Methanogenic population dynamics and performance of an anaerobic membrane bioreactor (AnMBR) treating swine manure under high shear conditions

Sudini I. Padmasiri, Jiangzhao Zhang, Mark Fitch, Birgir Norddahl, Eberhard Morgenroth, Lutgarde Raskin

A 6-L, completely mixed anaerobic bioreactor with an external ultrafiltration membrane module was operated for 300 days to evaluate the startup and performance of an anaerobic membrane bioreactor (AnMBR) treating swine manure. The reactor had a successful startup at the initial loading rate of 1 g volatile solids (VS)/L/day. After a two-fold increase in loading rate followed by a sudden, two-fold increase in flow velocity through the membrane module on day 75, the performance of the AnMBR deteriorated as measured by volatile fatty acid (VFA) accumulation, decrease in pH, and decrease in biogas production. The methanogenic population dynamics in the reactor were monitored with terminal restriction fragment length polymorphism (T-RFLP). Changes in the relative levels of Methanosarcinaceae and Methanosaetaceae were consistent with changes in VFA concentrations, i.e., high and low levels of acetate corresponded to a high abundance of Methanosarcinaceae and Methanosaetaceae, respectively. The levels of hydrogenotrophic methanogens of the order of Methanomicrobiales increased during decreased reactor performance suggesting that syntrophic interactions involving hydrogenotrophic methanogens remained intact regardless of the degree of shear in the AnMBR.

1. Introduction

Anaerobic digesters with membrane separation units (anaerobic membrane bioreactors [AnMBRs]) facilitate retention of microorganisms and allow operation with high biomass concentrations. Thus, AnMBRs are expected to provide more efficient digestion, higher methane production, and better effluent quality, and can be smaller in size than conventional anaerobic digesters. These advantages, together with increased stringency in waste disposal for animal production facilities, have led to pilot scale testing of AnMBRs to treat swine waste (du Preez et al., 2005; Lee et al., 2001). The main challenge for AnMBRs has been fouling of membrane units (Choo and Lee, 1996; Elmaleh and Abdelmoumni, 1997; Choo...
et al., 2000; He et al., 2005). Membrane fouling is the result of adsorption of organic matter, precipitation of inorganic matter, and adhesion of microbial cells to the membrane surface (Choo and Lee, 1996). High fluid flow velocities resulting in high shear rates at the surface of the membrane can be used in AnMBRs with external membrane units to help reduce membrane fouling caused by adhesion of biomass and colloidal organic matter to the membrane surface (Stephenson et al., 2000). However, the digestion efficiency in AnMBRs may be negatively affected by exposure of biomass to high shear conditions.

High mixing and shear intensities can negatively influence anaerobic digestion performance. Brockmann and Seyfried (1996) demonstrated a 50% loss in the specific activity of anaerobic biomass treating potato starch wastewater in an AnMBR with cross-flow microfiltration following re-circulation of the entire reactor content through a membrane unit 20 times. Similar results were presented in a study by Stroott et al. (2001) and McMahon et al. (2001), who demonstrated that vigorously and continuously mixed solid waste digesters (power input of 1.5 W/L) exhibited unstable performance, whereas minimally mixed digesters (thoroughly shaken by hand for 2 min each day) performed very well. They also demonstrated that continuously mixed unstable digesters were quickly (within 3 weeks) stabilized by reducing the degree of mixing. Kim et al. (2002) obtained similar performance results in anaerobic digesters under minimal and vigorous mixing conditions. They compared the stability of mesophilic and thermophilic anaerobic digesters under different loading rates and mixing conditions. During startup, both the minimally mixed mesophilic and thermophilic reactors showed superior performance and achieved stable operation in shorter time periods compared to continuously mixed reactors. Under steady-state conditions with a feed of 4% solids and a hydraulic retention time (HRT) of 20 days, these two minimally mixed reactors produced more biogas and less volatile fatty acid (VFA) in each temperature category than the corresponding continuously mixed reactors. When the loading rate was increased, the thermophilic minimally mixed reactors were the last to fail at an organic loading rate of 20 g volatile solids (VS)/L/day.

While it is clear from the above discussion that high mixing and shear intensities have a negative impact on anaerobic digestion performance, the reasons behind such observations are less obvious. Brockmann and Seyfried (1996) suggested that the observed reduced performance in their AnMBR may be the result of a physical interruption of the syntrophic association between proton reducing acetogenic bacteria and their methanogenic partners. This suggestion seems logical since high shear rates may result in break-up of microbial aggregates and a disruption of the juxtaposition of relevant microbes. Similarly, Kim et al. (2002) concluded that their results imply that minimal mixing resulted in microbial consortia proximity, and that this strategy can be used to alleviate the problem of poor effluent quality in thermophilic systems. Likewise, Stroott et al. (2001) and McMahon et al. (2001) hypothesized that vigorous mixing may have interrupted syntrophic associations. In contrast to the other studies, their hypothesis was supported by microbial population dynamics results, which indicated that the relative abundance of syntrophic propionate oxidizing bacteria, saturated fatty acid-β-oxidizing syntrophs, and various groups of methanogens was lower under vigorous mixing conditions (McMahon et al., 2001, 2004). Alternatively, their observations may be explained by differences in the rates of hydrolysis and fermentation between vigorously and minimally mixed systems. Vigorous and continuous mixing may have promoted rapid hydrolysis and fermentation, resulting in production of fermentation products at rates greater than the utilization rates of such products by syntrophs, whereas minimally mixed conditions may have resulted in slower hydrolysis and fermentation, allowing immediate consumption of fermentation products by syntrophic bacteria and methanogens (Stroot et al., 2001). This second hypothesis assumes interactions between syntrophic bacteria and methanogens are not disrupted, but implies these microorganisms are not able to keep up with the high production of fermentation products. Their population dynamics data (i.e., lower abundance of syntrophic bacteria and methanogens under vigorously mixed conditions) also support this second hypothesis.

From the above discussion, it can be concluded that the degree of shear to which the biomass in an AnMBR is exposed can impact the performance and stability of anaerobic digestion. This may be even more critical when complex waste streams containing substantial levels of suspended solids, such as swine manure, are to be treated. The overall objective of this study was to evaluate whether high shear in AnMBRs with external membrane units can decrease process efficiencies and whether these changes are reflected in the microbial community structure. This paper presents results collected with a laboratory-scale AnMBR and focuses on reactor and membrane performance and characterization of methanogenic population dynamics with terminal restriction fragment length polymorphism (T-RFLP).

2. Materials and methods

2.1. AnMBR operation

A 6-L, completely mixed anaerobic digester (supplied by Odense College of Engineering, Odense M, Denmark) was operated to treat swine manure (Fig. 1). Solid–liquid separation was achieved using a tubular ultrafiltration membrane of 1-m length and 12-mm diameter with a total surface area of 0.0377 m². The membrane was made of polyethersulfone (Weir Envig, Paarl, South Africa) with a molecular weight cut-off of 20,000 Da. The reactor content was pumped through the membrane module using a progressing cavity pump (NM021, Netzsch, Germany) controlled with a variable frequency converter (Danfoss, Loves Park, IL, USA). The trans-membrane pressure (TMP) was varied using a back pressure valve (Tru-Trol BCCA-CAT2, Tru-tech, Mars, PA, USA). Reactor operation and data acquisition were controlled using Lab View (National Instruments, Austin, TX, USA) on a PC with a FieldPoint external input/output card (National Instruments, Austin, TX, USA). The reactor temperature was kept constant at 37 ± 1 °C with a water jacket.
The AnMBR was inoculated with a 1:1:1 (v:v:v) mixture of sludge dredged from a swine lagoon (near Vichy, MO, USA), anaerobic granules from an upflow anaerobic sludge blanket (UASB) reactor treating brewery wastewater (Anheuser-Busch, Baldwinsville, NY, USA), and anaerobic sludge from the primary anaerobic sludge digester of the Urbana Champaign Sanitary District North-East wastewater treatment plant (Urbana, IL, USA). Swine manure was collected from finisher buildings (University of Illinois at Urbana-Champaign, Swine Research Farm, Urbana, IL, USA) and blended to ensure break up of large particles. The homogenized waste was stored in containers (one for each 24-h period of operation) at \(20^\circ C\)

Every day, a new batch of feed was thawed overnight and diluted with tap water to the desired concentration of 6 g VS/L before addition to an influent storage tank kept at \(4^\circ C\) which was continuously mixed. The reactor was fed 125 mL every 3 h from the influent storage tank, which corresponds to a loading rate of 1 g VS/L/day (1 g VS corresponds to approx. 1 g COD). The HRT of the system was controlled at 6 days by returning excess permeate back to the digester. The startup-loading rate was doubled to 2 g VS/L/day on day 53 and tripled to 3 g VS/L/day on day 186.

To characterize membrane fouling, the initial water flux of the clean membrane unit was measured. The permeate flux \((J)\) through the membrane was measured with a graduated cylinder and a timer under different values of TMP. The TMP was controlled by adjusting the backpressure valve (Fig. 1) and was measured online with a pressure sensor (Red valve series 42, Red Valve Co., Inc., Carnegie, PA, USA). During operation, the permeate flow rate was measured with a variable area flow meter (Gilmont Instruments, Racine, WI, USA). The cross flow velocity through the membrane was measured with a magnetic flow meter (Endress+Hauser Promag 50 H, Greenwood, IN, USA). The total membrane resistance \((R_{\text{total}})\) was calculated as follows:

\[
R_{\text{total}} = \frac{\text{TMP}}{\mu J}, \tag{1}
\]

where \(\mu\) is the viscosity of the permeate estimated using the viscosity of pure water.

### 2.2. Chemical analyses

The biogas production was measured with a wet-test gas meter (Schlumberger Industries, Dordrecht, The Netherlands) and the methane composition in the biogas was determined using a gas chromatograph (Perkin Elmer AutoSystem gas chromatograph, Norwalk, CT, USA) equipped with a GS-Q column and a flame ionization detector operated isothermally at 40 °C using helium at a flow rate of 26 mL/min. The suspended solids (SS), volatile suspended solids (VSS), and soluble chemical oxygen demand (SCOD) in the reactor, and total COD (TCOD) and SCOD in the effluent were determined according to Standard Methods (APHA, WEF, AWWA, 1998). The total VFA concentration and bicarbonate alkalinity in the reactor were measured by a simple titration technique (Anderson and Yang, 1992). A parameter \(z\), defined as the ratio of VFA concentration over bicarbonate alkalinity, was also calculated. This parameter has been found useful to monitor reactor upsets or failures (Poggi-Varaldo and Oleszkiewicz, 1992). The individual VFAs were analyzed using a high-pressure liquid chromatograph (Waters 486 Tunable Absorbance detector, Millipore Corporation, Milford, MA, USA) with an ion exclusion column of 300 mm × 7.8 mm (BIO-RAD, Aminex HPX-87 H, Hercules, CA, USA) the mobile phase was 0.005 M H2SO4 at flow rate of 0.6 mL/min. The ammonia+ammonium (NH3–N) concentration in the effluent was determined using a HACH test kit (Method 8038, Hach, Loveland, CO, USA).

### 2.3. DNA extraction and PCR

DNA was extracted using a combined detergent and bead beating procedure (UltraClean Soil DNA Kit, Mo Bio Laboratories Inc., Solana Beach, CA, USA) according to the manufacturer’s instructions. The polymerase chain reaction (PCR) targeting 16S ribosomal RNA (rRNA) genes of Archaea was performed using the A109f (S-D-Arch-0109-a-S-17) and A912r (S-D-Arch-0912-a-A-20) archaela primer pair described pre-
viously (Lueders and Friedrich, 2000). The 5’ end of the forward primer was labeled with 6-carboxyfluorescein (FAM) for terminal restriction fragment length polymorphism (T-RFLP) analysis. The PCR reaction mixture contained 1× PCR buffer, 2 mM MgCl₂, 0.2 mM of each deoxynucleoside triphosphate (dNTP), 0.2 μM each of forward and reverse primers, approximately 10 ng of the DNA template and 2.5 U of Ex Taq DNA polymerase (Takara Biomedicals, Otsu, Shiga, Japan) in a final volume of 50 μl. The PCR was performed in a thermal cycler (PTC-200 DNA Engine, MJ Research Inc., Reno, NV, USA). The amplification was done with one denaturation step at 94°C for 2 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min with a final extension of 8.5 min.

### 2.4. T-RFLP and data analysis

Amplicons from duplicate PCR reaction mixtures were pooled using a PCR purification kit (UltraClean PCR clean-up Kit, Mo Bio Laboratories Inc., Carlsbad, CA, USA). The purified fluorescently labeled PCR products were digested with restriction enzymes AluI (New England Biolabs Inc., Beverly, CA, USA), MaeI (Fermentas Inc., Hanover, MD, USA), and MseI (New England Biolabs Inc.) for 3 h at 37°C followed by an enzyme inactivation step at 65°C for 20 min. The digested samples were treated by ethanol precipitation to remove excess salt. The fluorescently labeled terminal restriction fragments (T-RFs) obtained in this manner were separated by capillary electrophoresis (ABI Prism 3730xl Analyzer, Applied Biosystems, Foster City, CA, USA) at the W.M. Keck Center for Comparative and Functional Genomics at the University of Illinois at Urbana-Champaign Biotechnology Center (Urbana, IL, USA) to determine the number and size of T-RFs obtained from each sample. Fragment analysis was conducted using GeneMapper™ Version 3.7 software (Applied Biosystems, Foster City, CA, USA). The three restriction enzymes were selected using a model developed by Klein (2003) to allow coverage of the majority of the known groups of methanogens and to target individual groups of methanogens. The model allows in silico screening of a collection of downloaded 16S rRNA sequences for populations corresponding to T-RFs obtained with specified restriction enzymes for a selected primer pair. The 16S rRNA sequences of the domain Archaea used in the in silico analysis were downloaded from the National Center for Biotechnology Information (NCBI) database (Bethesda, MD, USA) in September 2004. The coverage and specificity of each of the three restriction enzymes selected are presented in Table 1.

<table>
<thead>
<tr>
<th>Target group</th>
<th>No of species in NCBI database</th>
<th>Size (bp)</th>
<th>Coverage (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanobacteriales</td>
<td>122</td>
<td>341</td>
<td>76.2</td>
<td>51.8</td>
</tr>
<tr>
<td>Methanococcales</td>
<td>26</td>
<td>341</td>
<td>76.2</td>
<td>87.1</td>
</tr>
<tr>
<td>Methanomicrobiales</td>
<td>73</td>
<td>494</td>
<td>87.6</td>
<td>90.9</td>
</tr>
<tr>
<td>Methanosaetaceae</td>
<td>61</td>
<td>494</td>
<td>87.6</td>
<td>94.3</td>
</tr>
<tr>
<td>Methanosarcinales</td>
<td>14</td>
<td>474</td>
<td>54.1</td>
<td>94.3</td>
</tr>
<tr>
<td>Methanospirillales</td>
<td>14</td>
<td>474</td>
<td>54.1</td>
<td>97.0</td>
</tr>
</tbody>
</table>

Each peak was given an allowance of ±5 nucleotides during T-RF assignment and only T-RFs resulting in peaks greater than 50 fluorescence units were considered as operational taxonomic units (OTUs). The relative abundance of each OTU was calculated as the ratio of the peak area of that OTU over the sum of the peak areas of all OTUs. Only the OTUs that were found to have a relative abundance greater than 10% at least once during reactor operation are presented in the results.

An experiment was carried out to check the precision and reproducibility associated with the T-RFLP analysis. The experiment consisted of two separate DNA extractions.
of the same reactor sample (collected on day 291), triplicate PCR for each extraction, triplicate enzyme digestion for each PCR, followed by duplicate runs by the capillary electrophoresis unit. The de-salting step following enzyme digestion was not replicated and, as a result, the variation associated with restriction enzyme digestion also includes the variation associated with de-salting. The reproducibility was calculated as the portion of total variance that could be attributed to each T-RFLP step in a particular fragment size. For example, the % total variance of the PCR step \( \frac{s_{PCR}^2}{s_{DNA	ext{ extraction}}^2 + s_{PCR}^2 + s_{Digestion}^2 + s_{Capillary	ext{ electrophoresis}}^2} \). Variance components were estimated with the MIVQUEO option of the VARCOMP procedure of SAS (Version 8.1, SAS Institute Inc.).

3. Results and discussion

3.1 Reactor performance

3.1.1 Day 0–52
The AnMBR was started with a loading rate of 1 g VS/L/day. A few days after startup, the specific biogas production increased to 2–3 L/g VS/day (Fig. 2a). The decreases in biogas production observed on day 19 and days 27–38 were due to problems with the gas collection system. After an initial increase in effluent SCOD soon after startup, the effluent SCOD decreased and remained stable between 200 and 300 mg/L (Fig. 2b). The pH and α decreased during the startup phase but remained stable between 6 and 7. The VFA concentrations, especially formic, acetic, and propionic acids, increased during the first 30 days and then decreased (Fig. 2d).
250 mg/L (Fig. 2b). The effluent TCOD was very similar to the effluent SCOD (data not shown) and the average TCOD and SCOD removal efficiencies were over 96% and 86%, respectively, during this period. The total VFA concentrations in the reactor were also low and averaged 250 mg/L as acetic acid without significant variations in the individual VFA concentrations (Fig. 2c and d). The excellent performance of the reactor during startup also was reflected in stable pH (average of 7.5) and 0 values (average of 0.2) (Fig. 2c). Poggi-Varaldo and Oleszkiewicz (1992) determined that an 0 value of 1.0 corresponded to the threshold of stability for anaerobic digestion of the organic fraction of municipal solid waste (OFMSW) and sewage sludge and Ripley et al. (1986) observed that an 0 value less than 0.3 was required for successful digestion of poultry manure.

The observed performance suggests that the inoculum, which consisted of biomass collected from three different anaerobic environments, provided sufficient levels of the relevant populations for the anaerobic degradation of swine manure and that these populations exhibited suitable levels of activity. In addition, the performance results indicate that anaerobic digestion, at least for the loading rate of 1 g VS/L/day, was not affected by the high shear conditions in the membrane recirculation loop (the average cross flow velocity was 1.6 m/s).

3.1.2. Day 53–90
The loading rate of the system was doubled to 2 g VS/L/day on day 53 and a two-fold increase in biogas production was observed soon thereafter. The biogas production and the methane content in the biogas remained high for a period of 21 days (up to day 75) (Fig. 2a) following the increase in loading rate. The SCOD removal was more than 96% during this 21-day period and the reactor VFA concentrations were below 1000 mg/L as acetic acid, indicating successful treatment of swine manure. These results indicate that anaerobic digestion of swine manure in an AnMBR remained feasible for a loading rate of 2 g VS/L/day and shear conditions in the membrane recirculation loop corresponding to an average cross flow velocity of 1.1 m/s.

The stator in the progressing cavity pump (P1 in Fig. 1) was replaced on day 75 due to declining pump performance. As a result of the stator change, the cross flow velocity in the membrane recirculation loop increased from approximately 0.9–1.9 m/s. The system performance began to deteriorate soon after these changes. VFAs accumulated, the 0 value increased, and the SCOD in the reactor and the effluent SCOD (data not shown) and the average TCOD and total VFAs in the reactor following the second dose was calculated to be approximately 1030 mg/L, which is well below reported inhibitory levels of 3500–5500 mg/L (McCarty, 1964).

Following the second addition of NaHCO3, the reactor started recovering. The 0 value decreased to below 1.0 and the pH increased to above 7.0 (Fig. 2c). The methane content in the biogas also increased and the SCOD and total VFAs in the reactor decreased. Acetate, propionate, and butyrate concentrations decreased from day 103 to 114, but the decrease in propionate concentrations was considerably slower (approximately 34% reduction from day 103 to 110) compared to reductions in acetate (approximately 81% reduction from day 103 to 110) and butyrate (100% reduction from day 103 to 110) concentrations.

3.1.4. Day 116–300
After the system recovered (i.e., returned to low 0 and VFA values), the feeding was resumed on day 116 at a loading rate of 1 g VS/L/day. On day 142, the loading rate was increased to 2 g VS/L/day and on day 186–3 g VS/L/day. We observed an increase in VFA concentrations approximately 2 weeks after each increase in loading rate (Fig. 2c). However, by reducing the loading rate for a short period of time, the VFA levels returned to low values. The reactor performance was relatively stable for the remainder of the operational period at a loading rate of 3 g VS/L/day. However, continuous operation beyond day 300 at this loading rate of 3 g VS/L/day proved difficult (data not reported) and it did not seem possible to increase the loading rate beyond 3 g VS/L/day without affecting the performance of the AnMBR. This could be because of the high C:N ratio present in the raw swine manure. Similar problems were encountered in pilot plant AnMBRs treating swine manure for which the loading rate could not be increased beyond 2.4 g VS/L/day (unpublished data).

3.2. Membrane performance
Membrane performance was characterized using membrane flux and total membrane resistance (Fig. 3). The target TMP for membrane operation was 0.4 bar, but during reactor operation measured TMPs varied between 0.3 and 0.7 bar during the first 135 days. Membrane flux using a clean membrane rapidly declined from a clean water flux of 100 L/m²/h to values in the range of 5–10 L/m²/h with a corresponding increase in total membrane resistance. Details of membrane fouling and cleaning have been described elsewhere (Zhang et al., 2007).

During reactor operation, the stator of the progressing cavity pump in the membrane recirculation loop (Fig. 1) was gradually wearing out and needed to be replaced on days 22, 75, 209, and 269. The relatively fast deterioration of the stator could be due to abrasion caused by sand particles found in the swine waste. The initial stator change, with resulting rapid increase in the cross flow velocity from 0.9 to 1.9 m/s, did not have any effect on the total membrane resistance. The second stator was replaced on day 75, which resulted in a significant
reduction in total resistance (Fig. 3). This reduction in total membrane resistance can be explained by the removal of a fouling layer, which had accumulated during the first 75 days of operation and was removed by the sudden increase in shear. This decrease in total membrane resistance was followed by a steady increase in total membrane resistance during subsequent operation. For subsequent stator changes (days 209 and 269), the flow velocity was gradually increased after each stator change to avoid negative impacts on biological conversion processes as observed with the second stator change.

The lack of influence on membrane resistance due to the initial stator change on day 22 can be explained by the shorter operational period (there was less build up of fouling layer) and the low solids content in the reactor due to the low loading rate: approximately 20 g SS/L on day 22 compared to 40 g SS/L on day 75. The initial membrane was used for over 100 days without any form of cleaning and was replaced on day 134 for fouling analysis. The satisfactory long-term membrane performance suggests that maintaining high cross-flow velocities was critical to reduce the rate of membrane fouling. However, sudden changes in shear can have a negative effect on biological conversion processes in the AnMBR, although it had a positive effect on membrane performance. The membrane flux of the second membrane was not measured due to problems related to partial clogging of the flow meter by microbial growth in the effluent tubing resulting in unreliable data. Microbial growth in the effluent tubing was not surprising since the effluent SCOD during this period averaged 1272 mg/L.

3.3. Microbial community analysis

3.3.1. Archaeal community dynamics with T-RFLP

Using T-RFLP analysis, four archaeal populations belonging to the orders Methanomicrobiales and Methanosarcinales were found to be abundant in the AnMBR throughout the 300-day operational period. A fifth group, belonging to the family of Methanobacteriales, was only present at substantial levels during the first 60 days of the reactor run (Fig. 4, Table 2).

At the start of operation, Methanosaeta spp. (T-RF MseI 103), which have a high affinity for acetate (i.e., able to grow under low acetate concentrations) (Jetten et al., 1992), were abundant (Fig. 4). Methanosaeta spp. were present in the reactor at startup due to their presence in the inoculum, which consisted of 1/3 (v/v) of anaerobic granules. Methanosaeta spp. have been found to act as nuclei for granulation in UASB reactors (Zheng et al., 2006) and anaerobic migrating blanket reactor systems (Angenent et al., 2004). However, the granules used to inoculate the AnMBR in the current study were crushed at the time of inoculation and conditions in the AnMBR did not promote granule formation. Thus, the increase in the levels of Methanosaeta spp. during the initial operational period was likely due to the low acetate concentration during this period (Fig. 2d). With the increase in loading rate (day 53), the acetate concentration in the reactor began to increase and the abundance of Methanosaeta spp. decreased (Fig. 4). Following the increase in cross flow velocity through the membrane module (day 75, Fig. 3), the Methanosaeta spp. remained low, but did not decrease further (Fig. 4), suggesting that the increase in shear did not contribute to the reduction in Methanosaeta spp. levels. Consistent with these observations, the abundance of Methanosarcina spp. (T-RFs AluI 474 and MseI 474), which are able to proliferate under high acetate concentrations (Jetten et al., 1992), increased from day 93 onwards; the acetate concentration in the reactor was at its maximum on day 92. The Methanosarcina spp. continued to dominate in the reactor for the rest of the operational period.

Methanogens belonging to two hydrogen utilizing methanogenic groups (Methanobacteriales and Methanomicrobiales) were present in the AnMBR. The levels of Methanobacteriales were high soon after startup, but decreased to below 5% after approximately 30 days of operation. Methanomicrobiales became the dominant hydrogen utilizers after the levels of Methanobacteriales had decreased and their relative abundance
**Fig. 4** – Methanogenic population dynamics in the AnMBR monitored with T-RFLP. (a) Hydrogenotrophic methanogens, (b) aceticlastic methanogens.

**Table 2** – Groups of Archaea identified in the AnMBR using T-RFLP following PCR with A109f-A912r primers and digestion with restriction enzymes AluI, MaeI, and MseI

<table>
<thead>
<tr>
<th>Archaea population present in the AnMBR and the T-RF used for relative abundance calculations</th>
<th>Terminal restriction fragment (T-RF) size (bp)</th>
<th>Specific genera identified by combining information obtained from digestions with three restriction enzymes (number of species in each genus)</th>
<th>Primary substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanosetaeae - MseI 103</td>
<td>AluI 108, MseI 169</td>
<td>Methanosaeta (7), Methanolobus (1)</td>
<td>Acetate, C-1 compounds</td>
</tr>
<tr>
<td>Methanosarcinaceae - MseI 474</td>
<td>AluI 108, MseI 169</td>
<td>Methanomethylovorans (4), uncultured Methanosarcina (4), Thermoplasma volcanium (2), uncultured Methanoseta sp. (1)</td>
<td>Acetate, C-1 compounds</td>
</tr>
<tr>
<td>Methanobacteriales – AluI 341</td>
<td>AluI 341, MseI 474</td>
<td>Methanobrevibacter (79)</td>
<td>Hydrogen</td>
</tr>
<tr>
<td>Methanomicrobiales – AluI 434</td>
<td>AluI 434, MseI 169</td>
<td>Methanospirillum (4), Methanocalculus (2)</td>
<td>Hydrogen</td>
</tr>
<tr>
<td>Methanosarcinaceae - AluI 474</td>
<td>AluI 474, MseI 169</td>
<td>Methanosarcina (20), Methanolobus (2), Methanococcoides (3)</td>
<td>Acetate, C-1 compounds</td>
</tr>
</tbody>
</table>

* Theses fragment sizes were not included in Table 1 since the specificity and coverage are very low when considered by themselves. However, when the use of MseI was combined with information obtained by using AluI and MaeI, specific groups of species were identified.
remained above 10% throughout the operational period. With the increase in loading rate and increase in shear in the system, the abundance of the Methanomicrobiales population increased (days 59–95). During this time period, the reactor performance deteriorated with increased VFA concentrations and decreased methane production. The increase in the abundance of the Methanomicrobiales population suggests that this population became more active due to an increase in hydrogen production during this period. Although hydrogen levels were not be measured in the biogas, the observed increase in propionate concentration after the increase in loading rate on day 52 (Fig. 2d) is consistent with an increase in hydrogen production. With the change in stator on day 75, the propionate concentration rose even further. The concurrent increases in propionate and hydrogen production could be the result of a faster hydrolysis rate due to increased shear conditions, leading to higher production of fermentation products, including hydrogen, as well as an increased production of hydrogen by syntrophic bacteria through conversion of other fermentation products. This would indicate that the increase in shear was not sufficient to break up the syntrophic interactions between the syntrophic bacteria and their methanogenic partners, as suggested by Brockmann and Seyfried (1996). However, the syntrophic bacteria were not able to convert fermentation products at the rate they were produced during this time period, which led to the acidification of the reactor. Reactor performance recovered after addition of NaHCO₃ and termination of feeding for 2 weeks. The abundance of the Methanomicrobiales population decreased with the termination of feeding (days 98–115), and subsequently increased when feeding was resumed. It should be noted that some strains of Methanosarcina also can utilize hydrogen. However, the importance of hydrogen as a growth substrate for Methanosarcina spp. in complex microbial communities has not been studied extensively. A few studies have suggested that Methanosarcina spp. are not able to compete for hydrogen with other hydrogen utilizing methanogens in most anaerobic digesters (Ahriing et al., 1991; Zinder, 1993).

The reactor VFA concentration again increased each time the loading rate was increased. The first incident occurred between days 200 and 205 (Fig. 2c and 2d). During this short time period and a few days thereafter, an increase in the levels of Methanosarcina spp. was observed (Fig. 4b), which could be the result of the increase in acetate concentration. With decreasing acetate concentrations, the Methanosarcina spp. relative abundance also decreased until day 244. VFAs accumulated again between days 246 and 253, which again resulted in an increase in the relative abundance of Methanosarcina spp. Their relative abundance remained high for the rest of the operational period (except for days 291 and 295) consistent with the relatively high VFA concentration (except for days 274–291) observed in the reactor. As the relative abundance of Methanosarcina spp. decreased between days 205 and 244, a corresponding increase in the abundance of the Methanomicrobiales population was observed (Fig. 4a and b). These changes could be the result of high turnover of propionate leading to increased hydrogen production in the reactor, and a corresponding increase in the levels of hydrogenusers. It should be noted that the observed changes in population levels may be the result of changes in the levels of populations not targeted by the T-RFLP analysis since relative population levels, rather than absolute population levels, were determined.

3.3.2. Precision and reproducibility associated with T-RFLP for AnMBR samples

T-RFLP has become a powerful tool for the rapid analysis of microbial community structure in multiple samples. It is often considered a semi-quantitative technique due to biases associated with DNA extraction and PCR amplification. However, the output can be used to evaluate microbial diversity and, in combination with sequence information, to monitor changes in the abundance of specific populations.

The experiment conducted to gain confidence in the data consisted of a variance component analysis performed on four dominant peak areas (peak area >10%) observed in the AnMBR sample of day 291 with restriction enzyme AluI (Table 3). Peaks 104 and 472 had relative peak areas of approximately 25%, whereas peaks 355 and 432 exhibited relative peak areas of approximately 10%. The variance component analysis indicated that most of the variance (70–90%) was associated with the enzyme digestion step, which also includes the de-salting step. The coefficient of variation (CV) involved when considering the absolute peak area was also high (>34%, Table 3). However, when relative peak area (i.e., relative abundance) was considered, less variation (CV <20%) was observed among the samples (Table 3).

<table>
<thead>
<tr>
<th>Fragment size (bp)</th>
<th>% of total variance in absolute peak area associated with different T-RFLP steps</th>
<th>CV (%) for absolute peak area</th>
<th>CV (%) for relative peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>104</td>
<td>0.0</td>
<td>11.2</td>
<td>34</td>
</tr>
<tr>
<td>355</td>
<td>20.0</td>
<td>79.6</td>
<td>45</td>
</tr>
<tr>
<td>432</td>
<td>17.1</td>
<td>68.0</td>
<td>53</td>
</tr>
<tr>
<td>472</td>
<td>0.0</td>
<td>89.2</td>
<td>37</td>
</tr>
</tbody>
</table>

Table 3 – Percent variance associated with different T-RFLP steps in AnMBR sample collected on day 291 using restriction enzyme AluI, and coefficient of variation (CV) for relative peak area and absolute peak area.
4. Conclusions

- Stable anaerobic digestion of swine manure can be achieved in an AnMBR with external membrane filtration using cross flow velocities of up to 2 m/s.
- An increase in cross flow velocity was found to benefit membrane performance, but resulted in poor anaerobic digestion performance.
- T-RFLP results indicated that hydrogen utilizing methanogens increased in abundance during a period of poor reactor performance, caused by a sudden increase in shear conditions.
- The decrease in reactor performance likely can be explained by an increase in the rate of hydrolysis caused by an increase in shear leading to a buildup of fermentation products. It is unlikely that the decrease in reactor performance was due to breakup of microbial aggregates and interruption of syntrophic interactions.

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References


