Electronic Supplementary Material (ESI) for Green Chemistry.

Supplementary materials

Tailor-made Enzyme Consortium Segregating Sclerenchyma Fibre Bundles from Willow Bark

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Table S1. The chemical compositional differences between grass (ramie or flax) and wood (willow and spruce) bark.[1-4]

	ramie or flax	willow bark	spruce bark
Holocellulose %	85-87	50	40
Pectin %	2	3	13
Lignin %	0.2-0.5	24-31	20
Extractive %	5-10	10-15	20
Suberin %	0	5	1.3
Ash %	0	5-10	4-5
Starch %	0	1.3	—

Table S2. Overview of the determined enzyme activities.

crude enzymes	Pectate lyase activity (polygalacturonic acid, U/mL)	Pectate lyase activity (apple pectin, U/mL)	Xylanase activity (U/mL)	Mannanase activity (U/mL)	FPA activity (U/mL)
7-3-3	2.06	1.32	0.3	1.45	0.15
PelA	2215.48	111.59	0.66	3.64	0.23
PelB	2.14	130.44	0.23	1.6	0.1
PelC	19.53	4.45	0.21	1.36	0.12
PelAPelC	2245.66	121.01	0.48	3.45	0.23
AbnA	2.17	1.25	0.24	1.63	0.09
RhgW	2.25	1.33	0.24	1.25	0.1
GmuG	2.32	1.44	0.23	489.17	0.11
XynA	3.31	1.55	247.55	1.59	0.12

Table S3. The characteristics of the studied strains or plasmids.

category	Strain or plasmid	Characteristics	Source or reference	Fermentation medium
Plasmids	pNW33n	Chloramphenicol resistance, *Escherichia coli -Bacillus subtilis* shuttle plasmid	5	
Strains	*Bacillus subtilis* 7-3-3	wild type	5	production medium II (2× Super-rich)
	BSpelA	*pelA*-overexpressed by pNW33n	5	production medium I
	BSpelB	Overexpression of *pelB* by P43 promoter	This study	production medium II (2× Super-rich)
	BSpelC	Overexpression of *pelC* by P43 promoter	This study	production medium I
	BspelApelC	Coexpression of *pelA* and *pelC*	This study	production medium I
	BSabnA	Overexpression of *abnA* by P43 promoter	This study	production medium II (2× Super-rich)
	BSrhgW	Overexpression of *rhgW* by P43 promoter	This study	production medium II (2× Super-rich)
	BSgmuG	Overexpression of *gmuG* by P43 promoter	This study	production medium II (2× Super-rich)
	BSxynA	Overexpression of *xynA* by P43 promoter	This study	production medium II (2× Super-rich)

Table S4. The sequence of primers used in this study. The strains and plasmids used in this study are shown in **Table S3**. Gene pelB, *pelC*, *abnA*, *rhgW, gmuG, xynA* and P43 promoter were cloned from the genome DNA of *B. subtilis* 7-3-3 using gene specific primers (as shown in this table). Purified PCR products were cloned into plasmids using a one-step cloning kit (TransGene Biotech, Beijing, China) and transformed into *Escherichia coli* DH5α. The positive clones were verified by sequencing. Then the correct recombinant vector was transformed into *B.subtilis* 7-3-3 by electrotransformation[5].

primer	sequence
P43-F	TTGAATTCGAGCTCGGTAGGTATGTTTTCGCTTGAGCTTTT
P43-R	CATGTGTACATTCCTCTCTTACCT
PelB-F	GAGAGGAATGTACACATGAAACGACTTTGTTTATGGTTC
PelB-R	TACGCCAAGCTTGCATGCCAGTAGCCAAATACCAGAGTTGTA
PelC-F	GAGAGGAATGTACACATGAAAAAAATCGTGTCTATCCTATTT
PelC-R	TACGCCAAGCTTGCATGCTTAAAATTGAGTGTTGTTGTTTTCG
AbnA-F	GAGAGGAATGTACACATGAAAAGAAAAAAACATGGAAACG
AbnA-R	TACGCCAAGCTTGCATGCTCAATAGGACGGCCAGCCCGAAC
RhgW-F	GAGAGGAATGTACACATGAGAAGGAGCTGTCTGATGATTAG
RhgW-R	TACGCCAAGCTTGCATGCTTAAGGCGTATACATATTTGGTTTT
Gmug-F	GAGAGGAATGTACACATGGGGGAGTTGCATTTG TTTAAGA
Gmug-R	TACGCCAAGCTTGCATGCTCATTCAACGATTGGCGTTAAAG
XynA-F	GAGAGGAATGTACACATGTTTAAGTTTAAAAAGAATTTC
XynA-R	TACGCCAAGCTTGCATGCTTACCACACTGTTACGTTAGAACT

Table S5. The experimental recipes for the media preparation between the substrate (willow bark), enzymes (see **Table 2**) and the buffer (pH 9.6 0.2M Glycine -NaOH). For NAWIB and WIB**,** see **Fig. 1**.

substrate/ 1g	PelAPelC/ml	733/ml	PelB/ml	AbnA/ml	GmuG/ml	XynA/ml	PelA/ ml	PelC/ml	RhgW/ml	Enzyme total/ml	Buffer/ ml	Fibre bundle	Pectin
NAWIB	1		0.5	0.5						2	28.0	PN-F	PN-P
NAWIB	1		0.5	0.5	0.5	0.5				3	27.0	PHN-F	PHN-P
WIB	1		0.5	0.5						2	28.0	PWI-F	PWI-P
WIB	1		0.5	0.5	0.5	0.5				3	27.0	PHWI-F	PHWI-P
WIB							2.5			2.5	27.5	WIB-PelA	-
WIB			0.5							0.5	29.5	WIB-PelB	-
WIB	1									1	29.0	WIB-PelAPelC	-
WIB				0.5						0.5	29.5	WIB-AbnA	-
WIB								0.5		0.5	29.5	WIB-PelC	
WIB									0.5	0.5	29.5	WIB-RhgW	
WIB		0.5								0.5	29.5	WIB-733	
WIB		0.5					0.5			1	29.0	WIB-PelA-733	
WIB							0.5		0.5	1	29.0	WIB-PelA-RhgW	
WIB				0.5			0.5			1.5	28.5	WIB-PelA-AbnA	-
NAWIB										0	30.0	Na-buffer	-
WIB										0	30.0	WIB-buffer	-

Table S6. XPS fitting parameters & resulting relative concentration.

Sample	Name	Position	FWHM	Line Shape	%At Conc
a) PN-F	C-C	284.8	1.4	GL(30)	19.7
	C-O	286.6	1.4	GL(30)	61
	C=O	288	1.4	GL(30)	15.4
	O-C=O	289.2	1.4	GL(30)	3.9
b) PHN-F	C-C	284.8	1.5	GL(30)	15.2
	C-O	286.6	1.5	GL(30)	59.8
	C=O	288	1.5	GL(30)	20.8
	O-C=O	289.3	1.5	GL(30)	4.2
c) PWI-F	C-C	284.8	1.4	GL(30)	18.3
	C-O	286.6	1.4	GL(30)	61.3
	C=O	288	1.4	GL(30)	16.4
	O-C=O	289.2	1.4	GL(30)	3.9
d) PHWI-F	C-C	284.8	1.4	GL(30)	15.8
	C-O	286.6	1.4	GL(30)	61.4
	C=O	287.9	1.4	GL(30)	18.7
	O-C=O	289.3	1.4	GL(30)	4.1
e) $NaHCO_3$ 20wt% FB	C-C	284.8	1.4	GL(30)	26.1
	C-O	286.5	1.4	GL(30)	54.8
	C=O	288	1.4	GL(30)	15.8
	O-C=O	289.3	1.4	GL(30)	3.4
f) Whatman cellulose	C-C	284.8	1.2	GL(30)	12
	C-O	286.5	1.2	GL(30)	69.4
	C=O	288	1.2	GL(30)	17.2
	O-C=O	289.3	1.2	GL(30)	1.4

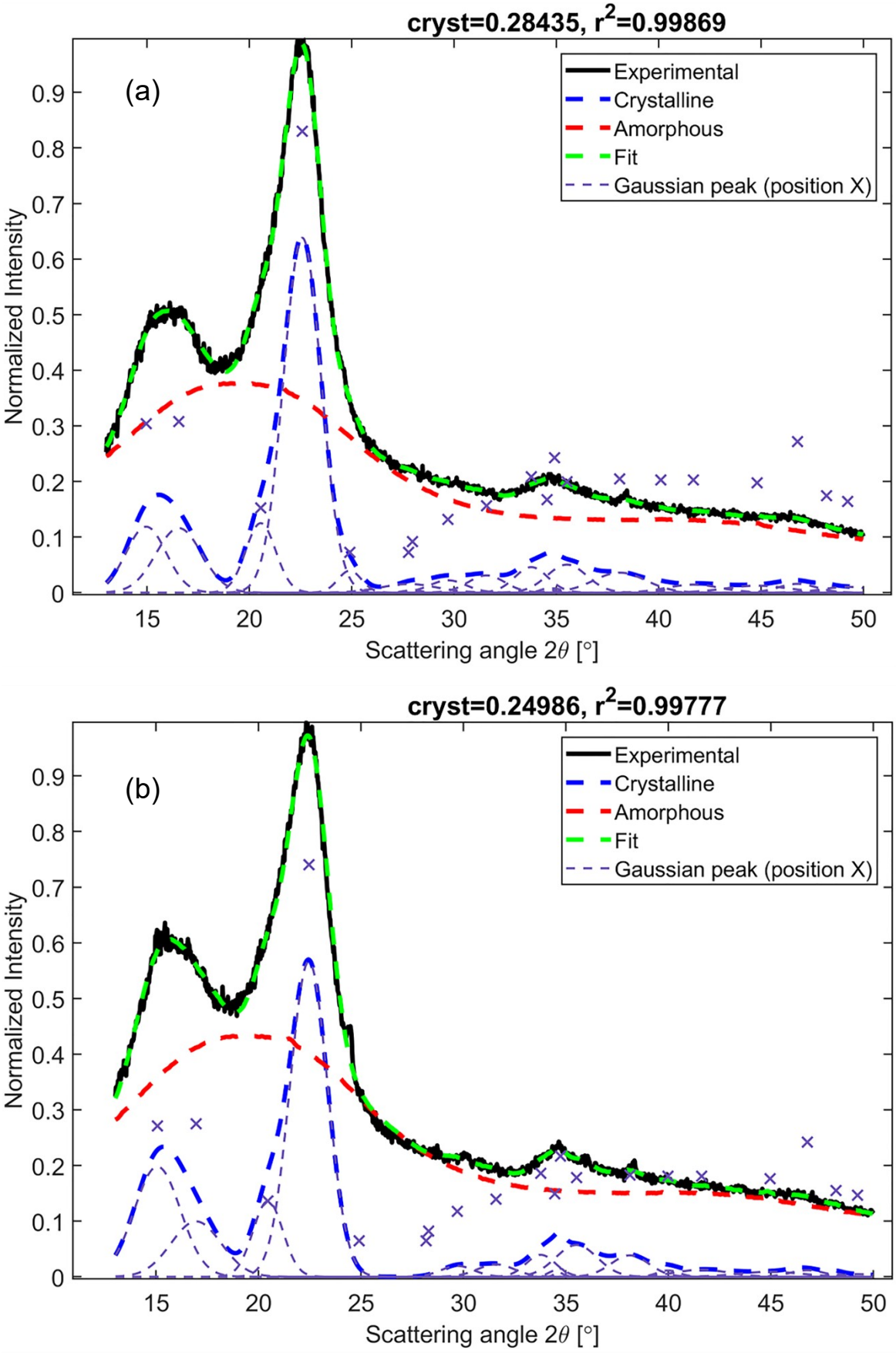


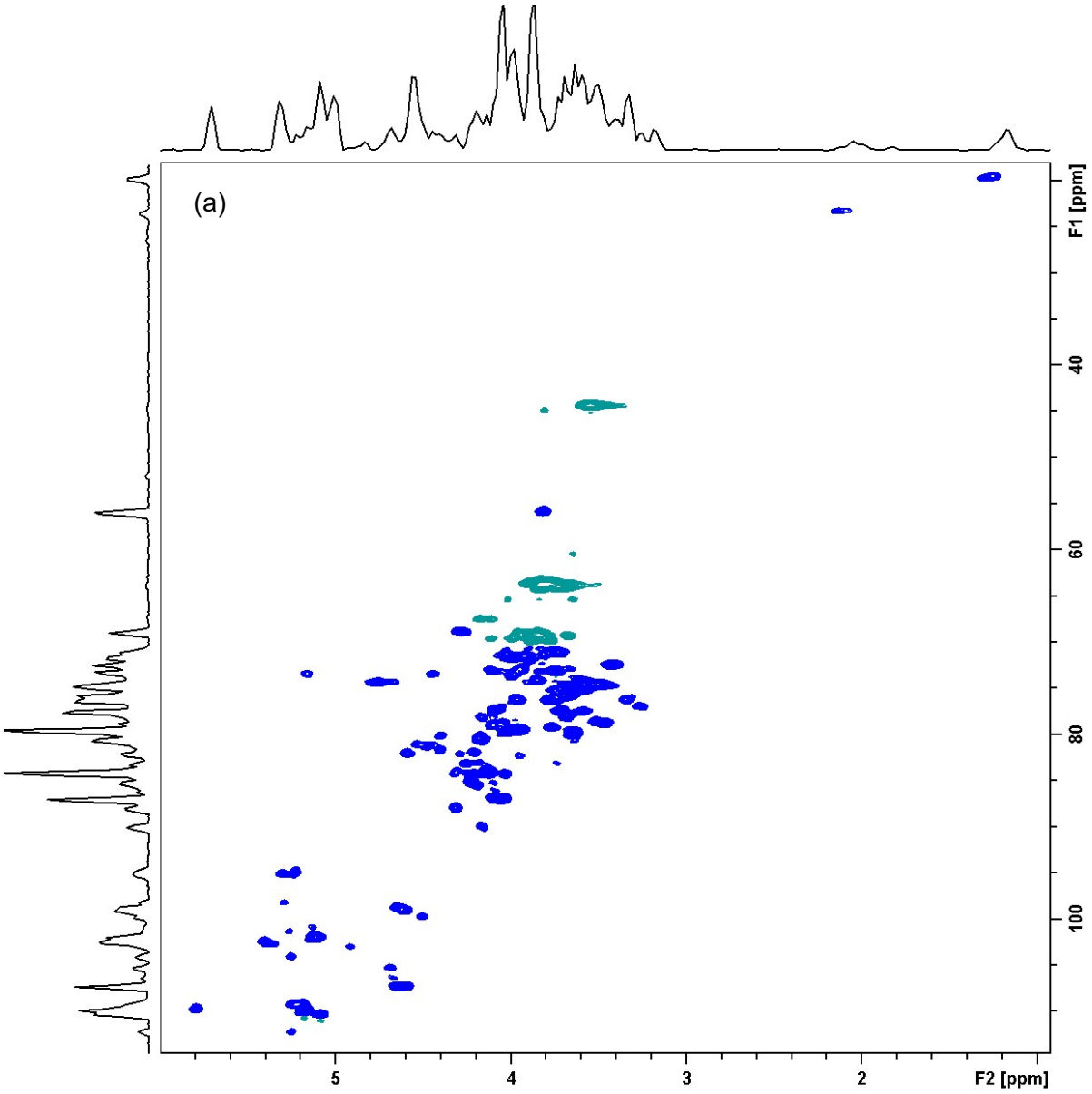
Fig. S1. crystallinity analysis results for fibre bundle samples: (a) PWI-F; (b) PN-F based on the amorphous background fitting.

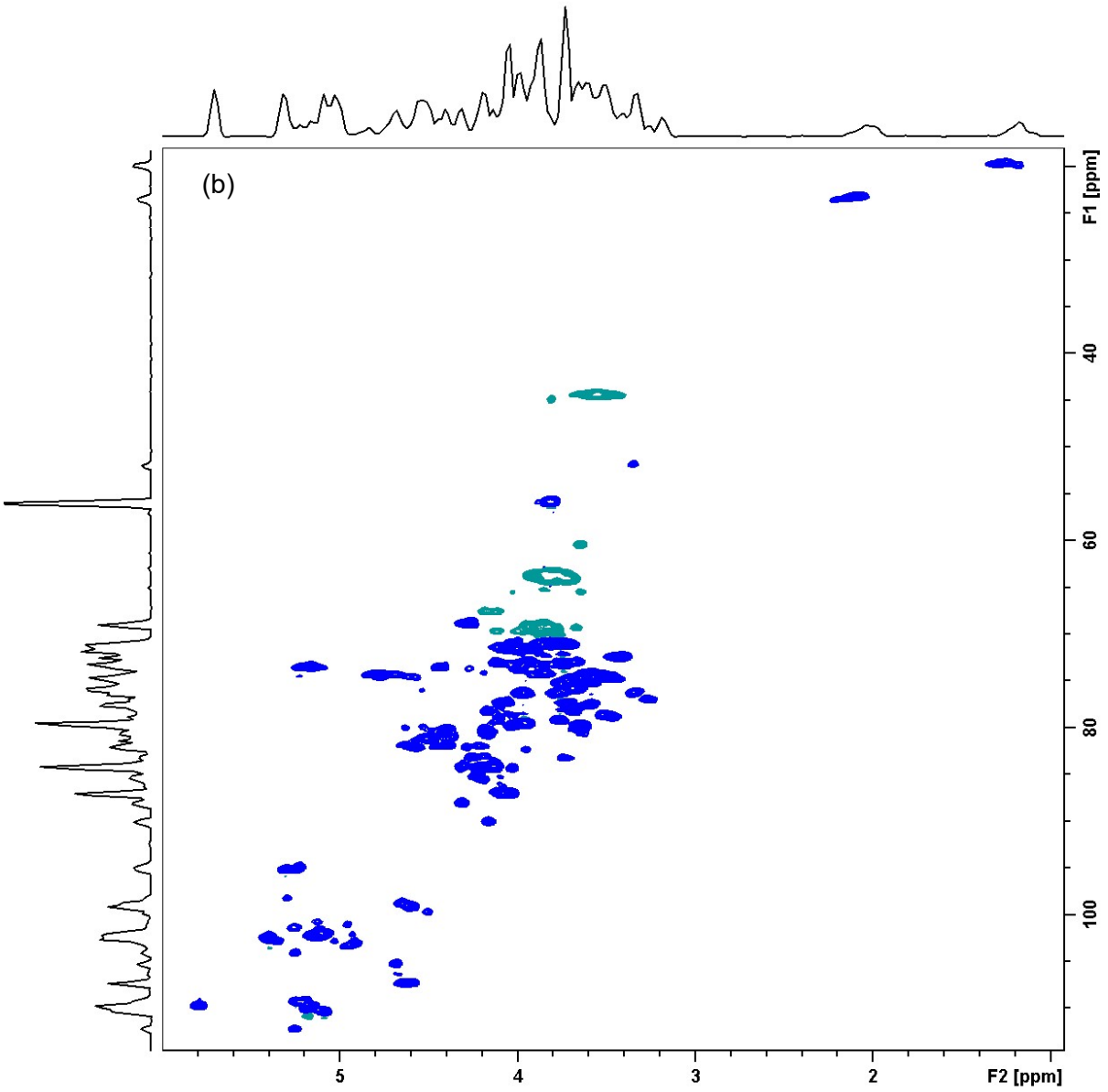
Table S7. Yield (% willow bark) of pectin obtained by three different solvents (i.e. citric acid, hydrogen chloride and sodium hydroxide). Standard deviations are shown in the parentheses.

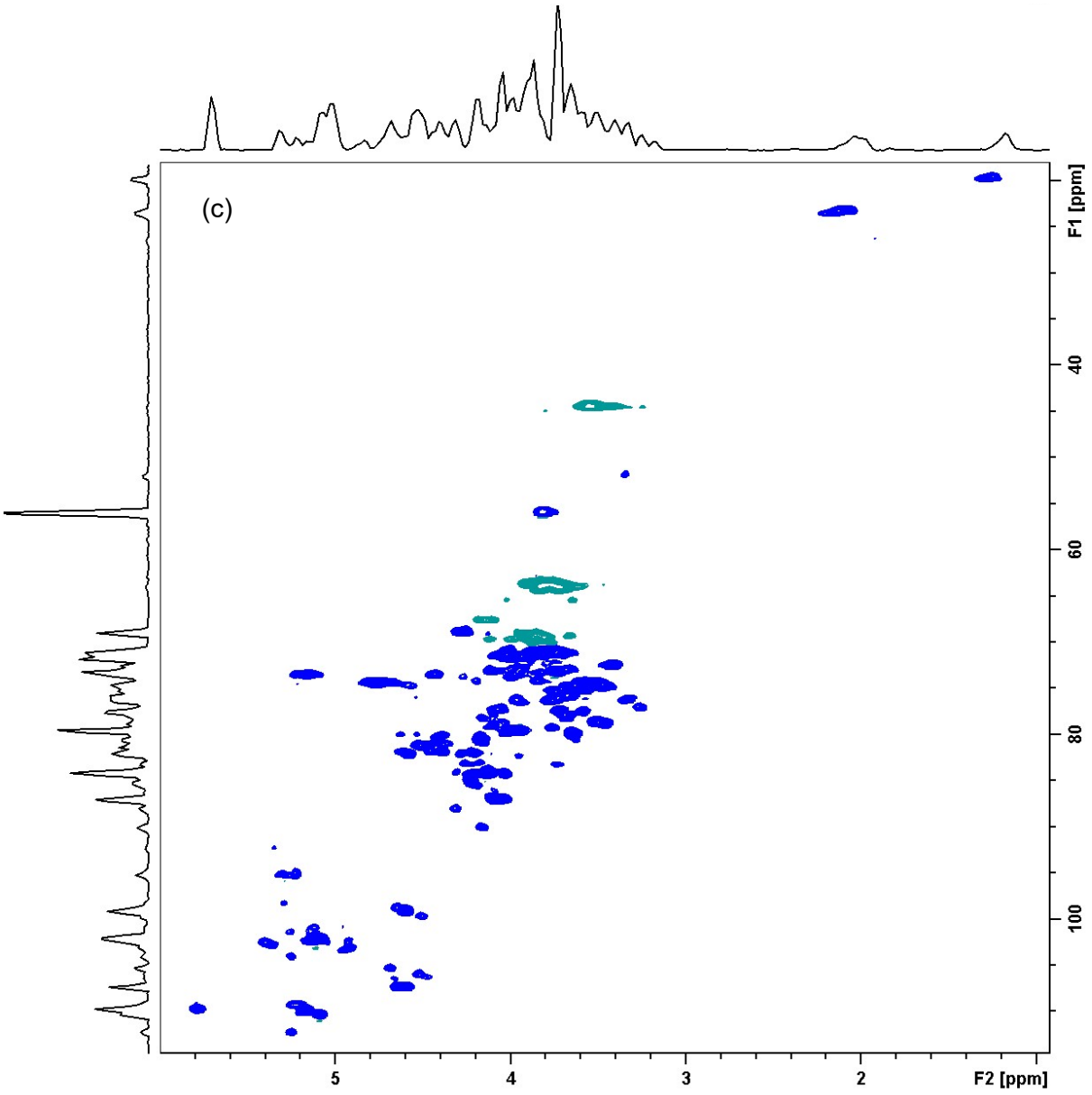
	citric acid	HCl	NaOH
Yield	2.8 (0.2)	1.5 (0.4)	1.5 (0)

Table S8. Chemical shift assignments for dialyzed citric acid extracted pectin (DCA-P, **Fig. 2**) of willow bark, referenced to TSP-*d*4.

Residue	Label	C-1 *H-1*	C-2 *H-2*	C-3 *H-3*	C-4 *H-4*	C-5 *H-5;5'*	C-6 *H-6;6'*	$\underline{C}H_3CO$	$CH_3\underline{C}O$	$\underline{C}H_3O$
→4)-α-Gal*p*A-6-*O*Me-(1→	Gal*p*A (*OMe*)	102.9 *5.4*	70.8 *3.7*	71.6 *4.11*	82.1 *4.31*	73.5 *5.1*				55.9 *3.82*
→4)-α-Gal*p*A-3-*O*Ac-(1→	Gal*p*A (OAc)	102.9 *5.4*	*71.6* *4.11*	*73.7* *5.14*	*n.d.* *n.d.*	73.5 *5.1*		23.6 *2.16*		
→4)-α-Gal*p*A-(1→	Gal*p*A	102.9 *5.4*	69.3 *3.83*	*69.7* *3.9*	*83.2* *4.27*	74.2 *4.73*				
→5)-α-Ara*f*-(1→	$A_{1,5}$	110.5 *5.09*	84.2 *4.15*	79.5 *3.98*	85.4 *4.22*	69.4 *3.81*				
→3)-α-Ara*f*-(1→	$A_{1,3}$	109.4 *5.26*	84.2 *4.31*	87 *4.07*	84.5 *4.23*	64.1 *3.84;3.73*				
→2,3,5)-α-Ara*f*-(1→	$A_{2,3,5}$	109.5 *5.19*	88.03 *4.33*	83.3 *4.26*	85.2 *4.3*	69.2 *3.95*				
→2,5)-α-Ara*f*-(1→	$A_{2,5}$	109.6 *5.19*	89.9 4.18	n.d. n.d.	n.d. n.d.	n.d. n.d.				
β-Ara*f*-(1→	A_1-β	102.7 *5.42*	79.7 *4.03*	76.5 *3.98*	85.2 *3.91*	65.5 *3.82*				
α-Ara*f*-(1→	A_1-α	*112.3* *5.25*	79.7 *4.03*	76.5 *3.98*	85.2 *3.91*	65.5 *3.82*				
→2)-α-Rhap-(1→	$R_{1,2}$	112.2 5.26	78.3 4.18	69.7 3.89	74.9 3.45	72.9 3.67	19.6 1.26			
→2,4)-α-Rhap-(1→	$R_{1,2,4}$	112.2 5.26	78.3 4.18	72.7 4.06	83.3 3.73	69.9 3.87	19.6 1.26			
β-Gal*p*-(1→	Ga	106.6 *4.47*	74.7 *3.53*	75 *3.65*	n.d. *n.d.*	77.6 *3.72*	63.6 *3.84*			
→4)-β-Gal*p*-(1→	$Ga_{1,4}$	107.4 *4.65*	75.9 *3.69*	76.4 *3.78*	80.7 *4.17*	79.8 *3.67*	63.7 *3.83*			
→4)-α-D-Glc*p*-(1→	$St_{1,4}$	102.5 *5.41*	74.5 *3.64*	76.5 *3.98*	79.7 *3.66*	74.2 *3.84*	63.7 *3.83*			


(a)
F1 [ppm]
40
60
80
100
5
4
3
2
F2 [ppm]


(b)
F1 [ppm]
40
60
80
100
5
4
3
2
F2 [ppm]


(c)
F1 [ppm]
40
60
80
100
5
4
3
2
F2 [ppm]

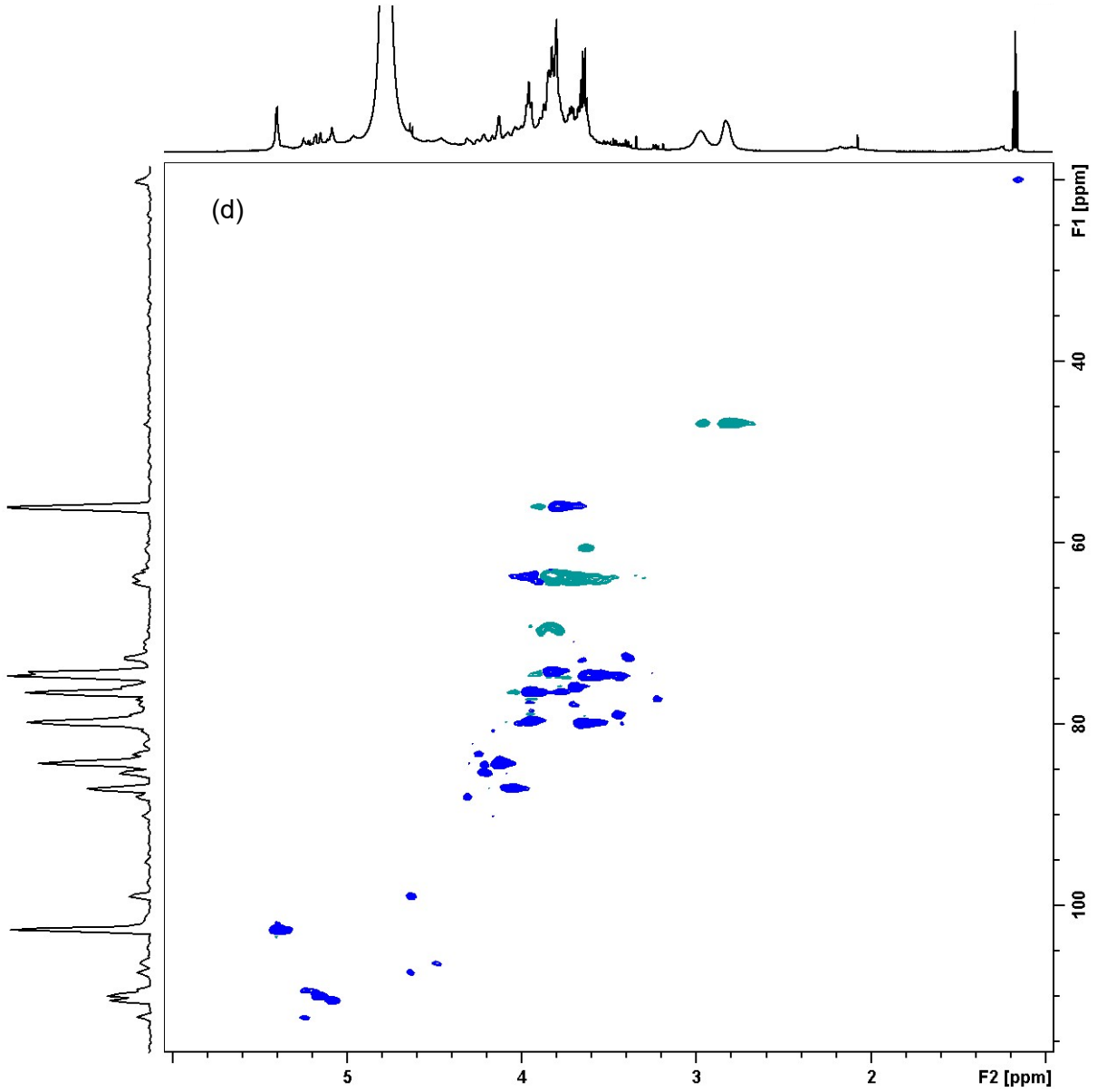


Fig. S2. δC/δH (18.4−114.2/1.0−6.0 ppm) regions of 2D HSQC NMR spectra of PN-P (a), PWI-P (b), PHWI-P (c), and CA-P (d). For abbreviations, see **Table S5** and **Fig. 1**.

Table S9. Chemical compositional analysis of the samples from the preliminary study: WIB-PelA (PelA treated WIB); WIB-PelB (PelB treated WIB); WIB-PelAPelC (PelAPelC treated WIB); WIB-AbnA (AbnA treated WIB); WIB-PelC (PelC treated WIB); WIB-RhgW (RhgW treated WIB); WIB-733 (733 treated WIB); WIB-PelA-733 (PelA + 733 treated WIB); WIB-PelA-RhgW (PelA + RhgW treated WIB); WIB- PelA- AbnA (PelA + AbnA treated WIB); NA-b ($NaHCO_3$ 3% treated WB with buffer); WIB-b (WIB with buffer). For abbreviations, see **Table S5**. Standard deviations are shown in the parentheses based on three independent experiments.

		WIB-PelA	WIB-PelB	WIB-PelAPelC	WIB-AbnA	WIB-PelC	WIB-RhgW	WIB-733	WIB-PelA-733	WIB-PelA-RhgW	WIB-PelA-AbnA	NA-b	WIB-b
Fibre bundle	fibre bundle yield (% WB)	22.4 (1.0)	25.5 (2.3)	20.4 (1.9)	33.6 (0.5)	23.4 (0.8)	33.7 (0.7)	33.9 (0.03)	22.6 (1.06)	22.5 (0.4)	21.3 (1.05)	32.0 (0.5)	38.1 (0.8)
	Monosaccharide composition (mg/g)												
	d-Galacturonic acid (GalA)	11 (1)	15 (0)	9 (0)	25 (2)	—	—	—	—	—	—	22 (4)	31 (3)
	glucose	81 (3)	91 (5)	73 (2)	109 (2)	—	—	—	—	—	—	99 (6)	97 (6)
	xylose	15 (4)	19 (1)	17 (1)	20 (3)	—	—	—	—	—	—	26 (5)	19 (5)
	galactose	2 (1)	4 (0)	4 (1)	6 (0)	—	—	—	—	—	—	0 (0)	4 (5)
	mannose	8 (5)	7 (0)	3 (0)	12 (3)	—	—	—	—	—	—	8 (6)	14 (1)
	rhamnose	3 (0)	4 (0)	3 (0)	5 (0)	—	—	—	—	—	—	5 (0)	6 (1)
	arabinose	5 (0)	7 (0)	5 (0)	11 (1)	—	—	—	—	—	—	12 (2)	14 (1)
Pectin	pectin Yield (% WB)	2.6 (0.3)	1.6 (0.1)	3.7 (0.2)	0.9 (0.1)	2.1 (0.3)	1.0 (0.1)	1.1 (0.1)	2.76 (0.23)	2.5 (0.5)	2.80 (0.31)	0.5 (0.04)	0.6 (0.1)
	d-Galacturonic acid (GalA) mg/g	268 (29)	312 (14)	277 (12)	414 (123)	—	—	—	—	—	—	—	—

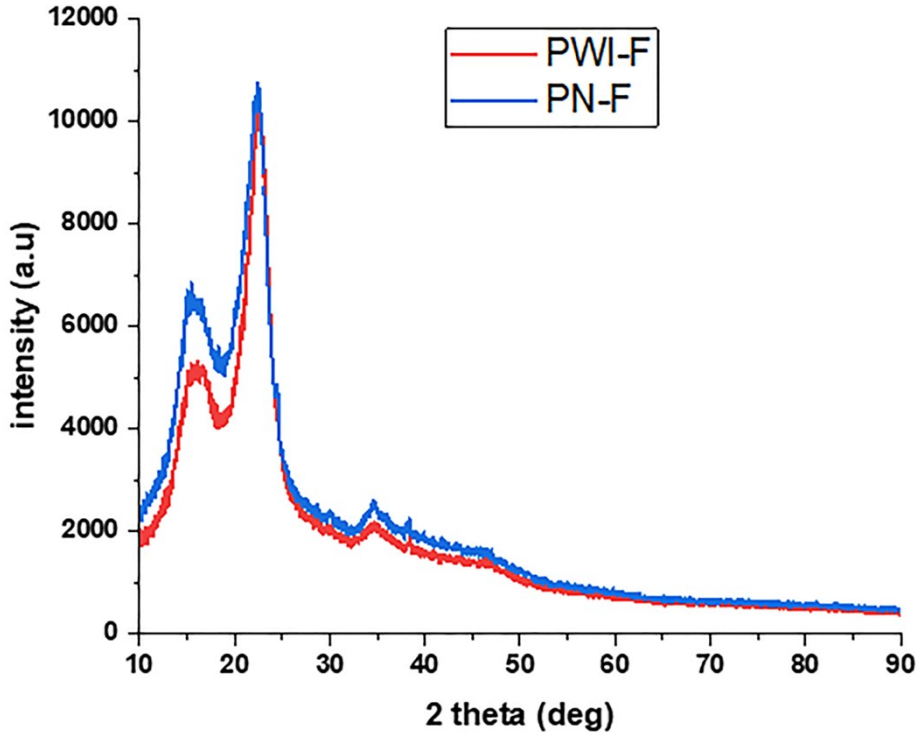


Fig. S3. X-ray diffraction (XRD) patterns for fibre bundle samples from PWI-F and PN-F.

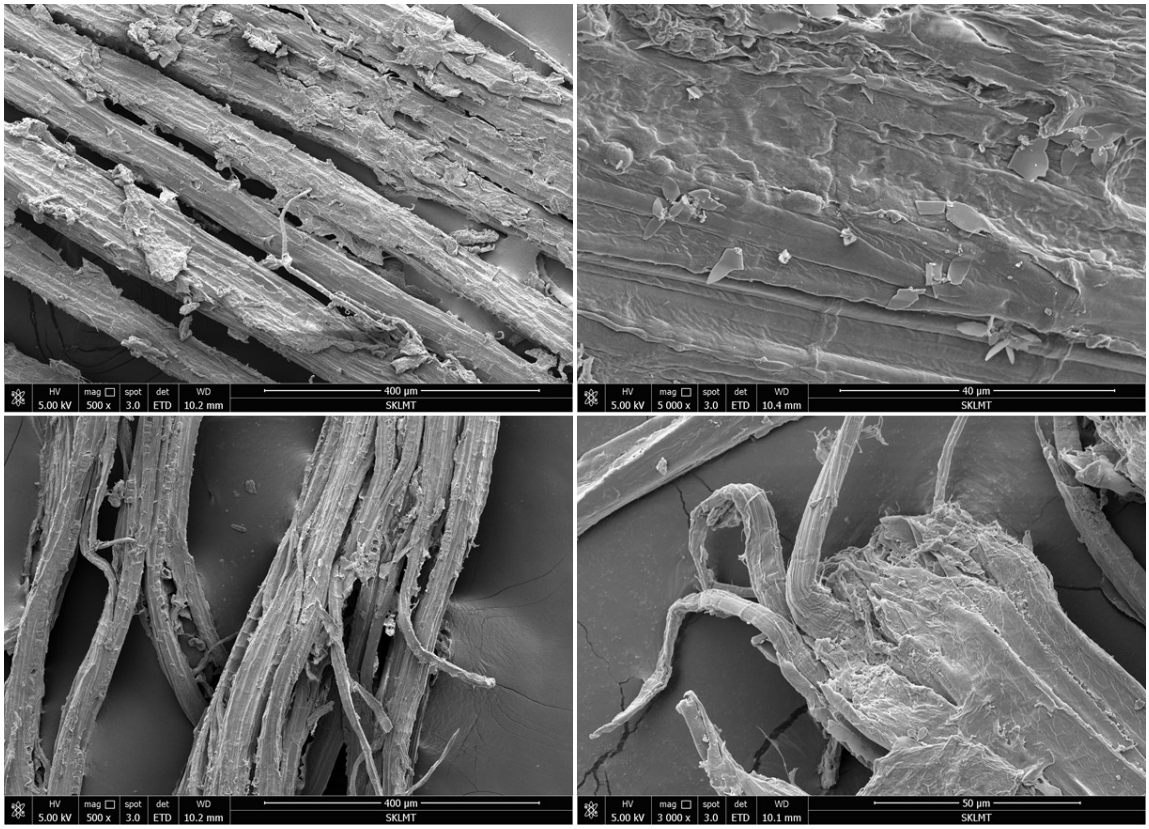


Fig. S4. SEM imaging of the isolated fibre bundles from treatment of NAWIB with pectinases (PN-F).

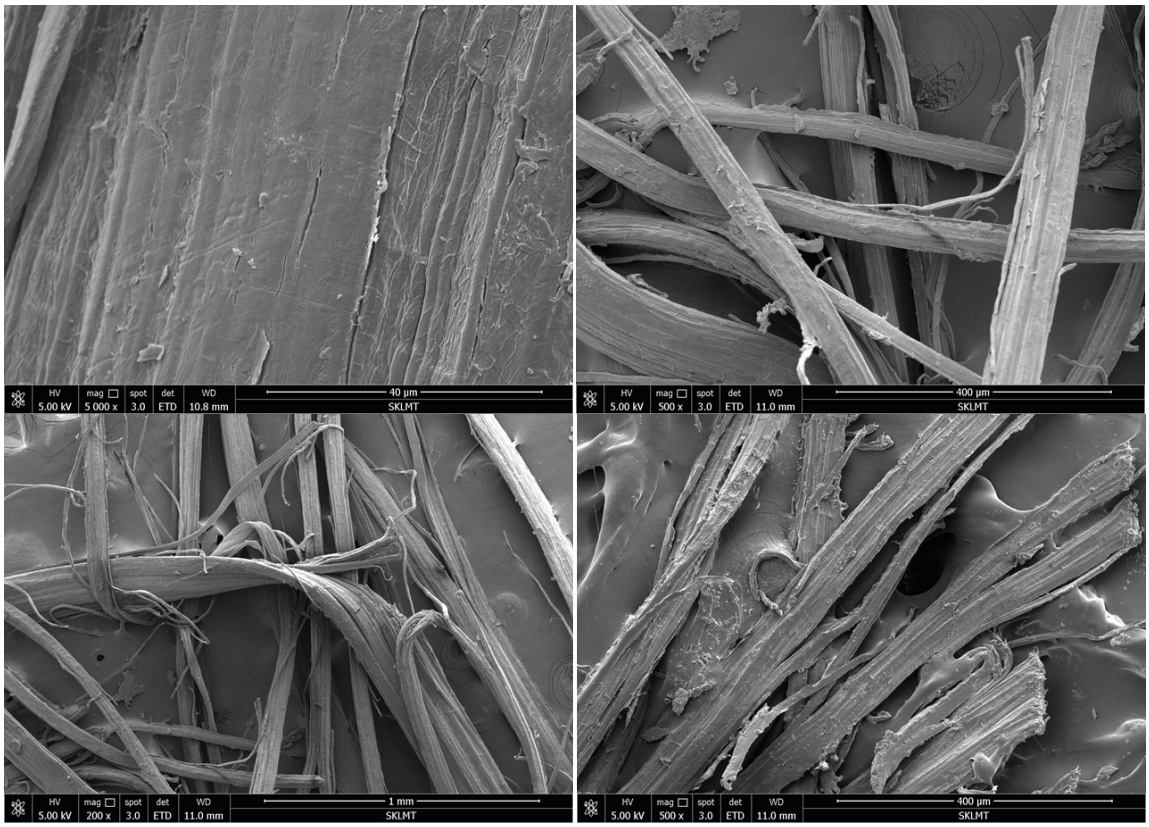


Fig. S5. SEM imaging of the isolated fibre bundles from treatment of NAWIB with a mixture of pectinases and hemicellulases (i.e. PHN-F).

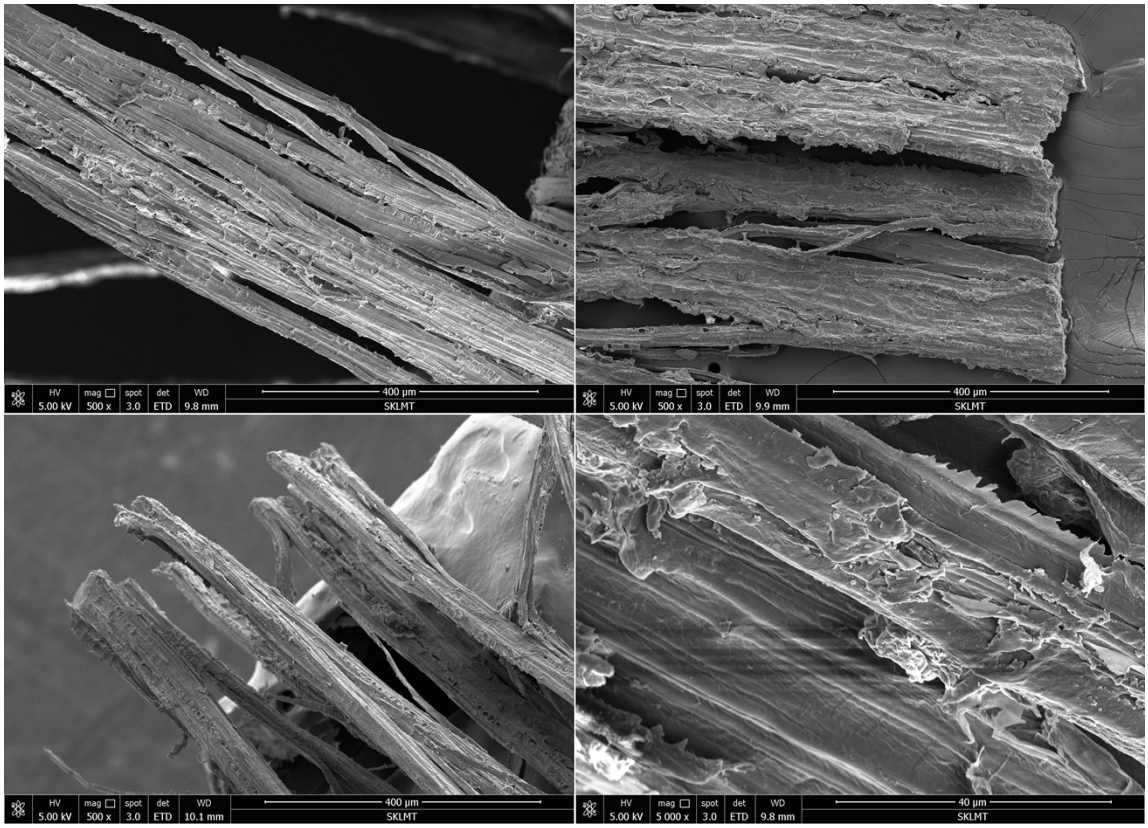


Fig. S6. SEM imaging of the isolated fibre bundles from treatment of WIB with pectinases (PWI-F).

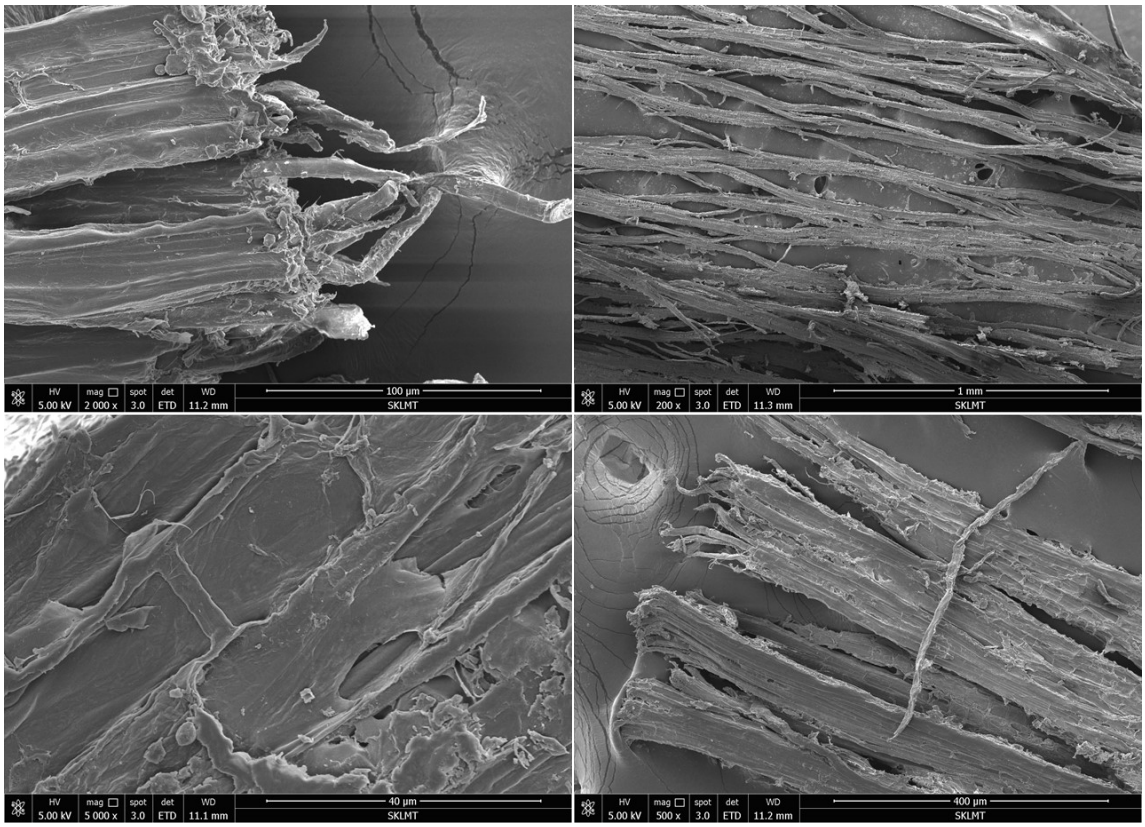


Fig. S7. SEM imaging of the isolated fibre bundles from treatment of WIB with a mixture of pectinases and hemicellulases (i.e. PHWI-F).

Table S10. Analysis of surface atomic compositions of investigated samples by X-ray-photoelectron spectroscopy (XPS). For abbreviations, see **Table S5** and **Fig.1**.

	CC %	CO %	COO %	COOO %
PN-F	19.7	61.0	15.4	3.9
PHN-F	15.2	59.8	20.8	4.2
PWI-F	18.3	61.3	16.4	3.9
PHWI-F	15.8	61.4	18.7	4.1
$NaHCO_3$ 20wt% FB	26.1	54.8	15.8	3.4
reference cellulose	12.0	69.4	17.2	1.4

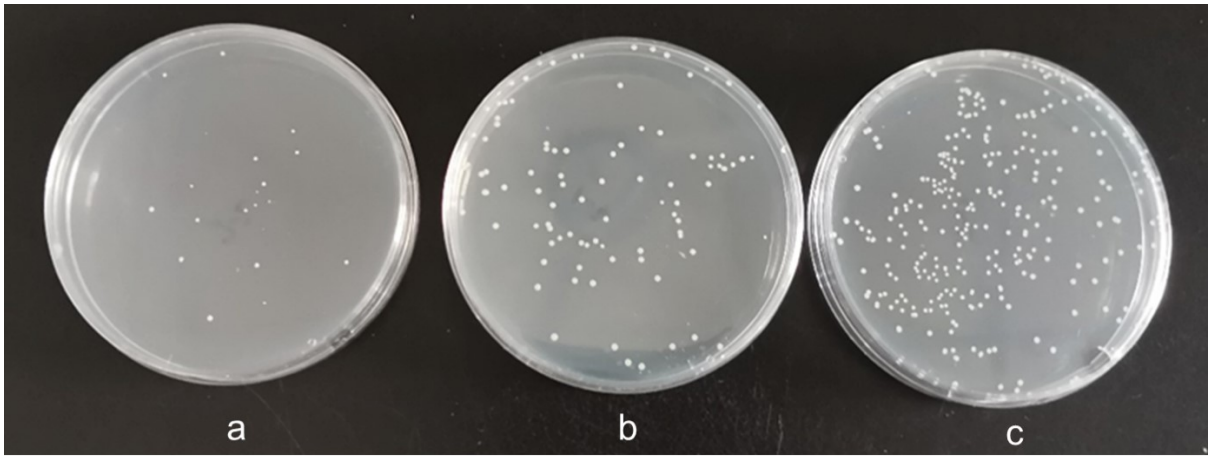


Fig. S8. Photograph of the antibacterial experiment of different fabrics against *Staphylococcus aureus* ATCC 29213: a) $NaHCO_3$ (20wt % dosages) fibre bundle; b) PWI-F; c) cotton (reference sample).

Table S11. CIELab coordinates (Elrepho, Lorentz & Wetter) measured from the obtained fibre bundles of this study in comparison to the fibre bundles obtained by the thermochemical method (i.e. $NaHCO_3$ 20wt% FB)[6]. Standard deviations are included in the parenthesis.

CIELab coordinate	fibre bundle [this study]	$NaHCO_3$ (20 wt% dosages) fibre bundle
ISO brightness	8.8 (0.4)	7.3 (1.0)
a*	7.7 (0.1)	12.2 (0.6)
b*	19.0 (0.2)	19.8 (1.4)

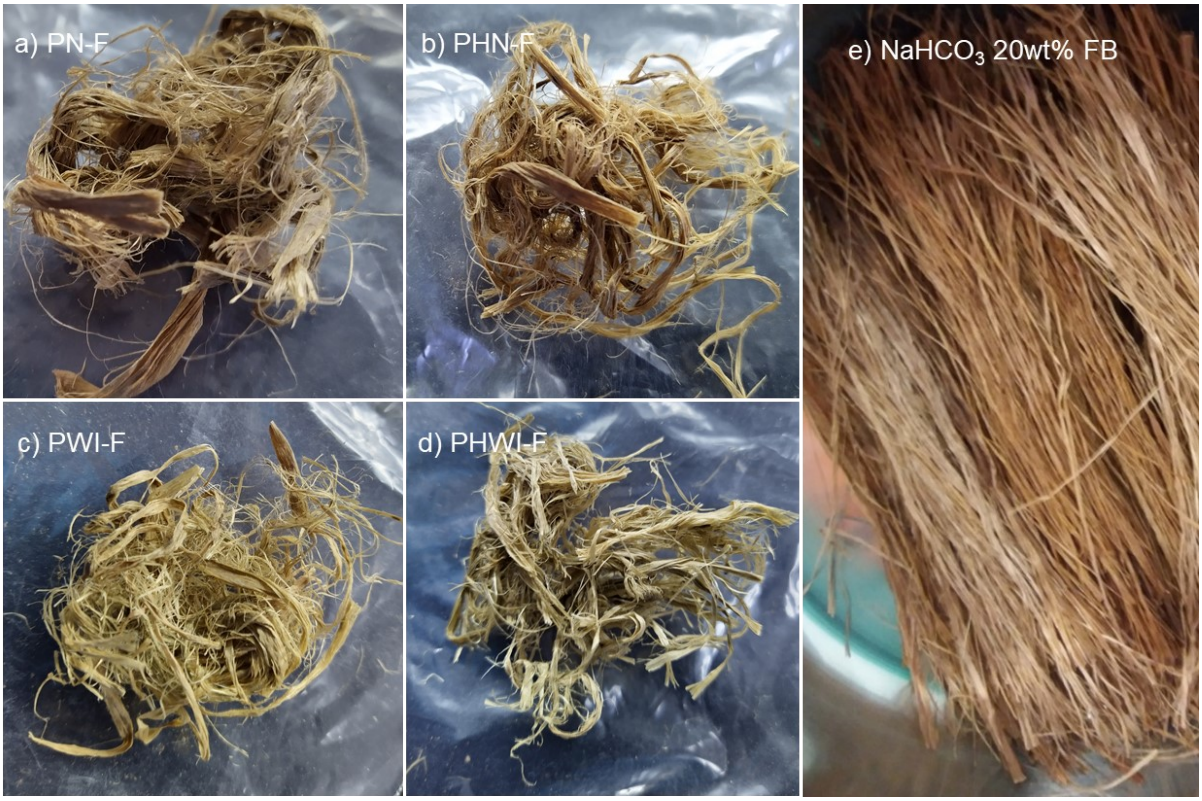


Fig. S9. Photograph of both the fibre bundles from present study (a-d) and the $NaHCO_3$ (20 wt% dosage) treatment (e)[6]. For abbreviations see **Fig.1**.

Table S12. The weight-average molecular weight (Mw) and polydispersity (Mw/Mn) of different ethanol precipitated pectin samples from treatments with aqueous citric acid before (CA-P) and after dialysis (DCA-P), aqueous $NaHCO_3$ (NA-P) and pectinases after hot water (PWI-P) and aqueous $NaHCO_3$ (PN-P). MF refers to the share of each fraction on total weight of the sample. Standard deviations are shown in the parentheses. Molar mass distribution chromatograms of different willow pectin are displayed at **Fig. S10**.

	Whole range		Peak 1			Peak 2			Peak 3			Peak 4		
	Mw (10^5 g/mol)	Mw/Mn	Mw (10^5 g/mol)	Mw/Mn	MF (%)	Mw (10^5 g/mol)	Mw/Mn	MF (%)	Mw (10^5 g/mol)	Mw/Mn	MF (%)	Mw (10^5 g/mol)	Mw/Mn	MF (%)
CA-P	2.63 (0.3%)	5.3 (2%)	3.49 (0.3%)	3.5 (0.4%)	72.9	0.31 (3%)	1.5 (4%)	27.1						
DCA-P	2.64 (0.5%)	3.4 (1%)	2.82 (0.5%)	3.2 (0.7%)	92.0	0.55 (2%)	1.8 (3%)	8.0						
NA-P	0.16 (2%)	4.3 (15%)	0.17 (2%)	3.4 (6%)	89.1	0.01 (31%)	2.6 (51%)	10.9						
PN-P	0.51 (1%)	4.3 (5%)	1.35 (0.6%)	2.8 (0.8%)	32.6	0.23 (1%)	1.0 (2%)	11.9	0.14 (4%)	1.0 (5%)	20.3	0.07 (6%)	1.2 (9%)	35.2
PWI-P	0.42 (2%)	3.6 (7%)	1.38 (0.7%)	2.5 (2%)	24.2	0.16 (5%)	1.1 (8%)	41.1	0.08 (7%)	1.2 (11%)	34.7			

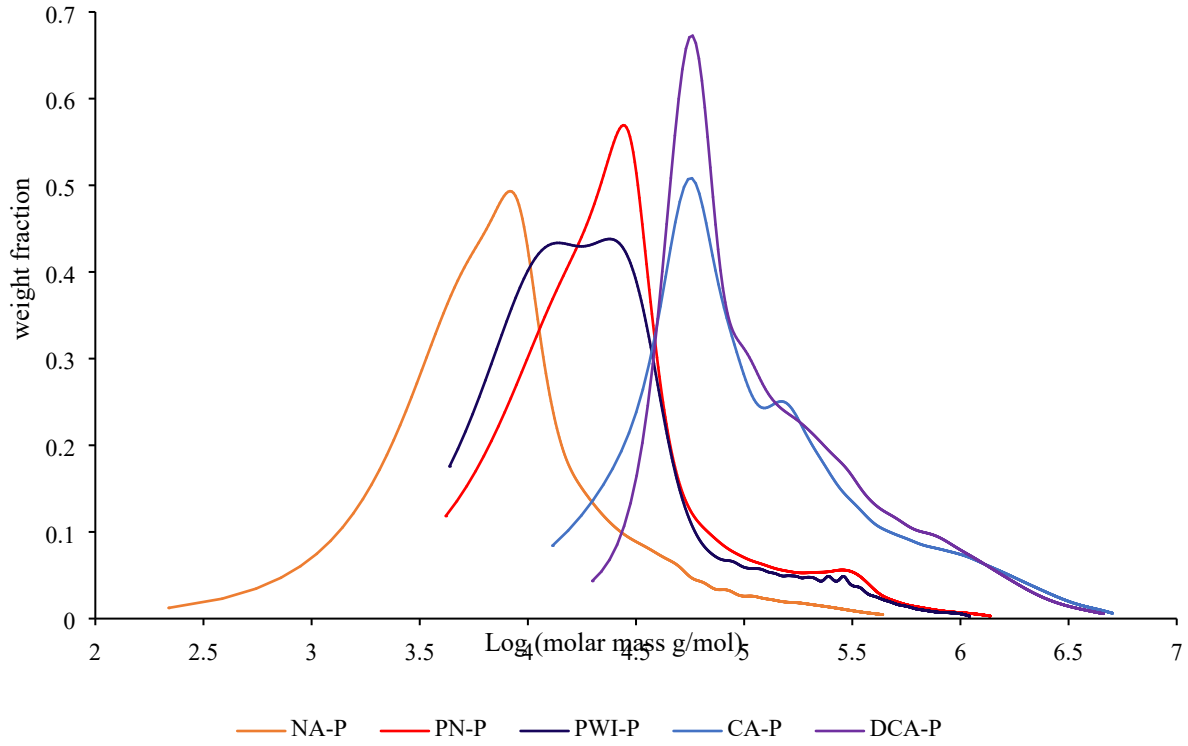


Fig. S10. The elution profile of different pectin (i.e. NA-P; PN-P; PWI-P; CA-P and DCA-P) from MALLS-SEC. For abbreviations, see **Table S5** and **Fig.1**.

Reference

1 R. Thakur, C. R. Sarkar and R. Sarmah, *Indian J. Fibre Text. Res.*, 1999, **24**, 276−278.

2 J. Dou, D. V. Evtuguin and T. Vuorinen, *J. Agric. Food Chem.*, 2021, **69**, 10848−10855. https://doi.org/10.1021/acs.jafc.1c04112.

3 J. Dou, H. Kim, Y. Li, D. Padmakshan, F. Yue, J. Ralph and T. Vuorinen, *J. Agric. Food Chem.*, 2018, **66**, 7294–7300. https://doi.org/10.1021/acs.jafc.8b02014.

4 D. M. Neiva, J. Rencoret, G. Marques, A. Gutiérrez, J. Gominho, H. Pereira and J. C. del Río, *ChemSusChem*, 2020, **13**, 4537–4547. https://doi.org/10.1002/cssc.202000431.

5 M. Zou, X. Li, W. Shi, F. Guo, J. Zhao and Y. Qu, *Process Biochem.*, 2013, **48**, 1143−1150. https://doi.org/10.1016/j.procbio.2013.05.023.

6 J. Dou, M. Rissanen, P. Ilina, H. Mäkkylä, P. Tammela, S. Haslinger and T. Vuorinen, *Ind. Crops Prod.*, 2021, **164**, 113387. https://doi.org/10.1016/j.indcrop.2021.113387.