Lignocellulose as raw material in fermentation processes

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Lignocellulose in the form of forestry, agricultural, and agro-industrial wastes is accumulated in large quantities every year. These materials are mainly composed of three groups of polymers, namely cellulose, hemicellulose, and lignin. Cellulose and hemicellulose are sugar rich fractions of interest for use in fermentation processes, since microorganisms may use the sugars for growth and production of value added compounds such as ethanol, food additives, organic acids, enzymes, and others. Submerged and solid-state fermentation systems have been used to produce compounds of industrial interest from lignocellulose, as an alternative for valorization of these wastes and also to solve environmental problems caused by their disposal. When submerged fermentation systems are used, a previous stage of hydrolysis for separation of the lignocellulose constituents is required. This work is an overview about the potential uses of lignocellulosic materials in fermentation processes. Aspects related to submerged and solid-state fermentation systems will be described focusing on the raw materials, hydrolysis processes, fermentation conditions, microorganisms, and products that can be obtained.

Keywords lignocellulose; cellulose; hemicellulose; lignin; submerged fermentation; solid-state fermentation

1. Lignocellulose structure

Lignocellulosic biomass comprising forestry, agricultural and agro-industrial wastes are abundant, renewable and inexpensive energy sources. Such wastes include a variety of materials such as sawdust, poplar trees, sugarcane bagasse, waste paper, brewer’s spent grains, switchgrass, and straws, stems, stalks, leaves, husks, shells and peels from cereals like rice, wheat, corn, sorghum and barley, among others. Lignocellulose wastes are accumulated every year in large quantities, causing environmental problems. However, due to their chemical composition based on sugars and other compounds of interest, they could be utilized for the production of a number of value added products, such as ethanol, food additives, organic acids, enzymes, and others. Therefore, besides the environmental problems caused by their accumulation in the nature, the non-use of these materials constitutes a loss of potentially valuable sources.

The major constituents of lignocellulose are cellulose, hemicellulose, and lignin, polymers that are closely associated with each other constituting the cellular complex of the vegetal biomass. Basically, cellulose forms a skeleton which is surrounded by hemicellulose and lignin (Fig. 1).

Cellulose is a high molecular weight linear homopolymer of repeated units of cellobiose (two anhydrous glucose rings joined via a β-1,4 glycosidic linkage) [1]. The long-chain cellulose polymers are linked together by hydrogen and van der Walls bonds, which cause the cellulose to be packed into microfibrils [2]. By forming these hydrogen bounds, the chains tend to arrange in parallel and form a crystalline structure. Therefore, cellulose microfibrils have both highly crystalline regions (around 2/3 of the total cellulose) and less-ordered amorphous regions. More ordered or crystalline cellulose is less soluble and less degradable [3, 4].

Hemicellulose is a linear and branched heterogeneous polymer typically made up of five different sugars - L-arabinose, D-galactose, D-glucose, D-mannose, and D-xylose - as well as other components such as acetic, glucuronic, and ferulic acids. The backbone of the chains of hemicelluloses can be a homopolymer (generally consisting of single sugar repeat unit) or a heteropolymer (mixture of different sugars). According to the main sugar residue in the backbone, hemicellulose has different classifications e.g., xylans, mannans, glucans, glucuronoxylans, arabinoxylans, glucomannans, galactomannans, galactoglucomannans, β-glucans, and xyloglucans. When compared to cellulose, hemicelluloses differ thus by composition of sugar units, by presence of shorter chains, by a branching of main chain molecules, and to be amorphous [5], which made its structure easier to hydrolyze than cellulose.

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Lignin is a very complex molecule constructed of phenylpropane units linked in a large three-dimensional structure. Three phenyl propionic alcohols exist as monomers of lignin: p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol. Lignin is closely bound to cellulose and hemicellulose and its function is to provide rigidity and cohesion to the material cell wall, to confer water impermeability to xylem vessels, and to form a physic-chemical barrier against microbial attack [5]. Due to its molecular configuration, lignins are extremely resistant to chemical and enzymatic degradation [6].

The amounts of carbohydrate polymers and lignin vary from one plant specie to another. In addition, the ratios between various constituents in a single plant may also vary with age, stage of growth, and other conditions. However, cellulose is usually the dominant structural polysaccharide of plant cell walls (35–50%), followed by hemicellulose (20–35%) and lignin (10–25%) [7]. Average values of the main components in some lignocellulose wastes are shown in Table 1.

<table>
<thead>
<tr>
<th>Lignocellulose waste</th>
<th>Cellulose (wt %)</th>
<th>Hemicellulose (wt %)</th>
<th>Lignin (wt %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley straw</td>
<td>33.8</td>
<td>21.9</td>
<td>13.8</td>
</tr>
<tr>
<td>Corn cobs</td>
<td>33.7</td>
<td>31.9</td>
<td>6.1</td>
</tr>
<tr>
<td>Corn stalks</td>
<td>35.0</td>
<td>16.8</td>
<td>7.0</td>
</tr>
<tr>
<td>Cotton stalks</td>
<td>58.5</td>
<td>14.4</td>
<td>21.5</td>
</tr>
<tr>
<td>Oat straw</td>
<td>39.4</td>
<td>27.1</td>
<td>17.5</td>
</tr>
<tr>
<td>Rice straw</td>
<td>36.2</td>
<td>19.0</td>
<td>9.9</td>
</tr>
<tr>
<td>Rye straw</td>
<td>37.6</td>
<td>30.5</td>
<td>19.0</td>
</tr>
<tr>
<td>Soya stalks</td>
<td>34.5</td>
<td>24.8</td>
<td>19.8</td>
</tr>
<tr>
<td>Sugarcane bagasse</td>
<td>40.0</td>
<td>27.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Sunflower stalks</td>
<td>42.1</td>
<td>29.7</td>
<td>13.4</td>
</tr>
<tr>
<td>Wheat straw</td>
<td>32.9</td>
<td>24.0</td>
<td>8.9</td>
</tr>
</tbody>
</table>

2. Pre-treatments for selective separation of lignocellulose constituents

Selective and effective separation of the main fractions present in lignocellulose biomass is of great interest, mainly when submerged fermentation process will be subsequently performed, because the by-products obtained during hydrolysis of these materials may affect the fermentation yield, and also because valuable sources are lost if other fractions are partially hydrolyzed. Due to the structural differences among these fractions, separation of cellulose, hemicellulose and lignin from lignocellulose biomass requires the use of specific processes, which may be physical, physico-chemical, chemical or biological. There is a variety of methods that have been proved to be efficient for the lignocellulose hydrolysis. The most commonly used are briefly described below.

2.1 Cellulose hydrolysis

Chemical and enzymatic methods are the most common techniques for hydrolyzing cellulose. The chemical method, also known as concentrated acid hydrolysis, is conducted with mineral acids such as H$_2$SO$_4$ or HCl (in the range of 10–30%), at temperatures of about 160 ºC and pressures of about 10 atm [9, 10]. These harsh conditions (high temperature and acid concentration) are needed to liberate glucose from the tightly associated chains, because most cellulose is crystalline. In this process, acid concentration, temperature and time are crucial factors, and must be controlled to avoid the sugars and lignin degradation to by-products [11]. If present in the raw material to be hydrolyzed, hemicellulose is also removed by using concentrated acids [12].

Enzymatic hydrolysis has attracted increasing attention as an alternative to concentrated acid hydrolysis because the process is highly specific, can be performed under milder reaction conditions (pH around 5 and temperature less than 50 ºC) with lower energy consumption and lower environmental impact. In addition, it does not present corrosion problems, and gives high yield of pure glucose with low formation of by-products that is favorable for the subsequent hydrolysate use in fermentation processes [13–15]. Enzymatic hydrolysis of cellulose is a reaction carried out by cellulase enzymes, which correspond to a mixture of several enzymes, among which at least three major groups are involved in the hydrolysis of cellulose: (1) β-1,4-endoglucanase (EC 3.2.1.4.), which attacks regions of low crystallinity in the cellulose fiber creating free chain ends; (2) β-1,4-exoglucanase or cellobiohydrolase (EC 3.2.1.91.), which degrades the molecule further by removing cellobiose units from the free chain ends; (3) β-glucosidase (EC 3.2.1.21.), which hydrolyzes cellobiose to produce glucose [9, 16, 17]. A schematic representation of the cellulase enzymes over the cellulose structure is shown in Fig. 2. A wide variety of cellulolytic fungi and bacteria have been reported. Among them, the enzyme system secreted by the filamentous fungus *Trichoderma reesei* has been intensively studied. Usually, lignocellulosic materials must be pretreated prior to the enzymatic hydrolysis in order to make the cellulose more accessible to enzymes [14, 18, 19].
2.2 Hemicellulose hydrolysis

Dilute acid hydrolysis is the most employed technique for the hemicellulose breakdown. In this process, the use of diluted acids (1-4%), under moderate temperatures (120 to 160 °C), has proven to be adequate for hemicellulose hydrolysis, promoting little sugar decomposition [11, 20]. H$_2$SO$_4$ is the usual acid employed although HCl, HNO$_3$, and H$_3$PO$_4$ are also used. The mechanism involved in the acid hydrolysis of hemicellulose is similar to that for cellulose: acid catalyzes the breakdown of long hemicellulose chains to form shorter chain oligomers and then to sugar monomers. However, because hemicellulose is amorphous, less severe conditions are required to release hemicellulose sugars. Other important advantages are the generation of lower degradation products as well much less corrosion problems in hydrolysis tanks.

Steam explosion is also a method commonly used for hemicellulose hydrolysis. In this method, the biomass is heated using high-pressure saturated steam (0.69–4.83 MPa, 160–260 °C) for a short period (from seconds to few minutes). Steam condenses under high pressure, thereby wetting the material, and then the pressure is suddenly reduced, which makes the material undergo an explosive decompression [10, 12]. Combination of acetic acid with sudden depressurization, promote the hemicellulose hydrolysis and solubilization. Although avoiding acid catalysts is stated as an advantage of this method, addition of an acid catalyst improves hemicellulose hydrolysis and decreases production of degradation compounds [9, 21]. Limitations of steam explosion include an incomplete disruption of the lignin–carbohydrate matrix, and generation of compounds that may be inhibitory to microorganisms [9].

Autohydrolysis is a process similar to the steam explosion, but in this case, the explosion does not occur. This process uses compressed liquid hot water (≈ 200 °C; pressure > saturation point) and the acids resulting from hydrolysis of acetyl and uronic groups, originally present in hemicelluloses, catalyze hydrolysis of links between hemicellulose and lignin as well as between the carbohydrates. This process is able to hydrolyze hemicellulose in minutes, with high yield, low by-products formation and no significant lignin solubilization [12].

Specific microorganisms or a suitable cocktail of enzymes known as hemicellulases can also promote the hemicellulose hydrolysis. Hemicellulases are produced by many species of bacteria and fungi, as well as by several plants. A number of hemicellulases, including xylanases and mannanases, have been identified in *Trichoderma reesei* [22]. Today, most commercial hemicellulase preparations are produced by genetically modified *Trichoderma* or *Aspergillus* strains. Biological treatments have some advantages such as the high specificity, low energy consumption, no chemical requirement, and mild environmental conditions thus avoiding sugar degradation and resulting in high sugar yields [9].

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**Fig. 2** Schematic representation of the cellulase enzymes over the cellulose structure.
2.3 Lignin hydrolysis

Alkaline treatments, ozonolysis, peroxide and organosolv treatments are some of the methods usually employed for lignin removal from lignocellulose biomass. Such methods are effective for lignin solubilization but in most of them, part of the hemicellulose is also hydrolyzed.

Alkaline treatments refers to the application of alkaline solutions such as NaOH, Ca(OH)\(_2\) or ammonia. Among these, treatment with NaOH is one of the most used for delignification of agricultural residues [23]. The alkali treatment causes swelling, leading to an increase in internal surface area, a decrease in the degree of polymerization, a decrease in crystallinity, separation of structural linkages between lignin and carbohydrates, and disruption of the lignin structure [24]. As a consequence, the lignin is dissolved from the raw material, being separated in the form of a liquor rich in phenolic compounds that represents the process effluent [5, 25]. The inconvenient of this technique is that it also degrades part of the hemicellulose.

Hydrogen peroxide treatment utilizes alkaline solutions at temperatures higher than 100 °C, which promote a fast decomposition of H\(_2\)O\(_2\). As a consequence, more reactive radicals such as hydroxyl radicals (HO) and superoxide anions (O\(_2^-\)) are produced, which are responsible for lignin degradation. This technique is commonly used in paper and pulp industries for bleaching and delignification purposes (to improve the brightness of pulp as it reacts with colored carboxyl-containing structures in the lignin). However, delignification by this process on a large scale can be costly. Similarly to the alkaline treatment, during the lignin solubilization by hydrogen peroxide part of the hemicellulose is also removed from the material structure [8].

Treatment with organosolvents involves the use of an organic liquid (for example, methanol, ethanol, acetone, ethylene glycol or triethylene glycol) and water, with or without addition of catalysts such as oxalic, salicylic, and acetylsalicylic acid. This mixture hydrolyzes lignin bonds and lignin-carbohydrate bonds, but many of the carbohydrate bonds in the hemicellulose components are also broken. Lignin is dissolved as a result of the solvent action and the cellulose remains in the residual solid material [4, 9].

Ozone treatment is another way of reducing the lignin content of lignocellulosic materials. Lignin attacks as a scavenger during this pre-treatment because it consumes most of ozone during the degradation of the carbohydrate content. As a consequence, low ozone amounts are available for cellulose degradation. However, this treatment may also attacks the cellulose and hemicellulose components besides the lignin molecule. Cellulose degradation has been attributed partly to a direct reaction of ozone with the glycosidic linkage and partly to a free radical mediated oxidation of hydroxyl groups in glucose [8, 26]. Some advantages of this treatment are that ozonolysis are carried out at room temperature and normal pressure. Furthermore, since ozone can be easily decomposed by using a catalytic bed or increasing the temperature, this process can be designed to minimize environmental pollution [10].

Biological treatments, based on the use of brown-, white- and soft-rot fungi have been commonly used to degrade the lignin, being considered a cheap and effective method of delignification. Degradation of lignin by fungi such as *Phanerochaete chrysosporium*, *Trametes versicolor*, *Trametes hirsuta* and *Bjerkandera adusta*, may be used to allow better access to the cellulose and hemicellulose components, besides to be also considered as an effective biological detoxification alternative. Main problems in using biological methods are that fungi may also attacks cellulose and hemicellulose, and hydrolysis rate in most biological materials is very low [9]. Three main enzymes are involved in the lignin biodegradation, namely lignin peroxidise, manganese peroxidise, and laccase. These enzymes have gained large attention by their industrial applications in pulp and paper industries, for biochemical pulping and decolorization of bleach plant effluent.

Although each treatment is more efficient to remove a specific fraction of lignocellulose, in some cases, a combination of them is required to achieve an efficient conversion of the fraction of interest. Biological or enzymatic treatments, for example, cannot be applied directly on the raw materials because lignin hinders the attack of fungi or enzymes to the material cell wall. Therefore, for the enzymatic hydrolysis of cellulose is crucial a previous hydrolysis step to promote a partial removal of lignin and hemicellulose, so that the cellulose fibers become more accessible to the enzymes attack. In addition, the best method and conditions of treatment depend greatly on the type of lignocelluloses, varying thus, with the raw material used. It is important emphasizing that the selection of a treatment method affects the cost and performance in the subsequent hydrolysis and fermentation stages. Ideal hydrolysis process should achieve high yields of fermentable reducing sugars, avoid degradation or loss of yielded sugars and the formation of inhibitors to the subsequent fermentation, and require minimal energy, chemicals and equipments [27].

3. Fermentation processes for value added compounds production from lignocellulose

3.1 Submerged fermentation systems

Submerged fermentation (SmF) systems can be defined as the cultivation of microorganisms in a liquid medium containing soluble carbon source and nutrients, maintained or not under agitation. Several characteristics make these systems attractive for the microorganisms cultivation and production of biological products, which include: i) the mixture of the medium is easy and allows uniform conditions for the microorganism growth; ii) modification of the
cultivation conditions like pH, dissolved oxygen, temperature, agitation, and nutrient concentration are easy and fast; iii) the temperature control is favored by the high specific heat and thermal conductivity; iv) efficient technologies have already been developed, with high automation grade, diversity and availability of equipments. Countless microorganism strains have been used in fermentation processes by submerged cultivation, including bacteria, yeasts, fungi and algae. Fermentation media used in these systems may be synthetically formulated or produced by hydrolysis of lignocellulose.

Sugars released from cellulose and hemicellulose structures can be microbiologically converted into various products of industrial interest by SmF systems, including organic acids, ethanol, glycerol, food additives, butanol, etc.... However, bioconversion of these hydrolysates is not a simple process, but requires considerable attention in many points so that high yields and productivities may be attained. One of these points is related to the hydrolysates composition. Usually the hydrolysis processes not only extract sugars from the lignocellulose structure, but a broad range of compounds proceeding from the lignin or originated from the sugars degradation can also be found in the hydrolysates, being their concentration dependent of the raw material and hydrolysis process used. Such compounds are toxic for the microorganisms and therefore, previous the use as fermentation medium, lignocellulosic hydrolysates should be submitted to a detoxification process to become a more suitable medium for the microorganisms’ cultivation [28]. A number of detoxification methods, including biological, physical, and chemical ones, have been proposed to transform inhibitors into inactive compounds or to reduce their concentration. The effectiveness of a detoxification method depends both on the type of hemicellulose hydrolysate and on the species of microorganism employed, because each type of hydrolysate has a different degree of toxicity, and each species of microorganism has a different degree of tolerance to inhibitors. In some cases, the addition of nutrient sources to the hydrolysate may be necessary to improve the bioconversion yield. Another point requiring considerable attention is when hemicellulosic hydrolysates are used as fermentation medium. Such hydrolysates contain a mixture of pentose and hexoses sugars, and thus, for an efficient bioconversion process, the fermenting microorganism should be able to consume and/or convert both kinds of sugars.

3.1.1 Cellulose bioconversion

Currently, ethanol production is one of the most studied and promising alternative for cellululosic biomass conversion, due to the large incentive that has been given to biofuels use in replacement of gasoline. In addition, ethanol production from lignocellulosic wastes is very attractive because of their low cost and abundance, and non-competition with foodstuffs. Therefore, a large variety of lignocellulosic materials including wood, straws, agricultural wastes, and crop residues have been evaluated for use in this bioconversion process. The basic process steps in producing ethanol from cellulose biomass consist in an initial treatment (for example, diluted acid, alkaline or steam explosion) to render cellulose more accessible to the subsequent step of enzymatic hydrolysis, which break down polysaccharides to simple sugars [29]. The glucose solution obtained is fermented to ethanol by microorganisms; *Saccharomyces cerevisiae* being the most currently used since it gives high ethanol yields from glucose.

Together with bioethanol, biogas production from lignocellulosic biomass has shown enormous potentialities for sustainable bioenergy production. The H2 production from lignocellulosic materials can be performed by anaerobic fermentation process. However, direct conversion of lignocellulosic biomass to hydrogen needs a previous treatment to hydrolyze the cellulose crystalline structure. The cellulose present in the raw material may be hydrolyzed by using acids such as HCl, for example. The hydrolysate obtained (rich in glucose and some hemicellulose sugars) is thus converted to biogas by natural anaerobic microflora. Butyrate and acetate may be obtained as by-products of the hydrogen fermentation [30]. Biogas rich in H2 and CH4 may be also produced by fermentation of glucose from lignocellulose. In this case, it is necessary to treat the raw material with chemicals such as NaOH solution to destroy and remove the solid lignin. Then, the released cellulose is hydrolyzed with cellulase into reducing sugars, which can be fermented with anaerobic bacteria to produce biogas [31].

Besides biofuels, several organic acids, including lactic, citric, acetic, and succinic acids, may be produced by cellulose conversion. Lactic acid may be produced from lignocellulosic materials by sequential steps of chemical processing (in order to make the cellulose more accessible to the enzymes), enzymatic saccharification (for obtaining solutions containing glucose as main sugar) and finally, the hydrolysate fermentation by microorganisms, especially *Lactobacillus species* [32]. The conventional process for cellulose biomass conversion to acetic acid includes also an initial stage of acid or enzymatic hydrolysis of the substrate, followed by yeast fermentation and oxidation to acetic acid by *Acetobacter* sp [33]. Similarly to these two cases, citric acid can also be produced by fermentation of glucose rich hydrolysates produced from cellulose [34]. Several moulds such as *Penicillium luteum*, *P. citrinum*, *Aspergillus niger*, *A. wentii*, *A. clavatus*, *Mucor piriformis*, *Citromyces pfefferianus*, *Paecilomyces darcariatum*, and *Trichoderma viride*, and yeasts such as *Yarrowia lipolytica* and *Candida guilliermondii* are able of producing this acid. Succinic acid is another acid that can be produced by fermentation of cellulolic hydrolysates. Many agricultural and industrial wastes, for example, whey, wheat, straw and wood, have been reported as raw carbon materials for the production of succinic acid. Wood hydrolysate prepared by steam explosion followed by enzymatic hydrolysis of the cellulose rich residue was successfully fermented to succinic acid by *Mannheimia succiniciproducens* bacteria [35]. Corn stalk and cotton stalk hydrolysates produced by a sequence of steam explosion and enzymatic hydrolysis treatments were fermented to succinic acid by *Actinobacillus succinogenes* [36]. Several microorganisms have ability to produce this acid, among of which, *Anaerobiospirillum succiniciproducens* is considered the most efficient producer. This microorganism only
Aspergillus niger is a promising microorganism for this bioprocess since this yeast is able to transform both pentoses and hexoses sugars.

Production of carotenoids (natural pigments with a variety of applications in food technology) such as astaxanthin has already been performed by fermentation of wood hydrolysate. Hydrolysates containing glucose and cellobiose or glucose only, were obtained by alkaline- or acid-processed wood treated with a cellulase complex. Such hydrolysates were suitable as fermentation media for carotenoids production by Xanthophyllomyces dendrorhous [37, 38].

3.1.2 Hemicellulose bioconversion

Fermentation of hemicellulosic hydrolysates is usually more complicated than fermentation of cellulosic hydrolysates, due to the presence of pentoses sugars such as xylose, which is not metabolized by large number of microorganisms. Even though, efforts have been directed to the use of this fraction from lignocellulose. Currently, one of the technologies that have been strongly investigated for conversion of hemicellulose sugars is for ethanol production. During the process for ethanol production from cellulose, a hemicellulosic hydrolysate is generated in the first step of raw material treatment (usually performed with dilute acids or steam explosion). Utilization of the sugars released during this stage (hemicellulosic sugars) is considered essential for efficient and cost-effective conversion of lignocellulose to ethanol. The main challenge of this process is that Saccharomyces cerevisiae, which is the most widely used microorganism for ethanol production, does not utilize pentose sugars. Pichia stipitis has been described as a promising microorganism for this bioprocess since this yeast is able of transforming both pentoses and hexoses sugars into ethanol, which is an important advantage since both kinds of sugars are currently found in hemicellulosic hydrolysates [39]. Some bacteria, such as Escherichia coli, Klebsiella, Erwinia, Lactobacillus, Bacillus, and Clostridia, can utilize mixed sugars but produce no, or only a limited quantity of ethanol [7]. Some attempts have also been made to genetically modify S. cerevisiae and other microorganisms in order to produce ethanol with high yields from both, hexoses and pentoses [40].

Butanol is another biofuel that can be produced from hemicellulose bioconversion. As a product of ABE (acetone butanol ethanol) fermentation, butanol is an excellent feedstock chemical and a superior fuel compared to ethanol. This compound can be produced by fermentation of dilute sulfuric acid hemicellulosic hydrolysates produced from agricultural wastes such as barley straw, corn stover and switchgrass. A variety of microorganisms are able of converting hemicellulose sugars to butanol, but the most commonly used strains are Clostridium acetobutylicum and Clostridium beijerinckii [41, 42].

Xylitol, a five-carbon sugar alcohol that can be used as a natural food sweetener, a dental caries reducer, and as a sugar substitute for diabetics, may be produced by fermentation of xylose present in hemicellulosic hydrolysates [43]. Production of this polyalcohol may be carried out by fermentation of hemicellulosic hydrolysates generated by acid hydrolysis or autohydrolysis from different raw materials, including rice and wheat straw, sugarcane bagasse, eucalyptus wood, corn cobs, brewer’s spent grains and several others. Many microorganisms are able of producing xylitol from xylose, among of which, Candida guilliermondii yeast has been one of the most employed due to its high conversion efficiency. Besides xylitol, arabitol (another polyalcohol) may also be produced by fermentation of hemicellulosic hydrolysates. Yeasts like Candida entomaea and Pichia guilliermondii have demonstrated ability to convert both pentoses, xylose and arabinose, to xylitol and arabitol respectively, when cultivated in hemicellulosic hydrolysates. However, xylose is assimilated preferentially than arabinose [44]. The arabinose utilization by microorganisms is of great interest for the economic conversion of lignocellulose biomass, since this sugar is present in significant amounts in many hemicelluloses.

2,3-Butanediol, also known as 2,3-butylen glycol, is a valuable chemical feedstock because of its application as a solvent, liquid fuel, and as a precursor of many synthetic polymers and resins. This compound may be produced by fermentation of hemicellulosic hydrolysates by several microorganisms, including Bacillus polymyxa, Klebsiella pneumoniae (Aerobacter aerogenes), Bacillus subtilis, Seratia marcescens and Aerobacter hydrophilia [7]. Among these strains, two bacterial species, namely Bacillus polymyxa and Klebsiella pneumoniae, have demonstrated potential for butanediol fermentation on a commercial scale [45]. Klebsiella pneumoniae is also a microorganism of interest due to its ability of utilizing all the major sugars (hexoses, pentoses, certain disaccharides) and uronic acid derived from the hydrolysates of hemicellulose and cellulose materials [46, 47]. Sugars present in wood hemicelluloses could be efficiently utilized by this microorganism after they had been released by either acidic [46] or enzymatic [47] hydrolysis.

Lactic acid production from lignocellulosic materials has been mostly reported using the cellullosic fraction, because there are few microorganisms capable of fermenting hemicellulosic sugars. However, Lactobacillus pentosus has been reported as able to produce lactic acid by fermentation of hemicellulosic sugars (mainly xylose, arabinose, and glucose) from acid hydrolysates of most agricultural residues [48]. Hemicellulosic hydrolysates of wet-oxidized wheat straw was successfully used as substrate for lactic acid production by Lactobacillus brevis and Lactobacillus pentosus. A lactic acid yield of 95% and complete substrate utilization were achieved in this process using a mixed culture of these microorganisms [49]. Sugars from hemicellulosic hydrolysates may also be used for citric acid production by Aspergillus niger. Operational variables including agitation, aeration, and the time of spores cultivation influence this
bioconversion process and must be taken into consideration to maximize the product yield [50]. Besides lactic and citric acids, butyric acid can also be produced by fermentation of hemicellulosic hydrolysates. *Clostridial* species are known to ferment xylose, and are thus of interest for the production of butyric acid from plant biomass. Conversion of corn fiber hydrolysate produced by dilute acid hydrolysis, for example, gave high butyric acid yield when fermented with *Clostridium tyrobutyricum* [51].

### 3.2 Solid-state fermentation systems

Solid-state fermentation (SSF) can be defined as any fermentation process allowing the growth of microorganisms on moist solid materials in the absence of free-flowing water [52]. The low moisture content means that fermentation can only be carried out by a limited number of microorganisms, mainly yeasts and fungi, although some bacteria have also been used. Among these microorganisms, fungi have been considered to be the most adapted to SSF because their hyphae can grow on particle surfaces and penetrate into the inter particle spaces and thereby colonizing solid substrates [53].

In the last decades there has been an increasing trend towards the utilization of these systems instead of SmF, because they have promoted higher yields and better product characteristics than cultivations in liquid medium. In addition, costs are much lower due to the efficient utilization and value-addition of wastes [54]. The main drawback of this type of cultivation concerns the scale up of the process, largely due to heat transfer and culture homogeneity problems [55, 56]. Several researches have been directed towards the development of bioreactors for SSF systems; however, the available information has not indicated an ideal bioreactor yet. Although this obstacle to be overcome, many studies have been performed using SSF systems for the production of different compounds of interest, including flavors, organic acids, enzymes and others.

The nature of the solid substrate is one of the most important factor affecting SSF processes and its selection depends on several factors mainly related with cost and availability. Therefore, many studies have been performed involving the screening of several agro-industrial wastes as solid substrate in SSF. Some examples of products obtained by SSF of lignocellulosic materials are described below.

#### 3.2.1 Enzymes

SSF systems have been considered as a promising technology for the production enzymes and therefore, this is a topic that has been strongly studied. Many works report the production of a variety of enzymes by SSF, using different microorganisms and solid substrates. Some examples of enzymes that may be produced by SSF from lignocellulosic materials include α-amylase, cellulase, xylanase, protease, fructosyl transferase, chitinase, pectinase, among others.

Production of α-amylase is usually performed by bacteria strains such as *Bacillus subtilis*, *B. polymyxa*, *B. mesentericus*, *B. vulgaris*, *B. megaterium* and *B. licheniformis* [57]; however, some fungi strains are also able of producing this enzyme. A variety of lignocellulosic wastes have been used as substrate materials for α-amylase production by SSF. For example, rice husks were used as substrates for the production of α-amylase by *B. subtilis* [58]. Rice straw, rice bran, red gram husk, jowar straw, jowar spathe and wheat bran were used as substrates for the production of α-amylase by the fungus *Gibberella fujikuroi* [59], and spent brewing grains were used for α-amylase production by *Aspergillus oryzae* [60].

Lignocellulolitic enzymes, more specifically cellulases and xylanases, have been produced by SSF on various agricultural residues. Cellulases production has been extensively studied using sugarcane bagasse as raw material. Production of this enzyme by a strain of *Streptomyces* sp. HM29 occurred with higher yields when the strain was grown on bagasse in comparison to rice straw, rye straw or corn cobs [61]. Production of xylanases by *Aspergillus terreus* and *Aspergillus niger* was performed in different lignocellulosic wastes, including sugarcane bagasse, rice straw and soya bean hulls [62]. *Thermoascus aurantiacus* was able to produce a high level of thermostable xylanase from sugarcane bagasse [63], while *Bacillus* sp. was good producer of this enzyme from rice bran [64].

Protease production has also been studied using different lignocellulosic wastes as solid substrate. In a study on the production of this enzyme by *Aspergillus oryzae* from wheat bran, rice husk, rice bran and spent brewing grains, wheat bran provided the best production results [65]. Green gram, chick pea, red gram and black gram husks, and wheat bran were used as solid substrate for protease production by *Bacillus* sp. Among the substrates, green gram husk gave the maximum protease production [66].

Fructosyl transferase, enzyme responsible for the fructooligosaccharides production from sucrose, was produced by *Aspergillus oryzae* using corn cobs, coffee husk, spent coffee, spent tea, sugarcane bagasse, cassava bagasse and cereal brans (from wheat, rice and oat) as solid substrates [67]. Production of chitinase has been reported using *Penicillium aculeatum* grown on wheat bran–crude chitin mixture [68], whereas the production of tannase has been reported using *Aspergillus* sp. grown on different lignocellulosic wastes, including ber leaves (*Zyzphyus mauritiana*), jamun leaves (*Syzygium cumini*), amla leaves (*Phyllanthus emblica*), jawar leaves (*Sorghum vulgaris*), wheat bran, rice bran, sawdust, rice straw dust and sugarcane pith [69, 70].

Most of agro-industrial wastes have also been reported as containing inducers of laccase synthesis, and therefore, they ensure an efficient production of laccase by SSF. Furthermore, agro-wastes have shown to produce higher laccase
activities than inert supports for the same fungal strain and culture conditions [71]. Use of SSF for pectinase production has been proposed using different agro-industrial wastes, such as coffee husk and sugarcane bagasse. Commercial pectinase preparation is produced from fungal microorganisms, mainly by *Aspergillus niger* strains [72].

### 3.2.2 Flavors

Natural flavors are chemical substances with aroma properties that are produced from feedstock of plant or animal origin by means of physical, enzymatic or microbiological processing [71]. Flavor synthesis by biotechnological processes plays an increasing role in the food, feed, cosmetic, chemical and pharmaceutical industries. SSF has been used for the production of flavor compounds by cultivating yeasts and fungi. The production of flavor compounds is related to the low oxygen availability in the medium, which results in production of odor compounds including alcohols, aldehydes and ketones [73, 74]. Coffee husk is one of the lignocellulosic materials that have already been used for the production of flavors. Cultivation of *Ceratocystis fimbriata* in coffee husk under SSF conditions proportioned the production of fruity aroma, with ethyl acetate, ethanol and acetaldehyde being the major compounds produced [75]. When cultivated in cassava bagasse, wheat bran, and sugarcane bagasse, this microorganism produced banana flavor and fruity complex flavors [76]. *Kluveromyces marxianus* produced aroma compounds, such as monoterpene alcohols and isomyl acetate (responsible for fruity aromas), in SSF using cassava bagasse or giant palm bran as a substrate [74].

### 3.2.3 Organic acids

Several organic acids have already been produced by SSF from lignocellulose wastes. Citric acid is one of these examples. Almost the entire production of this acid has been obtained using crops and crop residues as substrates and *Aspergillus niger* as production strain. When comparing sugarcane bagasse, coffee husk and cassava bagasse as solid substrate for citric acid production by *Aspergillus niger*, cassava bagasse led to the highest production results [77]. An important point to be considered in the production of citric acid by SSF is that the synthesis appears to be directly influenced by the nitrogen source and its consumption leads to a pH decrease [78]. It has also been generally found that addition of methanol increases citric acid production in SSF [52].

Lactic acid may be produced by SSF using fungal as well as bacterial cultures. Strains of *Rhizopus* sp. have been common among the fungal cultures and that of *Lactobacillus* sp. among the bacterial cultures. When cassava bagasse and sugarcane bagasse were used to produce L(+)-lactic acid by *Lactobacillus delbrueckii*, more than 99% of the total sugar available was converted into this acid [79]. L(+)-lactic acid production by *Rhizopus oryzae* in solid-state conditions operating with sugarcane bagasse as a support gave slightly higher productivity than in SmF [80].

### 3.2.4 Single cell protein

Some processes have focused on the direct conversion of lignocellulosic wastes to single-cell protein. The microorganisms’ strains *Chaetomium cellulolyticum* mutant, *Pleurotus sajor-caju*, strains of *Aspergillus* and *Penicillium* spp., may be used for this purpose. This co-cultivation of fungi has the ability to utilize cellulose and hemicellulose, after lignin degradation, for single cell protein production. There is no need for any treatment in the raw material before use in this system as together these fungi are capable of separating lignocellulose into its individual components. The cellulose obtained may be also used for paper production or as single cell protein for animal or human feed [8].

### 3.2.5 Bioactive compounds

Several bioactive compounds may be produced by SSF from different lignocellulose wastes. Some examples include i) the production of gibberellic acid by *Gibberella fujikuroi* and *Fusarium moniliforme* from corn cobs, ii) the production of tetracycline from cellulose substrates, iii) production of oxytetracycline by *Streptomyces rimosus* from corn cobs, iv) the production of destrucxins A and B (cyclopediapeptides) by *Metarhizium anisopliae* from rice husk, and v) production of ellagic acid by *Aspergillus niger* from pomegranate peel and creosote bush leaves [52, 81].

### 4. Conclusion

Lignocellulosic materials including forestry, agricultural and agro-industrial wastes contain several high value substances such as sugars, minerals and protein. Disposal of these wastes to the soil or landfill causes serious environmental problems, besides to constitute loss of these value added substances. Therefore, the development of processes for reuse of these wastes is of great interest. Since these wastes are rich in sugars, which are easily assimilated by microorganisms, they are very appropriate for use as raw materials in the production of industrially relevant compounds by fermentation. Products of interest for food, pharmaceutical and biofuels industries may be produced by fermentation of lignocellulose, both by submerged or solid-state fermentation systems. Submerged fermentation
systems require a previous step for fractionation of the lignocellulose to produce a sugar rich hydrolysate fermentable by microorganisms. Different treatments may be used for the lignocellulose fractionation and the selection of the best one must be done considering the product that will be produced and economical aspects. Solid-state fermentation systems are other interesting option to reuse lignocellulose and have as advantage the no need of raw material fractionation previous the use in the fermentation stage. Besides to serve as low-cost raw materials for the production of important metabolites, the lignocellulose reuse in fermentation processes is an environment friendly method of waste management.

References


Silva JPA, Mussatto SI, Roberto IC. The influence of initial xylose concentration, agitation, and aeration on ethanol production by Pichia stipitis from rice straw hemicellulosic hydrolysate. Applied Biochemistry and Biotechnology. 2010;162:1306-1315.


