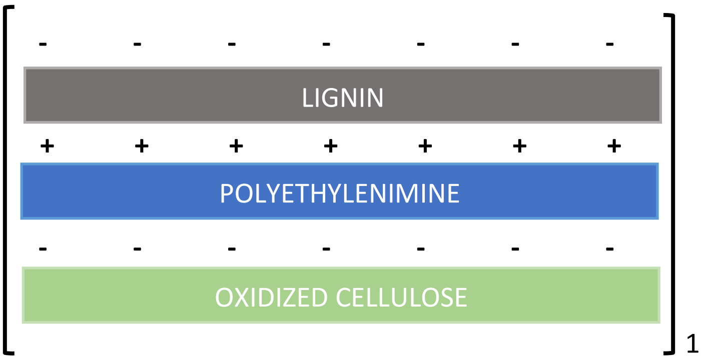
Scientific Report

The activity carried out during the stage in Tecnalia in Azpeitia (Spain) consisted in investigating a layer-by-layer process to deposit water-soluble lignin (L^-) on dicarboxylic acid cellulose fibres (DCC^-), coated with polyethylenimine (PEI^+). An illustration of the work is reported in the image below:



Example of mono-layer $[DCC^- \text{-} PEI^+ \text{-} L^-]_1$, pH 10.

The process is based on alternating layers with opposite charge in order to obtain a compound kept together by electrostatic interactions. The obtained compound will be characterized through TGA and contact angle measurements. If the process will be performed successfully, a multilayer of $(DCC^- \text{-} PEI^+ \text{-} L^- \text{-} PEI^+ \text{-FireRetardant}^-)_n$ will be prepared. The compound will be used to produce composites with white glue through compression moulding at mild conditions. The fire performance of the samples will be tested at cone calorimeter. Lignin should enhance the matrix fire performance by promoting char formation.

The first activity was based on the oxidation of cellulose fibres, while the second on the preparation of cellulose fibrous compounds by means of Layer-by-Layer deposition method along with thermal and contact angle measurements.

- Sodium Periodate ($NaIO_4$) Cellulose Oxidation

A cellulose sheet from pine (Figure 1a) (227 g, theoretical 12 wt.% moisture, 88 wt.% cellulose) was reduced into pieces of different size (Figure 1b) and dispersed in 5L of deionized water at a concentration of 4 wt./v%. The cellulose was dispersed in a disintegrator for 1h 30 min (Figure 1c), but after the milling the sample was not completely disintegrated. For this reason the cellulose was diluted adding 1L of deionized water, reaching in this way the concentration of 3.3 wt.%. The system was left in these conditions for other 30 min. Then, when the cellulose resulted to be well dispersed, was treated with 132 g of $NaIO_4$ (66 wt.% respect to g of plain cellulose without moisture) and 480 g of NaCl (5.8 wt.% with respect to the total amount of water) dissolved in 2 L of deionized water (Figure 1d). The oxidation reaction was performed for 2h, at room temperature in the dark to avoid the $NaIO_4$ photodegradation. The system was stirred mechanically. After the reaction the oxidized pulp was filtered (filter porosity = 500 μm) and washed with deionized water several times. The oxidized pulp was re-dispersed in water and stored in fridge at 5 °C. 4 pans of cellulose suspensions were collected and dried in oven over night at 105 °C (Figure 1e , 1f) The dried cellulose suspensions were put in a dryer at room temperature and then weight (Table 1). The product is coded as dialdehyde cellulose (**DAC**). All the phases of the process are illustrated in Figure 1.

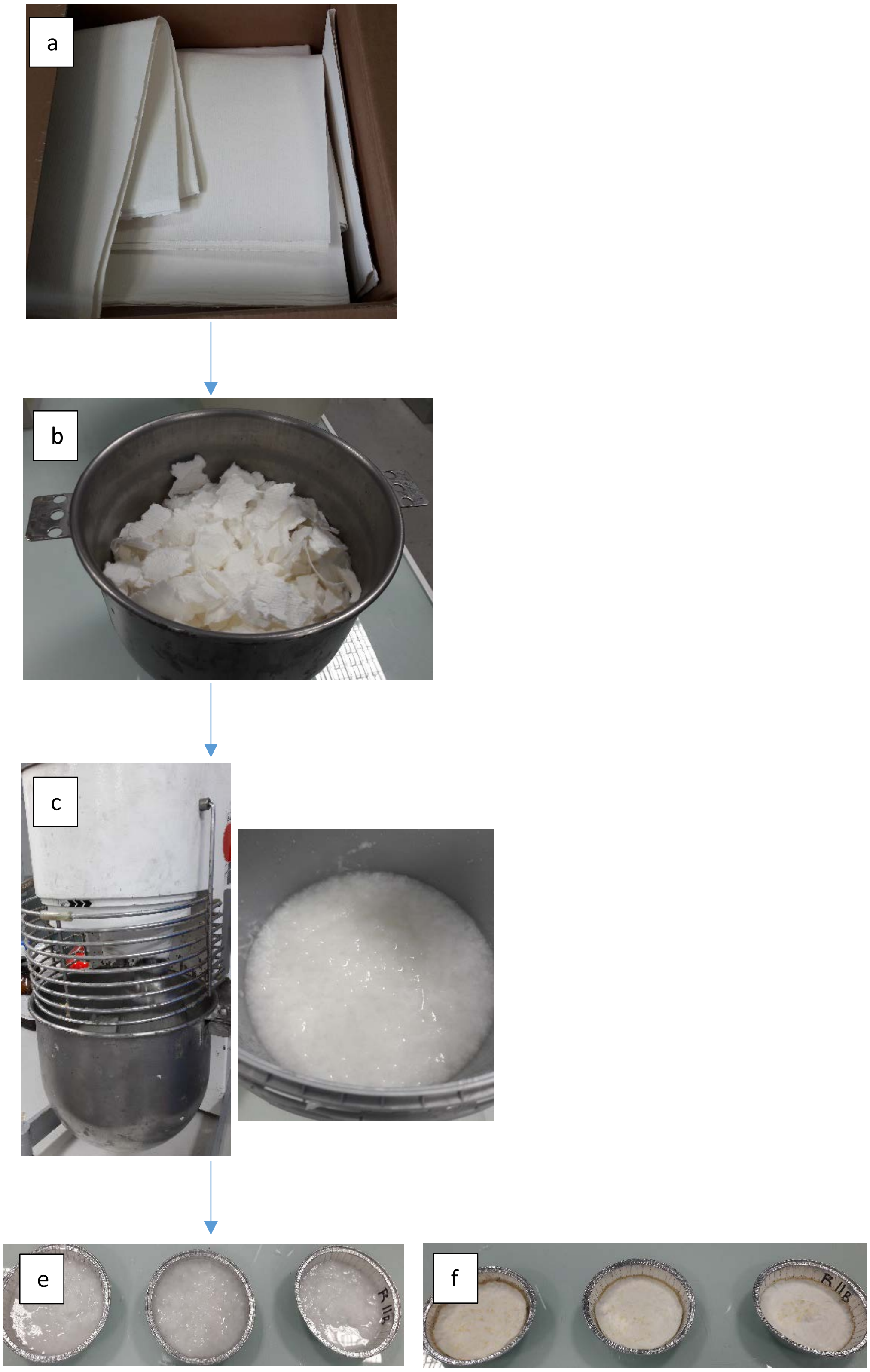


Figure 1. Phases of cellulose disintegration and oxidation: from pine cellulose sheet to DAC.

The samples' weight is indicated below in Table 1:

Table 1. Weight and concentration of four cellulose suspension specimens.

Sample	Suspension, (g)	Dried cellulose, (g)	Concentration, (wt.%)
1	61.86	1.66	2.68
2	33.41	0.97	2.90
3	35.65	1.06	2.97
4	47.11	1.49	3.16
Average	44.51	1.29	2.92

- Mechanism of cellulose oxidation by $NaIO_4$

Sodium periodate ($NaIO_4$) breaks apart 1,2-diols (vicinal diols) to form aldehydes and ketones. It is possible to obtain aldehydes or ketones, depending on whether $NaIO_4$ is breaking down secondary or tertiary alcohols. In the case of cellulose, $NaIO_4$ breaks down secondary alcohols and in particular the bond between C-2 and C-3 of the glucosidic unit. The mechanism is reported below in Figure 2:

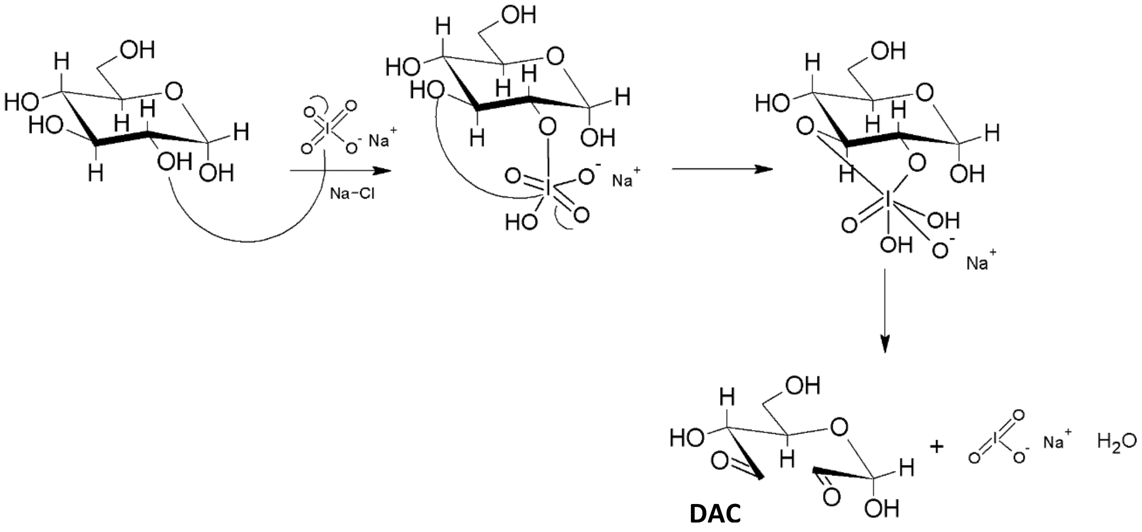


Figure 2. Mechanism of cellulose oxidation by sodium metaperiodate.

- Determination of aldehyde content

The determination of aldehyde groups was performed by the hydroxylamine-hydrochloride titration method, by which the hydroxylamine-hydrochloride reacts with the carbonyl compound and produces oxime and HCl. The reaction between aldehydes and hydroxylamine-hydrochloride is reported below in Figure 3:

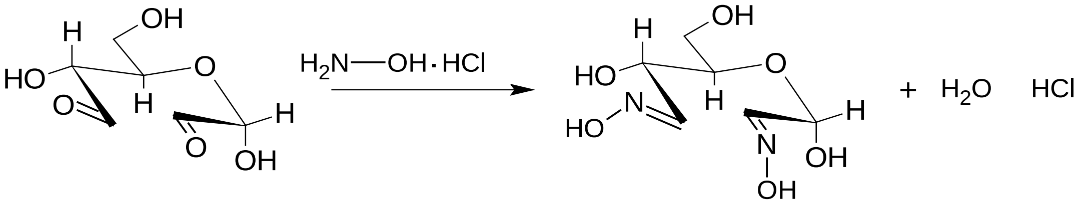


Figure 3. Mechanism of reaction between DAC and $NH_2OH \cdot HCl$.

The HCl released from its reaction with aldehydes is back titrated with a known concentrated NaOH solution. The determination was performed three times. All the determinations were performed according to the titration method. Firstly, the cellulose suspensions were mixed with water (1/2 v/v) and then magnetically stirred. The pH of the mixtures was adjusted to 2-2.5 by adding few drops of HCL 25 wt.% and then carefully adjusted to 3.5 with NaOH 0.1 N. 10 mL of 10 wt.% $NH_2OH \cdot HCl$ solution were added to this mixture, allowing it to react for 10 min. Finally, the HCl released from the reaction was titrated with 0.5 N NaOH titrant solution until pH 3.5 was reached again (Figure 4).

Figure 4. Titration of aldehyde content in DAC.

The aldehyde content was calculated using the following equation:

$A_{ald}=V_{NaOH} \text{ X } 0.5N/w_{cell}$

Where:

A_{ald}= aldehyde content (mmol/g)
V_{NaOH}= volume of NaOH 0.5 N (mL)
w_{cell}= weight of dried cellulose (g)

The data related to the aldehyde content determinations are reported in Table 2.

Table 2. Data from aldehyde content determinations.

Repetition	Suspension Weight, (g)	Theoretical Cellulose content, (g)	pH after HCl addition	NaOH 0.1 N, (mL)	NaOH 0.5 N, (mL)	Theoretical A_{ald}, (mmol/g)
1	29.89, pH 4.90	0.86	2.37	9.9	1.00	0.58
2	25.62, pH 5.21	0.74	2.45	5.9	0.80	0.54
3	19.30, pH 5.33	0.56	2.44	4.5	0.65	0.53
Average						0.55

After the determinations, the samples were dried in oven first at 80 °C, then at 105 °C over night. Finally, the samples were dried in oven under vacuum at 105 °C and kept into a dryer.

The effective cellulose weights and the aldehyde content, determined using the effective dried cellulose weight (w_{cell}), are reported in Table 3.

Table 3. Data from aldehyde content determinations using the effective cellulose weight and comparison with the theoretical.

Repetition	Suspension Weight,	Theoretical Cellulose	Theoretical A_{ald}, (mmol/g)	Effective Cellulose	Effective A_{ald},
1	29.89, pH 4.90	0.86	0.58	0.83	0.60
2	25.62, pH 5.21	0.74	0.54	0.82*	0.48
3	19.30, pH 5.33	0.56	0.53	0.69*	0.46
Average			0.55		0.51

** Potential not effective drying or higher cellulose concentration*

- Sodium Chlorite ($NaClO_2$) Cellulose Oxidation

284 g of cellulose suspension (theoretical cellulose content = 8 g; 2.8 wt.%) were filtrated and the obtained pulp was suspended in water (1:100 w/w). Firstly, 46.4 g of NaCl (5.84 wt.% with respect to the calculated total amount of water) were dissolved in water. Then, 5.7 g of $NaClO_2$ (71.2 wt.% with respect to theoretical cellulose content) were dissolved in water. Then, the chlorite solution was added to the NaCl one. Both the solutions were mixed by mechanical stirring. The cellulose pulp was added and soon after 17.6 g of H_2O_2 aqueous solution 30 wt.% (220 wt.% with respect to estimated dried cellulose) were added to the mixture. The calculated total amount of water was equal to 800 g. The amounts of water in H_2O_2 (70 wt.%) and in cellulose (100 wt.%) were removed from the calculated 800 g. So, the amount of water for the reaction was equal to 503 g. The mixture was mechanically stirred and the pH was allowed to decrease from 7 to 5 by adding HCl 25 wt.%.

The reaction was let to go for 2 h when the pH started to decrease. The pH was kept in the range 5-3 by adding 0.5 M NaOH solution. The mixture turned from white to yellow along the reaction occurred and after the reaction a pH equal to 3.74 was recorded (Figure 5).

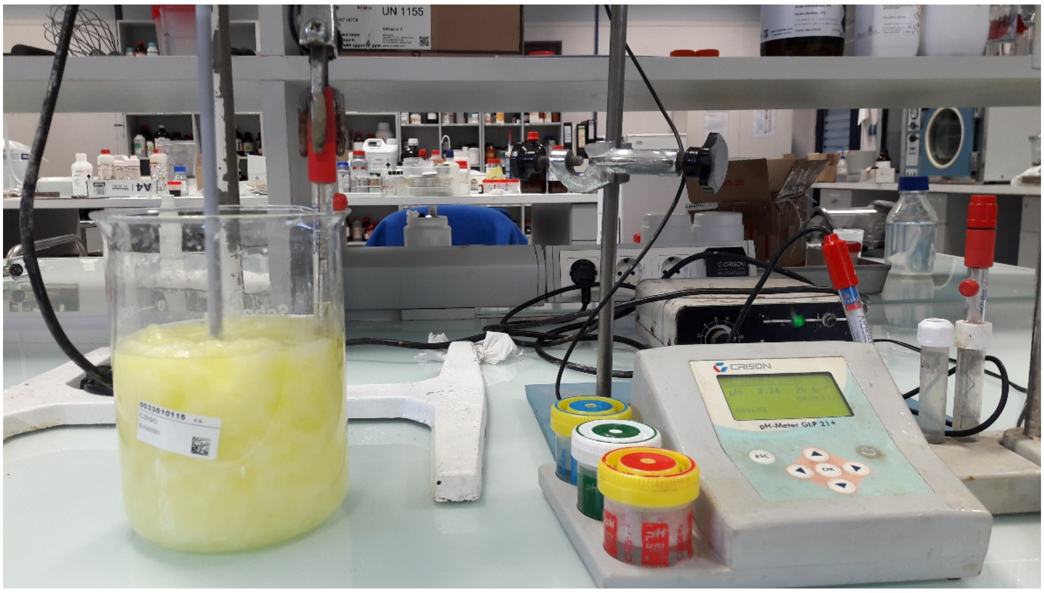


Figure 5. Cellulose oxidation reaction by $NaClO_2$.

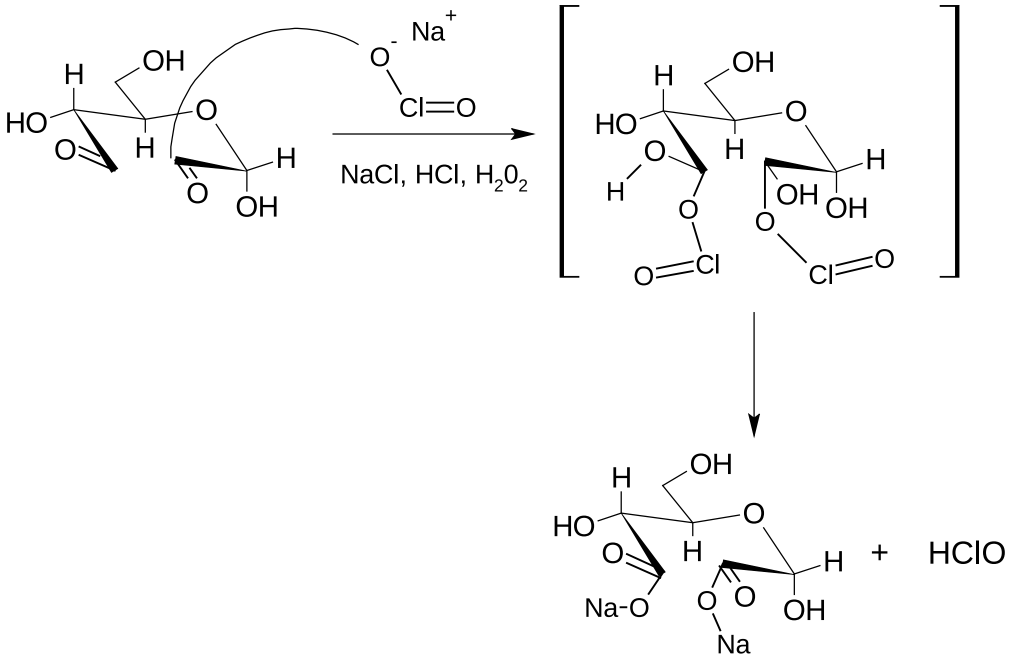
The mixture was filtrated (filter porosity= 500 μm) and washed with deionized water 8 times. The cellulose suspension was dispersed in water. The cellulose dispersion was stored in fridge at 5 °C. Two pans of cellulose dispersion were put in oven and dried at 105 °C for 3 hours. The weight of dried cellulose and the relative concentrations are reported in Table 4. The product is coded as dicarboxylic acid cellulose (**DCC**). It was worth to note that the most the DCC was washed with water, the softest and most swelled it appeared. It was a confirmation of the positive outcome of the reaction.

Table 4. DCC concentration.

Repetition	Suspension, (g)	Dried Cellulose, (g)	Concentration, (wt.%)
1	55.20	1.73	3.14
2	38.71	1.07	2.81
Average	46.95	1.40	2.97

- Mechanism of cellulose oxidation by $NaClO_2$

$NaClO_2$ is a strong and common oxidant reactive of cellulose. The hydrogen peroxide is necessary to quench the ipochlorite that forms along the reaction. The mechanism of oxidation is reported in Figure 6:



$$HClO + H_2O_2 \longrightarrow HCl + H_2O + O_2$$

Figure 6. Oxidation of DAC by $NaClO_2$ in order to obtain DCC.

- **Determination of carboxylate content**

The carboxylate content of DCC was determined by conductivity titration analysis by using a conductivity meter (Figure 7).

Figure 7. Conductivity meter.

The titration is performed on a solution of strong acid (HCl) and strong base (NaOH). Firstly, in the solution there are H_3O^+ and Cl^- ions. The high initial conductivity depends on ion high mobility. During the titration, the addition of Na^+ and OH^- affects the ionic content of the solution. At the beginning H_3O^+ ion content decreases, Cl^- ion content remains stable, Na^+ ion content increases step by step and OH^- ion contenti is $< 10^{-7}$. H_3O^+ ions are replaced by Na^+. The last one are less able to conduct, so the conductivity decreases progressively by adding the titrant NaOH. Then the solution reaches the equivalence point, that is the point at which chemically equivalent quantities of acid and base have been mixed. This point refers to the COOH groups present on DCC that do not react. After the equivalence point, H_3O^+ ion content is < 10^{-7}, Cl^- ion content remains stable, Na^+ ion content increases step by step and OH^- ion content increases. The presence in the solution of OH^- ions makes the conductivity value increase. A conductivity curve is obtained and the volume of titrant added, necessary to determine the COO^- ion content, is recorded drawing three tangents to the curve. The conductivity curve and the drawn tangent lines are reported in the Figure 8.

The cellulose dispersion was diluted from 2.97 wt.% to 0.02 wt.%. To a 154 mL of 0.02 wt.% cellulose dispersion (32 mg of dried cellulose), 2.5 mL of a 0.02 M NaCl were added and the mixture was gently stirred. Then 0.1 M HCl was added to the mixture to set the pH value to 2.91. 0.005 M NaOH solution was used to make the mixture reach pH 10.8-11 adding each time 0.5 mL. The carboxylate content was determined from the conductivity curve (Figure 8) using the following equation:

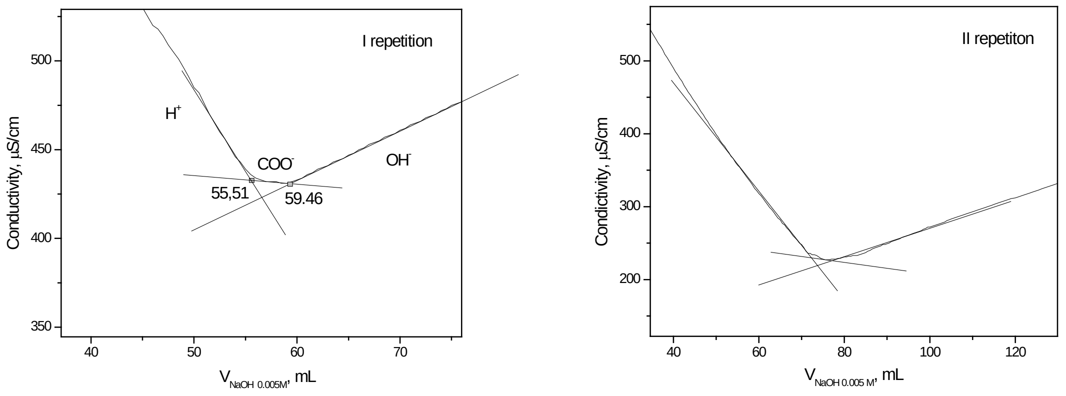


Figure 8. Conductivity curve (strong acid vs strong base).

$$[COO^-] = V_{NaOH} \text{ X } M_{NaOH}/w_{cell}$$

Where:

$[COO^-]$= carboxylate content (mmol/g)
V_{NaOH}= volume of NaOH 0.005 M (mL)
M_{NaOH}= molarity of NaOH (mol/L)
w_{cell}= weight of dried cellulose (g)

Another oxidation reactions was performed on the residual DAC (Figure 9) and the carboxylate determination was performed according to the same method discussed above.

Figure 9. Residual DAC oxidation by $NaClO_2$.

The determination of carboxylate groups from I and II batch are reported in Table 5.

Table 5. Data obtained after carboxylate group determination.

Sample	Cellulose, (g)	$V_{NaOH\ 0.005\ M}$, mL	Carboxylate content, (mmol/g)
I batch			
1	0.032	3.95	0.61
2	0.035	4.66	0.66
Average			0.63
II batch			
1	0.030	3.87	0.64

The content of carboxylate groups is higher than that of aldehydes one because plain cellulose contain carboxylic groups, that from literature data, amount around 0.05 mmol/g.

Another batch of DCC was prepared in order to have enough material for the preparation of composites with white glue. This time, all the filtration and washing cycles were performed using a filter with porosity equal to 63 μm in order to reduce drastically the mechanical material losses.
Amount of plain cellulose: 203 g. Total amount of obtained DCC: 183 g (losses 9.8 wt.%, concentration 3.5 wt.%) - Theoretical aldehyde content: 0.54 mmol/g - Effective aldehyde content: 0.42 mmol/g – Carboxylate content (NaOH added=3.79 mL, wcell=0.032g): 0.59 mmol/g.

- $NaIO_4$ and $NaClO_2$ Cellulose Oxidation, t=24h

The same oxidation reactions were performed on cellulose sheets from pine for a prolonged time, that were 24h. The aim is to test the effect of a more prolonged degree of oxidation on cellulose properties.
220 g of pine sheets (8wt.% moisture) were disintegrated in 6 L of deionized water reaching a concentration of 3.3. wt.%. The disintegration was performed in a planetary stirrer for 2h. Then, the obtained pulp was mixed with NaCl (5.8 wt.% respect to the total amount of water) and $NaIO_4$ (66 wt.% respect to cellulose without moisture), dissolved separately in 2 L of deionized water. The reaction was let to go for 24h in the dark. The sample was washed and filtered (MESH 63 μm) several times. It is labeled as **DAC'** (losses 27 wt.% with respect to the feed, average concentration 4.64

wt.%) - Average theoretical aldehyde content: 1.64 mmol/g - Average effective aldehyde content: 1.45 mmol/g.

DAC’ was carboxylated through chlorite oxidation in order to obtain dicarboxylic cellulose, labelled as **DCC’**. The oxidation was performed for 24 h and the pH trend from the beginning of the reaction was followed. The pH trend of the first 3h 20’ is reported in Figure 10.

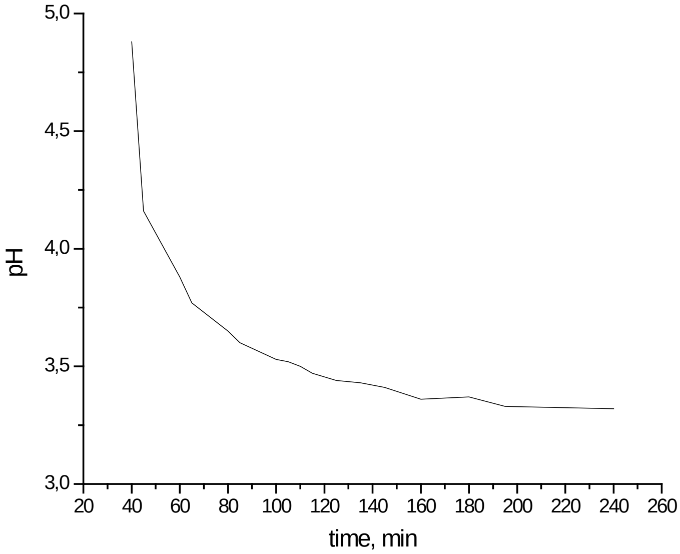


Figure 10. pH trend of chlorite oxidation reaction in the first 3 h 20‘.

The pH was tentatively corrected by adding NaOH 2 N solution in order not to have a value minor than 3. After 24 h of reactions the pH reaches a stationary state and the value stabilizes around 3.32. The cellulose dispersion does not appear yellow as in the first hours of reaction and the chlorine smell has nearly disappeared.

The sample was washed and filtered using a filter (MESH 63 μm). The sample was labelled as **DCC’**. Total amount of obtained DCC’: 108 g (concentration 3.2 wt.%); losses 32 wt.% with respect to DAC’; Carboxylate content: 1.41 mmol/g (NaOH added= 9.05 mL, wcell= 0.032 g).

DCC’ appears as a very soft and swellable gel-like material. The long reaction produces a more viscous and degraded cellulose than the material produced after only two hours of reaction.

- Morphological characterization

A small amount of Pine, DAC, DAC’, DCC and DCC’ were dispersed in water and a drop of each one was put on microscope slides. The water was left to evaporate under extractor hood and the fibers were observed through stereo microscope. Photos of the samples were taken at different magnifications by using lens 6.5, 10, 16, 25 and 40X (Figure 11). The length and width of the cellulose fibers were measured and listed in Table 5.

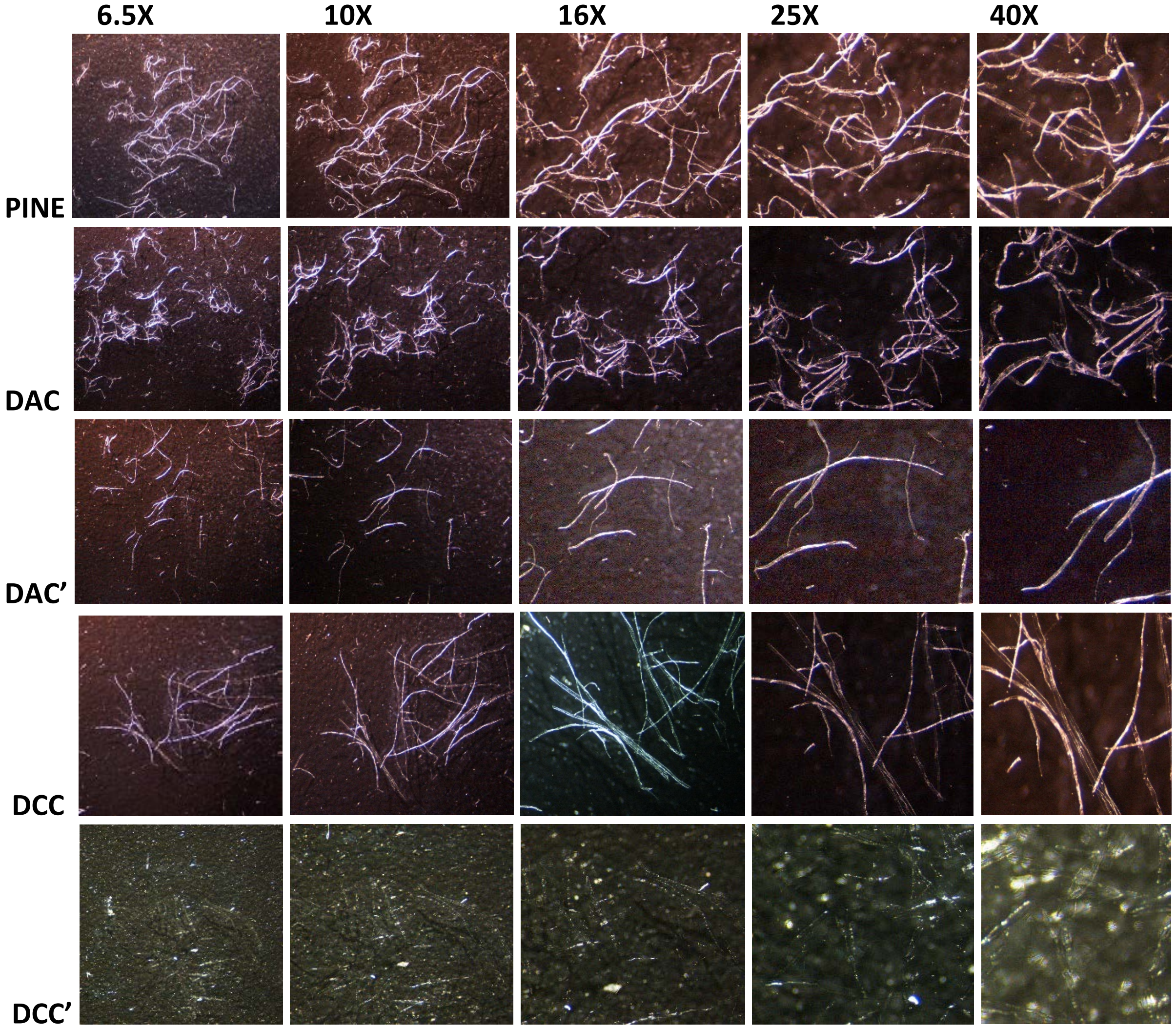


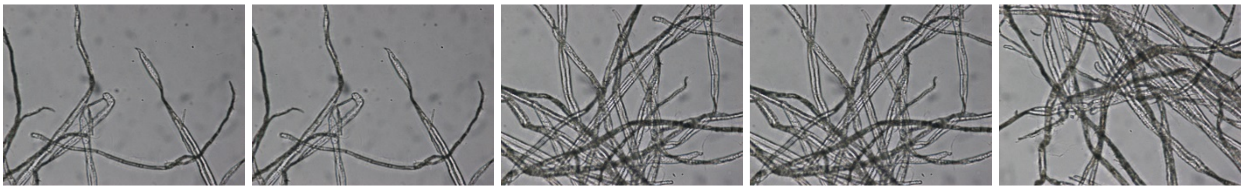
Figure 11: Optical Images of Pine, DAC, DAC’, DCC and DCC’; Lens 6.5, 10, 16, 25, 40X.

Table 5. Average length and width of fibers.

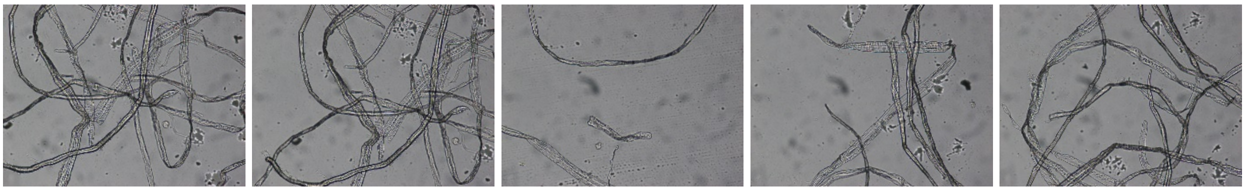
Sample	Average Length (μm) lens 10X	Average Width (μm) lens 40X
PINE	3023	45
DAC	4100	38
DAC’	2356	47
DCC	3162	57
DCC’	2442	100

A small amount of Pine, DAC, DAC’, DCC and DCC’ were dispersed in water and a drop of each one was put on microscope slides and covered with another microscope slide. The fibers were observed through a polarized optical microscope. Photos of the samples were taken using lens 10X (Figure 12).

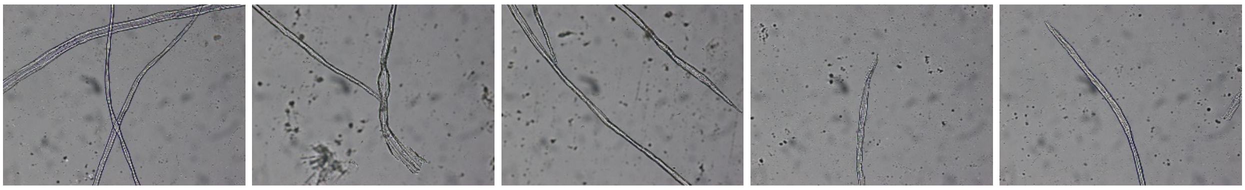
Pine



DAC



DAC’



DCC

DCC’

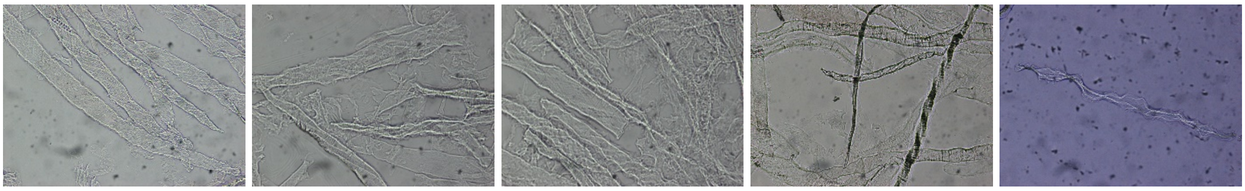


Figure 12. Optical photos of Pine, DAC, DAC’, DCC and DCC’ through polarized optical microscope.

A detail of sample DAC’ is reported in the sequence of photos illustrated in Figure 13. In this sequence it is possible to observe the aspect of the oxidized cellulose fiber that, along its length, opens and defibers.

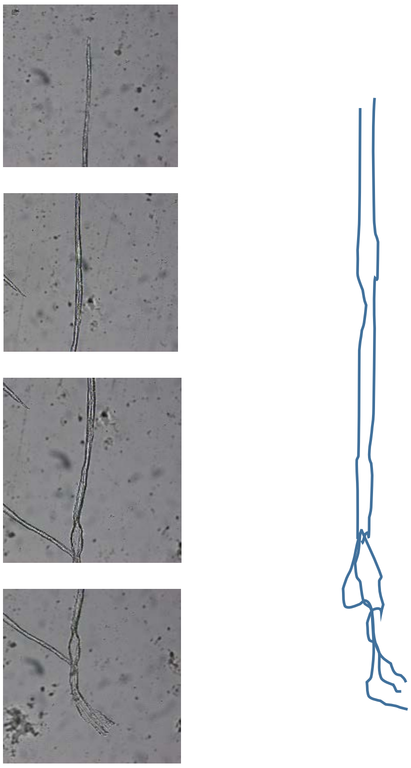


Figure 13. Aspect of a DAC’ cellulose fiber.

A detail of helicoidal morphology of DCC’ fibers is reported in Figure 14 where an optical image taken by lens 20X is shown.

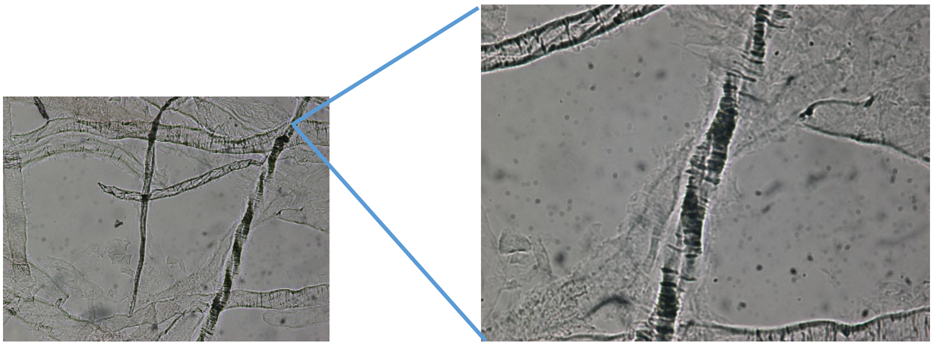


Figure 14. Aspect of a DCC’ cellulose fiber.

The distribution of fiber length was measured by means of an optical fiber analyzer and the distribution is reported in the Figure 15.

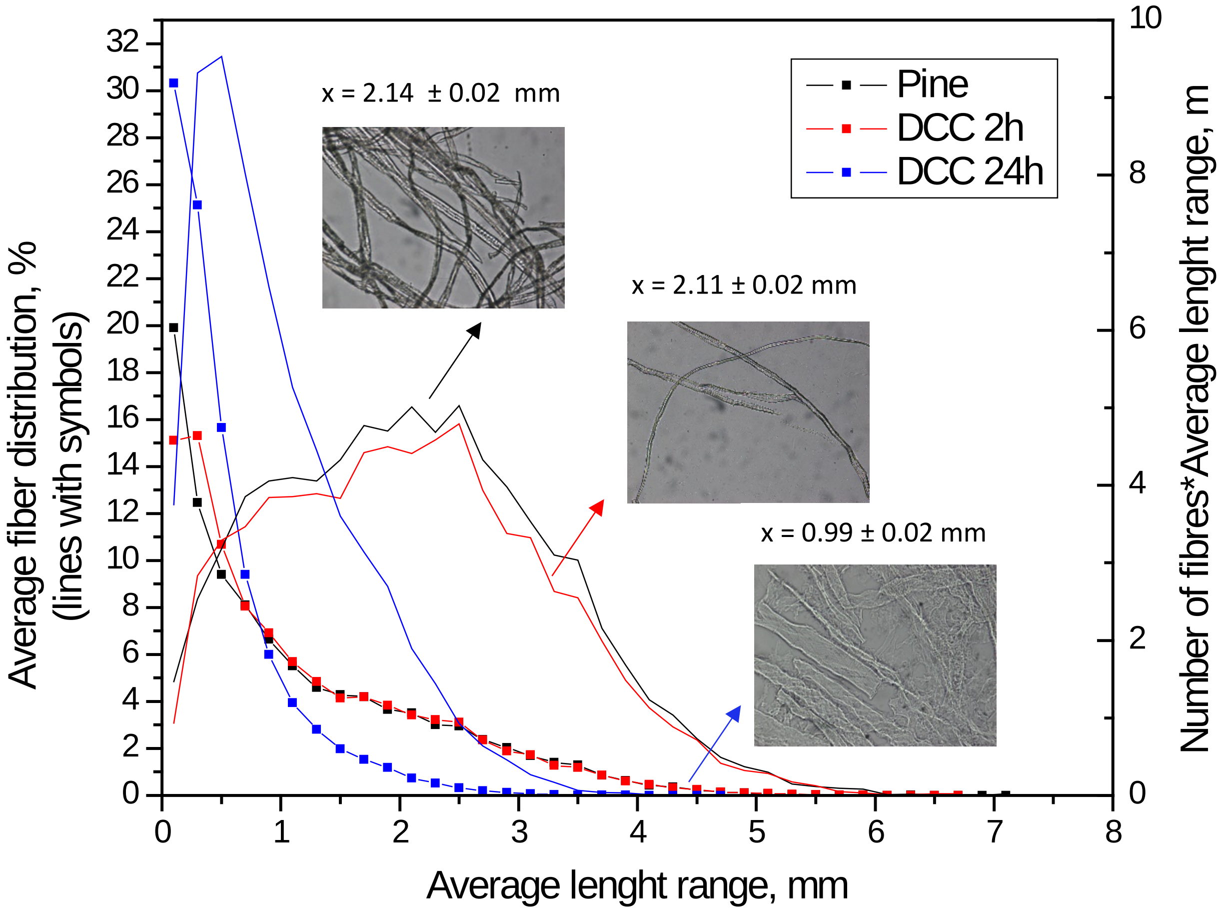


Figure 15. Fiber lenght distribution in Pine, DCC and DCC'.

- **Layer-by-Layer Deposition on DCC**

DCC was treated through Layer-by-Layer deposition in order to prepare cellulose fibres coated with lignin (L^-) and ammonia polyphosphate (APP^-). According to this method, DCC should have most char after thermal treatment and be more hydrophobic.

The samples were prepared by submerging DCC aqueous suspension (average concentration 2.9 wt.%), contained in a nylon bag, firstly in PEI^+ solution, then in water to wash it, then in plain lignosulfonates aqueous solution (L^-) and at the end in water to wash it (Figure 16). The pH of cellulose suspension and solutions was kept at 10 by adding NaOH 0.5 M. Firstly, the plain lignosulfonates were chosen among the other lignin and lignocellulosic samples because they were completely soluble in water. Then, after performing further solubility tests, it was observed that also lignin from SE-MAE treatment was soluble in water, when increased the pH at 12. Therefore, samples of DCC with LbL deposition and SL as lignin were prepared. Details about lignin and lignocellulosic samples solubility are reported in the supplementary section.

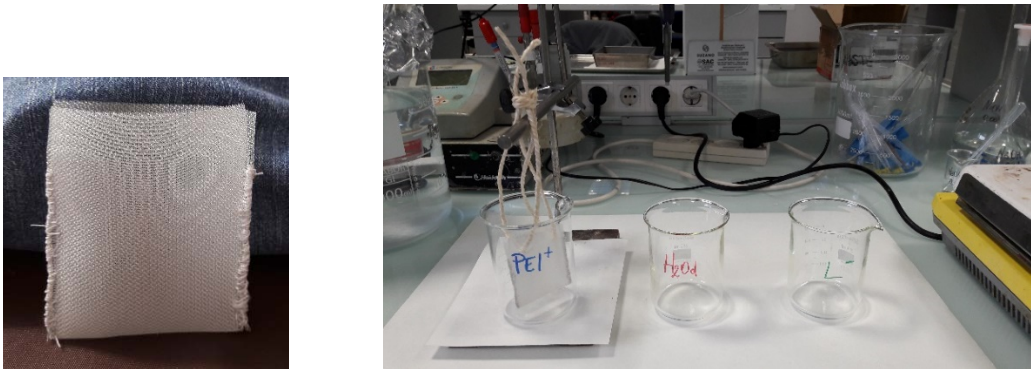


Figure 16. Setting of tools for LbL deposition.

The amount of PEI^+ (solid content 23-25.5 wt.%) and L^- was equal to 5 wt.% with respect to cellulose weight. Therefore, an adequate amount of PEI^+ was dissolved in 100 mL of water and the pH was increased at 10.21 by adding carefully NaOH 0.5 M. The bag with DCC was submerged in the PEI^+ solution and kept for 20 min under magnetic stirring. Then, the bag was submerged in deionized water at pH 10 and kept for 5 min in order to wash it. Finally, plain lignosulfonates were dissolved in water and the pH was increased from 4.83 to 10.22 by carefully adding NaOH 0.5 M. The bag was submerged in the lignosulfonates solution and kept for 20 min. Finally, the cellulose was further washed in deionized water for 5 min. All the phases of the preparation are illustrated in Figure 17. The final product is indicated as DCC $(PL)_1$.

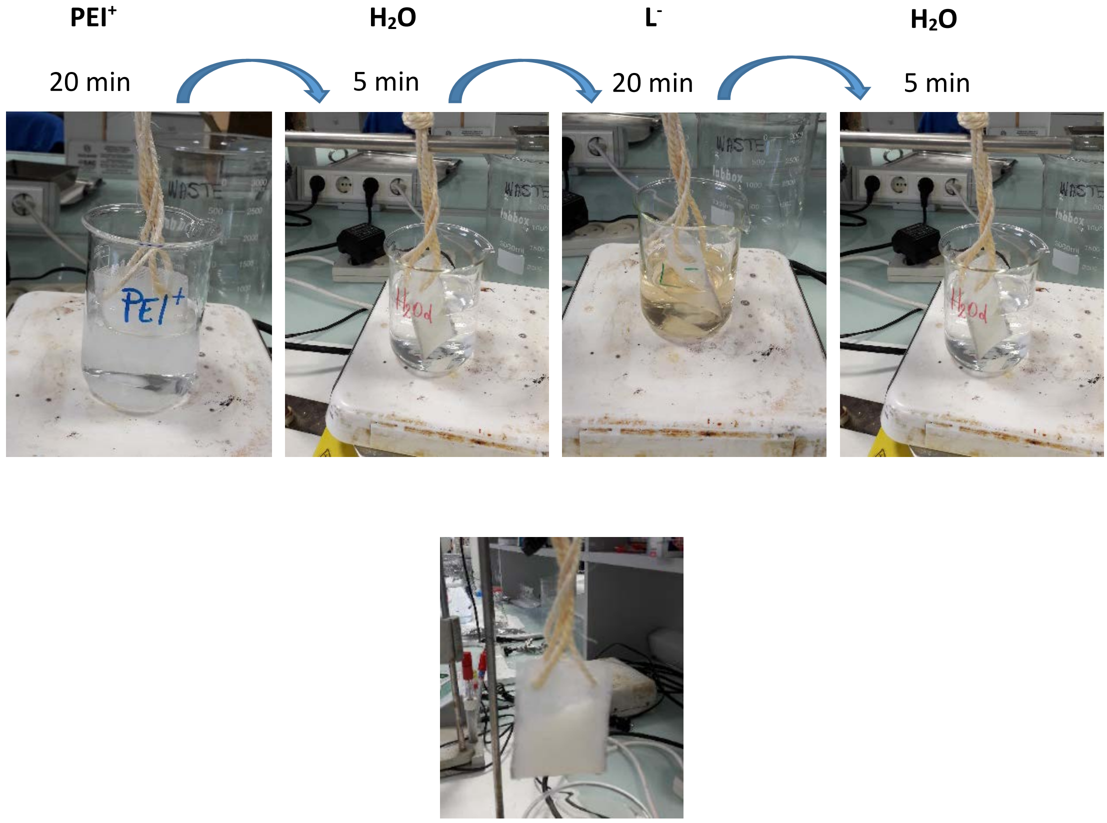


Figure 17. Layer-by-Layer deposition and preparation of DCC $(PL)_1$.

The DCC $(PL)_1$ in the bag was dried in oven under vacuum at 60 °C.

The same procedure was applied to deposit ten layers of PEI^+ L^- on DCC. The final product is indicated as DCC $(PL)_{10}$. Moreover, samples with ammonia polyphosphate, used as fire retardant, were prepared. A layer of PEI^+ L^- was alternated with one of PEI^+ APP^-. Two samples were obtained depositing one layer and five layers of PEI^+ L^- PEI^+ APP^- on DCC, thus obtaining DCC $(PLPA)_1$ and DCC $(PLPA)_5$. The procedure is shown in Figure 18.

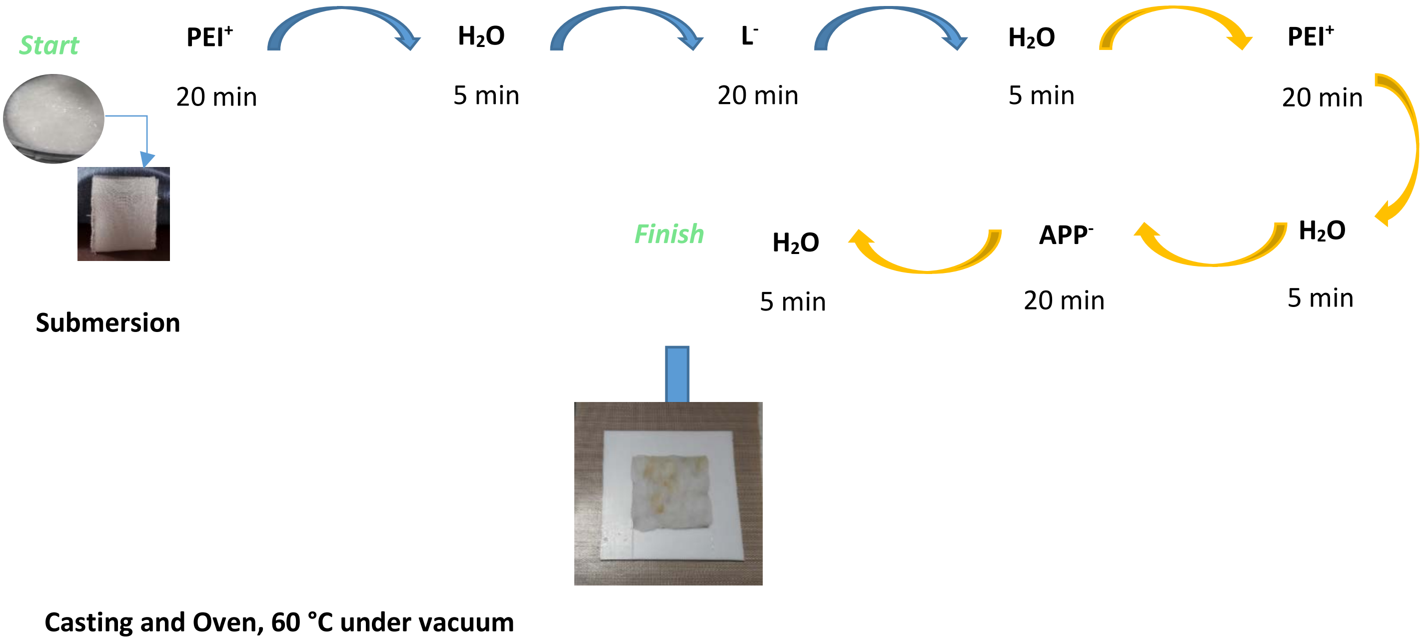


Figure 18. Preparation of DCC PLPA.

Then the samples were transferred in a Teflon mold (thickness 2 mm) and sandwiched between two Teflon sheets. A portion of the samples was re-dispersed in water and stored in fridge at 4 °C and all the rest was dried in oven at 60 °C under vacuum until complete drying was reached. Finally, the dried samples were pressed in order to decrease the thickness around 0.8-1 mm and try to compact the fibers to perform angle contact measurements. Both samples are characterized by a not too rough surface and are whitish. An example of dried and pressed sample is illustrated in Figure 19.

Figure 19. Dried DCC after LbL deposition.

The same procedure was followed to prepare DCC samples using SL lignin instead of LS (Figure 20), with the aim to obtain a more hydrophobic surface. SL was dissolved in water at pH 12 (conc. 0.25 wt.%) and two samples were prepared alternating PEI^+ SL^- and PEI^+ SL^- PEI^+ APP^-, thus obtaining:

- DCC $(PSL)_1$;
- DCC $(PSLPA)_1$

Figure 20. Submersion of DCC in SL (right); Aspect of DCC (PSL)₁ after LbL deposition.

- Thermal analysis (TGA)

TGA analysis under nitrogen and air flow (flow rate 60 mL/min) was performed on all the dried samples in oven at 50 °C under vacuum. Pine, DAC and DCC were analyzed as references. Moreover, samples of Pine and DCC were adjusted to pH 10 and then stirred for 100’ in order to reproduce the same conditions used to obtain layer-by-layer treated samples. All the thermal analysis were performed according to the following thermal program:

- 1° Heating RT-100 °C, 20 °C/min;
- Isotherm 100 °C, 30 min;
- 2° Heating 100-900 °C, 10 °C/min.

Thermograms and DTG signals of pine, pine pH 10, 100’, DAC and DAC’ are reported in Figure 21 and thermal data in Table 6.

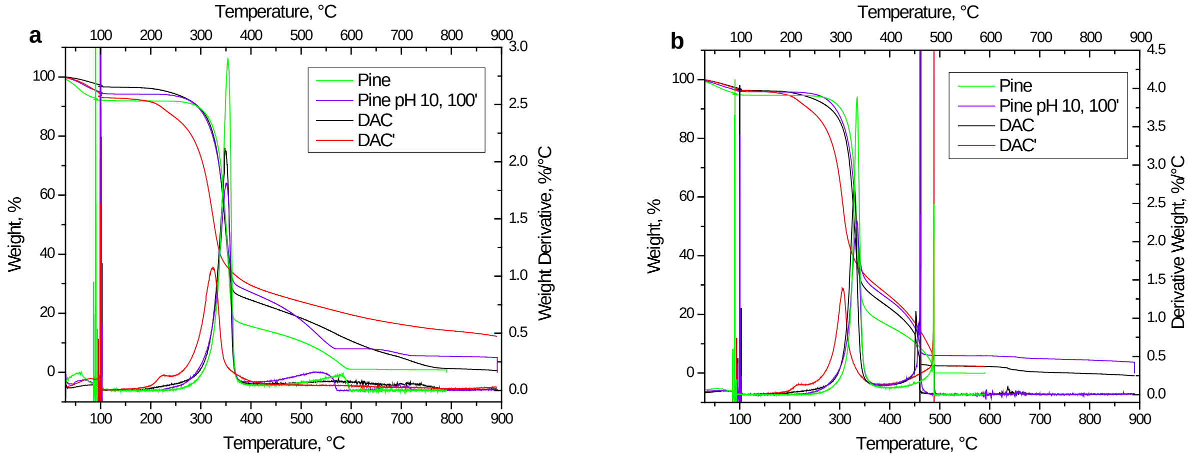


Figure 21. TGA thermograms of Pine, Pine pH 10, 100’, DAC and DAC’; a) nitrogen, b) air.

Table 6. Thermal parameters from TGA of Pine, Pine pH 10, 100’, DAC and DAC’.

	NITROGEN			AIR		
Sample	**T$_{max}$, °C**	**Residue$_{500\ °C}$, %**	**Char$_{900\ °C}$, %**	**T$_{max}$, °C**	**Residue$_{500\ °C}$, %**	**Char$_{900\ °C}$, %**
Pine	353; 581	10.5	0.8	334; 484	0	*n.a*
Pine pH 10, 100’	351; 540	16.6	5.0	332; 458	6.0	3.7
DAC	346; 564 (very low)	18.4	0.6	328; 455; 654	2.4	-
DAC’	224; 323	23.9	12.2	224; 313; 493	2.6	-

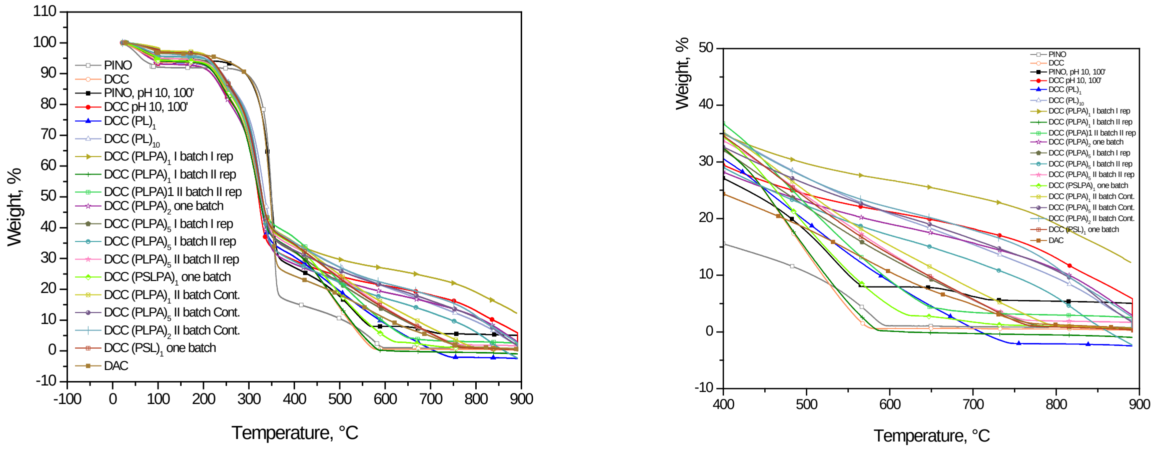
n.a. not analyzed

DAC obtained after 2h of oxidation reaction seems to thermally behave as untreated pine cellulose. On the contrary, the initial weight loss of DAC’, obtained after 24h of oxidation reaction, occurs at lower temperature (around 220 °C) than untreated pine.Therefore, DAC thermal stability is lower than pine. It is explained assuming that the oxidised cellulose is degraded with a large fraction of oligomers and less energy is necessary to decompose them. At higher temperatures DAC’ seems to become more stable than pine due to the higher residue at 500 °C.

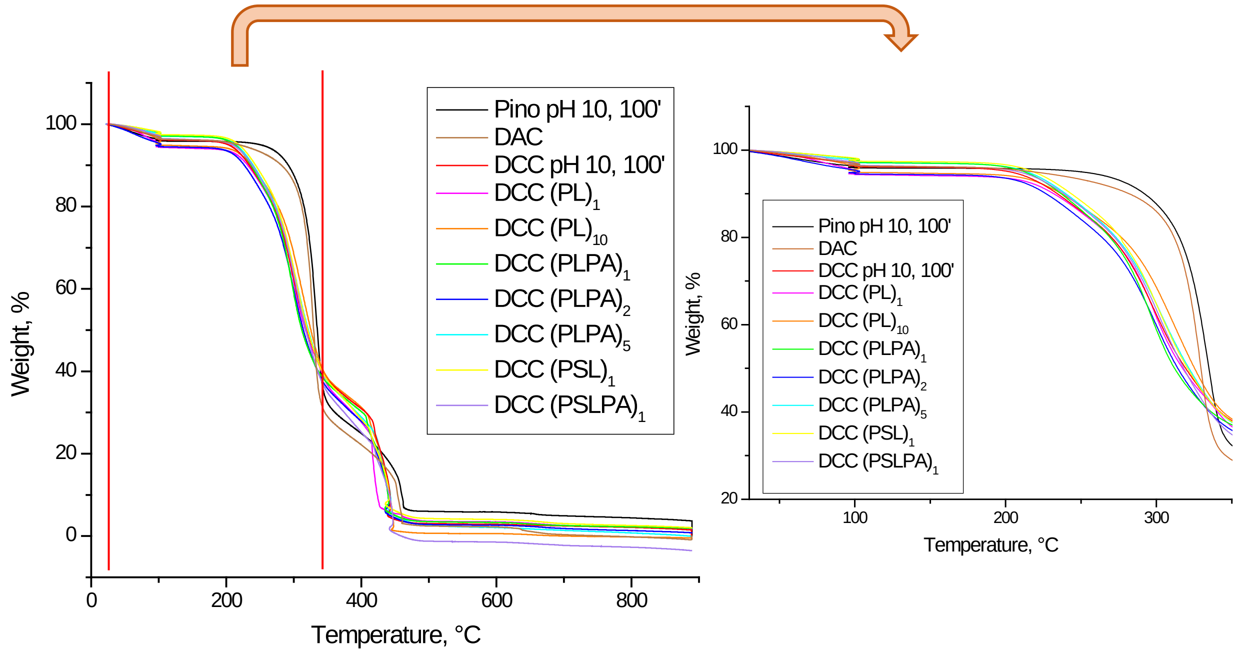
The thermograms and DTG signals of samples treated by layer-by-layer deposition are illustrated in Figure 22. Thermal data are listed in Table 7. The samples analyzed in nitrogen atmosphere were tested in duplicate.

N.B. The samples labelled CONT. refer to ones after contamination of a solvent (probably dodecane).

a



b



N.B. All the TGA in air were performed on the II batch of the samples with PLPA layers.

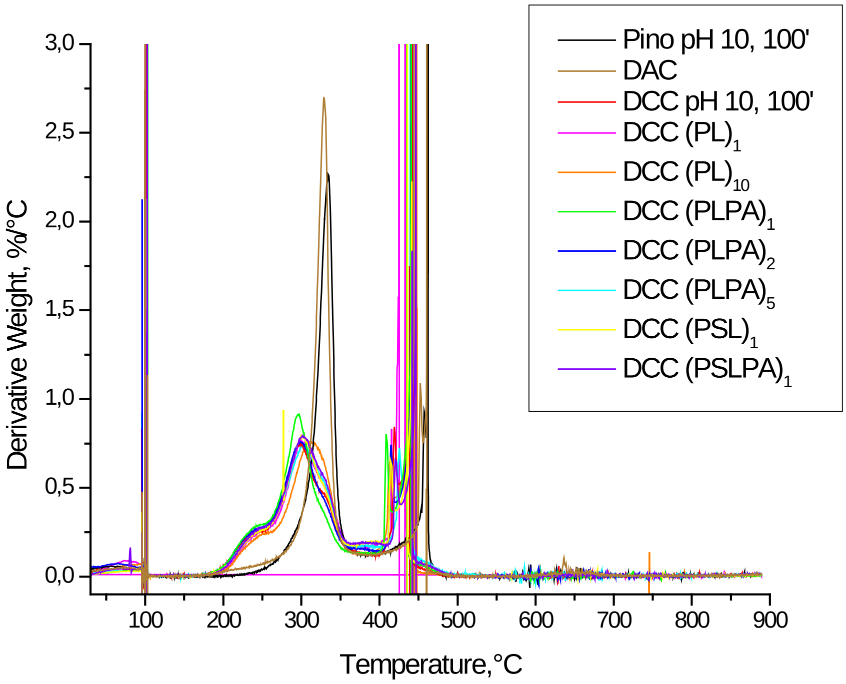


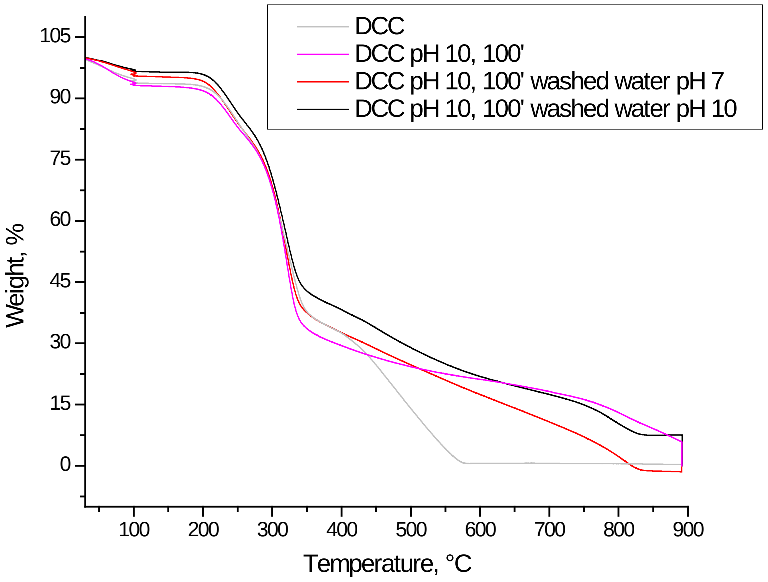
Figure 22. Thermograms and DTG curves; a) nitrogen, b)air.

Table 7. Thermal parameters from TGA analysis.

	NITROGEN		**AIR**	
Sample	**T_{max} (°C)**	**Char $_{900\ °C}$, %**	**T_{max} (°C)**	**Char $_{900\ °C}$, %**
PINE	353; 581	0.8	334; 484	*n.a.*
DAC	346; 564 (very low)	0.6	328; 455; 654	-0.9
DCC	247; 322; 461	0.3		
PINO, pH 10, 100’	351; 540	5.0	332; 458	3.7
DCC, pH 10, 100’	239; 321; -	5.9	239; 297; 421; 438	1.5
DCC (PL)$_1$	243; 324; 441	-2.0	244; 299; 418; 435	1.8
DCC (PL)$_{10}$	250; 332; 441	2.0	246; 311; 414; 443	0
DCC (PLPA)$_1$ I batch I replica	240; 322; -	12.4		
DCC (PLPA)$_1$ I batch II replica	242; 319; 462	-0.9		
DCC (PLPA)$_1$ II batch II replica	243; 320; 449	2.5		
DCC (PLPA)$_1$ II batch CONT.	241; 319; 398	0.7	242; 295; 410; 435	1.9
DCC (PLPA)$_2$ one batch, I replica	242; 326; -	2.9		
DCC (PLPA)$_2$ one batch, CONT.	238; 320; 405	2.6	240; 298; 416; 433	0.8
DCC (PLPA)$_5$ I batch I replica	247; 327; 427	0.8		
DCC (PLPA)$_5$ I batch II replica	247; 329; -	-2.3		
DCC (PLPA)$_5$ II batch II replica	248; 324; 466	1.7		
DCC (PLPA)$_5$ II batch CONT.	242; 326; -	1.8	243; 302; 426; 439	0
DCC (PSL)$_1$ one batch	243; 321; 405	0.4	243; 301; 415; 434	2.1
DCC (PSLPA)$_1$ one batch	247; 325; 461	0.4	243; 302; 423; 440	-3.5

n.a. not analyzed

In order to understand if the method of deposition is effective or not, DCC was washed with water at pH 7 and pH 10. In particular 13.35 g of DCC were well dispersed in 40 mL of water and then they were treated with NaOH 0.5 N to increase the pH up to 10. The DCC dispersion was magnetically stirred for 1 h 40’ and then the cellulose was filtered in the same nylon bag used to perform the layer-by-layer deposition. Then, the cellulose in the nylon bag was submerged in water at pH 10 for 20’. The cellulose was dried in oven under vacuum at 50 °C. The same procedure was carried out washing cellulose in water at pH 7. TGA analysis under nitrogen flow was performed on the samples according to the abovementioned thermal program and the thermograms along the DTG signals are illustrated in Figure 23.



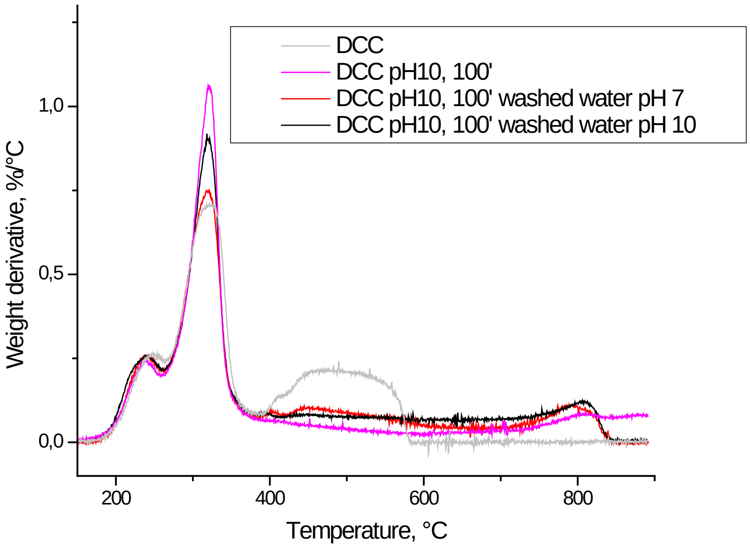


Figure 23. TGA (up) and DTG (down) thermograms of DCC samples with and without washing; nitrogen.

Table 8. Thermal parameters from TGA of DCC with and without washing.

Sample	**T_{max} (°C)**	**Char $_{900\,°C}$,%**
DCC	247; 322; 461	0.3
DCC pH 10, 100’	239; 321; 418 (shoulder); 801	5.9
DCC pH 10, 100’ washed water pH 7	239, 319	-1.5
DCC pH 10, 100’ washed water pH 10	239; 319; 446; 792	7.6

The samples after compression were tested to measure the contact angle. All the samples were very hydrophilic and it was not possible to measure contact angle. An example is shown in Figure 24.

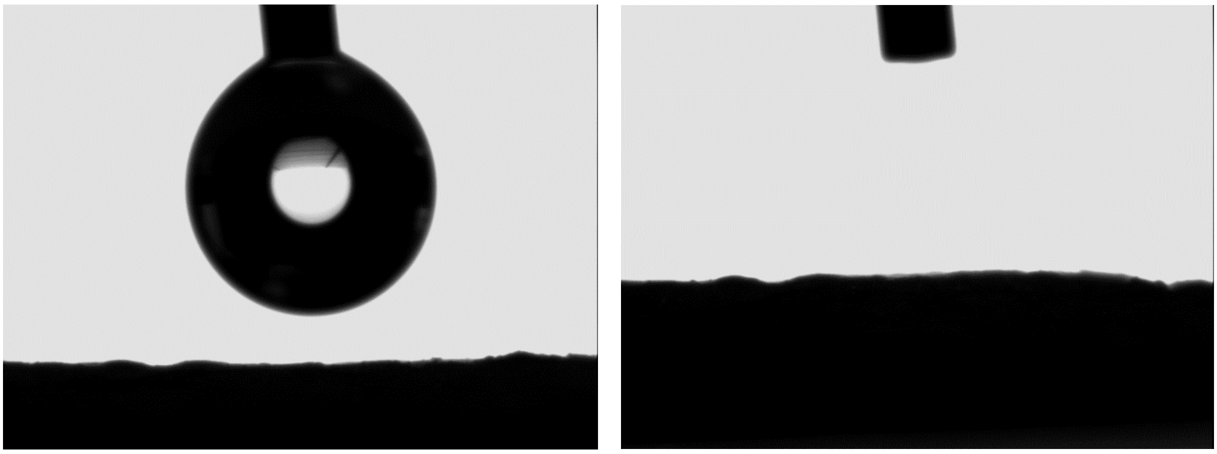


Figure 24. (left) Deposition of water drop on DCC $(PL)_{10}$ surface. (right) Absorbed drop by the surface.

- Chemical Characterization (FT-IR ATR)

Some of the samples (Pine, DAC, DCC, DCC $(PL)_1$ and DCC $(PL)_{10}$) were sent to IPCB (Pozzuoli, Italy) in order to characterize them through SEM, EDX, ^{13}CP MAS NMR and FT-IR ATR. The samples without drying were chemically characterized through FT-IR ATR and the spectra are illustrated in Figure 25.

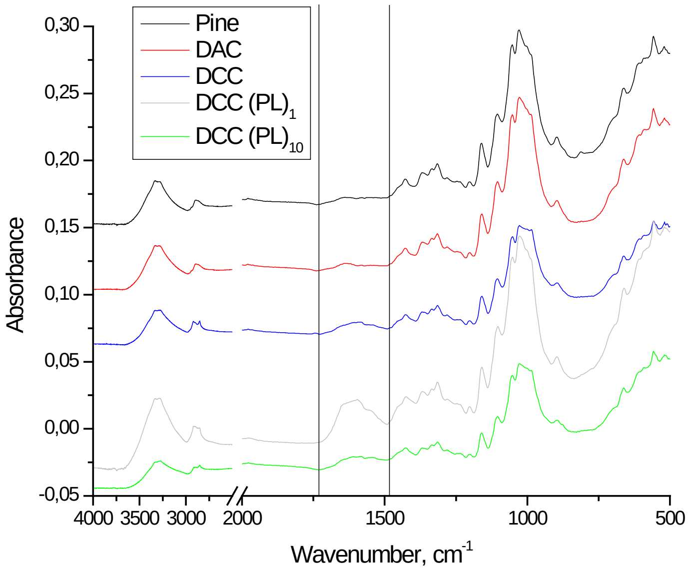


Figure 24. FT-IR ATR spectra of Pine, DAC, DCC, DCC $(PL)_1$ and DCC $(PL)_{10}$.

It is possible to observe in the region between 1733 and 1490 cm^{-1} a broad band whose intensity increases after carboxylation and deposition of only one layer of PL on DCC. This band is in the typical region of absorbed and bonded water, but also of carboxyl groups (1720 cm^{-1}). Generally, it is also possible to observe a peak at 880 cm^{-1}, attributed to hemiacetal and hydrated forms, that should increase when the oxidation degree increases. In this case we observe a peak at 890 cm^{-1}, but the intensity remains the same from pine to DAC to DCC. Probably we are not able to see any peaks related to caroxyl units due to the low degree of oxidation. Anyway,in order to remove water from the samples, ATR measurements on dried (oven °C, 5 h and 12 h) Pine and DAC were performed and the spectra are illustrated in Figure 25. The spectra of the ones without drying treatment are illustrated for comparison.

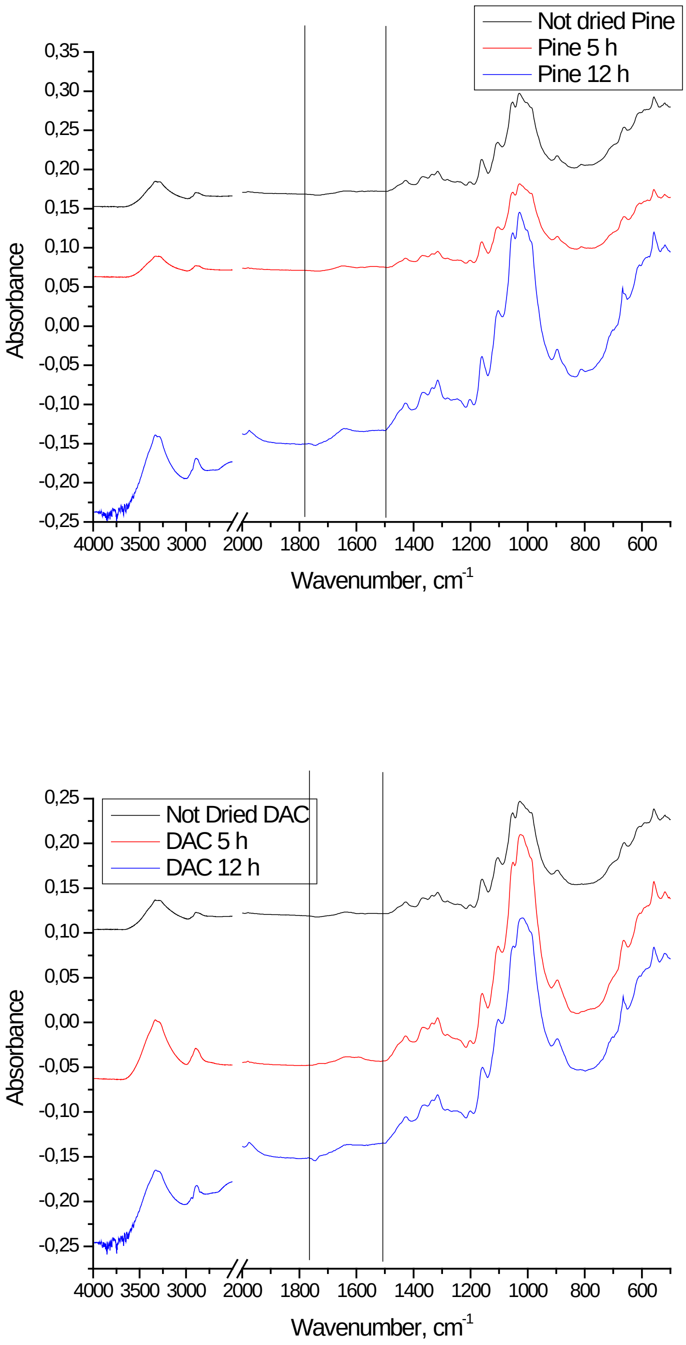
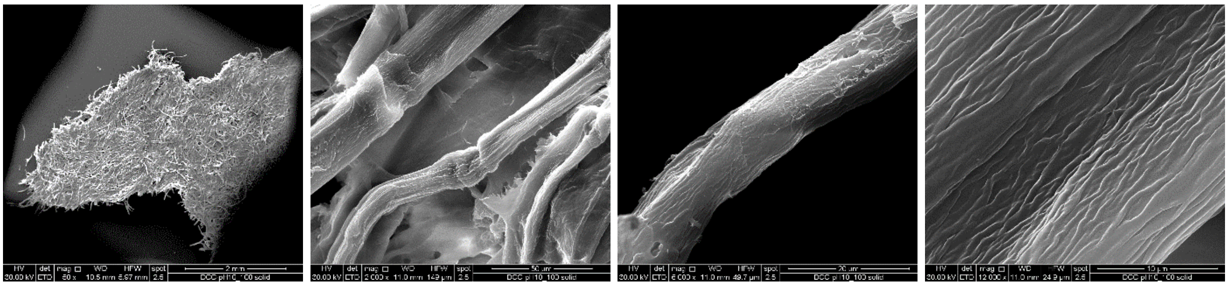


Figure 25. FT-IR ATR spectra of Pine and DAC after drying for 5 and 12 h.

- Morphological analysis (SEM - EDS)

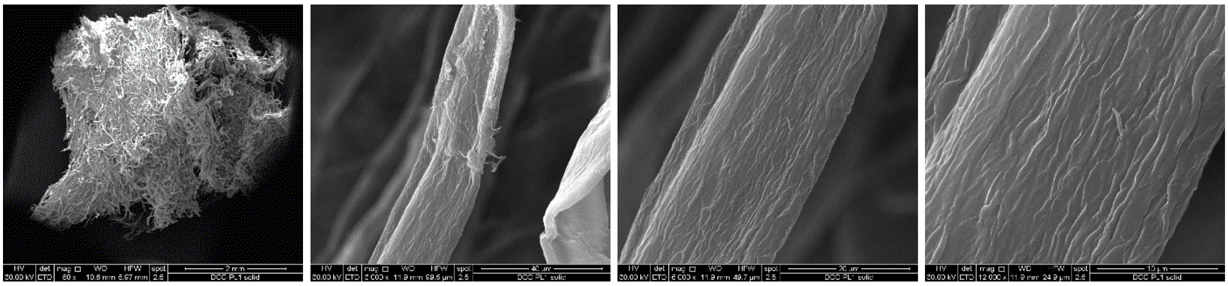
Dried samples of DCC pH 10, 100’, DCC (PL)$_1$ and DCC (PL)$_{10}$ were characterized through scanning electron microscopy. The micrographs are reported in Figure 26.

DCC pH 10, 100’



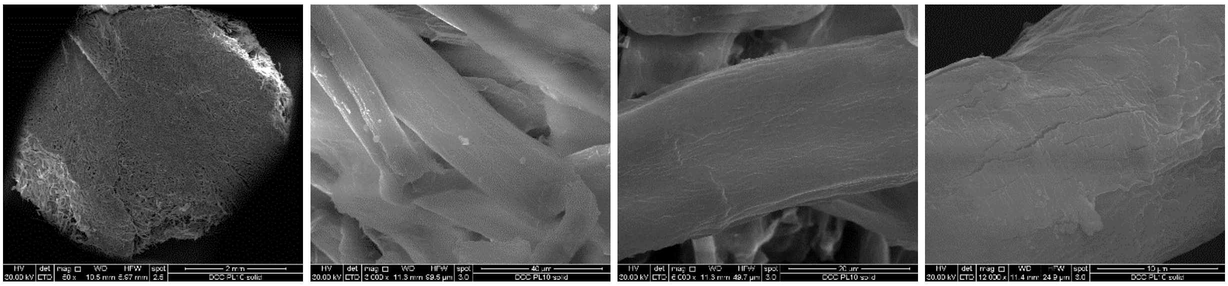
50X *2000X* *6000X* *12000X*

DCC (PL)$_1$



50X *3000X* *6000X* *12000X*

DCC (PL)$_{10}$

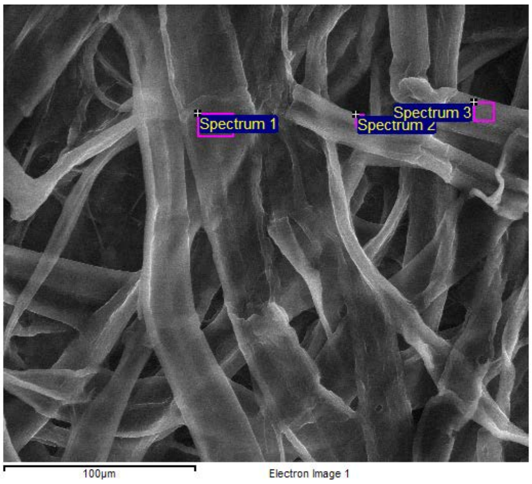


50X *3000X* *6000X* *12000X*

Figure 26. SEM micrographs of DCC pH 10, 100’, DCC (PL)$_1$ and DCC (PL)$_{10}$.

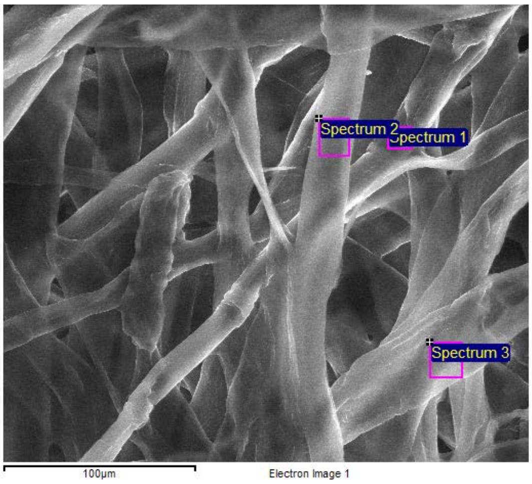
The same samples were analyzed through Energy Dispersive X-Ray Spectroscopy (EDS) in order to determine the elemental composition and to, eventually, detect the presence of sulfur atoms in the samples treated by layer-by-layer deposition. The elemental analysis is reported below.

DCC pH10, 100'



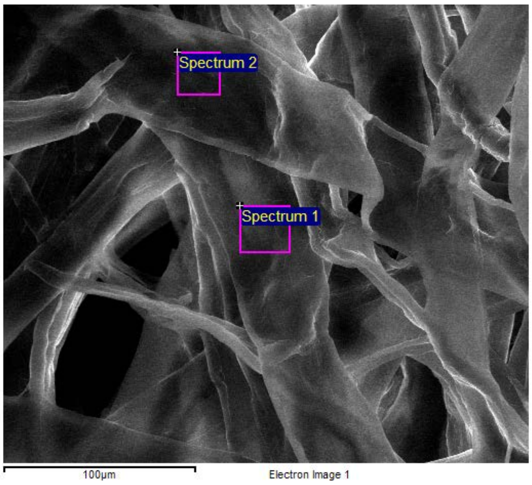
Spectrum	In stats.	C, %	O, %	Na, %	Al, %
Spectrum 1	Yes	53.36	40.55		6.09
Spectrum 2	Yes	47.19	51.14	0.68	1.00
Spectrum 3	Yes	45.43	52.90	0.54	1.12
Max.		53.36	52.90	0.68	6.09
Min.		45.43	40.55	0.54	1.00

DCC $(PL)_1$



Spectrum	In stats.	C, %	O, %	Na, %	Al, %	Total
Spectrum 1	Yes	46.77	51.90	0.80	0.54	100.00
Spectrum 2	Yes	45.71	53.53	0.49	0.27	100.00
Spectrum 3	Yes	46.41	52.46	0.76	0.36	100.00
Mean		46.29	52.63	0.68	0.39	100.00
Std. deviation		0.54	0.83	0.17	0.14	
Max.		46.77	53.53	0.80	0.54	
Min.		45.71	51.90	0.49	0.27	

DCC $(PL)_{10}$



Spectrum		In stats.	C, %	O, %	Al, %	Total
Spectrum 1		Yes	46.48	50.90	2.61	100.00
Spectrum 2		Yes	46.50	51.82	1.68	100.00
Mean			46.49	51.36	2.14	100.00
Std. deviation			0.01	0.65	0.66	
Max.			46.50	51.82	2.61	
Min.			46.48	50.90	1.68	

DCC’ aqueous suspension (30.7397 g average concentration 3.2 wt.%, 0.980 g), contained in a nylon bag, was submerged firstly in PEI^+ solution, then in water to wash it, then in plain lignosulfonates aqueous solution (L^-) and at the end in water to wash it. The pH of cellulose suspension and solutions was kept at 10 by adding NaOH 0.5 M. The amount of PEI^+ (solid content 23-25.5 wt.%) and L^- was equal to 5 wt.% with respect to cellulose weight. The layer-by-layer deposition was performed in such a way to obtain 10 layers of PL. During the procedure of deposition the cellulose absorbs a lot of water and it swells as to appear as a gel. The sample was labelled as DCC’ $(PL)_{10}$. A part of the sample was stored in fridge and the other was dried in oven at 105 °C. DCC’ $(PL)_{10}$ photos are reported in Figure 27. The dried sample appears as a discontinuous brittle film.

Figure 27. DCC’ $(PL)_{10}$

The thermal stability of the samples was analyzed through TGA under nitrogen according to the aforementioned thermal program. The thermograms and the thermal data are reported in Figure 28 and Table 9.

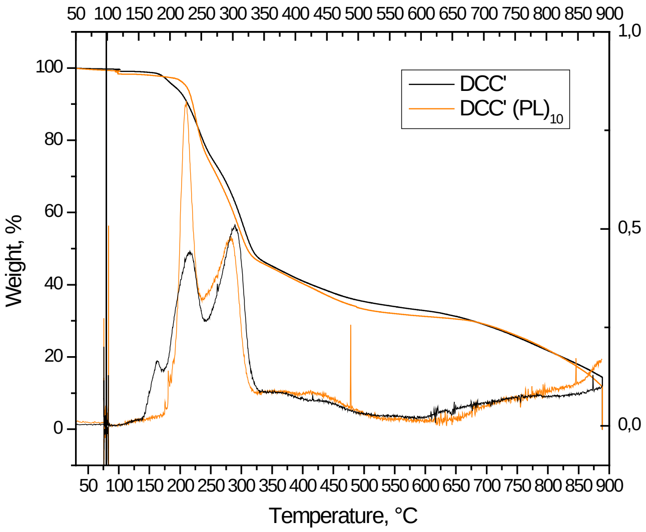


Figure 28. TGA thermograms of DCC’ and DCC’ $(PL)_{10}$.

Table 9. Thermal data from TGA experiments.

Sample	**Tmax, °C**	**Char, 900 °C**
DCC’	179.7; 230.6; 304.07	14.4
DCC’ $(PL)_{10}$	226.3; 295.9	11.8

The measurement were performed in duplicate.

- Preparation of Cellulose Composites

A pine dispersion (3 wt.%) was prepared, disintegrating cellulose sheets for 2 hours under a planetary stirring. The cellulose dispersion was treated with NaOH 0.5 M to increase the pH up to 10. The pine dispersion (1L) containing around 29 g of cellulose was treated with standard white glue (solid content 48 wt.%) at 1wt.% with respect to cellulose. The system was gently mechanically stirred and then filtered on a nylon membrane (25 μm). The filtrate appeared white and turbid. 200 g and 100 g of pine pulp after the removal of water were placed into a mould (10X10X2 cm) and compressed. The system was left in oven at 60 °C for 4 hours. Two bricks of 16.8 and 8.8 g were obtained (Pine/Glue). It was estimated a loss of around 11 wt.%. The aspect of the samples is shown in Figure 29.

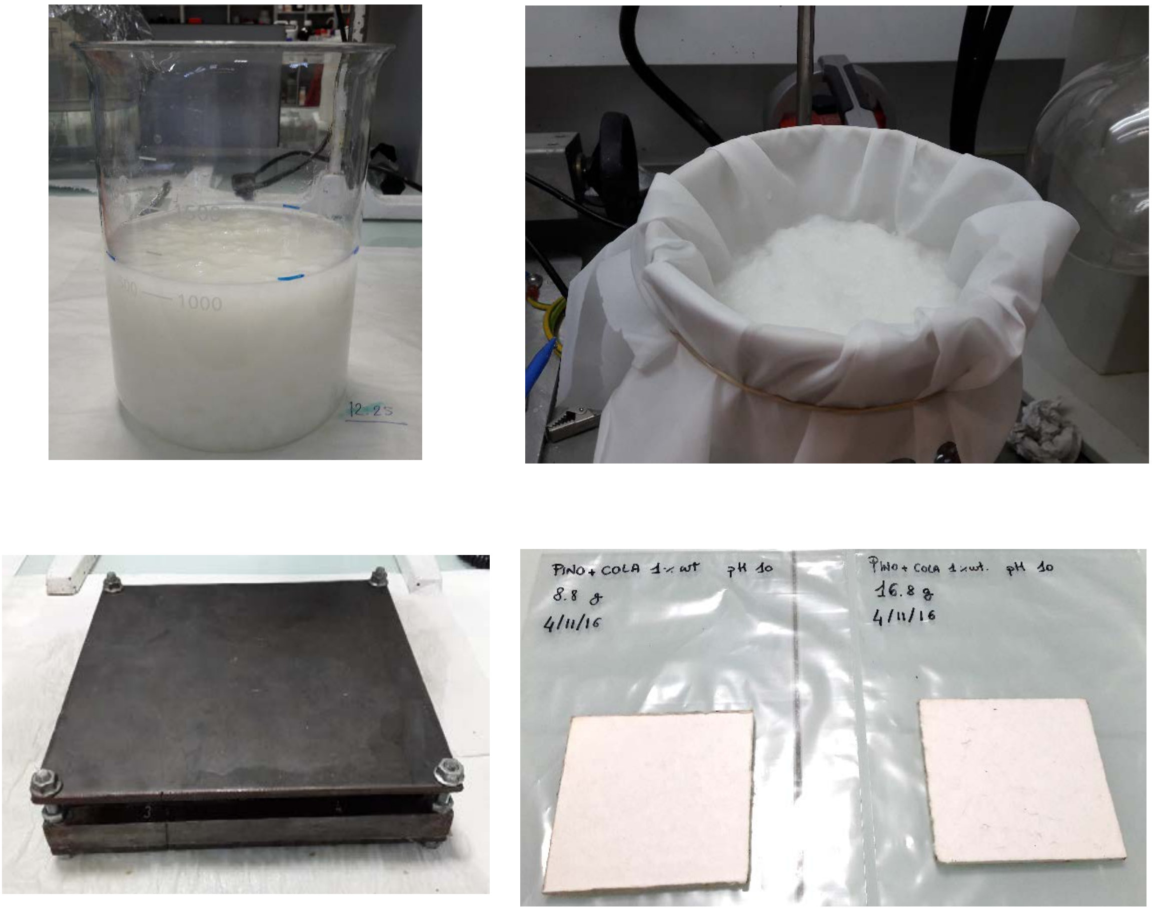


Figure 29. Preparation of pine-white glue composites.

In order to avoid the loss of white glue during the filtration, samples of pine were prepared adding the glue (1 wt.%) on the filtered cellulose pulp (Pine/Glue after filtration). The glue was tentatively dispersed mixing by hands and then the casting was performed. Composites of pure cellulose from pine (Pine) were prepared according to the same method for comparison. The properties of the composites are listed in Table 10.

Table 10. Properties of cellulose composites.

Sample	Mass, g	Thickness, cm	Density, g/cm^3	Average Density, g/cm^3
Pine 1	19.60	0.59; 0.64	0.34; 0.32	0.33
Pine 2	21.29	0.67	0.40	-
Pine/Glue 1	16.80	0.54; 0.48	0.32; 0.36	0.34
Pine/Glue 2	8.80	0.33; 0.43	0.26; 0.20	0.23
Pine/Glue after filtration 1	15.96	0.69; 0.65	0.23; 0.24	0.24
Pine/Glue after filtration 2	16.88	0.65	0.27	-

- Conclusions

The activity carried out in Tecnalia allowed to obtain cellulose systems that were coated by different layers of polyethylenimmine and lignosulfonates, in some cases a fire retardant was used, the ammonium polyphosphate. The experimental design to obtain these systems must be optimized and the formulations must have chemically characterized.

Supplementary Section

- Lignin and Lignocellulosic Samples Solubility

Tests of lignin and lignocellulosic samples solubility were performed. Samples solubility was tested in water at pH 4-6 and pH 10. The tested samples were:

1. **SE-MAE LIGNIN (SL):** This lignin was isolated from *Phragmites australis* residue (common reed, Lake Trasimeno, Italy) through steam explosion and microwave-assisted organosolv extraction. The solvent used in the organosolv extraction method was γ-valerolactone. The lignin was recovered by water precipitation and then it was treated by means of Soxhlet extraction to remove all the contaminants, as γ-valerolactone traces. The contaminants were equal to 8.7 wt.%. From HPLC analysis the material is composed by around 98 wt.% of lignin.

2. **LIGNOSULFONATES (LS):** This sample is made of conventional calcium lignosulfonates, obtained after cooking with aqueous calcium sulfur dioxide. It is the conventional bleaching treatment aimed at recovering cellulose to produce paper.

3. **DIALYZED LIGNOSULFONATES (LD):** This sample is made of dialyzed calcium lignosulfonates. The calcium lignosulfonates (10 wt./v.%) were dialyzed (cutoff dialysis tubes= 3500 Da) until the conductivity of the water was around 10 μS/cm (plain water conductivity=1.2 μS/cm). After each cycle of dialysis an amount of material equal to 42.2 wt.%, with respect to the inlet, was recovered. From ^{13}C MAS NMR it was observed that dialysis reduce dramatically the content of hemicelluloses in lignosulfonates. Therefore, dialyzed lignosulfonates are composed by lignin and a minor amount of hemicelluloses.

4. **LIGNOCELLULOSE, NO WATER EXTRACTION (LC):** This sample is a lignocellulosic biomass that is composed by 59 wt% of Klason lignin (acid-insoluble lignin) and 36 wt% of holocellulose, that comes from the second generation bioethanol production (*Arundo donax* biomass).

5. **LIGNOCELLULOSE, WATER EXTRACTION (W):** This sample is (4) after water treatment. The sample at 2% w/v was treated at room temperature for 5 h in order to remove all the water-extractives. The water-extractives were determined by subtraction with the residue and were equal to 14.7 wt%.

The ratio lignin samples/water was 1:20 (g/v). The solubility of the samples is illustrated in Figure S1 and the data are listed in Table S1.

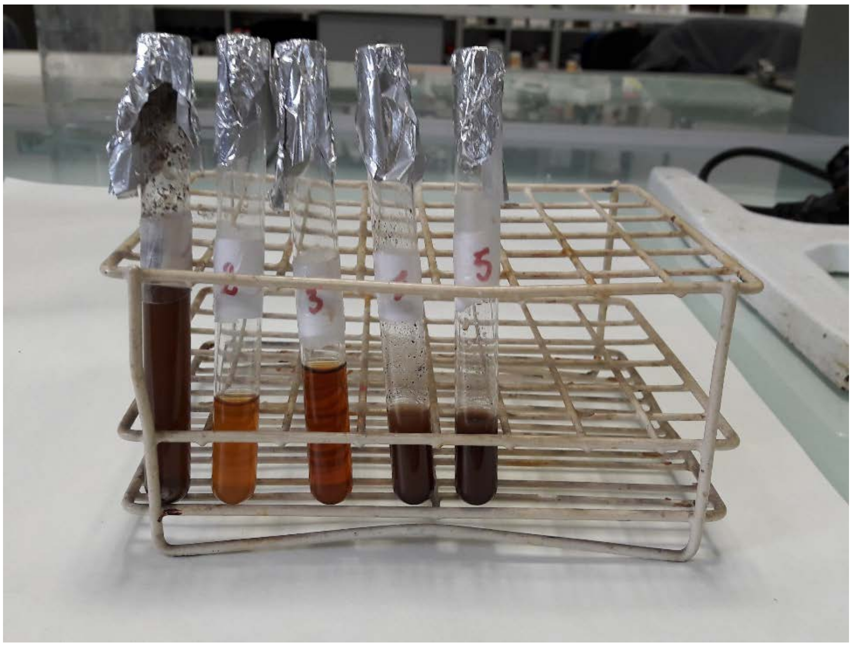


Figure S1. Lignin and Lignocellulosic samples solubility.

Table S1. Solubility of lignin and lignocellulosic sample at pH 4-6 and pH 10.

Sample	*Solubility pH 4-6*		*Solubility pH 10*		*Solubility pH 12*	
	Yes	*No*	*Yes*	*No*	*Yes*	*No*
SL		**X**	**X**		**X**	
LS	**X**		**X**			
LD	**X**		**X**			
LC		**X**		**X**		
W		**X**		**X**		

The completely soluble samples at the tested both pH were plain and dialyzed lignosulfonates. The lignocellulosic samples were completely not soluble at both pH. The SL sample was completely not soluble in water at neutral pH, but when increased the pH, its solubility resulted to improve. Therefore, a SL aqueous dispersion (1 wt.%) was prepared. The pH was increased by adding NaOH 0.5 M until 10 and it was observed that the solubility improved. Then the dispersion was diluted to 0.5, 0.3 and 0.25 wt.%. The dilutions were performed to try to observe a complete dissolution of SL in water at pH 10. The most diluted dispersion appeared brownish and with suspended particles. The dispersion was filtered on a cellulose filter and the filtrated, that presented not soluble material, was filtrated again several times. At the end the material on the filter was dried in oven under vacuum at 80 °C and the insoluble fraction was estimated to be equal to 68.6 wt.%. Due to the data obtained from solubility tests, it was concluded to use plain lignosulfonates to prepare DCC through Layer-by-Layer deposition.

The test to verify SL solubility in water at pH 12 was performed. It was observed that SL is soluble at pH 12 at concentration equal to 0.25 wt.%. The test of solubility at neutral and alkaline pH is illustrated in Figure S2.

Figure S2. SL solubility test at pH 6 (left) and 12 (right).

Due to the complete solubility of SL in water at pH 12, SL was used to prepare DCC samples with Layer-by-Layer deposition, in order to obtain a more hydrophobic cellulose surface.

- Differential Scanning Calorimetry

Lignin SL was characterized through differential scanning calorimetry (DSC) in order to record its glass transition temperature. The thermal program was the following and the graph relative to second heating is illustrated in Figure S3.

I heating up to 150 °C; 20°C/min
Cooling up to 0 °C; 20 °C/min
II heating up to 250 °C, 20 °C/min

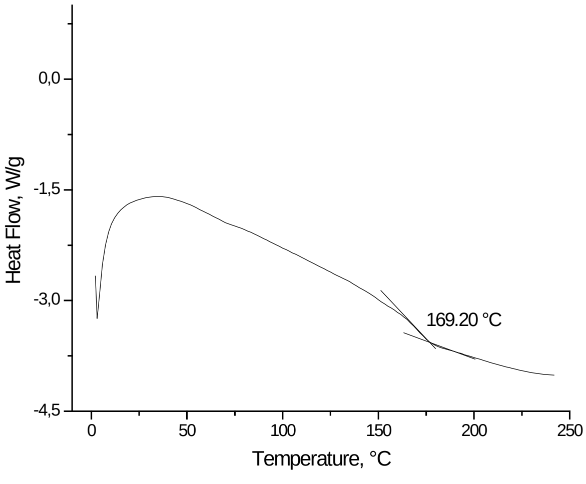


Figure S3. DSC second heating scan and T_g value.