Rapid one-step solvent-free acid-catalyzed mechanical depolymerization of pine sawdust to high-yield water-soluble sugars

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Abstract

One-step absolute solvent-free acid-catalyzed mechanical depolymerization of pine sawdust (PSD) and commercially available α-cellulose to water-soluble sugars was carried out using ball milling. For comparison purposes, the commonly reported "solvent-free" mechanocatalytic depolymerization of lignocellulose method, which normally involves three steps (acid impregnation in solvent, vacuum drying, and mechanical depolymerization of lignocellulose), was performed. The 3,5-dinitrosalicylic acid (DNS) method was used to measure the total reducing sugar (TRS) of the obtained sugar solution, and major monosaccharides in the solution were analyzed by capillary electrophoresis (CE). More than 90% of the PSD became water-soluble through milling. Furthermore, most of the PSD was converted into TRS in approximately 30 min, and the highest TRS yield obtained was 31%, based on the dry mass. Interestingly, the TRS solutions obtained from the processed PSD were much darker than those obtained from α-cellulose due to the chromophores that formed during the depolymerization of lignin.

1 Introduction

Lignocellulose-based solid residuals, such as sawdust, bark, and peat, have long been used as major wood fuels in the forest industry in Finland [1] [2]. In general, approximately 500,000–600,000 tons of ash are generated annually by the forest industry and power plants in Finland, with most of this ash traditionally disposed of in landfills [1]. However, the Landfill of Waste Directive [3], which comes into force in 2016, limits biodegradable or organic biowaste with a maximum of 35% by weight at landfills. In addition, the Renewables Directive [4] encourages industries to avoid combustion and reuse or recycle lignocellulose-based solid residuals in a more energy-efficient and economical way.

Cellulose, hemicellulose, and lignin, which consist of 60–70% carbohydrates/sugars, account for about 90% of the dry matter in lignocellulose. The conversion of lignocellulose into biofuel and more available chemicals have been widely reported [5]. Mechanocatalytic depolymerization of lignocellulose into sugars, mainly C_6 and C_5, using a ball mill has emerged as a novel "solvent-free" approach in the last decade [5] [6]. The widely used method of acid-impregnated mechanocatalytic depolymerization (AIMD) of biomass involves three steps: 1) acid impregnation of the biomass in diethyl ether, 2) removal of the solvent by vacuum evaporation, and 3) a ball mill process involving the depolymerization/decrystallization of lignocellulose and conversion of water-insoluble

51 lignocellulose into water-soluble oligosaccharides and lignin at room temperature (RT)

52 [7] [8].

53

54 In this study, we introduced an absolute solvent-free process through direct acid-catalyzed

55 mechanical depolymerization (DAMD) of pine (*Pinus sylvestris*) sawdust (PSD) that

56 converted lignocellulosic biomass into sugars without requiring a prior pretreatment step.

57 The focus of the study is to explore the industrial potential of such a methodology for the

58 valorization of forest industry waste into reducing sugars that can be used for further

59 production of bioenergy and valuable chemicals.

60

61 Experiments were performed with different acid/substrate (A/S) concentrations and

62 milling times. DAMD of commercially available α-cellulose was carried out as a

63 reference sample. For a comparison with the DAMD method, the commonly used

64 "solvent-free" AIMD method was also performed. DAMD and AIMD of PSD were

65 performed with a fixed (0.3 mol kg^{-1}) A/S concentration to provide a comparable

66 overview of these mechanocatalytic depolymerization processes. The major

67 monosaccharides obtained from the depolymerization of lignocellulose were analyzed by

68 capillary electrophoresis (CE). The sum of disaccharides, oligosaccharides, and certain

69 polysaccharides was analyzed according to the 3,5-dinitrosalicylic acid (DNS) method,

70 in addition to which analysis of the produced total reducing sugars (TRS) was carried out.

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72

73

2 Materials and Methods

2.1 Samples

Pine is a softwood, which is a major lignocellulosic resource in Northern Europe. In softwood, lignin is mainly composed of guaiacyl units, which are less susceptible to be depolymerized in contrast to grass or hardwood. The delignification process of softwood leads to large amounts of C-C linked dimers at the C_5 position of the ring, which renders it difficult to be hydrolyzed by acids or bases. The major sugar in softwood hemicellulose is mannose, followed by xylose [5] [7].

PSD was obtained from the Biofuel Technology Centre in Umeå. It was derived from approximately 30-year-old pine tree stumps that originated from the coastal area of Västerbotten in northern Sweden. The collected stumps were crushed and transferred to chipper. The chipped sample was then sieved with 1 mm sieve.

The composition of the PDS is given in Table 1. Commercially available α-cellulose powder was obtained from Sigma-Aldrich and used as a reference sample for PSD. The major difference between cellulose and PSD in composition is the presence of additional lignin and hemicellulose in PSD.

(Table 1 Here)

94 ## *2.2 Methods*

95 DAMD and AIMD of PSD were performed (Fig. 1) in a planetary micro mill (FRITSCH

96 Planetary Micro Mill PULVERISETTE 7 *premium line*). Figure 1 provides an overview

97 of the two different methods.

98 (Figure 1 Here)

99 ## *2.3 DAMD of PSD*

100 DAMD of the PSD was performed using different concentrations (0.45 mol kg^{-1}, 0.3 mol

101 kg^{-1}, and 0.2 mol kg^{-1}) of sulfuric acid (A, mol)/substrate (S, kg), Approximately 2 g of

102 PSD, equal to 15 cm^3 (measured in a measuring cylinder), was roughly premixed with

103 H_2SO_4 in a 45 cm^3 stainless steel bowel. The premixed sample was then milled with

104 approximately 8 cm^3 (46.4g) of 3 mm diameter grinding balls [9]. The temperature of the

105 milling process was controlled by a "1-min milling/1-min pause" alternation mode at 13.3

106 Hz (Fig. 1a). The sample was then transferred to a further hydrolysis process at 100° C.

107 The water solubility of the processed PSD was determined at RT, as described in Section

108 2.6.

109

110 ## *2.4 AIMD of PSD*

111 The AIMD of the PSD (Fig. 1b) was performed according to the procedure of Schüth et

112 al. [10]. It involved three steps: 1) acid impregnation in a mixture of H_2SO_4 and diethyl

113 ether, 2) vacuum evaporation drying, and 3) mechanocatalytic depolymerization. The

114 PSD was immersed in the solution of H_2SO_4/diethyl ether, with an A/S concentration of

115 0.3 mol kg^{-1}. The resulting mixture was shaken in an incubator at 3.3 Hz at RT for 2 h,

116 followed by drying in a rotary evaporator at approximately 50 kPa, and 40º C for 2 h.

117   Mechanocatalytic depolymerization and the same procedures as followed in the DAMD

118   process were then carried out (Section 2.3.).

119

120   ## 2.5   Determination of the remaining acid in the acid-impregnated PSD

121   To obtain data on the effect of the acid impregnation on the biomass, acid impregnation

122   of PSD and α-cellulose was performed using the same A/S concentration of 0.8 mol kg^{-1}.

123   Three layers, based on the color differences, were observed in the rotary evaporator flask

124   after the vacuum evaporation drying. The processed PSD was carefully collected in a

125   round flask, according to the color of the PSD on the top, middle, and bottom. The acid

126   concentration of each layer was then determined by classical acid-base titration using 0.1

127   mol dm^{-3} sodium hydroxide (NaOH).

128

129   ## 2.6   Determination of the water solubility of processed PSD at RT

130   The temperature of the grinding balls was measured immediately after depolymerization.

131   The water solubility of the processed PSD was determined by adding 15 cm^3 of distilled

132   water in a 500 mg sample, followed by shaking the resulting aqueous mixture in an

133   incubator at RT at 3.3 Hz for 1 h. The water solubility of the processed sample was then

134   calculated based on the dried remaining solid residuals [9].

135

136   ## 2.7   Determination of total reducing sugars (TRS)

137   The DNS method was used to determine the TRS produced during DAMD and AIMD

138   [2].

139

140   *2.8   Determination of monosaccharides by capillary electrophoresis (CE)*

141   The samples were filtered with a 0.45 μm GHP Acrodisc syringe filter (Pall Corporation,

142   USA) prior to the CE analysis. Monosaccharides were analyzed using a CE method

143   modified from the original method [11] with a P/ACE MDQ CE instrument (Beckman-

144   Coulter, Fullerton, CA, USA) and diode array detection at 270 nm. Uncoated fused-silica

145   capillaries with an I.D. of 25 μm and a length of 30/40 cm (effective/total length) were

146   used. The samples were injected at a pressure of 0.5 psi for 10 s. The used separation

147   voltage was +16 kV, and it was raised linearly within a 1-min ramp time, followed by a

148   separation time of 15 min. Calibration curves for the external quantification of

149   monosaccharides were prepared using standard solutions. In addition, each sample was

150   spiked with standard solution for reliable identification of peaks in the electropherogram.

151   All samples were duplicated.

152

153   **3   Results**

154   *3.1   Yields of remaining acid in the acid-impregnated PSD*

155   Three different colored layers appeared after acid impregnation and vacuum drying.

156   Approximately equivalent amounts of light brown and yellow (natural color of PSD)

157   colored PSD appeared on the top and middle layers of the flask, respectively. In addition,

158   a small amount of dark brown PSD accumulated at the bottom of the flask. The three

159   colored layers were collected separately from the flask, and the remaining acid in each

160   layer was determined by classical acid-base titration (Fig. 2).

161   (Figure 2 Here)

7

162   The highest acid concentration was found in the darkest colored PSD (bottom layer), and

163   the lowest acid concentration was found in the lightest colored PSD (middle layer).

164   Unlike PSD, the impregnation of α-cellulose with an A/S concentration of 0.8 mol kg^{-1}

165   did not produce obvious color changes in the sample after the acid impregnation and

166   vacuum drying. The overall remained acid was 89% of the original added amount using

167   the AIMD method [9].

168

169   ### 3.2   *Yields of the water solubility processed PSD at RT and elevated temperature*

170   The water solubility of processed PSD was determined at RT and elevated temperature

171   100° C which was after acid hydrolysis. The water solubility of the processed PSD using

172   an A/S concentration of 0.2 mol kg^{-1} and 0.45 mol kg^{-1} is shown in Table 2. More than

173   90% of the processed PSD was dissolved in water with 75 min milling process at both

174   RT and elevated temperature. At both concentrations, the water solubility increased as

175   the total milling duration increased at RT. At the same milling conditions, more water-

176   insoluble PSD was cleaved and converted into water-soluble products at higher A/S

177   concentration, than at the lower A/S concentration at RT.

178   (Table 2 Here)

179   Table 2 indicates that the processed PSD has higher water solubility when the temperature

180   is elevated. However, opposite phenomenon is observed when the total milling time is

181   increased to 75 min (A/S concentration of 0.2 mol kg^{-1}) and 45 min (A/S concentration

182   of 0.45 mol kg^{-1}). Appearance of a dark brown precipitate was found when the water

183   solubility of processed sample was lower at elevated temperature than at RT. When the

184   processed PSD was hydrolyzed in the acidic solution, the appearance of dark brown

185   precipitate was observed earlier in the higher acidic solution (0.45 mol kg^{-1} A/S

186   concentration) than in the lower one (0.2 mol kg^{-1} A/S concentration). The precipitate

187   was analyzed by FTIR and exhibited together with PSD Figure 3. The inset in Figure 3

188   illustrates the filtrated solid remaining after hydrolysis at 100° C with 75-min milling of

189   PSD and an A/S concentration of 0.45 mol kg^{-1}.

190

191   (Figure 3 Here)

192

193   **3.3   Obtained sugar solutions after depolymerization of biomass and yields of**
194   **produced sugars**

195

196   Figure 4 illustrates the obtained sugar solutions after depolymerization and acid

197   hydrolysis of both PSD and α-cellulose. Interestingly, the colors of the sugar solutions of

198   α-cellulose (Fig. 4a) and PSD (Fig. 4b) became darker when the total milling time was

199   increased. The colors of the TRS solutions obtained from cellulose turned from light

200   yellowish to brown yellowish while increasing the milling time. Whereas, the colors of

201   obtained TRS solutions from PSD could turn much darker than that from cellulose.

202

203   (Figure 4 Here)

204   The obtained sugar solutions were analyzed by two types of measurements. DNS method

205   provides a rapid estimation of TRS, such as, monosaccharides, disaccharides and

206   oligosaccharides. CE analysis offers more detailed analysis of monosaccharides which

207   were converted from biomass, such as glucose, xylose and galactose.

208

209   The DNS method was performed three times on each sample and the mean values are

210   given in Figure 5. The highest yield of TRS (31%) was obtained using an A/S

211   concentration of 0.2 mol kg^{-1} in the 60-min direct acid-catalyzed process, whereas the

212   yield of TRS showed a decreasing trend with 75 min of milling. Similar to the other A/S

213   concentrations milling, the TRS yields showed an increasing trend at the beginning and a

214   decreasing trend at the end of the milling process. With high A/S concentration (0.45 mol

215   kg^{-1}) milling, the trend of TRS reduction occurred earlier than other A/S concentration.

216

217   (Figure 5 Here)

218   Based on the yield of TRS, DAMD of PSD using an A/S concentration of 0.45 mol kg^{-1}

219   showed the most rapid depolymerization at the beginning of the milling (15 min). At an

220   A/S concentration of 0.45 mol kg^{-1}, the maximum TRS yield (30%) was determined after

221   30 min of milling, and the extended duration decreased the recovery of TRS. In the AIMD

222   of PSD using an A/S concentration of 0.3 mol kg^{-1} with 30 min milling, the obtained TRS

223   yield was as high as with an A/S concentration of 0.45 mol kg^{-1}. With slightly longer

224   milling (45 min), AIMD of PSD achieved the highest TRS yield.

225

226   It is clear that the depolymerization of PSD using a higher A/S concentration (0.45 mol

227   kg^{-1}) required a shorter time (< 30 min) to achieve the highest TRS production. However,

228   the reduction of TRS was observed directly after 30 min milling at the high acid

229   concentration. Conversely, at a lower A/S concentration (0.2 mol kg^{-1}), a longer milling

230   time (> 60 min) was required to reach high TRS concentration, but the reduction of TRS

231   happened much more slowly.

232

233   The sugars obtained from DAMD of α-cellulose differed from those of PSD. A

234   significantly longed milling time (> 60 min) was required to achieve optimal

235   depolymerization of α-cellulose. Furthermore, DAMD of α-cellulose resulted in a huge

236   reduction of TRS (3.6% in 15 min), Interestingly, the reduction in the TRS yield following

237   PSD depolymerization (between 0.5% and 2.1% in 15 min) was not as much as observed

238   with α-cellulose depolymerization, even at an A/S concentration of 0.45 mol kg^{-1}.

239

240   (Figure 6 Here)

241   The major monosaccharides obtained from depolymerized lignocellulose were glucose,

242   xylose, galactose, mannose, and arabinose, as shown by the CE analysis (Fig. 6). In the

243   depolymerization of α-cellulose, glucose was the only monosaccharide that was detected.

244   Figure 6 inset shows a clear view of produced glucose from the conversion of PSD and

245   α-cellulose. The conversion of α-cellulose into sugars using depolymerization produced

246   the least total amount of monosaccharides in comparison to other reaction conditions.

247   However, the inset in Figure 6 shows the highest amount of glucose is obtained from the

248   depolymerization of cellulose. In addition, at longer milling times, more biomass and

249   sugar oligomers were cleaved, and more monosaccharides were released. The least

250   obtained total monosaccharides from PSD is with AIMD of PSD at a milling time of 30

251   min (Fig. 6), although the TRS yields of AIMD of PSD is as high as DAMD of PSD at

252   A/S concentration of 0.45 mol kg^{-1} (Fig. 5).

4 Discussion

The impregnation process which was performed according to the procedure of Schüth et al. [10] with slight modification which was only involved in AIMD process. The results of acid-base titration of processed PSD presented above show that the acid was not enough homogeneously loaded into the biomass during acid impregnation. The impregnation of α-cellulose did not show the obvious color change. This indicates that the color change in the PSD after vacuum evaporation may be exclusively due to lignin oxidation, as chromophores were formed [12] [13] [14]. The appearance of the three different color layer might be caused during the solvent removal process. When the solvent was removed by vacuum evaporation, the acid remained mostly on the bottom of the flask. Some of the acid was carried by the vapor to the surface of the PSD. Therefore, the color on the bottom and top was darker than the color in the middle.

Approximately 11% of the acid in the sample was lost after removing the solvent. Certain amount of the acid was removed via water vapor during the vacuum evaporation [9]. In addition, H_2SO_4 is a strong acid, which is expected to cleave diethyl ether to alcohol. As the alcohol was easily removed during the vacuum drying, and therefore it could be a potential carrier for acids to the upper layer of the flask. Hence, the actual amount of acid in the further depolymerization reaction in AIMD was less than in DAMD.

In the same reaction conditions, the obtained TRS solutions of α-cellulose and PSD showed significant color difference, especially, when the total milling time was longer than 30 min. It is well known that cellulose and hemicellulose are easier to be cleaved than lignin. Hemicellulose and amorphous cellulose have weaker hydrogen bonding than

278   crystalline cellulose. In the first 15 min of milling, mainly existing amorphous cellulose

279   and hemicellulose in the PSD were most likely cleaved. As the amount of cleaved lignin

280   was negligible, the colors of the TRS solutions obtained from α-cellulose and the PSD

281   were similar. It is likely that lignin started to be cleaved after 30 min of milling, given the

282   appearance of chromophores in the solution at this point. Increasing the milling time

283   amplified the dark color of the TRS solution.

284

285   Most likely, the sugars released by acid-catalyzed milling were slightly heated up due to

286   the increased reaction temperature ($> 70°$ C) caused by collision-induced energy transfer

287   in the bowel. The milling time affected the color of the TRS solution, with an increase in

288   the milling time resulting in a more yellowish or yellow-brownish color. In contrast to

289   commercially available α-cellulose, native PSD contains mainly cellulose, hemicellulose,

290   and lignin. The dark color in the TRS solution may have been derived from the

291   chromophores in the lignin and extract or from products of these chromophores. $C{=}C$

292   stretching of the aromatic ring in lignin, in addition to free radicals and metal/organic

293   complexes, may also have exerted effects on the change of color. For instance, in acidic

294   conditions, β-1 structures in lignin can be converted to stilbene structures, and red-colored

295   stilbene quinones can be obtained after oxidation of stilbene in the presence of Fe^{3+} or/and

296   Cu^{2+} in native lignin. Furthermore, leucochromophores can be converted to

297   chromophores by oxidation during milling [12] [13] [14].

298

299   A dark brown precipitate was found when the water solubility of processed PSD showed

300   significant higher at RT than elevated temperature. It is obvious that the precipitation

301   occurs when the temperature is elevated in Table 2. Figure 3 provides The Fourier

302   transform infrared spectroscopy (FTIR) spectrum of PSD used in this study and found

303   precipitate after acid hydrolysis at elevated temperature. In the spectrum of PSD, strong

304   signals point to the presence of lignin in the obtained precipitate. For instance, the

305   absorption peak 1510 cm^{-1} is assigned to the aromatic skeletal vibration C═C of the

306   benzene ring in lignin. Therefore, lignin is one of the components in the precipitate that

307   was found after acid hydrolysis process.

308

309   Lignin is a three-dimensional amorphous polymer, which is rich in aromatic rings. Lignin

310   and carbohydrates are linked by covalent bonds, which consist of benzyl ether, benzyl

311   ester, and phenyl glycosides [15] [16] [17]. During depolymerization, lignin is milled to

312   lignin fragments and then to low-molecular-weight unstable monomeric products, such

313   as phenolic products, which are water soluble [7] [18]. When the sample was hydrolyzed

314   at 100° C for 1 h, the water-soluble lignin was re-polymerized and became water insoluble

315   again, which was considered as lignin condensation. However, lignin condensation

316   occurs during hydrolysis and is difficult to prevent. The condensation of lignin is thought

317   to occur through nucleophilic attack on its benzylic carbon, leading to high-molecular-

318   weight lignin intermediates during acid hydrolysis at 100° C. The condensation of lignin

319   is associated with the acidity of the hydrolysis and the reaction time [18] [19] [20] [21].

320   This finding explains why at elevated temperature the water solubility of processed PSD

321   is lower than at RT in certain reaction conditions.

322

323   Figure 5 provides an overview of the yields of produced TRS which includes

324   monosaccharides, disaccharides and oligosaccharides. The best obtained TRS yield is 31%

325   which is obtained from DAMD of PSD with 60 min milling at A/S concentration of 0.2

326   mol kg^{-1}. However, the best reaction conditions of converting PSD to TRS are considered

327   to be DAMD and AIMD of PSD with 30 min milling at A/S concentration of 0.45 mol

328   kg^{-1} and 0.3 mol kg^{-1}. Both reactions produced 30 % of TRS yield within a shorter

329   reaction time, although the actual A/S concentration in AIMD of PSD (0.3 mol kg^{-1}) was

330   less than original added. According to the amount of produced monosaccharides in Figure

331   6, the most amount of monosaccharides is produced by DAMD of PSD with 75 min

332   milling at A/S concentration of 0.2 mol kg^{-1}. In addition, the least amount of

333   monosaccharides is produced by AIMD of PSD (A/S concentration of 0.2 mol kg^{-1}) with

334   30 min milling. This result demonstrates that the impregnation process "softens" the

335   biomass and the "softened" PSD is only easily cleaved to the sugar oligomers, but

336   monosaccharides. In addition, longer milling time enhances the conversion of

337   monosaccharide.

338

339   The findings of reduction in TRS yields can be explained by the decomposition of sugars

340   occurring while the biomass and sugar oligomers were depolymerized/decrystallized to

341   monosaccharides in the processing system. Since α-cellulose contains only crystalline

342   cellulose, which is more difficult to depolymerize than hemicellulose or amorphous

343   cellulose, both of which are components of PSD. It needed 60 min milling for α-cellulose

344   to achieve the highest yield of TRS and glucose was the only detected sugar in the TRS

345   solution. The decomposition of sugar in α-cellulose obtained TRS solution can be

346   assumed due to the formation of humins that was mainly caused by the decomposition of

347   C_6 sugars. Therefore, lignin and humins could be the two major components in the dark

348   brown precipitate.

349

350

5 Conclusions

This study examined mechanocatalytic depolymerization of lignocelluosic biomass in two different approaches: AIMD and DAMD of PSD. Both AIMD and DAMD of PSD provided fast approaches to convert PSD into TRS. The acid-impregnated "presoftened" PSD easily cleaved to sugars. Nevertheless, this "presoftening" process did not enhance the depolymerization of sugar oligomers. Depolymerization of PSD with a higher A/S concentration resulted a high yield of TRS in a short time (30 min). The conversion of monosaccharides from biomass required a longer milling time in general. Moreover, A/S concentration also affected the speed of sugar decomposition, with higher concentrations of A/S resulting in faster sugar decomposition and lignin condensation. Sugar decomposition and Lignin condensation are hardly avoidable in acid hydrolysis at elevated temperature. Sugar decomposition and lignin condensation can be minimized, especially in the acidic reaction and at elevated temperature, by minimization of A/S concentration and milling time in the process.

6 Acknowledgments

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