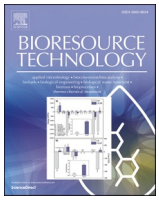
Bioresource Technology 408 (2024) 131180

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech



Biomethanization of rigid packaging made entirely of poly (3-hydroxybutyrate-co-3-hydroxyhexanoate): Mono- and co-digestion tests and microbial insights

Octavio García-Depraect [a,b], Leonardo J. Martínez-Mendoza [a,b], Rosa Aragão Börner [c], Johannes Zimmer [c], Raúl Muñoz [a,b,*]

[a] Institute of Sustainable Processes, Dr. Mergelina s/n, 47011 Valladolid, Spain
[b] Department of Chemical Engineering and Environmental Technology, School of Industrial Engineering, University of Valladolid, Dr. Mergelina, s/n, 47011 Valladolid, Spain
[c] Nestlé Research, Société des Produits Nestlé S.A., Route du Jorat 57, 1000 Lausanne, Switzerland

HIGHLIGHTS

- PHBH-made rigid packaging can be mesophilically digested, yielding 400 L-CH_4/kg.
- Size reduction of rigid packaging is needed and feasible with commercial shredders.
- Successful co-digestion of PHBH with food waste, sewage sludge or swine manure.
- PHBH was kinetically compatible with other organic waste.
- Hydrogenotrophic methanogenesis was key in all co-digestion scenarios.

ARTICLE INFO

Keywords:
Anaerobic digestion
Biodegradability
Bioplastics
Organic recycling
Packaging
PHBH

ABSTRACT

This study evaluates the anaerobic mesophilic mono- and co-digestion of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBH) plastic bottles as a proxy for rigid packaging materials. Initial tests showed a 97.3 ± 0.2 % reduction in weight and an observable alteration in the surface (thinning, color fading and pitting) of the PHBH bottles after eight weeks. Subsequent tests showed that PHBH squares (3 × 3 cm) produced 400 NmL-CH_4/g-VS_{fed}, at a slower rate compared to powdered PHBH but with similar methane yield. Co-digestion experiments with food waste, swine manure, or sewage sludge showed successful digestion of PHBH alongside organic waste (even at a high bioplastic loading of 20 % volatile solids basis), with methane production comparable to or slightly higher than that observed in mono-digestion. Molecular analyses suggested that the type of co-substrate influenced microbial activity and that methane production was mainly driven by hydrogenotrophic methanogenesis. These results suggest the potential for integrating rigid PHBH packaging into anaerobic digesters.

1. Introduction

The development and use of bioplastics has become increasingly important in recent years due to their inherently sustainable and environmentally friendly nature, as well as changing societal trends such as increased environmental awareness, social responsibility and green marketing drivers. Bioplastics are versatile materials with tailored properties and a wide range of applications in packaging, food service, agriculture, consumer electronics, automotive and transportation, consumer goods, and others (European Bioplastics, 2023a,b). According to the latest market data compiled by European Bioplastics and the nova-Institute, global bioplastics production capacity was ~2.2 million tons in 2022 and is expected to increase up to 6.3 million tons by 2027 (European Bioplastics, 2023a,b). In particular, biodegradable bioplastics, such as polyhydroxyalkanoates (PHAs), have a tremendous potential for circularity because they can be produced from renewable

* Corresponding author at: Department of Chemical Engineering and Environmental Technology, School of Industrial Engineering, University of Valladolid, Dr. Mergelina, s/n, 47011 Valladolid, Spain.
E-mail address: raul.munoz.torre@uva.es (R. Muñoz).

https://doi.org/10.1016/j.biortech.2024.131180
Received 7 April 2024; Received in revised form 25 July 2024; Accepted 29 July 2024
Available online 3 August 2024

biomass and can be bio-upcycled into value-added molecules, such as biodegradable plastics and chemicals (Blank et al., 2020). In addition, the biodegradable properties of some bioplastics have opened up new options for end-of-life waste management, such as organic recycling (Briassoulis et al., 2021). In this context, compost for soil amendment is produced by composting compostable plastics with other organic wastes. In contrast, biogas (a renewable energy carrier) and digestate (used as a soil fertilizer) can be produced from some biodegradable bioplastics via anaerobic co-digestion (Abraham et al., 2021).

In the anaerobic digestion (AD) process, organic matter is converted mainly into biogas by anaerobic bacteria and methanogenic archaea through four sequential metabolic steps, namely, hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Although AD is a cleaver option for the disposal of some bioplastics contaminated with food waste, it is currently limited by its low hydrolysis rate, which entails longer hydraulic retention times (HRT) than those typically applied in industrial digesters (Yasin et al., 2022). In this context, it has been reported that the specific surface area available for microbial attack determines the rate of anaerobic biodegradation (García-Depraect et al., 2022a). The higher the specific surface area (reduced particle size), the higher the rate of anaerobic biodegradation. In this sense, commercial shredders can be used to reduce bioplastics to pieces of a few centimeters in size. Pretreatment of bioplastics has also been proposed and studied to increase the methane production rate from biodegradable bioplastics, mainly by increasing the specific surface area and surface porosity while reducing the molecular weight and the degree of crystallinity (Calabro' et al., 2020; Yasin et al., 2022).

It is worth noting that most studies on the AD of bioplastics have used unprocessed bioplastic pellets/powders (Ryan et al., 2017; Bátori et al., 2018; Abraham et al., 2021; García-Depraect et al., 2022a). However, manufactured bioplastics may contain additives (e.g., plasticizers, stabilizers, colorants, etc.) and fillers that can affect their methanization behavior (Thomas et al., 2023). Thus, manufactured biodegradable bioplastics can influence the AD performance due to their nature and additive content, thickness, polymer crystallinity, and product shape (Bracciale et al., 2023). Although several works on AD using commercial bioplastics have been recently reported (Calabro' et al., 2020; Cazaudehore et al., 2021; Cucina et al., 2022a,b; Álvarez-Méndez et al., 2023; Bracciale et al., 2023; Üveges et al., 2023; Yu et al., 2023), there are still research gaps. For instance, the influence of the type of organic co-substrate and the composition of the microbiome involved on bioplastic methanization remains an open question. In addition, only a few studies have investigated the co-digestion of bioplastics to date. Most of these studies have used highly biodegradable food waste or the organic fraction of municipal solid waste (OFMSW), as most bioplastic products currently available on the market are used to safely protect or serve food (Zhang et al., 2018; Hobbs et al., 2019; Cucina et al., 2021; Hegde et al., 2021; Dolci et al., 2022; Kakadellis et al., 2022; Peng et al., 2022; Yu et al., 2023). The methanization of commercial bioplastics (starch-based choppers and polylactic acid (PLA)-based cutlery and dishes) has been recently investigated in co-digestion with sewage sludge under full-scale conditions (Cucina et al., 2022a,b). Furthermore, although C-rich substrates such as PHAs are suitable co-substrates for manures (Mata-Alvarez et al., 2014), to the best of the authors' knowledge, the co-digestion of biodegradable bioplastics and manure has not been studied.

This study aims at investigating, for the first time, the anaerobic mono- and co-digestion of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBH) plastic bottles. First, a low-solids anaerobic disintegration test was performed for the PHBH bottle alone. Then, a methanization assessment was performed using biochemical methane potential (BMP) tests with three different co-substrates, i.e., household food waste (FW), swine manure (SM), and mixed sewage sludge (MS). The microbiome enriched at the end of the co-digestion tests was also studied.

2. Materials and methods

2.1. Bioplastic material

Bioplastic bottles made entirely of PHBH were used in the disintegration and BMP tests. Three different bottles (hereafter referred to as A, B and C), differing only in the colorant used, were tested as a proxy for rigid packaging. All the PHBH bottles had the following thickness: 0.93 mm (body), 1.95 mm (bottom), 1.70 mm (neck), 0.65 mm (cap). For the disintegration test, complete bioplastic bottles (PHBH B randomly selected) were cut horizontally into 3 pieces, i.e., bottom, middle, and top with the cap attached. For BMP tests in mono-digestion, the body of the bioplastic bottles (A, B and C) was manually cut in 3 cm × 3 cm squares. On the other hand, only PHBH B was evaluated in the co-digestion tests. The methanization of raw PHBH in powder form with an average particle size of 500–1000 μm was also evaluated for comparison with mono-digestion tests carried out with 3 cm × 3 cm bioplastic bottle pieces. For this purpose, PHBH pellets containing <10 mol % of 3-hydroxyhexanoate (3HHx) were mechanically ground with dry ice, then sieved, dried at room temperature, and finally stored in the dark (García-Depraect et al., 2023).

2.2. Disintegration test of complete PHBH bottles

The biodegradability of the rigid PHBH packaging (PHBH B) was measured in duplicate using the low-solids anaerobic disintegration test, which was based on the international standard ISO 13975 Plastics − Determination of the ultimate anaerobic biodegradation of plastic materials in controlled slurry digestion systems − Method by measurement of biogas production (2019). The test was performed in two identical 4-L glass vessel digesters for 8 weeks at mesophilic temperature (37 ± 2 °C). The bottom, middle and top parts (total mass of 49.65 g) of the rigid bottle were fed to each of the digesters containing 2450 g of anaerobic sludge as inoculum, entailing an inoculum to substrate (I/S) ratio of ≈ 0.9 on a VS basis. The inoculum was collected from a full-scale anaerobic digester treating sewage sludge at a wastewater treatment plant. Prior to use, the inoculum was filtered through a 2 mm sieve and allow to stabilize at 37 °C for 7 days. The physicochemical characteristics of the inoculum were as follows: total solids (TS) 3.5 %, volatile solids (VS:TS) 51.4 %, ash content 48.6 % on TS, pH 8.1, total ammonia nitrogen (TAN) 0.71 g/kg, volatile fatty acids (VFA, g/kg) below the detection limit (0.14 g VFA/kg). Lastly, the reactors were flushed with nitrogen gas to ensure anaerobic conditions. After 1, 2, 3, 4, 6 and 8 weeks of incubation, 3 pieces of the bottle (bottom, middle and top part with the cap still attached) were removed from the digesters. The test item was carefully rinsed with tap water, dried with some paper, and then visually assessed. The weight of the bottle was also determined at each sampling point to determine the percentage of disintegration (Eq. (1). Finally, the test item was readded to the digester, which was again flushed with nitrogen gas to ensure anaerobic conditions. Methane production from the PHBH rigid packaging was not measured in this anaerobic disintegration test, but was measured in the mono- and co-digestion BMP tests.

$$\text{Weight loss}\,(\%) = \frac{initialweight - presentweight}{initialweight} \times 100 \quad (1)$$

2.3. BMP tests in mono-digestion

Mono-digestion BMP tests were conducted to evaluate the anaerobic biodegradation behavior of PHBH. Particular attention was paid to evaluating the differences in the short-term methanization of the PHBH bottle (PHBH B) and powdered PHBH. These tests were carried out in 2.1-L screw-capped glass digesters (0.5 L working volume) containing 500 mL of anaerobic sludge as inoculum and 3.18 g of test material (rigid packaging and powdered PHBH), which entailed an I/S ratio of 2 on a VS basis. Each digester also contained 5 g/L of sodium bicarbonate

to ensure proper buffering capacity. The anaerobic inoculum, obtained from the municipal wastewater treatment plant of Valladolid, Spain, had a pH of 7.3, with TS and VS contents of 2.3 % and 1.3 %, respectively, where VS constituted 56.5 % of the TS, ash content was 43.5 % of the TS, and VFA concentration was below the detection limit. Prior to use, the inoculum was preincubated for 1 week at 36 ± 1 °C under anaerobic conditions to reduce endogenous methane production, which was measured by a blank test containing only the inoculum. The digesters were capped tightly with a butyl rubber stopper and an aluminum screw cap, then flushed with helium gas (Abello Linde, Barcelona, Spain) for 5 min, and finally incubated in a Wheaton® roller apparatus (Scientific Products, USA). The temperature and agitation rate were set at 36 ± 1 °C and 4.5 rpm (gently mixing conditions). The test materials included PHBH A, PHBH B, PHBH C and raw PHBH in powder form. All conditions (including the blank) were assessed in triplicate.

The standard manometric method was employed to record the cumulative methane production (García-Depraect et al., 2022b). Headspace methane and carbon dioxide concentrations were recorded by GC-TCD along with headspace overpressure measurements. The methane amounts presented here have already been normalized to 1 atm and 0 °C. The duration of this series of BMP tests was of 62 days, which was the time required to observe a plateau in methane production for all conditions tested. The cumulative methane yield was expressed as NmL CH_4/g VS fed. At the end of the test, the pH of the digestate was measured to verify that it remained within the proper range for AD. Additionally, the final concentration of soluble total organic carbon (TOC) and dissolved inorganic carbon (DIC) was also measured in the digesters containing the inoculum alone and the mixture of target material and inoculum. The ultimate biodegradability was calculated based on the total net mass of gaseous carbon and DIC recorded at the end of the test (by subtracting blank concentrations from those measured in the test material), expressed as a percentage of the initial mass of carbon relative to PHBH (García-Depraect et al., 2023).

2.4. BMP tests for the co-digestion of PHBH and organic waste

A second series of BMP tests was carried out to assess the compatibility of PHBH rigid packaging (PHBH B) with various organic wastes. Three different organic feedstocks were evaluated, namely FW, SM, and MS. The FW was prepared with 78 % potato, 14 % chicken, 4 % pork lard, and 4 % cabbage (on wet weight basis), which mimics the typical composition (carbohydrate, protein, lipid, and fiber) of household FW. This simulated FW is referred to herein as either FW1 or FW2, depending on the condition tested. (see Table 1). Fresh SM was collected from a nearby swine farm located at Segovia (Spain). Likewise, MS was collected from the sewage treatment plant of Valladolid (Spain). FW was frozen (−20 °C) while SM and MS were preserved at 4 °C until use. This series of BMP tests was conducted in 0.5-L crew-cap glass digesters with a working volume of 0.2 L. The BMP of the bioplastic and of organic waste alone was also assessed with the aim of inferring potential synergistic/deleterious effect induced by the presence of PHBH. Blanks (without substrate) were always run in parallel for background methane estimation. As shown in Table 1, 12 different runs were performed using 3 inocula. The inocula were i) mesophilic anaerobic sludge from the wastewater treatment plant of Valladolid (Spain); ii) mesophilic anaerobic sludge from a lab-scale continuously stirred tank reactor treating SM; and iii) anaerobic sludge from a large-scale mesophilic digester treating OFMSW. These inocula were selected as per use/adaptation to the tested co-substrate. To decrease the background methane production, all inocula were pre-incubated at 36 ± 1 °C for 7 days. The I/S ratio was set at 2 (on a VS basis) for all the conditions evaluated. All co-digestion conditions consisted of 80 % organic waste and 20 % PHBH (on a VS basis). Helium flushing was applied at approximately 0.5 bar for 5 min to ensure anaerobic conditions. The experiments were performed over 75–112 days (depending on the condition assessed) at 37 °C, and under orbital shaking conditions (approximately 100 rpm). All experiments were conducted in triplicate. The methodology described in section 2.4 was employed for evaluating the BMP. At the end of the experiment, the pH and the concentration of VFAs in the anaerobic broth were measured. Finally, the structure of the microbial community enriched at the end of the co-digestion tests was analyzed by 16S rRNA amplicon sequencing as an initial attempt to the explore the microbiology involved in the anaerobic co-digestion of the PHBH bottle.

Table 1
Summary of the experimental mesophilic co-digestion assays of PHBH with mixed sewage sludge (MS), food waste (FW1 and FW2), and swine manure (SM).

Run	Feedstock	Anaerobic sludge source	Duration
1	- MS	Full-scale mesophilic digester treating sewage sludge	75 days
2	- 3 cm × 3 cm PHBH		
3	- 80 % MS and 20 % PHBH		
4	- FW1	Full-scale mesophilic digester treating sewage sludge	85 days
5	- 3 cm × 3 cm PHBH		
6	- 80 % FW and 20 % PHBH		
7	- FW2	Full-scale mesophilic digester treating OFMSW	97 days
8	- 3 cm × 3 cm PHBH		
9	- 80 % FW and 20 % PHBH		
10	- SM	Lab-scale mesophilic digester treating SM	112 days
11	- 3 cm × 3 cm PHBH		
12	- 80 % SM and 20 % PHBH		

Note: Co-digestion blend expressed on a VS basis. FW1 and FW2 refer to the same formulation of food waste; their different nomenclature indicates that they do not share the same type of methanogenic inoculum.

2.5. Analytical methods

The determination of TS, VS, and pH was performed according to the standard methods for the examination of water and wastewater (APHA, 2005). The headspace overpressure was measured by using a pressure transducer (IFM electronic PN7097, Germany). TOC and DIC concentration in digestate samples previously centrifuged (10000 rpm for 10 min) and prefiltered (0.7 μm) was measured using a TOC analyzer (TOC-L series, Shimadzu, Japan). VFAs were analyzed by a gas chromatograph (GC) Agilent 7820A (Agilent, USA) equipped with a packed column (10 % SP-1000 + 1 % phosphoric acid on Chromosorb® W acid washed 100/120 mesh size, 2 m × 3.175 mm; Teknokroma, Spain) and a flame ionization detector (FID) (García-Depraect et al., 2022b). The composition of biogas was determined by gas chromatography (GC-TCD) in an Agilent 8860 GC (California, EEUU), following the procedure outlined by García-Depraect et al. (2022a).

2.6. Microbial diversity analysis

For the microbial analysis, triplicate digestate samples (which were then mixed together for each condition tested) were collected at the end of the BMP tests for the co-digestion of organic waste with PHBH (i.e., SW, MS, FW1, and FW2) to obtain composite samples that were preserved at −80 °C. The composite samples were then sent to ADM-Biopolis (Valencia, Spain) for 16S rRNA amplicon sequencing. Total genomic DNA was isolated using the QIAsymphony PowerFecal Pro DNA Kit (Qiagen, Germany) according to the manufacturer's instructions. The 341F-805R primer set was used for bacterial community analysis, targeting the V3-V4 hypervariable region. The V4 region was amplified to study archaeal communities, using the primer pair combination 344F-1041R/519F-806R. Amplicon libraries (300 bp paired-end reads) were prepared following the 16S Metagenomic Sequencing Library Illumina 15,044,223 B protocol (Illumina 1.9). The raw sequences were merged and trimmed using the BBMerge package of BBMap V.38 software with Cutadapt v 1.8.1, with the default parameters. Subsequently, the quality-checked (Q20 threshold) reads were processed

using the DADA2 denoise-single command. The error rates were determined from a set of subsampled reads using the "learnErrors" function, and the sample inference algorithm was applied with the "dada" function. Chimeric amplicon sequence variants (ASVs) were removed using the "removeChimeraDenovo" function. The clean ASVs were annotated against the NCBI 16S rRNA database (version 2022) using BLAST (version 2.2.29) at a 97 % similarity threshold. Taxonomy of ASVs with lower percentage identity than 97 % was reassigned using the NBAYES algorithm. The NBAYES classifier was trained on the V3-V4 region of the 16S rRNA gene from the SILVA v.138 database. Data were normalized using the rarefaction technique from the Phyloseq R package to perform alpha diversity analysis. The raw sequences were deposited in the Sequence Read Archive (SRA) database of the NCBI GenBank with the accession BioProject number PRJNA1034484.

2.7. Data analysis

The mean values of the disintegration and BMP tests, which were performed in duplicate and replicate, respectively, were reported. Error bars were included in all figures, and standard deviations were presented in the table.

3. Results and discussion

3.1. Anaerobic disintegration of PHBH rigid packaging

When compared to the initial mass of the bottle, the finished PHBH rigid packaging at the end of the assays was characterized by an average disintegration percentage of 97.3 ± 0.2 % after 8 weeks of testing (Fig. 1). The disintegration degree after 1 week of anaerobic degradation was 12.6 ± 0.2 %. At that time, the color of the bottle was slightly faded, while the entire surface of the bottle presented small dimples, which suggested an initial biotic breakdown on the bioplastic's surface (Ruggero et al., 2019). The disintegration percentage rose to 46.4 ± 3.9 % after 2 weeks, wherein the thickness of the bottle was clearly decreasing, and small holes started to appear on all 3 pieces (bottom, middle, and top part of the PHBH bottle). The disintegration of the bottle averaged 63.6 % following 3 weeks of incubation. However, one replicate clearly showed more disintegration than the others, resulting in a higher standard deviation among replicates (18.2 %). In the initial replicate, only the lower and upper portions (with the screw still attached) could be retrieved by the end of the third week. In contrast, in the second replicate, substantial portions of the central region of the bottle remained identifiable. Small dimples and holes were observed over the entire surface of the bottle. The color of the cap continued fading. After 4 weeks, the average disintegration degree of the PHBH bottle was 75.7 %, with a reduction in the standard deviation of the disintegration degree from 18.2 to 11.0 %. Another interesting observation after 4 weeks incubation was the breakdown of the ring of the cap. The disintegration of the bottle reached an average value of 88.3 ± 7.0 % by week 6 of incubation. For the one of the replicates only a small piece of the bottom and the screw-piece of the top part could only be retrieved after 6 weeks. The cap was loose from the bottle and showed a hole on the top. Similarly, some small pieces of the middle part of the bottle were still found in the other replicate. At the end of the test (8 weeks), only the thickest part of the bottom and the screw-piece of the top part could be retrieved for both replicates. Most of the cap was disintegrated by the end of the experiment.

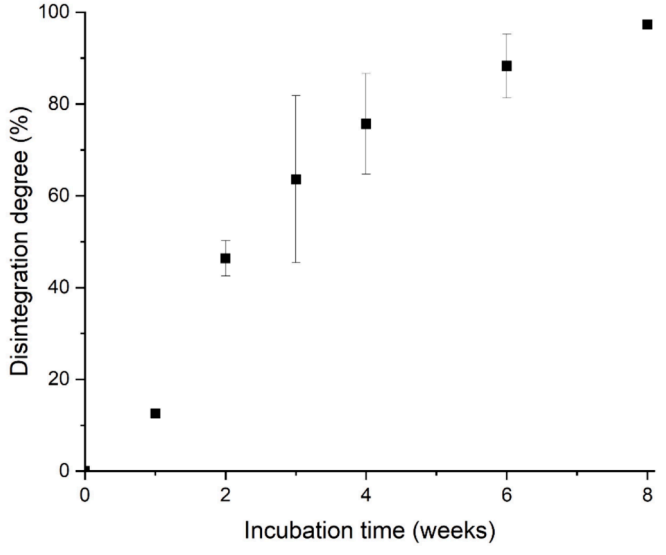


Fig. 1. Degree of disintegration (by weight loss) of the PHBH bottle "B" over time under anaerobic, mesophilic and low-solids conditions. Error bars represent the standard deviation of duplicate experiments.

A visual inspection of the remaining bioplastic during the test period is useful to provide a qualitative assessment of the biodegradation phenomena such as consistency, shape, thickness, discoloration, and erosion (Ruggero et al., 2019). In general, the visible biodegradation phenomena recorded in the present study are similar to those reported by Cucina et al. (2022a,b), who evaluated the biodegradation of starch-based and PLA-based bioplastic products under high-solids, thermophilic anaerobic conditions. Similar results were also reported by Zhang et al. (2018), who observed pits, holes, and color fade of the surface during the biodegradation of cellulose-based films via mesophilic anaerobic co-digestion with food waste. In that study, microscopic examination of the cellulose films also allowed to observe the growth of bacterial colonies at the bottom of pits.

3.2. Anaerobic biodegradability of PHBH: Rigid packaging made of PHBH versus powdered PHBH

The methane yield of the PHBH bottle A, B, and C after 62 days incubation time was 402.3 ± 4.9, 406.5 ± 10.5, and 400.5 ± 3.5 NmL CH_4/g VS fed, respectively. It should be noted that the only difference between the manufactured PHBHs tested was the coloring agent used, which should be present at a very low percentage by weight and should not alter the chemical structure of the PHBH bottles. This explains the similar methanization behavior observed for PHBH bottle A, B, and C. The methane yields herein obtained were in agreement with the 462.3 ± 5.5 NmL CH_4/g VS fed and 397.0 ± 15.6 NmL CH_4/g VS fed achieved after 75 days of mesophilic AD for unpretreated and alkali-pretreated PHBH, respectively (García-Depraect et al., 2023). The average methane content in the biogas generated was ~53 % (*v*/*v*) regardless of the material type, which matched well with the theoretical methane content (~56 %) of PHBH with 3–10 mol% 3HHx (Reischwitz et al., 1997). The kinetics of methane production were very similar for all PHBH rigid materials tested, but significantly slower than that of the powdered PHBH (Fig. 2). It has been consistently shown that a low particle size (higher specific surface area) increases the rate of anaerobic biodegradability of polyhydroxyalkanoates (PHAs) (García-Depraect et al., 2022a). In this context, the methanization of flexible PHBH packaging, which is generally thinner and lighter than its rigid counterparts, should be evaluated in future research. Here, the bottle-shaped PHBH materials also showed a slightly lower methane yield than that recorded for the powdered PHBH (440.8 ± 19.3 NmL CH_4/g VS fed) during the same period, suggesting that the ultimate methane production for 3 cm × 3 cm PHBH was not reached under the time and conditions tested. Nonetheless, there was no evidence of residual pieces of PHBH bottles after a visual inspection of sieved digestates (1 mm sieve). On the other hand, the average pH at the end of the test was 7.5 ± 0.1, which is favorable for AD. The final biodegradability of the bottles A, B and C accounted for 75.3 ± 0.1, 75.8 ± 1.6 and 75.3 ± 1.8 %, respectively. The final concentration of dissolved TOC in the tests supplied

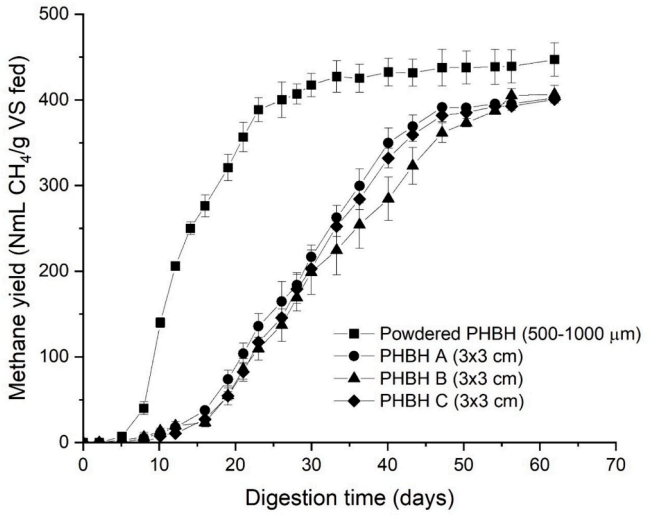


Fig. 2. Time course of the methane yield of manufactured PHBH-made bottles and powdered raw PHBH (500–1000 μm particle size).

with PHBH bottles was always lower than that of the blank, indicating very little or no accumulation of PHBH degradation intermediates. The PHBH powder used for comparison purposes showed a final degree of biodegradability of 84.8 ± 4.8 %. It should be noted that the share of initial bioplastic carbon diverted towards growth and maintenance was not measured, but typically accounts for 10–15 % (Chernicharo, 2007). This fact would imply the achievement of higher final biodegradability values than those herein estimated only based on the carbon release in the headspace and DIC.

3.3. Co-digestion tests of PHBH bioplastic and organic waste

In the second series of BMP tests performed, it was observed that the mono-digestion of PHBH rigid packaging was highly dependent on the type of inoculum used (Fig. 3). For instance, the anaerobic inoculum previously adapted to sewage sludge (run 2 and run 5) supported methane yields ranging from 380.5 ± 23.1 to 406.3 ± 6.6 NmL CH_4/g VS fed after 85 and 75 days of incubation. The use of the anaerobic inoculum derived from a mesophilic digester treating OFMSW (run 8) resulted in a slightly lower methane yield of 341.0 ± 20.3 NmL CH_4/g VS fed following 97 days digestion time, while the anaerobic inoculum adapted to SM (run 11) only yielded 112.8 ± 12.3 NmL CH_4/g VS fed even after a long incubation period of 112 days. When testing the mono-digestion of organic waste, the methane yields recorded were (in NmL CH_4/g VS fed) 237.7 ± 5.2 for MS (run 1), 417.7 ± 12.4 for FW1 (run 4), 291.5 ± 3.3 for FW2 (run 7), and 606.0 ± 12.6 for SM (run 10). In general, all the methane yields achieved for the organic wastes were in agreement with the typical values previously reported for similar substrates (Grosser, 2018; Santos et al., 2022). All tests carried out exhibited suitable final pH values (7.3–8.2) for methane production and accumulated low concentrations of VFAs in the order of few mg/L (see Supplementary material). Butyrate, acetate and propionate were the only VFAs detected, while hexanoate was not identified. As the methanogenic sludge used herein enabled a relatively good methanization performance of the organic wastes, it was therefore concluded that the short-term methanization of the rigid packaging entirely made of PHBH

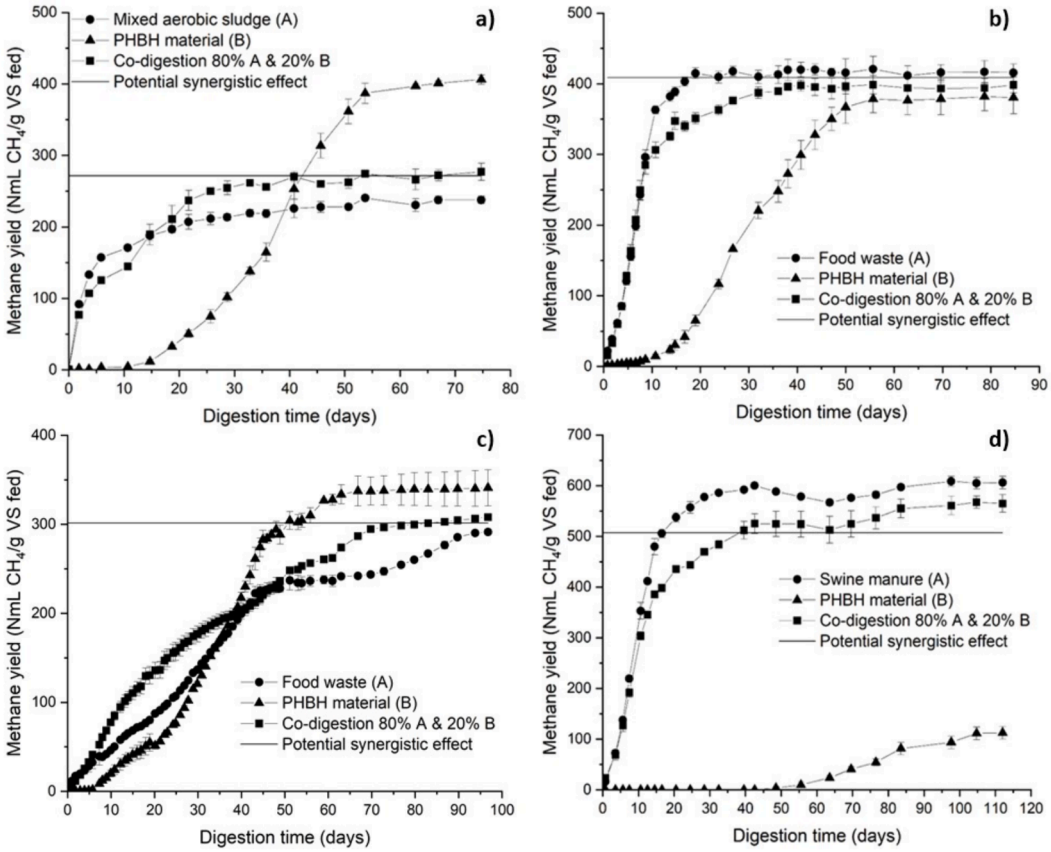


Fig. 3. Time course of methane yield for anaerobic co-digestion of a) MS, b) FW1, c) FW2 and d) SM with rigid packaging made of PHBH. The co-digestion mixtures consisted of 80% organic waste and 20% PHBH (based on MS). The horizontal lines represent the theoretical methane yield that would be achieved by co-digestion. This theoretical reference value was calculated from the maximum methane yields recorded in the mono-digestion tests. A potential synergistic effect on methane production can be inferred if the co-digestion test showed a final methane yield above the horizontal line.

was directly associated with the metabolic capacity of the microbiome originally present in the inoculum. Section 3.4 discusses the microbiology of the co-digestion of organic wastes and PHBH.

The ultimate methane yields were 277.1 ± 12.0, 398.2 ± 11.2, 308.0 ± 0.3, and 564.8 ± 17.4 NmL CH_4/g VS fed for the co-digestion of PHBH with MS (run 3), FW1 (run 6), FW2 (run 9), and SM (run 12), respectively. Potential synergistic or deleterious effects of PHBH supplementation on methane production were not evident from the data gathered, except for SM as co-substrate, where the methane yield recorded was 10 % higher than that of SM alone. The co-digestion blend showed a similar, or even higher in the case of MS and FW2, production of methane during the initial stages of the BMP tests (Fig. 3). In contrast, the bioplastic always exhibited longer lag times and slower methane production rates compared to the methanization of organic waste alone (Fig. 3). The fact that methane production in the co-digestion tests tied or overtook the mono-digestion of organic waste is important for practical application. There is consistent evidence that the kinetics of methane production of PHAs tends to be slower than that of typical organic wastes (Bátori et al., 2018; Abraham et al., 2021). Indeed, the effective methanization of PHAs needs longer HRTs than those typically implemented in most of the full-scale anaerobic digesters. Hence, the presence of PHBH (at 20 % solids load) in all the organic wastes tested did not adversely affect neither the extent nor the kinetics of methane production.

Most plastics and bioplastics discarded are currently landfilled after being mechanically separated at an early stage in waste treatment facilities (Cucina et al., 2022a). Based on the co-digestion results obtained in this study, the PHBH bottle used as a proxy of rigid packaging showed good compatibility with the AD of MS, FW, and SM, leading to additional generation of methane. It has been estimated that about 45 % more methane can be obtained through the thermophilic anaerobic co-digestion of sewage sludge with bioplastics like starch-based shoppers and PLA-based products in comparison with the scenario without the bioplastics (Cucina et al., 2022a). Based on the VS content of the organic wastes (i.e., MS, FW1, FW2, and SM) and of the PHBH bottle, and on the methane yields achieved herein for all of them, the mono-digestion of 1 ton (wet weight) of MS, FW or SM was estimated to produce 3.5, 53.4–71.2, or 12.0 cubic meters of methane, respectively. In contrast, the use of 20 % PHBH (VS basis) as co-substrate would produce up to 4.7, 67.6–85.4, or 13.6 cubic meters of methane per ton of MS, FW or SM treated, resulting in up to 34 %, 20–27 %, and 13 % increase in methane production, respectively. On this calculation basis, and considering that PHBH would be completely degraded in the digester, each ton of MS, FW and SM treated would accommodate up to 3, 35, and 4 kg of PHBH, respectively, which is equivalent to a bioplastic loading of 20 % (VS basis).

3.4. Microbial communities

As shown in Fig. 4, high microbial diversity was observed in all cases of co-digestion. Prokaryote richness estimates at genus level were relatively high for all the assays, i.e., 187, 154, 102, and 136 in run 3, run 6, run 9, and run 12, respectively. The Shannon and Simpson indices were 2.69 and 0.75 for run 3, 2.70 and 0.80 for run 6, 2.71 and 0.88 for run 9, and 2.34 and 0.79 for run 12, respectively. In general, the bacterial community structure was found to be mainly dependent on the co-substrate and inoculum used. The bacterial community using the co-substrates FW1, FW2, and SM was, at the phylum level, predominantly represented by Firmicutes, with a relative abundance of 24.3, 79.9 and 89.8 % of the total ASV, respectively. The relative abundance of Firmicutes in run 3 was 11.9 %. Actinobacteria were detected in run 6 (11.0 %), run 9 (11.7 %), run 12 (2.8 %) and run 3 (13.0 %). Bacteroidetes was also a representative bacterial phylum in all the conditions tested, with a relative abundance of 8.7, 3.3, 3.6 and 9.0 % for run 6, run 9, run 12 and run 3, respectively. Proteobacteria, Chloroflexi, Synergistetes and Planctomycetota were only dominant bacterial phyla in run 6 (8.2, 18.4, 4.3 and 5.9 %, respectively) and run 3 (11.3, 19.2, 4.4 and 8.4 %, respectively). According to a clustering analysis (paired group UPGMA) performed for bacteria (see Supplementary material), run 3 and run 6 had a similarity of more than 0.75, probably because they shared the same anaerobic inoculum. As discussed above, the co-digestion of MS and FW1 with PHBH showed a good methanization. Members of Chloroflexi, Synergistetes and Planctomycetota are capable of perfoming the hydrolysis-acidogenesis of organic matter (Lim et al., 2018). Thus, it was suggested that in addition to Firmicutes, Actinobacteria and Bacteroidetes, other bacterial phyla such as Chloroflexi, Synergistetes and Planctomycetota were also important in preparing the tested organic waste and PHBH for the subsequent methanogenic step.

Previous studies analyzing the microbiology involved in the anaerobic biodegradation of bioplastics have shown similar bacterial phyla to those found in the present work. The enrichment of Bacteroidetes, Firmicutes, Chloroflexi and Proteobacteria phyla during mesophilic AD of biodegradable plastic coffee capsules was reported by Cazaudehore et al. (2021). Similarly, Peng et al. (2022) found Firmicutes, Bacteroidetes and Proteobacteria to be the dominant phyla in anaerobic biodegradation tests conducted under mesophilic conditions for biopolymer bags made of polybutylene adipate terephthalate (PBAT) and PLA. Venkiteshwaran et al. (2019) observed that the microbial communities in continuous mesophilic digesters treating synthetic municipal primary sludge changed transiently when the digesters were co-fed with polyhydroxybutyrate (PHB). However, the authors observed that Bacteroidetes and Firmicutes were consistently the two most dominant phyla in all mono- and co-digestion tests. Firmicutes and Bacteroidetes were also the dominant phyla during the mesophilic co-digestion of food waste with PLA, PBAT, a PBAT/PLA/starch blend, or with polyethylene (Yu et al., 2023).

At the genus level, each condition had its own bacterial community structure, confirming that both the co-substrate and the inoculum source shaped the microbial community. *Clostridium* appeared in run 6 (9.9 %), run 9 (0.2 %), run 12 (39.6 %) and run 3 (3.9 %). *Turicibacter* and *Terrisporobacter* were mainly detected in run 12 (12.1 and 15.6 % respectively). *Rombustia* was another bacterium found in run 6 (7.9 %), run 12 (4.9 %), and run 3 (4.9 %). *Fastidiosipila* (19.9 %), *Gallicola* (8.4 %), *Lactobacillus* (6.8 %), *Glemia* (6.3 %), *Syntrophaceticus* (5.4 %), *Limosilactobacillus* (3.4 %), *Streptococcus* (3.3 %), and *Leuconostoc* (2.6 %) were genera mainly associated with run 9. The run 3 and run 6, using the same methanogenic inoculum (Table 1), shared some secondary bacteria such as *Candidatus Cloacimonas*, *Mycolicibacterium*, *Leptolinea*, *Candidatus Bipolaricaulis*, *Thermovirga*, and *Candidatus Caldatribacterium*, all with a relative abundance of less than 5 %.

In contrast to the bacterial richness, the richness of methanogens was lower, i.e., 8, 22, 22, and 16 in run 6, run 9, run 12, and run 3, respectively. Shannon and Simpson indices for archaea were 1.42 and 0.66 in run 6, 2.16 and 0.85 in run 9, 1.73 and 0.73 in run 12, and 1.43 and 0.69 in run 3, respectively. Euryarchaeota showed the highest relative abundance regardless of the co-substrate used (79.2 % run 6, 78.7 % run 9, 80.3 % run 12, and 96.6 % run 3). The second most dominant phylum was Candida Thermoplasmatota (18.1 % run 6, 9.7 % run 9, 5.2 % run 12). Another important phylum was Halobacterota (1.5 % run 6, 0.8 % run 9, 4.5 % run 12 and 0.3 % run 3), while Crenarchaeota was only found in run 9 and run 12, with relative abundances of 8.9 and 5.6 %, respectively. Finally, the phylum Thaumarchaeota was detected to a lesser extent in run 12 (2.8 %) and in run 6 (1.1 %). More specifically, using the co-substrate FW1 (run 6), *Methanolinea* (51.9 %) was the dominant archaea detected, followed by Methanomassiliicoccus (18.3 %), Candidatus *Methanofastidiosum* (12.3 %), and *Methanobacterium* (9.2 %). In run 9, the most representative archaea were Candidatus *Methanofastidiosum* (22.2 %), *Methanobrevibacter* (19.8 %), *Methanolinea* (19.0 %), *Methanomassiliicoccus* (9.8 %), *Methanobacterium* (8.4 %), and *Methanosphaera* (4.2 %). The use of SM (run 12) as co-substrate showed an enriched microbial community with the genera *Methanosphaera* (30.6 %), *Methanoculles* (39.8 %), *Methanomassiliicoccus*

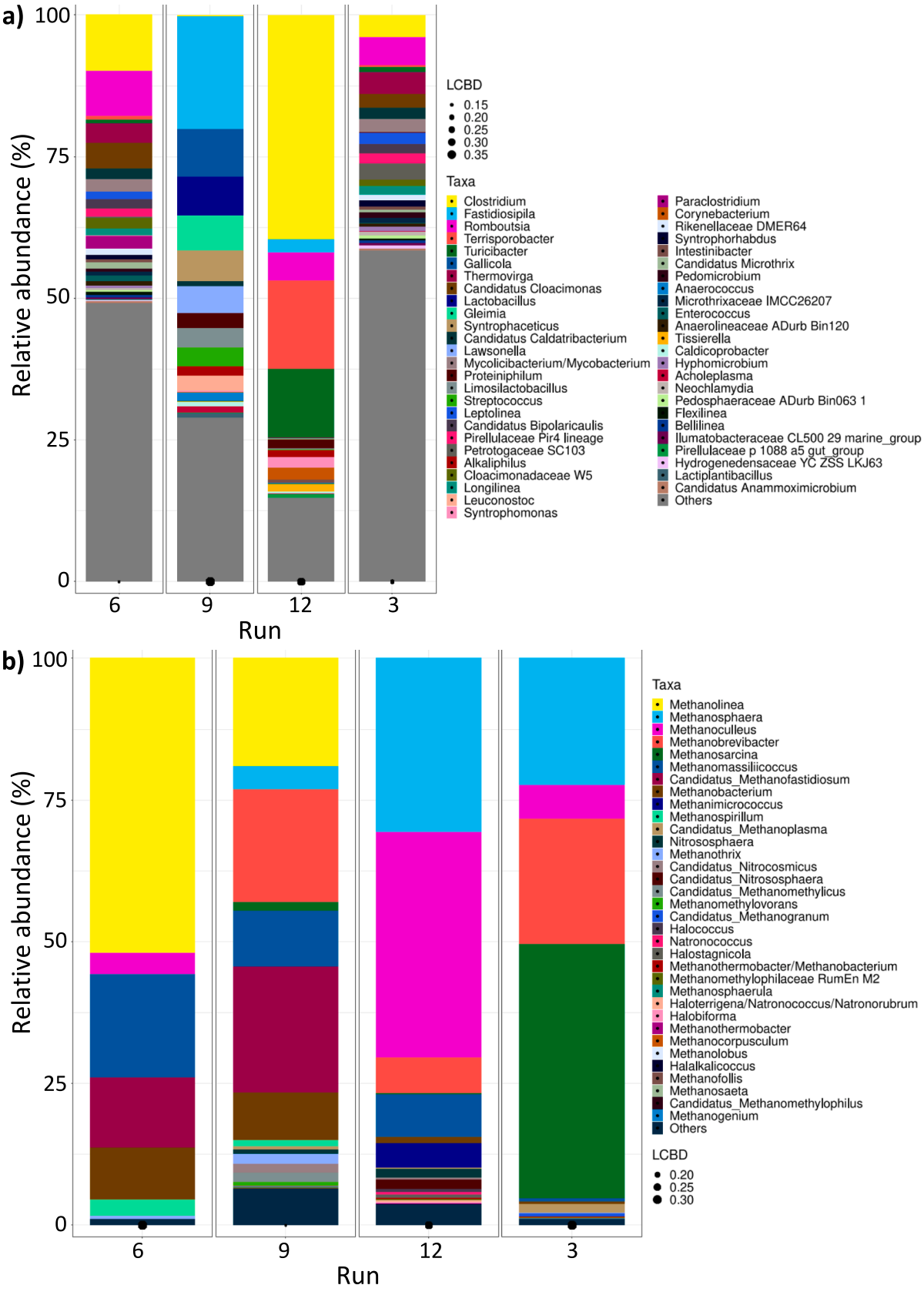


Fig. 4. Bacteria (a) and archaea (b) microbial communities at the genus level detected at the end of mesophilic, low-solids methanization of rigid PHBH packaging with FW1 (run 6), FW2 (run 9), swine manure (run 12) and mixed sludge (run 3). Local contribution to beta-diversity (LCBD) is a dissimilarity coefficient; large LCBD values indicate samples with a higher dissimilarity. For interpretation of the runs, the reader is referred to Table 1.

(7.5 %), *Methanobrevibacter* (6.2 %), and other minor archaea. Finally, the most dominant archaea detected in run 3 was *Methanosarcina* (44.9 %), followed by *Methanosphaera* (22.3 %), *Methanobrevibacter* (22.0 %), and *Methanoculleus* (6.0 %). *Methanolinea*, *Methanobacterium*, *Methanobrevibacter, Methanoculles* and *Methanosphaera* are classified as hydrogenotrophs (Astals et al., 2020). *Methanomassiliicoccus* has been reported to be a methylotrophic methanogen (Wu et al., 2016). Therefore, the hydrogenotrophic pathway was hypothesized to be responsible for the methane production during the mesophilic co-digestion of PHBH with FW, SM or MS. Furthermore, a good PHBH methanization was observed with all the co-substrates tested, despite the fact that the microbial communities, both bacteria and archaea, detected in all the assays differed from each other. Such flexible communities were attributed to the intrinsic biodegradable nature of PHBH (Eraslan et al., 2022). It is worth noting that the microbial community in BMP tests is significantly influenced by the initial microbial population. Additionally, the microbial community in BMP tests is dynamic, as changes in carbon availability over time lead to shifts in the microbiome. The microbial communities enriched during long-term co-digestion of organic waste with anaerobically biodegradable bioplastics, such as those from the PHA family, should be investigated in future studies.

4. Conclusions

The methanization of rigid PHBH packaging was evaluated for the first time in mono- and co-digestion BMP tests. After an 8-week incubation, the PHBH bottles degraded significantly. Mono-digestion of different PHBH bottles yielded consistent methane production, averaging ~ 400 NmL CH_4/g VS fed after 62 days. Compared to powdered PHBH, the 9 cm^2 size PHBH had a slightly lower methane potential and a longer digestion time. However, at 20 % co-substrate loading, PHBH required similar HRTs as FW, SM or MS, suggesting good compatibility in digesters. The hydrogenotrophic pathway was hypothesized to be responsible for methane production during the co-digestion of PHBH.

CRediT authorship contribution statement

Octavio García-Depraect: Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Leonardo J. Martínez-Mendoza:** Investigation, Formal analysis. **Rosa Aragão Börner:** Writing – review & editing, Validation, Conceptualization. **Johannes Zimmer:** Writing – review & editing, Validation, Conceptualization. **Raúl Muñoz:** Writing – review & editing, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: R. A.B. and T.B. report a relationship with Société des Produits Nestlé S.A. that includes: employment. The authors declare the following financial interests relationships which may be considered as potential competing interests: O.G.-D., L.J.M.-M., and R.M. reports financial support was provided by Société des Produits Nestlé S.A.

Data availability

Data will be made available on request.

Acknowledgments

This project was financed by Société des Produits Nestlé S.A. Octavio García-Depraect is supported by Grant RYC2021-034559-I funded by MCIN/AEI/10.13039/501100011033 and by European Union NextGenerationEU/PRTR. The regional government of Castilla y León and the European FEDER Programme (CL-EI-2021-07 and UIC 315) are also acknowledged.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2024.131180.

References

Abraham, A., Park, H., Choi, O., Sang, B.I., 2021. Anaerobic co-digestion of bioplastics as a sustainable mode of waste management with improved energy production – A review. Bioresour. Technol. 322, 124537 https://doi.org/10.1016/j.biortech.2020.124537.

Álvarez-Méndez, S.J., Ramos-Suárez, J.L., Ritter, A., Gonzalez, J.M., Pérez, Á.C., 2023. Anaerobic digestion of commercial PLA and PBAT biodegradable plastic bags: Potential biogas production and ^{1}H NMR and ATR-FTIR assessed biodegradation. Heliyon 9, e16691.

APHA, AWWA, WEF, 2005. Standard Methods for the Examination of Water and Wastewater, 21st ed. APHA, Washington DC.

Astals, S., Koch, K., Weinrich, S., Hafner, S.D., Tait, S., Peces, M., 2020. Impact of storage conditions on the methanogenic activity of anaerobic digestion inocula. Water 12, 1321. https://doi.org/10.3390/w12051321.

Bátori, V., Åkesson, D., Zamani, A., Taherzadeh, M.J., Sárvári Horváth, I., 2018. Anaerobic degradation of bioplastics: A review. Waste Manag. 80, 406–413. https://doi.org/10.1016/j.wasman.2018.09.040.

Blank, L.M., Narancic, T., Mampel, J., Tiso, T., O'Connor, K., 2020. Biotechnological upcycling of plastic waste and other non-conventional feedstocks in a circular economy. Curr. Opin. Biotechnol. 62, 212–219. https://doi.org/10.1016/j.copbio.2019.11.011.

Bracciale, M.P., De Gioannis, G., Falzarano, M., Muntoni, A., Polettini, A., Pomi, R., Rossi, A., Sarasini, F., Tirill, J., Zonfa, T., 2023. Anaerobic biodegradation of disposable PLA-based products: Assessing the correlation with physical, chemical and microstructural properties. J. Hazard. Mater. 452, 131244 https://doi.org/10.1016/j.jhazmat.2023.131244.

Briassoulis, D., Pikasi, A., Hiskakis, M., 2021. Organic recycling of post-consumer/industrial bio-based plastics through industrial aerobic composting and anaerobic digestion - Techno-economic sustainability criteria and indicators. Polym. Degrad. Stab. 190, 109642 https://doi.org/10.1016/j.polymdegradstab.2021.109642.

Calabro', P.S., Folino, A., Fazzino, F., Komilis, D., 2020. Preliminary evaluation of the anaerobic biodegradability of three biobased materials used for the production of disposable plastics. J. Hazard. Mater. 390, 121653 https://doi.org/10.1016/j.jhazmat.2019.121653.

Cazaudehore, G., Monlau, F., Gassie, C., Lallement, A., Guyoneaud, R., 2021. Methane production and active microbial communities during anaerobic digestion of three commercial biodegradable coffee capsules under mesophilic and thermophilic conditions. Sci. Total Environ. 784, 146972 https://doi.org/10.1016/j.scitotenv.2021.146972.

Chernicharo, C.A.L., 2007. Biological wastewater treatment. London. In: Publishing, I.W.A. (Ed.), Aanerobic Reactors, pp. 1–4.

Cucina, M., Nisi, P.D., Trombino, L., Tambone, F., Adani, F., 2021. Degradation of bioplastics in organic waste by mesophilic anaerobic digestion, composting and soil incubation. Waste Manag. 134, 67–77. https://doi.org/10.1016/j.wasman.2021.08.016.

Cucina, M., Carlet, L., Nisi, P.D., Alexandre, C., Giordano, A., Adani, F., 2022a. Degradation of biodegradable bioplastics under thermophilic anaerobic digestion: A full-scale approach. J. Clean. Prod. 368, 133232 https://doi.org/10.1016/j.jclepro.2022.133232.

Cucina, M., Soggia, G., Nisi, P.D., Giordano, A., Adani, F., 2022b. Assessing the anaerobic degradability and the potential recovery of biomethane from different biodegradable bioplastics in a full-scale approach. Bioresour. Technol. 354, 127224 https://doi.org/10.1016/j.biortech.2022.127224.

Dolci, G., Venturelli, V., Catenacci, A., Ciapponi, R., Malpei, F., Romano Turri, S.E., Grosso, M., 2022. Evaluation of the anaerobic degradation of food waste collection bags made of paper or bioplastic. J. Environ. Manage. 305, 114331 https://doi.org/10.1016/j.jenvman.2021.114331.

Eraslan, K., Aversa, C., Nofar, M., Barletta, M., Gisario, A., Salehiyan, R., Goksu, Y.A., 2022. Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBH): Synthesis, properties, and applications - A review. Eur. Polym. J. 167, 111044 https://doi.org/10.1016/j.eurpolymj.2022.111044.

European Bioplastics, 2023. Applications for bioplastics. Available at: https://www.european-bioplastics.org/market/applications-sectors/ accessed on July 12th 2023.

European Bioplastics, 2023. Bioplastics market data. Available at: https://www.european-bioplastics.org/market/ accessed on July 12th 2023.

García-Depraect, L.R., Rodriguez-Vega, S., Bordel, S., Santos-Beneit, F., Martínez-Mendoza, L.J., Aragão Börner, R., Börner, T., Muñoz, R., 2022a. Biodegradation of bioplastics under aerobic and anaerobic aqueous conditions: Kinetics, carbon fate and particle size effect. Bioresour. Technol. 344 https://doi.org/10.1016/j.biortech.2021.126265.

García-Depraect, O., Lebrero, R., Rodriguez-Vega, S., Börner, R.A., Börner, T., Muñoz, R., 2022b. Production of volatile fatty acids (VFAs) from five commercial bioplastics via acidogenic fermentation. Bioresour. Technol. 360, 127655 https://doi.org/10.1016/j.biortech.2022.127655.

García-Depraect, O., Lebrero, R., Martínez-mendoza, L.J., Tim, B., Rodriguez-vega, S., Aragao, R., Muñoz, R., 2023. Enhancement of biogas production rate from bioplastics by alkaline pretreatment. Waste Manage. 164, 154–161. https://doi.org/10.1016/j.wasman.2023.04.009.

Grosser, A., 2018. Determination of methane potential of mixtures composed of sewage sludge, organic fraction of municipal waste and grease trap sludge using biochemical methane potential assays. A comparison of BMP tests and semi-continuous trial results. Energy 143, 488–499. https://doi.org/10.1016/j.energy.2017.11.010.

Hegde, S., Diaz, C.A., Dell, E.M., Trabold, T.A., Lewis, C.L., 2021. Investigation of process parameters on the anaerobic digestion of a poly(hydroxyalkonate) film. Eur. Polym. J. 148, 110349 https://doi.org/10.1016/j.eurpolymj.2021.110349.

Hobbs, S.R., Parameswaran, P., Astmann, B., Devkota, J.P., Landis, A.E., 2019. Anaerobic codigestion of food waste and polylactic acid: Effect of pretreatment on methane yield and solid reduction. Adv. Mater. Sci. Eng. 2019 https://doi.org/10.1155/2019/4715904.

Kakadellis, S., Lee, P.H., Harris, Z.M., 2022. Two birds with one stone: Bioplastics and food waste anaerobic co-digestion. Environments 9, 1–14. https://doi.org/10.3390/environments9010009.

Lim, J.W., Ting, D.W.Q., Loh, K.C., Ge, T., Tong, Y.W., 2018. Effects of disposable plastics and wooden chopsticks on the anaerobic digestion of food waste. Waste Manag. 79, 607–614. https://doi.org/10.1016/j.wasman.2018.08.033.

Mata-Alvarez, J., Dosta, J., Romero-Güiza, M.S., Fonoll, X., Peces, M., Astals, S., 2014. A critical review on anaerobic co-digestion achievements between 2010 and 2013. Renew. Sustain. Energy Rev. 36, 412–427. https://doi.org/10.1016/j.rser.2014.04.039.

Peng, W., Wang, Z., Shu, Y., Lü, F., Zhang, H., Shao, L., He, P., 2022. Fate of a biobased polymer via high-solid anaerobic co-digestion with food waste and following aerobic treatment: Insights on changes of polymer physicochemical properties and the role of microbial and fungal communities. Bioresour. Technol. 343, 126079 https://doi.org/10.1016/j.biortech.2021.126079.

Reischwitz, A., Stoppok, E., Buchholz, K., 1997. Anaerobic degradation of poly-3-hydroxybutyrate and poly-3-hydroxybutyrate-co-3-hydroxyvalerate. Biodegradation 8, 313–319. https://doi.org/10.1023/A:1008203525476.

Ruggero, F., Gori, R., Lubello, C., 2019. Methodologies to assess biodegradation of bioplastics during aerobic composting and anaerobic digestion: A review. Waste Manag. Res. 37, 959–975. https://doi.org/10.1177/0734242X19854127.

Ryan, C.A., Billington, S.L., Criddle, C.S., 2017. Assessment of models for anaerobic biodegradation of a model bioplastic: Poly(hydroxybutyrate-co-hydroxyvalerate). Bioresour. Technol. 227, 205–213. https://doi.org/10.1016/j.biortech.2016.11.119.

Santos, A.D., Silva, J.R., Castro, L.M., Quinta-Ferreira, R.M., 2022. A biochemical methane potential of pig slurry. Energy Reports 8, 153–158. https://doi.org/10.1016/j.egyr.2022.01.127.

Thomas, A.P., Kasa, V.P., Dubey, B.K., Sen, R., Sarmah, A.K., 2023. Synthesis and commercialization of bioplastics: Organic waste as a sustainable feedstock. Sci. Total Environ. 904, 167243 https://doi.org/10.1016/j.scitotenv.2023.167243.

Üveges, Z., Damak, M., Klátyik, S., Ramay, M.W., Fekete, G., Varga, Z., Gyuricza, C., Székács, A., Aleksza, L., 2023. Biomethane potential in anaerobic biodegradation of commercial bioplastic materials. Fermentation 9, 261. https://doi.org/10.3390/fermentation9030261.

Venkiteshwaran, K., Benn, N., Seyedi, S., Zitomer, D., 2019. Methane yield and lag correlate with bacterial community shift following bioplastic anaerobic co-digestion. Bioresour. Technol. Rep. 7, 100198 https://doi.org/10.1016/j.biteb.2019.10019.

Wu, Y., Wang, C., Liu, X., Ma, H., Wu, J., Zuo, J., Wang, K., 2016. A new method of two-phase anaerobic digestion for fruit and vegetable waste treatment. Bioresource technology 211, 16–23.

Yasin, N.M., Akkermans, S., Impe, J.F.M.V., 2022. Enhancing the biodegradation of (bio) plastic through pretreatments: A critical review. Waste Manag. 150, 1–12. https://doi.org/10.1016/j.wasman.2022.06.004ç.

Yu, C., Dongsu, B., Tao, Z., Xintong, J., Ming, C., Siqi, W., Zheng, S., Yalei, Z., 2023. Anaerobic co-digestion of three commercial bio-plastic bags with food waste: Effects on methane production and microbial community structure. Sci. Total Environ. 859, 159967 https://doi.org/10.1016/j.scitotenv.2022.159967.

Zhang, W., Heaven, S., Banks, C.J., 2018. Degradation of some EN13432 compliant plastics in simulated mesophilic anaerobic digestion of food waste. Polym. Degrad. Stab. 147, 76–88. https://doi.org/10.1016/j.polymdegradstab.2017.11.005.