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Citation	Kunacheva, C., Soh, Y. N. A., Trzcinski, A. P., & Stuckey, D. C. (2017). Soluble microbial products (SMPs) in the effluent from a submerged anaerobic membrane bioreactor (SAMBR) under different HRTs and transient loading conditions. Chemical Engineering Journal, 311, 72-81.
Date	2016
URL	http://hdl.handle.net/10220/43777
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Soluble Microbial Products (SMPs) in the Effluent from a Submerged Anaerobic Membrane Bioreactor (SAMBR) under Different HRTs and Transient Loading Conditions

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Abstract

This study investigated the performance of a submerged anaerobic membrane bioreactor (SAMBR) fed with synthetic wastewater (544 ± 22 mgCOD/L) operating at different hydraulic retention times (HRTs -12 h, 8 h, 6 h, 4 h, 2 h, and 1 h) at both steady state, and under transient load conditions (2 and 1 h), and the SMPs produced under these conditions. COD removal at decreasing HRTs (12 h, 8 h, 6 h, 4 h, and 2 h) was high (>94%), but decreased to 80% when operating at 1 h HRT. VFAs accumulated when the HRT was decreased to 2 h and 1 h, accounting for 69% and 89% of the effluent COD, respectively. Effluent SMPs accounted for an average of 14±2 mgCOD/L at steady state, but this fluctuated more during transient conditions (12±6 mgCOD/L). The COD

equivalent of dissolved methane in the effluent was 17% at 4 h HRT, exceeding the saturation value of methane. Low MW compounds were identified using gas chromatography-mass spectrometry (GC-MS), with solid phase extraction (SPE) as the pre-treatment. 120 compounds were identified in the effluent at steady state, and were alkanes (39), alkenes (3), esters (11), alcohols (7), nitrogenated compounds (11), phenols (11), and others (9). Increases in cyclooctasulfur, N-butyl-benzenesulfonamide, alkanes, 1-naphthalenol, camphor, 2-methylphenol, and (Z)-9-octadecenamide were also found during transient conditions, and these compounds were not found in the feed; hence it is possible that these compounds were produced by microorganism as by-products from substrate utilization.

Keywords: anaerobic; GC-MS analysis; HRT; membrane bioreactor; soluble microbial products; wastewater

Abbreviations

BOD	biochemical oxygen demand
COD	chemical oxygen demand
CSTR	continuous stirred tank reactor
EI	electron ionisation mode
F/M	food to microorganism ratio
GC-MS	gas chromatography-mass spectrometry
HPLC	high-performance liquid chromatography
HRT	hydraulic retention time
IDL	instrument detection limit

LC-MS	liquid chromatography–mass spectrometry
LMH	litres per square meter per hour
MLVSS	mixed liquor volatile suspended solids
MW	molecular weight
OLR	organic loading rate
ORP	oxidation reduction potential
SAMBR	submerged anaerobic membrane bioreactor
SCOD	soluble chemical oxygen demand
SMA	specific methanogenic activity
SMPs	soluble microbial products
SPE	solid phase extraction
SRT	solids retention time
TCD	thermal conductivity detector
TMP	transmembrane pressure
TSS	total suspended solids
UASB	upflow anaerobic sludge blanket
VFA	volatile fatty acid

1. Introduction

Interest in Submerged Anaerobic Membrane Bioreactors (SAMBR) for the treatment of domestic wastewater has increased in the past decade due to its small footprint, and low energy consumption and solids production compared to existing conventional aerobic domestic wastewater treatment processes [1]. Using SAMBRs is a promising solution

since it offers independent control of the solids (SRT) and hydraulic retention times (HRT) which allows larger volumes of wastewater to be treated on a smaller footprint. In addition, the use of membranes keeps the effluent free from suspended solids, which is a significant benefit when considering water reuse. Many researchers have reported that most of the chemical oxygen demand (COD) in the effluent from biological systems has been identified as soluble microbial products (SMPs) [2, 3]. “SMPs” have been defined as the pool of organic compounds that are released into solution from substrate metabolism and biomass decay other than key intermediates such as VFAs [4], and their presence affects the performance of most biological treatment systems.

The majority of the SMPs in biological effluents are degradable over time in both aerobic and anaerobic processes, however, conventional HRTs are usually not long enough for them to be totally degraded [5]. It has been reported from previous studies, in both aerobic and anaerobic biological processes, that around 2% of the incoming feed COD is present in the effluent as SMPs [6]. However, under transient conditions including nutrient limitations, the presence of toxicants, or when the feed flow or composition is changed radically, the effluent SMPs can be as high as 17% of the influent COD [6]. The presence of SMPs influences the performance of biological processes through changes in microbial community composition [7], and by fouling the membranes in membrane bioreactors [1]. Therefore, it is important to evaluate the production and composition of SMPs in a SAMBR in order to understand what these compounds are, how they are produced, so we can start to find a solution for controlling the system and membrane fouling during different HRTs and transient load conditions. The objective of this study was to evaluate the performance of a SAMBR under different operating HRTs, to test its HRT limits, and, most importantly, to evaluate the

effects of transient load conditions in the reactor on SMP production and composition which in turn influence effluent COD and membrane fouling. The HRT was decreased from 12 h to 8 h, 4 h, 2 h and 1 h. Effluent from the SAMBR was analysed for COD, carbohydrates and protein-like compounds, and volatile fatty acids (VFA). Lower molecular weight SMPs (<600 Da) were identified using gas chromatography-mass spectrometry (GC-MS) with solid phase extraction (SPE) as a pre-concentration step. There is no work in the literature in this area, and the depth of analysis of SMPs in this paper has never been carried out before.

2. Materials and methods

2.1 Reagents and chemicals

Acetone, chloroform, dichloromethane, and n-hexane (GC-MS grade or equivalent) were purchased from Merck. Methanol (LC-MS grade) was purchased from Sigma-Aldrich. Other solvents such as diethyl ether, ethyl acetate, n-heptane, methyl tert-butyl ether and toluene were of chromatographic grade and purchased from Fisher. Formic, acetic, propionic, iso-butyric, butyric, iso-valeric and valeric acid (analytical grade) and the alkane standard mixture (C10 - C40, all even, 50 mg/L each) were purchased from Sigma-Aldrich. Deionised water was obtained from a MilliQ water treater (Millipore Advantage A10).

2.2 Submerged Anaerobic Membrane Bioreactor (SAMBR)

Figure 1 shows a diagram of the experimental set-up used for this study. The SAMBR was made from polymethyl methacrylate (Plexiglas®) and had a working volume of 3 L. A microfiltration flatsheet membrane (size: 222mm × 315mm × 6mm, Chlorinated Polyethylene) from Kubota with a surface area of 0.116 m^2 and a maximum pore size of 0.4 and average of 0.2 μm was used. The flux of the membrane was set at 15 litres per square meter per hour (LMH) for most conditions (HRT 12, 8, 6, 4 and 2 h) using a membrane flux pump to keep it below the critical flux (24 LMH), although at an HRT of 1 h it had to be set to 27.4 LMH to allow for this short HRT. The HRT was set using another pump on the effluent line after the flux pump. The sludge inoculum was obtained from an anaerobic digester in a WWTP in Singapore. The mixed liquor volatile suspended solids (MLVSS) in the reactor were 6,000 mg/L at the start of the experiment at each HRT, and the SAMBR was operated at a 200 days SRT. The pH in the system was maintained in the range of 6.8 and 7.2 using 1M $NaHCO_3$. Oxidation-reduction potential (ORP) was in the range of -451 ± 6 mV throughout the study.

The reactor was designed with a baffle to direct the liquid in an upward direction past the membrane, and then down the downcomer after gas disengagement, and was placed in a water bath at 35±1°C. Biogas was re-circulated through a stainless steel tube diffuser with four holes which generated coarse bubbles in order to mix the biomass in the reactor and clean the surface of the membrane (minimize membrane fouling), and the gas flow rate was controlled at 8 L/min (4.14 $m^3/m^2.h$). The reactor was continuously fed with a synthetic feed (544 ± 22 mgCOD/L) comprised of glucose, peptone, meat extract, and essential nutrients which had a similar COD to domestic wastewater in Singapore. The SAMBR was operated under stable conditions for each

HRT, starting at 12 h which was then decreased to 8 h, 6 h, and 4 h. Hydraulic shock loads were performed in two phases; the 1st phase was when the HRT decreased from 4 h → 2 h (for 12 h) → 4 h, while the 2nd phase was when the HRT decreased from 4 h → 2 h (6 days) → 1 h (12 h) → 4 h. MLVSS were controlled at 6,000 mg/L at the start of the 1st phase and at 7,000 mg/L at the start of the 2nd phase in order to cope with the high organic loading rates (OLRs) at 2 h and 1 h HRT.

In common with most literature work in this area, the biological "control" was internal, ie. our SMP data was compared to the results obtained before the shock. This is because even using a stock "seed" culture to reseed the reactor after every shock load to nominally obtain the "same" starting culture, the microbial ecology in the seed reactor would have changed over time [8], and at present there is nothing in the literature to link changes in microbial ecology to changes in SMP production due to the complexity of this relationship, although this is a very interesting question.

2.3 General parameters

All samples were filtered through 0.45μm glass fibre filters to separate any residual biomass, and then analysed in duplicate for glucose, VFAs and soluble chemical oxygen demand (SCOD). The amount of SMPs is typically estimated by subtracting the COD due to intermediate VFAs and residual substrate, from the soluble effluent COD (not including methane in the dissolved phase).

After changes in HRT, the composition and concentration of SMPs and COD were monitored over time (in some cases every 4 hours) to ensure that "steady state" had been reached in SMP production, and hence the data reported here is the stable composition of the SMPs after HRT changes. These measurements also ensure that any hydrophobic SMPs have time to equilibrate with the biomass in terms of partitioning and gas transfer.

The measurement of pH (Mettler-Toledo) was accurate to within ±0.01 units. Total suspended solids (TSS), MLVSS and SCOD were measured as described in Standard Methods APHA [9], while the Biochemical Oxygen Demand (BOD) was measured using the OxiTop® system (WTW, Germany). Total nitrogen was measured using a multi N/C 2100s analyser from Analytikjena, Germany, while ammonia nitrogen was measured using an ammonia nitrogen ion selective electrode (Hanna instruments, U.S.A). VFAs were measured using a Shimadzu high-performance liquid chromatography (HPLC, SPD-20AD) with a UV diode array detector (DAD, SPD-M20A) at 210 nm using an Aminex® HPX-87H (300×7.8mm) column. Analysis time was 25 min for each sample operating under isocratic and isothermal conditions using 0.005M H_2SO_4 as the mobile phase at a flowrate 0.8mL/min at 55^oC [10]. A total of seven VFAs including formic acid, acetic acid, propionic acid, iso-butyric acid, butyric acid, iso-valeric acid and valeric acid were quantified with this method. Coefficients of variation (COV=SD/average value) for all VFAs were below ±4%. Size exclusion chromatography (SEC) was carried out using two columns (PolySep GFC-P1000 and 4000, Phenomenex) connected in series, with detection using UV-DAD and refractive

index (RI) detectors (Shimadzu). EasiVial polymer standards (Agilent, U.S.A.) were used for molecular weight (MW) calibration.

The composition of biogas (methane, oxygen, nitrogen, and carbon dioxide) from the SAMBR was determined using a Shimadzu GC-2010plus gas chromatograph with a thermal conductivity detector (TCD) (COV below ±3%). A select permanent gases/CO_2 (CP7429) column from Agilent was used for gas separation. Gas volume was measured with a gas-sampling bag using a gas pump with a flow meter. Soluble methane in the effluent was measured by headspace analysis of a serum bottle partially filled with effluent and left to come to equilibrium for 24 h (no agitation). The amount of soluble methane was then calculated based on assuming equilibrium between the gas and liquid phase in the serum bottle using Henry's law. Specific methanogenic activities (SMAs) were conducted in triplicate for anaerobic sludge at 35°C using 37 mL serum bottles containing acetic acid as a carbon source. The food to microorganism ratio (F/M) was set at 0.5, and the method was based on Ho and Sung [11]. The COD mass balance was calculated using the following equation;

$$COD_{influent} = COD_{effluent} + COD_{methane}\ (gas) + COD_{methane}\ (dissolved\ in\ the\ effluent) + COD_{biomass\ waste} + COD_{biomass\ growth} \quad \text{Equation 2}$$

Note: $COD_{methane}$: 395mL CH_4/g COD (35°C and 1 bar)

$COD_{biomass}$: 1.42g COD/g VSS

2.4 Sample pretreatment for identifying compounds in SMPs

Organic compounds were extracted using an SPE procedure [12]. Collected samples were filtered through a 0.45 µm glass fibre filter to remove TSS. SPE cartridges (Waters Oasis®HLB) were conditioned using 10 mL of LC/MS-grade methanol followed by 20 mL of ultrapure water, and then one litre of filtered sample was loaded onto two cartridges connected in series using a peristaltic pump (Watson-Marlow 120U) at a flowrate of 10 mL/min. The tubing of the pump was rinsed with methanol (5 min) followed by ultrapure water (10 min) prior to use, and the filtrate bottle was rinsed using ultrapure water to pass the entire sample through the cartridge. Finally, the compounds were eluted with 2 mL of the selected solvents (methanol, acetone, dichloromethane, n-hexane) in sequence into individual glass sample vials. The eluent from each SPE cartridge was collected and analysed separately. Plasticware was avoided during the elution procedure since plastic in contact with solvent can cause leaching of contaminants into our samples. Ultrapure water was used as the control to identify any contamination during pre-treatment.

2.5 Gas chromatography-mass spectrometry (GC-MS)

Eluted samples from SPE were then analyzed using a GC-MS system (GCMS-QP2010ULTRA, Shimadzu); the sample (3 µL for acetone, dichloromethane, and n-hexane samples; 2 µL for methanol) was injected into an RTX®-5MS (30 m × 0.25 mm ID, Restek) column for the separation of low to mid polarity compounds. Splitless injection was used with a controlled temperature at 280°C, and Helium was used as a carrier gas at a column flow rate of 1 mL/min. The total runtime per sample was 60 minutes, and the temperature program was: 50°C, hold 7 min, rate 7°C/min to 325°C, and hold for 14 min. This temperature program was modified based on the alkane

standards (C10 – C40). The mass spectrometer was operated in the electron ionisation mode (EI) with the ion source temperature at 230°C. Mass spectra were acquired from *m*/*z* 30 to 580 after a 10 min solvent cut time. The chromatographic peaks were identified using the NIST11 library (National Institute of Standards and Technology, Gaithersburg, MD, USA, http://www.nist.gov/srd/ mslist.htm) and the compound was considered identified if the match percentage was higher than 80%. Compounds that had a match percentage below 80% were mentioned as unknown peaks. Similarity index, mass spectrum and retention index were all used as selection criteria for compound identification from the NIST library list of suggested compounds. Method blanks (deionized water) were run through the same pretreatment and analysis, while feed samples were also run to identify compounds in the feed. Finally, the reactor and tubing were soaked in DI water for a month to provide a blank for compounds potentially leaching from the reactor and system components; all these blanks were then subtracted from the SMP results to identify microbially produced compounds.

2.6 Quantification

Alkanes were selected as "representative" compounds for the approximate quantification of SMPs based on literature findings, availability, and cost. One important factor is that alkanes have widely variant chain lengths (C10 – C40), and hence are able to cover most of the volatility range of the RTX®-5MS column. The calibration curve for each compound was plotted with concentration points 0.1, 0.25, 0.5, 1 and 2 mg/L, and the coefficients of multiple correlation (R^2) values were above 0.99 for all compounds except for C38 and C40 which had lower intensities due to their low volatility (Table 1).

Quantification was done separately for each unknown compound using the alkane with the closest retention time. A set of standards was run in and between every batch of analyses to minimise instrumental error, and blank solvents (methanol, acetone, dichloromethane, n-hexane) were also run with every batch for background subtraction. The instrumental identification limit (IDL) of alkane standards was evaluated for each compound based on the maximum blank concentration, and the signal-to-noise ratio of 3. We appreciate that this is not a perfect solution to identify and quantify the compounds (SMP), however, it is a useful tool to start understanding what compounds are produced as SMPs and their approximate concentration, although there is a clearly a considerable degree of uncertainty surrounding the concentration of identified compounds beside alkanes.

3. Results and discussion

3.1 Performance of the SAMBR under different HRTs at steady state (HRT 12 h, 8 h, 6 h and 4 h)

The SAMBR was operated for 168 days, starting from an HRT of 12 h and subsequently decreasing it to 8 h, 6 h, 4 h, and 2 h, and then to 1 h. Samples were collected during transient conditions and also under stable conditions, and Table 2 shows the performance of the SAMBR operated under different HRTs under stable conditions. COD removal at HRTs (12 h, 8 h, 6 h and 4 h) was excellent (> 97%). The COD removal values are consistent with previous research in anaerobic membrane

bioreactors [13-15]. Hu and Stuckey [13] operated a SAMBR with the lowest HRT at 3 h and still obtained very good performance (90% COD removal).

Permeate COD was very low, and in the range of 12 -16 mg/L (HRT 12 h, 8 h, 6 h and 4 h). BODs in the effluent at an HRT of 4 h were also very low, and averaged around 1 mg/L, indicating that there was very little aerobically biodegradable organics left in the effluent. Carbohydrates were also very low at all HRTs indicating minimal residual biodegradable substrate in the effluent. The effluent SMPs were an average of 2.5% of the incoming COD under stable conditions. The percentage of methane in the gas was more than 70%, and about 10% carbon dioxide under most HRTs (the balance being nitrogen), and this is typical of short retention time anaerobic digesters. The system was operating at an SRT of 200 days, and hence the sludge production and wastage was very low compared to aerobic biological processes. SMA assays were conducted at HRTs of 12, 8, 6, and 4 h, and the activity gradually increased as the HRT was reduced, with values of 29, 109, 120, and 122 mL CH_4/g.VSS.day, respectively. Hence, as the organic load increased by 3 times (HRT 12 to 4 h), the methanogens grew by over 4 times (29 to 122), and yet the effluent COD remained constant. One advantage of a membrane reactor is that biomass is retained and is not washed out during perturbations.

3.2 Analysis of SMPs in the reactor effluent

SMP concentrations in the effluent were low (12 – 16 mg/L), and HPLC-SEC was used to investigate the MW distribution of the effluent (Figure S1). The results show that the majority of compounds had MWs higher than 60 kDa; the high MW compounds were

probably from biomass associated products (BAP) which were not hydrolysed or degraded into smaller compounds [13], while lower MW compounds were not detected using SEC, despite the technique being able to detect very low MWs. The MW distributions of the compounds were similar at HRTs of 8 and 6 h, while higher MW compounds were observed at 4 h HRT. The effluent samples were then analysed using SPE and GC-MS to identify the lower MW compounds present as SMPs, and a total of 91 compounds with a similarity index above 80% were identified in the SAMBR effluent. The compounds identified were categorized as alkanes (39), alkenes (3), esters (11), alcohols (7), nitrogenated compounds (N-compounds) (11), phenols (11), and others (9). There were 29 compounds which could not be identified, and were designated as "unknown". These compounds must have come mostly from bacterial metabolism and/or bacterial degradation since they were not found in the raw feed. Alkanes, alkenes and alcohols were also found in a study using a SAMBR treating municipal solid waste [16], and in the effluent of a pilot-scale upflow anaerobic sludge blanket (UASB) [17].

As expected, the total number of compounds increased from 44 to 70 when the HRT was reduced from 8 h to 4 h (Table 3) because some of these compounds probably needed longer time to degrade within the reactor, and under increased OLRs (stress) more SMPs could have been produced [5]. The combined concentration of compounds found in the effluent was in the range of 7-25 μg/L with the highest concentration detected at an HRT of 1 h, and more alkanes, N-compounds, and phenols were found in the samples collected at shorter HRTs. Only about 0.1% of the effluent COD was identified as low MW (<580 Da) compounds. At steady state (HRT 4 h) 25% was

accounted for as organic nitrogen, although the major portion of the effluent COD was still unidentified (74.9% of total COD). During transient conditions (HRT 1 h), the major part of the effluent COD was VFAs (89%), while only 4% was organic nitrogen, and hence only 6.9% of the total effluent COD was still unidentified. Hence, there are still considerable challenges to being able to identify the unknown portion of the effluent COD.

3.3 Performance of the SAMBR under transient hydraulic shock load conditions (HRT 2 h, 1 h)

This study was the first time that a SAMBR had been operated at HRTs as low as 2 h and 1 h. Hydraulic shock loads were performed in two distinct phases; with the 1st phase when the HRT decreased from 4 h → 2 h (for 12 h) → 4 h, the performance of the SAMBR decreased from 97% COD removal to about 81% indicating the SAMBR's relative tolerance to hydraulic shock loads. VFAs started to accumulate immediately within the first hour after the HRT was changed, and reached a peak at 85 mgCOD/L after 11 hours. The SMP doubled (26 mg/L) within the first hour, but gradually decreased to about 14 mg/L 12 hours later. After the HRT was changed back to 4 h, the VFAs decreased by 31% in 2 h, although the SMPs increased again by 56%, which indicated that bacteria were probably producing more SMPs during the adaptation phase.

In the 2nd phase, the reactor was operated at an HRT of 2 h for 6 days, and then at an HRT of 1 h for the next 12 h. After the HRT was changed from 4 h → 2 h, performance

of the SAMBR was better than during the 1st phase. COD removal decreased but was still effective at 94%, indicating that bacterial ecology in the reactor might have adapted to the hydraulic shock by then by increasing the number of archaea since the g COD removed/g biomass.d changed from 2.0 (1st phase) to 2.3 (2nd phase) at 12 h after the HRT was reduced from 4 h to 2 h (Table 2). Analysis showed an instant accumulation of VFAs (Figure 2), however, the concentration of SMPs did not appear to have increased during the transition. The sole contributor to the increase in effluent COD was VFAs, which were mainly acetate and propionate. Surprisingly, the amount of SMPs decreased over time at 2 h HRT and was almost completely depleted after 120 h. When the HRT was changed from 2 h to 1 h at 144 h, the COD removal almost immediately dropped substantially to about 80% (Figure 2). Propionate increased rapidly but became stable after 148 h, while acetate was constant during that time. Acetate concentration started to rise at 150 h and reached about 30 mg/L at 12 h. SMPs increased from 2 to 14 mgCOD/L in 5 h after the HRT was changed to 1 h, but this gradually decreased to 4 mgCOD/L at 12 h (both changes significantly different). Increases in the SMP level under transient conditions was also reported in previous studies such as during fluctuations in the feed, in the presence of toxicants, and under nutrient limitations [18]. The MLVSS in the reactor increased substantially from 6,900 mg/L to about 11,500 mg/L during the transition; this increase in MLVSS was related to the amount of biogas produced, which also increased over time (Figure S2).

Transmembrane pressure (TMP) was monitored during operation (Figure 3), and was about 3.3 kPa when the membrane flux was maintained at 15 LMH during the 2 h HRT. The membrane flux had to be increased at 1 h HRT to 27.4 LMH to accommodate such

a short HRT, and the TMP increased to 4.3 kPa. However, the TMP did not increase over time at this high flux indicating that the reactor was able to operate under these stressed conditions without leading to excess membrane fouling, despite the increase in SMPs.

3.4 Analysis of SMPs in the reactor effluent during transient conditions

HPLC-SEC was used in this study to investigate the MW distribution of compounds in the effluent from the SAMBR under transient conditions (Figure S3). The high MW compounds (>60k Da), which dominated steady state, decreased dramatically. Two large peaks were observed under transient conditions in the lower MW range (<1k Da), and these compounds could be intermediates (UAPs) and/or the easily degradable metabolic products. However, the retention time of the reactor may be too short to degrade these compounds before they exit in the effluent.

Figure 4 shows the combined concentration of compounds found in the effluent over time after the HRT was reduced from 4 h to 2 h (2^{nd} phase SMPs increased substantially during transient conditions). In contrast, the SMP concentration (as COD) decreased during this period. At 120 h, the concentration of low MW compounds accounted for 0.3 mg COD/L (calculated from 3.46 g O_2/g docosane), which is 30% of the total SMPs. Various compounds were found during transient conditions which were not detected under stable conditions. The concentration of compounds such as cyclooctasulfur (92% -similarity index), N-butyl-benzenesulfonamide (93), alkanes (95), 1-naphthalenol (93), camphor (95), 2-methyl-phenol (92), and (Z)-9-octadecenamide (91) all increased

significantly during the HRT transition period (Figure 5). Alkanes were the most common compounds which were found in the effluent of a SAMBR treating solid waste leachate [16] and UASB effluent [17]. Two alkanes, 2-methyl-nonane and dodecane, were found at 0.010 mg/L and 0.001 mg/L, respectively, 24 h after the HRT was changed from 4 h to 2 h (Figure 5b). It has been shown that alkanes can be produced by bacterial metabolism [19], and that bacteria appear to be able to degrade alkanes under both aerobic and anaerobic conditions [20]. However, the retention time of the reactor may be too short to degrade these solutes, which might be one of the reasons why fewer alkanes were detected in the samples at longer HRTs.

N-butyl-benzenesulfonamide, a type of plasticizer, was detected at an extremely high concentration of 0.282 mg/L accounting for 0.6% of the effluent COD (calculated from 1.73 g O_2/g N-butyl-benzenesulfonamide) in the 1st phase. This compound has also been found in a batch anaerobic stirred tank reactor which was left decaying for one month to produce SMPs [2], in a landfill leachate [21], and also in a SAMBR operating at low temperature (20°C) [16], and there is evidence that *Streptomyces* sp. TN262 can produce this compound [22]. This is not the first time that a plasticizer has been found in the effluent of biological systems, and since control samples of the plastic reactor and tubing were run by soaking them in deionised water for a month, these compounds are not likely to come from the plastic components used in the reactor system. Bis-(2-ethylhexyl) phthalate was also found in very high concentrations in previous research where wastewater was treated using anaerobic reactors [2, 23]. Cyclooctasulfur (S_8), which is a central intermediate in the biotic or abiotic oxidation of sulfides [24], was also found in elevated concentrations during transient loads (Figure 5a and 5d). Its

concentration was 0.018 mg/L and 0.028 mg/L during the shocks of HRT 4 h → 2 h and 2 h → 1 h, respectively. The cause of an increase in this compound during transient loads is unclear at the moment, but may be due to cell leakage of intermediate metabolites. However, despite the reasons for the production of this compound being uncertain, cyclooctasulfur was found to be degraded by deltaproteobacteria such as *desulfobulbaceae and desulfuromonadales* [25]. This could be the reason for the absence of S_8 after the HRT was changed back to 4 h. However, further study is needed to understand how these compounds can be produced in complex bacterial communities such as those present in anaerobic reactors.

3.5 COD mass balance

Overall COD mass balances for the SAMBR were conducted at HRTs of 4 h and 2 h (2^{nd} phase) (n=4 at each HRT-Figure 6). Permeate COD was quite similar for the HRTs of 4 h and 2 h at 3% and 6% of the feed COD, respectively. Dissolved methane in the effluent converted to COD were also similar at 17% at HRTs of 4 h (0.0281 mL CH_4/mL water), and 19% at an HRT of 2 h (0.0303 mL CH_4/mL water), which far exceeded the saturation value of methane in water by 104 - 113% (solubility of methane in water: 0.0269 mL CH_4/mL water at 35°C [26]). Over saturation of dissolved methane in the effluent was also found in the pilot plant of a staged anaerobic fluidized membrane bioreactor, where Shin, McCarty, Kim and Bae [27] found 15-23% oversaturation in the permeate. Dissolved methane is currently one of the topics of concern in anaerobic treatment due to the importance of methane recovery from the effluent, and fugitive greenhouse gas emissions. The biggest difference was the higher portion of methane in the gas phase (67%) with an HRT of 4 h, compared to 52% under

an HRT of 2 h. At an HRT of 2 h, the COD to biomass conversion (calculated from 1.42 g O_2/g cells) was higher than at an HRT of 4 h (18% vs. 3%), and the reactor MLVSS at 2 h also increased rapidly during operation (6,901 mg/L to about 10,975 mg/L in 6 days). Higher biomass concentrations at short HRTs was also observed in a previous study [14]. Higher organic loading rate (OLRs) at shorter HRTs obviously induced more biomass growth, and more carbon conversion to methane. The unknown fraction in the COD balances (8% at 4 h, 5% at 2 h) is probably be due to cumulative or measurement errors, and are good balances for these type of reactors.

4. Conclusions

The results showed that:

- The SAMBR was capable of operating at low HRTs, and thus has a high tolerance to hydraulic shock loads. This study was the first time that a SAMBR was operated at an HRT as low as 2 h and 1 h. The COD removal of the SAMBR was above 94% at HRTs of 12 h, 8 h, 6 h, 4 h, and 2 h, while it decreased slightly to 80% when operating at an HRT of 1 h. The reactor produced a maximum effluent of 16 mg/L COD at HRTs of 4 – 12 h, and BODs in the effluent at an HRT of 4 h were also very low, and averaged around 1 mg/L.

- VFAs began to accumulate when the HRT was decreased to 2 h and 1 h, accounting for 69% and 89% of the effluent COD, respectively. The majority of the VFAs detected were acetate and propionate.

- Calculated SMPs accounted for an average of 14±2 mgCOD/L at steady state, but this fluctuated more during transient shock load conditions to average 12±6 mgCOD/L. However, the effluent SMPs were always less than 22 mgCOD/L.

- A total of 120 compounds were identified in the effluent from the SAMBR under steady state conditions. The compounds identified were alkanes (39), alkenes (3), esters (11), alcohols (7), N-compounds (11), phenols (11), and others (9). The total number of compounds increased from 44 to 70 when the HRT was reduced from 8 h to 4 h. This increment was expected because some of the compounds produced needed a longer period of time to degrade within the reactor. The presence of nitrogenated and phenolic compounds were detected at lower HRTs.

- Many compounds such as cyclooctasulfur, N-butyl-benzenesulfonamide, alkanes, 1-naphthalenol, camphor, 2-methylphenol, and (Z)-9-octadecenamide were found at significant concentrations during transient conditions, and hence it is possible that these compounds were produced by microorganisms as by-products of substrate utilization.

5. Acknowledgements

The writers would like to acknowledge the financial support from Environmental & Water Industry Programme Office of Singapore [grant number PUB IDD 21100/36/6].

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Table 1. Alkane standards for quantification.

Compound name	Formula	Molecular weight	Retention time (min)	R^2	*IDL* (mg/L)	*IQL* (mg/L)	Retention Index
Decane	$C_{10}H_{22}$	142	12.15	0.999	0.026	0.087	1015
Dodecane	$C_{12}H_{26}$	170	17.16	0.999	0.017	0.056	1214
Tetradecane	$C_{14}H_{30}$	198	21.96	0.999	0.019	0.064	1413
Hexadecane	$C_{16}H_{34}$	226	25.70	0.997	0.020	0.066	1612
Octadecane	$C_{18}H_{38}$	254	29.03	0.997	0.015	0.050	1810
Eicosane	$C_{20}H_{42}$	282	32.04	0.996	0.020	0.068	2009
Docosane	$C_{22}H_{46}$	310	34.78	0.997	0.022	0.073	2208
Tetracosane	$C_{24}H_{50}$	338	37.30	0.991	0.017	0.055	2407
Hexacosane	$C_{26}H_{54}$	366	39.62	0.995	0.013	0.042	2606
Octacosane	$C_{28}H_{58}$	394	41.77	0.993	0.014	0.046	2804
Triacontane	$C_{30}H_{62}$	422	43.78	0.994	0.016	0.054	3003
Dotriacontane	$C_{32}H_{66}$	450	45.66	0.990	0.015	0.051	3202
Tetratriacontane	$C_{34}H_{70}$	478	47.51	0.991	0.010	0.033	3401
Hexatriacontane	$C_{36}H_{74}$	506	49.72	0.990	0.014	0.046	3600
Octatriacontane	$C_{38}H_{78}$	534	52.59	0.981	0.011	0.038	3800
Tetracontane	$C_{40}H_{82}$	562	56.45	0.984	0.054	0.182	3997

Note: IDL= Instrumental Detection Limit, IQL= Instrumental Quantification Limit

Table 2. Performance of the SAMBR under different HRTs.

n = 4

HRT (h)	Period (day)	COD_{inf} (mg/L)	COD_{eff} (mg/L)	Eff (%)	MLVSS[a] (mg/L)	Methane in gas (%)	Mass COD removed (g COD/d)	g COD removed/ g biomass.d	CH_4(mL) /g.COD[d]	VFAs (mg/L)	Carbohydrate (mg/L)	Organic nitrogen[c] (mg/L)
12	69	533±68	14±8	97±2	4010	69±2	3.1	0.8	252±27	ND	2±2	2
8	23	502±34	12±4	98±1	5305	72±2	4.4	0.8	236±27	ND	ND	2
6	46	520±35	16±4	97±0	6097	74±1	6.0	1.0	249±29	ND	ND	3
4	23	484±62	12±4	97±2	7533	75±3	8.5	1.1	264±46	ND	ND	1
2 (1st)	12 h	471±20	88±10	81±3	6921	72±1	13.8	2.0	134±23	82±3	1±0	0
2 (2nd)	6	458±73	26±6	94±2	10975	80±2	15.6	1.4 (2.3[b])	205±24[b]	18±4	ND	4
1	12 h	432±29	85±3	80±1	11357	78±3	25.0	2.2	187±39	76±5	1±0	2

Note: Eff = efficiency

[a]MLVSS on the last day of each HRT.

[b]The number was calculated at 12 h after changed HRT of 4 h to 2 h (MLVSS = 6898 mg/L).

[c]Organic nitrogen was calculated by total nitrogen minus ammonia nitrogen

[d]Methane was calculated based on gas phase only.

Table 3. Number of low MW compounds found in SMPs at HRTs of 8 h, 4 h, and 1 h under steady state conditions.

Sample	Number of low MW compounds									
	Alkane	**Alkene**	**Ester**	**Alcohol**	**Nitrogenated compounds**	**Phenols**	**Others**	**Unknown**	**Total**	**Total Concentration (µg/L)**
HRT 8 h	18	1	7	0	0	1	2	15	44	7
HRT 4 h	31	2	9	5	3	1	4	15	70	11
HRT 1 h	28	2	7	2	7	9	7	21	83	25

Figure 1

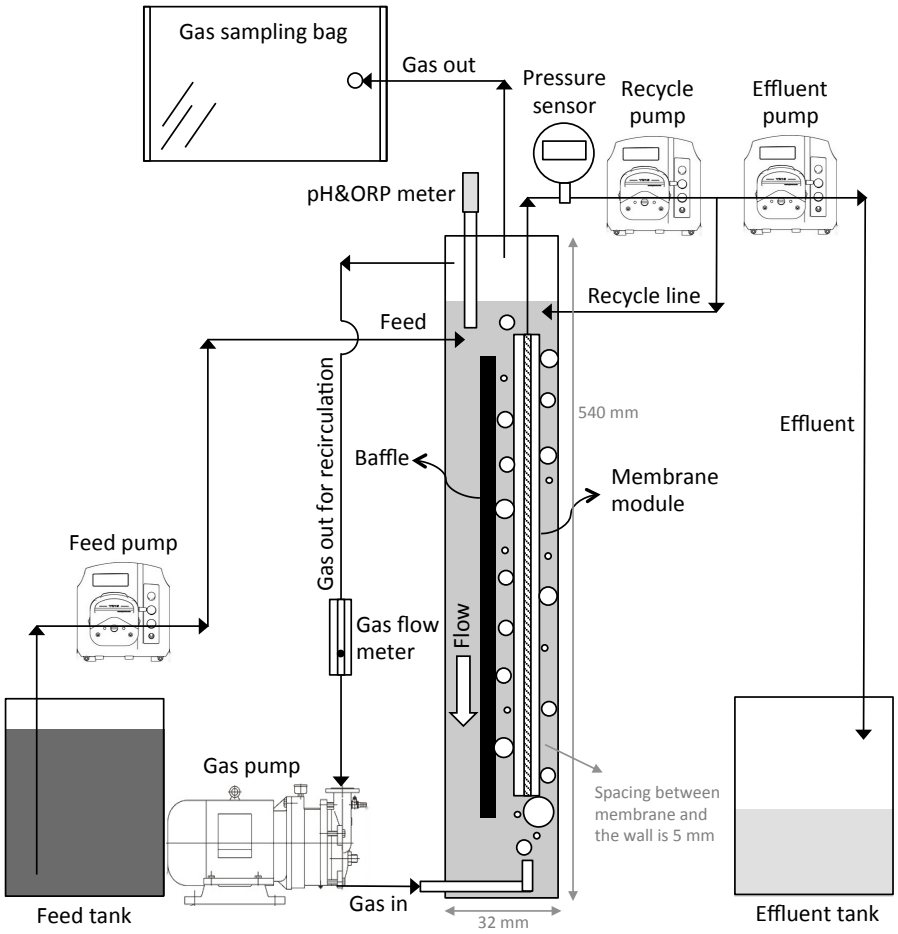

Figure 1. Diagram of the SAMBR.

Figure2

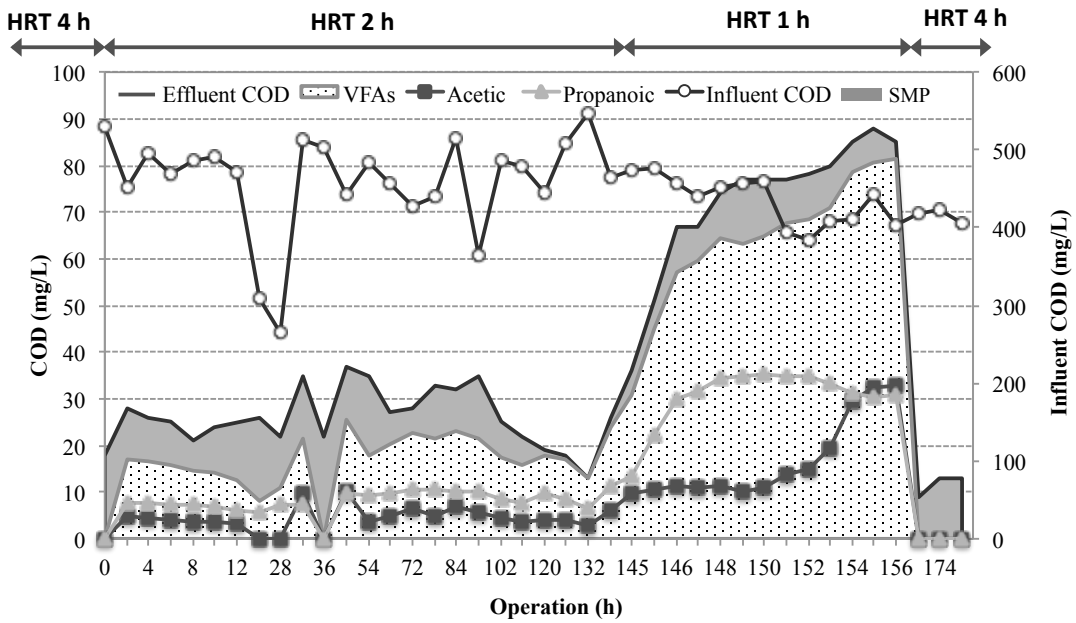

Figure 2. COD, VFAs, and SMP in the effluent during transient conditions (HRT 4 h → 2 h → 1 h → 4 h).

Figure3

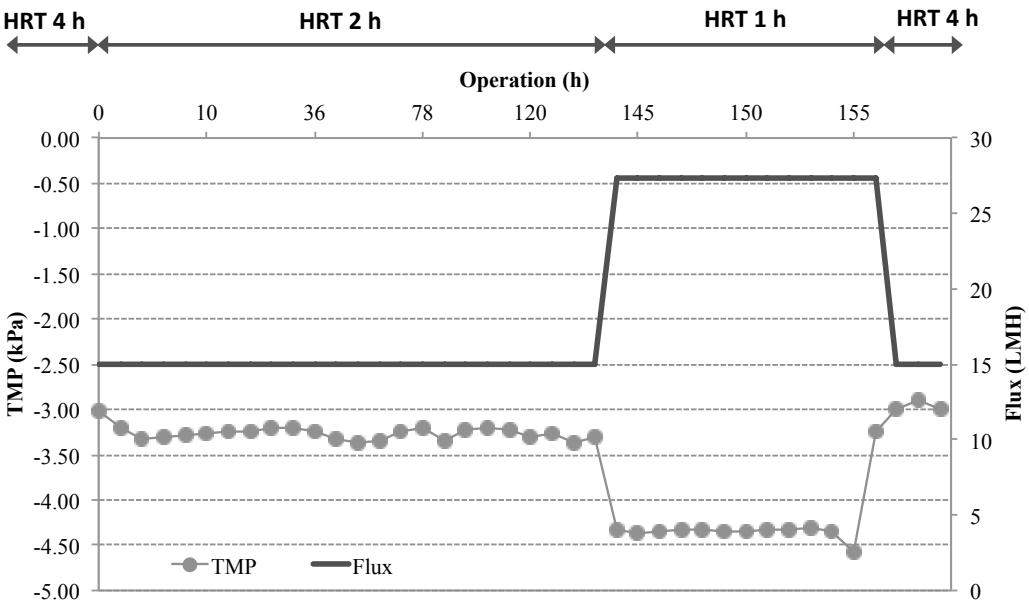

Figure 3. Transmembrane pressure (TMP) of the SAMBR under transient conditions (HRT 4 h → 2 h → 1 h → 4 h).

Figure4

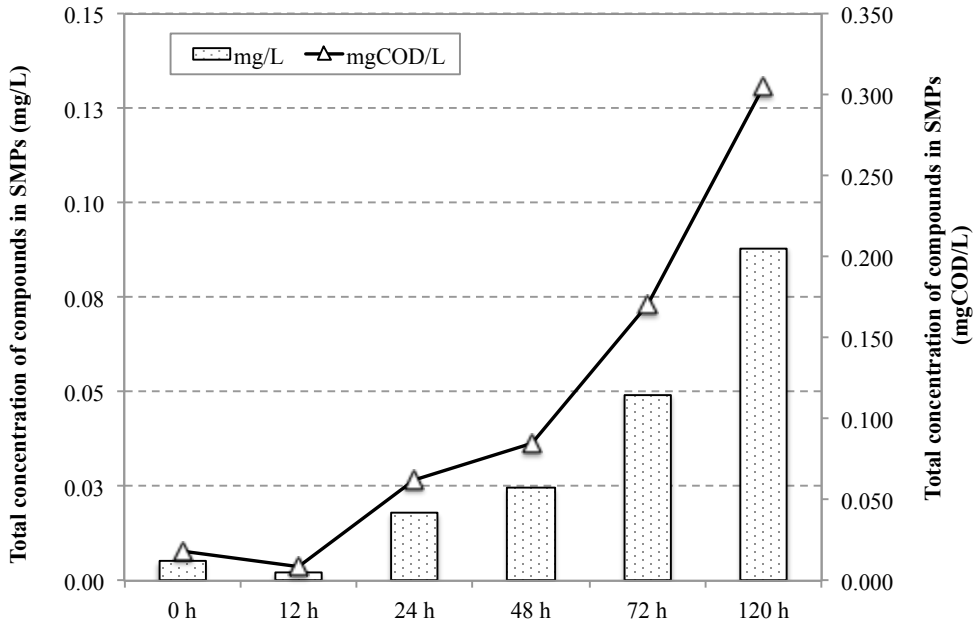

Figure 4. The combined concentration of compounds found in the effluent over time during transient conditions (HRT of 4 h to 2 h (2^{nd} phase)).

Figure5

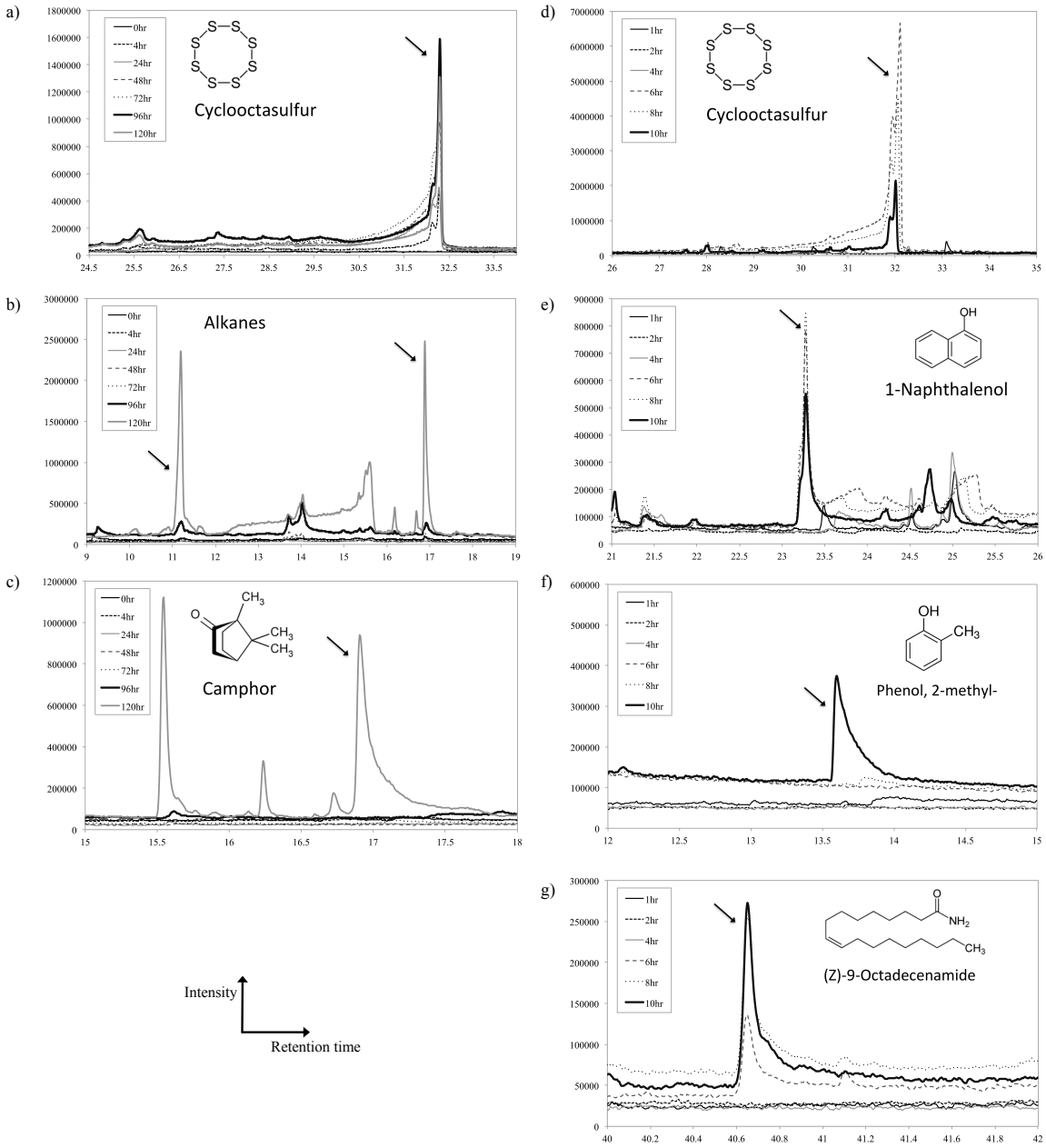

Figure 5. Chromatograms of compounds found in SMPs during transient conditions under HRT 4 h → 2 h (2nd phase) (a, b, c) and HRT 2 h → 1 h (d, e, f, g).

Figure6

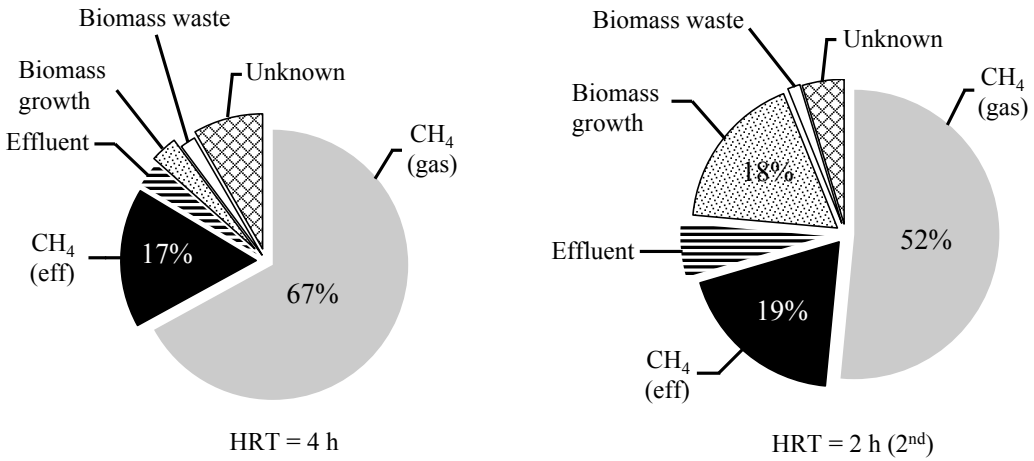

Figure 6. COD mass balance of the SAMBR at HRT 4 h and 2 h (2nd phase).

Supplementary section

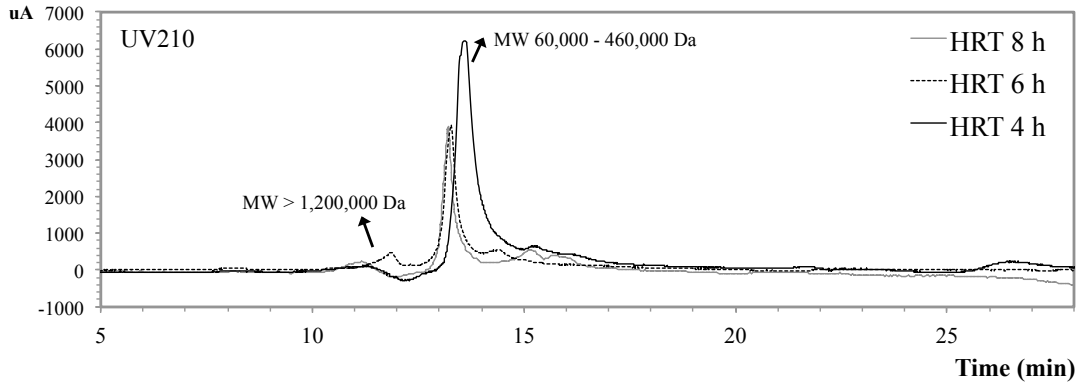

Figure S1 Size exclusion chromatograms of effluent under different HRTs.

FigureS2

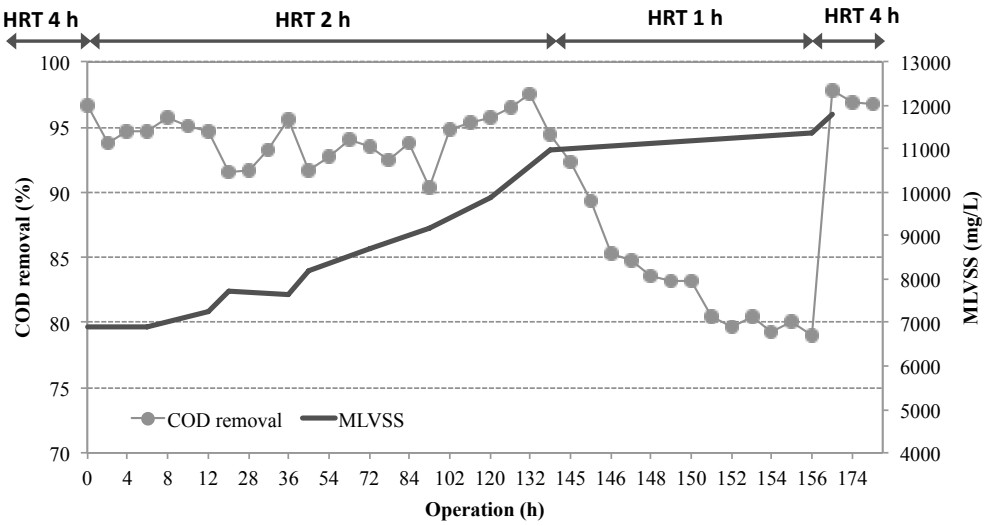

Figure S2 MLVSS concentration (mg/L) and COD removal (%) during transient conditions (HRT 4 h → 2 h → 1 h → 4 h).

FigureS3

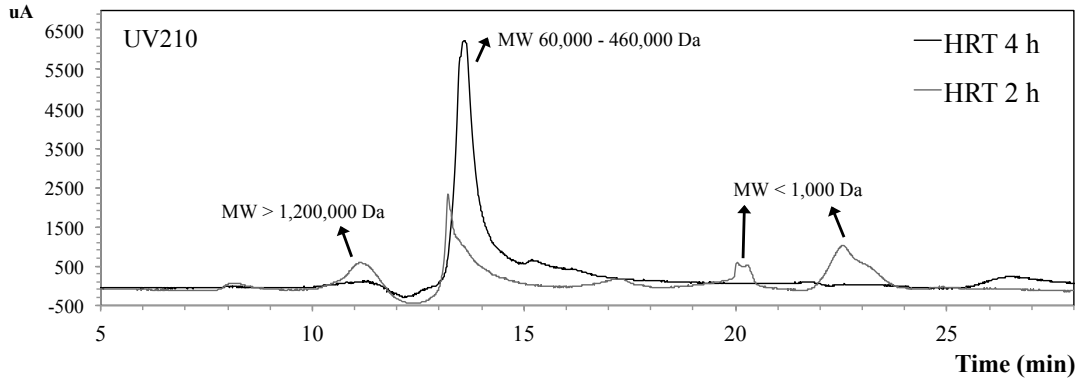

Figure S3 Size exclusion chromatograms of effluent under transient condition.

Supplementary Material

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