Biofuel redux

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Yet another increase in the price of petrol is on the cards, reminding us that hydrocarbon fossil fuel reserves have an expiry date. The need to develop replenishable biofuel that is made from starch or cellulose in biomass by capturing sunlight by photosynthesis is increasingly becoming acute. In a move to reduce the dependency on depleting oil reserves, successful test flights of airliners on a blend of aviation fuel and ethanol, derived from fermentation of sucrose in sugar-cane juice or starch in corn kernels, have been demonstrated though this is embroiled by the criticism that precious land for growing food crops is diverted for burning as fuel for cars or jet planes. Even in Brazil – a biofuel pioneer – 41 of the country's roughly 400 sugar-cane ethanol plants have closed[1]. In some countries a blend of petrol with 10% or 20% ethanol called the E10 and E20 fuel respectively, derived from fermentation of corn starch or sucrose is already being used for automobiles with modified flexible fuel engines. Research in USA has identified a fast-growing, non-edible tall grass species *Miscanthus giganteus* as an 'energy crop' – a source of cellulose for conversion into ethanol. It is a hardy, perennial woody grass with C4 photosynthesis and will grow for 20 years producing an annual harvest from year two onwards. In India, The International Crops Research Institute for the Semi-Arid Tropics in Hyderabad has developed sweet sorghum *Sorghum bicolor* (Figure 1) as an energy crop – which offers grain for food, sugar in stalks for distillery and leftover cellulosic cell wall for conversion into biofuel. To distinguish that the source of ethanol is plant cellulose, it is called cellulosic ethanol. Several companies in USA and Europe have set up biorefineries to produce transportation fuels from plant biomass. However, despite the apparently simple scheme of conversion of insoluble cellulose into ethanol, there is yet only a single report of cellulosic ethanol manufactured from wheat straw (http://www.iogen.ca/news_events/press_releases/2008_10_25.html). While the details of this process have not been made public, presumably it was based on the scheme given in Figure 2.

Figure 1. A potential biofuel grass, sweet sorghum (photo courtesy: ICRISAT-IN, Patancheru, India). Reprinted from *Current Science*, 2008, **95**, 599–602.

The status of biofuel for use as liquid transportation fuel has been previously reviewed[2,3]. In a move to reduce the dependency on oil reserves, the focus of research is on manufacture of ethanol from cellulose – the world's most abundant naturally occurring and replenishable raw material. On an average, the dried grass stems contain approximately 70% cellulose – a homopolysaccharide of glucose. To distinguish that the source of the derived ethanol is cellulose, it is called cellulosic ethanol.

According to *Biofuels International*, a bimonthly, most of the companies that were set up in USA and Europe for manufacturing cellulosic ethanol are closing down because of unsustained availability of raw material and the high cost of pretreatment of raw material for removing lignin – a highly branched phenylpropanoid polymer which covers cellulose in cell walls of all land plants. The presence of lignin in terrestrial plants gives the plant the rigidity to grow erect for trapping light for photosynthesis, as also making them resistant to infection by moulds which contain cellulolytic enzymes. Yet it is the lignin polymer deposited between the cellulose microfibrils and cross-linked to hemicelluloses which makes the plant cell walls (biomass) recalcitrant to break down into simple sugars for fermentation to yield biofuel ethanol. Researchers at the Weizmann Institute of Science, Israel[4], selectively removed lignin from the cell wall by chlorite oxidation. The cellulose in the delignified cell walls was rendered susceptible to fungal cellulase or bacterial cellulosome enzyme system showing that removal of lignin from biomass is the crucial step in its conversion into fermentable sugars and fuel ethanol.

Two cellulolytic systems of commercial relevance are: (i) multienzyme cellulosomes from the anaerobic bacterium *Clostridium thermocellum* and (ii) free enzyme from the fungus *Trichoderma reesei*. Using high-resolution microscopy, the Israeli researchers showed that the digestibility of corn stem cell walls of which cellulose comprises about 80%, is restricted to the hydrophobic cellulose face with lignins physically impending accessibility to cellulolytic enzymes. Thus a pretreatment of native biomass for its enzymatic conversion into fermentable sugars will be a crucial pretreatment step in manufacturing ethanol. In the pretreatment step, combination of chemicals (water, acid, caustics and/or ammonia) and heat to remove lignin and partially breakdown the cellulose or convert it into a more reactive form. However, this alters the orientation of glucose molecules in the cellulose microfibrils. This architectural change affects attachment of fungal or bacterial cellulosomes to the hydrophobic cellulose face in the cell wall and the saccharification of biomass by cellulolytic enzymes for further conversion into biofuel ethanol. To circumvent this problem, a possibility is the

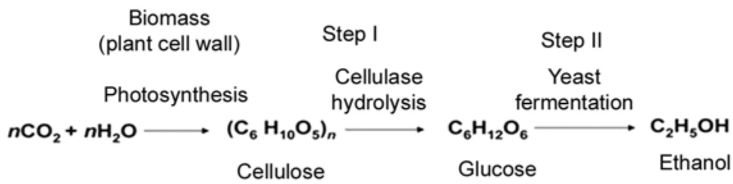

Figure 2. A scheme for conversion of biomass into liquid fuel ethanol using fungal cellulolytic enzyme and yeast fermentation.

Box 1. Glossary

The development of cellulosic ethanol has been slow, because it is harder to make alcohol from nonagricultural biomass than from corn starch or sugar cane. That is because the leaves and stalks of plants have a more complex molecular structure made of a mixture of the biopolymers cellulose, hemicelluloses and lignin – a material composed of phenylpropanoid units – that prevents microbes from digesting plants as they grow. To make the sugars available for fermentation, cellulosic ethanol manufacturers must first grind the plant matter and separate out the lignin with acids and other chemical additives. They then need to add cocktails of different enzymes to break apart the different sugar chains, with each feedstock needing a slightly different cocktail. Finally, many cellulosic ethanol producers have had to engineer specialized microbes to convert the broader mixture of sugars in cellulosic material into ethanol. All these extra steps add to the cost.

Advanced biofuel: Ethanol manufactured from cellulosic material.

Biomass: Any plant-derived organic matter. Biomass available for energy on a sustainable basis includes herbaceous and woody energy crops, agricultural food and feed crops, agricultural crop wastes and residues, wood wastes and residues, aquatic plants, and other waste materials, including some municipal wastes. Biomass is a heterogeneous and chemically complex renewable resource.

Biofuel: Fuel produced from stored as starch or cellulose in biomass.

C4 plant: A plant in which the first product of photosynthetic carbon fixation is a four-carbon molecule in contrast to the 3-carbon molecule in C_3 plants; and is more efficient under some conditions.

First generation biofuel: Biofuel from food sources such as corn starch and sugar-cane juice.

Second generation biofuel: Biofuel manufactured from non-food feedstock.

Cellulosic (bio) ethanol: Ethanol manufactured from holocellulose.

Lignocellulosic ethanol: Ethanol produced from lignocellulose following pretreatment step to remove lignin.

Fermentation: Anaerobic digestion of glucose to yield ethanol.

Cellulosome: Multienzyme complexes in anaerobic bacteria attached on cell wall surface.

Cellulolytic enzyme: Cellulase, β,1-4 glucanase.

Pretreatment: Processing done to the plant material before it is broken down into simple sugars (hydrolysis).

Trichoderma reesei (*Hypocrea jecorina*): One of the most powerful cellulolytic fungi.

Lignin: The major polyphenolic structural constituent of wood and other native plant materials that encrusts the cell walls and cements them together.

Wood: Solid lignocellulosic material made of up to 40–50% cellulose, 20–30% hemicelluloses and 20–30% lignin.

breeding of lignin-free plants (raw material). The authors[4] say, 'The challenge now is to effectively and economically modify lignins via strategies that maintain the integrity of fermentable sugars'. How practical will this approach be is a moot question, for it is the presence of lignin in plants that gives the cell wall the rigidity for the plants to grow erect and capture sunlight for photosynthesis and form carbon compounds. The authors[4] conclude, 'In the foreseeable future, we expect that lignins in genetically modified energy plants will be extracted under mild conditions and used as valuable chemicals; the remaining cell wall architecture could be left intact with minimum alteration of the polysaccharides'. In India, biofuel ethanol manufactured from corn starch or sugar-cane juice is unlikely to be available in the foreseeable future. Neither will be the cellulosic ethanol available until snags in the utilization of cellulose in plant cell walls are removed. Do we have any option but to keep coughing up the increasing cost of petrol? Now more than ever it is necessary to convey that the 'dead' plant cell wall covering demands high priority.

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2. Maheshwari, R., *Curr. Sci*., 2008, **95**, 594–602.
3. Maheshwari, R., *Curr. Sci*., 2009, **97**, 1710–1711.
4. Ding, S.-Y. *et al*., *Science*, 2012, **338**, 1055–1059.

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