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Biomass Refining

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Because there is no shortage of petroleum at present, there is less apparent pressure to develop alternative chemical feedstocks. But the respite may be brief as OPEC (the Organization of Petroleum Exporting Countries) is attempting to cut production and to dissipate oil stockpiles. Having alternatives to petroleum may soon be essential to economic survival. While complicated technologies for producing renewable energy from the sun's rays may someday be affordable, biomass represents captured solar energy that is ready to be exploited now. Carbohydrate polymers in biomass can be hydrolyzed to their monomers: fermentable sugars. In the past, when petroleum was abundant and inexpensive, fermentation was used mainly to produce antibiotics, enzymes, and other molecules that chemists could not synthesize easily. However, the cost of biomass has risen less steeply than that of oil and, despite the present decline in oil prices, there may well be sufficient margin for profit in biomass refining.

The present administration's policy is that the U.S. Department of Energy should concentrate on support for long-range projects and leave it to private industry to commercialize technology with immediate potential. However, the stimulus of several years of governmental funding has advanced some processes to the stage where private groups have been willing to invest in further development. This is one of the Department of Energy's infrequent success stories, and it has created opportunities for new industry, new jobs, and increased tax revenues.

Summary. Processes for refining biomass from trees or from agricultural residues are fast approaching commercialization. Advances have been made in acidic and enzymatic hydrolysis of the cellulose component of biomass and in some newer processes for biomass refining which leave the lignin fraction relatively intact. Rather than low-priced fuels, which have received the most publicity, initial targets will be products with more profit potential, such as chemicals and molasses. Curtailment of government support for alcohol fuel programs is not crucial, because private investment is taking biomass refining from the pilot plant to the demonstration scale.

There are many options for processing biomass, but the aim in most cases is to produce cheap fuels such as methanol or methane or an intermediate such as synthesis gas (hydrogen plus carbon monoxide). Profit margins tend to be small, and large-scale operations are essential since the value of the products is scarcely more than the cost of the feedstock. It is far more practical to compete with the products made from fossil feedstocks than to produce substitutes for fossil fuels. Refining of biomass to more valuable products has few barriers to commercialization, and factories may be built in a few years.

Lignocellulosic materials are promising feedstocks for bioconversions. The composition of various woods and agricultural residues is 30 to 50 percent cellulose, 18 to 35 percent hemicellulose, and 15 to 25 percent lignin. While cellulose and hemicellulose are polysaccharides that can be hydrolyzed to fermentable sugars, lignin has a three-dimensional phenolic structure that is highly resistant to microbial attack or to chemical hydrolysis. Indeed, it may be practical to use intact lignin in plastics, adhesives, and various formulations that make use of these properties.

Much of the research and development related to alcohol fuels has concentrated on the hydrolysis of cellulose. But converting only the cellulose to alcohol fuels is uneconomic and can consume a high percentage of the energy represented by the alcohol. The feedstock cost alone would bring the cost of fuel-grade ethanol to about the present cost of gasoline, and biomass refining is relatively expensive. If the lignin and hemicellulose are wasted or used as very cheap fuel to power an ethanol factory, economic prospects are poor indeed, as these fractions represent opportunities for additional products. Nonetheless, the best way to power a biomass factory is with biomass. Tree species not suitable for lumber or paper pulp can be burned and are much less costly than oil and natural gas; cheap grades are, in fact, competitive with coal in terms of cost per unit of energy. Materials handling is simplified by using the same stockpile for the process and the power plant. Furthermore, biomass is low in sulfur and its combustion requires less pollution control equipment than does the burning of coal.

Cellulose Hydrolysis

There are many approaches to cellulose hydrolysis that have been discussed in some detail (*1*). In this section they are reviewed briefly, with emphasis on processes with more immediate economic potential.

Acidic hydrolysis of cellulose to glucose is an old technology that may be revitalized with the achievement of better glucose yields. Sulfuric acid has been used because of its low cost, but the glucose yield is usually only about 55 percent of the theoretical amount. Superior performance is obtained with other,

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more expensive acids, and methods are being developed for more economic recovery of these acids.

Cellulose can also be hydrolyzed by enzymes, yielding glucose by way of the disaccharide cellobiose. Glucose yields of more than 90 percent of the theoretical amount have been obtained by enzymatic hydrolysis of cellulose, and prospects are excellent for the recovery of additional sugars from hemicellulose and for producing undamaged lignin. Although enzymes are very expensive and acid hydrolysis may appear economically more attractive, costs of enzymes have been dropping dramatically and enzymatic hydrolysis is likely to become highly profitable in the future.

A process developed at U.S. Army Laboratories, Natick, Massachusetts, was the first significant advance in enzymatic hydrolysis of cellulose. E. T. Reese, M. Mandels, and their co-workers at Natick intensively studied the molds that produce cellulase, in particular *Trichoderma reesii*. These efforts, plus the contributions of other groups, especially at Rutgers University, have led to strains that produce excellent enzyme titers. The U.S. Army has discontinued its work on biomass energy projects, and the Natick team of scientists and engineers has been disbanded and reassigned. A modification of the Natick process, developed at the University of California, Berkeley, has contributed engineering improvements and alternative treatments.

It is generally true that intermediate process streams represent an investment that far outweighs their fuel values. A process developed at Massachusetts Institute of Technology (MIT) is an exception to this rule. In one step, cellulose and hemicellulose are hydrolyzed to sugars that continue on to ethanol, leaving a residue that is dried and burned. Two synergistic organisms (*Clostridia*) are added directly to coarsely shredded biomass and utilize much of the polysaccharides present. The first organism hydrolyzes cellulose and hemicellulose and ferments the resulting glucose to ethanol. The second organism, which is added after the first organism has nearly finished hydrolyzing cellulose and fermenting the glucose, ferments the remaining sugars from hemicellulose.

This project illustrates the importance of genetics and strain selection. The organisms initially isolated had poor tolerance to ethanol and produced some lactic acid and about as much acetic acid as ethanol. Through mutation and strain selection organisms with greater ethanol tolerance have been developed that produce about 3 to 4 percent ethanol with little acetic or lactic acid (*2*).

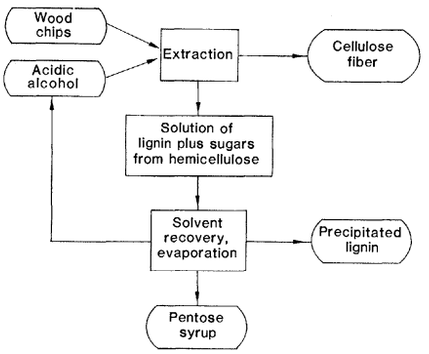

Fig. 1. Solvent refining of wood.

Corn stover is a promising feedstock for the MIT process because of its high cellulose content and its ease of shredding. Unfortunately, although the process works extremely well with pure cellulose, there is considerable variability in performance with real feedstocks (*3*). With corn stover, inhibitory products slow hydrolysis rates and impair enzyme stability, and acetate accumulates.

The remarkable feature of the MIT approach is the low investment in processing that it requires; the residue has roughly the same value as the biomass feedstock and burning becomes feasible. However, as the fuel value of the residue exceeds the needs of the factory, steam or electricity would be products about equal in importance to the ethanol. Since this very promising process has acquired industrial sponsorship, less information about it has been released.

Another process employing simultaneous saccharification and ethanol fermentation originated at the Gulf Oil Chemicals Company and is now being developed at the University of Arkansas. The advantage of simultaneous saccharification and fermentation is that sugars do not accumulate to slow enzyme rates by feedback inhibition. Part of the feedstock for this process is to be municipal solid wastes, and credits are taken for waste disposal when estimating costs. There seems to be some difficulty in reducing the feedstock to a size suitable for bioconversion because in one design for a factory, pretreatment by ball milling accounted for 37.8 percent of the capital cost (*4*). For waste paper, Natick workers showed that two-roll wet milling is effective and relatively inexpensive. The Arkansas workers have fed residues from the process to animals, but regulatory agencies would be slow to approve human consumption of animals grown on materials derived from municipal wastes.

The Solar Energy Research Institute (SERI) has assembled teams to work on several aspects of processing of lignocellulosic materials. The genetic engineering group has isolated a plasmid for xylose isomerase that could enable alcohol-tolerant organisms to ferment sugars from the hydrolysis of hemicellulose. Several other groups worldwide are also applying recombinant DNA techniques to improvement of the organisms involved in ethanol fermentation. A SERI team has obtained from Spain a yeast that grows rapidly and tolerates 30 percent ethanol (*5*). Also of interest are some thermophilic organisms isolated from hot springs in Yellowstone Park. These organisms can tolerate 7 percent alcohol and can be used at elevated temperatures, where fermentations occur more rapidly and the ethanol can be easily recovered by vacuum distillation directly from the fermentation broth.

Understanding of the mechanisms of cellulose hydrolysis has been advanced by research at the Laboratory for Renewable Resource Engineering at Purdue University. The demonstration of cellulose solvents for pretreatments that allowed nearly quantitative hydrolysis was also a key contribution from this laboratory. In a Purdue process in which sulfuric acid is used as a solvent to reduce the crystallinity of cellulose, glucose yields of about 95 percent of the theoretical value are achieved with enzymes, although the 70 percent yields obtained with acid hydrolysis will be more economic until the cost of the enzymes drops further. It is interesting that destroying the crystallinity of cellulose facilitates both enzymatic and acidic hydrolysis; other pretreatments usually have a large effect on one type of hydrolysis but little effect on the other. Products based on lignin have not yet been studied at Purdue, and the lignin may be burned to power the factory if the acid damages it too badly.

Processes That Focus on Lignin

In three recently announced processes for biomass refining several products are obtained. The treatments are mild, and the lignin fractions are relatively unaffected and remain reactive. Such relatively intact lignin is much superior to the damaged, substituted material obtained from paper pulping by the conventional kraft or sulfite processes. The molecular weight of this lignin is low rela-

tive to many of the values reported for lignin in the literature, and there may have been some cleavage. However, there has already been sufficient preliminary testing by potential customers to indicate the suitability of the lignin for adhesive formulations.

One process, developed by the Biological Energy Corporation (a collaboration among the University of Pennsylvania, Hahneman Medical Center, Lehigh University, and the General Electric Company) in Philadelphia, is analogous to the solvent pulping of wood chips (*6*). Extraction of wood chips with acidic or alkaline alcohol dissolves the lignin and hydrolyzes the hemicellulose to soluble sugars (see Fig. 1). The remaining cellulose has long fibers and should command a premium price as paper pulp. During alcohol recovery, the lignin precipitates and is diverted to chemical uses, probably in plastics such as phenol-formaldehyde. The aqueous fraction is a solution of mixed sugars rich in pentoses that are suitable for feeding to cattle. This process converts all the main components of wood to high-value products, some of which can substitute for chemicals that are now derived from petroleum or require fossil energy for their production. Another possible product is the leafy residue, if the trees are harvested while green. The leaves are rich in protein, and similar material is sold in Europe as cattle feed. However, when entire trees are collected, soil fertility will decline quickly unless fertilizers are used.

The second process, developed by Battelle-Geneva in Switzerland, is quite similar to the scheme of the Biological Energy Corporation except that the solvent for lignin is phenol (*7*). At 100°C and atmospheric pressure, phenol is miscible with water, and at the low *p*H of the solvent hemicellulose is hydrolyzed as lignin dissolves. The solid residue is cellulose with a fibrous structure. The most valuable use for this cellulose is probably as pulp, but other potential applications are in making cellulose derivatives such as cellulose acetate and in making glucose by hydrolysis.

The aqueous phenol extract from the Battelle-Geneva process separates into two phases when cooled. The phenol-rich phase contains the lignin, and the water-rich phase contains the sugars from hydrolysis of the hemicellulose. Phenol is recovered and recycled. Cracking of the lignin in reductive conditions produces benzene, phenol, cresols, and other aromatic compounds, so this is a means of furnishing phenol for the process. However, preliminary discussions with companies interested in hydrocracking have indicated that the lignin may command a higher price in other applications. Most uses for the sugars from hemicellulose will require very efficient removal of residual phenol.

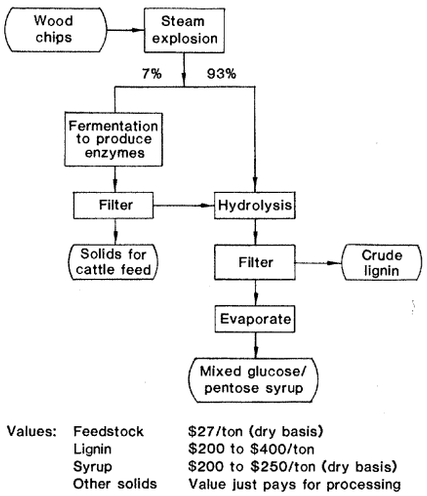

Fig. 2. Steam explosion of wood.

The third process, developed by the Iotech Corporation, Ottawa, is entirely aqueous (*8*). Commercial wood chips are impregnated with high-pressure steam, and sudden release of the pressure disintegrates the structure and potentiates enzymatic hydrolysis of the cellulose. About 7 percent of the exploded wood goes to the production of cellulase enzymes. Cellulase broth is added directly to the rest of the wood, and the resulting hydrolysis of cellulose yields more than 85 percent of the glucose theoretically available. The hemicellulose is readily hydrolyzed to be with the glucose after saccharification; this solution is to be evaporated to a syrup for feeding cattle (see Fig. 2). The solid residue from enzymatic hydrolysis is rich in lignin, which can be recovered in quite pure form by extraction with dilute alkali; alternatively, the residue can be sold directly for adhesive applications such as binding of plywood or chipboard, where some inert fillers are desirable in the resin.

Table 1. Economics of biomass refining of trees.

Feedstock cost	$30 per ton (1000 kg)*
Plant size	700 tons per day
Capital investment	$34 million†
Labor and materials	$14 million per year†
Product values	
Cellulose pulp	$350 per ton‡
Pentose syrup	$150 per ton
Lignin	$350 per ton
Costs per year	
Feedstock	$7.4 million
Operations	$14 million
Interest	$5.2 million
	$26.6 million
Revenues per year	
Pulp (or glucose syrup)	$31.5 million‡ (or $15 million)
Pentose syrup	$7 million
Lignin	$20 million
	$58.5 million with pulp ($42 million with glucose syrup)

*Based on quoted costs in several states. †From (*7*). ‡Actual value of pulp has not been verified; lignin value has been verified from letters of intent from prospective customers.

Tests of the Iotech process at a batch size of 1000 liters scale at the Gulf pilot plant in Kansas achieved about 90 percent of the theoretical glucose from cellulose, and the fermentation to ethanol was satisfactory. Recipes for wood binders using the lignin have received favorable evaluations by potential customers. Financial backing is now being sought for the construction of a demonstration plant with full-sized modules that could be extended to a prototype factory. In order to keep down capital costs and facilitate plant start-up, Iotech plans to have the initial factory stop at the glucose-pentose syrup (molasses) stage and to hold fermentation products such as ethanol as projects for the future.

An overview of the economics of biomass refining is shown in Table 1. It is clear that the sale of high-value lignin is a key to profitability. However, the projected markets for lignin could become saturated by the outputs of several large factories. Therefore, as the processes improve, profits may remain constant as lignin is sold at a lower price for other applications. Should deep markets develop because of the availability of abundant lignin at a reasonable price, biomass refining could also produce large amounts of fuel-grade ethanol. If unsold lignin must be burned as a fuel, however, sales of alcohol alone may not generate enough revenue to make ethanol production by this method profitable.

Cellulase Recycling

In addition to continued improvements in cellulase fermentation, efforts to recycle cellulases are finding success. Recycling saves on the cost of producing fresh enzymes, and it can also result in an enzyme concentration in the hydrolysis mixture which is higher than the concentration supplied by only the broth from the enzyme-producing fermentation. Recycling is based on the affinity of cellulases for cellulose and on ways of reducing this affinity. Of the dozen or so components of cellulase, several bind strongly to cellulose. These can be re-

covered easily from the hydrolysate by adsorption on cellulosic material, which can be blended with the solids added at the hydrolysis stage. Enzymes on the solid residue left after hydrolysis can be desorbed by *p*H adjustment (*9*) and the extract used to supplement the fresh enzyme. Beta-glucosidase, a key component of cellulase which splits cellobiose to glucose, is not very stable and cannot be recovered by either of the methods just presented. However, low-cost beta-glucosidase can be obtained by a separate fermentation. There are indications that about 50 percent of the cellulase can be recycled, and the effect on costs should be dramatic.

Conclusion

Biomass refining is approaching commercialization. The first factories are likely to emphasize high-value products such as lignin, molasses, and paper pulp rather than ethanol. With products from all the major fractions of biomass very little waste will be generated, so these factories will have few problems in meeting environmental standards. Establishment of refining technologies will open the way for factories producing alcohol fuels. An excellent return on investment is likely, and guarantees and tax incentives may not be needed to stimulate the construction of factories.

References and Notes

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Translational Efficiency of Transfer RNA's: Uses of an Extended Anticodon

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A transfer RNA (tRNA) efficiently translates its codon (or codons) despite the apparent fact that its principal device for doing so, the anticodon trinucleotide, seems to function weakly and inaccurately when not a part of a tRNA molecule. We therefore expect that the detailed architecture of tRNA's will account for their superiority. But while some of the important contributions of the tRNA structure are known (*1*, *2*), there has been no unifying scheme that connects tRNA structure with translational performance. The extended anticodon hypothesis is such a scheme. It suggests that the structure of the anticodon loop and the proximal anticodon stem are related to the sequence of the anticodon. Thus, it is as if the anticodon itself were extended into the nearby structure. This extension can therefore be used to specifically enhance the selected, accurate pairing with a cognate codon.

Summary. Transfer RNA's are probably very strongly selected for translational efficiency. In this article, the argument is presented that the coding performance of the triplet anticodon is enhanced by selection of a matching anticodon loop and stem sequence. The anticodon plus these nearby sequence features (the extended anticodon) therefore contains more coding information than the anticodon alone and can perform more efficiently and accurately at the ribosome. This idea successfully accounts for the relative efficiencies of many transfer RNA's.

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The tRNA's (as well as the rest of the translational apparatus) of free-living, fast-growing microorganisms are probably highly selected for translational efficiency. More than half the dry weight of a bacterium can be protein, and the rate at which that mass can be generated is quite critical to evolutionary success. Even a tiny difference in growth rate can quickly be amplified by a period of exponential growth into a decisive advantage in numbers. It is therefore plausible to begin by treating the tRNA's functional in *Escherichia coli* as a set selected for good (if not optimal) translational efficiency.

Order in Natural Sequences

In Fig. 1, the sequences of 42 wild-type tRNA's functional in *E. coli* are abstracted and grouped so as to allow comparison of their anticodon loop and stem regions. The sequences are presented linearly, beginning at the first (5′) anticodon loop nucleotide, running 3′ through the anticodon loop, and extending through the five 3′ nucleotides of the anticodon helix (see the drawing in Table 1). Thus, the figure allows one to visualize the entire loop, and the helix by inference.

The sequences are grouped so that translators with the same 3′ anticodon nucleotide (same 5′ or "first" codon nucleotide) are written together; 3′ pyrimidines are at the top of the figure, 3′ U or A is on the left, 3′ C or G is on the right of the groupings (*3*). This helps to make the design of the sequences most evident, because all sequences having the same 3′ anticodon nucleotide also have a similar set of anticodon region sequence features. The constrained features include the sequence of the 3′ nucleotides in the anticodon loop, and some of the base pairs of the anticodon stem (Table 1). A comparable set of shared features does not appear when sequences are grouped by first [wobble; (*4*)] or second (middle) anticodon positions.

These relationships are summarized

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