Investigation on Enzymic Hydrolysis of Lignified Cellulosic Materials

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Abstract. Investigations on enzymic hydrolysis of cotton cellulose and spruce groundwood pulp with the multicomponent enzyme Onozuka SS have shown that lignin causes an almost total inhibition of the enzyme action. Breaking of lignified covers of cell walls and fibrillar bundles by grinding increases the accessibility toward the enzyme very effectively. Depolymerization of lignin is less important for improving the accessibility. The compact fibrillar structure of both cotton and wood cellulose also have an inhibitory effect on the penetration of the enzyme into the fiber. Beating loosens the compactness of the fibrillar structure and increases the accessibility. Only about 10% of the cellulose, probably highly crystalline, requires more drastic treatment e.g. ball-milling. Last units of polysaccharides linked to lignin in the lignin carbohydrate complex are resistant toward enzyme hydrolysis and require chemical cleavage. Total enzymic solubilization of lignified polysaccharides can therefore be attained only after a combined mechanical and chemical pretreatment.

Introduction

The effect of isolated cellulolytic enzymes on lignified cellulose, e.g. in wood, is rather low. However, after vigorously grinding the wood or cellulose fibers both the rate and yield of enzymic hydrolysis substantially increase [Pew, Weyna 1962; Katz, Reese 1968; Azhar, Polčin, Rapson 1971; Polčin, Bezúch 1973; Caulfield, Moore 1974]. Some increase in digestibility after exposing the wood or cellulose to strong swelling agents had been also reported [Tarkow, Feist 1969; Vamos, Rose, Gajzago 1972, 1973; Dietrichs, Hennecke 1974; Garves, Dietrichs 1975; Millet, Baker, Satter 1975]. These observations led to the conclusion that the limited action of cellulolytic enzymes is caused by a low accessibility of the rather compact structure of lignocellulosic materials.

The aim of this work was to investigate to which extent the crystalline structure of cellulose and/or lignification of the cell walls are responsible for decreasing the susceptibility to enzymic hydrolysis. For this purpose, the enzymic process was studied first with cotton fibers as a model of pure cellulose, and then with spruce wood and spruce groundwood pulp as a model of lignified cellulose.
Experimental

Material: Bleached cotton linters (α-cellulose 98.7 %, DP 1000); spruce wood (Picea abies) and a commercial stone-ground-wood pulp subsequently extracted with aqueous EDTA (removal of metallic cations), benzene + ethanol (2 : 1), acetone + water (1 : 1) and dried at room temperature (lignin 27.3 %, hexosan hemicelluloses 14 %, pentosans 9 %).

Milling equipment: [a] Wiley mill (Brabender); [b] Valley hollander beater (Tappi St. T 200-os-70); [c] rotary porcelain ball-mill [Brownell, West 1961], volume 1.2 dm$^3$, 1 kg balls of 25 mm diameter, 70 r.p.m., 25 g of a sample.

Enzymic hydrolysis: 1.5 g of Onozuka SS enzyme (Kanematsu-Gosho, Ltd, Tokyo) was added to 100 ml of a 3 % polysaccharide suspension in an acetate buffer (pH 4.5). The hydrolysis was performed at 45°C under continuous shaking. After a chosen time the undissolved residue was removed by filtration, washed with water, and dried to constant weight. Onozuka SS is a multicomponent “polyosolytic” enzyme, able to hydrolyze all wood polysaccharides, as was proved when a ball-milled spruce holocellulose was completely degraded by Onozuka (Table 3).

Analytical methods: Sugars were determined according to Somogyi [1952], lignin by the Tappi Standard Method T-13m-54. Determination of accessibility was carried out by the iodine sorption method according to Schwertassek [1953]. X-ray crystallinity was determined with a Mikrometa diffractometer and calculated according to Hermans [Hermans 1949; Polčin et al. 1966].

Electron microscopy: The electron microscope TESLA BS 242 A was used for obtaining electron micrographs of surfaces of fibers under study (atomic replicas) and Jeol VSM-V for scanning electron micrographs.

Preparation of holocellulose: The sodium chlorite method at 35 °C/40 h was applied [Klauditz 1957].

Preparation of cellulose: By extraction of holocellulose with 5 % NaOH at 25 °C/24 h.

Pretreatment with NaOH solutions: Groundwood pulp or cellulose was dispersed in a NaOH solution of the corresponding concentration at 25 °C. Liquid-to-solid ratio for groundwood pulp was 8 : 1 and for cotton 50 : 1. After 20 min the sample was removed by filtration, washed with ethanol and water, and used for analysis and enzymic digestion. The portion of the sample used for determination of x-ray crystallinity was dehydrated by subsequent washing with methanol, benzene and diethyl-ether and drying in vacuo over P$_2$O$_5$.

Pretreatment with liquid ammonia: The sample was submerged in liquid ammonia in a Dewar flask and after 1 h drained, washed and treated similarly as after the NaOH mercerization.