SO_2-ETHANOL-WATER FRACTIONATION OF FOREST BIOMASS AND IMPLICATIONS FOR BIOFUEL PRODUCTION BY ABE FERMENTATION

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Received November 17, 2009

The study proposes an economic process for the production of commodity chemicals from forest biomass and recycled fibers. This so-called Bioforest process uses tree tops, limbs, twigs, stumps and recycled paper as feedstock for fermentation to butanol, ethanol and acetone/isopropanol. This mixture of solvents can be sold as chemicals or used to replace gasoline in internal combustion engines. The SO_2-ethanol-water fractionation method affords efficient fractionation of forest biomass, under moderate conditions, yielding a high amount of fermentable sugars. Subsequently, sugars are subjected to ABE fermentation. Preliminary trials with conditioned hemicellulose solutions produced by the fractionation process show that they are fermentable by bacteria, such as the *Clostridia* species, but further work is needed to optimize the production of butanol, ethanol and acetone or isopropanol.

Keywords: biorefinery, forest biomass, SEW pulping, ethanol pulping, ABE fermentation, *Clostridia*

INTRODUCTION

The search for alternative transportation fuels, such as ethanol, produced from non-food feedstocks is ongoing around the world. Several promising production routes have been studied, and some are now being considered for large-scale implementation. Butanol is considered to be an even better replacement fuel for gasoline than ethanol, due to its better qualities, such as lower volatility, octane number improvement power and higher energy content. Another benefit of butanol is the possibility to use the existing transportation fuel pipelines and infrastructure.[1,2] A promising technology for the production of butanol is the acetone-butanol-ethanol (ABE) fermentation that has been used for almost a century.[3-5] The renewed interest in this process has focused on cheap feedstocks, for instance lignocellulosic biomass and recycled wood residues.

SO_2-ethanol-water (SEW) pulping, introduced in 1957 by Schorning,[6] can be considered a hybrid between acid sulfite and organosolv pulping and it is a promising fractionation process for lignocellulosic biomass. The advantages over conventional pulping methods include: simplified chemical recovery due to the absence of base (*i.e*. NaOH, $Ca(OH)_2$ or MgO) in the process, lower capital costs and rapid impregnation of the feedstocks, due to the presence of ethanol in the cooking liquor. Moreover, the process is omnivorous and can be applied to all lignocellulosic raw materials. SEW pulping

is also a highly suitable fractionation method for biorefineries, since a high yield of fermentable sugars can be produced already at moderate temperatures and relatively short cooking time periods.[7] However, for process economics, it is essential to recover the solvent ethanol at very high yields, since it has been claimed that solvent losses exceeding 2% make organosolv processes non-economical.[8] Losses could occur at each fractionation stage, for example by volatilization or incomplete removal of ethanol from pulp and by-products. Distillation as a separation process to recover ethanol is energy intensive, constituting 20 to 40% of the operational costs of fractionation.[9] Furthermore, the recovery of SO_2 is crucial not only for economic reasons, but also to minimize environmental impact.

The aim of the present research project is to create an economical process for producing chemicals and biofuels from low-quality forest biomass by means of SEW fractionation and ABE fermentation technologies. The flow diagram of the Bioforest process, presented in Figure 1, clarifies the process steps. Besides utilizing sugars from hemicelluloses, the target of the proposed process is to fully hydrolyze cellulose into oligomers and fermentable sugars. Acetic acid and lignosulphonates produced as by-products can be sold to increase the revenue. Moreover, combustion of the precipitated lignin may be used to fulfill the energy requirements of the process. Additional earnings can be also obtained by selling the hydrogen and CO_2 derived from fermentation. This process, aimed at producing transportation fuels, has important advantages over other fermentation processes, such as: 1) the low fractionation temperature minimizes the production of furfurals and other inhibitory products for fermentation; 2) it eliminates the need for conventional pretreatment and enzymatic hydrolysis of the pretreated lignocellulosic biomass; 3) *Clostridia* bacteria can naturally ferment C5 and C6 hemicellulose monosugars and oligomers.

The paper presents the experimental principles of the SEW fractionation and ABE fermentation procedures. Some preliminary results of the feedstock fractionation and fermentation tests are also included.

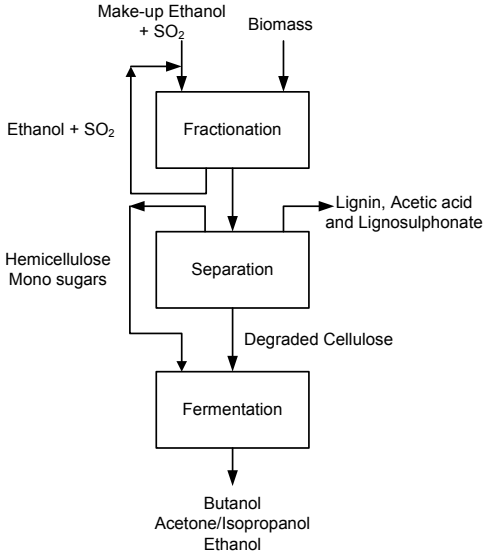

Figure 1: Flow diagram of the Bioforest process

EXPERIMENTAL

Raw materials and their chemical composition

The feedstocks used in the Bioforest process are green softwood (SW) and hardwood (HW) biomass, and deinked pulp (DIP). SW biomass was screened according to SCAN-CM 40:01, for removing the humus and needles (rejected through a 7 mm hole screen). In the initial fermentation trials, the spent liquor from pulping of industrial spruce chips (air-dried, screened to 2-4 mm thickness) has been used.

The chemical composition of the feedstocks was determined after milling in a Wiley mill, on a 30 mesh screen. The extractive content was determined according to SCAN-CM 49:03, using acetone as an extractant. The content of lignin and of the structural carbohydrates was analyzed by a two-stage acid hydrolysis procedure (72% H_2SO_4 at 30 °C for 1 h and 4% H_2SO_4 at 121 °C for 1 h), known as "Determination of structural carbohydrates and lignin in biomass" (NREL/TP-510-42618). An exception from the cited procedure was the determination of the acid soluble lignin (ASL) with a UV-VIS spectrophotometer at 205 nm wavelength, according to the following equation:

$$ASL = \frac{Absorbance \cdot Volume_{filtrate} \cdot Dilution}{Absorbtivity_{biomass} \cdot ODW_{sample}} \cdot 100 - 0.2$$

where ODW_{sample} – oven dry weight of the sample. The absorbtivity values used were 128 L $g^{-1}cm^{-1}$ for SW biomass, and 110 L $g^{-1}cm^{-1}$ for HW biomass and DIP. The ash content was determined at 550 °C, according to the "Determination of ash in biomass" procedure (NREL/TP-510-42622).

SEW fractionation of wood and spent liquor upgrading for fermentation

Fractionation of the feedstocks was carried out by the SO_2-ethanol-water (SEW) pulping process, also termed AVAP™, by the American Process Inc. (API). Fractionation was done in a silicon oil bath using 220 mL bombs. The fresh fractionation liquor was prepared by injecting gaseous sulfur dioxide into an ethanol-water solution. Deionized water and ethanol ETAX A (96.1 v/v %) were used. In the actual cooking liquor (including the water coming from the chips), the volume fraction of ethanol was of 0.55 and the concentration of SO_2 was 12% by weight. The liquor-to-wood ratio was of 6 L/kg. Fractionation, carried out at 135 °C (±1 °C), was continued for 80 min, including the heating-up period (about 10 min). After fractionation, the bombs were rapidly removed from the oil bath and cooled in cold water. The spent liquors were collected by squeezing the pulp suspensions contained in the washing socks and then analyzed for their carbohydrate composition through methanolysis, followed by gas-liquid chromatographic analysis.[10] The furfural and hydroxymethylfurfural (HMF) contents of the spent fractionation liquors were analyzed by HPLC (Dionex Ultimate 3000, Sunnyvale, CA, USA).

Prior to fermentation, the spent fractionation liquor was treated in a rotary evaporator to remove SO_2 and ethanol. About 60% of the original weight was evaporated to remove 98-99% of the ethanol and most of the free SO_2 present. SO_2 was captured in the vacuum gas line by absorption and oxidation to sulfate, using a solution of 1M NaOH and 0.5M H_2O_2. After evaporation, the concentrated spent liquor was neutralized by addition of $Ca(OH)_2$. The precipitate was removed by centrifugation (6000 rpm for 15 min) and washed twice with deionized water. Finally, the collected washing water was added to the neutralized upgraded liquor, which was then used in the fermentation stage.

Fermentation with corn starch

Fermentations were performed in a 2 L Braun Biostat MD fermenter (Germany), connected to a computer software (MFCS version 2.1), to monitor the process parameters. The corn starch (80 g/L of corn flour) was sterilized by autoclaving for 60 min at 126 °C. After the temperature was set to 37 °C and the stirring rate at 100 rpm, the fermenter was inoculated[11] with 100 mL of *Clostridium acetobutylicum* DSM 792 strain grown for 24 h in MSS media. Fermentations were run for 72-96 h, samples being taken over at regular intervals, to monitor product formation and substrate consumption. Several control tests were performed, to study the progress of solvent production. For these control tests, rye starch, as well as a mixture of pure monomer sugars (arabinose, galactose, glucose, mannose, xylose), were used. The corn and rye flours were commercial products from Venezuela and Russia. The monomers used for the sugar mixture were also of commercial origin. The control tests provided essential information on the selection of the proper conditions for further studies with wood liquors.

Fermentation with wood fractionation liquors

The liquor samples were autoclaved to achieve sterile conditions. The exponentially growing *C. acetobutylicum* bacterial culture on a MSS medium with glucose as a substrate was used to inoculate the wood liquor (in a 1:10 ratio). The studies were carried out with pure liquor, a mixture of liquor and corn starch, or a mixture of liquor and MSS medium, in different ratios (pure liquor, 1:1 liquor and MSS medium, 1:2 liquor and MSS medium). The fermentation recipients consisted of 125 mL airtight anaerobic glass bottles or glass test tubes, placed in an anaerobic chamber and incubated at 37 °C for 48-72 h. After this period, it was possible to determine – on the microscope – the evolution or lack of bacteria growth. The chemical composition of the liquor was determined by gas chromatography (Hewlett Packard HP 6850 Series, GC System, USA) and high pressure liquid chromatography (Alliance, Waters 2690 and 2695 Separations

Modules, Singapore), both prior to and after fermentation, to verify the fermentation product formation.

RESULTS AND DISCUSSION

Chemical composition of raw materials

The chemical composition after duplicate analysis of each feedstock is presented in Table 1. Overall mass balances of the chemical composition are close to 100%. The HW biomass has a higher content of acetyl groups and extractives compared to other feedstocks but, otherwise, the chemical compositions are somewhat comparable. The cellulose/hemicellulose ratio of the hardwood and softwood biomass is clearly lower than that of regular softwood. Also, the carbohydrate compositions of HW and SW are different, due to the different hemicelluloses present in each type of wood (*e.g.* galactoglucomannan is dominant in SW and arabino-4-O-Me-glucuronoxylan in HW). The ratio of pentoses and hexoses is, however, not so critical since *Clostridia* bacteria can ferment both. DIP differs from the other feedstocks, notably due to the high ash content derived from the inorganic fillers used in paper production. In addition, the lignin content of DIP is clearly lower compared to that of untreated wood biomass.

Since the chemical composition of the forest biomass varies considerably, both the fractionation and fermentation processes should be able to tolerate changes in feedstock composition. Moreover, the target of the process is to simultaneously fractionate biomass from different sources, so that an adequate feedstock supply can be obtained within an economic transport radius. However, separate experiments carried out with industrial spruce chips, SW and HW biomass and DIP have shown that, despite the differences in their chemical composition, they can be all efficiently fractionated under the same conditions. Regardless of the variation in feedstock composition, bacterial cells can also produce ABE solvents efficiently, as long as the amount of inhibitors is low enough. However, the variation in feedstock sugar composition affects the amount and ratio of the ABE solvents produced.

SEW fractionation of feedstocks

In SEW fractionation of spruce chips, the yield of the separated cellulosic fibers was of 51.8%. When fractionation is carried out under the above-presented conditions, these fibers represent a high quality pulp for papermaking.[12,13] However, the target in the present Bioforest process is to further decrease the solid yield by also degrading cellulose to oligomers, which are amenable to biochemical conversion by *Clostridia* bacteria. The results of the spent liquor carbohydrate composition analyses for each feedstock, presented in Figure 2, are calculated as averages of two to four spent liquors prepared under the same fractionation conditions, while the total carbohydrate amounts are presented as monosugars. After liquor upgrading, the total carbohydrate concentrations increase by a factor, as due to volume reduction after ethanol removal.

Table 1
Chemical composition of the feedstocks

Component	Spruce chips	SW biomass	HW biomass	DIP
	Average, %	Average, %	Average, %	Average, %
Extractives	2.5	2.7	3.9	0.8
Lignin	27.7	30.6	26.8	17.7
Acid insoluble	27.3	29.8	23.9	17.1
Acid soluble	0.3	0.8	2.8	0.6
Ash	0.4	1.4	0.9	14.8
Acetyl groups	1.2	1.5	4.1	0.4
Carbohydrates	71.9	64.4	62.9	69.5
Glucan	47.3	40.0	36.0	49.8
Mannan	14.2	10.3	2.2	8.7

Xylan	6.5	7.8	21.3	7.8
Galactan	2.9	4.3	2.1	2.0
Arabinan	1.0	2.0	1.3	1.3
TOTAL	103.6	100.5	98.5	103.0

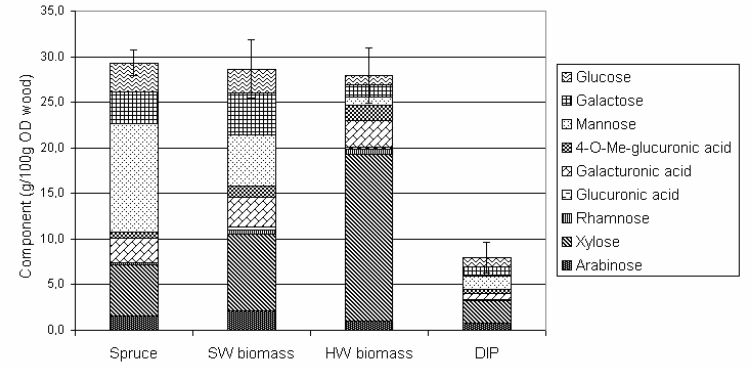

Figure 2: Chemical composition of SEW process spent liquors after fractionation at 135 °C for 80 min (SO_2 concentration – 12%)

Table 2
Chemical composition of SW biomass spent fractionation liquors produced under different fractionation conditions

Component (g/100 g OD wood)	SW biomass spent liquor		
	135 °C, 80 min	135 °C, 180 min	155 °C, 40 min
Arabinose	2.2	2.3	2.3
Xylose	8.4	8.7	7.7
Rhamnose	0.4	0.5	0.4
Glucuronic acid	0.4	0.4	0.4
Galacturonic acid	3.2	3.0	2.9
4-O-Me-glucuronic acid	1.2	1.6	1.5
Mannose	5.7	10.1	8.2
Galactose	4.6	6.2	5.6
Glucose	2.6	4.5	3.9
Total carbohydrates	28.7	37.4	32.9
STDEV	3.2	0.5	5.4
Furfural	0.21	0.39	0.34
Hydroxymethylfurfural	0.03	0.07	0.12

The biomass chemical composition differs somewhat from that of the reference spruce chips, yet, under the same fractionation conditions, a comparable sugar yield is obtained. The compositions of the spent liquors correlate well with those of the corresponding feedstocks. For example, the higher content of xylose, galactose and arabinose present in SW biomass, compared to spruce chips, is evidenced both in Table 1, presenting the chemical composition of the feedstocks, and in Figure 2, plotting the composition of the spent liquor. However, the standard deviation observed in the analyses and shown in Figure 2 was rather high, up to 11%, based on the average value.

The intensification of the fractionation process leads to the partial dissolution of cellulose, which results in further improvements in sugar yield. However, raising temperature and increasing pulping duration is known to increase the formation of unwanted hemicellulose degradation products, such as furfural, from pentoses, and hydroxymethylfurfural (HMF), from hexoses.[14] A few examples of intensified fractionation conditions and resulting spent

liquor compositions, including furfural and HMF contents, are provided in Table 2.

Expectedly, an increase in fractionation time or temperature results in an improved carbohydrate yield in the spent liquors; consequently, the content of furfural and HMF also increases. These compounds inhibit fermentation and furfural is stated[1] to be toxic for *Clostridia* above a concentration of 0.25% (v/v). Other presently unknown inhibitors may also originate from the bark and from other contaminants present in the biomass. Therefore, an adsorbent may be needed to permit the removal of inhibitors to an acceptable level prior to fermentation.

The techno-economical viability of the Bioforest process has been assessed and recommended as a profitable method for the production of biofuels. This technology is suitable especially for repurposing old pulp mills as biorefineries, thus notably reducing the investment costs of the process. Techno-economical analysis was based on the utilization of all sugars present in wood, with a solvent yield up to 40% of the total sugars. In ABE fermentation, a solvent concentration of 22-28 g/L is considered the threshold for an economical production.[15] Therefore, the *Clostridia* strain has to be developed to tolerate overall solvent concentrations up to 30 g/L.

Fermentation with corn starch and wood fractionation liquors

The results obtained from the fermentation on corn starch are displayed in Figure 3 and a comparison between different substrates used in the fermentations with *C. acetobutylicum* is shown in Table 3. The highest product yield attained, using corn starch, was of 39.6%. It may be concluded that this bacterial strain exhibits enzymatic (amylase) activity, capable of breaking the glucosidic bonds holding the starch subunits together. Therefore, glucose concentration first rises and then drops, as due to bond breaking and consumption, respectively. The highest production of solvents was achieved with corn starch.

Preliminary shake flask trials with wood liquor have indicated that the *Clostridia* cells will survive in the wood liquors. However, so far, they could not perform the required two-step fermentation under such growing conditions, which is a prerequisite for acetone-butanol-ethanol production by the *Clostridia* strain. Inhibition studies indicate that *Clostridia* cells can tolerate ethanol and acetate concentrations of up to 20 and 8 g/L, respectively (data not shown). Further studies on upgrading of liquors, regarding liquor compositions, product formation and inhibitor removal, are underway.

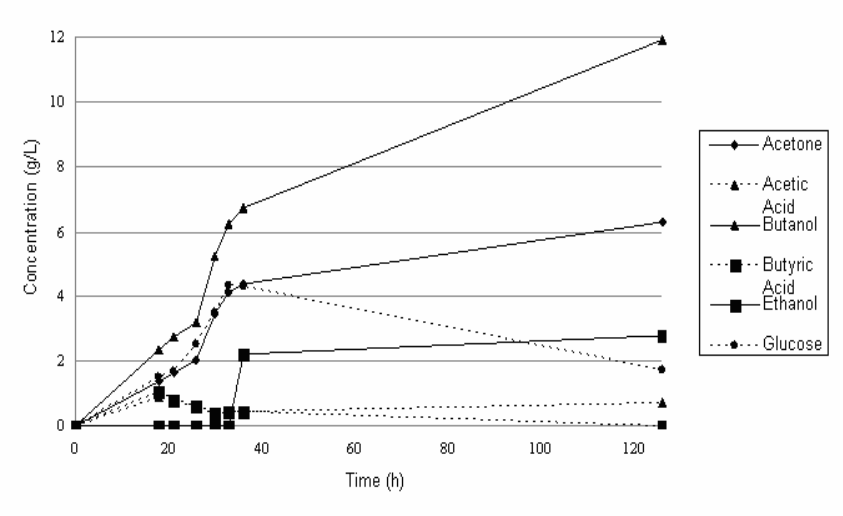

Figure 3: ABE fermentation with *C. acetobutylicum* on corn starch; product and substrate concentrations *vs*. time (solid lines represent ABE products)

Table 3
Comparison of final product concentrations using different substrates in ABE fermentation with *C. acetobutylicum*

Substrates	Final concentrations of products					
Substrate type and initial concentration	Acetic acid, g/L	Acetone, g/L	Butanol, g/L	Butyric acid, g/L	Ethanol, g/L	Total solvents, g/L
Glucose, 50 g/L	3.2	0.0	0.5	3.7	3.4	3.9
Mixture of sugars, 50 g/L	6.6	0.0	0.2	6.1	0.0	0.2
Rye flour, 90 g/L	0.9	6.5	11.0	0.4	4.3	21.9
Corn flour, 80 g/L	0.0	6.3	11.9	0.0	2.8	21.0

CONCLUSIONS

SEW fractionation is a promising technology for biorefineries based on lignocellulosic biomass. Initial experiments show that forest biomass can be efficiently fractionated under moderate conditions, and also that hemicelluloses are dissolved in the spent fractionation liquor at a high yield. Further feedstock development work to improve the sugar yield is in progress.

The bacterial strain, *C. acetobutylicum*, is capable of achieving high yields of solvents with different fermentation substrates; their ability to grow in the wood fractionation liquor and to utilize the sugar components has been also demonstrated. Further work is ongoing to optimize the fermentation of the conditioned liquor. During the fermentation of starch substrates, the highest solvent yield attained was of 39.6%. Further increases are expected after applying a product removal system. Also, extended studies will be performed with larger volumes of liquor, to determine the behavior of this bacterial strain, as well as its productivity on sugars derived from lignocellulosic biomass.

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