

SOLUBLE MICROBIAL PRODUCTS (SMPS) IN A SUBMERGED ANAEROBIC MEMBRANE BIOREACTOR (SAMBR) UNDER DIFFERENT HRTS AND TRANSIENT LOAD 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. COD removal at decreasing HRTs (12 h, 8 h, 6 h, 4 h, and 2 h) was high, and always above 94%, while the performance of the reactor decreased to 80% when operating at 1 h HRT. VFAs started to accumulate when the HRT was decreased to 2 h and 1 h, accounting for 69% and 89% of the effluent COD, respectively. The effluent SMP accounted for an average of 2.5% of the incoming COD at steady state, but this increased a little to 3.1 – 3.6% during transient conditions. The equivalent COD representing the dissolved methane in the effluent was 17% at 4 h HRT, which exceeded the saturation value of methane in water. Low MW compounds were identified using gas chromatography-mass spectrometry (GC-MS), with solid phase extraction (SPE) as the pre-treatment. A total of 120 compounds were identified in the effluent at steady state, and were categorized as 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, soluble microbial products, wastewater

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 (Stuckey 2012). Using SAMBRs is a promising solution since they offer independent control of the solids (SRT) and hydraulic retention times (HRT), which allows for 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) (Aquino 2004, Mesquita *et al.* 2010). It has also 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 (Barker and Stuckey 2001, Aquino 2004). However, under transient conditions including nutrient limitations, the presence of toxicants, or when the feed flow or composition is changing radically, the effluent SMPs can be as high as 17% of the influent COD (Barker and Stuckey 1999). Therefore, it is important to evaluate the production and composition of SMPs in a SAMBR in order 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 to evaluate the effects on the reactor under transient load conditions. 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 (<580Da) were identified using gas chromatography-mass spectrometry (GC-MS) with solid phase extraction (SPE) as a pre-concentration step.

2. Materials and methods

Submerged Anaerobic Membrane Bioreactor (SAMBR)

The submerged anaerobic membrane bioreactor (SAMBR) was made from polymethyl methacrylate (Plexiglass®) and had a working volume of 3 L. A microfiltration flat-sheet membrane (Chlorinated Polyethylene) from Kubota with a surface area of 0.116 m² and a maximum pore size of 0.4 with an average of 0.2 µm was used. The flux of the membrane was maintained at 15 litres per square meter per hour (LMH) for most conditions (HRT 12, 8, 6, 4 and 2 h), although during an HRT of 1 h it was set at 27.4 LMH. The mixed liquor volatile suspended solids (MLVSS) in the reactor were 6,000 mg/L at the start of the experiment, and the SAMBR was operated at a 200 days sludge retention time (SRT). The pH in the system was maintained in the range of 6.8 and 7.2 using 1M NaHCO₃. 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 m³/m².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 for 168 days, starting from an HRT of 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 went from 4 h → 2 h (for 12 h) → 4 h, while the 2nd phase was when the HRT went from 4 h → 2 h (6 days) → 1 h (12 h) → 4 h.

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). Total suspended solids (TSS), MLVSS and SCOD were measured as described in Standard Methods APHA (Eaton and Franson 2005), while the Biochemical Oxygen Demand (BOD) was measured using the OxiTop® system (WTW, Germany). 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 (Guerrant *et al.* 1982). 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). A select permanent gases/CO₂ (CP7429) column from Agilent was used for gas separation. Gas volume was measured from 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. The amount of soluble methane was then calculated based on assuming equilibrium between the gas and liquid phase in the serum bottle.

Sample pretreatment for identifying compounds in SMPs

Organic compounds were extracted using a solid phase extraction (SPE) procedure (Kunacheva and Stuckey 2014) with Waters Oasis®HLB cartridges. 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. Ultrapure water was used as the control to identify any contamination during pre-treatment.

Gas chromatography-mass spectrometry

Eluted samples from SPE were then analysed using a GC-MS system (GCMS-QP2010ULTRA, Shimadzu); 2-3 μL of a sample was injected into an RTX[®]-5MS (30 m \times 0.25 mmID, Restek) column for the separation of low to mid polarity compounds. Alkanes were used as a reference standard for the analysis. 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%. Similarity index, mass spectrum and retention index were all used as selection criteria for compound identification from the NIST library list of suggested compounds.

3. Results and discussion

3.1. Performance of the SAMBR under different HRTs at stable condition

The SAMBR was operated for 168 days, starting from an HRT of 12 h and subsequently decreased 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 1 shows the performance of the SAMBR operated under different HRTs under stable conditions. COD removal at HRTs (12 h, 8 h, 6 h, 4 h, and 2 h (2nd phase)) was excellent at above 94%, while the performance of the reactor decreased a little to 80% when operating at an HRT 1 h indicating the SAMBR's relative tolerance to hydraulic shock loads. Permeate COD was very low, and in the range of 12 -16 mg/L (HRT 12 h, 8 h, 6 h and 4 h), which is lower than most wastewater discharge standards in most countries. 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. SMP was calculated to be an average of 2.5% of the incoming COD under stable conditions. Specific methanogenic activity (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 mLCH₄/g.VSS.day, respectively.

Table 1: Performance of the SAMBR under different HRTs.

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	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	ND	2±2	2
8	23	502±34	12±4	98±1	5305	72±2	4.4	0.8	ND	ND	2
6	46	520±35	16±4	97±0	6097	74±1	6.0	1.0	ND	ND	3
4	23	484±62	12±4	97±2	7533	75±3	8.5	1.1	ND	ND	1
2 (1st)	12 h	471±20	88±10	81±3	6921	72±1	13.8	2.0	82±3	1±0	0
2 (2nd)	6	458±73	26±6	94±2	10975	80±2	15.6	1.4 (2.3 ^b)	18±4	ND	4
1	12 h	432±29	85±3	80±1	11357	78±3	25.0	2.2	76±5	1±0	2

Note: Eff = efficiency

^aMLVSS on the last day of each HRT.

^bThe number was calculated at 12 h after changed HRT of 4 h to 2 h (MLVSS = 6898 mg/L).

^cOrganic nitrogen was calculated by total nitrogen minus ammonia nitrogen

The effluent samples were then analysed using SPE and GC-MS to identify the compounds present as SMPs. 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). 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 (Trzcinski and Stuckey 2010).

3.2. Performance of the SAMBR under transient hydraulic shock load conditions

Hydraulic shock loads were performed in two distinct phases. 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.

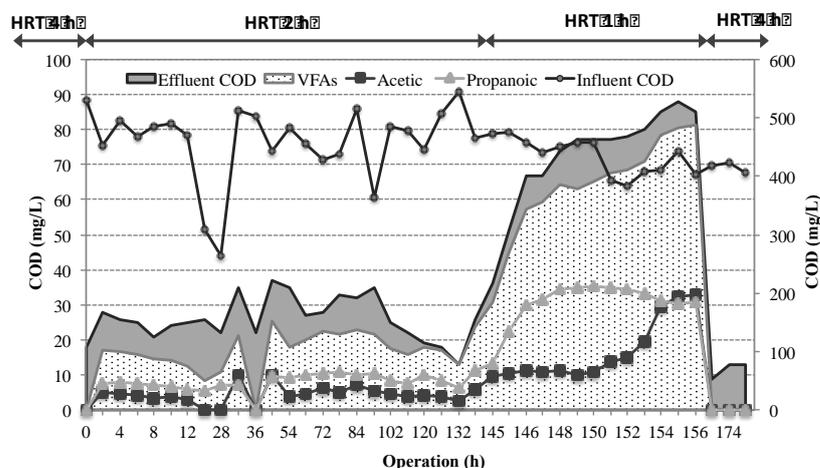


Figure 1: COD, VFAs, and SMP in the effluent during transient conditions

Analysis showed an instant accumulation of VFAs (Figure 1), 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 1). 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. SMP increased a little to 3.1% of the incoming feed during 1 h HRT; when the HRT was changed back to 4 h after 156 hours of operation VFAs were not detected after this time. However, the SMPs further increased to about 3.6% of the incoming feed. 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 (Barker and Stuckey 2001).

4. Conclusions

- The SAMBR is capable of operating at low HRTs, and thus has a high tolerance for hydraulic shock loads. The COD removal 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.
- Calculated SMPs accounted for an average of 2.5% of the incoming COD under stable conditions, but increased a little to 3.1 – 3.6% during transient shock load conditions. However, the effluent SMPs were always less than 15 mgCOD/L.
- A total of 120 compounds were identified from the SAMBR under steady state conditions. The compounds identified were categorized as alkanes (39), alkenes (3), esters (11), alcohols (7), N-compounds (11), phenols (11), and others (9).
- 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.

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