**Developing Multi-Enzyme Metal-Organic Framework Nanocrystals for Rapid Soybean Biomass Conversion**

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**Technical Report**

**Introduction**

Soybean is among the most abundant agricultural products in North Dakota (ND). Thus far, the major use of soybean is extracting soybean oil, which has become an important income of ND soybean farmers. After extracting the oil, the residual components, although containing food proteins, valuable polysaccharides, and chemicals of energy resources, are often utilized as

animal foods. This is a tremendous waste given the huge market on food and energy industries

and the high soybean yield in ND. The cause of such a waste is the difficulty in extracting these

valuable components from the intense soybean cellulose network. By far, little success has been

achieved, due to the lack of proper approaches to efficiently and rapidly degrade the cellulose

with low-cost, minimal damage to the valuable portions, and no unwanted side-products. Here,

we will develop a unique nanoscale materials that can overcome these barriers. The key is to

immobilize all enzymes that can degrade the cellulose network on the surface of a recently

developed crystal called Metal-Organic Framework (MOF) as shown in our recent works.1-5 MOFs offer enhanced stability to the trapped enzymes so that the reaction can be carried out under elevated temperatures and acidic pHs to enhance the efficiency of enzymes. The use of enzymes generates no damage to any valuable components. The developed nanoscale crystals are easily recovered for reuse, which increases the cost-efficiency, and produce no pollution, contaminant, or metal-toxicity (because food-grade metals will be utilized). The work will potentially maximize the use of soybean crops for industry and improve the incomes of soybean growers.

**Results and Discussions**

The central goal of this project is to simultaneously immobilize three key enzymes responsible for degrading cellulose network on a Metal-Organic Framework (MOF) so that their close proximity can help rapidly degrade native cellulose, while the MOF scaffolds can offer certain degree of protection to the enzymes so that elevated temperature can be applied to speed up the cellulose degradation. Furthermore, all enzymes are stabilized on the MOF so that it may be possible to reuse the enzyme@MOF composites after degrading one batch of cellulose samples. The three enzymes are cellulase, hemocellulase, and xylanase. Toward this goal, we have conducted the following, step-wise investigation: I) demonstration of the enzyme activity on free enzymes, II) proving enzyme activity upon immobilization on 3 MOFs, III) proving all enzymes are active when combined on MOFs, IV) determining the long-term stability of the composites, and V) improving the reusability of the composites. This technical report is organized following this order.

I. Positive and negative controls: free enzymes are active in solution.

 Commercial activity assay kits for cellulase, hemicellulose, and xylanase were purchased to confirm that the commercial enzymes are active. The activity of the cellulase is measured as the optical absorption at 340 nm (A340) which indicate the degradation of cellulose and generation of a product that can react with a coloring agent. The activity of cellulase is shown in Figure 1. As is evident from Figure 1, the cellulase itself is active as judged by the increase in A340 due to cellulose degradation (traces labeled as Cell 1, 2 and 3). Interestingly, the presence of hemicellulose (traces labeled as Cell/Hemi 1, 2, and 3 of Figure 1) increases the activity of cellulase. Lastly, the presence of three enzymes in solution resulted in significantly enhanced overall activity (traces labeled as Cell/Hemi/Xyl 1, 2, and 3 of Figure 1).

**Figure 1.** Activity of three enzymes in solution.

II. Activity of each individual enzyme confined in MOFs.

 We have attempted 3 kinds of MOFs using Ca2+ as the metal and two organic ligands, namely BDC and BPDC, as well as magnetic nanoparticles (MNP): CaBPDC, CaBDC, and CaBDC-MNP. These are investigated to determine the best way to immobilize our enzymes for cellulose biodegradation; MNPs are employed to carry out magnetic separation after a catalytic cycle. CaBPDC was the first MOF we attempted. The advantage is that all three enzymes are active (be aware that a lot more composites are needed as the enzymes are only partially exposed to the surface of the CaBPDC MOF as compared to the complete free enzymes in solution). The activity data of cellulase on CaBPDC is shown in Figure 2. However, the separation of cellulase@CaBPDC after a reaction from unreacted cellulose substrate is difficult, because the composites are smaller in size than the substrates.

**Figure 2.** Cellulase activity on CaBPDC MOF at different MOF concentrations (x1 to x10).

 CaBDC is known to form larger crystals (micrometer scale), which may help separation. Therefore, we attempted to immobilize enzymes on CaBDC. While the activity was relatively lower, the particle size is larger (see our recent paper probing the particle size).1 Using the same activity kit, we also found the cellulase is active (Figure 3). However, the enzyme@CaBDC composites were mixed with the unreacted substrates, making it difficult to separate and reuse the composites.

Figure 3. Cellulase on CaBDC at different composite concentrations.

**Figure 4.** Hemicellulase activity on CaBPDC.

 Interestingly, we found it possible to incorporate magnetic properties in CaBDC if MNPs are doped during the formation of enzyme@CaBDC composite.4 We therefore prepared such a complex and found it possible to separate the composite from the substrate. The xylanase activity was found to be reusable (see below).

III. Activity of three enzymes on one MOF.

 We have then immobilized three enzymes together on CaBPDC and CaBDC. The activity of each enzyme was tested using the same commercial kits and each enzyme was found active.

IV. Activity over time.

 The cellulase activity was tested as a pilot to show the long-time activity. Basically, we initiated a reaction and let the reaction go for a long time. Over a period of 5 days, we found the activity stabilized at 2-3 days. This indicates that our composites were not disassembled during the reaction.

**Figure 5.** Longevity of our composites.

V. Reusability of the composites.

 As discussed above, the most challenge of our project is the reusability test, which requires separation of the composites from the substrates, cellulose. We have attempted gravimetric separation which did not work out because the composites and substrates are both large in size and heavy. We have also attempted the magnetic separation using enzyme@CaBDC-MNP composites but this strategy did not result in cellulase reusability data. The Xylanase, on the other hand, was found reusable after the magnetic separation. As shown in Table 1, the xylanase seems to be reusable after 4 rounds.

**Table 1.** Reusability tested using the xylanase assay on CaBDC-MNP.

|  |  |  |
| --- | --- | --- |
| Trial | BDC-MOF sample 1 (A400) | BDC-MOF sample 2 (A400) |
| 1 | 0.136 | 0.175 |
| 2 | 0.119 | 0.112 |
| 3 | 0.085 | 0.079 |
| 4 | 0.066 | .071 |

 We rationalize the loss of reusability of cellulase from the CaBDC-MNP test to the large, viscous unreacted cellulose substrates that even magnetic separation was not able to recycle the composites. One potential solution is to use large magnetic nanoparticles so that the magnetic interaction is stronger to help the separation. The other is to heat the mixture to unwrap the unreacted cellulose from the CaBDC-MNP particles.

**Impact of COVID-19 and future plans**

 We have submitted samples for the characterization of all particles to the facility centers of SEM/TEM, TGA, and powder X-ray diffraction. However, due to COVID-19 and some instrument repairment, our data acquisition was not as expected. We are waiting for 10-12 data from each of these facility centers. As soon as we obtained all the data, a manuscript will be constructed for submission.

**References**

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