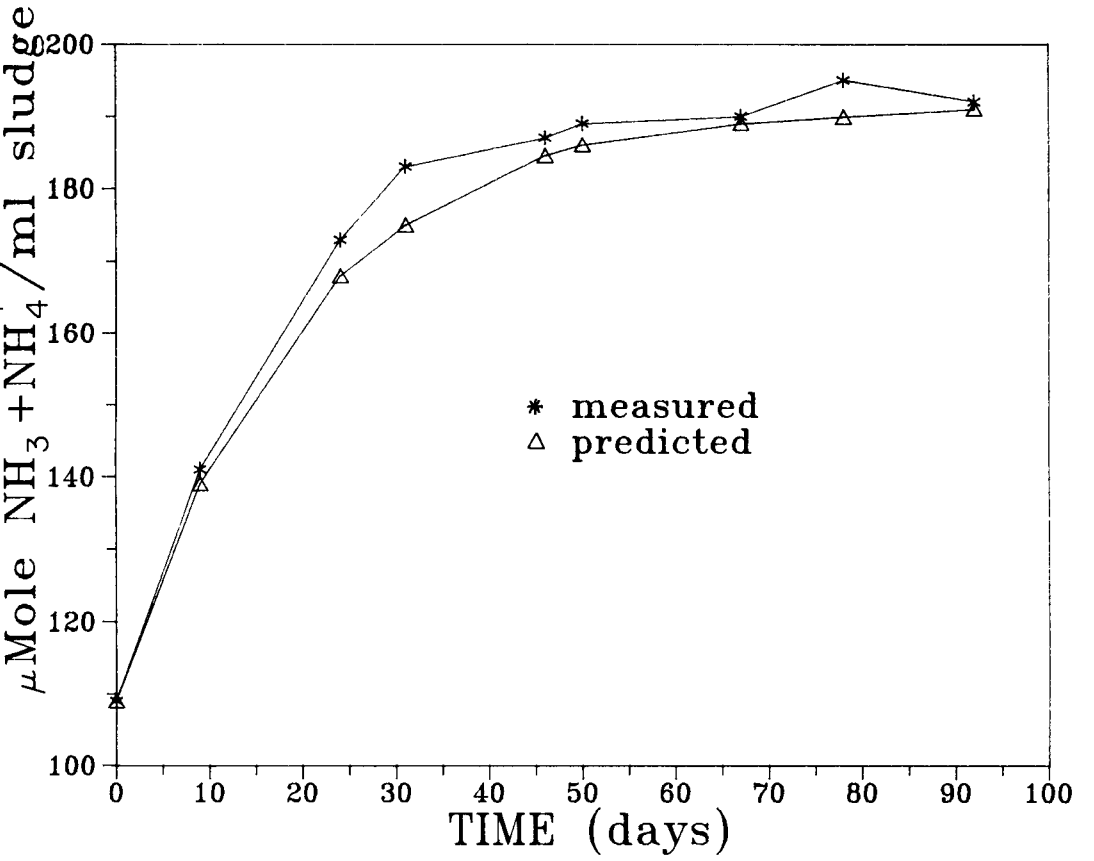

Fig. 2. Comparison of measured and predicted ammonium pools in the digester continuously amended with nitrate at the rate of 0.4 µmol/mL sludge/h.

higher gas production and lower volatile fatty acid levels. The ammonia levels resulting from nitrate reduction were not responsible for inhibition as determined by the performance in the ammonium chloride amended digester.

Analysis of the digester effluent indicated no statistically significant (1 SD) differences in acid detergent fiber, cellulose + cutin, and lignin between in the nitrate amended and control digester (Table 2). Higher levels of volatile solids in the nitrate amended digester sludge may be caused by greater microbial mass production. Microbial mass is largely solubilized in the acid detergent fiber analysis.

Enumeration of microbial populations indicated greater numbers of nitrate reducing and cellulose degrading bacteria with lower levels of methane-producing and sulfate-reducing bacteria in the nitrate-amended digester sludge (Table 3).

The activities of the enumerated populations of sulfate- and nitrate-reducing microbes (enumerations that require extensively long incuba-

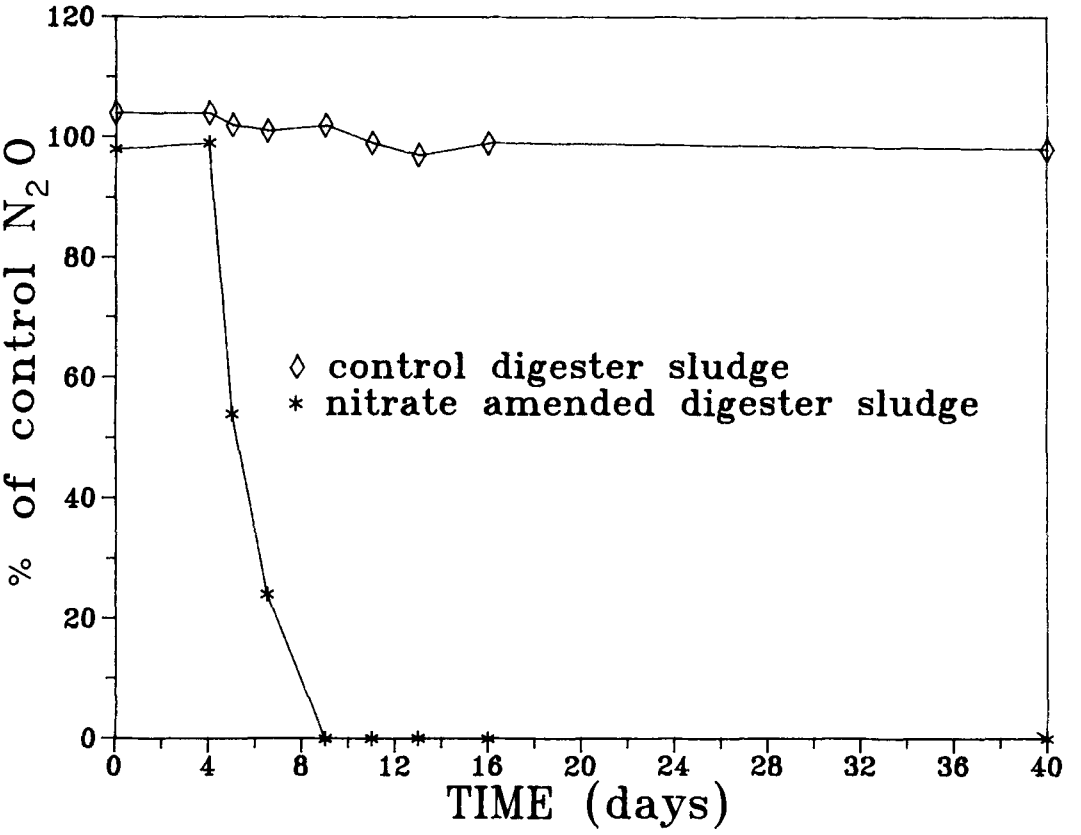

Fig. 3. Fate of nitrous oxide added to control and nitrate-amended digester sludge.

tion times, i.e., 2–3 wk) were tested by addition of sulfate and nitrate alone and together to control digester sludge (Fig. 6). In all cases, whether added alone or with an equivalent concentration of sulfate (data not shown), the nitrate was reduced within 18 h, and the sulfate levels remained unchanged in 24 h. Using calcium nitrate amended sludge resulted only in faster nitrate reduction (within 7 h), with the sulfate levels unchanged after 24 h (data not shown).

The effect of continuous sulfate addition on control and nitrate amended sludge is shown in Fig. 7. Continuous sulfate addition to a control digester resulted in a sharp drop in gas production (within 8–10 d) with no sulfate accumulation (levels below 0.1 μmol SO_4/mL sludge). Addition of sulfate to the nitrate amended digester resulted in sulfate accumulation to levels consistent with the addition rate, and no appreciable effect on gas production was evident until sulfate levels of approx 35–40 μmol SO_4/mL sludge were attained. At higher levels, the sulfate was reduced with increases in sulfide levels and reduction in gas production.

The level of inhibition of control digester gas production was determined for sulfate and the intermediates in the reduction to sulfide (Fig.

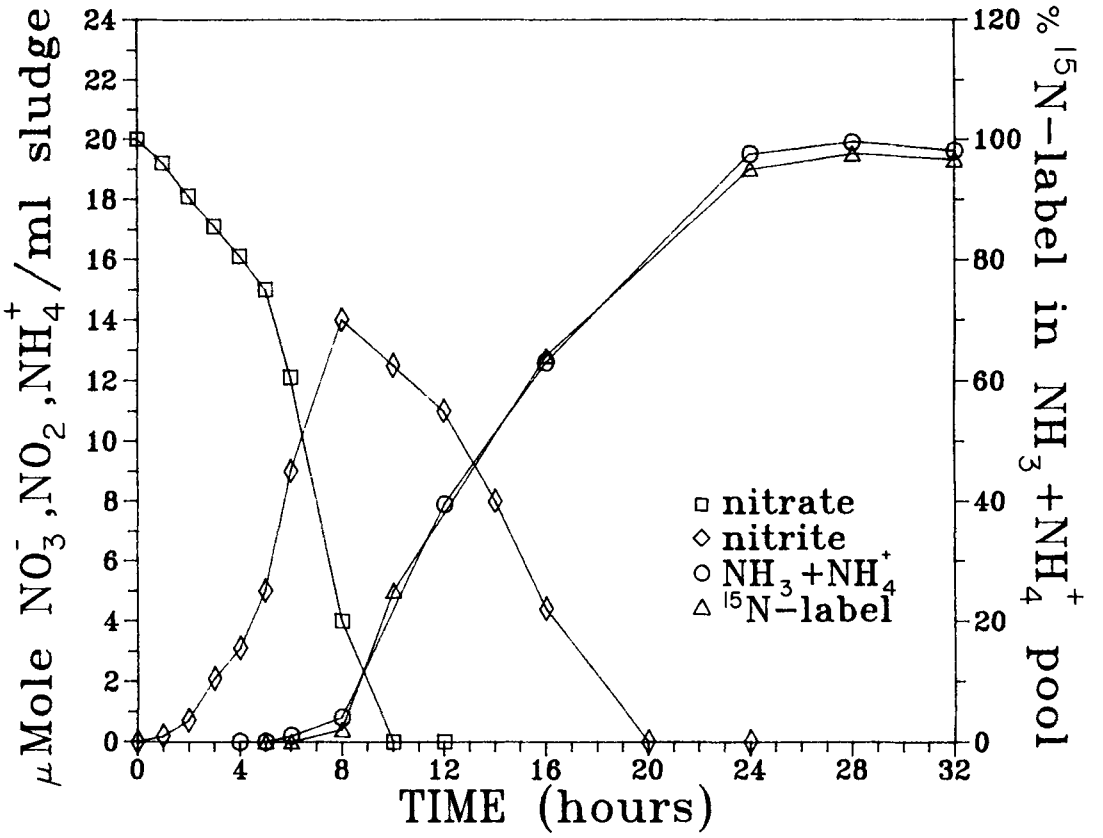

Fig. 4. Fate of added ^{15}N-labeled nitrate in nitrate amended digester sludge. The ^{15}N-label was determined only for the ammonium ion pools and are represented as a percent of the ^{15}N-label added as nitrate.

8). Sodium sulfite and sodium hydrosulfite were determined to be most inhibitory, with 92–95% inhibition of gas production at 5 μmol addition of either /mL sludge. Sodium sulfide inhibition was concentration dependant with complete inhibition of gas production at 100 μmol sulfide/mL of sludge. Sodium sulfate, although stimulatory in small additions, resulted in minor inhibition at concentrations of 200 μmol/mL sludge.

DISCUSSION

Initial addition of nitrate is inhibitory to gas production in the thermophilic anaerobic digestion system tested. Upon subsequent additions, the digestion microbial consortium readily acclimates to the nitrate addition, and high doses may be added, resulting in decreased gas production without digester failure. Unlike anaerobic sediments, the reduction of nitrate in the thermophilic anaerobic digester resembles that ob-

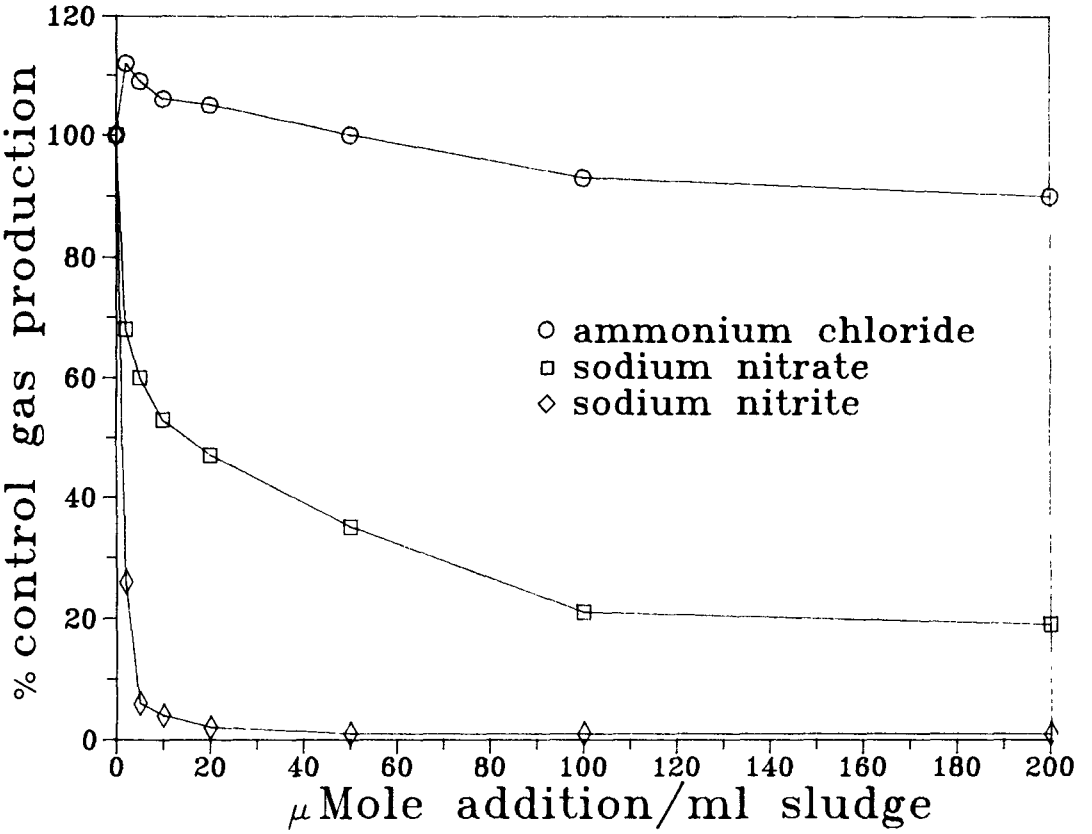

Fig. 5. Effects of various levels of added nitrate, nitrite, and ammonium on gas production in control digester sludge.

served in the rumen of animals. Essentially all of the nitrate is reduced to ammonia in the digester, as determined from accumulated ammonia pools in the digester and ^{15}N-labeled nitrate studies. Reduction of nitrate is by the dissimilar pathway with nitrite as the intermediate. The denitrification pathway is not responsible for reduction of nitrate as determined by the 4-d lag period required for nitrous oxide reduction. Ammonia buildup is not responsible for the reduced rates of gas production in the nitrate amended digester as determined by the performance of the ammonium chloride amended digester. The decrease in gas production is attributable to the competition for (and removal of) electrons for nitrate reduction instead of carbon dioxide reduction to methane. The competition for electrons results in methane formation from carbon dioxide only after all available nitrate and sulfate have been reduced (*16,23–30,54,55*). The toxic effect of excess nitrate on the digestion microbial consortium is caused by the nitrite produced as an intermediate in the reduction pathway. Added sulfate is reduced in control digester sludge after induction of activity in the resident population of sulfate reducers in the micro-

Table 1
Effects of Continuous Nitrate or Ammonia Additions to Thermophilic Anaerobic Digesters on Parameters Measured[a]

Digester designation:	E-1	D-1	D-2	F-2
Solution added[b]:	None	$Ca(NO_3)_2$	$NaNO_3$	NH_4Cl
% Control gas production	100	71.8 ± 1.6	51.3 ± 2.0	92.7 ± 6.2
pH	7.2 ± 0.05	7.6 ± 0.08	7.8 ± 0.05	7.0 ± 0.15
Volatile fatty acid pools				
Acetate[c]	1.6 ± 0.8	58.1 ± 16.1	107.8 ± 35.0	1.2 ± 0.7
Propionate[c]	1.1 ± 2.4	10.2 ± 1.2	12.5 ± 1.5	1.3 ± 1.4
Ammonia pools[c]				
$NH_3 + NH_4$	31.3 ± 2.3	194.0 ± 4.2	192.2 ± 3.8	195.0 ± 4.8

[a]Data represents measurements recorded over a 30-d period in which all digesters remained stable.

[b]Continuous addition rate was maintained at 0.4 μmol NO_3 or NH_4/mL sludge/h.

[c]Pools are reported in μmol/mL sludge.

Table 2
Analysis of Effluent Composition from Control and Nitrate Acclimated Digesters[a]

Digester designation:	E-1	D-1
Solution added[b]:	None	$CaNO_3$
% Dry matter[c]	3.26 ± 0.15	3.35 ± 0.11
% Volatile solids[c]	81.64 ± 0.39	88.13 ± 0.59
% Acid detergent fiber	32.21 ± 2.03	29.35 ± 1.05
% Lignin	8.86 ± 0.54	8.13 ± 0.42
% Cellulose + cutin	19.84 ± 1.09	17.26 ± 0.99

[a]Values represent data from two analyses conducted on sludge collected approximately three weeks apart.
[b]Continuous addition rate of 0.4 μmol NO_3/mL sludge/h.
[c]Calculations were based on weights corrected for calcium input.

bial consortium and results in immediate reductions in gas production rates and eventual digester failure (presumably from sulfide accumulation). The accumulation of the reduced end-product in sulfate reduction, sulfide, is inhibitory to the digestion microbial consortium, as opposed to ammonia accumulation resulting from nitrate reduction. Intermediates in sulfate reduction (such as sulfite and hydrosulfite) are inhibitory to the digester microbial consortium and have been confirmed from other research work (*56*). When sulfate is added to the nitrate amended digester the sulfate accumulates while nitrate is continually reduced and only when higher sulfate levels accumulate is the sulfate reduced, confirming the competition for electrons formed in the anaerobic digestion process by the various electron acceptors to be in the order of nitrate > sulfate > carbon dioxide (bicarbonate). Further, since nitrate has been shown to be preferentially reduced in the presence of sulfate and nitrate, nitrate

Table 3
Enumeration of Various Important Groups of Microorganisms in Control and Nitrate Acclimated Sludge[a]

Digester designation:	E-1	D-1
Solution added[b]:	None	$Ca(NO_3)_2$
Cellulose degraders	5.6×10^6	6.5×10^6
Nitrate reducers	3.9×10^8	6.9×10^8
Methanogens		
Hydrogen utilizing	1.1×10^9	1.4×10^7
Acetate utilizing	1.7×10^5	6.4×10^3
Sulfate reducers	2.1×10^7	7.4×10^6

[a]All enumerations are CFU/mL sludge and represent the average of duplicate roll tubes from three separate enumerations conducted three weeks apart during which digester parameters were stable.
[b]Continuous addition rate of 0.4 μmol NO_3/mL sludge/h.

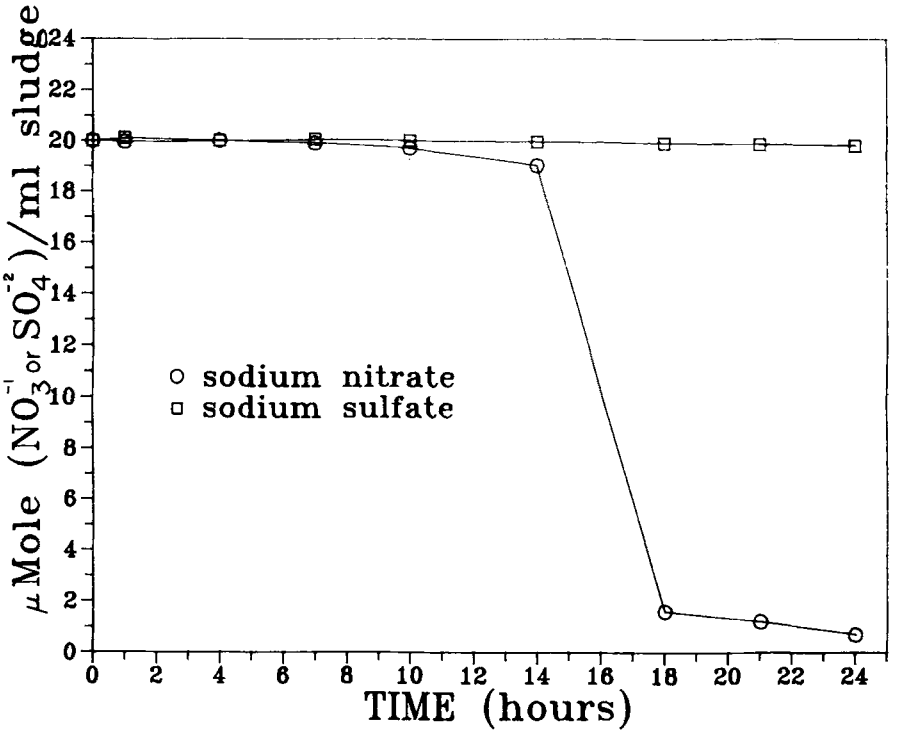

Fig. 6. Fate of nitrate and sulfate added to control digester sludge.

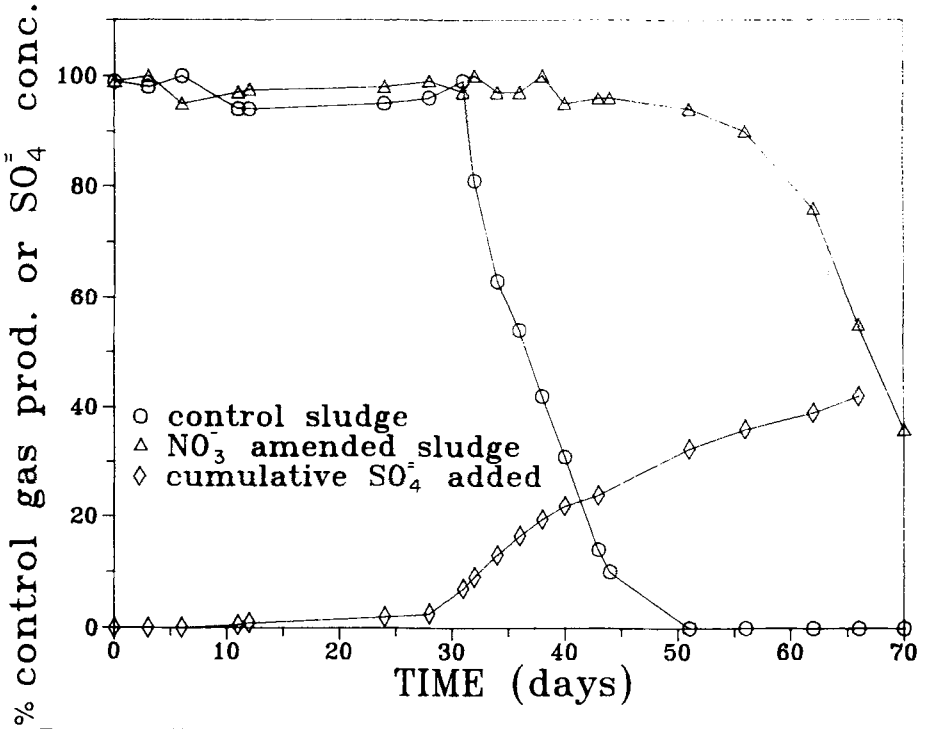

Fig. 7. Effects of continuous additon of sulfate on gas production in control and nitrate amended digester sludge.

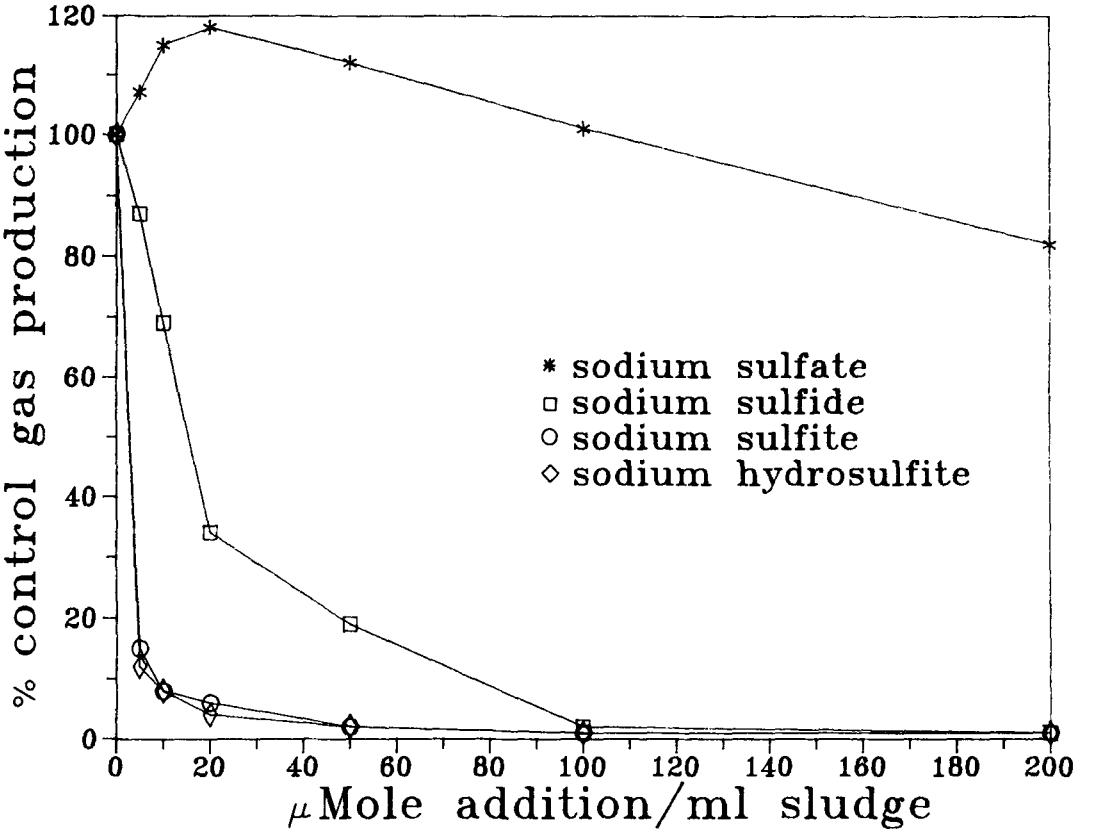

Fig. 8. Effect of various levels of added sulfate, sulfite, hydrosulfite, and sulfide on gas production in control digester sludge.

amendment may offer possibilities for the regulation and control of inhibitory sulfide concentrations.

ACKNOWLEDGMENTS

This paper reports results from a project that contributes to a cooperative program between the Institute of Food and Agricultural Sciences (IFAS) of the University of Florida and the Gas Research Institute (GRI), entitled "Methane from Biomass and Waste."

The authors thank Jeanette Player for her assistance.

Journal article no. 8238 of the Florida Agricultural Experiment Station.

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