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

Abstract

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

The first AD test showed a methane yield equal to 229 Nm³CH₄/tVS (27% lower than that measured during the batch test). During the second AD test, the specific production was 272 m³CH₄/tVS. Both tests showed a similar methane content of methane in the biogas, equal to about 57%.

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

Keywords

Biomethane; Anaerobic Digestion; Bioeconomy; Biomasses; Advanced biofuels.

1. Introduction

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

CO₂ emissions and about 15% of CH₄ emissions, are the most responsible in global warming (Williams et al., 2012; Hosseini and Wahid, 2014; Rahimnejad et al., 2015).

According to the Kyoto protocol the key factor to both reduce CO₂ and other GHG emissions and improve the living standard of developing countries is to produce cost effective energy as well as use bioenergy efficiency (Changua et al., 1999; Garnier, 2014; Ebner et al., 2015). In this context, renewable bioenergy is a promising alternative to achieve the world energy requirements by avoiding extra economic burden and any significant environmental impacts (Morero et al., 2015; Raimondo et al., 2018; Valenti et al., 2018a). Renewable energy supply chains were documented by Ingrao et al. 2018a as important for transitioning to equitable, sustainable, post fossil-carbon societies. In this context, biomass has become one of the most interesting input for sustainable processes.

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

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

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

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

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

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

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

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

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

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

2. Materials and Methods

2.1 Feedstocks characteristics

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

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

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

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

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 (CO₂) and methane (CH₄) 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 (CH₄), dioxide carbon (CO₂), ammonia (NH₃), hydrogen sulphide (H₂S) and water (H₂O). The process involves several families of bacteria: fermentative bacteria, acetogenic bacteria that produce H₂, Acetogenic bacteria using H₂, 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
272 Each reactor was provided with a 'syringe' to extract digestate and a transducer to measure the
273 pressure generated in the digester head space during the process. The digestate was weekly
274 monitored and was chemically characterized according to the parameters of TS and VS for
275 evaluating the organic matter degradation rate.

276
277 The first test started by filling the reactor with an inoculum taken from a digester that used citrus
278 pulp and olive pomace in order to make the microbial flora suitable for degrading as far as possible
279 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 m³ 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 m³ 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

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

298 2.3 Analytical methods

299

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

306

307 The calculation of the methane yield, as biochemical methane potential (BMP), was carried out
 308 in accordance with the standard ISO 11734. FOS/TAC ratio computation consists of weighting
 309 about 5.0 grams of fresh sample, added in a plastic container suitable for titration 50 mL of distilled
 310 water. The first titration is carried out with H₂SO₄ 0.1 N titrator until reaching pH value of 5.0 to
 311 complete bicarbonate titration, then to reach pH value of 4.4 by titrating the alkalinity.

312

313 Volatile fatty acids (VFAs) are important elements in controlling the anaerobic digestion
 314 process. They are important to decompose organics and to generate gasses, methane and carbon
 315 dioxide. Oxygen demand decreases when both decomposing and generating occur
 316 continuously and completely. The BMP test was performed by following the method set by
 317 CRPA Lab and the obtained results were expressed in normal cubic meters of methane per ton
 318 of VS (Nm³CH₄/tVS). The VS reduction, in terms of degradability of the organic matter, was
 319 calculated considering the ratio between the amount of the produced biogas and the amount of
 320 VS loaded. By using a gas chromatographic (GC) method, VFAs were measured during the semi-
 321 continuous process. 10 mL of the collected AD effluent was centrifuged at 7025 times gravity (xg)
 322 for 15 min using a centrifuge

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 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 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 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 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 Nm³CH₄/tVS (Figure 1) with a VS reduction of about 67.8%. Moreover, the peak value of the production, about 62.4 Nm³CH₄/t, which corresponds to the maximum degradation speed (K_{max}) 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 Nm³CH₄/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_{max} 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

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

395
396

Table 5. FM characteristics for daily load.

398

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

402

403

3.2.2.1 First anaerobic digestion test

405

The methane specific production recorded during the entire AD test was equal to 229 Nm³ CH₄ / t VS. In Figure 3 the accumulated biogas production for the first test is shown. The daily percentage of methane recorded in the biogas is shown in Figure 4 with the average value for the entire test of 57.8%. The hydrogen sulphide (H₂S) content, measured in biogas is shown in Figure 5. During the first test, H₂S concentration reached the maximum value of 800 ppm after 40 days.

411

412

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

414

415

Figure 4. Biogas composition in terms of CH₄ content from the first AD test.

417

418

Figure 5. Biogas composition in terms of H₂S content from the first AD test.

420

421

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

426

427

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

428

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

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

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

By analysing the entire process, from 39th day until 54th day the biological process has shown several inhibition signs. The acetic acid was continuously increasing, an increase in the FOS/TAC ratio was also recorded, with consequent lowering of methane production. A deterioration in the quality of biogas was observed with methane content of about 50% and high values of H₂S of about 800 ppm were found.

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

3.2.2.2 Second anaerobic digestion test

Due to the inhibition of the process a second batch test was performed, by considering the same feedstock mixture analysed during the first AD test but using a different inoculum. The adopted inoculum was taken from a biogas plant located in Southern Italy which typically uses the analysed 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 38th, 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 54th 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/m³ day. The test lasted ad 54th 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 Nm³ CH₄ / 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 (H₂S) content, was also measured in
 483 produced biogas. During the test H₂S concentration reached the maximum value of 700 ppm at 27th
 484 day.

485
 486
 487 **Figure 8.** Accumulated biogas production during second AD test.

488
 489
 490 **Figure 9.** Biogas composition in terms of CH₄ content during the second AD test.

491
 492
 493 The FOS-TAC ratio (volatile organic acid and buffer capacity ratio) measured in the digested daily
 494 extract was linear and constant, as shown in Figure 10, with a mean value of the entire test of 0.27
 495 indicating process equilibrium. Acidity values (FOS) fall within the stability range, with values
 496 ranged from 3100 to 4800 mg/kg and alkalinity (TAC) ranged from 11000 to 17000 mg/kg. The pH
 497 values recorded during the AD test were sometimes slightly higher than the neutral value, about
 498 8.00.

499
 500
 501 **Figure 10.** FOS/TAC ratio monitoring during the second AD test.

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

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

In this second AD test the recorded values of acetic acid remain below the critical threshold, with values that exceed slightly 500 mg kg⁻¹. The values of the other VFAs are negligible.

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

4. Conclusions

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

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

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

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

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

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

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

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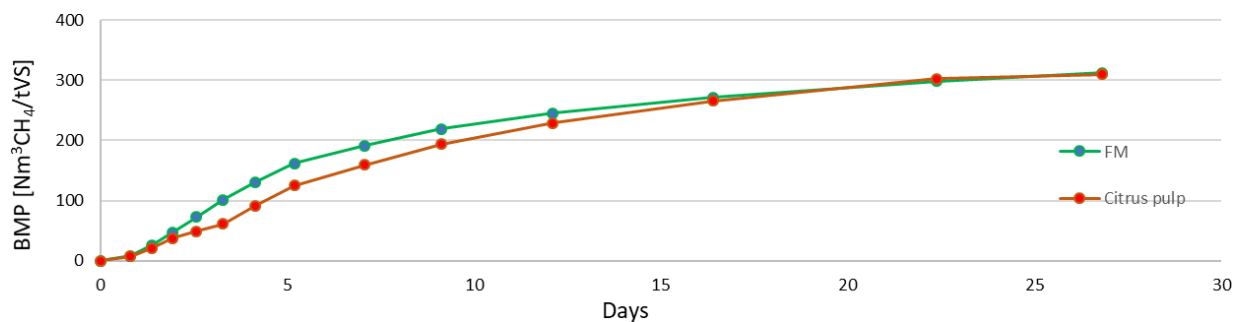


Figure 1. Accumulated methane production during BMP test.

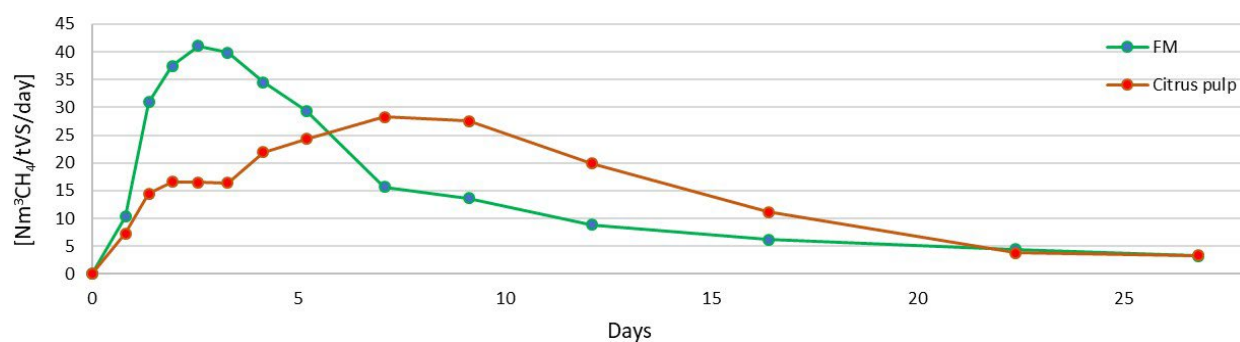


Figure 2. FM and citrus pulp daily methane production.

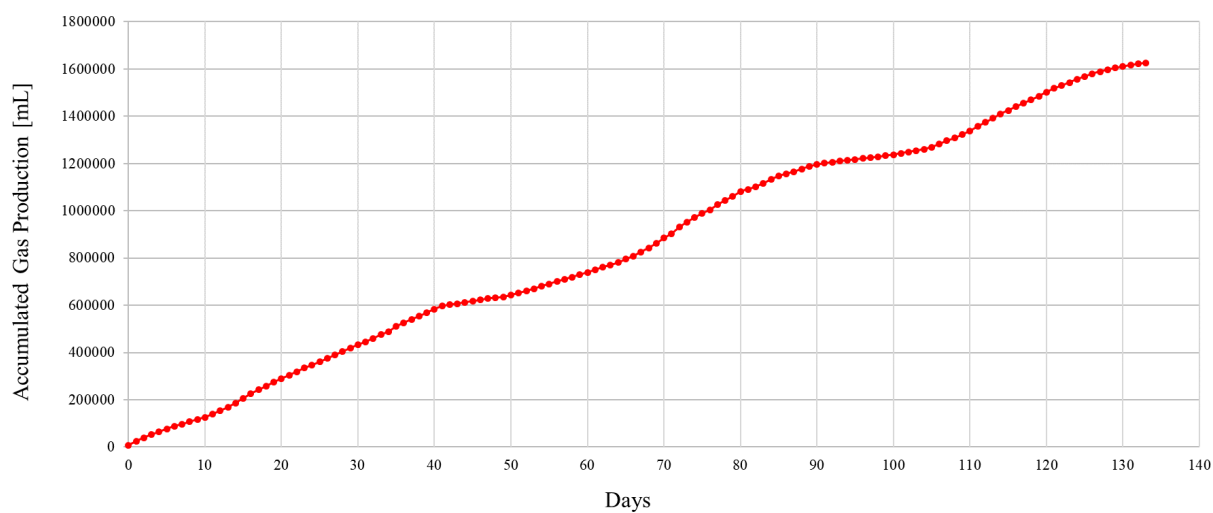


Figure 3. Accumulated biogas production during the first AD test.

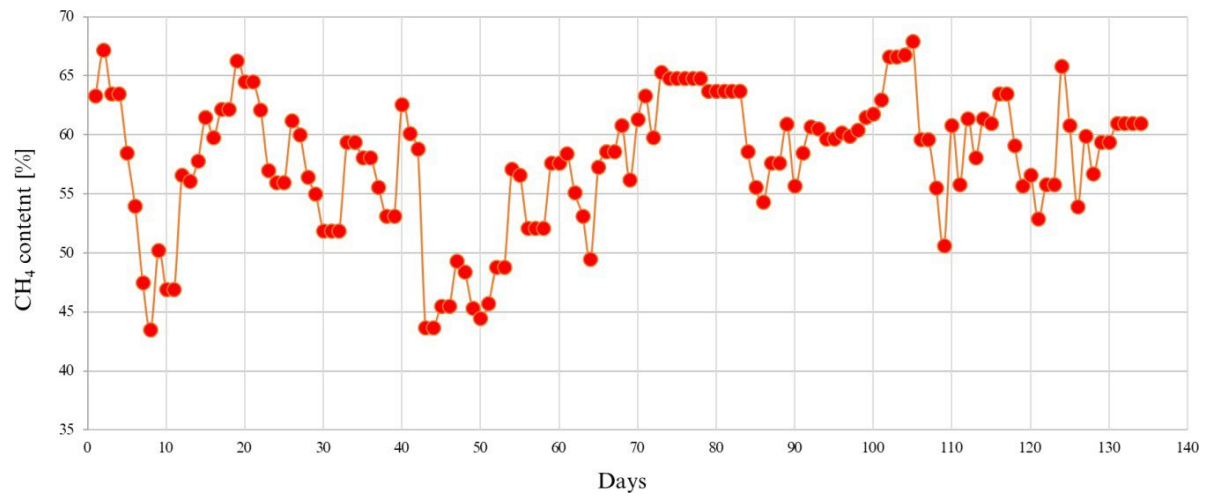


Figure 4. Biogas composition in terms of CH₄ content from the first AD test.

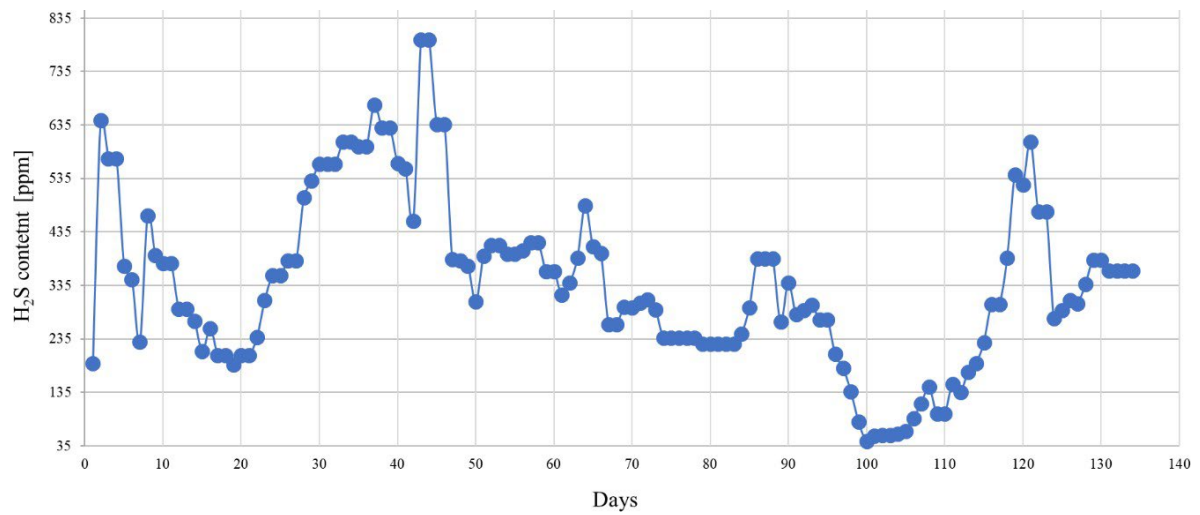


Figure 5. Biogas composition in terms of H₂S content from the first AD test.

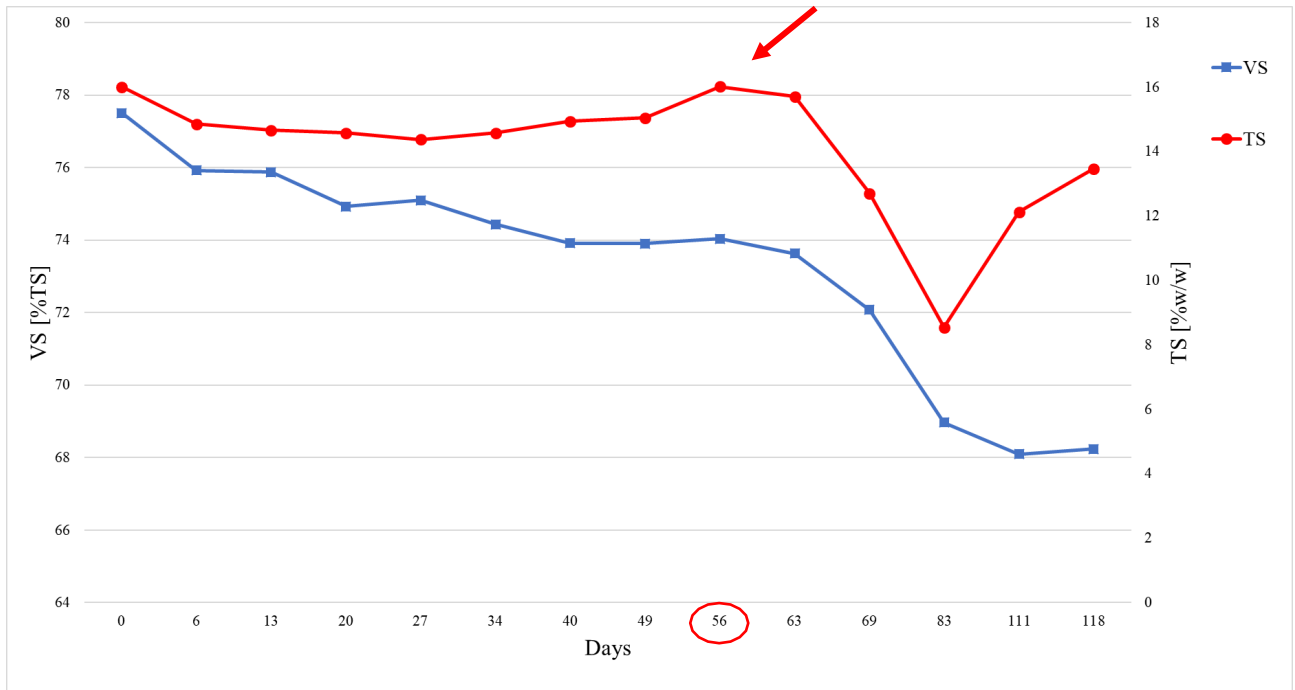


Figure 6. TS and VS trend during the first AD test.

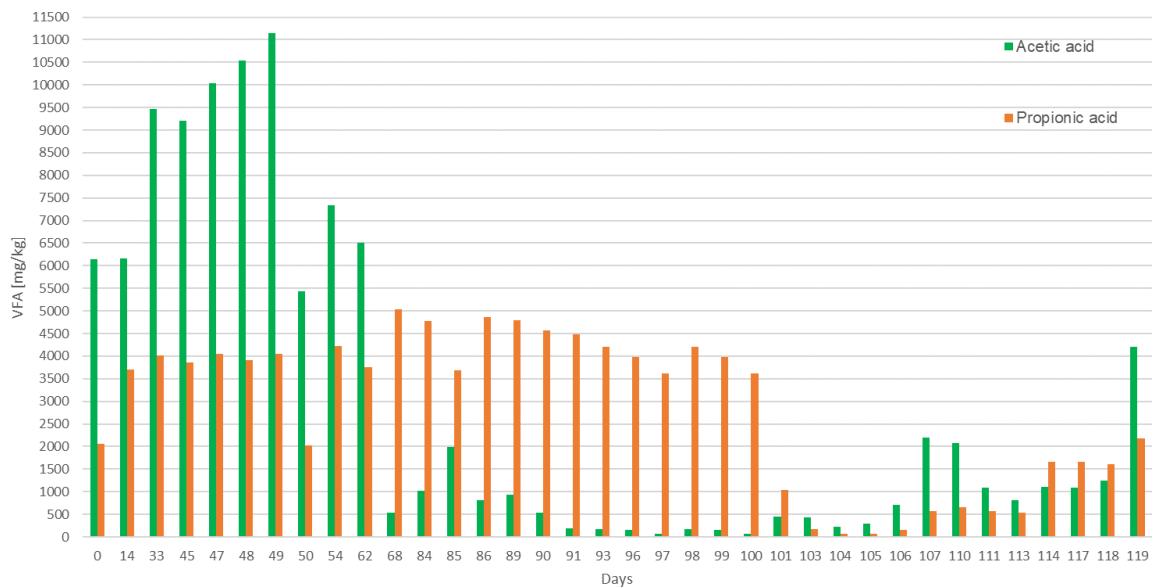


Figure 7. Acetic and propionic acids concentrations in digestate samples, monitored during the first AD test.

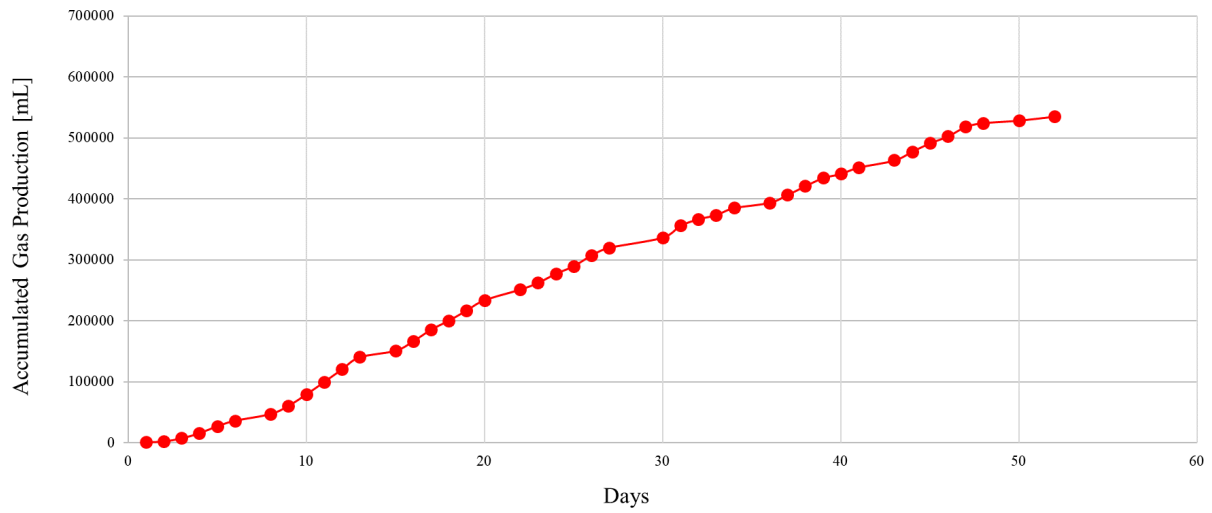


Figure 8. Accumulated biogas production during second AD test.

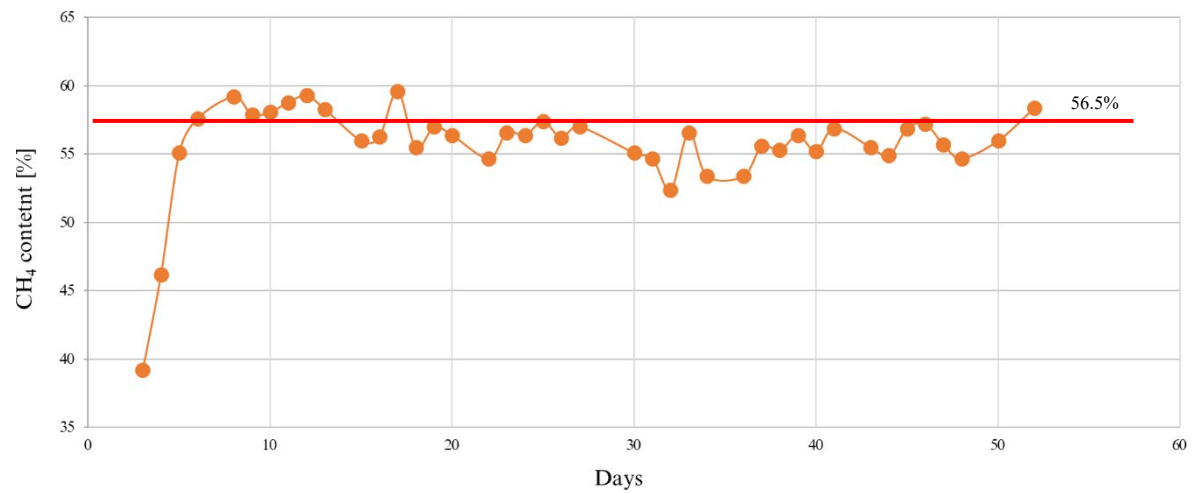


Figure 9. Biogas composition in terms of CH₄ content during the second AD test.

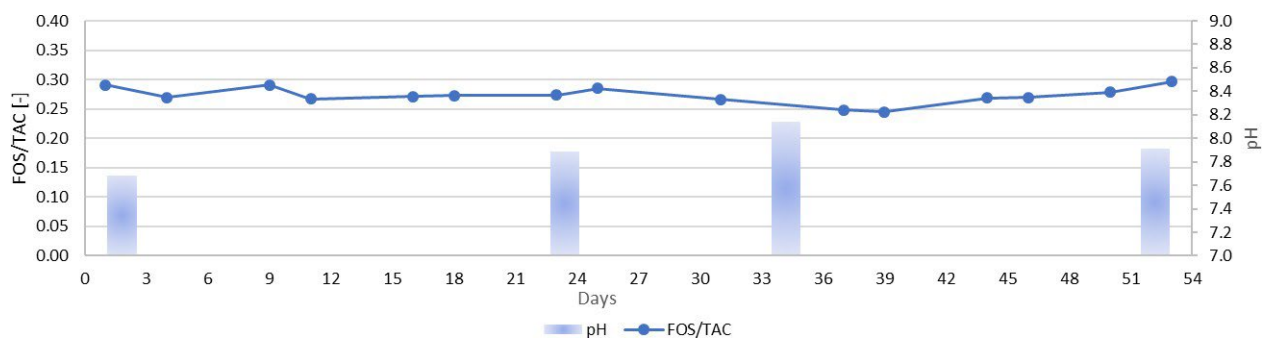


Figure 10. FOS/TAC ratio monitoring during the second AD test.

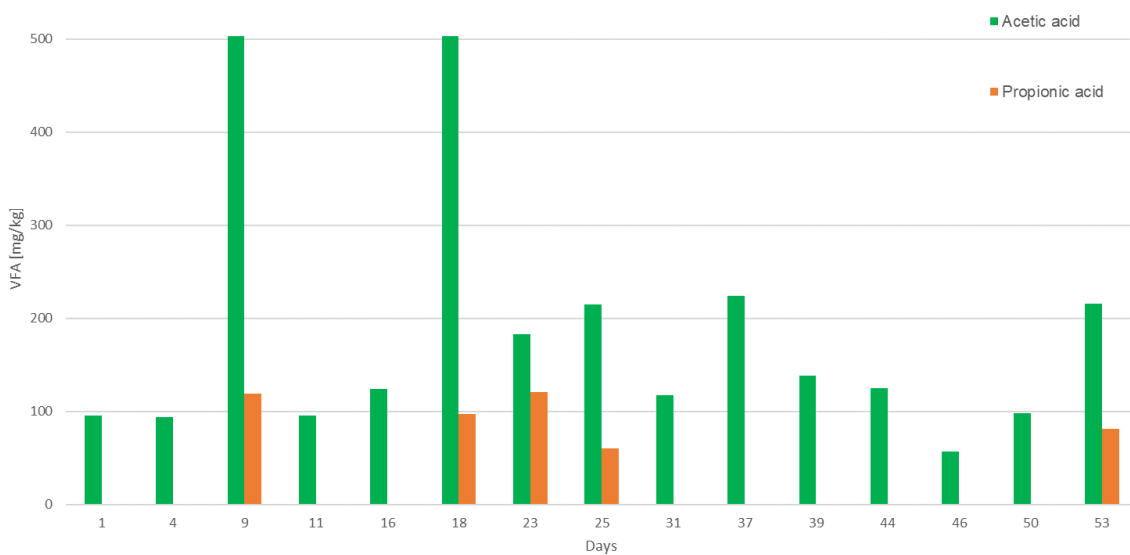


Figure 11. Acetic and propionic acid concentrations in digestate samples, monitored during the second AD test.

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

Matrices	TS [% w/w ^a]	VS [% TS]	FM composition [%w/w, DM ^a]
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
Total	-	-	100

^a DM means dry matter.

Table 2. Characteristics of adopted inoculum.

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

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

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

Matrices	TS	VS	BMP	BMP Peak value	Kmax ¹	VS reduction	CH ₄	H ₂ S
	[g/kg]	[g/kg]	[Nm ³ CH ₄ /t]	[Nm ³ CH ₄ /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

¹ Kmax: maximum degradation rate of volatile solids.

Table 5. FM characteristics for daily load

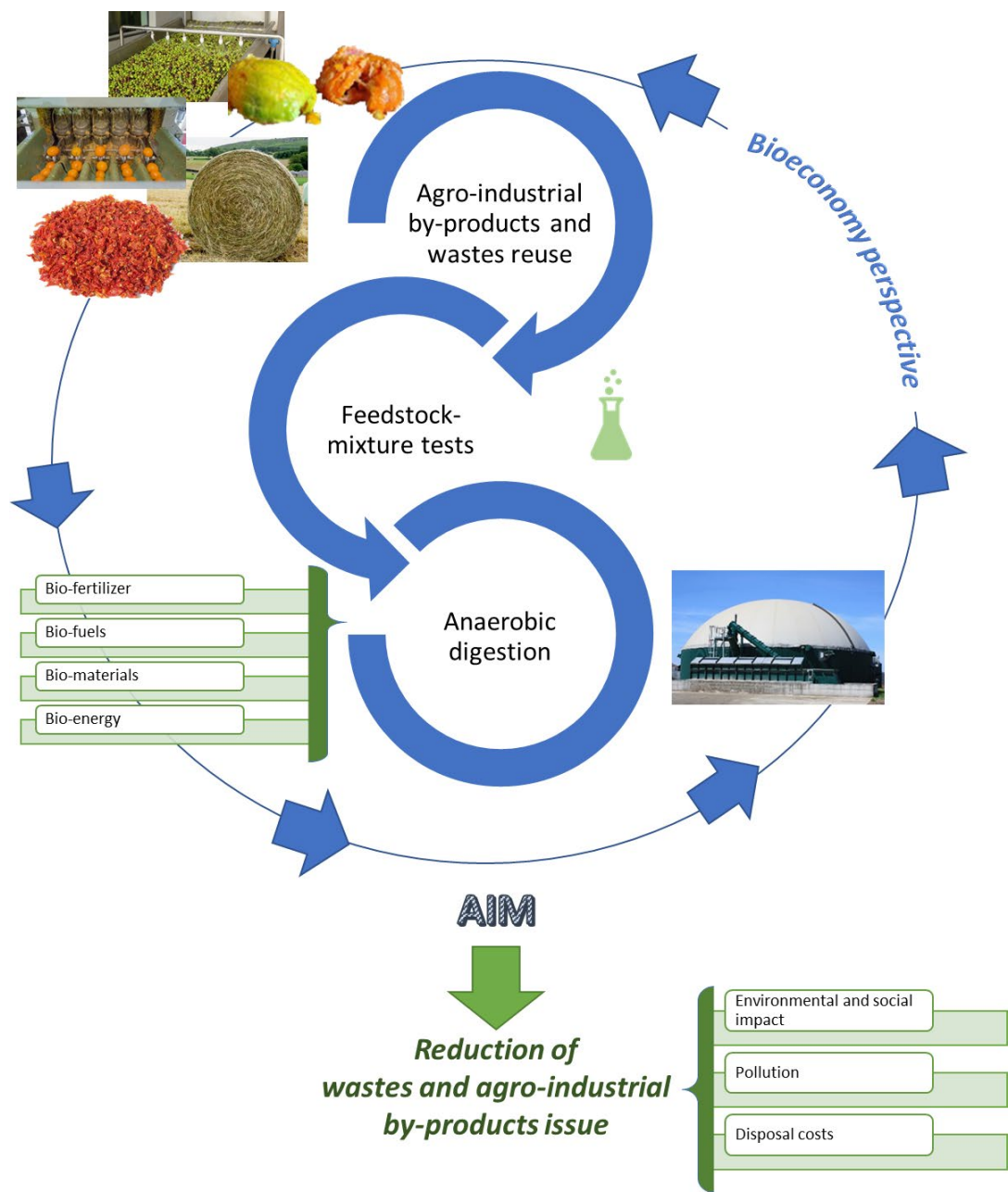
Feedstock	Amount on FM	Daily amount loaded*	Daily amount of TS loaded	Daily amount of VS loaded	VS on FM
	[%]	[g]	[g]	[g]	[%]
Citrus pulp	40	149.20	26.00	19.20	27
Olive mill wastewater	15	18.70	3.00	2.70	4
Poultry manure	10	37.30	12.60	8.50	12
Triticale silage	9	29.80	22.30	19.10	27
Poultry litter	8	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.

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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