MINI-REVIEW

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A review of the production of ethanol from softwood

Abstract Ethanol produced from various lignocellulosic materials such as wood, agricultural and forest residues has the potential to be a valuable substitute for, or complement to, gasoline. One of the major resources in the Northern hemisphere is softwood. This paper reviews the current status of the technology for ethanol production from softwood, with focus on hemicellulose and cellulose hydrolysis, which is the major problem in the overall process. Other issues of importance, e.g. overall process configurations and process economics are also considered.

Introduction

At the beginning of the 1970s, OPEC decreased the output of oil, which resulted in a dramatic increase in oil prices and the world's interest in alternative fuels increased significantly. The aim then was to become less dependent on oil and to reduce the cost of expensive oil imports. Still today, an interest in production of gasoline-substituting fuels is maintained in many countries all over the world. However, the emphasis today is on reducing pollution and helping to satisfy the Kyoto protocol, established in 1997, by limiting the global net emission of carbon dioxide (CO_2). In December 1997, The European Commission adopted a white paper for a community strategy and action plan, "Energy for the Future", with the given objective of a contribution of renewable sources of energy to the European Union's gross inland energy consumption of 12% by 2010. One of the objectives is to reach 5 million metric tons of liquid biofuels by 2003.

During the past 150 years, human activities have caused a dramatic increase in the emission of a number of greenhouse gases, e.g. CO_2, which has led to changes in the equilibrium of the earth's atmosphere. The content of CO_2 in the air has increased from 280 ppm to 365 ppm during this period. The OECD countries contribute more than half of the world's total emission of CO_2. The United States is one of the countries with the highest rates of discharge, emitting more than 20,000 kg CO_2 per capita per year (The Swedish Energy Administration 1999). The corresponding CO_2 emission for Sweden for the same period was about 6,000 kg per capita (The Swedish Energy Administration 1999). The transport sector is responsible for the greatest proportion of CO_2 emission, and it is increasing from year to year.

One way of reducing environmental effects and the dependence on fossil fuels is to use renewable bioethanol. Apart from a very low net emission of CO_2 to the atmosphere, the combustion of bioethanol in general results in the emission of low levels of non-combusted hydrocarbons, carbon monoxide (CO), nitrogen oxides and exhaust volatile organic compounds (Bailey 1996; Wyman 1996). However, of environmental concern regarding the increased use of ethanol fuels is the increased exhaust emission of reactive aldehydes, such as acetaldehyde and formaldehyde. Thus, a key factor with respect to the possible effects of ethanol on urban air quality will be the durability and effectiveness of catalyst systems for aldehyde control.

Today, all cars with a catalyst can be run on a mixture of 90% gasoline and 10% ethanol without adjusting the engine. New cars can even use mixtures containing up to 20% ethanol. There are also new engines available that can run on pure ethanol, and so-called flexible fuel vehicles that are able to use mixtures of 0–85% ethanol in gasoline (E85). Ethanol can also replace diesel fuel in compression-ignition engines. However, to be able to mix diesel with ethanol an emulsifier is needed.

Fuel ethanol is used in a variety of ways; however, the major use of ethanol today is as an oxygenated fuel additive (Wheals et al. 1999). Mixing ethanol and gasoline has several advantages. The higher octane number of ethanol (96–113) increases the octane number of the mixture, reducing the need for toxic, octane-enhancing additives. Ethanol also provides oxygen for the fuel,

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which will lead to the reduced emission of CO and non-combusted hydrocarbons. Bailey (1996) has summarised the pros and cons of replacing spark-ignition and compression-ignition engines with optimised ethanol engines. His conclusions were that ethanol has about the same overall transport efficiency as diesel in compression-ignition engines, but is about 15% more efficient than gasoline in optimised spark-ignition engines (Bailey 1996). So, even though ethanol has only about two-thirds of the volumetric energy content of gasoline, it will still be possible to drive 75–80% of the distance on a given volume of ethanol (Wyman 1996).

Despite the advantages of bioethanol, Brazil and the United States are still the only two countries that produce large quantities of fuel ethanol (from sugar cane and maize, respectively Wheals et al. 1999). Brazil produces 12 million m^3 ethanol per year and the United States about half this amount. Fuel ethanol production is considerably more modest in the European Union, where France and Spain are the largest producers; each country producing about 200,000 m^3/year, used for production of ethyl tertiary butyl ether. However, countries such as The Netherlands, Italy and Portugal have also shown interest in ethanol. In Sweden, an ethanol plant based on grain, with a capacity of 50,000 m^3/year, came into operation in 2001. The ethanol is used as a 5% blend in gasoline.

The efficiency of ethanol production from biomass has steadily increased, but tax relief will be required to make fuel ethanol commercially viable compared to oil (Wheals et al. 1999). To attain an economically feasible process, the production cost must be reduced so as to approach the corresponding cost for fossil fuels. When using sugar- or starch-containing feedstocks, such as sugar cane or maize, the raw material accounts for 40–70% of the total ethanol production cost and the production cost is also dependent on animal feed as a by-product (Claassen et al. 1999). To achieve lower production costs, the supply of cheap raw materials is thus a necessity. When lignocellulosic raw materials are used, the main by-product is lignin, which can be used as an ash-free solid fuel for production of heat and/or electricity for which there are no foreseeable market limits. Accordingly, it will only be possible to produce large amounts of low-cost ethanol if lignocellulosic feedstocks such as fast-growing trees, grass, aquatic plants, waste products (including agricultural and forestry residues) and municipal and industrial waste are used (Wheals et al. 1999). The potential of using lignocellulosic biomass for energy production is even more apparent when one realises that it is the most abundant renewable organic component in the biosphere. It accounts for approximately 50% of the biomass in the world, with an estimated annual production of 10–50×10^{12} kg (Claassen et al. 1999).

Softwoods are the dominant source of lignocellulosic materials in the Northern hemisphere. During recent years, softwoods have been the subject of great interest in Sweden, Canada and the western United States as a renewable resource for ethanol production (Vallander 1998; Nguyen et al. 1999; Wu et al. 1999). The ecologically sustainable potential of woody biomass for fuel production is estimated to be 130 TWh/year in Sweden around 2020 (Parrika 1997). The total consumption of woody biomass for fuel production in Sweden in 1995 was 42 TWh/year. This is assumed to increase to about 90 TWh/year by 2020 due to the need for increased heat and power generation from biomass as a consequence of the shutdown of some of Sweden's nuclear power plants. Even so, there would be sufficient amounts of woody biomass for the production of bioethanol to be able to replace more than 20% of the gasoline and diesel used today.

Softwoods, e.g. pine and spruce, contain around 43–45% cellulose, 20–23% hemicellulose and 28% lignin. The hemicellulose is mainly made up of mannose, which is a hexose that can be fermented by normal baker's yeast, and the content of pentoses is only around 6–7% of the total wood. Theoretically, around 410 l of ethanol can be produced per metric ton dry raw material using only the hexose fraction and 455 l if all carbohydrates are considered.
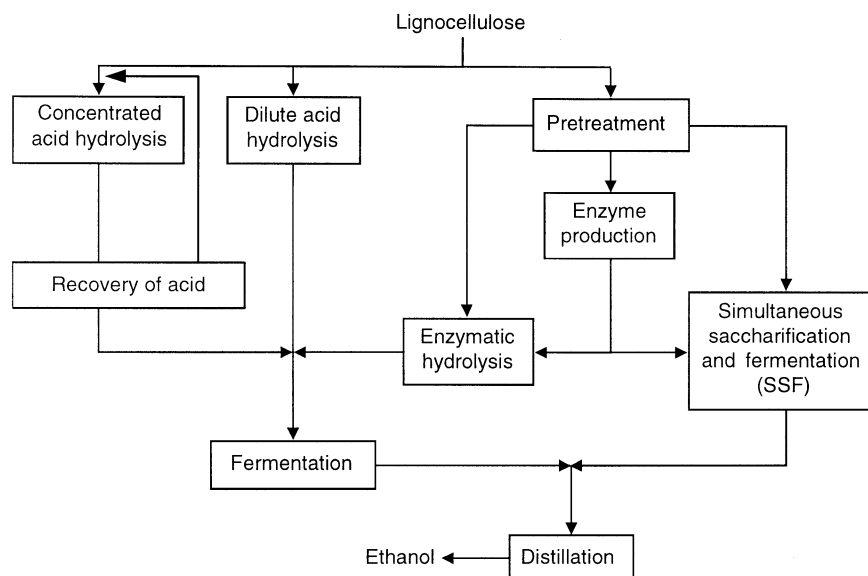
This review presents the recent status of the technology for production of fuel-ethanol from softwood.

Production of ethanol from lignocellulosics

Lignocellulose consists of three main components: cellulose, hemicellulose and lignin, the first two being composed of chains of sugar molecules. These chains can be hydrolysed to produce monomeric sugars, some of which can be fermented using ordinary baker's yeast. To attain economical feasibility a high ethanol yield is a necessity. However, producing monomer sugars from cellulose and hemicellulose at high yields is far more difficult than deriving sugars from sugar- or starch-containing crops, e.g. sugar cane or maize. Therefore, although the cost of lignocellulosic biomass is far lower than that of sugar and starch crops, the cost of obtaining sugars from such materials for fermentation into bioethanol has historically been far too high to attract industrial interest. For this reason, it is crucial to solve the problems involved in the conversion of lignocellulose to sugar and further to ethanol. However, the heterogeneity in feedstock and the influence of different process conditions on microorganisms and enzymes makes the biomass-to-ethanol process complex.

Ethanol can be produced from lignocellulosic materials in various ways. The main features of the different ethanol processes are outlined in Fig. 1. All processes comprise the same main components: hydrolysis of the hemicellulose and the cellulose to monomer sugars, fermentation and product recovery and concentration by distillation. The main difference between the process alternatives is the hydrolysis steps, which can be performed by dilute acid, concentrated acid or enzymatically. Some of the process steps are more or less the same, independent of the hydrolysis method used. For example, enzyme production will be omitted in an acid-hydrolysis process; likewise, the recovery of acid is not necessary in an enzyme-hydrolysis process.

Acid hydrolysis

Acid hydrolysis of plant lignocellulosic biomass has been known since 1819 (see Harris and Beglinger 1946). Examples are the modified Bergius process (40% HCl) operated during World War II in Germany, and the more recently modified Scholler processes (0.4% H_2SO_4) in the former Soviet Union, Japan and Brazil (Keller 1996). Acid hydrolysis can be performed with several types of acids, including sulphurous, sulphuric, hydrochloric, hydrofluoric, phosphoric, nitric and formic acid. These acids may be either concentrated or diluted.

Processes involving concentrated acids are operated at low temperature and give high yields (e.g. 90% of theoretical glucose yield), but the large amount of acids used causes problems associated with equipment corrosion and energy-demanding acid recovery (Jones and Semrau 1984). Furthermore, when sulphuric acid is used the neutralisation process produces large amounts of gypsum (Keller 1996). However, the process has attracted some new interest due to novel economic methods for acid recovery proposed by the companies Masada Resource Group (Birmingham, Ala), Arkenol (Mission Viejo, Calif.) and APACE (New South Wales, Australia) (Katzen 1997). To our knowledge there is no on-going development of concentrated acid hydrolysis of softwood.

The main advantage of dilute acid hydrolysis is the relatively low acid consumption. However, high temperatures are required to achieve acceptable rates of conversion of cellulose to glucose, and high temperatures also increase the rates of hemicellulose sugar decomposition and equipment corrosion (Jones and Semrau 1984). Sugar degradation products can also cause inhibition in the subsequent fermentation stage (Larsson et al. 1999a). The maximum yield of glucose is obtained at high temperature and short residence time, but even under these conditions the glucose yield is only between 50% and 60% of the theoretical value (Wyman 1996).

A two-stage process has been developed to decrease sugar degradation. In the first hydrolysis stage, the relatively easily hydrolysed hemicellulose is released under rather mild conditions. This enables the second acid hydrolysis step to proceed under harsher conditions without degrading the hemicellulose sugars to furfural, hydroxymethylfurfural and other degradation products. Using a two-stage dilute acid hydrolysis process, recovery yields of as much as 70–98% of the xylose, galactose, mannose and arabinose from softwood have been reported (Nguyen et al. 1999). However, the yield of glucose was still low at 50%.

In the United States, BCI (Wyman 1999) is commercialising the technology based on two-step dilute acid hydrolysis. In the first step, hemicellulose is hydrolysed under milder conditions (170–190°C) and in the second step the cellulose is hydrolysed under more severe conditions (200–230°C). However, as mentioned earlier, the high temperatures required for hydrolysis of the cellulose also result in sugar decomposition, which may cause some inhibition in the fermentation step, as well as a lower yield of sugars and ethanol. The Swedish Ethanol Development Foundation (formerly SSEU, now: the Bioalcohol Fuel Foundation) developed a process for two-stage dilute-acid hydrolysis of wood in cooperation with St. Lawrence reactors, Canada and Tennessee Valley Authority in the United States, which is known as the CASH process (O'Boyle et al. 1991). The process was based on hydrolysis at moderate temperature using a sulphur dioxide impregnation step, followed by a second hydrolysis step where diluted hydrochloric acid was used for impregnation. This concept was concluded in 1995; since then the development has focused on a two-step dilute-acid hydrolysis process for softwood employing sulphuric acid.

Pretreatment prior to enzymatic hydrolysis

The biodegradation of lignocellulosics was in its infancy as recently as the 1960s (Keller 1996). It is performed at

Table 1 Pretreatment conditions for acid-catalysed steam-pretreated softwoods

Substrate	Pretreatment conditions			Reference
	Catalyst	Temperature	Time	
Pine	4.44% SO_2	200 C	10 min	(Dekker 1987)
Spruce + pine	2.0–2.6% SO_2	188–204 C	2 min	(Wayman et al. 1986)
Pine	0.5–12% SO_2	182–248 C	0.5–18 min	(Clark and Mackie 1987)
Pine	2.0–2.6% SO_2	150–208 C	2–20 min	(Wayman and Parekh 1988)
Spruce	0.5–5% SO_2	190–220°C	50–250 s	(Schwald et al. 1989)
Spruce	0.35% H_2SO_4	215°C	140 s	(Schell et al. 1998)
Fir + pine	0.4% H_2SO_4	200–230°C	125–305 s	(Nguyen et al. 1998)
Spruce + pine	1–6% SO_2	190–230°C	2–15 min	(Stenberg et al. 1998b)
Spruce	0.5–4% H_2SO_4	180–240°C	1–20 min	(Tengborg et al. 1998)
Fir + Pine[a]	0.6–2.4% H_2SO_4	180–215°C	100–240 s	(Nguyen et al. 2000)
	2.5% H_2SO_4	210 C	100–120 s	
Spruce[a]	0.5% H_2SO_4	180 C	10 min	(Söderström et al. 2002a)
	1–2% H_2SO_4	180–220°C	2–10 min	

[a]Two-stage pretreatment

low temperature using enzymes as catalysts, thus providing a very specific conversion of cellulose. It is therefore considered to have the potential of higher yields and reduced formation of toxic compounds than acid hydrolysis. However, if cellulolytic enzymes are added to the native cellulose, the conversion of cellulose to sugar will be extremely slow since the cellulose is well protected by the matrix of hemicellulose and lignin. Therefore, pretreatment of the raw material is necessary to expose the cellulose or modify the pores in the material to allow the enzymes to penetrate into the fibres and hydrolyse the cellulose to monomeric sugars.

The most investigated pretreatment method is steam explosion (or steam pretreatment) with and without addition of acid catalyst. Of the acids, H_2SO_4 has been most extensively studied, (Torget et al. 1990, 1991, 1996; Nguyen et al. 1998, 1999, 2000; Tengborg et al. 1998) apparently because it is inexpensive and effective. SO_2-catalysed steam pretreatment has also been thoroughly investigated (Clark and Mackie 1987; Clark et al. 1989; Schwald et al. 1989; Fein et al. 1991; Ramos et al. 1992; Stenberg et al. 1998b). Pretreatment using gaseous SO_2 does not induce corrosion in the same manner as does pretreatment with H_2SO_4, and it is also easier and faster to introduce into the material. The main drawback of SO_2 is its high toxicity, which may pose safety and health risks. However, SO_2 is used in various industrial processes using established techniques. Other pretreatment methods include alkaline and solvent pretreatment techniques, which are basically delignification processes (Schell et al. 1991). Alkaline pretreatment is more effective on agricultural residues and herbaceous crops than on wood materials. This is also the case for the ammonia freeze explosion process (Holtzapple et al. 1991). Wet oxidation pretreatment involves the treatment of the biomass with water and air, or oxygen, at temperatures above 120°C, sometimes with the addition of an alkali catalyst (McGinnis et al. 1983). This method is suited to materials with a low lignin content, since the yield has been shown to decrease with increased lignin content, and since a large fraction of the lignin is oxidised and

solubilised (Schmidt and Thomsen 1998). As with many other delignification methods, the lignin cannot be used as a fuel, which considerably reduces the income from by-products in large-scale production.

Softwoods are generally recognised as being much more refractory than hardwoods or agricultural residues. This is due to the fact that softwoods have a more rigid structure and contain more lignin. Also, the content of acetylated groups is lower than in hardwoods and autohydrolysis cannot occur to the same extent. Acid-catalysed steam pretreatment is therefore the best available pretreatment method. Various conditions for steam pretreatment of softwoods have been investigated (Table 1).

The effect of the pretreatment is usually assayed by measuring the amount of hemicellulose and cellulose sugars liberated during the pretreatment, and the digestibility of the cellulose in the following enzymatic hydrolysis step. The latter is usually performed with washed material at low dry matter content to avoid end-product inhibition. Some recent results on sugar yields from softwood are shown in Table 2, obtained with one- and two-stage pretreatment using H_2SO_4 or SO_2. The yields are presented as a percent of the theoretical value based on the sugars available in the raw material. One problem in trying to maximise the total sugar yield in the process is that the sugars from hemicellulose and cellulose exhibit their maxima under different pretreatment conditions (Tengborg et al. 1998; Stenberg et al. 1998b).

The highest overall yield of hemicellulose and cellulose sugars obtained so far with one-stage pretreatment of a mixture of 70% fir and 30% pine is 75% (Nguyen et al. 2000), which was reached after impregnation with 0.65% H_2SO_4 followed by pretreatment at 215°C for 100 s. For spruce, the highest overall yields are 66 and 67% of the theoretical value using impregnation with SO_2 and H_2SO_4, respectively (Stenberg et al. 1998b; Tengborg et al. 1998). The most significant difference between pretreatment using H_2SO_4 and SO_2 as a catalyst is the fermentability (Tengborg et al. 1998). Material that has been impregnated with H_2SO_4 is often poorly fermented after being treated at high severities while this is

Table 2 Sugar yields from pretreatment, under optimal conditions, of softwoods using SO_2 and H_2SO_4 followed by enzymatic hydrolysis. *P* Pretreatment stage, *EH* enzymatic hydrolysis stage

Reference	Yields of sugars as % of the theoretical in raw material					
	(Stenberg et al. 1998b)	(Tengborg et al. 1998)	(Nguyen et al. 2000)	(Nguyen et al. 2000)	(Söderström et al. 2002a)	(Söderström et al. 2002a)
Pretreatment stages	1	1	1	2	2	2
Catalyst	SO_2	H_2SO_4	H_2SO_4	H_2SO_4	H_2SO_4	SO_2
Conditions	a	b	c	d	e	f
Glucose, P	13	33	21	57	41	35
Hemicellulose sugars, excluding glucose, P	52	55	79	84	96[g]	95[g]
Glucose, EH	58	39	31	17	36	45
Glucose and hemicellulose sugars, P + EH	66	67	75	82	77	80

[a]210 C, 5.5 min, 3.6% SO_2
[b]210 C, 1 min, 2.25% H_2SO_4
[c]215 C, 100 s, 0.65% H_2SO_4
[d]1st stage: 180°C, 4 min, 2.66% H_2SO_4; 2nd stage: 210 C, 1.5 min, 2.5% H_2SO_4

[e]1st stage: 180 C, 10 min, 0.5% H_2SO_4; 2nd stage: 200°C, 2 min, 2% H_2SO_4
[f]1st stage: 190°C, 2 min, 3% SO_2; 2nd stage: 220 C, 5 min, 3% SO_2
[g]Mannose only

not the case for SO_2. The difference in fermentability indicates that H_2SO_4 impregnation leads to either higher concentrations of by-products or the formation of additional inhibitory compounds.

To recover the maximum amount of sugars from both hemicellulose and cellulose, different pretreatment conditions are required. Two investigations by Stenberg and Tengborg (Stenberg et al. 1998b; Tengborg et al. 1998) suggest that two-stage pretreatment using H_2SO_4 impregnation in the first stage under mild conditions, followed by SO_2 impregnation in the second stage under harsher conditions, could be advantageous. Nguyen et al. (2000) used a two-stage dilute acid process to hydrolyse a mixture of 70% fir and 30% pine. The first stage was performed at 180°C for 4 min with 2.66% H_2SO_4, and the second stage at 215°C for 1.5 min with 2.5% H_2SO_4. The overall yield of hemicellulose sugars and glucose, after enzymatic hydrolysis, was 82% of the theoretical value (Table 2), which is an improvement of 7% over the one-stage pretreatment.

Söderström et al. (2002a, b) investigated two-stage pretreatment of spruce with impregnation with either SO_2 or H_2SO_4. When using SO_2, the optimal conditions were found to be impregnation with 3% SO_2 and a first pretreatment step at 190 C for 2 min and a second pretreatment step at 220°C for 5 min. The overall sugar yield was 80% of the theoretical (Table 2), which is a significant improvement over that obtained using one-stage pretreatment (66%; Stenberg et al. 1998b). When H_2SO_4 was used in the two-stage pretreatment of spruce, the overall yield was only 77%. The yields obtained for spruce are slightly lower than that obtained by Nguyen et al. (2000) for a mixture of fir and pine. This could be due to the different substrate or because a lower cellulase activity was used in the enzymatic hydrolysis; 25 FPU/g cellulose compared with the 60 FPU/g cellulose used by Nguyen et al. (2000).

Two-step steam pretreatment of softwood has some attractive advantages, such as high ethanol yield, better utilisation of the raw material and lower consumption of enzymes. However, further evaluation is required to determine whether these advantages outweigh the extra cost involved for the addition of another steam pretreatment step to the process.

Enzymatic hydrolysis and fermentation

After pretreatment, the cellulose is hydrolysed using cellulases. Cellulase production among fungi is common, and can be found in a large variety of species, including *Trichoderma*, *Penicillum* and *Aspergillus*. A book edited by Harman and Kubicek presents the most important research on *Trichoderma* and *Gliocladium* (Harman and Kubicek 1998). Compared with fungi, cellulolytic bacteria produce low amounts of cellulolytically active enzymes. A pioneering work was performed by Mandels and Sternberg (1976), who collected and screened 14,000 fungi. From this immense number of organisms, by far the most efficient cellulose-hydrolysing organism was the fungus *Trichoderma*. It produces a complex mixture of cellulase enzymes. These enzymes share a common feature, which is their specificity towards β-1,4-glucosidic bonds. The action of the cellulases is synergistic, which means that the combined activity of the enzymes is greater than the sum of the individual components. Liberated water-soluble cellobiose is cleaved into two glucose molecules by the enzyme β-glucosidase. Strictly speaking, β-glucosidase is not a cellulase; however, it has a very important role in hydrolysis since cellobiose is an end-product inhibitor of many cellulases (Mandels and Reese 1963; Sternberg et al. 1977). On the other hand, β-glucosidase is inhibited by glucose (Holtzapple et al. 1990). Since the enzymes are inhibited by the end products, the build-up of any of these products negatively affects cellulose hydrolysis. The maximum cellulase activity for most

fungally derived cellulases and β-glucosidase occurs at 50±5°C and a pH of 4.0–5.0 (Saddler and Gregg 1998). However, the optimal conditions change with the hydrolysis residence time (Tengborg et al. 2001a) and are also dependent on the source of the enzymes.

The enzymatic hydrolysis process can be designed in various ways. The steps following pretreatment, i.e. hydrolysis and fermentation, can be run as separate hydrolysis and fermentation (SHF) or as simultaneous saccharification and fermentation (SSF). The advantage of SHF is the ability to carry out each step under optimal conditions, i.e. enzymatic hydrolysis at 45–50°C and fermentation at about 30°C. It is also possible to run fermentation in continuous mode with cell recycling. The major drawback of SHF is that the sugars released inhibit the enzymes during hydrolysis. In SSF, the glucose produced is immediately consumed by the fermenting microorganism, e.g. *Saccharomyces cerevisiae* (ordinary baker's yeast), which avoids end-product inhibition of β-glucosidase. The ethanol produced can also act as an inhibitor in hydrolysis but not as strongly as cellobiose or glucose. Another advantage of SSF compared with SHF is the process integration obtained when hydrolysis and fermentation are performed in one reactor, which reduces the number of reactors needed The temperature of about 35°C in SSF is a compromise, but the development of thermotolerant yeast strains is expected to improve the performance of SSF. A major disadvantage of SSF is the difficulty in recycling and reusing the yeast since it will be mixed with the lignin residue. An often-claimed advantage is a reduction in the sensitivity to infection in SSF (Szczodrak and Targonski 1989; Wyman et al. 1992; Grohmann 1993). However, it was demonstrated by Stenberg et al. (2000b) that this is not always the case. Quite the contrary, SSF is, at least in some cases, more sensitive to infections than SHF.

The overall ethanol yield and the ethanol production rate depend not only on the sugar yield, but also on the fermentability of the solution. This is influenced by the concentration of the soluble substances released during pretreatment, present in the original raw material, or formed in the pretreatment step. The concentrations of these substances in the fermentation step depend on the configuration of the preceding process steps. To increase the sugar concentration in a future large-scale operation, it is assumed that the whole slurry after pretreatment would be used without introducing separation steps that would dilute the process stream. Furthermore, the initial substrate loading in enzymatic hydrolysis would probably be maintained at around 10% fibrous material or higher to maintain the energy required for ethanol recovery at an acceptable level (Zacchi and Axelsson 1989).

It is not possible to define a single optimum for enzymatic hydrolysis since this may shift depending on factors such as dry matter content, pH, temperature and the desired residence time. In general, lower solids concentrations result in higher hydrolysis yield, especially for dry matter concentrations below 5%. The concentration of cellulases has a high impact on the conversion of the cellulose. When softwood is hydrolysed, extremely high enzyme loadings can be used without signs of approaching saturation. Tengborg (Tengborg et al. 2001a) added 220 FPU/g cellulose, to SO_2-impregnated and steam-pretreated spruce, diluted to 5% dry matter, but still reached only about 78% conversion of the cellulose in the hydrolysis step after 72 h.

The optimum temperature and pH is not only a function of the raw material and the enzyme source, but is also highly dependent on the hydrolysis time. It has often been suggested that a temperature of 50°C is optimal (Martín et al. 1988; Krisa et al. 1997; Saddler and Gregg 1998). This was, however, not the case when the residence time was included as an optimisation parameter (Tengborg et al. 2001a). When longer residence times were employed (>24 h), a temperature of 38°C was found to be optimal. Likewise, a higher pH (around 5.3) reduced the differences between the various temperatures. This implies that it is of high importance to make an overall economic analysis when setting hydrolysis conditions.

The most frequently used microorganism for fermenting ethanol in industrial processes is *S. cerevisiae*, which has proved to be very robust and well suited to the fermentation of lignocellulosic hydrolysates (Olsson and Hahn-Hägerdal 1993). *Zymomonas mobilis* can ferment glucose to ethanol with higher yields, due to the production of less biomass, but is less robust (Rogers et al. 1979; Lawford and Rousseau 1998). Since lignocellulosic hydrolysates contain pentoses, which are not readily fermented by these microorganisms, several attempts to genetically engineer *S. cerevisiae* (Walfridsson 1996; Tonn et al. 1997), *Z. mobilis* (Lawford et al. 1997) and the bacteria *Escherichia coli* (Ingram et al. 1997) have been performed. Although significant advances have been made this is essentially still at the laboratory stage.

Since softwood hydrolysates contain much more mannose than xylose, the need for a xylose-fermenting organism is less when this raw material is used. Regarding softwood hydrolysates, *S. cerevisiae* has a great advantage over *Z. mobilis*, since *S. cerevisiae* ferments mannose and, after adaptation, also galactose. SSF has also been performed using thermotolerant yeasts (Szczodrak and Targonski 1988; Spindler et al. 1989a; Ballesteros et al. 1991), and cellobiose-fermenting yeasts (Spindler et al. 1989b; Spindler et al. 1992).

When hardwood is used as raw material, both SHF and SSF are usually rapid processes, resulting in high yields of ethanol. Softwood, and especially spruce, behaves differently. If steam pretreatment is performed in one step, SSF has some advantages, such as higher yield of ethanol and higher productivity. When SHF and SSF of SO_2-impregnated and steam-pretreated (215 C, 5 min) spruce were compared using the same dry matter content (5%) and enzyme dosage (21 FPU/g cellulose), the overall ethanol yield was 60% of the theoretical for SSF and 40% for SHF. When the enzyme concentration was increased to 32 FPU/g cellulose, the overall ethanol yield in the SSF was 280 l/metric ton raw material, corre-

sponding to 68% of the theoretical based on the content of hexose sugars in the raw material (Stenberg et al. 2000a). However, this might change for two-stage pretreatment. Söderström et al. (2002a, b) found no difference in overall ethanol yield when assaying two-stage pretreatment of spruce with SHF and SSF. It should, however, be noted that the assay with SHF was performed using washed pretreated material at 2% dry matter while SSF was performed with the whole slurry from the pretreatment at 5% dry matter.

Whether SHF or SSF is used to produce ethanol, some of the critical issues are the performance and production cost of the cellulases. Today, cellulases are produced by a small number of large enzyme companies, e.g. Novozymes and Genencor, in small quantities for applications other than cellulose hydrolysis. It will be necessary to develop more effective enzymes, either by selection or by using genetic tools, and to lower the production cost. The latter includes on-site production of enzymes using the carbon sources available in the ethanol production process. For softwood, that may very well be the pentose fraction, which constitutes about 7% of the raw material and which cannot be fermented by normal baker's yeast. Szengyel et al. (Szengyel 2000; Szengyel et al. 2000) investigated the possibility of using various carbon sources based on steam-pretreated spruce for production of cellulases using *Trichoderma reesei* RUT C30. They found that it was possible to use either the pretreated softwood or the liquid stream for enzyme production although the maximum enzyme activity obtained was rather low. The volatiles in the liquid stream were shown to be inhibitory to the fungus indicating that the use of the stillage stream, where the volatiles have been removed by distillation, should be suitable for enzyme production.

Recirculation of process streams

To reduce the emission of CO_2 it is necessary to utilise non-fossil fuels for production of the heat and electricity required to run the process. This can be achieved using the lignin fraction, obtained as a solid residue, and the concentrated stillage residue from the evaporation plant. However, the lignin is a valuable by-product, which is essential for the process economics. It is thus necessary to minimise the energy demand in the process and thereby increase the fraction of lignin that can be sold as a by-product. This can be achieved by energy integration, i.e. running the distillation integrated with a multiple effect evaporation unit (Larsson et al. 1997) and by utilising the flash vapour from the pretreatment step(s) to supply heat to units requiring low-pressure steam. A mass and energy balance shows that up to 50% of the energy in the raw material can theoretically be made available in the ethanol and another 36% in the solid fuel by-product (lignin). A feasible energy recovery in the products could be in the range of 50–60% of that in the original raw material. The rest of the energy is used to supply the
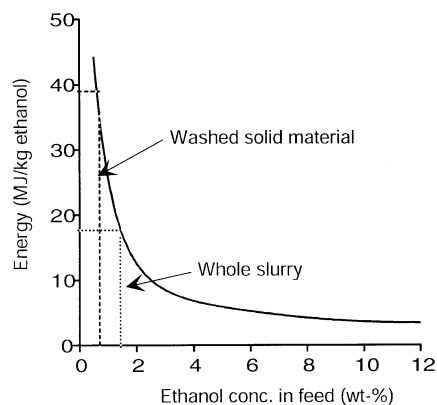
Fig. 2 Energy demand in a single distillation unit for concentration of the dilute ethanol stream to 94.5% (w/w)

process with heat and electricity and a fraction is dissipated as energy losses in the process.

Another way to reduce the energy consumption is to run the process at higher solids consistency or to recirculate process streams to maintain a high concentration of ethanol and dissolved solids. This would reduce the energy requirements in the distillation and evaporation units. In most laboratory scale investigations, the solid material is separated from the liquid after pretreatment, and washed prior to enzymatic hydrolysis (Clark and Mackie 1987; Schwald 1989; Gregg and Saddler 1996; Stenberg et al. 1998b; Tengborg et al. 1998). From an economic point of view, it is preferable to include the prehydrolysate in the enzymatic hydrolysis step since this reduces the need for fresh water in the subsequent enzymatic hydrolysis step. As a consequence, the concentration of fermentable sugars increases, provided that the cellulose conversion in the enzymatic hydrolysis step is unchanged. This results in a higher ethanol concentration in the fermentation step and reduces the cost of subsequent distillation (Zacchi and Axelsson 1989) and evaporation (Larsson et al. 1997). Zacchi and Axelsson have previously investigated the influence of the ethanol concentration in the feed on the energy consumption in a single distillation unit (Fig. 2). When washed solid material (5% dry matter) was used in enzymatic hydrolysis, the energy demand for the concentration of the dilute ethanol stream is twice as high as when the feed obtained using the whole slurry is used (Fig. 2).

Stenberg (1999) investigated recirculation of process streams for ethanol production from spruce based on SSF. The costs were calculated for a plant size corresponding to 200,000 metric tons spruce per year. Figure 3 shows that the ethanol production cost could be reduced significantly by recirculation of either the liquid after SSF (Fig. 3 configuration 1) or the stillage stream after distillation (Fig. 3 configuration 2). This is based on the assumption that the concentrations of non-fermentable dissolved substances, which accumulate during recirculation, have no effect on yield or productivity in SSF.

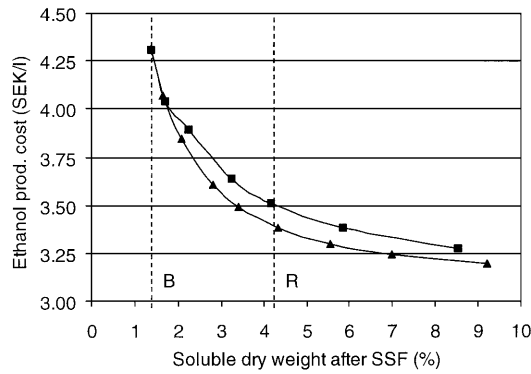However, the increased concentration of compounds originating from the wood or formed during pretreatment

Fig. 3 Ethanol production cost as a function of the soluble dry weight after simultaneous saccharification and fermentation (SSF). Configuration 1 (*triangles*) and configuration 2 (*squares*) with 5% dry matter fibrous material concentration in SSF. *R* Fresh water reduction by 60% compared with *B* Basecase

Fig. 4 The ethanol production cost broken down into individual costs. The SSF step is further subdivided into the cost of: enzymes (*black*), yeast (*dark grey*), capital (*light grey*) and other (including nutrients and electricity) (*white*)

might be toxic to the enzymes and/or yeast and thereby reduce the overall ethanol yield. The effect of process stream recirculation on ethanol production from steam-pretreated softwood has been investigated both for separate enzymatic hydrolysis and fermentation (Stenberg et al. 1998a) and for SSF (Alkasrawi et al. 2002). In the latter study, two process configurations were studied. In the first configuration a part of the stillage stream after distillation was recycled and in the second configuration the liquid after SSF was recycled. The aim was to minimise the energy consumption in the distillation of the fermentation broth and in the evaporation of the stillage, as well as the use of fresh water. When 60% of the fresh water was replaced by stillage, the ethanol yield and the productivity were the same as for the configuration without recirculation. The ethanol production cost was reduced by 17%. In the second configuration, up to 40% of the fresh water could be replaced without affecting the final ethanol yield although the initial ethanol productivity decreased. The ethanol production cost was reduced by 12%. At higher degrees of recirculation fermentation was clearly inhibited, resulting in a decrease in ethanol yield while hydrolysis seemed unaffected.

The inhibition is due to the accumulation of non-fermentable substances and inhibitors present in the process (Galbe and Zacchi 1993). Degradation products from sugars and lignin are formed during pretreatment (Olsson and Hahn-Hägerdal 1996), and prehydrolysate has been shown to inhibit the enzymatic hydrolysis of pretreated spruce (Tengborg et al. 2001b). A substantial increase in the conversion of cellulose can be obtained by washing the solid fraction with water prior to enzymatic hydrolysis, which, however, results in dilution of the hydrolysate and increased energy demand as discussed earlier. One theory that has been presented is that phenolic compounds may be responsible for the inhibition observed (Mandels and Reese 1963; Asiegbu et al. 1996; Tengborg et al. 2001b). Phenolic compounds have been found in prehydrolysates from woody substrates (Ando et al. 1986; Buchert et al. 1990; Tanahashi 1990; Fenske
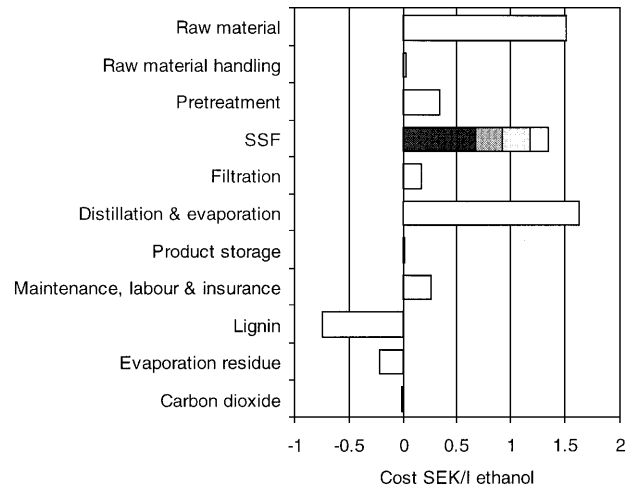
et al. 1998; Larsson et al. 1999b; Tengborg et al. 2001b). Laccase, which is an enzyme that oxidises phenolic compounds, was used to treat the prehydrolysate after pretreatment of spruce prior to enzymatic hydrolysis. This form of treatment was found to be a very specific method of detoxification since it decreased the amount of polyphenolic compounds by more than 90%, but had only a minor effect on other compounds. Overliming with calcium hydroxide, i.e. increasing the pH to about 10 and separating the precipitate from the liquid prior to reducing the pH, has been found to be an efficient detoxification method when used in the fermentation step (Larsson et al. 1999b).

Economics

The estimated cost of producing ethanol from wood varies widely between different investigations, with production costs in the range US$ 0.32–1.0/l ethanol (Nystrom et al. 1985; Lynd et al. 1996; von Sivers and Zacchi 1996). The lower costs are usually obtained for rather large plant capacities – above 600,000 metric tons of dry raw material per year. Most cost analyses are performed for hardwoods although some are for softwoods (Wayman and Dzenis 1984; Manerson et al. 1989; von Sivers and Zacchi 1995; Gregg et al. 1998; Kadam et al. 2000). The cost of raw material is a major contributor (about 25–40%) to the total production cost, although it varies between different studies due to differences in the local price of raw material (US$ 22–61/metric ton dry matter).

Most cost estimations are based on lab-scale and, to some extent, pilot-scale data for individual process steps and should be treated with caution and not used to obtain an absolute production cost. The cost estimations are useful, though, for identification of bottlenecks and to compare the relative costs of different process strategies

and the effect of changes in process configurations, e.g. plant capacity, ethanol yield, energy efficiency, etc.

As shown in Fig. 3, the production cost for the base case without recirculation of any process streams was SEK 4.30/l ethanol. This was based on an income corresponding to about SEK 1.0/l for solid fuel as by-product. A breakdown of the production cost (Fig. 4) into various process parts showed that the raw material constituted about 30%, distillation and evaporation about 30% and SSF about 25%. Half the cost for the SSF was due to cost of the cellulase enzymes, which is highly variable. The high cost for the distillation and evaporation steps is due to the low concentration of suspended solids in the SSF (5% dry matter used in the base case), which results in an ethanol concentration in the distillation of around 2 wt%.

Future needs for development

Much improvement has been achieved during the last 10 years regarding pretreatment, enzymatic hydrolysis, fermentation and process integration. The next step is to implement all these improvements in a pilot scale process where all steps are integrated into a continuous pilot plant to verify the technology and to obtain data for scale-up to a demonstration or full-scale process. Currently, there are no full-scale or demonstration plants for the production of bioethanol from wood using the enzymatic hydrolysis process. However, Iogen (Ottawa, Ontario, Canada) is building a commercial demonstration plant based on this technology for the conversion of agricultural residues to ethanol (Foody 1999).

In Sweden, a fully integrated pilot plant for ethanol production from softwood, comprising both two-stage dilute acid hydrolysis and the enzymatic process, is now being constructed in Örnsköldsvik. The pilot is mainly financed by the Swedish Energy Administration and will be in operation by mid-2003.

With the technology available today, at least at lab-scale, it should be possible to obtain around 280 l ethanol per metric ton spruce, which is about 70% of the theoretical overall yield based on the hexose sugars, at a production cost around SEK 4/l.

However, to make the ethanol production process economically feasible, several research challenges remain in order to further improve the overall yield of both ethanol and solid fuel, increase the productivity in the conversion steps and to reduce the production cost. The main challenges are:

- to improve enzymatic hydrolysis and especially the production of cellulases
- to improve fermentation of all sugars available in wood and to make the fermenting organism more tolerant to inhibitors
- to increase process integration to reduce the number of process steps, the energy demand and to re-use process streams in order to minimise the use of fresh water and reduce the amount of waste streams.

An interesting possibility that may reduce the production cost is to integrate ethanol production with a combined heat and power plant or with a pulp and paper mill. In 1994, the environmental consulting company ÅF-IPK AB performed a feasibility study on integration of an ethanol plant with a pulp mill, a combined power and heating plant and a sawmill with production of solid fuels (Ångpanneföreningen-IPK 1994). The result showed that the integration could reduce the ethanol production cost by up to 20%. A similar study (Kadam et al. 2000) was recently performed for co-production of ethanol and electricity from softwood, based on the conditions in California. This study also showed that co-production is more favourable than stand-alone production of ethanol.

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