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

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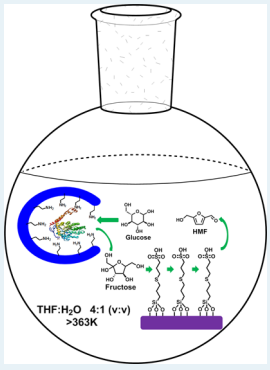
Supporting Information

ABSTRACT: Conversion of cellulosic biomass to renewable chemicals such as 5-hydroxymethylfurfural (HMF) is of high current interest. Herein, we report a rare example of one-pot synthesis of HMF from glucose by tandem catalysis. The system is composed of a thermophilic glucose isomerase enzyme for glucose isomerization to fructose and a solid acid catalyst for fructose dehydration to HMF. A base ($-NH_2$) functionalized mesoporous silica (aminopropyl-FMS) with large pore size was deployed successfully to immobilize and protect the thermophilic glucose isomerase in organic solvents at high temperature. The combination of this catalyst with a Brønsted acid ($-SO_3H$) functionalized mesoporous silica (propylsulfonic acid-FMS) allowed us to conduct a one-pot transformation of glucose to HMF directly in a monophasic solvent system composed of tetrahydrofuran (THF) and H_2O (4:1 v/v) with 61% yield of fructose and 30% yield of HMF at temperatures >363 K in 24 h.



KEYWORDS: *biomass conversion, tandem catalysis, biocatalysis, one-pot reaction, functionalized ordered mesoporous silica*

In recent decades, because of the depletion of and environmental concerns about the use of fossil fuels, the transformation of cellulosic biomass to 5-hydroxylmethylfurfural (HMF), a potentially important chemical intermediate, has been of great interest.[1] Simple carbohydrates such as fructose can be dehydrated to HMF with various liquid/solid acids in different solvents;[2] however, the direct conversion of glucose, a more abundant monosaccharide than fructose, to HMF in a one-pot fashion has the potential of minimizing energy and solvent spent on product isolation and purification.

Several approaches have been successfully developed to integrate glucose isomerization and fructose dehydration catalysts into a one-pot system to transform glucose to HMF through fructose. For example, Nikolla et al. combined tin-beta zeolite (solid Lewis acid) with aqueous hydrochloric acid in a biphasic water/THF reactor system to convert glucose to HMF with 110 mol HMF/mol Sn-Beta/h at 453 K.[3] Peng et al. demonstrated HMF production by combining both acid ($-SO_3H$)- and base ($-NH_2$)-functionalized mesoporous silica in an ionic liquid to obtain up to 0.54 mol HMF/mol LPMSN-NH_2/h at 393 K.[4] Enzymes have also been employed for glucose isomerization, but the integration of an enzyme in HMF production is confined to a two-step operation. In one case, Huang et al. reported HMF production by using an immobilized glucose isomerase (Sweetzyme IT), followed by treatment with aqueous hydrochloric acid.[5] A similar strategy was used by Grand et al.[6] and Simeonov et al.[7] Here, we report a rare example of a one-pot synthesis of HMF from glucose by combining an immobilized enzyme and a solid acid catalyst.

Because of the orthogonality of the reaction environments for the enzymatic isomerization of glucose and the acid-catalyzed dehydration of fructose, a well-studied, SBA-15-type solid acid (SO_3H-FMS) was chosen to simplify the reaction system to minimize the interaction between the two catalysts. This acid-functionalized ordered mesoporous solid acid was reported to continuously convert fructose into HMF at 403 K for periods up to ∼150 h.[2a,8] Fructose dehydration by a solid acid could avoid the high acidity in the reaction system and afford easier separation during the work-up relative to using homogeneous catalysts. Next, a thermophilic glucose/xylose isomerase from *Thermotoga neapolitana* (TNXI) was chosen to meet the high temperature requirements of the dehydration reaction, since this enzyme has a half-life of 64 min at 363 K as well as a high glucose specificity (k_{cat} = 1139 min^{-1}, K_m = 88.5 mM).[9]

Finally, an appropriate solvent medium was chosen to accommodate both reactions in the tandem catalytic sequence. Enzymes prefer aqueous solutions, whereas the acid-catalyzed

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fructose dehydration shows low selectivity to HMF in water, although it can be highly selective in ionic liquids and some polar aprotic organic solvents.[10] Several studies have highlighted the special advantages of monophasic cosolvents in semiaqueous mixtures, including improved HMF yield and specificity, both by increasing the active furanose tautomers of fructose and by suppressing the further transformation of HMF in aqueous solution.[2b,11] Several cosolvents reported in the literature that benefit fructose dehydration were screened with the TNXI. This preliminary study revealed that the wild-type enzyme has the highest tolerance for dimethyl sulfoxide (DMSO) and ethanol (EtOH), moderate tolerance for tetrahydrofurfuryl alcohol (THFA) and tetrahydrofuran (THF) while being least tolerant of *N*-methylpyrrolidinone (NMP) and γ-valerolactone (Figure 1). However, in all cases, the addition of the cosolvent led to a loss of some enzyme activity.

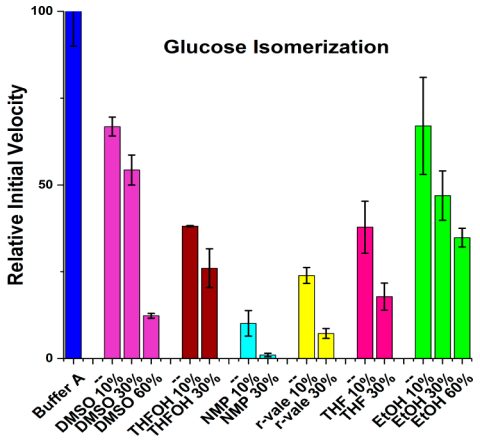

Figure 1. Relative activity of free TNXI in various cosolvents (the activity in pure aqueous buffer A is defined as 100). Free enzyme (0.6 mg/mL) in cosolvent/buffer A (v/v) and 100 mg/mL glucose were incubated at 363 K for 10 min. THFOH, tetrahydrofurfuryl alcohol; NMP, *N*-methylpyrrolidinone; γ-vale, γ-valerolactone.

Enzyme immobilization within a mesoporous silica was explored both to enhance its tolerance for high concentrations of organic cosolvents and to avoid the interference with the solid acid catalyst. Because of their rigid and controllable pore sizes, unfunctionalized and functionalized mesoporous silicas (UMS and FMS) have been widely used to improve enzyme thermostability, tolerance for hostile conditions, or both, according to the rules of electrostatic complementary and size match.[12] In this work, we prepared materials with various pore sizes and surface terminations (Suppporting Information (SI) Figure S1). Unlike FMS8.9, which has a narrow pore size distribution, FMS20 and FMS30 have a broad range of pore sizes (SI Figure S2). The protein loading density (P_{LD}, w/w %) and immobilization efficiency (I_e, defined as the ratio of the specific activity of the immobilized enzyme to the specific activity of the free enzyme in solution) vary considerably (Figure 2).

TNXI (MW: 50 893) is a homotetramer with a unit cell 16.2 × 12.2 × 9.9 nm (PDB: 1A0E). Its isoelectric point (pI) is 5.69 (Protein Calculated V3.4); thus, the overall charge of the protein is negative at pH 7.0. As shown in Figure 2, the negatively charged TNXI is barely immobilized by either UMS (P_{LD} = 0.6%) or COOH-FMS (P_{LD} = 0.15%) because of

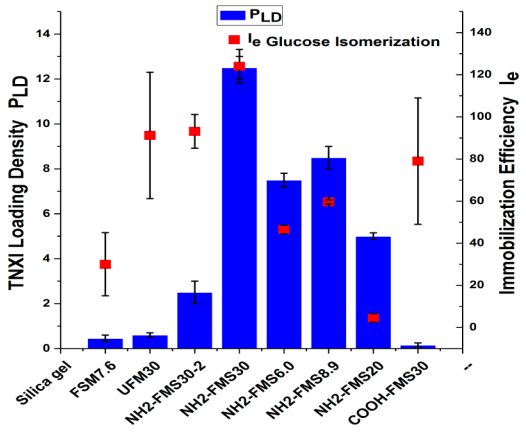

Figure 2. TNXI immobilization in different solid supports (P_{LD}, blue); immobilization efficiency (I_e, red). FSM, folded-sheet mesoporous silica with pore size 7.6 nm; UFM, unfunctionalized mesoporous silica; NH_2-FMS, aminopropyl-fuctionalized mesoporous silica; COOH-FMS, propylcarboxylic acid-functionalized mesoporous silica.

electrostatic repulsion with the negatively charged silanolate-terminated silica surface (≡SiO^-) and the surface functionalized with carboxylate groups. In contrast, positively charged NH_2-FMS shows a much higher protein loading density (>5%). Pore size is another important factor in TNXI immobilization. NH_2-FMS30 (4.9% coverage, SI Figure S3) shows the highest enzyme loading density (P_{LD} = 12.5%) and efficiency (I_e = 120%) as well as the best enzyme protection ($t_{1/2}$ = 117 min at 363 K in buffer A). The immobilized enzymes included the enzymes adsorbed on the surface (P_{LD} ~ 7%) and the enzymes entrapped in pores over 20 nm (P_{LD} ~ 5%). In contrast, NH_2-FMS20 was inferior due to the shortage of large pores (SI Figure S3).

The entrapped TNXI in NH_2-FMS30 experienced superior protection from the deleterious effect of high concentrations of THF (Figure 3). Unexpectedly, the mesoporous support failed

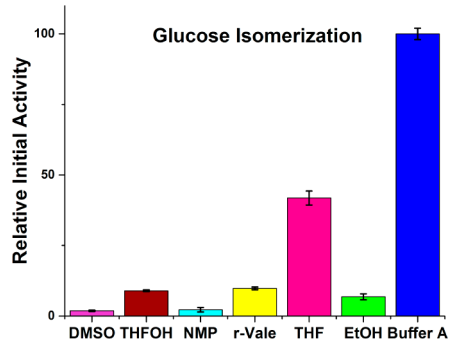

Figure 3. Relative activity of TNXI immobilized in NH_2-FMS30 in the presence of different cosolvents (80% in water; the activity in pure buffer A is defined as 100). Immobilized TNXI (0.5 mg/mL) was incubated in cosolvent/buffer A (4:1 v/v) and 100 mg/mL glucose at 363 K for 10 min.

to protect the enzyme from DMSO and EtOH. The immobilized enzyme has an activity up to 4 U/mg and a half-life of $t_{1/2}$ = 12.8 min in 80% THF/buffer A at 363 K.

To further improve the stability and activity of the enzyme, two engineered TNXIs—CBD-TNXI (TNXI fused with a chitin binding domain)[13] and TNXI-1F1 (V185T/F186S/L282P)[14]—were prepared. Compared with the WT, each has

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its own special features: CBD-TNXI shows higher thermostability and activity in buffer A, but much lower enzyme yield and binding affinity for glucose, whereas TNXI-1F1 possesses a higher activity and moderate substrate binding affinity at the compensation of its thermostability. However, all of the free enzymes are sensitive to THF, and the immobilized enzymes show improved thermostability in buffer A and similar half-lives in THF/H_2O (SI Table S1).

In parallel, fructose dehydration by the solid acid catalyst (SO_3H-FMS) was tested in the monophasic cosolvent systems. All activities are on the same scale as the highest dehydration activity in DMSO/H_2O. Further tests confirmed that addition of Co^{2+}, the cofactor required for TNXI catalysis, has little effect on the solid acid catalyst, and the presence of the buffer deactivates it completely (Figure 4).

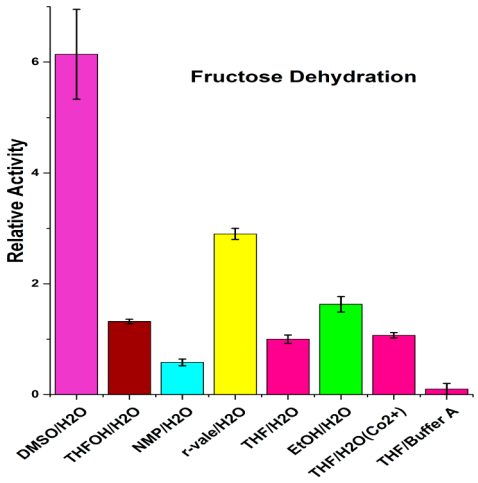

Figure 4. Relative activity of SO_3H-SBA in different cosolvents (the activity in THF/H_2O, 2.7 × 10^{-4} U/mg, is defined as 1.0). A 2 mg portion of solid acid was suspended in 500 μL of cosolvent/H_2O (4:1 v/v) containing 10 mg of glucose at 363 K for 24 h.

Upon combining the two heterogeneous catalysts in the THF/H_2O solvent system, we observed their mutual suppression. On one hand, the presence of the basic support (NH_2-FMS) caused deactivation of the solid acid catalyst (SO_3H-FMS), presumably as a result of neutralization (SI Figure S4).[15] On the other hand, the immobilized enzyme lost its activity quickly in the presence of the solid acid catalyst, presumably as a result of acid-induced deactivation (SI Figure S5). Therefore, 1 equiv weight of immobilized TNXI-1F1 and 2 equiv weights of the solid acid were used to catalyze the tandem reaction sequence in one pot under separately optimized conditions by holding the temperature first at 363 K for 1 h, then at 403 K for 24 h, without product isolation. The enzyme-catalyzed glucose isomerization occurred in the first hour to reach up to 61% fructose, then fructose dehydration accelerated at the elevated temperature (Figure 5) to obtain 30% HMF with 64% specificity. The control experiment by replacing immobilized enzyme with solid support did show up to 7% fructose yield and 2% HMF yield. After the reaction, no free enzyme and 1-propanesulfonic acid were observed in solution by SDS–PAGE and GC/MS analysis (SI Figure S6). However, the recycled enzyme completely lost its activity and the solid acid remained active. Some known impurities such as levulinic acid and insoluble humins were observed.[16]

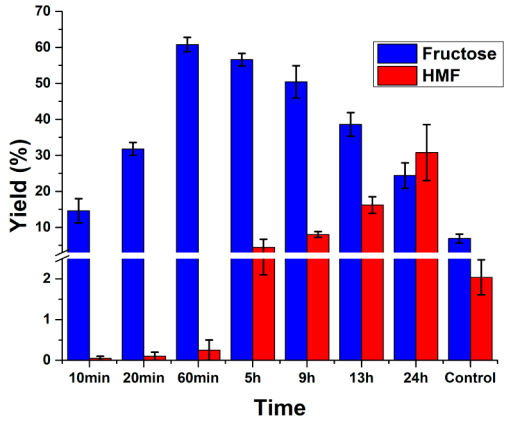

Figure 5. Time course for the conversion of glucose to HMF in one-pot reactions consisting of immobilized TNXI-1F1 and solid acid. An 8 mg portion of NH_2-FMS (TNXI-1F1) was combined with 16 mg of SO_3H-FMS in 800 μL of THF/200 μL of H_2O containing 1 mM Co^{2+} and 10 mg glucose at 363 K for 60 min, then the temperature was raised to 403 K for different time intervals. Control: 8 mg of NH_2-FMS and 16 mg of SO_3H-FMS under the same conditions.

In summary, we successfully immobilized a thermophilic glucose isomerase enzyme within an amine (−NH_2)-functionalized mesoporous silica. The alkylamine provides high affinity and a favored environment for the enzyme, which not only results in a higher enzyme activity and stability in buffer, but also affords moderate protection in organic solvents at 363 K. By combining this immobilized enzyme catalyst with an acid (−SO_3H) functionalized mesoporous silica, we achieved one-pot synthesis of HMF from glucose with up to 61% fructose yield and 30% HMF yield. Nevertheless, the enzyme could not be recycled because its short half-life ($t_{1/2}$ = 15.8 min) in the organic solvent and the efficiency of the tandem system is greatly hindered by the large discrepancy in rates for the two catalysts. Studies to further improve the enzyme stability and the efficiency of fructose dehydration are in progress.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Details of the experimental procedures; characterization of the solid supports by powder X-ray diffraction, nitrogen adsorption−desorption isotherm and solid NMR spectra; enzyme properties comparison. This material is available free of charge via the Internet at http://pubs.acs.org

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Notes

The authors declare no competing financial interest.

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