Supporting Information

Tandem Catalytic Conversion of Glucose to 5-Hydroxymethylfurfural with an Immobilized Enzyme and a Solid Acid

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1. Materials and methods

Unless otherwise specified, all solvents and chemicals were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. Restriction enzymes were purchased from New England Biolabs (Ipswich, MA). Isopropyl-β-d-thiogalactoside (IPTG) and ampicillin were purchased from GoldBio (St. Louis, MO). Primers were ordered from Integrated DNA Technologies (Coralville, IA). The nucleotide sequence was determined by Genewiz (South Plainfield, NJ). Proteins were purified with 5 mL HiTrap Q HP column by AKTA Pure from GE Healthcare (Fairfield, CT), and protein concentrations were determined using the Bradford assay kit from Thermo Scientific (Waltham, MA). Glucose was assayed using a Glucose (GO) assay kit from Sigma-Aldrich, fructose was assayed using the resorcinol-ferric ammonium sulfate-hydrochloric acid method, while 5-(hydroxymethyl)furfural (HMF) was analyzed by HPLC (Agilent 1100 series) with UV detection at 285 nm (Column: Zorbax SB-C18, 3.0 x 15 mm; solvent: MeOH : H₂O = 1: 9). 1-Propanesulfonic acid was analyzed by GC-MS (Agilent 6890N/GC 5973 MS) with a capillary column (Agilent 19091J-416 HP-5 5% Phenyl Methyl Siloxane) after derivatized by (trimethylsilyl)diazomethane. The temperature for the program was started at 100 °C for 3 min, then elevated to 300 °C at a speed of 20 °C/min, and finally held at 300 °C for 10 min. Nitrogen adsorption isotherms of the mesoporous silicas were measured at 77 K using a Quantachrome Nova 2200e (Boynton Beach, FL), after drying the samples in a flow of N₂ gas at 403 K overnight. Specific surface areas were calculated by the BET method using adsorption data range from P/P₀ = 0.05 to 0.35, and the pore diameter distribution curve was derived from the adsorption branch by the BJH method. X-ray diffraction (XRD) patterns of all mesoporous silica were obtained on a Siemens D5000 diffractometer (Bruker, Billerica, MA) with Cu Kα radiation (40 kV, 30 mA, λ = 0.15418 nm) at 0.5°/min over the range 1.0° < 2θ < 30°. Solid-state MAS NMR spectra (1D) were recorded with a Varian 300 MHz wide-bore Solid-State NMR spectrometer using a 4 mm 15N-31P MAS probe (Foster City, CA).

Enzyme immobilization was performed by mixing 0.50 mg enzyme with 4 mg of the corresponding supports in 100 μL Buffer A (25 mM MOPS pH 7.0, 2.5 mM MgSO₄, 0.25 mM Co²⁺), followed by shaking at 1200 rpm and 313 K for 2.5 h. The immobilized enzyme was collected by centrifugation at 4000 rpm. The enzymatic reaction assays in buffer were performed in PCR tubes and heated with a PCR machine (MJ Research, Quebec, Canada), while reactions in THF/buffer were performed in pressure-relief reaction vials from Chemglass (Vineland, NJ). One-pot reactions were conducted in Kinesis microwave vials (Malta, NY). One unit (U) represents the conversion of 1.0 μmole of substrate at 1min. Unless specified otherwise, the enzyme initial activity assays were carried out at 363 K for 10 min. Enzyme/solid acid one-pot reactions were performed in THF/H₂O at 363 K for one hour, followed by 403 K for a further 24 h. In a typical reaction, 200 μL H₂O containing 1 mM CoCl₂ and 10 mg glucose was mixed with 800 μL THF, then 8 mg immobilized enzyme solid (containing 1.0 mg enzyme) and 16 mg solid acid were added. The reaction mixture was quenched by cooling in ice and then diluted with 600 μL H₂O. After centrifuging the solid catalysts, the clear solution was injected directly into HPLC for HMF detection. In the solid acid (1-propanesulfonic acid) leaching test, water was firstly removed by drying with anhydrous Na₂SO₄, and the THF solution was derivatized with (trimethylsilyl)diazomethane for one hour at room temperature before being injected into GC-MS. While in the fructose assay with resorcinol-ferric method and the enzyme leaching assay with SDS-PAGE gel, THF was firstly evaporated by bubbling the solution with N₂ gas.
Scale up reaction. To a 20 ml Kinesis microwave vial: 80 mg immobilized enzyme-NH$_2$-FMS30(1F1), 1.5 ml DI H$_2$O, 500 µl glucose aqueous solution (200 mg/ml in DI H$_2$O), 2 µl CoCl$_2$ solution (1M in DI H$_2$O), 140 mg of solid acid (SO$_3$H-FMS) and 8 ml THF were successively added. The vial was sealed and stirred at 90 °C for one hour, then 130 °C for 24h. After that, the reaction was quenched by cooling on ice and diluted with 6 ml DI H$_2$O. The same methods described above were used to determine the concentrations of fructose and HMF. Fructose yield: 16.2%; HMF yield: 34.3%.

2. Preparation of WT-TNXI, CBD-TN XI and TNXI-1F1 in E. coli

WT-TN XI was prepared as described elsewhere. Briefly, the plasmid carrying the *Thermotoga neapolitana* glucose/xylose isomerase gene (PET22b-TN XI, a gift from Robert Kelly at North Carolina State University, with TN XI gene flanked by N-terminal NdeI and C-terminal HindIII) was transformed into BL21 (DE3) for protein expression. Cells harboring the plasmid were cultured in TB media containing 100 µg/mL ampicillin at 310 K until OD~0.7, at which point IPTG was added to give a final concentration of 1 mM. Cells were harvested by centrifugation (5,000 rpm) after 4 h (OD~2.0). Cell pellets were suspended in buffer A and then lysed by French Press (Thermo Scientific, Waltham, MA). Cell debris was removed by centrifugation (18,000 rpm) for 30 min. The lysate was heated at 363 K for 45 min in an oil bath with stirring and then centrifuged again (12,000 rpm) for 12 min to remove precipitated lysate components. The clear, light yellow solution was purified on a HiTrap Q HP column using a salt gradient (0.1 – 0.3 N NaCl). The positive fractions (~0.20 N NaCl) by SDS-PAGE were combined and concentrated by Amicon (EMD Millipore, Billerica, MA). The pure enzyme was stored in buffer A with 50% glycerol at -253 K.

CBD-TN XI was prepared as described elsewhere. Briefly, the gene encoding a chitin-binding domain (CBD, PDB: 2cwr) based on sequence analysis of chiB (PF1233) in *Pyrococcus furiosus* with NdeI (CATATG) on both ends was ordered from Integrated DNA Technologies. The WT-TN XI plasmid (PET22b-TN XI) was digested with NdeI followed by treatment with alkaline phosphatase. The dephosphorylated plasmid was then ligated with NdeI digested CBD gene and transformed into DH5α. The resulting colonies were sequenced to check for the correct orientation.

The plasmid for TNXI-1F1 (V185T/F186S/L282P) was prepared using a Quickchange multi-site-directed mutagenesis kit (Agilent Technologies) with the following primers: 5’-GGAGAAGGTACACCTCTGTGGGTGGAAGAG-3’, 5’-CTTCCAGCAGACGGAGAAA TGGCAAGGAT-3’, in which the mutation sites are underlined. The WT-TN XI (PET22b-TN XI) plasmid was used as the template. The CBD-TN XI and TNXI-1F1 protein preparations were conducted in exactly the same way as the WT-TN XI preparation described above.

3. Representative procedures for the preparation of solid supports

3.1 Preparation of amine-functionalized mesoporous silica for enzyme immobilization

Functionalized mesoporous silica was prepared according to a protocol described elsewhere. In a typical procedure for the preparation of the unfunctionalized mesoporous silica UMS30, 2.0 g of Pluronic P-123 (MW = 5800) was dissolved in 1.6 M HCl solution (75 mL) at 303 K overnight. Then 3.0 g mesitylene and 4.2 g tetraethylorthosilicate (TEOS) were added to the
solution slowly and stirred for 20 h at 313 K. The mixture was aged statically in a sealed bottle at 373 K for 24 h in an oven. The white precipitate was collected by filtration, washed with distilled H$_2$O three times, and dried successively in air overnight, at 413 K for 12 h, and at 823 K for 3 h. A similar protocol was used to prepare other mesoporous materials. In the preparation of UMS20, the procedure was exactly the same as above except that 2.0 g mesitylene was added, while for UMS8.9, no mesitylene was added. In the preparation of UMS6.0, the solution was stirred at 308 K for 20 h after the addition of TEOS (no mesitylene added), and the ageing temperature was 353 K.

In a typical procedure to prepare amine-functionalized mesoporous silica-FMS, 1.0 g mesoporous silica prepared above was suspended in toluene (60 mL) and pretreated with 0.32 mL water in a three-necked flask fitted with a stopper and reflux condenser. This suspension was stirred vigorously for 2 h to distribute the water throughout the mesoporous matrix, then 169 mg tris(ethoxy)aminopropylsilane (TEAPS) was added, and the mixture was refluxed for another 6 h. At the end, the mixture was allowed to cool to room temperature and the product was collected by vacuum filtration. The functionalized mesoporous silica was washed with ethyl alcohol repeatedly and dried at 413 K under vacuum overnight.

3.2 Preparation of the sulfonic acid-functionalized mesoporous silica catalyst for fructose dehydration

SO$_3$H-FMS was prepared as described elsewhere. Briefly, Pluronic P123 (2.00 g) was dissolved in 1.6 M aqueous HCl (69 mL) and heated to 308 K. TEOS (4.13 mL, 18.5 mmol) was slowly added to the surfactant solution. After 30 min, 425 mg NaCl (7.33 mmol) was added to the reaction mixture. One hour later, 424 µL (3-mercaptopropyl)trimethoxysilane (2.06 mmol) was added in 106 µL increments every 15 min for 1 h. Afterward, 6 equivalent H$_2$O$_2$ (30 wt% in water; 669 µL, 12.36 mmol) was added to the reaction mixture. The mixture was stirred at that temperature for 20 h, and then aged at 373 K for 24 h. The resulting suspension was filtered, washed with water, and air-dried. The surfactant was removed by ethanol extraction under mild refluxing overnight twice. Finally, the solid was suspended in 2 M HCl (100 mL) for 3 h, then filtered and dried at 413 K for 20 h.

3.3 Preparation of Folded Sheet Mesoporous Silica (FSM-7.6)

FSM-7.6 was prepared as described elsewhere. Kanemite was prepared by mixing amorphous silica and NaOH (SiO$_2$ : NaOH = 1:1) in 100 mL water and then dried at 373 K overnight. The dry material was calcined at 973 K for 5.5 h. FMS-7.6 was prepared by dispersing kanemite (5 g) in an aqueous solution (250 mL) containing 1,3,5-trisopropylbenzene (0.14 M) and docosyltrimethylammonium chloride (0.1 M). The mixture was stirred rigorously at 343 K for 2 h at pH 11.5, then the pH was adjusted to 8.5 by adding aqueous HCl slowly. The mixture was stirred for another 3 h at that temperature. The resulting products were filtered and washed with warm deionized water repeatedly. Finally, the obtained gel was dried at 353 K overnight and then calcined at 823 K for 6 h.
4. Supporting figures

Figure S1: A: Powder X-ray diffraction patterns of functionalized mesoporous silicas; B: Nitrogen adsorption-desorption isotherms recorded at 77 K for dry, unfunctionalized mesoporous silicas.
Figure S2: Pore size distributions derived from nitrogen adsorption-desorption isotherms for dry, functionalized mesoporous silicas using the BJH/BH method.
Figure S3: $^{29}$Si single-pulse solid-state NMR spectra of functionalized mesoporous silicas (R.I. is the relative integrated intensity). std-UFM: unfunctionalized mesoporous silica 6.0; FMSC30: COOH-FMS30; FMSN06: NH$_2$-FMS6.0; FMSN20: NH$_2$-FMS20; FMSN30: NH$_2$-FMS30; FMSN30-2: NH$_2$-FMS30 lower coverage; FMSN89: NH$_2$-FMS8.9.
Figure S4: Fructose dehydration activity of the solid acid catalyst (SO$_3$H-FMS, variable amounts) in the presence of the solid base support (2 mg NH$_2$-FMS) in THF/H$_2$O (4:1 v/v), upon incubation with 100 mg/mL fructose at 363 K for 24 h.

Figure S5: Glucose isomerization activity of the immobilized enzyme (2 mg NH$_2$-FMS30 (TNXI)) in the presence of the solid acid catalyst (SO$_3$H-FMS, varied amounts) in aqueous solution, upon incubation with 100 mg/mL glucose at 363 K for 60 min.
Figure S6: Enzyme and 1-propanesulfonic acid leaching tests. Left: SDS-PAGE analysis of leaching enzymes in solution after different time points and temperatures; Right: GC-MS analysis of 1-propanesulfonic acid standard (plot above) and the solution after one-pot tandem reaction (plot below).

Table S1: Characteristics of free and immobilized WT-TNXI, CBD-TNXI and TNXI-1F1. \(P_{LD}\): protein loading density on NH\(_2\)-FMS30; \(I_e\): the ratio of the specific activity of the immobilized enzyme to the specific activity of the free enzyme in buffer; \(I_{A_{THF}}\): initial activity of immobilized enzyme in THF/Buffer A (4:1 v/v); \(T_{1/2F}\): half life of free enzyme in buffer; \(T_{1/2I}\): half-life of the immobilized enzyme at 90 °C. a: 80°C

<table>
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<tr>
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<th>WT-TNXI</th>
<th>CBD-TNXI</th>
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<td>Yield (mg/L)</td>
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<td>6.0</td>
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<tr>
<td>pI</td>
<td>5.7</td>
<td>5.4</td>
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<td>Mass Calc. (Da)</td>
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<td>Mass Measured (Da)</td>
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<td>Initial activity (U/mg)</td>
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<td>(K_m) (mM(^{48, 10}))</td>
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<td>536±17.3</td>
<td>52±7.1(^a)</td>
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<td>(P_{LD}) (w/w)</td>
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<td>(I_e)</td>
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References