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Stimulation of Batch Mesophilic Anaerobic Digestion by Cellulose- and Polysaccharide-Derived Polymers in Landfill Leachates

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Abstract: The fate of biobased and biodegradable cellulose-derived plastics in landfills represents an important topic from economic and environmental points of view. Anaerobic digestion is a cost-effective waste-to-energy technology. The behaviour of six polymer types—that is, cellulose (C), cellulose acetate (CA), viscose (V), nanocellulose (NC), acetate textile (AT), and heteropolysaccharide pectin (P)—was studied under anaerobic batch mesophilic conditions in a landfill leachate for 147 days. The cumulative biogas production was as follows: C>V=CA>>AT>>NC=P. Metagenomic analysis revealed notable variations in the proportion of bacterial and archaeal domains with the highest archaeal abundance in the presence of CA (80.2%) and C (78.5%). At the end of digestion, cellulolytic, hydrolytic, and dehydrogenase activities were measured in the intact samples, as well as the liquid and solid fractions, under aerobic and anaerobic conditions. Cellulolytic activity in P was detected only in the pellet, while in NC, activity was mostly in the supernatant under both aerobic and anaerobic conditions. Scanning electron microscopy and confocal scanning laser microscopy showed a defragmentation and degradation of polymeric substrates as well as microbial colonisation. Based on the results, landfill leachate is appropriate for the anaerobic biodegradation of cellulose-derived polymers; however, the process is polymer specific.

Keywords: biogas; cellulase; cellulose acetate; landfill leachate; viscose; scanning electron microscopy; shotgun sequencing

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1. Introduction

Cellulose is a major constituent of municipal solid waste (MSW) [1] due to biobased and biodegradable cellulose-derived plastics and textile in landfills. The global production of bioplastics in 2027 is predicted to rise to 6.3 million tons [2]. The major textile type production is synthetic (65%), which is followed by plant, manmade cellulosic, and animal fibres. Polyester represents the highest proportion of textile production (54%), which is followed by cotton (22%), viscose (5%), and wool (1%) [3]. Therefore, rapid changes in the amount and composition of plastic wastes in landfills require further research to optimise technological solutions and thus reduce the negative impact on life cycle assessment issues.

Anaerobic digestion is a cost-effective and sustainable waste-to-energy technology [4]. Most studies on plastic biodegradation, which mimic the situation in landfills, have focused on anaerobic conditions [5–7]. Many types of biodegradable plastics exhibit low biodegradability under anaerobic conditions due to limitations of hydrolysis [8]. Nevertheless, there is a great variation in the plastic composition and operation conditions that

require additional studies to elucidate more specific data on the optimal conditions for plastic biodegradation. Ishigaki et al. [9] studied the biodegradability of different types of plastics in a landfill model reactor under aerobic and anaerobic conditions [9]. According to their results, the degradation of polyhydroxybutyrate and hydroxyvalerate plastic was higher in the aerobic environment, while a blend of starch and polyvinyl alcohol (SPVA) plastic and cellulose acetate (CA) were not degraded in these conditions [9]. Fricko and Fellner [10] compared the removal of organic waste in a landfill simulation reactor. Compared with the aerobic conditions, the anaerobic conditions stimulated mineralisation to a considerable degree [10].

The degradation of cellulose may result in different fermentation products because of flexibility in the fermentative degradation pathways. Hydrogen, propionate, butyrate, acetate, ethanol, and methane are among the most abundant products of cellulose degradation [11]. Nevertheless, the fate of cellulose-derived polymers during the anaerobic digestion of organic wastes differs greatly and has been poorly explored [12]. The degradation behaviour of a broad spectrum of biopolymers and commercial blends requires specific knowledge of the multiple factors that influence the degradation process. The most important are the physicochemical properties of polymers and external factors such as the temperature, oxygen availability, pH, microbial community structure, operation mode, retention time, pretreatment, additives, inoculum acclimation, and food/microorganism ratio, among others [13–16]. The increased crystallinity and a high carbon-to-nitrogen ratio of the polymer decrease its biodegradability [17,18]. The degradation of CA and other biobased plastic is more efficient in batch conditions compared with semi-continuous systems [12,19]. Researchers have reported that thermophilic conditions increase plastic degradation under anaerobic conditions [20–22].

Several early studies demonstrated a very promising approach of the use of landfill leachate in the degradation of MSW. Specifically, Nopharatana et al. [23] proposed that the leachate should be trickled through a bed of anaerobically stabilised waste to beds of fresh MSW, utilising formate and cellulose as indicators to optimise the operational strategy [23]. Our group has described some aspects regarding the potential of landfill leachate for the biodegradation of cassava-derived plastics under aerobic conditions [24] and CA (cigarette filters) under anaerobic conditions [25]. The leachates sampled at the Getliņi MSW (Latvia) contained a broad spectrum of microorganisms with target functions. Specifically, the relative abundance of the gene families containing plastic-degrading enzyme groups varied in the range of 1.69×10^{-5} (lipases) to 7.63×10^{-4} (proteases). Among other relevant enzymes, ureases, alkane hydrolases, laccases, and esterases were detected in the leachate [24].

In the present study, the 147-day anaerobic digestion process of five cellulose-derived polymer types—cellulose (C), CA, acetate textile (AT), viscose (V), nanocellulose (NC)—and heteropolysaccharide pectin (P) was assessed in landfill leachate in mesophilic conditions. The combined use of metagenomics and enzyme assays, as well as scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM), provided novel findings regarding C and heteropolysaccharide polymers subjected to anaerobic digestion in landfill leachate. Finally, the correlation between biogas production and cellulolytic, hydrolytic, and dehydrogenase activities highlights new important aspects of microbial activity under the tested conditions.

2. Materials and Methods

2.1. Materials

Batch anaerobic digestion (BAD) used the following: CA with an average Mn of ~30,000 (GPC); Sigmacell Cellulose Type 20, 20 µm, and pectin from apple (Sigma-Aldrich, St. Louis, MO, USA); nanocrystalline cellulose CNC (diameter 10–20 nm, length 300–900 nm) from Nanografi Nano Technology (Ankara, Türkiye, NG01NC0101; and C and AT from Abakhan Fabrics Eesti Latvijas Filiāle (Riga, Latvia).

2.2. Landfill Leachate

Landfill leachate was obtained from the Getliņi municipal solid waste landfill, which is managed by SIA Getliņi EKO (Riga, Latvia) [26]. Leachate samples were taken using a collector manhole.

2.3. Physicochemical Testing of Landfill Leachate

A composite sample was obtained from 10 individual samples taken over a period of 1 h. The pH was measured using a WTW Multi9620 IDS with the SenTix 940 P. Electrical conductivity (EC) was measured using a TetraCon 925 electrode. The total nitrogen concentration was measured by Koroleff's method using a photoLab S12 photometer and a CR 420 thermoreactor (WTW) as well as the Spectroquant Nitrogen (Total) 10–150 mg L^{-1} N Kit (Sigma-Aldrich, 1.14763.0001). The N-NH_4^+ concentration was measured using the Spectroquant Ammonium Cell Test 4.0–80.0 mg L^{-1} N-NH_4^+ Kit (Sigma-Aldrich, 1.14559.0001). The process involved reactions with hypochlorites in a strong alkaline solution, which was followed by photometric determination with sodium nitroprusside using a photoLab S12 photometer (WTW). The chemical oxygen demand (COD) was measured using the Spectroquant COD Cell Test 300–3500 mg L^{-1} Kit (Sigma-Aldrich, 1.14691.0001). It involved a reaction with potassium dichromate, sulfuric acid, and mercury (II) sulfate at 148 °C for 2 h, using the photoLab S12 photometer and CR 420 thermoreactor (WTW). Total solids (TS) were determined by drying a sample at 105 °C.

2.4. BAD Experiment

The six types of polymers were digested with landfill leachate in 100 mL anaerobic bottles supplied with butyl rubber stopper and an aluminium seal (Chemglass Life Sciences, Asbach, Germany). Each bottle contained 20 mL of leachate containing 500 mg of a polymer. Additionally, one blank (landfill leachate alone) was digested. The samples were incubated at 37 °C for 147 days in duplicate. Biogas production was measured by 50 mL medical syringes. The head space gas pressure was measured every day during the first two weeks and then once every 4 days. Before measuring, the bottle was shaken for 1 min. The following acronyms refer to the samples: RL—raw leachate; BAD-0—without polymers; BAD-C—with cellulose; BAD-V—with viscose; BAD-CA—with cellulose acetate; BAD-AT—with acetate textile; BAD-NC—with nanocellulose; and BAD-P—with pectin.

2.5. Detection of Microbial DNA in the Landfill Leachate Samples

2.5.1. DNA Extraction and Shotgun Sequencing

DNA was extracted from the landfill leachate samples using a FastDNA Spin Kit for Soil (MP Biomedicals, Irvine, CA USA) according to the manufacturer's protocol. DNA samples were sheared to 400-base pair (bp) fragments using a S220 focused-ultrasonicator (Covaris, Woburn, MA USA). Libraries were prepared with the MGIEasy Universal DNA Library Prep Set V1.0 (MGI Tech Co., Shenzhen, China) according to a standard workflow. Quality control was performed using a Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA USA) and a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Samples were sequenced on a DNBSEQ-G400 sequencer with the DNBSEQ-G400RS High-Throughput Sequencing Set PE150 (MGI Tech Co., Shenzhen, China), following the standard protocol. Each sample was expected to produce at least 20 million 150 bp paired-end reads.

2.5.2. Shotgun Sequencing Data Analysis

The raw reads were quality trimmed using Trimmomatic v.0.39 [27] with the leading quality of Q30 and trailing quality of Q30. Following quality filtering, the sequences were classified using Kraken2 [28] along with the RefSeq database release 98 [29], which includes taxonomic reference data for bacterial, fungal, viral, and protozoan domains. Taxonomic aggregation was conducted using the R-based tool Pavian [30] v1.0. The Shannon diversity index was calculated using the vegan v.2.6-6.1 package and visualised with ggplot2 v.3.5.1. The significance of the Shannon diversity index values was assessed using the Wilcoxon

rank-sum exact test with Holm *p*-value adjustment, which is also from the vegan package. Principal component analysis, based on Hellinger-transformed abundance counts, was performed using the MicrobiotaProcess v.1.14.0 package [31].

2.6. Biochemical Testing

The activity of enzymes was measured in intact landfill leachate samples after the 147-day BAD as well as its supernatant and pellet, which were obtained by centrifugation at 10,000 rpm for 5 min. Furthermore, enzyme assays were performed under aerobic and anaerobic conditions. The GasPak™ EZ pouch system (BD, Franklin Lakes, NJ, USA) was used to generate the anaerobic conditions.

2.6.1. Cellulolytic Activity

Each landfill leachate sample (100 µL) was added to a well (diameter of 7 mm) in the centre of agarised medium in Petri plates. The medium contained the following (g L^{-1}): 10.0 carboxymethylcellulose, 10.0 peptone, 2.0 K_2HPO_4, 0.3 $MgSO_4 \cdot 7\ H_2O$, 2.5 $(NH_4)_2SO_4$, and 15 agar [32]. The plates were incubated at 30 °C for 5 days under aerobic and anaerobic conditions. After incubation, the plates were stained with 1% (*w*/*v*) Congo Red for 20 min at 23 °C and then rinsed with 2 × 15 min with 1 M NaCl. The clear zone diameter around the well was measured.

2.6.2. Fluorescein Diacetate (FDA) Hydrolysis

Each sample (150 µL) was placed in a well of a 96-well plate containing 150 µL of the FDA reaction mixture (4 mg of FDA, 2 mL of acetone, and 48 mL of 60 mM phosphate buffer, pH 7.6). The plate was incubated at 30 °C for 60 min. After incubation, 150 µL of the reaction mixture was transferred to the well of another plate containing 150 µL acetone to stop the reaction. The concentration of hydrolysed FDA was determined photometrically by measuring the optical density at 492 nm [33].

2.6.3. Dehydrogenase Activity (DHA)

Each sample (150 µL) was placed in a well of a 96-well plate containing 150 µL of the DHA reaction mixture (1.97 g of Trizma, 0.1 g of 2-*p*-iodo-3-nitrophenyl-5-phenyltetrazolium chloride [INT], 25 mg of glucose, and 50 µL deionised water). The plate was incubated at 30 °C for 24 h. DHA was determined photometrically by measuring the optical density at 492 nm [34].

2.7. Microscopy

2.7.1. SEM

Scanning electron micrographs were taken with a TM300 Tabletop Microscope SEM (Hitachi High Technologies Corporation, Tokyo, Japan) with the following parameters: magnification of 1500×, a vacuum of 10^{-2} Torr, an ion coater with a current of 6 mA, a gold (Au) cover, and a coating thickness of 10 nm.

2.7.2. CLSM

Samples were fixed with 70% ethanol and analysed using a DM RA-2 confocal laser scanning microscope (Leica, Wetzlar, Germany) equipped with a TCS-SL confocal scanning head. Propidium iodide (PI) was excited at a 488 nm, and fluorescence was detected between 600 and 640 nm.

2.8. Statistical Analysis

The data are presented in the figures are presented as the mean ± standard deviation. Differences between treatments were assessed by one-way analysis of variance (ANOVA) in Excel, Office365 Version 2409 (Microsoft, Redmond, WA, USA).

3. Results

3.1. Physicochemical Characteristics Before and After BAD

The main characteristics of the landfill leachate used in the present study are shown in Table 1. These samples were compared with other landfill leachate samples that originated from different sites at Getliņi landfill (from the mature and young dumping sites as well as the leachate collection pond) from a previous study [26]. There were no notable differences in the tested parameters among the landfill leachates. Therefore, the landfill leachate tested in the present study is typical for the Getliņi landfill.

Table 1. The physicochemical characteristics of raw leachate. COD—chemical oxygen demand, EC—electrical conductivity. The composite sample was obtained from 10 individual samples taken over 1 h.

Parameter	Temperature	pH	EC (mS cm^{-1})	COD (mg L^{-1})	P_{tot} (mg L^{-1})	N_{tot} (mg L^{-1})	NH_4-N (mg L^{-1})	NO_2^- (mg L^{-1})	NO_3^- (mg L^{-1})
Value	26.2	7.94	33	6860	35	2760	>2400	20–30	90–100

The BAD of the landfill leachate samples altered the TS, pH, and EC depending on the type of added polymer. In particular, BAD-NC produced the highest TS content (9.51%); for the other samples, the TS content was 1.67%–2.69% (Table 2). The EC of the landfill leachate after 147 days of BAD varied from 1.17 to 8.92 mS/cm; it was highest in BAD-V and BAD-AT. The pH mostly increased after BAD compared with RL (7.94) except for BAD-0 and BAD-AT, which showed decreases in pH to 7.01 and 7.65, respectively (Table 2).

Table 2. The total solids, pH, and electrical conductivity (EC) in the landfill leachate samples after the 147-day batch anaerobic digestion (BAD) in the presence of polymers. RL—raw leachate; BAD-0—without polymers; BAD-C—with cellulose; BAD-V—with viscose; BAD-CA—with cellulose acetate; BAD-AT—with acetate textile; BAD-NC—with nanocellulose; BAD-P—with pectin.

Parameter	Type of Treatment						
	BAD-0	BAD-C	BAD-V	BAD-CA	BAD-AT	BAD-NC	BAD-P
Total solids, %	1.98	1.67	1.68	2.03	2.69	9.51	1.75
pH	7.01	8.50	8.40	8.09	7.65	8.82	9.01
EC, mS cm^{-1}	1.74	1.17	8.91	1.52	8.82	1.72	1.73

3.2. Biogas Production

Monitoring biogas production during BAD revealed considerable differences among the samples. Specifically, BAD-C produced the highest biogas volume (252.5 mL), which was followed by BAD-V (212.5 mL) and BAD-CA (205.6 mL). In turn, BAD-AT presented relatively low biogas production (115.5 mL). The lag phase in BAD-C, BAD-CA, and BAD-AT lasted for 5, 6, 7, and 10 days, respectively. The overall biogas production period was polymer specific and varied from 14 days (BAD-AT) to 140 days (BAD-C). BAD-0, BAD-NC, and BAD-P did not produce biogas under the tested conditions (Figure 1).

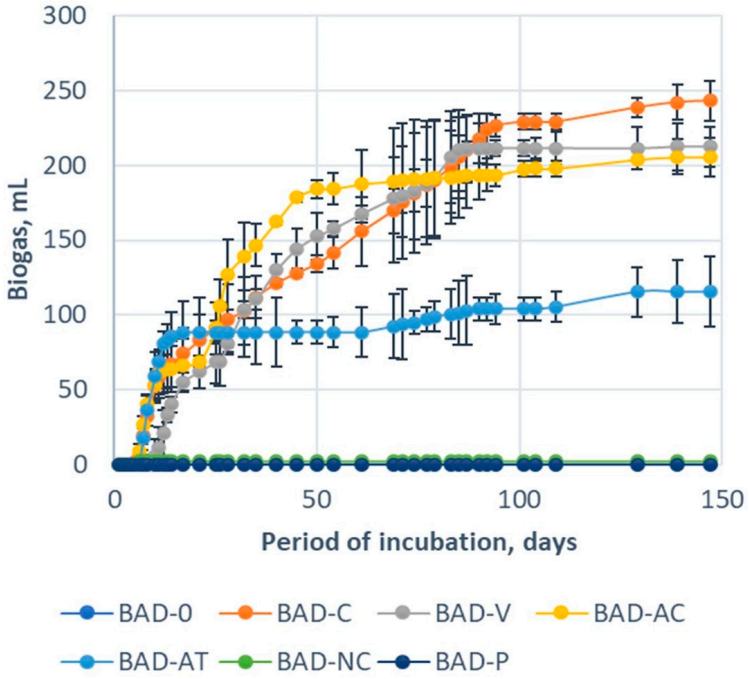

Figure 1. Cumulative biogas production from the landfill leachate samples containing different types of polymers during the 147-day batch anaerobic digestion (BAD). RL—raw leachate; BAD-0—without polymers; BAD-C—with cellulose; BAD-V—with viscose; BAD-CA—with cellulose acetate; BAD-AT—with acetate textile; BAD-NC—with nanocellulose; BAD-P—with pectin. At the end of the experiment, the biogas volume of BAD-0, BAD-NC, and BAD-P was 0.0, 2.0, and 0.2 mL, respectively.

3.3. Taxonomic Profile of the Landfill Leachate Samples

Metagenomic analysis revealed that the proportion of bacterial and archaeal domains in the landfill leachate samples after the 147-day BAD varied greatly depending on the type of polymer added (Table 3). BAD-CA (80.2%) and BAD-C (78.5%) showed the highest relative abundance of archaea. BAD-V (40.4%) and BAD-P (16.2%) also showed a relatively high archaeal abundance, but it was lower than RL (52.0%; Table 3). BAD-0 (99.1%), BAD-AT (99.2%), and BAD-NC (98.0%) had the highest relative abundance of bacteria (Table 3). These samples had comparatively lower cumulative biogas production (Figure 1), suggesting a relationship between the dominance of archaea and biogas production. Nevertheless, BAD-P demonstrated a different trend: the archaeal abundance increased to 83.4% (compared with 47.3% for RL), while biogas production was not detected (Figure 1).

Table 3. The microbial community structure at the domain level in the landfill leachate samples after the 147-day batch anaerobic digestion (BAD). RL—raw leachate; BAD-0—without polymers; BAD-C—with cellulose; BAD-V—with viscose; BAD-CA—with cellulose acetate; BAD-AT—with acetate textile; BAD-NC—with nanocellulose; BAD-P—with pectin. The values represent the relative abundance (%).

	BAD-0	BAD-C	BAD-V	BAD-CA	BAD-AT	BAD-NC	BAD-P	RL
Bacteria	99.913	21.480	59.596	19.813	99.156	97.995	83.369	47.286
Archaea	0.077	78.516	40.387	80.174	0.836	1.424	16.153	52.045
Eukaryota	0.007	0.003	0.005	0.012	0.002	0.052	0.006	0.472
Viruses	0.004	0.002	0.012	0.001	0.006	0.529	0.472	0.198

At the phylum level, archaea were represented by *Euryarchaeota* with relative abundances of 82.9% (BAD-CA), 79.4% (BAD-C), 17.7% (BAD-P), and 57.5% (RL). Among anaerobic bacteria, *Thermotogae* dominated in BAD-AT (relative abundance of 83.4%). Other

bacteria were mainly represented by *Bacteroidetes* (96.0% in BAD-0) and *Proteobacteria* (81.5% in BAD-NC, 35.5% in BAD-P, and 15.5% in RL; Figure 2A). At the class level, *Methanomicrobia* prevailed in RL (60.5%), BAD-CA (84.8%), BAD-V (42.8%), and BAD-C (79.3%; Figure 2B). At the order, family, and genus levels, this taxon was represented by *Methanomicrobiales*, *Methanomicrobiaceae*, and *Methanoculleus*, respectively (Figure 2C–E). At the species level, *Fermentimonas caenicola* dominated (>20%) in BAD-0, BAD-NC, and BAD-P; *Methanoculleus sp.* MAB1 dominated in BAD-C, BAD-V, BAD-CA, and RL; and *Defluviitoga tunisiensis* dominated in BAD-V and BAD-AT (Figure 2F).

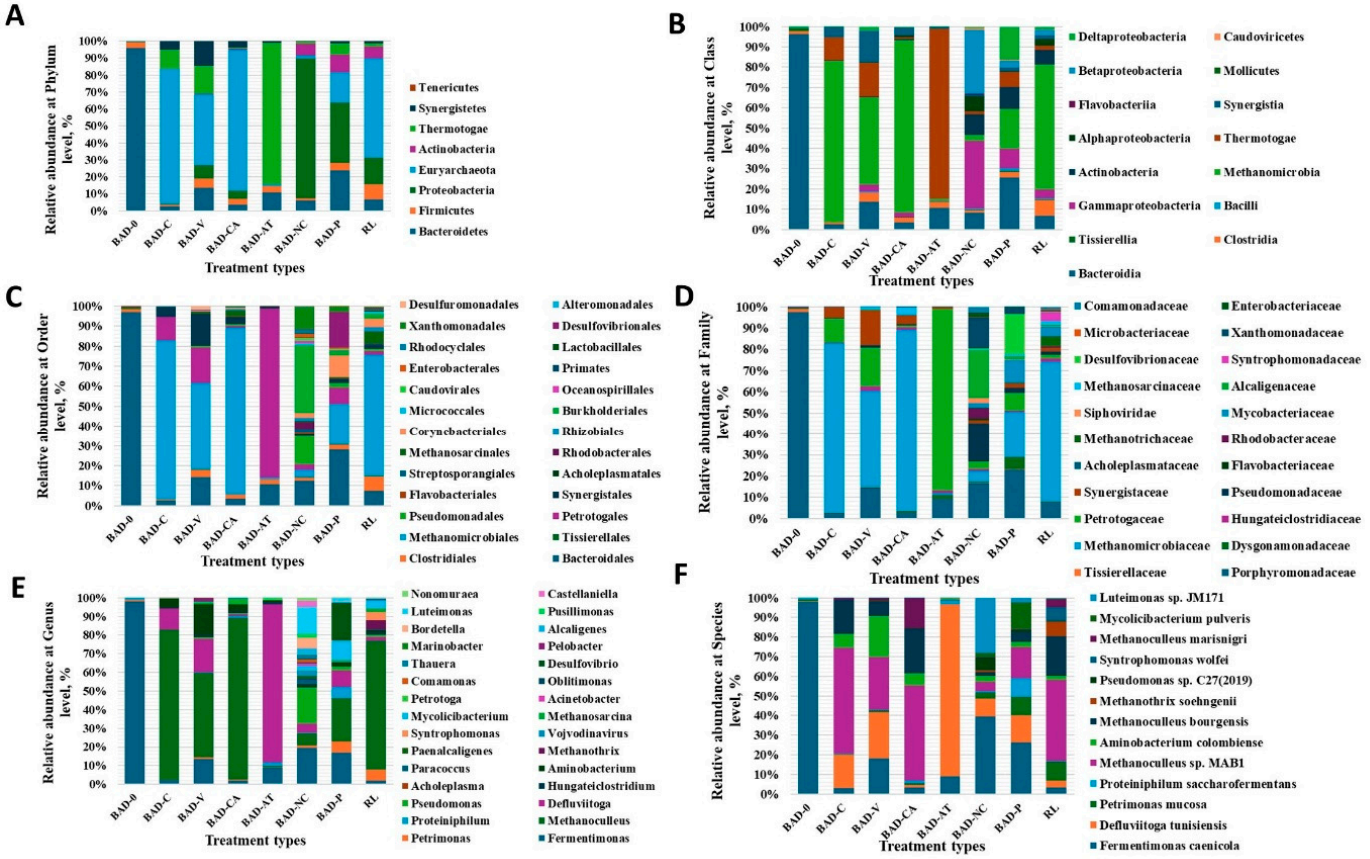

Figure 2. The microbial community structure at the (**A**) phylum, (**B**) class, (**C**) order, (**D**) family, (**E**) genus, and (**F**) species levels in the landfill leachate samples after the 147-day batch anaerobic digestion (BAD). RL—raw leachate; BAD-0—without polymers; BAD-C—with cellulose; BAD-V—with viscose; BAD-CA—with cellulose acetate; BAD-AT—with acetate textile; BAD-NC—with nanocellulose; BAD-P—with pectin. Only taxon yields ≥1% (**A**–**E**) and ≥5% (**F**) in at least one sample are shown.

BAD-NC had the highest Shannon diversity index (2.67), while BAD-0 and BAD-AT had the lowest (0.85 and 1.06, respectively; Figure 3A). However, the Shannon diversity index did not differ significantly between the samples ($p > 0.05$). Beta diversity analysis (Figure 3B), based on the Hellinger transformation, showed that BAD-0, BAD-NC, and BAD-P tended to cluster in the same quadrant, which was characterised by the abundance of *Fermentimonas caenicola*. In contrast, RL and BAD-CA clustered near each other and were represented by *Methanoculleus marisnigri*, *Methanoculleus bourgensis* and *Methanoculleus* sp. MAB1. Lastly, BAD-AT and BAD-V were represented by *D. tunisiensis*.

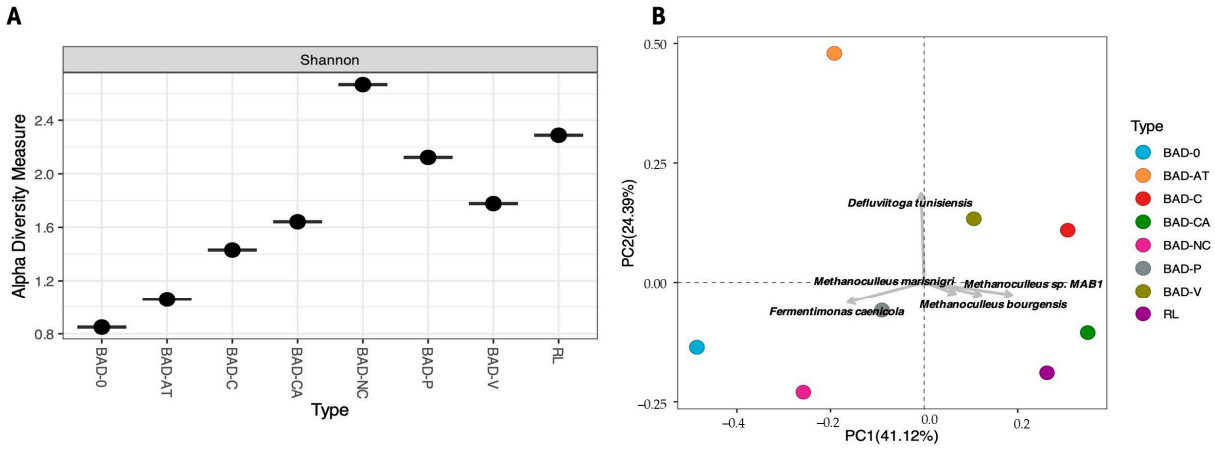

Figure 3. Diversity analysis of the landfill leachate samples. (**A**) The Shannon index values for the various landfill leachate samples. (**B**) Principal component analysis based on the Hellinger-transformed abundance data. RL—raw leachate; BAD-0—without polymers; BAD-C—with cellulose; BAD-V—with viscose; BAD-CA—with cellulose acetate; BAD-AT—with acetate textile; BAD-NC—with nanocellulose; BAD-P—with pectin.

3.4. Enzyme Activity

The cellulolytic activity was tested in the intact samples as well as its liquid and solid phases, which were obtained by centrifugation. Enzyme activity in the supernatant is expected to reveal an abundance of extracellular enzymes. As shown in Figure 4, the cellulolytic activity in the intact landfill leachate samples and their pellets and supernatants differed depending on the type of polymer added. Furthermore, aerobic versus anaerobic conditions during the enzymatic reaction influenced the final clear zone diameter. Specifically, the intact samples under aerobic conditions exhibited similar cellulolytic activity (i.e., the diameter of the clear zone was 46–56 mm) except for BAD-NC, which demonstrated the lowest activity with a zone diameter of 22 mm. BAD-NC demonstrated contrasting behaviour in cellulolytic activity in the pellet and supernatant compared with the other samples. In particular, only BAD-NC did not show cellulolytic activity in the pellet fraction. Only two samples—BAD-NC and BAD-AT—demonstrated activity in the supernatant (Figure 4A). In turn, under anaerobic conditions, more samples exhibited cellulolytic activity in the supernatant with the highest values in BAD-NC and BAD-CA (38 and 36 mm, respectively). The BAD-C, BAD-P, and BAD-0 supernatants did not show any cellulolytic activity (Figure 4B).

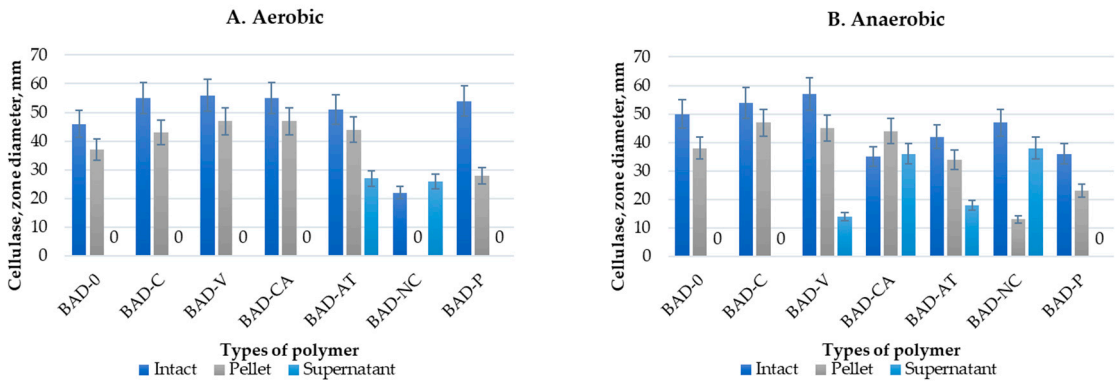

Figure 4. The cellulolytic activity in the landfill leachate samples after the 147-day batch anaerobic digestion (BAD). BAD-0—without polymers; BAD-C—with cellulose; BAD-V—with viscose; BAD-CA—with cellulose acetate; BAD-AT—with acetate textile; BAD-NC—with nanocellulose; BAD-P—with pectin. The values are the average of two measurements.

DHA also broadly varied depending on the polymer that was added. Under both aerobic and anaerobic conditions, the BAD-CA pellet had the highest DHA (Figure 5). The BAD-C, BAD-V, and BAD-CA supernatants exhibited the lowest DHA among the samples (Figure 5).

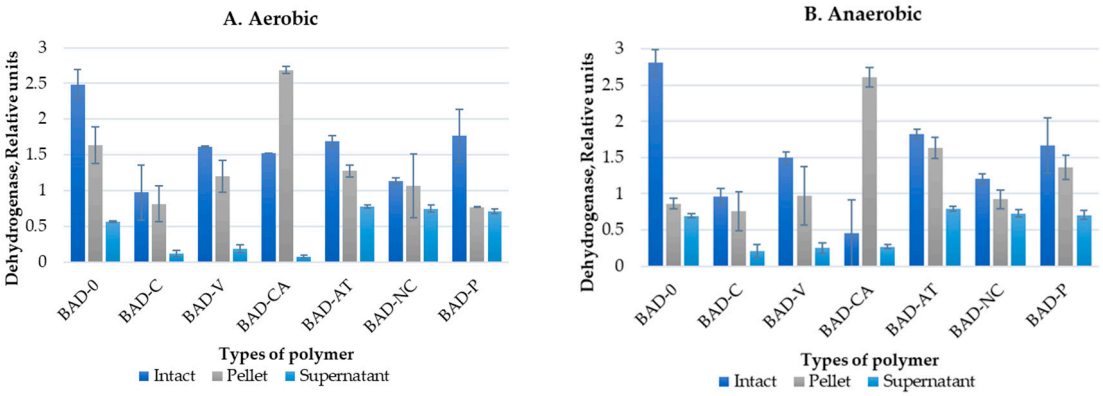

Figure 5. The dehydrogenase activity in the landfill leachate samples after the 147-day batch anaerobic digestion (BAD). BAD-0—without polymers; BAD-C—with cellulose; BAD-V—with viscose; BAD-CA—with cellulose acetate; BAD-AT—with acetate textile; BAD-NC—with nanocellulose; BAD-P—with pectin. The values represent the mean ± standard deviation of three measurements ($p < 0.05$).

The data on the FDA hydrolysis activity of after BAD are summarised in Figure 6. Overall, there was higher activity in the supernatant compared with the pellet except for BAD-AT, where both polymer-associated and extracellular activity showed similar results. There was unexpectedly low hydrolytic activity in BAD-C, BAD-V, and BAD-CA (Figure 6).

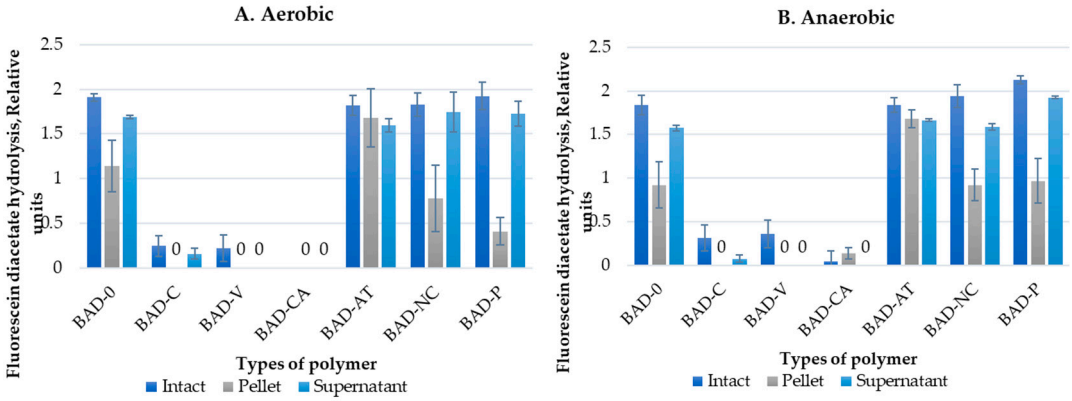

Figure 6. The fluorescein diacetate hydrolysis activity in the landfill leachate samples after the 147-day batch anaerobic digestion (BAD). BAD-0—without polymers; BAD-C—with cellulose; BAD-V—with viscose; BAD-CA—with cellulose acetate; BAD-AT—with acetate textile; BAD-NC—with nanocellulose; BAD-P—with pectin. The values represent the mean ± standard deviation of three measurements ($p < 0.05$).

Considering the enzyme activities, there was only a correlation between FDA hydrolysis and DHA in the supernatant under aerobic and anaerobic conditions (R^2 = 0.93 and 0.96, respectively;). Correlation analysis between enzyme activity and biogas production is shown in Table 4. Cellulase activity in the pellet correlated positively with biogas production (R^2 = 0.66). There was a high negative correlation between biogas production and FDA hydrolysis and DHA in the supernatant (Table 4).

Table 4. Coefficient of determination (R^2) between biogas production and enzyme activity, which was determined either under aerobic or anaerobic conditions. (*)—negative correlation.

Enzyme Group	Fraction	Coefficient of Determination R^2	
		Aerobic	Anaerobic
DHA	Intact	0.22 *	0.37 *
	Pellet	0.06	0.06
	Supernatant	0.73 *	0.78 *
FDA hydrolysis	Intact	0.85 *	0.86 *
	Pellet	0.32 *	0.48 *
	Supernatant	0.87 *	0.84 *
Cellulase	Intact	0.36	0.09
	Pellet	0.49	0.66
	Supernatant	0.10	0.01

3.5. Visualisation of Polymers and Microorganisms

The changes in polymer morphology after the 147-day BAD are presented in Figure 7 (I and II). There was a defragmentation of all six tested polymers. After treatment, the CA surface appeared eroded. CLSM provided additional information regarding microbial colonisation on the surface of the polymers (Figure 7, III). All samples presented an abundance of microorganisms (red colour) in the pellets, which were previously concentrated from digested samples and thoroughly rinsed (Figure 7, III).

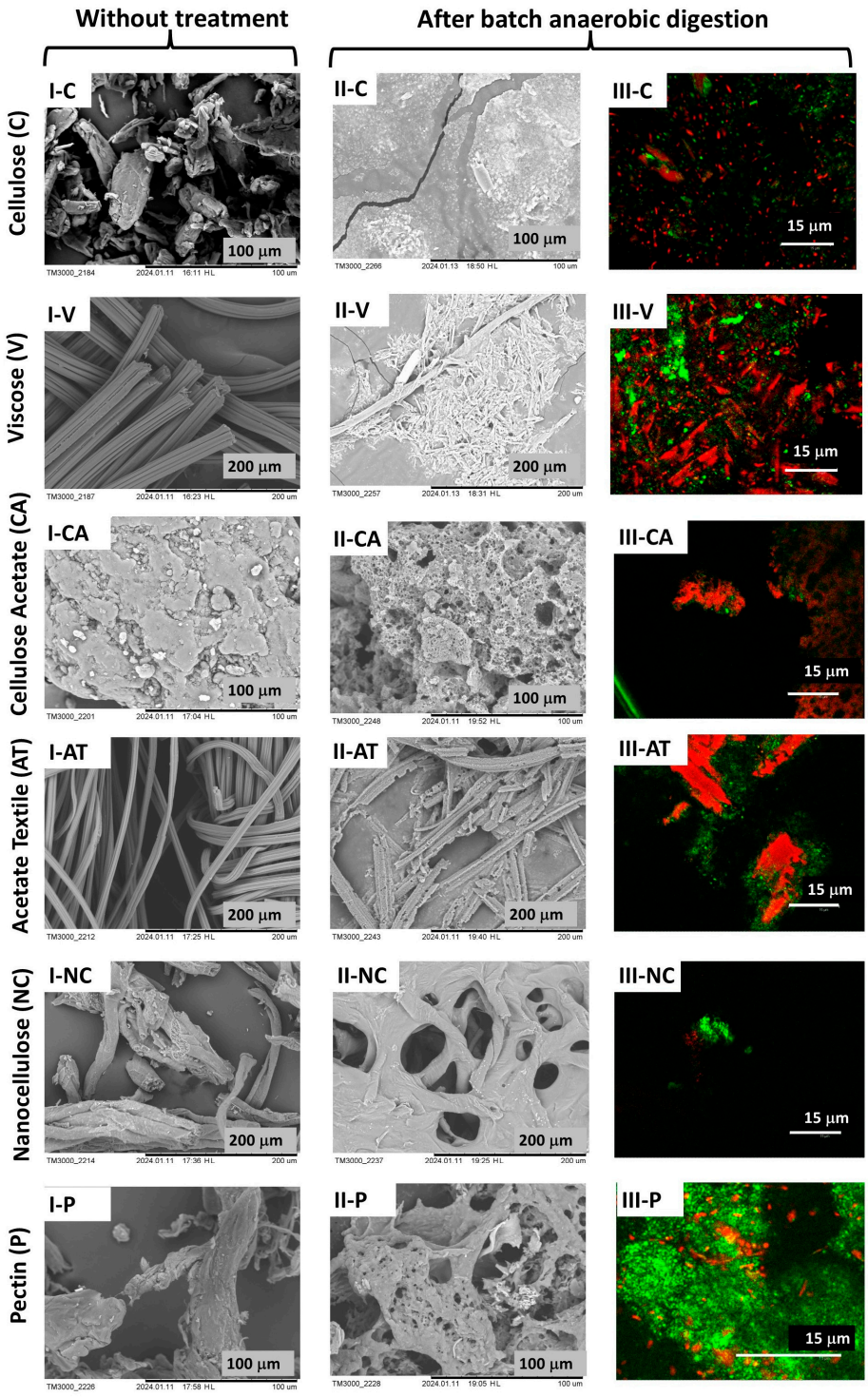

Figure 7. (**I**, **II**) Scanning electron micrographs and (**III**) confocal laser scanning micrographs of polymers without treatment (**I**) and after the 147-day batch anaerobic digestion (**II** and **III**). For confocal laser scanning microscopy, cells were fixed in 70% ethanol followed by staining with propidium iodide (PI) at a concentration of 20 mM. The excitation wavelength was 488 nm, and emission was observed at 600–640 nm. The field of view was scanned in three dimensions, using 20 optical sections separated at a depth of 10 µm. Visualisation used a 100× objective lens with a numerical aperture of 1.25. Green corresponds to the abiotic part and red corresponds to the biotic part of the specimen. The scale bars in the micrographs indicate the length.

4. Discussion

The data showed that among the six tested polymers, digestion of cellulose (BAD-C, 20 μm) for 147 days resulted in the highest biogas volume (252.5 mL). Azcona et al. [35] investigated the anaerobic digestion of several fabrics [35]; the anaerobic degradation of crystalline cellulose depended on the cellulose particle size. Hu et al. [36] reported that the hydrolysis and acidogenesis rates at a particle size of 50 μm were higher than those at 100 μm [36]. The lower cellulose particle size resulted in decreased methane production and an increased concentration of soluble products [36]. The role of the particle size of crystalline cellulose is a key factor that determines the kinetics of crystalline hydrolysis [37]. The authors developed two surface-related models for the anaerobic digestion of microcrystalline cellulose based on spherical and cylindrical particles, which they compared with the first-order kinetics model. For example, two fractions of cellulose—20 ÷ 32 mm and 212 ÷ 300 mm—differ by a specific surface area (0.801 and 0.730 m^2 g^{-1}, respectively) and biogas production potential (726 and 619 mL g^{-1}, respectively) [37].

In the present study, CA (average molecular weight of 30,000), C (20 μm), and NC (diameter of 10–20 nm and length of 300–900 nm) showed distinct differences in biogas production under the tested conditions, with CA and C being relatively efficient, while NC was completely inert. The biodegradability of NC depends on surface chemistry, namely the large surface area-to-volume ratio. This may limit surface interactions with microorganisms, which are necessary for biodegradation [38]. This property is useful in the context of using NC as a nanofiller in polymers, which should be persistent. To increase NC recalcitrancy toward biodegradation, researchers have proposed surface modification by hydrophobic functional groups [39]. Nevertheless, the nano-size range of cellulose does not always inhibit biodegradation. This process involves different microorganisms and pathways [40].

Along with the highest biogas production, C degradation resulted in the lowest TS and EC as well as relatively high pH compared with the initial landfill leachate (8.50 and 7.94, respectively; Table 2). The effect of pH on C degradation has been studied [36]. Specifically, C degradation by ruminal microbes increased from pH 6.0 to 7.5, whereas at pH $\leq$5.5, there was no degradation [36].

A higher ratio of C in food and paper waste influences the microbial community composition during thermophilic anaerobic co-digestion and leads to the direct formation of acetate and hydrogen from monosaccharide by syntrophs rather than propionate and butyrate. In that case, cellulose-degrading bacteria prevail—for example, *D. tunisiensis* [41]. Interestingly, the present study showed the highest prevalence of *D. tunisiensis* in BAD-AT (84.24%), which was followed by BAD-V (21.46), BAD-C (16.52%), and BAD-P (11.70%). RL and BAD-0 contained only 2.40% and 0.05%, respectively, of this microorganism (Figure 2F). These data were confirmed by the beta diversity analysis (Figure 3B). The shift in microbial community structure upon the anaerobic digestion of biobased plastic depends on temperature. Thus, under mesophilic conditions, *Anaerolineales*, *Bacteroidales*, *Clostridiales*, *SBR1031*, and *Synergistales* appear to play an important role. In turn, under thermophilic conditions, the hydrolysis, acidogenesis, and methanogenesis of most biobased plastics are mainly conducted by *Coprothermobacter* and the archaea *Methanothermobacter* [22]. In another study on the anaerobic digestion of biobased plastic, archaea exhibited different biodiversity at distinct temperatures and prevailed under thermophilic conditions (*Methanothermobacter*, 56.0%) [17]. Therefore, different conditions of anaerobic digestion, as well as the type of polymer(s) and inoculum, are expected to result in distinct shifts in the microbial community structure, which adapts to the syntrophic degradation of polymers. The production of biogas in the presence of cellulose-based fibres is stimulated by the partial breakage of cellulose bonds and easy solubilisation of acetate [35]. In this respect, cellulases play a crucial role in the hydrolysis stage. According to Jin et al. [42], the anaerobic digestion of 11 textile wastes was initially supported by predominant bacteria, mostly *Clostridium*, which attached to the surface of the textiles and converted cellulose and hemicellulose into

acetate as the core intermediate. Afterwards, acetate was utilised by methanogens via the acetoclastic methanogenesis pathway to produce methane [42].

In the present study, the type of polymer added to the landfill leachate specifically influenced the microbial enzyme activity at the end of anaerobic digestion. Cellulolytic, hydrolytic, and dehydrogenase activities behaved differently depending on the polymer. Furthermore, distinguishing the extracellular versus biofilm enzyme activity provided additional data on mechanisms of substrate conversion in anaerobic digestion. Mazzotta et al. [43] recently studied the efficiency of marine microorganisms to degrade a cellulose diacetate plastic used in textiles and single-use consumer products [43]. The disintegration of this plastic in a continuous sea water mesocosm lasted for months, which was accompanied by increased esterase and cellulase activity in the biofilm community products [43]. Based on the present study, cellulases were mostly concentrated in biofilm on the polymer surface, and their activity was comparable among all samples except BAD-NC and BAD-P, which showed decreased cellulase activity in the pellet, which was probably due to the surface area-to-volume ratio, as mentioned above (Figure 4). CLSM revealed the lower extent of colonisation on NC and P compared with the other polymers, where the abiotic part prevailed (Figure 7, III).

DHA was mostly associated with polymer fragments (pellet; Figure 5). In a previous study, there was a positive correlation between DHA and methane yield in an anaerobic digestion system, using wheat straw and sheep manure as substrates [44]. In the present study, extracellular DHA correlated negatively with biogas production (R^2 = 0.78), while polymer-associated DHA did not show any correlation with biogas production. Moreover, hydrolytic activity (e.g., FDA hydrolysis) in the supernatant also demonstrated a high negative correlation with biogas production (R^2 = 0.84). Of note, there was a drastic decrease in FDA hydrolysis in the pellets and supernatants of the samples with the highest biogas production (Figure 6 and Table 4). SEM revealed the defragmentation of all six types of polymers, findings that corroborate the data on the surface erosion of biobased plastics upon anaerobic digestion, which have been reported previously [22]. Nevertheless, the extent of morphological changes of textile upon anaerobic digestion can vary notably depending on the experimental conditions. For example, the degradation of cellulose-based fibres during mesophilic anaerobic digestion at the initial pH of 7.0–7.5 and a TS of 1%–4% was higher in the samples with a lower solid concentration [35].

The valorisation of textile waste by anaerobic digestion is preferable because of its economic and environmental benefits. Landfill leachate is appropriate for the anaerobic biodegradation of cellulose-derived polymers. In the present study, NC and P resisted biodegradation under the tested conditions. Earlier studies reported a positive role of P for increasing anaerobic digestion, describing its function like that of powdered activated carbon. P may provide adsorption sites where a substrate can be accumulated and a more favourable growth environment for bacteria–substrate systems can be produced [45]. Another study on the anaerobic digestion of P in freshwater lakes revealed the high activity of pectinolytic anaerobes, with *Clostridium butyricum* being prevalent [46]. In the present study, the low activity of P as a substrate for biogas production could be associated with the specific characteristics of the landfill leachate. This effect requires further investigation. Pectin solubilisation can be facilitated by alkaline and thermal pretreatment [47].

Due to polymer/textile complexity, some substrates pose a significant challenge to microbial degradation and anaerobic digestion. Additional pretreatment technologies (e.g., hydrothermal) are suggested to improve biogas production [48]. The further optimisation of digestion conditions for the degradation of biobased plastics/textiles should evaluate environmental performance and the ability to avoid greenhouse gas emissions (which would have negative impact values) [49]. Nevertheless, the environmental impact of incomplete biodegradation requires further investigation. The risks of unintended consequences related to biodegradable plastics introduced into the environment remain largely unexplored [50].

5. Conclusions

The results of this study allowed the following conclusions to be drawn:

- The anaerobic digestion of polymers in landfill leachate is an appropriate model to study the mechanisms of biogas production. The leachate provides a diverse inoculum that is inert in terms of biogas production. At the same time, some of the results differ from what has been reported in the literature; hence, the specific physicochemical characteristics of leachates should be considered.
- The biodegradability of CA and AT differed, even though both polymers represent acetate cellulose. In turn, biogas production from C and V degradation was similar. More initial characteristics of fabrics should be considered when predicting their efficiency to produce biogas.
- Regarding the shifts in microbial community structure in the presence of the tested polymers, the beta-diversity analysis showed that BAD-0, BAD-NC, and BAD-P tended to cluster in the same quadrant. These samples did not show biogas production. Conversely, BAD-AT and BAD-V, which produced biogas, clustered near each other and were represented by D. tunisiensis. BAD-CA was similar to RL with a domination of Methanoculleus spp. Thus, the type of polymer considerably influences the development of a microbial community.
- Analysis of microbial enzyme activities after the 147-day BAD revealed notable changes related to the specificity of each enzyme group. The cellulase activity and DHA were mostly associated with polymers, while FDA hydrolysis was higher in the supernatant and negatively correlated with biogas production. More data are necessary to bridge the knowledge gap in understanding the mechanisms of polymer biodegradability upon anaerobic fermentation.

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