Enzymatic degradation of a commercial bio-based polyester and evaluation of the mechanism involved

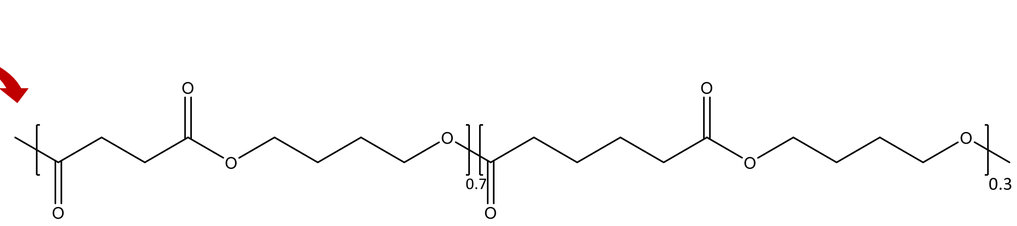
TERMINUS — in-built Triggered Enzymes to Recycle Multi-layers: an Innovation for Uses in plastic-packaging

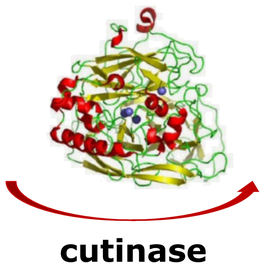
Angela Romano, Antonella Rosato, Grazia Totaro, Annamaria Celli, Giulio Zanaroli, Laura Sisti

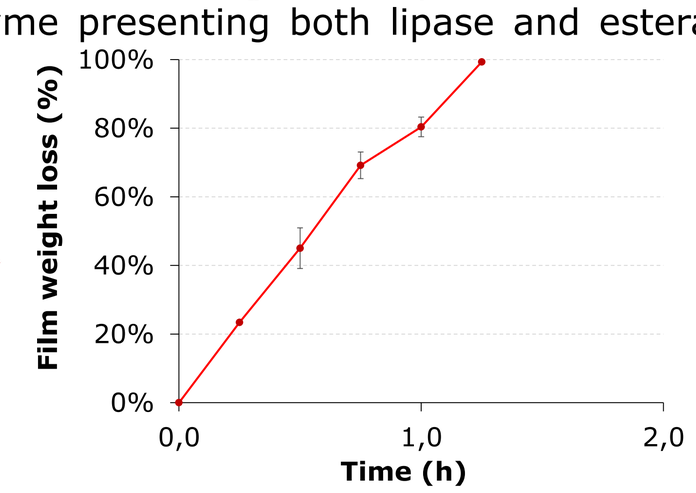
Dept. of Civil, Chemical, Environmental and Materials Engineering, Alma Mater Studiorum - University of Bologna

angela.romano6@unibo.it

Aliphatic polyesters are the most promising biodegradable plastics because of their high susceptibility to the attack of hydrolytic enzymes and of many microorganisms naturally occurring in the environment. Poly(butylene succinate-*co*-adipate) (PBSA) is a bio-based semicrystalline copolymer of poly(butylene succinate) (PBS), with adipic acid as co-monomer. It is highly biodegradable because of its lower crystallinity and higher flexibility of polymer chains compared to PBS. Its biodegradability mechanism was evaluated using cutinase from *Humicola insolens*, which is a commercial hydrolytic enzyme presenting both lipase and esterase features.



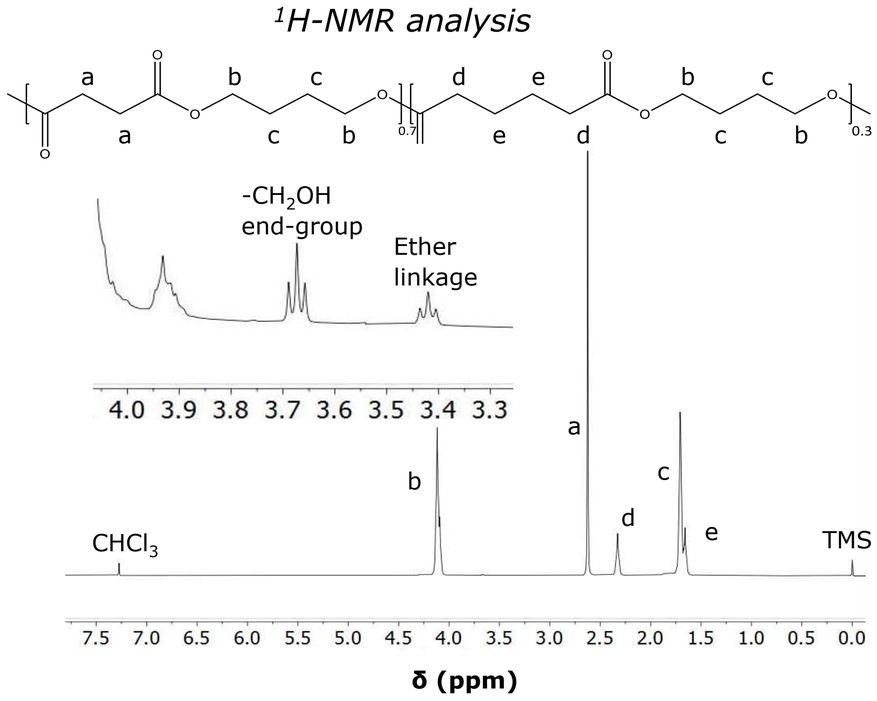




PROPERTIES OF RESIDUAL PBSA FILM AFTER ENZYMATIC INCUBATION

Molecular characterization

^{1}H-NMR analysis



- **^{1}H-NMR spectra** of degraded films did not show significant differences compared to pristine PBSA. Alcoholic end-groups increase over time.
- **GPC characterization**: M_n values decreased of about 20% in the early stages of incubation but successively, no further significant changes were detected.

			^{1}H-NMR	Gel permeation cromatography (GPC)		
	Time (h)	**Weight loss (%)**	**OH end-groups (mol%)**	**M_n (x10^3 g/mol)**	**M_w (x10^3 g/mol)**	**PD**
PBSA	-	-	1.1	81	195	2.4
PBSA-Cutinase	0.5	45	1.6	68	166	2.4
	1	80	2.2	72	177	2.5

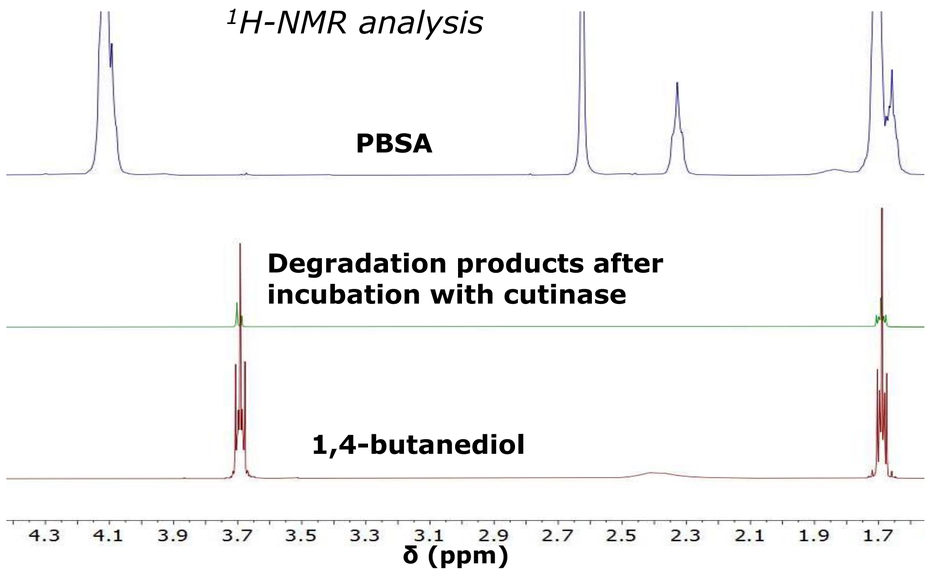
		Cooling scan		Heating scan			
	Time (h)	**T_c (°C)**	**ΔH_c (J/g)**	**T_g (°C)**	**T_m (°C)**	**ΔH_m (J/g)**	**X_c (%)**
PBSA	-	43	39	-45	86	37	32
PBSA-Cutinase	0.5	46	36	-48	85	33	28
	1	39	39	-48	85	37	32

Thermal characterization

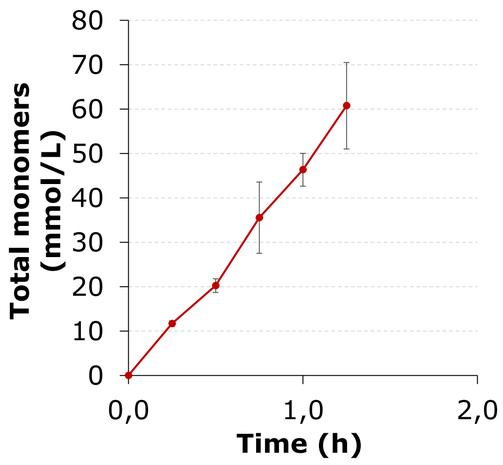
- Thermal properties of residual film samples remain fairly constant. A small change can be observed only in crystallization temperature T_c, and T_g values, due to the molecular weight reduction.
- The non perfect linear trend between the 0,5 and 1h results can be related to the fact that each characterization value is obtained from different sacrificial film samples, characterized by small variation in thickness and surface area.

ANALYSIS OF RELEASED DEGRADATION PRODUCTS

^{1}H-NMR analysis



HPLC Analysis



- All monomers, detected by HPLC analysis (succinic acid, adipic acid and 1,4-butanediol) were released over time during degradation.
- A large excess of 1,4-butanediol and traces of oligomers were detected by ^{1}H-NMR analysis.
- Cutinase acted mainly as an exo-type enzyme, cleaving the ester bonds mainly from the end of the polymeric chains.

CONCLUSIONS

Enzymatic degradation mechanism of cutinase proceeded by degrading the polymer from the surface of the film. An exo-type cleaving action mode was proposed based on the identification of the oligomers and monomers released as degradation products.

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