

Potential and prospects of cellulosic ethanol in the world

Clifford Louime and Hannah Uckelmann

There is a general understanding that the potential and prospects of bioenergy in the world depend heavily on advances in cellulosic research. It is however expected that any significant progress on that front will be driven by major technological innovations. Advances in feedstock development and conversion processes will indeed propel the bioenergy industry into a well-secured and competitive future. A diversified feedstock source will provide sustainability well beyond our current fossil-fuel market. Based on the industry estimates, the present energy sources dominated by mineral fuels though limited, are predicted to last well into the next decades. Therefore, turning low-value cellulosic materials into high energy products will maintain the viability of the bienergy industry, as these materials are renewable and abundantly available.

As crude-oil prices continue to rise and researchers keep sounding alarms on global warming, the search for viable alternatives to fossil fuels will intensify. Lately, first-generation biofuels or grain ethanol, widely accepted as a first step toward energy independence, have been the subject of severe criticism by 'food vs fuel' debaters¹. Not only will the use of corn as substrates for fermentation generate higher prices for this commodity but the share amount of it required for biofuel generation will lead to major expansion and fertilizer application. The latter has been associated with elevated temperature (climate change) and groundwater contamination. An alternative and potentially less climate-threatening approach is the use of cellulosic waste as potential biomass substrate for fuel and chemical development.

Cellulosic ethanol is known to be the most abundantly produced biomaterial on earth. In addition, it is constantly being replenished by photosynthetic respiration, which makes it sustainable. Using cellulosic waste as substrate in the fermentation process will not only double ethanol production yield, but will also have distinct environmental advantages, such as a smaller carbon footprint compared to grain ethanol or first-generation biofuels. On a life-cycle basis, ethanol produced from agricultural residues has significantly lower greenhouse gas (GHG) emissions and higher sustainability rating than ethanol produced from grain². The amount of energy-intensive fertilizers and fungicides will remain the same, for a higher output of usable material. Cellulosic ethanol can also be produced in most regions of the world; therefore, this renewable fuel has been widely advertised as a significant resource capable of improving national energy

security in countries without fossil-fuel reserves, mitigating GHG and promoting rural economic development.

Cellulosic ethanol, also known as second-generation biofuel, promises to circumvent some of the limitations of first-generation biofuel, such as threat to food supply and biodiversity. However, certain important issues regarding cellulosic ethanol need to be addressed. Among them is the price competitiveness to existing fossil fuels. It is well understood that cellulosic ethanol offers greater environmental benefits and can be sustainable, but the question remains whether it is affordable.

Second-generation biofuels rely heavily on major technological innovations centered on feedstock and conversions processes. While first-generation biofuels are produced by fermenting plant-derived sugars such as sugarcane, corn and sugar beet to ethanol, cellulosic ethanol production uses either a biological or thermochemical approach. The former, also known as cellulolytic method, is hydrolysis followed by fermentation of the generated free sugars. The latter is gasification, which produces synthesis gas that can be converted to ethanol by fermentation or thermochemical catalysis. Both methods are followed by a distillation process to separate pure ethanol.

In the biological process, the cellulosic material is usually physically or chemically pretreated to accelerate hydrolysis, where the cellulose molecules are broken down into their monomer components. This cellulolysis step, which can be either enzymatic or chemical (acid), is followed by a separation process where lignin, for example, is removed from the sugar solution. Lignin is known to be extremely resistant to enzymatic degradation. The separation phase allows microbial

fermentation of the sugar solution into ethanol, which then can be distilled to produce a solution of 99.5% pure alcohol.

During the thermochemical conversion process, the cellulosic material is broken down to release carbon monoxide, carbon dioxide and hydrogen, which undergo a fermentation process. The fermentative organisms convert the carbon and hydrogen molecules into ethanol, which then has to be separated from water through a distillation process. Alternatively, the synthesis gas can be fed to a catalytic reactor where ethanol and other higher alcohols are produced through a thermochemical process. This process can also generate other types of liquid fuels.

Hydrolysis is well recognized as a key bottleneck in the processing of lignocellulosic materials. Most microorganisms known to degrade cellulosic wastes release a battery of enzymes with different specificities. The enzyme system that converts cellulose to glucose consists of at least three enzymes working synergistically: exoglucanase, endoglucanase and β -glucosidase. Exoglucanases, also known as cellobiohydrolase, are exo-acting enzymes which release cellobiose from the reducing and non-reducing ends of the cellulose fiber. Endoglucanases cleave internal β -1,4-glycosidic bonds, while β -glucosidase hydrolyses cellobiose to glucose³. The activities of the exo- and endoglucanases are often inhibited by the products of β -glucosidases, making β -glucosidases the rate-limiting factor in cellulose hydrolysis. Generally, β -glucosidases are considered as being responsible for the regulation of the whole cellulolytic process, as they release not only glucose from cellobiose, but also reduce cellobiose inhibition, allowing exo- and endo-acting enzymes to function more efficiently.

In the past few years, the research focus has been on metabolic engineering of microorganisms used in fuel-ethanol production. By focusing on the ethanol-producing pathway, some microorganisms have been genetically engineered to optimize ethanol production⁴. Besides *Saccharomyces cerevisiae*, other microorganisms have been targeted as well, such as *Thermomonospora fusca*, *Trichoderma reesei* and *Cellulomonas fimi*. Cellulase researchers all over the globe have worked on the aerobic fungus, *Trichoderma reesei* for more than 50 years. Through a combination of enzyme engineering and fermentation process development, scientists were able to achieve more than ten-fold cost reduction, making *T. reesei* the most commonly used cellulase in laboratory- and pilot-scale biofuels production today.

Nowadays, the isolation and characterization of novel cellulase enzymes from both bacterial and fungal sources continue to be extensively investigated⁵ leading to substantial reduction in the cost of cellulase production. This decline in cost is mainly due to improvements in the performance of cellulase enzymes. For example, improving the efficiency of the cellulase enzymes, the amount of enzyme needed to complete cellulose hydrolysis decreases drastically. To achieve this goal, several approaches have been used, such as site-directed mutagenesis and DNA shuffling. Several of the constructed variants also responded with improved characteristics, in terms of their thermostability and reversibility. Another common approach leading to cost reduction is the development of fusion protein,

where a heterologous enzyme is introduced into an existing system so that the overall performance of the system can be enhanced. Efficiency increase as high as 20% has been reported.

A breakthrough in the investigation of cellulose digestion processes will indeed have an enormous impact on the world food supply and economy. In this regard, an understanding of the molecular mechanisms underlying cellulose degradation in combination with new and superior enzymes may facilitate increased usage of this valuable renewable resource. This in turn will have tremendous social and economic impacts. As communities around the world strive to reduce their dependence on foreign oil and expand the development of alternative fuels, biofuel plants are springing up all over the place, mostly in rural communities. Such plants often are sold to the general public as economic drivers, although the real benefits have yet to be assessed. One of the major challenges remains the integration of technological solutions into a bioenergy platform that is in line with societal expectations and fiscal responsibilities.

Concluding remarks

The potential and prospects of cellulosic ethanol in the world rests on the drastic reduction in costs for both of the main unit operations, namely feedstock development and the conversion processes. This can only be achieved by significant technological innovations. Although substantial improvements in both aspects

have been achieved over the past several years, additional progress is needed in order to keep pace with the current fossil fuel market. The rising price of fuels in the past few years and the global climate changes should facilitate capital flow into the bioenergy sector. However, unless prices are highly competitive, the industry may not survive. Today, cellulosic ethanol still lies around US\$ 4 per gallon, based on the best estimates. This price, still over the present gasoline market price, needs to be cut by more than half for cellulosic ethanol to be economically sustainable. In order to overcome the technical and economical barriers, significant future improvements will have to lead to an enhancement of the specific activity of target cellulase enzymes along the ethanol-production pathways as well as to a clear and basic understanding of the molecular mechanisms underlying hydrolytic processes.

1. Rosillo-Calle, F. and Hall, D. O., *Biomass*, 1987, **12**, 97–128.
2. Williams, J., *Ethanol Producer Magazine*, April 2005.
3. Bhat, M. K. and Bhat, S., *Biotechnol. Adv.*, 1997, **15**, 583–620.
4. Aristidou, A. and Penttilä, M., *Curr. Opin. Biotechnol.*, 2000, **11**, 187–198.
5. Glick, B. R. and Pasternak, J. J., *Biotechnol. Adv.*, 1989, **7**, 361–386.

Clifford Louime and Hannah Uckelmann, College of Engineering Sciences, Technology and Agriculture, Florida A&M University, Tallahassee, FL 32317, USA.
e-mail: clifford.louime@famu.edu