Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

Supporting Information for:

Assessing the substrate specificity of a micropollutant degrading strain: generalist or specialist?

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S1 Supplementary Methods

a) ABT Mineral medium

Cells were grown in ABT mineral medium which consists of four mineral solutions (Table S1) as well as a trace element solution based on Sørensen and Aamand (2003). All solutions were stored individually at 4 ºC and assembled and diluted in milliq water before the experiment. The final (100%) mineral solution contained 10% of solution 1, 0.1% of each solution 2-4 and 0.1% of trace elements and was sterile filtered before usage.

Table S1. Mineral solution 1-4.

Substance	Amount (g/L)	CAS
Solution 1		
$(NH_4)_2\,SO_4$	20	7783-20-2
$Na_2\,HPO_4$	60	7558-79-4
KH_2PO_4	30	7778-77-0
NaCl	30	7647-14-5
Solution 2		
MgCl2*6H2O	2030	7791-18-6
Solution 3		
CaCl2	111	10043-52-4
Solution 4		
Fe-EDTA	34.4	-

b) Selective plating

Correlations between cell concentrations estimated from different plating media are shown in Fig. S1a-d). The correlation between cell concentrations estimated by plating and by DIC microscopy is shown in Fig. S1e).

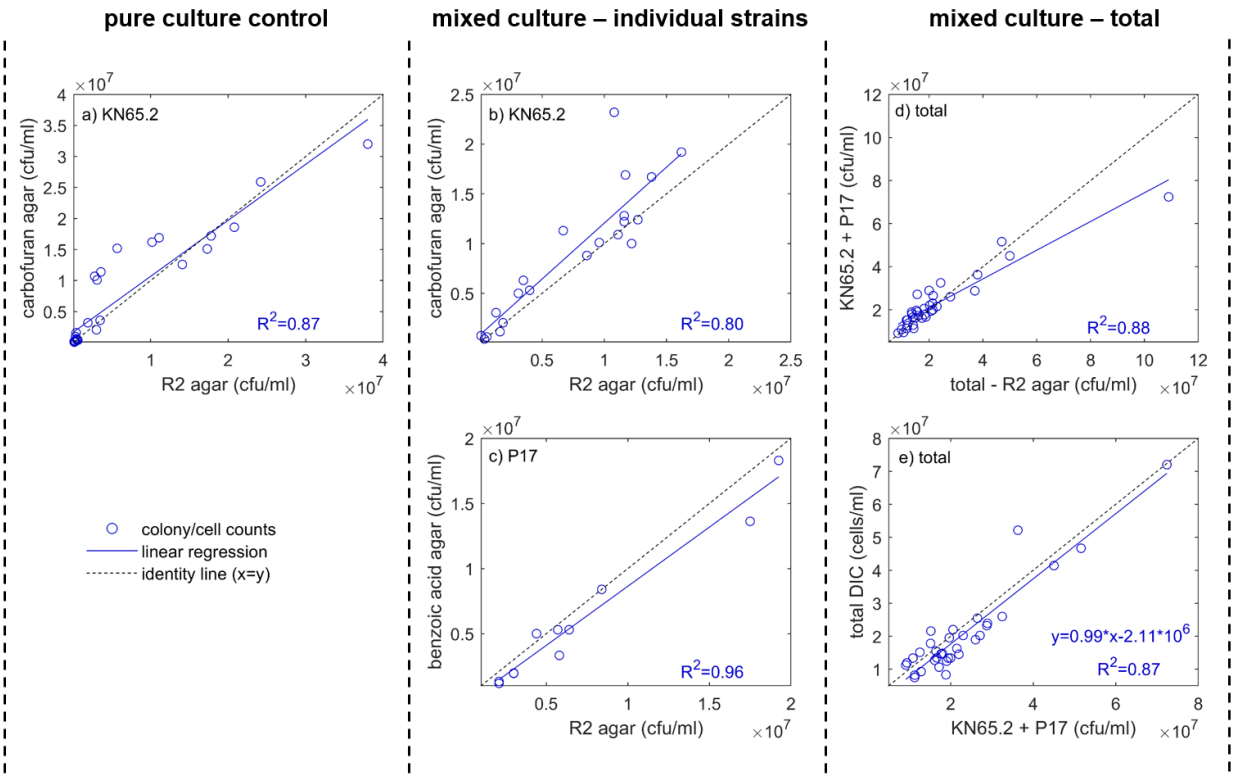

Figure S1. a) Correlation between R2 agar and carbofuran agar for colony counts of pure *KN65.2* controls; b-c) correlation between R2 agar and selective medium (carbofuran -/benzoic acid - agar) for colony counts of individual strains in mixed culture (individual strains on R2 agar were identified based on physical characteristics); d-e) correlation plots for total colony/cell counts of mixed cultures, determined with different methods. *KN65.2 + P17* refers to the total concentration (cfu/ml) of the mixed culture, determined by summing up *KN65.2* concentration, measured on selective carbofuran agar, and *P17* concentration, measured on selective benzoic acid agar.

c) Calculation of the interaction coefficient (I)

The interaction coefficient I was calculated as shown in equation SEq1.

$$\text{SEq1.}\quad W=\frac{\ln\left(\frac{N(t_{max})_{\text{OMP degrader}}}{N(t_0)_{\text{OMP degrader}}}\right)}{\ln\left(\frac{P(t_{max})_{\text{OMP degrader}}}{P(t_0)_{\text{OMP degrader}}}\right)} \qquad (1)$$

where N refers to cell concentrations of mixed culture experiments, while P refers to cell concentrations of pure culture experiments. Index t_0 refers to the starting cell concentration, while index t_{max} refers to the plating point showing the maximum specific cell concentration of the OMP degrader *KN65.2* (cfu/ml) in the mixed culture (N) or the pure culture (P).

S2 Supplementary Results

a) Abiotic controls

Abiotic controls confirmed the absence of abiotic transformations (Table S2), since they showed stable carbon concentrations over time. Average carbon concentrations of abiotic controls throughout experiments are shown in Table S2 for the different compounds.

Table S2. Average carbon concentration and standard deviation for abiotic controls throughout experiments (from time = 0 h to the end sampling point), for each test compound, including data from pure (*KN65.2*, *P17*) and mixed (*KN65.2* + *P17*) culture experiments.

compound	average concentration [mg C /L]
4-hydroxybenzaldehyde	8.08 ± 0.41
4-hydroxybenzoic acid	7.56 ± 0.29
L-tyrosine	8.31 ± 0.60
p-coumaric acid	8.77 ± 0.78
ferulic acid	8.29 ± 0.41
vanillic acid	8.60 ± 0.35
benzoic acid	8.84 ± 0.90
L-phenylalanine	9.13 ± 0.72
L-tryptophan	9.24 ± 0.55
cinnamic acid	9.09 ± 0.61
piceol	7.17 ± 1.95
indole	10.30 ± 1.17
salicylic acid	9.12 ± 0.48
carbofuran	8.81 ± 0.75
total average & variance	**8.67 ± 0.77**

b) Growth of the OMP degrader strain *KN65.2*

Fig. S2 shows carbofuran biodegradation curves for *KN65.2* after pre-culturing the strain on different auxiliary carbon sources.

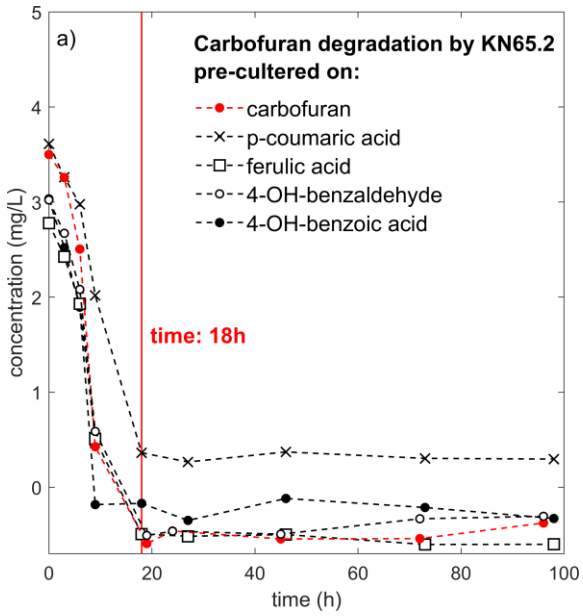

Figure S2. Carbofuran biodegradation curves for *KN65.2* after pre-culturing the strain on different carbon sources.

Fig. S3 and Fig. S4 show results from pure culture experiments with *KN65.2* (standard experimental conditions: pre-growth of *KN65.2* on carbofuran). Biodegradation curves for the pesticide carbofuran and for all six DOM constituents (referred to as auxiliary compounds) that supported the growth of the OMP degrader *KN65.2* are shown in Fig. S3. Corresponding growth curves are shown in Fig. S4. Degradation- and growth curves for the six compounds (cinnamic acid, salicylic acid, benzoic acid, piceol, indole, L-phenylalanine, L-Tryptophan) that did not support the growth of *KN65.2* are not shown.

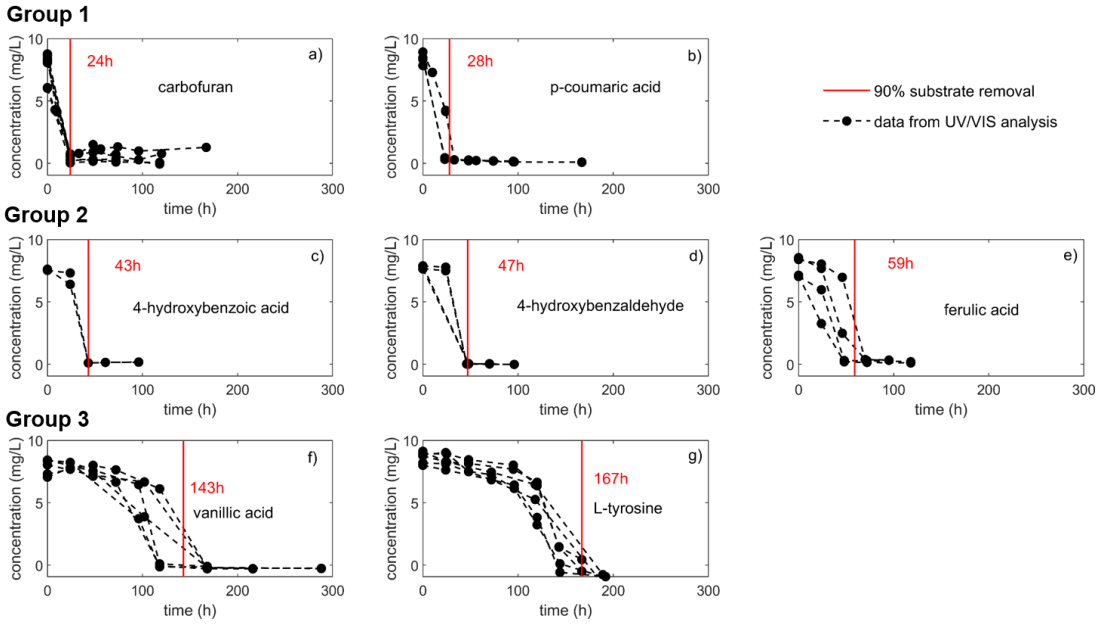

Figure S3. Biodegradation curves for the pesticide carbofuran and for auxiliary substrates, for strain *KN65.2*. Curves are sorted by the time passed to remove more than 90% of the substrate and the according biodegradation groups are indicated (group 1: degradation ≤ 28 h, group 2: degradation between 43 h and 59 h, group 3: degradation ≥ 143 h).

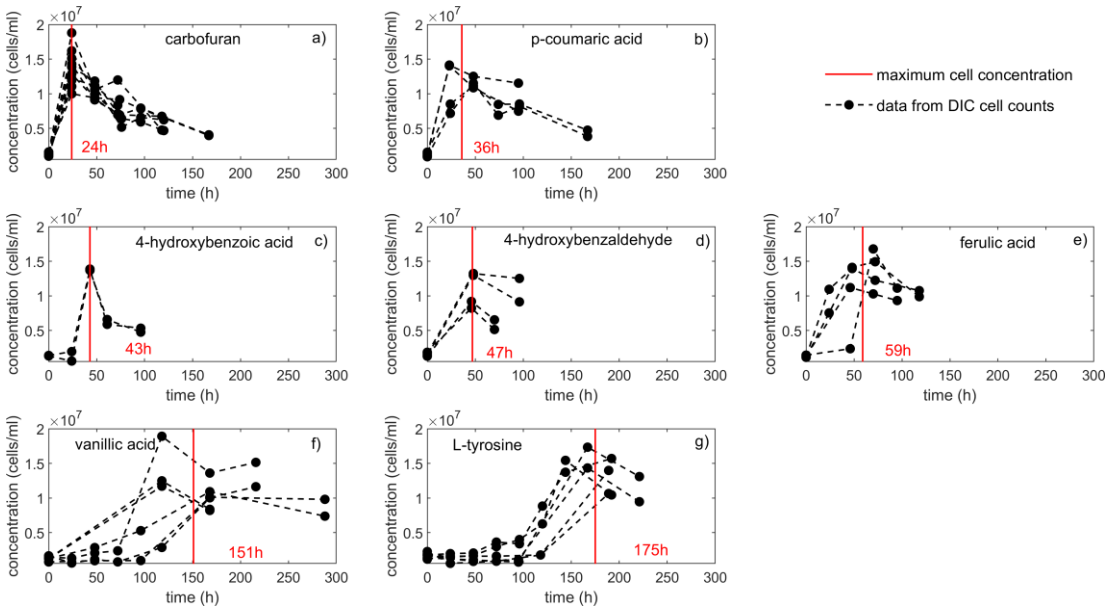

Figure S4. Growth curves for the pesticide carbofuran and for degradable auxiliary substrates, for strain *KN65.2*. Curves a-g correspond to the according biodegradation curves in Fig. S3 (a-g).

c) Cell size of *KN65.2* and *P17*

KN65.2 and *P17* have approximately the same cell size (Fig. S5). This allowed us to directly compare yields (cells/mg C) obtained for experiments with *KN65.2* and with *P17*.

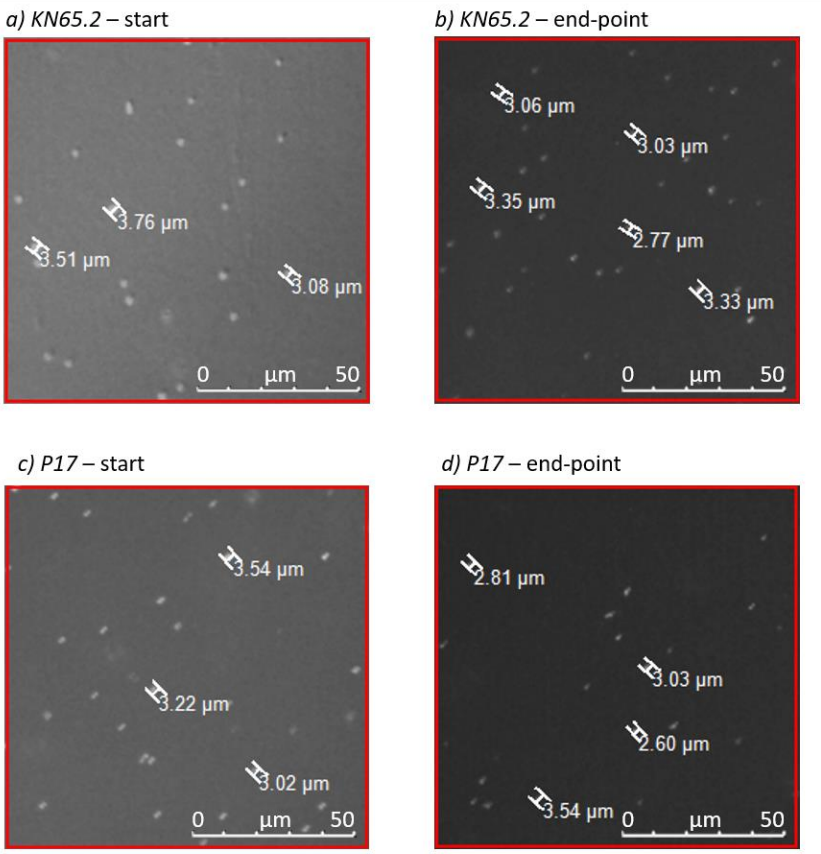

Figure S5. Microscopic images showing the cell diameter of *KN65.2* and *P17* before (a, c) and after growth on 4-hydroxybenzaldehyde (b, d).

d) Growth of the generalist *P17*

Biodegradation curves for all six compounds that support the growth of the generalist *P17* in pure culture are shown in Fig. S6. Corresponding growth curves are shown in Fig. S7. Degradation- and growth curves for the six compounds (cinnamic acid, *p*-coumaric acid, ferulic acid, vanillic acid, salicylic acid, piceol, indole) that did not support the growth of *P17* are not shown.

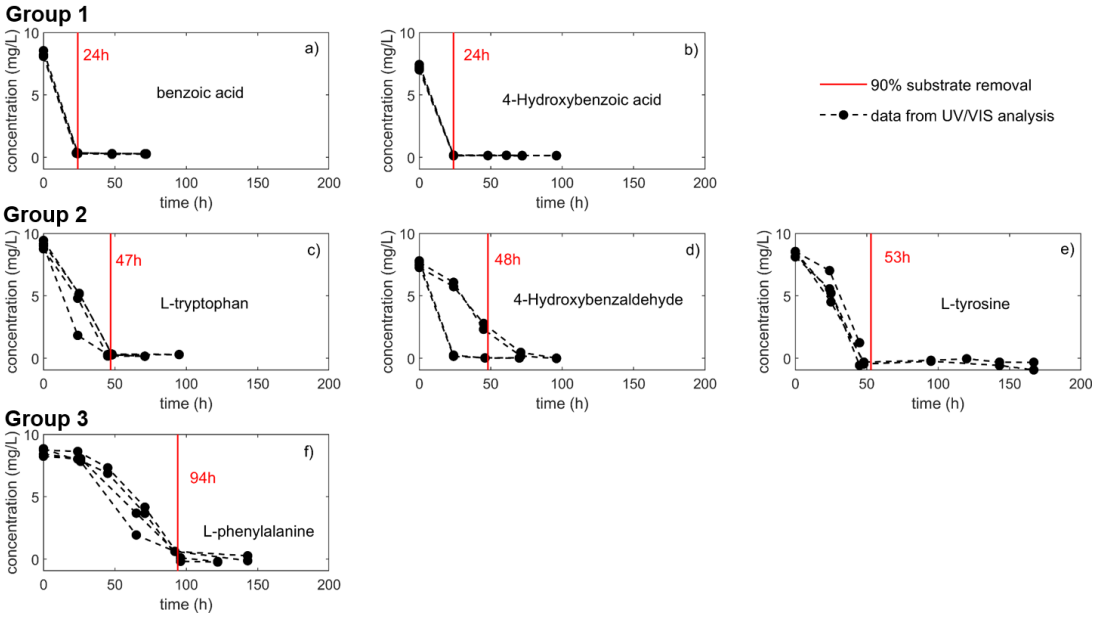

Figure S6. Biodegradation curves for auxiliary substrates for the generalist, *P17*. Curves are sorted by the time passed to remove more than 90% of the substrate and the according biodegradation groups are indicated (group1: degradation ≤24 h, group 2: degradation between 47 h and 53 h, group3: degradation ≥94 h).

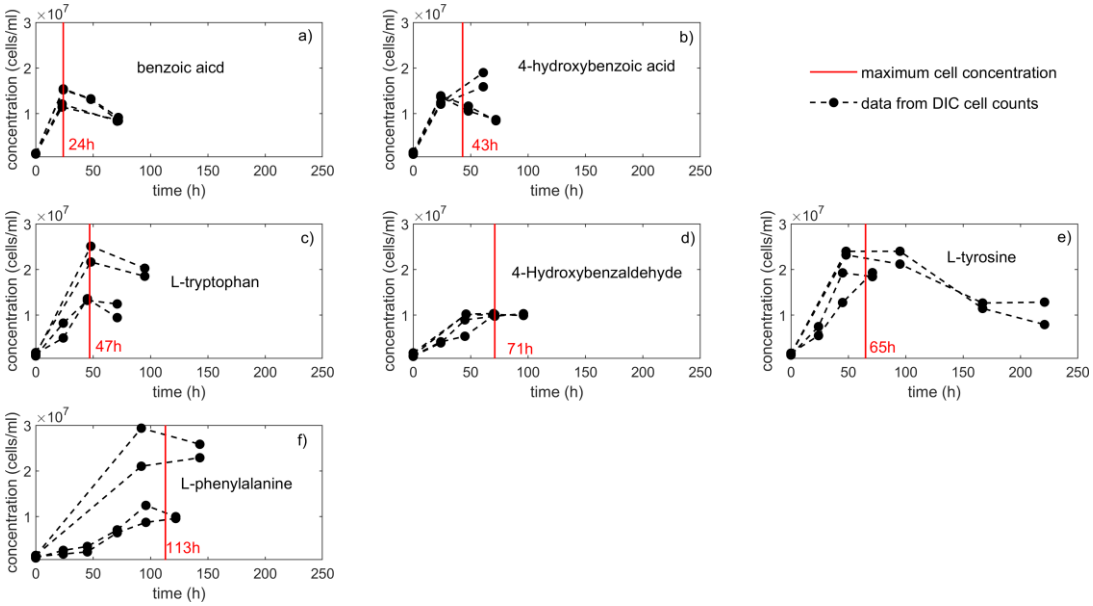

Figure S7. Growth curves for the growth of *P17* on auxiliary substrates. Curves a-f correspond to the according biodegradation curves in Fig. S6 (a-f).

e) Mixed culture experiments

The comparison between the maximum total yields of mixed culture experiments and pure culture experiments is shown in Fig. S8.

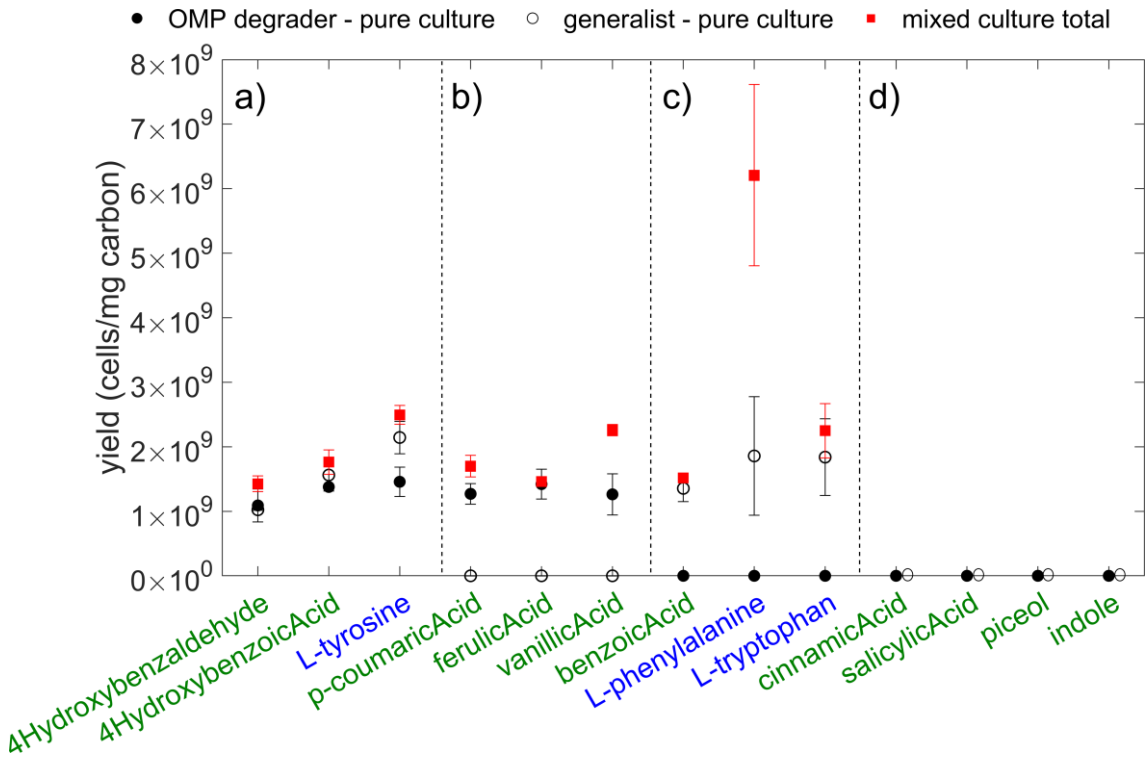

Figure S8. Maximum total yields of the mixed culture (red, square) compared to the OMP degrader *KN65.2* in pure culture (black, filled circle), and the general degrader *P17* in pure culture (black, empty circle), for compounds supporting the growth of both strains (a), only the growth of *KN65.2* (b), only the growth of *P17* (c), do not support the growth of any of the two strains (d). Starting cell concentrations were 2 x 10^6 cells/ml for mixed cultures and 1 x 10^6 cells/ml for pure cultures. Starting substrate concentrations were 0.01 g C/L for all experiments. Compounds not containing N are shown in green, while compounds containing N are shown in blue.

Biodegradation curves for all compounds tested in mixed culture experiments (*KN65.2 + P17*) are shown in Fig. S9. Corresponding growth curves are shown in Fig. S10.

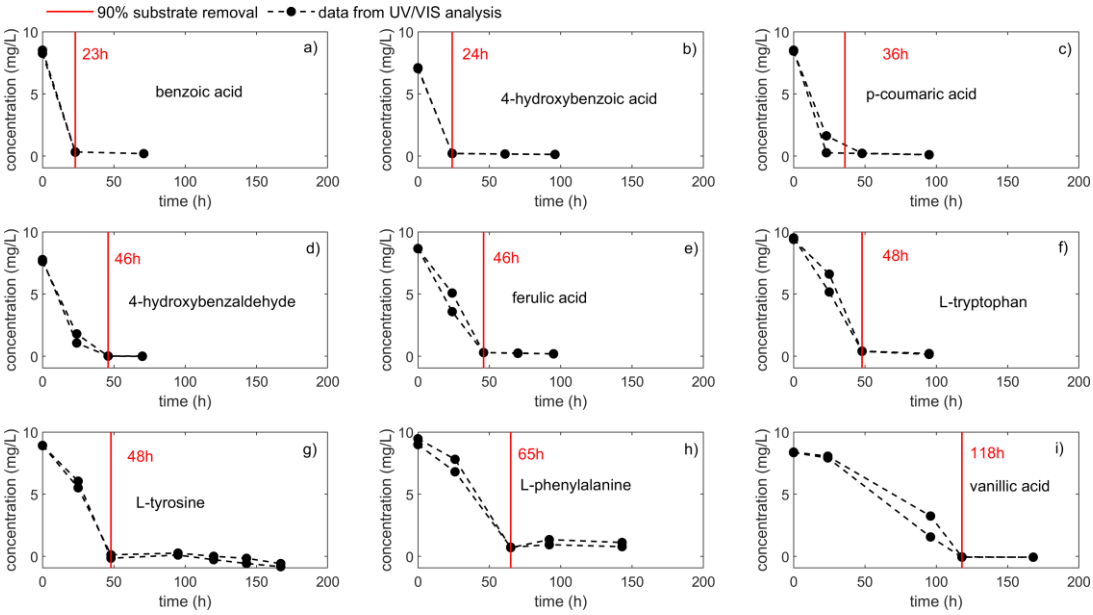

Figure S9. Biodegradation curves for auxiliary substrates for mixed culture experiments (*KN65.2* and *P17*). Curves are sorted by the time passed to remove more than 90% of the substrate.

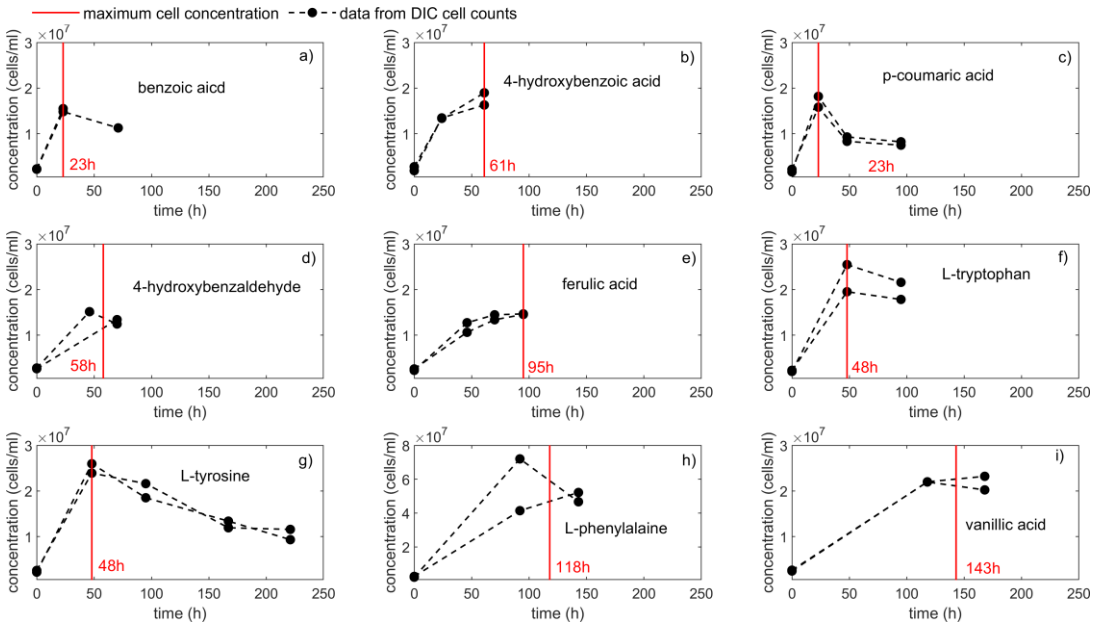

Figure S10. Growth curves for the growth of the mixed culture (*KN65.2* and *P17*) on auxiliary substrates. Curves a-i correspond to the according biodegradation curves in Fig. S9 (a-i).

Comparison of all biodegradation curves (pure - OMP degrader, pure - generalist, mixed culture) is shown in Fig. S11. Corresponding growth curves are shown in Fig. S12.

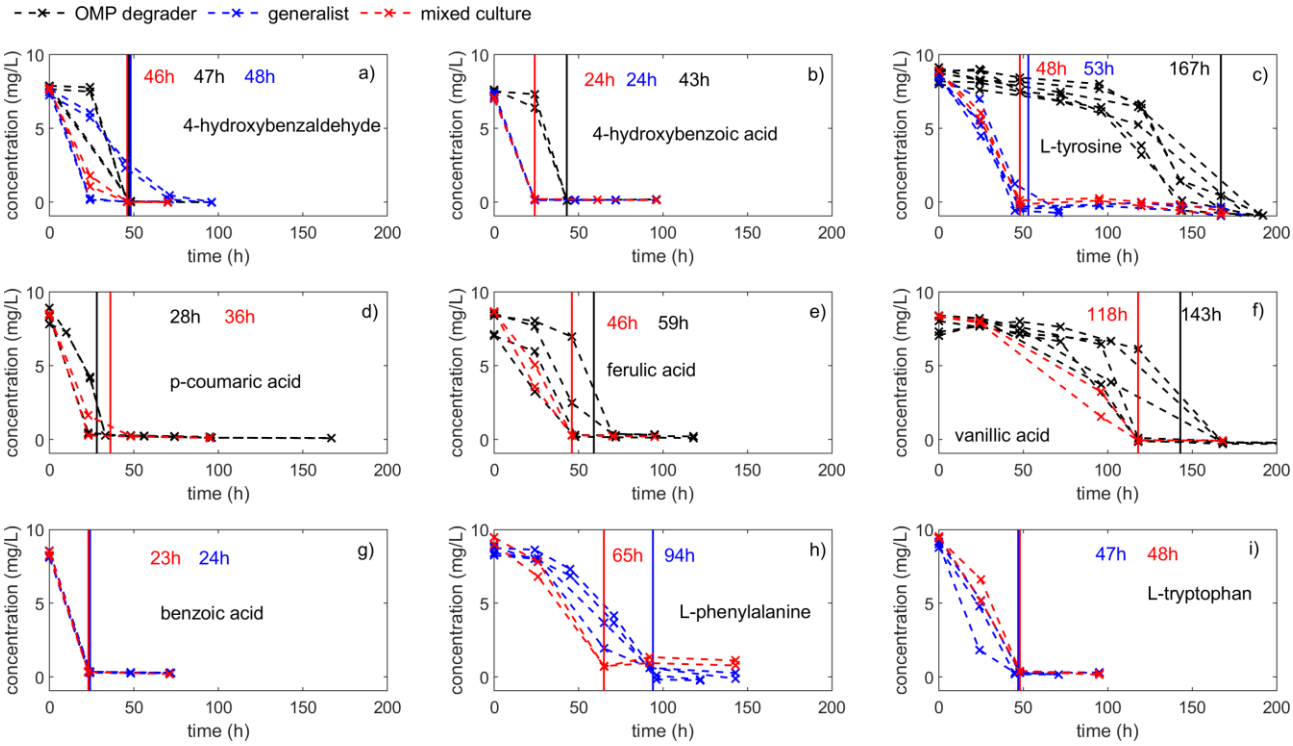

Figure S11. Biodegradation curves for auxiliary substrates for pure culture experiments (OMP degrader – black, generalist – blue) and mixed culture experiments (OMP degrader + generalist – red). a-c) Auxiliary substrates which are degraded by both strains. d-f) Auxiliary substrates which are only degraded by the OMP degrader. g-i) Auxiliary substrates which are only degraded by the generalist. Vertical lines indicate the time at which 90% of the substrate was removed.

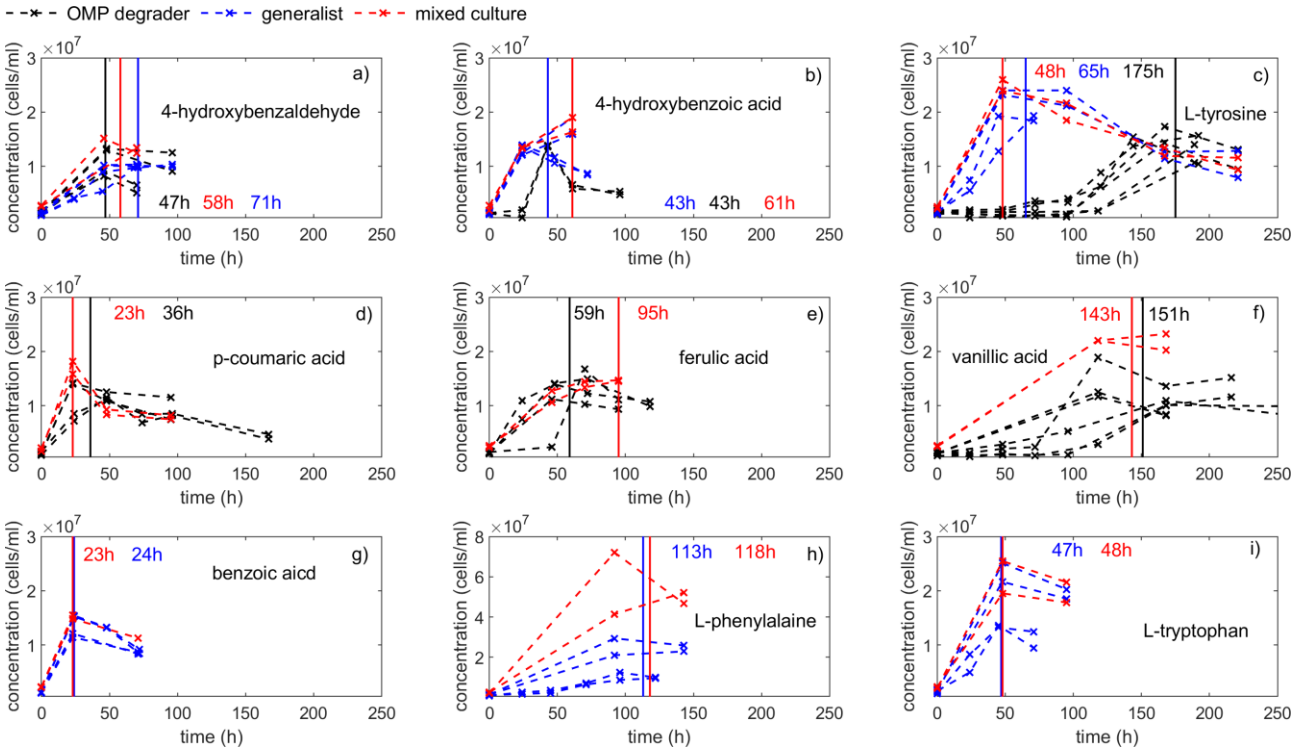

Figure S12. Growth curves for the growth of pure strains (OMP degrader – black, generalist – blue) and for the mixed culture (OMP degrader + generalist – red) on auxiliary substrates. Curves a-i correspond to the according biodegradation curves in Fig. S11 (a-i).

Pure culture control experiments, carried out in parallel to mixed culture experiments, confirmed no growth of the generalist, *P17* on *p*-coumaric-, ferulic- and vanillic acid (Fig. S13).

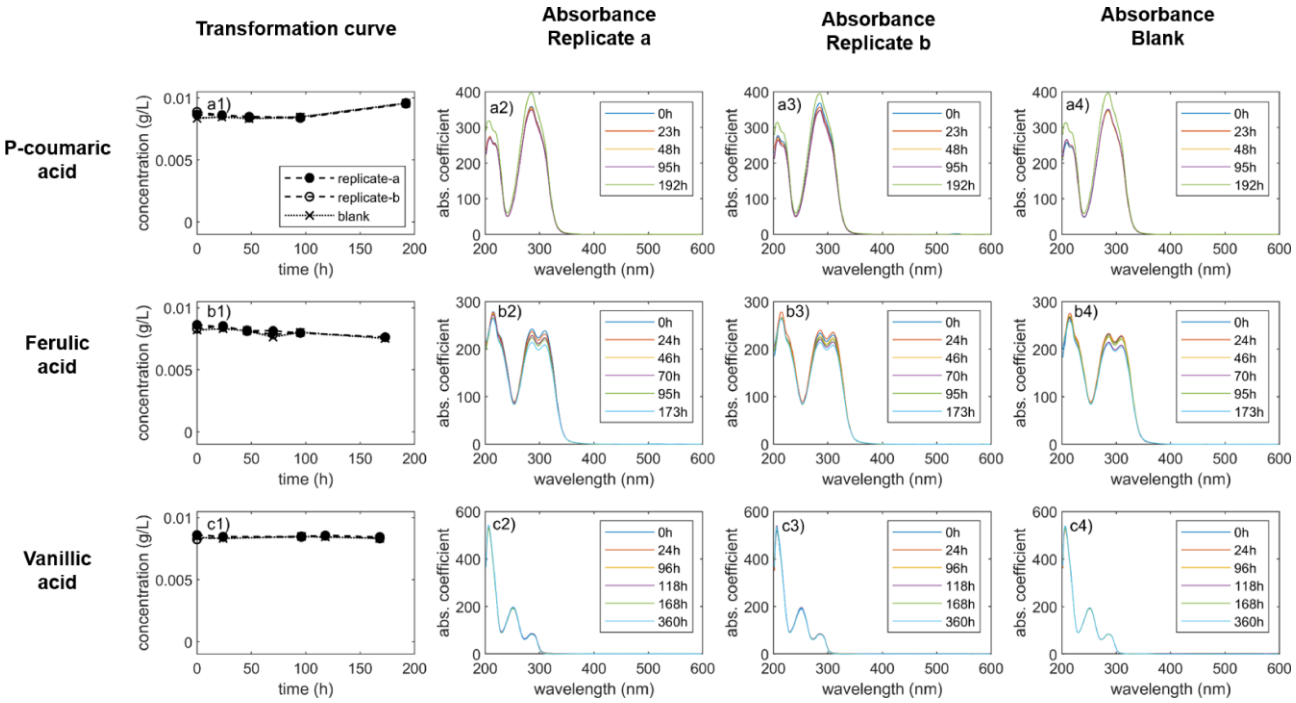

Figure S13. Biodegradation curves and absorbance data for pure culture controls containing the generalist, *P17* and a) *p*-coumaric acid, b) ferulic acid, and c) vanillic acid. Stable substrate concentrations and absorbance signals in all cases confirm no growth of *P17* on the three represented compounds.

References

Sørensen, S.R., Aamand, J., 2003. Rapid mineralisation of the herbicide isoproturon in soil from a previously treated Danish agricultural field. Pest Manag. Sci. 59, 1118–1124. https://doi.org/10.1002/ps.739