Streamlining biological recycling of poly(ethylene terephthalate) via pre-treatment methods

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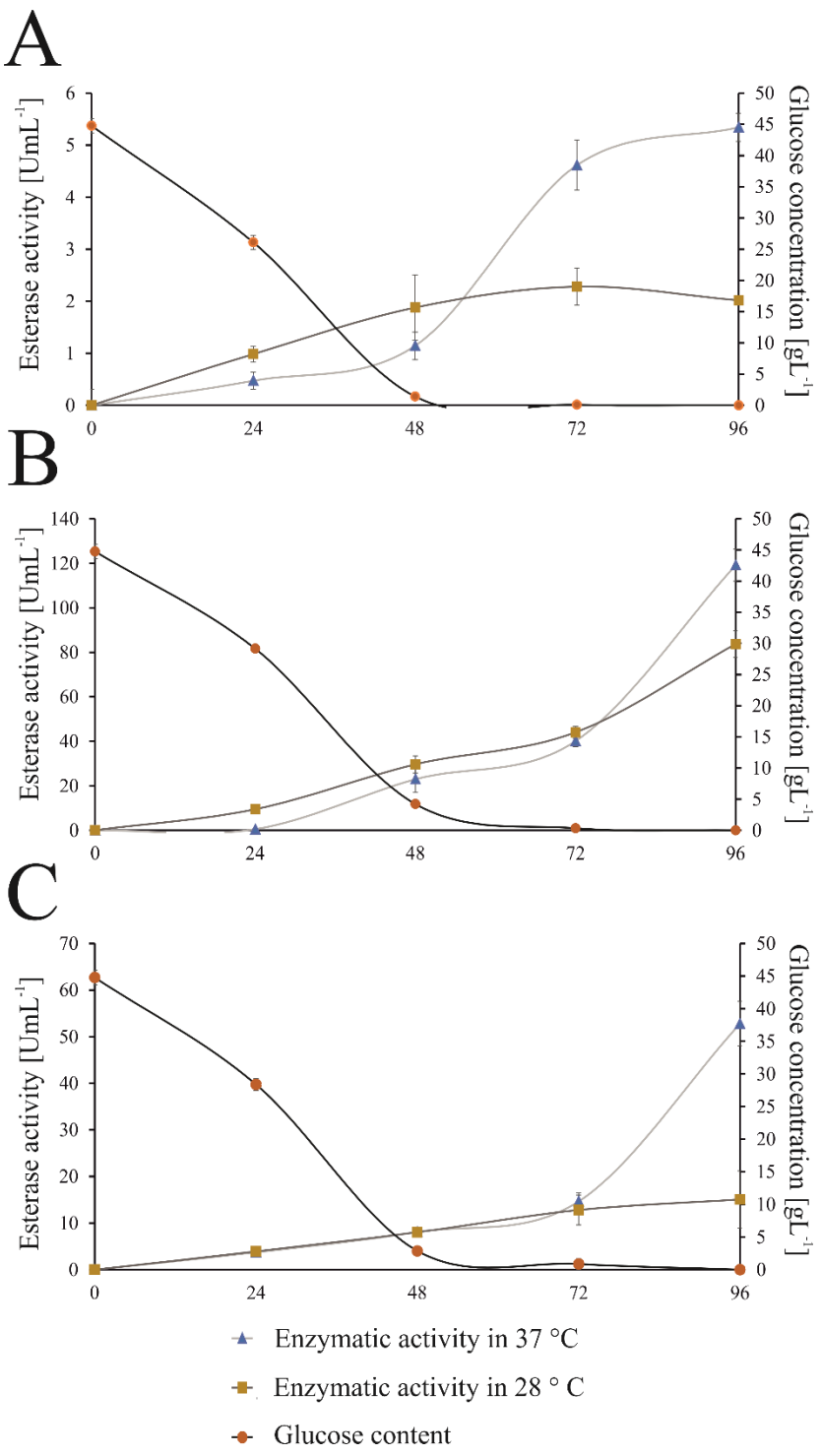
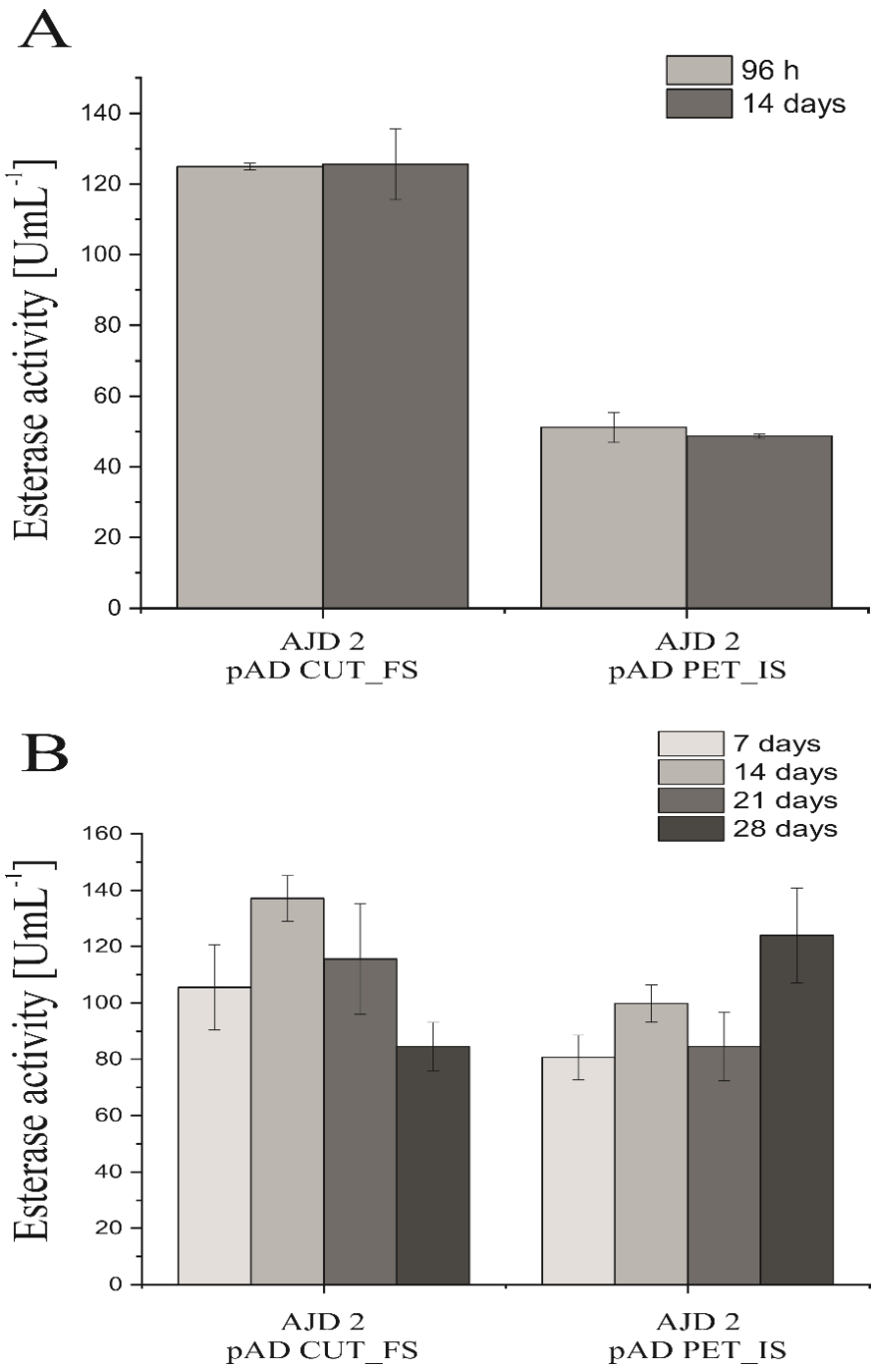


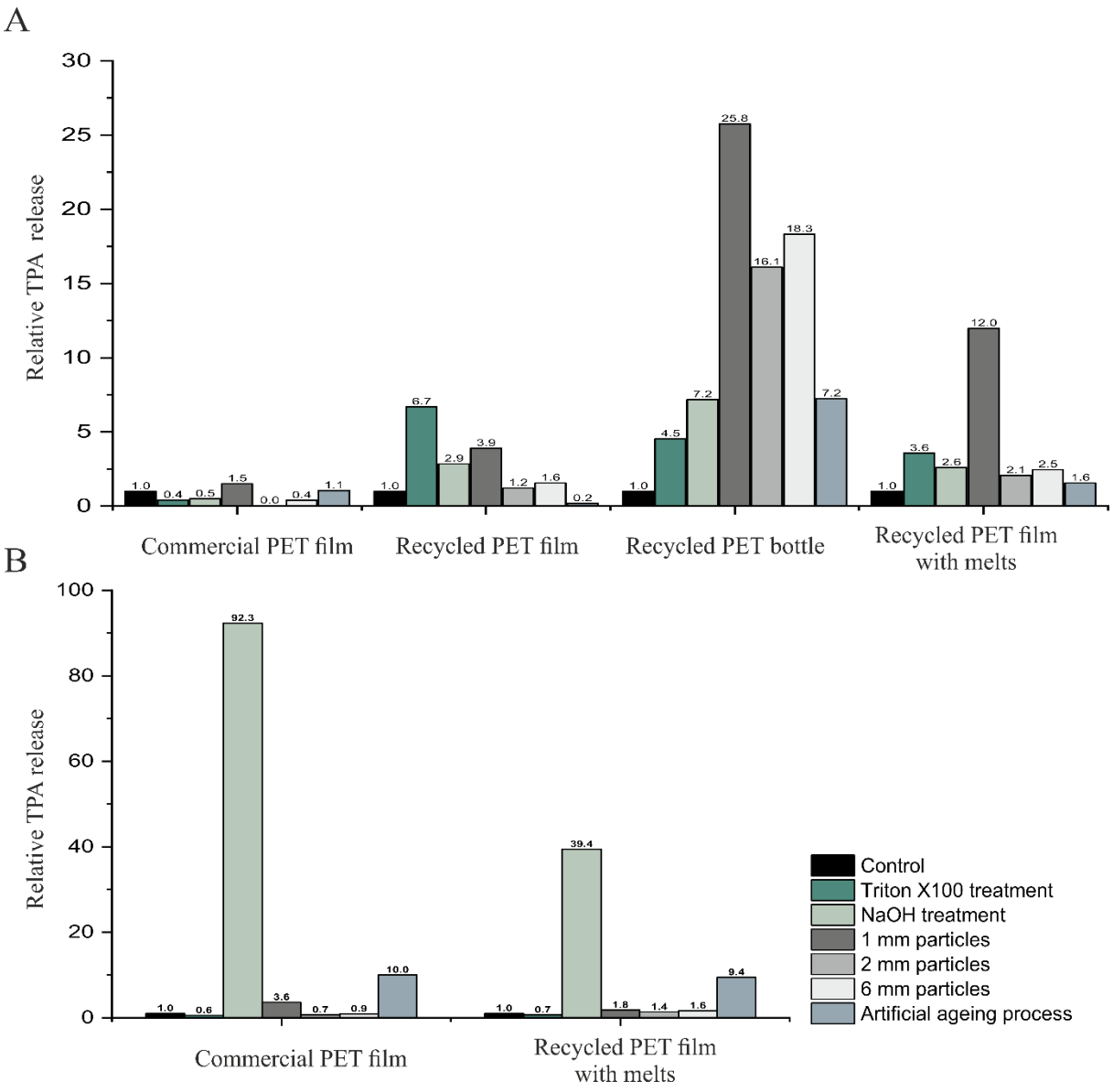
Supplementary Fig. 1

Correlation of the esterase activity in the supernatant and glucose consumption by strain AJD 2 (A), AJD 2 pAD CUT_FS (B) and AJD 2 pAD PET_IS (C). The esterase activity was measured at both the optimal temperature for enzyme action (37 °C) and the temperature used in yeast culture (28 °C).



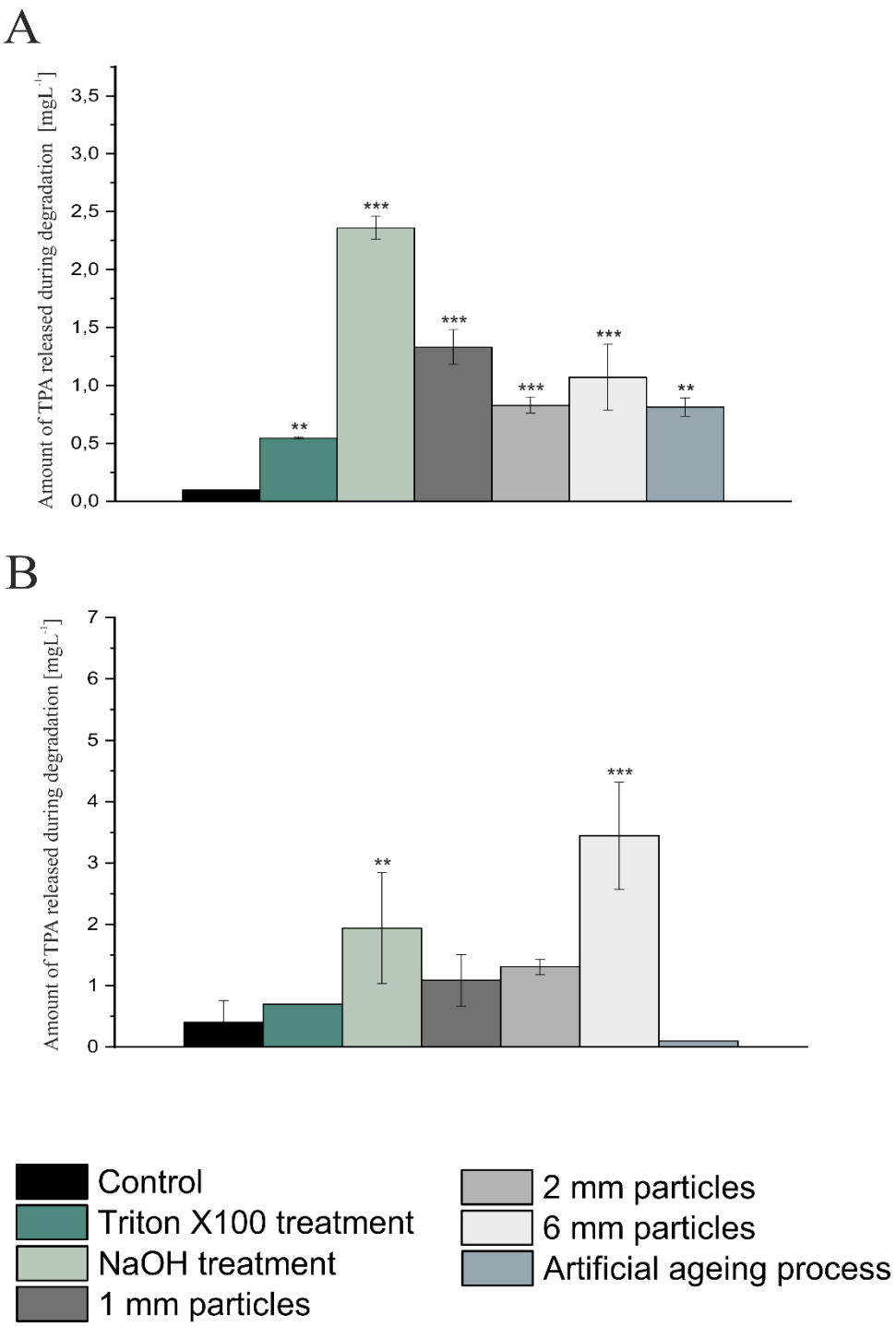
Supplementary Fig. 2

Enzyme stability during degradation process in supernatant (A) and long-term cultures (B).



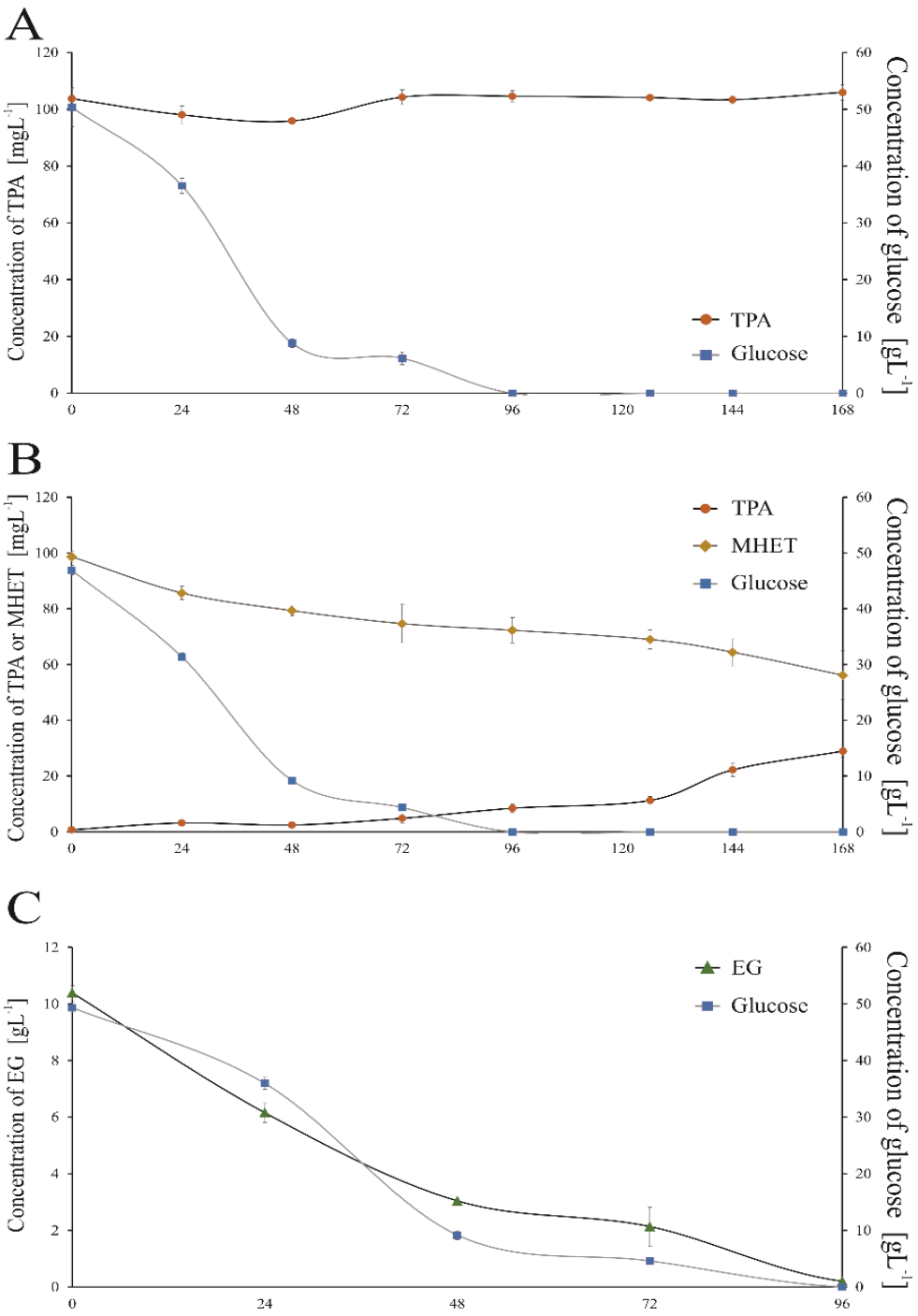
Supplementary Fig. 3

Amount of TPA released during degradation of pre-treated PET films relative to the number of products released when incubated with untreated films (control) with supernatants from AJD 2 pAD CUT_FS (A) and AJD 2 pAD PET_IS (B).



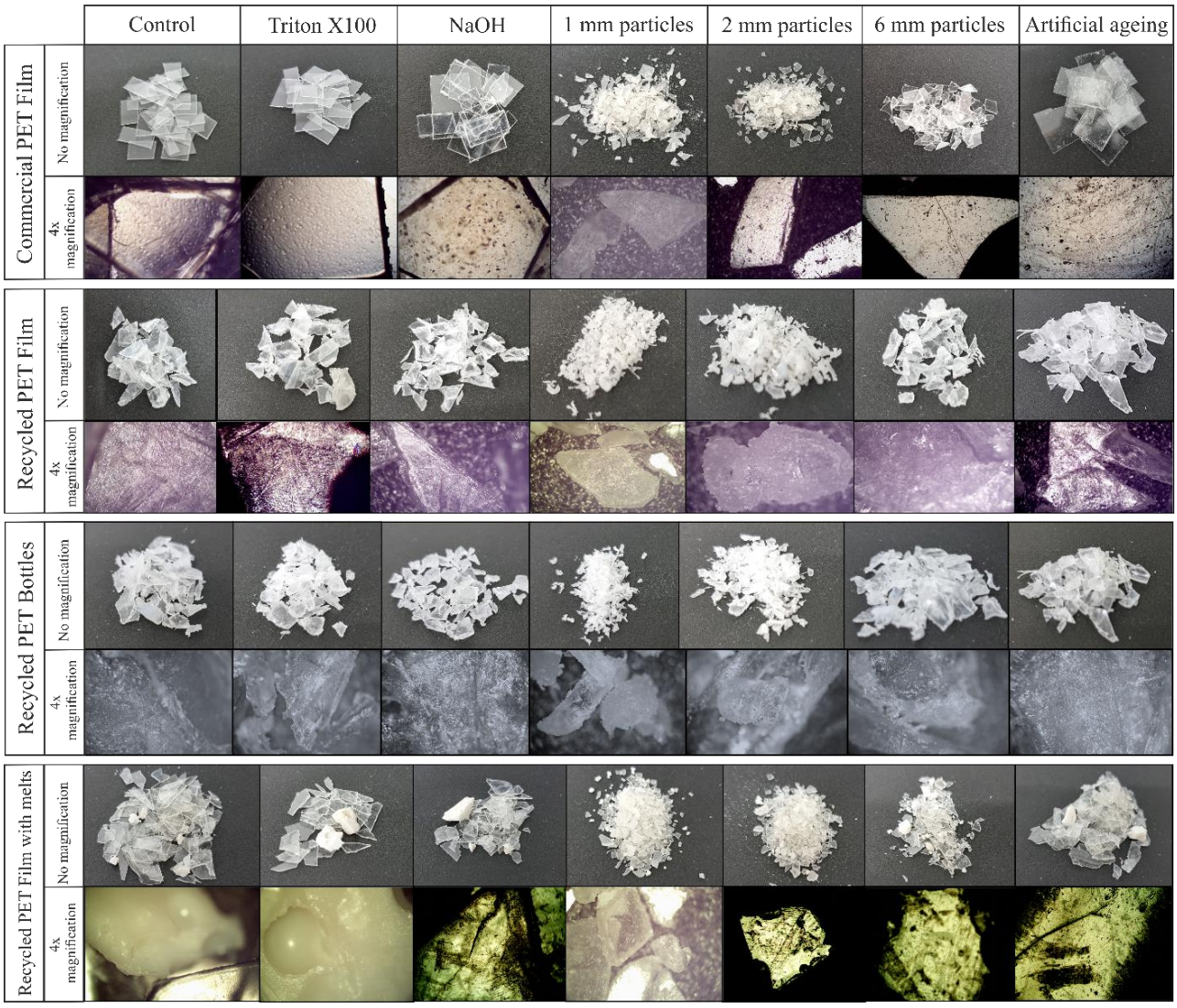
Supplementary Fig. 4

Amount of TPA released during 2-week incubation of pre-treated recycled films (A) and recycled bottles (B) with supernatant of AJD 2 pAD PET_IS.



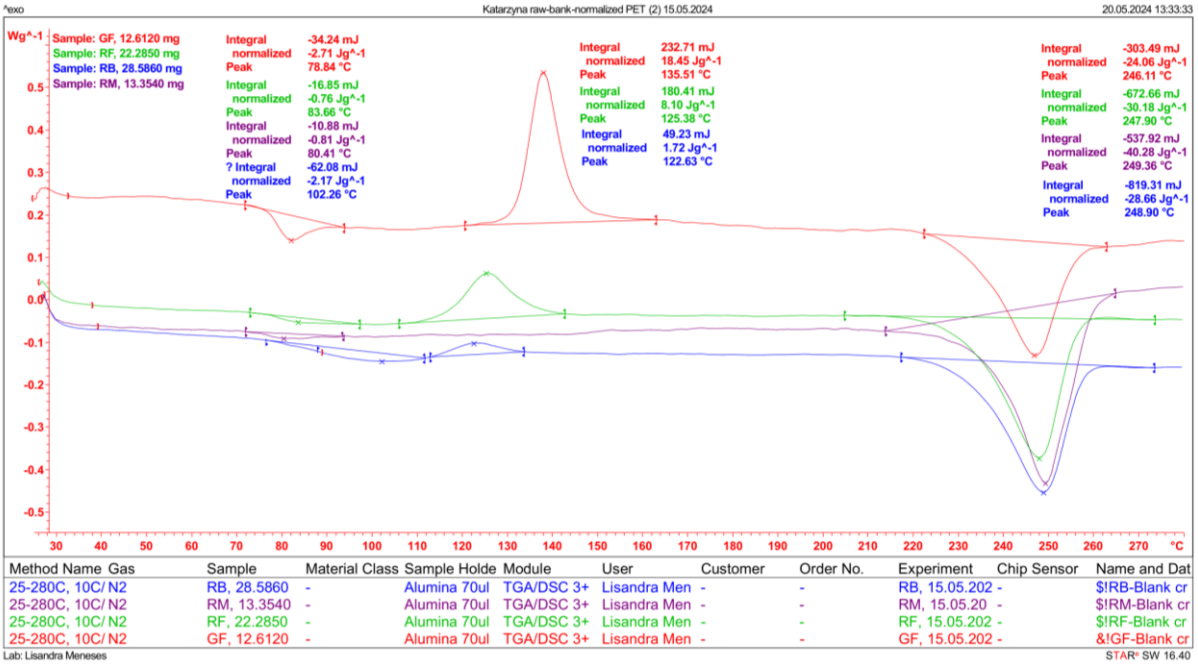
Supplementary Fig. 5

Examination the ability to assimilate TPA (A), EG (C), and MHET hydrolysis (B) by the AJD 2 pAD CUT_FS strain.



Supplementary Fig. 7

PET material after pre-treatment. The images show a visual representation of the appearance of the PET without magnification and with 4x magnification. The microscope images were taken with a Delta Optical MET-200-TRF metallographic microscope, using the EPI (transmitted light) observation technique. Lens used: 4x, total magnification 40x, microscope camera: DLT-Cam PRO 6.3 MP USB 3.0, software: DLTCamViewer.



Supplementary Fig. 8

TGA-DSC analysis of PET material used in this study.

Supplementary Table 1

Esterase activity of enzymes measured in supernatant after 96 h of cultivation AJD 2 pAD CUT_FS and AJD 2 pAD PET_IS strains.

	Esterase activity		K_m	v_{max}
	[UmL^{-1}]	[Umg^{-1}]	[mM]	[$\mu molmin^{-1}mg^{-1}$]
Cutinase	124.9 ± 0.91	42.72 ± 0.31	6.48 ± 0.29	447,89 ± 35,2
PETase	51.13 ± 4.2	23.43 ± 1.92	4.31 ± 0.64	170,31 ± 26,3

Supplementary Table 2

Amount of released PET degradation products [mgL^{-1}] during 28-day culture of AJD 2 pAD CUT_FS and AJD 2 pAD PET_IS with commercial PET film.

Strain		**AJD 2 pAD CUT_FS**		**AJD 2 pAD PET_IS**
Compound		**TPA**	**MHET**	**TPA**
Control	7 days	17,14 ± 1,04	0 ± 0	19,19 ± 3,18
	14 days	30,56 ± 4,72	0 ± 0	54,54 ± 10,56
	21 days	95,95 ± 1,76	23,76 ± 1,71	299,57 ± 9,45
	28 days	151,20 ± 5,01	19,97 ± 1,33	496,55 ± 36,94
NaOH treatment	7 days	N/A	N/A	46,70 ± 6,29
	14 days	N/A	N/A	87,58 ± 0,97
	21 days	N/A	N/A	282,69 ± 7,71
	28 days	N/A	N/A	644,86 ± 21,28
1 mm particles	7 days	59,07 ± 2,9	2,66 ± 0,15	N/A
	14 days	131,31 ± 5,33	1,49 ± 0,13	N/A
	21 days	263,38 ± 4,15	2,81 ± 1,72	N/A
	28 days	377,77 ± 21,53	4,37 ± 0,46	N/A
Artificial ageing process	7 days	35,64 ± 2,18	9,07 ± 1,51	163,74 ± 15,73
	14 days	85,25 ± 4,16	5,70 ± 1,16	595,26 ± 59,25
	21 days	127,56 ± 6,20	11,50 ± 2,85	1203,27 ± 114,25
	28 days	230,11 ± 9,87	22,94 ± 2,44	2181,12 ± 181,24

Supplementary Table 3

Amount of released PET degradation products [mgL^{-1}] during 28-day culture of AJD 2 pAD CUT_FS with pre-treated recycled PET film.

Strain		**AJD 2 pAD CUT_FS**	
Compound		**TPA**	**MHET**
Control	7 days	3,83 ± 0,89	0 ± 0
	14 days	17,46 ± 1,27	21,90 ± 2,91
	21 days	20,45 ± 3,80	9,88 ± 2,38
	28 days	33,89 ± 1,42	4,11 ± 0,58
Triton X100 Treatment	7 days	0 ± 0	0 ± 0
	14 days	9,17 ± 2,59	1,49 ± 2,59
	21 days	12,74 ± 2,75	24,98 ± 2,75
	28 days	53,32 ± 1,31	24,44 ± 1,31
NaOH treatment	7 days	44,09 ± 2,81	0 ± 0
	14 days	67,02 ± 10,41	4,01 ± 0,56
	21 days	155,49 ± 2,94	23,87 ± 5,20
	28 days	263,13 ± 20,48	3,58 ± 0,74
1 mm particles	7 days	4,05 ± 2,35	33,29 ± 13,28
	14 days	23,81 ± 7,99	30,38 ± 3,61
	21 days	79,55 ± 2,05	29,28 ± 8,74
	28 days	199,65 ± 16,15	25,68 ± 2,91

Supplementary Table 4

Amount of released PET degradation products [mgL^{-1}] during 28-day culture of AJD 2 pAD CUT_FS with pre-treated recycled PET bottles.

Strain		**AJD 2 pAD CUT_FS**	
Compound		**TPA**	**MHET**
Control	7 days	18,13 ± 1,14	0 ± 0
	14 days	11,12 ± 0,14	10,76 ± 5,00
	21 days	21,78 ± 20,09	5,82 ± 5,60
	28 days	33,91 ± 5,12	1,71 ± 1,51
NaOH treatment	7 days	2,07 ± 0,26	1,69 ± 0,23
	14 days	8,10 ± 0,38	2,21 ± 1,94
	21 days	16,02 ± 0,62	3,63 ± 3,19
	28 days	29,26 ± 3,48	3,72 ± 0,31
1 mm particles	7 days	27,60 ± 1,96	22,86 ± 1,85
	14 days	116,52 ± 3,50	44,02 ± 4,66
	21 days	121,04 ± 8,38	42,53 ± 2,72
	28 days	588,41 ± 71,23	37,39 ± 7,99
2 mm particles	7 days	23,19 ± 4,38	2,80 ± 0,13
	14 days	104,15 ± 1,76	7,00 ± 0,52
	21 days	172,63 ± 17,52	117,04 ± 3,91
	28 days	171,58 ± 3,33	127,71 ± 12,26
6 mm particles	7 days	15,509 ± 1,40	2,54 ± 0,06
	14 days	116,83 ± 5,38	4,89 ± 0,67
	21 days	159,30 ± 2,53	82,61 ± 27,24
	28 days	173,63 ± 9,35	120,47 ± 6,36
Artificial ageing process	7 days	0 ± 0	0 ± 0
	14 days	5,92 ± 0,45	41,95 ± 1,27
	21 days	16,54 ± 0,66	21,17 ± 3,26
	28 days	24,00 ± 2,57	42,23 ± 6,46

Supplementary Table 5

Amount of released PET degradation products [mgL^{-1}] during 28-day culture of AJD 2 pAD CUT_FS and AJD 2 pAD PET_IS with pre-treated recycled PET films with melts.

Strain		**AJD 2 pAD CUT_FS**		**AJD 2 pAD PET_IS**
Compound		**TPA**	**MHET**	**TPA**
Control	7 days	2,62 ± 0,28	3,45 ± 0,42	14,40 ± 0,39
	14 days	8,79 ± 3,44	7,04 ± 1,54	17,99 ± 1,26
	21 days	16,23 ± 1,36	1,72 ± 2,12	23,25 ± 5,30
	28 days	18,39 ± 3,26	0,90 ± 0,48	26,12 ± 0,23
Triton X100 Treatment	7 days	6,02± 0,34	2,65 ± 0,17	N/A
	14 days	7,49 ± 1,43	3,80 ± 3,30	N/A
	21 days	28,37 ± 0,82	5,49 ± 0,40	N/A
	28 days	43,85 ± 0,60	8,30 ± 2,20	N/A
NaOH treatment	7 days	2,20 ± 0,14	2,17 ± 1,88	19,01 ± 1,91
	14 days	13,01 ± 0,36	4,72 ± 4,27	36,14 ± 3,08
	21 days	17,60 ± 0,93	6,74 ± 1,23	84,45 ± 2,07
	28 days	25,73 ± 1,22	8,30 ± 2,25	133,42 ± 8,06
1 mm particles	7 days	2,62 ± 0,28	3,45 ± 0,423	N/A
	14 days	67,83 ± 4,68	51,05 ± 11,55	N/A
	21 days	69,21 ± 1,06	4,12 ± 1,57	N/A
	28 days	99,39 ± 2,76	43,18 ± 2,37	N/A
2 mm particles	7 days	15,55 ± 1,48	4,85 ± 0,18	N/A
	14 days	36,09 ± 1,03	8,21 ± 0,61	N/A
	21 days	45,95 ± 2,12	5,02 ± 1,22	N/A
	28 days	60,19 ± 2,1	4,31 ± 2,2	N/A
6 mm particles	7 days	18,56 ± 1,30	4,38 ± 0,32	N/A
	14 days	37,33 ± 1,71	6,32 ± 5,70	N/A
	21 days	46,94 ± 5,42	2,06 ± 1,57	N/A
	28 days	62,19 ± 4,67	2,26 ± 0,58	N/A
Artificial ageing process	7 days	3,91 ± 0,14	0 ± 0	0 ± 0
	14 days	18,41 ± 0,21	13,41 ± 1,40	53,35 ± 0,67
	21 days	34,54 ± 1,45	2,94 ± 0,72	69,97 ± 3,05
	28 days	46,22 ± 3,39	4,21 ± 1,32	171,69 ± 12,11