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Production of volatile fatty acids (VFAs) from five commercial bioplastics via acidogenic fermentation

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Abstract

The feasibility of producing volatile fatty acids (VFAs) from five commercial bioplastics via acidogenic fermentation by a non-pretreated anaerobic sludge was investigated. Mesophilic, anaerobic, acidogenic batch assays at 1, 10 and 20 g/L feed concentrations revealed the feasibility of producing VFAs from polyhydroxyalkanoates (PHA), i.e., PHB and PHBV, but not from PBS, PCL and PLA under the test conditions and time. However, only high PHA substrate concentrations (10–20 g/L) resulted in organic overloading and decreasing the pH of the culture broth down to 4–5, which in turn induced the accumulation of VFAs via kinetic imbalance between acidogenesis and methanogenesis. Gaseous carbon (C-CO$_2$ and C-CH$_4$) accounted for 8–35% of the total initial carbon, while C-VFAs represented 10–18%, mainly as acetate and butyrate. This study represents the first systematically assessed proof-of-concept to produce VFAs from PHA, which is key for the design of bioplastic-to-bioplastic recycling (bio)technologies.

Keywords: Biodegradation; Biorefinery; Microbial upcycling; Plastic waste; Polyhydroxyalkanoates; VFAs.

1. Introduction

Plastic pollution is one of the largest environmental issues facing society today at a global scale. The global plastic production was estimated at 367 million tonnes in 2021, of which 22% is mismanaged and only 9% is appropriately recycled (OECD, 2022; European Bioplastics, 2021). Currently, the disposal of plastic waste through either managed or unmanaged landfills is the least favoured but most applied (~ 50%) end-of-life method, which results in multiple social, economic and environmental problems (OECD, 2022). Moreover, it is estimated that 5–13 million tonnes of plastic end up in the oceans every year (Filho et al.,
Bio-based and/or biodegradable plastics, commonly referred to as bioplastics, have become attractive materials that can offer circularity and a reduced ecological footprint compared to the recalcitrant fossil-based plastics (García-Depraect et al., 2021; Jehanno et al., 2022; Rosenboom et al., 2022). The bioplastic market is experiencing a rise in the number of bioplastic manufacturers, end-user acceptance and demand, regulatory policies (e.g., incentives, directives and bans), and the emergence of new materials and products with advanced features and applications. However, the global production of bioplastics was still limited to 2.4 million tonnes in 2021 and is forecasted to 7.6 million tonnes by 2026, representing nowadays less than 1% of the total plastic production (European Bioplastics, 2021). The development and broader uptake of new competitive value chain concepts for post-consumer bioplastics is therefore needed to push the bioplastics industry forward.

Recently, the biotechnological conversion of (bio)plastics into oligomers and monomers that can be further used as carbon sources to produce chemicals with high value, or alternatively new biodegradable polymers, has received increasing attention (Blank et al., 2020; Ru et al., 2020; Ballerstedt et al., 2021; Gao et al., 2022; García-Depraect et al., 2022; Liu et al., 2021; Tiso et al., 2022). In this context, Tiso et al. (2022) summarized and discussed the potential of biochemically upcycling different (bio)plastics as an alternative end-of-life approach, producing plenty of chemicals of industrial interest, such as glycerol, succinic acid, phenol, ethylene, etc., from plastic monomers that can be produced either...
through biological or thermochemical methods. By using the genome scale metabolic model iJN1462 of Pseudomonas putida, several polymers such as PLA (polylactic acid), PHA (polyhydroxyalkanoate), PBS [poly(butylene succinate)], among others, showed encouraging potential to be precursors of value-added chemicals depending on the target product (Tiso et al., 2022). Bio-upcycling of (bio)plastics is a timely and disruptive circularity-focused research field, albeit it is still in its infancy. On the one hand, most attention has been given to the bio-upcycling of mass-produced plastics such as PET (polyethylene terephthalate), PP (polypropylene), PE (polyethylene) and PUR (polyurethanes) (Ru et al., 2020; Ballerstedt et al., 2021; Dissanayake and Jayakody, 2021; Liu et al., 2021; Gao et al., 2022; Jehanno et al., 2022). On the other hand, bio-upcycling technologies for bioplastics are at present limited but will be very relevant based on the increasing growth of bioplastics industry.

In this context, mixed culture fermentation allows to produce carboxylic acids from low-cost feedstocks. The production of volatile fatty acids (VFAs) from organic waste is an attractive topic in the current research because these building blocks can be readily used by a plethora of microorganisms to produce a wide portfolio of marketable bioproducts including chemicals (e.g., surfactants, flocculants, fertilizers, preservatives, etc.), biofuels (e.g., hydrogen, gasoline, diesel, jet fuel), and bioplastics (e.g., PHAs) (Kumar et al., 2019; Sekoai et al., 2021; Szacherska et al., 2021), with applications in sectors such as chemical, food, textile, pharmaceutical, cosmetics, agricultural and wastewater treatment (Varghese et al., 2022). To date, VFAs production from organic wastes via anaerobic acidification is in its early stages of development, with a few pilot-scale studies (Atasoy et al., 2018). The main goal is to maximize the yields and rates of bioconversion of a given organic feedstock to selectively produce and recover VFAs in a cost-efficient and environmentally friendly way. Extensive research has been focused on the evaluation of different waste streams, biomass pre-treatments, reactor configurations, process parameters (i.e., temperature, pH, hydraulic
retention time, organic load, etc.) on the process and its microbiology, as well as on the
recovery and use of the produced VFAs (Varghese et al., 2022). However, to the best of the
authors' knowledge, the valorization of bioplastics into VFAs via acidogenic fermentation
technology is a breakthrough concept so far unexplored.

In the quest of fostering bioplastics circularity, this study aimed at investigating the
feasibility of producing VFAs from five different commercial bioplastics via acidogenic
fermentation by non-pretreated anaerobic sludge. Emphasis was paid on investigating the
effect of the initial polymer concentration on the product yield and spectrum of VFAs. The
bioplastics tested included PHB [poly(3-hydroxybutyrate)], PHBV [poly(3-hydroxybutyrate-
co-3-hydroxyvalerate], PCL (polycaprolactone), PLA, and PBS. To the best of the authors'
knowledge, the present study validates a new circularity-oriented biotechnological approach
for the bio-upcycling/recycling of bioplastics. Finally, conclusions on practical implications
and further research needs to improve the production of VFAs from bioplastics were outlined.

2. Materials and methods

2.1 Polymers

Acidogenic fermentation tests were carried out for PHB (ENMAT™ Y3000P), PHBV
(ENMAT™ Y1000P, 3 mol% HV), PCL (Capa® 6500D), PLA (LUMINY® L105), and PBS
(BioPBS™ FZ91PM/FZ91PB), which were supplied by the Technological Institute of
Packaging, Transportation and Logistics (ITENE, Spain). Each pelletized material was
initially blended using a commercial Titanium 2000 pro blender (Cecotec, Spain) and then
screened through stainless steel sieves with a mesh size of 100, 250, 500 and 1000 µm using
an electromagnetic sieve (CISA RP-20, Spain), according to García-Depraect et al. (2022).
All bioplastics tested exhibited a particle size distribution of 100–250 µm. Microcrystalline
cellulose powder purchased from Merck Ltd. (CAS number 9004–34-6), with a particle size
distribution of 20–160 μm, was used as the sole carbon source in parallel acidogenic tests for comparison purposes.

2.2 Inoculum

The acidogenic fermentation tests were performed with non-pretreated anaerobic sludge. The anaerobic sludge was kindly supplied by the sewage treatment plant of Valladolid (Spain). The inoculum was initially degassed by preincubation at 36 ± 1 °C for 6 days, without addition of any nutrient and carbon source, to reduce the background gas production as low as possible. The total suspended (TSS) and volatile (VSS) solids concentration of the sludge was determined as 21.8 and 12.3 g/L, respectively. The raw anaerobic sludge was washed twice and resuspended in a mineral salt medium following the procedure reported by García-Depraect et al. (2022). This medium was composed of (in g/L): KH$_2$PO$_4$, 0.27; Na$_2$HPO$_4$·12H$_2$O, 1.12; NH$_4$Cl, 0.53; CaCl$_2$·2H$_2$O, 0.075; MgCl$_2$·6H$_2$O, 0.1; FeCl$_2$·4H$_2$O, 0.02; resazurin, 0.001; Na$_2$S·9H$_2$O, 0.1, and 10 mL of a stock solution of trace elements containing (in g/L): MnCl$_2$·4H$_2$O 0.05, H$_3$BO$_3$, 0.005; ZnCl$_2$, 0.007; CuCl$_2$, 0.004; Na$_2$MoO$_4$·2H$_2$O, 0.002; CoCl$_2$, 0.055; NiCl$_2$·6H$_2$O, 0.017; Na$_2$SeO$_3$, 0.003; Na$_2$WO$_4$·2H$_2$O, 0.002. All reagents were of analytical grade.

2.3 Experimental set up and operational conditions

A series of acidogenic fermentation tests was performed to evaluate the feasibility of producing marketable VFAs from bioplastics. The assays were carried out in 2.1 gas-tight glass bioreactors with a working volume of 1 L. Each material was tested at three different substrate concentrations, namely, 1, 10 and 20 g/L. It is known that the feed-to-microorganism (F/M) ratio is one of the critical parameters affecting the efficiencies and conversion rates of organic wastes into VFAs. Higher F/M-ratios can lead to inhibition of
methanogenesis, thus boosting the accumulation of VFAs (Raposo et al., 2011). The inoculum was supplied at a fixed VSS concentration of 1.1 g/L, constituting F/M ratios of 0.9, 9.1 and 18.2 for an initial material concentration of 1, 10 and 20 g/L, respectively. After inoculation, the bioreactors were filled-up with the fresh mineral salt medium (see section 2.2) and closed with rubbers septa and aluminium caps. The headspace of the bioreactors was flushed with helium gas (Abello Linde, Barcelona, Spain) for 5 min to provide anaerobic conditions. Finally, the bioreactors were incubated at 36 ± 1 °C in a roller shaker set at ≈ 4.5 rpm (Wheaton Scientific Products, USA). Parallel blank tests containing only inoculum were also run to subtract the endogenous carbon dioxide (CO$_2$) and methane (CH$_4$) production from those generated by the materials tested. All assays were conducted in triplicate. The initial pH of the culture broths was 7.28 ± 0.01. The duration of the experiment, mainly determined by the stabilization of the pH of the culture broth, was 56 days. During the incubation, 3-mL liquid samples were withdrawn periodically for pH and VFAs analysis. After pH measurement, 2 mL of sample were returned to the bioreactor while the remaining volume of collected supernatant was centrifuged at 10000 rpm for 10 min and then filtered through a 0.22 μm filter, acidified with sulfuric acid 96% (20 μL of concentrated acid per one mL of sample), and finally frozen at -20 °C for further analysis.

For the sake of comparison, VFA concentrations were expressed in mg VFA/L, mg acetic acid-equivalent/L and mg COD-equiv./L. The COD equivalence (g COD/g VFA) was 1.06, 1.51, 1.82 and 2.04 for acetate (HAc), propionate (HPr), butyrate (HBu) and valerate (HVal), respectively. The VFAs yield was calculated according to Eq. (1). Soluble total organic carbon (TOC) concentration was also determined at the end of the fermentations and compared to the equivalent carbon estimated from the final VFAs concentration recorded.

\[
Y_{\text{VFAs, material}} = \frac{C_{\text{VFAs}} - C_{\text{VFAs}}^0}{C_{\text{material}}}
\]

Eq. (1)
Where, $Y_{VFAs,material}$ is the yield of VFAs produced from the test material (mg COD-equiv./g material or g $C_{VFAs}/g \, C_{material}$); $C_{VFAs}$ is the sum of the concentrations of COD-equiv. (or TOC) calculated from the individual VFAs measured at the end of the fermentation (in mg COD-equiv./L or mg $C_{VFAs}/L$); $C_{0VFAs}$ is the sum of the concentrations of COD-equiv. (or TOC) calculated from the individual VFAs measured at the beginning of the fermentation (mg COD-equiv./L or mg $C_{VFAs}/L$); $C_{material}$ is the initial concentration of the test material (or equivalent carbon contained in the material fed; in g material/L or mg $C_{material}/L$).

The concentration of CH$_4$ and CO$_2$ in the headspace was also measured periodically, while the overpressure was recorded followed by biogas venting before each measurement. Biogas volume was normalized to 1 atm and 0 °C. The degree of biodegradation was calculated according to Eq. (2):

$$D_T = \sum \left( \frac{C_{m_{biogas}}}{m_v} \right)_{Test} - \sum \left( \frac{C_{m_{biogas}}}{m_v} \right)_{Blank} \times 100$$

(2)

where, $D_T$ is the degree of biodegradation (%) at time $t$ (in days); $\sum (C_{m_{biogas}})_{Test}$ and $\sum (C_{m_{biogas}})_{Blank}$ represent the cumulative mass of carbon evolved in the headspace as CO$_2$ and CH$_4$ (in mg) in the bioreactors containing the test material and in the blank tests, respectively, between the start of the test and time $t$; $m_v$ is the mass of carbon of the test material (in mg). Note that $D_T$ estimated the bioconversion of the test material into gaseous carbon products, thus it is only a measure of the partial biodegradation and not of the final biodegradation degree.

2.4 Analytical procedures
The amount of VFAs produced was determined using a gas chromatograph (GC) Agilent 7820A (Agilent, USA) equipped with a flame ionization detector (FID) and a packed column (10% SP-1000 + 1%H₃PO₄ on Chromosorb® W acid washed 100/120 mesh size, 2 m × 3.175 mm; Teknokroma, Spain). The temperature of the injection port and FID was kept at 350 °C. The oven temperature was initially maintained at 135 °C for 10 min, then it was increased to 151 °C at a rate of 3 °C/min, and finally ramped at 8 °C/min to 180 °C and held for 5 min. Nitrogen, at a flow rate of 45 mL/min, was employed as the carrier gas. The flow rates of hydrogen and air were 45 and 350 mL/min, respectively. A VFA standard mix (Sigma-Aldrich part number CRM46975, USA) was used to calibrate the GC-FID. The VFA standard contained (in mM) 10.0 formic acid (HFor), 10.0 HAc, 10.0 HPr, 10.0 isobutyric acid (i-HBu), 10.0 HBu, 10.0 isovaleric acid (i-HVal), 10.0 HVal, 10.0 isocaproic acid (i-HCa), 10.1 hexanoic acid (HHex) and 10.1 n-heptanoic acid (HHep). Finally, biogas production (CO₂ and CH₄) was estimated by manometric and gas-chromatographic methods, as previously reported elsewhere (García-Depraect et al., 2022).

3. Results and discussion

3.1 Production of VFAs through acidogenic fermentation is feasible but strongly dependent on polymer type

Out of the five different bioplastics tested for VFAs production via acidogenic fermentation, only PHB and PHBV were biodegraded to a certain extent in 56 days. As expected, microcrystalline cellulose was also assimilated by microbial communities due to its biodegradable and fermentable nature. As a result of the biodegradation, VFAs, mainly HBu and HAc, were produced and their final concentration depended on the type of material as well as on the initial substrate concentration (Figure 1). For instance, HAc and HBu accounted for ~38% and 55%, respectively, of the total organic acids measured (as COD
equivalents) when fermenting PHBV at an initial concentration of 10 g/L (Table 1). The
effect of substrate concentration on pH and VFAs production will be presented and discussed
in detail in section 3.2. In contrast, PLA, PBS and PCL did not show any measurable
biodegradation in the form of CH₄ and/or CO₂ production throughout the duration of the
experiment. Indeed, the final pH of the culture broth for PBS, PCL and PLA averaged 6.92 ±
0.06, 6.77 ± 0.19 and 6.89 ± 0.08, respectively, regardless of the substrate concentration,
while the final pH for PHAs and cellulose dropped below 5.5, yet only at high substrate
concentrations (10 and 20 g/L) (Figure 2). The culture broths collected using PBS, PCL and
PLA as feedstocks were therefore not analysed for VFAs production.
PLA, PBS and PCL have been found to be non-digestible bioplastics when exposed (for 77
days at 36 °C) to anaerobic, mesophilic conditions by non-pre-exposed anaerobic sludge
(García-Depraect et al., 2022), which could explain the observed biodegradation behaviour in
this study. The non-biodegradable nature of such materials can be attributed to their chemical
structure, which limits polymer accessibility, and/or to the lack of suitable metabolic machinery
needed to hydrolyse the polymer and metabolize the hydrolysis-derived products by the
inoculum used (García-Depraect et al., 2022). On the other hand, Yagi et al. (2009)
demonstrated that the anaerobic, mesophilic (35 °C) digestion of PLA grade H-400 (with a
particle size similar to this study) featured a pronounced lag phase of about 50–60 days during
which hardly any methane was produced but started to increase thereafter. This indicates that
the exact fermentation conditions, inoculum, and polymer grade (PLA Luminy L105 from
Total Corbion was used herein) may impact the outcome of PLA digestibility. The constitutive
monomer of PLA is lactic acid, which can be readily metabolize by acidogens. At this point it
should be noted that, if lactic acid was produced, the pH of the culture broth certainly should
have dropped, indicating in this case that the depolymerization of PLA was the rate limiting
step. Likewise, the monomer of PCL is 6-hydroxyhexanoic acid, which can be activated by
CoA transfer to be further metabolized through the β-oxidation cycle like 3-hydroxybutyric acid and 3-hydroxyvaleric acid (Janssen and Schink, 1993). The latent VFAs conversion for PBS seems more challenging due to its constitutive monomers. Thus, the catabolism of 1,4-butanediol (1,4-BDO) remains unclear, requiring an oxidation step of 1,4-BDO to 4-hydroxybutyrate which can be further metabolized via β-oxidation, while the metabolization of succinate requires the tricarboxylic acid (TCA) cycle (Li et al., 2020; Tiso et al., 2022). On the other hand, PHAs are regarded as biodegradable bioplastics while microcrystalline cellulose is indeed employed as a reference material to carry out positive controls devoted to assessing the quality of a given inoculum in standard testing methods determining the biodegradability of plastic materials (García-Depraect et al., 2022, ISO 14853).

PHAs are aliphatic polyesters that can be hydrolysed by extra- and intracellular PHAs depolymerases, including enzymes degrading native/denatured intracellular/extracellular short- and medium-chain length PHAs (García-Depraect et al., 2021). The resulting PHA depolymerization products vary depending on the type of PHA, for instance, 3-hydroxybutyric acid is produced from PHB while 3-hydroxybutyric acid and 3-hydroxyvaleric acid are released from PHBV (Meereboer et al., 2020). The hydrolysis of PHAs can also result in the formation of oligomers and dimmers (Reischwitz et al., 1998). The resulting (R)-3-hydroxyalkanoic acids can be metabolized mainly to HAc and HBu, but also to HPr and/or HVal depending on the type of PHA, via the β-oxidation pathway with crotonyl-CoA as the metabolic intermediate as demonstrated with single strain cultures (Janssen and Schink, 1993). That acidogenic pathway involved in the acidogenic fermentation of PHAs could explain why HAc and HBu were the major soluble end products observed in this study.

Additionally, the control material cellulose, which is a linear polymer of β-(1-4)-linked glucose molecules, is insoluble, crystalline, and heterogeneous in nature, and is
completely hydrolysed to glucose by the cooperative action of cellulases such as endo- and exo-glucanases and β-glucosidases (Arantes and Saddler, 2010; Lakhundi et al., 2015). Glucose can be anaerobically metabolized to pyruvate via the Embden-Meyerhof-Parnas (EMP) pathway, which can be further converted into a wide range of products (e.g., HFor, HPr, HAc, HBu, lactate, H₂, CO₂) depending on the prevailing fermentation pathway(s) (Zhou et al., 2018). Based on the metabolic profile observed at the end of the fermentation, the use of cellulose at low (1 g/L) and high (10 and 20 g/L) concentrations resulted in the prevalence of acetic-type and mixed-type fermentation, respectively (Table 1).

3.2 Effect of polymer concentration (substrate-to-inoculum ratio) on VFAs production efficiency

The influence of polymer concentration (1 to 20 g material/L) on the production of VFAs via mixed culture acidogenic fermentation was systematically assessed. The results obtained showed that the initial concentration of substrate not only affected the net production of VFAs but also their distribution (Figure 1 and Table 1). The concentration of polymer selected also determined the F/M ratio since the anaerobic microbial load was kept constant. Thus, the highest substrate concentration of 20 g/L constituted the highest F/M ratio (VS basis) of ~ 18. At a substrate concentration of 1 g/L, the net total production of VFAs (difference between the final and initial total VFA concentration) after a 56-day incubation was negative regardless of the material tested. The profile of VFAs for PHBV and cellulose in the assays conducted at 1 g/L showed a sharp accumulation (up to 200 mg/L) of HAc and HPr, respectively, within the first 10 days of fermentation, but both were gradually degraded during days 10 to 20 and remained at very low levels (< 20 mg/L) from day 20 onwards. Thus, the acidogenic rate did not exceed that of methanogenesis, generating a biogas rich in CO₂ and CH₄ in lieu of VFAs. It should be note that, in the present study, the non-pre-
exposed anaerobic sludge used as inoculum showed evidence of harbouring the hydrolytic bacteria and acidogens needed to depolymerize and transform the polymer into VFAs, but also contained undesirable acetogens and methanogens that readily degraded the excreted VFAs. Anaerobic sludge, which is commonly used as biocatalyst to perform acidogenesis (Wang et al., 2014; Yin et al., 2016), was not subjected to any pre-treatment (e.g., heat-shock) in order to preserve a high microbial diversity encoding the metabolic machinery needed for polymer metabolism (García-Depraect et al., 2022). Thus, the strategy to produce VFAs from bioplastics herein investigated relied on the inherent acidification of the culture broth by VFAs accumulation, which in turn may inhibit or halt the methanogenesis (Reischwitz et al., 1998; Braz et al., 2019; Wang et al., 2020). In the present study, the lowest concentration of polymer tested did not bring about kinetic imbalance between acidogenesis and methanogenesis. Indeed, the final pH of the anaerobic broth remained at 6.3 ± 0.04, 6.4 ± 0.06 and 7.1 ± 0.1 for PHBV, PHB and cellulose, respectively, while the pH when using the other polymers (which showed no sign of biodegradation) was ~ 6.97 (Figure 2). The total alkalinity of the culture broth, estimated at 408 ± 6 mg CaCO₃/L, was sufficient to buffer polymer biodegradation at 1 g/L. The implications of substrate concentration (F/M ratio) on biogas production are extensively discussed in section 3.3.

The increase in polymer concentration to 10 and 20 g/L resulted in a marked accumulation of VFAs, implying that acidogenesis outcompeted, to a certain extent, methanogenesis due to organic overload (Figures 1 and 3). In these assays, the culture pH dropped down to 4.1 on average, thus causing bioreactor acidification (Figure 2). At a substrate concentration of 10 g/L, the net production of VFAs for PHBV was 2310 ± 823 mg HAc-equiv./L (2694 ± 948 mg COD-equiv./L), while for PHB was computed as 1860 ± 234 mg HAc-equiv./L (2158 ± 286 mg COD-equiv./L). Likewise, net VFAs productions of 3109 ± 304 mg HAc-equiv./L (3808 ± 318 mg COD-equiv./L) and 3940 ± 536 mg HAc-equiv./L (4745 ± 788 mg COD-equiv./L)
were computed for PHBV and PHB, respectively, at a substrate concentration of 20 g/L.
Interestingly, the resulting VFAs mixture was over-represented by HAc and HBu when using
PHAs as the carbon and energy source. HVal accumulated at much lower levels, owing to its
low content (3%) in PHBV test material, albeit it exhibited an inconsistent trend among
fermentations (Table 1). The highest substrate concentration (20 g/L) also entailed higher
variability in the final VFAs distribution compared to assays conducted at 10 g/L, likely due to
the different effects that PHA concentration induced on the microbiota (Braz et al. 2019; Basak
et al., 2021; see section 3.3). Indeed, when compared at the same polymer concentration (10
g/L), PHBV fermentation yielded $0.16 \pm 0.03 \text{ g C}_{\text{VFAs}}/\text{g C}_{\text{material}}$ or $269.4 \pm 94.8 \text{ mg VFA COD-equiv./g material}$, while PHB fermentation yielded $0.18 \pm 0.02 \text{ g C}_{\text{VFAs}}/\text{g C}_{\text{material}}$ or $215.8 \pm 28.6 \text{ mg VFA COD-equiv./g material}$. The VFAs yields achieved were slightly lower at a
polymer concentration of 20 g/L regardless of the type of PHA, i.e., $0.10 \pm 0.01 \text{ g C}_{\text{VFAs}}/\text{g C}_{\text{material}}$ (or $190.4 \pm 15.9 \text{ mg VFA COD-equiv./g material}$) for PHBV and $0.13 \pm 0.02 \text{ g C}_{\text{VFAs}}/\text{g C}_{\text{material}}$ (or $237.2 \pm 39.4 \text{ mg VFA COD-equiv./g material}$) for PHB.
The attainable VFAs yield is greatly influenced by the type of substrate used as well as the
operational (e.g., volumetric organic loading rate, hydraulic retention time) and environmental
(e.g., pH, temperature) conditions at which the acidogenic fermentation process takes place.
The degree of acidification herein achieved (10–18%) was comparatively lower than the 40%
exhibited by readily fermentable feedstocks like the organic fraction of municipal solid waste
(OFMSW), cheese whey, and molasses, but similar to the 11–13% reached when using
glycerol, olive mill effluent and winery waste (Atasoy et al., 2018). Overall, despite the
promising results herein obtained, the recorded VFAs yields from PHAs need further
optimization.

Cellulose was used as a model substrate for the sake of comparison, which must
undergo hydrolysis and acidogenesis before any VFA can be produced. The use of cellulose
at an initial concentration of 10 g/L resulted in an average net VFAs production of 633 ± 134 mg HAc-equiv./L (or 776 ± 161 mg COD-equiv./L), yielding 0.06 g $C_{\text{VFA}}$/g $C_{\text{material}}$ or 77 mg VFA COD-equiv./g material. Such yields were comparable to those reached at a substrate concentration of 20 g/L, while the net VFAs production was approximately doubled when testing cellulose at 20 g/L (Figure 3). The VFAs yields derived from cellulose were comparatively lower than those attained for PHAs (Figure 3). One possible explanation for the lower VFAs yields recorded for cellulose could be associated to the fact that the relatively fast hydrolysis and acidogenesis of cellulose might inhibit not only acetogens and methanogens but also acid producers by sudden reduction in pH of the fermentation medium. Interestingly, a weak alkalinization with a rise in pH from 4.9 to 5.5 was observed at 10 g/L cellulose as a result of VFAs consumption (Figure 1h and Figure 2b), indicating somewhat relief of acid-induced inhibition. Figure 2 indeed showed that the pH of the assays containing cellulose always decreased at a higher rate compared to those with PHAs. It reinforces the hypothesis that depolymerization was the rate-limiting step in the PHA-to-VFAs bioconversion.

### 3.3 Effect of initial substrate concentration (F/M ratio) on biogas formation

In the quest of maximizing VFAs production from PHA, the degradation of VFAs via biogas formation must be prevented. In this study, natural acidification from neutral to slightly acid pH values (4–5) led to the accumulation of VFAs but did not halt biogas formation, indicating the need of pre-treating the inoculum to eliminate methanogens. As discussed in section 3.2, a low initial polymer concentration of 1 g/L enabled a balanced acidogenesis and methanogenesis. Under such a condition, major reducing equivalents were diverted toward biogas formation. About 80 ± 2, 79 ± 2 and 75 ± 1% of the total carbon initially contained in the material was transformed into gaseous carbon as $\text{CH}_4$ and $\text{CO}_2$ for PHBV, PHB and
cellulose, respectively (Figure 4a). Higher polymer loading for PLA, PCL and PBS did not change the insignificant biodegradation into VFAs.

At higher initial substrate concentrations, the generation of CH$_4$ and CO$_2$ as carbon sinks represented 27 ± 18 and 35 ± 28%, 26 ± 12 and 12 ± 4%, and 24 ± 5 and 8 ± 5% of the total initial carbon present in PHBV, PHB and cellulose at 10 and 20 g/L, respectively. The carbon estimated from the VFAs concentrations recorded at the end of the fermentations accounted for 16 ± 3 and 10 ± 1%, 18 ± 2 and 13 ± 2%, and 6 ± 1 and 6 ± 2% of the initial carbon present in PHBV, PHB and cellulose at 10 and 20 g/L, respectively (Figure 4b,c). Such carbon distributions suggested that a significant fraction of the test material remained non-biodegraded. Previous literature reports have shown that syntrophic and methanogenic populations are commonly impaired by VFAs accumulation along with low pHs in overloaded digesters (Braz et al., 2019; Basak et al., 2021). Yet, some communities such as hydrogenotrophic methanogens could withstand those adverse conditions (Taconi et al., 2008; Wang et al., 2020), explaining the biogas production and incomplete bioconversion of the test material observed.

TOC analyses of the supernatant of the culture broth were conducted at the end of fermentation as an attempt to investigate whether depolymerization and biotransformation of PHBV, PHB and cellulose would result in other organic intermediate compounds in addition to VFAs. The concentration of organic carbon determined by soluble TOC analysis (C-TOC) was then compared to the concentration of carbon equivalent calculated from the measured VFAs (C-VFAs) by a simple linear regression analysis. The correlation coefficient ($R^2$) between C-TOC and C-VFAs was 0.9823, which confirmed that VFAs overwhelmingly prevailed as metabolic intermediates during the acidogenic fermentation of the materials tested (Figure 5).
To the best of the authors' knowledge, the feasibility of producing VFAs from bioplastics has not been previously systematically investigated from a circular economy approach but reported as an anaerobic biodegradation pathway. Reischwitz et al. (1998) studied the anaerobic biodegradation of PHB and PHBV (19.1 mol% HV) at 1 g/L, 35 °C and pH 7.2, both in powdered form with a mean particle size of 9.8 and 46.4 μm, respectively, using a methanogenic sludge (at a F/M ratio of 4) derived from a sugar industry wastewater treatment plant. Incomplete mineralization (39–55%) was observed for both PHAs and the main organic acids detected were HAc, HBu and i-HBu for PHB, and HAc, HPr, HBu, i-HBu, and HVal for PHBV. In a second experiment, Reischwitz and co-workers (1998) evaluated the fermentation of 1 g/L PHBV (8.4 mol% HV) at 35 °C and pH 7.2 in the dark for 14 days using an enriched methanogenic culture (2% v/v) previously exposed to the polymer powder. Under such conditions of pre-conditioned sludge, the transformation of PHBV to HAc, n-HBu, HPr and n-HVal was reported to be 87%. The accumulation of VFAs was attributed to the high concentration of substrate employed in relation to the concentration of biomass inoculated, thus leading to the total inhibition of acetogens and methanogens. As an attempt to measure intermediate products in the culture supernatant, the authors also performed gas chromatography-mass spectrometry (GC-MS) analyses, particularly in a third set of experiments evaluating the anaerobic biodegradation of 10 g/L PHBV (8.4 mol% HV) with 10% (w/w) methanogenic sludge (0.25% VSS) at 35 °C and pH 7.2. The results of that study showed 3-hydroxybutyrate, 3-hydroxyvalerate and other four related dimeric esters as intermediates during hydrolysis. VFAs such as HAc, n-HBu, HPr and n-HVal were also produced during the early stage of biodegradation but were further degraded as biogas production proceeded.

In another study conducted by Wang et al. (2013), the anaerobic production of VFAs from a sludge with a high PHA content (116 mg/g VSS; 52.8% PHB and 41.8% PHV;
derived from an aerobic/extended-idle biological phosphorous removal process) was investigated in batch and long-term semi-continuous experiments at 21 °C and pH 10. This study revealed that intracellular PHAs were effective precursors to produce VFAs, which sustained a good anaerobic hydrolysis rate, even faster than that of protein and carbohydrates. *Clostridium* sp. and *Alkaliflexus imshenetskii* were the dominant bacterial species, as shown by PCR-DGGE analysis. The anaerobic sludge fermentation resulted in up to 304.6 and 143.4 mg VFA COD-equiv./g VSS under batch and semi-continuous operation, respectively, with HAc as the most abundant (> 44%) VFA under both feeding regimes, followed by n-HBu, HPr, HVal, i-HVal, and i-HBu, which is consistent with the VFAs production results herein reported.

### 3.4 Practical implications and targets for the future

The outcome of the present study provides the background for future works aiming at upcycling biodegradable bioplastics through the production of VFAs. VFA production from bioplastics is relevant since they serve as building blocks for the manufacture of valuable chemicals and biofuels (Sekoai et al., 2021; Varghese et al., 2022). HAc and HBu, the major acids produced in this study, have a market size and price of 14000–17000 kton/year and 400–800 €/ton and 90–105 kton/year and 1500–1650 €/ton, respectively (Atasoy et al., 2018). HAc is employed to produce polymers, adhesives, dyes, food additive, solvents, and other chemicals, while HBu can be applied as animal and human food additive, chemical intermediate, solvent, flavouring agent, among other applications (Atasoy et al., 2018). In addition, VFAs can be used to produce new biodegradable polymers (Kumar et al., 2019; Szacherska et al., 2021). PHAs are commercially produced by biosynthesis using renewable feedstocks and their global production capacity in 2021 accounted for 43560 tonnes (European Bioplastics, 2021). Owing to their biocompatibility and biodegradability, PHAs...
are used in various applications such as packaging, medical devices, agricultural films, among many others. Thus, the novel concept of bioplastics to VFAs herein proposed could help in fostering bioplastic circularity, thereby closing the recycling loop. In this regard, closing the loop is an urgent need for bioplastics as their current share is still limited.

Here, we systematically addressed the production of VFAs from bioplastics from an easily accessible anaerobic sludge. However, this study employed anaerobic sludge without any pre-treatment and the selective pressure that carbon overloading rendered in the acidogenic fermentation was not enough to completely stop the generation of biogas. Thus, further studies with pre-treated anaerobic sludge or other selective factor(s) inhibiting methanogenesis are needed to enhance the VFAs yield. Moreover, in-depth studies are also needed to shed light into the microbial communities involved and their associated metabolism including the enzymatic pathways. In this context, the development of robust, cost-effective, and efficient bioplastics pretreatments is also of utmost importance to improve their intrinsic low hydrolysis rate, which is obviously the rate-limiting step in the process. The cost-effective downstream (i.e., extraction and purification) of the produced VFAs also deserves attention in futures studies. This challenging task could be addressed via engineering novel selective membranes (Pervez et al., 2022). Further work should also evaluate the use of commercial products containing bioplastics, e.g., packaging material available for instance at supermarkets (Cucina et al. 2022). Finally, process automation to effectively control key operational variables such as pH or bioplastic load will certainly improve VFA yields.

4. Conclusions

The feasibility of producing VFAs from bioplastics via acidogenic fermentation was systematically explored for the first time. The results obtained confirmed the feasibility of bioconverting PHAs into VFAs, mainly into HAc and HBu. High polymer concentrations (10–20 g/L) boosted the accumulation of VFAs (up to 0.18 Cmol product/Cmol substrate) by
partially arrested methanogenesis due to overloading and further acidification broth. Conversely, no noticeable VFAs production was observed for PCL, PLA and PBS after 56-days of incubation, which remained non-biodegradable. Overall, the PHAs-to-VFAs bioconversion herein validated represents a promising breakthrough in the field of biotechnological upcycling of plastic waste.

Acknowledgments

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References


Figure captions:

**Figure 1.** Time course of the VFAs concentration for PHBV (a, b, c), PHB (d, e, f) and microcrystalline cellulose (g, h, i) at an initial concentration of 1 (a,d,g), 10 (b, e, h) and 20 (c, f, i) g/L. All data are mean and standard deviation values of triplicate fermentations (n = 3), except for PHBV at 10 and 20 g/L (n = 2) where one outlier fermentation was removed.

**Figure 2.** Time course of the culture broth pH at 1 g/L (a), 10 g/L (b) and 20 (c) g/L of initial substrate concentration. Error bars are the standard deviation values of triplicate fermentations (n = 3), except for PHBV at 10 and 20 g/L (n = 2), where one outlier fermentation was removed.

**Figure 3.** Effect of initial substrate concentration and material type on the net production of VFAs via acidogenic fermentation by non pre-treated anaerobic sludge. The yield of carbon as VFAs achieved in relation to the initial carbon contained in the material (in g $C_{VFAs}$/g $C_{material}$ or C mol product/C mol substrate) is shown above bars. All data are mean and standard deviation values of triplicate fermentations (n = 3), except for PHBV at 10 and 20 g/L initial concentration (n = 2) where one outlier was removed.

**Figure 4.** Time course of the biodegradation degree (ratio between the gaseous carbon measured as CO$_2$ and CH$_4$ and the carbon derived from material) at 1 (a), 10 (b) and 20 (c) g/L initial substrate concentration. Error bars are the standard deviation values of triplicate fermentations (n = 3), except for PHBV at 10 and 20 g/L initial concentration (n = 2) for which one outlier was removed.
Figure 5. Linear correlation between the average concentration of carbon equivalent of the VFAs (C-VFAs) determined by GC-FID and the average concentration of organic carbon determined by TOC analysis (C-TOC), both these measurements were performed at the end of the acidogenic fermentation.
Table 1. VFAs distribution at the end of the fermentation as a function of material type and initial substrate concentration.

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<td>20</td>
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<td></td>
<td>Avg.</td>
<td>Stand. dev.</td>
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Note: Values stand for the per cent (%) of the total VFAs concentration expressed in COD equivalents. All data are mean and standard deviation values of triplicate fermentations (n = 3), except for PHBV at 10 and 20 g/L initial concentration (n = 2), where data from one outlier test was removed.
Fig. 1
Fig. 2
Fig. 3
Fig. 4
Fig. 5

**CRediT author statement**

**Octavio García-Depraect:** Conceptualization, Methodology, Investigation, Writing – Original Draft. **Raquel Lebrero:** Conceptualization, Supervision, Project administration, Writing – review & editing. **Sara Rodríguez-Vega:** Investigation, Writing – review & editing. **Rosa Aragão Börner:** Conceptualization, Funding acquisition, Writing – review & editing. **Tim Börner:** Conceptualization, Funding acquisition, Writing – review & editing. **Raúl Muñoz:** Conceptualization, Funding acquisition, Project administration, Methodology, Supervision, Writing – review & editing.

**Declaration of interests**

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Graphical abstract:

Highlights:

- This study assessed the feasibility of producing VFAs from 5 commercial bioplastics
- Acidogenic fermentation of PHA by non-pretreated anaerobic sludge was achieved
- PCL, PLA and PBS did not support VFA production via microbial fermentation
- PHB and PHBV were selectively bioconverted towards acetate and butyrate
- First systematic validation of PHAs to VFAs as precursors of high-value products