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BIOFUEL PRODUCTION FROM ACID-IMPREGNATED WILLOW AND SWITCHGRASS

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ABSTRACT: As part of a broader technical and economic feasibility study, we studied production of bioethanol from two types of lignocellulosic biomass by way of concentrated acid impregnation at low temperature. Willow chips and switchgrass were submitted to various impregnation techniques with concentrated sulfuric acid at varying acid: biomass ratios and impregnation times. Goal of the experiments was to investigate the technical feasibility of concentrated acid pretreatment technology as part of an industrial process that employs recycling of acid through biological means. Experimental results showed that significant amounts of fermentable sugars including glucose (up to 78% of max. obtainable glucose) and xylose can be obtained by relatively simple impregnation techniques at room temperature. Fermentation of willow-derived hydrolysates with *S. Cerevisiae* yielded 0.45 - 0.49 g ethanol/g glucose. Ethanol production rates however were 38% lower compared to standard glucose fermentation, prompting the need for further optimization to reduce the formation of acetic acid and furfural, two fermentation inhibitors. Novel impregnation techniques, including employment of sulfur trioxide, were also investigated but require more work to assess technical feasibility

Keywords: bio-ethanol, acid hydrolysis, biomass conversion

1 INTRODUCTION

The European market in biofuels for transportation is expected to increase considerably in the near future. Current EU policies stipulate a wider use of renewable transportation fuels within the next decade. In the Netherlands government policies are taking shape that aim at a 10% substitution of fossil fuels by the year 2020. As a result, there is a rapidly growing interest of the industry in the use of alternative feedstocks for ethanol production. As part of a broader technical and economic feasibility study, we studied production of bioethanol from two types of lignocellulosic biomass by way of concentrated acid impregnation at low temperature. Concentrated acid hydrolysis has gained renewed interest because of perceived disadvantages of other feedstock pretreatment routes including high enzyme costs, high energy use and considerable investment costs. Goal of the experiments was to investigate the technical feasibility of concentrated acid pretreatment technology as part of an industrial process that employs recycling of acid through biological means. Key research questions included optimal acid-to-biomass ratio required for significant conversion of (hemi-) cellulose into monomeric sugars, effect of acid hydrolysis techniques on fermentation yield and –kinetics, and technical feasibility of novel impregnation techniques.

2 MATERIALS AND METHODS

2.1 Feedstocks

Two feedstocks, willow chips and switchgrass, were submitted to various impregnation techniques with concentrated sulfuric acid at varying acid: biomass ratios and impregnation times. The willow was derived from a 3 year stand at the Oostwaardhoeve research farm in province of North Holland, the Netherlands and consisted of chips of 10-15 mm length en 5 mm thickness. Switchgrass *(Panicum Virgatum L.)* was derived from a commercial farm in the province of Groningen and consisted of stems that were cut by hand into 30 mm long parts. Samples of willow and switchgrass were also ground through a 2mm screen by using a knife mill. Compositional analysis using a modified TAPPI T249 cm-85 test method [1] showed that the willow consisted of 26.3% lignin, 38.5% glucan, 14.7% xylan, 5.7% uronic acids, 3.4% extractives and 1.5% ash. Switchgrass consisted of 21.6% lignin, 37.0% glucan, 28.2% xylan, 2.8% uronic acids, 2.3% extractives and 4.5% ash. The equilibrium moisture content of the feedstocks used were 14.4% and 9.4% (wet weight basis) for willow and switchgrass, respectively.

2.2 Concentrated acid hydrolysis

Two series of impregnation experiments were carried out with liquid sulfuric acid, with impregnation at room temperature and dry biomass at two particle sizes

(willow chips and switchgrass stems, and ground biomass samples as indicated above). In the first series, samples of dry biomass were placed in a 50ml polypropylene vial and a known quantity of 12 M (72% w/w) sulfuric acid was poured on top of the biomass. Subsequently, the vials were vibrated on a laboratory vortex-shaker for 1 minute to allow for spreading of acid through the biomass, and the vials were closed and left standing at room temperature for the duration of the impregnation period (24h). Following the impregnation, demineralized water was added to a dry matter content of 6.67%, and the vials were placed in a hot water bath (95°C) for three hours to complete hydrolysis of depolymerized biomass to monomeric sugars. After completion of the hydrolysis, the resulting hydrolysate was centrifuged, and samples of supernatant were analyzed for monomeric sugars on a Dionex HPLC equipped with a PA1 column. In addition, samples of supernatant were submitted for analysis of organic acids and 5-hydroxymethylfurfural (5-HMF) and furfural, two known fermentation inhibitors. The second series of experiments was carried out in a similar manner as the first series except that a larger amount of biomass was used, impregnation was performed in 500ml glass erlenmeyers and at varying times (1h, 4h, 16h), and hydrolysis was carried out at a dry matter content of 12%. No stirring was performed during impregnation and hydrolysis, except for the first minute after adding acid to the biomass to allow for spreading of acid through the material

A third series of impregnation experiments was carried out with sulfur trioxide. Samples of ground, dry biomass were sprayed with demineralized water to reach a moisture content of 50% (wet weight basis) and biomass was left standing a room temperature for 2 hours to allow for the water to impregnate throughout the biomass. Subsequently, the biomass was placed in a 50ml glass reaction vial and an open, 4ml hplc vial was placed on top of the biomass. After closing the 50ml reaction vial, a known quantity of liquid sulfur trioxide was injected through the cover of the reaction vial into the hplc vial, making sure that no direct contact was made between the biomass and the acid. The 50ml reaction vial was then placed in a heating block set at 35°C to allow for evaporation of the sulfur trioxide. At selected times intervals, the reaction vials were taken out of the heating block and photographed to inspect the extent of impregnation of sulfur trioxide into the wet biomass. After completion of the impregnation (6d), demineralized water was injected through the cover and hydrolysis was carried out as indicated above.

2.3 Fermentation

Eight 200ml samples of hydrolysate from the second series of impregnation experiments were used for ethanol fermentation tests with *Saccharomyces cerevisae*. Following pH adjustment of the hydrolysates with $Ca(OH)_2$ to 5.0, the hydrolysates were conditioned by using the method of Verduyn [2,3]. Per 100 ml hydrolysate the following components were added: 5 g/L $(NH_4)_2SO_4$, 3 g/L KH_2PO_4, 0,5 g/L $MgSO_4.7H_2O$, vitamins, trace elements, Tween 80 and ergosterol. Following the conditioning 6,3 g/L wet baker's yeast (=1,7 g Cell Dry Weight) was added. All fermentations were carried out at 32°C and 300 rpm stirring speed by employing a 'Biological Activity Monitor' fermentation system (Halotec, Delft, the Netherlands). During fermentation, cumulative CO_2 production volume and production rate was monitored, which was used as a measure of conversion rate of glucose into ethanol.

Table I: Glucan conversion, 1st impregnation series

Feedstock	Acid: biomass	Coarse [a] % [c]	Fine [b] % [c]
Willow	2 : 1	64.2	67.3
	3 : 1	64.9	69.7
	4 : 1	65.8	67.1
Switchgrass	2 : 1	47.5	78.8
	3 : 1	60.2	73.0
	4 : 1	59.0	67.9

[a] willow chips; switchgrass stems; [b] ground samples [c]

3 RESULTS

3.1 Conversion yield

Conversion yields of the 1st series of experiment at 24h impregnation time (Table I) show that 65-70% of glucan contained in the willow is converted into monomeric glucose, and that reducing the particle size of the wood chips (<2mm particle size) results in a minor increase in conversion yield. Glucan conversion for switchgrass ranged from 48 to 60% for stems, and 68 to 79% for ground switchgrass (<2mm). The lower conversion yield for switchgrass stems is believed to be due to sub-optimal contact of acid and biomass, as the switchgrass has a high volume to weight ratio in its native form. Glucan conversion yields also show that for both biomass feedstocks, increasing the acid-to-biomass ratio (on weight basis) does not increase the glucan conversion significantly. Conversion of xylan (Table II) at acid-to-biomass ratio of 2:1 ranged from 59 to 64% for willow and 57 to 71% for switchgrass. Results also show that at higher acid:biomass ratio, xylose conversion drops down which is likely due to decomposition of the monomeric sugar under very low pH conditions. These results show

Table II: Xylan conversion, 1st impregnation series

Feedstock	Acid: biomass	Coarse	Fine
Willow	2 : 1	59.1	63.6
	3 : 1	62.0	58.8
	4 : 1	54.7	44.0
Switchgrass	2 : 1	57.2	70.8
	3 : 1	46.0	56.7
	4 : 1	37.5	43.0

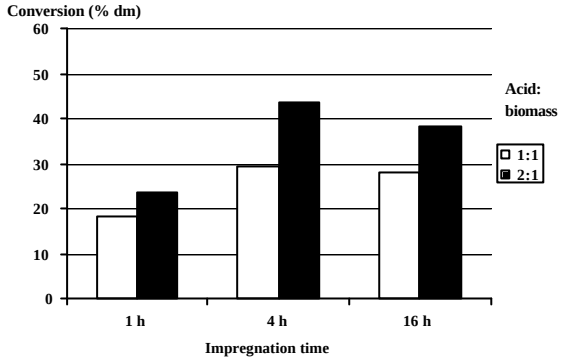

Figure 1: Dry matter conversion of acid impregnated willow chips into monomeric sugars

that the simple acid impregnation techniques lead to extensive conversion of cellulose and hemicellulose in both biomass types. Total dry matter conversion for the second series of impregnation experiments with willow chips at varying impregnation times (Figure 1) show that conversion yields vary from 18 to 24% for 1h impregnation, and 30 to 44% conversion (50 to 75% of total polysaccharides in the biomass) for 4hr impregnation. In addition, extending the impregnation time to 16h does not lead to higher conversion, but rather a small decline which is possibly due to sugar decomposition. In all cases the conversion yields are higher at 2:1 acid:biomass ratio.

3.2 Inhibitor concentrations

Analysis of the first series of experiments showed that acetic acid, levulinic acid and furfural concentrations in the hydrolysates all increased with increasing acid:biomass ratio. The highest concentrations (at acid:biomass of 4:1) were 3.3 g/L acetic acid and 1.95 g/L furfural derived from 67 g/L dry biomass. Acetic acid and furfural for the experiments with varying impregnation times (Table III; 120 g/L dry biomass) show concentrations of 5 to 8 g/L and 0.25 to 0.95 g/L, respectively. Here increasing the impregnation times did not lead to significant increase in acetic acid concentrations, but higher furfural concentrations.

3.3 Fermentation

Fermentation tests with the hydrolysates of willow gave good results indicated by complete conversion of glucose to ethanol, and ethanol yields (0.45 to 0.49 g ethanol per g glucose) were comparable to that of standard glucose fermentation. Measurements of ethanol production speed as indicated by on-line CO_2 monitoring showed slowing down of production rate for most hydrolysates, with the exception of willow impregnated for 4 h at 1:1 acid:biomass ratio. In all cases, initial CO_2 production rate was lower for willow hydrolysates which resulted in the fermentation taking longer to complete in comparison with standard glucose fermentation. The cause of ethanol production rate slowing down is believed to be due to the

Table III: Acetic acid and Furfural concentrations in willow hydrolysate, 2nd impregnation series

Acid: biomass	Impreg-nation time	Acetic acid (g/L)	Furfural (g/L)
1:1	1 h	4.8	0.31
	4 h	3.7	0.25
	16 h	8.2	0.55
2:1	1 h	5.0	0.96
	4 h	4.4	0.60
	16 h	5.0	0.94

inhibiting compounds in the hydrolysates. This hypothesis was tested by performing a similar fermentation of diluted hydrolysate where glucose concentration was increased upon dilution to its original concentration (i.e. resulting in lower concentration of inhibitors, but same concentration of glucose). In all cases, the diluted hydrolysates showed a higher production speed of ethanol compared to its undiluted counterpart. Although no conclusions can be drawn from these exploratory experiments on the level of inhibition from specific inhibiting compounds, fermentation speed generally increased from 25 to 78% by lowering the fermentation inhibitor concentrations.

3.4 Novel impregnation techniques

Inspection of the vials containing moist biomass and sulfur trioxide showed that in general, all SO_3 evaporated in a 16h period. Brown-black decoloration of the biomass indicated that SO_3 did impregnate into the biomass, however at a much lower rate of several days. Analysis of sugars after 6 days impregnation and 3 h hydrolysis did not show significant conversion into monomeric sugars, indicating that further development of this technique is required to assess its technical feasibility for complete hydrolysis of lignocellulose. It is likely that the exploratory experiments as described here did not lead to the required acid concentrations (12M) that is generally believed to be necessary for depolymerization of the lignocellulose.

4 CONCLUSIONS

Simple impregnation techniques with liquid sulfuric acid led to considerable conversion of lignocellulose into monomeric sugars, including glucose and xylose. The most optimal acid-to-biomass ratio from these experiments appears to be 2:1, although further optimization of the technique could lead to a decrease in acid consumption. Impregnation of willow chips for periods exceeding 4h did not lead to a higher conversion rate. In all experiments, formation of fermentation inhibiting components occurred, and acetic acid and furfural concentrations increased with increasing

acid:biomass ratio. Hydrolysates from acid-impregnated willow chips were fermentable under current inhibitor and yeast concentrations, although acetic acid and furfural concentrations did lead to some inhibition of Saccharomyces Cerevisae. More experimental work is required to assess the technical feasibility of novel acid impregnation techniques. The results as described in this paper provide key parameters for the design of a impregnation reactor for lignocellulosic biomass.

5 ACKNOWLEDGEMENT

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