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THE STRUCTURE OF A CELLULOSE SHEET IN THE NASCENT FIBRIL PRODUCED BY ACETOBACTER XYLINUM

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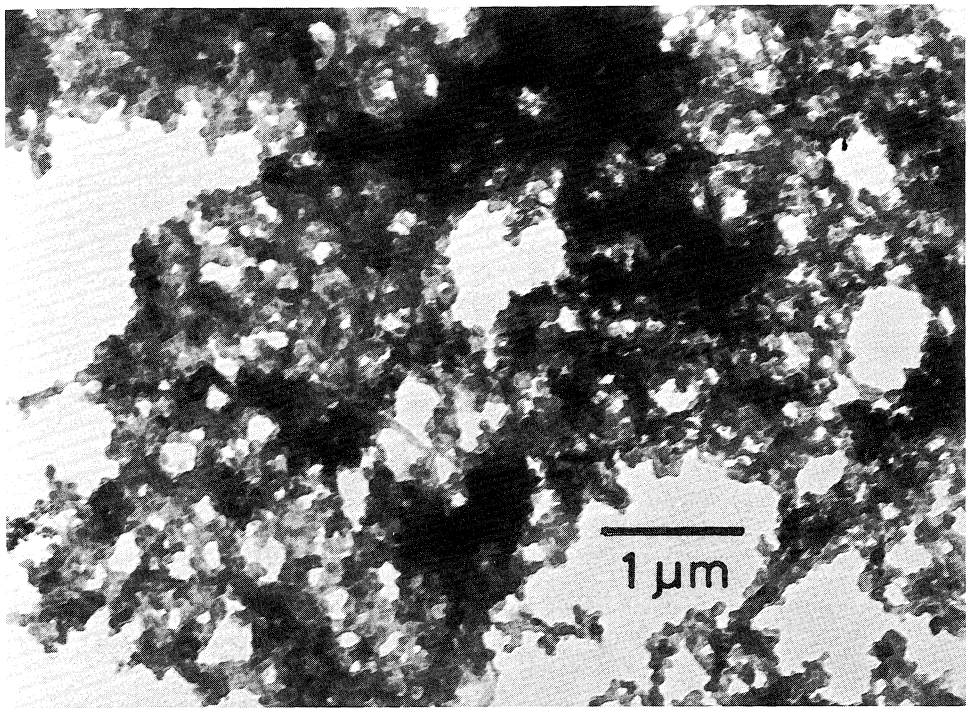
It became clear that the nascent fibril produced by Acetobacter xylinum crystallizes into cellulose II, when treated with alkali of a very low concentration ranging from 1×10^{-3} to 1.0×10^{-2} wt%. This fact shows that the bonding strength of inter-molecules of a cellulose sheet, as well as the bonding strength between the cellulose sheets in the nascent fibril, is very weak.

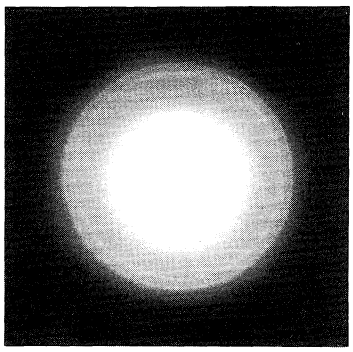
It has became clear that Acetobacter xylinum extrudes the cellulose chains from the cell in the form of monomolecular layers corresponding to the $(1\bar{1}0)$ plane of cellulose I.[1,2] In the nascent fibril composed of boundles of the cellulose sheets, they inevitably remain bound each other, and form cellulose I crystallites, although they may also carry traces of the medium.[2] This position makes it essential to determine the structure and properties of a nascent cellulose sheet, as a part of the mechanism of formation of a bacterial cellulose fibril.

We report on the interesting results obtained from an examination of the resistance to alkali of the nascent fibril from the incubated glucose medium, as a part of the elucidation of the structure and properties of a nascent cellulose sheet.

The methods of the incubation of Acetobacter xylinum (IFO 13693) and the alkali-treatment of the nascent fibril are as follows. An amount of 4 ml of 3 wt% glucose medium was added to 20 ml of cellulose-free cell suspension[1] and the mixture (pH 6.8) was then incubated at 28°C for a given time. To the incubated medium was added dropwise an equal volume of aqueous NaOH, two times as dense as the given concentration. After gently stirring a homogeneous



a

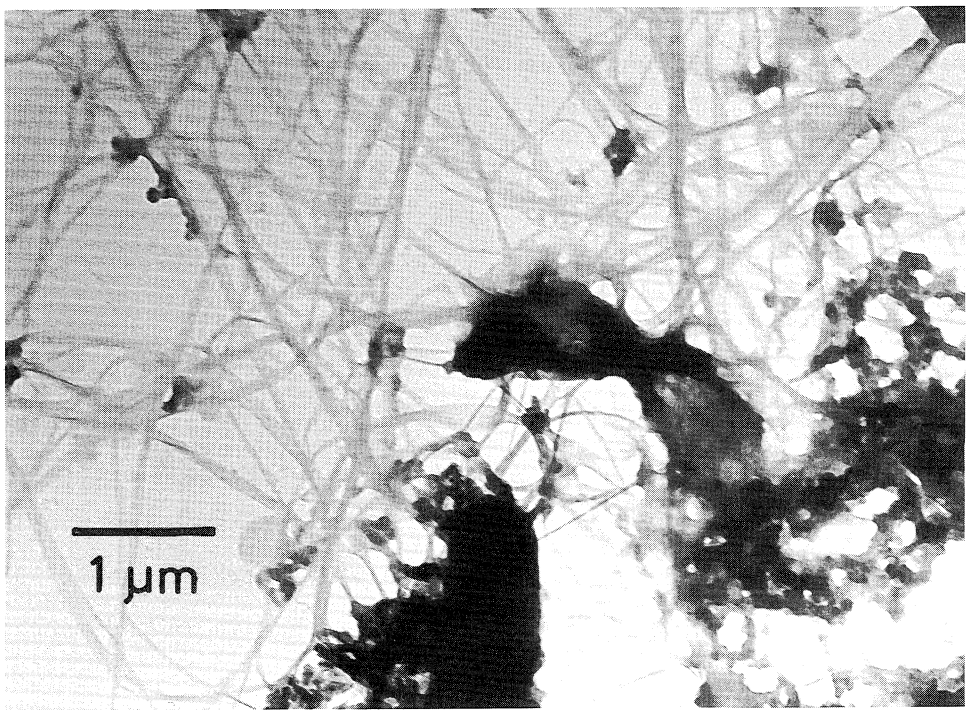


b

Fig. 1. Morphology and electron-diffraction patterns of 0.06 wt% alkali-treated cellulose, which was obtained after a 5-min glucose incubation period at 28 °C.

The diffraction patterns correspond to the (110) and (020) planes of cellulose II respectively.

alkaline solution, we kept it at 20 °C for 1 h and then added aqueous acetic acid solution to neutralize it. The solution was dialyzed through Cellulose Dialyzer Tubing (Nakarai Chemical Ltd, through M. W. cut-off: 8000) until it became sodium acetate free. A drop of the cellulose suspension was placed on a sheet mesh covered with a collodion membrane. The specimens were stained with addition of a drop of 0.5 wt% aqueous sodium tungstophosphate solution for 15 min before drying except for the specimens for the electron diffraction. The NaOH concentration of less than 0.1 wt% was determined from the relation between the weight percentage of the alkali and the pH of the solution to which NaOH was added to the glucose medium. For electron microscopy, all preparations



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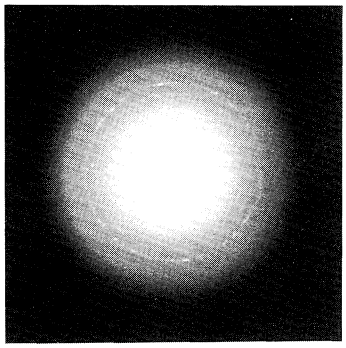

b

Fig. 2. Morphology and electron-diffraction patterns of 4.6 wt% alkali-treated cellulose, which was obtained after a 30-min glucose incubation period at 28 °C.

Figure b is the diffraction patterns of fibrils. They correspond to the $(1\bar{1}0)$,(110),and (020) planes of cellulose I respectively.

were examined with a JEOL JEM-100U electron microscope operating at 80 kV.

Judging from the staining behavior of cellulose fibril produced by Acetobacter xylinum, toward sodium tungstophosphate, the structure of nascent fibril seems to remain intact for about 30 min, in the case of the 28°C incubation.[3] Accordingly, the resistance to alkali of the cellulose obtained from the incubation of within 30 min was examined.

The never-air-dried cellulose obtained from the 5-min incubation period, when treated with 4×10^{-4} wt% alkali, maintained the fibrous shape. The electron diffraction patterns of the specimen indicated those of cellulose I. However, when the cellulose obtained from the 5-min incubation period was treated

with 1×10^{-3} wt% alkali, about 50% of fibrils underwent morphological change and the remaining did not change. In the case of alkali-treatment of more than 1.0×10^{-2} wt%, as shown in Fig. 1a, almost all fibrils changed in the morphology. The electron diffraction patterns of the sample indicated those of cellulose II as shown in Fig. 1b. The fibril seems to maintain the nascent state until about 30 min after the production as described above. However, although the never-air-dried cellulose obtained from the 30-min incubation period was treated with 4.6 wt% alkali, as in Fig. 2a, about 70 - 80% of cellulose maintained the fibrous shape and only about 20 - 30% of it changed in the morphology. The electron diffraction patterns of the 4.6 wt% alkali-treated fibrils still indicated those of cellulose I as shown in Fig. 2b. Even when the sample from the 30-min incubation period was treated with 9.6 wt% alkali, about 30% of it maintained the fibrous shape, and about 70% of it changed in the morphology. The electron diffraction patterns of the fibrils indicated those of cellulose I and the cellulose changed in the morphology showed the diffraction patterns of cellulose II. The above results were found to be reproducible.

It became clear that the never-air-dried cellulose from the 5-min incubation period crystallizes into cellulose II, when treated with alkali of a very low concentration ranging from 1×10^{-3} to 1.0×10^{-2} wt%. Furthermore, it became clear that, although the fibril seems to maintain the nascent state for until about 30 min after production, the resistance to alkali of the fibril after the 30-min incubation period would show a extreme increase. The resistance to alkali of a fibril just after production indicates that the bonding strength of inter-molecules of a nascent cellulose sheet, as well as the bonding strength between the nascent cellulose sheets, is very weak.

References

1) A. Kai, Makromol. Chem. Rapid Commun., 5, 307(1984)

2) A. Kai, Makromol. Chem. Rapid Commun., 5, 653(1984)

3) A. Kai, Bull. Chem. Soc. Jpn., 57, 836(1984)

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