Effect of an Organic Shock Load on the Stability of an Anaerobic Migrating Blanket Reactor

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Abstract: The goal of this study was to examine the effect of an organic shock load on the performance and stability of a laboratory-scale anaerobic migrating blanket reactor (AMBR). To accomplish an organic shock load, nonacidified sucrose solution was almost doubled in concentration, while maintaining a constant hydraulic retention time. The volumetric loading rate (VLR) was increased from 27 to 50 g chemical oxygen demand (COD) L^{-1} day^{-1} for a period of six hydraulic retention times (42 h). This resulted in an increase in the standard methane production rate (liters of methane at standard temperature and pressure per liter reactor volume per day) from 7 to 12 L L^{-1} day^{-1}. The pH levels stayed favorable and biomass washout was limited during the shock load due to the damping effects of a compartmentalized configuration. During the shock load, the propionate production in the initial compartments of the AMBR remained at the same level as before the shock load, while the acetate production rose sharply. Because propionate is the most difficult volatile fatty acid to be removed, unstable conditions due to excessive propionate accumulation during the shock load were prevented. Meanwhile, the acetate concentration in the liquid phase and hydrogen content in the headspace of the final compartments remained low, which ensured propionate degradation. Due to these intrinsic characteristics of the AMBR, the soluble COD removal efficiency stayed above 87% under these stressed conditions. Moreover, the performance of the AMBR reached pre-shock-load levels immediately after the VLR was restored to 25 g COD L^{-1} day^{-1}.

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Introduction

During the last 30 years, anaerobic treatment of industrial and domestic wastewater has proven sustainable, especially after the development of the upflow anaerobic sludge blanket (UASB) reactor. The UASB process and its derivatives have demonstrated excellent performance in numerous full-scale operations worldwide (Lettinga 1995; Hulshoff Pol et al. 1997). Nevertheless, the need for other self-immobilized biomass processes became obvious for a variety of reasons. One is that there is a need for simpler and more economical technologies for wastewater treatment at small and medium sized industries (Hulshoff Pol et al. 1997). Another reason is that the loss of biomass with the effluent due to excessive bed expansion posed problems to noncompartmentalized reactors, such as the UASB process, especially during increases in loading rates (Guiot et al. 1995).

Compartmentalization in anaerobic reactors was first described by Bachman et al. (1982), who developed the anaerobic baffled reactor (ABR). A gas-solids separator system was eliminated due to the compartmentalized configuration, which simplified the process (Bachman et al. 1985). More recent studies have characterized the ABR to be very stable during and after step increases in the feed chemical oxygen demand (COD) concentration or the feed flow (shock loads) (Nachaiyasit and Stuckey 1997a,b). Shock loads for industrial wastewater occur frequently, either as a transient or a long-term change. Therefore, reactor stability to shock loads is one of the most important design characteristics. Several factors, such as moderate washout of the biomass, stable hydrogen concentration and pH level in the final compartments, and the ability to use the buffer zone in the reactor to absorb the overload, contributed to the stability of the ABR during a long-term shock load. Moreover, a lower pH level and a high substrate concentration in the first compartments during shock loads selected for a microbial community that primarily produced acetate and butyrate rather than propionate. This resulted in a stable operation, because acetate and butyrate are degraded faster than propionate (Nachaiyasit and Stuckey 1997a). Despite these advantages, it is difficult to maintain a pH level sufficiently high to sustain methanogenesis in the initial compartments of the ABR, and hence addition of vast amounts of alkalinity or recycling of effluent is needed. Moreover, baffle systems between the compartments are required to prevent biomass from migrating towards the final compartment.

The anaerobic migrating blanket reactor (AMBR) was developed as a high-rate anaerobic treatment system that combines compartmentalization, continuous flow, and a simple design (Angenent and Sung 2001). It is operated without a hydraulic upflow pattern for mixing and granular development, which eliminates a feed-distribution system. Intermittent, mechanical mixing is required to accomplish sufficient contact between the substrate and

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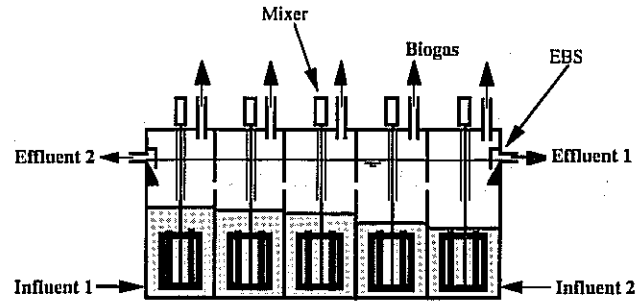

Fig. 1. Schematic diagram of the AMBR; EBS=effluent baffle system

biomass. The flow is reversed periodically to prevent the biomass from accumulating in the final compartment (Fig. 1), which makes baffles between the compartments superfluous. A reversing flow scheme also prevents total phase separation and low pH levels in the initial compartment for long periods of time. Hence, effluent recycling is not required to control pH levels in the initial compartments. This is advantageous, because effluent recycling changes the compartmentalized reactor from a system that approaches plug-flow conditions into a system that approaches completely mixed conditions, which tends to reduce removal efficiencies (Barber and Stuckey 1999).

It was previously described that the COD removal efficiency in ABRs declines rapidly for a volumetric loading rate (VLR) that exceeds 20 COD L^{-1} day^{-1} (Barber and Stuckey 1999). For example, at a VLR of 32 g COD L^{-1} day^{-1} a total COD (TCOD) removal efficiency of 55% was achieved (Barber and Stuckey 1999). A study with a five-compartment AMBR achieved TCOD removal efficiencies of 87% at a VLR of 42 g COD L^{-1} day^{-1} (Angenent et al. 2002). Due to reversing the flow for the AMBR and an absence of effluent recycling, a higher removal efficiency for the AMBR was achieved at this higher loading rate compared to for the ABR. Because the ABR had shown advantageous behavior under shock loading conditions due to its compartmentalized reactor configuration, we decided to study the AMBR under a shock loading rate that would stress the system severely, with the objective to examine the effect on performance, dynamic behavior, and stability. To accomplish an organic shock load, the feed concentration was almost doubled, while maintaining a constant hydraulic retention time (HRT). The shock load was designed to mimic a transient change in the organic wastewater concentration, which is common for industrial wastewater.

Methodology

Reactor

A rectangular shaped AMBR (inside dimensions: length=65 cm, height=33 cm, width=13 cm) made of Plexiglas had an active volume of 20 L and consisted of five compartments of 4 L each (Fig. 1). Round openings (2.5 cm in diameter) were placed in the Plexiglas sheets between the compartments at a height of 17.5 cm from the bottom (one opening: 6.5 cm from the back), while the liquid level in the reactor was 23.6 cm. Sample ports for all compartments were placed 3 cm from the bottom of the reactor. The headspaces of the compartments were separated and the biogas quantity and composition were measured in separated collection systems. The gas collection systems consisted of an observation bottle, a gas sampling port, and a wet-test gas meter (type 1 L, Schlumberger Industries, Dordrecht, The Netherlands). Timers (ChronTrol Corporation, San Diego) were used to regulate the operation. Sufficient contact between the substrate and biomass was maintained by mechanical mixing. The mixers (model 5vb, EMI Inc., Clinton, Conn.) were equipped with paddles (four vertical bars 1.25 cm in width and 10 cm in height were mounted on two horizontal bars of the same width and a length of 9 cm). An effluent baffle system was placed in front of the effluent ports to prevent floating granules from washing out with the effluent. The effluent baffle system was covered to separate the headspaces of the outside compartments from air, so that the effluent was able to flow out of the reactor by gravity without losing biogas from the headspace (Fig. 1). Two automatic ball valves, with internal diameters of 2.5 cm, were used to open and close effluent ports (True blue electric actuator model EBV-6, Plast-o-matic Valves Inc., Cedar Grove, N.J.). The temperature in the AMBR was kept constant at 35±1°C by circulating warm water through a jacket around the reactor (Polyscience heating recirculator, model 210, Niles, Ill.).

Inoculum

The AMBR was inoculated with granules obtained from an UASB reactor at the Heileman brewery in La Crosse, Wis. At start-up, the mixed liquor volatile suspended solids (MLVSS) concentration, which is an indication of the amount of biomass in the reactor, was 25 g L^{-1} [the volatile suspended solids (VSS) to total suspended solids (TSS) ratio was 0.85]. The specific methanogenic activity of these granules was 1.25 g COD g^{-1} VSS day^{-1} (SD=±0.03; n=3).

Substrate

A substrate stock solution consisted of approximately 100 g COD L^{-1} nonacidified sucrose, essential nutrients (C/N ratio of 16), alkalinity, yeast extract, and trace elements, which was modified from work by Zehnder et al. (1980) and van Lier (1995). The components of the stock solution are given in mg added per g of COD sucrose and consisted of 960 mg sucrose, 550 mg bicarbonate, as $NaHCO_3$, 3 mg yeast extract, 100 mg NH_4Cl, 20 mg K_2HPO_4, 17 mg $NaH_2PO_4 \cdot H_2O$, 10 mg $FeCl_2 \cdot 4H_2O$, 2 mg $CoCl_2 \cdot 6H_2O$, 1 mg EDTA, 0.5 mg $MnCl_2 \cdot 4H_2O$, 0.2 mg Resazurin, 0.142 mg $NiCl_2 \cdot 6H_2O$, 0.123 mg Na_2SeO_3, 0.090 mg $AlCl_3 \cdot 6H_2O$, 0.050 mg H_3BO_3, 0.050 mg $ZnCl_2$, 0.050 mg $(NH_4)_6Mo_7O_{24} \cdot 4H_2$, 0.038 mg $CuCl_2 \cdot 2H_2O$, and 0.001 mL HCl (37.7% solution). This stock solution was kept at 4°C to prevent preacidification, and was mixed during feeding to maintain a constant loading rate. To maintain the feed concentrations given in Table 1, make-up water of 35±1°C was added to the substrate just before the inlet point. The make-up water (city of Ames, Iowa, tap water) contributed additional essential nutrients such as calcium, magnesium, and sulfate.

Analyses

Concentrations of N_2, CH_4, and CO_2 in the headspaces of the AMBR were measured using a gas chromatographer (model 350, Gow-Mac Instruments, Co., Bridgewater, N.J.) with a thermal conductivity detector (column: 1.7 m×3 mm stainless steel Porapack Q 80/100 mesh; carrier gas: helium). H_2 gas concentrations were measured with a reduction gas analyzer (model RGA3, Trace Analytical, Menlo Park, Calif., with a HgO assembly bed

Table 1. Operating Parameters

Operating parameters	Units	Before Shock Load			Shock load	After shock load
		Period 1	Period 2	Period 3		
Time after start	days	0–26	27–34	35–74	75–76	77–98
HRT	h	13	8	7	6.5	7
VLR	$g\,L^{-1}\,day^{-1}$	12	21	27	50	25
COD conc. at inlet	$g\,L^{-1}$	6.6	7.1	7.9	13.3	7.1
Cycle length	h	4	4	4	4	4

and Hg detector; carrier gas: nitrogen). The individual volatile fatty acid (VFA) concentrations were measured with an ion chromatographer [model DX-500, Dionex, Sunnyvale, Calif., with a critical dimension (CD) 20 conductivity detector and an anion micromembrane suppresser; column: Ion Pac ICE-As1; effluent: 0.8–1.0 mM heptafluorobutyric acid]. For VFA analysis, samples were first acidified with HCl. The total alkalinity and concentrations of total VFA, TCOD, soluble COD (SCOD), TSS, and VSS were determined according to procedures described in the American Public Health Association (APHA) Standard Methods (APHA 1995). The pH of the reactor contents was measured with a pH probe outside the reactor. To measure the pH, mixed liquor samples were taken from the reactor and were measured immediately to prevent the pH levels from rising. Effluent samples of the AMBR processes were taken midway between two reversals of flow (3 h after switching the flow direction) or were taken during an indicated point in time of the cycle (i.e., the time period during which the reactor is fed in one flow direction).

Assessment of Reactor Performance

To obtain information on the degradation of propionate in individual compartments, the $[CO_2]_{aq}$ was, first, estimated from the partial carbon dioxide pressure using Henry's law (Perry et al. 1997). Second, the bicarbonate concentration was calculated from the carbonate species equilibrium (Snoeyink and Jenkins 1980), and finally the free energy change for propionate conversion was estimated according to the method reported by Thauer et al. (1977). To obtain information on the performance of the system, COD removal efficiencies were based on COD measurements on the influent and effluent, and methane production. For the individual compartments, the SCOD removed ($g\,COD\,day^{-1}$) was calculated by the reduction in SCOD levels times the flow, while the SCOD removal efficiency (%) was calculated by dividing the reduction in SCOD levels by the total influent COD concentration. The VLR was calculated as the mass of the COD fed to the system per wet volume of the reactor (20 L) per day ($g\,COD\,L^{-1}\,day^{-1}$). The food to micro-organism (F/M) ratio of the reactor was calculated by dividing the mass of the COD fed each day by the total VSS in the reactor, while the F/M ratio of the initial compartment was calculated by dividing the same mass of the COD by the VSS that was present in the initial compartment. Data for the SCOD and TCOD removal efficiencies were obtained by measuring the COD of the feed and the effluent. Methane production was determined as follows: (1) the biogas production (measured with the gas meters) was corrected to standard temperature and pressure (STP) using the ideal gas law; (2) the standard methane production rate (SMPR) was obtained after converting the biogas production with the wet volume of the reactor and the methane percentage that was present in the biogas. Therefore, the SMPR was expressed as L of methane per reactor volume per day ($L\,L^{-1}\,day^{-1}$). Theoretically, 0.35 L methane for each g COD utilized is produced by the methanogens at STP, when biomass growth is ignored (0.35 L of methane reacts with 1 g of oxygen in complete oxidation, and thus represents 1 g COD). Hence, the methane-based COD (MCOD) removal efficiency can be calculated by the methane production using the following formula:

$$\text{MCOD removal efficiency, } \% = \frac{\text{SMPR}}{\text{VLR} \cdot 0.35} \times 100$$

Results and Discussion

Operating Conditions

In a previous study, we observed granules to float when contact was made with a new substrate at a high food to micro-organism (F/M) ratio in the initial compartment (Angenent et al. 2002). Hence, before start-up we crushed the inoculated granules by mixing the reactor contents at high rotational speed for 1 day. At the initial VLR of 12 $g\,COD\,L^{-1}\,day^{-1}$ [Table 1; Fig. 2(a)], a complete granular blanket was formed within 2 weeks. A similar time period for granular formation with crushed granules was found in a previous study with an AMBR (Angenent et al. 2002). After granules were formed, accumulation of biomass was observed and surplus biomass was wasted constantly from the reactor to maintain a granular blanket at a constant level of 25–30 $g\,MLVSS\,L^{-1}$ during operation.

During the development of the AMBR, reversing the flow was initiated with the goal to prevent accumulation of most biomass in the final compartment (Angenent and Sung 2001). Because of this approach, baffles between the compartments were not needed and the biomass was able to migrate with the flow over the horizontal plane of the system (this promoted granulation and eventually gave the AMBR its name). It was soon realized that an additional benefit of reversing the flow was the prevention of phase separation (phasing) and the promotion of staging. In staged processes all phases of anaerobic digestion are present, but acidogenic activities are higher in the initial compartments. For reaction equations and graphs of the pathways of anaerobic digestion, see the work of Thauer et al. (1977) and of Guyer and Zehnder (1983). Fox and Pohland (1994) and Lettinga (1995) already postulated advantages of slight pre-acidification in staged processes. In addition, due to migration of biomass through the compartments of the AMBR (and hence mixing and recycling of biomass between the compartments) in combination with reversing the flow, the biomass in the outside compartments harbored high levels of methanogens despite partial acidification in the initial compartment (Angenent and Sung 2001). This biomass recycling between a high and low F/M ratio reactor (feast and famine conditions, respectively) was studied by Duran and Speece (1998), who called this phenomenon staging. In this study reversing the flow

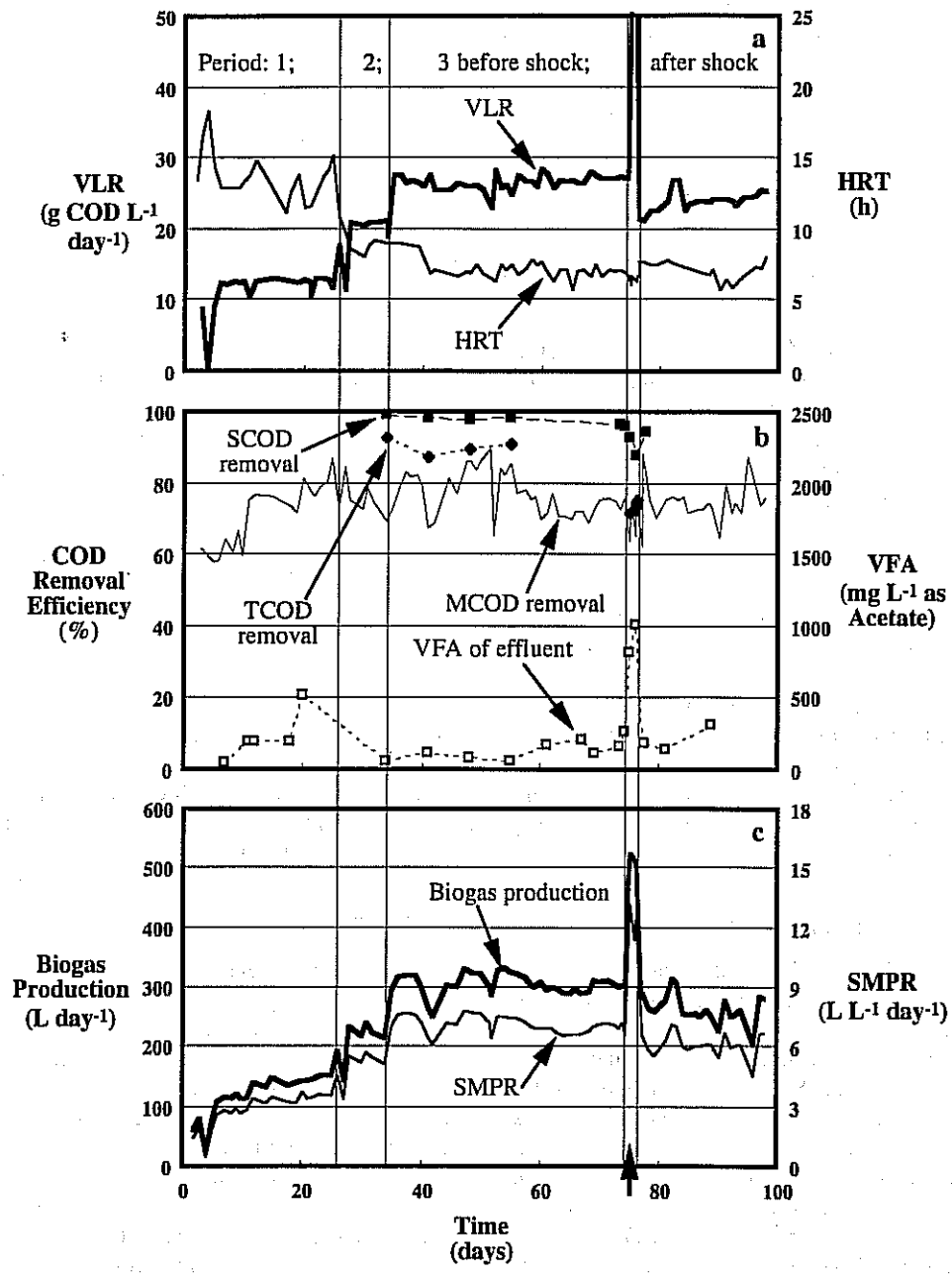

Fig. 2. Reactor operating conditions and performance of AMBR (arrow indicates the period of shock load). Volumetric loading rate and hydraulic retention time (a), COD removal efficiencies and VFA concentration of effluent; samples were taken midway between reversals of flow (b), and biogas production and standard methane production rate (c).

four times a day made effluent recycling superfluous, while avoiding relatively low pH levels (lower than 6) in the initial compartment for extended periods of time. Four cycles/day resulted in a cycle period of 6 h. First, one of the outside compartments (the initial compartment) was fed for 4 h. Next, the adjacent, second compartment was fed for 2 h to prevent a breakthrough of substrate in the effluent just after the flow was reversed (high concentrations of substrate would have otherwise been released from the reactor after the instantaneous change from initial to final compartment). Then, the same process was repeated in a reversed flow scheme. Because the reactor consisted of five compartments, the middle (third) compartment was never fed.

The persistence of acidogens attached to granules in UASB reactors can create bulking, which is defined here as low-buoyancy rising of the blanket as clumps of granular biomass. Therefore, carbohydrate wastewater is required to be pre-acidified before feeding at high loading rates to UASB reactors (Alphenaar 1994). Due to the higher shear of mechanical mixing compared to, for example, the shear generated by a hydraulic upflow pattern and biogas production in the UASB reactor, nonacidified carbohydrate wastes were treated successfully in the AMBR at high loading rates (Angenent and Sung 2001). Higher shear stresses sloughed off acidogens that were attached to the outside layers of granules, and acidogens were eventually washed out of the system. To promote the sloughing off of acidogens, but to prevent granular destruction, intermittent mixing was performed by mixing the compartments equally for 10 s every 10 min at a rotational speed of 100 min^{-1}. At a rotational speed of 100 min^{-1}, the paddles used produced a root mean square velocity gradient, G, of

232 s^{-1} in a 4-L compartment, as determined by a rotating torque meter (Bex-O-Meter, model 38, The Bex Company, San Francisco) described in the work of Sajjad and Cleasby (1995).

Reactor Performance before Shock Load

The VLR was increased from 12 to 21 g COD L^{-1} day^{-1} on day 27 [Table 1; Fig. 2(a)]. This increase in VLR showed no noticeable change in performance. Eight days later on day 35, the VLR was increased again from 21 to 27 g COD L^{-1} day^{-1} [Table 1; Fig. 2(a)]. Fig. 2(b) shows that during the latter increase, the SCOD removal efficiency decreased slightly from 99.0% on day 34 to 98.3% on day 41 and the effluent VFA concentrations increased from 57 to 120 mg L^{-1} as acetate for the same days. The TCOD removal efficiency decreased from 92.5% on day 34 to 87.1% on day 41 [Fig. 2(b)] due to an increase in the biomass level in the effluent from 30.2 to 38.6 g VSS day^{-1}. The biogas production and SMPR increased immediately after the VLR increase on day 35 [Fig. 2(c)], which shows the ability of the AMBR to maintain high removal efficiencies. The reactor was operated at a VLR of 27 g COD L^{-1} day^{-1} for 1 month (between days 45 and 75) as a baseline condition. This resulted in stable operation between days 45 and 75 during which the SCOD removal efficiencies exceeded 97% [Fig. 2(b)], and the MCOD removal efficiency was on average 76.0% [standard error (SE) =6.6; $n=30$]. The biomass level in the reactor increased slightly during this period from 27 to 30 g VSS L^{-1} (biomass was wasted continuously). This resulted in a constant F/M ratio of 0.94 g COD g^{-1} VSS day^{-1} (SE=0.05; $n=4$) for the overall reactor volume during days 45–75.

The increases in VLR during the start-up period were within the range of acceptable loading rates, which resulted in stable performance and high removal efficiency. This was not a surprise, since we previously achieved a maximum VLR of 42–45 g COD L^{-1} day^{-1} with satisfying COD removal efficiencies for a similar AMBR being fed with nonacidified sucrose substrate, but with a higher MLVSS concentration of 45 g L^{-1} (Angenent et al. 2002). To our knowledge, no other system able to treat nonacidified sucrose at these loading rates has been reported. Because the anticipated VLRs exceeded 25 g COD L^{-1} day^{-1} for the AMBR fed a nonacidified carbohydrate substrate, no single-vessel anaerobic system fed the same substrate could be used as a comparison for the stability study of the AMBR. Previous studies with nonacidified sucrose as the only substrate had found maximum VLRs for a three-compartment AMBR, an UASB reactor, and an anaerobic sequencing batch reactor of 30, 21, and 19 g COD L^{-1} day^{-1}, respectively (Angenent and Sung 2001). Even in the ABR study mentioned in the "Introduction," a maximum VLR of 18 g COD L^{-1} day^{-1} was achieved during a step increase of the COD in the feed (Nachaiyasit and Stuckey 1997a).

Shock-Load Event

The organic shock load was designed to stress the AMBR for a short period of time (six HRTs; 42 h) at a VLR that was higher than the maximum VLR for which long-term satisfying performance was expected. The VLR was 50 g COD L^{-1} day^{-1} [Table 1; Fig. 2(a)] at which the F/M ratio doubled for the overall reactor volume from 1.0 to 2.0 g COD g^{-1} VSS day^{-1} (on days 74 and 75, respectively). Meanwhile, the F/M ratio in the initial compartment during feeding was increased from 5.0 to 10.0 g COD g^{-1} VSS day^{-1}. During the organic shock load, the SCOD removal efficiency decreased from 96 to 87% [Fig. 2(b)], which showed that the AMBR was still able to remove most SCOD despite the anticipated reduction in effluent quality. The biogas production increased to more than 500 L day^{-1} immediately after the VLR increase, which resulted in a SMPR of 12 L L^{-1} day^{-1} [Fig. 2(c)]. Because of increased biogas production during the shock-load event, biomass levels in the effluent increased from 67 to 189 g VSS day^{-1} (on days 74 and 76, respectively). This resulted in a noticeable decrease of the biomass levels in the reactor from 30 to 22 g VSS L^{-1}, and indicated that the laboratory-scale AMBR is sensitive to biomass loss at these extreme situations. Based on work by Grobicki and Stuckey (1991), Guiot et al. (1995), and Nachaiyasit and Stuckey (1997a,b) we believe, however, that biomass loss at extreme conditions would have been higher in single-vessel systems. More importantly, biomass loss occurred slowly instead as a single event due to excessive bed expansion, which we have observed several times for UASB reactors that were under shock loading conditions (data not shown).

To determine if the system was able to achieve stable performance again, we monitored reactor performance after restoring the VLR to 25 g COD L^{-1} day^{-1} [Table 1; Fig. 2(a)]. The SCOD removal efficiency increased from 87 to 94% 1 day after the VLR was restored (due to a technical error TCOD removal efficiency data after the shock load do not exist). An immediate response to the decrease of the VLR after the shock load is indicated by the VFA concentrations in the effluent, which decreased from 1,010 to 180 mg L^{-1} (as acetate) a day after the shock load was completed. Hence, despite biomass loss, the AMBR was able to achieve stable performance and high removal efficiencies after the VLR decrease from 50 to 25 g COD L^{-1} day^{-1} [Fig. 2(b)]. Moreover, the SMPR reached pre-shock-load levels within a day after the initial VLR was restored [Fig. 2(c)]. This shows that the AMBR is able to handle changes in feed concentrations at high VLRs.

We showed in a previous study with a five-compartment AMBR operated under similar conditions that the cyclic high F/M ratio in the initial compartments provided conditions suitable for the development of high levels of hydrogenotrophic methanogens, such as those from the family *Methanobacteriaceae*, and syntrophic propionate-oxidizing bacteria, such as *Synthrophobacter* spp. In individual compartments the levels of hydrogenotrophic methanogens and syntrophic propionate-oxidizing bacteria were as high as 13 and 6%, respectively (the sum of relative 16S rRNA levels from the orders *Methanobacteriaceae* and *Methanomicrobiales*, and the family *Methanococcaceae* for hydrogenotrophic methanogens and the family *Syntrophomonadaceae*, *Syntrophobacter* spp., and *Desulfobulbus* spp. for syntrophic propionate-oxidizing bacteria) (Angenent et al. 2002). Interactions between these micro-organisms are necessary for hydrogen and propionate removal and, because of their abundance in the AMBR, they may have been responsible for the stable conditions during increased VLRs. This hypothesis is in accordance with that of McMahon et al. (2001). Those authors found a previously unstable digester (the unstable start-up resulted in high levels of hydrogen and propionate, and therefore developed a biomass that consisted of high levels of *Methanobacteriaceae* and *Syntrophobacter wolinii*) to reduce propionate faster, and thus stabilize sooner, than a previously stable digester. The stable operation of continuously fed single-vessel anaerobic reactors results in low levels of hydrogen and propionate and the virtual absence of propionate- and hydrogen-oxidizing microorganisms (Harper and Pohland 1986; McCarty and Mosey 1991). Consequently, without adequate removal of hydrogen and propionate during a sudden increase in the VLR, single-vessel anaerobic reactors accumulate

Table 2. Characteristics of Compartments over Time of Operation; Samples taken Midpoint in Time between Reversals of Flow (Initial Compartment Fed for 3 h). The VLR for Days 73 and 74 was 27 g COD L^{-1} day^{-1}. The VLR for days 75 and 76 was 50 g COD L^{-1} day^{-1}, and the VLR was 25 g COD L^{-1} day^{-1} for days 77 and 80.

Operation time (days)	Parameter[a]	Compartment				
		Initial	Second	Middle	Fourth	Final
73	Formate	8	4	0	0	0
	Acetate	788	388	197	79	49
	Propionate	619	346	147	62	46
	T butyrate	358	122	50	<15	0
	SCOD level	2,679	1,393	664	278	234
	SCOD removed	17.3	4.1	2.3	1.2	0.1
	SCOD efficiency	67.0	15.8	9.0	4.7	0.5 (97.1)
	pH	6.0	6.5	6.8	6.8	6.7
	Methane	30	56	61	59	60
74	Formate	7	0	0	0	0
	Acetate	855	413	169	150	87
	Propionate	523	238	99	83	63
	T butyrate	354	<75	<37	<15	0
	SCOD level	2,746	1,185	537	372	318
	SCOD removed	14.6	4.6	1.8	0.5	0.2
	SCOD efficiency	64.6	20.4	8.0	2.1	0.7 (95.9)
	pH	6.1	6.6	6.8	6.8	6.8
	Hydrogen	3.30×10^{-2}	4.78×10^{-4}	6.0×10^{-5}	8.6×10^{-5}	2.76×10^{-4}
	ΔG, propionate	12.9	−20.6	−36.9	−33.8	−25.6
	Methane	31	57	62	59	60
75	Formate	11	0	0	0	0
	Acetate	1,179	767	295	168	155
	Propionate	593	412	160	92	119
	T butyrate	644	255	<75	0	0
	SCOD level	4,628	2,651	1,329	656	682
	SCOD removed	26.1	5.9	4.0	2.0	−0.1
	SCOD efficiency	65.3	14.8	9.9	5.0	−0.2 (94.9)
	pH	6.2	6.7	7.0	7.1	7.0
	Hydrogen	3.90×10^{-2}	1.35×10^{-3}	1.06×10^{-4}	1.17×10^{-4}	3.97×10^{-4}
	ΔG, propionate	15.0	−12.3	32.6	−31.6	−23.0
	Methane	21	54	66	62	61
76	Formate	6	3	5	2	0
	Acetate	1,859	1,388	774	432	353
	Propionate	579	404	265	192	225
	T butyrate	409	329	125	26	0
	SCOD level	5,109	3,674	2,142	1,292	1,417
	SCOD removed	24.7	4.3	4.6	2.3	−0.4
	SCOD efficiency	61.7	10.8	11.5	6.4	−0.9 (89.4)
	pH	6.2	6.6	6.9	6.9	6.8
	Hydrogen	2.90×10^{-2}	3.73×10^{-3}	1.46×10^{-4}	9.10×10^{-5}	2.72×10^{-4}
	ΔG, propionate	13.9	−2.7	−28.9	−32.9	−25.3
	Methane	23	50	65	61	59
77	Formate	5	5	3	1	1
	Acetate	512	692	354	177	157
	Propionate	117	164	93	49	38
	T butyrate	108	73	28	7	11
	SCOD level	2,692	1,503	777	452	412
	SCOD removed	11.0	3.1	1.9	0.8	0.1
	SCOD efficiency	61.1	17.2	10.5	4.7	0.6 (94.1)

Table 2. (*Continued*)

Operation time (days)	Parameter[a]	Compartment				
		Initial	Second	Middle	Fourth	Final
	pH	6.0	6.4	6.7	6.8	6.7
	Hydrogen	3.40×10^{-2}	3.22×10^{-4}	4.0×10^{-5}	3.0×10^{-5}	1.84×10^{-4}
	ΔG, propionate	15.7	−21.1	−37.8	−40.0	−25.9
	Methane	28	52	60	58	60
80	Formate	6	NA	3	1	1
	Acetate	815	NA	305	150	79
	Propionate	400	NA	134	65	32
	T butyrate	293	NA	42	8	0
	pH	6.2	6.3	6.7	6.6	6.5
	Methane	24	50	60	58	61

Note: NA=not available.

[a]Hydrogen (partial pressure) and methane were measured in separated headspaces and are expressed in atm and %, respectively; VFA and SCOD levels in the liquid phase as mg L^{-1}; T butyrate=total butyrate; SCOD removed as g h^{-1}; SCOD efficiency is the SCOD removal efficiency that the individual compartments removed as a percentage of the total influent COD, hence the sum of the individual SCOD efficiencies gives the total SCOD removal efficiency of the system (given in parens). The free energy change for propionate conversion (ΔG, propionate) is given in kJ mol^{-1}.

VFAs quickly, which subsequently inhibit methanogenesis. Ultimately, inhibition of methanogenesis leads to further VFA buildup and unstable operation. Hence, the observed stability of the AMBR may be partly the result of exposure of the microbial community to alternating substrate concentrations (feast and famine conditions) due to the reversing flow scheme in the AMBR.

Gradients in AMBR Before, During, and After Shock Load

To further explain the operation of the AMBR, to determine SCOD removal efficiencies, and to obtain free energy changes for propionate conversion, we measured pH levels, individual VFA and SCOD concentrations in the liquid phase, and hydrogen and methane levels in the headspace of the five compartments. Table 2 shows gradients in the reactor of these parameters before, during, and after the shock load midway between reversals of flow (t =3 h). Table 2 shows that the VFA (except for propionate in the initial compartments; see below) and SCOD concentrations during the shock load (days 75 and 76) increased sharply in all compartments compared to before the shock load (days 73 and 74), but decreased again after the shock load (day 77). For example, the acetate, propionate, and total butyrate concentrations in the fourth compartment were 150, 83, and <15 mg L^{-1}, respectively (day 74). During the shock load, these levels increased to 432, 192, and 26 mg L^{-1}, respectively (day 76), while these levels decreased again to 177, 49, and 7 mg L^{-1}, respectively (day 77) as shown in Table 2. Hence, these VFA levels in the fourth compartment were similar on days 74 and 77 (1 day before and 1 day after the shock load, respectively). The SCOD concentrations in the compartments during the shock load period indicate a similar pattern. The methane content in the initial compartment decreased from approximately 30 to 20% during the shock load. However, no change in methane content was noticed for the final two compartments during and after the shock load compared to before the shock load (Table 2). A slightly higher methane content was observed for the middle compartment during the shock load (an increase from approximately 60 to 65%). Grobicki and Stuckey (1992) and van Lier (1995) showed that for compartmentalized anaerobic configurations the actual number of compartments correlates closely with an equal number of perfectly mixed compartments, and that, as such, plug-flow conditions were approached

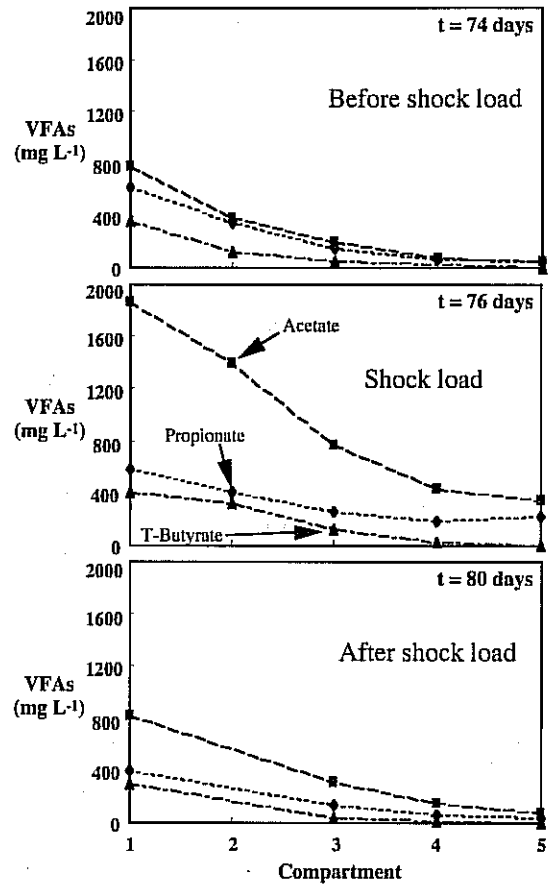

Fig. 3. VFA concentrations in separate compartments before (t =74 days), during (t=76 days), and after (t=80 days) shock load; samples were taken midway between reversals of flow

(biogas production improved the mixing conditions). It needs to be noted that plug-flow conditions were only "approached" for the AMBR due to a reversing flow scheme, which prevented plug-flow conditions during the change in flow direction. To show, however, the occurrence of a substrate gradient as one would expect in a plug-flow approached AMBR midway between the

Table 3. VFA Concentrations and pH of the Liquid Contents of the Compartments during One Flow Cycle at Day 74 (before Shock Load at a VLR of 27 g COD L^{-1} day^{-1}); Samples Taken every h during One Cycle of 6 h. At $t=0$ h, the Initial Compartment Had Been Fed for 1 min. At $t=4$ h, the Second Compartment Was Fed. At $t=6$ h, the End of the Cycle Was Reached after Which the Flow Direction Was Changed and the Final Compartment Became the Initial Compartment.

Elapsed time of cycle (h)	Parameter[a]	Compartment				
		Initial	Second	Middle	Fourth	Final
0	Formate	6	2	0	4	0
	Acetate	96	88	160	402	376
	Propionate	52	40	80	214	249
	T butyrate	NA	NA	27	227	137
	pH	6.6	6.8	6.7	6.4	6.4
1	Formate	5	3	NA	0	0
	Acetate	448	237	NA	172	261
	Propionate	182	97	NA	109	189
	T butyrate	113	66	NA	<37	27
	pH	6.4	6.6	6.9	6.7	6.6
2	Formate	8	2	0	0	0
	Acetate	769	423	160	110	177
	Propionate	386	228	75	71	148
	T butyrate	311	123	<37	<15	<15
	pH	6.2	6.6	6.8	6.7	6.7
3	Formate	7	0	0	0	0
	Acetate	855	413	169	150	87
	Propionate	523	238	99	83	63
	T butyrate	354	<75	<37	<15	0
	pH	6.1	6.6	6.8	6.8	6.8
4	Formate	5	0	0	0	0
	Acetate	660	327	157	96	49
	Propionate	381	184	103	60	31
	T butyrate	264	<75	<37	<15	0
	pH	6.1	6.6	6.8	6.8	6.8
5	Formate	0	2	0	0	0
	Acetate	516	375	182	100	49
	Propionate	381	226	97	56	23
	T butyrate	204	115	<37	<15	0
	pH	6.3	6.4	6.7	6.8	6.8
6	Formate	0	0	0	0	0
	Acetate	351	365	346	150	53
	Propionate	303	207	201	104	25
	T butyrate	78	<75	<37	24	0
	pH	6.5	6.4	6.7	6.8	6.8

Note: NA=not available.

[a]VFA concentrations in mg L^{-1}; T butyrate=total butyrate.

reversals of flow, the VFA concentrations at $t=3$ h are illustrated in Fig. 3. This staging pattern was not lost during and after the shock load (Fig. 3), which shows that during the entire operating period most treatment of the substrate was achieved by the initial compartments.

Dynamic Behavior

To study the dynamic environmental conditions in the five compartments, the VFA concentrations and pH were monitored before the shock load (day 74) during a flow cycle of 6 h (Table 3). At $t=0$ h of this flow cycle, the flow had just been reversed and the initial compartment had been fed for 1 min. Prior to this point, the fourth compartment had been fed for 2 h, which can be seen in Table 3 by the relatively high levels of VFA and relatively low pH of 6.4. During the flow cycle, the VFA concentrations in the fourth compartment decreased due to VFA consumption, which resulted in a pH increase to 6.8. Concurrently, the VFA concentrations in the initial compartment increased, and hence the pH level decreased to 6.1 at $t=4$ h. If the biomass were subjected to this pH level for a long time period, methanogenic activity would be inhibited severely (and phased conditions would be imminent). However, at $t=4$ h the substrate was fed to the second compartment and the pH in the initial compartment increased to 6.5 within 2 h. Meanwhile, the effluent quality improved during the flow cycle, as shown by the VFA levels in the final compartment.

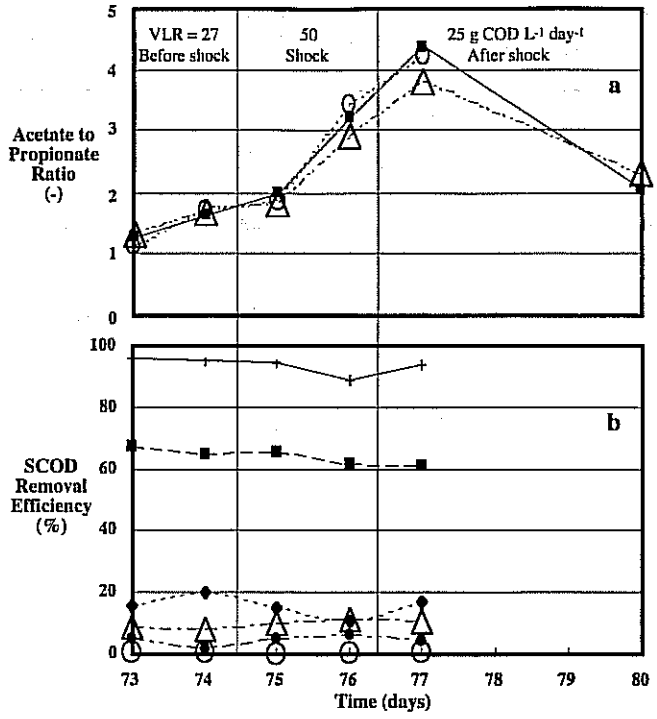

Fig. 4. Environmental conditions and treatment in the individual compartments before, during, and after shock load. Acetate to propionate ratio before, during, and after shock load; samples were taken from the initial compartment (■), second compartment (○), and middle compartment (△) midway between flow reversals (a), and SCOD removal efficiencies as a percentage of the total influent COD for the initial compartment (■), second compartment (♦), middle compartment (△), fourth compartment (●), final compartment (○), and for the overall system (+) midway between flow reversals (b).

The most undesirable conditions for methanogens were seen in the outside compartments of the AMBR during feeding (Table 3). The middle compartment, which was never fed during the time of operation maintained a pH level above 6.7, and thus higher levels of methanogens were expected to be present in this compartment. Indeed, staging of biomass was found in a recent study with a similar AMBR for which the methanogen abundance was lower in the outside compartments than in the middle compartment (Angenent et al. 2002). It must be realized that, despite less methanogen abundance in the outside compartments, a larger amount of methane was produced in the initial compartments compared to in the middle compartment due to being close to plug-flow conditions (Angenent et al. 2002). This can also been seen in Table 2 and Fig. 4(b) of this study by the SCOD efficiencies of the initial compartment, which achieved a SCOD removal efficiency exceeding 60% as a percentage of the total influent COD even during the shock load.

To follow the dynamic behavior during the shock load on day 76, VFA concentrations and pH were monitored during a flow cycle similar to that described above (Table 4). The VFA concentrations were much higher in all compartments during the shock load compared to the levels before the shock load for the entire cycle (except for propionate in the initial compartment; see below). This resulted in a maximum acetate concentration of up to 1,930 mg L^{-1} in the initial compartment and a decreased effluent quality (Table 4). The pH in the initial compartment reached a similar minimum of 6.1 at $t=4$ h of the flow cycle during the shock load and before the shock load (Tables 3 and 4). The minimum pH was similar in the initial compartment during the shock load despite a higher VFA level, because of a two times higher sodium bicarbonate concentration in the feed solution (the sodium bicarbonate to feed COD ratio was maintained at 0.55 g g^{-1}). The pH did not, however, increase as fast in the 2 h after termination of the feed to the initial compartment during the shock-load event (compared to before the shock load). Meanwhile, the pH of the final compartment was almost 7 during the shock load compared to a maximum of 6.8 before the shock load (Table 4). Hence, most of the biomass in the AMBR was protected from low pH levels that are commonly seen in overloaded anaerobic systems.

Hydrogen Management

During the shock load, the maximum hydrogen partial pressure of the biogas in the initial compartment was 3.9×10^{-2} atm (3.9%) as seen in Table 2. Accumulation of hydrogen in the headspace of the initial compartment was expected, because of the lower pH, which inhibits the hydrogen uptake by methanogens (McCarty and Mosey 1991). Because of the compartmentalized nature of the AMBR, excess amounts of hydrogen can be vented from the system and enhance the stability (Harper and Pohland 1986). The hydrogen content in the headspace of the initial compartment before and after the shock load was in the same range as during the shock load (Table 2). During the shock load, however, the hydrogen content in the headspace of the second compartment was higher compared to before and after the shock load (Table 2). Concurrently, formate levels stayed low during the entire operational time, which suggests that reducing equivalents were channeled through hydrogen instead of formate (Table 2). This phenomenon is in agreement with work by van Lier (1995) and by Nachaiyasit and Stuckey (1997a), who also saw low levels of formate in anaerobic systems that contained a granular biomass. In addition, acidogens that grow under acidic conditions (which is the case in this study) are predominantly hydrogen producers (Voolapalli and Stuckey 2001). However, it cannot be excluded that the formate turnover was high, which resulted in undetected increases in formate production.

Propionate Conversion

Propionate is one of the most difficult to be removed intermediates in anaerobic systems due to a positive Gibbs free energy change for propionate conversion (ΔG°) of +76.1 kJ mol^{-1} (Thauer et al. 1977; Stams 1994). The free energy change needs to be negative 17–20 kJ mol^{-1} to sustain growth of propionate-oxidizing bacteria (Schink 1992). Hence, conversion of propionate is only possible when products are taken away, which dictates that hydrogen levels (and formate levels) must be kept low. In stable anaerobic systems, hydrogen-utilizing methanogens keep the hydrogen partial pressure below 10^{-4} atm to maintain sufficient propionate degradation (interspecies hydrogen transfer) (Thauer et al. 1977; Gujer and Zehnder 1983; Harper and Pohland 1986; Schmidt and Ahring 1993). To predict propionate conversion in the AMBR, we estimated the free energy change for propionate conversion in the individual compartments. Table 2 shows these values. Free energy changes for propionate conversion in the initial compartment were positive before, during, and after the shock load, which indicated that according to our thermodynamic calculations propionate was not removed in the initial compartment of the AMBR during the period of operation. Propionate conversion in the second compartment, on the other hand, was possible before and after the shock load due to sufficiently nega-

Table 4. VFA Concentrations and pH of Liquid Contents of the compartments during One Flow Cycle at Day 76 (during the Shock Load; at $t=0$ h, the VLR Had Been 50 g COD L^{-1} day^{-1} for Five HRTs); Samples Were Taken every h during One Cycle of 6 h, Similar to the Cycle Described in Table 3.

Elapsed time of cycle (h)	Parameter[a]	Compartment				
		Initial	Second	Middle	Fourth	Final
0	Formate	NA	NA	NA	NA	NA
	Acetate	553	366	686	1,309	1,232
	Propionate	182	189	347	556	584
	T butyrate	75	0	<75	427	405
	pH	6.6	7.0	6.8	6.3	6.3
1	Formate	8	9	8	8	5
	Acetate	1,389	557	532	1,040	669
	Propionate	299	203	231	422	259
	T Butyrate	308	59	30	215	189
	pH	6.4	6.8	7.0	6.6	6.5
2	Formate	7	6	8	2	0
	Acetate	1,930	930	396	576	754
	Propionate	541	254	140	267	392
	T butyrate	483	137	0	55	138
	pH	6.3	6.8	7.0	6.9	6.7
3	Formate	6	3	5	2	0
	Acetate	1,859	1,388	774	432	353
	Propionate	579	404	265	192	225
	T butyrate	409	329	125	26	0
	pH	6.2	6.6	6.9	6.9	6.8
4	Formate	NA	6	NA	2	NA
	Acetate	NA	1,454	NA	514	NA
	Propionate	NA	428	NA	209	NA
	T butyrate	NA	291	NA	37	NA
	pH	6.1	6.4	6.8	7.0	7.0
5	Formate	0	4	6	0	NA
	Acetate	1,854	1,342	970	470	NA
	Propionate	679	371	352	191	NA
	T butyrate	391	311	161	47	NA
	pH	6.3	6.3	6.7	6.9	7.0
6	Formate	0	5	8	6	2
	Acetate	1,647	1,923	1,172	603	352
	Propionate	694	580	368	268	178
	T butyrate	365	470	187	43	38
	pH	6.3	6.2	6.0	6.9	7.0

Note: NA=not available.

[a]VFA concentrations in mg L^{-1}; T butyrate=total butyrate.

tive free energy changes of -20.6 and -21.1 kJ mol^{-1} for days 74 and 77, respectively. In contrast, we predicted that propionate conversion was severely inhibited in the second compartment during the shock load due to the higher hydrogen partial pressures (free energy changes of -12.3 and -2.7 kJ mol^{-1} for days 75 and 76, respectively). The shock load reduced the free energy change for propionate conversion in the middle compartment from -36.9 (day 74) to -28.9 kJ mol^{-1} (day 76), but did not stop propionate from degrading. Interestingly, the shock load did not alter the free energy change for propionate conversion in the final two compartments compared to before the shock load, which predicted that despite a shock load propionate degradation remained possible in the AMBR. It is important to note that calculations were made with species concentrations in the bulk liquid, and that these concentrations may be different in the granules.

Despite sufficiently negative free energy changes for propionate conversion in the final compartments, degradation of propionate for the AMBR during the shock load seemed to be inhibited somewhat, because the propionate levels remained higher in the individual compartments during the shock load compared to before the shock load (Fig. 3). Propionate degradation in anaerobic systems is also inhibited by elevated acetate concentrations (van Lier 1995). Hence, the inhibition of propionate degradation in the final compartments during the shock load was most likely due to the inhibition of syntrophic propionate-oxidizing bacteria by higher acetic acid concentrations. Table 4 shows, however, that

propionate still degraded in the final compartments during the shock load, which validates our thermodynamic calculations. This leads us to conclude that compartmentalized anaerobic systems, such as the AMBR, have an intrinsic advantage over single-vessel systems, because hydrogen levels and acetic acid concentrations remain relatively low in the final compartments. Hence, propionate can be degraded in part of the reactor under extreme loading conditions.

Acetate to Propionate Ratio

Fig. 3 reveals that the acetate concentration in the initial compartment rose during the shock load, while propionate concentrations remained similar to those before the shock load. Notably, the propionate concentration was lower in the initial compartment for the first 2 h of feeding the initial compartment during the shock load compared to before the shock load (Table 4). The subsequent increased acetate to propionate (A/P) ratio in the initial compartment during the shock load is shown in Fig. 4(a). To elucidate if a shift in propionate production from the initial compartment to the second compartment, or even to the middle compartment during the shock load was the cause of low propionate levels in the initial compartment, we show A/P ratios in the second and middle compartments [Fig. 4(a)]. A similar trend of these A/P ratios compared to the A/P ratios in the initial compartments rejected the possibility of a shift. Because we also did not expect propionate degradation in the initial and second compartments (as shown above), it became likely that propionate production was decreased during the shock load. Lower propionate production in the initial compartment is in agreement with shock-load studies utilizing the ABR (Nachaiyasit and Stuckey 1997a) and with McCarty and Mosey's (1991) hypothesis that predicted a decrease in propionate production during periods of low pH and high substrate concentrations. Channeling the electrons through acetate instead of propionate is very important for enhancement of the stability of anaerobic systems, as propionate is the most difficult VFA to remove from the system.

Buffer Capacity

Nachaiyasit and Stuckey (1997a) showed that during shock-load conditions, the final compartments of the ABR absorbed relatively more treatment (buffer capacity) compared to before shock-load conditions. Since the initial compartments of the AMBR produced most of the methane and achieved the highest SCOD removed (Table 2), and hence performed most of the treatment, the final compartments were potentially able to act as a buffer for treatment of VFAs during the shock-load event. To further elucidate this buffer capacity of the final compartments, SCOD removal efficiencies and the amount of SCOD that was removed for the individual compartments are given in Table 2. Despite increases in the SCOD removed by the middle and fourth compartments during the shock load, the relative SCOD efficiency of these compartments increased only marginally during the shock load compared to before the shock load [Table 2 and Fig. 4(b)]. For example, while the SCOD removed for the middle compartment increased from 1.8 to 4.6 g day^{-1} (days 74 and 76, respectively), the SCOD removal efficiencies as a percentage of the total substrate COD only increased from 8.0 to 11.5% [Fig. 4(b)]. This relatively small increase was in agreement with the increase of the relative methane production for the middle compartment during the shock load compared to before the shock load (data not shown). Hence, the initial compartment still achieved most of the treatment during the shock load (61.7% SCOD efficiency, day 76), which was similar to before the shock load (64.6% SCOD efficiency, day 74). The lack of a large shift in treatment to the final compartments of the AMBR was, therefore, different from the ABR system behavior during shock-load events.

Conclusions

The AMBR showed no noticeable deterioration of effluent quality during the start of the operation in which the VLR was increased from 12 to 21 g COD L^{-1} day^{-1} and then again 8 days later from 21 to 27 g COD L^{-1} day^{-1}. These VLRs were achieved with nonacidified sucrose as a substrate. During a subsequent organic shock load in which the VLR almost doubled from 27 to 50 g COD L^{-1} day^{-1} for 42 h (six HRTs), the pH levels stayed favorable and biomass washout was limited due to the compartmentalized configuration of the anaerobic system. Meanwhile, the propionate concentration in the initial compartment of the AMBR remained at the same level as before the shock load, whereas the acetate concentration rose significantly. In addition, due to the compartmentalized reactor configuration, excess amounts of H_2 were vented from the headspace of the initial compartments. During shock-load conditions, acetate concentrations in the liquid phase and hydrogen content in the headspace of the final compartments remained low, which ensured propionate degradation. Due to these intrinsic characteristics of the AMBR, the SCOD removal efficiency stayed above 87% midway between the flow reversals during the shock load. Moreover, the stability of the AMBR was excellent, since the AMBR reached pre-shock-load performance levels immediately after the VLR was restored to 25 g COD L^{-1} day^{-1}.

This study with the AMBR shows that compartmentalized anaerobic systems have intrinsic characteristics that promote stability during extreme loading conditions. This was also shown by van Lier (1995) and by Nachaiyasit and Stuckey (1997a, b). Increased knowledge of the stability of anaerobic systems during high loading conditions is of utmost importance for the application of anaerobic treatment to industrial and domestic wastewater, because they tend to have large load fluctuations.

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