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PRODUCTION OF ETHANOL FROM LIGNOCELLULOSIC MATERIALS

Mats Galbe and *Guido Zacchi*

INTRODUCTION

Bioethanol has been introduced in large scale in Brazil, the US and some European countries and is projected to be one of the dominating renewable biofuels in the transportation sector within the coming 20 years. At present bioethanol is produced almost solely from either sugar – or starch-based raw materials (e.g, cane sugar, corn, wheat etc) often called first generation (1G) bioethanol. However, it is a general opinion that future expansion has to be based on bioethanol from lignocellulosic materials, i.e. second-generation (2G) bioethanol such as agricultural residues (e.g. wheat straw, sugarcane bagasse, corn stover) and forest residues (e.g. sawdust, thinning rests), as well as from dedicated crops (salix, switch grass). These raw materials are sufficiently abundant and also available world-wide. They generate very low net greenhouse gas emissions, thus reducing environmental impacts. To achieve systems that are economic and sustainable it is necessary to efficiently utilize all parts of the raw materials, mainly cellulose, hemicellulose and lignin. This requires a high overall yield of ethanol produced by hydrolysis and fermentation of the carbohydrate fraction (hemicellulose and cellulose), as well as a high yield of the main co-product (lignin). Another option is to utilize the hemicellulose for other products like biogas. 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. sugarcane or corn. Therefore, the conversion process for lignocellulosic materials is more complex than are the other two processes.

Ethanol production from lignocellulose comprises the following main steps: hydrolysis of hemicellulose, hydrolysis of cellulose, fermentation, separation of lignin, recovery and concentration of ethanol and wastewater handling, see Figure 1. A process based on enzymatic hydrolysis and fermentation is currently regarded as the most promising option for the conversion of carbohydrates in lignocellulosic materials into ethanol in an energy-efficient way, resulting in high yields and low production cost[1, 2]. The enzymatic hydrolysis and fermentation can either be run separately (SHF) or combined into a simultaneous saccharification and fermentation (SSF). The latter has been shown to result in higher ethanol yields than does SHF. Some of the most important factors to reduce the production cost are: efficient utilization of the raw material by high ethanol yields, high productivity, high ethanol concentration in the feed to distillation and process integration in order to reduce capital cost and energy demand. The key steps for success are the conversion steps, i.e. pretreatment, enzymatic hydrolysis and fermentation (or SSF) of all sugars. It is also crucial to have a highly integrated process working at high consistency to minimize the energy demand in the downstream processing, e.g. distillation and evaporation. Pilot-scale production plants and pre-commercial demonstration facilities have recently been brought into operation in several places world-wide[3, 4, 5, 6]. However, the process concept has not yet been demonstrated on an industrial scale.

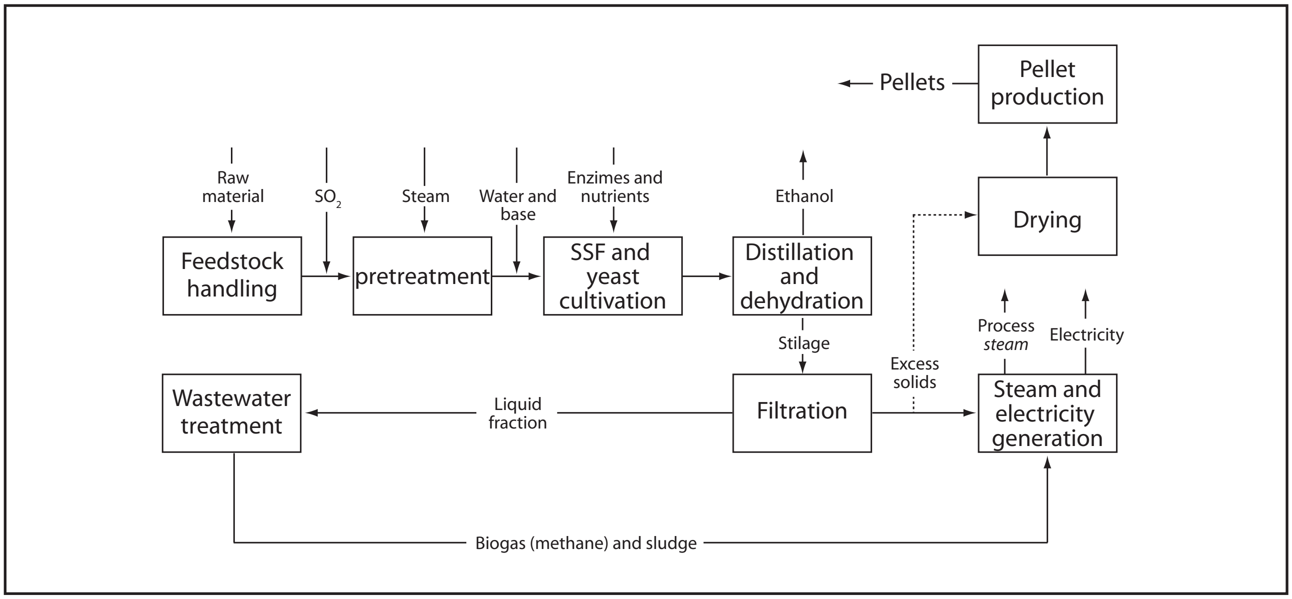

FIGURE 1 Schematic flow of the production of ethanol from lignocellulosic meterials.

External integration, with other production units, like a heat and power plant, a pulp mill or a first-generation ethanol production plant may also reduce the energy demand and the production cost. This type of studies is still only performed at lab scale and by computer simulations and the feasibility has also to be proven at pilot or demo scale.

This chapter summarizes recent developments of bioconversion processes and discusses the individual process steps aiming at fuel ethanol production with emphasis on process integration mainly based on results obtained at Lund University.

PRETREATMENT

Enzymatic hydrolysis, using cellulases, is regarded to be the most attractive way to convert cellulose to glucose[7, 8, 9]. However, due to the recalcitrant nature of most biomass species, the enzymatic hydrolysis is very slow and it is difficult to reach high sugar yields if the raw material is not pretreated prior to enzymatic hydrolysis. The pretreatment is perhaps the single most crucial step as it has a large impact on all the other steps in the process, e.g. enzymatic hydrolysis, fermentation, downstream processing and wastewater handling in terms of digestibility of the cellulose, fermentation toxicity, stirring power, energy demand in the down-stream processes and waste water treatment demands.

An effective pretreatment should have a number of features[10]. It has to:

- Result in high recovery of all carbohydrates.
- Result in high digestibility of the cellulose in the subsequent enzymatic hydrolysis.
- Produce no or very limited amounts of sugar and lignin-degradation products[11]. The pretreatment liquid should be possible to ferment without detoxification.
- Result in high solids concentration as well as high concentration of liberated sugars in the liquid fraction.
- Require a low energy demand or be performed in a way so that the energy can be re-used in other process steps as secondary heat.
- Require low capital and operational cost

Several pretreatment methods have been investigated during the last two decades. The various methods can be classified in different ways, e.g. physical (e.g. milling, grinding, and irradiation), chemical (e.g. alkali, dilute acid, oxidizing agents and organic solvents), physico-chemical (e.g. steam pretreatment/autohydrolysis, hydrothermolysis, and wet oxidation) and biological, or combinations of these. Several reviews on pretreatment have been written during the last few years[10, 12, 13, 14, 15] and the classification is not always consistent between these. It is difficult to clearly assign a pretreatment method to one group, since several mechanisms may be involved to break down the material.

PHYSICAL METHODS

Physical methods comprise chipping, milling and grinding. The biomass is turned into a fine powder, which increases the surface area of the solid material and to some extent also improves the decrystallization of cellulose. In order to achieve a high digestibility in the enzymatic hydrolysis step very small particles are required which calls for prohibitively high power consumption. It can be even higher than the theoretical energy content that is available in the biomass[16]. However, physical treatment in an extruder combined with heating and addition of chemicals could be an interesting option[17].

CHEMICAL METHODS

Dilute acid pretreatment is performed by soaking (or by spraying) the material using a dilute acid solution and then by heating to temperatures between 140-200 °C for a certain time (from several minutes up to an hour). Sulfuric acid, at concentrations usually below 4 wt-percent, has been of most interest in such studies as it is inexpensive and effective. The hemicellulose is hydrolyzed and the main part is usually obtained as monomer sugars. It has been shown that materials that have been subjected to acid hydrolysis may be harder to ferment because of the presence of toxic substances[18, 19, 20].

Alkaline pretreatment is performed at lower temperature and pressure than acid hydrolysis. Soaking of the material in an alkaline solution, like sodium, potassium or ammonium hydroxide, followed by heating leads to swelling of the pores in the material. This results in an increase in the internal surface area, and a decrease in the degree of polymerization and crystallinity. Alkaline pretreatment breaks the bonds between lignin and carbohydrates and disrupts the lignin structure, which makes the carbohydrates more accessible to enzymatic attack. This pretreatment method is more effective on agricultural residues and herbaceous crops than on wood materials, as these materials in general contain less lignin. For woody materials the concentration of alkali has to be increased considerably, thus the procedure is more like a Kraft pulping process.

Pretreatment using lime instead of sodium hydroxide is an alkaline method, especially suited for agricultural residues, e.g. corn stover, or hardwood materials, such as poplar[21, 22]. Lime has attained more attention due to low cost and the possibility to recover it from water when reacted with CO_2 to yield almost insoluble $CaCO_3$. The latter can then be converted to lime using the lime kiln technology.

Addition of calcium to the system may however result in problems like fouling in other parts of the process as calcium salts have rather low solubility. In the process the pH and the temperature vary from unit to unit which means that precipitation may occur in some unexpected places especially if process streams are recirculated. This is a well-known problem in the pulp and paper industry.

Another approach is to use an organic solvent, like methanol, ethanol, acetone, ethylene glycol, triethylene glycol and phenol, with addition of inorganic acid catalysts (H_2SO_4 or HCl). These so called organosolv processes[23] dissolves the lignin which is recovered in the organophilic phase. These methods require total recovery of the solvent both for economic and environmental reasons and also as the solvent may be inhibitory to the enzymatic hydrolysis and fermentation steps. A special case is the use of ethanol as solvent as this is already produced in the process which facilitates the recovery.

PHYSICO-CHEMICAL METHODS

This category comprises methods that combines a physical and a chemical effect like steam pretreatment with addition of a catalyst (acid or alkaline), hydrothermolysis, wet oxidation and ammonia fibre explosion (AFEX).

Steam pretreatment is one of the most widely used methods for pretreatment of lignocellulose materials. In reality, it is a chemical method very similar to dilute-acid hydrolysis although usually performed at much higher dry matter content in a steam environment. The raw material is usually treated with high-pressure saturated steam at typical temperatures between 160 and 240 °C for 1-20 minutes, after which the pressure is released. The acid can either be present in the raw material or be added, such as H_2SO_4 or SO_2, to enhance the hydrolysis. Most agricultural residues and some types of hardwood contain enough organic acids (mainly acetic acid) to act as catalysts for the hemicellulose hydrolysis, so called auto-hydrolysis. The latter usually starts at neutral pH and ends at a pH around 3.5-4 depending on how much acid is released. Addition of an acid to reduce the pH considerably, often below 2, results in an increased recovery of hemicellulose sugars, and also improves the subsequent enzymatic hydrolysis of the solid residue but may also cause further degradation if too severe. It has been widely tested in pilot scale equipment, for example, in the NREL pilot plant in Golden, Co (USA)[24], in the SEKAB pilot plant in Örnsköldsvik (Sweden)[5] and is also used in a demonstration scale ethanol plant at Iogen in Ottawa (Canada)[4] and is considered to be close to commercialization.

Hydrothermolysis is similar to steam treatment but is performed in liquid hot water at somewhat lower temperatures and lower dry matter content. This results in solubilization of diluted sugars in oligomer form[25, 26] which results in energy-demanding down-stream processing. In cases where an acid is added, the method becomes similar to dilute-acid pretreatment.

Wet-oxidation pretreatment involves the treatment of the biomass with water and air, or oxygen, at temperatures between 120-200 °C, sometimes with the addition of an alkali catalyst. This method is suited for materials with low lignin content, since the yield has been shown to decrease with increased lignin content, and since a large fraction of the lignin is oxidized and solubilized[27]. As with many other delignification methods, the lignin cannot be used as a solid fuel, which considerably reduces the income from by-products in large-scale production.

Ammonia fibre explosion (AFEX) is also an alkaline method, which similarly to the steam pretreatment process operates at high pressures. The biomass is treated with liquid ammonia about 10-60 minutes at moderate temperatures (below 100 °C) and high pressure (above 3 MPa)[28, 29]. Up to 2 kg of ammonia is used per kg of dry biomass. The ammonia is recycled after pretreatment by reducing the pressure, as ammonia is very volatile at atmospheric pressure. During pretreatment only a small amount of the solid material is solubilized, i.e. almost no hemicellulose or lignin is removed. The hemicellulose is degraded to oligomer sugars and deacetylated[30], which is a probable reason for the hemicellulose not becoming soluble. However, the structure of the material is changed resulting in an increased water-holding capacity and a higher digestibility. Like the other alkaline pretreatment methods Afex performs best on agricultural waste, but has not proven to be efficient on wood, due to its higher lignin content[31, 32]. According to SUN *et al*. the Afex process does not produce inhibitors that may affect downstream biological processes[11].

Another type of process utilizing ammonia is the ammonia recycle percolation (ARP) method[33, 34]. In the process aqueous ammonia (10-15 wt-%) passes through biomass at elevated temperatures (150-170 °C) after which the ammonia is recovered. ARP is an efficient delignification method for hardwood and agricultural residues, but is somewhat less effective for softwood.

BIOLOGICAL METHODS

Biological pretreatment can be performed by applying either enzymes or microorganisms to the lignocellulose material. In most cases investigated the purpose has been to degrade the lignin fraction by use of some white – and soft-rot fungi[32, 35]. Biological pretreatment has not attracted much attention as the rate of biological pretreatment processes is far too low for industrial use. However, the method could be used as a first step followed by some of the other pretreatment methods.

ASSESSMENT OF PRETREATMENT

For evaluation of the various pretreatment methods one important thing is the effect the pretreatment has on the constituents of the biomass, i.e. cellulose, hemicellulose and lignin, and the properties of the solid fraction remaining which is to be hydrolysed by enzymes. Based on this the chemical and physico-chemical methods can be divided in classes depending on the pH of the pretreatment.

- *Low pH methods*, i.e. addition of acids, e.g. dilute acid hydrolysis and steam treatment with addition of acids. Most of the hemicellulose is usually hydrolysed to monomer sugars and to some extent oligomer sugars available in the liquid fraction after pretreatment. Depending on the severity, i.e. temperature, acid concentration and residence time, also a part of the cellulose may be hydrolysed. Also, a minor part of the lignin is solubilized as phenolic compounds, but the major part remains in the solid fraction although redistributed. These pretreatment methods usually also result in production of sugar degradation products, like furfural and HMF.
- *High pH methods*, e.g. alkaline pretreatment, ammonia fibre explosion and wet oxidation with addition of alkali. These methods result in partial solubilization of hemicellulose and solubilization of the major fraction of the lignin. An exception to this is the Afex method where a fractionation is obtained but both hemicellulose and lignin are still in the solid fraction. The hemicellulose sugars that are solubilized are however obtained mainly as oligomer sugars. This then requires hemicellulases acting both on solid and dissolved hemicellulose.
- *Methods working close to neutral conditions* at the start of the pretreatment e.g. steam pretreatment and hydrothermolysis. Most of the hemicellulose is solubilized due to the acids released from the hemicellulose, e.g. acetic acid. However, the sugars are obtained as a mixture of monomer and oligomer sugars. This thus requires hemicellulases or acids acting on soluble oligomer fractions of the hemicellulose.

In all methods above the cellulose fraction mainly remains in the solid fraction and is made more accessible for the cellulase enzymes used during enzymatic hydrolysis. The digestibility of this material, as well as the amount of the hemicellulose sugars that are solubilized and the extent of degradation that occurs is dependent on the severity of the pretreatment. The severity increases with increased temperature and residence time and with increased catalyst (acid or alkaline) concentration.

A high severity in the pretreatment is often required to enhance the enzymatic digestibility of cellulose[36]. The reason why cellulose becomes more accessible for enzymatic attack is still not fully understood. Many structural parameters have been studied, like cristallinity, pore size distribution etc. but there are no clear relations between digestibility and these factors. It is however established that the removal of hemicellulose enhances the enzymatic digestibility of the cellulose fibers. However, more severe conditions during pretreatment will cause greater degradation of hemicellulose sugars[37, 38, 39]. The optimum conditions are often a compromise between very high digestibility and high yield of hemicellulose sugars, i.e. low sugar degradation.

Assessment of pretreatment is usually done by using some standard method for enzymatic hydrolysis at low substrate concentration, very often at 2 wt-percent water-insoluble solids (WIS), or alternatively at 1% cellulose, to avoid end-product inhibition[40]. It is also common to wash the solids from the pretreatment in order to avoid inhibition from water-soluble compounds released or formed during pretreatment. In some cases enzymatic hydrolysis is replaced by SSF. The pretreatment efficiency is then assessed by measuring the amount of sugars released during pretreatment and enzymatic hydrolysis (or indirectly from ethanol produced in SSF). Fermentation of the pretreatment liquid to assess inhibition of the fermentative microorganism is also performed in some cases. Enzymatic hydrolysis or SSF can be performed using various conditions, e.g. enzyme dosage and yeast concentration. Usually the enzyme dosage is rather high in the assessment procedure, 25 FPU per g substrate or even higher. The cellulase load in industrial scale must be much lower.

This type of assessment gives the maximum achievable digestibility or glucose yield but it does not reflect the yield obtained in a full-scale process where the enzymatic hydrolysis would be performed at other conditions. Such a process could involve performing SSF on the whole slurry from pretreatment at high substrate concentration (above 10 wt-%) and low enzyme dosage and low concentration of yeast in order to reach high ethanol concentration and low production cost.

The overall ethanol yield depends also on the concentration of inhibitors, which influence the fermentability. These compounds include both substances present in the raw material, e.g. acetic acid from the hemicellulose, extractives, or compounds formed during pretreatment, e.g. the sugar degradation products furfural and 5-hydroxymethylfurfural (HMF) and lignin degradation products. The concentrations of these and all other inhibitory substances in the fermentation step depend on the configuration of the preceding process steps.

PRETREATMENT RESULTS

There are a lot of raw materials that have been investigated using various pretreatment methods and this is well summarized in several recent review papers on pretreatment[10, 12, 13, 14, 15]. We have at Lund University mainly being working with acid-catalysed steam pretreatment. A summary of results obtained with softwood is given in the review by GALBE and ZACCHI 2007[10] with sugar yields up to 80% of the theoretical based on the sugar content in the raw material. Table 1 summarizes the maximum sugar yields obtained for some of the other materials investigated[41, 42, 43, 44, 45, 46]. For

TABLE 1 Pretreatment conditions and sugar yields for various raw materials after pretreatment and enzymatic hydrolysis expressed in g/100 g raw material (ODM). The pretreatment conditions are chosen for maximum yield in glucose. Yield as % of theoretical in brackets.

	Salix			Wheat straw	Corn stover	Sugarcane bagasse
Pretreatment conditions	200 ºC, 8 min. 0.5% H_2SO_4 [41]	205 ºC, 4 min. 2.5% SO_2 [42]	210 ºC, 14 min. no catalyst [42]	190 ºC, 10 min. 0.2% H_2SO_4 [43]	190 ºC, 5 min. 2.5% SO_2 [44]	190 ºC, 5 min. 2% SO_2 [45]
Glucose in liquid after pretreatment	5.2 g (11.2%)	8.4 g (18.3%)	1.8 g (4%)	1.8 g (4.7%)	5.8 g (14%)	2.3 g (4.7%)
Glucose in EH	37.4 g (81.3%)	34.1 g (74%)	40.1 g (87.2%)	37.6 g (95.7%)	29.8 g (72.9%)	42 g (87.2%)
Xylose in liquid after pretreatment	11.6 g (68%)	10.4 g (61%)	3.4 g (20%)	17.1 g (75%)	14.7 g (58.6%)	13 g (47.1%)
Xylose in EH*	1 g (5.8%)	1.1 g (6.5%)	1.7 g (10%)	4.6 g (20.2%)	4.9 g (19.3%)	1.1 g (4%)
Overall yield of glucose + xylose	55.2 g (87.5%)	54 g (85.6%)	47 g (74.6%)	61.1 g (98.2%)	55.2 g (83.6%)**	58.4 g (77.1%)

* EH: Enzymatic hydrolysis at standard conditions (2% WIS, 40 ºC, 15 FPU/g WIS, 96 hours).

** The sugar yield obtained without another batch of corn stover, from Italy, pretreated at the same conditions resulted in higher yields of sugars, 41.0 g glucose (90% of theoretical) and 24.4 g xylose (85% of theoretical)[46] in spite of the fact that this straw had a higher content of glucan and xylan than the corn stover in the table (of Hungarian origin).

TABLE 2 Typical composition of various lignocellulosic materials (% of dry material) and theoretical ethanol yield (L/ton DM) based on available carbohydrates (given as anhydrous sugars).

	Salix	Wheat straw	Corn stover	Sugarcane bagasse
Glucan	41.4	35.5	36.8	43.4
Xylan	15	20.1	22.2	24.3
Arabinan	1.2	3.3	5.5	1.5
Galactan	2.3	0.8	2.9	0.4
Mannan	3.2	–	–	–
Lignin*	26.4	26.5	23.1	22.3
Others**	10.5	13.8	9.5	8.1
Ethanol from hexoses	332	257	280	310
Ethanol from pentoses	117	169	200	187

* Both acid soluble and acid-insoluble.
** Ash, extractives, protein etc.

these materials the hemicellulose consists mainly of xylan, see Table 2, so the sugar yields are given for xylose and glucose only. For most materials the glucose yield is above 90% of the theoretical while the xylose yield varies from 50% for sugarcane bagasse to 95% for wheat for most of the raw materials the pretreatment conditions resulting in the highest glucose yield differs from those yielding the maximum yield of xylose. For instance, for salix the overall xylose yield could be increased to 14.6 g/100 g raw material corresponding to 86% of theoretical for pretreatment conditions 0.5% H_2SO_4, 180 °C and 12 min. However at these conditions the glucose yield decreased to 73% of theoretical compared with 92.5% obtained at 0.5% H_2SO_4, 200 °C and 8 min. The same pattern is valid for the sugarcane bagasse. For pretreatment using 2.0% SO_2, 180 °C and 5 min the xylose yield increased to 18 g/100 g raw material, corresponding to 67% of the theoretical. However, also in this case the glucose yield decreased to 80.5% of theoretical compared with 92% obtained at 2.0% SO_2, 190 °C and 5 min.

This would suggest two-stage steam pretreatment, in which the first stage is performed at low severity to hydrolyse the hemicellulose, and the second stage at a higher degree of severity, in which the solid material from the first step is pretreated again. This would result in a high yield of both hemicellulose sugars and of high digestibility of cellulose. Major drawbacks are, however, the higher capital cost and the higher energy demand. In a study by WINGREN *et al.*[47], on two-stage steam pretreatment of softwood, the overall ethanol production cost was shown to be very much dependent on the way the two pretreatment steps are performed. The key issue is if the pressure is released or not between the two steps, but also on the dry matter concentration after the second step.

It must once again be emphasized that the yields obtained are affected by the method of assessment, especially the enzymatic hydrolysis. ÖHGREN[48] obtained a higher yield of both glucose and xylose when the enzymatic hydrolysis was performed with a small addition of xylanase enzymes in the hydrolysis vessel. The overall glucose yield after enzymatic hydrolysis increased from around 83% to near 100% and the xylose yield from 71% to 96% for pretreatment with 3% SO_2 at 190 °C for 5 min. When pretreatment was performed without catalyst at 190 °C for 5 min addition of xylanases had an ever higher effect. The glucose yield increased from 69 to 94% and the xylose yield from 74.6 to 85% of theoretical. It should be noted that the addition of xylanases had a higher effect on the improvement of cellulose hydrolysis than on the increase of hemicellulose sugars. This means that the pretreatment severity can be decreased in case xylanases are added to the enzymatic hydrolysis. It must be pointed out that most assessments of pretreatment of various raw materials found in literature are based on enzymatic hydrolysis (or SSF) without the addition of xylanases.

Also the origin of the raw material, especially for agricultural residues, may affect the pretreatment results. The data on corn stover in Table 1 shows that the same pretreatment conditions resulted in higher sugar yields when using Italian corn stover than when using Hungarian corn stover, which was the material used in optimization of the pretreatment conditions. This is important to keep in mind when comparing results from different studies on the same type of raw material. It is more adequate to compare different pretreatment methods using the same raw material, which was done in a study undertaken in the U.S., where the same batch of corn stover was pretreated using various pretreatment methods (dilute acid, Afex, hot water treatment etc.). The pretreated materials were then subjected to standard evaluation techniques and the total sugar yields were found to be more or less the same, around 90% or more, for all methods[49]. However, it is questionable if the standard assessment is the best way of doing the comparison as the different pretreatment methods yield different types of pretreated materials as discussed above.

To be successful pretreatment has to be developed as an integrated part of the whole process, including enzymatic hydrolysis, fermentation, downstream processing and wastewater treatment. Each pretreatment method has to be assessed based on the process configuration and process conditions suitable for this specific pretreatment method. For instance, the use of hemicellulases in the enzymatic hydrolysis, instead of only cellulases, will be beneficial to pretreatment methods that result in a large amount of oligomer hemicellulose sugars. In the same way, fermentability tests of pretreated slurries from methods generating inhibitors should be performed using adapted yeast.

Figure 2 shows some options of how the pretreated material may be utilized. The assessment

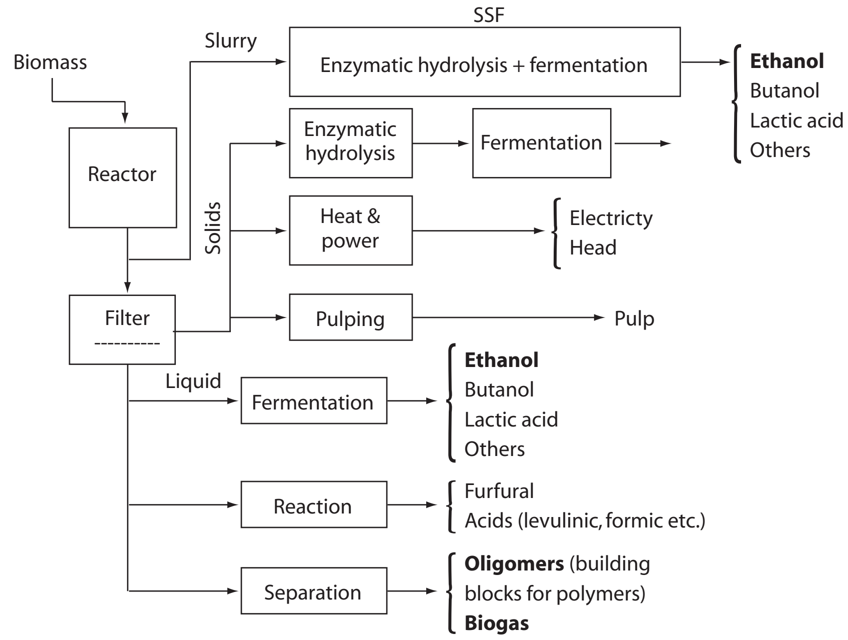

FIGURE 2 Some possible scenarios for utilization of pretreated biomass.

of the pretreated material should thus also reflect the process option that is used and what product is produced from the various parts of the fractionated raw material.

It is our conviction that there is no "best pretreatment" that is the most suitable for all kinds of raw materials or process configuration options. The choice of pretreatment depends mainly on what co-products are produced besides ethanol, the process configuration including process integration as well as how the ethanol production is integrated with external processes, e.g. heat and power production or first-generation ethanol production.

HYDROLYSIS AND FERMENTATION

Enzymatic hydrolysis is performed using cellulases, i.e. a mixture of various endoglucanases and cellobiohydrolases, which attack the amorphous areas of cellulose and cleave cellobiose units from both ends of the cellulose chain, respectively. They are supplemented with β-glucosidase, which cleaves cellobiose into two glucose molecules. The enzymes are end-product inhibited, i.e. most cellulases are inhibited by cellobiose[50, 51] and β-glucosidase is inhibited by glucose[52], so the build-up of any of these products affects cellulose hydrolysis negatively. The maximum cellulase activity for most fungal-derived cellulases and β-glucosidase occurs at 50 ± 5 °C and a pH of 4.0-5.0.

Fermentation is performed using a microorganism, usually yeast, which converts sugar to ethanol. The most commonly used yeast for ethanol fermentation today is *Saccharomyces cerevisiae*, also called baker's yeast. It has a high ethanol tolerance and has also been shown to be rather tolerant to inhibitors produced during pretreatment of biomass. However, it only ferments hexose sugars, i.e. glucose, mannose and under certain circumstances galactose, but it is not capable of fermenting pentose sugars, like xylose and arabinose, which are the main constituents of most hemicellulose variants.

Enzymatic hydrolysis and fermentation can be performed either separately, so called SHF, or combined, so called simultaneous saccharification and fermentation (SSF). The latter can also be preceded by a pre-hydrolysis to diminish the viscosity in the SSF step, as is practice in the starch-based first-generation ethanol production. Whichever configuration is chosen it is important to maintain a high concentration of carbohydrates in the hydrolysis step in order to reach a high concentration of ethanol in the fermentation vessel. This is important primarily to diminish the energy demand for distillation of ethanol and for evaporation of the stillage stream, in case this is included in the process. Figure 3 shows the energy demand for distillation as function of the ethanol concentration in the feed for a distillation system comprising 3 heat-integrated columns. The shape of the curve is the same also for other distillation configurations although the absolute value of the energy demand may vary. The curve starts to level off at around 5 wt-% ethanol so this concentration may be considered a minimum concentration to achieve in the fermentation or SSF step.

SHF

Separate enzymatic hydrolysis and fermentation (SHF) has the advantage that each of the two steps can be optimized separately concerning temperature and pH but also regarding the design of the equipment including stirring. Cellulases usually have a maximum activity around 50 °C or higher while most fermenting microorganisms, e.g. *S. cerevisiae*, do not tolerate temperatures above around 37 °C. Conventional ethanol fermentation is usually performed below 35 °C. It is thus obvious that running the enzymatic hydrolysis at 50 °C results in a higher productivity than when running it at 35 °C. However, at the temperature for maximum activity the enzymes are also deactivated faster than at lower temperatures. This means that although the enzymatic hydrolysis is faster at 50 °C it may very well be so that the sugar yield after 48 or 72 hours hydrolysis is higher at 40 °C, or even lower temperatures, due to the enzyme deactivation[53].

Another advantage of SHF is that the fermentation is performed with a liquid broth, instead of slurry containing solid material which is the case in

SSF, which facilitates the mass transfer and makes it possible to recycle the yeast after fermentation by filtration or centrifugation.

The main drawback for SHF is that the cellulases are end-product inhibited, i.e. the productivity decreases with increasing sugar concentration. This is especially noticeable when the hydrolysis is performed at high consistency, which is a prerequisite to obtain high ethanol concentration in the subsequent fermentation step. The enzymes may also be inhibited by the inhibitors present in the pretreated biomass slurry such as sugar – and lignin-degradation products. TENGBORG *et al.* (2001)[54] showed that inhibition from these compounds were even larger than the end-product inhibition in the hydrolysis of steam pretreated SO_2-impregnated spruce.

Another drawback is the loss of sugars in the separation of solids and liquids after enzymatic hydrolysis. This may be diminished by washing, but this will on the other hand lead to dilution of sugars even if a countercurrent washing system is used. This is avoided when SSF is employed as the ethanol is separated from the slurry by stripping in a distillation column.

SSF

The main advantage of SSF is that the sugars formed by enzymatic hydrolysis are converted by the yeast as soon as they are released. This maintains a low concentration of sugars in the broth which alleviates the end-product inhibition of the cellulases and also diminishes the risk for infections. Another advantage is the capability of the yeast to partly detoxify the slurry[54]. These two effects result in an increased enzymatic hydrolysis productivity also compared to enzymatic hydrolysis performed at higher temperatures. This leads to higher overall ethanol productivity, which means a lower total reactor volume. It has also been shown in several studies that the ethanol yield is higher

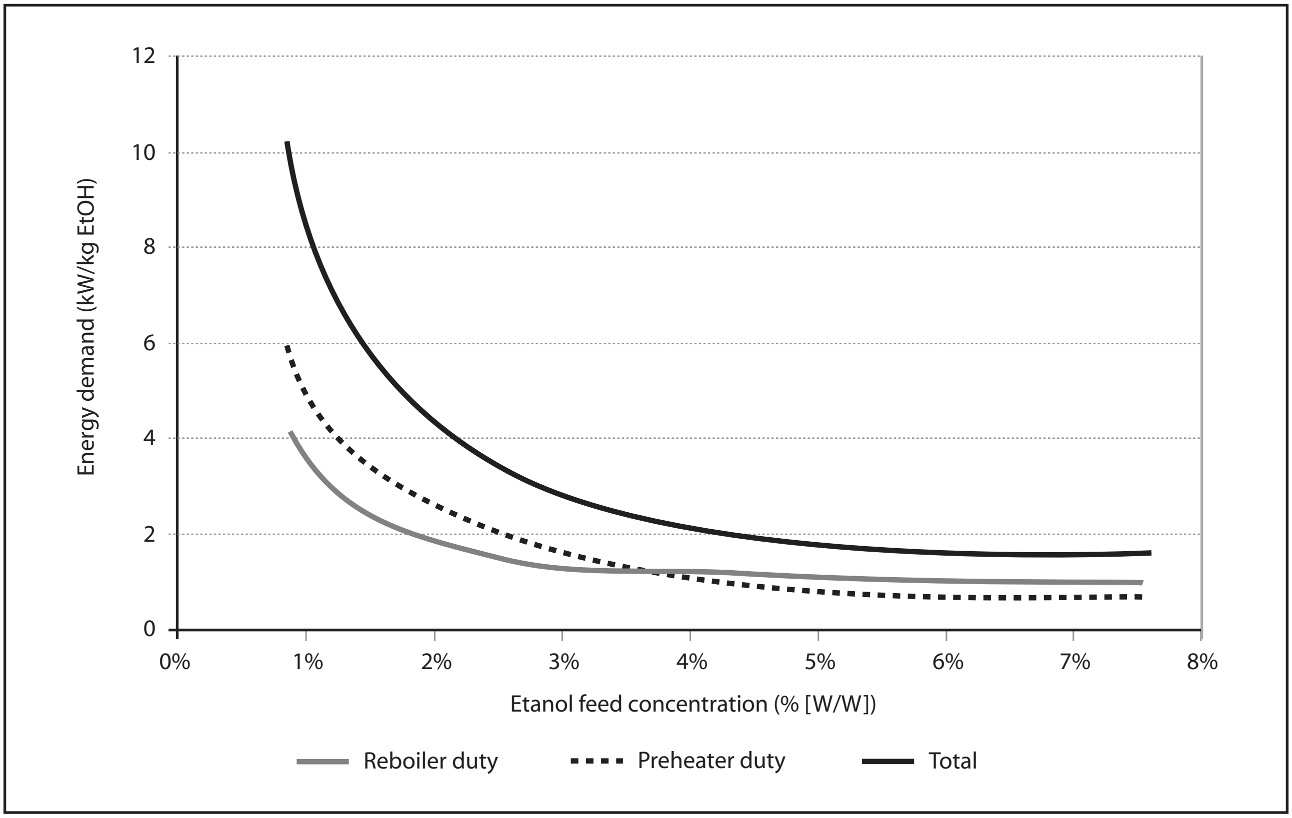

FIGURE 3 Energy demand in distillation of ethanol to 94 wt% in a distillation unit consisting of two stripper columns and one rectification column connected in series on the vapour side. The set-up is described more in detail in reference 58.

after SSF than after SHF both for softwood and agricultural residues[55, 56].

The main drawback with SSF is that the yeast after SSF is difficult to recover as it is mixed with the residual solid, i.e. mainly lignin. In spite of this we consider SSF as a better option than SHF for all raw materials we have investigated so far. The use of SSF is also cost-effective since it reduces the number of reactors[57].

As pointed out earlier one of the remaining challenges is to produce ethanol at a high concentration. This can be achieved in various ways:

I. The most obvious is to perform enzymatic hydrolysis or SSF at high dry matter concentration. Figures 4 and 5 shows a comparison of the energy demand and production cost as function of the solid concentration in the SSF for ethanol production from corn stover, salix and spruce[58, 59]. The capacity is 200,000 ton raw material (DM) per year for all cases and only the hexose sugars were assumed to be converted to ethanol. It is clear that the solid concentration is a crucial parameter. The higher ethanol production cost for salix and corn stover, compared with spruce is due to lower production of ethanol as the pentose fraction constitutes a high percentage of the sugars, see Table 2. High dry matter concentration also means high concentration of inhibitors, which requires a robust yeast that may be obtained by adaptation, e.g. cultivation on pretreatment hydrolysates[60] and by control of the fermentation process[61].

II. The other important factor is to utilize all the sugars available in the pretreated material, i.e. including pentose fermentation. This will lead both to a higher ethanol concentration and to a lower production cost. The production cost for the base case in Figure 4 would diminish from 5.49 to 4.54

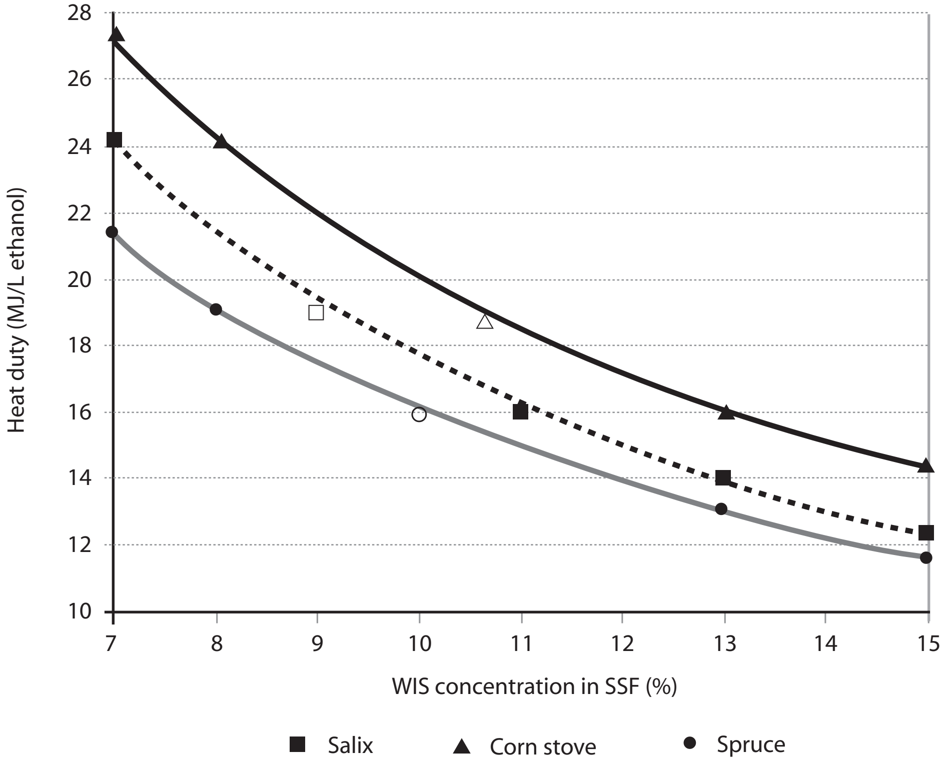

FIGURE 4 Overall process heat demand as function of WIS concentration in SSF. Ethanol yields are maintained at the same level as in the base cases, which are represented by open symbols. Adapted from reference 59.

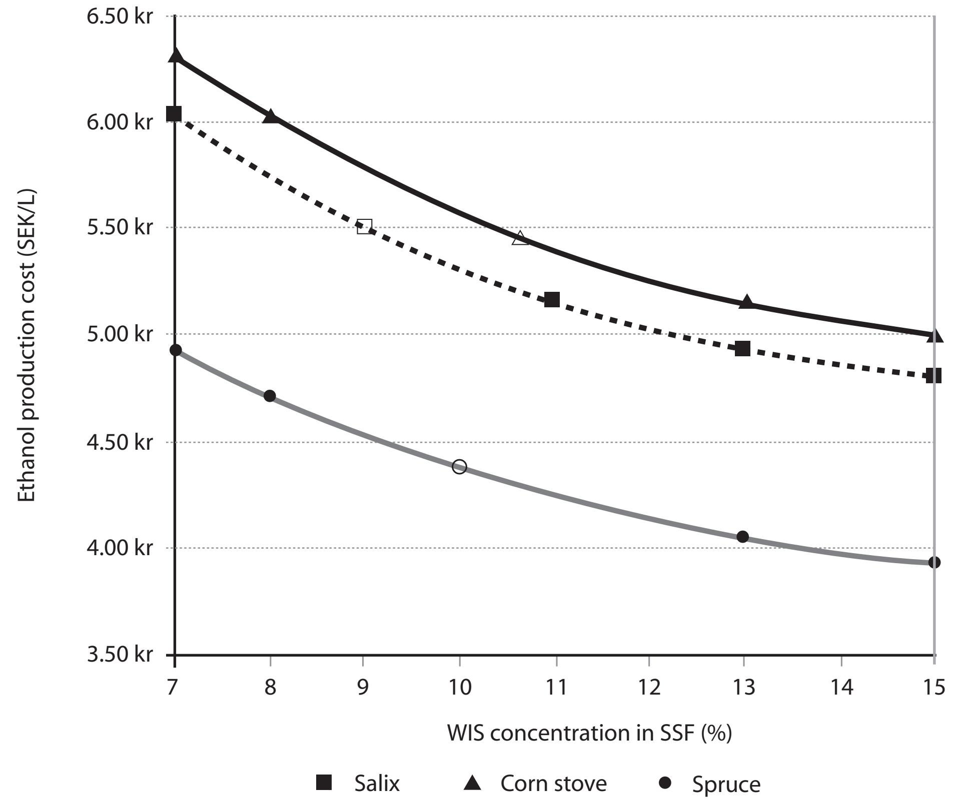

FIGURE 5 Ethanol production cost as function of the WIS concentration in SSF. Ethanol yields are maintained at the same level as in the base cases, which are represented by open symbols. Adapted from reference 59.

SEK/L for salix and from 5.45 to 4.25 SEK/L for corn stover[60].

III. In the study discussed in point I the ethanol yield was assumed to be constant for all WIS concentrations, equal to that in the base case, which was obtained experimentally. However, the increase in concentration of inhibitory compounds with increased dry matter may lead to a decreased ethanol yield. To cope with this, an option is to separate the solid and liquid fractions and only use the solid fraction, i.e. the cellulose, for ethanol production such as in the Ibus process[62]. The liquid could then be used for other applications, e.g. biogas production, where it may be diluted without negative effects in the product recovery as this would be a gas phase.

IV. Integration with first-generation ethanol production as outlined below.

PROCESS INTEGRATION

A large effort has been put in to genetically modify various microorganisms, e.g. *S. cerevisiae*, so that they can ferment xylose, which is the most abundant sugar in most hemicelluloses. The progress on genetically modified *S. cerevisiae* is presented in a review by HAHN-HÄGERDAL *et al.*, 2007[63] where it is also compared with other microorganisms. In some recent studies a recombinant *S. cerevisiae* strain has been used in SSF of steam-pretreated corn stover, see Figure 6[64] and on sugarcane bagasse[65] and wheat straw[66] with very promising results. However, some challenges remain, e.g. that the yeast's affinity for xylose is much lower than for glucose, and is more sensitive towards toxic substances.

Process integration is important especially to reduce the energy demand in the process but also to diminish capital cost. SSF could be seen as

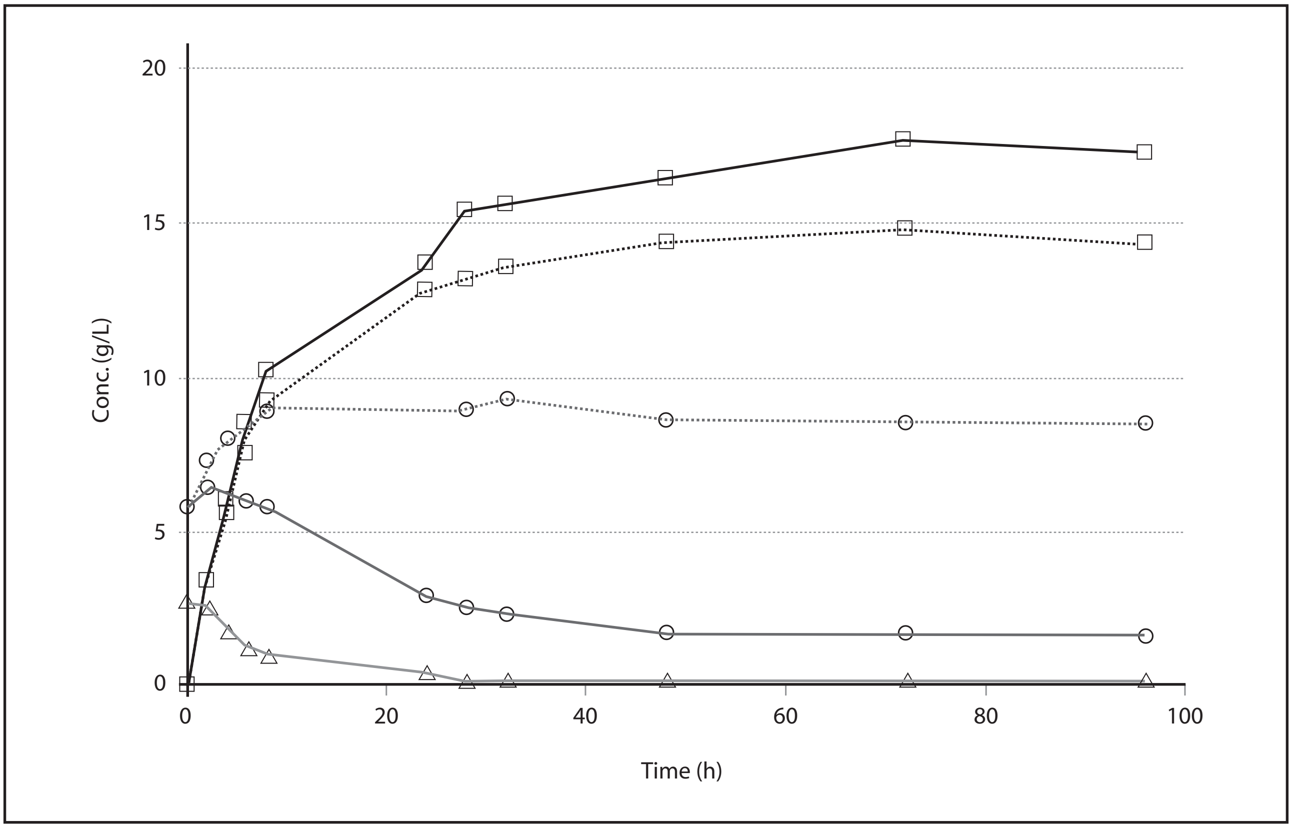

FIGURE 6 Time course of ethanol (□), glucose (△) and xylose (○) concentration during batch SSF of steam pretreated corn stover at 5% WIS using normal baker's yeast (dotted line) and the genetically modified yeast TMB3400 (solid lines).

one type of integration that results in both lower energy demand and lower capital cost. It can also lead to better utilization of sugars. Several studies[66, 68] have shown that the utilization of xylose, using pentose-fermenting yeast, was improved when SSF was used as slow release of glucose facilitated the uptake of xylose, compared with when all glucose is available from the start, which is the case in SHF. The slow release may be controlled either by the enzyme dosage, by the temperature during SSF or by a combination of both[67].

The most obvious way to reduce energy demand is by heat integration of various steam-requiring equipment by using the secondary steam obtained, e.g. by integration of the distillation with the evaporation plant or by increasing the amount of units in the multiple-effect evaporation unit[68]. A somewhat different concept is to introduce mechanical vapor recompression in distillation or evaporation. Another option is to replace energy-demanding process units with a less energy-demanding process step. The evaporation of the stillage is very energy demanding, see Figure 7, and is performed mainly as a waste-water treatment step. The non-volatiles in the stillage stream are concentrated to dry matter content above 50-60 wt-% and then burnt in a boiler to produce heat for the ethanol process. However, the energy obtained from the combustion of the concentrated stillage stream is in the same range as that required in the evaporation plant. By replacing the evaporation plant with anaerobic fermentation for production of biogas the energy demand for evaporation can be eliminated and also the capital cost. Both volatile and non-volatile organic compounds are converted to biogas, which can then be used in the boiler for production of heat and power to the process. Alternatively, the biogas can be upgraded to pure methane to be used as transportation fuel. In a study performed by WINGREN *et al.* (2008)[69] on production of ethanol from steam-pretreated spruce the evaporation plant was assumed to be

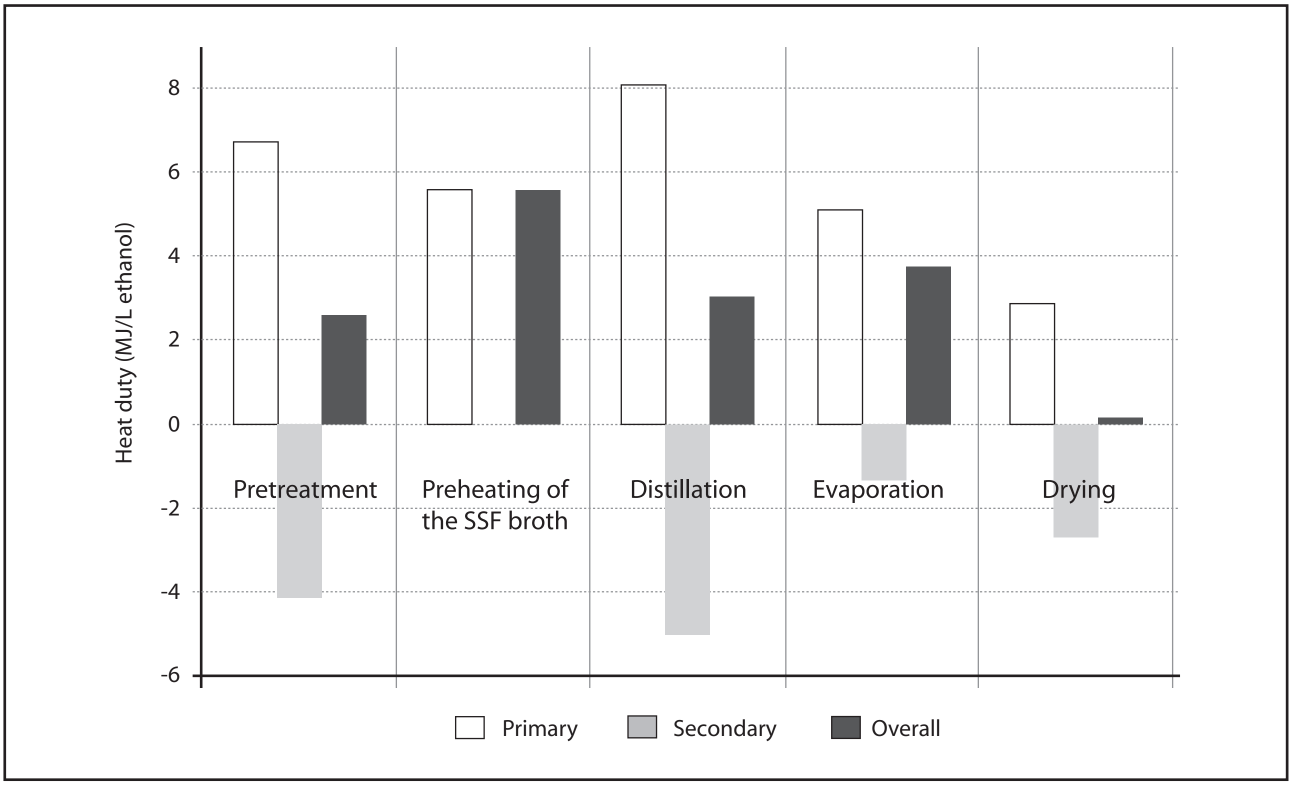

FIGURE 7 Heat duty of the most energy-demanding process steps in ethanol production from spruce based on steam pretreatment and SSF for a capacity of 200,000 ton spruce (DM) per year. The process is explained more in detail in reference 68. White bars = primary steam demand; grey bars = amount of secondary steam generated; black bars = difference between primary and secondary heat, i.e., the net heat demand for the process. Adapted from reference 69.

replaced by anaerobic digestion producing 0.35 m^3 methane per kg COD with removal of 50% of the total COD in the stillage stream. This resulted in a decrease in the energy demand in the process by 48% and the total production cost decreased by 8%. However, this process configuration has to be verified experimentally for each specific stillage stream to assure that most of the organics are fermented. Also the requirement of an aerobic fermentation step for final waste water handling needs to be evaluated. The sludge formed from the fermentation process also needs to be discharged.

EXTERNAL PROCESS INTEGRATION

One approach to reduce the production cost is integration of ethanol production with another suitable plant, e.g. a combined heat and power plant, a starch-based ethanol plant or a sugar based ethanol plant. Regarding the immediate future, we believe that these integrated plant concepts will be used in the first successful industrial-scale production of lignocellulosic fuel ethanol.

INTEGRATION WITH HEAT AND POWER PLANT

Integration of cellulose-based ethanol production with a combined heat and power plant has in a recent techno-economic study[70] been estimated to reduce the ethanol production cost by up to 20 percent for conditions prevailing in Sweden and it is the main strategy pursued in the Swedish cellulose-to-ethanol effort. The study was based on ethanol production from 200 000 ton of spruce per year. In all cases the live steam required in the ethanol process was generated by burning a part of the solid residue (together with the concentrated liquid from evaporation of the stillage and possibly some biogas generated in waste-water treatment). Five different scenarios were investigated where various combinations of co-products, i.e. pellets,

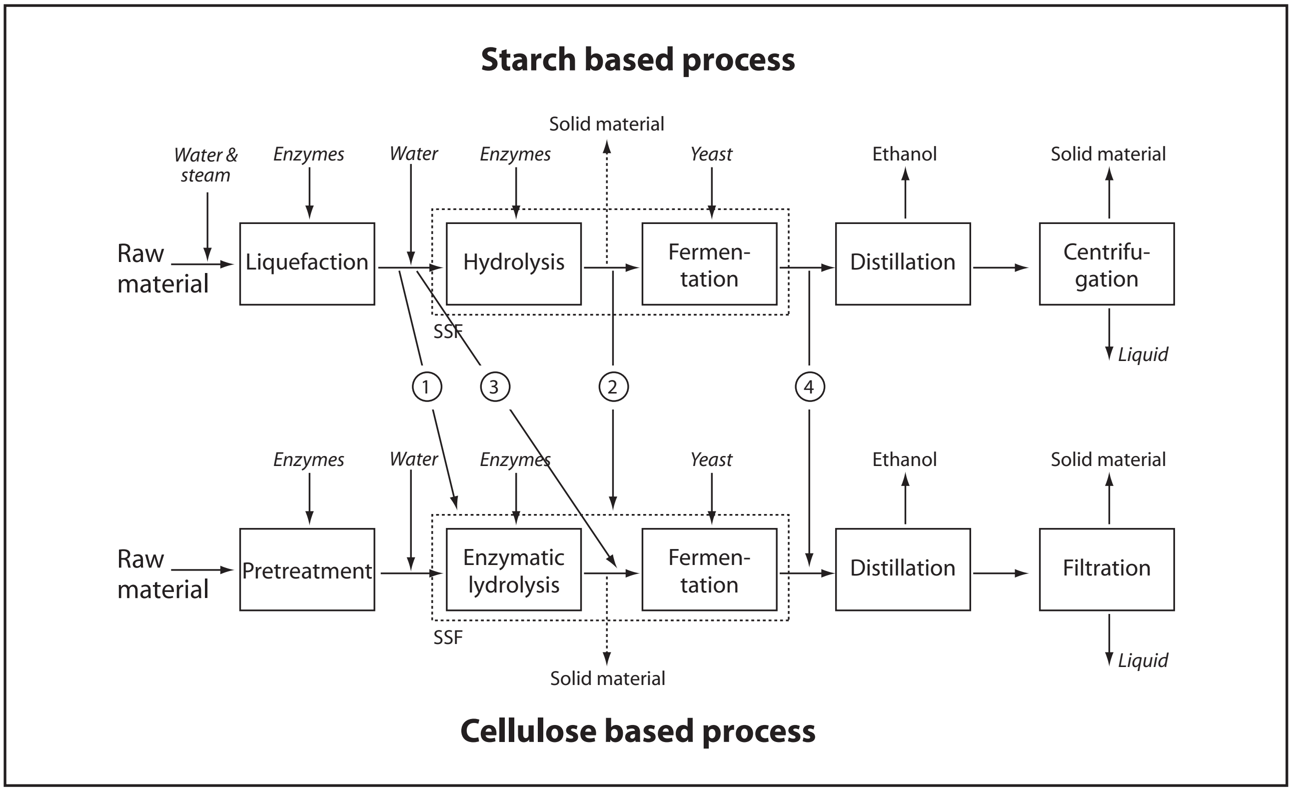

FIGURE 8 Schematic flowsheet for possible points of integration between first and second generation bio-ethanol production.

electricity and district heating were produced. The energy efficiency, defined as the energy output in the products (ethanol, pellets, excess electricity and/or district heating) divided by the energy input varied from 53% to 92%. The ethanol production cost varied from 4.73 SEK/L for the case where ethanol and pellets were produced to 3.87 SEK/L for the case of producing ethanol, electricity and district heating. The latter option restricts the location of the plant as there must be a demand for the surplus heat. Similar conclusions were reached in a study on co-production of ethanol and electricity from softwood, based on conditions in California[71]. One of the benefits is that the syrup or lignin residue can be used for steam production without prior drying.

INTEGRATION WITH FIRST GENERATION ETHANOL

Another option is to integrate 2G cellulosic ethanol production with 1G starch-based or sugar based ethanol production to use the whole agricultural crop. Examples of agricultural residues are corn stover, wheat straw and sugarcane bagasse and trash.

Taking it further the two methods could be integrated at some suitable point in a plant allowing the two methods of producing ethanol to share some common process equipment. Figure 8 shows some possible integration schemes for a starch-based 1G plant. Due to the similarities in the two processes several points of process integration exist. The easiest point would be after fermentation and solid residue separation before the distillation as the two processes would have separate and dedicated equipments for pretreatment, hydrolysis and fermentation. However, by combining material streams further upstream the equipment cost for adding a second-generation technology into an existing first-generation plant could be lower and the energy demand could be decreased.

Integration of the two concepts can be beneficial for both processes. As an example, the 2G ethanol production has an energy surplus in the form of lignin, which can be used in the whole

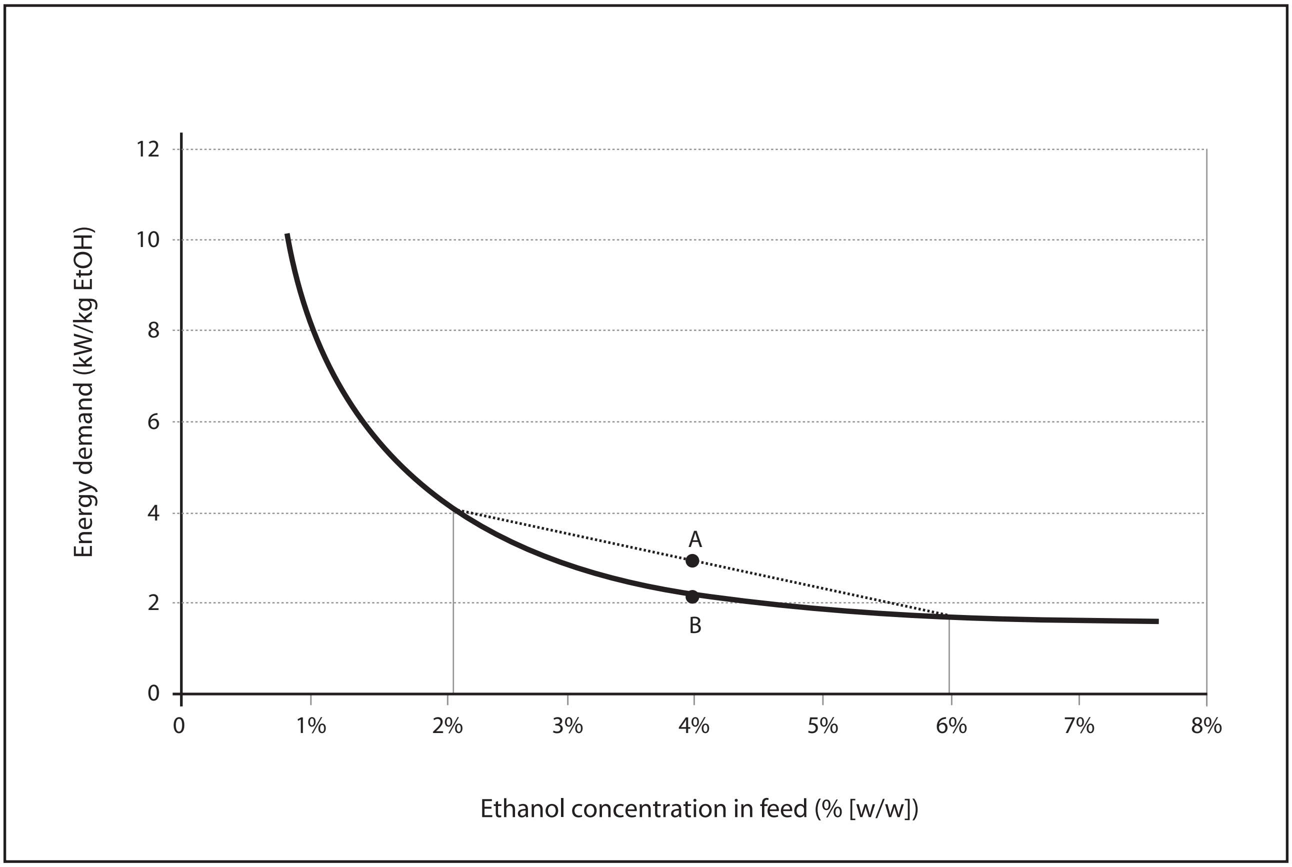

FIGURE 9 Energy demand in distillation of ethanol to 94 wt-% (see Figure 3). The dotted line show the energy demand when two streams containing 2 and 6 wt-% ethanol are distilled separately (for instance point A for a 50/50 mixture). This is always higher than the energy demand for distillation for the mixture, which is on the solid line (point B for a 50/50 mixture).

plant. It is also usually difficult to reach high sugar and ethanol concentrations in the 2G ethanol production while starch – or molasses – based ethanol production require dilution of the sugar. By combining the process flows at some point in the plant, the energy situation in the distillation can be improved compared to two stand-alone plants. The blended distillations feed solutions, for instance at 2 wt-% and 6 wt-%, has a lower energy demand compared to stand-alone processes, as can be seen in Figure 9. Also, the energy demand for evaporation of the stillage stream, not shown in the simplified process scheme in Figure 8, can be diminished for some of the process configurations. It might be a disadvantage if the residue cannot be used for animal feed (DDGS). However, it will still have a fuel value, which will help to improve the economics of the overall process. Also the investment cost may be diminished for some of the integration alternatiIntegration may also alleviate some of the inhibitory effects occurring from formation of toxic compounds in the pretreatment step. If the process streams are mixed prior to fermentation the lignocellulosic streams will be diluted by the starch-based streams.

Figure 10 shows one result from batch SSF of steam-pretreated wheat straw at 5% WIS and a 50/50 mixture of steam pretreated wheat straw and wheat meal at a total WIS of 5%. The overall ethanol yield in the SSF was 81% for the wheat straw case and 94% for the 50/50 mixture, based on theoretically possible from the available fermentable sugars.

To summarize, we believe that integration of first – and second – generation bioethanol production results in higher ethanol yield, lower energy demand and lower production cost than by using a stand-alone second-generation ethanol production.

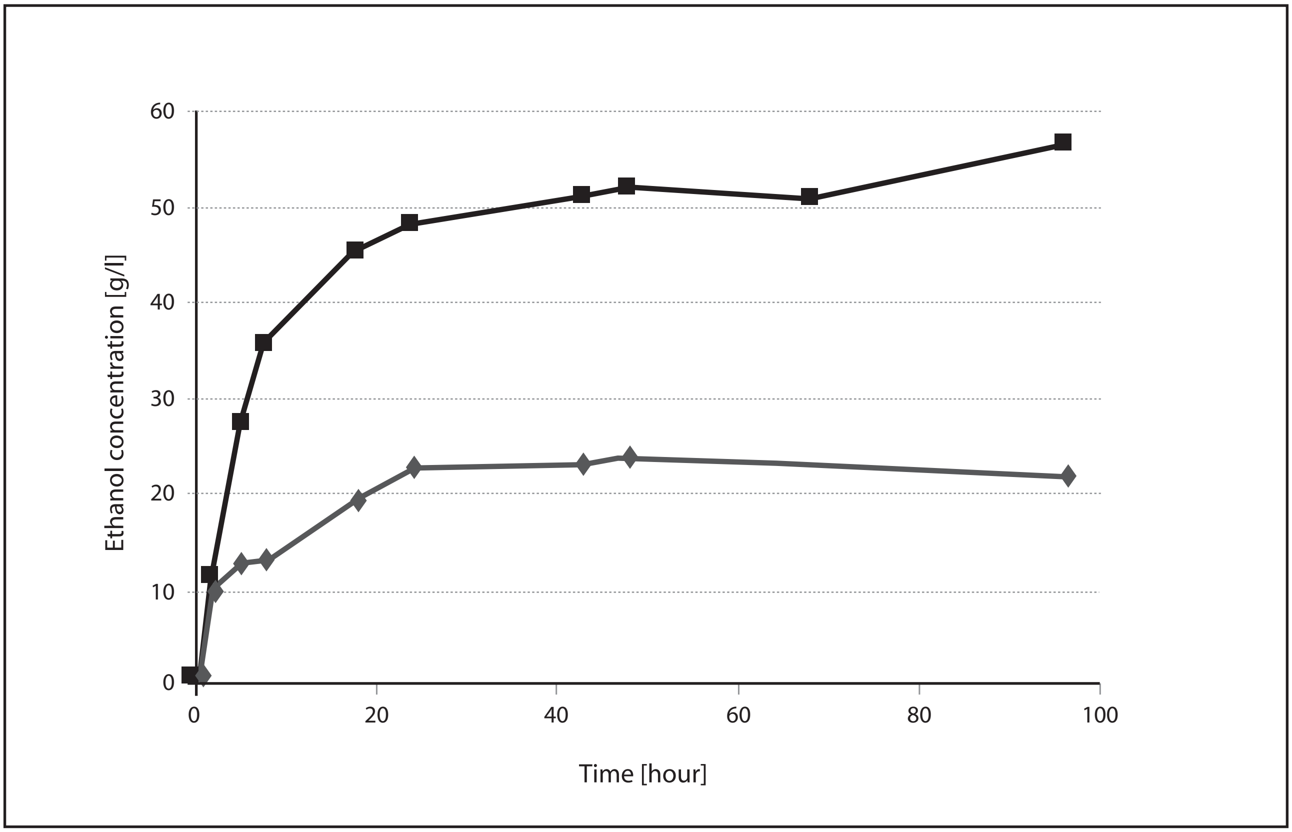

FIGURE 10 Time course of ethanol concentrations during batch SSF of steam pretreated wheat straw (♦) at 5% WIS and a 50/50 mixture (■) of steam pretreated wheat straw and wheat meal at total WIS of 5%.

To define the most optimal way of integration requires detailed studies, e.g. by flowsheeting calculations based on reliable experimental data. This work is at present in progress at Lund University using flowsheeting to evaluate various process concepts.

FINAL CONSIDERATIONS

In summary substantial progress has been achieved in the field lignocellulosic fuel ethanol production, especially within research. However, the transition into a mature industrial technology requires further research and development efforts to cope with the following major research challenges in the areas summarized below:

- Improvement of enzymatic hydrolysis with efficient enzymes, reduced enzyme production cost and novel technology for high solids handling.
- Development of robust fermenting organisms, which are more tolerant to inhibitors and ferment all sugars in the raw material in concentrated hydrolysates at high productivity and with high ethanol concentration.
- Extension of process integration to reduce the number of process steps and the energy demand and to re-use process streams to eliminate the use of fresh water and to reduce the amount of waste streams.
- Process integration with other types of industrial processes, e.g. a combined heat and power plant or a starch-based ethanol plant, which will reduce the production cost further.

Finally, one of the most important issues is to verify all process steps in an integrated way in pilot scale. Especially critical process steps as pretreatment and SSF have to be verified at large scale but also more technical issues like filtration of lignin and the influence of process integration and recycling of process streams on fouling.

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5 Pilot plant in Örnsköldsvik, Sweden [http://www.sekab.com/default.asp?id=1137&refid=1037]

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