Renewable Energy 98 (2016) 29–42

Contents lists available at ScienceDirect

Renewable Energy

journal homepage: www.elsevier.com/locate/renene

Cellulase adsorption on lignin: A roadblock for economic hydrolysis of biomass

Jitendra Kumar Saini [1], Anil Kumar Patel, Mukund Adsul, Reeta Rani Singhania*

DBT-IOC Centre for Advanced Bio-Energy Research, Indian Oil Corporation Ltd, R&D Centre, Sector-13, Faridabad 121007, Haryana, India

ARTICLE INFO

Article history:
Received 9 January 2016
Received in revised form
11 March 2016
Accepted 25 March 2016
Available online 4 April 2016

Keywords:
Cellulase
Lignin
Biomass
Pretreatment
Hydrolysis
Enzyme inhibition

ABSTRACT

Enzymatic saccharification of biomass is a crucial step in bioethanol production from lignocellulosic biomass through sugar platform which essentially requires attachment of cellulases onto cellulose which is affected by the presence of lignin in biomass. This article focuses on types of interaction between cellulase-lignin and the possible strategies to restrict or overcome it so as to allow maximum cellulases for cellulase-cellulose productive binding. By inhibiting cellulase-lignin binding the cellulase dosage could be reduced dramatically thereby reducing the cost of enzyme in bioethanol process. The techniques to study these interactions have also been discussed.

1. Introduction

Lignin is a polymer of cross-linked phenylpropane units which confers hydrophobicity, structural rigidity and microbial resistance to plant cell wall. In the lignocellulosic biomass, lignin remains in close association with cellulose and hemicelluloses chemically and physically, providing structural support and impermeability to the cell wall, thus creating a physical and chemical wall [1]. In plant cell wall, lignin content varies from 2 to 40% and provides strength to lignocellulosic biomass and forms shield on carbohydrate making it undesirable for enzymatic hydrolysis of biomass [2]. Both in nature and in industrial processes, cellulases usually act in an environment where lignin is an inseparable part of the substrate structure (lignocelluloses). In native lignocellulose, the cellulosic fibrils are embedded in a hemicellulose–lignin network, whereas in pretreated lignocellulosic feedstocks, the network is disrupted to improve enzyme accessibility to the cellulosic fibrils.

1.1. Chemical structure and types of lignin

The chemical structures of lignin have been mostly studied by chemical and spectroscopic methods. Lignin is an amorphous, polyphenolic substance formed by enzymatic dehydrogenative polymerization of *p*-coumaryl, coniferyl and sinapyl alcohols. The basic lignin structure consists of aromatic part and the C3 chain. Lignin consists of 4-hydroxyphenyl (1), guaiacyl (2), and syringyl (3) structures connected with carbon atoms in phenylpropanoid units (Fig. 1.). These three structural components of lignin conjugate to produce an irregular but three-dimensional lignin polymer, which is physically and chemically heterogeneous. The structure of lignin is highly affected by environmental factors during biosynthesis. Various combinations of side chain structures and monolignols increase lignin structural diversity. Almost all lignins have linkages to carbohydrates [3], sometimes forming lignin-carbohydrate complex (LCC), especially after pretreatment.

Based on the methods for lignin isolation from lignocellulosic materials, lignin has been categorized into different types. One of the methods involves acid based hydrolysis (acidolysis) of polysaccharide parts of lignocellulosics; while in another second method, lignin is extracted by ball milling or enzymatic treatments to yield milled wood lignin (MWL). Lignin is also isolated during industrial processes, such as pulping and bio-ethanol production.

Lignin can be divided into several classes according to their structural elements. Guaiacyl lignin is a largely polymerization product of coniferyl alcohol and occurs in almost all softwoods. Guaiacyl-syringyl lignin is typical of hardwood and is a copolymer of coniferyl and sinapyl alcohol in a ratio varying from 4:1 to 1:2. *p*-

* Corresponding author.
E-mail address: reetasinghania@gmail.com (R.R. Singhania).
[1] Present address: Department of Microbiology, Central University of Haryana, Jant-Pali, Mahendergarh, Haryana 123029, India.

http://dx.doi.org/10.1016/j.renene.2016.03.089
0960-1481/

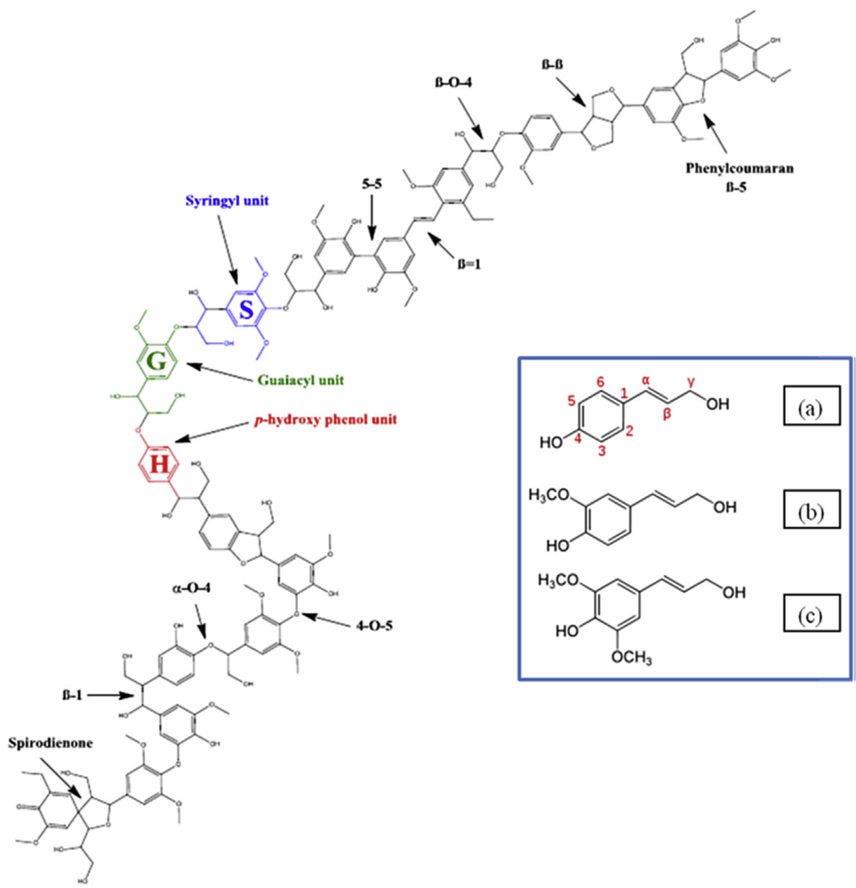

Fig. 1. Lignin structure showing syringyl (S), guiacyl (G), and *p*-hydroxyphenol (H) phenylpropanoid components and linkages within lignin. Inset shows three common monolignols: (a) paracoumaryl alcohol (*p*-hydroxyphenol (H)), (b) coniferyl alcohol (guaiacyl (G)) and (c) sinapyl alcohol (syringyl (S)).

Hydroxyphenyl lignin is present in compression wood and has a high proportion of phenylpropane units of the *p*-hydroxyphenyl type in addition to the normal guaiacyl units. In hardwood, there are still many uncertainties involved, because of the more heterogeneous nature of the wood and the presence of both quaiacyl and syringyl units in the lignin. The lignin present in the secondary wall of hardwood fibers contains higher syringl content; whereas middle lamella lignin contains higher guaiacyl units. The vessels in birch contain only guaiacyl lignin, whereas in parenchyma cell syringyl lignin is major content [4].

1.2. Physical properties of lignin

1.2.1. Glass transition

It is the reversible transition in amorphous materials (or in amorphous regions within semi-crystalline materials) from a hard and relatively brittle “glassy” state into a molten or rubber-like state, as the temperature is increased. The glass-transition temperature (T_g) of a material characterizes the range of temperatures over which this glass transition occurs and is always lower than the melting temperature. Phenylpropane units of lignin molecular chain characterize its molecular motion. Restriction of molecular motion and increase in T_g are due to cross-linking and presence of rigid groups of main chain. Bulky side chains enhance molecular mobility of lignin through the local mode relaxation. Differential scanning calorimetry (DSC) is the most common technique used to study glass transition properties of lignin [5]. DSC curves proved lignin to be an amorphous polymer and that cross linking of lignin molecules are not very dense. Other techniques are: dilatometry, viscoelastic measurements, dynamic mechanical analysis (DMA), thermomechanical analysis (TMA), solid state nuclear magnetic resonance spectrometry (NMR), spin probe method using electron spin resonance spectroscopy (ESR), temperature dependency of absorption bands by infrared spectroscopy (IR), and temperature dependency of x-ray diffractogram. At elevated pretreatment temperatures (>140 °C), lignin is above its glass transition phase and separates from the carbohydrate polymers of the cell wall due to lignin–lignin hydrophobic affinity following transition from solid to liquid [6].

1.2.2. Local mode relaxation

In addition to glass transition (α relaxation), two local mode relaxations (β and γ) for various types of isolated lignins (such as dioxane lignin (DL), lignosulfonate (LS), and MWL) may be seen when the relaxation is from the higher to lower temperature side. Previous studies revealed that β relaxation and γ relaxation are due to the hindered motion of methyl group and of the side chain, respectively [6]. Dynamic mechanical measurement, and broad-line nuclear magnetic resonance spectroscopy (b-NMR) have been used to study local mode relaxation of isolated lignin and its model compounds [6,7].

1.3. Lignin–water interaction

Inside plant tissue, lignin is surrounded by excess water and combines with carbohydrate polymers to form complex structures having chemical linking, suggesting effect of coexisting carbohydrate polymers and water molecules on molecular motion [8].