Biomass Research and Development Technical Advisory Committee Meeting
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"Back to Basics"
More Emphasis Needed on Catabolic Pathway and Enzyme Research
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When someone from outside of the biofuels and bioproducts community looks at the bioenergy websites of DOE or USDA or those of bioenergy firms trying to raise money, they are probably left with the impression that all the necessary discoveries have been made and commercialization is only being slowed by lack of mid or late stage funding. And while this overly optimistic approach may be useful when dealing with Wall Street or Congress, I hope the decision makers at these agencies and companies have a more realistic appreciation of our lack of understanding in critical areas and are plotting new paths to solve these core scientific problems.

Speaking from my experience in enzyme and catabolic pathway development I can clearly say we have a long way to go. In essence, the high performance cellulase enzymes such as the Novozyme Ctec3 are not the complete answer.

More importantly, the question they were built to answer wasn't the right one.

If I may, I'd like to offer three core research areas that need to be pursued if we are to conquer Biomass Recalcitrance and produce cost-effective biofuels and bioproducts.

Enzyme Recycling: As Weiss et al wrote in 2013[1] the recycling of enzymes offers a significant cost reduction in biomass pretreatment and saccharification costs. However, the major roadblock to efficient recycling is releasing the enzymes from the insoluble biomass[2] they attach to in order for the active sites of the enzymes to come in contact with the specific biomass bonds they are designed to break. In fact, some of the higher yielding enzymes compound this problem by improving or increasing the attachment points. In recent recycling work we have seen decreases of over 30 percent between runs when the biomass is in the early stages of processing. Improvements in one type of enzyme that address the attachment and related issues could result in the improvement of all categories of catabolic enzymes.

Non-Cellulose Synthesis/Catabolic Pathways: The biggest surprise I had entering the biomass processing business was how lacking our knowledge of plant cell structure is. The scale of the United States' plant cell research is laughable in comparison to the

billions spent by the US science establishment over the last half-century on animal plant cells. Without an improved knowledge of how pectin, xylose, lignin, and cellulose are formed[3] and how they intertwine into beautiful and incredibly strong matrixes we're kidding ourselves about economically producing "Total Biomass" fuels and products.

Pathway Enzyme Development: The consolidated organism approach to combining saccharification and fermentation was an interesting initial approach. Unfortunately in most cases the complexity of the pathways and the number of enzymes needed were greatly underestimated. Building on the original idea, such ideas as adapting catabolic organisms to specific biomass should be pursued to develop a complete catabolic/ saccharification pathway. The hypothesis is that by restricting the organisms to only the target carbon sources, the organism colonies would have two reactions: 1) currently used enzymes needed to break down the biomass would be overexpressed, and/or 2) currently non expressed genes would secrete "new" enzymes. After several generations the population would have moved to expressing a new set of enzymes specifically suited to the target biomass. Again, once this process had been developed in one organism and biomass source it could be used in multiple systems.

I hope that the Biomass Research and Development Initiative Funding Opportunity Announcement that was just released as well as those in future years will be focused on solving critical core science issues such as those just listed. This is especially critical since funding is limited, and spreading it over too many unrelated projects certainly dilutes it impact.

References

[1]Weiss, Noah, Börjesson, Johan, Pedersen, Lars Saaby, Meyer, Anne S, "*Enzymatic lignocellulose hydrolysis: Improved cellulase productivity by insoluble solids recycling,"* Biotechnology for Biofuels , 2013, 6:5 http://www.biotechnologyforbiofuels.com/content/6/1/5

[2]Xue, Ying, Jameel, Hasan, Park Sunkyu, Strategies to Recycle Enzymes and Their Impact on Enzymatic Hydrolysis for Bioethanol Production, BioResources, 2012.

[3]Mohnen, D, Bar-Peled, M., and Sommerville, C.: *Biosynthesis of Plant Cell Walls,* in Chapter 5, Biomass Recalcitrance, ed. Himmel, M., Blackwell Publishing, Oxford, 2008. P-113)