

1 **Identification of recalcitrant compounds in a pilot-scale AB system: an Adsorption (A) stage**
2 **followed by a Biological (B) stage to treat municipal wastewater.**

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22 **Abstract**

23 This manuscript presents a comparison of the A-stage and B-stage sludges in terms of anaerobic
24 biodegradability and low molecular weight compounds present in the supernatant using Gas
25 Chromatography-Mass Spectrometry (GC-MS). The GC-MS analysis of A-stage and B-stage
26 supernatants identified respectively 43 and 19 organic compounds consisting mainly of aromatics
27 (27.9% and 21%), alcohols (25.6% and 15%) and acids (30.2% and 15%). The methane potential
28 was found to be 349 ± 1 mL CH₄/g VS and 238 ± 12 mL CH₄/g VS, respectively. After anaerobic
29 digestion of these sludges, a greater proportion of aromatics (42% and 58%) and a lower
30 proportion of acids (10% and 10%) and alcohols (16% and 10%) was observed.

31

32 **Keywords** AB process, anaerobic biodegradability, dissolved organic compounds, Soluble
33 Microbial Products (SMP), sewage sludge, GC-MS

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35 **1. Introduction**

36 In the recent years, research efforts aiming to improve energy efficiency of wastewater treatment
37 processes in large centralized wastewater treatment plants (WWTPs) have increased. Concerns
38 over global warming impacts, energy sustainability, and biosolids generation are among several
39 key drivers towards the establishment of more energy-efficient WWTPs (Chai et al., 2015). The
40 biosolids management system is cost-intensive as it typically accounts for 25-60% of the total
41 operational costs of conventional activated sludge (CAS)-based WWTPs (Canales et al., 1994;
42 Verstraete & Vlaeminck, 2011). Innovative design and treatment strategies, therefore, are required
43 to achieve more cost-effective and energy self-sufficient WWTPs by minimizing energy
44 consumption while increasing its recovery.

45

46 An approach towards an energy-neutral, if not -positive, wastewater treatment process is to
47 recover the potential energy available in raw municipal wastewaters (Shizas & Bagley, 2004). A
48 well-structured strategy deploying a two-stage process, the so-called AB process, has been
49 suggested for the recovery of caloric energy content from sewage organics (Böhnke, 1977;
50 Meerburg et al., 2015; Versprille et al., 1984). The first stage is an extremely high loaded
51 biosorption stage (A-stage), which is subsequently followed by a low loaded biological stage (B-
52 stage) to ensure the removal of dissolved organics and ammonia. The A-stage treatment at the
53 entry of WWTP allows biological concentration of sewage with minimum oxidation of organics to
54 CO₂, and consequently producing a concentrated sludge stream to be channeled to the anaerobic
55 digester. The entrapped organics (chemical energy) can then be recovered through an efficient
56 conversion to biogas without significant energy losses (Verstraete et al., 2009). A characteristic
57 feature of the A-stage reactor is operation with high food to microorganisms (F/M) ratios, short
58 hydraulic retention times (HRTs), and short solid retention times (SRTs), to achieve high reduction
59 rate of sewage organics (Boehnke et al., 1997). Indeed, the treatment with short SRT has been
60 demonstrated to significantly improve the biodegradability of sludge in the downstream anaerobic
61 digester (Ge et al., 2013). The separation of excess sludge in the A-stage can be achieved through
62 an intermediate clarifier (henceforth referred to as ‘A-stage clarifier’) or dynamic membrane
63 filtration unit (Ersahin et al., 2012; Roest et al., 2012).

64

65 During the biosorption process, the A-sludge retains particulate and colloidal organic substances
66 within the biomass matrix, and therefore leaving mainly dissolved organics in the effluents. This
67 would mean reduced aeration energy requirement and lower sludge production in the following B-

68 stage (Versprille et al., 1984), and therefore may lead to considerable energy savings and overall
69 reduction in biosolids generation. There is currently little information available regarding the
70 biodegradability of the excess sludge and the types of dissolved organics leaving the A and B
71 stages. Effluents from biological processes contain a wide range of complex organic compounds,
72 including soluble microbial products (SMP) and extracellular polymeric substances (EPS),
73 released during bacterial metabolism in mixed culture in bioreactors. Generally, in order to
74 evaluate the performance of biological wastewater treatment processes, only the common generic
75 parameters are measured. These include measures such as chemical oxygen demand (COD),
76 biochemical oxygen demand (BOD), mixed liquor volatile suspended solids, and total organic
77 carbon (TOC), which are done according to Standard Methods from the American Public Health
78 Association (APHA) (Eaton and Franson, 2005). It is important to clearly identify the primary
79 components of SMPs and ECPs in order to understand the fundamental mechanisms of biological
80 activity that create these compounds, and how to reduce these compounds in the effluent.
81 Preliminary results from Aquino (2004) on the identification of SMPs using GC-MS surprisingly
82 revealed long chain alkenes and alkanes, as well as some aromatic compounds such as phthalates
83 in significant concentration (low mg/L). Shen et al. (2012) showed that the concentration of SMPs
84 in wastewater treatment plants ranged roughly from 5 to 25 mg TOC/L, with the major component
85 being polysaccharides (ca. 3–18 mg/L) followed by humic substances (ca. 2–10 mg/L); while the
86 protein concentration was relatively low (<5 mg/L). The SMPs presented a broad molecular weight
87 distribution from smaller than 1 kDa to over 100 kDa. In addition, these compounds constitute the
88 main foulants in membrane bioreactors which are being used more widely around the world (Mei
89 et al., 2014).

90 Thus so far, there is virtually no report on the A-sludge's biodegradability and its comparison with
91 the B-sludge, a more conventional type of sludge, and the type of organics and their concentration
92 in each stage.

93

94 In this study, gas chromatography coupled with mass spectrometry (GC-MS) was used to identify
95 recalcitrant low molecular weight (MW) organics (<580 Da) that were not adsorbed in the A-stage
96 and appeared in the influent to the B-stage. Moreover, the recalcitrant compounds and soluble
97 microbial products (SMPs) produced in the B-stage were also identified and compared with those
98 in the A-stage. These are the compounds that are most likely to foul the membrane when MBRs
99 are used in the B-stage, and that could also appear in the final effluent. There is therefore interest
100 to shed more light on these compounds, in particular from an AB process treating combined
101 industrial municipal wastewaters.

102

103 **2. Material and methods**

104 **2.1 Reactors configuration and operating conditions**

105 A pilot unit was operated with an AB process to treat real municipal wastewater from households
106 and small businesses in Singapore. The pilot plant was run in a continuous flow mode with an
107 average wastewater flow of 1000 m³/d. It consisted of an equalization tank, 2 coarse (5 mm) rotary
108 drum screen units, a high-rate A-stage contact tank, a primary/A-stage clarifier, 2 fine (2 mm)
109 rotary drum screen units, and an ultrafiltration membrane bioreactor (MBR) system which
110 comprised 5 biological tanks (2 anoxic tanks and 3 aerobic tanks), 1 membrane tank and 1
111 deoxygenation tank. A simplified schematic diagram of the pilot plant is shown in Figure 1. The
112 raw influent consisted of a mixture of incoming municipal wastewaters and dewatered digested

113 sludge and was drawn through submersible pumps operating in constant flowrate mode. Initial
114 screening was subsequently performed through 5 mm perforated screen units followed by a screw
115 conveyor type grit removal system. The A-stage was designed with an SRT of 0.5 d (calculated
116 over the entire contact tank and clarifier) and a total HRT of 2 h, consisting of 0.5 h and 1.5 h for
117 the contact tank and clarifier, respectively. To protect the downstream MBR process, 2 mm fine
118 screens were provided for the removal of smaller solid particles. The following B-stage was
119 operated with a 5-h HRT in the Modified Ludzack – Ettinger (MLE) configuration with a step-feed
120 of 50% influent to the first anoxic zone and the other 50% to the second anoxic zone. A target SRT
121 of 5 d was set in order to maintain the slow-growing nitrifying organisms for N removal. Dissolved
122 oxygen (DO) concentrations were maintained at 0.3 and 1 mg O₂/L in the corresponding contact
123 tank and aerobic tanks.

124

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127 **2.2 Physicochemical analyses**

128 Sludge samples were taken from the pilot plant on 26th March 2015. Physico-chemical parameters
129 such as Total Solids (TS), Volatile Solids (VS), Total Suspended Solids (TSS), Volatile Suspended
130 Solids (VSS) and COD concentrations were immediately analyzed in accordance with Standard
131 Methods for the Examination of Water and Wastewater (APHA, 1995). Calorific value was
132 determined using an oxygen bomb calorimeter (IKA, Malaysia) to measure the energy content in
133 the sludge. The calorimeter unit consisted of a stainless steel bomb, a water jacket, an ignition unit,
134 a thermometer, and a mechanical stirrer. Internal volume of the stainless steel bomb was
135 approximately 350 mL and the volume of water jacket surrounding the bomb was 2 L. The

136 mechanical stirrer was used to keep the water jacket uniformly mixed. After centrifugation, the
137 biomass pellet was frozen at -20°C and subsequently freeze-dried at 0.01 mbar vacuum and -45°C
138 overnight. Next, the dried samples were crushed into powder, weighed and combusted using high
139 pressure oxygen (30 bar) in bomb calorimeter. The temperature rise in the water jacket during
140 combustion was used to calculate the energy content of sludge samples. The heat capacity of the
141 bomb was determined using benzoic acid as a standard (Shizas & Bagley, 2004).

142

143

144 **2.3 Liquid-Liquid extraction**

145 Liquid-liquid extraction was performed on 100 mL of filtered supernatant (<0.45 m) using 70 mL
146 Dichloromethane (GC-MS grade, Merck). This solvent was chosen because it has been used by
147 other researchers for SMP analysis on GC-MS (Wu & Zhou, 2010). All glassware was washed
148 with acetone prior to the procedure. A blank containing only distilled water was run along as
149 control. Mixing was provided for 3 minutes by manually inverting the extraction funnel and
150 separation of the 2 phases was then allowed for 5 minutes. Traces of water were removed by
151 mixing the solvent phase with 2 spoons of Na₂SO₄. Solvent evaporation was then carried out at
152 50°C under vacuum until 1 mL of solvent phase was obtained.

153

154

155 **2.4 Gas Chromatography – Mass Spectrometry**

156 The samples (injection volume: 1 µL) were then analyzed using a Shimadzu gas chromatograph
157 equipped with an autosampler and a QP2010Ultra mass spectrometry detector (Shimadzu, Japan).
158 The analytes were separated using an Rtx® -5MS column of 30m x 0.25 mm with a film thickness

159 of 0.25 μm . The temperature program of the GC-MS oven was: 50°C, hold 7 min, rate 7°C min⁻¹ to
160 325°C, hold 14 min. Helium was used as a carrier gas at a column flowrate of 1 mL/min. The
161 injector temperature was set at 280°C (splitless injection mode), and the MS was operated in the
162 electron impact ionisation mode (70 eV). The transfer line and ion source temperatures were 280
163 and 230°C, respectively. Scan runs were made with a range from m/z 30 to 580. The
164 chromatographic peaks were identified either by direct analysis of the mass spectrum or/and
165 comparison with the NIST11 library (National Institute of Standards and Technology,
166 Gaithersburg, MD, USA, <http://www.nist.gov/srd/mslist.htm>). The retention indexes were
167 calculated by the library according to alkanes standards retention times (Trzcinski & Stuckey,
168 2010). Quantification was done separately for each unknown compound using the alkane with the
169 closest retention time.

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171

172 **2.4 Biochemical Methane Potential**

173 Biochemical methane potential (BMP) of the A-stage and B-stage sludges was determined in batch
174 assays using an Automatic Methane Potential Test System (AMPTS II, Bioprocess Control,
175 Sweden). The assay was performed to examine the biodegradability of substrate subjected to the
176 anaerobic incubation through the measurement of its cumulative methane production. The AMPTS
177 reactor was seeded with anaerobic sludge which was collected from a mesophilic digester at Ulu
178 Pandan Water Reclamation Plant in Singapore. The assay was conducted at 35°C for
179 approximately 28 days. Prior to the assay, the inoculum was degassed at 35°C for one week to
180 remove the residual carbon source. Biomedium containing nutrients and vitamin was prepared in

181 accordance with Owen et al. (1979). 200 mL of inoculum, 100 mL of substrate, and 50 mL of
182 biomedium were added to each reactor which was subsequently flushed with nitrogen gas at 5 psi
183 for approximately 5 min. Batch reactor without substrate addition was used as negative control and
184 its methane production was subtracted from the methane production in the test bottles. All assays
185 were performed in duplicate. The composition of biogas was analyzed with gas chromatography as
186 previously reported (Tian et al., 2014). The percentage of biodegradability was calculated through
187 stoichiometric conversion of CH₄ production from organic degradation as described in Speece
188 (1996). Sample preparation of the anaerobically digested sludge prior to GC-MS analysis was done
189 as described above. SMPs from the anaerobic inoculum used in the AMPTS were also analyzed
190 following the same procedure and is referred to as “AMPTS control” in results and discussion.

191

192 **3. Results and discussion**

193

194 **3.1 Physicochemical characteristics**

195 Table 1 shows the properties of A-stage and B-stage sludge collected in this study. The physical
196 properties were very similar and both sludges had similar organic content (VS/TS ratio). Although
197 the TCOD was around 4-6 g/L in both sludges, the B-stage had a significantly lower SCOD (38
198 mg/L) compared to the A-stage (153 mg/L). This is due to the biodegradation of dissolved organics
199 in the membrane bioreactor. The calorific value was higher in the A-stage sludge due to the
200 concentration of carbon including dissolved organics, and possibly cellulose and lignin from raw
201 sewage.

202

203 **3.2 GC-MS analysis of recalcitrant compounds and SMPs in AB process**

204

205 144 peaks appeared on the chromatograph from the A-stage sludge supernatant (Supplementary
206 material), but only 43 (30%) could be identified with a match percentage greater than 80% (Figure
207 2 top left). Their concentration was not higher than 5 µg/L, except for a few acid compounds
208 detected at a higher concentration such as dodecanoic (11.2 µg/L), hexadecanoic (28.5 µg/L), oleic
209 (21.1 µg/L) and octadecanoic acids (20.5 µg/L) (Table 2). Long chain fatty acids (LCFA) originate
210 from the degradation of fats, oils and grease present in raw sewage. LCFAs could have been taken
211 up by Poly-phosphate accumulating microorganisms (PAO) in the B stage.

212 It has been recently reported that LCFA can be used as sole carbon source for EBPR and were
213 found to enhance PAO activity (Tayà et al., 2015). It is also possible that some of the compounds
214 detected in this study by GC-MS were inhibitory or toxic to PAOs which can explain why the Bio-
215 P removal was not stable according to Qing (2015).

216

217 Aromatic compounds were found in the low MW range (<150 Da) as well as in the high MW
218 range (>300 Da) and bis(2-ethylhexyl) isophthalate was the largest aromatic compound in this
219 sample with a MW of 390 Da. Overall, it was found that the compounds were mainly aromatic
220 (27.9%), alcohols (25.6%) or acids (30.2%) (Figure 2 top right). The other compounds were
221 alkanes, amines and ester, but in much smaller proportions.

222

223 The total number of peaks was significantly greater in the A-stage supernatant (144) compared to
224 the B-stage supernatant (84) (Figure 2 bottom left). This is consistent with the A-stage
225 chromatograph that shows more peaks compared to the B-stage (Supplementary material). The B-
226 stage chromatogram also displayed a flatter baseline which is an indication that it had fewer peaks.

227 Similarly, the number of identified peaks with a match percentage greater than 80% was higher in
228 the A-stage with 43 peaks versus 19 peaks in the B-stage supernatant. However, the B-stage
229 supernatant was less characterized than the A-stage supernatant with 23% of the peaks being
230 identified versus 30% for the A-stage supernatant.

231 The A-stage supernatant contained high molecular weight (MW) compounds with Retention Index
232 (RI) greater than 3000 and the greatest molecular weight was 534 Da for 9-Octadecenoic acid (Z)-,
233 octadecyl ester. In contrast, the B-stage supernatant did not contain any compounds with RI greater
234 than 3000 indicating that high MW compounds from the A-stage were hydrolyzed. This is relevant
235 since membrane modules (ultrafiltration) are submerged in the B-stage membrane tank and the
236 type of organics, their concentration and molecular weight will affect the fouling because they are
237 the same size as the pore diameter (Mei et al., 2014). From this study, there were clear differences
238 between the A-stage and B stage in terms of number of compounds, the type of organics, their
239 concentration and molecular weight. The A-stage is a rapid physical separation step and the
240 compounds detected in the A-stage supernatant are therefore very likely to be recalcitrant from raw
241 sewage. In contrast, the B-stage is a biological step and soluble microbial products are more likely
242 to be dominant in that sample.

243 Zhou et al. (2009b) investigated SMPs in the effluent of a bench scale aerobic sequencing batch
244 reactor treating distillery wastewater and found only 13 components by GC-MS whereas in this
245 study 19 were found in the B-stage supernatant; They found that alkanes and esters such as
246 heneicosane (19.8%), hexadecanoic acid, butyl ester (18.4%) and tetratetracontane (10.4%) were in
247 significant percentage of the total compounds. Alkanes such as octacosane (3.3%), hentriacontane
248 (2.4%), dotriacontane (2.4%) and acids such hexadecanoic acid, trimethylsilyl ester (1.2%) and
249 acetic acid, octadecyl ester (3.8%) were also found but in lower proportions. Alkanes were the

250 most common compounds which were found in the effluent of a SAMBR-treated solid waste
251 leachate (Trzcinski & Stuckey, 2010) and UASB effluent (Zhou et al., 2009a). These long chain
252 carbohydrates (or alkanes) and esters are frequently found in the biological treatment effluent and
253 are known to be the main components of SMP in aerobic reactors (Janga et al., 2007; Liang et al.,
254 2007). In this study, aromatic, alcohols and acids were more dominant presumably due to the more
255 complex raw wastewater and also because of the short SRT applied in the pilot plant. It is known
256 that the accumulation of SMPs becomes more pronounced at short SRTs (Liang et al., 2007) .

257

258 Overall, there was a radical shift of compounds between the A-stage and B-stage. In fact, the B-
259 stage supernatant consisted of completely different compounds, except three: flutolanil (a common
260 pesticide), triacetine and n-Nonadecanol-1, and their concentrations decreased compared to the A-
261 stage supernatant, showing that indeed some compounds could be biodegraded in the process or
262 removed through adsorption to the B-stage sludge. The new compounds in B-stage were either
263 SMPs or biodegradation end-products of residual COD in the soluble phase.

264 The B-stage supernatant contained very diverse compounds such as aromatics (21%), alcohols
265 (15%), acids (15%), alkanes (10%), alkenes (15%), aldehydes (10%), amide (5%) and ester (5%)
266 as shown in Figure 2 (bottom, right).

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268

269 **3.3 Anaerobic Biodegradability**

270 The cumulative methane production is shown in Figure 3 where it can be seen that 349 ± 1 mL
271 $\text{CH}_4/\text{g VS}$ and 238 ± 12 mL $\text{CH}_4/\text{g VS}$ were produced from the A-stage and B-stage sludges,
272 respectively, showing the greater biodegradability (+47%) of the A-stage sludge. From the COD

273 mass balance and considering the theoretical COD equivalence of 395 mL CH₄ per gram COD
274 (Speece, 1996), it was derived that 53% and 42% of the COD in A-stage and B-stage sludges were
275 converted to methane gas, respectively.

276

277 Moreover, the respective methane content in the biogas were 64% and 54% showing the higher
278 energy content of the biogas obtained from the A-stage sludge. This is consistent with the calorific
279 value given in Table 1 which confirms that the A-stage yielded sludge with a greater carbon
280 content and biodegradability potential compared to the more conventional aerated waste activated
281 sludge. This indicates the capacity of the AB system to rapidly capture the carbon from raw
282 sewage and channel it to the existing anaerobic digester to increase energy production.

283

284 **3.4 GC-MS analysis of recalcitrant compounds and SMPs after anaerobic digestion (AD)**

285

286 After anaerobic digestion (AD) tests, SMPs and recalcitrant compounds in the supernatant of the
287 digested sludges were also analyzed using GC-MS. A few peaks (identified by ** in
288 Supplementary Table S2) were also found in the inoculum used in the anaerobic biodegradability
289 test, for instance p-cresol which was detected in relatively high concentration. It was found that the
290 number of peaks decreased from 144 to 124 in the digested A-stage sludge (Figure 4 top left). This
291 shows that some compounds were anaerobically degraded to methane, CO₂ or converted to new
292 biomass while new molecules appeared as end-product of the anaerobic process or SMPs produced
293 by anaerobic metabolism. Among these 124 peaks, only 31 (or 25%) were identified and only 6
294 were in common before and after the anaerobic biodegradability test (identified by *** in
295 Supplementary Table S2). These compounds originated therefore from the raw wastewater and not

296 from the anaerobic metabolism. One of them was oleic acid and its concentration had decreased
297 from 21.14 $\mu\text{g/L}$ before AD to 1.4 $\mu\text{g/L}$ after AD. However, the concentration of some of these
298 increased through the anaerobic digestion test which could be the result of biological degradation
299 of colloids and large molecules in the sludge sample.

300

301 The number of low molecular weight compounds (with RI lower than 1200) was 3 before AD
302 (Table 2), and this increased to 10 after AD (Supplementary Table S2) showing that high
303 molecular weight compounds were hydrolyzed to low molecular weights compounds during
304 anaerobic digestion tests. The number of compounds with $\text{RI} > 3000$ (chain with more than 30
305 Carbons) was 5 before AD and 4 after AD. In both B-stage supernatants (before AD in Table S1
306 and after AD in Table S3) no compounds with $\text{RI} > 3000$ was found showing a different molecular
307 weight distribution than in A-stage.

308 It was observed that the distribution of compounds also changed with a significantly greater
309 proportion of aromatic compounds: 42% after AD versus 28% before AD. This is because
310 aromatic compounds are generally more recalcitrant and therefore represent a major fraction of
311 residual compounds after AD. All the aromatic compounds detected after AD were smaller than
312 206 Da which is different than before AD where they were found in the low (< 150 Da) and high
313 ranges of MW (> 300 Da). From the results of the A-stage sludge, it can be added that aromatic
314 biodegradation end-products and SMPs were all smaller than about 200 Da (aromatics are shown
315 with † in Supplementary S2). Alcohols and acids were secondary compounds with 16% and 10%
316 of the total number of compounds, respectively (Figure 4 top right). These proportions were 25.6%
317 and 30% in the sample before AD (Figure 2 top right).

318 In conclusion, there were fewer compounds after AD with a higher proportion of aromatics and a
319 lower proportion of acids and alcohols.

320

321 In the B-stage supernatant the total number of compounds decreased from 84 to 76 before and after
322 AD, respectively, while the number of identified peaks remained 19 (Figure 4 bottom left). When
323 comparing before and after AD, only 2 compounds (2,4,7,9-Tetramethyl-5-decyn-4,7-diol and
324 propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester) were common in
325 both samples indicating that there was a radical shift of compounds during anaerobic digestion of
326 the B-stage sludge. The number of compounds with RI lower than 1200 was 2 before AD and
327 became 10 after AD showing that hydrolysis of larger molecular weight compounds was taking
328 place during the BMP tests (Supplementary materials Tables S1 and S3).

329 The proportion of various compounds significantly changed during the AD process. The
330 percentage of aromatic compounds increased to 58 % while the percentage of alcohols and acids
331 decreased to 10% each (Figure 4 bottom right). The further stabilization in the B stage due to the
332 process configuration was confirmed with a lower number of compounds compared to the A-stage
333 supernatant (19 versus 31) and also by a higher degree of aromaticity: 58% versus 42%. This was
334 expected since the SRT is longer in the B-stage (5 days) than in the A-stage (0.5 days) and
335 retention of bacteria by the membrane in the B-stage can also contribute to a better biodegradation
336 of SMPs. The role of the A-stage is also to provide protection to the B-stage and buffer any
337 organic shock that may occur. The higher number of compounds in the A-stage compared to the B-
338 stage showed that indeed the process configuration allowed for fewer contaminants ending up in
339 the B-stage. This provides protection for the biological process in the B-stage as fewer toxic or
340 inhibitory compounds were detected.

341

342 In this study aromatics were detected in both the aerobic sludges (from A-stage and B-stage) and
343 the anaerobically digested sludges, but the degree of aromaticity was greater in the anaerobically
344 digested sludge.

345 The concentrations were typically less than 5 $\mu\text{g/L}$ which is too low to explain the residual SCOD
346 given in Table 1: 153 mg/L and 38 mg/L in the A-stage and B-stage sludge, respectively.

347 This is because the use of GC-MS is limited to the identification of non-polar, volatile and
348 thermostable compounds and many peaks in the chromatograms could not be identified.

349 Techniques such as LC-MS or Matrix Assisted Laser Desorption Ionization-Time of Flight-Mass
350 Spectrometry (MALDI-ToF-MS) would certainly shed more light on the nature of the high MW
351 compounds that were not detected and could explain the residual COD in the effluent.

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356 **4. Conclusions**

357 This study showed that the supernatant of both A-stage and B-stages sludges contained aromatics
358 (27.9% and 21.1% of identified compounds), long chain alkanes (7% and 10.5%), alcohols (25.6%
359 and 15.8%), acids (30.2% and 15.8%) and esters (2.3% and 5.3%). More methane could be
360 produced from the A-stage sludge (349 ± 1 mL $\text{CH}_4/\text{g VS}$) compared to the B-stage sludge (238 ± 12
361 mL $\text{CH}_4/\text{g VS}$). After anaerobic digestion of these sludges, the total number of compounds
362 detected by GC-MS was lower, and there was a greater proportion of aromatic compounds (42%
363 and 58%).

364

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368

369 **6. References**

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372 APHA. 1995. *Standard Methods for the Examination of Water and Wastewater. 19th ed. ed.*

373 American Public Health Association, Washington, DC.

374 Boehnke, B., Diering, B., Zuckut, S.W. 1997. Cost-effective wastewater treatment process for

375 removal of organics and nutrients. *Water Engineering & Management*, **144**(7), 18-21.

376 Böhnke, B. 1977. Das Absorptions-Belebungs-Verfahren. *Korrespondenz Abwasser*, **24**(77), 33-42.

377 Canales, A., Pareilleux, R.J.L., Goma, G., Huyard, A. 1994. Decreased sludge production strategy

378 for domestic wastewater treatment. *Water Science and Technology*, **30**, 97-106.

379 Chai, C., Zhang, D., Yu, Y., Feng, Y., Wong, M.S. 2015. Carbon Footprint Analyses of

380 Mainstream Wastewater Treatment Technologies under Different Sludge Treatment

381 Scenarios in China. *Water* **7**, 918-938.

382 Ersahin, M.E., Ozgun, H., Dereli, R.K., Ozturk, I., Roest, K., van Lier, J.B. 2012. A review on

383 dynamic membrane filtration: Materials, applications and future perspectives. *Bioresource*

384 *Technology*, **122**, 196-206.

385 Ge, H., Batstone, D.J., Keller, J. 2013. Operating aerobic wastewater treatment at very short sludge
386 ages enables treatment and energy recovery through anaerobic sludge digestion. *Water*
387 *Research*, **47**, 6546-6557.

388 Janga, N., Ren, X., Kim, G., Ahn, C., Cho, J., Kim, I.S. 2007. Characteristics of soluble microbial
389 products and extracellular polymeric substances in the membrane bioreactor for water reuse.
390 *Desalination*, **202**, 90-98.

391 Liang, S., Liu, C., Song, L. 2007. Soluble microbial products in membrane bioreactor operation:
392 behaviors, characteristics, and fouling potential. *Water Research*, **41**, 95-101.

393 Meerburg, F.A., Boon, N., Van Winckel, T., Vercamer, J.A.R., Nopens, I., Vlaeminck, S.E. 2015.
394 Toward energy-neutral wastewater treatment: A high-rate contact stabilization process to
395 maximally recover sewage organics. *Bioresource Technology*, **179**, 373-381.

396 Mei, X., Wang, Z., Zheng, X., Huang, F., Ma, J., Tang, J., Wu, Z. 2014. Soluble microbial
397 products in membrane bioreactors in the presence of ZnO nanoparticles. *Journal of*
398 *Membrane Science*, **451**, 169-176.

399 Owen, W.F., Stuckey, D.C., Healy, J.B., Jr., Young, L.Y., McCarty, P.L. 1979. Bioassay for
400 monitoring MONITORING BIOCHEMICAL METHANE POTENTIAL AND
401 ANAEROBIC TOXICITY. *Water Research*, **13**, 485-492.

402 Qing, P.N. 2015. Integrated Validation Plant: Modelling and optimisation of the biological
403 treatment process. in: *Department of Civil & Environmental Engineering, Faculty of*
404 *Engineering*, Vol. Master of Science in Civil Engineering, National University of
405 Singapore. Singapore.

406 Roest, K., Daamen, B., de Graaff, M.S., Hartog, L., Zandvoort, M.H., Uijterlinde, C.A., Dilven, S.,
407 van Lier, J.B., van Loosdrecht, M.C.M. 2012. Energy production from wastewater -

408 Dynamic filtration of A-stage sludge. *IWA World Congress on Water, Climate and Energy*,
409 13 – 18 May 2012, Dublin, Ireland.

410 Shen, Y.-x., Xiao, K., Liang, P., Sun, J.-y., Sai, S.-j., Huang, X. 2012. Characterization of soluble
411 microbial products in 10 large-scale membrane bioreactors for municipal wastewater
412 treatment in China. *Journal of Membrane Science*, **415–416**, 336-345.

413 Shizas, I., Bagley, D.M. 2004. Experimental Determination of Energy Content of Unknown
414 Organics in Municipal Wastewater Streams. *Journal of Energy Engineering*, **130(2)**, 45-53.

415 Speece, R. 1996. *Anaerobic Biotechnology for Industrial Wastewaters*. Archae Press, Nashville,
416 Tennessee, U.S.A. .

417 Tayà, C., Guerrero, J., Suárez-Ojeda, M.E., Guisasola, A., Baeza, J.A. 2015. Assessment of crude
418 glycerol for Enhanced Biological Phosphorus Removal: Stability and role of long chain
419 fatty acids. *Chemosphere*, **141**, 50-56.

420 Tian, X., Trzcinski, A., Chong, W., Lin, L., Ng, W.J. 2014. Insights on the solubilization products
421 after combined alkaline and ultrasonic pre-treatment of sewage sludge. . *Journal of*
422 *Environmental Sciences*, DOI : **10.1016/j.jes.2014.07.024**.

423 Trzcinski, A.P., Stuckey, D.C. 2010. Treatment of municipal solid waste leachate using a
424 submerged anaerobic membrane bioreactor at mesophilic and psychrophilic temperatures:
425 Analysis of recalcitrants in the permeate using GC-MS. *Water Research*, **44(3)**, 671-680.

426 Versprille, A.I., Zuurveen, B., Stein, T. 1984. THE A-B PROCESS: A NOVEL TWO STAGE
427 WASTEWATER TREATMENT SYSTEM. *Water Science and Technology*, **17**, 235- 246.

428 Verstraete, W., de Caveye, P.V., Diamantis, V. 2009. Maximum use of resources present in
429 domestic “used water”. *Bioresource Technology*, **100(23)**, 5537–5545.

- 430 Verstraete, W., Vlaeminck, S.E. 2011. ZeroWasteWater: short-cycling of wastewater resources for
431 sustainable cities of the future. *International Journal of Sustainable Development & World
432 Ecology*, **18**(3), 253-264.
- 433 Wu, B., Zhou, W. 2010. Investigation of soluble microbial products in anaerobic wastewater
434 treatment effluents. *Chem. Technol. Biotechnol.*, **85**(12), 1597-1603.
- 435 Zhou, W.L., Wu, B.T., She, Q.H., Chi, L., Zhang, Z.J. 2009a. Investigation of soluble microbial
436 products in a full-scale UASB reactor running at low organic loading rate. *Bioresource
437 Technology*, **100**(14), 3471-3476.
- 438 Zhou, W.L., Wu, B.T., She, Q.H., Chi, L., Zhang, Z.J. 2009b. Investigation of soluble microbial
439 products in a full-scale UASB reactor running at low organic loading rate. *Bioresour.
440 Technol*, **100**(14), 3471-3476.

441