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Anaerobic membrane bioreactors enable high rate treatment of slaughterhouse wastewater

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Highlights

- AnMBRs are an effective technology for treatment of slaughterhouse wastewater

- COD removal was consistently over 95% and was independent of OLR and HRT
- Organic loading limit of 3-3.5 $gCOD.L^{-1}d^{-1}$ was imposed by active biomass inventory
- Biomass inventory in the AnMBR was limited to 40 $g.L^{-1}$ (TS) to manage fouling

ABSTRACT

Anaerobic Membrane Bioreactors (AnMBRs) enable high space loading by retaining solids selectively through microfiltration membranes. For organic industrial wastewaters, this offers an alternative to lagoons and granule based high-rate anaerobic treatment due to excellent effluent quality, high tolerance to load variations, and ability to produce a solids free effluent for the purposes of reuse. While there has been extensive work on low-strength and low solids effluent, there has been limited application in high-solids, high fats systems such as slaughterhouse wastewater, which are a key application. A 200L AnMBR pilot plant operated at 2 Australian cattle slaughterhouses consistently removed over 95% of chemical oxygen demand (COD) from the wastewater. Virtually all degradable COD was converted to biogas, 78- 90% of nitrogen and 74% of phosphorus in the wastewater were released to the treated permeate as ammonia and phosphate respectively; which would enable subsequent nutrient capture. The mass loading rate limit of 3-3.5 $gCOD.L^{-1}d^{-1}$ is imposed by the active biomass inventory, with this in turn limited to 40 $g.L^{-1}$ (TS) by the need to manage membrane fouling control.

KEYWORDS

Anaerobic Processes; Biogas; Membrane Bioreactors; Slaughterhouse; Resource Recovery; Waste-Water Treatment.

1 INTRODUCTION

Animal slaughterhouses generate large volumes of wastewater rich in organic contaminants and nutrients [1-3], and are therefore strong candidates for treatment processes aimed at recovery of both energy and nutrient resources. The current default treatment methods for removing organic contaminants, indicated by chemical oxygen demand (COD) from slaughterhouse wastewater vary widely. Anaerobic lagoons are commonly used in tropical and equatorial temperate zones and engineered reactor systems (including activated sludge and UASB reactors) are commonly used in polar equatorial temperate zones. Anaerobic lagoons are effective at removing organic material [4]; however lagoon based processes also have major disadvantages including large footprints, poor gas capture, poor odor control, limited ability to capture nutrients and expensive de-sludging operations. Daily biogas production from anaerobic lagoons may vary by an order of magnitude depending on temperature or plant operational factors [4]. While the organic solids in slaughterhouse wastewater is highly degradable [3, 5] reducing sludge accumulation and expensive desludging events, there are increased risks of scum formation [4] which can reduce methane recovery and damage lagoon covers. Therefore, even in warmer climates, there is an emerging and strong case for reactor based technologies.

High-rate anaerobic treatment (HRAT) is an effective method, with space-loading rates up to 100x that of lagoons, and the ability to manipulate input temperature. The most common is upflow anaerobic sludge blanket (UASB) but UASB and other granule based high-rate anaerobic treatment systems are highly sensitive to fats [6], and moderately sensitive to other organic solids [7], hence require considerable pretreatment (including dissolved air flotation) [8], and still operate relatively poorly, with COD removals on the order of 60%. In the last 5 years, a number of fat and solid tolerant processes have emerged, including the anaerobic baffled reactor [9], the anaerobic sequencing batch reactor [10], anaerobic membrane bioreactors (AnMBR) [11, 12] and the Anaerobic Flotation Reactor [13]. The AnMBR combines high rate anaerobic digestion with a membrane biomass retention system that is independent of sludge settleability [14]. AnMBRs in particular are probably the most appropriate HRAT technology suitable for slaughterhouse wastewater, particularly high-strength streams, due to excellent effluent quality, high tolerance to load variations, and ability to produce a solids free effluent for the purposes of final treatment an reuse [15]. However, they have most widely been applied to domestic and soluble industrial wastewaters, with a number of potential risk factors as outlined below.

Slaughterhouse waste risks include high proteins, causing release of ammonia (NH_3), and fats, causing release of long chain fatty acids (LCFA), both potential inhibitors of methanogenic activity [16]. Ammonia inhibition is related to its capacity to diffuse into microbial cells and disruption of cellular homeostasis [17], whereas LCFAs may exert a surface proportional toxicity to anaerobic biomass, similar to toxicity exhibited by surfactants and resulting in cell lysis [18]; or may suppress the sludge activity by adsorbing on to the anaerobic biomass and limiting transfer of substrate and nutrients across the cell membrane, interfering with membrane functionality [19, 20]. Release of ammonia and/or LCFA is a particular risk at high-strength and in high rate or intensified processes such as AnMBRs where increased OLR and shorter HRT may result in accumulation of substrate and/or inhibitory intermediates within the reactor volume. AnMBRs have been used successfully to treat raw snack food wastewater with high fat, oil and grease (FOG) concentrations (4-6 $g.L^{-1}$) reporting removal efficiencies of 97% in COD and 100% in FOG at a loading rate of 5.1 kg $COD.m^{-3}.d^{-1}$, without any biomass separation problems or toxic effects [21]. This suggests AnMBRs could be applied successfully to treat slaughterhouse wastewater.

The accumulation of particulates in the AnMBR vessel can increase membrane fouling due to cake accumulation [22]. Membrane fouling rate, and the ability to operate at an effective critical flux (the flux below at which the system can be operated without periodic cake dispersal) is the primary factor influencing economic feasibility of membrane processes [23], with membrane costs in the range of 72% of capital investment [24]. Fouling is potentially more severe in slaughterhouse applications due to the high protein content in the waste and the fouling propensity of mixtures with a high protein to polysaccharide ratio [25, 26].

While AnMBR systems have been widely applied to low strength, and soluble industrial wastewaters, particularly in the laboratory, risks around higher solids wastewater, which should be a key application, are not well known. The aim of the present study is to evaluate loading rates, retention times, and membrane performance for intensified anaerobic treatment of combined slaughterhouse wastewater through a longer term study, associated to achievable performance through biochemical methane potential (BMP) testing.

2 MATERIALS AND METHODS

2.1 Biomethane Potential Tests

Batch digestions were performed according to Angelidaki et al. [27] in 160 mL non-stirred glass serum vials (100 mL working volume) at 38°C. Inoculum was collected from mesophilic anaerobic digesters operating at 37°C and treating a mixture of primary and waste activated sludge at a domestic WWTP (Queensland, Australia). The average inoculum composition was 28.6 $g.L^{-1}$ COD, 26.1 $g.L^{-1}$ TS and 69% VS (as a fraction of TS). Specific methanogenic activity of the inoculum was 0.2 $gCOD.gVS^{-1}.d^{-1}$. The inoculum to substrate ratio (ISR) in the BMP tests was set at 2 (volatile solids basis) according to Jensen et. al [28]. Bottles were flushed with 100% N_2 gas for 3 min (1 L min^{-1}), sealed with a rubber stopper retained with an aluminum crimp seal and stored in temperature-controlled incubators (38±1°C). Tests were mixed by inverting once per day. Blanks containing inoculum without the substrate were used to correct for background methane. Separate positive controls were conducted using α- cellulose, casein or olive oil at 1 $g.L^{-1}$ resulting in biochemical methane potential (B_0) values of 373 $L.kg^{-1}$ VS, 537 $L.kg^{-1}$ VS and 1012 $L.kg^{-1}$ VS respectively (data not presented). All tests were carried out in triplicate, and all error bars indicate 95% confidence in the average of the triplicate based on a two-tailed *t*-test.

Biogas volume was measured by manometer at the start of each sampling event. Accumulated volumetric gas production was calculated from the pressure increase in the headspace volume (60 mL) and expressed under standard conditions (25°C, 1 atm).

Ultimate methane potential, and apparent first order kinetic coefficient were estimated through parameter estimation in a simple first order model through Aquasim 2.1d as shown in Eq. (1) and described previously [28].

$$B_t = B_0(1 - e^{-k_{hyd} t}) \qquad (1)$$

Where B_t is the cumulative methane production, t is the incubation time, B_0 is the ultimate methane potential and k_{hyd} is the hydrolysis rate coefficient. Parameters were estimated using a gradient search technique with the sum of squared errors as the objective function, and parameter uncertainty calculated from linear estimates in parameter standard error (95% confidence based on a two-tailed t-test).

2.2 Design of Anaerobic Membrane Bioreactor Pilot Plant

The AnMBR pilot plant (Figure 2) consists of a 200L stainless steel reactor (500 mm diameter x 1060 mm height) containing a vertical mounted submerged hollow fibre membrane (Zenon ZW-10, approx. 600 mm height and 100 mm diameter, 0.93 m^2 surface area).

During operation, wastewater flux through the membrane was controlled at a specific rate using a peristaltic pump on the permeate stream. Biogas in the AnMBR was continuously circulated across the membrane surface at a fixed flow rate of 35 $L.min^{-1}$ (2.3 $m^3.m^{-2}.h^{-1}$) for fouling control. The AnMBR temperature was measured using an resistance temperature detector (RTD) (model SEM203 P, W&B Instrument Pty.) and controlled at 37°C using a surface heating element. Biogas production volumes and Biogas recirculation rates were monitored using Landis Gyr Model 750 gas meters with a digital pulse output. Pressure transducers were used to monitor liquid level, headspace pressure and transmembrane pressure. Pressure and temperature (4-20 mA transmitter) were logged constantly via a process logic control (PLC) system. A detailed piping and instrument diagram for the AnMBR pilot plant is shown in Figure 2.

2.3 Pilot Plant Operation

The AnMBR pilot plant was operated at two Australian slaughterhouses processing cattle only. At each site the pilot plant was inoculated with digested sludge from a crusted anaerobic lagoon at the host site, the methanogenic activity of the inoculum was measured at both sites at the time of inoculation and was $0.15 gCOD.gVS^{-1}.d^{-1}$ for both sites. This activity is within the range expected for anaerobic digesters/lagoons and indicated a healthy inoculum.

At Site A, the AnMBR pilot plant was treating combined red wastewater after primary treatment using dissolved air floatation (DAF), which was only partially effective due to elevated temperatures, this wastewater contained material from cattle slaughter areas and rendering waste, but did not contain paunch or cattle manure. The plant was initially operated at a long hydraulic retention time of 7 days to allow for acclimatization of the anaerobic inoculum. During this initial operation, feed events occurred 2 per week, using a burst feed at relatively high membrane flux. This strategy was used to test if the membrane could operate sustainably at flux rates of $6.25 L.m^{-2}.h^{-2}$ required to achieve the eventual target of operating at a HRT of 1 day. Once the biomass was acclimatized and the performance was stable, the plant switched to a continuous operating mode. At Site B, the AnMBR pilot plant was treating raw combined red wastewater with no primary treatment to remove solids or fats. This wastewater contained material from cattle slaughter areas and rendering waste, but did not contain paunch or cattle manure. A summary of operating periods and strategies is summarized in Table 1. During Period 3 on Site B the sludge retention time was 50 days. During all other operating periods sludge was withdrawn for sample analysis only resulting in an SRT exceeding 1000 days. Detailed analysis of wastewater characteristics at Site A and B are presented in Table 2 and Table 3 respectively. Further details on AnMBR operation and organic loading rates are summarized in Figure S1.

2.4 Chemical Analyses:

Total solids (TS) and volatile solids (VS) were measured according to standard methods procedures 2540G [29]. Chemical oxygen demand (COD) was measured using Merck Spectroquant® cell determinations and a SQ 118 Photometer (Merck, Germany) for total (TCOD) and soluble fractions (SCOD). Total Kjeldahl nitrogen (TKN), total phosphorus (TP), ammonia-nitrogen (NH_4-N), and phosphate-phosphorus (PO_4-P) were measured using a Lachat Quik-Chem 8000 Flow Injection Analyser (Lachat Instrument, Milwaukee). Fat, oil and grease (FOG) were measured using S316 extraction and a Wilks InfraCal CVH (Wilks Texas). Sodium and potassium were measured using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). For measurement of SCOD, NH_4-N and PO_4-P, the slurry samples were filtered through a syringe filter (0.45 µm PES membrane) immediately after collection and stored prior to analysis.

Biogas quality (CH_4, CO_2, H_2) was determined using a Gas Chromatography-Thermal Conductivity Detection (GC-TCD). The system was a Perkin Elmer auto system GC-TCD with a 2.44 m stainless steel column packed with Haysep (80/100 mesh). The GC was fitted with a GC Plus Data station, Model 1022 (Perkin Elmer, Waltham, MA, USA). High purity nitrogen (99.99%) was used as carrier gas at a flow rate of 24.3 mL/min and a pressure of 220 kPa. The injection port, oven and detector were operated at 75°C, 40°C and 100ºC, respectively. The GC was calibrated using external gas standards from British Oxygen Company (Sydney, Australia).

2.5 Membrane Critical Flux

Filtration or flux-step experiments were conducted in accordance with the protocol described by Le Clech et al [30]. Flux was incrementally increased in steps of 2 $L.m^{-2}.h^{-2}$, and time intervals of 15 minutes. As flux step is increased, the resulting transmembrane pressure (TMP) is recorded. Fouling rate, dP/dt, is taken as the gradient of the line at each flux step and is plot against its flux value. The behavior of the dP/dt vs. flux curve can be used to comment on the fouling propensity of the substrate tested, with higher rate of increase in dP/dt (fouling rate) indicating greater fouling propensity. The flux at which dP/dt exceeds 0.01 kPa/min is taken as the critical flux [30].

3 RESULTS AND DISCUSSION

3.1 Biomethane potential of slaughterhouse wastewater

Biomethane potential tests (Figure 3) were used to determine wastewater degradability and degradation rate constant, as a baseline to assess performance of the AnMBR pilot plants. B_0 at Site A was estimated at 661 L CH_4 $kgVS^{-1}{}_{fed}$, hydrolysis rate constant was estimated at 0.35 d^{-1} and degradable fraction was estimated as 0.98 (as a fraction of COD fed). At Site B, the B_0 lower, estimated at 570 L CH_4 $kgVS^{-1}{}_{fed}$, hydrolysis rate constant was similar at 0.38 d^{-1} and degradable fraction was estimated as 0.75 (as a fraction of COD fed). The reduced degradability of the wastewater at Site B is likely the result of solids and grit in this stream, which are removed during primary treatment prior to wastewater collection at Site A. The B_0 of both streams is consistent with substrates containing a high fraction of protein (~600 L CH_4 $kgVS^{-1}$) and lipids (~1000 L CH_4 $kgVS^{-1}$) and agrees with values previously reported for slaughterhouse wastewater [3, 5].

Anaerobic lagoons in Australian slaughterhouses are typically designed with a HRT of approximately 20 days resulting in ponds that occupy very large footprints and operate with variable energy recovery and organic removal efficiency [4]. The BMP results from the current study emphasize the unsuitability of mixed digesters for slaughterhouse wastewater treatment, since a HRT of 20 days would achieve 85% conversion (based on application of the hydrolysis coefficient to a CSTR). This would result in an OLR of 0.3 $gCOD.L^{-1}.d^{-1}$, and hence relatively inefficient use of reactor volume (compared with conventional digesters at 1-3 $gCOD\ L^{-1}d^{-1}$) [31, 32]. AnMBRs are an effective technology to address this limitation with successful operation at HRTs as low as 2 days.

The slow methane production in the first several days of the biomethane potential tests indicates minor inhibition or acclimatization. Inhibition from slaughterhouse wastewater may be caused by LCFA accumulation from FOG digestion or ammonium inhibition from protein hydrolysis. Inhibition constants (K_{I50}) for FOG in slaughterhouse wastewater, representing the concentrations where substrate uptake rates are reduced to 50% of the maximum, have previously been reported in the range of 1-1.5 $g.L^{-1}$ [5], this is similar to the initial FOG concentrations in the slaughterhouse wastewater in this study and suggests that the minor inhibition was the result of LCFA accumulation. In this case the inhibition appeared to be minor, and relatively quickly overcome and was likely more related to acclimatization or biostatic inhibition than to the loss of metabolic function or cell death [33].

3.2 AnMBR Process Performance

Reactor performance was assessed by comparing COD added to the process as feed, with COD removed as biogas and COD removed in the treated permeate, the results from Site A are shown in Figure 4 (top). At Site A, the COD removal efficiency and methane yields were not impacted by HRT or OLR. COD removal from the wastewater was over 95%. i.e less than 5% of COD from the wastewater feed remained in the treated permeate while over 95% of COD was converted to biogas. The biogas composition was typically 70% methane (CH_4) and 30% carbon dioxide (CO_2); during full and steady operation methane production (expressed at 25°C and 1 atm) was approximately 760 $L.kg^{-1}$ VS added, corresponding to 365 $L.kg^{-1}$ COD added (96% of COD added). The quality of permeate effluent is shown in Figure 4 (bottom), generally the effluent quality was very good with COD concentrations less than 100 $mg.L^{-1}$ and total VFA concentrations less than 50 $mg.L^{-1}$. In particular, the process completely removed oil and grease. The combination of biogas production and low VFA concentrations in the digester effluent were a good indication of a healthy and stable process.

Performance at Site B is shown in Figure 5 and was more variable. COD removal efficiency at Site B was still greater than 95%. i.e less than 5% of COD from the wastewater feed remained in the treated permeate, the methane yields were lower with only 77% of COD converted to biogas, indicating a consistent accumulation of COD within the reactor. The pilot plant at Site B experienced 2 major failure events, the first failure occurred after approximately 100 days and was a membrane limitation caused by in-reactor solids concentration accumulating to 40.2 $g.L^{-1}$. The sludge inventory was reduced to 20 $g.L^{-1}$ and the plant was re-started, after which it was operated with an SRT of 50 days to minimize sludge accumulation. A second failure event occurred between Day 140 and Day 150 and was a biological failure due to overload inhibition. The OLR at Site B at the time of overload was 3-3.5 $gCOD.L^{-1}.d^{-1}$ and was similar to the OLR successfully achieved at Site A. While the concentration of FOG in wastewater at Site B was higher than wastewater at Site A, FOG was a similar fraction of the COD and therefore FOG loads were similar between the plants.

However, the OLR of 3-3.5 $gCOD.L^{-1}.d^{-1}$ at Site A was achieved with a sludge inventory of 25 $g.L^{-1}$ (20 $g.L^{-1}$ VS) while the sludge inventory at Site B was only 17 $g.L^{-1}$ (13 $g.L^{-1}$) at the time of overload. The reduced sludge inventory required for effective fouling control likely increased the risk of overload inhibition.

Table 2 and 3 show a summary of the AnMBR performance and compares the wastewater feed with the treated AnMBR permeate for each site. The results confirm COD removal at both sites was over 95%. At Site A, 90% of N is released to the permeate as NH_3 while 74% of P is released to the permeate as PO_4. At Site B, 78% of N is released to the permeate as NH_3 while 74% of P is released to the permeate as PO_4. This is potentially recoverable as struvite given the concentrations are well above limit values for precipitation [34].

The cumulative COD balance for the AnMBR pilot plant is shown in Figure S2. At Site A, there was initially some accumulation of COD within the AnMBR, likely due to some anaerobic sludge production. However, COD balance converged over time, demonstrating there was virtually no accumulation of COD within the process. This very high COD-to-biogas conversion would suggest that the AnMBR could operate with a near infinite sludge age, however the concentration of N (90%) and P (74%) in the AnMBR permeate was lower than the concentrations in the feed, this demonstrates that nutrients were accumulating in the AnMBR and it is therefore likely that non-degradable or inert solids were also accumulating. At Site B, the pilot plant was accumulating COD approximately 20% of COD added to the reactor when operating with an infinite SRT and this was due to the lower degradability of the feed. Where non-degradable solids are added to the AnMBR, sludge removal is the only mechanism for removal therefore required during operation.

Biological operating limits of the AnMBR pilot plant were estimated as an organic loading rate of 3-3.5 $gCOD.L^{-1}.d^{-1}$ and the maximum sludge inventory for fouling control estimated at 40 $g.L^{-1}$ estimated for the sludge inventory. Higher organic loads and/or shorter retention times may be possible but increase the risk of failure due to membrane fouling; mitigating this risk through continuous removal of sludge will also reduce the inventory of active biomass in the process and increase the risk of organic overload. The AnMBR operating limits identified in the current study are conservative compared to Saddoud and Sayadi (2007) who reported successful operation of an AnMBR treating slaughterhouse wastewater at OLR in the range of 4-8 $gCOD.L^{-1}.d^{-1}$ [35], however the sCOD content of the feed was much higher suggesting a more readily degradable material. However, Saddoud and Sayadi (2007) also reported lower methane yields in the range of 200 to 300 $L.kg^{-1}$ sCOD removed, this demonstrates that at high OLR solids and COD were accumulating in the reactor and complete biological degradation was not occurring.

The OLRs of the AnMBR achieved in the present study were significantly higher than OLRs achieved for anaerobic lagoons treating municipal sewage [36-38], slaughterhouse effluent [4], or other agri-industrial wastes, and on the order of that achieved by UASB reactors [39, 40]. UASBs operate by retaining solids in the process volume, the AnMBR is not dependent on sludge settleability and therefore the COD removal and effluent quality were also substantially higher in the AnMBR compared to lagoon processes and UASBs. Importantly, the COD removal efficiency from the AnMBR process were not impacted by HRT or OLR within the identified limits, this demonstrates that AnMBRs may be tolerant to variations in flow with minimal risk of sludge washout or impacts on effluent quality. Methane yields from the AnMBRs were consistent during the operating period demonstrating stable performance, due to temperature regulation. Again, this trend is not observed in lagoon based processes where process performance is impacted by environmental conditions and daily biogas production can vary by an order of magnitude depending on temperature or plant operational factors [4], and where temperature management is not possible.

In this study, an AnMBR was operated successfully at HRTs as low as 2 days, an order of magnitude lower than the HRTs expected for a conventional CSTR style digester (20 days, based on hydrolysis rate constant of 0.35 day^{-1}), the reduction in HRT required for treatment would significantly reduce both the footprint and capital cost of the treatment process.

3.3 Membrane Performance and Fouling

Transmembrane pressure (TMP), logged using a PLC is shown in Figure 6. The TMP is an indication of membrane fouling; with fouling rates calculated from an increase in TMP over time and used to schedule corrective maintenance such as shut down/cleaning events. Figure 6 demonstrates no observable increase in TMP over time, indicating that membrane fouling is sustainable and below critical flux. Gas sparging provides surface shear and therefore controls particle deposition [22]. At Site A, there were two notable exceptions with fouling events on Day 30 and Day 170, these fouling events were represented by a rapid increase in TMP (Figure 6) and coincided with failure of the biogas recirculation pump and in both occurrences, the gas sparging rate reduced from 35 $L.min^{-1}$ to approximately 10 $L.min^{-1}$. At Site B, there was a major fouling event around Day 100, corresponding with an increase in the sludge concentrations in the reactor from 30 $g.L^{-1}$ to 40 $g.L^{-1}$, under these conditions the gas sparging (35 $L.min^{-1}$) was no longer sufficient for fouling control and rapid fouling resulted in a complete disruption of permeate flow. The sludge inventory was reduced to 20 $g.L^{-1}$, and gas sparging was again effective for fouling control. The results demonstrate that gas sparging is critical for fouling control, but loses effectiveness at higher solids concentrations.

A critical flux test was conducted after 200 days of AnMBR operation. Figure 7 shows the evolution of TMP at flux steps between 0 to 11 $L.m^{-2}.h^{-2}$, incremented every 15 minutes. The results show that the fouling rate (dP/dt) was less 0.01 $kPa.min^{-1}$ at flux as high as 9 $L.m^{-2}.h^{-2}$, however this methodology is based on short-term testing and may not be a reliable representation of long-term sustainable flux. Submerged AnMBRs are characteristically low shear systems compared with cross-flow MBRs, which is generally desirable to maintain lower shear conditions and minimize harm to slow growing anaerobic consortia. However, low membrane shear leads to lower operating fluxes, and therefore higher membrane areas and higher capital costs to maintain the reactor HRT.

At a sludge inventory of 30 $g.L^{-1}$ or lower, sustainable permeate flux achieved in the submerged AnMBR in this study was between 3 and 7 $L.m^{-2}.h^{-2}$ (Figure 6 and is similar to fluxes of 5 to 10 $L.m^{-2}.h^{-2}$ [41] and 2 to 8 $L.m^{-2}.h^{-2}$ [35] previously achieved in AnMBRs treating slaughterhouse wastewater. The reactors operated by Fuchs et al (2003) and Saddoud (2007) operated with lower overall TS (8 to 25 $g.L^{-1}$) compared to the current study (30 $g.L^{-1}$) but had higher organic loading rates (6 to 16 $gCODL^{-1}.d^{-1}$). Similar membrane flux from AnMBRs treating slaughterhouse waste and from AnMBRs treating municipal wastewaters [42] suggest that membrane fouling is not more severe in slaughterhouse applications and is therefore not a strong or unique barrier against application of AnMBRs to slaughterhouse wastes. However, critical flux and management of membrane fouling remain key factors influencing economic feasibility of membrane processes [23], with membrane costs in the range of 72% of capital investment [24]. Therefore, optimization and control of membrane fouling will be a core area for ongoing research and development.

4 CONCLUSIONS

This study has successfully demonstrated that Anaerobic Membrane Bioreactors (AnMBRs) are an effective technology for high rate treatment of cattle slaughterhouse wastewater. This is based on a stable OLR of 3-3.5 $gCOD.L^{-1}d^{-1}$ at a HRT of 2 days, with the operating limit being defined by in-reactor active biomass inventory. An upper limit on the inventory is imposed by the inability to manage fouling at very high solids concentrations (>20 g L^{-1}). The pilot plants consistently removed over 95% of COD from the wastewater. Methane yields were closely related to waste biodegradability established from reference batch tests; 78-90% of nitrogen and 74% of phosphorus in the wastewater were released to permeate respectively enabling subsequent capture of nutrient resources, again nitrogen release was linked to waste biodegradability. The sustainable permeate flux in this study was consistent with values previously reported for AnMBRs treating municipal and industrial wastewaters and demonstrates that membrane fouling with high-solids, high fats wastewater is not a substantial barrier to application of AnMBRs to slaughterhouse wastes.

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6 REFERENCES

[1] M.R. Johns, Developments in wastewater treatment in the meat processing industry: A review, Bioresource Technology 54 (1995) 203-216.
[2] Y.Y. Liu, R.J. Haynes, Origin, nature, and treatment of effluents from dairy and meat processing factories and the effects of their irrigation on the quality of agricultural soils, Critical Reviews in Environmental Science and Technology 41 (2011) 1531-1599.
[3] P.D. Jensen, T. Sullivan, C. Carney, D.J. Batstone, Analysis of the potential to recover energy and nutrient resources from cattle slaughterhouses in Australia by employing anaerobic digestion, Applied Energy 136 (2014) 23-31.
[4] B.K. McCabe, I. Hamawand, P. Harris, C. Baillie, T. Yusaf, A case study for biogas generation from covered anaerobic ponds treating abattoir wastewater: Investigation of pond performance and potential biogas production, Applied Energy 114 (2014) 798-808.
[5] S. Astals, D.J. Batstone, J. Mata-Alvarez, P.D. Jensen, Identification of synergistic impacts during anaerobic co-digestion of organic wastes, Bioresource Technology 169 (2014) 421-427.
[6] M. Carballa, W. Vestraete, Anaerobic Digesters for Digestion of Fat-Rich Materials, in: K. Timmis (Ed.) Handbook of Hydrocarbon and Lipid Microbiology, Springer, Berlin Heidelberg, 2010, pp. 2631-2639.
[7] D.J. Batstone, J. Keller, L.L. Blackall, The influence of substrate kinetics on the microbial community structure in granular anaerobic biomass, Water Research 38 (2004) 1390-1404.
[8] N.T. Manjunath, I. Mehrotra, R.P. Mathur, Treatment of wastewater from slaughterhouse by DAF-UASB system, Water Research 34 (2000) 1930-1936.
[9] W. Cao, M. Mehrvar, Slaughterhouse wastewater treatment by combined anaerobic baffled reactor and UV/H2O2 processes, Chemical Engineering Research and Design 89 (2011) 1136-1143.
[10] D. Martinez-Sosa, M. Torrijos, G. Buitron, P. Sousbie, P.H. Devillers, J.P. Delgenès, Treatment of fatty solid waste from the meat industry in an anaerobic sequencing batch reactor: Start-up period and establishment of the design criteria, Water Science and Technology, 2009, pp. 2245-2251.
[11] R.K. Dereli, M.E. Ersahin, H. Ozgun, I. Ozturk, D. Jeison, F. van der Zee, J.B. van Lier, Potentials of anaerobic membrane bioreactors to overcome treatment limitations induced by industrial wastewaters, Bioresource Technology 122 (2012) 160-170.
[12] G. Skouteris, D. Hermosilla, P. López, C. Negro, Á. Blanco, Anaerobic membrane bioreactors for wastewater treatment: A review, Chemical Engineering Journal 198–199 (2012) 138-148.
[13] T. Hulsen, E. van Zessen, C. Frijters, Production of valuable biogas out of fat and protein containing wastewaters using compact BIOPAQ AFR and the THIOPAQ-technology IWA Water and Energy conference IWA Conference Proceedings, Amsterdam, 2010.
[14] C. Ramos, A. García, V. Diez, Performance of an AnMBR pilot plant treating high-strength lipid wastewater: Biological and filtration processes, Water Research 67 (2014) 203-215.
[15] S. Judd, The MBR Book : Principles and Applications of Membrane Bioreactors for Water and Wastewater Treatment, A Butterworth-Heinemann Title, Burlington, 2011.
[16] M.J. Cuetos, X. Gómez, M. Otero, A. Morán, Anaerobic digestion of solid slaughterhouse waste (SHW) at laboratory scale: Influence of co-digestion with the organic fraction of municipal solid waste (OFMSW), Biochemical Engineering Journal 40 (2008) 99-106.

[17] M. Kayhanian, Ammonia inhibition in high-solids biogasification: An overview and practical solutions, Environmental Technology 20 (1999) 355-365.
[18] C.S. Hwu, S.K. Tseng, C.Y. Yuan, Z. Kulik, G. Lettinga, Biosorption of long-chain fatty acids in UASB treatment process, Water Research 32 (1998) 1571-1579.
[19] X. Chen, R.T. Romano, R. Zhang, H.S. Kim, Anaerobic co-digestion of dairy manure and glycerin, American Society of Agricultural and Biological Engineers Annual International Meeting 2008 8 (2008) 5053-5070.
[20] J. Palatsi, M. Laureni, M.V. Andrés, X. Flotats, H.B. Nielsen, I. Angelidaki, Strategies for recovering inhibition caused by long chain fatty acids on anaerobic thermophilic biogas reactors, Bioresource Technology 100 (2009) 4588-4596.
[21] V. Diez, C. Ramos, J.L. Cabezas, Treating wastewater with high oil and grease content using an Anaerobic Membrane Bioreactor (AnMBR). Filtration and cleaning assays, Water Science and Technology 65 (2012) 1847-1853.
[22] A. Boyle-Gotla, P.D. Jensen, S.D. Yap, M. Pidou, Y. Wang, D.J. Batstone, Dynamic multidimensional modelling of submerged membrane bioreactor fouling, Journal of Membrane Science 467 (2014) 153-161.
[23] S.-m. Lee, J.-y. Jung, Y.-c. Chung, Novel method for enhancing permeate flux of submerged membrane system in two-phase anaerobic reactor, Water Research 35 (2001) 471-477.
[24] H. Lin, J. Chen, F. Wang, L. Ding, H. Hong, Feasibility evaluation of submerged anaerobic membrane bioreactor for municipal secondary wastewater treatment, Desalination 280 (2011) 120-126.
[25] M. Yao, K. Zhang, L. Cui, Characterization of protein-polysaccharide ratios on membrane fouling, Desalination 259 (2010) 11-16.
[26] S. Arabi, G. Nakhla, Impact of protein/carbohydrate ratio in the feed wastewater on the membrane fouling in membrane bioreactors, Journal of Membrane Science 324 (2008) 142-150.
[27] I. Angelidaki, M. Alves, D. Bolzonella, L. Borzacconi, J.L. Campos, A.J. Guwy, S. Kalyuzhnyi, P. Jenicek, J.B. Van Lier, Defining the biomethane potential (BMP) of solid organic wastes and energy crops: A proposed protocol for batch assays, 2009, pp. 927-934.
[28] P.D. Jensen, H. Ge, D.J. Batstone, Assessing the role of biochemical methane potential tests in determining anaerobic degradability rate and extent, Water Science and Technology 64 (2011) 880-886.
[29] M.A.H. Franson, A.D. Eaton, A.P.H. Association., A.W.W. Association., W.E. Federation., Standard methods for the examination of water & wastewater, 21st ed. ed., American Public Health Association, Washington, DC :, 2005.
[30] P. Le Clech, B. Jefferson, I.S. Chang, S.J. Judd, Critical flux determination by the flux-step method in a submerged membrane bioreactor, Journal of Membrane Science 227 (2003) 81-93.
[31] R.E. Speece, Anaerobic Biotechnology and Odor/Corrosion Control for Municipalities and Industries, Archae Press, Nashville, TN., 2008.
[32] D.J. Batstone, P.D. Jensen, Anaerobic processes., in: P. Wilderer, P. Rogers, S. Uhlenbrook, F. Frimmel, K. Hanaki (Eds.) Treatise on Water Science, Academic Press, Oxford, U.K., 2011, pp. 615-640.
[33] D.G. Cirne, X. Paloumet, L. Björnsson, M.M. Alves, B. Mattiasson, Anaerobic digestion of lipid-rich waste-Effects of lipid concentration, Renewable Energy 32 (2007) 965-975.
[34] C.M. Mehta, D.J. Batstone, Nucleation and growth kinetics of struvite crystallization, Water Research 47 (2013) 2890-2900.
[35] A. Saddoud, S. Sayadi, Application of acidogenic fixed-bed reactor prior to anaerobic membrane bioreactor for sustainable slaughterhouse wastewater treatment, Journal of Hazardous Materials 149 (2007) 700-706.
[36] M.R. Peña, D.D. Mara, High-rate anaerobic pond concept for domestic wastewater treatment: Results from pilot scale experience, (2003) 68.

[37] H. Toprak, Temperature and organic loading dependency of methane and carbon dioxide emission rates of a full-scale anaerobic waste stabilization pond, 29 (1995) 1111-1119.
[38] B. Picot, J. Paing, J.P. Sambuco, R.H.R. Costa, A. Rambaud, Biogas production, sludge accumulation and mass balance of carbon in anaerobic ponds, 48 (2003) 243-250.
[39] S. Sayed, L. van Campen, G. Lettinga, Anaerobic treatment of slaughterhouse waste using a granular sludge UASB reactor, Biological Wastes 21 (1987) 11-28.
[40] I. Ruiz, M.C. Veiga, P. De Santiago, R. Blázquez, Treatment of slaughterhouse wastewater in a UASB reactor and an anaerobic filter, Bioresource Technology 60 (1997) 251-258.
[41] W. Fuchs, H. Binder, G. Mavrias, R. Braun, Anaerobic treatment of wastewater with high organic content using a stirred tank reactor coupled with a membrane filtration unit, Water Research 37 (2003) 902-908.
[42] M. Xu, X. Wen, Z. Yu, Y. Li, X. Huang, A hybrid anaerobic membrane bioreactor coupled with online ultrasonic equipment for digestion of waste activated sludge, Bioresource Technology 102 (2011) 5617-5625.

ABBREVIATIONS

AnMBR	Anaerobic Membrane Bioreactor
COD	Chemical Oxygen Demand
CSTR	Continuous Stirred Tank Reactor
DAF	Dissolved Air Flotation (tank)
FOG	Fat, Oils and Grease
HRAT	High rate anaerobic technology
HRT	Hydraulic Residence Time
LCFA	Long Chain Fatty Acids
OLR	Organic Loading Rate
PLC	Process Logic Controller
RTD	Resistance Temperature Detector
SRT	Sludge Retention Time
TKN	Total Kjehldahl Nitrogen
TMP	Transmembrane pressure
TP	Total Phosphorus
TS	Total Solids

UASB	Upflow Anaerobic Sludge Blanket
VFA	Volatile Fatty Acids
VS	Volatile Solids
WWTP	Waste Water Treatment Plant

TABLES

Table 1: Summary of operating strategies for the AnMBR pilot plant

Table 2: Composition of feed wastewater and permeate from AnMBR Pilot Plant at Site A

Table 3: Composition of feed wastewater and permeate from AnMBR Pilot Plant at Site B

FIGURES

Figure 1: Anaerobic Membrane Bioreactor Pilot Plant installed at an Australia Beef processing facility (left) and hollow fiber membrane module (right).

Figure 2: Detailed piping and instrument diagram of anaerobic membrane pilot plant.

Figure 3: Results from replicate biomethane potential tests on slaughterhouse wastewater used as feed to the AnMBR pilot plants (expressed at 25°C and 1atm). Line represents best model fit with parameter.

Figure 4: COD loading to the AnMBR pilot plant at Site A with corresponding COD removal in the permeate and biogas (top); and composition of the AnMBR permeate (bottom).

Figure 5: COD loading to the AnMBR pilot plant at Site B with corresponding COD removal in the permeate and biogas (top); and composition of the AnMBR permeate (bottom).

Figure 6: Transmembrane pressure (○) in AnMBR pilot plant at Site A (top) and Site B (bottom).

Figure 7: Analysis of critical flux using digested sludge in AnMBR after 200 days at Site A. Critical flux assessed using flux step method.

Figure S1: Effective hydraulic retention time (HRT) and Organic Loading Rate (OLR) during the pilot plant operation.

Figure S2: Chemical oxygen demand balance in the AnMBR pilot plant during extended operation at Site A (top) and Site B (bottom). The black line indicates that the Feed COD is equal to the product COD. Where the data is below the black line, the reactor may have been accumulating sludge.

Table 1: Summary of operating strategies for the AnMBR pilot plant

Site	Period	HRT (days)	membrane flux ($L.m^{-2}.h^{-1}$)	Operation
A (DAF separated wastewater)	1	7	6.25	75L fed twice weekly over 12 hours per feed
	2	4	3.5	40L fed daily over 12 hours per feed

	3	2	3.5	80 $L.d^{-1}$ fed continuously
	1	7	0.9	22 $L.d^{-1}$ fed continuously
B (combined, no pre-treatment)	2	4	1.6	38 $L.d^{-1}$ fed continuously
	3	4	1.6	38 $L.d^{-1}$ fed continuously. Sludge withdrawn for 50 d SRT

Table 2: Composition of feed wastewater and permeate from AnMBR Pilot Plant at Site A

		TS	VS	tCOD	sCOD	FOG	VFA	TKN	NH_3-N	TP	PO_4-P
		mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
	Minimum	1,200	900	2,084	470	266	11	107.6	12.0	8.9	3.7
Feed	**Average**	**3,378**	**2,834**	**5,919**	**1,187**	**1,407**	**159**	**190.2**	**24.4**	**19.1**	**7.9**
	Maximum	7,000	6,200	13,381	2,778	5,953	566	294.8	59.6	34.6	17.3
	Minimum	ND	ND	23	23	ND	6	139.6	124.0	8.4	8.3
Permeate	**Average**	**ND**	**ND**	**71**	**71**	**ND**	**15**	**172.6**	**170.2**	**14.1**	**12.8**
	Maximum	ND	ND	379	379	ND	67	207.2	209.0	38.3	37.1

TS - total solids; VS - volatile solids; tCOD - total chemical oxygen demand; sCOD - soluble chemical oxygen demand; FOG – fat, oil, grease; VFA – volatile fatty acids; TKN – total Kjeldahl nitrogen; NH_3-N – ammonia nitrogen; TP – total phosphorus; PO_4-P phosphate phosphorous

Table 3: Composition of feed wastewater and permeate from AnMBR Pilot Plant at Site B

		TS	VS	tCOD	sCOD	FOG	VFA	TKN	NH_3-N	TP	PO_4-P
		mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
	Minimum	2036	1782	3163	156	11	52	130.0	8.0	16.8	3.0
Feed	**Average**	**5162**	**4472**	**10604**	**1778**	**1881**	**481**	**373.7**	**59.2**	**36.3**	**22.7**
	Maximum	15485	14395	31600	4512	5540	1282	1163.2	274.0	172.8	76.0
	Minimum	ND	ND	20	20	ND	5	44.0	27.9	2.0	1.4
Permeate	Average	ND	ND	183	183	ND	100	297.2	294.1	25.2	27.0
	Maximum	ND	ND	2034	2034	ND	1577	747.2	618.0	62.4	76.0

TS - total solids; VS - volatile solids; tCOD - total chemical oxygen demand; sCOD - soluble chemical oxygen demand; FOG – fat, oil, grease; VFA – volatile fatty acids; TKN – total Kjeldahl nitrogen; NH_3-N – ammonia nitrogen; TP – total phosphorus; PO_4-P phosphate phosphorous

Figure 1 .

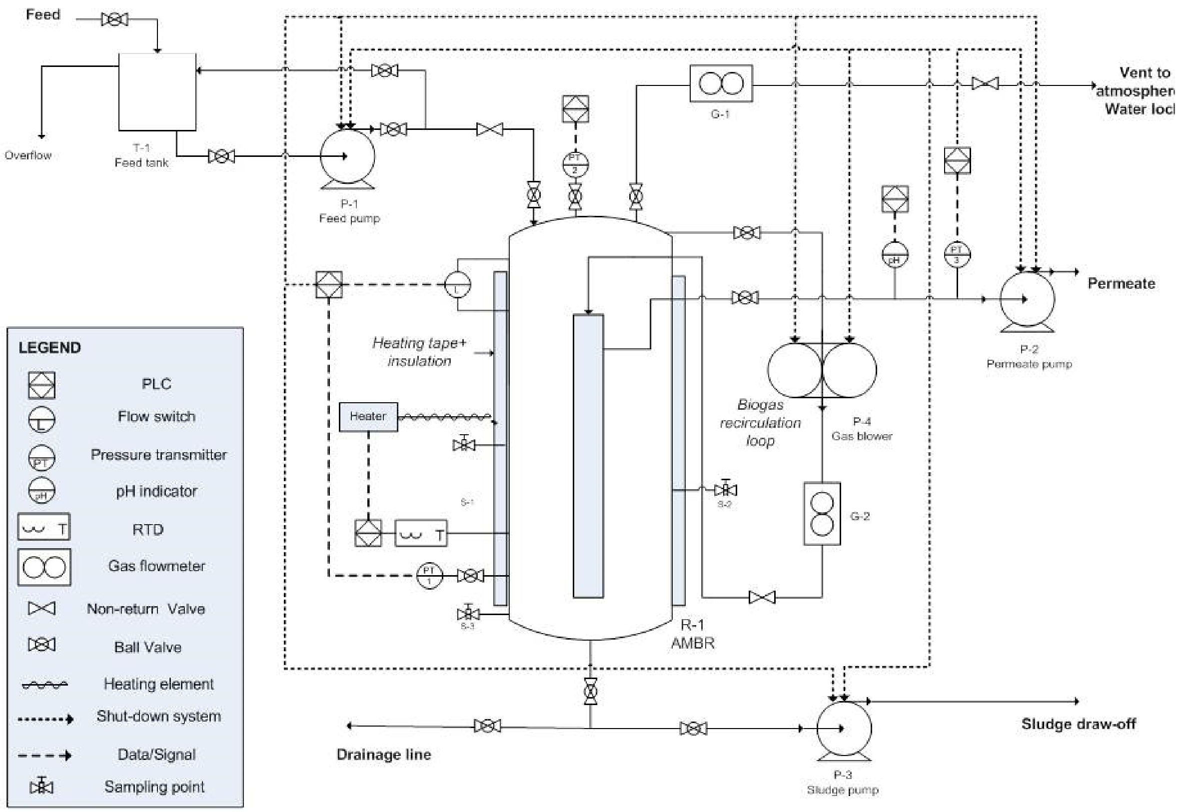
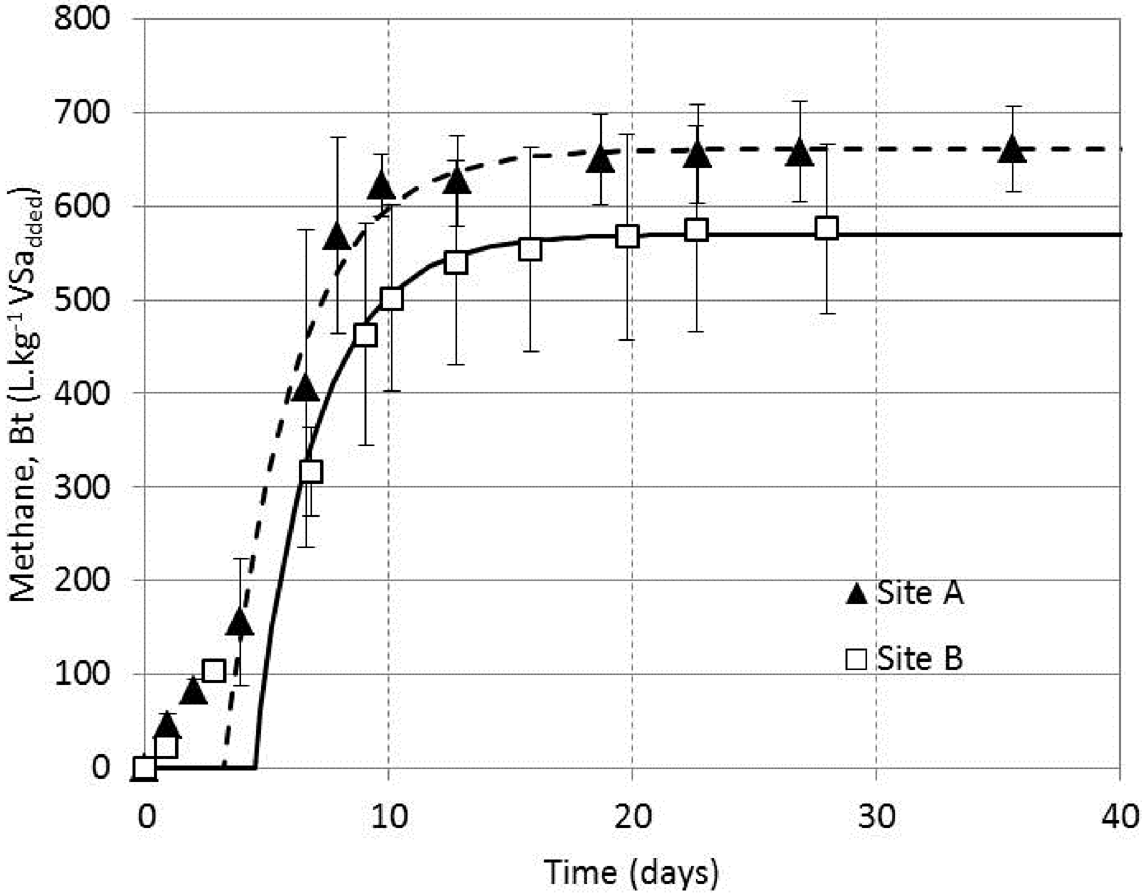

Feed
Overflow
T-1
Feed tank
P-1
Feed pump
G-1
Vent to
atmospher
Water locl
Permeate
P-2
Permeate pump
Heating tape+
insulation
Heater
Biogas
recirculation
loop
P-4
Gas blower
G-2
S-1
S-2
S-3
R-1
AMBR
Drainage line
Sludge draw-off
P-3
Sludge pump
LEGEND
PLC
Flow switch
Pressure transmitter
pH indicator
RTD
Gas flowmeter
Non-return Valve
Ball Valve
Heating element
Shut-down system
Data/Signal
Sampling point

Figure 2 .



800
700
600
500
400
300
200
100
0
Methane, Bt (L.kg-1 VSadded)
0
10
20
30
40
Time (days)
Site A
Site B

Figure 3 .

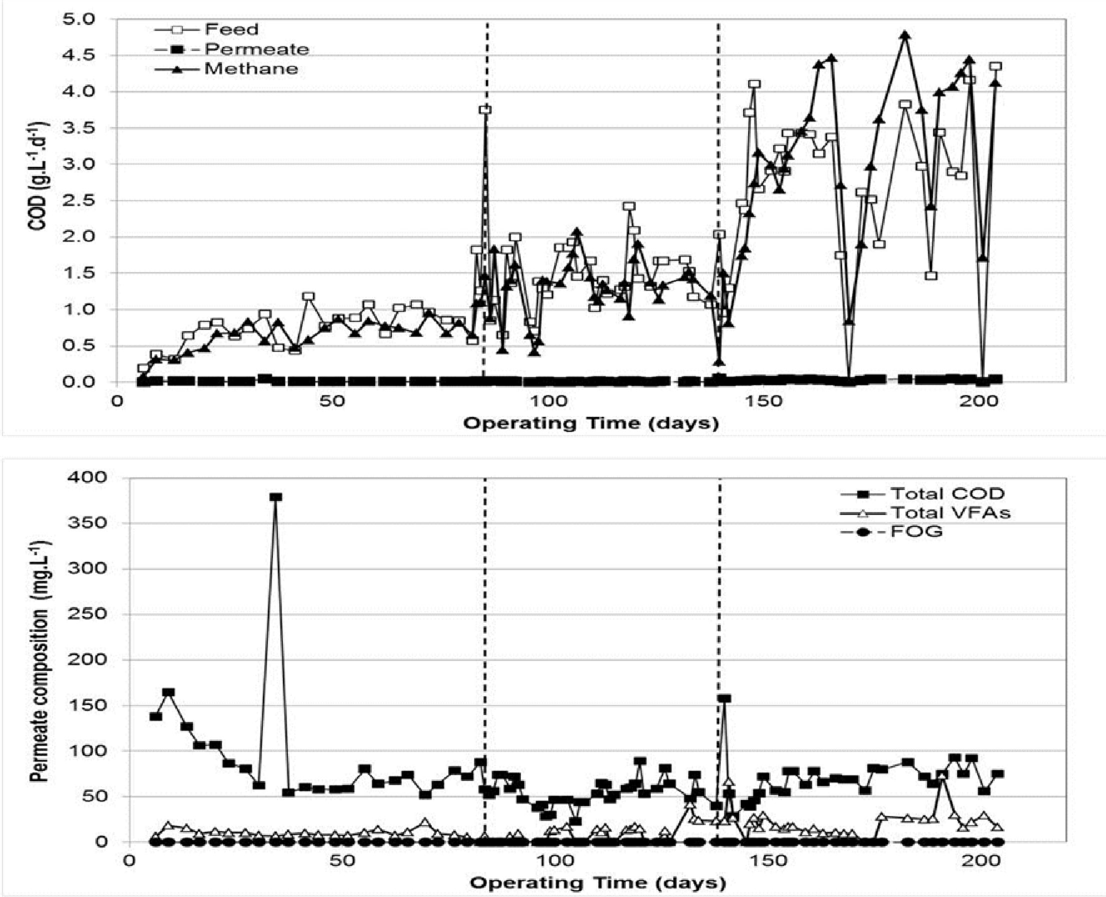

Feed
Permeate
Methane
COD (g.L-1.d-1)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
50
100
150
200
Operating Time (days)
Total COD
Total VFAs
FOG
Permeate composition (mg.L-1)
400
350
300
250
200
150
100
50
0
0
50
100
150
200
Operating Time (days)

Figure 4 .

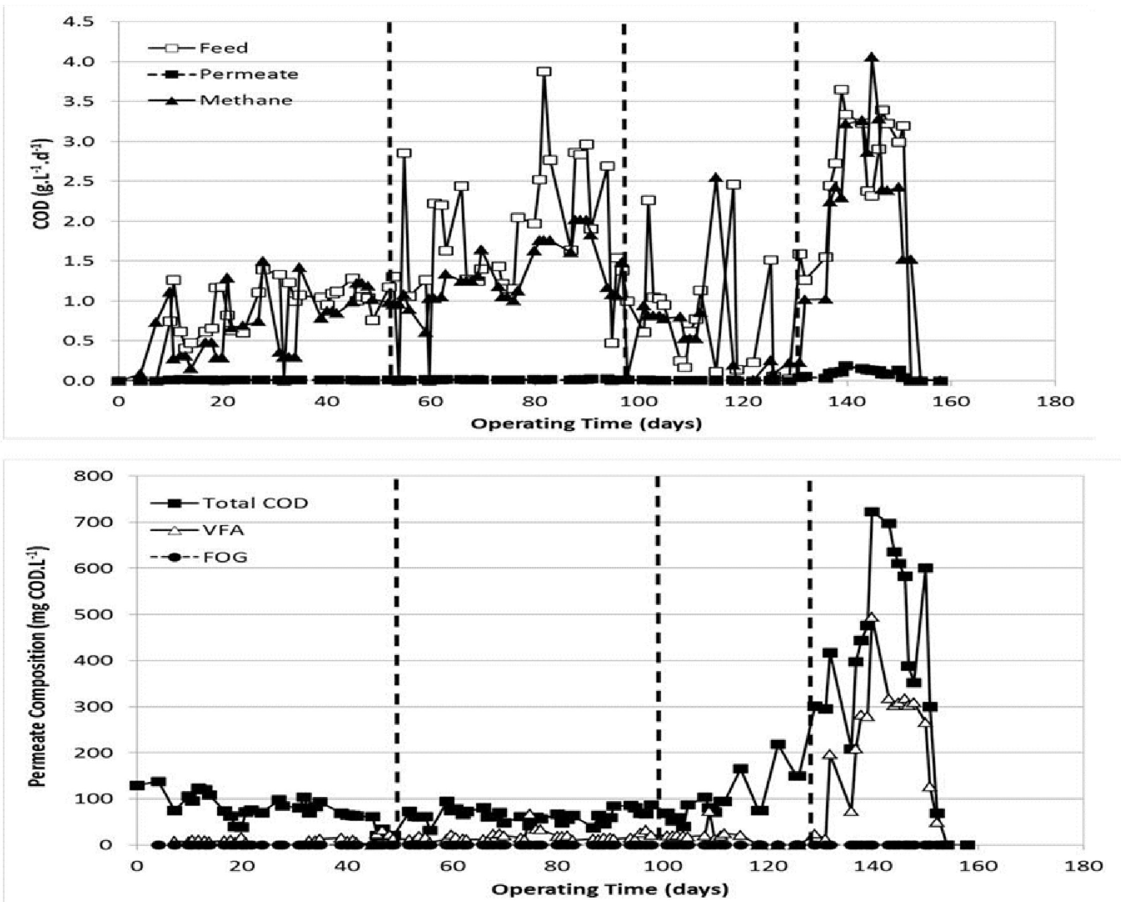

Feed
Permeate
Methane
COD (g.L-1.d-1)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
20
40
60
80
100
120
140
160
180
Operating Time (days)
Total COD
VFA
FOG
Permeate Composition (mg COD.L-1)
800
700
600
500
400
300
200
100
0
0
20
40
60
80
100
120
140
160
180
Operating Time (days)

Figure 5 .

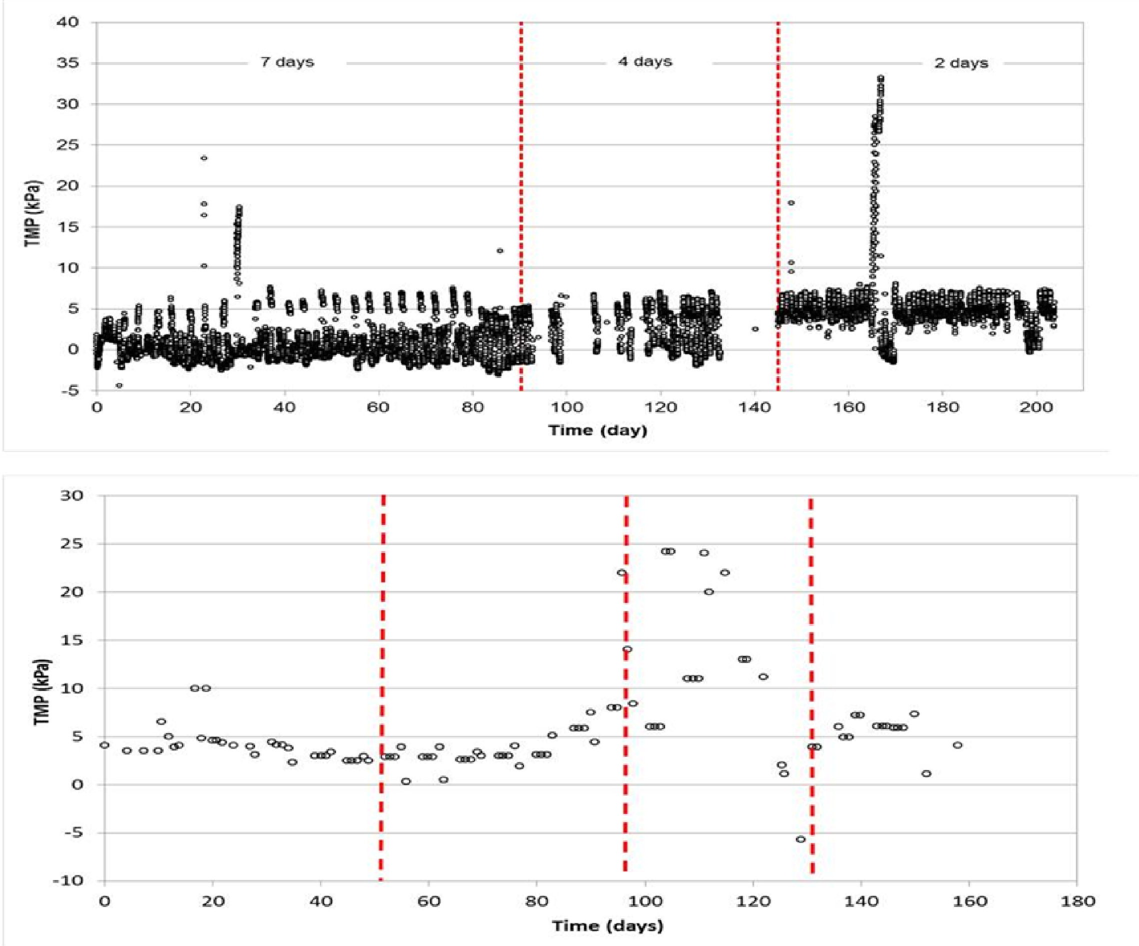
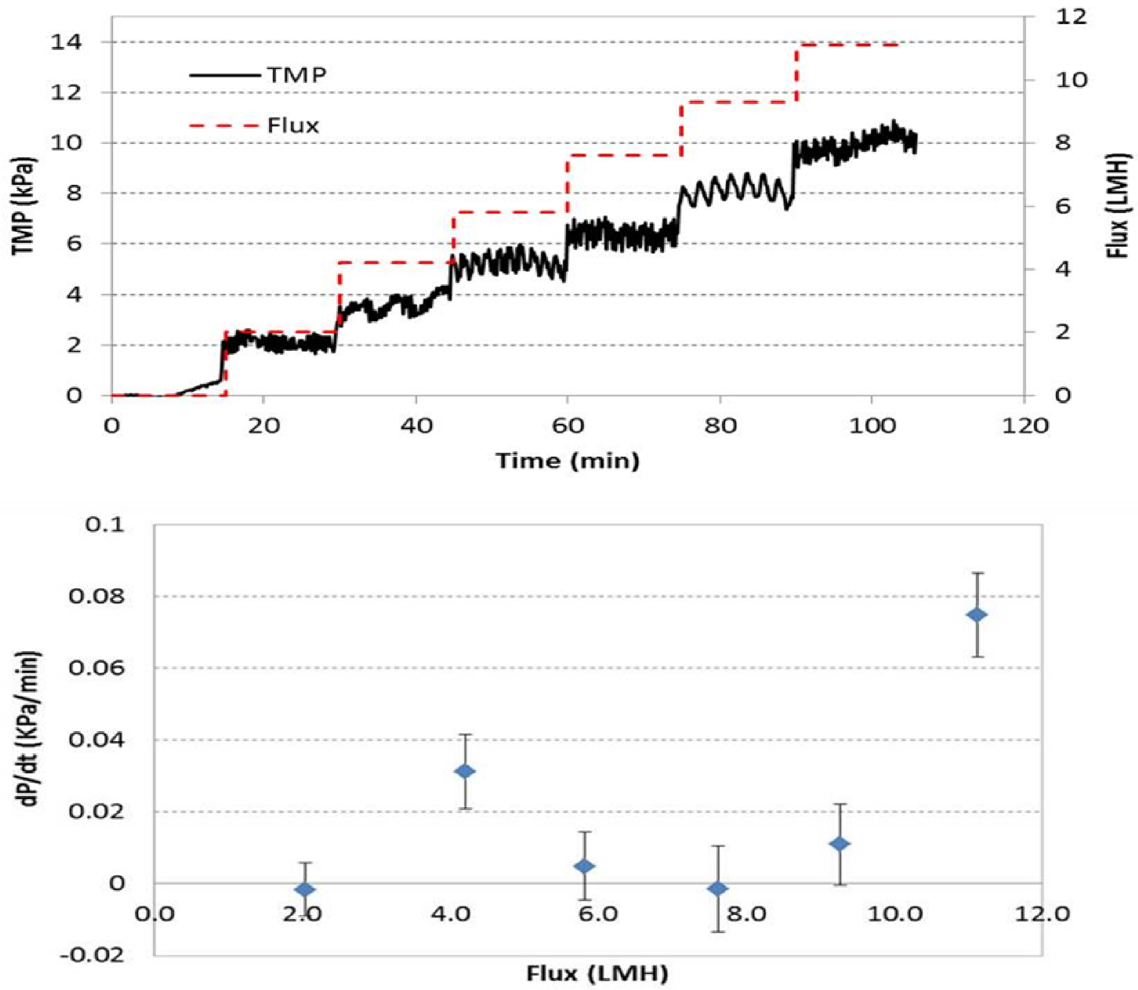


Figure 6 .



14
12
10
8
6
4
2
0
TMP
Flux
TMP (kPa)
Flux (LMH)
0
20
40
60
80
100
120
Time (min)
0.1
0.08
0.06
0.04
0.02
0
-0.02
dP/dt (KPa/min)
0.0
2.0
4.0
6.0
8.0
10.0
12.0
Flux (LMH)

Figure 7rev .

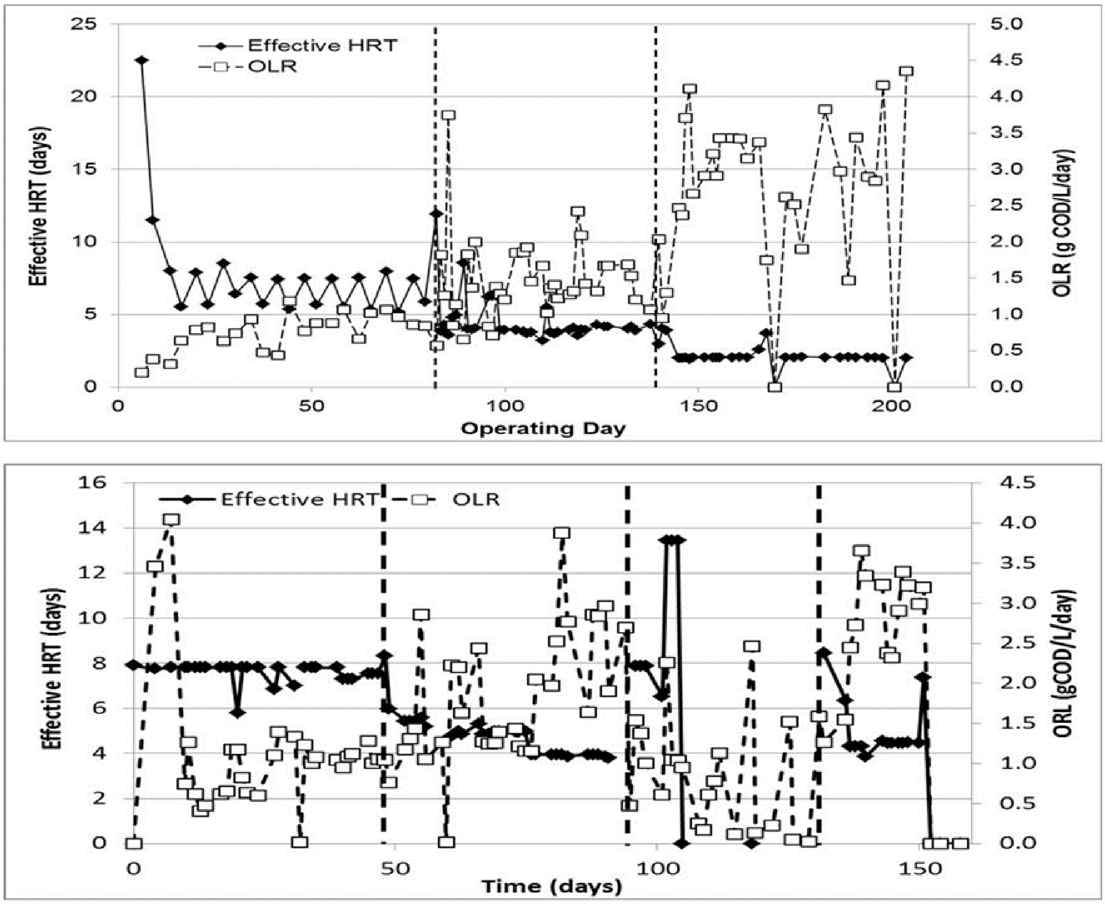
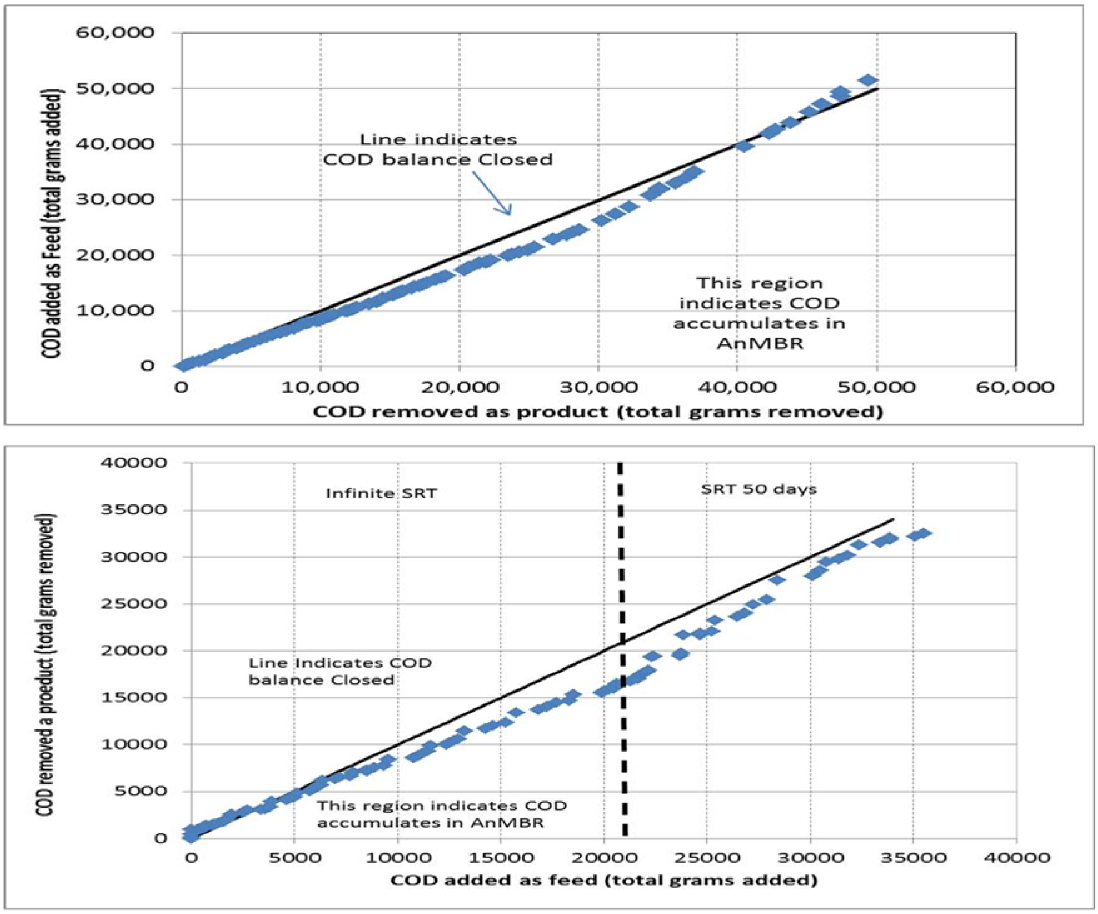

Effective HRT
OLR
Effective HRT (days)
OLR (g COD/L/day)
Operating Day
Effective HRT
OLR
Effective HRT (days)
ORL (gCOD/L/day)
Time (days)

Figure S1 .



60,000
50,000
40,000
30,000
20,000
10,000
0
0
10,000
20,000
30,000
40,000
50,000
60,000
COD added as Feed (total grams added)
COD removed as product (total grams removed)
Line indicates
COD balance Closed
This region
indicates COD
accumulates in
AnMBR
40000
35000
30000
25000
20000
15000
10000
5000
0
0
5000
10000
15000
20000
25000
30000
35000
40000
COD removed a proeduct (total grams removed)
COD added as feed (total grams added)
Infinite SRT
SRT 50 days
Line Indicates COD
balance Closed
This region indicates COD
accumulates in AnMBR

Figure S2 .