Supplementary Information

A microbial factory for bio-indigo synthesis: demonstration of sustainable denim dying

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1 Supplementary data

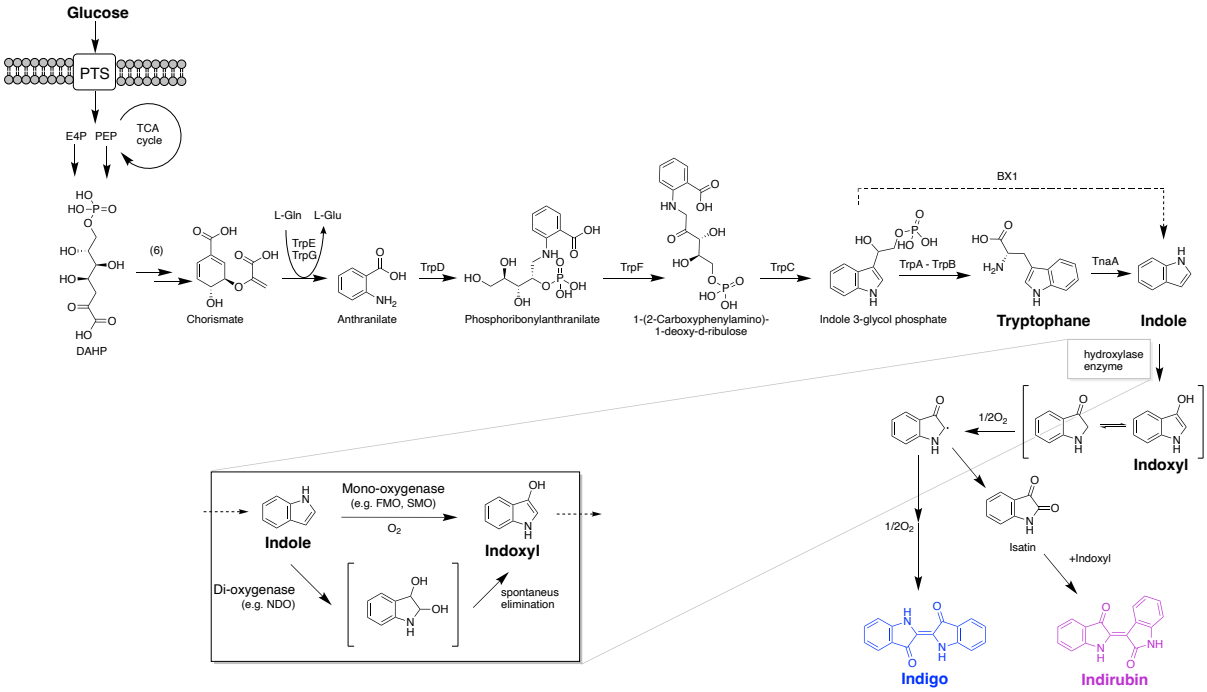

Fig. S1| Key reactions steps employed for recombinant synthesis of indigo from glucose. Simplified scheme of the main steps involved in glucose conversion to indigo through the tryptophan-shikimate pathway. Hydroxylation-steps of indole to indoxyl are depicted in detail in the inset, illustrating the two potential pathways to indoxyl either via enzyme-catalysed monooxygenation or dioxygenation followed by chemical decay. NDO: naphthalene dioxygenase; FMO: flavine monooxygenase; SMO; styrene monooxygenase; BX1: indole-3-glycerol phosphate lyase *Zea mays*; DAHP: 3-deoxy-D-arabino-heptulosonic acid 7-phosphate; E4P: erythrose 4-phosphate; PEP: phosphoenolpyruvate.

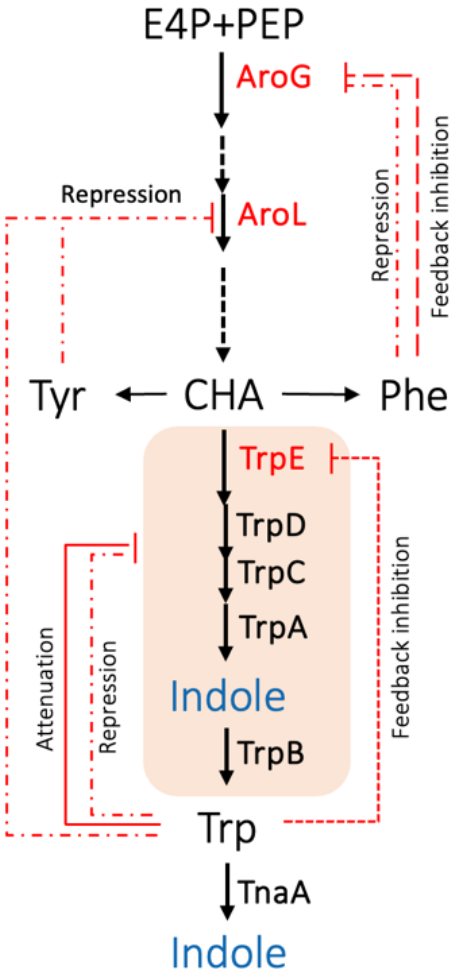

Fig. S2| Tryptophan-shikimate regulation. Simplified scheme of the kinetic and genetic regulation of the enzymes involved in the production of indole from erythrose 4-phosphate (E4P) and phosphoenolpyruvic acid (PEP). Highlighted in red are the targets that were modified by us. CHA: chorismite; Tyr: tyrosine; Phe: phenylalanine.

Table S1| Tryptophan (Trp) producing strains. Trp production of two *E. coli* strains BL21(DE3) and W3110 harbouring J23119-*aroG*^{S180F} and J23119-*trpE*^{S40F} modifications. Experiments were performed in flask 1:10 filling volume at 30°C with fermentation medium containing 1% glucose (Table S5).

Strain code	***E. coli***	**Max Trp titre (g L-1)**
G0b	**BL21(DE3)** J23119-*aroG*^{S180F} J23119-*trpE*^{S40F}	**0.7**
G0w	**W3110** J23119-*aroG*^{S180F} J23119-*trpE*^{S40F}	**0.5**

Table S2| *In vivo* hydroxylation activity. O_2 consumption rate of resting *E. coli* BL21(DE3) cells harbouring one of four different plasmids (P2, P4, P5, P6), each expressing a different indole-hydroxylating enzyme, in the presence of indole. O_2 consumption rate was measured in reaction chamber equipped an oxygen electrode. U: µmol min^{-1}, CDW: cell dry weight, NDOb: Naphthalene dioxygenase from *Pseudomonas balearica* DSM 6083; NDO: Naphthalene dioxygenase form *Pseudomonas putida*; SMO: styrene monooxygenase from *Pseudomonas sp.*; mFMO: flavine monooxygenase from *Methylophaga sp*.

***E. coli* BL21(DE3) +**	**Whole-cell activity for O_2 (U/gCDW)**
NDOb	55
NDO	43
SMO	47
mMFO	41

Cell preparation: *E. coli* BL21(DE3) cells (harbouring the appropriate expression plasmid; see Table S8) were cultivated in M9 mineral medium supplemented with 10 g L^{-1} glucose and the appropriate antibiotic. Cultures were grown at 30 °C in baffled flasks filled to 1/10 of their volume and shaken at 200 rpm (2.5 cm amplitude). When the culture reached an OD_{600} of 0.6, protein expression was induced by adding 0.1 mM IPTG, followed by overnight incubation. Cells were then harvested by centrifugation, washed twice with an equal volume of PBS (pH 7.4), and resuspended in PBS to an OD_{600} of 2.0.

Assay condition: Resting cells of *E. coli* BL21(DE3) (OD_{600} = 2.0) were resuspended in PBS (pH 7.4) that had been saturated with 240 µM O_2 by bubbling air through it for 10-15 minutes. The suspension, supplemented with 10 mM glucose, was maintained at 30 °C with gentle stirring (100 rpm) using a magnetic stirring bar. The cell suspension was then transferred to the chamber of an oxygen electrode (Hansatech Instruments Ltd). After a 1-minute equilibration period, the baseline (substrate-free) oxygen consumption rate was recorded for 1 minute. Subsequently, indole (final concentration: 1 mM) was added, and the change in oxygen consumption was measured to assess the cell-specific respiratory activity in the presence of the substrate.

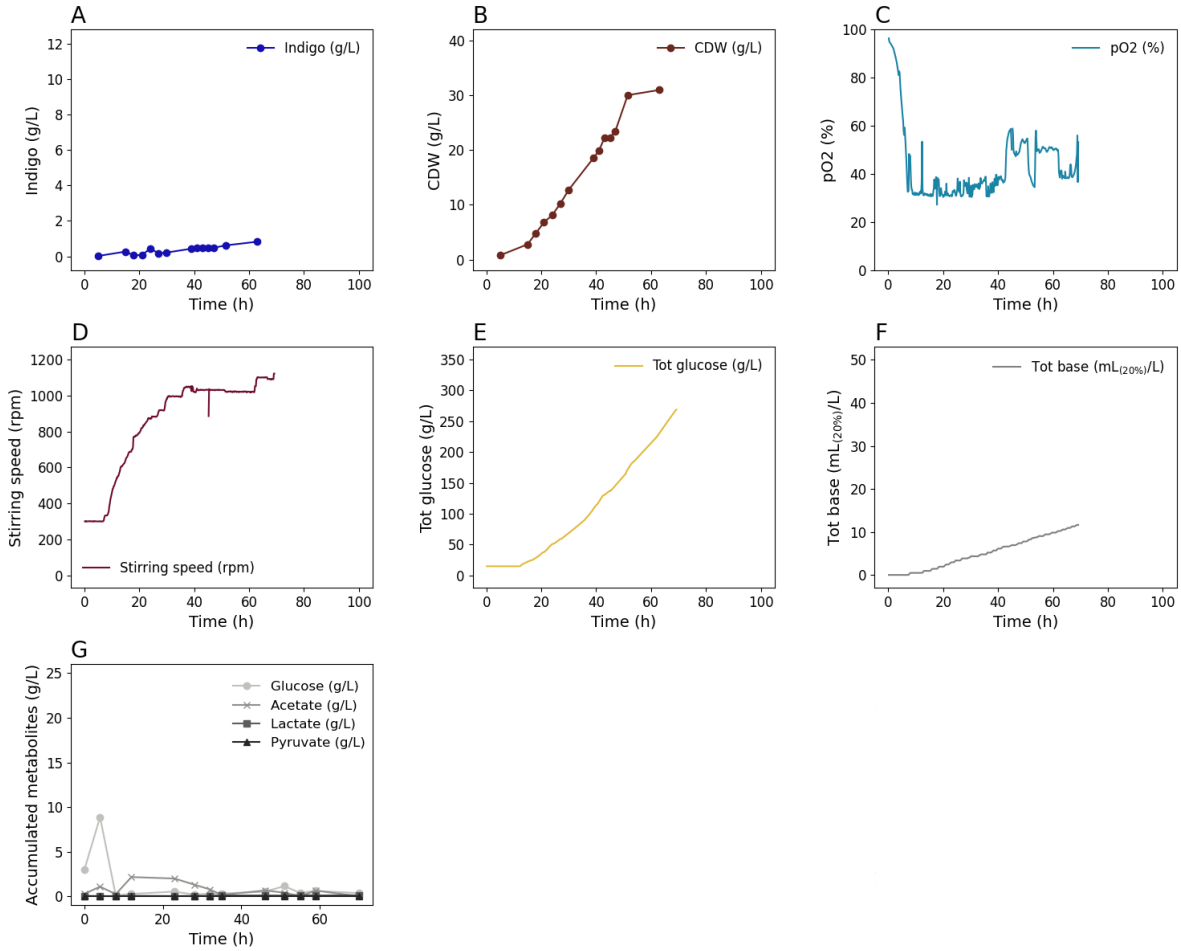

Fig. S3| Fed-batch cultivation with strain G1. Representative fed-batch cultivation with *E. coli* BL21(DE3) J23119-*aroG*^{S180F} J23119-*trpE*^{S40F} (strain G1) after process optimization. **a.** Indigo accumulation (g L-1); **b.** biomass accumulation as cell-dry-weight (CDW, g L-1); **c.** pO_2 (dissolved oxygen, %); **d.** stirring speed profile (rpm); **e.** total amount of glucose (g L-1 of liquid volume) added to the bioreactor; **f.** total amount of base (mL of 20 % NH_4OH per L of liquid volume) added to the bioreactor; **g.** accumulation of glucose and overflow metabolites as quantified in clarified supernatants(g L-1).

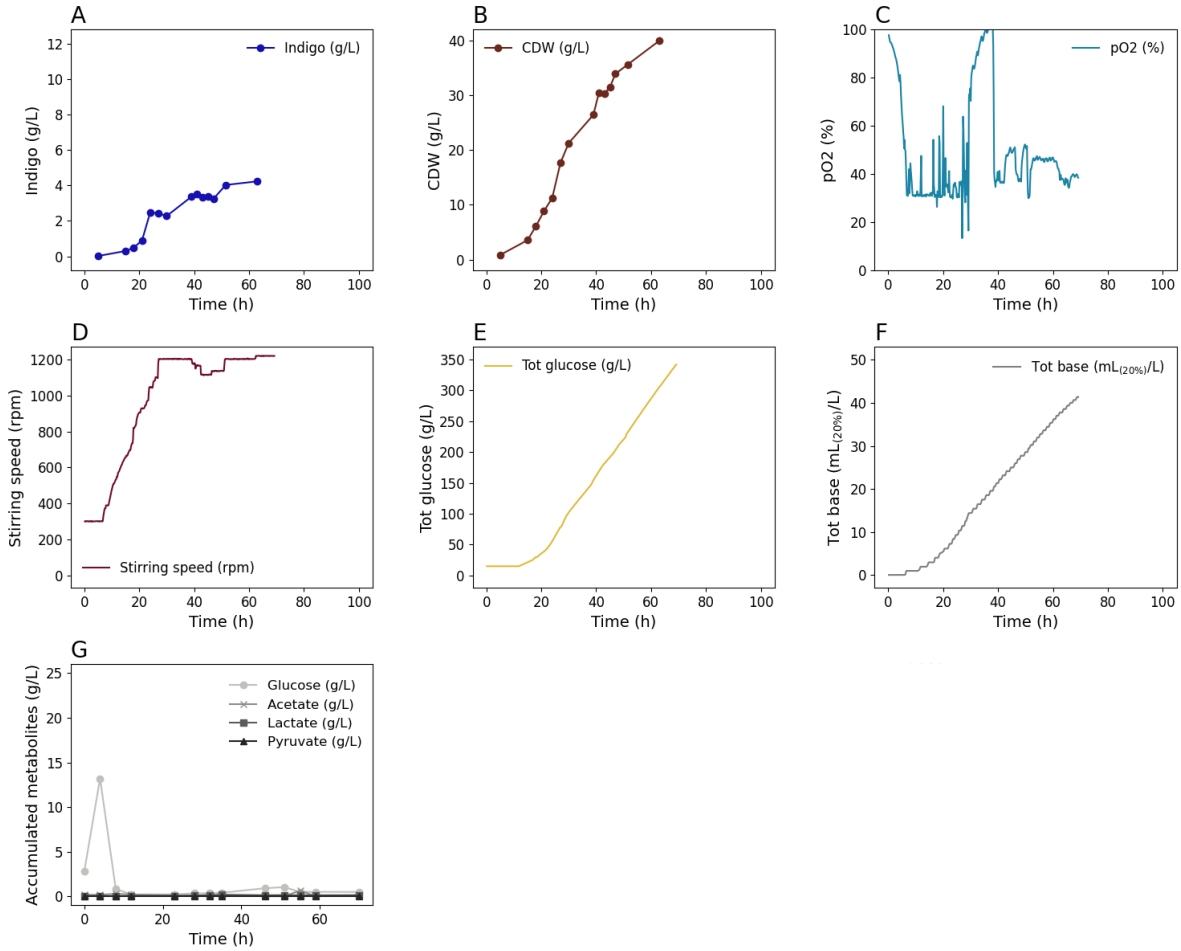

Fig. S4| G2 strain fermentation. Results obtained with the G2 strain after process optimization. **a.** indigo accumulation (g L-1); **b.** biomass accumulation as cell-dry-weight (CDW, g L-1); **c.** pO_2 (dissolved oxygen, %); **d.** steering speed profile (rpm); **e.** total amount of glucose (g L-1) added to the bioreactor; **f.** total amount of base (mL of 20 % NH_4OH per L) added in the bioreactor; **g.** glucose and overflow as quantified in clarified supernatants (g L-1).

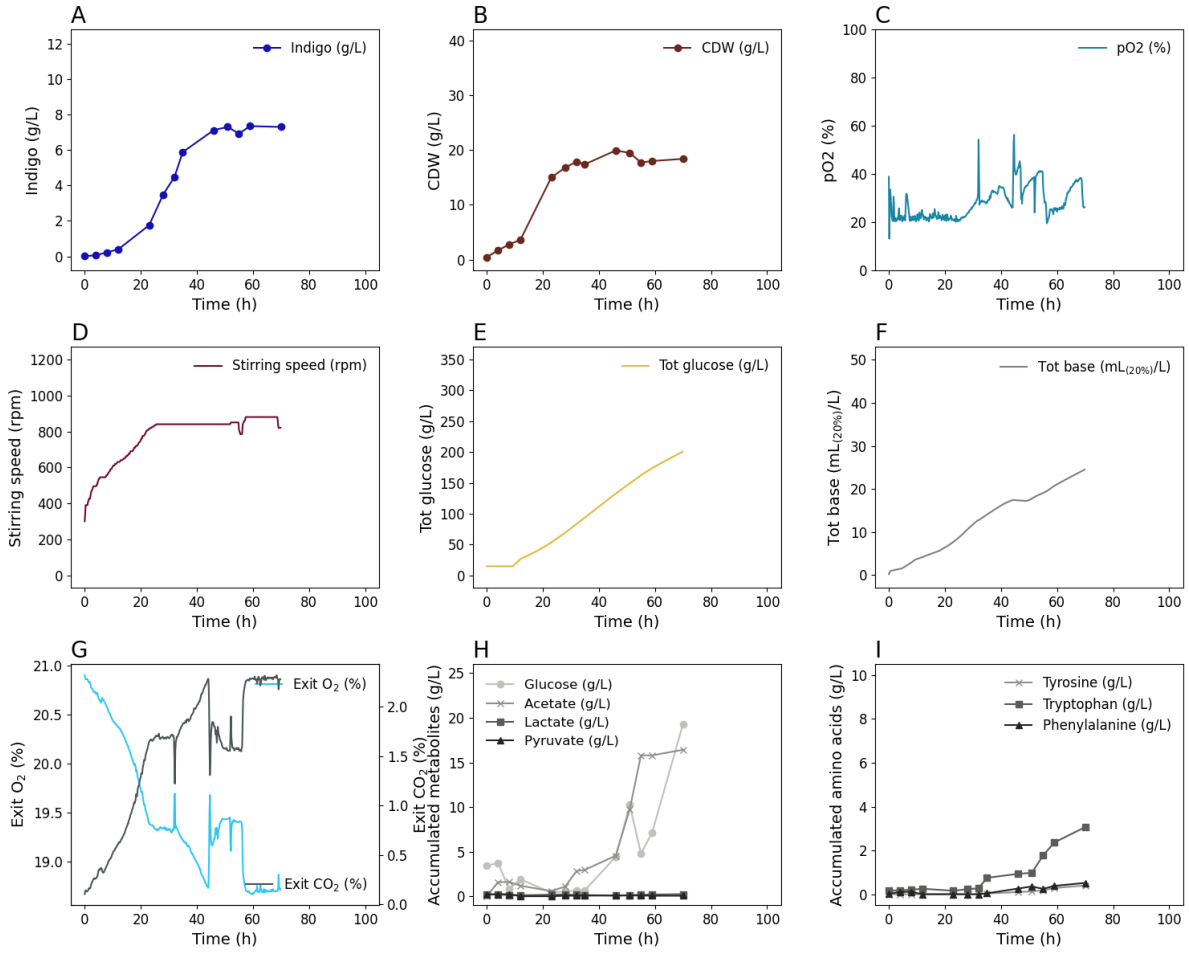

Fig. S5| G3 strain fermentation. Results obtained with the G3 strain after process optimization. **a.** indigo accumulation (g L-1); **b.** biomass accumulation as cell-dry-weight (CDW, g L-1); **c.** pO_2 (dissolved oxygen, %); **d.** steering speed profile (rpm); **e.** total amount of glucose (g L-1) added to the bioreactor; **f.** total amount of base (mL of 20 % NH_4OH per L) added to the bioreactor; **g.** O_2 and CO_2 air concentration at the off-gas (entry composition 20.8% and 0%, respectively); **h.** glucose and overflow metabolites accumulation (g L-1); **i.** aromatic amino acids as quantified in clarified supernatants (g L-1).

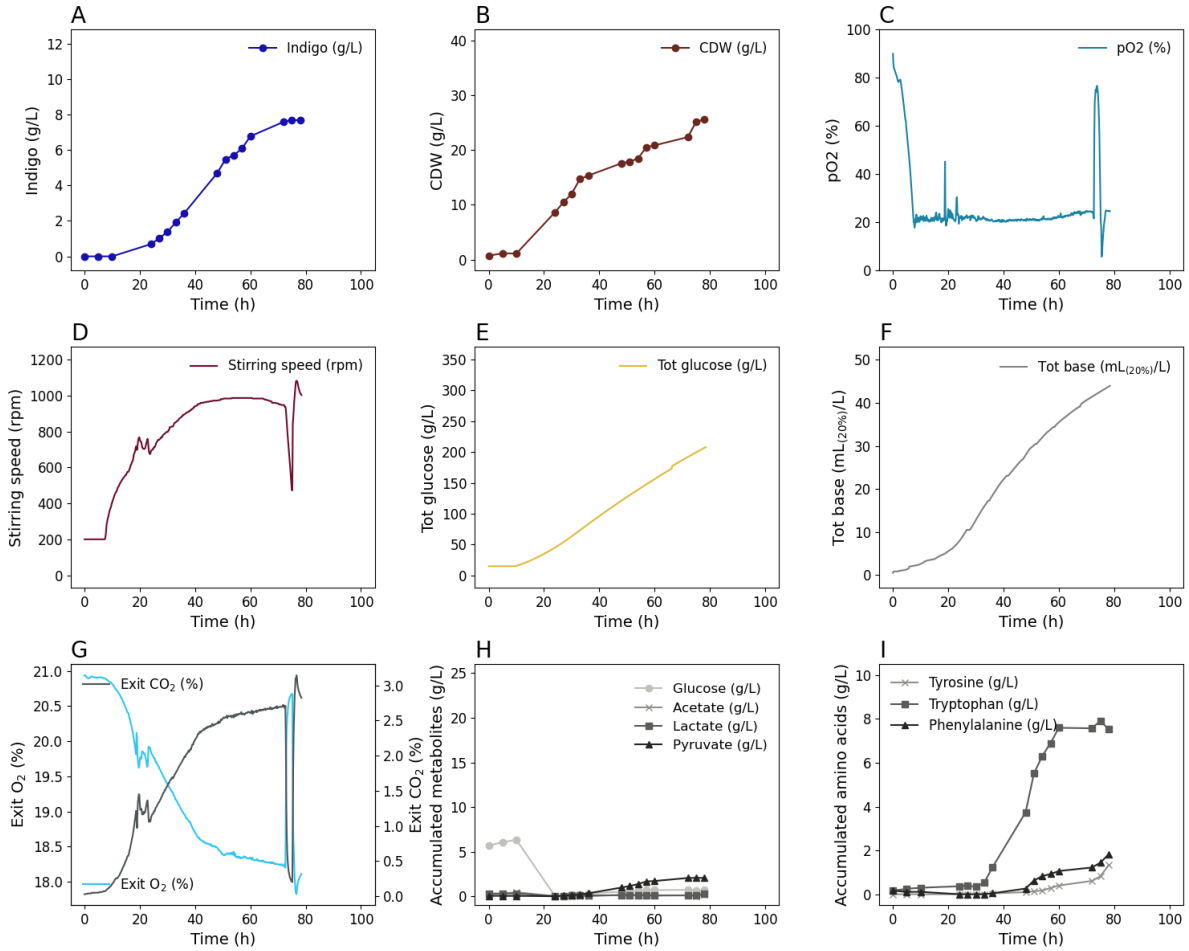

Fig. S6| G4 strain fermentation. Results obtained with the G4strain after process optimization. **a.** indigo accumulation (g L-1); **b.** biomass accumulation as cell-dry-weight (CDW, g L-1); **c.** pO_2 (dissolved oxygen, %); **d.** steering speed profile (rpm); **e.** total amount of glucose (g L-1) added to the bioreactor; **f.** total amount of base (mL of 20% NH_4OH per L) added in the bioreactor; **g.** O_2 and CO_2 air concentration at the off-gas (entry composition 20.8% and 0%, respectively); **h.** glucose and overflow metabolites accumulation (g L-1); **i.** aromatic amino acids accumulation as quantified in clarified supernatants (g L-1).

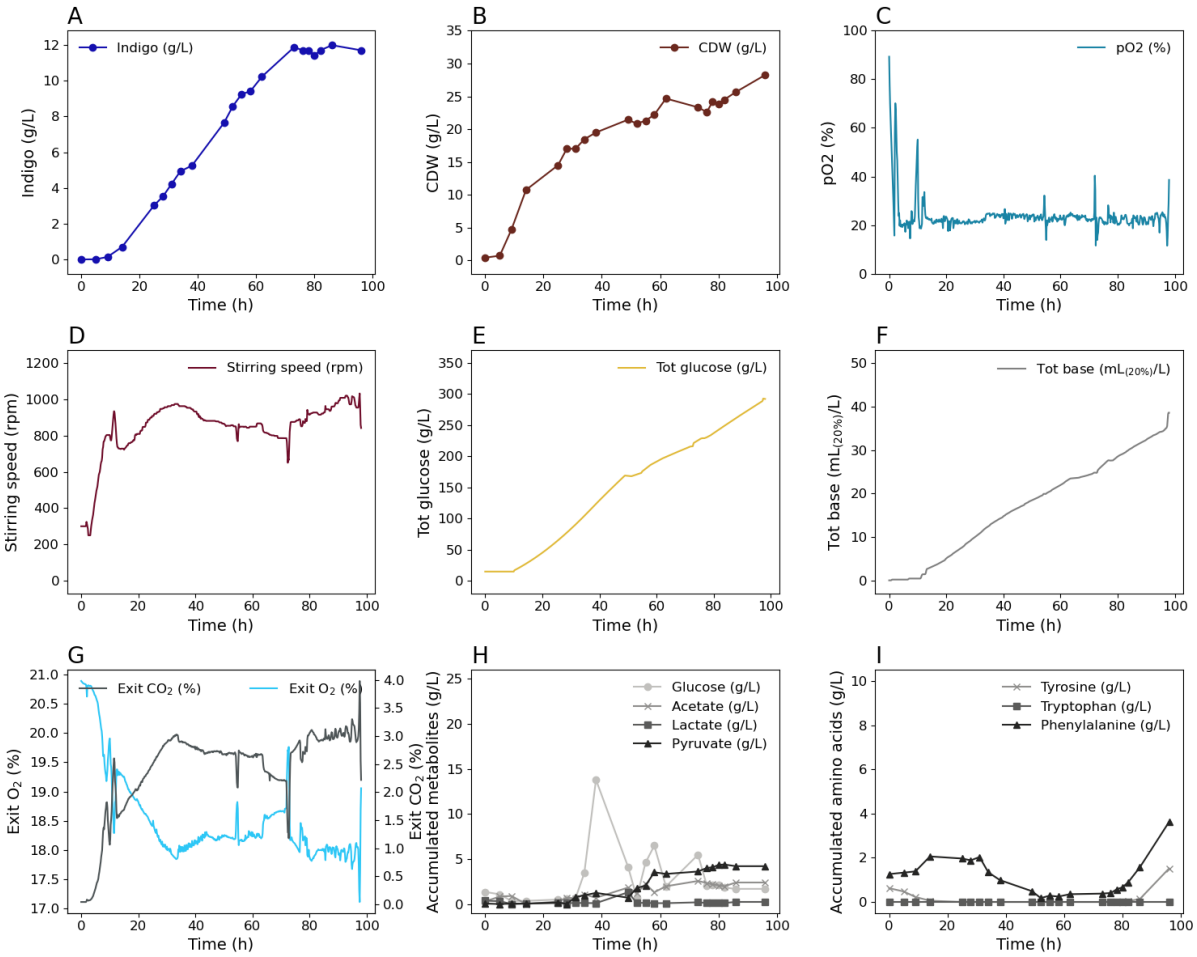

Fig. S7| G5 strain fermentation. Results obtained with the G5 strain after process optimisation. **a.** indigo accumulation (g L-1); **b.** biomass accumulation as cell-dry-weight (CDW, g L-1); **c.** pO_2 (dissolved oxygen, %); **d.** steering speed profile (rpm); **e.** total amount of glucose (g L-1) added in the bioreactor; **f.** total amount of base (mL of 20 % NH_4OH per L) added in the bioreactor; **g.** O_2 and CO_2 air concentration at the off-gas (entry composition 20.8% and 0%, respectively); **h.** glucose and overflow metabolites accumulation (g L-1); **i.** aromatic amino acids accumulation as quantified in clarified supernatants (g L-1).

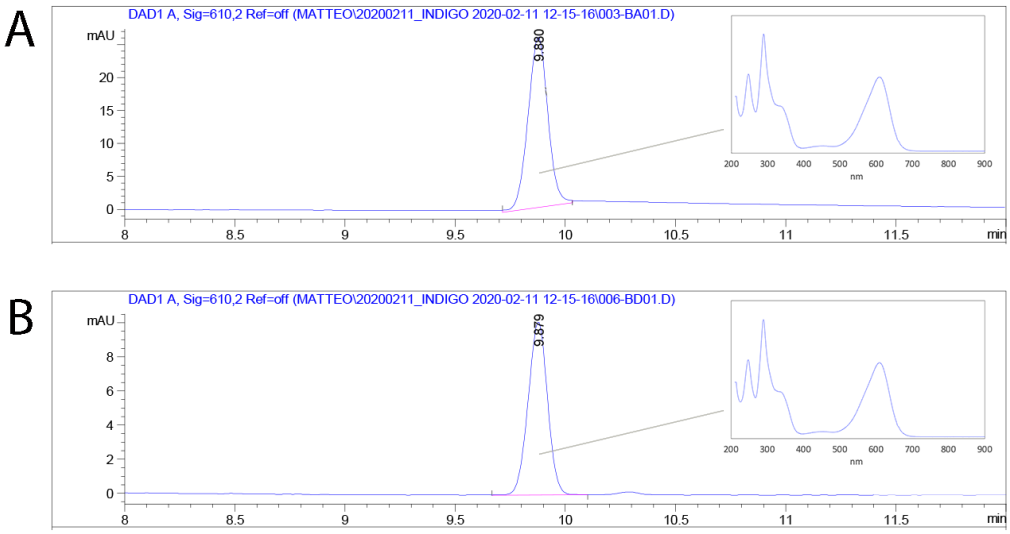

Fig. S8 | RP-HPLC analysis of indigo samples. Representative LC chromatograms of indigo analysed by reverse-phase HPLC and corresponding UV spectra recorded at the apex of the indigo peak. **a.** Indigo chemical standard (retention time [RT] = 9.880 min); **b.** Biologically produced indigo (RT = 9.879 min). HPLC method: indigo powder dissolved in DMSO at ca. 0.1 mg L-1 (5 µL) was loaded onto an Supersil AQ-C18, 5 µm, ID 4.6 mm x 150 mm; flow rate: 1 mL/min. Gradient: 7 min from 80:20 to 30:70 H_2O (0.01 % TFA)/MeCN; 4 min isocratic at 30:70 H_2O (0.01 % TFA)/MeCN; 1 min gradient back to 80:20 H_2O (0.01 % TFA)/MeCN (610 nm 1000 $AI_{9.88min}$ = 0.014 g_{indigo} L^{-1}).

Table S3| Indigo purification and fabric dying. Results obtained during screening of different purification protocols. The last condition (entry 6) selected for further studies and tested in replicates (n >3). For details on the purification procedure, refer to main text.

Entry	Acid	Acid conc. (% w/w)	Time (min)	Temperature (°C)	Purity (% w/w)	Dye strength (%)[a]	CMC DE[b]
1	-	-	-	-	68	57	n/a
2	-	-	-	-	91	102	n/a
3	HCl	4	1440	90	79	108	1.3
4	Sulfuric	10	1440	90	84	107	0.7
5	Citric	10	1440	90	80	111	1.4
6	Citric[c]	0.5	20	121	84 ± 5	105 ± 9	1.3 ± 0.2

[a] commercial synthetic indigo with 96% purity was used as a reference compound

[b] CMC-factor Delta E: Colour measurement committee factor variation value of the ellipsoid around a standard colour corresponding to hue, chroma and lightness. Commercial factor (cf) tolerance set at 1.0.

[c] technical replicate

n/a: not available

Table S4| Elemental analysis. Comparison of elemental composition of chemically synthesized indigo and indigo produced by microbial cultivation after biomass removal and three sedimentation-washing cycles.

Sample / Element	Carbon %	Hydrogen %	Nitrogen %	Oxygen %	Sulphur %
Chemically synthesized indigo (99.8 %)	74.8	4.3	10.6	12.1	0.0
Indigo by fermentation after sedimentation & washing (70 %)	64.7	5.1	11.4	16.5	**0.3**

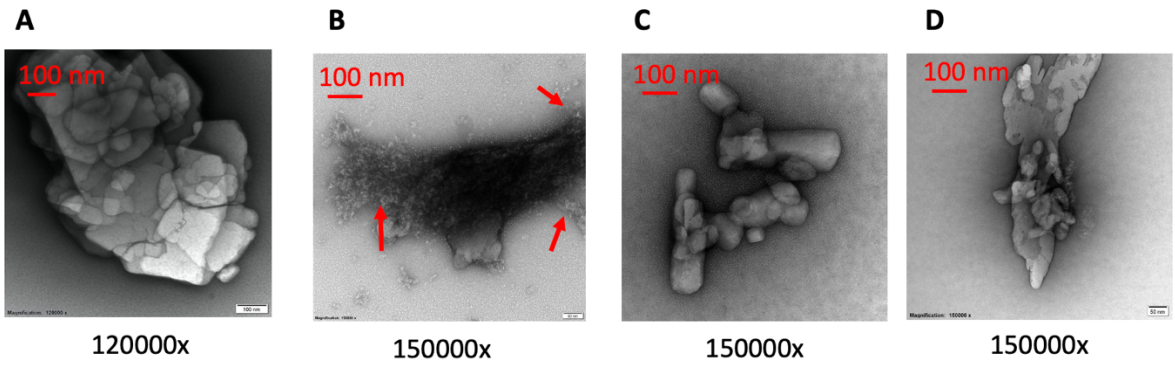

Fig. S9| Electron microscopy pictures of indigo particles. a. Chemically synthesized indigo; **b.** to **d.**: Indigo obtained from microbial production. **b.** Indigo particles after from microbial production after three washing-sedimentation cycles. Putative proteinaceous impurities adhered to indigo particles are marked with red arrows); **c.** indigo purified as in (b) but followed by reduction to leucoindigo, filtration, and re-oxidation; **d.** indigo purified as in (b) followed by temperature / citric acid treatment. Uranyl acetate was used as negative stain.

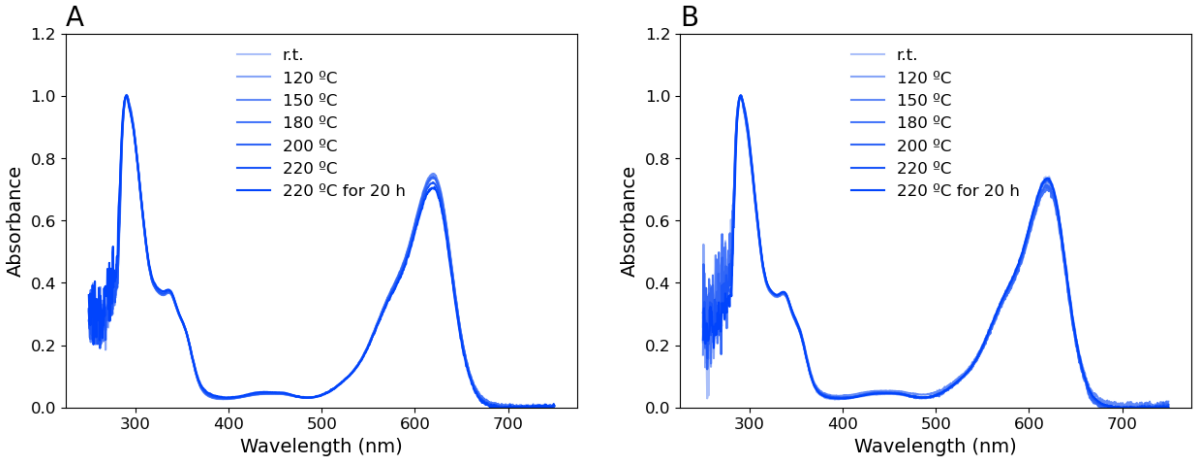

Fig. S10| Indigo stability at elevated temperature in acids. UV-Vis spectrum illustrating the stability of indigo treated for 10 hours (or 20 hours, if separately mentioned) at temperatures ranging from 120 to 220°C with **a.** 5 % sulfuric acid and **b.** 5 % citric acid. The experiments were carried out in a pressurized stainless-steel reactor, with samples for analysis taken after cooling of the indigo mixture to room temperature (r.t. = room temperature as control).

Table S5| Aromatic amine content in different indigo preparations. Quantification of aromatic amines aniline and N-methylaniline. Chemical indigo 96% routinely used for dyeing was test as benchmark (GC method details were not disclosed by Archroma's QC to us but the protocol described by Tahara *et al.*[1] may serve as a reference).

Entry	Batch name	Aniline (ppm)	N-methylaniline (ppm)
1	Chemical indigo 95% (reference in dying experiment)	5130.8	2237.6
2	Only decantation step (entry 1 Table S3)	n.d.	n.d.
3	Indigo-white method (entry 2 Table S3)	n.d.	n.d.
4	4% Hydrochloric acid (entry 3 Table S3)	n.d.	n.d.
5	10% Sulfuric acid (entry 4 Table S3)	n.d.	n.d.
6	10% Citric acid (entry 5 Table S3)	n.d.	n.d.
7	0.5 % Citric acid (entry 6 Table S3)	n.d.	n.d.

n.d.: not detectable (< 6.3 ppm)

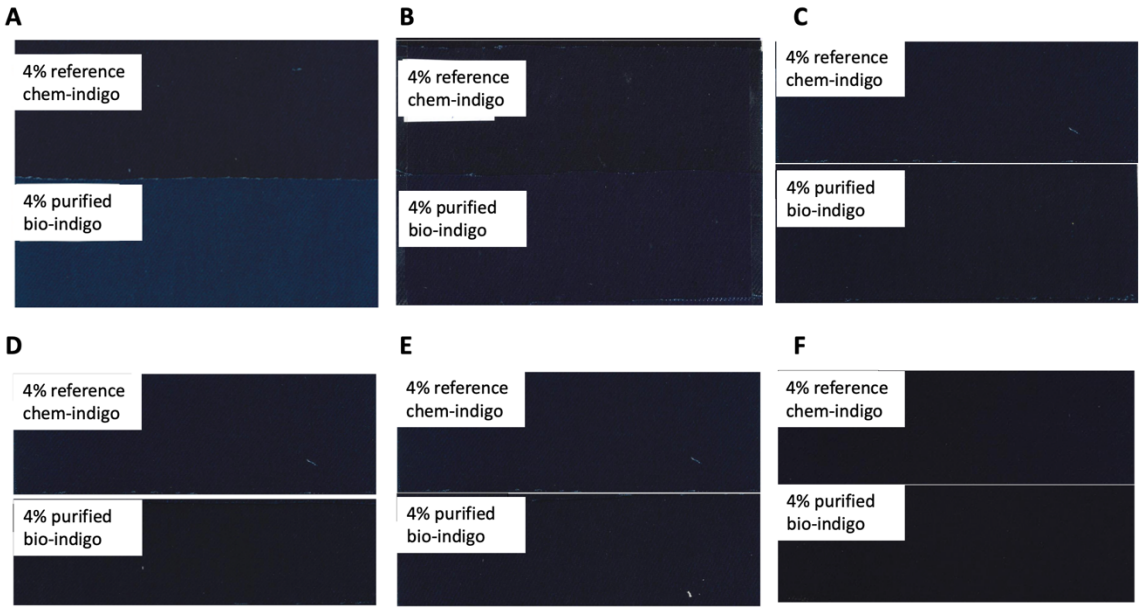

Fig. S11| Cotton fibre dying. Dyeing experiment on cotton fibre using **a.** Indigo after three sedimentation and washing cycles (refer to Table S3, entry 1); **b.** Indigo purified by the leucoindigo method (Table S3, entry 2); **c.** Indigo purified by HCl treatment (Table S3, entry 3); **d.** Indigo purified with H_2SO4 treatment (Table S3, entry 4); **e.** Indigo purified with 10% citric acid treatment (Table S3, entry 5); **f.** Indigo purified with 0.5 % citric acid treatment. c. - f. were all done at elevated temperature (Table S3, entry 6).

[1] M. Tahara, T. Kawakami and Y. Ikarashi, *J. AOAC Int.*, **2024**, *107*, 61–68.

Fig. S12| 500 L Pilot Scale. Image of the isolated indigo obtained from the 500 L pilot fermentation. A total of 5 kg was isolated after purification.

2 Media

Table S6| Fermentation media. Receipt for the preparation of the media: yeast extract and peptone were autoclaved in the reactor vessel in 0.45 L distilled water and the remaining components, separately sterilised by filtration (0.2 μm), and added prior inoculation. pH was adjusted to 7.00 in the bioreactor with NH_4OH

	Components	**Final concentration**
1	Yeast extract	24 g L^{-1}
2	Peptone	20 g L^{-1}
3	Glucose[b]	50 g L^{-1}
4	KH_2PO_4[b]	2.3 g L^{-1}
5	K_2HPO_4[b]	126 g L^{-1}
6	$MgSO_4$[b]	8 mM
7	VB1[b]	0.01 g L^{-1}
8	Trace elements[a, b]	2x
9	Antibiotics[b]	1x

[a] Trace elements (1000x): 1.5 g L^{-1} $MnCl_2$, 1.05 g L^{-1} $ZnSO_4$, 0.3 g L^{-1} H_2BO_4, 0.25 g L^{-1} Na2MoO4, 0.15 g L^{-1} $CuCl_2$, 0.84 g L^{-1} in NaEDTA, 4.87 g L^{-1} $FeSO_4$, 4.12 g L^{-1} $CaCl_2$, in 1 M HCl[3]

[b] mixed post individual sterilisation by filtration (0.2 μm)

Table S7| Feeding solution composition. Receipt for the preparation of the feeding solution: glucose was sterilised at pH 5 (0.01 mM HCl) by autoclaving, while the remaining components were separately sterilized and added prior to utilisation.

	Components	**Final concentration**
1	Glucose	600 g L^{-1}
2	Salts[a, c]	1x
3	Yeast Extract	5 g L^{-1}
4	Peptone	5 g L^{-1}

5	$MgSO_4$[c]	8 mM
6	VB1[c]	0.05 g L^{-1}
7	Trace elements [b, c]	6x
8	Antibiotics [c]	1x

[a] Feeding salts (20x): 140 g L^{-1} K_2HPO_4, 80 g L^{-1} $(NH_4)_2SO_4$, 50 g L^{-1} NH_4Cl, 60 g L^{-1} NaCl
[b] Trace elements (1000x): 1.5 g L^{-1} $MnCl_2$, 1.05 g L^{-1} $ZnSO_4$, 0.3 g L^{-1} H_2BO_4, 0.25 g L^{-1} Na2MoO4, 0.15 g L^{-1} $CuCl_2$, 0.84 g L^{-1} in NaEDTA, 4.87 g L^{-1} $FeSO_4$, 4.12 g L^{-1} $CaCl_2$, in 1 M HCl[2]
[c] mixed post individual sterilisation by filtration (0.2 µm)

3 Protein sequences

NDOb: Naphthalene dioxygenase from *Pseudomonas balearica* DSM 6083 (3 domains)

MTEKWIDAVALYEIPEGDVLGVTVEGKELALYEVEGEIYATDNLCTHGAARMSDGFLEGREIECPLHQGRFDVCTGRALCAPVTQN
IKTYPVKIEGQRVMIDLS

MNYKNKNLVSESGLTQKHLIHGDEELFQRELETIFARNWLFLTHDSLIPSPGDYVTAKMGVDEVIVSRQNDGSIRAFLNVCRHRGK
TLVHAEAGNAKGFVCSYHGWGFGANGELQSVPFEKELYGEALDKKCMGLKEVARVESFHGFIYGCFDEEAPSLKDYMGDAGWY
LEPMFKHSGGLELIGPPGKVIIKANWKAPAENFVGDAYHVGWTHAASLRTGQSVFSSLAGNAALPPEGAGLQMTSKYGSGMGV
LWDGYSGVHSADLVPELMAFGGAKQERLNKEIGEVRARIYRSHLNGTVFPNNSFLTCSGVFKVWHPIDANTTEVWTYAMVEKD
MPEDLKRRLVDAVQRTFGPAGFWESDDNDNMETESQNAKKYQSRDGDLVSNLGFGGDVYGDEVYPGIVGKSAIGETSYRGFYR
AYGAHISSSSWAEFEDVSKNWHTELAKTTDR

MMINIQEDKLVSAHDAEEFLRFFNSGDEALQQEATTLLTREAHLLDIQAYRAWLEHCVDSEVKYQIISRELRSASERRYQLNETMNI
FNENYEQLEVRVAHQLDPQNWGNSPKVRFTRFITNIQAAMDENEDLLHIRSNLIVHRARRGNQVDVFYATREDKWKRGEDGAR
KLVQRLIDYPERTFQTHNVMIFM-

IH: Isatin hydrolase from *Pseudomonas putida*

MTSIKLLAESLLKDKIKIVDLSHTLRSEFPTLTLPPQFGQTWAFKKEEISRYDDRGPAWYWNNFSCGEHTGTHFDAPVHWVTGESV
PENSVDRIEPQRFMAPAVVIDASKEVLENPDWVLEPEFIQEWEKLHGRIEAGSWFLLRTDWSKKINNPLEFANLIDGAPHTPGPS
QRTVEWLIAERDVVGFGVETINIDAGLSGRWEVPYPCHNKMLGAGRFGLQCLNNLDLLPPTGAVIISAPLKIEDGSGSPLRVLAIFD
RE

BX1: Indole-3-glycerol phosphate lyase *Zea mays*

MAFAPKTSSSSSLSSALQAAQSPPLLLRRMSSTATPRRRYDAAVVVTTTTTARAAAAAVTVPAAPPQAPAPAPVPPKQAAAPAERR
SRPVSDTMAALMAKGKTAFIPYITAGDPDLATTAEALRLLDGCGADVIELGVPCSDPYIDGPIIQASVARALASGTTMDAVLEMLRE
VTPELSCPVVLLSYYKPIMSRSLAEMKEAGVHGLIVPDLPYVAAHSLWSEAKNNNLELVLLTTPAIPEDRMKEITKASEGFVYLVSVN
GVTGPRANVNPRVESLIQEVKKVTNKPVAVGFGISKPEHVKQIAQWGADGVIIGSAMVRQLGEAASPKQGLRRLEEYARGMKNA
LP

TnaA: Tryptophanase from *E. coli*

MENFKHLPEPFRIRVIEPVKRTTRAYREEAIIKSGMNPFLLDSEDVFIDLLTDSGTGAVTQSMQAAMMRGDEAYSGSRSYYALAESV
KNIFGYQYTIPTHQGRGAEQIYIPVLIKKREQEKGLDRSKMVAFSNYFFDTTQGHSQINGCTVRNVYIKEAFDTGVRYDFKGNFDLE
GLERGIEEVGPNNVPYIVATITSNSAGGQPVSLANLKAMYSIAKKYDIPVVMDSARFAENAYFIKQREAEYKDWTIEQITRETYKYAD

[2] H. Preusting, et al., *Biotechnol. Bioeng.*, **1993**, *42*, 550–556

MLAMSAKKDAMVPMGGLLCMKDDSFFDVYTECRTLCVVQEGFPTYGGLEGGAMERLAVGLYDGMNLDWLAYRIAQVQYLVD
GLEEIGVVCQQAGGHAAFVDAGKLLPHIPADQFPAQALACELYKVAGIRAVEIGSFLLGRDPKTGKQLPCPAELLRLTIPRATYTQTH
MDFIIEAFKHVKENAANIKGLTFTYEPKVLRHFTAKLKEV

NDO: Naphthalene dioxygenase from *Pseudomonas putida* (4 domains)

MELLIQPNNRLISFSPGANLLEVLRENGVAISYSCMSGRCGTCRCRVTDGSVIDSGAGSGLPNLVDEHYVLACQS
VLTHNCAIEIPETDEIVTHPARIIKGTVVAVESPTHDIRRLRVRLAKPFEFSPGQYATLQFSPEHARPYSMAGLP
DDQEMEFHIRKVPGGRVTEYVFEHVREGTSIKLSGPLGTAYLRQNHTGPMLCVGGGTGLAPVLSIVRGALKLGMT
NPILLYFGVRSQQDLYDAERLHKLAADHPQLTVHTVIAMGPINESQRAGLVTDVIEKDIISLAGWRAYLCGAPAM
VEALCTVTKHLGISPEHIYADAFYPGGI

MTEKWIEAVALSDIPEGDVLGVTVEGKELALYEVEGEIYATDNLCTHGAARMSDGYLEGREIECPLHQGRFDVCT
GRALCAPVTENIKTYAVKIENLRVMIDLSGEF

MNYKNKILVSESGLTQKHLIHGDEELFQHELRTIFARNWLFLTHDSLIPSPGDYVTAKMGIDEVIVSRQSDGSIR
AFLNVCRHRGKTLVNAEAGNAKGFVCSYHGWGFGSNGELQSVPFEKELYGESLNKKCLGLKEVARVESFHGFIYG
CFDQEAPPLMDYLGDAAWYLEPIFKHSGGLELVGPPGKVVIKANWKAPAENFVGDAYHVGWTHASSLRSGESIFA
SLAGNAVLPPEGAGLQMTSKYGSGMGVLWDGYSGVHSADLVPELMAFGGSKQERLNKEIGDVRARIYRSHLNCTV
FPNNSMLTCSGVFKVWNPIDANTTEVWTYAIVEKDMPEDLKRRLADSVQRTFGPAGFWESDDNDNMETASQNGKK
YQSRDSDLLSNLGFGKDVYGDAVYPGVVGKSAIGETSYRGFYRAYQAHVSSSNWAEFEDASSTWHTELTKTTDR

MMINIQEDKLVSAHDAQEFLRFFNCHDAALQQEATTLLNREAHLLDIQAYRAWLEHCVGSEVQYQVISRELRAAS
ERRYKLNEAMNVYNENFQQLKVRIEHQLDPQNWSNSPKLRFTRFITNVQAARDVDDEELLHIRSNVILHRARRGN
QVDVFYAAREDKWKRGEGGVRKLVQRFVDYPERILQTHNLMVFL

SMO: Styrene monooxygenase from *Pseudomonas sp.* (2 domains)

MKKRIGIVGAGTAGLHLGLFLRQHDVDVTVYTDRKPDEYSGLRLLNTVAHNAVTVQREVALDVNEWPSEEFGYFG
HYYYVGGPQPMRFYGDLKAPSRAVDYRLYQPMLMRALEARGGKFCYDAVSAEDLEGLSEQYDLLVVCTGKYALGK
VFEKQSENSPFEKPQRALCVGLFKGIKEAPIRAVTMSFSPGHGELIEIPTLSFNGMSTALVLENHIGSDLEVLAH
TKYDDDPRAFLDLMLEKLGKHHPSVAERIDPAEFDLANSSLDILQGGVVPAFRDGHATLNNGKTIIGLGDIQATV
DPVLGQGANMASYAAWILGEEILAHSVYDLRFSEHLERRRQDRVLCATRWTNFTLSALSALPPEFLAFLQILSQS
REMADEFTDNFNYPERQWDRFSSPERIGQWCSQFAPTIAA

MTLKKDMAVDIDSTNFRQAVALFATGIAVLSAETEEGDVHGMTVNSFTSISLDPPTVMVSLKSGRMHELLTQGGR
FGVSLLGESQKVFSAFFSKRAMDDTPPPAFTIQAGLPTLQGAMAWFECEVESTVQVHDHTLFIARVSACGTPEAN
TPQPLLFFASRYHGNPLPLN

mFMO: Flavine monooxygenase from *Methylophaga sp.*

MATRIAILGAGPSGMAQLRAFQSAQEKGAEIPELVCFEKQADWGGQWNYTWRTGLDENGEPVHSSMYRYLWSNGP
KECLEFADYTFDEHFGKPIASYPPREVLWDYIKGRVEKAGVRKYIRFNTAVRHVEFNEDSQTFTVTVQDHTTDTI
YSEEFDYVVCCTGHFSTPYVPEFEGFEKFGGRILHAHDFRDALEFKDKTVLLVGSSYSAEDIGSQCYKYGAKKLI
SCYRTAPMGYKWPENWDERPNLVRVDTENAYFADGSSEKVDAIILCTGYIHHFPFLNDDLRLVTNNRLWPLNLYK
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PVAKEA

4 Plasmids

Table S8| Plasmid summary

Name	Purpose	Origin of replication (ORI)	Resistance marker
P1	Over-expression of BX1 and IH	p15A	Kan
P2	Over-expression of NDOb	pMB1	Amp
P3	Over-expression of TnaA	pUC	Cm
P4	Over-expression of NDO	pBR322	Amp
P5	Over-expression of SMO	pBR322	Kan
P6	Over-expression of mFMO	pBR322	Kan
GM1	pCas (λ-red recombinase and Cas9), plasmid	p15A	Kan
GM2	pTarget (sgRNA and template) plasmid for introducing the gene modification J23119-*aroG*^{S180F}	pBR322	Amp
GM3	pTarget (sgRNA and template) plasmid for introducing the gene modification J23119-*trpE*^{S40F}	pBR322	Amp
GM4	pTarget (sgRNA and template) plasmid for introducing the gene modification pL-*aroL*	pBR322	Amp
GM5	pTarget (sgRNA and template) plasmid for introducing the gene modification WG219-*ahpC*	pBR322	Amp
GM6	pTarget (sgRNA and template) plasmid for introducing the gene modification *pta*^{P69L}	pBR322	Amp

P1

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P2

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P3

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