

## **Supplementary Information**

### **Metabolic engineering of *Zymomonas mobilis* for high-gravity fermentation to co-produce bioethanol and fatty acids**

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Table S1. CCD experiment to find the optimum fermentation conditions.

Experiment No.	Coded setting values			Actual values			Experimental values for ethanol production (g/kg)
	Hexose concentration (X <sub>1</sub> )	Temperature (X <sub>2</sub> )	Initial pH (X <sub>3</sub> )	Hexose concentration (X <sub>1</sub> , g/L)	Temperature (X <sub>2</sub> , °C)	Initial pH (X <sub>3</sub> )	
1	0	0	-1.68	305	33	5.0	126.1
2	1	1	-1	340	36	5.6	129.1
3	1.68	0	0	364	33	6.5	126.1
4	1	-1	-1	340	30	5.6	124.2
5	0	0	0	305	33	6.5	141.6
6	1	-1	1	340	30	7.4	127.9
7	-1	-1	1	270	30	7.4	119.6
8	0	0	1.68	305	33	8.0	135.5
9	1	1	1	340	36	7.4	132.3
10	-1	1	1	270	36	7.4	125.1
11	0	-1.68	0	305	28	6.5	122.6
12	-1	1	-1	270	36	5.6	121.4
13	0	1.68	0	305	38	6.5	131.4
14	0	0	0	305	33	6.5	142.1
15	-1	-1	-1	270	30	5.6	115.5
16	-1.68	0	0	246	33	6.5	112.9
17	0	0	0	305	33	6.5	141.3

Results represent the mean of three experiments.

Table S2. Analysis of Variance (ANOVA) of the CCD experiment on optimum fermentation conditions

Source	Sum of squares	Degree of freedom	Mean square	F-value	P-value
Model	1201.76	9	133.53	237.98	< 0.0001 (significant)
•Hexose concentration ( $X_1$ )	214.31	1	214.31	381.95	< 0.0001
•Temperature ( $X_2$ )	92.28	1	92.28	164.46	< 0.0001
•Initial pH ( $X_3$ )	68.16	1	68.16	121.47	< 0.0001
• $X_1^2$	708.78	1	708.78	1263.2	< 0.0001
• $X_2^2$	313.99	1	313.99	559.6	< 0.0001
• $X_3^2$	174.47	1	174.47	310.95	< 0.0001
• $X_1 X_2$	0.55	1	0.55	0.98	0.3546
• $X_1 X_3$	0.1	1	0.1	0.18	0.6837
• $X_2 X_3$	0.1	1	0.1	0.18	0.6837
Lack of fit	3.6	5	0.72	4.41	0.1951 (not significant)
$R^2$				0.9967	
$R^2$ Adjusted				0.9926	

Table S3. CCD experiment of hexose-xylose coupled fermentation at 33.5 °C and initial pH 6.75.

Experiment No.	Coded setting values		Actual values		Experimental values for ethanol production (g/kg)
	Total sugar concentration (X <sub>4</sub> )	Xylose concentration (X <sub>5</sub> )	Total sugar concentration (X <sub>4</sub> , g/L)	Xylose concentration (X <sub>5</sub> , g/L)	
1	0	-1.41	305	1	143.1
2	0	0	305	100	137.9
3	-1.41	0	255.5	100	115.2
4	0	0	305	100	137.6
5	0	1.41	305	199	111.9
6	1	-1	340	30	134.8
7	0	0	305	100	138.2
8	1	1	340	170	115.3
9	-1	-1	270	30	131.2
10	1.41	0	355	100	122.3
11	-1	1	270	170	109.9

Results represent the mean of three experiments.

Table S4. Analysis of Variance (ANOVA) of the CCD experiment on hexose-xylose coupled fermentation.

Source	Sum of squares	Degree of freedom	Mean square	F-value	P-value
Model	1511.02	5	302.2	792.2	< 0.0001 (significant)
•Total sugar concentration ( $X_4$ )	45.32	1	45.32	118.8	0.0001
•Xylose concentration ( $X_5$ )	901.5	1	901.5	2363.21	< 0.0001
• $X_4^2$	526.55	1	526.55	1380.31	< 0.0001
• $X_5^2$	157.51	1	157.51	412.89	< 0.0001
• $X_4 X_5$	0.81	1	0.81	2.12	0.2049
Lack of fit	1.73	3	0.58	6.4	0.1382 (not significant)
$R^2$				0.9987	
$R^2$ Adjusted				0.9975	

Table S5. Ethanol fermentation results of *Z. mobilis* CP4, *Zm*-PMXFWTY and angel yeast (Corresponding to Figs 3C,D, Figs 4B,C, Fig. 5A, Figs 6B,C,D, and Figs 7F,G in the text).

Fermentation parameters			Measurement items		
1.0 g/L urea, 0.5 g/L MgSO <sub>4</sub> ·7H <sub>2</sub> O, 1.0 g/L KH <sub>2</sub> PO <sub>4</sub> , 100 rpm			<i>Zm</i> -PMXFWTY	<i>Z. mobilis</i> CP4	Angel yeast ( <i>S. cerevisiae</i> S288C)
10 g/L yeast extract, initial pH 6.5, 33 °C, 72 hours	Initial	100	48.8 <sup>a</sup>	48.7	47.5
	glucose (g/L)	150	72.5	72.9	70.8
		200	96.9	91.8	89.8
		230	110.7	100.4	98
		250	119.6	106.1	101
		280	132.1	113.9	89.7
		300	140.4	104.8	78
		310	144.9	95.3	
		320	144.5	80.5	
		330	148.8		—
		350	122.1	—	
		370	109.8		
250 g/L initial glucose, 10 g/L yeast extract, 33 °C, 72 hours	Initial pH	4.5	92.7	65.3	82.5
		5.0	107.4	80.0	92.2
		5.5	114.8	93.3	97.7
		6.0	119.2	102.2	101.3
		6.5	118.8	106.2	101.5
		7.0	119.6	106.1	101.0
		7.5	119.1	105.9	101.3
		8.0	115.5	102	92.9
		8.5	105.8	93.6	75.1

		9.0	74	75.3	56.8
250 g/L initial	Temperat	25	110.8	99.7	96.6
glucose, 10 g/L	ure (°C)	28	118.8	104.8	100.6
yeast extract, initial		30	119.0	105.1	99.7
pH 7.0, 72 hours		33	120.8	107.1	102.0
		35	120.0	102.8	100.5
		38	110.3	96.3	92.8
		40	103.8	80.6	86.6
		43	83.2	56.9	77.4
100 g total medium	Time	8	6.9 g/kg EtOH (pH 5.7; 233.2 g/L residual	5 (pH 5.81; 237.2 g/L	7.4 (pH 5.6; 230.5 g/L
(250 g/L initial	(hours)		glucose; 0.64 g CO <sub>2</sub> weight loss)	residual glucose; 0.46 g	residual glucose; 0.46 g
glucose, 10 g/L				CO <sub>2</sub> weight loss)	CO <sub>2</sub> weight loss)
yeast extract,		16	19.8 (4.1; 205.9; 1.82)	13.2 (4.6; 220; 1.19)	23.2 (3.64; 197.9; 2.12)
33 °C, initial pH		24	40.2 (3.19; 163.5; 3.62)	22.9 (3.63; 200; 2.05)	43.4 (3.22; 154.6; 3.9)
7.0)		32	74.2 (2.91; 97.8; 6.48)	34.5 (3.26; 176.2; 3.06)	71.7 (2.99; 98.8; 6.28)
		40	97.9 (2.82; 54.8; 8.38)	58 (3.15; 131.7; 5.04)	88.5 (2.89; 67.7; 7.64)
		48	111.9 (2.75; 30.2; 9.46)	79.2 (3.07; 93.3; 6.76)	96.3 (2.83; 53.7; 8.26)
		56	118.1 (2.68; 18.9; 9.94)	97.3 (3.02; 61.2; 8.18)	99.7 (2.79; 47.8; 8.52)
		64	120.8 (2.63; 14.1; 10.14)	107.1 (2.99; 44.3; 8.93)	102 (2.8; 43.4; 8.7)
		72	122.1 (2.61; 12.1; 10.26)	109.8 (2.92; 36.4; 9.21)	103.2 (2.76; 40.1; 8.8)
		80	121.9 (2.6; 11.7; 10.28)	110 (2.93; 36; 9.25)	103.4 (2.77; 39.7; 8.83)
250 g/L initial	Time	0	Biomass (g/kg)	0.25	0.30
glucose, 10 g/L	(hours)	4		0.31	0.39
yeast extract,		8		0.46	0.83
33 °C, initial pH		12		0.71	1.47
7.0		16		1.04	2.33

		20		2.15	1.46	3.58
		24		2.88	1.98	4.78
		28		3.41	2.36	5.99
		32		3.8	2.63	6.55
		36		4.02	2.89	6.80
		40		4.06	3.13	6.87
		48		4.09	3.15	6.91
		56		4.12	3.18	6.92
		64		4.14	3.21	6.93
		72		4.16	3.24	6.96
		80		4.16	3.23	6.95
10 g/L yeast extract, 33 °C, initial pH 7.0, 24 hours	Initial glucose (g/L)	230	Colony-forming units (CFU, × 10 <sup>9</sup> /mL, total/survival)	16.2/15.1		
		250		17.3/16.1		
		280		19.3/17.8		
		300		20.5/18.7		
		310		21.6/19.0		
		320		21.7/19.1		
	Initial xylose (g/L)	350		16.3/13.5	—	—
		370		15.4/12.3		
		230		11.6/10.7		
		250		12.8/11.7		
312 g/L initial glucose, 10 g/L	Time (hours)	280	0.25 g/kg biomass 9 g/kg EtOH (291.6 g/L residual glucose;	14.2/12.3		
		300		11.3/9.2		
		310		10.1/7.9		
		0				
		8			—	—



yeast extract,			0.6 g/kg biomass)			
33.5 °C, initial pH		16	26 (258.4; 1.68)			
6.75		24	51.7 (206.7; 3.47)			
		32	92.6 (126.8; 4.61)			
		40	119.7 (74.4; 4.93)			
		48	135.1 (44.4; 4.97)			
		56	142 (30.7; 5)			
		64	144.9 (25.3; 5.03)			
		72	146.7 (21.9; 5.05)			
		80	146.8 (21.6; 5.05)			
180 g/L initial	Time	0	0.25 g/kg biomass			
glucose, 90 g/L	(hours)	8	8.8 g/kg EtOH (168.7 g/L residual glucose;			
initial xylose, 42			84.4 g/L residual xylose; 39.3 g/L residual			
g/L initial			mannose; 0.59 g/kg biomass)			
mannose, 10 g/L		16	25.3 (150.2; 75; 35; 1.64)			
yeast extract,		24	50.3 (121.4; 60.6; 28.3; 3.39)			
33.5 °C, initial pH		32	90.1 (76.9; 38.5; 17.9; 4.49)			
6.75		40	116.4 (47.8; 23.9; 11.2; 4.8)			
		48	131.5 (31.1; 15.5; 7.3; 4.84)			
		56	138.2 (23.4; 11.7; 5.5; 4.87)			
		64	141 (20.4; 10.2; 4.7; 4.9)			
		72	142.8 (18.5; 9.3; 4.3; 4.92)			
		80	142.9 (18.3; 9.2; 4.2; 4.92)			
10 g/L yeast	Initial	150	72.2	Initial	150	51.2
extract, initial pH	mannose	200	96.6	xylose	200	68.3
6.5, 33 °C, 72	(g/L)	230	110	(g/L)	230	77.2

hours	250	119.5		
	280	132.5	250	83.9
	300	140.3		
	310	144.7	280	88.7
	320	144.1		
	330	143.8	300	75.4
	350	131.5		
	370	117.1	310	70.3

<sup>a</sup>Unless otherwise specified, the data tested defaults to ethanol production (g/kg), all results represent the mean of three experiments.