ORIGINAL RESEARCH

Stoichiometry of reactions of ozone and hypochlorous acid with lignin and hexenuronic acid and its chlorination

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Abstract The stoichiometry of ozone and hypochlorous acid reactions with lignin and hexenuronic acid (HexA) was measured in bleaching experiments of Eucalyptus sp. kraft pulp. The progress of the reactions was followed by UV Resonance Raman spectroscopy that can quantify lignin and HexA based on the Raman scattering intensities of the carbon–carbon double bond in HexA and the aromatic ring in lignin. Here, one mol of ozone converted 0.16 mol of lignin (C9 monomer units) and 0.28 mol of HexA, whereas 1 mol of hypochlorous acid converted 0.09 mol of lignin and 0.23 mol of HexA. The use of a tertiary amine catalyst with the hypochlorous acid treatments did not affect these stoichiometries. The stoichiometric ratios showed that ozone was more efficient in oxidizing lignin than hypochlorous acid, while both electrophiles reacted with HexA to a similar extent.

HexA reaction by hypochlorous acid was concluded to involve initial electrophilic chlorination of the carbon–carbon double bond, contributing to significant organochlorine (OX) formation in the pulp. Evidence on this was the linear correlation between the initial HexA content and OX (0.59 mol OX per mol HexA) and the high OX content in the xylan extracted from the bleached pulp. The 2D NMR HSQC and TOCSY spectra of the isolated xylans showed the disappearance of HexA signals after the treatment with hypochlorous acid and the appearance of a new spin system, yet to be fully identified.

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Introduction

Ozone (O_3) and hypochlorous acid (HOCl) are well known electrophilic agents that are widely used in water treatment and disinfection applications because they efficiently oxidize a wide range of organic substrates (Jyoti and Pandit 2004; Gombas et al. 2017; Ye et al. 2019). O_3 is also utilized in the wood pulp industry, more specifically in pulp bleaching, where it is a well-established bleaching chemical, whereas HOCl has never been used directly for this application. However, recent laboratory studies on bleaching with HOCl have shown

that, even at low dosages, HOCl can be an extremely efficient oxidant (Isaza Ferro et al. 2021), generating the question of how different the efficiencies of these two bleaching chemicals are.

O_3 is the main oxidant for totally chlorine free pulp bleaching technologies. It degrades lignin, hexenuronic acid (HexA) and chromophores, but it can also depolymerize cellulose (Table 1) (Alen et al. 2011). More specifically, O_3 reacts two times faster with HexA than lignin, and a 1000 times faster with lignin than with cellulose (Eriksson 1985; Wennerström 2005; Ventorim et al. 2008). Ozone's reaction with HexA generates oxalic acid and hydroxyl radicals (Vuorinen et al. 1997; Pouyet et al. 2014). With lignin, it reacts by 1,3-dipolar cycloaddition across a double bond forming a primary ozonide, which leads to hydrogen peroxide, muconic acid analogues and structures with carbonyl groups (Gierer 1982, 1997)·Direct cellulose depolymerization by O_3 only occurs when HexA and lignin amounts are low, by attacking cellulose anomeric carbon atoms or by reacting with the glycosidic oxygen atom (Pan et al. 1981). Additionally, the hydrogen peroxide and hydroxyl radicals (formed in situ) contribute to carbohydrate degradation in Z stage (Chirat and Lachenal 1997; Magara et al. 1998; Pouyet et al. 2013). The stoichiometry of lignin degradation with O_3 has been studied in bleaching experiments with straw and model compounds, where it was identified that 0.2–0.3 mmol lignin were degraded per mmol O_3 (Ben'ko et al. 2003, 2020). Whereas, for the reaction of O_3 with HexA there are no stoichiometric ratios available in literature.

On the other hand, as stated earlier, HOCl has not been used directly in industrial pulp bleaching. It is an unstable compound that raises environmental concerns regarding the formation of organochlorinated compounds (Suess 2010). Instead, HOCl is an intermediate in chlorine dioxide (ClO_2) bleaching, the most common wood pulp bleaching technology. HOCl is produced when ClO_2 reacts with lignin (Kolar et al. 1983). Once produced, it plays a crucial role in chlorine dioxide regeneration reaction, increasing the overall delignification of the bleaching stage (Tarvo et al. 2009; Lehtimaa 2010). The reactions of HOCl with lignin and HexA, have not been studied in detail but as a part of other bleaching treatments. However, as an electrophile, HOCl could react with lignin leading to muconic acid, quinones, lignin depolymerization and chlorination (Table 1) (Gierer 1982; Alen et al. 2011). In contrast, with HexA, HOCl is expected to react via chlorination, contributing to the OX of bleached pulps and AOX of bleaching filtrates (Ventorim et al. 2005).

Only recently, the direct application of HOCl in wood pulp bleaching has gained attention from scholars. Short laboratory bleaching sequences, which include a first stage with low amounts of HOCl, produced fully bleached pulps in nearly 1 h (Afsahi et al. 2015). The stages with HOCl may be, uncatalyzed (H_{mild}) or catalyzed (H_{cat}). H_{mild} is a 10 min bleaching stage that uses HOCl (1% active chlorine (Act. Cl) on dry mass of pulp), pH 5 and 60 °C (Isaza Ferro et al. 2021). H_{cat} is similar to H_{mild}, but it is a faster stage due to the addition of a tertiary amine catalyst, and the consequential

Table 1 Main reaction types of lignin and HexA with ozone and hypochlorous acid

Oxidant	O_3	HOCl
Primary reactions	Oxidative cleavage of carbon–carbon double bond (HexA)[a] Oxidation of aromatic ring to muconic acid type of esters (lignin)[a]	Electrophilic addition of chlorine on carbon–carbon double bond (HexA)[a] Oxidation of aromatic ring to quinone and muconic acid type of structures (lignin)[a] Chlorination of aromatic ring (lignin)[b]
Secondary reactions	Release of oxidized HexA and lignin structures, e.g., oxalic acid, through ester hydrolysis	Release of aliphatic OX through nucleophilic substitution (HexA)
Side reactions	Hydroxyl radical formation and subsequent oxidation of cellulose	

[a]The progress of these reactions can be monitored by UV Resonance Raman spectroscopy, which detects the C=C bond of HexA and the aromatic ring in lignin

[b]Chlorination by HOCl leads to OX formation in the pulp or release of AOX in the filtrate

formation of a highly active chloroammonium cation (Afsahi et al. 2015).

Previous studies have shown the efficiency of H_{cat} and H_{mild} compared to D stage (Isaza Ferro et al. 2021). H_{cat} and H_{mild} stages were more efficient and selective towards delignification, even with smaller Act. Cl dosages, than D stage (Isaza Ferro et al. 2021). The pulp-bound organochlorinated compounds (OX) in H_{cat}/H_{mild} pulps were degraded in posterior Z and P bleaching stages, producing fully brightened pulps with low OX values (Isaza Ferro et al. 2021). The Adsorbable Organic Halides (AOX) produced in H_{cat}/H_{mild} stages were treated with aerobic bacteria and degraded to similar extend than the AOX formed in a D stage, reaching values accepted by the industry for AOX emissions (Isaza Ferro et al. 2021). Seeing that H_{cat} and H_{mild} stages delivered pulps and effluents with similar characteristics, it was proposed that the reaction routes and stoichiometries were similar in both bleaching stages (Isaza Ferro et al. 2021). However, more direct evidence is required to prove this hypothesis.

Nowadays, there is no clear comparison between efficiencies of HOCl and O_3 for the degradation of lignin and HexA in wood pulp. Hence, this article intends to identify the stoichiometry of HOCl and O_3 reactions with lignin and HexA, in bleaching experiments. These values will provide a more objective perspective on the efficiencies of these chemicals for pulp bleaching, which is interesting considering that both chemicals react fast even at low temperatures. This data will also show if H_{cat} and H_{mild} have the same stoichiometric ratios for the degradation of lignin and HexA. In this article degradation or conversion refers to changes in the molecular structure of lignin and HexA, not only to the breaking of the molecules into smaller ones. More specifically, the term is used to express the change on lignin's aromatic character and the breaking of C=C or C=O bonds of HexA, measured with Ultraviolet Resonance Raman (UVRR) spectroscopy. Finally, a new hypothesis is proposed, stating that HOCl chlorinates HexA without releasing it from the xylan chain in pulp, and assessed by OX measurements and Nuclear Magnetic Resonance (NMR) of the extracted xylans.

Materials and methods

Materials

An oxygen-delignified *Eucalyptus sp.* kraft pulp with kappa number (KN) 11.9, 50.9% ISO brightness and 1100 ml/g viscosity, provided by a Brazilian pulp and paper mill, was used in the bleaching experiments.

Commercial 10% sodium hypochlorite (NaOCl) solution, sodium thiosulfate, potassium iodide, 1 M sulfuric acid and citrate pH 5 buffer were purchased from VWR.

Dimethyl sulfoxide (DMSO), ≥ 97.5 sulfuric acid and sugar monomers > 99% (D-(+)-xylose, D-(+)-galactose, D-(+)-glucose and L-rhamnose monohydrated) were obtained from Sigma Aldrich. D-(+)-mannose > 99% was acquired from Fluka. Copper Ethylenediamine (CED) solution (SCAN 16:62) was obtained from OY FF-Chemicals Ab. NMR solvents, deuterated dimethylsulfoxide (DMSO-d6) 99.8% and deuterium oxide were bought from Merck. 1-Ethyl-3-methylimiddazolium acetate (EMIM OAc) ≥ 95% was acquired from Proionic.

Ozone (O_3) was produced via oxygen electrolysis in a Wedeco GSO 30 reactor at a rate of 0.117 ± 0.01 g O_3/min. The tertiary amine catalyst, CEM-DABCO (1-carboethoxymethyl-1-azonia-4-aza-bicyclo[2.2.2] octane chloride) was synthetized according to Isaza Ferro et al. (2020). ^{1}H NMR (400 MHz, D_2O): δH 1.20 (3H, t, $^3J_{HH}$ = 7.2, $COOCH_2CH_3$); 3.16 (6H, t, $^3J_{HH}$ = 7.6, $(CH_2)_3N^+$); 3.59, (6H, t, $^3J_{HH}$ = 7.6, $(CH_2)_3N$); 4.17 (2H, s, N^+CH_2COOEt); 4.21 (2H, q, $^3J_{HH}$ = 7.2, $COOCH_2CH_3$). ^{13}C NMR (400 MHz, D_2O): 13.10, 43.97, 52.79, 61.67, 63.42, 164.70.

Bleaching experiments and pulp characterization

The molar ratio method was used to identify the stoichiometry of the reactions of O_3 and HOCl with lignin and HexA. Hence, the eucalyptus pulp (42 mmol HexA and 44 mmol lignin C9 monomeric units per kg pulp) was bleached with varying oxidant dosages. The residual lignin and HexA contents were plotted against the oxidant dosage, with the maximum slope or slope in the first part of the plot corresponding to the stoichiometric ratio of the reaction.

Lignin and HexA quantities were measured using Ultraviolet Resonance Raman (UVRR) spectroscopy according to (Montet 2021). This method quantifies

lignin and HexA by using the normalized (relative to cellulose) intensity of lignin's aryl band (symmetric aryl ring stretch) at 1605 cm^{-1}, and of HexA's C=C and C=O bonds at 1655 cm^{-1} (Montet 2021). Montet's quantitative method is an improved procedure based on Jääskeläinen et al. (2005). In comparison with the original method, Montet's method is more accurate because it removes the effect of the mutual overlapping of lignin and HexA signals by deconvolution of the UVRR spectra. The method provides HexA in μmol/g pulp, and lignin in % (mg/g pulp). Hence, the equivalent moles of lignin were calculated by using molar mass of 230 g/mol for hardwood lignin C9 units $(C_9H_{10.52}O_{3.36}(OCH_3)_{1.20})$ (Jahan et al. 2012).

Pulp bleaching experiments with HOCl (expressed as Act. Cl or Cl$_2$), included: a bleaching stage at mild conditions (H$_{mild}$) and a catalytic pulp bleaching stage (H$_{cat}$), with 0.1% addition of a tertiary amine catalyst (CEM-DABCO) on pulp. H$_{mild}$ and H$_{cat}$ stages were made in plastic bags at pH 5–5.5, 60 °C and 10% weight pulp consistency, with NaOCl dosages from 0.2 to 2.5% Act. Cl on pulp. The plastic bags, containing the pulp and reagents, were placed inside a water bath at 60 °C and mixed manually for 10 to 30 min, until all the HOCl was consumed. Bleaching with O$_3$ (Z stage) was carried out in a glass balloon flask at 30% consistency, 20 °C and constant agitation. The O$_3$ dosages varied from 0.5 to 1.5% O$_3$ on pulp, and the initial pH was 2.9. NaOCl and O$_3$ were titrated daily before the experiments according to SCAN-C 29:72. The HOCl and O$_3$ amounts presented in this article correspond to the amounts dosed into the system.

Pulps with different initial HexA content were produced by exposing the eucalyptus pulp to hot acid treatments (A stage) in plastic bags, at 90 ℃, 10% pulp consistency and pH 2.9. The pH was controlled by the addition of sulfuric acid, and the duration of the stage varied from 2 to 10 h. These pulps were used to identify possible correlation between initial HexA content and pulp-bound organochlorinated compounds (OX) production by HOCl.

The OX content was determined according to ISO 11480 using a Trace Elemental Instruments Xplorer with duplicates. The standard error was ± 30 mg OX/kg.

The bleached pulps were washed with deionized water, centrifuged, homogenized, and stored in the fridge. Hand-sheets were made according to the standard method SCAN-CM 11:95 and used in ISO brightness and UVRR measurements. Viscosity was measured according to SCAN-CM 15:99. Structural carbohydrates were quantified following the NREL/TP-510-42618 laboratory analytical procedure, using a Thermo Scientific Dionex ICS 5000$^+$ DC detector coupled with a Dionex ICS-500$^+$ single pump and a Dionex ASP-AP autosampler.

Extraction of hemicelluloses

Hemicellulose samples from eucalyptus oxygen delignified and HOCl treated (H$_{mild}$ 2% Act. Cl) pulps were extracted with the ionic liquid EMIM OAc. The extraction was done in a closed stainless-steel reactor using a EMIM OAc to distilled water ratio of 85:15, 5% weight pulp consistency, 30 rpm, 60 °C for 3 h. The pulp was filter pressed, using a 5 μm pore size metallic mesh, and the dissolved hemicelluloses in EMIM OAc were recovered. The pulp was washed again with a mix of EMIM OAc and distilled water (85:15) and filtered to collect the remaining solubilized hemicelluloses. The collected solutions were combined, and hemicelluloses were precipitated by distilled water addition and centrifugation. Thereafter, the samples were collected and freeze dried according to Roselli et al. (2014).

Hemicellulose characterization

The hemicellulose samples were characterized with FT-IR (Fourier transform infrared), UVRR (Ultraviolet Resonance Raman), 2D NMR (Nuclear Magnetic Resonance) spectroscopy as well as OX measurements.

The FT-IR spectra were measured with a PerkinElmer FT-IR instrument (Spectrum Two model). The UVRR spectra were measured as described earlier (Montet 2021). The NMR spectra of the extracted hemicelluloses were measured in DMSO-d$_6$ at 298 K using a Bruker Avance NEO 600 spectrometer equipped with a 5 mm He-cooled TCI cryoprobe operating at 600.13 and 150.90 MHz for ^{1}H and ^{13}C, respectively. Chemical shifts were referenced to DMSO at 2.5 ppm (^{1}H) and 39.52 ppm (^{13}C). ^{1}H spectra were acquired using a 1 s relaxation delay, 64 transients, a spectral width of 16 ppm, and 64 K data points. ^{13}C spectra were recorded using 2 s relaxation

delay, a spectral width of 235 ppm, 5 K transients, and 64 K data points. Phase-sensitive 2D $^1H,^{13}C$ HSQC spectra were acquired with spectral widths of 11 ppm for ^1H and 215 ppm for ^{13}C using 2 s relaxation delay, 1 K data points, 256 t_1 increments, and 36 transients. An adiabatic version of the HSQC experiment was used (pulse program: hsqcetgpsisp.2). Phase-sensitive 2D $^1H,^1H$ TOCSY spectra were acquired with spectral width of 13 ppm in both dimensions using 2 s relaxation delay, 2 K data points, 256 t_1 increments, and 16 transients. All TOCSY data were obtained with a mixing time of 80 ms using the DIPSI-2 spin-lock sequence (pulse program: dipsi2gpphzs).

Results and discussion

Stoichiometry of hexenuronic acid degradation by ozone and hypochlorous acid

The stoichiometric ratios of HexA degradation by ozone (O_3) and hypochlorous acid (HOCl) were calculated from the slopes of Fig. 1a–c. Here, oxygen delignified eucalyptus pulp with an initial HexA content of 42 mmol HexA/kg pulp was treated with variable amounts of O_3 (in Z stage) or HOCl (during H_{mild} and H_{cat} stage). The change of HexA content as a function of added oxidant dosage revealed the stoichiometric ratios of the reactions. One mmol of O_3 degraded 0.28 mmol HexA (Fig. 1a). Whereas one mmol of HOCl degraded 0.22 and 0.23 mmol HexA, in the catalyzed and uncatalyzed reactions, respectively (Fig. 1b, c). Hence, O_3 was only 20% more

efficient for HexA degradation, breaking of its C=C and C=O bonds, than HOCl.

HexA's degradation continued linearly until most of it was consumed. Additionally, the R^2 for all the linear regressions, used to identify the slopes or stoichiometric ratios, were > 0.99 suggesting a high accuracy of the measurements (Fig. 1a–c).

The residual, 'unreactive' HexA content was 3–5 mmol/kg depending on the treatment (Fig. 1). This low residual and unreactive HexA relates to previous studies, which proposed that it is not HexA and it actually corresponds to other structures with UVRR shifts similar to HexA's (Pouyet et al. 2012; Montet 2021).

Stoichiometry of lignin degradation with ozone and hypochlorous acid

The stoichiometry of the reactions of O_3 and HOCl with lignin were identified in a similar manner than for HexA. Eucalyptus pulp, with initial C9 lignin content of 44 mmol /kg of pulp, was treated with variable dosages of oxidants (Fig. 2). The stoichiometric ratios of lignin degradation by O_3 and HOCl were identified from the slopes in Fig. 2a–c. One mmol of O_3 degraded 0.16 mmol lignin units (C9), while 1 mmol of HOCl degraded 0.09 mmol lignin units, in both H_{mild} and H_{cat} treatments. The stoichiometric ratios of 0.16 mmol lignin/mmol O_3 and 0.09 mmol lignin/ mmol HOCl, suggest that O_3 is a 70% more efficient than HOCl for lignin degradation (or disappearance of lignins aryl group) (Fig. 2). The measured stoichiometric ratio of lignin degradation by O_3 of 0.16:1
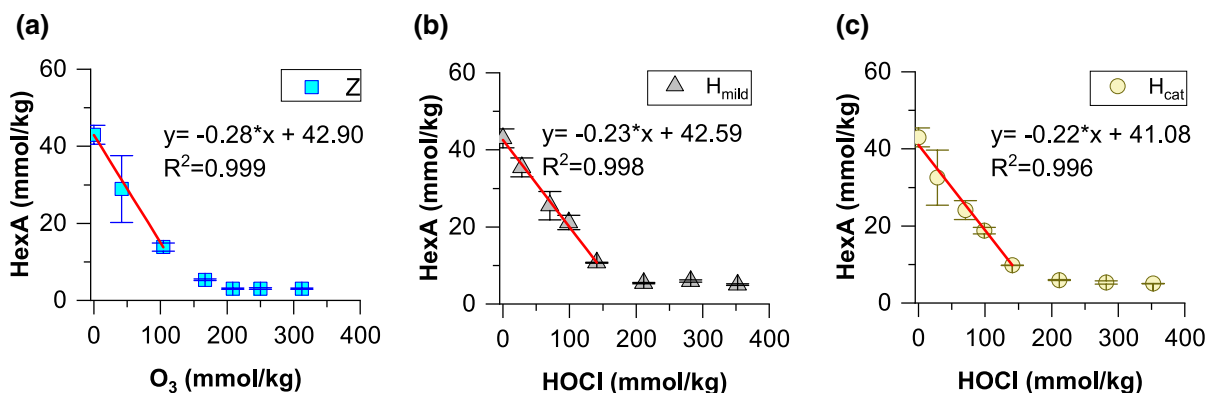
Fig. 1 HexA degradation with increasing dosage of: **a** O_3 during Z stage, **b** HOCl during H_{mild} stage and **c** HOCl during catalytic stage H_{cat}

(a)

y= -0.16*x + 44.70
R^2=0.999

(b)

y= -0.09*x + 44.64
R^2=0.986

(c)

y= -0.09*x + 45.11
R^2=0.976
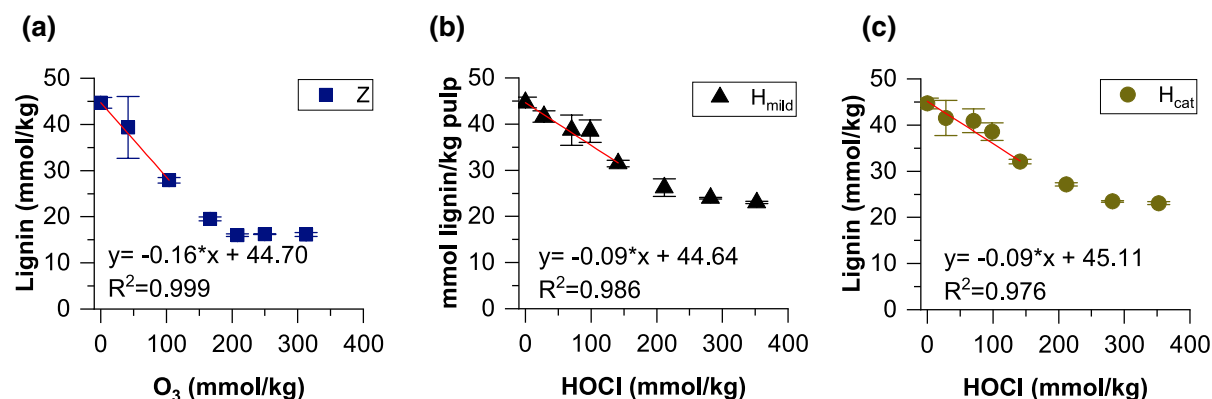
Fig. 2 Lignin degradation with increasing dosage of: **a** O_3 during Z stage, **b** HOCl during H_{mild} stage and **c** HOCl during catalytic stage H_{cat}

is lower than the stoichiometric ratio of 0.2–0.3:1 reported by Ben'ko et al. (2003, 2020). This apparent lower stoichiometric ratio of lignin degradation by O_3 is due to the presence of HexA in the sample, which also consumed O_3.

O_3 degraded lignin to a higher extent than HOCl did, probably due to the presence of hydroxyl radicals formed in Z stage (Ek et al. 1989; Pouyet et al. 2013). However, the residual lignin amounts remained constant even at large oxidant dosages for both oxidants. The residual lignin content for Z pulps was 16 mmol/kg pulp, whereas for HOCl treated pulp it was 23 mmol/kg pulp. These plateaus are well known in pulp bleaching, and they are one of the main reasons to have bleaching sequences with alternating oxidizing and alkaline stages, which solubilize the oxidized lignin.

Overall, it is evident that the catalyst used in the HOCl treatments did not affect the stoichiometry of the reactions with HexA and lignin (Figs. 1, 2). This confirms the observation from previous studies, that the catalyst only increases the speed of reaction of the HOCl with the substrates (Isaza Ferro et al. 2021). Therefore, only the data from H_{mild} will be taken into consideration from this point onwards.

Reactions taking place in Z and H_{mild} stage, including lignin degradation, are complex and vary widely depending on the oxidant used. O_3 can react further with the muconic acid like structures initially formed (Gierer 1982). Whereas HOCl, may chlorinate lignin, decreasing its reactivity for further oxidation (Dence et al. 1996; Tarvo et al. 2010; Alen et al. 2011). But

overall is evident that both electrophiles were more selective towards HexA than lignin degradation.

Additionally, identifying possible cellulose degradation, especially at low oxidant dosages, where the stoichiometric ratios were calculated (0–150 mmol oxidant/kg pulp), is important because it could affect the stoichiometric values presented above. This was assessed by measuring the intrinsic viscosity of the samples (Fig. 3). To avoid underestimating the intrinsic viscosity of pulps with CO groups (Z and possibly H_{cat} and H_{mild}), the pulps were treated with an alkaline (E) stage before the viscosity measurement with CED (Pouyet et al. 2014). CO groups cause carbohydrate chains scissions in the CED measurements via β-elimination (Lewin et al. 1997).

The viscosity of the pulp decreased rapidly even with low O_3 dosages (Fig. 3a), which can be mostly attributed to the reaction of the formed hydroxyl radicals with cellulose, just like proposed by Pouyet et al. (2013). The oxygen-delignified unbleached pulp (KN 11.9) contained abundantly HexA and lignin, which reacted with O_3 producing hydroxyl radicals that lead to a sharp viscosity loss. A direct cellulose depolymerization by O_3 is possible but unlikely at this low O_3 dosages, due to the faster reaction of O_3 with HexA and lignin than with cellulose (Eriksson 1985; Wennerström 2005). Increasing the ozone dosage from 200 to 300 mmol/kg didn't lower the pulp viscosity further which confirms that O_3 did not directly react with cellulose (Fig. 3a).

The viscosity loss in H_{cat} and H_{mild} experiments was very minor (Fig. 3b–c), and there was no change
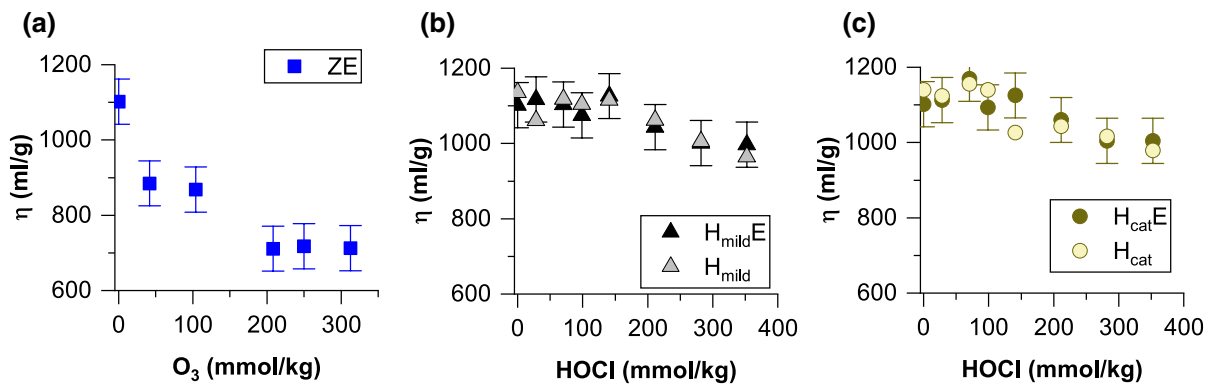
Fig. 3 Intrinsic viscosity (η) of: **a** ZE, **b** H_{mild}/E and **c** H_{cat}/E treated pulps

in viscosity at low HOCl dosages (at < 200 mmol HOCl/kg pulp). Also, there was no difference between the viscosity of the H_{mild} and H_{cat} treated samples and the pulps with posterior E stage, suggesting that the formation of CO groups was minimal in these treatments.

Overall, from the decrease in viscosity of the Z, H_{mild} and H_{cat} samples, at low oxidant dosages, it can be assumed that cellulose degradation did not consume significant amounts of O_3 or HOCl directly, and hence, the lignin and HexA degradation stoichiometric ratios were not substantially affected.

On the other hand, HOCl is known to chlorinate HexA, during chlorine dioxide bleaching, and to form soluble organochlorinated compounds (AOX) in the bleaching filtrate (Ventorim et al. 2005). Nearly 80% of the AOX from D stage can be traced to soluble chlorinated HexA compounds. For example in a traditional D stage, 53 out of 60 mg AOX/kg pulp could be attributed to HexA (Freire et al. 2003). Instead, this article proposes that a significant part of the chlorinated HexA may initially remain in the pulp as OX, which has not been proved before. Hence, the next sections aim to finding enough evidence to prove this chlorination hypothesis and attempting to characterize the chlorinated compound.

Chlorination of HexA

The hypothesis that HOCl converts HexA, while forming OX, was studied by exposing oxygen delignified eucalyptus pulps with different initial HexA contents to a constant dosage of HOCl (1% Act. Cl). Through plotting the OX content of the treated pulps against their initial HexA content (Fig. 4), a linear correlation was found. The slope of the linear fitting indicates that the molar ratio between HexA content and OX formation was 0.59, indicating that OX formation was a major reaction in H_{mild} treatment. From the linear equation presented in Fig. 4, it is evident that only a minor part of OX was not related with HexA content, indicating the smaller role of lignin chlorination in the initial OX formation.

Characterization of H_{mild} extracted hemicelluloses

To identify the mechanism of OX formation from HexA, hemicelluloses from the unbleached eucalyptus oxygen delignified and H_{mild} (2% Act. Cl) pulps were extracted using EMIM OAc as solvent. Sugar analysis of the pulps before and after the extraction
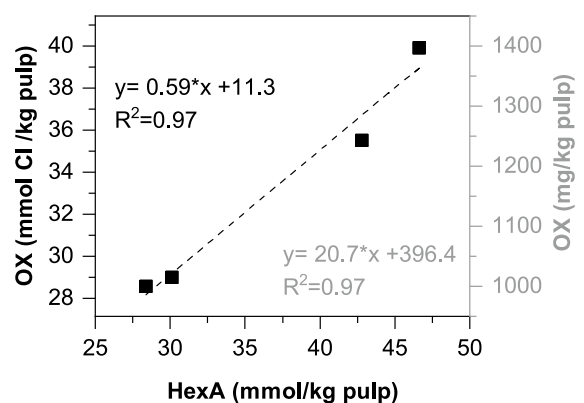
Fig. 4 OX content of kraft pulps after H_{mild} stage (1% act. Cl per gram of pulp) according to their initial HexA content

was done to identify the efficacy of the extraction process.

According to the sugar analysis, the H_{mild} and oxygen delignified pulps consisted mainly of cellulose (glucose) and xylan (xylose). The EMIM OAc treatment extracted most ($> 70\%$) of the hemicelluloses from the pulps. However, only ~24% of the extracted hemicelluloses was precipitated and prepared for further analyses. Hence, the overall yield of the recovered hemicelluloses was 3.3% of the pulp.

OX of extracted hemicelluloses and pulps before and after the extraction

Because HexA is bound in xylan, the extracted hemicelluloses should have a higher OX content than the original pulp. Hence, the OX of the extracted hemicelluloses and pulps before and after the extraction were measured (Table 2). The extraction treatment of the H_{mild} treated pulp decreased its OX content by 77%, from 958 mg/kg to only 218 mg/kg, which relates nicely to the 70% hemicellulose extraction mentioned above. The OX content of the isolated hemicelluloses from the H_{mild} treated pulp was 7 times higher or 1749 mg/kg. Unfortunately, it was not possible to close the mass balance of OX because the applied conditions degraded part of OX during the extraction and purification steps, and as explained earlier, a large part of the hemicelluloses originally in H_{mild} pulp were lost in the precipitation process.

The high OX content (958 mg/kg pulp) in H_{mild} pulp before the extraction is due to the large HOCl dosage used in the bleaching treatment (2% Act. Cl). This large HOCl dosage was used to guarantee the abundance of the chlorinated HexA. Consequently, this OX content is much higher than the reported OX from traditional D stages (125 mg/kg pulp) and from H_{mild} (1% Act. Cl) ZP bleaching sequence (with final OX of 148 mg/kg pulp) (Isaza Ferro et al. 2021).

FT-IR and UVRR spectroscopy

FT-IR and UVRR spectroscopy were used to characterize the extracted hemicelluloses and to detect possible changes in them caused by the H_{mild} treatment.

The FT-IR spectra of the extracted hemicelluloses from oxygen delignified and H_{mild} pulps presented similar bands at 895, 984, 1040, 1164, 1241, 2886, 2913, and 2270 cm^{-1} (Fig. 5). These bands are characteristic of xylan structures (Table 3) and confirmed that the extracted hemicelluloses from both pulps were mainly xylans. The only difference the FT-IR spectra of the pulps, is the presence of a band of very low intensity at 734 cm^{-1}. This can represent the C–Cl bond (Feng et al. 2016), suggesting some chlorination of xylan during the H_{mild} treatment.

The wide UVRR band region of 1250–1500 cm^{-1}, present in the spectra of the extracted hemicelluloses, is non-specific and may have contributions from lignin, HexA and carbohydrates in general (Loureiro et al. 2013). In contrast, the band at 1606 cm^{-1} is characteristic of aromatic structures (Jääskeläinen et al. 2005), suggesting that the extracted hemicelluloses contained some lignin. Additionally the sample from the oxygen delignified pulp showed the characteristic HexA band at 1655 cm^{-1} (Jääskeläinen et al. 2005). The lack of the HexA band in the spectrum of H_{mild} treated sample can relate to the breaking of the C=C and C=O of HexA by HOCl. Hence, the extractions were successful, and the samples were used to identify the possible chlorination of HexA.

Table 2 OX of pulps and EMIM OAc extracted and precipitated hemicelluloses

| Pulp	OX (mg/kg)			
	Pulp before extraction	Removed during extraction	Pulp after the extraction	Extracted hemicelluloses[c]
Oxygen delignified pulp	0[a]	0	0[b]	5
H_{mild} (2% Act. Cl) pulp	958[c]	740	218[d]	1749

[a]Oxygen delignified pulp before (77.8% Glucose and 17.6% xylose)

[b]Oxygen delignified pulp after EMIM OAc extraction (92.6% glucose and 3.8% xylose)

[c]H_{mild} pulp before the extraction (77.3% glucose and 19.1% xylose)

[d]H_{mild} pulp after EMIM OAc extraction (91.9% glucose and 4.6% xylose)
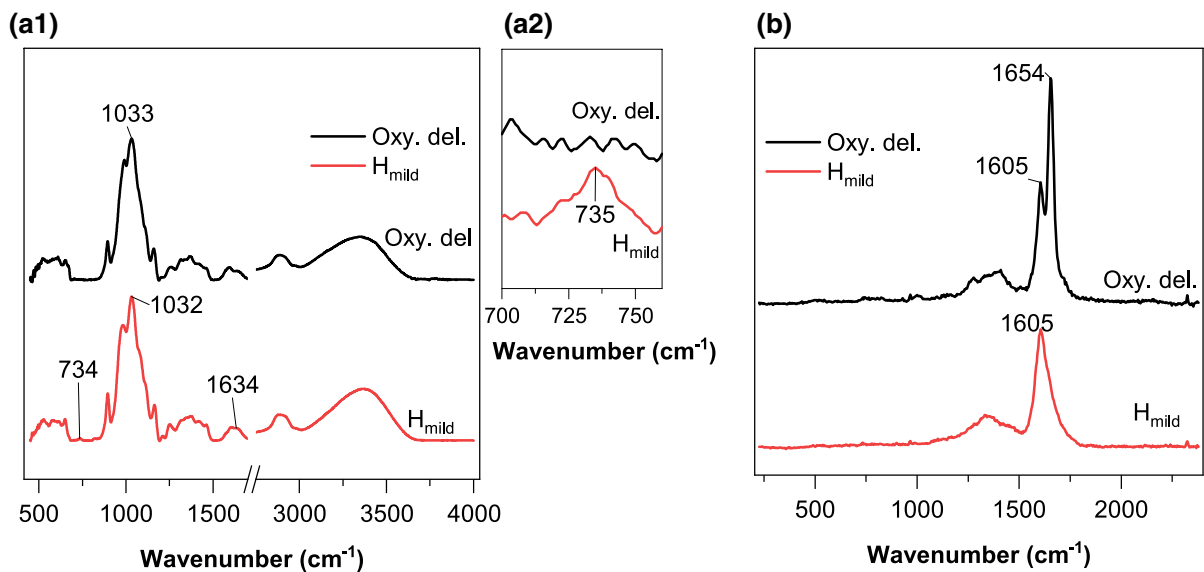
Fig. 5 a (**1–2**) FT-IR and **b** UVRR spectra of the extracted xylans from oxygen delignified and H$_{mild}$ treated pulps

Table 3 FT-IR bands in the extracted hemicelluloses from oxygen delignified and H$_{mild}$ pulps

FT-IR band wave-number (cm^{-1})	Type of bond	Source
895	β-glucosidic linkages between xylose units	Gao et al. (2015)
984	CH$_3$ and C–OH bending of xylose	Kacurakova et al. (1998)
1040	C–O, C–C and C–OH bending	Chaikumpollert et al. (2004) and Corradini et al. (2018)
1164	C–O and C–C stretching, characteristic of the C–O–C glucosidic linkages	Fu et al. (2018)
2913	C-H structure from xylose	Fu et al. (2018)
3380	water O–H stretch	Corradini et al. (2018)

2D HSQC and TOCSY NMR spectra

The 2D NMR HSQC spectra of the extracted hemicelluloses (Fig. 6) confirmed that they consisted mainly of xylans. The characteristic signals for xylan (X-I 1 to 5) (Fu et al. 2018) remained unchanged during the H$_{mild}$ treatment, which confirms that xylan backbone was not modified during the treatment. The xylan ^{1}H and ^{13}C shifts were: 4.3/101.8 (X-I$_1$), 3.9/63.3 (X-I$_{5e}$); 3.2/63.3 (X-I$_{5a}$); 3.5/75.5 (X-I$_4$); 3.3/74.0 (X-I$_3$) and 3.0/72.7 (X-I$_2$) ^{1}H/^{13}C ppm which coincide with previous literature (Fundador et al. 2012).

On the other hand, the HSQC spectra of HexA was present only in hemicelluloses extracted from the oxygen delignified pulp. The following

HexA signals were identified: 4.89/99.8 (HexA-I$_1$), 3.47/71.0 (HexA-I$_2$), 3.9/66.4 (HexA-I$_3$) and 5.36/105.37 (HexA-I$_4$) ^{1}H/^{13}C ppm. The signals were upfield shifted compared to the reported HexA shifts (Teleman et al. 1996b, a), probably due to the use of DMSO-d6 instead of D$_2$O as NMR solvent. Zooming the HSQC spectra of the hemicelluloses from H$_{mild}$ treatment revealed some possible new signals, and hence, TOCSY was used to identify possible new spin systems.

The TOCSY spectra of both hemicelluloses presented strong xylan signals, with all the protons being visible, including the hydroxyl protons C2–OH and C3–OH (Fig. 7). The HexA spin system was easily recognized only in the spectrum of hemicelluloses

Fig. 6 HSQC 2DNMR spectra of the extracted hemicelluloses **a** from oxygen delignified pulp and **b** from H_{mild} treated pulp
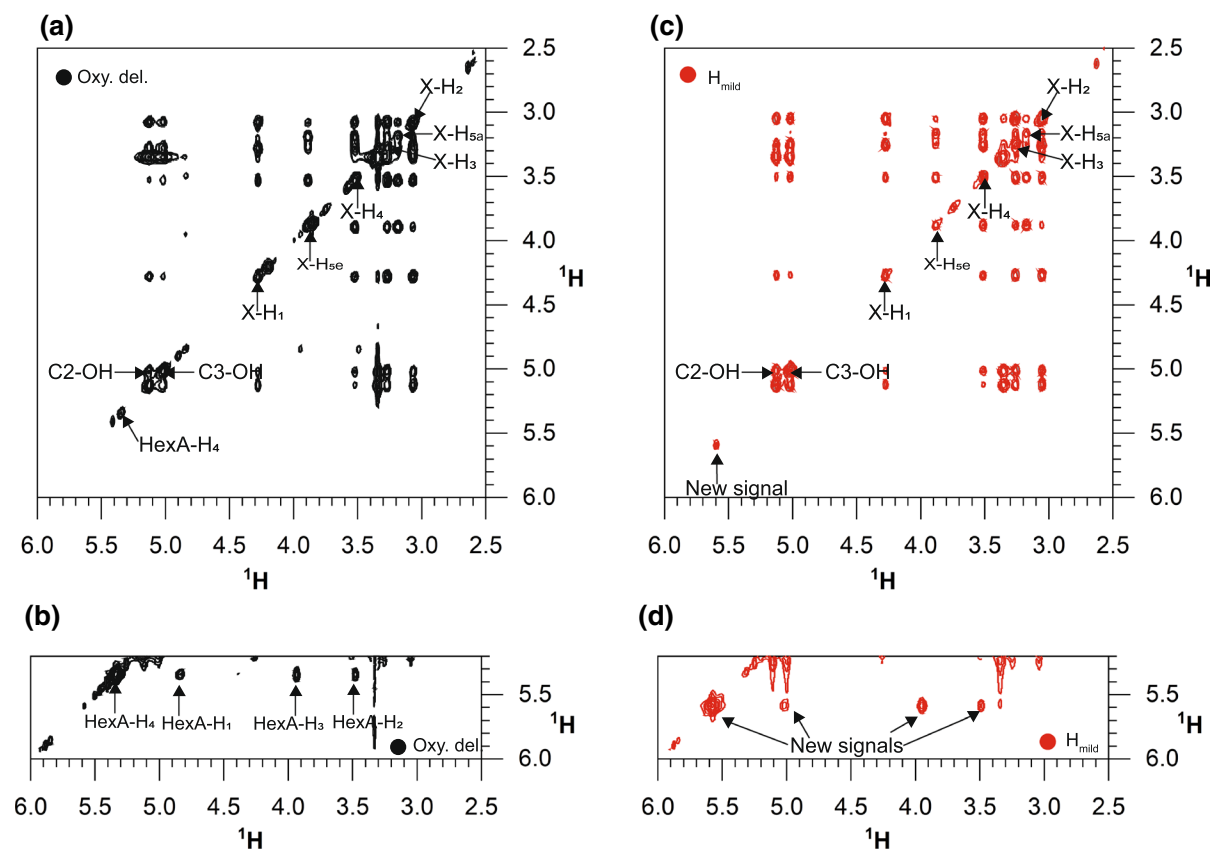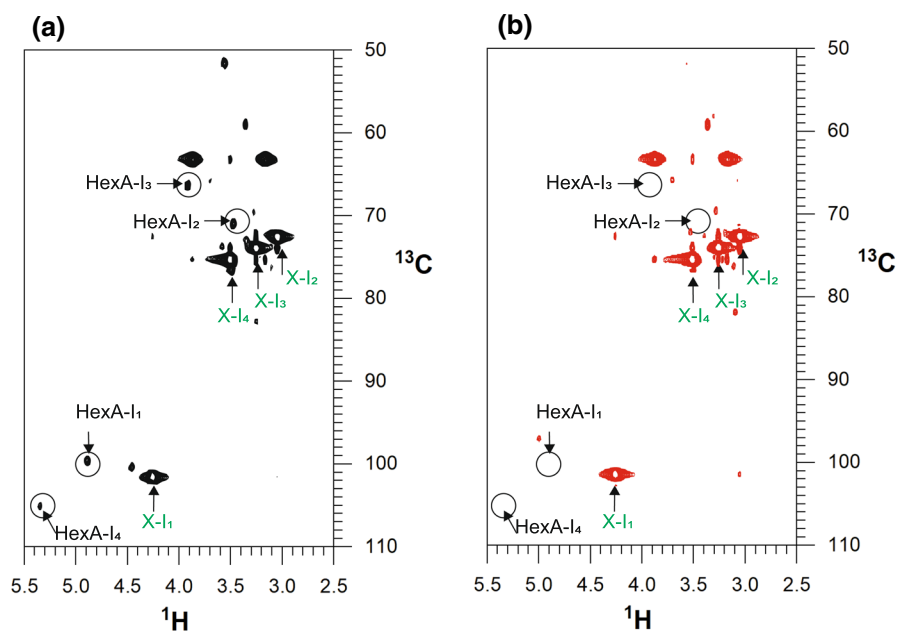
Fig. 7 TOCSY spectra of the extracted hemicelluloses in DMSO-d_6. **a**, **b** Extracted hemicelluloses from oxygen delignified pulp and **b** section of A at higher intensity. **c-d** Extracted hemicelluloses from H_{mild} pulp and **d** a section of C at higher intensity

from oxygen delignified pulp, confirming the previously identified signals at: 4.89 (H1), 3.47 (H2), 3.9 (H3) and 5.36 (H4) ^{1}H ppm (Fig. 7a–b). In the TOCSY spectrum of hemicelluloses from the H_{mild} treated pulp an unidentified spin system of four (4) protons was present (Fig. 7c–d). Two of the signals were close to HexA's H2 (3.47 ppm) and H3 (3.9 ppm) shifts. The two other signals were downfield relative to HexA's H1 and H4 shifts, at 5.03 and 5.58 ppm. Hence, the four new signals most likely relate to the structure formed after HexA was chlorinated. The de-shielding effect of chlorine atom in proton chemical shifts of organic compounds was reported by Sprengel et al. (2019).

The four new signals from the TOCSY spectrum were searched in the HSQC spectrum of the hemicelluloses from H_{mild} pulp. Three (3) of them were identified at 3.47/72.11 3.9/67.15 and 5.03/97.21 ^{1}H/^{13}C ppm. The signal at 5.58 ^{1}H ppm was not located, and it would correspond to the chlorinated carbon. Unfortunately, it remains unclear why this signal cannot be seen in the HSQC spectrum.

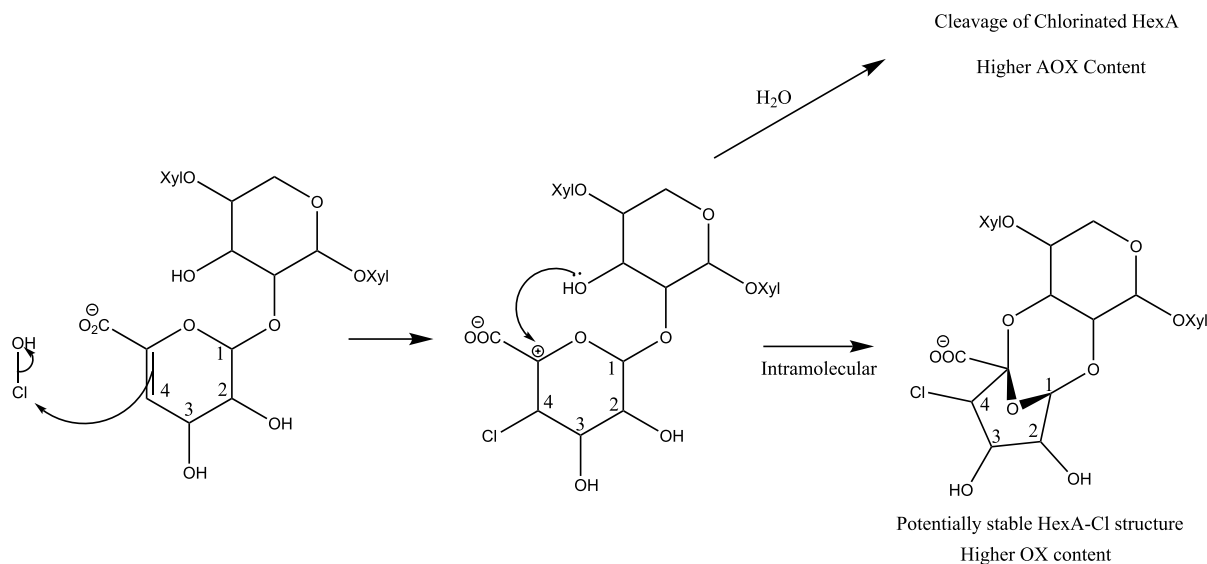Proposed HexA chlorination mechanism

The electrophilic addition of chlorine on C4 of HexA yields C5 carbonium ion, which is stabilized by the vicinity xylan ring oxygen atom (Fig. 8). Addition of water to the carbonium ion forms a hemiacetal that would ultimately liberate from the xylan chain yielding HexA derived AOX as proposed by earlier studies (Chenna et al. 2016). The formation of stable OX structures requires the nucleophile adding on the carbonium ion to be an alcohol group in xylan. Nevertheless such reaction has not been observed in previous studies with methyl 4-deoxy-ß-L-threo-hex-4-enopyranosiduronic acid (Chenna et al. 2016), and hence, the nucleophile is likely the O3 atom of the xylose unit on which HexA is attached with 1,2-glycosidic bond. The formed structure is an acetal that is relatively stable under mildly acidic and alkaline conditions.

This proposed structure is partly consistent with the observed new NMR spin system. However, we cannot confirm its assignment fully due to the low signal intensity deriving from the ca. 150:1 molar ratio between xylose units and OX in the extracted hemicellulose.

Conclusions

The stoichiometric ratios of lignin and HexA degradation, in eucalyptus wood pulp, with ozone (O_3) and hypochlorous acid (HOCl) were identified. O_3 oxidized 70% more lignin compared to an equimolar

Fig. 8 Possible chlorination mechanism of HexA during treatment with HOCl

amount of HOCl. Surprisingly, both electrophiles had quite a similar efficiency for HexA conversion, which is reported for the first time. O_3 degraded 0.16 and 0.28 equivalents of lignin (C9 units) and HexA, respectively. For HOCl the corresponding stoichiometric values were 0.09 and 0.23 equivalents. Also, both electrophiles were more selective towards HexA than lignin conversion.

The HexA conversion by HOCl was accompanied with chlorination (during H_{mild} stage), with the chlorinated structure staying mostly attached to the pulp. Evidence on this HexA related pulp chlorination includes a linear correlation between initial HexA content and the formed OX content (0.59 mmol OX/mmol HexA) and a eightfold OX content in the extracted xylan in comparison with the extracted HOCl treated pulp. Hence, this article showed that chlorinated HexA is a main component of OX in pulps treated with HOCl. Unfortunately, the proposed chlorinated structure could not be fully identified, and it requires further studies.

Additionally, this study brings a new way of looking at the chemical reactions taking place in chlorine dioxide bleaching stage, where HOCl is present and plays an important role in the delignification and HexA degradation. The proposed chlorinated OX structure is certainly formed in D stage, contributing to OX of the bleached pulp.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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