Hydrogen and Carbon Monoxide as Early Warning Indicators of Upsets in Anaerobic Digestion

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ABSTRACT

The role of two novel trace intermediate gases (hydrogen and carbon monoxide) was evaluated as monitoring parameters of the anaerobic methane fermentation process. H_2 and CO were monitored for indiction of toxic upsets in batch serum bottle reactors fed a soluble substrate and for organic overloading in an on-line reactor. Toxicants investigated included heavy metals (Zn, Ni, and Cd) and organic compounds (formadehyde and bromoethanesulfonic acid).

The results of these experiments are compared to earlier experiments with particulate substrates. Results indicate that the trace intermediate gases show potential as early warning indicators of several upset conditions. H_2 and CO used together with the rate of methane production as a single parameter may be useful to indicate the behavior of an anaerobic system under stress.

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1. INTRODUCTION

The advantages of anaerobic digestion technology for the treatment of organic residues are well known among environmental engineers [1]. Significant advances in reactor configurations have been achieved in recent years, broadening the spectrum of applications of the process to municipal wastewaters [2], and xenobiotic substances [3] as well as the traditional applications to wastewater sludges and industrial wastewaters. Nevertheless, the process control strategies available are still the same as those which have long been used for sludge digesters.

The principal causes of process upsets in anaerobic digestion are toxic substances (e.g. heavy metals and some organics) [4] and organic overloads. The posibility of using the trace gases carbon monoxide and hydrogen as indicators of toxic upsets in the anaerobic digestion of a soluble substrate is explored in this paper. Results of batch inhibition studies and organic overloading on anaerobic digestion of a soluble substrate ,sucrose, and a particulate substrate, waste activated sludge (WAS), are compared. More detailed discussion of the results of toxic studies on WAS is presented elsewhere [5] [6]. The heavy metals copper, zinc, nickel and cadmium and the organics formaldehyde and bromoethanesulfonic acid, BES, were tested. Metals and formaldehyde are found as common causes of operational difficulties in anaerobic digestion. BES is a specific inhibitor of methanogenesis [7].

2. BACKGROUND

The good performance of an anaerobic digester depends on the coordinated action of several populations of microorganisms (Figure 1). Thus, one of the most important variables in anaerobic digestion process control is the accurate characterization of the metabolic status of the microorganisms involved. Different parameters have been proposed for the characterization of anaerobic digestion (e.g., volatile fatty acids/ alkalinity ratio, methane and carbon dioxide composition, methane yield, redox potential, DNA content, ATP content, dehydrogenase activity, phosphatase activity, specific cofactors of methanogens such as F-420, etc.); nevertheless, few of these parameters are suitable for on-line real-time process monitoring. Additionally, it is not clear whether the information that these parameters provide under stress situations of the process is a final result of an upset as opposed to a warning of it.

Monitoring of trace gases such as hydrogen and carbon monoxide present several potential advantages as early warning upset indicators. They are slightly soluble gases and so they partition preferentially into the gaseous phase. Hydrogen is present in trace ammounts and its theoretical turnover time (e.g. concentration divided by the rate of consumption-production) is very short and should present a quick response to environmental upsets. Monitoring of the gas phase is suitable to on-line real-time data acquisition; the physical and chemical conditions of the gas phase of the digester are less severe for the sensors than in the liquid phase; and the gas phase gives a better composite picture of the conditions in the reactor [5][6].

Several researchers have proposed hydrogen as a monitoring parameter for anaerobic digesters [8] [9] [10] [11]. There are few studies that look

at the behavior of hydrogen during the occurrence of a toxic upset and/or overload condition in a digester [5] [6] [8] [12] [13]. Hydrogen responses were observed to vary depending upon the toxicant added, organic or inorganic. It was not clear whether or not hydrogen could give a distinct response from natural variations and an inhibitory situation where less than total inhibition ocurred [5][6].

Carbon monoxide was recently proposed as a monitoring parameter by Hickey [14]. Carbon monoxide behavior in anaerobic digesters treating WAS was investigated. Under non toxic situations carbon monoxide was found to be directly related to acetate concentrations during an organic overload [15]. Under toxic situations carbon monoxide was observed to be a more predictable process indicator than hydrogen [6].

Potential sources and sinks of hydrogen and carbon monoxide in anaerobic digestion are presented in Figure 1. A recent review of the role of hydrogen in anaerobic digestion was presented by Harper and Pohland [16]. Hydrogen can be produced by the acid forming bacteria during the fermentation of carbohydrates or proteins, and by the acetogenic bacteria during the degradation of intermediate compounds such as organic acids and alcohols. Hydrogen is consumed by hydrogen utilizing methanogens principally when the sulfate concentration in the environment is low. Thus, it is expected that hydrogen will be present at high levels in situations of organic overload or in toxic events that specifically inhibit the hydrogen consuming methanogens. On the other hand, a bound carbon monoxide moiety is an intracellular intermediate in the recently proposed Acetyl-CoA pathway for autotrophic growth [17]. Several microorganisms present in anaerobic digestion ecosystems use this pathway as shown in Figure 1. Of these microorganisms, some sulfate reducers and the acetoclastic methanogens use

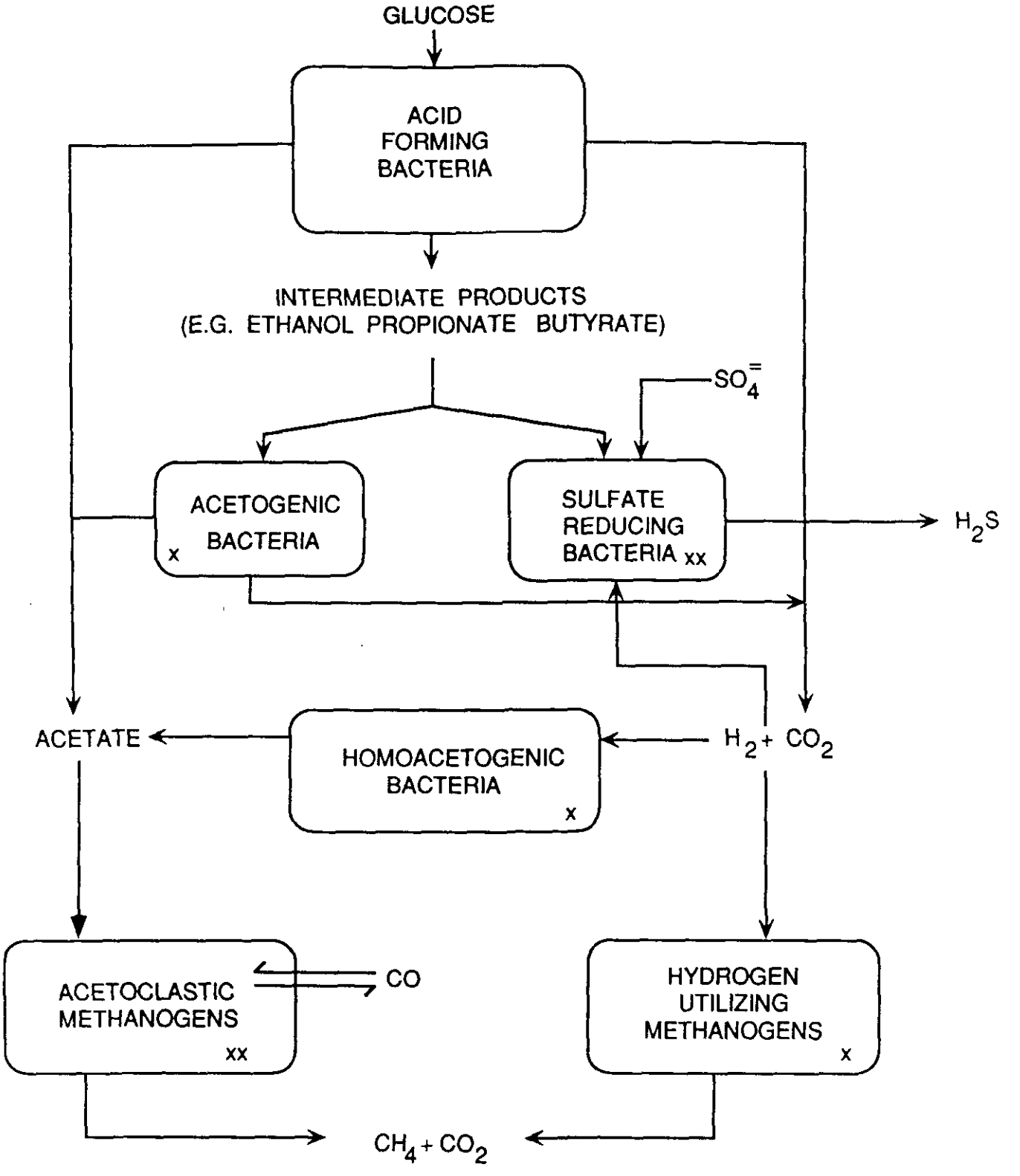


Figure 1.

DIFFERENT MICROBIAL POPULATIONS IN AN ANAEROBIC DIGESTER. BACTERIAL SPECIES MARKED WITH X USE ACETYL-CoA PATHWAY IN AN ANABOLIC WAY. THOSE MARKED WITH XX USE THE PATHWAY IN A CATABOLIC WAY

the pathway in a catabolic way. This means that the levels of carbon monoxide dehydrogenase, the key enzyme of the pathway, are higher in these two groups than in the other groups indicated in Figure 1 [18][19]. In the anaerobic ecosystems reported in this paper, the sulfate concentration was negligible, and since approximately 70% of the electron and carbon flow to methane goes through the acetoclastic methanogens [20], it is probable that the main pool of carbon monoxide dehydrogenase existed in the acetoclastic methanogens. Thus, the principal source of carbon monoxide in the experiments reported here are most likely the acetoclastic methanogens as proposed originally by Hickey [14].

3. METHODOLOGY

A. Toxicity Studies

Behavior of trace gases was tested using serum bottle assays developed by Miller and Wolin [21]. The protocol of the assays is based on Hickey's modification [14] of the anaerobic toxicity assay, ATA, developed by Owen et al. [22].

Two different set of assays were done. One used sucrose as substrate and the other one used acetate as substrate. During the acetate fed assays it was expected that the major active population were the acetoclastic methanogenic.

Inoculum for both kind of toxicity assays was provided by a sucrose-fed fill and draw digester. The digester was operated with a solids retention time of 20 days and a temperature of 35^{o} C. This reactor was brought to steady-state prior to the initiation of the serum bottle assays. The synthetic feed used sucrose as carbon and electron source and nutrient and buffer salts. The composition of the synthetic feed is reported elsewhere [23][24]. The daily mixed-liquor waste from the reserve digester was anaerobically transferred into a sealed bottle with a 70% N_2, 30% CO_2 atmosphere and resarzurin indicator to detect the presence of oxygen. Serum bottles were gased out with the gas mixture for several minutes with the aid of a gas manifold. Inoculum from the digester was then transferred to the serum bottle using a repipeter. Serum bottles were sealed with rubber septa and aluminum seals and transferred to a 35^{o} C incubator for thermal equilibration. Serum bottles were fed with sucrose or acetate as electron and carbon source, and the corresponding nutrient salts and buffer. After 15 minutes headspace pressure of the serum bottles was equilibrated to atmospheric with a lubricated glass syringe. Gas space was tested for

content of CO_2, CH_4, CO and H_2 and these values taken as starting concentrations. The bottles were then spiked with the desired ammount of toxicant (all metals were added as the chloride salt) from concentrated stock soultions and incubated at 35°C with constant mixing using a pulley driven mixer to avoid heating effects. It was assumed that hydrogen and carbon monoxide in the gaseous phase were in equilibrium with the concentrations in the liquid phase. Several pieces of evidence, not presented here, suggested that this was the case [23]. The concentration of the above mentioned gases in the headspace of the serum bottles was measured and the amount of gas produced was recorded at 2, 5, 9, 24, 48 hours after the initiation of the assays. At the conclusion of the assay pH was measured. For a given toxicant four different dosages were tested. All the assays for a selected dosage were done in duplicate and the controls in triplicate. Methane and carbon dioxide were measured by gas chromatography using a thermal conductivity detector. Hydrogen and carbon monoxide were measured by gas chromatography using a mercury reducing detector [23] [24].

B. Organic Overload Study

The organic overload study was conducted in a continuous on-line system which was developed by Hickey (14). A schematic of the test digester used for the continuous on-line monitoring is presented in Figure 2. The test digester is connected to a sampling control and data capture system which allows continuous sampling of the total gas production and head space composition.

The test digesters are monitored in an on-line mode. Methane and carbon monoxide are continuously analyzed via a dual cell infra red detector (ADC Limited). Hydrogen and carbon monoxide are determined with a mercury

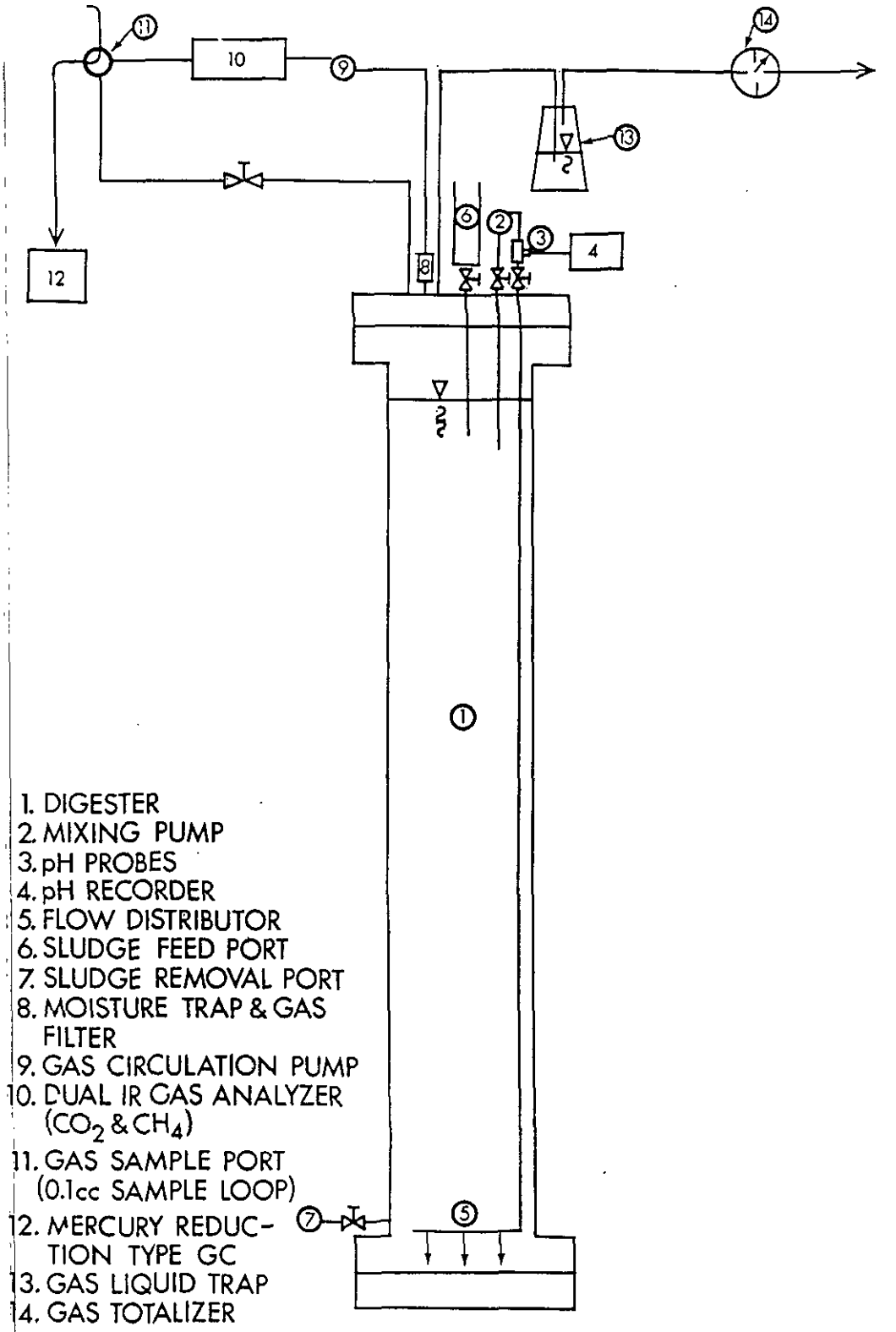


Figure 2 Schematic representation of the 5 liter on-line test digester.

reduction chromatographic system equipped with a 10 port auto sampling valve (Valco). A column packed with Spherocarb was used to separate hydrogen and CO and retain methane and heavier gases. These gases are subsequently backflushed from the system prior to the next sample time .

Gas production is continuously tracked using a liquid displacement technique. The weight of acidified salt solution displaced is monitored with an Ohaus Port-o-gram balance equipped with RS-232 computer interface.

All gas sampling equipment is interfaced with an Apple II+ computer and Isaac 91A data acquisition system. Sampling operations are controlled by the Apple-Isaac system.

For these organic overloading experiments, a 10 day HRT test digester was operated in a semi-continuous mode (batch daily feeding) for approximately three weeks after transfering of inoculum from the sucrose reserve reactor. During this period the feed sucrose concentration was maintained at 900 mg/l. Mineral salts and vitamins were also added as has been reported elsewhere (23,24). Daily monitoring of the digester was initiated 1 -2 weeks prior to the beginning of the organic overloading portion of the experiment. During this period a rather constant operation was observed. After collection of baseline information, the real-time data aquisition system was used to investigate the response of this digester to a series of continuous organic overloads (2.5, 4.0, and 6.0 times the baseline concentration of sucrose at the same HRT). Two sets of overloading experiments were performed. The first was done with sufficient alkalinity in the reactor and the second set was done with no pH control.

To begin the overload, the digester feed was switched to a more concentrated influent (2.5, 4, and 6 X), but the reactor was maintained at the same 10 day HRT.

Immediately before feeding the reactor a 50 ml sample was taken for analysis of volatile suspended solids (VSS), pH, chemical oxygen demand (COD), alkalinity and volatile fatty acids (VFA). After the reactor was fed 0.5 ml samples were taken at defined intervals during each daily batch. These samples were centrifuged and the supernatant immediately analyzed for individual volatile fatty acids by gas chromatography. Details of this analysis has been reported elsewhere (14).

4. RESULTS

A. Toxicity Studies

The response patterns of hydrogen and carbon monoxide were similar for all the metals tested in this study. A similar observation was made for the studies with WAS [6]. In this report data for cadmium are presented as typical. Cumulative methane production, expressed as percent of control, as a function of cadmium dosage and time for the sucrose assay is presented in Figure 3. It can be seen that inhibition in methane production develops with time and increases with applied dosage. No apparent recovery in methane production was observed for cadmium inhibited samples. For other metals such as copper, recover was observed.

The behavior of hydrogen concentration in the headspace as a function of cadmium dosage and time, using sucrose as substrate, is shown in Figure 4. Two different responses can be distinguished. Cadmium dosages of 4 and 8 mg/l do not produce a significant variation in the behavior of hydrogen with respect to the controls. Higher dosages induced a definite response; hydrogen accumulates in the headspace with time to levels way above the control by 24 hours. This dual pattern may reflect an important characteristic of the behavior of the microbial ecosystem in an anaerobic digester under toxic stress generated by heavy metals.

Results for carbon monoxide are presented in Figure 5. Again, a dual pattern is observed here. At low cadmium dosages carbon monoxide was consistently higher than the control, whereas at high dosages carbon monoxide accumulation was less than the controls.

BES is a specific inhibitor of methanogens [7]. Thus it can be used as a reference to illustrate the behavior of hydrogen when the hydrogen consuming methanogens are inhibited. The effect of BES dosage on methane

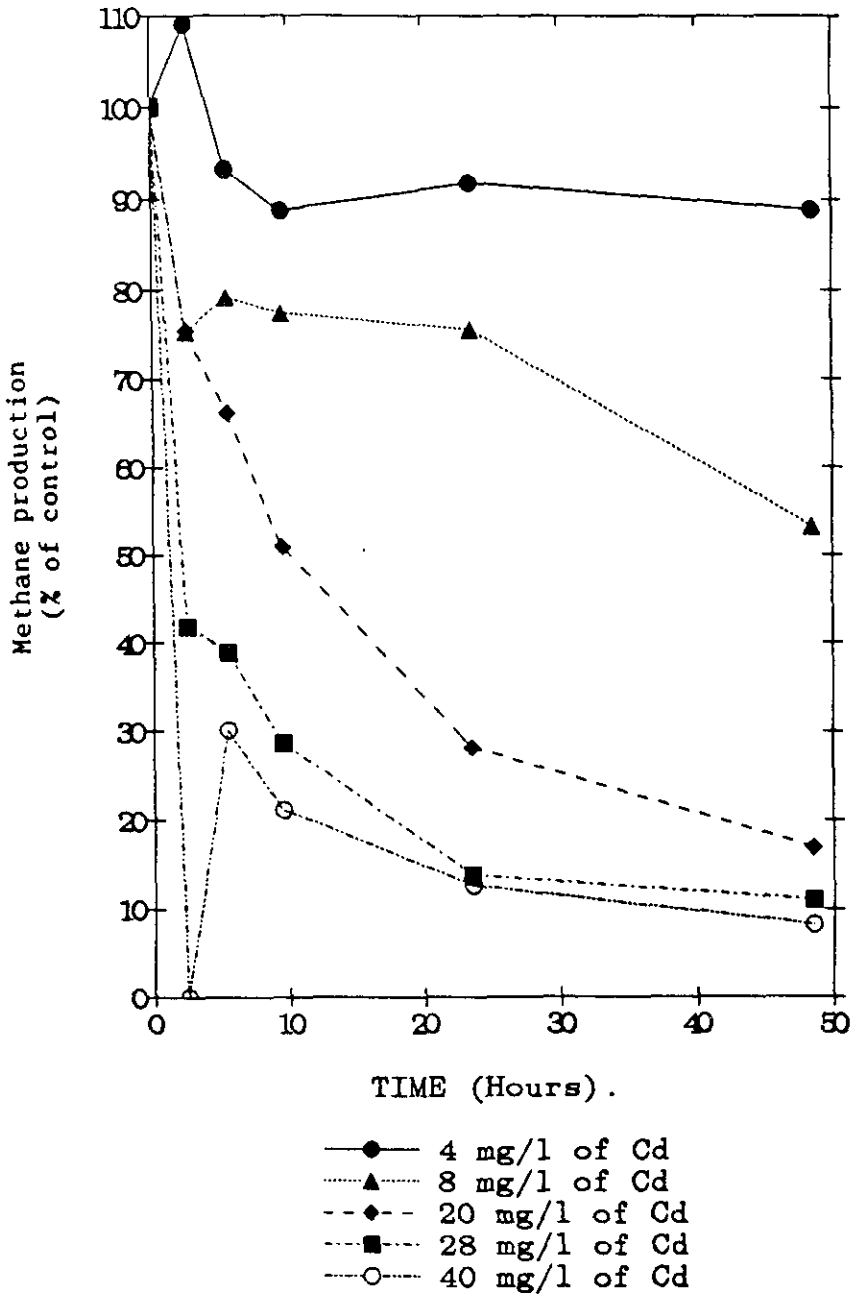


Figure 3 Effect of cadmium (Cd^{+2}) on the cumulative methane production as percent of control. Sucrose as electron donor.

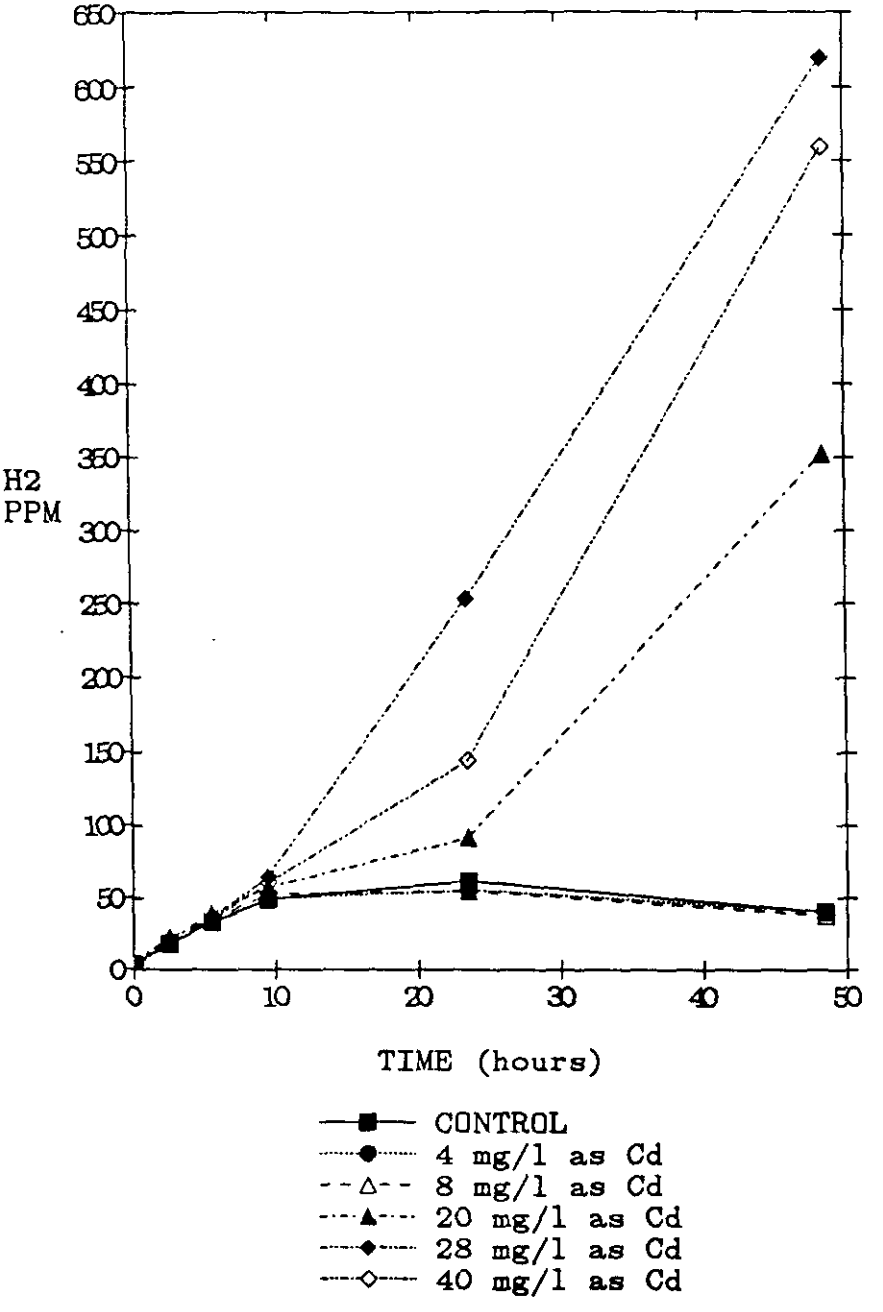


Figure 4 Effect of cadmium dosage on the headspace concentration of hydrogen over time. Sucrose as electron donor.

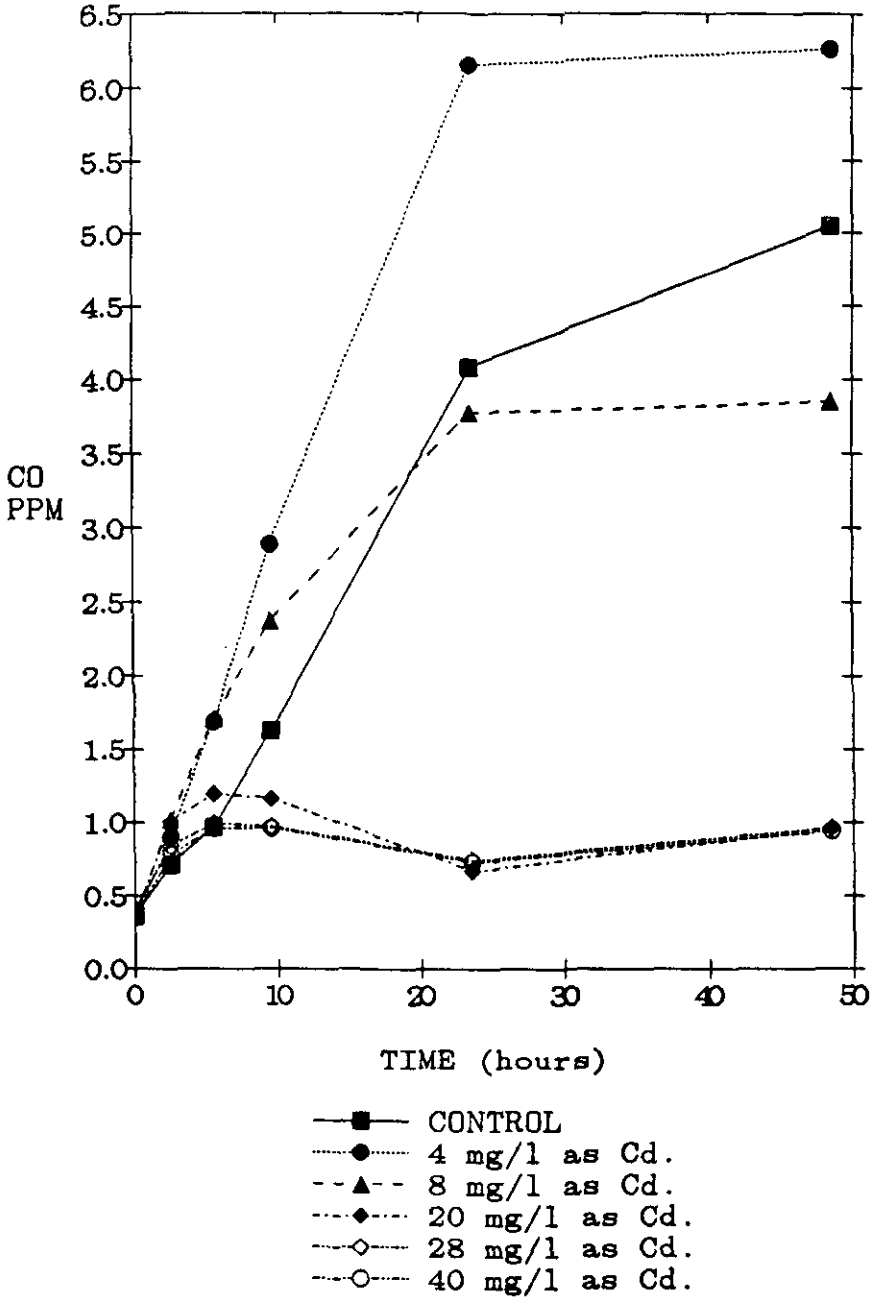


Figure 5 Effect of cadmium dosage on the headspace concentration of carbon monoxide over time. Sucrose as electron donor.

production, expresed as percent of control, is presented in Figure 6. It can be seen that increasing BES dosages cause increasing inhibition of methane production. The variation of hydrogen, expressed as percent of control, and as function of percent of methane production is presented in Figure 7 for the sucrose run and in Figure 8 for the acetate run when BES is the toxicant. For all levels of inhibition of methanogenesis, hydrogen accumulates in the headspace of the bottle. When acetate was used as the substrate, and as a result the main active population is the methanogenic acetoclastic, accumulation of hydrogen in the headspace is significantly less (Figure 7). These observations are expected from the ecosystem structure presented in Figure 1. If hydrogen consuming methanogens are selectively inhibited hydrogen should accumulate in the system. When there is little or no activity of hydrogen producing populations the accumulation of hydrogen on the system due to inhibition of methanogenesis should be less.

Data for hydrogen in the sucrose assay are presented as percent of control in Figure 9 for cadmium and in Figure 10 for zinc. From a comparison of the results for BES in Figure 7, and Cd in Figure 9, it can be seen that hydrogen does not accumulate in the gas phase when cadmium is added as it accumulates when methanogenesis is inhibited by BES. This pattern is better observed in Figure 10 for zinc. In this case hydrogen was below control values for all levels of methane production inhibition. This observation suggests that other populations different from the methanogens are more severe inhibited by cadmium and zinc (e.g., hydrogen producing populations). Similar behavior is observed for other metals. Based on the above observations the dual trend observed in Figures 4 and 5 can now be explained

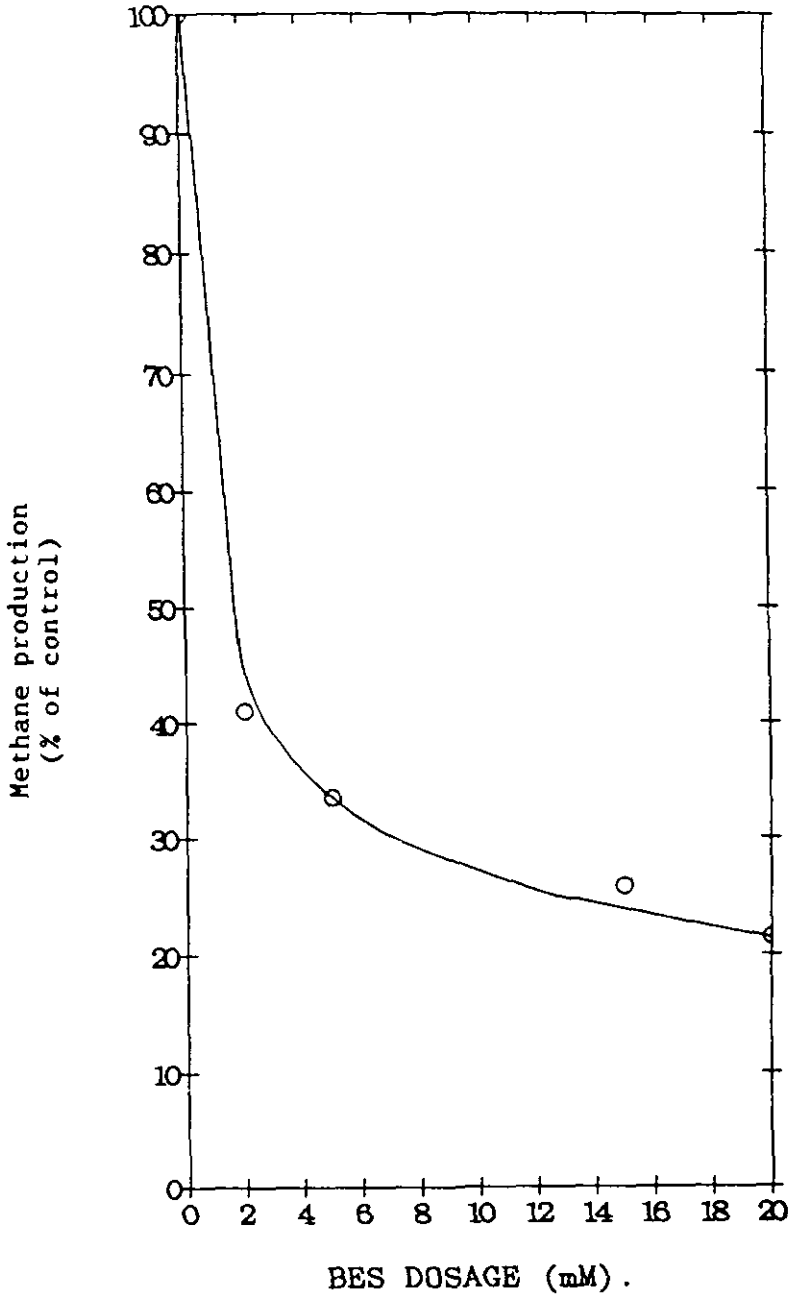


Figure 6 Effect of BES dosage on cumulative methane production as percent of control at t=48 hours. Sucrose as electron donor.

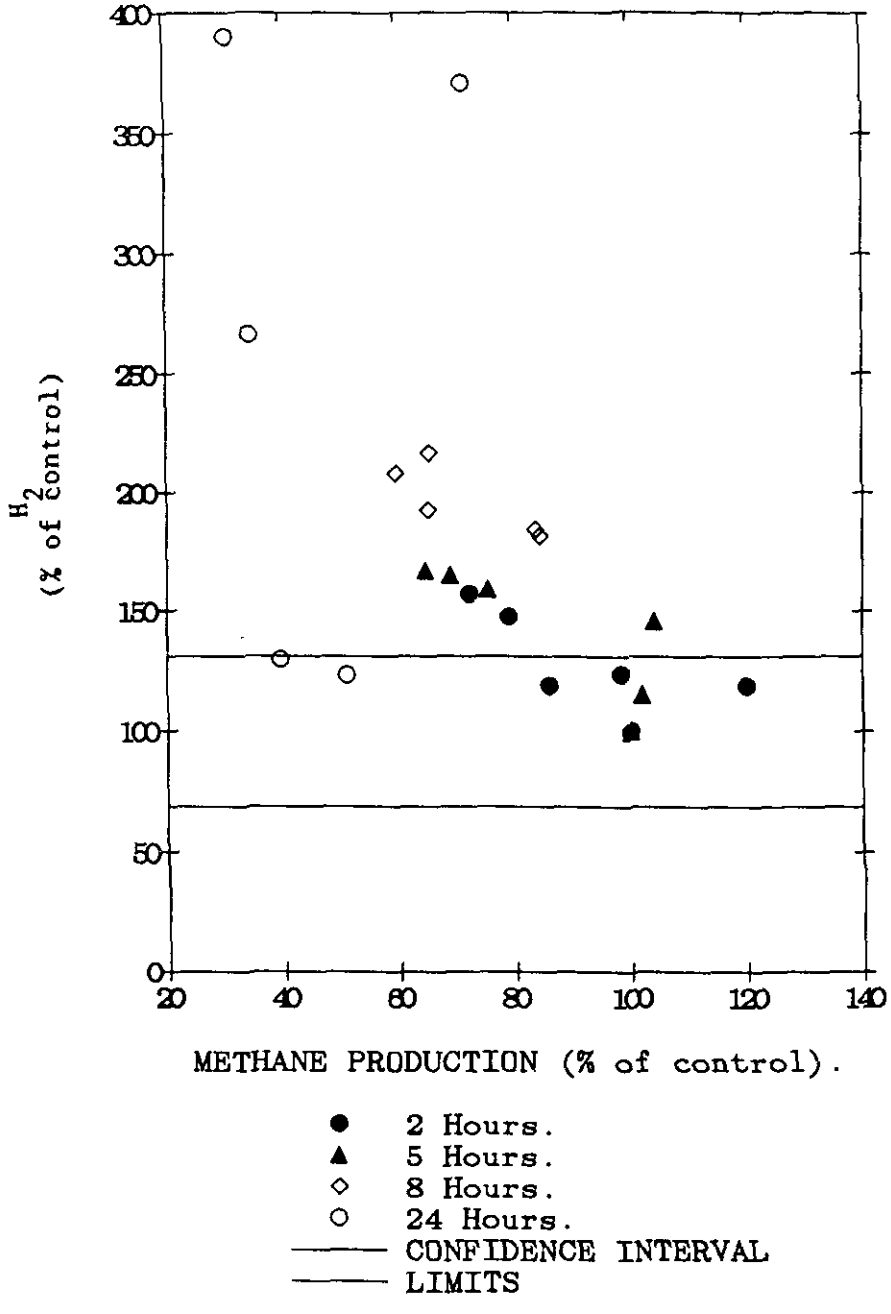


Figure 7 Comparison of hydrogen headspace concentration and cumulative methane production for BES inhibited samples. Sucrose as electron donor.

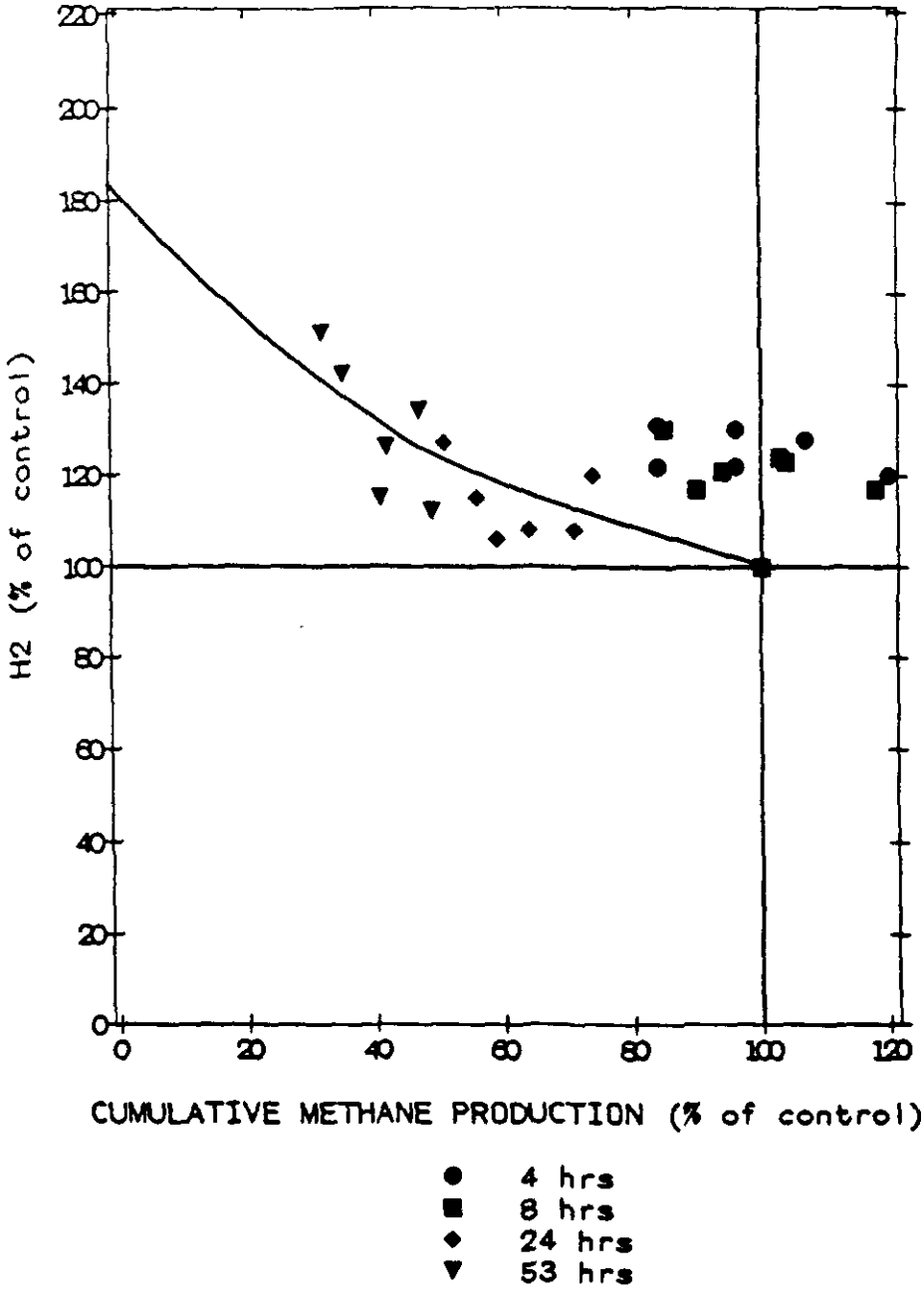


Figure 8 Comparison of hydrogen headspace concentration and cumulative methane production for cadmium inhibited samples. Acetate as electron donor.

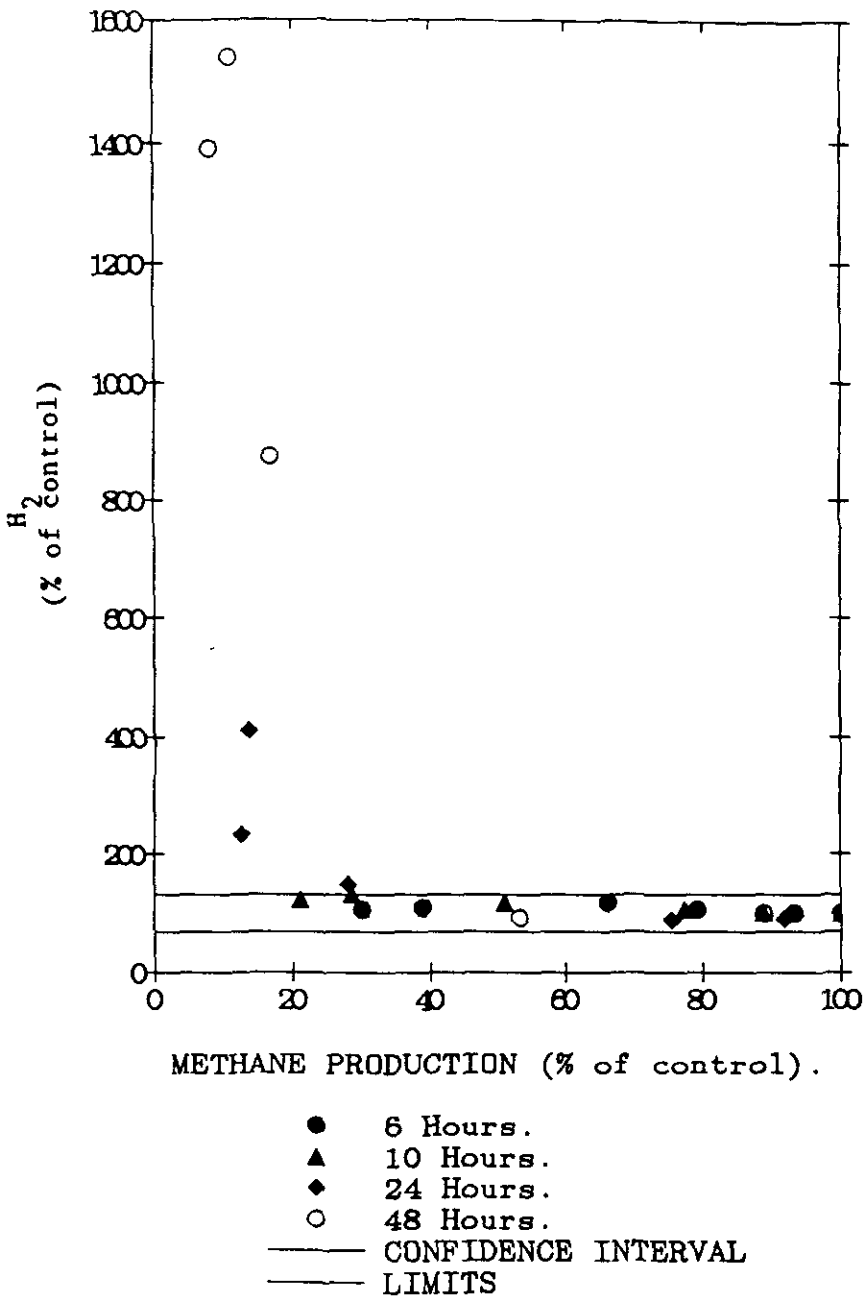


Figure 9 Comparison of hydrogen headspace concentration and cumulative *methane production for cadmium inhibited samples.* Sucrose as electron donor.

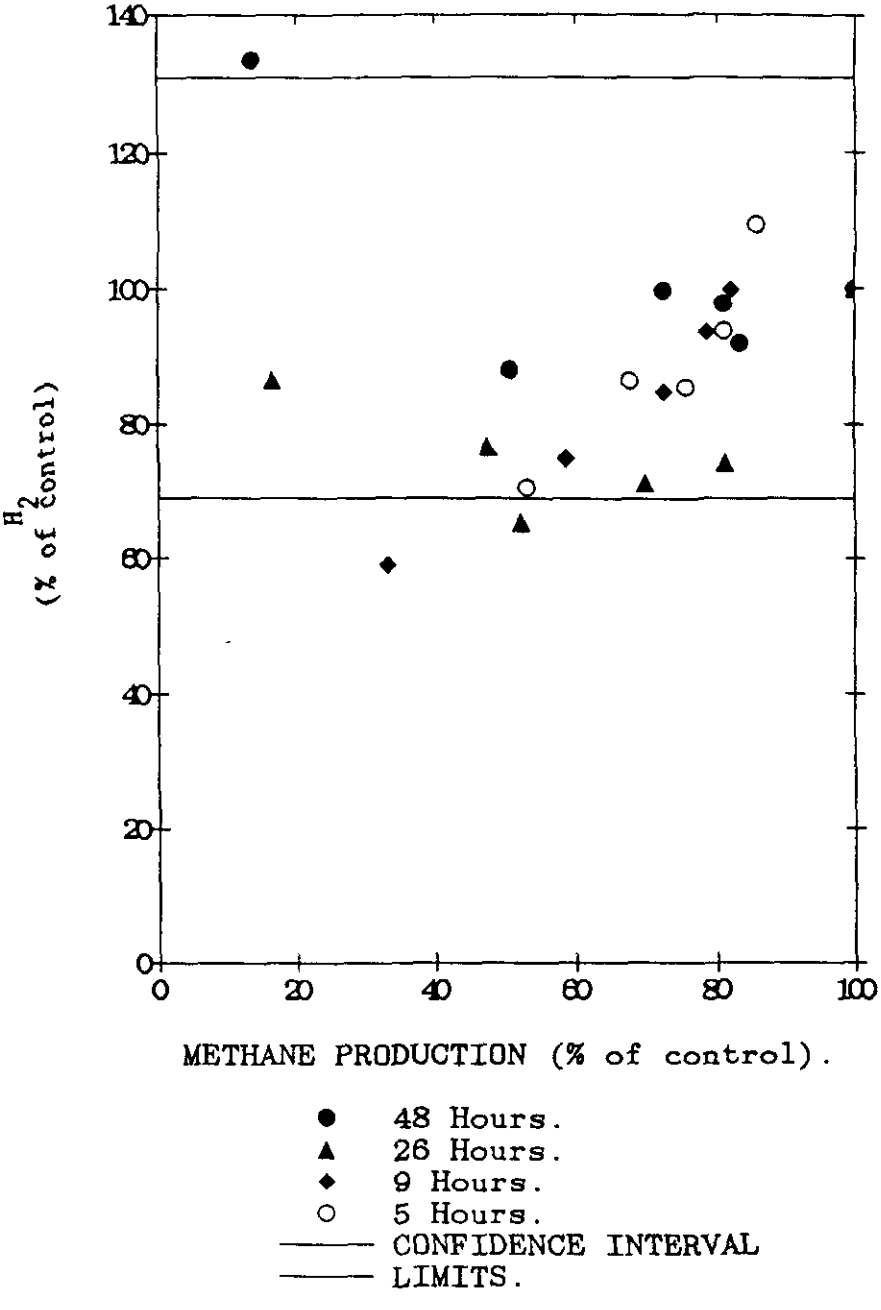


Figure 10 Comparison of hydrogen headspace concentration and cumulative methane production for zinc inhibited samples. Sucrose as electron donor.

as a specific inhibition phenomenon. At low metal dosages some non-methanogenic populations are first inhibited and the hydrogen headspace concentration follows control values. At higher cadmium dosages methanogenic populations are also significantly affected and changes of hydrogen and carbon monoxide with respect to control are observed. Similar results were obtained for the WAS as reported elsewhere [6].

Comparison of toxic dosages required to inhibit methane production for sucrose, waste activated sludge and acetate serum bottles assays is presented in Table 1. These results agree with the previous observations. Dosages of metals necessary to get 50% inhibition of methane production are in general higher for the acetate system. This situation means that for the same dosages of a metal, non-methanogenic populations are more inhibited than the methanogenic population. These results are in contradiction with the general belief that methanogenic populations are more suceptible to toxicants than the other populations of microorganisms in an anaerobic digester.

The specific inhibition phenomena such as the one observed here have important effects on the type of parameters that should be used to monitor toxic upsets in anaerobic digesters. Typical parameters such as methane yield, volatile fatty acids or alkalinity may show toxic stress in a digester when the situation is well on the way to complete inhibition. Preliminary results with a mathematical model that simulates specific inhibition suggest that this is the case [23].

B. Organic Overload Study

Data for the organic overloading experiments is presented in Figures 11 through 20. Figures 11 -15 present data for the first set on experiments

TABLE 1. COMPARISON OF TOXICANT DOSAGES CAUSING 50% INHIBITION OF METHANE PRODUCTION AFTER 24 HOURS.

DIGESTER SYSTEM	Cu mg/gVSS	Cd mg/gVSS	Ni mg/gVSS	Zn mg/gVSS	BES mM	HCHO mg/l
ACETATE	10[1]	20	100	350[2]	2.0[3]	35
SUCROSE	10	9	380	150	1.0[2]	30
WASTE ACT.[4] SLUDGE	15	28	-	70	1.8	40

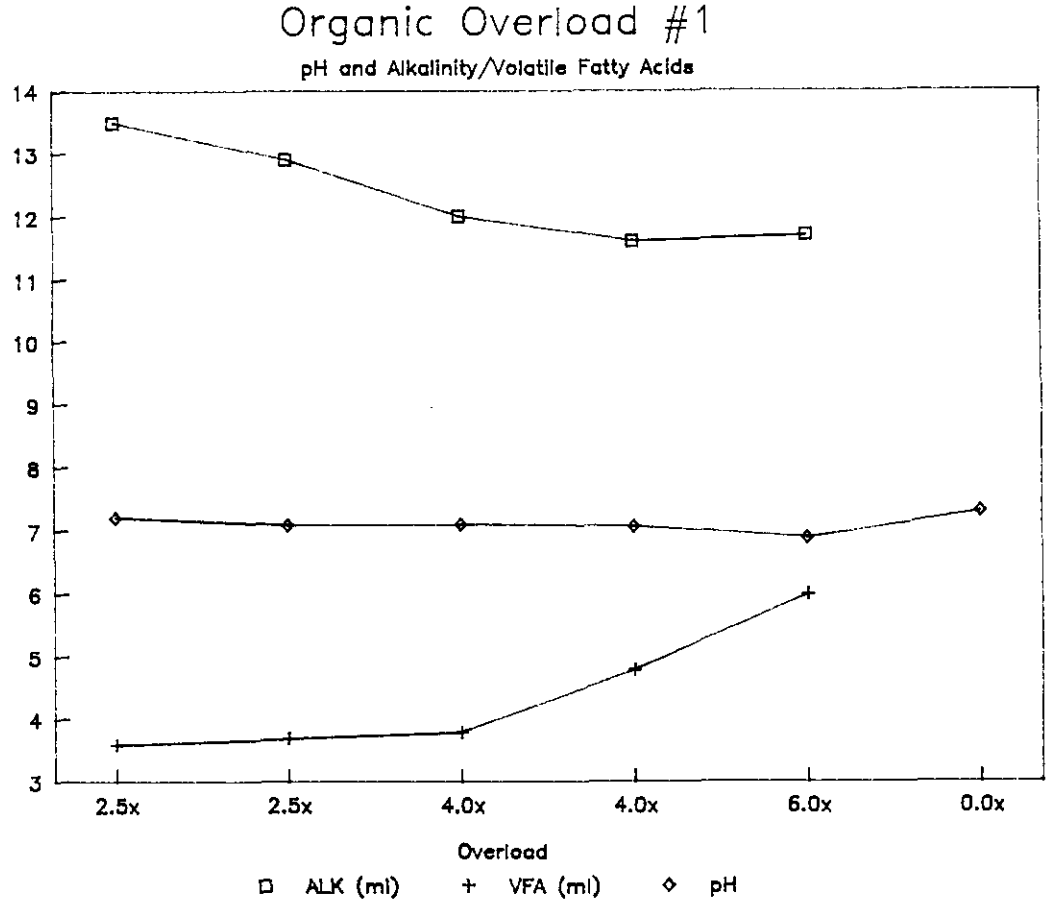
(1) Time= 23 hours.
(2) Time= 48 hours.
(3) Time= 53 hours.
(4) Data from Hickey [8].

(alkalinity added for pH control and Figures 16 -20 present data for the second set of experiments (no pH control).

Results obtained for alkalinity, pH, and VFA for the first set of experiments are presented in Figure 11. pH remained essentially unchanged through the experiment, while VFA accumulated and alkalinity was consumed with increasing loading of the reactor. Individual VFA daily averages are presented in Figure 12. After the initial accumulation of propionic and acetic acids from the baseline behavior, these acids show slow accumulation, indicating that although the reactor was under stress it was coping with the extra load. It is interesting to notice too, the appearence of valeric and isovaleric acids as the overload develops. Gas daily averages are presented in Figure 13. Hydrogen shows an increassing concentration through the overload, much in the same way as the increase in the propionic acid. At the end of the overload the reactor was not fed for one day, and the hydrogen concentration drops in a similar way to the propionic acid concentration. Carbon monoxide shows a more complicated pattern. After the initial overload of 2.5x, carbon monoxide shows a stable pattern of behavior much in the same way as acetate does. At this point carbon monoxide averages exhibit a declining trend with increasing loading. It is worth noting that acetate is accumulating during the same period. When the reactor is left idle at the end of the overload, acetate returns to baseline levels and CO exhibit a sharp decline in the concentration. Methane and carbon dioxide daily averages concentrations do not exhibit significant variation through the overload.

Total and differential gas production are plotted in Figure 14. It can be seen that differential gas production exhibit a quick response after the daily feed, generating a characteristic curve. Total gas production

Figure 11. pH, alkalinity, and VFA data (overload #1).



Alkalinity and VFA are expressed in relative units.

Figure 12. Individual VFA daily averages (overload #1).

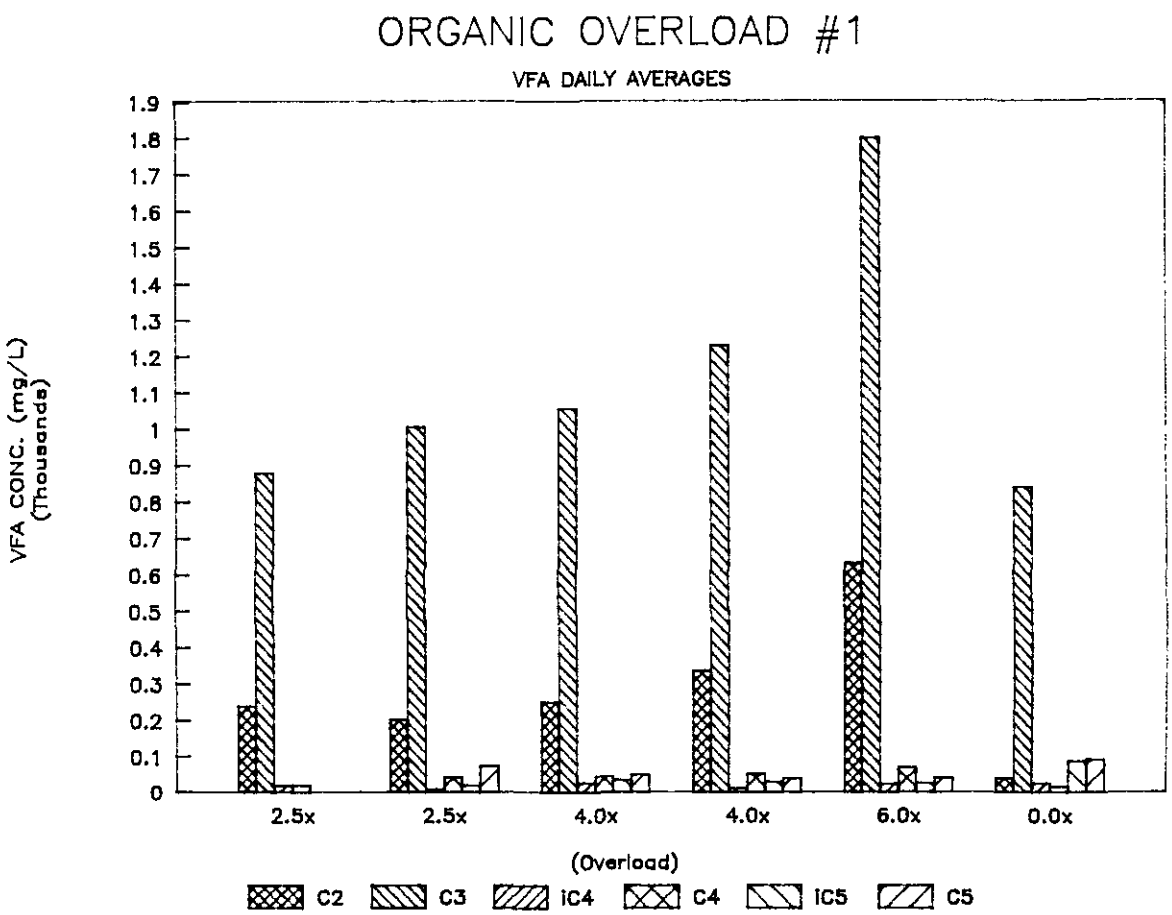


Figure 13. Gas daily averages (overload #1).

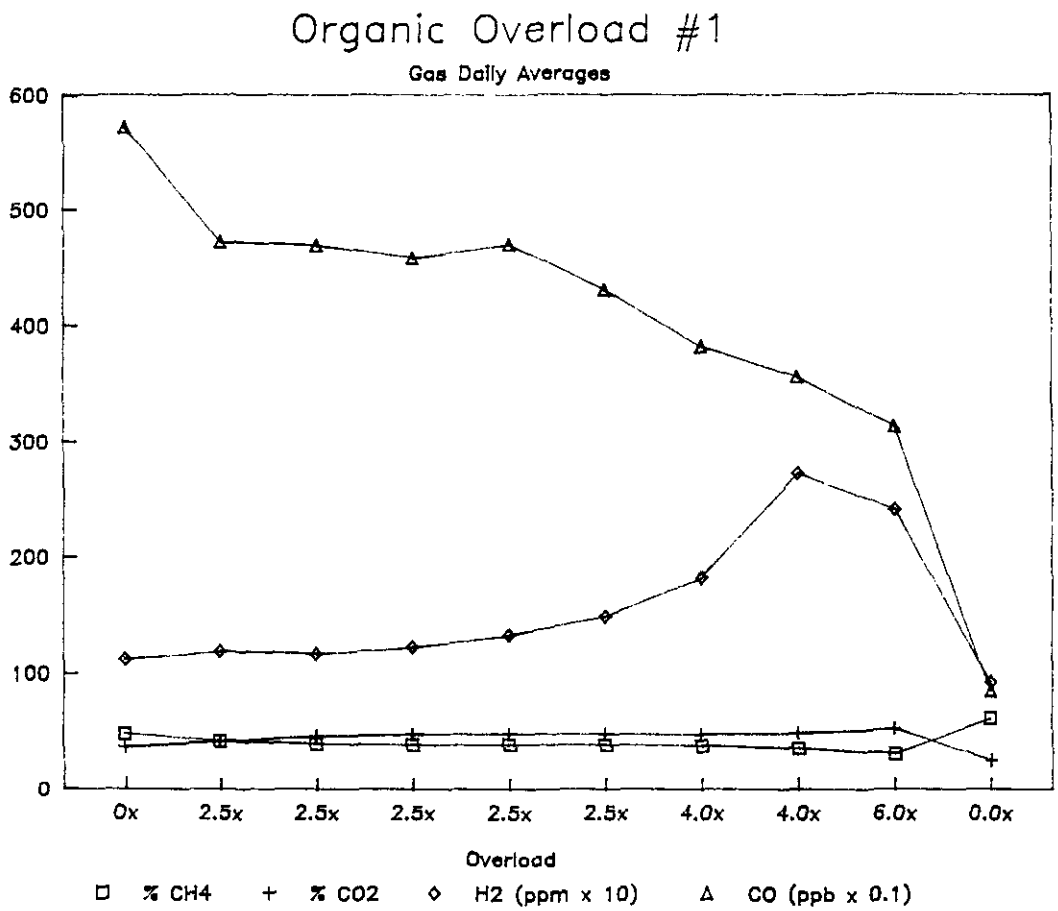
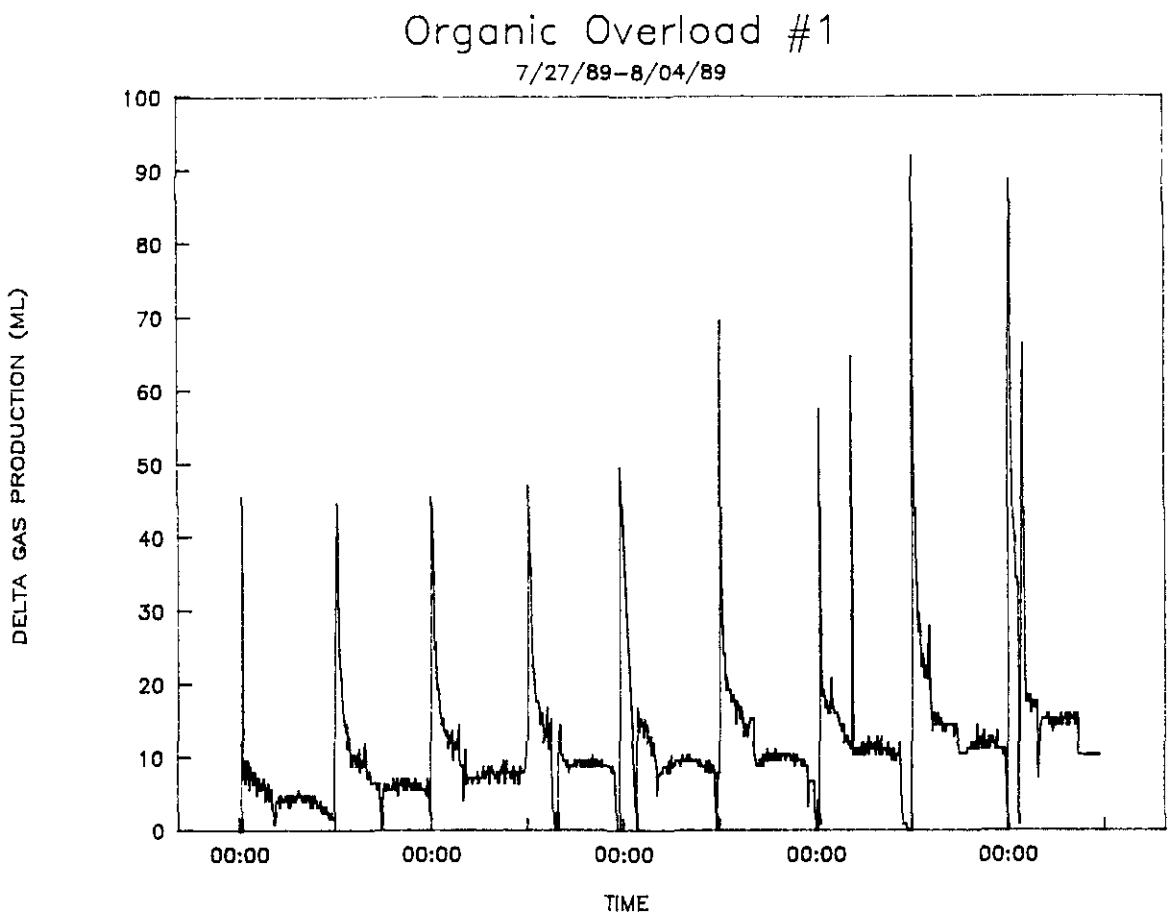
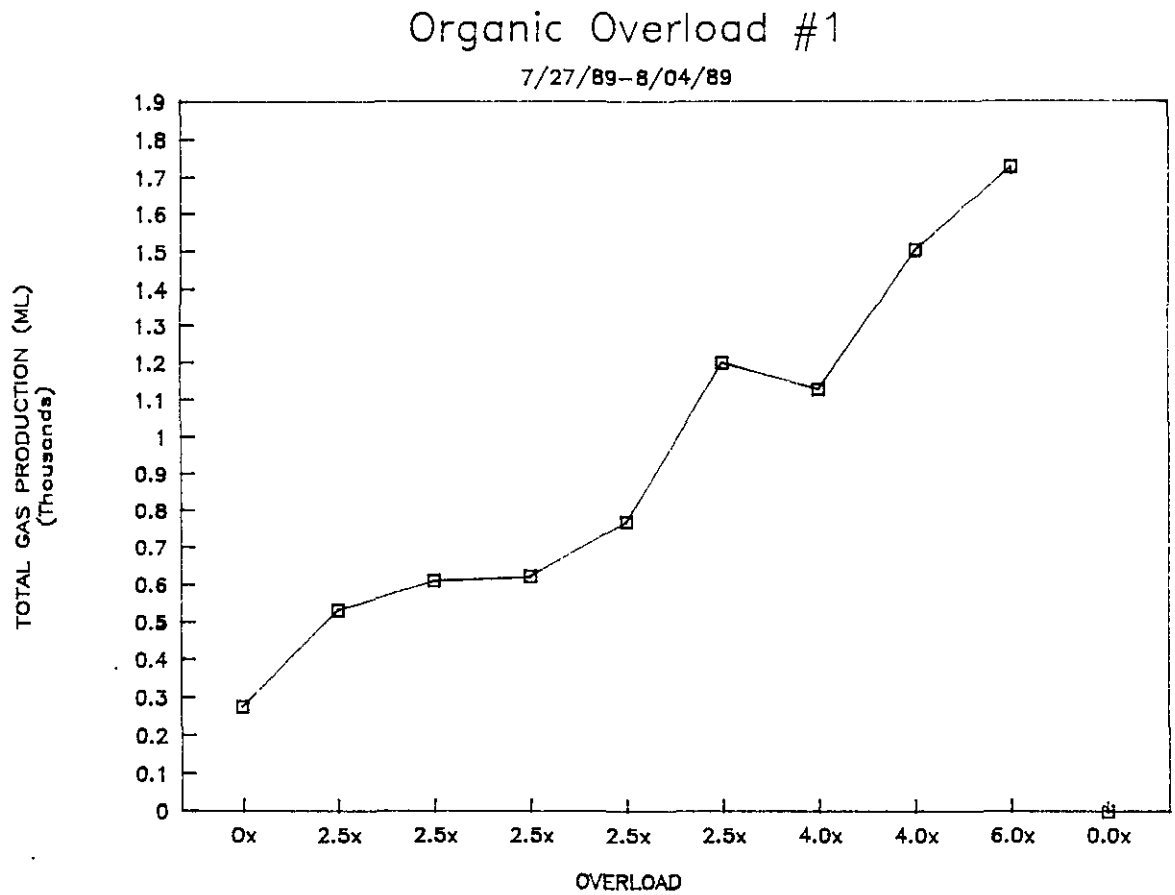


Figure 14. Total and differential gas production (overload #1).


Organic Overload #1
7/27/89-8/04/89
DELTA GAS PRODUCTION (ML)
100
90
80
70
60
50
40
30
20
10
0
00:00
00:00
00:00
00:00
00:00
TIME

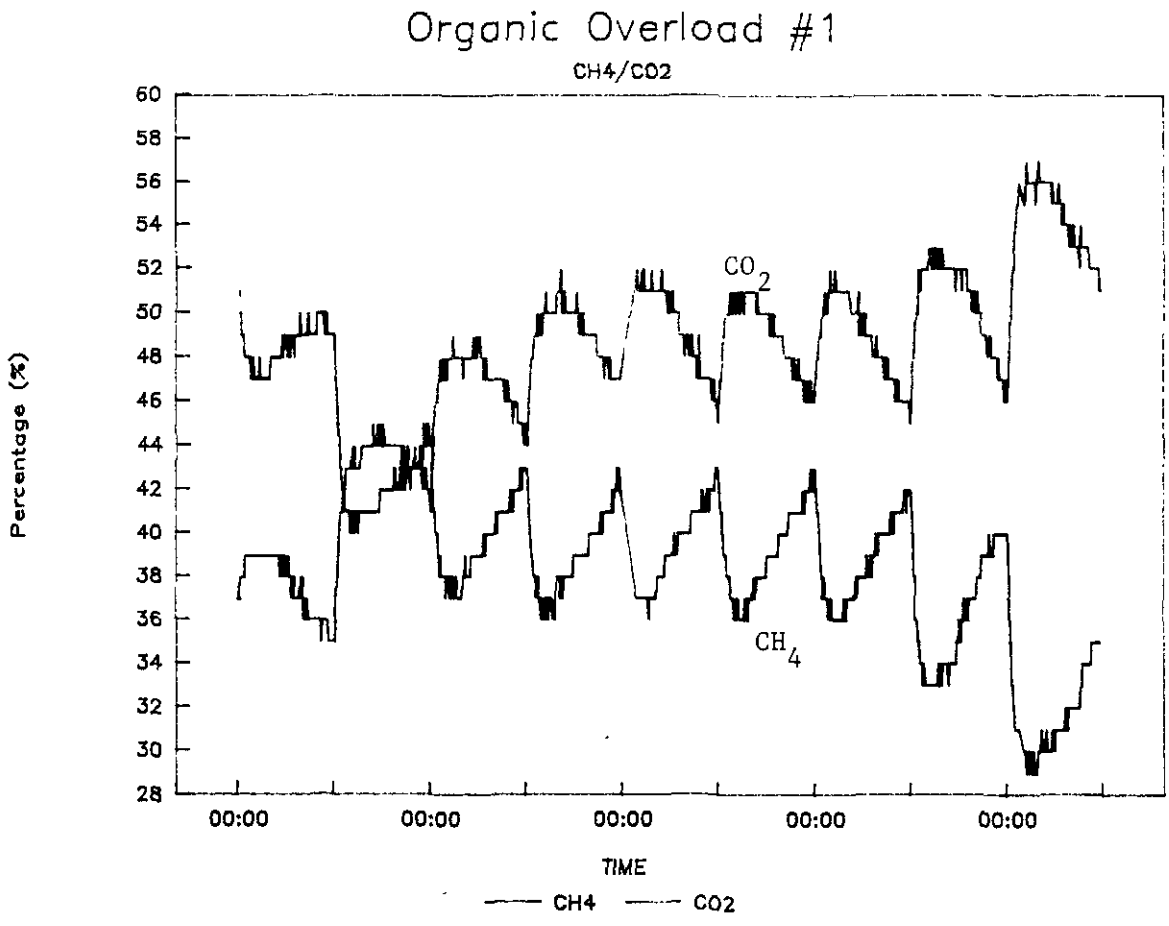

Organic Overload #1
7/27/89-8/04/89
TOTAL GAS PRODUCTION (ML)
(Thousands)
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0x
2.5x
2.5x
2.5x
2.5x
2.5x
4.0x
4.0x
6.0x
0.0x
OVERLOAD

increases consequently with organic loading indicating that the microorganisms never really reached an absolute inhibition situation, in spite of the significant acid accumulation. On-line methane, hydrogen, carbon monoxide and carbon dioxide data are presented in Figure 15. Methane and carbon dioxide composition exhibit a typical pattern of behavior. The concentration of one seems to be the mirror image of the other. It is important to notice that after the initial increase in load, carbon dioxide concentration becomes higher than methane concentration; but the concentrations return to the same level that existed the previous day at the end of the cycle. Similarly, this behavior demonstrates the capacity of the system to cope with the organic overload.

Results from the second set of experiments, without pH control, are presented in Figures 16 to 20. Results from pH, alkalinity and VFA measurements are plotted in Figure 16. pH, at the end of the cycle, varied from a value of 6.7 during normal feed to 5.9 at the end of the experiment. Alkalinity presents a more pronounced decline than in the previous experiment; acids accumulate in a similar way. Individual VFA are presented in Figure 17. Propionate exhibits a higher accumulation than acetate which remains at relatively low levels until the 6.0x load is applied. The absolute concentrations of acids accumulated in this experiment are slightly lower than the ones observed in the previous experiment. In a similar way as the previous experiment, butyric, isobutyric, valeric and isovaleric acids start to accumulate as the overload develops.

Gas daily averages are presented in Figure 18. Due to failure in the on-line system occuring when applying the 4.0x load, the average daily values for hydrogen exhibit a decline. Nonetheless, from Figure 20, it can be inferred that hydrogen increases with increasing loading of the system.

Figure 15. On line CH_4, CO_2, H_2, and CO data (overload #1).


Organic Overload #1
CH4/CO2
Percentage (%)
60
58
56
54
52
50
48
46
44
42
40
38
36
34
32
30
28
CO2
CH4
00:00
00:00
00:00
00:00
00:00
TIME
CH4
CO2

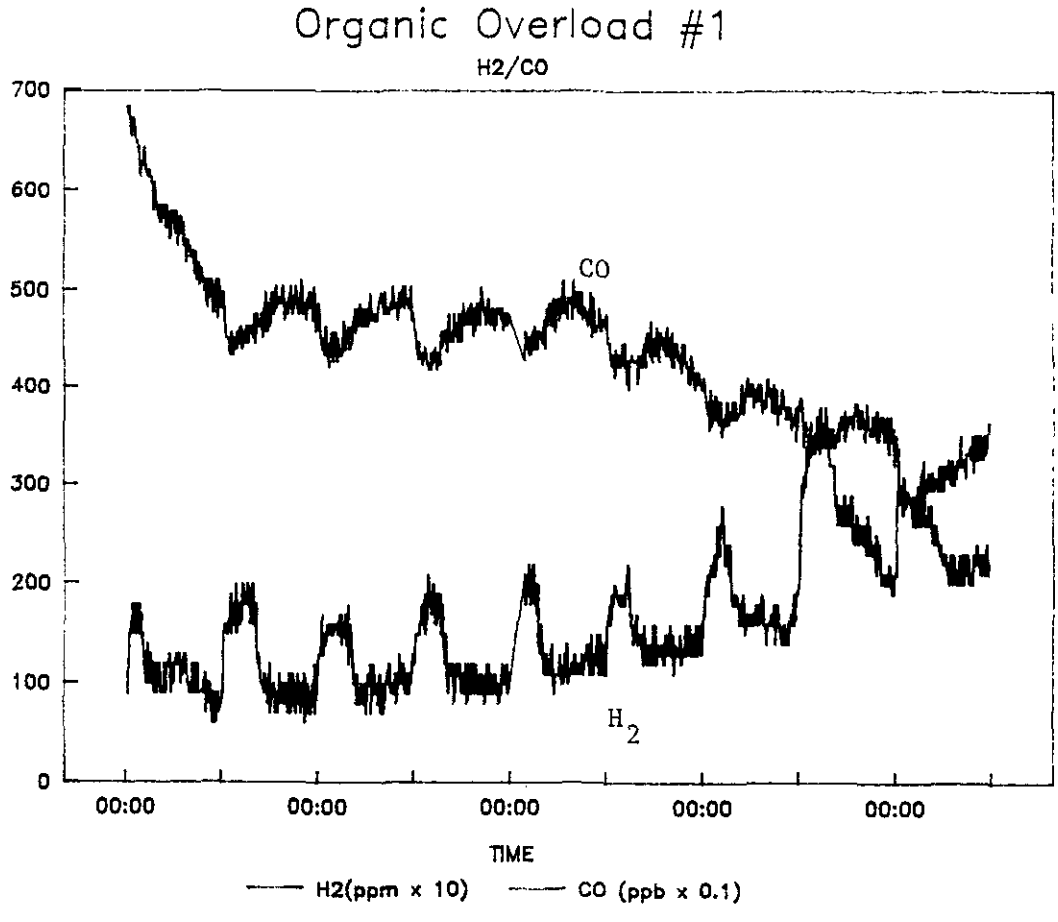
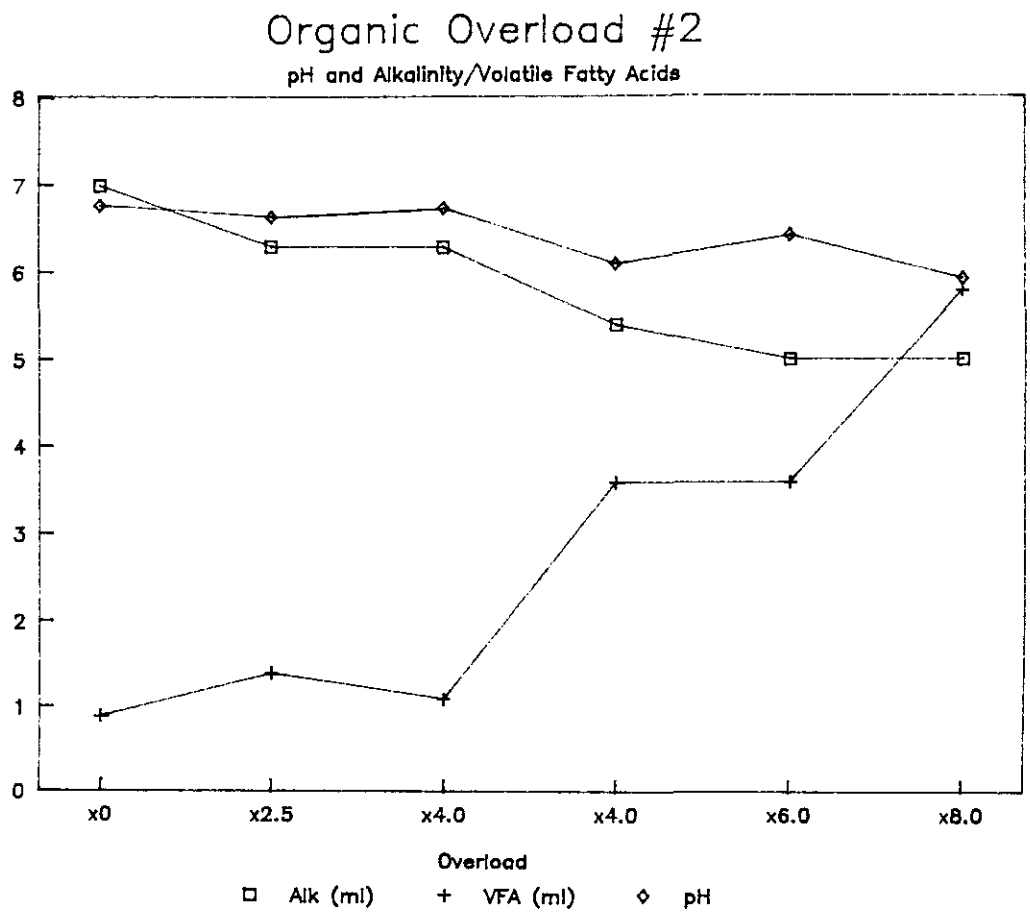

Organic Overload #1
H2/CO
700
600
500
400
300
200
100
0
CO
H2
00:00
00:00
00:00
00:00
00:00
TIME
H2(ppm x 10)
CO (ppb x 0.1)

Figure 16. pH, alkalinity, and VFA data (overload #2).



Alkalinity and VFA are expressed in relative units.

Figure 17. Individual VFA daily averages (overload #2).

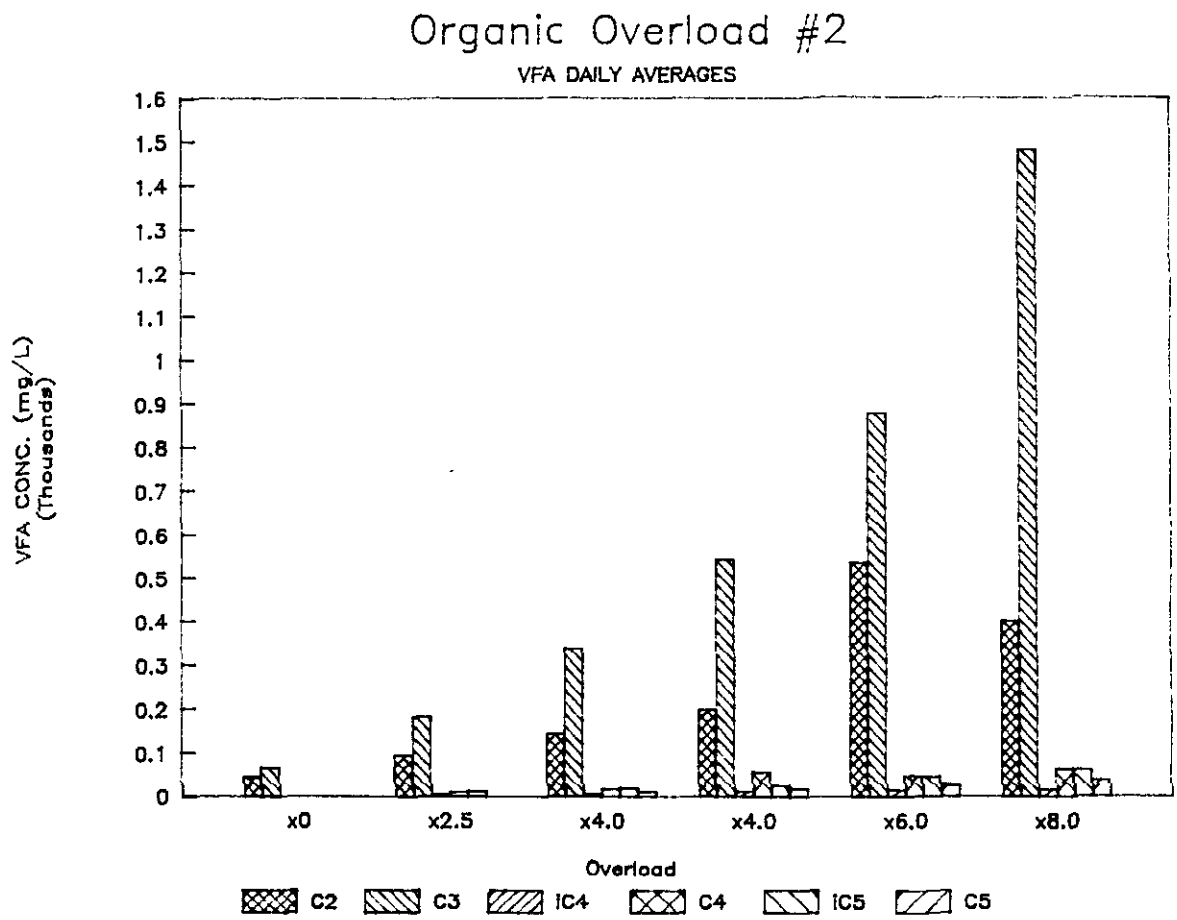
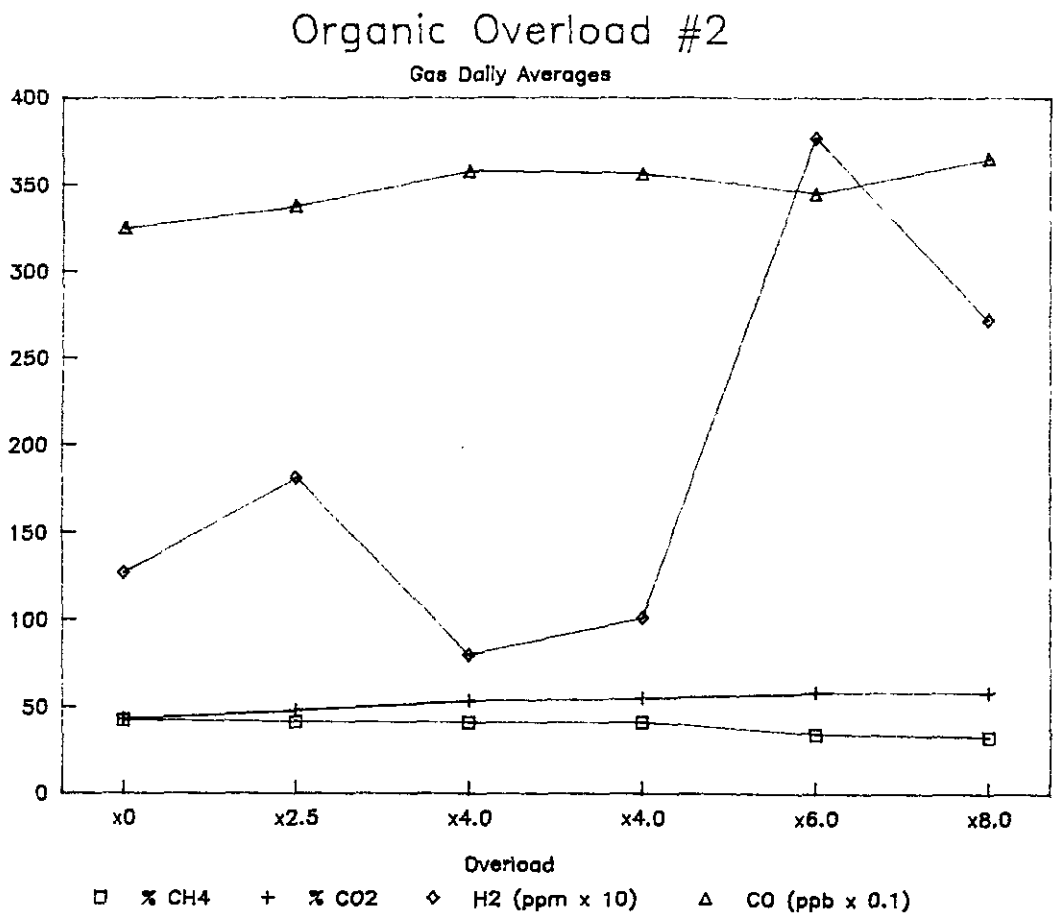


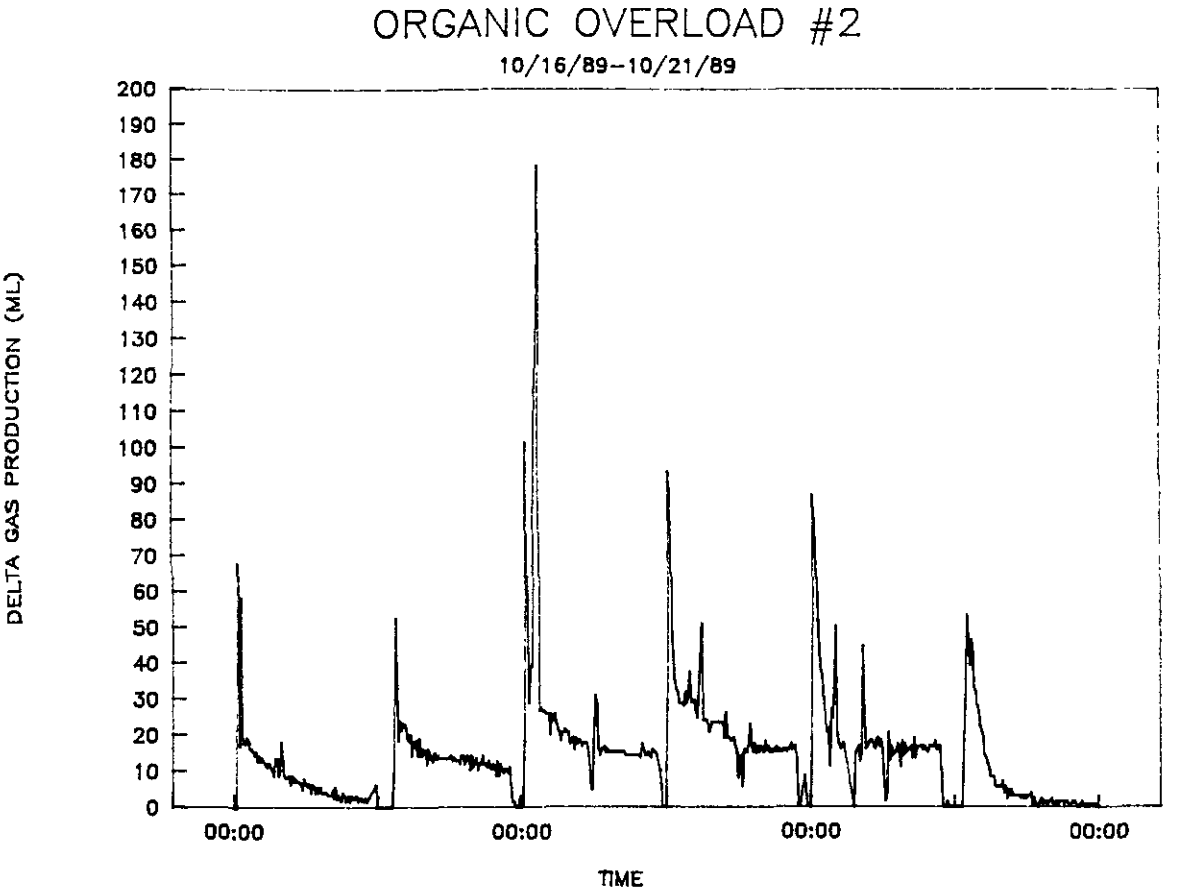
Figure 18. Gas daily averages (overload #2).



Carbon monoxide concentration exhibit a slow increase, and in contrast with the results from the previous experiment, does not exhibit a decrease in concentration at the end of the overload. Methane and carbon dioxide concentrations show a permanent divergence as the organic loading increases. Total and differential gas production are presented in Figure 19. Daily gas production show the typical pattern already observed in the previous experiment. Nonetheless, the total gas production, in contrast with the previous results, does not increase steadily as the load increases. After the 4.0x load, the total gas production remains constant, and decline abruptly when the load is increased from 6.0x to 8.0x, indicating a failure of the reactor.

On-line data are presented in Figure 20. Methane and carbon dioxide behave similarly to the previous experiment, with one important difference. The concentrations of methane and carbon dioxide continue to be mirror images, but the end-of-cycle concentrations diverge as the organic loading increases. Absolute hydrogen concentration variations are higher in this experiment than in the previous one. Hydrogen seems to track the concentrations of propionic acid in this system. Carbon monoxide remains relatively constant trough the experiments with a slight increment from day to day, and a mild pattern through the day. This is in contrast with the previous experiment where carbon monoxide exhibited a defined pattern through the day and a decline at the end of the experiment. It is important to notice that acetate concentrations remained also relatively low thoughout the entire experiment.

Figure 19. Total and differential gas production (overload #2).


ORGANIC OVERLOAD #2
10/16/89-10/21/89
DELTA GAS PRODUCTION (ML)
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
00:00
00:00
00:00
00:00
TIME

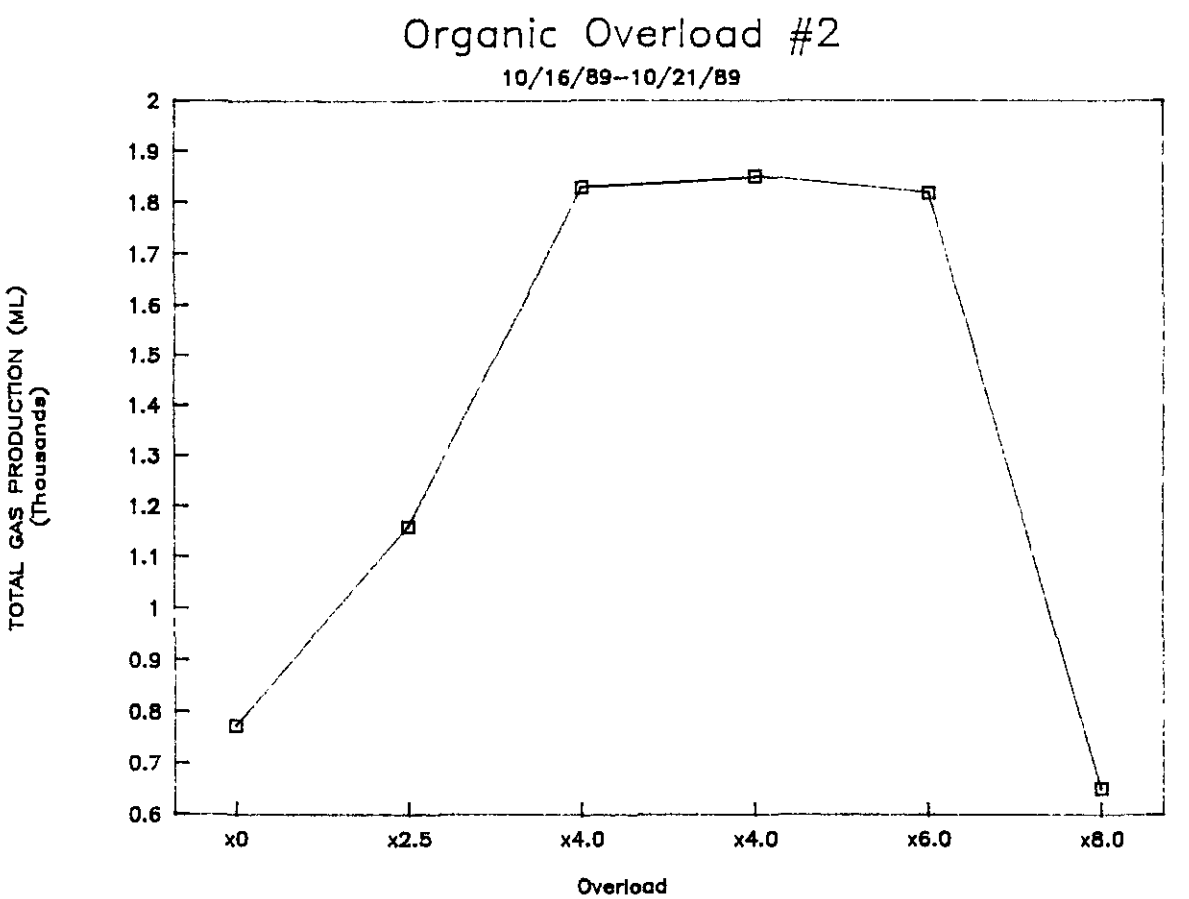
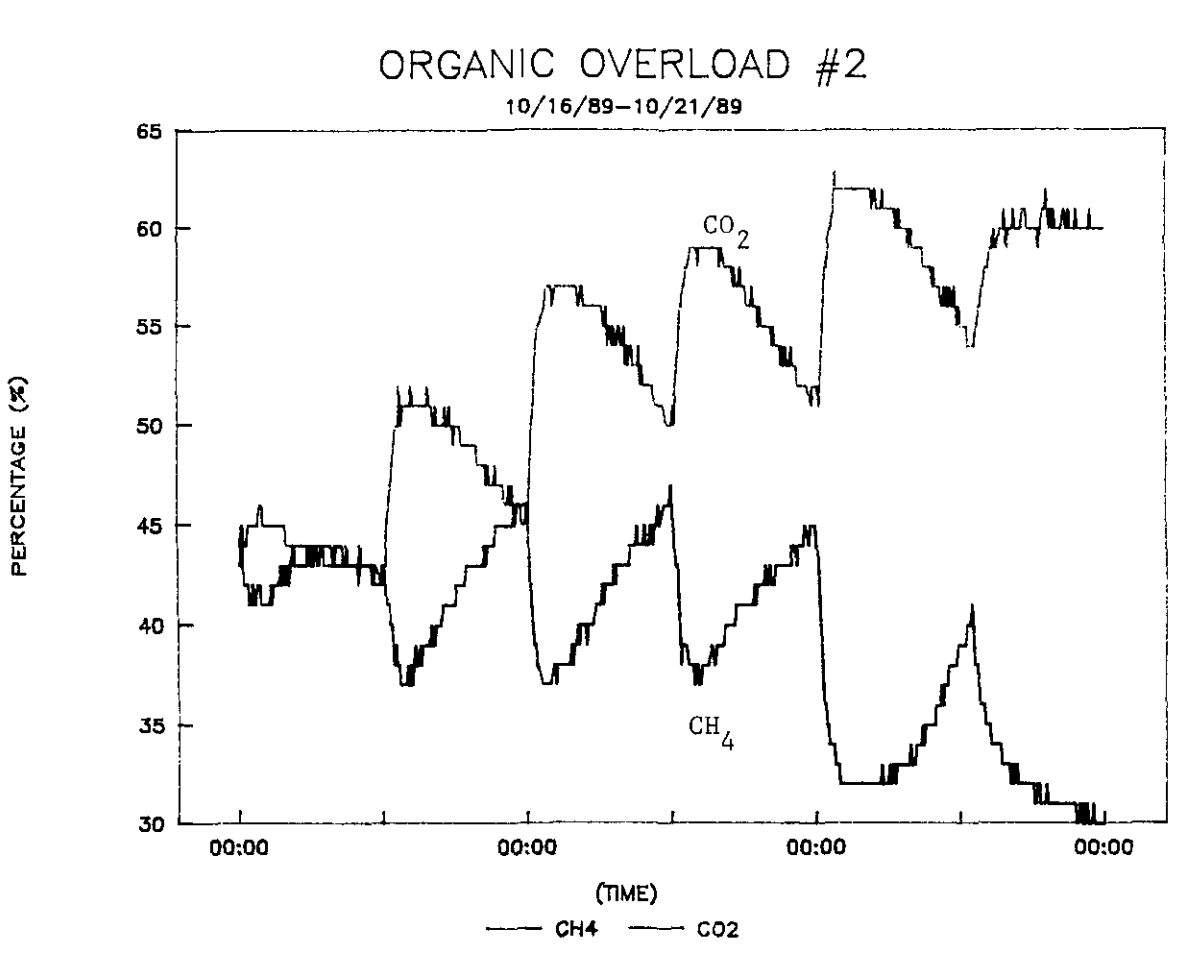
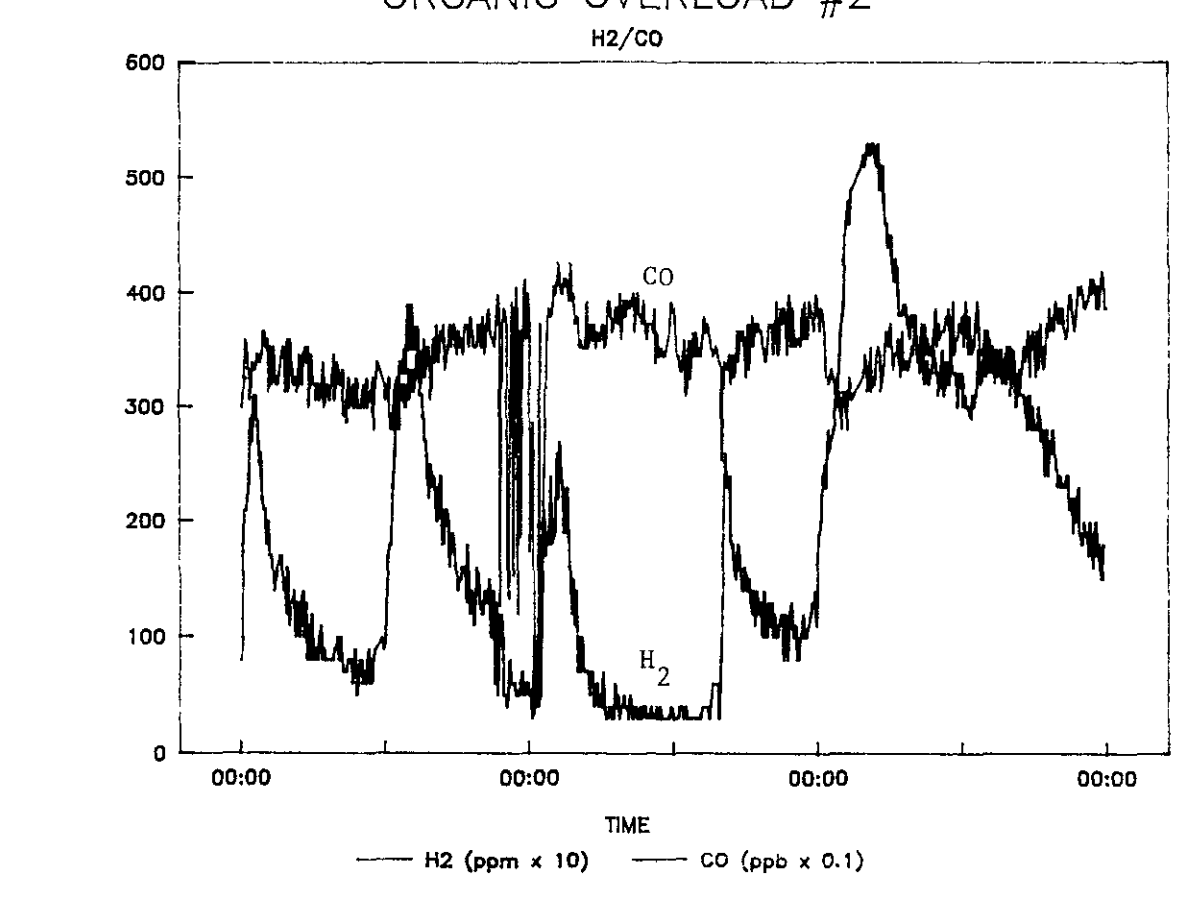

Organic Overload #2
10/16/89-10/21/89
TOTAL GAS PRODUCTION (ML)
(Thousands)
2
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
x0
x2.5
x4.0
x4.0
x6.0
x8.0
Overload

Figure 20. On line CH_4, CO_2, H_2, and CO data (overload #2).


ORGANIC OVERLOAD #2
10/16/89-10/21/89
PERCENTAGE (%)
65
60
55
50
45
40
35
30
CO_2
CH_4
00:00
00:00
00:00
00:00
(TIME)
CH4
CO2


ORGANIC OVERLOAD #2
H2/CO
600
500
400
300
200
100
0
CO
H_2
00:00
00:00
00:00
00:00
TIME
H2 (ppm x 10)
CO (ppb x 0.1)

5. DISCUSSION

A good toxicity monitoring parameter should present a response in toxic situations that can be easily distinguished from normal natural variations. In order to evaluate the natural variability of hydrogen carbon monoxide and methane production in the toxicity experiments reported here a statistical analysis was performed. The analysis was done on the triplicate controls (no toxicant added) from all of the different assays (each assay corresponding to a different toxicant and performed independently of the other ones) in this study. These results cover a period of seven months during which the experiments of this study were performed. An important assumption in this analysis is that each assay is independent of the other ones. Analysis of variance (ANOVA) was done in order to distinguish between variations within the control bottles in the test, and variations among the different assays. Results for the sucrose experiments are presented in Table 2 and those for waste activated sludge in Table 3. These results are based on batch assays and may not represent reallystically the behaviour of trace gases under continuous flow applications of anaerobic digestion technology.

It can be seen that methane and hydrogen show a smaller coefficient of variation (relative standard variation) than carbon monoxide. From the standard deviation a 95% confidence interval can be constructed based on the t-distribution [25]. A 95% confidence interval can be interpreted as the range of values over which 950 out of 1000 random observations of the parameter in consideration under nontoxic conditions will lie. Based on this approach, a tentative criterion to distinguish between natural variations in the behavior of hydrogen and variations resulting from toxic stresses, in the assays reported here, is a difference greater than 31% between a measured value and an expected value. For carbon monoxide a 95% confidence

TABLE 2. STATISTICAL PARAMETERS OF CO, H_2 AND CH_4 PRODUCTION (SUCROSE ENRICHMENT SERUM BOTTLES)

TIME (hours)	$\mu^{(1)}$ CH_4 ml	$Cv^{(2)}$ CH_4	μ H_2 ppm	Cv H_2	μ CO ppm	Cv CO
0	0	0	5	0.44	0.379	0.12
2.5	0.6	0.46	16	0.19	0.705	0.26
5	1.2	0.28	28	0.17	1.06	0.32
9	2	0.21	43	0.21	1.76	0.38
24	4.6	0.14	63	0.12	4.19	0.28
48	7.3	0.14	35	0.29	3.96	0.44
Cv MEAN		0.24		0.24		0.30
t statistic confidence interval		0.31		0.31		0.39

(1) μ = mean value of the observations.
(2) Cv = coefficient of variation (standard deviation/mean).

TABLE 3. STATISTICAL PARAMETERS OF CO, H_2 AND CH_4 PRODUCTION (WASTE ACTIVATED SLUDGE SERUM BOTTLES)

TIME (hours)	$\mu^{(1)}$ CH_4 ml	$Cv^{(2)}$ CH_4	μ H_2 ppm	Cv H_2	μ CO ppb	Cv CO
0.5	-	-	24	0.86	560	0.21
4.5	-	-	91	0.34	840	0.23
8.5	-	-	99	0.20	910	0.23
12.5	-	-	103	0.11	920	0.17
24.5	16.5	0.065	71	0.06	560	0.21
Cv MEAN		0.065		0.31		0.21
T STATISTIC CONFIDENCE INTERVAL		0.080		0.37		0.25

(1) μ = mean value of the observations.
(2) Cv = coefficient of variation (standard deviation/mean).

interval based on the t-distribution is range of ± 39% of an average value for a specific set of conditions (e.g. time, volumetric load)

Statistical confidence limits for hydrogen and carbon monoxide are plotted in Figures 7, 9, 10, 21, 22, and 23. Figures 8,9,10 and 21 refer to data obtained for the sucrose enrichment culture and Figures 22 and 23 to data obtained for waste activated sludge serum bottle runs. Data points lying inside the statistical confidence boundaries do not exhibit any difference from the value that would have being expected under non-toxic conditions- in other words, a toxic effect could not be detected in terms of that indicator. It can be seen that carbon monoxide is a good indicator of toxic events when methane production is only partially inhibited, that is, levels of methane production that otherwise would be considered as acceptable. Hydrogen on the other hand seems more indicative of toxic events for high levels of methane production inhibition. It should be also noted that carbon monoxide reaches statistically significant levels in the first 4.5 hours for waste activated sludge and in the first 6 hours for the sucrose assays. It must be pointed out that in order to apply the criteria given here, it is necessary to compare a measured value with an expected value. In this research the comparison was done between the values obtained for the control values and the values obtained when toxicant was added. In a real world situation a treatment plant operator should compare the measured value with an expected value based on historical records or the forecast of a mathematical model.

Carbon monoxide exhibited a consistent pattern of behavior with respect to methane production inhibition caused by heavy metals (Figure 21). This pattern has been reported before [6][14].The reason why carbon monoxide concentration rise during low inhibition of methane production generated by

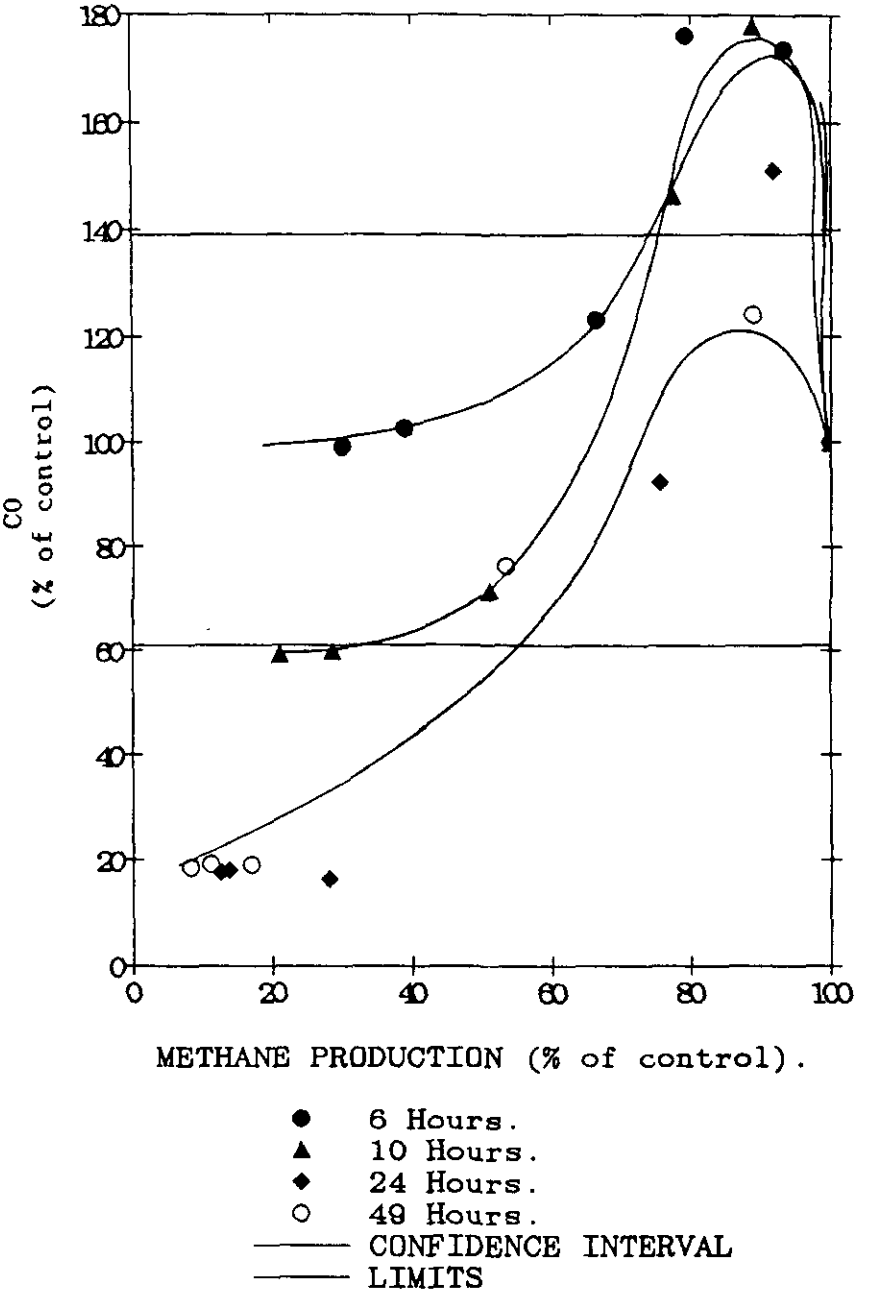


Figure 21 Comparison of carbon monoxide headspace concentration and cumulative methane production for cadmium inhibited samples. Sucrose as electron donor.

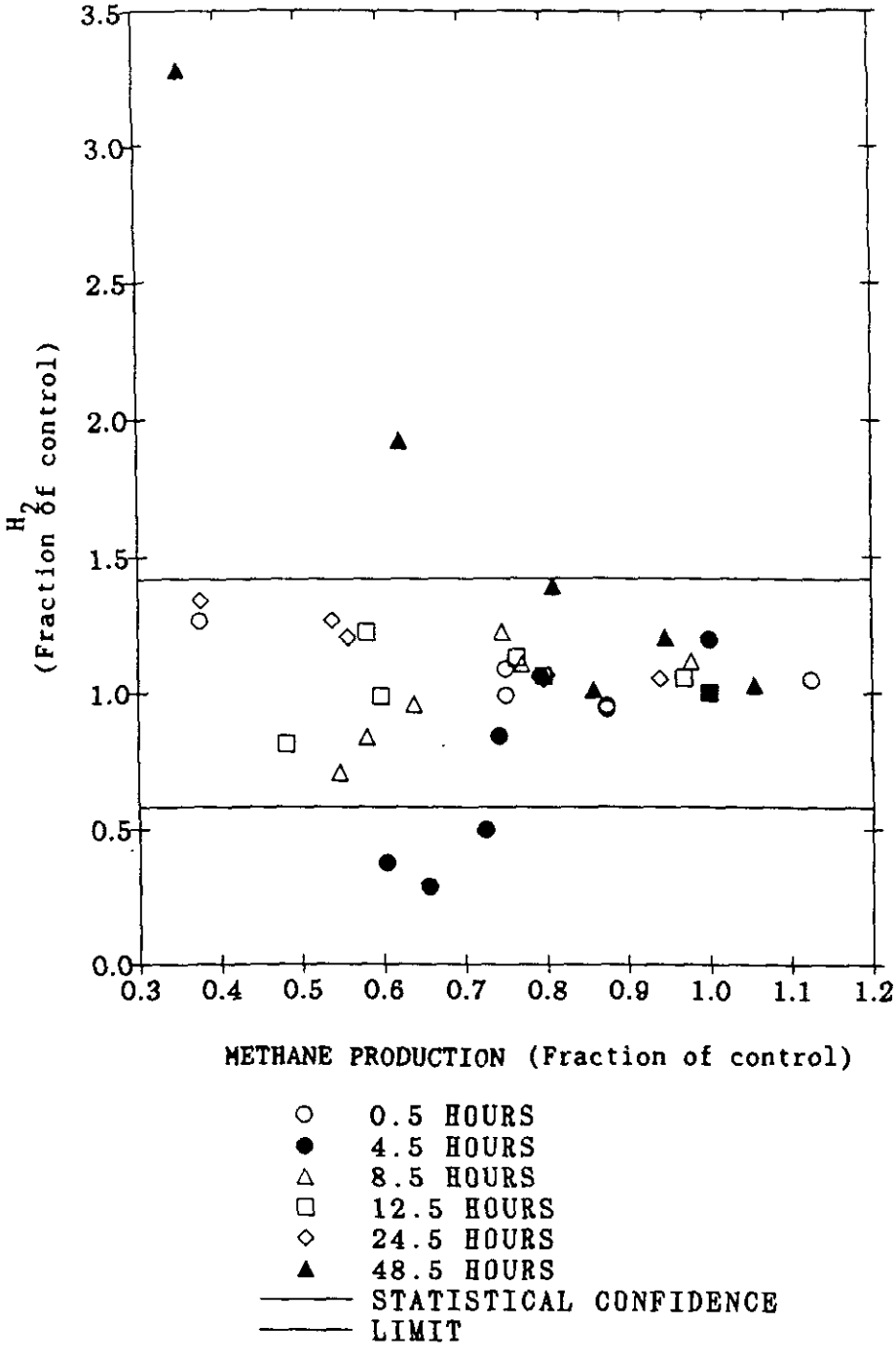


Figure 22 Comparison of hydrogen headspace concentration and cumulative methane production for cadmium inhibited samples. WAS as electron donor.

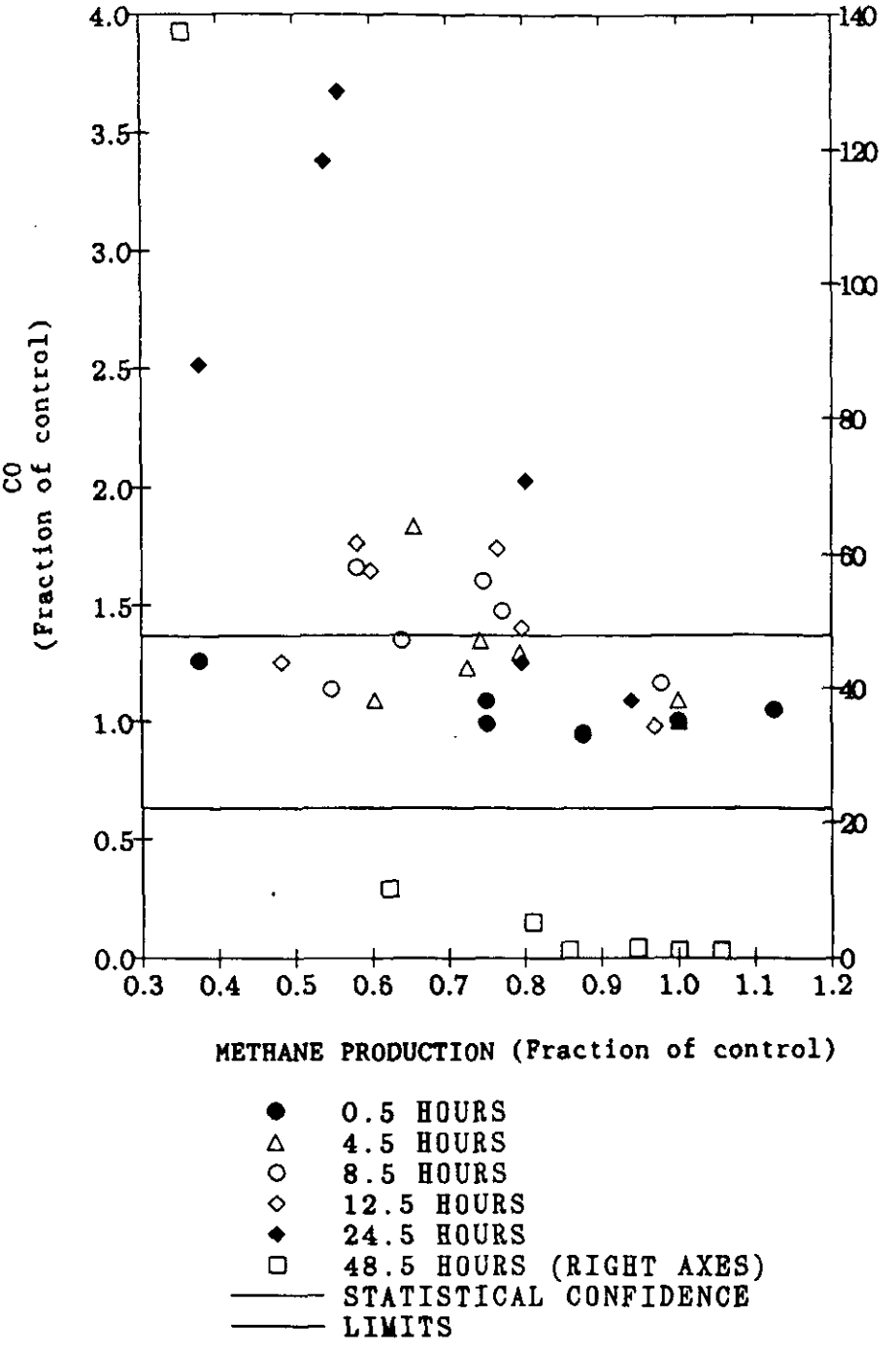


Figure 23 Comparison of carbon monoxide headspace concentration and cumulative methane production for cadmium inhibited samples. WAS as electron donor.

heavy metals is not clear. Heavy metals may be interacting with carbon monoxide dehydrogenase, the key enzyme in the pathway, and cause a change in the affinity of the enzyme for the bound carbon monoxide moiety. Further research is necessry to elucidate this observation.

The two organic overload experiments behaved as expected with regard to the effects of pH control on the stability of the reactor. During the first experiment, with pH control, the reactor withstood the effects of increasing loading and increasing gas production rate was always observed as a consequence of the increment in the influent concentration. During the second experiment, the reactor failed since gas production did not show an increase when the load was changed from 4.0x to 6.0x, and finally gas production droped to low levels when the load was increased to 8.0x. This effect in itself was expected, but it is interesting to observe the differences between the behavior of the monitoring parameters during the two experiments.

Hydrogen behaved in a somewhat unexpected way in these experiments. With a soluble carbohydrate such as sucrose, it was expected that hydrogen would reach considerable higher concentrations than the ones observed here (10). Hydrogen always remained below 50 ppm independently of the organic load. Additionally, the time response of hydrogen was slower than what would be expected from theoretical calculations based on reported kinetic parameters and assuming that hydrogen would be produced during the fermentation of sucrose (23). This phenomenon can be explained if it is postulated that hydrogen is generated from propionate and not from glucose (sucrose is hydrolized into glucose and fructose). There are several observations that suggest that this is the case. First, in both experiments hydrogen tracks the concentrations of propionate; propionate is a product of

the fermentation of sucrose as can be seen in the daily profiles of individual acids in Figure 17. If hydrogen would be produced from glucose fermentation it would show a sharp accumulation immediately after the daily batch-fed. Second, hydrogen concentrations are in agreement with thermodynamic calculations on the energetics of propionate degradation (10)(11). Propionate degradation turns endergonic at hydrogen partial pressures higher than 100 ppm. It has been reported by several researchers that hydrogen accumulate beyond the thermodynamic boundaries for propionate degradation when hydrogen is generated during the fermentation of sugars (12)(13). Third, the main products of the fermentation of sucrose in this reactors are propionate, acetate and CO_2, as it is suggested on the daily concentration profiles for fermentation acids presented in Figures 12 and 17, and the pronounce variation of carbon dioxide after the daily feed. A fermentation of the type:

$$1.5\ \text{Glucose} \dashrightarrow 2\ \text{Propionate} + \text{Acetate} + CO_2$$

is widely documented for a variety of anaerobic bacteria e.g. Clostridium propionicum, Megasphaera elsdenii (26). An interesting feature of this type of bacteria is that they lack of the presence of dehydrogenase enzymes, thus hydrogen can not be produced although could be energetically favorable due to the low partial pressure of this gas prevailing in anaerobic digesters.

It is interesting to observe that the absolute daily variations of hydrogen in both experiments are different. In the experiment with limited pH control, hydrogen variations are more pronounced in a daily cycle than in the initial experiment. Thus pH seems to affect the patterns of hydrogen production and consumption in syntrophic associations.

The behavior of carbon monoxide in these experiments is also somewhat unexpected at a first look. Hickey (14) has observed that carbon monoxide tracks acetate during hydraulic and organic overloads in a sludge anaerobic digester. In that system acetate acumulates from a baseline concentration of approximately 50 mg/l to 2000 mg/l in a prolonged organic overload; with steadily increasing end-of-cycle concentrations. In the experiments reported here, acetate behavior was different. Acetate concentrations at the end of the cycle remained very low in the first experiment, and moderately low in the second experiment, see Figures 12 and 17. Carbon monoxide concentrations reflect this behavior, see Figures 13, 15, 18 and 20. During the first experiment, carbon monoxide pattern tracks the acetate pattern but a time delay can be observed. The cause of this time delay may be explained by mass transfer limitations or in the thermodynamic interplay among the different gases. Towards the end of the experiment carbon monoxide concentrations start to decline altough end-of-cycle acetate concentrations remain at the same level as before. This behavior may be a result of the thermodynamic interplay among methane, carbon dioxide, hydrogen and carbon monoxide observed by Hickey (14). It shold be noticed that at the end of the experiment the concentrations of methane, carbon dioxide and hydrogen are significantly different than in previous days. This possibility deserves a more careful analysis. Another possibility may be that carbon monoxide reflect not just acetate concentrations but metabolic activities related to acetoclastic methanogenesis. During conditions of healthy catabolic activity, carbon monoxide is linked to acetate concentrations, when specific catabolic activities decrease, so does carbon monoxide in spite of acetate concentrations. In any case a further exploration of this phenomenom is necessary.

Based on the previous discussion we can see that carbon monoxide and hydrogen provide relevant information on the performance of the anaerobic digester. Hydrogen tracks hydrogen-producing-substrates while carbon monoxide tracks acetate (no hydrogen producing substrate). Thus between both of them it is possible to obtain a accurate picture of the loading status of the reactor. Nevertheless, while the digester with good pH control was able to withstand the overloading without a complete failure, the lower buffered ractor could not. This was evident from the decrease in gas production in the latter, in spite of the increase in the loading indicators hydrogen and carbon monoxide. Thus it seems logical to conclude that a monitoring parameter that relates the rate of methane production to the concentrations of hydrogen and carbon monoxide would provide a good indication of the stress situation of the reactor. When the increase in the loading on the reactor, as evidenced by the behavior of hydrogen and carbon monoxide, occurs with an increase in the methane production rate, then the reactor is coping with the increased load. This is the behavior in the first experiment. When this is not the case, as in the second experiment, the indicator will warn of the unbearable stress in the system. Of course additional analysis is necessary to identify the ranges of stress that a system can handle.

6. CONCLUSIONS

The following conclusions can be drawn from the data presented:

- Carbon monoxide exhibited potential as an early warning indicator of toxic upsets generated by heavy metals. Statistics might be used to give a clear criterion for distinction between natural variations and variation generated by toxicity. The time necessary for carbon monoxide to achieve statistically significant levels is between 4 and 6 hours depending on the system under consideration. Further studies on the use of carbon monoxide in anaerobic toxicity monitoring are recommended.
- The methanogenic population in the systems described here seem to be more resistant than the non-methanogenic population to toxicity generated by heavy metals. A selective inhibition pattern was clearly seen. Selective inhibition might help to explain the failure of conventional indicators such as VFA or alkalinity for the successful operation of anaerobic digesters subject to toxic inhibition.
- Hydrogen did not show a statistical significant response, that allow to distinguish between natural variations and toxic induced variations, under conditions of low toxic inhibition of methane production by heavy metals.
- The data presented here is based in batch assays and may not represent realistically the behavior of the trace gases under continuous flow applications of anaerobic digestion technology. Nevertheless the results obtained here are encouraging and the study of toxic events with other reactor configurations and real world systems are recomended.
- On line monitoring of hydrogen and carbon monoxide allows to obtain an accurate picuture of the loading status of the anaerobic digestion process.

Hydrogen tracks the concentration of hydrogen generating substrates, while carbon monoxide tracks acetate concentration.

- The combination of hydrogen and carbon monoxide with the rate of methane production in a single parameter, may provide a very useful indicator of the behavior of an anaerobic digester under stress.

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