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MANAGEMENT AND POSSIBLE VALORIZATION OF BIOPLASTICS SEPARATED FROM ORGANIC FRACTION OF MUNICIPAL SOLID WASTE

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Abstract

Italian law requires bioplastics to meet precise degradability criteria under aerobic conditions to be treated with Organic Fraction Municipal Solid Waste (OFMSW) but does not provide specific controls on anaerobic degradation capacity. The result is longer composting times for bioplastics than OFMSW. The marketing and use of biodegradable single-use products, such as shopper bags, sidesteps the problem of using common plastics without completely solving it. Therefore, there is a need to identify technological solutions that allow for the complete degradation of biodegradable plastics without the need to modify the process in place in organic waste treatment plants. For this purpose, on biodegradable plastics composed mainly of starch and PBAT, anaerobic digestion tests were carried out to assess whether the degradation kinetics are consistent with the digestion hydraulic retention time of the organic fraction. Alongside this process, pre-treatment tests involving chemical hydrolysis and commercial enzymes were conducted. The tests were performed on laboratory and pilot scales, using organic matter and culture medium for methanogenic bacteria growing also in comparison with Clostridia consortia and both in mesophilic and thermophilic conditions. During the tests, samples were collected after 30 and 55 days of reaction. Microplastic content was analysed in terms of weight loss and presence of PBAT building blocks: adipic acid, terephthalic acid, and 1,4-butanediol. The results showed that both types of pre-treatments increase the degradation yield of bioplastics at different processing times. These results lead the way for the prospect that pre-treated biodegradable plastics can be delivered along with organic waste to existing anaerobic digestion and could increase the quantities normally handled in the aerobic composting plants.

Key words: anaerobic digestion, bioplastic, chemical pre-treatment, enzyme, PBAT

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1. Introduction

According to European Bioplastics, an association representing the interests of the bioplastics industry in Europe, a plastic material is defined as bioplastic if it is "biobased, biodegradable, or has both properties" (https://www.european-bioplastics.org/bioplastics/). For a bioplastic to be "biobased," it must come, even partially, from renewable sources, while for it to be biodegradable, it is sufficient for the polymer to be digestible into simpler monomers by microorganisms in the environment (Comanita et al., 2015; Venturelli et al., 2021). The two properties are not directly related: some polymers are 100% biobased but not biodegradable, and some are 100% obtained from fossil sources but are biodegradable. Indeed, biodegradability has little to do with the polymer source but more to do with the chemical bonds in the polymer structure.

When the biodegradation of the polymer occurs in a manner and time frame compatible with those of industrial or household degradation of organic waste, the bioplastic is also called "compostable". The

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spread of biodegradable and compostable plastics has gained momentum in some Countries after European Directive 2019/904 banned single-use plastic products. Bioplastic (BP) can be disposed of and processed with organic waste, but only if it meets the compostability criteria of EN 13432. The problem arises because the standard refers to the mode and time of aerobic digestion, which is much longer than that required by a standard municipal solid waste anaerobic digester (Kosheleva et al., 2023). Different pre-treatments can be applied to promote bioplastic degradation and their digestion by the microorganisms (Mohee et al., 2008). Calabro et al. (2020) conducted an experiment using Mater-Bi, previously treated by different pre-treatment methods: mechanical shredding, chemical pre-treatment with NaOH for 24 hours and aerobic digestion using an aerobic inoculum from a sludge plant activated. Mechanical trituration has no significant effect, while chemical pre-treatment with NaOH was the most effective because it makes the polymer more accessible to enzymes, increasing both methane yields and sample mass loss. In contrast, aerobic digestion slowed the growth of the aerobic consortium, inhibiting biogas production.

In the literature are mentioned several enzymes of the hydrolase class, synthesized by bacteria, adhere to the surface of the substrate and degrade it (Muroi et al., 2017; Suzuki et al., 2014; Zumstein et al., 2017). Among the enzymes readily available on the market are RoL lipase extracted from *Rhizopus oryzae* (SIGMA Catalog No. 80612) and an FsC-like cutinase isolated from *Fusarium solani* (NOVOZYM 51032) (Urbanek et al., 2020). These two enzymes completely degrade very thick films of Mater-Bi within 20 hours at a pH of 6 at a temperature of 20°C.

This paper studied the action of enzymatic and chemical pre-treatment on BP bags subjected to anaerobic digestion afterward.

2. Materials and methods

The chemical and enzymatic pre-treatment effects were studied on BP bags composed of 70 percent polybutylene adipate co-terephthalate (PBAT), 20 percent starch, and 10 percent additives (Venturelli et al. 2021). The weight decrease of plastics over time is the critical parameter studied to evaluate the effectiveness of pre-treatment.

2.1. Laboratory-scale chemical pre-treatment and anaerobic digestion

For this activity, were used HCl and NaOH supplied by Sigma Aldrich. The tests were conducted in 500-mL Duran bottles.

Carried out: nine tests with NaOH under different concentrations and process conditions; two pre-treatments with hydrochloric acid and one alkaline pre-treatment followed by anaerobic digestion. The Table 1 shows the operating conditions of the several tests performed. Having selected, thanks to the observation of weight decrease of the polymer, the best pre-treatment conditions among those tested, the experimentation continued with the replication of the test followed by anaerobic digestion. Was performed the pre-treatment test followed by anaerobic digestion by mixing 10 mL of 25% NaOH and 7g of BP in 320 mL of water, with a pH of 12.2 and a process temperature of 50°C. The test lasted for 96 hours. Then 10% v/v digestate was added and started the anaerobic digestion process. The anaerobic digestion lasted 16 days.

2.2. Laboratory-scale enzymatic pre-treatment

The enzymes used were amylase from *Bacillus licheniformis*, amylase from *Bacillus amyloliquefaciens*, and esterase from *Bacillus subtilis*. The enzymes were all supplied by Sigma Aldrich. The tests were conducted in 500-mL Duran bottles.

For the enzyme pre-treatment, were set up three bottles for each amylases enzymes (two for the test at different enzyme concentrations and one for the blank for each enzyme), two bottles for the esterase at varying BP content, and two bottles containing amylase from *Bacillus amyloliquefaciens* and esterase, added at two different times, to evaluate the synergy of the two enzymes in the pre-treatment process. Process parameters such as pH, temperature, buffer, and reaction times utilized are given in Table 2, while Table 3 shows a review of the test setup.

Each plastic bag was divided into equal parts weighed. The pieces were placed in the oven at 55 °C for 30 minutes; then, they were dried in the desiccator for 30 minutes and weighed again. In each bottle was placed a certain amount of bioplastic (Table 3). The bottles were placed in the shaken bath at 55 °C. After 24 hours, was sampled one piece of bioplastic from the test bottles and one from the blank bottle. The plastic pieces were rinsed in distilled water and placed in the oven at 55°C overnight; afterward, they dried again in the desiccator for 30 minutes. The sampling procedure was carried out every 24 hours and at the end of the tests. In each bioplastics sampling, 8 mL of liquid was taken from both the test bottle and the blank bottle to later test for the presence of sugars (Megazyme's K-MASUG kit) and stored in the freezer.

In tests conducted with both enzymes, 2.5 mg esterase (Test 3) and 20 µL amylase (Test 4), the bottles were placed in a shaker at 55 °C for an additional 48 hours, with sampling every 24 hours. After drying, the samples were weighed, and the weight loss was estimated. The bioplastic pieces were stored in a dry place.

2.3. Laboratory-scale anaerobic digestion tests

Following the initial screening on the effectiveness of enzymatic pre-treatments on the degradation of plastics, several anaerobic digestion tests were conducted to evaluate the effect of the pre-treatments performed on the degradation kinetics of bioplastics. These tests were carried out at 50 °C with consortia of methanigenic bacteria and at 37 °C with

consortia of Clostridia-type bacteria in the presence or absence of Organic Fraction Municipal Solid Wastes (OFMSW) and a specific medium (Perz et al., 2016; Yagi et al., 2014). Non-pre-treated BPs were used as a comparison. Again, as with the pre-treatments, degradation efficiency was studied by analyzing weight decreases as principal parameter. Table 4 shows the summary of the tests performed. The unpretreated bioplastic pieces were placed in the oven at 55°C for 30 minutes and then in the desiccator for 30 minutes.

After drying, the bioplastics were weighed. In the bottle test without pre-treatment, was added 0.6 g of bioplastic per bottle. In the test with pre-treatment, was added the amount of bioplastic remaining after enzymatic action. Fresh OFMSW consisting exclusively of fruit and vegetable waste was shredded. Medium for Clostridia and methanigenic bacteria were prepared, with the composition shown in the Table 5. Anaerobic digestion tests were carried out in 500 mL Duran bottles with a total filling volume of 375 mL.

Table 1. Operating conditions of chemical pre-treatments

Test (%)	*Base or Acid quantity (mL)*	*Bioplastic quantity (g)*	*Water (mL)*	*pH*	*Temperature (°C)*	*HRT (h)*
NaOH, 25	10	7	330	12.3	50	96
NaOH, 25	0.5	7	330	10	50	168
NaOH, 25	0.75	7	330	12	50	192
NaOH, 25	0.25	8.5	330	11	50	144
NaOH, 25	0.094	8.5	330	11.5	50	144
NaOH, 25	0.25	8.5	330	12	50	144
NaOH, 25	6	7	330	13.5	50	384
NaOH, 25	8	7	330	13.5	50	384
NaOH, 25	10	7	330	13.6	50	384
HCl, 37	0.028	8.5	330	3	25	144
HCl, 37	0.001	8.5	330	5	25	144

Table 2. Summary of the process parameters of the enzymatic tests performed

Enzyme	*Buffer*	*pH*	*Temperature (°C)*
Bacillus licheniformis	citrate	5	50
Bacillus amyloliquefaciens	phosphate	7	50
Bacillus subtilis	phosphate	7.5	30

Table 3. Tests carried out with the different enzymes studied

Test	*Type of enzyme*	*Enzyme quantity*	*Biopolymer quantity (g)*	*HRT (h)*
L1	Amilase (*B. licheniformis*)	2 mg	7	48
L2		4 mg	7	48
A1	Amilase (*B. amyloliquefaciens)*	20 µL	7	48
A2		40 µL	7	48
Test 1	Esterase (*B. subtilis*)	2.5 mg	3.5	65
Test 2		2.5 mg	1.7	65
Test 3	Amilase + Esterase	20 µL + 2.5 mg	3.5	48 + 48
Test 4	Esterase + Amilase	2.5 mg + 20 µL	3.5	48 + 48

Table 4. Summary of anaerobic digestion conditions tested

Test	*Colture*	*pH*	*T (°C)*	*Medium (mL)*	*OFMSW (mL)*	*Water (mL)*	*Digestate (mL)*	*Total volume (mL)*	*Pretreat-ment*	*HRT (d)*
D1	Clostridia	5	37	337.5	-		37.5	375	-	111
D2	Clostridia	5	37	-	-	337.5	37.5	375	-	111
D3	Methanigenic	7	50	337.5	-		37.5	375	-	76
D4	Methanigenic	7	50	-	-	337.5	37.5	375	-	76
FD1	Clostridia	5	37	37.5	300	-	37.5	375	-	111
FD1*	Clostridia	5	37	37.5	300	-	37.5	375	A1 o L1	111
FD2*	Methanigenic	7	50	37.5	300	-	37.5	375	A1 o L1	56
FD4*	Methanigenic	7	50	37.5	250	-	87.5	375	A2 o L2	86
FD5*	Clostridia	5	37	37.5	250	-	87.5	375	A2 o L2	86
FD6*	Clostridia	5	37	37.5	300	-	37.5	375	Prova 1	49
FD7*	Clostridia	5	37	37.5	300	-	37.5	375	Prova 3	49
FD8*	Methanigenic	7	50	37.5	300	-	37.5	375	Prova 2	49
FD9*	Methanigenic	7	50	37.5	300	-	37.5	375	Prova 4	49

Table 5. Medium for Clostridia and methanogenic bacteria

Reagent	*Medium Clostridia (g)*	*Medium methanigenic (g)*
Distillate water	675	675
$NaHCO_3$	0.324	3.375
NH_4Cl	0.206	0.675
KH_2PO_4	0.102	0.202
$FeCl_3$ $6H_2O$	0.007	-
Yeast extract	0.021	-
NaCl	-	0.405
Glucose	-	13.5

All bottles were placed in anaerobiosis (nitrogen flow for 15 min under a chemical fume hood). At the end of this step, the 0.5 L bottles were sealed with a cap. Was attached an anaerobiosis indicator, appropriately activated with distilled water. A one liter or 3 L Tedlar bag was attached to each bottle's cap to collect the gases produced during the fermentation process. The bottles were placed in the shaking bath at 37 °C or in the 50°C orbital shaker. Each time a bottle was opened for sampling, the pH was measured to monitor its trend and put back into anaerobiosis as described above. The bioplastic samples were rinsed and then placed in an oven at 55°C for 2-3 hours or overnight (in the case of enzymatically pre-treated bioplastics) so that they dry well; then, they are placed in a desiccator for 30 minutes. After drying, the samples are weighed, and the loss incurred over time is estimated.

2.4. Pilot-scale enzymatic pre-treatment and anaerobic digestion tests

Enzymatic pre-treatment testing of pilot-scale bioplastics took place in the pilot plant consisting of a 7l reactor (LAMBDA Minifor Fermenter). The reactor is placed on a housing equipped with a coil for temperature control and connected to two peristaltic pumps that control its pH by withdrawing controlled amounts of H_2SO_4 or NaOH from two bottles placed on the base of the reactor support. Temperature and pH are measured with the probe. After filling the reactor, temperature, pH, and stirring values are set via the control panel integrated into the reactor housing.

BP bags were cut into squares of about 10x10 cm, weighed (initial weight of 0.21 g each), and subjected to enzymatic pre-treatment to promote their degradation during anaerobic digestion tests by methanigenic bacteria. In the 7 L reactor, 35 g of so-cut bags (i.e., about 166 squares), 3.30 L of phosphate buffer (100 mM, pH 7), 200 µl of α-amylase from *Bacillus amyloliquefaciens* and 25 mg of an esterase from *Bacillus subtilis* were placed, according to the protocol developed after laboratory-scale enzymatic pre-treatment trials. The reactor was kept stirring at a constant temperature of 55°C for 48 hours. Samples of bioplastics were taken from the reactor after 18, 24, 36, and 48 hours to monitor their weight change.

After 48 hours of enzymatic pre-treatment, the reactor was emptied. The bioplastics were put back into the reactor while the buffer was discarded. The OFSMW, consisting mainly of fruit and vegetable waste, was shredded and slightly diluted with water. To the 5 L reactor, already containing the pre-treated bioplastics, 3 L of OFMSW, 375 mL of digestate containing the methanogenic bacteria, and 375 mL of the medium, whose recipe is given in Table 5, were added, with a final volume of about 4 L. Finally, was set a temperature of 50°C and constant stirring. To achieve anaerobiosis and to promote the initial stirring of the mixture, nitrogen was insufflate from the bottom of the reactor for about one hour. On samples was performed a FOS/TAC assay by titration with 0.1 N sulfuric acid to monitor and maintain this parameter in line with optimal values for anaerobic digestion.

After 30 and 55 days from the start of the test, digestate samples were taken and sent to a certified laboratory to quantify the weight change and to evaluate the degradation of PBAT into its three monomers: adipic acid, terephthalic acid, and 1,4-butanediol.

2.5. Quantification of weight loss and the presence of terephthalic acid, adipic acid and 1,4-butanediol

For weight loss quantification, samples were diluted with water and allowed to be decanted overnight. After filtering out the supernatant, the solid was rinsed several times with water and filtered; all the solid was then dried in an oven at 80°C and weighed.

For terephthalic acid quantification, the water in each sample was directly analysed through an HPLC-UV, employing a Phenomenex Sinergi Hydro 250 x 4.6 mm, 4µm column and eluting with a water +0.1% phosphoric acid/acetonitrile mixture. The wavelength of detection was 254 nm.

For adipic acid quantification, it was derivatised in acidic methanol and then determined in GC-MS, with a VF-17ms column.

For 1,4-butanediol, methanol was directly injected into a GC-MS system, equipped with a VF-17ms column, for sample dilution.

3. Results and discussion

3.1. Laboratory-scale chemical pre-treatment and anaerobic digestion

Due to the dissolution of the samples, it wasn't possible to proceed with evaluations of the effectiveness of the treatment in terms of weight decrease. Therefore, analytical evaluations were made

to assess whether some fundamental components of the polymer were separated as bond degradation results. Thus, 1,4 butanediol, adipic acid, and terephthalic acid were searched in the hydrolysate. The only quantifiable compound in the pre-treated hydrolysates was 1,4 butanediol. The percentage by weight of the compound found in the hydrolysate varied from a minimum of 0.265% to a maximum of 0.626%, depending on the pre-treatment performed. The maximum found corresponds to a conversion of about 30% w/w of the converted BP to 1,4 butanediol.

The NaOH addition in the bioreactor prevents acidification of the medium, prolongs the acidogenic for at least two weeks, and promotes hydrolysis of the bioplastic (Garcia-Aguirre et al., 2019). Comparable results were also achieved by Hobbs et al. (2019), using a PLA-based starting BP. In the study, alkali pretreatment at 21°C was critical to allow near-complete solubilization of the polymer (about 97%) after 15 days. In the present work, performing tests at a temperature of 50°C probably allowed the treatment time to be cut down, speeding up the breakdown of the polymer's internal bonds and resulting in BPs that were no longer visually detectable as early as 4 days. Treatments with hydrochloric acid proved to be less effective than those with caustic soda. The anaerobic digestion test proved effective in that, although already reduced to a powdery state in solution after chemical pre-treatment, the plastics were no longer visually detectable after anaerobic digestion.

3.2. Laboratory-scale enzymatic pre-treatment

Below are the trends of plastic weight decrease detected during the tests conducted; the blank tests were performed with the same reaction parameters but without the enzyme.

About the experiments involving *Bacillus licheniformis* (L1 and L2), it can be seen that the increase of the enzyme in solution does not result in a corresponding increase in the decrease in the weight of the bioplastics; moreover, there is not much difference between the decrease in the blank tests and those with the enzyme. Tests A1 and A2 (*Bacillus amyloliquefaciens*) show a decrease in weight that increases proportionately with the doubling of the enzyme concentration. In 48 h, 16% of the total bioplastic can be hydrolyzed. Tests with *Bacillus subtilis* esterase for cleavage of ester bonds (Test 1 and Test 2) do not denote direct efficacy in hydrolysing plastics. The trend outlined follows that of the blank tests. Tests 3 and 4 were carried out to assess whether esterases have an ester bond-breaking effect for improving the efficacy of amylases used in combination; the enzyme used was *B. amyloliquefaciens* since it was proved more efficient than *B. licheniformis*.

The tests showed that switching enzymes does not result in a different decrease in weight. The two effects add up to achieve a weight decrease of slightly less than 20%. The generated maltose concentration was evaluated for the pre-treatment tests. The analysis of maltose concentrations in the hydrolysates is consistent with the findings for weight decreases.

3.3. Laboratory-scale anaerobic digestion tests

It can be shown that enzymatic pre-treatment promotes higher degradation of bioplastic samples during anaerobic digestion trials by methanogenic bacteria: microbial enzymes, depolymerases or esterase, break down polymers into fragments easily assimilated for the microbial consortium in the digestate (Abraham et al., 2021).

Plastics that were pre-treated and processed in digestion with methanigens were all found to be unsampled after fermentation, in contrast to plastics that were not subjected to enzymatic pre-treatment, which achieved a maximum percentage reduction in weight of plastic samples of about 70%. However, this value represents an excellent result when compared with other data in the literature (Abraham et al., 2021). The degradation trend could be influenced by several factors attributable to different anaerobic digestion plants, for example, specific microbial consortium or the chemical characteristics of the digestate. In the future, it is relevant to study the influence of these factors to make it easier to reproduce the implemented method on an industrial scale.

In addition, trials in which pre-treatment had been done with esterases (in which the immediate result of pre-treatment did not seem significant) led in subsequent tests with methanogenic bacteria to a stepwise effect on kinetics. After 49 days, timing not too dissimilar to some industrial digestion processes (Ruggero et al., 2020), the plastics were no longer sampleable, an effect achieved with the other enzymatic pre-treatments after 56 and 86 days.

3.4. Pilot-scale enzymatic pre-treatments and anaerobic digestion tests

From the data shown in Table 6, it can be established that enzymatic pre-treatments caused a 27% reduction in the weight of bioplastics, a result in line with what was obtained during laboratory-scale tests. Furthermore, from these results, it could be assumed that an enzymatic pre-treatment equal to that carried out would present its process optimization after 24h. After ten days of the test, the digestate had a much more liquid consistency, while the bioplastics had a flakes appearance, evidence that the hydrolysis phase had been triggered. Another study achieved similar results after 30 days of anaerobic batch digestion (Kosheleva et al., 2023).

Infrared spectrometry analysis showed that the microparticles present were all PBAT microplastics. The results of the tests are shown in Table 7. As can be seen after 30 days, the concentration of PBAT was reduced by 70%, from 7000 mg/L to 2106 mg/L, degrading into its three monomers, mainly into adipic acid.

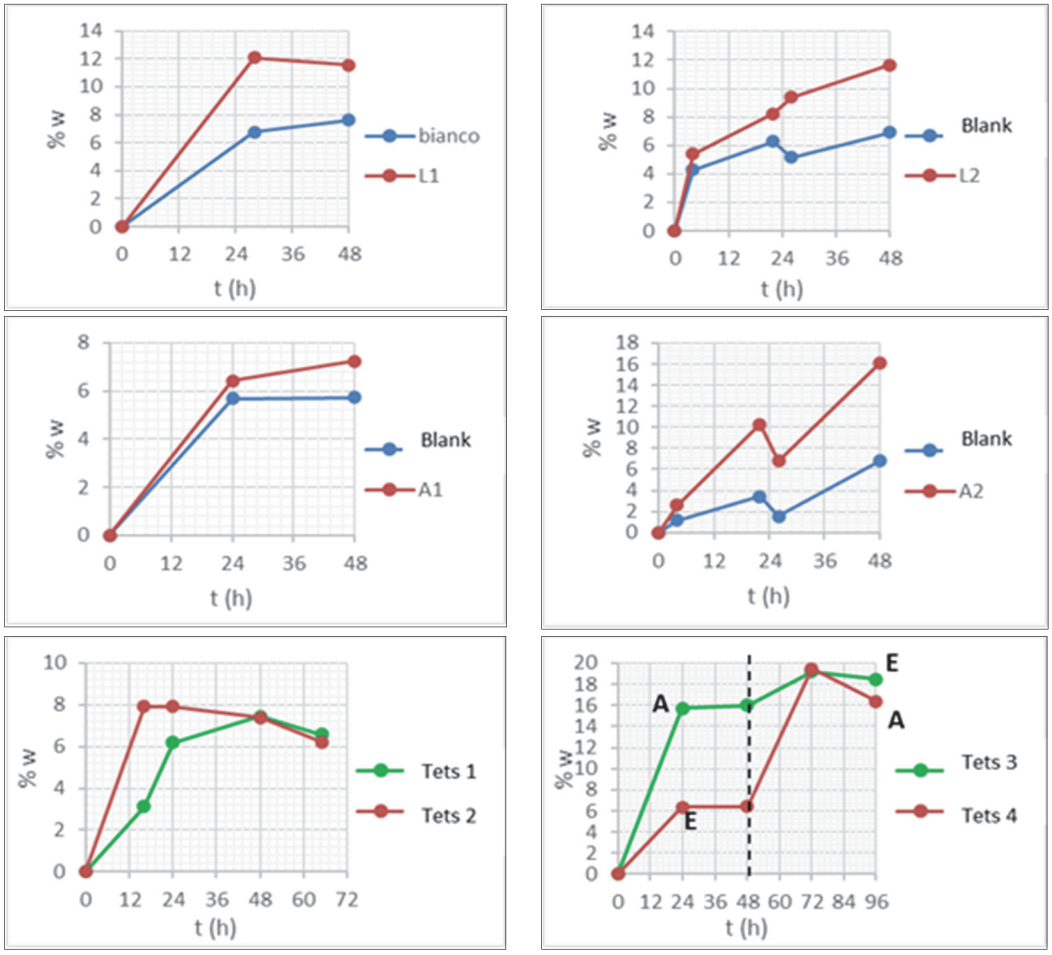

Fig. 1. Weight decreases over time

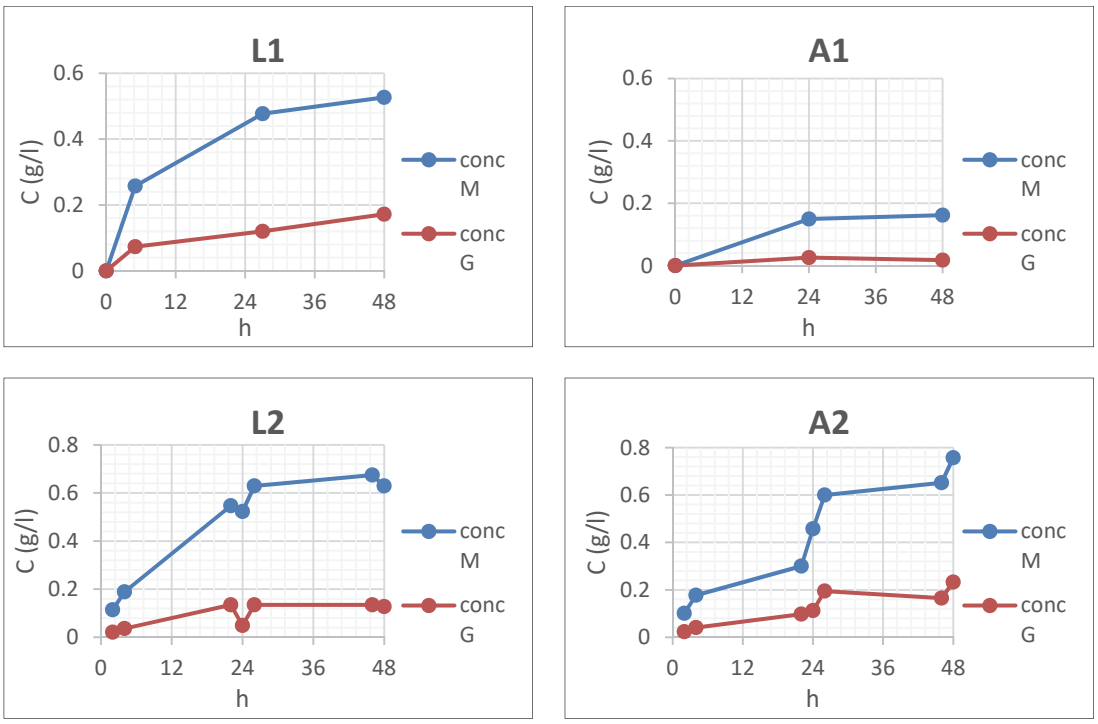

Fig. 2. Trend of maltose and glucose concentration in the hydrolyzate

Table 6. Weight variation during bioplastic enzymatic treatments

	Dry weight (g)	***Weight loss (%)***
18 h	0.2049	2.4
24 h	0.1595	24.0
36 h	0.1553	26.0
48 h	0.1530	27.1

Table 7. Bioplastics degradation rate and concentration of adipic acid, terephthalic acid, and 1,4-butanediol

	After 30 days	***After 55 days***
Degradation (%)	70	97
Adipic acid (mg/L)	130	167
Terephthalic acid (mg/L)	13.6	21.3
1,4-butanediol (mg/L)	33.8	76.6

In light of the results achieved and comparing the chemical and enzymatic pretreatment results, it is found that enzymes are more effective in breaking the polymer's internal bonds, allowing all three building blocks to be released, albeit in different ways, thus promoting the digestion of PBAT by methanigenic bacteria.

4. **Conclusion**

Anaerobic digestion seems to be a great way to dispose of BP-based waste. In addition, the efficiency of the process increases when chemical or enzymatic pretreatment is used. Both technologies have limitations, so the choice falls to the most efficient method. For the results achieved, it seems to be the enzymatic one.

The results show degradation efficiencies of up to 30 % with 96 hours of alkaline treatment and about 20 % with 72 hours of biological treatment. The results of the pilot tests confirmed the degrading ability of the enzymes and supported the hypothesis of possible industrial use: the effects can already be seen after only 24 hours of treatment by adding the enzymes all at once. Afterward, an anaerobic digestion test was performed with a degradation efficiency of 70 % after 30 days of digestion. Studies on the same polymer have obtained fewer exciting results, with degradations of up to 15% after 55 days at 50°C. These results are very positive and suggest that the retention time of industrial anaerobic digestion plants could be compatible with a high degradation rate of bio-plastics digested together with the OFMSW, provided that enzymatic pre-treatment is carried out beforehand.

Complete degradation of bioplastics should be allowed by enzymatic pre-treatment at the head of an anaerobic digestion process followed by aerobic digestion. They lead the way for further tests to confirm and refine the conclusions obtained and described in this paper and to develop complete and efficient industrial supply chains dedicated to the valorization of bio-plastics.

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