

CAN LIGNOCELLULOSE BIOSYNTHESIS BE THE KEY TO ITS ECONOMICAL DECONSTRUCTION?

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It is ironic to think that the venerable pulp and paper industry is now considering ways to degrade cellulose. This notion can be understood as a way that the industry can face a protracted downturn in profitability and ever-mounting socio-economic pressures to enhance the efficiency of biofuels production. Many approaches have been recently taken to deconstruct cellulosic biomass, but this Editorial explores one key that may start to explain the increasing momentum in the biofuels community – biotechnology. Two approaches appear to be possible as scientists search for an effective way to unzip cellulose to its key constituents through the use of biotechnology. On the one hand, there are efforts to re-engineer the chemical composition of the tree, rendering it more digestible by enzymes and decreasing the need for mechanical or chemical pretreatment. On the other hand, what we are learning about lignocellulose biosynthesis can be of potential help in designing more efficient systems to essentially reverse that process.

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Struggling with an Intractable Problem

Have you ever been plagued by a problem of an almost intractable nature and before you gave up on it, you came up with an unorthodox approach born of desperation? Well, the biofuels community appears to be at that crossroads in its short life, realizing with resolute stoicism that decomposing cellulosics is no trivial task, but more akin to the mythical Labors of Hercules.

Today, we are in a quagmire of divergent approaches to unzipping the most abundant polymer on the planet, namely cellulose, to its fermentable building blocks. However, none of the current approaches is anywhere near to being a viable process for surmounting the economic “energy barrier” of breaking lignocellulosic material down to its monomers. If we had an economically viable approach, then we would be inundated with an onslaught of companies clamoring to gain ascendancy in this nascent market by offering cheap grades of “gasohol.” Some readers may recall the oil embargo of the 1970s that led society to begin to insist on alternative fuels, including ethanol (gasohol). Yet, nature is far too smart for us. She allows us to degrade polymers such as starch found in abundance in food, but degrading cellulose does not appear to be anything she had in mind. Logically, starch should be easy to break down. It is amorphous in structure and hence a readily hydrolyzable (degradable) product. Cellulose is much more organized, and hence robust and suited for maintenance of vegetative living forms, more like the collagen and chitin building blocks in the animal kingdom. It is very stable, virtually impenetrable, and very strong. Furthermore, in wood the cellulose is encased

within layers of recalcitrant lignin and complex mixtures of hemicelluloses that defy easy chemical or enzymatic paths to their decomposition. Destroying the cellulose enzymatically is possible, after all, because nothing is built for total permanency, but it is a slow and tedious process. In short, the current paradigm of cellulose biosynthesis does not yet lend itself to clean and economically attractive solutions for saccharification, i.e., the process of decomposing cellulose to its glucose and other saccharide monomers.

A Radical Solution: Change the Tree

Such an *impasse*, as just described, calls for “desperate” thinking. What if we could manipulate the process of cellulose biosynthesis in a plant so that its eventual degradation could be facilitated? Such an idea would require manipulating the genetic pathways involved in cellulose biosynthesis. Forest biotechnologists including Profs. Vincent Chiang and Ron Sederoff (both of NC State University) have shown that not only is this possible, but they are doing it *now*. In short, they are attempting all kinds of amazing things such as down-regulating lignification (thus allowing easier access to cellulose by cellulose-degrading enzymes), playing with the polysaccharides to make them less crystalline, more labile, or changing their proportions, and finally, toying with the idea of including cellulose-degrading enzymes within the vicinity of the cellulose as the plant grows. Let’s analyze in piecemeal these revolutionary ideas.

First, the idea of affecting the relative proportion of lignin in a plant was a fundamental attempt at examining lignification’s pathways in a plant and manipulating them to facilitate pulping. Its current applicability in the biofuels arena is serendipitous and welcome. Lignin can be viewed as a waxy and brittle barrier that a plant uses to protect its polysaccharides, maintain its architecture, allow water transportation, and act as a barrier against attack by all kinds of foreign agents. Prof. Chiang was able to show that significant reductions in the proportion of lignin in wood can be achieved without compromise to the structural integrity of a tree. The modification also helped significantly in the process of pulping, *i.e.* the removal of lignin to make paper products. Now, his work is showing promise as a great way to improve enzyme penetration into the ultrastructure of the wood cell and thus expedite saccharification.

A second approach, based on a modification of the polysaccharides themselves, is relatively less novel, but no less important. It is an attempt to reduce their intractability so that they can be hydrolyzed. The disadvantage of this approach is that it has the chance of affecting plant metabolism and function. Yet, all in all, this is a very powerful model to pursue, because it facilitates downstream processing of the wood for biofuels.

The last approach, that of having a plant synthesize the very agents that will break it down, is risky and imaginative. Typically, all living systems require external agents to decompose their tissues, *i.e.*, bacteria, fungi, scavengers, etc. But what if a living system had the ability to self-catalyze its eventual decomposition from within? This poses a risk, since triggering the reaction too soon would be calamitous to its industrial processing. But, at least in the eye of the inventor, it may be that gentle heating in aqueous buffered solutions could activate the enzyme and begin the process of quite literally “dissolving” the plant.

A Less Radical Approach: Learn from Biology

The biotechnical approaches we have mentioned up to this point all share a common problem – they depend on whether or not society will accept the widespread planting of genetically engineered trees. In addition, the time required to grow a tree can serve as a barrier to implementation of the approaches just mentioned. For faster growing species, such as grasses, straws, stover, etc., the time issue is not as daunting. However, in either case, ethical questions and safety concerns need to be addressed by the world's populations and their governments when it is proposed to re-engineer DNA and place altered species in locations where their genes may spread into wild populations.

In view of this reality, we would argue that a form of “reverse bio-engineering” is a more probable approach to be employed worldwide in the coming decades. The idea is that by improving our understanding of the biosynthesis of lignocellulosic material, we can then improve our ability to reverse the process.

Some clues as to how reverse bioengineering may come about are revealed in a recent article in this journal (Ioelovich, 2008, *BioRes.* 3, 1403); the nanostructure of wood suggests that layers within cellulosic fibers are formed by a number of almost-simultaneous events, with the main molecules of the fiber wall all undergoing preparation in parallel with each other. One of the reasons that conventional pulping has posed great challenges over the years is that the process is not as selective as we would like it to be; pulping partially degrades polysaccharides into the same black liquor that is later burned for energy. This incineration of polysaccharides can be seen as a waste of valuable bioenergy precursors.

We propose that some of the inefficiency of conventional pulping schemes, in terms of recovering the value of the polysaccharide-derived chemicals in wood, can be attributed to a focus on removal of essentially all of the lignin in the first step. By noting the orchestrated way in which the components come together during their synthesis, it makes sense to use a different approach. Maybe if we employed strategies that methodically and successively target, peel, and collect the various polysaccharides at the same time, instead of just trying to remove all of the lignin at once, then we could succeed in a highly efficient way to unzip the fiber in layer-by-layer fashion, resulting in a stream of monomers that then could be fractionated efficiently. The separated monomers then could be further transformed by a variety of steps.

In short we envision a future biorefinery in which the processes mirrors that of the original synthesis – with many processes taking place in the reverse manner from the original synthesis, and many of the events taking place in parallel, rather than sequentially.