Journal of Industrial Microbiology, 5 (1990) 247–258
Elsevier

SIM 00233

The effect of sulfate reduction on the thermophilic (55°C) methane fermentation process

Michael J. McFarland[1] and William J. Jewell[2]

[1]*Department of Civil and Environmental Engineering, Water Research Laboratory, Utah State University, Logan, UT* and [2]*Department of Agricultural Engineering, Cornell University, Ithaca, NY, U.S.A.*

Received 19 December 1988
Revised 8 April 1989
Accepted 26 June 1989

Key words: Methanogenesis; Sulfate reduction; Competitive inhibition; Sulfide inhibition; COD

SUMMARY

The continuously operated suspended growth anaerobic contact system was utilized to estimate the effect of sulfate reduction on the thermophilic (55°C) methane fermentation process. Results indicated that reduction in methanogenesis in the presence of sulfate was due to two separate, but related, processes; *i.e.* competitive and sulfide inhibition. Although prevention of competitive inhibition would be difficult under normal fermenter operation, sulfide inhibition could be minimized by environmental selection of sulfide tolerant microbial populations through biomass recycle and pH control. Stable fermenter operation was achieved at soluble sulfide concentrations as high as 330 mg/l soluble sulfide. Using batch fermenters, a maximum thermophilic sulfate reduction rate of 3.7 mg SO_4^{2-}-S/g volatile solids (VS)-day was estimated. The importance of reporting sulfate reduction rates on a biomass basis is demonstrated by a simple population adjustment kinetic model.

INTRODUCTION

Thermophilic (55°C) methane fermentation has several advantages over the traditional mesophilic (35°C) and psychrophilic (15°C) processes. These include higher rates of methane production, lower required cell retention times, and improved dewater ability of the waste microbial sludges. Despite these

Correspondence: M.J. McFarland, Department of Civil and Environmental Engineering, Water Research Laboratory, Utah State University, Logan, UT 84322-8200, U.S.A.
This research study was conducted at the Department of Agricultural Engineering, Cornell University, Riley Robb Hall, Ithaca, NY 14853, U.S.A.

process improvements, little attention has been given to the understanding of the environmental conditions which affect the thermophilic methane fermentation process.

It is well known that the presence of sulfate in the methane fermenter can have adverse effects on microbial populations and result in the contamination of the effluent gases [22]. Some of the environmental problems caused by the presence of sulfate in anaerobic fermenters have been identified as: (a) fermentation inhibition; (b) metal corrosion; and (c) emanation of offensive odors [10,16,22].

In addition to the environmental concerns of hydrogen sulfide production, many studies have demonstrated that anaerobic treatment of sulfur rich wastes may result in the preferential consumption of organic matter and/or molecular hydrogen (H_2) in the reduction of oxidized forms of sulfur to sulfide at the expense of methane generation [5,9,17,29]. The reduction in methanogenesis due to this shunting of available electrons from methane generation to sulfate reduction has been termed competitive inhibition. In addition to competitive inhibition, a threshold soluble sulfide concentration of 200 mg/l S^{2-} has been reported to severely inhibit methanogenic activity [22]. However, the mechanism(s) of sulfide inhibition is, presently, unknown.

From previous investigations, it is clear that sulfate reduction and methanogenesis are not mutually exclusive bioreactions. However, none of the previous studies seem to have clarified the effects of sulfate reduction on thermophilic methane fermentation in terms of bacterial concentration or distribution. In addition, no study exists which focuses on the importance of microbial acclimation or nutrient/pH control in achieving stable thermophilic methane fermentation. Therefore, it is difficult to predict the quantitative effects of sulfate reduction on steady state thermophilic methane fermentation beyond that which can be calculated from stochiometry. However, even this may be presumptuous since the environmental conditions, which influence both concentration and activity of bacterial species, can affect the sulfate reduction efficiencies significantly [14,18,39].

To determine the practicality of thermophilic anaerobic conversion of a sulfate rich waste to energy, it is essential that the fundamental sulfate interactions be well understood. To provide baseline data on anaerobic sulfate interactions at thermophilic temperatures, the following study was conducted. The specific research objectives included:

(a) defining sulfate utilization in a series of anaerobic contact fermenters receiving varying sulfate loadings.

(b) relating the main fermenter operational characteristics (*e.g.* fermenter pH, methane production rate, COD removal efficiency) to the influent sulfate concentration.

(c) quantifying the influence of soluble sulfide on the accumulation of volatile fatty acids.

(d) estimating the maximum thermophilic sulfate reduction rates.

MATERIALS AND METHODS

The reactor system consisted of four ten liter plexiglass thermophilic (55°C) anaerobic contact units (Fig. 1). Due to the slow anaerobic bacterial

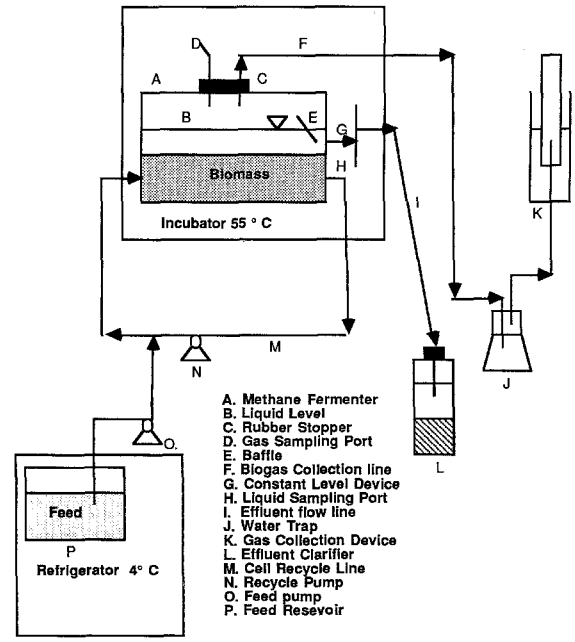

Fig. 1. Schematic of thermophilic anaerobic contact suspended growth reactor.

Table 1

Feed stock solution for thermophilic sulfate interaction study

	mg/l
Sucrose ($C_{12}H_{22}O_{11}$)	10 000
Yeast extract	200
Ammonium chloride (NH_4Cl)	190
Ammonium phosphate dibasic (NH_4HPO_4)	1500
Sodium bicarbonate ($NaHCO_3$)	9400

growth rates, intentional wasting of biological solids was not conducted. Volatile solids (VS) measurements were made periodically on both the discarded clarifier effluent and the reactor mixed liquor to calculate system solid retention times (SRT).

Sucrose was chosen as the principal carbon and energy source for all the experiments while sodium sulfate (Na_2SO_4) was the sulfate source. Both compounds were introduced as components of the feed solutions. The feed also contained nitrogen, phosphorus, alkalinity and miscellaneous nutrients (Table 1). Except for the sulfate concentrations, the feed stock solutions for the twelve conditions were identical.

Table 2

Tested and measured variables for the thermophilic (55°C) sulfide control experiments

Variables:		
Hydraulic retention time		~10 days
Influent substrate concentration		~10 g/l COD total
Organic loading rate		~1 g COD/l-d
Parameters:		
Influent:		
soluble COD		~10 g/l
soluble SO_4^{2-}-S		~0–800 mg/l
Effluent:		
alkalinity		
soluble COD		
soluble sulfide		
soluble SO_4^{2-}-S		
pH		
volatile fatty acids		
total solids		
% volatile solids		
Gas production:	liter/liter/day	
Gas quality:	% CH_4	
	% CO_2	
	% H_2S	

To insure biological acclimation during continuous operation, fermenter parameters were measured at pseudo-steady states called constant state operating conditions. A constant state operating condition was defined to occur when the measured methane fermenter operational parameters varied by less than ± 10% from the average value over one hydraulic retention time at a given set of experimental conditions. The reactors were allowed to operate for approximately twenty-five days at new experimental conditions before a constant state determination was made.

The constant state conditions were chosen in order to cover a wide range of sulfate loadings while maintaining constant hydraulic retention times and organic loading rates (Table 2). To compare the changes in reactor performance as a function of sulfate loadings, a control reactor was operated during the entire investigation.

Inoculum and reactor start up

To provide a viable source of thermophilic sulfate reducing and methane generating bacteria for start up of the microbial systems, each of the ten liter anaerobic contact reactors were inoculated with eight liters of reactor effluent from a preliminary thermophilic sulfate reduction study plus two liters of effluent from a full scale mesophilic methane fermenter. During this acclimation period, reactors were monitored closely to verify that all units were at the same constant state before introduction of sulfate.

Wet chemistry

Chemical Oxygen Demand (COD) was determined by the colorimetric method [19]. Soluble COD was determined by a COD analysis on supernatant samples obtained by decanting centrifuged raw samples. Centrifuging was done at 15 000 rpm for 20 min at 4°C.

Soluble sulfide was determined titrametrically [36] while the sulfate levels were measured turbidometrically [36].

Reactor alkalinity, total solids, and volatile solids were estimated according to standard procedures [36]. The pH of each reactor was determined by a Beckman Aeromatic SS-3 pH meter.

Volatile fatty acids (VFA) were measured every two days by a Flame Ionization Detector (FID) (Gow Mac Brand series 750). The specific fatty acids of interest were acetate (C2), propionate (C3), isobutyrate (IC4), *n*-butyrate (NC4), isovalerate (IC5), and *n*-valerate (NC5).

Gas analysis

Evolved biogas volumes were collected and measured in liquid displacement measuring devices every two days. Average production rates during constant state conditions are reported. Methane and carbon dioxide percentages in the biogas were determined using the Thermal Conductivity Detector (TCD) gas chromatograph (Gow Mac Brand 550 series). Gaseous hydrogen sulfide concentrations were measured colorimetrically by precalibrated lead acetate disposable glass tubes (Gastec Inc.).

RESULTS

The effect of sulfate on methane production and organic matter removal

The influence of sulfate on methane production over the entire range of sulfate concentrations tested is shown in Fig. 2. The maximum methane production rate of 0.27 l CH_4/l-d, which was observed at an influent sulfate concentration of 33 mg/l SO_4^{2-}-S, represented 85.2 percent of the maximum methane potential (based on organic matter reduction). At the largest influent sulfate concentration (*i.e.* 800 mg/l SO_4^{2-}-S), methane production decreased to 0.11 l CH_4/l-d or 30.0 percent of the maximum methane potential.

The reduction of one gram mol (32 g) of sulfate sulfur requires the shunting of 8 mol of electrons (or 64 g of chemical oxygen demand) from methane production according to Equation 1;

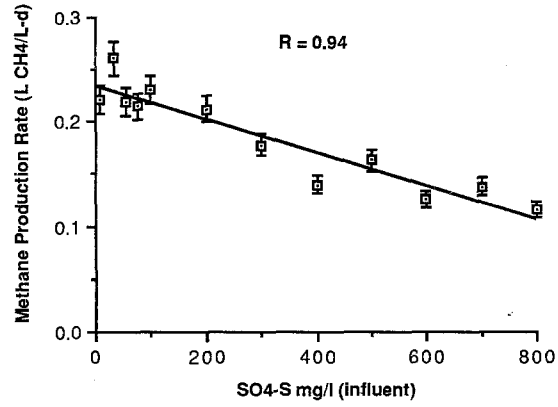

Fig. 2. The effect of influent sulfate concentration on the methane evolution rate when the organic loading rate was held constant at 1 g/l-d COD.

$$8H^+ + 8e^- + SO_4^{2-} \xrightarrow[\text{sulfate reducing bacteria}]{} S^{2-} + 4\,H_2O \qquad (1)$$

Thus, on an energy basis, the maximum sulfate loading rate of 800 mg/d SO_4^{2-}-S should have resulted in decreasing the methane production rate by 0.06 l CH_4/l-d (it should be noted that 0.35 liters of CH_4 is equivalent to one gram of chemical oxygen demand at standard temperature and pressure (273.15°K, 1 atm)). However, the observed reduction in methane production of 0.16 l CH_4/l-d suggested that competitive inhibition was not the only mechanism causing methanogenic inhibition.

Examination of organic matter utilization as a function of the influent sulfate concentration demonstrated that organic removal efficiency was affected adversely by sulfate additions (Fig. 3). This reduction became progressively worse with increasing sulfate loading. From a competitive inhibition standpoint, this reduction in organic matter removal efficiency was contrary to what would have been predicted. In other words, during competitive inhibition, the preferential consumption of organic matter by the faster growing sulfate reducing microorganisms should have led to greater organic matter utilization with increasing sulfate concentration [12].

It is well known that the toxic or inhibitory form

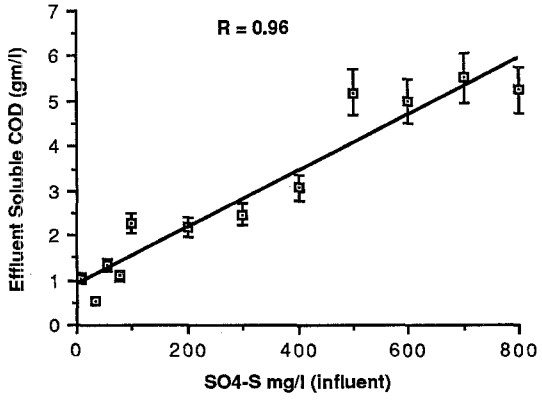

Fig. 3. The effect of sulfate on effluent chemical oxygen demand when the influent soluble COD was constant at 10 g/l and the hydraulic retention time remained fixed at ten days.

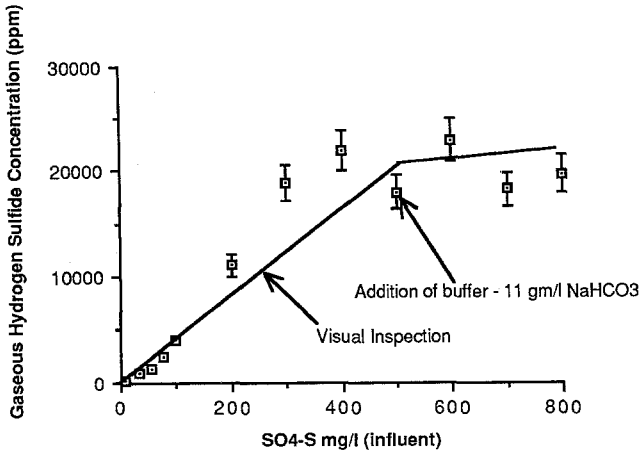

Fig. 5. Variations in gaseous sulfide concentration as a function of the influent sulfate concentration when the influent soluble COD was constant at 10 g/l and the hydraulic retention time remained fixed at ten days.

of sulfur within methane fermenters is not sulfate but, rather, soluble sulfide [19,20,22,33]. At constant state conditions, sulfate was completely reduced at all sulfate loading rates investigated. The rapid reduction of sulfate was reflected in both aqueous and gaseous sulfide concentrations (Figs. 4 and 5). As the influent sulfate concentration increased, soluble sulfide concentrations decreased slightly before reaching a maximum of 330 mg/l. Figs. 6 and 7 show the influence of soluble sulfide on the methane generation rate and organic matter removal efficiency, respectively. The asymptotic behavior of the data in the high soluble sulfide range suggests that microbial acclimation was occurring. It should be noted that this in the first study to report stable reactor operation of a suspended growth methane fermenter above 200 mg/l soluble sulfide. Until now, it was generally accepted that 200 mg/l soluble sulfide represented a threshold

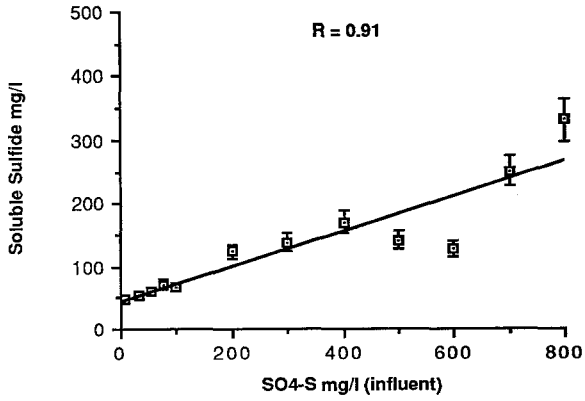

Fig. 4. Variation of the soluble sulfide levels as a function of the influent sulfate concentration when the influent soluble COD was maintained at 10 g/l and the hydraulic retention time remained fixed at ten days.

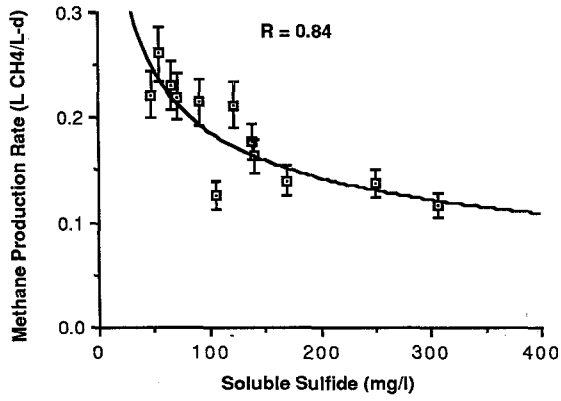

Fig. 6. Variation of methane production rate as a function of the soluble sulfide concentration. The influent soluble COD was constant at 10 g/l soluble COD while the hydraulic retention time was ten days.

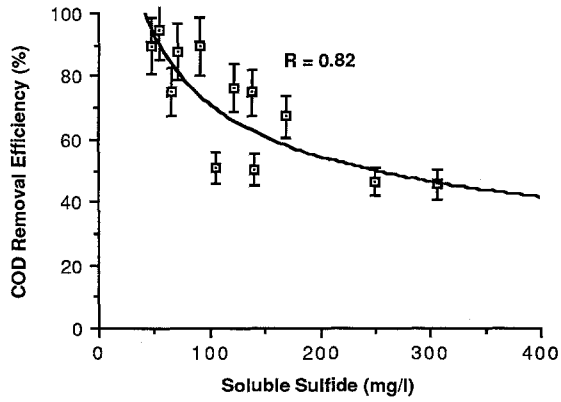

Fig. 7. Variation of COD removal efficiency as a function of the soluble sulfide concentration. The influent soluble COD was constant at 10 g/l while the hydraulic retention time maintained at ten days

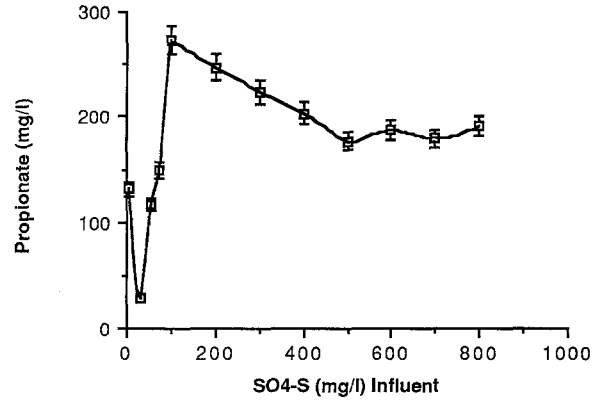

Fig. 9. Propionate accumulation as a function of the influent sulfate concentration when the influent soluble COD was maintained at 10 g/l and the hydraulic retention time was held constant at 10 days.

concentration, above which, total cessation of the methane fermentation process occurred in suspended growth systems [22]. To determine at which stage of the methane fermentation process inhibition is occurring (*i.e.* hydrolysis or methanogenesis), the behavior of some important volatile acids was investigated.

Volatile fatty acid (VFA) metabolism

Acetate is one of the main intermediates in the converion of organic matter to methane and carbon dioxide [12]. In a stable methane fermenter, acetate is utilized as soon as it is formed, thus, its concentration is normally low (below 1000 mg/l). When the methane fermentation process becomes unstable, acetate will accumulate together with other volatile fatty acids (*e.g.* propionate, butyrate, valerate). If not properly buffered, the increase in the organic acids will lead to pH depression which further imbalances the system. The accumulation of acetate as a function of the influent sulfate concentration is shown in Fig. 8.

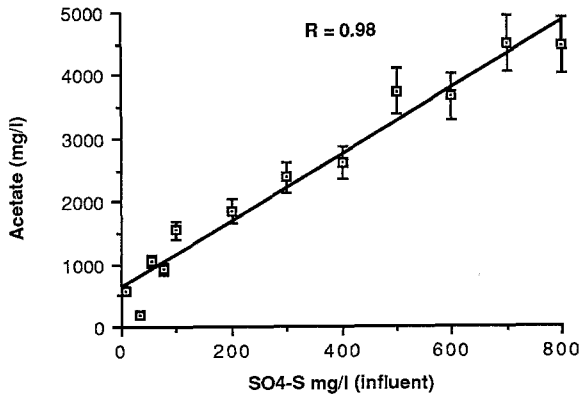

Fig. 8. Acetate accumulation as a function of the influent sulfate concentration when the influent soluble COD was constant at 10 g/l and the hydraulic retention time was held constant at ten days.

Below an influent sulfate concentration of 75 mg/l SO_4^{2-}-S, acetate levels remained between 500 and 1000 mg/l while methane production levels varied by approximately ± 12%. As the influent sulfate concentration increased above this level, acetate accumulation increased significantly. Acetate accumulations were reflected in decreases in both methane generation and fermenter pH. To prevent system failure, the sodium bicarbonate buffer concentration was increased to 11 g/l.

In addition to acetate, propionate accumulations also were significant (Fig. 9). In the low influent sulfate region (*i.e.* 6 to 100 mg/l SO_4^{2-}-S), the propionate concentration exhibited large fluctuations reaching a maximum of 270 mg/l at an influent sulfate concentration of 100 mg/l SO_4^{2-}-S. Above this influent sulfate level, propionate concentrations de-

creased and eventually reached a constant level of approximately 180 mg/l. The constant propionate concentration suggested that by proper pH maintenance and cell recycle, sulfide tolerant propionate utilizing bacteria developed within the reactor. Although this seems probable, identification of propionate utilizers was not included as part of the experimental program.

Butyrate and valerate also were detected during fermenter operation (data not shown). The concentrations of these acids varied from zero (at low influent sulfate concentrations *i.e.* <75 mg/l SO_4^{2-}) to approximately 40 mg/l at an influent sulfate concentration of 800 mg/l SO_4^{2-}. Due to the experimental error involved in measuring small concentrations of these acids, it was difficult to clarify their accumulation behavior. However, it is well known that their presence in methane fermenters reflects methanogenic biological stress [12].

Understanding the difference in fermenter response to low and high sulfate concentrations is a very important consideration in process control. At low sulfate concentrations, reduced methane production is due primarily to the shunting of electrons from methanogenic to sulfate reduction reactions. This does not represent a microbially stressed condition. Reestablishing prior methane production rates, under these conditions, can be achieved by increasing the electron donor concentration by an amount equal to the electron donor utilized by the sulfate reduction reactions. Conversely, at the high sulfate concentrations, competitive inhibition occurs, but it is the resulting sulfide which has the greater impact on methanogenic inhibition. This inhibition is characterized by an accumulation of intermediate fermentation products (*e.g.* volatile fatty acids) and declining pH. Under these conditions, increasing the electron donor concentration exacerbates fermenter inhibition.

Sulfate reduction efficiency during thermophilic methane fermentation

Because of the variations in sulfate reduction efficiencies reported in the literature [5,6,15,32], it was necessary to determine the degree of sulfate reduction in the thermophilic suspended growth system.

At constant state conditions, sulfate was reduced completely at all sulfate loading rates investigated. The rapid reduction of sulfate was reflected in both aqueous and gaseous sulfide concentrations (Figs. 5 and 6). As the influent sulfate concentration increased, soluble sulfide concentrations decreased slightly before reaching a maximum of 330 mg/l. The decrease in the soluble sulfide concentration above 400 mg/l SO_4^{2-} is probably due to the decrease in fermenter pH from 7.3 to 7.1 which shifted sulfide into its more volatile, unionized (H_2S) form. To prevent further pH suppression, the buffer capacity of the system was raised which resulted in stabilization of fermenter pH. The effect of the buffer addition was the retention of a greater portion of the influent sulfur as soluble sulfide in solution. This change in gaseous sulfide behavior is reflected by a change in the slope of the curve in Fig. 5.

Fermenters operating at high sulfate concentrations (*i.e.* ≥ 700 mg/l SO_4^{2-}) experienced significant losses in microbial solids in the effluent. Specifically, the solid retention time was observed to decrease from 129 to 38 days as sulfate feed increased from 400 to 700 mg/l SO_4^{2-} (Table 3). Over

Table 3

Effect of influent sulfate concentration on solid retention time and acetate concentration in continuously operated thermophilic (55°C) suspended growth anaerobic fermenter

COC constant operating condition	Influent sulfate concentration (mg/l)	Solid retention time (SRT)	Acetate concentration (mg/l)
I	6	179	507
II	33	160	201
III	55	151	1045
IV	75	160	926
V	100	122	1547
VI	200	120	1850
VII	300	135	2378
VIII	400	129	2603
IX	500	65	3740
X	600	63	3660
XI	700	38	4492
XII	800	35	4470

this same range of influent sulfate, the acetate concentration was observed to increase from 2603 to 4470 mg/l. Despite the increased loss in volatile solids, reduction of sulfate was complete over the entire range of influent sulfate concentrations. The increased loss of microbial solids appears to be the result of sulfide stress which reduced the solid separation efficiency of the microbial flocs. Despite the stressed fermenter conditions, maintenance of stable conditions (by pH control) resulted in continuous methane generation even at the high influent sulfate concentrations.

Kinetics of sulfate reduction

The maximum sulfate reduction rates achievable in a thermophilic (55°C) suspended growth system were determined using one liter batch reactors. The influent sulfate concentration of 200 mg/l SO_4^{2-}-S was chosen as the initial batch reactor concentration. The bacterial seed was taken from the continuously operating thermophilic fermenter receiving the same sulfate loading.

Due to the amount of time required to assay for sulfate relative to its expected utilization rate, an indirect sulfate analysis was formulated. The method involved equating the sulfate reduction rate to the total sulfide production rate. To use this method, it was assumed that sulfur losses due to microbial uptake and chemical precipitation were negligible.

Under these conditions, a maximum gaseous sulfide production rate of 0.039 ml H_2S/g VS-h (or equivalently, 0.056 mg S/g VS-h) was recorded four hours after the introduction of the feed solution. In addition to the gaseous sulfide production rate, a maximum soluble sulfide production rate of 0.098 mg S/g VS-h was determined after the same elapsed time. Combining the gaseous and soluble sulfide production rates gave a maximum total sulfate reduction rate of 0.154 mg SO_4-S/g VS-h (or 3.7 mg SO_4-S/g VS-day). These kinetic values represent an average of duplicate analyses measured over a twenty-four hour period.

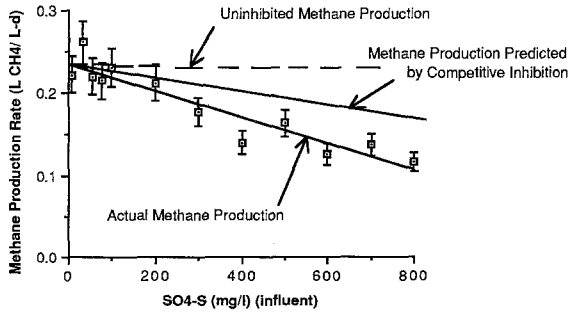

Fig. 10. The variation of methane production as a function of the influent sulfate concentration when the influent is held constant at 1 g/l-d soluble COD.

DISCUSSION

The existence of thermophilic sulfate reducing microorganisms has been known for some time [3,11,24]. However, few studies were found which investigated the influence of the behavior of these organisms on the thermophilic (55°C) methane fermentation process. This is the first study, to this author's knowledge, which documents the effects of sulfate reduction on the methane fermentation process in which effluent fermenter biomass was recycled and pH levels were controlled.

In the range of influent sulfate loading rates investigated (*i.e.* 0.6 to 80.0 mg/l-d SO_4^{2-}-S), the effluent sulfate concentrations were zero indicating complete reduction of sulfate. This result was not surprising when the sulfate reduction rate in the continuous system was compared with the maximum rate obtained in the batch tests. For example, when put on a volatile solids basis, the influent sulfate concentration of 200 mg/l SO_4^{2-}-S resulted in a specific sulfate loading rate of 0.90 mg SO_4^{2-}-S/mg VS-day to the continuous flow system. This loading rate was less than 25% of the maximum batch sulfate reduction rate of 3.7 g SO_4^{2-}-S/gm VS-day.

Fig. 10 demonstrates that the reduction in methane production increased from that predicted by stochiometry with increasing sulfate loading. Thus, sulfate inhibition of methane production was due to, at least, two separate phenomena; (1) competitive inhibition; and (2) sulfide inhibition. Competitive inhibition could be estimated from theoretical

considerations (Equation 1). Although it can lead to an appreciable loss in methane production, competitive inhibition is not characterized by the accumulation of intermediate fermentation products. On the other hand, sulfide inhibition leads to a large concentration of volatile fatty acids together with suppression of fermenter pH.

Despite the reduction in microbial activity during sulfide inhibition, fermenter stability could be maintained by biomass recycle and pH control. These process adjustments encouraged the colonization of sulfide tolerant organisms in the fermenter.

Comparison of maximum thermophilic sulfate reduction rates found in the present study to those recorded in previous studies demonstrates the importance of reporting reaction rates on a microbial solids basis. For example, a maximum volumetric sulfate reduction rate of 68 mg SO_4^{2-}-S/l-d had been reported in a thermophilic CSTR unit [38]. If it were shown that this suspended growth system contained a 2% mixed liquor volatile solids concentration (*i.e.* 20 g volatile solids/liter), the reported reaction rate would be identical to the one reported in this study (*i.e.* 3.7 mg SO_4^{2-}-S/g VS-d).

Because few studies in the literature reported sulfate reduction rates on a microbial mass or volatile solids basis, it was also difficult to compare the maximum thermophilic sulfate reduction rate found in this study to those reported at lower temperatures. Moreover, since sulfate reduction kinetics are dependent on the presence of viable sulfate reducing bacteria, it is important to consider the difference in sulfate reducer bacterial concentrations when comparing sulfate reduction rates of various systems [39]. The example given in Table 4 illustrates how the concentration of sulfate reducing

Table 4

Estimation of the percentage of sulfate reducing bacteria contained in measured biomass: hypothetical example

EXAMPLE 1	
Continously operating thermophilic (55°C) methane fermenter	
Data:	
Influent sulfate concentration	100 mg/l SO_4^{2-}-S
Influent biodegradable COD concentration	10 000 mg/l COD
Hydraulic retention time	10 days
Sulfate reducer bacterial yield coefficient	0.12 mg VS/mg SO_4^{2-}-S [24]
Methanogenic + hydrolytic yield coefficient	0.16 mg VS/mg COD [35]
A. Steady state production of sulfate reducing bacteria:	0.12 mg VS/mg SO_4^{2-}-S × (10 mg/l-d SO_4^{2-}-S) 1.2 mg VS/l-d
B. Steady state production of methanogenic plus hydrolytic bacteria:	
To approximate this value, the COD equivalent of the reduced sulfate (*i.e.* 200 mg/l COD) is first subtracted from the total influent COD removed from the system. The remaining fraction of COD utilized then can be multiplied by the appropriate yield coefficient to give an estimate of the steady state concentration of methanogenic and hydrolytic bacteria. The following demonstrates the calculations assuming that 100% COD removal was obtained;	
C. Steady state production of methanogenic and hydrolytic bacteria:	0.16 mg VS/mg COD × (9800 mg/l-d COD) 157 mg VS/l-d
Thus, the percentage of volatile solids which are sulfate reducing bacteria is determined as follows (assuming that the different populations are retained in proportion to their yields);	
D. Percentage of volatile solids produced which are sulfate reducing bacteria at steady state:	1.2/(157 + 1.2) × 100 0.8%

bacteria may be estimated in an anaerobic suspended growth system.

Results from this study are applicable directly to the design and operation of high rate thermophilic digesters treating sulfate laden organic wastes. Large volumes of such wastes are generated daily in the molasses and sugar processing industries [9,38].

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to the research personnel of the Agricultural Engineering Department of Cornell University, Ithaca, NY for their assistance in conducting this research. This study was supported in part by Gas Research Institute Contract 5083-226-0848.

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