

1 **Optimization of urban waste fermentation for volatile fatty acids production**

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24 **Abstract**

25 The problem of waste disposal has recently focused on practices for waste recycling and
26 bio-resources valorization. Organic waste produced in urban context together with
27 biological sludge produced in wastewater treatment plants (WWTPs) can be used as
28 renewable feedstock for the production of building blocks of different products, from
29 biopolymers to methyl esters. This paper deals with the optimization of the fermentation
30 process in order to transform urban organic waste (a mixture of pre-treated food waste
31 and biological sludge) into added-value volatile fatty acid (VFA) rich stream, useful for
32 biological processes within a biorefinery technology chain. Different temperatures, pH,
33 hydraulic retention times (HRTs) and organic loading rates (OLRs) were tested both in
34 batch and continuous trials. Batch tests showed the best working conditions at 37°C and
35 pH 9, using the bio-waste feedstock thermally pre-treated (76 h at 72°C). These
36 conditions were applied in continuous process, where higher HRT (6.0 d) and lower
37 OLR [7.7 kg VS/(m³ d)] gave the best performances in terms of process yield and
38 maximum VFA level achieved: 0.77 COD_{VFA}/VS₍₀₎ and 39 g COD_{VFA}/L. An optimized
39 fermentation process is crucial in a biorefinery perspective since it has to give a final
40 stream of constant composition or tailored products suitable for further applications.

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48 **Keywords**

49 Organic fraction of municipal solid waste (OFMSW); Volatile Fatty Acid (VFA);

50 Waste Activated Sludge (WAS); Biorefinery; Anaerobic fermentation

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52 **Abbreviations**

53 COD, chemical oxygen demand; COD_{SOL}, soluble COD; CSTR, continuous stirred tank

54 reactor; HRT, hydraulic retention time; MMC, mixed microbial cultures; OFMSW,

55 organic fraction of municipal solid waste; OLR, organic loading rate; PHA,

56 polyhydroxyalkanoates; TKN, Total Kjeldahl Nitrogen; TS, Total Solids; VFA, volatile

57 fatty acids; VS, Volatile Solid; WAS, waste activated sludge; WWTP, wastewater

58 treatment plant; 3HB, 3-hydroxybutyrate; 3HV, 3-hydroxyvalerate.

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72 **1. Introduction**

73 Waste management and disposal is one of the most pressing issues mainly due to the
74 exponential population increase in the last decades, especially in urban areas where the
75 majority of the population is located (Pfaltzgraff et al., 2013). The main organic refuses
76 produced in this context are food waste, also known as the organic fraction of municipal
77 solid waste (OFMSW), and biological sludge coming from the wastewater treatment. In
78 Europe, the average organic matter production expressed as total solid (TS) per day (d)
79 *pro capita* (person equivalent, PE) is approximately 55 g TS/(PE) d of OFMSW and 39
80 g TS/(PE) d of biological sludge (Colombo et al., 2017; IPCC, 2006). It is quite clear
81 that these kinds of organic waste are highly available especially in urban areas and at
82 present time they are handled and treated separately. The sludge is typically digested,
83 dewatered and then sent to final disposal, and the OFMSW is sent to composting. The
84 separate treatment of biological sludge and OFMSW is generally not convenient from
85 both energetic and environmental point of view. As a matter of fact, anaerobic digesters
86 treating sludge in wastewater treatment plants (WWTPs), especially waste activated
87 sludge (WAS), are often low loaded and underperforming (Bolzonella et al., 2005),
88 while aerobic composting is a highly energy consuming process. The advantages of a
89 combined treatment of OFMSW and WAS have already been exploited by the
90 anaerobic co-digestion approach, with the experiences reported in previous studies
91 (Mata-Alvarez et al., 2014). This technology directs all the organic matter into a single
92 step, in which the conversion into biogas and energy is realized (Scarlat et al., 2018).
93 Digestate is also produced together with biogas and energy, with environmental
94 concerns regarding its further stabilization and disposal and the possible presence of
95 heavy metals, pharmaceuticals and/or other pathogens (Mata-Alvarez et al., 2014).

96 OFMSW mixing with WAS generates several benefits, such as dilution of potential
97 toxic compounds and improved nutrient balance (Zahedi et al., 2016). A more effective
98 application of this approach can be found in urban contexts where the OFMSW is
99 coming from a source separate collection or a door-to-door collection, since the
100 biodegradability of the collected waste improves substantially due to the increase of
101 organic matter content and the decrease of inert materials (Bernstad et al., 2013).
102 Novarino and Zanetti (2012) reported the application of a mechanical pretreatment
103 method to further separate the inert material and homogenize the organic matter,
104 enhancing the anaerobic co-digestion process. The integration of OFMSW and WAS
105 treatment can be easily realized in existing WWTPs, where anaerobic digesters are in
106 most cases already present, in order to improve the WWTP energy balance. Authors
107 reported some co-digestion experiences in full-scale plants, such as the Rovereto
108 WWTP, located in the Trento province in northern Italy, in which the OFMSW and
109 mixed sludge co-digestion was implemented in 2014 (Mattioli et al., 2017). In the
110 Treviso province (northeast Italy) the co-digestion approach of OFMSW and WAS in
111 the full-scale WWTP was proposed and implemented since 1999 (Bolzonella et al.,
112 2006; Pavan et al., 2000). In this specific urban scenario, the waste separate collection is
113 very efficient and reaches 87.9% on the total wastes (ISPRA, 2017) making the organic
114 waste treatment integration an eligible way for valorization and recovery of the organic
115 matter. A possible and innovative approach for the realization of this treatment
116 integration can be found in an urban biorefinery. The urban biorefinery represent a
117 technology chain in which the organic material of urban waste can be converted into
118 new added-value bio-based products (Valentino et al., 2018). Bio-based products
119 obtainable in an urban biorefinery through the exploitation of organic waste are biofuels

120 (Stephen and Periyasamy, 2018), platform chemicals (Kiran et al., 2014), and
121 bioplastics (Valentino et al., 2017). Some of the most important intermediates that allow
122 the conversion of organic waste into these valuable bio-based products are volatile fatty
123 acids (VFA), which in most cases are the direct precursors for biopolymer synthesis,
124 such as polyhydroxyalkanoates (PHA) obtained from pure and mixed microbial culture
125 (MMC) (Valentino et al., 2014). VFA are produced during the anaerobic fermentation
126 process of almost all kinds of biodegradable organic waste (Strazzera et al., 2018).
127 Previous studies reported experiences of anaerobic fermentation on dewatered sludge
128 (Hao & Wang, 2015), cellulosic substrates (Keating et al., 2013), cheese whey
129 (Colombo et al., 2017; Valentino et al., 2015) and OFMSW (Chen et al., 2017; Girotto
130 et al., 2017; Korkakaki et al., 2016). In a platform where anaerobic and aerobic
131 processes are combined, VFA obtained from waste fermentation are intermediate
132 chemicals for the conversion of organic matter into the aforementioned biodegradable
133 added-value products (Koller et al., 2017). Indeed, in a scenario where the OFMSW is
134 coming from a highly efficient source separate collection and the anaerobic co-digestion
135 of OFMSW and WAS is already implemented, the urban biorefinery concept finds its
136 perfect integration. The municipality of Treviso (northeast Italy) is a representative
137 example where the OFMSW-WAS mixture is currently sent to anaerobic co-digestion.
138 Indeed, the hypothesis of driving part of this organic source into an anaerobic
139 fermentation step for the VFA production could be an eligible way for an efficient
140 urban organic waste management. Within this route, the combined VFA and methane
141 production from OFMSW-WAS mixture has been recently demonstrated (Valentino et
142 al., 2019) in the same urban context. However, in order to enhance the PHA synthesis
143 and productivity, the fermentation of OFMSW-WAS mixture needs to be optimized,

144 maximizing the VFA production and, as a consequence, the PHA potentially obtainable.
145 In this study, different fermentation conditions were tested by means of batch tests on a
146 mixture of OFMSW and WAS. These batch trials were conducted in order to find the
147 best working conditions for the fermentation process, namely optimum pH value and
148 temperature. Once the best condition was found, a continuous lab scale trial in a
149 continuous stirred tank reactor (CSTR) was set-up to better represent an acidogenic
150 fermentation process under different applied HRT and OLR.

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152 **2. Materials and Methods**

153 *2.1. Substrate characterization*

154 The substrates used in this study were thickened WAS and pre-treated OFMSW both
155 available inside the Treviso WWTP. The WAS has been collected from the static
156 thickener of the full-scale plant; the OFMSW came from the source sorted collection in
157 50 districts of the Treviso Province and was transferred to the full-scale WWTP after its
158 pre-treatment (squeezing and homogenization) in a dedicated plant. The mixture used
159 for all trials was composed by volumetric fractions of 65-70% thickened WAS and of
160 30-35% squeezed OFMSW. Thickened WAS and squeezed OFMSW had TS contents
161 of 30 ± 1 g TS/kg and 139 ± 7 g TS/kg respectively, with a volatile solid (VS) fraction
162 of 70% VS/TS for WAS and 90% VS/TS for OFMSW. The high VS/TS ratio of the
163 OFMSW was favoured by the quality of the source-sorted collection, which effectively
164 increased the readily biodegradable organic content. On the other hand, the relatively
165 low nutrients (nitrogen and phosphorus) level in the squeezed OFMSW was
166 counterbalanced by the high volumetric fraction of WAS, which contributed to the final
167 COD:N:P ratio of 100:2.5:0.7 in the mixture. Table 1 shows the average values obtained

168 from the chemical-physical characterization of both separated streams, of the final
 169 urban waste mixture and of the waste mixture after thermal pre-treatment.

170

171 **Table 1**

Parameter	Unit	Thickened WAS	Pre-treated OFMSW	Urban waste mixture	Thermally pre-treated urban waste mixture
Total Solid (TS)	g/kg	30 ± 1	139 ± 19	62 ± 7	58 ± 6
Volatile Solid (VS)	g/kg	21 ± 1	125 ± 14	50 ± 2	46.4 ± 0.9
VS/TS	%	70 ± 4	90 ± 2	81 ± 2	83 ± 1
COD _{SOL}	g COD/L	0.15 ± 0.06	81 ± 7	32 ± 3	38 ± 3
VFA	g COD/L	-	8.3 ± 0.6	2.4 ± 0.3	2.2 ± 0.2
pH	-	6.6 ± 0.2	4.3 ± 0.3	5.1 ± 0.3	5.0 ± 0.2
COD _{VFA} /COD _{SOL}	-	-	0.11 ± 0.02	0.08 ± 0.02	0.06 ± 0.01
Total Kjeldahl Nitrogen (TKN)	g N/kg TS	42 ± 3	27 ± 1	35 ± 2	36.0 ± 0.8
Phosphorus (P)	g P/kg TS	15 ± 1	7.0 ± 0.4	10 ± 1	10 ± 2

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174 2.2. Batch fermentation tests

175 Batch tests were performed in order to investigate the effects of different combinations
 176 of temperature and initial pH on urban waste fermentation. Mesophilic (37°C) and
 177 thermophilic (55°C) conditions were tested. In parallel, another mesophilic trial was
 178 performed with the same urban waste mixture after a thermal pre-treatment (72°C for
 179 76 h). For each of the three conditions, three different initial pH values (5.0, 7.0 and
 180 9.0) were investigated. Sodium hydroxide (NaOH) was added to reach both initial pH
 181 values of 7.0 and 9.0; no chemicals addition was necessary for the tests at initial pH
 182 value of 5.0 since it was the typical pH of the urban waste mixture. Each test was
 183 performed in duplicate, in 1.0 L glass bottles sealed with a silicon plug and an
 184 aluminium seal (working volume 0.75 L). No inoculum was added since more than a

185 sufficient amount of fermentative microorganisms were already present in the initial
 186 waste mixture. The investigated conditions are displayed in Table 2.
 187 Batch fermentation tests were monitored up to ten (trials A-B) and five (trial C) days.
 188 Liquid samples (15.0 mL) were collected each day for VFA analysis and pH
 189 measurements as well as for ammonium (N-NH₄⁺), phosphate (P-PO₄³⁻) and soluble
 190 COD (COD_{SOL}). The slurry was manually mixed twice a day, one hour before and 6
 191 hours after each sampling. The bottles were opened for the sampling and then
 192 maintained under N₂ flux for 20 min to re-establish anaerobic conditions.

193
 194

Table 2

Temperature (°C)	Thermal pre-treatment	Initial pH	Test name
55	-	5.0	A1
55	-	7.0	A2
55	-	9.0	A3
37	-	5.0	B1
37	-	7.0	B2
37	-	9.0	B3
37	72°C, 76 h	5.0	C1
37	72°C, 76 h	7.0	C2
37	72°C, 76 h	9.0	C3

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196 2.3. Urban waste fermentation process in a CSTR

197 The best performing condition obtained in batch tests was further investigated by means
 198 of a fermentation process in a 6.0 L CSTR that was maintained at a fixed temperature
 199 (37°C) by an external thermostatic jacket and stirred with a mechanical impeller at 80
 200 rpm. No inoculum was added. The reactor was fed once per day, in a semi-continuous
 201 manner. Acidogenic fermentation was started up and initially conducted with an OLR of
 202 7.7 kg VS/(m³ d), which was then increased up to 9.3 and 11.3 kg VS/(m³ d). Each
 203 condition was characterized under steady state and compared in terms of VFA yields

204 and composition. The increasing OLR was combined with a corresponding decrease of
205 HRT, from 6.0 to 5.0 and 4.1 days. The reactor was not equipped with pH control;
206 however, alkaline fermentation was performed by adjusting the pH of the feedstock at
207 pH 9.0. The effluent was collected before the feeding for pH measurements and VFA
208 analysis (up to five times a week) as well as TS, VS, ammonium, phosphate and
209 COD_{SOL} analysis (twice per week).

210

211 *2.4. Analytical methods*

212 Analyses were conducted according to Standard Methods (APHA, AWWA, WEF,
213 1998). Volatile fatty acids were determined using an Agilent 6890N gas chromatograph
214 equipped with a flame ionization detector (FID) (T = 250°C). Samples were analysed
215 through an Agilent J&W DB-FFAP fused silica capillary column (15 m length, 0.53
216 mm i.D. x 0.5 mm film) using hydrogen as carrier. The inlet was working in split mode,
217 with a split ratio of 20:1. The instrument was programmed with a ramp temperature
218 from 80°C to 100°C (10°C/min). Before GC analyses, samples were centrifuged at
219 4,500 rpm for five minutes and the supernatant was filtered at 0.2 µm using acetate
220 cellulose syringe filters (Whatman).

221

222 *2.5. Data analysis*

223 According to the sampling described above, data were collected daily and analysed at
224 the end of each test batch. In the CSTR experiment, data were processed according to
225 the adopted frequency of sampling (paragraph 2.3). In order to better represent VFA
226 distribution, the molar ratio between odd numbered acids and the total concentration
227 was determined. The nutrient concentration, related to the release of ammonium and

228 phosphate in the medium, was also quantified by means of the COD:N:P ratio expressed
229 in grams. The substrate solubilisation was calculated by the ratio between the final
230 soluble COD (as net concentration subtracted to the initial COD_{SOL} ; $COD_{SOL(0)}$) and the
231 initial VS of the feedstock ($VS_{(0)}$), as it follows:

$$232 \quad Solubilisation = \frac{(COD_{SOL} - COD_{SOL(0)})}{VS_{(0)}}$$

233 The VFA yield was determined by the ratio between the produced VFA (as net
234 concentration subtracted to the initial VFA; $COD_{VFA(0)}$) and the initial VS of the
235 feedstock ($VS_{(0)}$), as it follows:

$$236 \quad Yield = \frac{(COD_{VFA} - COD_{VFA(0)})}{VS_{(0)}}$$

237 Substrate solubilisation and VFA yield were calculated for each batch test and for the
238 CSTR process. In batch tests with the thermal pre-treated substrate (C1, C2 and C3) and
239 in the CSTR process both substrate solubilization and VFA yield were calculated
240 considering the substrate after thermal pre-treatment as starting point.

241

242 **3. Results and Discussion**

243 *3.1. Batch fermentation tests*

244 All batch tests were started with the same urban waste mixture; indeed, the initial solids
245 as well as the macronutrients (nitrogen and phosphorus) content were similar in all
246 trials. The urban waste mixture was extremely homogeneous since the OFMSW was
247 squeezed in the pre-treatment and resulted as liquid slurry after mixing with thickened
248 WAS. VFA concentrations were monitored daily for each batch test. The main results
249 are summarized in Table 3.

250

251 *3.1.1. Effect of temperature on the urban waste fermentation in batch tests*

252 Figure 1 shows the VFA concentrations in the thermophilic (A), mesophilic (B) and
253 pre-treated mesophilic (C) batch tests, which have been performed at initial pH value of
254 5.0 (1), 7.0 (2) and 9.0 (3). In terms of VFA production rates and yields, the mesophilic
255 condition was better performing. Some literature studies report that mesophilic
256 temperature ranges are preferable to thermophilic ones, not only for OFMSW (Garcia-
257 Aguirre et al., 2017) but also for sewage sludge (primary or WAS) fermentation process
258 (Morgan-Sagastume et al., 2015).

259 Under thermophilic condition, VFA production lagged in the first two days and started
260 noticeably only from the third day, especially in those trials started under alkaline pH
261 value (9.0). This observation was in line with previous studies (Peces et al., 2016; Jiang
262 et al., 2013), where the initial lower acidification yields observed at 55°C was due to the
263 slower adaptation of the thermophilic culture. In the tests performed under mesophilic
264 condition, with or without thermal pre-treatment, the fermentation activity was
265 immediately observed both at pH 7.0 and 9.0. Overall, at the same initial pH
266 investigated, VFA concentrations obtained under condition B (18.6-30.0 g COD_{VFA}/L)
267 and C (22.5-41.0 g COD_{VFA}/L) are higher than those obtained under condition A (4.7-
268 27.5 g COD_{VFA}/L), as most of literature studies suggest (Jiang et al., 2013; Morgan-
269 Sagastume et al., 2015; Wan et al., 2016). Thermophilic temperatures favoured the
270 organic matter solubilisation (0.22-0.36 g COD_{SOL}/g VS₍₀₎) but a substantial part of the
271 solubilized COD was not efficiently converted into VFA (0.06-0.52 g COD_{VFA}/g VS₍₀₎).

272 Indeed, thermophilic tests A1-A2-A3 were characterized by higher COD_{SOL} (39-46 g
273 COD_{SOL}/L) than mesophilic tests B1-B2-B3 (34-37 g COD_{SOL}/L), but lower final VFA
274 content independently from the chosen initial pH. On the other hand, thermal pre-
275 treatment (tests C1-C2-C3) increased the solubilisation yield (0.07-0.15 g COD_{SOL}/g

276 VS₍₀₎) in the mesophilic fermentation process compared to tests B1-B2-B3 performed
277 without thermal pre-treatment (0.06-0.09 g COD_{SOL}/g VS₍₀₎). The mesophilic
278 fermentation process applied to an already solubilized substrate allowed increasing the
279 VFA content (22.5-41.0 g COD_{VFA}/L), much more than tests B1-B2-B3 where the
280 thermal pre-treatment was not applied (19.2-30.0 g COD_{VFA}/L), being VFA yields
281 comparable in the fermentation series B (0.29-0.49 g COD_{VFA}/g VS₍₀₎) and C (0.26-0.57
282 g COD_{VFA}/g VS₍₀₎). Moreover, VFA production rate was substantially improved after
283 the organic matter pre-treatment at 72°C: approximately 35 g COD_{VFA}/L were achieved
284 in less than 5 days (at least under initial neutral and alkaline pH), which reasonably
285 meant a doubled production rate compared to the thermophilic alkaline test A3 and
286 mesophilic neutral-alkaline tests B2-B3.

287 Usually, thermophilic temperatures enhance the hydrolysis process in which complex
288 molecules are solubilized and become more available for the microorganisms'
289 metabolism (Dahiya et al., 2015; Morgan-Sagastume et al., 2015). Indeed, process
290 temperature strongly affected the nutrients release from the substrate. Ammonium and
291 phosphate levels quantified at the end of tests C (0.94-1.22 g N-NH₄⁺/L; 0.56-0.67 g P-
292 PO₄³⁻), where bio-waste was thermally pre-treated, were higher than those achieved in
293 thermophilic series A (0.41-1.04 g N-NH₄⁺/L; 0.28-0.43 g P-PO₄³⁻) and mesophilic
294 series B (0.43-0.54 g N-NH₄⁺/L; 0.25-0.33 g P-PO₄³⁻).

295

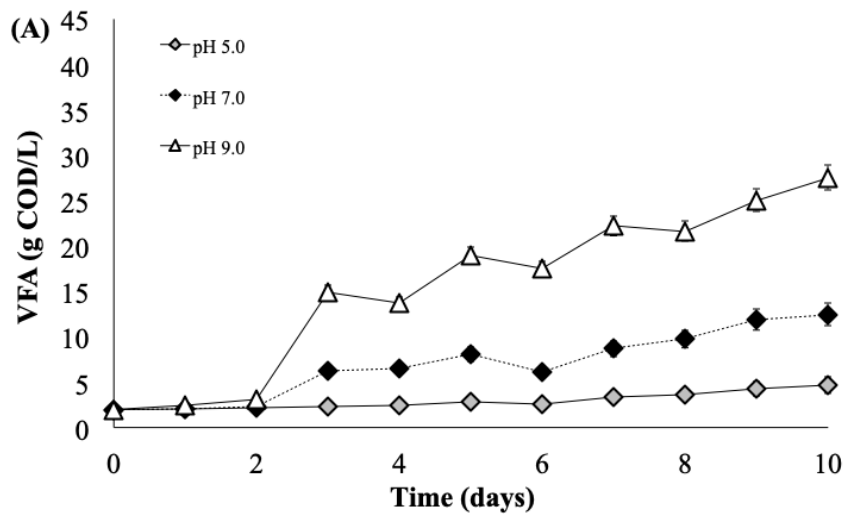
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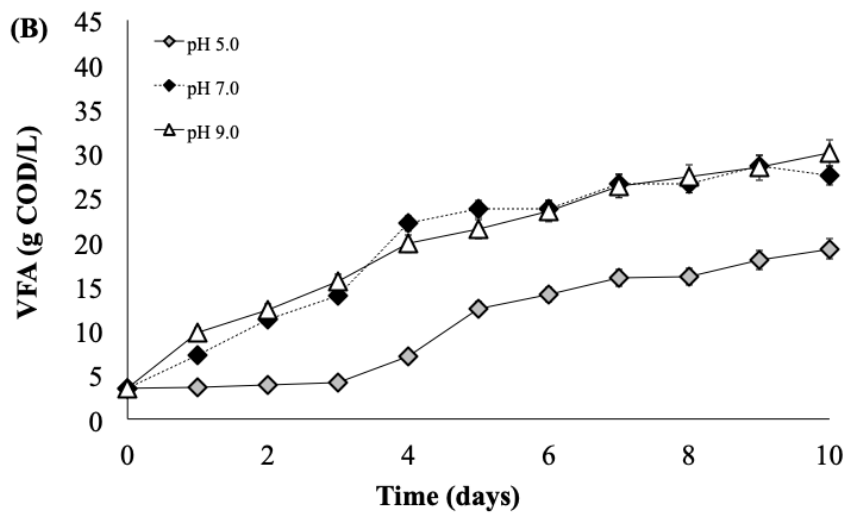
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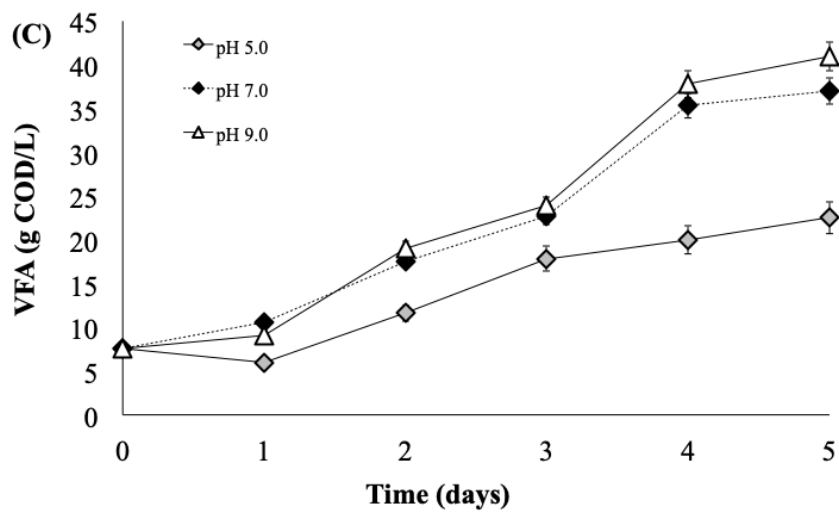
300 **Figure 1**



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304 **Table 3**

Parameter	Unit	Test name								
		A1	A2	A3	B1	B2	B3	C1	C2	C3
Total VFA	g COD/L	4.7 ± 1	12.5 ± 0.7	27.5 ± 2	18.6 ± 0.9	27.5 ± 2	30 ± 2	22.5 ± 0.7	37 ± 2	41 ± 2
Acetic acid	g COD/L	3.4 ± 0.5	5.4 ± 0.3	7.1 ± 0.5	4.8 ± 0.2	7.1 ± 0.2	7.1 ± 0.3	6.4 ± 0.3	7.3 ± 0.6	10.0 ± 0.8
Propionic acid	g COD/L	1.2 ± 0.4	2.5 ± 0.2	6.8 ± 0.2	0.8 ± 0.1	3.8 ± 0.3	6.9 ± 0.4	3.2 ± 0.1	9.6 ± 0.1	13.0 ± 0.3
Isobutyric acid	g COD/L	0	0	0.6 ± 0.1	0	0	0	0	0	0
Butyric acid	g COD/L	0	3.1 ± 0.1	6.9 ± 0.4	3.9 ± 0.1	3.8 ± 0.2	4.0 ± 0.3	6.3 ± 0.1	7.6 ± 0.3	8.2 ± 0.4
Isovaleric acid	g COD/L	0	0	1.2 ± 0.1	1.3 ± 0.1	1.0 ± 0.4	1.2 ± 0.1	0	0	0
Valeric acid	g COD/L	0	0	4.2 ± 0.4	1.4 ± 0.1	5.0 ± 0.5	4.2 ± 0.5	1.8 ± 0.1	5.0 ± 0.5	5.8 ± 0.3
Caproic acid	g COD/L	0	1.5 ± 0.1	0.7 ± 0.3	3.5 ± 0.4	2.9 ± 0.1	2.8 ± 0.3	3.3 ± 0.2	3.0 ± 0.2	1.4 ± 0.1
Heptanoic acid	g COD/L	0	0	0	2.9 ± 0.1	3.9 ± 0.4	3.9 ± 0.1	1.6 ± 0.1	4.6 ± 0.3	2.4 ± 0.1
Soluble COD	g COD/L	39 ± 2	40 ± 3	46 ± 1	34 ± 2	35 ± 2	37 ± 3	41 ± 3	44 ± 4	45 ± 3
Ammonium	g N-NH ₄ ⁺ /L	0.41 ± 0.05	0.88 ± 0.06	1.04 ± 0.05	0.43 ± 0.04	0.47 ± 0.06	0.54 ± 0.08	0.94 ± 0.07	1.07 ± 0.09	1.22 ± 0.09
Phosphate	g P-PO ₄ ³⁻ /L	0.28 ± 0.04	0.37 ± 0.05	0.43 ± 0.07	0.25 ± 0.05	0.28 ± 0.05	0.33 ± 0.04	0.56 ± 0.05	0.62 ± 0.08	0.67 ± 0.07
Solubilisation	gCOD _{SOL} /gVS ₍₀₎	0.22 ± 0.02	0.24 ± 0.04	0.36 ± 0.03	0.06 ± 0.01	0.06 ± 0.02	0.09 ± 0.01	0.07 ± 0.01	0.13 ± 0.02	0.15 ± 0.02
Yield	gCOD _{VFA} /gVS ₍₀₎	0.06 ± 0.03	0.22 ± 0.03	0.52 ± 0.03	0.29 ± 0.04	0.44 ± 0.05	0.49 ± 0.05	0.26 ± 0.03	0.50 ± 0.04	0.57 ± 0.03
COD:N:P*	g	100/1.1/0.7	100/2.2/0.9	100/2.3/0.9	100/1.3/0.7	100/1.3/0.8	100/1.5/0.9	100/2.2/1.4	100/2.4/1.4	100/2.7/1.5

305 *COD refers to COD_{SOL}; N and P refer to ammonium and phosphate

306 *3.1.2. Effect of initial pH on the urban waste fermentation in batch tests*

307 Another crucial parameter investigated in this work is pH. It is well known from
308 literature that in anaerobic processes the pH affects the hydrolysis and fermentation rate,
309 substrate solubilisation and methanogenic activity (Chen et al., 2007; Li et al., 2018;
310 Maspolim et al., 2015). Generally, at relatively low reaction time methanogenic bacteria
311 do not show remarkable activity and VFA can be accumulated without being consumed.
312 However, hydrolytic and fermentative bacteria can be strongly inhibited if pH drops
313 below 4.5-5.0, limiting the organic matter solubilisation and VFA accumulation (Zou et
314 al., 2018). VFA productions caused a constant drop in pH during the tests, especially in
315 those starting from alkaline conditions. In test A1 and B1, performed under initial pH 5,
316 pH dropped from 5 (day 0) to 4.2 (day 10) and from 5 (day 0) to 4.13 (day 10)
317 respectively. Test C1 showed a quicker drop from 5 (day 0) to 4.25 (day 5) in less time.
318 Tests A2 and B2, performed under initial pH 7, were affected by a pH drop from 7 (day
319 0) to 5.88 (day 10) and from 7 (day 0) to 5.24 (day 10) respectively. In test C2, pH
320 dropped from 7 (day 0) to 5.18 (day 5). In tests A3 and B3, both performed under initial
321 alkaline conditions, pH dropped from 9 (day 0) to 6.51 (day 10) and from 9 (day 0) to
322 5.86 (day 10) respectively. In test C3, pH dropped from 9 (day 0) to 6.18 (day 5) in five
323 days. Tests performed with the thermal pre-treated substrate showed an overall quicker
324 pH drop, reaching almost the same final pH value in half of the time respect to other
325 tests, in accordance with quicker VFA productions.

326 Independently from the temperature range or pre-treatment adopted, the final VFA
327 concentration reached its maximum value under initial alkaline pH. In condition A, B
328 and C the maximum VFA concentrations achieved under alkaline pH were 27.5, 30.0
329 and 41.0 g COD_{VFA}/L respectively. At neutral pH, the maximum VFA concentrations

330 achieved were 12.5, 27.5 and 37.0 g COD_{VFA}/L in condition A, B and C respectively.
331 Apart from condition A, where the process temperature negatively affected the VFA
332 production yield (in particular at initial pH 5.0 and 7.0), conditions B and C showed
333 similar values both under initial neutral and alkaline pH. The drop in pH seemed to
334 affect adversely VFA production only in tests A1, B1 and C1 starting from pH 5, since
335 lower pH values were reached (around 4.2) due to the initial lower pH value. Hence, the
336 fermentation process appeared to be more sensitive to the initial pH value at which tests
337 were performed. These results revealed an interesting outcome about the optimum
338 initial pH value for urban waste fermentation, also in agreement with previous literature
339 studies. Considering the two separated sources, literature reports an optimum pH around
340 5.5 and 6.0 for the OFMSW fermentation (Jiang et al., 2013; Lim et al., 2008; Valentino
341 et al., 2018), and an optimum pH ranging between 10-11 for the WAS fermentation
342 (Wu et al., 2016). Dogan and Demirer (2009) found that an increase in pH value from
343 5.5 to 6.5 decreased hydrolysis performance and VFA production in a CSTR fed with
344 OFMSW at HRT 2 days and OLR 15 g VS/(L d). Since the substrate used for these
345 fermentation batches is a mixture of OFMSW and WAS, both initial neutral and
346 alkaline pH could be eligible working conditions in order to reach high VFA
347 concentrations or high acidification yields, especially related to the solubilized COD
348 (COD_{SOL}). In fact, one of the most relevant factors in the utilization of fermented
349 organic feedstock for biopolymers synthesis (e.g. PHA) is the COD_{VFA} content in
350 relation to COD_{SOL}. High VFA production associated with high COD_{SOL} could not be
351 suitable for PHA production since the non-VFA COD_{SOL} stimulates the growth of non-
352 PHA storing bacteria (Valentino et al., 2017). In order to ensure an appropriate PHA
353 storing bacteria selection process, especially with nutrient rich carbon sources (e.g. the

354 one studied in the present research) high COD_{VFA}/COD_{SOL} ratio is the key element to
355 obtain a good PHA storing biomass selection process (Albuquerque et al., 2010).
356 Therefore, the COD_{VFA}/COD_{SOL} ratio is a useful parameter to understand the
357 fermentation performances. At constant temperature, the initial alkaline fermentation
358 process increased the substrate solubilisation compared to initial acidic and neutral
359 condition. The higher COD_{SOL} achieved (46, 37 and 45.0 g COD_{SOL}/L in test A3, B3
360 and C3 respectively) was also associated to the higher VFA production. VFA increased
361 from 4.7 to 27.5 g COD_{VFA}/L in series A, from 18.6 to 30 g COD_{VFA}/L in series B and
362 from 22.5 to 41 g COD_{VFA}/L in series C as initial pH increased from 5 to 9. In general,
363 all tests under acidic pH showed lower COD_{SOL}/COD_{VFA} ratio. The lowest
364 COD_{VFA}/COD_{SOL} ratio of 0.12 was obtained in test A1, where the activity of
365 fermentative bacteria was strongly inhibited. In the other thermophilic trials, the final
366 COD_{VFA}/COD_{SOL} ratio was equal to 0.31 (A2) and 0.60 (A3). In condition B, the
367 COD_{SOL} concentrations were lower than those obtained in other conditions, but the
368 remarkable VFA production increased the COD_{VFA}/COD_{SOL} ratio up to 0.55, 0.79 and
369 0.81 with initial acidic (B1), neutral (B2) and alkaline (B3) initial pH, respectively. The
370 combined effect of thermal pre-treatment and initial alkaline condition in test C3
371 allowed achieving the highest value of 0.91 COD_{VFA}/COD_{SOL} , due to the enhanced
372 substrate solubilisation combined with a subsequent mesophilic temperature range.
373 Fermentation at initial neutral pH (test C2) also produced a VFA-rich stream with high
374 COD_{VFA}/COD_{SOL} ratio (0.84).
375 In terms of nutrients, ammonium and phosphate were higher in the tests conducted
376 under alkaline fermentation. In the three series, independently from the applied
377 temperature, nutrients concentration increased at increasing initial pH from 5.0 to 9.0.

378 As observed for VFA production, in test C3 the ratio of nutrients related to the COD_{SOL}
379 reached their maximum values (COD:N:P = 100:2.7:1.5).

380

381 *3.1.3. VFA distribution*

382 The distribution of VFA in the fermented feedstock is an important aspect of the
383 process, especially if the production needs to be addressed to a specific group or single
384 VFA. The effect of pH on the VFA composition and production has been widely
385 investigated on different kind of waste (Cavinato et al., 2017; Girotto et al., 2017; Lee et
386 al., 2014; Morgan-Sagastume et al., 2015). In general, the optimum pH level is strictly
387 dependent on the type of waste. In addition, in the context of MMC-PHA production,
388 the content of even numbered C-atoms VFA (3-hydroxybutyrate precursors; 3HBp) and
389 the odd numbered C-atoms VFA (3-hydroxyvalerate precursors; 3HVp) affects
390 monomers synthesis and consequently the polymer composition (Valentino et al.,
391 2017).

392 In the tests conducted in the present study, the qualitative VFA spectrum appeared to be
393 regulated by the feedstock characteristics more than from the fermentation conditions.

394 A dominance of acetic, butyric and propionic acid was observed, with a not negligible
395 amount of valeric, caproic and heptanoic acid. Similarly, acetic, butyric and propionic
396 acid were the most abundantly observed (respectively 27-32%, 18-23% and 15-17%
397 COD basis) in the acidogenic fermentation of thermally pre-treated sludge (Morgan-
398 Sagastume et al., 2011), or WAS fermentation (Feng et al., 2009). Valeric and caproic
399 acid were in some cases also reported at lower concentrations in WAS fermentation
400 (Morgan-Sagastume et al., 2011). Butyric acid was usually predominant (40-60%) in

401 food waste fermentation followed by acetic acid (25-40%) (Giroto et al., 2017;
402 Valentino et al., 2018).

403 The relative VFA percentage seemed to be affected also by the initial pH, more than
404 temperature. Series A showed a net dominance of acetic acid at pH 5.0 (72%) and 7.0
405 (43%); the initial alkaline condition increased the production of propionic, butyric and
406 valeric acid, whose percentages were 25%, 25% and 15% of total VFA respectively.

407 Acetic acid still remained relatively high at 26% but the molar ratio between
408 $[3HVp/(3HB+3HV)p]$ was more than doubled (0.36 mol/mol), if compared to the molar
409 ratio quantified at acidic (0.17 mol/mol) and neutral (0.16 mol/mol) condition.

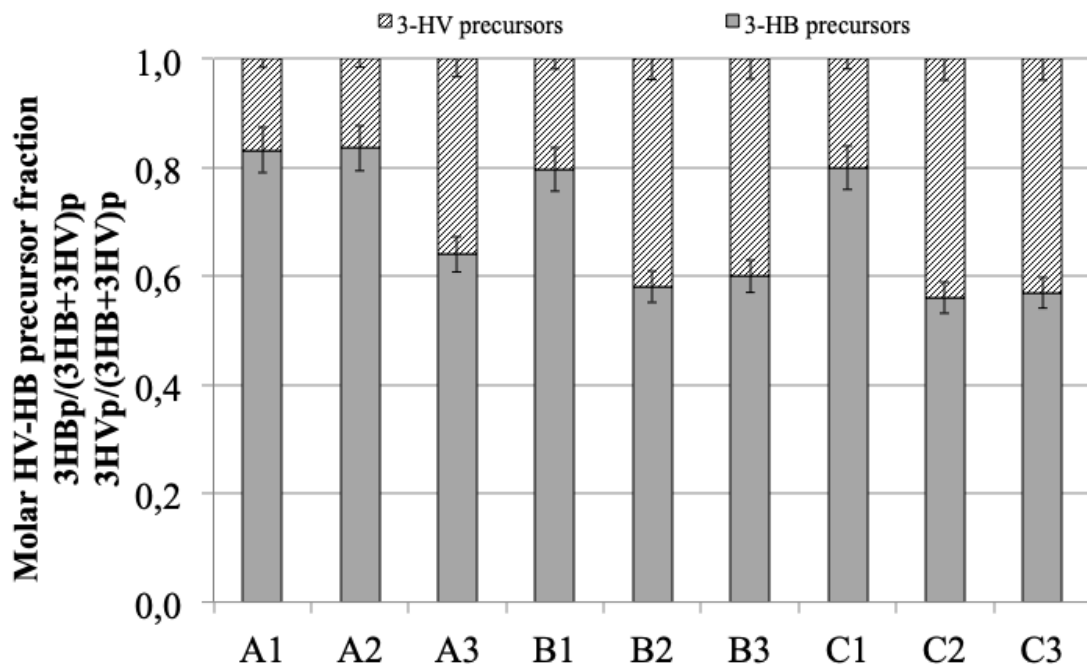
410 In both mesophilic fermentation tests (series B and C), the $[3HVp/(3HB+3HV)p]$ ratio
411 similarly changed as pH increased from 5.0 (0.20 mol/mol) to 7.0 (0.40 mol/mol) and
412 9.0 (0.44 mol/mol) (Figure 2). These results were mainly related to the progressive
413 increase of propionic (up to 13.0 g COD/L, 31% COD basis) and valeric acid (up to 5.8
414 g COD/L, 14% COD basis). Under initial neutral and alkaline conditions, the
415 percentage of butyric acid was constant at 25% in the tests A2-A3, at 14-13% in the
416 tests B2-B3, and at 21-20% in the tests C2-C3. The acidic fermentation seemed to
417 enhance its production up to 3.9 g COD/L (21%) and 6.3 g COD/L (28%) in the tests
418 B1 and C1 respectively, with the only exception of test A1, where the fermentative
419 bacteria were inhibited and the fermentation process was only partially observed.

420 Caproic acid was also abundant under the same conditions: 19% (3.5 g COD/L) and
421 10% (3.3 g COD/L) in tests B1 and C1 respectively. Some variability was observed in
422 the percentage of acetic acid, even though it remained one of the most predominant
423 VFA in all tests performed. Its percentage ranged between 20-28% with two maximum
424 peaks of 43% (5.4 g COD/L, test A2) and 72% (3.4 g COD/L, test A1). Heptanoic acid

425 was particularly affected by the temperature more than pH: it was detected only in the
 426 mesophilic tests with relative higher content in those trials performed without thermal
 427 pre-treatment: 16% (2.9 g COD/L, test B1), 14% (3.9 g COD/L, test B2) and 13% (3.9 g
 428 COD/L, test B3).

429 Initial alkaline fermentation of squeezed OFMSW and thickened WAS mixture
 430 enhanced the production of some acids, propionic in particular, while lowering the
 431 production of butyric and caproic acid, which achieved higher level at acidic pH, as
 432 confirmed by previous studies (Horiuchi et al., 2002).

433 **Figure 2**



434

435

436 *3.2. Effect of different HRTs and OLRs on urban waste alkaline fermentation at*
 437 *mesophilic temperature in a CSTR*

438 The best performing condition C3 (thermal pre-treatment followed by alkaline
 439 mesophilic fermentation) was reproduced in a CSTR for further investigations on the
 440 effects of different HRTs and OLRs on VFA production. The reactor was started with

441 Run I; then, the OLR was increased by decreasing the HRT. The narrow OLR range
442 [7.7 - 11.3 kg VS/(m³ d)] was chosen in order to find a threshold or a limit OLR value,
443 above which the process may be considered not feasible in terms of stable VFA
444 production. Table 4 resumes all the parameters and the process yields analysed in the
445 three conditions. The COD_{SOL} in the effluent reached similar values in the range 41 - 43
446 g COD/L in all the three trials and it was not correlated to the progressive increase of
447 the OLR. Probably, the increase of OLR from 7.7 to 9.3 kgVS/m³ d may not have been
448 so high to justify an increase of the COD_{SOL}. At the highest OLR (11.3 kgVS/m³ d, Run
449 III) the expected increase of COD_{SOL} was not observed most likely due to the lower VS
450 solubilisation (0.07 g COD_{SOL}/g VS₍₀₎), reduced by almost 50% if compared to Run I
451 and II (0.12 g COD_{SOL}/g VS₍₀₎).

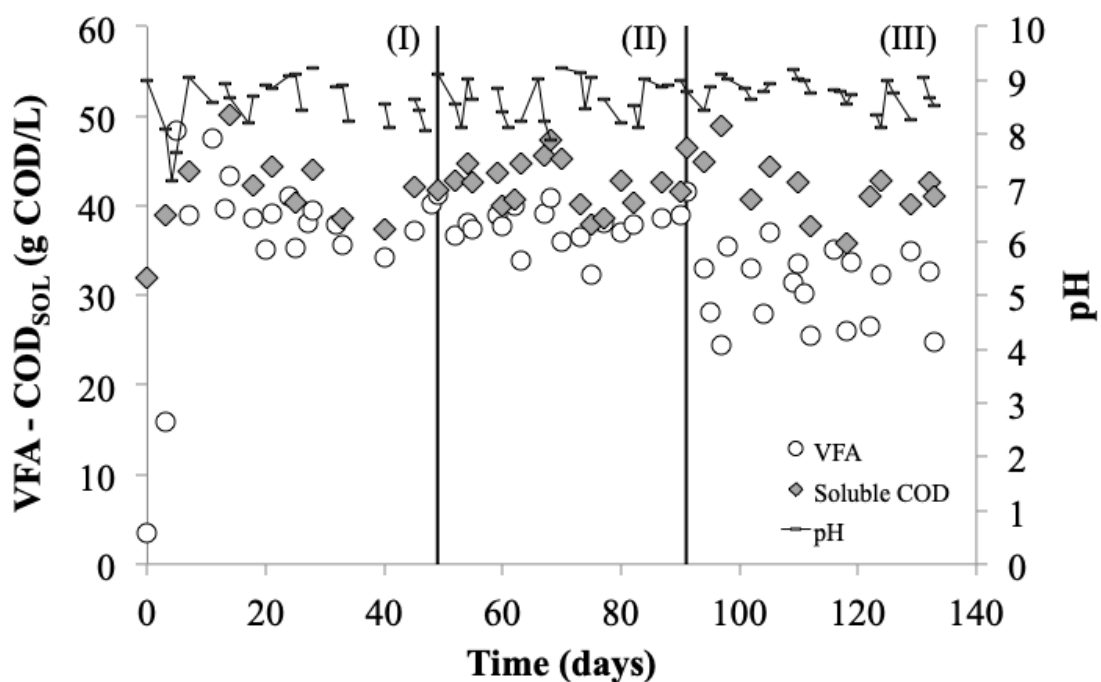
452 The mixture acidification was remarkable from the 5th day (less than 1.0 HRT) and it
453 was steadily maintained at high level, with VFA concentration above 35 g COD_{VFA}/L,
454 for the whole Run I and II. In the last Run III, the reactor seemed to be overloaded,
455 especially in the first part (immediately after the OLR increase), where the VFA
456 dropped from 40 to 24 g COD_{VFA}/L. Over the course of the process, the VFA
457 concentration was affected by frequent fluctuations (Figure 3) and in general the
458 process suffered the imposed high rate condition. Other authors found out that VFA
459 production was similarly affected by high substrate concentration and high applied OLR
460 in a CSTR fed with OFMSW, while high HRT favoured substrate solubilization and
461 VFA production (Argelier et al., 1998). The average VFA concentrations achieved were
462 39 ± 3, 38 ± 2 and 31 ± 4 g COD_{VFA}/L in Run I, II and III respectively. Run I exhibited
463 the highest COD_{VFA}/COD_{SOL} ratio of 0.91. This high value makes this VFA-rich stream
464 suitable for those applications that strictly required this feature (e.g. PHA from MMC;

465 Valentino et al., 2017); Run II was also promising, providing a fermented stream with a
466 ratio of 0.88. Both runs exhibited the highest VFA yields of 0.74 g (Run I) and 0.71
467 (Run II) g COD_{VFA}/g VS₍₀₎; while Run III showed the lowest yields equal to 0.60 g
468 COD_{VFA}/g VS₍₀₎. In terms of VFA composition, acetic and propionic acid were the most
469 predominant in all three conditions (22-25% acetic and 22-28% propionic acid, COD
470 basis), representing together the 50% of all species, in line with the observation of the
471 previous C3 batch test. Butyric (20-22%) and valeric acid (10-13%) were the other
472 predominant species in all three runs. The highest molar [3HVp/(3HB+3HV)p] ratio
473 was obtained in Run II with 0.39 mol/mol, followed by 0.38 mol/mol in Run I, and 0.35
474 mol/mol in Run III. It seems that no significant correlation exists between those ratios
475 and the applied OLR (or HRT). Nutrient concentrations were investigated in terms of
476 ammonium and phosphate quantification. The highest concentrations of both nutrients
477 (1.02 g N-NH₄⁺/L; 0.28 g P-PO₄³⁻/L) were found at higher HRT and lower OLR (Run
478 I), with a slight difference respect to Run II, probably due to the sufficiently high
479 process stability and the available prolonged time for the substrate solubilisation and
480 degradation (Lee et al., 2014). The combined effect of higher HRT and lower OLR in
481 an alkaline system (Run I) resulted as the best operating condition, in terms of VFA
482 production and process stability (39 g COD_{VFA}/L; 0.91 COD_{VFA}/COD_{SOL}). The slight
483 decrease in HRT and increase in OLR (Run II) resulted also as eligible process
484 condition for the soluble COD acidification and further VFA utilisation (38 g
485 COD_{VFA}/L; 0.88 COD_{VFA}/COD_{SOL}). Indeed, every process configuration can have
486 different optimum condition for VFA production. In previous studies focused on
487 OFMSW fermentation, where a different process configuration was applied (leach-bed
488 reactor), an increase in leachate recycle rate favoured VFA production (Yesil et al.,

489 2014). Depending on their composition and characteristics, each feedstock has its
490 optimum fermentation conditions that can be tuned on the scope of future applications
491 (Atasoy et al., 2018). Since the feedstock used in this study was composed by a mixture
492 of OFMSW and WAS, the optimum fermentation conditions in terms of VFA level
493 maximization can be intermediate between the one for OFMSW and WAS. Authors
494 found out that increasing OLR lead to increasing VFA productions in OFMSW
495 fermentation; however, OLR higher than 13 kg VS/m³ d made the process unstable
496 (Lim et al., 2008). Generally, high rate processes for OFMSW fermentation are
497 considered unsuitable since the easy putrescible matter may lead to a quick VFA
498 production, not properly buffered unless an efficient pH control system is applied
499 (Valentino et al., 2018). On the other hand, prolonged HRTs (up to a maximum of 12
500 days) can enhance VFA production and process stability with complex substrates such
501 as OFMSW and WAS (Lim et al., 2008; Zhou et al., 2018). Hong and Haiyun (2010)
502 investigated the effects of the interactions of different parameters (food waste
503 composition as co-substrate, HRT, OLR and pH) on food waste and dewatered excess
504 sludge fermentation process through the application of experimental design methods.
505 They observed significant correlations between pH and OLR, pH and HRT, pH and
506 food waste composition, showing that with increasing OLR, VFA production decreased
507 above 7.9 g VSS/(L d). VFA production also decreased with too long HRT, above 8.92
508 days, in accordance to the findings of the present study. Other authors reported VFA
509 concentrations up to 60 g/L (Giroto et al., 2017) with food waste fermentation.
510 However, such high value was obtained in batch test and presumably a continuous
511 process implementation could require effective strategies to maintain the pH at the
512 desired value. Indeed, this high rate VFA production could probably cause a drastic

513 drop in the pH with the risk of system imbalance. On the contrary, WAS fermentation
 514 generates less VFA than OFMSW (no more than 6 g COD/L) in the same temperature
 515 range (Chen et al., 2007; Girotto et al, 2017; Hao, Wang, 2015; Horiuchi et al., 2002;
 516 Strazzera et al., 2018). A total VFA concentration close to 40 g COD_{VFA}/L with limited
 517 amount of non-VFA soluble COD, as herein obtained (Run I and II) is quite promising
 518 since it would be a valuable carbon source for those applications that require high VFA
 519 productions in high organic loading rate processes (e.g. MMC-PHA production). In
 520 fact, higher VFA production would translate in higher volumetric PHA productivity [kg
 521 PHA/(L d)], which is defined on the overall PHA storage yield over the utilised VFA
 522 (Valentino et al., 2018). Economic viability of this approach needs to be addressed for
 523 the evaluation of associated costs of heating requirements and chemical supply, in a
 524 specific biorefinery scenario where the MMC-PHA production technology is intended
 525 to be integrated in existing WWTP facilities.

526 **Figure 3**



527

528 **Table 4**

Operating condition	Unit	CSTR trial		
		Run I	Run II	Run III
HRT	d	6	5	4.1
OLR	kg VS/(m ³ d)	7.7	9.3	11.3
Parameter	Unit	Run I	Run II	Run III
TS	g/kg	54 ± 4	52 ± 4	57 ± 5
VS	g/kg	40 ± 3	41 ± 3	42 ± 4
VFA	g COD/L	39 ± 2	38 ± 2	31 ± 4
COD _{SOL}	g COD/L	43 ± 4	43 ± 3	41 ± 3
COD _{VFA} /COD _{SOL}	-	0.91 ± 0.04	0.88 ± 0.06	0.76 ± 0.07
[3HVp/(3HB+3HV)p]	mol/mol	0.38 ± 0.03	0.39 ± 0.02	0.35 ± 0.04
Solubilisation	g COD _{SOL} /g VS ₍₀₎	0.12 ± 0.07	0.12 ± 0.06	0.07 ± 0.06
Yield	g COD _{VFA} /g VS ₍₀₎	0.74 ± 0.05	0.71 ± 0.03	0.60 ± 0.06
Ammonium	g N-NH ₄ ⁺ /L	1.02 ± 0.04	0.87 ± 0.05	0.82 ± 0.05
Phosphate	g P-PO ₄ ³⁻ /L	0.28 ± 0.08	0.27 ± 0.02	0.17 ± 0.02

529

530 **4. Conclusions**

531 This study assessed how the fermentation process of a specific waste mixture of urban
532 origin could be optimized in terms of organic matter solubilisation and VFA production.
533 For this work, a mixture composed by thickened WAS (65-70% v/v) and squeezed
534 OFMSW (30-35% v/v) was used as renewable feedstock. A first screening of batch
535 trials revealed that alkaline pH (9.0) and mesophilic temperature (37°C), coupled with
536 thermal pre-treatment (72°C, 76 h) gave the best performances in terms of maximum
537 VFA concentration and COD_{VFA}/COD_{SOL} ratio. The same working conditions were
538 proposed in a CSTR. The effluent VFA-rich stream was characterized by a stable
539 production close to 40 g COD_{VFA}/L with consistent composition and limited amount of
540 non-VFA soluble COD (0.91 COD_{SOL}/COD_{VFA}), at HRT of 6 days and OLR of 7.7 kg
541 VS/m³ d. The outcomes of this work could be used as a beneficial tool for the
542 development of a biorefinery platform dedicated to the valorisation of urban organic

543 waste and recovery of added-value bio-products (such as biopolymers) through a
544 combined anaerobic/aerobic multi-steps process.

545

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567 **Figures and Tables captions**

568 Figure 1. VFA concentration trends in the thermophilic batch tests A1-A2-A3 (a) under
569 initial pH 5, 7 and 9; mesophilic batch tests B1-B2-B3 (b) under initial pH 5, 7 and 9;
570 mesophilic batch tests with thermal pre-treatment C1-C2-C3 (c) under initial pH 5, 7
571 and 9.

572 Figure 2. VFA distribution expressed as 3HB (even numbered C-atoms VFA) and 3HV
573 (odd numbered C-atoms VFA) precursors obtained in batch tests.

574 Figure 3. Soluble COD, VFA concentrations and pH obtained during the continuous
575 lab-scale trial in the three conditions tested.

576 Table 1. Chemical-physical characteristics of the thickened WAS, pre-treated OFMSW,
577 urban waste mixture and thermally pre-treated substrate used for batch tests and
578 continuous trial.

579 Table 2. Summary of the operating conditions investigated in the nine batch tests.

580 Table 3. Final concentrations and fermentation performances obtained in all batch tests.

581 Table 4. Final concentrations and fermentation performances obtained in the three
582 CSTR trials.

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1 | **Optimization of urban ~~bio-waste (food waste and waste activated sludge)~~**
2 | **fermentation for volatile fatty acids production**

3

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24

25 **Abstract**

26 The problem of waste disposal has recently focused on practices for waste recycling and
27 bio-resources valorization. Organic waste produced in urban context together with
28 biological sludge produced in wastewater treatment plants (WWTPs) can be used as
29 renewable feedstock for the production of building blocks of different products, from
30 biopolymers to methyl esters. This paper deals with the optimization of the fermentation
31 process in order to transform urban organic waste (a mixture of pre-treated food waste
32 and biological sludge) into added-value volatile fatty acid (VFA) rich stream, useful for
33 biological processes within a biorefinery technology chain. Different temperatures, pH,
34 hydraulic retention times (HRTs) and organic loading rates (OLRs) were tested both in
35 batch and continuous trials. Batch tests showed the best working conditions at 37°C and
36 pH 9, using the bio-waste feedstock thermally pre-treated (76 h at 72°C). These
37 conditions were applied in continuous process, where higher HRT (6.0 d) and lower
38 OLR [~~7.7~~ 7.7 kg VS/(m³ d)] gave the best performances in terms of process yield and
39 maximum VFA level achieved: 0.77 COD_{VFA}/VS₍₀₎ and 39 g COD_{VFA}/L. An optimized
40 fermentation process is crucial in a biorefinery perspective since it has to give a final
41 stream of constant composition or tailored products suitable for further applications.

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49 **Keywords**

50 Organic fraction of municipal solid waste (OFMSW); Volatile Fatty Acid (VFA);

51 Waste Activated Sludge (WAS); Biorefinery; Anaerobic fermentation

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53 **Abbreviations**

54 COD, chemical oxygen demand; COD_{SOL}, soluble COD; CSTR, continuous stirred tank

55 reactor; HRT, hydraulic retention time; MMC, mixed microbial cultures; OFMSW,

56 organic fraction of municipal solid waste; OLR, organic loading rate; PHA,

57 polyhydroxyalkanoates; TKN, Total Kjeldahl Nitrogen; TS, Total Solids; VFA, volatile

58 fatty acids; VS, Volatile Solid; WAS, waste activated sludge; WWTP, wastewater

59 treatment plant; 3HB, 3-hydroxybutyrate; 3HV, 3-hydroxyvalerate.

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73 **1. Introduction**

74 Waste management and disposal is one of the most pressing issues mainly due to the
75 exponential population increase in the last decades, especially in urban areas where the
76 majority of the population is located (Pfaltzgraff et al., 2013). The main organic refuses
77 produced in this context are food waste, also known as the organic fraction of municipal
78 solid waste (OFMSW), and biological sludge coming from the wastewater treatment. In
79 Europe, the average organic matter production expressed as total solid (TS) per day (d)
80 *pro capita* (person equivalent, PE) is approximately 55 g TS/(PE) d of OFMSW and 39
81 g TS/(PE) d of biological sludge (Colombo et al., 2017; IPCC, 2006). It is quite clear
82 that these kinds of organic waste are highly available especially in urban areas and at
83 present time they are handled and treated separately. The sludge is typically digested,
84 dewatered and then sent to final disposal, and the OFMSW is sent to composting. The
85 separate treatment of biological sludge and OFMSW is generally not convenient from
86 both energetic and environmental point of view. As a matter of fact, anaerobic digesters
87 treating sludge in wastewater treatment plants (WWTPs), especially waste activated
88 sludge (WAS), are often low loaded and underperforming (Bolzonella et al., 2005),
89 while aerobic composting is a highly energy consuming process. The advantages of a
90 combined treatment of OFMSW and WAS have already been exploited by the
91 anaerobic co-digestion approach, with the experiences reported in previous studies
92 (Mata-Alvarez et al., 2014). This technology directs all the organic matter into a single
93 step, in which the conversion into biogas and energy is realized (Scarlat et al., 2018).

94 Digestate is also produced together with biogas and energy, with environmental
95 concerns regarding its further stabilization and disposal and the possible presence of

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96 | [heavy metals, pharmaceuticals and/or other pathogens \(Mata-Alvarez et al., 2014\).](#)
97 | [OFMSW mixing with WAS generates several benefits, such as dilution of potential](#)
98 | [toxic compounds and improved nutrient balance \(Zahedi et al., 2016\).](#) A more effective
99 | application of this approach can be found in urban contexts where the OFMSW is
100 | coming from a source separate collection or a door-to-door collection, since the
101 | biodegradability of the [food-collected](#) waste improves substantially due to the increase
102 | of organic matter content and the decrease of inert materials (Bernstad et al., 2013).
103 | Novarino and Zanetti (2012) reported the application of a mechanical pretreatment
104 | method to further separate the inert material and homogenize the organic matter,
105 | enhancing the anaerobic co-digestion process. The integration of OFMSW and WAS
106 | treatment can be easily realized in existing WWTPs, where anaerobic digesters are in
107 | most cases already present, in order to improve the WWTP energy balance. Authors
108 | reported some co-digestion experiences in full-scale plants, such as the Rovereto
109 | WWTP, located in the Trento province in northern Italy, in which the OFMSW and
110 | mixed sludge co-digestion was implemented in 2014 (Mattioli et al., 2017). In the
111 | Treviso province ([northeast](#) Italy) the co-digestion approach of OFMSW and WAS in
112 | the full-scale WWTP was proposed and implemented since 1999 (Bolzonella et al.,
113 | 2006; Pavan et al., 2000). In this specific urban scenario, the waste separate collection is
114 | very efficient and reaches 87.9% on the total wastes (ISPRA, 2017) making the organic
115 | waste treatment integration an eligible way for valorization and recovery of the organic
116 | matter. A [possible and innovative](#) approach for the realization of this treatment
117 | integration can be found in [an](#) urban biorefinery. The urban biorefinery represent a
118 | technology chain in which the organic material of urban waste can be converted into
119 | new added-value bio-based products (Valentino et al., 2018). Bio-based products

120 obtainable in an urban biorefinery through the exploitation of organic waste are biofuels
121 (Stephen and Periyasamy, 2018), platform chemicals (Kiran et al., 2014), and
122 bioplastics (Valentino et al., 2017). Some of the most important intermediates that allow
123 the conversion of organic waste into these valuable bio-based products are volatile fatty
124 acids (VFA), which in most cases are the direct precursors for biopolymer synthesis,
125 such as polyhydroxyalkanoates (PHA) obtained from pure and mixed microbial culture
126 (MMC) (Valentino et al., 2014). VFA are produced during the anaerobic fermentation
127 process of almost all kinds of biodegradable organic waste (Strazzera et al., 2018).
128 Previous studies reported experiences of anaerobic fermentation on dewatered sludge
129 (Hao & Wang, 2015), cellulosic substrates (Keating et al., 2013), cheese whey
130 (Colombo et al., 2017; Valentino et al., 2015) and OFMSW (Chen et al., 2017; Giroto
131 et al., 2017; Korkakaki et al., 2016). In a platform where anaerobic and aerobic
132 processes are combined, VFA obtained from waste fermentation are intermediate
133 chemicals for the conversion of organic matter into the aforementioned biodegradable
134 added-value products (Koller et al., ~~2016~~2017). Indeed, in a scenario where the
135 OFMSW is coming from a highly efficient source separate collection and the anaerobic
136 co-digestion of OFMSW and WAS is already implemented, the urban biorefinery
137 concept finds its perfect integration. The municipality of Treviso (northeast Italy) is a
138 representative example where the OFMSW-WAS mixture is currently sent to anaerobic
139 co-digestion. Indeed, the hypothesis of driving part of this organic source into an
140 anaerobic fermentation step for the VFA production could be an eligible way for an
141 efficient urban organic waste management. Within this route, the combined VFA and
142 methane production from OFMSW-WAS mixture has been recently demonstrated
143 (Valentino et al., 2019) in the same urban context. However, in order to enhance the

144 PHA [synthesis and productivity](#), the fermentation of OFMSW-WAS [mixture](#) needs to
145 be optimized, maximizing the VFA [production and, as a consequence, the PHA](#)
146 [potentially obtainable](#). In this study, different fermentation conditions were tested by
147 means of batch tests on a mixture of OFMSW and WAS. These batch trials were
148 conducted in order to find the best working conditions for the fermentation process,
149 namely optimum pH value and temperature. Once the best condition was found, a
150 continuous lab scale trial in a continuous stirred tank reactor (CSTR) was set-up to
151 better represent an acidogenic fermentation process under different applied HRT and
152 OLR.

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154 **2. Materials and Methods**

155 *2.1. Substrate characterization*

156 The substrates used in this study were thickened WAS and pre-treated OFMSW both
157 available inside the Treviso WWTP. The WAS [has been](#) collected from the static
158 thickener of the full-scale plant; the OFMSW came from the source sorted collection in
159 50 districts of the Treviso Province and was transferred to the full-scale WWTP after its
160 pre-treatment (squeezing and homogenization) [in a dedicated plant](#). The mixture used
161 for all trials was composed by volumetric fractions of 65-70% thickened WAS and of
162 30-35% squeezed OFMSW. Thickened WAS and squeezed OFMSW had TS contents
163 of 30 ± 1 g TS/kg and 139 ± 7 g TS/kg respectively, with a volatile solid (VS) fraction
164 of 70% VS/TS for WAS and 90% VS/TS for OFMSW. The high VS/TS ratio of the
165 OFMSW was favoured by the quality of the source-sorted collection, which effectively
166 increased the readily biodegradable organic content. On the other hand, the relatively
167 low nutrients (nitrogen and phosphorus) level in the squeezed OFMSW was

168 counterbalanced by the high volumetric fraction of WAS, which contributed to the final
 169 COD:N:P ratio of 100:2.5:0.7 in the mixture. Table 1 shows the average values obtained
 170 from the chemical-physical characterization of both separated streams, ~~and~~ of the final
 171 ~~urban bio~~-waste mixture and of the waste mixture after thermal pre-treatment.

172
 173 **Table 1**

<u>Parameter</u>	<u>Unit</u>	<u>Thickened WAS</u>	<u>Pre-treated OFMSW</u>	<u>Urban waste mixture</u>	<u>Thermally pre-treated urban waste mixture</u>
<u>Total Solid (TS)</u>	<u>g/kg</u>	<u>30 ± 1</u>	<u>139 ± 19</u>	<u>62 ± 7</u>	<u>58 ± 6</u>
<u>Volatile Solid (VS)</u>	<u>g/kg</u>	<u>21 ± 1</u>	<u>125 ± 14</u>	<u>50 ± 2</u>	<u>46.4 ± 0.9</u>
<u>VS/TS</u>	<u>%</u>	<u>70 ± 4</u>	<u>90 ± 2</u>	<u>81 ± 2</u>	<u>83 ± 1</u>
<u>COD_{SOL}</u>	<u>g COD/L</u>	<u>0.15 ± 0.06</u>	<u>81 ± 7</u>	<u>32 ± 3</u>	<u>38 ± 3</u>
<u>VFA</u>	<u>g COD/L</u>	<u>-</u>	<u>8.3 ± 0.6</u>	<u>2.4 ± 0.3</u>	<u>2.2 ± 0.2</u>
<u>pH</u>	<u>-</u>	<u>6.6 ± 0.2</u>	<u>4.3 ± 0.3</u>	<u>5.1 ± 0.3</u>	<u>5.0 ± 0.2</u>
<u>COD_{VFA}/COD_{SOL}</u>	<u>-</u>	<u>-</u>	<u>0.11 ± 0.02</u>	<u>0.08 ± 0.02</u>	<u>0.06 ± 0.01</u>
<u>Total Kjeldahl Nitrogen (TKN)</u>	<u>g N/kg TS</u>	<u>42 ± 3</u>	<u>27 ± 1</u>	<u>35 ± 2</u>	<u>36.0 ± 0.8</u>
<u>Phosphorus (P)</u>	<u>g P/kg TS</u>	<u>15 ± 1</u>	<u>7.0 ± 0.4</u>	<u>10 ± 1</u>	<u>10 ± 2</u>

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 175

176 2.2. Batch fermentation tests

177 Batch tests were performed in order to investigate the effects of different combinations
 178 of temperature and initial pH on ~~urban bio~~-waste fermentation. Mesophilic (37°C) and
 179 thermophilic (55°C) conditions were tested. In parallel, another mesophilic trial was
 180 performed with ~~the same urban waste mixture after a~~ thermal pre-treatment (72°C for
 181 76 h) ~~of bio-waste~~. For each of the three conditions, three different initial pH values
 182 (5.0, 7.0 and 9.0) were investigated. Sodium hydroxide (NaOH) was added to reach
 183 both initial pH values of 7.0 and 9.0; no chemicals addition was necessary for the tests
 184 at initial pH value of 5.0 since it was the typical pH of the ~~bio-urban~~ waste mixture.
 185 Each test was performed in duplicate, in 1.0 L glass bottles sealed with a silicon plug

186 and an aluminium seal (working volume 0.75 L). No inoculum was added since more
187 than a sufficient amount of fermentative microorganisms were already present in the
188 ~~squeezed OFMSW~~initial waste mixture. The investigated conditions are displayed in
189 Table 2.

190 Batch fermentation tests were monitored up to ten (trials A-B) and five (trial C) days.

191 Liquid samples (15.0 mL) were collected each day for VFA analysis and pH
192 measurements as well as for ammonium (N-NH₄⁺), phosphate (P-PO₄³⁻) and soluble
193 COD (COD_{SOL}). The slurry was manually mixed twice a day, one hour before and 6
194 hours after each sampling. The bottles were opened for the sampling and then
195 maintained under N₂ flux for 20 min to re-establish anaerobic conditions.

196
197

Table 2

Temperature (°C)	Thermal pre-treatment	Initial pH	Test name
55	-	5.0	A1
55	-	7.0	A2
55	-	9.0	A3
37	-	5.0	B1
37	-	7.0	B2
37	-	9.0	B3
37	72°C, 76 h	5.0	C1
37	72°C, 76 h	7.0	C2
37	72°C, 76 h	9.0	C3

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199 2.3. Urban waste fermentation process in a CSTR~~CSTR~~

200 The best performing condition obtained in batch tests was further investigated by means
201 of a fermentation process in a 6.0 L CSTR that was maintained at a fixed temperature
202 (37°C) by an external thermostatic jacket and stirred with a mechanical impeller at 80
203 rpm. No inoculum was added. The reactor was fed once per day, in a semi-continuous
204 manner. Acidogenic fermentation was started up and initially conducted with an OLR of

205 | 7.7 kg VS/(m³ d), which was then increased up to 9.3 and 11.3 kg VS/(m³ d). Each
206 | condition was characterized under steady state and compared in terms of VFA yields
207 | and composition. The increasing OLR was combined with a corresponding decrease of
208 | HRT, from 6.0 to 5.0 and 4.1 days. The reactor was not equipped with pH control;
209 | however, alkaline fermentation was performed by adjusting the pH of the feedstock at
210 | pH 9.0. The effluent was collected before the feeding for pH measurements and VFA
211 | analysis (up to five times a week) as well as TS, VS, ammonium, phosphate and
212 | COD_{SOL} analysis (twice per week).

213

214 | 2.4. Analytical methods

215 | Analyses were conducted according to Standard Methods (APHA, AWWA, WEF,
216 | 1998). Volatile fatty acids were determined using an Agilent 6890N gas chromatograph
217 | equipped with a flame ionization detector (FID) (T = 250°C). Samples were analysed
218 | through an Agilent J&W DB-FFAP fused silica capillary column (15 m length, 0.53
219 | mm i.D. x 0.5 mm film) using hydrogen as carrier. The inlet was working in split mode,
220 | with a split ratio of 20:1. The instrument was programmed with a ramp temperature
221 | from 80°C to 100°C (10°C/min). Before GC analyses, samples were centrifuged at
222 | 4,500 rpm for five minutes and the supernatant was filtered at 0.2 µm using acetate
223 | cellulose syringe filters (Whatman).

224

225 | 2.5. Data analysis

226 | According to the sampling described above, data were collected daily and analysed at
227 | the end of each test batch. In the CSTR experiment, data were processed according to
228 | the adopted frequency of sampling (paragraph 2.3). In order to better represent VFA

229 distribution, the molar ratio between odd numbered acids and the total concentration
230 was determined. The nutrient concentration, related to the release of ammonium and
231 phosphate in the medium, was also quantified by means of the COD:N:P ratio expressed
232 in grams. The substrate solubilisation was calculated by the ratio between the final
233 soluble COD (as net concentration subtracted to the initial COD_{SOL}; COD_{SOL(0)}) and the
234 initial VS of the feedstock (VS₍₀₎), as it follows:-

$$235 \quad \text{Solubilisation} = \frac{(COD_{SOL} - COD_{SOL(0)})}{VS_{(0)}}$$

236 The VFA yield was determined by the ratio between the produced VFA (as net
237 concentration subtracted to the initial VFA; COD_{VFA(0)}) and the initial VS of the
238 feedstock (VS₍₀₎), as it follows:-

$$239 \quad \text{Yield} = \frac{(COD_{VFA} - COD_{VFA(0)})}{VS_{(0)}}$$

240

241 Substrate solubilisation and VFA yield were calculated for each batch test and for the
242 CSTR process. In batch tests with the thermal pre-treated substrate (C1, C2 and C3) and
243 in the CSTR process both substrate solubilization and VFA yield were calculated
244 considering the substrate after thermal pre-treatment as starting point.

245

246 **3. Results and Discussion**

247 *3.1. Batch fermentation tests*

248 All batch tests were started with the same bio-urban waste mixture; indeed, the initial
249 solids as well as the macronutrients (nitrogen and phosphorus) content were similar in
250 all trials. The bio-urban waste mixture was extremely homogeneous since the OFMSW
251 was squeezed in the pre-treatment and resulted as liquid slurry after mixing with

252 | thickened WAS. VFA concentrations were ~~daily~~-monitored daily for each batch test.
253 | The main results are summarized in Table 3.
254 |
255 | *3.1.1. Effect of temperature on the ~~bio-urban~~ waste fermentation in batch tests*
256 | Figure 1 shows the VFA concentrations in the thermophilic (A), mesophilic (B) and
257 | pre-treated mesophilic (C) batch tests, which have been performed at initial pH value of
258 | 5.0 (1), 7.0 (2) and 9.0 (3). In terms of VFA production rates and yields, the mesophilic
259 | condition was ~~more-better~~ performing. Some literature studies report that mesophilic
260 | temperature ranges are preferable to thermophilic ones, not only for OFMSW (Garcia-
261 | Aguirre et al., 2017) but also for sewage sludge (primary or WAS) fermentation process
262 | (Morgan-Sagastume et al., 2015).
263 | Under thermophilic condition, VFA production lagged in the first two days and started
264 | noticeably only from the third day, especially in those trials ~~performed-started~~
265 | alkaline pH value (9.0). This observation was in line with previous studies (Peces et al.,
266 | 2016; Jiang et al., 2013), where the initial lower acidification yields observed at 55°C
267 | was due to the slower adaptation of the thermophilic culture. In the tests performed
268 | under mesophilic condition, with or without thermal pre-treatment, the fermentation
269 | activity was immediately observed both at pH 7.0 and 9.0. Overall, at the same initial
270 | pH investigated, VFA concentrations obtained under condition B (18.6-30.0 g
271 | COD_{VFA}/L) and C (22.5-41.0 g COD_{VFA}/L) are higher than those obtained under
272 | condition A (4.7-27.5 g COD_{VFA}/L), as most of literature studies suggest (Jiang et al.,
273 | 2013; Morgan-Sagastume et al., 2015; Wan et al., 2016). Thermophilic temperatures
274 | favoured the organic matter solubilisation (0.22-0.36 g COD_{SOL}/g VS₍₀₎) but a
275 | substantial part of the solubilized COD was not efficiently converted into VFA (0.06-

276 0.52 g COD_{VFA}/g VS₍₀₎). Indeed, thermophilic tests A1-A2-A3 were characterized by
277 higher COD_{SOL} ([39-46 g COD_{SOL}/L](#)) than mesophilic tests B1-B2-B3 ([34-37 g](#)
278 [COD_{SOL}/L](#)), but lower final VFA content independently from the chosen initial pH. On
279 the other hand, thermal pre-treatment (tests C1-C2-C3) increased the solubilisation yield
280 (0.07-0.15 g COD_{SOL}/g VS₍₀₎) [in the mesophilic fermentation process](#) compared to tests
281 B1-B2-B3 performed without thermal pre-treatment (0.06-0.09 g COD_{SOL}/g VS₍₀₎). The
282 mesophilic fermentation process applied to an already solubilized substrate allowed
283 increasing [the](#) VFA content (22.5-41.0 g COD_{VFA}/L), much more than tests B1-B2-B3,
284 where the thermal pre-treatment was not applied (19.2-30.0 g COD_{VFA}/L), being VFA
285 yields comparable in the fermentation series B (0.29-0.49 g COD_{VFA}/g VS₍₀₎) and C
286 (0.26-0.57 g COD_{VFA}/g VS₍₀₎). Moreover, ~~the~~ VFA production [rate](#) was substantially
287 improved after the organic matter pre-treatment at 72°C: approximately 35 g COD_{VFA}/L
288 were achieved in less than 5 days (at least under [initial](#) neutral and alkaline pH), which
289 reasonably meant a doubled production rate compared to the thermophilic alkaline test
290 A3 and mesophilic neutral-alkaline tests B2-B3.

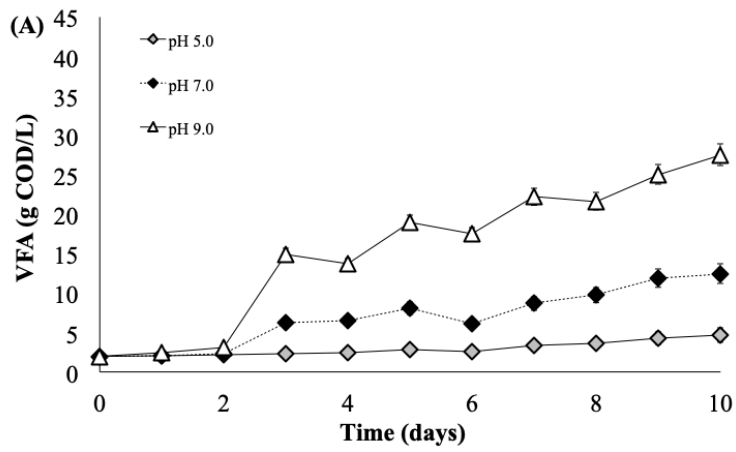
291 Usually, thermophilic temperatures enhance the hydrolysis process in which complex
292 molecules are solubilized and become more available for the microorganisms'
293 metabolism (Dahiya et al., 2015; [Morgan-Sagastume et al., 2015](#)). Indeed, process
294 temperature strongly affected the nutrients release from the substrate. Ammonium and
295 phosphate levels quantified at the end of tests C (0.94-1.22 g N-NH₄⁺/L; 0.56-0.67 g P-
296 PO₄³⁻), where bio-waste was thermally pre-treated, were higher than those achieved in
297 thermophilic series A (0.41-1.04 g N-NH₄⁺/L; 0.28-0.43 g P-PO₄³⁻) and mesophilic
298 series B (0.43-0.54 g N-NH₄⁺/L; 0.25-0.33 g P-PO₄³⁻).

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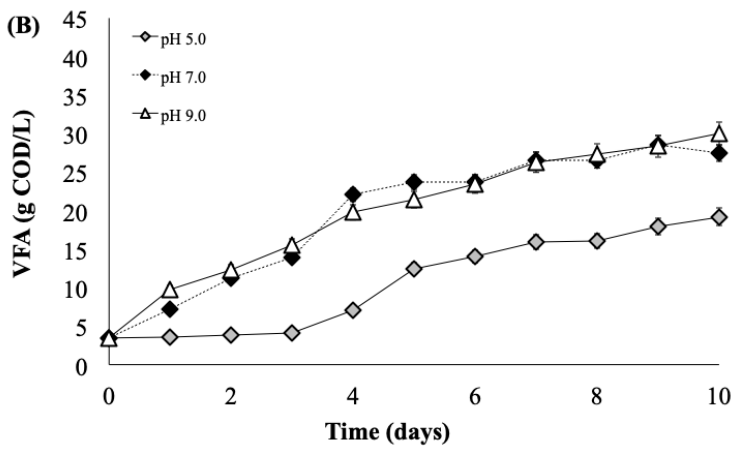
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Figure 1

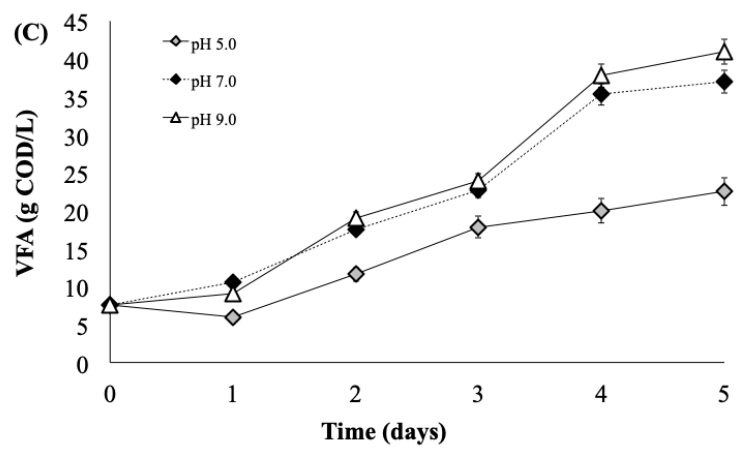
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306



307



308 **Table 3**

Parameter	Unit	Test name								
		A1	A2	A3	B1	B2	B3	C1	C2	C3
Total VFA	g COD/L	4.7 ± 1	12.5 ± 0.7	27.5 ± 2	18.6 ± 0.9	27.5 ± 2	30 ± 2	22.5 ± 0.7	37 ± 2	41 ± 2
Acetic acid	g COD/L	3.4 ± 0.5	5.4 ± 0.3	7.1 ± 0.5	4.8 ± 0.2	7.1 ± 0.2	7.1 ± 0.3	6.4 ± 0.3	7.3 ± 0.6	10.0 ± 0.8
Propionic acid	g COD/L	1.2 ± 0.4	2.5 ± 0.2	6.8 ± 0.2	0.8 ± 0.1	3.8 ± 0.3	6.9 ± 0.4	3.2 ± 0.1	9.6 ± 0.1	13.0 ± 0.3
Isobutyric acid	g COD/L	0	0	0.6 ± 0.1	0	0	0	0	0	0
Butyric acid	g COD/L	0	3.1 ± 0.1	6.9 ± 0.4	3.9 ± 0.1	3.8 ± 0.2	4.0 ± 0.3	6.3 ± 0.1	7.6 ± 0.3	8.2 ± 0.4
Isovaleric acid	g COD/L	0	0	1.2 ± 0.1	1.3 ± 0.1	1.0 ± 0.4	1.2 ± 0.1	0	0	0
Valeric acid	g COD/L	0	0	4.2 ± 0.4	1.4 ± 0.1	5.0 ± 0.5	4.2 ± 0.5	1.8 ± 0.1	5.0 ± 0.5	5.8 ± 0.3
Caproic acid	g COD/L	0	1.5 ± 0.1	0.7 ± 0.3	3.5 ± 0.4	2.9 ± 0.1	2.8 ± 0.3	3.3 ± 0.2	3.0 ± 0.2	1.4 ± 0.1
Heptanoic acid	g COD/L	0	0	0	2.9 ± 0.1	3.9 ± 0.4	3.9 ± 0.1	1.6 ± 0.1	4.6 ± 0.3	2.4 ± 0.1
Soluble COD	g COD/L	39 ± 2	40 ± 3	46 ± 1	34 ± 2	35 ± 2	37 ± 3	41 ± 3	44 ± 4	45 ± 3
Ammonium	g N-NH ₄ ⁺ /L	0.41 ± 0.05	0.88 ± 0.06	1.04 ± 0.05	0.43 ± 0.04	0.47 ± 0.06	0.54 ± 0.08	0.94 ± 0.07	1.07 ± 0.09	1.22 ± 0.09
Phosphate	g P-PO ₄ ³⁻ /L	0.28 ± 0.04	0.37 ± 0.05	0.43 ± 0.07	0.25 ± 0.05	0.28 ± 0.05	0.33 ± 0.04	0.56 ± 0.05	0.62 ± 0.08	0.67 ± 0.07
Solubilisation	gCOD _{SOL} /gVS ₍₀₎	0.22 ± 0.02	0.24 ± 0.04	0.36 ± 0.03	0.06 ± 0.01	0.06 ± 0.02	0.09 ± 0.01	0.07 ± 0.01	0.13 ± 0.02	0.15 ± 0.02
Yield	gCOD _{VFA} /gVS ₍₀₎	0.06 ± 0.03	0.22 ± 0.03	0.52 ± 0.03	0.29 ± 0.04	0.44 ± 0.05	0.49 ± 0.05	0.26 ± 0.03	0.50 ± 0.04	0.57 ± 0.03
{(HV)/(HB+HV)}	mol/mol	0.17 ± 0.03	0.16 ± 0.03	0.36 ± 0.02	0.20 ± 0.04	0.42 ± 0.03	0.40 ± 0.03	0.20 ± 0.01	0.44 ± 0.01	0.43 ± 0.02
COD:N:P*	g	100/1.1/0.7	100/2.2/0.9	100/2.3/0.9	100/1.3/0.7	100/1.3/0.8	100/1.5/0.9	100/2.2/1.4	100/2.4/1.4	100/2.7/1.5

309 *COD refers to COD_{SOL}; N and P refer to ammonium and phosphate

310 | 3.1.2. Effect of *initial pH* on the *bio-urban* waste fermentation in batch tests

311 | Another crucial parameter investigated in this work is pH. It is well known from
312 | literature that in anaerobic processes the pH affects the hydrolysis and fermentation rate,
313 | substrate solubilisation and methanogenic activity (Chen et al., 2007; Li et al., 2018;
314 | Maspolim et al., 2015). Generally, at relatively low reaction time methanogenic bacteria
315 | do not show remarkable activity and ~~the~~ VFA can be accumulated without being
316 | consumed. However, hydrolytic and fermentative bacteria can be strongly inhibited if
317 | pH drops below 4.5-5.0, limiting the organic matter solubilisation and VFA
318 | accumulation (Zou et al., 2018). ~~Independently from the temperature range or pre-~~
319 | ~~treatment adopted, the final VFA concentration reached its maximum value under~~
320 | ~~alkaline pH. VFA productions caused a constant drop in pH during the tests, especially~~
321 | ~~in those starting from alkaline conditions. In test A1 and B1, performed under initial pH~~
322 | ~~5, pH dropped from 5 (day 0) to 4.2 (day 10) and from 5 (day 0) to 4.13 (day 10)~~
323 | ~~respectively. Test C1 showed a quicker drop from 5 (day 0) to 4.25 (day 5) in less time.~~
324 | ~~Tests A2 and B2, performed under initial pH 7, were affected by a pH drop from 7 (day~~
325 | ~~0) to 5.88 (day 10) and from 7 (day 0) to 5.24 (day 10) respectively. In test C2, pH~~
326 | ~~dropped from 7 (day 0) to 5.18 (day 5). In tests A3 and B3, both performed under initial~~
327 | ~~alkaline conditions, pH dropped from 9 (day 0) to 6.51 (day 10) and from 9 (day 0) to~~
328 | ~~5.86 (day 10) respectively. In test C3, pH dropped from 9 (day 0) to 6.18 (day 5) in five~~
329 | ~~days. Tests performed with the thermal pre-treated substrate showed an overall quicker~~
330 | ~~pH drop, reaching almost the same final pH value in half of the time respect to other~~
331 | ~~tests, in accordance with quicker VFA productions.~~
332 | ~~Independently from the temperature range or pre-treatment adopted, the final VFA~~
333 | ~~concentration reached its maximum value under initial alkaline pH.~~ In condition A, B

334 and C the maximum VFA concentrations achieved under alkaline pH were 27.5, 30.0
335 and 41.0 g COD_{VFA}/L respectively. At neutral pH, the maximum VFA concentrations
336 achieved were 12.5, 27.5 and 37.0 g COD_{VFA}/L in condition A, B and C respectively.
337 Apart from condition A, where the process temperature negatively affected the VFA
338 production yield (in particular at initial pH 5.0 and 7.0), conditions B and C showed
339 ~~quite~~-similar values both under initial neutral and alkaline pH. The drop in pH seemed
340 to affect adversely VFA production only in tests A1, B1 and C1 starting from pH 5,
341 since lower pH values were reached (around 4.2) due to the initial lower pH value.
342 Hence, the fermentation process appeared to be more sensitive to the initial pH value at
343 which tests were performed. These results revealed an interesting outcome about the
344 optimum initial pH value for ~~bio-urban~~ waste fermentation, also in agreement with
345 previous literature studies. Considering the two separated sources, literature reports an
346 optimum pH around 5.5 and 6.0 for the OFMSW fermentation (Jiang et al., 2013; Lim
347 et al., 2008; Valentino et al., 2018), and an optimum pH ranging between 10-11 for the
348 WAS fermentation (Wu et al., 2016). Dogan and Demirer (2009) found that an increase
349 in pH value from 5.5 to 6.5 decreased hydrolysis performance and VFA production in a
350 CSTR fed with OFMSW at HRT 2 days and OLR 15 g VS/(L d). Since the substrate
351 used for these fermentation batches is a mixture of OFMSW and WAS, both initial
352 neutral and alkaline pH could be eligible working conditions in order to reach high VFA
353 concentrations or high acidification yields, especially related to the solubilized COD
354 (COD_{SOL}). In fact, one of the most relevant factors in the utilization of fermented
355 organic feedstock for biopolymers synthesis (e.g. PHA) is the COD_{VFA} content in
356 relation to COD_{SOL}. High VFA production associated with high COD_{SOL} could not be
357 suitable for PHA production since the non-VFA COD_{SOL} stimulates the growth of non-

358 PHA storing bacteria (Valentino et al., 2017). In order to ensure an appropriate PHA
359 storing bacteria selection process, especially with nutrient rich carbon sources (e.g. the
360 one studied in the present research) high COD_{VFA}/COD_{SOL} ratio is the key element to
361 obtain a good PHA storing biomass selection process (Albuquerque et al., 2010).
362 Therefore, the COD_{VFA}/COD_{SOL} ratio is a useful parameter to understand the
363 fermentation performances. At constant temperature, the initial alkaline fermentation
364 process increased the substrate solubilisation compared to ~~initial~~the acidic and neutral
365 condition. The higher COD_{SOL} achieved (46, 37 and 45.0 g COD_{SOL}/L in test A3, B3
366 and C3 respectively) was also associated to the higher VFA production. VFA increased
367 from 4.7 to 27.5 g COD_{VFA}/L in series A, from 18.6 to 30 g COD_{VFA}/L in series B and
368 from 22.5 to 41 g COD_{VFA}/L in series C as initial pH increased from 5 to 9. In general,
369 all tests under acidic pH showed lower COD_{SOL}/COD_{VFA} ratio. The lowest
370 COD_{VFA}/COD_{SOL} ratio of 0.12 was obtained in test A1, where the activity of
371 fermentative bacteria was strongly inhibited. In the other thermophilic trials, the final
372 COD_{VFA}/COD_{SOL} ratio was equal to 0.31 (A2) and 0.60 (A3). Condition A showed the
373 ~~lowest COD_{VFA}/COD_{SOL} ratio in all the exploited pH values, especially under acidic pH~~
374 ~~where the activity of fermentative bacteria was strongly inhibited~~alkaline pH, the
375 ~~COD_{VFA}/COD_{SOL} ratio increased up to 0.6.~~In condition B, the COD_{SOL} concentrations
376 were lower than those obtained in other conditions, but the remarkable VFA production
377 increased the COD_{VFA}/COD_{SOL} ratio up to 0.55, 0.79 and 0.81 with initial acidic (B1),
378 neutral (B2) and alkaline (B3) initial pH, respectively. The combined effect of thermal
379 pre-treatment and initial alkaline condition in test C3 allowed achieving the highest
380 value of 0.91 COD_{VFA}/COD_{SOL}, due to the ~~initial~~enhanced substrate solubilisation
381 combined with a subsequent mesophilic temperature range. Fermentation at initial

382 | ~~neutral~~ Neutral-pH fermentation (test C2) also produced a VFA-rich stream with high
383 | COD_{VFA}/COD_{SOL} ratio (0.84).

384 | In terms of nutrients, ammonium and phosphate were higher in the tests conducted
385 | under alkaline fermentation. In the three series, independently from the applied
386 | temperature, nutrients concentration increased at increasing initial pH from 5.0 to 9.0.
387 | As observed for VFA production, in test C3 the ratio of nutrients related to the COD_{SOL}
388 | reached their maximum values (COD:N:P = 100:2.7:1.5).

389

390 | 3.1.3. VFA distribution

391 | The distribution of VFA in the fermented feedstock is an important aspect of the
392 | process, especially if the production needs to be addressed to a specific group or single
393 | VFA. The effect of pH on the VFA composition and production has been widely
394 | investigated on different kind of waste (Cavinato et al., 2017; Girotto et al., 2017; Lee et
395 | al., 2014; Morgan-Sagastume et al., 2015). In general, the optimum pH level is strictly
396 | dependent on the type of waste. In addition, in the context of MMC-PHA production,
397 | the content of even numbered C-atoms VFA (HydroxyButyrate-3-hydroxybutyrate
398 | precursors; 3HBp) and the odd numbered C-atoms VFA (HydroxyValerate-3-
399 | hydroxyvalerate precursors; 3HVp) affects monomers synthesis and consequently the
400 | polymer composition (Valentino et al., 2017).

401 | In the tests conducted in the present study~~In these tests~~, the qualitative VFA spectrum
402 | appeared to be regulated by the feedstock characteristics more than from the
403 | fermentation conditions. A dominance of acetic, butyric and propionic acid was
404 | observed, with a not negligible amount of valeric, caproic and heptanoic acid. Similarly,
405 | acetic, butyric and propionic acid were the most abundantly observed (respectively 27-

406 32%, 18-23% and 15-17% COD basis) in the acidogenic fermentation of thermally pre-
407 treated sludge (Morgan-Sagastume et al., 2011), or WAS fermentation (Feng et al.,
408 2009). Valeric and caproic acid were in some cases also reported at lower
409 concentrations in WAS fermentation (Morgan-Sagastume et al., 2011). Butyric acid was
410 usually predominant (40-60%) in food waste fermentation followed by acetic acid (25-
411 40%) (Giroto et al., 2017; -Valentino et al., 2018).

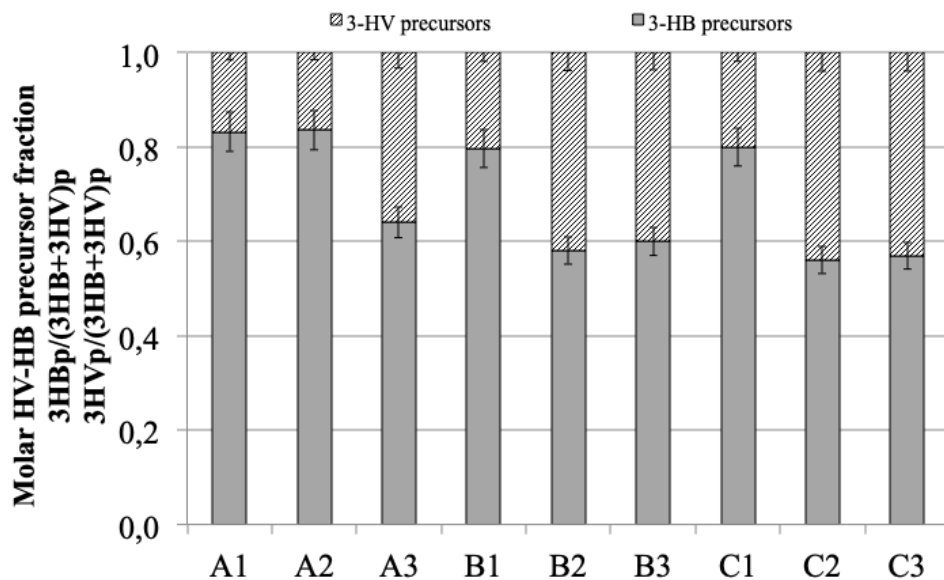
412 The relative VFA percentage seemed to be affected also by the initial pH, more than
413 temperature. Series A showed a net dominance of acetic acid at pH 5.0 (72%) and 7.0
414 (43%); the initial alkaline condition increased the production of propionic, butyric and
415 valeric acid, whose percentages were 25%, 25% and 15% of total VFA respectively.

416 Acetic acid still remained relatively high at 26% but the molar ratio between
417 $\frac{[3\text{HV}_p]}{[3\text{HB}+3\text{HV}]_p}$ precursors was more than doubled (0.36 mol/mol), if compared
418 to the molar ratio quantified at acidic (0.17 mol/mol) and neutral (0.16 mol/mol)
419 condition.

420 In both mesophilic fermentation tests (series B and C), the $\frac{[3\text{HV}_p]}{[3\text{HB}+3\text{HV}]_p}$ ratio
421 similarly changed as pH increased from 5.0 (0.20 mol/mol) to 7.0 (0.40 mol/mol) and
422 9.0 (0.44 mol/mol) (Figure 2). These results were mainly related to the progressive
423 increase of propionic (up to 13.0 g COD/L, 31% COD basis) and valeric acid (up to 5.8
424 g COD/L, 14% COD basis). Under initial neutral and alkaline conditions, the
425 percentage of butyric acid was constant at 25% in the tests A2-A3, at 14-13% in the
426 tests B2-B3, and at 21-20% in the tests C2-C3. The acidic fermentation seemed to
427 enhance its production up to 3.9 g COD/L (21%) and 6.3 g COD/L (28%) in the tests
428 B1 and C1 respectively, with the only exception of test A1, where the fermentative
429 bacteria were inhibited and the fermentation process was only partially observed.

430 Caproic acid was also abundant under the same conditions: 19% (3.5 g COD/L) and
 431 10% (3.3 g COD/L) in tests B1 and C1 respectively. Some variability was observed in
 432 the percentage of acetic acid, even though it remained one of the most predominant
 433 VFA in all tests performed. Its percentage ranged between 20-28% with two maximum
 434 peaks of 43% (5.4 g COD/L, test A2) and 72% (3.4 g COD/L, test A1). Heptanoic acid
 435 was particularly affected by the temperature more than pH: it was detected only in the
 436 mesophilic tests with relative higher content in those trials performed without thermal
 437 pre-treatment: 16% (2.9 g COD/L, test B1), 14% (3.9 g COD/L, test B2) and 13% (3.9 g
 438 COD/L, test B3).
 439 Initial Alkaline-alkaline fermentation of squeezed OFMSW and thickened WAS
 440 mixture enhanced the production of some acids, propionic in particular, while lowering
 441 the production of butyric and caproic acid, which achieved higher level at acidic pH, as
 442 confirmed by previous studies (Horiuchi et al., 2002).

443 **Figure 2**



444

445

446 | 3.2. Effect of different HRTs and OLRs on ~~the bio-urban~~ waste alkaline fermentation at
447 | mesophilic temperature in a CSTR

448 | The ~~most best~~ performing condition C3 (thermal pre-treatment followed by alkaline
449 | mesophilic fermentation) was reproduced in a CSTR for further investigations ~~of on~~ the
450 | effects of different HRTs and OLRs on VFA production. ~~The three conditions can be~~
451 | ~~summarized as it follows: Run I was characterized by an HRT of 6 days and a~~
452 | ~~corresponding OLR of 7.7 kg VS/m³ d; in Run II, the HRT was 5 days and the OLR 9.2~~
453 | ~~kg VS/m³ d; Run III was performed at HRT of 4.1 days and OLR of 11.1 kg VS/m³ d.~~

454 | The reactor was started with Run I; then, the OLR was increased by decreasing the
455 | HRT. ~~The narrow OLR range [7.7 - 11.3 kg VS/(m³ d)] was chosen in order to find a~~
456 | ~~threshold or a limit OLR value, above which the process may be considered not feasible~~
457 | ~~in terms of stable VFA production.~~ Table 4 resumes all the parameters and the process
458 | yields analysed in the three conditions. The COD_{SOL} ~~in the effluent~~ reached ~~quite high~~
459 | ~~and~~ similar values in the range 41 - 43 g COD/L in all the three trials ~~and it was not~~
460 | ~~correlated to the progressive increase of the OLR. Probably, the increase of OLR from~~
461 | ~~7.7 to 9.3 kgVS/m³ d may not have been so high to justify an increase of the COD_{SOL}.~~
462 | ~~At the highest OLR (11.3 kgVS/m³ d, Run III) the expected increase of COD_{SOL} was~~
463 | ~~not observed most likely due to the lower VS solubilisation (0.07 g COD_{SOL}/g VS₍₀₎),~~
464 | ~~reduced by almost 50% if compared to Run I and II (0.12 g COD_{SOL}/g VS₍₀₎). As~~
465 | ~~demonstrated in the batch tests, the high solubilisation yield was mainly favoured by the~~
466 | ~~thermal pre-treatment step. Indeed, the solubilisation yield indicated similar~~
467 | ~~performances between the three runs, being in the range 0.29-0.32 g COD_{SOL}/g VS₍₀₎.~~

468 | The mixture acidification was remarkable from the 5th day (less than 1.0 HRT) and it
469 | was steadily maintained at high level, with VFA concentration above 35 g COD_{VFA}/L,

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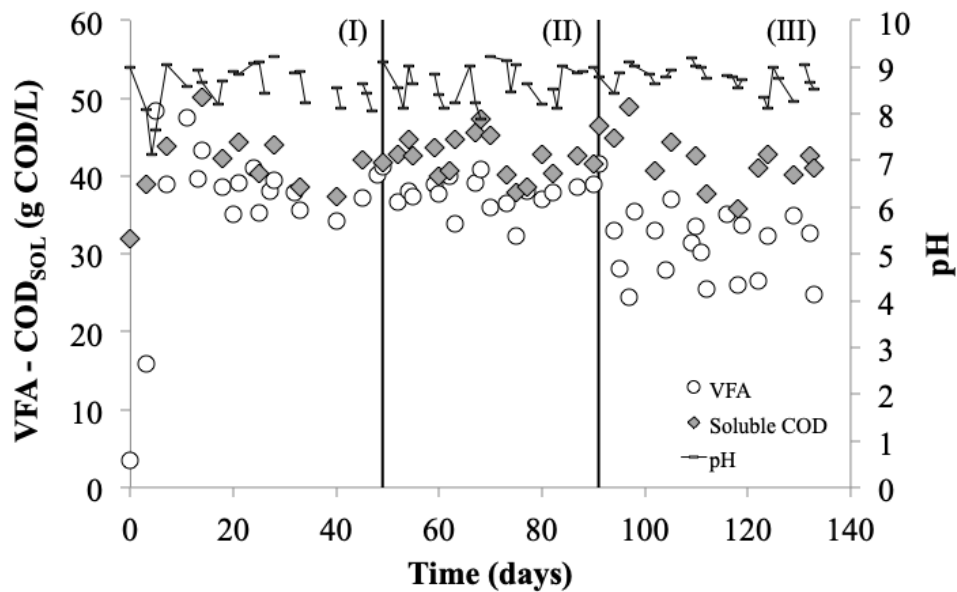
470 for the whole Run I and II. In the last Run III, the reactor seemed to be overloaded,
471 especially in the first part (immediately after the OLR increase), where the VFA
472 dropped from 40 to 24 g COD_{VFA}/L. Over the course of the process, the VFA
473 concentration was affected by frequent fluctuations (Figure 3) and in general the
474 process suffered the imposed high rate condition. Other authors found out that VFA
475 production was similarly affected by high substrate concentration and high applied OLR
476 in a CSTR fed with OFMSW, while high HRT favoured substrate solubilization and
477 VFA production (Argelier et al., 1998). The average VFA concentrations achieved were
478 39 ± 3 , 38 ± 2 and 31 ± 4 g COD_{VFA}/L in Run I, II and III respectively. Run I exhibited
479 the highest COD_{VFA}/COD_{SOL} ratio of 0.91. This high value ~~facilitates-makes~~ this VFA-
480 rich stream ~~suitability-suitable~~ for those applications that strictly required this feature
481 (e.g. PHA from MMC; Valentino et al., 2017); Run II was also promising, ~~giving~~
482 ~~providing~~ a fermented stream with a ratio of 0.88. Both runs exhibited the ~~higher~~
483 ~~highest~~ VFA yields of 0.74 g (Run I) and 0.71 (Run II) g COD_{VFA}/g VS₍₀₎; while Run
484 III showed the lowest yields equal to 0.60 g COD_{VFA}/g VS₍₀₎. ~~With respect to the~~In
485 terms of VFA composition, acetic and propionic acid were the most predominant in all
486 three conditions (22-25% acetic and 22-28% propionic acid, COD ~~basinbasis~~),
487 representing together the 50% of all species, in line with ~~the observation of what~~
488 ~~observed in~~ the previous C3 batch test. Butyric (20-22%) and valeric acid (10-13%)
489 were the other predominant species in all three runs. The highest molar ~~ratio-between~~
490 ~~[3HV_p/(3HB+3HV)_p] ratio precursors~~ was obtained in Run II with 0.39 mol/mol,
491 followed by 0.38 mol/mol in Run I, and 0.35 mol/mol in Run III. It seems that no
492 significant correlation exists between those ratios and the applied OLR (or HRT).
493 Nutrient concentrations were investigated in terms of ammonium and phosphate

494 quantification. The highest concentrations of both nutrients (1.02 g N-NH₄⁺/L; 0.28 g P-
495 PO₄³⁻/L) were ~~determined~~ found at higher HRT and lower OLR (Run I), with a slight
496 difference respect to Run II, probably due to the sufficiently high process stability and
497 the available prolonged time for the substrate solubilisation and degradation (Lee et al.,
498 2014). The combined effect of higher HRT and lower OLR in an alkaline system (Run
499 I) resulted as the best operating condition, in terms of VFA production and process
500 stability (39 g COD_{VFA}/L; 0.91 COD_{VFA}/COD_{SOL}). ~~the~~ The slight decrease in HRT and
501 increase in OLR (Run II) resulted also as eligible process condition for the soluble COD
502 acidification and further VFA utilisation (38 g COD_{VFA}/L; 0.88 COD_{VFA}/COD_{SOL}).
503 Indeed, every process configuration can have different optimum condition for VFA
504 production. In previous studies focused on OFMSW fermentation, where a different
505 process configuration was applied (leach-bed reactor), an increase in leachate recycle
506 rate favoured VFA production (Yesil et al., 2014). Depending on their composition and
507 characteristics, each feedstock has its optimum fermentation conditions that can be
508 tuned on the scope of future applications (Atasoy et al., 2018). Since the feedstock used
509 in this study was composed by a mixture of OFMSW and WAS, the optimum
510 fermentation conditions in terms of VFA level maximization can be intermediate
511 between the one for OFMSW and WAS. Authors found out that increasing OLR lead to
512 increasing VFA productions in OFMSW fermentation; however, OLR higher than 13 kg
513 VS/m³ d made the process unstable (Lim et al., 2008). Generally, high rate processes for
514 OFMSW fermentation are considered unsuitable since the easy putrescible matter may
515 lead to a quick VFA production, not properly buffered ~~provided that~~ unless an efficient
516 pH control system ~~was~~ is applied (Valentino et al., 2018). On the other hand, prolonged
517 HRTs (up to a maximum of 12 days) can enhance VFA production and process stability

518 with complex substrates such as OFMSW and WAS (Lim et al., 2008; Zhou et al.,
519 2018). Hong and Haiyun (2010) investigated the effects of the interactions of different
520 parameters (food waste composition as co-substrate, HRT, OLR and pH) on food waste
521 and dewatered excess sludge fermentation process through the application of
522 experimental design methods. They observed significant correlations between pH and
523 OLR, pH and HRT, pH and food waste composition, showing that with increasing
524 OLR, VFA production decreased above 7.9 g VSS/(L d). VFA production also
525 decreased with too long HRT, above 8.92 days, in accordance to the findings of the
526 present study. Other authors reported VFA concentrations up to 60 g/L (Giroto et al.,
527 2017) with food waste fermentation. However, such high value was obtained in batch
528 test and presumably a continuous process implementation could require effective
529 strategies to maintain the pH at the desired value. Indeed, this high rate VFA production
530 could probably cause a drastic drop in the pH with the risk of system imbalance. On the
531 contrary, WAS fermentation generates less VFA than OFMSW (no more than 6 g
532 COD/L) in the same temperature range (Chen et al., 2007; Giroto et al, 2017; Hao,
533 Wang, 2015; Horiuchi et al., 2002; Strazzera et al., 2018). A total VFA concentration
534 close to 40 g COD_{VFA}/L with limited amount of non-VFA soluble COD, as herein
535 obtained (Run I and II) is quite promising since it would be a valuable carbon source for
536 those applications that require high VFA productions in high organic loading rate
537 processes (e.g. MMC-PHA production). In fact, higher VFA production would translate
538 in means higher volumetric PHA productivity ($[kg\ PHA/(L\ d)]$), which is defined on the
539 overall PHA storage yield over the utilised VFA (Valentino et al., 2018). Economic
540 viability of this approach needs to be addressed for the evaluation of associated costs of
541 heating requirements and chemical supply, in a specific biorefinery scenario where the

542 MMC-PHA production technology is intended to be integrated in existing WWTP
 543 facilities.

544 **Figure 3**



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547 **Table 4**

Operating condition	Unit	CSTR trial		
		Run I	Run II	Run III
HRT	d	6	5	4.1
OLR	kg VS/(m ³ d)	7.7	9.32	11.31
Parameter	Unit	Run I	Run II	Run III
TS	g/kg	54 ± 4	52 ± 4	57 ± 5
VS	g/kg	40 ± 3	41 ± 3	42 ± 4
VFA	g COD/L	39 ± 2	38 ± 2	31 ± 4
COD _{SOL}	g COD/L	43 ± 4	43 ± 3	41 ± 3
COD _{VFA} /COD _{SOL}	-	0.91 ± 0.04	0.88 ± 0.06	0.76 ± 0.07
$\frac{[(C_3)3HVp]/(C_3+C_33HB+3HVp)}{VFA}$	mol/mol	0.38 ± 0.03	0.39 ± 0.02	0.35 ± 0.04
Solubilisation	g COD _{SOL} /g VS ₍₀₎	0.12 ± 0.07	0.12 ± 0.06	0.07 ± 0.06
Yield	g COD _{VFA} /g VS ₍₀₎	0.74 ± 0.05	0.71 ± 0.03	0.60 ± 0.06
Ammonium	g N-NH ₄ ⁺ /L	1.02 ± 0.04	0.87 ± 0.05	0.82 ± 0.05

Phosphate	g P-PO ₄ ³⁻ /L	0.28 ± 0.08	0.27 ± 0.02	0.17 ± 0.02
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549 4. Conclusions

550 This study ~~determined~~-assessed how the fermentation process of a specific waste
551 mixture of ~~bio-waste of~~ urban origin could be optimized in terms of organic matter
552 solubilisation and VFA production. For this work, a mixture composed by thickened
553 WAS (65-70% v/v) and squeezed OFMSW (30-35% v/v) was used as renewable
554 feedstock. A first screening of batch trials revealed that alkaline pH (9.0) and
555 mesophilic temperature (37°C), coupled with thermal pre-treatment (72°C, 76 h) gave
556 the best performances in terms of maximum VFA concentration and COD_{VFA}/COD_{SOL}
557 ratio. The same working conditions were proposed in a CSTR. The effluent VFA-rich
558 stream was characterized by a stable production close to 40 g COD_{VFA}/L with consistent
559 composition and limited amount of non-VFA soluble COD (0.91 COD_{SOL}/COD_{VFA}), at
560 HRT of 6 days and OLR of 7.7 kg VS/m³ d. The outcomes of this work could be used as
561 a beneficial tool for the development of a biorefinery platform dedicated to the
562 valorisation of urban organic waste and recovery of added-value bio-products (such as
563 biopolymers) through a combined anaerobic/aerobic multi-steps process.

564

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586 **Figures and Tables captions**

587 Figure 1. VFA concentration trends in the thermophilic batch tests A1-A2-A3 (a) under
588 initial pH 5, 7 and 9; mesophilic batch tests B1-B2-B3 (b) under initial pH 5, 7 and 9;
589 ~~and~~ mesophilic batch tests with thermal pre-treatment C1-C2-C3 (c) under initial pH 5,
590 7 and 9.

591 Figure 2. VFA distribution expressed as 3HB (even numbered C-atoms VFA) and 3HV
592 (odd numbered C-atoms VFA) precursors obtained in batch tests.

593 Figure 3. Soluble COD, VFA concentrations and pH obtained during the continuous
594 lab-scale trial in the three conditions tested.

595 | Table 1. Chemical-physical characteristics of the thickened WAS, pre-treated OFMSW,
596 | ~~and bio-urban~~ waste mixture ~~and thermally pre-treated substrate~~ used for batch tests and
597 | continuous trial.

598 | Table 2. Summary of the operating conditions investigated in the nine batch tests.

599 | Table 3. ~~Main results~~Final concentrations and fermentation performances obtained in all
600 | batch tests.

601 | Table 4. ~~Main results~~Final concentrations and fermentation performances obtained in
602 | the three CSTR trials.

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