Lignocellulosic Feedstocks for Ethanol Production: The Ultimate Renewable Energy Source

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Lignocellulosic Feedstocks for Ethanol Production: The Ultimate Renewable Energy Source

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Production of ethanol from lignocellulosic feedstocks is of growing interest worldwide. Potential utilization of large volumes of primary and secondary lignocellulosic wastes, as well as renewable biomass (Table 1), to produce fuel and chemical feedstocks presents significant technical and economic challenges (Cowling *et al.*, 1976; Ferchak *et al.*, 1980; Klass and Emert, 1981; Tsao, 1978; Zermetz, 1979; GAO, 1981). The solutions to these problems can aid present and future generations. Production of fuel ethanol from biomass is the primary target for a commercial product. However, it is obvious that sugars produced for fermentation, or synthesis gas derived from biomass, can be utilized to produce other useful fuel and chemical products.

Table 1. Lignocellulosic feedstocks

Wood
Primary (native)
Plantation
Primary forest waste
Secondary processing waste
Agricultural residues
Straws (wheat, barley, rice)
Bagasse (sugarcane, sweet sorghum)
Stover (corn, milo)
Municipal waste
Waste paper

CHEMISTRY OF LIGNOCELLULOSIC BIOMASS

The term 'holocellulose' is often used to describe the total carbohydrate contained in a plant or microbial cell. Holocellulose is comprised of cellulose and hemicellulose. Cellulose is further categorized into long-chain α-cellulose and the short-chain forms of β-cellulose with a degree of polymerization (DP) of 15-90 units, and γ-cellulose with a DP of less than 15 units. Following is a description of terms used in lignocellulosic chemistry.

Cellulose is a structural material formed by a plant or microbial cell from glucose, a common six-carbon sugar. Glucose is a carbohydrate and therefore has the composition of

carbon plus hydrogen and oxygen (CH_2O). Although glucose ($C_6H_{12}O_6$) is the smallest unit (monomer) that can be isolated from cellulose degradation, the basic building block of cellulose is actually cellobiose, a two-glucose anhydride unit (dimer). As glucose units are linked together into polymer chains, a molecule of water is lost, which makes the chemical formula for each monomer unit (glucan) $C_6H_{10}O_5$.

The cellulose molecular structure is an unbranched linear polymer. The length of a polymeric cellulose molecule is determined by the number of glucan units in the polymer, usually expressed as $(C_6H_{10}O_5)_n$, where n is the degree of polymerization. The DP of cellulose depends on the type of plant or microorganism from which it is isolated, as well as the method of isolation. Typical numbers for DP of native (nondisturbed) cellulose are estimated to be from 2000-14,000 glucan units, with commercial wood pulp DP in the range of 650-1500 units per glucan chain.

Due to the potential for each monomer unit of cellulose to form three hydrogen bonds with a monomer in a neighboring chain, the chains fit tightly together to form larger units known as microfibrils. The result is a very stable configuration—essentially free of interstitial spaces, making it anhydrous and quite recalcitrant to hydrolysis by acid, base or enzyme action. Chain regions with no special disturbances form crystalline areas known as micelles. Regions without extensive inter-chain hydrogen bonding are consequently less structured (amorphous) and are relatively more susceptible to hydrolysis. Native cellulose consists of cellulose fibrils bound together by an amorphous matrix comprised of pectin, hemicellulose and lignin.

Pectin and hemicellulose are macromolecular polysaccharides and are the most soluble fraction of native plant biomass. Pectin consists of mostly D-galacturonic acid with some residues of rhamnose, galactose and arabinose. Hemicellulose is not a form of cellulose, but is a mixture of straight-chain and branched forms of both 5- and 6-carbon sugars with varying types of linkages. Hemicellulose can be hydrolyzed to the 6-carbon sugars of glucose, mannose and galactose, and the 5-carbon sugars of xylose and arabinose.

Lignin is a very complex molecule constructed of phenyl propane units linked in a three-dimensional structure (Smook, 1982). Lignin is considered to be the glue holding the cellulose fibrils together, and as such is difficult to completely remove. Lignin confers stiffness to the cell and may also provide protection against microbial attack. Because lignin molecules have varying types of chemical bonds, the particular extraction method will determine what types of chemicals result.

RESEARCH

Although research on the production of ethanol and related products from cellulosic and lignocellulosic feedstocks is developing rapidly, there is a substantial record of prior research, development and commercialization that can be utilized in planning for the introduction of improved and more economic technology. The following will relate the prior history and long-established art of biomass conversion, particularly of lignocellulose to ethanol, with ongoing research and development in this area.

Concentrated acid hydrolysis

Hydrolysis with concentrated acid is relatively old art, harking back to the early days of lignin and cellulose chemistry (Table 2). The Klason lignin determination, developed in 1890 and still a standard analysis in the wood conversion industry, utilizes cold, concentrated (72%) sulfuric acid (H_2SO_4) to dissolve the cellulosic fraction of the raw material, leaving the lignin as a residue. The dissolved cellulose, separated from the lignin, is then hydrolyzed by dilution of the sulfuric acid and heating to yield sugars (Locke et al., 1961). This basic technology is part of the two stage acid hydrolysis process developed at Purdue University's Laboratory of Renewable Resources Engineering (Ladisch, 1979).

Table 2. Acid hydrolysis technologies

Sulfuric
Concentrated: one stage
Dilute: one stage
Dilute/concentrated: two stage
Dilute/dilute: two stage
Hydrochloric
Concentrated: liquid phase
Concentrated: vapor phase
Dilute
Organic (autohydrolysis)
Steam pressure
Steam and mechanical pressure

A second method of concentrated acid hydrolysis is the Bergius process, which uses cold, concentrated (40%) hydrochloric acid (HCl) to dissolve the cellulosic fraction. Water dilution and heating then permits hydrolysis of the cellulose to sugars. Commercially, the acidified lignocellulosic material from this process was neutralized with caustic soda and used as animal feed in Germany during World War II. More recent research on cellulose hydrolysis with concentrated HCl has been carried out in Japan (Locke et al., 1961).

The concentrated acid processes generally give higher sugar and ethanol yields than dilute acid processes, but the relatively mild corrosiveness of the concentrated acid at essentially ambient temperature is superseded by more corrosive conditions as the acids are diluted and heated to hydrolyze the dissolved cellulose to sugars. The intermediate acid concentrations are extremely corrosive, requiring either expensive alloys or specialized non-metallic construction such as ceramic or carbon brick lining, resistant both to corrosion and temperature. The resulting high investment and maintenance costs have greatly reduced the commercial potential for these processes.

Dilute acid hydrolysis

The technology for dilute sulfuric acid hydrolysis and extraction of sugars at elevated pressures and temperatures was developed by Scholler before World War II and employed in Germany for production of ethanol. Plants were also built in Russia, during and after the war, to produce ethanol or yeast from wood sugars. Another plant was built and operated at Ems, Switzerland. As part of the war effort, a plant was also designed and built in the US at Springfield, Oregon utilizing the US Forest Products Laboratory (Madison) variation of the Scholler process. The plant operated for only a short time because construction was not completed until after World War II ended.

Test runs in Oregon indicated serious corrosion, erosion, tar and pitch formation and mineral scale formation, problems that have bedeviled all of these installations. These problems create high investment and high cleaning and maintenance costs and have limited the commercialization of these processes (Hajny, 1981; Harris *et al.*, 1946; Saeman, 1979; Underkofler and Hickey, 1954; Hokanson *et al.*, 1978).

A Brazilian government agency contracted with the USSR for the design and construction of a demonstration plant utilizing dilute sulfuric acid technology with 30,000 liter/day capacity for conversion of Brazilian wood feedstocks to ethanol. This plant was put into operation in the 1980s, but results were apparently disappointing.

New acid hydrolysis technology

Newer approaches to acid hydrolysis technology that may lead to increased yields and reduced investment are under development in many countries (Gilbert *et al.*, 1952; Goldstein, 1981; Kamiyama *et al.*, 1979; Porteous, 1972; Anonymous, 1981). Some of these, such as the Swiss Inventa technology (Mendelsohn *et al.*, 1981), and the New Zealand Forest Service technology (Whitworth *et al.*, 1980), appear to be improvements on the US Madison-Scholler technology mentioned above.

Other newer technologies, such as the Purdue two stage process (Ladisch, 1979), utilize combinations of dilute and concentrated acid. The dilute acid is used under mild conditions for pre-hydrolysis of the hemicellulose while the concentrated acid is used to extract the cellulose from the lignocellulose residue after hemicellulose removal. Another version is the technology being developed at Dartmouth University (Grethlein *et al.*, 1980; Thompson *et al.*, 1979; Lynd *et al.*, 1991), which provides for a two stage dilute acid hydrolysis at high temperatures for short times. The first stage has temperature and time adjusted to hydrolyze hemicellulose; while the second stage is set at higher temperatures and longer retention times to hydrolyze the residual cellulose. Pressurized dilute acid extrusion technology was under development at New York University in the 1970s (Brenner *et al.*, 1978). At Georgia Tech, various combinations of dilute and concentrated acid hydrolysis have been tested in a multipurpose pilot plant.

None of these two stage processes have advanced beyond a relatively modest pilot plant scale. Longer term operation in demonstration plants will be required before firm

investment and operating costs, as well as technical and economic feasibility, can be established.

Enzymatic processes

No enzymatic system for cellulose hydrolysis has yet been proven commercially without first removing (pretreating) the lignin present in lignocellulosic feedstocks (Table 3).

Table 3. Enzymatic hydrolysis technology pretreatment options

Autohydrolysis
Steam pressure (Dietrichs)
Steam and mechanical pressure (Stake)
Steam explosion (Iogen)
Acid pre-hydrolysis
Dilute sulfuric acid
Dilute hydrochloric acid
Acetic acid
Concentrated sulfuric acid (cold)
Concentrated hydrochloric acid (cold)
Alkali
Sodium hydroxide
Ammonia
Organosolv
Methanol
Ethanol
Butanol
Hexamethylenediamine
Phenol
Mechanical
Attrition mill
Roller mill
Vibratory rod mill
Extruder

Failure of cotton clothing during military campaigns in the Pacific during World War II sparked research by the US military into cellulose degradation. Early work at the US Army Natick Laboratories (Katz *et al.*, 1968; Mandels *et al.*, 1974; Nystrom *et al.*, 1976; Reese, 1976) identified the Trichoderma fungus and its multiple enzyme system as responsible for the cotton (cellulose) failure (hydrolysis). Further research showed it essential to use delignified cellulose to enable the enzymes to produce reasonable yields of sugar within a moderate time

frame. The long time element and low yield of the early work at Natick indicated relatively high investment and production costs. New research worldwide has advanced this basic technology (Cysewski *et al.*, 1976; Emert *et al.*, 1980a; Lyness *et al.*, 1981; Nisizawam, 1973; Puls *et al.*, 1977; Shoemaker *et al.*, 1981; Rosenberg *et al.*, 1980).

Research is under way at several laboratories to develop microorganisms that will secrete enzymes to break down lignin (Kirk et al., 1977) that act as a protective coating for, and are apparently chemically bonded to, the hemicellulose. To date, the organisms under development have not demonstrated appreciable lignin removal without substantial degradation of the cellulose and with little improvement in sugar yield. Dilute acids, both sulfuric and sulfurous, have been tested in pretreatment methods to break the lignin-hemicellulose bond (Mandels, 1976; Millet *et al.*, 1974; DOE, 1978; Wilke *et al.*, 1979) and make the cellulose more accessible to enzyme attack. Initial results indicate potential feasibility and improvement in yields. However, the hemicellulose hydrolyzate produced by the dilute acid pretreatment will be more useful with suitable microorganisms developed for fermentation of the C_5 sugars to ethanol. Organisms such as Thermonospora and genetically-modified bacteria show potential in this regard (Ingram, 1991).

Alkali pretreatment has also been utilized on certain materials, particularly straws (Detroy *et al.*, 1981; Millet *et al.*, 1974; Toyama, 1976). This treatment cleaves the lignin-hemicellulose bond and at least partially solubilizes the lignin and decrystallizes the cellulose. Sodium hydroxide and ammonia have been used in such systems. Such pretreatment has indicated improved susceptibility of cellulose to enzyme hydrolysis. However, the C_5 sugars are modified by the alkaline extraction and may not be accessible for fermentation by the new organisms. The Ammonia Freeze Explosion pretreatment process for woody feedstocks has been developed by Dale and his associates at Texas A&M University (Dale *et al.*, 1989).

A non-chemical reaction route (organosolv) using organic solvents to remove lignin to provide a clean cellulose suitable for enzyme hydrolysis has been under development. Solvents such as methanol, ethanol, butanol, phenol and hexamethylenediamine, with water, have reportedly been used (Holzapple *et al.*, 1981). In each system, the solvent action is accompanied by partial pre-hydrolysis effected by the presence of water and organic acid catalysts (acetyl groups), producing hemicellulose sugars. Such solvent-delignified celluloses have proven susceptible to enzyme hydrolysis. The main advantage of using solvents over chemical pretreatment is that relatively pure, low molecular weight lignin can be recovered as a by-product. The C_5 sugars solubilized during organic solvent delignification are relatively clean and could be fermented to ethanol with appropriate microorganisms, such as the recombinant bacteria developed by Ingram and co-workers at the University of Florida (Ingram, 1991).

Another group of pretreatment methods can be described as thermomechanical, with the thermal energy in these systems generated by the mechanical action. This covers a wide variety of pretreatments such as vibratory rod milling (Millet *et al.*, 1979), the Natick roller mill system (Tassinari *et al.*, 1980), and the Gulf Oil/University of Arkansas thermomechanical treatment in attrition mills (Emert *et al.*, 1980a). The Stake and Dietrichs technologies involve steaming under moderate pressures, plus mechanical attrition in a screw-propelled device (Dietrichs *et al.*, 1978; Taylor *et al.*, 1980). The Iogen technology (Foody, personal communication) involves steaming of wood chips at pressures up to 500 psig followed by explosion through a slotted die to provide both cleavage of the lignocellulosic linkage and

shortening of the cellulose fibers. The Rugg technology developed at New York University (Gilbert *et al.*, 1952) involves a combination of acid, heat and pressurized operations in an extruder (Werner-Pfleiderer), which gives moderate yields of sugars. However, this device apparently combines the corrosion and polymerization problems of acid treatment with the erosion and corrosion problems of an extruder discharge (pressure reduction) mechanism. These latter operations indicate some promise, but involve substantial investment in mechanical pretreatment plus ongoing maintenance due to corrosion and erosion caused by processing the wood under pressure and discharging through a pressure-reducing device.

In some of these developments, experimental work has only been conducted through the hydrolysis stage. There is some indication that high temperature and acid combinations can lead to serious toxicity problems in subsequent fermentation due to the non-specific hydrolysis that can produce a variety of inhibitory materials such as furfural and its derivatives. Solutions to this problem are claimed; but actual methods of operation have not been defined, and the economics of such treatments are not identified. Energy cost is a critical factor in determining feasibility of these technologies (Datta, 1981).

Ethanol from biomass synthesis gas

Production of methanol from synthesis gas (carbon monoxide plus hydrogen) derived from wood is under development, and has been carried through the pilot plant stage (Feldman, 1980). This technology could be economic in areas where natural gas is too expensive and coal is not readily available as a basic feedstock for synthesis gas. Current research and recently issued patents have indicated development of catalytic systems for homologation reactions, which yield alcohols higher than methanol (Bartish, 1979; Koermer *et al.*, 1978). Thus, methanol produced in a primary synthesis can be converted to ethanol by reaction with additional carbon monoxide and hydrogen.

At this stage of development the catalytic systems are not highly selective, but yield a mixture of ethanol and higher alcohols as well as some unreacted methanol. This is not a problem where the ethanol is to be used as a fuel or octane improver in gasoline. However, this can lead to complications in production of industrial ethanol, which must be of high purity. It should be recognized that technical and economic evaluations of ethanol derived from lignocellulosic substrates should be compared with the ongoing developments in production of ethanol from biomass synthesis gas (Figure 1).

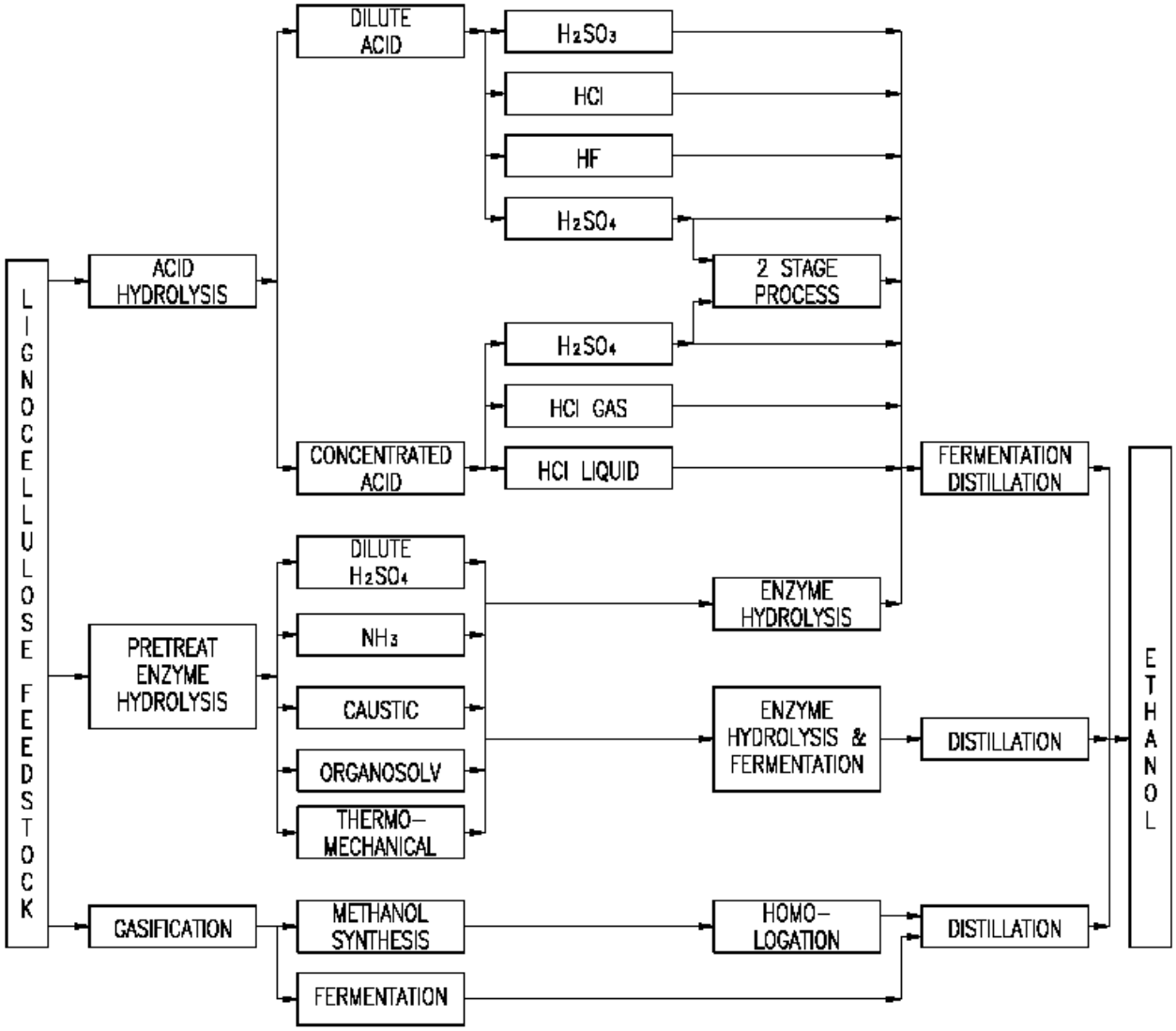

Figure 1. Ethanol from lignocellulose process matrix.

Development of cellulose-to-ethanol technology

Although modern technology for the acid hydrolysis of cellulose (particularly with two-stage operation) can lead to improved yields, there are not sufficient data available to define firm investment requirements. The optimistic estimates of low investment and operating costs indicated by some research groups have not been verified by engineers and contractors experienced in design and construction.

Enzymatic hydrolysis appears more promising, particularly in that the operating conditions are near ambient and corrosion, polymerization and fouling problems are relatively minor. These problems can be solved with conventional moderate alloy construction in certain parts of the process. The time element in the production of the enzyme mix and in the enzymatic hydrolysis have been overcome to a substantial extent by development of mutations

of Trichoderma reesei, use of continuous process technology and development of the Simultaneous Saccharification and Fermentation (SSF) process (Emert et al., 1980a).

Ongoing work in developing either improved mutations or genetically modified microorganisms promises to yield more economic hydrolysis of cellulose, possibly even in the presence of substantial amounts of lignin. Combining hydrolytic enzyme production with fermentation in a single organism could result in improvements in this technology. Furthermore, a combination of dilute acid (or alkali) pretreatment, as an option to mild thermomechanical pretreatment, and secondary enzymatic hydrolysis of the cellulose could result in an overall process with improved yields and better operating economics.

Among the groups conducting cellulolytic enzyme research, the partnership of Gulf Oil (now Chevron) and Nippon Mining (Bio-Research Corporation) of Japan led not only to advances in technology for production of the cellulolytic enzymes, but also the critical and major advance of developing the SSF process (Katzen, 1990; Mandels *et al.*, 1976; Gauss *et al.*, 1976).

A one-ton-per-day pilot plant built by Gulf Oil Chemicals at Pittsburg, Kansas operated with both SSF and continuous cascade fermentation starting in the late 1970s. Feedstocks included wood waste, pulp and paper mill waste, municipal solid waste and various agricultural residues. The key results of SSF application were a major decrease in retention time required for both the hydrolysis and fermentation operations and a major increase in yield relative to other known technologies. This was due to the fact that as fast as the cellulolytic enzymes produced glucose, the yeast converted the glucose to ethanol. Thus, the feedback inhibitory action of glucose on enzyme activity was essentially eliminated. Also, the Gulf researchers developed a continuous process for producing the Trichoderma fungus and the cellulolytic enzymes from the base feedstocks for the fermentation operation (Huff *et al.*, 1976). Coupled with a novel enzyme recycle process, enzyme cost was dramatically reduced.

Although the Gulf pilot plant operated for several years, there were shortcomings limiting its application to continuous SSF operation. In 1981, Gulf turned over this program to the University of Arkansas for further research and development. This included the improved enzyme production process, recovery and recycling of cellulolytic enzymes and continued evaluation of lignocellulosic feedstocks. Considerable emphasis was placed on pretreatment methods for breaking the lignin-hemicellulose-cellulose linkages to permit access of the enzymes to the cellulose. Such pretreatments included organosolv delignification, caustic, acid and thermomechanical processing.

Another opportunity arose to advance this technology when Procter & Gamble decided to apply this process to their pulp and paper waste, which had become a waste disposal problem. A one-ton-per-day pilot plant was erected at their mill complex in Pennsylvania. Six months of intensive experimentation in this pilot plant with both primary pulping waste and paper-making waste resulted in substantial improvement in the efficiency of conversion of cellulose to glucose and ethanol (Easley *et al.*, 1989).

Key improvements resulting from the operation of this pilot plant related to application of a mild pretreatment (since most of the lignin had been removed by the pulping process) and use of fed-batch techniques in the SSF process. As indicated in Table 4, runs of more than

180 hrs were made before a significant reduction in enzyme activity was indicated. Specific yields obtained during a typical extended fed-batch run of Table 4 are indicated in Figure 2. A key item to be noted is the feasibility of operating the SSF system at temperatures as low as 37°C, previously considered too low for economic enzymatic hydrolysis. Also, this work showed it was not necessary to use expensive vacuum recovery of ethanol to achieve good yields of ethanol per unit of enzyme activity.

Table 4. Fed-batch SSF conversion of cellulose to ethanol

Conditions	*Organism*		
	Candida brassicae	*Saccharomyces cerevisiae*	*Saccharomyces cerevisiae*
Bench scale tests			
Total fiber, g (dry basis)	6804	1600	1000
Enzyme dose, g	183.7	43.2	27
Enzyme:fiber ratio	0.027	0.027	0.027
Vacuum-recovered ethanol, g	1427	NONE	NONE
Final ethanol concentration, g/L	17.3	36.3	32.7
Final volume, L	24	11.42	8.12
Total ethanol produced, g	1569	415	265
Ethanol yield, gal/ton	69.8	78.5	80.2
Enzyme:ethanol ratio	0.117	0.104	0.102
Fed-batch pilot operation			
Fermentation time, hr	192	168	188
Volumetric production rate, g/L/hr	0.34	0.22	0.17
Average glucose concentration, g/L	2.4	1.05	0.82
Aeration rate, $ft^3/min/ft^3$	0.02	NONE	0.002
Temperature, °C	40	37	37

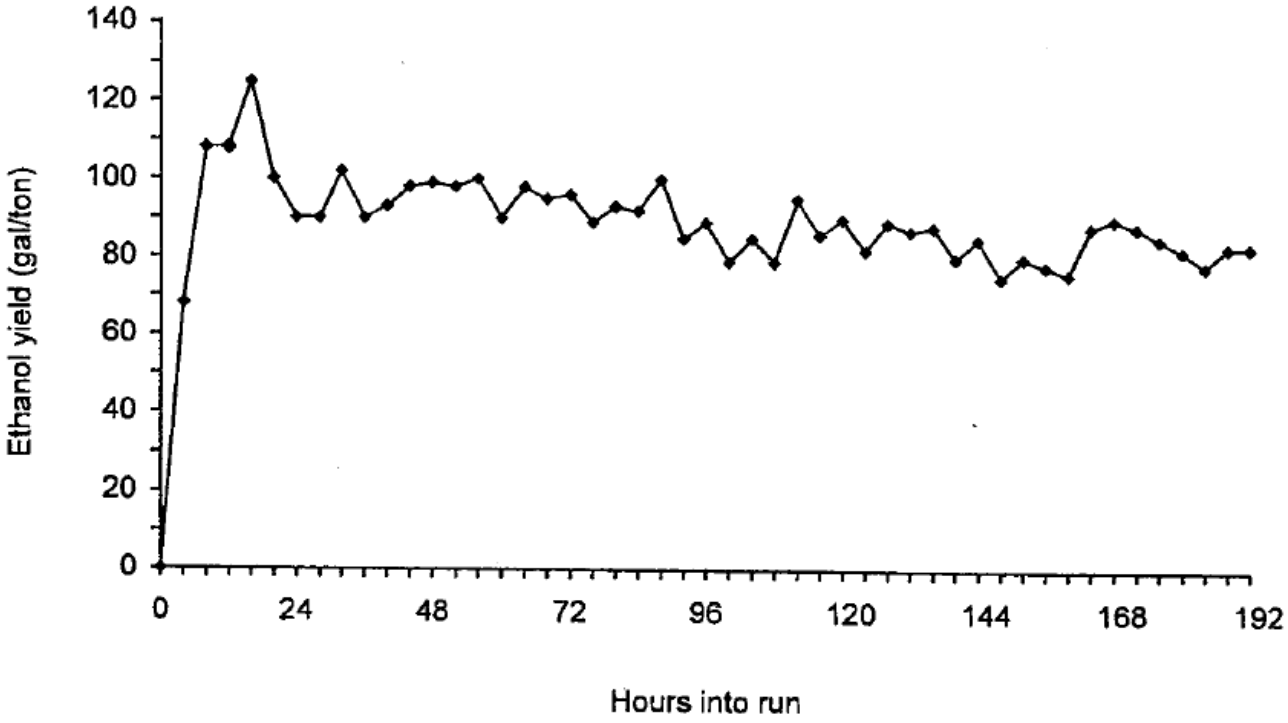

Figure 2. Procter and Gamble's SSF fed-batch performance.

An interesting development during this pilot plant operation involved the addition of a surfactant. The surfactant acts to facilitate access of the cellulolytic enzymes to the cellulose fiber (Katzen *et al.*, 1994). Yield of ethanol per unit of feedstock increased by more than 10%.

Work conducted for organizations interested in conversion of bagasse led to substantial pilot plant testing of a mild acid pre-hydrolysis pretreatment. Such pretreatment, carefully controlled with respect to acid use, temperature and retention time, led to essentially complete hydrolysis of the hemicellulose and recovery of the hemicellulose sugars in a near quantitative manner. The residue from this mild acid hydrolysis was processed in the SSF system, with high yields of ethanol achieved based on cellulose content.

The accumulated data and knowledge from the Gulf and Procter & Gamble pilot plants (Easley *et al.*, 1989; Emert *et al.*, 1980a; b), along with recent advances in improved cellulolytic enzyme systems have made it feasible to extend this technology by construction of commercial demonstration facilities using recombinant bacteria or other select organisms to ferment both C_5 and C_6 sugars (Ingram, 1991) for production of ethanol from lignocellulosic feedstocks.

Recent advances in gas phase fermentation via selected bacteria (Clostridium ljungdahlii) along with new gasification technology show promise for the production of ethanol from a variety of feedstocks including biomass. This technology has been successfully demonstrated on a pilot plant scale by Bioengineering Resources, Inc. (Gaddy *et al.*, 1992). This technology is independent of the sugar molecule and is adaptable to a wide variety of non-sugar based carbon sources.

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