Supplementary Information (SI) for Green Chemistry.

Bioconversion of ethylene glycol to glycolic acid for waste polyethylene terephthalate upcycling

Table S1. Strains and plasmids used in this study.

Resource	Description	Resource
Strains		
E. coli BL21(DE3)	F^{-}*ompT* *hsdS*(rB^{-},mB^{-})gal dcm (DE3)	Purchased from Vazyme
E. coli DH5α(DE3)	*deo*R *end*A1 *gyr*A96 *hsd*R17 (rk-mk+)*rec*A1 *rel*A1 supE44 thi-1 Δ(*lac*ZYA-*arg*F)U169 Φ80*lac*Z ΔM15F-λ-	Purchased from Vazyme
E. coli MG1655	Wild-type	Laboratory preservation
GA00	*E. coli* BL21(DE3) with pET29a(+)	This study
GA01	*E. coli* BL21(DE3) with pET-*fucO*^{Mut}	This study
GA02	*E. coli* BL21(DE3) with pET-aldA	This study
GA03	*E. coli* BL21(DE3) with pET-*fucO*^{Mut}-*aldA*	This study
GA041	*E. coli* BL21(DE3) with pET-*fucO*^{Mut}-*aldA*-*ycdW*	This study
GA042	*E. coli* BL21(DE3) with pET-*fucO*^{Mut}-*aldA*-T7-*ycdW*	This study
GA043	*E. coli* BL21(DE3) with pET-*fucO*^{Mut}-T7-*aldA*-*ycdW*	This study
GA044	*E. coli* BL21(DE3) with pET-*fucO*^{Mut}-*ycdW*-*aldA*	This study
GA045	*E. coli* BL21(DE3) with pET-*fucO*^{Mut}-*ycdW*-T7-*aldA*	This study
GA046	*E. coli* BL21(DE3) with pET-*fucO*^{Mut}-T7-*ycdW*--*aldA*	This study
GA05	*E. coli* BL21(DE3) with pET-*fucO*^{Mut}-*aldA* and pETDuet- *ycdW*-*nox*	This study
GA06	*E. coli* BL21(DE3) with pET-*fucO*^{Mut}-*aldA*-T7-*ycdW* and pMAL-c5X-*nox*	This study
Plasmids		
pET-*fucO*	pET-29a(+); P_{T7} promoter, *E. coli* MG1655 *fucO* gene, Km^{R}	This study

pET-*fucO*^{Mut}	pET-29a(+); P_{T7} promoter, *E. coli* MG1655 *fucO*^{Mut} gene, Km^R	This study
pET-*aldA*	pET-29a(+); P_{T7} promoter, *E. coli* MG1655 *aldA* gene, Km^R	This study
pET-*fucO*^{Mut}-*aldA*	pET-*fucO*^{Mut}; P_{T7} promoter, RBS-*aldA* gene of pET29a-*aldA* inserted into pET-*fucO*^{Mut}, Km^R	This study
pET-*ycdW*	pET-29a(+); P_{T7} promoter, *E. coli* MG1655 *ycdW* gene, Km^R	This study
pET-*fucO*^{Mut}-*aldA*-*ycdW*	pET-*fucO*^{Mut}; P_{T7} promoter, *aldA* gene of pET-*aldA* and *ycdW* gene of pET-*ycdW* inserted into pET-*fucO*^{Mut}, Km^R	This study
pET-*fucO*^{Mut}-*aldA*-T7-*ycdW*	pET-*fucO*^{Mut}-*aldA*; P_{T7} promoter, PT7-*ycdW* gene of pET-*ycdW* inserted into pET-*fucO*^{Mut}-*aldA*, Km^R	This study
pET-*fucO*^{Mut}-T7-*aldA*-*ycdW*	pET-*fucO*^{Mut}; P_{T7} promoter, PT7-*aldA* gene of pET-*aldA* and *ycdW* gene of pET-*ycdW* inserted into pET-*fucO*^{Mut}, Km^R	This study
pET-*fucO*^{Mut}-*ycdW*-*aldA*	pET-*fucO*^{Mut}; P_{T7} promoter, *ycdW* gene of pET-*ycwW* and *aldA* gene of pET-*aldA* inserted into pET-*fucO*^{Mut}, Km^R	This study
pET-*fucO*^{Mut}-*ycdW*-T7-*aldA*	pET-*fucO*^{Mut}; P_{T7} promoter, *ycdW* gene of pET-*ycwW* and PT7-*aldA* gene of pET-*aldA* inserted into pET-*fucO*^{Mut}, Km^R	This study
pET-*fucO*^{Mut}-T7-*ycdW*-*aldA*	pET-*fucO*^{Mut}; P_{T7} promoter, PT7-*ycdW* gene of pET-*ycwW* and *aldA* gene of pET-*aldA* inserted into pET-*fucO*^{Mut}, Km^R	This study
pMAL-c5X-*nox*	pMAL-c5X P_{T7} promoter, *E. faecalis Nox* gene, Amp^R	Synthesized by GenScript
pETDuet-*ycdW*-*nox*	pETDuet-1; Double P_{T7} promoter, *E. coli* MG1655 *aldA* gene and *E. faecalis Nox* gene, Amp^R	This study

Table S2. Primers for strains construction in this study.

primer	Sequence (5'→3')
fuco-F	CCATGGCTGATATCGGATCCATGGCTAACAGAATGATTCTGAACG
fuco^{Mut}-F	CCATGGCTGATATCGGATCCATGGCTAACAGAATGCTGGTGAAC
fuco^{Mut}-R	GTGGTGGTGGTGCTCGAGCCAGGCGGTATGGTAAAG
aldA-F	CATGGCTGATATCGGATCCATGTCAGTACCCGTTCAAC
aldA-R	GGTGGTGGTGGTGCTCGAGTTAAGACTGTAAATAAACCAC
ycdW-F	CGGTTCCATGGCTGATATCGGATCCATGGATATCATCTTTTATCACCC
ycdW-R	GTGGTGGTGGTGCTCGAGTTAGTAGCCGCGTGCGCG
GA03- *fuco*^{Mut}-R	GAACGGGTACTGACATGGATCCCTCCTTTTACCAGGCGGTATGGTAAAG
GA03- *aldA*-F	CCATACCGCCTGGTAAAAGGAGGGATCCATGTCAGTACCCGTTCAAC
GA041-*aldA*-R	GATAAAAGATGATATCCATGGATCCCTCCTTTTAAGACTGTAAATAAACCACCTGG
GA041-*ycdW*-F	CCAGGTGGTTTATTTACAGTCTTAAAAGGAGGGATCCATGGATATCATCTTTTATC
GA042-*ycdW*-F	GGTTTATTTACAGTCTTAACTCGAGTAATACGACTCACTATAGG
GA043-*aldW*-F	GCTTTACCATACCGCCTGGTAACTCGAGTAATACGACTCACTATAGG
GA044-*ycdW*-F	CATACCGCCTGGTAACTCGAGAAGGAGGGATCCATGGATATCATC
GA044-*ycdW*-R	CTGACATGGATCCCTCCTTTTAGTAGCCGCGTGCGCG
GA044-*aldA*-F	CGCGCACGCGGCTACTAAAAGGAGGGATCCATGTCAG
GA045-*ycdW*-R	CCTATAGTGAGTCGTATTATTAGTAGCCGCGTGCGCG
GA045-*aldA*-F	CGCGCACGCGGCTACTAATAATACGACTCACTATAGG
pEDuet-*ycdW*-F	CACCACAGCCAGGATCCGAATTCATGGATATCATCTTTTATCACCC
pEDuet-*ycdW*-R	GCATTATGCGGCCGCAAGCTTTTAGTAGCCGCGTGCGCG
pMAL-nox-F	CGAGGGAAGGATTTCACATATGAGATCTCATGAAAGTTAC
pMAL-nox-R	TTAATTACCTGCAGGGAATTCTTAGTGATGATGGTGGTGG

Table S3. Genes used in this study.

gene	NCBI accession numbers or gene sequence (5'-3')
fucO (Gene ID: 947273)	ATGGCTAACAGAATGATTCTGAACGAAACGGCATGGTTTGGTCGGGGTGCTGTTG GGGCTTTAACCGATGAGGTGAAACGCCGTGGTTATCAGAAGGCGCTGATCGTCAC CGATAAAACGCTGGTGCAATGCGGCGTGGTGGCGAAAGTGACCGATAAGATGGA TGCTGCAGGGCTGGCATGGGCGATTTACGACGGCGTAGTGCCCAACCCAACAATT ACTGTCGTCAAAGAAGGGCTCGGTGTATTCCAGAATAGCGGCGCGGATTACCTGA TCGCTATTGGTGGTGGTTCTCCACAGGATACTTGTAAAGCGATTGGCATTATCAGC AACAACCCGGAGTTTGCCGATGTGCGTAGCCTGGAAGGGCTTTCCCCGACCAATA AACCCAGTGTACCGATTCTGGCAATTCCTACCACAGCAGGTACTGCGGCAGAAGT GACCATTAACTACGTGATCACTGACGAAGAGAAACGGCGCAAGTTTGTTTGCGTT GATCCGCATGATATCCCGCAGGTGGCGTTTATTGACGCTGACATGATGGATGGTA TGCCTCCAGCGCTGAAAGCTGCGACGGGTGTCGATGCGCTCACTCATGCTATTGA GGGGTATATTACCCGTGGCGCGTGGGCGCTAACCGATGCACTGCACATTAAAGC GATTGAAATCATTGCTGGGGCGCTGCGAGGATCGGTTGCTGGTGATAAGGATGCC GGAGAAGAAATGGCGCTCGGGCAGTATGTTGCGGGTATGGGCTTCTCGAATGTTG GGTTAGGGTTGGTGCATGGTATGGCGCATCCACTGGGCGCGTTTTATAACACTCC ACACGGTGTTGCGAACGCCATCCTGTTACCGCATGTCATGCGTTATAACGCTGAC

	TTTACCGGTGAGAAGTACCGCGATATCGCGCGCGTTATGGGCGTGAAAGTGGAAG GTATGAGCCTGGAAGAGGCGCGTAATGCCGCTGTTGAAGCGGTGTTTGCTCTCAA CCGTGATGTCGGTATTCCGCCACATTTGCGTGATGTTGGTGTACGCAAGGAAGAC ATTCCGGCACTGGCGCAGGCGGCACTGGATGATGTTTGTACCGGTGGCAACCCG CGTGAAGCAACGCTTGAGGATATTGTAGAGCTTTACCATACCGCCTGG
fucO^{Mut}	ATGGCTAACAGAATGCTTGTGAACGAAACGGCATGGTTTGGTCGGGGTGCTGTTG GGGCTTTAACCGATGAGGTGAAACGCCGTGGTTATCAGAAGGCGCTGATCGTCAC CGATAAAACGCTGGTGCAATGCGGCGTGGTGGCGAAAGTGACCGATAAGATGGA TGCTGCAGGGCTGGCATGGGCGATTTACGACGGCGTAGTGCCCAACCCAACAATT ACTGTCGTCAAAGAAGGGCTCGGTGTATTCCAGAATAGCGGCGCGGATTACCTGA TCGCTATTGGTGGTGGTTCTCCACAGGATACTTGTAAAGCGATTGGCATTATCAGC AACAACCCGGAGTTTGCCGATGTGCGTAGCCTGGAAGGGCTTTCCCCGACCAATA AACCCAGTGTACCGATTCTGGCAATTCCTACCACAGCAGGTACTGCGGCAGAAGT GACCATTAACTACGTGATCACTGACGAAGAGAAACGGCGCAAGTTTGTTTGCGTT GATCCGCATGATATCCCGCAGGTGGCGTTTATTGACGCTGACATGATGGATGGTA TGCCTCCAGCGCTGAAAGCTGCGACGGGTGTCGATGCGCTCACTCATGCTATTGA GGGGTATATTACCCGTGGCGCGTGGGCGCTAACCGATGCACTGCACATTAAAGC GATTGAAATCATTGCTGGGGCGCTGCGAGGATCGGTTGCTGGTGATAAGGATGCC GGAGAAGAAATGGCGCTCGGGCAGTATGTTGCGGGTATGGGCTTCTCGAATGTTG GGTTAGGGTTGGTGCATGGTATGGCGCATCCACTGGGCGCGTTTTATAACACTCC ACACGGTGTTGCGAACGCCATCCTGTTACCGCATGTCATGCGTTATAACGCTGAC TTTACCGGTGAGAAGTACCGCGATATCGCGCGCGTTATGGGCGTGAAAGTGGAAG GTATGAGCCTGGAAGAGGCGCGTAATGCCGCTGTTGAAGCGGTGTTTGCTCTCAA CCGTGATGTCGGTATTCCGCCACATTTGCGTGATGTTGGTGTACGCAAGGAAGAC ATTCCGGCACTGGCGCAGGCGGCACTGGATGATGTTTGTACCGGTGGCAACCCG CGTGAAGCAACGCTTGAGGATATTGTAGAGCTTTACCATACCGCCTGGTAA
aldA (Gene ID: 945672)	ATGTCAGTACCCGTTCAACATCCTATGTATATCGATGGACAGTTTGTTACCTGGCG TGGAGACGCATGGATTGATGTGGTAAACCCTGCTACAGAGGCTGTCATTTCCCGC ATACCCGATGGTCAGGCCGAGGATGCCCGTAAGGCAATCGATGCAGCAGAACGT GCACAACCAGAATGGGAAGCGTTGCCTGCTATTGAACGCGCCAGTTGGTTGCGCA AAATCTCCGCCGGGATCCGCGAACGCGCCAGTGAAATCAGTGCGCTGATTGTTGA AGAAGGGGGCAAGATCCAGCAGCTGGCTGAAGTCGAAGTGGCTTTTACTGCCGA CTATATCGATTACATGGCGGAGTGGGCACGGCGTTACGAGGGCGAGATTATTCAA AGCGATCGTCCAGGAGAAAATATTCTTTTGTTTAAACGTGCGCTTGGTGTGACTAC CGGCATTCTGCCGTGGAACTTCCCGTTCTTCCTCATTGCCCGCAAAATGGCTCCC GCTCTTTTGACCGGTAATACCATCGTCATTAAACCTAGTGAATTTACGCCAAACAAT GCGATTGCATTCGCCAAAATCGTCGATGAAATAGGCCTTCCGCGCGGCGTGTTTA ACCTTGTACTGGGGCGTGGTGAAACCGTTGGGCAAGAACTGGCGGGTAACCCAA AGGTCGCAATGGTCAGTATGACAGGCAGCGTCTCTGCAGGTGAGAAGATCATGGC GACTGCGGCGAAAAACATCACCAAAGTGTGTCTGGAATTGGGGGGTAAAGCACCA GCTATCGTAATGGACGATGCCGATCTTGAACTGGCAGTCAAAGCCATCGTTGATTC ACGCGTCATTAATAGTGGGCAAGTGTGTAACTGTGCAGAACGTGTTTATGTACAGA AAGGCATTTATGATCAGTTCGTCAATCGGCTGGGTGAAGCGATGCAGGCGGTTCA ATTTGGTAACCCCGCTGAACGCAACGACATTGCGATGGGGCCGTTGATTAACGCC GCGGCGCTGGAAAGGGTCGAGCAAAAAGTGGCGCGCGCAGTAGAAGAAGGGGC

	GAGAGTGGCGTTCGGTGGCAAAGCGGTAGAGGGGAAAGGATATTATTATCCGCC GACATTGCTGCTGGATGTTCGCCAGGAAATGTCGATTATGCATGAGGAAACCTTTG GCCCGGTGCTGCCAGTTGTCGCATTTGACACGCTGGAAGATGCTATCTCAATGGC TAATGACAGTGATTACGGCCTGACCTCATCAATCTATACCCAAAATCTGAACGTCG CGATGAAAGCCATTAAAGGGCTGAAGTTTGGTGAAACTTACATCAACCGTGAAAAC TTCGAAGCTATGCAAGGCTTCCACGCCGGATGGCGTAAATCCGGTATTGGCGGCG CAGATGGTAAACATGGCTTGCATGAATATCTGCAGACCCAGGTGGTTTATTTACAG TCTTAA
ycdW (Gene ID: 946431)	ATGGATATCATCTTTTATCACCCAACGTTCGATACCCAATGGTGGATTGAGGCACT GCGCAAAGCTATTCCTCAGGCAAGAGTCAGAGCATGGAAAAGCGGAGATAATGAC TCTGCTGATTATGCTTTAGTCTGGCATCCTCCTGTTGAAATGCTGGCAGGGCGCG ATCTTAAAGCGGTGTTCGCACTCGGGGCCGGTGTTGATTCTATTTTGAGCAAGCTA CAGGCACACCCTGAAATGCTGAACCCTTCTGTTCCACTTTTTCGCCTGGAAGATAC CGGTATGGGCGAGCAAATGCAGGAATATGCTGTCAGTCAGGTGCTGCATTGGTTT CGACGTTTTGACGATTATCGCATCCAGCAAAATAGTTCGCATTGGCAACCGCTGCC TGAATATCATCGGGAAGATTTTACCATCGGCATTTTGGGCGCAGGCGTACTGGGC AGTAAAGTTGCTCAGAGTCTGCAAACCTGGCGCTTTCCGCTGCGTTGCTGGAGTC GAACCCGTAAATCGTGGCCTGGCGTGCAAAGCTTTGCCGGACGGGAAGAACTGT CTGCATTTCTGAGCCAATGTCGGGTATTGATTAATTTGTTACCGAATACCCCTGAA ACCGTCGGCATTATTAATCAACAATTACTCGAAAAATTACCGGATGGCGCGTATCT CCTCAACCTGGCGCGTGGTGTTCATGTTGTGGAAGATGACCTGCTCGCGGCGCT GGATAGCGGCAAAGTTAAAGGCGCAATGTTGGATGTTTTTAATCGTGAACCCTTAC CGCCTGAAAGTCCGCTCTGGCAACATCCACGCGTGACGATAACACCACATGTCGC CGCGATTACCCGTCCCGCTGAAGCTGTGGAGTACATTTCTCGCACCATTGCCCAG CTCGAAAAAGGGGAGAGGGTCTGCGGGCAAGTCGACCGCGCACGCGGCTACTAA
nox	AGATCTcATGAAAGTTACTGTAGTCGGATGTACACACGCGGGCACCTTCGCTATTA AGCAAATTCTAGCGGAGCACCCGGACGCCGAAGTTACCGTGTATGAACGTAACGA TGTCATTAGCTTCCTGAGCTGTGGCATCGCGCTGTACCTGGGCGGCAAGGTGGC CGACCCGCAAGGTTTGTTTTATTCCTCACCGGAAGAATTACAAAAATTAGGCGCGA ACGTGCAGATGAATCACAATGTTCTGGCGATCGACCCAGACCAGAAAACCGTCAC GGTTGAGGACCTGACCTCGCACGCCCAAACCACAGAATCTTACGACAAGCTGGTG ATGACCAGTGGTAGCTGGCCTATTGTTCCGAAAATCCCGGGTATCGACTCTGATC GTGTTAAGCTGTGCAAGAACTGGGCGCACGCACAAGCACTAATCGAAGATGCGAA AGAGGCTAAACGTATCACCGTGATTGGTGCAGGCTATATTGGCGCTGAGCTCGCA GAGGCCTATAGCACCACCGGTCATGATGTCACCCTTATCGACGCAATGGATCGCG TTATGCCGAAGTACTTCGACGCAGATTTTACTGACGTGATTGAACAAGATTACCGT GATCATGGTGTTCAGCTGGCTCTGTCTGAAACTGTGGAGAGCTTCACCGATTCCG CAACCGGTTTGACGATCAAAACTGACAAGAACAGCTACGAGACGGACTTGGCGAT CCTGTGCATCGGTTTTAGACCGAACACGGACTTACTGAAAGGCAAAGTAGATATG GCACCGAATGGCGCGATCATTACCGACGACTACATGCGTAGCTCTAACCCGGATA TCTTTGCTGCCGGAGATAGCGCGGCGGTGCATTATAACCCGACCCACCAGAACGC ATACATCCCGCTGGCGACCAACGCGGTTCGTCAGGGCATCTTGGTTGGTAAGAAC CTGGTGAAACCGACGGTTAAGTACATGGGTACGCAGTCCAGCTCCGGTTTGGCGT TGTACGACCGCACCATCGTCAGTACCGGCCTGACGCTGGCCGCGGCCAAACAGC

	AGGGTGTGAATGCGGAGCAAGTAATTGTGGAGGACAATTACCGCCCTGAGTTCAT GCCATCGACCGAGCCGGTCTTGATGAGCCTGGTTTTTGACCCGGATACCCATCGT ATTCTGGGCGGTGCGCTGATGAGCAAATATGATGTGAGCCAGAGCGCTAACACGC TGTCCGTGTGCATCCAGAATGAAAACACCATTGACGATCTGGCTATGGTTGATATG CTGTTCCAGCCGAACTTCGATCGCCCGTTTAACTATTTGAATATTCTCGCTCAAGC GGCGCAGGCGAAGGTTGCGCAAAGCGTTAATGCGCACCACCACCATCATCACTAA CTCGAG

Table S4. Yearly operating cost breakdown (Scenario 1)[1].

Operating Parameters			
Metric/Parameter	**Value**	**Units**	
Annual operating hours	7,884	hrs/yr	
On stream factor	0.9	%	
Feedstock contaminants	5.0%	wt %	
Feedstock PET	46,811	Tonnes/yr	
Total rTPA rate	31,112	Tonnes/yr	
rTPA yield	0.66	Tonnes rTPA / tonne PET feed	
PET flake feed mass flow	6,250	kg/h	
TPA production rate	3,946	kg/h	
Variable Operating Costs			
Process Hierarchy/Section	**Raw Material/Utility**	**Mass Flow, kg/hr**	**$M/yr**
Raw Materials			
Feedstock	PET flake feedstock	6,250	32.59
PET Depolymerization	Caustic (as pure)	2,133	2.78
	Water Makeup	22,683	0.03
	Enzyme Consumption	28	3.34
Clarification	Ultrafiltration unit replacement	-	0.14
	Activated carbon replacement	-	0.25
Crystallization	Sulfuric Acid, 93 wt. %	2,702	2.05
	Caustic (as pure), reneutralize	51	0.07

OSBL Utilities	Membrane replacement	-	0.01
	MP Steam, 175°C/114 psig	10,381	1.19
	HP Steam, 250°C/560 psig	5,513	0.63
	Cooling water	1,162,662	0.36
	Chiller water, 4°C	2	0.17
	Grid electricity	6,929	2.64
	Subtotal		46.25
Waste Streams			
Clarification	Solids disposal	4,649	1.26
Crystallization	Brine disposal, permeate	17,330	1.45
Crystallization	Brine disposal, SS wastewater	3,707	0.31
	Subtotal		3.02
Co-Products and Credits			
Crystallization	SS co-product	2,769	3.21
EG Recovery	EG co-product	8,199	6.20
	Subtotal		9.41
Total Variable Operating Costs			39.86
Fixed Operating Costs			4.42
Total Operating Costs			44.28

Table S5. Yearly operating cost breakdown (Scenario 2).

Operating Parameters		
Metric/Parameter	Value	Units
Annual operating hours	7,884	hrs/yr
On stream factor	0.9	%
Feedstock contaminants	5.0%	wt %
Feedstock PET	46,811	Tonnes/yr

Total rTPA rate	31,112	Tonnes/yr	
rTPA yield	0.66	Tonnes rTPA / tonne PET feed	
Total GA rate	13,083	Tonnes/yr	
GA yield	0.28	Tonnes GA / tonne PET feed	
PET flake feed mass flow	6,250	kg/h	
TPA production rate	3,946	kg/h	
EG production rate	1,474	kg/h	
GA production rate	1,659	kg/h	
Variable Operating Costs			
Process Hierarchy/Section	Raw Material/Utility	Mass Flow, kg/hr	$M/yr
Raw Materials			
Feedstock Pretreatment	PET flake feedstock	6,250	32.59
	Caustic (as pure)	2,133	2.78
PET Depolymerization	Water Makeup	22,683	0.03
	Enzyme Consumption	28	3.34
Clarification	Ultrafiltration unit replacement	-	0.14
	Activated carbon replacement	-	0.25
Whole Cell Catalytic Transformation	Water Makeup	9,894	0.01
	Catalyst Acquisition*	950	18.67
Crystallization	Sulfuric Acid, 93 wt. %	2,702	2.05
	Caustic (as pure), reneutralize	51	0.07
	Membrane replacement	-	0.01
OSBL Utilities	Steam	12,469	1.43
	Cooling water	1,162,662	0.36
	Chiller water, 4°C	2	0.17
	Grid electricity	6,929	2.47
	Subtotal		64.37
Waste Streams			

Clarification	Solids disposal	4,649	1.26
Crystallization	Brine disposal, permeate	17,330	1.45
Crystallization	Brine disposal, SS wastewater	3,707	0.31
	Subtotal		3.02
Co-Products and Credits			
Crystallization	SS co-product	2,769	3.21
EG Recovery	GA co-product	9,231	32.11
	Subtotal		35.32
Total Variable Operating Costs			32.46
Fixed Operating Costs			4.42
Total Operating Costs			36.49

***This cost includes the costs related to facilities, raw materials, utilities, waste treatment, consumables, labor and other costs for catalyst Acquisition**[2]

Table S6. Culture media used in the catalyst production process and cost of raw materials.

Component	Batch Medium (mg/L)	Feed Solution 1 (mg/L)	Feed Solution 2 (mg/L)	Feed Solution 3 (mg/L)	Others	Price (US$/kg)
Glucose	25×10^3	670.0×10^3				0.16
$MgSO_4 \times 7H_2O$	1.5×10^3	19.8×10^3				0.50
K_2HPO_4	16.6×10^3					0.80
Citric acid	2.1×10^3					0.60
$(NH_4)_2HPO_4$	4.0×10^3		227.0×10^3			0.25
$(NH_4)H_2PO_4$			169.5×10^3			0.50
Fe(III) citrate hydrate	75.00			5.0×10^3		1.20
H_3BO_3	3.80			250.00		0.70
$MnCl_2 \times 4H_2O$	18.80			125.00		1.60
EDTA $\times$ $2H_2O$	10.50			700.00		1.00
$CuCl_2 \times 2H_2O$	1.90			125.00		1.80
$Na_2MO_4 \times 2H_2O$	3.10			213.00		5.00
$CoCl_2 \times 6H_2O$	3.10			213.00		0.90
$Zn(CH_3COO)_2 \times 2H_2O$	10.00			668.00		0.54
Aqueous NH_3 (NH_4OH)					25% w/w	0.30
Kanamycin Sulfate					50 mg/L*	35.00
Ampicillin Sulfate					100 mg/L*	20.00
IPTG					0.2 mmol/L*	300.00

Except where indicated, the prices of all raw materials were market prices obtained at the Molbase Chemical E-Commerce platform (www.made-in-china.com, accessed February 2025). It is required to harvest 120g of bacteria per 1L of medium

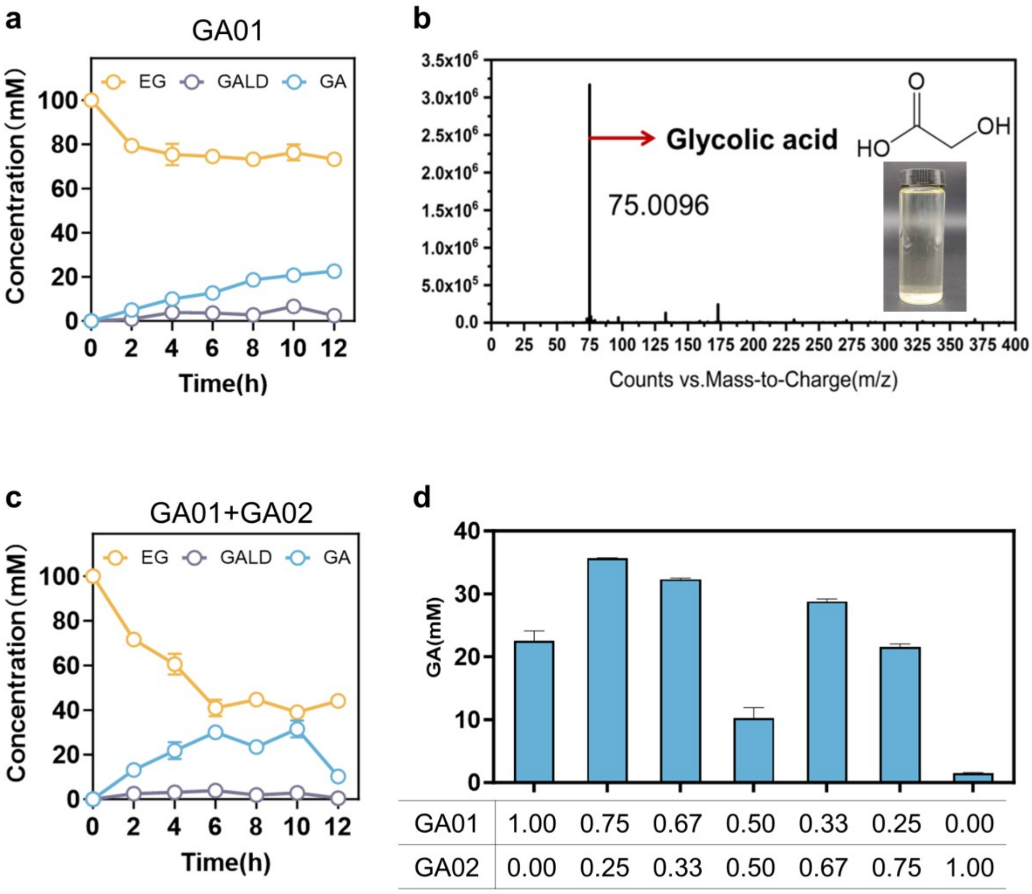

Figure S1. (a) Catalytic performance of strain GA01 at 100 mM EG. (b) Mass spectrum of the reaction products obtained from strain GA01 after catalysis. (c) Biocatalytic conversion by the mixed culture of GA01 and GA02 at 100 mM EG. (d) GA production by seven enzyme cascade systems composed of strains mixed at different ratios. (d) Catalytic profile of engineered strain GA03. The bar chart represents GA yield, while the line chart indicates pH variation during the catalytic process.

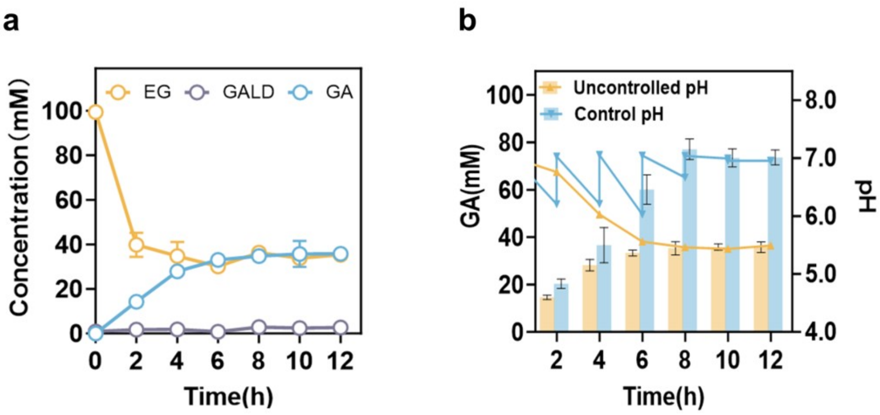

Figure S2. (a) Catalytic performance of strain GA03 at 100 mM EG. (b) Catalytic profile of engineered strain GA03. The bar chart represents GA yield, while the line chart indicates pH variation during the catalytic process.

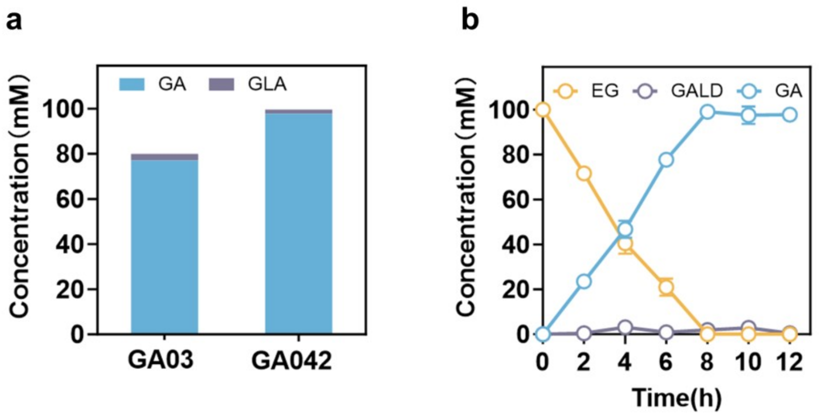

Figure S3. (a) Accumulation of GA and GLA of biotransformation at 100 mM EG. (b) Catalytic performance of strain GA042 at 100 mM EG.

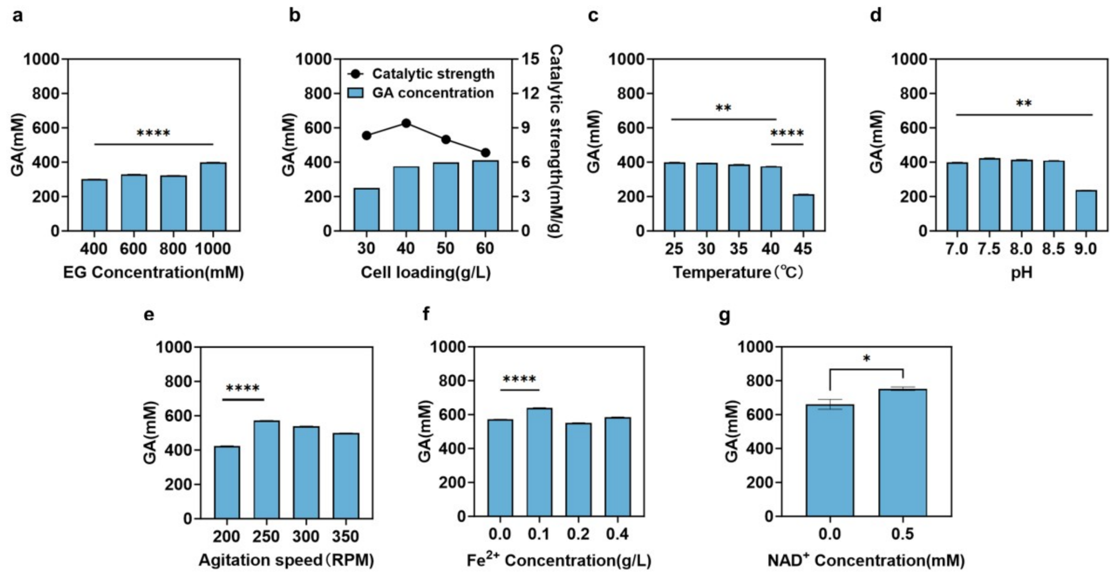

Figure S4. Optimization of catalytic system conditions of strain GA042.

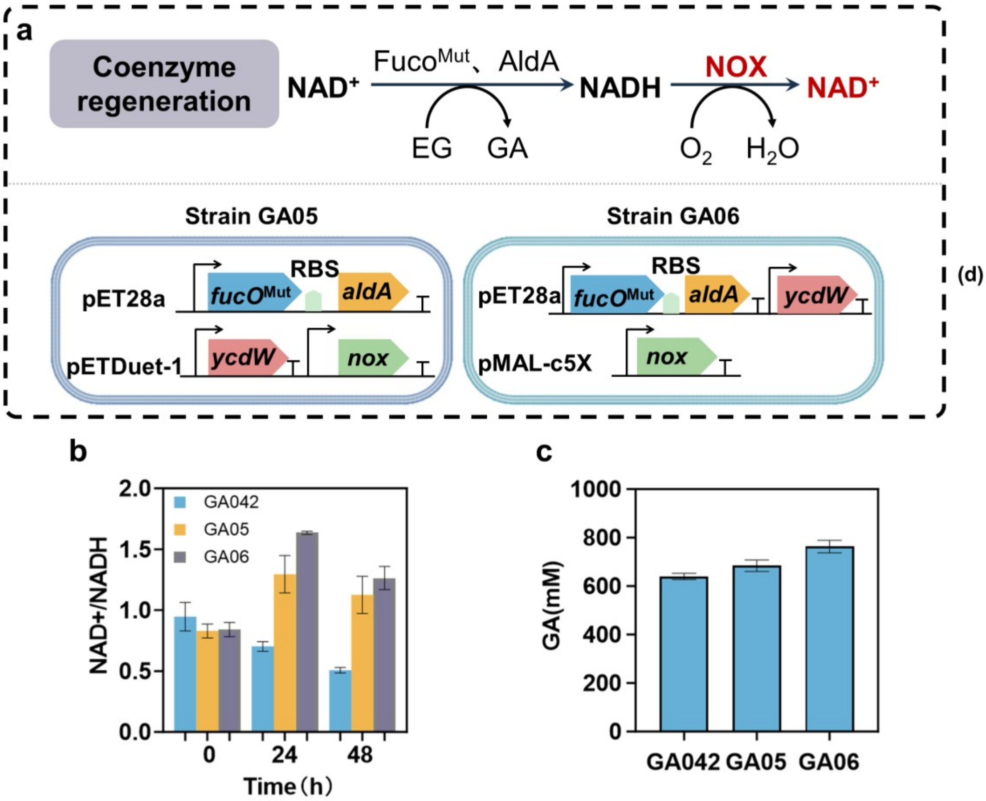

Figure S5. (a) Mechanism of NAD$^+$ coenzyme recycling. (b) Intracellular NAD$^+$/NADH content during 0-48 hours catalysis by GA042, GA05 and GA06. (c) GA yields of various engineered strains under coenzyme recycling at 1000 mM EG concentration.

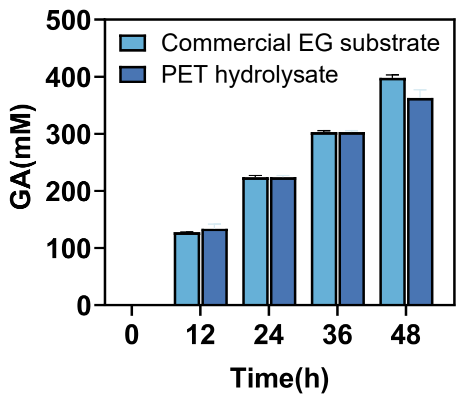

Figure S6. (a) GA production from PET Hydrolysate and commercial EG with equivalent EG loading.

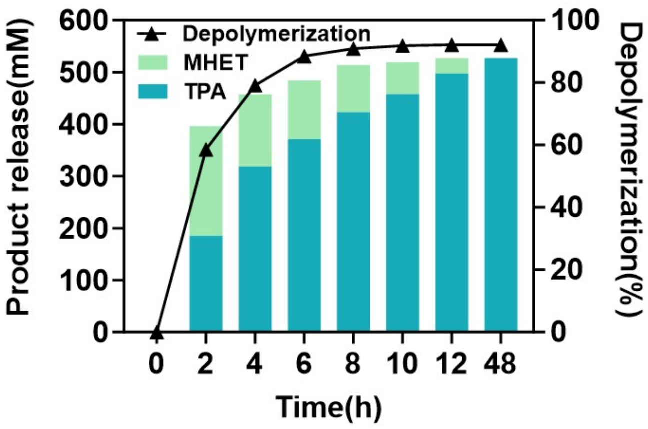

Figure S7. Enzymatic hydrolysis of PET by LCC[ICCG] enzyme.

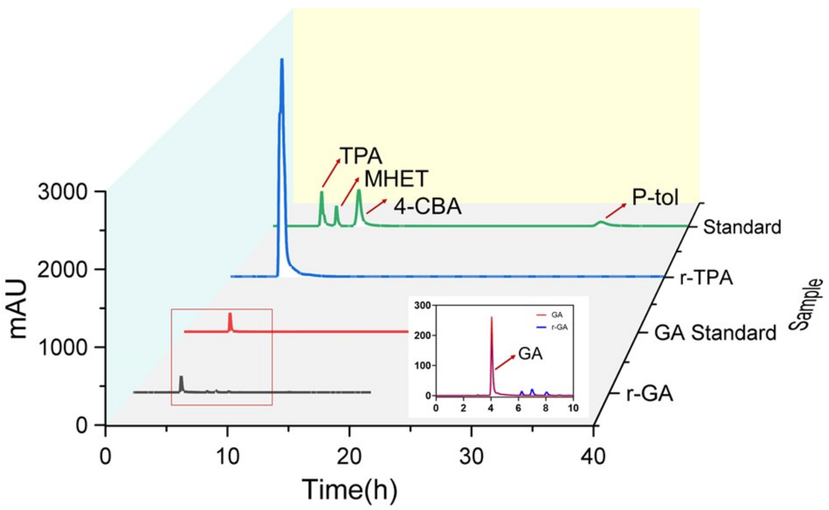

Figure S8. Testing of recovered samples.

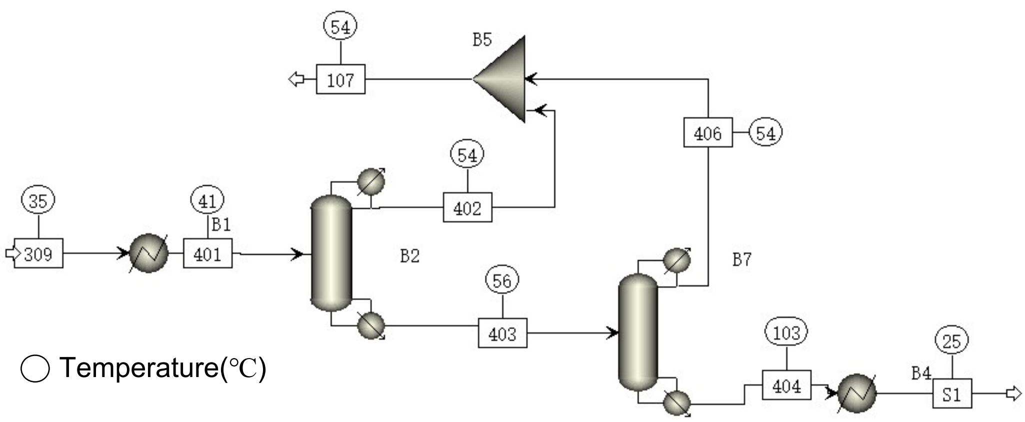

Figure S9.Detailed PFD of GA recovery process section