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# Together Is Better: The Rumen Microbial Community as Biological Toolbox for Degradation of Synthetic Polyesters

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Microorganisms, like bacteria and fungi, are becoming an emerging resource for the development of eco-sustainable plastic degradation and recycling processes. In this study, the rumen content from cattle (*Bos taurus*) was investigated regarding synthetic polyester hydrolyzing enzymes based on the fact that the diet of ruminants may contain natural plant polyesters. A screening with model substrates demonstrated hydrolytic activities of rumen fluid on *p*-NP-esters with four to eight carbon atoms. Rumen fluid hydrolyzed synthetic aromatic polyesters with higher amounts of terephthalic acid released from poly(butylene adipate-co-terephthalate) (PBAT) (0.75 and 0.5 mM for polymer powder and film, respectively) and thus exceeded when compared to the hydrolysis of the second terephthalic acid-based polymer—poly(ethylene terephthalate) (PET) (0.6 and 0.15 mM, for powder and film, reciprocally). Additionally, rumen fluid hydrolyzed the bio-based polyester poly(ethylene furanoate) (PEF) according to HPLC and SEM analysis. Shotgun metagenome analysis of the rumen microbiome revealed the real proportion of all domains of life, showing the dominance of bacteria (98%), followed by Eukaryota (1%) and finally Archaea. Among the most abundant genera encountered in this study, polyester hydrolysis activity has already been proven (e.g., *Pseudomonas*).

**Keywords:** synthetic polyesters hydrolysis, recycling, microbial communities, rumen, polyester degrading enzymes

## INTRODUCTION

In Europe, the widespread consumption of plastic waste led to the accumulation of 25.8 million tons of waste in the terrestrial and marine environment, with polyesters accounting for approximately 15% thereof (Plastics Europe and EPRO, 2019). Recently, increasing public awareness and changing legislation have pushed the development of new strategies to reduce this huge environmental problem. Moreover, in the last two decades, the concept of bio-economy motivated the scientific community to focus on production of bio-based and/or biodegradable

polyesters and/or technologies for microbial or enzymatic recycling (Pellis et al., 2016b). Thereby, the application of highly specific enzymes allows for a stepwise recovery of highly valuable building blocks from blended plastics or mixed waste streams. Previous studies from our group and others have demonstrated the potential of enzymes for the hydrolysis of poly(ethylene terephthalate) (PET), the most important synthetic polyester used in numerous applications including textiles and packaging (Herrero Acero et al., 2011; Pellis et al., 2016b; Quartinello et al., 2017). Apart from PET, two further polyesters recently gaining interest were assessed in this study, namely, the biodegradable poly(butylene adipate-*co*-terephthalate) (PBAT) and the bio-based poly(ethylene furanoate) (PEF). PBAT is a copolymer consisting of an aliphatic part (units of adipic acid and 1,4-butanediol) and the aromatic unit (terephthalic acid) (Biundo et al., 2016). Thanks to its properties, PBAT finds applications mostly as food packaging material and compostable plastic bags and, as a result of the presence of the aliphatic chains, renders the material compostable. On the other hand, PEF is based on 2,5-furandicarboxylic acid which is produced from renewable resources (Weinberger et al., 2017a). Several studies defined that mechanical properties (e.g., gas barrier of oxygen, carbon dioxide, and water vapor) of PEF are comparable to PET, while the bio-based origin can drastically reduce greenhouse emissions (Weinberger et al., 2017a). While enzymatic hydrolysis of these polyesters has been reported, there is still a strong demand for more efficient enzymes allowing industrial implementation. In addition to cultivation-based (Yoshida et al., 2016) or *in silico*-based screening of polyester-degrading microorganisms and their enzymes eventually followed by genetic engineering (Biundo et al., 2018), metagenomic approaches allow for the assessment of enzymes produced by microorganisms living almost in any niche in the biosphere with distinct roles in the ecosystem.

Using the above approach, microbial polyester hydrolyzing enzymes have been identified from different ecosystems including leaf-branch compost (Sulaiman et al., 2012), marine (Pinnell and Turner, 2019), and moss-associated environments (Müller et al., 2017). The identified enzymes are mainly microbial cutinases able to hydrolyze the natural plant polyester cutin, a major component of the plant cell wall.

Following from metagenomic screening, different hydrolases with polyester-degrading abilities have been isolated from Actinobacteria (e.g., *Thermobifida* spp.) and from fungi (*Fusarium solani*, *Humicola insolens*, *Aspergillus* spp., and *Rhizopus* spp.) (Ribitsch et al., 2013; Wei and Zimmermann, 2017). Subsequently, some of the novel hydrolases were engineered to further improve their activities on synthetic polyesters (Ribitsch et al., 2013). However, many ecosystems still remain to be exploited for polyester-hydrolyzing activities.

Ruminants are a well-studied group of herbivorous mammals. They have evolved a forestomach, the rumen (or more precisely the reticulorumen), which is a large chamber (50–100 L capacity in adult cattle) where the ingested feed is subjected to microbial degradation before the real animal digestion. The content in the rumen is quite heterogeneous, consisting of a mass of float (forage), sediment, liquids, and microbial cells (Gijzen et al., 1987). The rumen ecosystems, in this sense, can be defined as a

“chemostat” where different biochemical reactions are occurring. Among these, enzymatic hydrolysis, e.g., of (hemi)cellulose and the responsible enzymes have been studied intensively, while other polymer-degrading enzymes potentially present in rumen remained under-explored. Yet the diet of ruminants may contain natural plant polyesters (cutin), and thus the enzymes with hydrolytic activities may be present in the rumen (Mao et al., 2013). As a consequence, the main objective of this study therefore was to explore the microbial community of the rumen from ruminant animals regarding polyester-degrading enzyme activities.

## MATERIALS AND METHODS

### Chemicals and Substrates

Buffer components, bovine serum albumin (BSA), *para*-nitrophenyl-esters (*p*-NP-esters), terephthalic acid (Ta), and formic acid were purchased from Sigma-Aldrich (United States). Fresh rumen liquid was obtained from a slaughterhouse (Mayr, Natters, Austria), which originated from an alpine pasture-fed young ox. Within 2 h of sampling, the rumen was sieved to <2 mm and immediately stored at  $-20^{\circ}\text{C}$ , till the start of the experiments.

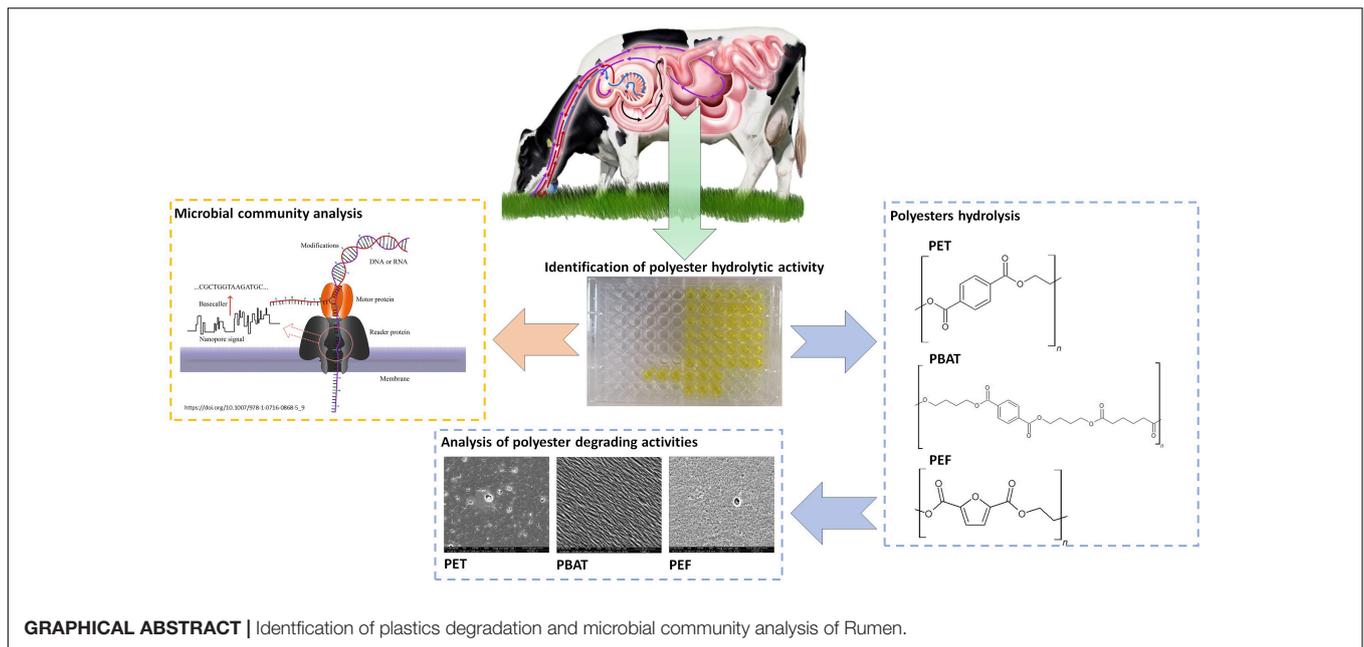
Poly(ethylene terephthalate) (PET) powder and film were purchased from Goodfellow (United Kingdom). Polybutylene adipate-*co*-terephthalate (PBAT) powder and film were provided from BASF (Germany). Poly(ethylene furanoate) (PEF) powder and film were supplied by Corbion (Netherlands). Molecular weight (kDa) are listed in **Supplementary Table 1**. All other chemicals and reagents were purchased from Sigma-Aldrich and used at reagent grade, without further purification if not otherwise specified.

### Esterase Activity Assay and Protein Concentration Assays

The pH in rumen ranges between 6 (in case of animal acidosis) and slightly higher than 7 under normal conditions (Mao et al., 2013). As the exposure to oxygen causes a fast rumen acidification, thawed rumen samples were diluted in 100 mM pH 7 potassium phosphate buffer with a ratio of 1:10 and stirred for 2 h at 150 rpm. Esterase activities were measured with different *p*-nitrophenyl (*p*-NP)-esters as substrates: *p*-NP-acetate (*p*-NPA, C<sub>2</sub>), *p*-NP-butyrate (*p*-NPB, C<sub>4</sub>), *p*-NP-hexanoate (*p*-NPH, C<sub>6</sub>), *p*-NP-octanoate (*p*-NPO, C<sub>8</sub>), *p*-NP-decanoate (*p*-NPD, C<sub>10</sub>), and *p*-NP-laureate (*p*-NPL, C<sub>12</sub>) as described by Biundo et al. (2016). Protein concentration was determined according to the Bio-Rad protein assay (Bio-Rad Laboratories GmbH, Vienna, Austria, catalyst no.: 500-0006, Austria) (Pellis et al., 2016a).

### Enzymatic Hydrolysis of Polymer Powders and Films

Briefly, 5.0 mg of the polymer powders (PET, PBAT, and PEF) were incubated with 2.0 ml of rumen solution. Incubations were conducted for 24, 48, and 72 h in an orbital shaker set



at 150 rpm and mimicking the rumen temperature (i.e., 40°C). Blank reactions were carried out in buffer. All reactions were performed in triplicates (Quartinello et al., 2017).

PET, PBAT, and PEF amorphous film samples of 0.5 × 1.0 cm were washed three times as previously described by Vecchiato et al. (2017). After washing, the films were incubated with 2 ml of sieved rumen samples for 24, 48, and 72 h. Incubations were performed with the same settings as the powder's incubation. To test activity of various microorganisms on polyesters from *Penicillium citrinum*, *Beauveria bassiana*, and *Pseudomonas pseudoalcaligenes* were isolated and cultivated as previously described (Liebminger et al., 2007; Almansa et al., 2009). Following incubation, the culture supernatants were collected and centrifuged at 4°C and 3,700 rpm for 25 min (Eppendorf Centrifuge 5920 R) and thereafter incubated at the same conditions performed as earlier described for rumen solution.

## High-Performance Liquid Chromatography (HPLC-DAD)

After enzymatic treatment of polyester powders/films, proteins were precipitated with ice-cold MeOH. Samples were centrifuged at 12,700 rpm at 0°C for 15 min. The resulting supernatant was filtered (0.45 μm PA filters) and filled in an HPLC vial (Quartinello et al., 2017).

HPLC coupled with reversed-phase column C18 was used for analysis of hydrolysis products using a H<sub>2</sub>O/MeOH/HCOOH gradient (**Supplementary Table 2**). The flow rate was set to 0.85 mL min<sup>-1</sup>, and the column was maintained at a temperature of 40°C. The injection volume was 10 μl. Detection of the analytes was performed with a photodiode array detector at a wavelength of 245 nm (for PET and PBAT) and 260 nm (for PEF) (Weinberger et al., 2017b).

For analysis of PET hydrolysis products, Ta, mono-2-hydroxyethyl terephthalate (MHET), and bis(2-hydroxyethyl)terephthalate (BHET) calibration curves were prepared, while Ta, mono(hydroxybutyl)terephthalate (BTa), and bis(4-hydroxybutyl) terephthalate (BTaB) calibrations were prepared for PBAT released product detection (Biundo et al., 2016). A standard 2.5-furandicarboxylic acid (FDCA) calibration curve was used for quantification of PEF hydrolysis products (Perz et al., 2016).

## Scanning Electron Microscopy (SEM)

For the SEM investigations, a FEI Quanta 250 FEG (Thermo Fisher Scientific, Hillsboro, OR, United States) was used under high-vacuum conditions and a variable high tension from 5 to 10 kV. The micrographs were recorded with the Everhart-Thornley detector in secondary electron (SE) mode. The fracture surface was sputter-coated with a 10-nm-thin layer of gold in order to provide sufficient electrical conductivity (Quartinello et al., 2019).

## Microbial Community Analysis

### DNA Extraction

Total DNA was isolated from a 1 ml rumen sample. Debris and liquid fraction were briefly vortexed for sample homogenization, split in two aliquots, and thereafter centrifuged. The pellets were then resuspended in 100 μl 0.01 M phosphate buffer. The DNA extraction was performed using the FastDNA SPIN kit for soil (MP Biomedicals, Illkirch, France) according to the manufacturer's recommendations. In order to determine DNA purity and concentration to match the Nanopore workflow's requirement, the extracts were pooled and purified using the QIAamp Viral RNA Mini Kit (Qiagen). The DNA concentration was assessed by using the Qubit 2.0 fluorometer (Invitrogen Corporation), with the Qubit dsDNA BR Assay Kit.

## Library Construction, Template Preparation, and Sequencing

A shotgun metagenome analysis was performed with the extracted DNA without any step of amplification of target sequences. To this end, DNA libraries were constructed following the “1D Genomic DNA by Ligation” guideline available in the Oxford Nanopore community using the Ligation Sequencing kit SQK-LSK109 and the Flow Cell Priming Kit EXP-FLP001 (Oxford Nanopore, United Kingdom). All steps for library construction were performed following the specifications of the protocol and using 1  $\mu$ g input of extracted DNA. Following the preparation of the DNA ends for adapter attachment using the NEBNext FFPE DNA repair mix (M6630) and the NEBNext End Repair/dA-Tailing Module (E7546) (New England Biolabs), the attachment of sequencing adapters (supplied in the kit) to the DNA ends was achieved by means of the NEBNext Quick Ligation Module (E6056, New England Biolabs, Ipswich, MA, United States).

After each step of library preparation, the sample cleanup was performed using the AMPure XP beads (Agencourt, Beckman). A flow cell quality control (SpotON Flow Cell Mk I R9 Version, FLO-Min 106D) was run prior to the start of sequencing. The MinKNOW™ software was used to check the number of active pores in the flow cell. Finally, the priming and the loading of the DNA library into the flow cell were performed following protocol recommendations.

The Nanopore device (MinION) was connected to a portable computer, and the software MinKNOW was launched after the appropriate protocol script was selected. The sequencing run was performed for 36 h.

## Data Analyses

The quality of the run was monitored by checking the duty time plot showing the distribution of channel states—sequencing, pore, recovering, inactive, and unclassified—over time. The resulting fastq data files were base-called live using the Nanopore GPU with the GUPPY base-calling software 3.0.3 on UBUNTU 16.04 (Nanopore Community Platform). GUPPY is a data processing toolkit that contains the Oxford Nanopore Technologies’ base-calling algorithms and several bioinformatic post-processing features. The fastq files generated in the sequencing run were compared with databases using one of the available pipelines for data analyses of the Nanopore Community Platform, following the steps recommended by manufacturers. The selected workflow chosen was “What’s in my pot” (WIMP), which is an EPI2ME workflow for taxonomic classification of base-called sequences (reads) generated by Nanopore sequencing. WIMP initially filters fastq files with a mean q-score below a minimum threshold (defaults to 7). For reads above the quality threshold, the Centrifuge classification engine is executed to assign each read to a taxon in the NCBI taxonomy. The Centrifuge classification results are then filtered and aggregated to calculate and report counts of reads at the species rank. For reads without a reliable assignment at the species rank, higher ranks of the taxonomy tree are used for the assignment. If no placement is reliable enough (below a scoring threshold), the sequence is labeled as

“Unclassified.” The run report, including identification data and associated number of reads presented as NCBI taxonomy trees, is accessible in the EPI2ME section of the Nanopore community website.

## RESULTS AND DISCUSSION

### Identification of Polyester-Degrading Activities

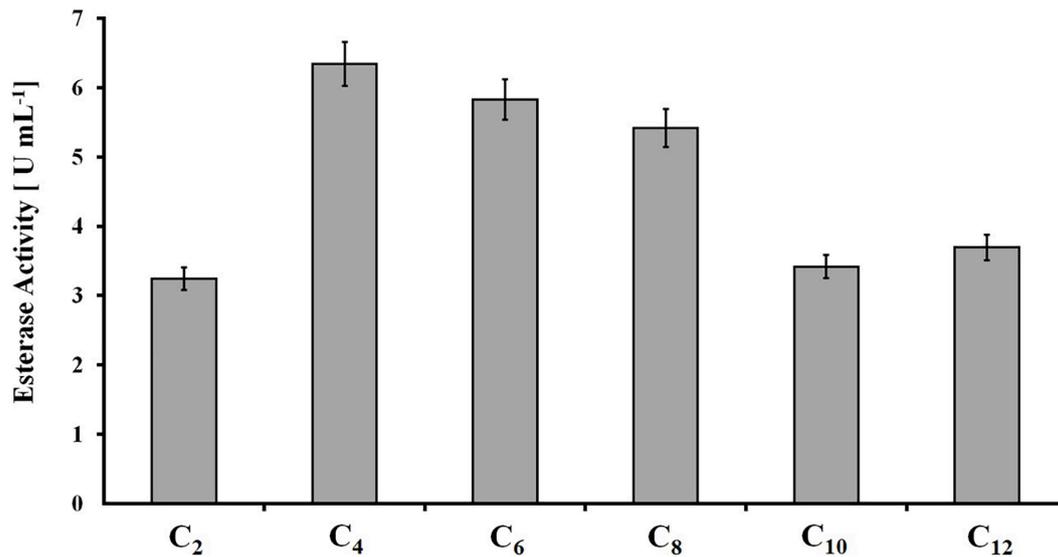
Despite the potential of microbial communities from different environments to contain novel polyester-degrading enzymes (Danso et al., 2019), the plenitude of polyester-degrading enzyme activities present in rumen has not yet been fully exploited. Yet rumen from alpine pasture-fed animals could contain polyester-degrading enzymes needed for the digestion of polyesters such as cutin from berries (Kallio et al., 2006).

The protein concentration in rumen samples was 2.5 mg ml<sup>-1</sup> ( $\pm 0.1$  mg ml<sup>-1</sup>). All short-chain *p*-NP-esters were hydrolyzed into *p*-nitrophenol, with slightly higher activity on those esters based on C<sub>4</sub>, C<sub>6</sub>, and C<sub>8</sub> acids (6.5, 5.8, and 5.5 U ml<sup>-1</sup>, respectively) (Figure 1). Therefore, rumen content demonstrated considerably higher esterase activity in comparison with polyester-induced fungal supernatants, where esterase activity accounted for approximately 0.4 U ml<sup>-1</sup> with *p*-NPB (C<sub>4</sub>) as a model substrate (Weinberger et al., 2020).

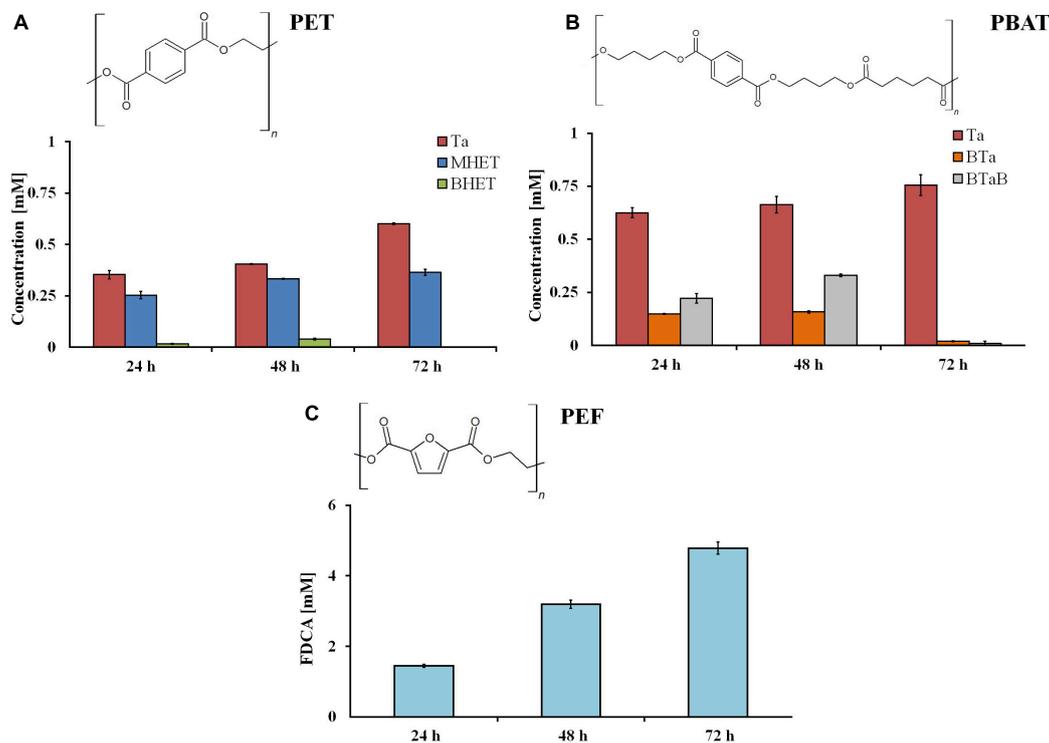
The investigation of hydrolysis efficiencies revealed that rumen samples were able to degrade all three tested polyesters. As expected, the hydrolysis of powders was faster in comparison to films, due to the higher surface-to-volume ratio. After 72 h, 0.6 and 0.75 mM of hydrolysis products were released from the aromatic polyesters PET and PBAT, respectively. Considerably higher amounts (4.8 mM) of hydrolysis products (namely, FDCA) were released from PEF (Figure 2).

In comparison to polymer powder, much lower amounts of hydrolysis products were released from all polyester films when compared to the powders. After 72 h, 0.15 mM of Ta were measured from PET (Figure 3A), while from PBAT, a higher amount (0.5 mM) of Ta was released most likely due to its aliphatic component. In case of PBAT, it can be clearly seen that oligomers released are subsequently hydrolyzed after prolonged incubation (Figure 3B). Like for the other polyesters, a lower amount of hydrolysis products (0.78 mM) was released from PEF films when compared to powders (Figure 3C). Nevertheless, in case of both films and powders, the rumen enzymes were more active on PEF when compared to PET. Apart from the chemical composition (FDCA versus Ta as acid components), other properties can have a strong influence on enzymatic hydrolysis. The polymer chain must be flexible enough to allow access for and sorption of the enzymes. Furthermore, the degree of crystallinity, chain length, or possible intermolecular bonds can have an impact on hydrolysis (Wei and Zimmermann, 2017).

Since the enzymes are unable to diffuse into the polymer matrix due to their size, hydrolysis starts with surface erosion. While a quite uniform hydrolysis with small cavity formation was observed for PET, both PBAT and PEF formed rougher surfaces



**FIGURE 1** | Hydrolysis of *para*-nitrophenyl-esters with different fatty acid chain lengths (C<sub>2</sub> to C<sub>12</sub>) by rumen samples. Data are mean values of triplicate experiments, and bars represent the standard deviation.

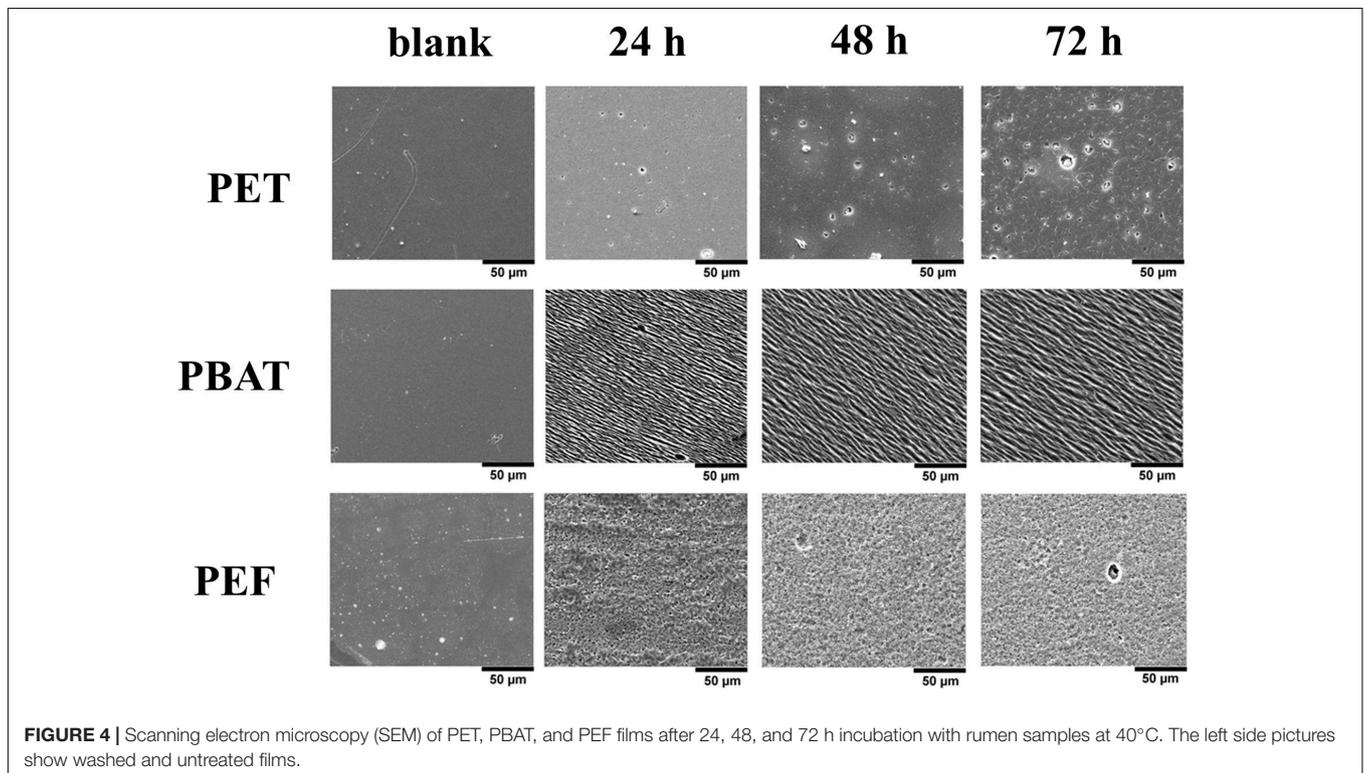
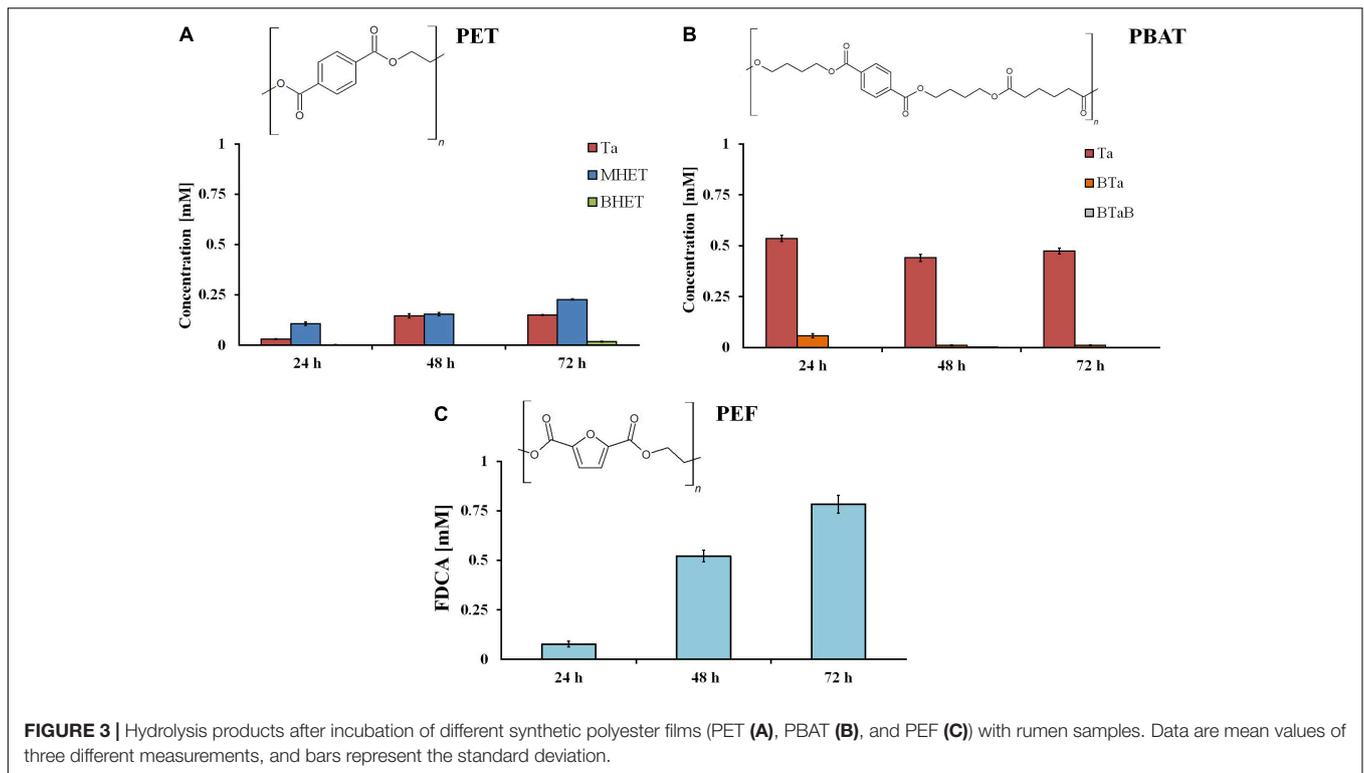


**FIGURE 2** | Hydrolysis products after incubation of different synthetic polyester powders (PET **(A)**, PBAT **(B)**, and PEF **(C)**) with rumen samples. Ta, terephthalic acid; MHET, mono-2-hydroxyethyl terephthalate; BHET, bis(2-hydroxyethyl)terephthalate; BTa, mono(hydroxybutyl)terephthalate; BTaB, bis(4-hydroxybutyl) terephthalate; FDCA, 2,5-furandicarboxylic acid. Data are mean values of three different measurements, and bars represent the standard deviation.

already after 1 day. In PBAT films, groove-like structures were seen while for PEF crater-like structures were formed (**Figure 4**).

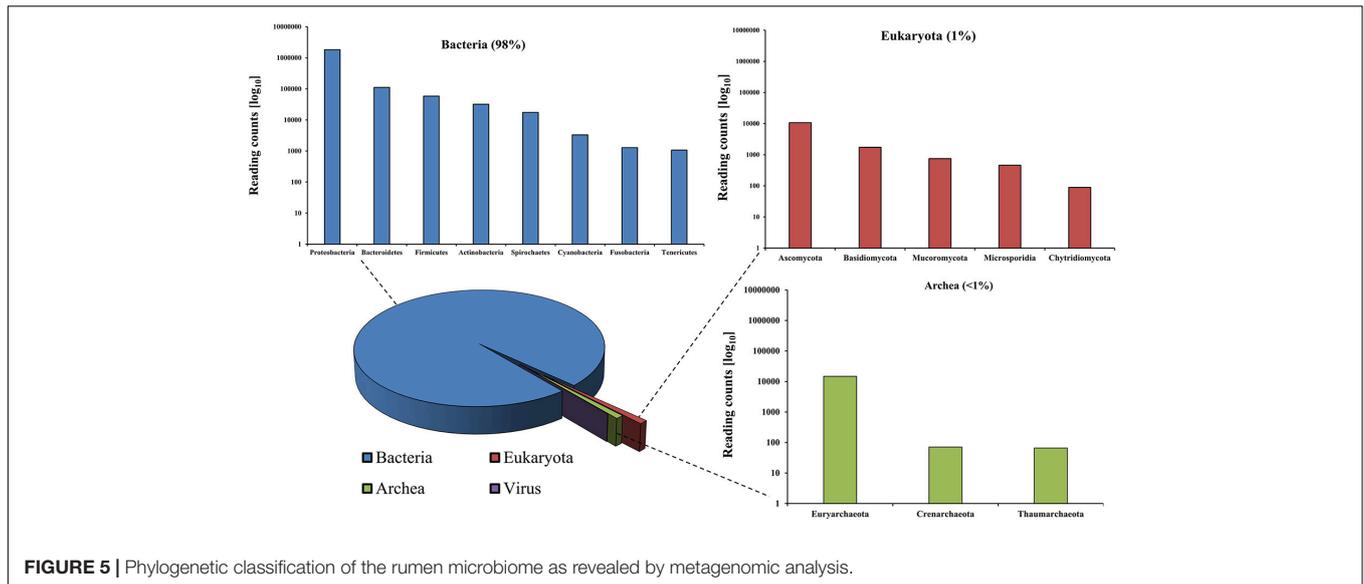
Previous studies have investigated the potential of synthetic polyester degradation using metagenomic libraries combined,

e.g., with agar plate-based activity assay (Haernvall et al., 2017; Bollinger et al., 2020b; Molitor et al., 2020). This study was designed based on incubation with polymers, in order to assign polyester hydrolysis activity in the rumen microbiome to



identified possible candidates within this community. Despite this difference, most of the previous investigations on polyester degradations were conducted using singular microorganisms. For

example, *Yarrowia lipolytica* has been investigated for amorphous PET film degradation, where 0.06 mM Ta was released after 72 h (da Costa et al., 2020). Recently, the black fungus *Knufia*



**FIGURE 5 |** Phylogenetic classification of the rumen microbiome as revealed by metagenomic analysis.

**TABLE 1 |** Analysis of the rumen microbial community related to the production of enzymes potentially capable of polyester degradation.

Microorganism	Kingdom	Enzymes				References
		Esterase	Lipase	Cutinase	Protease	
<i>Pseudomonas</i> spp.	Bacteria	Gray	Gray	Gray	Gray	Haernvall et al., 2017; Bollinger et al., 2020a
<i>Stenotrophomonas maltophilia</i>	Bacteria				Gray	Wu et al., 2015
<i>Acinetobacter</i> spp.	Bacteria		Gray	Gray	Gray	Suzuki et al., 2002
<i>Thauera</i> spp.	Bacteria				Gray	Yu et al., 2018
<i>Delftia tsuruhatensis</i>	Bacteria				Gray	Shigematsu et al., 2003
<i>Acidovorax</i> spp.	Bacteria	Gray				Teeraphatpornchai et al., 2003
<i>Exophiala</i> spp.	Eukaryota		Gray			Song et al., 2017
<i>Fonsecaea</i> spp.	Eukaryota		Gray			Publishers KA, 1989
<i>Aspergillus</i> spp.	Eukaryota	Gray			Gray	Weinberger et al., 2020
<i>Penicillium</i> spp.	Eukaryota	Gray			Gray	Weinberger et al., 2020
<i>Talaromyces</i> spp.	Eukaryota		Gray			Romdhane et al., 2010
<i>Trichophyton</i> spp.	Eukaryota		Gray			Niaz et al., 2013
<i>Botrytis cinerea</i>	Eukaryota		Gray			Reis et al., 2005
<i>Sclerotinia sclerotiorum</i>	Eukaryota	Gray				Bashi et al., 2012
<i>Pseudogymnoascus</i> spp.	Eukaryota		Gray			Sahay and Chouhan, 2018
<i>Colletotrichum</i> spp.	Eukaryota	Gray	Gray		Gray	Balaji and Ebenezer, 2008
<i>Beauveria bassiana</i>	Eukaryota				Gray	Vici et al., 2015
<i>Trichoderma</i> spp.	Eukaryota	Gray	Gray			Roussel et al., 2014
<i>Purpureocillium lilacinum</i>	Eukaryota				Gray	Cavello et al., 2013
<i>Scedosporium</i> spp.	Eukaryota				Gray	Han et al., 2017
<i>Chaetomium thermophilum</i>	Eukaryota	Gray				Tong et al., 2014
<i>Thermothelomyces thermophilus</i>	Eukaryota				Gray	Zerva et al., 2018
<i>Thielavia terrestris</i>	Eukaryota	Gray				Meng et al., 2019
<i>Candida</i> spp.	Eukaryota		Gray			Weinberger et al., 2020
<i>Yarrowia lipolytica</i>	Eukaryota		Gray			Kim et al., 2007
<i>Ustilago maydis</i>	Eukaryota	Gray	Gray			Katsivela et al., 1995
<i>Natrialba magadii</i>	Eukaryota				Gray	Giménez et al., 2000
<i>Methanocorpusculum labreanum</i>	Archaea				Gray	Nam et al., 2013
<i>Methanoregula</i> spp.	Archaea	Gray				Bayer et al., 2010

The gray bar is correlated to specific hydrolytic activity described in the reference.

*chersonesos* showed the presence of polyesterases, able to cleave PBAT (0.6 mM Ta released), while from enzymatic treatment with rumen, 0.75 mM of the same monomer was released (Tesei et al., 2020). These preliminary results can confirm that different enzymatic activities and/or microorganisms (like rumen microbiome) can break down polymeric structures, as also demonstrated by Barth et al. (2016). On the other hand, PET surface erosion was comparable with previous investigations using different purified variants of the *Thermobifida cellulosilytica* cutinases 1 and 2 (Thc\_Cut1 and Thc\_Cut2) (Vecchiato et al., 2017) but was lower when compared with purified engineered PETase from *Ideonella sakaiensis*, which was able to release 0.5 mM Ta from PET film, whereas 0.15 mM of the monomer was obtained from rumen incubation (Yoshida et al., 2016). Recently, the thermophilic *Clostridium thermocellum* was engineered for the extracellular secretion of LC cutinase, which resulted in around 1 mM terephthalic acid released (Yan et al., 2021). This is more than that found in this study for the wild-type rumen consortium and indicates the potential of further engineering of promising candidate microbes/enzymes from the rumen consortium.

## Microbial Community Analysis

The analysis of extracted DNA from rumen content was performed via shotgun metagenomics using sequencing platform, namely, the MinION Nanopore technology (Brown et al., 2017). Around 3.6 million reads and a total 14.8 Gb of DNA were obtained, with an average length of 4,084 bases (Supplementary Figure 1). More than two million, out of the total number of reads obtained, could be taxonomically classified. The shotgun metagenomic approach applied in this study allowed us to infer the actual proportions of all domains of life and thus an overall identification of the rumen microbial community, which revealed 98% of Bacteria, 1% of Eukaryota, around 0.9% of Archaea, and finally less than 0.1% of viral origin (Figure 5).

Specifically, among the bacterial kingdom, *Proteobacteria* were predominant, followed by *Bacteroidetes*, *Firmicutes*, and *Actinobacteria*. Species of the *Pseudomonas* genus accounted for the highest number of reads of all identified genera within the rumen microbiome (Supplementary Figure 2). *Pseudomonas* spp. are typically present in food and feed and are responsible for enzymatic spoilage (Caldera et al., 2016). Interestingly, *Pseudomonas veronii* ( $>10^6$  reads), which is known to be involved in degradation of alkyl methyl ketones (Onaca et al., 2007), was the most dominant species in the rumen microbiome. *Acinetobacter* was demonstrated to be the second most common species in this microbial community, which is involved in enhancing the intestinal digestive activities and improving feed utilization (Mao et al., 2013).

Rumen Eukaryotes can be mostly considered to belong to the *Opisthokonta* which comprise metazoans (animals), fungi, and other microbial Eukaryotes, including the *Choanoflagellida*, *Ichthyosporae*, *Nucleariida*, and *Capsaspora* (Supplementary Figure 3) (Karpov et al., 2014). Fungi comprised *Ascomycota* and *Basidiomycota* phyla, which are important for the degradation and the use of fibrous cellulose feed. Another detected eukaryotic

phylum was *Mucoromycota*, which is often associated with plant matter and therefore introduced with the ruminant's diet.

Expectedly, members of the domain of *Archaea*, which are responsible for ruminant methanogenesis (Patra et al., 2017), were found, including *Euryarchaeota* and, within this phylum, methanogenic organisms like members of the genus *Methanobrevibacter*, which are able to reduce acetic acid into carbon dioxide and methane, as the most abundant genus (Supplementary Figure 4).

## Potential Polyester-Degrading Microorganisms

Among hydrolases, esterases, lipases, and cutinases have been described to hydrolyze synthetic polyesters while proteases may hydrolyze polyamides. Indeed, such activities have been reported for abundant species identified in the rumen microbiota (Table 1). Considering the number of generated and taxonomically classified reads in this study, *Pseudomonas* spp. in rumen samples may play a major role in polyester degradation. These bacteria are well known to be able to produce different hydrolytic activities: esterases, lipases, and cutinases (Bollinger et al., 2020a,b), for all of which hydrolysis of PET has been previously reported. For example, an arylesterase from *P. pseudoalcaligenes* was recently demonstrated to hydrolyze ionic phthalic polyesters and PBAT (Wallace et al., 2017) while enzymatic polyester-polyurethane hydrolysis was reported for *Pseudomonas fluorescens* (Jia et al., 2021). On the other hand, *P. veronii* has been characterized for potential application toward bioremediation of crude oil, hydrocarbons, and aromatic and other hydrophobic compounds (McCarthy et al., 2017). Moreover, different bacterial species (like *Pseudomonas*) can utilize polyester hydrolysis products such as terephthalic acid as carbon sources (Wilkes and Aristilde, 2017). *Acinetobacter* spp. are well-known bacteria able to utilize different hydrophobic compounds as nutrients, including triglycerides and waxy esters. Therefore, in different studies, *Acinetobacter* spp. were successfully used for biodegradation of synthetic polyesters in the environment (Dong et al., 2017).

Regarding fungi, different hydrolases (lipase and esterase) from *Aspergillus*, *Penicillium*, and *Candida* species are already exploited at industrial level and found applications in different sectors like detergents, textile industry, dairy, and food preparation (Weinberger et al., 2020). Hydrolysis of polyesters and other synthetic polymers was previously described for *Penicillium* and *Beauveria* species (Heumann et al., 2006; Liebminger et al., 2007; Almansa et al., 2009). Fungi involved in plant cell wall degradation, like *Thermothelomyces thermophilus*, *Talaromyces wortmannii* (Zerva et al., 2018), and *Thielavia terrestris* (Meng et al., 2019), produce feruloyl esterases. This class of hydrolases cleaves the ester bonds between hydroxycinnamic acid esterified with arabinoxylans (Wong, 2006). As shown by Tesei et al. (2020) the black fungus *K. chersonesos* is able to secrete an esterase which shows high similarity with either a feruloyl esterase (i.e., AoFaeB from *Aspergillus oryzae*) or MHETase from *I. sakaiensis*, suggesting therefore that it can be possible that this class of hydrolase is involved in synthetic polyester degradation.

On the other hand, enzymes from Archaea are generally under-explored for biotechnological applications. Since the

microorganisms belonging to this domain can live and tolerate extreme conditions (e.g., high temperature, salinity, alkalinity, or acidity), identification of polyester-degrading enzymes could have interesting properties for industrial applications. Among the Archaea identified in the rumen samples, thermophilic esterases have been described for *Methanocorpusculum labreanum* and *Methanoregula* sp. (Bayer et al., 2010; Nam et al., 2013).

As a proof of principle, the activity of selected microorganisms (i.e., their enzymes) listed in **Table 1** was assessed. Among fungi, indeed *P. citrinum* (i.e., culture filtrate) was able to hydrolyze PET while no significant activity was found for *B. bassiana* in terms of release of Ta. Among bacteria, *P. pseudoalcaligenes* was able to hydrolyze aromatic polyesters.

## CONCLUSION

The rumen microbial community has demonstrated the ability to degrade three different synthetic polyesters (PET, PBAT, and PEF) as demonstrated by quantification of solubilized molecules and SEM analysis of surface erosion. In nature, polyester-hydrolyzing activity of rumen microbes may be involved in the digestion of polyesters, such as cutin present in the diet of ruminants. Among the bacteria, fungi and archaea identified in this study by microbial community analysis as two of the most abundant species have been described to produce enzymes potentially capable of polyester hydrolysis. Compared to published data for pure enzymes and/or supernatants of single microorganisms, the polyester-hydrolyzing activity of rumen fluid was relatively high. Apparently, not only one type of enzymes is present in the rumen mixture, but rather a synergistic action of different esterases, lipases, or cutinases may occur.

This is not surprising since there are many examples in nature of biopolymers (e.g., cellulose) where different enzymes are involved in synergistic degradation. Despite the fact that rumen fluid could be a cheap source for polymer-degrading enzymes, future studies should aim at identification and cultivation of

the microbes and enzymes involved in synergistic hydrolysis of polyesters as well as on possible community changes during incubation with polyesters.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

## AUTHOR CONTRIBUTIONS

FQ, KK, and HS performed the experiments. FQ, DR, and GG planned the experiments. LP performed the SEM measurements. DT, GP, and KS performed the DNA extraction and MinION measurement. FQ and DR wrote the manuscript. DR and GG supervised the work. All authors discussed the collected data and corrected the manuscript before submission.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fbioe.2021.684459/full#supplementary-material>

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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