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EFFECT OF PROCESS CONFIGURATION AND SUBSTRATE COMPLEXITY ON THE PERFORMANCE OF ANAEROBIC PROCESSES

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Abstract—The roles of substrate complexity (molecular size of the substrate) and process configuration in anaerobic wastewater treatment were investigated to determine optimal methanogenic technology parameters. Five substrates (glucose, propionate, butyrate, ethanol, and lactate) plus a mixed waste (60% carbohydrate, 34% protein, and 6% lipids) were studied under five reactor configurations: batch-fed single-stage continuous stirred tank reactor (CSTR), continuously fed single-stage CSTR, two-phase CSTR, two-stage CSTR, and single-stage upflow anaerobic sludge blanket (UASB). The substrate feed concentration was 20,000 mg/L as COD. The solids retention time (SRT) and hydraulic retention time (HRT) in the CSTR reactors were 20 d, while HRT in the UASB was 2 d. All reactors were operated for at least 60 d (equal to 3SRT).

Substrate complexity was observed to be less significant under two-phase, two-stage and UASB reactor configurations. Two-phase CSTR, two-stage CSTR, and single-stage UASB configurations yielded the lowest effluent chemical oxygen demands (130–550, 60–700, and 50–250 mg/L, respectively). The highest effluent chemical oxygen demands were detected when feeding glucose, propionate, and lactate to continuously fed single-stage CSTRs (10, 400, 9900, and 4700 mg/L COD, respectively) and to batch-fed single-stage CSTRs (11, 200, 2500, and 2700 mg/L COD, respectively). Ironically, the one stage CSTR — most commonly utilized in the field — was the worst possible reactor configuration. © 2001 Elsevier Science Ltd. All rights reserved

Key words—process configuration, two-phase, two-stage, anaerobic treatment, wastewater, volatile fatty acids

INTRODUCTION

Over the past 30 years the popularity of anaerobic wastewater treatment has increased as public utilities and industries have utilized its considerable benefits. Low biomass production, low nutrient requirements and the energy production of methane gas are all significant advantages over aerobic processing. Due to early failures, however, in spite of its many successes anaerobic biotechnology in general is still considered by many to be an unstable process incapable of producing a high-quality effluent.

On the contrary studies have now shown that anaerobic treatment is in fact a stable process when properly operated. But parameters such as process configuration, temperature, biomass immobilization, pH, nutrient supplementation, and substrate complexity must be carefully scrutinized in order to make possible successful anaerobic treatment. Pohland and Ghosh have long advocated the merits of two-phase anaerobic treatment. Therefore, this study will explore the role of process configuration and substrate complexity in the performance of anaerobic treatment.

The role of process configuration

Most COD in the effluent of anaerobic processes is composed of readily degradable organics (Duran and Speece, 1999). Therefore, in this research a protocol to exploit this advantage was sought to enable anaerobic treatment to give the same high effluent quality as aerobic treatment. A sequential approach was chosen to research more stable operation in the form of phased or separated systems. This approach enabled profoundly differing effects to be observed in anaerobic process performance caused by variations in reactor configuration. Multiple stage configuration vs. single stage, plug flow as opposed to a continuous stirred tank reactor (CSTR) with less favorable kinetics, and fixed film biomass immobilization vs.

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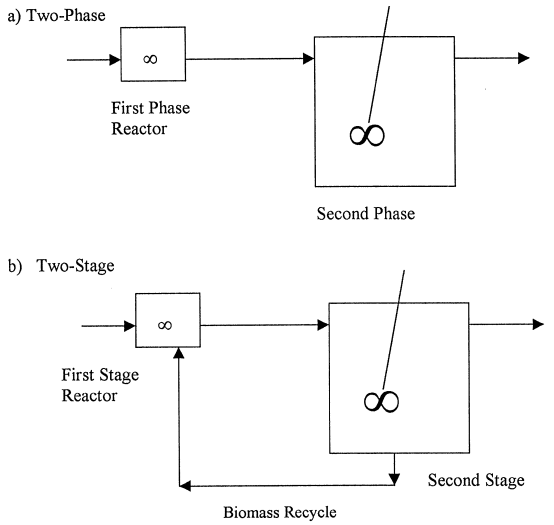


Fig. 1. Two-phase and two-stage process configurations.

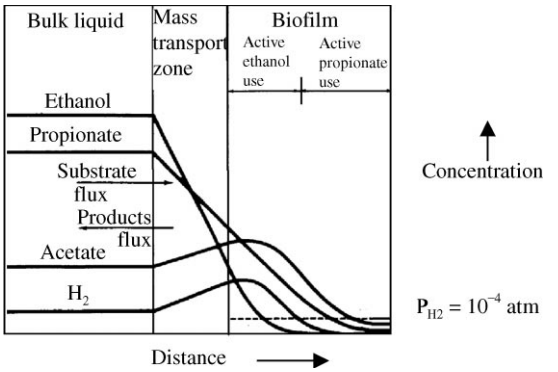


Fig. 2. Substrate and product diffusion (McCarty and Smith, 1986).

dispersed growth were found to produce the greatest improvements.

For the purpose of this article phasing (two-phase configuration) will refer to the development of *different biomasses in separate reactors* while staging (two-stage configuration) will refer to recycling the *same biomass in various environmental conditions,* such as pH, reactor types and concentrations, but also *in separate reactors*. Fig. 1 displays typical schematics of two-phase and two-stage process configurations. Massey and Pohland (1978), Ghosh and Klass (1978), Cohen *et al*. (1980), and Anderson *et al*. (1994) have shown improved performance with the implementation of phasing when compared to a single-stage process. Wiegant *et al*. (1986) also compared single- and two-stage upflow anaerobic sludge blanket (UASB) reactors and found that two-stage configuration yielded significantly better results.

Granular biomass systems

McCarty and Smith (1986) found that dense packing of microorganisms as found in granules has several advantages over dispersed growth systems. Reactant concentration gradients and pH gradients across a granule (characterized by variable pH and reactant concentrations) were seen to enhance granular biomass systems significantly when contrasted to dispersed growth systems which are prone to failure. Fig. 2 depicts substrate and intermediate metabolite distribution across a biofilm, as hypothesized by McCarty and Smith (1986).

The UASB with its pseudo-plug flow kinetics is a good example of a wastewater treatment system employing granular biomass. Because there is limited spatial mixing under plug flow conditions, a spatial concentration gradient is established along the length of the reactor. Likewise a spatial concentration gradient also evolves through the depth of the granules.

Biomass concentration gradient provision

Subjecting biomass to concentration gradients has the potential of enhancing process efficiency by optimizing substrate utilization rates. This increased success is provided for by the consortium of bacteria present in the reactor, each of which is characterized by a different biological kinetic parameter. Consequently, for a given reactor substrate or metabolic intermediate concentration, each variety of bacteria utilizes the substrate at a different rate. By introducing concentration gradients either spatially or temporally, optimal substrate utilization rates may be achieved for various classes of bacteria within the reactor.

Phased and staged systems apply this principle to some degree by operating at a high F/M in the contact reactor and a low F/M in the main reactor, which is the second reactor of a two-reactor system. Conditions in the contactor (first reactor of a two-reactor system) favor biomass with large K_s (half-saturation concentration) and k (substrate degradation rate) values while the methanogenic main reactor favors biomass with lower K_s and k values.

The use of a selector in the activated sludge process also employs this principle. High substrate concentration and short hydraulic retention time (HRT) in the activated sludge selector shift the microbial predominance from filamentous bacteria (with poor settling characteristics) to flocculent bacteria (with good settling characteristics).

Since the substrate utilization rate is greater at higher substrate concentrations for Monod kinetics (assuming K_s values of 20–300 or more mg/L), anaerobic systems can be manipulated to maximize use of higher utilization rates.

Batch feeding (or pulse feeding) introduces a temporal substrate concentration gradient into the reactor. In the batch-fed reactor the substrate concentration is initially at a maximum level after

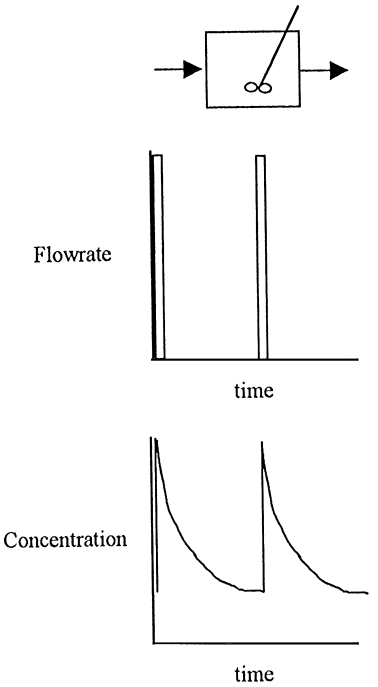


Fig. 3. Temporal flow and concentration profile for single-stage batch-fed CSTR.

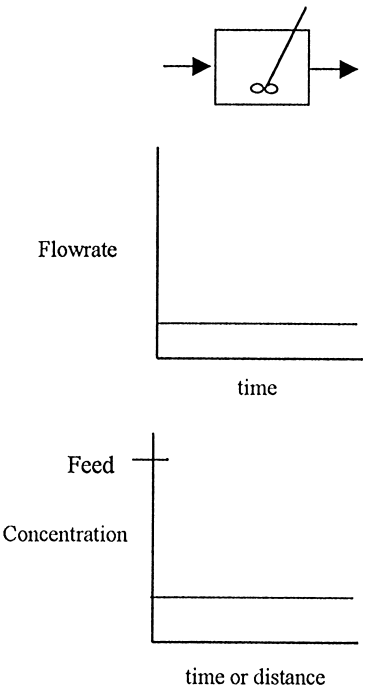


Fig. 4. Flow and concentration profile for single-stage continuously fed CSTR.

feeding and exponentially decreases until the next feeding, as shown in Fig. 3. However, the substrate concentration in the continuously fed CSTR reactor is nearly constant and equal to the effluent concentration.

Feeding mode also governs reactant concentrations. A continuously fed reactor receives a constant influent substrate concentration and has a constant concentration vs. time in the reactor, as shown in Fig. 4. Dispersed growth systems, on the contrary, with small floc and nil diffusional resistance, keep all of the biomass in contact with the same *low* substrate concentration all the time.

As mentioned, the biomass in a continuously fed dispersed growth CSTR is exposed to the same reactor concentration over time and space whereas a plug flow reactor configuration (see Fig. 5) encourages a spatial concentration gradient over time and a batch-fed CSTR design provides a temporal concentration gradient.

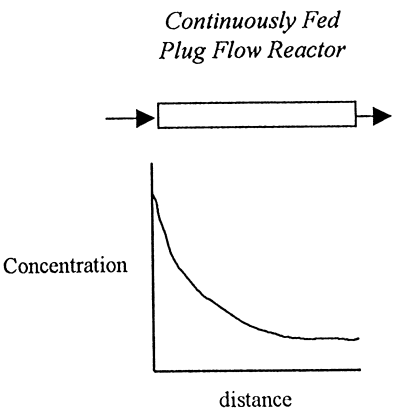


Fig. 5. Concentration profile for a continuously fed plug flow reactor.

Metabolic pathways for more favorable intermediates

Studies have also suggested that the choice of anaerobic process configuration may influence the metabolic pathway by which a contaminant is biodegraded. Thus an anaerobic system could be engineered to produce different intermediates more favorable to the methanogens. Pipyn and Verstraete (1981) noticed that formation of ethanol and lactic acid in a two-phase system enhanced methanogenesis, since these metabolic intermediates provided greater free energy for the methanogens. Inanc *et al.* (1996) showed that when an acidogenic contact reactor was fed with a carbohydrate waste stream and operated at a pH less than or equal to 5.0, butyric acid and acetic acid became the primary intermediates, which were subsequently more easily metabolized in the methanogenic reactor of the two-phase system. Bull *et al.* (1984) operated a glucose-fed two-phase reactor system and noted that ethanol was the primary intermediate in the contactor when the pH was maintained between 3 and 5, while butyrate was the major intermediate at a pH of 5.7.

Limiting H_2 accumulation

Acetate, propionate, butyrate, lactate, and ethanol among others are commonly observed intermediate

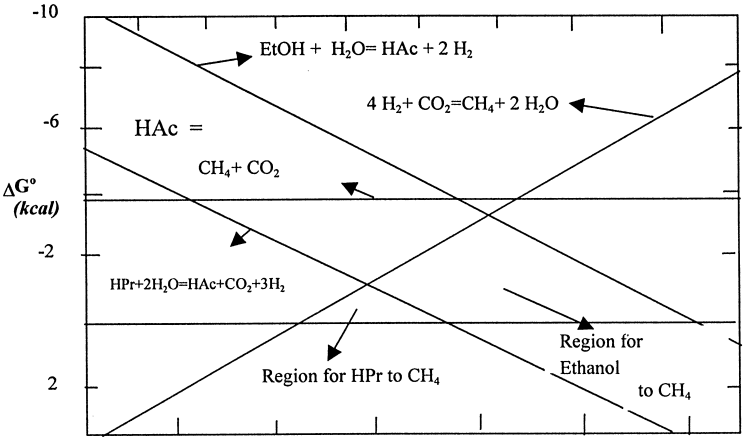


Fig. 6. Effect of H_2 partial pressure on the free energy of conversion of propionate, ethanol, acetate, and H_2 during methane fermentation (adapted from Smith and McCarty, 1989).

fermentation products. According to thermodynamics, propionate can only be converted to acetate if the H_2 partial pressure in the anaerobic reactor is between 10^{-6} and 10^{-4} atm (see Fig. 6). The means for limiting H_2 accumulation is governed by the choice of biomass immobilization technique, gas-phase management methods, and kinetic separation of H_2 production. McCarty and Smith (1986) hypothesized that close proximity of H_2 producers and H_2 consumers found in dense agglomerates of biomass such as biofilms and granules promotes more rapid turnover of H_2. Harper and Pohland (1987) demonstrated that removal of gaseous products from the early stages of substrate conversion improved degradation in the later stages.

Thermodynamic restrictions

It is obvious that thermodynamics play an important role in the degradation of various organics. All oxidation–reduction reactions can be described in thermodynamic terms by Gibbs free energy change values available from substrate catabolism at unit activity and pH 7 (Nichols, 1982). Overall reaction is favorable only if the net free energy change, ΔG, has a negative value. If this value is positive, the reaction under question is not favorable under standard conditions and in order for that reaction to proceed it is necessary for the reaction products (mainly H_2) to be reduced sufficiently in concentration to yield a negative value for the free energy change.

Gibbs free energy change values for various substrates are given in Table 1. It is obvious that conversion of glucose to propionate yields the most free energy change to the acid formers of all the intermediates. Ironically, propionate presents the most difficulty in further conversion to acetate and H_2. Propionate conversion is also most sensitive to H_2 partial pressure while butyrate, ethanol, and lactate conversions are increasingly tolerant of H_2 partial pressure (see Fig. 7).

Table 1. Important fermentation reactions in the absence of sulfate and nitrate[a]

Reaction	ΔG (kJ/mol)
$C_6H_{12}O_6+3H_2O \equiv 3CH_4+3HCO_3^-+3H^+$	−404
Acetate intermediate	
$C_6H_{12}O_6+4H_2O \equiv 2acetate^-+2HCO_3^-+4H^+ + 4H_2$	−206 (HF)
$2acetate^-+2H_2O \equiv 2CH_4 + 2HCO_3^-$	−62 (M)
Ethanol Intermediate	
$C_6H_{12}O_6+2H_2O \equiv 2ethanol + 2HCO_3^-+2H^+$	−226 (HF)
$2ethanol + 2H_2O \equiv 2acetate^- + 2H^+ + 4H_2$	+19 (SA)
$Ethanol + H_2O \equiv acetate^- + 2H_2 + H^+$	+9.6 (SA)
Lactate Intermediate	
$C_6H_{12}O_6 \equiv 2lactate^- + 2H^+$	−198 (HF)
$2lactate^- \equiv 2acetate^- + 2HCO_3^- + 2H^+ + 4H_2$	−8.4 (SA)
$3lactate^- \equiv 2propionate^- + acetate^- + HCO_3^- + H^+$	−165 (HF)
$2lactate^- + 2H_2O^- \equiv butyrate^- + 2HCO_3^- + 2H_2$	−56 (HF)
Butyrate Intermediate	
$C_6H_{12}O_6+2H_6 \equiv butyrate + 2HCO_3^- + 3H^+ + 2H_2$	−255 (HF)
$Butyrate + 2H_2O \equiv 2acetate^- + H^+ + 2H_2$	+48 (SA)
Propionate Intermediate	
$C_6H_{12}O_6+2H_2 \equiv 2propionate + 2H_2O + 2H^+$	−358 (HF)
$Butyrate + 2H_2O \equiv 2acetate^- + H^+ + 2H_2$	+152 (SA)
Acetate to CH_4	
$2acetate + 2H_2O \equiv 2CH_4 + 2H_2CO_3^-$	−62 (M)
H_2 to CH_4	
$4H_2 + HCO_3^- + H^+ \equiv CH_4 + 3H_2O$	−136 (M)

[a] HF: hydrolytic fermentative bacteria; SA: syntrophic acetogenic bacteria; M: methanogenic bacteria.

Multiple intermediate biodegradation of complex substrates

Compounds with larger molecular weight are defined as more complex substrates in this study. Large compounds are typically more difficult to degrade than small compounds. Ease of degradation may be related to the potential metabolic pathway that the substrate follows. For example glucose, a

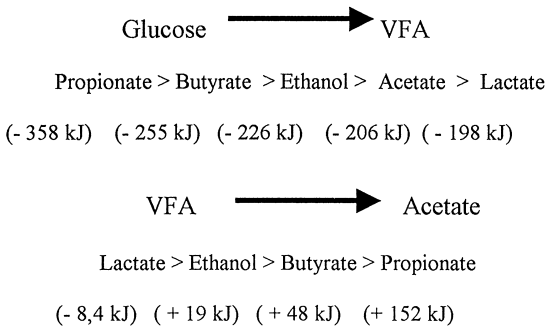


Fig. 7. Gibbs free energy changes for various substrates at different stages of fermentation reactions.

more complex compound, could be converted to a variety of metabolic intermediates including propionate, acetate, butyrate, lactate, and ethanol. Thus multiple intermediates must also be degraded for complete glucose conversion to methane. However, only acetate and H_2 would be the primary intermediates for a relatively small molecule such as ethanol. It can be hypothesized that the most complex compounds present more difficult degradation problems since they involve a more intricate series of biological reactions during the overall conversion to methane.

MATERIALS AND METHODS

As outlined in Table 2, five different reactor configurations with six different substrates and a mixed waste were studied. For this purpose 28 bench-scale reactors were built and used throughout the study. Batch-fed single-stage CSTR, continuously fed single-stage CSTR, batch-fed two-phase CSTR, batch-fed two-stage CSTR, and continuously fed single-stage UASB were the five reactor configurations examined. Glucose, propionate, butyrate, lactate, ethanol, and a mixed waste (60% carbohydrate, 34% protein, and 6% lipids) were the substrates evaluated. The UASB and continuously fed CSTR were not studied in regard to the mixed waste. Experiments with each different substrate were run at least 60 d (3SRT, solids retention time).

All experiments with the exception of the UASB experiments utilized 500 mL serum bottles for the main reactors. The operating volume was set at 200 mL and the bottles were capped with natural rubber sleeve stoppers while the contact reactors were 40 mL glass vials with plastic screw caps and rubber septum. The operating volume was 17 mL for the two-stage system contactors and 20 mL for the two-phase system contactors (the first reactor of the phased or staged system). Sixteen-inch long glass columns with a 2 in diameter were used for the UASB experiments and the operating volume was 650 mL.

All reactors were cleaned with a 10% HCI solution and rinsed with distilled water prior to starting the experiments. All experiments were performed at $35 \pm 1^{\circ}C$ in a temperature-controlled room. Figure 8 shows the experimental setup as well as a summary of HRT, SRT, operating volume, and F/M ratio.

Vanderbilt media constituents are shown in Table 3 with their respective concentrations. Each substrate was

Table 2. Summary of reactor configurations and substrates

Substrate	Configuration	SRT = HRT(d)	Influent COD (mg/L)
Glucose	Two phase	20	20,000
Glucose	Two stage	20	20,000
Glucose	Single stage[a]	20	20,000
Glucose	Single stage[b]	20	20,000
Glucose	UASB	2	20,000
Propionate	Two phase	20	20,000
Propionate	Two stage	20	20,000
Propionate	Single stage[a]	20	20,000
Propionate	Single stage[b]	20	20,000
Propionate	UASB	2	20,000
Butyrate	Two phase	20	20,000
Butyrate	Two stage	20	20,000
Butyrate	Single stage[a]	20	20,000
Butyrate	Single stage[b]	20	20,000
Butyrate	UASB	2	20,000
Lactate	Two phase	20	20,000
Lactate	Two stage	20	20,000
Lactate	Single stage[a]	20	20,000
Lactate	Single stage[b]	20	20,000
Lactate	UASB	2	20,000
Ethanol	Two phase	20	20,000
Ethanol	Two stage	20	20,000
Ethanol	Single stage[a]	20	20,000
Ethanol	Single stage[b]	20	20,000
Ethanol	UASB	2	20,000
Mixed waste	Two phase	40–20–7	20,000
Mixed waste	Two stage	40–20–7	20,000
Mixed waste	Single stage[a]	40–20–7	20,000

[a] batch feeding (once a day).
[b] continuous feeding.

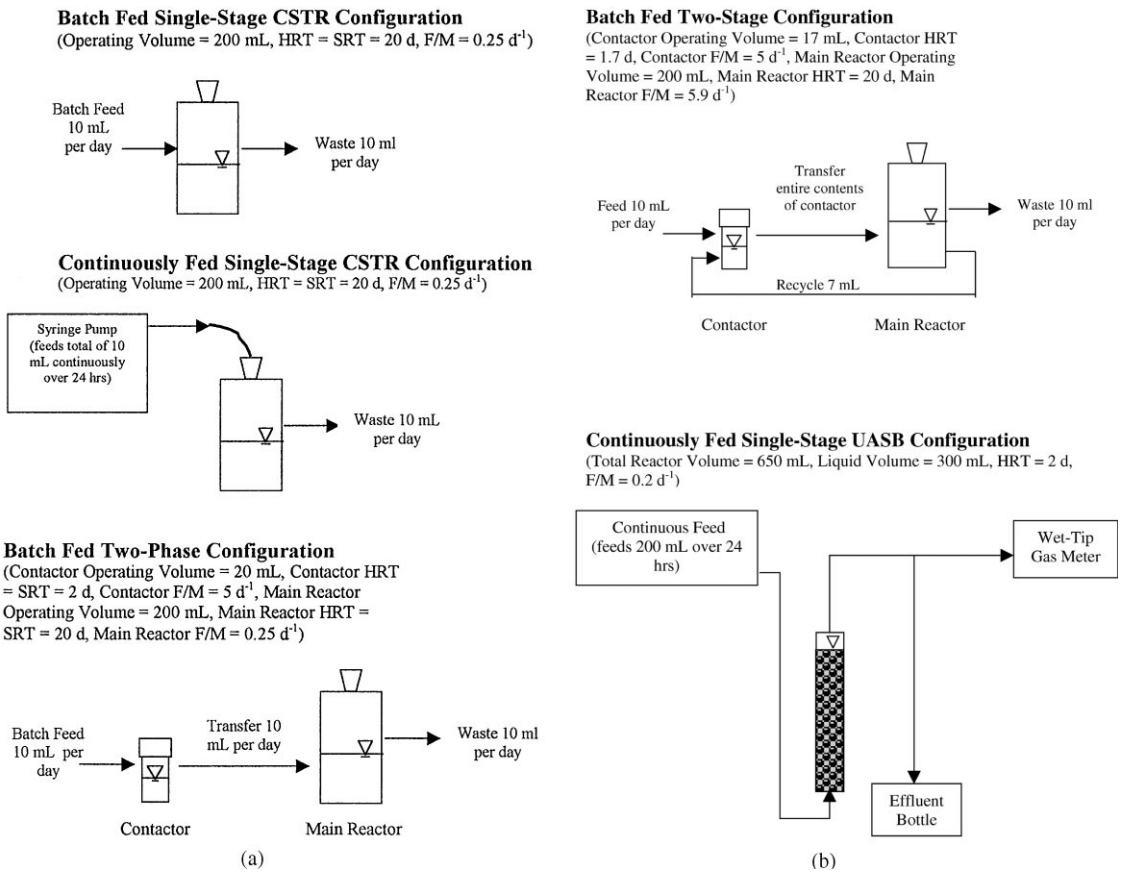


Fig. 8. Experimental setup and daily feeding/wasting routine.

Table 3. Vanderbilt media constituents

Chemical	Concentration (mg/L)
$NaHCO_3$	6000
NH_4Cl	500
$MgCl_2 \cdot 6H_2O$	200
KCl	150
$(NH_4)_2HPO_4$	80
Na_2SO_4	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_4VO_3	0.5
$Na_2WO_4 \cdot 2H_2O$	0.5
Cysteine	10
$Na_2S \cdot 9H_2O$	10

supplemented with the Vanderbilt media before feeding. Additional alkalinity (as sodium bicarbonate) was added directly to reactors to maintain a minimum pH of > 5.0 in the contact reactors and > 6.5 in the main reactors.

The main CSTR reactors were seeded with a methanogenic culture, which was enriched on dry milk and Similac baby formula (60% carbohydrates, 34% protein, and 6% fat) for one year in a 40 L CSTR with $pH = 7.2 \pm 0.4$, $OLR = 580 \pm 70$ mg/L d, $SRT = 40$ d, and $VSS = 3000 \pm 200$ mg/L. The contact reactor for the two-phase system was seeded with an acidogenic culture enriched on glucose in a 2 L CSTR operated for 8 months with an SRT of 2 d. Phase separation was accomplished by kinetic controls (Massey and Pohland, 1978) by using the differences in growth rates of acidifying organisms. Low sludge ages in the reactor tended to wash out the slower-growing methanogenic organisms while the acidifying organisms propagated. The lack of methane production, existence of significant levels of VFA concentration in the reactor, and maintenance of a low pH were the evidence of phase separation. The UASB reactors contained granular biomass enriched on a carbohydrate wastewater with granules obtained from the Smuckers Jelly Company UASB.

Daily gas production in all reactors except the UASB reactors was measured using a water displacement apparatus. Wet-tip gas meters were used to measure gas production in the UASBs. pH was measured daily in all reactors using a pH probe (Corning Co., pH meter 220).

A gas chromatograph (Shimadzu Model GC-6AM) was utilized to measure volatile acids. It was equipped with a flame ionization detector and a 1.7 m glass column packed with 0.3% Carbowax 20M/0. 1% H_3PO_4, 60/80 Carbopack C (Supelco, Inc.). Temperature for the column was kept at 150°C and at 200°C for the injector/detector. The carrier gas nitrogen had a flow rate of 50 mL/min. A Dionex 4290 integrator was used for data integration. Samples were

prepared by centrifuging (Beckman Instruments, Model GP) at 4000 rpm for 15 min and filtering approximately 3 mL of the supernatant through 0.45 μm filter paper (cellulose acetate, Micron Separations Inc.). The filtered samples were then acidified with 10% H_3PO_4 to lower the pH below 3 and ensure that acids were unionized and able to volatilize.

Standard solutions and reagents were prepared and COD concentrations were measured using the closed reflux, colorimetric method (5220 D Standard Method). A spectrophotometer (Milton Roy Co., Spectronic Model 20D) was used to measure absorbency. COD samples were centrifuged and filtered in the same manner as volatile acid samples.

Single-stage CSTR reactors

The daily feeding/wasting routine of the single-stage CSTR reactors was a batch process using a batch-feeding mode. First a 10 mL aliquot was withdrawn from the inlet/outlet tubing via a plastic syringe after measuring the daily gas production, and then the reactor pH was measured using this sample. A 10 mL volume of feed solution having the substrate and nutrient media was then injected into the reactor via inlet/outlet tube.

CSTR reactors with continuous feeding mode were fed using a syringe pump. The syringe pump was set to deliver a total volume of 10 mL of feed daily. No effluent was collected until the end of the 24 h feeding period. A total of 10 mL effluent was withdrawn the next day.

Two-stage CSTR reactors

A batch feeding mode was always utilized for the two-stage CSTR reactors. After a 10 mL aliquot was withdrawn from the main reactor and either wasted or kept for further COD and VFA analysis, another 7 mL of aliquot was withdrawn from the same main reactor to be mixed with the incoming substrate in the contact reactor. After removing the necessary amount of aliquot from the main reactor, the entire contents of the contact reactor were transferred to the main reactor. Then the contact reactor was filled with a mixture of 10 mL fresh substrate nutrient media and 7 mL of returned biomass from the main reactor.

Two-phase CSTR reactors

Similarly pH and gas production measurements were carried out in both contact and main reactors in the two-stage CSTR tests before any feeding action was taken. Ten mL of aliquot was wasted from the main reactor and 10 mL volume of contact reactor contents was transferred from the contact reactor to the main reactor. Subsequently, 10 mL of fresh substrate nutrient solution mixture was added to the contact reactor. No recycle of biomass from the main reactor was applied in this case. The rapid growth rate of the acidogens maintained the existence of the acidifiers in the short HRT/SRT contact tank.

UASB reactors

The UASB reactors were initially filled to 80% of the total volume with granules while the void volume was occupied with the Vanderbilt media. The UASB reactors were fed continuously. Gas production was measured daily using a wet-tip meter and the effluent pH was also measured daily.

RESULTS AND DISCUSSION

In every experimental run the systems were considered to be in steady state when the effluent characteristics showed approximately steady values (less than 10% variation in concentration). To ensure that reasonable steady-state conditions were established, experiments were operated for at least 60 d with an SRT of 20 d.

Role of process configuration

Five different reactor configurations were studied to evaluate the effects of staging, phasing, plug flow kinetics, CSTR kinetics, batch feeding, and continuous feeding on overall process efficiency. The results clearly showed that multiple stage processes and plug flow reactor arrangements outperformed single-stage CSTR systems in terms of COD removal efficiency.

Table 4 and Figs. 9a–f compare the steady-state effluent COD of the various process configurations for each substrate. Error bars in Fig. 9 represent two standard deviations above and below the mean. Effluent VFA concentrations (as COD) are also reported in Figs. 9a–f (effluent VFAs were not measured for the UASBs). Data in Fig. 9 represent the average of the last five data points at the end of 60 d operation time.

The Empirical Rule states that 95% of the observations of a given variable will fall within two standard deviations above and below the mean. For the purpose of comparing data, the authors assume that if the standard deviation error (± 2) bars do not overlap for effluent from two reactors, then their effluent CODs are statistically different.

In nearly all cases, the two-phase, two-stage, and UASB configurations yielded significantly lower effluent COD than the single-stage processes. This result is particularly evident for glucose as a

Table 4. Effluent COD (mg/L) concentrations for various substrates and process configurations at the end of 60 d operation time ($S_o = 20,000$ as mg COD/L)[a]

Substrate	Batch-fed single-stage CSTR	Continuously fed single-stage CSTR	Two-phase CSTR	Two-stage CSTR	Single-stage UASB
Glucose	11,000 (1250)	10,000 (1600)	280 (280)	340 (20)	250 (60)
Propionate	2500 (50)	9900 (1350)	140 (40)	680 (50)	160 (50)
Butyrate	70 (100)	760 (300)	130 (80)	60 (90)	50 (10)
Lactate	2700 (250)	4700 (170)	230 (10)	240 (70)	240 (120)
Ethanol	500 (150)	400 (30)	300 (20)	400 (50)	50 (15)
Mixed waste	1000 (300)	—	550 (100)	700 (170)	—

[a] Numbers in parenthesis two standard deviation above and below the mean.

substrate, as seen in Fig. 9a–d. The single-stage CSTRs removed less than 50% of the influent COD (batch-fed $S = 11,200$ mg/L and continuously fed $S = 10,400$ mg/L), while the two-phase, two-stage, and UASB configurations removed >98% (S= 280, 340, and 250 mg/L, respectively). Lactate as substrate similarly yielded distinctly different effluents (see Table 4). The UASB COD removal efficiency was greater than 96% ($S < 300$ mg/L) for all substrates. These data partially support the hypothesis that processes utilizing concentration gradients produce a better quality effluent.

The results also showed that batch feeding produces a higher quality effluent than continuous feeding. The batch-fed propionate, butyrate, and lactate CSTRs gave significantly lower COD effluents (S= 2500, 70, and 2700 mg/L, respectively) than the continuous fed CSTRs (S = 9900, 760, and 4700 mg/L, respectively) as shown in Figs. 9a–d. Feeding mode (batch or continuous to a single-stage CSTR) yielded similar COD removal efficiency when feeding ethanol or glucose.

The continuously fed *single-stage CSTR* study revealed that

- propionate and glucose yielded higher effluent COD (9900 and 10,000 mg/L, respectively) than all other substrates;
- lactate yielded higher effluent COD (4700 mg/L) than butyrate (760 mg/L) and ethanol (410 mg/L)

The *two-phase CSTR* process configuration resulted in:

- the mixed waste yielding higher effluent COD (550 mg/L) than all other substrates except glucose (error bars overlapped);
- propionate and butyrate yielding lower effluent COD (140 and 130 mg/L, respectively) than ethanol (320 mg/L) and lactate (230 mg/L).

The *two-stage CSTR* process configuration resulted in:

- the mixed waste yielding higher effluent CODs (680 and 700 mg/L, respectively) than all other substrates;
- butyrate yielding an effluent COD (60 mg/L) lower than all other substrates;
- ethanol and glucose yielding higher effluent CODs (400 and 340 mg/L, respectively) than lactate (240 mg/L).

In the study using the *single-stage UASB* configuration:

- glucose yielded a higher effluent COD (250 mg/L) than all other substrates except lactate;

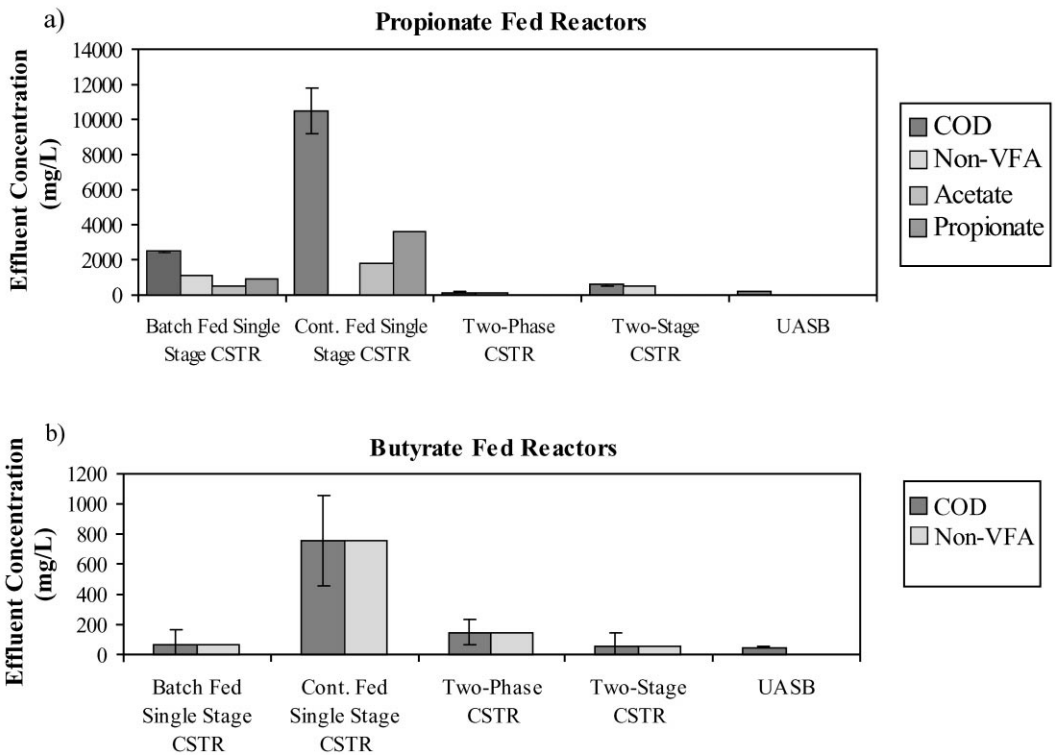
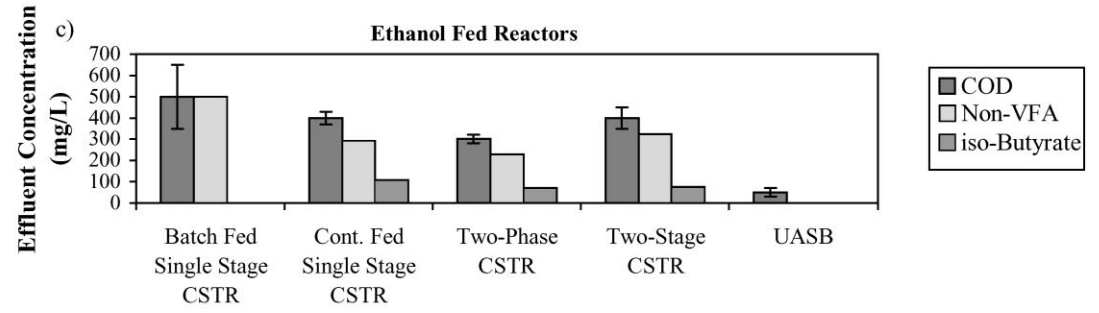
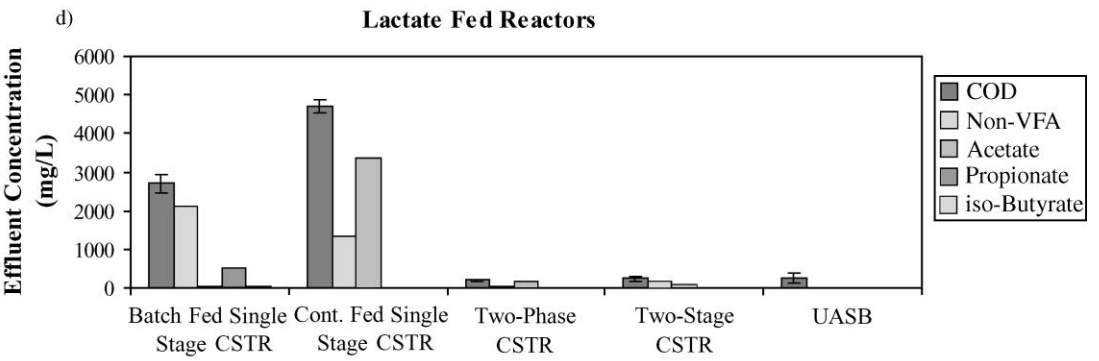
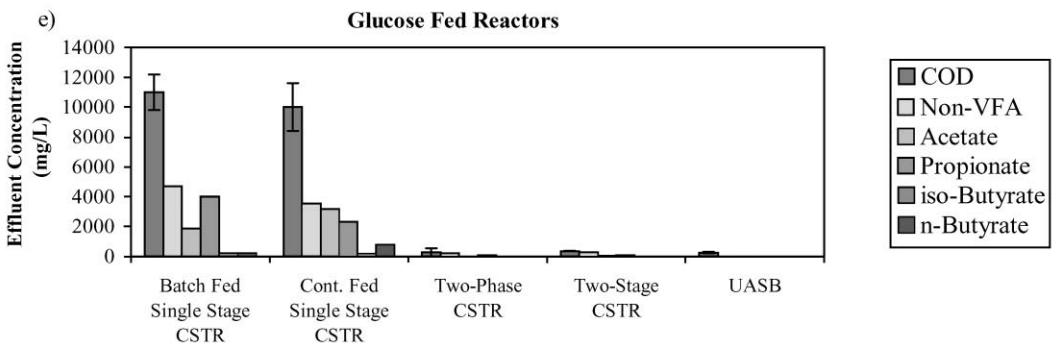


Fig. 9. Effluent COD and VFA at the end of 60 d operation time for various process configurations: (a) lactate-fed reactor, (b) glucose-fed reactor, (c) mixed waste fed reactor (d) propionate-fed reactor, (e) butyrate-fed reactor, and (f) ethanol-fed reactor.







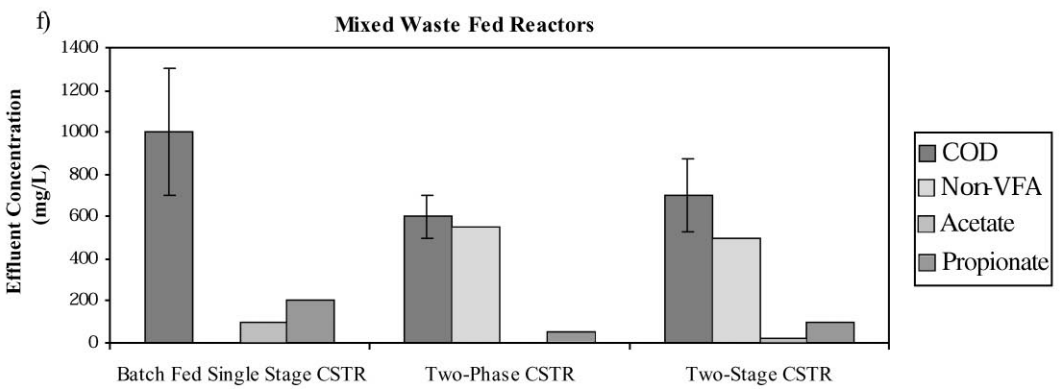


Fig. 9 *Continued*

- ethanol yielded a lower effluent COD (50 mg/L) than all other substrates except butyrate.

Results in Table 4 show that in most cases two-phase configuration resulted in lower effluent COD values, although these results except for propionate and ethanol were not significantly different.

Role of substrate complexity

A variety of substrates were studied to explore the effect of substrate complexity on process efficiency for each process configuration. The results suggest that substrate complexity impacts single-stage CSTR process efficiency, but is less significant under two-phase, two-stage, and UASB configurations.

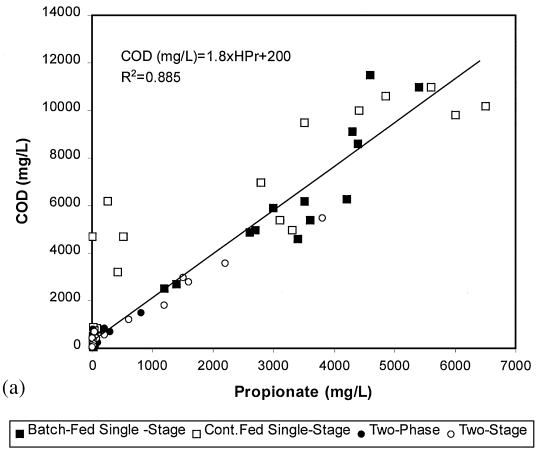
On the other hand, the potential metabolic pathway of a substrate may influence overall process efficiency regardless of reactor configuration. Note that glucose, a larger molecule, or lactate, has the potential to form a variety of intermediates including the sometimes problematic propionate, whereas ethanol which is a smaller compound, typically forms fewer intermediates, the most common being acetate. Elevated VFA concentrations were observed under single-stage CSTR configurations when feeding lactate (continuously fed CSTR acetate concentration = 3400 mg/L) and glucose (batch-fed CSTR propionate concentration = 4000 mg/L, continuously fed CSTR acetate concentration = 3200 mg/L). Elevated VFA concentrations may have inhibited VFA utilization leading to poor effluent COD quality.

The authors also observed a clear statistical difference in COD removal for three-carbon substrates (propionate and lactate) vs. two- and four-carbon substrates (ethanol and butyrate) under single-stage CSTR configurations.

To determine the correlation between effluent COD and effluent VFA concentrations, all the effluent COD values were plotted against corresponding VFA data for each process configuration (see Figs. 10a and b). The results indicated that effluent COD was mostly composed of propionate and acetate, especially for the single-stage CSTRs.

Coefficients (R^2) accounting for correlation between COD and propionate concentration in the effluent were significantly high for the batch-fed CSTRs and the two-stage reactors ($R^2 = 0.94$, and 0.97, respectively). Correlation data from the two-phase configuration and continuously fed reactors were also high enough to indicate that significant portions of the effluent COD were composed of propionate ($R^2 = 0.70$ for the two-phase CSTRs, with $R^2 = 0.82$ recorded for the continuously fed single-stage CSTRs).

Similar evaluation was also carried out for acetate data. Only the two-phase and the batch fed CSTRs yielded reasonably high correlation coefficients ($R^2 = 0.70$ and 0.80, respectively). Data from continuously fed reactors and the two-stage reactors resulted in low correlations between effluent COD and acetate concentrations, $R^2 = 0.40$ and 0.30, respectively.



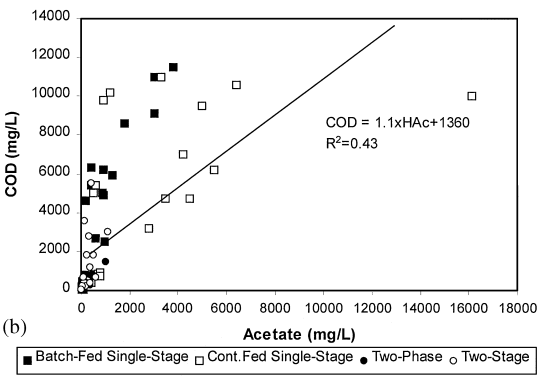


Fig. 10. Correlation between effluent COD and effluent VFA for all reactor configurations: (a) propionate and (b) acetate.

Substrate gradients and improved reactor performance

There appears to be something profoundly beneficial to phasing and staging of anaerobic treatment processes for the substrates studied. Provision for a substrate gradient may be closely related and this substrate gradient may occur in space or time. A spatial gradient occurs in phased, staged or plug flow configurations as well as in granules, biofilms and large diameter flocs and a temporal substrate gradient occurs in batch-fed reactors.

We may hypothesize that the advantage of substrate gradient producing configurations is to be found in the yielding of metabolic intermediates which are more advantageously converted by the methanogens to methane.

Improved reactor performance from substrate gradients may potentially be caused by:

- the reduced pH due to VFA accumulation providing an optimal environment for the

acetogens and in addition to the subsequent neutral pH in the second phase which also provides optimal conditions for the methanogens;
- the alteration of metabolic pathway and intermediates;
- the alteration of H_2 production and consumption;
- the reduced pH associated with increased VFA concentration which may enhance bioavailability of essential inorganic ions;
- the reduced pH associated with increased VFA concentration which may stress the acidogens, causing them to produce an intermediate that is energetically less favorable to them but more favorable to the subsequent methanogenic conversion to methane;
- the action, competition and inhibition of sulfate reducers.

The relative efficiency and robustness of the acidogens in fermenting common soluble substrates, in the opinion of the authors, is not generally overall rate-limiting for the process and does not require that their activity be optimized with respect to their own energetics, i.e., producing intermediates which provide them with the most energy. Rather it would appear to be more beneficial to the overall process to tailor it to enhance the activity of the commonly overall rate-limiting propionate and acetate conversion steps. Both of these two volatile fatty acids are often found at elevated concentrations in the effluent of anaerobic processes and constitute the major fraction of degradable COD in the effluent. Thus the production of intermediates more easily converted by methanogenesis may account for the improved efficiency of phased/staged processes.

The metabolism of propionate also appears to be favored by a concentration gradient, apparently causing the propionate metabolism pathway to be altered in a way which is more favorable for efficient conversion to methane.

Although there is a very high concentration of propionate in the first phase ($>10{,}000$ mg/L) of the two-phase and two-stage processes, the very positive result of the sequential processing by these reactors is that the propionate concentration fell below detection limit after 24 h. A similar beneficial phenomenon was observed in the batch-fed CSTR in spite of the 1000 mg/L propionate concentration increase at the beginning of each day following the feeding. The concentration at the end of the day fell to approximately only 800 mg/L vs. 3800 mg/L in the continuously fed CSTR, evidencing a very inefficient metabolism rate in the latter. This poor performance may indicate a strong necessity for providing a substrate gradient in the reactor design criteria (Fig. 9a).

Since biodegradation of propionate to acetate and H_2 is often problematic, it appears to be desirable to route complex substrates through ethanol or butyrate, which are more efficiently metabolized to methane. This has been observed in phased systems. As noted in Table 1, this routing results in less energy available to the acidogens, yet more energy to the methanogens. It would appear that some environmental stress, such as a more acid pH environment, is required to force a microorganism to produce an intermediate which reduces the net energy available to itself.

It has been reported that side reactions 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, we did not detect significant butyrate or higher VFAs in our systems.

Trace metal supplementation and "glass floor" inhibition

In a phased or staged system with a substrate concentration gradient in space or time, there will be an associated pH gradient due to VFA changes. This gradient will have an impact on the solubility and/or bioavailability of essential inorganic ions that may be sufficient to stimulate microbial activity significantly. For instance, the anaerobic reactor environment with its high alkalinity concentrations precipitates calcium effectively.

Increased propionic acid concentration reduces the pH and this in turn increases the solubility of heavy metals, potentially resulting in stimulation of microbial activity. On occasion we have observed in other experiments that propionate and/or acetate concentrations would not decrease below an elevated "glass floor" concentration of >2000 mg/L, even when substrate feeding was stopped.

The MINEQL model predicts soluble Ca^{++} concentrations of only 2 mg/L for the Vanderbilt Media in an anaerobic environment at pH 8.0. Similarly, the essential presence of low concentrations of sulfide tends to precipitate all of the heavy metals except for chromium. However, there appear to be chelators produced by the microorganisms which facilitate higher heavy metal solubilities than predicted by sulfide precipitation alone.

The MINEQL model predicts the following soluble heavy metal solubilities:

$$Fe^{++}\ 1.5\times10^{-8}\ \text{mM}$$

$$Co^{++}\ 1.9\times10^{-13}\ \text{mM}$$

$$Ni^{++}\ 4.9\times10^{-12}\ \text{mM}$$

In one test when propionate was the only substrate being added and was removed rapidly after feeding, gas production ceased whenever the propionate concentration reached the "glass floor"

concentration of approximately 1000 mg/L. Supplementation of Fe^{++} to the reactor, however, eliminated the "glass floor" inhibition and the concentration subsequently decreased to approximately 100 mg/L.

Regarding the essential role of trace metals in acetate conversion to methane, it is plausible that propionate metabolism may have a similar dependence on trace metal bioavailability. If this is the case, reduced pH may play a role in improving propionate degradation through enhancing trace metal bioavailability. Possibly, pH reduction due to phasing/staging plays a partial role in the observed enhancement of anaerobic performance.

The role of gas venting

There are reports in the literature suggesting that gas release (including hydrogen gas) from the first phase may increase performance (Harper and Pohland, 1987; Rhen *et al.*, 1997). Coincidentally, in this study gas produced in the first reactors of both of our two-phase and two-stage configurations was vented. This phenomenon may have contributed to better performances for the two-phase and two-stage configurations when contrasted to single-stage systems.

Another aspect of the importance of elevated H_2 gas in the headspace was reported by Mamouni *et al.* (1992). They hypothesized that high H_2 partial pressure in the headspace may cause acetate-consumers (which are also capable of metabolizing H_2) to prefer H_2 over acetate, causing an acetate increase because H_2 has a greater free energy change (up to -136 kJ/mol depending on the H_2 concentration) than acetate (-31 kJ/mol). In this manner venting H_2 might also have helped to avoid acetate accumulation which could otherwise cause elevated COD in the effluent. Hydrogen gas in the headspace was not measured in this study.

The manipulation of fermentation products due to the use of specific reactor configurations such as two-phase might be another strong reason for an improved performance. Among the main products of fermentation acetate, ethanol and butyrate are more favorable intermediates. It is ironic that the reactor configuration most commonly utilized in the field — the single-stage CSTR — was the worst of all reactors in performance.

CONCLUSIONS

The results of this study indicate that the two-phase or two-stage systems and the UASB process configuration outperform single-stage CSTR configurations significantly in terms of COD removal efficiency when glucose, propionate or lactate were the substrates. Batch-fed single-stage CSTRs were also shown to produce a higher quality effluent than continuously fed single-stage CSTRs for all substrates except ethanol and glucose, in which there was no statistical difference. The two-phase process configuration resulted in lower average effluent COD values than the two-stage configuration for all substrates tested except butyrate. For the even-numbered carbon compounds, ethanol and butyrate, there was very little difference in effluent COD between batch-fed CSTR and two-phase or two-stage reactor process configurations.

Molecular size and carbon types in a compound or its intermediates significantly influenced substrate degradability efficiency. Under single-stage CSTR configurations, glucose (a larger molecule capable of producing three-carbon intermediates), propionate (three-carbon compound), and lactate (three-carbon compound) substrates yielded the worst performance, i.e., the highest effluent COD. Propionate comprised most of the residual COD found in the effluent.

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