Processing Pine Wood into Vanillin and Glucose by Sequential Catalytic Oxidation and Enzymatic Hydrolysis

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Pine wood was processed into vanillin (up to 18 wt. % based on the lignin) and cellulose (typically 84–93 % of the initial amount in the wood) by one-stage catalytic oxidation, followed by enzymatic hydrolysis of the resulting cellulose into glucose (reducing sugar yield up to 70 % based on the cellulose). Correlation between the cellulose conversion in hydrolysis and the lignin content in the post-oxidation lignocellulosic material was established, conforming to the general trend for the products of various delignification methods. The obtained results demonstrate the practical possibility of efficient two-step processing of wood into vanillin and glucose.

INTRODUCTION

Wood consists of three principal polymeric components: lignin, cellulose, and hemicelluloses. Processing them into fine chemicals can substitute petroleum as the feedstock for organic syntheses by renewable plant matter, and possibilities of such processing are being actively researched.[1] Oxidation of native softwood lignins in alkaline media yields up to 23–28 wt. % of vanillin (depending on the wood species), from hardwood lignins up to 30–40 % of vanillin and syringaldehyde mixture can be obtained.[2] During chemical pulping of wood, lignin molecules undergo significant changes in structure, including those of condensation nature. Thus obtained lignins yield 2–3 times less of the aromatic aldehydes in oxidation when compared to native lignins.[2]

Softwood hemicelluloses comprise primarily hexose carbohydrates, whereas in hardwood ones pentoses dominate.[3] The former hemicelluloses can be hydrolyzed into hexoses which can be further converted into bioethanol or levulinic acid, by microbiological or acid-catalyzed processes respectively.[4,5] Cellulose can also be similarly processed. Pentoses are converted into furfural and its derivatives at industrial scale.[3]

The vast majority of the studies on oxidation of lignins into the aromatic aldehydes focus on processing the industrially obtained lignins (Kraft lignin, lignosulfonates, steam explosion lignin, etc.).[2,6–9] Oxidation of native lignins (i.e., contained in wood) is studied much less extensively.[2,10] This difference is probably due to the relative affordability of technical lignins, and the need of solving the environmental problems caused by these materials.

Native lignins have the herein above explained advantage of high aromatic aldehydes yield in comparison to technical lignins. However, in the case of softwood versus lignosulfonates, if vanillin yield based on reaction feedstock total weight is considered, then the wood is not favored (4 wt. % as opposed to 10 wt. % from lignosulfonates). This comparison shows that lignins still in timber can only be viably used for vanillin and syringaldehyde production if comprehensive processing of the timber's carbohydrate portion is also performed.

The classical oxidation of wood by nitrobenzene in strongly alkaline medium at 160 °C takes three hours,[11] and the subject of the cellulose remaining in the reaction mass is not touched at all. This is not surprising as under such conditions cellulose can completely dissolve, just like it happens under the harsh conditions of catalytic oxidation by oxygen at high alkali concentration.[12]

Modern research into the production of vanillin, ethanol, and other products from lignocellulosic feedstock generally addresses three-stage processing: alkaline delignification, cellulose hydrolysis, and oxidation of the alkaline lignin.[13] Apparently, the only publications declaring the possibility of the combined production of the aromatic aldehydes and cellulose by oxidation of softwood and hardwood are the patents.[14,15] This approach enables simplification of the softwood processing into vanillin along with the products of cellulose's chemical or microbial conversion (levulinic acid, or glucose and ethanol, respectively).

The goal of the present paper is experimentally evaluating the possibility of pine wood processing by sequential oxidation into vanillin and cellulose, and by enzymatic hydrolysis of the latter into reducing sugars.

EXPERIMENTAL

All reactants were of “pure for analysis” grade, supplied by Reachem (Russia). Air-dried extracted powdered pine wood (*Pinussilvestris*) of size fraction < 0.1 mm was used for the experiments. Removal of wood extractives was carried out according to the standard alcohol–toluene extraction method.[16] Lignin content in the studied materials was measured as the Klason lignin remaining after hydrolyzing the carbohydrate components with 72 vol.% sulfuric acid (as per a standard technique[17]). Cellulose content was measured in the form of Kuerschner cellulose obtained by refluxing a studied sample three times for 1 h with a 1:4 volume mixture of concentrated nitric acid and ethyl alcohol.[18] The lignin content in the extracted timber was found to be 28.7 wt. %, cellulose content was 46.3 wt. %.

Oxidation of the wood was performed in a stainless steel PTFE-sealed batch reactor of 1 l capacity at 160–170 °C and at the oxygen partial pressure 0.2–0.3 MPa(g). Reaction mass stirring was carried out with a magnetic stirrer (800–900 min^{-1}, reactor i.d. 85 mm, stir bar diameter 10 mm, stir bar length 60 mm, PTFE stir bar housing, Reynolds number for pure water $Re = 2.9–3.2 \cdot 10^5$). Oxygen was fed into the reactor from a buffer volume, the feed manually controlled by a valve in order to keep a constant pressure in the reactor, compensating for the oxygen consumption. The reactor was heated by an externally wound electric resistive element (1 kW). The heating element temperature was controlled by an OWEN TRM-502 (Russia) on-off temperature regulator with a type L thermocouple (hysteresis band 2 °C). The heating element temperature setpoint was chosen based on the desired reaction mass temperature, the latter monitored by a TM-902C (China) digital thermometer connected to a type K thermocouple located in the reactor's thermowell.

A reaction mass was prepared by sequentially adding the desired amounts of distilled water, pine wood, NaOH aqueous solution, and aqueous solution of the catalyst ($CuSO_4 \cdot 5H_2O$), all while stirring.

The assembled reactor was flushed by argon with its contents stirred at ambient temperature. Then, with approx. 0.05 MPa(g) of argon (ambient temperature) sealed in it, the reactor was heated to the desired temperature at the rate of 3–8 °C/min.

Upon reaching the desired reactor content temperature, oxygen was introduced into the reactor, and its desired partial pressure was manually maintained along the course of the reaction. After a certain reaction duration, the gas phase was throttled from the reactor into the atmosphere until the saturation pressure of pure water at the reaction temperature remained. Then the reactor was flushed with argon (also throttling it into the atmosphere), and was allowed to cool down by passive heat exchange with the ambient air until the reaction mass temperature 80–90 °C was reached (cooling duration 50–105 min). Finally, the reactor was opened.

Then, an aliquot of 10 ml was taken out of the liquid phase of reaction mass to determine its pH (Mark-901 digital pH meter, Russia). Consequently, the reaction mass was filtered, its solid portion was thoroughly rinsed with distilled water. This solid was mixed with 500 ml of distilled water, the mixture was acidified to pH 2 by 32 wt. % hydrochloric acid while stirring (in order to dissolve the copper oxide). Thus obtained solid was again mixed with 500 ml of water, neutralized by an excess of sodium bicarbonate under stirring, and filtered; while still on the filter, the solid was rinsed with water till neutral pH was achieved. This final solid was dried in ambient air (relative humidity 50–70 %) until a constant weight was obtained, then analyzed for cellulose and lignin content according to methods described elsewhere.[17,18]

Liquid phase from the initial filtering of the reaction mass was acidified by hydrochloric acid to pH 2. Precipitated tars were filtered, the filtrate was sequentially extracted by three chloroform portions (the volume of each equal to the volume of the aqueous phase). The extract was evaporated in a rotary evaporator decreasing its volume below 100 ml. After cooling, chloroform was added to the resulting extract to give the volume of 100 ml. The vanillin content in the extract was then determined by gas chromatography. Chromatographic analyzes were performed with Kristall-2000M chromatograph (Russia), Agilent DB-1 30 m x 0.32 mm column with SE-30 immobilized phase, internal standard—anthracene.

CrI values of the obtained cellulose samples were determined as the peak ratios from their powder X-ray diffractograms, as described elsewhere;[19] the background for each diffractogram was assumed to be a straight line connecting the intensities at 2θ values 7 and 47 ° in the respective recorded pattern. The diffractograms were recorded with a DRON-3 diffractometer (LOMO, Russia), Cu Kα radiation source.

Enzymatic hydrolysis of the LCMsamples obtained in the wood oxidation was performed using a multienzyme preparation comprising the products BrewZyme-BGX by PolfaTarchomin Pharmaceutical Works S.A., Poland (0.1 g per 1 g of the substrate), and CelloLux-A by Sibbiopharm, Russia (0.04 g per 1 g of the substrate). This preparation was previously used for assessing the enzymatic hydrolysis efficiency of technical grade cellulose from nontimber feedstock.[20]

Enzymatic hydrolysis was carried out in acetate buffer (pH 4.7) in Erlenmeyer flasks on a rotary platform shaker (PE-6410 from Ecros-Analytika, Russia) at 150 min^{-1}, temperature 40 °C, for 72 hours. The substrate (total LCM) concentration was 33.3 g/l. After the 72 hours of hydrolysis, another batch of the enzyme preparation (the same amount as was added initially) was again added to the sample, and the hydrolysis process was allowed to continue for 20 hours more in order to exclude the possible influence of the enzyme degradation on the maximum attainable yield.

Concentrations of RS (expressed as g/l of glucose) were determined spectrophotometrically with dinitrosalicylic acid (UNICO UV-2804 spectrometer, USA), relative error of determination 3.5 %. For all the samples, the RS yields at the 72 h and 92 h time points were found to be equal within the error margin. Maximum attainable RS yields are represented as the molar amount of the glucose (based on the average of the RS concentrations at the 72 h and 92 h time points, 1 mol RS assumed to be 1 mol glucose) divided by the molar amount of glucose units in the pre-hydrolysis cellulose contained in the hydrolyzed sample.

RESULTS AND DISCUSSION

Table 1 details the wood oxidation procedures performed in order to obtain the LCM samples for further hydrolysis. The vanillin yield in the oxidation of powdered pine wood catalyzed by copper oxide attains 18.6 wt. % based on the initial lignin. This is quite high, but still lower than the data (23 %) for a similar process performed in a reciprocating shaking reactor with considerably higher mass exchange rates.[2]We link this yield difference to the lower intensity of oxygen transfer into the bulk of the liquid phase observed in the magnetically stirred reactor. In the case of slow transfer of oxygen from the liquid's near-surface region, the produced vanillin can undergo oxidative destruction by the oxidant accumulating in the said region.[21]

It should be noted that focusing on the patterns and relationships between the oxidation conditions and the results thereof falls outside the scope of the present research. Moreover, many of the dependences that could be described here (like the influence of the reaction mass pH on the vanillin yield) have been studied in the past.[21–23] The purpose here is the production of different LCM samples for hydrolysis, and estimating the optimal conditions for producing both vanillin and hydrolysis products.

Table 1. The procedures for oxidation of powdered extracted pine wood catalyzed by in situ produced copper oxide. Unless otherwise stated, the conditions are: 300 ml of water, 160 °C, 0.3 MPa(g) O_2.

Experiment number	Wood[g]	Sodium hydroxide [g]	Copper (II) sulfate pentahydrate[g]	Reaction duration[min]	Final reaction mass pH	Vanillin yield[wt. % of the initial lignin]
1	10.16	6.01	8.03	15	8	5.83
2	10.32	8.3	15.1	20	11	8.47
3	10.04	10.06	10.06	15	>12	12.6
4[a]	20.12	15.07	15.03	15	>12	15.5
5	13.41	10.05	10.02	25	>12	17.1
6	10.07	12.04	8.01	15	>12	11.1
7	13.20	13.21	10.04	25	>12	18.6

[a] 450 ml water, 0.2 MPa(g) O_2.

Table 2 contains the data on the yield and composition of the LCM residues remaining after the oxidation procedures outlined in Table 1, the results of their hydrolysis by the multienzyme preparation are also presented. The cellulose yield after the oxidation experiments 1–6 is 84–93 % based on the wood's initial cellulose, the vanillin yield at the same time is also relatively high, 12–17 wt. % based on the lignin. The best compromise is achieved in the experiment # 5: 17 % vanillin and 84 % cellulose yields based on the corresponding initial substances. Therefore, there exist certain optimal conditions of vanillin production from wood under which the catalytic oxidation is essentially oxidative delignification, and it is quite selective. For comparison, it can be noted that the cellulose yield in the sulfate delignification at the stage of unbleached technical product attains 90 % of the wood's cellulose.[24] In case the catalytic oxidation is targeted at the maximum vanillin yield (18 %), the cellulose yield decreases to 64 % of the initial compound.

Table 2. The yield and properties of the LCM samples produced in catalytic wood oxidation. For the LCM preparation conditions, please see Table 1.

Experiment number	Vanillin yield [wt. % of the initial lignin]	LCM yield[wt. % of the initial wood]	Cellulose content in the LCM[wt. %]	Lignin content in the LCM [wt. %]	Cellulose yield[% of the w ood's initial cellulose]	CrI[%]	RS yield in hydrolysis based on the LCM cellulose[%]
1	5.83	50.0	83.0	13.3	89.6	78.2	48.2
2	8.47	48.9	82.0	15.7	86.6	76.6	38.5
3	12.6	46.5	87.0	4.79	87.5	79.7	57.9
4	15.5	69.9	61.5	21.7	92.9	75.4	17.6
5	17.1	49.5	78.6	5.90	84.0	77.9	48.3
6	11.1	45.3	89.0	1.68	87.0	81.4	69.6
7	18.6	32.3	91.6	1.49	63.9	82.7	64.9
8[a]	-	100	46.3	28.7	100	64.2	16.2

[a] the initial pine wood.

The dependences of the RS concentrations versus the duration of the enzymatic hydrolysis of the samples are presented in Figure 1a. The highest cellulose hydrolysis rates as well as eventual yields (57–70 % of the loaded compound hydrolyzed) are attained for the samples with the highest alkali to wood ratios during the oxidation step (Table 1, experiments 3, 6–7). Obviously, this is because higher alkali content leads to more intense destruction of the lignin and its removal from the cellulose surface.

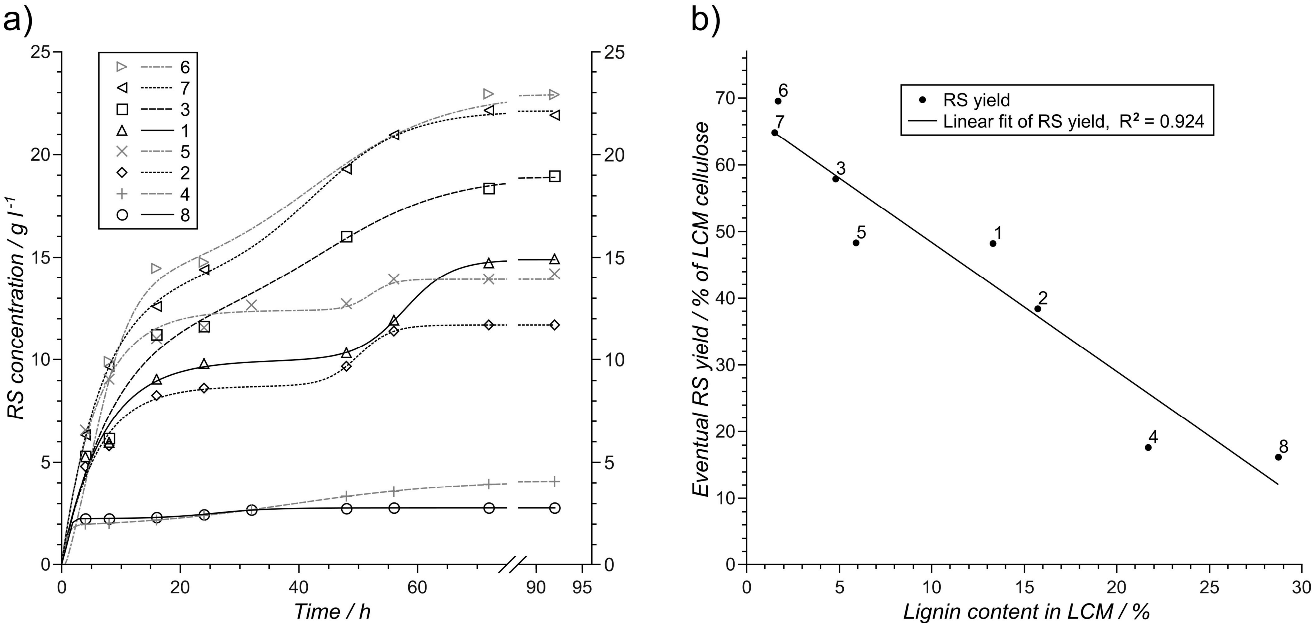

Figure 1. (a) The dependence of the RS concentration versus the duration of the enzymatic hydrolysis of the LCM samples obtained in vanillin synthesis from wood. The symbols represent experimental data; the lines are only for the ease of perception and do not mean to represent the hydrolysis dynamics in any way. (b) The correlation between the eventual RS yield in enzymatic hydrolysis and the lignin content in the samples before hydrolysis. Numbers in the legend in (a) and data labels in (b) correspond to the procedures for obtaining LCMs, as detailed in the Table 1.

The role of lignin presence in LCM can be further emphasized by the correlation between the eventual RS yield in cellulose enzymatic hydrolysis and the lignin content in the LCM samples before hydrolysis (Figure 1b). Such correlations are well known[25] and could be related to considerable adherence between cellulose and the noncellulosic timber portion. This results in the cellulose becoming obstructed during enzymatic hydrolysis, higher lignin content in the lignocellulosic matrix leading to stronger hydrolysis hindrance. Different approaches to breaking down this matrix are known (chemical, physicochemical, or mechanical treatment[26,27]), but using catalytic oxidation for this purpose has not been described in literature.

Figure 2a presents the correlation between the CrI values of cellulose in the LCM samples after wood oxidation and the eventual RS yield in their enzymatic hydrolysis. The crystallinity data are obtained from X-ray diffractograms (Figure 2b). It appears that the hydrolysis yield increases concordantly with the cellulose CrI. Such correlation is the opposite of what could be expected based on prior studies (e.g., this source[25]). To better comprehend the relation obtained here, it should be noted that CrI increases as the content of lignin in the LCM samples decreases, as can be seen in Figure 2a. Therefore, it appears that lignin content plays the major role on the enzymatic hydrolysis outcome, whereas cellulose crystallinity does not considerably matter for the samples produced by the oxidative delignification presently utilized. In this context, quite noteworthy is a paper that describes independence between the cellulose crystallinity and its conversion in enzymatic hydrolysis, i.e., the enzymatic hydrolysis rates for the amorphous and crystalline cellulose portions do not meaningfully differ.[28]

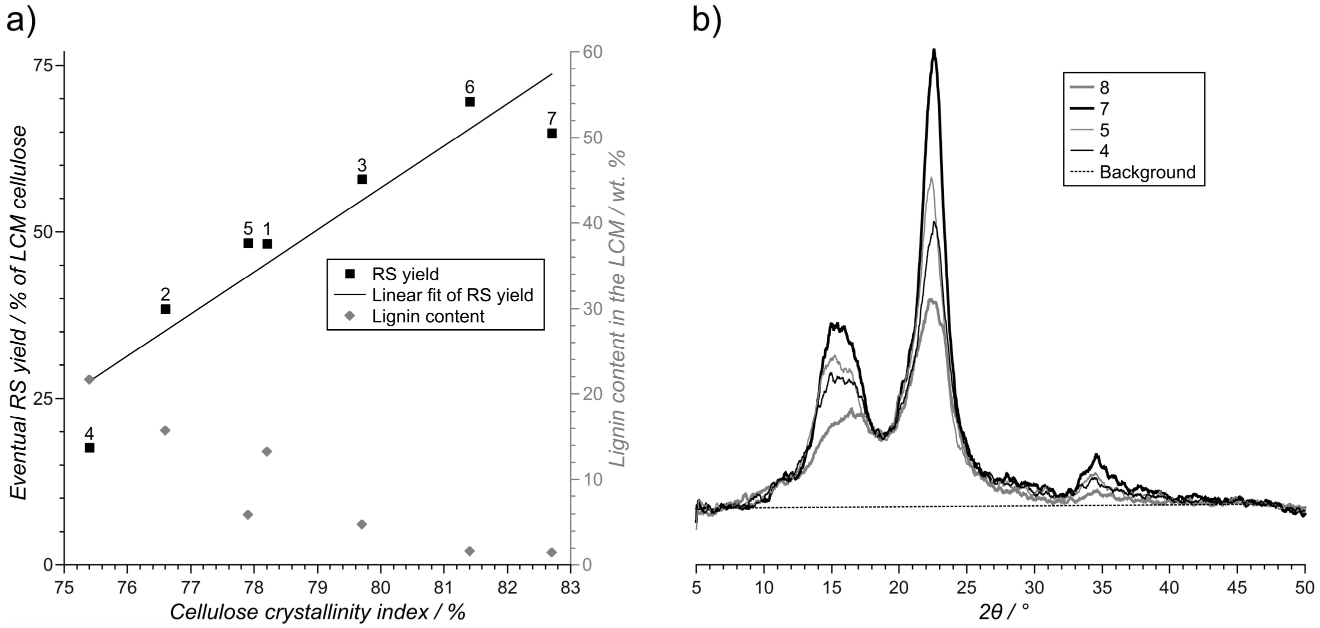

Figure 2. (a) The correlation between the CrI of cellulose in the LCM samples after wood oxidation and the eventual RS yield in their enzymatic hydrolysis (for the hydrolysis data, please see Figure 1a). The lignin content values for the LCM samples are also present to emphasize the role of lignin content on the hydrolysis outcome. (b) Powder X-ray diffractograms of several LCM samples after wood oxidation. Data labels in (a) and numbers in the legend in (b) correspond to the procedures for obtaining LCMs, as detailed in the Table 1.

The obtained results can be compared to literature data. For example, even bleached eucalyptus Kraft cellulose after one freeze-thaw cycle is hydrolyzed with only 40 % yield by the Novozym 476 endoglucanase preparation (Novozymes A/S).[29] In our experiments with the best vanillin output, RS yields in the range 48–65 % were obtained. Another interesting fact is that higher enzymatic hydrolysis efficiency in LCM samples prepared by catalytic oxidation can be achieved without considerable cellulose loss during the delignification: Kraft pulping yields approx. 90 % of the initial wood cellulose.[24] Similar figures were obtained for most of our oxidation procedures, but the resulting cellulose is hydrolyzed at least 1.5 times better than the aforementioned eucalyptus cellulose. I.e., delignification by catalytic oxidation “softens” the cellulose for enzymatic hydrolysis without sacrificing any considerable portion of it as a side effect.

On the other hand, some lignocellulosic materials of different origins are hydrolyzed by enzymes almost completely. Such result is obtained, e.g., for the timber treated by the solution of sodium bisulfite combined with sulfuric acid.[30] However, this success of enzymatic hydrolysis can be caused by the preceding cellulose partial hydrolysis during the acid treatment at 180 °C. Indeed, the timber activation by sodium bisulfite without the acid allows achieving only 68 % yield in enzymatic hydrolysis.[30]This comparison suggests that the cellulose enzymatic hydrolysis outcome strongly depends on the conditions of the LCM preparation. Overall, the preparation of pine wood for enzymatic hydrolysis by catalytic oxidation into vanillin as studied in this paper is efficiency-wise on par with many other approaches described in literature.

CONCLUSION

The present study demonstrates the possibility of single-step catalytic oxidation of pine wood into cellulose and vanillin, with the yields exceeding correspondingly 84 and 70 % of the theoretical limit for the substance in question.[21]This process can be viewed as catalytic alkali–oxygen delignification of wood also producing vanillin as a side product with considerable yield.

The cellulose obtained in this oxidation process can be enzymatically hydrolyzed with up to 70 % yield of RS which can serve as feedstock for bioethanol production. In terms of enzymatic hydrolysis, this cellulose performs favorably compared to the product of the industrially dominant Kraft pulping. The RS yield in the enzymatic hydrolysis of the studied cellulose samples increases with the increase of the actual cellulose content in the material undergoing hydrolysis (i.e., with the decrease lignin content). This corresponds to the general trend observed for cellulose enzymatic hydrolysis. Cellulose crystallinity appears to not play any considerable role in determining the enzymatic hydrolysis outcome for the lignocellulosic samples obtained by the catalytic wood oxidation, at least when compared to the factor of the lignin content in the samples.

Using the same enzyme preparation, it was shown that the cellulose obtained in the catalytic wood oxidation hydrolyzes much easier than the initial wood. Together with the data on the maximum attainable vanillin yield from native lignins and the vanillin yields actually obtained in this work, this means that out of the two possible sequential methods for processing wood into vanillin and hydrolyzed sugars—enzymatic hydrolysis with subsequent lignin oxidation as opposed to oxidation first with subsequent cellulose hydrolysis—the latter is preferable.

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ABBREVIATIONS

CrI, crystallinity index; LCM, lignocellulosic material;MPa(g), megapascal (gauge pressure); RS, reducing sugars.

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