



# Enzymatic hydrolysis of hardwood and softwood harvest residue fibers released by sulfur dioxide–ethanol–water fractionation



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## HIGHLIGHTS

- SEW fractionated SW and HW harvest residues are efficiently hydrolyzed by enzymes.
- SW harvest residues require higher enzyme dosage than HW due to high lignin content.
- Polyphenolic acids in bark notably impair the delignification of SW harvest residue.
- Optimized enzyme mixtures containing mannanase are needed for improved SW hydrolysis.
- SW hydrolyzate was fermented through ABE fermentation with Clostridia.

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## ABSTRACT

The enzymatic hydrolysis of hardwood and softwood harvest residues treated by SO<sub>2</sub>–ethanol–water (SEW) fractionation was studied. The target was to convert these fibers with high yield into glucose monomers which could be further converted into biofuel by a subsequent fermentation stage. Hardwood biomass residues were efficiently digested at low enzyme dosage (5 FPU/g cellulose) whereas the softwood residues required notably higher enzyme dosage (20 FPU) for sufficient conversion. However, cellulase dosage of softwood could be reduced mannanase supplementation. Especially the high lignin content of softwood biomass pulps impairs the digestibility and thereby, improved delignification could notably enhance the hydrolysis yields. It was shown that inferior delignification of SW biomass is due to persistent polyphenolic acids present in coniferous bark, whereas no evidence of the negative effect of inorganics and acetone extractives was observed. Additionally, SW hydrolyzate was successfully converted into a mixture of butanol, acetone and ethanol through ABE fermentation.

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## 1. Introduction

Global concerns on climate change, energy security and depletion of fossil resources have promoted the research on and production of biofuels from renewable resources (Balan et al., 2013). Additionally, in many countries the legislation requires gradual transfer towards more sustainable fuels, preferably produced from non-food resources.

Forest harvest residues offer abundant, sustainable and cost-effective feedstock for biofuel production. Advantages over agricultural biomass include possibility of all year round harvest and

higher bulk density, which reduces the transportation costs and improves the logistics (Zhu and Zhuang, 2012). Availability of forest biomass is also less affected by annual fluctuations in weather. Previously, harvest residues have mainly been used for energy production by combustion or alternatively, left to forest in order to retain the nutrition balance of the forest land. The effect of whole-tree harvesting on the soil properties is evident but further long-term research is still required to better understand the changes in site productivities (Karakka et al., 2014). Nevertheless, even partial harvest of stump wood, branches and tree tops offers valuable raw material source for the production of liquid biofuels. Additionally, there is surplus forest growth in many areas of the world which could be used more efficiently and for example, allocated to biofuel production.

Several pathways exist for the conversion of forestry residues into biofuels. Dried bark, stumps and other residues could be

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treated by thermochemical processes, such as gasification or pyrolysis. Another promising pathway for the conversion of forest residues to biofuel includes biochemical approaches using enzymes and fermentation processes. Pretreated or fractionated biomass is converted into soluble sugars by cellulolytic enzymes and the released pentose and hexose sugars are fermented by yeast or bacteria, either subsequently or simultaneously, to produce fuels, such as ethanol or butanol. Bioethanol fuel production from starch sources via biotechnological pathway is widely applied especially in USA and Brazil. However, there is constant political pressure and ethical concerns to move towards non-food raw materials, such as forestry or agricultural residues. Still, only few studies have demonstrated biofuel production from harvest residues via biochemical route.

Currently, combination of SO<sub>2</sub>-Ethanol-Water (SEW) fractionation, enzymatic hydrolysis and Acetone-Butanol-Ethanol (ABE) fermentation has been studied for the production of biofuel from forestry residues (Yamamoto et al., 2014b; Sklavounos et al., 2013b; Survase et al., 2011). SEW technology is also part of a patented process termed AVAP<sup>®</sup> by American Process Inc., a member of the industrial consortium supporting the present research (Retsina and Pylkkanen., 2011).

SEW fractionation has been successfully demonstrated for several types of lignocellulosics, including hardwood (HW) and softwood (SW) harvest residues (Yamamoto et al., 2014b; Iakovlev et al., 2011, 2014). In the fractionation, approximately 30% of the carbohydrates in wood, mainly hemicelluloses, are dissolved in spent fractionation liquor whereas cellulose and some residual hemicelluloses remain in the solid fraction. Sugar degradation and formation of inhibitive compounds is very low due to relatively low temperatures and rapid treatment. The liberated cellulosic fibers are hydrolyzed by enzymes, and the glucose stream is combined with the conditioned spent liquor, to be followed by fermentation (Sklavounos et al., 2013b).

High enzymatic hydrolysis yields have earlier been demonstrated for deeply delignified spruce SEW pulps, while at high lignin content the conversion was impaired (Yamamoto et al., 2014a). Lignin content correlated strongly with the digestibility, whereas other features, such as cellulose DP or hemicellulose content, had weaker effect. Nevertheless, no results are reported for enzymatic hydrolysis of SEW pulps derived from harvest residues. One of the main difficulties here is inferior delignification of SW harvest residues having high bark content (Yamamoto et al., 2014b). It was suspected that either the extractives, ash or polyphenolic acids present in the coniferous bark impair the delignification.

SEW process was developed based on commercial acid sulfite pulping by replacing the base (Ca, Mg, Na or NH<sub>4</sub>) with ethanol. It is well known that acid sulfite pulping is extremely sensitive to certain extractives. Acid sulfite process is not capable of delignifying pine, larch, Douglas fir and bark-damaged wood due to condensation involving extractives (notably, pinosylvin located in pine heartwood and tannins located in bark) (Sixta et al., 2006). Acetone extraction allows removal of these extractives. High amount of ash is assumed to cause negative effect on delignification through decreasing the acidity as well as formation of thiosulfate anions capable of condensation with lignin. Acidic leaching is used to remove ash from biomass.

Polyphenolic acids are compounds abundantly present in bark, preferentially in cork, amounting for up to 40–50% of bark weight (Erman and Lyness, 1965; Goldstein, 1975). The acids were found in the cork cells of Scott's pine, Douglas fir, spruce, Loblolly pine, white fir, Mediterranean oak, etc. (Fang and McGinnis, 1975; Fengel and Wegener, 1989). They are supposedly derived from flavonoids and their structure resembles that of humic acids. In contrast to lignin, polyphenolic acids are characterized by substantially lower amounts methoxyls (1.5–3.5%) and high amounts of

carboxylic acid groups (4.5–13.5%). The latter explains their high solubility in alkaline solutions, which form the basis for their quantification. 1% NaOH is conventionally used (at 25 or 100 °C), as lignin is said to be insoluble during such a mild treatment. No common organic solvent is capable of extracting these compounds (Jensen et al., 1963; Hergert et al., 1965).

Another critical aspect of the process is the efficient ABE fermentation of the combined sugar stream. The earlier reports demonstrated successful ABE fermentation of the conditioned spent SEW liquors supplemented by pure glucose, for both spruce and SW harvest residues (Survase et al., 2011; Sklavounos et al., 2013b). The addition of glucose accounted for the sugar stream obtained from enzymatic hydrolysis. However, the fermentability of SW biomass enzymatic hydrolyzate was not verified.

Therefore, the objective of this study was to investigate enzymatic digestibility of SW and HW forest residues treated by SO<sub>2</sub>-ethanol-water (SEW) fractionation. Potential to convert the obtained carbohydrate monomers into biofuel was demonstrated by ABE fermentation. Additionally, SEW fractionation process was further investigated in order to clarify the causes of inferior delignification since especially the high lignin content has been identified to impair the enzymatic hydrolysis of SW biomass.

## 2. Methods

### 2.1. Raw materials and their pretreatments

The raw materials studied were softwood (SW) and hardwood (HW) forest harvest residues, which consist of stump wood, tree tops, branches and twigs. They are later referred to as SW and HW biomass. Green biomass chips were screened (SCAN-CM 40:01, accepted particles from screens Ø7 mm and Ø13 mm) and air-dried before fractionation to decrease the heterogeneity of the feedstocks, as well as to remove humus and needles. Iakovlev et al. (2014) have shown that the dry matter content of the feedstock does not affect the efficiency of SEW treatment and this finding has also been confirmed on SW biomass. Bark content of the biomass used was 28.0% and 7.2% for SW and HW biomass, respectively (Yamamoto et al., 2014b).

To evaluate the relative effect of ash, extractives and polyphenolic acids on delignification, ground SW biomass was pretreated by three different methods before SEW fractionation. For the removal of ash, ground biomass was treated with 0.1 M HCl at a liquor-to-wood (L:W) ratio of 20 L kg<sup>-1</sup> for 30 min at room temperature with vigorous stirring. After the treatment, the biomass was washed three times with deionized water at a liquor-to-wood (L:W) ratio of 20 L kg<sup>-1</sup> at room temperature and air-dried. For the removal of extractives, ground biomass was extracted with acetone for 6 h (SCAN 49:03). The removal of polyphenolic acids was carried out on acetone extracted SW biomass and spruce stem wood by 1% NaOH extraction at a L:W ratio of 100 L kg<sup>-1</sup> at 25 °C and 98 °C for 1 h with vigorous stirring. Washing was performed at room temperature at L:W 100 L kg<sup>-1</sup> (5 times with deionized water, 1 time with 1% acetic acid, 3 times with deionized water).

### 2.2. SEW fractionation

The pulps which were used as substrates for enzymatic hydrolysis were prepared from SW and HW biomass by SO<sub>2</sub>-ethanol-water fractionation technology. SEW fractionation was done in a thermostated silicon oil bath using bombs of 220 mL each filled with 25 g (o.d. basis) biomass chips or 15 g ground biomass. Pretreated biomass was dosed based on the weight of the original biomass before any treatments (the material removed by pretreatment deducted from 15 g). The composition and charge of the

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