Supporting Information

Hybrid synthesis of polyhydroxybutyrate bioplastics from carbon dioxide

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Materials and Methods

Chemicals and agents

Common chemicals were bought from Sigma-Aldrich (Shanghai, China), SolarBio (Beijing, China), Zhenzhun Biotech (Shanghai, China) and Yuanye Biotech (Shanghai, China). Standard acetic acid, formaldehyde (FALD), glycoaldehyde (GALD), dihydroxyacetone (DHA), and acetyl phosphate (AcP) were purchased from Yuanye Biotech (Shanghai, China). Standard Poly(3-hydroxybutyrate) (PHB) was purchased from SHANGHAI ZZBIO CO., LTD. Restriction enzymes and DNA polymerase were purchased from Thermo Fisher Scientific (Shanghai, China), and TransGen Biotech (Beijing, China). Kits for DNA manipulation were purchased from Axygen (Shanghai, China), BL21(DE3) and DH5α cells were purchased from TransGen Biotech (Beijing, China). Primers and synthesized genes were obtained from Genecreat (Wuhan, China) or GENEWIZ (Suzhou, China). Materials and equipment for protein purification were obtained from GE Healthcare (Beijing, China) and BioRad (Beijing, China). HPX-87H column were purchased from BioRad (Beijing, China).

Plasmids construction

Plasmids used in the study are listed in Supplementary Table 4. The plasmids of mutagenesis libraries were constructed by Gibson DNA assembly. The plasmids for protein expression were constructed into the pET28a plasmid. The genes were inserted between the *NdeI* and *XhoI* restriction sites. There was an 6 × His tag in N-terminal for purification.

Bacterial strains and growth condition

E. coli BL21 (DE3) (TransGen^{TM}) was grown at 37 ºC and induced at 16 ºC in 2YT medium for protein expression. 2YT medium contains 1.6 % peptone, 1 % yeast extract, 0.5 % NaCl. Antibiotics for selection purposes were used accordingly at 50 μg

ml^{-1}.

Purification of alcohol oxidases

Transformed *E. coli* BL21 (DE3) was grown in 2YT medium at 37 °C. Protein expression was induced when the cultures reached $OD_{600} \approx 0.6$-0.8 by adding 0.5 mM isopropyl β-D-1-thiogalactopyrano (IPTG). Next, the cells were incubated at 16 °C until late stationary phase and then harvested by centrifugation at 5500 rpm for 10 min at 4 °C. Cells were resuspended in 35 mL lysis buffer (50 mM potassium phosphate pH 7.5, 400 mM NaCl, 100 μM FAD). The high-pressure homogenizer cracked the bacteria to release proteins. After removal of cellular debris by centrifugation (10000 rpm, 4 °C, 60 min), the supernatant was loaded onto nickel affinity columns (GE Healthcare) pre-equilibrated using the lysis buffer. The elution of proteins was performed using a 50-400 mM imidazole gradient. Fractions containing the pure proteins as indicated by SDS-PAGE were pooled and then desalted and concentrated using 30 kDa centrifugal filter units and 50 mM potassium phosphate buffer (pH 7.5).

Other protein synthesis and purification

Catalase was purchased from Aladdin. All genes were transformed into *E. coli* BL21 (DE3) for expression and cultured with 800 mL 2YT medium. 0.5 mM IPTG was added and induced for 16 h. Cells were collected at 5500 rpm and resuspend in pre-cooled buffer. The high-pressure homogenizer cracked the bacteria to release the proteins. Proteins were purified by His-Spin protein mini-prep columns (Zymo Research). 50 mM and 100 mM imidazole was used to wash out impurities. Then used 200 mM imidazole to elute target proteins. And we used Amicon Ultra-15 ultrafiltration tubes to concentrate proteins. The proteins concentration was determined using a BCA Protein Assay Reagent Kit (Pierce, USA) with 2 mg/mL BSA as the standard. Purified enzymes were stored at -80 °C before use.

Demonstration of the module III in vitro

The module III used glycolaldehyde as the initial substrate: The assay was set up in a final volume of 1 mL at 37 °C, 5 h, containing 50 mM HEPES buffer (pH 7.5), 5 mM $MgSO_4$, 5 mM K_3PO_4, 1 mM ThDP, 0.5 mM CoA, 0.5 mM $NADP^+$, 2 mg·mL^{-1} ACPS, 1 mg·mL^{-1} PTA, 2 mg·mL^{-1} PhaA, 1 mg·mL^{-1} PhaB, 2 mg·mL^{-1} PhaC, 2 mg·mL^{-1} FDH.

The complete process in vitro from methanol to PHB

Firstly we used methanol as the initial substrate, the assay was set up at 37 °C in a final volume of 1 mL containing 50 mM HEPES buffer (pH 7.5), 5 mM $MgSO_4$, 1 mM ThDP, 0.2 mg·mL^{-1} AOX, 300U mL^{-1} CAT, 2 mg·mL^{-1} $GALS_{F397YC398M}$ (or 10 mg·mL^{-1} GALS). Reaction was taken place in an enzyme reactor at 900 rpm, 37 ℃ for 1.5 h. The reaction solution was fed into micro-ultrafiltration tubes (3 kDa) to intercept proteins, at 3500 rpm, 0.5 h. The filtrate was used as the initial substrate for next step.

The second stage was initiated by supplementing the remaining enzymes and auxiliary components, containing final concentration 5 mM K_3PO_4, 0.5 mM CoA, 0.5 mM $NADP^+$, 2 mg·mL^{-1} ACPS, 1 mg·mL^{-1} PTA, 2 mg·mL^{-1} PhaA, 1 mg·mL^{-1} PhaB, 2 mg·mL^{-1} PhaC, 2 mg·mL^{-1} FDH. The reaction volume was filled to 1 mL by HEPES buffer (pH 7.5), 5 mM $MgSO_4$, 5 mM K_3PO_4. The assay was set up at 37 °C, 900 rpm for 5 h.

Assay of formaldehyde

Preparation of 0.25% acetylacetone solution: weigh 25 g of ammonium acetate and dissolve in a little ddH_2O, then add 3 mL glacial acetic acid and 0.25 mL acetylacetone, after mixing, make up to 100 mL and adjust the pH to 6.0.

Detection of formaldehyde: take 50 μL of samples and add 150 μL acetylacetone solution to reaction at 60 ℃ for 10 min, then detect the absorbance value at 414 nm.

The amount of formaldehyde was calculated from the standard curve.

Assay of formate, glycoaldehyde, acetate and acetyl-phosphate

Formate was detected by high performance liquid chromatography (HPLC). HPLC conditions: column, Aminex HPX-87H (Bio-Rad); detection wavelength, 210 nm; mobile phase, 5 mM sulphuric acid; flow rate, 0.6 mL·min^{-1}; sample volume, 20 μL; column temperature, 40 ℃.

Glycoaldehyde was detected by HPLC. HPLC conditions: column, Aminex HPX-87H (Bio-Rad); detection wavelength, 210 nm; mobile phase, 5 mM sulphuric acid; flow rate, 0.6 mL·min^{-1}; sample volume, 20 μL; column temperature, 40 ℃.

Acetate was detected by HPLC. HPLC conditions: column, Aminex HPX-87H (Bio-Rad); detection wavelength, 210 nm; mobile phase, 5 mM sulphuric acid; flow rate, 0.6 mL min^{-1}; sample volume, 20 μL; column temperature, 40 ℃

Acetyl-phosphate was detected by HPLC: add equal volume of 5% sulfuric acid to the sample for completely decomposing AcP into acetic acid. Acetic acid was then detected by HPLC. HPLC conditions: column, Aminex HPX-87H (Bio-Rad); detection wavelength, 210 nm; mobile phase, 5 mM sulphuric acid; flow rate, 0.6 mL min^{-1}; sample volume, 20 μL; column temperature, 40 ℃.

Analytical method of PHB

Samples after reaction were centrifuged and dried before digestion in 99.99% sulfuric acid for 30 min at 95 ℃. The acid-digested samples were then allowed to cool to room temperature for 30 min prior to filtering the samples through a 0.2 μm PVDF syringe filter. The content of PHB was detected by HPLC equipped with an HPX-87H column. HPLC conditions: detection wavelength, 210 nm; mobile phase, 5 mM sulphuric acid; flow rate, 0.6 mL·min^{-1}; sample volume, 20 μL; column temperature, 40 ℃. Different weights of PHB standards were processed in the same way and calculated to obtain a standard curve.

Screening candidates of Alcohol oxidases

We blasted the PcAOX from *Phanerochaete chrysosporium* to NR database in the NCBI database, and downloaded all potential AOX proteins with the amino acid identity more than 60. We divided the AOX proteins into two sets: one included the proteins with identity >= 80, the other included the proteins with identity > 60 and < 80. For each set, the AOX proteins were classified different groups by using OrthoMCL with the amino acid identity more than 90 in a group. For each group, we selected a AOX protein, which is closest approximation to supposed optimal sequence, consisting of the highest frequency residues in multiple sequences alignment. we selected eight AOX proteins from the first set, and two AOX proteins from the second set for next functional evaluation (Figure S6).

Then, we blasted the PpAOX from *Komagataella phaffii* to NR database in the NCBI database. We used the above method to select two AOX proteins from set for functional evaluation (Figure S6).

Iterative Saturation Mutagenesis of GALS

The determination of glycolaldehyde is as follows: 30 μL different concentrations of glycolaldehyde were prepared, and then 150 μL spetrophotometric chromogenic reagent (1.5 g diphenylamine was dissolved into 100 mL acetic acid, then added 1.5 mL concentrated sulfuric acid) was added, keeping at 90 °C for 15 min. At last, product concentration was measured by spectrophotometrically monitoring at 652 nm.

In order to obtain the desired saturation mutagenesis, oligonucleotide primers were designed with degenerate codon NNK. For 95% library coverage, the screening of 96 transformants for single-site saturation mutant was required by using NNK codon degeneracy. Each single-site saturation mutant library was generated according to the PCR-based Quick Change method. PCR reaction was performed with Fastpfu DNA Polymerase (Transgen, China) under the following conditions: the reaction was started at 94 °C (5 min), followed by 30 cycles 94 °C (20 s), 58 °C (20 s), 72 °C (3.5 min), with a final extension at 72 °C (5 min). The PCR product was digested with

*Dpn*I restriction enzyme and transformed into *E. coli* BL21 (DE3) competent cells to create the library for screening.

Each of the mutant colonies was picked and incubated 24 hours in 200 μL LB medium with 100 μg mL^{-1} kanamycin while shaking at 37 °C in 96-well microplate, and then scaled up to 1 mL LB medium for protein expression as well as GALS. The cell pellets were harvested by centrifugation at 3,300 g for 10 min and lysed by the re-suspension in 150 μL lysis buffer with 1 U DNase I and 1 mg mL^{-1} lysozyme, followed by 1 hour at 37 °C. Subsequently, 150 μL lysis buffer with 30 mM L^{-1} formaldehyde was added directly to the crude lysates for condensation assay and the plates were further incubated at 37 °C at 750 rpm for 90 min. After removing cells by centrifugation, 30 μL of the samples was used for a coloration assay.

Kinetic properties of glycolaldehyde synthase

An initial continuous assay included 50 mM potassium phosphate buffer (pH 7.4), 5 mM $MgSO_4$, 0.5 mM ThDP, 50 μg mL^{-1} glycerol dehydrogenase, 1 mM NADH, and different concentrations of formaldehyde. The reaction was initiated by the addition of purified GALS or mutants at 37 °C, and then an initial linear decrease in absorbance at 340 nm was observed. Enzyme kinetics were determined with formaldehyde as substrate. The concentrations ranged from 0 to 160 mM. Kinetic parameters k_{cat} and K_m were estimated by measuring the initial velocities of enzymic reaction and curve-fiting according to the Michaelis-Menten equation, using GraphPad Prism 5 software.

Four-site combination of mutations

Two double-sites saturation mutant library (N27, E28 and F397, C398) was constructed by consecutive degenerate codon NNK. Two rounds of directed evolution were implemented. In each round, the best double-sites mutation was obtained. Then, four beneficial substitutions were randomly fixed by single-point mutation. Finally, a library of 16 mutants was screened for the best mutants.

The computational analysis of glycolaldehyde synthase

To analyze the structural differences between $GALS_{F397YC398M}$ and GALS, we built a $GALS_{F397YC398M}$ structure model based on GALS crystal structure (PDBID: 6A50). Then we performed an energy minimization and equilibrium on the structure model of $GALS_{F397YC398M}$ and the GALS crystal structure with Amber force field. After that the POVME [1] package was used to calculate the shape and volume of the binding pockets. The POVME calculation was run default settings, a voxel grid spacing of 1.0 Å and the ConvexHullExclusion option set to 'first'.

When citing Amber22 or AmberTools22 please use the following: D.A. Case, H.M. Aktulga, K. Belfon, l.Y. Ben-Shalom, J.T. Berryman, S.R. Brozell, D.S. Cerutti, T.Echeatham. I. G.A. Cisneros. VwD. Cruzeiro. TA. Darden. R.E. Duke. G. Giambasu. M.k. Gilson.HGohlke, A.W. Goetz, R. Harris, S. lzadi, S.A. lzmailov, K. Kasavajhala, M.C. Kaymak, E. King, A.Kovalenko, T. Kurtzman, T.s. Lee, S. LeGrand, P. Li, C. Lin, J. Liu, T. Luchko, R. Luo, M. Machado, VMan, M. Manathunga, K.M. Merz, Y. Miao, 0. Mikhailovski, G. Monard, H. Nguyen, K.A. OHeamn, AOnufriev, F. Pan, S. Pantano, R. Qi, A. Rahnamoun, D.R. Roe, A. Roitberg, C. Sagui, s. Schott-VerdugoA. Shajan, J. Shen, C.L. Simmerling, N.R. Skrynnikov, . Smith, J. Swails, R.C. Walker, J. Wang, jWang, H. Wei, R.M. Wolf, X. Wu, Y. Xiong, Y. Xue, D.M. York, S. Zhao, and P.A. Kollman (2022), Amber2022. University of California, San Francisco.

Concentration of reaction solution

The reaction solution was fed into parallel to several ultrafiltration tubes to intercept proteins. The filtrate was collected centrally and then was concentrated by Vacuum Concentrator at ambient temperature and pressure. Precipitated salts were removed during the concentration process.

Assay oxidative capacity of AOX and CAT for NADPH

The oxidative ability to NADPH is demonstrated by the reduction value of OD_{340}. The reaction was carried out in a 200 μL system contains 50 mM potassium phosphate

buffer (pH 7.5),1 U/mL AOX or CAT, 2 mM NADPH.

Energy efficiency calculation

The theoretical energy efficiency was calculated based on the Gibbs free energy gain (ΔrG^o) as previous work [2]. The Gibbs free energy gain (ΔrG^o) of chemicals, along with the corresponding chemical reactions, are listed in the following:

Hydrogen formation:

$H_2O(l) \rightarrow H_2\ (g) + 0.5\ O_2\ (g)$

$\Delta rG^o_{hydrogen} \sim -\Delta cH^o_{hydrogen} = 285.8\ kJ\ mol^{-1}$

Methanol formation:

$CO_2\ (g) + 2\ H_2O\ (l) \rightarrow CH_4O\ (l) + 1.5O_2\ (g)$

$\Delta rG^o_{methanol} \sim -\Delta cH^o_{methanol} = 726.1\ kJ\ mol^{-1}$

PHB formation:

$4n\ CO_2\ (g) + 3n\ H_2O\ (l) \rightarrow (C_4H_6O_2)_n\ (s) + 4.5n\ O_2\ (g)$

$\Delta rG^o_{3HB} \sim -\Delta cH^o_{3HB} = 1903\ kJ\ mol^{-1}$ per monomer

Theoretical hydrogen-to-methanol energy efficiency (ηHME):

$CO_2\ (g) + 3H_2 \rightarrow CH_4O\ (l) + H_2O\ (l)$

Output energy = $1 \times \Delta rG^o_{methanol}$

Input energy = $3 \times \Delta rG^o_{hydrogen}$

The theoretical ηHME is 85%.

Theoretical methanol-to-PHB energy efficiency (ηMPE):

$4n\ CH_4O\ (l) + NADPH\ (l) + 1.5\ O_2 \rightarrow (C_4H_6O_2)n + NADP^+ + 5\ H_2O + H^+$

In this study, the NADPH consumed for PHB synthesis is derived from methanol. Thus, the stoichiometry of converting methanol to PHB is 5:1. Thus, the theoretical η MPE is 52.4%.

Output energy = $1\times \Delta rG^{o}_{3HB}$

Input energy = $5 \times \Delta rG^{o}_{methanol}$

The E-factor calculation

The classical E-factor was calculated using equation [3]:

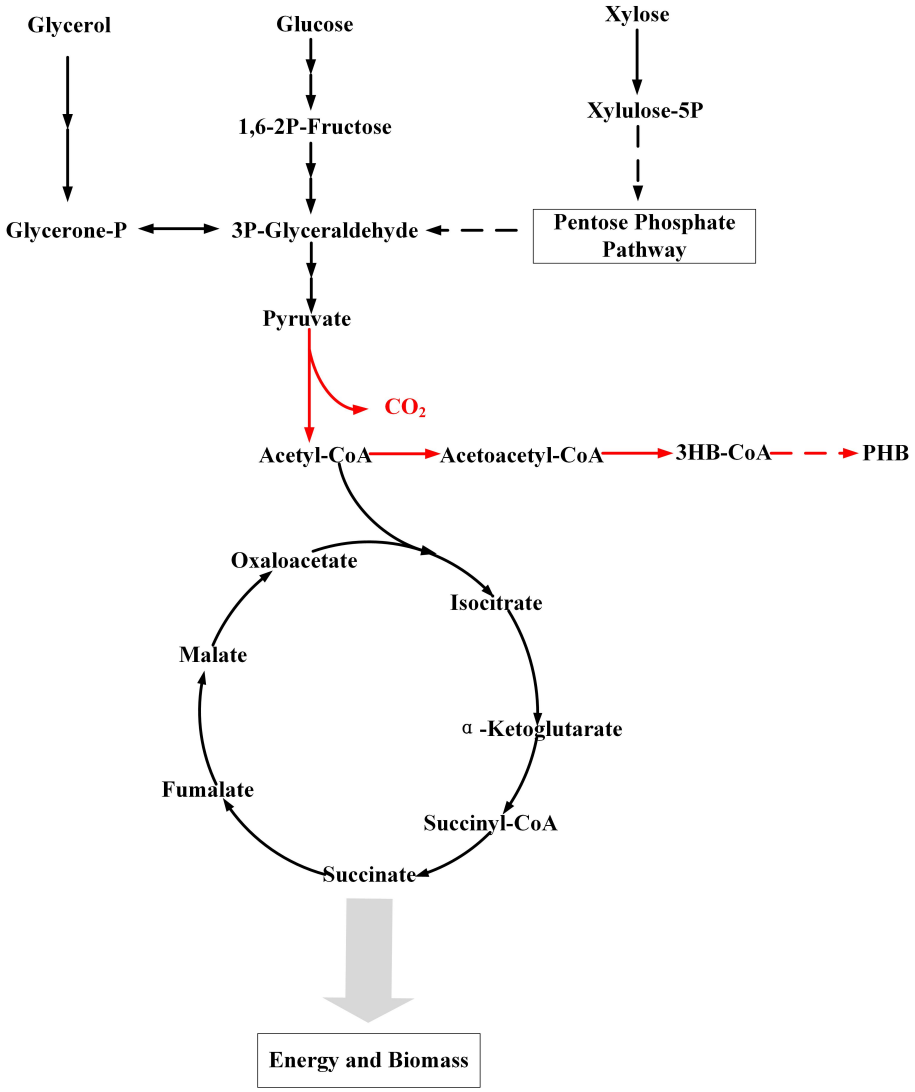
$$\boldsymbol{E} = \frac{\sum \boldsymbol{m(wastes)}\ \ [\boldsymbol{Kg}]}{\boldsymbol{m(product)}\ \ [\boldsymbol{Kg}]}$$

The m(wastes) represents the mass of the wastes including enzymes, vapour, solvents (except water) and chemical catalyst. The reaction volume of Module II was 1 L. The enzyme mass in Module II were 0.2 g of PcAOX and 2 g of GALS. The enzyme CAT was used in negligible amount due to its high activity. The reaction volume of Module III was approximately 58.8 mL. The enzyme mass in Module III were 0.059 g of ACPS, 0.059 g of PTA, 0.12 g of PhaA, 0.059 g of PhaB and 0.12 g of PhaC. The total mass of inorganic salts was approximately 12.97 g including HEPES, $MgSO_4$ and ThDP. The methanol produced in the chemical module was accompanied by an equal amount of vapour consumption (Approximately 0.64g). The amount of chemical catalyst was 1.1 g.

The m(product) represents the mass of PHB produced in the initial 1 L reaction system. The final titer of the PHB was 5.96 g/L. According to the final volume after concentration, the mass of PHB was 0.36 g.

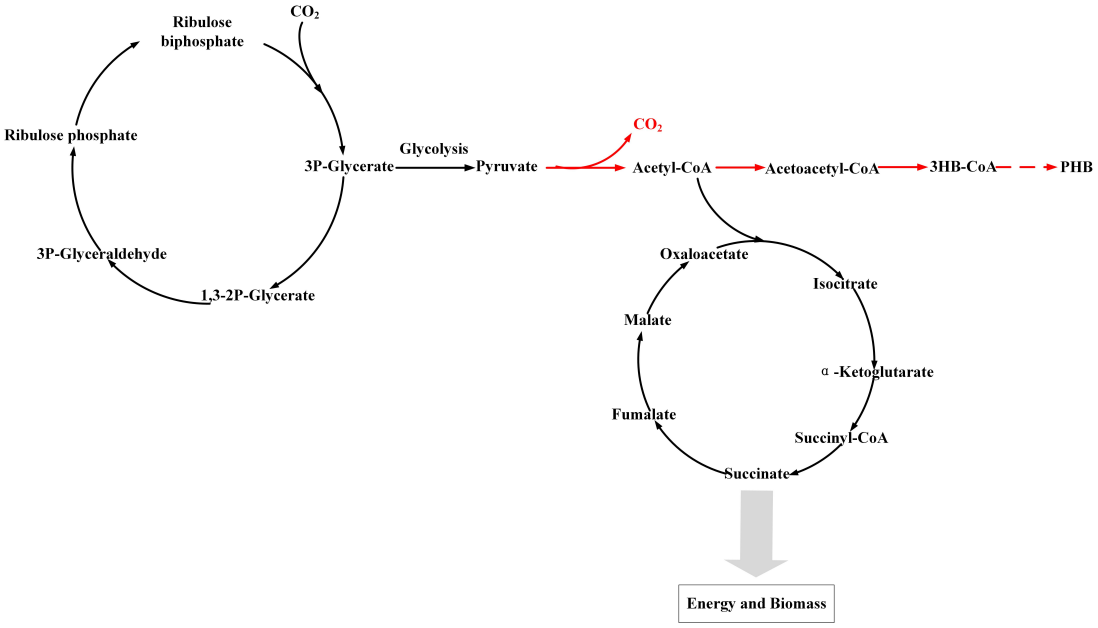
Supplementary Figure 1

A



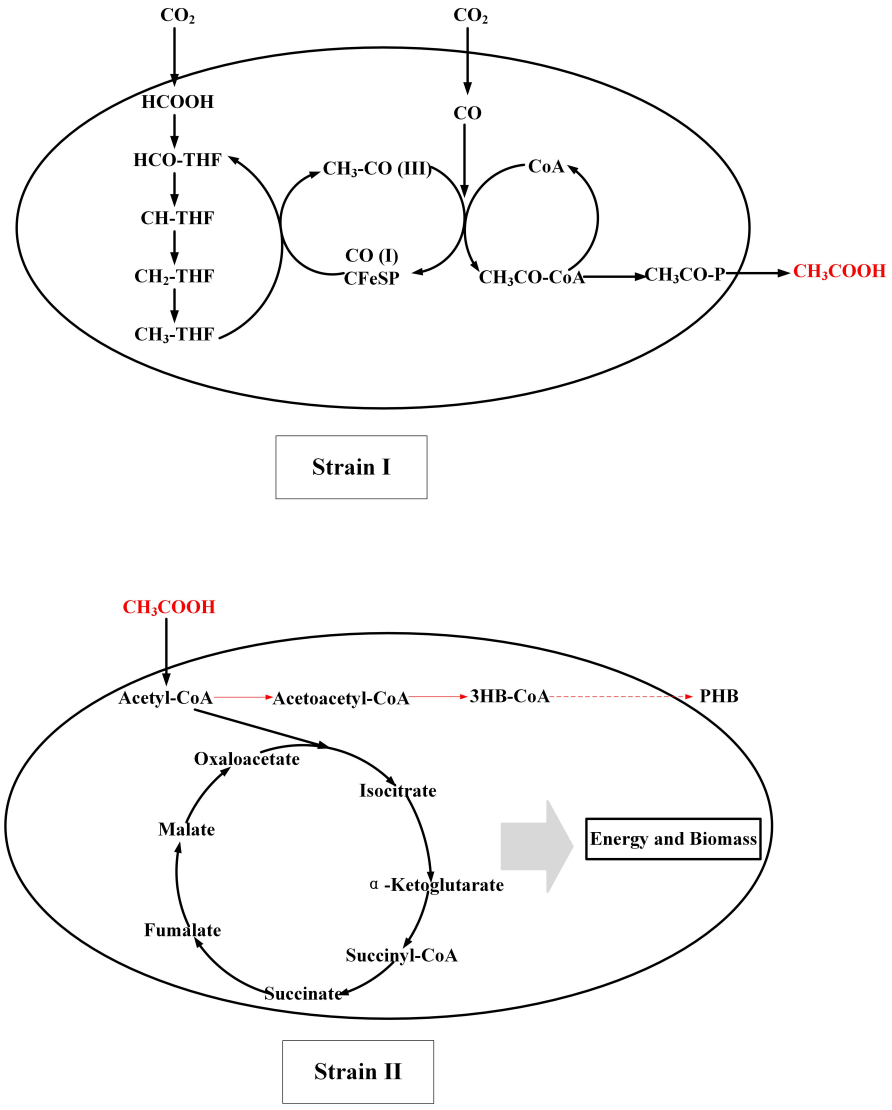
Supplementary Figure 1A. Sugar-based feedstock metabolic pathway for carbon assimilation. The molar carbon yield of PHB is 0.67 because of the irreversible CO_2-releasing reactions from pyruvate decarboxylation. One C6 substrate generate two acetyl-CoA molecules. One C3 substrate generate one acetyl-CoA molecule. Three C5 substrates generate five acetyl-CoA molecules.

B



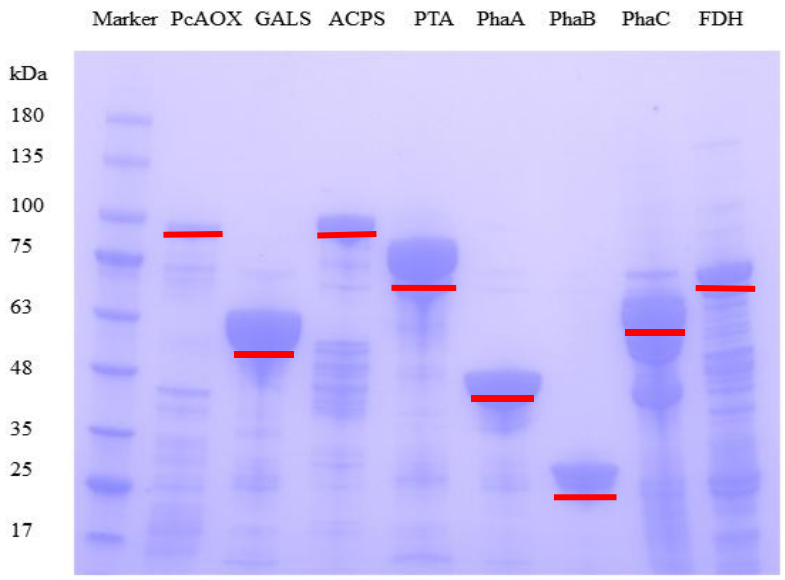
Supplementary Figure 1B. CBB cycle for CO_2 assimilation. Three CO_2 molecules enter the CBB cycle to generate one acetyl-CoA with one carbon loss. The molar carbon yield of PHB is 0.67 because of the irreversible CO_2-releasing reactions from pyruvate decarboxylation.

C



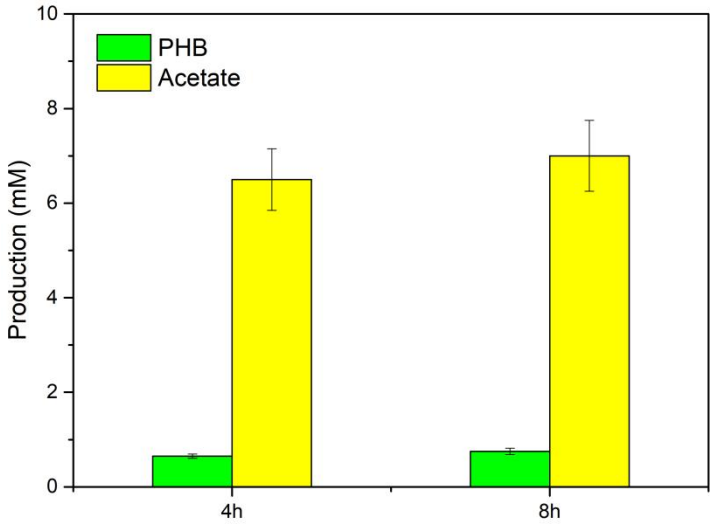
Supplementary Figure 1C. An integrated two-module process for the production of PHB from CO_2. Strain I:Wood-Ljungdahl pathway for CO_2 assimilation, two CO_2 molecules enter the Wood-Ljungdahl Pathway to generate one acetyl-CoA, which then enters acetogenesis. Strain II: Acetate assimilation in this strain, one acetate is assimilated to generate one acetyl-CoA, which can enter the TCA cycle.

Supplementary Figure 2



Supplementary Figure 2. SDS-PAGE analysis of the recombinant enzymes. PcAOX: Alcohol oxidase from Phanerochaete chrysosporium, GALS: glycolaldehyde synthase, ACPS: acetyl-phosphate synthase, PTA: phosphate acetyltransferase, PhaA: Acetyl-CoA acetyltransferase, PhaB: Acetoacetyl-CoA reductase, PhaC: PHB synthase, FDH: formate dehydrogenase.

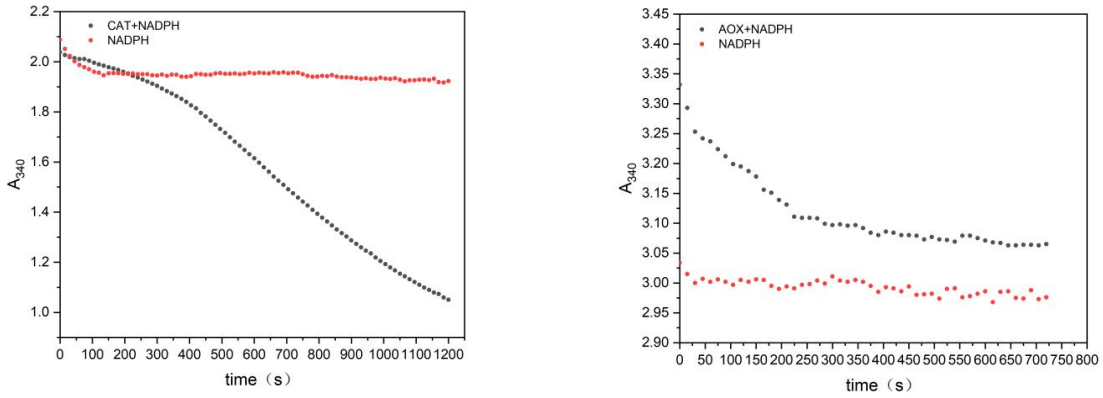
Supplementary Figure 3



Supplementary Figure 3. One-pot enzymatic synthesis of PHB in vitro. The reaction mixture (0.5 mL) was performed under the condition of 20 mM methanol, at 37 ℃, 4 h and 8 h. The enzyme loading was 2 g/L each and the glucose-6-phosphate was supplemented to regenerate reduced nicotinamide adenine dinucleotide phosphate (NADPH) by glucose-6-phosphate-dehydrogenase (G6PD). All values shown are means of triplicate measurements. The error bars represent standard deviations.

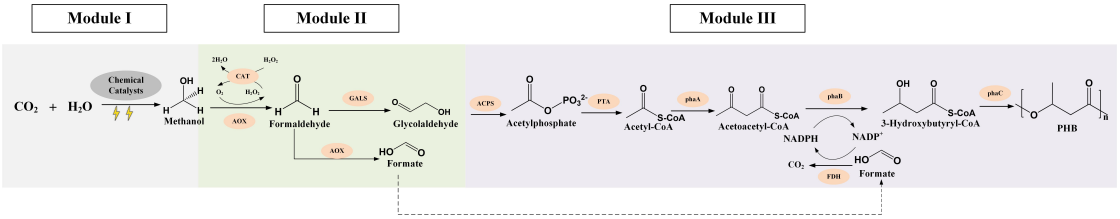
Supplementary Figure 4

(A) **(B)**



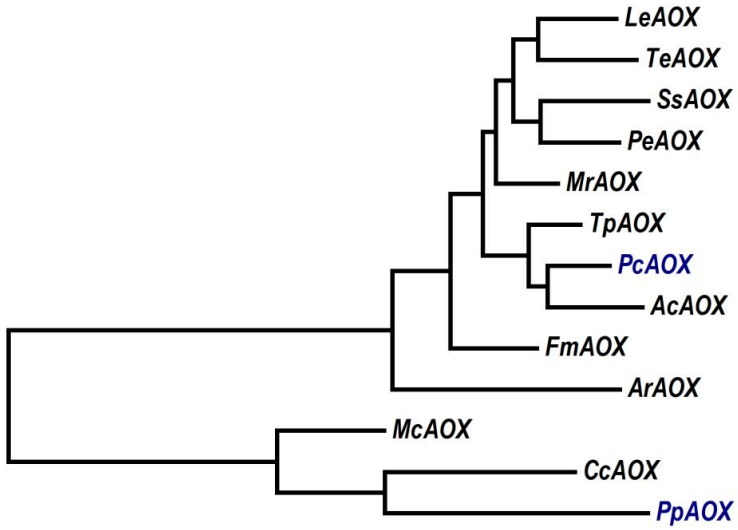
Supplementary Figure 4. Identified the activity of AOX (A) and CAT (B) in response to NADPH. The oxidative ability to NADPH is demonstrated by the reduction value of OD_{340}. The reaction was carried out in a 200 μL system contains 50 mM potassium phosphate buffer (pH 7.5), 1 U/mL AOX or CAT, 2 mM NADPH.

Supplementary Figure 5



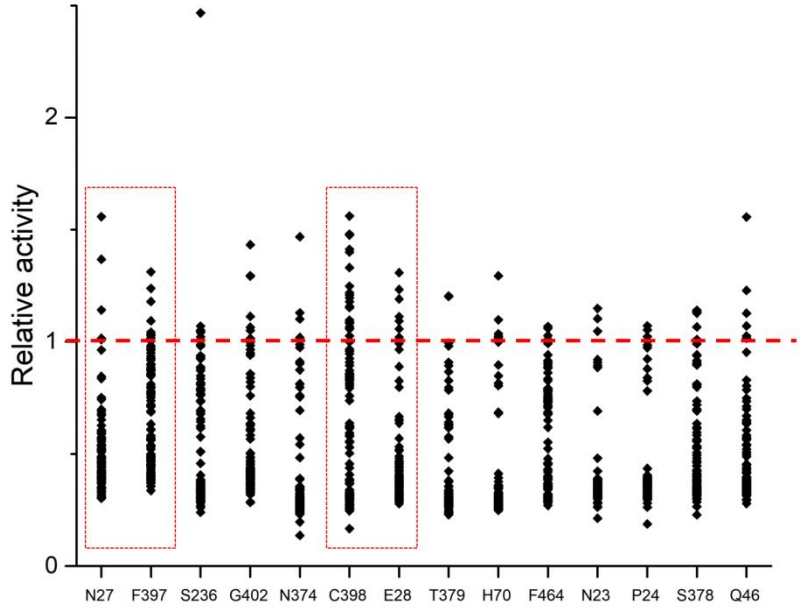
Supplementary Figure 5. Modular pathway for PHB synthesis from CO_2. Schematic of modular pathway design, with individual modules colored. The colored arrows and structures indicate the artificially designed pathways for production of PHB from CO_2 and H_2O. All enzymes and chemicals are indicated.

Supplementary Figure 6



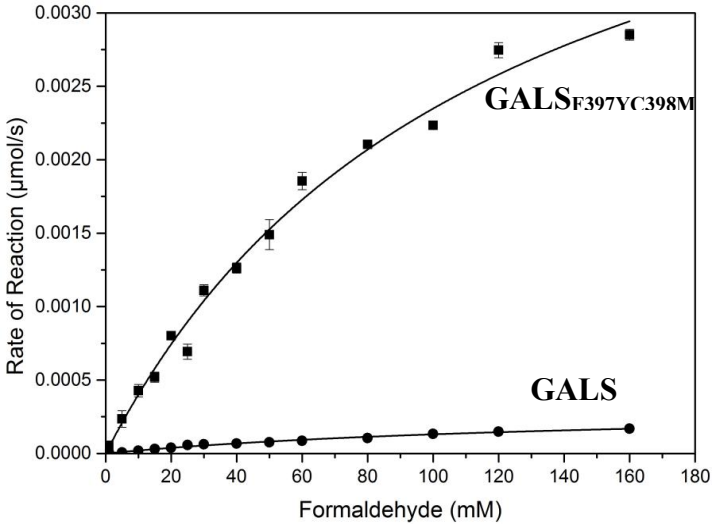
Supplementary Figure 6. Phylogenetic tree of selected AOX genes. The Maximum likelihood tree was constructed by MEGA 7.0 based on the protein sequences. Accession ID: LeAOX: XP_046079723, TeAOX: KAG5730165, SsAOX: KAG2364097, PeAOX: KAF9487399, MrAOX: ESK84078, TpAOX: KAI0637534, PcAOX: UXW61383.1, AcAOX: KAI0915540, FmAOX: XP_007269069, ArAOX: KAH8919511, McAOX: RPB07876, CcAOX: OLN87382, PpAOX: XP_002494271

Supplementary Figure 7



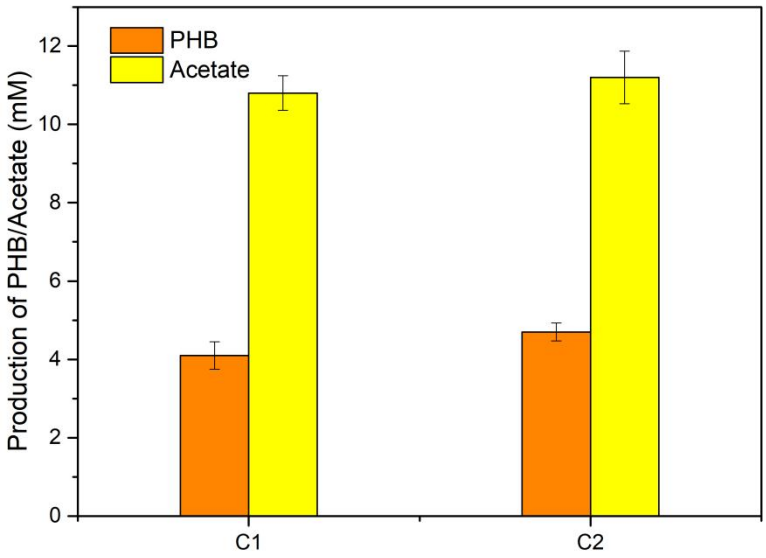
Supplementary Figure 7. Experimental validation of single-point saturation mutant. The x-axis label represents the selected positions in GALS. The single-point saturation mutation assays were carried out for each selected position. The y-axis label represents the relative catalytic activity of different mutants. The relative activity was defined as the ratio of the production of glycolaldehyde in the mutants to that in GALS. The yields of glycolaldehyde were determined by the chromogenic reaction. 4 positions containing higher activity mutants were selected in the red rectangle.

Supplementary Figure 8



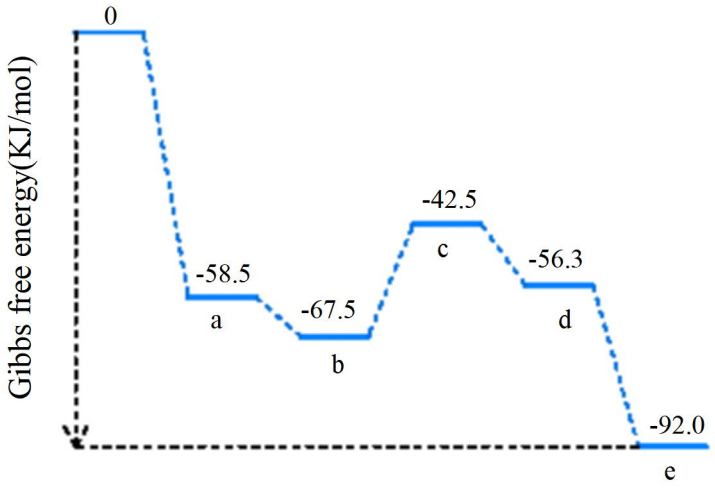
Supplementary Figure 8. Michaelis-Menten graphs of GALS and GALS$_{F397YC398M}$. Enzyme kinetics were determined with 0.1 mg mL^{-1} GALS and GALS$_{F397YC398M}$. The concentration of formaldehyde ranged from 1 to 160 mM. Error bars represent standard deviation, *n*=3.

Supplementary Figure 9



Supplementary Figure 9. PHB production from glycolaldehyde by increasing enzyme loadings. The reaction mixture (0.5 mL) was performed under the condition of 20 mM glycolaldehyde, at 37 ℃, 5 h. Group C1 indicates 2 g/L of enzyme loading in this pathway. Group C2 indicates 4 g/L of enzyme loading in this pathway. All values shown are means of triplicate measurements. The error bars represent standard deviations.

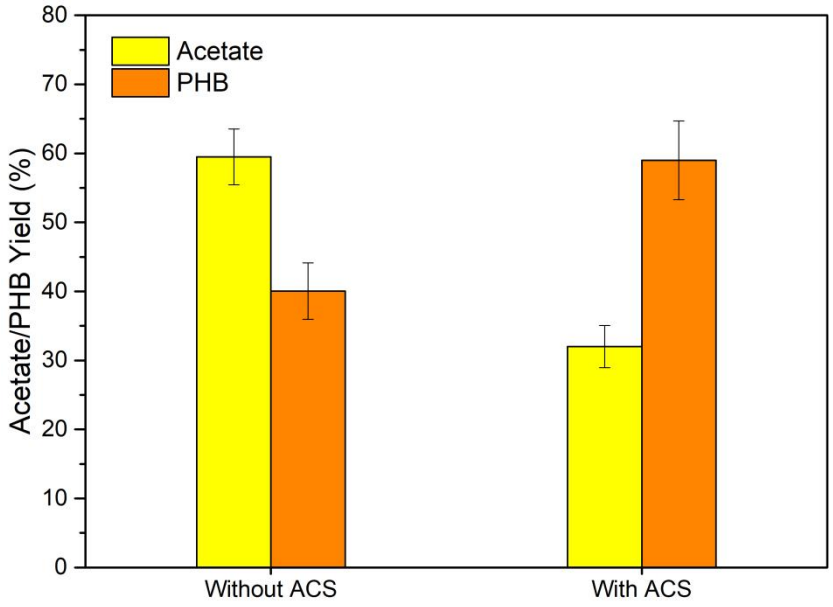
Supplementary Figure 10



a) Glycolaldehyde+phosphate <=> acetyl-phosphate
b) Acetyl-phosphate+CoA <=> acetyl-CoA
c) 2Acetyl-CoA <=> Acetoacetyl-CoA+CoA
d) Acetoacetyl-CoA+NADPH <=> 3-hydroxybutyryl-CoA+$NADP^+$
e) 3-hydroxybutyryl-CoA <=> polyhydroxybutyrate+CoA

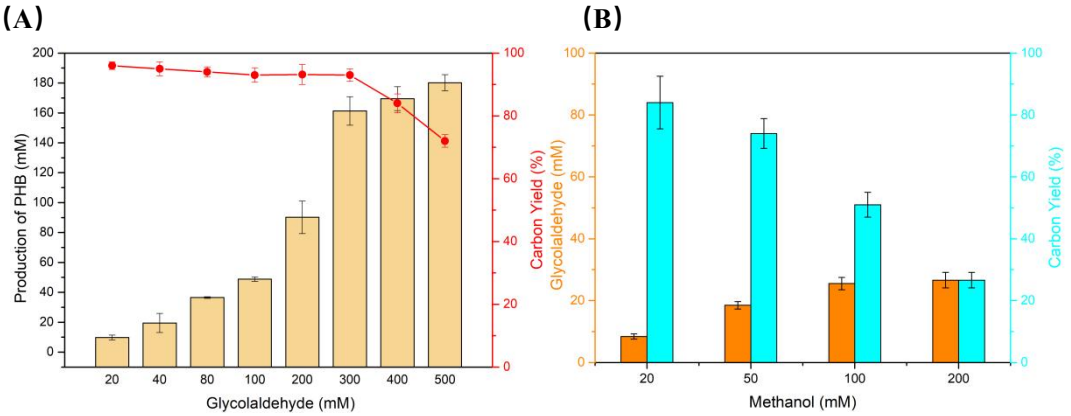
Supplementary Figure 10. Standard Gibbs free energy changes of each and the overall reaction steps under conditions of pH 7.5 and ionic strength of 0.25 M.

Supplementary Figure 11



Supplementary Figure 11. The introduction of acetyl-CoA synthetase (ACS) to decrease the accumulation of acetic acid. The reaction mixture (0.5 mL) was performed under the condition of 20 mM glycolaldehyde, at 37 ℃, 5 h. Group with ACS added 2 g/L acetyl-CoA synthetase, 1mM ATP , 1mM polyphosphoric acid and 2g/L ATP-polyphosphate phosphotransferase additionally. All values shown are means of triplicate measurements. The error bars represent standard deviations.

Supplementary Figure 12



Supplementary Figure 12. Increasing substrate concentration for module II and module III. (A) Increasing glycolaldehyde concentration for module III. The reaction mixture (0.5 mL) was performed under the condition of 20-500 mM glycolaldehyde, at 37 ℃, 5 h. **(B)** Increasing methanol concentration for module II. The reaction mixture (0.5 mL) was performed under the condition of 20-200 mM methanol, at 37 ℃, 1.5 h. All values shown are means of triplicate measurements. The error bars represent standard deviations.

Supplementary Table 1. Enzymes used in this hybrid system

Enzyme	Name	Plasmid	Tag	Organism	references
AOX	Alcohol oxidases	pet28a	N-His	*Phanerochaete chrysosporium*	[4]
CAT	catalase	purchased from Aladdin			
FDH	formate dehydrogenase	pet28a	N-His	*Mycolicibacterium vaccae*	[5]
GALS	glycolaldehyde synthase	pet28a	N-His		[6]
ACPS	Phosphoketolases	pet28a	N-His	*Bifidobacterium*	[6]
PTA	phosphate acetyltransferase	pet28a	N-His	*E. coli*	
PhaA	Acetyl-CoA acetyltransferase	pet28a	N-His	*R. eutropha*	[7]
PhaB	Acetoacetyl-CoA reductase	pet28a	N-His	*R. eutropha*	[7]
PhaC	Polyhydroxybutyrate synthase	pet28a	N-His	*R. eutropha*	[7]

Supplementary Table 2. The thermodynamic data of all reactions

Enzyme	Reaction	△G（kJ/mol）
AOX	Methanol+O_2 <=> formaldehyde+H_2O_2	-98.9 ± 10.3
CAT	$2H_2O_2$ <=> $2H_2O$+O_2	-174.8 ± 13.2
GALS	2Formaldehyde <=> glycolaldehyde	-28.7 ± 6.4
ACPS	Glycolaldehyde+phosphate <=> acetyl-phosphate	-58.8 ± 3.8
PTA	Acetyl-phosphate+CoA <=> acetyl-CoA	-8.7 ± 1.2
PhaA	2Acetyl-CoA <=> Acetoacetyl-CoA+CoA	25.0 ± 1.7
PhaB	Acetoacetyl-CoA+NADPH <=> 3-hydroxybutyryl-CoA+$NADP^+$	-13.8 ± 2.3
PhaC	3-hydroxybutyryl-CoA <=> polyhydroxybutyrate+CoA	-35.7
FDH	Formate+$NADP^+$ <=> CO_2+NADPH	-14.4 ± 6.4

Supplementary Table 3. Plasmids used in this study.

Plasmids	Relevant characteristics	Source
pET28a	pBR322 ori with pT7; *KanR*	Novagen
pET28a-*PcAOX*	pET28a vector, *Nde*I-*PcAOX*-*Xho*I	This study
pET28a-*GALS*	pET28a vector, *Nde*I-*GALS*-*Xho*I	This study
pET28a-*ACPS*	pET28a vector, *Nde*I-*ACPS*-*Xho*I	This study
pET28a-*PTA*	pET28a vector, *Nde*I-*pta*-*Xho*I	This study
pET28a-*PhaA*	pET28a vector, *Nde*I-*phaA*-*Xho*I	This study
pET28a-*PhaB*	pET28a vector, *Nde*I-*phaB*-*Xho*I	This study
pET28a-*PhaC*	pET28a vector, *Nde*I-*phaC*-*Xho*I	This study
pET28a-*FDH*	pET28a vector, *Nde*I-*FDH*-*Xho*I	This study

Supplementary Table 4. The DNA sequences of used in this study

Gene	Sequence
pcaox	ATGGGTCACCCCGAGGAGGTCGATGTCATCGTATGCGGTGGTGGTCCTGCTGGATGC GTTGTGGCGGGCAGGCTCGCTTACGCGGACCCTACACTGAAGGTCATGCTCATTGAA GGTGGTGCCAACAACCGCGATGACCCATGGGTGTACCGCCCAGGCATCTACGTCCGC AACATGCAGAGGAACGGCATCAACGACAAGGCGACGTTCTACACCGACACCATGGC TTCTTCGTATCTCCGTGGCCGTCGCAGCATCGTCCCCTGCGCCAACATCCTCGGTGGT GGTTCTTCGATCAACTTCCAGATGTACTTTCGCGCGTCTGCTTCCGATTGGGACGACT TCAAGACCGAAGGGTGGACCTGCAAGGATCTTCTGCCTCTTATGAAGCGGCTCGAG AACTACCAGAAGCCATGCAACAACGACACCCACGGCTACGATGGTCCGATTGCCATC TCCAACGGTGGACAGATCATGCCTGTCGCCCAAGACTTCCTGAGGGCTGCCCATGCG ATCGGTGTGCCATACAGCGATGATATCCAGGATCTTACCACTGCACATGGTGCTGAGA TTTGGGCCAAGTATATCAACCGTCACACCGGCCGTCGTAGCGATGCTGCGACCGCAT ACGTGCACTCCGTCATGGACGTCCAGGATAACCTCTTCCTACGCTGCAACGCCCGCG TCAGCCGCGTTCTATTCGACGACAACAACAAGGCAGTCGGCGTAGCCTATGTCCCGT CCCGCAACCGGACACACGGCGGTAAGCTCCATGAGACCATTGTCAAGGCTCGCAAG ATGGTCGTCCTCAGCTCCGGCACTCTTGGTACTCCTCAGATCCTCGAGCGCTCCGGT GTCGGCAACGGCGAGCTCCTCCGCCAACTTGGCATCAAGATCGTCAGCGACCTCCCG GGTGTTGGTGAGCAGTACCAGGACCACTACGCAACGCTGTCCATATACCGTGTCTCC AACGAGTCCATTACCACCGATGACTTCCTCCGTGGTGTCAAGGACGTGCAGCGCGAG CTTTTCACAGAGTGGGAGGTTTCGCCCGAGAAGGCTCGTCTGTCGTCCAACGCCATC GACGCTGGCTTCAAGATCCGCCCAACGGAGGAAGAGCTGAAGGAGATGGGCCCTGA GTTCAACGAACTCTGGAACCGCTACTTCAAGGACAAGCCCGACAAGCCCGTCATGT TCGGCTCCATCGTCGCTGGTGCCTACGCCGACCACACACTCCTGCCGCCCGGCAAGT ACATTACGATGTTCCAATTTCTCGAGTACCCGGCGTCGCGTGGCAAGATCCACATCAA GTCGCAGAACCCCTACGTCGAGCCATTCTTCGACTCCGGCTTCATGAACAACAAGGC CGACTTTGCGCCCATCCGCTGGAGCTACAAGAAGACCCGTGAGGTCGCGCGCCGCA TGGATGCATTCCGTGGTGAACTGACGTCGCACCACCCGCGCTTCCACCCTGCCTCCC CCGCGGCATGCAAGGACATTGACATCGAGACTGCCAAGCAGATCTACCCCGACGGC CTCACGGTCGGCATCCACATGGGCTCGTGGCACCAGCCGTCCGAGCCGTACAAGCA CGACAAGGTCATCGAGGACATCCCCTACACCGAGGAGGATGACAAGGCTATCGACG ACTGGGTCGCCGATCACGTTGAGACCACCTGGCACTCGCTCGGTACCTGCGCCATGA AGCCGCGCGAGCAGGGCGGTGTTGTCGACAAGCGCCTCAACGTCTACGGCACGCAG AACCTCAAGTGTGTTGACCTGTCGATCTGCCCCGACAACCTCGGCACGAACACCTAC TCGTCTGCGCTCCTCGTCGGCGAGAAGGGCGCTGATCTCATCGCTGAGGAACTCGGC CTCAAGATCAAGACCCCGCATGCTCCCGTCCCGCACGCACCCGTCCCGACCGGCAG GCCCGCTACCCAGCAGGTCCGG
gals	ATGGCTTCTGTTCACGGTACCACCTACGAACTGCTGCGTCGTCAGGGTA TCGACACCGTTTTCGGTAACCCGGGTTCTAACGAACTGCCGTTCCTGAA AGACTTCCCGGAAGACTTCCGTTACATCCTGGCTCTGCAGGAAGCTTGC GTTGTTGGTATCGCTGACGGTTACGCTCAGGCTTCTCGTAAACCGGCTTT CATCAACCTGCACTCTGCTGCTGGTACCGGTAACGCTATGGGTGCTCTG TCTAACGCTCGTACCTCTCACTCTCCGCTGATCGTTACCGCTGGTCAGCA GACCCGTGCTATGATCGGTGTTGAAGCTGGTGAAACCAACGTTGACGCT GCTAACCTGCCGCGTCCGCTGGTTAAATGGTCTTACGAACCGGCTTCTG CTGCTGAAGTTCCGCACGCTATGTCTCGTGCTATCCACATGGCTTCTATG GCTCCGCAGGGTCCGGTTTACCTGTCTGTTCCGTACGACGACTGGGACA AAGACGCTGACCCGCAGTCTCACCACCTGTTCGACCGTCACGTTTCTTC TTCTGTTCGTCTGAACGACCAGGACCTGGACATCCTGGTTAAAGCTCTG AACTCTGCTTCTAACCCGGCTATCGTTCTGGGTCCGGACGTTGACGCTG CTAACGCTAACGCTGACTGCGTTATGCTGGCTGAACGTCTGAAAGCTCC GGTTTGGGTTGCTCCGTCTGCTCCGCGTTGCCCGTTCCCGACCCGTCAC CCGTGCTTCCGTGGTCTGATGCCGGCTGGTATCGCTGCTATCTCTCAGCT GCTGGAAGGTCACGACGTTGTTCTGGTTATCGGTGCTCCGGTTTTCCGT TACGTTTTTTACGACCCGGGTCAGTACCTGAAACCGGGTACCCGTCTGA TCTCTGTTACCTGCGACCCGCTGGAAGCTGCTCGTGCTCCGATGGGTGA CGCTATCGTTGCTGACATCGGTGCTATGGCTTCTGCTCTGGCTAACCTGG TTGAAGAATCTTCTCGTCAGCTGCCGACCGCTGCTCCGGAACCGGCTAA

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acps	ATGACGAGTCCTGTTATTGGCACCCCTTGGAAGAAGCTGAACGCTCCGG TTTCCGAGGAAGCTATCGAAGGCGTGGATAAGTACTGGCGCGCAGCCA ACTACCTCTCCATCGGCCAGATCTATCTGCGTAGCAACCCGCTGATGAA GGAGCCTTTCACCCGCGAAGACGTCAAGCACCGTCTGGTCGGTCACTG GGGCACCACCCCGGGCCTGAACTTCCTCATCGGCCACATCAACCGTCTC ATTGCTGATCACCAGCAGAACACTGTGATCATCATGGGCCCGGGCCACG GCGGCCCGGCTGGTACCGCTCAGTCCTACCTGGACGGCACCTACACCG AGTACTTCCCGAACATCACCAAGGATGAGGCTGGCCTGCAGAAGTTCTT CCGCCAGTTCTCCTACCCGGGTGGCATCCCGTCCCACTACGCTCCGGAG ACCCCGGGCTCCATCCACGAAGGCGGCGAGCTGGGTTACGCCCTGTCC CACGCCTACGGCGCTGTGATGAACAACCCGAGCCTGTTCGTCCCGGCCA TCGTCGGCGACGGCGAAGCTGAGACCGGCCCGCTGGCCACCGGCTGGC AGTCCAACAAGCTCATCAACCCGCGCACCGACGGTATCGTGCTGCCGAT CCTGCACCTCAATGGCTACAAGATCGCCAACCCGACCATCCTGTCCCGC ATCTCCGACGAAGAGCTCCACGAGTTCTTCCACGGCATGGGCTATGAGC CGTACGAGTTCGTCGCTGGCTTCGACAACGAGGATCACCTGTCGATCCA CCGTCGTTTCGCCGAGCTGTTCGAGACCGTCTTCGACGAGATCTGCGAC ATCAAGGCCGCCGCTCAGACCGACGACATGACTCGTCCGTTCTACCCGA TGATCATCTTCCGTACCCCGAAGGGCTGGACCTGCCCGAAGTTCATCGA CGGCAAGAAGACCGAGGGCTCCTGGCGTTCCCACCAGGTGCCGCTGGC TTCCGCCCGCGATACCGAGGCCCACTTCGAGGTCCTCAAGAACTGGCTC GAGTCCTACAAGCCGGAAGAGCTGTTCGACGAGAACGGCGCCGTGAA GCCGGAAGTCACCGCCTTCATGCCGACCGGCGAACTGCGCATCGGTGA GAACCCGAACGCCAACGGTGGCCGCATCCGCGAAGAGCTGAAGCTGCC GAAGCTGGAAGACTACGAGGTCAAGGAAGTCGCCGAGTACGGCCACG GCTGGGGCCAGCTCGAGGCCACCCGTCGTCTGGGCGTCTACACCCGCG ACATCATCAAGAACAACCCGGACTCCTTCCGTATCTTCGGACCGGATGA GACCGCTTCCAACCGTCTGCAGGCCGCTTACGACGTCACCAACAAGCA GTGGGACGCCGGCTACCTGTCCGCTCAGGTCGACGAGCACATGGCTGT CACCGGCCAGGTCACCGAGCAGCTTTCCGAGCACCAGATGGAAGGCTT CCTCGAGGGCTACCTGCTGACCGGCCGTCACGGCATCTGGAGCTCCTAT GAGTCCTTCGTGCACGTGATCGACTCCATGCTGAACCAGCACGCCAAGT GGCTCGAGGCTACCGTCCGCGAGATTCCGTGGCGCAAGCCGATCTCCTC CATGAACCTGCTCGTCTCCTCCCACGTGTGGCGTCAGGATCACAACGGC TTCTCCCACCAGGATCCGGGTGTCACCTCCGTCCTGCTGAACAAGTGCT TCAACAACGATCACGTGATCGGCATCTACTTCCCGGTGGATTCCAACAT GCTGCTCGCTGTGGCTGAGAAGTGCTACAAGTCCACCAACAAGATCAA CGCCATCATCGCCGGCAAGCAGCCGGCCGCCACCTGGCTGACCCTGGA CGAAGCTCGCGCCGAGCTCGAGAAGGGTGCTGCCGAGTGGAAGTGGG CTTCCAACGTGAAGTCCAACGATGAGGCTCAGATCGTGCTCGCCGCCA CCGGTGATGTTCCGACTCAGGAAATCATGGCCGCTGCCGACAAGCTGG ACGCCATGGGCATCAAGTTCAAGGTCGTCAACGTGGTTGACCTGGTCA AGCTGCAGTCCGCCAAGGAGAACAACGAGGCCCTCTCCGATGAGGAGT TCGCTGAGCTGTTCACCGAGGACAAGCCGGTCCTGTTCGCTTACCACTC CTATGCCCGCGACGTGCGTGGTCTGATCTACGATCGCCCGAACCACGAC

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phaB	ATGACTCAGCGCATTGCGTATGTGACCGGCGGCATGGGTGGTATCGGAA CCGCCATTTGCCAGCGGCTGGCCAAGGATGGCTTTCGTGTGGTGGCCGG TTGCGGCCCCGAAGATCCGAATCAGGAAAAGTGGCTGGAGCAGCAGAA GGCCCTGGGCTTCGATTTCATTGCCTCGGAAGGCAATGTGGCTGACTGG GACTCGACCAAGACCGCATTCGACAAGGTCAAGTCCGAGGTCGGCGAG GTTGATGTGCTGATCAACAACGCCGGTATCACCCGCGACGTGGTGTTCC GCAAGATGACCCGCGCCGACTGGGATGCGGTGATCGACACCAACCTGA CCTCGCTGTTCAACGTCACCAAGCAGGTGATCGACGGCATGGCCGACC GTGGCTGGGGCCGCATCGTCAACATCTCGTCGGTGAACGGGCAGAAGG GCCAGTTCGGCCAGACCAACTACTCCACCGCCAAGGCCGGCCTGCATG GCTTCACCATGGCACTGGCGCAGGAAGTGGCGACCAAGGGCGTGACCG TCAACACGGTCTCTCCGGGCTATATCGCCACCGACATGGTCAAGGCGAT CCGCCAGGACGTGCTCGACAAGATCGTCGCGACGATCCCGGTCAAGCG CCTGGGCCTGCCGGAAGAGATCGCCTCGATCTGCGCCTGGTTGTCGTCG GAGGAGTCCGGTTTCTCGACCGGCGCCGACTTCTCGCTCAACGGCGGC CTGCATATGGGCTGA
phaC	ATGGCGACCGGCAAAGGCGCGGCAGCTTCCACGCAGGAAGGCAAGTC CCAACCATTCAAGGTCACGCCGGGGCCATTCGATCCAGCCACATGGCTG GAATGGTCCCGCCAGTGGCAGGGCACTGAAGGCAACGGCCACGCGGC CGCGTCCGGCATTCCGGGCCTGGATGCGCTGGCAGGCGTCAAGATCGC GCCGGCGCAGCTGGGTGATATCCAGCAGCGCTACATGAAGGACTTCTCA GCGCTGTGGCAGGCCATGGCCGAGGGCAAGGCCGAGGCCACCGGTCC GCTGCACGACCGGCGCTTCGCCGGCGACGCATGGCGCACCAACCTCCC ATATCGCTTCGCTGCCGCGTTCTACCTGCTCAATGCGCGCGCCTTGACCG AGCTGGCCGATGCCGTCGAGGCCGATGCCAAGACCCGCCAGCGCATCC

	GCTTCGCGATCTCGCAATGGGTCGATGCGATGTCGCCCGCCAACTTCCT TGCCACCAATCCCGAGGCGCAGCGCCTGCTGATCGAGTCGGGCGGCGA ATCGCTGCGTGCCGGCGTGCGCAACATGATGGAAGACCTGACACGCGG CAAGATCTCGCAGACCGACGAGAGCGCGTTTGAGGTCGGCCGCAATGT CGCGGTGACCGAAGGCGCCGTGGTCTTCGAGAACGAGTACTTCCAGCT GTTGCAGTACAAGCCGCTGACCGACAAGGTGCACGCGCGCCCGCTGCT GATGGTGCCGCCGTGCATCAACAAGTACTACATCCTGGACCTGCAGCCG GAGAGCTCGCTGGTGCGCCATGTGGTGGAGCAGGGACATACGGTGTTT CTGGTGTCGTGGCGCAATCCGGACGCCAGCATGGCCGGCAGCACCTGG GACGACTACATCGAGCACGCGGCCATCCGCGCCATCGAAGTCGCGCGC GACATCAGCGGCCAGGACAAGATCAACGTGCTCGGCTTCTGCGTGGGC GGCACCATTGTCTCGACCGCGCTGGCGGTGCTGGCCGCGCGCGGCGAG CACCCGGCCGCCAGCGTCACGCTGCTGACCACGCTGCTGGACTTTGCC GACACGGGCATCCTCGACGTCTTTGTCGACGAGGGCCATGTGCAGTTGC GCGAGGCCACGCTGGGCGGCGGCGCCGGCGCGCCGTGCGCGCTGCTGC GCGGCCTTGAGCTGGCCAATACCTTCTCGTTCTTGCGCCCGAACGACCT GGTGTGGAACTACGTGGTCGACAACTACCTGAAGGGCAACACGCCGGT GCCGTTCGACCTGCTGTTCTGGAACGGCGACGCCACCAACCTGCCGGG GCCGTGGTACTGCTGGTACCTGCGCCACACCTACCTGCAGAACGAGCTC AAGGTACCGGGCAAGCTGACCGTGTGCGGCGTGCCGGTGGACCTGGCC AGCATCGACGTGCCGACCTATATCTACGGCTCGCGCGAAGACCATATCG TGCCGTGGACCGCGGCCTATGCCTCGACCGCGCTGCTGGCGAACAAGC TGCGCTTCGTGCTGGGTGCGTCGGGCCATATCGCCGGTGTGATCAACCC GCCGGCCAAGAACAAGCGCAGCCACTGGACTAACGATGCGCTGCCGGA GTCGCCGCAGCAATGGCTGGCCGGCGCCATCGAGCATCACGGCAGCTG GTGGCCGGACTGGACCGCATGGCTGGCCGGGCAGGCCGGCGCGAAAC GCGCCGCGCCCGCCAACTATGGCAATGCGCGCTATCGCGCAATCGAACC CGCGCCTGGGCGATACGTCAAAGCCAAGGCATGA
fdh	CATATGGCAAAAGTGCTGTGCGTGCTGTATGATGATCCGGTGGATGGTTA TCCGAAAACCTATGCACGTGATGATCTGCCGAAAATTGATCATTATCCGG GTGGCCAGATTCTGCCGACCCCGAAAGCAATTGATTTTACCCCGGGCCA GCTGCTGGGTAGTGTTAGTGGCGAACTGGGCCTGCGCGAATATCTGGAA AGCAATGGTCATACCCTGGTGGTGACCAGTGATAAAGATGGTCCGGATA GTGTGTTTGAACGTGAACTGGTGGATGCAGATGTGGTGATTAGTCAGCC GTTTTGGCCGGCCTATCTGACCCCGGAACGCATTGCCAAAGCCAAAAAT CTGAAACTGGCACTGACCGCAGGCATTGGTAGTGATCATGTGGATCTGC AGAGCGCCATTGATCGTAATGTTACCGTTGCAGAAGTGACCTATAGTAAT AGTATTAGCGTGGCCGAACATGTTGTTATGATGATTCTGAGCCTGGTGCG TAATTATCTGCCGAGCCATGAATGGGCACGCAAAGGTGGTTGGAATATT GCCGATTGCGTTAGTCATGCCTATGATCTGGAAGCCATGCATGTGGGCAC CGTGGCCGCAGGCCGCATTGGTCTGGCCGTGCTGCGCCGTCTGGCCCCT TTTGATGTTCATCTGCATTATACCCAGCGTCATCGTCTGCCGGAAAGTGT GGAAAAAGAACTGAATCTGACCTGGCATGCAACCCGTGAAGATATGTAT CCGGTGTGTGATGTGGTGACCCTGAATGTTCCGCTGCATCCGGAAACCG AACACATGATTAATGATGAAACCCTGAAACTGTTTAAGCGCGGTGCATAT ATTGTGAATACCGCACGTGGTAAACTGTGTGATCGCGATGCAGTGGCCC GCGCCCTGGAAAGTGGTCGTCTGGCAGGTTATGCCGGCGATGTTTGGTT TCCGCAGCCGGCACCGAAAGATCATCCGTGGCGCACCATGCCGTATAAT GGCATGACCCCGCATATTAGCGGCACCACCCTGACCGCCCAGGCACGTT ATGCCGCCGGCACCCGTGAAATTCTGGAATGTTTTTTTGAAGGCCGTCC GATTCGCGATGAATATCTGATTGTTCAGGGCGGTGCACTGGCCGGCACC GGTGCACATAGCTATAGTAAAGGTAATGCAACCGGTGGTAGCGAAGAAG CCGCAAAATTTAAAAAAGCCGTTCTCGAG

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