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Disturbance, starvation, and overfeeding stresses detected by microbial lipid biomarkers in high-solids high-yield methanogenic reactors

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SUMMARY

Microbial biomass and community structure of methanogenic anaerobic biomass reactors can be quantitatively monitored by signature lipid analysis. The eubacterial and eukaryotic polar lipid fatty acids and the methanogen polar lipid ethers are reliable measures of their respective biomasses. The pattern of polar lipid fatty acids yields information on the community structure and metabolic state of the eubacteria and eukaryotes. These biomarker methods were applied over a 2-day feeding cycle of a highly productive batch-fed high-solids anaerobic biomass reactor. It was sampled before feeding, 6 h after feeding (disturbed), at maximum gas production (healthy, 24 h), and after feedstock utilization (starved, 48 h). Relative to the healthy condition, the disturbance of feeding significantly decreased eubacterial biomass and the proportion of unsaturated fatty acids, and increased branched fatty acids and the eubacterial stress biomarker, *trans/cis* 16 : 1ω7. The starved condition was not significantly different from the healthy in biomass or proportions of fatty acids, but did show a significant increase in the proportion of the eubacterial stress biomarker *trans/cis* 18 : 1ω7. This reactor was compared to a second of the same design which had been overfed and showed significantly less productivity. The overfed reactor had a significantly lower methanogenic biomass, *iso*-branched fatty acids, and higher eubacterial stress markers Cy17 : 0 and *trans/cis* 18 : 1ω7 than the highly productive reactor.

INTRODUCTION

Anaerobic digestion of biomass by methanogenic fermentation has been proposed as an alternative to fossil fuels and as a method of disposing of municipal solid waste [15]. Methane is a non-polluting, renewable energy source whose economics are not dependent upon international markets as are fossil fuels. Its use would permit dwindling supplies of petroleum to be reserved for petrochemicals. Anaerobic degradation of the cellulosic fraction of municipal solid waste would greatly decrease the volume of landfill space required.

A critical parameter for economic biomass-to-methane energy systems is the yield of methane per unit of feedstock [5], which is limited by the ability to hydrolyze recalcitrant polymers such as lignin and hemicellulose. The greatest difficulty in anaerobic digestion of municipal solid waste is maintaining process stability [14]. Toxic compounds in municipal solid waste may inhibit the critical microbial populations of biopolymer hydrolyzers, volatile fatty acid syntrophs, and/or the methanogens, inducing process instability. A deeper understanding of the microbial ecology of anaerobic methanogenic fermentations will help facilitate the development of economically viable processes.

Membrane lipid analyses have been used to estimate the in situ microbial biomass, community structure, and metabolic status of natural environments [23], and methanogenic anaerobic reactors [10,18,20]. The polar fraction of the total lipid extract contains the eubacterial and eukaryote membrane polar lipid fatty acids (PLFA). PLFA is an accurate measure of viable microbial biomass, and has a rapid turnover in natural environments [1]. Membrane polar lipid ethers (PLEth) are characteristic of the archaebacterial methanogens [22], a key component of the methanogenic community. The pattern of individual PLFA provides a 'fingerprint' of the microbial community structure of the environment sampled. Community structure is defined as the pro-

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portions of distinguishable groups of organisms within the viable biomass. Specific lipid markers are known which reflect changes in the metabolic status of members of the microbial community. For example, the ratio of *trans* to *cis* mono-unsaturated PLFA increases with unbalanced growth [7] and the amount of cyclopropyl PLFA increases in the stationary phase of bacterial growth [16]. In this work, lipid biomarker analysis was applied to understanding the changes in the microbial community over the 2-day feeding cycle of a pair of bioreactors, operated at a thermophilic temperature.

MATERIALS AND METHODS

High-solids methanogenic reactors. The Cornell University Department of Agricultural and Biological Engineering constructed the high-solids reactors using 20-l polypropylene carboys containing 5 kg of reactor material statically incubated at 55 °C [12]. Reactors were fed every Monday, Wednesday, and Friday by removing 5–15% of the reactor contents and replacing it with a 1 : 1 (w/w) mixture of α-cellulose (Sigma Chemical Co.) and milled dried sorghum (*Sorghum bicolor*, Stanford Seeds variety X9204) moistened with a trace mineral solution [11]. The amount of material replaced depended upon the feeding interval, the amount of mass lost to carbon dioxide and methane, and the loading rate. Ammonia was supplemented at the rate of 32 to 40 mg N $kg^{-1}day^{-1}$. The reactors were mixed by rapid rotation before sampling in order to minimize differences within the carboy. The samples were approximately 5 g. Wet weight was obtained as the difference between the container's weight before and after adding the sample. Samples were frozen immediately and kept frozen until they were lyophilized. Activity measurements, gas production, and radiolabel incorporation are detailed in Hedrick et al. [9].

'Disturbed', 'healthy' and 'starved' reactor conditions. High-Solids Reactor no. 1 (HS1) had been operated under stable operating conditions for efficient conversion of biomass to methane for 60 days at the time of sampling, with an organic loading rate (OLR) of 18 g volatile solids per kg wet weight reactor contents per day (gVS $kg^{-1}day^{-1}$) [12]. It was sampled at several times over the feeding cycle to observe changes in the microbial community as the feed was colonized and consumed. These were named the before feeding (0 h), disturbed (6 h), healthy (24 h), and starved (48 h) reactor conditions, after their biogas production rates [11] and microbial activities [9].

'Overfed' reactor condition. High-Solids Reactor no. 2 (HS2) was operated in the same way as HS1, except that it had been operated at 24 gVS $kg^{-1}day^{-1}$ OLR for 36 days. Feedings had been suspended for 10 days, but were resumed the day before sampling at the high rate. This reactor displayed instability due to overfeeding, as indicated by variable and falling gas production, and increasing volatile fatty acid and ammonia concentrations [11]. HS2 was sampled at its time of maximum gas production (24 h after feeding) for comparison of the overfed condition with the healthy condition of HS1.

Lipid analyses. All solvents were nanopure grade from Burdick and Jackson (Muskegon, MI). The water used was Barnstead reverse osmosis (Boston, MA) at 18 MΩ/cm or better. Water was stored over chloroform to remove lipid contaminants and inhibit microbial growth. All glassware and glass wool used was fired at 450 °C overnight to remove lipid contaminants.

The lyophilized samples were ground in a mortar and pestle, and approximately 250 to 300 mg weighed for extraction. The total lipid was extracted from the samples by the method of Bligh and Dyer [2] as modified by White et al. [25]. The total lipid extract was separated into neutral lipid, glycolipid, and polar lipid fractions by silicic acid column chromatography [6]. Neutral lipids were eluted with 10 ml of chloroform, glycolipids with 10 ml of acetone, and polar lipids with 10 ml of methanol. The three fractions were collected separately and the solvent was removed with a stream of dry nitrogen at 37 °C. All subsequent steps were carried out with the polar lipid fraction. Mild alkaline transesterification [24] was used to cleave and methylate the ester-linked PLFA. The lipid recovered from the mild alkaline transesterification was again fractionated by silicic acid column chromatography, omitting the acetone elution step, to obtain the PLFA in the neutral lipid fraction as fatty acid methyl esters, and the alkali-stable polar lipids, including the archaebacterial PLEth, in the polar lipid fraction.

PLFA were analyzed by capillary gas chromatography, and the peak identities verified by gas chromatography-mass spectroscopy [24]. The gas chromatograph used was a Schimadzu GC-9A equipped with a flame ionization detector and an HP-1 50 m non-polar column, 0.2 mm i.d. and 0.11 μm film thickness. Gas chromatography-mass spectroscopy was performed on a Hewlett-Packard 5995A system with the same column and under the same operating conditions.

The fatty acid nomenclature used was as follows: the number of carbons; ':' (colon), the number of unsaturations; 'ω' (omega), the distance of the first unsaturation from the methyl end of the fatty acid; and 'c' or 't' for the *cis* or *trans* geometric isomers of the unsaturation. The prefixes 'i' and 'a' denote iso- and anteiso-branching, and 'Cy' a cyclopropyl moiety. Therefore, '16 : 1ω9c' indicates *cis*-7-hexadecenoic acid and 'i14 : 0' indicates 11-methyl-tridecanoic acid in the IUPAC nomenclature [19]. 'a17 : 0/17 : 1ω8' represents unresolved a17 : 0 and 17 : 1ω8 fatty acids, '10Me16 : 0'

represents 16 : 0 with a methyl branch 10 carbons from the carboxyl terminus, and 'Unk' signifies fatty acids of unknown structure.

The PLEth were cleaved from the phosphate moiety by strong acid methanolysis [17] and analyzed by supercritical fluid chromatography (SFC) [8]. The SFC apparatus was constructed from an ISCO LC 2600 syringe pump (ISCO, Inc., Lincoln, NE) and a Varian 3700 gas chromatograph with a flame ionization detector (Varian Instrument Group, Palo Alto, CA). SFC-grade CO_2 (Scott Specialty Gasses, Plumbsteadville, PA) was used as the mobile phase. The syringe pump was controlled by a BASIC language program (available upon request from DBH) written in this laboratory for a Commodore 64 computer. Details of SFC construction, operation, and performance are presented in Hedrick et al. [8].

Statistical analyses. The fatty acid profiles were analyzed by applying an arcsin square root transformation [26] to the mol percents (mol%) and clustering the PLFA with the 1-Pearsons r correlation coefficient metric [21] using the Systat statistical package (Systat, Inc., San Diego, CA). The algorithm clusters fatty acids together which varied together (covariance). The biomass measures, fatty acid clusters, and microbial stress biomarkers were tested for homogeneity of variance over all samples by the method of Cochran's C [26]. Analysis of variance at the $P = 0.05$ significance level was used to determine whether a parameter differentiated between treatments.

RESULTS

Microbial biomass

In reactor HS1, the measures of microbial biomass – PLFA and PLEth (Table 1) – decreased between before feeding and 6 h after feeding (disturbed). At 24 (healthy) and 48 h (starved) after feeding, these biomass markers had risen to approximately their initial level. Both PLFA and PLEth were lower in the overfed HS2 samples than in the healthy HS1 samples at 24 h after feeding. Analysis of variance was applied to the disturbed, starved, and the overfed conditions, compared to the healthy condition (Table 2). The PLFA content was significantly different between the disturbed and healthy conditions, and PLEth was significantly different between the overfed and healthy conditions.

Cluster analysis of polar lipid fatty acid patterns

The PLFA clustered into five biosynthetically related groups, and four PLFA that joined no cluster (Fig. 1). The same five groups were formed using either the complete, average, median, or centroid linkage methods, indicating that the clusters obtained were robust with respect to the statistical method used.

The iso-branched cluster (Fig. 1) contained all the iso- and anteiso-branched fatty acids greater than 15 carbons, as well as 16 : 1ω9c and an unknown fatty acid, possibly a19 : 0. a17 : 0 co-eluted with 17 : 1ω8, so the identity of this peak as the anteiso-fatty acid was not confirmed. The mol% of the PLFA in this cluster was the largest of the five clusters, and was stable over the time course of feeding, varying from 36 to 41.5 mol% (Fig. 2a). No statistically significant difference was found over the time course. At maximum gas production (24 h), the value for the healthy HS1 was significantly higher than for the overfed HS2.

The Long cluster contained all the even numbered straight chain fatty acids from 16 to 24 carbons, plus three minor, incompletely defined PLFA of 20 carbons (Fig. 1). This cluster had the second highest mol% due to palmi-

TABLE 1

Measures of activity and biomass in the high-solids reactors

Methane production rates in l/kg per day and acetate turnover rates in units per hour (from ref. 9). Polar lipid fatty acids (eubacterial and eukaryote biomass) and polar lipid ethers (archaebacterial biomass) in μg/g wet weight. All expressed as average (standard deviation), $n = 3$.

Sample name	Sampling time (h)	Methane production	Acetate turnover	Polar lipid	
				Fatty acids	Ethers
High solids reactor 1					
Before feeding	0	5.75 (1.17)	0.42 (0.02)	75.5 (13.1)	4.0 (0.9)
disturbed	6	5.68 (0.70)	0.37 (0.06)	52.5 (17.4)	2.9 (0.4)
healthy	24	6.26 (0.84)	0.58 (0.10)	89.2 (20.8)	4.9 (1.6)
starved	48	1.77 (0.28)	0.12 (0.01)	87.1 (4.0)	4.4 (0.2)
High solids reactor 2					
Overfed	24	1.92 (0.12)	0.12 (0.04)	53.2 (19.0)	2.4 (0.8)

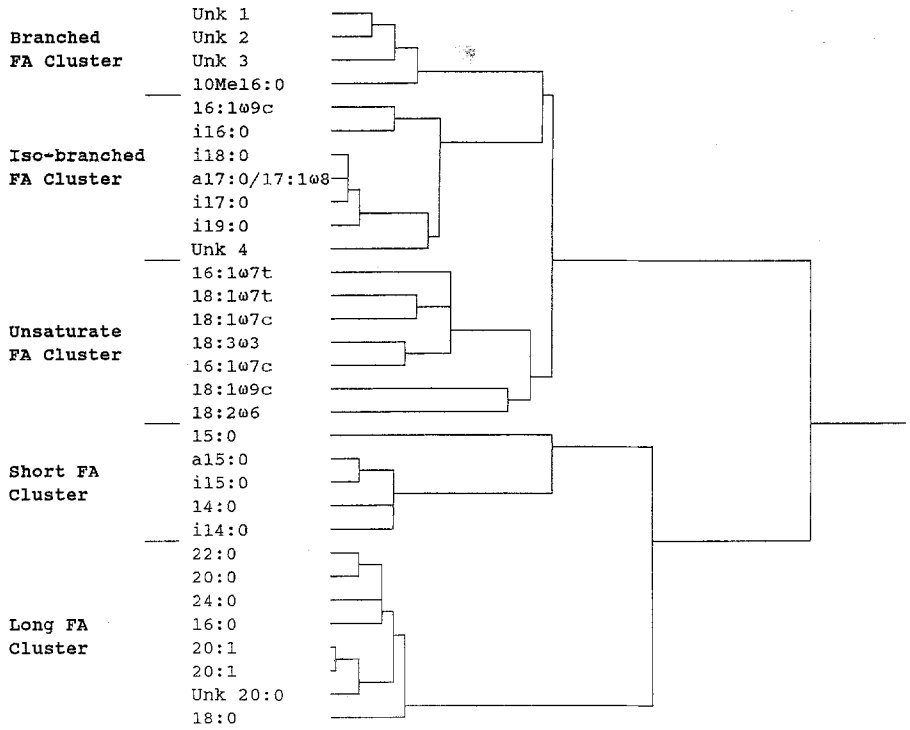

Fig. 1. Fatty acid cluster dendrogram. The distance metric used was 1-Pearson correlation coefficient, complete linkage method. Fatty acid nomenclature is defined in MATERIALS AND METHODS.

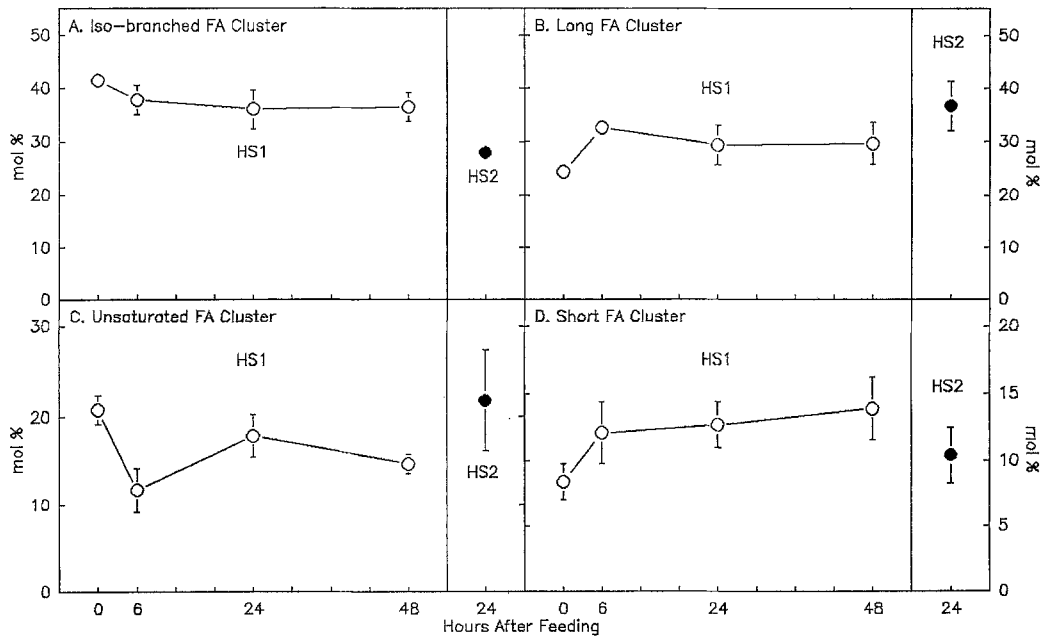

Fig. 2. Shifts in mol percent of polar lipid fatty acids in the Iso-branched, Long, Unsaturated, and Short clusters during a growth cycle of HS1 and compared to HS2. Reactor conditions: HS1 at 6 h, Disturbed; 24 h, Healthy; 48 h, Starved; HS2 at 24 h, Overfed. The fatty acids comprising each cluster are listed in Fig. 1.

TABLE 2

Statistical significance of shifts in measures of activity, biomass, community structure, and metabolic status, relative to the healthy state of HS1

Symbols: ↑↑ = significantly greater than in the Healthy reactor; ↓↓ = significantly less than; ↑ = greater than; ↓ = less than; ≈ = approximately equal to the healthy reactor.

	Difference from the healthy reactor for:		
	Disturbed	Starved	Overfed
Microbial activities			
Methane production	≈	↓↓	↓↓
Acetate turnover	↓↓	↓↓	↓↓
Microbial biomass			
PLFA (eubacteria)	↓↓	≈	↓
PLEth (methanogens)	↓	≈	↓↓
Fatty acid clusters			
Iso	≈	≈	↓↓
Long	≈	≈	↑
Unsaturates	↓↓	↓	↑
Short	≈	≈	↓
Branched	↑↑	↑	↓
Stress measures			
Cy17:0	↑	≈	↑↑
trans/cis 16:1ω7	↑↑	↑↑	↑
trans/cis 18:1ω7	≈	≈	↑↑

tate (16:0), by far the most common component of this group. This group was a stable component of the fatty acids over the time course of HS1, and was slightly greater in HS2 (Fig. 2b). None of the conditions was significantly different for this cluster by analysis of variance.

The Unsaturate cluster consisted of most of the unsaturated fatty acids. This cluster varied over the time course of feeding, decreasing significantly from a maximum of 20.8 ± 1.6 mol% before feeding to 11.7 ± 2.5 mol% 6 h later, and then increasing again (Fig. 2c). The disturbed condition was significantly less than the healthy for this cluster. The difference between the healthy HS1 and the overfed HS2 was not statistically significant.

The Short cluster contained all of the fatty acids containing 14 or 15 carbons: iso- and normal 14:0 and iso-, anteiso-, and normal 15:0. The contribution of the Short cluster to the total fatty acids ranged about 10%, with i15:0 the largest contributor (Fig. 2d). The differences in this cluster were not significant.

The Branched cluster contained 10Me16:0 and three unknown PLFA (Table 2) which could also have mid-chain branching, based upon relative GC retention times. This cluster is a small contributor to the fatty acid pattern, less than 5%, with a very different pattern over the time course of feeding than any of the other groups (Fig. 3a). The only statistically significant contrast was between the disturbed and healthy conditions.

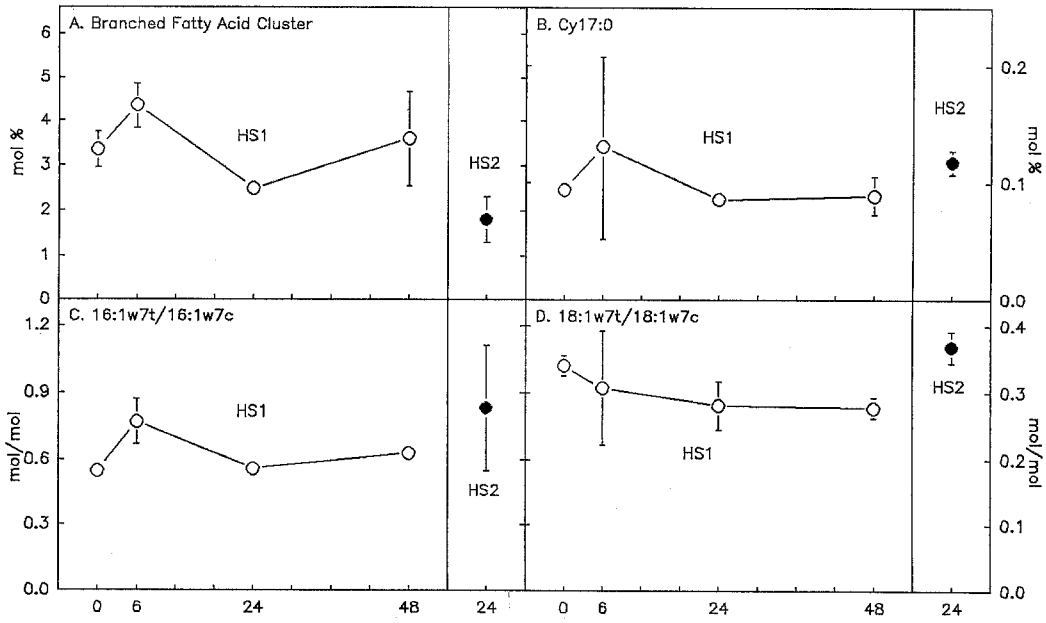

Fig. 3. Shifts in mol% of polar lipid fatty acids in the Branched fatty acid cluster, mol% Cy17:0, and the *trans/cis* ratios for 16:1ω7 and 18:1ω8. Abbreviations are as in Figs. 1 and 2.

There were four fatty acids which joined none of the clusters. The unsaturates 17 : 1ω6 and 18 : 1ω5c were each reported in less than half the samples and in very low quantities. The mol percent of 17 : 0 varied only slightly over all the samples. These will not be considered further. Cy17 : 0 will be discussed under microbial stress markers.

Stress markers

Cyclopropyl fatty acids accumulate in many bacteria in the stationary phase of growth [13] and when environmentally stressed [16]. The mol% of Cy17 : 0 (Fig. 3b) was much more variable in the disturbed samples and was significantly greater in the overfed HS2 than in the healthy HS1.

The ratio of *trans* to *cis* geometrical isomers has been used as a marker for bacterial stress [7]. The *trans/cis* ratio for 16 : 1ω7 (Fig. 3c) was significantly greater for the disturbed and starved conditions than for the healthy state. *Trans/cis* for 18 : 1ω7 (Fig. 3d) was significantly greater in the overfed HS2 than in the healthy HS1.

DISCUSSION

Microbial community structure and PLFA cluster profiles

A PLFA profile is a multivariant description of a viable microbial community's structure and physiological status [24]. Changes in PLFA content due to physiological differences are generally minor compared to those due to microbial population shifts [3,4,7,24]. For the present data set, we generated groups (clusters) of co-varying PLFA based on a multivariant cluster analysis. The PLFA in Fig. 1 are clustered into biosynthetically related groups, similar to a priori groupings and to other cluster analyses in the microbial ecology literature [3,4], indicating that the clustering of PLFA was due to the digesters' microbial community structure rather than an analytical or statistical artifact. The principal difference between the present results and previous work, most of which was of marine sediment microbial communities, is the lack of a separate microeukaryote group. In this work, the eukaryotic polyunsaturated PLFA clustered with the monounsaturated PLFA. The Unsaturated cluster contained only unsaturated PLFA, with 7 of the 10 unsaturates identified. Within it was the 'vaccenic' acid group, typical of eubacteria, containing *cis* and *trans* 16 : 1ω7 and 18 : 1ω7. The other three fatty acids had biosynthetic precursor → product relationships, that is, 18 : 1ω9c → 18 : 2ω6 → 18 : 3ω3, by introduction of successive unsaturations. The polyunsaturated and 'ω9' PLFA are typical of eukaryotes [13]. Also, this digester community included a saturated long-chain cluster not common to marine sediments.

Lipid markers of metabolic status

High values for the lipid measures of metabolic stress (Cy17 : 0, and the *trans/cis* ratios for 16 : 1ω7 and 18 : 1ω7 (Fig. 3b,c,d, respectively) indicate bacteria in a state of unbalanced growth. That is, cell growth was limited by a toxic chemical, inhibitory pH, or the lack of some nutrient. *Trans/cis* 16 : 1ω7 was significantly greater in the disturbed and starved conditions, and Cy17 : 0 and 18 : 1ω7 were significantly greater in the overfed condition than in the healty HS1 (Table 2), indicating that the lipid measures of metabolic stress responded to changes in reactor conditions, and that the response observed depended upon the type of change.

The highly active community – HS1 at 24 h

The healthy reactor condition had the highest biomass (equivalent to the starved condition), the highest methane production, and the highest acetate turnover rate of all conditions (Table 1). It also had the lowest values for the metabolic stress markers. The statistical significance of the shifts in the lipid biomarkers were tested relative to the healthy community (Table 2) in order to delineate the differences caused by the disturbance of feeding, starvation, and overfeeding.

Disturbance due to feeding – HS1 at 6 h

The reactor HS1 was sampled at 6 h after feeding in order to determine the effect of the disturbance of feeding on the microbial community. For the two measures of microbial biomass – PLFA and PLEth – the disturbed sample was the lowest of the four time points of HS1 sampled. This was due to removal of reactor contents and dilution of microbial population with feed. Methane production was not significantly lower than in the healthy condition, but the acetate turnover rate was (Table 2). This indicates that there must have been a larger acetate pool 6 h after feeding than at 24 h, consistent with the high soluble sugar content of the sorghum feed.

The Unsaturated PLFA cluster was significantly less and the Branched cluster was significantly greater than in the healthy community (Table 2). This is either a shift in the lipids produced by the community during the stress of disturbance, or a shift in the population towards opportunistic types.

One of the stress measures – the *trans/cis* ratio for 16 : 1ω7 – was significantly greater in the disturbed than in the healthy community, indicating that the disturbance of feeding did stress a portion of the microbial community. The other two stress measures, Cy17 : 0 and *trans/cis* for 18 : 1ω7, were much more variable for the disturbed sample than for any other sample. This could be an effect of incomplete mixing of the feed with reactor contents and

resulting patchy colonization of the feed material causing local starvation for nutrients.

The starved community – HS1 at 48 h. In the starved condition, HS1 at 48 h after feeding, there was no loss in biomass relative to the healthy digester (Table 2), although the methane production and acetate turnover rates had fallen dramatically. This indicates that the microbes were still present at 48 h after feeding and that the reduction in methane production was due to lower activity. None of the fatty acid clusters were significantly different between the healthy and starved communities, so there was no change in the microbial community composition with starvation, either. The ratio of *trans/cis* 16 : 1ω7 was significantly higher than in the healthy condition but not nearly so high as at the disturbed condition sampling point. The microbial community was more stressed by the disturbance of feeding than by starvation. The variability in the stress ratios was much less than in the disturbed samples: the feed was by now completely colonized, the local regions of stressed microbial populations eliminated.

The overfed reactor – HS2

Overfeeding of HS2 lead to substrate inhibition of the microbial community, accumulation of ammonia from feed, low uptake of ammonia due to inhibition, and further ammonia release upon cell death [11]. The result was toxic levels of ammonia and volatile fatty acids, notably propionate, and portions of the microbial community eliminated.

The biomass measures were all lower in the overfed reactor HS2 than in the healthy HS1 (Table 2), but only PLEth was significantly so, even though the methane production was much less. The Iso-branched fatty acid cluster was significantly less than in the healthy condition, while it was essentially unchanged over the time course of feeding HS1. The Long and Unsaturated clusters were greater, and the Short and Branched clusters were less in the overfed than in the healthy condition. The microbial community structure was very different between the overfed and healthy reactors. The stress markers *trans/cis* 18 : 1ω7 and Cy17 were both significantly greater in the overfed than in the healthy condition, while *trans/cis* for 16 : 1ω7 was not significantly different, but was more variable.

Overview of reactor performance

Differences between the disturbed, healthy, starved, and overfed reactor conditions have been found in the PLFA profiles and the metabolic state markers, as well as indications of the micro-heterogeneity of the reactor contents in the disturbed condition 6 h after feeding but not in the starved condition.

The reactor HS1 presents an example of a microbial community selected to efficiently utilize the iterative batch feeding cycle. The biomass was mechanically removed at feeding, as observed in the lower biomass in the disturbed condition. However, the biomass was not less in the starved condition relative to the healthy. The microbial community was not dying off in the starved condition, but waiting to be fed again.

The overfed reactor HS2 was much less active, had a slightly lower biomass, a different community structure, and higher stress markers than the healthy condition of HS1 (Table 2). How the overfed condition selected for a community that could survive such conditions warrants further study. Further, the overfed reactor was very difficult to bring back to a highly productive condition. Reduced feeding and ammonia supplementation rates, replacement of 30% of the reactor contents with healthy inoculum, and trace mineral supplementation did not restore performance [11,12]. It should be determined whether members of the community were eliminated that were required for rapid fermentation and conversion of products to methane; or whether another community had been selected to survive and maintain the high acetate, propionate, and ammonia concentrations, the low pH and productivity environment.

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REFERENCES

1 Balkwill, D.L., F.R. Leach, J.T. Wilson, J.F. McNabb and D.C. White. 1988. Equivalence of microbial biomass measures based on membrane lipid and cell wall components, adenosine triphosphate, and direct counts in subsurface sediments. Microbial Ecol. 16: 73–84.

2 Bligh, E.G. and W.J. Dyer. 1959. A rapid method of lipid extraction and purification. Can. J. Biochem. Physiol. 35: 911–917.

3 Dobbs, F.C. and J.B. Guckert. 1988. Microbial food resources of the macrofaunal-deposit feeder *Ptychodera bahamensis* (Hemichordata: Enteropneusta). Mar. Ecol. Prog. Series 45: 127–136.

4 Findlay, R.H., M.B. Trexler, J.B. Guckert and D.C. White. 1990. Laboratory study of disturbance in marine sediments: response of a microbial community. Mar. Ecol. Prog. Series 62: 121–133.

5 Frank, J.R. and W.H. Smith. 1987. Ch. 27. Perspectives on biomass research. In: Methane From Biomass: A Systems Approach (Smith, W.H. and Frank, J.R., eds.), Elsevier, Amsterdam.
6 Guckert, J.B., C.P. Antworth, P.D. Nichols and D.C. White. 1985. Phospholipid ester-linked fatty acid profiles as reproducible assays for changes in prokaryotic community structure of estuarine sediments. FEMS Microbiol. Ecol. 31: 147–158.
7 Guckert, J.B., M.A. Hood and D.C. White. 1986. Phospholipid ester-linked fatty acid profile changes during nutrient deprivation of *Vibrio cholerae*: increases in the *trans/cis* ratio and proportions of cyclopropyl fatty acids. Appl. Environ. Microbiol. 52: 794–801.
8 Hedrick, D.H., J.B. Guckert and D.C. White. 1990. Archaebacterial ether lipid diversity: analysis by supercritical fluid chromatography. J. Lipid Res. 32: 659–666.
9 Hedrick, D.B., A. Vass, B. Richards, W.J. Jewell, J.B. Guckert and D.C. White. 1990. Starvation and overfeeding stress on microbial activities in high-solids high-yield methanogenic digesters. Biomass Bioenergy. Submitted.
10 Henson J.M., P.H. Smith and D.C. White. 1985. Examination of thermophilic methane-producing digesters by analysis of bacterial lipids. Appl. Environ. Microbiol. 50: 1428–1433.
11 Jewell, W.J., R.J. Cummings, B.K. Richards and F.G. Herndon. 1990. Engineering design considerations for methane fermentation from energy crops: Fundamental investigations. Annual Report, 1989 – Year 2. Gas Research Institute, Contract Number 6083-226-0848.
12 Jewell, W.J. and B.K. Richards. 1989. Engineering design considerations for methane fermentation of energy crops: Fundamental investigations. Annual Report, 1988 – Year 1. Gas Research Institute, Contract Number 6083-226-0848.
13 Knivett, V.A. and J. Cullen. 1965. Some factors affecting cyclopropane acid formation in *Escherichia coli*. Biochem. J. 96: 771–776.
14 Kotze, J.P., P.G. Thiel and W.H.J. Hattingh. 1969. Anaerobic digestion II. The characterization and control of anaerobic digestion. Water Res. 3: 459–494.
15 Large, P.F. 1983. Methylotrophy and Methanogenesis, pp. 65–82, American Society for Microbiology, Washington, DC.
16 Lepage, C., F. Fayolle, M. Hermann and J.-P. Vandecasteele. 1987. Changes in membrane lipid composition of *Clostridium acetobutylicum* during acetone-butanol fermentation: effects of solvents, growth temperature, and pH. J. Gen. Microbiol. 133: 103–110.
17 Mancuso, C.A., G. Odham, G. Westerdahl, J.N. Reeve and D.C. White. 1985. C_{15}, C_{20}, and C_{25} isoprenoid homologues in glycerol diether phospholipids of methanogenic archaebacteria. J. Lipid Res. 26: 1120–1125.
18 Mikell, A.T. Jr., T.J. Phelps and D.C. White. 1987. Phospholipids to monitor microbial ecology in anaerobic digesters. In: Methane From Biomass: A Systems Approach (W.H. Smith and J.R. Frank, eds.), Elsevier Pub. Co., pp. 413–444, New York, NY.
19 Morrison, R.T. and R.N. Boyd. 1973. Organic Chemistry, 3rd Edn, Allyn and Bacon, Inc., Boston.
20 Schropp, S.J., T.J. Phelps, A.T. Mikell and D.C. White. 1988. The relationship of eubacterial and methanogenic community structure in anaerobic digesters. Proceedings of 10th Annual Conference on Energy from Biomass and Waste, Washington, DC, April 7–10, 1986.
21 Sokal, R.R. and F.J. Rohlf. 1987. Introduction to Biostatistics, pp. 270–279, W.H. Freeman, New York.
22 Tornebene, T.G. and T.A. Langworthy. 1979. Diphytanyl and dibiphytanyl glycerol ether lipids of methanogenic archaebacteria. Science 203: 51–53.
23 White, D.C. 1986. Quantitative physical-chemical characterization of bacterial habitats. In: Bacteria in Nature, Vol. 2 (Poindexter, J. and Leadbetter, E., eds.), pp. 117–203, Plenum Press, New York.
24 White, D.C., R.J. Bobbie, J.D. King, J.S. Nickels and P. Amoe. 1979. Lipid analysis of sediments for microbial biomass and community structure. In: Methodology for Biomass Determinations and Microbial Activities in Sediments, ASTM 673 (Litchfield, C.D. and Seyfried, P.L., eds.), pp. 87–103, American Society for Testing Materials, Washington, DC.
25 White, D.C., W.M. Davis, J.S. Nickels, J.D. King and R.J. Robbie. 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia 40: 51–62.
26 Winer, B.J. 1971. Statistical Principles in Experimental Design, p. 208, McGraw-Hill, New York.