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From vapour to gas: optimising cellulose degradation with gaseous HCl

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A cellulose degradation technique utilizing a pressurized HCl gas (up to 100 kPa) device is introduced. High pressure HCl quickly degraded cellulose in purified cotton linters, reaching the so-called levelling-off degree of polymerisation (LODP) in less than 1.5 h. LODP marks the point where the disordered portions of cellulose microfibrils have been degraded and only the crystalline portions remain, generally signalling the end of cellulose degradation unless remarkably high concentrations are used. In the present high pressure system, however, continued hydrolysis following the LODP was detected by incremental release of sugars from the hydrolysate after its exposure to water, supposedly caused by erosion from the cellulose crystallite ends. With minimal water consumption and the ease of recycling the gaseous acid, the technique could be a potential candidate for pre-treatment considering the future production of cellulose nanomaterials, particularly cellulose nanocrystals.

Introduction

Cellulose is the major structural ingredient in plant fibres. Its degradation is important for two reasons: (i) for obtaining sugars for fermentation^{1,2} or commodity chemical production via HMF, CMF, and levulinic platforms³⁻⁵ and (ii) for extracting nano-sized components, i.e., nanocellulose, from native fibers.⁶⁻⁹ Many other polysaccharides, such as starch, are easily degraded but cellulose is designed by evolution to be mechanically strong and chemically inert over prolonged time periods. Multiple ways for degradation have been examined, the main route being hydrolysis, involving cleavage of the glycosidic bond by water addition (Fig. 1).

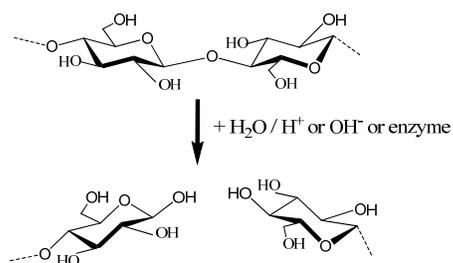


Fig. 1. Hydrolysis of cellulose is usually catalysed by acid, base, or enzymes.

Cellulose hydrolysis is usually a heterogeneous system consisting of solid fibres and an aqueous liquid with a catalyst. Considering acid-catalysed hydrolysis (Fig.1), increased acid concentration is required to degrade cellulose efficiently. For example, a form of nanocellulose called cellulose nanocrystals (CNCs) is generally produced at 64–65% aqueous H₂SO₄¹⁰⁻¹⁶ and total hydrolysis to monosaccharides is reached at 72% aqueous H₂SO₄¹⁷⁻¹⁹. Because of such high concentrations, full acid recovery is nearly impossible and it is laborious to purify the desired product from the reaction mixture. In consequence, acid hydrolysis has been met with a mixed response from the industry within the recent decades.

Recently, our group showed how a gas/solid system can be applied in efficient cellulose hydrolysis, leading to CNC production²⁰. HCl gas is adsorbed on fibres covered by a thin water layer under ambient conditions as the hydrolysis commences. The benefit of this approach lies in easy separation and purification of the hydrolysed product, as the only mass transfer is due to HCl adsorption on the fibres before the hydrolysis and desorption from the fibre surface afterwards. The recycling of the acid is also a far easier task compared with the liquid/solid system. Furthermore, we demonstrated a proof of concept on how the gas/solid system can be used for production of CNCs with abnormally high (>97%) yields²⁰.

The previous efforts were done with HCl vapour, i.e., utilizing the fuming of an aqueous HCl solution at room temperature. Here, we drive the system a step further and demonstrate the use of pure HCl gas for the hydrolysis reaction by building a small reactor for this purpose. The relatively low HCl pressure originates from the vapour pressure applied, while the reaction can be shown to follow the rules of heterogeneous catalytic systems in a more rigorous manner, as shown by kinetic data of the hydrolysis reaction.

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Experimental

Materials. HCl gas (99.8 %, 10 dm³, 6 kg) was purchased from AGA (Sweden). 50% aqueous sodium hydroxide, diluted to neutralise acid gas residues, was purchased from AKA Chemicals, Finland. Whatman 1 filter paper (Cat No 1001 150, 150 mm diameter) was used as cellulose source. Whatman 1 is a product of purified cotton linters (measured cellulose content > 99 %¹⁹). Cupri-ethylenediamine (CED) solution was purchased from FF-Chemicals (Finland). *N,N*-Dimethylacetamide was purchased from VWR Chemicals (EC) and lithium chloride from Merck (Darmstadt, Germany). The water content of filter papers was measured by drying the sample at 105 °C (overnight) and weighing it before and after. The average water content over 15 measurement points was 5.0±0.7%. For dilution, washing and rinsing steps, Milli-Q water (Millipore Corporation) was used. Acetone extraction (6 h) was conducted in a Soxhlet system.

Hydrolysis with HCl gas was performed in a custom-built reactor (see below). The cellulosic samples were weighed and placed in the reactor which was degassed (addition of pressurized HCl gas and releasing the pressure to a neutralizing system) 5 times with HCl gas. The HCl gas pressure was adjusted to the desired level after degassing. The gas pressure in the reactor was released after the desired reaction time had elapsed. The sample was immediately weighed and washed with Millipore water (2 x 15 min, 300 ml) afterwards. Subsequently, it was dehydrated to > 90 % dry matter content in fumehood overnight, RT) prior to the analyses.

Degree of polymerization (DP_v) of cellulose. The limiting viscosity of cellulose dissolved in cupri-ethylenediamine solution (CED) was determined according to the standard ISO 5351:2004. The limiting viscosity ([η]) was converted to DP_v with the Mark-Houwink equation ([η]=Q'DP_v^α where Q'=2.28 and α=0.76 when DP > 950, and Q'=0.42 and α=1 when DP < 950).²¹

The molar mass distribution (MMD) of selected samples was determined by gel permeation chromatography (GPC). First, the sample was activated for dissolution by a water–acetone–*N,N*-dimethylacetamide (DMAc) solvent-exchange sequence. 50 mg sample was mixed with 4 mL of milliQ-water for 6 h. The sample was dewatered prior to the addition of 2 mL of acetone which was subsequently removed in vacuum. Thereafter, the sample was maintained in approximately 4 ml acetone for more than 2 hours, after which the acetone was removed in vacuum. The sample was then immersed in ca. 4 ml DMAc overnight which was again removed in vacuum. The activated sample was then dissolved in DMAc with 90 g/l LiCl at room temperature under gentle stirring and the solution was diluted to 9 g/l LiCl/DMAc, filtered through 0.2 μm syringe filters and fed to a Dionex Ultimate 3000 system equipped with four PLgel MIXED-A 7.5 x 300 mm columns and a refractive index (RI) detector Shodex RI-101. LiCl/DMAc was used as the eluent. Pullulan standards (343 Da – 708 kDa, Polymer Standard Service GmbH, Mainz, Germany, and 1600 kDa, Fluka GmbH, Germany) were used as calibrants. The molar masses of pullulan standards were converted to the corresponding ones for cellulose, using the equation $M_{\text{cellulose}} = q \times (M_{\text{Pullulan}})^p$.²²

X-ray photoelectrons spectroscopy (XPS). A Kratos Analytical AXIS 165 electron spectrometer with a monochromatic Al_{Kα} X-ray source under neutralisation was applied for chemical composition analysis of the cellulose surfaces. All spectra were collected with an electron take-off angle of 90° from a sample area of less than 1 mm diameter. Survey spectra were recorded at 1 eV intervals at pass energy of 80 eV. Higher resolution regional spectra were recorded at 0.1 eV intervals at pass energy of 20 eV. The spectra were recorded at three different spots on each sample and the vacuum analysis was monitored throughout the measurement, with an in-situ reference sample. No X-ray induced degradation was detected during the characterization.

Scanning electron microscopy (SEM). JEOL JSM-7500F FEG (Japan) was used for SEM imaging of the filter paper samples, with an acceleration voltage of 1-5 kV, varied depending on the sample. All samples were coated with a Au-Pd mixture for preventing the surface charging effect.

Reactor design. The reactor was designed to implement safe additions of HCl gas to cellulose samples (Figs. 2 and 3). HCl gas is added at a default pressure to the glass bottle (Duran pressure plus bottle (-1 – 1.5 bar), Sigma Aldrich) which can be referred to as the sample reactor. Default HCl gas pressure was acquired with degassing process, *i.e.*, releasing air/HCl mixture to the flush line after HCl addition prior to the repeating HCl gas addition. Alternatively, vacuum in the sample reactor can be induced prior to the HCl gas addition. The sample bottle was detached after the HCl addition. NSH coupling valves (Fig.4, Colder Products Company, USA) were applied for fast detachment/attachment and to ensure the preservation of the gas pressure in the sample reactor (Figs. 2 and 3). Compressed air and nitrogen gas were used to thoroughly flush the gas lines (PTFE) after HCl gas addition. The HCl gas was eventually neutralized by a system consisting of two alkaline solution containers (the first container being closed). Sirai D105V31 (Asco Numatics Sirai SRI, Italy) dry solenoid valves (body material – PVDF, denoted with red colour in Fig. 2) were applied to control the gas flows. The gas release valve is a safety measure which releases the gas pressure at 4 bar.

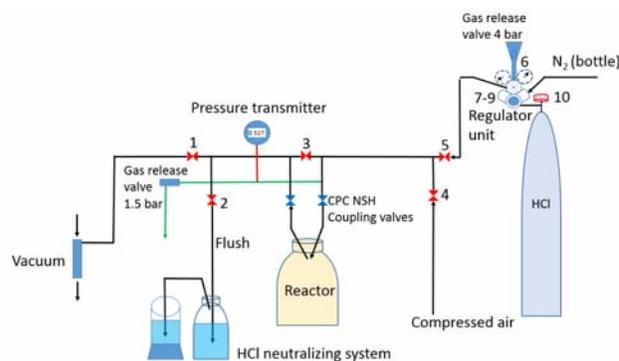


Fig. 2. Schematic illustration of the cellulose degradation system with HCl gas. Solenoid valves: (1) vacuum valve, (2) Flush line valve, (3) bypass valve, (4) compressed air valve, (5) HCl gas valve. Control valves (6-9) are applied to adjust HCl gas pressure and to control N₂ gas flow (applied to flush the gas regulator unit). The HCl gas bottle main valve is denoted as 10.

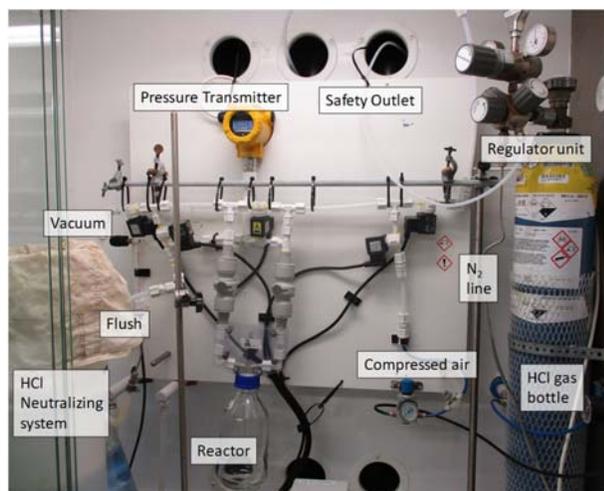


Fig. 3. The snapshot of the cellulose degradation system with the markings of the components.



Fig. 4. The sample reactor. Duran pressure plus bottles (0.25 dm³, 0.5 dm³ and 1.0 dm³) were used as reaction bottles.

Results and discussion

Degradation of cellulose with gaseous HCl

Fig. 5 shows the degradation of pure cotton linter cellulose (Whatman 1 filter paper) at different pressures and reaction times. The degradation patterns follow the general trend for acid catalysed hydrolysis of cellulose: first, disordered segments in a semi-crystalline cellulose microfibril are degraded in a rapid phase, leaving intact only the crystallites, while the DP value stays virtually constant at what is called levelling off degree of polymerisation (LODP). The LODP (240), which is quite similar to the one reported for cotton linters in a solid/liquid system (220)²³, was reached in 1.5 h at 100 kPa pressure (Fig. 5). The lowest CED-viscosity level of dissolved cellulose at LODP (~ 240)

was 100–102 ml/g with all the used pressures of gaseous HCl (10, 40, 80, 100 kPa, Fig. S1 in ESI), while the corresponding value of CED-viscosity was 110 ml/g at the highest partial pressure of HCl vapour (maximum reaction time)²⁰. This observation indicates that gaseous HCl technique can be applied to degrade cellulose to a similar DP_v level which has been reported with the HCl vapour technique¹⁹. Furthermore, an additional trial was performed with hydrolysing a sample dried overnight at 105°C prior to the hydrolysis at 100 kPa HCl pressure (Fig. 5, start-shaped data point). While the fibres were presumably not totally devoid of water, their water content after the drying was very low, which clearly affected the extent of hydrolysis. Further experiments to illuminate the role of the water content in HCl (g) hydrolysis are underway and will be published in a future study.

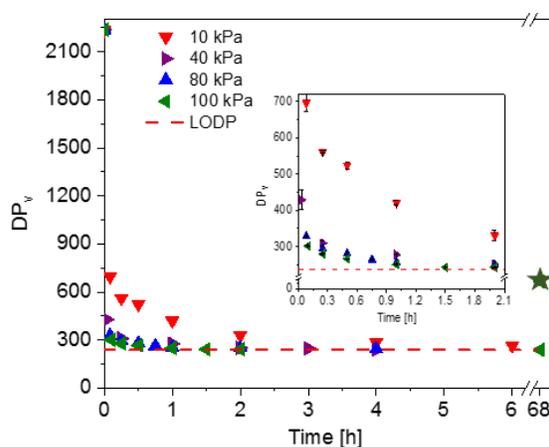


Fig. 5. Reduction of the degree of polymerization determined by CED-viscosity (DP_v) as a function of reaction time with different pressures. The dashed line represents the LODP determined for cotton linters with the novel method introduced here (lowest DP_v with highest HCl gas pressure and practically unlimited reaction time). Dry matter content of all samples was 95 % excluding sample which was dried in the oven overnight (green star, 100 kPa).

While the LODP values are expectedly identical, the applied high pressure HCl gas degradation is evidently faster than the HCl vapour technique where partial pressures of more than 8.2 kPa cannot be reached because of the solubility limit of HCl in water.²⁰ Fig. 6 shows the kinetic treatise. Here, the average number of scissions per cellulose chain, as calculated from the DP_v values, was used as a measured quantity – a normal procedure in kinetic analysis of cellulose degradation.

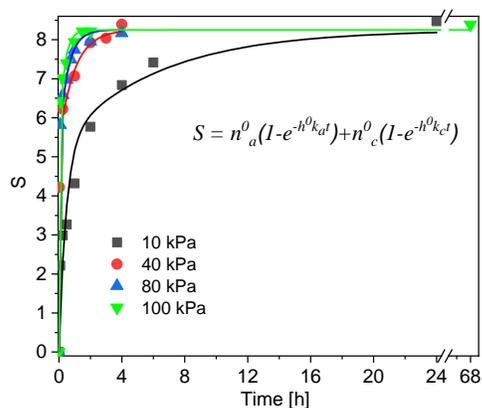


Fig. 6. Kinetic fits to the degradation data (DP_v values). S denotes the number of chain scissions per cellulose chain (calculated from $S = DP_0 / DP - 1$). Two term-model was applied. Easily cleavable weak links of cellulose (w) were degraded first. The glycosidic bonds in amorphous or disordered regions (a) were not cleaved as easily as the weak links. The terms of used kinetic equation: initial amount of weak links in a cellulose chain (n_a^0), initial amount of glycosidic bonds in amorphous cellulose (n_c^0), the initial acidity of cellulose (h^0), hydrolytic rate constants (k_a , k_c), and time as hours (t). The acidity of the acid degradation is supposed to remain at default level during the process due to the extensive HCl gas dosage (pressurized HCl gas).

The kinetic fit in Fig. 6 is performed according to the Calvini equation which takes into account the different degradation regimes due to the semi-crystalline nature of native cellulose. The fitting parameters are listed in Table 1. The hydrolytic rate constant estimates ($h^0 k_a$, $h^0 k_c$) at 10 kPa pressure correspond to the values acquired with solid/liquid acid hydrolysis (1 M HCl) at temperature between 50 - 80 °C (1.69 - 13.07, 0.04 - 0.5).²⁴ By contrast, at higher HCl pressures like 100 kPa, the rate constant exceeds the reported ones for solid/liquid systems by several decades. Moreover, the estimated weak link - glycosidic bond ratio (0.65) is clearly higher than the reference values with cotton cellulose (0.11-0.4).²³⁻²⁶

Table 1. Parameter estimating the fitted kinetics of degradation data (Fig. 5).

$p(\text{HCl}) / \text{kPa}$	n_a^0	$h^0 k_a$	n_c^0	$h^0 k_c$	$n_a^0 / (n_a^0 + n_c^0)$
10		2.16		0.16	
40	5.33	45.0	2.92	1.04	0.647
80		15600		1.81	
100		11400		3.13	

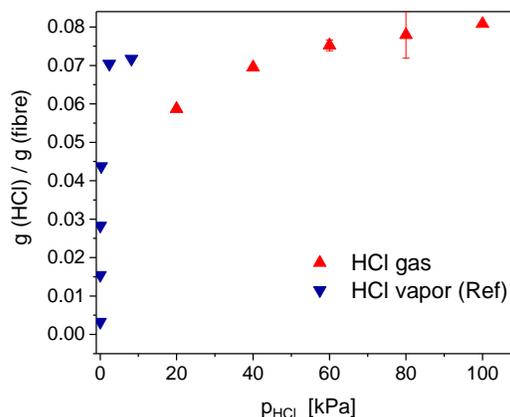


Fig. 7. Adsorption of HCl on fibres. HCl adsorption was analysed by weighting the papers before and immediately after the hydrolysis. Whatman 1 filter paper was hydrolysed with the HCl device at adjusted pressure (default 1 h reaction time).

The physical model for degradation was presented in our previous study: HCl adsorbs on fibres that are always covered by a thin water layer (5.0 ± 0.7 w-%) under ambient conditions. The water dissociates HCl, enabling the acid catalysed hydrolysis to commence. Because of the small absolute amount of water, the HCl concentration rapidly increases, and the reaction is able to proceed fast, even at relatively low partial pressures. Therefore, the amount of HCl adsorbed is an important parameter in this system (Fig. 7). The plateau level of adsorption is quite similar for both HCl gas (0.07 - 0.08 g HCl / g fibre) and HCl vapour (0.07 - 0.072 g HCl / g fibre²⁰). As stated in the vapour study,²⁰ the adsorption of HCl conforms to a Langmuir type monolayer. The plateau appears to be reached at slightly lower pressures with vapour than with gas (Fig. 7). However, already at relatively low gas pressures (~ 40 kPa), the plateau level of the monolayer is reached. It is important to note here that the HCl/water ratio on fibres is already well above the solubility limit of HCl, *i.e.*, not all adsorbed HCl is in the dissolved state.

As a result, it is clear that both the adsorption of HCl and the degradation proceed in a similar manner in the gas reactor as they do in a simpler setup of HCl vapour. Only the faster kinetics under elevated pressures represents a significant difference. Another major advantage of the gas reactor - not visible from the data - is that the reactions can be performed at a much faster pace. With the vapour setup in a desiccator, one had to wait for days for the vapour to reach equilibrium and displace the air inside the vessel before the reaction could be started.

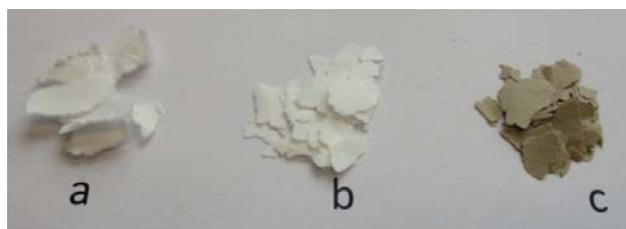


Fig. 8. Cellulosic samples before and after degradation with gaseous HCl. a) Untreated Whatman 1 paper, b) Whatman 1 paper after 1.5 h treatment time with 100 kPa HCl pressure, c) Whatman 1 paper after 68 h treatment time with 100 kPa HCl pressure. The samples b ($DP_v = 242$) and c ($DP_v = 238$) were degraded to LODP level. The darkening of cellulose occurred after reaction of tens of hours (sample c).

In conclusion, the outcome of the HCl gas reaction was similar to that of HCl vapour. However, that applies only for short reaction periods. By increasing the reaction time to several days, a noticeable discoloration was observed (Fig. 8). Interestingly, the DP_v remained at LODP level despite the colour darkening and extended reaction times. A set of experiments to investigate the discoloration was performed. The gravimetric yield after immersing the hydrolysed sample in water was measured, as water dissolves the soluble reaction products from the cellulose substrate. The yield of the degraded filter papers decreased as a function of reaction time and HCl pressure (Fig. 9). It was typically >95% at the point where LODP was reached. However, the yield decreased down to ~90% at longer reaction times, which is in accordance with earlier findings with extended solid/liquid hydrolysis (2.5 N HCl, 100 °C, lowest yield ca. 88 %).²² The carbohydrate analysis of the washing filtrates (HCl hydrolysis) indicates that soluble glucose was formed as the major product, as expected from acid hydrolysis of cellulose (Table 2). The other sugars in Table 2 originate from trace amounts of hemicellulose, still left in the substrate after purification of the cotton linters for filter paper production. It is curious that M_w distributions as determined by GPC showed nearly identical profiles with short and long reaction times after reaching the LODP (Fig. 10). The major MMD shift took place within 15 minutes (100 kPa) degradation time (Fig. 10) while the degradation to LODP was still ongoing. All in all, the GPC data correlated well with DP_v values from viscosity data (Fig. S1 in ESI) but they failed to reveal any of the phenomena taking place during the long exposure times to high pressure HCl. Similarly, the FTIR spectra did not exhibit any alterations between short and long exposure times to HCl gas (Fig. S2 in ESI). In XPS data (Table 3), however, there was a clear increase in the non-cellulosic components at the sample surface after a long degradation time (68 h and 100 kPa).

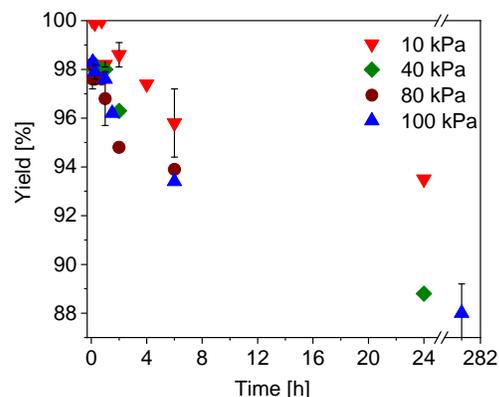


Fig. 9. Yields of HCl gas degraded filter paper samples. After HCl hydrolysis (gas), all samples were washed with Milli-Q water, dried overnight at RT, and homogenized prior to the yield analysis and DP_v determination.

Table 2. Monosugar compositions of the water used to rinse the hydrolysed cellulose substrates. Cellulose cotton linters (Whatman 1) were hydrolysed (A - B) with long reaction time (100 kPa, (A) 281h, (B) 68h), and hydrolysed (C) with short reaction time (100 kPa, 0.25 h). The portion of dissolved sugars (%) of the original fibre weight is calculated. All analyses were conducted with first washing filtrate (excluding A which is a sum of first and second washing filtrate). The default washing procedure was 2 x 15 min with 300 ml Milli-Q water (1 % pulp consistency).

Dissolved carbohydrate (%)	A	B	C
Arabinose	0.06	0.04	0.01
Galactose	0.05	0.02	0.01
Glucose	10.6	6.0	1.0
Xylose	0.26	0.3	0.5
Total	11.0	6.3	1.5

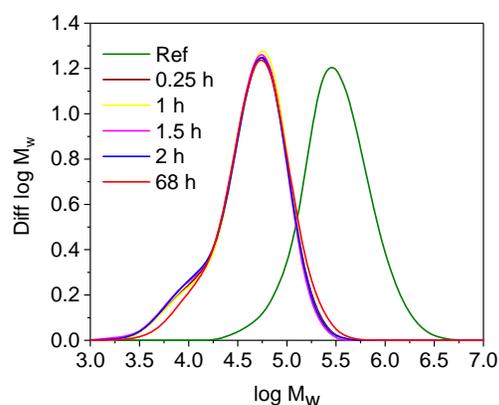


Fig. 10. Molecular mass distributions determined by GPC system. Cellulose cotton linters (Whatman 1) were degraded with 100 kPa pressure of gaseous HCl.

Table 3. XPS data of untreated (A), HCl hydrolyzed with long reaction time (100 kPa, 68h) (B), and HCl hydrolyzed with short reaction time (100 kPa, 0.25 h) (C) cellulose cotton linters (Whatman 1).

Sample	XPS elemental analysis				C 1s components			
	O 1s (%)	C 1s (%)	Cl 2p (%)	S 2p (%)	C-C (%)	C-O (%)	O-C-O/C=O (%)	O-C=O (%)
A	60.4	39.6			4.3	74.9	18.8	2.0
B	63.6	35.3	0.4	0.7	14.0	67.5	16.4	2.0
C	61.0	38.9	0.1	0.0	7.0	72.9	18.1	2.1

In conclusion, the spectroscopic data with FTIR and XPS rule out the occurrence of any extensive oxidation reaction that would lead to discoloration at longer exposure to HCl gas. Furthermore, the samples reach the LODP after 2 h reaction with high pressure HCl and additional degradation after LODP can scarcely be detected by DP analysis (Figs. 5 and 10). The only sign of further action is found in the sugar analysis of the water extract of the cellulose substrate after the hydrolysis where increased amounts of glucose were detected (Table 2). What was apparent after all hydrolyses down to LODP, however, was the brittleness of the substrate. The papers were tangibly brittle directly after reaching the LODP, but the brittleness increases significantly upon longer exposure times to HCl gas. Morphological analysis by SEM (Fig. 11) showed some visible breakage in fibres, directly after reaching the LODP (Fig. 11B). Still, at this point, most of the fibres appear morphologically intact and the physical integrity of the cellulose substrate is retained.²⁰ After further hydrolysis, the fibres are clearly shattered and broken after 68 h exposure to 100 kPa HCl (Fig. 11C). More severe degradation of fibres can be observed after 281 h exposure at corresponding conditions (Fig. S4 in ESI). Fig. 12 represents a schematic hypothesis for the sequence of events. After reaching LODP, the disordered segments in a cellulose microfibril have been degraded. Although the crystallites are no longer covalently connected, they are virtually touching each other, especially when taking into account that the disordered ends have crystallized upon degradation.¹⁹ At this point, the hydrolysed paper is already brittle because of the reduced DP, but the fibres are able to retain their integrity. When the hydrolysis is further prolonged, the crystallites are subjected to slow end-wise degradation, according to the Sharples model.^{27,28} The crystallite ends are gradually eroded, leading to generation of increased amounts of glucose. A physical gap emerges between the crystallites in a microfibril and the microfibril easily breaks down, making the whole fibre susceptible to breakage. The erosion of the crystallites can be detected by HPLC (Table 2) due to increased amount of glucose in the water extract of the hydrolysis product (11 w-%). At LODP of 240, 11 w-% of glucose released corresponds to a DP reduction of ca. 20-25 in cellulose. This reduction indicates that some of the smaller crystallites disappear completely (Fig. 12), which accounts for the disappearing shoulder in the low M_w region of the M_w distribution pattern (Fig. 10). The overall M_w , as detected by GPC, is not decreased, precisely because some of the low M_w crystals are totally degraded: the other crystallites are eroded

and the M_w goes down but at the same time the overall M_w increases as some of the small M_w components are removed, resulting in unchanged average M_w .

Furthermore, to probe whether the detached glucose from the crystallite ends reacts further, the presence of furfural and hydroxymethyl furfural was investigated with UV/Vis spectroscopy and HPLC chromatography from filtrates of hydrolyses (S5). Formation of humins from the furfural compounds is likely the origin of discoloration of hydrolyzed cellulose cotton linters²⁹⁻³⁰. The phenomenon will be investigated in detail in a future study.

Conclusions

A novel device to degrade cellulosic samples with pressurized HCl was introduced. Cellulose cotton linters (in filter paper) can be effortlessly and reproducibly degraded down to LODP within 1.5 hours reaction time (100 kPa) at room temperature. The technique consumes minimal amounts of water, and the gaseous HCl catalyst is easy to recycle when the method is developed further. The LODP remains apparently stable after several days of hydrolysis. However, when the hydrolysed substrate was washed with water, an increased amount of glucose was found in the filtrate, suggesting that the hydrolysis continued after reaching the LODP. The continued hydrolysis leads hypothetically to breakage of fibres, visible in SEM images and tangible in the increased brittleness of the cellulose substrate that has been exposed to 100 kPa HCl gas for several days. This suggests that long exposures to high pressure HCl may be beneficial for the production of CNCs which aims at complete disintegration of fibres.

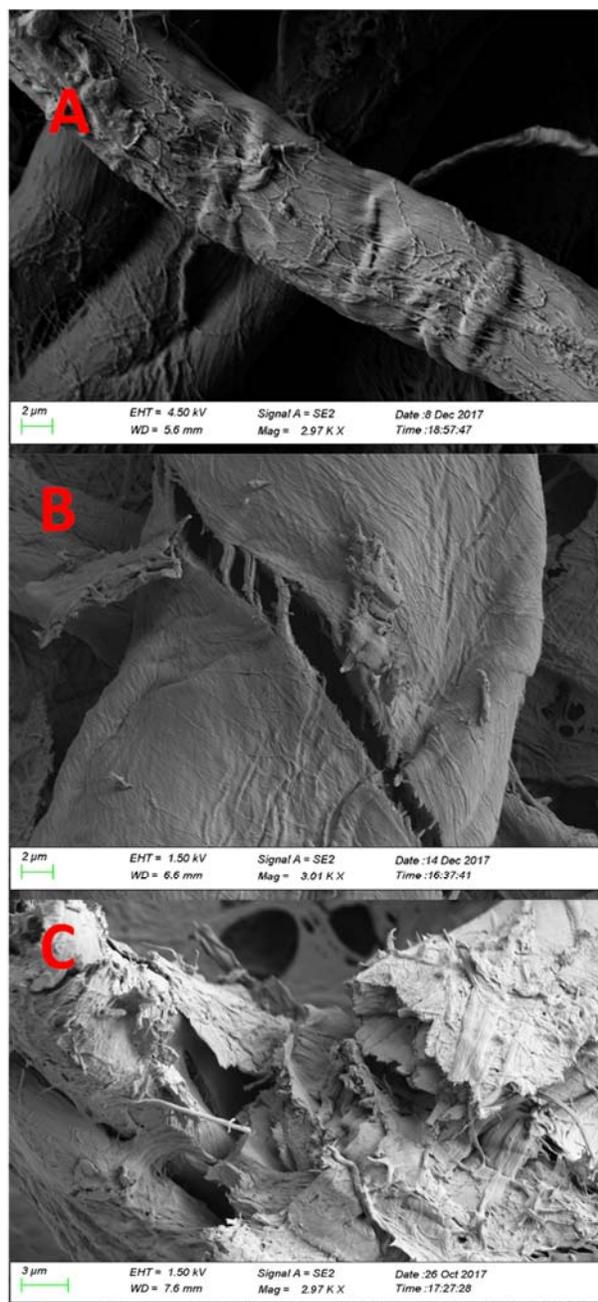


Fig. 11. SEM images of reference (A), HCl degraded sample to LODP level (B, 100 kPa, 2h) and degraded sample of prolonged hydrolysis time (C, 100 kPa, 68 h). The reference sample (A, 2.97 Kx) is compared with the hydrolyzed ones. Fiber cleavage (B, 3.01 Kx) and severe fiber degradation (C, 2.97 Kx) are observed.

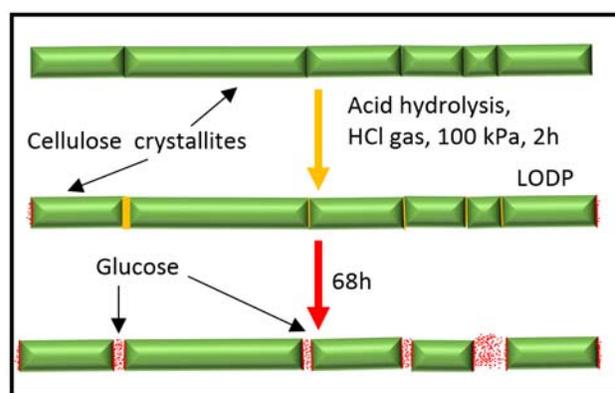


Fig. 12. Schematic illustration of cellulose microfibrils bearing longitudinal disorder with crystalline and disordered segments (top). The short disordered segments can be cleaved by HCl gas, leading to crystallites that are virtually touching each other. Further exposure to high pressure HCl gas leads hypothetically to end-wise erosion of the crystallites and consequent generation of additional glucose (Table 2) as well as breakage of fibres.^{26,27}

Conflicts of interest

There are no conflicts to declare.

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