Detailed protocol for series A — Large samples for LSC measurements

Protocol name	Deionized water	Mercerizing		Acid-Base-Acid						Bleaching			Alpha-cellulose extraction					Drying
		Base	Rinse	Acid	Rinse	Base	Rinse	Acid	Rinse	Chlorination	Ultrasonic bath	Rinse	10% NaOH	17% NaOH	Rinse	1% HCl	Rinse	
d. water	overnight, 25 °C																	5 days, 75 °C
BABA	overnight, 25 °C	2% NaOH, 15 h, 75 °C	4 days	2% HCl, 1 h, 75 °C	1 day	2% NaOH, 30 min, 75 °C	2 days	2% HCl, 1 h, 75 °C	1 day									6 days, 75 °C
ABA	overnight, 25 °C			4% HCl, 1 h, 75 °C	1 day	2% NaOH, 1 h, 75 °C	2 days	2% HCl, 1 h, 75 °C	1 day									5 days, 75 °C
BABABl	overnight, 25 °C	2% NaOH, 15 h, 75 °C	4 days	2% HCl, 1 h, 75 °C	1 day	2% NaOH, 30 min, 75 °C	2 days	2% HCl, 1 h, 75 °C	1 day	5% $NaClO_2$ + HCl added to pH ~2, 2 h, 75 °C, 5 times	1 min, 25 °C	4.5 h, the sample had to be centrifuged						1 day, 75 °C
BABABlB	overnight, 25 °C	2% NaOH, 15 h, 75 °C	4 days	2% HCl, 1 h, 75 °C	1 day	2% NaOH, 30 min, 75 °C	2 days	2% HCl, 1 h, 75 °C	1 day	5% $NaClO_2$ + HCl added to pH ~3, 2 h, 75 °C, 2 times	1 min, 25 °C, after each $NaClO_2$	1 h, the sample had to be centrifuged	10% NaOH, 45min, 75 °C	17% NaOH, 45min, RT	1 day	1% HCl, <5 min, RT	1 day	1 day, 75 °C
Bl	overnight, 25 °C									5% $NaClO_2$ + HCl added to pH ~2, 2 h, 75 °C, 4 times	after chlorination: 1^{st}: 25 min, 25 °C 2^{nd}: 10 min, >25 °C	6 h, the sample had to be centrifuged						1 day, 75 °C
BlB	overnight, 25 °C									5% $NaClO_2$ + HCl added to pH ~2, 2 h, 75 °C, 7 times	after chlorination: 1^{st}: 25 min, 25 °C 2^{nd}: 10 min, 25 °C 5^{th}: 5 min, 25 °C 7^{th}: 10 min, >25 °C	1 h, the sample had to be centrifuged	10% NaOH, 45min, 75 °C	17% NaOH, 45min, RT	1 day	1% HCl, <5 min, RT	1 day	1 day, 75 °C

Abbreviations: RT — room temperature.

1. In the case of BABABlB, chlorination was repeated only twice (as planned), and in other methods (BABABl, Bl, BlB) it was repeated from 4 to 7 times, due to the low extraction rate of holocellulose. In the BABABlB pretreatment, well-preserved larger pieces of wood were chosen from the sample, and for them, the extraction of holocellulose was much more efficient than for other samples.
2. In the samples where mercerization was performed, the base step in ABA pretreatment was shortened to 30 min from the planned 1 hour.
3. In all samples that were bleached, the material had to be centrifuged with the MPW-350 laboratory centrifuge.
4. Sample BlB had to be chlorinated more times than Bl, because sample BlB was larger, as additional losses were expected in the alpha-cellulose stage.

Detailed protocol for series B — Small samples for AMS measurements

Protocol name	Deionized water	Mercerizing		Acid-Base-Acid						Bleaching			Alpha-cellulose extraction					Freeze-drying
		Base	Rinse	Acid	Rinse	Base	Rinse	Acid	Rinse	Chlorination	Ultrasonics bath	Rinse	10% NaOH	17% NaOH	Rinse	1% HCl	Rinse	
d. water	overnight, 75 °C																	✓
BABA	overnight, 75 °C	2% NaOH, 17.5 h, 75 °C	✓	4% HCl, 1 h, 85 °C	✓	2% NaOH, 1 h, 85 °C	✓	4% HCl, 1 h, 85 °C	✓									✓
ABA	overnight, 75 °C			4% HCl, 1 h, 85 °C	✓	2% NaOH, 1 h, 85 °C	✓	4% HCl, 1 h, 85 °C	✓									✓
BABABl	overnight, 75 °C	2% NaOH, 18 h, 75 °C	✓	4% HCl, 1 h, 85 °C	✓	2% NaOH, 1 h, 85 °C	✓	4% HCl, 1 h, 85 °C	✓	5% $NaClO_2$ 5 ml + 1 M HCl 200 µl, 1.5 h, 75 °C		✓ the sample had to be centrifuged						✓
BABABlB	overnight, 75 °C	2% NaOH, 18 h, 75 °C	✓	4% HCl, 1 h, 85 °C	✓	2% NaOH, 1 h, 85 °C	✓	4% HCl, 1 h, 85 °C	✓	5% $NaClO_2$ 5 ml + 1 M HCl 200 µl, 1.5 h, 75 °C		✓ the sample had to be centrifuged	10% NaOH, 45min, 75 °C	17% NaOH, 45min, 75 °C	✓	1% HCl, <5 min, RT	✓	✓
Bl	overnight, 75 °C									5% $NaClO_2$ 5 ml + 1 M HCl 200 µl, 2 h 45 min, 75 °C	1 min, RT	✓ the sample had to be centrifuged						✓
BlB	overnight, 75 °C									5% $NaClO_2$ 5 ml + 1 M HCl 200 µl, 4 h, 75 °C	1 min, RT	✓ the sample had to be centrifuged	10% NaOH, 45min, 75 °C	17% NaOH, 45min, 75 °C	✓	1% HCl, <5 min, RT	✓	✓

Abbreviations: RT — room temperature.

The rinse time was relatively short compared to the pretreatment of LSC samples, so it is not given in the table.

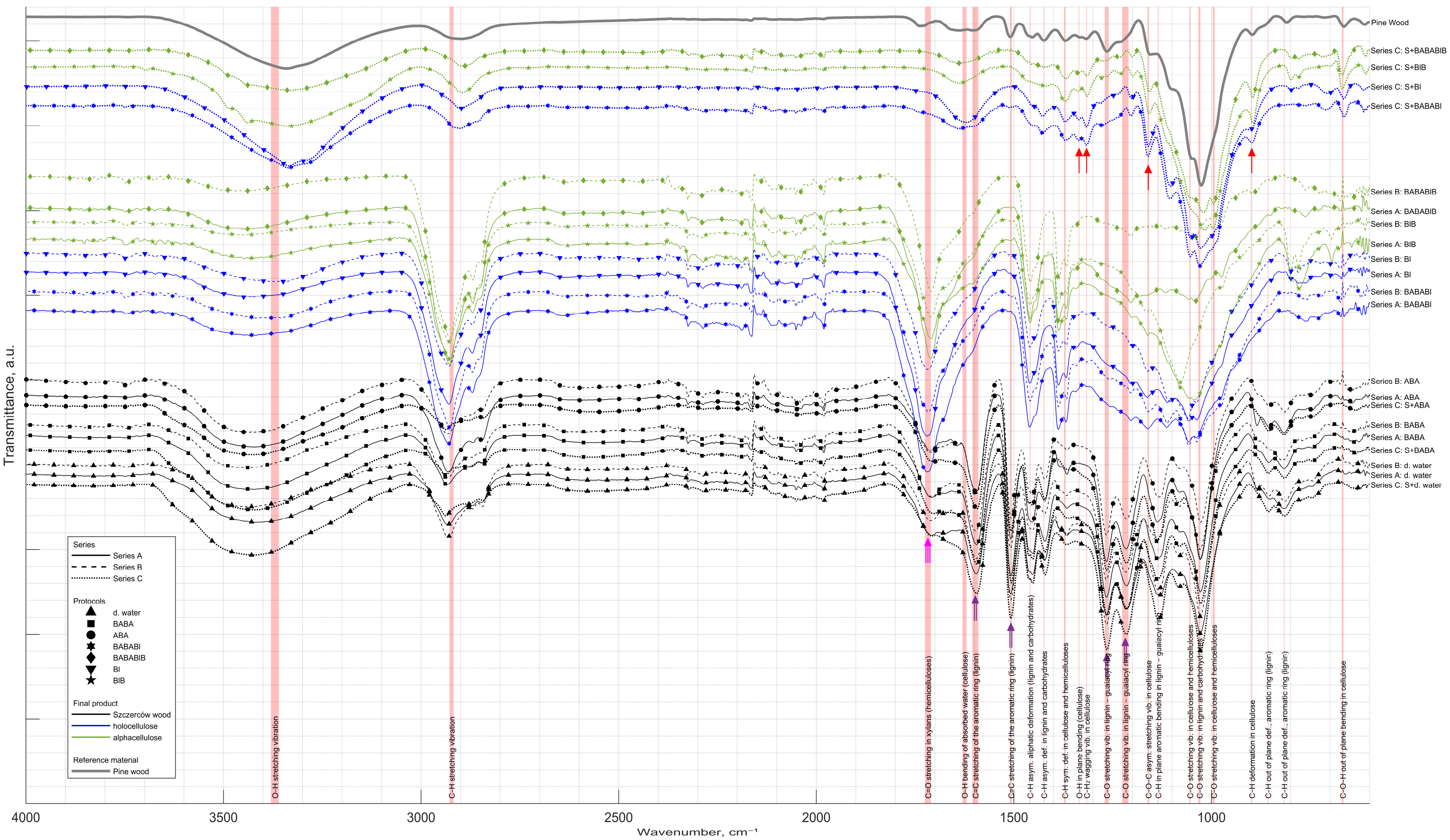
Detailed protocol for series C — Medium sized samples for AMS measurements

Protocol name	Soxhlet extraction				Mercerizing		Acid-Base-Acid						Bleaching			Alpha-cellulose extraction					Drying
	1[st] step	2[nd] step	3[rd] step	Rinse	Base	Rinse	Acid	Rinse	Base	Rinse	Acid	Rinse	Chlorination	Ultrasonic bath	Rinse	10% NaOH	17% NaOH	Rinse	1% HCl	Rinse	
d. water	$C_2H_5OH + C_7H_8$, 4 h, BT	C_2H_5OH, 4 h, BT	H_2O, 1 h, BT	✓																	✓
BABA	$C_2H_5OH + C_7H_8$, 4 h, BT	C_2H_5OH, 4 h, BT	H_2O, 1 h, BT	✓	4% NaOH, 12 h, 75 °C	✓	0.5 M HCl, 1 h, 60 °C	✓	2% NaOH, 30 min, 60 °C	✓	0.5 M HCl, 1 h, 60 °C	✓									✓
ABA	$C_2H_5OH + C_7H_8$, 4 h, BT	C_2H_5OH, 4 h, BT	H_2O, 1 h, BT	✓			0.5 M HCl, 1 h, 60 °C	✓	2% NaOH, 30 min, 60 °C	✓	0.5 M HCl, 1 h, 60 °C	✓									✓
BABABl	$C_2H_5OH + C_7H_8$, 4 h, BT	C_2H_5OH, 4 h, BT	H_2O, 1 h, BT	✓	4% NaOH, 12 h, 75 °C	✓	0.5 M HCl, 1 h, 60 °C	✓	2% NaOH, 30 min, 60 °C	✓	0.5 M HCl, 1 h, 60 °C	✓	5% $NaClO_2$ + HCl added to pH ∼2, 2 h, 75 °C		✓						✓
BABABlB	$C_2H_5OH + C_7H_8$, 4 h, BT	C_2H_5OH, 4 h, BT	H_2O, 1 h, BT	✓	4% NaOH, 12 h, 75 °C	✓	0.5 M HCl, 1 h, 60 °C	✓	2% NaOH, 30 min, 60 °C	✓	0.5 M HCl, 1 h, 60 °C	✓	5% $NaClO_2$ + HCl added to pH ∼2, 2 h, 75 °C		✓	10% NaOH, 45min, 75 °C	17% NaOH, 45min, RT	✓	1% HCl, 5 min, RT	✓	✓
Bl	$C_2H_5OH + C_7H_8$, 4 h, BT	C_2H_5OH, 4 h, BT	H_2O, 1 h, BT	✓									5% $NaClO_2$ + HCl added to pH ∼2, 2 h, 75 °C		✓						✓
BlB	$C_2H_5OH + C_7H_8$, 4 h, BT	C_2H_5OH, 4 h, BT	H_2O, 1 h, BT	✓									5% $NaClO_2$ + HCl added to pH ∼2, 2 h, 75 °C		✓	10% NaOH, 45min, 75 °C	17% NaOH, 45min, RT	✓	1% HCl, 5 min, RT	✓	✓

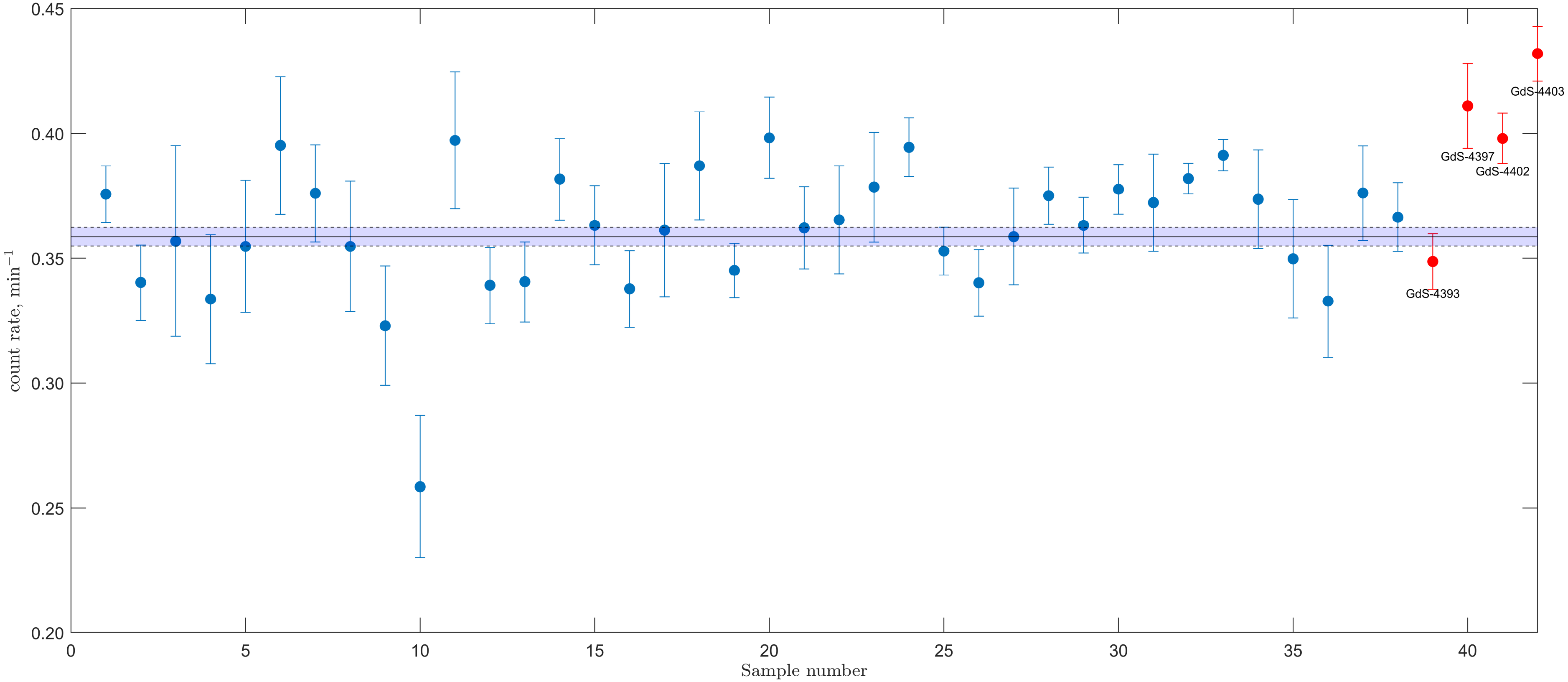
Abbreviations: BT — boiling temperature, RT — room temperature.

The rinse time was relatively short compared to the pretreatment of LSC samples, so it is not given in the table.

Full spectra FTIR



Background samples vs samples from subseries A_1 (LSC technique)

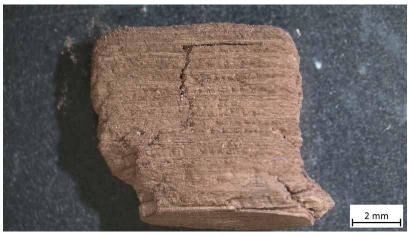
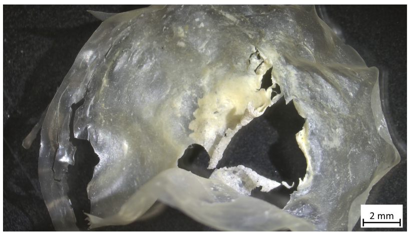
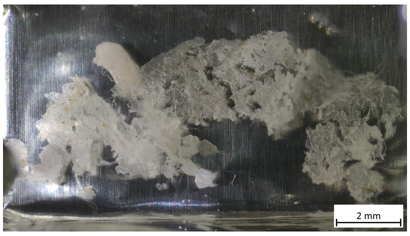


FTIR assignments

Wavenumber, cm^{-1}	Description	Series A							Series B							Series C							References (selection)
		d. water	BABA	ABA	BABABI	BABABIB	BI	BIB	d. water	BABA	ABA	BABABI	BABABIB	BI	BIB	S+d. water	S+BABA	S+ABA	S+BABABI	S+BABABIB	S+BI	S+BIB	
1734–1740	C=O stretching in xylans (hemicelluloses)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓					Colom et al. 2003 Pandey and Pitman 2003 Richard et al. 2014
1623–1650	O–H bending of absorbed water (cellulose)																		✓	✓	✓	✓	Fan et al. 2012 Pandey and Pitman 2003 Pandey and Theagarajan 1997 Richard et al. 2014
1595–1610	C=C stretching of the aromatic ring (lignin)	✓	✓	✓					✓	✓	✓					✓	✓	✓					Colom et al. 2003 Drobniak and Mastalerz 2006 Pandey and Theagarajan 1997 Richard et al. 2014 Stark et al. 2015
1505–1510	C=C stretching of the aromatic ring (lignin)	✓	✓	✓					✓	✓	✓					✓	✓	✓					Colom et al. 2003 Drobniak and Mastalerz 2006 Fan et al. 2012 Pandey and Pitman 2003 Pandey and Theagarajan 1997 Richard et al. 2014 Stark et al. 2015
1458–1466	C–H asym. aliphatic deformation (lignin and carbohydrates)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓		Colom et al. 2003 Pandey and Pitman 2003 Pandey and Theagarajan 1997 Richard et al. 2014 Stark et al. 2015
1423–1425	C–H asym. def. in lignin and carbohydrates	✓	✓	✓					✓	✓	✓					✓	✓	✓	✓		✓		Colom et al. 2003 Fan et al. 2012 Pandey and Pitman 2003 Pandey and Theagarajan 1997 Richard et al. 2014

Wavenumber, cm^{-1}	Description	Series A							Series B							Series C							References (selection)
		d. water	BABA	ABA	BABABI	BABABIB	BI	BIB	d. water	BABA	ABA	BABABI	BABABIB	BI	BIB	S+d. water	S+BABA	S+ABA	S+BABABI	S+BABABIB	S+BI	S+BIB	
1367–1375	C-H sym. def. in cellulose and hemicelluloses	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	Drobniak and Mastalerz 2006 Fan et al. 2012 Pandey and Pitman 2003 Pandey and Theagarajan 1997 Richard et al. 2014
1335	O–H in plane bending (cellulose)				✓	✓	✓	✓				✓	✓	✓	✓				✓	✓	✓	✓	Colom et al. 2003
1313–1316	C–H_2 wagging vib. in cellulose																		✓	✓	✓	✓	Colom et al. 2003 Fan et al. 2012
1265–1275	C–O stretching vib. in lignin – guaiacyl ring	✓	✓	✓					✓	✓	✓					✓	✓	✓					Drobniak and Mastalerz 2006 Pandey and Pitman 2003 Pandey and Theagarajan 1997
1210–1226	C–O stretching vib. in lignin – guaiacyl ring	✓	✓	✓					✓	✓	✓					✓	✓	✓					Drobniak and Mastalerz 2006 Pandey and Theagarajan 1997
1158–1162	C–O–C asym. stretching vib. in cellulose				✓		✓	✓				✓		✓	✓				✓	✓	✓	✓	Colom et al. 2003 Drobniak and Mastalerz 2006 Fan et al. 2012 Pandey and Pitman 2003 Pandey and Theagarajan 1997
1139–1141	C–H in plane aromatic bending in lignin – guaiacyl	✓	✓	✓					✓	✓	✓					✓	✓	✓					Drobniak and Mastalerz 2006
1055–1058	C–O stretching vib. in cellulose and hemicelluloses				✓		✓					✓		✓					✓		✓		Pandey and Theagarajan 1997
1040–1048	C–O stretching vib. in lignin and carbohydrates	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓		✓		✓	✓	✓	✓	✓	✓	✓	Pandey and Pitman 2003 Pandey and Theagarajan 1997
995–1000	C–O stretching vib. in cellulose and hemicelluloses																			✓		✓	Pandey and Theagarajan 1997
896–900	C–H deformation in cellulose																		✓	✓	✓	✓	Colom et al. 2003 Pandey and Pitman 2003 Pandey and Theagarajan 1997 Richard et al. 2014
863	C–H out of plane def., aromatic ring (lignin)	✓	✓	✓					✓	✓	✓					✓	✓	✓					Richard et al. 2014 Stark et al. 2015
823	C–H out of plane def., aromatic ring (lignin)	✓	✓	✓					✓	✓	✓					✓	✓	✓					Stark et al. 2015
667–670	C–O–H out of plane bending in cellulose																		✓	✓	✓	✓	Pandey and Theagarajan 1997

Microscope photographs of the final product

Protocol code Dating material	Series A	Series C (with Soxhlet step)
d. water degraded wood		
BABA degraded wood		
ABA degraded wood		
BABABl holocellulose		
BABABlB alpha-cellulose		
Bl holocellulose		
BlB alpha-cellulose		

Microscope photographs were captured using a ZEISS Stemi 508 optical microscope equipped with an Axiocam 208 Color camera at the Gliwice Laboratory. The photographs reveal noticeable distinctions between the cellulose-related products (holocellulose and alpha-cellulose) prepared with and without the utilization of the Soxhlet apparatus. These observations align with the findings from FTIR analysis, which suggest that the application of the Soxhlet apparatus during pretreatment yields a distinct type of cellulose.

A plausible explanation for these differences is that the cellulose, which is a polysaccharide composed of numerous glucose units, undergoes damage in the old wood. This damage entails the breaking of molecular bonds, leading to a reduction in the degree of polymerization. Consequently, the chemical pretreatment exerts a significant influence on the ultimate product obtained.

Reagents required for pretreatment of subfossil wood

In the conducted multi-criteria analysis, one of the criteria considered was the cost of pretreatment, including factors such as the expenses associated with chemical reagents and electricity. Throughout the pretreatment process, the quantities of water and reagents used were meticulously recorded. The table below presents the calculated values (normalized) required to obtain 10 g of the final product (wood, holocellulose, or alpha-cellulose) using the LSC technique and 5 mg of the final product using the AMS technique. These mass values were chosen to ensure sufficient quantities of benzene and graphite could be obtained, even in cases of low yields in benzene synthesis or graphitization. Centrifugation was employed for the samples prepared for measurements with the LSC technique, while the samples prepared for the measurements with the AMS technique did not involve centrifugation.

LSC technique — values calculated in order to obtain 10 g of the final product

Protocol code	Dated material (final product)	Dry wood mass, g	Deionized water, l	35-38% HCl, ml	NaOH, g	$NaClO_2$, g
d.water	degraded wood	11	0.1			
BABA	degraded wood	20	25	40	10	
ABA	degraded wood	15	10	40	5	
BABABl	holocellulose	75	100	120	25	150
BABABlB	alpha-cellulose	350	350	460	325	500
Bl	holocellulose	90	10	50		200
BlB	alpha-cellulose	190	30	120	250	400

AMS technique — values calculated in order to obtain 5 mg of the final product

Protocol code	Dated material (final product)	Dry wood mass, mg	Deionized water, ml	35-38% HCl, ml	NaOH, g	$NaClO_2$, g
d.water	degraded wood	5.5	3			
BABA	degraded wood	7	45	0.2	0.04	
ABA	degraded wood	6	30	0.2	0.02	
BABABl	holocellulose	1 000	7 000	35	6	10
BABABlB	alpha-cellulose	2 700	19 000	90	30	25
Bl	holocellulose	200	70	0.6		2
BlB	alpha-cellulose	550	230	1.8	0.9	5