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Review

Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects

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HIGHLIGHTS

- By-products of lignocellulose pretreatment inhibit microbial and enzymic biocatalysts.
- Groups of inhibitors from components of lignocellulose are reviewed.
- The review covers different strategies to alleviate inhibition problems.
- Industrial implementation increases the relevance of inhibitor management.

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ABSTRACT

Biochemical conversion of lignocellulosic feedstocks to advanced biofuels and other commodities through a sugar-platform process involves a pretreatment step enhancing the susceptibility of the cellulose to enzymatic hydrolysis. A side effect of pretreatment is formation of lignocellulose-derived by-products that inhibit microbial and enzymatic biocatalysts. This review provides an overview of the formation of inhibitory by-products from lignocellulosic feedstocks as a consequence of using different pretreatment methods and feedstocks as well as an overview of different strategies used to alleviate problems with inhibitors. As technologies for biorefining of lignocellulose become mature and are transferred from laboratory environments to industrial contexts, the importance of management of inhibition problems is envisaged to increase as issues that become increasingly relevant will include the possibility to use recalcitrant feedstocks, obtaining high product yields and high productivity, minimizing the charges of enzymes and microorganisms, and using high solids loadings to obtain high product titers.
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1. Introduction

Dwindling fossil resources and environment pollution related to the exploitation of petroleum and coal make it necessary to consider a gradual transition towards a bio-based economy. While the future supply of energy is likely be based on a wide range of alternative platforms, such as wind, water, solar fuels, and biomass, among others, the production of chemicals will increasingly depend on plant biomass (FitzPatrick et al., 2010). Lignocellulosic biomass from agriculture and forestry, which includes agro-industrial residues, forest-industrial residues, energy crops, municipal solid waste, and other materials, is the most abundant bioresource to consider as feedstock for biorefineries that complement oil refineries as sources of fuels and platform chemicals.

Utilization of lignocellulosic materials for biochemical conversion in biorefineries requires pretreatment for disrupting the close inter-component association between main constituents of the plant cell wall (Yang and Wyman, 2008). Pretreatment clears away the physical and chemical barriers that make native biomass recalcitrant and makes cellulose amenable to enzymatic hydrolysis, which is a key step in biochemical processing of lignocellulose based on the sugar platform concept. This effect is achieved by increasing the accessible cellulose surface area through solubilization of hemicelluloses and/or lignin, which are coating the cellulose of the native biomass.

While the aims of pretreatment are to uncover the cellulose for enzymatic saccharification and fractionating the main components of the feedstock, pretreatment often involves side reactions resulting in lignocellulose-derived by-products that are inhibitory to downstream biochemical processes. Inhibition problems become more significant as the by-products accumulate as a result of recirculation of process water, or as their concentration increases when

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high solids loadings are used to achieve concentrated sugar streams and high product titers.

There are several older reviews on inhibitors, for example Palmqvist and Hahn-Hägerdal (2000) and Klinke et al. (2002), as well as more recent ones, such as Pienkos and Zhang (2009), Jönsson et al. (2013), and Ko et al. (2015). The aims of this review are to provide, in brief, an updated overview of the origin and characteristics of different groups of inhibitory substances, and, with focus on the more recent literature, examine different remedies that can be used to alleviate inhibition problems. We provide a new scheme of groups of inhibitory substances, and pay attention to inhibition of both microbial cells and cellulolytic enzymes.

2. Pretreatment

Most lignocellulose-derived inhibitors form during pretreatment when hemicelluloses and/or lignin are solubilized and degraded (Fig. 1). Extractives and cellulose that is unintentionally affected by the pretreatment are other sources (Fig. 1). Since the formation of inhibitory substances is much dependent on the pretreatment process, this review includes a brief discussion on the most commonly used pretreatment techniques, as summarized in Table 1. Only pretreatment methods that are relevant with respect to formation of inhibitors and that are of interest for industrial implementation are covered.

2.1. Acid-based methods

Acid hydrolysis is one of the most promising pretreatment methods with respect to industrial implementation. It is usually performed with mineral acids, but organic acids and sulfur dioxide are other options. Dilute sulfuric acid pretreatment has been studied for a wide range of lignocellulosic biomass (Yang and Wyman, 2008; Hu and Ragauskas, 2012). It results in high recovery of the hemicellulosic sugars in the pretreatment liquid, and in a solid cellulose fraction with enhanced enzymatic convertibility. Acid pretreatment has also some drawbacks, such as high cost of the materials used for construction of the reactors, gypsum formation

Table 1
Overview of pretreatment methods for lignocellulosic feedstocks prior to enzymatic hydrolysis of cellulose.

Pretreatment methods	Main effect	Used chemicals	By-product formation
Acid-based methods	Hydrolysis of hemicelluloses to monosaccharides	Involve catalysts such as H_2SO_4, SO_2, HCl, H_3PO_4	Aliphatic carboxylic acids, phenylic compounds, furans, etc. (see Fig. 1)
Hydrothermal processing	Solubilization of hemicelluloses without complete hydrolysis	No additives	Acetic acid, minor amounts of furan aldehydes
Mild alkaline methods	Removal of lignin and a minor part of hemicelluloses	Involve alkali such as NaOH, $Ca(OH)_2$, NH_3	Acetic acid, hydroxy acids, dicarboxylic acids, phenolic compounds
Oxidative methods	Removal of lignin and part of hemicelluloses	Involve oxidants such as H_2O_2 and O_2 (alkaline conditions), and O_3	Aldonic and aldaric acids, furoic acid, phenolic acids, acetic acid
Chemical pulping processes	Methods that target lignin and to some extent hemicelluloses	Kraft pulping, sulfite pulping, soda pulping, organosolv pulping	Aliphatic acids
Alternative solvents	Dissolution of specific lignocellulosic components or the whole biomass	Ionic liquids	Dependent on solvent and conditions

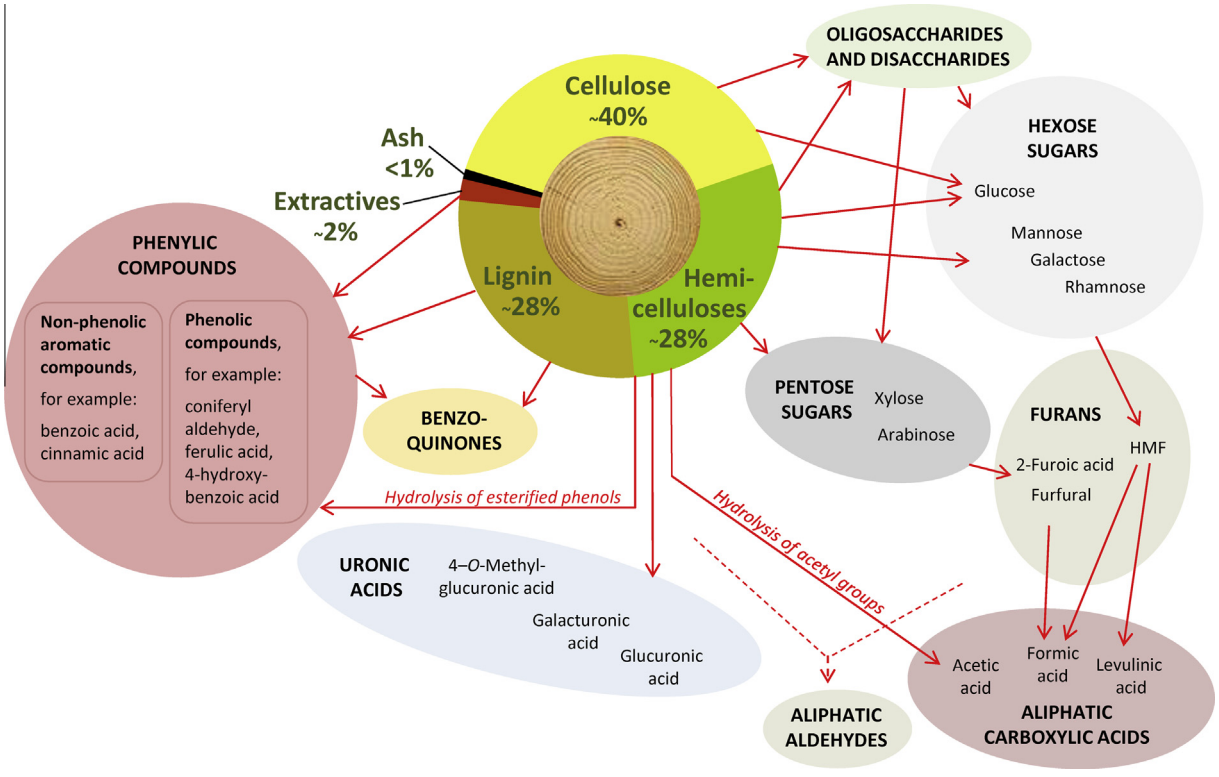

Fig. 1. Degradation products from lignocellulose as a result of pretreatment under acidic conditions. Numbers indicate fractions of constituents of wood of Norway spruce. Red arrows indicate tentative formation pathways. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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