CELLULOSIC ETHANOL VIA BIOCHEMICAL PROCESSING POSES A CHALLENGE FOR DEVELOPERS AND IMPLEMENTORS

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In the future liquid biofuels will need to be renewable, sustainable, as well as technically and economically viable. This paper provides an overview of the challenges that the biochemical production of cellulosic ethanol process still faces. The main emphasis of the paper is on challenges that emerge from the scale of liquid biofuel production. These challenges include raw material availability, other consumables, and side stream handling. The pretreatment, C5 fermentation, and concentration of sugars in processing need improvements, too. Sustainability issues and greenhouse gas reduction also pose a challenge for implementation and require development of internationally recognized sustainability principles and standards, and certification of sustainable operation. Economics of cellulosic ethanol processes are still also an area under development and debate. Yet, the Energy Independence and Security Act mandate together with the European Union Renewable Energy Directive and other local targets are driving the development and implementation forward towards more significant contribution of biofuels in the transportation sector.

Keywords: Liquid biofuels; Biochemical processing; Cellulosic ethanol; Biomass; Sustainability; Side products

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INTRODUCTION

The recent rapid and very large fluctuations in oil prices have made energy cost predictions even more precarious than before. Yet, some facts remain: as oil reserves are being used with an increasing rate, and as the reserves are limited, society's primary dependence on oil will end within some decades. The prices of other main fossil energy sources such as natural gas and coal, albeit having a clearly longer time span to contribute to the future energy mix, will follow the price of oil. As of yet, tar sands are expensive to exploit in comparison to other fossils, but do also offer an alternative longer-term source for fuels and energy.

Approximately three-quarters of fossil fuels is currently used for heat and power generation, about one-quarter for transportation fuel, and just a few percent for chemicals and materials (EIA 2006). Replacing the fossil energy with renewable energy - which is only partly biomass based - creates competition of biomass between primary energy and transportation biofuels.

As our global markets and local societies are relying on the mobility of people and goods, we will need suitable energy carriers for transportation. Of the options 1) electricity produced from renewables, 2) hydrogen from renewables, and 3) liquid biofuels from renewables, liquid biofuels fulfill best the present requirements of transportation. The others need more significant changes in the vehicle fleet and fuel distribution systems, and thus can be regarded as potential longer-term solutions.

Liquid biofuels must be renewable, sustainable, as well as technically and economically viable. Renewability in liquid biofuels usually corresponds to using biomass as raw material. Technical feasibility for the near future means fuels that require only minor, if any, changes in the vehicle fleet and the fuel distribution systems. The economical feasibility is more elusive, but eventually the production cost of biofuels must be comparable to other available fuels without subsidies. The technical and economical requirements still pose a significant challenge to developers and implementors.

This paper accordingly provides an overview of the challenges that the biochemical production of cellulosic ethanol process still faces, while striving to economic production of liquid biofuels. Cellulosic ethanol can be produced by biochemical or thermochemical routes, and the main emphasis of this paper is on biochemical processing, and the thermochemical approach is addressed on a comparative basis only. In the order to discuss the challenges brief descriptions of the processes including raw material supply are given below.

RAW MATERIALS

Economical feasibility requires that the raw material must be plentiful, inexpensive, and preferably in low demand for other applications. The second-generation technologies are versatile in terms of the usable feedstock and facilitate efficient conversion of a variety of non-food raw materials and wastes, ranging from wood chips, wood waste, corn stover, wheat straw, switch grass, sugar cane bagasse, or special energy crops. In many scenarios raw materials for cellulosic ethanol are presented with low or even with negative price. In real life this may not hold: collection and transport costs are significant issues for many raw materials, for example straw price at mill gate ranges between 65 and 130 USD per tonne (unpublished).

Type of Raw Materials

Wood and woody residues

The world production of cellulose in biomass is estimated to be $100*10^9$ tonnes (Bozell 2001). Wood is thus available in large quantities, in principle, but competing uses may make the availability/price less attractive for fuel purposes. Half of the woody biomass is used for heat generation.

At a yield level of 300 liters of ethanol per tonne of dry spruce chips, the amount of spruce to produce 32 billion liters of ethanol is some 107 million tonnes, which converts to approx. 20% of world pulp production or a mere 0.1% of natures cellulose production. This 32 billion liters refers to the amount of cellulosic ethanol in 2019 in the

Energy Independence and Security Act of 2007 (signed by President Bush December 19, 2007), and Expansion of the Renewable Fuel Standards demand for advanced biofuels year 2017 therein.

Corn stover

Corn stover is considered as an important feedstock for cellulosic ethanol production. The National Renewable Energy Laboratory (NREL) estimated that a harvest of 80 to 100 million dry tonnes per year of corn stover can be achieved in a sustainable fashion (Lau *et al*. 2008). This amount of corn stover would yield 38 billion liters, assuming a yield of 430 l/dry tonnes (DOE Biomass Program Theoretical Ethanol Yield Calculator and Biomass Feedstock Composition and Property Database), meaning 110% of annual US target of advanced biofuels in 2017.

Straw

For straw – as for corn stover – the collection logistics exist, but the low bulk density of straw makes the transportation costly. Inbicon A/S and Colusa Inc. have announced their plans to utilize straw for manufacturing ethanol. Inbicon has advanced further in their development, since their first pilot plant has been in operation from 2003, and a demonstration plant with a capacity of 4 tonnes straw per day is under construction (http://www.inbicon.com/Projects/Kalundborg). When using straw, silica, ash, and soil depletion are issues to be considered and solved.

Bagasse

Sugar cane bagasse, the outer fibrous residue of sugar cane, is produced in large quantities in the sugar and ethanol industries. The Brazilian bagasse production is over 120 million tonnes/a (Undata 2006). An enzyme producer estimates that Brazil could produce 8 billion liters ethanol from bagasse in 2020 (Anon, 2009a). The collection logistics are also in place.

Bagasse is typically used in boilers to provide steam and electricity to the sugar cane (first generation) ethanol plant, and thus its use as solid fuel for the ethanol process is competing with the second generation ethanol production. In all, 93% of the bagasse produced is used as fuel in cane processing. Additionally, 85% of sugar cane leaves (trash) are burned prior to cane harvesting to reduce costs, while the remaining 15% of harvested unburned trash is left on the ground to decay (Falabella Souza-Aguiar *et al.* 2007).

By improving the efficiency of bagasse and trash use in energy use, significant amounts of bagasse could instead be used as a raw material for cellulosic ethanol processes, thereby substantially increasing the yields per ha of sugar cane plantation.

Other agricultural wastes

Many other kinds of agricultural wastes also are potentially available, but usually in limited quantities when compared to corn stover or bagasse. However, the USDA assessment indicates that the total agricultural residues can amount to over 200 million tonnes annually (corn stover and wheat straw 200 million tonnes, too). With an assumed yield of 380 litres per ton, this would mean 150 billion litres of ethanol per year (USDA 2005).

Other agricultural wastes include: brown juice, green juice, corn steep liquor, potato waste water, molasses, rice hull, wine shoots, sorghum bagasse, olive cake, citrus waste, banana waste, and peanut shell (Hedegaard Thomsen 2005).

Energy crops

There is potential also for specific developed energy crops, but economical commercial scale growing of switch grass, energy poplar, willow, or energy cane is yet to be confirmed. In the US, experimental plantations are ongoing with switch grass and miscanthus in large scale with yields for switch grass between 4.9 and 6 tonne/ha and for miscanthus between 22 and 35 tonne/ha (Owens, 2009). Assuming 380 liters/tonne and an average yield of 30 tonnes/ha the 32 billion liters correspond to 2.8 million ha. The land use change and biodiversity are issues that require very careful consideration with energy crop production.

Municipal solid waste

For municipal solid waste (MSW), logistics for collection and handling already exist. Although the raw material seems cheap or at least inexpensive, biofuel processes are to some extent competing with biogas production for the raw material.

A couple of projects already are underway: for example PERSEO in Spain and Bluefire and Fulcrum Biofuels in USA. The PERSEO-project claims to achieve an ethanol yield of 160 liter/tonne and aims towards production of 220 liter/tonne MSW (Castañeda *et al*. 2009).

In addition, a levulinic acid plant is under construction in Caserta, Italy (http://biodevelopment.us/crnt_lecal.html). This plant will use carbohydrates from paper mill sludge to produce levulinic acid, and now the same process has been applied for MSW. Thus, the competition for MSW as a raw material could also increase: either for direct burning, for gasification and for fuel production, for levulinic acid, or for cellulosic ethanol.

Raw Material Sustainability

Sustainability is a key element in the future development of biofuels. Biofuels do offer a mitigation opportunity for climate change, but the rapid increase of biomass use for biofuels and energy production puts new pressure on land use (Searchinger *et al*. 2008). Increasing unsustainable land use for biofuel crop production may aggravate deforestation and socio-economic issues in different parts of the world. In response to avoiding adverse effects of biomass use for energy production, sustainability criteria and certification schemes are evolving (Cramer 2007). One example is the Roundtable on Sustainable Biofuels (RSB), which is an international initiative bringing together various stakeholders to develop the sustainability of biofuels production and distribution.

In the European Union, the Renewable Energy Directive (RED) established a 10% binding target for biofuels by the year 2020 for all the European Union member states. The RED also incorporates sustainability criteria for the production of biofuels and obliges the sector to fulfill sustainability criteria set out in the RED if they are to be counted against national biofuel targets. The sustainability criteria include: a sufficiently positive greenhouse gas balance, no competition with foodstuff or other local uses such as medicines or building materials, no adverse effects to the vulnerable biodiversity, no adverse effects to the environment, contribution to local prosperity, and contribution to the welfare of the employees and the local population (Panoutsou 2009). Several of these criteria are difficult to measure, and care is needed when interpreting published reports.

Advanced biofuels are promising from an emission and sustainability perspective. Second-generation technologies under development for the production of e.g. lignocellulosic ethanol and renewable diesel fuels use non-food renewable raw material resources and offer substantially higher greenhouse gas reductions than most conventional biofuels. Advanced biofuels are expected to meet the current and future GHG saving thresholds imposed by the EU RED, which will benefit the development of next generation biofuels at the expense of conventional biofuels, with low saving potential for GHG emissions.

Sustainability assessment related to the socio-economic impact of feedstock and biofuel production is a broad and complex matter. While the relatively straightforward concept of GHG emission saving can turn into a multifaceted topic, socio-economic aspects that involve e.g. lifestyles and values inherent in a society are far more difficult to evaluate. Socio-economic sustainability aspects, due to their local characteristics, need to be evaluated in a regional context involving several stakeholders. Evolving sustainability schemes are increasingly taking also socio-economic aspects into account. This can be witnessed by e.g. the Roundtable on Sustainable Biofuels “Version Zero” document, (Uneptie 2009) which outlines the initiative’s proposed standards for sustainable biofuels. Several of the 12 proposed principles include socio-economic aspects.

The sustainability debate will certainly continue, and it will shape the future biofuels industry. The discrimination between biofuels based on the sustainability of the feedstock and the GHG balance of the biofuels is likely to intensify – the gradually increasing GHG emission targets for biofuels imposed by the EU RED is a good example of this direction. Biofuel sustainability policies are not global, but the development is towards internationally recognized sustainability principles and standards, and certification of sustainable performance. The obligatory minimum Greenhouse Gas (GHG) savings for biofuels imposed by the RED is an important consideration in the development of biofuel technologies and in the installation of new biofuel production capacity. One tangible criterion is the GHG emission saving of at least 35%, which a given biofuel has to achieve to comply with the RED. With effect from 2017, the 35% threshold will rise to 50% in 2017 for existing plants, while new facilities whose production has started in 2017 and later will need to achieve a 60% GHG emission saving.

PRETREATMENT

Cellulosic biomass is harder to hydrolyze than starch, and this fact is the reason for cellulosic ethanol being more expensive to produce than starch (or sugar cane) ethanol. At the moment, a pretreatment step is regarded as the bottleneck in the production of cellulosic ethanol. Whether or not plant crop breeding with or without genetic engineering will resolve the pretreatment challenges remains to be seen.

Pretreatment is necessary to facilitate the access of hydrolyzing agent to the cellulose. A very important aspect of the pretreatment – as well as of hydrolysis – is the concentration of biomass in this processing step. It is rather straightforward to calculate that if one is to achieve e.g. 5 w/w% ethanol prior to distillation, then we need approx. 20 w/w% dry biomass in the pretreatment step or its output stream. In Table 1, some commonly used pretreatment techniques and conditions are listed. It is evident that some of these do not fulfill the ethanol concentration requirement and are using very large amounts of water. A biomass concentration of 4% equals to 4 million liters per hour in a 570 million liters per year plant (assuming 380 litres/tonne).

Table 1. Different Pretreatment Techniques and Conditions*

Technology	Chemicals	Temp °C	P atm	Reaction times, min	Concentration of solids, wt.%
Steam explosion	none	190 - 240	6 - 34	3 - 8	not known
Steam explosion + SO_2	2.5% SO_2	190- 215	6 - 30	3 - 8	not known
Dilute sulphuric acid	0.5–3.0% sulphuric acid	130–200	3 – 15	2 – 30	10 – 40
Flowthrough pretreatment	0.0–0.1% sulphuric acid	190–200	20 – 24	12 – 24	2 – 4
pH controlled water pretreatment	water or stillage	160–190	6 – 14	10 – 30	5 – 30
Ammonia fibre explosion (AFEX)	100% (1:1) anhydrous ammonia	70–90	15 – 20	<5	60 – 90
Ammonia recycle percolation	10–15 wt.% ammonia	150–170	9 – 17	10 – 20	15 – 30
Lime	0.05–0.15 g $Ca(OH)_2$/g biomass	70–130	1 – 6	1 – 6 h	5 – 20
Lime + air	0.05–0.15 g $Ca(OH)_2$/g biomass	25–60	1	2 weeks– 2 months	10 – 20
Liquid hot water	none	150 -200	4-39	15	2-10

* Data: Wyman *et al.* 2005; Glasser and Wright 1998; Ballesteros *et al.* 2006; Boussaid *et al*. 2000, Carvalheiro *et al.* 2008.

Dilute Acid

During recent years the dilute acid pretreatment has been studied quite extensively, as it enhances the subsequent enzymatic hydrolysis. As is shown in Table 1, the concentration of biomass can be high enough for cost efficient separation of ethanol. The down side of dilute acid treatment is the combination of high pressure, high temperature, and low pH that imposes very demanding requirements for the materials of construction and contributes heavily to the cost of pretreatment. The low pH brings in also the need of neutralization. The dilute acid hydrolysis is used, for instance, by Verenium and SEKAB.

Hot Water

Pretreatment in hot water at 200 to 230°C for up to 15 minutes can result in extensive autocatalyzed hydrolysis of hemicellulose. However, a high lignin content in the biomass can reduce subsequent cellulose hydrolysis (Liu and Wyman 2004). The production of possible inhibitors such as furfural and hydroxymethyl furfural was reported to account for less than 3% loss of carbohydrates (Allen *et al.* 1997).

Hot water pretreatment followed by enzymatic hydrolysis of cellulose has been reported to yield glucose in 25 to 95% yields, with the latter only being accomplished with the aid of physical milling. With hardwoods the hot water pretreatment has been reported to result in 90% conversion of glucose to ethanol after simultaneous saccharification and fermentation (SSF) (van Walsum *et al*. 1996).

Allen *et al*. (2001) compared a hot-water treatment to a dilute-acid pretreatment and concluded that both yield comparable conversion to ethanol under optimized conditions, although the severity of the former pretreatment had to be much higher.

Although very tempting cost-wise (no chemicals, no extreme pH, therefore chemical and equipment material costs are low) the very dilute conditions may lead to costly production of ethanol.

Steam Explosion

In steam explosion, the raw material after size reduction is treated with high pressure saturated steam. Typically temperatures between 160 and 240°C are used together with pressure of 6-34 bar for a period ranging from few seconds to a few minutes. Thereafter, the pressure is rapidly released. Via this treatment, hemicellulose can be partly solubilized.

Acid catalysts such as H_2SO_4 or SO_2 have been found to improve the enzymatic hydrolysis of the solid cellulose fraction in the steam explosion. The addition of acid catalyst benefits especially softwood, since the hemicellulose in softwood contains less acetylated groups, and therefore autohydrolysis cannot occur to the same extent as in hardwood.

Steam explosion has advanced to the demonstration scale at Iogen (Canada), and to the pilot scale at the Iotech Pilot Plant (Canada), the Souston pilot plant (France), and in Örnsköldsvik (Sweden). KL Energy has applied a somewhat modified steam explosion pretreatment process.

Alkaline Treatments that Depolymerise Lignin

Calcium hydroxide (lime) is one of the alkaline reagents used to depolymerize lignin. The long reaction times and low biomass concentration are severe economical obstacles of this technique. The benefit of this approach is that it proceeds at atmospheric pressure and at low temperatures.

In the Ammonia Fiber Explosion (AFEX) process biomass is heated with concentrated liquid ammonia at 14 to 50 bar (200 to 700 PSI), temperature range 100 to 200°C for 5 to 45 min. Thereafter, the pressure is rapidly released, and ammonia evaporates from biomass, thus breaking the biomass matrix.

The AFEX pretreatment has led to very good yields: nearly 95% (Wyman 2005) when followed by enzymatic hydrolysis. For example, Lau (2008) found that enzyme properties limited sugar yield more than the inherent reactivity of the AFEX treated corn stover material.

In AFEX, the biomass concentration is high enough and ammonia consumption seems not to be a costly issue. Over 99% of the ammonia is claimed to be recovered, compressed, and reused, while the rest is to serve as a nitrogen source for fermentation. This indicates that 10 kg ammonia is consumed per one ton of biomass. The 32 billion liters of ethanol require therefore approx. one million tonnes of ammonia (less than 1% of world ammonia production).

The ammonia compression, on the other hand, is costly, and NREL has estimated that the minimum ethanol selling price for AFEX process is higher than for the dilute acid pretreatment process.

Ethanol Pretreatment

This process uses a mixture of ethanol and water at about 50:50 (w/w) at 200°C and 28 bar to extract most of the lignin from wood chips or other lignocellulosic biomass (the Lignol process). Pulps with residual lignin 6 to 27% (w/w) have been produced under different processing conditions (temperature, 185 to 198°C; time, 30 to 60 min; liquor pH, 2.0 to 3.4; liquor:wood ratio, 7–10:1 by Pan *et al*. (2005).

The fermentability of sugars after ethanol pretreatment is reported to be very high compared to steam explosion (Berlin *et al*. 2005), or dilute acid pretreatment (www.lignol.com), as these the latter two do not remove lignin prior the hydrolysis.

It should also be noted that in all of the pretreatment scenarios the effect of subsequent steps must be analyzed carefully with regard to xylose. All xylose (C5 sugars) should be released simultaneously, if the C5 sugars are to be fermented separately.

HYDROLYSIS

Concentrated Acid Hydrolysis

The concentrated acid hydrolysis has been used for years, meaning that it is technically feasible, but economics have not been very favorable. Nevertheless, in Japan during the early 2000s this process was used at a pilot scale to produce cellulosic ethanol from construction wood waste. A larger demonstration plant is being constructed in the USA. (www.bluefire.com). The advantage of concentrated acid over dilute acid is the higher glucose yield and the lower formation of fermentation inhibitors. Taherdazeh and Karimi (2007a) provides an excellent review of chemistry of acid - both concentrated and dilute - hydrolysis of lignocellulosic materials.

The challenges of concentrated acid hydrolysis lie in the consumption of hydrolyzing acid and subsequent neutralization, combined with the formation of gypsum, when using sulphric acid. If the 3 billion liters of ethanol were to be produced from wood/wood waste with concentrated acid hydrolysis, it would require 2% of world’s sulphuric acid production. It should also be noted that for the dilute acid process the figures for acid consumption are almost the same. Another cost item is related to the materials needed to construct the equipment: low pH at high temperatures implies that expensive material solutions will be required.

Concentrated acid hydrolysis is being demonstrated by Arkenol, BlueFire, and Weyland.

Dilute Acid Hydrolysis

This hydrolysis approach has lately been under intense research, but so far without a breakthrough. The main obstacle seems to be glucose degradation proceeding under the hydrolysis processing conditions. The high temperature and low pH used induce the degradation of the formed glucose to furfural and 5-hydroxymethyl-2-furaldehyde, acetic acid, formic acid, and levulinic acid (Almeida *et al*., 2007). See also Taherdazeh and Karimi (2007a).

As indicated above for concentrated acid hydrolysis, the consumption of acid, of neutralizing agent (lime), subsequent formation of gypsum and costly equipment materials are amongst the issues to be solved.

Dilute acid hydrolysis is pursued by at least SEKAB, BioEnergy, and Verenium.

Enzymatic Hydrolysis

Enzymatic hydrolysis offers milder process conditions, fewer inhibitors, a longer reaction times, and more expensive catalyst than acid-based processes. For a detailed review of enzymatic process see, e.g., Taherdazeh and Karimi (2007b).

Enzyme costs were long regarded as the prohibiting factor in enzymatic hydrolysis, but over the recent years the coordinated research funded by DoE has led to a 20-fold decrease in enzymes costs; from 0.53 USD/liter to 0.03 - 0.05 USD/liter (Greer 2005). If the development of cellulosic enzyme technology follows the route observed for the amylolytic or protease enzymes, further reductions in enzyme cost are to be expected.

The use of 25 mg enzyme/g cellulose leads to the need of 1,500,000 tonnes of enzyme protein for the 32 billion liters of cellulosic ethanol. If this amount of enzyme is produced from the same cellulosic raw material as ethanol, it would require about 5% of the incoming cellulose. This reasoning also implies that some 300 pieces of 1000 m^3 fermenters are required for the production of 32 billion litres of ethanol. This number is roughly twice the number of 190 MLPY cellulosic ethanol plants needed to produce the ethanol (all calculations by the authors). The leading enzyme producers and for example least Abengoa, Iogen, Verenium and Poet are developing enzymatic hydrolysis.

FERMENTATION

Inhibitors

The pretreatment and especially the dilute acid hydrolysis can create inhibitors to the fermenting microbe (Almeida *et al*. 2007). The tolerance towards inhibitors is different between fermenting species and even within species, as is reported by e.g., Modig *et al*. (2008). The same authors also noted that the fermentation mode affects the tolerance. This is an important factor, as fed-batch type solutions may be needed to increase the ethanol concentration prior to separation. The cost of inhibitor removal can be as high as 22% of the total ethanol production cost (Lau 2008).

Hexoses and Pentoses

The pretreatment and hydrolysis sequence produces C6 (hexoses) and C5 (pentoses) sugars. The C6 fermentation is very straightforward, with *Saccharomyces* yeast or with *Zymomonas* bacterium (Huber *et al*. 2006), but the C5 sugars pose a challenge. The C5 sugar fermentation is needed, as the potential ethanol yield from these sugars, depending on the raw material, corresponds to between 13 and 70% of that of C6 sugars.

There are two basic ways to ferment C5 sugars: 1) separately from C6 sugars or 2) together in the same tank. Acid pretreatment tends to hydrolyze hemicellulose into sugars, of which a large part is C5 sugars (especially xylose and arabinose). If the fermentation is carried out separately, a naturally C5 fermenting microorganism or a GMO species can be used. In either case, the conditions can be set to the optimum for C5 fermentation. If C5 sugars are fermented together with C6 sugars, the fermentation is more challenging. A microorganism tends to ferment sugars sequentially, starting usually with glucose. The rate of C5 sugar fermentation is often lower. The use of two microorganisms leads to a compromise in fermentation conditions for the microorganisms, thus leading to a decreased rate of fermentation. The result is that the fermentation time is longer, and therefore the required tank volumes increase. On the other hand, the number of fermentation tanks will be increased if the fermentation is carried out separately.

In order to use only one microorganism, e.g. *Saccharomyces* (Jeffries 2006) or *Zymomonas* (Aden *et al*. 2002; Deanda *et al*. 1996) for both C5 and C6 sugars, the microorganism has to be modified to ferment C5 sugars by inserting pentose fermentation pathways through genetic engineering. Another way is to insert an ethanol pathway into microbes that naturally use C5 sugars (for example see Altherum and Ingram 1989).

Simultaneous Hydrolysis and Fermentation

In order to limit the number of tanks, and more importantly to avoid glucose inhibition of fermentation, a simultaneous hydrolysis and fermentation can be used. In this approach the same tank is used both for the hydrolyzing enzymes to break down the cellulose into sugars (glucose) and for the fermenting microorganism to convert the formed sugars into ethanol at the same time. The pH and temperature are probably a compromise between the optimum for the enzymes and the optimum for fermenting microorganism. Therefore, the advantage of separate processes is more optimal conditions for each of the processes.

The advantage of simultaneous processing is the avoidance of the end-product inhibition of the enzymes: the released sugars inhibit the hydrolysis. This inhibition can result in lower sugar - and consequently ethanol - concentrations prior to distillation. A disadvantage of simultaneous processing is that solid residues are present during fermentation and are mixed with the fermenting microorganism(s), which makes recycling difficult. In the separate processing option solid residue can be separated prior fermentation. A recent review of the simultaneous saccharification and fermentation is given by Olofsson *et al*. (2008).

Combined Processing Option

Combined bioprocessing is a process where the hydrolytic enzymes are produced by the fermenting microorganism. The microbe is engineered to contain both cellolytic enzymes and fermentation ability of C5 and C6 sugars (Lynd *et al*. 2005). The capital cost would be lower, due to a lower number of tanks, and operations should be less complicated (elimination of microorganisms that only produces enzymes – or eliminating the need to buy enzymes from a supplier).

Very recent news from the company pursuing this approach reveal high expectations of cost lowering by increased enzyme production, higher ethanol concentrations after fermentation, and elimination of side products (Mascoma 2009).

SEPARATIONS, RECYCLING, AND BYPRODUCTS

Solid-Liquid Separation

Solid-liquid separation is needed in various phases, depending of the process design, to separate hydrolyzed hemicellulose sugars from solids, to separate hydrolyzed lignin from the rest, to separate hydrolyzed cellulose sugars, to separate yeast and other solids from the fermented liquid, or in dealing with bottoms from the first distillation column. Of these, lignin separation, especially from steam explosion pretreated biomass can provide some challenges. Viscosity and stickiness of high concentration pretreated biomass hinders and lowers the separation of sugar solutions and decreases the yield if the amount washing liquid is minimized.

Ethanol Separation and Dehydration

The ethanol concentration is a significant factor in distillation. If the ethanol concentration is below 4%, the distillation energy demand rises very sharply (see Fig. 1), as shown by Hasanen (2009) by an Aspen Plus simulation. Additionally, if the ethanol production capacity is kept the same, the distillation column size must be increased, thereby increasing the investment cost per ethanol capacity.

However, the ethanol separation process is very well established technology and could be considered as mature technology. The energy integration over the whole plant or possible next-door processes is the challenge, and there will be room for innovative approaches, depending the specific situation on site.

Widgren *et al*. (2008) showed that by engineering the ethanol recovery, significant savings in energy can be achieved. Their study had a low ethanol concentration after fermentation (3.5%), but still the energy used could be almost halved from 19.0 MJ/liter ethanol to 9.8 MJ/liter by mechanical vapor compression and using an anaerobic digester.

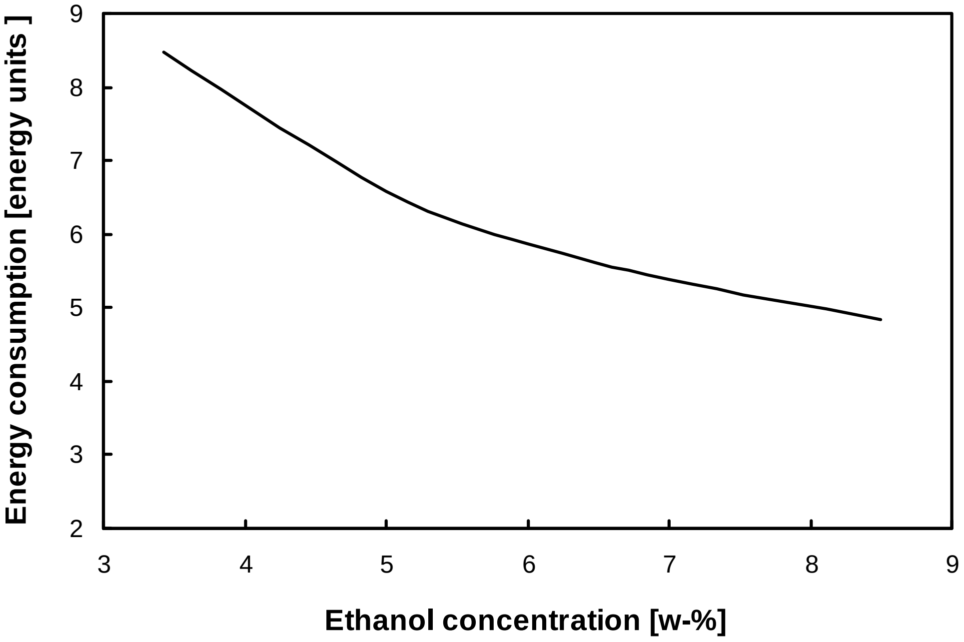

Fig 1. Energy consumption in distillation of ethanol as a function of ethanol concentration prior to distillation (Hasanen 2009)

Recycling and Byproducts

Efficient water usage is not only an energy and cost factor, but probably will be, to an increasing extent, a sustainability factor, too. Lignocellulosic ethanol processes are not yet in commercial scale, and integrated to existing industrial environment the differences between technological options are hard to determine. The accumulation of hydrolysis or fermentation inhibitors must be avoided when recycling water. Any buffering solutes should be avoided, too, as they increase pH control chemical usage and increase osmotic stress for the fermenting microbe(s). The sustainable, economical production of lignocellulosic ethanol requires good engineering solutions for water usage.

Cellulosic ethanol via a biochemical route produces lignin as a byproduct. In the Lignol process lignin is even considered as a main product. Lignol estimates the lignin market to be 2 billion dollars (www.lignol.com), providing a route to replace e.g., phenol in phenol-formaldehyde resins. On the other hand, lignin can be used as fuel to provide energy to run the ethanol production process.

Gypsum, as discussed earlier, forms a large side product of the acid hydrolyses process. The use of thus produced gypsum is questionable and largely remains to be resolved. The amount of gypsum is very large. For example for 190 MLPY will produce via concentrated acid hydrolysis 71,000 tonnes of gypsum, 180,000 tonnes of CO_2, and 173,000 tonnes of lignin (if not used for energy) (data from Weyland). On the other hand, the process uses 43,000 tonnes of acid.

COSTS

Economics of cellulosic ethanol processes is an area under development and debate. Open literature publishes a variety of data for lignocellulosic ethanol, as is illustrated in Table 2 (Wright and Brown 2007). Another, more recent comparative report indicates that a two-staged acid hydrolysis process with fermentation, a two staged enzyme hydrolysis process with fermentation, and a syngas fermentation process have investment costs of 1.32, 2.11, and 1.85 USD per liter capacity, respectively. The operating costs are estimated to be >0.79, 0.66 and 0.4 USD per liter (Sklar 2009). On diesel side a fast pyrolysis process is estimated to have 0.53 USD/liter capacity cost and 0.26 USD/liter operating cost (Sklar 2009).

Table 2. Capital and Operating Costs for 570 Million Liters Liquid Biofuels per Year Plants (Wright and Brown 2007).

Product	Total capital cost	Capital per capacity liter	Operating costs per liter
Grain ethanol	111	0.20	0.32
Cellulosic ethanol	756	1.33	0.46
Methanol	606	1.07	0.34
Hydrogen	543	0.96	0.28
Fischer –Tropsch diesel	854	1.50	0.45
Note 1: liter = liter of gasoline equivalent Note 2: dollar = 2005 US dollar			

As the fossil fuel reserves diminish, and energy consumption rapidly increases, alternative solutions will be both necessary and economically viable in future. Some deeper insight to the future of cellulosic biofuels is provided, e.g. by Dr. Bruce E. Dale. (www.everythingbiomass.org).

CONCLUDING REMARKS

Despite all the efforts, the production of cellulosic ethanol via biochemical process is not yet practiced at a commercial scale. However, a few industrial-scale plants are under construction: Verenium (140 MLPY), KL Energy (6 MLPY), POET (95 MLPY), Iogen (68 MLPY), Mascoma (150 MLPY), and Inbicon (5 MLPY). Recently TAPPI published that 11 demonstration plants are running, with total production capacity over 30 MLPY, and in total 19 pilot scale units exist with total production capacity less than 4 MLPY (Anon. 2009b).

The amounts of raw material to produce the aimed volumes of biofuels are large, and only few of the many possible raw materials exist in affordable prices and amounts. Sustainability issues and greenhouse gas reductions can most likely be kept under control when the raw material is grown and collected correctly. The EU is currently establishing sustainability criteria, which will help in streamlining the assessments for sustainability and greenhouse gas reductions.

Pretreatment seems to be an unsolved problem. The progress made in optimizing different techniques will lead in the near future to a process that will economically make cellulose available for hydrolysis without producing fermentation inhibitors that require extensive detoxification process steps. Technological issues to be improved remain in C5 fermentation, and concentration of sugars. The improvements are likely to appear in the near future.

The more seldom discussed issues that relate to the production of liquid biofuels are the need of large amounts of cellulose degrading enzymes and/or of acid, the water consumption in the processes, and the huge amount of gypsum produced. These issues as well as raw material related side streams need careful attention and sound engineering solutions. The aim of this paper is to point out these seldom mentioned challenges.

All of the above will have a significant effect on the economics of liquid biofuels, which is hard to evaluate. Yet, all activity in the research and engineering will lead, sooner or later, depending of oil prices and other global economy factors, to economical production of liquid biofuels. As Wyman nicely stated: what is needed, is a commercial plant (Wyman 2007).

Glucose from lignocellulosic origin can be fermented into butanol, too, which is from the point of view of an engine a better fuel, but this technology may be a few years behind lignocellulosic ethanol, as the yields, fermentation rates, and inhibitor tolerances of these microorganisms are not yet at the level of ethanol producing microorganisms. Recently a concentration of 30 g/l was reached by Ohio State University researchers (Lane 2009). If ethanol will stay as the main biochemical end product remains to be seen; iso-butanol is being actively researched by British Petroleum, Dupont, Cobalt Biofuels, and Gevo.

The vehicle fleet is being developed towards hybrid vehicles, plug-in-drives, and/or fuel cell powered vehicles (Nylund *et al.* 2008). As it is now, the electrical vehicles capable of running a hundred miles without recharge may be further away than we think. For example, in passenger vehicles, presently a 50 liter tank full of gasoline (38 kg) can be replaced by 860 kg of Li metal hybrid batteries, the theoretical minimum being 350 kg based on the charge capacity of the $LiFePO_4$-anode. Yet, it should also be noted that the heavy-duty fleet and air traffic will also in future rely on liquid fuels. Biofuels are thus likely to increasingly contribute in the transportation sector for the coming decades.

The Energy Independence and Security Act mandate, together with the European Union Renewable Energy Directive and other local targets, are driving the global development and implementation of biofuels forward, and cellulosic ethanol will play a significant role in future biofuels.

Disclaimer:
All opinions are of the authors and do not reflect Pöyry Plcs or its subsidiaries opinions.

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