

Supporting Information

Anaerobic Fermentation of Poly(3-Hydroxybutyrate-co-3-hydroxyvalerate) plasticized with Glycerol Trilevulinate into Volatile Fatty Acids

Elena Togliatti ^{a,b,1}, Yong Jin ^{c,1}, Luca Lenzi ^{b,d}, Davide Morselli ^{b,d}, Micaela Degli Esposti ^{b,d}, Paola Fabbri ^{b,d}, Daniel Milanese ^{a,b}, David P. B. T. B. Strik ^{c,*}, Corrado Sciancalepore ^{a,b,**}

a. Department of Systems and Industrial Technologies Engineering, Università di Parma, Parco Area delle Scienze 181/A, 43124 Parma, Italy

b. INSTM, National Interuniversity Consortium of Materials Science and Technology, Via G. Giusti 9, 50121 Firenze, Italia

c. Environmental Technology, Wageningen University & Research, 6708 WG Wageningen, The Netherlands

d. Department of Civil, Chemical, Environmental and Materials Engineering, Università di Bologna, Via Terracini 28, 40131 Bologna, Italy

1 Authors contributed equally

* Corresponding author at: Environmental Technology, Wageningen University & Research, 6708 WG Wageningen, The Netherlands

** Corresponding author at: Department of Engineering for Systems and Industrial Technologies, Università di Parma, Parco Area delle Scienze 181/A, Parma 43124, Italy.

Table S1. Twin screw extrusion conditions.

Head	Zone 6	Zone 5	Zone 4	Zone 3	Zone 2	Zone 1	Hopper	Screw speed
150±1 °C	158±1 °C	160±1 °C	162±1 °C	162±1 °C	155±1 °C	150±1 °C	40±1 °C	30 rpm

Table S2. Composition of the stock solutions used to prepare the nutrient medium for the mixed culture fermentation.

	Compound	Concentration (g/L)	Dilution factor
	NH ₄ H ₂ PO ₄	180	
Stock solution I – Minerals	MgCl ₂ · 6H ₂ O	16.5	50x
	MgSO ₄ · 7H ₂ O	10	
	CaCl ₂ · 2H ₂ O	10	
Stock solution II – Minerals	KCl	7.5	50x
	FeCl ₂ · 4H ₂ O	30	
Trace metals	MnCl ₂ · 4H ₂ O	0.6	2000x
	H ₃ BO ₄	6.0	
	CoCl ₂ · 6H ₂ O	4.0	
	CuCl ₂ · 2H ₂ O	0.2	
	NiCl ₂ · 6H ₂ O	0.4	
	ZnSO ₄ · 7H ₂ O	2.0	
	Na ₂ MoO ₄ · 4H ₂ O	0.6	
	Na ₂ SeO ₃	0.2	
	EDTA	12.4	
	Vitamins	Biotin	
Folic acid		0.005	
Pyridoxal-HCl		0.0025	
Lipoic acid		0.015	
Riboflavin		0.0125	
Thiamin-HCl		0.413	
Ca-D-panthotene		0.0125	
Cyanocobalamin		0.0125	
P-aminobenzoic acid		0.0125	
Nicotinic acid		0.0125	
Supplements	Yeast extract	0.1	
	KOH	4M	

Notes: After all trace metals were added, the pH in the trace metals stock solution was adjusted to 1.0 by adding 37% HCl.

Figure S1. Temperature and pressure profiles during the hydrothermal processing (HTP).

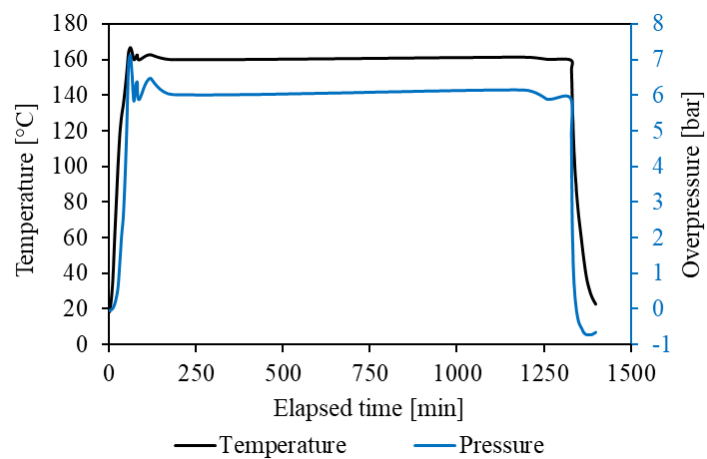


Figure S2. Full DSC scans of PHBV and plasticized PHBV

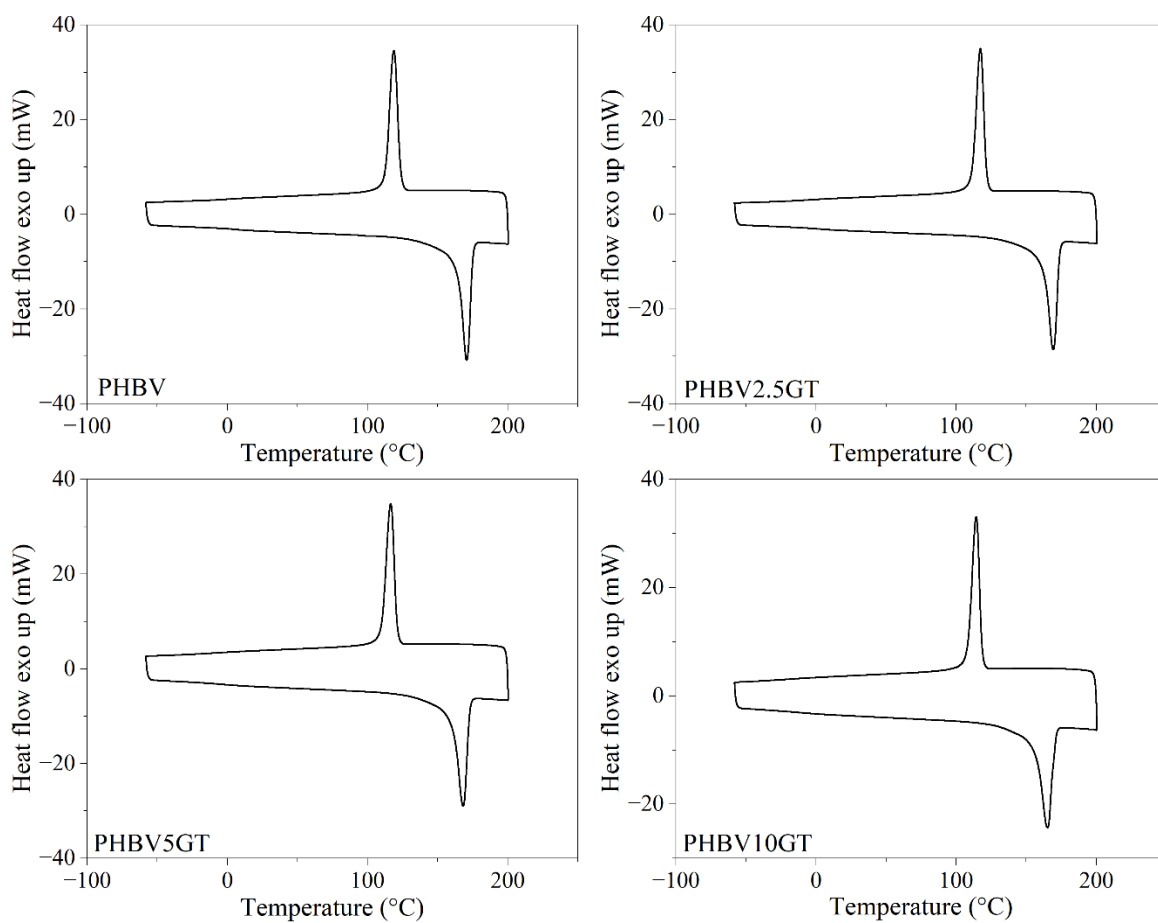
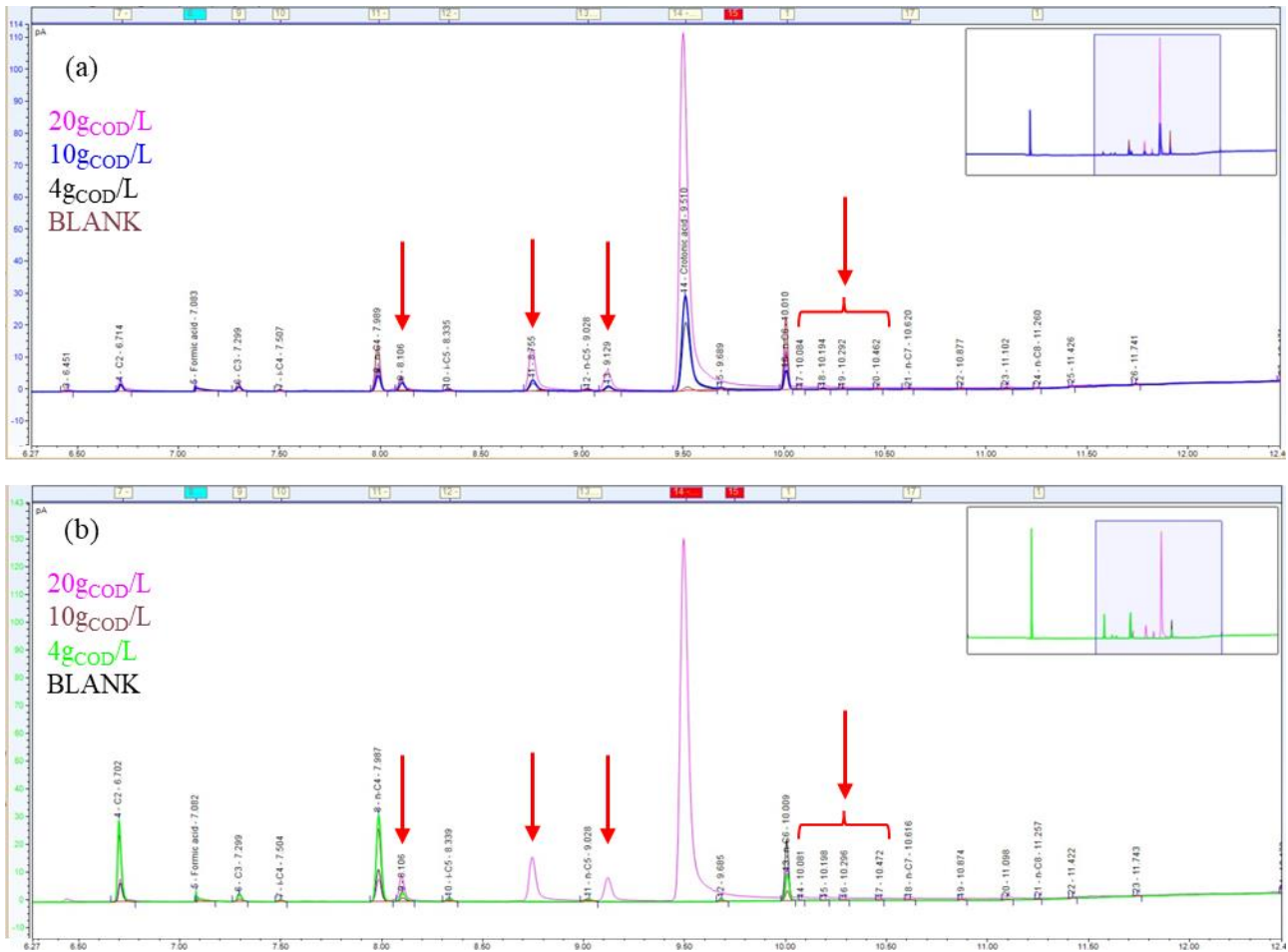


Table S3. Thermal properties extrapolated from DSC thermograms.

	T_g (°C)	T_m (°C)	ΔH_m (J/g)	T_c (°C)	ΔH_c (J/g)	χ_c (%)
PHBV	4.8 ± 0.7	170.7 ± 0.2	108.8 ± 2.5	118.7 ± 0.1	99.3 ± 2.7	75 ± 2
PHBV2.5GT	3.3 ± 0.7	169.2 ± 0.1	103.0 ± 3.5	117.2 ± 0.2	93.9 ± 2.9	72 ± 2
PHBV5GT	-1.6 ± 3.1	168.1 ± 0.2	105.9 ± 3.9	116.5 ± 0.1	96.2 ± 3.2	76 ± 3
PHBV10GT	-9.1 ± 1.1	165.1 ± 0.1	94.2 ± 5.0	114.2 ± 0.1	84.8 ± 4.7	72 ± 4

Figure S3. Chromatograms of the hydrolysate fermentation (HF) samples (a) before (day 0) and (b) after (day 15) the fermentation. Unidentified peaks are highlighted with red arrows.



Calculation S1. COD calculations for a) PHBV10GT supplied for the hydrothermal processing (HTP) and b) PHBV10GT supplied for the solids fermentation, as representative example.

The PHBV utilized in this work has a composition of 97% PHB and 3% PHV comonomers.

$$\text{COD}_{\text{PHB}} = 1.6744 \text{ g COD /g}_{\text{PHB}}$$

$$\text{COD}_{\text{PHV}} = 1.92 \text{ g COD /g}_{\text{PHV}}$$

$$\text{COD}_{\text{PHBV}} = 0.97 \cdot \text{COD}_{\text{PHB}} + 0.03 \cdot \text{COD}_{\text{PHV}} = 0.97 \cdot 1.6744 \text{ g COD/g}_{\text{PHB}} + 0.03 \cdot 1.92 \text{ g COD/g}_{\text{PHV}} = 1.6816 \text{ g COD/g}_{\text{PHBV}}$$

$$\text{COD}_{\text{GT}} = 1.9877 \text{ gCOD/g}_{\text{GT}}$$

- a) The supplied material for HTP was 15g of plasticized PHBV10GT: 13.5g are of PHBV and 1.5g are of glycerol trilevulate bio-plasticizer.

$$\text{COD}_{\text{PHBV10GT}} = 13.5\text{g} \cdot 1.6816 \text{ g COD/g}_{\text{PHBV}} + 1.5\text{g} \cdot 1.9877 \text{ g COD/g}_{\text{GT}} = 25.6815 \text{ g COD}_{\text{PHBV10GT}}$$

The concentration of the COD in the reactor volume is: $25.6815 \text{ g COD}_{\text{PHBV10GT}} / 0.7 \text{ L} = 36.6879 \text{ COD}_{\text{PHBV10GT}}/\text{L}$.

- b) The supplied material for SF was 2.5g of plasticized PHBV. In the case of PHBV10GT: 2.25g are of PHBV and 0.25g are of glycerol trilevulate bio-plasticizer.

$$\text{COD}_{\text{PHBV10GT}} = 2.25\text{g} \cdot 1.6816 \text{ g COD/g}_{\text{PHBV}} + 0.25\text{g} \cdot 1.9877 \text{ g COD/g}_{\text{GT}} = 4.2805 \text{ g COD}_{\text{PHBV10GT}}$$

The concentration of the COD in the reactor volume is: $4.2805 \text{ g COD}_{\text{PHBV10GT}} / 0.05 \text{ L} = 85.61 \text{ g COD}_{\text{PHBV10GT}}/\text{L}$.

Figure S4. Chromatograms of the solid fermentation (SF) samples at day 120. Unidentified peaks are highlighted with red arrows.

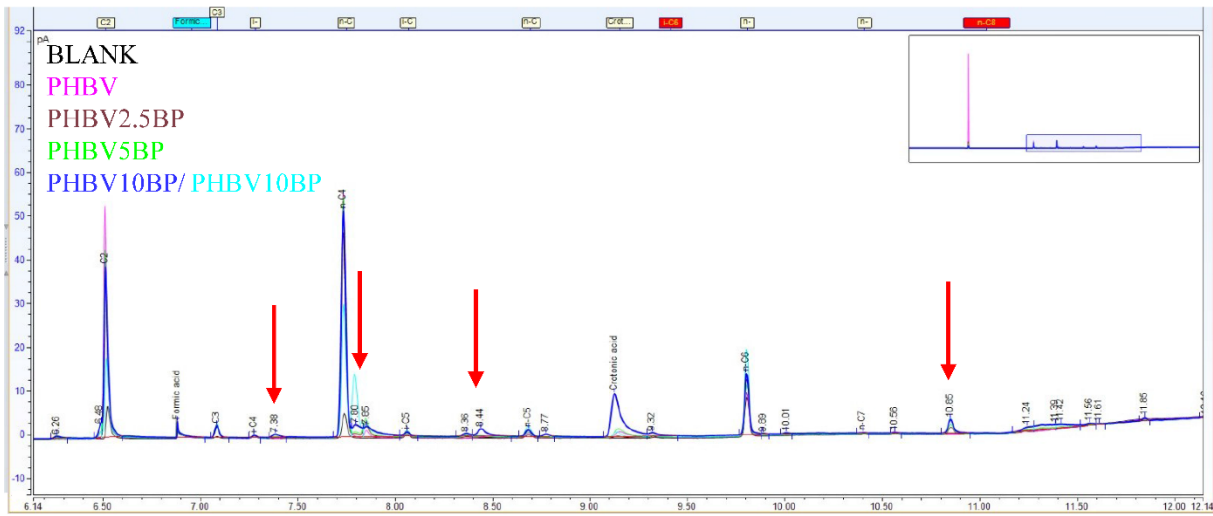
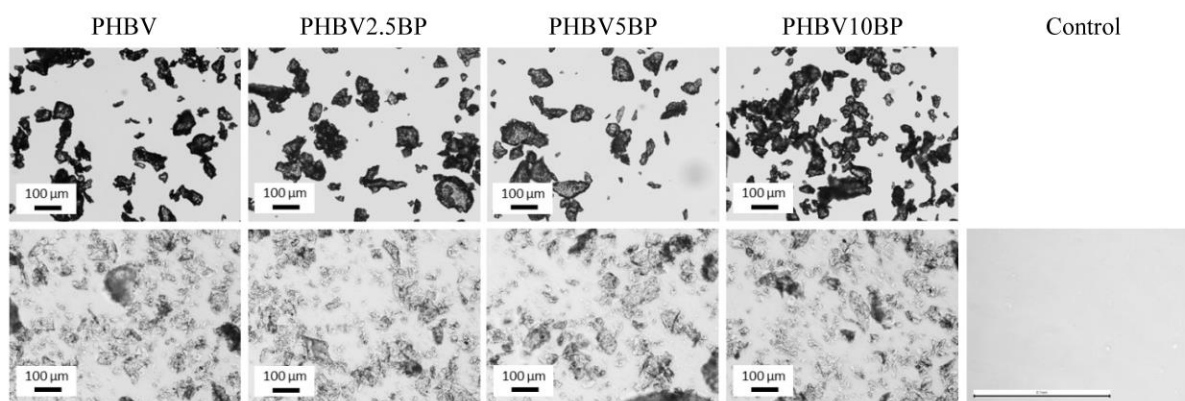


Figure S5. PHBV/GT solid particles not converted at the end of the fermentation process (day 120) as visible by eye.



Figure S6. Optical microscopy of the powder materials before (day 0, top line) and after (day 120, bottom line) the anaerobic fermentation (SF experiment).



The images displayed in the top row illustrate the dry, milled materials in their original state before their introduction in to the reactors. The bottom row depicts particles immersed in the reaction liquid at the end of the experiment. The alteration in opacity may be attributed to the different imaging conditions, with the top row images captured in the absence of a liquid medium, while the bottom row images depict particles within the fermentation liquid.