

Unspecific peroxygenase immobilization in 3D-printed microfluidics: Towards tailor-made screening platforms

Elena Gkantzou^a, Theofilia Koulopoulou^a, Hannah Brass^b, David Schönauer^b, Anton Glieder^c,
and Selin Kara^{a,d*}

^a Institute of Technical Chemistry, Leibniz University Hannover, Callinstrasse 5, DE-30167, Hannover, Germany.

^b Aminoverse B.V., Daelderweg 9, NL-6361HK Nuth, Netherlands.

^c Bisy GmbH, Wünschendorf 292, 8200 Hofstätten an der Raab, Austria.

^d Biocatalysis and Bioprocessing Group, Department of Biological and Chemical Engineering, Aarhus University, Gustav Wieds Vej 10, DK-8000 Aarhus, Denmark.

* corresponding author: selin.kara@iftc.uni-hannover.de

SUPPLEMENTARY INFORMATION

Table S1: Investigation of UPO substrates ABTS and H₂O₂ concentration under flow conditions. Enzymatic activity measurement conditions: citrate phosphate buffer (100 mM at pH 4.4), T = 25°C, flow rate = 10 μL/min, reactor volume = 20 μL. One unit (U) of enzymatic activity is the amount of enzyme that converts one μmol of substrate per minute, under the specified conditions.

ABTS (mM)	H ₂ O ₂ (mM)	U (μmol·min ⁻¹)
0.3	3	0.0008
1	3	0.0026
3	3	0.0044
5	3	0.0033
1	0.5	0.0018
1	1	0.0024
1	3	0.0027
1	5	0.0017

Table S2: Supplementary data to Figure 3a. Analytical yields of the UPO-immobilized microreactor for different substrate concentrations (0.3 – 5 mM). Yields are calculated by applying the Beer-Lamberts law for ABTS⁺ concentration calculation. Measurement conditions: c(ABTS) = 1 mM, c(H₂O₂) = 3 mM, citrate buffer (100 mM at pH 4.4), T = 25°C, reactor volume = 20 μL.

Flow rate (μL/min)	Analytical Yield (%)			
	0.3 mM	1 mM	3 mM	5 mM
20	57.583	32.851	34.501	17.536
10	30.333	25.196	14.642	6.621
5	12.939	20.283	62.273	67.934
2.5	3.946	3.270	10.258	8.3579

Table S3: Supplementary data to Figure 2b. Application of different substrate concentrations and different flow rates for kinetic constants determination. Measurement conditions: c(ABTS) = 1 mM, c(H₂O₂) = 3 mM, citrate buffer (100 mM at pH 4.4), T = 25°C, reactor volume = 20 μL.

Flow rate (μL/min)	K _{M,app} (mM)
20	0.979
10	1.677
5	2.981
2.5	3.944

Table S4: Supplementary data to Figure 4. Operational stability of the UPO-immobilized microreactor. Activity measurement conditions: $c(\text{ABTS}) = 1 \text{ mM}$, $c(\text{H}_2\text{O}_2) = 1 \text{ mM}$, citrate buffer (100 mM at pH 4.4), $T = 25^\circ\text{C}$, flow rate = 10 $\mu\text{L}/\text{min}$, reactor volume = 20 μL .

Cycles of use	Remaining activity (%)
1	100
2	104.267
3	101.067
4	94.133
5	97.867
6	94.4
7	93.067
8	91.2
9	77.867
10	80.267
11	77.867
12	74.667
13	72.267
14	69.333
15	68.267
16	64.8
17	60
18	54.4
19	54.133
20	53.067
21	46.667
22	45.867
23	46.133
24	43.467
25	42.133
26	39.2
27	38.667
28	38.933
29	34.667
30	38.133
31	28.8
32	30.933
33	31.733
34	32
35	29.867
36	28.267
37	28.267
38	28.267
39	25.481
40	25.473
41	25.356
42	20.483
43	19.371
44	18.489
45	17.499
46	16.355
47	13.754
48	12.258
49	9.577
50	5.1