

1 **Revealing the role of supernatant and granular sludge**  
2 **fractions on granular anaerobic membrane bioreactor fouling**

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11 **ABSTRACT**

12 In order to design efficient fouling mitigation strategies in granular anaerobic membrane  
13 bioreactors (G-AnMBR), foulant characteristics and their role have to be thoroughly  
14 investigated. Raw mixed liquor of G-AnMBR was split by sieving into granules and  
15 supernatant fractions at 0.125 mm. Then, the fouling potential and reversibility of the  
16 different samples (granules, supernatant and raw mixed liquor) were assessed by  
17 measuring critical fluxes and through filtration tests. Various hydrodynamic conditions,  
18 i.e. gas sparging and recirculation, were applied to evaluate the impact of shear stress on  
19 fouling propensity. Results revealed that the supernatant fraction, composed of fine  
20 compounds and micro-particles, had a strong fouling potential, whilst the granule  
21 fraction led to minor fouling filtration resistance. Three-dimensional excitation emission  
22 fluorescence spectroscopy emphasised the prominent role of colloidal proteins in G-  
23 AnMBR membrane fouling. During the filtration test of raw mixed liquor, the fouling

24 propensity of the micro-particles was lowered, since the structural cake layer was  
25 modified. Gas sparging allowed for the mitigation of cake formation, but excess of  
26 shear forces may lead to granule break-up and more irreversible fouling. Liquid  
27 recirculation led to a higher filtration resistance, but almost all the membrane  
28 permeability was recovered by physical cleaning. A short filtration cycle without gas  
29 sparging followed by a short period of relaxation and gas sparging could be a suitable  
30 fouling mitigation method. In this way, release of micro-particles from granule break-up  
31 could be limited, the cake build-up would be mostly reversible by physical cleaning,  
32 and the energy demand of gas sparging would be greatly reduced, thereby improving the  
33 energy neutrality of the G-AnMBR biotechnology.

#### 34 **KEYWORDS**

35 membrane; granular sludge; membrane fouling; gas sparging; membrane cleaning.

### 36 **1. Introduction**

37 The anaerobic membrane bioreactor (AnMBR) is an emergent biotechnology that  
38 combines anaerobic digestion and membrane filtration. This hybrid technology is  
39 drawing attention for domestic wastewater treatment due to its competitiveness in terms  
40 of (i) conversion of organics into methane, (ii) effluent quality and (iii) reactor  
41 compactness [1,2]. Many lab- and pilot-scale studies have proven that AnMBR is a  
42 sustainable and efficient alternative to conventional energy-intensive processes, which  
43 could be suitable for low-energy, water-scarce, low-income and space-limited areas  
44 [3,4].

45 Nonetheless, a major hindrance to AnMBR scale-up and implementation in mainstream  
46 wastewater treatment is membrane fouling, as this reduces process productivity and  
47 increases energy, operational and maintenance costs (e.g. chemicals, membrane

48 replacement, etc.) [1,5]. Among strategies to reduce AnMBR fouling, the granular-  
49 sludge-based anaerobic membrane bioreactor (G-AnMBR) has gained prominence in  
50 the last decade, since granules simultaneously boost the biomass activity and reduce  
51 membrane fouling [4,6,7]. Granular sludge is characterised by a self-immobilisation of  
52 biomass into compact and dense aggregates which form well-established micro-  
53 ecosystems. The structural arrangement of the granules imparts high settling capacity,  
54 efficient methanogenic activity and high strength to loading rates changes and shocks  
55 [2]. Zhang et al. (2021) found that 39.9% of fouling mitigation in a granular membrane  
56 bioreactor (MBR) was due to the scouring effect of granules over the membrane  
57 surface, while 50.3% was attributed to granule structure [8]. The mechanical scouring  
58 effect of the granular material expanded by gas sparging has been reported effective in  
59 diminishing membrane fouling in MBR by friction with the membrane and by  
60 enhancing the collision between granules and suspended sludge, thus reducing their  
61 deposition [8,9]. Due to the higher density of granules, granular sludge is less easily  
62 pushed towards the membrane surface than suspended sludge. It is further hypothesised  
63 that the large size and solid structure of granular biomass and the immobilisation of  
64 extracellular polymeric substances (EPS) within granule structure limit fouling (i.e. pore  
65 blocking, deposition and thickness of the cake layer on membrane surface) compared to  
66 conventional flocculated sludge MBR [9,10]. Actually, Martin-Garcia et al. (2013)  
67 measured a concentration of soluble microbial products (SMP) at least twice as high in  
68 a flocculated AnMBR than in a G-AnMBR. Moreover, some solid and colloidal  
69 organics are adsorbed and biodegraded inside the granular sludge bed, which is  
70 supposed to cause less membrane fouling [11,6]. [12] found that large granules ( $d_p \geq 1.2$   
71 mm) and small granules ( $d_p \leq 1$  mm) were associated to high flux and low membrane  
72 fouling because of loose cake layer structure and less EPS-membrane adhesion,

73 respectively. Conversely, they found that granular sludge with intermediate size ( $1 \leq d_p$   
74  $\leq 1.2$  mm) was responsible of more severe fouling due to both compact cake layer and  
75 higher adhesion of EPS to membrane surface. Hence, the size of granules has been also  
76 identified as a determining factor in the extent of membrane fouling.

77 The granular sludge matrix is a complex mixture. Based on the size distribution, the  
78 granular sludge matrix is generally divided into various fractions, such as (i) granules,  
79 (ii) sludge flocs, (iii) micro-particles – including free bacteria and micro-organisms,  
80 colloidal and sub-visible particles ( $0.45 - 15 \mu\text{m}$ ), and (iv) dissolved compounds, e.g.  
81 biopolymers, salts and SMP [13,14]. All these fractions could be of influent origin, the  
82 result of the bacterial activity, or process dependent and they all might cause membrane  
83 fouling [15,14,2]. Several studies have focused on the characterisation of the fouling  
84 phenomena in conventional AnMBR. In AnMBR studies, micro-particles were found to  
85 dominate the membrane fouling phenomenon [14,15]. Yao et al. (2020) suggested that  
86 cake layer formation and biofouling occurred concurrently within the AnMBR, since  
87 analogous organics and micro-organisms were found in micro-particle fraction and  
88 foulant components. Subsequently, even though granular sludge partly helps membrane  
89 fouling mitigation compared to conventional flocculated sludge, fouling concerns  
90 remain and need to be better understood to define effective fouling mitigation and  
91 cleaning strategies.

92 Based on the most common MBR fouling mitigation strategy, some studies have  
93 investigated different permeate fluxes (from 5 to 20  $\text{L}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$  (LMH)) and specific gas  
94 demand (SGD) ( $0.1 - 2.0 \text{ m}^3\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ ) to identify the best operating and hydrodynamic  
95 conditions for G-AnMBR to maintain high membrane permeability with low energy  
96 requirements and treatment costs [16,17]. Vinardell et al. (2022) stated that operating at  
97 moderate fluxes and gas sparging rates ( $J_{20} = 7.8$  LMH;  $\text{SGD} = 0.5 \text{ m}^3\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ ) could be

98 the most favourable membrane fouling control strategy in G-AnMBR and balance  
99 process productivity and process economics. Wang et al. (2018a) tested continuous and  
100 intermittent gas sparging regimes and filtration cycles and stated that shear rate, gas  
101 sparging frequency and filtration cycle length are of high importance for delivering  
102 sustained membrane filtration. Intermittent filtration associated with intermittent gas  
103 sparging has been identified as the best fouling mitigation method in G-AnMBR, since  
104 low residual fouling resistance and energy neutrality can be achieved [17]. However, the  
105 authors suggested further investigations to properly manage operating conditions.

106 To implement suitable and affordable fouling management, it is essential to understand  
107 the inherent G-AnMBR fouling phenomenon, since membrane fouling characteristics  
108 are matrix-dependent. To the best of the authors' knowledge, no previous studies have  
109 explicitly explored the fouling potential of an anaerobic granular sludge matrix. The aim  
110 of this study is therefore to reveal the fouling potential and mechanisms of an anaerobic  
111 granular sludge. To provide an in-depth assessment, the G-AnMBR mixed liquor was  
112 fractionated by sieving into two parts, the granules fraction and the supernatant fraction.  
113 The threshold size that distinguishes a granule from a flocculated sludge varies from 0.1  
114 mm to 1 mm, depending on the study [18–20]. In this study, bioparticles above 0.125  
115 mm were regarded as granules. Filtration tests were conducted on the raw granular  
116 mixed liquor, the granules fraction and the supernatant fraction to evaluate their impact  
117 on membrane fouling. Since hydrodynamic conditions and the resulting shear stress are  
118 a key driver in membrane fouling mitigation, two gas sparging conditions and a liquid  
119 recirculation condition were tested in each filtration test. The critical flux concept and  
120 resistance-in-series model were used to determine fouling rate and reversibility of each  
121 fraction for the three different hydrodynamic conditions. Three-dimensional  
122 excitation/emission fluorescence analyses were conducted to characterise the foulants.

123 Specific objectives are to (i) make a direct and systematic comparison of the fouling  
124 behaviour of the different fractions, (ii) determine the main compounds responsible for  
125 membrane fouling, (iii) find out the possible interactions between granules and  
126 supernatant fractions and their effect on fouling, and (iv) identify the impact of the  
127 hydrodynamic conditions on the granular sludge and fouling behaviour to increase  
128 understanding and help decision making about fouling strategies.

## 129 **2. Materials and methods**

### 130 **2.1 Anaerobic granular sludge fractions**

131 Raw granular anaerobic sludge (called the “raw mixed liquor”) was taken from a  
132 mesophilic (35-38°C) industrial Upflow Anaerobic Sludge Blanket (UASB) reactor  
133 treating the process water from the manufacturing of recycled paper (Saica Paper  
134 Champblain-Laveyron, France). Four litres of raw mixed liquor at a constant total  
135 suspended solids (TSS) concentration of 10 g/L were split through a standard sieve of  
136 0.125 mm mesh size. Granules retained on the sieve ( $d_p \geq 0.125$ ) were resuspended into  
137 four litres of deionised water and represent the granules fraction from now on. The four  
138 litres of liquid and particles that flowed through the sieve were regarded as the  
139 supernatant fraction ( $d_p < 0.125$ ). Fouling propensities of (i) granules, (ii) supernatant,  
140 and (iii) raw mixed liquor were systematically assessed.

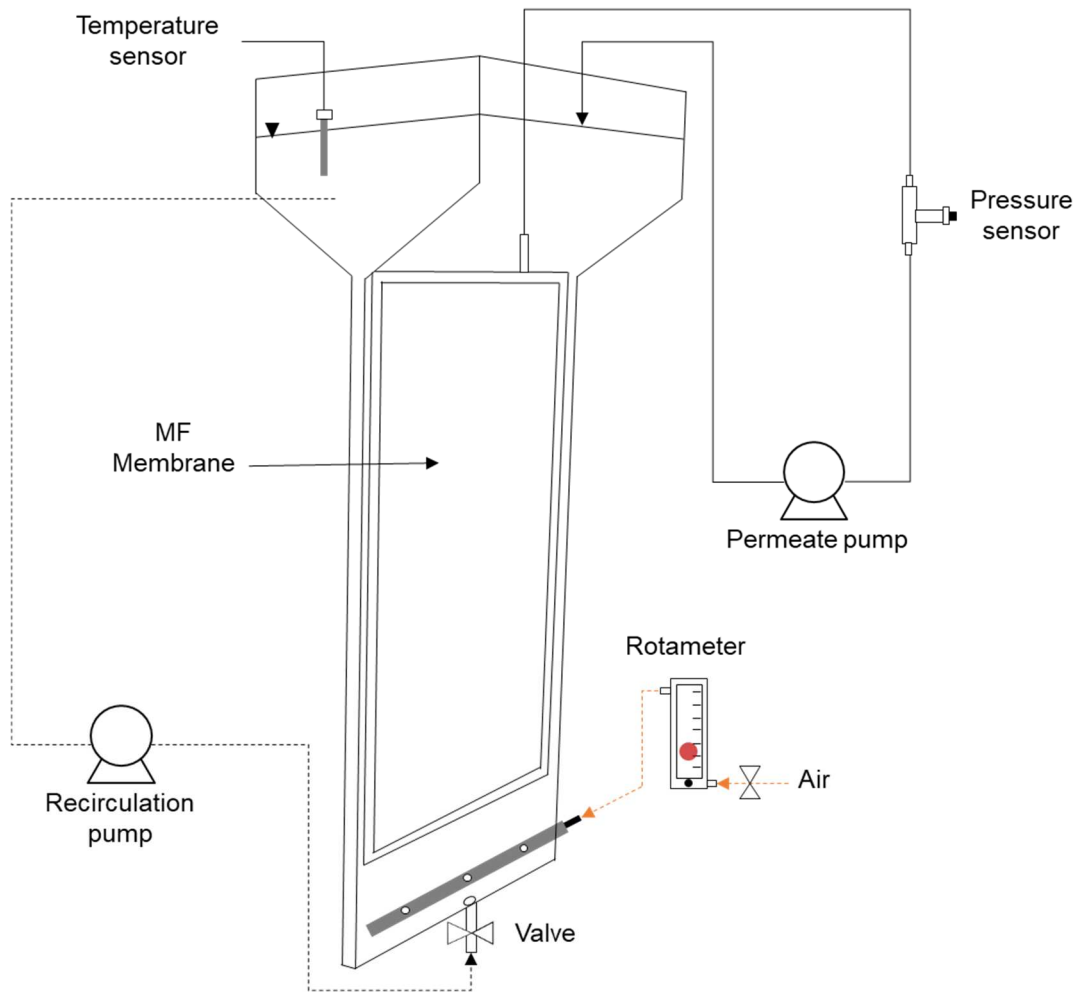
### 141 **2.2 Experimental set-up**

142 The experimental lab-scale system, shown in Fig. 1, was composed of two sections with  
143 an effective volume of 4 L. The lower part of the reactor consisted of a thin  
144 parallelepiped-shaped zone (215 x 24 x 405 mm) where the membrane cartridge was  
145 immersed and the upper part was a flared section. The bottom of the reactor included  
146 both a liquid recirculation and an aeration diffuser used separately according to the

147 operating conditions applied. The liquid recirculation was carried out by a peristaltic  
148 pump (Watson Marlow (WMFTG), UK). Supernatant was pumped from the top of the  
149 reactor and reintroduced under the membrane through a 10 mm hole placed in the  
150 middle of the section. The gas sparging was done by a hollow tube with three drilled  
151 holes (1 mm diameter) distributed along the length and controlled by a gas flowmeter.  
152 The microfiltration membrane used was a flat sheet module from KUBOTA Membrane  
153 Europe (UK) in Polyethylene Terephthalate (PET) and Chlorinated Polyethylene (PE-C)  
154 with a nominal pore size of 0.4  $\mu\text{m}$ , 0.11  $\text{m}^2$  surface area and 6 mm cartridge width. The  
155 permeate was suctioned through a peristaltic pump (Watson Marlow (WMFTG), UK)  
156 and returned to the reactor to maintain a constant volume. The transmembrane pressure  
157 (TMP) was obtained through a pressure gauge installed on the permeate line. The water  
158 temperature (T) in the reactor was monitored. Pressure and temperature data were  
159 recorded using a Bluetooth-based system provided by Instrument Works (Waterloo,  
160 Australia). Visualisation, acquisition and storage of all data was realised thanks to the  
161 Dataworks software (Instrument Works, Waterloo, Australia). Membrane flux ( $J_T$ ) was  
162 set at 20  $\text{L}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$  (LMH) and the corresponding normalised flux at 20°C ( $J_{20}$ ) was  
163 recalculated using Equation (1).

$$J_{20} = \frac{J_T \cdot \mu_T}{\mu_{20}} \quad (1)$$

164 where  $J_{20}$  is the normalised flux at 20°C ( $\text{m}^3\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) and  $\mu_{20}$  and  $\mu_T$  (in Pa.s) are the  
165 viscosity of water at 20°C and at the working temperature T (°C) respectively.



166

167 Fig. 1 – Schematic representation of the experimental set-up

168

169 **2.3 Filtration tests**

170 Filtrations tests were conducted for the three sludge fractions (raw mixed liquor,  
 171 granules and supernatant). Each experiment consisted of filtration tests based on five  
 172 consecutive operating cycles composed of 45 min of filtration and 90 s of relaxation.

173 Three hydrodynamic conditions were investigated with the aim of evaluating the  
 174 influence of turbulence on filtration performances. Hence, a liquid recirculation of 24  
 175 L/h (RE) and two aeration flow rates of 25 L/h (A25) and 100 L/h (A100) were applied  
 176 resulting in shear stress of 15, 205 and 409  $s^{-1}$  respectively (see equations in  
 177 supplementary data). For RE, the crossflow velocity was about 11 m/h and the specific



178 gas demand was 0.23 and 0.91 m<sup>3</sup>.m<sup>-2</sup>.h<sup>-1</sup> for A25 and A100, respectively. Before each  
 179 filtration test, the permeability of the clean membrane was measured with deionised  
 180 water through flux-stepping increments and determined as follows:

$$J_{20} = L_p \cdot TMP \quad (5)$$

181 where  $L_p$  is the permeability (L.m<sup>-2</sup>.h<sup>-1</sup>.bar<sup>-1</sup>),  $J_{20}$  is the normalised flux at 20°C (L.m<sup>-2</sup>.h<sup>-1</sup>) and TMP is the transmembrane pressure (bar).

## 183 2.4 Critical flux

184 Critical fluxes ( $J_c$ ), corresponding to the onset of prominent fouling, were assessed by  
 185 the flux-step method [21] for each fraction and hydrodynamic condition tested. The  
 186 permeation rate was increased stepwise from 2 LMH to 30 LMH and then incrementally  
 187 decreased. The corresponding TMP was continually recorded. The step duration was  
 188 fixed at 10 minutes and a step height of 2 LMH and 4 LMH was chosen for the  
 189 ascending and descending phases, respectively. Le Clech et al. (2003) established three  
 190 key TMP-based parameters to determine the critical flux, namely: (i) the initial TMP  
 191 increase ( $\Delta P_n$ ), (ii) the TMP increase rate  $(dTMP/dt)_n$ , and (iii) the average TMP  
 192 ( $P_{average_n}$ ). All these parameters are depicted below:

$$\Delta P_n = TMP_i^n - TMP_f^{n-1} \quad (2)$$

$$(dTMP/dt)_n = \frac{(TMP_f^n - TMP_i^n)}{t_f^n - t_i^n} \quad (3)$$

$$P_{average_n} = \frac{TMP_i^n + TMP_f^n}{2} \quad (4)$$

193 where  $TMP_i^n$  and  $TMP_f^n$  are the initial and final TMP, respectively, of the n flux step,  $t_i^n$   
 194 is the starting time and  $t_f^n$  is the ending time of this step.

195 The three parameters were calculated for each flux step. When the TMP-based  
196 parameters were no longer constant between flux steps and deviated from clean water  
197 values, the critical flux was considered to have been reached. The critical flux values  
198 given in this study are the average of the critical flux obtained through each parameter.  
199 Hence, the critical flux mentioned in this study is not in its zero-rate strict form, but  
200 corresponds to the flux level under which a sustainable filtration can be achieved.

## 201 **2.5 Fouling propensity and fouling reversibility**

202 Filtration resistances were determined following Darcy's law (Equation (6)).

$$R_t = \frac{TMP}{\mu_{20} \cdot J_{20}} \quad (6)$$

203 where  $R_t$  is the resistance ( $m^{-1}$ ) and TMP is the transmembrane pressure (Pa).

204 Membrane fouling was characterised by means of the resistance-in-series model. In this  
205 study, the total resistance ( $R_t$ ) is defined as the sum of the intrinsic membrane resistance  
206 ( $R_m$ ) and the fouling resistance ( $R_f$ ) which, in turn, was divided into the resistances  
207 caused by reversible fouling, irreversible fouling and residual fouling ( $R_{reversible}$ ,  
208  $R_{irreversible}$ ,  $R_{residual}$  respectively) as described in Equation (7) and (8).

$$R_t = R_m + R_f \quad (7)$$

$$R_f = R_{reversible} + R_{irreversible} + R_{residual} \quad (8)$$

209 The above-mentioned resistances were determined by filtering deionised water in the  
210 same hydrodynamic conditions as the filtration tests, using the following experimental  
211 procedure: (i)  $R_m$  was measured by filtering deionised water through the clean  
212 membrane; (ii)  $R_t$  was evaluated using the fouled membrane at the end of the filtration  
213 test; (iii)  $R_f$  was deduced from Equation (7); (iv) superficial cleaning with water was  
214 undertaken, taking the fouled membrane out of the reactor and flushing the surface with  
215 1 litre of deionised water, after which the remaining resistances ( $R_{irreversible} + R_{residual}$ )

216 were measured by filtering deionised water;  $R_{\text{reversible}}$  was then calculated using  
217 Equation (8); finally, (v) a two-hour chemical cleaning by soaking in a 0.2% sodium  
218 hypochlorite solution was carried out under aeration, and the leftover resistance  $R_{\text{residual}}$   
219 was measured;  $R_{\text{irreversible}}$  was then deducted from Equation (8).

## 220 **2.6 Analytical methods**

### 221 **2.6.1 Particle size distribution**

222 Particle size distribution (PSD) was performed on the raw mixed liquor before and after  
223 filtration tests at A25, A100 and RE. Size fractionation was done by wet sieving. The  
224 standard sieves used were of 1.0, 0.63 and 0.125 mm mesh sizes, resulting in four  
225 fractions: large granules ( $d_p \geq 1$ ), medium granules ( $d_p 1-0.63$ ), small granules ( $d_p 0.63-0.125$ ),  
226 and flocs and fines ( $d_p < 0.125$ ). Total solids of each fraction were measured according to  
227 Standard methods [22]. The PSD was expressed as a fraction's mass distribution [2].

### 228 **2.6.2 Three-dimensional excitation emission matrix fluorescence**

229 Three-dimensional Excitation Emission Matrix (3DEEM) fluorescence was used to  
230 characterise and semi-quantify the dissolved and colloidal organic matter (DCOM), as  
231 3DEEM samples were filtered at 0.45  $\mu\text{m}$ . The cleaning water from the physical  
232 cleaning and the raw mixed liquor before and after filtration tests were analysed. Three-  
233 dimensional excitation emission matrices were obtained using a Perkin-Elmer FL6500  
234 spectrometer (USA). Excitation and emission scan ranges were fixed at 200-500 nm and  
235 280-600 nm, respectively, while scan speed was set at 12,000 nm/min, incremented to  
236 10 nm. The slit width was 5 nm for both excitation and emission. Every sample was  
237 associated with a Milli-Q water blank analysed in the same conditions. To circumvent  
238 the over-quantification caused by Raman and Rayleigh water scatter peaks, all spectra  
239 were scatter-corrected by the blank sample [23]. From the 3DEEM spectra, four regions  
240 were distinguished, based on their specific fluorophores [24]. Region I+II was

241 associated with protein-like fluorophores (tyrosine) ranging from  $\lambda_{ex} = 200-$   
242 250nm to  $\lambda_{em} = 280-380$ nm, Region III ( $\lambda_{ex} = 200-250$ nm /  $\lambda_{em} = 380-600$ nm)  
243 corresponded to fulvic acid-like molecules, Region IV ( $\lambda_{ex} = 250-350$ nm /  $\lambda_{em} = 280-$   
244 380nm) was associated with soluble microbial product (SMP)-like molecules  
245 (Tryptophan), and Region V ( $\lambda_{ex} = 250-500$ nm /  $\lambda_{em} = 380-600$ nm) corresponded to  
246 humic acid-like molecules. Region III and IV were merged into a single region III+IV,  
247 called humic substances. The normalised volume of fluorescence (in arbitrary unit per  
248 nm<sup>2</sup> (A.U/nm<sup>2</sup>)) beneath each area was calculated as a function of the fluorescence  
249 intensity at each excitation-emission pair.

### 250 **2.6.3 Proteins and polysaccharides**

251 Protein (PN) and polysaccharide (PS) contents were used to characterise the different  
252 fractions and to follow any modification or release of these organic compounds during  
253 the experiments. The colorimetric Lowry and Dubois methods were used for PN and  
254 PS, respectively [25,26]. Bovine Serum Albumin (BSA) and glucose were used as  
255 calibration solutions. All samples were pre-filtered through a 0.45  $\mu$ m acetate cellulose  
256 filter before dosing.

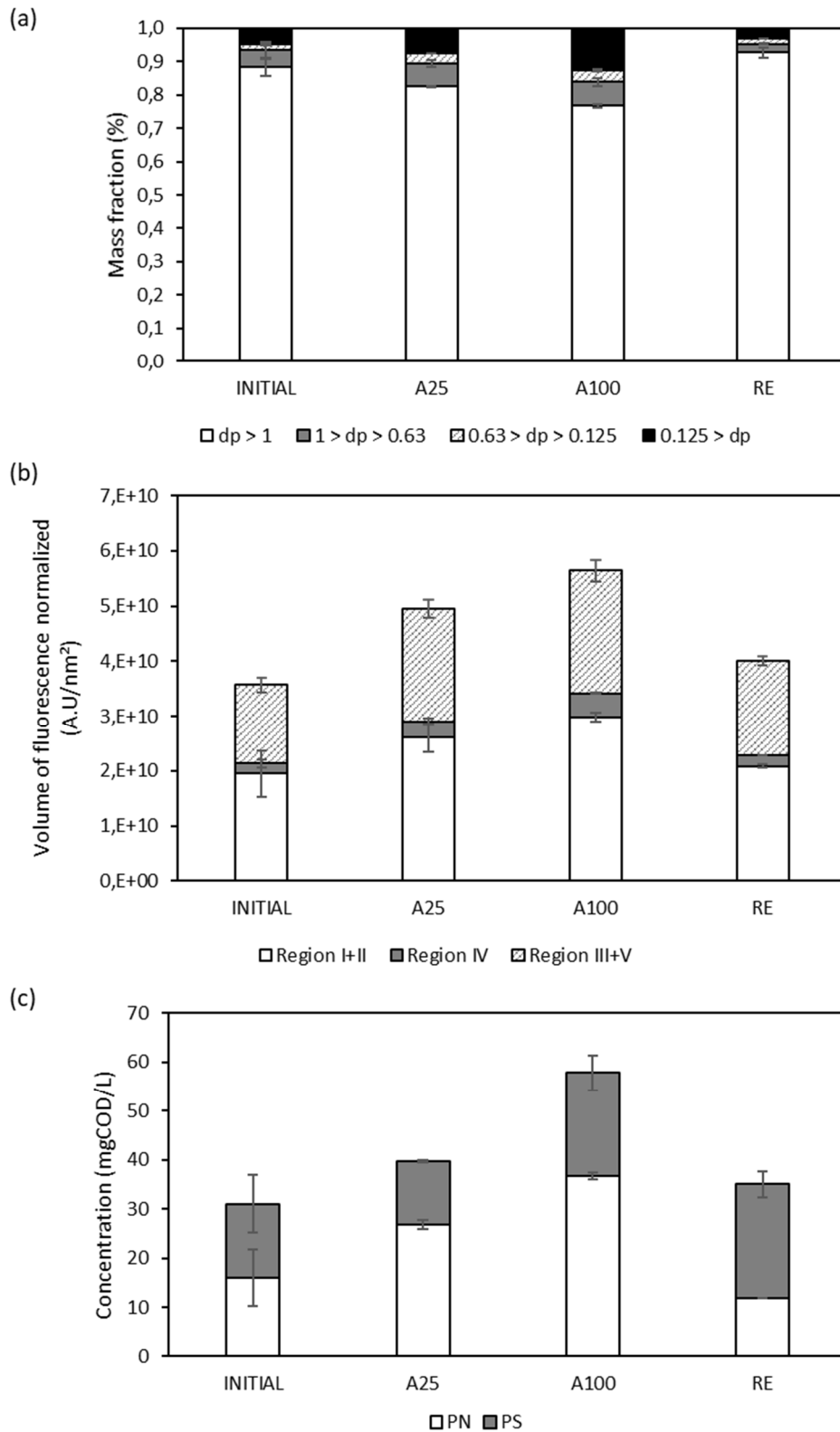
## 257 **3. Results and discussion**

### 258 **3.1 Granular sludge characteristics' behaviour during the filtration test**

259 In order to follow the raw mixed liquor trends during the filtration test, particle size  
260 distribution (Fig. 2a), total volume of fluorescence (Fig. 2b), and PS and PN  
261 concentrations (Fig. 2c) were measured. The initial raw mixed liquor was mainly  
262 composed of large granules, i.e. around 95% of mass fraction of particles over 0.125  
263 mm in diameter ( $d_p \geq 0.125$ ). Initially, the DCOM of the raw mixed liquor was  
264 predominantly composed of proteins ( $54.2 \pm 4.0$  %) and humic substances

265 (40.9 ± 5.0 %) and with a total volume of fluorescence of  $3.6 \pm 0.5 \cdot 10^{10}$  A.U.nm<sup>-2</sup>. The  
266 recirculation (RE) condition had a slight effect on the PSD with a lower proportion of  
267 smaller compounds, probably due to the aggregation of particles resulting from the low  
268 shear rate, however, no significant change in organic composition was observed. In  
269 contrast, in the A25 and A100 conditions, the large granule content ( $d_p \geq 1.0$ ) decreased  
270 from 88% to 83% and 77%, respectively, showing that higher shear stress increased the  
271 number of smaller granules. In the same way, the volume of fluorescence reached  $4.9 \pm$   
272  $0.5 \cdot 10^{10}$  and  $5.6 \pm 0.3 \cdot 10^{10}$  A.U/nm<sup>2</sup> for A25 and A100, respectively, that is, +40% and  
273 +58% more than the initial value. Similarly, the concentration of PN increased  
274 especially for both aeration rates A25 and A100 (+67% and +129%, respectively)  
275 confirming the release of protein substrates from the granules. The greater the forces of  
276 attrition, the higher the total volume of fluorescence and proteins became, underlining  
277 granule degradation and its release of fines and DCOM. This confirmed the pre-  
278 established positive correlation between hydrodynamic forces, attrition forces and  
279 granule disruption. Granule attrition created crevices on the granule surface and pushed  
280 surface bacteria and DCOM off the granule [27]. Moreover, soluble COD (sCOD)  
281 membrane removals are shown in supplementary materials. The sCOD removal rate was  
282 globally not affected by the hydrodynamic conditions, as the sCOD rejection was  
283 constant and about  $27.2 \pm 10.5$  %.

284



285

286 Fig. 2 - Evolution of (a) Particle Size Distribution (PSD), (b) relative percentage volume of fluorescence  
 287 from 3DEEM and (c) concentration of PN and PS of the raw mixed liquor before the experiment (initial)  
 288 and after each hydrodynamic operating condition applied (A25, A100, RE).

289

290

### 3.2 Effect of sludge fraction and operating conditions on critical flux

291

The critical flux results are given in Fig. 3. In all conditions, the granule fraction

292

showed lower fouling potential, with the lowest TMP in all conditions applied, which

293

resulted in highest critical flux values ( $J_c > 22$  LMH) (Fig. 3d). The TMP profiles also

294

showed that no incremental effect occurred when the supernatant and granules were

295

both present in the raw mixed liquor. Hence, the supernatant was found to be the major

296

foulant, emphasised by the lower and similar critical fluxes of both the raw mixed liquor

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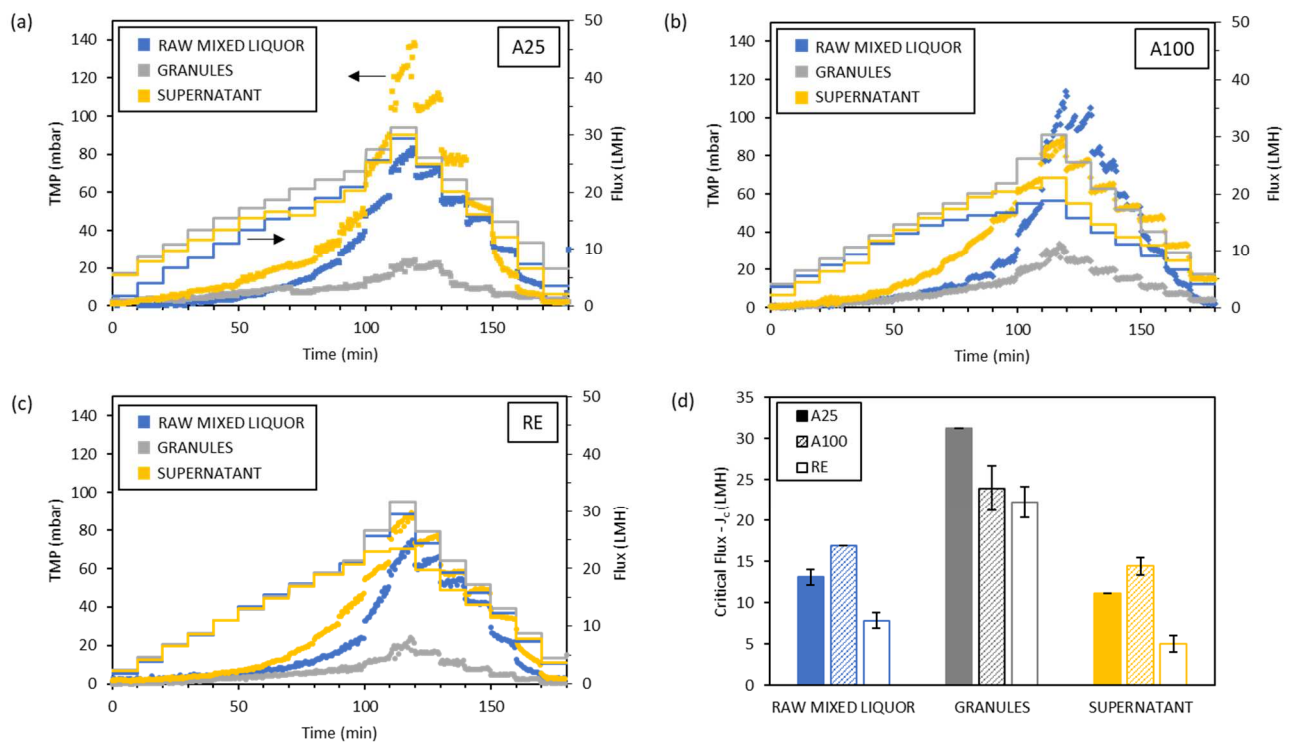
and supernatant fractions. These results support those observed in recent studies which

298

stated that micro-particles ( $0.45\text{-}10\mu\text{m}$ ) – including some colloids – were mainly

299

responsible for membrane fouling in AnMBR [13,14,28].



300

301

Fig. 3 – Evolution of TMP and flux for the raw mixed liquor and the two fractions at the different

302

operating conditions (a) A25, (b) A100, (c) RE and the (d) critical flux obtained through the TMP-based

303

indicators.

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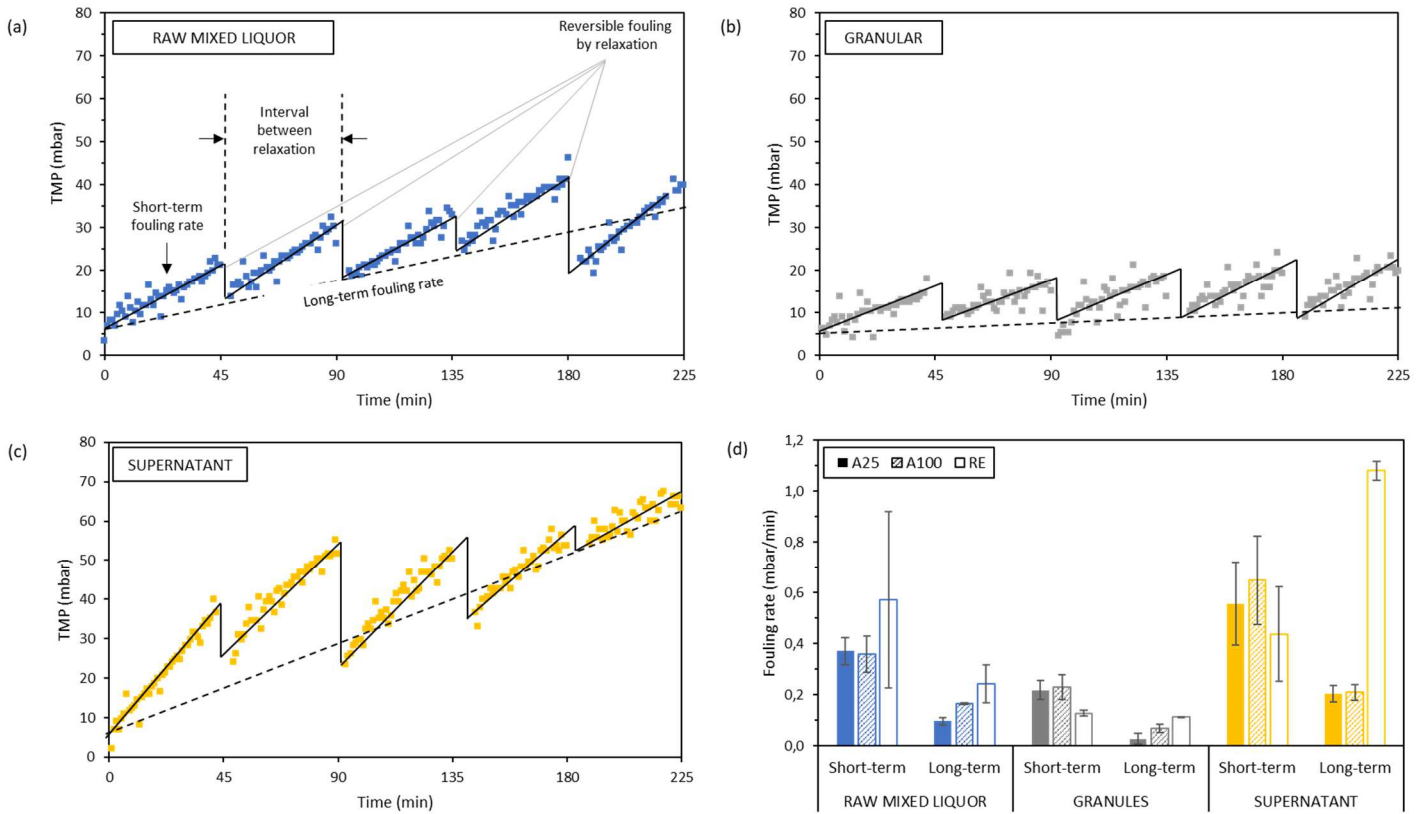
305 The increase in hydrodynamic conditions, induced by gas sparging, had a beneficial  
306 effect on filtration performance, since the critical flux values were positively related to  
307 the shear stress, except for the granule fraction (Fig. 3d). In the latter case,  
308 hydrodynamic conditions were too high and detrimental for the granule filtration  
309 capacity with a drop in critical flux from 31.3 LMH in A25 condition to 24 LMH for  
310 A100. This phenomenon can be linked to the granule disruption mentioned earlier  
311 which led to a rise of fine particles and colloidal and dissolved compounds (Fig. 2).  
312 These findings support that shear forces are of high importance in mitigating membrane  
313 fouling but the use of gas scouring as a fouling mitigation method has to be precisely  
314 adapted to avoid the detrimental effect of the shear stress, such as granular biomass  
315 disruption, membrane fouling by fines, and energy overspending.

316 In addition, while comparing the TMP reached during the flux increasing phase and  
317 decreasing phase, several phenomena were observed. An apparent hysteresis was  
318 observed, for the supernatant fraction and raw mixed liquor especially, suggesting non-  
319 reversible fouling by the tested turbulence (see supplementary data). Interestingly,  
320 below the critical flux, the gap between the ascending TMP and the descending TMP  
321 was reduced in conditions A25 and A100. This phenomenon highlights that above the  
322 critical flux, there was still an accumulation of foulant on the membrane surface.  
323 Conversely, below the critical flux, no significant deposition of particles occurred, so  
324 during the decreasing phase, the TMP declined because of the flux reduction and the  
325 cake layer detachment under the aeration effects. Nevertheless, the granule fractions  
326 showed no apparent hysteresis under both aeration conditions, meaning that particle  
327 deposition was mostly reversible regardless of flux. Finally, in the RE condition, a  
328 strong level of hysteresis was observed for all fractions. During the decreasing flux



329 steps, the TMP dropped linearly, which emphasised the absence of additional  
 330 accumulation and a lack of foulant detachment [29].

331 **3.3 Membrane filtration behaviour**



332

333 Fig. 4 – Evolution of the TMP over time during the five cycles of filtration with aeration at 25L/min for  
 334 (a) the raw mixed liquor, (b) granules, (c) supernatant and (d) the corresponding average reversible fouling  
 335 rate for each filtration condition (A25, A100, RE) and for raw mixed liquor and the two fractions.

336

337 The change in TMP during the filtration tests of raw mixed liquor and the two fractions  
 338 for the A25 condition are presented in Fig. 4. An overview of the A100 and RE  
 339 conditions is provided in the supplementary data. From these TMP profiles, two fouling  
 340 rates were determined: (i) the average short-term fouling rate, which corresponds to the  
 341 mean fouling rate observed between intermittent relaxation steps and (ii) the long-term  
 342 fouling rate (Fig. 4a and Fig. 4d). First, the lowest fouling capacities of the granule  
 343 fraction were confirmed, whatever the hydrodynamic conditions, with a negligible TMP

344 increment ( $\sim 0.1\text{-}0.2\text{ mbar}\cdot\text{min}^{-1}$ ) during operation cycles. In comparison, the supernatant  
345 presented a TMP increase almost three times higher ( $\sim 0.45\text{-}0.65\text{ mbar}\cdot\text{min}^{-1}$ ),  
346 highlighting its stronger fouling propensity. This is in accordance with previous results  
347 which stated that the membrane permeability declines with the decrease of the particle  
348 size deposition which forms a more compact fouling deposit and leads to higher pore  
349 blocking [8,30]. Interestingly, under aeration conditions (A25 and A100), the raw mixed  
350 liquor sample, which combines granules and supernatant fractions, exhibited TMP  
351 profiles and fouling rates below the supernatant ones, with a short-term fouling rate  
352 around  $0.35\text{ mbar}\cdot\text{min}^{-1}$ , confirming the benefit effect of granules upon the supernatant  
353 fouling behaviour. Indeed, it has been reported that the granular sludge structure is  
354 largely favourable for membrane mitigation due to the larger particle diameter than  
355 membrane pore, leading to low pore blocking. Moreover, when combined with gas  
356 sparging, the granules had an additional scouring effect, which helped to diminish the  
357 flocs accumulation on the membrane surface and decrease the penetration driving force  
358 of fine particles on membrane pores [8].

359 With regards to the hydrodynamic conditions applied, no significant differences were  
360 observed for short-term fouling rates between the A25 and A100 conditions, regardless  
361 of the fraction filtered. Numerous studies have shown that there is a critical gas velocity  
362 above which the gas sparging flow rate no longer impacts the fouling rate [11,17,31].  
363 Above the threshold gas sparging rate, the increase of shear stresses has no additional  
364 effect on particle deposition mitigation and the coalescence of the air bubbles can even  
365 reduce the shear events in the vicinity of the membrane [32]. Furthermore, the back-  
366 transport resulting from the hydrodynamic conditions has been positively correlated to  
367 the particle size, meaning that smaller particles face lower shear-induced diffusion

368 [33,34]. Hence, it is likely that membrane fouling in G-AnMBR is mainly a result of  
369 smaller compounds and dissolved matter.

370 Moreover, the long-term fouling rate values (Fig. 4d) for the A100 condition were  
371 found to be higher than the A25 for the granules and raw mixed liquor (0.07 vs 0.03  
372 mbar.min<sup>-1</sup> and 0.16 vs 0.10 mbar.min<sup>-1</sup> respectively). This phenomenon is almost  
373 certainly due to the attrition of the granules that increased the amount of smaller  
374 compounds (see Fig. 2a), as well as extracellular polymeric substances [31], which  
375 might contribute to membrane fouling that is less responsive to the relaxation step.  
376 Moreover, for the granule fraction with RE condition, the short-term and long-term  
377 fouling rates were almost similar, suggesting that the relaxation steps did not have a  
378 significant fouling mitigation effect. Conversely, the RE condition is the least effective  
379 solution for fouling management when fine compounds ( $d_p < 0.125$ ) are in abundance (i.e.  
380 raw and supernatant samples), probably due to a lack of shear events at the water-  
381 membrane interface. These filtration tests support the careful consideration that should  
382 be given to the hydrodynamic parameters in G-AnMBR. Shear conditions have to be  
383 great enough to prevent membrane fouling and provide a long-lasting filtration, but not  
384 too high to avoid excessive energy consumption and adverse effects on granular  
385 biomass. This is of great importance, because the damage of granular sludge does not  
386 only have a detrimental effect on membrane fouling, but it also reduces the organic  
387 removal efficiency, since biomass activity and syntrophic associations are hindered  
388 [4,35].

### 389 **3.4 Filtration resistance**

390 Fig. 5 shows the filtration resistances measured at the successive stages of the filtration  
391 tests and the different types of fouling deducted from the resistance-in-series. A  
392 significant difference was observed between the resistance recorded at the end of the

393 filtration test and the total resistance measured on the fouled membrane by filtering  
394 deionised water, notably for the A25 and A100 conditions. Therefore, the average total  
395 resistances measured during the 10 last minutes of fifth filtration cycle are also  
396 presented in Fig. 5. This difference may be due to the concentration polarisation  
397 occurring during the filtration of fractions but mainly to the manipulation of the  
398 membrane and aeration shear stresses during the permeability measurement, which  
399 unintentionally contributed to removing the fouling during the total resistance  
400 measurement. In fact, it is as if a membrane cleaning by gas sparging had been  
401 performed. Nonetheless, these facts show that the filtration resistances caused by cake  
402 build-up were easily suppressed by gas sparging and, therefore, counted as reversible  
403 fouling.

404 The higher fouling potential of the supernatant fraction and the synergetic effect  
405 between granules and supernatant fractions were confirmed by the total resistance,  
406 measured at the end of the fifth filtration cycle, which diminished from  $14.2 \cdot 10^{11} \text{ m}^{-1}$  for  
407 supernatant filtration to  $7.5 \cdot 10^{11} \text{ m}^{-1}$  for raw mixed liquor in A25 condition, from  $13.6$   
408  $10^{11}$  to  $8.5 \cdot 10^{11} \text{ m}^{-1}$  at A100, and from  $60.7 \cdot 10^{11}$  to  $33.3 \cdot 10^{11} \text{ m}^{-1}$  at RE. In the presence  
409 of granular sludge, it has been reported that the cake layer built on the membrane  
410 surface had a lower filtration resistance by means of a high cake porosity and the  
411 collision of granules with flocs, biopolymers and membrane surface (Zhang et al., 2020;  
412 Zhang et al., 2021). These results clearly show that the lack of shear stress in the vicinity  
413 of the membrane (e.g. RE condition) led to a stronger increase in fouling resistance,  
414 since the applied shear forces were not sufficient to counteract the penetration driving  
415 forces [8] and to promote the scouring effect of the granules. It should be noticed that in  
416 the RE condition, constant filtration fluxes were difficult to sustain and thus high  
417 resistance variabilities were observed in the experiment (see supplementary material).

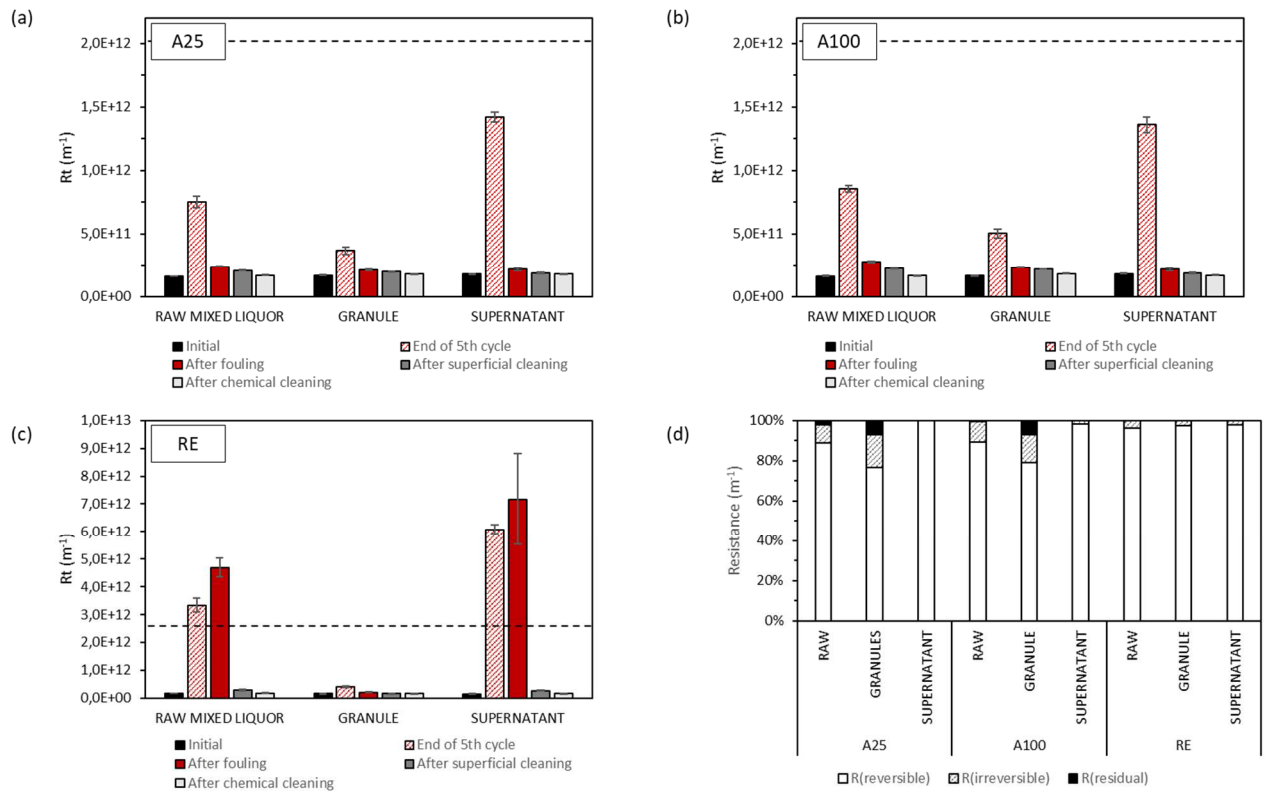
418 Fig. 5d describes the relative reversibility of the fouling deposit for each experiment. In  
419 any hydrodynamic condition, the filtration resistance built up during the filtration of the  
420 supernatant was predominantly reversible ( $\geq 98\%$ ), meaning that almost all of the  
421 fouling was removed by the physical cleaning. Interestingly, for the granule fraction, in  
422 both aeration conditions, around 20% of the total resistance remained after superficial  
423 cleaning ( $R_{\text{irreversible}} + R_{\text{irrecoverable}}$ ), and 7% of the filtration resistance was not even  
424 recovered after chemical cleaning ( $R_{\text{irrecoverable}}$ ). Regarding the raw mixed liquor, about  
425 10% of the fouling resistance remained after physical cleaning. Based on these findings,  
426 it could be suggested that different fouling mechanisms were implicated relative to the  
427 fraction filtered. During the filtration of the supernatant fraction, the rapid accumulation  
428 of micro-particles and fines led to the build-up of a cake layer. Then, under the  
429 continuous filtration, the cake layer was compressed, causing the change in cake  
430 structure and a shift in particle size distribution to a larger size next to the membrane  
431 surface [36]. The cake consolidation allowed for an easier and effective cake removal,  
432 since it allows the detachment of large agglomerates and large layer fragments [37]. In  
433 contrast, when the supernatant was combined with the granule fraction (i.e. raw mixed  
434 liquor), the cake layer was more porous because of larger particle size, which was  
435 beneficial for the filtration performance. However, soluble and smallest substances can  
436 pass through the loose cake layer and attach to the membrane surface or block the  
437 membrane pores [10]. A lower reversibility of small and single particles which interact  
438 with the membrane surface has been reported [37] which could explain the measured  
439 irreversible and residual resistances.

440 These distributions of the nature of the fouling should be interpreted with caution,  
441 because although it describes the relative repartition of the resistances, it does not  
442 highlight their effective resistance to the filtration. Hence, even if a part of the fouling

443 caused by granules (i.e. raw and granule fractions) was not removed by the cleaning  
444 methods employed, the filtration resistance caused by the persistent foulants was still  
445 low compared to the initial filtration resistance ( $R_m = 16.5 \pm 1.1 \cdot 10^{10} \text{ m}^{-1}$ ).

446 In contrast, for all fractions in the RE conditions, the initial resistance was restored  
447 almost entirely with the physical cleaning ( $\geq 96\%$ ). In this case, the low hydrodynamic  
448 conditions resulted in a barely fixed granular sludge bed, so that the granule fraction  
449 was not in the vicinity of the membrane and did not take part of the cake layer  
450 formation. Therefore, the membrane fouling that took place during the raw and granule  
451 fractions must have been composed of micro-particles and non-settable compounds  
452 similar to the supernatant fraction and thus had the same compact structure which  
453 induced the same degree of reversibility.

454 Based on these results, a combine fouling mitigation method can be suggested with  
455 short intermittent cycles composed of filtration without gas sparging intersected by  
456 relaxation and gas sparging periods. In this way, the granules would not be degraded,  
457 the fouling deposition would be mostly reversible and the gas sparging energy demand  
458 would be lowered. This is in accordance with a previous G-AnMBR study in which, in  
459 absence of gas sparging, high fouling resistance was observed, but almost all the  
460 accumulated cake was removed by simultaneous use of relaxation and gas sparging  
461 [17].



462

463 Fig. 5 – Evolution of the filtration resistance at different operating stages: (i) the initial stage (i.e.  $R_t =$   
 464  $R_m$ ), (ii) the end of the fifth cycle of filtration, (iii) the fouled membrane, (iv) after the superficial  
 465 cleaning, and (v) after the chemical cleaning for (a) the 25L/min aeration rate, (b) the 100 L/min aeration  
 466 rate, (c) the recirculation, and (d) repartition of the different type of fouling resistance for raw mixed  
 467 liquor and the two fractions at different operating conditions.

468

### 469 3.5 Membrane fouling characteristics

470 Table 1 presents the repartition of the volume of fluorescence obtained through 3DEEM  
 471 for rinsing water collected from each superficial cleaning – i.e. the reversible foulant. In  
 472 all cases, the largest amount of fluorescence appeared in region I+II, accounting for 68-  
 473 85% of the total fluorescence. According to Jacquin et al. (2017), region I+II from the  
 474 3DEEM fluorescence is associated with colloidal proteins and, consistent with the  
 475 present results, they appeared to be the major foulants in G-AnMBR. It appears that  
 476 micro-particles (0.45-10  $\mu m$ ) play a key role in G-AnMBR, as already observed in  
 477 classical AnMBR studies [14], and organic foulant compounds are mainly protein-like  
 478 substances [38]. The critical role of proteins has already been underlined due to their

479 greater hydrophobicity, which induces a higher adhesion capacity of protein-rich  
480 compounds to the polymeric membrane surface [17,39]. The total volume of  
481 fluorescence of every fraction in RE conditions was definitely higher than under  
482 aeration conditions. It seems that the higher filtration resistance is due to a larger  
483 amount of foulants on the membrane surface rather than a different and harsher type of  
484 foulant. Moreover, the volume of fluorescence increased in the A100 condition relative  
485 to A25 which described a stronger organic matter deposition, certainly linked to the  
486 granule disruption and protein release mentioned previously (see Section 3.1).

#### 487 **4. Conclusion**

488 From the present study, the following conclusions can be drawn:

- 489     ▪ Granules ( $d_p \geq 0.125$ ) had a negligible fouling potential with a fouling rate below  
490     0.2 mbar.min<sup>-1</sup> whatever the hydrodynamic conditions used.
- 491     ▪ The supernatant fraction, composed of fine compounds and flocs ( $d_p < 0.125$ ), was  
492     the key driver of membrane fouling in G-AnMBR. Nevertheless, the related  
493     membrane fouling was reversible with more than 98% of the fouling resistance  
494     recovered by simple water cleaning. Moreover, the compression of the cake  
495     layer under the drag forces led to a denser layer, which enables its complete  
496     removal.
- 497     ▪ In the raw mixed liquor, where supernatant and granules are mixed, the fouling  
498     rate was lower compared to the supernatant fraction. It is suggested that granules  
499     diminished the impact of the fines and micro-particles over membrane  
500     permeability through mechanical scouring action and the formation of a more  
501     porous and loose cake layer structure. Interestingly, around 20% of the total  
502     fouling resistance remained after the superficial cleaning, suggesting that the



503 loose cake layer does not prevent small and adherent foulants from entering the  
504 membrane pore, causing residual fouling.

505     ▪ Based on the 3DEEM analysis, at least 68% of the fluorescent organic matter  
506 from the reversible fouling came from the protein-*like* region regardless of the  
507 fraction. Hence, colloidal proteins seemed to be the main organic foulant in G-  
508 AnMBR.

509     ▪ Hydrodynamic conditions were of high importance in mitigating membrane  
510 fouling. In tested conditions, gas sparging was more efficient in limiting  
511 membrane fouling than recirculation. However, a plateau was reached in gas  
512 sparging rate, above which the increase gas flow does not lead to a decrease in  
513 fouling rate. Moreover, higher shear stress led to stronger granule disruption,  
514 releasing smaller compounds which in turn increased membrane fouling.

515 Based on the results, it is evident that well-shaped and high-strength granules have to be  
516 privileged in G-AnMBR. The induced shear forces have to be sufficient to scour the  
517 membrane surface whilst not damaging the granular biomass, nor incurring unnecessary  
518 energy consumption. The fouling mitigation-energy nexus could lie at an intermittent  
519 filtration cycle associated with the threshold sparging rate. Further investigation and  
520 technical-economic analysis have to be conducted to define the most favourable  
521 filtration cycle which maximises the net energy balance of the G-AnMBR process. In  
522 addition, long-term experiments need to be studied to see the potential composition  
523 change of the mixed liquor and fractions over time, and its consequences on fouling  
524 behaviour.

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530 Table 1 – Repartition of the volume of fluorescence within 3DEEM regions of the superficial cleaning for the three fractions and hydrodynamics conditions studied.

Region	Units	RAW MIXED LIQUOR			GRANULES			SUPERNATANT		
		A25	A100	RE	A25	A100	RE	A25	A100	RE
I+II	$\times 10^9$ (A.U.m <sup>-2</sup> .L <sup>-1</sup> )	2,0 (82.1%)	3,0 (84.8%)	18,3 (81.3%)	1,2 (68.0%)	2,3 (78.4%)	8,3 (78.6%)	1,7 (81.7%)	2,8 (76.0%)	18,0 (85.0%)
IV	$\times 10^9$ (A.U.m <sup>-2</sup> .L <sup>-1</sup> )	0,1 (4.6%)	0,2 (4.4%)	1,5 (6.8%)	0,1 (3.5%)	0,1 (3.6%)	0,4 (3.6%)	0,1 (3.8%)	0,2 (4.1%)	1,3 (6.0%)
III+V	$\times 10^9$ (A.U.m <sup>-2</sup> .L <sup>-1</sup> )	0,3 (13.3%)	0,4 (10.8%)	2,7 (11.8%)	0,5 (28.5%)	0,5 (18.0%)	1,9 (17.8%)	0,3 (14.5%)	0,7 (19.9%)	1,9 (9.0%)
Total	$\times 10^9$ (A.U.m <sup>-2</sup> .L <sup>-1</sup> )	2,4 (100%)	3,6 (100%)	22,6 (100%)	1,8 (100%)	2,9 (100%)	10,6 (100%)	2,0 (100%)	3,7 (100%)	21,1 (100%)

531

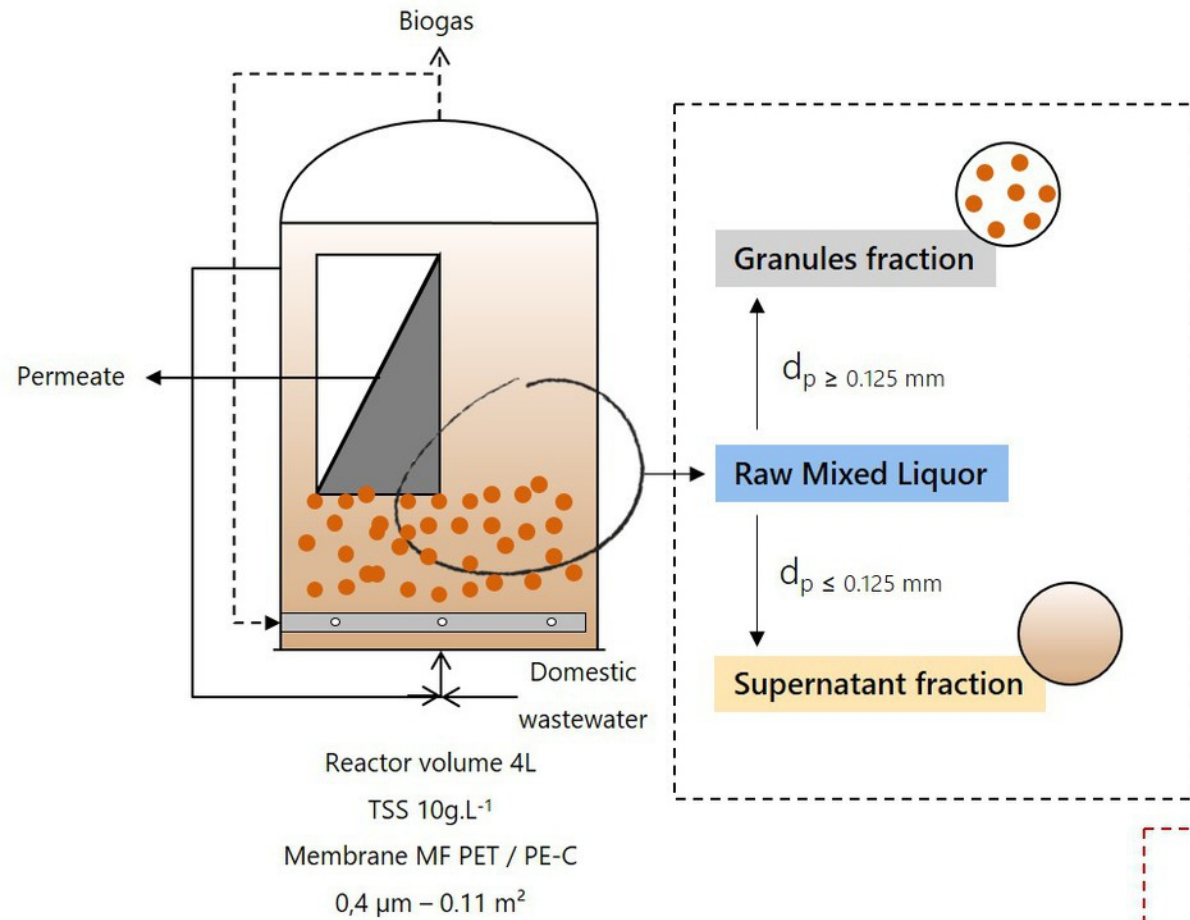
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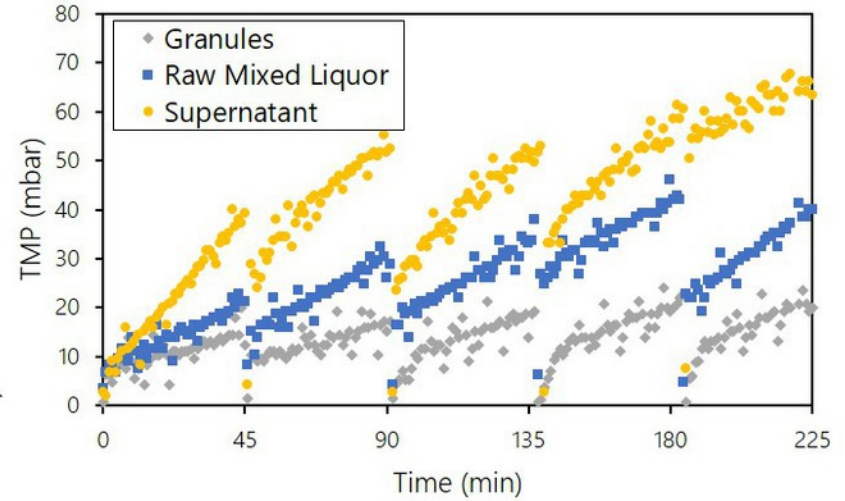
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# Granular Anaerobic Membrane Bioreactor (G-AnMBR)



Filtration tests



## MEMBRANE FOULING MITIGATION STRATEGY PROPOSED

Intermittent filtration cycle  
+  
Intermittent gas-sparging

↓ granules break-up  
↑ reversible fouling behavior  
↓ energy-demand

