

Lignocellulosic biomass processing: A perspective

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Abstract

As biorefineries replace oil refineries, society and the environment will benefit from a switch from hydrocarbon feedstocks to renewable carbohydrates as a source of energy, materials and chemicals. Biomass-based ethanol technologies are rapidly evolving and bottlenecks are being identified that need to be overcome to achieve widespread commercialization. Current research is driven by the need to reduce the cost of biomass-ethanol production. The preferred method is to thermochemically pretreat the biomass material and subsequently, enzymatically hydrolyze the pretreated material to fermentable sugars that can then be converted to ethanol. Pretreatment research is focused on developing processes that would result in reduced bioconversion time, lower cellulase enzyme usage, and/or higher ethanol yields. Cellulase research efforts are focused on developing a cost-effective, highly thermostable, synergistically acting enzyme mixture that would meet the end user's needs. Robust fermentation microorganisms are also being developed for conversion of biomass sugars to ethanol and other bioproducts. An integrated research and development approach is paying off and commercial plants for the production of biomass-ethanol are close to becoming a reality.

Procesamiento de biomasa lignocelulósica: Una perspectiva

A medida que las biorefinerías reemplazan las refinerías de petróleo, la sociedad y el ambiente se irán beneficiando por el cambio de materia prima proveniente de hidrocarburos, a carbohidratos renovables como fuente de energía, materiales y productos químicos. Las tecnologías de etanol basadas en biomasa se están desplegando rápidamente aunque ciertos obstáculos en el progreso han sido identificados y necesitan ser superados para poder lograr una comercialización amplia y generalizada. Las investigaciones actuales están impulsadas por la necesidad de reducir el costo de la producción de etanol de biomasa. El método preferido es el pretratamiento termoquímico del material de biomasa y la subsiguiente hidrólisis enzimática del material pretratado a azúcares fermentables, que luego pueden ser convertidas en etanol. La investigación sobre pretratamiento se concentra sobre la elaboración de procesos que puedan ayudar a la reducción del tiempo necesario para la bioconversión, a un menor uso de la enzima celulasa y lo a rendimientos más altos de etanol. Los esfuerzos de investigación sobre la celulasa están concentrados sobre el desarrollo de una mezcla sinérgica de enzimas de costo económico y altamente termoestable y que pueda satisfacer las necesidades del usuario final. También se está estudiando la fermentación fuerte de microorganismos que convierten los azúcares de biomasa a etanol y otros bioproductos. La investigación integrada y el enfoque sobre el desarrollo han dando resultados y las plantas comerciales para la producción de etanol de biomasa están por convertirse en realidad.

Introduction

The benefits of developing biomass-to-ethanol technology have been previously stated: increased national energy security, reduction in greenhouse gas emissions, use of renewable resources, foundation of a carbohydrate-based chemical process industry, macro-economic benefits for rural communities and society at large (1). Despite realizing these benefits at bench scale, commercialization and widespread application of lignocellulosic biomass utilization has not yet occurred. Decades of research have demonstrated that biomass requires extensive processing--hydrolysis of the raw material into fermentable sugars, and its subsequent biological conversion into a myriad of fuels and chemicals. Feedstock availability, its location and transport to the site of treatment, pretreatment strategies, efficient hydrolytic agents, availability of robust fermentative microorganisms and process options all impact the production cost of ethanol. Recent technology developments have the potential to remove these economic performance obstacles and make commercialization possible. Many government/university/industry partnerships have been formed to develop compatible processes and enable

an integrated approach towards biomass conversion to achieve the required cost reduction. For biomass-to-ethanol conversion to become a reality, biomass processors must prove their technology. This article will provide a perspective on biomass processing by highlighting the key elements required for commercializing lignocellulosic biomass conversion, with particular emphasis on some technological advances in cellulase development.

Biomass offers an abundant and inexpensive source of renewable resources. For example, sugarcane residue, called bagasse, is generated during the milling of sugarcane and is plentiful in tropical and subtropical regions such as Brazil, India, Thailand, Hawaii and the southern U.S. Theoretically, one dry ton of bagasse can generate 112 gallons of ethanol. Alternative lignocellulosic feedstocks include agricultural residues such as corn stover, wheat and rice straw and forestry residue; industrial residue such as pulp and paper processing waste; and energy crops such as switchgrass. But, unlike starch, which contains homogenous and easily hydrolyzed polymers, lignocellulose plant matter contains cellulose (23-53%), hemicellulose (20-35%), polyphenolic lignin (10-25%) and other extractable components.

Commercialization of biomass conversion

Although feedstock is available in large quantities, the main challenge for commercialization is to reduce the major operating costs of biomass conversion processes, primarily pretreatment and enzymes.

Efficient and cost-effective pretreatment technology

Most pretreatment approaches do not hydrolyze significant amounts of the cellulose fraction of biomass. Pretreatment enables more efficient enzymatic hydrolysis of the cellulose by removal of the surrounding hemicellulose and/or lignin along with modification of the cellulose microfibril structure. Current pretreatment research and development activities are geared towards identifying, evaluating, developing, and demonstrating promising approaches that primarily support the subsequent enzymatic hydrolysis of the treated biomass. Universal pretreatment process is difficult to envision owing to the diverse nature of biomass. Thus, several physical, chemical and biological treatments are under evaluation. The resulting composition of the treated material is dependent on the source of the biomass and the type of treatment, but, in general, is much more amenable to enzymatic hydrolysis by cellulases and related enzymes than native biomass.

Physical treatments, including high temperature, freeze/thaw cycles and radiation, are aimed at size reduction and mechanical decrystallization. Most of these methods are limited in their effectiveness and often expensive. Biological pretreatments, where natural organisms are allowed to grow on the biomass, result in cellulose and lignin degradation but are not very effective and require long treatment times. Thus, chemically based approaches have gained significant attention, and different chemical methods have been explored. Those using dilute acid (primarily utilizing sulfuric acid) and steam or pressurized hot water result in hydrolysis of a significant amount of the hemicellulose fraction of biomass. Thus, high yields of soluble sugars from the hemicellulose fraction can be achieved. The hot-wash process, a variation of the dilute acid pretreatment, involves high-temperature separation and washing of the pretreated solids, which is thought to prevent re-precipitation of lignin and/or xylan that may have been solubilized under pretreatment conditions. Re-precipitation of lignin can negatively affect the subsequent enzymatic hydrolysis of the pretreated solids. On the other hand, alkaline-based methods are generally more effective at solubilizing a greater fraction of lignin while leaving behind much of the hemicellulose in an insoluble, polymeric form. Ammonia freeze explosion (AFEX) disrupts lignocellulose and reduces the cellulase requirement (2) but removes neither hemicellulose nor lignin.

A limitation of all pretreatment processes is their capital-intensive nature. For example, requirement of costly reactor materials and additional process steps for waste treatment and recovery of pretreatment catalysts presents additional costs for the hydrolysis process. Some pretreatments, like AFEX, offer potential advantages in operating costs such as low waste generation. Thus, criteria for successful pretreatment can be narrowed to high cellulose digestibility, high hemicellulose sugar recovery, low capital and energy cost, low lignin degradation, and recoverable process chemicals.

Advanced enzymes for efficient biomass hydrolysis

The greatest potential for ethanol production from biomass lies in enzymatic hydrolysis of cellulose using cellulase enzymes. Even after decades of research on cellulases, the costs of these enzymes have remained high. To make biomass processing competitive would require substantial reductions in the current cost of producing cellulase enzymes. Cellulases are relatively slow-acting enzymes, primarily because of the complex, insoluble, and semi-crystalline nature of their substrate. In addition, maximal cellulase activity requires multiple, related enzymes such as endoglucanases, exoglucanases and beta-glucosidases to act synergistically for complete conversion of cellulose into glucose.

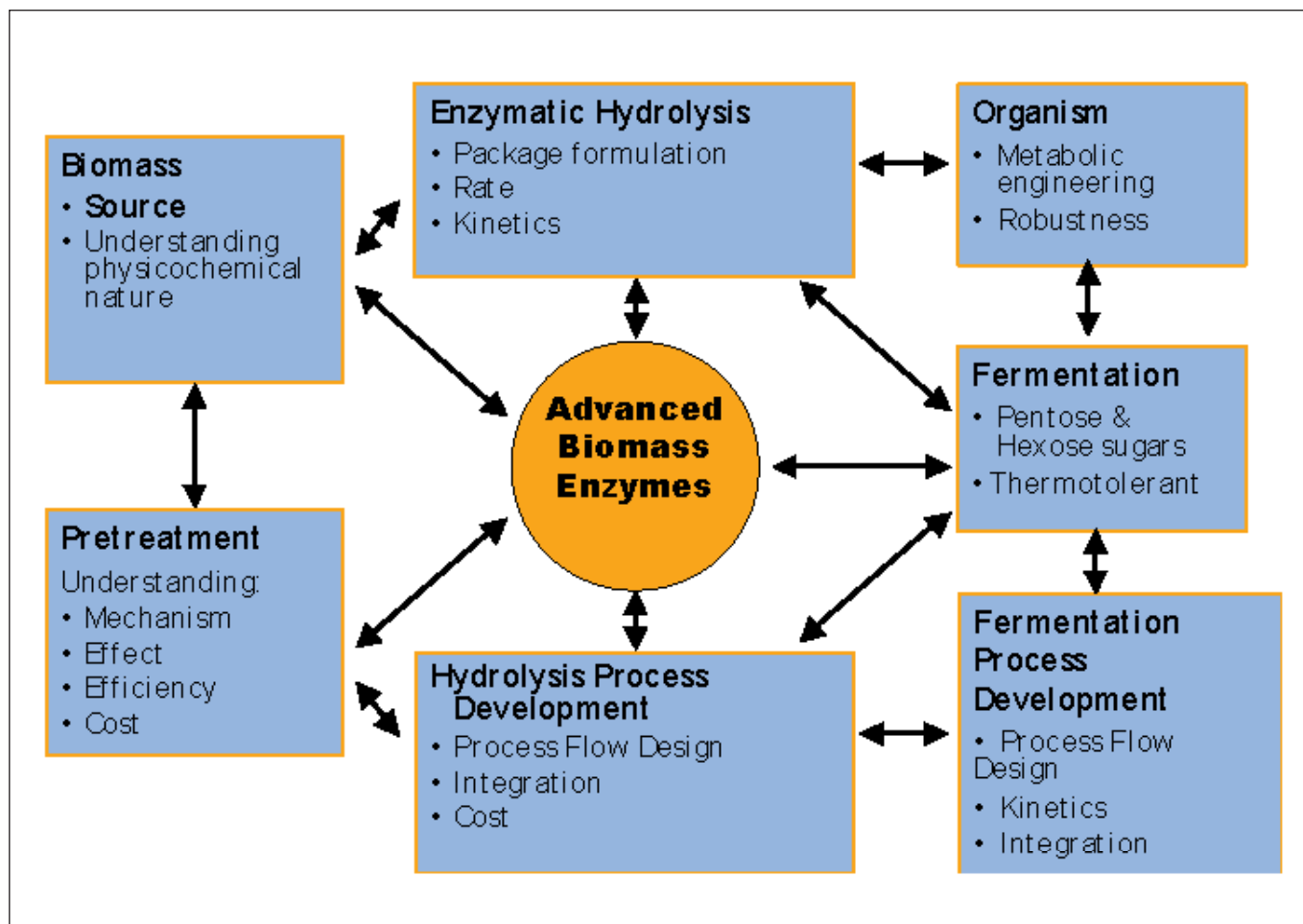
The engineering of cellulase enzymes for biomass processing thus faces numerous challenges in light of the different parameters that need to be considered (Figure 1). Not only do advances in enzyme development have to achieve classical enzyme improvements such as stability, yield, and increased specific activity but they also need to be effective in harsh environments generated by the pre-treatment process. The physico-chemical nature of the feedstock itself impacts which bonds, and how many, need to be

enzymatically hydrolyzed. Secondly, different pretreatment processes pre-hydrolyze biomass in a unique way, resulting in matter with different composition. Thus, enzymes that break down the components have to be tailored to the unique composition of the pretreatment process. In addition to the extent of crystallinity and degree of polymerization of the cellulose, deacetylation and hydrolysis of hemicellulose influence enzyme requirements. For example, higher accessibility of beta-1,4-glycosidic bonds on the substrate surface improves the rate of attack by endo- and exoglucanases, while a lower degree of polymerization favors a higher ratio of exo- to endoglucanases. Higher lignin content blocks enzyme accessibility, adsorbs enzyme non-productively, causes end-product inhibition and reduces rates and yields of cellulose conversion. In addition to lignin, cellobiose and glucose also act as strong inhibitors of cellulases. Moreover, fermentation parameters such as pH, temperature, cellobiose and pentose sugar utilization, all influence the optimal enzyme system for each feedstock-process combination.

In 2000, Genencor International was awarded a \$17 million grant from the U.S. Department of Energy, administered by the National Renewable Energy Laboratory (NREL), to develop low-cost cellulases and other enzymes for the production of ethanol and other products from biomass. The three-year goal was to achieve a ten-fold reduction in the cost of enzymes in NREL's model assay. This aggressive but achievable goal was approached from two directions by making significant improvements in: (i) cellulase production economics (reduced \$/gram enzyme) and (ii) cellulase enzyme performance (reduced grams of enzyme needed). At the end of the three-year period, the 10-fold goal was not only met, but exceeded (3), resulting in the development of a cellulase enzyme system (tailored to dilute acid pretreated corn stover, in NREL's model process), a production organism, and a process to produce the enhanced cellulase system.

The technological approach involved studying known enzymes by expressing and purifying a variety of cellulases from different species; finding new activities, identifying novel proteins through genomics, transcript profiling and proteomics; making improved

Figure 1. Enzyme engineering challenges



enzymes by protein engineering and directed evolution to improve cellulase performance under preferred process conditions; and optimizing the mix for maximum biomass degradation. These efforts led to the discovery of several novel glycosyl hydrolases. Moreover, many genes encoding previously unknown *Trichoderma reesei* proteins were found, for example: glucanases, chitinases, proteases, etc. These studies also resulted in determining the response of known and novel glycosyl hydrolases to induction by sophorose, the most potent inducer of *T. reesei* cellulases, and to the discovery of many unknown genes that are co-regulated with glycosyl hydrolases (4). These findings and further characterization of the genes will likely have significant implications for the design of industrial processes for commercial production of biomass-degrading enzymes.

An optimized organism and process to ferment mixed sugars

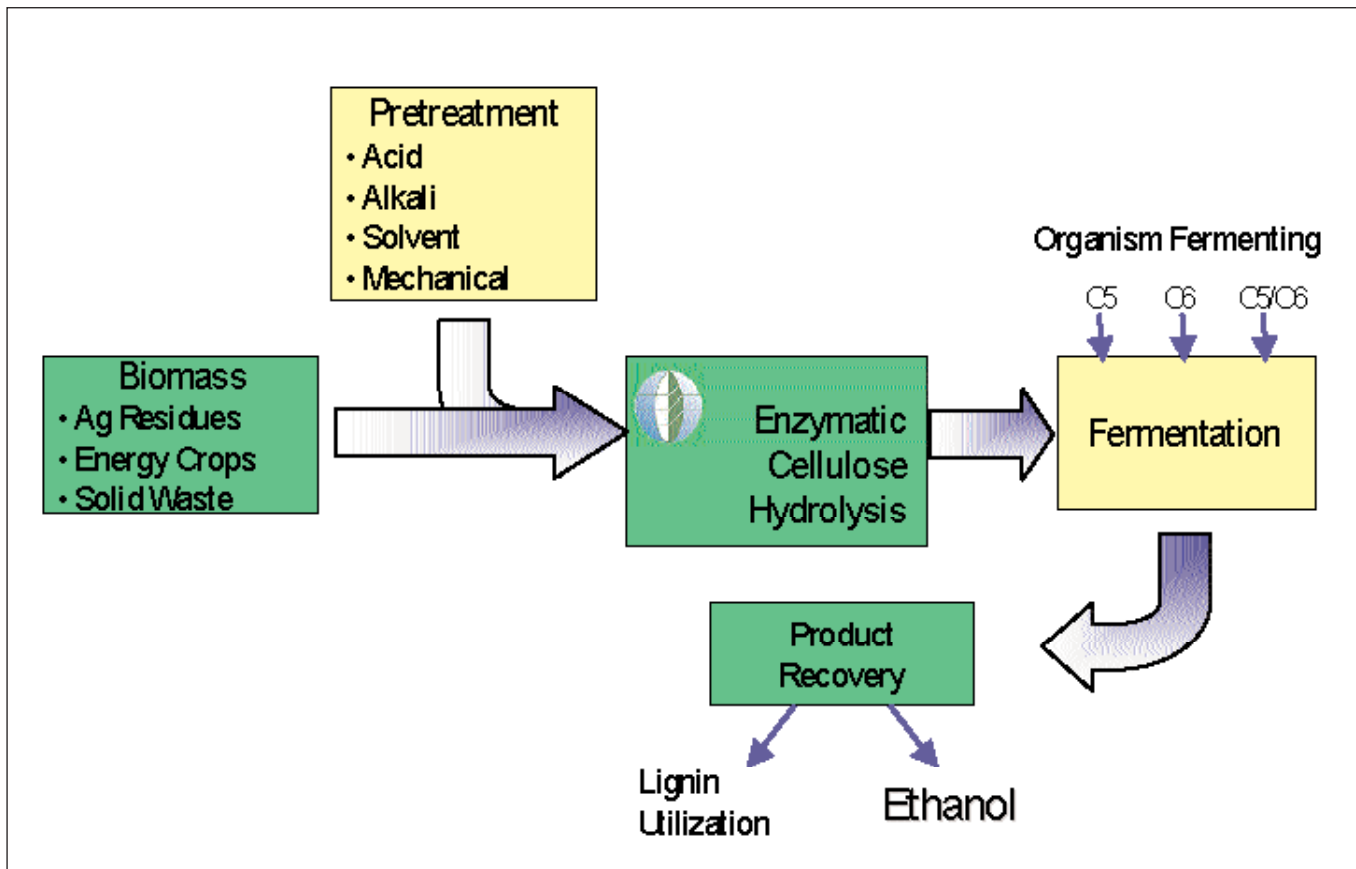
Following enzymatic hydrolysis of cellulose and hemicellulose to sugars, the next challenge lies in fermentation of the “unusual” sugars to ethanol and other chemicals. The glucose produced from cellulose hydrolysis can be easily fermented with existing organisms as is currently done. However, hydrolysis of hemicellulose from biomass produces both hexose (C6) and pentose (C5) sugars: mannose, galactose, xylose and arabinose. As with cellulase development, fermentation microorganisms

such as *Saccharomyces*, *E.coli*, and *Zymomonas* that can utilize C5 substrates have been engineered through genetic modifications and improved. But ethanol yields are still not sufficient to make the process economically attractive. Several degradation products, such as furfural, hydroxymethylfurfural (HMF), phenols, and formic, acetic and other acids produced during the pretreatment and hydrolysis, can inhibit the fermentation process and affect ethanol yields, and thus have to be removed or mitigated. The lignin-rich stream produced during hydrolysis can be incinerated and converted into electricity or steam. Researchers have also developed microbial technology that is capable of simultaneous hydrolysis and fermentation of amorphous cellulose. Such advanced ethanol producer strains are capable of secreting endoglucanases along with utilizing dimers and trimers of glucose and xylose and metabolizing C5 sugars (5).

Integration of process steps for process design and scale up

Successful integration of all the unit operations of biomass conversion is the key element to enabling commercialization (Figure 2). With extensive knowledge of the individual unit operations, various researchers are focusing on linking unit operations together for industrial application and demonstrating integrated processes at the mini-pilot and pilot scales.

Figure 2. Process integration for biomass commercialization



Biomass processing timeline prediction

Today, several biomass processing pilot facilities are being designed, in late-stage development, or operating. Within the next two years, construction of the first modern commercial-scale biomass processing facilities will likely begin; side stream biomass processing will also be incorporated into existing grain ethanol plants in this timeframe. It is anticipated that within 5 to 8 years, cost-effective biomass conversion technologies will lead to significant market expansion for ethanol and other fermentation products.

Conclusion

The importance of continuing cellulosic biomass process development investment cannot be understated. Although significant progress has been made, commercialization of lignocellulosic conversion to ethanol has been difficult, not only due to the heterogeneous nature of biomass itself, but also due to multiple treatments required for effective processing. It is quite apparent that development of advanced enzyme technologies is critical, as today’s commercial cellulases are inadequate for cost-effective biomass processing. Successes from pilot projects have clearly demonstrated that understanding interaction between cellulase action and pretreatment can facilitate and accelerate progress in this area. Such integrated approaches are enabling superior multi-component cellulase systems to be developed. Further improvements can be achieved by integrating these processes with fermentation. As teams of major industrial partners and technology leaders work

together, commercialization of enzyme-catalyzed biomass processing will soon lead to new biorefineries.

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