PRELIMINARY EVALUATION OF THE ANAEROBIC BIODEGRADABILITY OF THREE BIOBASED MATERIALS USED FOR THE PRODUCTION OF DISPOSABLE PLASTICS

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Abstract

Biodegradable plastics have been introduced to the market to substitute "traditional", non-biodegradable, petro-based plastics to alleviate plastic pollution. Biochemical methane potential tests were carried out on compostable bags made of MaterBi®, biodegradable bottle wine corks and cellulosic plates to examine the anaerobic biodegradability of those materials. The impact of four factors: type of pretreatment (predigestion, mechanical, alkaline, predigestion and alkaline), digestion duration, type of inoculum and temperature were statistically evaluated through regression modeling. Anaerobic tests on compostable and polyethylene bags (control) were carried out in mesophilic (35 °C) and thermophilic (55 °C) conditions, while tests on bottle wine corks and cellulosic plates were carried out in mesophilic conditions only. After 15 days of digestion, a dry mass reduction of 22.8±6.2% and 27.6±14.0% for mesophilic and thermophilic tests respectively was recorded for MaterBi®. Chemical pretreatment with NaOH led to a mass reduction of 78.2±7.2% and was the only statistically significant factor to affect both methane yields and dry mass loss. A higher digestion temperature led to an increased mass loss without a concurrent increase in methane production. The cellulosic plates were completely degraded (99.9±0.03% mass reduction), while the wine bottle corks weight did not change.

Keywords

Anaerobic digestion, bioplastics, organic waste, biodegradable plastics, BMP

1. INTRODUCTION

Pollution of the marine environment by plastics is one of the most severe environmental threats humanity has to cope with [1], since around 7.8–8.2 million tonnes of discarded plastics enter the oceans every year [2]. Plastic fragments, varying in size from macrodebris (>20 mm) to microdebris (<5 mm) are one of the main causes of pollution of the world's oceans [3]. Seabirds, seals, whales, and turtles are just a few in the long list of affected wildlife species suffering from exposures to plastic fragments [4,5]. Moreover, it has been recently outlined how microplastics originating from plastic debris can enter the human food chain [1].

Ban/reduction of single use plastic items, separate collection and valorisation through material or energy recovery are the main options for solving the problem [1]. In addition to these policies, a more recent option is to substitute the "traditional" non-biodegradable polymers with biodegradable ones that are less persistent in the environment. These polymers and plasticizers should be readily degradable to prevent the continuous accumulation of plastic debris in terrestrial and aquatic environments [6,7].

Biodegradable plastics can have similar applications to fossil fuel based plastics [7] but their biodegradability renders them a sustainable solution [8,9] when discarded.

A plastic that can be biologically decomposed during a composting process at a similar rate to other compostable organic materials, without leaving visible toxic remainders, is classified as "compostable" [10]. Therefore, a compostable plastic is biodegradable whereas a biodegradable plastic is not always compostable [11,12].

The main factors affecting the biodegradation of plastics in the natural environment are their chemical structure, their polymer chain and the crystallinity and complexity of their polymeric formula. Generally, biodegradation of polymers with a short chain, an amorphous part, and a simple formula is relatively easy. Moreover, the environmental conditions in which the polymers are placed or disposed of (in particular pH, temperature, moisture and the oxygen content) play an essential role in their biodegradation [13].

Although biodegradable plastics, like Polylactic Acid (PLA) or Polyhydroxyalkanoate (PHA), require few years for degradation, their manufacturing cost is still high compared to that for conventional plastics. A feasible way to minimize cost is to blend them with natural bio-materials such as cellulose [2].

In Italy, since 01.01.2018, non-biodegradable lightweight plastic carrier bags are banned and have been substituted for compostable bags. Moreover, compostable bags are now required in Italy to deliver kitchen waste to the separate collection systems. The bags' compostability was considered sufficient to avoid any problem in the facilities treating organics and to ensure the quality of the digestate and compost that would be applied to farmland.

These bags must comply with the EN 13432 norm according to which lightweight plastic carrier bags need to possess the following characteristics:

- the material must be degraded (weight loss) by at least 90% in 6 months in an environment rich in carbon dioxide;
- at least 90% of the mass of the selected material must be reduced in fragments of less than 2 mm if in contact with organic materials for a period of at least 3 months;
- the presence of the material does not imply negative effects on the composting process;
- the amount of heavy metals present in the composted material must not exceed specified standards.

For anaerobic degradation, the rate of conversion of the substance to biogas has to be at least 50% of the theoretical value over a maximum period of two months.

Despite the sufficient knowledge on the aerobic degradation of biopolymers [12,14,15], research on their anaerobic degradability is still limited [16].

Therefore, we cannot be sure that the organic waste treatment facilities (composting and anaerobic digestion plants) already in operation can effectively manage bioplastics labelled as compostable. In addition, we do not know if the quality of compost/digestate is affected by the presence of bioplastic items, and if it fulfils the farmers' expectations (e.g. lack of visible pieces of bioplastics in compost/digestate). On the contrary, recently all these concerns have been raised by composting/anaerobic digestion plant managers in Italy [17].

The efficiency of biodegradation can be analysed through a number of tests (e.g. weight loss, analysis of the surface morphology of polymer after microbial degradation); these tests can be performed under both anaerobic or aerobic conditions [10].

Mohee et al. (2008) [39] examined the anaerobic degradation of MaterBi: the authors measured the cumulative methane production over 32 days of batch digestion assays and compared it to that of the reference cellulose filter paper (CFP). The plastic and CFP samples were cut finely to film sizes of 0.5–1.0 mm. The results showed methane yields equal to 125 and 126 NmL/g_{VS} for MaterBi and cellulose, respectively. In another study [40], a Y class (injection moulded rigid items made of thermoplastic starch and cellulose derivatives) MaterBi product was degraded by 90% under anaerobic conditions after 30 days.

Yagi et al. (2014) [41] evaluated the anaerobic biodegradability of four bioplastics powders (125-250 μm), polycaprolactone (PCL), poly(lactic acid) (PLA), polyhydroxybutyrate (PHB) and poly(butylene succinate) (PBS) under mesophilic condition (37 °C). The biodegradability of PHB, PLA, and PCL was 90% in 9 days, 29% and 49% in 277 days, respectively. PBS could not be anaerobically biodegraded by the sludge used as inoculum in that study.

98 The objective of this paper was to study the anaerobic degradation of some items designed to
99 substitute petro-based (i.e. fossil fuel based) conventional plastics (i.e. carrier bags, wine corks and
100 single use plates) under anaerobic conditions found in typical full-scale anaerobic plants. Some
101 experiments were specifically designed in such a way as to verify several management possibilities
102 for bio-plastic bags, since they are very common in Italy and are used to collect food waste.
103

104 **2. MATERIALS AND METHODS**

105 **2.1. Substrates, inocula and sampling**

106 The experiments were carried out in four series, using different substrates and inocula (Table 1). The
107 substrates used (Figure 1.SI) were conventional carrier bags made of polyethylene (PE), compostable
108 bags made of MaterBi® designed for delivering organics to household waste collection systems
109 (composed of at least 60% starch and starch derivatives and of approximately 40% synthetic resin
110 that is hydrophilic and biodegradable [16]) and are compliant with the EN 13432 norm. Additional
111 substrates were wine bottle corks, marked as produced with a biobased materials, and cellulosic
112 plates. Substrates were characterized in terms of total and volatile solids and COD [18], as shown in
113 Table 1.
114 To verify several management possibilities, the compostable bags were pre-treated either
115 mechanically (shredding) or chemically (alkali addition) or were subject to anaerobic digestion for
116 15 days and then dried and used over a second cycle of digestion. Mechanical pre-treatment
117 (Experiment 2) consisted of shredding so that to reach particles with a final dimension of 1x1 cm.
118 Chemical pre-treatment (Experiment 2) was performed using a solution of NaOH (50%, Sigma-
119 Aldrich) with a dosage of 5% of total solids and a duration of 24 hours. The tests carried out on pre-
120 digested bags (Experiment 3) were aimed to simulate a new cycle of anaerobic digestion of MaterBi
121 bags remainders that are recovered from digestate by physical means (e.g. screening). Finally,
122 chemical (alkaline) treatment was also applied to pre-digested bags (Experiment 3).

123 **Table 1.** Characterization of substrates and inocula

124

Experiment 0 – PE and compostable bags				
	TS[a] (%)	VS[b] (%)	pH[c]	COD[d] (mg/gTS)
Mesophilic Inoculum (MES_RAW_A)	5.3 ± 0.05	74.5 ± 0.01	7.9	n.a.
Thermophilic inoculum (TERM_A)	4.7 ± 0.01	72.2 ± 0.01	8.3	n.a.
LDPE carrier bags	99.9 ± 0.01	92.4 ± 0.01	n.a.	n.a.
Compostable bags (MaterBi)	97.7 ± 0.35	99.5 ± 0.02	n.a.	1421 ± 110.09
Experiment 1 - compostable bags				
Mesophilic Inoculum (MES_RAW B)	5.0 ± 0.001	61.8 ± 0.53	8.5	n.a.
Thermophilic inoculum (TERM_B)	2.3 ± 0.04	58.6 ± 0.36	8.8	n.a.
Compostable bags (MaterBi)	97.7 ± 0.35	99.5 ± 0.02	n.a.	1421 ± 110.09
Experiment 1 - bio-based wine corks and cellulosic plates				
Mesophilic Inoculum (MES_RAW C)	5.23 ± 0.01	72.9 ± 0.01	8.1	n.a.
Bio-based corks	99.9 ± 0.01	95.5 ± 0.14	n.a.	90.4 ± 6.36
Cellulosic plates	97.6 ± 0.10	99.6 ± 0.04	n.a.	1185[e]
Experiment 2 - compostable bags				
Mesophilic Inoculum (MES_RAW D)	4.20 ± 0.002	69.7 ± 0.003	8.0	n.a.
NaOH pre-treated compostable bags	99.9 ± 0.04	98.7 ± 0.19	12.3	n.a.
Experiment 3 - compostable bags				
Mesophilic Inoculum (MES_RAW E)	4.98 ± 0.001	74.28 ± 0.003	7.7	n.a.
Acclimated inoculum from exp. 2 (ADAPT)	2.83 ± 0.001	67.28 ± 0.024	7.9	
Pre-digested compostable bags	97.42 ± 0.003	99.45 ± 0.003	n.a.	n.a.
NaOH pre-tr. pre-digest. comp. bags	100.0 ± 0.1	97.8 ± 0.04	12.5	n.a.

125 [a]Total Solids; [b]Volatile Solids; [c]Chemical Oxygen Demand; [d]COD of pure cellulose

126

The mesophilic inoculum used in the experimental activity (MES_RAW_A/B/C/D/E; Table 1) was mainly represented by liquid digestate taken at different times from a full-scale plant fed with manure and other agro-waste or residues operating under mesophilic conditions. The digestate from this source has been being used as inoculum for the anaerobic batch tests, since years and has proved to be apt for digesting diverse substrates [19–23]. During the mesophilic tests, inoculum conditioning was reduced to the minimum; after collection, it was sieved to remove the remainders of fibrous materials (e.g. straw) and was then kept for 5-10 days under anaerobic conditions at 35°C to reduce non-specific biogas production before the experiments.

During the thermophilic tests, the inoculum (TERM_A/B) that was obtained from a real scale plant was subject to the same pre-treatment but was progressively acclimated to thermophilic conditions by increasing temperature from 35 to 55 °C at a rate of 1 °C per day.

In addition, during experiment 3, another inoculum (ADAPT) was used to verify a possible adaptation of the microbial consortium to the specific substrate. The digestate of experiment 2 was used as the ADAPT inoculum..

2.2. Biochemical methane potential (BMP) tests

Biochemical methane potential (BMP) tests were performed under mesophilic (35±0.5°C) and thermophilic conditions (55±0.5°C). They were carried out using a method based on Schievano et al. [24] that has been extensively used in previous experiments [20,25] and is compliant with the UNI/TS 11703:2018 norm recently introduced in Italy. The norm requires the use of three different nutrient solutions defined as Solution A, B and C respectively.

148 Solution A contains specified quantities of KH_2PO_4, $Na_2HPO_4 \cdot 12H_2O$, NH_4Cl, distilled water while
149 the amount to be used is 5% of the final volume of the mixture subjected to BMP test. Solution B
150 contains $CaCl_2 \cdot 2H_2O$, $MgCl_2 \cdot 6H_2O$, $FeCl_2 \cdot 4H_2O$, distilled water and the amount to be used is 5% of
151 the final volume. Solution C contains $MnCl_2 \cdot 4H_2O$, H_3BO_3, $ZnCl_2$, $CuCl_2$, $Na_2MoO_4 \cdot 2H_2O$,
152 $CoCl_2 \cdot 6H_2O$, $NiCl_2 \cdot 6H_2O$, Na_2SeO_3, distilled water and the amount to be used is 1% of the final
153 volume of the blend. Each set of experiments included blanks (used to assess the methane/biogas
154 production of inoculum, $V_{CH4,blank}$) and, as per UNI/TS 11703:2018 norm, a control comprised a
155 batch fed with pure cellulose only. The methane yield of the latter must be 325±25% NmL/g_{VS},
156 otherwise the experimental results are considered unreliable. Experimental conditions are
157 summarized in Table 2.
158 Bottles with three necks (two side necks, equipped with septa and the central main neck, volume 1.1
159 L, WTW-Germany), filled with the appropriate amounts of substrate and inocula (see Table 2), were
160 placed in a thermostatic cabinet at the appropriate temperatures (either 35±0.5 °C or 55 ±0.5 °C).
161 The contents (substrate and inoculum) were mixed by a magnetic stirrer throughout the test period.
162 Three times per week, biogas was slowly transferred with a 100 mL syringe into a second bottle (an
163 alkaline trap) containing 1 L of a 3M NaOH solution to capture the CO_2 present in the biogas. The
164 pressure increases in the alkaline trap provoked the displacement of an amount of the alkaline solution
165 that was transferred by a tube connected to another side opening in the bottle to a graduated
166 volumetric cylinder. The total volume of the alkaline solution displaced by the gas was considered
167 equal to the volume of methane present in the biogas. The volume of the carbon dioxide was
168 calculated by the difference of the methane volume from the total biogas volume.
169
170
171
172

Table 2. Experimental program (n=number of replicates)

		Inoculum Type	T[a] (°C)	F/M ratio[b] (VS_{sub}/VS_{in})	Working Volume (mL)	Expected duration (days)	TS[c] at the beginning of experiment(%)	Substrate added (gTS)	pH at the beginning of experiment
Experiment 0	PE bags (n=3)	MES_RAW A	35	0.5	400	15	4.7	13.3	8.0 ± 0.01
	Compostable bags (n=3)	MES_RAW A	35	0.5	400	15	4.6	13.3	8.0 ± 0.01
	PE bags (n=3)	TERM_A	55	0.5	400	15	4.0	11.6	8.3 ± 0.01
	Compostable bags (n=3)	TERM_A	55	0.5	400	15	4.0	11.6	8.3 ± 0.01
	PE bags (n=3)	MES_RAW A	35	0.5	400	30	4.7	13.3	8.0 ± 0.01
	Compostable bags (n=3)	MES_RAW A	35	0.5	400	30	4.6	13.3	8.0 ± 0.01
	PE bags (n=3)	TERM_A	55	0.5	400	30	4.0	11.6	8.3 ± 0.01
	Compostable bags (n=3)	TERM_A	55	0.5	400	30	4.0	11.6	8.3 ± 0.01
Experiment 1	Compostable bags (n=3)	MES_RAW B	35	0.5	400	15	2.6	2.48	8.5 ± 0.01
	Compostable bags (n=3)	MES_RAW B	35	0.5	400	30	2.6	2.48	8.5 ± 0.01
	Compostable bags (n=3)	TERM_B	55	0.5	400	15	2.6	2.37	8.7 ± 0.01
	Compostable bags (n=3)	TERM_B	55	0.5	400	30	2.6	2.48	8.5 ± 0.01
	Bio-based Corks (n=3)	MES_RAW C	35	0.3	800	44	3.5	4.27	8.1 ± 0.02
	Cellulosic plates (n=2)	MES_RAW C	35	0.3	800	44	3.5	5.05	8.0 ± 0.01
Experiment 2	Raw comp. bags (n=3)	MES_RAW D	35	0.5	400	15	3.5	3.68	8.4 ± 0.20
	NaOH pre-treated comp. bags (n=3)	MES_RAW D	35	0.5	400	15	3.5	3.68	8.7 ± 0.12
	Mechanically pre-treated comp. b.(n=3)	MES_RAW D	35	0.5	400	15	3.5	3.68	8.3 ± 0.08
Experiment 3	Raw comp. bags (n=3)	MES_RAW E	35	0.3	262	15	4.0	1.90	8.3 ± 0.02
	Pre-digested comp. bags (n=3)	MES_RAW E	35	0.3	262	15	4.0	1.91	8.2 ± 0.03
	NaOH pre-tr. pre-digest. comp. bags (n=3)	MES_RAW E	35	0.3	262	15	4.0	1.91	8.2 ± 0.04
	Raw comp. bags (n=3)	ADAPT	35	0.5	250	15*	2.0	1.90	8.4 ± 0.05
	Pre-digested comp. bags (n=3)	ADAPT	35	0.5	250	15*	2.0	1.91	8.4 ± 0.02
	NaOH pre-tr. pre-digest. comp. bags (n=3)	ADAPT	35	0.5	250	15*	2.0	1.91	8.5 ± 0.03

[a]Temperature; [b]Substrate to inoculum (Food to Microorganism) ratio; [c] Total Solids;

*The experiment was terminated on day 8, since no biogas was generated from day 3 to 8..

178 The following expression was used to obtain the net specific methane production after 30 days:

179

180 $$BMP_{30}\left[\frac{NmL\ CH_4}{g\ VS}\right] = \frac{\left(V_{CH_{4,s}} - V_{CH_{4,blank}}\right)[NmL\ CH_4]}{VS_s\left[\frac{g\ VS}{L}\right] \cdot V_s\ [L]}$$

181 where the difference represents the net methane production measured at the end of the test; VS_s is the
182 concentration of volatile solids of the substrate present in the bottle at the beginning of the test, and
183 V_s is the overall volume (L).
184 The duration of the batch tests was set either at 30 or 44 days, to quantify the final biomethane yield
185 of raw bags, plates and corks or to 15 days, since this latter is the most common hydraulic retention
186 time for anaerobic digestion plants accepting food waste in Italy.
187 The results of the experiments were the biogas and methane yields and the substrate dry mass losses.
188 That is, after 15 days, or at the end of the experiments (30 or 44 d), the substrates were removed,
189 sieved, rinsed, dried and weighed to quantify the amount of substrate biodegraded.
190 Only for Experiment 3 and for the batches carried out using MES_RAW_D volatile fatty acids (VFA)
191 at the end of experiment were evaluated [26]
192

193 **2.3 Experimental design and regression modelling**

194 The experimental design for the tests carried out on biodegradable MaterBi bags is included in Table
195 3.
196 Outliers were removed prior to statistical analysis. Outliers were considered the experimental
197 measurements that were outside the Q1-3xIQ to Q3+3xIQ range (with IQ being the interquartile
198 range, which is defined as the difference Q3 (75% percentile) - Q1 (25% percentile).

As a result of that removal, from an initial number of n=48 experimental measurements for methane yield, 40 data point were finally used in the statistical analysis (8 outliers were removed). In the case of the dry mass loss measurements, there were no outliers, so all n=42 data points were used to perform statistics. The following generic linear regressions model was used to estimate significant parameters:

$$\text{Response} = a + b\cdot\text{Sub\_Raw} + c\cdot\text{Sub\_Chem} + d\cdot\text{Sub\_Mech} + e\cdot\text{Sub\_Pred} + f\cdot\text{Sub\_Pred/Chem} + g\cdot\text{Temp} + h\cdot\text{Duration} + i\cdot\text{Inoculum} \quad (1)$$

where:

Response is the dependent variable, which is either the methane yield (NmL/g_{VS}) or the bioplastic dry mass loss (%); Sub_Raw: categorical variable which takes the value of 1 if the substrate is raw without any pretreatment and 0 in any other case; Sub_Chem: categorical variable which takes the value of 1 if the substrate was chemically pretreated (alkali) and 0 in any other case; Sub_Mech: categorical variable which takes the value of 1 if the substrate was mechanically pretreated (shredding) and 0 in any other case; Sub_Pred: categorical variable which takes the value of 1 if the substrate was predigested and 0 in any other case; Sub_Pred/Chem: categorical variable which takes the value of 1 if the substrate was predigested and chemically pretreated and 0 in any other case; Temp: categorical variable to indicate digestion temperature (mesophilic, thermophilic); Duration: categorical variable to indicate experiment duration (15 d, 30 d); Inoculum: takes values of 0, 1,2,3 , respectively, for the four types of inocula mentioned earlier. ; a-h: regression coefficients; a: constant; b, c, d, e, f, g, h, i: term coefficients.

During regression modelling, the best reduced models were calculated by sequentially removing the terms that were statistically insignificant (at $p<0.05$) from the initial most complex model.

224 **Table 3**. Experimental design used for biodegradable bags only

Substrate type	Inoculum type	Temperature	n*
Raw MaterBi bags	1 - (MES_RAW A)	Mesophilic	6
Raw MaterBi bags	2 – TERM_A	Thermophilic	6
Raw MaterBi bags	2 - (MES_RAW B)**	Mesophilic	6
Raw MaterBi bags	3 – TERM_B**	Thermophilic	6
Raw MaterBi bags	4 - (MES_RAW D)	Mesophilic	3
Raw MaterBi bags	5- (MES_RAW E)	Mesophilic	3
Chemically pretreated MaterBi bags	4 - (MES_RAW D)	Mesophilic	3
Mechanically pretreated MaterBi bags	4 - (MES_RAW D)	Mesophilic	3
Predigested MaterBi bags	5 - (MES_RAW E)	Mesophilic	3
Predigested chem. pretreated MaterBi bags	5 - (MES_RAW E)	Mesophilic	3

225 *: n=number of replicates per experiment
226 ** For these batches methane productions after both 15 and 30 days were used for statistical analysis.
227

228 **3. RESULTS AND DISCUSSION**

229 No degradation of the conventional LDPE bags was recorded under both mesophilic and thermophilic
230 conditions (Table 4) as was assessed by the negligible mass loss; the slight cumulative biogas
231 production that recorded, however, was most probably attributed to the inoculum.

232 Cellulose based plates (Experiment 1) presented the highest specific methane production (Table 4),
233 since their degradation was fast and complete. In fact, 75% of the total methane production occurred
234 over 10 days. The behaviour of the corks was completely different (Experiment 1), since their
235 methane production was negligible.

236 Methane production from the compostable bags indicates that probably microbial consortium
237 acclimation is an issue. In fact, in the tests (24 different batches) carried out using raw or mechanically
238 treated compostable bags as substrate, seven (7) batches had a zero methane production or well below
239 the average of the other replicates. This was evident after analysing the coefficient of variation
240 indicated in Table 4 (i.e. Exp. 0, raw bags; Exp. 1, raw bags; Exp.2 mechanically pretreated bags;
241 Exp. 3).

242

243 **Table 4**. Experimental results
244

	Regimen - Substrate	Inoculum	Cumulative CH_4 prod.[I] [NmL/g_{VS}]				Mass loss [%]	
			15 days	30 days	44 days	Final pH	15 days	30 days
Experiment 0	Mesophilic - LDPE bags	MES_RAW_A	-11±24.1[II]	-17±26.3[II]	n.a.	7.6±0.01	-0.3±0.6[III]	-0.1±0.1
	Thermophilic - LDPE bags	TERM_A	19±4.6	54±13.0	n.a.	8.0±0.02	-0.2±0.1[III]	-0.4±0.1
	Mesophilic - Raw comp. bags	MES RAW_A	132±35.7	152±32.6	n.a.	7.5±0.03	24.4±0.1	28.7±0.4
	Thermophilic - Raw comp. bags	TERM_A	67±62.6	67±62.6	n.a.	7.9±0.03	36.5±9.2	44.7±11.3
Experiment 1	Mesophilic - Raw comp. bags	MES_RAW_B	111±51.0	95±93.7	n.a.	7.3±0.01	19.5±9.43	24.1±1.6
	Thermophilic - Raw comp. bags	TERM_B	60±39.3	186±11.8	n.a.	7.8±0.01	28.5±1.91	37.0±12.1
	Mesophilic – Corks	MES_RAW_C	32±9.5	51±14.7	52±20.8	7.3±0.04	-1.6±0.02 [III]	1.5 ±0.2 [IV]
	Mesophilic - Cellulosic Plates	MES_RAW_C	276±22.5	304±29.2	311±37.6	7.3±0.04	99.9±0.03	100±0.0[IV]
Experiment 2	Mesophilic raw comp. bags	MES_RAW_D	144±18.4	n.a.	n.a.	7.6±0.02	27.5±1.3	n.a.
	Mesophilic - NaOH pre-treat. comp. bags	MES_RAW_D	203±7.3	n.a.	n.a.	7.6±0.03	78.2±5.9	n.a.
	Mesophilic - Mechanically pre-treat. comp. bags	MES_RAW_D	117±46.5	n.a.	n.a.	7.5±0.02	29.3±2.2	n.a.
Experiment 3	Mesophilic raw comp. bags	MES_RAW_E	-2±58.4	n.a.	n.a.	7.5±0.00	19.7±1.9	n.a.
	Mesophilic - Pre-digested comp. bags	MES_RAW_E	33±12.2	n.a.	n.a.	7.6±0.00	4.8±2.4	n.a.
	Mesophilic - NaOH pre-tr. pre-digest. comp. bags	MES_RAW_E	27±26.2	n.a.	n.a.	7.6±0.02	- 0.3±2.4	n.a.
Experiment 3 [V]	Mesophilic raw comp. bags	ADAPT	42±5.8	n.a.	n.a.	n.a.	n.a.	n.a.
	Mesophilic - Pre-digested comp. bags	ADAPT	66±18.7	n.a.	n.a.	n.a.	n.a.	n.a.
	Mesophilic - NaOH pre-tr. pre-digest. comp. bags	ADAPT	70±6.6	n.a.	n.a.	n.a.	n.a.	n.a.

[I] The cumulative production is net, inoculum production was subtracted
[II] A negative value indicates a production lower of that of the control (inoculum)
[III] A negative value indicates a weight increase probably due to imperfect rinsing after digestion
[IV] 44 days
[V] Experiments with adapted inoculum stopped at day 8 since no biogas production was registered in days 3-8. Only biogas production at day 3 is reported.

In Experiment 1 (Table 4, Figure 1) the methane production of the compostable bags under mesophilic conditions was completed in about 20 days. Then, the production was kept lower that the blank's (inoculum) and therefore the accumulated net methane production after 30 days ended up to be lower than that at 15 days. The error bars clearly indicate the high variability of methane production, which was due to the negligible amount measured in one of the batches.

The highest methane production was recorded for Experiment 2 (Table 4, Figure 1).

In Experiment 3, the CH_4 production from the batches inoculated with MES_RAW_E was low, although the blank's production was quantitative; the negative net cumulated production reported in Table 4 (-2 Nml/g_{VS}) was a result of the fact that two of the three replicates produced less methane than that of the inoculum. This might be a result of an inhibition of the microbial consortia present in the runs with the substrate.

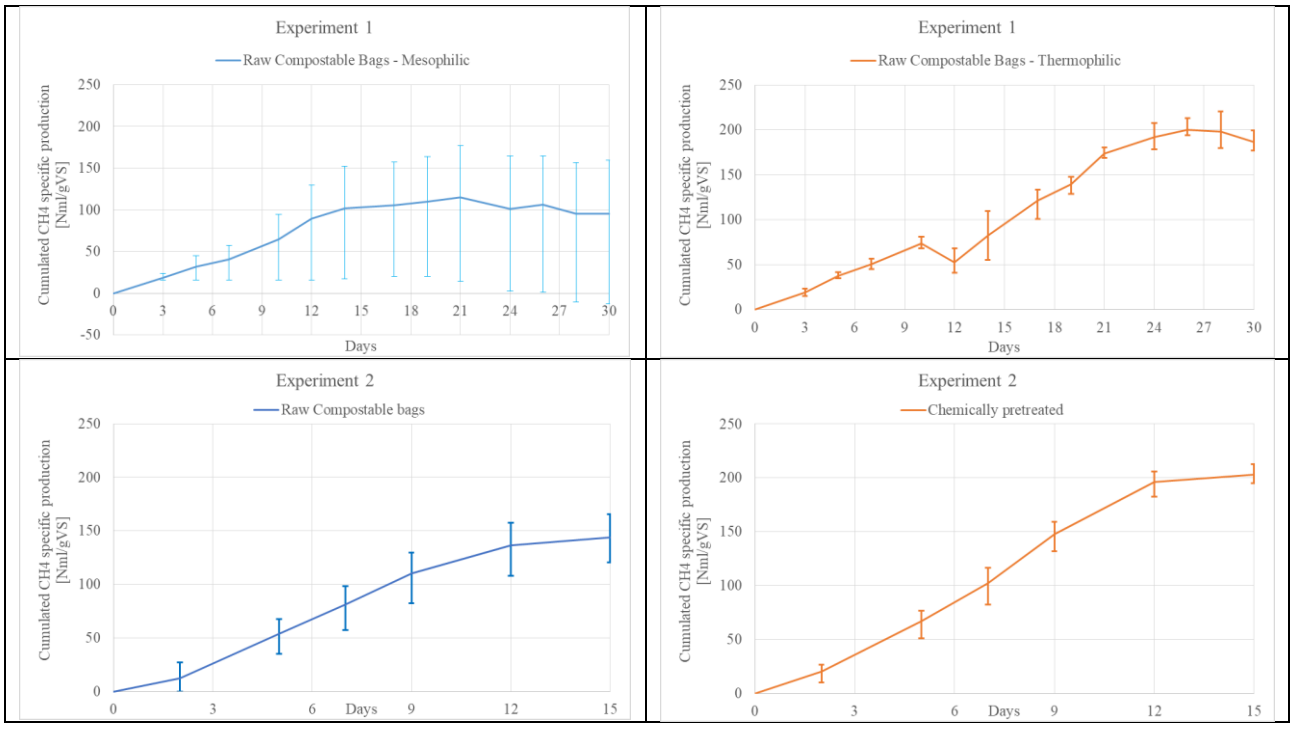

Figure 1. Cumulative methane production for some of the batch tests

The behaviour of the batches inoculated with the digestate coming from Experiment 2 (ADAPT) was peculiar. This can be explained by the fact that methane production occurred in the first three days, while in days 3-8, none of the batches produced biogas. The equipment and the procedures were reviewed and the only explanation was some toxic effect probably somewhat similar to that recently found by [27]; this was likely attributed to compounds derived from the bioplastic bags, such as plastic additives and non-intentionally added compounds possibly present in Mater-bi constituents or due to other compounds derived from its degradation. The batches were terminated at day 8 and digestate was not analysed.

The mass of substrate recovered after sieving was negligible for the cellulosic plates. On the contrary, no mass loss was detected for the corks and for the conventional LDPE bags (the small mass increase could be due to imperfect rinsing of the materials extracted from batches). The compostable bags in thermophilic conditions had lost 28% of their mass after 15 days and 41% after 30 days. Under mesophilic conditions, bags dry mass reduction was 23% (4 experiments) after 15 days. The mass reduction of shredded bags was similar to that of raw bags. The chemical treatment was very effective in reducing the dry mass by over 78%.

A new digestion cycle (15 days) on pre-digested bags was ineffective even when a chemical treatment was applied. It is possible that the synthetic resin present in MaterBi, although an alleged biodegradable compound, needs a long residence time for effective degradation.

Biogas and methane production from the raw bags was practically null in Experiment 3. In fact, the VFA concentration at the end of the experiment was very low for all the batches (range: 203 – 421 mg/L, mean: 272±61 mg/L) and comparable to that of the blank (234 mg/L) while mass reduction was similar to the other two experiments; it is likely that an inhibition of the process occurred preventing the conversion of the substrate to VFAs and then to biogas.

281 This confirms that the acclimation of the microbial consortia [28] and the potential
282 release/accumulation of inhibiting compounds [27] are crucial during the anaerobic digestion of
283 bioplastics in full scale anaerobic digestors.
284

285
286 **Figure 2.** Digested compostable bags visual inspection; a) raw - mesophilic conditions – 15 days, b)
287 raw - mesophilic conditions – 30 days, c) raw - thermophilic conditions – 15 days, d) raw -
288 thermophilic conditions – 30 days; e) NaOH pre-treated - mesophilic conditions – 15 days, f) pre-
289 digested - mesophilic conditions – 15 days.
290
291

292 Figure 2 shows the rinsed and dried compostable bags removed from the reactors run at mesophilic
293 conditions. The samples digested for 15 days show some holes as they begun to disintegrate but even
294 after 30 days in mesophilic conditions their appearance did not change significantly. After 30 days in
295 thermophilic conditions, the bags were completely disintegrated.

296 It is evident that in full scale digesters with typical hydraulic residence times of around 15 days, the
297 bags are expected to be fully recognizable in the digestate, which is unacceptable for farmers [17] .
298 This situation can be different at longer residence times and under thermophilic conditions, but
299 unfortunately these conditions are rarely found in real digesters. The aforementioned results
300 (influence of temperature on fragmentation) are fully in agreement with those reported by [37].
301 Better results, based on the visual inspection of disintegration, were obtained with NaOH pretreatment
302 while the second cycle of digestion (Figure 2f) confirmed its inefficacy (compare with Figure 2b).
303
304 **3.1 Regression modelling**
305 The statistical analysis carried out using the generic regression modelling of equation (1) indicated
306 that from the pretreatment techniques, only the chemical pretreatment (see Table 5), when applied
307 alone, significantly increased both methane yields and dry mass loss of the bioplastics. That is, the
308 sole chemical pretreatment appeared to render the biodegradable organic matter of the bioplastics
309 more accessible for hydrolyzation to microorganisms compared to the raw substrate thus leading to
310 higher degradation extents and methane generation. The predigested and predigested/chemically
311 pretreated bioplastics, on the other hand, resulted in a significant reduction of the methane yields and
312 mass losses; this is reasonable, as predigestion (even when combined with chemical pretreatment)
313 removes organic matter from the substrate so that less organic matter becomes eventually available
314 during the anaerobic stage that follows. The mechanical pretreatment alone did not affect methane
315 yields or mass losses at all. Conflicting temperature effects were noted. The mesophilic temperature
316 led to a significant increase of the methane yield, while, on the other hand, it did not lead to a
317 concurrent increase of the dry mass loss. That is, dry mass loss was higher at thermophilic
318 temperatures than the mesophilic ones; this could be a result of an increased solids hydrolysis rate at
319 the thermophilic temperature. This increased hydrolysis may lead to a dry mass reduction but not to
320 a concurrent conversion to methane which was higher at the mesophilic range. The higher

experimental duration (30 d) led to a significantly higher methane yield and dry mass loss compared to the 15 d duration, which is an expected finding. The different inocula types did not affect any of the two responses.

Table 5. Regression coefficients (only coefficients statistically significant at $p<0.05$ are shown)

Variable	Regression Coefficient	CH_4 Yields NmL/g_{VS}	Dry Mass Loss (%)
Constant	a	110	0.36
Sub_Raw	b	-	-
Sub_Chem	c	99	0.52
Sub_Mech	d	-	-
Sub_Pred	e	-71	- 0.22
Sub_Pred / Chem	f	-61	-0.26
Temp	g	+15 (Meso) -15 (Thermo)*	-0.059 (Meso) +0.059 (Thermo)*
Duration		+22 (30 d)** -22 (15 d)	+0.03 (30 d) -0.03 (15 d)
Inoculum	h	-	-

*: coefficients +15 or -0.059 apply to mesophilic conditions and coefficients -15 or +0.059 applies to thermophilic conditions;
**: coefficient +22 or +0.03 apply to a 30 d duration and coefficients -22 or -0.03 apply to a 15 d duration.

4. CONCLUSIONS

The experiments carried out here indicate that:

- After anaerobic digestion, cellulose based samples were completely dissolved while the others were partially degraded (compostable bags mass loss in 15 days was 23% and 28% in mesophilic and thermophilic conditions respectively) or completely untouched (corks).

- The highest mass loss was recorded for the chemically pretreated bags digested under mesophilic conditions; the mass loss was 344% and 283% higher than that recorded for the raw bags in mesophilic and thermophilic environments, respectively.
- Compostable bags, except of those that were digested for long treatment times (30 days) under thermophilic conditions, were fully recognizable in the digestate after the end of the digestion process.
- The statistical analysis indicated that the chemical and the predigestion pretreatments statistically increased and decreased, respectively, the methane production and the mass loss compared to those of raw bioplastics. On the other hand, the mechanical pretreatment and the inocula type had no significant effects on both responses.
- The statistical analysis provided conflicting results on the the temperature effects. That i, the mesophilic temperature led to a significant increase of the methane yield, but did not lead to a concurrent increase of the dry mass loss. This effect could be a result of an increased solids hydrolysis rate at the highest temperature range.

For waste management systems already in operation, the substitution of petrol-based plastic items with biodegradable ones would be a sustainable solution. It is, however, certain that compostable plastics cannot be simply fed to conventional anaerobic reactors unless there is a preliminary treatment (e.g. chemical treatment) or some type of a post treatment (e.g. sieving to remove partially degraded bioplastics to be thermally treated before re-digestion or composting, digestate composting under thermophilic conditions), so that to minimize their visual presence in the digestate.

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