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GASDISCHARGE ETCHING APPLIED ON SECTIONS OF BEECH AND ASH

B. J. SPIT

(*Technical Physics Department T.N.O.—T.H., Delft*)

AND

S. M. JUTTE

(*Forest Products Research Institute T.N.O., Delft*)

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INTRODUCTION

In the last few years several investigators have drawn attention to the possibility of etching in a low voltage gasdischarge. In general etching is a very widely spread technique applied to specimens studied both in optical and electron microscopy. As far as this is done chemically, swelling often occurs, especially with organic materials with a polymer nature.

When gasdischarge etching is applied this swelling is avoided (ISINGS and SPIT, 1964) and it seems to be very suitable for high polymers (SPIT, 1963). Two types of discharges have been used viz. a high frequency discharge by JAKOPIĆ (1961) and GRASENICK (1961a) and a direct voltage discharge by SPIT (1961).

The materials investigated were all synthetic polymers. Latex particles and filler materials in rubber were studied by JAKOPIĆ (1961), GRASENICK (1961b); ANDERSON and HOLLAND (1960) studied nylon fibres etched in an argon discharge, ANDERSON (1963) did the same for polyethylene, SPIT (1961, 1963) has given some results obtained with different gases on regenerated cellulose sections and on sections of high impact polystyrene. DLUGOSZ (1962) studied two components in a high polymer mixture. Other applications, such as specimen thinning and "Hülle" replication are not discussed here.

METHOD AND MATERIALS

The materials used were thin sections of authentic samples from *Fagus sylvatica* L. (Beech) and *Fraxinus excelsior* L. (Ash). Sections of normal wood as well as of tension wood from both species were etched. The method of etching has been described previously (SPIT, 1961), and therefore only a short summary of the main points of this technique is given here.

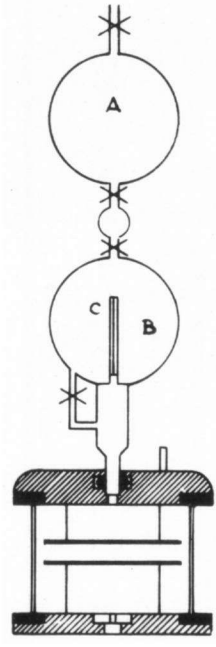

Fig. 1. Scheme of the gasdischarge chamber with glass bulbs. A is filled with pure oxygen. B is the expansion bulb. C capillary for regulation of the pressure in the discharge chamber.

A scheme of the discharge chamber is given in Fig. 1. Ultra thin sections of methacrylate embedded material (JUTTE and SPIT, 1963), thickness approx. 800 Å, were cut on a Reichert microtome using a diamond knive of 51° and they were picked up on a formvar coated grid. A carbon film was evaporated over the sections in such a way that they are sandwiched between the carbon film and the formvar substrate. The next step is dissolving the formvar film and the methacrylate embedding in chloroform. After this the sections fasten themselves on the carbon film on the grid. The grids were laid upside down on the anode of the gasdischarge apparatus, which means the grid on top.

The discharge chamber was filled with pure oxygen and the pressure was installed in such a way that a stable discharge is obtained at about 300 V between the aluminium electrodes with a current of 3 mA. An etching time of 3 minutes is sufficient. At last the specimens are shadowed with platinum or tungstenoxide.

RESULTS

Figures 2 and 3 show transverse sections of normal fibres in Beech and Ash after etching. It is striking that the layered structure of the cell wall becomes particularly clear. In electron micrographs of

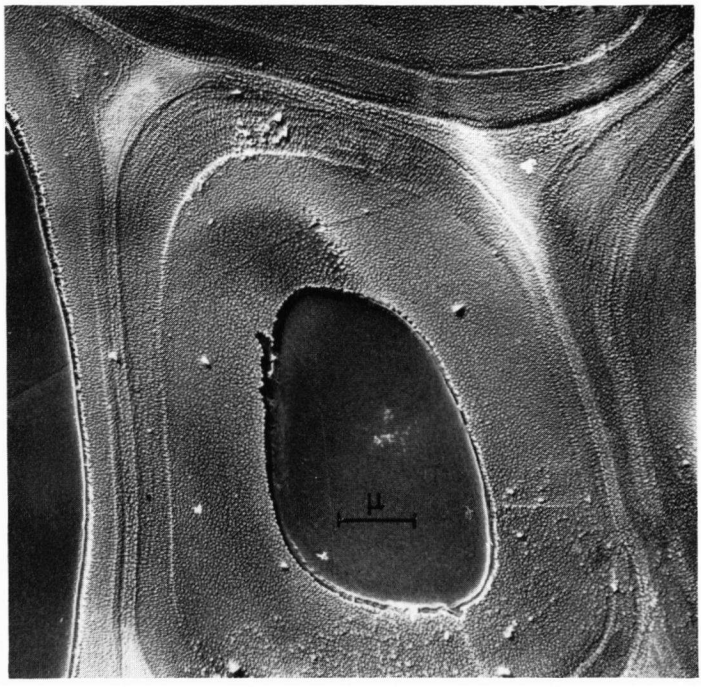

Fig. 2. Transverse etched section of a normal wood fibre in *Fagus sylvatica* L. The S 1 layer shows concentric circles and a radial striation.

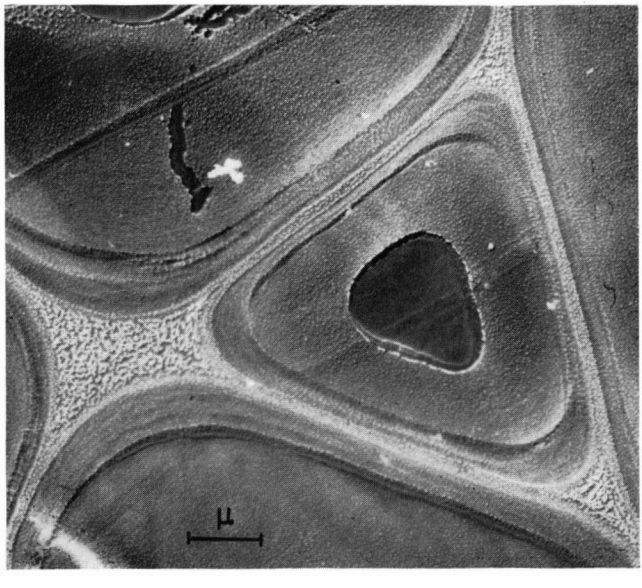

Fig. 3. Transverse etched section of normal wood fibres in *Fraxinus excelsior* L. The intercellular space is bordered by the compound middle lamella. On the lumen side a very thin S 3 layer is visible.

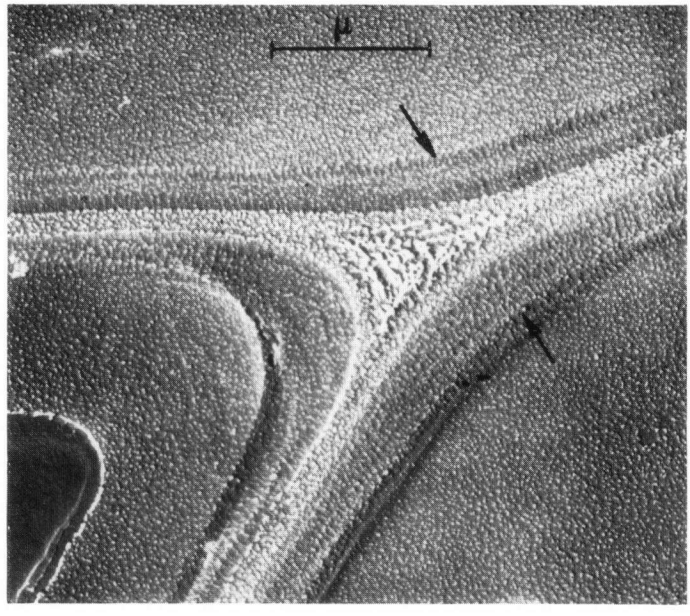

Fig. 4. Intercellular space with adjacent cells in Ash. Enlargement of a part of Fig. 3.

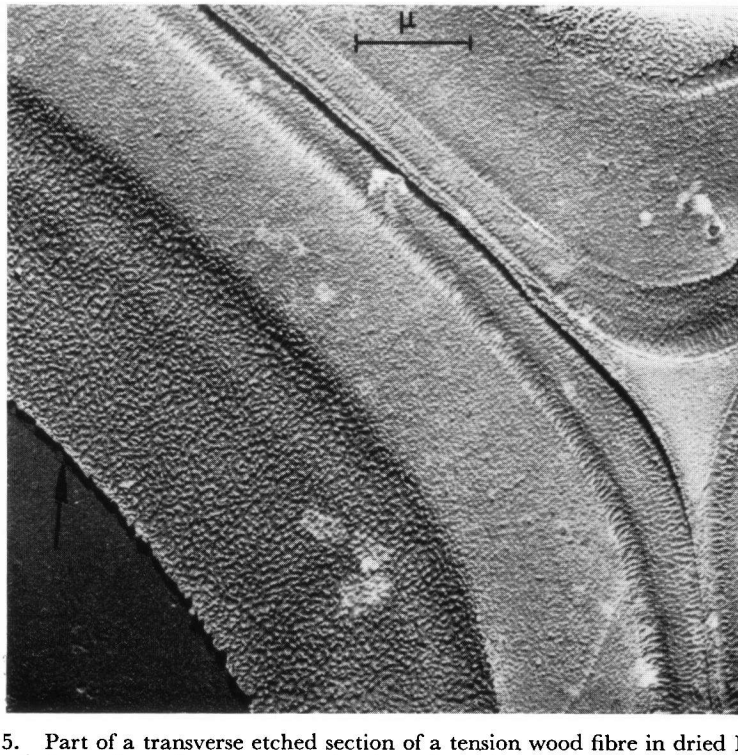

Fig. 5. Part of a transverse etched section of a tension wood fibre in dried Beech. The gelatinous layer has a coarser structure than the normal S 2 layer. On the lumen side is an extremely thin layer partly visible (arrow).

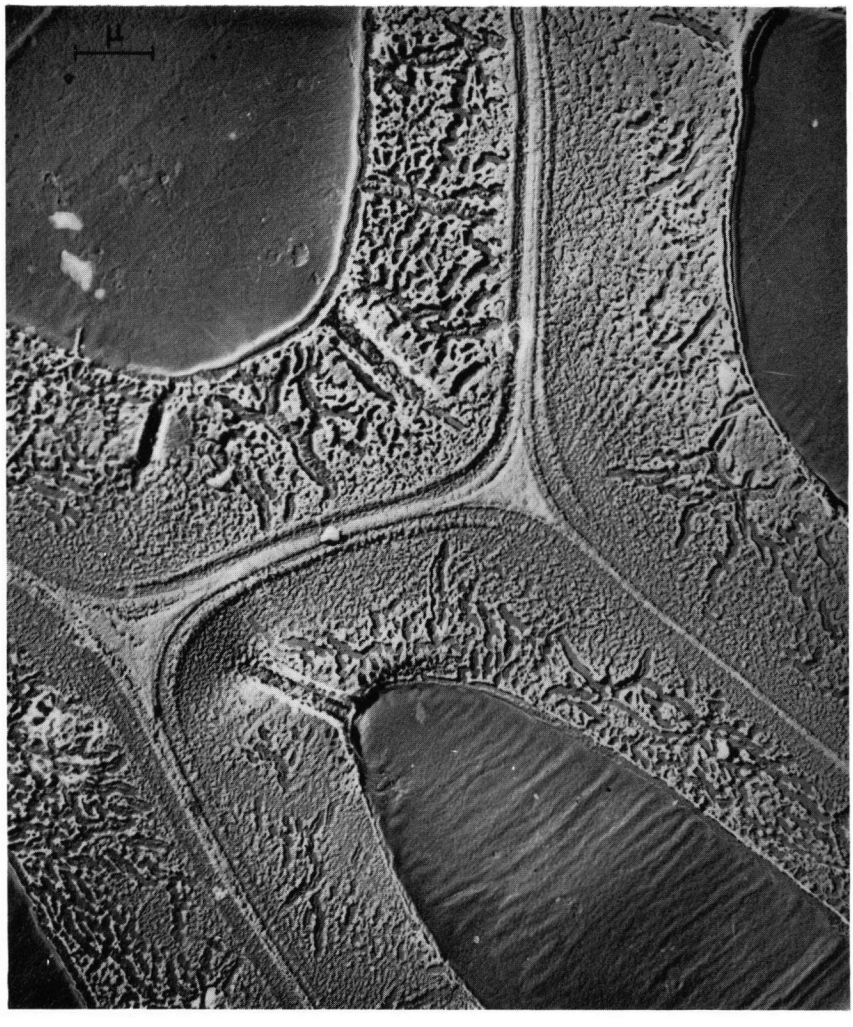

Fig. 6. Transverse etched section of tension wood fibres in fresh Ash. There is no gelatinous layer. Radial running fissures in the S 2 and the lumen bordered by a thin layer.

unetched sections the compound middle lamella (middle lamella and attached primary walls) of the cell cannot clearly be distinguished. In the pictures of etched sections the compound middle lamella, however, can be seen as a separated, defined layer with a thickness of 0.1–0.2 μ. In a few of our pictures a thickness of 0.06 μ was measured. In these cases two or three distinct points could be observed. In the thicker ones 6 to 8 points are visible.

Further we observe a differentiation in the material filling up the intercellular space between adjacent cells. The middle lamellae are split up and run as separated layers together with the primary walls along the cells, enclosing a triangle with a coarser structure (Fig. 3). The etching pattern in the intercellular region is in sharp contrast with that of the compound middle lamellae.

Looking at the remaining cell wall at first a rather broad layer (S 1) is observed which is usually thicker in the cell edges. The etching pattern in the layer shows a point structure, whereby the points are arranged in radially running lines (Fig. 4, arrows) as well as in concentric circles (Fig. 2). In unetched sections the same thick band becomes mostly visible as a darker or lighter zone, which is not differentiated. In consequence of the spiral arrangement of the microfibrils in these layers the sections become thicker on one side and thinner on the other side of the cell by cutting (PREUSSER *et al.*, 1961). Both in etched and unetched sections a shadow layer can be distinguished in this zone. But in the etched sections the S 1 is sharply defined between the adjacent walls. Moreover, in comparison with the etching pattern in the next layer (S 2) the etching pattern in S 1 denotes a different orientation of the microfibrils with respect to the cell axis. After etching, the S 2 layer shows a point shaped structure over its entire breadth. These individual points are very regular in dimensions as well as in their distribution over the wall. A third important observation is the bead-shaped enclosure of the lumen (Figs. 2 and 3), that corresponds with the S 3 layer. It is noted that in our Beech material seldom a warty layer was observed. The observations described above are equal for the normal wood fibres of Ash and Beech. With respect to the tension wood fibres of these two wood species it is known that only the gelatinous layer in the fibres of Beech can clearly be distinguished.

In a recent study on these tension wood fibres SPIT and JUTTE (1964) found that the microfibrils in Ash are much thinner than those in Beech and that there is a different behaviour of the gelatinous layer with respect to a treatment with sodium hypochlorite. In our etched transverse sections of tension wood fibres of Beech and Ash differences were also visible.

For the etching of tension wood fibres of Beech the dried material was chosen. In sections of fresh material mostly the gelatinous layer is swollen and shows the honeycomb structure or "Wabenstruktur" (SACHSSE, 1962 and 1963). By the way we remark that in our opinion this structure has something to do with an embedding artefact as consequence of a special microfibrillar habitus. In sections of dried

Beech the gelatinous layer remains intact and so the etching pattern can better be studied. In this etched material the middle lamella, primary wall and S 1 show the same picture as in etched sections of normal wood fibres. The thick S 2 is divided into two layers; adjacent to this S 1 a normal S 2 with a closely packed point-like structure is found and the gelatinous layer with an etching pattern that shows also a point structure which is much coarser than with the normal S 2 (Fig. 5). Finally on the lumenside the cell is limited by an extremely thin layer, partly visible in this micrograph.

Fresh tension wood fibres of Ash have much coarser S-layers in comparison with normal Ash fibres. This phenomenon is characterized by the point-like structure in the S 2 of normal fibres and by a rope like structure in the S 2 of tension wood fibres (Fig. 6). This means that there must be another microfibrillar structure in these S 2 layers. Like in the unetched sections branched fissures run in these layers, with their main direction radially. Further in many micrographs the S 1 layers seem to be thicker in the normal fibres than in the tension wood fibres. Close examination showed, however, that on one side of the cell the S 1 layer had been compressed during cutting the sections. On the lumen side the fibres from both normal and tension wood were bordered by a very thin layer, which is in sharp contrast with the S 2 layer.

In this species no extra gelatinous layer has been formed.

Discussion

Gasdischarge etching has proved to be a valuable aid to get a differentiation in materials which consist of chemical components of polymers.

Up till now the mechanism of the interaction of the ions with the material is unknown. As was pointed out previously and confirmed by Anderson (1963) ion bombardment produces a true etching by removing material preferentially from the surface, revealing structural units, which must be in relation to the submicroscopic structure of the material.

In previous experiments whereby cellulose was etched by ion bombardment, it appeared that the microfibrils split up into grainlike particles and that these particles sometimes together with adjacent parallel running microfibrils, form a lateral striation. With these two points in mind we will try to give an explanation of the etching patterns found in the cell walls. In a transverse section of wood the microfibrils stick into the surface under different angles with the axial direction. These angles correspond with the spiral structure in the layers of the cell wall. The microfibrils of S 2 with the steep spiral structure are almost perpendicularly sticking into the surface. After etching a point structure is found. The regular distribution of the points represents the fact that there is no lateral ordening of the microfibrils, as was the case in the regenerated cellulose fibre Super-

cordura (SPIT, 1961). The points are said to represent the crystalline parts in the surface. The etching pattern of the S 1 resembles very clearly the picture of an etched longitudinal fragment of regenerated cellulose, Fibre G (SPIT 1963, micrograph 6). The microfibrils in this layer run in a transverse direction and there must be a considerable amount of lateral ordening between the microfibrils in adjacent layers as the points form a radially running striation.

Usually S 3 is very thin, a thickness of a few microfibrils, and is also running in a transverse direction. After etching this layer a bead-like structure is found caused by the particles in which the microfibrils have been split up.

In the case of the compound middle lamella a variable quantity of points lying across this layer is distinguished. About 6–8 points could be observed. This means that the two primary walls have a thickness of about 4 microfibrils each. The triangle in the intercellular space is separated from the compound middle lamella. The latter belongs clearly to the cell wall. This triangle is the space which is filled up in a later stadium of the cell development. This ion etching technique makes it possible to differentiate between the compound middle lamella and the material in the intercellular space.

The coarse point structure in the etched gelatinous layer of Beech tension wood fibres confirmed the X-ray findings of an axial orientation of the microfibrils in this layer. The packing density is lower than in the adjacent S 2. It is striking that the etching pattern of the gelatinous layer in Ash tension wood fibres gives another picture, however, from blending experiments (SPIT and JUTTE, 1964) it is known that the microfibrils in Ash are much thinner than those in Beech. The points in the S 2 of normal fibres of Ash are also smaller than those in Beech. Contrary to this the etching pattern in tension wood fibres gives more islands or aggregates of points, which means that the microfibrils are more connected to each other. This is in agreement with macerated tension wood material. In the case of Beech we found bundles of hydrolised-like short microfibrils, tightly packed in bundles, this was not the case with Ash, however.

Finally the conclusion can be drawn that this etching technique gives a useful possibility to distinguish the different cell wall layers and the microfibrillar orientation in these layers. In a recent study on the cell plate formation FREY–WYSSLING *et al.* (1964) have shown, that in the stadium that the cell plate contacts the longitudinal walls of the mother cell, the width of the vesicles is about 0.07 μ. In later stadia, until the covering of the plasmalemma, there is an increase in width till 0.1 μ. In view of this and of our observations mentioned before, it may be possible that in some cases not the compound middle lamella, but the middle lamella is observed. If this is true, we must conclude that by this etching technique the middle lamella is split up into three points, which resemble the outer sides and the dark centre in the vesicles seen by FREY–WYSSLING *et al.* (1964).

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