Anaerobic Migrating Blanket Reactor Treatment of Low-Strength Wastewater at Low Temperatures

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ABSTRACT: The feasibility of the compartmentalized anaerobic migrating blanket reactor (AMBR) was studied for the treatment of low-strength soluble wastewater under low-temperature conditions. During an operating period of 186 days, a 20-L AMBR was fed nonfat dry milk substrate as a synthetic wastewater at low temperatures (15 and 20 °C). The concentration of the influent was constant at chemical oxygen demand (COD) and 5-day biological oxygen demand (BOD_5) concentrations of 600 and 285 mg/L, respectively. The soluble COD (SCOD) removal efficiency was 73% at the end of the operating period (15 °C) at a 4-hour hydraulic retention time (HRT), while the total COD (TCOD) removal efficiency was 59%. At a 4-hour HRT, staged conditions promoted complete removal of propionic acid in the final compartments of the reactor. The specific methanogenic activity of granules increased slowly until the end of the operating time, improving the removal rate. Biomass was retained effectively, as evidenced by the solids retention time (SRT) that was always greater than 50 days even during step decreases of the reactor HRT from 12 hours to 4 hours. A long SRT also promoted system stability during changes in flow, which was observed by SCOD removal efficiencies staying greater than 70%. During a hydraulic stress test, the HRT was reduced from 4 hours to 1 hour for one day (24 HRTs) in which volatile suspended solids (VSS) in the effluent increased from an average background level of 8.7 g/d to 35 g/d and the SRT decreased from 50.5 days to 12.6 days. However, mixed liquor volatile suspended solids concentration decreased only by 1 g/L, and hence a similar COD removal efficiency and biogas production was found one day after the hydraulic stress (as compared to one day before the hydraulic stress). *Water Environ. Res.,* **73,** 567 (2001).

KEYWORDS: anaerobic treatment, low-strength wastewater, low-temperature conditions, compartmentalized reactor, granules, staged treatment, anaerobic migrating blanket reactor, methanogenesis.

Introduction

Advantages of anaerobic pretreatment over aerobic treatment for low-strength wastewater include decreased sludge production and lower energy requirements, which result in decreased operating costs (Mergaert et al., 1992). Low-strength wastewater, such as domestic and food-processing wastewater, is often discharged at ambient temperatures (15 to 20 °C) and heating the wastewater to maintain mesophilic conditions (35 °C) for anaerobic treatment introduces large energy requirements. This would eliminate savings in operating cost for the anaerobic system completely. Therefore, pretreatment of low-strength wastewater is more attractive under low-temperature conditions. Recently, several laboratory- and pilot-scale studies revealed promising results for high-rate anaerobic treatment of low-strength wastewater at temperatures as low as 3 to 5 °C (Dague et al., 1998; Lettinga et al., 1999; and Rebac et al., 1997). Most often an aerobic polishing (posttreatment) step is needed after the anaerobic system (pretreatment) to meet effluent quality standards, such as for effluent nitrogen and phosphorus levels (Rodrigues et al., 2001).

For full-scale treatment of low-strength wastewater, the upflow anaerobic sludge blanket (UASB) reactor was used widely (Hulshoff Pol et al., 1997, and Lettinga et al., 1993). The UASB reactor consists of a single vessel with a hydraulic upflow pattern, and hence a gas–solids separator system and a feed-distribution system are required to retain biomass and to distribute influent evenly, respectively. Kato (1994) showed in laboratory-scale studies that the expanded granular sludge bed (EGSB) reactor, which resembles a UASB reactor with a higher upflow velocity, was more efficient compared with the UASB reactor for this type of wastewater. Evidently, a higher mixing intensity in EGSB reactors because of a higher upflow velocity decreased transport diffusion limitations of substrate into the granules. Feasibility of this technology was confirmed with pilot-scale studies treating low-strength malting wastewater at 13 °C and 20 °C (Rebac et al., 1997).

Mechanically mixed, anaerobic sequencing batch reactors (ASBRs) were also thought to be an attractive option for the treatment of low-strength, low-temperature wastewater, because increased mixing conditions are combined with ideal conditions for biomass settling and retention in the reactor. In addition, because of the absence of a hydraulic upflow pattern, gas–solids separator and feed-distribution systems are superfluous, which simplified the reactor configuration compared with UASB and EGSB systems. Despite eliminating a hydraulic upflow pattern, the ASBR was able to develop and grow granular biomass (Sung and Dague, 1995, and Wirtz and Dague, 1996), which protects the strict anaerobes from oxygen toxicity in low-strength, low-temperature wastewater treatment (Kato, 1994). Studies by Dague et al. (1998) on ASBR treatment of nonfat dry milk wastewater at temperatures ranging from 5 to 25 °C showed that solids retention times (SRTs) were always exceeding 25 days and hence could offset the low growth rates of biomass at these low temperatures. At a temperature of 5 °C and a 6-hour hydraulic retention time (HRT), the ASBR still achieved a total chemical oxygen demand (TCOD) removal efficiency of 60%. An advantage of the ASBR technology was the alternating higher and lower substrate levels (feast and famine conditions) during the operating cycle, which resulted in increased biomass settling and retention before effluent decanting and increased substrate utilization rates just after feeding (Dague et al., 1998). Increased substrate utilization is preferred, as for low-strength wastewater substrate utilization rates are generally low, dictated by Monod kinetics (Monod, 1949).

The kinetic advantage of relatively high substrate levels can also be obtained with a continuously fed staged reactor configuration. Lettinga et al. (1999), for example, used two EGSB reactors in series to gain from higher substrate concentrations in the first EGSB reactor, when feeding a low-strength volatile fatty acid (VFA) synthetic wastewater at a 1.5-hour HRT and a temperature of 8 °C. These researchers found also that staging was beneficial for the following reasons: (1) more favorable conditions for propionic acid degradation in the staged-reactor configuration because of low acetic acid and hydrogen levels in the second stage; and (2) relative high levels of bacteria in the first stage of the system (while methanogens were still present) and relative high levels of methanogens in the second stage (biomass staging), which improved biodegradation kinetics due to each major group of microorganisms existing in an optimal environment (Lettinga et al., 1999, and van Lier et al., 1997).

Another continuously fed staged reactor, the anaerobic migrating blanket reactor (AMBR) was developed, because there is still a need for simple and cost-effective high-rate anaerobic treatment systems for small and medium sized communities and industries, especially for treating low-strength wastewater (Hulshoff Pol et al., 1997). The AMBR utilizes the advantages of the ASBR, such as mechanical mixing, biomass retention, a simple design (no gas–solids separation and feed-distribution systems required because of the absence of a hydraulic upflow pattern), and granulation (Angenent and Sung, 2001). In addition, the HRT in a continuously fed AMBR can be shortened (and thus the reactor volume can be decreased) compared with the batch-fed ASBR. Relatively long HRTs due to physical limitations of the system (a combination of a batch-fed operation and a relatively tall granular blanket) are required for the ASBR, because a reduction of the 6-hour HRT for ASBRs treating low-strength wastewater was not anticipated (Dague et al., 1998).

Because of the staged and continuously fed configuration of the AMBR (and thus a potential for short HRTs) in combination with a simple design, the feasibility of the AMBR system for the treatment of highly soluble, low-strength wastewater was studied. The AMBR treatment was evaluated under low-temperature conditions (15 °C) by monitoring reactor performance over a period of 6 months, during which the HRT was reduced from 12 hours to 4 hours in a stepwise manner. Biomass retention, granular size, and methanogenic activity of granules were also monitored during this period. In addition, a sudden drop of HRT from 4 hours to 1 hour was introduced to study the behavior of the AMBR under hydraulic-stress conditions, which are common for low-strength wastewater.

Materials and Methods

Anaerobic Migrating Blanket Reactor. The AMBR, with an active volume of 20 L (inside width: 140 mm; inside length: 580 mm), was divided into four compartments (Figure 1). Baffles were placed between the compartments to reduce short-circuiting of substrate. The space between a baffle and the inside wall was 10 mm to prevent clogging by large granules. The temperature of the AMBR was kept constant at 20 ± 1 °C for the first 87 days of operation. At day 88, the temperature was decreased to 15 ± 1 °C, and precooling of influent became necessary. The flow over the horizontal plane of the reactor was reversed once a day. A daily change in flow direction was chosen to prevent a pH drop due to VFA buildup in the initial compartment and to prevent unequal biomass levels due to anticipated biomass migration between compartments.

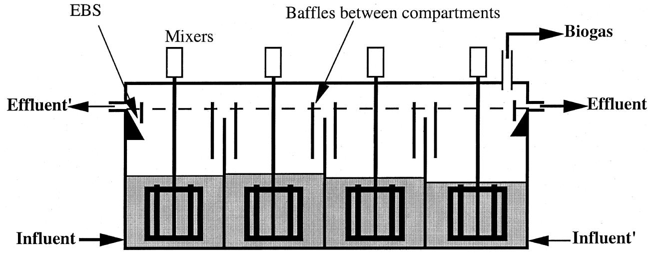

Figure 1—Schematic diagram of AMBR (EBS = effluent baffle system).

Before the flow was reversed, the second compartment was step fed for 4 hours (after termination of feeding the initial compartment) to prevent a breakthrough of substrate. Sufficient contact between substrate and biomass was maintained using intermittent, gentle mixing. Mixers (model 5vb, EMI Inc., Clinton, Connecticut) were mounted with paddles to ensure gentle mixing. At a rotational speed of 60 r/min, these paddles produced a root mean square velocity gradient (G) of 77 s^{-1} in a 5-L compartment, as determined by a rotating torque meter (Bex-O-Meter, model 38, The Bex Company, San Fransisco, California) described in Sajjad and Cleasby (1995). The compartments were mixed equally for 10 seconds every 4 minutes at 60 r/min for the first 155 days of operation. At day 156, the mixing frequency was doubled to once every 2 minutes in the three initial compartments, and the final compartment was mixed every 4 minutes to prevent excessive biomass loss. Increasing the mixing frequency of the initial compartments was implemented to decrease transport limitation and short-circuiting of substrate at higher organic loading rates. All pumps used were Masterflex pumps (Cole Parmer Instrument Co., Chicago, Illinois).

The gas collection systems consisted of an observation bottle, a gas sampling port, and a wet-test gas meter (GCA, Precision Scientific, Chicago, Illinois). The biogas was directly discharged from the reactor to the gas collection system. A water head was installed on the effluent tubes to prevent biogas from escaping directly through the effluent ports. Timers (ChronTrol Corporation, San Diego, California) regulated the operation. An effluent baffle system was placed in front of the effluent ports to prevent floating granules from leaving with the effluent (Figure 1).

Inoculum. Granular inoculum was obtained from laboratory-scale ASBRs fed with the same substrate (Dague et al., 1998) and was stored for 3 months at 5 ± 1 °C and for 1 month at 20 ± 1 °C before operation. These granules were acclimated to psychrophilic temperatures as low as 5 ± 1 °C. At start-up, the mixed liquor volatile suspended solids (MLVSS), which is an indication of the amount of biomass in the reactor, was 20 g/L.

Substrate. A concentrated substrate stock solution, consisting of nonfat dry milk (962 mg/g chemical oxygen demand [COD]), sodium bicarbonate (962 mg/g feed COD) and trace elements (ferrous chloride, $FeCl_2{\cdot}4H_2O$: 17.1 mg/g COD, nickel chloride, $NiCl_2{\cdot}6H_2O$: 1.9 mg/g COD, cobaltous chloride, $CoCl_2{\cdot}6H_2O$: 1.9 mg/g COD, manganese chloride, $MnCl_2{\cdot}4H_2O$: 1.7 mg/g COD, and zinc chloride, $ZnCl_2$: 1.3 mg/g COD), was stored at 4 ± 1 °C and was mixed during feeding to obtain a constant loading rate. Makeup water was added to the concentrated substrate just before introduction to the reactor to obtain a COD and 5-day biochemical oxygen demand (BOD_5) concentration of 600 mg/L and 285 mg/L, respectively (the BOD_5/COD ratio was 0.49). The soluble fraction of COD (SCOD) of the nonfat dry milk substrate solution was on average 8.4% smaller than the TCOD fraction. The sulfate concentration of the influent (mainly from the city of Ames, Iowa, tap water) was approximately 110 mg/L (COD/sulfate ratio was 5.5).

Analyses. Concentrations of nitrogen, methane, and carbon dioxide in the headspaces of the AMBR and activity bottles were measured using a gas chromatograph (model 350, Gow-Mac Instruments, Co., Bridgewater, New Jersey) with a thermal conductivity detector (column: 1.7 m x 3 mm stainless steel Poropack Q 0.177/0.149 mm [80/100 mesh]; carrier gas: helium). The individual VFA levels were measured with an ion chromatograph (model DX-500, Dionex, Sunnyvale, California) containing a CD 20 conductivity detector and an anion micromembrane suppressor (column: Ion Pac ICE-As1; eluant: 0.8 to 1.0 mM heptafluorobutyric acid). For VFA analyses, samples were first acidified with hydrochloric acid. The total alkalinity, total VFAs, TCOD, SCOD, total suspended solids (TSS), volatile suspended solids (VSS), and sludge volume index tests were performed according to procedures described in *Standard Methods* (APHA et al., 1995). Effluent samples were taken at the midpoint of the time interval between reversals of flow.

Biomass Characteristics. The specific methanogenic activity of biomass was assessed at 35 $\pm$ 1 °C using the "headspace method" according to tests described by Rinzema et al. (1988). To analyze the change in size of the granules during the operational time, the arithmetic mean diameter [Sum (d/n), where d is the granule diameter and n is the number of granules] and area-weighted mean diameter [Sum(d^3)/Sum(d^2)] were calculated by automated image analysis. Mixed liquor samples were collected six times throughout the course of the study and diluted to obtain fully suspended granular biomass samples for image analysis. Next, 1.75 mL of this sample was added to a specially prepared slide consisting of two, 3-mm thick glass sheets cemented together, with a 25-mm diameter hole in the top sheet. The image analysis setup contained a black and white video camera (series 68, Dage-MTI, Michigan City, Indiana), a microscope (model SZH, Olympus, Melville, New York), and a personal computer with Quartz PCI Imaging software (Quartz Imaging Corporation, Vancouver, British Columbia, Canada). Some manual editing of the image was necessary to separate adjacent granules. Particles smaller than 0.1 mm were not included in the calculations of the size distribution (Grotenhuis et al., 1991).

The biomass migration rate was determined by the decline in MLVSS from the initial compartment during the time of feeding. The food/microorganism (F/M) ratio was calculated by dividing the mass of COD fed per day with the total VSS in the reactor. The SRT was determined by dividing the total VSS in the reactor with the daily loss of VSS in the effluent.

Assessment of Reactor Performance. To obtain information on the performance of the system, COD removal efficiencies were based on COD concentrations measured in the influent and effluent and methane production. The volumetric loading rate (VLR) was calculated as the mass of COD fed to the system per reactor volume per day (g COD/L·d). Data for the SCOD and TCOD removal efficiencies were obtained by measuring the COD concentrations of the feed and the effluent. Effluent samples and samples from individual compartments were taken at the midpoint of the time interval between two reversals of flow. Methane production was determined as follows. First, the biogas production (gas meter) was corrected to standard temperature and pressure (STP). Next, the standard methane production rate (SMPR) was obtained after dividing the biogas production with the wet volume of the reactor and converting it to the methane percentage that was present in the biogas. Therefore, the SMPR was expressed as volume of methane production per reactor volume per day (L/L·d). The methane that left the reactor in a soluble form with the effluent was then calculated. First, the solubility of methane in water at 15 or 20 °C was estimated using Henry's law (Perry et al., 1997). Second, the methane quantity per day that left the reactor through its effluent was calculated and was corrected to STP and converted to SMPR. Theoretically, 0.35 L of methane has 1 g of COD, which also means 1 g of COD can potentially generate 0.35 L of methane at STP (when biomass growth is ignored). Hence, the methane-based COD (MCOD) removal efficiency can be calculated by the total SMPR (methane that left the reactor through the gas meter and the effluent) using the following formula:

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

Results and Discussion

Operating Conditions. At the start of the operating period, the reactor was fed at a 12-hour HRT and a VLR of 1.25 g COD/L·d. These loading rates at a temperature of 20 °C were possible, because the inoculum was already acclimated to similar environmental conditions. The concentration of the influent was kept constant at 600 mg COD/L (as TCOD) during the entire operating period and decreasing the HRT resulted in an increase in the VLR. At the end of operating time, the HRT was 4 hours, which resulted in a VLR of 3.5 g COD/L·d (Figure 2a). The operating length of each different HRT was chosen based on TCOD, SCOD, and MCOD removal performance. If two or more data points were within 5% margin after the system was operated for at least 10 HRTs, a quasi steady state was determined. True steady-state conditions were not achieved at the HRTs of 12, 8, and 4 hours, because the MLVSS and SRT were still increasing slowly while the HRT was decreased again (Figure 2c). It needs to be emphasized that the AMBR is a dynamic process in which substrate concentrations and environmental conditions change over time because of reversing the flow.

Figure 2 illustrates the operating conditions during the operating period. Biomass levels in the reactor stayed at approximately 23 g/L during this period (Figure 2b). Meanwhile, VSS losses with the effluent oscillated around a mean of 4.4 g/d (standard error, SE = 1.9; number of datapoints, n = 18) during the operating period (not including the hydraulic stress data). The oscillation pattern was the result of temporary solids loss in the effluent after a temperature decrease at day 88 and after the increased flows due to HRT changes. Even during the temporary biomass loss increases, biomass retention was high and the SRT remained greater than 50 days. After a period of acclimation, VSS levels in the effluent decreased and the SRT increased to values greater than 100 days. The HRT could then be decreased again (by increasing the flow), which resulted in temporarily lower SRTs of 50 days (Figure 2c). Because the MLVSS levels remained constant, F:M during the operating period increased from 0.05 to 0.18 g COD/g VSS·d, depending on VLR (Figure 2c). Meanwhile, pH levels in the initial compartment measured at the midpoint between reversals of flow (12 hours of feeding the initial compartment) were constant over the operating period with a mean of 6.7 (SE = 0.1; n = 30). The alkalinity in the effluent remained constant as well (mean = 0.53 g/L as calcium carbonate; SE = 0.04; n = 18).

Therefore, the increase in F:M showed no decrease in pH level in the initial compartment, which indicates that stable conditions prevailed in the reactor.

Anaerobic Migrating Blanket Reactor Performance. Figure 3 illustrates the reactor performance over the operating period. At a 12-hour HRT, biogas production (measured with the gas meter) reached a plateau within a month of feeding the reactor at a temperature of 20 °C (Figure 3a). At this period (days 24 to 43), the AMBR achieved SCOD, TCOD, and MCOD removal efficiencies of 93.9% (SE = 1.8; n = 3; average effluent SCOD concentration, eSCOD = 34.6 mg/L), 81.3% (SE = 5.0; n = 3; average effluent TCOD concentration, eTCOD = 105.5 mg/L), and 61.0% (SE = 7.9; n = 20), respectively (Figure 3b). The MCOD removal efficiencies were lower than the TCOD removal efficiencies primarily because of biomass accumulation in the reactor (Figure 2b). The lower MCOD removal efficiency can also be partly explained

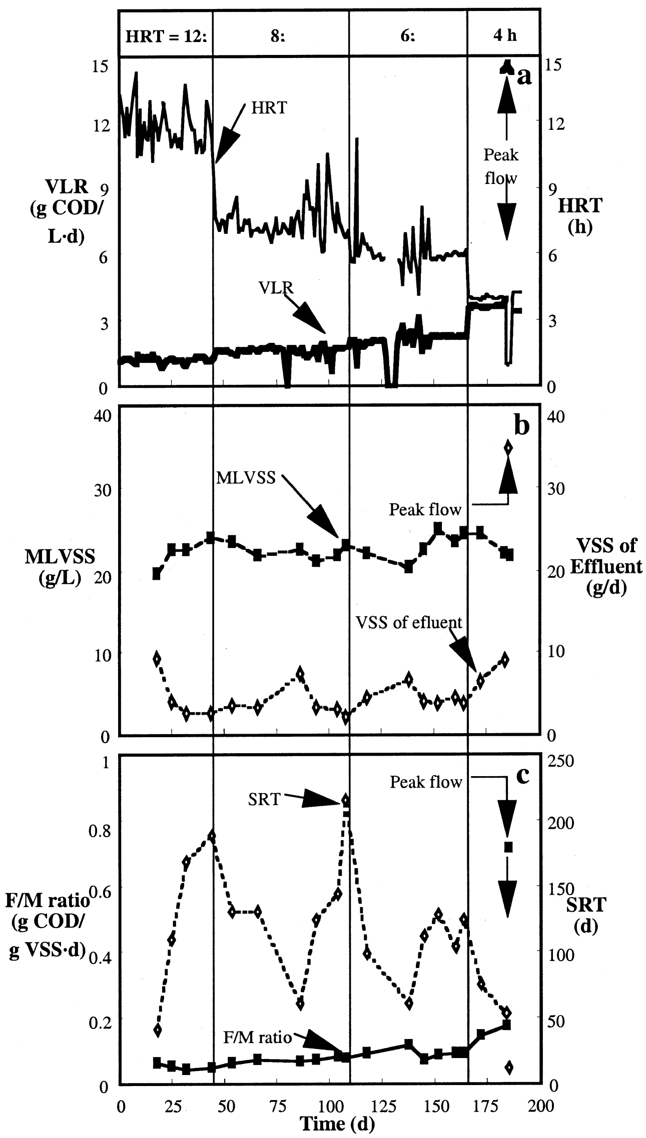

Figure 2—Operating conditions: (a) loading conditions, (b) biomass concentrations in reactor and effluent, and (c) SRT and F:M. Results during the hydraulic stress test are indicated by peak flow.

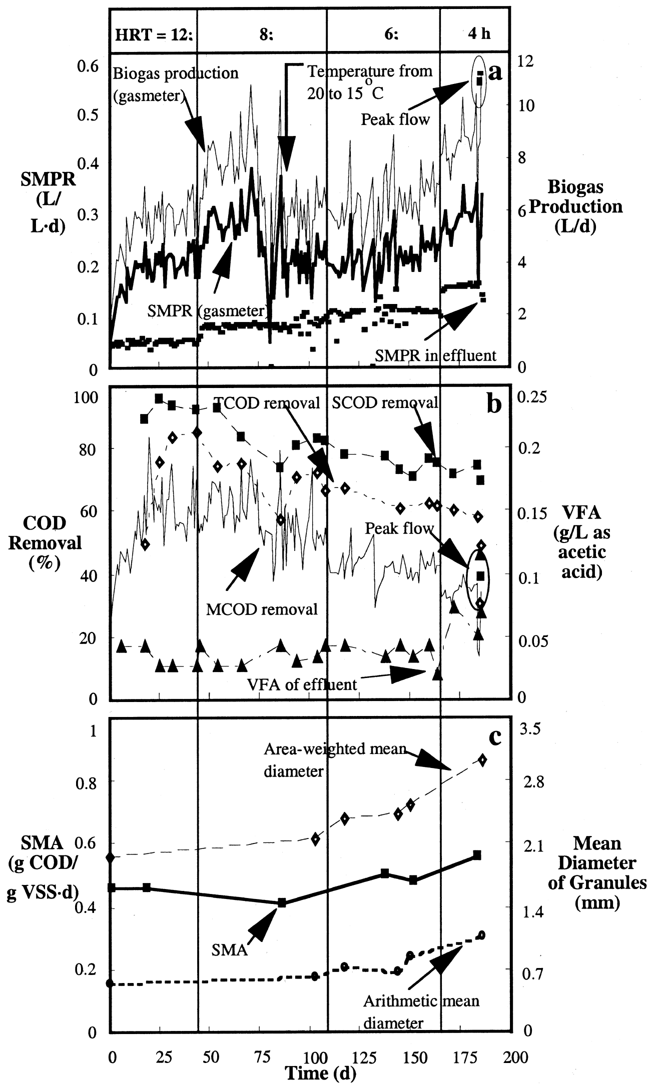

Figure 3—Reactor performance: (a) biogas and methane production, (b) COD removal efficiency, and (c) biomass activity and granular size. Results during the hydraulic stress test are indicated by peak flow.

by sulfide formation by sulfate-reducing bacteria and the subsequent sulfide loss, and thus COD loss, through stripping of hydrogen sulfide or precipitation of metal sulfides. A theoretical maximum of 10% of the SCOD removed can be attributed to this phenomenon when 100 mg/L sulfate was reduced and all sulfide was stripped or precipitated. An increased VLR resulting from the HRT decrease from 12 hours to 8 hours increased the biogas production slowly until day 78. Feeding was terminated for 2 days from day 78, which resulted in a reduced biogas production. After feeding the system again for 7 days, the biogas production reached a level similar to that before the termination of feed.

Reducing the operating temperature from 20 °C to 15 °C on day 88, decreased the biogas production (as measured with the gas meter) from approximately 8 L/d to 6 L/d and the SMPR (gas meter) from approximately 0.3 L/L·d to 0.2 L/L·d (Figure 3a). The increased level of soluble methane in the effluent at 15 °C compared with 20 °C did not correct entirely for the decrease in measured methane and biogas production. After decreasing the temperature, the SMPR for the soluble methane fraction accounted for one third of the total SMPR at a temperature of 15 °C (Figure 3a). The biogas production remained at a similar level for the rest of the 8-hour HRT period (days 94 to 108) during which the SCOD, TCOD, and MCOD removal efficiencies were 82.1% (SE = 1.0; n = 3; eSCOD = 96.3 mg/L), 70.3% (SE = 3.0; n = 3; eTCOD = 159.8 mg/L), and 53.4% (SE = 7.3; n = 13), respectively. These removal efficiencies decreased further when the HRT was reduced from 8 hours to 6 hours. At the end of the 6-hour HRT period (days 157 to 165), the biogas production increased slowly to approximately 7 L/d (as measured with the gas meter). During this period, the AMBR achieved SCOD, TCOD, and MCOD removal efficiencies of 75.9% (n = 2; eSCOD = 136.1 mg/L), 62.1% (n = 2; eTCOD = 213.7 mg/L), and 42.8% (SE = 2.1; n = 9), respectively, which were lower than those at the 8-hour HRT and temperature of 15 °C (Figure 3b).

The biogas production increased to approximately 8 L/d (gas meter) when the HRT was further decreased to 4 hours, indicating that the system was increasing its removal rates. An increase in removal rate was also determined by the specific methanogenic activity of the granules, which increased slowly for the final 100 days of operation (Figure 3c). The increase in removal rate resulted in only slightly lower COD removal efficiencies at a 4-hour HRT compared to the 6-hour HRT. The SCOD, TCOD, and MCOD removal efficiencies for the two data points (days 172 and 184) at a 4-hour HRT before the hydraulic stress test were 73.0% (n = 2; eSCOD = 163.9 mg/L), 59.2% (n = 2; eTCOD = 248.4 mg/L), and 35.6% (SE = 1.7; n = 11), respectively. The lower SCOD removal efficiencies were a result of an increase in the VFA concentrations of the effluent from 30 mg/L as acetic acid (n = 2) to 60 mg/L (n = 2) during the HRT decrease.

The operating performance showed an SCOD removal efficiency greater than 70% throughout the operating period, while the TCOD removal efficiency remained at approximately 60% for a 4-hour HRT. These values did not dramatically decrease after HRT changes, and hence the system showed stability after flow increases. During each period of a 12, 8, and 6-hour HRT, the performance of the AMBR improved slightly. Therefore, the performance at a 4-hour HRT was expected to have improved at a longer operating period, because the biogas production and specific methanogenic activity of the granules were increasing slowly at the end of the operating time. Dague et al. (1998) performed similar studies with ASBRs fed with a similar nonfat dry milk substrate. At a 6-hour HRT and a temperature of 15 °C, these workers found an SCOD removal efficiency of 81.5%. A slightly lower SCOD removal efficiency of 75.9% at similar conditions was achieved during the current study. Although the ASBR achieved a higher SCOD removal efficiency, the HRT could not be reduced from 6 hours to 4 hours because of physical limitations of the batch-fed system. In contrast, the HRT of the AMBR was reduced to 4 hours and was even decreased to 1 hour during the hydraulic stress test.

Pretreatment of raw domestic wastewater by a UASB system at ambient temperatures was studied by Barbosa and Sant' Anna (1989). Although solids were present in their wastewater, the BOD_5 and COD concentrations were 357 and 627 mg/L, respectively, which were close to those of the current study (285 and 600 mg/L, respectively). Similar to this previous study, current results suggest that the AMBR as a pretreatment step can be effective in removing COD, and thus can reduce energy requirements and add stability to an aerobic posttreatment facility. Moreover, sludge production can be reduced greatly, as the data suggest a true growth yield of 0.16 g biomass COD formed/g SCOD removed over the final three data points at a 6-hour HRT (15 °C). This is in agreement with ASBR data that showed a true growth yield of 0.145 g COD/g COD at 15 °C (Banik et al., 1998). In contrast, typical activated-sludge growth yields are greater than (0.6 g COD/g COD) (Grady et al., 1999). In contrast to the procedures used by Barbosa and Sant' Anna (1989), the AMBR of the current study was operated with a highly soluble synthetic wastewater. For nonsettled domestic wastewater that contains relatively high levels of suspended solids, the AMBR can be operated at a longer HRT to facilitate primary solids separation and the possibility to maintain flocculent biomass instead of granular biomass to promote suspended and colloidal solids destruction. For this type of wastewater, hydrolysis and acidogenesis rather than methanogenesis become the rate-limiting steps, and hence to provide for solids destruction, the entrapment of suspended solids and long SRTs become important (Switzenbaum and Grady, 1986). An AMBR operated with (partly) flocculent biomass may provide for sufficient biomass and solids retention, because this study with granular biomass showed an SRT that was longer than 100 days during pseudo-steady-state conditions. However, further studies are needed to elucidate the feasibility for AMBR treatment of nonsettled domestic wastewater.

Besides reducing nutrient levels in the effluent, posttreatment of anaerobic reactor effluent is needed to reduce the mean SCOD concentrations of 106 mg/L (SE = 50; n = 19) as found in this study. The soluble organic material in the effluent consisted of VFAs with a mean total concentration of 39 mg/L as acetate (SE = 14; n = 21). The remaining soluble organic material may have partly consisted of soluble microbial products (Langenhoff and Stuckey, 2000). Most soluble microbial products are not, or slowly, biodegradable and, therefore, are not measured with BOD_5 tests. Hence, BOD_5 measurements and removal efficiencies would have given an additional evaluation of the performance of the AMBR, especially as the nonfat dry milk substrate was not fully degraded under aerobic conditions within 5 days (BOD_5/COD ratio of 0.49).

Biomass Characteristics. An increase in the size of the granules over time is illustrated in Figure 3c. At the end of the operating period, the arithmetic and area-weighted mean diameter were 1.1 and 3.0 mm, respectively. Also, the area distribution of the granules in Figure 4 shows an increase in granular diameter over the operating time, during which the graph at day 185 had shifted to the right compared with the graph of the seed granules and the graph at day 105. At day 185, 40% of the projected granular area was due to particles between 3.16 and 5.62 mm in diameter. For the seed sludge no particles with a diameter between 3.16 and 5.62 mm were found (Figure 4). A selection for larger granules occurred because of growth and an increasing washing out of smaller biomass at higher flows. It needs to be realized that selection of larger granules occurred despite the absence of a hydraulic upflow pattern. At the end of the operation, the migration rate of large granules was virtually zero because of their good settling characteristics (sludge volume index was 18.7 mL/g) and a compartmentalized configuration of the AMBR. This absence of migration became ubiquitous because a difference in granular structure was observed between the compartments. The surfaces of granules from the outside compartments showed an abundance of white colonies (75 μm in diameter), consisting of microorganisms with a different morphology (microscopic observations of the colonies showed a homogeneous population), while the granules from the inside compartments continued to have a smooth black surface. Because of high concentrations of sulfate in the influent (110 mg/L), these microcolonies, presumably, consisted of sulfate-reducing bacteria as was also described by Sekiguchi et al. (1999), who discovered colonies consisting of *Desulfobulbus* spp. on granules by using fluorescence in situ hybridization.

The specific methanogenic activity of biomass for the outside compartments from the AMBR was 0.53 g COD/g VSS·d (standard deviation, SD = 0.01; number of measurements, $m = 2$) and was 0.58 g COD/g VSS·d (SD = 0.01; $m = 3$) for the inside compartments. Hence, at the end of the study a small difference in specific methanogenic activity was found between the granules of the inside and outside compartments (*t*-test: 95% significance level), which resulted in "biomass staging". This nomenclature indicates that different microbial communities developed over the length of a plug-flow reactor or in a sequence of compartments. For example, biomass staging in a two-stage EGSB reactor fed with partly acidified substrate at temperatures as low as 8 °C, resulted in a dominant acidogenic population in the first stage and dominant acetogenic and methanogenic populations in the second stage (van Lier et al., 1997). Results of the current study suggest, however, a much smaller difference in biomass between the compartments. The reversing flow scheme is the most likely reason why biomass staging was limited for the AMBR compared with the unidirectionally fed, two-stage EGSB system. The above given specific methanogenic activity was measured at 35 °C, which verified that mesophilic methanogens remain active over the operating period despite low-temperature conditions, as was also found by Lettinga et al. (1999), Banik et al. (1997), and Langenhoff and Stuckey (2000).

Hydraulic Stress. At day 185, the HRT was decreased from 4 hours to 1 hour to study the effect of a temporary increase in wastewater flow (hydraulic stress). During this 24-hour period (24 HRTs), a total of 480 L of feed was applied to both flow directions (240 L was fed to each direction for 12 hours). One day before the hydraulic stress test, at day 184, staged environmental conditions in the AMBR were apparent with relatively high concentrations of acetic acid and SCOD in the initial compartment (compartment 1) and low concentrations in the final compartment (Figure 5a). The appearance of formic acid and propionic acid in the initial compartment indicated the occurrence of "substrate staging". In this paper, substrate staging indicates that different substrate levels (and thus environmental conditions) are present over time or over a spatial arrangement of the reactor configuration, regardless of whether biomass staging occurs. Propionic acid concentrations that were below the detection limit illustrated favorable propionic acid degradation in compartments 2 to 4. Grobicki and Stuckey (1992), van Lier et al. (1996), and Langenhoff and Stuckey (2000) 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. The resulting SCOD and VFA concentration gradients at a 4-hour HRT were used as a baseline to compare reactor performances during and after the hydraulic stress.

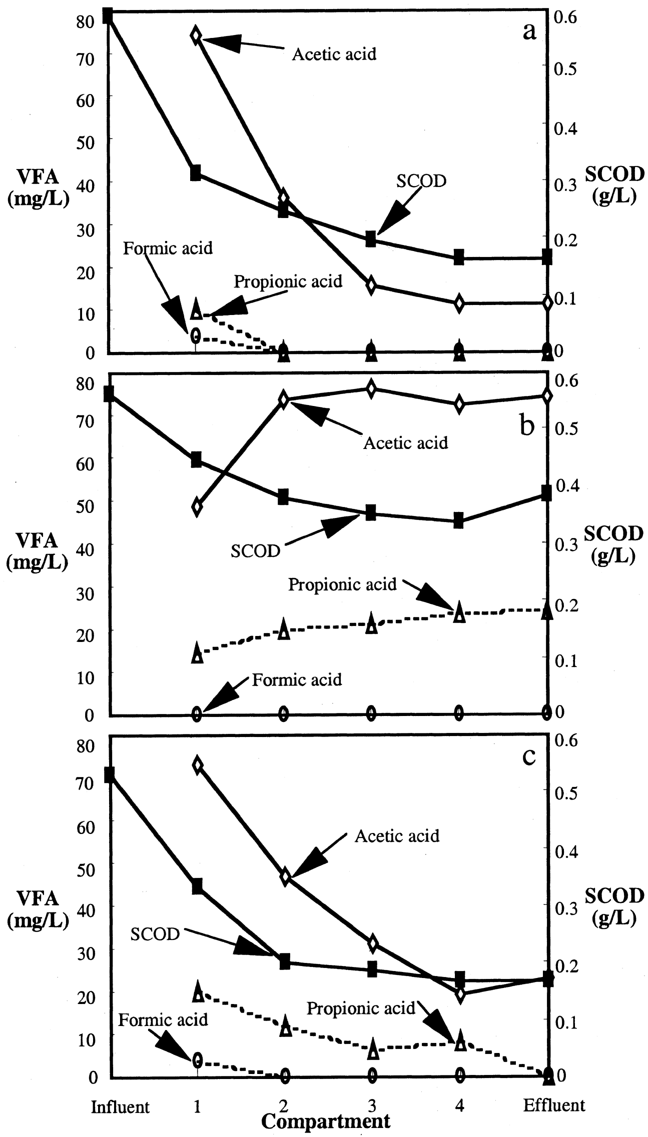

Figure 5—Volatile fatty acid and SCOD concentration at the midpoint between intervals of flow per compartment: (a) *t* = 184 days and general operating conditions at a 4-hour HRT; (b) *t* = 185 days and hydraulic stress at a 1-hour HRT; and (c) *t* = 186 days and day after hydraulic stress at a 4-hour HRT.

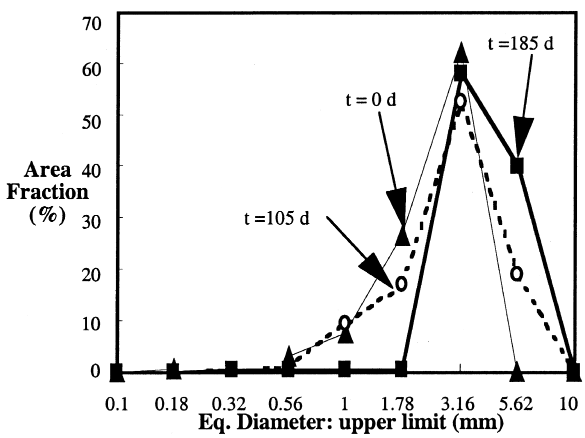

Figure 4—Area distribution of granules from the start, middle, and end of the operating period.

The 4-hour to 1-hour HRT drop at day 185 increased the VLR from 3.5 g COD/L·d to 14.4 g COD/L·d (COD level of the feed was kept constant). Consequently, the F/M ratio was increased from 0.18 to 0.72 g COD/g VSS·d (Figure 2c). The SCOD, TCOD, and MCOD removal efficiencies decreased to 39.3% (eSCOD = 384.3 mg/L), 30.3% (eTCOD = 441.2 mg/L), and 14.8% (SE = 0.8; n = 4), respectively, while the VFA concentration of the effluent increased to 110 mg/L as acetic acid (Figure 3b). Despite lower removal efficiencies, the AMBR removed more SCOD during than before the hydraulic stress test (123.4 and 58.5 g/d, respectively). The hydraulic stress did not upset the reactor in terms of a pH drop in the initial compartment, but reactor performance (COD removal efficiencies) was deteriorated severely. Substrate staging that was apparent at a 4-hour HRT was lost completely at day 185 (Figure 5b). For example, the acetic acid concentration in compartment 1 was lower than that in compartments 2 to 4. Moreover, propionic acid levels that were lower than the detection limit in compartments 2 to 4 at a 4-hour HRT, had reached a level as high as 22 mg/L during the hydraulic stress. Clearly, a shift in degradation of nonfat dry milk substrate had occurred from the initial compartment to the middle and final compartments because of the flow increase from 120 to 480 L/d.

The biomass migration rate during the hydraulic stress increased from virtually zero to approximately 13.2 g VSS/L·d, and hence more granules were migrating through the system. Also, the wash out of biomass with the effluent increased from 8.7 to 35 g VSS/d (Figure 2b), which decreased the SRT from 50.6 to 12.6 days (Figure 2c). However, after 1 day of feeding the AMBR at a 1-hour HRT, the MLVSS had decreased only by 1 g/L. Because of the retention of biomass in the system, the reactor performed almost similarly the day after the hydraulic stress as compared to the day before the hydraulic stress, with SCOD, TCOD, and MCOD removal efficiencies of 69.6% (eSCOD = 168.1 mg/L), 49.2% (eTCOD = 280.5), and 32.8%, respectively (Figure 3b). Figure 5c illustrates that the AMBR showed substrate staging at day 186 similar to that before the hydraulic stress. Only slightly elevated acetic and propionic acid concentrations of approximately 10 mg/L in compartments 2 to 4 indicated that the system had been stressed (Figure 5c). Studies with an alternative compartmentalized anaerobic system, called the anaerobic baffled reactor, also showed a compartmentalized system to be very stable during large changes in flow (Nachaiyasit and Stuckey, 1997). The anaerobic baffled reactor recovered to its baseline performance shortly after the period of hydraulic stress ended. Hence, anaerobic systems with a configuration that promotes high biomass retention are required in off-setting hydraulic stress. This is especially important for low-strength wastewater at low-temperature conditions, because of the low biomass growth rate.

Conclusions

A laboratory-scale AMBR, which consisted of four compartments and was fed with a nonfat dry milk synthetic wastewater at a concentration of 600 mg COD/L (BOD_5 of 285 mg/L) at a temperature of 15 °C, achieved SCOD, TCOD, and MCOD removal efficiencies of 73%, 59%, and 36%, respectively, at a 4-hour HRT. The reactor removal rate improved over the operating time, during which the specific methanogenic activity of the granules increased slowly. The improvement in removal rate was possible because of long biomass SRTs in the AMBR resulting from effective granular retention. Biomass retention also promoted stability of the system during changes in flow, as was demonstrated during a temporary drop in HRT from 4 hours to 1 hour.

The results of this study indicate that the AMBR is an attractive option for treating highly soluble industrial and domestic low-strength wastewater at low-temperature conditions, because the AMBR showed high biomass retention and stability during changes in flow. Moreover, at a 4-hour HRT it showed substrate staging with relatively high SCOD and acetic acid concentrations in the initial compartment and low SCOD and acetic acid concentrations in the final compartment. These conditions were beneficial in promoting a complete removal of propionic acid in the final compartments of the reactor. We did not study the dynamic behavior of the AMBR because of the flow reversals over the horizontal plane of the reactor and the possible advantages this might have on reactor stability. Also, we did not optimize the frequency of reversing the flow over the horizontal plane of the reactor. Hence, further studies should include optimization of the AMBR operation, while feeding real domestic or low-strength industrial wastewater.

Acknowledgments

Credits. This research was made possible by a grant from the U.S. Department of Agriculture (Washington, D.C.), through the Iowa Biotechnology Byproducts Consortia (Ames, Iowa). The authors acknowledge Richard Dague, who initiated anaerobic research with low-strength wastewater at Iowa State University (Ames) and Warren Straszheim (Civil and Construction Engineering Material Analyses Research Laboratory, Iowa State University) for his assistance with image analyses. This paper was presented in part at WEFTEC 2000, the Water Environment Federation's 73rd Annual Conference and Exposition held in Anaheim, California, October 14–18, 2000.

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Submitted for publication November 16, 2000; revised manuscript submitted May 14, 2001; accepted for publication August 28, 2001

The Deadline to submit Discussions of this paper is February 15, 2002.

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