Perspective

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Toward a sustainable biorefinery using high-gravity technology

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Abstract: The realization of process solutions for a sustainable bioeconomy depends on the efficient processing of biomass. High-gravity technology is one important alternative to realizing such solutions. The aims of this work were to expand the knowledge-base on lignocellulosic bioconversion processes at high solids content, to advance the current technologies for production of second-generation liquid biofuels, to evaluate the environmental impact of the proposed process by using life cycle assessment (LCA), and to develop and present a technically, economically, and environmentally sound process at high gravity, i.e., a process operating at the highest possible concentrations of raw material. The results and opinions presented here are the result of a Nordic collaborative study within the framework of the HG Biofuels project. Processes with bioethanol or biobutanol as target products were studied using wheat straw and spruce as interesting Nordic raw materials. During the project, the main scientific, economic, and technical challenges of such a process were identified. Integrated solutions to these challenges were proposed and tested experimentally, using wheat straw and spruce wood at a dry matter content of 30% (*w*/*w*) as model substrates. The LCA performed revealed the environmental impact of each of the process steps, highlighting the importance of the enzyme dose used for the hydrolysis of the plant biomass, as well as the importance of the fermentation yield. © 2016 The Authors. *Biofuels, Bioproducts, and Biorefining* published by Society of Chemical Industry and John Wiley & Sons, Ltd.

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Introduction

Biochemical conversion of lignocellulosic feedstocks to liquid biofuels is increasing in importance, with a few commercial plants to produce second-generation ethanol already under construction.[1] These plants will use agricultural residues, energy crops, and wood, and will have an annual production capacity ranging from 1.1 to 95 million liters of ethanol per year.[1] A plant with a capacity of 95 million liters requires approximately 290 000 tons of dry biomass per year, assuming a maximum theoretical conversion rate. On this scale, the dry matter (DM) content in each of the operation units (pre-treatment, enzymatic hydrolysis, and fermentation) has a significant influence on the capital and operating costs. However, there are technological challenges that need to be addressed in order to realize a biofuel production process at high DM content (from here on referred to as high gravity). The HG Biofuels project, the overall results of which are presented in this paper, aims to identify, study, and propose solutions to these challenges using Scandinavia's most representative agricultural and woody biomass as feedstocks (wheat straw and spruce, respectively).

As an example, operating at 30% (*w/w*) DM content as opposed to 10% (*w/w*) would reduce the number of 300 m^3 reactors required for hydrolysis and fermentation from 36 to 12 in order to produce 95 million liters of ethanol per year. Accordingly, the capital and operating costs of bioreactors are significantly higher when operating at low gravity.[2] Operating at high gravity would also give a high sugar concentration and therefore possibly a high ethanol concentration. Since distillation is one of the energy- and cost-intensive process steps, only ethanol concentrations above 4% (*w/w*) are economically feasible.[3] To achieve this ethanol concentration, the process must be operated at a minimum DM content of 20% (*w/w*).[4] A problem encountered with high-gravity conditions is that a paste-like slurry – or even a semi-solid pulp – is formed after the pre-treatment step, when the DM content exceeds 15% (*w/w*). Consequently, the pre-treated material is very viscous and difficult to handle in the subsequent processing steps,[4-6] leading to inefficient mixing with enzymes and micro-organisms, and ultimately to low yields and productivities.[7] Depending on the type of pre-treatment, compounds that are inhibitory to the fermenting, micro-organisms are formed due to degradation of hemicellulose and lignin. The kinds and the amounts of different inhibitors depend on the raw material used and on the conditions of the pre-treatment.[8] Performing the pre-treatment at high gravity results in the generation of higher amounts of inhibitors in the pre-treated material and therefore higher concentrations of these in the fermentation medium. When hydrothermal or steam explosion pre-treatments (in the presence of acids) are employed, the generation of inhibitors is especially common at high gravity.[8]

Butanol is another biofuel that can be produced from lignocellulosic feedstocks by means of fermentation.[9,10] The production of butanol and associated solvents used to be the second-largest industrial fermentation process. For instance, in China the production of acetone, butanol, and ethanol via the so-called acetone–butanol–ethanol (ABE) fermentation process reached a peak in the 1980s, but declined and finally disappeared in the 1990s.[9] Due to the high cost of crude oil, as well as China's increasing energy consumption, butanol production by fermentation has gained renewed interest, and a number of plants have been rebuilt and constructed in recent years with a total expected annual production capacity of about 1 million tonnes.[9] The development of a butanol production process at high gravity can be justified with the same arguments as given in the case of ethanol, although strong product inhibition of microbial action will limit the butanol concentration that can be achieved. However, in a production system with continuous product removal and fermentation, the use of high-gravity conditions will be of benefit in order to achieve high sugar utilization. This will directly influence the operating costs and carbon efficiency.

In the HG Biofuels project, we combined different competences from the project partners in order to enable the development of a high-gravity process concept for ethanol and butanol production. Intensive collaboration between all partners took place in order to face the main challenges in each process step, and to achieve a feasible high-gravity process. The industrial partners Inbicon and SEKAB supplied the pre-treated biomass, which was pre-treated wheat straw and spruce, respectively. Enzymatic hydrolysis was studied by the Biomass Group at the University of Copenhagen in various process configurations. The study of the ethanol fermentation process step was carried out at Chalmers University and the University of Copenhagen, while butanol fermentation was performed by Statoil. Selected experimental results were used as a basis for performing life cycle assessment (LCA) for the production of ethanol under high-gravity conditions, from feedstock cultivation to final product, in order to evaluate its performance from an environmental point of view.

Process description

Two possible process concepts, one for ethanol production and one for butanol production, were designed as a

foundation for an increased understanding of the effects of high-gravity conditions at the process level, and as part of a more encompassing, larger system (from raw material extraction until the products leave the production process) for carrying out the LCA.

The ethanol production process

The process concept for ethanol production, including the production of co-products, is based on the Integrated Biomass Utilization System (IBUS) process developed by Inbicon in the case of wheat straw,[11] and the e-Tech process developed by SEKAB in the case of spruce (Fig. 1). Both processes are continuous, with the feedstocks first being prepared and then being pre-treated (refer to Table 1 for the process conditions of each of these pre-treatment steps). The wheat straw is hydrothermally pre-treated in the Inbicon process, while the wood is processed using SO_2-catalyzed steam

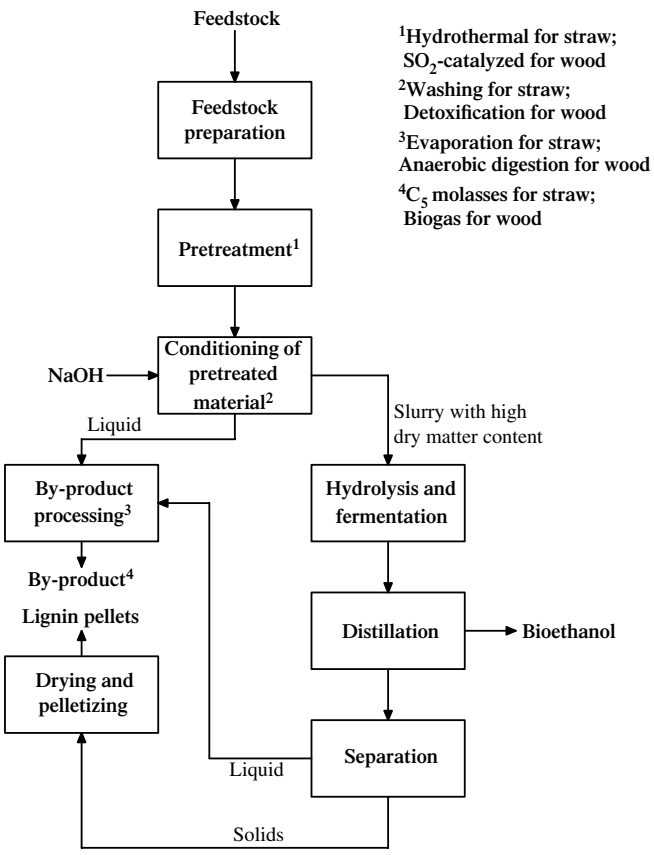

Fig. 1. Conceptual process flow diagram for the production of ethanol from lignocellulosic feedstock. Differences in the process because of the use of straw or spruce are indicated by numbers.

Table 1. Process conditions during the pre-treatment of wheat straw and spruce.

	Wheat straw	Spruce
Temperature (°C)	195	190–210
Residence time (min)	18.5	5–8
pH	2-4	1.5–1.8
Catalyst loading (% of DM)	n/a	2.5
Type of catalyst	no	SO_2

pre-treatment in the SEKAB process. The pre-treated material leaves the pre-treatment unit at approx. 30% DM for both feedstocks.

The pre-treated straw in the Inbicon process is washed to separate the solubilized hemicellulose from the fibers. The solubilized hemicellulose is concentrated by evaporation to 65% DM content and leaves the process as the by-product C_5 molasses. The pre-treated wood in the SEKAB process is separated, detoxified, or washed, and the liquid fraction after this step goes to the waste-water treatment where it is anaerobically digested in order to produce biogas (containing 60% (*w/w*) CH_4 and 40% (*w/w*) CO_2) as a by-product. The biogas produced is assumed to cover part of the energy demands of the wood-based ethanol production process.

Next, the pre-treated fibers, now mainly consisting of cellulose and lignin for both feedstocks, are enzymatically hydrolyzed and fermented to produce ethanol. During the downstream processing of the fermentation broth, ethanol is separated and purified up to 99.5% (*v/v*) using distillation and molecular sieves, and the solids in the bottom product of the distillation are dried. These solids consist mainly of lignin and are made into lignin pellets. The pellets are assumed to fulfil the energy demands of both the straw-based and the wood-based production process, and they are exported if a surplus is generated. If the incineration of the pellets (and the biogas, in the case of the wood-based process) does not provide enough energy to meet the energy demands of the process, then it is assumed that a representative fossil fuel mix can be burned to make up for this shortage.

The butanol production process

Butanol produced by ABE fermentation has conventionally been a batch process. In industrial plants, the individual fermentors will be put in a different stage of the fermentation process whereby the overall process can be considered semi-continuous. In recent years, several attempts at developing a real continuous butanol process have been

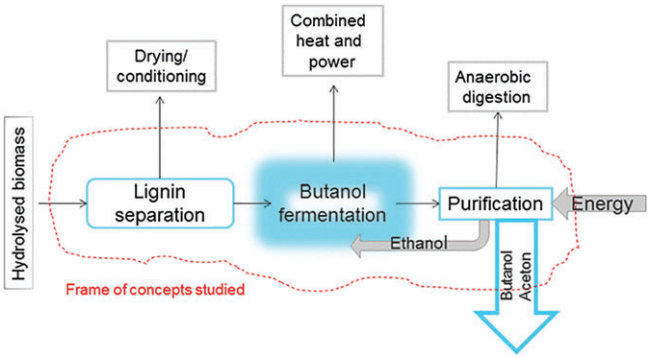

Fig. 2. Conceptual process flow diagram for butanol production from lignocellulosic feedstock. The red dotted line indicates the process steps that were considered in the study.

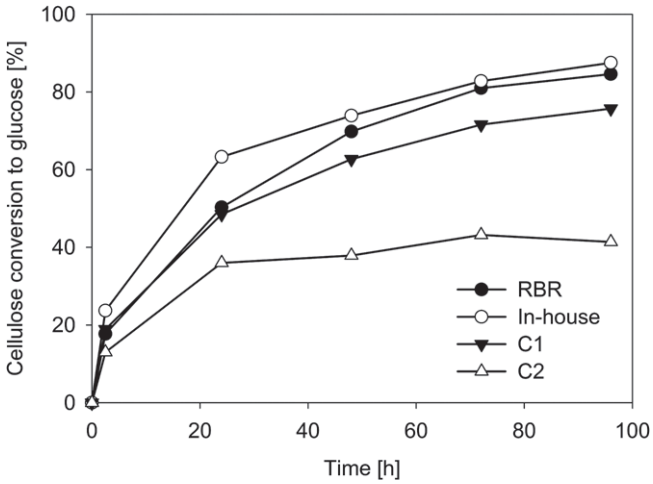

Fig. 3. Comparison of enzymatic hydrolysis of pretreated wheat straw at 30% DM with enzyme loading of 7.5 FPU/g DM in laboratory- (0.1-L to 10-L scale) and pilot-scale reactors (400 L). RBR, roller-bottle reactor; In-house, in-house produced reactor combining RBR and internal stirrer; C1 and C2, two commercially available reactors for high solids operation. Final glucose concentration: 143 g/kg for RBR, 151 g/kg for in-house, 128 g/kg for C1, and 73 g/kg for C2.

reported, both by academia and by industrial players.[10,12] In the present work, a process concept where the products (acetone, butanol, and ethanol) are continuously extracted from the fermentation broth was investigated. This can be achieved by continuously removing a fraction of the fermentation broth and extracting the fermentation products before recirculating the raffinate, or the fermentation products may be extracted from the broth *in situ* using, for example, gas stripping. In the first concept, the unreacted carbohydrates, organic acids, etc., can either be used for production of biogas in an anaerobic digester or recirculated to the fermentor. A conceptual flow diagram of the process is given in Fig. 2.

Enzymatic hydrolysis of lignocellulose at high solids concentrations

Operation of the enzymatic hydrolysis at high initial solids concentrations is complicated by the nature of the biomass. Pre-treatment will cause removal and/or relocation of hemicellulose and lignin, defibrillation of the biomass, reduction of fiber length, and swelling of the fibers. The extent to which this happens depends on the type of pre-treatment and its severity. Entangling of the curled fibers, swelling of fibers, and the high water-binding capacity result in a high-viscosity material at more than 10–15% DM, which complicates homogeneous mixing.[13] For this reason, enzymatic hydrolysis was rarely performed above 15% DM until a new mixing principle termed free-fall mixing was introduced in 2006,[6] which is now operated on a demonstration scale by Inbicon.[11] The need for adequate (laboratory-scale) reactors for high solids fermentations was emphasized during the HG Biofuels project, especially in the case of pre-treated wheat straw, which – due to less severe pre-treatment conditions – had much more intact fiber structure and very high initial viscosity. Model reactors were designed using the principle of free-fall mixing, either as roller-bottle reactors (RBRs) or with an in-house custom-made horizontal reactor combining the RBR with internal stirring.[14,15] In addition, some commercial reactors capable of operating at high solids concentration and vertical reactors with various impellers were tested. The experimental results showed that reactor type and the efficiency of mixing clearly had an effect on hydrolysis (and fermentation) yields (Fig. 3).

Despite being able to operate the process at high initial solids concentrations, it has been shown that the enzymatic decomposition of cellulosic biomass decreases almost linearly with increasing solids concentration.[16] Several topics have been investigated in numerous studies in the attempt to explain the causes of the effects of solids: lignocellulose structure and chemical composition, mixing and mass-transfer, end-product inhibition, water content, and enzyme adsorption characteristics. However, none of these factors alone can fully explain the solids effect. Recently, studies have revealed that the state in which water is present in the plant cell wall structure – as well as water activity in general – has a crucial role during hydrolysis of insoluble substrates and is affected by the

presence of soluble species.[17] Monosaccharides formed during hydrolysis have a negative effect on cellulases. This is a problem particularly when operating at high initial solids concentrations, as final glucose concentrations above 150 g/L may occur (Fig. 3).[15] Interestingly, in addition to end-product inhibition by glucose, all soluble monosaccharides restrict water availability and affect the water available for hydrolysis, contributing to a diminished hydrolysis yield.[18] Due to the negative effect of high concentrations of monosaccharides on enzyme performance, it has commonly been accepted that operating the ethanol production process as a simultaneous saccharification and fermentation (SSF) gives better performance and yield than separate hydrolysis and fermentation (SHF).

With the introduction of new, improved cellulase preparations containing not only the 'classic' cellulases (cellobiohydrolase, endoglucanase, and β-glucosidase) but also the recently discovered lytic polysaccharide monooxygenases (LPMOs) – or AA9/AA10 – it was of interest to determine whether the previous conclusion was still valid when operating at initial DM of 20–30%.[19,20] It was found that during hydrolysis at high solids concentration, significant amounts of monosaccharides (up to 4% of released glucose) were found as gluconic acid, which is indicative of the action of LPMOs.[21, 22] To investigate the optimum process design, an extensive experimental series was designed, testing SHF, SSF, and an intermediate option (pre-hydrolysis, simultaneous saccharification and fermentation, P-SSF) at 30% DM using either the traditional enzyme combination Celluclast and Novozym 188 or the newer product Cellic® CTec2. The results showed that under optimum conditions – 30% DM – up to 95% of cellulose from wheat straw was hydrolyzed, yielding ethanol concentrations up to almost 90 g/kg slurry. Interestingly, the optimum process configuration using the cellulase preparation Cellic® CTec2 was SHF, which could be attributed to high levels of β-glucosidase and the presence of LPMO enzymes. LPMO enzymes work synergistically with cellulases and boost cellulose hydrolysis, but LPMO enzymes require oxygen in order to function, so the highest yield was obtained with SHF, where the hydrolysis is performed in the presence of oxygen.[15]

The optimization experiments also included testing of the addition of non-ionic surfactant, in this case polyethylene glycol (PEG). The presence of PEG is believed to reduce non-productive adsorption of enzyme onto lignin and to stabilize enzymes.[23,24] The results confirmed that conversion yields could be significantly increased, or in other words a similar conversion could be obtained with reduced enzyme loading. In the case of wheat straw hydrolysis, the addition of 0.01 g PEG3000 per g DM could reduce the enzyme loading by 30%.[15] The effect of this on the environmental impact of the process was further analyzed in the LCA study.

Fermentation to ethanol and butanol

There are significant differences in composition and structure between different lignocellulosic materials that are commonly used as feedstock for fermentation applications. The two substrates studied in this project, spruce wood and wheat straw, represent differences which will affect the design of the fermentation process:

- The hemicelluloses in the case of spruce are mainly (galacto)glucomannans, while in the case of wheat straw they are mainly arabinoxylan.
- In general, spruce pre-treated under acidic conditions is much more toxic to micro-organisms than wheat straw, which has been hydrothermally pre-treated due to the presence of higher concentrations of HMF, furfural, weak acids, and phenolic compounds. In particular, phenolics tend to be the most problematic group of inhibitors in spruce-derived streams.
- The pre-treated wheat straw showed a significantly higher viscosity than the pre-treated spruce at the same content of water-insoluble solids.

It is therefore clear that the challenges in each case are different, and different strategies must be adopted to achieve a successful process.

Ethanol fermentation studies

For both pre-treatment methods used in this project, hemicellulose is partly solubilized while cellulose and lignin remain in the solid fraction. Besides solubilized hemicellulose sugars, the liquid fraction will also contain all the inhibitory compounds generated during pre-treatment (HMF, furfural, weak acids, and phenolic compounds).

In the case of spruce, due to the presence of naturally fermentable sugars in the liquid fraction of the slurry (dissolved glucose, mannose, and galactose represent about one third of the total sugars in the slurry generated), major efforts were made to overcome the inhibitory effects during fermentation. Thus, fermentation of the whole slurry generated after pre-treatment was thoroughly investigated as the main fermentation option. Nevertheless, 'washing' of solids and therefore exclusion of the liquid fraction

from the fermentation medium was also part of the investigation.

In the case of wheat straw, the solubilized sugars in the pre-treated material were mainly pentoses (xylose and arabinose). In the Inbicon case, pentose sugars go to molasses and are used as a biogas booster or as animal feed.[11] Thus, the wheat straw fermentation study in this project focused only on the cellulose-rich fraction (the fiber fraction). Due to the separation of the two fractions during the Inbicon process, the level of inhibitors was significantly lower during the fermentation of the pre-treated wheat straw than during fermentation of spruce.

Operating the process at 30% (*w/w*) DM inevitably increases the problems associated with inhibitors formed during the pre-treatment, as they will be present in higher concentrations. Fermentation experiments with the pre-treated spruce showed severe inhibition of yeast. Pre-treated spruce slurry with 30% DM was not directly fermentable. Fermentation experiments performed in this project represented three distinct strategies to overcome or minimize this problem:

- Development of yeast strains tolerant to inhibitors and short-term adaptation of yeast cells prior to fermentation in order to acquire a yeast strain or population strong and robust enough to cope with the harsh conditions.
- Detoxification of the medium before fermentation in order to eliminate inhibitors.
- Supplementation with nutrients with the purpose of helping the yeast cells during fermentation.

During this study, we redefined the propagation of yeast before the SSF process. An earlier study has clearly shown improved ethanol yield and productivity in an

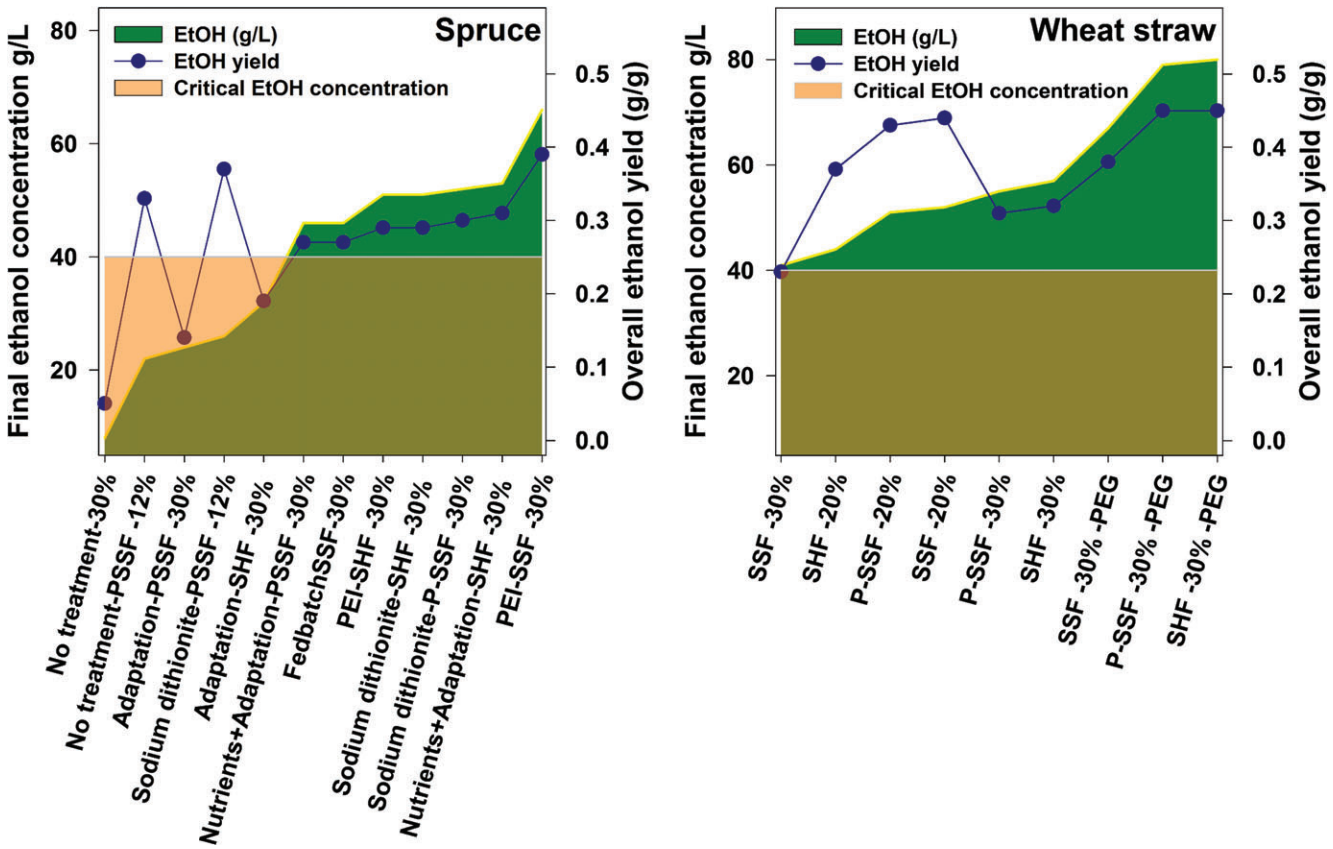

Fig. 4. Fermentations of dilute acid-pretreated spruce and hydrothermally treated wheat straw under high-gravity conditions (initial solids 30%), from the HG Biofuels project. Fermentations at lower solids concentrations are shown for comparison (12% for spruce and 20% for wheat straw). The blue dots represent the ethanol yields obtained in each case while the upper edge of the green area represents the ethanol concentration achieved in each case. The color change indicates the ethanol concentration above which the fermentation is likely to be economically feasible, as the cost of the process is significantly reduced when the final ethanol concentration is above 40 g/L. As can be seen, under high-gravity conditions, one can have low-cost fermentation processes without achieving very high ethanol yields.

SSF process when using yeast propagated in a fed-batch mode and using the liquid fraction from the pre-treated slurry.[25] Although this procedure worked relatively well at a lower DM content, the results of the HG Biofuels project clearly showed that feeding of the yeast suspension to the SSF reactor from the outflow of the cultivation reactor significantly enhanced the cell viability, which contributed to the improved ethanol yield and concentration.[26] Such an adaptation allowed the cells to adapt at sub-lethal concentrations of inhibitors and to be able to cope with inhibitors during fermentation.[5] Strain development was attempted by evolutionary engineering. These experiments focused on the effects of inhibitor mixtures in order to develop yeast strains that would be tolerant to inhibitory slurries. Strains with significantly higher specific growth rates in the presence of inhibitor mixtures were obtained at the end of the evolution experiments (unpublished data). The evolutionary engineering approach has been shown to be very efficient in many cases where the knowledge base of the molecular traits that need to be improved is lacking.

Two different detoxification methods were evaluated regarding the ability of the cells to overcome the inhibitory issues during the fermentation stage: (i) detoxification of spruce slurry using sodium dithionite ($Na_2S_2O_4$),[5,27,28] and (ii) detoxification of the medium using polyethylenimine.[29] The detoxification of the medium either by use of sodium dithionite or by applying a soluble polyelectrolyte polymer (PEI) to absorb inhibitory compounds in the material before the fermentation stage resulted in an impressive improvement in the final ethanol yield, with conversion of almost 60% of the total sugars in the slurry to ethanol.[5,29] When only the solids from spruce slurry were fermented, the ethanol yield per g of available fermentable sugars was significantly increased (~68%), indicating the detrimental effect of inhibitors present in the liquid stream.[5] However, because of the absence of the soluble sugars, the ethanol concentration did not reach levels as high as in the case of slurry detoxification.

Lignocellulosic hydrolysates are generally deficient in nutrients that maintain the viability and growth of the yeast. Supplementation of wheat straw hydrolysates with nutrients has previously been shown to significantly improve the fermentation rate, and in some cases also the final yield of ethanol.[30] As shown in Fig. 4, in the case of the more toxic spruce material, supplementation of the fermentation medium with nutrients significantly only improved the ethanol yields when combined with adaptation of the cells to the inhibitory conditions before the fermentation. This approach led to an ethanol yield of 0.40 g/g, as compared to 0.01 g/g in the base case. When yeast extract was used as supplement (using adapted *S. cerevisiae* cells), yields of more than 60% of the theoretical yield were achieved, whereas the material was not fermentable at all without addition of nutrients.[5]

Adaptation of the yeast (in combination with addition of yeast extract) was therefore an efficient method of avoiding the use of chemicals such as detoxification agents. In both detoxification and nutrient supplementation-adaptation strategies, very low concentrations of fermentable sugars were detected after the fermentation stage, showing that the enzymatic hydrolysis remained the main bottleneck of the process – and the reason that higher ethanol yields were not achieved (Fig. 4).

Throughout the project, four different operation modes were tested to overcome inhibitory effects: (i) a fed-batch process, (ii) an SSF process, (i) an SSF process coupled to a short hydrolysis step (P-SSF), and (iv) an SHF process in combination with the different strategies. As shown in Fig. 4, the strategies described above gave similar ethanol yields when used in both fermentation modes (P-SSF and SHF), indicating that novel enzymes (for reasons mentioned in the section on enzymatic hydrolysis) are not as affected by end-product inhibition (mainly by glucose or cellobiose) as earlier enzyme preparations, making the SHF process also attractive for production of biofuels.[7] The thermotolerant *S. cerevisiae* strain used in the HG Biofuels project also allowed us to evaluate the different strategies at different fermentation temperatures. The tolerance to inhibitors decreased as the temperature increased. The results showed that a high-gravity process at high temperatures was possible only when detoxified material was used.

The particular characteristics of different cellulosic raw materials suggest that a tailored process technology is needed, maximizing the final conversion yield using a different strategy in each case. Hydrothermally pre-treated wheat straw gave outstanding results with the SHF technology using the latest generation of cellulolytic enzymes. After the hydrothermal pre-treatment, the liquid fraction containing inhibitors and C_5 sugars was removed – providing a material ready to be hydrolyzed and fermented. Different process strategies tested at 30% DM highlighted the importance of a dedicated enzymatic hydrolysis step. Spruce is promising Nordic feedstock with high C_6 sugar content, thus avoiding the need for C_5 fermentation (or alternatively, the latter can be used as a side-stream as in the case of the IBUS process). However, it was clear that if we wanted to exploit this high biotechnological potential, it would be necessary to solve the major inhibitory issues.

Butanol fermentation

Fermentation to butanol was done using a bacterium, *Clostridium acetobutylicum,* under anaerobic conditions in batch or continuous mode, with glucose at a concentration of 60 g/L as feedstock. A butanol concentration of 16–17 g/L was obtained whereas addition of a redox compound (neutral red)[31] increased the butanol content to 18–19 g/L in batch fermentations.

Continuous fermentation with *in situ* solvent removal using gas stripping showed that the fermentation could be sustained for more than 800 h if the concentration of butanol was kept below 10–12 g/L. The average volumetric productivity was about 0.4 g solvent/Lh. It was observed that the fermentation progresses in cycles/oscillations, which may be a reflection of the well-known switch between the acidogenic phase and the solventogenic phase.[32]

A gradual substitution of the glucose feedstock with a hydrolysate prepared from wheat straw was studied. A substitution of up to about 10 wt% of the glucose with hydrolysates apparently did not deteriorate the fermentation performance significantly in the long run, even though a certain decline was observed initially (Fig. 5). This behaviour may indicate that the bacteria may be able to adapt to moderate concentrations of inhibitors in the hydrolyzate. At higher concentrations, the system stopped producing butanol. However, it should be mentioned that the observed ability to adapt to some extent to the new feedstock may indicate that the strain could be adapted to even higher concentrations if the system was allowed to operate for a longer time at the lower concentrations of hydrolyzates. It is tempting to believe that this behaviour is due to the presence of known inhibitors such as HMF, furfural, levulinic acids, or acetic acid. However, separate studies using controlled addition of these did not show the same response at similar concentrations. We can therefore assume that our observations were either due to synergetic effects of two or more inhibitors, or that the behaviour observed was due to presence of other inhibitory compounds.

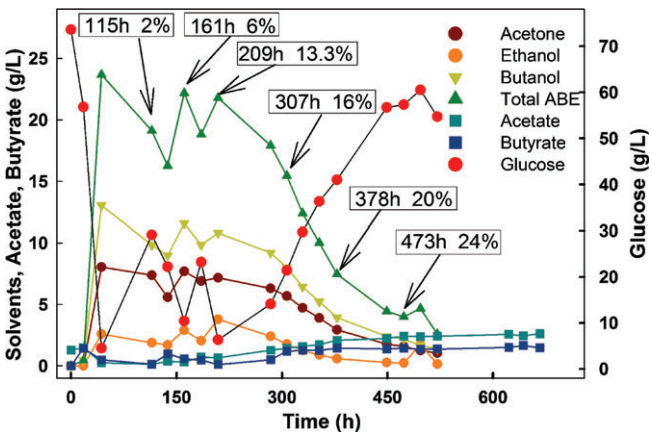

Fig. 5. Continuous fermentation with an increasing amount of wheat straw hydrolysate added to the broth. The concentrations of carbohydrates supplied by the hydrolysate are given in the figure, i.e. "24% hydrolysate" indicates that 24% of the carbohydrates originate from the hydrolysate; the remaining 76% was added as glucose. ABE is the sum of the solvents (acetone, butanol, ethanol).

A simplified techno-economic analysis for the process using three different concepts has been performed. The schemes investigated were (i) batch process with distillation, (ii) continuous fermentation with distillation, and (iii) continuous fermentation combined with gas stripping. According to the evaluation, all these concepts suffered from a relatively low yield of 0.2–0.3 g butanol per g of sugar consumed, with a corresponding low energy efficiency of 16–24% (i.e., the ratio between the energy content of the butanol produced and the energy used for its production). For a fairer comparison with the ethanol process, one should also include the production of acetone, which is also recovered. Including this leads to an increase in the yield to about 0.4 g butanol + acetone per g sugar. The main causes of the low energy efficiency of the process are the large energy demand for the separation process and the relatively low yield of butanol from the process.

Life cycle assessment

LCAs using the results of the experimental work described in the previous sections were performed in order to establish the influence of high-gravity conditions on the environmental impact of the production of ethanol from the two feedstocks tested.[33,34] The LCAs were set up using a cradle-to-gate attributional approach (from the cultivation and harvesting of the feedstocks to the ethanol production plant gate) in order to identify possibilities for improvement in the technologies being developed. The functional unit of the assessment was 1 L of ethanol produced. The mass and energy balances of the system analyzed were calculated based on the conceptual process flow diagram depicted in Fig. 1. Next, the results of these balances were combined with information about the upstream processes (e.g. production of chemicals and enzymes) in order to complete the inventory analysis. This was followed by performing the life cycle impact assessment. The environmental impact categories that were selected for this assessment were global warming potential (GWP), eutrophication

potential (EP), acidification potential (AP) and photochemical ozone creation potential (POCP). Furthermore, energy analyses were done in order to assess the renewable and non-renewable energy use (REU and NREU), respectively.[33,34]

For both feedstocks, the ethanol yield is the main factor that determines the environmental impact of the process under development. Yield affects both REU (the amount of feedstock needed) and NREU (mainly the use of fossil energy in enzyme production) and their related emissions, which therefore ultimately determine the environmental impact of a process configuration. For both feedstocks, the predominant contributor to all environmental impact categories studied was the production of the enzyme preparations used for hydrolyzing the lignocellulosic material (Fig. 6). This is due to the use of significant amounts of non-renewable energy during enzyme production. This indicates that reducing the effect of enzyme use by enzyme recycling and/or more sustainable enzyme production are interesting areas to focus on.[33,34] Furthermore, the results for straw ethanol production suggest that the enzyme load should be carefully chosen due to the trade-off between a reduced environmental impact (thanks to increased yield) and an increased environmental impact due to enzyme production. On-site production of enzyme may also be a solution to reduce the environmental impact of the process.[34,35] It was demonstrated for the wood ethanol production that on-site enzyme production may lead to a reduction of GWP by approx. 60%. This is mostly due to the elimination of fossil energy use during enzyme production.[34]

The pre-treatment and distillation steps (Fig. 1) also use significant amounts of energy, but this gives much lower impacts because this is renewable energy generated by incineration of lignin in the case of wheat straw, and of lignin and methane in the case of spruce wood. It should be noted that running the process at a lower DM content (10%, indicated as base cases in Fig. 6) resulted in the use of additional fossil fuel for both feedstocks in order to provide enough energy for the process. This indicates that at high yields, the concentration of ethanol in the fermentation broth must be sufficiently high to avoid additional use of fuel.

In the case of wheat straw,[33] other contributors (besides enzyme production and use) were straw cultivation and harvesting (to EP, due to the leakage of fertilizers and NO_x emissions) and the combustion of lignin for process energy (to AP, due to SO_2 emissions). The process configuration with the lowest GWP (Fig. 6) used the Celluclast enzyme cocktail, which needs significantly less fossil energy in its production than the Cellic Ctec2 enzyme cocktail. This indicates once more that a cleaner enzyme production is beneficial from an environmental point of view, even if it leads to a lower yield. The LCA results showed that having high-gravity process conditions during the hydrolysis and fermentation has a higher environmental impact than when running the process at lower DM content, due to the lower conversion at increasing solids content (solids effect). Technically, this higher impact could be compensated for by the addition of polyethylene glycol (PEG), and this highlights the importance of further research and development regarding reactor and process design for these processes.

In the case of spruce wood,[34] other contributors (apart from enzyme production and use) were $Na_2S_2O_4$ production and SO_2 production (to AP, due to SO_2 emissions; to POCP, due to emissions of volatile organic compounds (VOCs)). The process configuration with the lowest GWP (Fig. 6) used washing of the pre-treated spruce slurry as the detoxification strategy. Apart from an increased fermentation yield due to the removal of inhibitors, significant amounts of biogas are produced because the free sugars that are generated during the pre-treatment are now fed to the anaerobic digestion (Fig. 1). The biogas produced is subsequently incinerated to provide the process with energy, which then leads to lower consumption of lignin for this purpose. Besides ethanol, a larger amount of lignin pellets is therefore produced as a by-product, which then represents a larger share of the environmental burden of the process. Addition of sodium dithionite as a detoxification strategy leads to similar yields, but less biogas is produced with these configurations. This therefore leads to a larger allocation of the environmental burden to the ethanol product, because more lignin has to be incinerated to provide the process with sufficient energy. This indicates that in the case of wood ethanol production presented here, it is not only the yield of ethanol production that should be improved; the production of biogas is of importance from an environmental point of view.

Conclusions and outlook

During the course of the HG Biofuels project, significant progress has been made toward technical solutions for efficient high-gravity ethanol production. Important insights into further technical improvements and the results of the life cycle assessment have laid the foundation for further development of such processes to allow sustainable bioeconomic solutions.

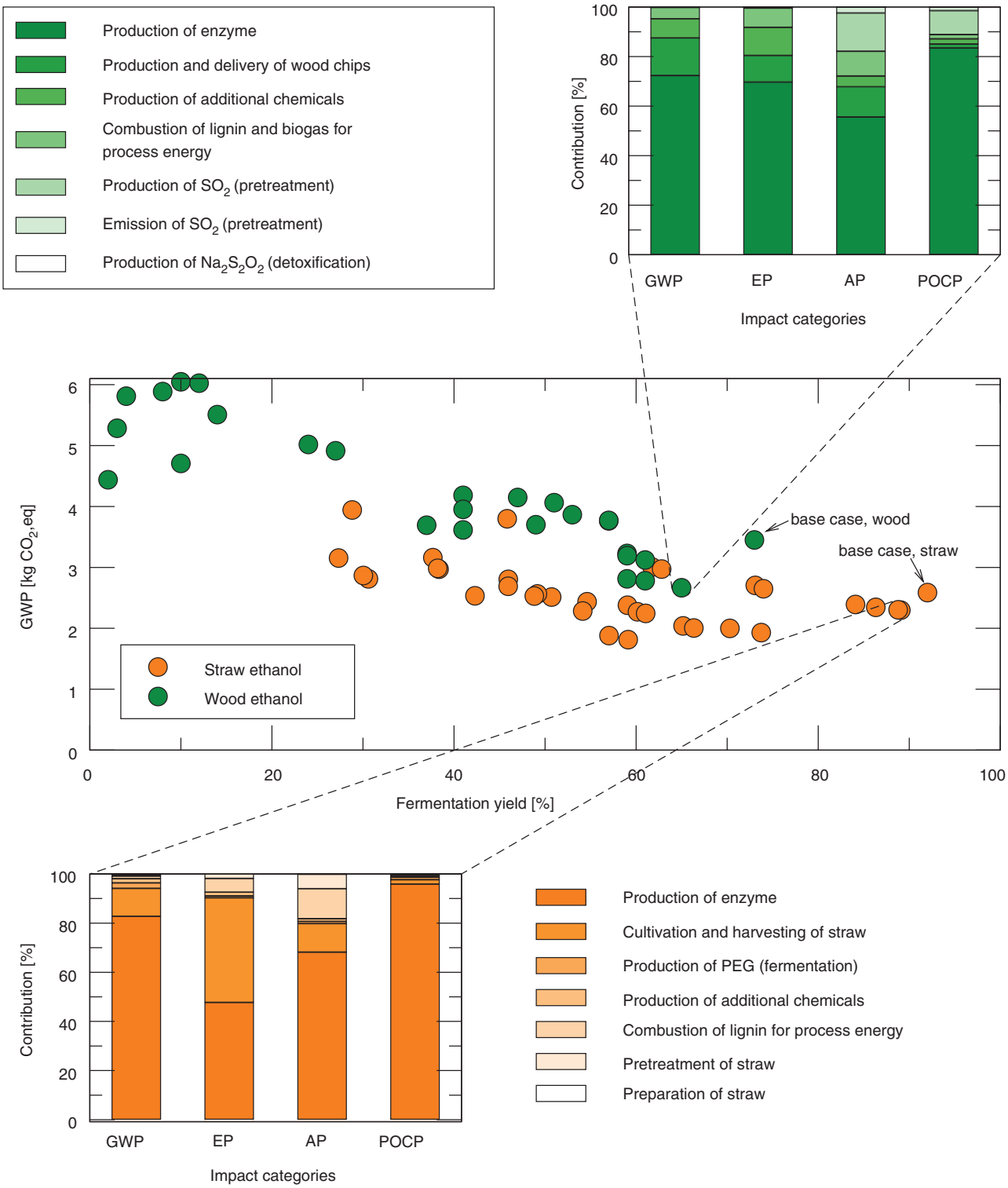

Fig. 6. Trend in global warming potential (GWP) per L of straw and wood ethanol produced, with fermentation yield for all experimental set-ups. The base cases for both feedstocks are configurations with low DM content (10% DM). Contribution analyses are depicted for the cases with the highest yields at high gravity for both feedstocks. The contributory processes are ranked according to their contribution to GWP. In the case of straw ethanol, PEG was not used in all cases. In the case of wood ethanol, $Na_2S_2O_4$ was not used in the case with the highest yield. EP: eutrophication potential; AP: acidification potential; POCP: photochemical ozone creation potential.

The results revealed that process design and choice of hydrolysis/fermentation strategy are critical factors that significantly affect the yield – and thereby the environmental impact. Operation of processes at high solids concentrations is still technically challenging, especially on a laboratory scale. This study offers approaches to improve high-gravity fermentations, and several of these have been proven in demonstration-scale trials. Of special interest would be studies of continuous processes for enzymatic hydrolysis and fermentation. Increasing the solids loading may reduce energy consumption for the separation, but the efficiency of the hydrolysis must be improved (or the environmental footprint for enzyme production reduced) to allow a sustainable production.

LCA is usually done based on mature industrial processes. In this case, however, experimental laboratory data were used to assess the environmental impact of a technology in the very early stages of development. This results in methodological challenges that have not been sufficiently addressed to date. For example, the inclusion of scale – both in terms of the process as such (e.g. yield changes when processes are scaled up) and in terms of the total scale of production (e.g. the total amount of biomass available being limited and competition for it increasing) – is an issue that must be addressed in future work.

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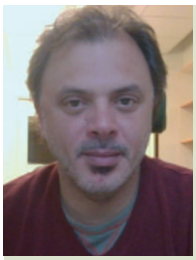

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