# Enzymatic Saccharification of Lignocellulosic Materials

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Reflecting the shortages of petroleum with rising prices and of protein resources in relation to world population growth, an increasing attention has been paid to the potential uses of lignocellulosic materials for chemicals, food, and fuel in the future. Lignocellulosic materials are the most important component of the renewable biomass and constitute a large proportion of agricultural, industrial and municipal wastes. Vast amounts are available throughout the world.

Enzymatic saccharification of woody waste has received considerable interest with the recent development in cellulase technology. The recovered sugars can be converted to ethanol and/or single cell protein by fermentation using yeasts. For large scale commercial application of this process, there are, however, quite many problems to be solved. For example, some pretreatments are necessary to enhance the susceptibility of the substrates to enzymatic attack. The cost for this process is one of the major economic factors. Efficient and economical techniques for pretreatment must be developed. A considerable amount of works has been carried out in the past, aiming at improving the susceptibility of lignocellulosic materials. The purpose of this paper is to briefly review the effect of various pretreatments on enzymatic saccharifiction.

## Components and ultrastructure of wood cell wall

The structural components of wood consist of three natural polymers, cellulose, hemicellulose, and lignin. Cellulose is a major component (40–55%) and linear polymer of β-(1–4)-linked D-glucopyranose residues. Main hardwood hemicellulose is a partly acetylated xylan consisted of β-(1–)-linked D-xylopyranose residues. Every tenth xylose residue, on an average, is substituted at C–2 by 4-O-methyl-α-D-glucopyranosyluronic acid residue. In addition, it has recently been suggested that galacturonic acid and rhamnose residues are integral parts of xylan[12]. Glucomannan, another hemicellulose, is found only in small amount. In softwood, partly acetylated galactoglucomannan amounts to 15–20%, while xylan is in the range of 5–10%. Galactoglucomannan consists of β-(1–4)-linked D-glucopyranose and D-mannopyranose residues in a ratio of 1:3. Some of the hexose units carry a terminal residue of α-D-galactopyranose attached to C-6. Softwood xylan has the framework similar to that of hardwood xylan. Unlike the latter, however, softwood xylan contains α-L-arabinopyranose residues, directly linked to C-3 of the xylose. One arabinose occurs per eight to nine xylose residues. Softwood xylan is more acidic than hardwood xylan, having five to six xylose residues per uronic acid side-chain. A large proportion of the uronic acid side-chains is located on two contiguous D-xylose resideus in the backbone of the softwood xylan[11].

Lignins are complex three-dimensional polymers formed from ciniferyl alcohol units in the case of softwood and both coniferyl and syringyl units in the case of hardwood.

Cellulose is present in the form of microfibrils. Most of the lignin encrusts the cellulose microfibrils and hemicelluloses like cement in ferro-concrete. The remaining part is located in middle lamella, where it serves the purpose of cementing the fiber cells together.

## Pretreatments for enhancing the susceptibility

Adsorption of enzymes on the substrates is a prerequisite step for their hydrolysis. The intimate association of the polysaccharides with lignin and crystalline nature of cellulose prevent the direct physical contact between cellulolytic enzymes and their substrates[1]. As a result, efforts have been exerted to develop the effective pretreatment to enhance the accessibility of the polysaccharides in wood to the enzyme. Various pretreatments have been reported[9], which are classified into chemical, physical and biological treatments. Combinations of several treatments have been also tried. The chemicals used are NaOH, $NH_4OH$, $Na_2S$, $NO_2$, $SO_2$, acetic acid, peracetic acid, various mineral acids and so on. The physical treatments include ball milling, gamma irradiation, high pressure steaming etc. The biological treatment aims to delignify by the use of wood-rotting fungi, especially, white-rot fungi which are able to decompose lignin[3].

### 1) *Effect of delignification*

Holocelluloses delignified to various extents were prepared from two hardwoods and two softwoods by acid chlorite procedure, and incubated with a commercial cellulase preparation derived from *Trichoderma viride*. This enzyme preparation contains cellulase, xylanase, mannanase, various glycosidase etc. The extent of hydrolysis was evaluated by determining the loss in weight and by measuring the total amount of the reducing sugar formed.[14] The result is shown in Fig. 1. In the hardwoods, the hydrolysis extent increases rapidly after the first 60% removal of lignin and reaches 70–80% as the delignification approaches completion, whereas in the softwoods it rapidly increases after 50% delignification and reaches more than 90%. These facts indicate that more than 80% delignification is necessary to hydrolyse the entire polysaccharide portion of woods with cellulolytic enzymes.

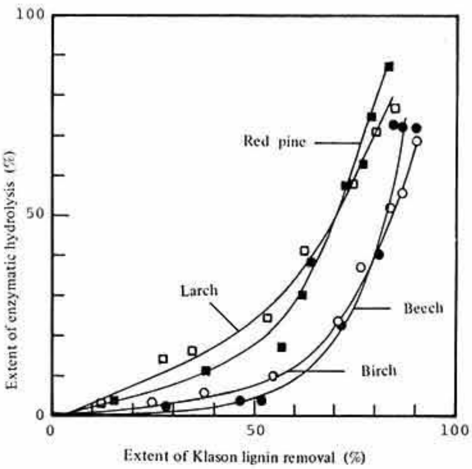

Fig. 1. Relationship between extents of enzymatic hydrolysis and delignification for partly delignified woods

Millet et al.[9] have shown that treatment of moist wood with gaseous $SO_2$ results in a almost quantitative conversion of the polysaccharides to sugars. This process only involves the reaction of moist sawdust with $SO_2$ under pressure for 2–3 hr at 120°C, evacuation of excess $SO_2$ and neutralization of the products to pH 7. Toyama used peracetic acid as a delignifying agent for sawdust and rice straw.[15]

All commercial delignification procedures are, at present, based on the removal of lignin through the selective action of chemical pulping and bleaching. In Japan, approximately 10 million tons of papers are produced annually by various pulping processes which are finally discarded and constitute a major part of municipal trash. Table 1 shows the hydrolysis extent of various papers with enzyme and relative sugar composition of a typical example of the hydrolysates[10]. Printing paper A is composed of 100% chemical pulp. Printing papers B and C contain chemical pulp more than 70% and 40–70%, respectively. Printing paper D is mixtures of

Table 1. Enzymatic saccharification of printing and writing papers

| Sample | | Extent of hydrolysis (%) | Sugar composition (%) | | | | |
|---|---|---|---|---|---|---|---|
| | | | Man | Ara | Gal | Xyl | Glu |
| Printing papers | A | 35 — 81 | 1.0 | 0.9 | 1.6 | 24.1 | 72.5 |
| | B | 44 — 49 | 1.9 | 0.7 | 1.6 | 20.4 | 75.5 |
| | C | 24 — 45 | 2.8 | 1.3 | 1.1 | 26.3 | 68.5 |
| | D | 6 — 27 | 6.0 | 1.7 | 1.9 | 21.9 | 68.4 |
| Gravure papers | | 26 — 44 | 10.8 | 0.7 | 1.2 | 7.4 | 79.9 |
| Writing papers | | 44 — 62 | 2.0 | 0.2 | 0.7 | 19.5 | 77.5 |
| Coat papers | | 30 — 48 | 1.8 | 0.9 | 1.2 | 20.3 | 75.8 |
| Newsprint papers | | 14 — 36 | 8.7 | 1.6 | 1.3 | 17.6 | 70.9 |

groundwood pulp and chemical pulp less than 40%. The hydrolysis extent is over a wide range (6–81%) depending on the lignin content. The main sugars in the hydrolysates are glucose and xylose.

### 2) *Fine grinding*

It is well known that grinding and gamma irradiation are very effective to increase the susceptibility of polysaccharides in wood to enzymatic attack[9]. Vibratory grinding severely destroys the cell wall structure and decreases the crystallinity of the cellulose with the reduction of particle size. Most importantly it increases the total surfase area accessible to the enzyme. Cellulose of woods ground to particle size of less than 30 μm is hydrolysed nearly 90%[8] Gamma irradiation is also a simple method to increase the reactivity of wood. About $5 \times 10^7$ rad is needed to attain substantial extent of hydrolysis.

These treatments are, however, estimated to be too costly for commercial application. The cost might be reduced by combination of other methods.

### 3) *Alkali treatment*

Alkali treatment with NaOH and $NH_4OH$ is a well-known method to enhance the reactivity of wood. Alkali saponifies the ester bonds and acetyl groups in the woods resulting in a loosening of the incrustation of carbohydrate by lignin network and an opening up of cell wall structure. The removal of hemicellulose with alkali possibly results in the porous structure of cell wall facilitating the penetration of enzymes into it. More than 10% aqueous NaOH brings about swelling of cellulose in wood. But the effect of alkali treatment varies with different substrates, for example, hardwoods are much more responsive than softwoods[4,8,9]. Other disadvantages are that it requires large amount of wash water, and recoveries of NaOH and dissolved hemicellulose from the spent liquor.

### 4) *Steaming*

The susceptibility of some species of hardwoods is remarkably improved by steaming at elevated temperature,[2] but, softwoods do not respond at all. By this process, most of hemicellulose is solubilized and significant quantities of lignin become soluble in a subsequent aqueous dioxane or caustic extraction step[7]. Furfural and acetic acid can be recovered from the vapor phase and xylose from the spent liquor. The lignin extracted and/or obtained as residue after hydrolysis is rather reactive and could be a favourable material for chemicals.

### 5) *Treatment with organic solvents*

Recently, delignification with organic solvents has been studied aiming at the pollution-free and economical pulping process[6]. Treatment with aqueous acidic methanol at elevated temperature results in delignification and hydrolysis of hemicellulose portion simultaneously[13]. The residue is pure cellulose and very susceptible to enzymatic attack. The hemicellulose and lignin dissolved can be separated from each other after evaporation of methanol by filtration, where the lignin is obtained as precipitates. Xylose can be predominantly recovered from the aqueous phase in the case of hardwoods and mannose in the case of softwoods. The lignin recovered should be useful raw materials for various chemicals.

### 6) *Biological treatment*

The hydrolysis extent of polysaccharides in wood increases with the progress of delignification on decay caused by the action of white-rot fungi³⁾. Edible mushrooms are produced as by-products. Unfortunately, white-rot fungi degrade cellulose and hemicellulose as well as lignin resulting in the loss of a large amount of fermentable sugars. This loss may be reduced by the production of less-cellulase and less-hemicellulase-mutant, which is capable of degrading lignin more selectively.

## General remarks

A wide variety of pretreatments have been tried to make wood polysaccharides more susceptible to enzymatic attack. Some of the pretreatments are effective in radically increasing the susceptibility, but their costs seem to be relatively expensive for application to large-scale commercial operation[16)]. What kind of pretreatment is feasible from economical and technological points of view?

An ideal case is that the pretreatment could produce some by-products from wood components to compensate for the cost and/or energy to be used for it. Total utilization of wood components has been one of the most important subjects for wood researchers for a long time. The development of an integrated processing for using all of the components must be again emphasized to make the process economically feasible. Xylose is converted to xylitol and furfural. Xylitol is a diabetic and non-cariogenic sweetner which has good taste and the same sweetening power and caloric value as sucrose. Lignin is also useful as raw materials for phenols, adhesive, plastics, carbon fibers etc.

The production of cellulase enzymes is also a major part of the overall process cost. Wilke et al. have reported that much of the enzyme is lost by adsorption on the noncellulosic fraction of the substrate[16)]. It is desirable to save the amount of enzyme by removing lignin by pretreatment as much as possible. Recently, Karube et al.[5)] have reported on the immobilization of cellulase on collagen beads and saccharification by a fluidized bed reactor. The continuous hydrolysis of cellulose can be performed without loss of cellulase by using cellulase-collagen beads.

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(Received for publication, October 4, 1980)