

Supporting Information

Tables

Table S1 Strains and plasmids used in this work

Strains or plasmids	Relevant features	Reference or source
Plasmids		
pEASY-E1	Vector for protein expression in <i>E. coli</i> , Amp ^R	TRANSGEN Inc., Beijing, China
pYES-pgk	pYES2-derived expression vector in yeast controlled by a 3-phosphoglycerate kinase promoter from <i>S. cerevisiae</i> , Amp ^R , G418 ^R	Laboratory collection
pYES-pgk-ADH4	<i>ADH4</i> gene amplicons cloned in pYES-pgk, Amp ^R , G418 ^R	This work
pEASY-E1-ADH4	<i>ADH4</i> gene amplicons cloned in pEASY Blunt E1, Amp ^R	This work
Strains		
<i>E. coli</i> Trans-T1	Host for cloning	TRANSGEN Inc., Beijing, China
<i>E. coli</i> DH5 α	Host for cloning	TIANGEN Inc., Beijing, China
<i>E. coli</i> BL21 (DE3)	Host for protein expression	TIANGEN Inc., Beijing, China
<i>E. coli</i> ADH4-BL21	<i>E. coli</i> BL21 harboring pEASY-E1-ADH4, Amp ^R	This work
<i>S. cerevisiae</i> S288c (ATCC 204508)	<i>MATα SUC2 gal2 mal mel flo1 flo8-1 hap1 ho bio1 bio6</i> ; donor of <i>ADH4</i> gene	Laboratory collection
<i>S. cerevisiae</i> S0	<i>S. cerevisiae</i> S288c harboring pYES-pgk, G418 ^R	9
<i>S. cerevisiae</i> S4	<i>S. cerevisiae</i> S288c harboring pYES-pgk-ADH4, G418 ^R	This work

Table S2 Primers used in this work

Primers	Sequences(5'-3')
RT-P1 (F-ADH1)	CTTGATTGGAGACTTGAC
RT-P2 (R-ADH1)	TCTATTGTTGGTTCTTACG
RT-P3 (F-ADH2)	TCTAAGGCTTCTCTGGTA
RT-P4 (R-ADH2)	TCCTCTGATGTCTTCAAC
RT-P5 (F-ADH3)	AGAGTTCTAGGTATTGAT
RT-P6 (R-ADH3)	GTCTAACATATTCCGTAG
RT-P7 (F-ADH4)	TTCAACAATGCTTCTCTA
RT-P8 (R-ADH4)	TCACCTAATCTCTTCTTG
RT-P9 (F-ADH5)	TGGTGTGTTGTTGTTAAGTTG
RT-P10 (R-ADH5)	TAAGGACATTGAGATTCATTAC
RT-P11 (F-ADH6)	CTGGTATAGAGATTGAGA
RT-P12 (R-ADH6)	ATTAGAAGAAGGTGATTG
RT-P13 (F-ADH7)	TCGTAAAGATTATGAAGATTGG
RT-P14 (R-ADH7)	ACTGCTTGAGATTGATACT
RT-P15 (F- ACT1)	GCCAAGATAGAACCACCAATCC
RT-P16 (R- ACT1)	CTGATGTCGATGTCCGTAAGG
P17 (F-ADH4- <i>Bam</i> H I)	CGGGATCCATGTCTTCCGTTACTG
P18 (R-ADH4- <i>Eco</i> R I)	CGGAATTCTTAATATTCATAGGCTTTCTTG
P19 (F-ADH4)	ATGTCTTCCGTTACTGGG
P20 (R-ADH4)	ATATTCATAGGCTTTCTTGATA
P21 (F-T7)	CGAAGGAAGACTCTCCTCCG
P22 (R-CYC1)	TTCTCAAGCAAGGTTTTTCAGTAT

Table S3 Factors and levels for L₉ (3³) orthogonal experimental design

Factor	Level		
	1	2	3
A (inducing temperature)	16	20	25
B (inducing time)	7	9	11
C (IPTG concentration)	0.2	0.2	0.4

Table S4 The design matrix and experimental data for optimization of induced expression of Adh4p

Run	Factors			Dehydrogenase activity (U/g)
	A	B	C	
1	1	1	1	1.55
2	1	2	2	1.68
3	1	3	3	1.75
4	2	1	2	1.83
5	2	2	3	1.88
6	2	3	1	2.09
7	3	1	3	1.87
8	3	2	1	1.47
9	3	3	2	1.64
k1	1.66	1.75	1.70	
k2	1.93	1.68	1.72	
k3	1.66	1.83	1.83	
R	0.27	0.15	0.13	
Q	A ₂ B ₃ C ₃			

K, the average value of each level; R, the range value for each factor; Q, the best group.

Identification of methionol and MMP by GC-MS

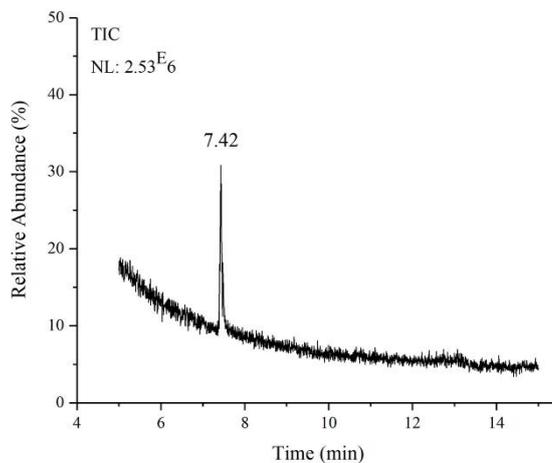


Figure S1. GC-MS chromatograms of the standard substance of MMP (7.42 min)

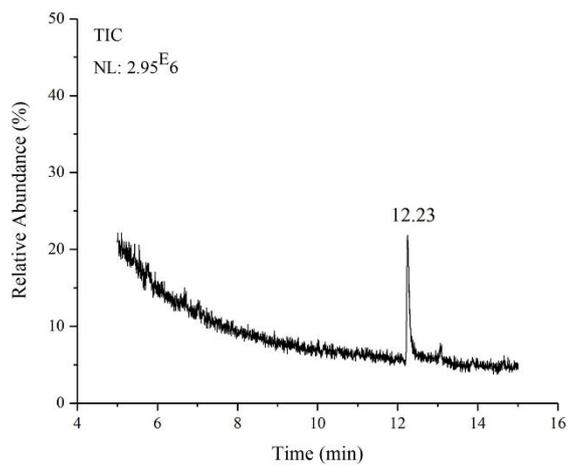


Figure S2. GC-MS chromatograms of the standard substance of methionol (12. 23 min)

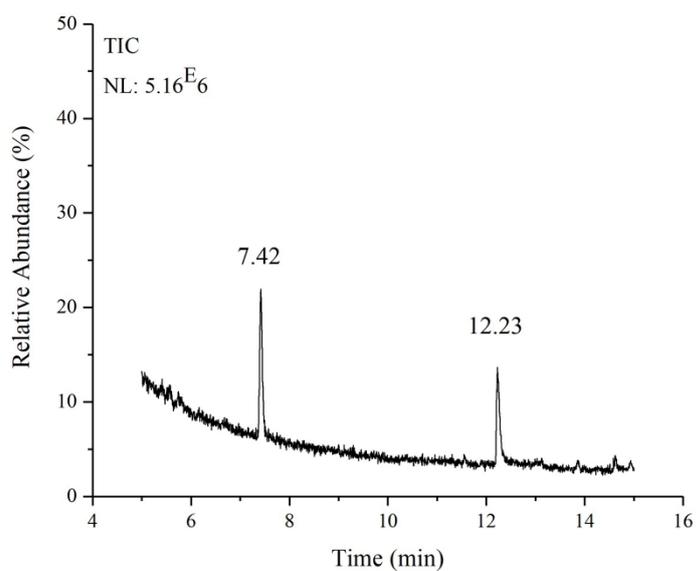


Figure S3. GC-MS chromatograms of MMP (7.42 min) and methionol (12. 23 min) in the fermentation sample

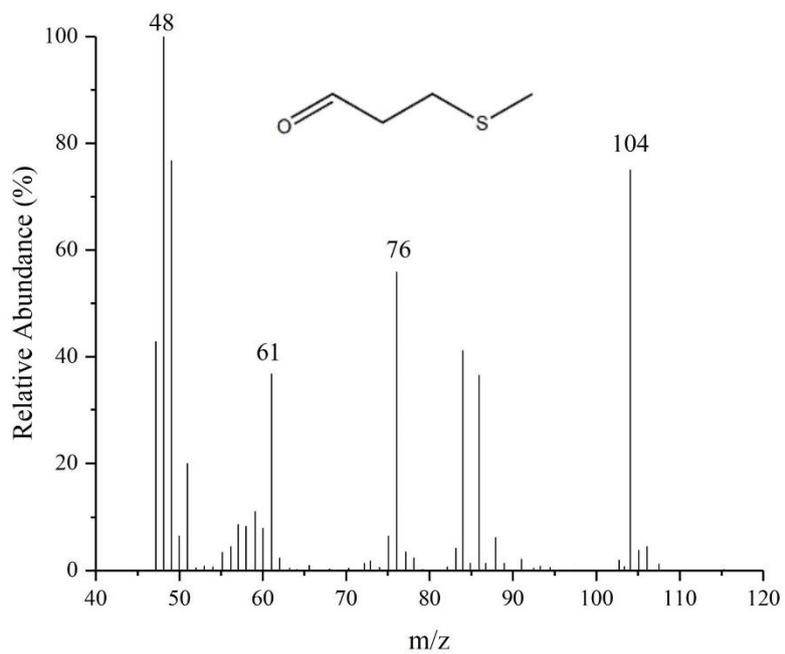


Figure S4. Full-scan MS spectra recorded at 7.42 min on MMP

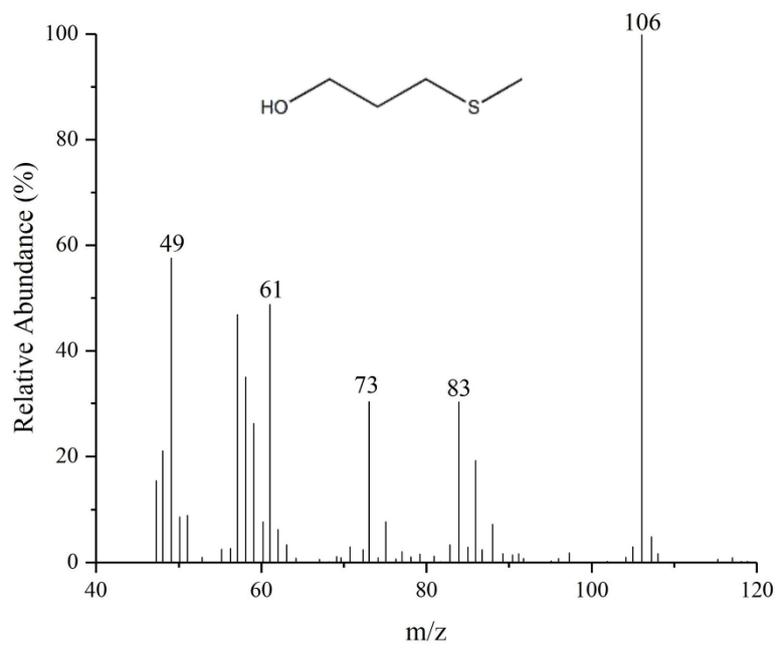


Figure S5. Full-scan MS spectra recorded at 12.23 min on methionol