

Influence of Acetic Acid as a Catalyst in Ethanol Organosolv Pretreatment of Wheat Straw for the Production of Colloidal Lignin Particles and Enzymatic Hydrolysis

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Ethanol organosolv pretreatment of lignocellulosic biomass produces high quality lignin and an enriched cellulose fraction while enabling a completely renewable-based process. However, optimization of this process is still necessary to be economically competitive. During autocatalytic pretreatment, acetic acid is released from hemicellulose and acts as catalyst for delignification. In this study, up to 4.4 wt% of acetic acid was added to ethanol organosolv pretreatment of wheat straw to determine its influence on delignification, enzymatic hydrolysis, and lignin yield, and compare the results to autocatalytic pretreatment. The results show that the addition of acetic acid significantly improves all of these parameters. Up to 48.5 % of delignification were achieved when 4.4 wt% of catalyst was added compared to 40.5 % in autocatalytic pretreatment. Glucose release in enzymatic hydrolysis slightly increased from 52.0 to 56.7 %, and a maximum lignin yield of 36.1 % was reached compared to 23.2 % when no catalyst was added. However, the addition of more than 0.6 wt% catalyst resulted in significantly darker lignin and a drastic increase in particle size after precipitation of the extracts into colloidal lignin particles. It can be concluded that acetic acid offers a promising possibility to improve ethanol organosolv pulping while maintaining a completely renewable-based process.

1. Introduction

The transition away from fossil resources requires a more holistic utilization of renewable resources for materials to replace fossil-based materials, as well as consideration of raw materials that are currently only marginally used. Wheat straw is a lignocellulosic raw material that is abundantly available, showing potential as a feedstock in a lignocellulose biorefinery (G et al., 2021). One of the major components of lignocellulosic biomass is lignin, which is currently underutilized as a material, but has shown potential for material applications like carbon fibres (Souto et al., 2018) or polymer blends (Choi et al., 2021). Conversion of lignin into colloidal lignin particles opens up further applications like sunscreens, Pickering emulsions, or food additives (Beisl et al. 2017). Compared to conventional pulping processes, ethanol organosolv pretreatment has the benefits of producing sulphur-free lignin of high purity (Gordobil et al., 2018) and biomass suitable for enzymatic hydrolysis to produce fermentable sugars (Zhao et al., 2009). However, the process faces some obstacles due to the comparatively expensive solvent and necessary ethanol recovery (Galbe and Wallberg, 2019). New ways for process improvement are necessary to improve its economic competitiveness. Sulfuric acid is commonly added as an acidic catalyst, increasing delignification efficiency, but introducing sulphur back into the process, while also enhancing production of sugar degradation products that are inhibitory to fermentation by microorganisms (Palmqvist et al., 1999). In auto-catalytic pretreatment, acetic acid derived from hemicellulose side-chains acts as catalyst hydrolyzing carbohydrates and lignin (McDonough, 1993). Consequently, addition of acetic acid as a catalyst in ethanol organosolv pretreatment could potentially improve delignification and enzymatic digestibility in a sulfur-free, renewable based process, while still providing high quality lignin.

Mondylaksita et al. (2020) compared the performance of sulfuric acid and acetic acid as catalysts and found that significantly higher concentrations of acetic acid are required for results comparable to sulfuric acid

concerning lignin recovery and purity. The present study investigates how the addition of acetic acid as a catalyst in ethanol organosolv pretreatment affects the composition of residual solids and liquor, as well as enzymatic hydrolysis and production of colloidal lignin particles. Wheat straw was pretreated using ethanol organosolv extraction with addition of varying concentrations of acetic acid as a catalyst. The solids and liquids were characterized on composition, specifically carbohydrates, lignin, degradation products in the liquor, and ash in the solids. Enzymatic hydrolysis of solids was carried out, as well as production of colloidal lignin particles from the liquors by solvent-shifting precipitation, which was only carried out from autocatalytic extracts in previous works (Beisl et al., 2018).

2. Materials and Methods

2.1 Materials

The wheat straw used in this work was harvested in Lower Austria in 2015. The particle size was reduced by milling in a cutting mill equipped with a 2 mm round hole sieve. Ethanol (96 %), acetic acid (99.8 %) and distilled water were used in the pretreatment. The composition of the wheat straw was 19.24 % lignin, 32.51 % glucan, 19.77 % xylan, 2.68 % arabinan, and 9.39 % ash.

2.2 Extraction

A 600 mL stainless-steel reactor (Parr Instruments Co, Moline, IL, USA) was used for the organosolv extractions. 30 g of wheat straw (based on dry matter) and 330 g of solvent were used for every extraction. The solvent consisted of 60 wt% aqueous ethanol and four different concentrations of acetic acid (0, 0.6, 2.2 and 4.4 wt% of the solvent); two extractions were carried out for every level of acetic acid. The mixture of biomass and solvent was heated up to 180 °C for 60 min and then quickly cooled to room temperature with a water bath and cold water circulating through an internal serpentine coil. After the pretreatment, the liquid fraction was separated from the straw with a hydraulic press. The solid fraction was washed with 330 g of 60 wt% aqueous ethanol followed by a second washing step with 660 g of water to remove both phenolic compounds and residual sugars. The extracts were centrifuged at 6,000 rpm for 20 min to remove suspended solids, after which the pH value of the solids-free extracts was determined.

2.3 Analytics

Analysis of dry samples

A sample of the washed and pressed solids was dried at 105 °C to determine the moisture content, another sample was taken and stored at 4 °C for enzymatic hydrolysis. The rest of the solids was dried at 45 °C for 48 h to determine the pulp yield as the difference of dry straw mass before and after pretreatment. Afterwards, the solids were analysed according to methods from the National Renewable Energy Laboratory (NREL) on their content of lignin, carbohydrates (Sluiter et al., 2008b), and ash (Sluiter et al., 2008a). The acid soluble lignin (ASL) was determined by measuring the absorption of the filtrate after the hydrolysis at 320 nm.

Enzymatic hydrolysis

Enzymatic hydrolysis of the solids was carried out according to a method from the NREL (Selig et al., 2008). The pretreated solids were incubated at an initial solids content of 5 wt% with 0.05 M citric acid and tri-sodium citrate buffer at pH 5.5 and an enzyme dosage of 10 % (w/w biomass) (Cellic CTec2, Novozymes A/S, Bagsværd, Denmark). For all extractions, enzymatic hydrolysis was carried out in duplicate, including a biomass and enzyme blank test. As results, the percentage of glucose and xylose released from the biomass after 72 h of enzymatic hydrolysis at 50 °C and 180 rpm are reported.

Extract analysis

The extract was analyzed on its dry matter content. The content of monomeric sugars, acetic acid, formic acid, levulinic acid, hydroxymethylfurfural (HMF), and furfural was determined by HPLC (Agilent 1110 series, Waldbronn, Germany) with a Aminex HPX-87H column (Bio-Rad, Hercules, USA) using 5 mM sulfuric acid as eluent. For the determination of the total sugars, the extract was hydrolyzed for 1 h at 121 °C at a sulfuric acid content of 4 wt%. Glucose, xylose, arabinose, and acetic acid were detected by a refractive index detector, furfural and HMF were determined by a UV-detector at 280 nm wavelength.

Lignin precipitation

Lignin was precipitated from the extracts by addition of four amounts of water. The precipitate was incubated for 2 h at 30 °C and shaken at 100 rpm for equilibration, afterwards the solids were separated by centrifugation for 20 min at 6,000 rpm. The supernatant was decanted, the solids were dried for 72 h at 45 °C and weighed to

determine the precipitation yield. The ethanol organosolv lignin (EOL) yield was calculated based on the lignin content of the original wheat straw. Additionally, the extracts were precipitated into colloidal lignin particles in a T-mixer set-up as described by Beisl et al. (2018). In short, the extracts were mixed with deionized water in a volumetric ratio of 1:5 at a flow rate of 112.5 mL/min. The hydrodynamic diameter of the resulting particles was determined by dynamic light scattering using an Anton Paar Litesizer 500 (Graz, Austria).

3. Results and Discussion

Ethanol organosolv pretreatment was applied to wheat straw at four levels of acetic acid concentration. The composition of the original and pretreated straw is shown in Table 1.

Table 1: Composition of wheat straw before and after pretreatment

Acetic acid concentration (wt%)	Lignin (%)	Glucan (%)	Xylan (%)	Arabinan (%)	Ash (%)
0 %	15.25	44.03	24.16	2.57	4.78
0.6 %	15.64	45.67	24.08	2.15	4.27
2.2 %	15.16	47.65	23.03	1.78	3.99
4.4 %	14.75	49.82	22.60	1.62	3.94
Untreated straw	19.24	32.51	19.77	2.68	9.39

While the lignin content is significantly lower after pretreatment, there is no significant correlation of the residual lignin content of the pretreated straw and the catalyst concentration. The results also show that a higher content of acetic acid in the solvent leads to lower pulp yields (Figure 1). Combined, this results in higher delignification at increased acetic acid concentrations. However, there is only small change in both pulp yield and delignification when 0.6 wt% of catalyst are added compared to auto-catalytic pretreatment. This indicates that acetic acid only becomes effective for delignification at higher concentrations, suggesting a threshold concentration below which the catalyst has little effect. Goh et al. (2011) found that sulfuric acid only showed a strong effect on delignification above a content of 1 wt% based on solids, which suggests that significantly higher levels are necessary if a weaker acid like acetic acid is used instead. Mondylaksita et al. (2020) compared sulfuric acid and acetic acid as catalysts in ethanol organosolv pretreatment. They found that there was no significant difference in lignin recovery when the pH in the solvent was set to the same value, while sulfuric acid led to higher recovery when the same concentration of catalyst was added. This fits together with the findings of this work that a higher threshold concentration of acetic acid is necessary for a significant impact on pulp yield or delignification.

The composition of the pulps shows that the content of the hemicellulose-based carbohydrates xylose and arabinose significantly increases after pretreatment compared to the original straw but decreases with the concentration of acetic acid (Table 1). Consequently, the glucan content of the pulps, which significantly increases after pretreatment, shows a positive correlation with the catalyst concentration.

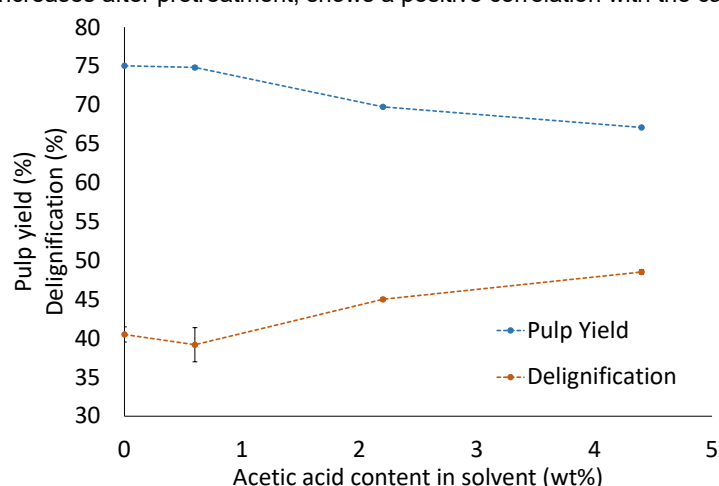


Figure 1: Pulp yield and delignification at different acetic acid contents

3.1 Enzymatic hydrolysis

Enzymatic hydrolysis of the pretreated straw was carried out to determine the potential as a carbohydrate source. The results show that the saccharification yield of both glucan and xylan is improved by addition of acetic acid to the solvent (Figure 2). The correlation has a similar trend to the delignification, which further indicates a threshold concentration of acetic acid and shows that effective delignification is beneficial for enzymatic hydrolysis. However, there is only a small increase in the percentage of both glucan and xylan from 2.2 to 4.4 wt% of acetic acid. This suggests that addition of higher acetic acid concentrations would not lead to a significant improvement in enzymatic digestibility, despite the increased delignification, making the application of acetic acid questionable if enzymatic hydrolysis of glucan is the main objective.

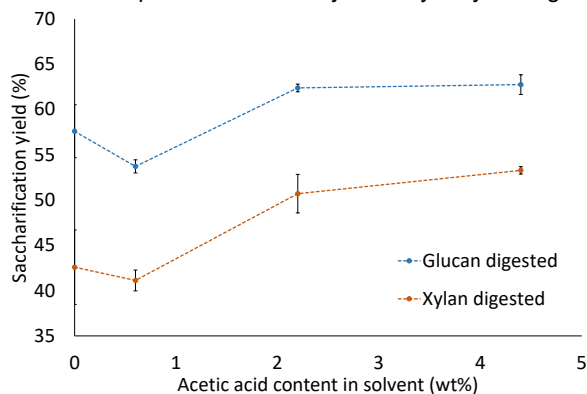


Figure 2: Saccharification yield of glucan and xylan at different levels of acetic acid in the solvent

3.2 Liquor composition

Figure 3 shows the concentration of carbohydrates and furfural in the liquors obtained after pretreatment. The content of HMF was below the quantification level in all samples, which fits together with previous works finding significantly less HMF than furfural in wheat straw organosolv extracts (Adamczyk et al., 2021), and can be connected to the prevalence of the C5 carbohydrates xylose and arabinose in the hemicellulose of the straw. Concerning furfural, there is a linear increase with the amount of acetic acid added, which can be explained with the acid catalysed formation of furfural (Choi et al., 2019). Contrary to that, the content of solubilized carbohydrates has a considerable increase from autocatalytic pretreatment to 0.6 wt% of acetic acid but seems to flatten at higher levels of catalyst. The concentration of glucose is significantly lower than those of xylose and arabinose and only has a small increase, which can be attributed to the higher resistance of cellulose to hydrolysis.

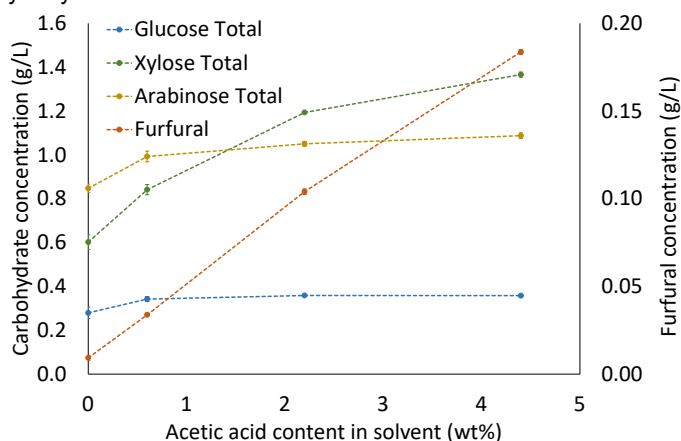


Figure 3: Liquor composition at different acetic acid contents

3.3 Lignin Precipitation

Figure 4a shows that the addition of acetic acid in pretreatment results in a significant increase in the yield of lignin precipitating when water is added to the liquor. This can be attributed to both the higher lignin content in

the extracts due to increased delignification, and the lower pH value resulting in significantly lower lignin solubility after the addition of water. Interestingly, the yield of precipitated lignin (based on the lignin present in the original straw) reaches its highest value at 2.2 wt% of acetic acid in the solvent mixture and is slightly lower when 4.4 wt% are added. This contrasts with the continuous increase in delignification with the acetic acid concentration. A possible explanation could be increased acid catalyzed depolymerization of lignin (McDonough, 1992), which could result in smaller lignin molecules with higher solubility (Adamczyk et al., 2021) at the highest acetic acid concentrations, and a lower yield of precipitating lignin.

The lignin dissolved in the extracts was also precipitated into colloidal lignin particles by addition of water in a T-Mixer. The diameter of the resulting particles shows a strong dependence on the content of acetic acid in the solvent (see Figure 4a). Addition of 0.6 wt% acetic acid to the solvent already resulted in an increase in particle diameter by 83 %, further increase of the catalyst content results in an increase by an order of magnitude. A previous work (Beisl et al., 2020) has shown that the zeta-potential of lignin particles increases with decreasing pH, resulting in agglomeration and bigger particles, which can explain the strong increase in particle size with increasing catalyst concentration.

Figure 4b shows that the precipitated lignin also becomes darker with increased acetic acid content, which can be undesired depending on the intended application. A previous study (Adamczyk et al., 2021) investigated the influence of increased pretreatment temperature on organosolv lignin and found that the lignin became darker at higher temperatures, which was attributed to an increase in condensation reactions at higher severities. Since a higher catalyst concentration also results in higher pretreatment severities, this could also explain the darker lignin at higher acetic acid contents found in this work. Reverberi et al. (2020) studied formation of carbon nanoparticles using carbohydrates as precursors and found increased colorization of the resulting suspensions with increased reaction times. They attributed this to ongoing caramelization, which could also be a contributing factor in the present study.

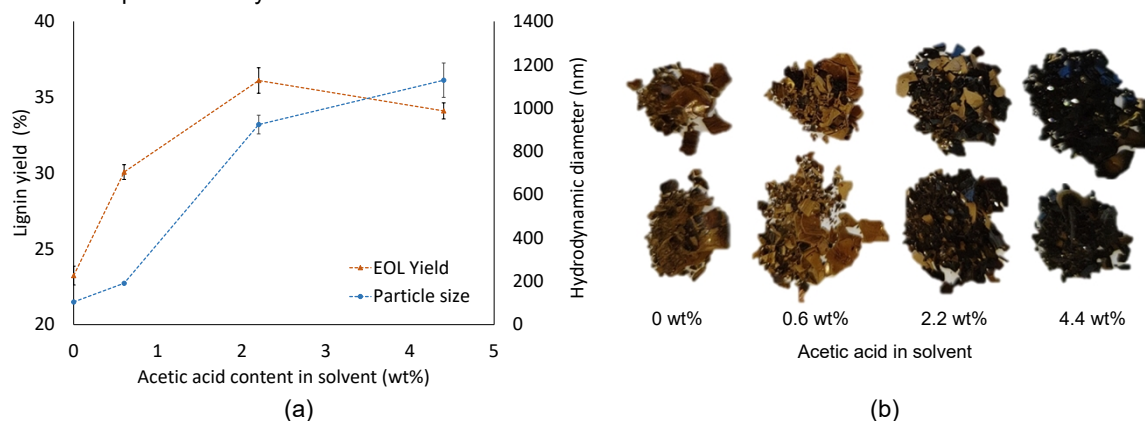


Figure 4: Lignin yield and particle size (a) and color (b) of EOL with increasing acetic acid content

4. Conclusion

It was shown that the addition of acetic acid as a catalyst in ethanol organosolv pretreatment of wheat straw increases delignification, enzymatic digestibility of the pretreated biomass, and EOL yield. On the other hand, the production of furfural also increases directly proportional with the amount of acid added. The results suggest that acetic acid content needs to meet a threshold level above 0.6 wt% in the solvent to act as an effective catalyst for delignification. The largest improvement (from 39.2 to 45.0 %) happened from 0.6 to 2.2 wt% of acetic acid, while the change towards the highest content was less substantial, and EOL yield decreased. It can be concluded that an acetic acid concentration of 2.2 wt% are optimal for improved delignification, enzymatic hydrolysis, and EOL yield. However, this concentration also results in a big increase in particle size when precipitating the extract into colloidal lignin particles, and a significantly darker lignin. Depending on the focus of the pretreatment, an acetic acid concentration of 0.6 wt% could be preferable, since this results in a brighter color and only a moderate increase in particle diameter, while the lignin yield increases substantially.

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References

- Adamczyk J., Beisl S., Amini S., Jung T., Zikeli F., Labidi J., Friedl A., 2021. Production and Properties of Lignin Nanoparticles from Ethanol Organosolv Liquors-Influence of Origin and Pretreatment Conditions. *Polymers*, 13, 384.
- Beisl S., Adamczyk J., Friedl A., 2020. Direct Precipitation of Lignin Nanoparticles from Wheat Straw Organosolv Liquors Using a Static Mixer. *Molecules*, 25, 1388.
- Beisl S., Friedl A., Miltner A., 2017. Lignin from Micro- to Nanosize: Applications. *International Journal of Molecular Sciences*, 18, 2367.
- Beisl S., Loidolt P., Miltner A., Harasek M., Friedl A., 2018. Production of Micro- and Nanoscale Lignin from Wheat Straw Using Different Precipitation Setups. *Molecules*, 23, 633.
- Choi J.-H., Cho S.-M., Kim J.-C., Park S.-W., Cho Y.-M., Koo B., Kwak H.W., Choi I.-G., 2021., Thermal Properties of Ethanol Organosolv Lignin Depending on Its Structure. *ACS Omega*, 6, 1534–1546.
- Choi J.-H., Jang S.-K., Kim J.-H., Park S.-Y., Kim J.-C., Jeong H., Kim H.-Y., Choi I.-G., 2019. Simultaneous production of glucose, furfural, and ethanol organosolv lignin for total utilization of high recalcitrant biomass by organosolv pretreatment. *Renewable Energy*, 130, 952–960.
- G G., S K., R Y.K., Bhatia S.K., S A.K., M R., Kumar G., Pugazhendhi A., Chi N.T.L., J R.B., 2021. Valorization of agricultural residues: Different biorefinery routes. *Journal of Environmental Chemical Engineering*, 9, 105435.
- Galbe M., Wallberg O., 2019. Pretreatment for biorefineries: a review of common methods for efficient utilisation of lignocellulosic materials. *Biotechnology for Biofuels*, 12, 294.
- Goh C.S., Tan H.T., Lee K.T., Brosse N., 2011. Evaluation and optimization of organosolv pretreatment using combined severity factors and response surface methodology. *Biomass and Bioenergy*, 35, 4025–4033.
- Gordobil O., Herrera R., Yahyaoui M., İlk S., Kaya M., Labidi J., 2018. Potential use of kraft and organosolv lignins as a natural additive for healthcare products. *RSC Advances*, 8, 24525–24533.
- McDonough T.G., 1993. The Chemistry of Organosolv Delignification. *TAPPI Journal*, 76, 186-193.
- Mondylaksita K., Ferreira J.A., Millati R., Budhijanto W., Niklasson C., Taherzadeh M.J., 2020. Recovery of High Purity Lignin and Digestible Cellulose from Oil Palm Empty Fruit Bunch Using Low Acid-Catalyzed Organosolv Pretreatment. *Agronomy*, 10, 674.
- Palmqvist E., Grage H., Meinander N.Q., Hahn-Hägerdal B., 1999. Main and interaction effects of acetic acid, furfural, and p-hydroxybenzoic acid on growth and ethanol productivity of yeasts. *Biotechnology and Bioengineering*, 46–55.
- Reverberi A., Vocciante M., Salerno M., Lauciello S., Fabiano B., 2020. Thermal Decomposition of Sugars as an Eco-friendly and Inherent Safe Process for Carbon Nanoparticles Production. *Chemical Engineering Transactions*, 82, 337–342.
- Selig M., Weiss N., Ji Y., 2008. Enzymatic saccharification of lignocellulosic biomass: laboratory analytical procedure (LAP), Technical Report NREL/TP-510-42629 Standard
<<https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&ved=2ahUKEwj3yvjdIYz4AhWN16QKHUinBywQFnoECCYQAQ&url=https%3A%2F%2Fcanvas.umn.edu%2Fcourses%2F72625%2Ffiles%2F2908627%2Fpreview%3Fverifier%3DhD2DZvOqD1jEh5SrbaTW7plmap5zVWcnPS3KsOs4&usg=AOvVaw0qbNzSO2eXsCePTowybquj>>, accessed 01.06.2022.
- Sluiter A., Hames B., Ruiz R., Scarlata C., Sluiter J., Templeton D., 2008a. Determination of Ash in Biomass: Laboratory Analytical Procedure (LAP); Issue Date: 7/17/2005, Technical Report NREL/TP-510-42622.
<<https://www.nrel.gov/docs/gen/fy08/42622.pdf>>, accessed 01.06.2022.
- Sluiter A., Hames B., Ruiz R., Scarlata C., Sluiter J., Templeton D., Crocker D., 2008b. Determination of Structural Carbohydrates and Lignin in Biomass: Laboratory Analytical Procedure (LAP), Technical Report NREL/TP-510-42618. <<https://www.nrel.gov/docs/gen/fy13/42618.pdf>>, accessed 01.06.2022.
- Souto F., Calado V., Pereira N., 2018. Lignin-based carbon fiber: a current overview. *Materials Research Express*, 5, 72001.
- Zhao X., Cheng K., Liu D., 2009. Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis. *Applied Microbiology and Biotechnology*, 82, 815–827.