Supporting Information

Continuous oxyfunctionalizations catalyzed by unspecific peroxygenase

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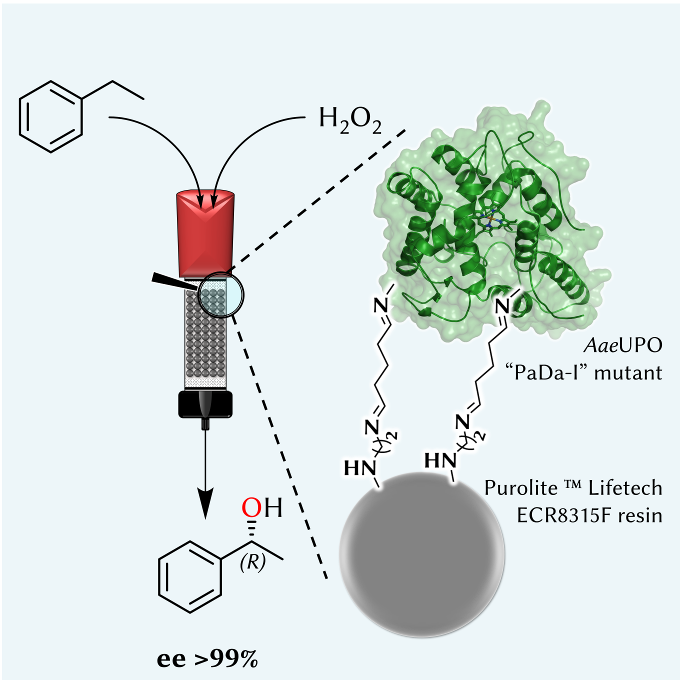

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1 Enzyme membrane reactor (EMR)

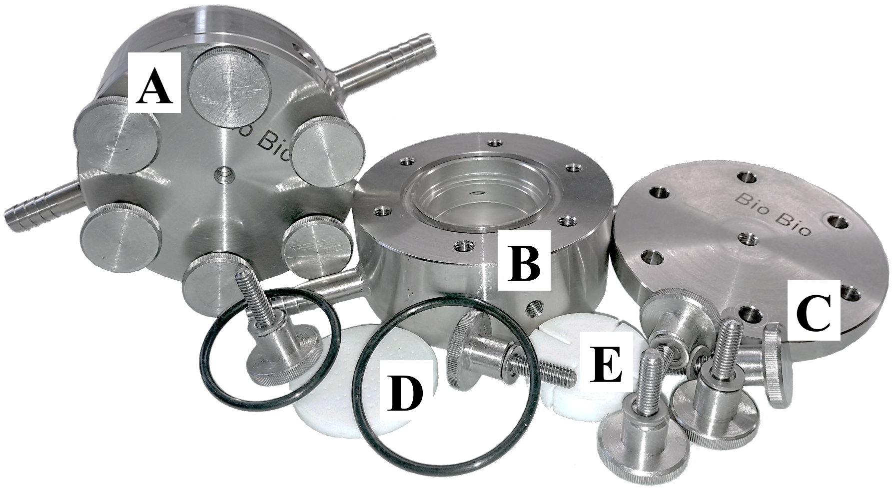
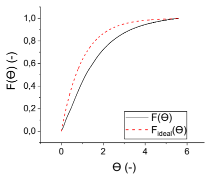
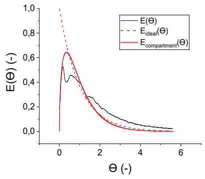
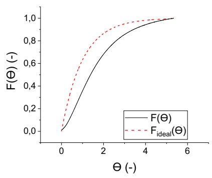
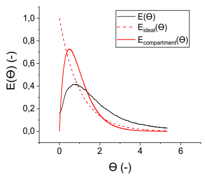
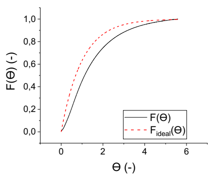
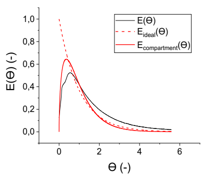
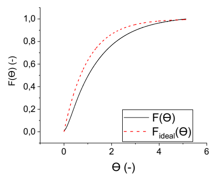
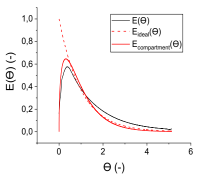
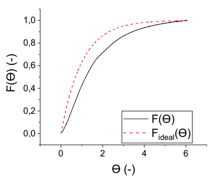
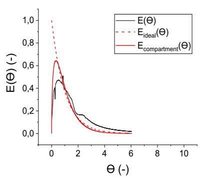
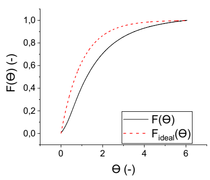
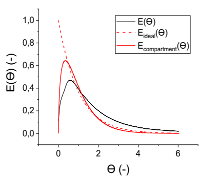
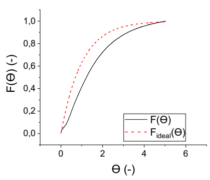
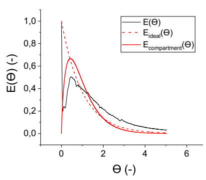
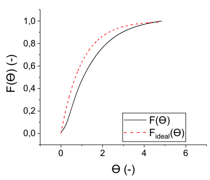
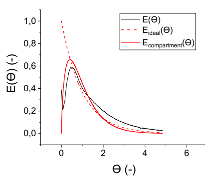


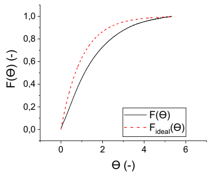
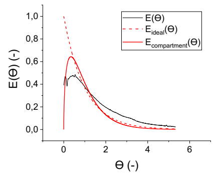
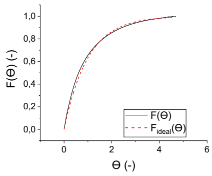
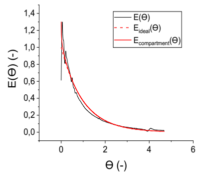
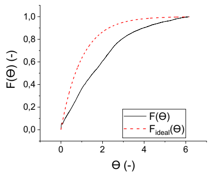
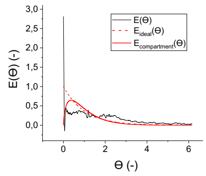
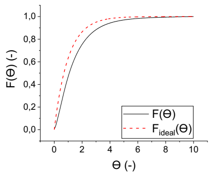
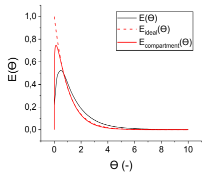
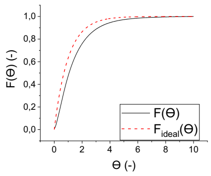
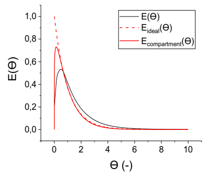
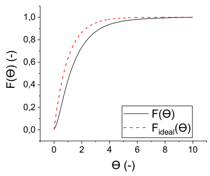
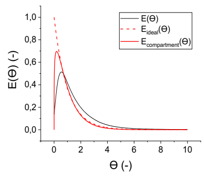
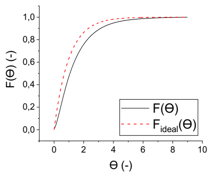
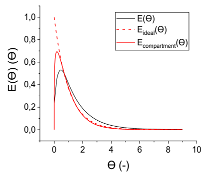
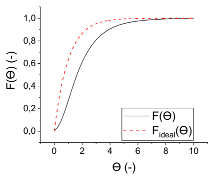
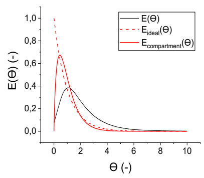
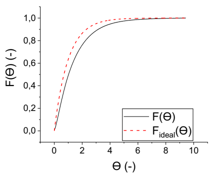
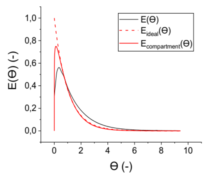
Figure S1. Membrane reactor used within this study (fully assembled, A) consisting of a lower part (B) and an upper part (C) together with the membrane holder for stabilization of the membrane (D) and the magnetic stirrer (E).

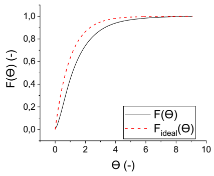
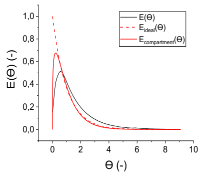
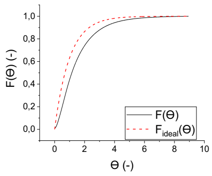
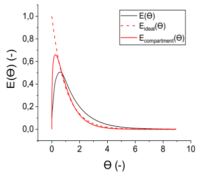
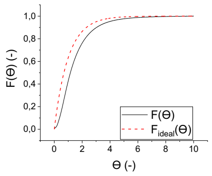
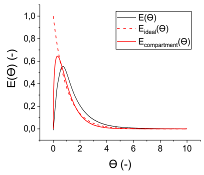
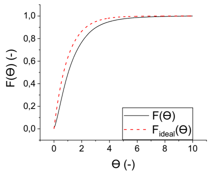
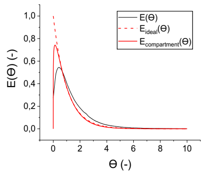
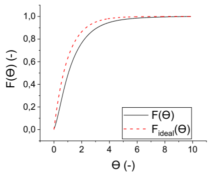
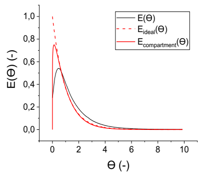
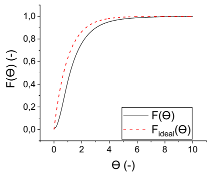
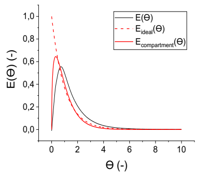
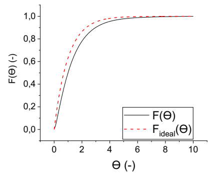
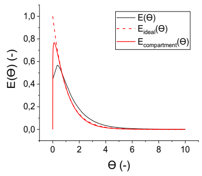
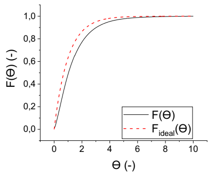
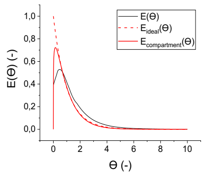
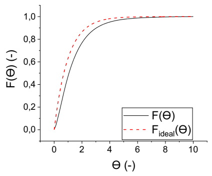
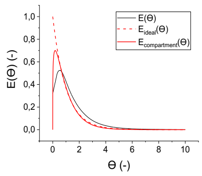
Figure S2. Top-down view of the membrane reactor after the first enzymatic run with a flow rate of 0.5 mL·min^{-1} (with a flow-proportion of 90% 0.3 mmol·L^{-1} ABTS and 10% of 3.5% (v/v) H_2O_2 solution). Purple crystals were formed and in direct contact with the membrane (left) and dispersed throughout the entire volume of the reactor (middle). Due to resulting high pressures, the membrane was damaged (right).

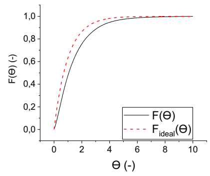
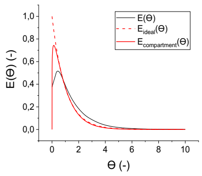
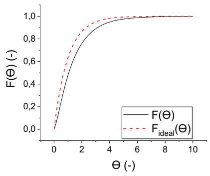
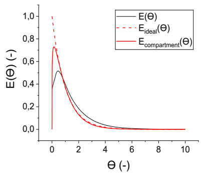
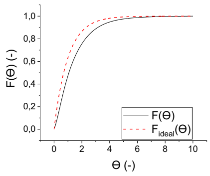
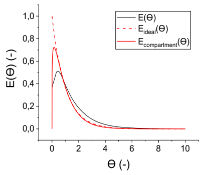
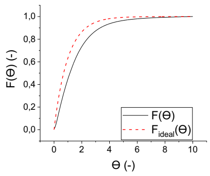
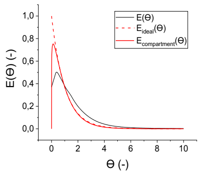
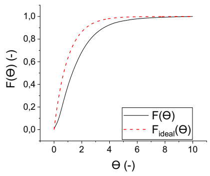
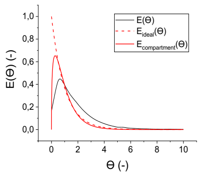
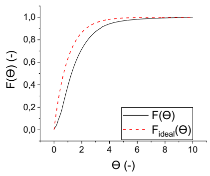
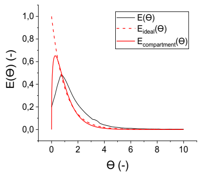
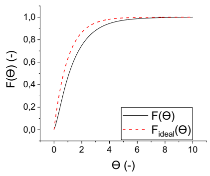
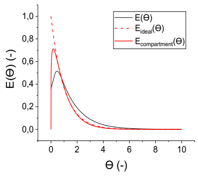
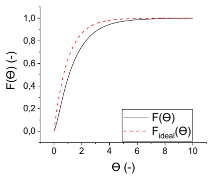
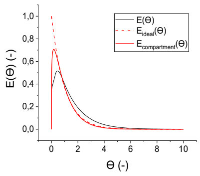
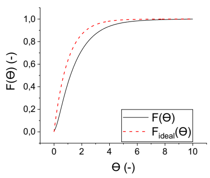
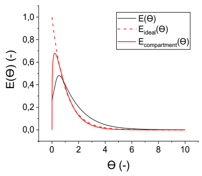
Figure S3. Top-down views of the reactor. Total flow of 0.5 mL·min^{-1} (with a flow-proportion of 90% KPi and 10% of 0.35% (v/v) H_2O_2 solution) with 0.1 mL neat ethylbenzene being manually injected every 30 minutes with 0.1 g BSA added before injection of PaDa-I (left) and the same conditions as previous image but without addition of BSA (middle). Total flow of 0.5 mL·min^{-1} (with a flow-proportion of 80% KPi, 10% of 0.35% (v/v) H_2O_2 solution and 10% of neat ethylbenzene) with 0.1 g BSA added before injection of enzyme (right).

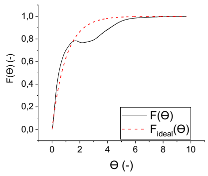
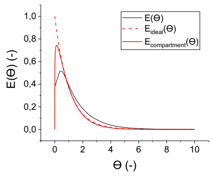
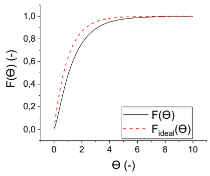
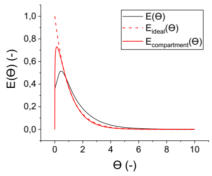
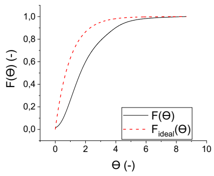
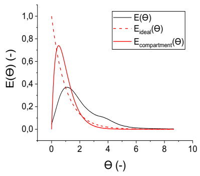
Table S1. Residence time distribution experiments for the membrane reactor. B: bottom-inlet, S: side-inlet. The cumulative distribution and the residence time distribution are plotted against the ideal behavior (column "F(Θ) vs Θ" and "E(Θ) vs Θ"). The plot of the residence time distribution also shows the similarity between the E-function (Equation (7)) and the E-function plotted with the number of vessel compartments ("tant-in-tank model").

Entry	Flow rate [mL·min⁻¹]	Stirring speed [RPM]	Inlet [–]	F(Θ) vs Θ	E(Θ) vs Θ	τ_{theo} [min]	$\tau_{exp, E(t)}$ [min]	$\tau_{exp, E(t)} \cdot \tau_{theo}^{-1}$ [–]	$N_{tank\text{-}in\text{-}tank}$ [–]
1	0.1	100	B			30	45.145	1.505	1.516
2*	0.1	100	B			30	49.675	1.656	1.973
3	0.1	100	B			30	43.775	1.459	1.560
4	0.1	100	S			30	39.582	1.319	1.447
5	0.1	100	S			30	46.013	1.534	1.487
6	0.1	100	S			30	47.245	1.575	1.526
7	0.1	700	B			30	45.096	1.503	1.752
8	0.1	700	B			30	41.013	1.367	1.686

9	0.1	700	B			30	43.782	1.459	1.518
10	0.1	700	S			30	28.985	0.966	0.959
11	0.1	700	S			30	54.870	1.829	1.599
12	0.5	100	B			6	8.921	1.487	1.149
13	0.5	100	B			6	8.783	1.464	1.170
14	0.5	100	B			6	9.436	1.573	1.232
15	0.5	100	S			6	8.694	1.449	1.239
16	0.5	100	S			6	11.966	1.994	1.788
17	0.5	100	S			6	8.569	1.428	1.144

18	0.5	700	B			6	9.187	1.531	1.284
29	0.5	700	B			6	9.180	1.530	1.346
20	0.5	700	B			6	9.025	1.504	1.463
21	0.5	700	S			6	8.508	1.418	1.153
22	0.5	700	S			6	8.636	1.439	1.144
23	0.5	700	S			6	9.025	1.504	1.463
24	1.0	100	B			3	4.129	1.376	1.124
25	1.0	100	B			3	4.227	1.409	1.182
26	1.0	100	B			3	4.309	1.436	1.224

27	1.0	100	S			3	4.289	1.430	1.151
28	1.0	100	S			3	4.255	1.418	1.171
29	1.0	100	S			3	4.278	1.426	1.182
30	1.0	700	B			3	4.669	1.556	1.140
31	1.0	700	B			3	5.167	1.722	1.384
32	1.0	700	B			3	4.914	1.638	1.391
33	1.0	700	S			3	4.448	1.483	1.200
34	1.0	700	S			3	4.445	1.482	1.209
35	1.0	700	S			3	4.750	1.584	1.276

36	0.5	0	B			6	4.289	1.430	1.151
37	0.5	0	B			6	4.255	1.418	1.171
38	0.5	0	B			6	11.797	3.932	2.008

2 Packed-bed reactor (PBR)

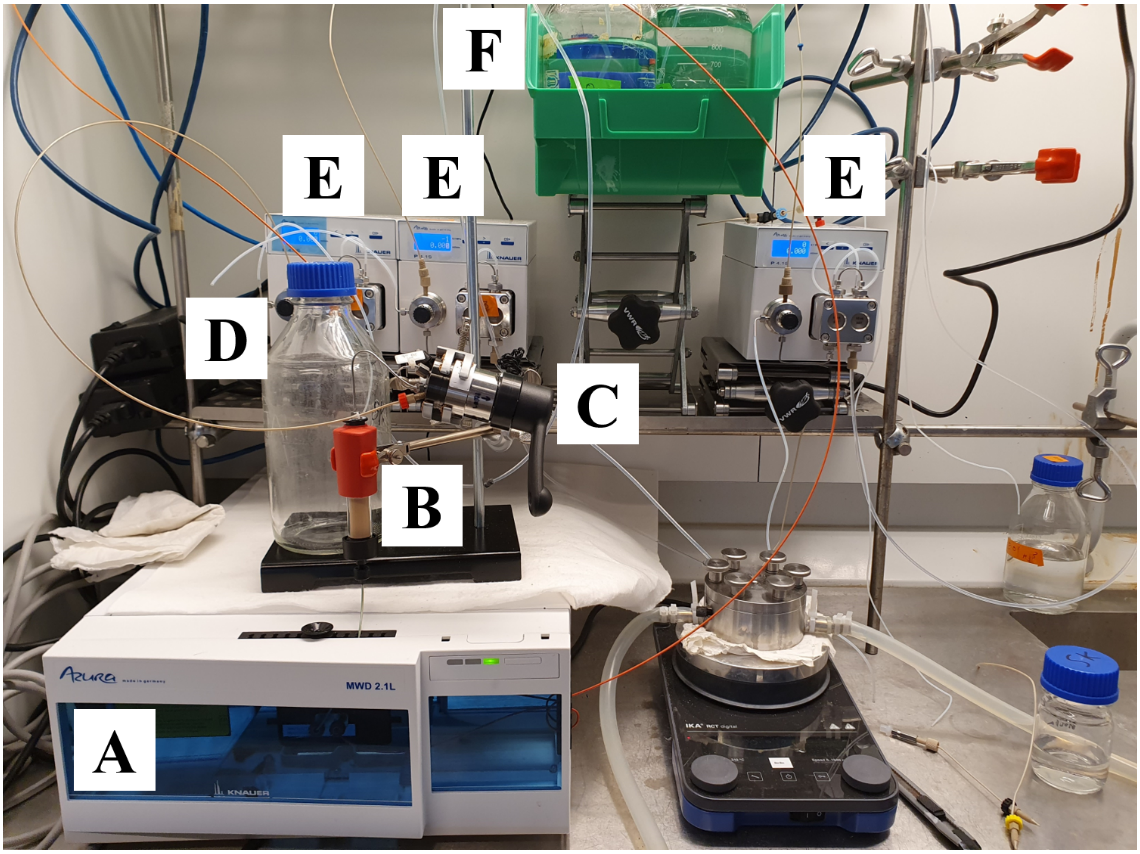
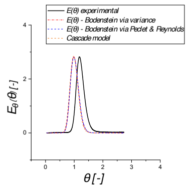
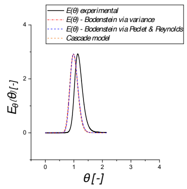
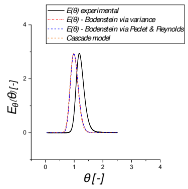
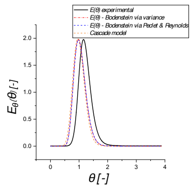
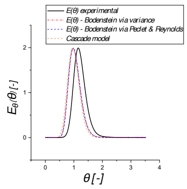
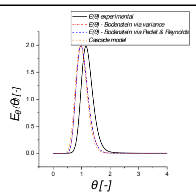
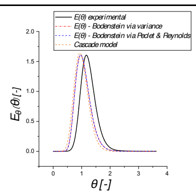
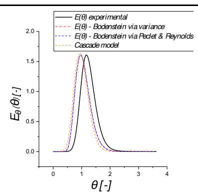
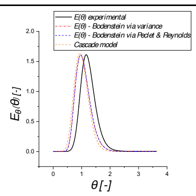


Figure S4. Flow set-up used within this study consisting of the UV/vis detector (A), self-packed bed reactor (B), six port valve (C), waste container (D), three individually operated HPLC pumps (E), and product containers (F).

Table S2. Residence time distribution experiments for the packed bed reactor with a bed height of 30 mm (2 g of ECR8315F:NC).

Entry	Flow rate [mL·min^{-1}]	E(Θ) vs Θ	τ_{theo} [min]	$\tau_{exp, E(t)}$ [min]	$\tau_{exp, E(t)} \cdot \tau_{theo}^{-1}$ [–]	$N_{tank-in-tank}$ [–]	Bodenstein [–]	Reynold [–]
1	0.1		23.6	29.4	1.25	50	100	1.5×10^{13}
2	0.1		23.6	28.0	1.19	53	107	3.0×10^{13}
3	0.1		23.6	29.0	1.23	54	108	3.0×10^{13}
4	0.5		4.71	5.80	1.23	24	49	5.0×10^{10}
5	0.5		4.71	5.75	1.22	24	49	6.0×10^{10}
6	0.5		4.71	5.79	1.23	24	49	6.0×10^{10}
7	1.0		2.36	2.94	1.25	16	32	1.9×10^{9}
8	1.0		2.36	2.95	1.25	16	32	1.9×10^{9}
9	1.0		2.36	2.93	1.24	16	32	1.9×10^{9}

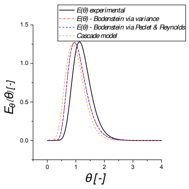
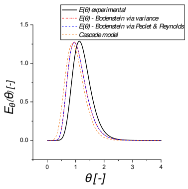
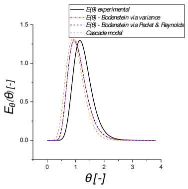
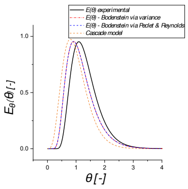
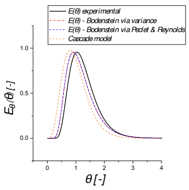
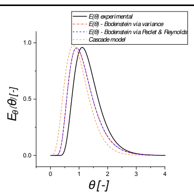
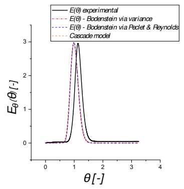
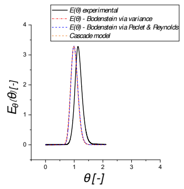
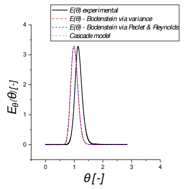
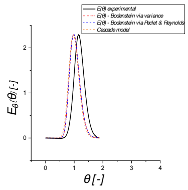
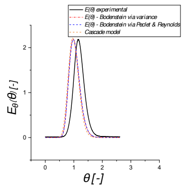
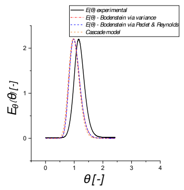
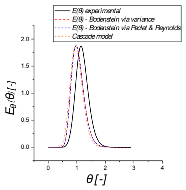
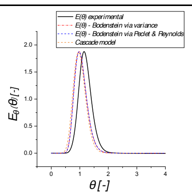
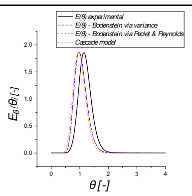
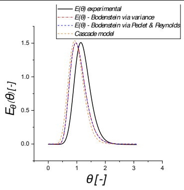
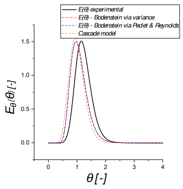
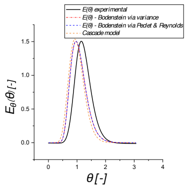
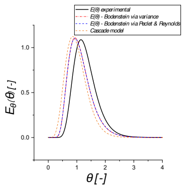
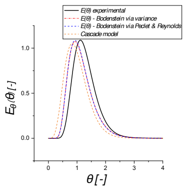
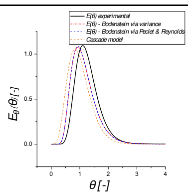
10	2.0		1.18	1.50	1.27	9	20	4×10^7
11	2.0		1.18	1.48	1.25	9	20	4×10^7
12	2.0		1.18	1.49	1.26	10	21	5×10^7
13	5.0		0.47	0.63	1.34	5	11	3.5×10^5
14	5.0		0.47	0.60	1.28	5	11	3.5×10^5
15	5.0		0.47	0.63	1.34	5	11	3.5×10^5

Table S3. Residence time distribution experiments for the packed bed reactor with a bed height of 45 mm (3 g of ECR8315F:NC).

Entry	Flow rate [mL·min^{-1}]	E(Θ) vs Θ	τ_{theo} [min]	$\tau_{exp, E(t)}$ [min]	$\tau_{exp, E(t)} \cdot \tau_{theo}^{-1}$ [–]	$N_{tank-in-tank}$ [–]	Bodenstein [–]	Reynold [–]
16	0.1		35.4	40.6	1.15	126	53	4.5×10^{12}
17	0.1		35.4	41.0	1.16	135	67	8.0×10^{12}
18	0.1		35.4	41.3	1.17	135	67	8.0×10^{12}
19	0.5		7.07	8.39	1.19	66	32	2.5×10^{10}
20	0.5		7.07	8.47	1.20	60	30	1.0×10^{10}
21	0.5		7.07	8.41	1.19	61	30	1.2×10^{10}
22	1.0		3.54	4.21	1.19	44	21	9.0×10^{8}
23	1.0		3.54	4.13	1.17	44	21	9.0×10^{8}
24	1.0		3.54	4.04	1.14	43	21	8.0×10^{8}

25	2.0		1.77	2.14	1.21	28	14	2.5×10^7
26	2.0		1.77	2.12	1.20	28	14	2.5×10^7
27	2.0		1.77	2.15	1.21	28	14	2.5×10^7
28	5.0		0.71	0.93	1.31	15	7	1.5×10^5
29	5.0		0.71	0.91	1.28	14	6	1.2×10^5
30	5.0		0.71	0.90	1.27	14	6	1.2×10^5

3 ABTS$^{\bullet+}$-radical product in continuous production

3.1 Correlation between absorbance readings of the flow detector with actual concentration

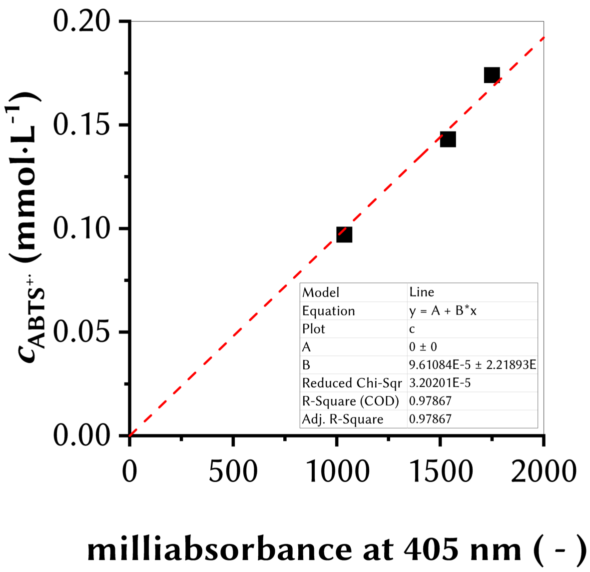


Figure S5. Correlation of the readings from the flow-through UV/vis detector (abscissa) to the concentration of the ABTS$^{\bullet+}$-radical concentration (ordinate) being determined via photometric measurements in a 1 mL cuvette.

3.2 Calculations with respect to continuous ABTS$^{\bullet+}$ radical production

3.2.1 Continuous production I (1837-τ_{exp}, different conditions)

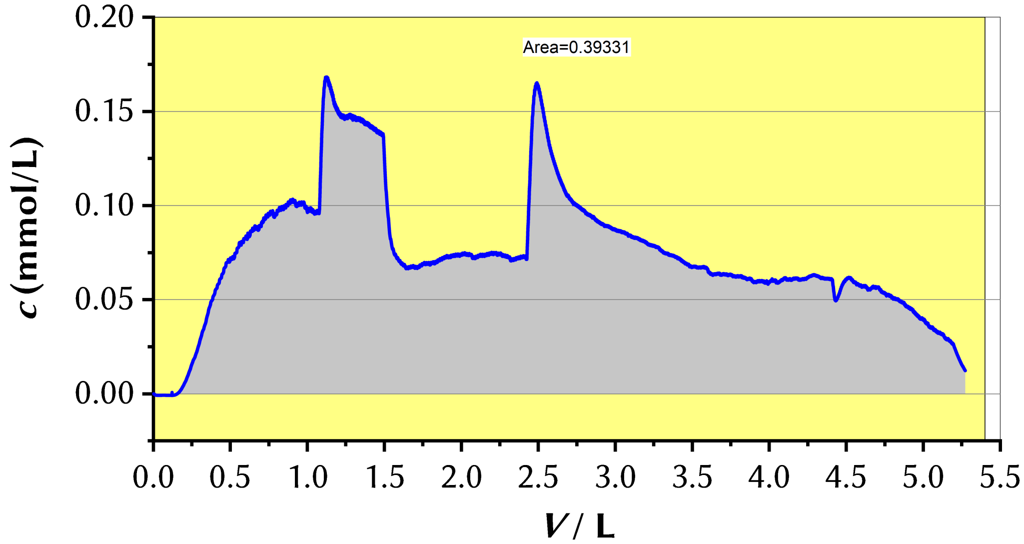


Figure S6. Integration of the first continuous run (see also Table 3, Figure 5). Conditions: 1 mL·min^{-1}, total produced volume: 5.4 L, total run time: 3.75 days, reactor volume (V_R): 2.36 mL, τ_{exp}: 2.94 min (0.049 h).

Based on the integration result (see Figure S6):

(1) $$n_{ABTS^{\bullet+}} = 0.393\ \text{mmol}$$

(2) $$c_{ABTS^{\bullet+}} = \frac{0.000393\ \text{mol}}{5.4\ \text{L}}$$

(3) $$c_{ABTS^{\bullet+}} = 7.3 * 10^{-5}\ \frac{\text{mol}}{\text{L}}$$

(4) $$m_{ABTS^{\bullet+}} = 0.000393\ \text{mol} \cdot 548.7\ \frac{\text{g}}{\text{mol}}$$

(5) $$m_{ABTS^{\bullet+}} = 0.2156\ \text{g}$$

(6) $$c_{ABTS^{\bullet+}} = \frac{0.2156\ \text{g}}{5.4\ \text{L}}$$

(7) $$c_{ABTS^{\bullet+}} = 0.0399\ \frac{\text{g}}{\text{L}}$$

Comment on Equation (3) and (7):

"Average steady-state concentration flowing out of the reactor" in mol·L^{-1} or g·L^{-1}.

STY f: 1 mL·min^{-1} (= 0.06 L·h^{-1}); V_R: 2.36 mL; [P] = 0.0399 g·L^{-1}

$$\mathrm{STY} = \frac{[\mathrm{P}] \times f}{V_\mathrm{R}}$$

$$\mathrm{STY} = \frac{0.0399\ \mathrm{g} \times 0.06\ \mathrm{L}}{\mathrm{L} \times \mathrm{h} \times 0.00236\ \mathrm{L}}$$

$$\mathrm{STY} = 1.014\ \frac{\mathrm{g}}{\mathrm{L \cdot h}}$$

P Flowrate: 1 mL·min^{-1}; m_P = 0.2156 g; overall run time: 90 h; overall reaction volume: 5.4 L

$$\mathrm{P} = \frac{0.2156\ \mathrm{g}}{90\ \mathrm{h} \times 5.4\ \mathrm{L}}$$

$$\mathrm{P} = 0.00044362\ \frac{\mathrm{g}}{\mathrm{L \cdot h}}$$

$$\mathrm{P} = 0.444\ \frac{\mathrm{mg}}{\mathrm{L \cdot h}}$$

Figure S7. Continuous production of ABTS$^{\bullet+}$-radical (see Table 3, Figure 5).

3.2.2 Continuous production II (3380-τ_{exp})

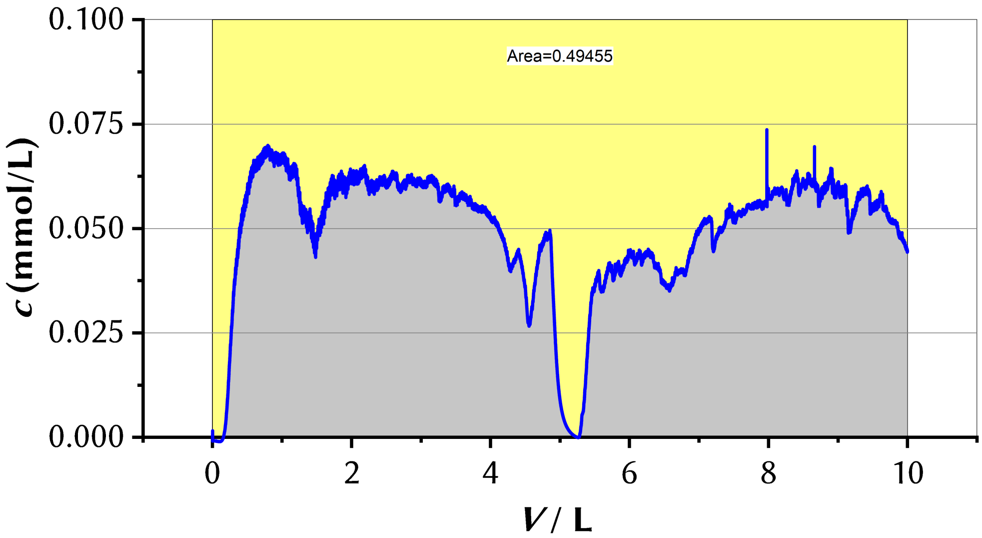


Figure S8. Conditions: 1 mL·min^{-1}, total produced volume: 10.2 L, total run time: 6.9 days, reactor volume (V_R): 2.36 mL, τ_{exp}: 2.94 min (0.049 h).

Based on the integration result (see Figure S8):

(1) $$n_{ABTS^{\bullet+}} = 0.495 \text{ mmol}$$

(2) $$c_{ABTS^{\bullet+}} = \frac{0.000495 \text{ mol}}{10.2 \text{ L}}$$

(3) $$c_{ABTS^{\bullet+}} = 4.9 \times 10^{-5} \ \frac{\text{mol}}{\text{L}}$$

(4) $$m_{ABTS^{\bullet+}} = 0.000495 \text{ mol} \cdot 548.7 \ \frac{\text{g}}{\text{mol}}$$

(5) $$m_{ABTS^{\bullet+}} = 0.2716 \text{ g}$$

(6) $$c_{ABTS^{\bullet+}} = \frac{0.2716 \text{ g}}{10.2 \text{ L}}$$

(7) $$c_{ABTS^{\bullet+}} = 0.0266 \ \frac{\text{g}}{\text{L}}$$

STY f: 1 mL·min^{-1} (= 0.06 L·h^{-1}); V_R: 2.36 mL; [P] = 0.0266 g·L^{-1}

$$\text{STY} = \frac{[\text{P}] \times f}{V_R}$$

$$\text{STY} = \frac{0.0266 \text{ g} \times 0.06 \text{ L}}{\text{L} \times \text{h} \times 0.00236 \text{ L}}$$

$$\text{STY} = 0.676 \ \frac{\text{g}}{\text{L} \cdot \text{h}}$$

P Flowrate: 1 $mL\cdot min^{-1}$; m_P = 0.2716 g; overall run time: 165.6 h; overall reaction volume: 10.2 L

$$P = \frac{0.2716\ \mathrm{g}}{165.6\ \mathrm{h} \times 10.2\ \mathrm{L}}$$

$$P = 0.000161\ \frac{\mathrm{g}}{\mathrm{L \cdot h}}$$

$$P = 0.161\ \frac{\mathrm{mg}}{\mathrm{L \cdot h}}$$

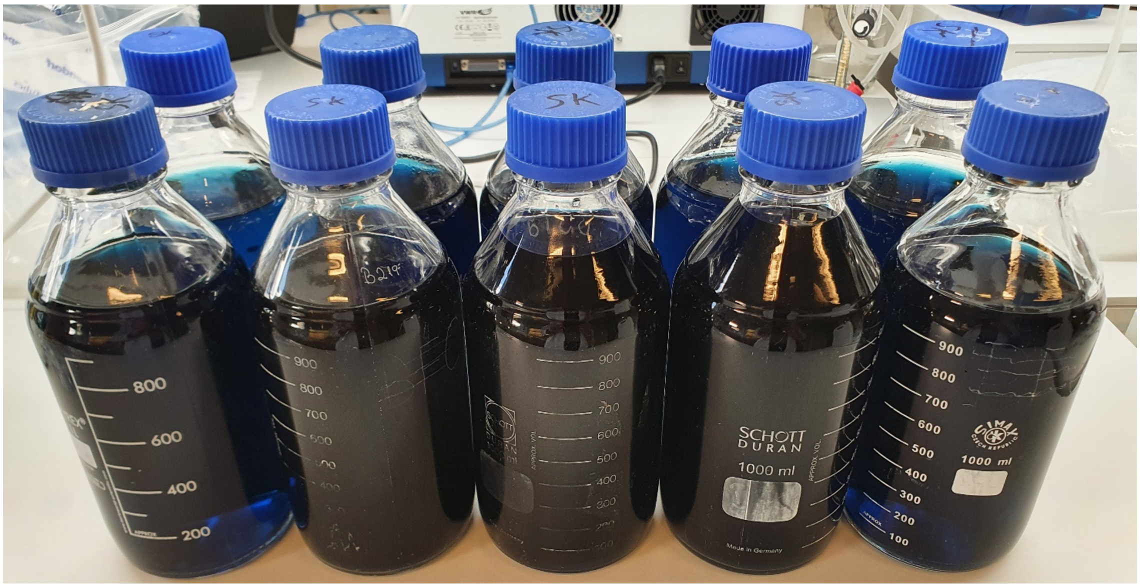


Figure S9. Continuous production of $ABTS^{\bullet+}$-radical (see also Figure 6). Conditions: 1 $mL\cdot min^{-1}$, total produced volume: 10.2 L, total run time: 6.9 days, reactor volume (V_R): 2.36 mL, τ_{exp}: 2.94 min (0.049 h).

4 Calculations with respect to continuous (*R*)-1-phenylethanol production

4.1 Continuous production I (506-τ_{exp})

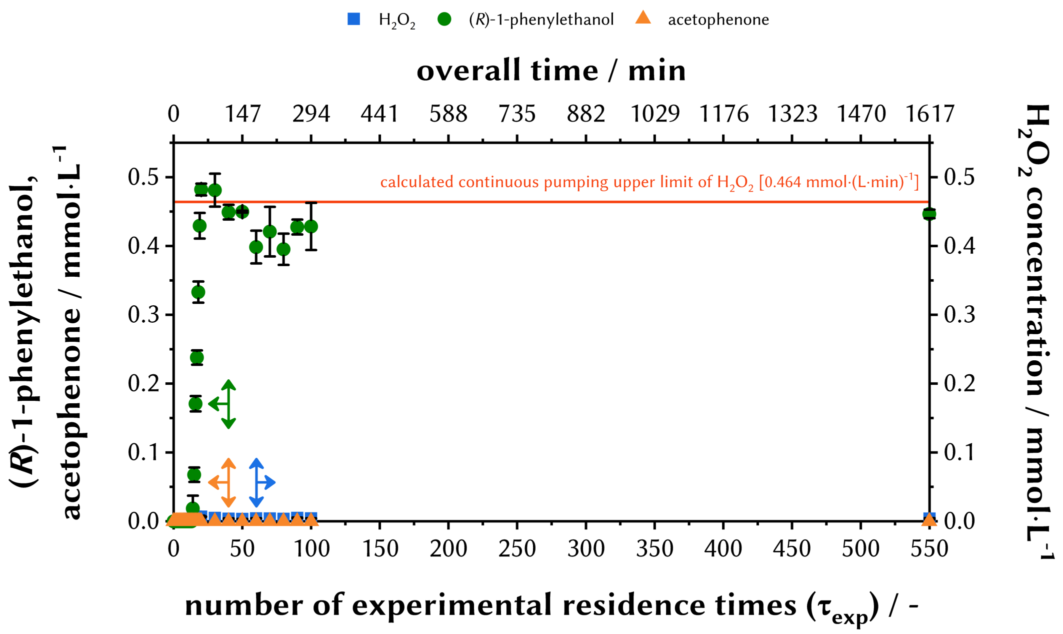


Figure S10. Conditions: 1 mL·min^{-1}, total produced volume: 1.617 L, total run time: 26.95 h, reactor volume (V_R): 2.36 mL, τ_{exp}: 2.94 min (0.049 h).

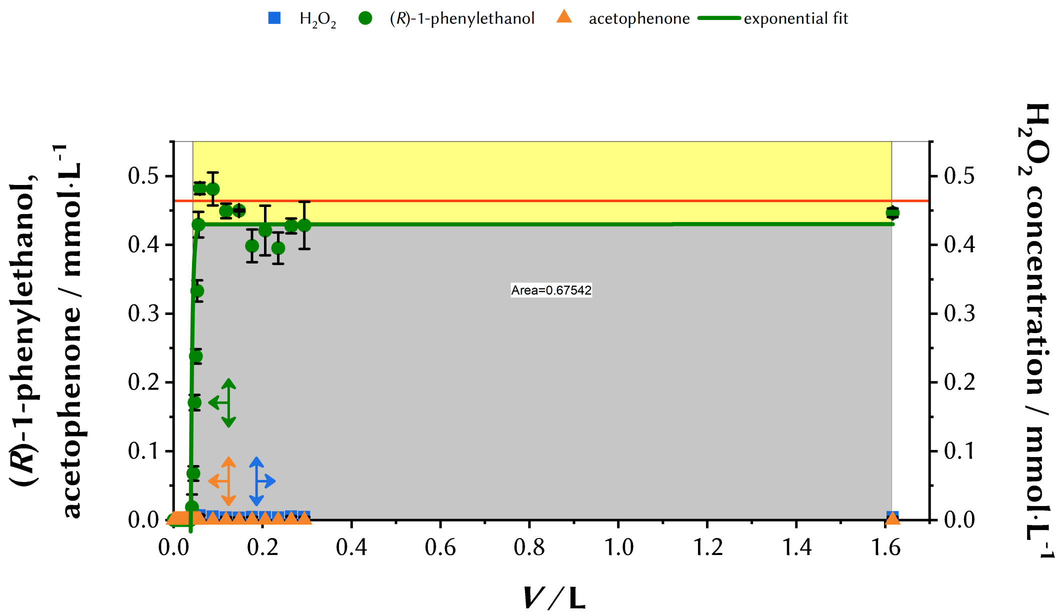


Figure S11. Integration result. Conditions: 1 mL·min^{-1}, total produced volume: 1.617 L, total run time: 26.95 h, reactor volume (V_R): 2.36 mL, τ_{exp}: 2.94 min (0.049 h).

Based on the integration result (see Figure S11):

(1) $$n_{(R)\text{-1-phenylethanol}} = 0.67542\ \text{mmol}$$

(2) $$c_{(R)\text{-1-phenylethanol}} = \frac{0.00067542\ \text{mol}}{1.617\ \text{L}}$$

(3) $$c_{(R)\text{-1-phenylethanol}} = 0.000417699\frac{\text{mol}}{\text{L}}$$

(4) $$m_{(R)\text{-1-phenylethanol}} = 0.000418\ \text{mol} \times 122.16\ \frac{\text{g}}{\text{mol}}$$

(5) $$m_{(R)\text{-1-phenylethanol}} = 0.083\ \text{g}$$

(6) $$c_{(R)\text{-1-phenylethanol}} = \frac{0.083\ \text{g}}{1.617\ \text{L}}$$

(7) $$c_{(R)\text{-1-phenylethanol}} = 0.051\ \frac{\text{g}}{\text{L}}$$

STY f: 1 mL·min^{-1} (= 0.06 L·h^{-1}); V_R: 2.36 mL; [P] = 0.051 g·L^{-1}

$$\text{STY} = \frac{[\text{P}] \times f}{V_\text{R}}$$

$$\text{STY} = \frac{0.051\ \text{g} \times 0.06\ \text{L}}{\text{L} \times \text{h} \times 0.00236\ \text{L}}$$

$$\text{STY} = 1.30\ \frac{\text{g}}{\text{L·h}}$$

P Flowrate: 1 mL·min^{-1}; m_P = 0.083 g; overall run time: 26.95 h; overall reaction volume: 1.617 L

$$\text{P} = \frac{0.083\ \text{g}}{26.95\ \text{h} \times 1.617\ \text{L}}$$

$$\text{P} = 0.00189336\ \frac{\text{g}}{\text{L·h}}$$

$$\text{P} = 1.893\ \frac{\text{mg}}{\text{L·h}}$$

4.2 Continuous production II (3050-τ_{exp})

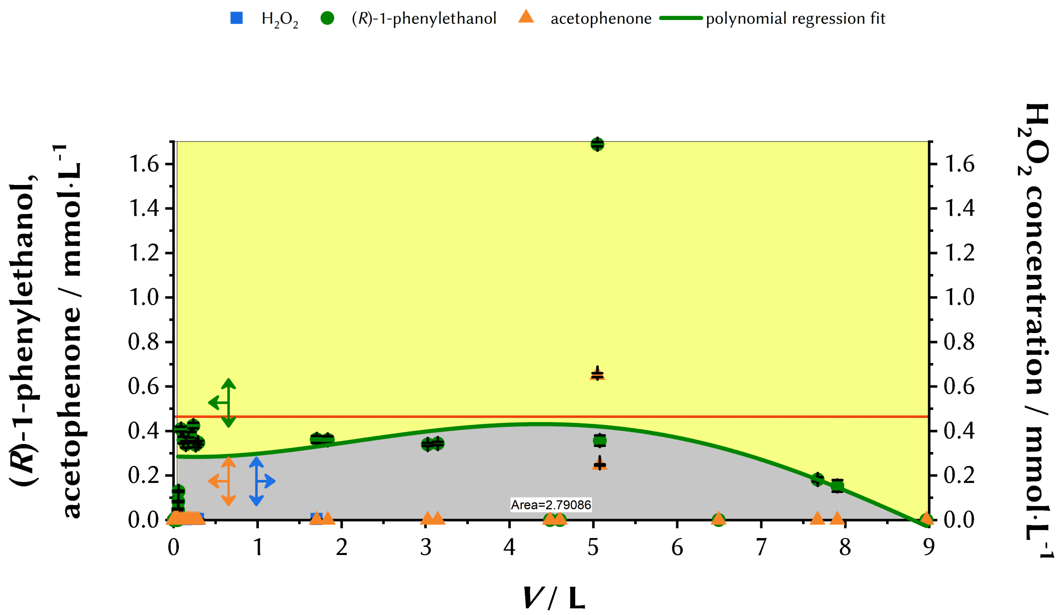


Figure S12. Integration result. Conditions: 1 mL·min^{-1}, total produced volume: 8.967 L, total run time: 149.45 h (6.23 days), reactor volume (V_R): 2.36 mL, τ_{exp}: 2.94 min (0.049 h).

Based on the integration result (see Figure S12):

(1) $$n_{(R)\text{-1-phenylethanol}} = 2.79086\ \text{mmol}$$

(2) $$c_{(R)\text{-1-phenylethanol}} = \frac{0.00279086\ \text{mol}}{8.967\ \text{L}}$$

(3) $$c_{(R)\text{-1-phenylethanol}} = 0.00031124\ \frac{\text{mol}}{\text{L}}$$

(4) $$m_{(R)\text{-1-phenylethanol}} = 0.00279086\ \text{mol} \times 122.16\ \frac{\text{g}}{\text{mol}}$$

(5) $$m_{(R)\text{-1-phenylethanol}} = 0.341\ \text{g}$$

(6) $$c_{(R)\text{-1-phenylethanol}} = \frac{0.341\ \text{g}}{8.967\ \text{L}}$$

(7) $$c_{(R)\text{-1-phenylethanol}} = 0.038\ \frac{\text{g}}{\text{L}}$$

STY f: 1 mL·min^{-1} (= 0.06 L·h^{-1}); V_R: 2.36 mL; [P] = 0.038 g·L^{-1}

$$\mathrm{STY} = \frac{[\mathrm{P}] \times f}{V_\mathrm{R}}$$

$$\mathrm{STY} = \frac{0.038\ \mathrm{g} \times 0.06\ \mathrm{L}}{\mathrm{L} \times \mathrm{h} \times 0.00236\ \mathrm{L}}$$

$$\mathrm{STY} = 0.97\ \frac{\mathrm{g}}{\mathrm{L{\cdot}h}}$$

P Flowrate: 1 mL·min^{-1}; m_P = 0.341 g; overall run time: 149.45 h; overall reaction volume: 8.967 L

$$\mathrm{P} = \frac{0.341\ \mathrm{g}}{149.45\ \mathrm{h} \times 8.967\ \mathrm{L}}$$

$$\mathrm{P} = 0.000254455\ \frac{\mathrm{g}}{\mathrm{L{\cdot}h}}$$

$$\mathrm{P} = 0.254\ \frac{\mathrm{mg}}{\mathrm{L{\cdot}h}}$$