Supplementary material

Enhancing plastic waste recycling: Evaluating the impact of additives on the enzymatic polymer degradation

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Tables

Table S1. List of substances used in the experiments, abbreviation, CAS number, purity and supplier.

Substance	Abbreviation	CAS number	Purity (wt%)	Supplier
Pretreatment to remove the additive				
(R)-(+)-Limonene	---	5989-27-5	97.0	Sigma-Aldrich
1,2,3-Propanetriol or Glycerol	---	56-81-5	≥ 99.5	Biochem
Enzymatic degradation of HDPE				
Laccase from *Trametes versicolor*	---	80498-15-3	---	Sigma-Aldrich
2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)	ABTS	30931-67-0	98.0	Sigma-Aldrich
1-hydroxybenzotriazole	HBT	123333-53-3	98.0	Sigma-Aldrich
Citric acid 1-hydrate	---	5949-29-1	99.5	PanReac
di-Sodium hydrogen phosphate anhydrous	---	7558-79-4	99.0	PanReac
Cholinium dihydrogencitrate	[Ch][DHC]	77-91-8	99.0	Aldrich Chemistry
Xylitol	Xly	87-99-0	99.0	Acros Organics

Table S2. Composition of the different systems tested on approaches A, B and C of enzymatic depolymerization of HDPE in terms of the polymer used and the percentages of each solution (biocatalytic medium, enzyme, mediator and water) regarding the 100 % volume of the liquid phase.

System	Polymer	Biocatalytic medium (v/v)%	Enzyme (v/v)%	Mediator (v/v)%	Water (v/v)%
1	HDPE with additive	90.0 (Buffer)	--	--	10.0
2	HDPE with additive	90.0 (Buffer)	--	5.0 (ABTS)	5.0
3	HDPE with additive	90.0 (Buffer)	5.0	--	5.0
4	HDPE with additive	90.0 (Buffer)	5.0	5.0 (ABTS)	--
5	HDPE with additive	90.0 (DES)	--	--	10.0
6	HDPE with additive	90.0 (DES)	--	5.0 (ABTS)	5.0
7	HDPE with additive	90.0 (DES)	5.0	--	5.0
8	HDPE with additive	90.0 (DES)	5.0	5.0 (ABTS)	--
9	HDPE with additive	90.0 (Buffer)	5.0	5.0 (HBT)	--
10	HDPE without additive	90.0 (Buffer)	--	--	10.0
11	HDPE without additive	90.0 (Buffer)	5.0	--	5.0
12	HDPE without additive	90.0 (Buffer)	5.0	5.0	5.0

Table S3. Weight loss (%) and laccase's average relative activity (%) of the different systems tested on approaches A, B and C of the enzymatic depolymerization of HDPE (N.A. – not applicable).

Approach	System	Weight loss (%)	Laccase's average relative activity (%)
A – Biocatalytic medium	1	0.05 ± 0.02	N.A.
A – Biocatalytic medium	3	1.98 ± 0.10	58.22 ± 4.66
A – Biocatalytic medium	4	3.26 ± 0.15	71.79 ± 7.75
A – Biocatalytic medium	5	0.04 ± 0.01	N.A.
A – Biocatalytic medium	7	1.57 ± 0.09	2.45 ± 0.12
A – Biocatalytic medium	8	0.99 ± 0.05	4.79 ± 0.23
B – Mediators	1	0.03 ± 0.01	N.A.
B – Mediators	3	1.15 ± 0.05	55.50 ± 4.11
B – Mediators	4	3.06 ± 0.13	63.74 ± 5.29
B – Mediators	9	1.24 ± 0.05	31.20 ± 1.97
C – HDPE with and without additive	1	0.01 ± 0.01	N.A.
C – HDPE with and without additive	3	2.01 ± 0.10	52.62 ± 3.10
C – HDPE with and without additive	4	2.87 ± 0.16	64.10 ± 4.29
C – HDPE with and without additive	10	0.08 ± 0.02	N.A.
C – HDPE with and without additive	11	25.06 ± 1.08	46.51 ± 6.46
C – HDPE with and without additive	12	32.90 ± 1.58	59.60 ± 8.76

Table S4. Temperature onset (T_{onset}) and maximum degradation temperature (T_{max}) of the recovered HDPE from the control and from the systems treated with laccase, and with laccase and ABTS, of HDPE with additive and of HDPE without additive for comparison purposes.

Polymer	Approach	T_{onset} (ºC)		T_{max} (ºC)	
		Solvent's degradation	**HDPE's degradation**	**Solvent's degradation**	**HDPE's degradation**
HDPE with additive	--	--	446.97	--	474.99
HDPE without additive	--	45.46	450.23	199.35	477.28
	Control	124.99	454.23	137.50	476.70
	Laccase	124.04	463.65	137.89	474.50
	Laccase + ABTS	124.36	444.94	139.34	478.39

Figures

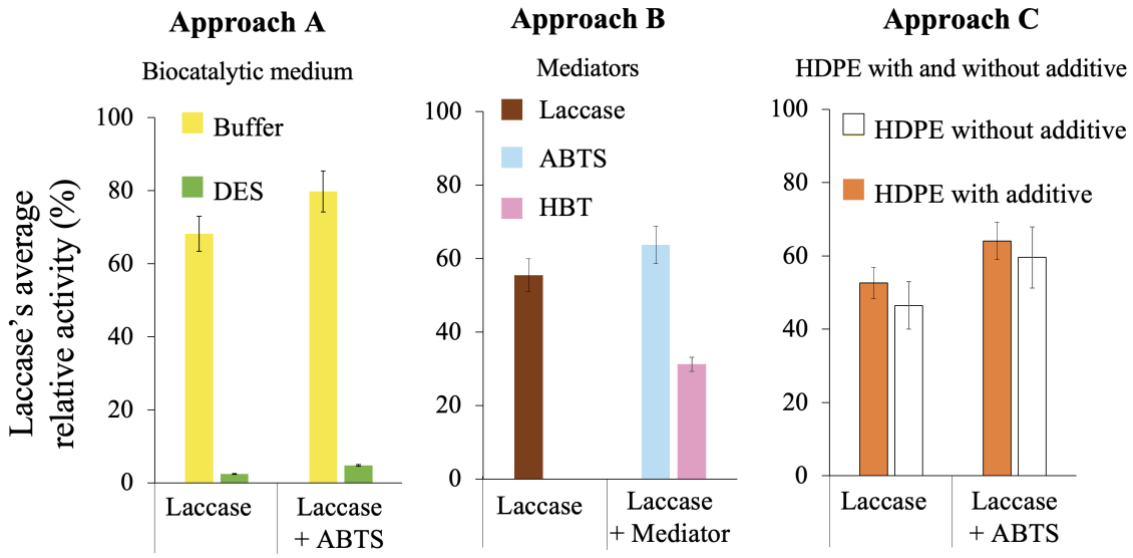

Figure S1. Average relative enzyme activity (%) in the different systems tested as a result of the different biocatalytic conditions on the enzymatic depolymerization of HDPE. Experiments conducted at 37°C and 100 rpm for 14 days with laccase from *T. versicolor*. In addition, approaches B and C used buffer as biocatalytic medium.

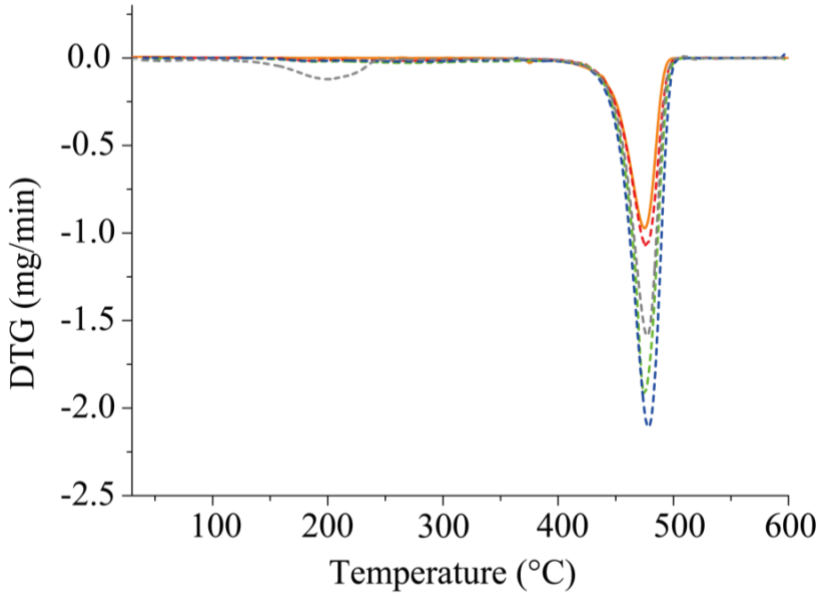

Figure S2. First derivative of thermogravimetric curves of the recovered samples of HDPE without additive followed by enzymatic degradation in different systems: (**red**) control; (**green**) laccase; (**blue**) laccase and ABTS. For comparison purposes the first derivative of thermogravimetric curves of (**orange**) HDPE with additive and (**grey**) HDPE without additive are included. For more details, see the Results section.

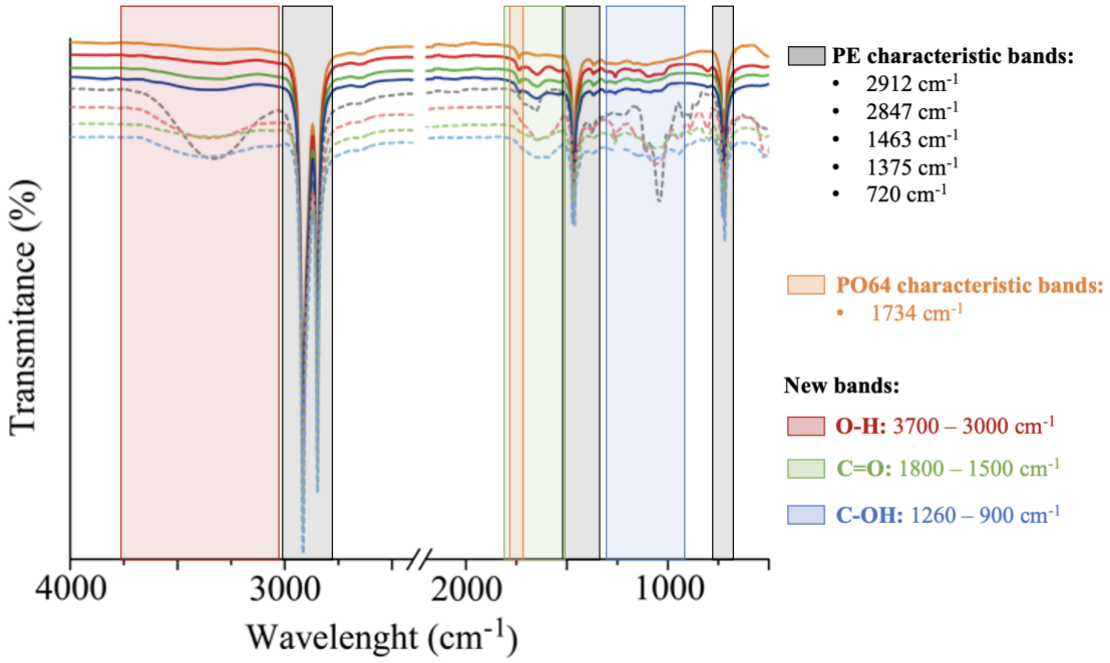

Figure S3. FTIR spectra of each system tested regarding approach C: (**Full line**) HDPE with additive and (**Dashed line**) HDPE without additive. FTIR spectra include: (**orange**) HDPE with additive; (**grey**) HDPE without additive; (**red**) control; (**green**) laccase; (**blue**) laccase and ABTS. All FTIR spectra have a gap between 2400 and 2200 cm^{-1}, which is due to the presence of a CO_2 band that is measured by instruments by default and is not relevant to this study. A band at 1734 cm^{-1} typical of the C=O associated with the orange pigment (PO64) for the colored samples is also visible.

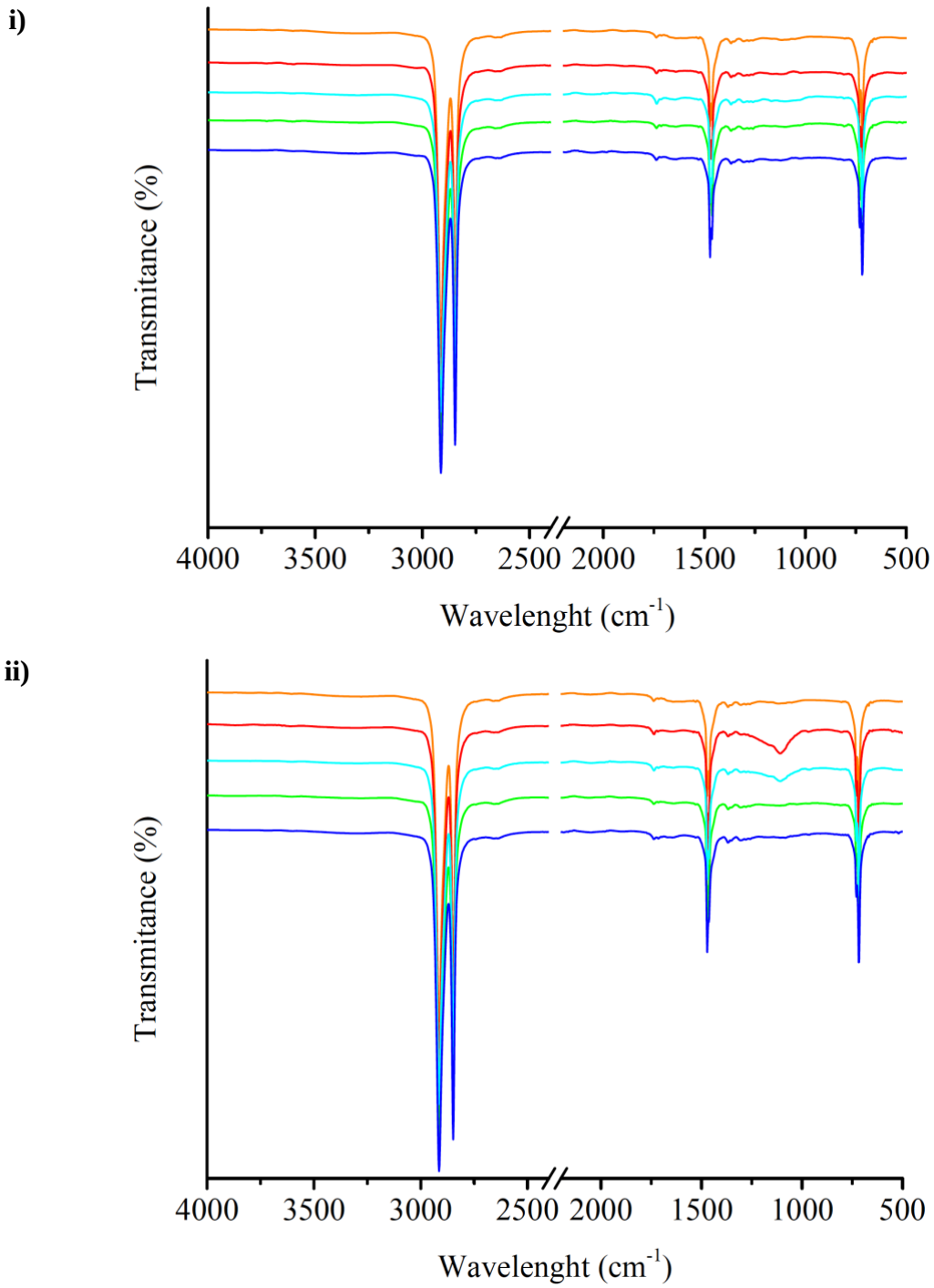

Figure S4. FTIR spectra of each system tested regarding approach A where **(i)** are the systems containing citrate-phosphate buffer pH 4.5 as the biocatalytic medium and **(ii)** are the ones with an aqueous mixture of DES [Ch][DHC]:Xly as the biocatalytic medium. (**orange**) HDPE with additive; (**red**) biocatalytic medium only (control); (**cyan**) biocatalytic medium and ABTS (control); (**green**) laccase; (**blue**) laccase and ABTS. All FTIR spectra have a gap between 2400 and 2200 cm^{-1}, which is due to the presence of a CO_2 band that is measured by instruments by default and is not relevant to this study.

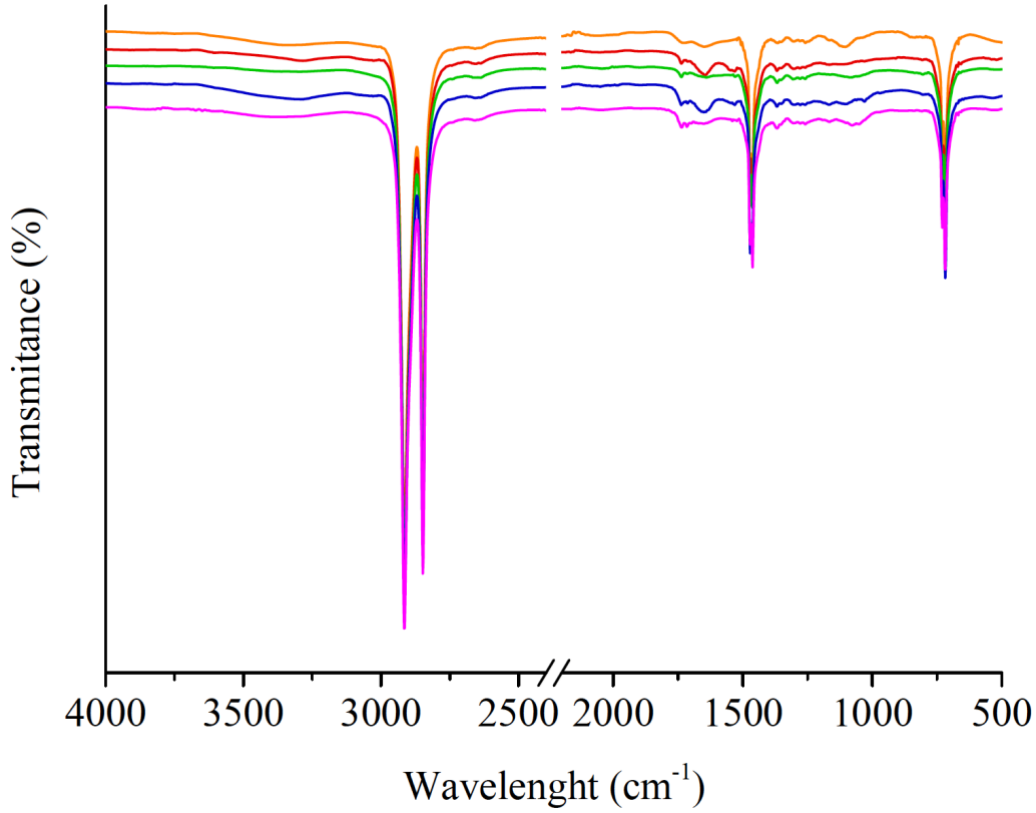

Figure S5. FTIR spectra of each system tested regarding approach B. (**orange**) HDPE with additive; (**red**) control; (**green**) laccase; (**blue**) laccase and ABTS; (**magenta**) laccase and HBT. All FTIR spectra have a gap between 2400 and 2200 cm^{-1}, which is due to the presence of a CO_2 band that is measured by instruments by default and is not relevant to this study.

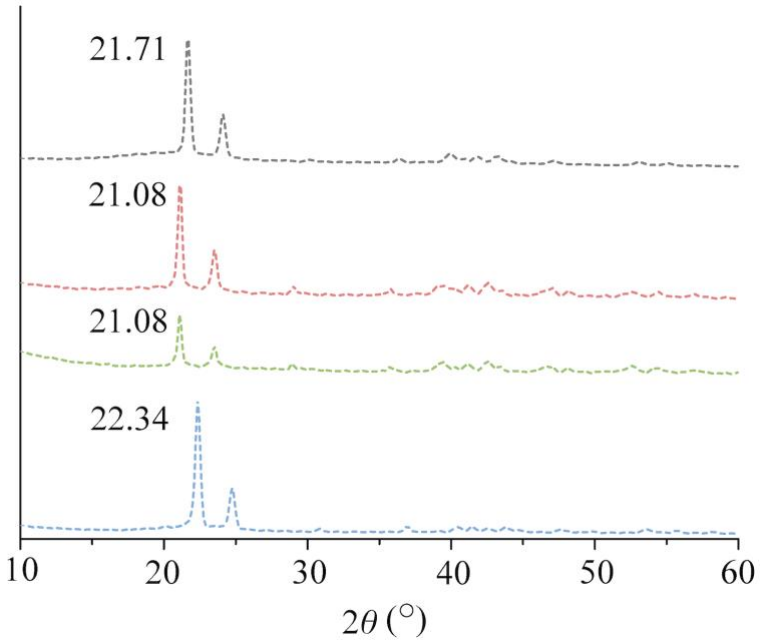

Figure S6. X-Ray Diffraction results of the HDPE recovered from the systems with HDPE without additive: (**red**) control, treated with (**green**) laccase, treated with (**blue**) laccase and ABTS, and of (**grey**) HDPE without additive, for comparison, and their diffraction angles of the maximum peaks defined.