DETOXIFICATION AND SEPARATION OF LIGNOCELLULOSIC BIOMASS PRIOR TO FERMENTATION FOR BIOETHANOL PRODUCTION BY REMOVAL OF LIGNIN AND HEMICELLULOSES

Run-Cang Sun

Lignocellulosic materials such as agricultural residues have been recognized as potential sustainable sources of mixed sugars for fermentation to bioethanol. To obtain a high overall ethanol yield and achieve an economically feasible production process, the removal of lignin and hemicelluloses improves the accessibility of cellulosic material to hydrolytic enzymes and avoids the degradation products that are inhibitory to the yeast used in the subsequent fermentation. Technological advances, e.g., environmentally friendly removal of lignin and hemicelluloses from lignocellulosic biomass prior to fermentation of the librated glucose from cellulose into bioethanol, has the potential to provide for sustainable and cost effective production of biofuel.

Keywords: Lignocellulosic materials; Removal; Lignin; Hemicelluloses; Bioethanol

Contact information: State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou China, and Institute of Biomass Chemistry and Technology, Beijing Forestry University, Beijing, China; E-mail: ynsun@scut.edu.cn, rcsun3@bjfu.edu.cn

Reasons to Remove Biomass Hemicellulose and Lignin Components

The main components in lignocellulosic biomass, such as cereal straw, sugarcane bagasse, and grass, are cellulose (28-40%) and hemicelluloses (25-38%) in close association with lignin (12-18%). The former two components are hydrophilic, and the latter is hydrophobic. These lignocellulosic materials are mostly insoluble in water and in organic solvents only partly because of the hydrogen bonds between polysaccharides and adhesion of lignin to the polysaccharides. However, these agricultural residues represent an abundant, inexpensive, and readily available source of renewable materials for production of biofuel and novel materials for industrial applications. In particular, lignocellulosic materials can be expected to be major feedstocks for bioethanol production in large scale, in which the ethanol yield and ethanol production rate depend not only on the sugar yield, but also on the fermentability of the solution. Currently, two hydrolysis processes are being developed in parallel for hydrolysis of lignocelluloses into the sugars, acid-based and enzyme-based processes. In addition, in order to release the polymer chains of cellulose and/or modify the pores in the materials to allow the enzymes or acids to penetrate into the fibers to render them amenable to hydrolysis, a number of pre-treatments of lignocellulosic materials by acid or alkali are performed and intended to disorganize the crystalline structure of macro- and microfibrils. However, although the pre-treatment disrupts the plant cell walls and improves enzymatic access to the cellulose, the by-products, e.g., acetic acid, formic acid, levulinic acid, furfural and its derivatives, ferulic and p-coumaric acids, and phenolic/aromatic compounds from the degradation of lignin and hemicelluloses in the hydrolyzates produced during the pre-treatment and acid hydrolysis inhibit the *in-vitro* activity of several important enzymes and can cause

inhibition in the subsequent fermentation stage, resulting in a lower yield of ethanol. This suggests that detoxification of the hydrolyzate prior to the fermentation might be the key stage for bioethanol production. Furthermore, conversion of pentose sugars into ethanol is less efficient than conversion of the hexose sugars. Although fermentation of both hexoses and pentoses have been nominally successfully, rates and yields on mixed sugar derived from biomass have not reached commercially viable targets. Obviously, to overcome the obstacles to complete enzymatic hydrolysis of biomass with a high sugar yield, the two main protective coats around cellulose, hemicelluloses and lignin, need to be removed. Several technologies have been developed during the past decades that allow this conversion process to occur, and the clear objective now is to make this process cost-competitive in today's markets. Therefore, developing an environmentally friendly method for removal of hemicelluloses and lignin from lignocellulosic materials prior to bioethanol production from cellulose is necessary. In this case, removal of lignin and hemicelluloses from the microfibrils is thought to expose the crystalline cellulose core, which can then be hydrolyzed by cellulose enzymes. It is probable that systematic removal of lignin and hemicelluloses will result in the marked reduction in cellulase loadings required to convert cellulose to cellobiose or glucose. Other studies have shown that a reduction in phenolic esters, such as those characteristic of the linkages between lignins and hemicelluloses, also will permit more efficient utilization of cellulases. In addition, the conversion of the isolated hemicelluloses and lignin into novel materials for industrial uses may also significantly increase the efficiency of biomass utilization.

Chemical Treatments and Separations

Removal or isolation of lignin and hemicelluloses typically involves alkaline hydrolysis of ester linkages to liberate them from the lignocellulosic matrix followed by extracting them into aqueous media. However, the liberation of lignin and hemicellulosic components from the cell wall of lignocellulosic materials such as cereal straws is restricted by the presence of the lignin network, as well as ester and ether lignin-hemicellulose linkages. Furthermore, extensive hydrogen bonding between the individual polysaccharide cell wall components may impede release of the lignin and hemicellulosic components. For quantitative isolation of lignin hemicelluloses from lignocellulosic biomass the material must first be, therefore, pre-extracted, preferably with ethanol-toluene (2/1, v/v), so as to remove all lipophilic and hydrophilic non-structural components. As a second step, the material can be extracted by an alkali, followed by hydrogen peroxide treatment and ultrafiltration. The hemicelluloses and lignin are then recovered by precipitation or spray drying.

The separation of lignin and hemicelluloses from biomass by steam explosion is another potential method in an industrial separation of the polymers from cereal straw and wood samples. The use of an extruder-type twin-screw reactor makes the extraction more feasible. The extractability of lignin and hemicelluloses from annual plants is easier than that of wood hemicelluloses due to the lower amounts and different structure of lignin. It can be affected by the alkali type and isolation conditions and improved by a multistep mechanical-chemical treatment. The mechanical and chemical effect of ultrasonication on the cell wall material during alkaline extraction of annual plants has been shown to be very effective. Higher yields of hemicelluloses can be achieved at

lower temperatures and shorter extraction times. The hemicelluloses, obtained by ultrasound-assisted extraction, appear to be more linear and less acidic than those of hemicelluloses that are extracted by alkali in the absence of ultrasonic irradiation. In addition, the hemicelluloses obtained by ultrasound-assisted extractions show a relatively lower content of associated lignin, but a higher molecular weight and a slightly higher thermal stability in comparison with the hemicelluloses isolated after alkali treatment without ultrasonic irradiation.

Characteristics of the Released Hemicelluloses and Lignin

The hemicelluloses isolated by aqueous alkali from wheat straw are, in general, brown, and this impedes their industrial utilization. The aim in our laboratory has been to develop a commercial process for fractionation of cereal straw components using an environmentally friendly procedure for the extraction of lignin and hemicelluloses in a large scale, and also achieving a light color. Based on our recent ten years' study of hemicelluloses and lignin, we have found that alkaline peroxide is an effective agent for liberation of both hemicelluloses and lignin from straws and grasses. It is generally accepted that the hydroperoxide anion (HOO^-), formed in alkaline media, is the principal active species in hydrogen peroxide bleaching systems. In contrast, hydrogen peroxide is unstable in alkaline conditions and readily decomposes into hydroxyl radicals (HO•) and superoxide anion radicals (O_2^-•). This is particularly true in the presence of certain transition metals such as manganese, iron, and copper. These radicals are thought to cause the oxidation of lignin structures that lead to the introduction of hydrophilic (carboxyl) groups, cleavage of some inter-unit bonds, and eventually, the dissolution of lignin and hemicelluloses. The results obtained from our laboratory have shown that alkaline peroxide is an effective agent for isolation of lignin and hemicelluloses from non-wood materials such as cereal straw and grass.

In lignocellulosic biomass hemicelluloses rank second to cellulose in abundance, comprising roughly one-fourth to one-third of most plant materials, and this amount will vary according to the particular plant species, such as maize stems (28.0%), barley straw (34.9%), wheat straw (38.8%), rice straw (35.8%), and rye straw (36.9%). They, unlike cellulose, which is a unique molecule differing only in degree of polymerization and crystallinity, are non-crystalline heteropolysacharides. Hemicelluloses, however, are the most complex components in the cell wall of woods, straws, and grasses. They form hydrogen bonds with cellulose, covalent bonds (mainly α-benzyl ether linkages) with lignins, and ester linkages with acetyl units and hydroxycinnamic acids. They are branched polymers of low molecular weight with a degree of polymerization of 80-200. Their general formulate are $(C_5H_8O_4)_n$ and $(C_6H_{10}O_5)_n$, and they are called pentosans and hexosans, respectively. Hemicelluloses consist of various different sugar units, arranged in different proportions and with different substituents. The principle sugars are D-xylose, L-arabinose, D-glucose, D-galactose, D-mannose, D-glucuronic acid, 4-*O*-methyl-D-glucuronic acid, D-galacturonic acid, and to a lesser extent, L-rhamnose, L-fucose, and various *O*-methylated neutral sugars. Hemicelluloses of Gramineae such as cereal straws have a backbone of (1-4)-linked β-D-xylpyranosyl units. The chain may be linear, but it is often branched and usually has other glycosidically bound sugar units. Some xylan chains have D-glucopyranosyluronic acid units attached, but the most impor-

tant acidic hemicelluloses are *O*-acetyl-4-*O*-methyl-D-glucuronoxylans and L-arabino (4-*O*-methyl-D-glucurono)xylans. Xylans from grasses and cereal straws have the same backbone as the wood xylans. However, they contain smaller proportions of uronic acids, but are more highly branched and contain large proportions of L-arabinofuranosyl units.

Utilization of Hemicelluloses and Lignin

The hemicelluloses are potentially very useful. Studies on utilization of hemicelluloses from cereal straws have shown them to be a potential fermentation feedstock in production of xylitol. Properties of hemicelluloses being worth exploiting are their ability to serve as adhesives, thickeners, and stabilizers, as well as film formers and emulsifiers. In addition, some important applications for xylans have been discovered. These include uses in chiral separations, cholesterol depressant, tablet disintegrant, and dietary fibre. Evidently, hemicellulosic biopolymers have a very wide variety of direct food and non-food applications. In particular, some hemicelluloses from higher plants and herbs represent a potential source of pharmacologically active polysaccharides. Glucuronic acid-containing (acidic) xylans isolated from annual plant residues, such as bamboo leaves and corn stalks, have been reported to markedly inhibit the growth of sarcoma-180 and other tumors, probably due to the indirect stimulation of the non-specific immunological host defense.

Lignin is a relatively intractable polymer of phenylpropanoid subunits. It is built up by oxidative coupling of three major C_6-C_3 units, namely, syringyl alcohol, guaiacyl alcohol, and p-coumaryl alcohol, which form a randomized structure in a tridimensional network inside the cell walls. Unlike other natural polymers such as proteins, polysaccharides, and nucleic acids, which have inter-unit linkages susceptible to enzymatic and chemical hydrolysis, lignin contains resistant carbon-carbon and biphenyl ether bonds. Besides the some 20 different types of bonds present within the lignin itself, lignin seems to be particularly associated with the hemicellulosic polysaccharides. Owing to its cross-linking, lignin *in-situ* is usually insoluble in all solvents, unless it is degraded by physical or chemical treatments. Several applications for the lignins isolated from fractionation of lignocellulosic biomass have been considered. One of its main uses so far has been as a phenol substitute in the formulation of phenol-formaldehyde resins for board manufacture. Chemical modification of lignin for use in the preparation of polyurethanes, acrylates, epoxies, polymer blends, and composites has also received considerable attention.