

1 **Treatment of Municipal Solid Waste leachate using a Submerged**  
2 **Anaerobic Membrane Bioreactor at mesophilic and**  
3 **psychrophilic temperatures: analysis of recalcitrants in the**  
4 **permeate using GC-MS**

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25 **ABSTRACT**

26 This study investigated the performance of two Submerged Anaerobic Membrane  
27 Bioreactors (SAMBRs) operating at a mean Solids Residence Time (SRT) of 30  
28 (SAMBR30) and 300 days (SAMBR300) at mesophilic and psychrophilic temperatures. At  
29 35°C results showed that SAMBR30 and 300 could achieve 95% Soluble Chemical Oxygen  
30 Demand (SCOD) removal at 1.5 and 1.1 days HRT, respectively, whereas at 20°C only  
31 SAMBR300 could maintain the same performance. Low temperatures were associated with  
32 higher bulk SCOD concentrations, which contributed to reducing the flux, but this was partly  
33 reversible once the SCOD was degraded. The utilization rate of compounds was affected  
34 differently by the drop in temperature with the concentration of some recalcitrants  
35 increasing, while for others such as bisphenol A it decreased when the temperature was  
36 decreased. Among the recalcitrants detected in SAMBR30 at 20°C there were long chain  
37 fatty acids such as undecanoic acid and dodecanoic acid, but also long chain alkanes such as  
38 tetracosane and heneicosane that could not be hydrolyzed at 20°C. In SAMBR300 these  
39 alkanes and acids only appeared at 10°C, whereas at 20°C complex compounds such as  
40 phenol,2-chloro-4-(1,1-dimethylethyl), 6-tert-butyl-2,4-dimethylphenol, benzophenone, and  
41 benzenesulfonamide, n-butyl were found.

42

43 **Keywords:** Anaerobic Digestion; Submerged Anaerobic Membrane Bioreactor;  
44 psychrophilic; recalcitrants; Gas Chromatography-Mass Spectrometry (GC-MS); Powdered  
45 Activated Carbon (PAC).

46 **List of abbreviations**

47

48 BPA	Bisphenol A
49 COV	Coefficient of Variation (%)
50 DW	Deionized Water
51 EGSB	Expanded Granular Sludge Bed
52 FWC	Fresh Water Consumption (mL.day <sup>-1</sup> )
53 GC-MS	Gas Chromatography – Mass Spectrometry

54	GPR	Gas Production Rate (L STP.day <sup>-1</sup> )
55	GW	Garden Waste
56	HR	Hydrolytic Reactor
57	HRT	Hydraulic Retention Time (days)
58	KW	Kitchen Waste
59	LMH	Flux (L.m <sup>-2</sup> .h <sup>-1</sup> )
60	MSW	Municipal Solid Waste
61	MLTSS	Mixed Liquor Total Suspended Solids (g.L <sup>-1</sup> )
62	MLVSS	Mixed Liquor Volatile Suspended Solids (g.L <sup>-1</sup> )
63	MTBE	Methyl Tertiary-Butyl Ether
64	OFMSW	Organic Fraction of Municipal Solid Waste
65	OLR	Organic Loading Rate (g COD or VS.L <sup>-1</sup> .day <sup>-1</sup> )
66	PAC	Powdered Activated Carbon
67	SAMBR	Submerged Anaerobic Membrane Bioreactor
68	SCOD	Soluble Chemical Oxygen Demand (mg.L <sup>-1</sup> )
69	SGP	Specific Gas Production (L CH <sub>4</sub> .g COD <sub>fed</sub> <sup>-1</sup> )
70	SPE	Solid phase extraction
71	SRT	Solids Retention Time (days)
72	STP	Standard Temperature and Pressure
73	TCOD	Total Chemical Oxygen Demand (mg.L <sup>-1</sup> )
74	TS	Total Solids
75	UASB	Upflow Anaerobic Sludge Blanket
76	VFA	Volatile Fatty Acids (mg.L <sup>-1</sup> )
77	VS	Volatile Solids

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## 79 **1. INTRODUCTION**

80

81 Many researchers have reported significant effects of temperature on microorganisms, and  
82 lowering temperature is known to lead to a decrease in the maximum specific growth and  
83 substrate utilization rates. Under psychrophilic conditions (10-15°C), chemical and  
84 biological reactions proceed much slower than under mesophilic conditions (30-40°C)  
85 (Lettinga *et al.*, 2001). Low temperatures were reported to affect biogas production,  
86 methanogenic activity and microbial community composition. Lower temperatures can also  
87 result in an exhaustion of cell energy, a leakage of intracellular substances or complete lysis  
88 (Kashyap *et al.*, 2003). COD removal efficiencies are typically in the range 93-99% at  
89 mesophilic temperatures, but when the temperature is decreased to ambient, a few studies  
90 have reported lower COD removals: 65-75% COD removal was achieved in an Upflow

91 Anaerobic Sludge Blanket (UASB) treating landfill leachate (Kettunen and Rintala, 1998).  
92 At 24°C COD removals of up to 75% were achieved with a 10 h HRT, and the highest  
93 organic loading rate (OLR) applied was 10 kg COD.m<sup>-3</sup>.d<sup>-1</sup> (Kettunen *et al.*, 1996).  
94 Connaughton *et al.* (2006) treated a synthetic VFA-based wastewater in an Expanded  
95 Granular Sludge Bed (EGSB) loaded with 24.64 kg COD.m<sup>-3</sup>.d<sup>-1</sup> at a HRT of 4.88h and an  
96 operating temperature of 18°C with COD removal of 82%. In a more recent study on the  
97 psychrophilic performance of an EGSB - anaerobic filter bioreactor treating a VFA-based  
98 wastewater, COD removal and methane concentration exceeded 80 and 65%, respectively, at  
99 an applied OLR of 10 kg COD.m<sup>-3</sup>.d<sup>-1</sup> between 9.5 and 15°C (McKeown *et al.*, 2009).  
100 Moreover, at a temperature in the range 4-10°C, an OLR of 3.75-5 kg COD.m<sup>-3</sup>.d<sup>-1</sup> could be  
101 applied with 82% COD removal, and 70% methane in the biogas. In a EGSB coupled with a  
102 hollow fibre membrane reactor treating domestic wastewater, Chu *et al.* (2005) obtained 85-  
103 96% COD removal at temperatures above 15°C and HRTs in the range 3.5 to 5.7 hours. At  
104 11°C, increasing the HRT from 3.5 to 5.7 h resulted in the total COD removal increasing  
105 from 76 to 81%.

106 Studying the response of anaerobic reactors to low temperatures is vital, especially when the  
107 temperature change affects the rate-limiting step by altering the growth rate of individual  
108 species, and hence changes the predominant group of microorganisms in the long run  
109 (Nachaiyasit and Stuckey, 1997). In addition, heating of anaerobic reactors requires energy  
110 and capital expenditure, and hence efficient digestion of wastewater at low temperatures (5-  
111 10°C) would be highly desirable to lower costs. The development of high rate anaerobic  
112 reactor designs such as the SAMBR containing high biomass concentrations should enable  
113 the biomass to operate at lower temperatures where the specific rate per unit of biomass is  
114 lower. To our knowledge, the best performance of a SAMBR treating sucrose-based  
115 wastewater was 98% COD removal at mesophilic temperatures (35 and 25°C), while the

116 performance dropped to 65% at 15°C at 20 hours HRT (Akram, 2006). Thus the purpose of  
117 this study was to investigate the operation of a SAMBR treating municipal solid waste  
118 leachate at ambient and psychrophilic temperatures. In particular, the effect of temperature  
119 and HRT on the COD removal and the flux was investigated. Also, in order to gain more  
120 insight into the effect of low temperatures the compounds that built up during low  
121 temperature shocks were identified using GC-MS.

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## 124 **2. MATERIALS AND METHODS**

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### 126 **2.1 Wet mesophilic Two-stage lab-scale process**

127 The hydrolytic reactor (HR, 10L working volume) producing the leachate was an acrylic  
128 cylinder with a stainless steel mesh which followed a concentric arrangement inside the  
129 cylinder, and had a grid of 1mm holes. A stirrer moved inside the mesh allowing two pieces  
130 of rubber to rub against the perforated mesh: the speed of the stirrer was 40 rpm (Heidolph)  
131 and was operated intermittently (15 min ON-15 min OFF). The HR was maintained at 37°C  
132 throughout the study and was fed daily with a simulated feedstock of the Organic Fraction of  
133 Municipal Solid Waste (OFMSW). The HR was inoculated with 10 L of anaerobic sludge  
134 treating the OFMSW and the initial TS and VS were 3.9 and 2.7%, respectively. Details of  
135 the HR, composition and properties of the OFMSW feedstock can be found elsewhere  
136 (Trzcinski and Stuckey, 2009). In addition to the screening in the HR, the leachate pumped  
137 from the HR was centrifuged (1500 rpm for 20 minutes) daily to avoid a build-up of  
138 recalcitrant suspended solids in the SAMBR, and the supernatant was poured into an  
139 intermediate tank from which the SAMBRs were fed. The OFMSW leachate was treated in  
140 two SAMBRs, SAMBR30 and SAMBR300, operating at mean SRTs of 30 days and 300

141 days, respectively. The SAMBRs were 3 litre reactors fitted with a Kubota polyethylene flat  
142 sheet membrane of 0.1 m<sup>2</sup> of total surface and a pore size of 0.4 micron (Trzcinski and  
143 Stuckey, 2009). The biogas sparging rate was set at 5 L/min to minimize cake formation on  
144 the membrane. SAMBR300 was inoculated with acclimatised anaerobic sludge treating  
145 leachate and its initial MLTSS and MLVSS were 5.2 and 3.9g/L, respectively, whereas in  
146 SAMBR30 it was 4.4 and 3.2 g/L, respectively. The small difference in the initial MLVSS in  
147 both reactors was due to technical difficulties, but was deemed to have a negligible effect on  
148 reactor performance. The low temperature of 10°C was achieved using a chiller (Model  
149 RC400, Grant). The permeates of SAMBR30 and SAMBR300 were recycled to the HR or  
150 used to moisten the fresh feedstock in order to minimise fresh water consumption. The effect  
151 of Powdered Activated Carbon (PAC) was investigated using a PAC (SAE2, Norit) having a  
152 surface area of 925 m<sup>2</sup>/g.

153

## 154 **2.2 Analytical Methods**

155 The measurement of pH (Jenway) was accurate to within  $\pm 0.02$  units. Total Solids (TS),  
156 Volatile Solids (VS), Mixed Liquor Total Suspended Solids (MLTSS), Mixed Liquor  
157 Volatile Suspended Solids (MLVSS) and Soluble Chemical Oxygen Demand (SCOD) were  
158 measured as described in Standard Methods (APHA, 1999). Their coefficient of variation  
159 (COV) for ten identical samples was 4, 4, 4, 3.1 and 2.6%, respectively. Volatile fatty acids  
160 (VFAs) were measured using a Shimadzu Gas Chromatograph with a flame-ionized detector  
161 and a SGE capillary column (12m $\times$ 0.53mm ID-BP21 0.5 $\mu$ m). The COV was 3% for ten  
162 identical samples. The gas production rate (GPR) was measured using the water (2% H<sub>2</sub>SO<sub>4</sub>,  
163 10% NaCl) displacement method. The composition of biogas was determined using a  
164 Shimadzu GC-TCD fitted with a Porapak N column (1500 $\times$ 6.35 mm). The COV for 10  
165 identical samples was 2%. For GC-MS analysis, the non-polar, volatile and thermostable

166 organic pollutants were extracted using a solid phase extraction (SPE) procedure. The Oasis  
167 HLB cartridge (Waters Corporation) was first conditioned with 3 mL methyl tertiary-butyl  
168 ether (MTBE), 3mL methanol and 3 mL deionized water (DW). A sample (500mL) at pH 2  
169 was then loaded onto the cartridge and filtered dropwise. The cartridge was then washed  
170 with 3mL of 40% methanol in DW to remove organic interferences, re-equilibrated with  
171 3mL DW, washed with 3mL 10% methanol/2% NH<sub>4</sub>OH to remove humic interferences and  
172 finally 6mL 10% methanol/90% MTBE. The final matrix (10% methanol/90% MTBE  
173 mixture containing the recalcitrants from the 500 mL sample) was then evaporated to 200  
174 µL. The samples were then analyzed using a 5890 Series gas chromatograph equipped with  
175 an autosampler and a 5970 mass spectrometry detector (Hewlett-Packard, USA). The  
176 analytes were separated using a SGE HT5 column of 25m x 0.22mm with a film thickness of  
177 0.1 µm. The temperature program of the GC-MS oven was: 50°C, hold 2 min, rate 8°C min<sup>-1</sup>  
178 to 350°C, hold 30 sec. Helium was used as a carrier gas at a flowrate of 2 mL/min. The  
179 injector temperature was set at 270°C, and the MS was operated in the electron impact  
180 ionisation mode (70eV). The transfer line and ion source temperatures were 290 and 220°C,  
181 respectively, and the quadrapole was not heated. Scan runs were made with a range from *m/z*  
182 33 to 500. The chromatograms were analyzed using the NIST05 library (*National Institute of*  
183 *Standards and Technology, Gaithersburg, MD, USA, <http://www.nist.gov/srd/mslist.htm>) and*  
184 a match percentage was obtained by comparing the mass spectrum of a peak with that of a  
185 known compound from the library. The compound was deemed identified and reported if the  
186 match percentage was higher than 70%.

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### 189 **3. RESULTS AND DISCUSSION**

190

### 191 **3.1 Performance of the Hydrolytic Reactor (HR)**

192 The performance of the HR was investigated under various OLRs and SRTs. The SRT and  
193 HRT were uncoupled due to the stainless steel mesh within the HR that retained large and  
194 partially hydrolyzed particles. Results are compiled in Table 1 where it can be seen that  
195 about 70% VS removal could be obtained at SRTs greater than 70 days and HRTs in the  
196 range 9-12 days. These high VS removals were associated with fresh water consumption in  
197 the range of 30-40 mL/day. If this value is expressed per unit of biowaste, it amounts to  
198 0.085 mL/g biowaste treated or 85 L/ton, which is similar to values reported for the dry  
199 thermophilic Dranco process of 80L/ton (Dierick, 2006). This shows that a wet mesophilic  
200 process does not necessarily require more fresh water as long as the stabilised leachate from  
201 the SAMBRs is recycled to moisten the feedstock. Moreover, the water requirement can be  
202 met with rainfall; if our process was scaled-up to a 1000 m<sup>3</sup> digester treating about 50 tons  
203 biowaste/day (OLR = 4-5 kg VS/m<sup>3</sup>.day), approximately 4 tons of fresh water would be  
204 required per day. If it is assumed that the plant occupies a total area of 5,000 m<sup>2</sup> which is  
205 similar to the Kompogas plant near Zurich (Kompogas website, accessed May 2009) (33  
206 ton/day, 900m<sup>3</sup> digester), and considering that annual rainfall is in the range 700-800 L/m<sup>2</sup> in  
207 the London area (Met Office website, accessed May 2009), or 1.9 L/m<sup>2</sup>.day, a surface of  
208 2,000 m<sup>2</sup> collecting rainfall or 40% of the total plant area would be sufficient to completely  
209 cover the daily fresh water requirements.

210

211 At a high OLR of 10 g VS/L.day, the solids started to build up in the HR due to the lignin in  
212 the feedstock resulting in mixing problems. In order to keep a stable TS, more solids were  
213 removed from the HR daily, which translated to lower SRT in the range 20-70 days. As a  
214 result, the extent of hydrolysis was considerably reduced resulting in a low VS removal of  
215 39%. This low VS removal was clearly attributed to the reduced amount of time given to the



216 solid waste to degrade/hydrolyse in the HR. This was associated with a drop in VFA  
217 concentration and COD in the leachate (data not shown). Hence, these results demonstrate  
218 that the SRT can not be lowered to 20 days as this resulted in VS removals below 40%. In  
219 our study the SRT had to be reduced to avoid a build up of TS in the HR because the OLR  
220 had been increased. This indicates that an OLR of 10 g VS/L.day was too high for the HR  
221 because the lignocellulosic waste could not be solubilised fast enough. Furthermore, the drop  
222 in the VS removal was linked to an increase in fresh water consumption because more solids  
223 were withdrawn daily which had to be compensated for by water to keep the volume  
224 constant.

225

226 Finally, from day 87 the HR was fed daily with kitchen waste and garden waste, but in such  
227 an amount that the OLR was kept constant at 10 g VS/L.day. The paper waste was omitted  
228 from the feedstock in order to increase the OLR to the SAMBR and investigate the limits of  
229 the SAMBR in terms of OLR. A SRT of 23 days with a HRT of 2.4 were sufficient to reach  
230 81% VS destruction (Table 1). The same removal was obtained at 1.8 days HRT that was  
231 applied from day 99 until the end of the experiment. The VFAs consisted of fatty acids  
232 usually not found in significant concentrations such as n-valeric, n-butyric and n-caproic  
233 acids as their concentration around day 100 increased to 710, 730 and 300 mg/L,  
234 respectively. This is likely to be due to the higher amounts of long chain fatty acids normally  
235 found in food waste.

236

237

### 238 **3.2 Performance of SAMBR30**

239 SAMBR30 was started up at a HRT of 3.7-4.6 days. Figure 1A shows that the start up at this  
240 HRT was successful in terms of VFA removal, but nevertheless, a temporary SCOD build up

241 occurred in the bulk. Moreover, the addition of PAC on day 21 helped to initially reduce the  
242 bulk SCOD during the start up. However, the effects on the permeate SCOD (Figure 1A) and  
243 the flux (Figure 1B) were negligible. SAMBR30 achieved a SCOD removal of between 79  
244 and 95% at a minimum HRT of 1.5 days and an OLR of 8 g COD/L.day. However, this  
245 performance was characterised by a residual SCOD in the bulk of greater than 2 g/L, while  
246 the permeate on the other hand had a SCOD concentration below 1g/L because of membrane  
247 rejection by the cake/gel layer. Unfortunately, these high bulk SCOD concentrations were  
248 responsible for a rapid drop in flux. Nghiem *et al.* (2006) also observed that a greater total  
249 organic carbon rejection coincided with the highest level of fouling which is consistent with  
250 our observations. The flux was zero after day 95, and as a result the SRT became equal to the  
251 HRT and a volume of sludge equal to the volume fed was removed daily, and the SCOD  
252 removal percentage was calculated using the bulk SCOD concentration instead of the  
253 permeate SCOD and the sludge.

254

### 255 **3.3 Effect of PAC addition to SAMBR30**

256 In order to gain more understanding of the PAC adsorption mechanisms a sample of the  
257 permeate of SAMBR30 was analysed by GC-MS before and after PAC addition on day 21.  
258 In order to let the PAC reach equilibrium, the sample after PAC addition was only taken on  
259 day 23. The abundances of the peaks detected by GC-MS were compared and an estimated  
260 removal due to PAC adsorption is given in Table 2. It can be seen that some compounds  
261 were completely removed by PAC as these were not detected on the chromatogram after  
262 PAC addition. Interestingly, 85 and 76% of Bisphenol A and Bis (2-ethylhexyl)phthalate,  
263 respectively, could be removed by adsorption onto PAC. Their adsorption onto PAC was  
264 favoured because of their high partition coefficients (log P equal to 3.4 and 8.7, respectively-  
265 SciFinder Scholar, 2007), meaning that they are very hydrophobic. The adsorbability of the

266 solutes is a function of the hydrophobicity of the solute; the more hydrophobic the solute the  
267 higher the tendency to be adsorbed onto the PAC to decrease the surface in contact with  
268 water, and hence lower the free energy of the system. Other researchers have observed high  
269 removals (over 90%) of endocrine disrupting chemicals using activated carbon (Bolong *et*  
270 *al.*, 2009, Westerhoff *et al.*, 2005). More recently their concentrations were found to increase  
271 during treatment in the SAMBRs because they are known to be non degradable  
272 anaerobically (Trzcinski and Stuckey, 2009).

273

274 The high removal of the phenanthrenecarboxylic acids (100 and 88%) can be explained by  
275 their structure which is made up of 3 rings, with one being aromatic. This structure results in  
276 a very high hydrophobicity ( $\log P = 6.3$  to  $6.75$ ), and a very low solubility of about 2 mg/L.  
277 (SciFinder Scholar, 2007). It was observed in Figure 1A that the addition of PAC caused a  
278 decrease in the bulk SCOD from 2380 to 1550 mg/L, but the SCOD then returned to the  
279 initial levels after 5 days suggesting that the PAC had reached saturation and could not  
280 adsorb anything more. Small quantities of PAC were removed daily because of the SRT set  
281 at 30 days, but these removals were likely to be negligible during the 5 days following PAC  
282 addition. Moreover, the adsorption capacity of the PAC could be estimated based on the  
283 mass of SCOD adsorbed. The SCOD concentrations were 2380 and 1645 mg/L on day 21  
284 and 22 respectively. Since approximately 4200 mg SCOD (1400 mg/L) were fed between  
285 day 21 and 22 (SCOD leachate = 5000 mg/L and HRT = 3.6 days) and assuming a 69%  
286 SCOD degradation (SCOD removal on day 18), there was an additional 430 mg/L  
287 recalcitrant SCOD in the bulk (31% of 1400 mg/L). So the total SCOD was thus 2380 + 430  
288 mg/L and because the residual SCOD was 1645 mg/L, there was thus 1170 mg/L adsorbed  
289 onto 2g PAC/L, thus achieving 585 mg SCOD adsorbed per gram PAC. The results showed  
290 that PAC was saturated due to high concentrations of recalcitrant SCOD found in the

291 leachate which contradicts previous studies that showed that PAC could adsorb SCOD in the  
292 bulk and improve the flux (Akram, 2006) in a SAMBR treating a sucrose-based synthetic  
293 wastewater. The authors stated that PAC (1.7 g/L) enhanced biodegradation of sorbed  
294 organics and that bioregeneration of PAC was taking place to further remove residual COD.  
295 In our study the low solid retention time of SAMBR30 may have resulted in the washout of  
296 slow-growing microorganisms responsible for the degradation of sorbed recalcitrants.  
297 Increasing the concentration of PAC will remove more SCOD, but will be expensive and  
298 increase the viscosity which can also result in flux drop (Akram, 2006).

299

### 300 **3.4 Performance of SAMBR300**

301 SAMBR300 was fed with centrifuged leachate from the HR from day 34 onwards because  
302 results obtained from SAMBR30 showed a high SCOD in the bulk (>2 g/L) due to the low  
303 sludge retention times of 30 days. SAMBR300 was therefore run at 300 days solid retention  
304 time to see if lower bulk SCODs could be attained. Permeate SCOD was omitted in Figure 2  
305 because it followed the same trend as in the bulk, but at a lower concentration due to  
306 membrane rejection. The initial HRT was set at 19 days to check that the inoculum was  
307 active and was then reduced gradually to low HRTs and temperatures. It can be seen from  
308 Figure 2A that the SCOD removal was over 90% at a HRT as low as 1.1 days even though  
309 the percentage initially dropped to below 80% and 70% during the shock to 2.7 and 1.6 days,  
310 respectively. These lower removals were, however, linked to a SCOD below 2000 mg/L in  
311 the HR effluent. It can be seen from Figure 2A and 2B that bulk SCODs lower than 1g/L  
312 gave rise to a stable flux of 4 LMH, whereas a bulk SCOD greater than 2 g/L in SAMBR30  
313 was very detrimental to the flux. The best performance of SAMBR300 is summarized in  
314 Table 3. The specific gas production (SGP) was found to be 0.25 L CH<sub>4</sub>/g COD<sub>fed</sub> on day 94

315 at an OLR of 11.7 g COD/L.day using the following equation where the units are in  
316 brackets:

$$317 \quad SGP \left( \frac{L \text{ CH}_4}{g \text{ COD}_{fed}} \right) = \frac{CH_4 (\%) \cdot GPR \left( \frac{L_{biogas}}{L.day} \right)}{OLR \left( \frac{g \text{ COD}}{L.day} \right)}$$

318 which implies that 63% (0.25/0.395) of the COD of the leachate fed to SAMBR300 was  
319 converted to methane. On day 95, the SCOD removal in SAMBR300 was 95.5% at 1.1 days  
320 HRT and OLR of 11.7 g COD/L.day. Later on the same day the heater was switched off to  
321 allow the SAMBR to reach ambient temperatures (20 to 22°C). It can be seen from Figure  
322 2A that on day 98 this temperature shock caused a sudden increase in acetate concentration  
323 from 0 to 500 mg/L, and in the bulk SCOD from 800 to 2000 mg/L. The SCOD removal  
324 dropped to 88% at an OLR of 13.4 g COD/L.day. This shows that acidogenic bacteria,  
325 acetoclastic methanogens and acetotrophs were affected by the temperature shock. However,  
326 these groups of bacteria and archaea recovered because the bulk SCOD and acetate  
327 concentrations went down to normal levels on day 100 achieving a SCOD removal of 95.3%  
328 at an OLR of 11.8 g COD/L.day. This indicates the adaptation and growth of acetate  
329 degraders at lower temperatures during the course of the experiment (McHugh *et al.*, 2004).  
330 The results demonstrate the benefits of maintaining a high SRT in the SAMBR to  
331 compensate for the low activity at lower temperatures. The absence of propionate during the  
332 shock at 20°C could be due to an enrichment and elevated activity of hydrogenotrophic  
333 methanogens at low temperature (Collins *et al.*, 2005). The specific gas production then  
334 became 0.14-0.16 L CH<sub>4</sub>/g COD, and the gas production rate (GPR) dropped from 4.5 L  
335 biogas/L.day at 35°C to 2.4-3 at 20°C. This shows that some of the particulate COD fed  
336 could not be hydrolyzed at 20°C to be converted to methane. Hence we showed that because  
337 of the high SRT, SAMBR300 could cope with the temperature shock down to 20°C.

338

339 On day 100 the temperature was further decreased to 10°C: this caused an immediate rise in  
340 bulk SCOD, acetate and propionate concentrations. Akram (2006) also observed that acetate  
341 and propionate were the dominant VFAs when a shock to 15°C took place. Other VFAs such  
342 as n-butyric, n-valeric and n-caproic acids also increased to maximum of 140 mg/L (data not  
343 shown). This shows that this time the acetogenic bacteria which degrade the VFAs into  
344 acetate were also affected in addition to acidogenic bacteria, acetotrophs and acetoclastic  
345 methanogens. This is consistent with Nachaiyasit and Stuckey (1997) who observed that  
346 acetogens and methanogens were the most affected groups.

347

348 As a result of the temperature drop the SCOD removal percentage dropped to 44% at an  
349 OLR of 14.1 g COD/L.day on day 106, and the specific gas production dropped to 0.015 L  
350 CH<sub>4</sub>/g COD; this is a ten-fold reduction from 20 to 10°C emphasizing the lower metabolic  
351 activity at 10°C. It is known that psychrophilic temperatures can decrease the number of  
352 viable microorganisms in addition to the reaction rate of biomass (Tian *et al.*, 1994). This  
353 can result in poor performance in cases where mesophilic bacteria do not grow well under  
354 psychrophilic temperatures. After day 106 the feeding was stopped, and in batch mode the  
355 SCOD and VFAs levels decreased, showing that the anaerobic degradation of leachate at  
356 10°C was possible, but at a lower rate. From days 106 to 113 the acetate concentration  
357 decreased from 2180 to 680 mg/L (-1500 mg/L) in 7 days whereas the propionate  
358 degradation rate was only 83 mg/L in 7 days demonstrating the lower degradation rate of  
359 propionate at 10°C. This is in line with Langenhoff and Stuckey (2000) who stated that  
360 propionate degrading bacteria are more affected by temperature than the acetate degrading  
361 archaea in the long term. Propionate degradation has also been identified as the rate-limiting  
362 step in psychrophilic anaerobic digestion (Collins *et al.*, 2003), particularly during acetate  
363 build-up. These persistently high acetate and propionate levels at low temperature, even after

364 long periods, has been observed before (Akram, 2006, Matsushige *et al.*, 1990). This is due  
365 to the  $K_s$  (half saturation constant) for acetate and propionate increasing dramatically at low  
366 temperatures, and hence there is a certain minimum concentration below which the acetate  
367 and propionate cannot go (Lawrence and McCarty, 1970). There is also the fact that the  
368 maximum specific substrate utilization rate, maximum microbial growth rate ( $\mu_{max}$ ) and the  
369 bacterial decay rate constant decrease with decreasing temperature in accordance with the  
370 Arrhenius equation. The increase in  $K_s$  and decrease in  $\mu_{max}$  results in a decline in the rate of  
371 reaction according to Monod's kinetics, and results in the build up of individual VFAs inside  
372 the SAMBR. Furthermore, the rise in VFAs was accompanied by a drop in pH to 6.6 on day  
373 105, and a drop in flux from 4.5 to 0.4 LMH (Figure 2B) due to the build up of SCOD in the  
374 bulk. Harada *et al.* (1995) stated that soluble organics were the primary factor that  
375 determined the formation of a gel layer and thus flux decline, and not the MLTSS. In our  
376 study the MLTSS remained around 10-11 g/l after day 90 while the bulk SCOD increased  
377 several fold which supports Harada's observations.

378 Moreover, the decrease in bulk SCOD in batch mode gave rise to an increase in flux from  
379 0.4 on day 105 to 1.1 LMH on day 113 demonstrating that the flux drop due to high bulk  
380 SCOD was partly reversible (a pump was continuously recycling the permeate to the  
381 SAMBR even during batch mode). It can be seen in Figure 2B that the flux was strongly  
382 influenced by the temperature which in turn governed the bulk SCOD degradation. Jin *et al.*  
383 (2009) who treated brackish water with reverse osmosis observed that colloids had a  
384 dramatically greater size at 15°C and this resulted in dramatically higher cake layer specific  
385 resistances such that the observed flux decline was more severe at 15 than at 35°C. From our  
386 observations and previous results (Trzcinski and Stuckey, 2009), it appears that the bulk  
387 SCOD was the most important parameter governing the flux at MLTSS concentrations  
388 below 20 g/L, and the flux drop was partly reversible once the bulk SCOD was degraded.

389

390

## 391 **3.5 Gas Chromatography - Mass Spectrometry (GC-MS)**

### 392 **3.5.1 Introduction**

393 In order to gain more insight into which molecules are recalcitrant and accumulate in the  
394 SAMBR, GC-MS analyses were carried out. The effect of low temperatures was also  
395 investigated in order to determine which compounds build up during the temperature shock  
396 that led to an increase in the bulk SCOD.

397

### 398 **3.5.2 Compounds from the reactor and equipment**

399 Firstly, a brand new SAMBR with new tubing and membrane was used and operated only  
400 with deionised water for 1 week at 35°C in order to determine which components if any  
401 leach from the reactor plastic and the tubing. The compounds that were detected are listed in  
402 Table 4. The alkanes detected are likely to come from the oils and waxes used to  
403 manufacture the pieces of acrylic sheets (Barkston Plastics, UK) in our workshop, whereas  
404 the phthalates and phenols are likely to come from the plastic itself. This was done to blank  
405 out any interferences from the reactor rather than the microbial system.

406

### 407 **3.5.3 Recalcitrant compounds in the HR effluent**

408 The HR effluent was analysed by the GC-MS, but unfortunately only 100 ml of effluent  
409 could be loaded on to the Oasis cartridge because of the high soluble organic content. As a  
410 result, there were only a few peaks detected, and these are listed in Table 5. The main  
411 compounds detected in the HR effluent were long chain fatty acids, as expected, because  
412 kitchen waste was being fed to the HR when the sample was taken (after day 87).

413



#### 414 **3.5.4 Recalcitrant compounds in SAMBR30 and SAMBR300**

415 The compounds detected at 35 and 20°C are shown in Table 6. There were more peaks  
416 detected in SAMBR30 compared to SAMBR300 (Table 7) probably due to the higher SCOD  
417 level in the former. Among the recalcitrants found at 35°C were tridecane that is considered  
418 as easily degradable because it is aliphatic. At 20°C, the compounds octanoic acid, nonanoic  
419 acid and n-decanoic acid that were present in the HR effluent could not be hydrolyzed in  
420 SAMBR30 because of the lower metabolic activity at 20°C and probably also because of the  
421 SRT equal to the HRT due to a blocked membrane. In contrast, in SAMBR300 they were  
422 completely hydrolyzed and only appeared when operating at 10°C in batch mode. Among  
423 the new molecules detected in SAMBR30 at 20°C there were long chain fatty acids such as  
424 undecanoic acid and dodecanoic acid, but also long chain alkanes such as tetracosane and  
425 heneicosane. Nevertheless, several compounds detected at 35°C were still partly degraded at  
426 20°C such as benzophenone, benzensulfonamide, n-butyl and bisphenol A. This could be  
427 due to the biomass attached to the membrane or simply because of the lower solubility of  
428 these compounds at 20°C.

429

430 It can be seen from Table 7 that several compounds started to build up when SAMBR300  
431 was operated at 20°C compared to 35°C; among these, phenol, 2-chloro-4-(1,1-  
432 dimethylethyl), 6-tert-butyl-2,4-dimethylphenol, benzophenone, tri(2-chloroethyl) phosphate  
433 and benzensulfonamide, n-butyl concentrations were found to increase at 20°C. In contrast,  
434 the concentration of 3,5-di-tert-butyl-4-hydroxyphenyl, bisphenol A and  
435 phenanthrenecarboxylic acid all decreased at 20°C. This shows that the utilization rate of  
436 compounds was differently affected by the drop in temperature. Nonetheless, the SCOD  
437 removal remained over 90% at 20°C indicating that the increase or decrease in concentration  
438 of the detected components had a negligible impact on the SCOD removal.

439

440 Interestingly, in this study the concentration of bisphenol A (BPA) was observed to decrease  
441 at 20°C and was not even detected at 10°C, while it was found to build up in the SAMBR in  
442 a previous study at constant temperature of 35°C (Trzcinski and Stuckey, 2009). Ogawa *et*  
443 *al.* (2005) found that BPA at an initial concentration in the range 0.05-10 µg/L could be  
444 biodegraded anaerobically in 30 days at 9-22°C. The aerobic biodegradation, on the other  
445 hand, took only 72 hours. A possible explanation, however, is that their solubility decreased  
446 with decreasing temperatures and hence more was adsorbed at lower temperatures onto  
447 sludge or reactor walls.

448

449 Some compounds such as nonanoic acid, n-decanoic acid and 1,19-Eicosadiene that  
450 appeared at 10°C, but not at mesophilic temperatures are marked “From HR” in the last  
451 column of Table 7 because they are long chain fatty acids also found in the HR effluent. This  
452 means that their hydrolysis could not take place in SAMBR300 at 10°C. The compounds  
453 2H-pyran-2-one,6-heptyltetrahydro, phthalic acid, butyl 4-octyl ester, 1,19-eicosadiene and  
454 hexadecanoic acid, hexadecyl ester were also not detected at mesophilic temperatures, and  
455 can thus be considered as recalcitrants appearing in SAMBR300 under psychrophilic  
456 conditions even when it was left in batch mode. In contrast, hexanedioic acid was not  
457 detected at 35°C, but appeared at 20°C and could be degraded at 10°C in batch mode. It is  
458 noteworthy to say that some compounds detected at 20°C were not detected at 10°C because  
459 the reactor was left in batch mode for 7 days before the sample for GC-MS analysis was  
460 taken, and degradation could have occurred during that time.

461

462

463 **4. CONCLUSIONS**

464

465 The results showed that:

- 466 • A SCOD removal of 95% was achieved at 1.1 days HRT in SAMBR300 and a biogas  
467 production rate of 4.5 L/L.day was observed at an OLR of 11.7 g COD/L.day at 35°C.
- 468 • SAMBR300 also achieved 95% SCOD removal at 1.1 days HRT at 20°C and at an OLR  
469 of 11.8 g COD/L.day, but the gas production rate dropped to 2.4-3 L/L.day. However, at  
470 10°C the SCOD and VFAs built up in the bulk due to lower degradation rates, especially  
471 for propionate.
- 472 • Low temperatures were associated with higher bulk SCOD which contributed to reduce  
473 the flux. Once SCOD was degraded the flux was partly restored.
- 474 • SAMBR30 operating at a mean SRT of 30 days could not be operated at 20°C.
- 475 • Some compounds became recalcitrant under psychrophilic conditions such as nonanoic  
476 acid, n-decanoic acid and 1,19-eicosadiene that are long chain acids also found in the HR  
477 effluent. Moreover, some new recalcitrants were found to appear only in the SAMBR300  
478 under psychrophilic conditions such as 2H-pyran-2-one, 6-heptyltetrahydro, phthalic acid  
479 butyl 4-octyl ester and hexadecanoic acid, hexadecyl ester.
- 480 • PAC addition (2 g/l) in the conventional SAMBR lowered the bulk SCOD for few days  
481 but then reached saturation and the SCOD increased back to the initial value  
482 demonstrating that the PAC was not biologically regenerated. Nevertheless, among other  
483 molecules PAC could remove were 85 and 76% of bisphenol A and bis (2-  
484 ethylhexyl)phthalate, respectively, that are two recalcitrants commonly found under  
485 anaerobic conditions.
- 486 • The number of peaks detected by GC-MS in SAMBR30 was considerably higher than in  
487 SAMBR300. At 20°C, the compounds octanoic acid, nonanoic acid and n-decanoic acid  
488 present in the HR effluent could not be hydrolyzed in SAMBR30. In contrast, in

489 SAMBR300 they were completely hydrolyzed and only appeared when operating at  
490 10°C.

491

492

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494

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**Figure 1.** A: Evolution of the SCOD, acetate and propionate concentration in the bulk and the permeate SCOD of SAMBR30 (left axis), and the SCOD removal percentage (right axis) under various HRTs and temperatures. B: Evolution of the flux and pH in SAMBR30. The error bars show the standard deviation.

**Figure 2.** A: Evolution of the SCOD, acetate and propionate concentration in the bulk of the SAMBR300 (left axis), and the SCOD removal percentage (right axis) under

600 various HRTs and temperatures. B: Evolution of the flux and pH in SAMBR300.  
601 The error bars show the standard deviation.

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622  
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