APPLICATION OF NEUTRON RADIOGRAPHY TO INVESTIGATE CHANGES IN PERMEABILITY IN BACTERIA TREATED PINUS RADIATA TIMBER*

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ABSTRACT

The permeability of softwoods can be enhanced by selective bacterial attack of the pit membranes. In this paper, green flat-sawn *Pinus radiata* sapwood boards were sprinkled for various exposure times with a nutrient solution containing a mixed bacterial population. The timber samples were subsequently dried and the tangential absorption of water was measured using neutron radiography to track the movement of moisture within the wood. There was a significant increase in water absorption after only two days of bacterial exposure, and the quantity of water absorption nearly doubled after a fortnight of bacterial exposure. These measurements showed that dried timber that has had a greater extent of bacterial exposure in the green condition has more void space available to store and conduct water. Scanning electron micrographs showed that bacteria had colonised the pits near the surface of the wood after only two days bacterial exposure. There was also clear evidence of damage to the margo-fibrils and tori of the pit membranes, which was attributed to enzymatic degradation by the bacteria. This study confirms the conclusions drawn by other investigators that bacteria degrade the pits in the green sapwood, so that many of the pathways for moisture flow remain unblocked once the wood is dried.

Keywords: bordered pit, pit aspiration, wood, lumber

INTRODUCTION

The effective processing of timber often hinges on the permeability of wood, which is determined by its anatomical structure. The principal feature of softwood structure is the system of longitudinal tracheids, which occupy up to 95 % of the wood volume and function as vertical conduits for the flow of sap in the living tree. Tracheids are hollow, relatively long and narrow, needle-shaped cells closely packed together so that a cross section through them resembles a honeycomb. Numerous bordered pits interconnect adjacent tracheids, acting as valves to regulate the flow of sap. When a living tree has been injured in some way, these bordered pits aspirate or close irreversibly to prevent the flow of sap between tracheids, so protecting wood in areas adjacent to the damaged regions. It is the phenomenon of pit aspiration which is primarily responsible for a progressive reduction in permeability as green timber is dried.

Bordered pits appear as disks with an impermeable central area, known as a torus, and a web-like region surrounding the torus called the margo (Figure 1a). The cell wall of the pit overarches the central membrane with dome-like structures on both sides of the pit. These domes have apertures, openings linking the lumen to the pit chamber, which are smaller than the diameter of the torus. The sap in the lumen of a tracheid can flow through the dome aperture, the margo, and the following dome aperture, and enter into the lumen of the adjacent tracheid. When greenwood is damaged or dried, the tori move to seal off completely the dome apertures, thus blocking the flow of sap between the tracheids, as shown in Figure 1b. Surface tension from a receding water meniscus is responsible for the displacement of the torus, which adheres to the pit dome by hydrogen bonding. One method that has been used successfully in the past to prevent the aspiration of bordered pits during drying involves the degradation of the fine pit membranes by bacteria when the wood is in the green condition (Bauch *et al.*, 1970, Allen *et al.*, 1993, Greaves, 1969).

Preferential attack of the pit membranes is commonly observed during water storage and sprinkling of logs. Bauch et al. (1970) have shown that liquid absorption of dried *Pinus silvestris* logs, previously stored in a pond for three weeks in the green condition, can be nearly double that of untreated logs. Allen *et al.* (1993) have measured a twofold increase in the liquid absorption of dried, kerfed Douglas-fir logs after one week of sprinkling in the green condition with bacteria-inoculated water. Both Greaves (1969, 1970) and Archer (1985) have used microscopy to study the phenomenon of bacterial migration into logs and subsequent pit degradation. Their investigations have shown that the pit membranes are usually degraded to some extent before bacteria can pass through them, because the spaces between the microfibrils of the margo are not much greater than 200 nm while the bacteria are generally no smaller than 400 nm. These bacteria produce enzymes capable of dissolving the fine membranes of the pits. In some cases, the margo of the pit is degraded by bacterial cellulases, causing the torus to be dislodged, which prevents aspiration in the bordered pits and consequently improves permeability. Degradation of the pectin-rich torus by bacterial pectinases has also been observed by Greaves (1969, 1970) and Archer (1985) with a similar effect on permeability.

In addition to longitudinal tracheids, ray parenchyma cells are also present in softwoods, although they comprise a very small fraction of the total wood volume. They are rectangular-shaped cells which occur in narrow, horizontal bands radiating outwards from the centre of the tree to the bark. Their function is to store nutrients and convey sap radially in the living tree. The longitudinal tracheids and ray parenchyma cells are closely interconnected by means of simple

cross-field pits. Unlike the bordered pits between tracheids, simple cross-field pits cannot aspirate. According to Greaves (1969), the ray parenchyma cells constitute the invading microorganism's main source of nutrients, and therefore they are the first structures to be colonised by the bacteria. Greaves (1969) has stated that the contents of the ray parenchyma cells become quickly depleted, which results in unblocking of intra-ray pits and tracheid-to-ray interconnecting pits, with a subsequent increase in the permeability of the wood.

Torus   Aperture

Mango   Dome

(a)   (b)
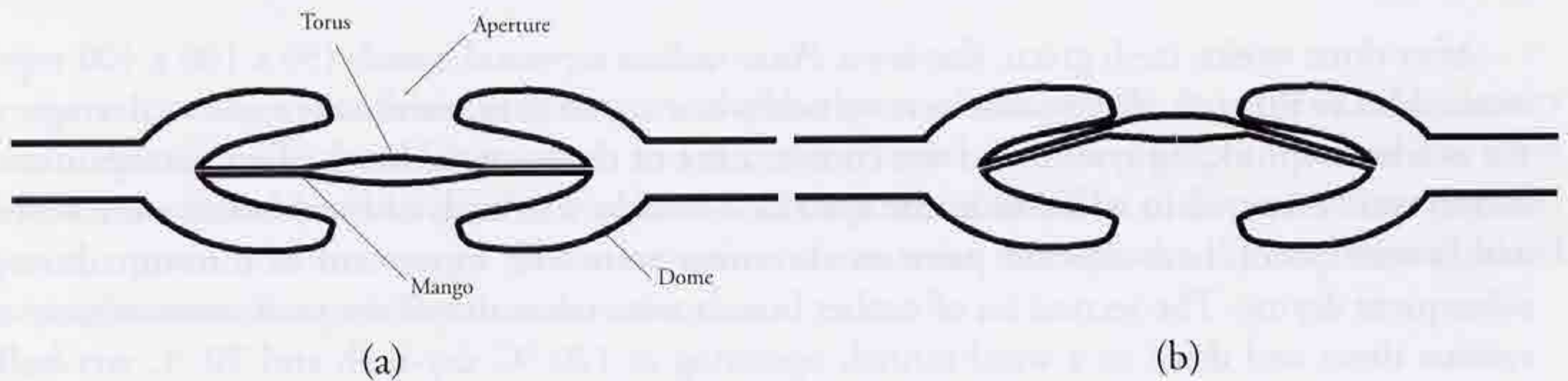
Figure 1. Schematic view of (a) an unaspirated bordered pit and (b) a bordered pit whose torus is being deflected upwards in the process of pit aspiration.

The purpose of this study is to examine the feasibility of a bacterial pre-treatment for *Pinus radiata* timber boards. Bacterial pre-treatments have often been investigated to improve the permeability and enhance preservative treatability of roundwood logs for refractory timber species (Allen *et al.*, 1993, Bauch *et al.*, 1970, Greaves, 1970). However, similar pre-treatments for less refractory *Pinus radiata* timber boards have received little attention. Archer (1987) has shown that it is possible to achieve total tangential penetration of a light organic solvent preservative (copper naphthenate in kerosene) into dried 50 x 100 mm *Pinus radiata* sapwood boards, using the Bethell preservative treatment (-85 kPa for 15 min followed by +70 kPa for 30 min), after a two-week bacterial treatment of the timber in the green condition. However, he did not optimise this process by investigating the effect of different exposure times on the permeability changes in the timber. In this paper, the exposure of *Pinus radiata* sapwood boards to a bacterial solution is described for experiments in which the moisture absorption and ease of drying are determined for various extents of bacterial exposure.

EXPERIMENTAL

The environmental factors that influence bacterial growth and metabolism in wood are aeration, the availability of nutrients, and temperature (Archer, 1985). Banks and Dearling (1973) have shown that the aerobic conditions of an aerated system induce permeability improvements more rapidly than anaerobic conditions, such as those that occur in a stagnant pond. Therefore, in this investigation, a recirculating sprinkling system was used to keep the wood saturated during water storage. Archer (1985) devised the following water-based sprinkling medium capable of sustaining optimal bacterial growth and enzyme production: 17 g/L K_2HPO_4, 4 g/L KH_2PO_4, 5 g/L $(NH_4)_2SO_4$, 0.5 g/L $MgSO_4$, 0.5 g/L NaCl, 0.1 g/L $CaCl_2$, 0.01 g/L $FeSO_4$.

A mixed population of bacteria was cultivated by continuously sprinkling green *Pinus radiata* sapwood timber boards (50 x 100 x 400 mm) in a tank for three weeks with the water-based

nutrient broth inoculated with bacteria from rotting *Pinus radiata* wood taken from a New Zealand sawmill. The advantage of using a mixed natural population of bacteria is that it is most likely to produce a wide range of enzymes capable of degrading the fine membranes of the pits, including the pectin-rich torus and the cellulose of the margo-fibrils. The bacterial broth was re-circulated by a pump and kept at a constant temperature of 24 °C using a 50 W heater. Archer (1985) has found that this temperature is ideal for promoting bacterial growth and enzyme activity.

After three weeks, fresh green, flat-sawn *Pinus radiata* sapwood boards (50 x 100 x 400 mm) were added to the tank. The boards were vertically orientated to maximise the surface coverage of the overhead sprinkling system and the contact time of the bacterial broth. Two non-sprinkled boards were prepared in addition to the sprinkled boards. The ends of every board were sealed with a waterproof, heat-resistant paint to encourage transverse movement of moisture during subsequent drying. The second set of timber boards were taken out of the tank consecutively at various times and dried in a wind-tunnel, operating at 120 °C dry-bulb and 70 °C wet-bulb temperature and a wind speed of 5 m/s, for approximately 20 hours. The non-sprinkled boards were similarly dried. Thin samples 20 mm thick in the lengthwise or longitudinal direction were cut from the dried boards, and these samples were subsequently tested for permeability.

Tangential permeability was determined indirectly by measuring water-absorption rates of the timber samples. The samples were placed in a water bath containing a thin layer of distilled water at the bottom. These samples were orientated so that water was wicked up the height of the timber sample in the tangential direction by capillary action. The rate of advancement of the rising front and the relative amounts of moisture in the wet zone were measured by neutron radiography using the radiography station NEUTRA at the Spallation Neutron Source Department at the Paul Scherrer Institute (PSI). Neutron radiography is very sensitive to moisture changes in wood because neutrons have a high interaction probability with hydrogen. Thus, even slight changes in the moisture content of a wood sample will generate a strong contrast between the initial and final neutron transmission images. NEUTRA generates a neutron beam with a circular cross-section of 40 cm. This beam was focused onto the timber samples in the dry condition and at various times as they subsequently absorbed water. The attenuation of neutrons was measured with a neutron-CCD camera positioned behind the timber samples. The application of this technique is described in more detail by Lehmann *et al.* (2000).

A scanning electron microscope (SEM), Leica F440, was used to examine the dry wood samples for evidence of colonisation and bacterial attack of the pits and ray parenchyma cells. Small specimens for examination were removed with a razor blade from areas near a corner and the centre of a transverse cross-section of each wood sample. These specimens were plated with gold palladium for 5 minutes at 1.2kV and 50mA prior to examination. An accelerating voltage and current of 15 kV and 30-50 pA, respectively, were used when scanning the samples to minimise electron beam damage to the fragile pit structures.

RESULTS

The weight of each wood sample was measured as it was high-temperature dried. This weight was normalised by subtracting the final weight after 20 hours drying and dividing by the change in weight. The loss-in-weight curves so obtained are displayed in Figure 2.

The intensity of a neutron beam after passing through a sample is given by the following equation (Bacon, 1969)

$$I = I_o \exp\left[-\sum_i (\rho\sigma)_i d\right] \tag{1}$$

where I_o is the initial beam intensity, is the thickness of the sample, ρ is the nuclear density (number of nuclei per unit volume), and σ is the effective cross-section per nucleus. The term inside the round brackets is otherwise known as the linear absorption coefficient of component i at the given neutron energy. The attenuation of neutrons through the dry and wetted wood samples were thus given by the expressions:

$$I_{dry} = I_o \exp\left[-(\rho\sigma)_{drywood} d\right] \tag{2}$$

$$I_{wet} = I_o \exp\left[-\left((\rho\sigma)_{drywood} + (\rho\sigma)_{H_2O}\right)d\right] \tag{3}$$

Equation (3) was divided by Equation (2) to give an expression for the density of water, or saturation index S, in the wet wood samples as follows:

$$S = (\rho\sigma)_{H_2O} d = -\ln\left(\frac{I_{wet}}{I_{dry}}\right) \tag{4}$$

The neutron CCD camera produced 16-bit, grey-scale images. Neutron-attenuation profiles taken from these images were normalised to filter out the effect of any background noise by dividing the profiles through by the average neutron attenuation measured away from the timber samples. The normalised attenuation profiles were then converted to saturation profiles using Equation (4).

Figure 3 shows the effect of the extent of bacterial exposure on the rate of the rising wet line (moving upwards in the tangential direction) and the amount of water S in the wet zone as the wood samples progressively absorbed moisture for 0 and 14 days bacterial exposure. Figure 4 shows the total water absorption against wetting time for all samples tested (after 0, 2, 4, 5, 6, 8, 14, and 21 days of bacterial exposure). Each profile was generated by integrating the curves of saturation versus height (tangential direction), such as those given in Figure 3, for each wetting time step. Figure 5 shows saturation profiles in the radial direction at approximately 10-mm above the water level in the bath for 0 and 14 days bacterial exposure.

Latewood pit membranes are more rigid than earlywood pit membranes and the cell walls thicker than those of earlywood; therefore, latewood pits are less likely to aspirate than earlywood pits (Petty, 1972). Consequently, dried latewood is generally found to be more permeable than

dried earlywood. Thus, the SEM micrographs taken were entirely confined to earlywood tissues because they are a greater hindrance to water absorption when dried, and therefore the effect of bacteria on the permeability of those tissues is more pronounced. The extent of bacterial degradation on the fine pit membranes was revealed only in bordered pits that were cleaved in half by the razor during sample preparation. Therefore, only cross-sectional micrographs of the bordered pits are presented here. Figure 6 compares an aspirated bordered-pit in the 0-day sample with a bordered pit colonised by rod-shaped bacteria in the 14-day sample. Figure 7 shows the effect of these bacteria on the structural integrity of the bordered-pit membranes.

DISCUSSION

The sprinkled timber samples dried more slowly than the non-sprinkled timber samples, as shown in Figure 2. Furthermore, the reduction in drying rate appears to be loosely related to the sprinkling time. The wood samples exposed to bacteria for two and three weeks dried more slowly than the samples exposed to bacteria for only a week, which dried more slowly than the timber that had no bacterial pre-treatment. Archer (1985) has found that kerfed Douglas-fir logs also dry more slowly for increasing extents of bacterial exposure of up to three weeks. He states that the decrease in the drying rate is most likely explained by the accumulation of bacterial biomass or the products of bacterial metabolism, such as polysaccharide slimes, which obstruct the pathways for moisture flow. However, he has not explained why the biomass does not similarly obstruct the flow of moisture into the dry wood, and we likewise can provide no satisfactory explanation for this phenomenon. Nevertheless, drying and wetting are not identical processes, and the primary pathways for moisture flow into the dry wood on wetting could differ from the primary pathways for moisture flow out of the greenwood during drying. Biomass debris may block one type of pathway but not the other, which could explain the apparent permeability difference when drying and wetting.
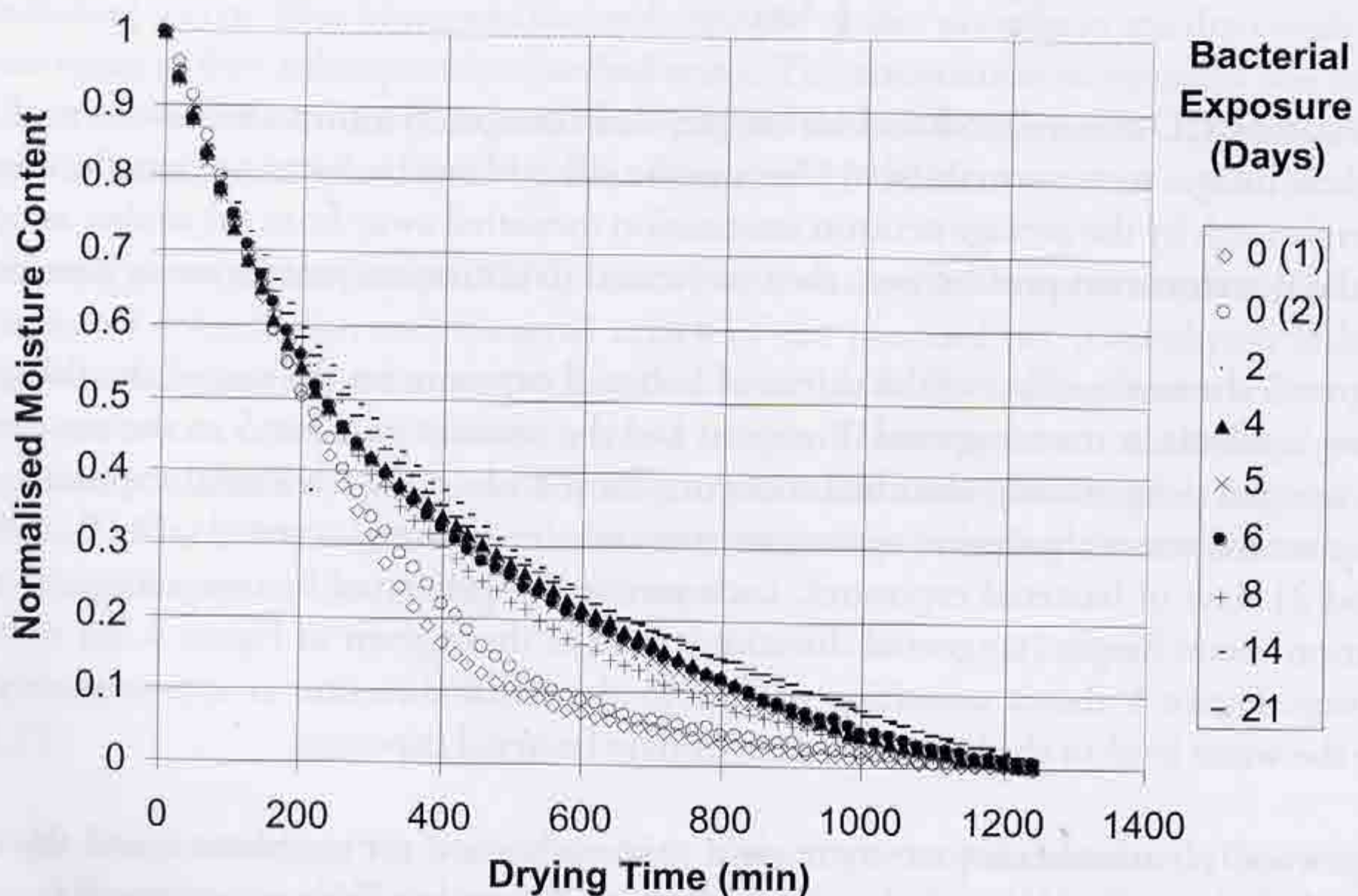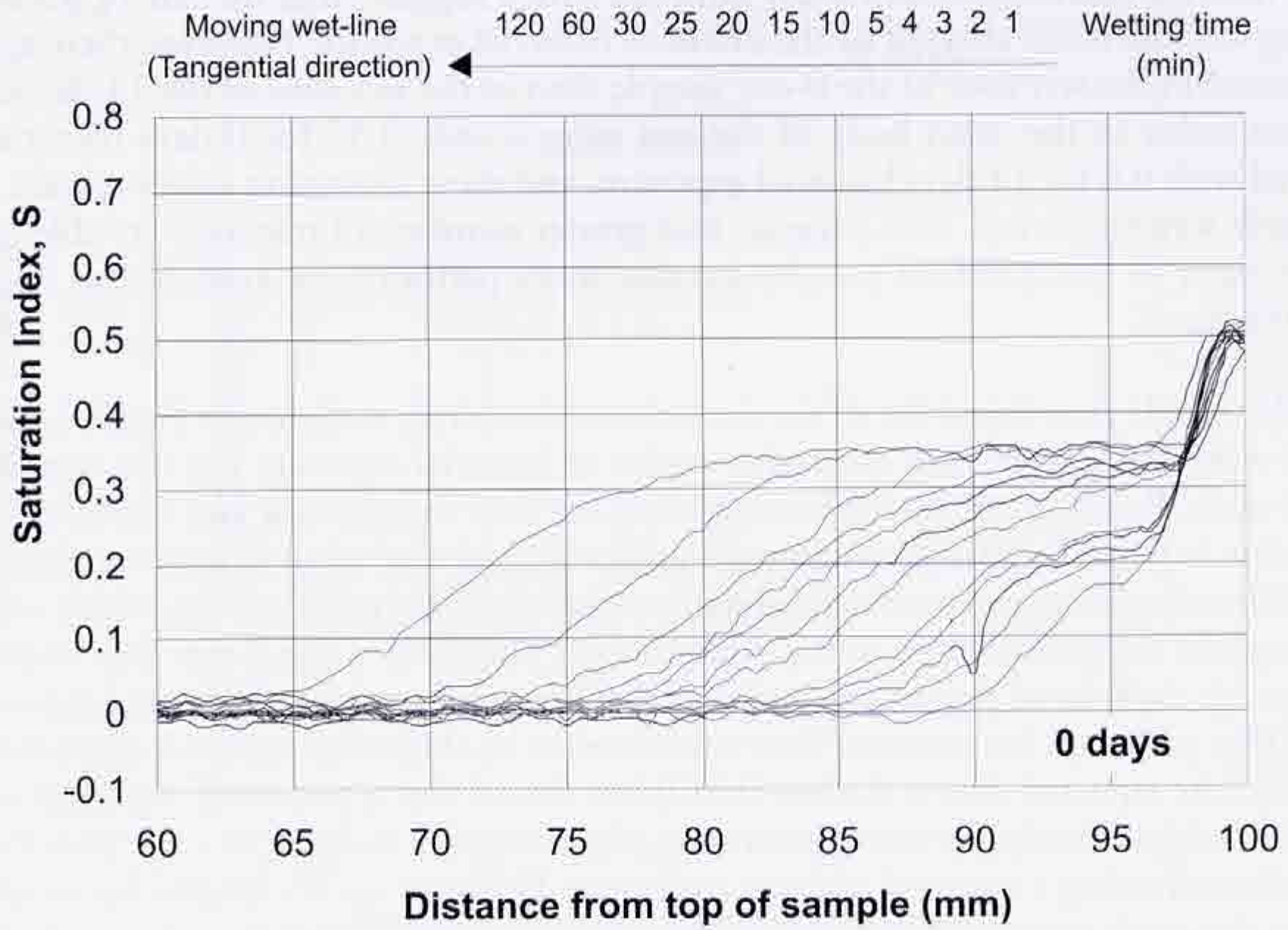

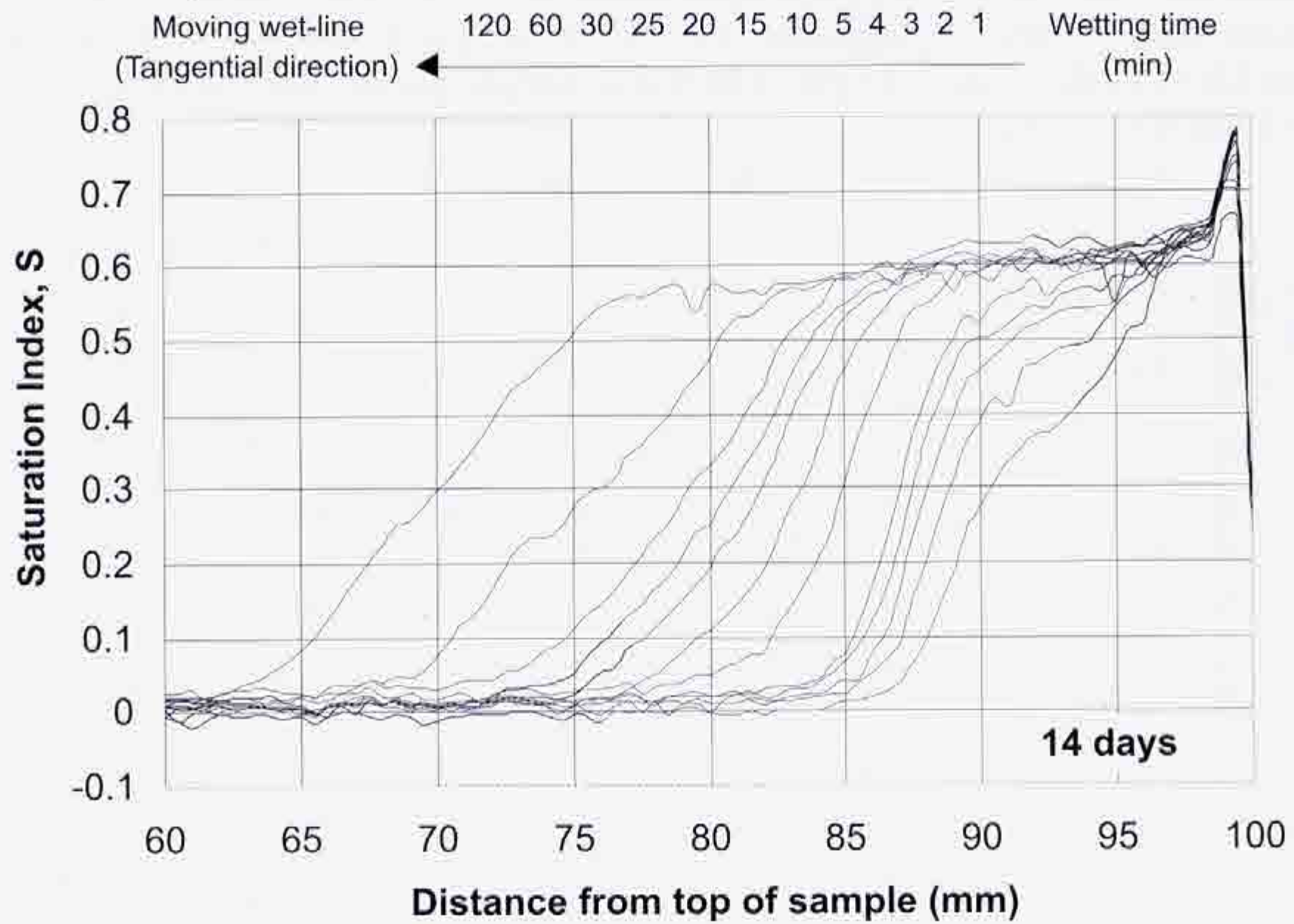Figure 2. Normalised drying curves for various extents of bacteria exposure.

(a)

(b)

Figure 3. Comparison between the tangential wetting curves of the non-sprinkled sample (a) and the sample sprinkled for a fortnight (b).

Figure 3 shows that the waterfront has moved upward approximately 40 mm after 120 minutes wetting time for both the 0 and 14-day samples, which suggests that the rate of advancement of the rising wet line is not affected by the extent of bacterial exposure. However, there appears to be less moisture in the wet zone of the 0-day sample than in the wet zone of the 14-day sample. The saturation index in the main body of the wet zone is only 0.35 for 0 days bacterial exposure compared with 0.6 for 14 days bacterial exposure, and these saturation values remain stable over a two-hour wetting period. This suggests that greater numbers of tracheids are able to store and conduct water in the sprinkled samples because more pathways are available for moisture flow within the wood.

Similar trends were found for all the timber samples tested, as shown in Figure 4, although the trend in water absorption with increasing extent of bacterial exposure was not very clear within the first week. Bauch *et al.* (1970) have pointed out that the amount and direction of bacterial degradation in wood is difficult to control. Furthermore, the inherent natural variability in wood, even within a single log, can cause considerable variations in bacterial activity, which will influence any changes in the permeability of the dried timber. There was a significant improvement in the water-storage capacity of the timber samples from one to two weeks of bacterial exposure, which suggests that pathways for moisture flow continued to be cleared by bacterial degradation during this period. As reported above, Archer (1987) has found that a two-week bacterial treatment is required to achieve total tangential penetration of preservative in dried 50 x 100 mm *Pinus radiata* sapwood boards using a standard pressure treatment. However, such a lengthy bacterial treatment precludes the application of this technique for enhancing preservative treatability of timber on an industrial scale, although there could be some scope for improvement by isolating a bacterial population that is more effective at increasing wood permeability.

The radial plots of saturation index show that more moisture has been absorbed by the 14-day sample than the 0-day sample at a plane approximately 10 mm above the height of the water level in the water bath (Figure 5). Moreover, the 14-day sample reaches full water-storage capacity more quickly than the 0-day sample. The 0-day sample potentially has further water-storage capacity; however, it appears that
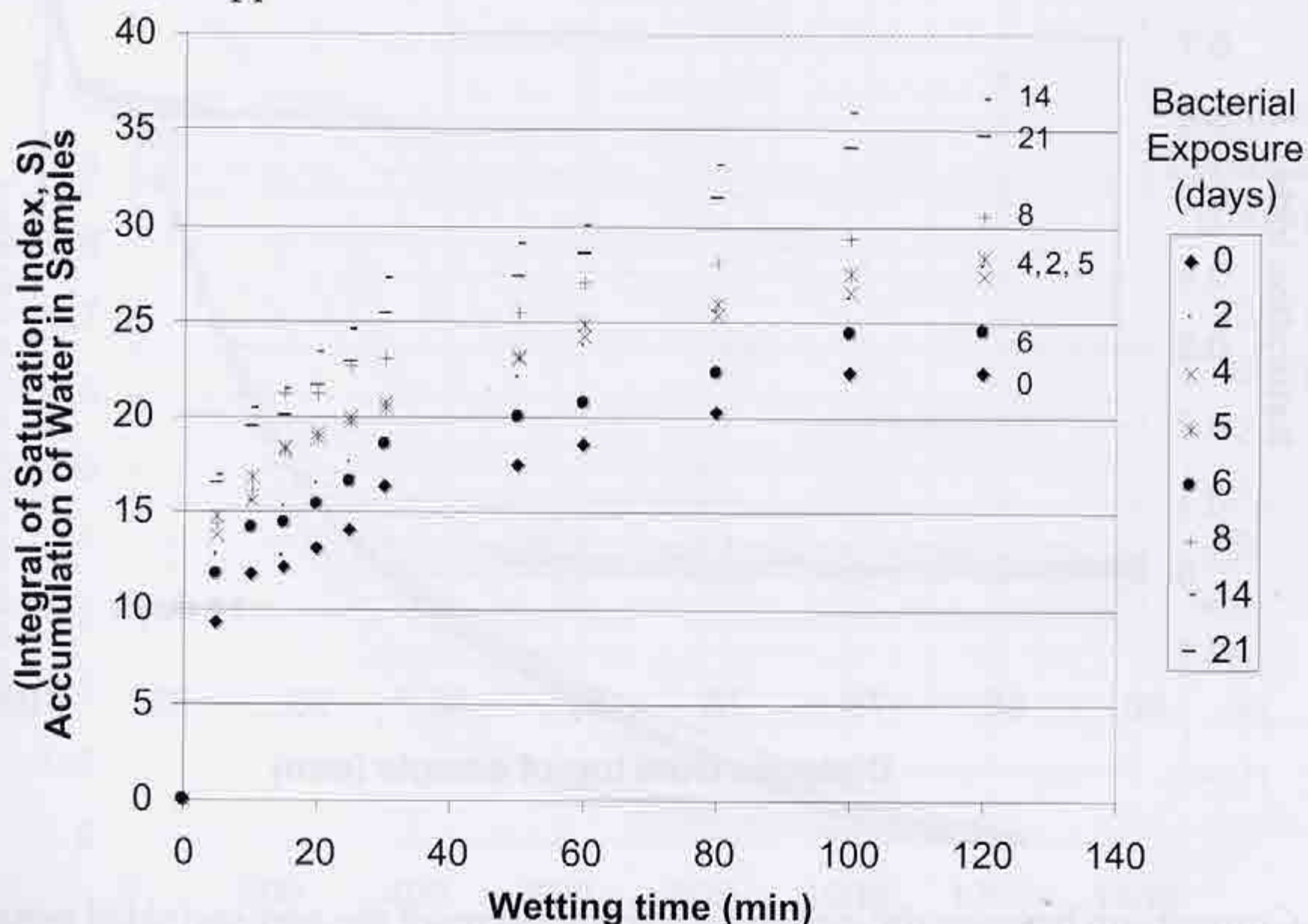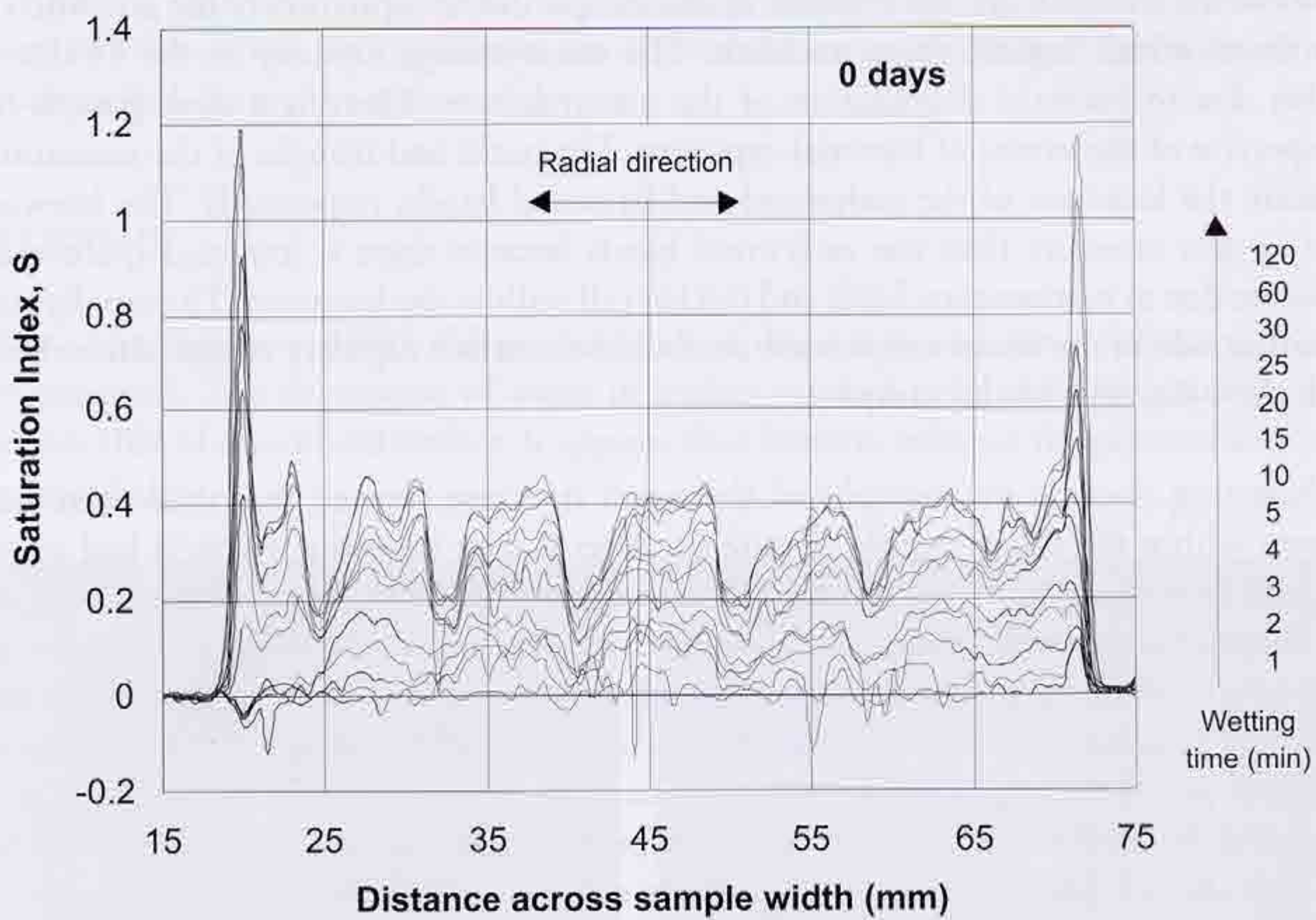

Figure 4. The absorption of water in dried timber samples with different extents of bacterial exposure in the green condition.
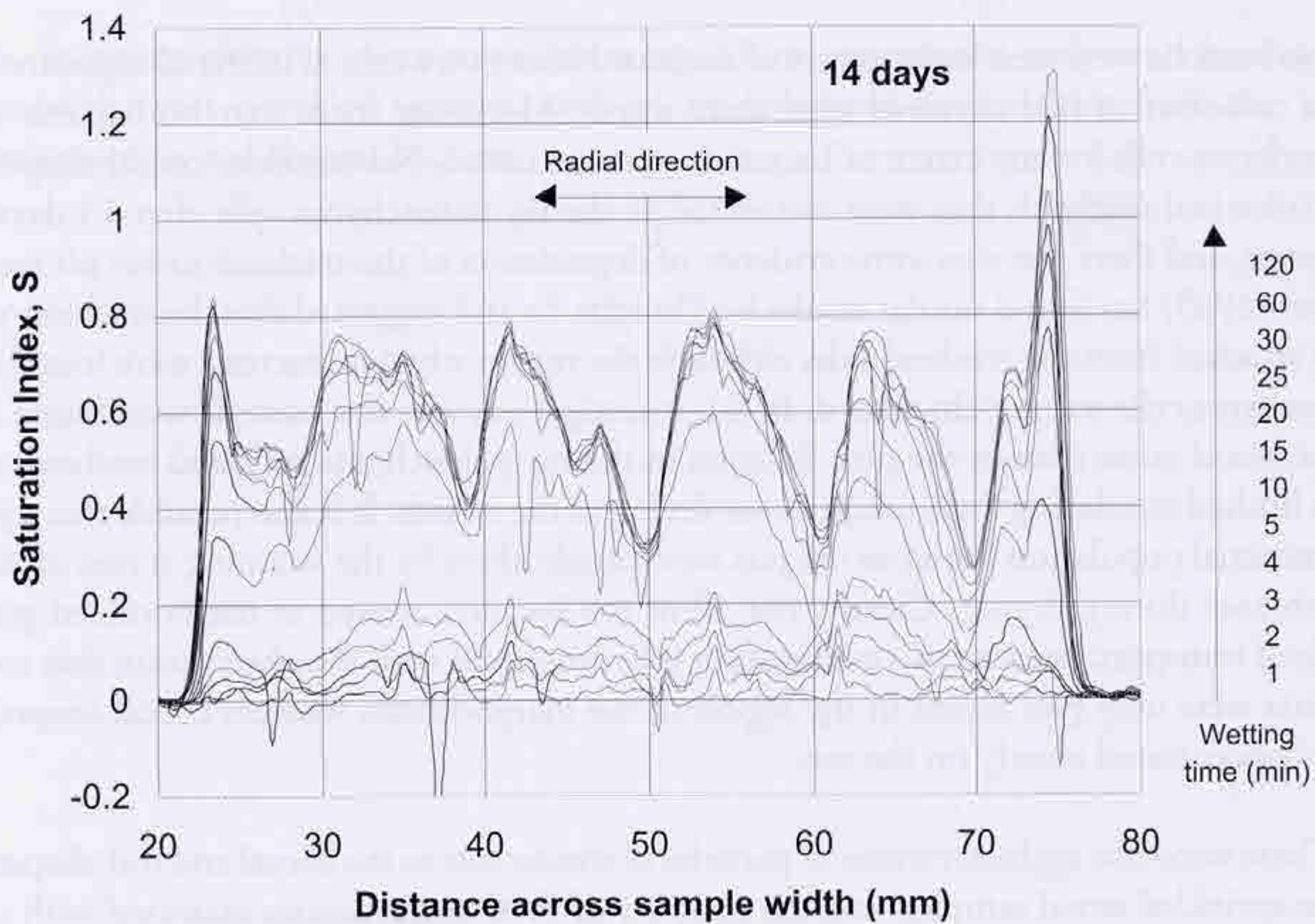
(a)

(b)

Figure 5. Comparison between the radial wetting curves of the non-sprinkled sample (a) and the sample sprinkled for a fortnight (b).

many of the tracheids are not available in this sample due to aspiration of the pits when the wood was dried, which isolates these tracheids. The water-storage capacity in the 14-day sample is higher due to bacterial degradation of the pit structures. There is a clear growth-ring effect irrespective of the extent of bacterial exposure. The peaks and troughs of the saturation profiles indicate the locations of the earlywood and latewood bands, respectively. The latewood bands take up less moisture than the earlywood bands because there is less void space available for moisture due to narrower tracheids and thicker cell walls in the latewood. The rapidly rising peaks on either side of the wood samples are attributed to surface capillary effects, since they occur in both the 0-day and 14-day samples.

Scanning electron micrographs of the wood structure showed that there were no bacteria present within the 0-day sample (Figure 6). After 2 days exposure, bacteria had colonised the tracheid-to-tracheid bordered pits near the exposed surface of the timber board. Both coccal and rod-shaped bacteria were evident in the exposed samples, as shown in Figure 7. The coccal bacteria were concentrated mostly on the tori, although they also colonised the margo-fibrils and domes, while the rod-shaped bacteria appeared to be confined to the margo-fibrils and domes of the bordered pits. This suggests that the coccal bacteria are capable of secreting both cellulase for dissolving the cellulose of the margo-fibrils and pectinase for dissolving the pectin-rich tori, whereas the rod-shaped bacteria can only produce cellulase. Both types of bacteria deteriorated predominantly the margo-fibrils of the pit membranes, although there was some evidence of degradation of the tori, as shown in Figure 7. Given sufficient time, the bordered pit membranes are eventually completely destroyed regardless of which type of bacterial attack prevails, which leaves open pathways for fluid flow between adjacent tracheids.

No bacteria were seen in the centre of the board after two weeks of bacterial exposure, although some colonisation had occurred after three weeks. Moreover, there were no bacteria in the ray parenchyma cells for any extent of bacterial exposure tested. Nevertheless, coccal-shaped bacteria had colonised tracheids that were connected to the ray parenchyma cells after 14 days bacterial exposure, and there was also some evidence of degradation of the tracheid-to-ray pit membranes. Archer (1985) has found similar results for Douglas-fir and suggested that the tracheid-to-ray pits were attacked from the tracheid side, although the reason why few bacteria were found in the ray parenchyma cells was not elucidated. In this investigation, very few bacteria were found anywhere in the wood other than in the pits. Bacteria in the ray parenchyma cells and tracheids may have been flushed out during high-temperature drying of the boards. It is also possible that a portion of the bacterial population found in the pits were caught there by the straining action of the fine pit membranes during drying. Clearly, not all of the bacteria arrived at the bordered pits by this mode of transport, because this explanation is incongruent with the observation that rod-shaped bacteria were only ever found in the region of the margo-fibrils, whereas coccal-shaped bacteria were concentrated mostly on the tori.

There were also agglomerations of particles of similar size to the coccal and rod-shaped bacteria in the sprinkled wood samples, and the quantity of these agglomerates increased with the extent of bacterial exposure. The agglomerated particles can be seen most abundantly on the torus of the bordered pit shown in Figure 6. It is doubtful that these agglomerates were waste products from bacterial degradation, since many were discovered in areas where the coccal and rod-shaped bacteria had never been found and where there was no evidence of bacterial attack. Although the bacteria may have been flushed from these areas during drying, this does not explain why the waste products

were not similarly removed. We propose that these agglomerates are crystalline formations from the nutrient solution, which having diffused into the wood during sprinkling of the timber samples, had been deposited during the course of drying.

CONCLUSIONS

The ability of bacteria to enhance the permeability of dried sapwood *Pinus radiata* timber has been demonstrated. The absorption of water in timber exposed to bacteria for a fortnight was nearly double that of unexposed timber. It appears that bacteria enhance the permeability of the timber primarily by

(a)   (b)
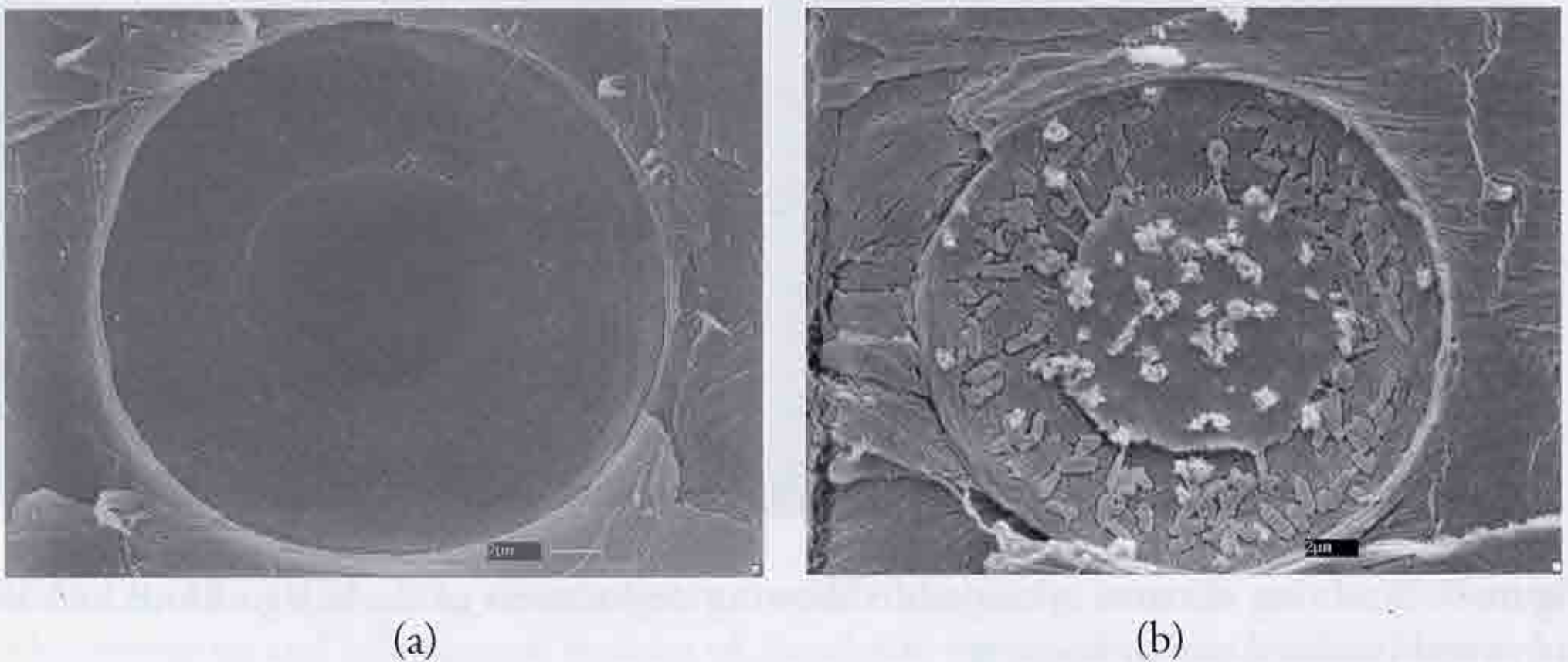
Figure 6. Scanning electron micrographs showing (a) an unaspirated earlywood bordered pit from one of the non-sprinkled samples, and (b) an earlywood bordered pit from the 14-day sample colonised by rod-shaped bacteria.
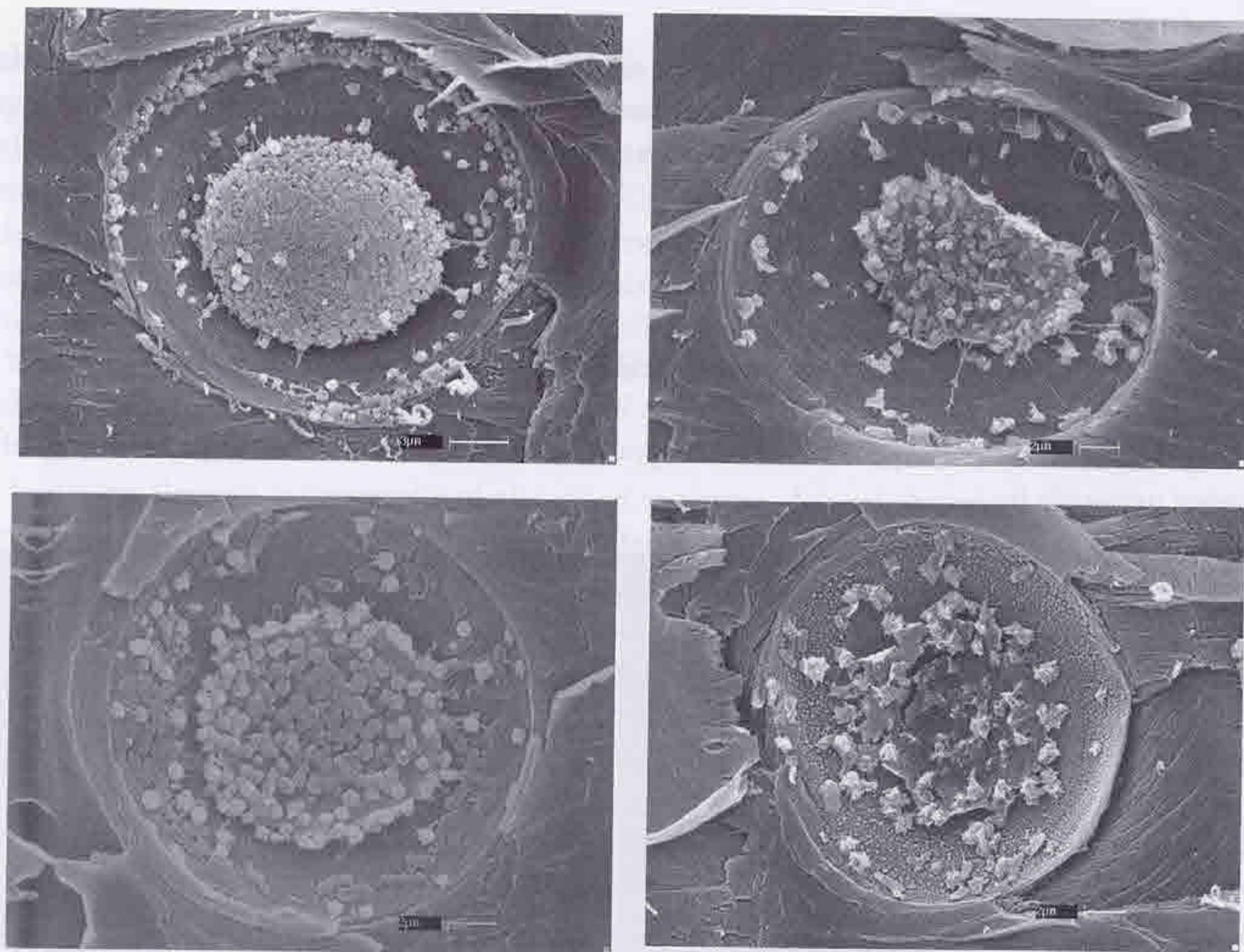
Figure 7. Scanning electron micrographs showing degradation of the margo-fibrils and tori of earlywood bordered pits by bacteria.

degrading the fine membranes of the bordered pits to prevent them from aspirating. This opens more pathways for moisture flow in the dry timber so that tracheids that would have previously been isolated due to the aspiration of bordered pits are thereafter able to conduct moisture, which effectively increases the storage capacity of the wood. Any improvement in permeability could be very beneficial for improved preservative impregnation in timber, although the bacterial population tested in this investigation was not sufficiently active for this technique to be commercially viable. An additional drawback of this technique is that green timber treated with bacteria appears to dry more slowly than untreated timber.

ACKNOWLEDGEMENTS

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