The science behind the biofuel controversy

Ramesh Maheshwari

At the current rate of use the crude oil reserves of the world are predicted to deplete in about 40 years. Therefore, it has become necessary to find and devise methods of processing a renewable raw material for conversion into transportation fuel. Some countries are manufacturing ethanol from sugarcane or cereal grains and blending it with petrol to reduce crude oil imports. This entails diverting agriculturally productive land for the production of fuel. An alternative is to use inedible plant biomass as raw material. Although plant biomass is mostly cellulose, it is covered and occluded by hemicelluloses and lignin. This greatly limits the access of microbial cellulases required to depolymerize cellulose and release glucose for production of alcohol by yeast-mediated fermentation. A possible solution may be based on the reactions that take place in the rumen of herbivores. These animals have symbiotic bacteria which have multienzyme particles called cellulosomes attached onto their surface. The cellulosomes tear down the ingested plant material into soluble compounds from which the host animal makes milk and meat. Genetic engineering techniques offer the possibility of enhancing the biodegradative action of cellulosomes by reconstituting cellulosomes with potent enzymes from different microbial species. Fast-growing species of grasses specifically grown on marginal land could provide ethanol feed stock for biorefineries.

Keywords: Bacteria, biofuel, biomass, cellulose, cellulosome, fungi.

'THE only form of solar energy harvesting that can contribute substantially to transportation fuel needs at costs competitive with fossil fuel is that captured by photosynthesis and stored in biomass[1].

'The most vital need to realize the great benefits of woods-to-wheels concept is to commercialize the technology at a large scale in India and to put extensive research efforts to overcome the recalcitrance of biomass through natural selection of microorganisms and development of recombinant microorganisms or microbial consortia[2].

'Cellulosic biofuels are part of an emerging US energy policy, from which other regions can learn . . . From 2016, refiners must begin to switch to cellulosic ethanol and other advanced biofuels that do not rely on corn sugars, and these fuels will have to meet new standards for reducing greenhouse gas emissions compared with standard petrol[3].

'Turning food crops such as corn into fuel is a crime against humanity[4].

'Food riots to worsen without global action: Increased food demand from rapidly developing countries such as China and India, the use of crops for biofuels, global stocks at 25 yr lows and market speculation are all blamed for pushing prices of staples like wheat, maize and rice to record highs[5].'

The above excerpts are indicative of a storm brewing over the use of plant crops for the production of transportation fuel. The spiralling price of petrol coupled to increase in prices of essential commodities requires alternative fuel, either as a substitute or for blending with non-renewable fossil fuel to reduce oil imports. Since more than 30 years Brazil has been manufacturing ethanol from sugarcane (sucrose) by yeast-mediated fermentation, which is blended with petrol and sold as gasohol as automobile fuel[6,7]. Currently, USA uses about 20% of the corn for manufacturing biofuel using α-amylase and glucoamylase from microbial sources to convert grain starch into alcohol via glucose. The ethanol yield is one l from 2.69 kg of corn grain[8]. In the midwest United States, where corn is a major crop, the E85 fuel containing 85% ethanol and 15% gasoline is becoming increasingly common[9]. However diverting 17% of the US corn crop to biofuel, 'drove corn prices to more than $4 per bushel, which has rippled throughout the economy – animal feed costs have soared, farmers have slaughtered their hogs and chickens, and prices for soft drinks and other products using corn syrup have increased' (http://www.genengnews.com/articles/chitem.aspx?aid=2141&chid=0). The decision to divert arable land with high agriculture productivity for biofuels is being criticized. In India, blending of petrol with 5% ethanol obtained from sugarcane molasses was mandated in the states of Andhra Pradesh,

Ramesh Maheshwari (formerly at Dept of Biochemistry, Indian Institute of Science, Bangalore) lives at 53A/2, SPL Sriteertha Apartments, 4th Main, 17th Cross, Malleswaram, Bangalore 560 065, India.
e-mail: ramesh.maheshwari01@gmail.com

Goa, Gujarat, Haryana, Karnataka, Maharashtra, Punjab, Tamil Nadu and Uttar Pradesh[10], and blending is to be increased[11] to 10%. It has been argued that 70% more energy – in harvesting, fertilizing and transporting of the corn to the refineries, and then refining it – is required to produce alcohol than the energy it contains[12]. There are those who advocate the use of ethanol as biofuel because it burns more cleanly than gasoline, has a higher octane rating and produces smaller amounts of ozone precursors, thus reducing carbon emission[13].

Since the bulk of biomass is comprised of the plant cell wall, of which cellulose is the major constituent, it is the earth's most abundant natural polymer. 'The biggest well in biofuels', viz. ethanol derived from cellulose 'is yet to be tapped'[14]. A research stint at bioconversion of cellulose into glucose had turned me into a 'biofuel watcher'. Here I reflect and review the problems in the manufacture of alcohol from cellulose and their possible solutions. Alcohol made from cellulose has the same formula (C_2H_5OH), but is referred to as 'cellulosic ethanol' to distinguish it from ethanol from sugar in grape or sugarcane juice or from starch in cereal grains.

A fungus fuels excitement

In 1972, a combination of luck and determination secured me a faculty position in a major research institute in India. The circumstances defined the research problem. Because of the oil crisis following the 1970 Gulf War, the funding agencies were soft on projects on bioconversion of cellulosic 'wastes' such as rice and wheat straw, stover (leftover maize leaves and stalks after harvest), bagasse (leftover fibrous waste after extraction of juice from sugarcane), waste from paper mills, etc., into alcohol. Incentives came from workshops and symposia. One of the first workshops was organized by T. K. Ghose at the Biochemical Engineering Research Centre, IIT Delhi. The high point of this workshop was a talk followed by a practical session by Mary Mandels from the US Army Laboratory in Natick, Massachusetts. During World War II, Mandels and Elwyn T. Reese had isolated a mold, *Trichoderma viride* from rotting military clothing and tents from Solomon Islands in the South Pacific. This filamentous fungus was chiefly responsible for stimulating research on how an insoluble polymeric substrate is utilized by microorganisms and whether this entails the secretion of extracellular (cell-free) enzymes. If so, what are these enzymes and could they be used to convert cellulosic materials into glucose for industrial production of alcohol and single cell protein[15]. After irradiation by high-energy electrons and UV, a strain of *T. viride* was selected that secreted 30–40 g protein/l of culture medium (which included cellulose both as a carbon source and as an inducer, peptone as an organic nitrogen supplement and Tween, a surfactant. The secreted protein contains a consortium of enzymes: ~70% is comprised of a class of enzymes called cellobiohydrolase that successively splits cellobiose (β, 1-4 diglucoside) from the crystalline regions in cellulose; ~30% by endoglucanases that randomly hydrolyse internal linkages, and ~1% by β-glucosidases that hydrolyse cellobiose and soluble cellodextrins into glucose. These enzymes work together synergistically to produce glucose as the final product. It was envisioned that concentrated culture filtrates of *T. reesei* containing the aforementioned classes of enzymes could break down cellulosic materials into glucose required for the manufacture of ethanol by yeast fermentation. In 1999, the commercial value of *Trichoderma* cellulase enzymes for worldwide scientific and industrial research was estimated to be 125 million USD, corresponding to 10% of all industrial enzymes produced. The high cost of imported cellulase required for research was an incentive to find indigenous strains of cellulase-producing fungi.

The Natick scientists had envisioned a scheme (Figure 1) for the manufacture of cellulosic ethanol. Skeptics regard this as 'easier said than done'[16]. I have vivid memory of a demonstration by Mandels, where strips of Whatman™ filter-paper (made from cotton) disintegrated and substantially dissolved upon incubation in a solution of *Trichoderma* cellulase, with concomitant release of glucose as measured by colour test. The second part, which is conversion of glucose into ethanol, had already been established in the 19th century when Louis Pasteur discovered the role of yeast in alcoholic fermentation of sugar in grape juice. Therefore, the mooted idea of manufacture of cellulosic ethanol using microbial sources of cellulase enzymes is sound.

Just as the discovery in 1929 of the fungus, *Penicillium notatum* by Alexander Fleming had initiated research on antibiotics, similarly, discovery of the cellulolytic fungus *T. viride* spawned research on cellulosic ethanol. The Natick scientists developed improved strains and procedures for large-scale cultivation of *T. viride* in relatively inexpensive nutrient media in fermentors (large vessels with on-line control of temperature, pH, dissolved oxygen, stirring and programmed addition of fresh nutrients). Originally identified as *T. viride*, the high cellulase-producing strain was renamed *T. reesei*, in recognition of E. T. Reese's vision and contributions to enzymatic conversion of plant biomass into ethanol (Figure 2).

Excitement

Henry Ford had built his first car in 1896 to run on ethanol. The production of cellulosic ethanol would bode well

$$\text{Cellulose} + H_2O \xrightarrow[\text{Step 1}]{\textit{Trichoderma}\ \text{Cellulase}} \text{Glucose} \xrightarrow[\text{Step 2}]{\text{Yeast}\ \text{Anaerobic fermentation}} \text{Ethanol} + CO_2$$

Figure 1. The basic scheme of manufacturing cellulosic ethanol.

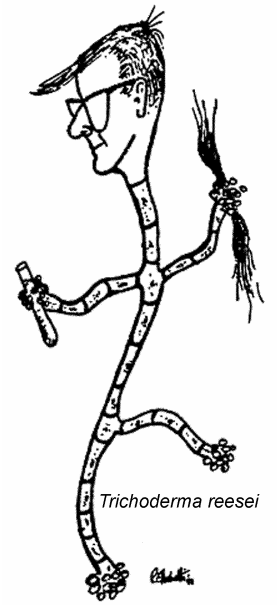

Figure 2. Elwyn T. Reese cartooned in the garb of the fungus, *Trichoderma reesei*[15].

for the world's dependency on Gulf oil. Future wars will be prevented. We, the new academics, jumped on the biofuel bandwagon. If the breeding of cereal crops ushered in the green revolution and earned a Nobel Peace Prize to the American plant breeder Norman Borlaugh for 'peace cannot be built on empty stomachs', would there not be one for the next big thing, i.e. the production of transportation fuel from cellulosic wastes? For, can there be peace if the fossil-fuel exporting countries are at war? Although the Natick programme was closed down following the retirement of Reese and Mandels, nonetheless the imagination of several scientists worldwide was fired-up. Ideas were generated about farming of non-edible, genetically-engineered, low lignin plants for cellulose; their harvesting and transport to cellulose refineries for physical, enzymatic or chemical pretreatment to expose cellulose; large-scale production of cellulase and other cell-wall degrading enzymes from microbial sources; enzymatic hydrolysis of cellulose in bioreactors; fermentation of glucose syrup by genetically engineered, ethanol-tolerant strains of yeast; recovery of ethanol from aqueous medium by distillation and finally, its distribution to petrol bunks!

Amazing cell factories

Half a century of search in terrestrial and aqueous habitats has led to the discovery of two other high cellulase producers. I was struck by the 'Indianish' name of the species and the authority of a fungus, *Chrysosporium lucknowense* Garg[17] (http://opsc.mediwire.com/main/Default.aspx?P=Content&ArticleID=321958). This fungus is said to have been isolated from a Siberian lake in Russia and deposited in the culture collection of the Russian Academy of Sciences. From the original strain, a mutant was derived that produces an incredible amount of protein, i.e. 50–80 g/l with 200–400 times more cellulase enzyme than the wild type. This amount of secreted protein by the mutant strain is an order of magnitude greater than that secreted by most wild species of fungi. Nearly 65–70% of the secreted protein is cellobiohydrolase – a crucial component of cellulase. The enzyme could convert nearly 60% of cotton into glucose at pH 5.0, 40°C in 140 h, if hydrolysis was carried out in the presence of a purified β-glucosidase from *Aspergillus japonicus* to overcome product inhibition due to cellobiose formed in the reaction mixture. The broad range of temperature and pH, low culture viscosity, and short cycle time offer numerous options to develop processes to produce proteins economically, that might otherwise be unstable or produced at lower yields in other production systems.

Strains of yet another newly studied fungus, *Penicillium verruculosum* secrete up to 47 g protein/l[18]. This fungus has been outsourced for strain improvement to Dyadic International, Inc, Florida, USA, and Moscow State University, Russia. Interestingly, the maximal yields of *T. reesei*, *C. lucknowense* and *P. funiculosum* are nearly similar, suggesting that these strains are amazing cell factories. In the absence of any published electron micrographs, we may imagine that their microscopic filamentous hyphae contain an abundance of endoplasmic reticulum, ribosomes and Golgi, which bud off cellulase-filled microscopic vesicles at amazing speed, with the vesicles fusing with the plasma membrane to secrete cellulase extracellularly. Moreover, the cell wall at the site of secretion must be rendered porous for the exit of cellulase proteins having molecular mass of 50–70 kDa[19]. The genome of *T. reesei* has been sequenced (C. P. Kubicek, pers. commun.). Surprisingly, there is no evidence of multiple copies of cellulase genes. Even more surprising is that it has only a limited set of genes for key enzymes known to be involved in degradation of plant cell-wall biopolymers.

Snags

The biodegradative potential of a fungus using complex substrates (for example, bagasse or straw) is rarely studied, even though these would be the feedstock in industrial-scale processes. From the available information, it appears that even if the cellulosic substrate is 'clean', i.e. it is not mixed with hemicellulose and lignin as in biomass, its conversion rate and extent of final conversion into glucose are low. For example, using *T. viride* enzyme, the Natick scientists obtained only 10% sugar syrup in 24 h at room temperature from Solka Floc™

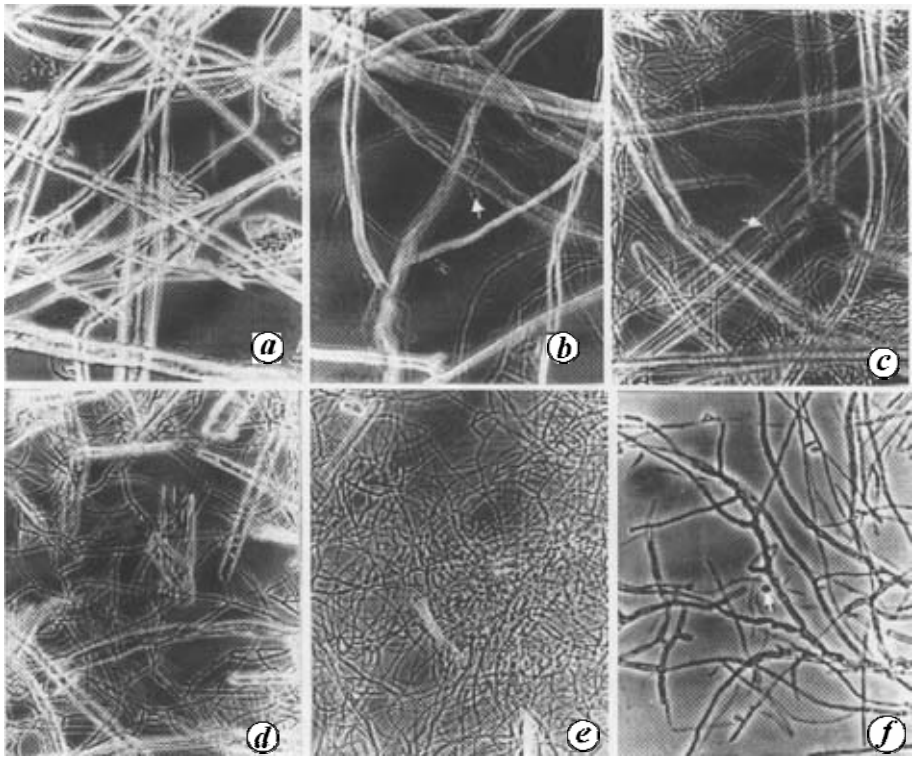

Figure 3. Photomicrographs showing utilization of cellulose by *Sporotrichum thermophile* in shake-flask cultures. ***a***, Initial appearance of cellulose fibres. ***b***, A 16 h culture showing germination of conidia. ***c***, A 30 h culture showing extensive fragmentation of cellulose fibres. ***d***, Magnified view of cellulose fibre from 30 h samples. ***e***, A 48 h culture showing extensive fungal growth and nearly complete utilization of cellulose fibres. ***f***, A 72 h culture showing total utilization of cellulose fibres[48].

(a wood cellulose)[20]. Ball-milled cotton cellulose yielded[21] only 5% sugar solution in 24 h upon treatment with cellulase at 50°C. This is rather small conversion of cellulose despite the high temperature used to increase the reaction rate and avoid contamination of the sugary solution! Cellulase from *C. lucknowense* has not performed any better either. It converted 6% cellulose into glucose in 70 h at the optimal temperature[17] of 40°C. Hidden inconvenient truth?

Unidentified factors

However, vast quantity of cellulosic material is constantly recycled in nature. Is there any clue that has been ignored or overlooked? One of the most powerful cellulolytic fungi known is *Sporotrichum thermophile*[22]. We compared *S. thermophile* and *T. reesei*. Surprisingly, under the optimal conditions of growth, *S. thermophile* completely and rapidly degraded cellulosic material (blotting paper; Figure 3) three times faster than *T. reesei*, although its endo- and exoglucanase activities are only one-tenth that of *T. reesei*. Surprisingly, the culture broth of *S. thermophile*, after it had completely utilized cellulose in the culture medium, did not duplicate the chemical feat of the growing fungus. When we tried to publish our findings, however, the paper was initially rejected – apparently because the observation challenged the notion that microbial dissolution of insoluble cellulose can only be through extracellular enzymes secreted in the environment, for how can an insoluble polymer enter the cell for metabolism? This implies that the growing fungus produces accessory factors to assist in cellulose degradation. Some had suggested that some fungi do Fenton chemistry – they produce oxidative enzymes (peroxidases) that generate highly reactive free radicals which, in the presence of iron, can depolymerize the biomass[23]. Not surprisingly, despite the basic steps behind the manufacture of cellulosic ethanol being straightforward, 'the world cannot yet boast a single commercial-scale cellulosic ethanol facility'[24]. The likely reasons are analysed below from the point of view of substrate recalcitrance.

Crystallinity

In cellulose some 10,000 glucose molecules are connected together in $\beta(1 \rightarrow 4)$ glycosidic linkage. Unlike the $\alpha(1 \rightarrow 4)$ glycosidic linkage in amylose (starch), the $\beta(1 \rightarrow 4)$ glycosidic linkages produce a glucan chain that is flat. There is strong inter-chain hydrogen bonding between the ring oxygen atom and the hydroxyl groups of glucose. As a consequence, approximately 30–36, β-glucan chains spontaneously aggregate, side by side and

above each other. The packing of the glucan chains is so ordered that cellulose exists largely in a crystalline state (Figure 4). Let alone cellulase enzyme molecules (mol wt 25,000–70,000), not even water molecules (mol wt 18) can penetrate into this crystalline structure, accounting for the insolubility of cellulose in water, its general resistance to microbial attack and to enzymatic degradation by cellulase. The glucan chains, interconnected by hydrogen bonds, form microfibrils which criss-cross to form a fine network in the cell wall (Figure 5). Moreover, several cellulose microfibrils are cross-linked by arabinoxylan and by linkages yet not fully characterized to generate a cross-linked three-dimensional structure. One can rationalize the requirement of several distinct classes of enzymes for pretreatment of biomass to free the cellulose from the stronghold of non-cellulosic polymers involving different linkages between the different sugar residues. The best sources of various classes of enzymes need to be identified for the formulation of a cocktail of enzymes for depolymering the cell wall into fermentable sugars by a microbial enzyme-mediated process of cellulosic ethanol.

Cell wall loosening

Clues to development of a commercial cellulosic ethanol process can come from unexpected sources. The network of polymers in the cell wall must be loosened as the cell expands in volume during plant growth for appropriate precursor molecules to be integrated. Cellulose may be most susceptible at this point. Cosgrove has found that during elongation growth plant cells produce trace amounts of proteins that he called expansins which disrupt non-covalent interactions between cell-wall polymers[25]. A gene with sequence similarity to plant expansins was discovered in the fungus *T. reesei*[26]. The swollenin gene was expressed in yeast. Concentrated swollenin-containing yeast supernatant disrupted the structure of the cotton fibres and filter paper without detectable formation of reducing sugars. Biofuel researchers must be broad-based. Swollenin or expansin-like proteins might be used as additives to improve accessibility of cellulase enzymes to cellulose in biomass[27].

Tailored biomass

In addition to cellulose (35–48%), plant biomass contains hemicelluloses (22–30%) and lignin (15–27%). The problem of lignin is more acute as few microorganisms can decompose lignin. Though eager to solve the fuel problem, many researchers were forced to shift focus elsewhere until molecular biology tools had become sophisticated, sequence resources were to become available and plants with reduced lignin could be produced. In recent work with tobacco and alfalfa using antisense constructs of any of the enzymes involved in its biosynthesis (cinnamate 4-hydroxylase, hydroxycinnamoyl CoA, shikimate hydroxycinnamoyl transferase, coumaroyl shikimate 3-hydroxylase, caffeoyl CoA 3-O-methyltransferase, ferulate 5-hydroxylase or caffeic acid 3-O-methyltransferase), the enzyme activity was inhibited[28,29]. The lignin content was reduced by 50% without differences in overall growth and development. The need for acid pretreatment in reduced-lignin alfalfa was obviated; the cell walls yielded nearly twice as much sugar as the control plants. Although modification of the lignin biosynthetic pathway enzymes by interfering with polymerization of monlignols has been demonstrated to decrease lignin content[30], it must be ensured that this modification will not interfere with the plant defence against invading pathogens and insects under field conditions.

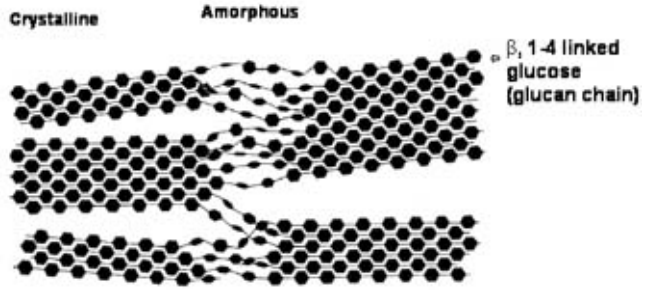

Figure 4. A drawing representing crystalline and amorphous regions in cellulose. The spacing between glucan chains is exaggerated to show the glucose molecules (filled hexagons)[49].

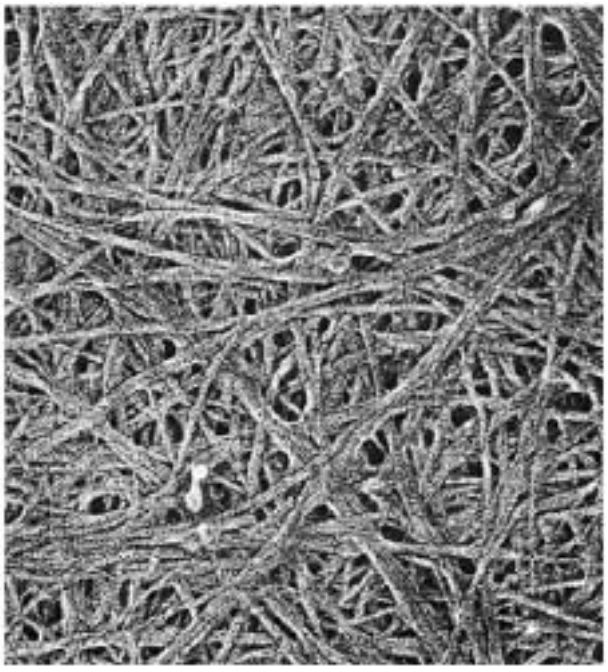

Figure 5. Electron microscope image of a network of cellulose microfibrils in the plant cell wall. The microfibrils are embedded in a matrix of hemicellulose and lignin which was removed by extraction[25].

Figure 6. Grasses as potential feedstock for bioethanol refineries. ***a***, Elephant grass, *Miscanthus giganteus* (photo courtesy: University of Illinois, Urbana-Champaign, USA). ***b***, Sweet sorghum (Photo courtesy: ICRISAT-IN (Patancheru, India).

Cellulose farming

Most experts are of the opinion that the cellulosic ethanol technology will require reduced lignin plants as raw material. I confess that I regard this with a tinge of glee, lest it leads to indiscriminate cutting of plants with disastrous consequences. Farming for cellulose is much talked about now. For example, farming of non-food, fast-growing grasses such as the elephant grass, *Miscanthus* (Figure 6 *a*), which contains ~17% lignin (http://miscanthus.uiuc.edu/) or the switch grass, *Panicum virgatum* (http://www.sciencedaily.com/releases/2008/01/080109110629.htm) which contains ~23.2% lignin. These grass species grow 6 m tall and can be grown on land of low agriculture value across a range of climatic conditions. In *Miscanthus sinensis* – a potential raw material for biofuels, addition of hemicellulase in delignified material increases the enzymatic hydrolysis of cellulose. When delignified *M. sinensis* was treated with cellulase, β-glucosidase and xylanase, hemicellulose was hydrolysed nearly completely into monosaccharides[31]. Another potential biofuel plant that is being developed at ICRISAT-IN is the sweet sorghum, *Sorghum bicolor* (Figure 6 *b*), which offers triple advantages: grain for food, sugar in stalks for distillery and leftover silage for biofuel. It is speculated that such a biomass may either obviate or reduce the need for microbial enzymes in bioreactors. Hitherto research on plant cell wall had been neglected. Tailor-made cell wall with appropriate chemical structure is likely to become one of the most important areas of plant biotechnology. An attractive idea is to engineer plants to self-produce microbial cellulase and ligninase in response to some externally applied signal[32].

Pretreatment

Even if lignin is removed by chemical treatment, the presence of hemicellulose enwrapping on the cellulose microfibrils hinders the action of cellulase. This was shown by quantitization of sugars from the delignified cell wall by sequential treatments with purified cellulase and xylanase[33]. More sugars were released from the delignified cell wall after sequential treatment of xylanase and cellulose – consistent with the view that xylan enwraps the cellulose microfibrils. Some thermophilic fungi have been identified that are exceptionally good producers of xylanases – the protein crystallized out in concentrated culture filtrates[34]. Presumably these can be used for selectively removing the hemicellulose barrier. Another means of removing hemicellulose is the ammonia freeze-explosion pretreatment, which simultaneously reduces lignin content and removes some hemicellulose while decrystallizing cellulose[35].

Microbial consortium

The quest to find the 'best' cellulase producer has made researchers overlook the fact that in nature recycling of biomass involves several species of microorganisms. This became clear when the biochemistry of a few individual species using pure cultures was investigated, particularly of the glycosyl hydrolases that produce monosaccharides. The enzymes differ in molecular sizes, pH and temperature optima, kinetic properties, regulation by substrate and in substrate specificities, suggesting that specific enzymes are optimized for variations in environmental conditions. The significance of an array of enzymes was understood when scientists in USA and Europe devised methods to understand the three-dimensional structure of the plant cell wall. Unlike the bacterial cell wall, the plant cell wall is a multilayered structure in which a repertoire of polysaccharides (pectin, cellulose, xylan, xyloglucans, glucuronoarabinoxylan, rhamnogalacturonan and other mixed-linked glucans) interact with each other through extensive glycosidic bonds[36,37]. The hemicellulose matrix is covalently linked to lignin, a highly complex organic

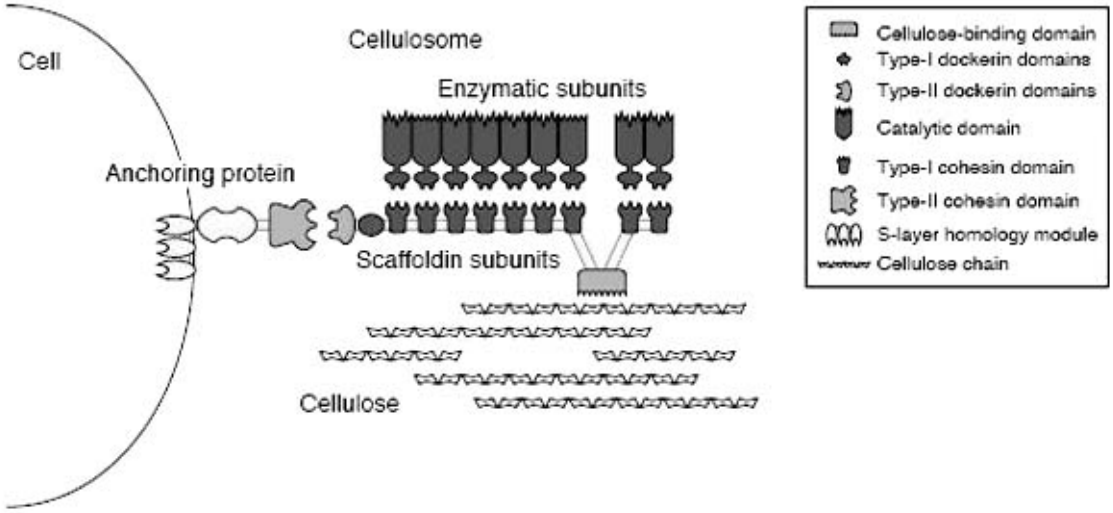

Figure 7. Cellulosome: An integrating scaffoldin subunit (yellow) organizes cellulosome into a cellulose-binding domain, and multiple copies of interconnected cohesin domains and catalytic subunits by cohesin–dockerin interactions. From Shoham *et al.*[41], with permission from Elsevier.

compound which is next only to cellulose in abundance. Because of the small genome size, no single microbial species is capable of producing the enzymes of different specificities required to break the different chemical bonds in the plant cell wall to be able to access cellulose and use it as a carbon and energy source for its own growth. Different microorganisms producing a large number of activities of endo- and exoglucanases, xylanases, acetyl-xylan esterases, feruloyl esterases, mannanases and ligninases are required to deconstruct the cell wall to derive sugars for utilization as carbon and energy source. Hence cellulosic ethanol technology would require the best enzymes of different specificities from a species of bacteria and fungi isolated by researchers for the formulation of a cocktail that can depolymerize the cell wall.

Molecular consortium of enzymes

Deus ex Machina

(Latin: 'God from the machine'. An agent who appears unexpectedly to solve an apparently insoluble difficulty)

An insight is provided by the cows, buffaloes and sheep which digest the ingested plant material in less than 24 h! These herbivores have a 'fermenter on four legs' called rumen that contains a mixed culture of anaerobic bacteria. These symbiotic bacteria help the animal to digest food, i.e. break it down into simple molecules for conversion into milk and meat. Each single microbial species has a 'consortium of enzymes' bound as a particle (or as an 'extracellular organelle') to its surface for the break down of the foraged matter. Johnson *et al.*[38] and Bayer *et al.*[39] showed that anaerobic rumen bacteria, exemplified by *Clostridium thermocellum*, break down ingested plant material but without secreting much cellulase. Despite vigorous stirring of cultures, the bacteria adhere tightly to cellulose added to the culture medium. The rate of solubilization of powdered crystalline cellulose by the bacterium *C. thermocellum* was about 2.5 times faster than the fungus *T. reesei*. Since this initial finding, several research groups are working on it[39–44]. The rumen bacteria break down the plant cell wall by multienzyme particles, called cellulosome, that are bound to the bacterial cell surface, allowing the bacterium to attach itself to the cell wall. Cellulosome (Figure 7) is composed of a protein, scaffoldin, that contains a cellulose-binding module and a number of cohesin modules which tightly bind to the complementary dockerin modules borne by the catalytic subunits. Cellulosome (2×10^6 Da) is a molecular consortium of several distinct cell-wall degrading enzymes associated as a protein complex on the cell wall of the bacterium. In other words, the rumen bacteria use a molecular consortium of multifunctional proteins on their cell walls, as a part of their cellular structure. Though bound on the cell surface, this structure enables the bacteria to rapidly degrade the complex structure of the plant cell walls comprising the bulk of herbivore diet, thereby enabling the host animals to obtain energy from simple compounds, mostly sugars. The advantage of extracellular cellulosome structure is in its positioning of the enzymes in an orientation, both with respect to each other and to the cellulosic substrate. The individual modules can be linked together genetically to form artificially designed chimeric cellulosome contain-

ing enzyme components from different species for more efficient solubilization of the plant cell walls comprising cellulose, xylan, mannan, pectin and other polysaccharides[45]. Chimeric cellulosomes incorporating cellulases or hemicellulases from different species of bacteria (or even a fungus), have been created for potential industrial use of *Clostridium cellulolyticum*[45–47]. This has opened up the possibility of using enzyme mixtures containing cellulosomal as well as non-cellulosomal enzymes for conversion of grass into simple sugars.

Concluding remarks

In plant cell walls, the intrinsic strength of cellulose fibres is augmented by enwrapping enmeshings with hemicellulose and lignin. Were it not for the sturdiness of the plant cell walls, we would not have fibres for clothing, ropes for tying, things, or wood for constructing shelter and construction of boats and ships, and innumerable other uses. Today, instead, interestingly, the sturdy plant cell walls have to be deconstructed to release fermentable sugars!

This article is an attempt to argue for the conversion of biomass into biofuels, which is far more complex than the green revolution. It requires knowledge of several scientific disciplines: How the plant cell wall is assembled and disassembled in nature, ways to alter this structure so that it becomes susceptible to microbial enzymes, the architecture and interior furnishings of the microbes themselves, recombinant DNA and protein engineering technology for designing and modifying 'cellwallase', still unknown factors that are apparently employed in transforming the cell wall into utilizable molecules, and the integration of clues into development of a workable technology. A new industry for converting plant lignocellulose into alcohol will require much basic research. I was therefore taken aback by a statement[14], 'Already several companies and government-funded laboratories have engineered enzymes and microorganisms to optimize lignocellulose degradation and help turn it into fuel'. However, a tantalizingly titled article[24] contains an attributed statement which gives a different picture: 'Researchers at some university centers and government-sponsored labs have incorrectly claimed that certain badly needed technologies are not yet ready for commercialization and that they won't be for at least five years. The effect is to keep research funds flowing to these organizations for an additional five years'. This is a barb aimed at the officials in the government to create a mission-oriented project, and at the researchers in universities to embolden themselves towards the solution of a problem facing not just a few, but the world population. Summing up, I wish to quote Sommerville[1], 'A national biofuels strategy will ultimately depend on massive support for basic curiosity-driven research in many aspects of nonmedical microbiology, plant biology, and chemical engineering'.

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