EFFECT OF ENZYME TREATMENT ON THE MECHANICAL PROPERTIES OF WOOD CELL WALLS BY NANOINDENTATION

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The objective of this research was to study the changes in hardness and elastic modulus of wood cell walls treated with enzymes. Such changes greatly influence the properties of paper and wood composites. Two enzymes, hemicellulase and lipase, were selected for the treatment. Poplar samples (*Populus euramevicana*) were treated with hemicellulase, while samples of southern yellow pine (*Pinus spp.*) and Mongolia scotch pine (*Pinus sylvestris L. var. mongolica Litv.*) were treated with lipase. Mechanical properties of both treated samples were investigated by nanoindentation. The results showed some changes in the hardness and elastic modulus of the poplar cell wall treated by hemicellulase. Hardness and elastic modulus values of southern yellow pine and Mongolia scotch pine cell walls treated by lipase decreased with increasing amounts of the enzyme.

Keywords: Cell wall; Enzyme; Mechanical properties; Nanoindentation

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INTRODUCTION

Enzymes have been widely used to treat wood fibers in many different research areas and for various purposes. For example, by enzymatic hydrolysis, poplar solids can deliver satisfactory sugar yields, and the release of glucose increases almost linearly with residual xylose removal when enzyme pretreatments are used (Kumar *et al*. 2009). Enzymatic activation of the middle-lamella lignin of wood fibers has also been investigated for the production of wood composites (Huttermann *et al*. 2001). The free-radical content of wheat straw has been shown to increase when wheat straw is treated with cellulase, and the benzene-ethanol or ether-extract content decreases when treated by lipase (Zhang *et al*. 2003). The chemical composition of the surface of fibers changes when fibers are treated with laccase, making better fiber-board (Felby *et al*. 2004). Jiang *et al.* (2009) have investigated the wettability of wheat straw before and after lipase treatment; the results showed that lipase treatment significantly changed the chemical group, the microscopic morphology, and the wettability of the outer surface of wheat straw. In summary, enzyme treatment appears to be a promising potential method for improving the wettability of natural fibers in composite materials and panel processing.

Mechanical investigations of plant fibers have been performed to compare fibers treated enzymatically with their untreated counterparts (Bourmaud *et al*. 2010). Konnerth (2010) found that red oak wood treated with a range of enzyme mixtures, including

hemicellulase, had diminished mechanical properties for different loading modes. The impact of the removal of wood cell-wall components (hemicellulose and lignin) on the mechanical properties of cell walls has also been shown by a study examining the effect of decreasing carbohydrate content on the properties of wood strands (Hosseinaei *et al*. 2011a). The study showed that only the most severe conditions (extraction at 170 ^{o}C) of extraction could result in some decrease in the mechanical properties of cell walls, mainly the elastic modulus. The bending properties of flakeboard (MOE and MOR) improved after extraction of hemicellulose, as shown in another study by Hosseinaei *et al*. (2011b). Further study is necessary to understand the mechanical properties of cell walls after hemicellulose extraction.

The use of enzymes to pretreat pulp in composite manufacture has several effects. Firstly, applying enzymes to wood chips can lower energy demand in the refining process (Kim *et al*. 2006; Maijala *et al*. 2008). In fact, some enzyme treatments can reduce the specific refining energy by at least 24 percent. Furthermore, enzyme treatments have the advantage of being mild and capable of high selectivity (Jiang *et al*. 2009).

Nanoindentation of wood fibre cell walls has been studied by several authors. Adusumalli *et al*. (2010) used nanoindentation technique to study the influence of fibre type and bleaching on hardness and elastic modulus. Gindl *et al*. (2002, 2004a and 2004b) extended the nanoindentation method, studying the unfluences of lignin content, microfibril angle and chemical wood modification on local mechanical properties. Xing *et al*. (2008) investigated the influence of thermomechanical refining pressure on pulp fibre properties using the nanoindentation technique. The nanoindetation technique was also suitable used to study the mechanical properties of pyrolysed wood (Zickler *et al*. 2006). This study focused on investigating the changes of hardness and elastic modulus of wood cell walls treated by lipase and hemicellulase under different loads; nano-indentation tests were performed to evaluate the changes in the wood cell walls. The objective of this research was to find a way to improve the surface properties of the wood cell wall by using environmental friendly treatments while preserving and improving the mechanical properties of the cell wall.

EXPERIMENTAL

Sample Preparation

Wood samples of Poplar (*Populus euramevicana*), southern yellow pine (*Pinus spp.*), and Mongolia scotch pine (*Pinus sylvestris L. var. mongolica Litv.*) were obtained from mills. The samples were cut into wood slices 20 mm (L) by 10 mm (W) by 0.5 mm (T). One batch of samples served as the control, whereas the other batch of samples was treated by enzymes. The enzymes used in the experiment were hemicellulase and lipase (Imperial Jade Bio Technology Co., Ltd., China). Both lemon acid and sodium phosphate were used in the buffer solution. Samples of poplar, southern yellow pine, and Mongolia scotch pine were mixed with the enzyme liquid and the buffer solution (at pH 4.8 for hemicellulase and pH 7.5 for lipase); all were placed in a reaction vessel. The hemicellulase dosages were 100 IU g^{-1}, 200 IU g^{-1}, 300 IU g^{-1}, and 400 IU g^{-1} (based on the dry wood and it is the same unit for IU g^{-1}, where "I" means international and it can

be omitted). The lipase dosages were 4 KU g^{-1}, 6 KU g^{-1}, 10 KU g^{-1}, and 12 KU g^{-1} (based on the dry wood, 1 KU g^{-1} equaling 1000 IU g^{-1}). Then, for all the treatments described above, the same treatment conditions were performed as follows: the mixture was incubated in a water bath at 50 ^{o}C and shaken at 200 rpm for 10 h. The enzyme reaction was terminated by filtering the mixture and washing the residual samples three times with distilled water. At last, the treated samples were dried under vacuum at 45 ^{o}C for 48 h and kept airtight in polyethylene bags and at room temperature for subsequent determinations.

Nanoindentation Testing Method

After oven-drying, the control and extracted specimens were cut into small pieces of about 5 mm (L) by 2 mm (W) by 0.5 mm (T) and sealed by FoodSaver polymer film, following the procedure of our previous research (Meng *et al*. 2011). Each small wood block was placed between two films and pressed with an electric iron with the temperature set at 160 ^{o}C. The pre-sealed samples were then embedded in epoxy resin. A TriboIndenter® system, manufactured by Hysitron, Inc., was used for all indentation tests. A Berkovich indenter with a three-sided pyramidal shape and with an area-to-depth function was loaded for all experiments (Oliver *et al*. 1992). All experiments were conducted with a closed-loop feedback control aimed at providing precise control of the nanoindentation probe in load-controlled modes. A drift monitoring time of 40 s was set up to measure the drift of the system before any of the tests. The single indentation procedure included four parts. Firstly, there was a 2 μN set-point force between the probe and sample surface. The indentation test did not start until the 2 μN pre-load force was detected by the transducer. Secondly, the peak load was achieved at a loading rate of 30 μN s^{-1}. Thirdly, at this peak load, the loading was held for 5 s to avoid the effect of creep occurring in viscous material during the unloading (Liu *et al*. 2006). Finally, the unloading was executed at the same loading rate as the loading, 5 s. The scanning probe microscopy (SPM) assembly in the Triboindentor system is capable of accurately positioning the wood cell walls' S2 layer. With a scanning size of 40 μm by 40 μm, interesting indent positions from surface to core layer of specimens were marked on the SPM image, and 44 indentations were implemented and checked by rescanning the image (as shown in Fig. 1). Only indentations in the middle of the fiber cell wall's S2 layer were selected as valid data. Any indentations performed in the embedding epoxy or at the border of the cell walls were expunged. On the basis of nanoindentation theory, the hardness and reduced elastic modulus were calculated from the valid data following the methods of Oliver and Pharr (1992) and Wu *et al*. (2009).

RESULTS AND DISCUSSION

Testing Data Distribution

In the nanoindentation testing, the data reflected that the hardness and elastic modulus of the cell wall S2 layer constituted about 80% of the total cell wall, which is the major contributor to the mechanical properties of wood cell walls (Tze *et al*. 2007). In order to assure the correct testing data, more than 30 points were tested for each sample,

which satisfied the normal distribution. The Anderson-Darling normality test was used to determine whether the data follows a normal distribution. The commonly chosen p-value of 0.1 (ranging from 0 to 1) was used to indicate how likely the data were to follow a normal distribution. If the p-value were lower than the criterion, the data would not follow a normal distribution. From Tables 1 and 2, it can be seen that all p-values of the nanoindentation testing results were larger than 0.1. Thus, the data tested by nanoindentation were reliable in this research.

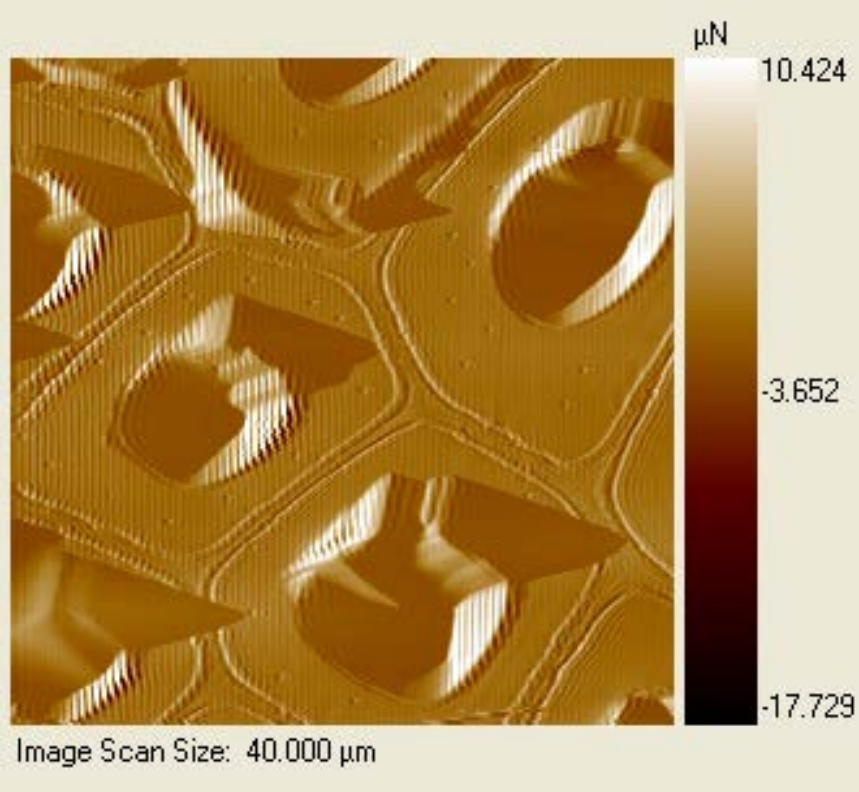

Fig. 1. Post indentation image-SPM

Effect of Hemicellulase on the Elastic Modulus of Poplar Cell Wall

The elastic moduli of the poplar cell wall in the control and in the extracted samples are shown in Table 1. It was found that there were some changes of elastic modulus of the poplar cell wall treated by hemicellulase with increasing hemicellulase loading. And the trends of changes were significant different at the 0.05 level. The elastic modulus increased from 17.4 GPa to 19.4 GPa as the amount of hemicellulase was increased from 0 to 100 IU g^{-1} in the control sample and treated samples and then decreased gradually, from 19.4 GPa to 17.6 GPa, as the amount of hemicellulase was increased from 100 IU g^{-1} to 400 IU g^{-1}. The elastic modulus of the extracted samples changed from being higher than that of the control samples to a level similar to that of the control. These results disagree with the report by Konnerth *et al*. (2010), who tested red oak samples with a commercial mixture of hemicellulases and found that the elastic

modulus obtained through indentation was significantly reduced in the treated wood. However, the tendency of the changes observed in this study was similar to that seen in the research of Hosseinaei *et al*. (2011a), who performed hot-water pretreatment on southern yellow pine and investigated the mechanical properties of wood cell walls before and after treatment by means of nanoindentation. According to their results, the elastic modulus at a low extraction temperature (140 ^{o}C) is slightly higher than the control sample but tends to decrease with extraction temperatures increasing from 155 ^{o}C to 170 ^{o}C. Generally, the two studies agreed that the cell wall elastic modulus decreased with the removal of hemicellulose. Here it should be noticed that different wood species were used in the three research studies; in this experiment, the samples were poplar, a fast-growing hardwood species. However, in the research carried out by Konnerth et al. (2010) the wood used was oak (a hardwood) and Hosseinaei *et al*. (2011a) used pine (a softwood), respectively.

Table 1. Elastic Modulus of Nanoindentation Testing for Poplar Treated by Hemicellulase

Enzyme amount (IU g^{-1})	Average of elastic modulus (GPa)	p-values
0	17.4±1.38	0.19
100	19.4±2.17	0.16
200	18.1±1.69	0.11
300	17.5±1.87	0.55
400	17.6±1.53	0.16

Note: "±" values are the standard deviations of measurements.

The significant effects on the mechanical behavior of enzymatically treated wood are still not well known, and the contribution of hemicel-lulose to modulus or hardness is still unclear. Therefore, the influence of hemicellulase on wood species is worthwhile to be investigated in future studies. For the poplar wood samples used in this study, the influence of partially removing the hemicellulose on the cell wall's elastic modulus and hardness (discussed in the next section) was small, which should be a positive result. One of the targets in this study was to use a hemicellulose treatment for selective degradation of the hemicelluloses in the cell wall without sacri-ficing the mechanical properties. The changes in the components and structure of the cell wall due to treatment, and the selective action effect of hemicellulase, were the main reason for the changes in elastic modulus.

Bergander and Salmén (2002) observed that among wood polymers, cellulose mainly dominates the properties of cell walls in the longitudinal direction, whereas hemicellulose dominates the modulus of cell walls in the transverse direction. At the early stage of the extraction, hemicellulose was partially extracted; coupled with the removal of some lignin, more cellulose microfibrils were exposed, which might account for the observed increase of the elastic modulus in the longitudinal direction. With further extraction of hemicellulose and the migration of lignin from the matrix surrounding the cellulose microfibrils, pores would be made in the matrix, and the elastic modulus would decrease. Otherwise, the action effect of hemicellulase on degrading mainly pentosan,

which was a main component of hemicelluloses, had little effect on cellulose and lignin of the cell wall for poplar. Therefore, the elastic modulus of the cell wall would not be impacted greatly by the partial removal of the hemicellulose. From this testing result, it is clear that the negative effect on the elastic modulus of the wood cell wall would not be produced if the amount of hemicellulase were controlled.

Effect of Lipase on the Elastic Modulus of Pine Cell Wall

The effects of lipase treatment on the elastic modulus of yellow pine and Mongolia scotch pine cell walls are shown in Table 2, which shows that the elastic modulus of the cell wall decreased generally as the amount of lipase was increased, and the tends of decreasing were significant different at the 0.05 level. In addition, the elastic modulus of the yellow pine samples experienced a bigger change than did the Mongolia scotch pine when treated by lipases at amounts lower than 12 KU g^{-1}. However, when 12 KU g^{-1} lipase was used, the elastic modulus values of both the yellow pine and Mongolia scotch pine decreased significantly. The changes illustrate that the lipase had an obvious influence on the cell wall. This is reasonable, since enzymes are able to penetrate all layers of cell wall, including the S1, S2, and S3 layers (Hart *et al*. 2009; Donohoe *et al*. 2009). Furthermore, there is a close relationship between the elastic modulus of the cell wall and that of its S2 layer, which accounts for 80 percent of the cell wall. Enzyme pretreatment affects the S2 layer of the cell wall so as to reform the mechanical properties of wood cell walls. Lipase treatments have been shown to reduce pulp triglycerides successfully and also act on other compounds, such as free and esterified sterols, resin acids, fatty alcohols, alkanes, *etc.* (Gutierrez *et al*. 2009). The lipase treatment also had the effect of deresination on different kinds of pine. Our previous research (Jiang *et al*. 2009) showed that lipases are able to effectively remove hydrophobic, lipophilic extractives and silica from the outer surface of wheat straw, which results in improved wettability of natural fiber. It has been proved that the strength of masson pine tends to decrease with deresinating (Yu *et al*. 2007), which agreed with the results of this study shown in Table 2.

Table 2. Elastic Modulus of Nanoindentation Testing for Yellow Pine (YP) and Mongolia Scotch Pine (MSP) Treated by Lipases

Enzyme amount (KU g^{-1})	Average of elastic modulus (GPa)		p-values	
	YP	MSP	YP	MSP
0	21.3±2.17	21.8±1.63	0.12	0.21
4	20.1±3.00	20.1±2.22	0.63	0.10
6	16.3±2.45	20.1±1.54	0.94	0.50
10	15.2±3.43	19.2±6.36	0.20	0.44
12	11.2±1.75	12.5±1.94	0.63	0.49

Effect of Enzymes on Cell-Wall Hardness

Figure 2 presents the change in hardness for the poplar cell wall treated with hemicellulase. The highest hardness was found of 0.56 GPa with hemicellulase dosage at 100 IU g^{-1}, and the values of hardness decreased with adding of hemicellulase from 100 IU g^{-1} to 300 IU g^{-1}. However, the hardness was observed to increase again when the hemicallulase was increased up to 400 IU g^{-1}. The change of hardness was significant at the 0.05 level when the amount of hemicellulase increased from 0 to 400 IU g^{-1}. The enzyme treatment could extract hemelluose from wood, and more or larger pores were created during extraction (Zhang *et al*. 2011), moreover, more lignin would be exposed, which could be result in increasing of hardness. However, to avoid the negative effect on the hardness of the wood cell wall, the proper dosage of hemicellulase was nessasary.

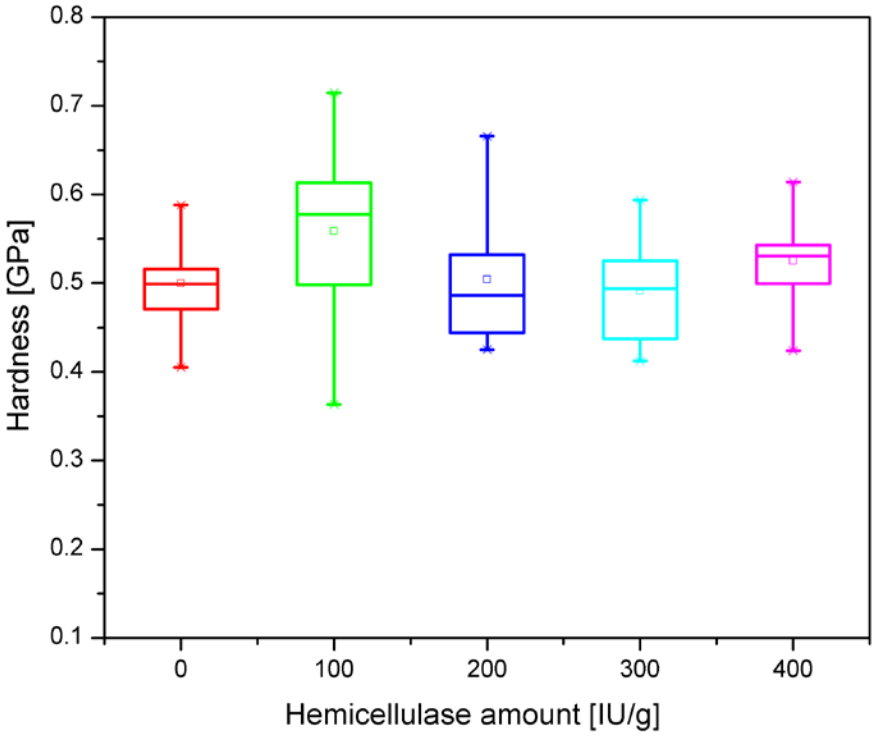

Fig. 2. Hardness of Poplar cell wall treated by hemicellulase

Figures 3 and 4 show the changes in hardness of yellow pine and Mongolia scotch pine cell walls treated with lipases. In general, hardness tended to decrease significantly at the 0.05 level with increasing amounts of lipase, but the change rate was different throughout the testing range. For example, the hardness of the cell wall for yellow pine had little change when the lipase usage increased from 0 to 6 KU g^{-1}, and then it went down from 0.59 GPa to 0.49 GPa as the loading rate increased from 6 KU g^{-1} to 12 KU g^{-1}.

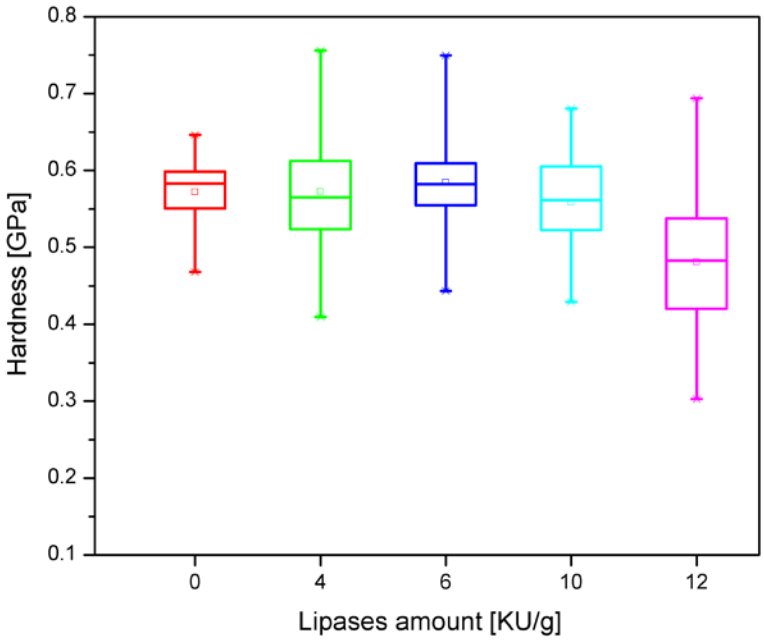

Fig. 3. Hardness of Yellow pine cell wall treated by lipases

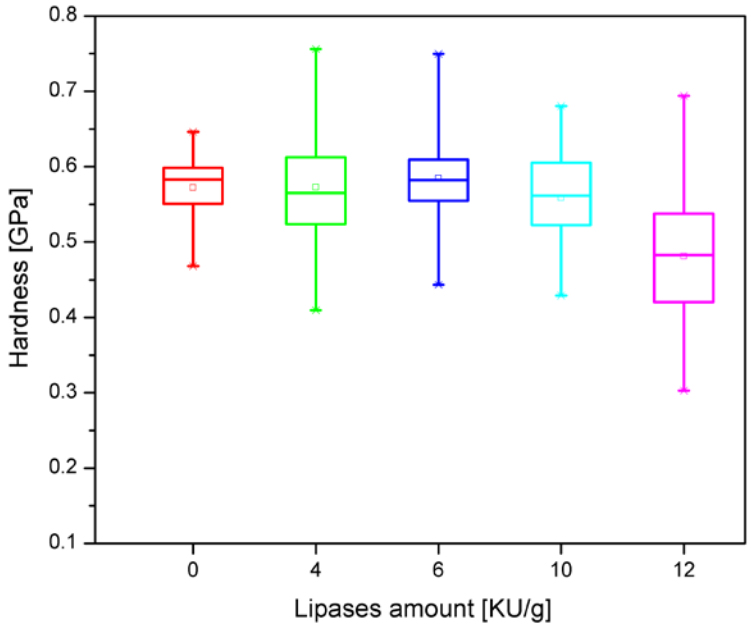

Fig. 4. Hardness of Mongolia Scotch pine cell wall treated by lipases

The hardness values (0.52 GPa - 0.40 GPa) for treated Mongolia scotch pine were all lower than the control (0.53GPa); the addition of lipase resulted in decreasing hardness. It was shown by Jiang *et al*. (2009) that the lipase could effectively remove the hydrophobic lipophilic extractives and silica from the wheat straw. Lignin (contributor of hardness) in wood cell wall could also be removed during lipase treatment, which might be the reason for reducing of hardness. Therefore, it would be preferable to apply less lipase in treating wood.

CONCLUSIONS

The effect of enzyme treatment on the mechanical properties of wood cell walls by nanoindention have been investigated. Elastic modulus and hardness show distinct changes with enzyme treatment.

1. The changes in the components and structure of the cell wall due to hemicellulase treatment and its selective action were the main reasons for the changes in the elastic modulus and hardness of the poplar cell wall.

2. The elastic modulus and hardness of southern yellow pine and Mongolia scotch pine cell walls treated with lipase tends to decrease with increasing the amount of enzymes.

3. The amount of lipase should be controlled to avoid deleterious effects on the mechanical properties of wood cell wall.

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