# Utilization Methods for Lignocellulosic Biomass

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Utilization of lignocellulosic biomass (LCB) for bioenergy production has been widely investigated in the previous decades, as it provides an excellent environmentally friendly alternative to non-renewable energy sources. There are numerous procedures to convert plant-based biomass into usable feedstock or valuable energy carriers, such as $C_5$ and $C_6$ sugars, organic acids, or more commonly, bioethanol and biogas.

Keywords: Lignocellulosic Biomass ; Utilization

## 1. General Characteristics of Lignocellulosic Biomass

Lignocellulosic biomass (LCB) is the most abundant raw material and biological resource on Earth, and is estimated to make up about 80% (450 Gt of carbon) of the total biomass [1][2]. Utilization of LCB for bioenergy production has been widely investigated in the previous decades, as it provides an excellent environmentally friendly alternative to non-renewable energy sources. There are numerous procedures to convert plant-based biomass into usable feedstock or valuable energy carriers, such as $C_5$ and $C_6$ sugars, organic acids, or more commonly, bioethanol and biogas. Lignocellulose itself represents a complex structural component of plant cell walls, composed primarily of three different biopolymers: cellulose, hemicellulose (collectively called holocellulose), and lignin. Cellulose, which is the most abundant, is a homopolysaccharide consisting of β-1,4-linked D-glucose units which are arranged into ordered fibrils. These fibrils form crystalline microfibrils with high chemical stability—this provides rigidity and strength to the plant cell wall [3]. Hemicellulose, in contrast, is a heteropolysaccharide, which acts as a filler material interfacing with the cellulose microfibrils. Hemicellulose is diverse in its monosaccharide composition and linkage patterns, depending on the species of the plant; however, the different sugar components are mainly xylose, arabinose, glucose, mannose, galactose, and rhamnose [4]. Lignin is a complex phenolic polymer, which provides resistance to microbial infections for the plant and contributes to water impermeability. It is composed of different phenylpropanoid units—among others, coniferyl, sinapyl, and p-coumaryl alcohols—connected through various linkages (primarily carbon–carbon and ether bonds), and its presence and abundance greatly vary among different species and even different tissues within the same plant. Due to its complex and unique structure, the degree of lignification can present difficulties when it comes to LCB utilization.

## 2. Utilization Methods for Lignocellulosic Biomass

Physical pre- or post-processing methods, such as mechanical milling, thermal treatments, microwave, or ultrasonic irradiation aim to disrupt the primary structure of the LCB fibers, improve the bioavailability of certain organic compounds, and/or increase the specific surface area of the biomass [5][6][7]. Chemical methods, like acid hydrolysis, alkaline treatment, or ionic liquids, aim to disrupt the lignin structure and thus make the polysaccharides more accessible [8][9]. Less intensive and more environmentally friendly biological methods harness the enzymatic capabilities of microorganisms or purified enzymes to convert the LCB material into fermentable sugars, which can be combined with physical and chemical treatment methods as well [10][11].

One of the major overall indicators of LCB utilization efficiency is bioethanol production, which can be achieved via four different major techniques: separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), consolidated bioprocessing (CPB), and simultaneous and saccharification co-fermentation (SSCF). Since CPB and SSCF usually grant a relatively low bioethanol yield and/or present difficulties in terms of optimization [12], the most commonly used methods are SHF and SSF. During SHF, the hydrolysis of the LCB material is performed first, followed by a separate fermentation step. This holds the advantage that the enzymes needed for the breakdown of cellulose strands and the microorganisms used for the ethanol fermentation can each be optimized for their own step, potentially leading to higher efficiency [13]. However, since these two sub-processes take place separately in place and time, SHF is considered time-consuming and more expensive in contrast to SSF. During the latter, hydrolysis and fermentation occur at the same time and place, which means that the produced glucose (and/or other sugars) cannot accumulate inside the reactor, thus

eliminating the possibility of feedback inhibition. Nevertheless, this approach means that the conditions (like pH, temperature) must be a compromise that allows both the hydrolysis enzymes and the fermentation organisms to function, which may not be optimal for either process [14].

In order to provide fermentable sugars to microorganisms to convert them further into ethanol, first the cellulose and hemicellulose need to be hydrolyzed into sugar monomers. This process is called saccharification, which is achieved by either using adequate microorganisms, purified enzymes, or chemicals. The former two are considered to be the most environmentally friendly approaches, and since enzymes can provide the same efficiency overall as acidic hydrolysis under certain conditions [15], the enzymatic hydrolysis is usually the most favorable method for saccharification. The enzymatic process primarily involves cellulases and hemicellulases, systematically breaking down complex carbohydrate polymers into fermentable sugars. The cellulase complex includes endoglucanases, exoglucanases, and β-glucosidases working synergistically to degrade cellulose. Likewise, hemicellulose is hydrolyzed by a mixture of enzymes, including xylanases, mannanases, and others, depending on its heterogenous composition. One of the greatest challenges in this process is due to the presence of lignin, and the overall rigidity and stability of the LCB fiber's components. Using standalone enzymes usually grants low yield and efficiency [16], and therefore a preliminary pre-processing step is needed to enhance the process [17]. The array of these pre-treatment methods is broad, as discussed above: a range of physical, thermal, chemical, biological, and combined treatments are available, and have been intensively investigated in the past several years. Among these, the utilization of microwave irradiation has shown promising results, due to its fast, selective, and volumetric heating capabilities. Microwaves (300 MHz–3 GHz electromagnetic waves), when absorbed in certain materials, are converted into heat through two major phenomena: dipole rotation and ionic conduction [18]. Molecules that have either permanent or induced dipolarity (like water) will arrange correspondingly to the polarity of the applied electromagnetic field. In case of a 2.45 GHz—the most commonly used microwave—frequency, this means $4.9 \times 10^9$ revolutions per second. The rapid rotational movement induces friction between the adjacent molecules, which ultimately leads to fast and excessive heat generation. For ionic molecules, the periodically changing electromagnetic field causes movement, or "migration", in the direction of the corresponding polarity, which also leads to friction and eventually heat formation [19]. Since plant-based biomass evidently contains plant cells with excessive free and bounded water content, microwave irradiation as a pre-processing method is certainly favorable [17]. The water content inside the plant cells under the influence of microwave irradiation rapidly heats up, which results in a great increment of pressure—to such an extent that the cell wall no longer can sustain, and, in the end, it disrupts [20]. Under this method, the enzymes used for LCB saccharification have better availability to the substrate molecules, which results in higher yields and/or shortening the time of hydrolysis [21][22][23]. Moreover, if microwave irradiation is coupled with chemical treatments (mostly alkaline addition), the degree of lignification can also be reduced [24].

The next step in bioethanol production after cellulose hydrolysis is the ethanol fermentation itself. Upon completion of the hydrolysis process, the resulting mixture consists primarily of glucose and xylose, the simplest forms of sugar derived from cellulose and hemicellulose, respectively. The most commonly used microorganisms, i.e., *Saccharomyces cerevisiae* or other yeasts, metabolize the simple sugars under anaerobic conditions and convert them into ethanol. The process of fermentation involves glycolysis, a biochemical pathway wherein one glucose molecule is converted into two molecules of pyruvate, yielding a small amount of energy. Pyruvate is then further metabolized anaerobically to ethanol and carbon dioxide. The fermentation process can take anywhere from hours to days, depending on the specific conditions and the strain of yeast used [25]. The end product, ethanol, can then be distilled and purified to produce bioethanol fuel. Meanwhile, the carbon dioxide released during fermentation can be captured and used in various industrial applications, thereby contributing to a closed carbon cycle and promoting environmental sustainability [26]. However, managing the by-products and potential inhibitors generated during the preceding steps of biomass pretreatment and hydrolysis remains a significant challenge in optimizing the efficiency of the SHF process [27].

Another possible and commonly used method of LCB utilization is biogas fermentation, especially in the form of co-fermentation with wastewater sludge [28], which presents a unique opportunity to enhance biogas production while also addressing waste-management issues [29]. As discussed above, the complex and resistant nature of LCB compounds usually necessitates the use of pre-processing methods to make the biomass components more accessible for subsequent microbial degradation. This means that using (appropriately prepared) bioethanol fermentation residues—which have already undergone pre-treatment—can be a promising alternative as a feed material for co-fermentation with sludge to achieve an optimal nutrient profile. The major downside of—especially industrial—sludge samples is the unfavorable C/N ratio, the sludge being relatively low on carbon content and usually high in nitrogen [30]. In contrast to this, plant-based biomass contains much more—typically organic—carbon, and most of the time only just a negligible amount of nitrogen. Thus, mixing these two feedstocks appropriately can create a favorable C/N ratio for biogas production, which is one of the most important factors in terms of anaerobic digestion (AD) [31]. The co-fermentation process occurs within an anaerobic digester, where the combined feedstock is broken down over several stages:

hydrolysis, acidogenesis, acetogenesis, and methanogenesis [32]. Co-fermentation offers several advantages over the standalone use of LCB. It allows increased overall biogas yield, reduced digestion time, and more efficient utilization of available resources [33]. Additionally, by integrating waste management with renewable energy production, co-fermentation also contributes to environmental sustainability.

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