Diversifying the products from the organic fraction of municipal solid waste (OFMSW) by producing polyhydroxyalkanoates from the liquid fraction and biomethane from the residual solid fraction.

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Abstract

This study describes the diversification of products obtainable from the organic fraction of municipal solid waste (OFMSW) by producing polyhydroxyalkanoates (PHA) from the liquid fraction and biomethane from the residual solid fraction. OFMSW samples were taken during the 2021 season from two full field scale plants treating wastes. After solid/liquid (S/L) separation, 80% of initial organic acids (OAs) were released in the liquid stream. OAs were then used as feed for PHA production and residual solid cakes were tested for biomethane production.

Complete mass balance and energy balance were calculated. PHAs production was of 115±23 (n =6) g kg^{-1} OFMSW (TS) and residual biomethane of 219 ±3 g kg^{-1} OFMSW TS, (n =6).

Energy balance indicated that nearly 40% of OFMSW energy was recovered as products. This value was lower than that obtained previously when AD was performed before OAs separation (i.e. 64%).

Keywords: Anaerobic Digestion (AD); Liquid and Solid Fractions; Organic Fraction of Municipal Solid Waste (OFMSW); Polyhydroxyalkanoates (PHAs); Volatile Fatty Acids (VFAs).

1. Introduction

In the European Union, about 88 million t of food waste are produced each year (Scarlat et al., 2019; Scherhaufer et al., 2018), of which about 6,071,512 t y^{-1} in Italy and 1.2 million t y^{-1} in the Lombardy Region (north Italy) are available as an organic fraction from separate collection (OFMSW) (Centemero, 2016).

The valorization of cheap, ubiquitous, renewable C sources, such as the OFMSW which contains large amounts of organic matter of high biological value, could enable the development of a biorefinery concept through the production of high added-value compounds, beside biomethane (bioCH_4) and/or electricity, produced through the anaerobic digestion process (AD).

Recently, biogas/biomethane production from OFMSW has been coupled with bioplastic-precursor molecules production, i.e. polyhydroxyalkanoates (PHA) (Valentino et al., 2019; Papa et al., 2020) by integrating different technologies and proposing a sustainable and viable closed-loop multistage biogas based biorefinery (Valentino et al., 2019).

Biopolymers such as polyhydroxyalkanoates (PHA) are a class of biodegradable polyesters of microbial origin that are attracting attention as replacements for petroleum-based plastics (Reddy et al., 2003).

PHA applications have been reported in the literature with respect to packaging, coating materials, in the medical and surgical field, as well for improving H_2 and biogas production during digestion of waste sludge treatment (Sethupathy and Sivashanmugam, 2021). Strategies that improve their industrial potential and applications represent a field of intense research (Sethupathy and Sivashanmugam, 2018). Different pathways for the fermentative production of PHAs have been explored to find microbial species which are capable of converting inexpensive renewable substrates, in order to decrease the cost of production that greatly depends on the cost of the C source (Koller, 2018). Low cost renewable sources such as organic acids (OAs) are promising C feedstock candidates for the development of an integrated "cradle to cradle" system biorefinery for PHA production (Valentino et al., 2019; Szacherska et al., 2021).

Many studies have reported the selection of PHA-storing cultures and product accumulation by using mixed microbial cultures (MMC) and OAs as substrates since they are the direct metabolic precursors of PHAs (Perez-Zabaleta et al., 2021; Fradinho et al., 2014; Albuquerque et al., 2011). OAs are largely produced during AD and they represent the biogas/biomethane precursors. Nevertheless, because of process parameters adopted at full scale, above all in treating OFMSW, i.e. low hydraulic retention time (HRT), not all OAs are completely converted to biogas/biomethane and they remain in the digestate, which is either used as a biofertilizer in agriculture or purified before being discharged.

66 In previous research (Papa et al., 2020), the possibility of recovering OAs from digestate
67 to produce PHAs was investigated and total energy balance as well as biogas/biomethane
68 and PHA production, were studied to maximize/optimize the overall process.
69 OAs are also produced during collection and storage of OFMSW before it is delivered to
70 the AD plant because of acidogenic fermentation which occurs under semi-anoxic
71 conditions.
72 With the aim to further exploring and developing strategies to convert a low-cost substrate
73 such as OFMSW into PHAs and with the intention of realizing an integrated biorefinery,
74 this work investigated a new process configuration. Specifically, the process scenario
75 included the recovery of the liquid fraction of OFMSW already rich in OAs to be used to
76 produce PHAs, leaving the depleted residue (solid filter cake) for the successive AD and
77 biogas production.
78 Biogas production proceeds by organic matter hydrolysis and its successive fermentation
79 to organic acids that are then transformed into methane. Therefore, producing PHA from
80 OAs results in competition with methane production, i.e. both processes used the same
81 substrates. Moreover, high organic acid concentrations can result in AD inhibitions:
82 previously it has been reported OFMSW containing 23 g L^{-1} of volatile fatty acids (Papa et
83 al., 2020).
84 Therefore, the idea to exploit OAs produced during waste storage (time between household
85 food trash and collection, and transportation to the AD plant) can be a win approach in
86 reducing competition with the successive biogas production in the AD plant, and in
87 reducing inhibition because of high organic acid concentration.

This biorefinery configuration was compared with a previously reported approach, in which a similar method was followed by using in that case, the residual OAs-rich stream (digestate) to produce PHA after that biogas production was ultimate. Comparative studies were carried out to understand and establish for each approach and scenario the effect on the overall performance in terms of bio-methane, PHA and energy balance, providing for the first time, a comprehensive analysis of material balance by testing different OFMSW sampled over different seasons and from two full-scale anaerobic digestion plants treating OFMSW located in North Italy.

2. Materials and methods

2.1. Materials and sample preparation

The OFMSW coming from door–to-door separate collection was obtained from two different full scale treatment plants located in Lombardy Region (northern Italy), specifically in the provinces of Lodi (L) and Cremona (C) and characterized for a processing capacity of 6–15 t of organic waste per day. The OFMSW, before being anaerobically digested, underwent a mechanical pulping process provided by a bio-pulper, to give a slurry-like final product. Waste sampling was done at intervals during a year: June (FP1), July (FP2), November (FP3), December (FP4), February (FP5) and March (FP6), to assess variability during the year. About 0.2 Mg of pulped waste was taken directly from the feeding pipelines of the AD tank reactors. The part of the feedstock that was used for this work was then stored at -20 °C before the experiments were set up.

111 *2.2. Chemical analyses - Analytical Methods*

112 The OFMSW pulps were analysed for total solid (TS) and volatile solid (VS) content
113 following a standard procedure (APHA, 1998) while pH, total carbon oxygen demand
114 (CODt), soluble COD (CODs), total nitrogen and organic acids detected according to
115 APHA Standard Methods (APHA, 1998). Volatile Fatty acids (VFAs), lactic acid and
116 ethanol content, were analyzed by Shimadzu HPLC using an Aminex HPX-87H as
117 described by (Papa et al., 2020). All analyses were performed in triplicate.

118

119 *2.3. Solid-liquid separation of OFMSW pulp by polyacrylamide cationic flocculant*

120 Solid liquid separation was carried out by using a flocculant added to the pulp slurry. The
121 cationic flocculant was provided as a solid powder by Kemira® (the Netherlands).
122 Flocculant solution was prepared by dissolving the powder in deionized water at a
123 concentration of 1 g L^{-1}. The pulp slurry suspension was mixed and diluted to achieve the
124 final flocculant concentrations of 150 mL L^{-1} and a final solid to solvent ratio of 10% wt.
125 Then, the slurry was filtered through 100 μm nylon mesh to separate solid and liquid
126 fractions. The separation efficiency was calculated as previously reported (Akhiar et al.,
127 2021), and was of 98.6±0.1%. Both the recovered solid biomass and liquid fractions were
128 stored at -20°C for later use. The liquid fraction was analyzed by HPLC for volatile fatty
129 acids (VFAs) and non-volatile organic acids detection as described in the previous section.
130 The solid fraction was characterized in terms of TS and VS prior to an anaerobic test
131 (anaerobic biogas potential - ABP-test).

132

133 *2.4 Polyhydroxyalkanoates (PHA) Production*

134 The organic acids-rich liquid fractions obtained after flocculation treatment from the four
135 OFMSW pulp substrates were used for subsequent enrichment of PHA accumulating
136 microorganisms (mixed microbial culture) (MMC) at the selection stage. For the
137 selection stage the C:N ratio was adjusted between 8 and 10 by using ammonium
138 sulphate and diluted to a final COD of about 40 mMol L^{-1} COD (i.e. 1,500 mg-COD L^{-1})
139 as previously described (Papa et al., 2020).
140 An inoculum composed of activated sludge collected from the secondary sedimentation
141 tank of a wastewater treatment plant, was used to set up both the selection and PHA-
142 enrichment of MMCs. The enrichment process consisted of an aerobic dynamic feeding
143 (ADF) strategy (Colombo et al., 2017), that involved several (about 30) sequential batch
144 bioreactor (SBR) cycles/runs lasting 12 h each (2 cycles per day) performed by carrying
145 out at least 3 sludge retention time cycles (SRT) of 5 days each (i.e. sequence of 30–40
146 feast and famine cycles). Bioreactors of 4 L with a working volume of 2 L were used.
147 The pH was automatically controlled at 8.5 by adding 3 Mol L^{-1} of KOH and the
148 temperature was kept at 25 °C. The bacterial biofilm formed was detached from the glass
149 wall of the reactor by manual means every other day.
150 The dissolved oxygen (DO) concentration in the selection media was measured daily by
151 an optical probe (FDO 925, WTW, Germany).
152 In the following fed-batch PHA accumulation tests, the selected/enriched MMC was
153 evaluated for PHA-storing efficiency during a series of six experimental trials for each of
154 the six substrates (i.e. L-FP1, C-FP2, L-FP3, C-FP4, L-FP5, C-FP6) using an Erlenmeyer
155 flask of 0.64 L working volume and filled with 63% (v/v) of culture broth; continuous
156 aeration and stirring was provided. The PHA accumulation experiments were carried out

157 by feeding a series of six pulses composed each by 200 mMol L^{-1} COD of the VFA-rich
158 substrate and controlled by respiration activity measured by the detection of the DO
159 concentration in the culture broth, which began to increase when carbon was consumed.
160 Each accumulation lasted about 2 hours.
161 At the end of each accumulation, the microbial biomass was kept under ice before
162 centrifugation at 8,000 rpm for 15 min; the supernatant was discarded, and the pellet
163 washed with 0.9% of NaCl solution. Finally, the wet PHA-rich biomass pellet was freeze-
164 dried and stored at room temperature before extraction/purification steps. The dried
165 biomass resulting from 36 runs of accumulation batches (six in each bioreactor) was
166 mixed and made homogeneous using a mortar and pestle.
167
168 *2.5 PHA characterization - PHA Content and Composition Determination*
169 The determination of PHA cell content and PHA composition were performed by GC
170 following the chloroform extraction method as described by Colombo et al. (2020).
171 Approximately 5 mg of dried biomass were suspended in 1 mL of acidified methanol
172 solution (at 20% v/v H_2SO_4) and 1 mL of chloroform with heptadecane (at 0.01% w/v) as
173 internal standard in a screw-capped test tube and vortexed thoroughly. Then, an acid-
174 catalyzed methanolysis of the PHA occurred by incubating the samples at 100 °C for 3.5
175 h. After cooling 1 mL of water was added. Samples were then vortexed to assure that
176 sufficient mixing occurred to allow all PHA to be transferred into the organic chloroform
177 phase. The organic bottom chloroform layer was removed using a pipette and transferred
178 to a GC vial for analysis. The released methyl esters were quantified by a gas
179 chromatograph equipped with mass spectrometry (MS) (7980, Agilent Technologies,

USA) as previously reported (Papa et al., 2020). Purity of the extracted PHA was assessed (>97%) by quantification of the PHA in the mass recovered through solvent extraction and precipitation such as previously reported (Ramsay et al., 1994). The resulting solid PHA, after the solvent recovery, showed a white/light brown color (see supplementary material).

The relative abundance of 3-hydroxy- butyrate (3HB) and 3-hydroxyvalerate (3HV) monomers was determined using a commercial P(3HB-co-3HV) copolymer with a 3HV content of P (HB-HV) (88%/12%) % (Sigma–Aldrich, Germany) as reference standard.

2.6 Anaerobic biogasification potential (ABP and BMP) assay

The anaerobic test (anaerobic biogas potential - ABP- test) was used to evaluate biogas production of the solid residue cake derived from S/L of pulp waste samples. The test was performed in 500 mL serum bottles by adding 3 g of dried biomass sample to 297 mL of inoculum. Inoculum in stable methanogenic activity (CH_4 in biogas 60 %, v/v) was obtained using the digestate of an anaerobic digester treating animal slurry plus crop energy.

Control blanks were prepared using 300 mL of inoculums. All batch reactors were sealed with Teflon hermetic caps, flushed with an N_2 atmosphere, and incubated at 37± 1 °C for 60 days. Assay bottles were analyzed, at regular intervals, for quantitative measurements of biogas production by withdrawing extra-pressure gas by passive flow with a syringe (atmospheric pressure). The sum of each single measurement (mL of biogas produced at atmospheric pressure) during the incubation period, represented the total amount of biogas produced by the samples, i.e. mL of biogas at atmospheric

pressure and 37 °C. The biogas production of the blank control was subtracted from the biogas production of each sample. Qualitative characterization of biogas was performed using a gas chromatograph (lGC; 3000A-lGC, AGILENT-SRA Instruments). All tests were run in triplicate.

2.7 Fourier-transform infrared spectroscopy (FT-IR)

FT-IR measurements were conducted using a Shimadzu IRAffinity-1S equipped with a Miracle Pike ATR device (Shimadzu Italia srl, Milano, Italy). Peak areas were determined using Shimadzu LabSolutions IR software. For each sample, 64 scans were accumulated between 4000 and 700 cm^{-1} at a resolution of 4 cm^{-1}. Commercial P(3HB-co-3HV) copolymer with a 3HV content of P (HB-HV) (88%/12%) % (Sigma–Aldrich, Germany) (Sigma-Aldrich) was used as a reference.

FTIR of PHA obtained in this work, being identical to those of the reference material confirmed the high purity of material produced (see supplementary material).

2.8 Energy density

The energy content of OFMSW samples was measured using an oxygen bomb calorimeter (IKA C6000, Wilmington, NC, USA). The bomb calorimeter was calibrated using a known amount of standard benzoic acid (Sigma-Aldrich, St Louis, MO, USA).

2.9 Mass balance and energy balance

Mass balance and energy balance were performed such as reported by Papa et al. (2020).

225 Referring to the total mass of the original non-separated OFMSW pulp samples, the
226 biogas yields in the solid residue were calculated taken in account the solid recovery (i.e.,
227 dry weight of solid after solid-liquid separation), which was directly weighted after the
228 solid-liquid separation process.
229 Following the approach described in Papa et al. (2020), the energy content of bio-
230 methane and PHA (31.6 MJ m^{-3} and 22 MJ kg^{-1} TS, respectively) were considered for
231 the calculation of the energy balance. The energy required for producing methane was
232 considered equal to 10% of the total energy produced by methane such as previously
233 calculated and reported for the full-scale plant studied (Papa et al., 2020).
234 Energy derived from biomethane and PHA were referred to values of energy content of 1
235 kg of OFMSW determined by bomb calorimeter (MJ kg^{-1} TS) as shown in Table 1.
236
237 *2.10 Statistical analysis*
238 All statistical analyses were carried out using SPSS statistical software (SPSS, Chicago,
239 IL).
240
241 **3. Results and Discussion**
242 *3.1 OFMSW composition and characteristics*
243 The chemical features of the OFMSW pulp feedstocks are presented in Table 1. Data
244 show that over the seasons considered, little or no significant difference was found
245 between the products of the two full-scale treatments plants located within the related
246 provinces of Lodi (L-FP1, L-FP3 and L-FP5) and Cremona (C-FP2, C-FP4 and C-FP6),
247 indicating the quality and the consistency of the sampling process.

248 The TS content ranged between of 231 and 302 g kg^{-1} ww and, on average, VS content of
249 215±56 g kg^{-1} ww (i.e., 810 ± 76 g kg^{-1} TS) was found.
250 The OFMSW contained on average 120±36 g L^{-1} and 108±33 g L^{-1} of total and soluble
251 COD, respectively, which corresponds to COD values in the range: 258-753 g COD kg^{-1}
252 TS. Regardless of the season, these values were found to be slightly higher from Lodi's
253 treatment plant compared to that in Cremona. Slightly different values were also
254 observed for the nitrogen contents (24 ± 2 g N kg^{-1} on average), which were slightly
255 higher in the OFMSW obtained from Lodi's treatment plant (L-FP) compared to those
256 obtained from the one in the province of Cremona (C-FP). Moreover, the characterization
257 of OFMSW and values obtained were overall in line with those reported for OFMSW
258 from previous reports and displayed the low variability mentioned among the sources in
259 different studies (Papa et al., 2020; Canul Bacab et al., 2020; Stylianou et al., 2020;
260 Pecorini et al., 2020; Demichelis et al., 2019).
261
262 *3.2. Evaluation of solid liquid separation, VFAs and lactic acid yields, i.e. OAs*
263 *characterization and mass balance*
264 The composition of the main VFAs and lactic acid in the OFMSW pulp material before
265 and after S/L treatment are summarized in Table 2 and the corresponding mass balance is
266 detailed in Figure 1.
267 OFMSW pulp showed a concentration of OAs of 20.322±4.319 mg L^{-1}: these data are
268 similar to those reported by Papa et al. (2020) working on a similar full scale plant (OAs
269 = 23.534 ± 1.778 mg L^{-1}).

The simultaneous presence in the pulp of VFAs, lactic acid and ethanol, indicated that different metabolic fermentation pathways had occurred during the collection (from house to waste treatment plant) and subsequent preservation of OFMSW in the storage tank. In particular, the presence of acetic acid revealed that fermentation occurred under strictly anaerobic conditions (Wang et al., 2020) while the presence of propionic acid and ethanol indicated facultative anaerobic microorganisms (Wang et al., 2020). Interestingly, lactic acid was the major fermentation product with concentrations in the range of 6.223 and 21.163 mg L^{-1}, indicating a high rate of conversion into lactic acid of the sugar molecules and of the easily hydrolysable poly- or disaccharides derived from partially hydrolyzed OFMSW (Dusselier et al., 2013; López-Gómez et al., 2020), as it was subjected to the pulping pretreatment process which enhanced solubilization of organic matter.

Although there was no clear seasonal trend, a high correlation ($r= 0.99$; $p < 0.02$; $n=3$) between pH and organic acids was found, when considering the average of the plants for the three different seasons, suggesting the possible link between organic acids concentration and acidity and confirming the occurrence of hydrolysis and acidogenic fermentation process in the OFMSW substrate (Cheah et al., 2019; Jiang et al., 2013). The total dry weight measured in the liquid fraction after the treatment was in the range of 3.5-3.9 g L^{-1} for all cases, whereas in the residual solid cake the moisture content was around 650±70g kg^{-1} ww.

The solid fraction recovered obtained after S/L separation did not vary significantly among samples and ranged from 582 g kg^{-1} to 770 g kg^{-1} (dry matter basis) of starting pulp material. Moreover, these values were positively correlated, ($r=0.91$, $p=0.03$; $n=6$) to

293 the total and soluble COD in the original pulp, confirming that most of the organic solids
294 were preserved in the cake residue.
295 Results showed no consistent changes between the OFMSW pulp materials sampled from
296 the two different treatment plants over different seasons, and similar trends were found in
297 the VFAs and lactic acid level as a function of flocculation treatment.
298 The mass balance reported in Figure 1 allowed us to reach some conclusions: i. 80% of
299 the starting organic acids (as averaged across all samples) were released in the liquid
300 stream, while in the solid fraction there remained only about 13±3 % of the initial organic
301 acids; ii. high organic acids recovery (relative to the original biomass) was obtained in
302 the liquid fraction after solid-liquid separation, however, as stated earlier, no clear trend
303 across seasons was found.
304
305 *3.2 Biogas production of the solid fraction obtained from solid-liquid separation.*
306 This work aimed principally at recovering the liquid fraction of OFMSW rich in organic
307 acids to be used to produce PHA, leaving the solid fraction for subsequent anaerobic
308 digestion to provide for biogas production. Therefore, solid fractions after S/L separation,
309 from now on called FP-AF, were tested for their potential biogas production in
310 comparison with the original non-separated OFMSW pulp samples, as previously
311 described in the M&M, and the results are presented in Table 3 and Figure 1 (see
312 supplementary material).
313 Specific biogas produced during trials for the original OFMSW pulp material was, on
314 average (Table 3), of 491±78 NL kg^{-1} VS (i.e. 395±59 NL kg^{-1} TS) which was similar to
315 the values obtained by Papa et al. (2020) and in agreement with ranges typically reported

316 in the literature for OFMSW, i.e. 300-600 NL kg^{-1} VS (Campuzano and González-
317 Martínez, 2016).
318 By comparing the biogas of OFMSW pulp (referred to TS content) (Table 3) from
319 different seasons and plants, unlike results from previous studies (Pecorini et al., 2020),
320 we found no trend and no higher methane production of OFMSW pulp materials sampled
321 in the seasons with lower temperatures compared to spring/summer.
322 As expected, the S/L separation led to a decrease of biomethane production from the
323 solid residue cake which was about 10% (as average data referring to kg of TS) lower
324 than that of non-separated pulp material (Table 3), i.e. it was about 60 % of the potential
325 biogas producible by 1 kg of non-separated OFMSW pulp, most likely due to the
326 migration of the easily degradable molecules (e.g. OAs) to the liquid fractions.
327 Furthermore, the results showed that the average concentration of methane content (%
328 v/v) in the biogas did not vary significantly throughout the different samples, with values
329 in the range of 65% to 67% (v/v), data similar to those previously reported by Papa et al.
330 (2020).
331
332 *3.3. PHA production: biomass selection and PHA accumulation*
333 The six liquids streams obtained after the S/L separation were employed as substrates to
334 select PHA storing bacteria, since these streams were rich in VFAs and lactic acid.
335 During the selection, the DO concentration was monitored and evolution of the feast
336 phase length of SBR cycles assessed.
337 The PHA selection and accumulation experiments were carried out by using substrates
338 rich in VFAs and lactic acid i.e., 0.8±0.1 g L^{-1} and of 4±0.6 g L^{-1} of total VFA and lactic

339 acid concentration, (40 mMol L^{-1} COD and 200 mMol L^{-1} COD) for the PHA selection
340 and PHA accumulation, respectively (Colombo et al., 2019)
341 In terms of PHA biomass recovery during PHA accumulation, an average of 3.65±0.4 g
342 L^{-1} was obtained with a maximum biomass yield of 5.2 g L^{-1} observed for C-FP4.
343 Table 4 summarizes the results of PHA concentration and content at the end of the
344 accumulation test, referred to total biomass pellets obtained and from the mass balance
345 assessment to OFMSW fresh and dry matter (g PHA kg^{-1} OFMSW).
346 PHA content in cells was referred to VS on a mass basis. The results showed that the
347 PHA content produced during the accumulation phase was between 15 to 32% of the
348 biomass pellet (dry basis) (i.e. 149 to 323 g PHA kg^{-1} TS pellet). In particular, the ADF
349 experiments led to an accumulation of PHA reaching levels close to 0.3 g PHA g VS,
350 which is the average quantity found in the literature when using carbon sources derived
351 from OFMSW (Colombo et al., 2019; Korkakaki et al., 2016; Sánchez Valencia et al.,
352 2021). The PHA storage data were in line with results reported in literature where the
353 overall yield of 110 g PHA kg^{-1} VS was found (Moretto et al., 2020b), as well in
354 agreement with values found previously by Papa et al., (2020) who working on a
355 digestate stream from food waste reported a PHA content ranging from 152 to 258 g
356 PHA kg^{-1} VS.
357 However, our results are intriguing because they differed from the previous ones as
358 regards the polymer composition. The 3HB represented the majority of the polymer
359 composition, whereas Papa et al., (2020) observed a higher 3HV fraction and different
360 distribution in terms of ΔHB/ ΔHV, most likely due to the different VFA profile
361 characterized by the higher presence of propionic acid compared to the VFA composition

362 obtained here. Moreover, there were no within-sample seasonal effects in terms of yields,
363 although the slight differences among samples could be derived from the variations made
364 at the dilution step to 200 mMol L^{-1} COD, as was also confirmed by the linear trend found
365 between PHA content and organic acids at these diluted values (data not shown).
366 The PHA concentration in the fermentation broth at the end of the accumulation stage
367 was found to range between 0.44 and 1 g L^{-1}, which was in line with the literature for
368 these bacterial feeding regimes (Papa et al., 2020; Lorini et al., 2020; Moretto et al.,
369 2020a).
370 The overall yield was calculated for the 36 runs, in terms of storage yield and maximum
371 PHA content achieved, and data were averaged.
372 In term of PHA produced, referring to 1 kg of $OFMSW_{TS}$ calculations were done taking
373 into consideration that from 1 kg of OFMSW pulp treated, it was possible to obtain about
374 60 L of 200 mMol L^{-1} COD feeding substrate, which represented 37.5% (i.e. 62.5 % is
375 the culture broth) of the entire volume during the accumulation. Since about 300 L of
376 broth are required to produce 1 kg of pellet (i.e. about 3.5 g L^{-1} TS) (see supplementary
377 material) the biomass pellets recovered ranged between 312-725 per 1 kg of initial
378 OFMSW pulp treated (dry basis).
379 In summary, the results indicated the total amounts of PHA, referring to total solids of
380 OFMSW ($OFMSW_{TS}$) comprised between 89 and 155 g PHA kg^{-1} $OFMSW_{TS}$ and 32±7 g
381 kg^{-1} $OFMSW_{FM}$ (on average), which means that approximately 6-11 kg $OFMSW_{TS}$ or 29-
382 41 kg $OFMSW_{FM}$ were required to produce 1 kg of PHAs (from Table 4). These values
383 were in line, although slightly lower in PHA yields, that those of the previous work,
384 which ranged between 117 and 199 g PHA kg^{-1} $OFMSW_{TS}$ (Papa et al., 2020).

385 *3.4 Energy balance*

386 Energy balance calculations were made taking into consideration flow mass data of the
387 full-scale AD plant of Lodi, which was also considered as the demo plant in previous
388 work (Papa et al., 2020).

389 This AD plant utilizes 25,000 t year^{-1} of pulp (i.e. average daily feeding of 68.4 t d^{-1}) with
390 hydraulic residence time (HRT) of 40 days (Papa et al., 2020). In order to evaluate the
391 energy output of the proposed biorefinery, the overall net energy balances were
392 calculated as previously proposed (Papa et al., 2020), i.e. energy content of products
393 (PHA and biomethane) and energy necessary for producing these products, which
394 allowed the calculation of net energy contents to be compared with the starting energy of
395 OFMSW pulp. Calculations were made for each of the samples collected (FP1-FP6) and
396 AD plants (L and C) (see supplemental material) and then data were reported as averages
397 for the entire season, because there were no differences significant between the two
398 plants studied. The energy density (ED) (in MJ kg^{-1}) for pulp biomass was, on average of
399 data reported in Table 1, of 18±0.6 MJ kg^{-1} , very close to data previously reported for
400 these organic biomass materials (Papa et al., 2020; Yang et al., 2018).

401 Results for energy balance are shown in Figure 2a, which reports the total net energy
402 recovered from the OFMSW pulp as the sum of biomethane and PHA produced from
403 OFMSW, to be compared with the same data previously calculated for a different
404 configuration, i.e. biomethane production and organic acids recovery from digestate,
405 previously studied and published in this journal (Papa et al., 2020). More interestingly,
406 and easy to follow, are data reported in Figure 2b, in which energy balances were
407 reported assuming the initial total energy content of OFMSW pulp samples as 100%. So,

408 the net energy recovered from OFMSW pulp in this work represented nearly 40% of
409 OFMSW total energy content, which was much lower than the 64% obtained previously
410 with the different configuration (Papa et al., 2020), indicating a lower efficiency.
411 In particular, 23± 1% was the energy recovered as CH_4, that was much lower than the 43
412 ± 3% obtained previously (Papa et al., 2020) (Figure 2b). On the other hand, no
413 differences were observed in terms of total energy recovered as PHA, i.e. 16.9± 2.7% and
414 17.9± 5% of OFMSW energy content for this work and for that of Papa et al., (2020)
415 respectively.
416 Therefore, it can be concluded that the AD configuration studied in this work was less
417 efficient because of the lower biomethane production which resulted from the migration
418 of the easily fermentable molecules to the liquid fractions after S/L separation, that was
419 about 33% of total COD.
420

421 **4. Conclusions**

422 The biorefinery approach in using OFMSW to produce biogas represents an interesting
423 approach to improving the number of products and total energy recovered. In this work
424 an AD configuration, i.e. solid/liquid separation followed by PHAs production from
425 liquid fraction and biomethane production from residual solid fraction resulted in a total
426 energy recovery of 40% that was lower than that reported (64%) for a different
427 configuration, i.e. biomethane production and subsequent isolation of a liquid fraction
428 containing OAs to produce PHAs. Independently of the configuration chosen, the
429 biorefinery approach confirmed the large increase in total energy recovered, making more
430 sustainable the OFMSW-AD.

E-supplementary data for this work can be found in e-version of this paper online

CREdiT authorship contribution statement

Gabriella Papa: Methodology, Investigation, Writing - original draft.; **Tommy Pepè Sciarria**: Methodology, Investigation; **Barbara Scaglia**: Investigation; **Fabrizio Adani**: Conceptualization, Methodology, Investigation, Writing - original draft, Funding acquisition.

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