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Assessing labelled carbon assimilation from poly butylene adipate-co-terephthalate (PBAT) monomers during thermophilic anaerobic digestion

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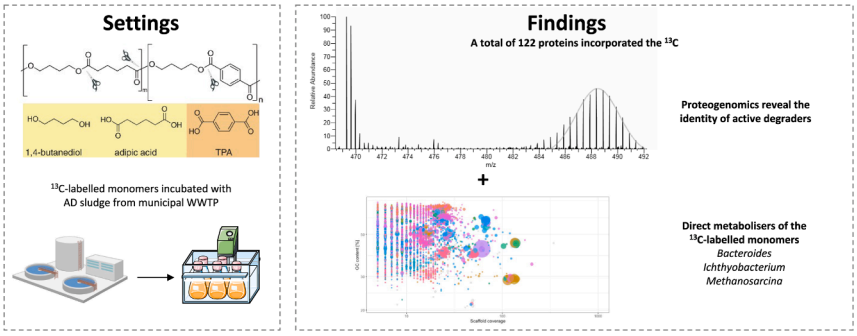
HIGHLIGHTS

- ^{13}C from PBAT monomers was tracked under anaerobic and thermophilic conditions.
- 1,4-butanediol and adipic acid were assimilated in the biomass.
- 3 genera were able to directly metabolise at least 1 of the 3 PBAT monomer units.
- A HQ-MAG was identified as *A. thermophila* and as an active degrader of butanediol.

GRAPHICAL ABSTRACT



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ABSTRACT

PBAT (poly butylene adipate-co-terephthalate) is a widely used biodegradable plastic, but the knowledge about its metabolization in anaerobic environments is very limited. In this study, the anaerobic digester sludge from a municipal wastewater treatment plant was used as inoculum to investigate the biodegradability of PBAT monomers in thermophilic conditions. The research employs a combination of ^{13}C-labelled monomers and proteogenomics to track the labelled carbon and identify the microorganisms involved. A total of 122 labelled peptides of interest were identified for adipic acid (AA) and 1,4-butanedio (BD). Through the time-dependent isotopic enrichment and isotopic profile distributions, *Bacteroides*, *Ichthyobacterium*, and *Methanosarcina* were proven to be directly involved in the metabolization of at least one monomer. This study provides a first insight into the identity and genomic potential of microorganisms responsible for biodegradability of PBAT monomers during anaerobic digestion under thermophilic conditions.

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1. Introduction

With more than 240 million tonnes of plastic being used annually, it is clear that the world is in the plastic age (Plastics Europe, 2021; Schyns & Shaver, 2021). Only a fraction of this is being recycled or recovered, and a significant part of plastic debris therefore accumulates in the environment (Geyer et al., 2017; Rillig, 2012). Displaying the wide and diverse daily life applications, more than 30 kinds of plastic polymers have been found in influents and effluents from wastewater treatment plants (WWTPs) (Sun et al., 2019). A single solution for the plastic problem appears unrealistic, and a combination of different approaches should likely be implemented to overcome this enormous challenge (Jian et al., 2020). The most straightforward strategy could involve the use of biodegradable plastics, combined with a high degree of recycling (Flury & Narayan, 2021).

Poly butylene adipate-co-terephthalate (PBAT) is the second-most produced biodegradable plastic after polylactic acid (PLA) (European Bioplastics, 2022), and from a total of 1.13 million tonnes of biodegradable plastics produced in 2022 in the global market, approximately 9% was PBAT (European Bioplastics, 2022). Its global demand is mainly for shopping and garbage plastic bag production, followed by compostable food packaging (Denial Mahata et al., 2021). It is a random copolyester composed of adipic acid (AA), 1,4-butanediol (BD), and terephthalic acid (TP), marketed as a fully biodegradable alternative to low-density polyethylene (PE). Its biodegradability comes mainly from breakage of ester linkages, as opposed to the carbon–carbon backbones seen in non-degradable polymers such as polyolefins. Nevertheless, the biodegradability of the different plastics has been shown to be strongly dependent not only on their characteristics, such as copolymer composition, structure, molecular weight, crystallinity, size, or shape (Thew et al., 2023; Tokiwa et al., 2009), but also affected by ambient conditions such as redox potential, temperature, or pH (Haider et al., 2019).

In aerobic environments, a discrete number of microorganisms obtained from agricultural soils have been identified to hydrolyse the raw PBAT: fungi from the genera *Cryptococcus* and *Trichoderma* (Jia et al., 2021) and bacteria affiliated with the genera *Stenotrophomonas*, *Bacillus,* and *Pseudomonas* (Jia et al., 2021; Morro et al., 2019; Muroi et al., 2017; Wallace et al., 2017). The release of AA, TP, or BD from PBAT by the action of hydrolases, lipases, and esterases has been confirmed (Jia et al., 2021), and *Bacillus subtilis* and *B. licheniformis* were able to metabolize all of them, yet with strain-specific differences on their efficiency (Morro et al., 2019).

PBAT treatment through composting has been shown to be possible, yet it requires prolonged time periods, in the range of weeks-months, (Zumstein et al., 2018), and releases microplastics and other pollutants (Liu et al., 2023). From an environmental point of view, landfilling is the least preferred option and the European Union (EU) have therefore developed landfill bans and imposed taxes to encourage a more sustainable management of plastic wastes (Schyns & Shaver, 2021; Thew et al., 2023). Industrial composting facilities also require months of processing (ASTM, 2021) as well as large surface areas. It is therefore urgent to explore more efficient and intensive biotechnological alternatives.

Anaerobic digestion presents the advantage of reducing space and aeration costs and potential for sustainable energy production. However, the limited number of studies published addressing this topic to date points towards a very low raw PBAT degradability in the absence of oxygen. A previous study showed that while other biodegradable plastics, such as polyhydroxybutyrate, polycaprolactone, or poly(lactic acid), significantly degrade under mesophilic and thermophilic anaerobic conditions, PBAT and polybutylene succinate biodegrade poorly or not at all under the same conditions (Cazaudehore et al., 2022). A more recent study of raw PBAT biodegradability showed only 13.4% ± 0.4% biodegradability in 500 days under mesophilic conditions and 1.7% ± 1.8% in 100 days in thermophilic range (Cazaudehore et al., 2023). Consequently, for those plastics that are not easily hydrolysed under anaerobic conditions, a two-step treatment could be proposed as a mean to accommodate this limitation. This could involve a hydrothermal pretreatment step as a strategy to enable the accessibility of carbon to the microorganisms. It presents several advantages as water is the only solvent used, it requires short time periods (hours), and it has been demonstrated to be successfully liberating monomers in the case of lignocellulosic biomass (Gallina et al., 2018; Mohanakrishna & Modestra, 2023). In the second step, the non-metabolised PBAT fractions would undergo anaerobic digestion to reduce cost of aeration and heating.

Moreover, anaerobic digestion of biodegradable monomers itself can be an interesting bioresource recovery strategy, leading to the generation of energy by methane conversions. The co-digestion of bioplastics which contain only carbon can lead to a higher C:N ratio during anaerobic digestion, resulting in an increased biomethane potential (Cazaudehore et al., 2022; Stroot, 2001), but only a few studies have covered this topic using PBAT (Abraham et al., 2021). In the context presented herein, it is necessary to establish if the PBAT monomers are easily metabolized under thermophilic and anaerobic conditions.

The aim of this study was to investigate the biodegradability of all PBAT monomers in anaerobic digesters operated under thermophilic conditions (55 °C). The biodegradability was assessed by tracking the carbon from a combination of incubations with ^{13}C-labelled monomers, metagenomics, and proteomic analyses. The approach allowed us to establish the identity and genetic repertoire of PBAT monomers. This workflow represents a high-throughput and culture-independent approach to link functional biodegradation with identity of the key microorganisms directly involved in complex ecosystems.

2. Materials and methods

2.1. Monomers

The ^{13}C-labelled monomers $^{13}C_6$-adipic acid and 1,4-$^{13}C_4$-butanediol were purchased from Merck, with more than 99% of the carbon atoms being ^{13}C. $^{13}C_8$-terephthalate was obtained by hydrolysis of dimethyl ($^{13}C_8$-terephthalate) purchased from Merck. The synthesis was done as previously described (Zumstein et al., 2018), and the final product was confirmed by ^{1}H- and ^{13}C NMR (see supplementary materials). NMR spectra were recorded on a BRUKER AVIII-600 MHz NMR spectrometer equipped with a cryogenic CPP-TCI probe. ^{1}H NMR of dimethyl($^{13}C_8$-terephthalate) was recorded in DMSO-d_6 and referenced to internal tetramethyl silane (TMS), ^{1}H and ^{13}C NMR of the resulting reaction product were recorded in D_2O and referenced to internal 2,2,3,3-tetradeutero-3-(Trimethylsilyl) propionic acid sodium salt (TSP-d_4).

2.2. Protein-stable isotope probing batch incubations

The batch reactors were set up in 60 mL serum bottles with a 50 mL liquid volume, incubated with ^{13}C-adipic acid, ^{13}C-butanediol, or ^{13}C-terephthalate as the sole added carbon source or without addition of any carbon source (control). The batch reactors were inoculated with sludge from a full-scale thermophilic anaerobic reactor (Aalborg West Urban Wastewater treatment plant, Denmark). The incubations were set up at a food-to-microorganism (F/M) ratio of 0.3 and the pH of the bulk was adjusted to 8.0 using 3 M HCl. The reactors were sealed with thick butyl rubber stoppers and aluminium crimps, and headspace was purged with nitrogen gas (N_2) for 2 min to ensure anaerobic conditions. The temperature was controlled and set to 55 °C, and to ensure full suspension, the reactors were placed on a rotary table at 500 rpm. The biomass was sampled after 0, 6, 24, 48, and 120 h for protein extraction. All handling and sampling were conducted using strict anaerobic techniques. Chemical oxygen demand (COD) (g/L) was determined at time zero and at the end of the experiment with COD cuvette test (LCK014, HACH), following the recommendations of the manufacturer. Total volatile solids (TVS) on the inoculum was determined with 1 mL aliquots by a

thermogravimetric balance (HB43-S, METTLER TOLEDO). CH_4 production was measured with AMPTS II (bioprocess control) equipment after bubbling the gas phase through a CO_2 trap (3 M NaOH). The COD of methane can be calculated using 1 g COD = 0.35 L CH_4 (Tauber et al., 2019), and was used to find the percentage of substrate being converted into methane.

2.3. Metagenome and bioinformatics

A metagenome was prepared from the inoculum of the batch incubation. Total DNA was extracted using FastDNA Spin Kit for Soil, following the recommendations of the manufacturer (MP Biomedicals, Denmark). One μg of non-size selected gDNA was used to create the library. DNA repair, end preparation, adapter ligation, clean-up, and priming were done following the manufacturer's protocol (MinION; Oxford Nanopore Technologies).

Base calling and sequence quality analysis were performed using Guppy v6.0.1 (https://community.nanoporetech.com). Sequence quality was assessed using NanoPlot v1.24.0 (De Coster et al., 2018), and hereafter filtered for quality (>Q9) and length (greater than 500 bp), using NanoFilt v3.8.6 (De Coster et al., 2018). Assembly of the reads was carried out using metaFlye v10.2.0 (Kolmogorov et al., 2020), and the following polish was carried out using minimap2 v2.17 (Li, 2016), racon v1.3.3 (Vaser et al., 2017), and medaka v1.0.1 (Oxford Nanopore Technologies Ltd, 2018). To generate a prediction of open reading frames in the metagenome, identifying essential genes, and classifying the contigs taxonomically, a script downloaded from https://github.com/Kirk3gaard/misc_scripts/tree/master/prepare_data_for_mmgenome2 was used in order to analyse the metagenome in R, using the mmgenome2 package (https://kasperskytte.github.io/mmgenome2/). Using Prokka (v.1.14) (Seemann, 2014), a metaproteome was generated from the metagenome and annotated to be used as the search database.

Metagenome-assembled genomes (MAGs) were extracted with the MetaBAT v2.12.1 (Kang et al., 2015) and checked for completeness and contamination using CheckM (Parks et al., 2015).

2.4. Protein extraction and protein-stable isotope probing analysis

Protein extraction was performed as previously described (Heyer et al., 2013), using TEAB (0.05 M TEAB buffer stock, 1.0 mg/L NaDOC, pH $\leq$ 8) as resuspension buffer. The extracted proteins were in-gel digested as previously described (Mosbæk et al., 2016), and the following desalting and analysis of tryptic peptides by automated liquid chromatograph-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) were performed as described elsewhere (Poulsen et al., 2021). In brief, tryptic peptides were desalted using a modified StageTip protocol and subsequently analysed by automated LC–electrospray ionization (ESI)–MS/MS, using a top 20 method to acquire the MS data, with an MS1 with an injection time of 50 ms and resolution of 60,000, an MS2 with an injection time of 45 ms, and resolution of 15,000, using an isolation window of 1.2 m/z, and the normalized collision energy was set to 28 eV.

MetaProSIP (Sachsenberg et al., 2015) was used to analyse the proteomic data in an OpenMS pipeline (https://www.openms.de), using standard settings, as described previously (Mosbæk et al., 2016).

2.5. Data availability

Metagenome data is available at the European Nucleotide Archive (ENA) under project accession number PRJEB58773. The mass spectrometry proteomics data has been deposited at ProteomeXchange Consortium via the PRIDE (Perez-Riverol et al., 2022) partner repository with the data set identifier PXD039265.

3. Results and discussion

3.1. Batch incubations

The pH changes during the incubations were negligible (Table S1). In AA, TP, and the control, a COD consumption of 3–5 g/L, were measured, while for BD, a higher COD consumption was observed (13.9 g/L).

Methane production was detected in all the microcosms, and at day 5, the total production was higher in the BD microcosm and the control, corresponding to 35% and 38% of the substrate being transformed into CH_4, respectively (see supplementary materials) (Tauber et al., 2019). A lower activity was detected in the case of AA and TP, corresponding to 26.5% and 18% of the AA and TP being transformed into CH_4 (see supplementary materials) (Tauber et al., 2019). These macroscopic data point to differences between the interactions of the different PBAT monomers and the biomass. The negative effect of specific aromatic compounds over sludge quality has been described previously (Trujillo-Reyes et al., 2023). Therefore, the reduced methanogenic activity in the presence of AA and TP compared to the control indicates a potential inhibitory effect from these monomers that deserves further research effort to be confirmed.

3.2. Metagenome

The metagenome generated from the biomass yielded a grand total of 8,060,000 reads, which when assembled resulted in 110,617,758 bp divided into 16,466 scaffolds with an N50 of 9,103 bp. The metagenome consisted of 88.9% *Bacteria*, 7.8% *Archaea*, 0.6% *Virus*, and 2.7% could not be classified. Within the metagenome, organisms are divided across a total of 45 different phyla, with *Proteobacteria* being the most abundant with 29% of all scaffolds (Fig. 1). The taxonomic diversity of the metagenome analysis is consistent with the expected diversity from anaerobic digester systems. *Proteobacteria* and *Firmicutes* has previously been shown to comprise the most abundant group of microbial communities in biogas reactors (Koeck et al., 2016).

3.3. Protein-stable isotope probing

Incubations with two of the PBAT monomers resulted in formation of ^{13}C-labelled peptides (Table 1 and Table S2), demonstrating that the microbial community in the sludge was able to assimilate the carbon from two of the three monomers in PBAT to gain energy. A higher number of ^{13}C-labelled peptides was detected for the incubation with BD (Table 1 and Table S1). This is in line with the highest COD consumption observed in the BD incubation.

Using a stringent filtering, the number of carbons replaced by their relative incorporation of heavy isotopes (defined as RIA) which were required to be equal to or higher than 10%, yielded a total of 122 labelled peptides of interest identified for two of the three substrates (Table S2). All 122 peptides were manually curated and showed clear shifts in their isotopic labelling profiles, both in RIA and the shape of isotope distribution. Already after 6 h of incubation, some labelled peptides were identified with an RIA of approximately 69%.

For the incubations with AA, a total of 3 labelled peptides were identified, belonging to *Bacteroides salanitronis* and *Ichthyobacterium seriolicida*. For the BD incubation, a total of 119 labelled peptides were identified, and the majority of these belonged to organisms representing the phylum *Euryarchaeota* (Fig. 3). Organisms representing *Firmicutes*, *Bacteroidetes*, and *Methanobacteriota* were also found to synthesize ^{13}C-labelled proteins, although in smaller numbers (Fig. 2).

The ^{13}C from TP was not found to be assimilated into any peptides when applying the stringent filtering criteria. This is not indicative of the substrate not being metabolised but reflects the more resilient nature with an aromatic structure which confers stability to the PBAT, and it is therefore not unexpected to be more difficult to degrade, while BD and AA are responsible for its biodegradability. However, 12 peptides were

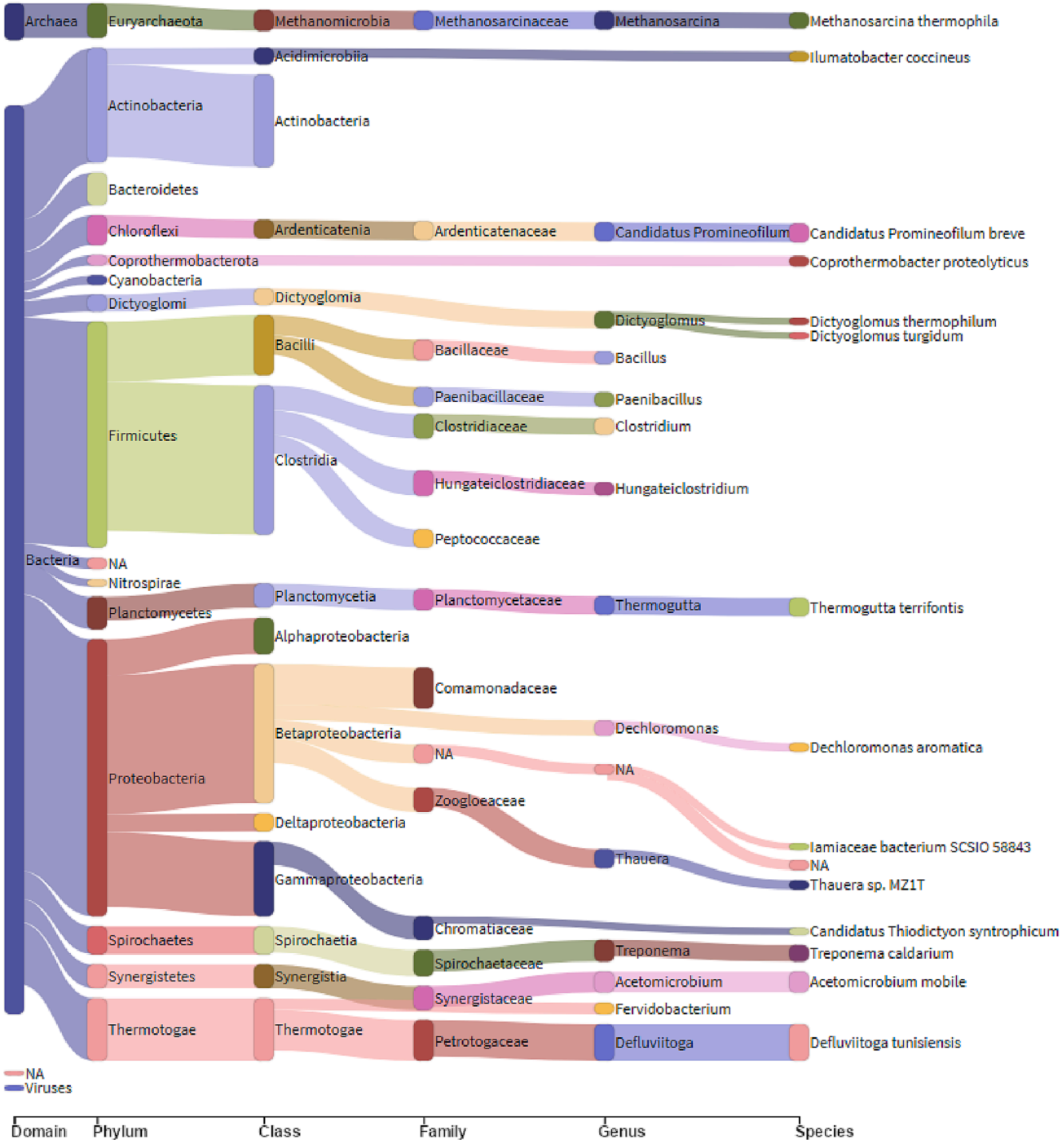

Fig. 1. Sankey diagram displaying the microbial composition of the metagenome (Top 15).

identified with an RIA greater than 2% for TP. In soil samples, aerobic microorganisms were found to mineralize all three monomers, even though with different mineralization rates (Morro et al., 2019). An isolated strain affiliated with *Bacillus subtilis* was found to degrade TP by 18%, AA by 50%, and BD by 11% (AA > TP > BD). In contrast, *B. licheniformis* biodegraded TP by 9% and AA by 20% and did not degrade BD during the same time interval. Therefore, the abilities to transform the different monomers under aerobic conditions (AA > TP > BD) are strain-dependent and mainly attributed to lipase-like enzymes (Morro et al., 2019). In the experiments, anaerobic, BD and AA were more readily mineralized relative to TP (BD > AA > TP), pointing to an effect of redox conditions over monomers preference.

Several enzymes from anaerobes, such as benzoyl-CoA reductase, have been linked to transform aromatic compounds, yet most of them under reducing conditions and associated to respiration of iron, nitrate, or sulphate reduction. Obligate anaerobic microorganisms capable of degrading aromatic compounds have been reported and mainly affiliated to *Deltaproteobacteria* and *Firmicutes* (Boll et al., 2014). Even one archaeon, *Ferroglobus placidus*, has been reported to be able to degrade aromatic compounds coupled to Fe(III) reduction (Tor & Lovley, 2001).

The genus *Bacteroides* was found to assimilate the labelled carbon after just 6 h of incubation with both AA and BD (Table 1). After 24 h *Ichthyobacterium* and *Bacteriodes* were identified as having assimilated labelled carbon when incubated with AA and BD, respectively. Looking

Table 1
Table of genera that have assimilated the labelled substrate and obtained RIA value above 10% for a number of peptides indicated for each sample.

Genera	AA6	AA24	AA48	BD6	BD24	BD120
Alkaliphilus						1
Bacteroides	1		1	1	1	
Candidatus Accumulibacter						1
Clostridium						2
Defluviitoga						1
Ichthyobacterium		1				
Laribacter						1
Methanosarcina						75
Methanothermobacter_A						5
NA						16
Sulfurihydrogenibium						3
Syntrophomonas						3
Thermincola						7
Thermomicrobium						1
Tumebacillus						1

AA: Adipic acid, BD: Butanediol, and the following number indicates the time at which the sample has been taken in hours.

into the samples taken at time 120 h for BD, the genus with the highest number of labelled proteins identified is *Methanosarcina*, followed by *Thermincola* (Table 1), and the total number of peptides labelled was the highest. The evolution along the time of ^{13}C-incorporation on BD incubation might point to a diauxic behaviour on the degraders (i.e.: first consuming another substrate such as VFA and then BD) or adaptation (lag phase elongation).

Based on the heavy isotope distribution of the proteins in the three organisms (*Bacteroides*, *Ichthyobacterium*, and *Methanosarcina*), it was possible to predict whether the degradation is due to a direct metabolization (primary consumption) (von Bergen et al., 2013). Most interestingly is that the *Methanosarcina* genus turn out to be able to metabolise the butanediol directly since the isotope pattern of nine of the labelled peptides showed a normal distribution. However, the largest number of the labelled peptides (40) from *Methanosarcina* was found to have a tailed isotope pattern, indicating that the labelling was due to cross-feeding of labelled intermediates from the degradation process (Fig. 3). *Methanosarcina* is known for being able to utilise all three pathways for methanogenesis and more than nine different substrates for methanogenesis (de Vrieze et al., 2012), making it a very robust methanogen against different impairments. Furthermore, more complex substrates will eventually be funnelled into the methanogens. *Methanosarcina* was the second archaeal genus most abundantly detected in a study of thermophilic reactors amended with biodegradable plastics (Cazaudehore et al., 2023), however, in that study, PBAT was not degraded.

Bacteroides turned out to be able to metabolise both AA and BD. For AA, the shape of the isotope pattern follows a normal distribution, giving a clear indication that this organism is directly metabolising the AA. *Ichthyobacterium* was also observed to have synthesised labelled proteins, and for this organism, the identified peptide's isotope pattern also followed a normal distribution and is therefore likely a primary degrader of AA.

A common feature for both *Methanosarcina* and *Bacteroides* is that they grow relatively fast compared to other methanogens or anaerobes in general, respectively (de Vrieze et al., 2012; Wexler, 2014). Therefore, they might have been able to outcompete other organisms and adapt to different substrates.

Peptides with the highest RIA (greater than80) include representatives of hypothetical proteins, corrinoid/iron-sulfur protein large subunit, NAD-specific glutamate dehydrogenase, and putative oxidoreductase YjmC (Table S2). Two proteins were detected in a higher quantity compared to the rest, these being hypothetical protein and

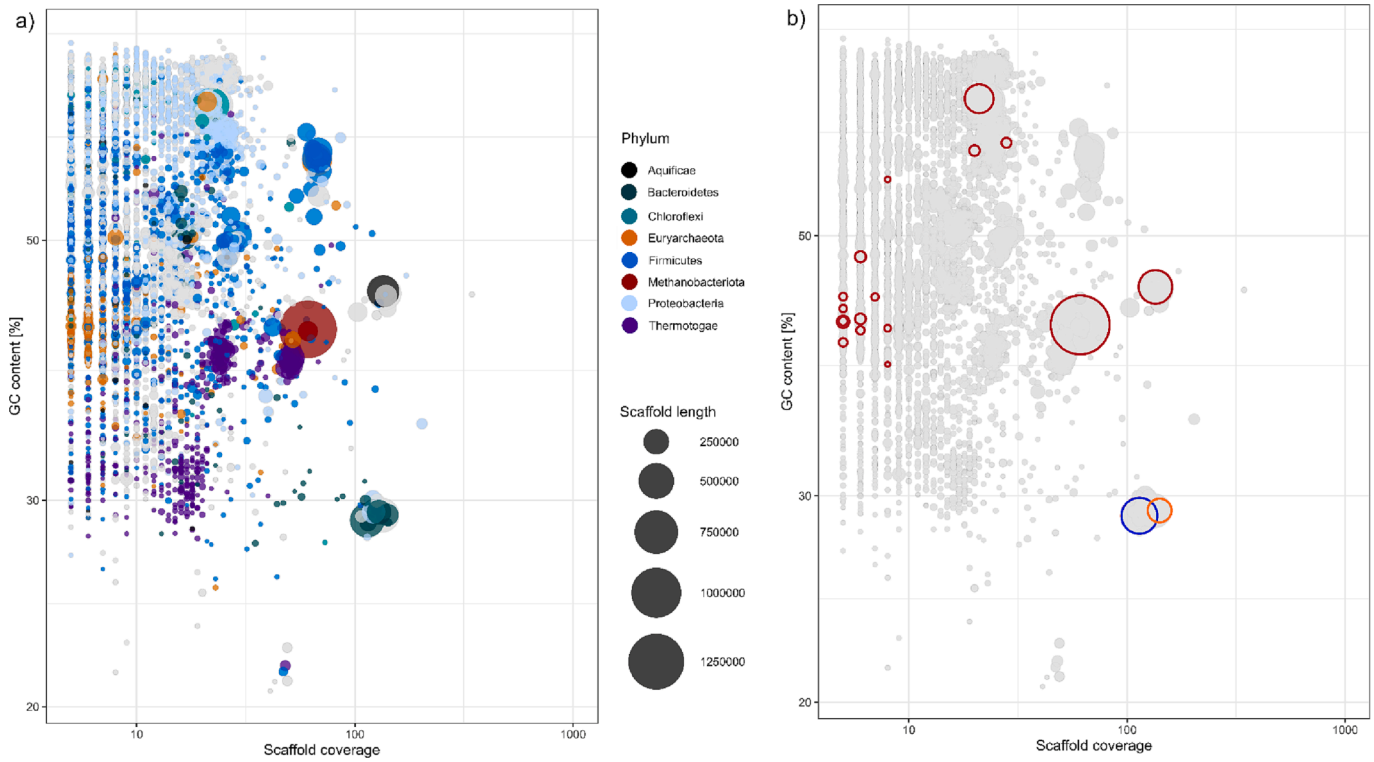

Fig. 2. The scaffold coverage and GC content from generated metagenome are plotted. The dot size indicates the scaffold length, and with a minimum length of 5000 bp. a) Colouring is according to the phylogeny (phylum). b) Scaffolds where ^{13}C-labelled proteins were found are marked. Red circles indicate that labelled proteins were only identified for the butanediol incubation, blue indicates that labelled proteins were identified for both butanediol and adipic acid, and orange indicates that the labelled proteins were only found in the adipic acid incubation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

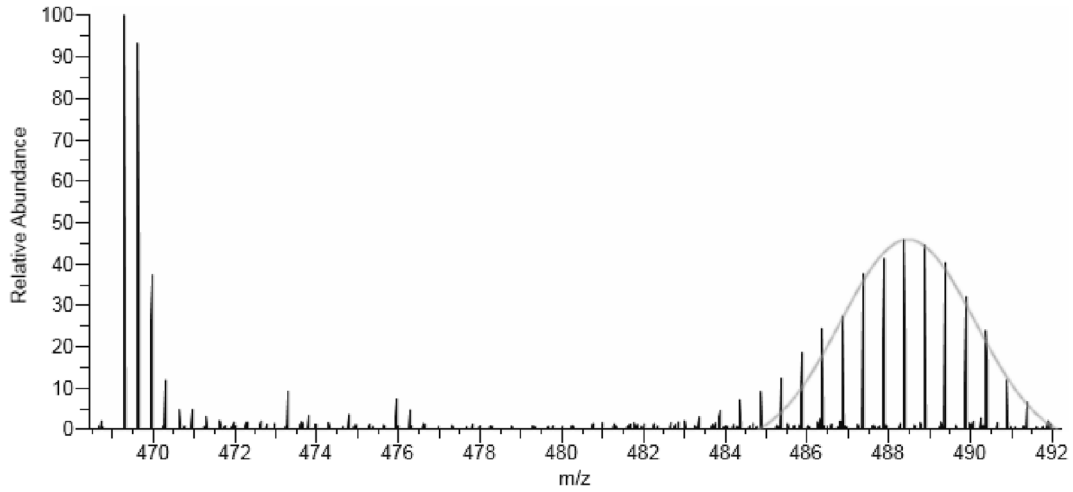

Fig. 3. Example of an MS1 isotope profile for the peptide [NILVNHIVAATLK + 4H]$^{4+}$ of a hypothetical protein from Methanothermobacter with a slightly negative skewness, indicating indirect ^{13}C substrate metabolization (von Bergen et al., 2013). A Gaussian curve has been plotted on top of the isotope profile to highlight the skewness. Example taken from the incubation with ^{13}C-labelled BD at timepoint 120 h.

corrinoid/iron-sulfur protein large subunit. Only the NAD-specific glutamate dehydrogenase was identified for both AA and BD (Table 2).

Table 2
Number of specific proteins identified as being labelled (RIA ≥ 10 %) in each sample.

Identified ^{12}C-labelled proteins	AA6	AA24	AA48	BD6	BD24	BD120
3-ketoacyl-CoA thiolase						1
60 kDa chaperonin						3
Acetate kinase						1
Autoinducer 2-binding protein LsrB						1
Carbon monoxide dehydrogenase/ acetyl-CoA synthase subunit alpha						2
Corrinoid/iron-sulfur protein large subunit						10
Electron transfer flavoprotein subunit alpha						7
Fatty acid oxidation complex subunit alpha						3
Formyltransferase/ hydrolase complex Fhc subunit C						1
Hypothetical protein						76
Methylcorrinoid: tetrahydrofolate methyltransferase						2
NAD-specific glutamate dehydrogenase	1		1	1	1	
Ornithine aminotransferase		1				
Phosphate acetyltransferase						3
putative oxidoreductase YjmC						1
Sulfate adenylyltransferase subunit 1						1
V-type sodium ATPase catalytic subunit A						2
V-type sodium ATPase subunit B						3

AA: Adipic acid, BD: Butanediol, and the following number indicates the time at which the sample has been taken in hours.

3.4. Metabolic potential of the identified poly butylene adipate-co-terephthalate degrader in anaerobic digesters

From the metagenome, a total of 40 MAGs were extracted, and of these, three MAGs are of high quality according to the stringent MIMAG high-quality draft requirements (Table S3) (Bowers et al., 2017).

One of the three HQ-MAGs, Bin.17, was of interest for this study, since it happens to contain the genomic material for one of the peptides that were found to be labelled from the incubation with BD as substrate. Bin 17 has been classified as the specie *Anaerolinea thermophila,* and the identity was further confirmed by the 16S rRNA gene sequence analysis. A cocultivation with hydrogenotrophic methanogens significantly stimulates the growth of this organism. Members of the *Methanosarcina* genera can use CO_2 to perform hydrogenotrophic methanogenesis (Lackner et al., 2018), therefore stimulating the growth of *Anaerolinea thermophila*.

Saccharomyces cerevisiae, the only known isolated organism able to grow on butanediol as sole carbon source (Gonzalez et al., 2000), contains a butanediol degradation pathway which is present in both bacteria and fungi (Caspi et al., 2018). Genomic comparisons of this isolate and the proteome of bin.17 identified two homologous enzymes, pointing to the possibility that they share the same function. These are alcohol dehydrogenase and inositol 2-dehydrogenase/D-chiro-inositol 3-dehydrogenase with sequence identities of 24% and 26% and e value of $4{\cdot}10^{-12}$ and 0.003, respectively. The alcohol dehydrogenase could be hypothesized to relate to the alcohol dehydrogenase protein family used in the degradation of the BD.

4. Conclusions

The protein-SIP approach provided evidence of the anaerobic and thermophilic metabolization of two of the three monomers (BD and AA) from PBAT, while TP conversion was less pronounced. The approach allowed specific identification of organisms metabolising the PBAT monomers, and it was possible to distinguish between direct and cross-feeding mechanisms. Direct metabolisers were affiliated to the genera *Bacteroides*, *Ichthyobacterium,* and *Methanosarcina*. An extracted HQ-MAG classified as *Anaerolinea thermophila* contains enzymes putatively responsible for degrading butanediol. This study provides a first insight into the identity and genomic potential of organisms responsible for biodegradability of PBAT monomers during anaerobic digestion under thermophilic conditions.

CRediT authorship contribution statement

Jan Struckmann Poulsen: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Alba Trueba-Santiso:** Conceptualization, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Juan M. Lema:** Validation, Writing – review & editing, Supervision. **Simon Gregersen Echers:** Validation, Formal analysis, Writing – review & editing. **Reinhard Wimmer:** Validation, Formal analysis, Investigation, Writing – review & editing, Visualization. **Jeppe Lund Nielsen:** Conceptualization, Methodology, Validation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jeppe Lund Nielsen reports financial support was provided by Horizon 2020 European Innovation Council Fast Track to Innovation.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2023.129430.

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