ETHANOL FROM LIGNOCELLULOSIC BIOMASS: TECHNOLOGY, ECONOMICS, AND OPPORTUNITIES

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Abstract

Production of ethanol from agricultural and forestry residues, municipal solid waste, energy crops, and other forms of lignocellulosic biomass could improve energy security, reduce trade deficits, decrease urban air pollution, and contribute little, if any, net carbon dioxide accumulation to the atmosphere. Dilute acid can open up the biomass structure for subsequent processing. The simultaneous saccharification and fermentation (SSF) process is favored for producing ethanol from the major fraction of lignocellulosic biomass, cellulose, because of its low cost potential. Technology has also been developed for converting the second largest biomass fraction, hemicellulose, into ethanol. The remaining fraction, containing mostly lignin, can be burned as boiler fuel to power the conversion process and generate extra electricity to export. Developments in conversion technology have reduced the projected gate price of ethanol from about US$0.95/liter (US$3.60/gallon) in 1980 to only about US$0.32/liter (US$1.22/gallon) in 1994. Technical targets have been identified to bring the selling price down to about US$0.18/liter (US$0.67/gallon), a level that is competitive when oil prices exceed US$25/barrel. However, at current projected costs, ethanol from biomass could be competitive with ethanol from corn, particularly if lower cost feedstocks or other niche markets are capitalized upon.

Key words: Lignocellulosic biomass, ethanol production, oil supply, air pollution.

INTRODUCTION

The US and other industrialized countries of the world are very dependent on imported oil, and oil imports continue to increase, threatening the strategic security of these countries. For instance, the US imports almost 50% of its current oil supply (US Congress, 1990). Furthermore, the transportation sector is particularly dependent upon oil, with approximately 97% of transportation energy being derived from petroleum in the US, and few substitutes exist for petroleum for transportation purposes. As a result, the transportation sector in many industrialized countries, such as the US, is strategically vulnerable to disruptions in the supply of oil.

In addition to strategic vulnerability, such large dependence upon foreign oil causes significant economic difficulties. For instance, a major fraction of the balance of trade deficit in the US is due to petroleum imports (Lynd *et al.*, 1991*a*). In addition, with the continuing decline in domestic economies and employment, new technologies are needed that can provide indigenous manufacturing capabilities for many of the industrialized nations.

Another concern is the increase in air pollution in major cities throughout the world, with transportation fuels accounting for a significant fraction of the problem. For instance, in the US, about two-thirds of carbon monoxide in major cities is due to transportation fuels. In addition, approximately one-third of the ozone formation experienced in cities within the US is attributed to transportation (US EPA, 1989). Furthermore, about one-third of the carbon dioxide released into the atmosphere in the US is the result of the utilization of transportation fuels, and carbon dioxide accumulation from the use of fossil fuels for transportation raises concern about the potential for global climate change (Intergovernmental Panel on Global Climate Change, 1990).

Production of ethanol from renewable sources of lignocellulosic biomass can improve energy security, decrease urban air pollution, and reduce accumulation of carbon dioxide in the atmopshere (Lynd *et al.*, 1991*a*). The production of ethanol from indigenous lignocellulosic biomass will stimulate new markets for the agriculture sector, thereby increasing domestic employment while reducing balance of payments deficits. The primary requirement is to reduce the cost of conversion of lignocellulosic biomass into ethanol to the point that it can compete with conventional transportation fuels without tax incentives. The benefits of this unique technology can then be realized.

ETHANOL PRODUCTION AND UTILIZATION

Currently, over 11 gigaliters (GL) (3 billion gallons) per year of ethanol are produced in Brazil from cane sugar. However, the price of sugar is controlled at about US$0.44/kg (US$0.20/lb) in the US and is far too expensive to be a viable feedstock for ethanol production (Commodity Research Bureau, 1991). Therefore,

about 3·8 GL (one billion gallons) per year of ethanol are produced from corn and other starch crops in the US. Since the cost of ethanol from starch crops still exceeds the value of the fuel, federal and various state tax incentives are employed to encourage its use as blends with gasoline in the US.

Ethanol is an excellent transportation fuel; it can be blended at about 10% levels with gasoline, as it is now in the US, or it can be used as 22% blends, as in Brazil. Blends realize three major benefits: (i) gasoline use is reduced, thereby lowering imported oil requirements; (ii) ethanol increases the octane of the gasoline to which it is added, improving the performance of the ethanol–gasoline blend, and (iii) ethanol provides oxygen for the fuel; thus more complete combustion results. This latter property is particularly desirable with the implementation of the Clean Air Act Amendments, which require the addition of oxygenates to gasoline to reduce the formation of carbon monoxide and ozone in non-attainment cities. Alternatively, ethanol can be reacted with isobutylene to form ethyl tertiary butyl ether (ETBE), which in addition to providing gasoline displacement, octane improvement, and oxygenate benefits of direct ethanol blends, reduces the vapor pressure of the gasoline to which it is added, and further improves the suitability of gasoline for meeting Clean Air Act Amendment requirements.

Table 1 provides some of the key properties of ethanol and ETBE.

Ethanol can be used as a nearly pure or 'neat' fuel. For example, hydrous ethanol containing about 95% ethanol and 5% water is used directly as a fuel for dedicated ethanol vehicles in Brazil (Wyman & Hinman, 1990). Alternatively, about 15% gasoline can be added to pure ethanol to form a mixture denoted as E85 for direct fuel use. In another formulation, E95, 5% gasoline is blended with pure ethanol for fuel use. Such nearly-pure ethanol fuels provide a number of environmental benefits, due to their low vapor pressure and reduced emissions of ethanol into the atmosphere, plus their clean-burning characteristics. In addition, ethanol has a low atmospheric photochemical reactivity, further reducing its impact on ozone formation.

Gasoline is added in E85 and E95 mixtures to improve cold start for these fuels. Alternatively, devices could be developed to facilitate cold starting. Engines designed for dedicated ethanol use can be tuned to achieve higher efficiencies than conventional gasoline engines. As a result, although ethanol would be expected to achieve two-thirds the range of gasoline on a volumetric basis based on its lower heating value, a vehicle can be expected to travel about 80% of the distance on a given volume of ethanol for the same volume of gasoline (Lynd et al., 1991a).

Table 1. Fuel properties for ethanol, methanol, ETBE, MTBE, isooctane, and gasoline

Property	Ethanol	Methanol	ETBE	MTBE	Isooctane	Unleaded regular gasoline
Formula	C_2H_5OH	CH_3OH	$(CH_3)_3COC_2H_5$	$(CH_3)_3COCH_3$	C_8H_{18}	C_4 TO C_{12}
Molecular weight	46·07	32·04	102·18	88·15	114	NA[a]
Density, kg/m³ @ 298 K	790	790	750	740	690	720–780
Air/fuel stoichiometric ratio						
Mole basis	14·29	7·14	42·86	35·71	59·5	57·28
Mass basis	9·02	6·48	12·10	11·69	15·1	14·6
Higher heating value, kJ/kg	26 780	19 919	36 031	35 270	44 420	41 800–44 000
Lower heating value, kJ/L	21 156	15 736	27 023	26 100	30 650	31 350–33 000
Research octane number (RON)	106	106	118	106	100	91–93
Motor octane number (MON)	89	92	102	99	100	82–84
(RON + MON)/2	98	99	110	103	100	88
Blending RON	114–141[b]	135	117–120[c]	118	NA[a]	NA[a]
Blending MON	86–97[b]	105	101–104[c]	101	NA[a]	NA[a]
(BLENDING RON + MON)/2	115	120	111	110	NA[a]	NA[a]
Atmospheric boiling pt., K	351·6	337·8	344·8	328·6	398	300–498
Heat of vaporization, kJ/kg	839	1 104	308	329	406	377–502
Flash pt., K	285	280	253	245	261	<233
Ignition pt., K	697	737	583	733	683	553–702
Reid vapor pressure, kPa						
Pure component	15·85		30·3	53·8		48·3 + 96·5
Blending	82·7–186	214 +	20·7–34·5	55·1		55·1–103·4
Water solubility, weight %						
Fuel in water	100	100	2	4·3	negligible	negligible
Water in fuel	100	100	0·6	1·4	negligible	negligible
Water azeotrope (atm b.p.), K	351·4	NA[a]	338·2	325·4	NA[a]	NA[a]
Water in azeotrope, wt%	4·4	NA[a]	4·0	3·2	NA[a]	NA[a]

[a]Not applicable.
[b]10% blends.
[c]Assumed 12·7% blend.
Sources: Wyman and Hinman (1990); Wyman and Goodman (1993b).

LIGNOCELLULOSIC BIOMASS COMPOSITION AND AVAILABILITY

Lignocellulosic biomass represents the major fraction of most plant matter. Common examples of lignocellulosic biomass include agricultural and forestry residues, the paper and much of the remaining organic fraction of municipal solid waste (MSW), industrial processing residues such as wastes in the paper and pulp industry, and herbaceous and woody plants grown as feedstocks for the production of fuels. As shown in Fig. 1, the major fraction of such materials, typically of the order of 35–50%, is a polymer of glucose known as cellulose. The next largest fraction, of the order of 20–35%, is hemicellulose. Hemicellulose is also a polymer of sugars, but the types and distributions of these sugars vary depending upon the particular biomass source. For many types of lignocellulosic biomass, the five carbon sugar xylose represents the predominant fraction of the hemicellulose component. The third largest fraction, of the order of 15 to about 25%, is typically lignin, a phenyl-propene polymer of complex composition that cannot be broken down to form sugar molecules. A number of other compounds such as plant oils, proteins, and ash make up the remaining fraction of the lignocellulosic biomass structure.

Lignocellulosic biomass represents a vast resource that could be used for production of fuels. As summarized in Table 2, it has been estimated that about 190 GL (50 billion gallons) of ethanol could be produced annually in the US from agricultural, forestry, and municipal waste materials alone. In addition, about 428–1130 GL (113–299 billion gallons) of ethanol could be produced from dedicated energy crops. The total of these sources could represent from 617–1320 GL (163–349 billion gallons) of ethanol production (Lynd *et al.*, 1991a). By way of comparison, it would require of the order of 510 GL (140 billion gallons) of

ethanol production per year to replace the current annual US gasoline market of approximately 410 GL (110 billion gallons), because of the somewhat lower energy content of ethanol as a fuel. Therefore, it is conceivable that more than sufficient ethanol from lignocellulosic biomass could be produced to replace all gasoline used in the US.

PRODUCTION OF ETHANOL FROM LIGNOCELLULOSIC BIOMASS

Acid hydrolysis of lignocellulosic biomass

As shown in Fig. 2, dilute or concentrated acids break down the cellulose and hemicellulose polymers in lignocellulosic biomass to form individual sugar molecules which can be fermented into ethanol. First, the biomass is milled to produce a sufficiently small particle size to facilitate the diffusion of acid into its structure. The acid breaks down the hemicellulose in the biomass to form xylose and other sugars which can be fermented into ethanol following neutralization. Acid also catalyzes hydrolysis of the cellulose fraction to produce glucose, which can be fermented into ethanol. The residual cellulose and hemicellulose plus the lignin and other solid materials left after hydrolysis can be burned as boiler fuel to provide energy to power the process, converted into octane boosters, or used as feedstocks for production of chemicals.

As an example, dilute sulfuric acid processes utilize of the order of 1·5% acid for the hydrolysis of biomass to form sugars (Wright, 1983; 1988c). Hemicellulose can be broken down at temperatures of around 160°C (320°F) to form xylose and other sugars with reasonable yields. However, hydrolysis of the crystalline cellulose requires temperatures of the order of 200–240°C (390–460°F). Unfortunately, these conditions are severe enough to degrade glucose into hydroxymethyl-

Agricultural Residues
— Cellulose 38%
— Hemicellulose 32%
— Lignin 17%
— Other 13%

Underutilized and Short Rotation Hardwoods
Cellulose ~50%
Extractives ~5%
Hemicellulose ~23%
Lignin ~22%

Sorted Municipal Solid Waste
— Cellulose 45%
Ash 15%
Lignin 10%
Hemicellulose 9%
Other carbohydrates 9%
Protein 3%
Other 9%

Herbaceous Energy Crops
— Cellulose 45%
— Hemicellulose 30%
— Lignin 15%
— Other 10%

Fig. 1. Although the physical appearance of various types of lignocellulosic biomass appears quite different, the composition is very similar with the major fraction being cellulose with a lesser amount of hemicellulose and lignin (Wyman, 1993; Wyman & Goodman, 1993a, b).

Table 2. Potential availability of lignocellulosic biomass and the corresponding ethanol production (Lynd et al., 1991a; Wyman & Goodman, 1993a, b)

	Productivity		Land available		Biomass availability		Ethanol potential		Energy potential	
	(Tonnes/ hectare/yr)	(Tons/ acre/yr)	(10⁸ hectares)	(10⁶ acres)	(Mega-tonnes)	(10⁶ tons)	(GL)	(Billions of gallons)	(GJ)[a]	(Quads)[a]
Waste materials										
Agricultural					203	224	84·8	22·4	1.8×10^9	1·7
Forestry					167	184	69·6	18·4	1.5×10^9	1·4
MSW					111	122	34·8	9·2	7.4×10^8	0·7
Lignocellulosic biomass										
Cropland										
Idled (1988)[b]	11/22	5/10	32	78	354–707	390–780	149·5–293·7	39·5–77·6	$3.2 \times 10^9 – 6.2 \times 10^9$	3·0–5·9
Excess (2012)[b]	11/22	5/10	61	150	680–1361	750–1500	283·9–567·8	75·0–150·0	$6.0 \times 10^9 – 1.2 \times 10^{10}$	5·7–11·4
Potential	7/18	3/8	61	150	408–1088	450–1200	169·2–453·4	44·7–119·8	$3.6 \times 10^9 – 9.6 \times 10^9$	3·4–9·1
Forest land	7	3	39	96	263	290	109·4	28·9	2.3×10^9	2·2
Total	132–161			324–396	1506–3193	1660–3520	617·3–1319·8	163·1–348·7	$1.3 \times 10^{10} – 2.8 \times 10^{10}$	12·4–26·5

[a] Ethanol rated at its lower heating value without consideration of possible efficiency improvements which would result in displacement of greater quantities of gasoline.
[b] Values are for biomass production potential in 1988 and 2012 based on expected availability of land and current and future biomass production (Lynd et al., 1991a).

furfural (HMF), which in turn degrades to form tars and other undesirable coproducts. Similarly, under these conditions, xylose degrades to form furfural, tars and undesirable compounds. Formation of substantial quantities of furfural, HMF, tars, and other by-products cannot be avoided by known processes at the harsh conditions required for dilute acid hydrolysis, and these degradation coproducts must be sold to achieve favorable economics. However, the size of the markets for these coproducts is too small to support large-scale ethanol production (Gaines & Karpuk, 1987). Thus, the low sugar and subsequent ethanol yields of 50–60% make it difficult for dilute acid processes to achieve low cost ethanol production (Wright, 1983; 1988).

Dilute acid processes were operated in the US during World War II for production of ethanol, but these technologies could not compete in peacetime. Such processes are also used in the former Soviet Union for the production of ethanol and single cell protein (Wenzl, 1970). Currently, the Tennessee Valley Authority (TVA) is developing technology for dilute acid conversion of MSW into ethanol (Buss et al., 1992; Lambert et al., 1990).

Strong acids, such as concentrated sulfuric acid or halogen acids, hydrolyze cellulose and hemicellulose at moderate temperatures with little sugar degradation (Goldstein et al., 1983; Goldstein & Easter, 1992). As a result, concentrated acid processes achieve the high yields of ethanol critical to economic success. On the other hand, because of the large quantities of sulfuric acid required and the relatively high cost of halogen acids, a substantial fraction of these acids must be recovered to achieve economic operation. The major challenge is to recover the acids at a cost significantly less than the price of these inexpensive materials (Wright et al., 1985). Several technologies are now being pursued for acid recovery which could make it possible to achieve application for some near-term markets. One of the primary benefits of concentrated acid processes is that they can convert a wide variety of biomass feedstocks into sugars for fermentation into ethanol (Goldstein & Easter, 1992).

Enzymatic hydrolysis of lignocellulosic biomass

Enzymes known as cellulase catalyze the breakdown of cellulose into glucose for fermentation into ethanol. Because enzymes are highly specific in the reactions that they catalyze, formation of by-products as evidenced in dilute acid hydrolysis is avoided, and waste treatment costs are reduced. Furthermore, enzymatic reactions take place at mild conditions and achieve high yields with relatively low amounts of catalysts. Enzymes have the further advantage in that they are naturally occurring compounds which are biodegradable and environmentally benign. Advances in enzyme-based technology for ethanol production have been substantial over the years, and as a result, ethanol production costs have been reduced considerably. Thus, large-scale application of ethanol produc-
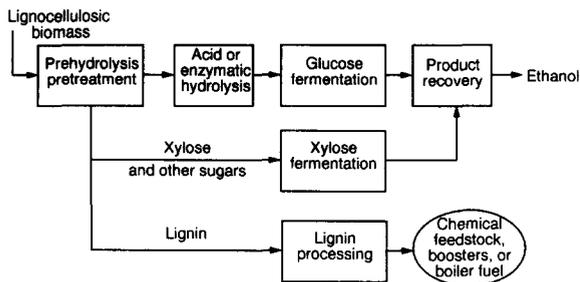
Fig. 2. Either acids or enzymes can catalyze the break-down of cellulose into glucose for fermentation to ethanol. The hemicellulose can be broken down to form xylose and other sugars that can also be converted into ethanol. The lignin can be burned as a boiler fuel or converted to octane boosters or chemicals (Wyman & Goodman, 1993*b*).

Fig. 3. For enzymatically catalyzed cellulose hydrolysis, a pretreatment step is used to open up the biomass structure to make it accessible to cellulase enzymes that convert cellulose into glucose for fermentation to ethanol. The xylose and other sugars from the hemicellulose are also fermented into ethanol. Enough lignin is available to provide all the process heat and electricity plus excess electricity to sell for added revenue when lignin is burned as a boiler fuel (Wyman, 1993).

tion through enzymatic hydrolysis of lignocellulosic biomass is now beginning to appear economically advantageous.

Pretreatment

Because lignocellulosic biomass is naturally resistant to breakdown to its component sugars, the pretreatment step shown in Fig. 3 is needed to open up the structure of the material, and to make it accessible for enzymes to hydrolyze the cellulose component at appreciable rates and acceptable yields. A number of options have been investigated for pretreatment of biomass, including acid-catalyzed steam explosion (Clark & Mackie, 1987), steam explosion (Brownell *et al.*, 1986; Brownell & Saddler, 1984), ammonia fiber explosion (Dale *et al.*, 1985; Holtzapple *et al.*, 1990), organosolv (Chum *et al.*, 1985), supercritical extraction (Chou, 1986; Reyes *et al.*, 1989), and dilute acid pretreatment. Currently, the dilute acid process appears to be in the best position for near-term commercial application (Knappert *et al.*, 1980; 1981; Schell *et al.*, 1991). In this process, about 0·5% sulfuric acid is added to the feedstock, and the mixture is heated to around 140–160°C (280–320°F) for 5–20 min (Grohmann *et al.*, 1985; 1986; Torget *et al.*, 1990). Most of the hemicellulose is hydrolyzed to form xylose and other sugars, leaving a porous structure of primarily cellulose and lignin that is more accessible to enzymatic action.

Dilute acid pretreatment has been applied to a number of agricultural residues, short rotation hardwoods and herbaceous energy crops successfully, and the digestibility of the pretreated material correlates well with the fraction of hemicellulose removed (Grohmann *et al.*, 1986; Torget *et al.*, 1988; 1990; 1991). Other pretreatment approaches open up the biomass structure in a similar fashion to dilute sulfuric acid. It would also be advantageous to develop economical techniques to remove the lignin from the biomass, since the solid lignin causes some processing difficulties in the downstream fermentation steps. In addition, it would be useful to develop pretreatment approaches that avoid the formation of salts during neutralization.

Minimization of by-product formation that could impede the subsequent fermentation steps is also desirable.

Enzyme production

Although a number of organisms, including bacteria and fungi, can produce cellulase, genetically altered strains of the fungus *Trichoderma reesei* are generally used for the cellulase production step in Fig. 3 because of their superior performance. Simple batch production of cellulase enzyme achieves satisfactory results. However, the best productivities and titers for enzyme production by fungal systems to date have generally been found for fed-batch operations in which the pre-treated biomass is slowly added to the enzyme production fermenter during the growth of the fungus and the production of cellulase (Watson *et al.*, 1984). Experiments with continuous enzyme production have suffered from lower cellulase productivities (Hendy *et al.*, 1984).

Cellulose hydrolysis and glucose fermentation

Several approaches have been examined for hydrolysis of cellulose and fermentation of glucose into ethanol as shown in Fig. 3. In one approach, typically termed separate hydrolysis and fermentation (SHF), cellulase from the enzyme production step is added to the bulk of the pretreated material to form glucose from the cellulose fraction. Upon completion of the hydrolysis reaction, yeasts ferment the glucose into ethanol. Thus, the SHF process involves distinct process steps for cellulase production, cellulose hydrolysis, and glucose fermentation, as shown in Fig. 4 (Mandels *et al.*, 1974; Wilke *et al.*, 1976).

A second cellulose conversion approach is termed direct microbial conversion (DMC). In this option, microorganisms simultaneously produce cellulase enzyme, hydrolyze cellulose, and ferment glucose into ethanol as shown in Fig. 4 (Veldhuis *et al.*, 1936; Ng *et*

al., 1977; Gauss et al., 1976; Lynd, 1989). In the most extensively studied version of the DMC process, an organism known as Clostridium thermocellum is used for enzyme production, hydrolysis, and glucose fermentation. Cofermentation with a second bacterium known as Clostridium thermosaccharoliticum simultaneously converts the hemicellulose sugars into ethanol. To date, these systems have suffered from formation of by-products such as acetic acid during the fermentation process, thereby limiting the yields achieved. Ethanol concentrations that such organisms will tolerate are also limited. However, the successful development of

the DMC process to achieve good performance could greatly simplify ethanol production from lignocellulosic biomass, and reduce ethanol production costs (Lynd et al., 1991b).

The cellulose conversion option that many currently favor is the simultaneous saccharification and fermentation (SSF) process shown in Figs 4 and 5 (Wright 1988; Wright et al., 1988). In this option, the cellulose hydrolysis and glucose fermentation steps are combined in a single vessel (Gauss et al., 1976; Takagi et al., 1977; Ghosh et al., 1982). Since cellulase is inhibited by glucose as it is formed, rapid conversion of
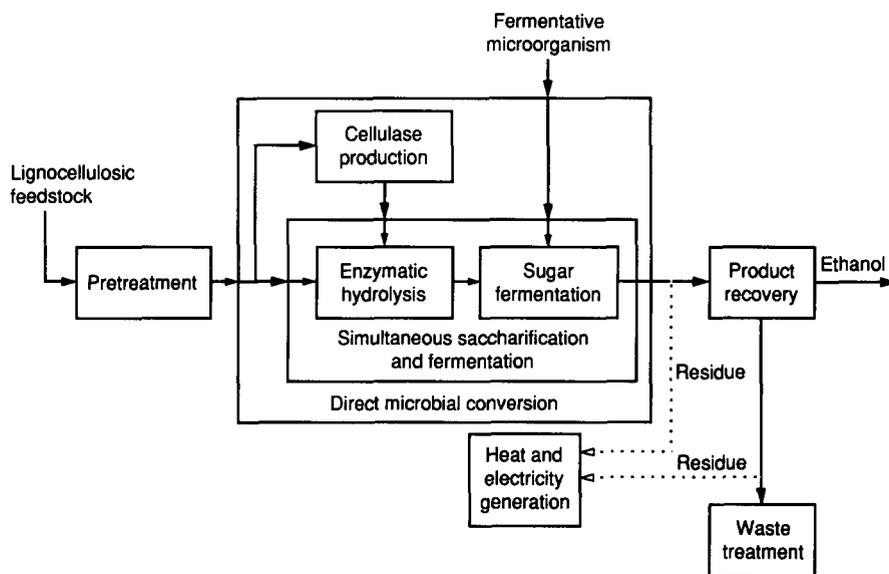
Fig. 4.   Distinct steps are involved for cellulase production, cellulose hydrolysis, and glucose fermentation for the separate hydrolysis and fermentation (SHF) process. The hydrolysis and fermentation steps are combined for the simultaneous saccharification and fermentation (SSF) process, while the direct microbial conversion (DMC) approach consolidates enzyme production with the hydrolysis and fermentation steps (Wyman et al., 1992).
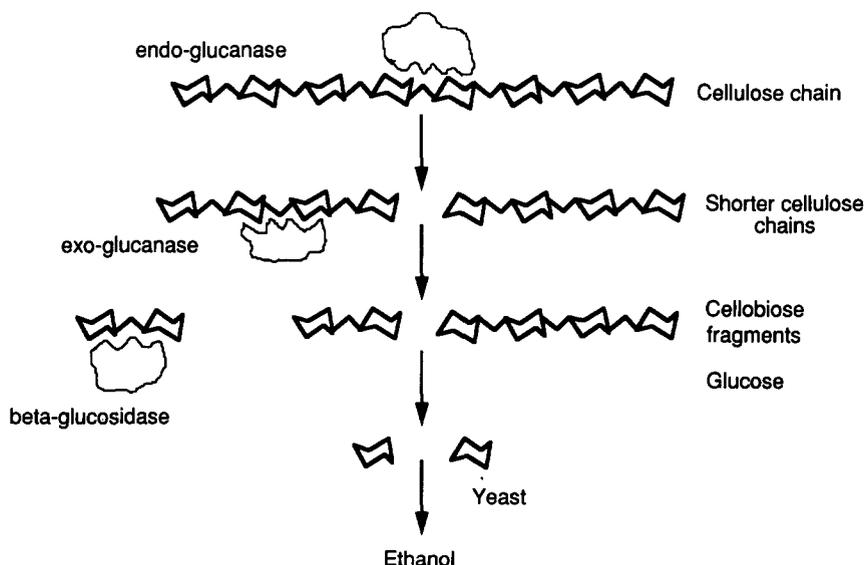
Fig. 5.   In the SSF process, the cellulose in pretreated biomass, cellulase enzyme, and yeast are all combined in one vessel to improve rates, yields, and concentrations of ethanol; reduce fermenter requirements; and reduce the probability of invasion by unwanted microorganisms. The three activities that comprise cellulase (endoglucanase, exoglucanase, and β-glucosidase) act synergistically to break down cellulose into glucose that yeast ferments to ethanol (Wright, 1988a, b; Wright et al., 1988).

the glucose into ethanol by yeast results in faster rates, higher yields, and greater ethanol concentrations than possible for SHF. Furthermore, by combining the hydrolysis and fermentation steps in one vessel, the number of fermenters required is approximately one-half that for the SHF process. The presence of ethanol in the fermentation broth also makes the mixture less vulnerable to invasion by unwanted microorganisms.

Over the years, substantial progress has been made in SSF technology for ethanol production (Lastick *et al.*, 1984; Wyman *et al.*, 1986; Spindler *et al.*, 1988; 1989; 1990; 1991). The hydrolysis of cellulose is carried out by a mixture of enzyme activities collectively known as cellulase. As shown in Fig. 5, one activity designated as endoglucanase breaks the cellulose somewhere along the chain. Then a second enzyme activity designated as exoglucanase breaks off two sugar units called cellobiose from the end of the chain. The third enzyme activity, β-glucosidase, cleaves these two sugar units apart to form glucose for fermentation into ethanol. Cellobiose is known to be a very powerful inhibitor of exoglucanase activities in particular, and minimization of this sugar results in higher rates of hydrolysis. Numerous experiments with the SSF process have clearly shown that high β-glucosidase activity minimizes the accumulation of cellobiose in the fermentation broth. Therefore, it is desirable either to employ enzymes with high β-glucosidase activity, add supplemental β-glucosidase to the fermentation broth, or employ yeast that has the capability of directly fermenting cellobiose into ethanol.

Several years ago, about 14 days were required to convert up to 70% of cellulose into ethanol at a concentration of about 2% by the SSF process (Lastick *et al.*, 1984). Through selection of improved cellulase and yeasts better suited to the SSF process, 90–95% yields with 4–5% ethanol concentrations can now be achieved in only 3–7 days for a variety of feedstocks (Spindler *et al.*, 1990; 1991). Furthermore, it has been found advantageous to add the whole cell broth from the enzyme production step containing not only the cellulase produced, but the fungal biomass and residual cellulose and lignin used in enzyme production into the SSF vessel (Takagi *et al.*, 1977; Schell *et al.*, 1990). Because some enzyme is trapped in the fungal cells, whole broth addition results in greater β-glucosidase activity essential to superior SSF performance, as well as more complete utilization of the substrate employed for enzyme production. The result is higher overall process yields than if enzyme is separated from the broth.

Hemicellulose conversion

It is interesting to note that the breakdown of cellulose to form glucose is quite difficult, while a number of organisms which have been adapted in the fermentation industry readily ferment glucose to ethanol. On the other hand, the hemicellulose fraction of many forms of biomass, such as hardwood and grasses, is rapidly

broken down to its component sugars in the dilute acid or other pretreatment step. Yet, conventional organisms cannot easily convert the five carbon sugars typically comprising hemicellulose into ethanol. If this portion is not used effectively, it adds substantial costs for waste disposal to the process and results in a significant loss of potential revenue from a fraction of the feedstock which was paid for.

Several options have been examined for the process of conversion of xylose into ethanol, shown in Fig. 3. A number of yeasts such as *Candida shehatae*, *Pichia stipitis*, and *Pachysolen tannophilis* will convert xylose into ethanol (Skoog & Hahn-Hagerdal, 1988; Prior *et al.*, 1989; Jeffries, 1990). However, such strains require that small amounts of oxygen be added in a microaerophilic operation to achieve reasonable xylose yields (Ligthelm *et al.*, 1988; Grootjen *et al.*, 1990); control of the oxygen at proper levels to achieve high yields and productivity is quite difficult. Furthermore, these strains often cannot tolerate such high ethanol concentrations as conventional glucose-fermenting strains.

Other microorganisms can ferment xylose and other five carbon sugars into ethanol. These include various bacteria and fungi (Carreira *et al.*, 1983; Asther & Khan, 1984; Antonopoulos & Wene, 1987; Slapack *et al.*, 1987; Lacis & Lawford, 1988; 1989; Buchert *et al.*, 1989; Lynd, 1989; Christakopoulos *et al.*, 1991). However, to date, high ethanol tolerance has not been demonstrated for these organisms, although there is some evidence that by proper process design, reasonable ethanol concentrations may be possible (Lynd *et al.*, 1991*b*). Furthermore, it is necessary to consistently demonstrate reasonable ethanol concentrations in such systems. These systems must also achieve high ethanol yields to be economically advantageous.

Another approach that has been used to convert xylose into ethanol is to produce an enzyme known as xylose isomerase that will convert xylose into an isomer called xylulose (Chaing *et al.*, 1981; Jeffries, 1981; Tewari *et al.*, 1985). This enzyme has been produced by genetically engineering the common intestinal bacterium *Escherichia coli* so that it can produce large quantities of xylose isomerase under controlled conditions. The xylose, the enzyme, and a yeast such as *Schizosaccharomyces pombe* are added to the vessel simultaneously because the isomerization reaction is equilibrium-limited to about 5–10% conversion levels (Lastick *et al.*, 1990). By having the yeast present to convert the xylulose into ethanol as xylulose is formed, yields of the order of 70% are possible. However, loss of xylose to form xylitol is still observed. Although this process has the advantage of employing anaerobic yeast on a large scale, the necessity of adding xylose isomerase enzyme to the broth, combined with different pH optima for the enzyme and yeast, complicates the technology (Hinman *et al.*, 1989).

A somewhat recent development has been the genetic engineering of several bacteria including *E. coli*

and *Klebsiella oxytoca* to allow them to directly ferment xylose and other five carbon sugars into ethanol (Ingram & Conway, 1986; Ingram *et al.*, 1987; Ohta *et al.*, 1991; Burnhardt & Ingram, 1992; Wood & Ingram, 1992). Key genes from the glucose-fermenting bacterium *Zymomonas mobilis* have been incorporated into these organisms to allow ethanol production. The result is a single organism that can ferment xylose into ethanol. Currently, bacteria modified by this approach must operate at neutral pH, necessitating that considerable amounts of base be added to the fermentation broth to counter the formation of some acids during the fermentation. Control of invasion by other organisms is also more difficult at neutral pH than at more acidic levels typical of most yeasts.

Lignin utilization

The third largest fraction of lignocellulosic biomass is typically lignin. To achieve economic viability, it is critical to derive value from the lignin fraction. Because lignin has a high energy content (Domalski *et al.*, 1987), it can be used as a boiler fuel to produce all the heat and electricity for the ethanol production process, as shown in Fig. 3 (Wright, 1988c; Hinman *et al.*, 1992). Furthermore, for most biomass feedstocks, the amount of lignin present is so high that excess electricity can actually be generated beyond that required for the process needs, and additional revenue can be gained from the sale of the excess electricity; about 8% of the energy content of the ethanol is available for sale as electricity from the process (Hinman *et al.*, 1992; US Department of Energy, 1993). Greater electricity revenues could be realized if more careful heat integration could be achieved beyond current process designs.

Because lignin is a complex phenolic polymer, it can be broken down to form a mixture of monomeric phenolic compounds and hydrocarbons (Johnson *et al.*, 1990). The phenolic fraction can be reacted with alcohols to form methyl or ethyl aryl ethers for use as octane boosters. This option could generate a more valuable product than using lignin as a boiler fuel, but the conversion technology must be sufficiently low in cost that a net income gain is achieved for the ethanol plant, relative to use of lignin as a boiler fuel.

Lignin can also be converted into a variety of chemical products including phenolic compounds, aromatics, dibasic acids, and olefins (Busche, 1985). These compounds could add significant revenue for the overall processing plant, but the cost of producing such chemicals must be low enough to insure a net revenue gain relative to lignin use in the boiler. In addition, because the market for lignin coproducts is limited relative to ethanol fuel markets, it is unlikely that lignin conversion into chemicals could support a large number of ethanol plants (Gaines & Karpuk, 1987).

Ethanol recovery

Typically, ethanol concentrations of 3–12% will result from fermentation of the hemicellulose and cellulose fractions into ethanol. In addition, there will be leftover solid materials such as lignin, enzymes, unreacted cellulose and hemicellulose, yeast, and various salts in the fermentation broth. The entire mixture can be fed to a distillation (beer) column to concentrate the ethanol in the overhead product, while water and solids exit from the bottom of the device. The somewhat enriched ethanol stream can then pass to a second rectification column for concentration of the ethanol-water mixture to the azeotrope composition of about 95% by weight ethanol. The bottoms from the first column can be further concentrated by centrifugation or other processes to provide a high solids content material that can be used as a boiler fuel. If the ethanol is used as a hydrous fuel, the azeotropic mixture would be suitable without further processing (Wyman & Hinman, 1990; Hinman *et al.*, 1992; US Department of Energy, 1993). However, since water has a low miscibility in gasoline, it is necessary to break the ethanol-water azeotrope if anhydrous ethanol is needed for blending with gasoline. This can be done by utilizing a third component, such as benzene or cyclohexane, in a ternary distillation column. Molecular sieves such as corn grits could also be used to preferentially absorb the ethanol or water phase of the mixture. Pervaporation membranes that are permeable to only one of the components, such as water, while retaining the ethanol, could be used to concentrate ethanol. At present, distillation with a third component and molecular sieves are typically used in commercial operations.

PROJECTED COST OF ETHANOL

Historical progress

In 1980, the projected cost of producing ethanol from lignocellulosic biomass was about US$0.95/liter (US$3.60/gallon) (Wright, 1988a,b). Since that time, substantial improvements have been made in cellulase for application to ethanol production. In addition, by utilizing the SSF instead of the SHF process, much better rates, yields, and concentrations have been achieved for ethanol production. Fermentative microbes have been identified that improve the performance of the SSF process further. Advances in microorganisms to convert the xylose fraction into ethanol have also improved the economics. As a result, the projected selling price of ethanol has been reduced to about US$0.32/liter (US$1.22/gallon), as shown in Fig. 6 (US Department of Energy, 1993).

Opportunities for technology improvement

Although considerable progress has been made in the technology for conversion of lignocellulosic biomass into ethanol, substantial opportunities still exist to lower the projected selling price (Hinman *et al.*, 1992; US Department of Energy, 1993). In the pretreatment area, these include reduction of the milling power by 25% through optimized approaches to size reduction.
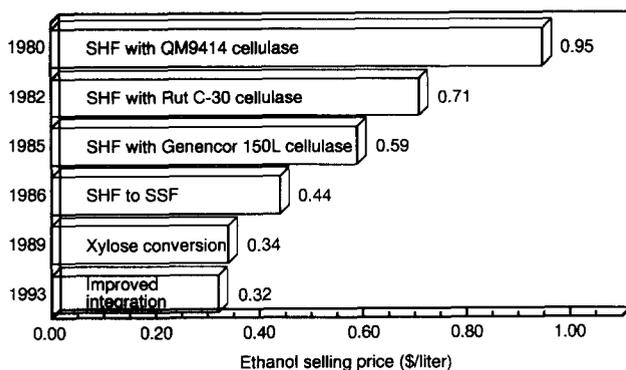
Fig. 6. The projected selling price of ethanol at the plant gate has dropped from about US$0.95/liter (US$3.60/gallon) in 1980 to about US$0.32/liter (US$1.22/gallon) now through improvements in cellulase enzymes, fermentative microorganisms, processing approaches, and xylose conversion technology.

Improving the yield of sugars from hemicellulose hydrolysis from about 80 to over 90% would also reduce the cost of ethanol production. Innovative pretreatment designs or alternative approaches could be used to increase the digestibility of the cellulose for conversion into ethanol, minimize the degradation of hemicellulosic sugars during pretreatment, and reduce energy requirements for pretreatment.

Another opportunity is to improve the utilization of the hemicellulose fraction of lignocellulosic biomass. This includes improving the yield of five carbon sugars from hemicellulose during the pretreatment step as mentioned above. In addition, it is desirable to improve the yields of ethanol from xylose, from the current levels of about 70 to over 90%, through better fermentative organisms. Improved ethanol productivity would also be beneficial in reducing the conversion times from two days to only one day or less. Another benefit would be gained by eliminating the requirement for inoculum preparation to seed hemicellulose conversion vessels. Use of organisms which can tolerate higher ethanol concentrations would improve the economics of xylose conversion into ethanol, by allowing smaller fermentation vessels and reduction in ethanol recovery costs. Xylose fermenting organisms must also tolerate various by-products created during the pretreatment process.

The cellulose conversion step could benefit from further technological improvements. First, it is desirable to develop technology to improve the yield of ethanol from cellulose from the current projected levels of 70–90%, through improvements in the pretreatment step to make the cellulose more accessible to enzymes, as well as through the enzymatic hydrolysis step by increasing enzyme activity. Such improvements could also decrease conversion times from the current levels of 5–7 to 2–3 days. Reductions in the cost of cellulase by higher productivities and titers would further improve the economics. Improve-

ments in cellulase specific activity would also benefit the process by reducing the amount of enzyme required to achieve a given sugar, and therefore ethanol, yield. If cost-effective methods could be developed for enzyme recovery in the conversion process, extended lifetime of enzymes could help to reduce ethanol production costs. Cellulose conversion would also benefit by insuring propagation of fermentative organisms in the ethanol production vessel, thereby eliminating the need for seed fermentation vessels for inoculum preparation. Improving the ethanol concentrations from the current levels of 4–5% to 8–12% would lower the cost of ethanol recovery somewhat, and reduce the size of fermentation vessels needed for the fermentation step.

Reductions in the power requirement for the overall process would lower ethanol costs. Targets include power needed for air compressors, vessel mixing, and size reduction. Decreasing the number of vessels, seed vessel requirements, and enzyme use would also reduce these costs. Power requirements for size reduction could be reduced either by utilizing materials that are more easily milled, such as herbaceous crops, or by developing equipment or processing sequences that more efficiently reduce particle size.

In addition, it is necessary to integrate the entire ethanol production process to understand the interactions among all the process steps from front to back. Feedstocks must be processed exactly as specified in process flow sheets developed through process engineering studies, and the performance of the integrated process determined. The flow sheet must then be modified and cost impacts determined to address problems identified. Substantial progress has been made in this direction, but full process integration is still needed, particularly at the pilot scale, to examine interactions among process steps and accurately measure material and energy balances. Furthermore, it is desirable to examine the performance of fully integrated continuous systems to determine their performance for lignocellulosic biomass conversion.

An area that could benefit from continuing advances in research and development is the feedstock production and collection activity. Biomass feedstocks which contain higher fractions of fermentable sugars are desirable. Feedstock costs under US$1.90/GJ (US$2/million Btu) could be achieved through enhanced biomass productivity. Lower cost feedstocks with a higher carbohydrate content decrease feedstocks costs, increase ethanol yields, and support a larger ethanol production plant within a given land area.

Process engineering studies have shown that a combination of improvements as mentioned above could reduce the projected selling price of ethanol from the current level of US$0.32/liter (US$1.22/gallon) to about US$0.18/liter (US$0.67/gallon) (Fig. 7). Such a price would be competitive with gasoline derived from oil at about US$25/barrel without tax incentives. With aggressive R & D, this goal could be
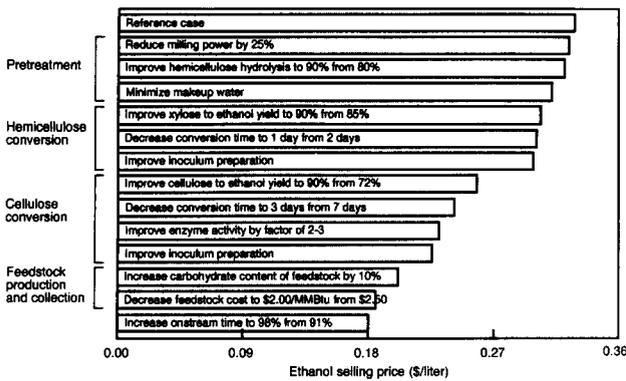
Fig. 7. A number of opportunities have been identified to reduce the selling price of ethanol to be competitive with gasoline from oil at US $26/barrel. One scenario for improvements in technology to achieve this goal is illustrated here.

Fig. 8. Substantial progress has been made on technology for ethanol production from biomass and continued improvements would make ethanol competitive with Department of Energy price projections for gasoline by about the year 2000. The range of ethanol prices shown depends on return on capital requirements (Lynd *et al.*, 1991a).

achieved just after the turn of the century, as shown in Fig. 8.

Near term applications

The current projected selling price of US$0.32/liter (US$1.22/gallon) for ethanol from lignocellulosic biomass is in the range of corn ethanol market prices. Thus, although the goal is to lower the cost of ethanol production to be competitive with gasoline through technology improvements, it may be possible to apply existing technology to produce ethanol from biomass for application in the current gasoline blending market. Furthermore, the projected selling price is based on feedstock costing US$46/dry tonne (US$42/ton) for a grass roots plant, and this feedstock cost translates into US$0.12/liter (US$0.46/gallon) of ethanol produced (Hinman *et al.*, 1992). The remaining US$0.20/liter (US$0.76/gallon) of the projected selling price covers operating, chemicals, and other costs, as well as return on capital invested. If an inexpensive material was used as the feedstock, the price of ethanol would be reduced accordingly. In addition, integration of the process into an existing plant, or recovery of higher value lignin coproducts, could lower the projected price of ethanol. Such opportunities would allow a possible higher rate of return to compensate for the risk of building the first plants. The lower projected selling price would also provide a greater margin to compensate for uncertainties in technology. As a result, an opportunity is presented to apply the technology in commercial markets now.

GLOBAL CLIMATE CHANGE

Even though carbon dioxide is released during the fermentation of sugars to form ethanol, the combustion of lignin to provide electricity and heat for the process, and the burning of ethanol to fuel a vehicle, the carbon dioxide is reutilized to grow new biomass to replace that harvested for ethanol production. As a result, no net carbon dioxide accumulates in the atmosphere. Thus, so long as renewable feedstocks are used
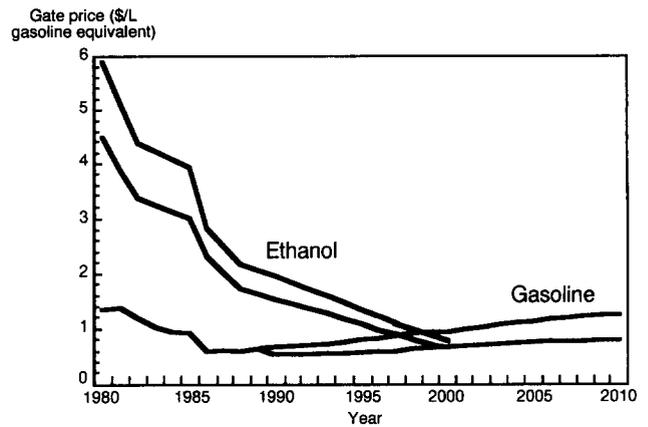
throughout the process, ethanol production does not lead to the potential global climate change. Even if fossil fuel must be used for biomass fertilizer, harvesting, fuel transport, and other steps, the total amount required is so modest that little impact on carbon dioxide accumulation is evidenced relative to conventional fuel use (Wyman & Hinman, 1990; Lynd *et al.*, 1991a); these sources could be derived from renewable resources, resulting in no net carbon dioxide build-up. Furthermore, when excess electricity from the ethanol plant displaces fossil-derived electricity, even further carbon dioxide benefits are gained.

CONCLUSIONS

Ethanol is an excellent transportation fuel that can be blended with gasoline either directly or as ETBE to reduce emissions of ozone-forming compounds and carbon monoxide, or used as a neat fuel to reduce the release of ozone-forming compounds. In both instances, ethanol use can improve urban air quality. Because enough waste materials and energy crops can be made available to produce sufficient ethanol to replace all gasoline used in the US, the potential ethanol supply from biomass is substantial, and large-scale application would thereby reduce the strategic vulnerability to disruptions in oil supply, while substantially improving the balance of trade deficit for imported oil. Technologies have advanced significantly for the conversion of lignocellulosic biomass into ethanol, so that the projected price of ethanol from lignocellulosic biomass is now competitive with ethanol derived from corn. Furthermore, opportunities have been identified which will reduce the cost of ethanol production to be competitive with gasoline, without the need for special tax incentives. Ethanol production from lignocellulosic biomass offers the added advantage in that no net accumulation of atmospheric carbon dioxide results.

REFERENCES

Antonopoulos, A. A & Wene, E. G. (1987). *Fusarium* strain development and selection for enhancement of ethanol production. *FY 1987 Biochemical Conversion Program Annual Review.* National Renewable Energy Laboratory, Golden, CO, p. C-51.

Asther, M. & Khan, A. W. (1984). Conversion of cellobiose and xylose to ethanol by immobilized growing cells of *Clostridium saccharolyticum* on charcoal support. *Biotech. Lett.*, 6(12), 809.

Brownell, H. H. & Saddler, J. N. (1984). Steam explosion pretreatment for enzymatic hydrolysis. *Biotech. Bioeng. Symp.*, 14, 55.

Brownell, H. H., Yu, E. K. C. & Saddler, J. N. (1986). Steam-explosion pretreatment of wood: effect of chip size, acid, moisture content and pressure drop. *Biotech. Bioeng.*, 28, 792.

Buchert, J., Pols, J. & Poutanen, K. (1989). The use of steamed hemicellulose as substrate in microbial conversions. *Appl. Biochem. Biotech.*, 20/21, 309.

Burnhardt, G. & Ingram, L. O. (1992). Conversion of xylan to ethanol by ethanologenic strains of *Escherichia coli* and *Klebsiella oxytoca*. *Appl. Environ. Microbiol.*, 58(4), 1128.

Busche, R. M. (1985). The business of biomass. *Biotech. Prog.*, 1(3), 165.

Buss, M. M., Shipley, T. M., Sheffey, L. L., Broder, J. D., Lambert, R. O. & Barrier, J. W. (1992). Design of a commercial plant for ethanol production from municipal solid waste. Poster presented at the 14th Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN.

Carreira, L. H., Wiegel, J. & Ljungdahl, L. G. (1983). Production of ethanol from biopolymers by anaerobic, thermophilic, and extreme thermophilic bacteria: I. Regulation of carbohydrate utilization in mutants of *Thermoanaerobacter ethanolicus*. *Biotech. Bioeng. Symp.*, 13, 183.

Chaing, L. C., Hsiao, H. Y., Ueng, P. P., Chen, L. F. & Tsao, G. T. (1981). Ethanol production from xylose by enzymatic isomerization and yeast fermentation. *Biotech. Bioeng. Symp.*, 11, 263.

Chou, Y. C. T. (1986). Supercritical ammonia pretreatment of lignocellulosic materials. *Biotech. Bioeng. Symp.*, 17, 18.

Christakopoulos, P., Koullas, D. P., Kekos, D., Koukios, E. G. & Macris, B. J. (1991). Direct ethanol conversion of pretreated straw by *Fusarium oxysporum*. *Biores. Technol.*, 35, 297.

Chum, H. L., Douglas, L. J., Feinberg, D. A. & Schroeder, H. A. (1985). Evaluation of pretreatments of biomass for enzymatic hydrolysis of cellulose. SERI/TP-231-2183, National Renewable Energy Laboratory, Golden, CO.

Clark, T. A. & Mackie, K. L. (1987). Steam explosion of the softwood *Pinus radiata* with sulfur dioxide addition, I. Process optimization. *J. Wood Chem. Tech.*, 7(3), 373.

Commodity Research Bureau (1991). 1991 CRB Commodity Year Book. Commodity Research Bureau, NY.

Dale, B. E., Henk, L. L. & Shiang, M. (1985). Fermentation of lignocellulosic materials treated by ammonia freeze explosion. *Dev. Ind. Microbiol.*, 26, 223.

Domalski, E. S., Jobe, T. L., Jr & Milne, T. A. (1987). *Thermodynamic Data for Biomass Materials and Waste Components*. American Society of Mechanical Engineers, NY.

Gaines, L. L. & Karpuk, M. (1987). Fermentation of lignocellulosic feedstocks: product markets and values. In *Proc. of the Energy from Biomass and Wastes X. Conference*, ed. D. L. Klass. Institute of Gas Technology, pp. 1395–416.

Gauss, W. F., Suzuki, S. & Takagi, M. (1976). Manufacture of alcohol from cellulosic materials using plural ferments. US Patent 3,990,944, 9 November.

Ghosh, P., Pamment, N. B. & Martin, W. R. B. (1982). Simultaneous saccharification and fermentation of cellulose: effect of β-D-glucosidase activity and ethanol inhibition of cellulases. *Enzyme Microbiol. Technol.*, 4, 425.

Goldstein, I. S. & Easter, J. M. (1992). An improved process for converting cellulose to ethanol. *Tappi J.*, 75(8), 135.

Goldstein, I. S., Pereira, H., Pittman, J. L., Strouse, B. A. & Scaringelli, F. P. (1983). The hydrolysis of cellulose with superconcentrated hydrochloric acid. *Biotech. Bioeng. Symp.*, 13, 17.

Grohmann, K., Torget, R. & Himmel, M. (1985). Optimization of dilute acid pretreatment of biomass. *Biotech. Bioeng. Symp.*, 15, 59.

Grohmann, K., Torget, R. & Himmel, M. (1986). Dilute acid pretreatment of biomass at high solids concentrations. *Biotech. Bioeng. Symp.*, 17, 135.

Grootjen, D. R. J., van der Lans, R. G. J. M. & Luyben, K. Ch. A. M. (1990). Effects of the aeration rate on the fermentation of glucose and xylose by *Pichia stipitis* CBS 5773. *Enzyme Microb. Technol.*, 12, 20.

Hendy, N. A., Wilke, C. R. & Blanch, H. W. (1984). Enhanced cellulase production in fed-batch culture of *Trichoderma reesei* C30. *Enzyme Microbiol. Technol.*, 6, 73.

Hinman, N. D., Wright, J. D., Hoagland, W. & Wyman, C. E. (1989). Xylose fermentation: an economic analysis. *Appl. Biochem. Biotech.*, 20/21, 391.

Hinman, N. D., Schell, D. J., Riley, C. J., Bergeron, P. W. & Walter, P. J. (1992). Preliminary estimate of the cost of ethanol production for SSF technology. *Appl. Biochem. Biotech.*, 34/35, 639.

Holtzapple, M. T., Jun, J.-H., Ashok, G., Patibandla, S. L. & Dale, B. E. (1990). The ammonia freeze explosion (AFEX) process: a practical lignocellulosic pretreatment. *Appl. Biochem. Biotech.*, 28/29, 59.

Ingram, L. O. & Conway, T. (1986). Expression of different levels of ethanologenic enzymes from *Zymomonas mobilis* in recombinant strains of *Escherichia coli*. *Appl. Environ. Microbiol.*, 54(2), 397.

Ingram, L. O., Conway, T., Clark, D. P., Sewell, G. W. & Preston, J. F. (1987). Genetic engineering of ethanol production in *Escherichia coli*. *Appl. Environ. Microbiol.*, 53(10), 2420.

Intergovernmental Panel on Climate Change (1990). *Climate Change — The IPCC Scientific Assessment*, eds J. T. Houghton, G. J. Jenkins & J. J. Ephraums. Cambridge University Press, Cambridge, UK.

Jeffries, T. W. (1981). Fermentation of xylulose to ethanol using xylose isomerase and yeast. *Biotech. Bioeng. Symp.*, 11, 315.

Jeffries, T. W. (1990). Fermentation of D-xylose and cellobiose. In *Yeast: Biotechnology and Biocatalysis*, eds H. Verachtert & R. De Mot. Marcel Dekker, NY, pp. 349–94.

Johnson, D. K., Chum, H. L., Anzick, P. & Baldin, R. M. (1990). Preparation of a lignin-derived pasting oil. *Appl. Biochem. Biotech.*, 24/25, 31.

Knappert, D., Grethlein, H. & Converse, A. (1980). Partial acid hydrolysis of cellulosic materials as a pretreatment for enzymatic hydrolysis. *Biotech. Bioeng.*, 22, 1449.

Knappert, D., Grethlein, H. & Converse, A. (1981). Partial acid hydrolysis of poplar wood as a pretreatment for enzymatic hydrolysis. *Biotech. Bioeng. Symp.*, 11, 67.

Lacis, L. S. & Lawford, H. G. (1988). Ethanol production from xylose by *Thermoanaerobacter ethanolicus* in batch and continuous culture. *Arch. Microbiol.*, **150**, 48.

Lacis, L. S. & Lawford, H. G. (1989). Analysis of the variation of ethanol yield from glucose or xylose with continuously grown *Thermoanaerobacter ethanolicus*. *Appl. Biochem. Biotech.*, **20/21**, 479.

Lambert, R. O., Jr, Moore-Bulls, M. R. & Barrier, J. W. (1990). An evaluation of two acid hydrolysis processes for the conversion of cellulosic feedstocks to ethanol and other chemicals. *Appl. Biochem. Biotech.*, **24/25**, 773.

Lastick, S., Spindler, D., Terrell, S. & Grohmann, K. (1984). Simultaneous saccharification and fermentation of cellulose. *Biotech.*, **84**, 277.

Lastick, S. M., Mohagheghi, A., Tucker, M. P. & Grohmann, K. (1990). Simultaneous fermentation and isomerization of xylose to ethanol at high xylose concentrations. *Appl. Biochem. Biotech.*, **24/25**, 431.

Ligthelm, M. E., Prior, B. A. & de Preez, J. C. (1988). The oxygen requirements of yeasts for the fermentation of D-xylose and D-glucose to ethanol. *Appl. Microbiol. Biotech.*, **28**, 63.

Lynd, L. (1989). Production of ethanol from lignocellulosic materials using thermophilic bacteria: critical evaluation of potential and review. In *Advances in Biochemical Engineering/Biotechnology*, ed. A. Fiechter, Vol. 38.

Lynd, L. R., Cushman, J. H., Nichols, R. J. & Wyman, C. E. (1991a). Fuel ethanol from cellulosic biomass. *Science*, **251**, 1318.

Lynd, L. R., Ahn, H.-J., Anderson, G., Hill, P., Kersey, D. S. & Klapatch, T. (1991b). Thermophilic ethanol production — investigation of ethanol yield and tolerance in continuous culture. *Appl. Biochem. Biotech.*, **28/29**, 549.

Mandels, M. L., Hontz, L. & Nystrom, J. (1974). Enzymatic hydrolysis of waste cellulose. *Biotechnol. Bioeng.*, **16**, 1471.

Ng, T. K., Weimer, P. J. & Zeikus, J. G. (1977). Cellulolytic and physiological properties of *Clostridium thermocellum*. *Arch. Microbiol.*, **114**, 1.

Ohta, K., Beall, D. S., Mejia, J. P., Shanmugam, K. T. & Ingram, L. O. (1991). Metabolic engineering of *Klebsiella oxytoca* M5A1 for ethanol production from xylose and glucose. *Appl. Environ. Micro.*, **57**(10), 2810.

Prior, B. A., Kilian, S. G. & du Preez, J. C. (1989). Fermentation of D-xylose by the yeasts *Candida shehatae* and *Pichia stipitis*. *Process Biochemistry*, Feb., pp. 21-32.

Reyes, T., Bandyopadhyay, S. S. & McCoy, B. J. (1989). Extraction of lignin from wood with supercritical alcohols. *J. Supercritical Fluids*, **2**, 80.

Schell, D. J., Hinman, N. D., Wyman, C. E. & Walter, P. J. (1990). Whole broth cellulase production for use in simultaneous saccharification and fermentation. *Appl. Biochem. Biotech.*, **24/25**, 287.

Schell, D. J., Torget, R., Power, A., Walter, P. J., Grohmann, K. & Hinman, N. D. (1991). A technical and economic analysis of acid-catalyzed steam explosion and dilute sulfuric acid pretreatment using wheat straw or aspen wood chips. *Appl. Biochem. Biotech.*, **28/29**, 87.

Skoog, K. & Hahn-Hagerdal, B. (1988). Xylose fermentation. *Enzyme Microbiol. Technol.*, **10**, 66.

Slapack, G. E., Russell, I. & Stewart, G. G. (1987). *Thermophilic Microbes in Ethanol Production*. CRC Press, Boca Raton, FL.

Spindler, D. D., Wyman, C. E., Mohagheghi, A. & Grohmann, K. (1988). Thermotolerant yeast for simultaneous saccharification and fermentation of cellulose to ethanol. *Appl. Biochem. Biotech.*, **17/18**, 279.

Spindler, D. D., Wyman, C. E., Grohmann, K. & Mohagheghi, A. (1989). Simultaneous saccharification and fermentation of pretreated wheat straw to ethanol

with selected yeast strains and β-glucosidase supplementation. *Appl. Biochem. Biotech.*, **20/21**, 529.

Spindler, D. D., Wyman, C. E. & Grohmann, K. (1990). Evaluation of pretreated herbaceous crops for the simultaneous saccharification and fermentation process. *Appl. Biochem. Biotech.*, **24/25**, 275.

Spindler, D. D., Wyman, C. E. & Grohmann, K. (1991). The simultaneous saccharification and fermentation of pretreated woody crops to ethanol. *Appl. Biochem. Biotech.*, **28/29**, 773.

Takagi, M., Abe, S., Suzuki, S., Emert, G. H. & Yata, N. (1977). A method of production of alcohol directly from yeast. In *Proc. Bioconv. Symp.*, IIT, India, pp. 551-71.

Tewari, Y. B., Steckler, D. K. & Goldberg, R. N. (1985). Thermodynamics of the conversion of aqueous xylose to xylulose. *Biophys. Chem.*, **22**, 181.

Torget, R., Himmel, M., Wright, J. D. & Grohmann, K. (1988). Initial design of a dilute sulfuric acid pretreatment process for aspen wood chips. *Appl. Biochem. Biotech.*, **17**, 89.

Torget, R., Werden, P., Himmel, M. & Grohmann, K. (1990). Dilute acid pretreatment of short rotation woody and herbaceous crops. *Appl. Biochem. Biotech.*, **24/25**, 115.

Torget, R., Walter, P., Himmel, M. & Grohmann, K. (1991). Dilute-acid pretreatment of corn residues and short-rotation woody crops. *Appl. Biochem. Biotech.*, **28/29**, 75.

US Congress (1990). *Replacing Gasoline: Alternative Fuels for Light-Duty Vehicles*. US Government Printing Office, Washington, DC, September.

US Department of Energy (1993). Assessment of costs and benefits of flexible and alternative fuel use in the US transportation sector. *Technical Report Eleven: Wood to Ethanol Process*, DOE/EP-0004. Prepared by Chem Systems for the US Department of Energy, Washington DC.

US EPA (Environmental Protection Agency) (1989). *EPA Lists Places Failing to Meet Ozone or Carbon Monoxide Standards*. Office of Public Affairs, Washington, DC.

Veldhuis, M. K., Christensen, L. M. & Fulmer, E. I. (1936). Production of ethanol by thermophilic fermentation of cellulose. *Ind. Eng. Chem.*, **28**, 430.

Watson, T. G., Nelligan, I. & Lessing, L. (1984). Cellulase production by *Trichoderma reesei* (RUT-C30) in fed-batch culture. *Biotech. Lett.*, **6**, 667.

Wenzl, H. F. (1970). *The Chemical Technology of Wood*. Academic Press, NY.

Wilke, C. R., Yang, R. D. & Stockar, U. V. (1976). Preliminary cost analysis for enzymatic hydrolysis of newsprint. *Biotech. Bioeng. Symp.*, **6**, 155.

Wood, B. E. & Ingram, L. O. (1992). Ethanol production from cellobiose, amorphous cellulose, and crystalline cellulose by recombinant *Klebsiella oxytoca* containing chromosomally integrated *Zymomonas mobilis* genes for ethanol production and plasmids expressing thermostable cellulase genes from *Clostridium thermocellum*. *Appl. Environ. Microbiol.*, **58**(7), 2103.

Wright, J. D. (1983). High temperature acid hydrolysis of cellulose for alcohol fuel production. SERI/TR-231-1714, National Renewable Energy Laboratory, Golden, CO.

Wright, J. D. (1988a). Ethanol from lignocellulosics: an overview. *Energy Prog.*, **8**(2), 71.

Wright, J. D. (1988b). Ethanol from biomass by enzymatic hydrolysis. *Chem. Eng. Prog.*, **84**(8), 62.

Wright, J. D. (1988c). Ethanol from lignocellulose: an overview. *Energy Progress*, **18**(2), 71.

Wright, J. D. & Power, A. J. (1985). Concentrated acid hydrolysis processes for alcohol fuel production. *Biotech. Bioeng. Symp.*, **15**, 511.

Wright, J. D., Power, A. J. & Bergeron, P. W. (1985). Evaluation of concentrated halogen acid hydrolysis processes for alcohol fuel production. SERI/TR-232-2386, National Renewable Energy Laboratory, Golden, CO.

Wright, J. D., Wyman, C. E. & Grohmann, K. (1988). Simultaneous saccharification and fermentation of lignocellulose: process evaluation. *Appl. Biochem. Biotech.*, **18**, 75.

Wyman, C. E. (1993). Cellulosic biomass conversion technology and its application to ethanol production from corn. *Fuel Reformulation*, March/April, pp. 67–74.

Wyman, C. E. & Goodman, B. J. (1993*a*). Biotechnology for production of fuels, chemicals, and materials. *Applied Biochemistry and Biotechnology*, **39/40**, 41.

Wyman, C. E. & Goodman, B. J. (1993*b*). Near term application of biotechnology to fuel ethanol production from lignocellulosic biomass. In *Opportunities for Innovation in Biotechnology*, ed. R. Busche. National Institute of Standards and Technology, Gaithersburg, MD (accepted for publication).

Wyman, C. E. & Hinman, N. D. (1990). Ethanol: fundamentals of production from renewable feedstocks and use as a transportation fuel. *Appl. Biochem. Biotech.*, **24/25**, 735.

Wyman, C. E., Spindler, D. D., Grohmann, K. & Lastick, S. (1986). Simultaneous saccharification and fermentation of cellulose with the yeast *Brettanomyces clausenii*. *Biotech. Bioeng.*, **17**, 221.

Wyman, C. E., Hinman, N. D., Bain, R. L. & Stevens, D. J. (1992). Ethanol and methanol from cellulosic biomass. *Fuels and Electricity from Renewable Resources*, ed. R. H. Williams, T. B. Johansson, H. Kelly, & A. K. N. Reddy. United Nations Solar Energy Group for Environment and Development (SEGED), 1992, UN Conference on Environment and Development in Brazil, pp. 865–924.