

## Chapter 21

# Potential for Fuels from Biomass and Wastes

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Current uncertainty about petroleum supplies and the third sharp rise in petroleum prices in the last 20 years have returned us to the fact that all modern, and many developing, countries have become utterly dependent on petroleum imports for their energy needs. No country has used fully the lessons of the recent past to develop an industrial base for the production of alternative fuels from domestic resources. Many liquid and several gaseous fuels can be produced from the fermentation of treated biomass. The commercial success of this approach appears increasingly assured due primarily to the aggressive application of research and engineering resources to key steps in conversion processes.

The annual consumption of petroleum in 1988 amounted to almost 50% of the total consumption of fossil fuels in the United States (1), with approximately 50% of this quantity imported from various countries around the world. The historical trend in annual production and consumption of petroleum in the United States does not provide hopeful trends for the future, because steadily decreasing domestic production and increasing consumption are balanced by ever-increasing imports. An even more ominous domestic trend is the sharp decrease of operating drilling rigs in the last decade, which reached their peak in 1980 and steeply plunged to a postwar low in 1986 (1). At the same time, Arab countries stepped up their production and depressed the world petroleum prices. While estimated current reserves can supply petroleum at current rates of production for another 20-30 years (1), most fields are past their peak primary production stage, and their output rate cannot be easily increased. Short of discoveries of new large petroleum fields in frontier areas, such as offshore or in Arctic regions, the lower 48 states do not offer great hopes for the abrupt reversal of the slowly declining domestic production trend. Because of complete dominance of petroleum-derived liquid fuels in the transportation and some heating markets, alternative sources of liquid fuels with similar properties need to be developed at an accelerated pace.

In any event, it must be noted that any alternative fuels industry will be a very large enterprise, and will take decades to develop. Its full production potential will be achieved after the research stage is largely completed and pilot and demonstration stages are initiated. For example, the spectacular growth of the fuel alcohol industry based on the fermentation of corn starch to alcohol, from almost zero production in 1980 to approx-

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imately 800 million gal/yr in 1988 (2), still accounts for less than 1% of the annual consumption of gasoline in the United States. Additionally, it did not require an extensive R&D effort to start. Commercial plants for the manufacture of ethanol from starch or molasses have been marketed for decades, and only minor modifications or improvements can convert them to production of fuel alcohol. While our supplies of petroleum do not appear adequate for our current and future needs, the United States has ample supplies of fossil or renewable solid fuels in the form of coal, oil shale, tar sands, and biomass (1). All of these resources can be converted to liquid fuels, but conversion of each resource has its unique aspects. Only biomass conversion falls within the scope of this chapter.

This chapter provides brief analyses of biomass resources and liquid fuels, which can be readily produced by combined biological and chemical processing steps. The key conversion steps, such as depolymerization of cellulose and related carbohydrates, are followed by brief overviews of fermentation processes, which are applicable to the production of liquid and gaseous fuels and their precursors. This chapter is closed with a summary of key engineering issues.

### **The Nature and Magnitude of Biomass Resources**

It has been estimated (3) that  $2 \times 10^{11}$  tons of carbon are fixed annually around the world by photosynthesis of higher plants. This renewable resource could, theoretically, supply approximately 10 times our energy needs and 100 times our food needs (3). While the plant kingdom encompasses tens of thousands of plants, only a few have been developed for large-scale cultivation by man (4). The world's agriculture is dominated by cultivation of grasses (4) (e.g., corn, wheat, rice, and sorghum), which produce starchy seeds necessary for our sustenance. These dense and storable sources of starch are supplemented by starchy tuberous crops (e.g., cassava and taro), which thrive in tropical lowlands unsuitable for grain production, except for rice. Potatoes are an analogous crop for temperate climate. Our sweet tooth is generally satisfied by sucrose crystallized from purified and evaporated juices of yet another grass (sugar cane), or sugar beets, cultivated in temperate zones. Starchy staples are supplemented by a few legumes (e.g., soybeans and peanuts), which produce seeds rich in both oil and protein, or in only protein (e.g., other beans). A few other plants (e.g., sunflowers and rapeseeds) are produced for their oily seeds, and a very small portion of land is devoted to specialty crops, such as vegetables, fruits, herbs, and spices. The major fiber crop is cotton, which is holding its own against synthetic fibers, while other fiber crops (e.g., flax, hemp and sisal) have declined. The remaining large tracts of agricultural land are divided into pasture, dominated again by grasses and herbaceous legumes, and forests where a variety of hardwoods and softwoods are grown.

Our inherent inability to digest polysaccharides other than starch, which as a plant storage polymer accumulates in seeds and tubers, leads to a tremendous wastage of total plant biomass during the harvesting of crops. Only seeds or tubers are removed, and the rest of the plant is usually left in the field as an "agricultural residue" of very little value. In the United States, the two major agricultural residues are corn stover and wheat straw with lesser amounts from minor grain crops, soybeans, and cotton (5,6). Because grain crops are harvested after the grasses have reached their maturity, the residues have very low digestibility and nutritional value, even for ruminants (i.e., cattle, sheep, and horses) that normally feed on grasses in younger stages of growth. Rapid changes in the composition of grass cell walls from germination to maturity were identified many years ago. These changes—mainly increases in xylans and lignins, and decreases in pectin content—were accompanied by a decrease in enzymatic digestibility by bacterial consortia in the rumen (7,8). Therefore, the only uses of grain crop residues, marginal at best, are as bedding and roughage for farm animals. Even if baled and collected, which is commonly done with wheat straw, a lot of residues are left to rot, or simply are burned in the fields. Because wasteful burning practices, whether they apply to forests, pastures, or cropland, are contributing to the greenhouse effect, with marginal recycling of nutrients and clearing of the land as the only discernible benefits, there is mounting pressure to

discourage plant burning practices in agriculture. Such a shift in our attitudes provides a strong incentive for the development of new end uses for agricultural residues.

Arguments have been made in the past that the removal of straw and corn stover will rapidly deplete the soil of needed nutrients and accelerate soil erosion. Such arguments represent an extreme view that is not strongly supported by facts. Current harvesting machinery for grain crops does not pull the plants by the roots (9). The stalks are cut at a predetermined height above the ground. Straw or stover, therefore, refers only to the upper, aerial parts of the plant that are cut and removed. Roots and the lower portions of the stems, stubble, are always left in the ground. Because the plants are harvested after maturity, many nutrients (e.g., nitrogen) are translocated to the grain and roots, and only small amounts are left in the stems and leaves (10).

It has been estimated (10) that wheat straw contains approximately 15–30 lb of nitrogen per acre and 35–60 lb of potassium per acre. Due to higher biomass yields, the removal rates are higher for corn stover, approximately 70 lb of nitrogen per acre and 95 lb of potassium per acre. It should be noted that approximately two-thirds of the total phosphorus and nitrogen are irretrievably removed from the field with grain, while only one-third of potassium is removed (10). There are additional trace elements needed by the plants, but nitrogen, potassium and phosphate are the major fertilizer requirements. The vastly increased yields of grain crops in recent decades are directly correlated with heavier fertilizer usage (10), which vastly exceeds the reservoir in plant residues. Therefore, the complete recycle of mineral nutrients in crop residues can only supply a fraction of the fertilizer needs of modern crop production. Only the mineral components, such as potassium and phosphorus, can be eventually recycled back to the soil. Decomposition of crop residues can actually deplete the soil of nitrogen (10), which is consumed by microorganisms decomposing these residues. While we should conserve present nonrenewable deposits of mineral fertilizers and recycle as much of these nutrients as possible back to the soil, there is no inherent conflict between processing of agricultural residues and mineral nutrient recycling. Thermochemical processing, such as combustion and gasification, produces mineral ash streams that can be readily applied back to the fields. In biochemical processing, recycle would be only slightly more complex. Microorganisms require the same major and micronutrients as plants. Therefore, the mineral nutrients released from plant residues during biochemical processing can support growth and maintenance of microorganisms, and after product separation either the aqueous stream or concentrated mineral nutrients can be recycled back to the soil in the vicinity of the fermentation plant. Fermentation plant processing would save on the mineral nutrients purchased by the plant, and the surplus contained in the incoming feedstock can be supplied back to the farmers. Our own experimental work in anaerobic digestion of wheat straw hydrolyzates indicated that only supplementation with nitrogen needed for maintenance of healthy populations of anaerobic bacteria. The remainder of the nutrients are released from the wheat straw substrate (11).

Another large source of residues in the United States is forestry. Approximately 50% of the standing tree biomass is actually harvested during a typical logging operation (12,13). Branches, crowns of trees, and crooked, diseased, or juvenile trees are not merchantable and are not generally removed from the forest. Because wood has extremely low (approximately 0.5%) ash content (14) and takes a long time to decompose, the benefits of mineral nutrient recycle are minimal. Additional residues are produced during lumber production. The current emphasis on minimizing landfill disposal fees creates incentives for any utilization of sawmill residues, which will decrease disposal problems. Thanks to the spectacular growth of the pulp and paper industry during the last hundred years and the equally fast increase in population, we are producing ever-increasing amounts of municipal solid waste, which has wastepaper as its major component. Because producers pay rather steep prices for disposal of this waste, this feedstock is available at negative or very low cost. In aggregate, the coproduction of agricultural, forestry, and municipal wastes is the large annual resource that is cellulosic in nature and has very low or no value at the present time. When augmented by harvest from

underutilized hardwood forests and energy crops on marginal lands and agricultural lands taken out of production, it has been estimated that a renewable fuel resource base twice as large as the total annual consumption of gasoline in the United States could become available, based on cellulosic biomass (2).

Thus, besides the starchy or sugar crops, part of which can be converted to biofuels during times of surpluses in agricultural production, by far the largest resource for fuel production is lignocellulosic biomass from softwoods, hardwoods, and grasses. Other crops, such as legumes, cotton, or oilseed plants, can be of interest in some localities, but they do not approach the magnitude of the first three resources.

By composition, the cell walls of grasses and hardwoods are similar, but they are significantly different from softwoods, see Table I (16-22; Torget, R., Solar Energy Research Institute, unpublished results). The major component in all substrates is cellulose, except in starchy organs such as corn kernels and cassava roots. Significant chemical differences occur in hemicelluloses and lignins (16,21,23-25). The major hemicelluloses in softwoods are glucomannans, which are replaced in angiosperms by acetylated xylans containing arabinose and glucuronic acid side groups. A significant shift also occurred during the evolution of hardwoods and grasses in lignin composition and content. The guaiacyl-type lignins present in softwoods are modified by syringyl groups in hardwoods and grasses. *p*-Hydroxyphenyl groups may also be present in the lignins of grasses. There is also a significant increase in the lignin content of softwoods when compared to grasses and hardwoods. Compositions of the major starchy crops are included for comparative purposes in Table I. The composition of newsprint and office paper are included to illustrate a range of paper products that are not uniform in composition, but contain mixtures of numerous pulp fibers from fiberized wood (i.e., mechanical pulp) to delignified and bleached chemical pulps that have a high cellulose content. Inspection of Table I indicates that cellulosic biomass and starchy grains contain very similar amounts of carbohydrates, but due to differences in structure and composition, they pose different challenges to conversion processes (discussed below).

### Summary of Fuel Properties of Biomass

Biomass, primarily wood, has been used as fuel from the times of prehistory. Even today, large amounts are used for this purpose around the world. Biomass has a reasonable heat of combustion when dry and it is usually low in sulfur, nitrogen, and ash. (Sulfur, nitrogen, and ash cause problems in the utilization of fossil fuels.) However, biomass is a solid fuel with energy content lower than fossil fuels, both on a weight-basis and especially on a volume-basis (Table II) (26-29). Plant cell walls occupy only a small fraction of total plant volume; therefore, the bulk packing density of biomass is very low (Table II). The low volumetric energy content is very severe for agricultural residues, which together with the fast rate of combustion, makes them undesirable even for a farm fuel source, except in times of emergency. The densification of the energy content of biomass by compression or conversion to liquid fuels should be an important economic driver for the development of biomass fuels industries in rural areas. Increased density and reduced perishability will make transportation to distant markets much more attractive. Another economic driver for the conversion of biomass and other solid fuels into liquid fuels is the significant premium customers pay for liquid fuels, both in transportation and in stationary heating markets.

### Alternative Fuels from Biomass

Gasoline and other liquid fuels derived from petroleum are extremely complex mixtures of aliphatic and aromatic hydrocarbons, which are characterized by a set of physical properties, such as boiling point range, volatility, ignition properties, flame propagation (antiknock), and heat of combustion. Because these liquid fuels are already complex blends, they can be blended with other organic liquids or replaced by them as long as these

TABLE I  
Approximate Chemical Composition of Selected Biomass Resources<sup>a</sup>

Compounds	Wood of hardwoods (wt%)	Wood of softwoods (wt%)	Wheat straw (wt%)	Newspaper (wt%)	Office paper (wt%)	Corn kernel (wt%)	Cassava roots (wt%)
Anhydrosugars							
glucose	45-55	45-55	40	45-52	71-75	0.07-0.17	82-93
xylose	15-25	5.0-7.0	21	4.9-5.3	6.5-8.9	6.2	0.1-1.1
mannose	0.5-3.0	10-12	1.0-2.0	4.9-6.2	2.7	ND	ND
galactose	0.3-1.0	1.0-1.4	1.7	0.5-1.0	0	1.0	ND
arabinose	0.3-0.5	0.5-1.5	1.0-2.0	0.6-1.1	0	4.2	ND
Glucuronic acids	2.0-5.0	2.0-4.0	1.5	ND	ND	0.8	ND
Acetyl groups	2.0-4.0	1.0-1.5	2.2	ND	ND	ND	ND
Lignins	19-28	27-34	18	25.5	0.5	0.2	ND
Crude protein	ND	ND	ND	ND	ND	8-14	2.1-6.2
Ash	0.5-0.7	0.5-0.7	8.0	0.5-3.5	7.7-15	1.1-3.9	0.9-2.4
Water soluble extractives	2.0-5.0	2.0-5.0	8.0-12	ND	ND	ND	ND

ND is not determined, or not available.

<sup>a</sup> refs. 16-24; Torget, R., Solar Energy Research Institute, unpublished data

TABLE II

Selected Physical Properties of Liquid and Solid Fuels <sup>a</sup>						
Fuels	Density		Higher heats of combustion			
	g/mL	lb/ft <sup>3</sup>	GJ/ton	Btu/lb x10 <sup>3</sup>	MJ/L	Btu/ft <sup>3</sup> x10 <sup>3</sup>
Gasoline	0.74	46.2	48.2	20.75	35.7	959
Diesel	0.85	53.0	46.0	19.81	39.1	1050
Vegetable oil	0.90	56.2	39.6	17.05	35.6	958
Methanol	0.79	49.3	22.3	9.6	17.6	473
Ethanol	0.79	49.3	29.8	12.8	23.5	632
Coal	0.6-0.9	40-58	17-35	7.4-15.2	11-33	300-880
Wood	0.16-0.4	10-25	19-22	8-9.7	3-9	80-240
Agricultural residues	0.05-0.2	3-12	16-19	7-8.2	0.8-3.6	21-99

<sup>a</sup> refs. 26-29; GJ = gigajoule, MJ = megajoule

liquids or their mixtures approximate the physical properties of gasoline or other fuels (e.g., jet and diesel). Gasoline extension or replacement is of highest interest in the United States because it dominates the U.S. refinery output (30). However, the higher boiling fractions, i.e., diesel fuel and heating oil, also account for a large fraction of refinery output and their replacement should be addressed as well. Because gasoline components must vaporize in the internal combustion engine, the boiling point of gasoline liquids has to be in the range of 60°–200°C. The boiling points of simple organic compounds (31) are compiled in Figure 1. Aliphatic, oxygenated compounds were selected for this figure, because many of them are produced directly by microbial fermentations, and the rest can be produced from the primary products by simple chemical or biological transformations. The single exception is methanol, which is not known to be produced by biological systems. However, methanol is produced as an intermediate during methane oxidation by methylotrophic bacteria, so possibilities for biological production of methanol may exist. Methanol is also a major contender for alternative liquid fuels produced by thermochemical methods and was included in Tables I and II for comparative purposes. This survey indicates that many simple alcohols, ethers, ketones, and esters are compatible with the boiling range of gasoline. Organic acids are included because of their value in the preparation of esters, ketones, and alcohols, but they are corrosive, have low heats of combustion, and are usually produced as nonvolatile salts. They are not, therefore, to be considered serious contenders for direct blending with gasoline. It must also be noted that with the exception of acids and two lower alcohols (i.e., methanol and ethanol), all other liquids are fully miscible with gasoline and the freezing points (31) of all of them are well below the lowest winter temperatures (-45°C).

The next important consideration is the energy contents (i.e., heats of combustion) of these liquids. They are summarized in Figure 2. This figure shows that numerous organic liquids that can be derived from biomass have significantly higher energy contents than methanol, with many reaching 80% of the energy content of the hydrocarbons in gasoline. Hydrocarbons can also be produced from fermentation products, but with significant weight loss penalty, which will be discussed below.

Another important fuel property needed for compatibility with current internal combustion engines is resistance to abnormal combustion (knocks), reflected by the octane number, which is usually displayed on the pump as an average of the research and motor octane numbers ( $R + M/2$ ). Both research and motor octane numbers are determined with specialized CFR (Cooperative Fuel Research) knock-test engines operated under ASTM specified conditions. Because the research octane number (RON) is determined under less stringent operating conditions than the motor octane number (MON), it is higher than MON for the same fuel. The oxygenated liquids are clearly superior to hydrocarbons in their antiknock properties (32-34). The research octane numbers of modern gasolines ranges from 90–98, but only due to extensive processing of oil fractions (34). The oxygenated compounds in Figures 1 and 2 show research octane numbers in the range of 108–125, with motor octane numbers correspondingly higher than those for gasoline. The octane numbers of gasoline were improved for many years by the addition of small amounts of tetraethyl lead, but due to its toxicity and poisoning of catalytic converters, its use was discontinued several years ago. Other newer organometallic compounds (35) may never be introduced to gasoline markets for the same reason. The industry is currently trying to balance the drop in octane values of gasoline with increased processing to aromatic hydrocarbons and branched aliphatic ones via isomerization, but even the standard for octane number measurements (i.e., isooctane) is a research octane number (i.e., 100) lower than many oxygenated compounds. Since fewer aromatic compounds (i.e., benzene, toluene, and xylene) have been identified as carcinogens, it is only a matter of time before political pressure builds to decrease or eliminate their inclusion in gasoline. Preemptive measures may be behind their decrease in "reformulated gasoline." While many oxygenated compounds are also toxic (32), they are biodegradable; and none has been identified as a carcinogen. The universal solution

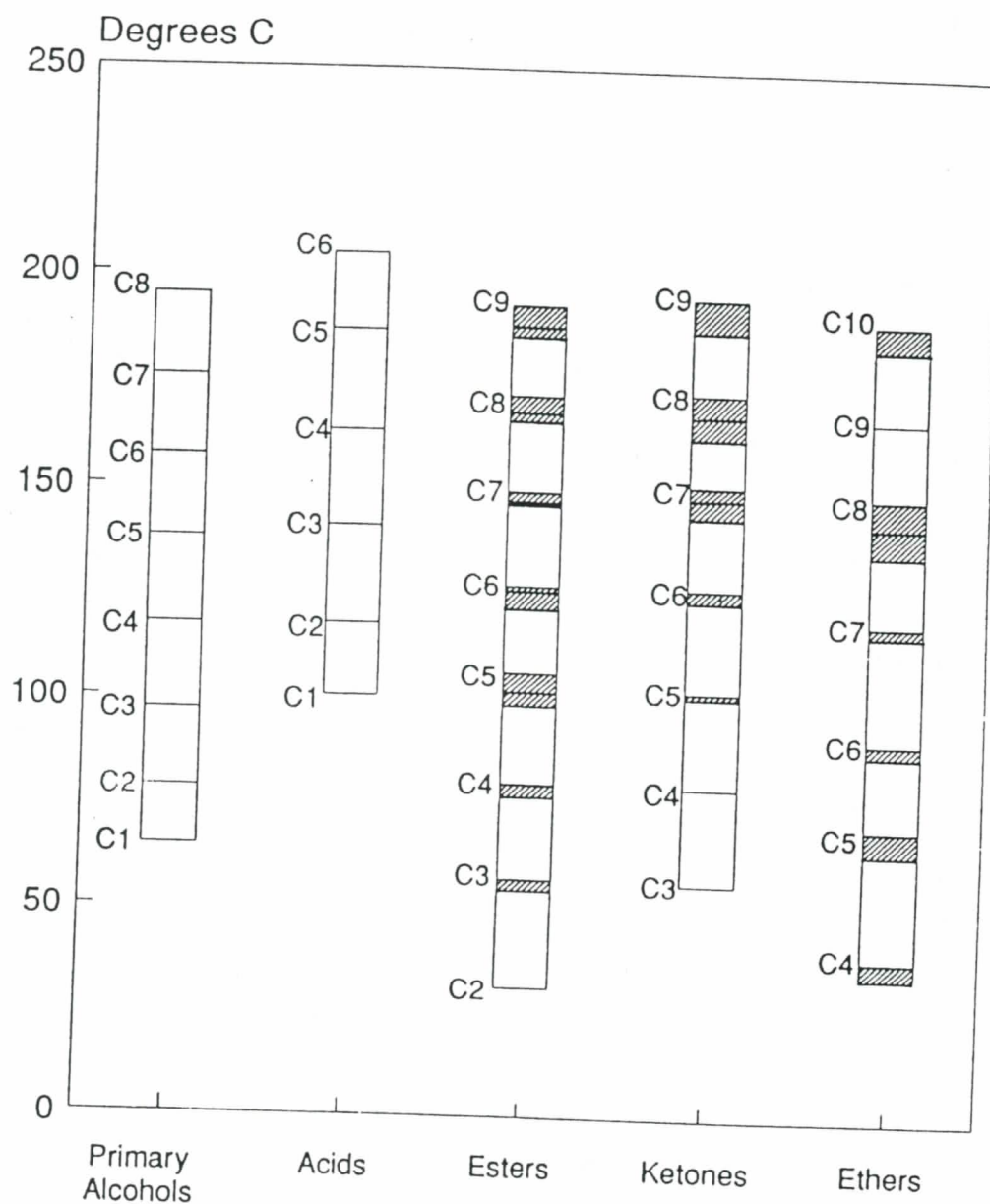


Figure 1. The boiling points of selected organic liquids. Compounds are labeled by the total number of carbon atoms and only straight-chain compounds were considered. Shaded areas depict the boiling point ranges of different compounds possessing the same number of carbon atoms. From reference (31). The boiling point range for gasoline is 60°–200° C.



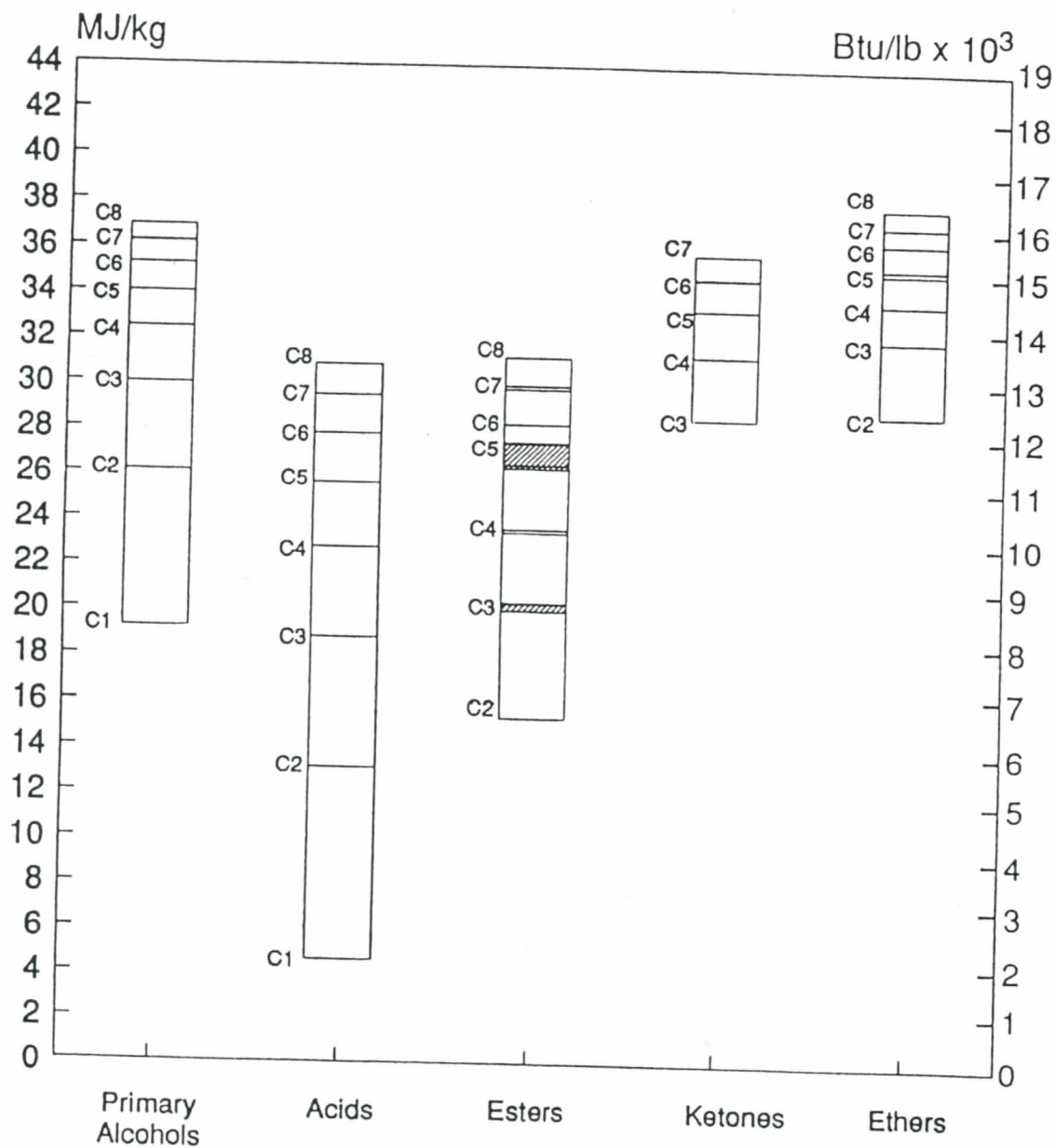


Figure 2. The net heats of combustion for selected organic liquids. From reference (31). The net heat of combustion for gasoline is 46 MJ/kg or  $20 \times 10^3$  Btu/lb.

to the toxicity problem could be an increase in the lower end of the boiling range of gasoline, or its substitute, to make it less volatile, or the development of better devices for prevention of vapor escape during fuel transfer and from fuel tanks. The higher octane numbers and improved combustion of gasoline are two important benefits of blending oxygenated liquids into gasoline. At least two of them [methyl-*t*-butyl ether (MTBE) and ethanol] already have been incorporated into commercial premium gasoline production, even though they have not achieved a full market penetration to date. The citywide tests carried out in Denver, Colorado, over the last three years, which involved a mandatory switch of all cars to gasoline blended with MTBE or ethanol during a smog-prone winter season, showed very positive results in terms of air pollution control. The carbon monoxide levels dropped by 30% and smog formation also decreased (36). In view of these positive results, it is to be expected that other smog-prone cities and states will try to improve their air quality in a similar fashion, and the demand for oxygenated liquid fuels will rise. California, the largest market in the country, is already showing signs of moving in that direction. Oxygenated liquids shown in Figures 1 and 2 also contain no sulfur or nitrogen. Therefore, their utilization in gasoline blends will decrease sulfate emissions, which are a major component of particulate smog and, as sole fuels, will eliminate sulfate emissions altogether.

There are other important properties of automotive fuels, such as heat of vaporization and others, but their full discussion is beyond the scope of the present chapter and interested readers can find them thoroughly discussed in a recent book (32).

### Production of Fuels from Cellulosic Biomass

While both thermochemical (e.g., pyrolysis and gasification) and biochemical (i.e., hydrolysis and fermentation) approaches can be used for the production of liquid fuels from biomass, the emphasis in this chapter will be on biochemical conversion, because thermochemical routes are covered elsewhere in the book. However, the current biochemical systems cannot directly convert all the substrate to liquid fuels, because lignin is very resistant to biological depolymerization and conversion (37). The range of organic products produced by fermentations is also very limited (38-40). These organic products may need to be upgraded to more valuable liquid fuels by using thermochemical routes. Therefore, a combination of thermochemical and biological routes may be necessary for the complete conversion of biomass to liquid and gaseous fuels.

The primary fermentation products produced during anaerobic metabolism by numerous microorganisms include a few simple alcohols, acids, a single ketone (acetone), and two potential gaseous fuels, methane and hydrogen. These products do not normally accumulate during aerobic respiration by microorganisms, yet two groups of highly reduced organic compounds, lipids and poly- $\beta$ -hydroxybutyrate, can accumulate in storage vesicles during aerobic growth (38-41). An interesting feature of some fermentations (see Table III) is that they proceed most efficiently not under strictly anaerobic conditions, but under limited aeration, i.e., in so-called "microaerophilic" mode of operation. Microaerophilic operation does not increase the complexity of fermentation reactors, but does require the accurate monitoring and control of dissolved oxygen levels and of oxygen transfer rates, if cultures are to be prevented from switching to aerobic respiration and production of cell mass instead of the desired products.

The rapid progress in elucidation of metabolic pathways, which occurred after World War II, allowed connection of all these seemingly unrelated products to a single intermediate, pyruvate (41). These microorganisms perform anaerobic glycolysis to derive a high energy source, adenosine triphosphate (ATP), for their growth and maintenance. The production of ATP is accompanied by the coproduction of reduced electron carriers (i.e., nicotinamide adenine dinucleotide [NADH] and nicotinamide adenine dinucleotide phosphate [NADPH]), which in the absence of respiration, must be reoxidized for recycle via the reduction of precursors to primary fermentation products. The final metabolic products are often secreted outside of the cell. There are other primary products secreted

TABLE III

Summary of Fermentations for Potential Fuel Production

Reduced Products	Favored Microorganisms	Usual Substrates	Oxygen Requirements	Optimal pH Range	Optimal Temperature Range (°C)	Products Concentration (wt%)	Product Yield (wt%)	Product Formation Rate (g/L/h)
Ethanol	Numerous species of yeasts	C <sub>6</sub> sugars and di- or trisaccharides	Anaerobic to microaerophilic	3-5	30-41	7-12	45-48	2-8 (5-100)
	<i>Pichia stipitidis</i>	Xylose and C <sub>6</sub> sugars	Microaerophilic	3-5	30-33	3-4	36-41	0.3-0.5
	<i>Candida shehatae</i>							
		<i>Pachysolen tannophilus</i>						
	<i>Zymomonas mobilis</i>	Glucose, fructose, sucrose	Anaerobic	3-5	35-39	10-12	46-49	6-10 (10-100)
Butanol Acetone Ethanol (Isopropanol)	Transformed <i>Escherichia coli</i>	C <sub>5</sub> and C <sub>6</sub> sugars	Anaerobic	6-8	35-37	3-4	41-48	1-3
	<i>Clostridium acetobutylicum</i> and related <i>Clostridia</i>	Starch, xylan, C <sub>5</sub> and C <sub>6</sub> sugars, disaccharides	Anaerobic	4-5	35-37	1-2	25-44	0.5-1 (1-3)
D(-)-2,3-Butanediol	<i>Bacillus polymyxa</i>	C <sub>5</sub> and C <sub>6</sub> sugars, disaccharides, starch, xylan, inulin	Microaerophilic	6-7	30-37	2-2.5	40-46	0.2-1.0
Meso-2,3-Butanediol	<i>Klebsiella pneumoniae</i>	C <sub>5</sub> and C <sub>6</sub> sugars, disaccharides	Microaerophilic	5-6	30-37	9-11	40-46	0.4-2.0 (2-4.5)

TABLE III (CONTD)

Reduced Products	Favored Microorganisms	Usual Substrates	Oxygen Requirements	Optimal pH range	Optimal Temperature Range (°C)	Product Concentration (wt%)	Product Yield (wt%)	Product Formation Rate (g/L/h)
Salts of acetic acid	<i>Clostridium acetivum</i> and related acetogenic bacteria	C <sub>5</sub> and C <sub>6</sub> sugars, syngas (CO <sub>2</sub> + H <sub>2</sub> or CO + CO <sub>2</sub> + H <sub>2</sub> )	Anaerobic	6-8	20-70	1.5-4.5	85	0.6-8
Salts of mixed organic acids	Numerous bacteria	C <sub>5</sub> and C <sub>6</sub> sugars, xylan, starch, cellulose, syngas	Anaerobic	6-8	20-70	2-3	66	0.5-2
Lipids	Yeasts, filamentous fungi	C <sub>5</sub> and C <sub>6</sub> sugars, disaccharides, n-alkanes	Aerobic	3-6	25-35	20%-70% of cell dry wt. (0.4%-3% of culture volume)	15-22	0.2-0.4
Methane	Numerous cocultures of anaerobic bacteria (usually undefined)	C <sub>5</sub> and C <sub>6</sub> sugars to polysaccharides and other organic compounds	Anaerobic	6.5-8	25-40 and 50-70	50%-70% CH <sub>4</sub> (v/v) in the gas phase	27	0.02-0.2 (0.2-2) or 0.1-10 v/v/d (10-100 v/v/d)

Note: Production rates in parentheses apply to advanced laboratory systems.

by microorganisms, notably di- and tri-carboxylic acids from the citric acid cycle and polyols, such as glycerol, sorbitol, or xylitol. These high-value chemicals are not of interest for liquid fuel production because they are nonvolatile and highly oxidized. Lactic acid is of marginal interest for the same reasons.

It must be noted that only three primary fermentation products of interest for fuel production are produced in the homo-fermentative mode (i.e., as the sole reduced product). They are acetic acid, ethanol, and butanediol, but butanediol is the sole product produced under only microaerophilic conditions. Ethanol is coproduced with butanediol during strictly anaerobic fermentations. The remaining products are secreted as mixtures (e.g., butanol/acetone/ethanol) of coproducts as in the hetero-fermentative mode. The important features and requirements of microbial fermentations are summarized in Table III. A survey of Table III clearly shows that the primary substrates for practically all these fermentations are sugars. The transport of substrates and other nutrients through cell membranes of microorganisms is tightly controlled and is limited to low molecular weight compounds. In the case of sugars, the largest molecules that can be transported through the cell membranes are di- and trisaccharides. The higher oligomers and polymers have to be depolymerized by chemical or enzymatic means before the microorganisms can ferment the resulting sugars of low molecular weight. Because biomass components are polymeric, the very first step must be their depolymerization to fermentable units.

### Depolymerization of Polysaccharides

Polysaccharides are carbohydrate polymers in which individual sugar units are joined by acetal groups (i.e., glycosidic linkages between hemiacetal or ketal) on one sugar unit and numerous hydroxy groups on the other. Since all acetal or ketal linkages are sensitive to acid-catalyzed hydrolysis, it is natural that acid hydrolysis of polysaccharides has been under development for a long time. Acid-catalyzed protonation and enzymatic hydrolysis of glycosidic linkages are common mechanisms of degradation of polysaccharides. Polysaccharides can also be degraded by the action of alkaline solutions, but this so-called "peeling" reaction leads to severe structural changes in cellulose and transformations of sugars to poorly fermentable hydroxy-acids. Therefore, this reaction is to be avoided if one is to produce sugars for subsequent fermentation. There are four families of polysaccharides of interest for large-scale production of fermentable sugars. Two are plant storage polymers, starch and inulin, and two are structural polymers in plant cell walls. There are numerous other polysaccharides produced by plants, but none of these are produced in the amounts needed for full production. Because behavior of storage and structural polysaccharides toward acid- or enzyme-catalyzed hydrolysis is significantly different, they are treated as separate categories.

**Hydrolysis of Starch and Inulin.** Starch is a storage polymer of  $\alpha$ -1,4-linked glucose units, which accumulates in granules in the cells of many organisms. The basic structural unit is the disaccharide, maltose (42). The starch chains are either linear (amylose) or branched (amylopectin), with natural starch granules containing various proportions of amylopectin and amylose. The chains of starch molecules appear to be only loosely bonded by interchain hydrogen bonds, which allow easy penetration of water into starch granules and make partially depolymerized starch soluble in water (i.e., maltodextrins and water soluble starch). The starch granules can also be liberated from plant tissues by simple mechanical or chemomechanical treatments (i.e., wet or dry milling). The properties of high surface area, swelling of starch granules in water, and solubility of large intermediate molecules (maltodextrins) in water make depolymerization and solubilization of starch a relatively easy matter (42). Acid-catalyzed hydrolysis in "starch cookers" has been practiced for many decades, and at high temperatures ( $150^{\circ}$ – $200^{\circ}$  C) it can proceed to completion in seconds to minutes. Dilute acid-catalyzed hydrolysis of starch has been recently supplanted by enzymatic catalysis, both at high ( $60^{\circ}$ – $100^{\circ}$  C) and low ( $20^{\circ}$ – $40^{\circ}$  C) temperatures. The thermophilic  $\alpha$ -amylases active at the boiling point of water became

commercially available in recent years and allows concurrent depolymerization by enzymes and gelatinization by steaming to occur. The strength of hydrogen bonds between starch molecules decreases rapidly with increasing temperatures; therefore, penetration of water and swelling of starch granules is aided by heating. However, enzyme systems that can depolymerize raw starch at lower temperatures are being developed in the laboratory, because their use could eliminate the energy consumption for steaming (43).

The ease of starch liberation and hydrolysis has led to its widespread industrial use for the production of high-fructose corn syrup and fuel alcohol. It must be pointed out that  $\alpha$ -amylase alone does not depolymerize starch to glucose, but a second enzyme, glucoamylase, is needed for liberation of glucose from maltodextrins by endwise (exo-) cleavage. The enzyme or acid consumption needed for starch hydrolysis is very low (< 1:100 by wt) and the cost of hydrolysis is a small portion of the total cost as well. Starch conversion, like many large chemical processes, is dominated by raw material cost.

Inulin, a polyfructosan, is a storage carbohydrate in tubers of numerous species of plants, notably dahlias and jerusalem artichokes (44). Like starch, it is very easy to hydrolyze and solubilize to oligofructosans by both acid- and enzyme-catalyzed hydrolysis. Its hydrolyzates could be easily fermented by microorganisms, but lack of widespread cultivation of inulin-producing crops and repolymerization (reversion) of fructose to unwanted oligofructosans, which interfere with the production of pure fructose syrups, are still important problems (44). However, these issues are not insurmountable and inulin-producing crops may see a brighter future.

**Polysaccharides in Plant Cell Walls.** The hydrolysis of polysaccharides in plant cell walls is a much more challenging problem than hydrolysis of storage polysaccharides. The plant cell walls can be simplistically depicted as "runs" of cellulose fibers embedded in the crosslinked matrix of lignin-hemicellulose complexes (16,45). These polymers provide not only structural strength to the plants but also protect them against destruction by physical, chemical, and biological agents. Plant cell walls do not turn over during the lifetime of a plant as do storage polysaccharides. Also, in many cases these structures have evolved to last for hundreds or thousands of years in a relatively harsh environment. Due to intimate and strong associations, the major cell wall polysaccharides cannot be liberated by simple mechanical treatments used successfully on starch granules. Very harsh and destructive chemical treatments used in chemical pulping are necessary to liberate even the cellulose fibers from plant cell walls (23-25). These treatments are usually accompanied by the destruction or severe modifications of hemicelluloses and lignin. The hydrolysis of cell walls of plants usually requires "pretreatments" before high yields of sugars can be achieved during the primary hydrolysis. The very first step or series of steps is disintegration of plant tissues into smaller particles to aid penetration of acidic and enzymatic catalysts. These steps are usually accomplished by efficient chipping and shredding machinery, which is commercially available. It must be noted that shredding and milling of herbaceous plants requires much less energy than the same operation for wood (46). The primary size reduction (i.e., through 1/8 in. screen) is quite efficient; only 50 KW·H/dry ton for aspen wood chips and 6 KW·H/dry ton for wheat straw. Milling to finer particles can be much more energy intensive, because energy consumption increases exponentially with a decrease in particle size. The chipping, shredding, and milling steps are an unavoidable part of the process for hydrolysis of polysaccharides in plant tissues and must be carefully optimized for minimal energy consumption while retaining rates and yields. The acid-catalyzed hydrolyses are more tolerant to larger particles because small acid molecules diffuse through cell walls and plant tissues much more rapidly than the relatively large enzyme molecules (47). However, even in acid hydrolysis, the use of large pulping chips will decrease the yields of sugars. An interesting new method for fiberization of large chips via explosive decompression of steam or gases from plant tissues (steam explosion) has been identified. However, the large pressures required for efficient steam or gas explosion increase the cost of the equipment.

While mechanical disintegration is usually the only pretreatment necessary for acid-catalyzed hydrolysis of polysaccharides in biomass, efficient enzymatic hydrolysis usually requires more dramatic changes in porosity and structures of cell walls. These issues will be discussed further below.

There are three basic methods for hydrolysis of polysaccharides in plant cell walls. The simplest one is hydrolysis with dilute mineral acids (usually sulfuric) at elevated temperatures. The second set of methods involves action of very concentrated strong mineral acids at low temperatures ( $20^{\circ}$ – $60^{\circ}$ C) and the last, most modern methodology involves pretreatment and hydrolysis with enzymes.

**Dilute Acid Hydrolysis.** Treatment of plant cell walls with dilute aqueous solutions of strong mineral acids releases oligomeric and monomeric sugars from polysaccharides. Due to a relatively high activation energy, hydrolysis is accelerated at elevated temperatures, so treatments are usually performed between  $100^{\circ}$  and  $160^{\circ}$ C for hemicelluloses, and between  $180^{\circ}$  and  $220^{\circ}$ C for cellulose (48-51). The primary drawbacks of this cheap and simple process include the simultaneous decomposition of sugars released from polysaccharides, the high cost of corrosion-resistant equipment, and the low concentrations of sugars produced by the percolation approach. The presence of toxins and microbial inhibitors is another well-documented drawback of utilizing dilute acid hydrolyzates (51). The chemistry and kinetics of dilute acid hydrolysis has been extensively studied. Indeed, the process using dilute sulfuric acid was commercialized in Germany before World War II and is in commercial practice in the Soviet Union today.

The dilute acid hydrolysis of polysaccharides is governed by two rate equations. The first equation describes the hydrolysis of polysaccharides to monomeric sugars (formation of intermediate oligomeric sugars is usually omitted) and the second equation describes the decomposition of monomeric sugars to furfuraldehydes. The yield of monosaccharides is controlled by the differences between the rates of hydrolysis and decomposition. This difference is quite high for hemicelluloses, and hemicellulosic sugars can be prepared by dilute acid hydrolysis in very high yields ( $>70\%$ ) (48-51). The problem appears with cellulose, which is semicrystalline and more resistant to hydrolysis than other polysaccharides. The rates of hydrolysis of cellulose and decomposition of glucose are similar and, therefore, yields of glucose are expected to be quite low ( $30\%$ – $50\%$ ). One simple engineering solution to decrease the rate of glucose decomposition has been to decrease the residence time of liquids in high-temperature reactors (51). The so-called "percolation reactors," where a stationary bed of biomass chips is leached with percolating dilute acid, are the accepted design of hydrolysis plants. While this stratagem leads to increased yields, the increased volumes of liquids pumped through the reactor dilute the sugars released from biomass. Therefore, dilute acid hydrolysis in percolation reactors produces very dilute ( $<4\%$ ) sugar streams that are unsuitable for fermentation to liquid fuels. The major stream from these reactors is a solid residue of condensed lignins and other polymers, which has little value except as a solid fuel or substrate for thermochemical processing. Two approaches have been followed recently that could potentially overcome the low glucose yields inherent in dilute acid hydrolysis of cellulose. The approach pursued by Chen and co-workers (52) attempts to exploit differences in activation energies for cellulose hydrolysis and glucose decomposition by increasing the temperature above  $200^{\circ}$ C. However, the reaction times become so short (a few seconds) that scale-up of this approach to industrial size may present difficulties. Another potential improvement is replacement of water with organic solvents, first studied by researchers in the Soviet Union (53). These results indicate that impressive increases in the rates of cellulose hydrolysis and in glucose yields can be achieved by replacing water with some organic solvents such as acetone (53-55). Unfortunately, this research is in a preliminary stage, and important issues such as decomposition and recovery of expensive organic solvents have not been thoroughly addressed.

**Hydrolysis with Concentrated Mineral Acids.** The concentrated acid hydrolyses are unique in solvation and dissolution of polysaccharides, which occur during hydrolysis. These transformations are particularly important for cellulose fibers that lose their crystalline character and are hydrolyzed efficiently under very mild conditions. Very high yields of monosaccharides by hydrolysis with concentrated mineral acids led to the adoption of these methods for quantitative analysis of carbohydrates in biomass, as well as to industrial scale-up in pre-war Germany. Two, strong mineral acids, hydrochloric acid and sulfuric acid, have always been main contenders for industrial development. Concentrated (60%–72%) sulfuric acid is very cheap, and it is both an efficient solvent and a hydrolyzing agent for cellulose. However, sulfuric acid has a very high boiling point (29), which severely limits the number of options for its recovery. The main recovery options that have been investigated are electrodialysis and solvent extraction (48).

Hydrochloric acid is much more volatile than sulfuric acid, which presents possibilities for its recovery by evaporation (48,56). Supersaturated (40%–45%) hydrochloric acid is needed for efficient dissolution and hydrolysis of cellulose, while lower concentrations (30%–35%) are required for hydrolysis of hemicelluloses at room temperatures. The recovery of HCl is simple evaporation, usually under vacuum, until the composition of a high boiling azeotrope (18% HCl, 120 °C) is reached. Overcoming this obstacle is a major problem in concentrated HCl hydrolysis. The cost of hydrochloric acid is also several-fold higher than for sulfuric acid. It is highly corrosive to many metals and alloys, and its recovery requires specialized and expensive equipment (56). The high consumption of acids, about equal to the weight of sugars produced, is the major problem in all concentrated acid processes (48,56). While recovery of acids is technically feasible, traditional approaches are too expensive for commercial use (56). Since their conception, attempts have been made to decrease the consumption of acids. Simple solutions are not yet available, because several criteria have to be fulfilled simultaneously: all tissues must be uniformly wetted with acid, the acid concentration must be maintained within prescribed limits, and certain amounts of water seem to be required for swelling and hydrolysis of cellulose. The uniform wetting of biomass is not a problem with volatile hydrochloric acid, but it is a serious obstacle with the nonvolatile, sulfuric acid. Two approaches seem to have been tested successfully in the laboratory. One centers on redistribution of acid by the high shear mixing of wetted biomass and another one relies on spraying the biomass with an excess of dilute sulfuric acid followed by the evaporation of water until a desired acid concentration is reached. While both approaches offer improvements in sulfuric acid consumption, neither decreases it to the point where acid recovery will become a minor issue. The concentration of sulfuric acid should also be maintained below 70%–75%, because at or above this strength, the acid sulfonates hydroxyl groups on sugar units, and care must be taken to desulfonate glucose with dilute acid during subsequent posthydrolysis.

**Pretreatment and Enzymatic Hydrolysis** Some of the problems plaguing the acid hydrolysis processes can be overcome by the relatively new technology of enzyme-catalyzed hydrolysis, which has been developed over the last 20 years. In simple terms, it is hoped that enzymatic hydrolysis can achieve high yields of sugars at low catalyst consumption under environmentally benign conditions.

The hydrolytic enzymes specific for polysaccharides do not require metal cofactors, are biodegradable, and should even be edible like many other proteins. Minimal environmental impact could also be an important consideration for future scale-up in an era of increased environmental concern. Enzymatic hydrolysis can also be made compatible with fermentations by judicious choice of enzymes and microorganisms. When a separately produced enzyme is added to the fermentation together with a polymeric substrate and microorganism, the system is called simultaneous saccharification and fermentation (SSF) (57). Rapid removal of sugars by fermenting microorganisms decreases the end product inhibition, thus permitting decreased enzyme consumption. This part of the process step also is simplified, because only one reactor is used instead of



the two required for separate hydrolysis and fermentation (SHF). An example of a successful application of this concept is the production of alcohol from starch, where glucoamylase is added to partially hydrolyzed starch together with yeast. The production of some oriental foods and beverages (e.g., soy sauce and rice wine) employs this concept as well. Enzymatic hydrolysis and fermentations can be simplified further in direct microbial conversion (DMC), where fermenting microorganisms also produce hydrolytic enzymes. Direct microbial conversions are in limited use at the present time, because very few fermenting microorganisms produce the required hydrolytic enzymes or have tolerance for high levels of the fermentation product (e.g., ethanol). One successful example of an industrial application is the anaerobic digestion of polymers in sewage sludge and agricultural wastes that creates a mixture of methane and carbon dioxide called biogas. Acid-catalyzed hydrolysis and microbial survival are, of course, incompatible and the steps must always be performed in separate reactors.

Despite the obvious advantages enzymatic hydrolysis offers, the enzymes are much more expensive on a weight basis than simple mineral acids; and cell walls of plants are very resistant to enzyme attack. Carbohydrates in native wood or agricultural residues are hydrolyzed slightly by available cellulase and hemicellulase enzymes. The enzymatic release of soluble sugars usually amounts to 5%–10% of the polysaccharides in biomass. Therefore, most biomass particles must be pretreated before significant enzymatic hydrolysis can occur.

Pretreatment methods are usually segregated into four main categories: mechanical, other physical, chemical, and biological (58-63). Mechanical methods, such as ball, rod, or compression milling achieve very high impact and shear forces and are effective pretreatment methods both for cellulose fibers and biomass particles. These methods can decrystallize cellulose and tear up the cell walls to such a degree that polysaccharides become more hydrolyzable by both acids and enzymes. However, intensive mechanical pretreatments suffer from high energy consumption, on the order of several hundred kilowatt hours per ton of biomass, and a low throughput (63). Low energy efficiencies rule out purely mechanical pretreatments for biochemical fuel production. Other physical pretreatments, such as bombardment with X-rays and other energetic particles can modify both cellulose and cell walls and improve yields in enzymatic hydrolysis, but they introduce radiation problems and are not very efficient (58-63).

Biological pretreatments with ligninase-producing microorganisms are still in their infancy, require long reaction times (i.e., weeks), and because ligninolytic microorganisms usually produce cellulases and hemicellulases and grow on resulting sugars, they suffer from diminished yields. Sugar loss could be decreased, however, by development of cellulase and hemicellulase negative mutants.

The pretreatment methods with the highest near-term potential appear to be chemical or chemomechanical. Some have a long research history, due to interest in the production of feeds for ruminant animals, such as cattle and sheep. All borrow heavily from chemical methods developed for other conversion processes, such as pulping and fiberboard production. The chemical pulping processes can also be used for the production of cellulose substrates for enzymatic hydrolysis. Large-scale feeding of wood pulp to cattle was practiced in Sweden during World War II. However, these processes are quite expensive and products have too high a value for utilization in biochemical fuel production. In addition, the dominant Kraft pulping process destroys hemicelluloses, which are a significant part of the total carbohydrates in biomass (16,25,48,64). The pulping and wood modification chemistry can be adjusted, however, to provide biomass pretreatments that are compatible with subsequent enzymatic hydrolysis.

The most important objective of pulping processes is the production of long, strong, and adherent fibers that can be matted (felted) into paper sheets (64). Better grades of paper also require bleached pulp. Long fibers are of no value for enzymatic hydrolysis, and their color is unimportant as well. Long fibers are actually detrimental to subsequent biochemical steps because their propensity for matting and bridging make pumping and mixing difficult. The minimal pretreatment requirement is the production of fine particles

with a high surface area accessible to cellulase enzymes. Small particles can be produced by mechanical milling or by explosive decompression, but the formation of cellulose surfaces accessible to cellulase enzymes requires dissolution and extensive modification of other components in plant cell walls. Lignin-hemicellulose complexes (59-63) and acetyl groups (20,65) in xylans were all identified as major barriers to enzymatic hydrolysis. These complexes can be broken by alkaline or acidic treatments, however. Solutions of alkali metal hydroxides are known to break lignin-hemicellulose bonds and dissolve both lignin and hemicelluloses. At very high concentrations (5%–20%) they also swell cellulose. The degradative and dissolving action of hot sodium hydroxide solutions is used in well-known soda and Kraft pulping processes. During these treatments acetyl groups are also hydrolyzed. The action of hydroxide solutions in pretreatments must be adjusted to lower the severity from that found in pulping in order to preserve hemicellulosic carbohydrates. This goal seems to be achievable with grass residues (e.g., wheat straw and corn stover) and some hardwoods, but has not been achieved for softwoods. While alkaline pretreatments can be performed with many substrates at ambient temperatures, they are not truly catalytic, and significant amounts of chemicals are consumed for neutralization of acidic carboxylic and phenolic groups in biomass. The recycle and regeneration of hydroxides are thus critical research issues. These pretreatments do not hydrolyze polysaccharides, at least not without simultaneous destruction of sugars. The enzyme mixtures for subsequent hydrolysis must contain both cellulase and hemicellulase enzymes and thus are more complex than enzyme mixtures required for hydrolysis of pure cellulose.

An alternative base used for alkaline pretreatment is ammonia. A whole variety of pretreatment techniques ranging from treatment with ammonia gas to treatment with liquid ammonia has been developed over the past 90 years. Agricultural residues seem to be the most susceptible to this type of pretreatment, with hardwoods giving a variable response and softwoods being very resistant. Treatment with liquid ammonia is rather unique, because cellulose fibers are swollen and recrystallized by this liquid.

Because hemicelluloses can be easily hydrolyzed to monomeric sugars by hot, dilute solutions of mineral and organic acids, the next set of pretreatment methods have evolved around acid-catalyzed prehydrolysis of hemicelluloses in biomass (19,66-73). Depending on hydrogen ion concentration, these pretreatments can be performed at temperatures between 100° and 220°C, and with reaction times ranging from minutes to hours. At very high temperatures (> 160°C) no acidic catalysts are required, because acidic compounds released from biomass provide the catalytic effect (68-69). This family of very simple pretreatments, because they involve only high-temperature steaming of biomass, is called "autohydrolysis" or, when explosive decompression is involved, "steam explosion." Conditions and equipment for steam explosion are similar to the commercial masonite process developed in the 1930s for the production of fiberboard. Hemicelluloses are partially hydrolyzed and solubilized during autohydrolysis, and lignin condenses into spherical particles. The pretreatment is very effective with some substrates, namely grasses and hardwoods, but softwoods are again resistant. The reaction times are short at temperatures higher than 190°C, and corrosion-resistant equipment is unnecessary. The main drawback of autohydrolytic pretreatments is the relatively low yield of hemicellulosic sugars (~50%) due to partial hydrolysis and pyrolytic decomposition at high temperatures (69). Baugh and coworkers (69) obtained evidence that alkaline degradation of sugars can extend to acidic pH values at high temperatures. The very high temperatures can be avoided by the addition of small amounts of inorganic acids, such as sulfuric acid or sulfur dioxide. These so-called "dilute acid pretreatments" (19,70) proceed rapidly anywhere between 140° and 200°C. Hemicelluloses are hydrolyzed to monomeric sugars at yields exceeding 70%. The removal of hemicelluloses and concurrent condensation of lignin creates numerous large pores in hardwoods and grasses, which allow penetration of cellulase enzymes to cellulose fibers (19,71). As in autohydrolysis, the creation of large pores is insufficient in softwoods to allow high enzymatic digestibility of cellulose. Reaction with sulfur dioxide seems to improve susceptibility of softwoods, however (72,73). The mechanism of its action is not quite clear, because extensive sulfonation of

lignin does not occur. Lignins are retained in pretreated solids, which shows that its removal is unnecessary for efficient enzymatic hydrolysis of some biomass substrates. The retention of lignins, however, dilutes the cellulose stream intended for enzymatic hydrolysis and fermentation. Lignins can also adsorb significant amounts of cellulase enzymes (74,75). Its removal can thus have beneficial effects for downstream processing. More or less selective removal of lignins can be accomplished by the addition of organic solvents (usually lower alcohols) to acidic or alkaline aqueous solutions in numerous variants of organosolv pulping and pretreatments (58-63), or by sulfonation of lignins with sodium bisulfite, as in bisulfite pulping. Lignins and lignosulfonates are not fermentable, so they should be removed from sugar solutions by precipitation or similar means. The high cost of organic solvents, in comparison to water, limits the choice of solvents in organosolv pretreatments and makes solvent recovery an important consideration. The acidic bisulfite pretreatment, in contrast, would be easy to scale up because it is very similar to commercial acid sulfite pulping processes.

All the efficient pretreatments listed above provide cellulosic substrates that are hydrolyzable by potent cellulase enzymes to glucose and cellobiose in very high yields, i.e., 80%–100%. However, these pretreatments seem to be limited to certain substrates, such as grasses and the woody tissue of hardwoods. Softwoods are responsive to delignifying pretreatments or require the partial acid hydrolysis of cellulose before high yields in enzymatic hydrolysis of cellulose can be achieved (72,73). The few reported attempts (76; Torget, R.; Himmel, M.E.; Grohmann, K. *Bioresource Technol.*, in press) at pretreatment of softwood and hardwood barks indicate that some barks are resistant to pretreatment methods that are effective with wood. Because bark is a very important component (10%–40% wt) of branches and juvenile stems, pretreatment methods need to be developed for enzymatic hydrolysis of the carbohydrates in bark, or selection of trees for fast rotation cultivation should take this factor into account. Herbaceous legumes also appear to be more resistant to some pretreatments (18; Torget, R.; Werdene, P.; Himmel, M.E.; Grohmann, K., *Appl. Biochem. Biotechnol.*, in press). Ultimately, the substrates, pretreatment methods, and cellulolytic enzymes need to be closely matched in the overall processes for biochemical conversion, because they show a strong interaction in terms of rates, yields, and enzyme consumption. Results from our own series of dilute acid pretreatments of various plants indicate that significant variations in rates of enzymatic hydrolysis can be observed for different substrates milled to the same particle size and pretreated under identical conditions (18,19,46,78). Cellulose in pretreated corn cobs hydrolyzes the most rapidly, followed by cellulose in various pretreated grasses. Cellulose in pretreated hardwoods was hydrolyzed at the lowest rates. Whether these differences in the rates of enzymatic hydrolysis reflect inherent differences in cellulose fibers and cell walls, or are a reflection of different rates of enzyme penetration into pretreated plant tissues, is unknown at the present time.

The need for careful matching of some pretreatment methods and cellulase enzyme systems used for subsequent hydrolysis results from the significant differences among cellulase enzyme complexes produced by various microorganisms. Microcrystalline cellulose is never hydrolyzed by a single enzyme. Cooperative hydrolysis by one or more endoglucanases (EC 3.2.1.4) and exoglucanases (EC 3.2.1.91) is needed for efficient hydrolysis of cellulose to soluble sugars, usually a mixture of glucose and cellobiose (77). Conversion of cellobiose to glucose requires the presence of  $\beta$ -D-glucosidases (EC 3.2.1.21) or exoglucosidases (EC 3.2.1.74). The complexity of cellulase systems is increased in many anaerobic bacteria that produce very large ( $>2 \times 10^6$  daltons) complexes called cellulosomes that are attached to outer cell walls. The penetration of individual enzymes to cellulose fibers and the rates and yields in enzymatic hydrolysis can thus be affected by selection of individual enzyme components. The commercial cellulase enzymes available today are mainly of fungal origin, and primarily from mutants of various *Trichoderma* and *Aspergillus* strains. The crude commercial preparations are complex mixtures of two or more endoglucanases and exoglucanases with additional hemicellulases,  $\beta$ -D-glucosidases and exoglucosidases, acetylxyln esterases, and other hydrolytic enzymes. There are

numerous other cellulase systems that have been investigated in recent years by biochemists and molecular biologists, but these cellulases have not been introduced to the industry yet. Their potential for cellulose saccharification remains undetermined.

Therefore, the following discussion of enzymatic hydrolysis will apply to *Trichoderma* cellulases, which have received the most R&D attention over the last 20 years and are available in commercial quantities. The various *Trichoderma* mutants are prolific producers of cellulases and other hydrolytic enzymes. The secreted protein concentrations of about 50 g/L have been observed (78), which is an unusually high concentration of secreted microbial enzymes. The *Trichoderma* enzyme complex is active in the pH range of 3-6 and at temperatures up to 55°C. The high retention of activity over a period of several days has been observed at a temperature of 45°C, and the stability increases as the temperature is decreased below this limit. The problems with *Trichoderma* cellulase systems, and perhaps with cellulases in general, are relatively low specific activity, sensitivity to end product (i.e., glucose and cellobiose) inhibition, and low levels of  $\beta$ -D-glucosidase enzymes (78). The low specific activity leads to high enzyme loading requirements. Even with newer commercial preparations of higher specific activity, approximately 1 kg of enzyme is needed for hydrolysis of 50 kg of cellulose fibers. By comparison, the consumption of more active amylases in starch hydrolysis is much lower. The severe end-product inhibition of *Trichoderma* exoglucanases by cellobiose makes this enzyme system unsuitable for the production of concentrated (10%–25%) sugar solutions. This inhibition can be overcome by the application of simultaneous saccharification/fermentation systems, where yeast or other fermenting microorganisms remove the sugar as soon as it is formed. The development of SSFs requires careful matching of components, or a compromise between the physical and chemical requirements of enzymes and microorganisms.

The low levels of  $\beta$ -D-glucosidase secreted by many mutants of *Trichoderma reesei* lead to a requirement for supplementation by  $\beta$ -D-glucosidases from other microorganisms (78). This expensive supplementation can be decreased or eliminated by utilization of microorganisms that ferment cellobiose.

The microcrystalline nature of cellulosic fibers, the restriction of hydrolysis to the surfaces of cellulose fibers, and changing porosity of pretreated materials all combine to decrease the rates of both enzymatic and dilute acid hydrolysis of cellulose. The tenfold decline between initial and final rates of cellulose hydrolysis has been frequently observed (62) and enzymatic hydrolysis of pretreated cellulosic substrates usually takes one to five days. Austrian and other researchers (62,79) obtained results indicating that perhaps two fractions of cellulose with different rates of enzymatic hydrolysis exist in pretreated cellulose fibers, but other explanations, such as enzyme inactivation and decreased surface area, are also possible. Diversity of cellulose fibers in cell walls and plant tissues may also manifest itself in different rates of enzymatic hydrolysis. The slow rates of enzymatic hydrolysis of cellulose cannot be simply increased by increasing the enzyme loading, because available surfaces become saturated with enzyme molecules. However, all pretreatments that increase surface area or simultaneously change both crystallinity and surface area (e.g., in ball-milled and reprecipitated celluloses) are effective in increasing the rates of enzymatic hydrolysis of cellulose fibers. While many of these methods are too expensive for industrial use, the preparation of clean cellulose fibers with high surface area remains one of the objectives for pretreatment research.

Due to a short research history, enzymatic hydrolysis of cellulosic materials requires significant R&D investment before it can become as efficient as enzymatic starch hydrolysis and can be adopted by the industry for commercial sugar and alternative fuel production. Key improvements are needed in decreasing enzyme consumption and enzyme cost. Enzyme recycle, increased specific activity, resistance to end-product inhibition, and increased productivity by genetically engineered microorganisms are obvious avenues to achieve this goal. The development of improved pretreatment methods and integration with naturally susceptible substrates needs to be addressed as well. Enzymatic hydrolysis of cellulosic substrates has already achieved significant

progress in the most important objective, high yields of sugars for fermentation. Modern methods in enzymology, genetics, and biochemical engineering make achievement of other goals possible as well. The sugars produced by enzymatic or acid hydrolysis can then be fermented by appropriate microorganisms to liquid or gaseous fuels. The fermentations of major interest for liquid fuel production can be grouped according to end products such as ethanol, acetone/butanol/ethanol, or organic acids. The major fermentation of organic materials to a gaseous fuel is an anaerobic digestion producing mixtures of methane and carbon dioxide, often called "biogas." The possibilities in the biological production of hydrogen are being explored as well (34).

### Ethanol Fermentations

Numerous yeast species (80) and two species of bacteria in the genus *Zymomonas* (81) efficiently ferment six-carbon sugars to ethanol and carbon dioxide. A few filamentous fungi, notably within the genera *Fusarium*, *Rhizopus*, and *Paecilomyces* can ferment both five- and six-carbon sugars to ethanol and CO<sub>2</sub>, but at lower rates and final concentrations of ethanol. Many bacteria ferment sugars to ethanol as a coproduct with lower organic acids, butanediol, and acetone/butanol (41). The yeasts and *Zymomonas* are of primary interest for industrial production of ethanol because the conversion yields and rates are high and ethanol can be accumulated in relatively high concentrations of 5%–12% (w/v). *Zymomonas* have even higher yields and rates of ethanol production than yeast strains, but they suffer from a very limited range of fermentable sugars (i.e., glucose and fructose). *Zymomonas* are also very ethanol-tolerant and produce ethanol in very high concentrations (Table III). The high ethanol concentrations (>4% w/v) are necessary for efficient separation of azeotropic (95%) or anhydrous ethanol by distillation of fermented media (82).

Yeast strains can ferment a variety of six-carbon sugars and their oligosaccharides. A few of them (notably *Saccharomyces diastaticus*, *Endomycopsis fibuligera*, and *Schwanniomyces castellii*) secrete amylolytic enzymes and can ferment starch (80). However, none of the yeasts (83) can ferment five-carbon sugars (i.e., xylose and arabinose) or uronic acids to ethanol under anaerobic conditions, and none of the known fermenting yeast strains produce cellulase enzymes in nature (80). A few cellulase producing yeast species were recently identified, but they are strictly aerobic and do not ferment sugars to ethanol. The inability of yeasts to directly ferment five-carbon sugars, cellulose, or hemicelluloses to ethanol poses unique problems for the development of fermentation processes using lignocellulosic biomass as a substrate. Xylose, and to a much lesser extent arabinose, are major building blocks of hemicelluloses in hardwoods and other angiosperms. Xylose and arabinose are thus very important components of total sugars produced by enzymatic or acid-catalyzed hydrolysis of numerous plants in the angiosperm family. They are of lesser importance only in hydrolyzates from softwoods and wastepaper because major hemicelluloses in softwoods are glucomannans and galactoglucomannans; and wastepaper is enriched in cellulose at the expense of hemicelluloses (Table I). Three major approaches are being pursued that provide systems for fermentation of xylose to ethanol (83). Isolation and identification of microorganisms fermenting xylose to ethanol led to identification of several yeast strains such as *Candida shehatae*, *Pichia stipidis*, *Pachysolen tannophilus*, and *Candida utilis*, which ferment xylose to ethanol under microaerophilic conditions (i.e., under limited and controlled supply of oxygen). The yields of ethanol are currently lower (approx. 80%), fermentation rates are significantly slower, and ethanol concentrations are also lower than those obtainable by fermentation of glucose by industrial yeast strains. The performance of these yeasts can be improved by genetic techniques, and research work in this direction is already in progress (84).

The second approach relies on isomerization of xylose to the keto-sugar, xylulose, which is fermentable by some yeast strains (83,85). The isomerization is performed by bacterial xylose isomerases, some of which are commercially available as glucose

isomerases and are used in large quantities for the production of high fructose corn syrups (85). The cost of xylose isomerase production can be decreased by overproduction in genetically engineered bacteria, and immobilization can increase pH compatibility between fermenting yeasts and immobilized xylose isomerase enzymes. The immobilized xylose isomerase enzyme and yeasts are usually combined with xylose substrate in one simultaneous isomerization and fermentation system (SFIX) because fermenting yeasts can remove xylulose as it is formed and drive the isomerization reaction to completion. The equilibrium between xylose and xylulose is 85:15, respectively, and is otherwise unfavorable to the production of xylulose. The yields of current SFIX fermentations (approx. 70%–80%) are similar to those obtainable with xylose fermenting yeasts, but the rates of ethanol production appear to be higher (85,86) and the ethanol tolerant yeast strains (e.g., *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*) can be used.

The third and newest approach involves construction of ethanologenic bacteria by transfer and expression of pyruvate decarboxylase and alcohol dehydrogenase genes from *Zymomonas* to other bacteria, such as *E. coli* (87-89). The activity of these two enzymes seems to be sufficient to change the metabolic pathway of *E. coli* from mixed acid production to ethanol as a major product. The reported yields and rates of these fermentations are very high, and arabinose can be used for ethanol production as well. The production of organic acids can be decreased by mutagenesis, and the whole enzyme system can potentially be transferred to other (e.g., cellulolytic) bacteria.

It can then be concluded that major improvements have been achieved in the fermentation of xylose to ethanol during the last 10 years, and the remaining obstacles can be overcome by genetic modifications. The fermentations of pretreated cellulosic substrates cannot be performed directly by yeasts because they are not cellulolytic, but require separate or simultaneous hydrolysis by cellulolytic enzymes produced by other microorganisms. Some fungal cellulases, such as those produced by *T. reesei*, are very compatible with yeast fermentation in terms of pH and fairly compatible with respect to temperature. The combination of *T. reesei* cellulase enzymes and thermotolerant yeast strains helped overcome the sensitivity of enzymes to end-product inhibition and decreased enzyme consumption in simultaneous saccharification and fermentation systems. High yields and reasonable final concentrations of ethanol (4%–7% v/v) have been achieved in this system using both wood pulp and pretreated cellulosic substrates. Measurements of transient sugar and ethanol concentrations clearly shows that the rates of SSF are limited by rates of cellulose hydrolysis, with yeast fermentation being a limiting factor only at the initial stage of SSF. Further improvements in the rates of SSF thus require continued development of more active enzymes and better pretreated substrates.

A significant difference between SSF of biomass and starch hydrolyzates is the presence of solid substrate throughout the SSF of pretreated cellulosic biomass because cellulose does not become solubilized until the very end of SSF. Because the higher rates of enzymatic hydrolysis are achievable by increasing the concentration of solid substrate, the higher rates of SSF should be achievable by increasing the substrate concentrations. This simple solution for increased rates and ethanol concentrations in SSF introduces mixing problems. Pretreated biomass particles, or fibers, can easily be stirred only at concentrations of less than 10% w/w. As concentrations increase above 10% w/w, the particle slurries rapidly change to wet solids and cannot be mixed by conventional impeller mixers. The improvements in SSF of biomass, which can be achieved by increasing the substrate concentration above 8%–10%, will require development of new large reactors equipped with high solids mixers. Our preliminary results in high solids SSF of pretreated wheat straw (90) indicate that significant increases in the rate of ethanol production and final ethanol concentration can be achieved by increasing the initial cellulose concentration from 8% w/w to an optimum value between 12.5% and 15% (w/w). Due to the presence of lignin in pretreated wheat straw, these concentrations of cellulose correspond to total solids concentrations between 12% and 23% (w/w). Above 12.5%–15% w/w cellulose concentrations, ethanol yields rapidly drop due to inhibition of yeast by ethanol.

### Acetone/Butanol/Ethanol Fermentation (38,39)

The acetone/butanol/ethanol (ABE) fermentation was conceived by Chaim Weirman circa 1904. Demand for industrial solvents during World War I led to its scale-up to industrial production both in the United States and in Europe. After decades of successful operation, the plants were commonly shut down in the 1940s and 1950s because the process could not compete with cheap petrochemical routes. However, under special circumstances, such as in South Africa, the process has survived even to this day.

The ABE fermentation is carried out by numerous bacterial species in the genus *Clostridium*, which are commonly known as "butyl" organisms. Two species were developed for industrial solvent production, namely *C. acetobutylicum* and *C. beijerinckii*. Some *Clostridia* can also reduce acetone and produce minor amounts of isopropanol. The ABE fermentation proceeds in two stages, controlled mainly by hydrogen ion concentration. During the first stage, which starts at neutral pH, organic (mainly acetic and butyric) acids are produced. As the pH drops below pH 5.5 and inhibitory concentrations of undissociated acids start to accumulate, the bacteria respond by switching to the solventogenic phase where neutral solvents are produced, partially by uptake and reduction of preformed organic acids. Strains of *C. acetobutylicum* are amyolytic and, thus, can ferment starch directly. They also ferment all five- and six-carbon sugars that are present in hemicellulose hydrolyzates. Therefore, ABE fermentation does not suffer from the five-carbon sugar problem, which are major obstacles for ethanol production. Numerous hemicellulolytic and weakly cellulolytic strains of *C. acetobutylicum* were recently identified and, after proper genetic improvement, could be used for direct conversion of pretreated cellulosic biomass to ABE solvents. The total solvent yield is approximately 37% (w/w) of sugar consumed and the approximate ratio of butanol:acetone:ethanol is 6:3:1, respectively, on a weight basis. These ratios can be changed by changes in fermentation conditions, strain differences, and mutagenesis; but usually butanol remains a dominant product. The rates and yields in ABE fermentations are comparable or slightly lower than yields in ethanolic fermentations, but high toxicity of butanol limits the final solvent concentrations to approximately 2% (w/w) and initial substrate concentrations to approximately 60 g/L. Ethanol and acetone are much less toxic than butanol. The production of liquid fuels by ABE fermentation requires low energy consumption in all steps of the process, and it is in product recovery (82) where ABE fermentation suffers most in comparison with ethanol production. The boiling point of butanol is higher and, due to its low concentration in fermented media, the energy consumption for its recovery by distillation is much higher than for ethanol. A whole array of methods from membrane separations to solvent extraction, has been investigated in recent years. Decreased energy consumption for butanol recovery and increased solvent production rates were the major aims. While some of these modern approaches appear promising, none have been scaled up and replaced traditional distillation. Attempts to increase the butanol tolerance of *C. acetobutylicum* by mutagenesis have not been highly successful; therefore, the development of energy-efficient separation methods appears to be a most important avenue for the improvement of ABE fermentation.

### 2,3-Butanediol/Ethanol Fermentation (39)

2,3-Butanediol fermentation is conducted by many strains of bacteria. The bacteria that received the most R&D attention are various strains of *Bacillus polymyxa* and *Klebsiella (Aerobacter) pneumoniae*. *B. polymyxa* produces optically active D-(-)-butanediol, while strains of *K. pneumoniae* produce racemic meso 2,3-butanediol. This fermentation is strongly controlled by aeration. Under anaerobic conditions, approximately equimolar amounts of ethanol and 2,3-butanediol are produced by all strains. Limited aeration decreases ethanol production and 2,3-butanediol becomes a major, or sole, product. Both five- and six-carbon sugars are fermented, and some strains of *B. polymyxa* are cellulolytic, hemicellulolytic, and/or amyolytic (39). The possibilities thus exist for direct microbial

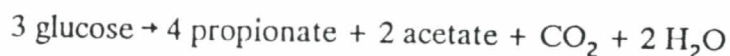
conversion of pretreated biomass to 2,3-butanediol, and all sugars in biomass can be utilized. The final solvent concentration is relatively low (2%–3% w/v) with *B. polymyxa*, but *K. pneumoniae* can accumulate much higher levels (6%–8% w/v). The rates of solvent production are comparable to ethanolic and ABE fermentations, but product recovery presents a major problem. 2,3-Butanediol has a very high boiling point and heat of vaporization. It forms complexes with water molecules and is very hydrophilic. This combination of properties makes efficient recovery very difficult. The only process tested on the pilot-plant level involved evaporation of water and vacuum distillation of 2,3-butanediol from evaporator bottoms. 2,3-Butanediol is also not a valuable liquid fuel. It has a high boiling point and relatively low heat of combustion. However, it can be easily rearranged and dehydrated to 2-butanone, which is an excellent liquid fuel. Experimental work indicates that this acid-catalyzed diol-rearrangement can occur in aqueous solutions acidified with sulfuric acid, while 2-butanone is being distilled off. The recovery of anhydrous 2,3-butanediol may not be necessary for the production of 2-butanone, and difficulties in recovery of 2,3-butanediol may be decreased.

### Fermentations to Volatile Organic Acids (33,41,82,91)

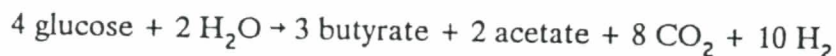
Several organic acids are produced during fermentation of sugars by numerous strains of bacteria. The acids of interest for fuel production are two- to five-carbon aliphatic acids from acetic to valeric. Lactic acid has a high boiling point and has a low heat of combustion; therefore, its production is not considered here. Some lower acids, namely acetic and propionic, can be produced as major or sole products, but most often mixtures of acids and alcohols are produced in "mixed acid" fermentations. The formation of mixed organic acids from pyruvate is accompanied by the formation of hydrogen and CO<sub>2</sub> if formate is decomposed by formate lyase or part of the carbon is secreted as formate. It must be emphasized that free organic acids are not produced during these fermentations because they are toxic to microorganisms. The fermentations are conducted near neutral pH, and salts of organic acids with added base are the actual products. Sodium, potassium, magnesium, calcium, and ammonium hydroxides, or carbonates, are usually added with the substrate or during fermentation to maintain the high pH values and neutralize organic acids as they are formed. This inherently high base consumption is an important feature of organic acid fermentations, unlike ethanol and ABE fermentations, where neutral solvents are produced and carbon dioxide is simply gassed off. All recovery schemes for products from organic acid fermentations should include recovery of basic cations which except for the calcium from limestone, are rather expensive. Production of acetic acid (i.e., vinegar) by strains of *Acetobacter* is a partial oxidation of ethanol to acetic acid and thus, is of limited value for fuel production. Homo-fermentative conversion of sugars to salts of acetic acid (82) is one of the recent additions to fermentation technology. This conversion is carried out by several species of "acetogenic" bacteria. *Clostridium thermoaceticum* and *Clostridium thermoautotrophicum* appear to have the highest potential because they can ferment both glucose and xylose to acetate. The acetogens can ferment fructose and a few other six-carbon sugars to three moles of acetate per mole of sugar utilized. Two moles of acetate are produced by decarboxylation of pyruvate, while the third mole is produced by reduction and incorporation of CO<sub>2</sub>. These bacteria are quite unique because they can also produce acetate from mixtures of hydrogen and CO or CO (i.e., syngas), formate, methanol, and other one-carbon compounds. The syngas conversion will be discussed later in the chapter. While fermentation of sugars is quite rapid and reasonable concentrations of acetate (i.e., 0.25 M) can be accumulated, the isolates studied so far suffer from a limited range of sugars utilized for acetate production and require rather complex media. Isolation of new strains and species, or transfer of genes for hydrolytic enzymes, may alleviate some of those problems. Propionate can also be produced by "propionic bacteria" and other bacterial species. The propionate is coproduced with acetic acid, and sometimes with smaller amounts of succinic acid.



according to the relationship:



Succinate is a usual precursor of propionate and can be secreted from some strains with minor amounts of other products. Propionic bacteria can use a variety of five- and six-carbon sugars and some disaccharides. Butyric acid is produced by many *Clostridia* and other bacteria according to the relationship:



Large amounts of hydrogen are thus produced in butyric acid fermentations. Some *Clostridia* can carry this fermentation farther and switch to ABE production in the second stage. *Clostridia* and other butyric acid-producing bacteria can utilize a wide variety of sugars, and some are cellulolytic and hemicellulolytic. Furthermore, the direct microbial conversion of cellulose and hemicelluloses to volatile organic acids occurs in rumens of animals and as a first stage in anaerobic digestion.

Many other bacteria, namely *Enterobacteriaceae*, ferment sugars to mixtures of organic acids, such as lactic, acetic, succinic, butyric, etc., with neutral coproducts, such as ethanol and butanediol. The coproduction of several acids is one of the drawbacks of acid fermentations, with the exception of acetic acid production. Another drawback for further conversion of organic acids to liquid fuels is their production in the form of salts, usually combined with inorganic cations. Three chemical methods have been investigated for the conversion of volatile organic acid salts to liquid fuels. A very simple thermochemical process for the production of valuable ketone fuels by pyrolysis of the calcium salts or esters of organic acids was investigated years ago (33). Low temperature (~ 300°C) pyrolysis of calcium salts of aliphatic organic acids proceeds according to the following equation:



Due to the formation of mixed calcium salts, a mixture of ketones from acetone to heptanone is produced by pyrolysis of calcium salts of mixed organic acids produced by anaerobic fermentations. These ketones have a high energy content (Figure 2), high octane values, and boiling range compatible with gasoline (Figure 1).

Another approach investigated for the production of fuels from organic acid salts was the Kolbe electrolysis, but this method consumed large amounts of expensive electrical energy and produced mainly gaseous lower hydrocarbons (92). Kolbe electrolysis has an advantage over other methods for the conversion of salts of volatile organic acids to liquid fuels because it does not require prior concentration of the salts or separation of the acids from the cations. Hydrocarbons produced during electrolysis of the salts of volatile organic acids can be removed by pervaporation. Alternatively, they will separate from the aqueous reaction mixture due to their immiscibility with water. Pyrolysis of the calcium salts will require concentration of the salts, which are quite soluble in water. The formation of esters by the classical reaction of carboxylic acids with alcohols requires not only concentration of the salts of organic acids, but also separation of the acids from the cations, production of highly concentrated organic acids, and preferably the recycle of the cations back to the fermentations. The formation of esters from carboxylic acids and alcohols is a simple dehydration reaction, which is unfortunately readily reversible in the presence of water. Mixed acid fermentations tend to become inhibited by end-products at concentrations of 20–30 g/L; therefore, the efficient separation and concentration of organic acids become the major problems. Salts of organic acids are not volatile and organic acids have high heats of vaporization, which negates their recovery by conventional distillation methods. Innovative separation concepts using solvent extraction, ion exchange, and membrane technology need to be developed (33) before these very simple

and attractive systems for direct microbial conversion of pretreated cellulosic materials can be utilized for liquid fuels production. Inspection of Figure 2 also indicates that the formation of esters from higher organic acids, such as butyric acid, and lower alcohols (e.g., methanol and ethanol) would be more attractive because the resulting esters will have higher energy contents than either of the starting reactants. The thermochemical formation of esters or ketones from organic acids or their salts will require additional input of thermal energy, but all or part of this energy can be recovered from the higher energy content of the upgraded products (see Figure 2).

### Lipid Production (38)

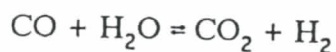
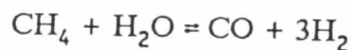
Many microorganisms, namely some aerobic yeasts and fungi, can accumulate large amounts of triglycerides of fatty acids (lipids) during the later stages of aerobic growth on sugars. The accumulation of lipids is usually triggered by exhaustion of nutrients other than the carbon source. The nutrient that is usually allowed to become exhausted is nitrogen, but with some microorganisms, depletion of other nutrients, such as phosphate sulfur, or iron also stimulates lipid accumulation. Very high fat content (50%–66% w/w) was achieved in some strains (38), but lipid production by microbial conversion of sugars is plagued by very low weight conversion yields. Fatty acids are highly reduced compounds, and overall conversions of 15%–24% of the weight of sugar to the weight of lipid were observed. Numerous lipid-producing strains can utilize a variety of sugars from biomass, but very low conversion yields on a weight basis will limit potential substrates to wastes of negative or very low (1¢–2¢/lb) cost. Direct production of lipids by photosynthetic plants and algae may be a better production route because lipid production is aided by photosynthesis (93). It must be noted that lipid production is rather unique among fermentation processes for liquid fuel production because it can accommodate relatively low substrate concentrations on the order of 10–40 g/L. Lipid droplets are sequestered in microbial cells and can be easily recovered by filtration or sedimentation of dilute cell suspensions. Production and recovery of other biofuels, such as ethanol, require much higher (10%–25% w/w) substrate concentrations.

The conversion systems discussed above all require sugars in one form or another as a substrate, with the exception of photosynthetic lipid production. These systems cannot convert phenolic compounds derived from lignins, tannins, and other phenolic components from biomass to liquid fuels. These processes thus impose a pressure on feedstock selection, requiring feedstocks with the highest possible carbohydrate contents and coupling with the chemical or thermochemical processing of lignin for complete substrate conversion. Biomass feedstocks with high carbohydrate content include some agricultural residues (see Table I) and the wood of hardwood trees. Tree bark, the wood of softwoods, and some forms of wastepaper are examples of substrates with high phenolic content (i.e., lignin, tannins, etc.) and correspondingly lower carbohydrate content. Two biological conversion processes, syngas fermentations and anaerobic digestion, can ultimately accommodate a wide range of biomass feedstocks and their components. Syngas conversion refers to a two-stage process where organic matter is first gasified in the presence of steam to syngas, a mixture of hydrogen, carbon monoxide, and carbon dioxide. Biomass, coal, and other organic materials can thus be converted to a fairly uniform gaseous feedstock, which can be biologically converted to liquid fuels or their precursors, disproportionated to hydrogen and carbon dioxide by photosynthetic bacteria, or converted to mixtures of methane and carbon dioxide (biogas) by anaerobic digestion consortia. The thermochemical conversion of biomass and steam to syngas has been investigated for many years and the technology should be adaptable to the gasification of lignin and mixtures of organic solids. Estimated capital costs are low and the gasification processes appear to be quite efficient. Depending on the feedstock cost, syngas can be produced at approximately 2¢–5¢/lb. Anaerobic digestion, an old set of technologies used primarily in waste treatment, can also convert a wide range of carbohydrates and other organic compounds to biogas without prior gasification.

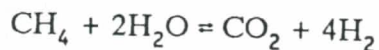
### Syngas Conversion to Liquid and Gaseous Fuels (78,82,94-97)

The anaerobic utilization of carbon monoxide or carbon dioxide-hydrogen mixtures was observed many years ago. Many methanogenic bacteria can disproportionate mixtures of  $H_2$  and CO or  $CO_2$  to mixtures of methane and carbon dioxide, usually called biogas. Likewise, a selected group of anaerobic bacteria, called acetogens, can convert mixtures of carbon monoxide or carbon dioxide with hydrogen to acetate, which can be converted by methanogens to biogas. Single- or two-stage microbial conversions of syngas to biogas are well documented in anaerobic bacterial systems. Additional conversions to alcohols and higher fatty acids were identified recently and will be discussed below.

Syngas, a mixture of carbon monoxide, carbon dioxide, and hydrogen, is being produced by the reaction of numerous carbonaceous substrates with steam at very high temperatures (97). Large-scale commercial processes for the production of ammonia and methanol utilize syngas production as a first step. Syngas is not a uniform mixture of gases at a fixed stoichiometric ratio. Depending on the carbon, hydrogen, and oxygen content of individual feedstocks, syngas mixtures of different hydrogen to carbon monoxide plus carbon dioxide ratios are produced. The syngas produced from natural gas is richest in hydrogen. Its formation proceeds according to two reactions:



yielding the following result:



The second reaction is usually called the water shift reaction. The equilibria are adjusted by choice of reaction conditions and the water shift reaction is promoted by decreased temperatures and use of catalysts. The ratios of hydrogen to combined carbon monoxide and carbon dioxide drop significantly if coal or biomass are used as a feedstock, with intermediate values produced by conversion of various petroleum fractions (94-97). Production of syngas from coal or biomass leads to molar ratios of carbon monoxide to hydrogen of approximately 1:1, or after complete water shift, carbon dioxide to hydrogen ratios of approximately 1:2. Because the composition of lignin is very similar to low ranking coals, the syngas composition from lignin is similar to syngas produced from coal. The low hydrogen content of syngas produced from coal or biomass makes it deficient in hydrogen for numerous chemical and biological syntheses (e.g., methanol and methane) and decreases the yields of many ultimate products. Simple solutions to hydrogen deficiency would be cogasification of methane and coal or biomass, or blending of syngas streams produced by separate gasification of hydrogen-poor substrates and methane. Because methane and water are the best natural substrates for hydrogen production and liquid or gaseous fuel production will involve hydrogenation in one fashion or another, we should be conserving natural gas for future fuel production processes.

The biological conversion of syngas to liquid or gaseous fuels received research attention only recently. The biomethanation of syngas using pure cultures of methanogens, mixed cultures of acetogens and methanogens, or enriched cultures of bacteria from natural populations has been studied (82). Very impressive rates and yields of methane production from coal syngas were observed, but methane concentrations are lower than usual due to the low hydrogen content of syngas. The conversions are also limited by low gas-liquid transfer rates of the relatively insoluble hydrogen and carbon monoxide, but rates have been improved by operating under pressure and decreasing aqueous film thickness (98). The biological conversion of syngas to methane proceeds under mild conditions (i.e., low temperatures and pressures) and is resistant to hydrogen sulfide, which poisons practically all chemical catalysts. Preliminary estimates (99)

indicate that biological methanation of syngas could be significantly cheaper than the chemical routes.

Some photosynthetic bacteria (i.e., *Rhodospirillum rubrum*) carry out a water shift reaction and convert carbon monoxide and water to CO<sub>2</sub> and hydrogen (98). The two-stage biomethanation reaction can also be converted to acetate production by selectively inhibiting the growth and metabolism of methanogens. The dominant acetogens will then produce acetate by the homo-fermentative conversion of syngas. Because the formation of higher organic acids has been observed in anaerobic bacterial cultures grown on syngas (100), the isolation of new strains that can convert syngas to higher and more valuable organic acids, such as propionic and butyric, should be relatively straightforward. A new species of *Clostridium* (*C. ljungdahlii*) has been recently isolated (101) that produces mixtures of ethanol and acetate from syngas. Researchers from the Michigan Biotechnology Institute (99) also demonstrated the two-stage conversion of syngas to acetone/butanol by converting syngas to organic acids in the first stage and performing ABE fermentation in the second stage.

The results obtained so far indicate that many bacterial fermentations may proceed using syngas as a substrate, and rapid development in this area can be expected. This route could allow biochemical conversion of lignin to additional liquid or gaseous fuels and complete utilization of all biomass components. The recovery problems for liquid fuel products or their precursors will be similar to those described previously under fermentations using sugars as substrate. Biological conversions of syngas must also compete with numerous chemical conversion processes which have been developed over many decades. Other alternatives for biochemical lignin conversion may occur in mixed anaerobic populations that convert depolymerized and solubilized coal and peat to biogas. Anaerobic fermentation of many organic compounds, for example phenols and furfural, is known to occur in anaerobic digesters, but bacteria responsible for these fermentations have not been isolated and identified. Anaerobic digestion is normally allowed to run its course and only the conversion of total organic carbon (TOC) to biogas is usually monitored. Although the direct anaerobic degradation of lignin to biogas does not seem to occur (37), the conversion of many phenolic and aromatic compounds is documented (102), so possibilities may exist for biological conversions of thermochemically depolymerized and solubilized lignins. Partial biological conversion of degraded lignin compounds from peat to biogas has been experimentally documented (82).

### Anaerobic Digestion (103)

Anaerobic digestion of biomass or its components can provide a major source of high quality gaseous fuel (i.e., methane or biogas). Anaerobic digestion is a sequential conversion process in which numerous organic compounds and biodegradable polymers are ultimately converted to biogas, i.e., gaseous mixtures of methane and carbon dioxide saturated with water vapor and containing small amounts of gaseous impurities, such as hydrogen and hydrogen sulfide. Anaerobic digestion processes are carried out by a consortia of anaerobic bacteria adapted to given substrate or mixture of substrates. Many diverse bacteria known to exist in anaerobic digestion systems have not been isolated or studied yet. Most of the information that has been gathered about bacteria involved in anaerobic digestion of cellulosic materials was derived from the rumen of grazing animals, such as sheep and cattle (104). Other important applications of anaerobic digestion (i.e., municipal sewage and wastewater treatments) did not support very extensive bacteriological studies. The broad and incomplete picture of steps involved in anaerobic digestion appears to be a primary fermentation of organic substrates to salts of organic acids and hydrogen plus carbon dioxide. Hydrogen, carbon dioxide, and the lowest organic acids (i.e., acetate and formate) can be directly converted to biogas by methanogens. Other organic acids are disproportionated to acetate, hydrogen, and carbon dioxide by "acetogenic" bacteria, which were mentioned previously in regard to organic acid production and syngas conversion. Some recent results indicate that the majority of

biogas is produced by disproportionation of acetate, with minor contribution from syngas (i.e., hydrogen and carbon dioxide) reduction.

Due to the diversity of bacterial consortia, anaerobic digestion is unique among the microbial fermentations in its adaptability to numerous organic compounds and their mixtures, which can be used as substrates. Because these consortia can contain numerous anaerobic bacteria producing hydrolytic enzymes, such as cellulases or hemicellulases, direct microbial conversion of biopolymers is usually practiced. The drawbacks of this simplistic approach are lower yields and rates of the conversions. Both pretreatments and supplementation with hydrolytic enzymes were shown to be effective methods for increasing rates and yields in the anaerobic digestion of some biomass substrates. Other productive approaches include increasing concentration of bacterial cells by immobilization on solid supports or flocculation for liquid feedstocks, and increasing the concentration of solid substrates for insoluble cellulosic feedstocks, such as municipal solid waste. Cellulosic feedstocks, such as municipal solid waste (MSW) and wheat straw, can be digested at concentrations of solids approaching 35%, and a severalfold improvement in the rate of gas production (over stirred tank reactors operated at low [ $<8\%$  w/w] concentrations of substrate) can be achieved. Anaerobic digestion of crop residues and food and feed waste is practiced on rather large scale in China, India, and other countries (105,106). In these countries, biogas is used for domestic cooking and also for local transportation. Residues from the digesters are recycled back to the fields as a fertilizer and as a solid soil amendment. Anaerobic digestion is a very suitable technology for fuel production from wet biomass and wastes in rural areas because the process is very simple, requires minimal attention and training of operators, and cycling of substrates and products can be easily done in a rural setting. Scaleup of anaerobic digestion in town- or city-size systems requires increased efficiency and attention to waste disposal problems. Similar to lipid production, the main problem of anaerobic digestion is the low weight yield of methane from substrate utilized. The theoretical weight yield of methane from glucose is 27% (w/w). The actual yield is less than that, due to the incomplete utilization of all components in biomass. Low yields on a weight basis and the low current cost of natural gas limit biogas production to waste treatment systems and hamper efforts for large-scale development. Otherwise, anaerobic digestion is very attractive. Product separation occurs automatically by outgassing, and biogas can be directly used as a fuel in the proximity of the plant. The heat of combustion of biogas ( $\sim 600$  Btu/scf) is lower than for natural gas (1,000 Btu/scf), but only minor adjustments are needed for replacement of natural gas by biogas in furnaces, boilers, and internal combustion engines. The acceptance of biogas by natural gas pipelines requires removal of carbon dioxide, water vapor, and hydrogen sulfide. The commercial technology for upgrading biogas to pipeline quality exists because it was developed for the upgrading of natural gas from selected gas fields, but such an upgrade will naturally increase the biogas costs. If excess biogas can be produced at prices competitive with natural gas, numerous liquid fuels can be produced from it by chemical processes; or it can serve as a source of hydrogen via reaction with steam.

The occurrence of anaerobic digestion during burial of organic wastes and its utilization in waste treatment can also lead to serious underestimates in potential productivities (rates) of anaerobic digestion. The usual volumetric rates of anaerobic gas production are summarized in Table III. The comparison of these rates indicates that gas production rates encountered in landfills and waste treatment systems are not even close to the maximal rates achieved in more advanced laboratory or pilot-scale systems. Anaerobic digestion is incidentally developed under some burial conditions, but anaerobic digestion in landfills is not an engineered production of biogas by any means. Likewise, the sewage and other waste treatment processes must be robust and degrade waste under variable conditions. Biogas production in these systems is not optimized because waste degradation and treatment is the main objective. The anaerobic digestion processes aimed at commercial biogas production require changes in emphasis from waste treatments. Dedicated feedstocks that are highly biodegradable with minimal pretreatments must be integrated with anaerobic digestion consortia and bioreactor design. High yields,

increased rates, and cheap reactors are key requirements for further development of anaerobic digestion as a fuel production system. Power consumption for mixing can also be a major energy drain, and efficient mixing systems with low power consumption must be developed.

### Biological Hydrogen Production (39,107)

Hydrogen and carbon dioxide are coproduced with organic acids during certain fermentations. If hydrogen is simply vented from the system, energy in the substrate is wasted. The hydrogen production and loss need to be suppressed, or hydrogen should be captured, separated from carbon dioxide, and used for reductions elsewhere in the liquid fuel production system. The only biological systems that produce hydrogen as a major primary product are photosynthetic microorganisms, namely some algae, blue-green algae (cyanobacteria), and other photosynthetic bacteria. Even with these systems, hydrogen is produced at the expense of other organic compounds formed by photosynthesis. The biological production of hydrogen thus does not appear to be very efficient at the present time, but can be useful in coproduction systems where hydrogen can find captive use in the reduction of organic compounds produced as a part of biomass conversion processes.

The biological shift reaction of syngas, performed by some photosynthetic bacteria, appears to have the highest potential for the production of pure hydrogen.

### Engineering Issues and Future Improvements

The biological conversions of sugars to liquid and gaseous fuels are very efficient (4) because between approximately 90% and 97% of heat of combustion in substrates can be converted to fuel products. Microorganisms derive only small amounts of energy by anaerobic fermentations and thus convert very small portions of the substrate into cell mass. However, significant losses in efficiency and yield can occur in other parts of the conversion system. The inability of current biological systems to convert lignin to liquid or gaseous fuels results in an obvious loss of energy in original substrate. While lignin is only a minor (10%–30% w/w) component of lignocellulosic biomass, it is a more significant component with respect to total energy content because the heat of combustion of lignin is significantly higher than that for carbohydrates.

The development of advanced biological, or preferably chemical, processes for the conversion of lignin to liquid or gaseous fuels is needed for the total conversion of biomass to other fuels. Simple alternative uses for lignin include its use as a boiler fuel to provide process energy or potentially the cogeneration of electricity. However, in these applications lignin must compete with coal, a relatively cheap solid fuel. Lignin streams also require dewatering to increase combustion efficiency.

Another source of potential losses resides in the pretreatment and depolymerization of polysaccharides in biomass to develop fermentable sugars. Mechanical steps for the disintegration of biomass to smaller particles must always be optimized with respect to energy consumption because tremendous amounts of energy can be dissipated in these steps. Mechanical pretreatments interact strongly with changes in plant cell walls and tissues produced by chemical pretreatments, but the effects of chemical pretreatments on the energy consumption in mechanical disintegration of plant tissues have been scarcely investigated to this day. The decrease in total energy consumption must always remain a goal of processes used for the conversion of biomass to other fuels.

The energy losses in pretreatments and hydrolysis are fairly specific to each of the three types one chooses to pursue, but operation at high concentration (> 10% w/w) of biomass solids can provide significant improvements in energy efficiency for all of them. Biomass is highly porous and cell walls occupy only about 30% of the total cell volume. Biomass thus has a very low bulk packing density (Table II) and absorbs large amounts of liquids. Biomass particles in liquids cannot be slurried at concentrations > 10%–12%, and free liquid disappears at about 18%–20% w/w. High solids processing thus provide:

unique challenges to the design and operation of processing equipment, which must handle wet solids rather than liquid slurries. The savings in energy consumption for heating biomass can be very large, because the concentration of biomass increases from 10% to 30% (108).

Two systems for hydrolysis of biomass (i.e., dilute acid hydrolysis and enzymatic hydrolysis) have low consumptions of catalyst, on the order of 2%–5% w/w of substrate. However, dilute acid hydrolysis gives low yields of fermentable sugars and enzymatic hydrolysis requires chemomechanical pretreatments, which add to the total consumption of chemicals. Enzymatic catalysts are also an order of magnitude more expensive than cheap inorganic acids, such as sulfuric acid. With the environmental policies shifting to waste minimization at the source, recycle of both chemical and biochemical catalysts, or reagents, is becoming an important part of process considerations. The recycle of enzymes can also be rewarding in an economic sense, because the enzyme consumption and cost is a significant portion of the total conversion cost. There are other obvious avenues for decreasing the enzyme cost: minimal or no purification, overproduction by genetically engineered microorganisms, and selection of enzymes with higher specific activities. However, even with improvements, enzyme production will remain a relatively complex process, consuming part of the substrate and requiring other nutrient input and energy input for mixing and, usually, aeration. Great strides have already been made in the development of cellulolytic enzymes, but a major R&D effort is still needed for the development of improved cellulolytic enzyme systems.

The key aspect of the overall conversion system for biological fuel production is the integration of plant substrates and pretreatments to provide easily hydrolyzable cellulose for subsequent enzymatic hydrolysis. High yields in enzymatic hydrolysis of cellulose have been achieved routinely for pretreated grasses and hardwoods, but little attention has been paid to the abundant softwoods that appear to be more resistant to enzymatic hydrolysis.

The development of efficient pretreatment methods needs to continue if the promise of efficient enzymatic hydrolysis of the carbohydrates in biomass is to be fulfilled. At a minimum, these methods need to yield porous cell walls or cellulose fibers with high surface area accessible to enzymes. The rates of enzymatic cellulose hydrolysis are still rather slow, requiring reaction times on the order of one to three days. Developments in plant substrate pretreatments and enzyme systems may speed up the rates, but the crystallinity of cellulose and the insolubility of celloextrin intermediates may provide upper limits for improvements that can be achieved. The long reaction times and insolubility of the substrate can lead to significant power consumption for mixing in fermentation vessels. Anaerobic fermentations do not require the intensive mixing for oxygen transfer as aerobic systems do, so the levels of mixing for efficient hydrolysis and fermentations of biomass need to be investigated and optimized.

Many fermentations of biomass to liquid fuels also require high concentrations of product for efficient product recovery. Due to the fixed stoichiometry of substrate to product conversion, correspondingly high substrate concentrations have to be achieved in saccharification-fermentation reactors, which points to the need for the development of new reactors for solid-state fermentations. High concentrations of substrate can also be achieved by fed-batch operation.

All biological processes targeting the conversion of carbohydrates in biomass must address the integration of carbohydrate processing with the conversion and utilization of lignin and other phenolic or nonphenolic components of biomass which are not readily amenable to biological conversion. These components, primarily lignin, contain a significant portion of the total energy content of biomass feedstocks. One obvious route for lignin is its recovery, dewatering, and utilization as a boiler fuel to provide energy for the rest of the conversion process. Such utilization will provide environmental benefits by minimizing emissions of carbon dioxide from fossil fuels input, but this fate for lignin provides only marginal economic benefits (i.e., 1¢–2¢/lb of lignin used as boiler fuel). Valorization of lignin and other organic components of biomass to liquid fuels, polymeric materials, and higher value organic chemicals can have significant economic benefits for

the overall production system. Pyrolytic cracking, hydrogenolytic deoxygenation, and dealkylation can be coupled with catalytic methylation to form anisole and other methylarylethers (112), which are excellent antiknocking agents for gasoline. Other large volume products which can be produced from lignin are asphalt and fuel oil replacements. Extensive R&D is needed for development of marketable products from the lignins of hardwoods and grasses, which are favorable feedstocks for biological fuel production. Lignin conversion research needs to be integrated with feedstocks selection and pretreatment methods, because lignins are not uniform polymers in biomass feedstocks and they are severely modified during pretreatment.

### Summary

Integrated biochemical and chemical methods are being developed for the conversion of biomass components to valuable liquid and gaseous fuels. These processes can initially tap vast reservoirs of agricultural, forestry, and municipal solid wastes, with additional supplies potentially available from energy crops.

The conversion of agricultural and related wastes is especially attractive because these organic materials are often burned before replanting or the rejuvenation of fields, forests, orchards, and pastures. Mass burning of biomass is an ancient agricultural and pastoral practice which releases large amounts of carbon dioxide and contributes to the greenhouse effect. Permitting the biomass residues to rot by the action of aerobic microorganisms achieves the same carbon dioxide release, only at a slower rate. We are thus needlessly oxidizing carbon fixed by current photosynthesis, while we are extracting energy from the carbon fixed by photosynthesis of ancient plants and other organisms. Liquid and gaseous fuel production from plant residues can capture part of this modern fixed carbon in a valuable fuel form and allows its utilization before terminal oxidation to carbon dioxide. The combined production and conversion system can also achieve a closed carbon dioxide cycle because carbon dioxide released during the conversion and combustion of biomass and fuels is reduced back to biomass substrates by photosynthesis.

Furthermore, the conversion of biomass to liquid fuels will provide a significant densification of the energy content in the original substrates and, thus, permits long distance export of fuel products from rural areas (see Table II). The development of processes for the conversion of lignocellulosic biomass also alleviates the food versus fuel controversy, because the conversion is concentrated on parts of the total plant biomass which are essentially undigestible by man and many farm animals.

Biomass production systems are also significant net producers of energy. The estimated energy output/input ratios range from approximately 5:1 for the production of corn and similar herbaceous crops, through the range of 10:1–40:1 for silviculture, to ratios of 50:1–150:1 for the harvesting of agricultural and forestry residues (109–111). Conversion processes for the production of liquid and gaseous fuels from biomass, whether thermochemical or biochemical, have to strive for the highest thermal efficiencies and product yields to decrease the dissipation of energy surplus from the production section. Biological conversion processes are eminently suitable for the conversion of moist feedstocks because they can utilize moisture in biomass as a reaction medium. They can also accommodate dry biomass equally well.

Several biological routes for the production of liquid fuels from carbohydrates in biomass have been reviewed in this chapter. Each projected process has its own unique features and limitations, but they share the common steps of depolymerization of complex carbohydrates, fermentation, and product recovery. Lower alcohols, such as ethanol, can be used directly as a fuel, while some other products, such as butanediol or salts of organic acids, will require additional chemical conversion steps to turn them into liquid fuels.

Great strides have been made over the last two decades in the pretreatment of biomass for enzymatic hydrolysis, especially the development of enzymes for the hydrolysis of cellulose and the development of microorganisms for fermentation of xylose to ethanol. High yields of sugars and fuel products have been achieved from a variety of pretreat-



substrates. Further improvements are needed in the integration of biomass feedstocks, pretreatment methods, and enzymes for the hydrolysis of polysaccharides. A decrease in the consumption of enzymes and the increased rates of cellulose hydrolysis will also have important benefits for economic viability of biological fuel production.

A serious obstacle to complete biological conversion of biomass to liquid or gaseous fuels is the inability of current biological systems to depolymerize lignin into fermentable units or to convert lignin and other phenolic compounds in biomass to liquid fuel products. Therefore, biological conversions of carbohydrates must be coupled to the recovery and chemical conversion of lignin. At a minimum, lignin must be recovered in a form suitable for boiler fuel, but this application does not provide much coproduct credit. Possibilities for combined chemical and biological conversion of lignin may exist in syngas conversion and anaerobic digestion systems, but both approaches have not been thoroughly investigated to date and will require significant research investment. There are many possibilities in the chemical conversion of lignins to valuable products, such as liquid fuels, polymeric materials, and organic chemicals, some of which are discussed in other chapters of this book. Increased attention should, however, be paid to the conversion of lignins from hardwoods and grasses, which are also modified by various pretreatment methods. Furthermore, the extensive research results from R&D on softwood lignins modified by pulping processes may not always be directly applicable to lignins from other plant families, some of which are different in structure and composition. Finally, the ultimate biomass refinery should operate with the same attention to marketable products as do current petrochemical refineries. R&D investment should also be made in recovery and utilization of such minor components as uronic acids, waxes, and acetic acid, all of which will be coproduced in significant quantities.

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