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The effect of bark on sulfur dioxide–ethanol–water fractionation and enzymatic hydrolysis of forest biomass

Minna Yamamoto[a,*], Tuomas Niskanen[a,b], Mikhail Iakovlev[a], Heikki Ojamo[b], Adriaan van Heiningen[a,c]

[a] Department of Forest Products Technology, Aalto University, FI- 00076 Aalto, Finland
[b] Department of Biotechnology and Chemical Technology, Aalto University, FI- 00076 Aalto, Finland
[c] Department of Chemical and Biological Engineering, University of Maine, 5737 Jenness Hall, Orono, ME 04469-5737, USA

HIGHLIGHTS

- SW bark impaired the SEW fractionation and increased the amount of wood rejects.
- Enzymatic hydrolysis was deteriorated with increasing SW bark content.
- HW bark was less harmful for both the fractionation and enzymatic hydrolysis.
- Surfactant Tween 20 notably improved the hydrolysis potential of bark containing SW.
- SEW lignosulfonates formed during fractionation seem to improve enzymatic hydrolysis.

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ABSTRACT

The focus of this study was to find out the effect of bark on SO_2–ethanol–water (SEW) fractionation and enzymatic hydrolysis of forest biomass. Softwood bark was found to be more harmful than hardwood bark in both processes. For softwood, the amount of undigested wood in SEW fractionation increased with the increasing bark content, whereas the hardwood bark did not impair the fractionation of wood. The higher the softwood bark content was the lower were the yields in enzymatic hydrolysis likely due to the unproductive binding of enzymes on lignin and other compounds. Addition of surfactant Tween 20 (2% w/w on substrate) prior to enzyme more than doubled the sugar yield of bark-rich softwood pulp. Hardwood bark impaired enzymatic hydrolysis when its share was over 28%. According to a preliminary study, lignosulfonates from the carry-over liquor seem to improve the sugar yield in the enzymatic hydrolysis by acting as a surfactant.

1. Introduction

In the selection of alternative sources to replace fossil based fuels and chemicals, forest biomass offer abundant, cost competitive and sustainable raw material. Especially forestry and wood processing residues are considered materials with high potential in bioenergy and fuel production while valuable stem wood is still directed to pulping, papermaking and timber industries. Nevertheless, in the conversion of forest biomass to liquid fuels, there are still several techno-economic challenges to overcome. Especially softwood (SW) biomass is recalcitrant and strongly resists the depolymerization (Galbe and Zacchi, 2002). Thus, robust and expensive pretreatments are required before bioconversion is viable. Also, harvest residues are very heterogeneous and the presence of bark is expected to considerably complicate the conversion processes since the chemical composition of bark notably varies from that of wood.

Tree stem has about 10–20% of bark depending on the species and growing conditions. In the branches, tree tops, stumps and roots, the share of bark is even higher (Fengel and Wegener, 1989). Generally, the bark obtained after debarking of the logs is burnt for energy at the mill site. Small scale use includes for example horticulture. Nevertheless, bark is rich especially in aromatic lignin and extractives compounds which might offer more value in specialized material and chemical applications (Feng et al., 2013). In biomass processing, the specific chemical characteristics of bark may complicate the processing compared to stem wood.

Bark contains notably less carbohydrate compared to normal wood and its lignin content is high. Also the content of various extractives is high in bark, although the amount and type of extractives varies a lot between different wood species (Sjöström, 1981). Extraction method also has a significant influence on the

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* Corresponding author. Tel.: +358 503440934.
E-mail addresses: minna.yamamoto@aalto.fi (M. Yamamoto), AvanHeiningen@umche.maine.edu (A. van Heiningen).

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extractives content and thus, a sequential extraction is required for comprehensive determination. Additionally, the inorganics content is higher in bark than in wood. (Fengel and Wegener, 1989) These differences influence the processing and feasibility in bioconversion of bark-containing biomass. First of all, high lignin content increases the biomass recalcitrance in both pretreatment and enzymatic hydrolysis. In addition, condensation reactions of lignin and extractives may occur. Persistent phenolic acids present in high amounts (40–50% by weight) especially in SW bark (Erman and Lyness, 1965; Goldstein, 1975) can be dissolved only by 1% NaOH treatment. Liberated extractives and phenolic compounds in the hydrolysate may inhibit subsequent fermentation, although at least ethanol fermentation has been successfully performed with bark containing feedstock (Robinson et al., 2002). Besides, the inorganic compounds in bark may neutralize the acid used in the pretreatment stage, causing impaired hydrolysis. Lower carbohydrate content inevitably leads to lower obtainable sugar yields. These difficulties have hindered the utilization of bark-rich biomass although the current demand on higher share of renewable fuels and energy has raised interest on exploitation of all types of lignocellulosics.

A potential method for the production of fermentable sugars from forest biomass is SO_2–ethanol–water (SEW) fractionation followed by enzymatic hydrolysis. SEW fractionation, originally developed as a pulping method (Schorning, 1957), is demonstrated to efficiently fractionate several types of lignocellulosic materials, including bark containing harvest residues (Iakovlev et al., 2011; Yamamoto et al., 2014). SEW technology is also part of a patented process termed AVAP® by American Process Inc., a member of the industrial consortium supporting the present research (Retsina and Pylkkanen, 2011). Advantages of the process are relatively low temperature, rapid impregnation of wood due to presence of ethanol and relatively simple chemical recovery due to absence of base. Sugar degradation and formation of inhibitive compounds is modest. However, like other pulping processes, SEW fractionation is negatively affected by the presence of bark. Especially SW harvest residues containing high amount of bark (28%) have inferior delignification which was found to be highly due to polyphenolic acids present in coniferous bark (Yamamoto et al., 2014). Since impaired delignification possesses problems also in the subsequent enzymatic hydrolysis and bark derived compounds might inhibit the fermentation, the influence of bark is crucial for the whole process and its feasibility. Thereby, further knowledge on the effects of bark has high importance.

This study investigated the influence of SW and hardwood (HW) bark on SEW fractionation and enzymatic hydrolysis of spruce and birch stem wood chips. The share of bark in raw material was rationally increased to observe its effect on the processing. Additionally, reduction of bark content by sedimentation and addition of surfactants before enzymatic hydrolysis were studied in order to improve the sugar yields. Since the lignosulfonates formed during pretreatment stage were earlier reported as potential enhancers of enzymatic hydrolysis (Zhou et al., 2013), the effect of reduced washing efficiency after SEW fractionation was also briefly investigated.

2. Methods

2.1. Raw materials

Five different biomass materials were included in this study: SW biomass (mixture of woody chips, bark and branches); bark-free stem wood spruce and birch chips; ground pine and birch bark. All raw materials were air-dried before the experiments. Spruce and birch chips were screened using the screens Ø45; //8; //6; //4; //2 mm and the fractions from the screens //2 and //4 mm were used for the experiments. To study the effect of bark, stem wood chips and ground bark were fractionated at different ratios (bark contents 0%; 9%; 28%, 60%; 100%). Spruce chips were combined with pine bark and birch chips with birch bark. The reason for using pine bark instead of spruce bark was the easier availability. Besides, the differences in the chemical composition of these barks were not considered crucial (Miranda et al., 2012). The bark content of SW biomass was determined by manually removing bark from the sample according to SCAN-CM 42:95.

2.2. SEW fractionation

SEW fractionation was performed in silicon oil bath (Haato model 43427, Finland) using 220 mL bombs each filled with 25 g (o.d. basis) raw material. SEW liquor was prepared by injecting gaseous sulfur dioxide into an ethanol–water solution (deionized water and ethanol ETAX A, 96.1% v/v). The composition and charge of the cooking liquor (SO_2:EtOH:H_2O = 12:43.5:44.5, by weight; liquor-to-wood (L:W) ratio 6.0–6.5 L kg^{-1}) and the temperature (150 °C) were kept constant in the experiments. Fractionation time was 30 min for HW and 60 min for SW, including 8–9 min of equivalent heat-up time. The fractionation duration corresponded to the optimal in terms of clean fractionation based on earlier kinetics studies (Iakovlev et al., 2011; Yamamoto et al., 2014). Fractionation was stopped by cooling the bombs in cold water. The spent liquors were collected by squeezing the pulp suspensions contained in washing bags. Pulp was washed twice with 50 mL of 40% v/v ethanol–water at 60 °C (L:W 2 L kg^{-1}) and twice with 500 mL of deionized water at room temperature (L:W 20 L kg^{-1}). Optimization of SEW treatment and sufficient pulp washing of stem wood chips have been determined by Iakovlev and van Heiningen (2012a, 2012b), Iakovlev et al. (2011, 2009). Pulps were preserved at +4 °C.

2.3. Analyses of the raw materials and pulps

The solid yield of fractionation was determined immediately after pulping by evaporation to dryness at 105 °C according to SCAN-C 3:78. Carbohydrate and lignin content of ground bark and pulps were determined according to the NREL/TP-510-42618. HPAEC-PAD (Dionex ICS-3000, CarboPac PA20 column, Sunnyvale, CA, USA) was used in the sugar analysis and cellulose content of the pulps was calculated based on the mannose-to-glucose ratio of 1.6 and 4.15 for HW and SW glucomannan, respectively. Acetone extraction was not performed before the analyses of carbohydrate and lignin content of pulps, which likely lead to moderate overestimation of lignin contents. Carbohydrates and lignin in bark were analyzed both with and without acetone extraction. Kappa number of pulp was determined according to SCAN-C 1:00. Content of acetone extractives and ash (575 °C) in ground bark were measured according to SCAN 49:03 and NREL/TP-510-42622, respectively. Analyses were performed in duplicate, with the exception that acetone extractives in bark were determined only once. Rejects of HW and SW pulps were removed by plate screening using 0.17 mm plate and the reject content was calculated based on the oven dry weight of the rejects.

2.4. Enzymatic hydrolysis

The enzyme preparation used was Cellic Ctec2 obtained from Novozymes. The filter paper unit (FPU) cellulase activity of Cellic Ctec2 was measured based on NREL/TP-510-42628 and the protein content of enzyme was defined by both Lowry and Bradford assays (Bio-Rad assay) (Bradford, 1976; Lowry et al., 1951).