УДК 663.5

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ETHANOL FROM CELLULOSE

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Анотація. У цій статті ми описуємо перспективи виробництва етанолу(в тому числі біоетанолу) з целюлози в якості сировини;

Ключові слова: целюлоза, попередня обробка, ферментація.

Аннотация. В этой статтье мы рассматриваем перспективы производства этанола (в том числе биоэтанола) из целюлозы в качестве сырья;

Ключевые слова: целюлоза, предварительная обработка, ферментация.

Abstract. In this artical we describe the perspectives of ethanol (including bioethanol) production from cellulose as a raw material;

Key words: cellulose, pretreatment, fermentation.

Introduction

The inevitable decline in petroleum reserves and the rise in demand for oil from rapidly growing economies have caused soaring oil prices, and coupled with climate change concerns have contributed to the current interest in renewable energy

resources. In some parts of the world this interest has resulted in the introduction of legislations promoting the use of renewable energy resources and increasing government incentives for commercialization of renewable energy technologies. Development of science and technologies for effi cient conversion of lignocellulosic biomass to renewable liquid transportation fuels has become one of the high priority research areas of the day, and bioethanol is the most successful biofuel to date.

Corn- and sugarcane-derived first generation bioethanol is currently in wide use as a blend-in fuel in gasoline sold in the United States, Brazil, and in a few other countries. However, there are a number of major drawbacks to these first generation fuels such as the effect on food prices as traditional food resources are utilized as raw materials, net energy balance, and poor greenhouse gas mitigation.

Cellulosic ethanol is a second generation biofuel produced from agricultural wastes, grasses, municipal wastes, and other feedstocks that do not double as food, so unlike traditional corn-based ethanol, it promises to avoid encroaching upon and destabilizing the human food supply. In addition, cellulosic ethanol can be produced from a variety of abundant lignocellulosic biomass feedstocks, and should be able to be produced in substantial amounts to meet the growing global energy demand. There are two fundamental routes to produce cellulosic ethanol from renewable biomass:

the aqueous-phase biomass saccharification-fermentation route, and thermochemical gasifi cation route. The thermochemical route can be divided into two paths as syngas produced from biomass can be converted to ethanol by chemical or enzymatic methods.

1. Cellulosic Ethanol Feedstock Types

Biomass feedstocks that can be used for cellulosic ethanol production can be broadly divided into fi ve categories:

1. **Agricultural wastes** — crop residues after taking the edible portion of the plant and can be in the form of stalks, leaves, trunks, branches, peels, or husks; all these parts of the plants are suitable as feedstock. In addition to this, edible agricultural products that are not suitable for human and animal consumption and rejected due to spoiling or contamination are also suitable as feedstock in the bioethanol production.

2. **Forestry residue** — logging and mill residues such as wood chips, sawdust, and pulping liquor.

3. **Grasses** — hardy, fast-growing grasses such as switchgrass grown specifi cally for ethanol production.

4. **Trees** — fast-growing trees such as poplar and willow grown specifi cally for ethanol production.

5. **Municipal and other wastes** — plant-derived wastes such as household garbage, paper products, paper pulp, and food-processing waste. Nevertheless, production of ethanol from starch- and sugar-containing food wastes requires fi rst generation bioethanol technologies, which are in wide use in the current corn and sugarcane ethanol industries.

1.1 Potential of Agricultural Wastes

Agricultural industry waste is the byproduct of industries which use agricultural products as raw materials. The major crops that produce large quantities of wastes on a global scale are rice, corn, barley, oat, wheat, sorghum, and sugarcane. The potential of producing ethanol from the crop residues as well as wasted agricultural products have been estimated. To avoid confl icts between human food use and industrial use of crops, only the wasted crop, which is defi ned as crop lost in distribution, is considered as feedstock.

There are about 74 Terra grams (Tg) of dry wasted crops in the world that could potentially produce 49 GL/ year of bioethanol.

Lignocellulosic biomass forms such as crop residues and sugarcane bagasse are the main components of agricultural waste, and about 1549 Tg /year of dry lignocellulosic biomass from these global crops is also available for conversion to bioethanol with a potential of producing up to 442 GL/ year of cellulosic bioethanol.

Thus, the total potential bioethanol production from crop residues and wasted crops is 491 GL /year.

Chemical composition of lignocellulosic feedstocks is a key factor affecting effi ciency of biofuel production during the complex conversion process. The structural

and chemical composition of lignocellulosic feedstocks is a highly variable factor, because of genetic and environmental infl uences and their interactions. Low lignin, globally abundant crop residues like rice and wheat straws are excellent biomass resources for the aqueous-phase cellulose hydrolysis-fermentation route. A comparison of major components: cellulose, hemicellulose, lignin and ash in major crop residues are shown in Table 1.1.

Table 1.1 A comparison of cellulose, hemicellulose, lignin and ash in major crop residues that can be used in cellulosic ethanol production (wt% on dry basis).

Crop residue	Cellulose	Hemicellulose	Lignin	Ash
Corn stover	38	26	23	5
Barley straw	42	28	7	11
Oat	40	20	18	8
Rice	40	23	15	13
Wheat straw	38	20	15	5
Sorghum	23	14	11	5
Soybean	33	14	14	6
Sugarcane bagasse	40	21	18	2

Dale and Kim [1] have studied the global potential in crop residues as well as agricultural wastes for bioethanol production, and the totals (Terra grams [Tg]) of seven major crops: corn, barley, oat, rice, wheat, sorghum, and sugarcane in fi ve continents are shown in Table 1.2.

Table 1.2 Total quantities of wasted crops and agricultural wastes in different continents, that are potentially available for bioethanol production

	Africa	Asia	Europe	America	Oceania	Subtotal
Wasted crop (Tg)						
Corn	3.12	9.82	1.57	6.17	0.01	20.70
Barley	0.17	1.23	2.01	2.04	0.09	3.66
Oat	0.004	0.06	0.43	0.06	0.001	0.55
Rice	1.08	21.8	6.02	2.45	0.02	25.44
Wheat	0.83	10.28	4.09	1.17	0.82	17.2
Sorghum	2.27	0.54	0.004	0.31	0.001	3.12

Sugarcane	0.46	1.64	0.00	1.10	0.00	3.20
Subtotal	**7.94**	**45.43**	**8.13**	**11.31**	**1.05**	**73.86**
Crop residues (Tg)						
Corn stover	0.00	33.90	28.61	140.86	0.24	203.62
Barley straw	0.00	1.97	44.22	10.3	1.93	58.45
Oat straw	0.00	0.27	6.83	3.04	0.47	10.62
Rice straw	20.93	667.59	3.92	37.23	1.68	731.34
Wheat straw	5.34	145.20	132.59	62.34	8.57	354.35
Sorghum straw	0.00	0.00	0.35	9.65	0.32	10.32
Sugarcane bagasse	1.73	74.88	0.01	87.62	6.49	180.73
Subtotal	**38.0**	**923.82**	**216.56**	**351.34**	**19.70**	**1549.42**

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The U.S. National Renewable Energy Laboratory (NREL) has estimated that 288–447 L of ethanol can be produced from a dry tonne of corn stover [2]. The ethanol yields from other forms of agricultural wastes can be calculated by using composition data of these materials and an "ethanol yield calculator" developed by the U.S. Department of Energy [3]. Even though the ethanol production effi ciency depends on the form of biomass, in many of these calculations they have assumed that the ethanol production efficiency of other crop residues is also similar to that of corn stover [1].

Potential for bioethanol production from crop waste and crop residues around the globe by different continents is shown in Table 1.3.

Table 1.3 Potential of bioethanol production from crop waste and crop residues around the globe in giga liters (GL) per year.

	Africa	Asia	Europe	America	Oceania	Subtotal
From waste crop (GL)						
Corn	2.17	6.82	1.09	4.29	0.01	14.40
Barley	0.12	0.83	1.35	0.03	0.13	2.46
Oat	0.002	0.04	0.30	0.04	0.001	0.38
Rice	0.71	14.4	0.02	1.61	0.02	16.80
Wheat	0.55	6.78	2.70	0.78	0.54	11.30
Sorghum	1.55	0.37	0.003	0.12	0.0004	2.14
Sugarcane	0.23	0.82	-	0.55	0.0001	1.59
Subtotal (A)	5.33	30.10	5.45	5.00	0.70	49.10
From Crop residues (GL)						
Corn stover	-	9.75	8.23	40.50	0.07	58.60

Barley straw	-	0.61	13.70	3.15	0.60	18.10
Oat straw	-	0.07	1.79	0.79	0.12	2.78
Rice straw	5.86	186.80	1.10	10.41	0.47	204.60
Wheat straw	1.57	42.60	38.90	18.40	2.51	103.80
Sorghum straw	-	-	0.10	2.61	0.09	2.79
Sugarcane bagasse	3.33	21.30	0.004	24.87	1.84	51.30
Subtotal (B)	10.80	261.00	63.80	100.82	5.70	442.00
Subtotal (A+B)	16.13	291.10	69.25	105.82	6.39	**491.10**

2 . Aqueous Phase Biomass Hydrolysis Route

2.1 Introduction – Two Ways to Produce Cellulosic Ethanol

There are two basic processes for conversion of lignocellulosic biomass to bioethanol:

1) cellulolysis process or aqueous-phase biomass saccharifi cation and fermentation process;

2) gasification or syngas to ethanol conversion process.

The first method of aqueous-phase biomass saccharification and fermentation involves the hydrolysis of cellulose and hemicellulose in biomass to sugars and then fermentation of the sugar solution with yeast to produce ethanol.

The basic steps of this route are shown in Figure 2.1.

Saccharification of the biomass or hydrolysis of polysaccharides to monosaccharides is the most challenging step in this process, and this can be accomplished by pretreatment of biomass followed by exposure to a cellulase enzyme cocktail, or by single-step direct acid hydrolysis using concentrated or dilute acid solution like aqueous sulfuric acid.

This route is known as biochemical process as well.

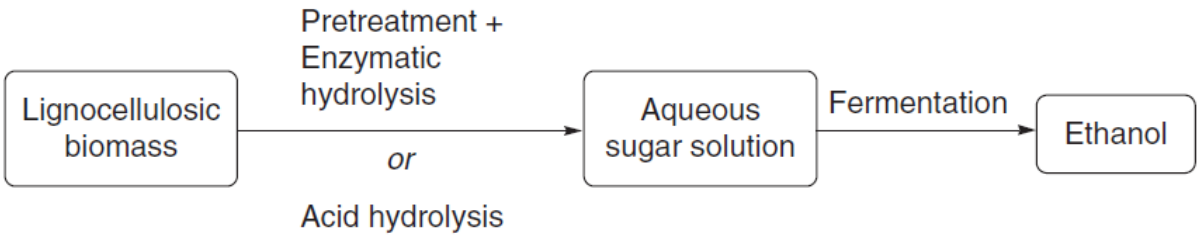

Figure 2.1 Basic steps of cellulolysis process or aqueous-phase biomass saccharifi cation and fermentation process.

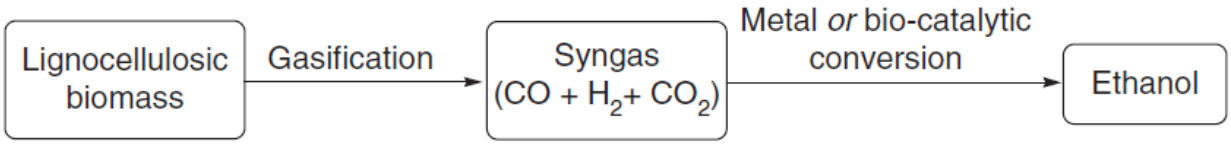

Figure 2.2 Basic steps of the gasifi cation-syngas-ethanol process.

The second method is the gasification or syngas to ethanol conversion process, and the key steps in this process are shown in Figure 2.2. This method is known as thermochemical process as well since pyrolysis of biomass to syngas (a mixture of carbon monoxide, carbon dioxide, and hydrogen) is the first step and an essential feature of the process. Then the gas mixture is converted to ethanol by using either a metal or biochemical catalyst.

2.2 Challenges in Aqueous-Phase Biomass Hydrolysis

In the cellulolysis processes or aqueous phase process, the most challenging step is the depolymerization of cellulose and hemicellulose in the biomass to a fermentable sugar solution, or the saccharification. The resistance of lignocellulosic biomass to the hydrolysis by enzymes or acid is one of the most formidable barriers for the production of cellulosic ethanol. This resistance or non-susceptible nature of the lignocellulosic structures, which does not allow other molecules to easily penetrate or interact with the molecular structure, is known as the *recalcitrance* of cellulose. Insolubility of cellulose and lignocellulosic biomass in most of the

common solvents is a direct consequence of recalcitrance property as well. Recalcitrance character is related to the structure of cellulose and lignocellulosic biomass.

Hence, a sound understanding of the molecular architecture of cellulose and lignocellulosic biomass is an extremely important aspect of cellulosic biomass science and a good launching point for biomass pretreatment and saccharifi cation research.

2.3 Major Components of Lignocellulosic Biomass

Lignocellulose or lignocellulosic biomass refers to the dry plant matter, which is the most abundant organic substance on earth. The three major components in lignocellulosic biomass and their typical percent compositions are:

1. *Cellulose* 35–50%
2. *Hemicellulose* 20–35%
3. *Lignin* 15–30%

The exact composition can vary in a wide range depending on the plant family, species and part of the plant. In addition to these, there are minor components like minerals, proteins, fats and oils in all plant materials.

2.3.1 Cellulose

Cellulose is a linear polymer of D-glucose molecules linked with β(1→4)-*glycosidic bonds*. The repeating unit of the polymer is D-cellobiose, which consists of two D-glucose molecules as shown in Figure 2.3.

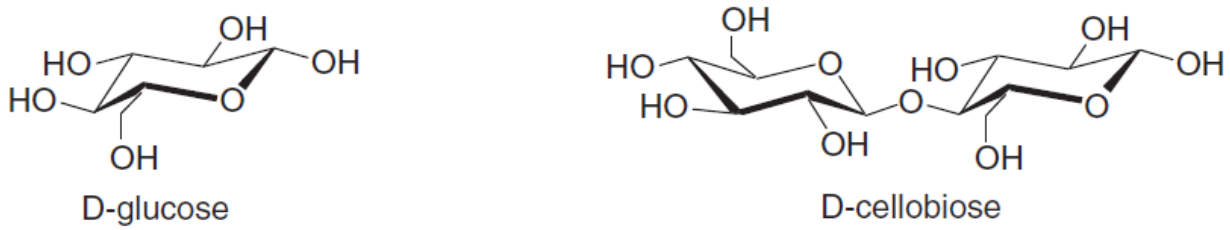

Figure 2.3 D-glucose, the basic unit in cellulose, and D-cellobiose, the repeating unit in cellulose.

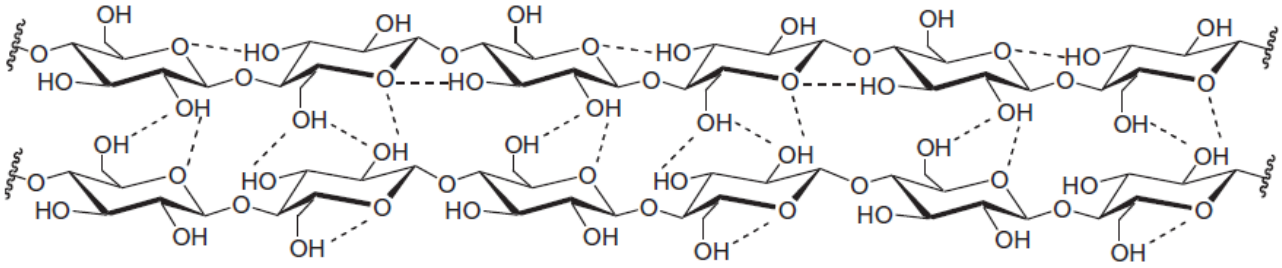

Figure 2.4 Linear polysaccharide chains in cellulose microfibrils. Inter- and intramolecular hydrogen bonds are shown in dashed lines.

The cellulose structure is composed of stacks of linear chains with D-cellobiose repeating units. These closely-packed chains form robust crystal structures with inter- and intramolecular hydrogen bonds, as shown in Figure 2.4.

This motifin cellulose contrasts with that of α(1→4)-glycosidic bonds present in starch, glycogen, and other carbohydrates. Unlike starch, no coiling or branching occurs in cellulose, and the molecule adopts an extended and rather stiff rod-like conformation aided by the equatorial conformation of all the D-glucose units in the linear chains as shown in Figure 2.4. The chain length of a polymeric cellulose molecule varies in a wide range depending on the plant source. However, a typical value of a number of glucose units in the polymer is in the range 100 to 14,000. Each cellulose molecule consists of a linear chain of glucose residues that are covalently linked to one another to form a ribbon-like structure, which is stabilized by hydrogen bonds within the chain. In addition, intermolecular hydrogen bonds between adjacent cellulose molecules cause them to adhere strongly, giving a high tensile strength to

the material. The bundles of linear cellulose chains are stacked along the axial direction of the microfi bril as shown in Figure 2.4.

Cellulose, which is the principle scaffolding component of all plant cell walls, exists in the form of a robust crystalline structure in solution or in solid state. This highly hydrogen-bonded complex molecular architecture of the cellulose molecules provides tensile strength to the primary cell wall. Such a cell wall polymer is neither soluble in water nor easily digestible in the gastrointestinal tract of humans. These cellulose microfi brils with a complex network of hydrogen bonding and van der Waals interactions resists deconstruction by solvent or by physical treatments.

2.3.2 Hemicellulose

Hemicelluloses are the second most abundant component in biomass and are composed of a combination of several heteropolymers.

The most common ones include xylan, glucuronoxylan, arabinoxyaln, glucomannan, and xyloglucan. These are often branched polysaccharides. In contrast to cellulose, which contains only D-glucose, hemicellulose contains many different sugar monomers. Most of the sugars in hemicelluloses are 5-carbon D-pentose sugars and occasionally small amounts of L-sugars as well. In most cases xylose is the sugar monomer present in the largest amount, although in softwoods mannose can be the most abundant sugar. Not only regular sugars like xylose, but also carboxylic acid group or their derivative containing sugars like glucuronic acid and galacturonic acid can also be present in hemicellulose.

Some common molecular motifs found in hemicellulose are shown in Figure 2.5

2.3.3 Lignin

Lignin is the third major component in biomass, which is a crosslinked macromolecular material based on phenylpropanoid monomer units *p*-coumaryl alcohol, coniferyl alcohol (guaiacyl), and sinapyl alcohol (syringyl). Typical

molecular masses of isolated lignins are in the range 1000–20,000 g/mol, but the degree of polymerization in nature is diffi cult to measure since lignin is invariably fragments during extraction and consists of several types of substructures which repeat in an apparently random manner.

A representative section of the lignin structure is shown in Figure 2.6.

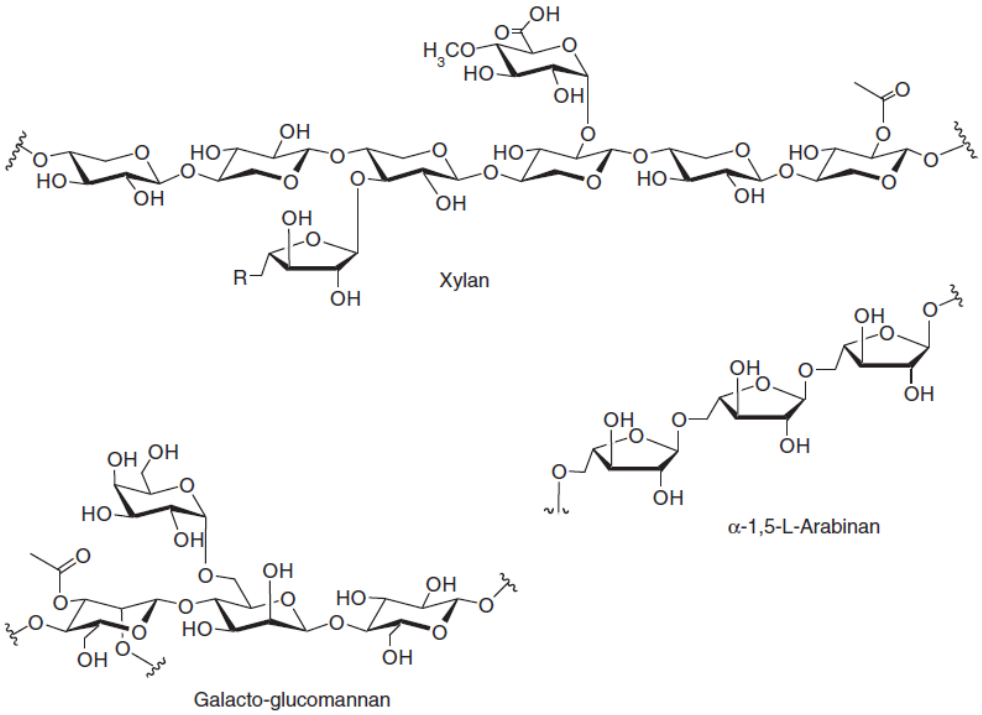

Figure 2.5 Some common molecular motifs found in hemicellulose.

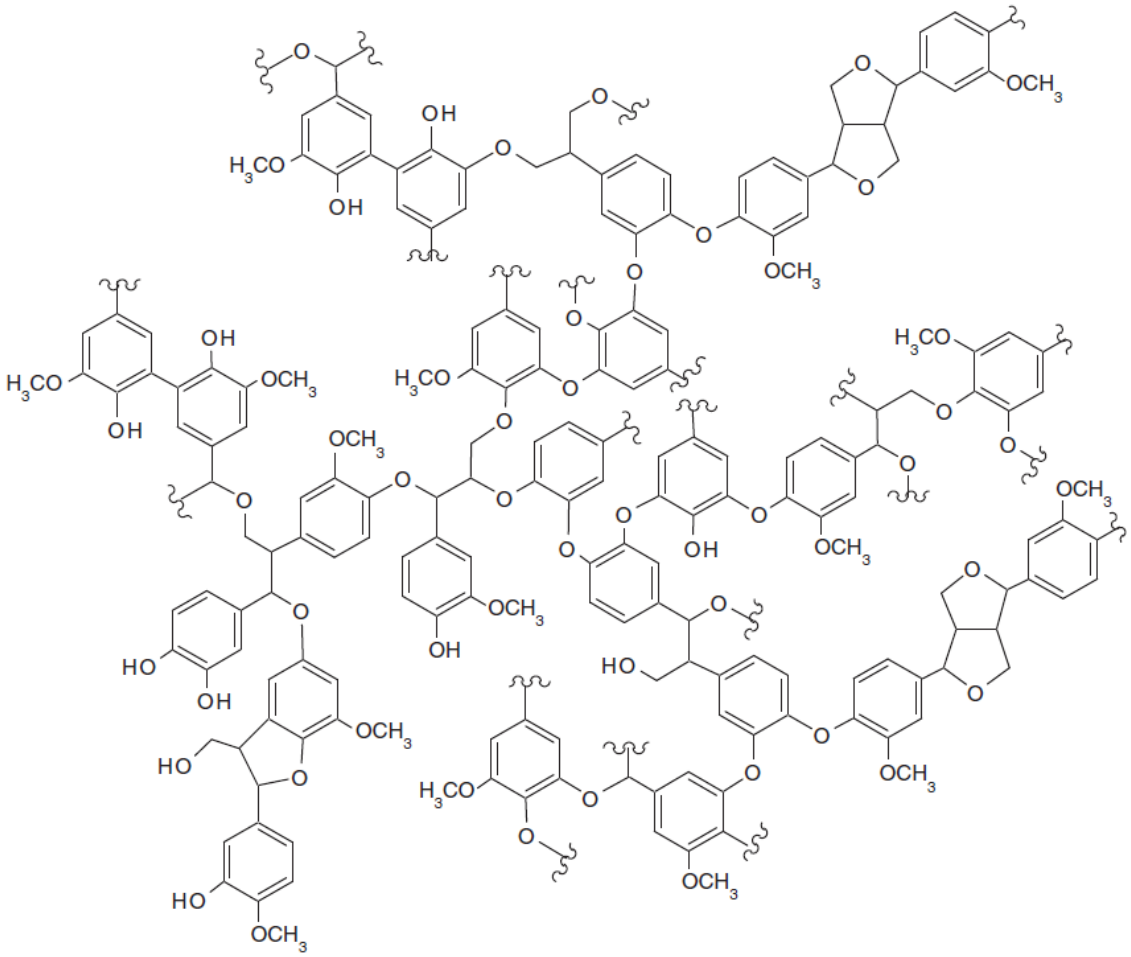

Figure 2.6 A representative section of the lignin structure.

3. Pretreatment of Lignocellulosic Biomass

3.1 Introduction

Pretreatment is the process used to liberate cellulose and hemicellulose from the lignin seal and its crystalline structure so as to render polysaccharides accessible for a subsequent hydrolysis step. The resistance of plant cell walls to deconstruction is known as the recalcitrance property, and pretreatment is the fi rst step in overcoming biomass recalcitrance.

The main factors that contribute to the recalcitrance of lignocellulosic biomass to hydrolysis are poor accessible surface area, protection of cellulose by lignin, the heterogeneous character of biomass particles, and cellulose sheathing as shown in the schematic representation of pretreatment in Figure 3.1. As illustrated in this fi gure,

pretreatment improves the accessibility to cellulose and hemicellulose by liberating them from the lignin shell.

In addition to this encapsulated arrangement, crystallinity of cellulose is also an important factor, because pure crystalline cellulose is diffi cult to hydrolyze without a pretreatment. Transformation between crystalline and amorphous forms of cellulose is reversible; both forms can break into glucose oligomers, however, the amorphous form degrades faster than the crystalline form, as shown in the kinetics schematic in Figure 5.2, with rate constant k2 >> k1. In principle, an effective pretreatment causes disruption of these barriers so that hydrolytic enzymes can penetrate and cause hydrolysis (Fig. 5.2) and also minimizes degradation of sugar to undesired degradation products shown in the last step of Figure 3.2.

Pretreatment of lignocellulosic biomass may produce degradation products with an inhibitory effect on the fermentation process. These undesired products are produced by the degradation of sugars as well as degradation of lignin. Pentose sugar monomers may dehydrate to the 5-carbon aldehyde furfural. Similarly, hexose sugars like glucose may degrade to 5-hydroxymethylfurfural (HMF). Furfural and HMF affect cell growth and respiration, and HMF is considered less toxic than furfural and its concentration in hydrolyzates is usually low. A variety of compounds like aromatics acids, phenols and aldehydes may be released from the degradation of lignin fraction. Phenolic compounds have a signifi cant inhibitory effect and are generally more toxic than furfural and HMF. Low molecular weight phenols are the most toxic. However, at temperatures lower than 180°C lignin degradation is not so signifi cant if no strong acid or alkaline conditions are present in the pretreatment medium. Some of the common inhibitory compounds formed during the pretreatment step are shown in Figure 3.3.

These inhibitors have toxic effects on the fermenting organisms, thus reducing the ethanol yield and productivity. The level of toxicity depends in part on fermentation variables including cell physiological conditions, dissolved oxygen concentration and pH of the medium. In many cases it is essential to remove these inhibitors before exposure to cellulose and hemicellulose hydrolyzing enzymes.

Pretreatment of biomass for cellulosic ethanol process has been the topic of a number of excellent review articles in recent years.

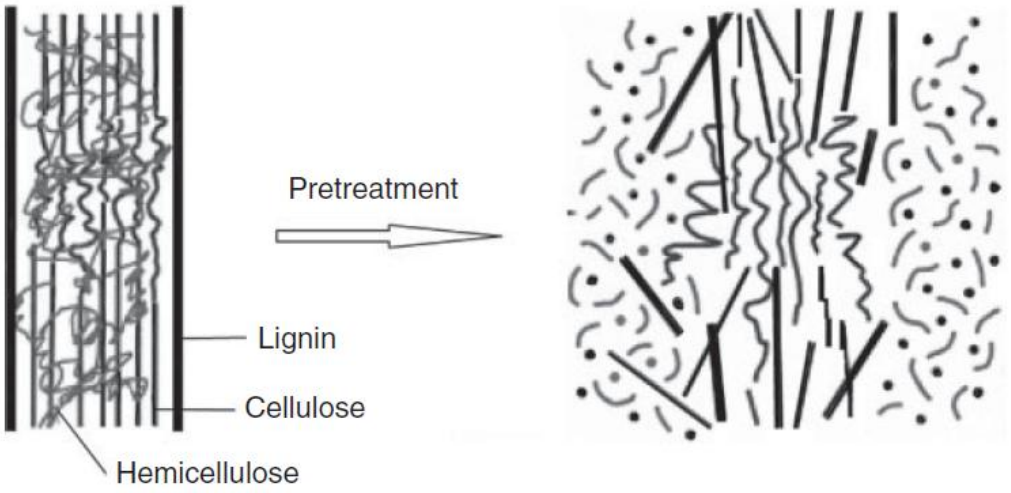

Figure 3.1 Schematic representation of the pretreatment process.

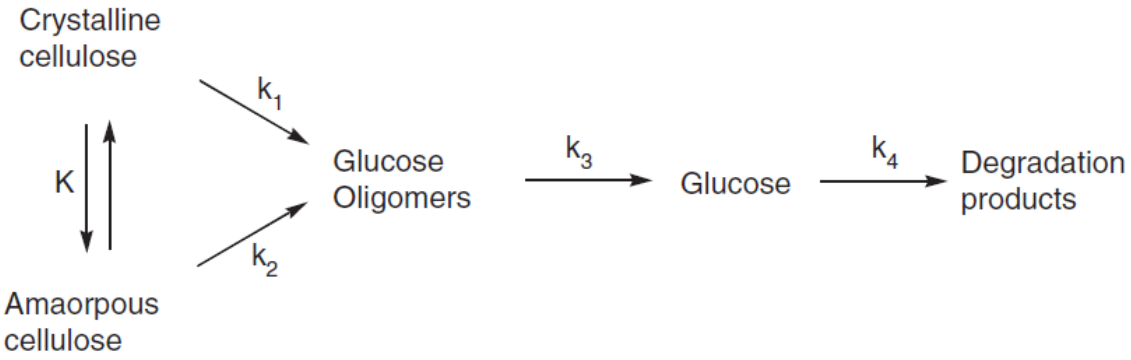

Figure 3.2 Schematic representation of transformations of crystalline and amorphous forms of cellulose to glucose oligomers, glucose, and to degradation products.

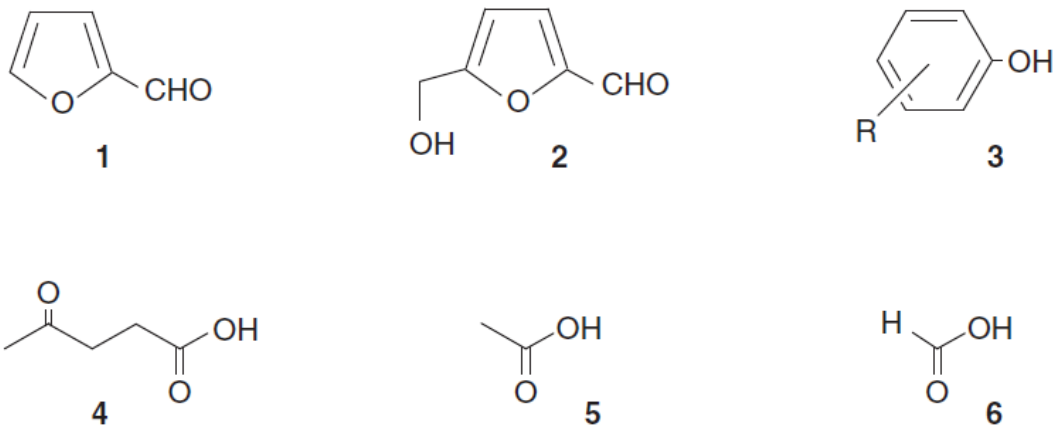

Figure 3.3 Common inhibitory compounds formed during the pretreatment of biomass: **1** - Furfural, **2**–5-Hydroxymethylfurfural, **3** - Phenols, **4** - Levulinic acid, **5** - Acetic acid, **6** - Formic acid.

There are a number of key factors in a good pretreatment method, which include the following:

1. Produces highly digestible solids that enhance sugar yields during enzyme hydrolysis.
2. Avoids the degradation of sugars, especially the pentose derived from hemicellulose.
3. Minimizes the formation of inhibitors for subsequent fermentation steps.
4. Is cost effective by operating in reactors of moderate size and by minimizing heat and power requirements.

3.2 Different Categories of Pretreatment Methods

Pretreatment technologies can be basically classifi ed into the following four categories:

1. Physical pretreatment
2. Physicochemical pretreatment
3. Chemical pretreatment
4. Biological pretreatment

Physical pretreatment is often called size reduction to reduce biomass physical size; it is also the first step in many other pretreatment processes as raw biomass comes in the form of larger pieces in most situations, except in a case like sawdust from a mill.

Chemical pretreatment utilizes chemical transformations to overcome the recalcitrance so that the enzymes can have access to cellulose for microbial depolymerization.

Biological pretreatment uses enzymes to achieve the accessibility for the hydrolysis step and is not as widely used as other methods.

Then there are multiple techniques within some of these classifi cations. In this chapter various pretreatment techniques are presented in detail under these four categories; however some of the techniques may have features of more than one category.

3.2.1 Biological Pretreatment

In biological pretreatment, microorganisms are used to degrade lignin and hemicellulose leaving cellulose, allowing cellulose to undergo facile hydrolysis when exposed to saccharifi cation enzymes. The most common type of microorganisms used in this pretreatment is fungi.

In the early 1990s Hatakka *et al*. reported the selective delignifi cation of wood and wheat straw by selected white-rot fungi such as *Phanerochaete chrysosporium, Phlebiaradiata, Dichmitus squalens, Rigidosporus lignosus, and Jungua separabilima*.

Lignin depolymerization by these fungi takes weeks to achieve signifi cant results but can be very selective and effi cient. White-rot fungi produce extracellular lignin-modifying enzymes, the best characterized of which are laccase (EC 1.10.3.2), lignin peroxidases (EC 1.11.1.7) and manganese peroxidases (EC 1.11.1.7).

Lignin biodegradation studies have been carried out mostly using the white-rot fungus *Phanerochaete chrysosporium,* which produces multiple isoenzymes of lignin peroxidase and manganese peroxidase but does not produce laccase. Many other white-rot fungi produce laccase in addition to lignin and manganese peroxidases and in varying combinations.

Based on the enzyme production patterns, white-rot fungi can be categorized into three groups:

1. Lignin-manganese peroxidase group (e.g., *P. chrysosporium*and *Phlebia radiata*)
2. Manganese peroxidase-laccase group (e.g., *Dichomitussqualens* and *Rigidoporus lignosus*)

3. Lignin peroxidase-laccase group (e.g., *Phlebia ochraceofulva*and *Junghuhnia separabilima*)

When compared to other methods, biological pretreatments are normally conducted at low temperatures and atmospheric pressures without using expensive equipment, chemical reagents, and additional energy for lignin removal and biomass structure destruction.

Therefore, it is a green, safe, and inexpensive method. However, the enzymatic reaction rates are slow, therefore long pretreatment times are required compared to other pretreatment methods.

Even though biological pretreatment technique is relatively new, it has been reported for the pretreatment of corn stover, rice straw, beech wood, *pinus densifl ora* and *eucalyptus globulus*.

White-rot fungi are mostly used for secreting ligninolytic enzymes in the biological pre-treatment process, and current research related to biological pretreatment is mainly focusing on the following five aspects:

1. Selection of white-rot fungi candidate strains for certain biomass materials.
2. Optimization of cultivation methods for white-rot fungi.
3. Characterization of fungal-treated materials.
4. Mutation breeding and crossbreeding of fungal mycelia to obtain engineered strains.
5. Integration of fungal pretreatment with simultaneous saccharification and fermentation to produce biofuels, and evaluation of combining bio pretreatment with chemical or physicochemical approaches.

Summary. Future Prospects of Cellulosic Ethanol

As of mid 2013, several indicators have shown a steady progress in the cellulosic ethanol industry, even though the earlier targets set in the United States have not been met, as expected. Technoeconomic analysis plays an important role in the realization of cellulosic ethanol. The overreaching goal for the DOE's office of the biomass program is to demonstrate the cost-competitiveness of cellulosic ethanol

with petroleum fuels. The 2011 NREL report on development targets predicted an *n*th-plant MESP of $2.15/gal by 2012, as modeled by the NREL process design for a corn stover-acid pretreatment-enzyme hydrolysis plant [4]. This MESP value is comparable to current gasoline prices in the US, since a kilogram of ethanol has about 66% of the energy in a kilogram of gasoline. With continuous R&D efforts in enzyme technologies and energy effi cient processing confi gurations, MESP value is expected go below $2.00/gal in the coming years, boosting investor confi dence.

The cellulosic biofuel industry 2012–2013 progress report is a more realistic and a vital indicator, which summarizes the global perspective of the industry [5]. This report gives a detailed snapshot of advancements made towards the commercial deployment of cellulosic ethanol. According to Sandia National Lab and the cellulosic biofuel industry 2012–2013 progress report, the United States could produce 75 billion gallons of cellulosic ethanol without displacing food and feed crops [5]. For comparison, the US consumed 134 billion gallons of gasoline in 2011. According to the advanced ethanol council's 2012–2013 progress report, there are about ten commercial-scale (>20 million gallons/year) cellulosic ethanol plants in operation or under construction around the world in 2013 [5]. Of course this is a very small fraction in comparison with fi rst generation corn ethanol production capacity in the United States, which is 13.9 billion gallons/year in 2011 [6]; clearly, cellulosic ethanol is an industry in its infancy. However, entering into a commercial operation phase is an encouraging sign and a testimony for investor confi dence on cellulosic ethanol technology. The future of cellulosic ethanol looks promising and the goal of large-scale production of fuel ethanol from abundant lignocellulosic biomass to meet the global energy demand is realizable in the near future.

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Статья отправлена: 07.11.2016 г.