Supplementary material

Production of PETase by engineered *Yarrowia lipolytica* for efficient poly(ethylene terephthalate) biodegradation

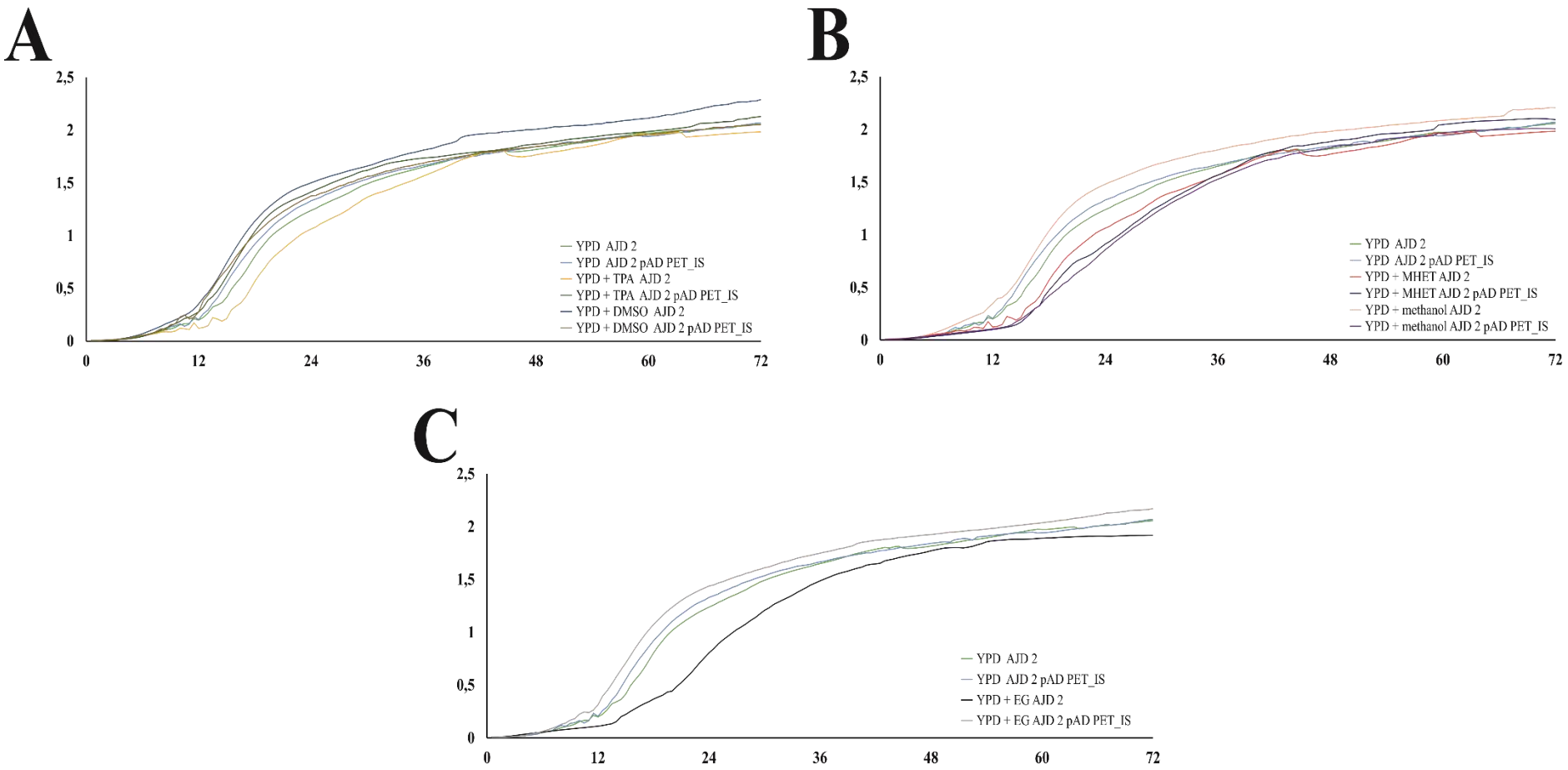
Katarzyna E. Kosiorowska[1], Antonio David Moreno García[2], Raquel Iglesias[2], Karol Leluk[3], Aleksandra M. Mirończuk[1]*

[1]Wrocław University of Environmental and Life Sciences, Department of Biotechnology and Food Microbiology, Chełmońskiego 37, 51-630, Wrocław, Poland

[2]Advanced Biofuels and Bioproducts Unit, Department of Energy, Research Centre for Energy, Environment and Technology (CIEMAT), Avda. Complutense 40, 28040 Madrid, Spain

[3]Wroclaw University of Science and Technology, Faculty of Environmental Engineering, Wybrzeże Wyspiańskiego 27, 50-370, Wrocław, Poland

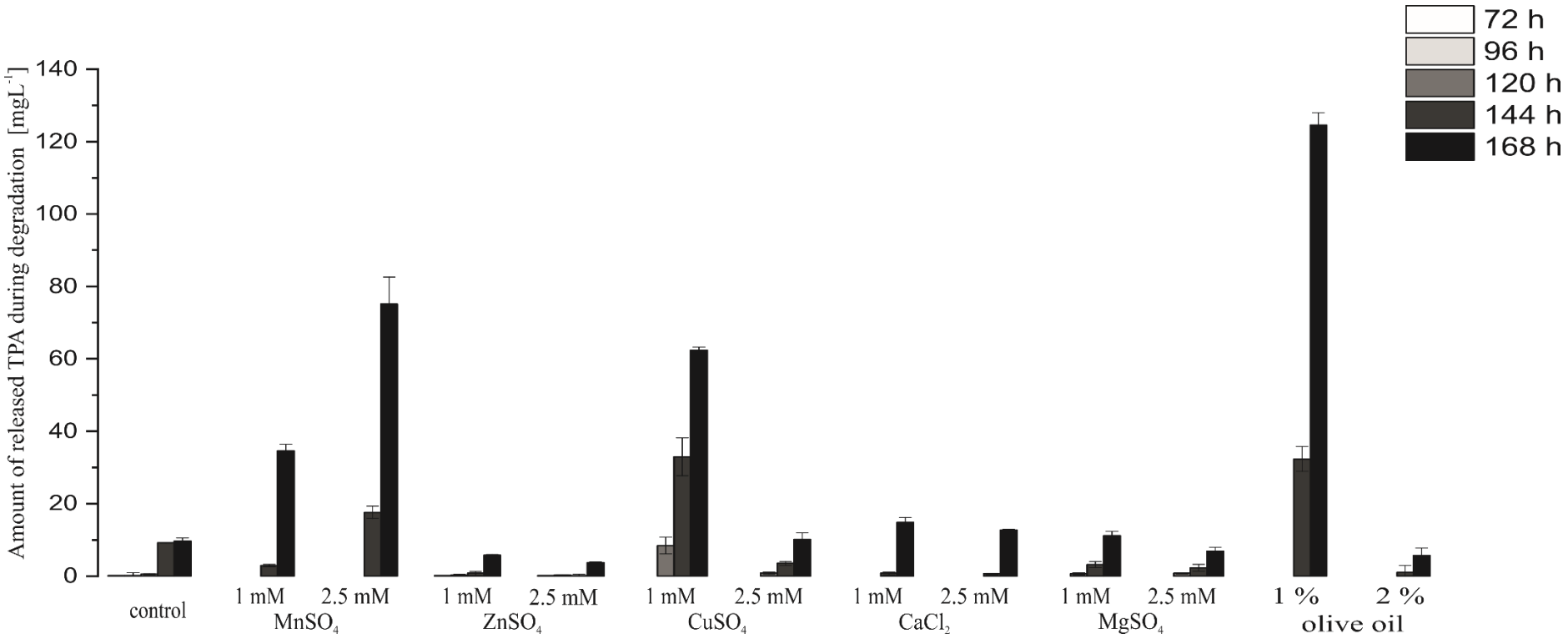
*Corresponding author: aleksandra.mironczuk@upwr.edu.pl



Supplementary Fig. 1

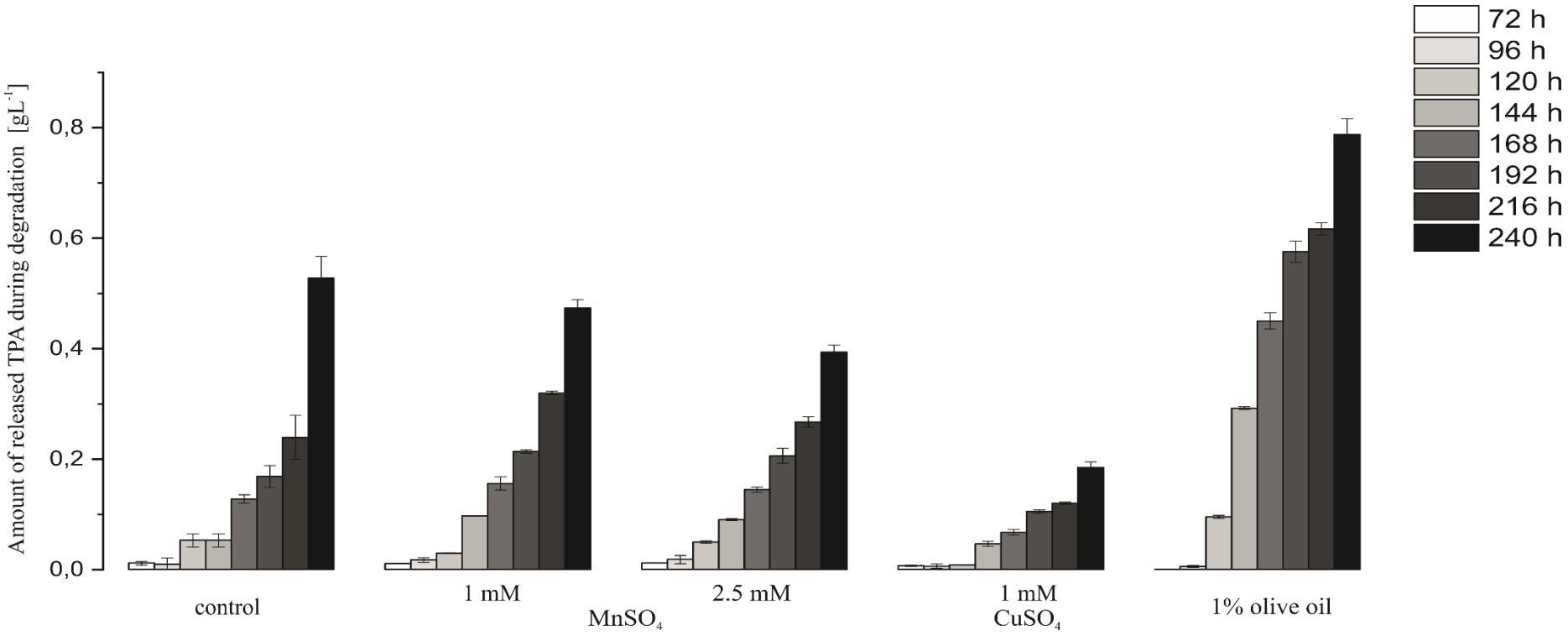
Effect of hydrolysis products and its solvents to *Y. lipolytica* growth.

A. YPD medium containing TPA and DMSO solvent.
B. YPD medium containing MHET and methanol solvent
C. YPD medium with EG.



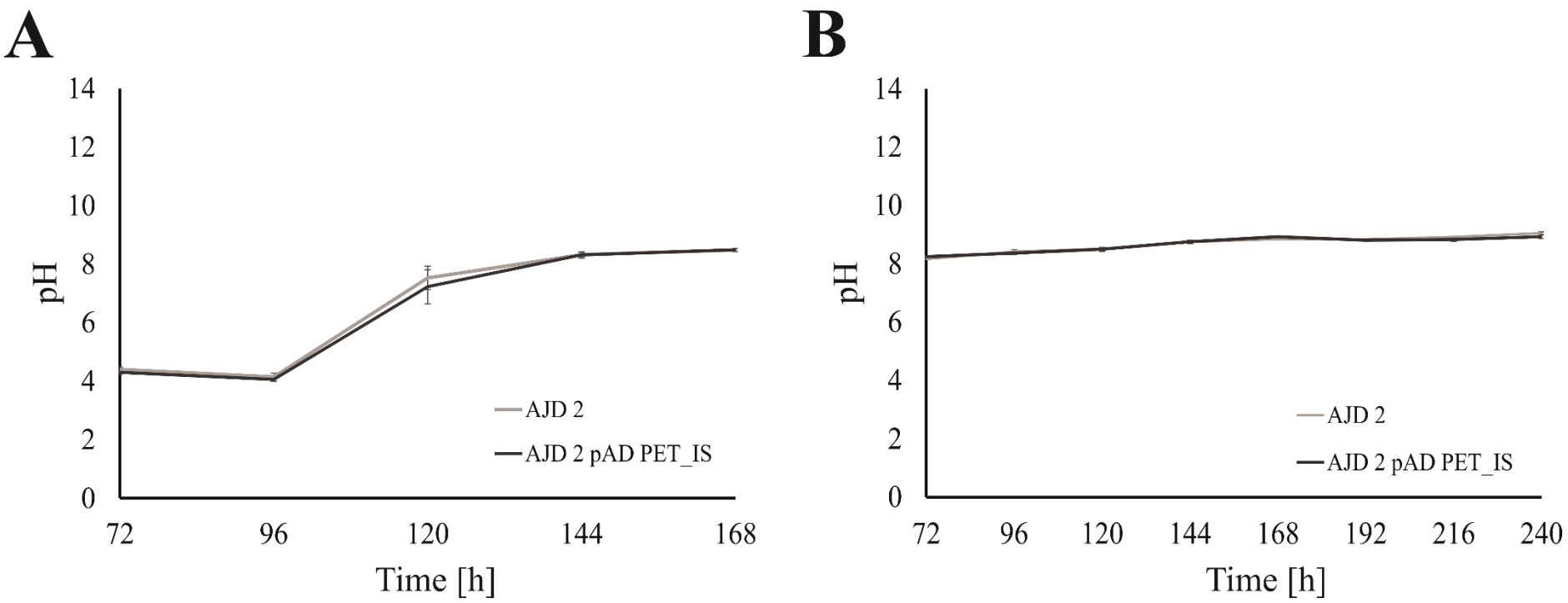
Supplementary Fig 2.

Daily increase of hydrolysis product measured in the supernatants during cultivation of AJD 2 pAD PET_IS in deep well plates.



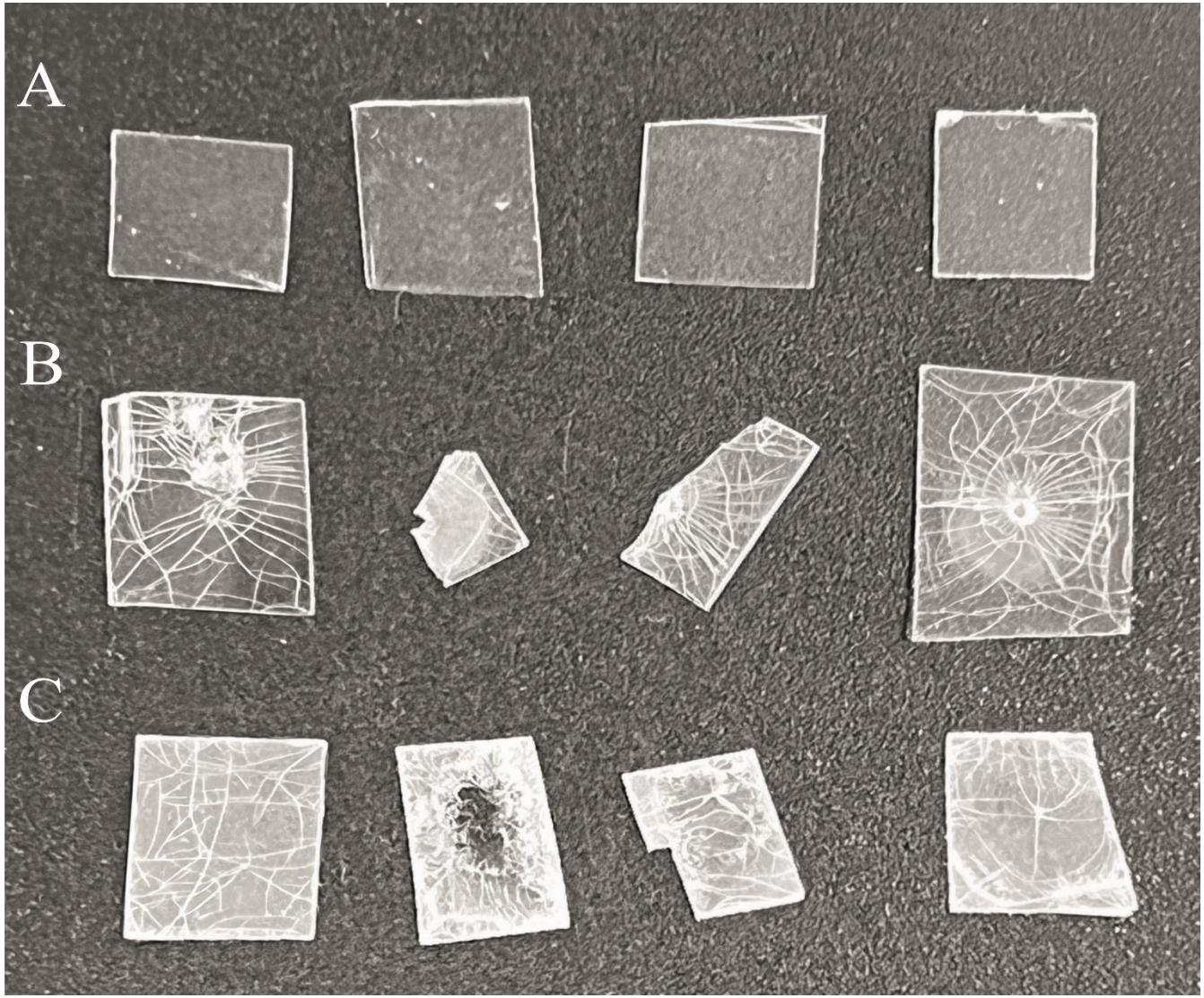
Supplementary Fig. 3

Daily increase of the PET hydrolysis product in AJD 2 pAD PET_IS culture carried out in 0.3-L Erlenmeyer flasks.



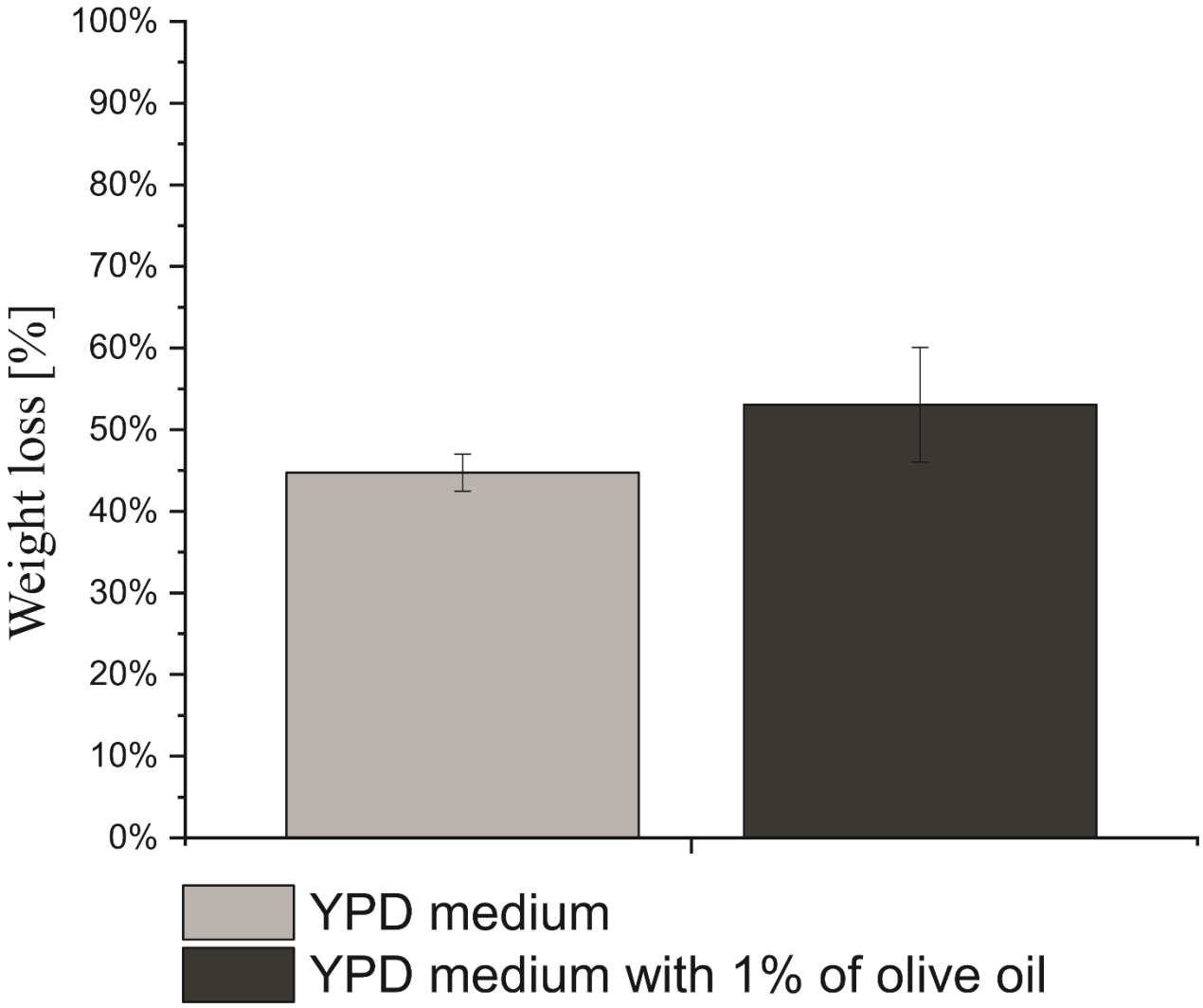
Supplementary Fig. 4

pH changes during AJD 2 and AJD 2 pAD PET_FS culture carried out in DPW (A) and 0.3 L Erlenmeyer shake flasks (B) in YPD medium containing 50 gL^{-1} of glucose and 0.266 g (A) or 2g (B) of PET powder. Error bars represent standard deviation



Supplementary Fig. 5

PET films images derived after culture of AJD 2 (A) in YPD media and AJD 2 pAD PET_IS cultivated in YPD media without (B) or with olive oil supplementation (C).



Supplementary Fig. 6

Estimated weight loss of PET film after long-term cultivation with *Y. lipolytica* AJD 2 pAD PET_IS.

Supplementary Table 1

Comparison of the amount of released PET hydrolysis products by various hydrolases and its mutants.

Host-organism	Enzyme	Substrate	Degradation conditions	Degradation time	Amount of PET degradation products released	References
Yarrowia lipolytica	PETase	Amorphous PET powder	Degradation during cultivation at 28 ºC at 200 RPM in YPD medium	240 h	TPA: 0.53 gL^{-1}	This study
			Degradation during cultivation at 28 ºC at 200 RPM in YPD medium supplemented with olive oil		TPA: 0.88 gL^{-1}	
Ideonella sakaiensis	PETase	PET film	Incubation with 50 nM of the purified enzyme at 30 º at pH 7.0	18 h	TPA: 0.09 mM MHET: 0.2 mM	(Yoshida et al., 2016)
		Highly crystallized PET			TPA: 0.007 mM MHET: 0.012 mM	
Escherichia coli	PETase	PET film	Incubation with 50 nM of the purified enzyme at 30 ºC at pH 9.0	144 h	TPA + MHET: 0.12 mM	(Chen et al., 2021)
			Incubation with 50 nM of the purified enzyme at 40 ºC at pH 9.0		TPA + MHET: 0.13 mM	
Escherichia coli	PETase	PET powder	Incubation with crude enzyme supernatant at 30 ºC at pH 9.0	18 h	TPA: 350 µM MHET: 160 µM	(Shi et al., 2021)
				24 h	TPA:360 µM MHET: 220 µM	
				48h	TPA: 400 µM MHET: 130 µM	

Escherichia coli	PETase	Commercial PET film	Incubation with the purified enzyme at 30 ºC at pH 9.0	72 h	TPA: 2.4 μM MHET: 9.1 μM	(Son et al., 2019)
			Incubation with the purified enzyme at 40 ºC at pH 9.0		TPA: 3.3 μM MHET: 5.4 μM	
Escherichia coli	PETase	PET film	Incubation with 5 μg of the purified enzyme at 30 ºC at pH 8.5	48 h	TPA: 2.5 mM	(Ma et al., 2018)
Chlamydomonas reinhardtii	PETase	PET powder	Incubation of 30 mg PET powder with the cell lysate	4 weeks	TPA: 9.12 mg	(Kim et al., 2020)
Fusarium solani	Cutinase	PET yarn	Incubation with 80 U crude enzyme with 2 gL^{-1} of substrate at 30 °C in pH 7.0	168 h	TPA: 9 μgmL^{-1}	(Nimchua et al., 2007)
Fusarium oxysporum					TPA: 16 μgmL^{-1}	
Thermobifida cellulosilytica Thc_Cut1	Cutinase	PET film	Incubation with 200 μgmL^{-1} of purified enzyme with PET film previously washed in Triton X-100 at 50 °C	48 h	TPA: 260 μM MHET: 20 μM	(Acero et al., 2013)
Thermobifida cellulosilytica Thc_Cut2					TPA: 85 μM MHET: 80 μM	
Humicola insolens	Cutinase	Post-consumer PET	Incubation with 1.0 $mg_{protein}mL^{-1}$ of purified enzyme at 70 °C in pH 7.0	96 h	TPA+MHET+BHET: 129 mM	(Eugenio et al., 2021)

Leaf-brach compost	LCC cutinase	PET film	Incubation of purified enzyme at 70 °C at pH 8.0	24 h	Data not shown	(Sulaiman et al., 2014)
Engineered *Pichia pastoris (Komagataella phaffii)*	LCC cutinase	PET film	Incubation with 1 μM purified enzyme at 50 °C in pH 8.0	48 h	Data not shown	(Shirke et al., 2018)
Microbacterium oleivorans	Synergic action of cutinase and other hydrolases	PET film	Combined treatment of *M. oleivorans* JWG-G2 at 5× 103 μLcm^{-2} and 120 μgmg^{-1} of cutinase from *T. Fusca* at 35 °C with constant agitation of 120 RPM	15 h	TPA: 47 nM MHET: 330 nM	(Yan et al., 2021)
Thermobifida fusca						
Engineered *Escherichia coli*	PETase $^{W159H/F229Y}$	PET tablets from bottle	Incubation with14 $mgmL^{-1}$ of purified engineered protein were compared to native enzyme at 40 °C	24 h	Data not shown	(Meng et al., 2021)
Engineered *Escherichia coli*	PETase	PET film	Incubation with 50 nM of purified PETase protein and its conjugates with various monomers at 40 °C in pH 9.0	4 days	TPA+MHET: 36.7	(Chen et al., 2021)
Conjugates	TBMA[1]-PETase				TPA+MHET: 82.5 μM	
	HEMA[2]-PETase				TPA+MHET: 55.0 μM	
	DMAEMA[3]-PETase				TPA+MHET: 77.5 μM	
	MA[4]-PETase				TPA+MHET: 46.7 μM	

[1] TBMA: tert-Butyl Methacrylate
[2] HEMA: Hydroxyethyl Methacrylate
[3] DMAEMA: 2-(dimethylamino)ethyl Methacrylate
[4] MA: Methacrylic acid

Engineered *Escherichia coli*	DuraPETase[5]	PET film	Incubation with 0.5 $mgmL^{-1}$ of purified engineered enzyme at 37 °C in pH 9.0	10 days	TPA+MHET+BHET: 3.1 mM	(Cui et al., 2021)
Engineered *Escherichia coli*	DuraPETase-4M[6]	PET powder	Incubation with 0.01 $mgmL^{-1}$ of purified enzyme at 60 °C in pH 9.0	96 h	TPA+MHET+BHET: 15.8 mM	(Liu et al., 2022)
		preformed PET film from PET powder			Data not shown	
Engineered *Escherichia coli*	LCC cutinase WCCG[7]	Post-consumer PET waste	Incubation with 1 $mg_{enzyme}g_{PET}^{-1}$ at 72 °C in pH 8.0	10.5 h	Data not shown	(Tournier et al., 2020)
	LCC cutinase ICCG[8]			9.3 h	Maximum productivity: 42.1 $g_{TPA}\ l^{-1}\ h^{-1}$	

[5] DuraPETase: *Is*PETase$^{S214H/I168R/W159H/S188Q/R280A/A180I/G165A/Q119Y/L117F/T140D}$
[6] DuraPETase-4M: DuraPETase$^{N233C/S282C/H214S/S245R}$
[7] WCCG: LCC$^{F243I/D238C/S283C/\ Y127G}$
[8] ICCG: LCC$^{F243W/D238C/\ S283C/Y127G}$