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Hydrodynamic cavitation pre-treatment of urban waste: integration with acidogenic fermentation, PHAs synthesis and anaerobic digestion processes

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Abstract

Urban waste can be valorized within a biorefinery approach, producing platform chemicals, biopolymers and energy. In this framework, hydrodynamic cavitation (HC) is a promising pre-treatment for improving biodegradability due to its high effectiveness and low cost. This paper deals with the effect of HC pre-treatment on the acidogenic co-fermentation process of thickened sewage sludge from a WWTP and seasonal vegetable waste from a wholesale market. Specifically, HC was assessed by testing two sets of parameters (i.e., treatment time of 30 and 50 min; vacuum pressure 1.4 and 2.0 bar; applied power 8 and 17 kW) to determine its effectiveness as a pre-treatment of the mixture. The highest increase in sCOD (+83%) and VFAs (from 1.93 to 17.29 gCOD_{VFA} L⁻¹) was gained after 50 minutes of cavitation. Fermentations were conducted with not cavitated and cavitated mixtures at 37°C on 4 L reactors in batch mode, then switched to semi-continuous with OLR of 8 kg_{TVS} m⁻³ d⁻¹ and HRT of 5-6.6 d. Good VFAs concentrations (12.94-18.27 gCOD_{VFA} L⁻¹) and yields (0.44-0.53 gCOD_{VFA} g_{VS(0)}⁻¹) were obtained, which could be enhanced by pre-treatment optimization and pH control. The organic acid rich broth obtained was then assessed as a substrate for PHAs storage by *C. necator*. It yielded 0.37 g g⁻¹ of polyhydroxybutyrate, such biopolymer resulted to have analogous physicochemical characteristics of commercial equivalent. The only generated side-stream would be the solid-rich fraction of the fermented effluent, which valorization was assessed through BMP tests, showing a higher SGP of 0.42 Nm³ kg_{TVS}⁻¹ for the cavitated.

Keywords: organic waste, sludge, cavitation, VFAs, PHAs

Acronym's list:

AC: Acoustic Cavitation

AD: Anaerobic Digestion

BMP: Biochemical Methane Potential

F/M: Food/Microorganisms

FW: Food Waste

- 31 FR: Fermentation Rate
- 32 GC: Gas-Chromatography
- 33 HC: Hydrodynamic Cavitation
- 34 HPLC: High Performance Liquid Chromatography
- 35 HRT: Hydraulic Retention Time
- 36 OAs: Organic Acids
- 37 OFMSW: Organic Fraction of Municipal Solid Waste
- 38 OL: Organic Loading
- 39 OLR: Organic Loading Rate
- 40 pCOD: particulate Chemical Oxygen Demand
- 41 PHAs: Poly-Hydroxy-Alkanoates
- 42 sCOD: soluble Chemical Oxygen Demand
- 43 SS: sewage sludge
- 44 tCOD: total Chemical Oxygen Demand
- 45 TKN: Total Kjeldahl Nitrogen
- 46 TS: Total Solids
- 47 TVS: Total Volatile Solids
- 48 VFAs: Volatile Fatty Acids
- 49 VW: Vegetable Waste
- 50 WWTP: WasteWater Treatment Plant

51 **1. Introduction**

52 The effects of climate change represent a global menace that requires action on an international level, thus emphasizing
53 the need for closing, among the others, the carbon cycle. In the form of CO₂, carbon is recognized as the main culprit
54 for climate change. The circular economy approach has been identified as a fundamental part of the solution at both the
55 international and european levels. For this reason, it became the core concept of the EU Green Deal, where waste
56 recovery and valorization into marketable products and energy represent a pivotal aspect (EC, 2019).

57 In the urban context, the two main waste streams are sewage sludge (SS) and food waste (FW), destined to grow with
58 the increasing world population. At present, in Europe, 13 million tonnes (dry matter) of sewage sludge and 78 million
59 tonnes of food waste are generated every year (Collivignarelli et al., 2019).

60 An innovative approach for the integrated management of SS and FW is represented by their valorization within a third-
61 generation bio-refinery approach. This consists of the combination of several processes to transform waste into high-

62 added value chemical compounds, such as volatile fatty acids (VFAs) and energy (Battista et al., 2020). VFAs are
63 starting molecules for bioenergy production and the synthesis of various products, such as reduced chemicals,
64 derivatives, and biopolymers. At the moment, 90% of VFAs are produced from non-renewable petrochemical
65 compounds through a process that has considerable environmental impacts (Atasoy et al., 2018). A sustainable
66 alternative can be VFAs production through the anaerobic fermentation of organic waste (Holtzapple et al., 2022). Co-
67 fermentation of SS and FW is reported to improve the fermentation performance, thanks to i) a higher organic material
68 content; ii) a stronger buffer capacity; iii) balanced macronutrients and micronutrients; iv) dilution of toxic and
69 inhibitory compounds; v) a more diverse microbial community (Fang et al., 2020; Vidal-Antich et al., 2021).

70 The VFAs-rich liquid fraction can be valorized into several routes in the frame of a biorefinery concept (Sivagurunathan
71 et al., 2018). The fermented effluent rich in VFAs is an ideal substrate for the cultivation of poly-hydroxy-alkanoates
72 (PHAs)-storing microorganisms, both with mixed microbial cultures (MMCs) (Valentino et al., 2019b) and with pure
73 cultures of microorganisms such as *R. eutropha*, also known as *C. necator* (Martinez et al., 2016). If the co-
74 fermentation and the PHAs production processes were coupled, the only waste overflow would be the solid-rich fraction
75 of the fermented effluent, which can be finally valorized through AD. The feasibility of this approach has been recently
76 demonstrated with similar substrates, i.e., the organic fraction of municipal solid waste (OFMSW) and SS (Moretto et
77 al., 2019; Valentino et al., 2019a).

78 The pre-treatment of the substrates is the first process to be performed in a biorefinery in order to i) reduce the size of
79 the substrate; ii) extract simpler chemical compounds, thus favouring the fermentation process; iii) remove the inert
80 material not applicable to the following bioprocesses (Li et al., 2017). Among pre-treatments, cavitation is a promising
81 physico-chemical process consisting of the formation, growth and collapse of vapor cavities due to a sharp pressure
82 drop. The pressure drop can be generated applying a sudden constriction (hydrodynamic cavitation, HC) or by using
83 ultrasound (acoustic cavitation, AC) (Bhat&Gogate, 2021). HC is a more promising technology from both the
84 environmental and economic point of view, since it has a higher potential of scalability and has been proved to be
85 orders of magnitude cheaper than AC (Bhat&Gogate, 2021). For this reason, it is fundamental to fill the gaps of
86 knowledge of this process. At present, both AC and HC have been tested on SS and wastewater, while few studies have
87 been conducted on FW, testing only AC. The sole study carried out on a mixture of SS and OFMSW showed a 24%
88 increase in the BMP (Cesaro et al., 2012). The best operating parameters have been identified only for the pre-treatment
89 of SS and are pressure of 2-4 bars and duration of 15-60 mins (Garuti et al., 2018; Zhao et al., 2019). This study aims at
90 evaluating the effects of HC of a mixture of organic wastes (SS and vegetable waste, VW) on its physico-chemical
91 characteristics (TS, TVS, sCOD, VFAs, and cations) and on the fermentative and AD processes by testing two sets of
92 operational parameters (pressure, power, duration). VFAs production from this mixture was assessed by batch and

93 semi-continuous fermentation tests, while methane production was quantified by Biochemical Methane Potential (BMP)
 94 tests. Finally, the suitability of the non-sterilized fermented effluent obtained for PHAs production by *C. necator* was
 95 evaluated.

96 2. Materials and methods

97 2.1 Substrates and anaerobic inoculum

98 The substrates used in this study were the biological sludge collected from a wastewater treatment plant (WWTP)
 99 located in Northern Italy and the seasonal vegetable waste from the fruit and vegetable wholesale market. The mixture
 100 was made from the two substrates in a 1:1 ratio on a TVS basis, at a volumetric fraction of 73-77% of sludge and 27-
 101 23% of vegetable scraps, according to Moretto et al. (2020b) and Valentino et al. (2019a). The anaerobic inoculum
 102 consisted of digestate collected from a WWTP, where wastewater sludge and OFMSW are anaerobically co-digested.
 103 All the three matrices were recurrently collected during the study period and were characterized in terms of total solids
 104 and volatile solids (TS and VS), sCOD, VFAs, total COD (tCOD), Total Kjeldahl Nitrogen (TKN), total phosphorus
 105 (P_{tot}), cations (Na⁺, NH₄⁺, K⁺, Mg²⁺, Ca²⁺), pH and alkalinity, showing stable values (table 1) (APAT, 2003; APHA,
 106 2012).

107 Table 1. Average chemical-physical characteristics of the vegetable waste, biological sludge, and anaerobic inoculum
 108 applied in this study.

Parameter	Unit	Biological sludge		Vegetable waste		Inoculum	
		Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
Total	g kg ⁻¹	36.0	±0.3	96	±13	18.2	±0.7
Solids (TS)							
Total	g kg ⁻¹	23.5	±0.4	89	±14	12.0	±0.3
Volatile Solids (TVS)							
VS/TS	%	65.4	±0.7	92	±2	66	±1
sCOD	gO ₂ L ⁻¹	0.58	±0.10	-	-	0.34	±0.01
pCOD	gO ₂ kg ⁻¹	26	±5	111	±20	17	±4

tCOD	gO ₂ kg ⁻¹	26	±5	-	-	18	±4
TKN	g _N kg ⁻¹	0.8	±0.1	1.1	±0.2	1.3	± 0.1
Phosphorus (P)	gP kg ⁻¹	0.8	±0.1	0.3	±0.1	0.4	± 0.02
COD:N:P	g	26:0.8:0.8		111:1.1:0.3		18:2.4:0.4	
VFA TOT	gCOD _{VFA} L ⁻¹	0.81	±0.01	-	-	0.23	±0.21
pH		7.7	±0.9	-	-	8.3	±0.1
Partial alkalinity	mgCaCO ₃ L ⁻¹	325	±48	-	-	2622	±208
Total alkalinity	mgCaCO ₃ L ⁻¹	606	±53	-	-	3091	±287
Na ⁺	mg L ⁻¹	2664	±732	-	-	2313	±235
N-NH ₄ ⁺	mg L ⁻¹	45	±64	-	-	1091	±194
K ⁺	mg L ⁻¹	418	±35	-	-	918	±293
Mg ²⁺	mg L ⁻¹	2438	±937	-	-	2004	±214
Ca ²⁺	mg L ⁻¹	6144	±1942	-	-	5110	±165

109

110 2.2 Hydrodynamic cavitation pre-treatment of the substrates

111 The HC reactor used to generate cavitation was a stator and rotor assembly (BioBang®, Three-es S.r.l.). The maximum
112 inlet pressure to the HC reactor was 2 bars. A closed-loop circuit was applied, where the mixture was recirculated for all
113 the duration of the pre-treatment. Electrical power (kW) was set as a fixed parameter, with rotation velocity varying
114 depending on the mixture's viscosity. HC was performed applying two different sets of parameters. Firstly, a power of
115 8 kW, P of 1.4-1.5 bar, Q_{mixture} of 25-30 L min⁻¹, and 1550-1650 rpm were applied for 30' on the mixture. In the second
116 pre-treatment, the operating parameters were kept at the lower values indicated above for the first 30 mins to avoid
117 pump overloading during the homogenization of the mixture and were then raised. A power of 17 kW, P of 2.0 bar,
118 Q_{mixture} of 80-100 L min⁻¹, and 1450-1550 rpm were applied for the last 20' on the mixture, for a total duration of the
119 HC pre-treatment of 50'.

120 To assess the effect of the HC pre-treatment, the physical-chemical properties of the mixture before and after cavitation
 121 were compared. The substrates' degree of disintegration (DD_{COD} %) was calculated as in Tonanzi et al. (2021). To
 122 evaluate the efficiency of the process and compare it with other pre-treatments, the specific energy input (SE) was
 123 calculated as in Gallipoli et al. (2014).

124 2.3 Anaerobic fermentation tests

125 Fermentation tests were conducted at uncontrolled pH and 37°C using a laboratory fermenter with 4L of working
 126 volume, automatically stirred at 14 rpm (RES Italia). The tests were carried out on the mixtures cavitated for 30' and
 127 50' and on the not cavitated mixture, which was considered as a blank.

128 Batch tests were performed; when the VFAs concentration was stable for at least three consecutive measurements, the
 129 reactors were fed in a semi-continuous manner. The parameters applied in the batch and semi-continuous tests were
 130 defined according to the experimental tests in literature, giving the best performances, and are reported in table 2
 131 (Moretto et al., 2019; Strazzera et al., 2021; Valentino et al., 2019a). The tests were inoculated with the anaerobic
 132 digestate (31-34% v/v) in order to maintain a high F/M ratio that allows to inhibit methanogens (González-Fernández
 133 and García-Encina, 2009; Greses et al., 2020). The slight variability of the parameters applied among the three
 134 conditions tested is due to the seasonal variability of the substrates used.

135

136

Table 2. Parameters applied in the fermentation tests.

	Parameter	Unit	Not cavitated	Cavitated 30'	Not cavitated	Cavitated 50'
Batch tests	OL	$kg_{iCOD} m^{-3}$	34.8	33.4	52.1	54.6
		$kg_{TVS} m^{-3}$	24.5	18.8	23.5	25
	F/M	$kg_{iCOD} kg_{TVS}^{-1}$	9.92	9.51	14.7	14.1
	F/M	$kg_{TVS} kg_{TVS}^{-1}$	7	5.4	6.4	6.7
Semi-continuous tests	OLR	$kg_{TVS} m^{-3} d^{-1}$	8	8	8	8
	HRT	days	6.6	5	6.0	6.4

137

138 Liquid samples were collected daily for VFA analysis, soluble chemical oxygen demand (sCOD), pH, alkalinity, and
 139 cations.

140 The VFAs yields were calculated according to Moretto et al. (2019). In the tests with the cavitated mixture, both the net
141 and total yields were calculated, respectively subtracting, and including, the VFAs generated during the HC pre-
142 treatment.

143 The fermentation rate (FR) was expressed as $\text{gCOD}_{\text{VFA}} \text{ gTVS}_{(0)}^{-1} \text{d}^{-1}$. For batch fermentations, it was calculated
144 following eq 1:

$$145 \quad \text{Fermentation rate}_{\text{batch}} = \frac{\text{g COD}_{\text{VFA}}}{\text{TVS}_{(0)} * \text{days}} \quad (1)$$

146 where gCOD_{VFA} indicates the VFAs in the volume of the reactor and $\text{TVS}_{(0)}$ refers to the quantity of total volatile solids
147 used to inoculate the fermenters at the beginning of the test.

148 The fermentation rate of the semi-continuous fermentations at steady state was calculated as it follows:

$$149 \quad \text{Fermentation rate}_{\text{semi-continuous}} = \frac{\text{g COD}_{\text{VFA}}}{\text{TVS}_{(0)} * \text{HRT}} \quad (2)$$

150 Where HRT is the hydraulic retention time of the fermenters.

151 Also, for the fermentation rate, the net, and the total rate were calculated in the tests with the cavitated mixture.

152 In order to describe the VFAs profile for the subsequent PHAs storage, the molar ratio between odd-numbered VFAs
153 and their total concentration (the $[\text{C3}/(\text{C3}+\text{C2})]_{\text{VFA}}$ ratio) was determined. The $[\text{C3}/(\text{C3}+\text{C2})]_{\text{VFA}}$ ratio is a pivotal
154 characteristic since it affects the composition of microbially synthesized biopolymers such as the
155 polyhydroxyalkanoates (PHAs), and, in turn, their market applications (Bengtsson et al., 2010).

156 **2.4 PHAs accumulation tests**

157 Production of PHAs was carried out in two bench-scale 2L fermenters (Infors- Minifors 2) by employing a pure culture
158 of *Cupriavidus necator* (DSMZ 545) in a fed-batch culture system. The experiment was carried out according to a dual-
159 phase process as reported previously when assessing other alternative substrates (Martinez et al., 2016). Briefly, 1) cells
160 were first grown under balanced conditions in batch mode and using glucose as substrate; then 2) polymer was
161 accumulated under imbalanced conditions (limiting N) by feeding the obtained VFAs-rich effluent without sterilization
162 (just filtrated at $0.45\mu\text{m}$) using a pO_2 -stat strategy. Air was fed at 0.5 VVM, and stirring was set at 600 rpm, allowing to
163 maintain $\text{pO}_2 > 20\%$ (Samorì et al., 2021). Moreover, two conditions were carried out, using *a*) the actual organic acid-
164 rich effluent of the cavitated 50' and *b*) using a simulated laboratory prepared solution containing just the detected
165 organic acids at the corresponding concentrations. This allowed to assess the presence of any inhibitor in the actual

166 anaerobic effluent matrix. Samples were performed periodically and treated as previously reported elsewhere (Martinez
167 et al., 2016).

168 **2.5 Biochemical Methane Potential (BMP) tests**

169 All the BMP tests were conducted at 42°C in bottles with a working volume of 0.5 L, with an OL of 4,5-5 kg_{TVS} m⁻³ and
170 an F/M of 0.36-0.48 VS/VS. The BMP tests were carried out on the single substrates (VW and SS), on the mixture of
171 organic waste and biological sludge not cavitated, cavitated 30' and cavitated 50', and finally on the solid-rich fraction
172 of the fermented effluent. The volume of biogas produced was measured by water displacement. Biogas composition
173 was determined with the portable biogas analyzer MCA 100 Bio-P (ETG risorse e tecnologia s.r.l). The parameters of
174 the BMP tests (SGP, SMP, k_h) were calculated according to the standard BMP methods (Holliger et al., 2020).

175 **2.6 Analytical methods**

176 All analyses were performed according to the APAT, IRSA-CNR (APAT, 2003), and APHA, AWWA, WET methods
177 (APHA, 2012). Volatile fatty acids were determined with an Agilent 1100 SERIES high-performance liquid
178 chromatograph (HPLC) equipped with an Acclaim™ Organic Acid 4x150 mm column (Thermo Fisher) and with a
179 diode array detector (DAD). The Volatile Free Acid Mix CRM46975 was used as standard. The lactic acid content was
180 determined for the fermented effluent after the pH drop with an HPLC-RID with the method described in Martinez et al.
181 (2015). PHAs content and the obtained polymer characterisation were carried out as previously reported by Martinez et
182 al. (2015) and (2021).

183 **3. Results and discussion**

184 **3.1 Hydrodynamic cavitation pre-treatment of the substrates**

185 HC pre-treatments did not impact on TS and TVS concentrations, indicating that no mineralization or evaporation
186 phenomena occurred. On the contrary, HC affected the sCOD, pCOD, and VFAs concentrations. The 30' HC pre-
187 treatment determined a 39% increase of the sCOD and raised VFAs concentration from 1.7 to 6.8 gCOD_{VFA} L⁻¹. These
188 results were enhanced in the 50' HC, where an 83% increase of the sCOD and a notable increase in VFAs concentration
189 from 1.9 to 17.3 gCOD_{VFA} L⁻¹ were observed, with gCOD_{VFA} gCOD⁻¹ of 0.67. The VFAs concentration of the mixture
190 cavitated for 50' was comparable with the one obtained during the fermentation process. This clearly indicates that a
191 high concentration of VFAs can be obtained only by HC, reaching values similar to or even higher than those obtained
192 through the fermentation processes. After both pre-treatments a 15-16% decrease in the pCOD of the mixture was
193 observed, due to the transfer of the organic material from the solid (pCOD) to the liquid (sCOD) phase (table 3).

194 The solubilization of the mixture increased with the intensity and the duration of the pre-treatment, with DD_{COD} of 6%
195 for the cavitated 30' (at SE of 2868 kJ kgTS⁻¹) and DD_{COD} of 17% for the cavitated 50' (at SE of 3734 kJ kgTS⁻¹). This

196 confirms that hydrodynamic cavitation can lead to the same or even higher DD_{COD} with a lower SE with respect to
 197 acoustic cavitation (Cesaro et al., 2012; Tonanzi et al., 2021). The highest DD_{COD} s of 27% and 72% achieved in the
 198 cited studies (SE of 33873 and 90692 kJ kgTS⁻¹, respectively) were not reached, but they can probably be obtained by
 199 increasing the SE.

200 A slight decrease in the pH after 30' HC was observed, while after 50' HC, the pH was comparable with the not
 201 cavitated mixture, despite the considerable increase in VFAs concentration. The pH stability of the mixture cavitated
 202 50' could be due to the increase in NH_4^+ concentration that contributed to the buffering of the mixture. The observed
 203 increase in NH_4^+ concentration after 50' of cavitation was probably due to cell lysis and was reported also elsewhere
 204 (Zhao et al., 2019). The concentrations of the other cations in the liquid phase after HC showed slight decrease
 205 concerning Na^+ , Mg^{2+} , and Ca^{2+} concentrations, as observed elsewhere (Laurent et al., 2009; Tonanzi et al., 2021). The
 206 divalent cations bridging theory states that Ca^{2+} and Mg^{2+} facilitate biofloculation, whereas Na^+ hinders it, especially at
 207 a ratio between monovalent and divalent cations $M/D > 2$ (Higgins and Novak, 1997; Sobek et al., 2002). In this study,
 208 the M/D ratio of the not cavitated mixtures was 0.33 and 0.26. Therefore, the decrease in Na^+ , Mg^{2+} and Ca^{2+}
 209 concentrations could be attributed to a slight refloculation phenomenon caused by the higher content in soluble organic
 210 matter (Laurent et al., 2009). After HC, an increase in the total alkalinity of the mixtures is observed due to the increase
 211 in VFAs concentration. Table 3 reports the impacts of the HC pre-treatments on the mixtures tested.

212 Table 3. Characterization of the mixture of SS and VW before and after HC pre-treatment.

Parameter	Unit	Not cavitated	Cavitated 30'	Not cavitated	Cavitated 50'
DD_{COD}	%		6		17
Total Solids (TS)	g kg ⁻¹	35.8 ± 0.4	37.3 ± 0.0	49 ± 6	46.1 ± 0.3
Total Volatile Solids (TVS)	g kg ⁻¹	27.2 ± 0.9	28.4 ± 0.0	38 ± 8	36.4 ± 0.2
VS/TS	%	76 ± 2	76.0 ± 0.0	79 ± 5	78.9 ± 0.2
sCOD	gO ₂ L ⁻¹	8.83 ± 0.10	12.28 ± 0.70	14.20 ± 0.29	25.99 ± 0.35
pCOD	gO ₂ kg ⁻¹	45 ± 5	38.0 ± 0.5	54.8 ± 0.2	47 ± 1

tCOD	gO ₂ kg ⁻¹	54 ± 4	50.3 ± 0.7	69.0 ± 0.3	73 ± 1
TKN	g _N kg ⁻¹	0.9 ± 0.1	0.8 ± 0.0	0.9 ± 0.0	0.9 ± 0.0
Phosphorus (P)	gP kg ⁻¹		0.8 ± 0.2	0.7 ± 0.0	0.8 ± 0.0
COD:N:P	g	54:1.2	50:1.1:0.8	69:0.9:0.7	73:1.2:0.8
VFA TOT	gCOD _{VFA} L ⁻¹	1.7 ± 0.2	6.8 ± 0.1	1.9 ± 0.4	17.3 ± 0.0
Formic acid	gCOD _{VFA} L ⁻¹	0.2 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.0
Acetic acid		1.0 ± 0.0	0.0 ± 0.0	0.0	4.4 ± 0.0
	gCOD _{VFA} L ⁻¹				
Propionic acid		0.0 ± 0.0	6.5 ± 0.1	0.1 ± 0.1	3.5 ± 0.0
	gCOD _{VFA} L ⁻¹				
Butyric and iso-butyric acids		0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.5	2.6 ± 0.0
	gCOD _{VFA} L ⁻¹				
Valeric acid		0.1 ± 0.0	0.2 ± 0.0	0.8 ± 0.0	0.4 ± 0.0
	gCOD _{VFA} L ⁻¹				
Iso-valeric acid		0.4 ± 0.1	0.2 ± 0.0	0.8 ± 0.1	3.5 ± 0.0
	gCOD _{VFA} L ⁻¹				
Hexanoic acid	gCOD _{VFA} L ⁻¹	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Heptanoic acid	gCOD _{VFA} L ⁻¹	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.7 ± 0.0
Iso-hexanoic acid	gCOD _{VFA} L ⁻¹	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.1 ± 0.1
pH		7.5 ±	6.6 ± 0.0	6.4 ± 0.0	6.5 ± 0.1
		0.0			
Partial alkalinity	mgCaCO ₃ L ⁻¹	100 ± 0	100 ± 0	106 ± 9	108 ± 12
Total alkalinity	mgCaCO ₃ L ⁻¹	525 ±	738 ± 0	738 ± 18	1017 ± 24
		106			
Na ⁺	mg L ⁻¹	2214 ± 33	2117 ± 27	3391 ± 67	2949 ± 66

N-NH ₄ ⁺	mg L ⁻¹	279 ± 13	264 ± 35	0.0	344 ± 33
K ⁺	mg L ⁻¹	948 ± 9	1092 ± 42	822 ± 10	942 ± 25
Mg ²⁺	mg L ⁻¹	1942 ± 27	1794 ± 33	3200 ± 11	3072 ± 31
Ca ²⁺	mg L ⁻¹	5139 ± 44	4939 ± 85	7783 ± 8	7496 ± 24

213

214 3.2 Anaerobic fermentation tests

215 Table 4 reports the values of process parameters and VFAs concentration obtained at the end of the batch tests. A high
 216 VFAs concentration was expected also in the not cavitated, since vegetable waste is quickly biodegradable. However, a
 217 higher VFAs concentration was expected from the cavitated due to the disgregation of the sludge flocs after the pre-
 218 treatment. This was not observed, therefore indicating that the HC pre-treatment could be further optimized.

219 Table 4. Final VFAs concentrations and fermentation performances achieved in the batch tests.

Parameter	Unit	Not cavitated	Cavitated	Not cavitated	Cavitated 50'
		(Blank)	30'	(Blank)	
pH		5.3 ± 0.0	5.4 ± 0.2	5.0 ± 0.0	5.4 ± 0.0
VFAs	gCOD _{VFA} L ⁻¹	15.75 ± 0.25	11.82 ± 0.45	18.25 ± 0.40	17.34 ± 0.62
Formic acid	gCOD _{VFA} L ⁻¹	0.0	0.0	0.0 ± 0.0	0.0
Acetic acid	gCOD _{VFA} L ⁻¹	4.21 ± 0.10	3.09 ± 0.05	4.67 ± 0.16	2.66 ± 0.08
Propionic acid	gCOD _{VFA} L ⁻¹	3.34 ± 0.06	1.69 ± 0.02	3.77 ± 0.06	1.60 ± 0.05
Butyric and iso-butyric acids	gCOD _{VFA} L ⁻¹	4.81 ± 0.09	4.90 ± 0.05	2.84 ± 0.07	5.15 ± 0.13
Valeric acid	gCOD _{VFA} L ⁻¹	0.60 ± 0.01	0.00	0.54 ± 0.03	0.29 ± 0.26
Iso-valeric acid	gCOD _{VFA} L ⁻¹	2.33 ± 0.07	1.79 ± 0.19	3.69 ± 0.07	2.10 ± 0.06
Hexanoic acid	gCOD _{VFA} L ⁻¹	0.00	0.00	0.00 ± 0.00	0.00

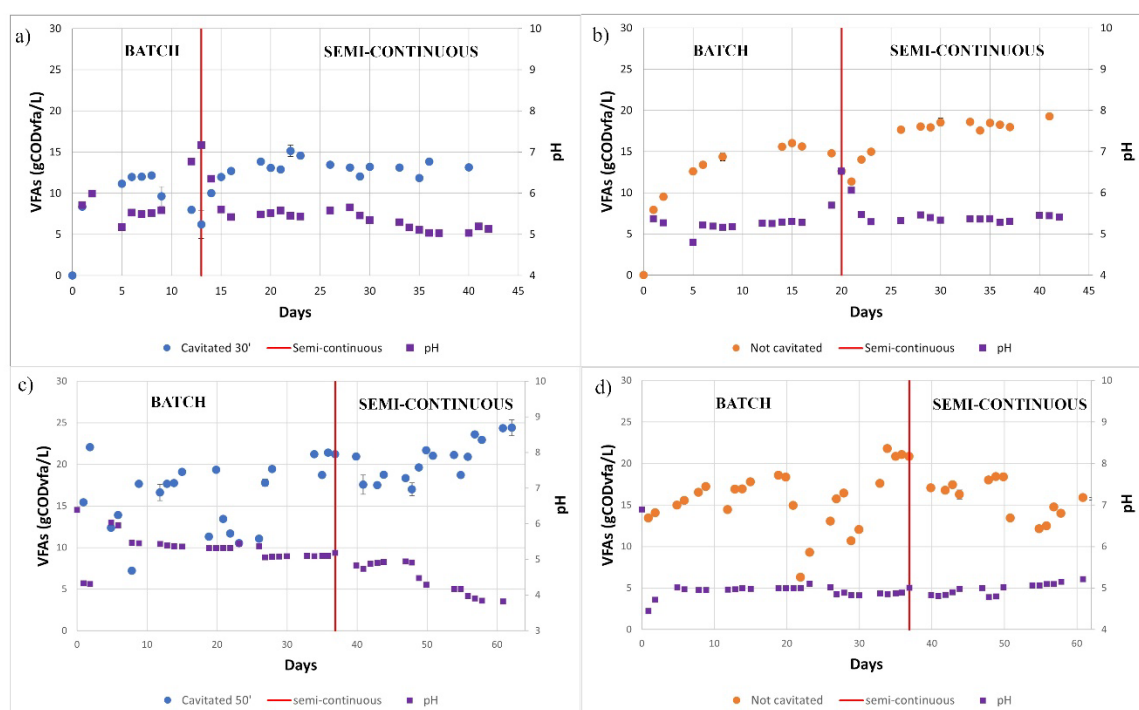
Heptanoic acid	$\text{gCOD}_{\text{VFA}} \text{L}^{-1}$	0.54 ± 0.01	0.60 ± 0.00	1.82 ± 0.02	4.84 ± 0.69
Iso-hexanoic acid	$\text{gCOD}_{\text{VFA}} \text{L}^{-1}$	0.00	0.00	0.91 ± 0.02	0.20 ± 0.12
$[\text{C}_3/(\text{C}_3+\text{C}_2)]_{\text{VFA}}$	mol mol^{-1}	0.36 ± 0.00	0.28 ± 0.02	0.41 ± 0.01	0.43 ± 0.01
Activity _{tot}	gCOD_{VFA}	2.17	2.30	3.6	4.2
	$\text{gTVS}_{(0)}^{-1}\text{d}^{-1}$				
Activity _{net}	gCOD_{VFA}	-	1.1	-	1.5
	$\text{gTVS}_{(0)}^{-1}\text{d}^{-1}$				
Net yield	$\text{gCOD}_{\text{VFA}} \text{gVS}_{(0)}^{-1}$	-	0.38 ± 0.02	-	0.20 ± 0.02
Total yield	$\text{gCOD}_{\text{VFA}} \text{gVS}_{(0)}^{-1}$	0.60 ± 0.01	0.60 ± 0.02	0.60 ± 0.01	0.62 ± 0.02
Test duration	days	20	13	37	37

220

221 Then, the reactors were fed in semi-continuous mode for 3.8 HRTs for the cavitated 30' and 2.6 HRTs for its blank and
222 for 4.0 HRTs for the cavitated 50' and 4.2 HRTs for its blank. Figure 1 shows the trend in VFAs concentration and pH
223 during the whole experiment for the cavitated 30' (1a) and its blank (1b) and the cavitated 50' (1c) and its blank (1d).
224 As observed in previous studies, mesophilic conditions resulted in a continuous fermentative activity that easily reached
225 a steady state (Moretto et al., 2019). Moreover, mesophilic temperatures are preferable in the perspective of
226 implementing the process in a full-scale plant since they require less amounts of energy (Valentino et al., 2019a). A
227 pseudo-stability was reached, where a lower VFAs concentration for the cavitated 30' ($12.94 \pm 0.63 \text{ gCOD}_{\text{VFA}} \text{L}^{-1}$) with
228 respect to its blank ($18.23 \pm 0.51 \text{ gCOD}_{\text{VFA}} \text{L}^{-1}$) was observed. On the contrary, VFAs concentration of the cavitated 50'
229 ($17.93 \pm 0.70 \text{ gCOD}_{\text{VFA}} \text{L}^{-1}$) was comparable with its blank ($18.27 \pm 0.23 \text{ gCOD}_{\text{VFA}} \text{L}^{-1}$). However, after 2 HRTs, the
230 cavitated 50' underwent a progressive drop of the pH to a value of 3.83 ± 0.02 with a concentration of organic acids
231 (OAs) of $15.0 \pm 0.4 \text{ gCOD}_{\text{OA}} \text{L}^{-1}$, represented for the 83% by lactic acid. The pH drop was likely due to the greater
232 initial sCOD concentration induced by the HC pre-treatment, which also raised the VFAs concentration from 1.93 to
233 $17.29 \text{ gCOD}_{\text{VFA}} \text{L}^{-1}$. pH control is needed if VFAs are kept as the target molecules for this process. However, the good
234 yield of $0.52 \text{ gCOD}_{\text{Lac}} \text{gVS}_{\text{fed}}^{-1}$ obtained at uncontrolled pH suggests that lactic acid production would be the most
235 advantageous way to exploit the mixture cavitated 50'. In fact, as observed in recent works (Pau et al., 2021; Strazzera
236 et al., 2021) and contrary to what was reported in most of the literature (RedCorn et al., 2016; Tang et al. 2016; 2017;
237 Yousuf et al., 2018; Zhang et al., 2017), lactic acid production became consistently higher at $\text{pH} < 4$. This is attributable
238 to the fact that at $\text{pH} < 4$, VFAs in the fermentation broth are in their undissociated form, thus being more liposoluble and

239 able to diffuse in the medium and penetrate the bacterial cells. In the cytoplasm, at neutral pH, VFAs dissociate, causing
 240 a drop in the intracellular pH. Thus, the cellular activities of the microorganisms are compromised, alongside their
 241 capability of producing VFAs (Palmqvist & Hahn-Hägerdal, 2000). On the contrary, lactic acid-producing bacteria were
 242 not inhibited at the pH reached in this study (3.82) since the pKa of lactic acid is lower (3.10). The enhancement of
 243 lactic acid production at low uncontrolled pH would be an advantage from an industrial point of view since it would
 244 decrease the overall cost of the process, the 14% of which is represented by alkalizing agents (usually NaOH, CaOH
 245 or lime) required to stabilize pH (López-Gómez et al., 2018; Joglekar et al., 2006). Moreover, the presence of lactic acid
 246 was not detrimental to the subsequent PHAs synthesis process, which has already been carried out successfully on
 247 mixtures of organic acids containing lactic acid (Dionisi et al., 2004; 2005; Gouveia et al., 2017).

248 The VFAs concentrations obtained show that good acidogenic performance took place concerning studies on similar
 249 substrates, such as those carried out by Valentino et al. (2019a) ($19.5 \text{ gCOD}_{\text{VFA}} \text{ L}^{-1}$), Strazzera et al. (2021) (18
 250 $\text{gCOD}_{\text{VFA}} \text{ L}^{-1}$), and Cheah et al. (2019) (3.65 ± 0.67 - $11.73 \pm 2.37 \text{ g}_{\text{VFA}} \text{ L}^{-1}$). The concentrations obtained were lower
 251 than $30 \pm 3 \text{ gCOD}_{\text{VFA}} \text{ L}^{-1}$ achieved by Moretto et al. (2020) by applying a thermal pre-treatment ($T=72 \text{ }^\circ\text{C}$ for 48 h) to a
 252 mixture of SS and OFMSW composed in a similar 70:30 volumetric ratio. This is attributable to the long duration of the
 253 thermal pre-treatment, which induced a greater solubilization of the organic matter.



254
 255 Figure 1. VFAs and pH trend during the whole experiment for a) cavitated 30' and b) blank of cavitated 30'; c)
 256 cavitated 50' and d) blank of cavitated 50'.

257 All the three mixtures tested showed good yields, higher than those obtained by Valentino et al. (2019a) (0.41-0.44
 258 $\text{gCOD}_{\text{VFA}} \text{gVS}_{(\text{fed})}^{-1}$) and by Strazzera et al. (2021) ($0.38 \text{gCOD}_{\text{VFA}} \text{gVS}_{(\text{fed})}^{-1}$), probably due to the lower protein content
 259 of the substrates used in this study respect to OFMSW+SS and synthetic household FW. The slight variation between
 260 the yields of the cavitated 30' and its blank and the cavitated 50' and its blank is attributable to the variability of the
 261 substrates used (tables 3 and 5). The net fermentation yields were lower for the cavitated mixtures because part of the
 262 VFAs was produced during the pre-treatment. The yields obtained were lower than $0.65 \text{gCOD}_{\text{VFA}} \text{gVS}_{(\text{fed})}^{-1}$ obtained by
 263 applying a thermal pre-treatment (Moretto et al., 2020b). The activity was 17% higher in the cavitated 30' with respect
 264 to its blank, while the activity of the cavitated 50' was 8% lower than its blank (table 5).

265 Therefore, our pre-treatment and fermentation processes could probably undergo further optimization, although the
 266 aforementioned studies used a richer substrate, i.e., OFMSW and FW.

267 Table 5. Final VFAs concentrations and fermentation performances achieved in the semi-continuous tests.

Parameter	Unit	Not cavitated (Blank)	Cavitated 30'	Not cavitated (Blank)	Cavitated 50'
pH		5.4 ± 0.0	5.5 ± 0.1	4.9 ± 0.1	4.9 ± 0.1
VFAs	$\text{gCOD}_{\text{VFA}} \text{L}^{-1}$	18.23 ± 0.51	12.94 ± 0.63	18.27 ± 0.23	17.93 ± 0.70
Formic acid	$\text{gCOD}_{\text{VFA}} \text{L}^{-1}$	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Acetic acid	$\text{gCOD}_{\text{VFA}} \text{L}^{-1}$	3.01 ± 0.36	1.76 ± 0.04	6.71 ± 1.51	4.35 ± 1.10
Propionic acid	$\text{gCOD}_{\text{VFA}} \text{L}^{-1}$	2.94 ± 0.39	2.49 ± 0.81	0.00 ± 0.00	0.26 ± 0.30
Butyric and iso- butyric acid	$\text{gCOD}_{\text{VFA}} \text{L}^{-1}$	3.41 ± 0.30	3.31 ± 0.81	1.76 ± 0.27	2.40 ± 0.45
Valeric acid	$\text{gCOD}_{\text{VFA}} \text{L}^{-1}$	1.25 ± 0.14	0.52 ± 0.90	0.61 ± 0.04	0.74 ± 0.25
Iso-valeric acid	$\text{gCOD}_{\text{VFA}} \text{L}^{-1}$	4.17 ± 0.71	3.31 ± 1.61	1.87 ± 0.26	1.64 ± 0.64
Hexanoic acid	$\text{gCOD}_{\text{VFA}} \text{L}^{-1}$	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Heptanoic acid	$\text{gCOD}_{\text{VFA}} \text{L}^{-1}$	2.61 ± 0.87	1.36 ± 0.26	5.97 ± 1.68	6.17 ± 1.92

Iso-hexanoic acid	$\text{gCOD}_{\text{VFA}} \text{L}^{-1}$	0.72 ± 0.75	0.21 ± 0.30	1.35 ± 0.16	0.94 ± 0.17
$[\text{C}_3/(\text{C}_3+\text{C}_2)]_{\text{VFA}}$	mol mol^{-1}	0.48 ± 0.04	0.53 ± 0.04	0.22 ± 0.1	0.32 ± 0.04
Activity _{tot}	$\text{gCOD}_{\text{VFA}} \text{gTVS}_{(0)}^{-1} \text{d}^{-1}$	0.69 ± 0.11	0.81 ± 0.15	0.60 ± 0.01	0.55 ± 0.02
Activity _{net}	$\text{gCOD}_{\text{VFA}} \text{gTVS}_{(0)}^{-1} \text{d}^{-1}$	-	0.54 ± 0.10	-	0.45 ± 0.02
Net yield	$\text{gCOD}_{\text{VFA}} \text{gVS}_{(0)}^{-1}$	-	0.33 ± 0.10	-	0.02 ± 0.02
Total yield	$\text{gCOD}_{\text{VFA}} \text{gVS}_{(0)}^{-1}$	0.52 ± 0.06	0.53 ± 0.07	0.48 ± 0.01	0.44 ± 0.02

268

269 The VFAs profiles observed in the semi-continuous tests were similar for the cavitated substrates with respect to their
270 blanks, as illustrated in the supplementary materials (figures S1 and S2). VFAs composition was stable for the not
271 cavitated and for the cavitated 30', with minor oscillations ascribable to the variability of the substrates used. The VFAs
272 profile was similar, with the sum of acetic, propionic, butyric and iso-butyric acid accounting for 50.8% and 59.9% of
273 the total for the not cavitated and cavitated 30', respectively. This profile is consistent with the ones obtained at pH=6 in
274 the fermentation process of SS (Moestedt et al., 2020) and FW, except for butyric acid (Feng et al., 2018). In our study,
275 butyric acid reached values comparable with Feng et al. (2018) only in the second batch test, representing 30.4% of the
276 not cavitated and 40.7% of the cavitated 30'. In the semi-continuous process, butyric and iso-butyric acids and
277 propionic acid were detected in similar percentages. The profile obtained in this study is similar to those obtained by
278 Moretto et al. (2019) and Valentino et al. (2019a) on similar substrates, i.e., OFMSW and SS. Acetic, propionic, and
279 butyric acids were identified as the most abundant in both studies, with some differences in the percentages due to the
280 different types of food waste, i.e., OFMSW instead of vegetable waste and the different pre-treatment (thermal). As in
281 this study, non-negligible amounts of valeric (11-13%), iso-valeric (2-3%), hexanoic (8-9%), and heptanoic (4-16%)
282 acids were detected (Valentino et al., 2019a; Moretto et al., 2019). Heptanoic acid was present in higher percentages in
283 the test on the not pre-treated mixture, as in Moretto et al. (2019), thus indicating that the HC pre-treatment enhanced
284 the conversion of the organic substrates into VFAs with shorter carbon chains. Heptanoic acid is known to be present
285 only in mesophilic conditions (Moretto et al., 2019), as those applied in this study. The higher concentration of iso-
286 valeric acid obtained in the not cavitated (20.4%) and cavitated 30' (25.8%) was found only in few previous studies on
287 SS fermentation, where it was attributed to protein fermentation (Hao&Wang, 2015; Jia et al., 2013; Xiong et al., 2012).

288 In fact, valeric acid was mainly associated with protein fermentation by reductive deamination of single amino acids or
289 oxidation–reduction between pairs of amino acids via Stickland reaction (Batstone et al., 2002).

290 The cavitated 50' kept a stable VFAs profile for the first 2 HRTs, after which lactic acid fermentation took over due to
291 the pH drop. At steady state, the VFAs profiles of the cavitated 50' and its blank were mainly represented by acetic
292 (26.3% and 36.7%), butyric and iso-butyric (14.8% and 9.7%) and heptanoic acid (37.4% and 32.6%), with non-
293 negligible amounts of valeric (4.4% and 3.4%), iso-valeric (10% and 10.2%) and iso-hexanoic acids (5.8% and 7.4%).
294 The profile obtained differs from the cavitated 30', probably because of the lower pH and the substrates' variability. In
295 particular, the relatively high concentration of heptanoic acid indicates that the fermentation process could undergo
296 further optimization.

297 3.3 PHAs accumulation tests

298 The OAs-rich effluent of the cavitated 50' without any further purification nor sterilization (just filtrated) and a
299 laboratory prepared simulating solution (Sim) were used as carbon sources for *C. necator* in the PHB-accumulation
300 phase after cells were grown with glucose (Figs. 2a and 2b). The effluent of the cavitated 50' was selected as a substrate
301 since it was the cavitated effluent with the highest concentration of OAs at the end of the experiment. A higher
302 concentration of OAs was reported to increase PHAs concentrations (Martinez et al., 2022).

303 The potential inhibition of OAs towards bacteria was avoided by keeping the concentration almost at zero all along the
304 accumulation phase through a pO₂-stat feeding strategy. Despite the cell dry weight (CDW) concentration profiles were
305 different between both conditions, the biopolymer (PHAs) concentration profiles were similar: in fact, PHAs increased
306 by a similar trend, from about 0.31 g L⁻¹ up to 2.1-2.9 g L⁻¹, confirming the equivalency of the two feeding solutions
307 used. The different CDW trends were due to the presence of suspended solids in the actual effluent, these were
308 centrifuged within the cells during sample treatment. During the accumulation phase the cell duplication did occur as a
309 consequence of PO₄³⁻ (0.577 g L⁻¹) and (0.252 g L⁻¹) NH₄⁺ occurring in the fed solution. This was effectively confirmed
310 when feeding the simulating solution which did not have suspended solids. The PHB content in *C. necator* at the end of
311 the accumulation phase was 57±5% and 44±2% for the simulated and actual effluents, respectively.

312 The PHAs yields here obtained with the actual and simulating feeding solutions ($0.37 \text{ g}_{\text{PHAs}} \text{ g}_{\text{Subs}}^{-1}$ and $0.25 \text{ g}_{\text{PHAs}} \text{ g}_{\text{Subs}}^{-1}$,
 313 respectively, Fig. 2b) were similar to those reached by feeding *C. necator* with a mixture of lactic and acetic acids (0.28
 314 and $0.15 \text{ g}_{\text{PHAs}} \text{ g}_{\text{Subs}}^{-1}$) as reported by Tsuge et al. (2001) and Schwartz et al. (2018), respectively. The PHAs yields were
 315 smaller to those obtained with VFAs from cheese whey ($0.54 \text{ g}_{\text{PHAs}} \text{ g}_{\text{VFA}}^{-1}$, Domingos et al., 2018).

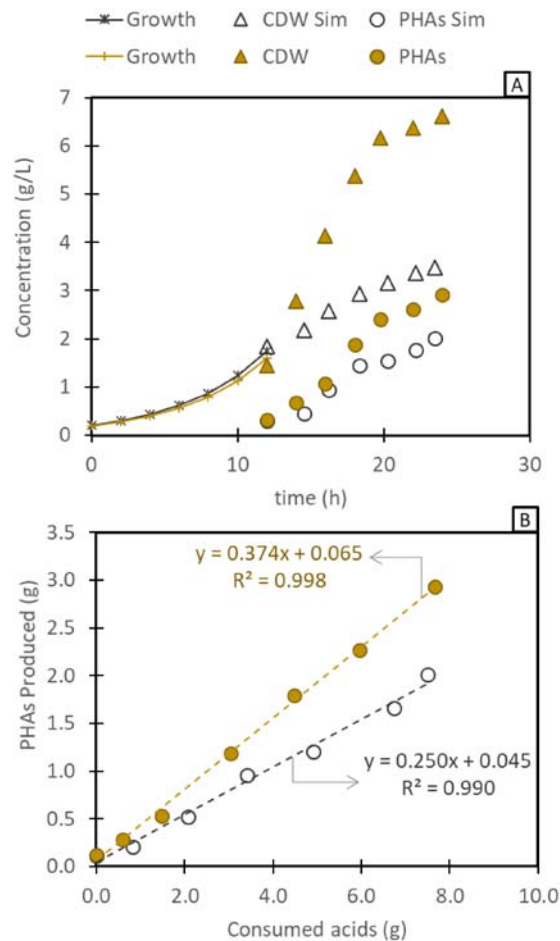


Figure 2. PHAs production from cavitated and anaerobic fermented substrate or laboratory prepared solution (Sim). Graph A shows the experimental concentrations of CDW and PHAs. Graph B shows the PHAs production yields with respect to acids consumed.

316 The PHB produced by *C. necator* fed with the actual effluent and simulating solution was finally extracted and
 317 characterized. Both conditions resulted in the production of an almost homopolymer of hydroxybutyrate (NMR spectres
 318 are shown in Figure S2). The molecular weights were 1.34-1.56 and 1.71-1.79 MDa for the actual and simulated
 319 effluent, respectively. This was in agreement with what is achievable by feeding the same bacterial strain with
 320 conventional carbon sources (e.g., glucose, 1.1 MDa, Samori et al., 2021). The polydispersity index (PDI) obtained for
 321 both conditions resulted significantly lower (1.6-1.8) than the values reported under analogous fermentation conditions
 322 (Schwartz et al., 2018). Moreover, T_m and T_c resulted 168-170 °C and 46-47 °C (DSC scans are shown in Figure S3)
 323 which are in agreement with commercial PHB characteristics (Schwartz et al., 2018).

324 3.4 BMP tests

325 The BMP tests on the solid-rich fermented effluent showed a higher SGP of $0.42 \pm 0.01 \text{ Nm}^3 \text{ kg}_{\text{TVS}}^{-1}$ for the
326 cavitated respect to the not cavitated ($0.34 \pm 0.01 \text{ Nm}^3 \text{ kg}_{\text{TVS}}^{-1}$), with a biogas production of $50.3 \text{ m}^3 \cdot 10^{-3} \text{ kg}$ of
327 substrate for the cavitated and $36.4 \text{ m}^3 \cdot 10^{-3} \text{ kg}$ of substrate for the not cavitated, respectively. This was probably due to
328 the disgregation of the lignocellulosic fraction of vegetable waste after the HC pre-treatment. The disgregated
329 lignocellulosic fraction was not consumed during the fermentation process, as shown by the absence of improvement in
330 the fermentation yields, but was degraded during the anaerobic digestion process in the BMP test. This improved the
331 overall biogas production from the solid-rich fraction of the fermented effluent. The SGP of the cavitated was
332 only slightly lower than the SGP of $0.44 \pm 0.02 \text{ m}^3 \text{ kg}_{\text{TVS}}^{-1}$ obtained by Moretto et al. (2020b) in a continuous
333 process. However, it should be considered that the author diluted the solid-rich fermentation effluent with SS, therefore
334 adding degradable organic material to the slowly degradable COD left in the solid-rich fermentation effluent.

335 4. Conclusions

336 This work demonstrated that hydrodynamic cavitation is a promising pre-treatment of vegetable waste and sewage
337 sludge, directly producing an interesting concentration of $17.29 \text{ gCOD}_{\text{VFA}} \text{ L}^{-1}$ after 50 minutes of pre-treatment, with an
338 SE of $3734 \text{ kJ kg}_{\text{TS}}^{-1}$ which is consistently lower respect to acoustic cavitation. The solubilization of the mixture
339 increased with the pre-treatment intensity and duration, with a DD_{COD} of 6% and 17% for the cavitated 30' and the
340 cavitated 50', respectively. The fermentation processes showed good VFAs concentrations ($12.94\text{-}18.27 \text{ gCOD}_{\text{VFA}} \text{ L}^{-1}$)
341 and yields ($0.44\text{-}0.53 \text{ gCOD}_{\text{VFA}} \text{ g}_{\text{VS}(0)}^{-1}$), which could be enhanced by pre-treatment optimization and pH control. After
342 the pH drop, the cavitated 50' showed a concentration of organic acids (OAs) of $15.0 \pm 0.4 \text{ gCOD}_{\text{OA}} \text{ L}^{-1}$, represented for
343 the 83% by lactic acid. This OAs-rich broth was routed into PHAs production, resulting a performant substrate for
344 PHBs storage with pure cultures of *C. necator*, with a PHB content of $44 \pm 2\%$ and a yield of $0.37 \text{ g}_{\text{PHAs}} \text{ g}_{\text{Subs}}^{-1}$.

345 Hydrodynamic cavitation pre-treatment enhanced the conversion into biogas of the solid-rich fermented effluent, with
346 an SGP of $0.42 \pm 0.01 \text{ Nm}^3 \text{ kg}_{\text{TVS}}^{-1}$ for the cavitated and $0.34 \pm 0.01 \text{ Nm}^3 \text{ kg}_{\text{TVS}}^{-1}$ for the not cavitated. In conclusion, the
347 feasibility of the integrated management of sewage sludge and vegetable waste in the frame of a biorefinery concept
348 was demonstrated. This paves the way for the optimization of these processes in future research.

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