Online Resource to:

Biorefining: The role of endoglucanases in refining of cellulose fibers

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Results of the activity assys of the enzyme formulations and purified endoglucanases in nkat ml^{-1}

Table S1 Results of the activity assays of enzyme formulations and purified endoglucanases in nkat ml-^{1} using 50 mM Citrate Buffer at pH 4.8

Enzyme	Beta Glucosidase [nkat ml^{-1}]	Endoglucanase activity on CellG5 [nkat ml^{-1}]	Endoglucanase activity on CMC [nkat ml^{-1}]	Xylanase activity [nkat ml^{-1}]	Activity on filter paper [nkat ml^{-1}]
EnzA	15.52	34.04	14.52	48.33	3.97
EnzB	55.71	923.93	164.85	10948.78	245.41
EnzC	342.05	3492.42	937.77	2085.13	478.58
Fiber Care R	0.28	467.13	78.93	0.96	136.40
EndoA	0.63	210.71	78.87	4.13	16.20
EndoB	0.81	21.96	29.72	145.89	2.88
EndoC	0.11	276.22	117.97	0.74	5.18

Table S2 Results of the activity assays of enzyme formulations and purified endoglucanases in nkat ml^{-1} using 50 % tap water + 50% deionized water, pH 7

Enzyme	Beta Glucosidase [nkat ml^{-1}]	Endoglucanase activity on CellG5 [nkat ml^{-1}]	Endoglucanase activity on CMC [nkat ml^{-1}]	Xylanase activity [nkat ml^{-1}]	Activity on filter paper [nkat ml^{-1}]	Activity on pulp [nkat ml^{-1}]
EnzA	0.89	13.55	5.06	11.05	5.00	2.24
EnzB	4.58	799.42	99.06	6336.44	109.13	40.81
EnzC	22.35	519.44	53.76	437.66	105.96	47.65
Fiber Care R	0.35	482.60	57.61	1.92	67.18	15.00
EndoA	0.70	35.51	11.55	5.25	7.92	1.21
EndoB	0.78	18.42	8.67	8.99	2.76	1.19
EndoC	0.51	54.96	11.11	0.22	7.03	2.10

SDS-PAGE of the enzyme formulations

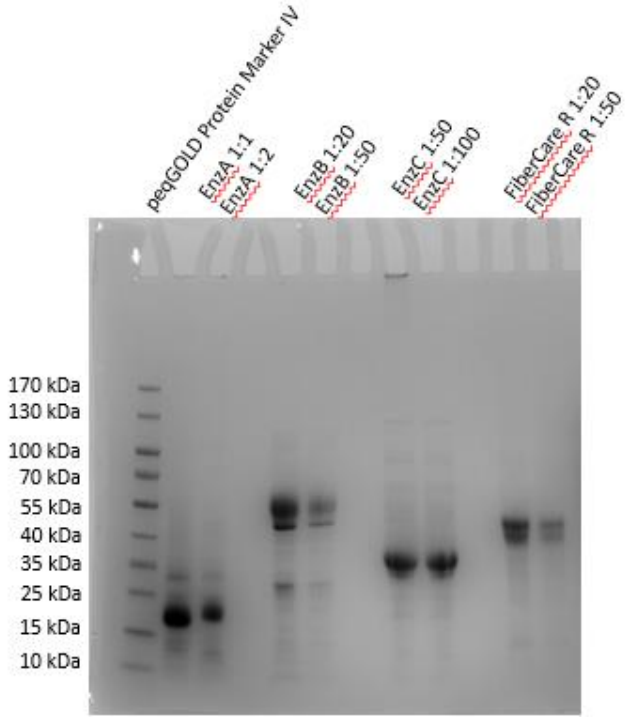

Fig. S1 SDS-PAGE of enzyme formulations including the EnzA, EnzB and EnzC used in the pulp and paper industry and the commercial FiberCare R endoglucanase

EnzA, EnzB and EnC enzyme formulations, obtained from the pulp and paper industry, as well as the commercial endoglucanase FiberCare R were analysed using SDS-PAGE **(Fig. S1)**. SDS-PAGE revealed that every enzyme formulation of the pulp and paper industry has one enzyme fraction with high concentration among a high number of proteins with a lower concentration.

Results of the Cellobiohydrolase I activity assay

Table S3 Results of the Cellobiohydrolase I activity assay of enzyme formulations and purified endoglucanases in nkat ml^{-1}

Enzyme	**Activity [nkat ml^{-1}]**
EnzA	0.0035
EnzB	0.0060
EnzC	0.083
FiberCare R	0.0035
EndoA	0.0024
EndoB	0.0083
EndoC	0.0011
Megazyme Cellobiohydrolase I	4.82

Additional measured parameters of the laboratory refining trials

Besides the degree of refining, fibre length and tensile strength parameters, also the fibril area and the air permeance according to Gurley were measured. The results show the same tendencies, with EnzC giving the highest effect regarding air permeance (increased to 79.2 s at 6000 U) and the caused fibrillation area (increased to 5.15 %

at 6000 U), while effects of EnzA and EnzB as well as their purified endoglucanases were lower. A clear distinction from the control without enzyme addition was achieved by all enzymes (air permeance according to Gurley: 56.8 s and fibril area 3.98 % at 6000 U).

Regarding the tensile strength EnzC led to the greatest improvement to 6.05 kN m^{-1} at 6000 U compared to a value of 5.73 kN m^{-1}in the absence of enzymes. In agreement with a lower effect in refining, EnzB and EnzA lead to a lower tensile strength. Likewise, the tensile strength improvement was lower for the endoglucanase FiberCare R, which is, however, in contrast one of the highest effects in the degree of refining. EndoA and EndoB led to a much higher tensile strength when compared to their corresponding enzyme formulations **(Fig. S2)**.

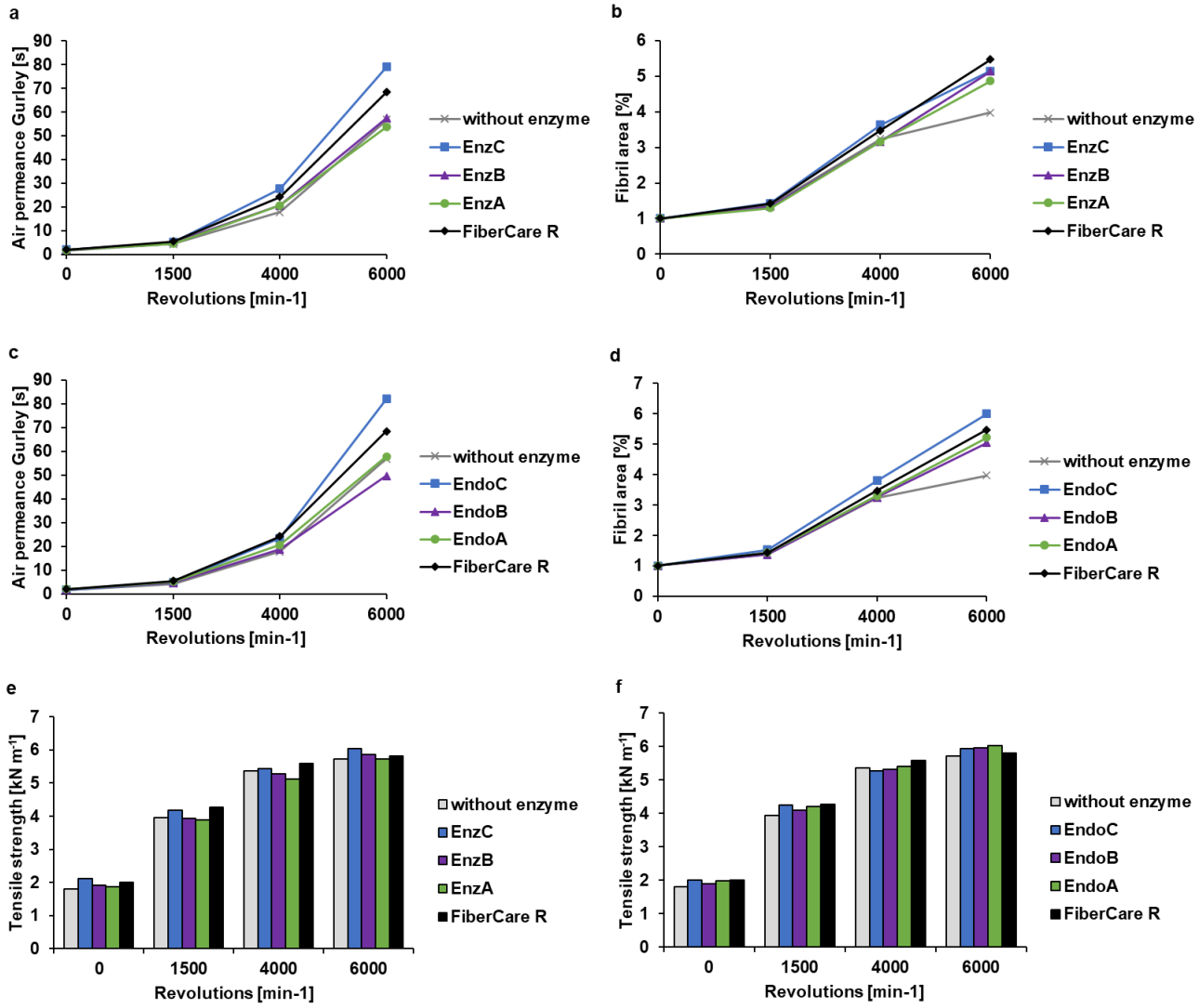

Figure S2 Determined air permeance values according to Gurley, measured fibril area and tensile strength of the enzyme formulations (a-c) and of the purified endoglucanases (d-f)

Scanning electron microscopy (SEM) pictures of refined sample sheets

Scanning electron microscopy pictures were acquired of the formed handsheets and compared with the control without enzyme treatment and with the results of the commercial endoglucanase formulation FiberCare R (***Fig.S4***, ***Fig.S5***, **Fig.S5**, **Fig.S6**). Pictures were acquired at 0 U. 1500 U, 4000 U and 1500 U to reveal any differences at high refining and low refining levels. Obtained pictures show only minor differences between the enzyme formulations and purified endoglucanases, which was expected due to the adjustment of the enzymes to the same activity according to the novel endoglucanase specific CellG5 substrate.

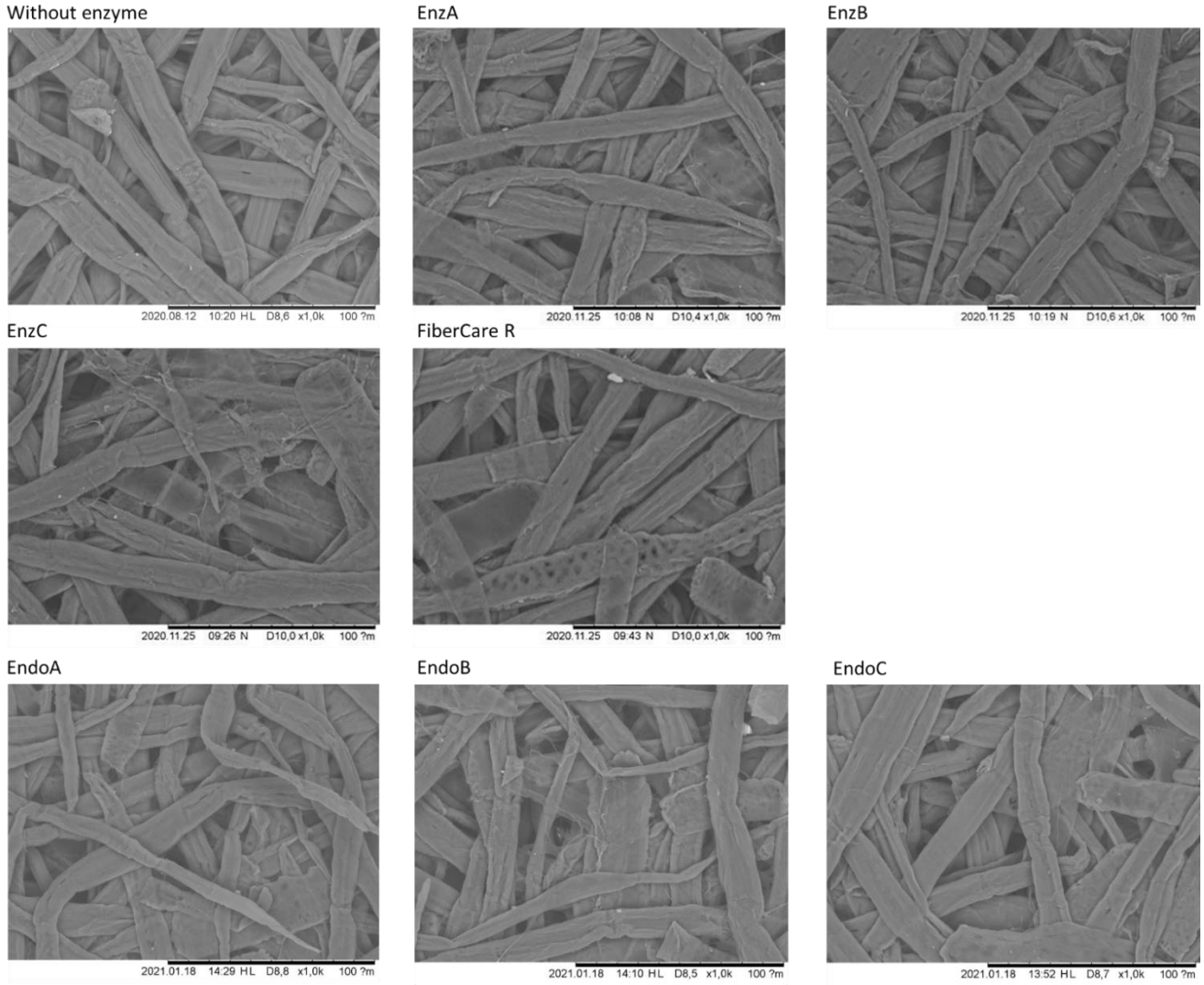

Fig.S3 Scanning electron microscope pictures of sample sheets at 1000x magnification. Comparison of the sample sheets refined at 0 U using the enzyme formulations EnzA, EnzB or EnzC and purified endoglucanases EndoA, EndoB and EndoC with sample sheets without enzyme treatment and with the commercial endoglucanase FiberCare R

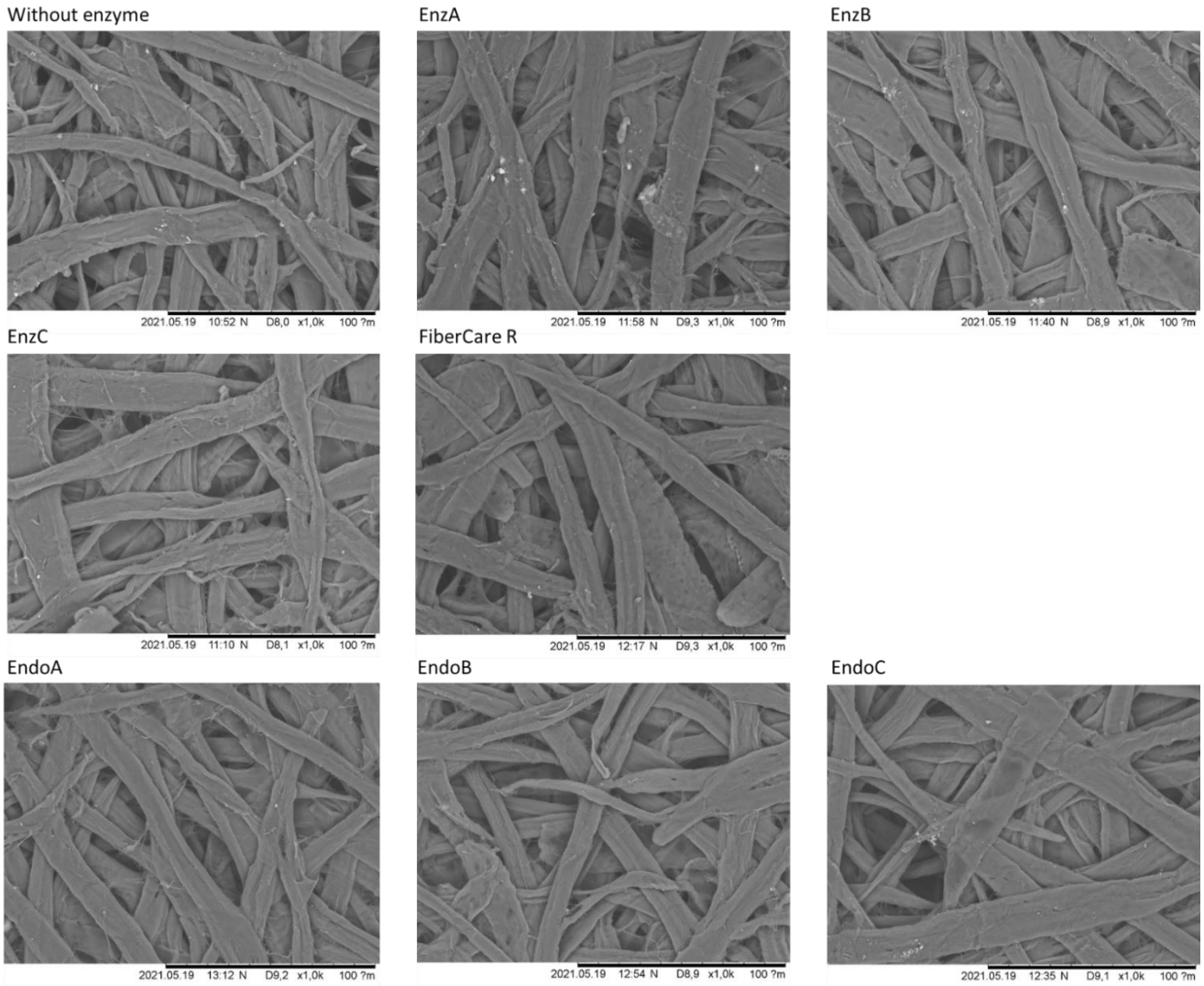

Fig.S4 Scanning electron microscope pictures of sample sheets at 1000x magnification. Comparison of the sample sheets refined at 1500 U using the enzyme formulations EnzA, EnzB or EnzC and purified endoglucanases EndoA, EndoB and EndoC with sample sheets without enzyme treatment and with the commercial endoglucanase FiberCare R

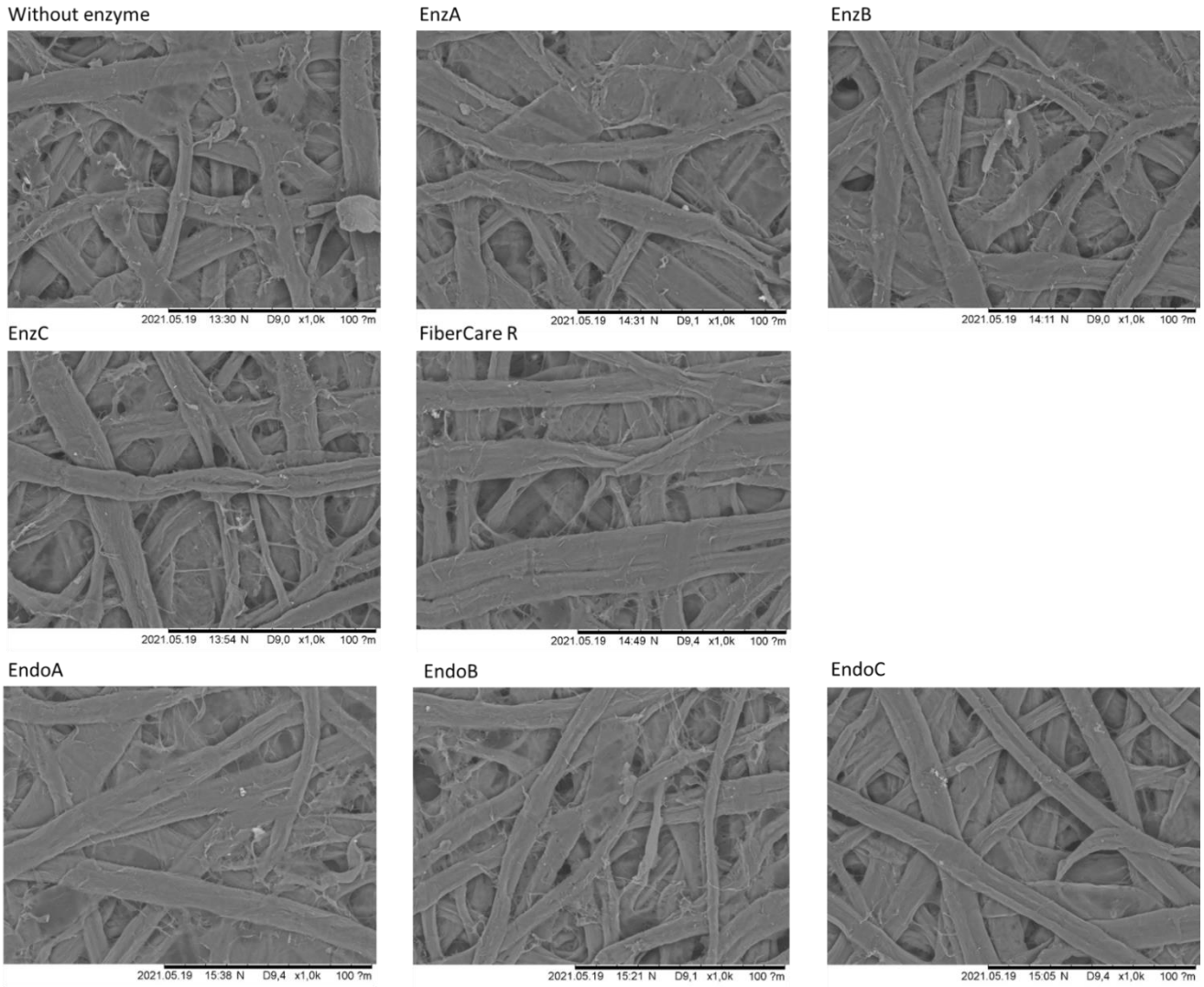

Fig.S5 Scanning electron microscope pictures of sample sheet at 1000x magnification. Comparison of the sample sheets refined at 4000 U using the enzyme formulations EnzA, EnzB or EnzC and purified endoglucanases EndoA, EndoB and EndoC with sample sheets without enzyme treatment and with the commercial endoglucanase FiberCare R

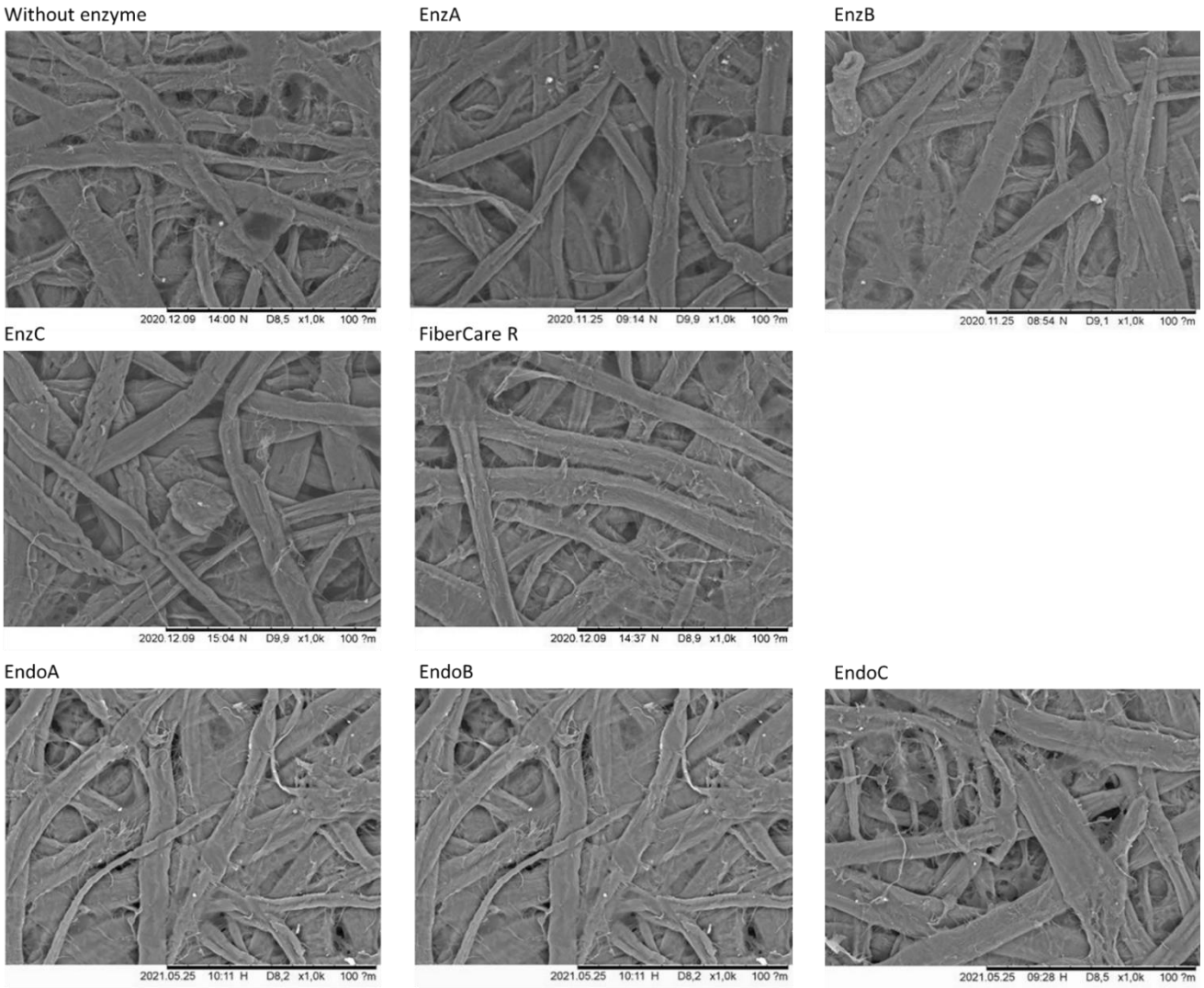

Fig.S6 Scanning electron microscope pictures of sample sheet at 1000x magnification. Comparison of the sample sheets refined at 6000 U using the enzyme formulations EnzA, EnzB or EnzC and purified endoglucanases EndoA, EndoB and EndoC with sample sheets without enzyme treatment and with the commercial endoglucanase FiberCare R