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Co-digestion of by-products and agricultural residues: A bioeconomy perspective for a Mediterranean feedstock mixture

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1 Co-digestion of by-products and agricultural residues: a  
2 bioeconomy perspective for a Mediterranean feedstock mixture

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11 Co-digestion of by-products and agricultural residues: a  
12 bioeconomy perspective for a Mediterranean feedstock mixture

13  
14 **Abstract**

15 This study focused on applying batch and continuous co-digestion approaches to investigate the  
16 effects of a feedstock mixture (FM) constituted by ten Mediterranean feedstocks highly  
17 available in the Mediterranean area (i.e., olive pomace, olive mill wastewater, citrus pulp,  
18 poultry litter, poultry and cattle manure, whey and cereal straw) on methane production for  
19 bioenergy generation. For the same feedstock mixture (FM), two different anaerobic digestion  
20 (AD) tests were carried out to evaluate the possible inhibitory effects of some biomasses on the  
21 biological process.

22  
23 The first AD test showed a methane yield equal to 229 Nm<sup>3</sup>CH<sub>4</sub>/tVS (27% lower than that  
24 measured during the batch test). During the second AD test, the specific production was 272  
25 m<sup>3</sup>CH<sub>4</sub>/tVS. Both tests showed a similar methane content of methane in the biogas, equal to  
26 about 57%.

27  
28 The first AD test showed an inhibition effect of the process: total conversion of the organic  
29 matter into biogas was not ended. The second batch test demonstrated that the selected FM  
30 could be viable to carry out the co-digestion and could provide a flexible solution to generate  
31 advanced biofuels in biogas plants located in the Mediterranean area.

32  
33 **Keywords**

34 Biomethane; Anaerobic Digestion; Bioeconomy; Biomasses; Advanced biofuels.  
35  
36

37  
38 **1. Introduction**

39 Among the greatest challenges human beings face in the 21st century, environmental pollution and  
40 energy instability are the most crucial. In detail, air pollution and global warming are the major  
41 concerns for the natural environment, which could be attributed to the large amount of greenhouse  
42 gases (GHG) from the continuous increasing combustion of fossil fuels (Abdshahian et al., 2010;  
43 Bansal et al., 2013; Hosseini et al., 2013). In this regard, it is well known that GHG, with 60% of

44 CO<sub>2</sub> emissions and about 15% of CH<sub>4</sub> emissions, are the most responsible in global warming  
45 (Williams et al., 2012; Hosseini and Wahid, 2014; Rahimnejad et al., 2015).

46  
47 According to the Kyoto protocol the key factor to both reduce CO<sub>2</sub> and other GHG emissions and  
48 improve the living standard of developing countries is to produce cost effective energy as well as  
49 use bioenergy efficiency (Changua et al., 1999; Garnier, 2014; Ebner et al., 2015). In this context,  
50 renewable bioenergy is a promising alternative to achieve the world energy requirements by  
51 avoiding extra economic burden and any significant environmental impacts (Morero et al., 2015;  
52 Raimondo et al., 2018; Valenti et al., 2018a). Renewable energy supply chains were documented by  
53 Ingrao et al. 2018a as important for transitioning to equitable, sustainable, post fossil-carbon  
54 societies. In this context, biomass has become one of the most interesting input for sustainable  
55 processes.

56  
57 The opportunity to disaggregate and re-aggregate the chemical components of biomass improves  
58 the value of resources usually considered only a cost, such as waste. The sectors which were born  
59 around this opportunity (e.g., bio-materials, specialized micro-organisms, fibers, new foods.) have  
60 shown important growth capacities and have developed new processes and products (Birner, 2018).  
61 The new knowledge gives multifunctional characteristics to agricultural byproducts. Therefore, a  
62 reformulation of the boundaries between sectors is required: a sustainable bioeconomy must  
63 prioritize the production of high-quality foods, but also the transformation of waste material into  
64 energy, as the last step in a series of use and reuse cycles (Ingrao et al., 2018b). As defined by Food  
65 and Agriculture Organization of the United Nations (FAO) the principal aim of the bioeconomy is  
66 not only to replace fossil raw materials, but also to develop completely new products and processes  
67 (FAO, 2018).

68  
69 In reports from a few years ago, a systemic approach to the circular economy and to the  
70 bioeconomy was applied, but recently these concepts were better defined considering also the  
71 aspects of bio-based products and the sustainable use of renewable resources (EFA, 2018).  
72 Moreover, both concepts were often used as substitutes but are very different.

73  
74 The concept of circular economy is based on rethinking industrial processes (Frosch and  
75 Gallopoulos, 1989) and draws from the ideas of industrial ecology and industrial metabolism  
76 formulated between 1970's and 1980's. The general framework of circular economy contemplates  
77 that, in opposition to linear economy, economic actors would exert no net effects on the  
78 environment (D'Amato et al., 2017). This involves a system to obtain net reductions at the

79 organizational supply chain and industrial levels (The Ellen MacArthur Foundation, 2012; Murray  
80 et al., 2015).

81  
82 Bioeconomy was defined by Georgescu-Roegenas in 1975, as a biophysical perspective to the  
83 economy. This concept is based on the idea that industrial inputs (e.g., material, chemicals, energy)  
84 should be derived from renewable biological resources, with research and innovation enabling the  
85 transformational process (Kleinschmit et al., 2014; Pfau et al., 2014; Bugge et al., 2016).

86  
87 More generally, the choice of the adoption of these two concepts as economic and environmental  
88 strategy is motivated by the fact that they all propose to adapt to or transform the current economy  
89 towards a more sustainable one. In this context forestry and the agriculture and forest industry can  
90 play a fundamental role in providing bio-based substitutes for non-renewables ones in different  
91 fields (Ollikainen, 2014; Roos and Stendahl, 2015).

92  
93 In energy field, several type of biomass can be reused as feedstock to produce biogas via the  
94 anaerobic digestion (AD) process, which is a biological treatment without oxygen to produce  
95 biogas, a mixture formed mainly of methane and carbon dioxide. AD is a well-established  
96 technology to treat organic-matter rich biomass and is increasingly gaining ground as one valid  
97 route to produce renewable energy in a sustainable manner (Ciriminna et al., 2019). So, it  
98 contributes to create the conditions for a closed circular economy to reduce environmental and  
99 economic costs resulting from food waste disposal agri-food supply chains (Borrello et al., 2016).

100  
101 Biogas is an environmentally derived energy source which attracts increasing attention (Esposito et  
102 al., 2012) due to its capabilities of both waste treatment and energy recover (Gebrezgabher et al.,  
103 2010). A relevant number of research studies was carried out on all aspects of biogas production,  
104 processing and utilization.

105  
106 AD has been applied worldwide as a biological process to reuse by-products and waste materials,  
107 by transforming them into energy sources through the treatment of various organic waste such as  
108 municipal solid waste, food waste, industrial waste, sewage sludge, animal manure and agricultural  
109 residues (Comparetti et al., 2015; Çelik and Demirer, 2015; Huang et al., 2015; Shen et al., 2015;  
110 Yong et al., 2015). In detail, AD of organic waste could help to reduce odour release, a decrement  
111 of pathogens and a low requirement for organic sludge. Furthermore, the digestate produced at the  
112 end of the process can be adopted as an organic fertilizer for arable land instead of mineral and  
113 organic fertilizer as well as an organic substrate for greenhouse cultivation (De Vries et al., 2012;  
114 Ounnar et al., 2012; Nasir et al., 2013; Hidalgo and Martín-Marroquín, 2015; Selvaggi et al., 2018a;

115 Katinas et al., 2019). In fact, in the context of a circular economy, the digestate can contribute to  
116 improve agronomical value of soils (Pappalardo et al., 2018a) and to reduce fertilizer costs (Amon  
117 et al., 2007; Pappalardo et al., 2018b).

118  
119 However, the AD process from different organic biomasses is a relatively sensitive process which  
120 mainly depends on the compounds of substrates that can be converted into biogas: chemical  
121 composition and biodegradability of the biomasses are the key factors for the biogas and  
122 biomethane productions (Salminen and Rintala, 2002). Many studies have been performed on the  
123 AD process using mono-substrates (Schittenhelm, 2008; Khalid et al., 2011; Babaei et al., 2013).

124  
125 In the last years there were many researches aiming to deepen AD knowledge and to broaden its  
126 application (Mata-Alvarez et al., 2000; Cavinato et al., 2010; Kacprzak et al., 2010; Astals et al.,  
127 2013; Sahito et al., 2014; Hubenov et al., 2015; Ohemeng-Ntiamoah and Datta, 2019). In most of  
128 them, co-digestion of agricultural waste and manure was investigated. In detail, Cavinato et al.  
129 (2010) carried out co-digestion of cattle manure, agro-wastes and energy crops; Kacprzak et al.  
130 (2010) analysed co-digestion of agricultural and waste; Hubenov et al. (2015) investigated a co-  
131 digestion of waste fruit and vegetables and swine manure; Sahito et al. (2014) carried out canola  
132 straw and buffalo dung co-digestion. In contrast, Schittenhelm (2008) analysed only maize  
133 digestion and Astals et al. (2013) carried out a pig manure co-digestion. As result the co-digestion  
134 process was adopted to overcome the difficulty of mixing agro-industrial by-products and livestock  
135 manure (Chen et al., 2013; Valenti et al., 2018b).

136  
137 Anaerobic co-digestion (AcoD) can establish good synergisms in the digestion reactor and it is  
138 economically feasible. Therefore, in the last decades, AcoD has been widely used to enhance the  
139 biogas production and several publications have dramatically increased becoming AcoD as the most  
140 relevant topic within research focused on AD process.

141  
142 Several researches studied the AcoD of livestock manure with different biomasses (i.e., municipal,  
143 industrial and agricultural by-products) to enhance biogas production (Callaghan et al., 2002;  
144 Giuliano et al., 2013; Sahito et al., 2014). In particular, Callaghan et al. (2002) focused on  
145 optimization of a co-digestion process by using three feedstocks of cattle and chicken manure, and  
146 fruit/vegetable wastes. Muradin and Foltynowicz (2014) carried out an economic analysis of a  
147 biogas plant which treated nine feedstocks (i.e., corn silage, potato pulp, spent vinassa waste, fruit  
148 and vegetable pomace, cereals, plant tissue waste, municipal sludge and soya oil). Wickham et al.  
149 (2016) analysed different mixing ratio of sewage sludge and organic waste co-digestion to evaluate  
150 their biomethane potential. Tasnim et al. (2017) showed a better gas production from the mixed co-

151 digestion of cow manure, sewage sludge and water hyacinth than the co-digestion of cow manure  
152 and kitchen wastes. Valenti et al. (2018c) investigated the effect of mixing six agro-industrial  
153 feedstocks (i.e., citrus pulp, olive pomace, whey, corn silage, cattle and poultry manure) on  
154 biomethane production, considering different analytical approaches. Valenti et al. (2018b)  
155 investigated six different feedstock-mixtures containing five Mediterranean biomasses such as  
156 poultry manure, Italian sainfoin silage (*Hedysarum Coronarium* L.) and opuntia fresh cladodes and,  
157 among the main available biomasses, citrus pulp and olive pomace by demonstrating a good biogas  
158 production from different organic matrices.

159  
160 As stated by all these authors, the main advantage of AcoD process is the improvement of biogas  
161 production and its methane content. Moreover, AcoD could help to improve the stabilization of the  
162 process, the dilution of inhibitory substances, the nutrient balance and the reduction of GHG  
163 emissions. Furthermore, AcoD could also contribute to achieve synergetic effect of  
164 microorganisms, and increase the load of biodegradable organic matter (Mata-Alvarez et al., 2000;  
165 Jagadabhi et al., 2008; Holm-Nielsen et al., 2009; Mata-Alvarez et al., 2011; Astals et al., 2014;  
166 Mata-Alvarez et al., 2014; Shah et al., 2015).

167  
168 Actually, the transport cost of the feedstocks to the biogas plants during the supply phase is the first  
169 selection criteria for considering new biomasses or selecting new location for biogas plants (Valenti  
170 at al., 2018d). Despite this fact, it is still important to select the best biomasses (i.e., by-products  
171 and agricultural waste) and feedstock-mixture with the aim of favouring synergisms and optimizing  
172 methane production. Yet, in-depth analyses of parameters, which could affect AcoD, and  
173 adjustment of operating parameters and optimisation strategies are still necessary. It is crucial to  
174 know the potential biogas production of a feeding organic mixture to achieve the correct approach  
175 in operating AD processes. By considering the availability of several agricultural residues and by-  
176 products and the absence of a correlation between BMP and single substrate properties (Rodrigues  
177 et al., 2019), increasingly digesters aim to adopt mix of different feedstocks to improve their  
178 digestion process performance. Lab-scale tests are required to determine the feasibility of such  
179 operations. So, in Sicily, the largest island in the Mediterranean basin highly characterised by  
180 agricultural activities, the development of the AD sector could be achieved by reusing and  
181 valorising the large amount of by-products available (Selvaggi et al., 2017 and 2018b). Therefore,  
182 the research of possible anaerobic digestion of multiple feedstocks is urgently needed to reduce  
183 disposal costs for companies that produce wastes and by-products and to increase their incomes.

184 In this context, the study reported in this paper focused on applying batch and continuous co-  
185 digestion approaches to investigate the effects of a feedstock mixture (FM) constituted by ten  
186 Mediterranean feedstocks highly available in the Mediterranean area (i.e., olive pomace, olive mill  
187 wastewater, citrus pulp, poultry litter, poultry and cattle manure, whey and cereal straw) on methane  
188 production for bioenergy generation. The novelty of this research is that the chemical tests were  
189 performed to put in evidence a new mix of biomasses: new limits for the contents of citrus pulp  
190 (most available by-product) were tested.

191

192

## 193 2. Materials and Methods

### 194 2.1 Feedstocks characteristics

195

196 Among the agricultural residues and by-product produced in the Mediterranean area, ten biomasses  
197 were selected as suitable feedstocks for the co-digestion process, based on their potential  
198 availability: citrus pulp, olive mill wastewater, Triticale silage, poultry litter, poultry and cattle  
199 manure, whey, tomato peels and cereal straw.

200 By considering the potential availability of the selected agricultural residues and by-products, a  
201 feedstock-mixtures (FM) of the selected ten biomasses was prepared based on the typical  
202 Mediterranean feedstock-mixture already used in digesters located in Sicily (Table 1). Citrus pulp  
203 was selected as main feedstock since it is highly available in the study areas where there is a  
204 relevant production of citrus fruits (Valenti et al., 2016; 2017a; 2017b; 2017c; Chinnici et al., 2018).

205 The biomasses used to carry out the co-digestion process have been partially provided from the  
206 Department of Agriculture, Food and Environment of the University of Catania (Sicily, Italy). In  
207 detail, citrus pulp and tomato peels were collected and shipped in coolers to the CRPA Lab  
208 (Research Center for Animal Production). The other feedstocks considered for the mixture, i.e.  
209 olive pomace (three phase), olive mill wastewater, poultry litter, poultry and cattle manure, whey,  
210 and cereal straw were collected by CRPA from farms located in Emilia-Romagna region (Italy).

211 Each individual sample was firstly chopped to reduce particle size by using a blender, and then  
212 were kept frozen prior to use. Every feedstock was chemically characterized according to the  
213 parameters of total solids (TS) and volatile solids (VS). The characteristics of individual feedstocks  
214 and of the selected feedstock-mixture are listed in Table 1.

215

216

217 **Table 1.** Characteristics of individual feedstocks and mass ratios of different feedstocks in FM.



## 2.2 Equipment and protocols of biomethane potential and semi-continuous anaerobic digestion of feedstock-mixture (FM)

### 2.2.1 Biomethane potential test

The Biomethane Potential (BMP) test, which allows the evaluation of the maximum content of methane and/or biogas that can be produced from biomasses, was modified based on methods reported in the UNI EN ISO 11734/2004 framework, described by Valenti et al. (2018b). Before starting the semi-continuous anaerobic digestion test the BMP static test was performed by simulating what usually could happen in a real-scale anaerobic plant.

The digester, a glass bottle with a total volume of about 2200 ml, was filled with the FM to about 70% and then placed in a thermostat cabinet (temperature of about 38 °C) for the entire digestion process. The BMP test was carried out also for citrus pulp, since it represents the main feedstock within the analysed FM. The content of produced biogas was monitored by adopting the mass method (Valenti et al., 2018b). In detail, during the gas analysis, the volume of the produced biogas was calculated, and the quality of the biogas was analysed.

During the test the biogas was analysed continuously, and the total amount of gas produced was reported in a cumulative production curve in order to provide also information about the degradation rate (Soldano et al., 2014]. Biogas quality, in terms of carbon dioxide (CO<sub>2</sub>) and methane (CH<sub>4</sub>) content, was determined using an infrared gas analyser (Geotech Instrument, Leamington Spa, UK).

### 2.2.2 Anaerobic digestion test

As well known, AD is a multistage process of biological reactions in series and in parallel, in absence of oxygen. The process can be traced back to 4 main phases: hydrolysis, acidogenesis, acetogenesis, methanogenesis. Methanogenic bacteria are only operational in the last phase. The families of bacteria mineralize the organic substance mainly in methane (CH<sub>4</sub>), dioxide carbon (CO<sub>2</sub>), ammonia (NH<sub>3</sub>), hydrogen sulphide (H<sub>2</sub>S) and water (H<sub>2</sub>O). The process involves several families of bacteria: fermentative bacteria, acetogenic bacteria that produce H<sub>2</sub>, Acetogenic bacteria using H<sub>2</sub>, Archea oxygen-reducing metanigenes, acetyl methanogens (using acetic acid). In addition to the transformations described above, other reactions may also trigger accumulation of high molecular weight fatty acids, alcohols, propionic acid and butyric acid. However, these reactions occur above all in case of management problems of the biological process. In normal mesophilic

253 conditions, acetic acid is the main precursor of methane (about 70% of methane is produced starting  
254 from acetic acid).

255 The activity was carried out at CRPA Lab by using anaerobic digestion reactors with continuous  
256 feeding to simulate the real-scale condition and monitor the biological process (Soldano et al.,  
257 2014). The experimental system developed by CRPA Lab consists of nine continuous-feed steel  
258 mini digesters, with a volume of 23L, mixed and heated (in mesophilic or thermophilic conditions).  
259 The system allows both the continuous recording of the amount of biogas produced (manometric  
260 system) and the periodic monitoring of the biogas quality (percentage of methane and carbon  
261 dioxide). The methodology involves the loading of feedstock-mixtures (FM) and the discharge of  
262 digestate (daily or even more frequently). Each reactor has an independent line and there are no  
263 common parts between them.

264 Each reactor was supervised continuously by means of a manometer in order to measure pressure  
265 augmentation generated in the headspace, due to the collection of gas produced and then released by  
266 venting. The conversion of the overpressure to biogas volume was calculated at standard pressure  
267 (1013.25 mbar) and standard temperature (0°C). The feeding of each digester involves the loading  
268 of the mixture and the daily discharge of the digestate, which was then analysed for the whole  
269 process control.

270 For the FM, two different AD tests were carried out in two different phases.

271 Each reactor was provided with a 'syringe' to extract digestate and a transducer to measure the  
272 pressure generated in the digester head space during the process. The digestate was weekly  
273 monitored and was chemically characterized according to the parameters of TS and VS for  
274 evaluating the organic matter degradation rate.

275  
276 The first test started by filling the reactor with an inoculum taken from a digester that used citrus  
277 pulp and olive pomace in order to make the microbial flora suitable for degrading as far as possible  
278 the organic substance used during the test and to reduce the start-up phase.

280 The adopted inoculum was chemically characterized according to the parameters of TS, VS, acidity  
281 (FOS) and alkalinity (TAC) (Table 2).

282 The digesters were set at 38°C (mesophilic conditions); the hydraulic retention time (HRT) was 50  
283 days and the organic loading rate (OLR) was 4.8kg of VS per day per m<sup>3</sup> of reactor. The test was  
284 performed for about 4 months including the start-up and the steady state phase. Stainless steel  
285 digester (CSTR, Completely Stirred Tank Reactor), 23L each (16L working volume), was fed daily.

286 The second test started by using a different inoculum, taken from a digester that used  
287 similar biomasses. The digesters were set at the same temperature of the first trial (38°C -  
288 mesophilic conditions); the HRT was 54 days and the OLR was 3.3kg of VS per day per m<sup>3</sup> of  
289 reactor. The test lasted about 54 days. Stainless steel digester (CSTR), 23L each (16L working  
290 volume), was fed daily. Biogas production was daily analysed and the obtained digestate was  
291 collected weekly and chemically characterised according to the parameters of TS, VS, FOS  
292 and TAC (Table 2) for evaluating the organic matter degradation rate.

293  
294  
**Table 2.** Characteristics of adopted inoculum.  
295  
296  
297

### 298 2.3 Analytical methods

299 Different parameters were measured for each feedstock and then for the considered FM before and  
300 during continuous test. The FM has been chemically characterized for the content of TS and  
301 VS. Total solids and ash contents were determined drying and incinerating the samples at 105 °C  
302 and 550°C, respectively, according to the standard methods (APHA-AWWA-WPCF, 2005).  
303 Following the Nordmann titration method, pH and FOS/TAC ratio of the digestate were  
304 performed using a Hach titrator, by adopting the TIM 840 titrator by HACH-LANGE.

305  
306 The calculation of the methane yield, as biochemical methane potential (BMP), was carried out  
307 in accordance with the standard ISO 11734. FOS/TAC ratio computation consists of weighting  
308 about 5.0 grams of fresh sample, added in a plastic container suitable for titration 50 mL of distilled  
309 water. The first titration is carried out with H<sub>2</sub>SO<sub>4</sub> 0.1 N titrator until reaching pH value of 5.0 to  
310 complete bicarbonate titration, then to reach pH value of 4.4 by titrating the alkalinity.

311  
312 Volatile fatty acids (VFAs) are important elements in controlling the anaerobic digestion  
313 process. They are important to decompose organics and to generate gasses, methane and carbon  
314 dioxide. Oxygen demand decreases when both decomposing and generating occur  
315 continuously and completely. The BMP test was performed by following the method set by  
316 CRPA Lab and the obtained results were expressed in normal cubic meters of methane per ton  
317 of VS (Nm<sup>3</sup>CH<sub>4</sub>/tVS). The VS reduction, in terms of degradability of the organic matter, was  
318 calculated considering the ratio between the amount of the produced biogas and the amount of  
319 VS loaded. By using a gas chromatographic (GC) method, VFAs were measured during the semi-  
320 continuous process. 10 mL of the collected AD effluent was centrifuged at 7025 times gravity (xg)  
321 for 15 min using a centrifuge  
322

323 to obtain the supernatant. Then, the supernatant was washed using 85% (w/w) orthophosphoric acid  
324 at a ratio of 1–5 (acid to sample) to remove remaining solids and prepare the sample for the GC  
325 analysis. A GC system (GC-Agilent 7820A), which was equipped with a capillary column (Colonna  
326 Agilent J&W DB) and a flame ionization detector (FID). The total VFA is the sum of six targeted  
327 VFAs (acetic acid, butyric acid, hexanoic acid, heptanoic acid, valeric acid, and propionic acid).

328  
329  
330

### 331 3. Results and discussion

#### 332 3.1 Feedstock-mixture characteristics

333  
334

334 The main characteristics of each analysed biomass were reported in Table 1. In detail, the highest  
335 TS content approximately equal to 94% was shown for cereal straw samples, and the analysed  
336 sample of tomato peels, reported the highest values of VS content, about 96% of TS. Regarding the  
337 lowest analysed TS and VS contents, they were recorded for whey and poultry manure samples,  
338 respectively for TS and VS contents. A dry substance of approximately 3% was found for whey  
339 samples, and the ash content of about 67% of TS was registered for poultry manure samples,  
340 coming from laying chicken farm. From the analyses of TS and VS contents related to the other  
341 selected feedstocks, the results showed a TS content range between 5% (olive mill wastewater) and  
342 75% (poultry litter), and VS content range between 69% (olive mill wastewater) and 91% (cereal  
343 straw).

344  
345

345 Then, based on different percentages of the analysed biomasses a FM was selected for anaerobic  
346 digestion test. In detail, the percentage of each adopted feedstock were listed in Table 1. Before  
347 starting AD test the FM was analysed by carrying out a BMP test.

348  
349

348 The FM was designed by taking into account the feedstocks availability and the diets currently  
349 adopted in the biogas plants located in Sicily.

350  
351

350 After the FM definition, before starting BMP test, the FM was characterised as reported in Table 3.  
351 The FM was chemical analysed several times during the test (Table 3).

352  
353  
354

**Table 3.** Characteristics of FM.

## 3.2 BMP and continuous anaerobic digestion test

### 3.2.1 BMP test

Before starting anaerobic digestion test on the selected FM, a BMP test was carried out. As reported in Table 1, since citrus pulp represents the highest concentration of the feedstock-mixture, the BMP test was carried out also for citrus pulp (Table 4). In particular, the specific yield of methane for the FM was equal to 312.2 Nm<sup>3</sup>CH<sub>4</sub>/tVS (Figure 1) with a VS reduction of about 67.8%. Moreover, the peak value of the production, about 62.4 Nm<sup>3</sup>CH<sub>4</sub>/t, which corresponds to the maximum degradation speed (K<sub>max</sub>) value, was observed after 2.6 days (Table 4). The specific production of methane obtained from BMP test on citrus pulp was equal to 310 Nm<sup>3</sup>CH<sub>4</sub>/tVS with a percentage of methane in the analysed produced biogas equal to 63.2%. Batch test results are listed in Table 4. Both the reactors were cultured at 38 ± 1 °C for 27 days. The total quantity of methane produced from both the analysed FM and citrus pulp was reported in Figure1.

**Table 4.** Batch test results of the analysed feedstock-mixture.

**Figure 1.** Accumulated methane production during BMP test.

**Figure 2.** FM and citrus pulp daily methane production.

As shown in Figure 1 no significant differences were reported by trend production of the FM and its main feedstock. The process was triggered quickly, due to the microbial flora contained in the adopted inoculum, and the production of methane immediately started, from the first days of the BMP test. The daily methane production curve allowed the identification of two different phases (Figure 2). The first phase was characterised by an intense growth, meanwhile, during the second phase a reduction of the speed production was recorded. Moreover, the peak value of the production, the K<sub>max</sub> value, was observed after three days for the analysed FM, and after eight days for citrus pulp (Table 4).

### 3.2.2 Continuous anaerobic digestion test

The characteristics of the adopted inoculum are listed in Table 2. The HRT was defined on 50 days based on the chemical analyses results, with a daily load of 373 g. In Table 5 the amount in terms of

393 grams, VS and TS of each feedstock is detailed. FM was characterized by high values of acidity; a  
394 high pH value of about 8.05 was recorded at the beginning of the test.

395  
396

397 **Table 5.** FM characteristics for daily load.

398  
399 As reported in Table 5, the citrus pulp (40%) is the main feedstock within the selected FM; in terms  
400 of organic matter the citrus pulp contributes as well as triticale silage about 27%, followed by whey  
401 and poultry manure with 13% and 12% of VS, respectively.

402  
403

#### 404 3.2.2.1 First anaerobic digestion test

405  
406 The methane specific production recorded during the entire AD test was equal to 229 Nm<sup>3</sup> CH<sub>4</sub> / t  
407 VS. In Figure 3 the accumulated biogas production for the first test is shown. The daily percentage  
408 of methane recorded in the biogas is shown in Figure 4 with the average value for the entire test of  
409 57.8%. The hydrogen sulphide (H<sub>2</sub>S) content, measured in biogas is shown in Figure 5. During the  
410 first test, H<sub>2</sub>S concentration reached the maximum value of 800 ppm after 40 days.

411  
412

413 **Figure 3.** Accumulated biogas production during the first AD test.

414  
415

416 **Figure 4.** Biogas composition in terms of CH<sub>4</sub> content from the first AD test.

417  
418

419 **Figure 5.** Biogas composition in terms of H<sub>2</sub>S content from the first AD test.

420  
421

422 Every day 373 g of the FM were fed to the reactor and the same amount of the AD effluent was  
423 removed from the reactor and stored in the refrigerator. Weekly, the stored samples were  
424 chemically characterized for TS and VS in order to evaluate the degradation of the organic matter  
425 inside the reactor. In Figure 6 the trend of the monitored TS and VS parameters was reported.

426  
427

428 **Figure 6.** TS and VS trend during the first AD test.

429 During the first 60 days of testing, a first increase in TS concentration was observed (Figure 6).  
430 Therefore, analyses on the stored samples aiming at determining the concentration of VFA were  
431 carried out in order to evaluate the stability of the biological process. Furthermore, acetic acid,  
432 butyric acid, hexanoic acid, heptanoic acid, valeric acid, and propionic acid were analysed. In  
433 Figure 7 acetic acid and propionic acid trends were reported.

434  
435 Day 0- sample refers to the original inoculum; in the next recorded samples during the beginning of  
436 the test a high value of acetic acid, 5300 mg/kg, was already observed, which increases until  
437 reaching a concentration of 11140 mg/kg. This concentration contributes to inhibit the  
438 methanogenic microorganisms (Figure 7). At the same time, an accumulation of propionic acid was  
439 also observed in the first month of the AD test, whose concentration reached about 4000 mg/kg up  
440 to day 103. From day 84 to day 101 an imbalance was also observed in the acetic - propionic ratio,  
441 with higher concentration of propionic acid.

442

443

444 **Figure 7.** Acetic and propionic acids concentrations in digestate samples, monitored during the first  
445 AD test.

446

447

448 By analysing the entire process, from 39<sup>th</sup> day until 54<sup>th</sup> day the biological process has shown  
449 several inhibition signs. The acetic acid was continuously increasing, an increase in the FOS/TAC  
450 ratio was also recorded, with consequent lowering of methane production. A deterioration in the  
451 quality of biogas was observed with methane content of about 50% and high values of H<sub>2</sub>S of about  
452 800 ppm were found.

453

454 The inhibition of the process avoided the total conversion of the organic matter into biogas, which  
455 has been very low with respect to expectations (Valenti et al., 2018b; 2018c) (about 25-30% less).  
456 Furthermore, the process inhibition led to a gradational accumulation of VFA (mostly acetic acid  
457 and propionic acid) that could be the main responsible of the high risk-acid processes.

458

459

#### 460 3.2.2.2 Second anaerobic digestion test

461

462 Due to the inhibition of the process a second batch test was performed, by considering the same  
463 feedstock mixture analysed during the first AD test but using a different inoculum. The adopted  
464 inoculum was taken from a biogas plant located in Southern Italy which typically uses the analysed  
465 feedstocks (i.e., citrus pulp, olive pomace, triticale silage, cattle and poultry manure and cereal

466 straw). Furthermore, to exclude that the high concentration of citrus pulp and olive mill wastewater  
467 lead to the inhibition of the process during the first AD, the second AD test started by adding  
468 gradually these two main feedstocks. Firstly, about 1/3 of citrus pulp and olive mill wastewater was  
469 replaced by cattle manure. After about 10 days, citrus pulp was gradually increased to replace  
470 partially the cattle manure used as feedstock-substitute, and subsequently, once the process became  
471 stable the amount of olive mill wastewater was increased. At the day 38<sup>th</sup>, the FM reached the same  
472 composition in terms of feedstock- percentages as that used in the first AD test. Then, for two  
473 weeks, until 54<sup>th</sup> day, the process was monitored with the daily full load. In this way, by introducing  
474 one variable at a time, the TS% was kept fix at 21%, and the organic loading rate (OLR) at 3.3 kg  
475 VS/m<sup>3</sup> day. The test lasted ad 54<sup>th</sup> days, only one HRT, just to try in different conditions the same  
476 FM analysed during the first AD test. It was decided to set this AD test with a lower organic  
477 loading rate (OLR) than the previous AD test.

478  
479 The methane specific production recorded during the entire test was equal to 297 Nm<sup>3</sup> CH<sub>4</sub> / t VS.  
480 The accumulated biogas production is shown in Figure 8. The percentage of methane content  
481 recorded in the biogas detected daily is shown in Figure 9 and the average value measured for the  
482 entire test was equal to 56.5%. The hydrogen sulphide (H<sub>2</sub>S) content, was also measured in  
483 produced biogas. During the test H<sub>2</sub>S concentration reached the maximum value of 700 ppm at 27<sup>th</sup>  
484 day.

485

486

487

**Figure 8.** Accumulated biogas production during second AD test.

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**Figure 9.** Biogas composition in terms of CH<sub>4</sub> content during the second AD test.

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**Figure 10.** FOS/TAC ratio monitoring during the second AD test.



502 Also in this AD test, analyses on the stored samples aiming at determining the concentration of  
503 VFA were carried out in order to evaluate the stability of the biological process. In Figure 11, acetic  
504 acid and propionic acid trends were reported.

505  
506 **Figure 11.** Acetic and propionic acid concentrations in digestate samples, monitored during the  
507 second AD test.  
508  
509

510  
511 In this second AD test the recorded values of acetic acid remain below the critical threshold, with  
512 values that exceed slightly 500 mg kg<sup>-1</sup>. The values of the other VFAs are negligible.

513  
514 The results clearly demonstrate the viability of the mixing ratio to carry out the AcoD to generate  
515 renewable energy. Under the stabilized culture condition, the FM demonstrates good performance  
516 on methane production due to the high citrus pulp content. Anaerobic co-digestion of different  
517 organic residues has been widely investigated, but only a few studies regarded multiple feedstocks  
518 investigation to demonstrate successful biogas production from multiple organic residues (Muradin  
519 et al., 2014; Wickman et al., 2016; Tasnim et al., 2017). In this context, the study described in this  
520 paper reports the effect on methane production of a diet obtained by mixing ten feedstocks typically  
521 available in the Mediterranean area. Such a diet has not been tested before in literature.  
522 Furthermore, the methane content produced from the analysed FM (56.5% and 57.38% for first AD  
523 and second AD respectively) is also in line with the results reported by Hobenov et al. (2015)  
524 (percentage of methane recorded range 57-62%) which regarded a FM of potatoes, tomatoes,  
525 cucumbers, apple wastes and swine manure, and the results reported by Giuliano et al. (2013)  
526 (percentage of methane recorded range 54-57%) which regarded a FM of cattle slurry, cow manure,  
527 triticale maize silage, onion and potatoes. As reported by Valenti et al. (2018c), which investigate  
528 the effect of mixing other Mediterranean feedstocks, the obtained results could be useful for  
529 developing biogas production in Mediterranean regions with similar sources of organic residues.

#### 530 531 4. Conclusions

532 In this study, in order to evaluate the technical feasibility of a FM constituted by ten Mediterranean  
533 feedstocks co-digestion (citrus pulp, olive pomace, tomato peels, olive mill wastewater, poultry  
534 litter, poultry and cattle manure, whey and cereal straw), both batch and semi-continuous anaerobic  
535 digestion approaches were applied. The FM was analysed for evaluating its methane production and  
536 verifying the possible inhibitory effects on the biological process. The analysed FM is energetically  
537 interesting in terms of methane potential. The batch digestion showed that the FM had potential to

538 be used for biogas production, in this regard, the test showed a production of biogas equal to 312  
539  $\text{Nm}^3\text{CH}_4/\text{t VS}$  with a VS reduction of about 68%; methane production of about 62.4  $\text{Nm}^3\text{CH}_4/\text{t}$  and  
540 57.7% of methane in the produced biogas. Two different AD tests were carried out. During the first  
541 AD test the specific methane production was equal to 229  $\text{Nm}^3\text{CH}_4/\text{tVS}$ , it was 27% lower than that  
542 measured during the batch test, with 56.5% of methane measured in biogas. Instead, during the  
543 second batch test, in which a different inoculum was used and the feeding plan was gradually  
544 introduced, the specific production of methane was 272  $\text{m}^3\text{CH}_4/\text{tVS}$ ; with 57.8% of methane  
545 measured in biogas, since the lower organic loading reduced inhibiting effects. On both AD tests,  
546 high values of hydrogen sulphide were recorded, therefore, desulfurization systems, in order to keep  
547 the biogas concentrations under control, should be considered. However, during the first AD test,  
548 the inhibition of the process avoided the total conversion of the organic matter into biogas, the  
549 second AD test demonstrated that the selected FM could be viable to carry out the co-digestion. In  
550 detail, the FM could be a flexible and suitable solution to generate sustainable bioenergy from  
551 diverse agricultural residues in Mediterranean area.

552  
553 The results of the research study proposed in this paper demonstrate that it is possible to develop a  
554 sustainable bioeconomy strategy by integrating the sustainable production of renewable natural  
555 resources and by converting these resources and waste streams into value added products such as  
556 food, feed, bio-based products and bio-energy.

557  
558 Moreover, the development of a sustainable bioeconomy for replacing non-renewable resources (i.e.  
559 reduction of greenhouses gas emissions), by supplying food to societies and preserving natural  
560 resources, will depend not only on innovations in biomass transformation processes, i.e., anaerobic  
561 digestion, but also on the organization of biomass feedstock production or biomass-based product  
562 consumption. These concepts are fundamental for a social sustainable development and to create a  
563 thriving economy based on the respects for the environment (BIT, 2017).

564  
565 According to the principles contained in the Juncker's Agenda for Jobs, Growth, Fairness and  
566 Democratic Change, it is essential to reduce the fossil fuels and finite materials dependence without  
567 the over-exploitation of renewable resources, preventing land use change, regenerating the  
568 environment and creating new economic growth and jobs and leveraging on local diversities and  
569 traditions in the rural areas (including those that are no usually cultivated).

570  
571 In this regard, to make real the obtained result at lab scale, further improvements of the research  
572 study are needed. The assessment of the analysed feedstocks availability could be obtained, by  
573 developing GIS-based model, which combine AD and BMP results with spatial analyses. This will

574 be relevant to improve the real reuse of by-products and agricultural residues for bioenergy  
575 production. In detail, a GIS-modelling approach could integrate information base suitable for the  
576 application of multi-criteria analysis methods that aim to optimize the biogas plant location from an  
577 economic and environmental point of view.

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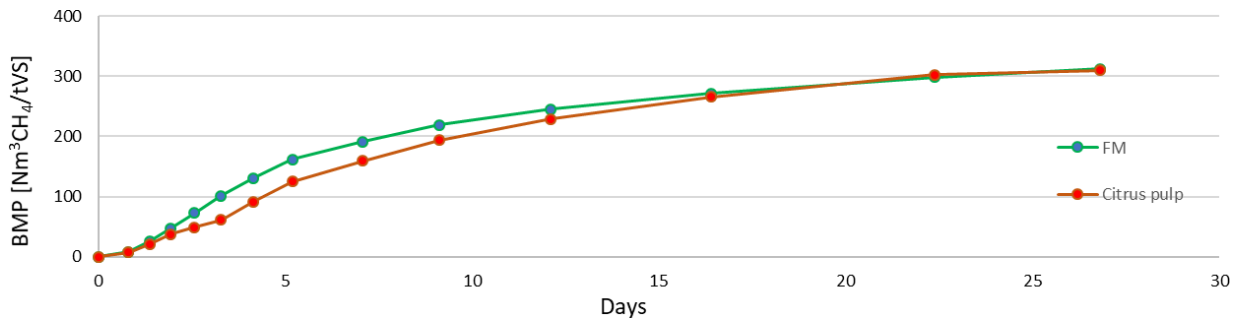
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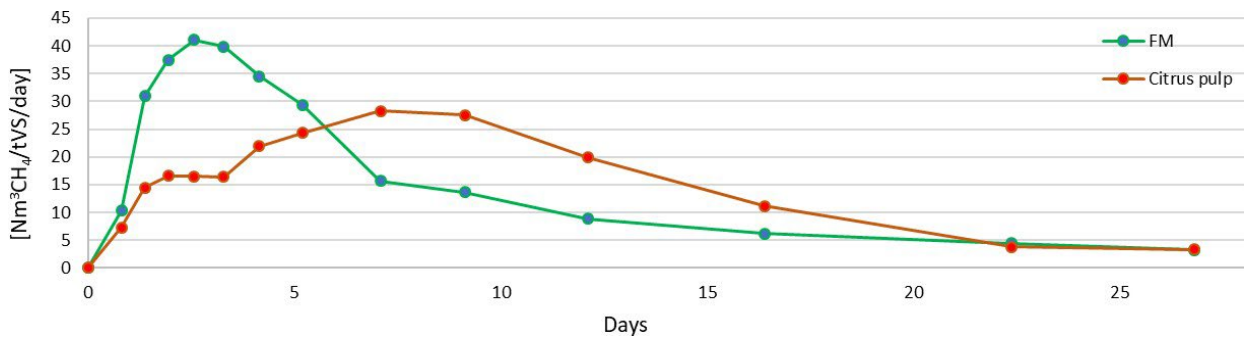
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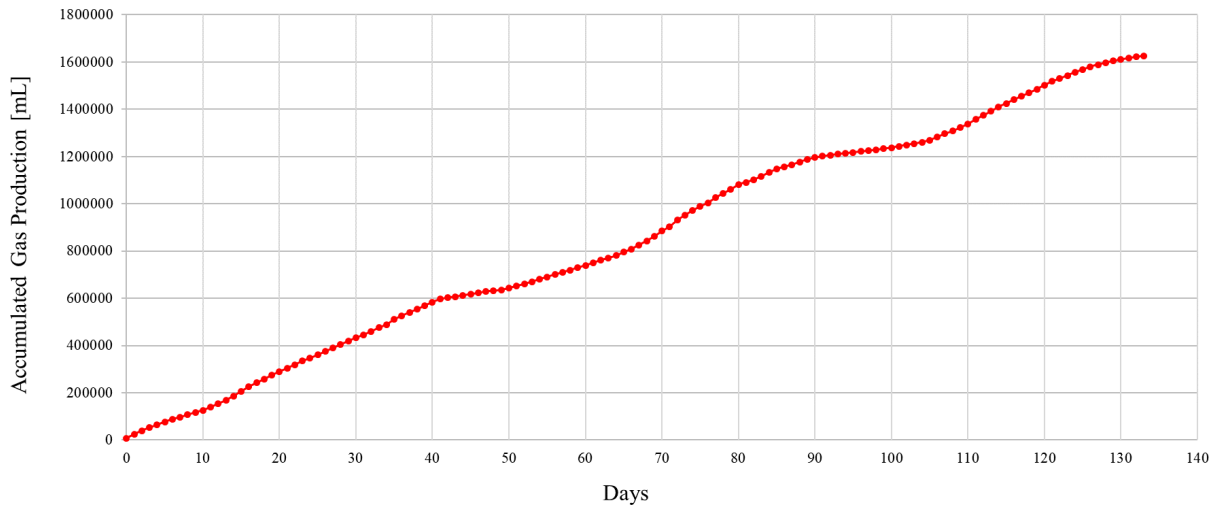
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**Figure 1.** Accumulated methane production during BMP test.



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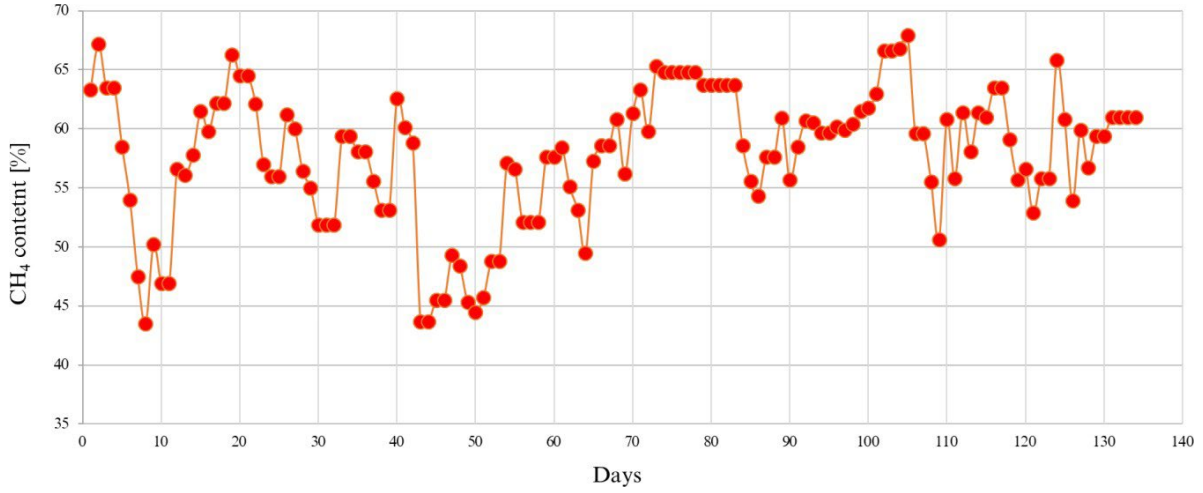
**Figure 2.** FM and citrus pulp daily methane production.



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**Figure 3.** Accumulated biogas production during the first AD test.

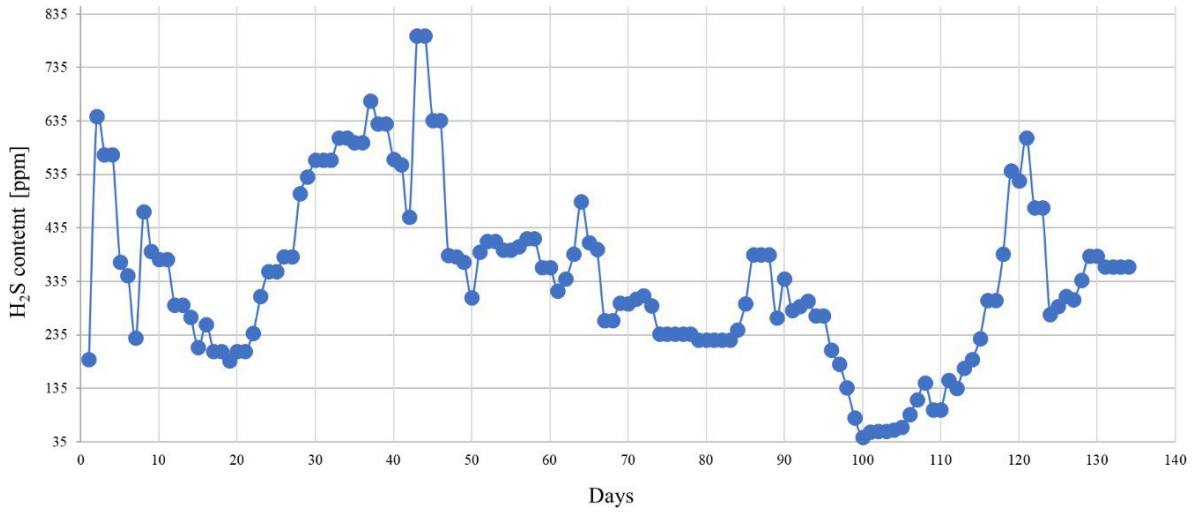
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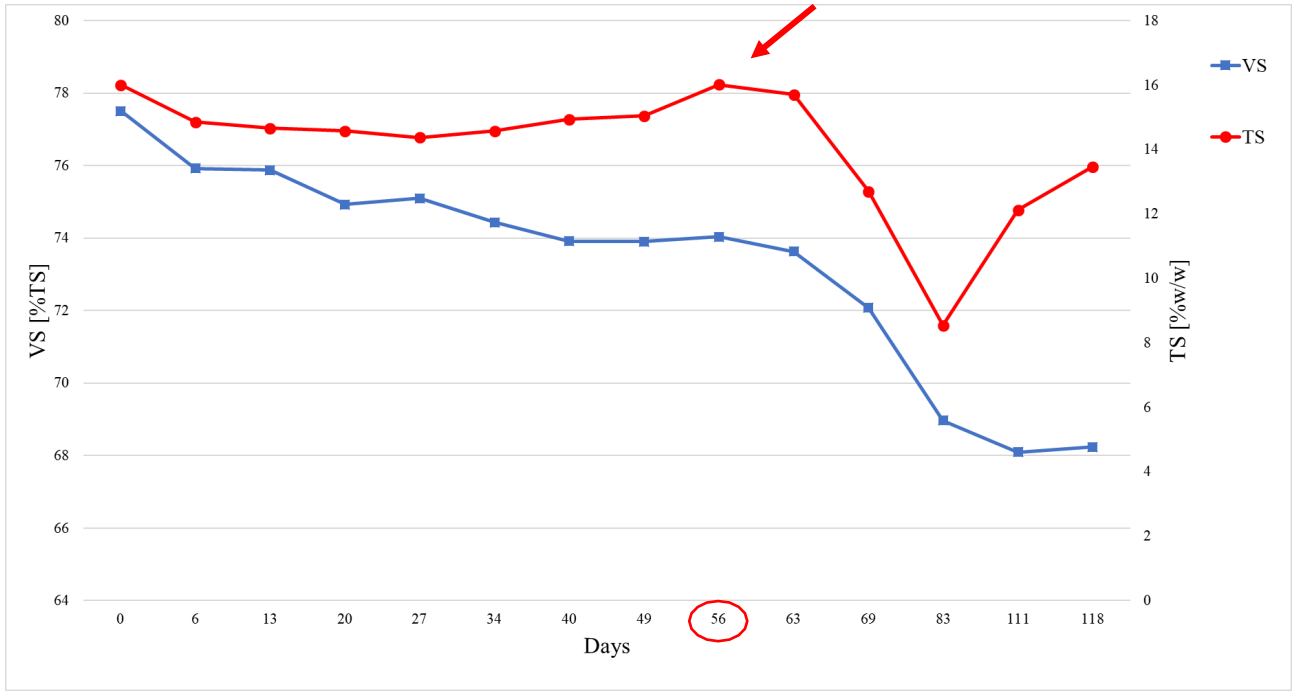
**Figure 4.** Biogas composition in terms of CH<sub>4</sub> content from the first AD test.

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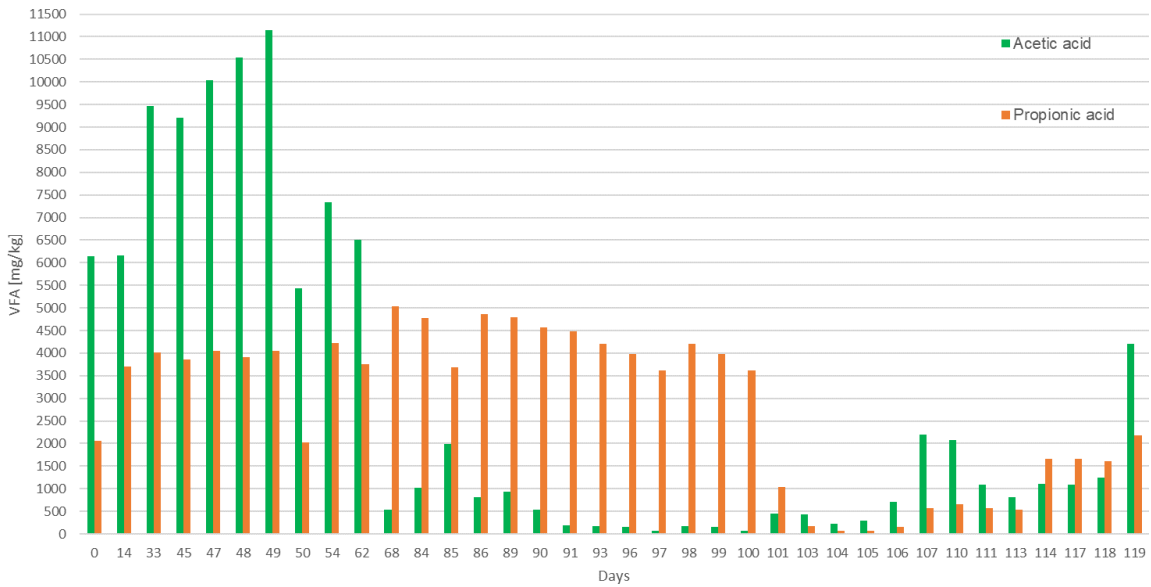


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**Figure 5.** Biogas composition in terms of H<sub>2</sub>S content from the first AD test.



**Figure 6.** TS and VS trend during the first AD test.

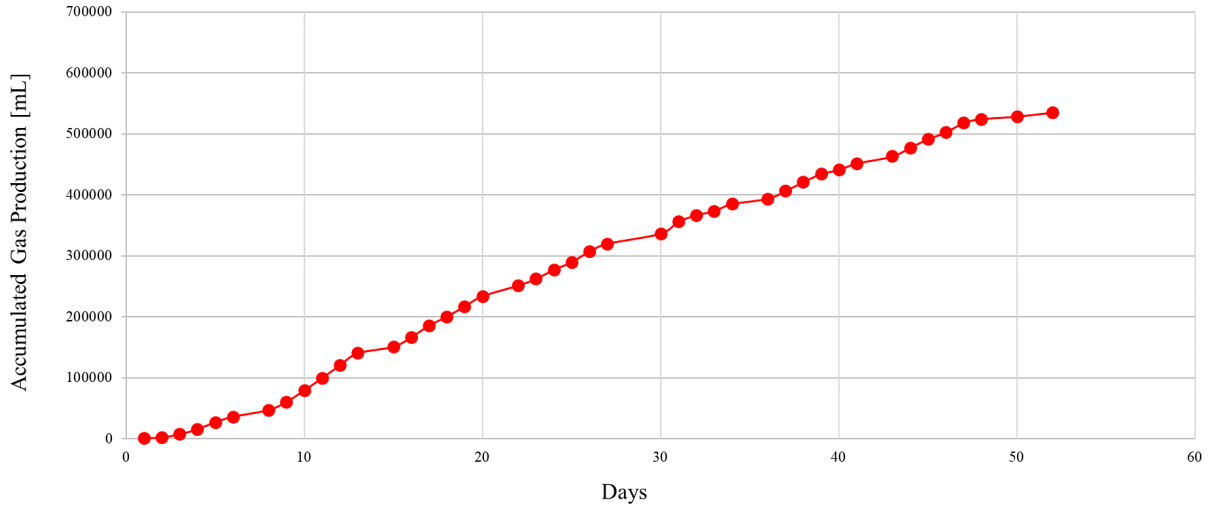


**Figure 7.** Acetic and propionic acids concentrations in digestate samples, monitored during the first AD test.

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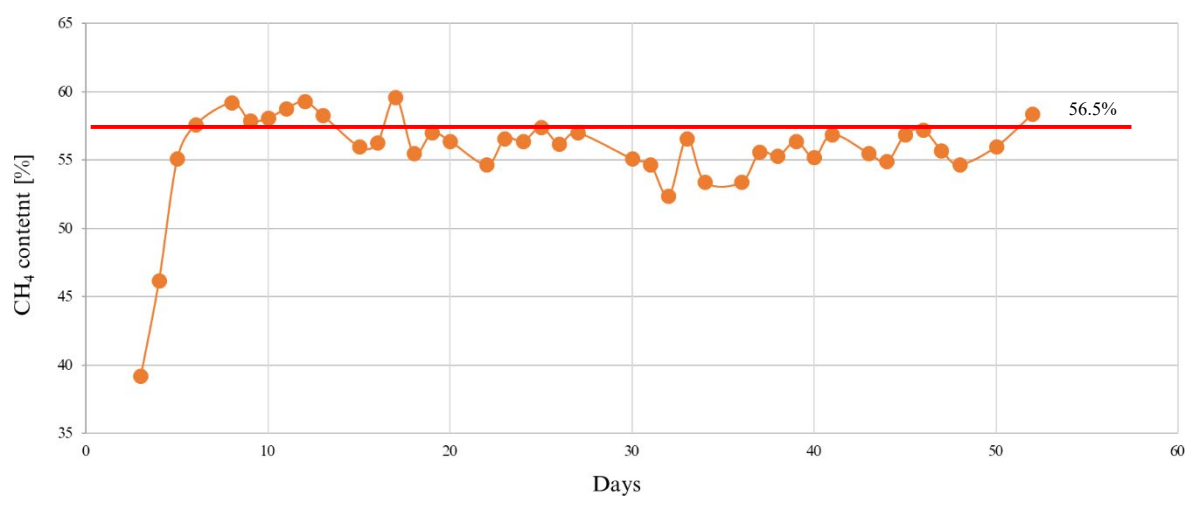
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**Figure 8.** Accumulated biogas production during second AD test.

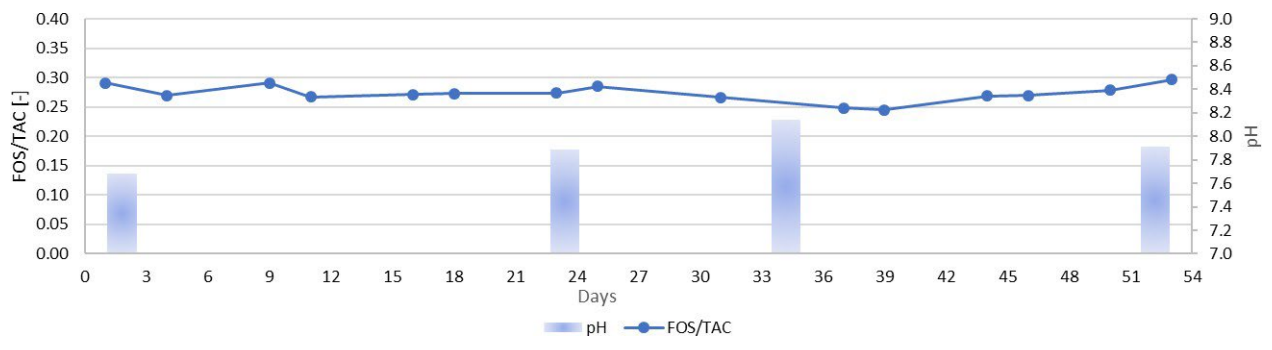
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**Figure 9.** Biogas composition in terms of CH<sub>4</sub> content during the second AD test.

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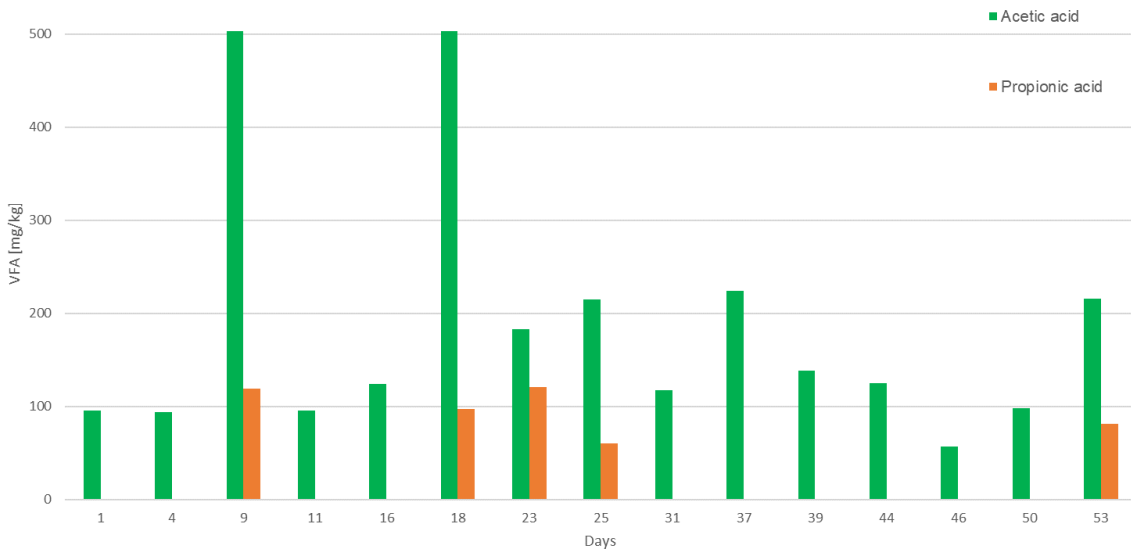


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**Figure 10.** FOS/TAC ratio monitoring during the second AD test.

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**Figure 11.** Acetic and propionic acid concentrations in digestate samples, monitored during the second AD test.

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**Table 1.** Characteristics of individual feedstocks and mass ratios of different feedstocks in FM.

Matrices	TS	VS	FM composition
	[% w/w <sup>a</sup> ]	[% TS]	[%w/w, DM <sup>a</sup> ]
Citrus pulp	17	74	40
Olive mill wastewater	5	69	15
Poultry manure	34	67	10
Triticale silage	30	92	9
Poultry litter	75	86	8
Olive pomace	16	90	5
Cattle manure	14	85	5
Whey	3	71	4
Cereal straw	94	91	2
Tomato peels	27	96	2
<b>Total</b>	-	-	100

<sup>a</sup> DM means dry matter.

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**Table 2.** Characteristics of adopted inoculum.

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Inoculum	TS	VS	FOS	TAC	FOS/TAC
	[%w/w]	[%TS]	[mgHAc <sub>eq</sub> /L]	[mgCaCO <sub>3</sub> /L]	[-]
First AD test	16.00	77.50	12184	23721	0.51
Second AD test	5.90	72.28	3195	10990	0.29

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**Table 3.** Characteristics of FM.

FM sample [date]	TS	VS
	[%]	[%TS]
10/10/17	24.30	82.30
10/24/17	23.20	82.80
11/15/17	24.60	82.40
01/02/17	23.00	82.20
02/01/18	23.40	83.30
Average	23.70	82.60
Standard deviation	0.70	0.00

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**Table 4.** Batch test results of the analysed feedstock-mixture.

Matrices	TS	VS	BMP	BMP Peak value	Kmax <sup>1</sup>	VS reduction	CH <sub>4</sub>	H <sub>2</sub> S
	[g/kg]	[g/kg]	[Nm <sup>3</sup> CH <sub>4</sub> /t]	[Nm <sup>3</sup> CH <sub>4</sub> /t]	[days]	[%]	[%]	[ppm]
FM	243	200	312.2	62.4	2.6	67.8	57.7	433
Citrus pulp	174	136	310.2	42.0	8.4	58.0	63.2	268

<sup>1</sup> Kmax: maximum degradation rate of volatile solids.

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**Table 5.** FM characteristics for daily load

Feedstock	Amount of FM [%]	Daily amount of FM loaded [g]	Daily amount of TS loaded [g]	Daily amount of VS loaded [g]	VS on FM [%]
Citrus pulp	40	149.20	26.00	19.20	27
Olive mill wastewater	15	18.70	3.00	2.70	4
Poultry manure	10	37.30	12.60	8.50	12
Triticale silage	9	29.80	22.30	19.10	27
Poultry litter	8	14.90	0.50	0.40	0.5
Olive pomace	5	56.00	2.60	1.80	3
Cattle manure	5	18.70	2.60	2.20	3
Whey	4	33.60	10.20	9.40	13
Cereal straw	2	7.50	7.00	6.40	9
Tomato peels	2	7.50	2.00	1.90	3
Total	100	373.00	89.00	72.00	100

\* Weekly amount based on six days.

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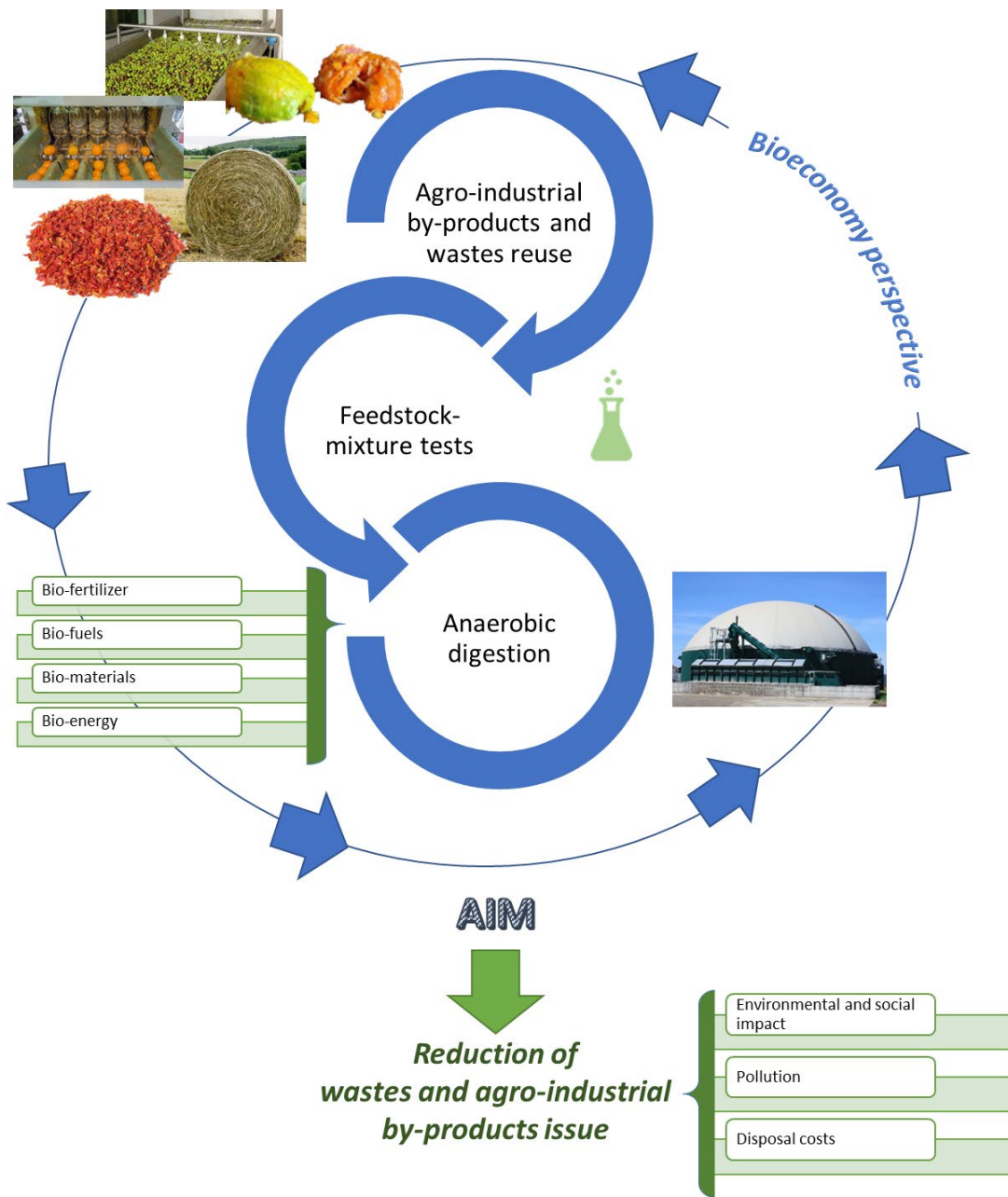


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**Highlights**

- Selected feedstocks mixture (FM) were evaluated by applying BMP and AD tests
- AD on Mediterranean FM was carried out to investigate advanced biofuels production
- Biomethane from agro-waste and by-products improves waste management sustainability
- A sustainable bioeconomy strategy can integrate preservation of natural resources
- ~~Digestate can improve agronomical soil value and reduce fertilizer costs~~
- AD contributes to create the conditions for a closed circular economy

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