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TWO-PHASE, TWO-STAGE, AND SINGLE-STAGE ANAEROBIC PROCESS COMPARISON

By Nuri Azbar[1] and Richard E. Speece[2]

ABSTRACT: In this research, three anaerobic process configurations—namely, two-phase dual sludge (TPDS), two-stage single sludge (TSSS), and single-stage—were evaluated for effluent COD concentration. The same temperature, SRT, and glucose substrate were used in all experiments. In every case, TPDS and TSSS configurations significantly outperformed the single stage. All experiments were carried out at a temperature of 35°C, and all reactors were operated as daily fill-and-draw with HRT = SRT. The following ranges for each design parameter were studied: pH (4.5, 5.5, and 6.5); first stage HRT (3, 8, and 24 h); and floc load (3, 9, and 27 gCOD/gVSS). The overall HRT/SRT of all systems was 30 days, and a pH-Stat system was used to control the pH in the acidification reactors at the desired value. Statistical evaluation of the results indicated that a floc load of 3 in the first reactor of TPDS yielded the lowest effluent COD concentrations under the studied range of parameters, while for the TSSS reactor configuration the staging of the system itself was the controlling phenomena responsible for reduced effluent COD.

INTRODUCTION

Anaerobic processes can operate as mixed cultures under widely varying environmental conditions. Consequently, there are often a great number of metabolic pathways available to the metabolism process, resulting in numerous potential metabolic intermediates that manifest varying degrees of difficulty in the subsequent biotransformation to methane. Up to the present, despite the advantages of anaerobic treatment, effluent produced by this process generally requires further aerobic treatment and therefore is not suitable for direct discharge to surface waters.

It is the writers' opinion, however, that anaerobic treatment should have the inherent capability to produce effluents of high quality comparable to that from aerobic processes, because readily degradable volatile fatty acids often comprise the major fraction of degradable COD in anaerobic effluents. Based on this premise, profoundly reduced effluent COD concentrations were achieved in the writers' laboratory by altering anaerobic reactor configurations, depending upon whether two-phase/stage, plug-flow, or single-state designs were chosen. Substrate characteristics may also have played important roles in achieving the higher quality anaerobic effluents, as evidenced by effluent soluble BOD_5 concentrations of 5–10 mg/L from pseudo-plug flow anaerobic reactors. The research described in this paper was undertaken to test the influence of pH, food-to-microorganism ratio (floc load), and HRT in acidification reactors upon the final effluent soluble COD from the overall process.

Two-Phase Dual Sludge (TPDS) Research Reported to Date

For the purpose of this paper, the TPDS process configuration (commonly called ''two phase'') refers to the development of unique biomasses in separate reactors. The first phase is referred to as ''acid fermentation'' and involves the production of volatile fatty acids (VFA), while the second phase is referred to as ''methane fermentation'' because in it the VFAs are converted to methane and carbon dioxide [Fig. 1(a)]. Due to a briefer solids retention time (SRT), only acidogens are found in the first phase, while both acidogens and methanogens are found in the longer SRT of the second phase. Because the acid-forming and methane-producing species widely differ in physiological and nutritional requirements, Pohland and Ghosh (1971) successfully employed two sequential reactors to separate the acid-forming phase from the methane-forming phase with improved performance.

Since that time a considerable amount of literature concerning the TPDS anaerobic process has been published. As early as 1979, Cohen et al. fed glucose to a TPDS system and demonstrated that under increased loading rates this configuration proved more stable than the single-stage conventional anaerobic process. Later, in 1980, the same team also demonstrated that with the anaerobic digestion of easily hydrolyzable carbohydrates, phase separation increased the maximum specific chemical oxygen demand (COD) turnover rate. At that time Cohen et al. also concluded that the COD turnover rate may amount to a threefold increase in phased systems during continuous feeding and to an average 6–8 fold increase subsequent to shock loading.

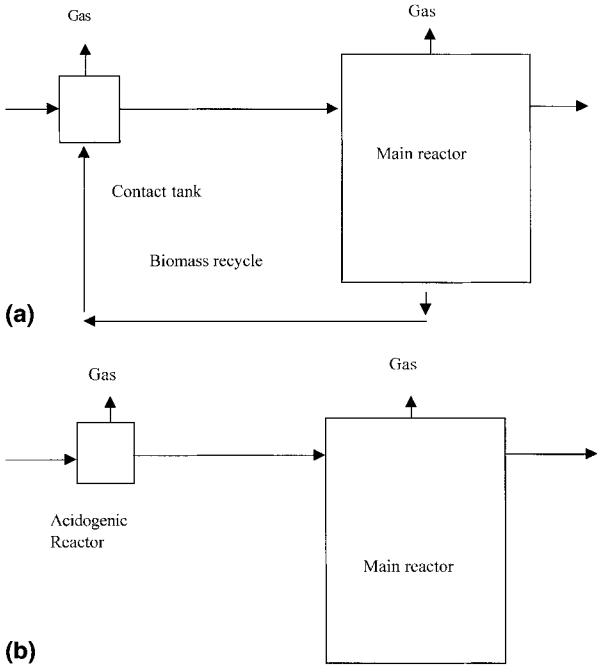

FIG. 1. (a) TSSS and (b) TPDS Configurations

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Zhang and Noike (1991) used both single-stage and two-phase anaerobic processes to compare the characteristics of substrate degradation and found that propionate effluent concentrations were 30–50% higher in the single-stage system. In addition, acetate-utilizing methanogens in the second phase of the TPDS system were established at 2–10 times higher rates than were present in the single-stage system.

Anderson et al. (1994) also studied the changes in microbial populations in TPDS systems and discovered that TPDS systems have several advantages over single-stage systems, such as the facilitation of the selection and enrichment of different bacteria in each reactor, increased process stability, and enhanced buffering of the methanogenic phase pH by the prior acid phase.

Massey and Pohland (1978), Ghosh and Klass (1978), and Cohen et al. (1980) have all demonstrated improved process performance by TPDS systems, which optimize environmental conditions for each phase when compared with single-phase processes, in which both classes of organisms are forced to operate in a common suboptimal environment.

The overall metabolic rate and operational robustness of the methanogenic phase depends heavily on the particular fermentation products formed (Rhen et al. 1997). Thermodynamically, the conversion of glucose to propionate yields the most free energy of all the intermediates including propionaldehyde to the acid-formers. Ironically, propionate yields the least free energy change in further conversion to acetate and H_2 (Fig. 2). Propionate conversion is also most sensitive to H_2 partial pressure while butyrate, propionaldehyde, ethanol, and lactate conversions become increasingly tolerant of H_2 partial pressure.

Pipyn and Verstraete (1981) proposed the production of ethanol and lactate as the best primary products of acidification for the second-phase reactor. It has also been recognized that only acetic, formic acid, methanol, and H_2/CO_2, as well as methyl amine and dimethylsulfide, can be directly used by the methanogens (Bhatia et al. 1985).

The effect of operational parameters on the content and composition of the acidification reactor effluent has also been studied by various researchers (Cohen et al. 1979; De la Torre and Goma 1981; Pipyn and Verstraete 1981; Breure and van Andel 1984; Dohanyos et al. 1985; Dinopoulou et al. 1988). According to Breure and van Andel (1984) and Zoetemeyer et al. (1982), product distribution is little influenced by the hydraulic retention time, but Cohen et al. (1982) maintained that it has a considerable effect on the composition of the effluent. Dinopoulou et al. (1987) found that longer hydraulic retention times resulted in increased acetic acid concentration, whereas the concentration of propionic acid did not change according to differing HRT. Similarly contradictory results have been reported for the effect of pH on the effluent composition, which was negligible in the range of pH 5–7 (Zoetemeyer et al. 1982), while Breure and van Andel (1984) and Dohanyos et al. (1985) found a more pronounced influence. Higher organic loading rates have been reported to result in the production of more propionic acid (Bull et al. 1984).

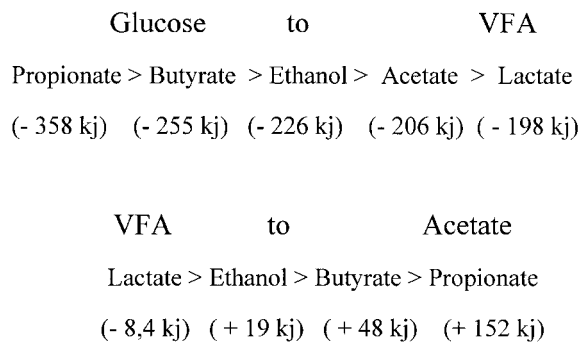


FIG. 2. Gibbs Free Energy Changes for Various Substrates at Different Fermentation Reaction Stages

Fox and Pohland (1994) pointed out that the substrate should not require a syntrophic relationship for acidification in TPDS treatment and that carbohydrates are very suitable for this design. Protein hydrolysis in domestic sludges occurred under methanogenic conditions, but in a recent study by Miron et al. (2000), amino acids such as proline were shown to resist acidification. On the other hand, aspartate and alanine were efficiently converted to methane, as reported by Jain and Zeikus (1989).

Some aromatic compounds can be inhibitory to methanogenesis. A partial conversion in an acidification-phase reactor, such as the conversion of phenol to benzoate (Kobayashi et al. 1989), might reduce the inhibitory effect of phenol in a wastewater. Acidification can detoxify inhibitory fatty acids by saturating double bonds and thus making a wastewater more amenable to methanogenesis. Lipids were satisfactorily degraded in a TPDS in contrast to inhibition caused by poor lipid degradation in the single stage system studied (Komatsu et al. 1991).

Since data on the overall performance of the TPDS process are scarce to date, a trial-and-error process is often used for the design of the acid phase in a TPDS.

Two-Stage Single Sludge (TSSS) Research Reported to Date

In this study, TSSS configuration will refer to two consecutive reactors in which a common microbial consortium is recycled between the second-stage methanogenic reactor and the first-stage acidification reactor [Fig. 1(b)]. The same microbes are thus exposed to different environmental conditions as well as to diverse substrate and metabolic intermediate concentrations in the acidification reactor of a TSSS system. Staging can be accomplished in both suspended growth (two consecutive continuously stirred tank reactors or CSTR) and attached growth systems (packing of dense granules or biofilms).

Published literature on the TSSS anaerobic systems is not as abundant as that on the TPDS, but subtle differences in process configuration such as staging, granules, gas phase management, and combinations have been demonstrated to profoundly improve process performance.

Harper and Pohland (1987) reported that venting of the gas phase from each stage resulted in more efficient and stable performance. They also demonstrated that staging of the gas phase in anaerobic treatment effectively alters the H_2 concentration and thus enables faster and more complete utilization of propionate. Wiegant et al. (1986) compared single- and two-stage upflow anaerobic sludge blanket (UASB) reactors and found 10–13% better treatment efficiency in the two-stage process. They concluded that the improvement was due to removal of the biogas evolved in the first stage.

Van Lier et al. (1994) reported very low effluent volatile acids concentrations, a high degree of biomass retention, and stable reactor performance by thermophilic anaerobic compartmentalized upflow reactors. Grobicki and Stuckey (1991) also documented that the anaerobic baffled reactor (ABR) could operate stably at high organic loading rates (OLR) while still ensuring low effluent VFA.

Duran (1997) observed a rapidly improved performance when he changed the operation of a molasses-fed reactor from single-stage to TSSS configuration. By providing a 24 h contact of the feed with 5–10% of the biomass from the main reactor in a first stage, lower effluent COD was obtained. Demirer (1997) demonstrated that acrylic acid, which is toxic at concentrations above 100 mg/L, nevertheless could be degraded very effectively by using two-stage UASB reactors.

Zhang (1998) studied anaerobic degradation of tetrachloroethylene (PCE) and trichloroethylene (TCE) and observed a decrease in PCE concentration from 6 μmol/L to zero in about 100 h in a two-staged system as compared with only a 50% reduction of the initial concentration in a single-stage system in 170 h. The TCE was essentially all converted to dichloroethylene (DCE) in the two-stage configuration by the end of 100 h, whereas only about 20% of the TCE was converted to DCE in a single stage reactor after 170 h.

Although extensive studies have been conducted on TPDS systems, the TSSS acidification reactor environmental condition impact upon overall performance has not been researched adequately. Data concerning the operational parameters such as pH, retention time, and floc loading in the acidification reactor to date are minimal. The aim of this study, therefore, was to determine the role of acidification reactor environmental conditions on the performance of TPDS and TSSS anaerobic systems as compared with the traditional single-stage system, using glucose as the substrate in all experiments.

MATERIALS AND METHODS

Two types of source inocula, acidogenic and methanogenic, were cultured in the writers' laboratory to provide the biomass needed for TPDS and TSSS experiments. The methanogenic source inocula reactor was initially seeded with digester sludge taken from the Murfreesboro TN Municipal Wastewater Treatment Plant and was fed baby formula (Similac) to support a wide range of microorganisms. The methanogenic inoculum source reactor operated on a daily fill and draw feed schedule under steady-state conditions (pH: 7.0 ± 0.4; MLVSS: 3,000 ± 200 mg/L; an organic loading rate of 1,200 ± 140 mg/L · d; and influent COD: 46 ± 2.7 g/L; HRT = SRT = 40 days) for more than one year. The acidogenic inoculum reactor was fed glucose and nutrient salts and was cultured under acidic conditions (pH 4.5) with HRT = SRT = 2 days, MLVSS = 1,500 ± 250 mg/L, and temperature (T) = 35°C. This acidogenic source inocula was also initially seeded with digester sludge taken from the Murfreesboro Municipal Wastewater Treatment Plant.

Phase separation was accomplished by kinetic controls (Massey and Pohland 1978) using the differences in growth rates of acidifying and methanogenic organisms. A short SRT in the acidfying reactor washed out the slower-growing methanogenic organisms while the faster-growing acidification organisms remained. The consequent lack of methane production was verified, and significant concentrations of VFA were noted in the acidification reactor. After the prescribed contact time in the acidification reactor, a sample of the contents was then transferred to a methanogenic reactor to observe gas production, COD reduction, and VFA concentration after a 30-day incubation period. Results were then compared with the effluent from a single-stage CSTR that had directly received (without prior treatment) the same glucose feed that the acidification reactors were fed.

Glucose was used as the carbon and energy sources throughout the experiments. Trace metals and nutrients were supplemented via Vanderbilt Media (VM), as shown in Table 1.

TABLE 1. Composition of Vanderbilt Media (VM)

Chemical (1)	Concentrations (mg/L) (2)
$NaHCO_3$	6,000
NH_4Cl	500
$MgCl_2 \cdot 6H_2O$	200
KCl	150
$(NH_4)_2HPO_4$	80
Na_2SO4	70
$CaCl_2 \cdot 2H_2O$	50
$FeCl_2 \cdot 4H_2O$	20
KI	10
$(NaPO_3)_6$	10
$CoCl_2 \cdot 6H_2O$	10
$MnCl_2 \cdot 4H_2O$	0.5
H_2BO_3	0.5
$ZnCl_2$	0.5
$CuCl_2$	0.5
$NaMoO_4 \cdot 2H_2O$	0.5
$NiCl_2 \cdot 6H_2O$	0.5
Na_2SeO_4	0.5
$AlCl_3 \cdot 6H_2O$	0.5
$NH_4WO_4 \cdot 2H_2O$	0.5
Cysteine	10
$Na_2S \cdot 9H_2O$	40

EXPERIMENTAL DESIGN

pH-Stat Experimental Procedure

A pH-Stat system capable of controlling and monitoring the pH individually in sixteen reactors was employed to keep the pH in the acidification reactors at a desired value throughout the experiments. The system also provided varying intervals of mixing, pump-on times, acid or base volume injection, pH recording intervals, and tightness of pH control. A schematic of this pH control system is illustrated in Fig. 3. A three-level factorial design was employed to evaluate the impact of three operational parameters (pH, floc load, and hydraulic retention time) within the acidification reactor of the two-reactor systems upon the effluent COD from the methanogenic reactors.

For the TSSS experiments an inoculum of 5–10% of the biomass from the methanogenic second reactor was transferred into the acidification reactor. The pH was manually adjusted initially in the acidification reactor to the desired value and then transferred to the controlled and monitored pH-Stat system. At the end of 3, 8, or 24 h time intervals the acidification reactor contents were analyzed for COD and VFAs; gas production was also recorded. Aliquots of the acidification reactor contents (5–30 mL, depending upon the F/M ratio in the reactors) were taken and injected into the second-stage methanogenic reactors which were 140 mL plastic syringes with a 100 mL operating volume.

Reactor Soluble COD and VFA Monitoring Procedure

The volume of the sample was predetermined to ensure that second-stage methanogenic reactors would start with identical influent COD concentrations and would be inoculated from the main methanogenic source inoculum reactor. During the methanogenic incubation time, the daily gas production was recorded. At the end of the 30-day incubation period, effluent samples were analyzed for soluble COD and VFAs. Similar procedures were employed for the TPDS experiments except that the acidification reactors were inoculated by biomass taken from the acidogenic source inoculum reactor.

Unfed control reactors were always run to account for background gas production. All control and test reactors were run in duplicate. A total of 108 batch experiments (including duplicates) were carried out for the TPDS, TSSS, and CSTR experiments, all performed at a temperature of 35°C.

The operational environmental parameters were applied only to the acidogenic reactor of both the TPDS and TSSS systems. Environmental conditions in the methanogenic second-phase/stage reactors remained constant throughout the entire investigation at a temperature of 35°C and a pH of 7.0 ± 0.5.

ANALYTICAL METHODS

Source inocula were analyzed for suspended and volatile suspended solids, background sCOD, VFA, and pH before

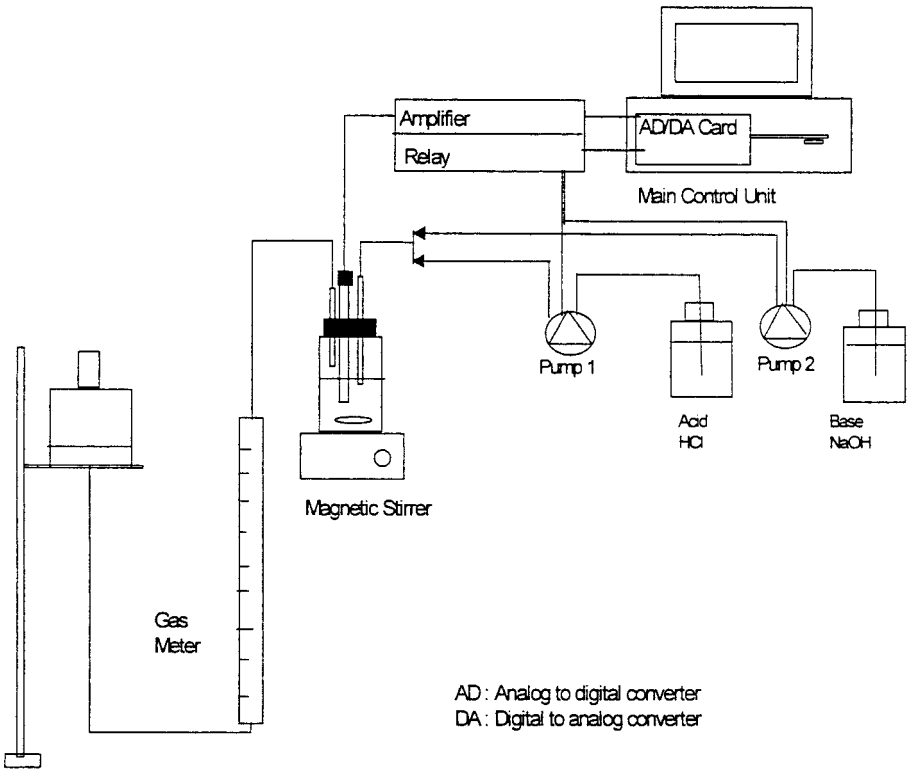


FIG. 3. pH-Stat System Diagram

each experiment. In addition, gas analyses were carried out before and after the glucose was added for the various contact times in both the acidogenic and methanogenic reactors. A Shimadzu gas chromatograph (GC-6AM) equipped with a flame ionization detector and a 1.7 m glass column packed with a 0.3% Carbowax 20M/0.1% H_3PO_4, 60/80 Carbopack-C (Supelco, Inc.), was used for both methane and VFA analyses. The column temperature was maintained at 150°C, and the injector/detector temperature was kept at 200°C with nitrogen as the carrier gas and a flow rate of 40 mL/min. The gas flow rates were gauged at 400 mL/min for air and 60 mL/min for hydrogen. Data integration was achieved with a Dionex 4290 integrator. The detection limit for acetic, propionic, and butyric acid was <10 mg/L.

Liquid samples were prepared by centrifuging (using a Model GP, Beckman Instruments Co.) for 10−20 min at 3,000−4,000 rpm and by filtering 5 mL of the supernatant through a 0.45 μm glass fiber filter (Whatman Co.). The filtered samples were acidified with 10% H_3PO_4 acid to a pH less than 3 to convert the fatty acids to their undissociated forms (i.e., acetic acid, propionic acid, butyric acid, etc.) before injecting 1 μL of the acidified samples into the GC. Biomass was measured as mixed liquor volatile suspended solids (MLVSS) according to Standard Methods 2540 E (APHA et al. 1992). COD measurements were conducted according to the Reflux Colorimetric Method described in Standard Methods 5220 D (*Standard* 1992).

Alkalinity measurements were taken as described in Standard Methods 2320 B (*Standard* 1992). Titration was stopped at pH = 5.8 to estimate the bicarbonate alkalinity and to exclude the VFA alkalinity (Jenkins et al. 1983).

RESULTS AND DISCUSSION

Comparative Results

The final methanogenic effluent COD results of the TPDS experiments after 30 days of incubation are depicted in Fig. 4. Bars in the figure represent two standard deviation values of replicates (with a 95% confidence level). It is noteworthy that reactors with TPDS configuration always resulted in much lower effluent COD concentrations than results from the conventional single-stage process, which yielded an average effluent COD of 2,100 ± 500 mg/L from 12 replicate runs (feed COD = 20,000 mg/L).

The lowest average effluent COD (100 ± 60 mg COD/L) from the two-phase experiments was obtained under the following operational conditions in the acidogenic reactor: floc load = 3; HRT = 8 h; pH = 5.5. There were also several other experiments of different combinations that yielded statistically comparable effluent COD.

The results of acidogenic reactor effluent VFA analyses are given in Table 2. It was observed that the concentration of acetic acid increased as the hydraulic retention time in the acidification reactor increased. All pH values of 4.5 and above resulted in the production of significant amounts of acetic acid. Notably, propionic and butyric acid were either very low or nondetectable.

Fig. 5 demonstrates that the TSSS configuration also produced much lower effluent COD concentrations than the conventional single-phase process, although in general the TSSS experiments yielded slightly higher effluent COD than the TPDS configuration. The TPDS process configuration gave lower effluent COD when compared with TSSS in 20 of 27 cases. Three of the five lowest average effluent sCOD in the TSSS studies were obtained from experiments run at pH = 5.5 and floc load = 3 but an HRT of 3−24 h for the acidification reactor did not result in much difference in effluent COD. Low effluent COD levels recorded for TSSS configuration reactors ranged from 420 to 950 mg/L.

When analyzed for VFA and COD, the main intermediates in the TSSS acidification reactor were acetic acid, propionic acid, and butyric acid (see Table 3). Maximum butyric acid concentrations occurred under an HRT of 24 h at a pH of 5.5. Propionic and butyrate were present in more significant concentrations in TSSS for this design.

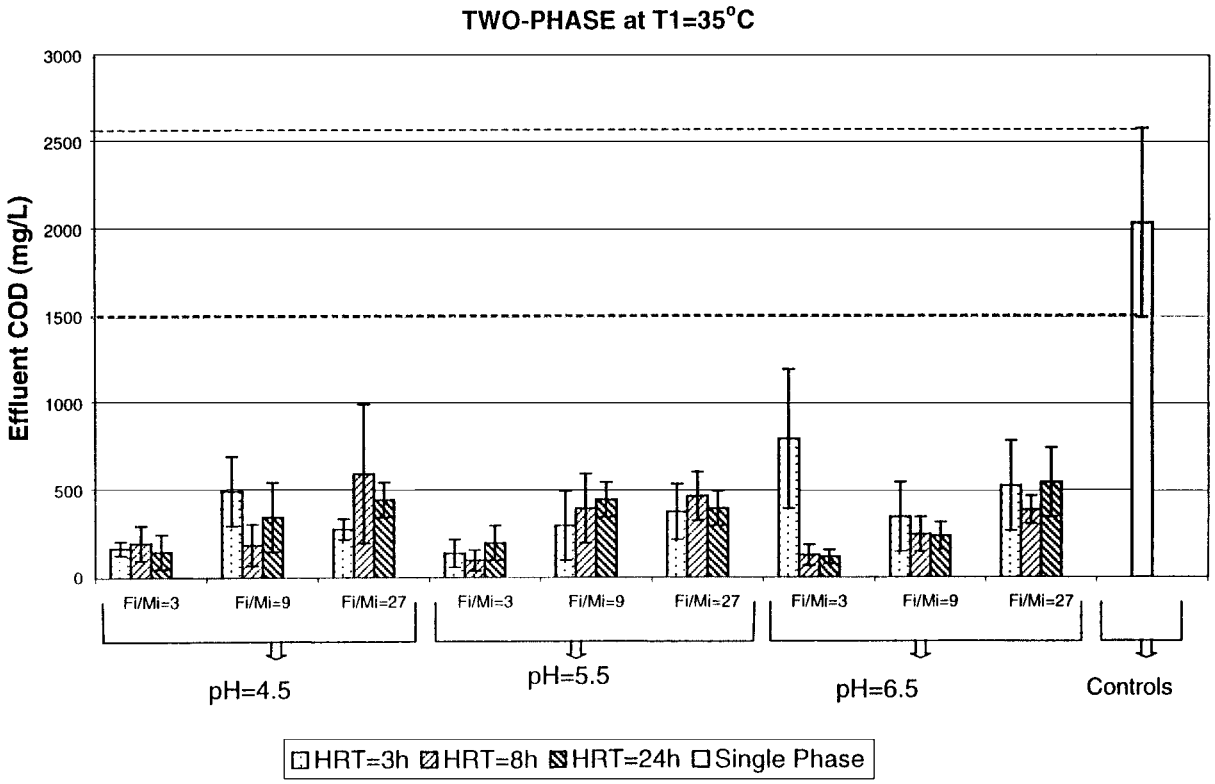


FIG. 4. Effluent sCOD versus pH, F/M, and HRT for TPDS

TABLE 2. TPDS Acidification Reactor VFA Effluent Analyses

Chemical (1)	pH (2)	Floc Load = 3			Floc Load = 9			Floc Load = 27		
		HRT = 3 h (3)	HRT = 8 h (4)	HRT = 24 h (5)	HRT = 3 h (6)	HRT = 8 h (7)	HRT = 24 h (8)	HRT = 3 (9)	HRT = 8 h (10)	HRT = 24 h (11)
Acetate (mg/L)	4.5	270	540	1,800	170	370	1,120	250	500	1,320
	5.5	290	980	1,730	520	1,290	3,700	440	1,220	3,350
	6.5	290	720	1,740	430	1,200	3,150	480	1,180	3,000
Propionate (mg/L)	4.5	ND	ND	90	ND	ND	ND	ND	ND	ND
	5.5	ND	30	ND	ND	ND	ND	ND	ND	ND
	6.5	ND	ND	10	ND	ND	ND	ND	ND	ND
Butyrate (mg/L)	4.5	ND	10	10	10	10	40	ND	ND	50
	5.5	10	10	20	ND	10	20	ND	ND	10
	6.5	ND	10	10	ND	ND	ND	ND	ND	20

Note: ND = not detected.

Both the TPDS and the TSSS experimental results were statistically evaluated using the Analysis of Variance Test (ANOVA) (Box and Hunter 1978). Increase in HRT and floc load in the acidification reactor of the TPDS system showed statistical improvement in effluent COD reduction. ANOVA analysis indicated that the controlling factor in TSSS effluent COD concentration was the staging itself.

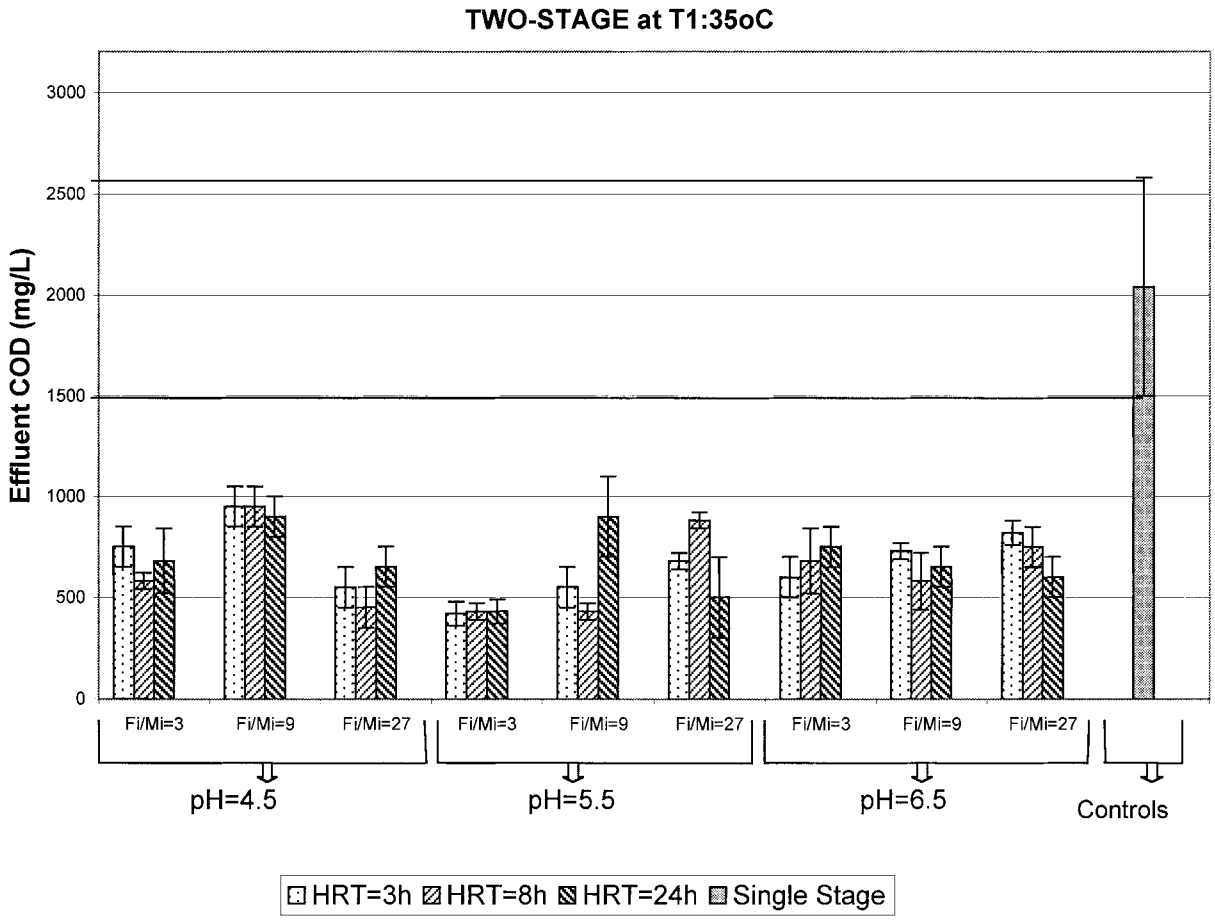
Substrate Gradient Impact on Overall Performance

There appears to be profound benefit accruing to the phasing and staging of anaerobic treatment processes that may be caused by the provision for a substrate gradient, particularly H_2 concentration or the H_2 turnover rate. This substrate gradient may occur in space, time, or both. A spatial gradient may be observed in phased, staged, or plug flow configurations as well as in granules, biofilms, and large diameter flocs, while a temporal substrate gradient is found in batch fed CSTR reactors.

Improved reactor performance associated with substrate gradients may be attributed to one or more of the following factors:

- The reduced pH due to VFA accumulation may provide an optimal environment for the acetogens, while the subsequent neutral pH in the second phase similarly may provide optimal conditions for the methanogens.
- The reduced pH associated with increased VFA concentration may enhance the bioavailability of essential inorganic ions.
- The reduced pH associated with increased VFA concentration may cause the acidogens to produce an intermediate that is energetically less favorable to them and more favorable to the subsequent methanogenic conversion to methane.
- The production of more favorable H_2 concentrations may influence lower COD concentrations.

The relative efficiency and robustness of the acidogens in fermenting common substrates excluding the obligate hydrogen producing acetogens (OHPA), in the opinion of the writers, is not generally overall rate limiting. For sludges, solubilization is often rate limiting, but for soluble substrates methanogenesis is often rate limiting. Thus, it would follow that the process would not require that acidogenic activity be



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		HRT = 3 h (3)	HRT = 8 h (4)	HRT = 24 h (5)	HRT = 3 h (6)	HRT = 8 h (7)	HRT = 24 h (8)	HRT = 3 (9)	HRT = 8 h (10)	HRT = 24 h (11)
Acetate (mg/L)	4.5	70	70	260	60	80	200	70	80	350
	5.5	150	350	960	110	240	2,300	120	300	2,600
	6.5	100	340	1,500	100	320	700	100	350	600
Propionate (mg/L)	4.5	10	ND	20	ND	ND	60	10	10	50
	5.5	30	110	530	30	100	190	30	100	190
	6.5	ND	80	1,600	40	90	170	20	110	200
Butyrate (mg/L)	4.5	10	ND	570	ND	ND	100	60	ND	300
	5.5	ND	ND	840	ND	ND	3,000	ND	ND	3,500
	6.5	ND	ND	ND	ND	ND	400	ND	ND	400

Note: ND = not detected.

optimized with respect to their own energetics, i.e., to produce intermediates that would provide them with the most energy. It would appear instead that the process should be optimized for the activity of propionate fermenters (OHPA) and methanogens.

Elevated propionate and acetate concentrations often found in anaerobic treated effluents also constitute a significant fraction of degradable COD in the effluent from single-stage systems. This raises the question that, since the effluent COD is inherently biodegradable, why should it persist in a single stage CSTR? Although the cause of persisting single stage CSTR effluent high COD is not yet evident, anaerobic series reactors clearly achieve lower effluent COD concentrations when compared with single reactor designs. Phasing/staging designs, which produce substrate gradients and in turn metabolic intermediates, enable better methanogenic conversions in the second reactors.

Since biodegradation of propionate to acetate and H_2 is often problematic, it may be postulated that routing complex substrates through ethanol or butyrate would facilitate more efficient metabolism to methane. As noted in Fig. 2, this phenomenon has been observed in phased systems and, although this strategy results in less energy available to the acidogens, more energy is available to the methanogens. It would appear that some environmental stress is required to force a microorganism to produce an intermediate, reducing the net energy available to it. Additional acid pH or the presence of a substrate gradient may be instrumental in producing this favorable factor in methanogenesis. However, in parallel experiments in our laboratory, the writers consistently noticed that the acidification reaction is significantly improved by maintaining a neutral pH.

The metabolism of propionate also appears to be related to the concentration gradient or H_2 turnover rate. In a companion

study, propionate was shown to be very inefficiently metabolized in a continuously fed single-stage CSTR having a 20-day HRT/SRT and being fed only HPr at 20,000 mg/L. Using this design the effluent propionate concentration remained relatively constant at 3,800 mg/L. However, in contrast a *batch*-fed single-stage CSTR, in which the propionate concentration increased by 1,000 mg/L at the beginning of each day due to an extra propionate spike feeding at the end of 24 h, effluent concentration levels dropped to approximately only 800 mg/L. It is noteworthy that high concentrations of propionate in the acidification phases (>10,000 mg/L) of TPDS and TSSS reactors in the same study decreased below detection limits after 24 h.

A main cause for this highly beneficial, more complete propionate metabolism may be its pathway being altered by series treatment, thus facilitating more efficient conversion to methane. It has been reported that side reactions do have an effect on anaerobic metabolism (Smith and McCarty 1989). Tholozan et al. (1990) demonstrated a reductive carboxylation of propionate to butyrate and then eventual metabolism of butyrate to acetate and methane. However, the authors did not detect significant butyrate or other VFAs in their companion studies.

In a phased/staged system with a substrate concentration gradient in space, time, or both, there will be an associated pH gradient due to VFA changes impacting the solubility and/or bioavailability of essential inorganic ions potentially stimulating microbial activity. For instance, the anaerobic reactor environment with its high alkalinity concentrations precipitates calcium very effectively. The MINEQL model—a chemical equilibrium computer program developed at the Massachusetts Institute of Technology—predicts soluble Ca^{++} concentrations of only 2 mg/L for the Vanderbilt media in an anaerobic environment at pH 8.0. Likewise, the essential presence of low concentrations of sulfide tends to precipitate all of the heavy metals except for chromium. The MINEQL model predicts the following heavy metal solubilities:

- Fe^{++} 1.5×10^{-8} mM
- Co^{++} 1.9×10^{-13} mM
- Ni^{++} 4.9×10^{-12} mM

However, there also appear to be chelators produced by the microorganisms, which facilitate higher heavy metal solubilities than predicted by sulfide precipitation alone.

"Glass Floor" Inhibition Factors

On occasion, the writers have observed that propionate and/or acetate concentrations do not decrease below a concentration of >2,000 mg/L even when substrate feeding is stopped. This phenomenon is being termed ''glass floor'' inhibition. In one case in the writers' research, when propionate was the only substrate added, rapid removal of the propionate after feeding was accompanied by stoichiometric methane production. In the study methane gas production was observed to cease when the propionate concentration reached a ''glass floor'' concentration of approximately 1,000 mg/L. However, supplementa-

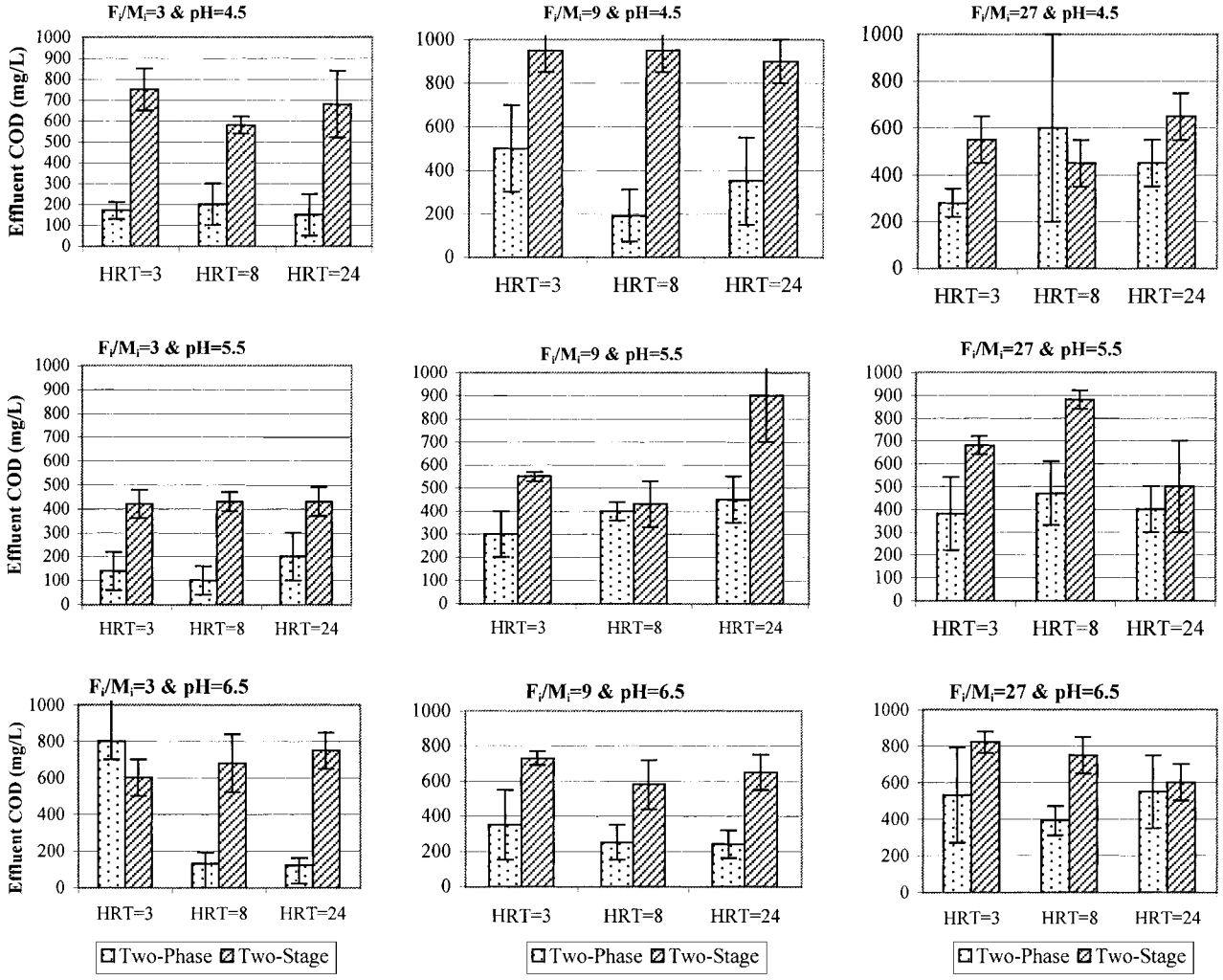

FIG. 6. Comparison of Effluent sCOD from TPDS and TSSS

tion of Fe^{++} to the reactor eliminated the ''glass floor'' inhibition and the propionate concentration subsequently decreased to the low level of approximately 100 mg/L.

Considering the essential role of trace metals in acetate conversion to methane, it appears likely that propionate metabolism similarly may be dependent on trace metal bioavailability, and this hypothesis has been confirmed by ongoing studies in the writers' laboratory. Possibly, pH reduction due to phasing/staging also plays an important role in the observed enhancement of anaerobic performance in phased/staged systems.

TPDS, TSSS, and Single-Stage CSTR Comparative Results

Fig. 6 compares TPDS and TSSS effluent COD. In this research, with only two exceptions out of 36 combinations, the TPDS process gave a lower effluent COD concentration than the TSSS at floc loadings of 3 and 9. At a floc loading of 27, there was no clear advantage to either configuration. The TSSS system recycle of methanogens in the first phase was capable of metabolizing H_2 to methane and therefore could cause lower H_2 concentrations in the first stage. The TPDS system has nil H_2-consuming microbes in the first stage and thus would not consume H_2.

Although both the TPDS and TSSS anaerobic process configurations had lower effluent COD concentrations than their single-stage counterpart, the experiments using the TPDS configuration resulted in slightly lower effluent COD values than the TSSS configuration. Manipulation of various environmental factors in the acidification reactor of two-phase systems apparently enhanced the biodegradative efficiency of the natural microbial populations.

The TPDS and TSSS reactor configurations, which employ two separate CSTR reactors in series, as a whole tend to behave more like a plug flow design. However a plug flow effect was not found to be significant in this study because there were relatively small volumes in the acidification reactors of both TPDS and TSSS configurations (3/24, 8/24, and 24/24 of a day), while the second stage or main reactor HRT was 30 days.

CONCLUSIONS

The results reported in this research apply only to glucose substrate. Phasing/staging designs, which produce substrate gradients and in turn metabolic intermediates, profoundly enhanced methanogenic conversion in the second reactors. Whether the same results would be noted using protein, lipid, or slurry substrates will have to be determined by additional experimentation. Using a glucose feed concentration of 20,000 mg/L, both the TPDS combinations (achieving 100–800 mg/L effluent COD) and the TSSS combinations (at 420–950 mg/L) consistently outperformed the single-stage systems, which produced much higher effluent COD levels of 2,100 $\pm$ 450 mg/L. ANOVA analysis showed HRT and floc load ratio in the acidification reactor of the TPDS to be statistically important parameters, while staging itself was the controlling factor for TSSS systems. Acetate was the dominant intermediate in TPDS acidification reactors under all conditions, while acetate was considerably less concentrated in TSSS acidification reactors, with propionate and butyrate dominating as intermediates instead. TPDS systems generally produced lower effluent COD than TSSS systems for most parameter combinations.

''Glass floor'' inhibition of propionate metabolism was remedied by adding Fe^{++} to the methanogenic reactor. Trace metal bioavailability was confirmed to positively impact phased/staged anaerobic system performance as well as manipulation of various environmental factors in the acidification reactor, which enhanced the biodegradative efficiency of the microbial populations.

APPENDIX. REFERENCES

Anderson, G. K., Kasapgil, B., and Ince, O. (1994). ''Microbiological study of two stage anaerobic digestion start-up.'' *Water Res*., 28, 2383–2392.

Bhatia, D., Vieth, W. R., and Venkatasubramaniat, K. (1985). ''Steady-state and transient behavior in microbial methanafication. I: Experimental results.'' *Biotechnol. Bioengrg*., 27, 1192.

Breure, A. M., and van Andel, J. G. (1984). ''Hydrolysis and acidogenic fermentation of a protein, gelatin in an anaerobic continuous culture.'' *Appl. Microbiol. Biotechnol*., 20, 40.

Box, G. E. P., and Hunter, J. S. (1978). *Statistics for experimenters, and introduction to design, data analysis, and model building*, Wiley, New York.

Bull, M. A., Steritt, R. M., and Lester, J. N. (1984). ''An evaluation of single and separated-phase anaerobic industrial wastewater treatment in fluidized bed reactors.'' *Biotechnol. Bioengrg*., 26, 1054.

Cohen, A., Breure, A. M., van Andel, J. G., and van Deusen, A. (1980). ''Influence of phase separation on the anaerobic digestion of glucose. I: Maximum COD-turnover rate during continuous operation.'' *Water Res*., 14, 1439.

Cohen, A., Zoetemeyer, R. J., Deusen, A. V., and Andel, J. G. (1979). ''Anaerobic digestion of glucose with separated acid production and methane fermentation.'' *Water Res*., 13, 571–580.

De La Torre, I., and Goma, G. (1981). ''Characterization of anaerobic microbial culture with high acidogenic activity.'' *Biotechnol. Bioengrg*., 23, 185.

Demirer, G. (1996). PhD thesis, Vanderbilt University, Nashville, Tenn.

Dinopoulou, G., Rudd, T., and Lester, J. N. (1988). ''Anaerobic acidogenesis of a complex wastewater. I: The influence of operational parameters on reactor performance.'' *Biotechnol. Bioengrg*., 31, 958–968.

Dohanyos, M., Kosova, J., Zabranska, J., and Grau, P. (1985). ''Production and utilization of volatile fatty acids in various types of anaerobic reactors.'' *Water Sci. and Technol*., 17, 191.

Duran, M. (1996). PhD thesis, Vanderbilt University, Nashville, Tenn.

Fox, P., and Pohland, F. G. (1994). ''Anaerobic treatment applications and fundamentals: Substrate specificity during phase separation.'' *Water Envir. Res*., 66(5), 716–723.

Ghosh, S., and Klass, D. L. (1978).''Two phase anaerobic digestion.'' *Proc. Biochem*., 15, 2.

Grobicki, A., and Stuckey, D. C. (1978). ''Performance of the anaerobic baffled reactor under steady state and shock loading conditions.'' *Biotechnol. Bioengrg*., 37, 344–355.

Harper, S. R., and Pohland, F. G. (1987). ''Enhancement of anaerobic treatment efficiency through process modification.'' *J. Water Pollution Control Fed*., 59, 152–161.

Jain, M. K., and Zeikus, J. G. (1989). ''Bioconversion of gelatin to methane by a coculture of *Clostridium collagenovorans* and *Methanosarcina barkeri*.'' *Appl. Envir. Microbiology*, 55, 366–371.

Komatsu, T., Hanaki, K., and Matsui, T. (1991). ''Prevention of lipid inhibition in anaerobic processes by introducing a two phase system.'' *Water Sci. and Technol*., 23, 1189–1200.

Mamouni, R. E., Rouleau, D., Mayer, R., Guiot, S. R., and Samson, R. (1992). ''Comparison of the novel multiplate anaerobic reactor with the upflow anaerobic sludge blanket reactor.'' *Proc., 46th Purdue Industrial Waste Conf*., Lewis Publishers, Chelsea, Mich.

Massey, M. L., and Pohland, F. G. (1978). ''Phase separation of anaerobic stabilization by kinetic controls.'' *J. Water Pollution Control. Fedn*., 50, 2204–2222.

McCarty, P. L., and Smith, D. P. (1986). ''Anaerobic wastewater treatment: Fourth part of a six-part series on wastewater treatment processes.'' *Envir. Sci. and Technol*., 20(12), 1200–1206.

Miron, Y., Zeeman, G., van Lier, J., and Lettinga, G. (2000). ''The role of sludge retention time in the hydrolysis and acidification of lipids, carbohydrates and proteins during digestion of primary sludge in CSTR systems.'' *Water Res*., 34, 1705–1713.

Pipyn, P., and Verstraete, W. (1981). ''Lactate and ethanol as intermediates in two phase anaerobic digestion.'' *Biotechnol. Bioengrg*., 23, 1145–1154.

Pohland, F. G., and Ghosh, S. (1971). ''Developments in anaerobic stabilization of organic wastes: The two phase concept.'' *Envir. Letters*, 1, 255–266.

Rhen, N., Wang, B., and Huang, H. (1997). ''Ethanol-type fermentation from carbohydrate in high rate acidogenic reactors.'' *Biotechnol. Bioengrg*., 54(5), 428–433.

Standard methods for the examination of water and wastewater. (1992). 18th Ed., American Public Health Association, Washington, D.C.
Sutton, P. C., and Li, A. (1983). ''Single phase and two phase anaerobic stabilization in fluidized bed reactors.'' *Water Sci. and Technol*., 15(8/9), 333–344.
Van Lier, J. B., Boersma, F., Debets, M. M., and Lettinga, G. (1994). ''High rate thermophilic anaerobic wastewater treatment in compartmentalized upflow reactors.'' *Water Sci. and Technol*., 30, 251–261.
Wiegant, W. M., Hennink, M., and Lettinga, G. (1986). ''Separation of the propionate degradation to improve the efficiency of thermophilic anaerobic treatment of acidfied wastewaters.'' *Water Res*., 20, 517–524.
Zhang, H. (1998). PhD thesis, Vanderbilt University, Nashville, Tenn.
Zhang, T. C., and Noike, T. (1991). ''Comparisons of one-phase and two phase anaerobic digestion in characteristics of substrate degradation and bacterial population levels.'' *Water Sci. and Technol*., 23, 1157–1166.
Zoetemeyer, R. J., Heuvel, V. D., and Cohen, A. (1982). ''pH influence on acidogenic dissimilation of glucose in an anaerobic digester.'' *Water Res*., 16, 303–311.