Short-Term and Long-Term Effects of Increasing Temperatures on the Stability and the Production of Volatile Sulfur Compounds in Full-Scale Thermophilic Anaerobic Digesters

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Abstract: This study compares the effect of a rapid increase of the digester temperature (from 54°C to 58°C in 2 weeks) with a slow increase (from 53.9°C to 57.2°C at a rate of 0.55°C per month) on full-scale thermophilic anaerobic digestion at Hyperion Treatment Plant. The short-term test demonstrated that rapidly increasing the digester temperature caused elevated production of volatile sulfur compounds, most notably methyl mercaptan, but volatile solids destruction and methane production were not significantly affected. The increase of the volatile fatty acid to alkalinity ratio from 0.1 to over 0.3 indicated a transient change in digester biochemistry, which was reversed by lowering the temperature. In the long term-test, a slow increase of digester temperature, the production of hydrogen sulfide increased above temperatures of 56.1°C, but was controlled by increased injection of ferrous chloride. Methyl mercaptan was detected in trace amounts at the highest temperature tested (57.2°C). This test showed insignificant effects on other digestion parameters, although some temperature-independent changes were observed that could have been seasonal effects over the year that the long-term test lasted. Thus a slow temperature increase was preferable. This observation contrasts with previous results showing the desirability of a rapid temperature rise to first establish a thermophilic culture when converting from mesophilic operation. Further research is warranted on temperature limits and process changes to optimize thermophilic anaerobic digestion. © 2005 Wiley Periodicals, Inc.

Keywords: temperature limits; thermophilic anaerobic digestion; Class A biosolids; volatile sulfur compounds; short-term effects; long-term effects

INTRODUCTION

In full-scale thermophilic anaerobic digestion, digester temperatures are usually in the lower end of the thermophilic temperature range, i.e., 50–55°C. The conviction that this is the preferable temperature range dates back at least to the work of Pohland and Bloodgood (1963), who carried out a thorough investigation that found much better results at 52°C than at 62°C. This has also been supported by the success of much later work in this temperature range, e.g., Aitken and Mullenix (1992). Anaerobic digestion above 60°C has only been investigated in lab-scale studies, focusing on process performance (Nozhevnikova et al., 1999; Varel et al., 1980), pathogen removal (Gabb et al., 2000), and microbial population dynamics (Ahring et al., 2001). Based on common practice criteria, it is usually recommended to keep the temperature below 60°C (van Lier, 1996; Wilson et al., 2004), although experiences on a full scale are very limited. These practice criteria also recommend a rapid temperature rise from mesophilic temperatures when thermophilic digestion is being established, to promote the development of a culture of true thermophiles, instead of the thermotolerant mesophiles that may dominate a culture in which the temperature has been raised slowly (Aitken and Mullenix, 1992).

The City of Los Angeles Bureau of Sanitation has conducted series of full-scale tests at the Hyperion Treatment Plant (HTP) for evaluation of Exceptional Quality (EQ) biosolids production by thermophilic anaerobic digestion that successfully followed the recommendation of a rapid initial temperature rise (Iranpour et al., 2002, 2003a,b, 2005a,b; Shao et al., 2002). A two-stage continuous-batch process has been implemented to comply with the Class A pathogen reduction requirements of U.S. EPA (1993) 40 CFR Part 503. In October 2002, the thermophilic digesters were temporarily operated with the temperature rapidly being increased from 54°C to 58°C. This was to evaluate the compliance with the time–temperature relationship for batch

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disinfection as specified in Alternative 1 of 40 CFR 503.32. Due to the limited batch digester capacity, performance was evaluated for a holding period of 16 h, which required a temperature equal or greater than 56.3°C. A deadline to demonstrate compliance by the end of 2002 required the temperature increase to be completed in about 2 weeks. This coincided with an increase of odor complaints from neighboring communities, and digester temperatures were reduced to around 53°C in November 2002.

Evidently, it was reasonable to hypothesize that the increase of odor emissions could have been a transient response to the quick increase of the temperature. Alternatively, these observations might also have been an intrinsic effect of the temperature being raised beyond the maximum temperature for thermophilic anaerobic digestion of wastewater sludge. To determine which of these possibilities were correct, a long-term test was conducted in 2003 with one digester in which the temperature was increased by 0.55°C (1°F) per month from 53.9°C to 57.2°C. The specific overall objectives of these investigations were:

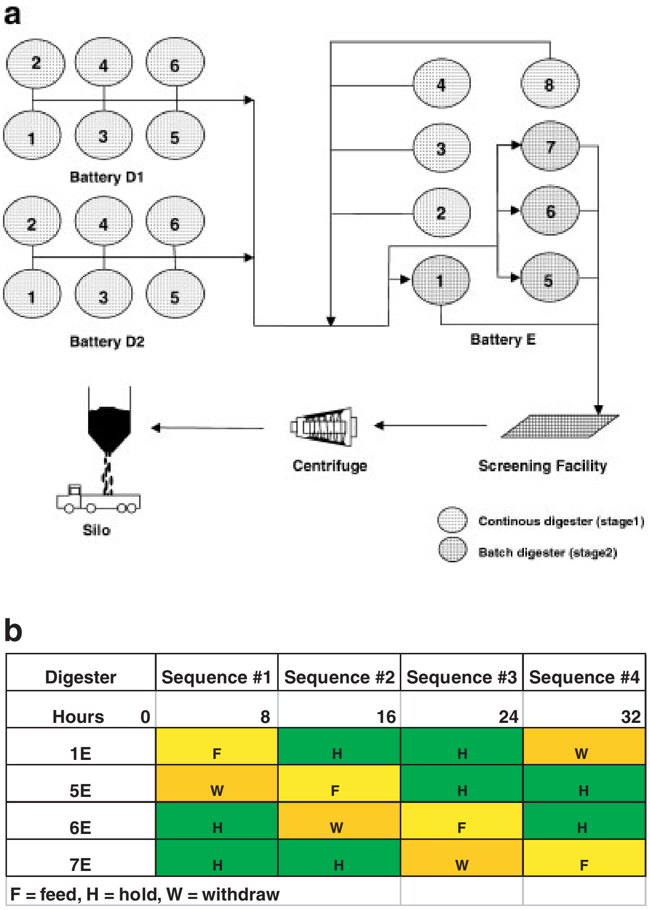
a) To determine the effect of a rapid temperature increase from 54°C to 58°C (the short-term test) on volatile sulfur compound (VSC) production and measures of digestion performance such as the volatile fatty acids (VFA) to alkalinity ratio, volatile solids destruction, and methane production.
b) To determine the effect of the temperature gradually increasing in steps of 0.55°C from 53.9°C to 57.2°C (the long-term test) on VSC production and digestion performance.
c) To compare the results of the short-term test with the long-term test.

Stable thermophilic operation was well established by the time of these studies, and HTP has consistently met the Class A pathogen reduction requirements since the complete conversion of the plant to thermophilic operation in September 2002. The start-up and disinfection results have been presented in previous studies (Haug et al., 2002; Iranpour et al., 2003a,b, 2005a,b).

MATERIALS AND METHODS

Hyperion Treatment Plant

HTP is located in Playa del Rey, California. Figure 1a shows that HTP has three batteries with 20 egg-shaped digesters. Each digester has a volume of 9500 m^3. The digesters were operated in a two-stage process. The first stage contained 16 digesters operated in a continuous mode at an average HRT of 11–12 days. The second stage contained 4 digesters operated in a batch mode with 32-h cycles of sludge feeding, holding, and withdrawal (Fig. 1b). The holding time was 16 h and each batch digester was fed/withdrawn for 8 h each, up to approximately 60%–70% of its total volume during one cycle.



Digester	Sequence #1	Sequence #2	Sequence #3	Sequence #4
Hours 0	8	16	24	32
1E	F	H	H	W
5E	W	F	H	H
6E	H	W	F	H
7E	H	H	W	F
F = feed, H = hold, W = withdraw				

Figure 1. **a**: HTP-Schematic of two-stage thermophilic anaerobic digestion. **b**: HTP-Digester cycles for batch operation. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

Digester Temperatures

The short-term test was conducted on plant-scale and involved all first- and second-stage digesters. Since almost all of the digestion process occurred in the first-stage digesters (HRT of about 11–12 days) rather than the second stage (holding time of 16 h), digester performance was related to the temperature in the first-stage. Second-stage digester temperatures were 1–2°C lower than first-stage digester temperatures. Figure 2a shows that in the last week of September 2002, the average temperature of the first-stage digesters was quickly increased from 54.4°C to around 58°C and held constant for a period of 3 weeks in October 2002. In November 2002, the average first-stage digester temperature was reduced to about 53°C.

The long-term test was conducted with Digester 1 in Battery D1, Digester 1D1, in the first stage. The temperature of Digester 1D1 was increased in steps of 0.55°C per month from 53.9°C in February 2003 to 57.2°C in September 2003, as shown in Figure 2b. The temperature of the other digesters was kept constant at around 53°C.

Operation Parameters

Table I summarizes the first-stage operation parameters during the short-term test. The monthly averages were

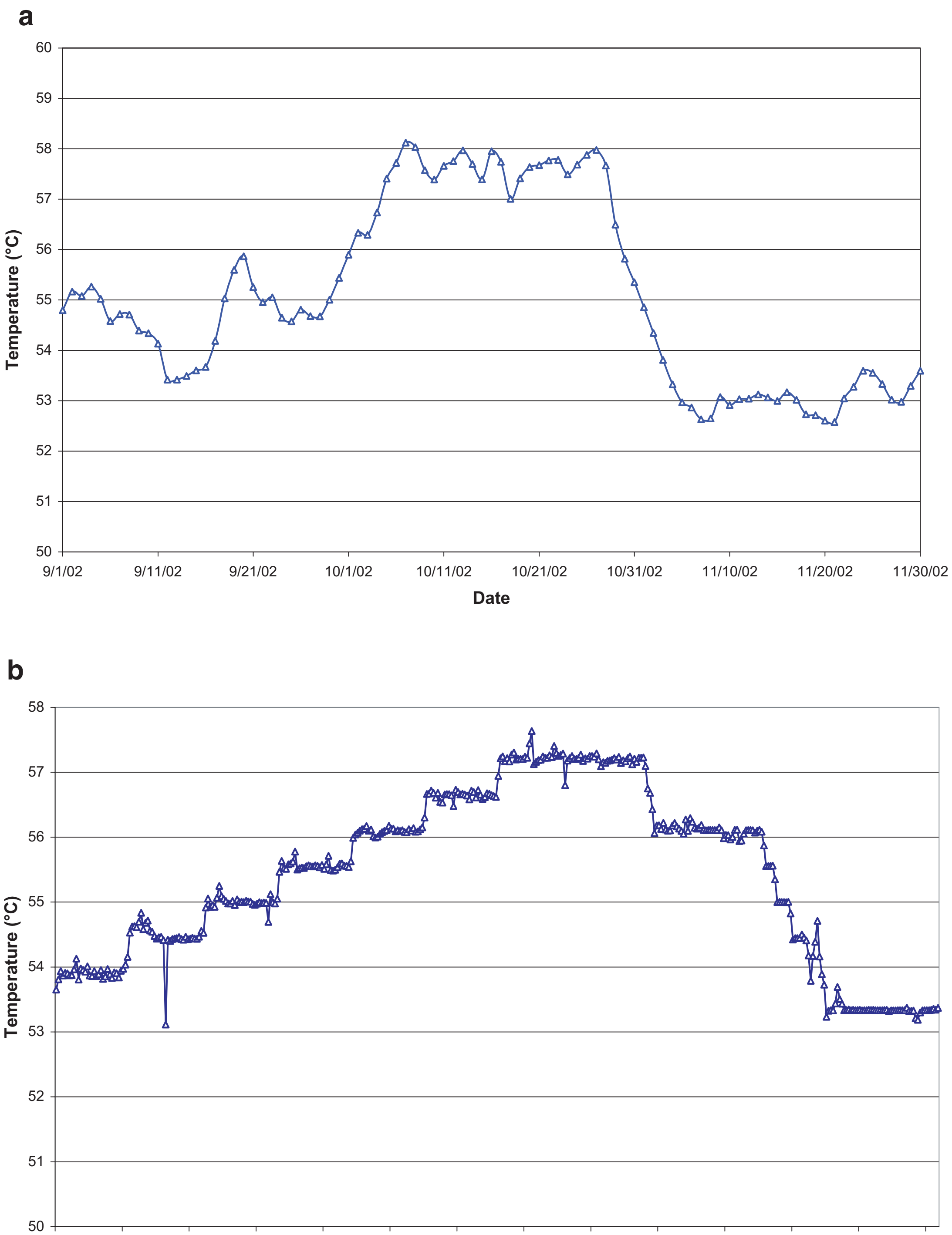


Figure 2. **a**: Short term test—Average temperature of first-stage digesters. **b**: Long term test—Average temperature of Digester 1 of Battery D1 (1D1). [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

very similar in October and November 2002, demonstrating that the temperature was the only input parameter with a significant change. Table II summarizes operation parameters of Digester 1D1 during the long-term test. Apart from the temperature, operation parameters did not change significantly and were comparable to those of the short-term test.

Sampling

For the short-term test, samples were taken from the combined digester outflow of the first stage and analyzed for VFA, total alkalinity, total solids, and volatile solids destruction (TSD and VSD, respectively). Digester gas samples were collected from the combined outflow of the first

Table I. Summary of feed operational parameters during short-term test (16 first-stage digesters; 4 second-stage digesters).

Parameter[a]	October 2002, ave. ± st. dev.	November 2002, ave. ± st. dev.
PS feed rate (m^3/d)	10166 ± 1365	9361 ± 805
TS in PS (%)	3.4 ± 0.3	3.7 ± 0.3
VS in PS (% of TS)	78.5 ± 1.3	78.9 ± 1.0
TWAS feed rate (m^3/d)	3049 ± 396	2993 ± 459
TS in TWAS (%)	5.7 ± 0.56	5.6 ± 1.0
VS in TWAS (% of TS)	83.1 ± 1.0	82.9 ± 0.5
First-stage HRT (d)	11.3 ± 1.2	12 ± 0.6
Second-stage holding time (h)	16	16

[a] PS, primary sludge; TWAS, thickened waste activated sludge; TS, total solids; VS, volatile solids; HRT, hydraulic retention time.

and second stage and analyzed for methane, carbon dioxide, and VSC.

For the long-term test, biosolids and digester gas samples were taken from sampling ports dedicated to Digester 1D1 and analyzed for the same parameters as in the short-term test. Sampling frequencies are shown in Table III.

Analytical Procedures

Analyses were conducted by the Environmental Monitoring Division at HTP according to the procedures summarized in Table III.

RESULTS

Short-Term Test with Rapid Temperature Increase

Figures 3–7 summarize the observations made over September through November, 2002. Each figure includes the temperature history for direct comparison with one or more other parameters.

VSC production

The methyl mercaptan concentration increased at a high rate when the temperature was rapidly increased to 58°C (Fig. 3). Lowering the digester temperature caused an immediate and sharp decline of the methyl mercaptan concentration. The changes of the hydrogen sulfide and dimethyl sulfide concentrations with temperature were minor. Production of carbonyl sulfide, ethyl mercaptan, 2-propyl mercaptan, or 1-propyl mercaptan was not observed.

Digestion

With a lag-time of about 1 week, the VFA concentration in first-stage digester biosolids rapidly increased to over 1000 mg/L (as acetic acid) after increasing the digester temperature (Fig. 4). The VFA concentration declined after decreasing the temperature. Alkalinity variations were always within ±10% of the average of 3400 mg/L (as $CaCO_3$). Since these variations were very minor compared to

Table II. Summary of feed operational parameters during long-term test (Digester 1D1).

	Ave. ± st. dev. of temperatures (°C), during increase							Ave. ± st. dev. of temperatures (°C), during decrease		
Parameter[a]	53.9 ± 0.09	54.5 ± 0.27	55 ± 0.12	55.5 ± 0.11	56.1 ± 0.09	56.6 ± 0.08	57.2 ± 0.13	56.1 ± 0.08	54.4 ± 0.23	53.3 ± 0.01
Period	2/10/03 to 3/14/03	3/15/03 to 4/16/03	4/17/03 to 5/19/03	5/20/03 to 6/21/03	6/22/03 to 7/24/03	7/25/03 to 8/26/03	8/27/03 to 9/28/03	11/06/03 to 12/23/03	1/6/04 to 1/17/04	1/29/04 to 2/29/04
PS feed rate (m^3/d)	657 ± 4	661 ± 11	661 ± 13	653 ± 42	662 ± 14	659 ± 15	643 ± 76	631 ± 55	635 ± 59	548 ± 60
TS in PS (%)	4.0 ± 0.5	3.8 ± 0.3	4.1 ± 0.6	3.9 ± 0.3	3.8 ± 0.2	3.3 ± 0.34	3.1 ± 0.3	3.4 ± 0.3	3.8 ± 0.2	3.7 ± 0.3
VS in PS (% of TS)	78.5 ± 2.6	78.7 ± 2.7	79.1 ± 1.1	79.8 ± 1.3	79.4 ± 0.69	78.6 ± 1.1	78.2 ± 1.0	79.1 ± 1.4	78.3 ± 1.7	79.2 ± 1.6
TWAS feed rate (m^3/d)	159 ± 12	167 ± 9	169 ± 14	169 ± 9	170 ± 11	167 ± 17	171 ± 9	193 ± 22	204 ± 26	234 ± 27
TS in TWAS (%)	5.6 ± 0.7	6.0 ± 0.7	6.3 ± 1.0	6.6 ± 0.8	6.0 ± 0.6	6.2 ± 0.6	5.6 ± 0.7	5.7 ± 0.8	5.5 ± 0.8	6.1 ± 0.8
VS in TWAS (% of TS)	82.8 ± 1.6	82.8 ± 2.7	83.3 ± 1.5	84.0 ± 1.1	83.4 ± 0.9	82.4 ± 0.5	81.5 ± 1.0	83.1 ± 1.2	83.1 ± 0.9	82.8 ± 0.8
HRT (d)	11.4 ± 0.2	11.2 ± 0.3	11.2 ± 0.3	11.4 ± 0.9	11.1 ± 0.22	11.2 ± 0.3	11.6 ± 1.7	11.3 ± 0.9	11.1 ± 0.8	11.9 ± 1.1

[a] Abbreviations as in Table I.

Table III. Laboratory procedures.

Parameter	Method	Sampling frequency	
		Short-term test	Long-term test
CH_4	EPA Method 18[a]	Daily	Twice weekly
CO_2	EPA Method 18[a]	Daily	Twice weekly
Total alkalinity	Titration, SM 2320 B[b]	Twice weekly	Daily to twice weekly
Volatile fatty acids	Distillation and titration, SM 5560 C[b]	Twice weekly	Daily to twice weekly
Total solids	Gravimetric, 1003-105 C, SM 2540 B[b]	Daily	Daily to twice weekly
Volatile solids	Gravimetric, 550 C, SM 2540 E[b]	Daily	Daily to twice weekly
pH	Electrometric, SM 4500-H^+ B[b]	Twice weekly	Daily to twice weekly
Ammonia	Nesslerization method following distillation, SM 4500-NH_3 B and C	Twice weekly	Daily to twice weekly
$CH_3SH/(CH_3)_2S$	SCAQMD Method 307-91[c]	Daily	Daily
H_2S	Colorimetric tube[d] or SCAQMD Method 307-91[c]	Daily	Daily

[a] U.S. EPA (1992).
[b] American Public Health Association; American Water Works Association; Water Environment Federation (1992).
[c] South Coast Air Quality Management District (1998).
[d] SCAQMD approved Draeger tubes as an alternative method for SCAQMD Method 307-91. No method number was assigned.

the changes in VFA concentration, the curve of the VFA to alkalinity ratio in Figure 5 was very similar to that of the VFA concentration in Figure 4. A small decrease in the methane concentration and a corresponding increase of the carbon dioxide concentration coincided with the increase of the digester temperature (Fig. 6), but these changes do not seem significant for overall digester performance. Variations were observed for VSD, but without a correlation with the temperature as the variations occurred during periods of high temperature and low temperature (Fig. 7). Although the pH increased from approximately 7.2 to 7.7, there was no apparent correlation with the digester temperature, neither was there an effect on the NH_3 concentration in the biosolids.

Long-Term Test with Gradual Temperature Increase

The parameters monitored during the long-term test were the same as those monitored during the short-term test. Figures 8–15 show monthly averages of the results, plotted with the temperature, from March 2003 to February 2004.

VSC production

H_2S production significantly increased at temperatures above 56.1°C. Figure 8a presents the H_2S concentrations in the digester gas, showing that in August, October, and December there were temporary rapid increases of H_2S production,

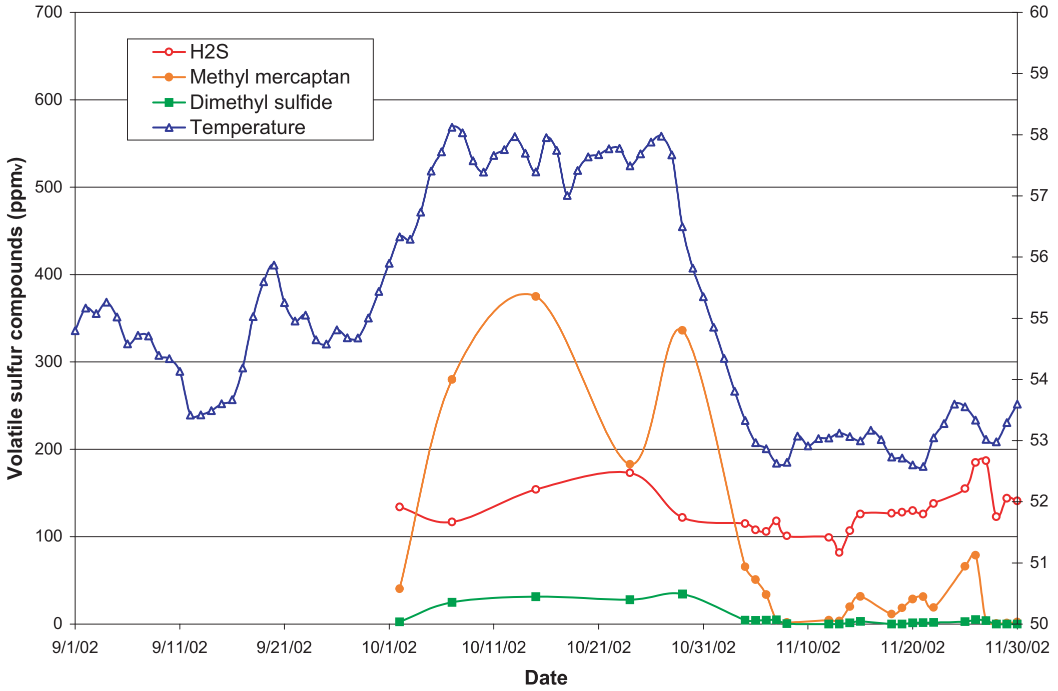


Figure 3. Short term test—Volatile sulfur compounds in combined gas from digesters in both stages. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

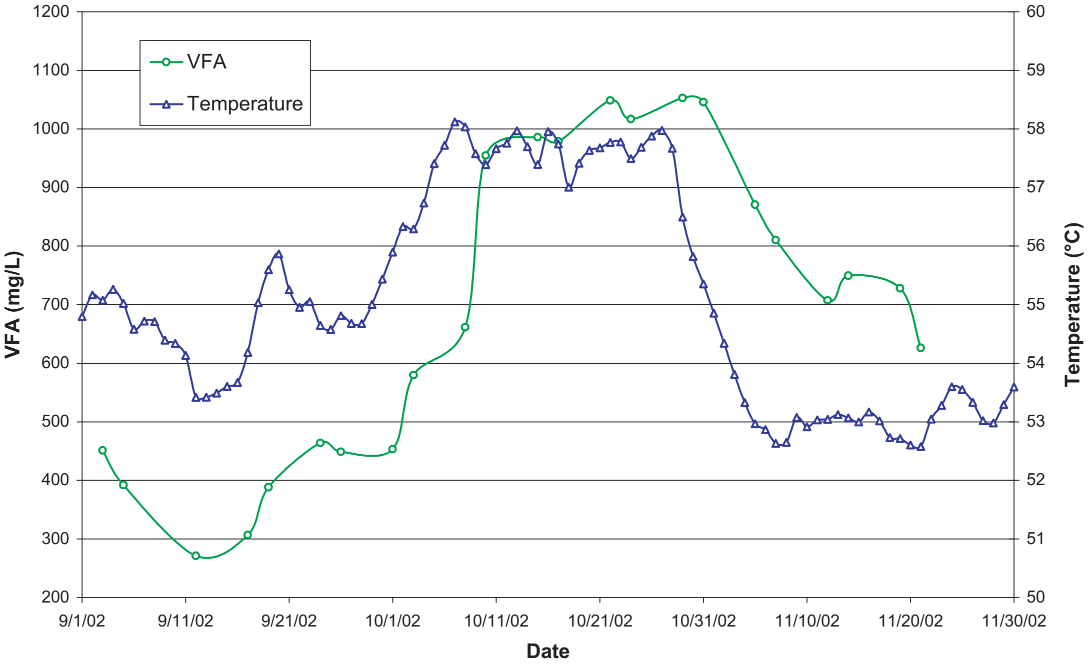


Figure 4. Short term test—Volatile fatty acids for first-stage digesters. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

followed by the injection of larger amounts of ferrous chloride to the incoming sludge, and consequent decreases of H_2S production. Production of methyl mercaptan was first observed at 57.2°C, albeit usually in trace concentrations (Fig. 8b). The injection of ferrous chloride had only little apparent effect on preventing the increase of the methyl mercaptan content. This was one of the reasons that the long-term test did not exceed a temperature of 57.2°C.

Digestion

The average VFA concentration in the biosolids of Digester 1D1 varied irregularly between about 150 and 450 mg/L (as acetic acid), as shown in Figure 9. The observed changes of the VFA concentration in Digester 1D1 were identical to those observed in other digesters, e.g., Digester 2D1, which was held at a constant temperature of 53.3°C (128°F) during

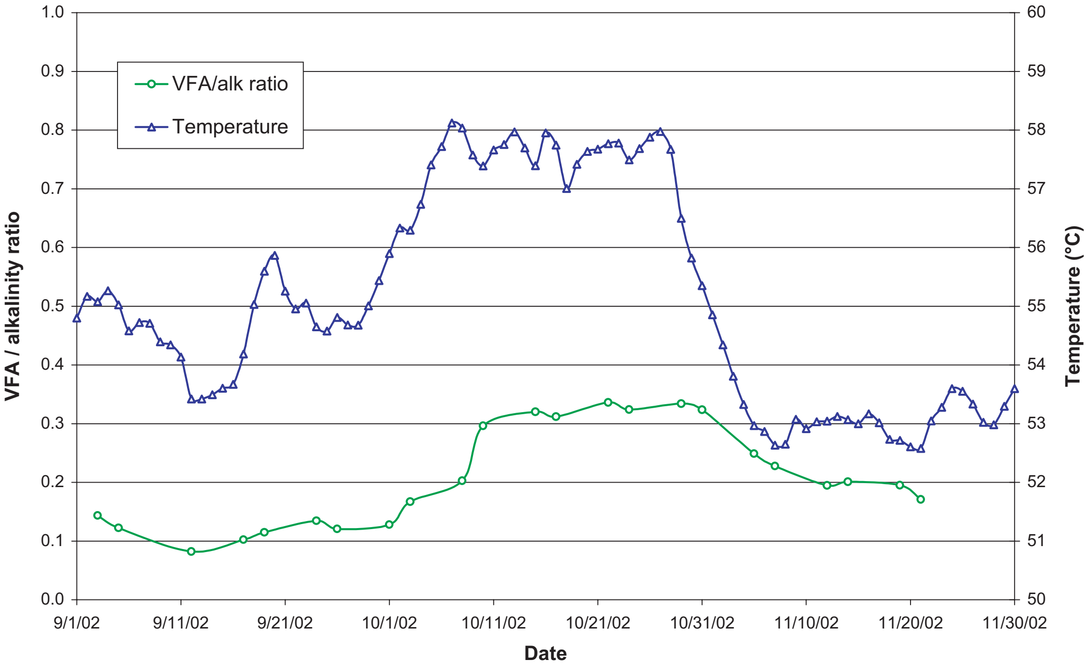


Figure 5. Short term test—VFA to alkalinity ratios for first-stage digesters. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

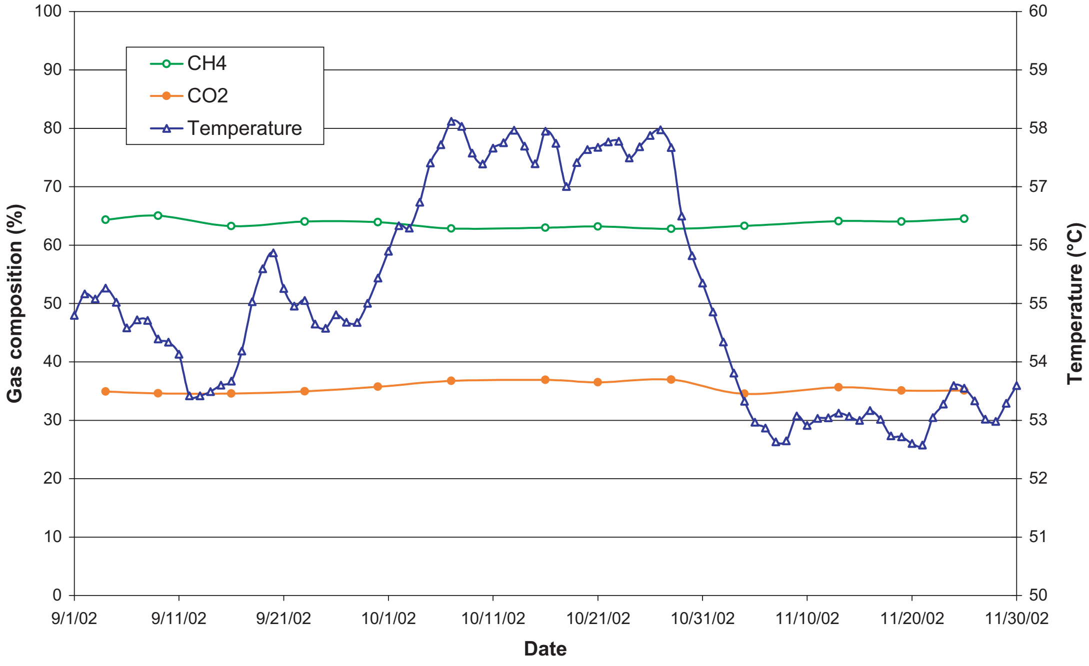


Figure 6. Short term test—Composition of combined gases for digesters in both stages. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

the entire period of the test. The alkalinity in the Digester 1D1 showed significant changes in the range of around 2500 and 4500 mg/L as $CaCO_3$ (Fig. 10), but these changes were identical to the patterns that were observed in digesters operated at a constant temperature. The changes of VFA concentrations and alkalinity in Digester 1D1 were, therefore, not related to the temperature, but probably to changing sludge characteristics. The VFA to alkalinity ratio, shown in Figure 11, was never more than about 0.1, and never reached the high levels of the short-term test. The digester gas composition is shown in Figure 12. The methane content was always in the range of 61.8%–63%, which was the same as during the short-term test, and although the data show some noise, there is no evidence of a dependence on temperature. The VSD declined slightly from 62% at 53.9°C to 58% at 57.2°C, as shown in Figure 13. There was not a steady trend

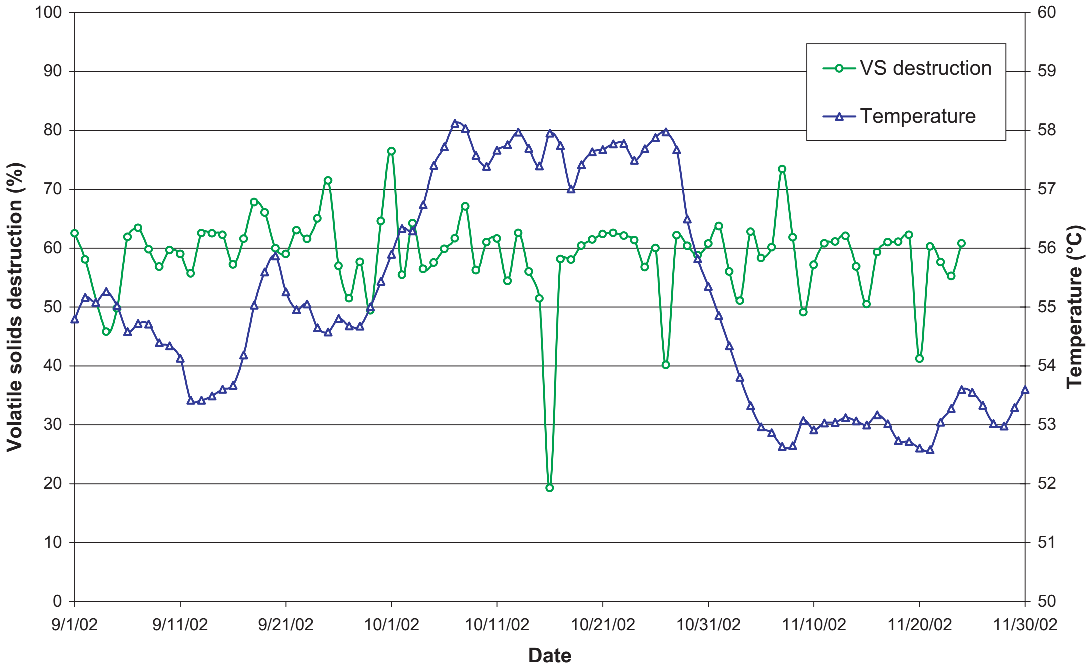


Figure 7. Short term test—Volatile solids destruction for first-stage digesters. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

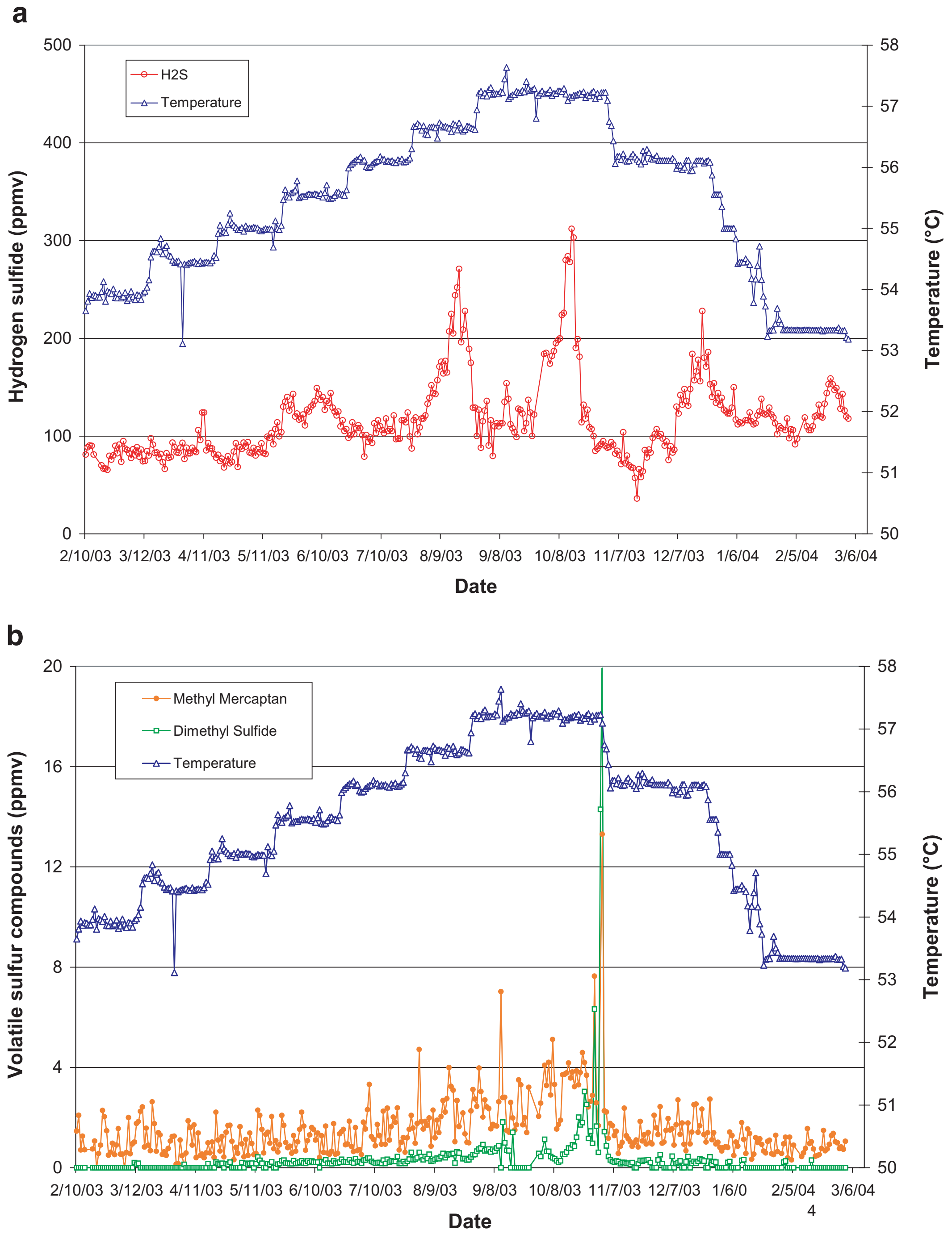


Figure 8. **a**: Hydrogen sulfide in gas from Digester 1D1. **b**: Methyl mercaptan and dimethyl sulfide in gas from Digester 1D1. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

with temperature, since the percentage stayed above 60% up to 56.1°C, but dropped below 60% at 56.6°C. Figure 14 shows the pH of the biosolids in the digester. Like the digester gas composition, this parameter shows random fluctuations, but no perceptible dependence on temperature. Figure 15 shows the ammonia concentration in the biosolids in Digester 1D1. Evidently, this plot closely resembles the variation with time of the VFA concentration in Figure 9 and it even more closely resembles the variation of the alkalinity in Figure 10.

DISCUSSION

Odor Emissions

The increase in odor nuisance during the short-term test can probably be attributed to elevated production of methyl mercaptan. Unlike hydrogen sulfide, methyl mercaptan is not removed by the addition of ferrous chloride to the incoming sludge. Hence, an increase of the methyl mercaptan production in the digester would result in a corresponding

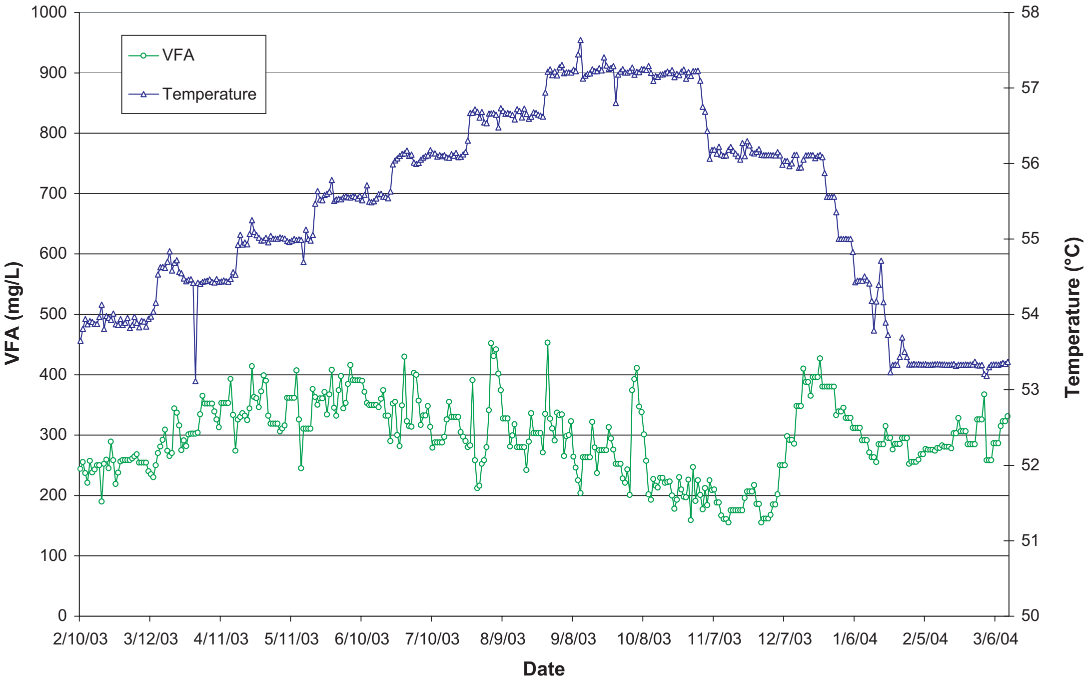


Figure 9. Long term-test—Volatile fatty acids for Digester 1D1. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

increase of methyl mercaptan in digester gas and in emissions to the atmosphere. Another factor contributing to the increase in odor nuisance could have been the elevated production of VFA. Although VFA were only analyzed in biosolids, it may be assumed that they partly volatize to air and, depending on their odor thresholds, may have increased the odor intensity.

Odor abatement at wastewater treatment plants has traditionally been focused on the capture and removal of hydrogen sulfide (Water Environment Research Foundation, 2003). However, the present results suggest the possible importance in some cases of methyl mercaptan, which, like hydrogen sulfide, has an odor threshold in the lower ppb range.

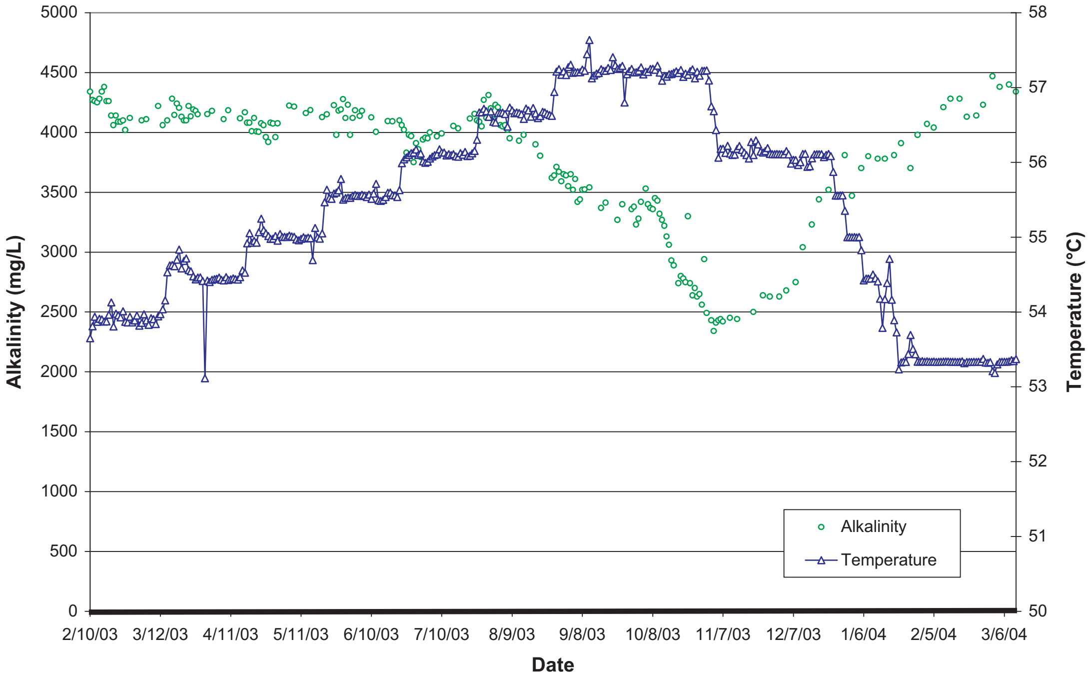


Figure 10. Long term test—Alkalinity in digested biosolids for Digester 1D1. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

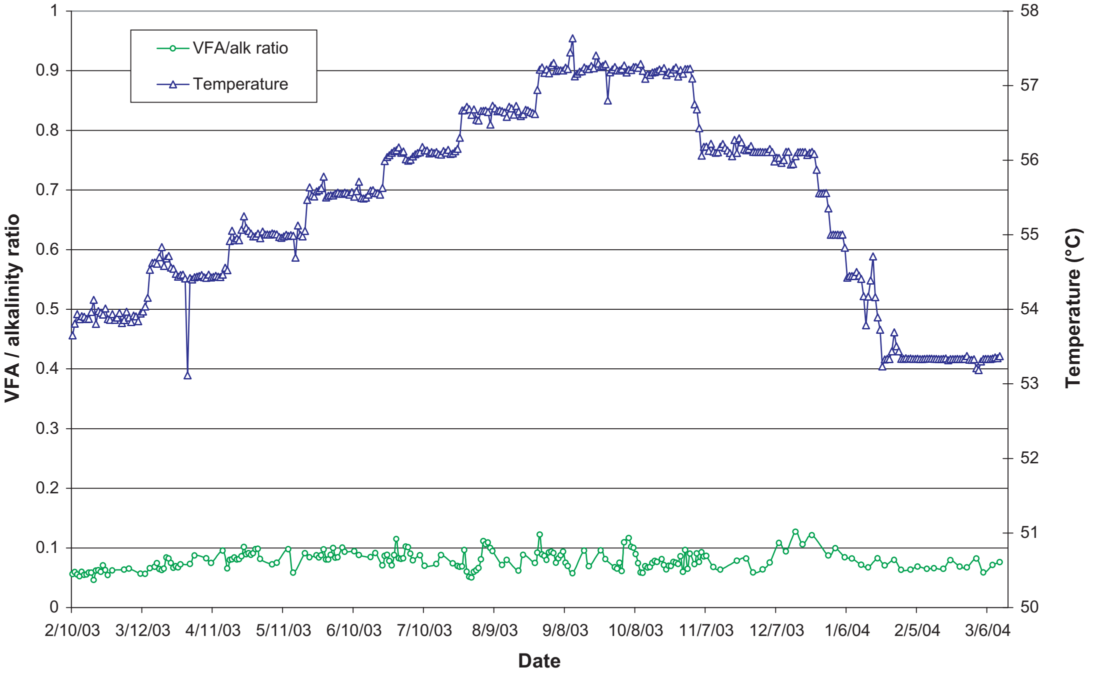


Figure 11. Long term test—VFA to alkalinity ratios for Digester 1D1. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

Microbiological Considerations

It may be postulated that some of the differences that were observed between the two tests may have been caused by imbalances of microbial populations in the short-term tests, which did not occur in the long-term test by allowing microbial populations to adapt to a gradually increasing temperature. This apparent specificity of an established thermophilic culture to a very narrow temperature range is consistent with observations dating as far back as the work of Heukelekian and Kaplovsky (1948), who observed that a culture acclimated to 40°C did not do well at 50°C, despite the fact that both were cultures of true thermophiles that went dormant at 20°C. The increase of the VFA to alkalinity ratio to 0.33 suggests that a rapid increase of the temperature may have resulted in changes of the relative metabolic activities of

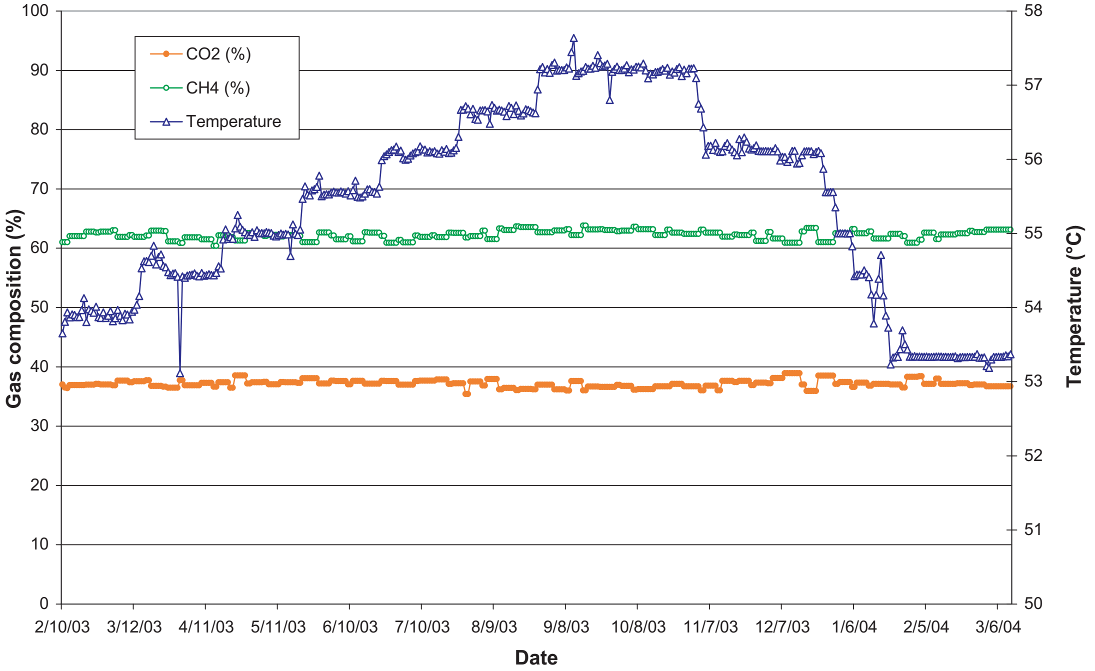


Figure 12. Long term test—Composition of gases for Digester 1D1. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

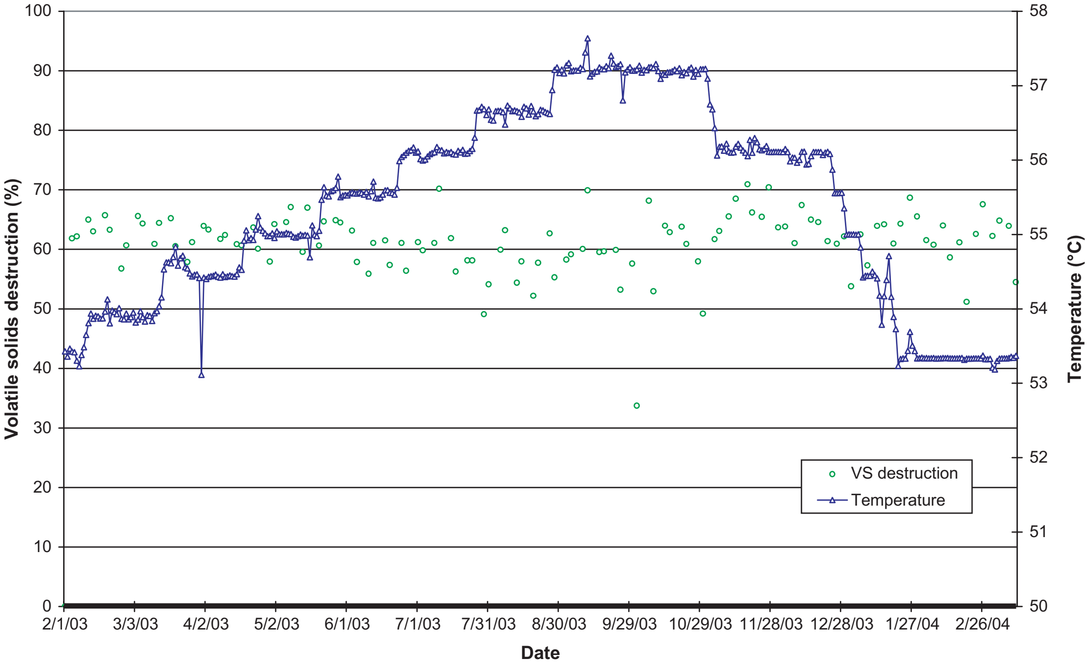


Figure 13. Long term test—Volatile solids destruction for Digester 1D1. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

acidogens and/or methanogens, thereby causing an increase of the VFA production. Likewise, reduced activity of autotrophic methanogens, which convert CO_2 to methane (Andrews and Pearson, 1965), may have caused the slight decrease of the methane content and the concomitant increase of the CO_2 content in digester gas during the short-term test. As both the digester gas composition and the VFA to alkalinity ratio during the long-term test were constant, it may be postulated that gradually increasing the temperature may have allowed a concurrent adaptation of microbial populations. Although these results suggest that a rapid increase of the digester temperature within the thermophilic range should be avoided, this appears to contrast with previous observations (e.g., Aitken and Mullenix, 1992) that a rapid temperature increase is favorable when converting mesophilic digesters to thermophilic operation. The results

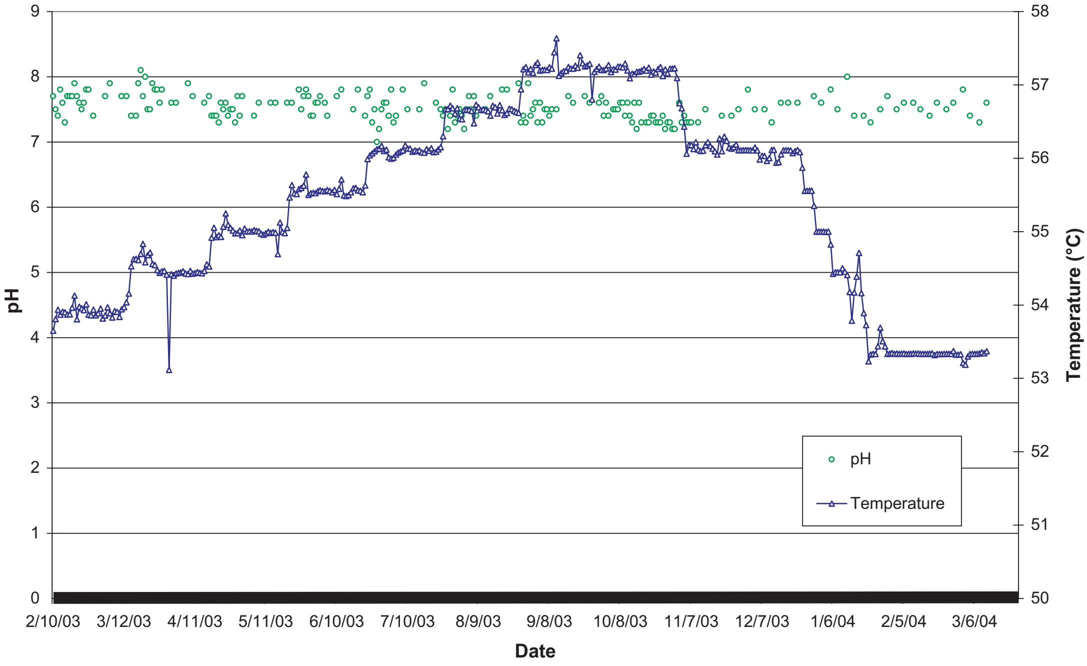


Figure 14. Long term test—pH of discharge for Digester 1D1. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

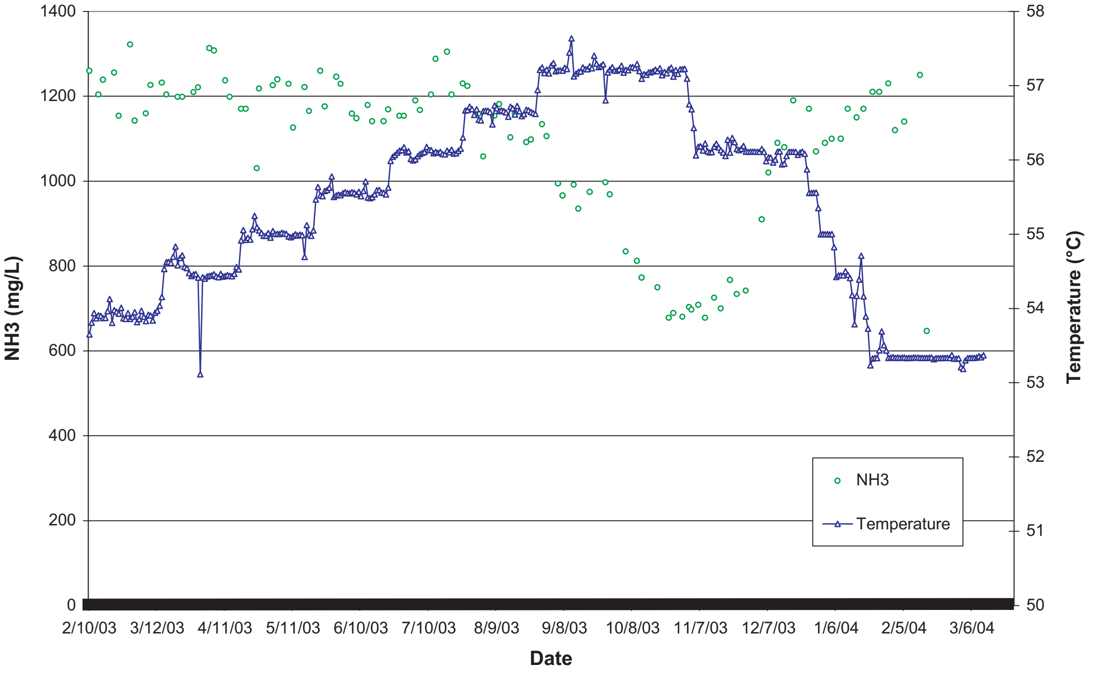


Figure 15. Long term test—Ammonia in biosolids for Digester 1D1. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

of the conversion to thermophilic digestion in the Los Angeles plants are consistent with the opinion of Aitken and Mullenix (1992) that a rapid initial rise is likely to establish a microbial population of true thermophiles instead of thermotolerant mesophilic species (Shao et al., 2002). However, once the digester is in stable operation at a thermophilic temperature, further temperature increases should only be made gradually.

The increase of the concentrations of hydrogen sulfide and methyl mercaptan occurred in both tests and may have been caused by imbalances in sulfur-metabolizing populations. Recent studies have shown that methanogens are involved in the biodegradation of methylated VSC such as methyl mercaptan (Higgins et al., 2003). Hence, inhibition of methanogenic activity would result in the accumulation of methyl mercaptan and other methylated VSC. This is also suggested by the studies of Speece (1996), Yerkes et al. (2002), and Zitomer and Speece (1995). Yerkes et al. (2002) observed that the concentrations of methyl mercaptan and dimethyl sulfide increased when toxic compounds such as chloroform or tetrachloro ethylene were added to an anaerobic sludge digester.

Digestion Performance

Although rapidly increasing the temperature had a significant effect on VFA production and some minor effect on VSD and the digester gas composition, these changes did not affect the overall digester performance during the short-term test. It could have been that the temperature rise to 58°C was too little to have a significant effect, because raising the digester temperature rapidly to 65°C at the Terminal Island Treatment Plant did have a pronounced effect on digester performance. The VFA to alkalinity ratio increased to 0.55, VSD became variable and the methane content of digester gas decreased from 60%–65% to 42% (Iranpour et al., 2004; Kearney et al., 2004). These clear signals of loss of methanogenic activity showed that the limits of the culture's adaptability had been exceeded, as expected from previous research, and thus provide additional perspective on the short-term and long-term studies at Hyperion.

Overall digester performance during the long-term test at Hyperion was not affected by temperatures in the range of 53.9–57.2°C. Since the observed variations in VFA concentration and alkalinity in Digester 1D1 were similar to those in Digester 2D1, which was held at a constant temperature of 53.3°C (128°F), some other explanation for them is needed. They occurred gradually over the months of the test, so it is reasonable to hypothesize that they resulted from unobserved seasonal changes in some aspect of the composition of the plant's sludge, perhaps reflecting changes in the influent to the plant. This possibility is supported by the observation of corresponding simultaneous changes in the ammonia concentration in 1D1, and may deserve future investigation, to provide additional understanding and predictability of system performance. However, its interest for the present study is that the VFA and alkalinity results evidently were not caused by the temperature changes in 1D1.

CONCLUSIONS

Since the hydrogen sulfide concentration did not change significantly, the present results imply that the increase in methyl mercaptan concentrations and perhaps also the elevated production of VFA are the most likely causes of the increased odor released during the short-term test with higher temperatures in late 2002. Since a temperature-related increase of the VFA to alkalinity was observed when the temperature was changed rapidly, but not during the period of slower temperature changes of the study conducted in 2003, these results appear to indicate that the temperature change in 2002 was too fast for the culture to make the adaptation that occurred during the slower temperature change.

Thus, the present results show that in a digester operating below 54°C with a well-established culture of true thermophiles, it is possible to increase the temperature to at least 57°C without an effect on performance, provided that the temperature increase allows sufficient time for microbial populations to adapt and that sufficient ferrous chloride is fed to suppress H_2S production. This is potentially significant for wastewater treatment plants that seek compliance with the time-temperature requirement for batch disinfection in Alternative 1 of 40 CFR 503.32, since they may need to operate their digesters at a relatively high thermophilic temperature if the batch digester holding capacity is limited. Since recent laboratory studies on the anaerobic digestion of manures have demonstrated biochemical instability and poor digester performance at temperatures over 60°C (Ahring et al., 2001; Gabb et al., 2000; Varel et al., 1980), it appears that there may be a maximum temperature somewhere near 60°C, or perhaps a little below it, beyond which thermophilic digestion is unsatisfactory. For the thermophilic anaerobic digestion of wastewater sludge, further research with full-scale digesters is warranted to determine whether this conclusion is correct, and, if so, why the temperature limit is there.

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