Supplementary material to

Enhanced energy savings in enzymatic refining of hardwood and softwood pulp

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1. Tensile index data of laboratory refining trials on softwood sulfate pulp

The tensile index increased the most with EnzC (84.8 Nm g^{-1}) after 6000 PFI revolutions, which is in accordance with the measured degree of refining. However, it is followed by the EnzB (82.2 Nm g^{-1}), which showed only a minor increase of the degree of refining value in relation to the blank. After which the endoglucanase formulation FiberCare R with a considerably high degree of refining value reached a tensile index value of 82.5 Nm g^{-1}. The enzyme formulation EnzA resulted in the lowest effect with a tensile index of 81.4 Nm g^{-1}, when compared to the results without enzyme addition with a value of 79.4 Nm g^{-1} (**Fig. S1a**).

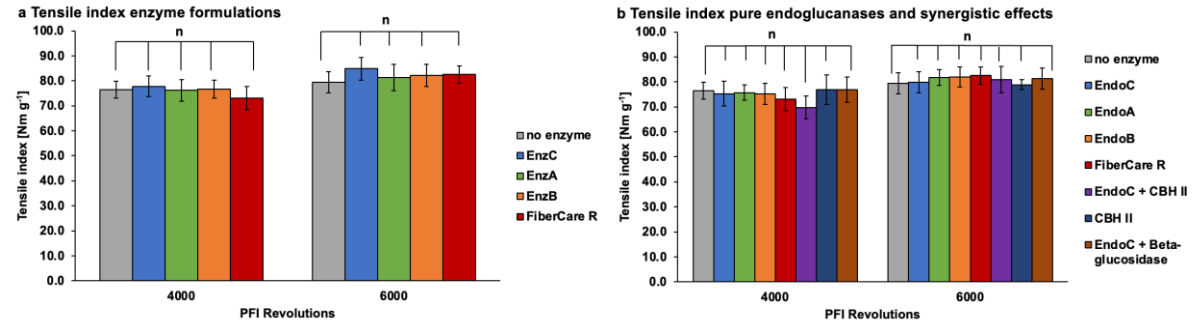

Fig. S1 Tensile index data after refining of softwood pulp after 4000 and 6000 PFI revolutions. (a) shows the data of the complex enzyme formulations and the commercial endoglucanase formulation FiberCare R, (b) the data of the pure endoglucanases and the investigation of synergistic effects through ß-glucosidase or cellobiohydrolase II. Statistical significancy was tested using a single factor ANOVA test with a significance level of 0.05 (significantly different results are indicated by the letter s, not significantly different results by the letter n).

When investigating the purified endoglucanases, the tensile index decreased with EndoC (79.9 Nm g^{-1}) after 6000 revolutions, while EndoA (81.8 Nm g^{-1}) and EndoB (82.0 Nm g^{-1}) showed similar values as the reference without enzyme addition with a value of 79.4 Nm g^{-1} (**Fig. S1b**). However, the addition of β-glucosidase to EndoC increased the tensile index to a value of 81.8 Nm g^{-1}, when compared to the treatment with EndoC alone (81.3 Nm g^{-1}) after 6000 PFI revolutions, but only to a minor extent. Similarly, the combination of cellobiohydrolase II with EndoC decreased the tensile index value to 80.9 Nm g^{-1}, suggesting a negative impact on tensile properties through synergistic action of both enzymes, possibly caused by degradation of cellulose fibers (**Fig. S1b**).

2. Cellobiohydrolase II activity

The activity of the Megazyme Cellobiohydrolase II (E-CBHIIM) was determined using 50% tap water + 50% deionized water (pH 7) to simulate refining conditions as well as 50 mM citrate buffer pH 4.8. The enzyme activity at 50% tap water + 50% deionized water (pH 7) consisted of 0.0016 nkat ml^{-1}, while its activity was higher using citrate buffer (0.0095 nkat ml^{-1}).

3. Scanning electron microscopy (SEM) pictures of refined paper sheets:

Obtained handsheets after laboratory PFI refining of softwood pulp were analysed using scanning electron microscopy for evaluation of morphological changes. As expected, higher fibrillation values could be observed after 6000 PFI refiner revolutions compared to only 4000 PFI refiner revolutions (**Fig. S2**/**Fig. S3** vs. **Fig. S4**/**Fig. S5**). When comparing the effect of the different enzymes, indeed shorter and thinner fibers could be observed for EnzC and FiberCare R at 4000 PFI revolutions, the enzymes with the highest refining performance, while the fibers were much looser for EnzA and EnzB. Especially the pure endoglucanases EndoA and EndoB showed lower fibrillation and fiber density compared to EndoC or EndoC with either ß-glucosidase or xylanase addition. As expected, the fibrillation levels were higher at 6000 refiner revolutions with EnzC, FiberCare R, EndoC + ß-Glucosidase and EndoC + CBH II exhibiting shorter and thinner fibers as well as higher fiber density. The generation of thinner and shorter fiber suggest a higher smoothness of the resulting paper sheets as it is expected during pulp refining.

Set 1

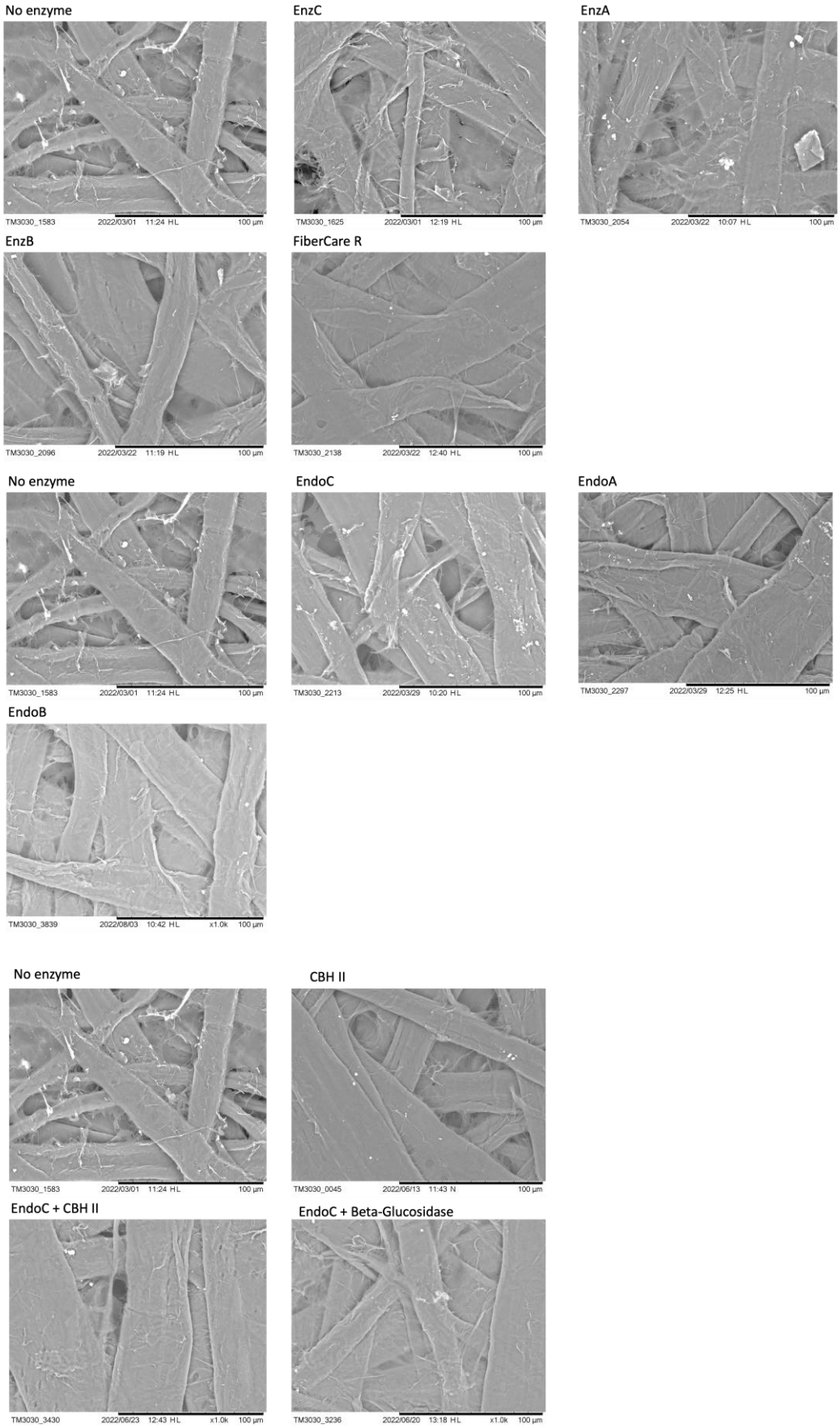

Fig. S2 First replicate of scanning electron microscopy pictures at 1000x magnification of handsheets obtained after refining of softwood sulfate pulp at 4000 PFI refiner revolutions.

Set 2

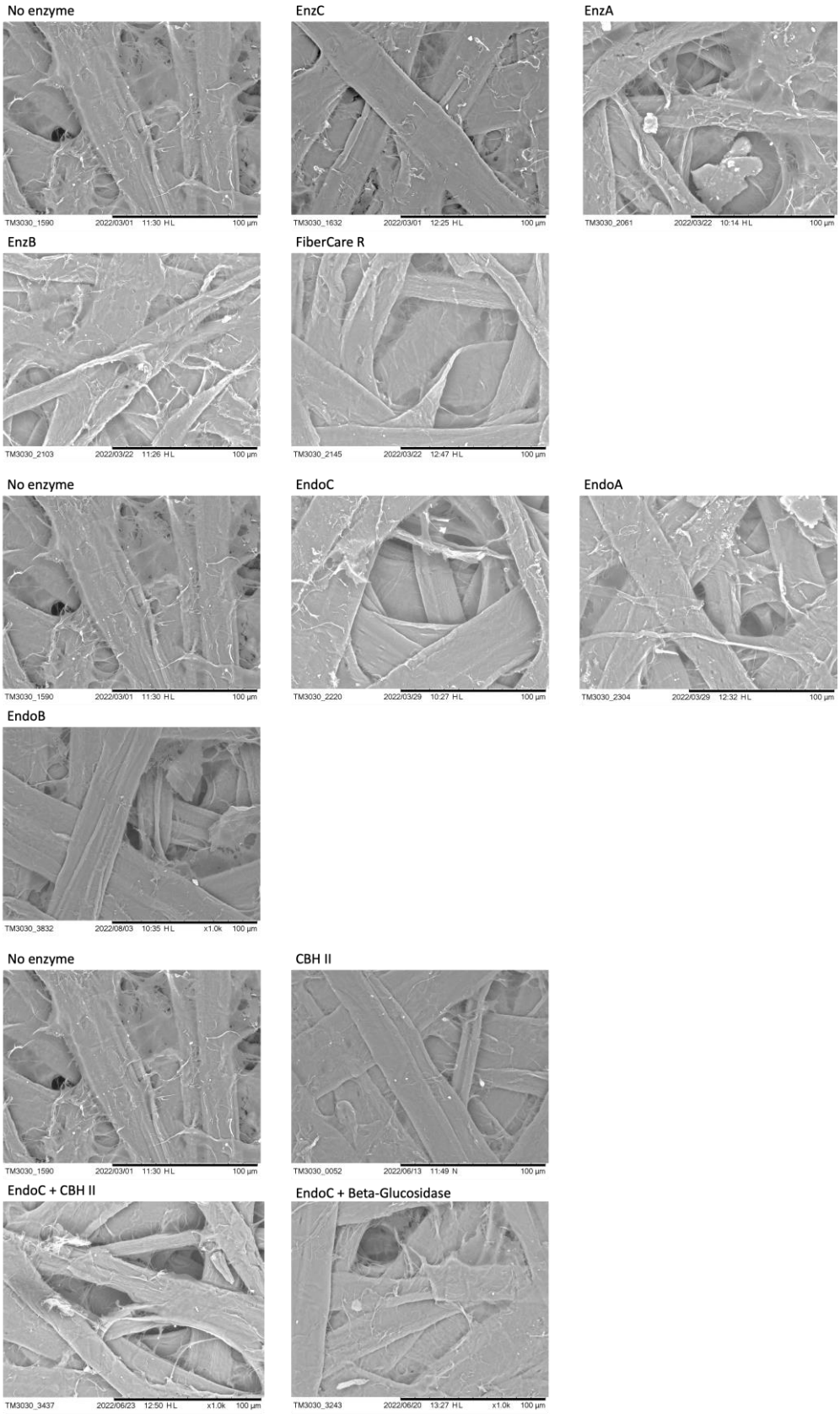

Fig. S3 Second replicate of scanning electron microscopy pictures at 1000x magnification of handsheets obtained after refining of softwood sulfate pulp at 4000 PFI refiner revolutions.

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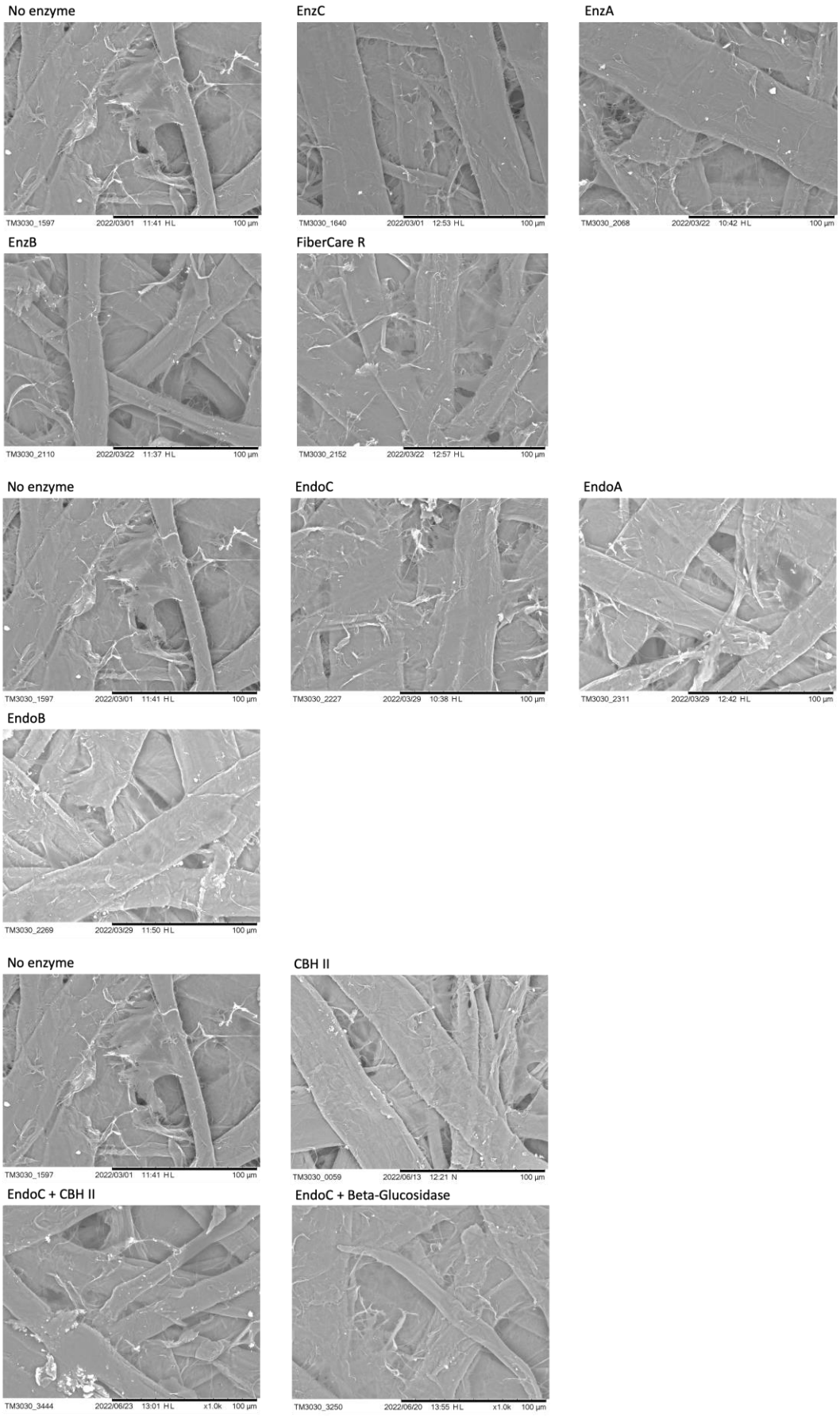

Fig. S4 First replicate of scanning electron microscopy pictures at 1000x magnification of handsheets obtained after refining of softwood sulfate pulp at 6000 PFI refiner revolutions**.**

Set 2

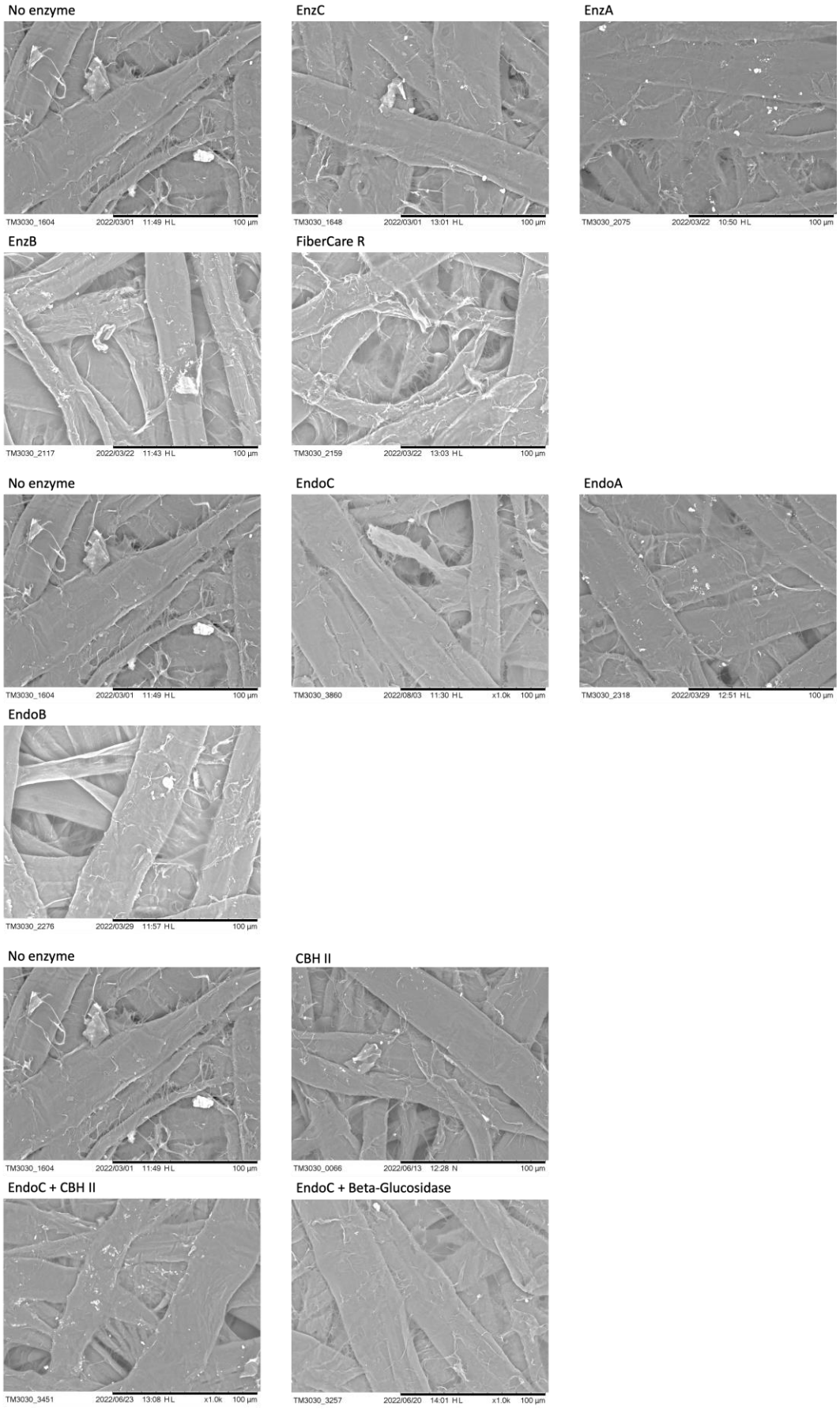

Fig. S5 Second replicate of scanning electron microscopy pictures at 1000x magnification of handsheets obtained after refining of softwood sulfate pulp at 6000 PFI refiner revolutions.