Supporting Information

One-pot enzymatic synthesis of L-threitol from C1 formaldehyde

Sanrunyi Gong,[#a,c] Tianzhen Li, [#b,c] Zijing Tang,[c] Zijian Tan,[c,d] Ruke Zhang,[c] Karsten Olsen, [e] Haifeng Liu,[d*] and Leilei Zhu [c*]

[a]Sino-Danish College, University of Chinese Academy of Sciences, Beijing 100049, China.
[b]Haihe Laboratory of Synthetic Biology, 21 West 15th Avenue, Tianjin Airport Economic Area, Tianjin 300308, China.
[c]Key Laboratory of Engineering Biology for Low-Carbon Manufacturing, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences,Tianjin 300308, China. E-mail: zhu_ll@tib.cas.cn.
[d]Jiangsu Collaborative Innovation Centre of Chinese Medicinal Resources Industrialization, School of Pharmacy, Nanjing University of Chinese Medicine, Nanjing 210023, China. E-mail: haifeng.liu@njucm.edu.cn.
[e]Department of Food Science, University of Copenhagen, Frederiksberg C, Denmark

‡These authors contributed equally to this work.
*Corresponding Authors: zhu_ll@tib.cas.cn; haifeng.liu@njucm.edu.cn.

1. Experimental Procedures

1.1. Materials and methods

Formaldehyde solution, L-threitol, glycerol, L-erythrulose, and erythritol were purchased from Aladdin (Shanghai, China); 1,3-dihydroxyacetone (DHA) was purchased from Bidepharm (Shanghai, China); Nicotinamide adenine dinucleotide disodium salt ($NADHNa_2$) was purchased from Kailian (Shenzhen, China); Thiamine pyrophosphate (TPP) was purchased from Solarbio (Beijing, China); Isopropanol was purchased from Macklin (Shanghai, China); Methanol was purchased from Sigma-Aldrich (Shanghai, China); Magnesium sulfate anhydrous was purchased from Sinopharm (Shanghai, China); The amount of protein was quantified by Sangon BCA protein assay kit (Beijing, China).

1.2. Genes, plasmids, and strains

The mutant (FSA^{A129S}) of D-Fructose-6-Phosphate Aldolase from *Escherichia coli* was synthesized and ligated into the expression vector pET28a via *NdeI* and *XhoI* restriction sites by GENEWIZ (Suzhou, China). The Glycerol dehydrogenase coding gene from *Escherichia coli* was synthesized and ligated into the expression vector pET21b via *BamHI* and *NotI* restriction sites by GENEWIZ. The methanol dehydrogenase coding gene from *Bacillus stearothermophilus* was synthesized and ligated into the expression vector pET28a via *NdeI* and *XhoI* restriction sites by GENEWIZ. The recombinant plasmid of pET28a-BFD-M6 was selected in our reported article. All the recombinant plasmids were transformed into *E. coli* BL21-Gold (DE3).

1.3. Analysis of DHA generated by $formolase_{BFD-M6}$

$Formolase_{BFD-M6}$ was quantified in phosphate buffer (50 mM KPi, 5 mM $MgSO_4$, pH 7.4). 0.13 mM $formolase_{BFD-M6}$ was incubated with different concentrations of formaldehyde (300 mM - 2100 mM) and 1 mM TPP with shaking (1000 rpm, 30°C) for 5 h. The enzymes in the reaction were removed by 3 kDa Millipore. DHA was detected by HPLC. The HPLC system was carried out on an Agilent 1260 system equipped with a refractory index (RI) detector, and fitted with a chromatographic column (ChromCore Sugar-Ca). Mobile phase: H_2O, flow rate: 0.4 mL/min, sample volume: 20 µL, column temperature: 55°C.

1.4. Analysis of L-erythrulose generated by BFD-M6 and FSA^{A129S}

$Formolase_{BFD-M6}$ and FSA^{A129S} in phosphate buffer (50 mM KPi, 5 mM $MgSO_4$, pH 7.4) were quantified. 0.13 mM $formolase_{BFD-M6}$ and 0.33 mM FSA^{A129S} were incubated in the presence of different concentrations of formaldehyde (900 mM - 2100 mM) and 1 mM TPP with shaking (1000 rpm, 30°C) in 6h. The enzymes in the reaction were removed by 3 kDa Millipore and the produced L-erythrulose was detected by HPLC. The HPLC system was carried out on an Agilent 1260 system equipped with a refractory index (RI) detector, and fitted with a chromatographic column (ChromCore Sugar-Ca). Mobile phase: H_2O, flow rate: 0.4 mL/min, sample volume: 20 µL, column temperature: 55°C.

1.5. Screening candidates of L-threitol dehydrogenase

To identify an enzyme that can produce L-threitol from L-erythrulose, xylitol dehydrogenase from *Gluconobacter oxydans* ATCC621 (pir: JC7939) was selected as a template, and candidate sequences were screened in the National Center for Biotechnology Information (NCBI) with the percent identity from 30% to 70%. Multiple sequence alignment was performed using MEGA11 and a Neighbor-joining phylogenetic tree was constructed and analyzed by all selected sequences. Finally, six candidate enzymes were confirmed for further expression and functional identification.

1.6. Expression and functional identification of six L-threitol dehydrogenase candidates

Six L-threitol dehydrogenase candidates were synthesized and ligated into the expression vector pET28a via *NdeI* and *XhoI* restriction sites by GENEWIZ (Suzhou, China). *E. coli* BL21-Gold (DE3) cells carrying different recombinant plasmids were inoculated into 5 mL LB liquid medium (50 μg/mL kanamycin) and cultured overnight at 37°C, and then transferred into 25 mL LB liquid medium (50 μg/mL kanamycin) with the ratio of 1:100. All the flasks were shaken at 220 rpm at 37°C. When the optical density at 600 nm (OD_{600}) reached 0.6-0.8, gene expression was induced using 0.1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) at 20°C for 24 h. Subsequently, 2 mL cell cultures were centrifugated at 12000 rpm for 10 min. The cells were harvested and resuspended in 500 μL phosphate buffer (50 mM KPi, 5 mM $MgSO_4$, pH 7.4), and subsequently followed by disruption with an ultrasonic cell disruptor (300 W, 1 s/3 s, 10 min; Scientz-ⅡD; Scientz, Ningbo, China). The disrupted cells were centrifuged at 12000 rpm, 4°C for 2 min, the reaction was initiated by adding 210 μL supernatant, 8 mM NADH, and 20 mM L-erythrulose and conducted for 19 h (30°C, 1000 rpm) and pET28a was added to the control group. The enzymes present in the reaction were removed using 3 kDa Millipore and the generated L-threitol was detected by HPLC. The HPLC system was carried out on an Agilent 1260 system equipped with a refractory index (RI) detector, and fitted with a chromatographic column (ChromCore Sugar-Ca). Mobile phase: H_2O, flow rate: 0.4 mL/min, sample volume: 20 μL, column temperature: 55°C.

1.7. Expression of target protein in shaking flask and purification

Except that the target proteins were eluted at ~300 mM imidazole using a 20-500 mM imidazole gradient, other methods are same as described in the article[1]. In this study, GldA is expressed using 100 μg/mL ampicillin, while other strains using 50 μg/mL kanamycin.

1.8. The optimum pH of NsTDH

Citric acid buffer (50 mM citric acid and trisodium citrate dihydrate, 5 mM $MgSO_4$) with different pH values (3.0, 4.0, 5.0), phosphate buffer (50 mM phosphate buffer, 5 mM $MgSO_4$) with different pH values (6.0, 7.0 and 8.0) and Glycine-NaOH buffer (50 mM Glycine and NaOH, 5 mM $MgSO_4$) with different pH values (9.0, 10.0, 11.0) were prepared. NsTDH was quantified by a BCA protein assay kit and diluted by different pH buffers. L-erythrulose was prepared by the above-mentioned buffer solutions with different pH. 200 μL reaction solution with 1.76 μM PHM, 8 mM L-erythrulose solution, and 2 mM NADH in 96-well microtiter plates. The reaction was initiated by adding NADH and subsequently was detected at 340 nm for 20 min using a microtiter plate reader (VersaMax; Molecular Devices, Sunnyvale, USA). The absolute value of the decreased rate of absorbance value at 340 nm under different pH buffers was calculated as the activity of NsTDH.

1.9. The optimum reaction temperature of NsTDH

NsTDH was quantified by BCA protein assay kit and diluted by phosphate buffer (50 mM KPi, 5 mM $MgSO_4$, pH 6). L-erythrulose was prepared with phosphate buffer (50 mM KPi, 5 mM $MgSO_4$, pH 6). 600 μL reaction solution with 1.76 μM L-threitol dehydrogenase, 5 mM L-erythrulose, and 2 mM NADH in a quartz cell. The reaction was initiated by adding NADH and subsequently was detected at 340 nm for 20 min using UV-Vis Spectrophotometers (Agilent 8463, California, USA). The absolute value of the decreased rate of absorbance value at 340 nm under different pH buffers was calculated as the activity of L-threitol dehydrogenase.

1.10. Kinetic parameters determination of NsTDH

Purified NsTDH was quantified by BCA protein assay kit and diluted with phosphate buffer (50 mM KPi, 5 mM $MgSO_4$, pH 6). Different concentration of L-erythrulose was prepared with phosphate buffer (50 mM KPi, 5 mM $MgSO_4$, pH 6). 200 μL reaction solution with 1.76 μM NsTDH, different

concentrations of L- erythrulose solution (0-80 mM), and 2 mM NADH in 96-well microtiter plates. The reaction was initiated by adding NADH and the decrease of NADH was subsequently detected at 340 nm for 20 min using a microtiter plate reader.

The initial velocity data was subjucted to a Michaelis-Menten equation ($v=V_{max}\times S/(K_m+S)$), where v refers to the initial enzyme velocity, S represents the substrate concentration, V_{max} represents the maximum enzyme velocity, and K_m represents the substrate concentration required to achieve a half-maximum enzyme velocity.

1.11. NADH regeneration by using NsTDH coupled with GldA

The standard reaction mixture consisted of 250 µL of phosphate buffer (50 mM KPi, 5 mM $MgSO_4$, pH 7.4) containing 75 mM glycerol, 75 mM L-erythrulose, 2 mM NADH, 0.11 mM NsTDH, and 0.15 mM GldA. The reactions were carried out in a 1.5 mL microcentrifuge tube at 30°C and 1000 rpm for 14 h. The enzymes present in the reaction were removed using 3 kDa Millipore, and the resulting L-threitol was detected by HPLC. The HPLC system was carried out on an Agilent 1260 system equipped with a refractory index (RI) detector, and fitted with a chromatographic column (ChromCore Sugar-Ca). Mobile phase: H_2O, flow rate: 0.4 mL/min, sample volume: 20 µL, column temperature: 55°C.

1.12. NADH regeneration by using NsTDH coupled with BsMDH

The standard reaction mixture consisted of 250 µL of phosphate buffer (50 mM KPi, 5 mM $MgSO_4$, pH 7.4) containing 75 mM methanol/isopropanol, 75 mM L-erythrulose, 2 mM NADH, 0.11 mM NsTDH, and 0.14 mM BsMDH. The reactions were conducted at 30°C and 1000 rpm in a 1.5 mL microcentrifuge tube for 14 h. The enzymes present in the reaction were removed using 3 kDa Millipore, and the resulting L-threitol was detected by HPLC. The HPLC system was carried out on an Agilent 1260 system equipped with a refractory index (RI) detector, and fitted with a chromatographic column (ChromCore Sugar-Ca). Mobile phase: H_2O, flow rate: 0.4 mL/min, sample volume: 20 µL, column temperature: 55°C.

1.13. The synthesis of L-threitol from formaldehyde with BsMDH/methanol system for NADH regeneration

For the synthesis of L-threitol, 750 µL standard reaction mixture with phosphate buffer (50 mM KPi, 5 mM $MgSO_4$, pH 7.4) containing 900 mM/1200 mM/1500 mM/2100 mM formaldehyde, 0.33 mM FSA^{A129S} and 0.13 mM $Formolase_{BFD\text{-}M6}$ as the first step. The reactions were carried out at 30°C and 1000 rpm in a 1.5 mL microcentrifuge tube for 8 h. And then 1mL reaction mixture with phosphate buffer (50 mM KPi, 5 mM $MgSO_4$, pH 7.4) containing 750 uL of the first step solution, 2 mM NADH, 0.11 mM NsTDH, 0.14 mM BsMDH and 170 mM/220 mM/280 mM/380 mM methanol as the second step. The reactions were carried out at 30°C and 1000 rpm in a 1.5 mL microcentrifuge tube for 12 h. The enzymes present in the reaction were removed using 3 kDa Millipore and the generated L-threitol was detected by HPLC. The HPLC system was carried out on an Agilent 1260 system equipped with a refractory index (RI) detector, and fitted with a chromatographic column (ChromCore Sugar-Ca). Mobile phase: H_2O, flow rate: 0.4 mL/min, sample volume: 20 µL, column temperature: 55°C.

1.14. The synthesis of L-threitol from formaldehyde with BsMDH/ isopropanol system for NADH regeneration

For the synthesis of L-threitol, 750 µL standard reaction mixture with phosphate buffer (50 mM KPi, 5 mM $MgSO_4$, pH 7.4) containing 900 mM/1200 mM/1500 mM/2100 mM formaldehyde, 0.33 mM FSA^{A129S} and 0.13 mM $Formolase_{BFD\text{-}M6}$ as the first step. The reactions were carried out at 30°C

and 1000 rpm in a 1.5 mL microcentrifuge tube for 8 h. And then 1mL reaction mixture with phosphate buffer (50 mM KPi, 5 mM $MgSO_4$, pH 7.4) containing 750 µL the first step solution 2 mM NADH, 0.11 mM NsTDH, 0.14 mM BsMDH and 170 mM/220 mM/280 mM/380 mM isopropanol as the second step. The reactions were carried out at 30°C and 1000 rpm in a 1.5 mL microcentrifuge tube for 12 h. The enzymes present in the reaction were removed using 3 kDa Millipore and the generated L-threitol was detected by HPLC. The HPLC system was carried out on an Agilent 1260 system equipped with a refractory index (RI) detector, and fitted with a chromatographic column (ChromCore Sugar-Ca). Mobile phase: H_2O, flow rate: 0.4 mL/min, sample volume: 20 µL, column temperature: 55°C.

1.15. L-threitol catalyzed by multienzyme synthetic system with one step based NsTDH

For the synthesis of L-threitol, 500 µL standard reaction mixture with phosphate buffer (50 mM KPi, 5 mM $MgSO_4$, pH 7.4) containing 0.13 mM $Formolase_{BFD\text{-}M6}$, 0.26 mM FSA^{A129S}, 0.11 mM NsTDH, 0.15 mM GldA, 2 mM NAD^+, 1 mM TPP, 600 mM/900 mM/1200 mM formaldehyde and 300 mM/450 mM/600 mM glycerol. The reactions were carried out at 30°C and 1000 rpm in a 1.5 mL microcentrifuge tube for 17 h. The enzymes present in the reaction were removed using 3 kDa Millipore and the generated L-threitol was detected by HPLC. The HPLC system was carried out on an Agilent 1260 system equipped with a refractory index (RI) detector, and fitted with a chromatographic column (ChromCore Sugar-Ca). Mobile phase: H_2O, flow rate: 0.4 mL/min, sample volume: 20 µL, column temperature: 55°C.

1.16. 50 mL scale reaction with BsMDH/ isopropanol system for NADH regeneration

To produce L-erythrulose, a 45 mL standard reaction mixture with phosphate buffer (50 mM potassium phosphate, 5 mM $MgSO_4$, pH 7.4) containing 1500 mM formaldehyde, 0.33 mM FSA^{A129S} and 0.13 mM $formolase_{BFD\text{-}M6}$ as the first step. The reactions were carried out at 30°C with shaking at 200 rpm in a 250 mL flask for 8 h. Subsequently, a 50 mL reaction mixture containing 37.5 mL of the first-step solution, 2 mM NADH, 0.11 mM NsTDH, 0.14 mM BsMDH and 280 mM isopropanol in potassium phosphate buffer was prepared and used for the second step, which was carried out for 12, 14 and 16 h. The enzymes present in the reaction were removed using 3 kDa Millipore and the generated L-threitol was detected by HPLC. The HPLC system was carried out on an Agilent 1260 system equipped with a refractory index (RI) detector, and fitted with a chromatographic column (ChromCore Sugar-Ca). Mobile phase: H_2O, flow rate: 0.4 mL/min, sample volume: 20 µL, column temperature: 55°C.

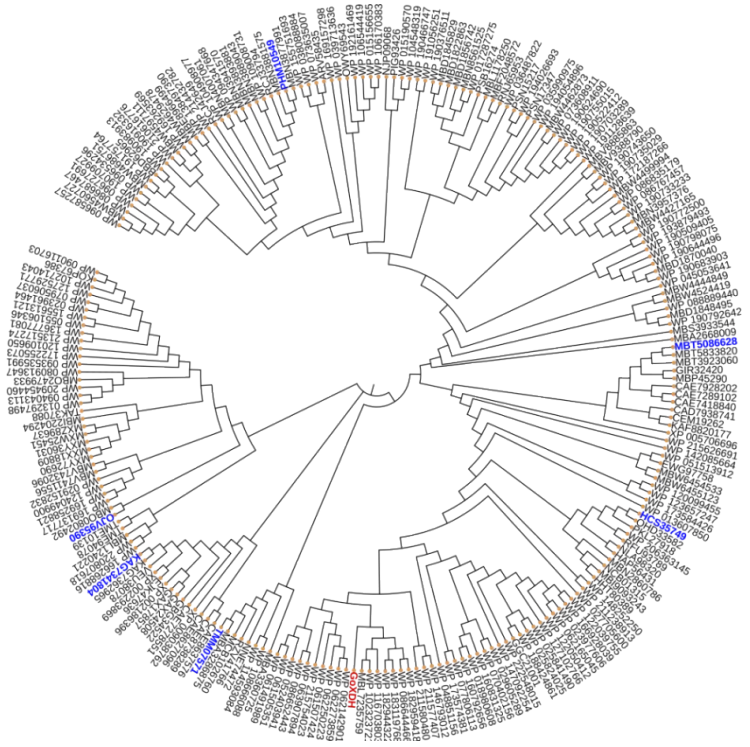

Figure S1. Evolutionary tree analysis of candidate L-Threitol dehydrogenase.

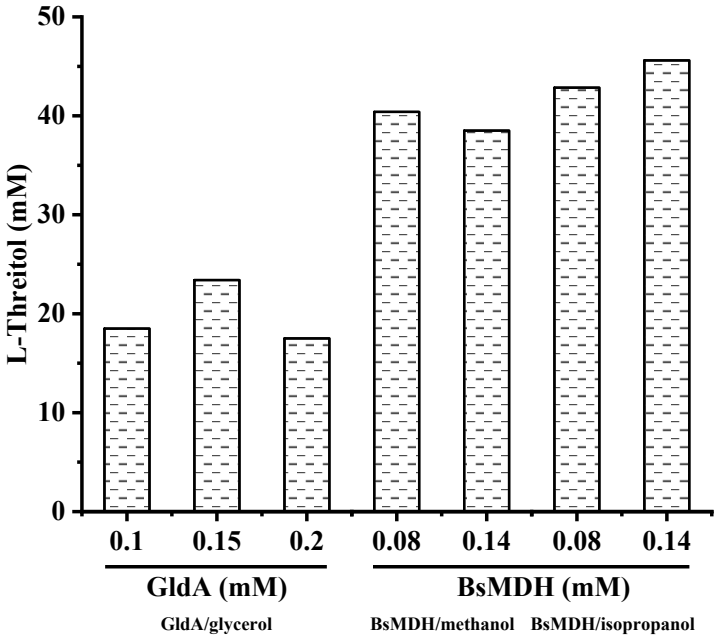

Figure S2. Reduction of L-erythrulose to L-threitol with different concentration of GldA or BsMDH for three NADH regeneration systems. The GldA/glycerol system employed GldA at 0.1, 0.13, and 0.15 mM. The BsMDH/methanol and BsMDH/isopropanol systems used BsMDH at 0.08 and 0.14 mM. All systems were performed in 50 mM potassium phosphate buffer (pH 7.4) containing 5 mM $MgSO_4$, 75 mM L-erythrulose, 75 mM isopropanol, 2 mM NADH, and 0.11 mM NsTDH, at 30°C with

shaking at 1000 rpm in a 1.5 mL microcentrifuge tube for 14 h.

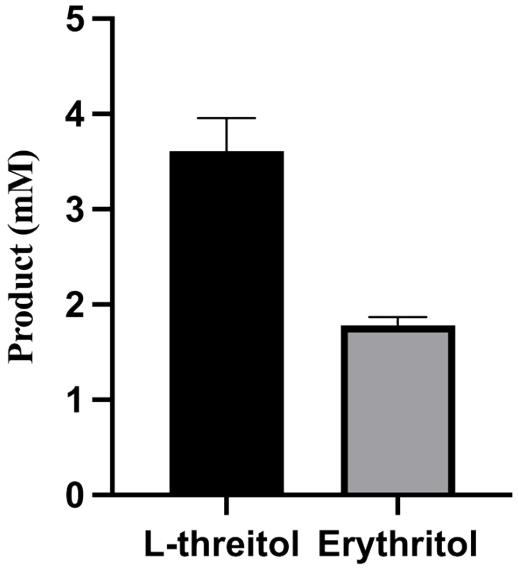

Figure S3. The products of the conversion of L-erythrulose by glycerol dehydrogenase.

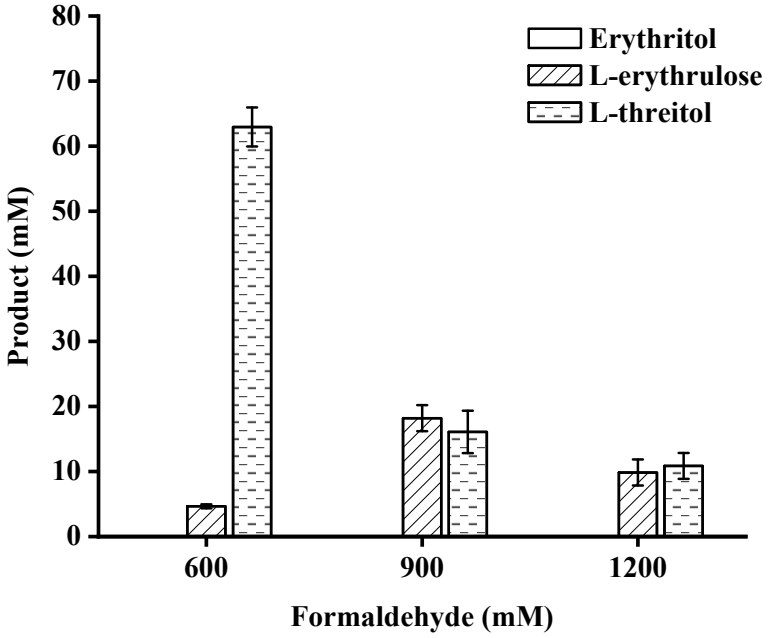

Figure S4. Enzymatic synthesis of L-threitol from formaldehyde using GldA/glycerol system for NADH regeneration without adding formolase$_{BFD-M6}$ as negative control. 0.5 mL standard reaction solution containing 50 mM phosphate buffer (50 mM phosphate buffer, 5 mM $MgSO_4$, pH 7.4), 2 mM NAD^+, 0.26 mM FSA^{A129S}, 0.15 mM GldA, 0.11 mM NsTDH, different concentrations of formaldehyde (600, 900 and 1200 mM) with the addition of 300, 450 and 600 mM glycerol to the system, respectively. The reactions were carried out at 30°C and 1000 rpm in a 1.5 mL

microcentrifuge tube for 17 h.

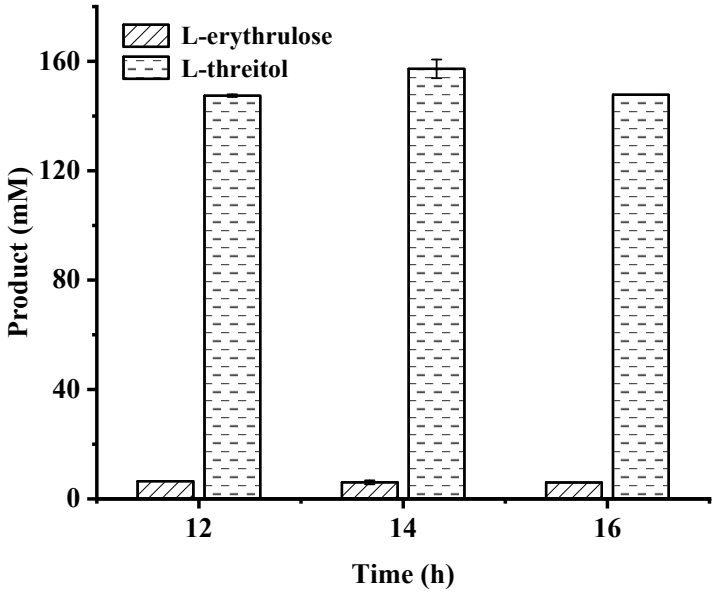

Figure S5. One-pot two-step synthesis of L-threitol from formaldehyde using BsMDH/isopropanol system for NADH regeneration in 50 mL scale. To produce L-erythrulose, the first step was performed in 45 mL contained 1500 mM formaldehyde, 0.33 mM FSA^{A129S} and 0.13 mM $formolase_{BFD\text{-}M6}$ in phosphate buffer (50 mM potassium phosphate, 5 mM $MgSO_4$, pH 7.4) at 30°C with shaking at 200 rpm in a 250 mL flask for 8 h. Subsequently, the second step was performed in 50 mL containing 37.5 mL of the first-step solution, 2 mM NADH, 0.11 mM NsTDH, 0.14 mM BsMDH and 280 mM isopropanol in potassium phosphate buffer was prepared and used for the second step. Samples were taken at 12, 14 and 16 h.

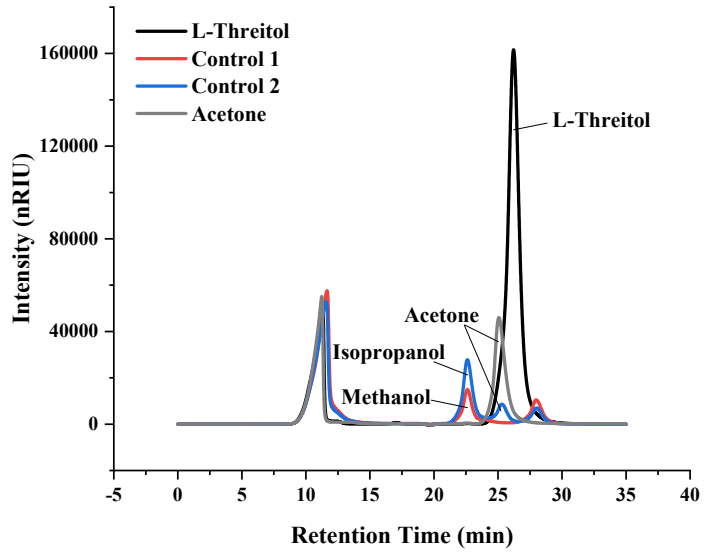

Figure S6. HPLC analysis of controls without formolaseBFD-M6 in the BsMDH/methanol and

BsMDH/isopropanol systems. Control 1 is the reaction using the BsMDH/isopropanol system for NADH recycling but in absence of formolase$_{BFD-M6}$. Control 2 is the reaction using the BsMDH/methanol system for NADH recycling but in absence of formolase$_{BFD-M6}$.

Table S1. Gibbs energy change (ΔrG') of the enzymatic cascade reaction for the synthesis of L-threitol from formaldehyde. The values were calculated under experimental conditions (Figure 1B) using eQuilibrator (pH 7.4, pMg 2.3, ionic strength 0.12 M).

Enzyme	Reactions	ΔrG' (kJ/mol)
Formolase$_{BFD-M6}$	3 Formaldehyde = 1,3-Dihydroxyacetone	-152.1 ± 9.2 [a]
D-fructose-6-phosphate aldolase FSA^{A129S}	Formaldehyde + 1,3-Dihydroxyacetone = L-Erythrulose	-28.4 ± 7.1 [b]
L-threitol dehydrogenase NsTDH	L-Erythrulose + NADH = Threitol + NAD^+	-137.9 ± 5.4 [c]

[a] Formaldehyde 1.5M

[b] Formaldehyde 1.5 M, 1,3-Dihydroxyacetone 0.001 nM

[c] L-Erythrulose 0.312 M, NADH 2 mM

Table S2. The source of the candidate sequence of L-Threitol dehydrogenase

Candidate sequence	Source
TDH-1	*Microbacterium sp.*
TDH-2	*Nitzschia inconspicua*
TDH-3	*Actinobacteria bacterium*
TDH-4	*Sphaerochaeta sp.*
TDH-5	*Deltaproteobacteria bacterium*
TDH-6	*Nostoc sp.*

Table S3. Gibbs energy change (ΔrG') of individual reactions for L-threitol production from 75 mM L-erythrulose with 75 mM glycerol/methanol/isopropanol as cosubstrates. The vaules calculated under experimental conditions (Figure 3D) using eQuilibrator (pH 7.4, pMg 2.3, ionic strength 0.12 M).

NADH regeneration system	Reactions	ΔrG' (kJ/mol)
GldA /glycerol	L-erythrulose + Glycerol + NAD^+ = Threitol + 1,3-Dihydroxyacetone + NADH	-120.8 ± 6.4
BsMDH/methanol	L-erythrulose + Methanol + NAD^+ = Threitol + Formaldehyde + NADH	-111.2 ± 8.3
BsMDH/isopropanol	L-erythrulose + Isopropanol + NAD^+ = Threitol + Acetone + NADH	-138.0 ± 5.4

Table S4. Amino acid sequences of the enzymes used for L-threitol production.

Enzyme	Amino acid sequence
Formolase$_{BFD-M6}$	MASVHGTTYELLRRQGIDTVFGNPGFNELPFLKDFPEDFRYILALQEACVVGIADG YAQASRKPAFINLHSAAGTGNAMGALSNARTSHSPLIVTAGQQTRAMIGVEASE TNVDAANLPRPLVKWSYEPASAAEVPHAMSRAIHMASMAPQGPVYLSVPYDD WDKDADPQSHHLFDRHVSSSVRLNDQDLDILVKALNSASNPAIVLGPDVDAANA NADCVMLAERLKAPVWVAPSAPRCPFPTRHPCFRGLMPAGIAAISQLLEGHDVV LVIGAPVFRYYQYDPGQYLKPGTRLISVTCDPLEAARAPMGDAIVADIGAMASAL ANLVEESSRQLPTAAPEPAKVDQDAGRLHPETVFDTLNDMAPENAIYLNESTSTT AQMWQRLNMRNPGSYYFCAAGGLGFALPAAIGVQLAEPERQVIAVIGDGSANY SISALWTAAQYNIPTIFVIMNNGTYGMLRWFAGVLEAENVPGLDVPGIDFRALAK GYGVQALKADNLEQLKGSLQEALSAKGPVLIEVSTVSPVK
FSA^{A129S}	MELYLDTSDVVAVKALSRIFPLAGVTTNPSIIAAGKKPLDVVLPQLHEAMGGQGR LFAQVMATTAEGMVNDALKLRSIIADIVVKVPVTAEGLAAIKMLKAEGIPTLGTAV YGAAQGLLSALAGAEYVSPYVNRIDAQGGSGIQTVTDLHQLLKMHAPQAKVLAA SFKTPRQALDCLLAGCESITLPLDVAQQMISYPAVDAAVAKFEQDWQGAFGRTSI
NsTDH	MTAKATYQFADKTILITGGAGDIGKATAHRFAANGAGIALLDLNEPKMADVAVEL KGYNVPVGTFRCDVTSSDDVAKAFTGAVKQLGRIDYVFNNAGYQGVFAKTDEYP EDDFQKVININVVGVFHILKAAAQQLRDAGGGAIVNMASYAGVVGPPNMLAYA ASKFAVIGITQTAAKDLAPYGIRVNALSPALIGPGFMWTRQTELQAAVGSQYFDA DPKVVEQQMIDSVPMRRLGSLEEVANGVAFLMSEEASYITGFNLEVTGG
GldA	MDRIIQSPGKYIQGADVINRLGEYLKPLAERWLVVGDKFVLGFAQSTVEKSFKDA GLVVEIAPFGGECSQNEIDRLRGIAETAQCGAILGIGGGKTLDTAKALAHFMGVPV AIAPTIASTDAPCSALSVIYTDEGEFDRYLLLPNNPNMVIVDTKIVAGAPARLLAAGI GDALATWFEARACSRSGATTMAGGKCTQAALALAELCYNTLLEEGEKAMLAAE QHVVTPALERVIEANTYLSGVGFESGGLAAAHAVHNGLTAIPDAHHYYHGEKVAF GTLTQLVLENAPVEEIETVAALSHAVGLPITLAQLDIKEDVPAKMRIVAEAACAEGE TIHNMPGGATPDQVYAALLVADQYGQRFLQEWEAAA
BsMDH	MKAAVVNEFKKALEIKEVERPKLEEGEVLVKIEACGVCHTDLHAAHGDWPIKPKL PLIPGHEGVGIVVEVAKGVKSIKVGDRVGIPWLYSACGECEYCLTGQETLCPHQLN GGYSVDGGYAEYCKAPADYVAKIPDNLDPVEVAPILCAGVTTYKALKVSGARPGE WVAIYGIGGLGHIALQYAKAMGLNVVAVDISDEKSKLAKDLGADIAINGLKEDPV KAIHDQVGGVHAAISVAVNKKAFEQAYQSVKRGGTLVVVGLPNADLPIPIFDTVL NGVSVKGSIVGTRKDMQEALDFAARGKVRPIVETAELEEINEVFERMEKGKINGRI VLKLKED

2. References

1. Li, T; Tan, Z; Tang, Z; Liu, P; Liu, H; Zhu, L; Ma, Y. Green Chemistry., 2022, **24**, 5064-5069.