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1 **Increased carboxylate production in high-rate activated A-sludge by**
2 **forward osmosis thickening**

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22 **Abstract**

23 Domestic wastewater represents a considerable feedstock for organics but the high dilution makes their
24 recovery typically unsuccessful. Here we investigated three routes to 10-fold concentrate the organics
25 using Forward Osmosis (FO) (Draw solution (DS) 2.2 M MgCl_2): directly on domestic wastewater, A-
26 sludge, or secondary sludge, with the end goal of increasing volatile fatty acid (VFA) yield from
27 subsequent 9-day fermentation tests. Forward osmosis concentrated the total COD by a factor of $8.2 \pm$
28 1.2 , 10.1 ± 2.4 and 4.8 ± 0.2 with respect to the raw streams of wastewater, secondary sludge and A-
29 sludge. The soluble fraction of the COD was concentrated up to 3.5 times in the A-sludge and 2.1 times
30 in the secondary sludge; the result of a combined effect of the chemical action of Mg^{2+} (diffused from
31 the DS) on sludge disaggregation and cell lysis, and the physical action of recirculation and air-scouring
32 of the A-sludge in the FO-unit.

33 The FO-concentrated A-sludge produced 445 ± 22 mg COD-VFA g^{-1} COD_{fed} , which was 4.4 times
34 higher than for the untreated A-sludge. No VFA were produced from untreated secondary sludge, but
35 after FO-concentration 71 ± 5 mg COD-VFA g^{-1} COD_{fed} could be reached. Due to the low organics in
36 wastewater even after FO-concentration (1.08 ± 0.08 g COD L^{-1}), no notable VFA production occurred.
37 The combination of A-stage technology and membrane technology for dewatering and COD
38 concentration could be a key advancement to increase VFA production from domestic wastewater,
39 whereby at least 45% of the COD can be recovered as valuable VFA.

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42 **Keywords:** *Carboxylate fermentation; Forward osmosis (FO); AB system; Volatile fatty acids (VFA);*
43 *Waste activated sludge (WAS).*

44 1. Introduction

45 The global domestic wastewater production is estimated at 330 billion m³ annually [1]. Significant
46 research efforts are focused on the recovery of water and nutrients [2] from this waste stream and
47 particularly in the conversion of the organic fraction into energy as biogas. Recently, an alternative route
48 was developed whereby these organics are converted into volatile fatty acids (VFA), which are building
49 blocks for a multitude of valuable products such as biopolymers [3], medium or long chain fatty acids
50 [4] and biofuels [5]. However, the generally low organic content of domestic wastewater (< 600 mg L⁻¹)
51 hampers efficient recovery, and is one of the main limitations in developing feasible bioproduction
52 platforms [6].

53 An evident means to concentrate organics is to work via sludge, generated from the wastewater as
54 substrate and separated via settling. The conventional activated sludge (CAS) process is typically
55 performed with high aeration energy, and at solid retention time (SRT) of 8 - 20 days, resulting in a very
56 efficient organic carbon removal, but yielding a sludge with poor digestion efficiency due to the high
57 sludge age and high sludge oxidation [7, 8].

58 An attractive alternative approach is the “Adsorption-Biooxidation” (AB) process [9]. The AB system is
59 a two-stage treatment system where the first stage (A-stage) is typically operated at low hydraulic
60 retention time (HRT 15 - 30 min) and low SRT (between 3 hours and 1 day). The soluble and colloidal
61 organic matter is removed in the A-stage through bioflocculation into microbial A-sludge. The A-sludge
62 is also separated in a clarifier, while the effluent is then taken to the subsequent B-stage to ensure
63 polishing of the wastewater [2, 9]. A-sludge is usually digested to biogas with high digestion efficiency
64 (higher than that of CAS) due to the remarkably low sludge age and high biodegradability [9, 10].

65 However, due to the poor settling performance of the A-sludge, the COD content is still too diluted to
66 ensure high VFA production [6, 11]. A-stage is operated at 2 to 10 kg BOD kg⁻¹ VSS d⁻¹ [9] while
67 optimum settling is obtained between 0.3 and 0.6 kg BOD kg⁻¹ VSS d⁻¹ [12]. CAS systems are typically
68 operated at 0.25 kg BOD kg⁻¹ VSS d⁻¹ [13], achieving good sludge settling but with a relatively high
69 water content. Thus, a further concentration of both A-sludge and secondary sludge is needed to ensure

70 sufficiently high VFA production for extraction ($> 5 \text{ g L}^{-1}$) and further valorization into high value
71 products [14, 15].

72 Forward osmosis (FO) is a potentially energy-efficient and low-cost technique for concentration of
73 challenging feed streams such as waste activated sludge (WAS) [16, 17] and domestic wastewater [18].
74 FO is based on the spontaneous process of osmosis, in which water is transported through a semi-
75 permeable membrane from a feed solution (FS) of relatively low solute concentration (low osmotic
76 pressure) to a draw solution (DS) of relatively high solute concentration (high osmotic pressure). The
77 membrane allows for water transport but displays a high rejection of solutes [19]. Draw solutes, such as
78 MgCl_2 , need to have a low molecular weight, a high diffusivity, produce high osmotic pressures, are
79 well-retained by the FO membrane, are non-toxic and cheap [20]. Concentrating any stream using FO
80 causes an increase in its dissolved solutes content, due to a build-up of rejected solutes from the feed
81 itself as well as the diffusion of solutes from the draw solution into the feed. The latter is referred to as
82 reverse salt diffusion (RSD).

83 Here, we applied FO to concentrate the organics of domestic wastewater, high-rate activated A-sludge
84 and secondary sludge to undergo a subsequent fermentation for VFA production purposes. FO
85 dewatering fluxes of the different streams were compared. MgCl_2 was chosen as draw solute because of
86 the high osmotic pressure generated by these solutions combined with the lower RSD compared to
87 monovalent salts such as NaCl [19]. VFA yields and composition of the concentrated streams were
88 measured and compared to the non-concentrated streams to assess the effect of concentration by FO.
89 The effect of salt leakage during FO was investigated by carrying out fermentation experiments with
90 and without addition of MgCl_2 .

91 2 Materials and Methods

92 2.1 Substrate collection

93 A-sludge was collected from the return flux to the A-tank in Nieuwveer WWTP (Breda, The
94 Netherlands). Domestic secondary sludge and domestic wastewater were collected from Destelbergen
95 WWTP (Destelbergen, Belgium). Characteristics of the substrates are summarized in Table 1. Mixed
96 liquors as collected were used as FO feed. Part of a second batch of A-sludge (7.76 ± 0.16 gCOD L⁻¹,
97 4.41 ± 0.14 gVSS L⁻¹) was stored at 4°C and gravity-concentrated (removing the liquid volume above
98 the settled sludge – resulting in a concentration of 3.8 times) to carry out fermentation tests to study the
99 effects of RSD.

100 2.2 Aerobic and Anaerobic Forward osmosis (FO) experiments

101 A schematic overview of the setup is given in Figure S1 (Supplementary information (SI)) and a
102 description of the set-up, cross-flow velocity, flow measurements and analysis are provided in the
103 methodology section of SI.

104 The FO membranes used in this study (supported in a membrane module, Figure S1), were commercial
105 thin film composite (TFC) membranes produced by Hydration Technology Innovations (HTI) (Albany,
106 Oregon, USA) [21]. The membrane orientation in this study was AL-FS (active layer facing the feed
107 solution). For each test, a new membrane coupon of 14 cm x 4 cm (56 cm²) was used. 10-fold
108 concentration of the FO feeds was done by recirculating a fixed volume of feed in batch mode.
109 Experiments were carried out in triplicate.

110 The DS was maintained at 2.2 M MgCl₂ by recirculating it over a bed of solid MgCl₂ when necessary
111 using a PID control based on conductivity measurements. Aeration (0.6 L min⁻¹) was applied to the FS
112 just before entering into the membrane cell to increase scouring and to reduce membrane fouling.

113 An anaerobic FO set-up was built to investigate the potential effect of microbial respiration during FO
114 treatment. In order to keep the feed compartment anaerobic and to mitigate concentration polarization
115 and fouling, an N₂/CO₂ (9:1 ratio) gas mixture was recirculated in the feed compartment (0.6 L min⁻¹).

116 **2.3 Fermentation experimental procedure**

117 Fermentation batch tests were carried out in triplicate with fermentative inoculum as described in SI.
118 The 3 feed substrates were fermented before and after FO-concentration. A-sludge was also fermented
119 after anaerobic FO-concentration. To avoid overloading of the fermentation tests by organics, different
120 substrate quantities were tested according each feed concentration as defined in Table S1.

121 To assess the reasons for changes in VFA production before and after FO-concentration, extra
122 experiments were carried out: the effect of sludge concentration, aeration during FO-concentration, RSD
123 or a combination of these effects was evaluated towards changes in soluble COD in the substrate and
124 VFA production. To this extent, the raw A-sludge and the gravity-concentrated A-sludge (obtained
125 removing the liquid volume above the sludge settled over night at 4 °C) were subjected to three different
126 types of treatments:

- 127 i) Addition of 7.1 g L⁻¹ of MgCl₂ (same concentration as in the FO-concentrate A-sludge
128 (Table 2));
- 129 ii) Addition of 28.4 g L⁻¹ of MgCl₂ (salt overdose to evaluate the effect of build-up salinity
130 and conductivity in acidogenesis)
- 131 iii) Air bubbling (flow 0.6 L min⁻¹, the same used during the FO);
- 132 iv) A combination of conditions i) or ii) with condition iii).

133 All conditions were applied for 37.3 hours (same time needed to FO-concentrate the A-sludge 10 times
134 (Table 2)). Fermentation tests were performed in each of the pre-treated substrates as indicated in Table
135 S1. The potential impact of RSD on cell lysis was determined by flow cytometry with live/dead staining
136 as described in SI.

137 All the analyses carried out during fermentation tests were performed as described in the SI.

138 **2.4. Statistical analysis**

139 Statistical significance between intact cell densities, cell proportions and VFA concentrations for the
140 different experiments were determined by generalized mixed model regression of the cell densities with

141 the experimental condition (e.g., GC-ASM, etc.) as a categorical predictor. A random intercept effect
142 was incorporated for each replicate analysis. All models were first checked for normal distributed
143 residuals (Shapiro Wilks test and QQ-plots) and homogenous variance (Levene's test). Parameters were
144 estimated by the maximum likelihood method. Posthoc analysis was performed in case of a significant
145 treatment effect ($p < 0.05$) by Tukey's all pair comparison method. Pair-wise comparisons were
146 considered significant for $p < 0.01$.

147 **3. Results and Discussion**

148 **3.1 Concentration of sewage and sludge by FO**

149 **3.1.1 Dewatering performance/flux behavior in FO**

150 The goal of the FO treatment was to concentrate the different substrates and produce a concentrate rich
151 in organic carbon for fermentative VFA production. To this end, A-sludge, secondary waste activated
152 sludge (WAS) and domestic wastewater were concentrated 10-fold by FO in batch mode.

153 During FO concentration, the water flux across the membrane decreases over time due to fouling and
154 increased salinity build-up on the concentrate side. The flux profile obtained for the different feed
155 solutions is shown in Figure 1. Repeated experiments for the respective feed solutions ($n = 3$) yielded
156 similar flux behavior (Figure S2). Despite the relatively similar conductivities of the different substrates,
157 the initial fluxes, defined as the average flux during the first 2 hours of FO concentration, were $11.4 \pm$
158 0.2 LMH ($L\ m^2\ h^{-1}$) for secondary sludge, 8.0 ± 0.7 LMH for A-sludge and 9.6 ± 0.4 LMH for
159 wastewater, respectively. We hypothesize that the different initial fluxes between the different streams
160 can be explained by the feed characteristics in terms of different solids (total solids (TS), volatile solids
161 (VS) and suspended solids (SS)) and organic content (soluble COD (sCOD) and total COD (tCOD))
162 (Table 1). Secondary sludge yielded the highest initial flux, approaching the DI water baseline. This was
163 consistent with an earlier study from Cornelissen et al. [16] that found similar flux values for secondary
164 activated sludge and DI water during 7 to 8 hour experiments (5.1 - 6.2 LMH for the DI water and 5.8
165 LMH for the activated sludge, respectively by using a TFC FO-membrane and 0.5 M NaCl draw
166 solution). Secondary sludge had a low content of soluble or colloidal VS (calculated as difference
167 between VS and VSS) compared to A-sludge. A-sludge contained the highest amounts of TS, VS and

168 SS of all feeds. Moreover, the sCOD content of A-sludge, and the sCOD/tCOD ratio ($1.25 \pm 0.02 \text{ g L}^{-1}$
169 and 15 %, respectively) were higher than that of secondary sludge ($0.22 \pm 0.01 \text{ g L}^{-1}$ and 5 %,
170 respectively) (Table 1). Thus, we hypothesize that a combination of a fast formation of a dense fouling-
171 layer on the membrane, combined with cake-enhanced concentration polarization caused the initial
172 lower flux during A-sludge FO-dewatering. The latter is due to the dominance of small, soluble/colloidal
173 organic compounds which potentially impart extra osmotic pressure in A-sludge, caused the initial lower
174 flux of A-sludge during FO-dewatering. In the case of secondary sludge, the sludge flocs could aid in
175 scouring the membrane surface and in sequestering the soluble organics, thus leading to less fouling (or
176 potentially a cake with less hydraulic resistance). Wastewater had the lowest VS/TS ratio (38 %) but the
177 highest sCOD fraction of tCOD (25 %) (Table 1). Similar conditions were found by Lutchmiah et al.
178 [18] who reported a 20% reduction of the initial dewatering flux from DI water to wastewater. It is likely
179 that the dominance of small, soluble organic compounds in wastewater caused the rapid formation of a
180 gel layer on the membrane and a higher osmotic pressure in the proximity of the membrane, resulting
181 in a lower initial flux compared with that of secondary sludge.

182 For both A-sludge and secondary sludge (containing higher solids concentration), the initial flux
183 remained stable for the first 5 hours of filtration, after which the flux declined linearly for all repetitions
184 (Figure S2). Flux decline rate for the secondary sludge ($0.302 \pm 0.005 \text{ LMH h}^{-1}$) was twice that of the
185 A-sludge ($0.114 \pm 0.029 \text{ LMH h}^{-1}$), which might be related to the higher initial flux of the secondary
186 sludge. It was visually observed that the secondary sludge thickened to a highly viscous mixture by the
187 end of the FO concentration. This caused the formation of a thick cake on the membrane, which inhibited
188 further sludge dewatering depicted by a profound flux decline in Figure 1. The A-sludge did not become
189 viscous and the final flux decline was less pronounced, but FO-dewatering lasted longer than that for
190 the secondary sludge to reach the same 10-fold concentration (28.1, 29.7 and 37.3 hours for secondary
191 sludge, wastewater and A-sludge, respectively (Table 2)) due to the lower average flux. Consistently
192 with what reported in literature [18], only limited flux decline occurred during wastewater concentration.
193 Most likely, the relatively low fouling propensity of the wastewater was related to the low total
194 concentration of organics in the wastewater (Table 1) and the low concentration of suspended solids.

195 3.1.2 Concentration of inorganic/organic compounds by FO

196 The concentration ratio of the organics during FO was determined as FO-concentrated COD to feed
197 COD. As a 10-fold volume reduction was reached, the same theoretical organics concentration ratio was
198 expected, assuming that no organics are transported through or adsorbed onto the membrane, converted
199 into biomass or mineralized. The COD concentration ratio was only 8.2 ± 1.2 for the domestic
200 wastewater and 4.8 ± 0.2 for the A-sludge, but 10.1 ± 2.4 for the secondary sludge. This indicates a
201 COD loss of 18 % for the domestic wastewater, 52 % for the A-sludge and no loss for the secondary
202 sludge. The high organic loss for the A-sludge might be due to biological degradation, given its high
203 biodegradability, low sludge age and high initial organic content [9, 22, 23] and the constant aeration
204 applied for membrane scouring. Its dewatering also took the longest, allowing more time for organic
205 loss through biological activity. Tests to demonstrate this are reported in the next section. Secondary
206 sludge contains less biodegradable organics as most of the SS and organics from sewages are removed
207 during upstream primary treatment [7] and due to the extent oxidation and high SRT of the secondary
208 treatment (CAS) itself. Hence, no detectable COD loss could be observed during secondary sludge FO
209 concentration. Wastewater showed a modest COD loss. The organic matter in wastewater is considered
210 relatively easy to degrade, as it is soluble and has not yet been incorporated in microbial biomass,
211 however, wastewater typically does not contain an enriched population of organisms able to degrade
212 organics, which are present in sludge [7, 9, 24].

213 FO concentration in general caused an increase in the dissolved compounds content of the feed streams,
214 which is caused both by concentrating the initial feed solutes in a smaller volume and by diffusion of
215 draw solutes into the feed. This can be seen in Table 2, where both conductivity and Mg^{2+} content of the
216 feed streams are shown. The conductivity of the feed streams increased from about 1.5 to about 30 mS
217 cm^{-1} in the case of wastewater and A-sludge, and to about 20 mS cm^{-1} in the case of secondary sludge.
218 The measured conductivity of the concentrates does not relate well to the $MgCl_2$ concentration measured
219 on filtered samples by ion chromatography (IC). Apparently, the different types of flocs in the different
220 types of sludge appeared to be specifically adsorbing different amounts of Mg^{2+} . This became clear by
221 freezing and thawing concentrated secondary sludge, which caused the conductivity to increase from

222 18.2 to 51.3 mS cm⁻¹ after thawing, indicating that MgCl₂ was sequestered by EPS or intracellular
223 uptake, which was subsequently released by lysis of the sludge structure when freezing-thawing. As a
224 result, no clear correlations can be seen between flux decline and measured Mg²⁺ concentrations.

225 *3.2 Identifying organic losses during FO concentration*

226 During FO concentration of A-sludge, half of the incoming COD was lost, almost twice as much as in
227 domestic wastewater. To elucidate if this loss was caused by bacterial respiration, 10-fold FO
228 concentration under anaerobic conditions was targeted with the A-sludge. Considerably lower FO flux
229 in anaerobic conditions caused that the concentration factor of 10 was not reached. The initial flux was
230 about 7.5 LMH (Figure S3), similar to the initial flux during aerobic A-sludge concentration (8.0 LMH
231 (Figure 1)). Flux declined steadily down to 0.5 - 1 LMH (after 30 hours) and then remained constant for
232 the rest of the FO treatment (Figure S3). After 95.4 hours, a concentration factor of only 3.9 was reached,
233 and the test was aborted in order to limit the salinity build-up in the feed due to RSD. In contrast to
234 aerobic FO experiments, the COD content increased from 5.5 ± 0.5 to 21.4 ± 1.7 g L⁻¹, equaling a 3.9 ±
235 0.1 -fold increase, equal to the concentration factor obtained. Chen et al. [25] studied the treatment of
236 low-strength wastewater using an anaerobic osmotic membrane bioreactors, reporting a flux decline to
237 3.5 LMH. The stable flux obtained during anaerobic FO concentration in this study is lower of that
238 obtained in literature [29], due to a higher initial COD of the A-sludge (8.40 gCOD L⁻¹ and 0.46 gCOD
239 L⁻¹, respectively).

240 The large extent of flux decline, starting already after few hours of anaerobic FO-concentration (Figure
241 S3), was most likely due to a major formation of gel-like substances (and deposition of these substances
242 on the membranes surface) by the high concentration of organics retained in the feed compartment,
243 which prevented dewatering. For concentration of A-sludge, especially under anaerobic conditions, it is
244 thus clear that a continuous scouring of the membrane, using alternative techniques in combination with
245 N₂:CO₂ (9:1) gas bubbling, is needed to prevent flux decline. However, a continuous flow of oxygen
246 free gases would increase the operational cost of the system, and just using air (as in the aerobic FO-
247 dewatering) will result in COD-loss. Alternative scouring systems, such as (osmotic) backwashing,
248 relaxation or periodic air bubbling should be considered.

249 **3.3 Effect of FO-concentration on fermentation**

250 *3.3.1 Fermentation yields of the different streams*

251 High initial organic matter content is needed to obtain high VFA production [6]. Fermentation batch
252 tests were performed on all the streams (domestic wastewater, secondary sludge and A-sludge) as well
253 as their FO-concentrates to assess if the application of an FO system after a CAS, an A-stage or directly
254 to the domestic wastewater could improve VFA production and yields. During fermentation tests,
255 maximum VFA production was obtained between day 4 and 7 followed by a decrease in product
256 concentrations due to conversion of VFA to methane.

257 No notable VFA production occurred for domestic wastewater, either before or after the FO
258 concentration. To maximize the available organics for VFA production from the domestic wastewater,
259 the organic load during fermentation was increased by 6 times compared to the initial test (Table S1),
260 but again no notable VFA production occurred (Figure 2). The tCOD after FO-concentration of the
261 domestic wastewater was $1.08 \pm 0.08 \text{ g L}^{-1}$ and the sCOD was $0.24 \pm 0.01 \text{ g L}^{-1}$. This concentration was
262 insufficient to sustain the growth of the microorganisms and, to a lesser extent, allow substantial
263 production of VFA or CH_4 . Higher concentrations still might be needed in order to produce VFA from
264 FO-concentrated domestic wastewater, however this would further increase contact time in the FO,
265 leading to more draw solutes leaking and increased conductivity. Such highly conductive feedstocks
266 could be used for VFA accumulation as conductivity above 30 mS cm^{-1} negatively affects
267 methanogenesis while acetogenic bacteria still produce VFA at high salt concentrations De Vrieze et al.
268 [26]. However, an economic assessment would be required as draw solute leakage comes at a cost. It is
269 unknown whether other factors, such as accumulation of toxic compounds, could also play a role in the
270 observed lack of VFA production.

271 Fermentation of the FO-concentrated secondary sludge led to a production of $71 \pm 5 \text{ mg COD-VFA g}$
272 $^1\text{COD}_{\text{fed}}$ after 7 days, corresponding to 7 % of COD converted into VFA. No notable VFA production
273 occurred for the raw secondary sludge. The digestion efficiency of the secondary sludge and the VFA
274 yields produced during fermentation are low compared to substrates typically used in fermentation. This

275 is mainly due to the high sludge age and the extent of oxidation [6, 8] of the secondary sludge, even
276 when the concentration is increased in the FO treatment.

277 For the FO-concentrated A-sludge, a VFA production of 346 ± 30 mg COD-VFA $g^{-1}COD_{fed}$ was reached
278 already after 1 day of fermentation (Figure S4), with a production up to 445 ± 22 mg COD-VFA $g^{-1}COD_{fed}$
279 after 4 days (Figure 2, Figure S4). For the raw A-sludge, the VFA production after 4 days of
280 fermentation was only 102 ± 3 mg COD-VFA $g^{-1}COD_{fed}$, which is 4.4 times lower ($p < 0.01$) than that
281 for the FO-concentrated A-sludge (Figure 2). This shows that FO-concentration increases fermentability
282 of A-sludge as higher VFA is produced compared to the same quantity of organics fed. Fermentation
283 was also performed with the anaerobic FO-concentrated A-sludge. VFA production already reached its
284 maximum after 2 days of fermentation with 444 ± 8 mg COD-VFA $g^{-1}COD_{fed}$ (Figure 2) (significantly
285 higher than A-sludge itself with $p < 0.01$). This fast start-up of VFA formation might be attributed to
286 hydrolysis of the sludge already starting during the 95.4 hours of anaerobic FO treatment.

287 *3.3.2 Factors affecting improvement in fermentation capacity*

288 FO-concentration of the two sludge types increased their overall COD as well as the sCOD concentration
289 (Table 3). For secondary sludge the increase corresponded to 10.1 ± 1.0 % sCOD/tCOD while for A-
290 sludge, this rose up to 30.4 ± 1.9 % sCOD/tCOD. Two main reasons why FO-concentration increases
291 the sCOD fraction might be a sort of physical pretreatment by the recirculation through the peristaltic
292 pump (for 37.3/95.4 hours), as well as the shear force of the continuous air bubbling in the feed
293 compartment. Because of this improvement on sCOD fraction and overall increased organics content,
294 specific VFA production improves after FO-concentration of sludge.

295 During FO, water molecules follow the osmotic gradient from the FS to the DS; likewise the ions
296 contained in the DS can migrate to the FS. One of the hypotheses we put forward in this manuscript, is
297 that the draw solute used for the dewatering might also have an impact on fermentation through
298 improved hydrolysis. A high concentration of cations such as Mg^{2+} (Table 2) facilitates the formation
299 of single-cells which are more sensitive to lysis by spontaneous disaggregation from the floc structure
300 [27] (hypothesis further evaluated in the next sections). Furthermore, several ions can also interact with
301 the negative charges of the EPS (extracellular polymeric substances) surrounding the bacterial cells,
302 breaking the bounds among these polymers and favoring cell lysis and release of organic matter [28].

303 Finally, an increased concentration of the sludge can enhance the hydrolysis due to overload shock [29].
304 The increased organic loading can also lead to the accumulation of carboxylates, which are crucial in
305 inhibiting methanogenesis, increasing the overall VFA accumulation [30].

306 3.3.3 Fermentation product speciation linked to stream composition

307 A different VFA speciation was found for the different streams and also for the same stream before and
308 after the FO-concentration (Figure 3). In case of the A-sludge before FO, acetate was the most abundant
309 VFA formed after 4 days of fermentation ($60 \pm 3 \%$) besides $26 \pm 3 \%$ propionate and $9 \pm 0 \%$ isovalerate.
310 In the FO-concentrated A-sludge, the proportion of acetate dropped to $24 \pm 4 \%$ after 4 days of
311 fermentation, with propionate increasing to $43 \pm 3 \%$. Moreover, a larger proportion of longer chain
312 fatty acids was produced after 4 days of fermentation for the FO-concentrated A-sludge compared to the
313 non-concentrated sludge, $4 \pm 0 \%$ isobutyrate, $7 \pm 0 \%$ butyrate, $8 \pm 0 \%$ isovalerate and $8 \pm 0 \%$ valerate
314 which shows an impact of higher loading.

315 Similar VFA speciation was found for the anaerobically FO-concentrated A-sludge, with $31 \pm 0 \%$
316 acetate and $35 \pm 0 \%$ propionate present after 2 days of fermentation. The proportion of longer chain
317 fatty acids also increased with $6 \pm 0 \%$ isobutyrate, $10 \pm 0 \%$ butyrate, $11 \pm 0 \%$ isovalerate and $5 \pm 0 \%$
318 valerate, for anaerobic FO-concentration of A-sludge, confirming again that the application of FO may
319 lead to the accumulation of longer chain fatty acids again due to increased loading. For the FO-
320 concentrated secondary sludge, acetate and propionate were the main VFA formed, at $46 \pm 3 \%$ and 18
321 $\pm 0 \%$ respectively, which is similar to the raw A-sludge with approximate sCOD fractions of that of
322 secondary sludge ($14.9 \pm 0.7 \%$ and $10.1 \pm 1.0 \%$ sCOD/tCOD, respectively (Table 3)). FO-concentrated
323 secondary sludge fermentation also led to the production of longer chain fatty acids, at $6 \pm 0 \%$
324 isobutyrate, $7 \pm 0 \%$ butyrate and $13 \pm 0 \%$ valerate after 4 days of fermentation (Figure 3).

325 Production of CH_4 at day 4 was relatively low for the FO-concentrated streams with $7 \pm 2 \text{ mg COD-}$
326 $\text{CH}_4 \text{ g}^{-1} \text{COD}_{\text{fed}}$ (9% initial COD) for secondary sludge and $29 \pm 3 \text{ mg COD-CH}_4 \text{ g}^{-1} \text{COD}_{\text{fed}}$ (5% of initial
327 COD) for the A-sludge, while $6 \pm 1 \text{ mg COD-CH}_4 \text{ g}^{-1} \text{COD}_{\text{fed}}$ (6% of initial COD) was obtained with
328 the raw A-sludge (Figure 3). The use of an FO-system to concentrate A-sludge and secondary sludge
329 thus seems to promote the production of more VFA with a higher proportion of longer chain fatty acids

330 (C₄-C₅), but not improving the conversion of VFA into methane. The increased organic loading together
331 with the increased conductivity reduces the acetoclastic activity of methanogens and thus leads to higher
332 VFA production and accumulation [28, 30, 31].

333 *3.4 Effect of FO operational conditions on fermentation*

334 FO-concentration of both secondary sludge and A-sludge increased VFA yields, from 0 to 7 % and 10
335 to 45 %, respectively. To understand whether the main reason for this was 1) the increased organics
336 concentration due to dewatering, 2) the enhanced hydrolysis of the organics by RSD, 3) enhanced
337 breakdown of the cells due to the air scouring, or 4) any combination of these phenomena, 9-days
338 fermentation batch tests were performed as described in section 2.3. The effect of the increase in
339 organics concentration was investigated by gravity-concentrating the sludge; the potential enhanced
340 hydrolysis by RSD was investigated by adding a 7.1 g L⁻¹ MgCl₂ (same concentration in the A-sludge
341 after aerobic FO-concentration) or 28.4 g L⁻¹ MgCl₂ (concentration needed in order to reach the same
342 conductivity after anaerobic FO-concentration of the A-sludge); and the potential breakdown of cells
343 due to air scouring was investigated by aerating at 0.6 L min⁻¹. All treatments were carried out for 37.3
344 hours, same time needed to reach a 10-fold by FO-concentration of A-sludge. Results are depicted in
345 Figure 4.

346 For the non-concentrated A-sludge, addition of MgCl₂ increased the sCOD/tCOD ratio from 19.0 % to
347 25.0 % (with low salt) and to 27.5 % (with high salt), respectively (Table 3). However, production of
348 VFA staid constant at 265 ± 3 mg COD–VFA g COD_{fed} with low salt addition and only 177 ± 5 mg
349 COD–VFA g COD_{fed} were produced by increasing the MgCl₂ up to 28.4 g L⁻¹ (Figure 4). For all the
350 other conditions tried VFA production was lower than that of untreated A-sludge. Thus, for the non-
351 concentrated A-sludge, neither MgCl₂ addition nor aeration nor the combination of these could explain
352 the increased VFA production resulting from the FO-concentration.

353 Gravity concentration of the A-sludge did not improve the VFA yield. Only 196 ± 13 mg COD -VFA g
354 COD_{fed} (corresponding to 20 % yield) were produced by fermenting gravity-concentrated (GC) A-
355 sludge, significantly lower (p < 0.01) than the yield obtained from non-concentrated A-sludge (around
356 26 %). This might be caused as gravity-concentrating the A-sludge 3.8 times was carried by removing

357 the supernatant rich in sCOD after settling (Table 3), but consequently the sCOD/tCOD ratio was
358 reduced from 19.0 to 13.1 % (Table 3). This is due to the fact that after gravity concentration the
359 wastewater rich in biodegradable organics (sCOD/tCOD 26.7 % (Table 3)) was removed and only the
360 sludge settled at the bottom, which is more difficult to hydrolyze, was used for the fermentation. Adding
361 MgCl_2 to the GC-A-sludge increased the sCOD/tCOD ratio somewhat from 13.1 % to 16.1 % (Table 3)
362 resulting in a VFA yield of 248 ± 7 mg COD -VFA g COD_{fed} (25 %), significantly higher ($p < 0.01$)
363 than that of GC-A-sludge (20 %) (Figure 4). This indicates that bringing the concentrated sludge in
364 contact with a relatively high concentration of salt (7.1 g L^{-1}) causes some disaggregation of the sludge
365 flocs and enhances the hydrolysis of the cells as reported by Chen et al. [28]. Again, although raising
366 the MgCl_2 up to 28.4 g L^{-1} increased the sCOD/tCOD ratio from 13.1 % to 14.2 %, only 158 ± 1 mg
367 COD -VFA g COD_{fed} were produced (significantly lower than that of GC-A-sludge with $p < 0.01$). High
368 conductivity ($< 40 \text{ mS cm}^{-1}$ (Table S2)) might be affecting batch fermentation.

369 Aerating the GC-A-sludge for 37.3 hours decreased the sCOD/tCOD ratio (from 13.1 % to 10.8 %) due
370 to COD uptake for biomass growth. Combination of aeration and salt addition ($7.1 \text{ g L}^{-1} \text{ MgCl}_2$) resulted
371 in increased sCOD/tCOD ratio to 12.6 %. Combining GC-A-sludge with aeration produced 197 ± 2 mg
372 COD -VFA g COD_{fed} , and with aeration and $7.1 \text{ g L}^{-1} \text{ MgCl}_2$ production was 210 ± 1 mg COD -VFA g
373 COD_{fed} (Figure 4). Further increase of the MgCl_2 concentration, yet again, resulted in a significantly
374 lower ($p < 0.01$) VFA production (132 ± 8 mg COD -VFA g COD_{fed}).

375 In all conditions tested, VFA production yield from GC-A-sludge was lower than for the FO-
376 concentrated A-sludge (between 16 and 25 %) (Figure 4), suggesting that none of these treatments nor
377 the combination of them – alone – could explain the increased VFA production obtained after FO-
378 concentration. The sCOD/tCOD ratio of the different treatments on GC-A-sludge varied between 10.4
379 % and 16.1%, while recirculating A-sludge throughout the FO unit increased the ratio up to 30.4 % for
380 the aerated system and 52.5 % for the anaerobic system (Table 3) (experiments run for 37.3 and 95.4
381 hours, respectively (Table 2)). Therefore, although the combination of sludge concentration, aeration
382 and salts leakage to the FS increased sCOD/tCOD, the main cause of VFA production enhancement was
383 probably due to the physical action of the peristaltic pump and the scouring of the bubbles that enter the

384 feed compartment. This leads to disaggregation of the sludge flocs promoting cell lysis. This hypothesis
385 was further investigated in the next section.

386 ***3.5 Effect of FO-concentration on cell lysis***

387 The number of intact planktonic cells and permeabilized cells, thus damaged by lysis, in the GC-A-
388 sludge and the FO-concentrated A-sludge was determined with a live/dead staining coupled to flow-
389 cytometry. These measurements were performed to confirm the hypothesis that FO facilitated breakage
390 of sludge flocs and cells (Figure 5) leading to enhanced fermentability. Intact and permeabilized cells
391 are defined as bacterial cells with a cellular membrane that is either impenetrable (intact) or penetrable
392 (permeabilized) by the nucleic acid binding compound propidium iodide (PI) [32]. (Table S3). Although
393 by gravity concentrating the A-sludge a 3.8-fold concentration could be reached, the concentration of
394 total intact cells only increased (significantly) from $4.11 \times 10^{+9} \pm 2.12 \times 10^{+7}$ to $9.41 \times 10^{+9} \pm 5.66 \times 10^{+7}$
395 cells mL⁻¹, corresponding to a 2.3-fold increase. Consequently, the intact cells/total cells ratio
396 significantly decreased from $68.2 \pm 0.4 \%$ to $56.1 \pm 2.2 \%$ (Figure 5) after gravity concentration
397 indicating that part of the intact cells (both sludge flocs and single cells) were present in the discarded
398 supernatant. The changes in intact cells/ total cells ratio followed the same behavior for both A-sludge
399 and GC-A-sludge under different treatments (Figure 5). Addition of $7.1 \text{ g L}^{-1} \text{ MgCl}_2$ resulted in a
400 significant decrease of the number of intact cells/ total cells ratio to $54.0 \pm 3.7 \%$ for the A-sludge and
401 down to $29.5 \pm 0.8 \%$ for the GC-A-sludge, respectively. These differences confirmed that Mg^{2+} and Cl^-
402 ions have a lytic effect on the sludge flocs and cells, which simultaneously disaggregates the flocs and
403 releases sCOD (Table 3). This was even more pronounced upon adding higher concentration of MgCl_2
404 (28.4 g L^{-1}), the intact cells/ total cells ratio significantly decreased to $29.5 \pm 0.8 \%$ for the A-sludge and
405 down to $15.9 \pm 0.4 \%$ for the GC-A-sludge. Aeration resulted in an increase of the intact cells/ total cells
406 ratio up to $69.0 \pm 1.2 \%$ for the A-sludge and up to $61.8 \pm 1.4 \%$ for the GC-A-sludge (Figure 5). This
407 was most likely due to biomass respiration and thus growth, with consequent reduction of sCOD.
408 Combining aeration and salt addition (7.1 g L^{-1}) the intact cells/ total cells ratio significantly increased
409 up to $75.7 \pm 2.9 \%$ for the A-sludge and to $65.9 \pm 5.6 \%$ for the GC-A-sludge when compared to the
410 mere aeration. In contrast, by increasing the concentration of the MgCl_2 to 28.4 g L^{-1} with active aeration,
411 the intact cells/ total cells ratio significantly decreased to $51.6 \pm 1.9 \%$ for the A-sludge and down to

412 35.2 ± 0.8 % for the GC-A-sludge. This supports the initial hypothesis that the lytic action of MgCl₂
413 increased the sCOD which was taken up to enhance biomass growth when aerating, only when
414 conductivity values (at low salt addition) did not affect the microbial performance. The intact cells/total
415 cells ratio for the aerobically FO-concentrated A-sludge was rather high (68.2 ± 11.9 %). For the
416 membrane-compromised fraction of FO-concentrated A-sludge (both anaerobically and aerobically
417 concentrated), permeabilised cell population overlapped with the background noise and was therefore
418 questionable. Thus the absolute value of intact cells has to be considered over the intact cells/total cells
419 ratio. 10-fold and a 3.4-fold concentrations could be reached by FO-concentrating the A-sludge aerobic
420 and anaerobically, respectively, while GC-concentration resulted in a 3.8-fold concentration of the A-
421 sludge. Nonetheless, FO concentration resulted in a significantly lower (p < 0.01) intact cells content
422 of 3.18 x 10⁺⁹ ± 8.39 x 10⁺⁷, compared to that of GC-A-sludge and GC-A-sludge treated with a
423 combination of salt addition and aeration (8.86 x 10⁺⁹ ± 9.53 x 10⁺⁸ and 6.96 x 10⁺⁹ ± 3.76 x 10⁺⁸,
424 respectively).

425 These findings are thus consistent with the hypothesis set above, that FO-concentration leads to a
426 combined effect of the chemical action of the salt on the sludge flocs and cells, and the physical action
427 of the recirculation and the scouring by bubbles of the A-sludge in the FO-cell. The more the A-sludge
428 was recirculated throughout the FO unit the more the flocs were broken down and their constituent cells
429 lysed (Figure 5). The number of intact cells found in the anaerobically FO-concentrated A-sludge was
430 2.13 x 10⁺⁸ ± 2.74 x 10⁺⁷. This was significantly lower than all of the values reported for the gravity
431 concentrated sludge and the aerobically FO-concentrated sludge, confirming the mechanical shear effect
432 of FO on the breakage of the sludge, which ultimately resulted in increased sCOD and increased VFA
433 production yield.

434 **3.6 Economical assessment**

435 Replacing thickening with a more compact FO-concentration will lead to higher VFA conversion
436 efficiency (from 20 % to 45 %) but also to additional cost. To assess the economic viability of the
437 proposed technology, three scenarios were considered, comparing the use of either 2.2 M MgCl₂ or
438 seawater (0.5 M NaCl) as DS in the FO, to the scenario with a settling (thickening) based AB-system.
439 The costs for the technologies and their references are listed in Table S4 and a description of the

440 assumptions made to calculate capital expenses (CAPEX) and operational expenses (OPEX) is given
441 in section S4 (SI).

442 Table 4 then gathers the scenarios, where it can be seen that the CAPEX for the 2.2 M MgCl₂ FO-
443 system is 0.79 € m⁻³sludge and for the seawater 1.06 € m⁻³sludge (due to the lower driving force and
444 higher membrane requirement). For the OPEX only the specific energy consumption (SEC) needed for
445 reverse osmosis (RO) reconcentration of the DS was considered (section S4, SI) and was as such
446 calculated only for the scenario with 2.2 M MgCl₂ (0.67 € m⁻³sludge) – it was assumed that when
447 using seawater as DS, the seawater is discharged back into the ocean. RO drinking water production
448 from this seawater might be possible, and might lead to a lower total cost (as the income of the
449 drinking water needs to be taken into account). Hence, the total costs (OPEX + CAPEX) for FO-
450 concentration of A-sludge amounted to 1.46 € m⁻³sludge and 1.06 € m⁻³sludge for 2.2 M MgCl₂ and
451 sea-water as DS, respectively. For the gravity concentration of A-sludge the CAPEX was 0.08 € m⁻³
452 sludge and the OPEX 0.06 € m⁻³sludge and thus the total costs (OPEX + CAPEX) were 0.14 € m⁻³
453 sludge (Table 4).

454 Profits obtained from the VFA production of FO-concentrated A-sludge (for both DS considered) and
455 the GC-A-sludge were calculated considering a conversion efficiency of 45 % for the former and 20 %
456 for the latter (sections 3.3.1 and 3.4) and amounted to 2.36 € m⁻³sludge and 1.05 € m⁻³sludge,
457 respectively. The net profits were comparable for the GC-A-sludge and for the A-sludge FO-
458 concentrated with 2.2 M MgCl₂, at 0.91 € m⁻³sludge (0.12 € kg⁻¹sludge) and 0.90 € m⁻³sludge (0.12 €
459 kg⁻¹sludge), respectively (Table 4). However, these calculations are based on current generation of FO
460 membranes in the market. Considerable research is currently aiming at novel FO-membranes with
461 increased water flux (LMH) during FO, reducing the CAPEX and therefore increasing the total profit.
462 In fact, several of these membranes are already close to commercialization [33]. In addition, it has to
463 be stated that, the use of FO system over conventional thickening for the sludge concentration has the
464 advantage of a smaller footprint at WWTP level.

465 When sea-water is used as DS, the total profit amounts to 1.30 € m⁻³sludge (0.17 € kg⁻¹sludge), higher
466 than that of the other scenarios considered but still underestimated. Indeed, for this scenario the same
467 conversion efficiency obtained for 2.2 M MgCl₂ was considered (45 %), but as in sea-water the

468 concentration of NaCl is 4.4 times lower, longer time would be needed to arrive to the same 10-fold
469 concentration. This would result in higher sCOD/tCOD of the FS compared to that reached with 2.2 M
470 MgCl₂ and therefore it would allow higher VFA production. On the other hand, the effect of Na⁺ on
471 cell lysis and sCOD increase and its effect on the subsequent fermentation should be further
472 investigated.

473 **3.7 Strategies to further increase VFA production after A-sludge FO-concentration**

474 FO-concentration of A-sludge increased the VFA conversion efficiency from 10 % to 45 % (4.4-fold),
475 which is higher than previously reported [11]. It has been described that high salinity and high
476 conductivity (above 30 mS cm⁻¹) negatively affect methanogenesis while acetogenic bacteria can
477 acclimatize to high salt concentration and produce VFA [34]. FO-concentration of A-sludge could be a
478 strategy to increase VFA production (sections 3.4 and 3.5) and, inhibit methanogens due to RSD, thus
479 allowing VFA accumulation in a continuous system.

480 Conversion efficiency up to 70 % has been reported during A-sludge digestion [10] suggesting that the
481 potential for VFA production from A-sludge should be higher. Methodologies such as the use of acid
482 or alkali pretreatment [35, 36] and/or thermal pretreatment [6] and the application of alkaline pH to the
483 fermentation are reported to increase VFA production from sludge [37, 38]. Particularly, alkaline
484 conditions increase both hydrolysis and acidification rates and also increase solubilization of
485 carbohydrates and proteins, main components of the sludge, resulting in higher VFA production [39].
486 Therefore, the combination of FO-concentration of the sludge with pretreatments or with fermentation
487 at alkaline pH might promote VFA production and should be further investigated.

488

489 **4. Conclusions**

490 Forward Osmosis enabled 10-fold dewatering of domestic wastewater, secondary activated sludge
491 and high-rate activated A-sludge, yet 18% and 52% of the COD were lost for the domestic
492 wastewater and A-sludge, respectively. Aeration, used to scour the fouling layer on the membrane,
493 promoted COD consumption, therefore other techniques to mitigate membrane fouling need to be
494 investigated. Forward osmosis increased the soluble COD (sCOD) fraction in the sludge enhancing
495 VFA production by 7 % for secondary sludge and by 35 % for A-sludge (from 10 % to 45 %) but

496 did not enable VFA production for the domestic wastewater due to the limited sCOD. This proves
497 the importance of an intermediate step to convert the sCOD into microbial sludge before the FO-
498 concentration. Increased VFA production for FO-concentrated streams is due to both chemical
499 action of the ions leaking from the draw solution and the physical effect caused by recirculation of
500 the stream and air scouring. Both effects together result in disaggregation of the sludge flocs and
501 lysis of the cells, increasing sCOD and VFA production.

502

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514

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611

612

Table 1. Characteristics of the streams. Analysis were carried out in triplicate.

	Domestic wastewater	Secondary sludge	A-sludge
pH	7.42	7.26	7.15
Conductivity (mS cm⁻¹)	1.4	1.3	1.8
Total solids, TS (g L⁻¹)	0.26 ± 0.04	4.89 ± 0.83	6.73 ± 0.59
Volatile solids, VS (g L⁻¹)	0.10 ± 0.03	2.83 ± 0.48	5.06 ± 0.62
Volatile solids, VS/TS (%)	38	58	78
Total suspended solids, TSS (g L⁻¹)	<0.01	4.01 ± 0.04	5.43 ± 0.52
Volatile suspended solids, VSS (g L⁻¹)	<0.01	2.51 ± 0.05	4.22 ± 0.48
Volatile suspended solids, VSS/TSS (%)	-	63	78
Kjeldahl nitrogen, TKN (mg N L⁻¹)	9 ± 0	974 ± 15	476 ± 40
Total ammonia nitrogen, TAN (mg N L⁻¹)	7 ± 5	56 ± 7	577 ± 20
Total chemical oxygen demand, tCOD (g L⁻¹)	0.132 ± 0.016	4.482 ± 0.979	8.398 ± 0.362
Soluble chemical oxygen demand, sCOD (g L⁻¹)	0.033 ± 0.003	0.215 ± 0.007	1.252 ± 0.023
Soluble chemical oxygen demand, sCOD/tCOD (%)	25	5	15
Fe (mg L⁻¹)	6 ± 0	141 ± 1	271 ± 7
P (mg L⁻¹)	463 ± 38	223 ± 1	200 ± 4
S (mg L⁻¹)	3111 ± 189	85 ± 9	56 ± 5
K (mg L⁻¹)	437 ± 29	47 ± 0	40 ± 2
Na (mg L⁻¹)	45 ± 2	85 ± 3	54 ± 2
Ca (mg L⁻¹)	387 ± 3	201 ± 7	233 ± 4
Mg (mg L⁻¹)	1658 ± 242	37 ± 0	41 ± 1

Table 2: Operational parameters changes during FO-concentration (FO) and anaerobic FO-concentration (AnaFO). t^0 stands for initial values.

	Time FO		Volume/concentration		COD concentration		Conductivity			Mg ²⁺ concentration	
	(hrs)		ratio		ratio		(mS cm ⁻¹)			(g L ⁻¹)	
	FO	AnaFO	FO	AnaFO	FO	AnaFO	t ⁰	FO	AnaFO	FO	AnaFO
Domestic waste water	28.1	-	10	-	8.2 ± 1.2	-	1.4	31.6	-	20.5 ± 0.6	-
Secondary sludge	29.7	-	10	-	10.1 ± 2.4	-	1.3	18.2	-	11.7 ± 0.2	-
A-sludge	37.3	95.4	10	4	4.8 ± 0.2	3.9 ± 0.1	1.8	29.6	41.7	7.1 ± 0.1	8.7 ± 0.3

Table 3: Total and soluble COD and its ratio (COD soluble/COD total) for inputs and outputs of the concentration tests. Acronyms stand for raw A-sludge (AS), secondary sludge (WAS), domestic wastewater (WW), FO-concentrated streams (FO-AS, FO-WAS, FO-WW), anaerobically FO-concentrated A-sludge (AnaFO-AS), gravity concentrated A-sludge (GC-AS), addition of 7.1 g L⁻¹ of MgCl₂ (ASM/ GC-ASM), aeration at 0.6 L min⁻¹ (ASA/ GC-ASA), a combination of salt addition and aeration (ASAM/ GC-ASAM) and the wastewater discharged after A-sludge gravity concentration (GC-WW). * estimated soluble COD according to typical COD ratios for A-sludge.

		COD total (g L⁻¹)	COD soluble (g L⁻¹)	COD ratio (%)
Forward Osmosis (FO) tests	AS	8.40 ± 0.36	1.25 ± 0.02	14.9
	FO-AS	40.49 ± 1.09	12.30 ± 0.70	30.4
	AS	5.46 ± 0.53	0.81 *	14.9 *
	AnaFO-AS	21.40 ± 1.65	11.23 ± 0.23	52.5
	WAS	4.48 ± 0.98	0.22 ± 0.01	4.8
	FO-WAS	48.53 ± 0.27	4.92 ± 0.50	10.1
	WW	0.13 ± 0.02	0.03 ± 0.00	25.2
	FO-WW	1.08 ± 0.08	0.24 ± 0.01	21.7
Gravity concentration (GC) tests	AS	7.76 ± 0.16	1.47 ± 0.04	19.0
	ASML	7.65 ± 0.16	1.91 ± 0.00	25.0
	ASMH	7.78 ± 0.01	2.14 ± 0.07	27.5
	ASA	6.84 ± 0.31	1.12 ± 0.03	16.4
	ASAML	6.50 ± 0.20	1.24 ± 0.02	19.0
	ASAMH	7.05 ± 1.10	1.28 ± 0.06	18.1
	GC-AS	29.42 ± 0.19	3.84 ± 0.01	13.1
	GC-ASML	29.14 ± 0.67	4.69 ± 0.01	16.1
	GC-ASMH	28.14 ± 0.01	4.01 ± 0.01	14.2
	GC-ASA	28.12 ± 0.15	3.04 ± 0.04	10.8
	GC-ASAML	29.25 ± 0.49	3.68 ± 0.08	12.6
	GC-ASAMH	28.18 ± 0.72	2.93 ± 0.09	10.4
	GC-WW	3.24 ± 0.04	0.86 ± 0.01	26.7

Table 4: Cost analysis for the Forward Osmosis (FO) concentration and of A-sludge (average concentration of 7.5 g COD L⁻¹) by using either 2.2 M MgCl₂ or sea-water (NaCl 0.5 M) as draw solution. For the former Reverse Osmosis (RO) was considered for the reconcentration of the DS and relative costs were included. Profit gained for the VFA production of either FO-concentrated or A-sludge concentrated by thickening are calculated considering a conversion efficiency (mg COD-VFA g⁻¹ COD_{fed}) of 45 % for the FO-concentrated A-sludge and of 20 % for the thickened A-sludge. Costs and profits are reported as ratio of € for m³ or kg of raw A-sludge (CAPEX capital expenses, OPEX operational expenses, SEC Specific Energy Consumption). Total profits (balance) are calculated as difference between the net for the VFA for the technology and the total costs (OPEX+CAPEX) of that technology.

		Forward Osmosis						Thickening
		2.2 M MgCl ₂			Sea-water (0.5 M NaCl)			
		FO	RO	Total	FO	RO	Total	
CAPEX	€ m ⁻³	0.53	0.26	0.79	1.06	-	1.06	0.08
OPEX								
SEC	€ m ⁻³	-	0.67	0.67	-	-	-	-
PE + Electricity	€ m ⁻³	-	-	-	-	-	-	0.06
Total costs (OPEX+CAPEX)	€ m ⁻³	0.53	0.93	1.46	1.06	-	1.06	0.14
	€ kg ⁻¹	0.07	0.12	0.19	0.14	-	0.14	0.02
VFA net	€ m ⁻³		2.36			2.36		1.05
	€ kg ⁻¹		0.32			0.32		0.14
Balance	€ m ⁻³		0.90			1.30		0.91
	€ kg ⁻¹		0.12			0.17		0.12

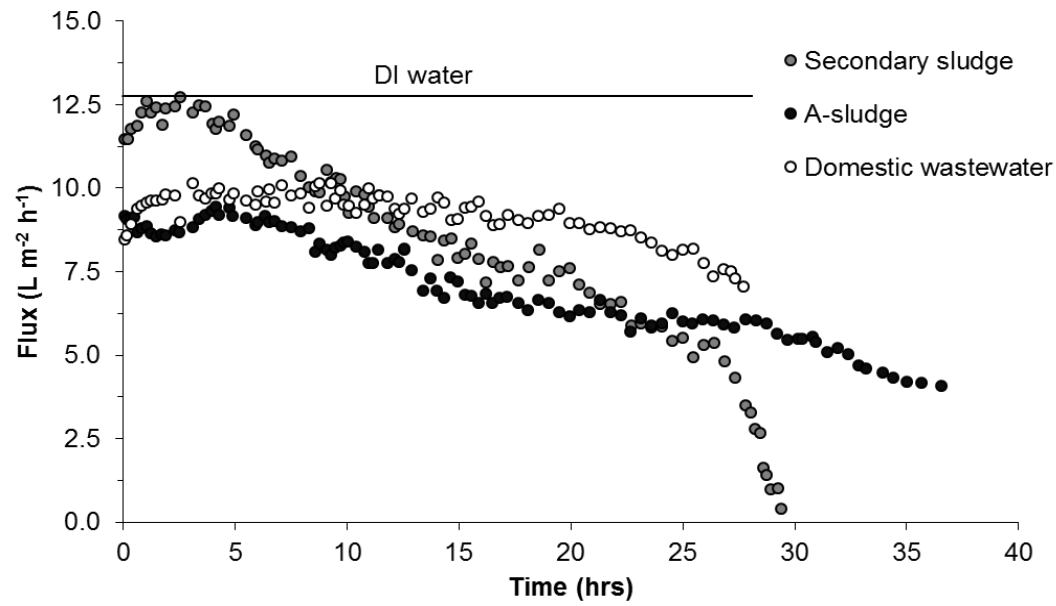


Figure 1. FO dewatering flux in $\text{L m}^{-2} \text{h}^{-1}$ for the domestic wastewater, secondary sludge or A-sludge (feed solution) when in contact with a solution of MgCl_2 2.2 M (draw solution) through a semipermeable TFC membrane. The streams were concentrated/dewatered with a factor 10.

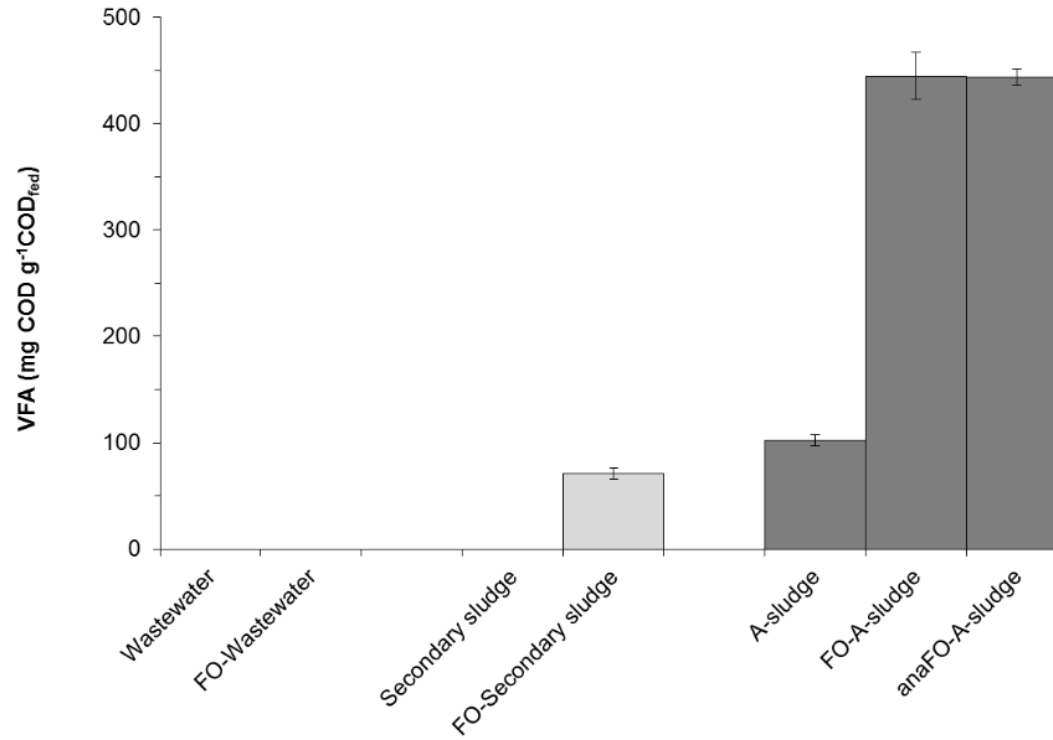


Figure 2. Specific VFA production for raw and FO-concentrated streams on their peak-production day. The test was carried out at 35 °C and pH 7. (n = 3).

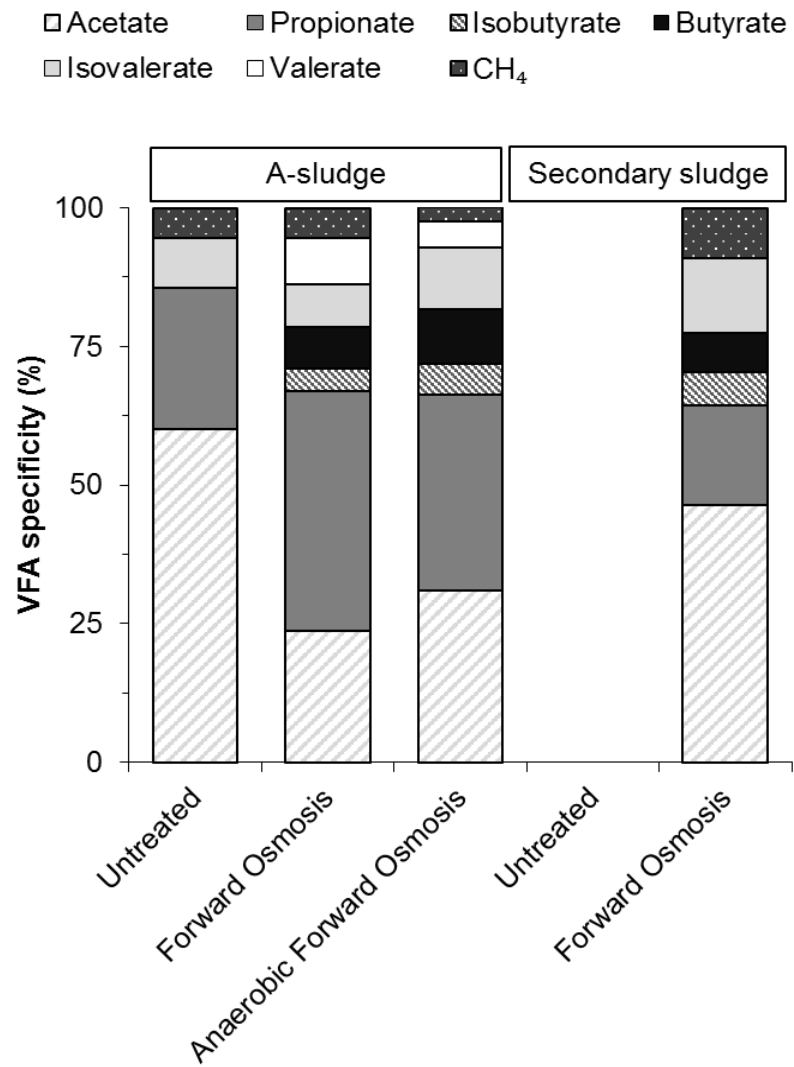


Figure 3. VFA composition in mg COD g⁻¹COD_{fed} on the peak production day. The test was carried out at 35 °C and pH 7 (n = 3).

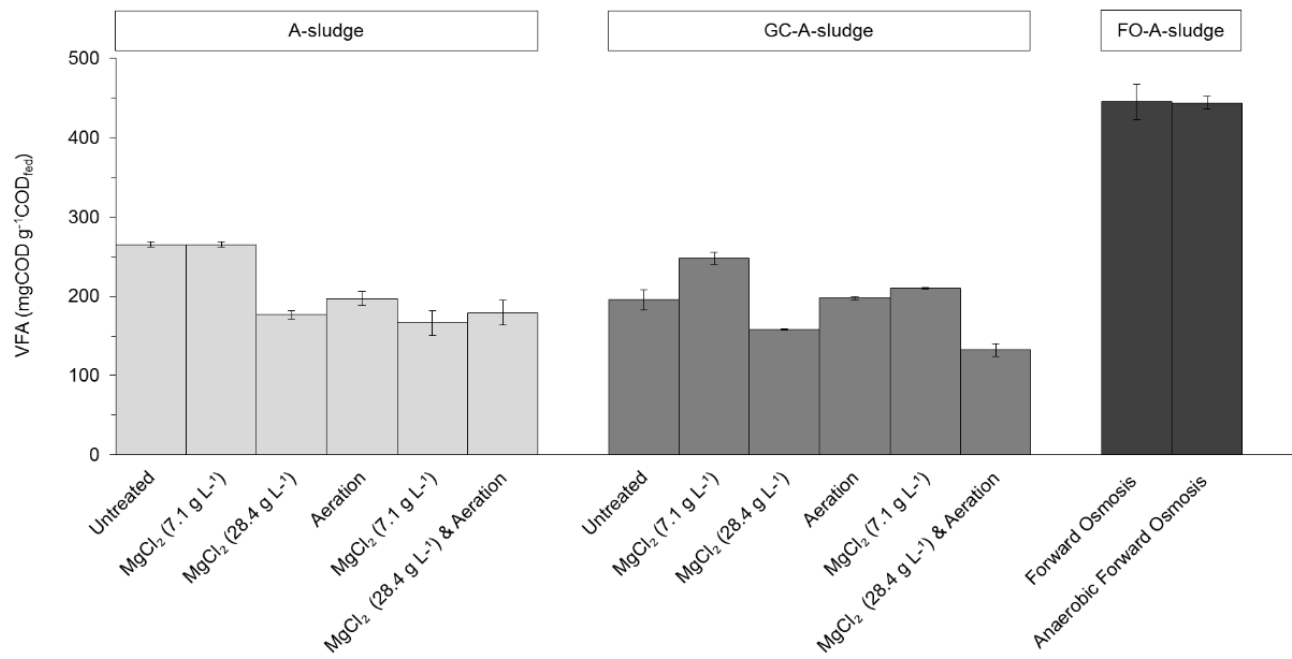


Figure 4. Specific VFA production on the peak-production day for A-sludge and gravity concentrated A-sludge (GC-A-sludge) with different treatments and A-sludge concentrated aerobically or anaerobically with forward osmosis (FO-A-sludge). The fermentation was performed at 35 °C and pH 7 (n = 3).

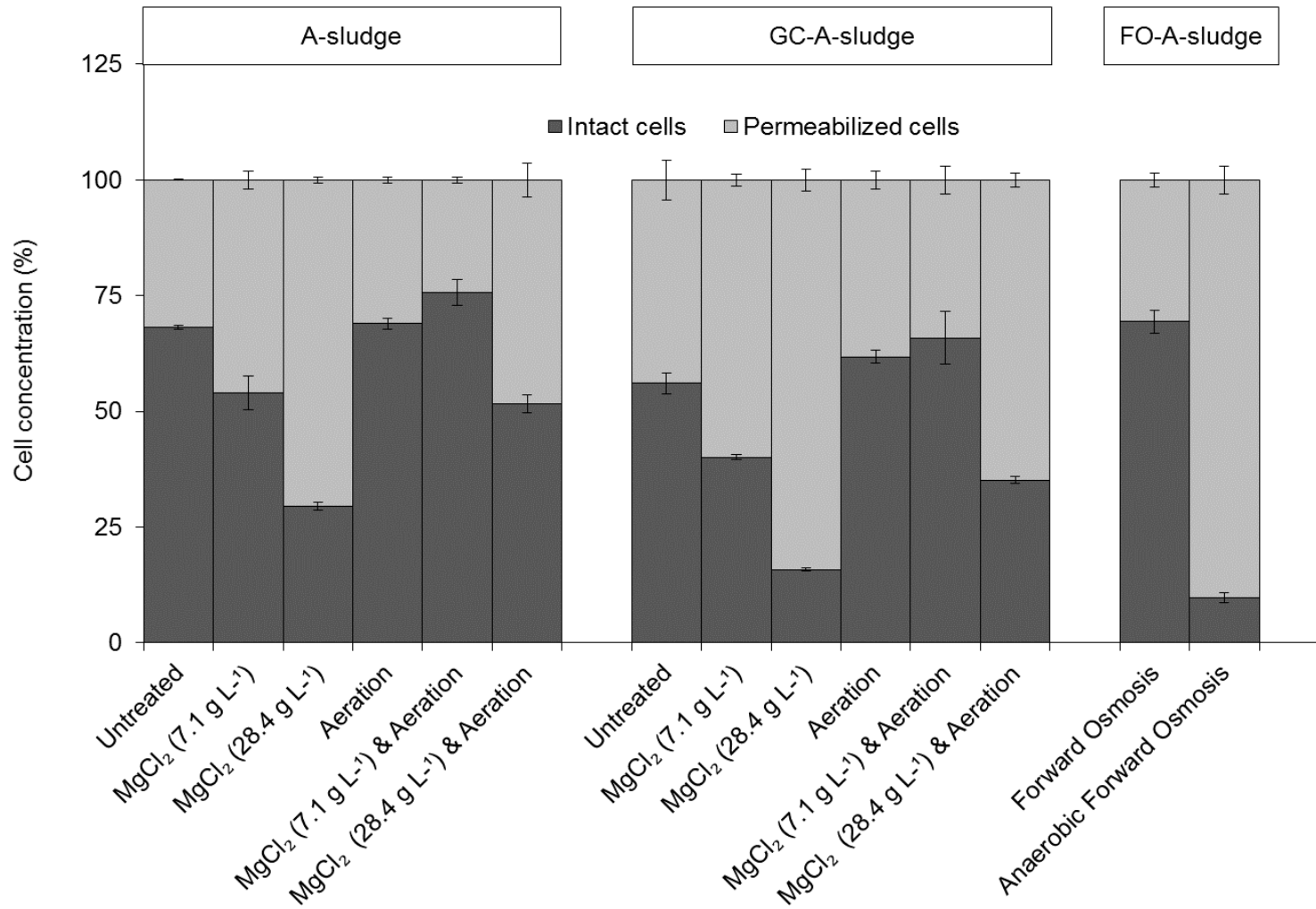


Figure 5. A. total intact cells/ permeabilized cells ratio (%) (n = 3) and B. total intact cells concentration for A-sludge and gravity concentrated A-sludge (GC-A-sludge) with different treatments and A-sludge concentrated aerobically or anaerobically with forward osmosis (FO-A-sludge) (n = 3).