Enzymic Isolation of Lignin from Wood and Pulps

J. Polčin and B. Bezúch
Pulp and Paper Research Institute, Bratislava, Czechoslovakia

Abstract. After enzymic hydrolysis of polysaccharides in milled wood or pulp the unhydrolysed lignin residue becomes soluble in certain polar solvents. Extensive disintegration of the wood is necessary to obtain a sufficient accessibility toward the enzyme. For milling a porcelain rotary ball mill is recommended. A relationship has been found between the milling energy, which is proportional to the size of the mill, the milling time, and the yield of ball-milled wood lignin (BMWL). Enzymatically isolated lignin (EIL) requires a critical milling time which is shorter than that for obtaining maximum yields of BMWL. A procedure for isolation of the total lignin content from wood and pulps in the form of three fractions has been developed, giving a water soluble, low molecular carbohydrate-lignin complex by water extraction of ball-milled wood, a BMWL-carbohydrate complex by subsequent extraction with dioxane, and a EIL-carbohydrate complex by dioxane extraction of the residue after enzymic hydrolysis.

Introduction

The isolation of an unchanged or at least not too much changed lignin from wood is still a very serious problem of lignin chemistry. By a simple extraction with organic solvents only about 1% of the original lignin can be obtained [Brauns 1952, 1960]. After a vigorous grinding the yield may be increased up to about 40% [Björkman 1956, 1957; Brownell 1961; Lai, Sarkanen 1971]. However, it is very difficult to say to which extent this lignin is representative of the remaining portion.

The liberation of lignin from plant tissue by acid-catalysed hydrolysis of the polysaccharidic portion yields lignin with a changed chemical structure, even when mild reaction conditions are used, e.g. acidolysis in ethanol or dioxane. From this point of view it might be promising to replace the acid-catalysed hydrolysis by an enzymatical one. Direct application of fungi producing cellulolytic enzymes [Schubert 1965], however, does not give reproducible results because of a simultaneous, uncontrolled production of enzymes which also attack lignin. Hydrolysis of wood polysaccharides with isolated cellulolytic enzymes is, therefore, preferrable. To

* The authors wish to thank the Slovak Academy of Sciences for interest in and financial support of this study.
achieve satisfactory penetration of the enzyme, the wood must be premilled in a vibratory ball mill [Pew 1957; Pew, Weyna 1962]. Premilling in a rotary ball mill has also been found satisfactory [Azhar, Polčín, Rapson 1971]. Among the common commercial cellulolytic enzymes, Onozuka SS was selected as the most effective in decomposing wood. Experiments with western hemlock have shown that for the isolation of the total content of lignin based on enzymic digestion of wood polysaccharides only half of the milling time was necessary than for the isolation of the highest possible yield (about 40 %) of ball-milled wood lignin [Pew, Weyna 1962]. The same was observed with spruce and some hardwood species [Polčín, Bezúch 1973, 1974]. Wayman and Obiaga [1974] investigated the molecular weights of hemlock and spruce lignins enzymatically isolated according to Azhar et al. [1971] and found that they contained more high-molecular weight material than did lignin premilled for a longer time. The same observation was also made by Chang et al. [1975] with MWL and lignins isolated from spruce wood and sweetgum sapwood premilled in a vibratory mill and digested with Onozuka SS enzyme. It is interesting that molecular weights of lignins isolated from a wood premilled in a rotary ball mill [Azhar et al. 1971; Wayman, Obiaga 1974] were higher than those milled in a vibratory mill [Chang et al. 1975]. The latter authors also reported that changing the type of the vibratory mill affected the yields, molecular weights and to some extent also chemical composition of MWL and enzymatically isolated lignins.

The change of milling condition e.g. the weight of milling balls, the ratio of sample to milling balls etc. in the same type of a vibratory mill has long been known to affect the yields of MWL significantly [Košikova et al. 1968].

Summarizing earlier observations, it is evident that premilling of the wood affects the yields and properties of both MWL and enzymatically isolated lignin. Therefore, the premilling process has to be performed under defined, optimum conditions. This paper reports an investigation on optimum milling conditions for porcelain rotary ball mills as well as other operation steps important for the enzymatic isolation of the total lignin content from wood and pulps.

Experimental

Material: Spruce wood (Picea abies) in the form of a commercial groundwood pulp; birch wood (Betula verrucosa) and poplar wood (Populus monilifera) in the form of sawdust (60—80 mesh) prepared in a Wiley mill. Metallic impurities were removed by extraction with 0.5 % aqueous EDTA (20 h at 40 °C). After drying at room temperature organic extractives were removed by a subsequent extraction with ethanol-benzene (2 : 1) in a Soxhlet apparatus (16 h) and acetone-water (1 : 1) at 25 °C for 8 hrs. After washing with distilled water the sample was dried at room temperature.

Milling: For milling of the extracted wood, porcelain rotary ball mills of different size were used with the jar volume (liter) : weight of balls (kg) : sample weight (g) ratio 1 : 1 : 20. Ball diameter was 25—30 mm, jar revolution 60 per minute.