Cellulosic ethanol as transportation fuel: hype no longer

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A recent editorial in *Science* issued a call for a mission-oriented project, similar to the Manhattan project, for processing lignocellulose (plant biomass) – nature's only renewable material – into fuel ethanol[1]. With amazing speed, an 'Energy Biosciences Institute (EBI)' has been set up, headquartered at University of California-Berkeley, with Chris Sommerville as its first director. EBI has a multimillion dollar partnership with BP Technology Ventures. With investments in manpower, innovation and technology deployment, EBI is charged to administer the transition from corn starch-based fuel (ethanol) to lignocellulose-based ethanol production in 10 years.

Setting the pace, a private biotechnology firm (Iogen) based in Ottawa, Canada, announced the first shipment of 100,000 l (26,417 gallons) of cellulosic ethanol processed from wheat straw to the Shell Company (http://www.iogen.ca/news_events/press_releases/2008_10_25.html). Though the protocol of the process is not made public, Figure 1 serves to convince us that marketing of gasoline blended with cellulosic ethanol has begun. Cellulosic ethanol (ceetol) is obtained from cellulose; it is chemically identical to ethanol (C_2H_5OH) obtained from sugarcane molasses or starch in corn[2]. According to an online brief, a Louisiana-based company (Verenium) has also begun producing cellulosic ethanol from sugarcane bagasse, a fibrous residue left after the crushing of canes[3]. The process begins when the cane is ground and cooked under high pressure with a mild acid to hydrolyse the hemicellulose and separate it from the cellulose. The five-carbon sugars in hemicellulose are then fermented using genetically modified *Escherichia coli*. The cellulose is broken down with enzymes and fermented with another type of bacteria called *Klebsiella oxytoca*. This bacterium also produces enzymes that break down cellulose, reducing the amount of enzymes from outside sources by 50%. The dilute ethanol produced from fermentation of both types of sugar is then distilled to make fuel. Verenium's process is claimed to ferment sugars from both cellulose and hemicellulose. Some 20 companies, almost all in USA, have pilot plants for the manufacture of ethanol from lignocellulose feedstocks[4].

Has the cellulose to ethanol conversion technology really come to the fore? I enquired with Sommerville[1], the need for a Manhattan-type project if a process for the production of cellulosic ethanol is under operation in USA. He responded to my e-mailed enquiry as follows: 'I think that to have a significant effect on the energy sector we need to get past the tinkering stage with biofuels and figure out what is sustainable and can be done on a large scale with optimized technology. I think we could easily see 50 cellulosic facilities each making 20 million gallons per year with "good enough" technology but not able to really scale up or replicate to a large scale. What we need is 600 plants working at 100 million gallon scale. Making that kind of capital investment will require compelling evidence that the technology has been optimized and the long-term sustainability issues are completely understood. Thus, I think we cannot rely on underfunded entrepreneurs but need a concerted R&D effort that will kill the problems. That is more or less what we are trying to do at the EBI with the $500M of BP funding.'

Research results published in peer-reviewed journals give the impression that cellulosic conversion technologies are at least a decade away from supplying a significant proportion of the world's liquid fuels (see Maheshwari[5] for a review). Repeatedly, these authors draw attention to three main bottlenecks in large-scale manufacture of cellulosic ethanol: (1) Lack of knowledge of the three-dimensional structure of the cell wall (composition and linkages between hemicelluloses, lignin, and cellulose microfibrils) from any plant source (raw material) – a reminder that the cell-wall composition varies from plant to plant, in different parts of the same plant, and from season to season. It follows therefore that the chemical composition of biomass feedstock can be quite variable, and preclude the formulation of enzyme mixtures for optimum biomass conversion efficiency and profitability. (2) Presence of hemicelluloses and lignin in the biomass which enwrap cellulose, thereby restricting the physical access of hydrolytic cellulase enzymes to cellulose for its depolymerization to occur. (3) Crystalline structure of cellulose – this prevents the entry of water, and therefore of high molecular weight enzyme proteins for catalysing cellulose depolymerization. However, the claims of process development for industrial conversion of biomass into alcohol cannot be farcical either, for in nature cellulose is constantly recycled, though slowly. The most important example of rapid biomass conversion occurs in herbivorous animals which digest the injested fodder and break it down into simple molecules that enter the metabolic pathways for conversion into milk and meat. These animals employ a consortium of symbiotic bacteria in their rumen, which are endowed with multienzyme complexes on their walls to breakdown the injested fodder. It therefore seems likely that a consortium of microorganisms, each with specialized biochemical ability, may be required to separate the cellulose microfibrils from hemicelluloses and lignin, and reduce the crystallinity of exposed cellulose before it can be enzymatically hydrolysed to yield glucose, for its conversion to ethanol (Figure 1). I wonder whether the success of Iogen and Verenium is based on a 'Superbug' that was genetically-engineered in the research laboratory, or if the success is due to artificial synthesis[6] of a 'Microbial Consortium' that produces a mixture of ligninase and heteropolysaccharide degrading enzymes? This would indeed be a breakthrough.

Another breakthrough is the possibility of producing hydrocarbons from plant matter by a chemical conversion method. A team of chemical engineers at the University of Wisconsin-Madison, report successful conversion of sugars in plant matter into hydrocarbons using a Pt–Re catalyst[7]. This process is based on the integration of several flow reactors, where the effluent from the one reactor is simply fed to the next reactor. A biofuel technology based on the chemical conversion process would reduce the requirement of expensive microbial enzymes for releasing fermentable sugars from the biomass and would thus cut costs. Could it be that processes developed by Iogen, Verenium and other companies are based

Table 1. Some key research objectives for sustained production of fuel ethanol from cellulosic biomass

Description	Selected references
Identify fast-growing, high biomass-producing plants capable of growth without irrigation	9, http://bioenergy.ornl.gov/papers/misc/switgrs.html
Determine molecular architecture of cell wall for designing enzymatic procedures for release of sugars from biomass	10
Engineer efficient Rubisco genes for increased biomass yield	11
Mutation breeding or genetic engineering of plants for reducing lignin sealing on cellulose microfibrils	12, 13
Genetically engineer plants for endogenous production of enzymes that remove the polysaccharide glue, freeing cellulose microfibrils in response to a trigger	14
Pretreatment of feedstock for lignin removal	15
Identify optimal mixtures from bacterial cellulosomes and fungal polysaccharide and lignin-degrading enzymes	16, 17
Engineer a yeast for direct fermentative conversion of cellulose into ethanol	18
Engineer yeast strains having key hydrolytic cellulase and lignin-peroxidase activities	19

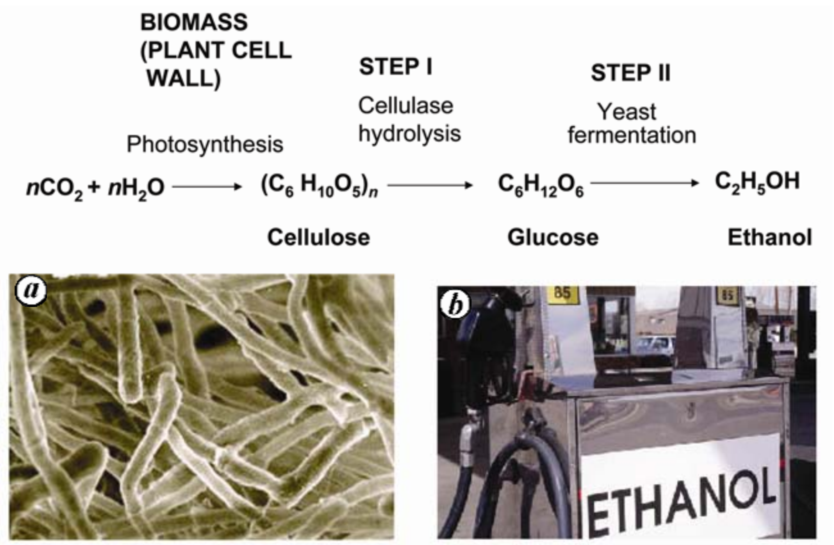

Figure 1. The basic scheme of manufacturing cellulosic ethanol from biomass using fungi. ***a***, *Trichoderma reesei* (mycelium) from Wikimedia Commons. ***b***, A cellulosic ethanol-filling pump in USA (http://www.broward.org/air/images/ethanol.bmp). Ethanol can be mixed with petrol at any blend. Blends are coded as E20 or E85, meaning that 20 or 85% of the fuel is ethanol by volume and the remaining is regular petrol derived from fossil fuel.

on a combination of both chemical and biological conversion of cellulose into sugars?

Behind the deceptively simple and logical scheme of making cellulosic ethanol by a biological conversion method (Figure 1), there are hidden complexities (Table 1) requiring much research. Since biofuel production implies diversion of land from 'food to fuel', or from 'wood to wheel', I doubt if any country has spare land for cultivating plants exclusively for biofuel production. Therefore, the only option is to use biotechnological methods to grow two blades of grass where one grew before, and improve the existing or evolve new technologies[8]. A national mission-oriented research needs to be started immediately to identify and improve the potential fuel crops, their cultivation methods, and improve conversion technologies in order that technologies are in operation before the supply of fossil fuel runs out. Some estimate that at the current rate of use, this will occur in less than 25 years.

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