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2 **Inorganic fouling of an anaerobic membrane bioreactor treating leachate from the Organic**

3 **Fraction of Municipal Solid Waste (OFMSW) and a polishing aerobic membrane**

4 **bioreactor**

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8 Antoine P. Trzcinski* and David C. Stuckey

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11 Department of Chemical Engineering and Chemical Technology, Imperial College of Science

12 and Technology and Medicine, Prince Consort Road, London SW7 2BY, UK

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20 * Corresponding author. E-mail: antoinetrzcinski@hotmail.com Tel.: +65 6592 7758

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26 **Abstract**

27 The treatment of leachate (Average TCOD=11.97 g/L, 14.4% soluble) from the organic fraction

28 of municipal solid waste was investigated using a Submerged Anaerobic Membrane BioReactor

29 (SAMBR), followed by an Aerobic Membrane BioReactor (AMBR) to polish this effluent. This

30 paper investigated the exact nature and composition of the inorganic precipitate in each of the

31 reactors in the process. The flux decreased due to precipitation of calcium as monohydrocalcite

32 ($CaCO_3.H_2O$) containing traces of metals onto the SAMBR membrane because of high CO_2

33 partial pressures. Precipitation of calcium in the AMBR was also observed due to a higher pH. In

34 this case, phosphorus also precipitated with calcium in two different phases: the background

35 layer contained calcium, oxygen, carbon and small amounts of phosphorus (2-6.7%), while

36 flakes containing calcium, oxygen and higher amounts of phosphorus (10-17%) were probably

37 hydroxyapatite ($Ca_5(PO_4)_3OH$).

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43 **1.   Introduction**

44 The main advantages of membrane bioreactors (MBR) include rapid start-up and a higher

45 loading rate than classical technologies (Stephenson et al., 2000), which combines in one unit the

46 removal of COD, solids and nutrients, thus giving rise to a small footprint and a very high

47 quality permeate with no suspended solids. Anaerobic MBRs have the added advantage of

48 producing energy in the form of biogas, and produce very little excess sludge reducing the

49 burden of sludge disposal. In a Submerged Anaerobic Membrane Bioreactor (SAMBR) the

50 membrane is submerged within the reactor, and membrane cleaning is accomplished by

51   recirculating the biogas as large bubbles underneath the membrane to scour it and alleviate

52   biofouling. Several researchers have also achieved fouling reduction by gas sparging (Hong et

53   al., 2002; Li et al., 2005), and other turbulence promoting techniques such as gas/liquid slug flow

54   (Mercier-Bonin et al., 2001) or polymeric particles (Imasaka et al., 1989). However, fouling

55   remains the main drawback of MBRs since it reduces flux through the membrane, and increases

56   trans-membrane pressure (TMPs) drops. Nevertheless, fouling can enhance COD removal since

57   the fouling layer acts as a secondary membrane and enhances the rejection of low molecular

58   weight (MW) solutes.

59

60   Fouling can occur by: adsorption or deposition of macromolecules onto the membrane surface;

61   by adsorption onto the pore surface; or, by complete pore-blocking. The phenomenon is

62   exacerbated by concentration polarisation as it increases the concentration of macromolecules

63   and particles in the vicinity of the membrane. Fouling can also be related to biological growth

64   due to cell attachment and extracellular polymeric substances (EPS) filling the void spaces

65   between the particles in the cake (Chu et al., 2005). Other fouling can be attributed to

66   components in the feed such as proteins, colloids and particulate materials. Colloids cover a wide

67   size range, from a few nanometers to a few micrometers, and can comprise clay minerals,

68   colloidal silica, iron, aluminium, and manganese oxides, organic colloids and suspended matter,

69   and calcium carbonate precipitates (Boussu et al., 2006; Mahvi & Razavi, 2005). Dissolved ions

70   may also precipitate in the form of struvite which is an inorganic precipitate with the chemical

71   formula: $MgNH_4PO_4 \cdot 6H_2O$, and has been reported by several investigators as playing a key role

72   in flux decline (Choo & Lee, 1996; Kang et al., 2002). Kang et al. (2002) reported that struvite

73   was found to have accumulated inside the pores of a zirconia skinned inorganic membrane, but

74   not in an organic polypropylene membrane. Choo and Lee (Choo & Lee, 1996) also found that

75   struvite plays a significant role in the consolidation of biomass cakes on the membrane surface.

76 Struvite is expected to be a problem in MBRs treating municipal solid waste (MSW) or the

77 leachate because it contains all the elements required to form struvite. Struvite precipitation is

78 also facilitated when the pH increases, and as a result acidic solutions are required to dissolve the

79 inorganic foulant (Kang et al., 2002). Other inorganic foulants in anaerobic environments can

80 also include $CaCO_3$ polymorphs such as calcite, aragonite, vaterite, monohydrocalcite, ikaite and

81 amorphous calcium carbonate (in order of decreasing stability) (Meiron et al., 2011; Salek et al.,

82 2016). However, the precipitation of inorganic salts can also be affected by module geometry

83 and membrane materials (Shih et al., 2005), and there is limited information on the effect of

84 these factors on fouling mechanisms. However, there is very little information available

85 describing inorganic fouling on membrane surfaces (Shirazi et al., 2010).

86

87 In the SAMBR, biogas is recirculated to scour the membrane surface which results in high CO_2

88 partial pressures. Information about inorganic fouling under these conditions in a SAMBR

89 treating leachate from the organic fraction of municipal solid waste (OFMSW) is poorly

90 documented. This paper presents a detailed investigation into the fate of inorganics and dissolved

91 ions in a novel process treating OFMSW leachate, and identifies the inorganic foulants on the

92 membranes.

93

94 **2.   Materials and methods**

95 *2.1. Leachate from the Organic Fraction of Municipal Solid Waste (OFMSW)*

96 The synthetic leachate used in this study was producing in a bench scale anaerobic reactor (20L)

97 from real components of Municipal Solid Waste: 41.3% kitchen wastes, 10.8% garden wastes

98 and 47.9% paper wastes on a wet basis as documented in a previous study (Trzcinski & Stuckey,

99 2009a). The leachate had the following properties: pH: 6-7.5, SCOD: 1,070-2,925 mg/L

100 (average: 1,730 mg/L), TCOD: 5.72-26.78 g/L (average: 11.97 g/L), Volatile fatty acids: 155-

101   1,290 mg/L as COD (average: 570 mg/L), Ammonia-nitrogen: 7-140 mg N/L (average: 44 mg

102   N/L), Phosphorus: 3.9-24 mg P/L as orthophosphates (average: 11 mg/L).

103

104   *2.2. Membrane BioReactors (MBRs)*

105   The Submerged Anaerobic Membrane BioReactor (SAMBR) was a three litre reactor fitted with

106   a Kubota polyethylene flat sheet membrane of 0.1 m^2 of total surface and a pore size of 0.4

107   microns. The SAMBR was maintained at $35 \pm 1°C$, and details of the reactor and inoculation can

108   be found elsewhere (Trzcinski & Stuckey, 2009b). The SAMBR was fed continuously at organic

109   loading rates in the range of 1-19.8 g $COD·L^{-1}·day^{-1}$ (average: 8.1 g $COD·L^{-1}·day^{-1}$).

110   One pump was used to set a constant flux through the membrane, while some of the permeate

111   was recycled back to the SAMBR in order to set the hydraulic retention time (HRT). The biogas

112   sparging rate was set at 5 L/min (LPM) to minimize cake formation on the membrane. The

113   permeate from the SAMBR was fed to an Aerobic Membrane BioReactor (AMBR) for

114   polishing. The AMBR was identical to the SAMBR except that it was maintained at ambient

115   temperature (21-22°C), and was inoculated with aerobic biomass from a wastewater plant at an

116   initial MLTSS and MLVSS of 3 and 2.3 g/l, respectively, and air was used to mix the reactor

117   contents at 2 LPM.

118

119   *2.3. Analytical methods*

120   The measurement of pH (Jenway 3020 pH Meter) was accurate to within ±0.02 units. The

121   measurement of Mixed Liquor Total Suspended Solids (TSS), Volatile Suspended Solids (VSS)

122   and Fixed Suspended Solids (FSS) was carried out weekly according to Standard Methods

123   (APHA, 2012). The coefficient of variation (COV) for ten identical samples was 4%, 3.1% and

124   7.1% for TSS, VSS and FSS, respectively.The Soluble Chemical Oxygen Demand (SCOD)

125   analysis was carried out biweekly as in Standard Methods (APHA, 2012) after filtration through

126 a 0.45 µm filter (Sartorius, Minisart), and the COV for ten identical samples was 2.6%. Volatile

127 fatty acids (VFAs) were measured biweekly using a Shimadzu Gas Chromatograph with a flame-

128 ionized detector and a SGE capillary column (12mx0.53mm ID-BP21 0.5µm); the COV was 3%

129 for ten identical samples. Ammonia-Nitrogen was measured biweekly using the Nesslerization

130 method by reading absorbance at 425 nm on a Shimadzu spectrophotometer; the COV was 6.6%

131 for 10 identical samples. The measurement of orthophosphates was carried out biweekly

132 according to the vanadomolybdophosphoric acid colorimetric method described in Standard

133 Methods (APHA, 2012). The absorbance was read on a spectrophotometer at 470 nm, and the

134 coefficient of variance for ten identical samples was ± 0.6%.

135 The method for alkalinity followed the procedure described in Standard Methods (APHA, 2012).

136 Weekly samples were titrated potentiometrically to an end-point of pH 4.5 with 0.1 N H_2SO_4.

137 The COV for 10 identical samples was within 2.7%. The calculation of alkalinity is shown below:

138 Alkalinity as mg $CaCO_3$/L = A × N × 50, 000/ mL sample

139 where: A= ml standard acid used, N= normality of standard acid. The ions Na^+, K^+, Mg^{++}, Ca^{2+},

140 Cl^-, PO_4^{3-}, were analyzed weekly using an Ion Chromatograph (Dionex) as described by the

141 American Public Health Association (APHA, 2012). The COV for the ions listed above were 2,

142 0.2, 0.5, 0.6, 1.1 and 3.4 % for 5 identical samples, respectively. The detection limit was between

143 50 and 100 ppb.

144 Fouled membranes were analyzed by scanning electron microscopy (SEM) and energy

145 dispersive X-ray (EDX) spectroscopy. SEM samples were fixed overnight at 4°C in 3%

146 glutaraldehyde and kept at pH 7.2 by a 0.1M phosphate buffer. Samples were then dehydrated in

147 a graded ethanol/water series (10-30-50-70-90-100%) for 20 minutes at each concentration, and

148 then dried for a day at 30 °C. Samples were sputtered-coated with gold or carbon (30mA for 2.5

149 minutes, vacuum 0.2 Torr) prior to SEM-EDX analyses. Specimens were examined and

150 photographed under a scanning electron microscopy (JEOL JSM-5610LV). Energy Dispersive

151 X-Ray analysis was conducted with an EDX-60 (Oxford instrument-incax-sight), and the EDX

152 analyzer was connected to a scanning electron microscope (model JSM-840A). More than one

153 point on the sample was analyzed by EDX. Powdered inorganic precipitates from the membranes

154 were also analyzed using a Bruker AXS using X-Ray Fluorescence technology (XRF). The exact

155 number of replicates is given along with the results.

156

157 *2.4. Membrane cleaning*

158 A cleaning procedure adapted from (Akram, 2006) was performed in order to determine the

159 relative contribution to fouling due to internal and external, organic and inorganic fouling of the

160 SAMBR and AMBR membranes. The virgin membrane flux of a new Kubota membrane was

161 determined by submerging it in distilled water under 1 meter of hydrostatic head. The flux, f_0,

162 was determined in triplicate, and the average $\pm$ standard deviation was $f_0 = 251 \pm 6$ LMH. At the

163 end of the experiment, the fouled membrane flux, f_1, was measured with distilled water under the

164 same conditions as described. The flux, f_1, was the result of all fouling types, i.e. organic and

165 inorganic, external and internal. The flux drop, FD1, due to all types of fouling was calculated as

166 $FD1 = f_0 - f_1$.

167 The membrane was then cleaned using different methods and chemicals in sequence. Firstly,

168 lukewarm water and a brush were used to remove the external organic fouling (biofilm) formed

169 on the membrane, assuming that any inorganic foulants would not be removed by this technique

170 due to their strong attachment. The measured flux, f_2, was then the residual flux due to combined

171 internal and external inorganic fouling. The flux drop, FD3, due to internal and external

172 inorganic foulants was then calculated as $FD3 = f_0 - f_2$. The flux drop due to organic external

173 foulants, or biofilm alone (FD2), was obtained as $FD2 = FD1 - FD3$.

174

175 The membrane was then soaked in 1% sodium hypochlorite for 2 hours to remove external and

176 internal organic foulants, and the flux was recorded as f_3. The flux drop due to inorganic foulants

177 only was then calculated as $FD4 = f_0 - f_3$. Finally, the membrane was soaked in 1% oxalic acid

178 for 2 hours to remove inorganic foulants, and the flux was recorded as f_4.

179

180 **3.   Results and discussion**

181 *3.1. Contribution of SAMBR and AMBR to overall COD removal in leachate*

182 The AMBR was started up at MLTSS and MLVSS concentrations of 3, and 2.3 g/L,

183 respectively, and was fed with the stabilized leachate, i.e. the permeate from SAMBR2. Hence

184 the feed contained no suspended solids. Figure 1 shows the contribution of both the SAMBR and

185 AMBR to the total COD removal from the OFMSW leachate at various HRTs from 5 days

186 during the start-up down to 0.4-0.6 days. The COD removal in the SAMBR was in the range of

187 89.7-98 % (Average: 94.5%), while the total COD removal increased into the range of 92.2-

188 98.4% (Average: 96.1%) due to the polishing action of the AMBR. The results demonstrated the

189 excellent resilience of the SAMBR to organic shocks at loading rates as high as 19.8 g COD·L⁻

190 ^{1}·day^{-1}, and HRTs as low as 0.4 days. Between 13.6% and 50.8% (Average: 26%) of the

191 SAMBR permeate COD was degraded in the AMBR. This illustrates the refractory nature of the

192 SAMBR permeate.

193

194 *3.2 Effect of the HRT on Flux and pH in the AMBR*

195 The pH in the AMBR started at 7.25 but then rapidly increased to 8.5 on day 4 and then

196 remained in the range 8.2-8.75 at all tested HRTs (Figure 2). The feed to the AMBR was

197 saturated in CO_2 because biogas was used in the SAMBR for mixing and scouring purposes. Due

198 to air sparging in the open AMBR, the CO_2 partial pressure dropped leading to lower dissolved

199 CO_2 according to Henry's Law. As a result of CO_2 stripping the carbonic acid equilibrium

200 shifted which caused the pH to rise.

201

202 The flux was found to decrease gradually from 3.5 LMH to 1.3 LMH towards the end of the

203 study indicating that considerable membrane fouling took place (Figure 2). It was found that

204 dissolved inorganic salts started to increase in the AMBR bulk, as evidenced by the mixed liquor

205 Fixed Suspended Solids (MLFSS) that increased from 0.7 g/L to 1.9 g/L during the study

206 (Supplementary material S1). Moreover, the MLVSS/MLTSS decreased accordingly from 76%

207 to 45% during the course of the study (Supplementary material S1) indicating a decrease in

208 organic content of the suspended solids in the AMBR. In contrast with the organic shocks

209 experienced in the SAMBR, the AMBR was found to experience inorganic shocks when the

210 HRT was reduced and this led to accumulation of dissolved solids in the bulk.

211 Figure 2 also shows that the flux in the AMBR was decreasing over time. Therefore, it is very

212 likely that calcium carbonate precipitating on the membrane was causing the flux to drop. Visual

213 inspection of the membrane indicated a hard, off-white, chalky deposit similar to limescale, and

214 contact with a drop of HCl provoked an immediate fizzing. Precipitation was also found to occur

215 in the permeate tubing; a sample was taken, rinsed with plenty of water and then dissolved in

216 deionized water with HCl. It was then filtered though a 0.2 micron filter and analysed on the ion

217 chromatograph which confirmed that the main element was calcium.

218

219 *3.3 Change in dissolved ion concentration in the process*

220 High salt levels can cause bacterial cells to dehydrate due to osmotic pressure, and Na^+ is more

221 toxic that any other salt on a molar basis. It is also known that cations determine the toxicity of

222 salts (McCarty & McKinney, 1961). Sodium, potassium, magnesium, calcium, phosphate and

223 sulphate are present in the influent of anaerobic digesters, and they are all required for microbial

growth. Moderate concentrations stimulate microbial growth, but excessive amounts can cause

inhibition or toxicity (Soto et al., 1993). Very little data is available for full-scale plants as ions

are not systematically measured to assess the health of anaerobic digesters.

Ion concentrations in the feed (OFMSW leachate), SAMBR permeate and AMBR permeate were

monitored to investigate the health of the process, as well as the fate of ions in the process and

the possibility of precipitation. The concentrations of NH_4^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, PO_4^{3-} are

plotted against time in Figure 3. The concentration of NH_4^+ was relatively low in the OFMSW

leachate, as most of the nitrogen was still in the proteinaceous form. The NH_4^+ concentration

increased significantly in the SAMBR permeate due to protein degradation to ammonia under

anaerobic conditions. In the AMBR, ammonia concentration was virtually zero as it was shown

in a previous study that nitrification took place in the AMBR (Trzcinski & Stuckey, 2009b).

In this study, the AMBR started with a sodium concentration over 1.5 g Na^+/L due to Owen et

al.'s biomedium (1979) added for the start-up, but over time the concentration stabilized to about

600 mg Na^+/L; this decrease could be due to sodium uptake for bacterial growth. In contrast,

there was a slight increase in Cl^- and K^+ concentrations during the study.

Excessive amounts of calcium can lead to the precipitation of carbonate which may result in the

scaling of reactors (Chen et al., 2008). It has been reported in the literature that calcium

concentrations in a real landfill leachate could be as high as 976 mg/L (Zayen et al., 2008).

Initially, calcium concentrations were in the range 20-60 mg/L, and the levels were similar in the

feed and both permeates suggesting that no precipitation occurred in the system. However, as the

level started to increase to 160 mg/L in the feed, significantly lower concentrations were

observed in both permeates showing that a loss of dissolved calcium occurred in the process. It

248 was therefore clear that calcium carbonates were precipitating in the SAMBR or on its

249 membrane.

250

251 High levels of magnesium and phosphate are not recommended because they can precipitate as

252 struvite ($NH_4MgPO_4 \cdot 6H_2O$). Because phosphate concentrations are usually lower than ammonia

253 and magnesium in an anaerobic digester, they will determine whether struvite precipitates or not.

254 The magnesium level remained between 100 and 200 mg/L in all the reactors, and then increased

255 to 200-250 mg/L in the OFMSW leachate and the two permeates. In fact, the Mg^{2+} concentration

256 was always slightly higher in the two permeates showing that it could not have precipitated to

257 form struvite as it was not removed from the liquid phase.

258

259 Phosphates (PO_4^{3-}) were found at a relatively low concentration in the feed and effluents, except

260 in the AMBR that started up at about 140 mg/L, presumably due to the level in the inoculum and

261 initial biomedium. The concentrations in the feed and permeate were then stable in the range of

262 20-40 mg/L, showing that it was not removed from the liquid phase. Using the solubility product

263 it is possible to predict if struvite can precipitate. The maximum concentration of NH_4^+, Mg^{2+}

264 and PO_4^{3-} in this study were 161, 246 and 25 mg/L, respectively, and the negative logarithm of

265 the solubility product ($pK_S = -\log K_S$) was equal to 7.6 which is lower than that of struvite

266 which is in the range 12.6 - 13.3 (Ohlinger et al., 1998). Thus in the present study, struvite did

267 not precipitate because of the low concentrations of phosphate, ammonia and magnesium, and

268 the neutral pH. This was confirmed by Stratful et al. (2001) who noticed that no struvite

269 precipitation occurred at pH 7 with concentrations of magnesium, ammonium and

270 orthophosphate equal to 187, 266 and 742 mg/L, respectively, while only a minute amount was

271 produced at pH 7.5.

272 This study suggests, therefore, that struvite precipitation is unlikely when treating a leachate

273 from real components of the organic fraction of municipal solid waste such as garden, kitchen

274 and paper waste; however, calcium is more likely to cause problems. In fact, direct treatment of

275 leachate containing calcium in an anaerobic membrane bioreactor should be avoided due to

276 inorganic precipitation on the membrane. Calcium precipitation should be carefully controlled in

277 a location (a separate precipitation unit) where it does not adversely affect the membrane

278 filtration process. Moreover, this research also demonstrated that CO_2 could be sequestrated in

279 the SAMBR by mineral carbonation. This offers an interesting option to remove CO_2 from the

280 biogas and increase its calorific value (Salek et al., 2016).

281

282 *3.4 Change in alkalinity in the process*

283 In theory the alkalinity should not change because the net reaction involving CO_2 stripping in the

284 AMBR removes the same number of equivalents of negatively contributing species (H^+) as

285 positively contributing species (HCO_3^-). However, the precipitation of calcium carbonate has a

286 strong influence on alkalinity; it will remove Ca^{2+} and CO_3^{2-} from solution, and CO_3^{2-} will

287 decrease the alkalinity by 2 times its molar concentration. Precipitation of calcium carbonate was

288 observed in our system, and the decrease in alkalinity was confirmed by titrating the feed,

289 SAMBR permeate and AMBR permeate. The alkalinity in OFMSW leachate was around 2,000-

290 3,000 mg eq. $CaCO_3$/L, except on day 67 where it reached 5,000 mg eq. $CaCO_3$/L. The SAMBR

291 and AMBR permeates were found to contain less alkalinity, in particular around day 67 when it

292 dropped to 2,250 and 750 mg eq. $CaCO_3$/L, respectively (Figure 1, bottom). Borzacconi et al.

293 (1999) have also observed that calcium ions can precipitate as $CaCO_3$ at a pH around 8. The

294 maximum concentration of calcium before precipitation can be predicted using the solubility

295 product assuming that the interference of other ions can be neglected. If $4.47 \cdot 10^{-9}$ is taken for K_{sp}

296 (Seely, 2008; Weast, 1963), the maximum solubility of Ca^{2+} in the AMBR is about 3.1 mg/L at

297   pH 8.5. Furthermore, this value shows a quadratic dependence with the concentration of H^+ so

298   that the maximum Ca^{2+} concentration is 100 times greater at pH 7 than at pH 8. In the SAMBR

299   at 35°C, the pH was 7 but the partial pressure of carbon dioxide was 0.2 atm (assuming 20% CO_2

300   in the headspace), and hence the CO_2 concentration in the liquid phase is $5.24 \cdot 10^{-3}$ M according

301   to Henry's law. The solubility of calcium at pH 7 is normally around 2,850 mg/L, but because of

302   the high partial pressure of carbon dioxide the solubility in SAMBR2 was only about 5.1 mg/L.

303   Thus precipitation can also occur in the SAMBR as well as in the AMBR if the calcium

304   concentration is greater than these theoretical values.

305

306   *3.5 Identification of the inorganic precipitate on the SAMBR membrane*

307   Scanning Electron Microscopy (SEM) was used to investigate the morphology and structure of

308   the inorganic precipitate found on the SAMBR membrane which appeared as a homogeneous

309   layer with little apparent porosity. This technique was coupled to Energy-Dispersive X-rays

310   (EDX) in order to determine the chemical composition of the precipitate, although the

311   inaccuracy of the technique increases with inhomogeneous and rough samples. Four sites were

312   scanned, and the weight percentages of the four spectra are given in Table 1 along with the

313   theoretical weight percentage of known minerals containing calcium: anhydrous calcium

314   carbonate or calcite, dolomite, monohydrocalcite and ikaite. Although very close to one another,

315   the four spectra showed some variability as indicated by the percentages varying within 4%, and

316   there was co-precipitation with magnesium and iron. In fact, gold (Au) from the sample

317   preparation was found to interfere with Phosphorus (P) in the elemental chromatograph because

318   both elements peak at 2 keV.

319

320   Some carbon and oxygen could also be attributed to dissolved organic matter; this means that the

321   oxygen percentage actually due to calcium carbonate could be overestimated. However, dolomite

322 is unlikely to have precipitated because of the low percentage of magnesium in the unknown

323 sample compared to the 13% theoretically present in dolomite. Calcite (an anhydrous form of

324 calcium carbonate) did not match well due its lower carbon and higher calcium content

325 compared to the sample, while the hydrated forms of calcium carbonate, such as

326 monohydrocalcite, had a better match.

327 Nonetheless, these forms contain hydrogen that cannot be detected by the EDS technique. As a

328 result, the percentage could be overestimated because they do not take hydrogen into account.

329 Further analysis using X-Ray Fluorescence technology (XRF) (Table 2) provided a very similar

330 percentage of calcium (~28%), but with a much smaller statistical error. The analysis also

331 showed that impurities such as phosphorus, silicon, magnesium, iron, sulphur, potassium,

332 manganese and aluminium co-precipitated, but most importantly, the analysis revealed that 29%

333 of the sample was inorganic, and hence 71% was organic (C, O and H). By comparing this with

334 the last column in Table 1, it was found that the mineral with the closest organic content was

335 indeed monohydrocalcite with 66%. This small discrepancy could be due to dissolved organic

336 matter trapped within the precipitate. Furthermore, monohydrocalcite is known for its bizarre

337 occurrences, which include inside the otoliths of the tiger shark, the bladder of a guinea pig

338 (Catherine et al., 1977), and the final stages of decomposition of the putrefying flesh of the giant

339 saguaro cactus (Garvie, 2003); these occurrences suggest a biochemical origin is possible. In

340 biological systems, some authors have reported that the precipitated minerals were related to the

341 bacterial metabolism and, consequently, could be considered biologically induced (Gonzalez-

342 Munoz et al., 2008). Amino acid metabolism results in the release of ammonia and CO_2 that

343 increase the pH and CO_3^{2-} concentration, creating an alkaline environment that favours

344 monohydrocalcite precipitation. This precipitation may also be related to heterogeneous

345 nucleation on negatively charged points of biological structures, and these functional groups are

346 able to attract positive ions such as Ca^{2+}. In aerobic granules, Zhou et al. (2013) characterized the

347   calcium compounds and tested by energy dispersive X-ray and X-ray diffraction (XRD) the

348   accumulation of calcium carbonate and certain magnesium compounds as $Ca_7Mg_2P_6O_{24}$. Mañas

349   et al. (2011) discovered calcium deposit in the inner part of aerobic granules as hydroxyl-apatite

350   $(Ca_5(PO_4)_3(OH))$. Juang et al. (2010) cultivated stable granules from continuous-flow reactors

351   and noted that large amounts of phosphates and hydroxides of calcium and iron were formed in

352   the granules to enhance the granule stability. In an anaerobic membrane bioreactor, Lin et al.

353   (2011) reported that the cake layer on the membrane was formed by organic substances and

354   inorganic elements such as 71.67% C, 9.03% O, 4.45% Ca, 1.94% Mg, 1.72% Al, 1.46% Si,

355   0.15% K, etc, but the low percentage of Calcium indicates that fouling was more organic in

356   nature.

357   These studies suggest strongly that a biofilm on the membrane of the SAMBR could have

358   triggered the precipitation of monohydrocalcite. Moreover, as the concentration of humic acids is

359   greater near the membrane in the concentration polarization layer, it is very likely that calcium

360   will form aggregates on the membrane because of the higher density of negatively charged

361   solutes near the membrane.

362

363   *3.5 Identification of the inorganic precipitate on the AMBR membrane*

364   SEM-EDX was also used to investigate the morphology and structure of the inorganic precipitate

365   found on the membrane in the AMBR. Compared to the SAMBR membrane, the precipitate was

366   much more heterogeneous with a rougher surface. Several random points on the samples were

367   analyzed by EDX, and all the spectra contained calcium, oxygen and phosphorus (2-6.7%) as the

368   main elements, with traces of metals (Fe, Al, Mg, Si, S, Na, Cl, Mn) (Table 3). It was found that

369   calcium could precipitate in two different phases; a background precipitate made of small

370   nodules, or as long and sharp flakes of about 70 microns growing in bundles and pointing

371   upwards. The former phase contained small amounts of manganese, iron, magnesium,

372   manganese, silicon, aluminium, sulphur, chloride and sodium (<1% by weight), whereas the

373   flakes contained almost no impurities, which indicates that the flakes were in a purer form than

374   the background (Table 3). Moreover, the flakes were characterized by significantly higher

375   amounts of phosphorus (10-17%) compared to the background (2-6%). Chesters (2009) obtained

376   very similar SEM pictures with flat needles which he assumed to be hydroxyapatite crystals.

377   Unfortunately, as the samples were coated with carbon to avoid interferences when using gold

378   coating, the carbon percentage was not available. Spectra of the flakes varied within a wide

379   range of calcium (19-59%), oxygen (31-64%) and phosphorus (10-17%) making its

380   identification particularly difficult. Several potential known minerals are listed in Table 3 with

381   their Ca, O and P composition for comparison.

382

383   Nonetheless, it is very likely that the background precipitate was comprised of calcium carbonate

384   containing many impurities. The calcite background may have acted as nucleation spots for the

385   formation of other amorphous calcium carbonate clusters. Occurrence of nucleation on the

386   calcite seed surface as a secondary nucleation site is a well accepted mechanism (Salek et al.,

387   2016). With respect to the flakes, their high P content suggests a calcium phosphate precipitate,

388   instead of calcium carbonate, such as calcium hydrogen phosphate (or brushite $CaHPO_4 \cdot 2H_2O$,

389     $K_{sp} = 10^{-6.69}$), dicalcium phosphate anhydrate ($CaHPO_4$, $K_{sp} = 10^{-6.9}$ (Montastruc, 2003)),

390   amorphous calcium phosphate ($Ca_3(PO_4)_2$, $K_{sp} = 10^{-24}$ (Mamais et al., 1994)), octacalcium

391   phosphate ($Ca_4H(PO_4)_3 \cdot 2.5H_2O$, $K_{sp} = 10^{-49.6}$ (Montastruc et al., 2003)), hydroxyapatite

392   ($Ca_5(PO_4)_3OH$, $K_{sp} = 10^{-46.8}$ (Barat et al., 2008)) or carbonated hydroxyapatite

393   ($Ca_{10}(PO_4)_6(CO_3)_x(OH)_{2-2x}$, $K_{sp} < 1 \cdot 10^{-100}$ (Ito et al., 1997)). Furthermore, the same authors

394   (Montastruc et al., 2003) found that both dicalcium phosphate dihydrate, and amorphous calcium

395   phosphate, can crystallize at a pH lower than 7.3, and only amorphous calcium phosphate at a pH

396   higher than 7.3. However, calcium phosphate precipitation follows the Ostwald rule, which

397   states that the least thermodynamically stable phase is the first one formed, which works as a

398   precursor of the most stable phase (Montastruc et al., 2003). In this case, carbonated

399   hydroxyapatite would be the least thermodynamically stable phase forming on the calcium

400   carbonate background as a precursor for a more stable calcium phosphate. Several authors

401   reported that the presence of phosphates inhibits calcite growth due to the adsorption of

402   phosphates on the calcite surface, which enables the formation of calcium phosphates (Lin &

403   Singer, 2005; Plant & House, 2002; Shintaro & Keisuke, 2012). Although it is a metastable

404   phase with respect to calcite and aragonite, monohydrocalcite ($CaCO_3 \cdot H_2O$) is a better adsorbent

405   than calcite and aragonite due to its greater surface area (Shintaro & Keisuke, 2012). This is in

406   line with Kim et al. (2007) who observed low levels of residual PO_4^{3-}, and they attributed this to

407   calcium that might preferentially react with orthophosphates to produce hydroxyapatite,

408   dicalcium phosphate or octacalcium phosphate. In abiotic batch adsorption tests, Shintaro and

409   Keisuke (2012) showed that at low phosphate concentrations, phosphate was removed by

410   sorption of phosphate during the transformation of monohydrocalcite to calcite, but at high

411   concentrations (>10.6 mg/L), amorphous calcium phosphate ($Ca_3(PO_4)_2 \cdot xH_2O$) was likely to be

412   formed.

413

414   Borzacconi et al. (1999) also observed that calcium can form complexes with organic matter,

415   orthophosphates and polyphosphates, which will diminish the phosphorus available for the

416   microorganisms or even for struvite. Sindelar et al. (2015) also reported that dissolved organic

417   carbon and phosphorus can co-precipitate with calcium at high pH.

418   Le Corre et al. (2005) and Doyle and Parsons (2002) observed that calcium ions can affect

419   struvite crystal growth, and leads to the formation of an amorphous calcium phosphate. This

420   study confirmed that struvite precipitation did not occur because the available phosphates

421 precipitated with calcium on the membrane, keeping the solution undersaturated with respect to

422 struvite.

423

424 From all these considerations, and from the light shed by other authors, it seems very likely that

425 the background precipitate was amorphous calcium carbonate, and the presence of phosphates

426 inhibited its growth due to the adsorption of phosphates, which enabled the formation of calcium

427 phosphates. The low level of impurities in these flakes suggests a mineral with a more crystalline

428 structure such as hydroxyapatite which is the most stable calcium phosphate.

429

430 *3.6 Quantification of flux drop due to inorganic fouling*

431

432 At the end of the study, a cleaning procedure (Akram, 2006) was performed in order to

433 determine the relative contribution to fouling due to internal and external, organic and inorganic

434 fouling in the SAMBR and AMBR; Table 4 documents the contribution of each component of

435 fouling. The flux drop due to an organic biofilm alone (FD2) was obtained by subtracting FD1

436 (due to combined internal, external, organic and inorganic fouling) and FD3 (external inorganic

437 layer and internal fouling). It can be seen that 98% of the clean water flux from the SAMBR

438 membrane was lost because of the combined effects of all types of foulants, i.e. external,

439 internal, organic and inorganic foulants. The flux f_2 (without the external biofilm removed by a

440 sponge and hot water) was only 13.6 LMH, which means that 94% of the flux drop was due to

441 external inorganics combined with internal fouling. This indicates that in the SAMBR the

442 external organic fouling or biofilm (FD2) played a minor role in total fouling of the SAMBR

443 (less than 4%). In contrast, the flux drop (FD2) due to external organic fouling or biofilm on the

444 AMBR membrane accounted for 30% of the total flux drop (FD1), indicating that adhesion of

445 bacteria onto the AMBR membrane was more pronounced.

446

447   Afterwards, the membrane was chemically cleaned in a 1% sodium hypochlorite solution for 2

448   hours to remove all organic foulants. These include organics left on the external surface and

449   organic foulants inside the membrane pores. The flux f_3 recovered after this cleaning was only

450   43 ± 1 LMH, which is considerably lower that the initial flux, and suggests that organic material

451   played a minor role in the internal and external fouling. In fact, the total flux drop due to

452   inorganic fouling regardless of internal or external material (FD4) can be calculated by

453   subtracting f_3 from f_0, which gives 208 LMH. Data in Table 4 also showed that inorganic fouling

454   accounted for 83% and 32% of the total flux drop in the SAMBR and AMBR, respectively. This

455   confirmed that precipitation of inorganics took place preferentially in the SAMBR due to high

456   CO_2 partial pressures and high calcium concentrations.

457

458   The membrane was then soaked in a 1% oxalic acid solution (pH<2) for 2 hours in an attempt to

459   remove inorganic foulants, as recommended by the membrane manufacturer. After 2 hours

460   soaking the flux measured afterwards was only 60 LMH, suggesting severe inorganic fouling

461   which would require longer soaking times and stronger chemicals. We then attempted to use 1%

462   hydrochloric acid for 2 hours, and then 1% hydrochloric acid with 1% sodium hypochlorite for 4

463   hours. The first cleaning attempt resulted in a flux of 94 LMH, while the last one resulted in a

464   flux of 106 LMH.

465

466   Similarly to the SAMBR membrane, the original flux in the AMBR membrane could not be

467   completely recovered, even with hydrochloric acid instead of oxalic acid. However, the flux after

468   HCl cleaning was 183 LMH which was still lower than the 250 LMH of the virgin membrane.

469   Using both the hydrochloric acid and the sodium hypochlorite solutions at 1% for 2 hours

470   resulted in a flux of 211 LMH, indicating that some residual fouling could be due to a close

471   association of organics and inorganics. The fact that it was easier to clean the AMBR membrane

472   also indicates that fouling in the AMBR was less severe, which is in line with Table 4.

473

474   **4.   Conclusions**

475   A Submerged Anaerobic Membrane BioReactor (SAMBR) and a polishing Aerobic Membrane

476   BioReactor (AMBR) achieved 96% COD removal at low HRTs of 0.4 days during the anaerobic

477   treatment of leachate. However, calcium (160 mg/L) in the leachate was found to precipitate on

478   the SAMBR membrane causing severe fouling, and the precipitate was found to be

479   monohydrocalcite. It was found that calcium precipitated in two different phases in the AMBR:

480   the inorganic layer on the membrane consisted of nodules of calcium carbonate with traces of

481   phosphorus. On top of this layer grew flake shaped precipitates made of pure hydroxyapatite

482    $Ca_5(PO_4)_3(OH)$.

483

484

485   **Supplementary materials**

486   Details of the inorganic precipitates can be found in electronic annex.

487

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491

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