Fate of Biodegradable Polymers Under Industrial Conditions for Anaerobic Digestion and Aerobic Composting of Food Waste

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Abstract

Biodegradable polymers were introduced in the past decades in order to address the issue of plastic pollutions, and these materials have thus required the development of methodologies to understand and evaluate their disintegration. The aim of this study was to simulate the organic fraction of municipal solid waste (OFMSW) treatment in laboratory-scale and to assess the biodegradation of poly(lactic acid) water bottles and starch-based bags under real industrial conditions of anaerobic digestion and aerobic composting. Methane production and loss of mass were determined to estimate the anaerobic degradation; whereas phytotoxicity tests were carried out to provide an evaluation of the compost quality. To visualize the effects on the materials, SEM analyses, differential scanning calorimetry (DSC) and FT-IR/ATR spectroscopy were performed. Different outcomes were found for the tested bioplastics products. Poly(lactic acid) bottles didn't biodegrade under anaerobic conditions and the pieces appeared wrap up at the end, while starch-based bioplastic bags performed 85.79% of disintegration degree. CH_4 production was between 40 and 50% for both the products. Phytotoxicity test on the final composts carried out negative effects on both selected seeds for poly(lactic acid) solutions. Water-soluble lactic acid from degraded poly(latic acid) bottle significantly reduced the pH of compost affecting Seed Germination and Germination Indexes. Both bioplastics showed chemical modification according to DSC and FT-IR/ATR analyses.

Keywords

anaerobic fermentation; bioplastic products; thermophilic digestion; PLA bottle; biogas

52

1.Introduction

Environmental pollution from plastic wastes has become a key issue the last decades, and policies have been implemented in many countries to reduce use of plastics (1). UK and France are enforcing procedures to ban the use of straws, plastic cutlery, stirrers and other disposable plastics. Additional measures have been devised to address this problem, such as the substitution of the traditional "petro-based" polymers with biodegradable ones, based on renewable resources, which are less persistent in the environment (2,3). According to the literature, these materials can be degraded by microorganisms under certain conditions of humidity, temperature and UV light (4). Among the bioplastics, starch-based and poly(lactic acid)-based ones are the most common nowadays. Due to its hydrophilic nature and its poor mechanical properties starch is not used on its own for bioplastic production, but it is modified chemically, physically or mechanically and/or blended with other polymeric compounds or plasticizers (5). The starch content of a typical blend can vary from 5 to 90 wt%. On the other hand, the bio-based and biodegradable thermoplastic polyester poly(lactic acid) (PLA) is produced by fermentation of the lactic acid monomer through polymerization. Despite of its biocompatible nature, PLA degradation in the environment is not easy because of its resistance to microbial attacks (6).

The amount of biodegradable plastics produced at the global level was less than 0.3% of the total amount of plastics produced in the same year (European bioplastics, 2018). In order to reduce plastic pollution, the EU Directive 2015/720 aims at limiting the annual number of lightweight plastic carrier bags consumed in Europe to a maximum of 90 units per person by the end of 2019. Moreover, the Directive addresses the issue of biodegradable and compostable plastic bags. Starting from 2011, organic fraction of municipal solid waste (OFMSW) must be contained in compostable bags made with biodegradable plastic resins or paper fibre (1). Due to increasing urbanization, the current global municipal solid waste production is very high, reaching approximately 1.3 billion tons per year (1.2 kg per capita per day) and is expected to increase to about 2.2 billion tonnes per year by 2025. Bio-waste comprises 46% of organic fraction (food debris, yard waste, wood, process residues), followed by 17% paper, 10% plastics, 5% glass 4% metal and 18% others (7). Due to its high moisture content

(approximately 80-95%, (8)) and organic matter content, municipal solid waste (MSW) management is typically associated with landfilling, thermal treatment, composting and open dumping. Anaerobic digestion is a method for the treatment of MSW that turns organic matter into easy to collect biogas (i. g. methane and carbon dioxide) and nutrient rich digestate that can be used directly or composted before use in agriculture. From an energy perspective, anaerobic digestion is a preferable method for treating the OFMSW, operating with a hydraulic retention time (HRT) of 15-30 days under thermophilic or mesophilic conditions (9). Composting is a post treatment which avoid possible health risks due to pathogens before land application (10). Therefore, bioplastic bags suitable for collecting food waste or compostable bioplastics should be digested within these conditions and include some requirements such as tensile strength and water resistance.

However, the Italian Composting Network (CIC) (Centemero et al., 2017) conducted a survey which has shown that almost half of the bags delivered to the anaerobic digestion and composting plants for OFMSW are still petro-based conventional plastics. Plastics adulterate the OFMSW and cause problems related to operation, maintenance and process efficiency (11,12), but also provide phthalates contamination which negative affects quality of the digestate (13). Plastic bags cause loss of fraction and lower biogas generation by wrapping around moving equipment parts, wearing out the pumps and valves or forming a top floating layer in the bioreactors (7). According to the literature, 1 to 4 mm of plastics (average 1.9% of compost dry weight) was found in samples of MSW compost after sieving, whereas for larger pieces the percentage was from 3.5 to 6.6% of the compost dry weight (14). Since the appearance of petro-based and bio-based plastics is similar it's difficult to clearly distinguish them and the widespread distribution makes them become part of other different and mixed wastes. On the other hand, further researches are needed to evaluate the fate of bioplastics delivered to other types of processing plants. According to the EN 13432 regulation, materials must indeed meet some prerequisites in order to be declared compostable. The most relevant ones are the following: (i) at least 90% degradation in weight in 6 months in carbon dioxide-rich environment; (ii) at least 90% of mass loss of the selected material, with fragments less than 2 mm if in contact with

105 organic materials for a period of 3 months; (iii) the materials must not cause negative effects on the
106 composting process; (iv) heavy metals present in the final compost must not exceed specified
107 standards.

108 ISO 13975 is a standard method to assess biodegradation of bioplastics under anaerobic
109 digestion system for a maximum of 90 days. On the other hand, ISO 20200, ISO 16929 and EN 14806
110 are standards to evaluate biodegradation under aerobic composting conditions which last from a
111 minimum period of 45 days or 12 weeks. The tests however cover long periods that not always reflect
112 real plants conditions. Time, in addition to temperatures and microbial strains involved, is a crucial
113 point that can effect biodegradation process (9). In other words, standards for biodegradability exist,
114 but more research is needed to assess the fate of compostable plastics under the conditions of an
115 active biogas plant and improve biodegradation processes.

116 The aim of this paper is to evaluate the fate of biodegradable and compostable bioplastics
117 products under real conditions of a typical anaerobic digestion and composting plant. Common
118 starch-based bags for the collection of OFMSW (SBB) and compostable water bottles made with
119 poly(lactic acid) (PLA) were employed in the final form of use in order to better simulate the real
120 situation. CH_4 production and mass loss were measured during 23 days of anaerobic digestion (55
121 °C) and the quality of the compost obtained after 21 days of aerobic treatment (65 °C) was assessed.
122 According to the literature, not many studies have been carried out on ecotoxicity of degraded
123 bioplastics (15).

124

125

126 **2. Materials and Methods**

127 *2.1 Materials*

128 The anaerobic sludge used as inoculum was collected from a thermophilic anaerobic digester working
129 as a continuous reactor for organic fraction of municipal solid waste (OFMSW) in the North of Italy.
130 The inoculum was used fresh immediately after sampling.

131 Food waste was collected from a local fruit and vegetable market. Food waste composed by
132 ripe lettuce and mouldy courgettes (1:3 ratio respectively) was blended with a blender and diluted to
133 slurry to obtain a concentration of 110.53 g of food per L of water (16).
134 Cellulose filter paper (Whatman No. 4, Thermo Fisher Scientific) was used as positive control
135 since its full biodegradability is well known (17,18), while low-density polyethylene (LDPE) film
136 from common packaging was used as non-biodegradable negative control. The investigated materials
137 were starch-based bioplastic (SBB) film from organic waste bags and polylactic acid (PLA) from
138 compostable water bottles. In order to simulate real conditions both degradable/biodegradable plastics
139 were commercially available, and all the materials were cut into pieces 20x20 mm. All tests were
140 carried out in biological triplicates.
141
142 *2.2 Anaerobic digestion*
143 Food waste blend was mixed with inoculum at a 1:10 ratio into 250 mL vials under anaerobic
144 conditions in a glove box (Ruskinn Concept 400, Baker, UK). Ten 20x20 mm pieces of material were
145 weighted and added separately in each vial (20 vials for every treatment for a total of 80 samples).
146 The ratio of the mass of the test materials to the total mass was approximately 0.32% for cellulose,
147 0.23% for LDPE, 0.010% for SBB and 1.55% for PLA. Although dimensions and number of pieces
148 were the same for all the thesis, differences in ratio resulted due to diverse masses of the materials
149 used. The inoculated flasks were left for 12 hours in the anaerobic chamber to replace the oxygen
150 with the gas mixture (10% CO_2, 5% H_2, 85% N) and hermetically closed with butyl rubber septum
151 and crimped aluminium caps. The vials were placed in a static incubator at 55 ± 2 °C for 23 days to
152 reproduce the real conditions of a thermophilic plant. This timing was chosen because, according to
153 the literature, a typical biogas plant treating the OFMSW works with a hydraulic retention time (HRT)
154 of 15-30 days under thermophilic or mesophilic conditions (9).
155
156 *2.3 Composting*

157 On the 23rd day of anaerobic digestion, the residual vials were opened and filtered to separate liquid
158 component (wastewater) from the solid component (muds). The composting process started by adding
159 1:4 w/w of lignocellulose component to the muds. This support additive function is to give structure
160 and porosity to the mixture and to reduce moisture content (19). Aerobic degradation was carried out
161 in glass bottles incubated at a constant temperature of 65 ± 2 °C for 21 days. The glass bottles were
162 periodically moistened with water to keep the humidity constant, stirred and aerated.
163
164 *2.4 Biodegradation analyses*
165 Biogas production and loss of mass were determined to assess the anaerobic biodegradation. The
166 amount of biogas was measured in the headspace of the vials with a pressure-lock precision analytical
167 syringe (Restek, LA, USA). CH_4, H_2 and O_2 were analysed on the 3rd, 7th, 13th, 17th and 23rd day of
168 the anaerobic digestion by a gas chromatograph GC 7820A (Agilent Technologies, Irving, TX, USA)
169 with a thermal conductivity detector (TCD) and packed column in UltiMetal tubing (1.83 m, Agilent,
170 USA). Each measurement was taken in triplicate. At every GC analysis, the digestate was filtered
171 through a gauze in order to recover the 10 pieces of the different test materials. They were washed
172 with distilled water, dried to constant mass and weighted. According to Regulation UNI EN ISO
173 20200:2005 the degree of disintegration (*D*) is calculated, as a percentage, using the formula:

175 $$D = \frac{m_i - m_r}{m_i} \times 100$$

174 (1)

176 where: m_i is the initial dry mass of the test material and m_r is the dry mass of the recovered test material
177 during anaerobic digestion (20).

178 In order to validate the disintegration results, the weight loss percentage between the initial
179 solution (inoculum, food waste and test material) and the final compost at the end of the process must
180 be greater than or equal to 30% (21). The volatile solids decreasing (R) was calculated with the
181 following equation:

$$R = \frac{[m_i(DM)_i(VS)_i] - [m_f(DM)_f(VS)_f]}{[m_i(DM)_i(VS)_i]} \times 100 \quad (2)$$

where m_i denotes the initial mass of the wet solution before anaerobic digestion, $(DM)_i$ is the initial dry mass of the solution (as % of total mass) and $(VS)_i$ represents the volatile solids of the solution (as percentage of *DM*). The term m_f corresponds to the final dry mass of the obtained compost, $(DM)_f$ represents the final dried mass of compost (as % of total mass) and $(VS)_f$ is the volatile solids value of the obtained compost (as % of *DM*). The weight loss due to organic matter conversion was performed on the positive control with cellulose as test material.

2.5 Phytotoxicity tests on compost

For the evaluation of the laboratory composting process, bioassays with seeds were performed to assess any presence of phytotoxic agents and to measure potential environmental risks (22). Seeds exposure to different concentrations of soluble elements in compost-water solutions allows to verify compost toxicity (23). Phytotoxicity test (24) was carried out on the final composts from cellulose, LDPE, SBB and PLA treatments. The compost was mixed with sterile distilled water (ratio 1:4 w/w) and well mixed (25). The solution was diluted 10 and 100 times and pH was measured on all the mixtures. Lettuce seeds (*Lactuca sativa* L.) and tomato seeds (*Lycopersicon esculentum* L.) were used separately for the phytotoxicity test. Ten seeds were placed in a Petri dish (90 mm diameter) with filter paper moistened with 5 mL of the three different dilutions and distilled water was used as a positive control. Petri dishes were sealed with Parafilm to ensure closed-system and incubated for 6 days at 24 °C in the dark.

The following parameters were analysed according to the guideline (25): Seed Germination (SG); Root Length (RL) and Gemination Index (GI). SG is the equivalent to the percentage of germinated seeds at the end of the experiment and was calculated using the formula:

$$\%SG = \frac{\overline{n}}{3} \times 100$$

(3)

where $\bar{n}$ is the average of triplicates of germinated seeds in each Petri dish. RL was estimated using the root measurement system WinRHIZO. According to UNICHIM (2003), the seed was considered to be germinated when radicle was over 0.5 mm long. GI is the relationship between the average of triplicates of root lengths (the amount for each Petri) and the number of germinated seeds as it follows:

$$GI_{si} = l_s \times n$$

(4)

where: $l_s = \frac{\Sigma root\ lengths}{n}$ and n is the number of germinated seeds for each replicate. Germination index was calculated according to the literature (26,27) with the following formula:

$$\%GI = \frac{GI_{si}}{GI_c} \times 100$$

(5)

where: $GI_{si} = l_s \times n$ in each Petri dish and GI_c is the germination index of the controls, similarly calculated earlier.

2.6 Physicochemical analyses

Total solids (TS) and volatile solids (VS) for each treatment were measured at the beginning and at the end of the experiment according to *Standard Methods* (28). Moisture content (%MC) was calculated gravimetrically with the following formula (24):

$$\%MC = \frac{M_w - M_d}{M_w - M_c} \times 100$$

(6)

where M_w corresponds to the total weight of the sample (including the mass of the container); M_d is the total weight of the dried sample (including the mass of the container); M_c is the weight of the

container. Humidity was measured only on cellulose treatment (positive control) in order to validate the correct process of composting.

To visualize the effects on the surface area, LDPE and PLA pieces were observed under high vacuum by scanning electron microscope (SEM, Fei 250Esen Quanta Feg, Hillsboro, OR, USA). Cellulose and SBB weren't available after anaerobic digestion and SEM analyses could not be performed. Samples were dehydrated in ethanol/water mixtures, with increasing ethanol concentration (75%, 85%, 95% and 100%). After critical-point drying in BAL-TEC CPD 030 dryer, samples were coated with gold. SEM analysis was performed at the beginning of the experiment, after anaerobic biodegradation and after composting.

In order to assess the thermal properties, differential scanning calorimetry (DSC) and FT-IR/ATR spectroscopy were performed on the pristine LDPE, SBB and PLA samples and after the two processes of anaerobic degradation and composting. The differential scanning calorimetry (DSC) analyses were done on a DSC Q20 (TA Instruments). The samples (about 8 mg) were heated at 10C/min under nitrogen from 0 to 200 °C Fourier Transform InfraRed (FT-IR) spectroscopy was used to monitor the chemical structure of the plastic materials using a Frontier FT-IR/ATR spectrophotometer (16 scans and 4 cm-1 resolution, Perkin Elmer).

3. Results and discussion

3.1 Lab-scale experiment setup

The variability of OFMSW's composition is a crucial factor that influences the digester operating parameters, making generalization about composition of the feedstock very difficult (29). *Moreover, food waste structure is heterogeneous, varying from region to region* (30) *and also depending on the seasonality* (31). *However, in Italy 82.7% of the waste sent to the anaerobic/aerobic integrated treatment of OFMSW consists of wet fraction from kitchens, canteens and markets* (32). *Here, the organic fraction was reproduced using lettuce and courgettes as representative of consumption*

256 *habits and mixed with anaerobic sludge as inoculum. The variability of the organic waste must be*
257 *taken into account as a limiting factor, especially for lab-scale tests.*
258 *As recently reported in the last Utilitalia's Position Paper, bioplastics production is moving towards*
259 *products other than bags for the collection of the OFMSW that introduce new issues in the treatments*
260 (33). *In this report, the need for tests and trials on these bioplastics under real-plant conditions is*
261 *stressed because the presence of these intact products in the organic waste management system is*
262 *one of the main challenges today. This was also reported in the literature* (1). *In this work, common*
263 *articles made of two different bioplastics were used. This resulted in different mass proportions due*
264 *to their different weight, which was much higher for the thicker PLA bottles. Equal dimensions and*
265 *equal surfaces were used to better estimate SBB and PLA biodegradation according to the literature*
266 (16,21,34,35). *The lab-scale reproduction and the necessity to avoid differences in available surfaces*
267 *did not allow to equal the mass proportions of tested bioplastics.*
268
269 *3.2 Biogas production and weight loss during anaerobic digestion*
270 Figure 1 shows the $\%CH_4$ production under anaerobic digestion related to time. The GC
271 measurements were taken in triplicates during static incubation at 55 ± 2 °C. The 4 tested materials
272 (cellulose, LDPE, SBB, PLA) blended with inoculum and food waste slurry are represented in
273 different colours. Bars followed by the same minor letter on each day were not statistically different
274 from each other (Tukey's test, $P < 0.05$). Cellulose (positive control) showed the highest percentage
275 of CH_4 production starting from 24.28% after 3 days, up to 48.39% at the end of the process. LDPE
276 (negative control) produced from 24.79% to 42.15% of CH_4 during the 23 days of anaerobic
277 digestion. SBB material was found in-between the two controls, producing 24.48% of CH_4 on the
278 third day until 44.56% on the twenty-third day. PLA showed a different evolution, starting from
279 13.13% after 3 days of incubation and proceeding slowly to 43.64% of CH_4. PLA treatment was
280 statistically different from the others on the 3rd and on the 7th day of anaerobic digestion, while on the
281 13th day was statistically equal to LDPE treatment. The $\%CH_4$ production for SBB and PLA

282 treatments is similar at the end of the process. As expected, LDPE production was statistically
283 different from cellulose. The methane production at the end of the process is between 40 and 50% for
284 all the test materials. In a similar study, from 58 to 62% of CH_4 was produced under the same
285 conditions (36). Anaerobic digestion involves a complex ecosystem of anaerobic bacteria and
286 methanogenic archaea (37). Microorganisms convert organic waste into 60-70% methane, 30-40%
287 carbon dioxide, traces of hydrogen and hydrogen sulphide as biogas (38). Since the OFMSW has
288 always been an attractive substrate for production of methane (39), biodegradation can be assessed
289 through the measurement of CH_4 production (40).

290 In order to recover the 10 pieces of the different test materials, the digestate was filtered through
291 a gauze at each GC analysis. Samples were washed with distilled water, dried to constant mass and
292 weighted. Table I presents the average ± the standard deviation of disintegration degree (%D)
293 calculated according to Eq. (1) during 23 days of anaerobic digestion. Data are expressed in
294 percentage as average of the three replicates for each test: cellulose, LDPE, SSB and PLA. Even at
295 three days test statistically significant differences were found between the materials. PLA
296 performance was similar to LDPE because the loss of weight didn't occur; quite the opposite they
297 increased their heaviness due to biofilm formation on the surface. As long as the digested residues
298 sticking to the surfaces, the disintegration degree for PLA and LDPE test materials was negative
299 (average of -0.42% and -1.69% respectively at the end of the anaerobic process) because the debris
300 could not be removed without damaging the remaining parts of the samples. LDPE was found
301 completely intact after 23 days of experiment, while PLA appeared wrap up and not biodegraded, in
302 accordance with literature evidence (41). As a matter of fact, PLA films shrink when the material is
303 close its melting temperature (42) and was previously found not to degrade under anaerobic
304 conditions (43,44). SBB behaviour during the anaerobic digestion was statistically different for each
305 measurement: the remaining pieces were recovered in the form of tiny powder at the end.
306 Disintegration degree of SBB had an average value of 85.79% after 23 days of anaerobic digestion.
307 According to literature, for temperatures lower than 37 °C the percentage of biodegradation does not

exceed 45%, independently on the test duration (43,45). Vice versa, at temperatures equal or higher than 58 °C biodegradation of tested bioplastics reaches between 80 and 95%. Moreover, for cellulose treatment no differences were found during the test because it was already digested at the first measurement (%D was 100%).

3.3 Phytotoxicity tests on compost

In order to provide a compost free of substances which could be a source of pollution for the environment, its safety must be assessed. Phytotoxicity tests were carried out on the final composts obtained from the 4 treatments and on their corresponding dilutions, while distilled water was used as control. Cellulose, LDPE and SBB initial solutions and respective dilutions had a pH close to neutrality (6.40 – 7.80), while PLA mixtures had an acid pH (2.72 – 3.40). pH values from cellulose, LDPE and SBB treatments were included in the range (6.0-8.5) indicated by Vaverková et al. (2012). Moreover, at the end of the process the values must fall nearby the neutral or alkaline pH according to the Norm (46). On the contrary, PLA solutions resulted in low pH values that certainly affected the characteristics of the compost, causing inhibition of microorganisms (Castro-Aguirre, Auras, Selke, Rubino, & Marsh, 2017). As is known, water-soluble lactic acid from degraded PLA can change the pH of the exposure environment affecting the rate of hydrolysis (48). Lettuce seeds (*L. sativa*) and tomato seeds (*L. esculentum*) were used to assess compost quality. Figure 2 represents the distribution of Seed Germination rate and Germination Index calculated according to Eq. (3), (4), (5). Bars followed by the same minor letter for each solution were not statistically different from each other (Tukey's test, $P < 0.05$). SG on tomato (Figure 2a) shows differences between undiluted solutions and the diluted ones. Especially for undiluted PLA treatment the negative effect on the number of germinated seeds was evident: SG clearly rose with increased dilutions values. PLA undiluted solutions resulted in 7% of germination rate while 1:10 and 1:100 dilutions resulted in 43% and 90% respectively. SBB treatment demonstrated a similar or even better response than cellulose with 70-93% of germination. SG on lettuce (Figure 2b) shows an evident negative response for PLA

compost (decreasing while the dilution increased). Indeed, for undiluted and 1:10 solution no germination occurred, whereas the most diluted one raised the number of germinated seeds up to 87%. Comparing each solution, composts from other treatments similarly influenced the germination rate, but differences between undiluted and diluted solutions were clear. The controls (distilled water) gave 93% of germination rate for tomato seeds and 77% for lettuce seeds (data not shown). Figure 2c shows the Germination Index on tomato seeds related to the control. This index is considered a strong measure of the phytotoxicity of compost (49). The only statistically significant difference was found for undiluted PLA treatment (0.44% GI). SBB performance for undiluted and 1:100 diluted was even better than positive control (cellulose) with 139.46% and 65.28% GI respectively. Germination Index on lettuce (Figure 2d) carried out better response for 1:100 diluted SBB treatment than cellulose. On the contrary, LDPE influenced negatively the compost, enhancing the GI with the increase of the dilution. PLA effect on seed germination was unfavourable for all the solutions, except for 1:100 dilution. Due to the low pH values, the solutions from PLA treatment performed negative effects on both selected seeds during the phytotoxicity test. Water-soluble lactic acid as degradation product from PLA can change the acidity of the environment affecting the rate of hydrolysis and also the growth of microorganisms. According to the literature, not many studies have been carried out on ecotoxicity of degraded bioplastics (15). Further analyses are needed to assess the impact of these increasing materials on the market and within the organic waste. As a matter of fact, a higher concentration of bioplastics in the organic waste could be a relevant issue in ecotoxicity and an important aspect to keep monitored.

The final composts were also visually observed. Visual analysis as inspection of the surface changes in the materials can confirm the results obtained with other methodologies (45). Cellulose (positive control) wasn't found at the end of the experiment, while 2x2 mm LDPE pieces (negative control) were recovered completely intact. Since plastics particles in compost is a major contamination problem, different separation mechanisms can be used. SBB wasn't distinguishable in the final compost, while PLA that was found in the form of tiny crystallized pieces (< 2 mm). PLA

360 is susceptible to hydrolysis due to atoms other than carbon, such as oxygen and nitrogen (41). For
361 this reason, random non-enzymatic chain scission of the ester groups leads to reduction in molecular
362 weight and biodegradation. The degradation rate increases as the relative humidity of the exposure
363 conditions increases (50). Indeed, the higher the rate of hydrolysis, the more available sites there are
364 for microbes to attack and faster is the biodegradation. The rate of diffusion of water in the
365 anamorphous regions controls polymer hydrolysis (51). For crystalline regions water diffusion is
366 insignificant, whereas PLA do not biodegrade without prior hydrolysis.
367
368 *3.4 Physicochemical analyses*
369 According to Eq. (2), the disintegration value of the positive control (cellulose) was 31.05% after 23
370 days of anaerobic digestion and 21 days of composting. The percentage of dry mass was adequate as
371 the relevant Norms recommended. This outcome suggested that the process developed properly
372 reducing the amount of organic matter.
373 Table II shows the values of total solids (TS) and volatile solids (TV) of the mixtures before the
374 anaerobic digestion and after the composting process. The final content of volatile solids for compost
375 from cellulose (positive control) was slightly lower than before anaerobic digestion, indicating that a
376 part of organic matter has been transformed into carbon dioxide. On the contrary, LDPE (negative
377 control), SBB and PLA treatment showed an increase in volatile solids, meaning that the organic
378 matter has not been transformed.
379 In order to demonstrate the successful composting process, moisture content was calculated
380 according to Eq. (6) on the final compost from cellulose treatment (positive control). %MC content
381 results was 62,40%, which is include in the range indicated by Adamcová & Vaverková (2014).
382 Humidity is a key factor in composting process and affect changes in oxygen diffusion, water
383 potential and water activity, and microbial growth rate (53).
384 Figure 3 shows SEM images of LDPE and PLA with and without biofilm at the initial time, after
385 23 days of anaerobic digestion and after 21 days of composting. LDPE pieces (negative control,

386 Figure 3a) with biofilm shows different microorganisms' colonization on the surface after anaerobic
387 and aerobic process. The plastic structure is not visible due to the high adhesion of the biofilm on the
388 material. Figure 3b shows LDPE without biofilm: no significant degradation phenomena were found
389 between the surface at the initial time and the final time (after composting). Moreover, some cellular
390 structures and microorganisms were still stick after anaerobic digestion and biofilm removal. PLA
391 with biofilm (Figure 3c) shows less microorganisms attached to the surface area than LDPE maybe
392 due to its resistance to microbial attacks in soil and sewage (6). The morphology of the microbes
393 seems to be also different from LDPE image (Figure 3a). After composting, no cells were found on
394 the PLA pieces, but the superficial structure showed crack formation, surface roughness and
395 degradation according to the literature (15). Relating these phenomena to the hydrolysis of the longer
396 polymeric chains, these was already observed on PLA after just 10 days of composting (54,55). Figure
397 3d shows PLA after biofilm removal: the surface presented cavities, erosion and lots of tiny holes,
398 whereas before degradation it was smooth.

399 Figures 4 a and b reported the DSC and FTIR of the LDPE materials. In LDPE T0 (Fig. 4a) it
400 is possible to see the presence of a single melting peak centered at 113 °C representative of a family
401 of chains with a certain distribution of lengths (the peak is a little widened), but nevertheless
402 attributable to very similar melting temperatures. In the FTIR spectrum (Fig.4b) the typical PE
403 absorptions were shown with only a small band at 1646 cm^{-1} related to a double bond (or aromatic
404 rings) probably due to some surface antioxidant additive. In fact, after anaerobic treatment this is
405 washed away or assimilated by bacteria and the spectrum becomes a pure PE. The DCS, on the other
406 hand, shows (green curve figure 4a) the appearance of a second melting peak at lower temperatures
407 (76 °C) evidence of the formation of a new family of chains shorter than the initial ones that were
408 ordered. It is likely that there may have been some fragmentation of shorter chains (probably side
409 chains) that then crystallized. The total crystallinity of PE in fact increases compared to the original
410 one. Finally, the aerobic treatment involves the verification of the two families of chains of different
411 length (melting T: 84 and 112 °C) while at the IR it is possible to see the appearance of wide bands

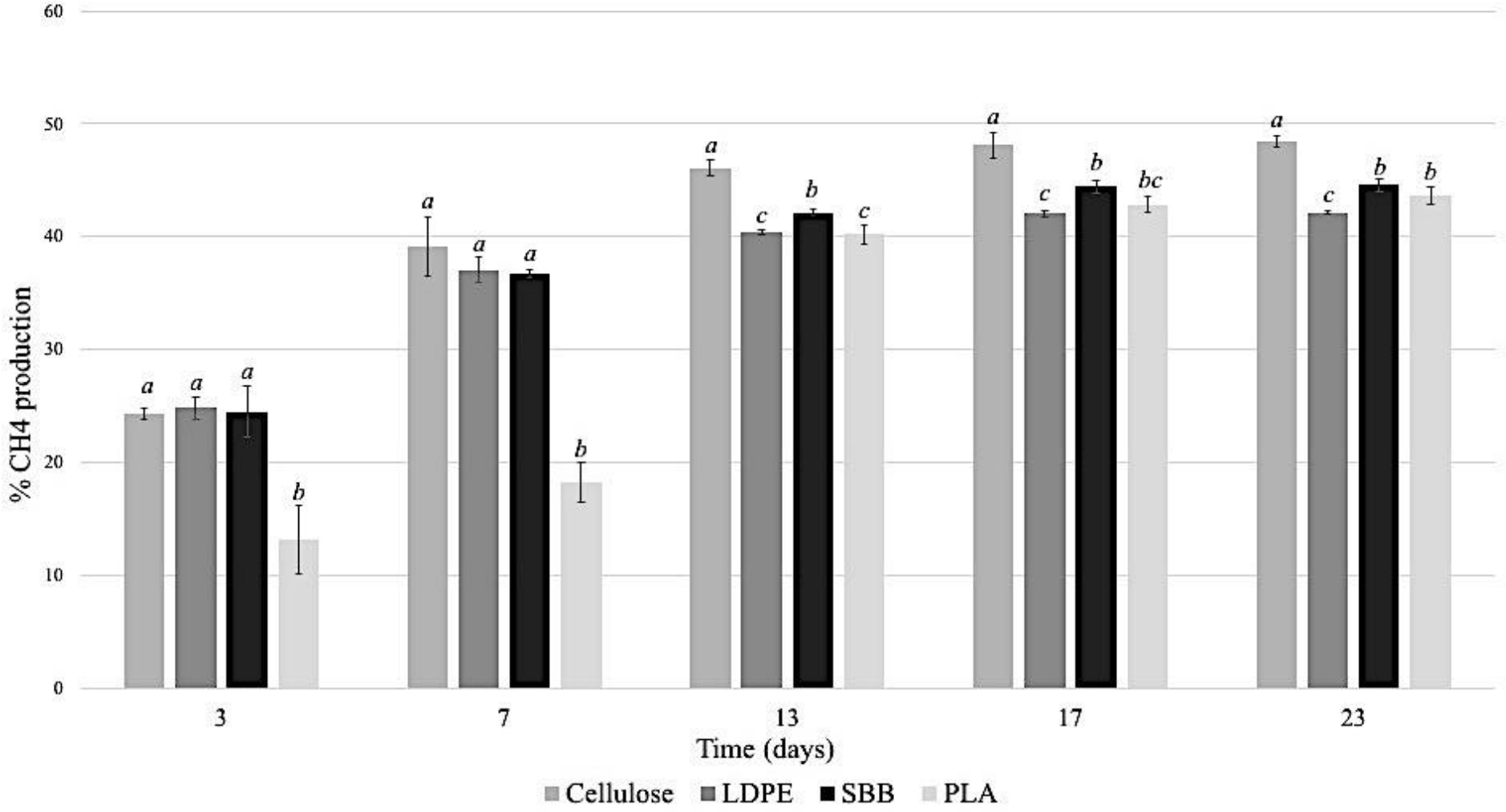
between 3400, 1700 and 1100 cm^{-1} that can be attributed to a small oxidation of the chains therefore with the formation of some C=O groups in the chain.

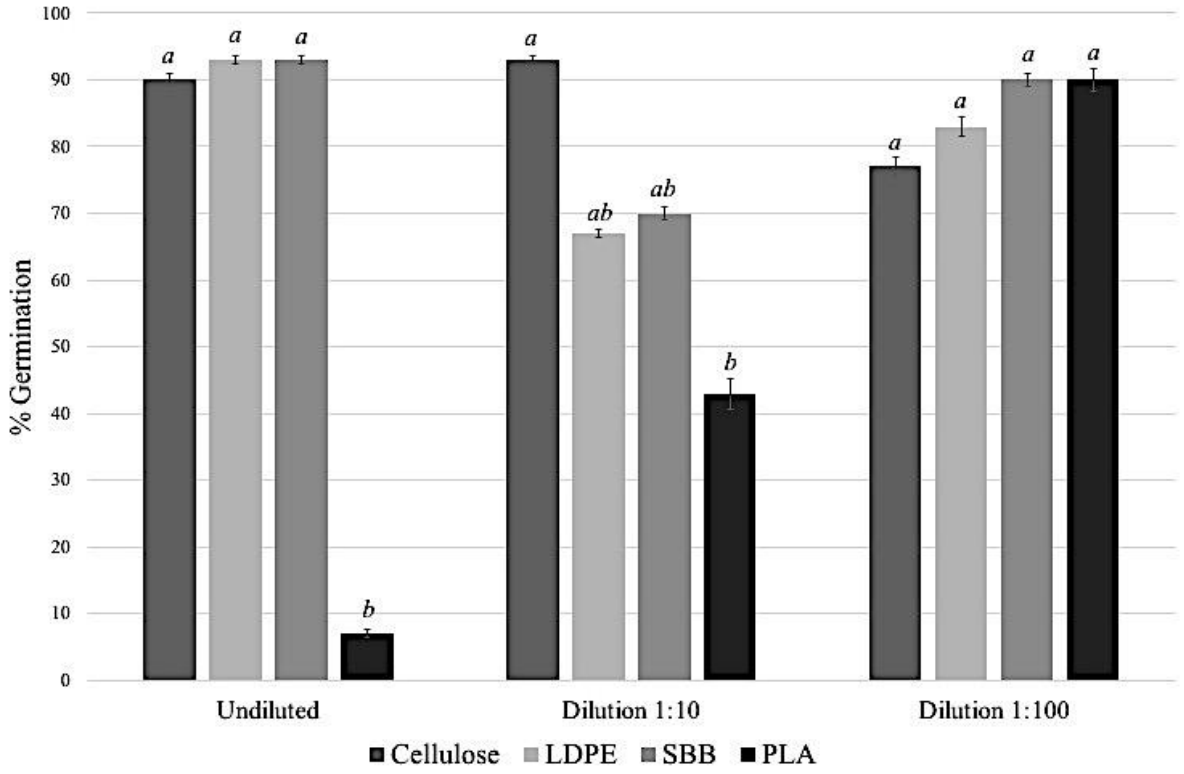
The DSC of the PLA at time 0 (Figure 5a) shows the polymer's Tg signal at around 65 °C, as expected, and melting at 153 °C. In this case a rather wide peak can be seen, symptom of a quite heterogeneous family in chain lengths however centered on that temperature. The ATR-FTIR spectrum (Figure 5b) shows the characteristic peaks of the polymer. After the anaerobic process in DSC there is a significant variation in the curve because, although the Tg remains at 65 °C, it can be seen that the melting peak is at a higher temperature of 156 °C and, above all, is much narrower, a symptom that there has been a reorganization of the chains with probably a degradation of the amorphous ones (and/or the shorter crystalline ones). The infrared spectra do not show any visible changes, so there should only have been a change in the length of the chains. After the aerobic treatment, the DSC shows an important change with the disappearance of the Tg (probably because many chains with different molecular weight have formed and each one vitrifies at slightly different temperatures so there is no unique Tg). But the most important change is certainly in the melting peak that drops to 131 °C, showing a significant decrease in chain length and also a very wide distribution of families. Obviously, since the chains are much shorter on average, they can be organized more easily and the total crystallinity of the system increases. From the FTIR investigation there are not so significant variations in the chemistry of the polymer chains (there are some small variations but in areas where different signals can fall and anyway these are minor vibrational modes). Generally, the whole spectrum decreases in intensity but this is mainly due to the fact that only a fragment and not a continuous film of the signal is missing.

The DSC of the SBB (Figure in Supporting Information) shows an almost amorphous material without a specific melting temperature. Also, after the anaerobic treatment the system remains predominantly amorphous even if the peak temperatures change a little bit. As far as the infrared spectra are concerned (Figure in Supporting Information), after the anaerobic process there is a

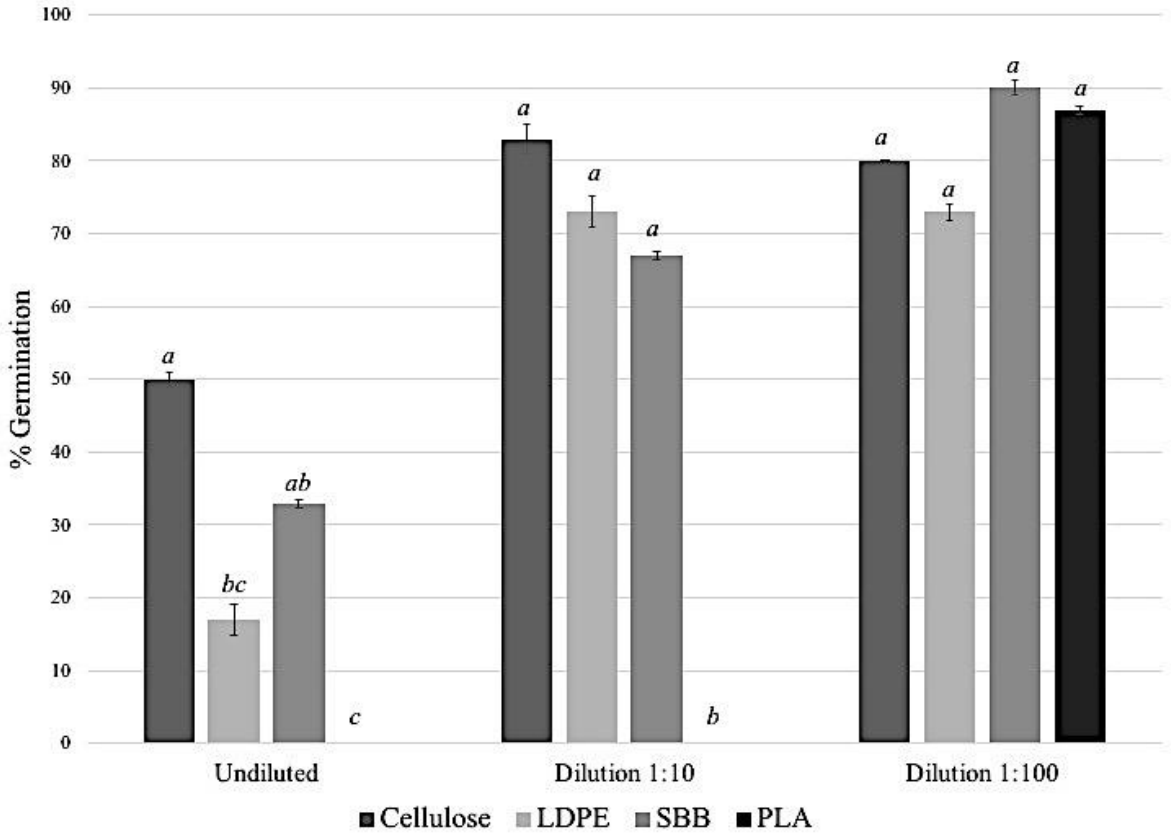
437 deconstruction of the system (many peaks lose resolution) both in the starch part (polysaccharide
438 rings around 1000 cm^{-1}) and in the polyester part (peak around 1700 cm^{-1}).
439
440
441 **4. Conclusions**
442 The results for methane production show differences for PLA treatment at the beginning of the
443 anaerobic digestion, whereas at the end of the process the $\%CH_4$ produced was between 40 and 50%
444 for all the tests materials. At the end of the anaerobic phase the disintegration degree (%D) for PLA
445 and LDPE was negative and similar due to biofilm formation on the surface which increased their
446 heaviness. PLA pieces appeared wrap up according to the literature, whereas the disintegration degree
447 of SBB had an average value of 85.79%. Test results obtained came to a different outcome for the
448 two bioplastics during anaerobic digestion. Due to the low pH values, phytotoxicity test on the final
449 composts showed negative effects on both selected seeds for PLA solutions. In fact, water-soluble
450 lactic acid as degradation product from PLA can change the acidity of the environment affecting the
451 rate of hydrolysis and also the growth of microorganisms. SBB treatment demonstrate a similar or
452 even better response than positive control. Chemical analyses confirmed significant results for PLA.
453 After the anaerobic process, the DSC showed a narrower and higher melting peak (156 °C) due to the
454 reorganization of the chains and the degradation of the anamorphous ones. After aerobic composting,
455 the DSC showed the disappearance of the Tg and the dropping of the melting peak to 131 °C. This
456 demonstrate a significant decrease in chain length and a very wide distribution of families. The
457 infrared spectra for SBB after anaerobic digestion showed a deconstruction of the system. Further
458 analyses are needed to assess the impact of these increasing materials within the organic waste. As a
459 matter of fact, the results here presented suggest that a higher concentration of bioplastics, in
460 particular PLA, in the organic waste can pose relevant issues in terms of materials recalcitrance to
461 biodegradation and ecotoxicity. Since the presence of PLA products is increasing on the market, the
462 effects of high concentrations of these manufacts on the OFMSW treatment, but especially on the

463 final compost, must be taken into account. It is thus important that these materials advertised by the
464 producers as “100% biodegradable” and/or “compostable” are tested under real plants conditions of
465 time and temperature and not only under standard ISO conditions.
466

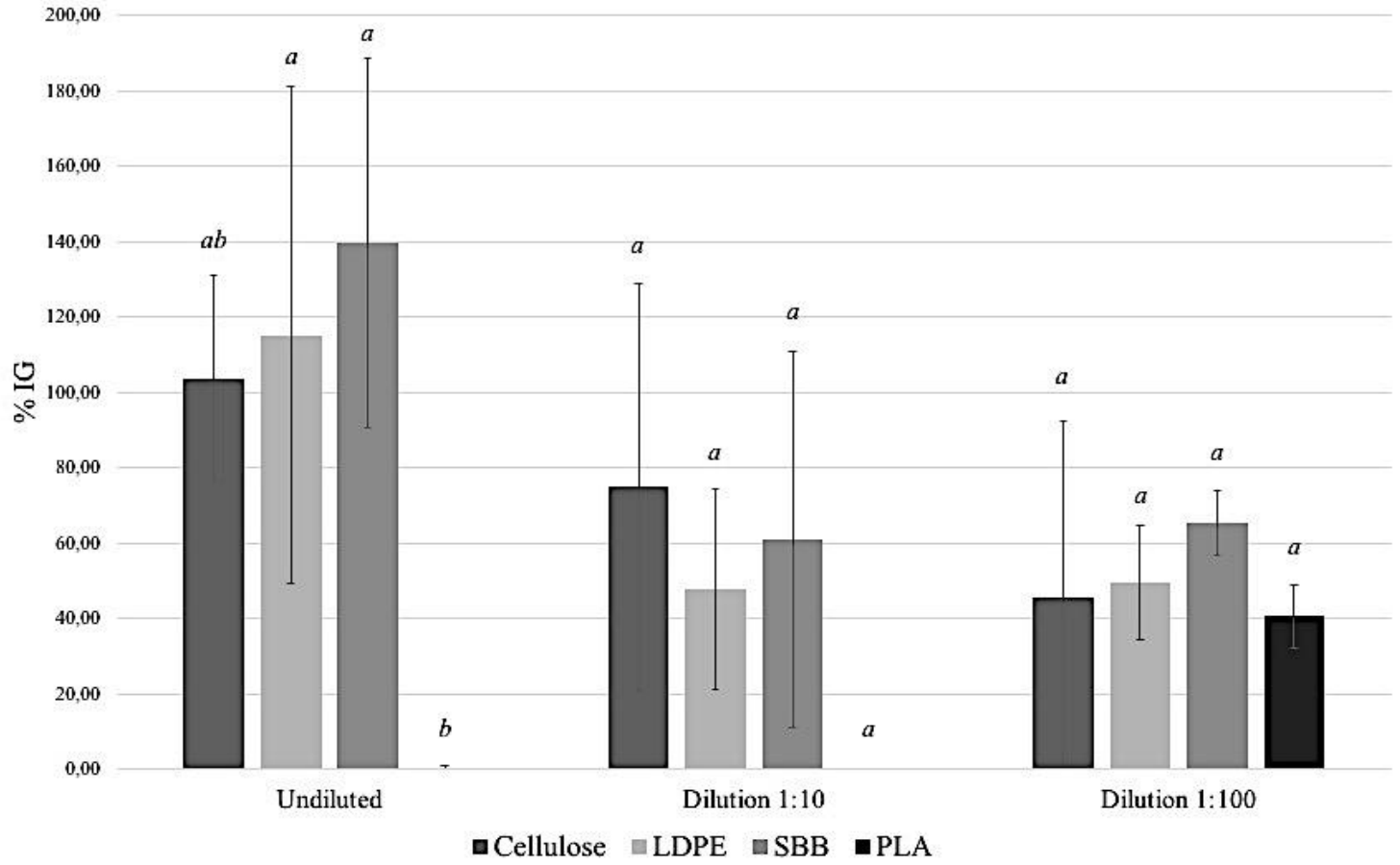

60
50
40
30
20
10
0
% CH4 production
a a a b
a a a b
a c b c
a c b bc
a c b b
3
7
13
17
23
Time (days)
Cellulose
LDPE
SBB
PLA


% Germination
100
90
80
70
60
50
40
30
20
10
0
a
a
a
b
a
ab
ab
b
a
a
a
a
Undiluted
Dilution 1:10
Dilution 1:100
Cellulose
LDPE
SBB
PLA

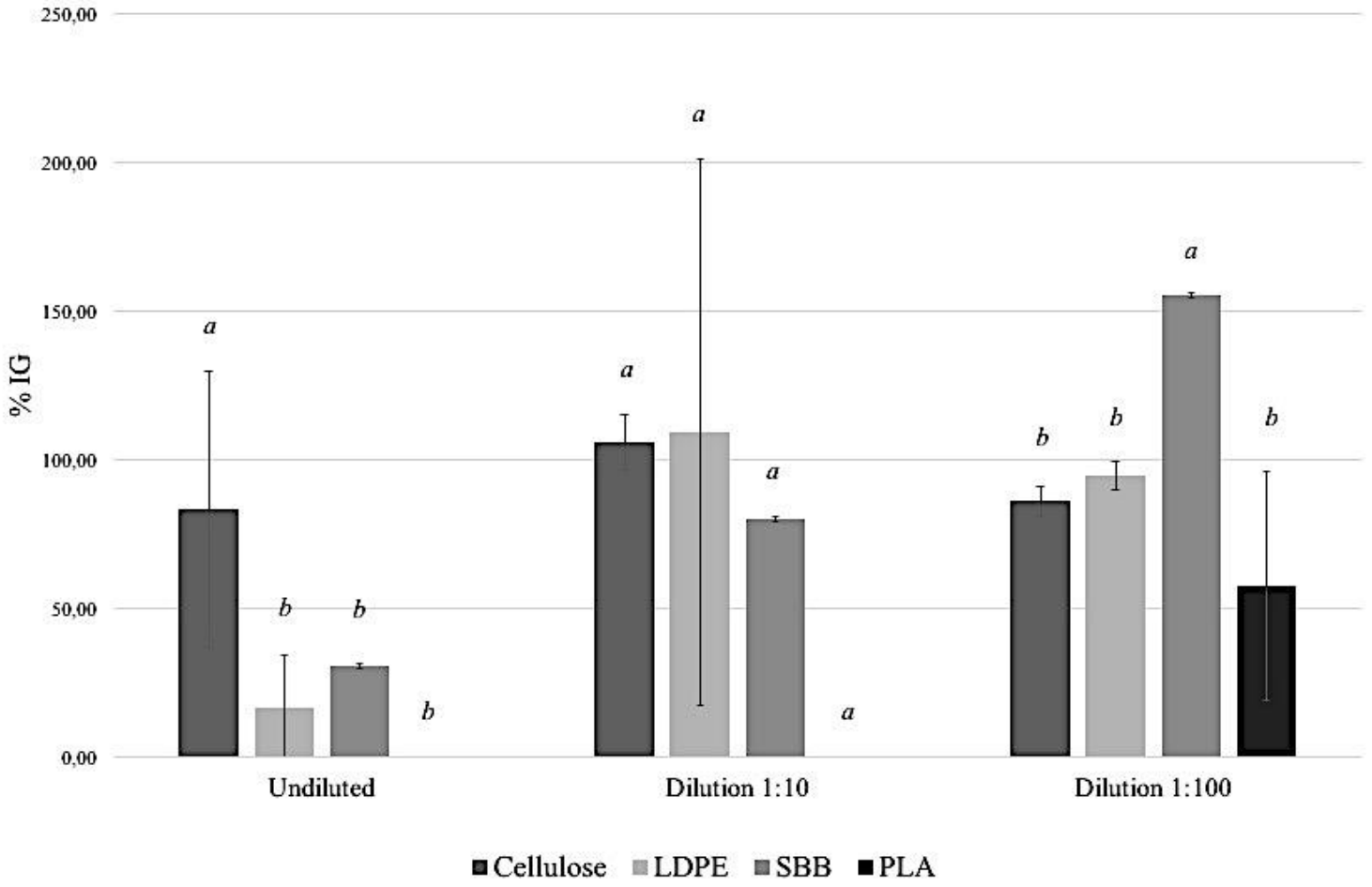
2a)


100
90
80
70
60
50
40
30
20
10
0
% Germination
a
bc
ab
c
a
a
a
b
a
a
a
a
Undiluted
Dilution 1:10
Dilution 1:100
Cellulose
LDPE
SBB
PLA

2b)


200,00
180,00
160,00
140,00
120,00
100,00
80,00
60,00
40,00
20,00
0,00
% IG
ab
a
a
b
a
a
a
a
a
a
a
Undiluted
Dilution 1:10
Dilution 1:100
Cellulose
LDPE
SBB
PLA

476 **2c)**

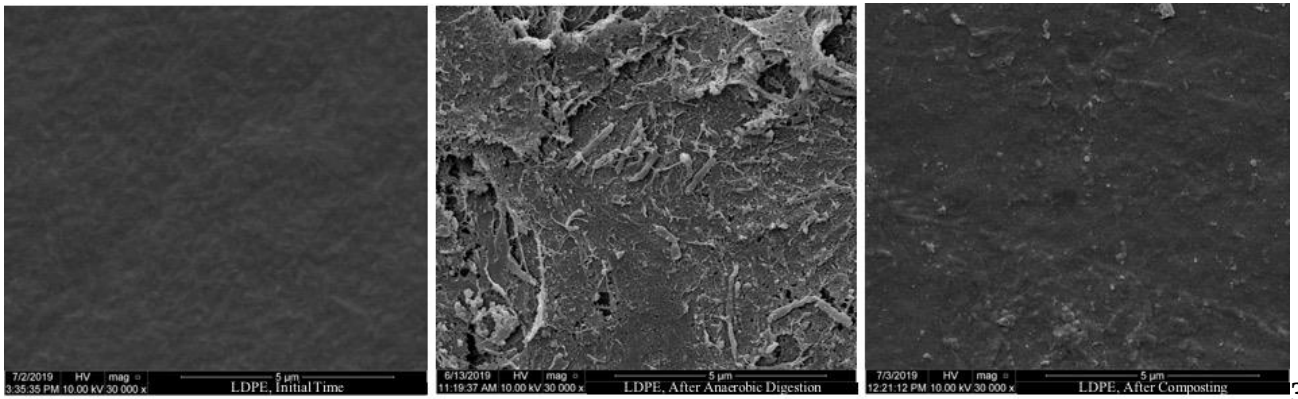

250,00
200,00
150,00
100,00
50,00
0,00
% IG
a
b
b
b
a
a
a
a
b
b
b
Undiluted
Dilution 1:10
Dilution 1:100
Cellulose
LDPE
SBB
PLA

2d)

480 

3a)

481

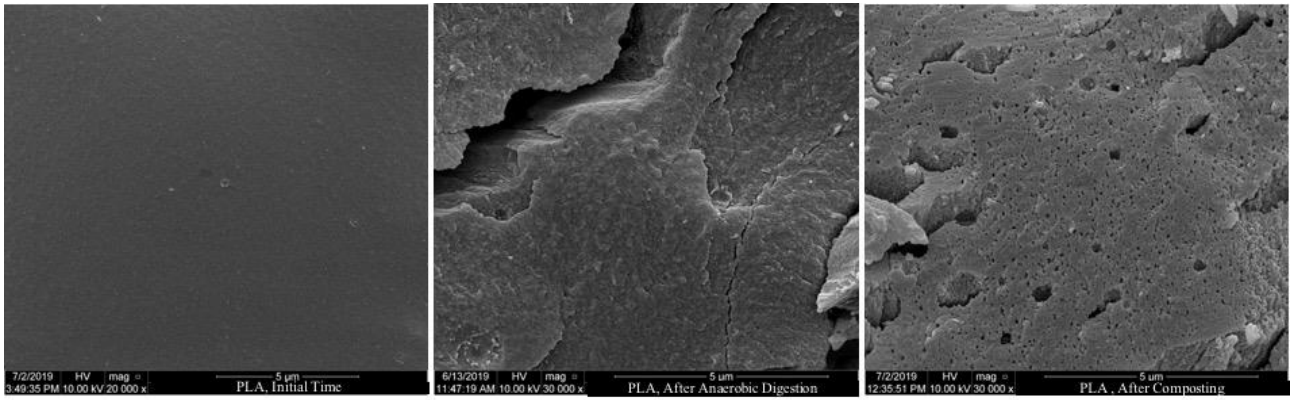
482 

3b)

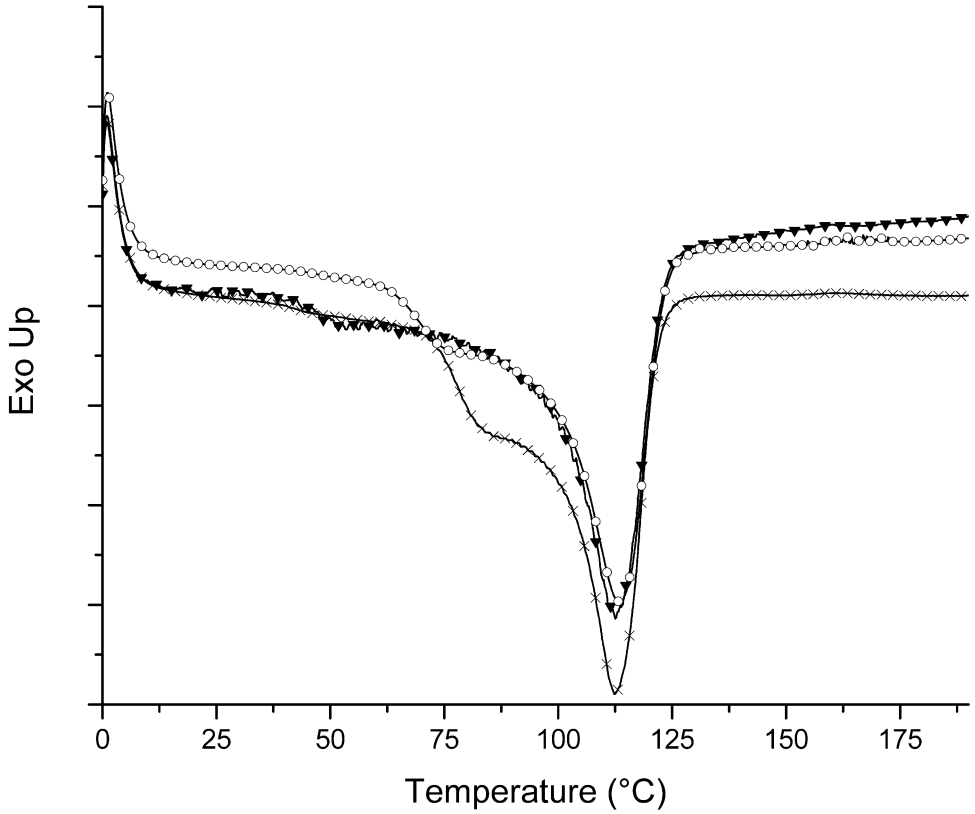
483


7/2/2019 HV mag
3:49:35 PM 10.00 kV 20 000 x
5 µm
PLA, Initial Time
6/13/2019 HV mag
12:26:59 PM 10.00 kV 20 000 x
5 µm
PLA, After Anaerobic Digestion
7/2/2019 HV mag
12:36:50 PM 10.00 kV 30 000 x
5 µm
PLA , After Composting

3c)

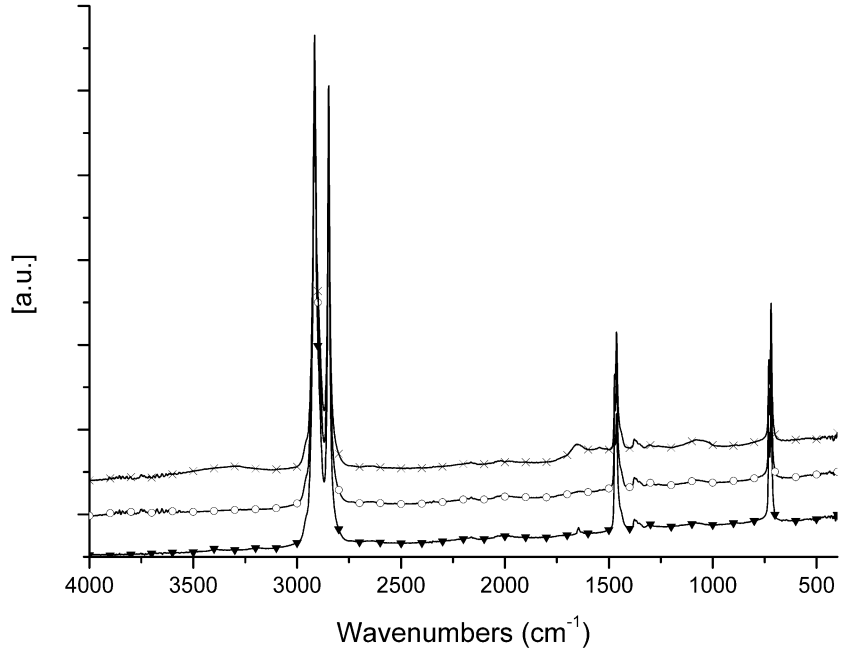

7/2/2019 HV mag
3:49:35 PM 10.00 kV 20 000 x
5 µm
PLA, Initial Time
6/13/2019 HV mag
11:47:19 AM 10.00 kV 30 000 x
5 µm
PLA, After Anaerobic Digestion
7/2/2019 HV mag
12:35:51 PM 10.00 kV 30 000 x
5 µm
PLA , After Composting

3d)



489

4a)



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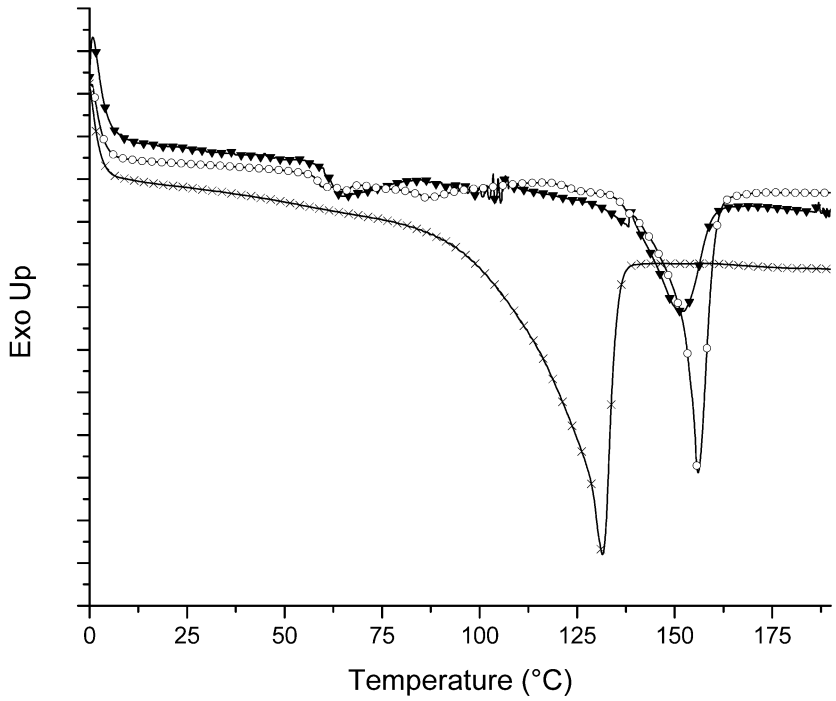
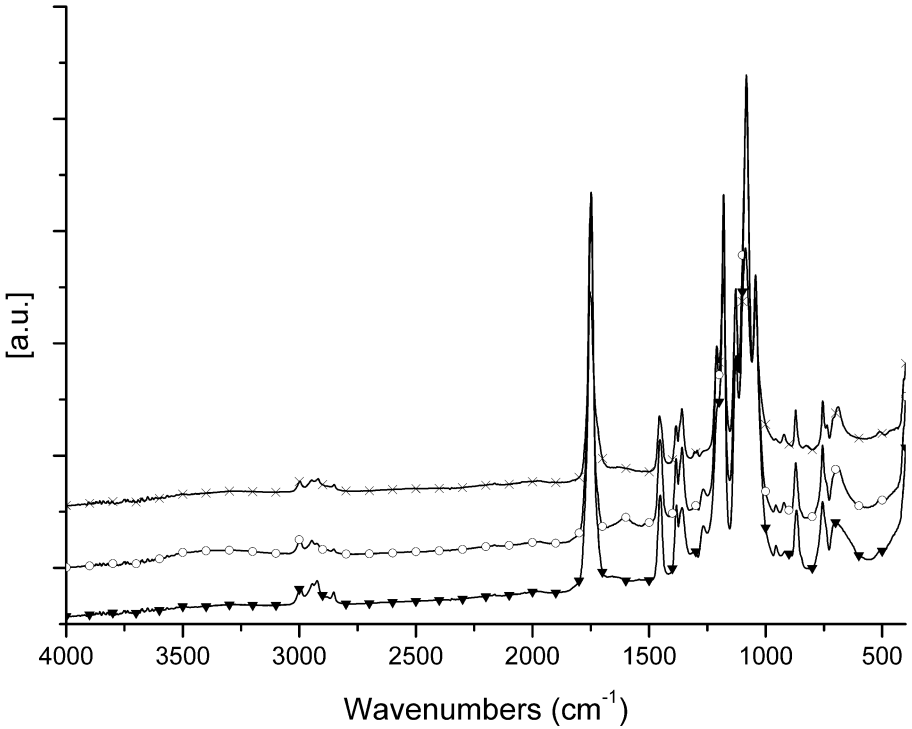
4b)



491 **5a)**

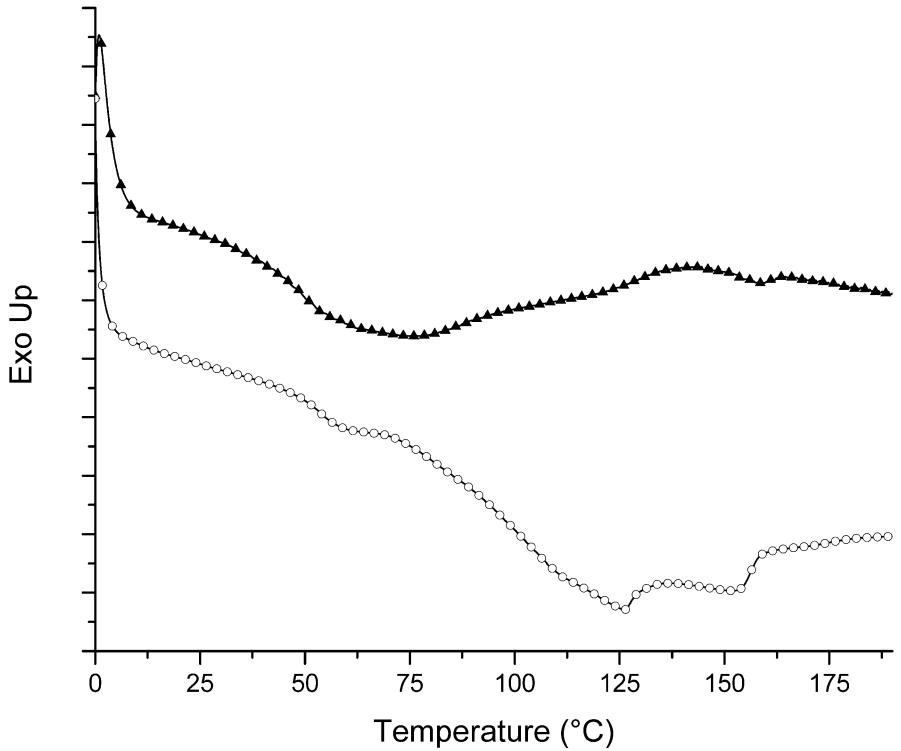
492



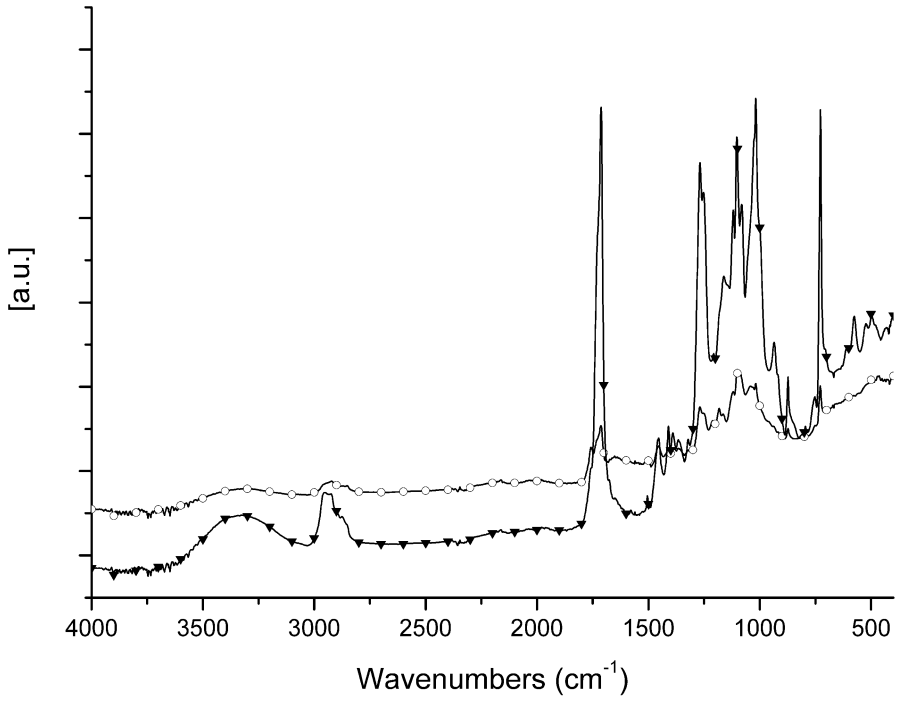
493

5b)

494 **Supporting information**



495 **a)**



496 **b)**

497

Figure captions

Figure 1. The %CH_4 production as a function of anaerobic digestion time for Cellulose (positive control), LDPE (negative control), SBB and PLA. Data are expressed as percentages of CH_4 produced. Bars followed by the same minor letter on each day are not statistically different from each other (Tukey's test, $P < 0.05$).

Figure 2. Seed Germination Rate (GR) and Germination Index (GI) on different composts obtained from cellulose (positive control), LDPE (negative control), SBB, PLA initial solution and diluted 10 and 100 times. a) GR on tomato seeds; b) GR on lettuce seeds; c) GI on tomato seeds d) GI on lettuce seeds. Data are expressed as percentage: bars followed by the same minor letter on each solution type are not statistically different from each other (Tukey's test, $P < 0.05$).

Figure 3. Scanning electron microscopy (SEM) images; pictures show representative fields selected among all the observations performed. a) LDPE (negative control) with biofilm at the initial time, after 23 days of anaerobic digestion and after 21 days of composting; b) LDPE (negative control) without biofilm at the initial time, after 23 days of anaerobic digestion and after 21 days of composting; c) PLA with biofilm a at the initial time, after 23 days of anaerobic digestion and after 21 days of composting; d) PLA without biofilm at the initial time, after 23 days of anaerobic digestion and after 21 days of composting. Cellulose and SBB weren't available after anaerobic digestion and SEM analyses were not performed.

Figure 4. Figure 4: DSC (a) and FTIR (b) curves of LDPE at initial time (▼), after anaerobic digestion (O) and after composting (X)

516 **Figure 5.** DSC (a) and FTIR (b) curves of PLA at initial time (▼), after anaerobic digestion (O) and after composting (X)**.**

517

518

Supporting information

Figure SI: DSC (a) and FTIR (b) curves of SBB at initial time (▼) and after anaerobic digestion (O)

Table I. Average ± standard deviation of degradation (expressed as percentage) during 23 days of anaerobic digestion. The reported data are the average of the three replicates for each test: cellulose, LDPE, SSB and PLA.

Data are expressed as percentages of degradation following Eq. (1). ANOVA significant differences were indicated by F values (*P < 0.05, **P < 0.01, ***P < 0.005) for comparisons between rows and columns for treatments and time. Data followed by the same minor letter on each column or by the same capital letter on each row are not statistically different from each other (Tukey's test, P < 0.05).

Days	**3**	**7**	**13**	**17**	**23**	***F-Value***
Cellulose	100 ± 0, aA	100 ± 0, aA	100 ± 0, aA	100 ± 0, aA	100 ± 0, aA	0***
LDPE	-0.16 ± 0.0005, cA	-0.24 ± 0, cA	-0.64 ± 0.003, cA	-0.54 ± 0.003, cA	-0.42 ± 0.001, cA	3.34
SBB	14.61 ± 0.012, bC	46.15 ± 0.254, bBC	75.95 ± 0.066, bAB	78.32 ± 0.045, bAB	85.79 ± 0.038, bA	18.13***
PLA	-1.05 ± 0.0039, cA	-0.87 ± 0.0045, cA	-1.43 ± 0.012, cA	-1.61 ± 0.002, cA	-1.69 ± 0.033, cA	0.13
F-Value	18132.58***	42.55***	730.26***	1669.67***	1395.97***	

Table II. Amounts (grams) of total solids (TS) and volatile solids (VS) for each treatment before anaerobic digestion and after composting.

	Treatments	Total Solids (g)	Volatile Solids (g)
Before Anaerobic Digestion	Cellulose	1.11	0.78
	LDPE	0.97	0.66
	SBB	0.82	0.51
	PLA	2.42	2.07
After Composting	Cellulose	0.63	0.58
	LDPE	0.97	0.91
	SBB	0.73	0.68
	PLA	2.27	2.21

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