

1 **Mesophilic and thermophilic anaerobic digestion of the liquid fraction of pressed**
2 **biowaste for high energy yields recovery**

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29 **Abstract**

30 Deep separate collection of the organic fraction of municipal waste generates streams
31 with relatively low content of inert material and good biodegradability. This material
32 can be conveniently treated to recovery both energy and material by means of simplified
33 technologies like screw-press and extruder. In this study, the liquid fraction generated
34 from pressed biowaste from kerbside and door-to-door collection was anaerobically
35 digested in both mesophilic and thermophilic conditions. Continuous operation results
36 obtained both in mesophilic and thermophilic conditions indicated that the anaerobic
37 digestion of pressed biowaste was viable at all operating conditions tested, with the
38 greatest specific gas production of $0.92 \text{ m}^3/\text{kgTVS}_{\text{fed}}$ at an organic loading rate of 4.7
39 $\text{kgTVS}/\text{m}^3\text{d}$ in thermophilic conditions. The energy returned on energy invested
40 (EROEI) per per ton of biowaste treated is therefore very favorable and around 8.9
41 $\text{kWh}_{\text{produced}}/\text{kWh}_{\text{consumed}}$, demonstrated the viability of this novel configuration with
42 respect to conventional ones.

43 The contents of heavy metals and pathogens of fed substrate and effluent digestates
44 were analyzed, and results showed low levels (below End-of-Waste 2014 criteria limits)
45 for both the parameters thus indicating the good quality of digestate and its possible use
46 for agronomic purposes.

47 It was demonstrated that biowaste pressing is a new alternative for mechanical
48 treatment plants that needs to be constructed or revamped.

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52 **Key words**

53 Anaerobic digestion, mesophilic, thermophilic, biogas, pressed biowaste, transient
54 conditions

55

56 *Abbreviations*

57 AD: Anaerobic Digestion, ALK tot: Total Alkalinity, BW: BioWaste, COD: Chemical

58 Oxygen Demand, CSTR: Continuous Stirred Tank Reactor, DW: Dry Weight, EoW:

59 End of Waste, GP: Gas Production, GPR: Gas Production Rate, HRT: Hydraulic

60 Retention Time, MSW: Municipal Solid Waste, OFMSW: Organic Fraction of

61 Municipal Solid Waste, OLR: Organic Loading Rate, P_{tot}: Total Phosphorus, SGP:

62 Specific Gas Production, SMP: Specific Methane Production, SSC: Steady State

63 Condition, TKN: Total Kjeldahl Nitrogen, TS: Total Solids, TVS: Total Volatile

64 Solids, VFAs: Volatile Fatty Acids, WW: Wet Weight

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66 **1. Introduction**

67

68 Anaerobic digestion is a proven and widespread technology for the management of
69 organic waste of different origin (Mattheeuws and De Baere, 2014). There are currently
70 more than 14,000 plants running in Europe, 28% of which are dedicated to the treatment
71 of wastewater sludge, municipal and industrial organic waste, while the remaining 72%
72 use agro-waste as feedstock (EBA, 2014).

73 With specific reference to municipal waste management, the success of this technology
74 in recent years has been determined by the implementation of deep separate collection
75 schemes: this determined the possibility of handling streams with a reduced amount of
76 inert material and high moisture content and biodegradability like segregated food waste
77 (Valorgas 2010).

78 Beside anaerobic digestion, the other biological process widely diffused within EU for
79 the management of organic wastes is the aerobic stabilization: at present a compost
80 production of around 10.5 million tonnes of organic waste is reported (COM(2008), 811
81 <http://eur-lex.europa.eu>). Noticeably, the two processes, i.e., anaerobic digestion and
82 composting, can be integrated together since the solid fraction of digestate can be
83 treated aerobically (Nayono et al., 2009) so to recovery both renewable energy and
84 nutrients from organic waste. At present some 8 million tons of biowaste are
85 anaerobically digested within EU Countries and normally the biowaste is pre-treated
86 and prepared for the AD process by means of several mechanical steps. A large number
87 of plants treating organic waste started their operations in the 1980s, when both the
88 amount and the composition of biowaste were quite different from the present situation.
89 This has resulted in the need for some modifications both in plant management and
90 operating conditions (Di Maria et al., 2012). In fact results showed that during
91 conventional pre-treatment methods around 30% of the initial wet material could be
92 rejected without any treatment (Pognani et al., 2012). The pre-treatment of the organic

93 fraction of municipal solid waste is in fact one of the main challenges in mechanical–
94 biological treatment plants equipped with anaerobic digesters (Romero-Güiza et al.,
95 2014).

96 Recent literature highlights the observations related to the loss of biodegradable organic
97 matter during the pre-treatment steps (Muller et al., 1998; Bolzonella et al., 2006; Ponsá
98 et al., 2010b). Moreover, these steps are time and energy consuming (Tonini et al.,
99 2014) and generally are not able to achieve high removal yields for inert materials like
100 small pieces of plastics and heavy materials like crashed glass (e.g., sea shells or
101 similar). These materials could then accumulate inside the reactor determining a
102 reduction of the reaction volume and a possible risk of process failure (Angelidaki and
103 Boe 2010).

104 Another important aspect to be considered is then the reduction of the energy demand
105 for pre-treatment processes and if possible enhance the biogas production of the
106 anaerobic digestion plants that treat the municipal biowaste (Morais et al., 2007).

107 In order to address all these issues an interesting option is the use of very simple pre-
108 treatment steps like presses and extruders: in these machines the size of the organic
109 material is reduced while inert material (mainly plastic) is eliminated.

110 Biowaste pressing, for example, produces of two streams: one semi-liquid to be
111 digested and a second one solid to be composted (Hansen et al., 2007).

112 Another fundamental aspect related to the treatment of properly pre-treated segregated
113 organic waste is that this results in a digestate or compost of good quality (Bernstad et
114 al., 2013). In fact Regulations in several EU countries only allows for use of compost or
115 liquid fertilizer (digestate) from food waste which is source segregated (as opposed to
116 co-mingled food waste with other waste from a materials recovery facility) to be used in
117 agricultural applications (Browne & Murphy, 2012; Lukehurst et al., 2010).

118 To avoid this problem, and because of the good quality of biowaste collected by means
119 of door-to-door and kerbside collection schemes, an advanced energy saving pre-

120 treatment approach has been developed: biowaste squeezing. This is a mechanical pre-
121 treatment process. The advantages of mechanical pretreatment include an easy
122 implementation, better dewaterability of the final anaerobic residue and a moderate
123 energy consumption (Ariunbaatar et al., 2014). Pretreatment and digester design are the
124 key techniques for enhanced biogas optimization (Shah et al., 2014).

125 Only few examples of such approach can be found in literature at the best of our
126 knowledge.

127 Satoto Nayono et al. (2009) studied AD of pressed off leachate from OFMSW and the
128 co-digestion of press water and food waste (Satoto Nayono et al. 2010) for
129 improvement of biogas production. The co-digestion of press water and food residues
130 with defibred kitchen wastes (food waste), operated at an OLR in the range 14-21
131 kgCOD/m³d, reported greater biogas production rates than sole biowaste. An increment
132 of the OLR of biowaste by 10.6% with press liquid fraction increased the biogas
133 production as much as 18%, with a biogas production rate of 4.2 m³/m³d at OLR of 13.6
134 kgCOD/m³d. These experimentations were conducted through laboratory scale reactors,
135 from 1 to 8 liters working volume.

136 According to this scenario, and the evidences of recent studies, the aim of the study was
137 to anaerobically digest the liquid fraction of pressed biowaste through a pilot-scale
138 investigation in order to identify bottlenecks, consumes and yields of interest for a
139 possible process up-scale. This research implements initiatives to support of improving
140 the quality of biowaste treated, to enhance energy production and reduce energy costs to
141 manage this bigger and bigger urban stream. It's a new paradigm for biogas plant pre-
142 treatment configuration.

143 This study deals with a pilot scale trial where biowaste from kerbside collection was
144 mechanically squeezed and the liquid fraction of the pressed material was anaerobically
145 digested. Both mesophilic and thermophilic conditions were tested, applying different
146 organic loading rates, in order to verify the capability of the systems to cope with

147 transient conditions.

148 Beside the operational parameters and yields, the digestate characteristics were
149 considered in detail also in order to respond to the requirements defined in the “End of
150 Waste Criteria” technical proposal by the Joint Research Center of Sevilla (2014).
151 Based on suggested criteria, pathogens and metals in the digestates were analyzed in
152 order to evaluate the necessity of further anaerobic digestate treatment, for example in a
153 co-composting process.

154

155 **2. Materials and Methods**

156 *2.1 Pretreatment strategy and experimental set up description*

157

158 A pilot-scale press, specifically designed for this experimentation, was used in order to
159 pre-treat separately collected biowaste and split it into two streams, one liquid to be
160 anaerobically digested and a second one solid to be composted.

161 Door-to-door collected biowaste from Treviso area (Italy) was first shredded into a
162 knife mill and treated in a press for solid-liquid separation. Only the liquid fraction was
163 then sent to the anaerobic process while the semi-solid part, characterized by a higher
164 content of dry matter, was suitable for aerobic stabilization process as composting.

165 The semi-liquid stream was then sent to two pilot scale CSTR anaerobic digesters, one
166 mesophilic ($37^{\circ}\text{C} \pm 0.1$) and the other thermophilic ($55^{\circ}\text{C} \pm 0.1$), working with an
167 organic loading rate in the range 3 - 6 kgTVS/m³, per day and a hydraulic retention time
168 of 20 days to simulate the best operating conditions expected at a full-scale treatment
169 plant. Organic matter degradation at increasing OLR and ultimately decreasing also the
170 HRT was investigated. The research was carried out using two pilot scale reactors
171 completely equal in terms of electro-mechanics, working volume (0.23 m³) and heating
172 system. The reactors were made of stainless steel AISI-304 and the mixing was ensured
173 by mechanical anchor-bars agitators in order to maximize the mixing degree inside the

174 reactor, thus avoiding the typical stratification of floating materials on the top and of
175 sinking heaviest materials on the bottom of the reactor. The temperature of 37 °C
176 (mesophilic thermal range) and 55 °C (thermophilic thermal range) of the reactors was
177 maintained constant by an external jacket, in which heated water was recirculated. The
178 biogas produced was sent to a hydraulic guard with the purpose of maintaining an
179 operating pressure of 0.1 m water column inside the reactor. Reactors were fed once a
180 day.

181

182 ***2.2 Analytical methods***

183

184 Biowaste commodity class was analyzed in accordance with the procedure reported by
185 MODECOMTM (1998). The reactor effluents were monitored 3 times per week in terms
186 of TS, TVS, COD, TKN and P total. For TS determination, 105°C drying temperature
187 was adopted and no losses were caused (Peces at al., 2014). The process stability
188 parameters, namely pH, volatile fatty acid (VFA) content and distribution, conductivity,
189 total and partial alkalinity and ammonia (NH₄⁺-N), were checked daily. All the analyses
190 performed according to the Standard Methods for Water and Wastewater Analysis
191 (1998). The analysis of the volatile fatty acids was carried out with a Carlo ErbaTM gas
192 chromatograph equipped with a flame ionization detector (T = 200 °C), a fused silica
193 capillary column Supelco NUKOLTM (15 m x 0.53 mm x 0.5 µm thickness of the film),
194 while hydrogen was used as carrier gas. The analysis was conducted using a
195 temperature ramp from 80 °C to 200 °C (10 °C / min). The samples were analyzed
196 before being centrifuged and filtered with a 0.45 µm filter. Biogas production was
197 monitored by a flow meter (Ritter CompanyTM), while methane, carbon dioxide and
198 oxygen in biogas were determined through a portable infrared gas analyzer GA2000TM
199 (Geotechnical InstrumentsTM) continuously and a Gas Chromatograph 6890N, Agilent
200 TechnologyTM, once a day.

201 The content of heavy metals and pathogens of fed substrate and digestates, in the two
202 experimentations (mesophilic and thermophilic), were analyzed (EPA 3051A 2007 +
203 EPA 6020A 2007).

204

205 **3. Results and discussion**

206 ***3.1 Biowaste pretreatment and composition***

207

208 The biowaste collected in Treviso area and used in this experimentation showed the
209 composition reported in table 1: fruit and vegetable waste were typically half of the
210 waste material, a result in line with previous studies on this topic (see Valorgas D2.1
211 <http://www.valorgas.soton.ac.uk/deliverables.htm>) while pasta/bread and meat/seafood
212 represented another 25% of the wasted food. Some 10% of the material was un-
213 classified (melt material).

214

215 Table 1. Components of biowaste

Composition	Fresh matter, %	Dry matter, %
Fruits & Vegetables	46-58	42-52
Other kitchen waste *	16-25	15-22
Paper & cardboard	9-14	7-12
Not classifiable	8-14	6-12

216 * Putrescible material non-vegetable (eg pasta, cakes, meat, etc..). WW = wet weight. DW = dry weight

217

218 Biowaste compositional analysis (of five samples) showed that food waste was more
219 than 82% of the total (on wet weight) while the remaining parts were paper (11%) and
220 inert materials (7%) like glass and metals or textiles. The different fractions for each

221 type of material in terms of wet and dry weight, and volatile fraction are presented in
 222 Table 2.

223

224 Table 2. Commodity characteristics of biowaste

Fractions	Wet weight	Dry matter	Volatile dry matter
	%	%	%
paper	10.75	11.25	12.87
plastic	0.85	1.20	1.31
inert	6.88	11.33	8.16
food waste	81.52	76.22	77.66

225

226 Table 3. Characteristics of biowaste, and pressed liquid and solid fractions

Substrate	TS g/kg	TVS g/kg	TVS/TS	COD g/kg	rbCOD	TKN	P
	biowaste	biowaste	%	TS	g/kg TS	g/kg TS	g/kg TS
biowaste	298.2±44.2	267.6±32.5	89.8±3	1,090±449	-	27±4	4.0±0.2
Liquid _{bw}	186.4±49.3	169.6±24.0	91±2	1,440±357	345±85	24±4	4.2±0.3
Solid _{bw}	378.2±34.3	343.4±18.9	89.6±1	1,389±498	-	23±6	3.9±0.4

227

228 As for the general chemico-physical characteristics, biowaste showed an average dry
 229 matter content of 298 gTS/kg, 90% volatile solids. The COD values were typically
 230 greater than 1090 gCOD/kg with a low nitrogen content.

231 The liquid phase obtained was particularly suitable for AD because of its total and
 232 volatile solids (91% of dry matter on average) very high COD content, and COD/N
 233 ratio.

234

235 **3.2 Performance of the pilot scale reactors**

236

237 The start-up phase of both mesophilic and thermophilic reactors was characterized by a
238 gradual increase of the organic loading rate (OLR) starting from 1 kgVS/m³_rd onwards
239 with a fixed hydraulic retention time of 20 days; when a steady state condition was
240 achieved the OLR applied was ranging between 3.0 and 3.5 kgTVS/m³_rd.

241 In order to verify the process resilience also transient conditions were tested: transient
242 conditions were obtained testing the system at different organic loading rates. In
243 particular, the OLR was tripled (from 2 to 6 kgVS/ m³_rd) on alternate days and stopping
244 the feed once a week. Every day pH, ammonia, alkalinity (partial and total) and VFAs
245 in the effluent as well as biogas production and its composition were analyzed before
246 addition of fresh substrate.

247

248 Table 1. Time division of the trial and organic loads applied

	RUN I – Start up	RUN II – SSC	RUN III – Transient
OLR (kgVS/m³_rd)	1-2	3.5	3 – 6
Average Value (M)			
RUNs time (d)	40	40	60
MESOPHILIC			started from (110day)
OLR (kgVS/m³_rd)	1	3.5	3 – 6
Average Value (T)			
RUNs time (d)	80	40	60
THERMOPHILIC			
HRTs	20 d	20 d	18/20 d
Meso and Thermo			

249

250 Reported values show how the two systems needed different time in order to conclude
251 the start up phase and reach a steady state condition. The transient period was the same
252 in both reactors, in order to make a good comparison of the results of the stressing tests.

253

254 *3.2.1 Performances of the mesophilic anaerobic digestion process*

255

256 The inoculum used for the mesophilic trials was anaerobically digested sludge
257 originated from a full-scale anaerobic digestion process (Treviso Municipal Wastewater
258 Treatment Plant). The pilot scale reactor was maintained at the operating temperature of
259 37°C with low loading rate (1 kgTVS/m³_rd) for a week, in order to acclimatize the
260 biomass to the liquid pressed organic stream. Initially the reactor was fed with an OLR
261 of 4,19 kgCOD m⁻³ d⁻¹.

262 Once the methanogenic biomass was active and responding appropriately in terms of
263 biogas quality, the reactor was fed daily, and the OLR was increased stepwise from 1
264 kgTVS/m³_rd to 3.5 kgTVS/m³_rd in 2 HRTs (RUN I, start up, from day 1 to day 40).

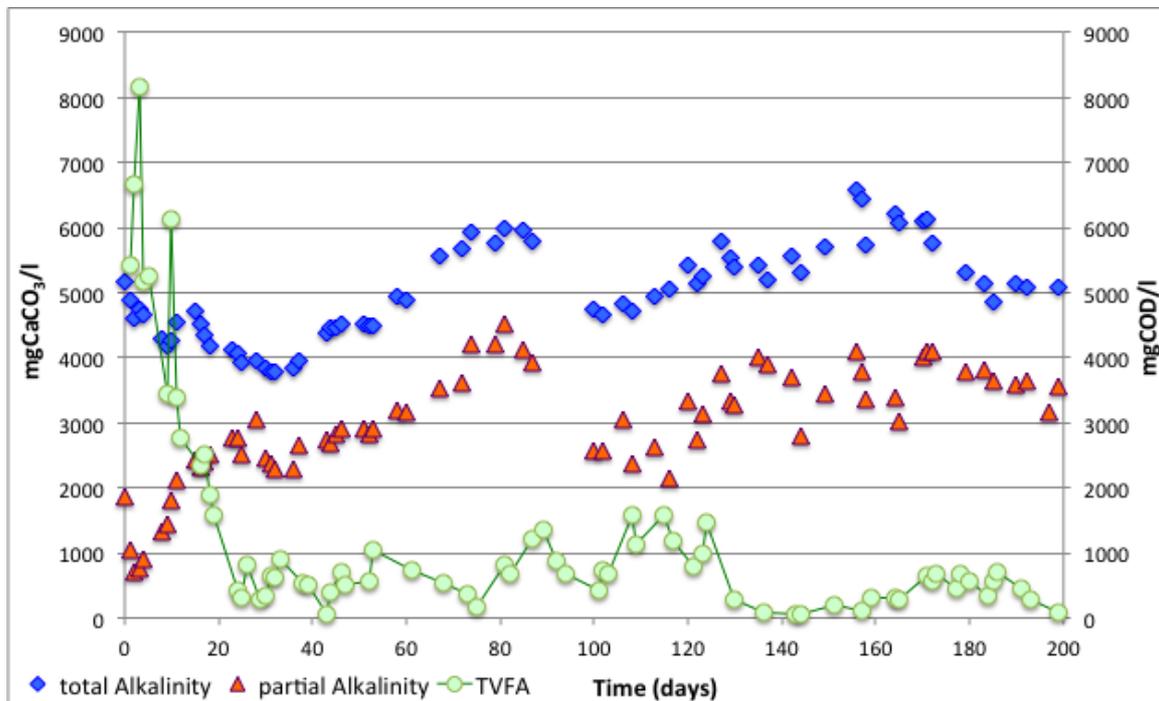
265 In steady state conditions the pH of the mesophilic AD was in the range 7.1 - 7.7
266 favoring the metabolic activities of fermentative bacteria and the growth of
267 methanogens. The pH drop observed during start up period (days 1-7) was related to the
268 high VFA concentration due to the sharp increase of acetate and propionate acids.

269 However, the overall anaerobic process and methane production is not inhibited
270 presumably because of the balanced presence of both acids (Zhang et al., 2014). After
271 day 7 VFA concentration decreased indicating the adaptation of biomass to the new
272 environmental conditions which signals the initiation of steady state phase (Gallert and
273 Winter, 2005) during which VFA concentration acquires an average value of 912

274 mgVFA/l (predominantly acetic acid). The system didn't show any upset to its stability,
275 thus indicating good robustness of the process also in transient conditions, a relevant
276 aspect for the full-scale implementation of the process. As for pH, this remained
277 constant, particularly in steady state conditions, with an average value around 7.7

278 because of the high buffer capacity of the system: this is highlighted by an average total
279 alkalinity value of 5,177 mgCaCO₃/l (figure 1). The ratio between VFA concentration
280 and alkalinity was also evaluated. Soluble COD (sCOD) showed an average value of
281 2,294 mgCOD/l. In steady state conditions the concentrations of the partial alkalinity

282 ranged between 2000 to 4500 mgCaCO₃/l (determined at pH 5.75), while the total
283 alkalinity was greater and in the range 4500 to 6000 mgCaCO₃/l (determined at pH 4).
284 These figures ensured sufficient buffer capacity: the ratio of VFA to total alkalinity (i.e.
285 the difference between total and partial alkalinity compared to total alkalinity) was
286 constantly below 0.3 indicating the stability of the AD processes in terms of volatile
287 fatty acid accumulation (Ripley et al., 1986). The concentration of volatile fatty acids
288 and alkalinity are the two parameters that show a more rapid variation when the system
289 tends to be upset (Ahring et al. 1995, Bolzonella et al, 2003) since in case of organic
290 overload, the concentration of fatty acids increases while the alkalinity tends to
291 decrease. The relationship between these two parameters (Cecchi et al., 2005) is a
292 useful stability indicator to be considered: ratio values around 0.3 indicate a stable
293 operation of the AD process, while greater values may indicate the inception of
294 instability. During organic loading increase the ratio was around 0.22, thus the system
295 achieved a perfect steady state even with the transient increasing of the organic loading
296 rate.
297



298

299 Figure 1. Alkalinity and VFAs trend during the mesophilic trial.

300

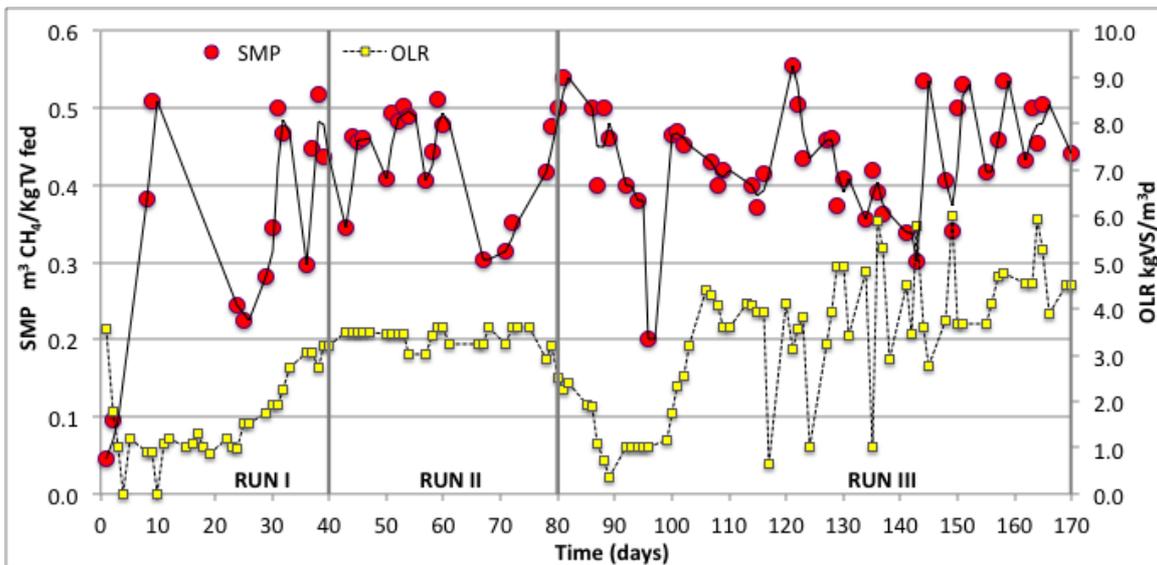
301 The use of a real substrate and the heterogeneity of biowaste, determined inevitable
 302 variations in the solid content in the reactor: the observed standard deviation was ± 39.3
 303 gTS/kg).

304 The monitoring of the total ammonia showed an average value of $878 \text{ mgN-NH}_4^+/\text{l}$
 305 (St.Dev. ± 79), $64 \text{ mgN-NH}_3/\text{l}$ free ammonia, well below the typical critical level for
 306 inhibition (Chen et al., 2008). The average content of total solids in the reactor remained
 307 almost constant with an average value of 22.5 gTS/kg (St.Dev. ± 1.9) and an average
 308 volatile solids content of 16.1 gTVS/kg (St.Dev. ± 0.7). The ratio between total solids
 309 and volatile shows an average value of 71.5% (TVS/TS), it is thus highlighted the
 310 capability of the system of converting the organic matter into biogas, leaving a residual
 311 dry matter content lower than 3% in digestate.

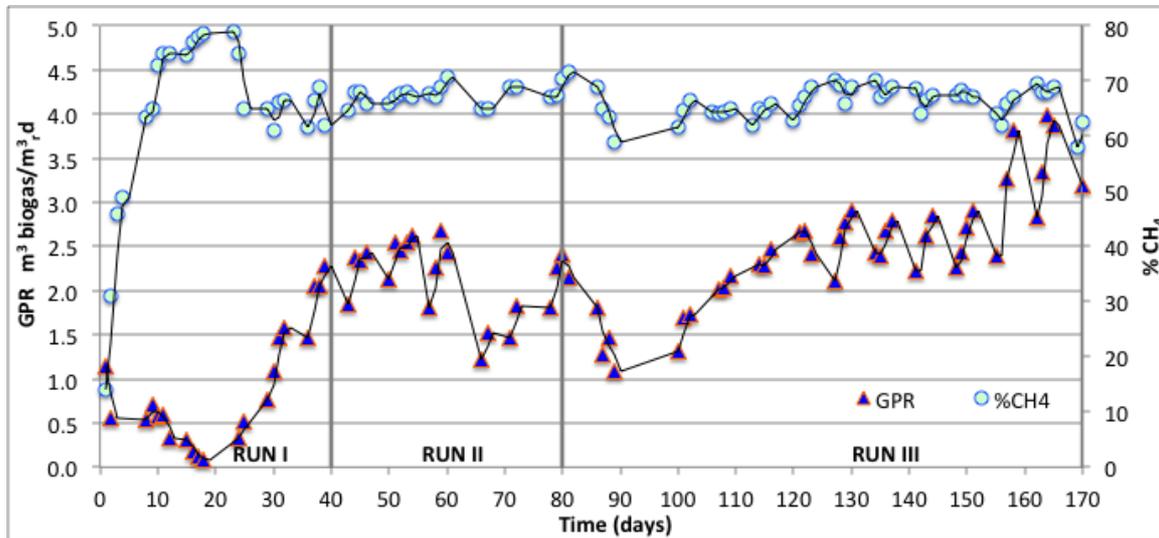
312 The biogas composition in terms of average percentage of methane detected in steady
 313 state condition (SSC) was 66% CH₄ and the remaining part CO₂. The average specific
 314 gas production (SGP) was found equal to 0.79 m³biogas/kgTVS and the average
 315 specific methane production (SMP) was 0.47 m³CH₄/kgTVS, while the average gas
 316 production rate (GPR) was 2.3 m³biogas/m³,d. Profiles of specific methane production
 317 (SMP) during the experimental trials are shown in figure 2.

318

319 Figure 2. Specific methane production and OLR variations in mesophilic anaerobic
 320 digestion and methane percentage compared to the Gas Production Rate



321



322

323

324 Overall the mesophilic digestion process of the semi-liquid fraction of biowaste in
 325 steady state conditions showed great strength and resilience with reference to the
 326 process parameters (pH, alkalinity and VFA concentration, and biogas composition).

327 Based on the aforementioned figures for each ton of biowaste semi liquid fraction, the
 328 biogas and methane production in AD mesophilic conditions equals to 148 and 98 m³
 329 respectively and corresponding to an expected power generation of around 300 kWh
 330 electrical energy (assuming biogas LHV=6.6 kWh/m³biogas and 30 % energy

331 conversion efficiency). A maximum value of 0.82 m³biogas/kgTVS in terms of SGP
 332 was observed at an OLR of 4.5 kgTVS/m³d. Interesting values in the velocity of biogas
 333 production were achieved in the latter period, with an average value of 3.1

334 m³biogas/m³d.

335 With specific reference to the mass balance of the AD system the average VS content of
 336 the influent was 790 gVS(in)/d, and the VS content of the output materials, namely
 337 biogas and digestate accounted for 512 and 259 gTS/d respectively. According to the
 338 above mentioned figures the mass balance accounts to 93%, with a 11.8% error, while

339 the VS reduction was approximately 65%. Also the resulting digestate contains less
 340 than 3% residue expressed as dry matter.

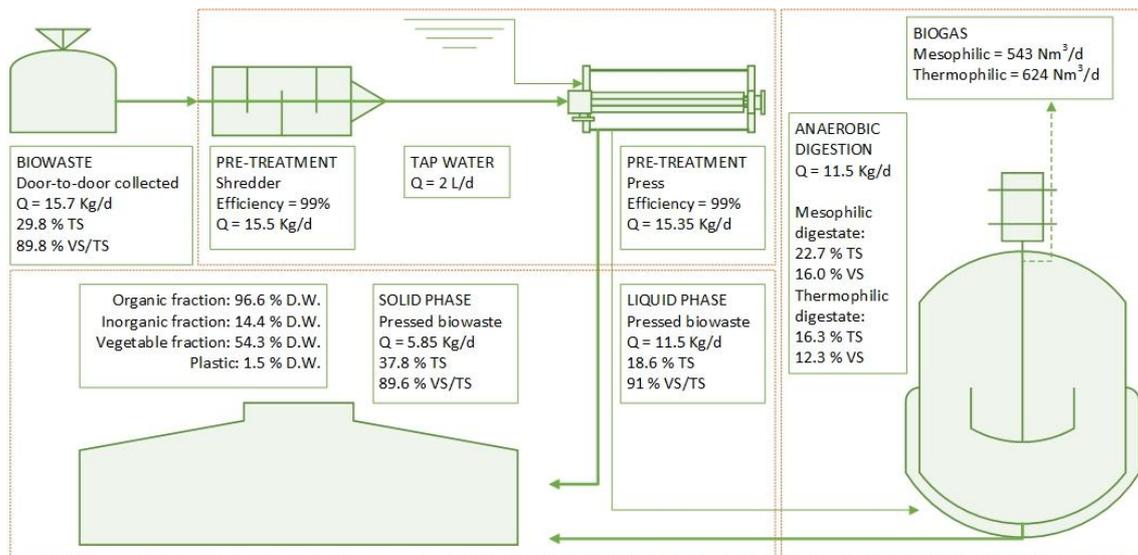
341 Similar results were found for dry matter and COD thus confirming the quality of the
 342 calculation. The COD balance showed a deficit of 9.8%.

343 Also the mass balances for nutrients (nitrogen and phosphorus) were good:
 344 nitrogen balance reached 89.5% with an error (deficit) of 11.5% while the
 345 ammonification degree of the mesophilic system was 56.2%.

346 Phosphorus was slightly higher in the digester output with a balance of 105% and an
 347 excess of 5%. Process scheme with material flow characterization is showed in figure 3.

348

349 Figure 3. Process scheme including the material flow characterization.



350

351

352 The removal efficiency of organic compounds was measured daily by determining the
 353 elimination of COD and Volatile Solids (VS) (Nayono et al., 2009). When SSC was
 354 reached, based on low residual fatty acids, stable biogas production, stable values for
 355 pH and COD elimination, TS and VS of the mesophilic reactor were also measured. In

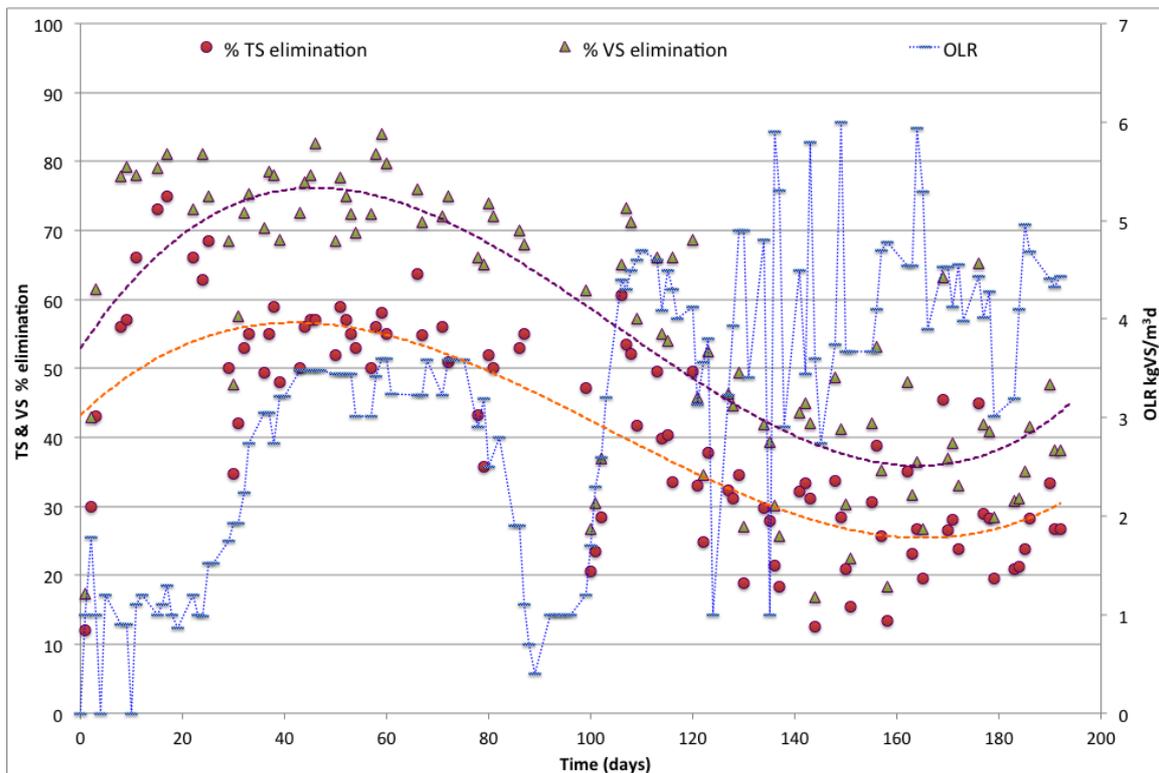
356 the first 22 days of operation the system reached a COD elimination more than 78% and
357 then around 74% during SSC.

358 In the figure 4 the relationship between solids elimination, total solids and volatile
359 solids compared to OLR trend are presented.

360 Compared to previous results of Nayono et al., (2009) we can underline that during SSC
361 with an OLR of 3.5 kgVS/m³d the mesophilic reactor showed a high efficiency on VS
362 removal and during transient conditions, raising the OLR from 4.5 to 6 kgVS/m³d the
363 anaerobic process appeared to be less efficient. This is due to the stress condition the
364 reactor had to endure, even if a VS elimination of 50% is considered close to the
365 optimum for anaerobic degradation of press water (Nayono et al., 2009).

366

367 Figure 4. Total solids and volatile solids elimination during all the trial in mesophilic
368 conditions.



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371 ***3.2.2 Performances of the thermophilic anaerobic digestion process***

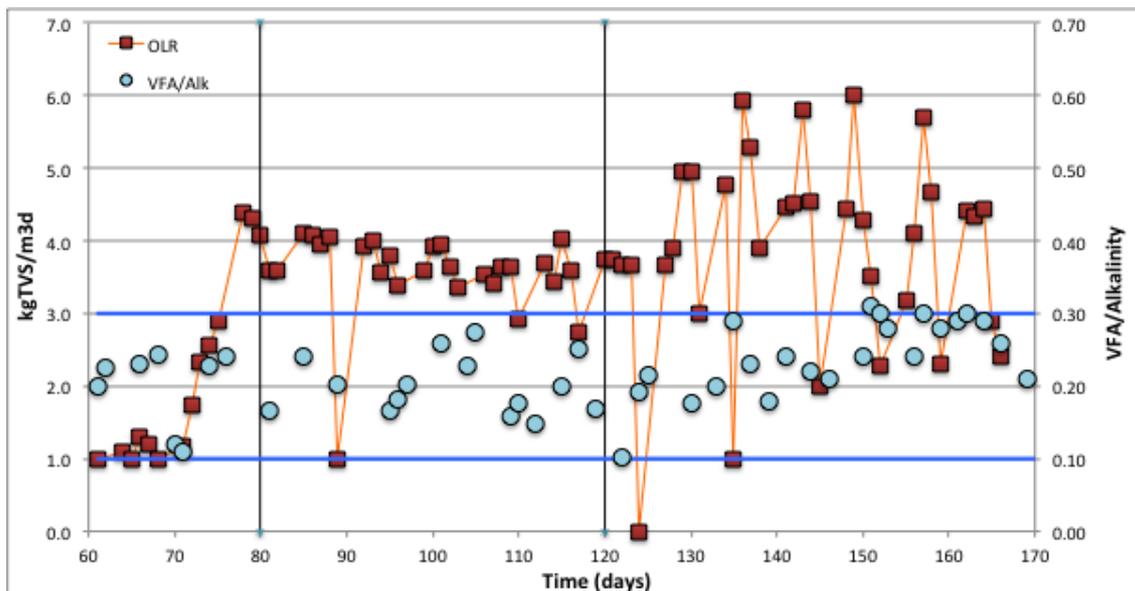
372

373 The inoculum used for the thermophilic reactor was the same of used for the mesophilic
374 tests. After the first days of operation in mesophilic conditions the working temperature
375 was increased from 37°C to 55°C using the single-step strategy (Cecchi et al., 1993) and
376 without feeding. The thermophilic conditions were reached in a couple of days and
377 maintained for about ten days without feeding. In order to acclimate the biomass to the
378 organic material, the reactor was started up with a low organic loading rate (1
379 kgTVS/m³_rd) for one week, then the OLR was increased to 3.5 kgTVS/m³_rd and
380 maintained for about 2 HRTs (RUN I from day 0 to day 80, see figure 5).

381 The short chain volatile fatty acids concentration remained constantly below 1000 mg/l,
382 with an average value of 489 mgVFA/l; acetate was the main compound found.
383 Maintaining VFA at this level prevents potential process inhibition due to VFA
384 accumulation which in turn leads to a decrease in pH and thereby declining
385 concentration of free ammonia (NH₃). Average pH was around 8.1. The low pH
386 fluctuations indicated the buffer capacity of the system which maintained pH at
387 compatible levels with the methanogenic thermophilic levels.

388 Average total alkalinity (determined at pH 4) in steady-state conditions was 5,380 mg
389 CaCO₃/l. Partial alkalinity (determined at pH 5.75) showed a profile in line with the
390 trend of the volatile fatty acids, and consequently the difference between partial and
391 total alkalinity, which is directly proportional to the concentration of VFA, remained
392 constant. The values of total and partial alkalinity were 5,200 and 3,900 CaCO₃/l
393 respectively, corresponding to a ratio value of 0.23 (VFA/alkalinity). Even in the
394 thermophilic reactor the value of this ratio was stable, thus it justifies the possibility to
395 increase the system to OLR of 4 - 4.5 kgTVS/m³_rd, in fact. During the transient period
396 (RUN III from day 120 to day 170) the best specific biogas productions were obtained

397 at OLR in the range 4 - 4.5 kgTVS/m³d, with a SGP average value of 0.9
 398 m³biogas/kgTVS. Transient OLR conditions from 2 to 6 kgTVS/m³d were evaluated.
 399 The VFA/alkalinity ratio of the transient OLR period had an average value of 0.27 as
 400 shown in Figure 4.
 401



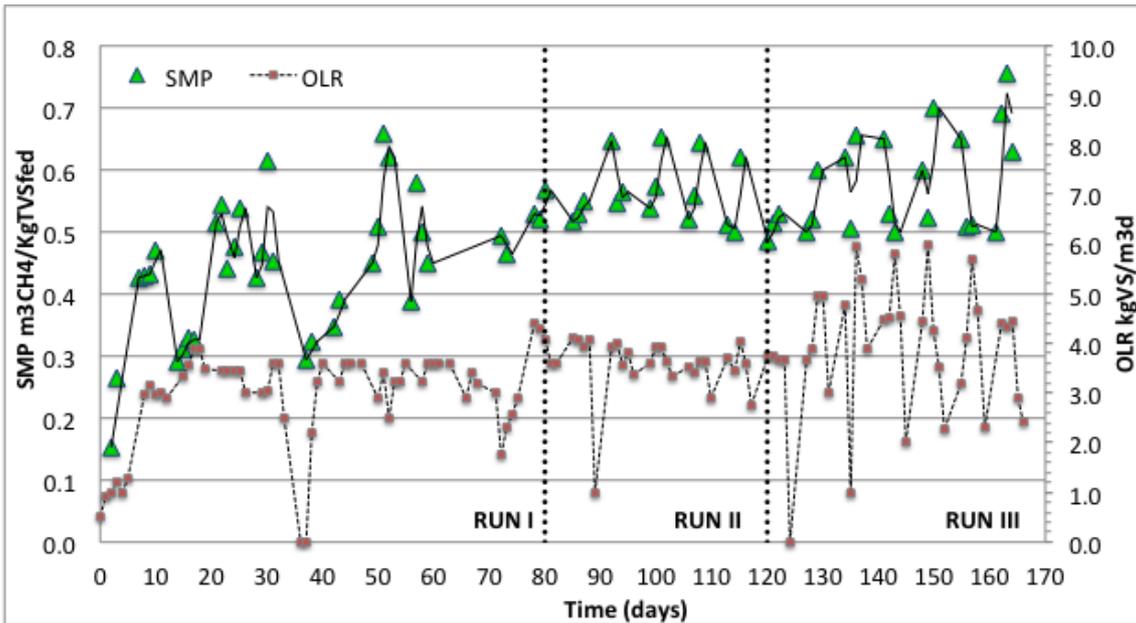
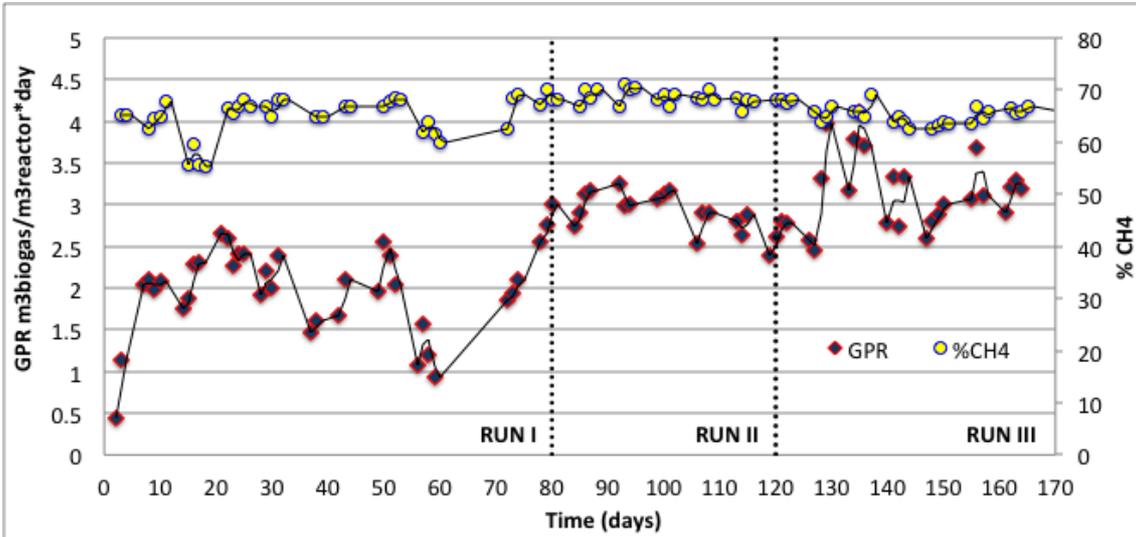
402
 403 Figure 4. Trend of the thermophilic ratio volatile fatty acids and alkalinity with OLR
 404 variations

405
 406 The average total ammonia concentration (as mgN-NH₄⁺/l) was 1,004 mgN-NH₄⁺/l,
 407 with maximum values of 1,086 mgN-NH₄⁺/l. When OLR was increased to 4-4.5 kgVS/
 408 m³d, the corresponding value of free ammonia was 374 mgN - NH₃/l, a value below the
 409 inhibition limit, normally reported in literature (about 700 mgN/l, Angelidaki et al.,
 410 1994).

411 The content of total solids in the reactor remained almost constant with an average
 412 value of 16.3 gTS/kg and a volatile solids content of 12.3 gTVS/kg. The ratio between
 413 total and volatile solids shows an average value of 76.5% (TVS/TS), it's thus

414 highlighted the large capacity of the system to convert the organic matter into biogas,
415 leaving a residue of dry matter lower than 2% in digestate.
416 The average composition of the biogas in terms of methane percentage was high, equal
417 to 68.8%, and the specific methane production (SMP) was $0.55 \text{ m}^3\text{CH}_4/\text{kgTVS}$ (Figure
418 5). With regard to biogas and energy yield, the average specific gas and methane
419 production equals to $0.90 \text{ m}^3\text{biogas}/\text{kgTVS}$ and $0.55 \text{ m}^3\text{CH}_4/\text{kgTVS}$ respectively, while
420 the average gas production rate was $3.0 \text{ m}^3\text{biogas}/\text{m}^3\text{r.d.}$ Based on the above figures for
421 each tonne of biowaste semi liquid fraction, the biogas and methane production in AD
422 thermophilic conditions equals approximately 166 and 113 m^3 respectively,
423 corresponding to around 350 kWh electrical energy (assuming biogas LHV= $6.9\text{kWh}/\text{m}^3$
424 biogas and 30% energy conversion efficiency). Overall the thermophilic digestion
425 process of biowaste semi-liquid fraction in steady state condition showed increased
426 buffer capacity and higher biogas production potential compared to mesophilic
427 digestion.
428 SGP reached a value as high as $0.94 \text{ m}^3\text{biogas}/\text{kgTVS}$ at an OLR $4.5 \text{ kg TVS}/\text{m}^3\text{r.d}$
429 transient period (RUN III, duration 4 HRTs), reporting an average increased value in
430 biogas production of $0.9 \text{ m}^3\text{biogas}/\text{kgTVS}_{\text{fed}}$.
431 These values indicated the capability of the system of converting most of the organic
432 material into biogas and are of particular significance.
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Figure 5. Specific methane production and OLR variations in thermophilic anaerobic digestion and methane percentage compared to the Gas Production Rate

440 During the steady state condition of the thermophilic reactor (RUN II from day 80 to
441 day 120), with OLR between 3 and 3.5 kgTVS/m³,d, the mass balance around the
442 system was calculated: the influent and effluent (as both digestate and biogas) quantity
443 of volatile solids accounted for 790, 141 and 578 g/d, respectively. The balance was
444 therefore 91%, with a 9% error. Similar results were found for dry matter and COD
445 (errors of 11% and 11.5%, respectively) thus confirming the accuracy of the monitoring
446 analysis data. The COD appeared to be in deficit-error of 11.4%.

447 Also the mass balances for nutrients (nitrogen and phosphorus) closed properly.
448 Nitrogen balance was 89.5% with a deficit of 10.5%. It is interesting to note here that
449 ammonification degree was 60.2% in thermophilic conditions.

450 Phosphorus balance was 104% with a surplus of 4%.

451 In conclusion we can highlight that the mass balances of the thermophilic anaerobic
452 system showed deficits or surpluses in matter transformation all within the margin of
453 error in order to consider the system trial to be admissible from scientific opinion.

454

455 ***3.3 Energetic considerations***

456

457 The mesophilic process allowed for the assessing of an average biogas production of
458 some 148 m³ per ton of pressed biowaste (99 m³ of methane).

459 The thermophilic anaerobic digestion experimentation allowed for the assessing of an
460 average production of some 166 m³ biogas per ton of pressed biowaste (115 m³ of
461 methane), 11% more than mesophilic production.

462 The thermophilic anaerobic production used in a unit for the co-generation of heat and
463 power this will give some 350 kWh of electric energy: the energy recovered from 1 ton
464 of treated biowaste is therefore some 270 kWh.

465 On the other hand, an advanced industrial press machine for the treatment of biowaste is
466 characterized by an installed power in the range 375-400 kW for a treatment capacity of

467 some 12 ton/h. The typical energy consumption is therefore some 33 kWh_{consumed} per
 468 ton of treated biowaste.
 469 The energy returned on energy invested (EROEI) per ton of biowaste treated is
 470 therefore very favorable and around 9 kWh_{produced}/kWh_{consumed}.
 471 Clearly, this figure drastically reduces when considering the energy used for the whole
 472 treatment train (preparation treatments, pumping, composting, treatment of the liquid
 473 fraction of digestate and of the off-gas streams).
 474 It is clear therefore how this kind of pressing pretreatment system permanently changes
 475 the paradigm of pre-treatment plants configuration: in fact two streams, one liquid and
 476 the other solid, of good quality are obtained with a low energy input.

477

478 *3.4 Digestate characteristics*

479

480 Heavy metals concentrations of biowaste and mesophilic and thermophilic digestate
 481 were checked five times during steady state conditions. The average concentrations of
 482 heavy metals in both the input matrix (biowaste) and in mesophilic/thermophilic
 483 digestates, were much lower than the limits of the technical proposals End-of-Waste
 484 criteria (EoW-2014) elaborated by the Joint Research Center of Sevilla, as showed in
 485 table 5. Concentrations referred to dry matter were typically greater in digestate
 486 because of the high conversion capability of the anaerobic process.

487

488 Table 5. Heavy metals concentrations (mesophilic and thermophilic conditions)

	EoW 2014	BIOWASTE	MESOPHILIC DIGESTATE	THERMOPHILIC DIGESTATE
Cu mg/kg d.m.	200	47±5	68.1±3.2	52.5±4.1
Zn mg/kg d.m.	600	112±28	155±13	129±17

Pb mg/kg d.m.	120	1.54±0.8	17.3±2.4	7.81±1.3
Ni mg/kg d.m.	50	43.7±3	42.1±1.6	27±0.5
Cr tot mg/kg d.m.	100	61.5±9	85.9±4.1	51.5±2.3
Cd mg/kg d.m.	1.5	0.4±0.2	0.23±0.14	0.26±0.08
Hg mg/kg d.m.	1	0.055±0.005	0.24±0.09	0.08±0.02
As mg/kg d.m.	10	0.24±0.1	0.25±0.09	0.19±0.03

489

490 Biowaste is known to contain pathogenic bacteria such as Salmonella and other
491 microorganisms that may be a health risk for both people and animals (Sahlström 2003).

492 The content of pathogens of fed substrate and both effluents digestates, in the two
493 experimentations, was analyzed through five replicates. While Salmonella spp was

494 never found, the limit of 1000 CFU/g for E.coli proposed in the End of Waste Criteria
495 technical report (2014) was reached only occasionally (Table 6). This suggests the

496 opportunity to treat digestate in a post-composting process in order to reduce the
497 presence of enteric bacteria (Cekmecelioglu et al., 2005). Several experimental

498 investigations demonstrated that rapid inactivation of Escherichia coli and Salmonella
499 spp. occurs by thermophilic digestion (Smith et al., 2005, Wagner et al., 2008).

500 As for fertilizing properties, AD allowed getting a final product (digestate) with very
501 good fertilizing properties because of the high nutrient content (C, N, P, K) in available
502 forms (Tambone et al., 2010).

503

504 Table 6. Pathogens analysis

SAMPLE	TBC 37°C ISS 004A	TBC 22°C ISS 004A	<i>E.coli</i>	total Coliform	<i>Salmonella spp</i> ISS 011A
Biowaste	$4 \cdot 10^8$ UFC/g	$8 \cdot 10^8$ UFC/g	$7 \cdot 10^5$ UFC/g	$6 \cdot 10^5$ UFC/g	absent
Thermophilic digestate	$1 \cdot 10^7$ UFC/g	$1 \cdot 10^7$ UFC/g	$4 \cdot 10^3$ UFC/g	$1 \cdot 10^3$ UFC/g	absent
Mesophilic digestate	$3 \cdot 10^6$ UFC/g	$4 \cdot 10^6$ UFC/g	$3 \cdot 10^3$ UFC/g	$2 \cdot 10^4$ UFC/g	absent

505

506 4. Conclusions

507

508 The liquid fraction of pressed biowaste was treated through anaerobic digestion. The
509 solid fraction should be used for composting with the dewatered digestate and some
510 bulking material. Adopting this technique the EROEI for ton of biowaste treated is
511 positive and at least around $8.9 \text{ kWh}_{\text{produced}} / \text{kWh}_{\text{consumed}}$.

512 Mesophilic digestion gave an average biogas production of $0.79 \text{ m}^3\text{biogas} / \text{kgTVS}$ with
513 66.0% methane content that has the potential to deliver approximately 300kWh_e per
514 tonne of semi-liquid biowaste, while in the case of thermophilic conditions the average
515 biogas production is $0.90 \text{ m}^3\text{biogas}/\text{kgTVS}$ with 68.8% methane content producing
516 theoretically about 350kWh_e per tonne of semi-liquid biowaste treated.

517 The application of press systems for the separation of segregated biowaste into semi-
518 liquid and semi-solid fraction can be beneficial for further optimizing biowaste
519 treatment in integrated anaerobic- aerobic systems. Steady states conditions in the AD
520 process were maintained even when subjected to transient OLR conditions from 3 to 6
521 $\text{kgTVS}/\text{m}^3\text{,d}$.

522 Heavy metals concentrations and pathogens were below the limits reported by “End-of-
523 Waste” criteria (2014) for future legislative developments, thus indicating the good
524 digestate quality and its possible use for agronomic purposes.

525

526 **5. Acknowledgments**

527

528 This work was carried out with the financial support of the LIFE+ programme (*LIFE10*
529 *ENV/GR/000610*). The hospitality of Treviso City Council and Alto Trevigiano Servizi
530 srl are gratefully acknowledged.

531

532

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684

685 **7. Figure Captions**

686

687 Table 1. Time division of the trial and organic loads applied

688 Table 2. Commodity characteristics of biowaste

689 Table 3. Organic fraction characterization

690 Table 4. Semi-liquid biowaste characterization, average values

691 Table 5. Heavy metals concentrations (mesophilic and thermophilic conditions)

692 Table 6. Pathogens analysis

693

694 Figure 1. Alkalinity and VFAs trend during the mesophilic trial.

695 Figure 2. Specific methane production and OLR variations in mesophilic anaerobic
696 digestion and methane percentage compared to the Gas Production Rate.

697 Figure 3. Process scheme including the material flow characterization.

698 Figure 4. Total solids and volatile solids elimination during all the trial in mesophilic
699 conditions.

700 Figure 5. Trend of the thermophilic ratio volatile fatty acids and alkalinity with OLR
701 variations.

702 Figure 6. Specific methane production and OLR variations in thermophilic anaerobic
703 digestion and methane percentage compared to the Gas Production Rate.

Highlights:

- Truly new and advanced low energy demand pre-treatment process of biowaste
- Anaerobic digestion of highly biodegradable liquid biowaste from kerbside collection
- Transient meso-thermophilic conditions by organic loading rate perturbations tested
- Heavy metals and pathogens of fed substrate and effluent digestates were analyzed
- High biogas yields, good quality of digestates, possible use for agronomic purposes