

Supplementary Material

DoE-based medium optimization for improved biosurfactant production with *Aureobasidium pullulans*

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1 Supplementary

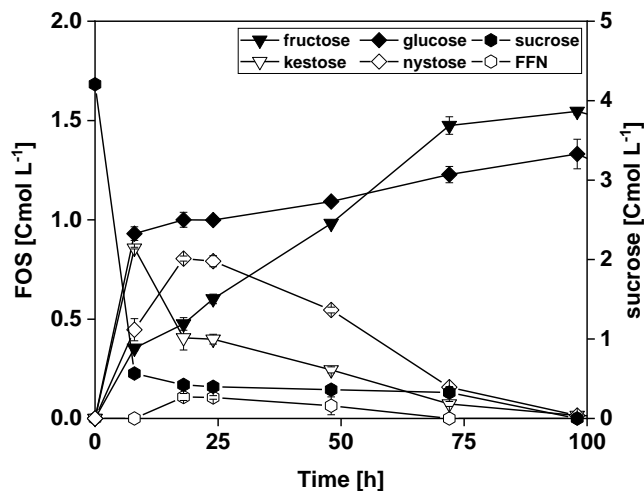


Figure S1: Fructooligosaccharide (FOS) formation and depletion using sucrose as carbon source: Concentration of sucrose, kestose, nystose, and fructofuranosylnystose (FFN) and their monomers glucose and fructose in the first 100 hours. Cultivations were performed with *A. pullulans* NRRL 62042 in 24 deep-well microtiter plates with 2 mL filling volume, 30 °C, and 300 rpm. Data show the mean of biological triplicates with \pm standard deviation.

Table S1: Two-level factorial design plan of experiments with factors C/N ratio, vitamin (vit.) solution, trace-element (TE) solution, K_2HPO_4 , $MgSO_4 \times 7 H_2O$, $MgSO_4$, NaCl, Glucose, and the response polyol lipid titer.

Run	C:N ratio	Vit.-Sol.	TE-Sol.	K_2HPO_4	$MgSO_4 \times 7 H_2O$	NaCl	Glucose	Polyol lipid
	[gC gN ⁻¹]	[ml L ⁻¹]	[ml L ⁻¹]	[g L ⁻¹]	[g L ⁻¹]	[g L ⁻¹]	[g L ⁻¹]	[g L ⁻¹]
1	200	0.5	5	0.5	2	0.5	150	28
2	50	2	15	2	2	0.5	50	7.95
3	50	0.5	15	0.5	0.5	0.5	50	9.45
4	50	2	5	2	2	2	150	27.05
5	50	2	15	0.5	0.5	2	150	18
6	200	2	5	2	2	0.5	50	5.75
7	200	2	15	2	0.5	2	50	6.6
8	50	2	15	0.5	2	2	50	8.3
9	50	2	5	0.5	2	0.5	150	21.85
10	200	2	15	2	2	2	150	27.9
11	200	0.5	5	0.5	0.5	0.5	50	9.5
12	50	0.5	5	2	2	0.5	50	8
13	50	2	5	0.5	0.5	0.5	50	7.95
14	200	2	5	2	0.5	0.5	150	32.65
15	50	2	15	2	0.5	0.5	150	25.95
16	200	0.5	15	0.5	0.5	2	150	25.7
17	50	2	5	2	0.5	2	50	7.1
18	50	0.5	15	2	2	2	150	29.75
19	200	0.5	5	2	0.5	2	50	8.85
20	125	1.25	10	1.25	1.25	1.25	100	22.9
21	50	0.5	5	2	0.5	0.5	150	31.6
22	200	2	5	0.5	2	2	50	9.4

23	200	2	15	0.5	2	0.5	150	29.35
24	200	2	15	0.5	0.5	0.5	50	7.3
25	200	0.5	15	2	2	0.5	50	7.9
26	200	2	5	0.5	0.5	2	150	25.85
27	125	1.25	10	1.25	1.25	1.25	100	20.5
28	50	0.5	15	2	0.5	2	50	6.65
29	125	1.25	10	1.25	1.25	1.25	100	20.7
30	50	0.5	5	0.5	0.5	2	150	23.8
31	50	0.5	15	0.5	2	0.5	150	19.1
32	200	0.5	15	2	0.5	0.5	150	29.3
33	200	0.5	15	0.5	2	2	50	8.7
34	125	1.25	10	1.25	1.25	1.25	100	20.05
35	50	0.5	5	0.5	2	2	50	8
36	200	0.5	5	2	2	2	150	26.15

Table S2 ANOVA analysis from two-level factorial design with the factors carbon-to-nitrogen ratio C/N ($\text{g}_{\text{carbon}} \text{g}_{\text{nitrogen}}^{-1}$), vitamin and trace-element solution, K_2HPO_4 , $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$, NaCl, and glucose.

Factor	Sum of squares	Degrees of freedom	Mean square	F-value	p-value
Model	2,981.05	26	114.66	17.50	< 0.0001
C/N ratio (A)	25.20	1	25.20	3.85	0.0815
Vit.-solution (B)	4.13	1	4.13	0.6308	0.4475
TE-solution (C)	5.78	1	5.78	0.8823	0.3721
K_2HPO_4 (D)	26.10	1	26.10	3.98	0.0771
$\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ (E)	0.3003	1	0.3003	0.0458	0.8352
NaCl	5.95	1	5.95	0.9084	0.3654
Glucose (F)	2,712.16	1	2,712.16	413.98	< 0.0001
AB	5.20	1	5.20	0.7938	0.3962
AC	1.45	1	1.45	0.2206	0.6498

AD	21.62	1	21.62	3.30	0.1027
AE	0.1378	1	0.1378	0.0210	0.8879
AF	1.71	1	1.71	0.2612	0.6216
AG	23.12	1	23.12	3.53	0.0930
BD	0.2813	1	0.2813	0.0429	0.8405
BE	7.41	1	7.41	1.13	0.3152
BF	0.3403	1	0.3403	0.0519	0.8248
CD	0.3403	1	0.3403	0.0519	0.8248
CE	16.68	1	16.68	2.55	0.1451
CF	0.6050	1	0.6050	0.0923	0.7681
CG	3.25	1	3.25	0.4963	0.4990
DE	5.61	1	5.61	0.8565	0.3789
DG	73.51	1	73.51	11.22	0.0085
ADE	12.75	1	12.75	1.95	0.1964
ADG	16.10	1	16.10	2.46	0.1514
BDE	5.70	1	5.70	0.8693	0.3755
CDE	5.61	1	5.61	0.8565	0.3789
Lack of Fit	54.12	6		5.58	0.0932
Pure Error	4.85	3			

Table S3: Central composite design plan of experiments with factors glucose and K_2HPO_4 , and the response polyol lipid titer and $Y_{P/S}$.

Run	Glucose	K_2HPO_4	Polyol lipid	$Y_{P/S}$
	$g L^{-1}$	$g L^{-1}$	$g L^{-1}$	$g g^{-1}$
1	120.00	8.00	24.80	0.21
2	300.00	1.40	27.95	0.09
3	120.00	1.40	29.80	0.25
4	210.00	4.70	45.80	0.22

5	300.00	8.00	35.45	0.12
6	210.00	4.70	45.70	0.22
7	120.00	1.40	27.15	0.23
8	300.00	1.40	30.75	0.10
9	120.00	8.00	23.15	0.19
10	120.00	8.00	21.15	0.18
11	300.00	1.40	32.80	0.11
12	120.00	8.00	25.70	0.21
13	120.00	1.40	25.45	0.21
14	120.00	1.40	24.75	0.21
15	300.00	8.00	31.20	0.10
16	300.00	1.40	25.75	0.09
17	120.00	8.00	20.45	0.17
18	300.00	8.00	39.90	0.13
19	300.00	8.00	41.50	0.14
20	300.00	1.40	31.20	0.10
21	210.00	4.70	36.15	0.17
22	120.00	1.40	26.20	0.22
23	300.00	8.00	37.30	0.12
24	210.00	4.70	46.70	0.22
25	210.00	4.70	50.10	0.24
26	82.72	4.70	18.85	0.23
27	82.72	4.70	18.00	0.22
28	210.00	0.03	2.20	0.01
29	210.00	9.37	41.75	0.20
30	210.00	9.37	44.55	0.21
31	82.72	4.70	17.80	0.22
32	337.28	4.70	36.85	0.11
33	337.28	4.70	35.15	0.10

34	82.72	4.70	18.65	0.23
35	210.00	4.70	49.70	0.24
36	210.00	9.37	44.20	0.21
37	210.00	0.03	3.15	0.01
38	210.00	0.03	3.15	0.01
39	337.28	4.70	36.90	0.11
40	337.28	4.70	35.85	0.11
41	210.00	9.37	39.50	0.19
42	82.72	4.70	19.10	0.23
43	210.00	0.03	2.95	0.01
44	210.00	9.37	42.80	0.20
45	337.28	4.70	36.85	0.11
46	210.00	4.70	47.00	0.22
47	210.00	0.03	2.65	0.01
48	210.00	4.70	50.00	0.24