

Pretreatment of Prevalent Canadian West Coast Softwoods Using the Ethanol Organosolv Process

Assessing Robustness of the Ethanol Organosolv Process

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ABSTRACT

The purpose of this master thesis was to examine the robustness of the ethanol organosolv process as a pretreatment method to produce ethanol from lignocellulosic material. A number of softwoods were pretreated at a single pretreatment condition shown to be effective on the pretreatment of Lodgepole pine (170°C, 60 min, 1.1% H₂SO₄ and 65% ethanol). The pulps were hydrolyzed with the enzyme loadings 5, 10 and 20 FPU/g cellulose at 2% consistency and with 10 FPU/g cellulose at 10% consistency. The results showed that the different consistencies in the examined range did not noticeably affect the hydrolysis yields. At 20 FPU the pulps hydrolyzed more or less the same and obtained near complete hydrolysis after 72 hours. At low enzyme loadings the pulps hydrolyzed different, indicating that at low enzyme loadings, the substrate was the limiting factor. The pulps had some differences in chemical composition concerning rejects and lignin content. The physical properties for the pulps were more or less the same. The pulp that hydrolyzed worst had the least amount of fermentation inhibitors indicating that the conditions should have been more severe for that pulp. To further examine why the pulps hydrolyzed different at low enzyme dosage they were washed in buffer and water at 50°C for 4 days. The hydrolysis yields from the washed pulps were improved significantly for all the pulps. The washes showed to contain significant amount of phenolic groups; however, these were not the reason for the improved conversion yields since Avicel was hydrolyzed in the washes from the pulps containing the phenolic compounds without having a reduced yield. A possible explanation for the improved yields is that the heat and the buffer removed surface physical barriers, thus increasing the accessibility for the enzymes.

INTRODUCTION

Biorefining to produce fuels from lignocellulosic biomass is viewed as a potential alternative and complement to the current use of petroleum oil. Ethanol fuel has shown to be particularly interesting mainly because of its ability to be used in internal combustion engines. The high consumption of fossil fuels has led to a dramatic increase in air pollution and CO₂ discharge. The CO₂ discharge from combustion of ethanol produced from trees is, on the other hand, being offset because of the growth of the trees.

Lignocellulose is an abundant, renewable and relatively cheap feedstock. Additionally, lignocellulosic material has no competitive use as human or animal foodstuffs, compared to other potential ethanol production feedstocks. Apart from cellulose, bioconversion to ethanol can be done using the feedstocks sugar or starch. The feedstocks need to be treated in their own different ways. Sugar can be fermented directly to ethanol. Starch and lignocellulosic material, on the other hand, need to be converted into

monomeric sugar before the fermentation to ethanol can occur. Today, Brazil and the USA are the two countries in the world that produce most ethanol for the transportation industry [1].

Currently the bioconversion of biomass to ethanol is only being performed at pilot-scale. One challenge that needs to be addressed before full-scale commercialization of bioconversion is the development of an effective and economical pretreatment method for the lignocellulosic feedstock [2]. The ethanol organosolv process is one of the methods investigated as a potential pretreatment for woody biomass. The process was originally designed to make clean biofuel for turbine generators and later developed into a pulping process. The process has not been extensively studied as a pretreatment for lignocellulose bioconversion but recent work has shown the method being suitable for especially softwoods.

The purpose of the project was to evaluate the robustness of the ethanol organosolv process to pretreat various softwood species from British Columbia, Canada. The organosolv process was

Table 1. The polymeric sugars, extractives and lignin content in the raw woods (% of dry material)

WOOD	Glu	Man	Xyl	Gal	Ara	Extractives	Acid-insoluble lignin	Acid-soluble lignin
Douglas fir (heartwood)	45.5	12.7	3.1	4.3	0.7	3.8	30.6	0.29
Douglas fir (sapwood)	43.1	11.8	2.6	2.4	0.8	1.0	30.7	0.30
Douglas fir (mixed)	44.6	12.5	3.4	2.5	1.0	2.0	29.1	0.31
Douglas fir (80 years old)	40.2	12.3	3.7	2.7	1.1	2.5	29.1	0.36
Western Hemlock (whole)	41.4	12.0	3.3	1.8	1.0	0.8	31.4	0.34
Western Hemlock (residual)	45.9	12.5	3.3	1.7	1.0	0.6	29.4	0.32
Lodgepole pine (beetle killed)	42.4	11.3	6.0	2.7	1.7	3.3	28.5	0.59
Mix spruce pine fir	52.0	13.8	7.7	3.2	1.9	3.2	27.0	0.35

performed under conditions that have shown to be effective for the pretreatment of Lodgepole pine [3].

MATERIAL AND METHOD

Raw Material

The trees being studied were Douglas fir, Western Hemlock, Lodgepole pine and a mixture of the softwoods spruce, pine and fir. The heartwood, sapwood and a mixture of the heartwood and sapwood from one Douglas fir were studied individually. That Douglas fir was around 150 years old. Another 80 year old Douglas fir tree was also studied. Two different Western Hemlocks were used, whole log and residual outer log, which is mostly sapwood. The Lodgepole pine being studied was killed by the mountain pine beetle and was in the grey phase. All the trees used were cultivated in British Columbia, Canada.

The woods were debarked, chipped and screened into a chip size of approximately $2.5 \times 2.5 \times 0.4$ cm. The chip size should be chosen as a compromise between the energy required and the cost to produce the chips and also the effectiveness of the pretreatment [2]. The sugar, extractive and the lignin content in the raw woods are shown in Table 1.

Pretreatment

Pretreatment of the lignocellulosic material is necessary to increase the accessibility of the cellulose to make it susceptible to enzymatic hydrolysis. A good pretreatment method should be effective on a wide range of lignocellulosic material. It should also require few preparation steps and ensure that the lignocellulosic material is recovered in a useable form. The organosolv process has not been developed significantly or optimized for softwoods but recent work has shown the method being suitable for the feedstock.

The raw wood chips were cooked in aqueous ethanol with sulfuric acid as catalyst. The ratio of liquor to wood was 1:7 (v/w). The cooking temperature was 170°C with the ramp time 54 minutes and the soak time 60 minutes. These conditions have shown to be effective for the pretreatment of Lodgepole pine [3]. The pretreatment conditions should be chosen as a compromise between separating the lignin and hemicellulose from cellulose and maximizing the recovery of the carbohydrates.

After the cooking the vessels were cooled down to room temperature in a water bath. The pulp and liquor were separated using a nylon mesh and the pulp was washed with ethanol and water. The washes were combined with the liquid fraction. The pulp was homogenized in a disintegrator for 5 minutes and passed through a laboratory flat screen with 0.203 mm slits to remove rejects. The pulp was then vacuum filtered and stored at 4°C for further analysis.

Enzymatic Hydrolysis

The hydrolysis is not only being influenced by the effectiveness of the enzymes but also by the chemical, physical and biological characteristics of the biomass substrates.

The hydrolysis reaction was performed at a total volume of 15 ml. Amounts of distilled water, depending on the moisture content of the pulps, were added to the hydrolysis flasks. 0.25 M buffer consisting of CH₃COOH and CH₃COONa with pH 4.8 and an amount of antibiotic stock solution to stop possible infections were also added. The hydrolysis were performed at the enzyme loadings 5, 10 and 20 FPU/g of cellulose in the substrate at 2% consistency and at the enzyme loading 10 FPU/g cellulose at 10% consistency. The enzymes being used were celluclast and beta-glucosidase and were provided by Novozymes. Samples for determination were taken at 12, 24, 48 and 72 hours.

Table 2. The sugar and lignin remaining in the pulps after the organosolv pretreatment (% of starting content)

Pulp	Glucan	Mannan	Galactan	Acid-insoluble lignin	Acid-soluble lignin
Douglas fir (heartwood)	82.1	7.1	0.3	33.5	55.1
Douglas fir (sapwood)	97.2	4.0	0.4	34.3	57.6
Douglas fir (mixed)	89.7	3.2	0.6	34.8	53.5
Douglas fir (80 years old)	99.3	5.3	1.2	53.6	58.3
Western hemlock (whole)	90.3	8.1	1.1	46.7	42.6
Western hemlock (residual)	87.2	5.1	0.9	44.6	51.3
Lodgepole pine (beetle killed)	94.0	5.8	0.8	43.8	35.0
Mix spruce pine fir	89.5	4.8	0.8	54.6	61.7

Analytical Procedure

The extractives in the woods were determined according to the procedure of TAPPI (Technical Association of Pulp and Paper Industry) standard method T280 pm-99 using acetone as solvent. The sugars and the lignin content of the untreated woods and the pretreated pulps were analyzed using a modified Klason method inferred from TAPPI standard method T222 om-88. The ash content in the woods and pulps was determined according to TAPPI standard method T211 om-85. Simons stain was used to determine the accessible area of the fibers and a fiber quality analyzer determined the fiber length and coarseness. The monomers and oligomers in the liquid fraction were analyzed according to a paper by Ruiz et al. [4]. The amounts of the fermentation inhibitors furfural and 5-hydroxymethyl furfural in the liquid fraction were analyzed using a Dionex HPLC system.

The lignin in the liquid fraction was also calculated after being precipitated, dried and weighted.

RESULTS AND DISCUSSION

Recovery

The key reasons of pretreatment are to recover the lignocellulosic material in a useable form and to break down the lignin to molecule fragments small enough to dissolve in the organic liquor. The amounts of sugar and lignin that are remaining in the pulps after the pretreatment are shown in Table 2. The amount of xylan and arabinan was negligible in all the pulps.

Concerning the recovery of the pretreatment,

the amounts of glucan being recovered in the solid fraction after the organosolv process varied between 82% and 100%. For the acid-insoluble lignin between 33% and 55% of the initial lignin was remaining in the pulps. For the acid-soluble lignin the remaining amounts varied between 35 and 62% of the initial amount. Concerning the hemicellulose, one drawback with the organosolv process today, is that a lot of hemicellulose is relegated to the liquid fraction, which also could be seen from this study.

Enzymatic Hydrolysis

The cellulose-to-glucose conversion yields during the hydrolysis at different enzyme loadings and consistencies are shown in Figure 1.

The hydrolysis at 5 and 10 FPU/g cellulose showed low cellulose-to-glucose conversion yields for the whole Western Hemlock (21% and 48% respectively after 72h) and the Douglas fir heartwood (19% and 62% respectively). The beetle killed Lodgepole pine showed high conversion yield (75% and 100% respectively after 72h) and so did the Douglas fir sapwood (58% and 93% respectively). At the enzyme loading 20 FPU/g cellulose the pulps hydrolyzed more or less the same and obtained near complete hydrolysis. This indicated that at low enzyme loadings, the substrate was the limiting factor. The hydrolysis at 10% consistency and 10 FPU/g cellulose showed more or less the same result as the hydrolysis at 2% consistency and the same enzyme loading. This showed that the consistencies in the examined range did not affect the hydrolysis conversion yields noticeable.

Table 3. The sugar and lignin content in the pulps (% of dry material)

Pulp	Glucan	Mannan	Xylan	Galactan	Acid-insoluble lignin	Acid-soluble lignin
Douglas fir (heartwood)	79.4	1.9	n.d	0.03	21.8	0.34
Douglas fir (sapwood)	82.4	0.9	n.d	0.02	20.7	0.34
Douglas fir (mixed)	82.2	0.8	n.d	0.03	20.8	0.34
Douglas fir (80 years old)	74.1	1.2	0.1	0.06	29.0	0.39
Western hemlock (whole)	74.8	2.0	0.4	0.04	29.3	0.29
Western hemlock (residual)	78.1	1.2	n.d	0.03	25.5	0.32
Lodgepole pine (beetle killed)	79.0	1.3	0.9	0.04	24.8	0.41
Mix spruce pine fir	77.6	1.1	0.5	0.04	24.6	0.36

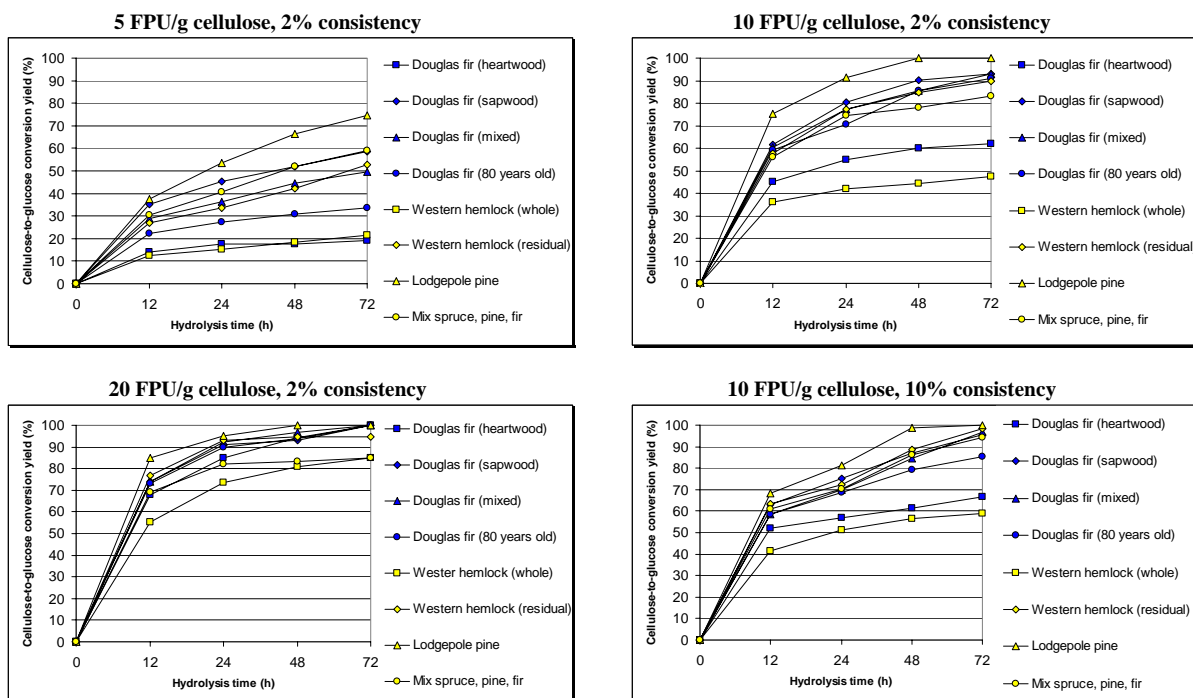


Figure 1. The cellulose-to-glucose conversion yields for the hydrolysis

Chemical Properties

The composition of the pulps is shown in Table 3.

The pulps were composed of between 74% and 83% glucan. The amount of glucan in the pulp should not have affected the hydrolysis yield since the enzyme dosage was added per gram cellulose. The highest amount of xylan was found in the beetle killed Lodgepole pine (0.9%), which showed the highest hydrolysis yield and therefore it is assumed that the small amounts of xylan did not affect the hydrolysis yield noticeable. The content of mannan differed among the pulps at the range from 0.8% to 2.0%. The Douglas fir heartwood and the whole Western Hemlock had the highest amounts with 1.9% and 2.0% respectively. How mannan affects enzymatic hydrolysis is not known but it could possibly work as an inhibitor and reduce the conversion yield. However, these differences were probably too small to have had any influence on the hydrolysis yields. Overall, the content of hemicellulose was similar for all the pulps. Lignin is known to inhibit enzymatic hydrolysis and to decrease the rate and yield. The amounts of acid-insoluble lignin in the pulps were varying between 20% and 30%. The whole Western Hemlock had the highest lignin content (29.3%) which might have been one of the reasons for its lower conversion yield. However, the Lodgepole pine and the residual Western

Hemlock, which hydrolyzed relatively well, had a lignin content of approximately 25%, which was not that much lower than for the whole Western Hemlock. Concerning the acid-soluble lignin, the amounts varied between 0.29% and 0.41%. The Lodgepole pine had the highest content and the whole Western Hemlock had the lowest, which indicated that the amount of acid-soluble lignin did not affect the hydrolysis conversion yield.

The content of the fermentation inhibitors furfural and 5-hydroxymethyl furfural in the liquid fraction is shown in Table 4.

Table 4. The content of furfural and HMF in the liquid fraction (g/100g raw material)

Liquor	Furfural	HMF
Douglas fir heartwood	0.21	0.56
Douglas fir sapwood	0.18	0.45
Douglas fir mixed	0.20	0.36
Douglas fir (80 years old)	0.21	0.41
Western hemlock whole	0.10	0.17
Western hemlock residual	0.21	0.38
Lodgepole pine	0.34	0.41
Spruce pine fir	0.37	0.43

The whole Western Hemlock, which hydrolyzed worst, had the lowest content of inhibitors. Since the pretreatment conditions are a compromise between getting a substrate with good fermentability and good material recovery (low severity) and a substrate that is well hydrolyzed

Table 5. The cellulose-to-glucose conversion yields for the washed and the unwashed pulps after 72 h

Pulp	Washed	Not washed	Difference
	5 FPU, 2% consistency	5 FPU, 2% consistency	
Douglas fir (heartwood)	45 %	19 %	26 %
Douglas fir (sapwood)	75 %	58 %	17 %
Douglas fir (mixed)	72 %	50 %	22 %
Douglas fir (80 years old)	54 %	34 %	20 %
Western hemlock (whole)	31 %	21 %	10 %
Western hemlock (residual)	74 %	53 %	21 %
Lodgepole pine (beetle killed)	92 %	75 %	17 %
Mix spruce pine fir	65 %	59 %	6 %

at low enzyme loadings (high severity), perhaps the pretreatment conditions for the whole Western Hemlock should have been more severe.

Concerning the compositions in the raw woods it is worth mentioning that the Douglas fir heartwood had the highest content of acetone extractives, which is known to inhibit enzymatic hydrolysis.

Physical Properties

The results from the Simons stain analyzing the accessible area of the fibers showed that almost the same amounts of orange and blue dyes were absorbed for all the pulps. The dyes compete for accessing the pores but since the larger orange dye has higher affinity, it displaces the blue dye if the pore is large enough. However, the results from the analysis showed that the accessible area of the substrates was similar and therefore it could be eliminated as a reason for the different hydrolysis yields. The fiber quality analyzer measured the fiber length and coarseness of the pulps. The results showed that the Douglas fir heartwood and Douglas fir sapwood had long fibers compared to the other pulps. The heartwood hydrolyzed poorly but the sapwood hydrolyzed well and therefore the fiber length could also be eliminated as a reason for different hydrolysis yields. Concerning the coarseness the results were more or less the same for the analyzed pulps.

Washed Pulp

Since the cellulose-to-glucose conversion yields differed a lot between the pulps at low enzyme loadings, the content of possible compounds that might have inhibited enzymatic hydrolysis were analyzed using an UV-visible spectrophotometer. The pulps were washed with 0.25 M buffer consisting of CH_3COOH and CH_3COONa with pH 4.8 for 4 days in an incubator at 50°C. The samples were then centrifuged and the supernatant and the solid were separated. The supernatant was detected with visible light at wavelengths from 200 to 800 nm. Between the wavelengths 200 and 300 nm the absorbance of

the liquors differed from each other indicating that the pulps contained different sorts and amounts of compounds that might have inhibited the enzymatic hydrolysis. Therefore, another hydrolysis of washed pulp was made to examine if the cellulose-to-ethanol conversion yields differed from the hydrolysis of unwashed pulp.

The pulps were washed for 4 days in buffer with subsequent water washing. The washed pulps were then prepared for hydrolysis at 2% consistency and the enzyme loading 5 FPU/g cellulose. For this hydrolysis the conversion yield was only analyzed after 72 hours. The results were compared to the former hydrolysis of the unwashed pulp at the same consistency and enzyme loading. The comparison is shown in Table 5. The hydrolysis yields showed to improve significantly after the pulps were washed. The Douglas fir heartwood showed the highest conversion improvement from 19% to 45% and the beetle killed lodgepole pine showed 92% cellulose-to-glucose conversion.

Softwoods are known for their high content of extractives, which can inhibit enzymatic hydrolysis. Therefore, the content of polyphenolic compounds, which are aromatic extractives, was analyzed in the washes using the Prussian blue method [5]. The results are shown in Table 6.

Table 6. The content of polyphenolic groups in the pulps (groups/g pulp)

Pulp	Polyphenols
Douglas fir heartwood	9.06×10^{18}
Douglas fir sapwood	8.36×10^{18}
Douglas fir mixed	7.47×10^{18}
Douglas fir (80 years old)	11.63×10^{18}
Western hemlock whole	11.41×10^{18}
Western hemlock residual	11.45×10^{18}
Lodgepole pine	10.37×10^{18}
Spruce pine fir	11.73×10^{18}

The determination showed that all the washed liquors contained significant amounts of polyphenolic groups. To determine whether the released compounds had any influence on the hydrolysis yield Avicel, which is pure microcrystalline cellulose, was hydrolyzed in the

liquor from washed pulps, containing the polyphenols and other compounds that might have been released during the wash. Avicel showed the same conversion yield independent of the liquor it was hydrolyzed in, which indicated that the polyphenolic compounds did not play a role in inhibiting hydrolysis. A possible explanation could be that the polyphenolic molecules are too large and could constitute a stereochemical hindrance.

A possible explanation or contribution to the improved yields is that during the wash, the buffer and the heat removed surface physical barriers, thus increasing the accessibility for the enzymes.

Final thoughts

The beetle killed Lodgepole pine did not show any remarkable data with respect to the chemical and physical properties explaining why it hydrolyzed so well. However, the pretreatment conditions chosen for the current study were based on a previous study by Pan et al. from 2007 where optimal conditions for beetle killed Lodgepole pine were examined, which might explain the high hydrolysis yields. The conditions in that study are probably not optimal for the other trees and that needs to be considered when choosing a pretreatment method for a number of different tree species.

CONCLUSIONS

A conclusion taken from the current study is that organosolv is robust considering that the pretreatment process created pulps from all the species tested, although there were differences in pulp yields, rejects and some of the chemical properties. The pulps had similar physical properties considering accessible area, fiber length and coarseness.

The organosolv process is not very robust considering the differences in hydrolysis yields at low enzyme loadings for the studied softwoods. At high enzyme loading all the substrates had near complete hydrolysis,

indicating that at the substrate was the limiting factor at low enzyme dosages. The whole Western Hemlock had a lot of rejects and high lignin content with lowest amounts of fermentation inhibitors indicating that the pretreatment conditions for that wood should have been more severe. The differences in hydrolysis due to lignin content were not completely evident as the beetle killed Lodgepole pine still hydrolyzed well although having relatively high lignin content. The pulps did not show any significant differences in hemicellulosic sugar content.

The pulps did contain significant amounts of polyphenolic compounds. However, the reason for the improved hydrolysis yields after the pulps were washed was not due to the removed phenolic groups but something else in the chemical or physical properties of the pulps.

REFERENCES

- [1] **Michael Himmel**, Enzymatic conversion of biomass for fuels production, American Chemical Society, (1994)
- [2] **R.P Chandra, R. Bura, W.E Mabee, A. Berlin, X. Pan and J.N Saddler**, Substrate pretreatment: The key to effective enzymatic hydrolysis of lignocellulosics, (2007)
- [3] **Xuejun Pan, Dan Xie, Richard W. Yu, Dexter Lam and Jack N. Saddler**, Pretreatment of lodgepole pine killed by mountain pine beetle using the ethanol organosolv process: Fractionation and process optimization, (2007)
- [4] **Raymond Ruiz and Tina Ehrman**, Dilute acid hydrolysis procedure for determination of total sugars in the liquid fraction of process samples, (1996)
- [5] **Horace D. Graham**, Stabilization of the Prussian blue color in the determination of polyphenols, (1992)