Chapter 2
Pretreatment Strategies to Enhance Value Addition of Agro-industrial Wastes

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2.1 Introduction

The utilization of agro-residues as substrate to generate new products of commercial interest through a bioprocess is considered an important strategy for the development of sustainable technologies that are essential, nowadays, for many reasons: (1) the use of a residue of an industrial process will demand less efforts and resources to treat and dispose the residue through conventional techniques, and (2) value addition of these wastes besides saving money on conventional treatment will provide a raw material at a relatively low cost. The proposal is ideally based on integrated processes, where the residue of one process will not be only treated and disposed but used as raw material for a new process mainly through biotechnological pathways. The use of agro-industrial residues as raw material for new bioprocesses usually deserves a study of many key points to evaluate the feasibility of the proposal. Among the points to be carefully analyzed is the residue nature, involving a complete characterization to determine the carbon source type, the presence of the appropriate nutrients and/or toxic compounds, and pretreatment steps to avoid deterioration of the material and/or to ensure the material accessible to chemical or biochemical transformation and the accessibility of the carbon source and nutrients to the microorganism metabolism. An appropriate pretreatment step may also be necessary for the detoxification of the residues that contains anti-metabolites. These pretreatments include (1) drying or concentration, (2) grinding and size classification, (3) improvement of the accessibility to the carbon sources through thermal or enzymatic treatment, (4) balancing the nutrient contents, (5) reduction of the concentration

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of toxic compounds, and (6) transformation of recalcitrant compounds. Consideration about transportation, drying, grinding and sieving, thermochemical and/or enzymatic hydrolysis, detoxification cases, analysis, and composition considerations, including technical and economical aspects, will be discussed. The characterization of the residue and the choice of suitable pretreatment strategies are essential as these steps directly affect the whole process.

2.2 Residue Characterization

The appropriateness of a residue to a particular application depends on its effective composition. Hence, the first step for a suitable choice of any alternative substrate is its composition characterization. Some important points to be evaluated are:

2.2.1 Physical State

It is practical to use the raw material in its original physical state. This will eliminate at least one operation to prepare the material for the new industrial processing. Among the alternatives available to use a residue as substrate, submerged fermentation (SmF) for liquid media and solid state fermentation (SSF) for solid media without free water and a range of fermentation alternatives between both strategies are used. Solid substrates, for example, can be used preferably through SSF. When it is not possible or viable, if the raw material is at solid state, to dissolve or hydrolyze the nutrients present at the solid matrix is necessary to develop a process through submerged fermentation. Glucose syrup, for example, to be used in SmF can be obtained from acid or enzymatic hydrolysis of potato starch residue or starch cassava bagasse. On the other hand, a liquid residue should be used preferably in submerged fermentation or while searching a solid support to be impregnated with the liquid medium if the target process is through SSF. Hence, to improve a process using a residue as raw material, the first alternative is to use the residue in its original physical state. In any case, technical and technological aspects and alternatives of the process must be studied.

2.2.2 Nature of Carbon Source

Each plant species has a particular composition in terms of structural molecules that depends on the variety, cultivar, climate, and soil where it is produced and time of the harvest. Each industrial process suitable to extract from the plant the main industrial product (starch, oil, protein) produces a residue besides the main product. The residue composition of each process varies depending on the processing itself, the technology used, the raw material processed, and the kind of main product recovered. Depending on the plant species, the carbon composition can also vary.

In relation to the nature of the carbon source, biomass organic matter can be classified into various types:

2.2.2.1 Phytobiomass

The biomass is produced through the photosynthesis using the solar energy and inorganic molecules, such as carbon dioxide and water, in the presence of chlorophyll. The plant species synthesize its biomass and acquire its energetic resources through photosynthesis. With a structural matrix composed of cellulose, hemicelluloses, and lignin, according to Carioca and Arora (1984) depending on the nature of the additional energetic molecule synthesized, phytobiomass can be classified into:

(a) Saccharide crops: Besides the cellulose, hemicelluloses, and lignin structure, this phytobiomass is characterized by the accumulation of energetic molecules in the form of a simple sugar. This directly fermentable sugar is the main energetic source of the species. The accumulated sugar depends on the species and can be a monosaccharide, such as glucose, fructose, and galactose; a disaccharide, such as sucrose; a polysaccharide, such as pectin, inulin, xylans, and galactoxylans; or a mixture of them in different proportions but at least one of them. Examples of saccharide species biomass are sugarcane juice, grape juice and grape skin, citric juice and citric pulp, apple juice and pomace, and sweet sorghum juice, among others. This kind of phytobiomass has a great importance due to a considerable part of the biomass being composed of directly fermentable or easily hydrolyzed sugar.
(b) Starchy crops: This phytobiomass species is characterized by an accumulation of energetic molecules in the amylaceous form at a certain part of the plant usually the root, but not only there. Crude starch is the main energetic source of the vegetal specie, such as cassava, potato, and corn, among others, and its processing residues, cassava bagasse and corn steep liquor, for example. This polymeric molecule (starch) can be hydrolyzed in acid or enzymatic medium to release mainly glucose from the starchy grain, producing a broth with large sugar concentration. Another strategy to use this phytobiomass or the residue of its industrial processing is to select an amylase-producing microorganism that will hydrolyze the starch during its growth and metabolism. Normally, this plant species is a substrate to produce and recover amylases. Among the microorganisms, amylase producers are filamentous fungi, such as *Aspergillus*, *Pleurotus*, *Rhizopus*, some yeasts, and bacteria.
(c) Oilseed biomass: Soya, sunflower, palm, and olive are examples of this phytobiomass species characterized by an accumulation of an oil energetic molecule, usually in its seed, grain, or pulp. The plant species is industrially processed in order to obtain the oil, for commercial aims, and the processing residue can be reprocessed to recover other molecules, such as protein, sugar, bioactive products, and others. For fermentative processes, specifically, these residues are of special interest due to the presence of large amounts of carbohydrates, nitrogen, and minerals besides residual oil.

(d) Lignocellulosic biomass: The most important phytobiomass available all over the world. It has a complex composition formed by cellulose fibers, hemicelluloses, protected by a lignin matrix in a very typical structure responsible for its mechanical, chemical, and biological resistance. Depending on the species, a specific extract and ashes complete its composition. These species include hardwood; wood from trees, such as broadleaves (oak, eucalyptus); and softwood, such as wood from coniferous (*Pinus*). It also includes agricultural residues and industrial residues, such as husks, bagasse, grass, straw, leaves, branches, shavings, and all kinds of trimmings and prunings. The amount, diversity, and presence all over the world of this source demonstrate its importance. Nowadays, the industrial exploration of this species is spotted mainly to produce cellulose pulp from the paper industry through some known thermochemical processes. Hemicellulose residues and lignin form the black liquor, residue of the process. Many processing alternatives are being studied to efficiently recover these fractions, mainly the sugars from the hemicellulosic fraction, due to its potentiality to be used as substrate for fermentative processes, producing a large number of biomolecules. Alternatives including mild thermochemical processes in acid medium are being studied to firstly hydrolyze the hemicelluloses followed by alkaline wash to remove the lignin and lastly recover and hydrolyze the cellulose. Nowadays, the search of processes to generate biofuels to substitute fossil fuels has urged the research groups around the world to improve technical and economic knowledge for a viable second-generation biofuel production.

Hemicelluloses are amorphous polysaccharides non-starchy and noncellulosic constituents, depending on the species, comprising hexoses (glucose, mannose, galactose), pentoses (xylose, arabinose), uronic acids (glucuronic, methylglucuronic, galacturonic), and deoxyhexoses (rhamnose, fucose). The hemicellulosic hydrolysate contains the simple sugar that constitutes the hemicelluloses of the plant species. This syrup can be potentially fermented by a selected microorganism to produce many biomolecules of industrial importance, including ethanol.

Cellulose is a crystalline homopolymer, constituted by glucose units linked by β-(1–4) linkage, generating a linear and flat structure, allowing the packing and superposition of the cellulose molecules, producing a compact and stable structure, which characterize the cellulose fiber. Acidic or enzymatic hydrolysis will furnish directly and easily fermentable glucose syrup.

Lignins are complex polymers, specific for each plant species; it is a three-dimensional network constituted by units derivated from phenyl propane with different degrees of methoxylation and acetylation linked by carbon–carbon or ether linkage. Lignin is linked to the hemicellulose structure through molecular bond. The physicochemical nature of the lignin and diversity of components are responsible for its recalcitrant characteristics.

2.2.2.2 Microbial Biomass

A complex carbon source due to its particular composition has been used as nitrogen source for many large-scale processes. For economic reasons, nitrogen source

compounds should be chosen among the low-price materials, for example, ammonium salts, urea, and nitrate salts, but depending on the microorganism, an organic nitrogen source must be tested. In these cases, some organic residues rich in nitrogen can be tested to compose the fermentative medium. Yeast extract is a nitrogen source and substrate for many microorganisms, containing many amino acids (alanine, arginine, cystine, glutamic acid, glycine, histidine, leucine, isoleucine, valine, serine, threonine, proline) and peptides, vitamins (thiamine, riboflavin, niacinamide, pantothenic acid), and carbohydrates (glycogen and trehalose) that can be hydrolyzed to glucose. Yeast extract can be produced through autolysis at 45–55 °C or plasmolysis by using high concentrations of a soluble salt, mainly NaCl. Both differ in quality depending on the process used, but in general, the first one possesses high preservative properties.

2.2.2.3 Animal Biomass

Other protein hydrolysates, peptones from meat, casein, gelatin, and keratin can also be used as organic nitrogen source to supply complex nitrogen components. They largely vary in composition depending on the origin; thus, the amino acid and oligopeptide composition must be carefully analyzed, and it is a good option when a specific substance is necessary to make up the fermentation medium. To supply proline, peptone from gelatin can be used, but it lacks sulfur-containing amino acids. Keratin peptone can supply proline and cystine, but has no lysine. Even being an option to specific amino acid supplementation, the utilization of peptones usually is limited by the price, due to its relatively high production cost, mainly for industrial fermentation, involving high substrate volumes.

2.2.3 Nutrient Sources

Media used as substrate in fermentative process must contain all components in a proper form and concentration to address the microorganism physiology. When properly balanced, it gives all conditions for the cell growth, energetic metabolism, and metabolic products production. At lab scale, pure substances can be used for a medium composition, but at industrial scale, less expensive yet balanced materials must be tested. Normally complex and undefined substances, but properly characterized, can be used.

2.2.4 Toxic Compounds

The presence of toxic compounds in the residue composition may demand some kind of special treatment in order to remove or previously degrade these toxic

compounds such as solvent extraction, lixiviation, sparging for volatile toxic compound release, and chemical or enzymatic degradation to generate two or more nontoxic substances or at least less toxic chemical products. If these strategies were not technical/economic possible, the selection of a resistant strain must be tried to run the fermentative process.

2.3 Transport and Storage Considerations

To use any agro-industrial residue, the place where it is generated, the place where it will be used, and its stability may be a concern, since agro-industrial residues, besides their organic nature, may have a high microbial load and usually are easily degraded. Transportation costs can be prohibitive to the whole process, due to distance and/or temperature control needs. In general, it is recommended to place the unit where the residue will be used near by the factory where it is generated, to be reused within a short period of time. If not possible, the logistic of the process must be studied in order to preserve the residue characteristics and consider all transportations costs.

Marrison and Larson (1995) developed the standard expression for transport costs as given in (2.1):

$$\text{Cost}(\text{in } \$/\text{ton}) = \text{fixed costs} + (\text{UTC} \times \text{distance}) \tag{2.1}$$

where UTC is the unitary transport cost, in $ per ton × km, and distance is in km. Most transportation of residual biomass is done by trucks, for which fixed costs may vary from 3.7 to 5.7 U$/t and variable costs may range from 0.085 to 0.146 U$/t.km for straw and stover or for wood chips, respectively, in Canada, data corrected for 2012 dollars (Searcy et al. 2007). Rail and barrage transportation have fixed costs of 17.1 and 34.1 and variable costs of 0.03 and 0.01, respectively (Montross and Crofcheck 2010). In Brazil, the fixed costs are around 7.5 U$/t and variable costs are around 0.06 U$/t.km for transportation by trucks.

Storage is usually cheap, but it should be as short as possible, because of the risk of degradation and infestation, and care must be taken about fire and for exposed heaps of wetting by rain.

2.4 Pretreatment Strategies

2.4.1 Drying and Concentration

Drying is the process of removal of a volatile substance, usually but not exclusively water, from a solid material through the evaporation. Humidity is the amount of water present in a material, and during the material drying, the humidity is reduced

to a final acceptable or an equilibrium value. In most cases, drying is a finishing product operation, but for agro-industrial residues, it is necessary to reduce the water activity, in order to prevent deterioration reactions, microbial growth, chemical redox reaction, and the enzymatic reactions (Pessoa and Kilikian 2005).

According to Coulson and Richardson (1991), drying process is essential for (1) reduction of volume and weight of the material, (2) improvement of storage and handling characteristics of the product, and (3) reduction of transportation, packing, and storage costs.

The liquid content of a residue may be reduced by mechanical processes, such as centrifugation or pressing, but the final solid still contains high humidity and water activity (a_w). Drying must be more effective and reduce the humidity to suitable values. Hence, thermal methods are more adequate and will be discussed in this chapter.

2.4.1.1 Drying Process

During the drying process, the liquid aggregated on the material is removed by evaporation, passing from the liquid to the gaseous phase. The evaporation happens at a temperature below the liquid boiling temperature at the internal system pressure. Thus, drying is a complex process that involves simultaneously heat and mass transfer, resulting in significant changes in the physical, chemical, and structural properties. Water loss can cause cellular structural stress, microstructure alteration, increasing porosity, and material shrinkage (Laopoolkit and Suwannaporn 2011).

When the material is put in contact with hot air, heat is transferred from the air to the material under the effect of temperature gradient between them. Simultaneously, the partial pressure difference of water vapor between the air and the material surface determines the mass transfer of water vapor from the material to the air (Perry et al. 1997).

According to Mc Cabe et al (1993) and Coulson and Richardson (1991), the material can be in different forms such as flakes, granules, crystals, powder, plates, or leaves showing different properties. Also, the liquid to be removed can be at the solid surface or at its interior, or partially outside and inside, in two basic forms: (1) free moisture, the water in excess relative to the equilibrium humidity content, and (2) bound moisture, the water retained in such way that it has a vapor pressure below the free water at the same temperature. It can be inside small capillaries, adsorbed on the surface, or inside the material cells. Aspects of the raw material, characteristics, and quality of the final product must be considered during the drying process.

2.4.1.2 Drying Rate

The drying rate or the variation of the humidity of each material along time is an important parameter for the process. The humidity variation depends on the differences of the moisture inside each material (free/bounded). The curve obtained during

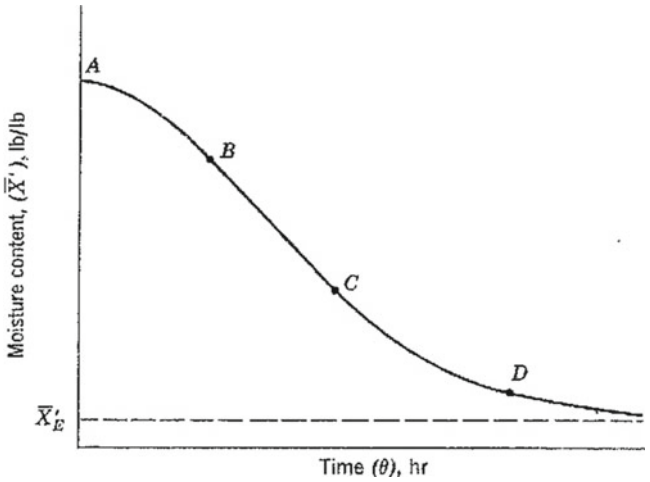

Fig. 2.1 Typical drying curve for constant drying conditions, moisture content as a function of time (Adapted from Foust et al. 1960)

monitoring of the variation of the moisture content with time allows the determination of the drying velocity for moisture content, and the curve shape varies with the structure, kind and granulometry of the material, thickness of the material layer, and kind of dryer (Coulson and Richardson 1991).

The main factors to be evaluated to correctly choose the drying method/equipment are directly related to the mass and heat transfer mechanism, to the physical material characteristics, and to the desirable final quality of the product.

Figure 2.1 shows a typical drying curve. AB segment represents the unsteady-state period. BC segment is the constant-rate drying part, where the entire exposed material surface is saturated with water and drying proceeds from a free liquid surface without the influence of the solid. CD segment is the period with a decreasing drying rate, where there is a lack of free liquid at the material surface as the liquid movement to the surface is slower than the movement of mass from the surface to the drying air. At point D and at lower moisture values, there is no significant amount of liquid at the material surface. The wet part of the surface dries by convective transfer of heat and mass to the drying gas stream, and vapor from inside of the material diffuses to the dry places of the surface and then into the gas stream. This mechanism is slower than the convective transfer from the saturated surface. All evaporation occurs from the interior of the solid, and the material moisture contents continue to fall until the equilibrium moisture content (EMC) is reached (X_E) and the drying process stops.

Figure 2.2 shows simulated drying curves for a 1,000 kg of *Miscanthus* grass. The initial biomass humidity is 30 %, reasonable for grasses and straw partially air-dried in the field. The drying temperature and the initial air moisture determine the drying rate and the equilibrium moisture content.

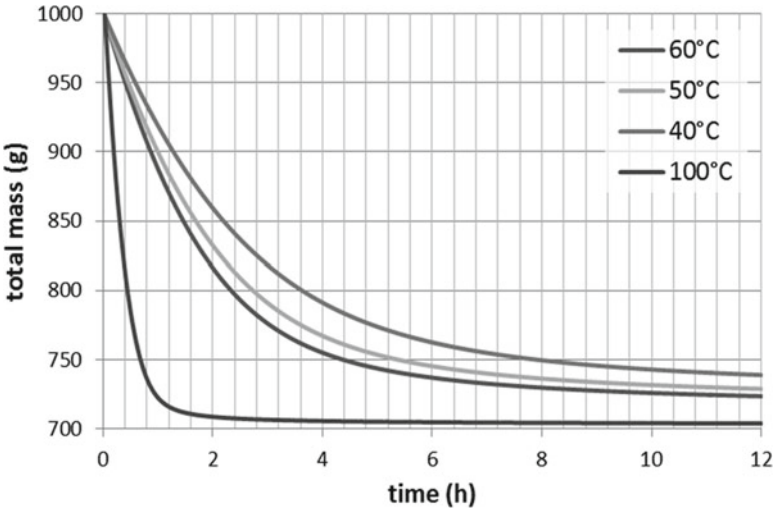

Fig. 2.2 Simulated drying curves for *Miscanthus* at several temperatures. Inlet air is at 20 °C and 50 % relative humidity. Data based on the equilibrium curve of Fig. 2.3 and literature thermodynamic data for water

Materials with free water, such as fruit residues, may have an initial lower drying rate than a constant drying rate period and finally an exponential decay of the drying rate as shown in Fig. 2.1; materials without free water (as is the case) show only the falling rate period.

The EMC of a material depends on the ambient temperature and relative humidity, being inversely proportional to the temperature and directly proportional to the relative humidity (RH). To know the behavior of a certain material is useful to determine the suitable humidity for storage, considering the usual weather.

Figure 2.3 shows EMC isotherms for selected biomasses. While *Jatropha* seeds, for example, show relatively stable moisture content for a wide range of RHs, the EMC of a grass such as *Miscanthus* grows from 10 to 20 % with RH going from 40 to 80 %. The RH in the isotherm may be used to predict the a_w of the solid, which should be below 0.8 to prevent the growth of most fungi.

2.4.1.3 Equipment

The classification of the drying equipment depends on the heat transfer method and the properties and characteristics of the material to be dried. In general, drying equipment can be divided into two groups: (1) direct dryers where the material is put in direct contact with the drying gas heat and (2) indirect dryers where the heat is supplied through other way, such as radiation, conduction, high-frequency electric field, and microwave (Mc Cabe et al. 1993; Perry et al. 1997). Both types can be used for residue drying.

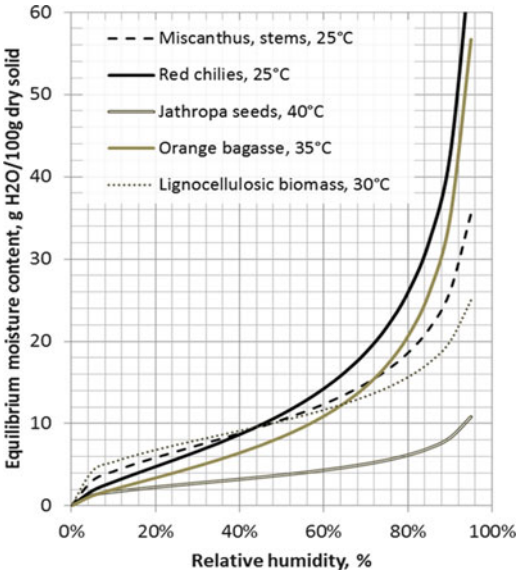

Fig. 2.3 Equilibrium moisture curves for 5 biomasses. Regression models using the Oswin modified equation and data from Arabhosseini et al. (2010), Kallemullah and Kailappan (2004), Kartikaa et al. (2012), Fioretin et al. (2010), Achargee et al. (2011)

There is a large variety of equipment and drying processes. The first criterion to be analyzed is the volume and characteristic of the material to be dried. Later, the heating method, material feeding mode, and cost must be evaluated (Al-Kassir et al. 2005).

For industrial scale, tray dryers, conveyors and tunnel dryers, rotatory dryers, and fluidized and fixed bed dryers can be used. According to Perry et al. (1997), the main dryers are:

Conveyors and tunnel dryers: The material is transported through a drying tunnel, where hot air circulates, transversal, countercurrent, or parallel to the material. The advantage of this equipment is the operational flexibility, and it can be used to dry materials of various sizes and forms, as the hot air velocity can be adjusted and the residence time does not depend on the particle characteristics, although it will determine the material final humidity.

Rotatory dryers: Consists of a big slightly inclined cylinder, rotating around an axis, with internal paddles that enhance the thermal exchange between the hot air and the material. Beyond the cylinder movement and the gravity action, the material is constantly rebutted favoring the drying and driven to the dryer discharge while the volatile mass is transported by the gas flow. Normally, dryers operate in countercurrent, where the hot air enters opposite to the material discharge, enhancing the process thermal yield. In this case, parameters such as density, form, particle size, and the equipment inclination significatively influence the biomass velocity along the dryer and the retention time.

Tray dryers: It consists of a thermal isolated camera, with heating systems and forced ventilation through trays placed in shelves. The air movement allows heat

conservation and improves drying efficiency. It is the simplest equipment, used mainly for discontinuous operation and in low scale.

2.4.2 Grinding and Size Classification

When searching a substrate for a fermentative process from a solid material or residue, the medium size of the particles must be defined, taking in view some particularities of the process; mainly about size, the substrate particles should be neither big nor small. Smaller particles provide bigger surface areas and consequently bigger transformation grade as far as its enhanced surface contact favors microbial growth but on the other hand can result in clumping and clogging of the fermentation medium, negatively affecting respiration/aeration, microbial growth, and process yield. Solid medium must have a proper granulometry to avoid medium compaction, preserving empty space among particles to allow air circulation through the mass, an efficient oxygen supply necessary, and an efficient dissipation of gas and heat produced during the microorganism activity, harmful to the process (Del Bianchi et al. 2001; Souza et al. 2007; Santos et al. 2005; Ruiz et al. 2012).

Particle material size and porosity will directly affect the superficial surface and the microorganism accessibility to the substrate, the enzyme activity, and the microorganism metabolism which are surface phenomena (Santos et al. 2005). In general, it is considered that the size reduction of a substrate is better for the fermentation process. However, there is a limit to decrease the particle size.

The ideal particle size is arbitrary and varies according to the substrate, microorganism, and process used. The particle uniformity in terms of size will provide a uniform process even for chemical and biochemical reaction (Izumi et al. 2010; Hendriks and Zeeman 2009).

Pandey et al. (2001) studied wheat bran and corn flour particles at the proportion of 9:1 with diameters between 425 and 500 μm and 500 and 600 μm, respectively, which reached the highest amyloglucosidase production, although they showed that diameter between 180 μm and 1.4 mm had presented similar yields.

Kumar et al. (2003) studied four different sugarcane bagasse granulometry varying between 0.64 and 2.0 mm for citric acid production. The best results were obtained with particle size between 1.2 and 1.6 mm. This particle size provided solid medium with high porosity, resulting in better heat and mass transfer. On the other hand, bigger particles (1.6–2.0 mm) provided smaller surface area to the microorganisms and presented lower citric acid production. For the test using smaller particles (0.64–1.2 mm), the production was affected by the low heat and mass transfer.

Studies of neomycin production using the *Streptomyces marinensis* mutant strain by Bapiraju et al. (2004), with three particles sizes of wheat flour (small, intermediate, and big), found that the best yield was obtained with the larger granulometry.

Yuan et al. (2011) investigated the influence of the wheat stem particle size (1–5 and 10 mm) to produce hydrogen, acetate, and butyrate using a microflora from a biogas plant; they obtained the best results with the smaller particle size.

2.4.2.1 Grinding Process

During grinding or particle size reduction, the average size of the solid particles is reduced by the application of an impact force, compression, or abrasion. Advantages of the particle size reduction are (1) increase of the ratio surface/volume of the material and (2) uniformity of the material particle size, helping the homogeneity of the substrate (Mc Cabe et al. 1993; Gauto and Rosa 2011).

The different methods for particle size reduction are classified according to the range of the particle size produced; crushers are used for the production of bigger and medium particles or mills that produce smaller particles and fine dust (Gauto and Rosa 2011; Coulson and Richardson 1991).

2.4.2.2 Equipment Used for Fragmentation

There is a large variety of equipment for solid size reduction that must be evaluated according to the finality, initial investment, and the operational cost. The proper equipment choice must take into account the nature, amount, and dimensions of the material to be treated. The main properties to be evaluated are hardness, structure, humidity, crushing strength, tendency to slip or impaste, dust production, and explosion risks (Coulson and Richardson 1991).

Large and medium particle sizes are produced using crushers or shredders, such as mandibles, swivels, hammer, rolls, discs, and conicals. They have little application to fragment industrial residues to biotechnological use, where smaller particle dimensions are necessary. In this case, mills are the best choice, which produce medium to fine particle size. The main types of mills are:

Disc mill: They are generally used to reach a fine granulation. They are constructed with two steel discs placed on horizontal axis, one or both movable. One of them is mounted on to an eccentric support, allowing that the two milling faces of the disc are continuously approaching and moving away. The material is fed at the center of the discs and discharged by centrifugal force. Fine material granulometry is obtained (Perry et al. 1997).

Roll mills: They are used for cereal milling; it provides a product with uniform texture. There are basically two or more heavy cylinders with the same diameter that spin in opposite directions with equal or different velocities. Fed particles are submitted to compression and cutting forces. The distance between the rolls is regulated and must be adjusted to the raw material conditions and desired granulometry (Coulson and Richardson 1991).

Knives and hammer mills: They are used to produce a material thinner than the rolls mill. This equipment is appropriate for cereal milling destined to extract any cereal component and/or to produce a fine powder. It is a mill of impact, constructed with a rotor that spins at high velocity inside a cylindrical camera. At the edge of the rotor is fixed a series of hammers or knives. The material is broken or cut under the impact of the hammers or knives and pulverized by the blades of the mill while

Fig. 2.4 Sieving

forced to pass through a screen placed at the opening between the hammer or knives and the camera. They are used for milling fibrous or brittle materials (Coulson and Richardson 1991).

Ball mills: It is basically a cylinder that spins supported on a horizontal or slightly inclined axis, with the internal space charged with balls of steel or porcelain. The size reduction takes place by the impact and friction of the material with the balls when the mill spins, producing a very homogeneous material even in liquid medium. They are recommended to obtain a very uniform liquid suspension mainly for the ceramic industry. The final size of the particles depends on the material properties, weight and diameter of the balls, spin cylinder velocity, time of the processing, and level of the material inside the cylinder (Coulson and Richardson 1991).

2.4.2.3 Granulometric Separation

Grinded material classification in different granulometries is required for better material homogenization and to assure a uniform process. Figure 2.4 shows the simplest and most common method in the granulometric separation that consists of passing the material through a series of sieves with meshes progressively smaller during vibration. Fractions are separated according to the meshes of the screens. The average particle size of each fraction and the amount of material of each fraction are determined. Later, it is possible to build a granulometric distribution curve

of each milling process (Gomide 1983). In lab scale, a set of sieves with screens progressively smaller can separate efficiently the size fractions of a milled material. However, at industrial scale, a continuous sieving process must be used.

For large-scale processes the available equipment are:

- *Slug sieve*: They are constituted of a long horizontal cylinder perforated (screen) with slight inclination, spinning at a low velocity. The screen opening along the cylinder increases progressively in the exit direction and allows the separation of the various material size fractions.
- *Shaking sieves*: In this case, the particle movement in the sieving surface allows the size separation. Normally, these sieves are horizontal but sometimes inclined to provoke the material displacement.
- *Vibrating sieves*: They are equipment of high capacity and efficiency, mainly for obtaining fine particles. They differ from the shaking sieves at the frequency and vibration amplitude and are bigger than the vibrating ones.

2.4.3 Thermochemical Hydrolysis

Many natural substrates, such as lignocellulosic biomass, have a complex microstructure that makes the material hard to digest. One approach to enhance further processing and/or to recover each constituent of the material as a partial hydrolysis or fiber expansion can be tried. Some thermochemical methods have been proposed for pretreating and hydrolyzing agro-industrial lignocellulosic wastes, such as dilute acid hydrolysis (Hernández-Salas et al. 2009; Balat et al. 2008; Zhang et al. 2007), steam explosion (Hernández-Salas et al. 2009; Hendriks and Zeeman 2009; Balat et al. 2008; Ramos et al. 1992; Ramos et al. 2000; Glasser and Wright 1997), alkali washing (Hernández-Salas et al. 2009; Hendriks and Zeeman 2009; Balat et al. 2008), and ammonia fiber expansion (Hendriks and Zeeman 2009; Balat et al. 2008), among others.

2.4.3.1 Acid Hydrolysis

Dilute acid hydrolysis can be an effective pretreatment to recover the sugars from the hemicellulosic part of the material, to improve further lignin separation, and to produce partially pure cellulose. Sulfuric acid can be used for pretreatment and hydrolysis (Lavarack and Griffin 2002), but other reagents, such as hydrochloric, nitric, and other acids, can also be used (Gámez et al. 2006; Rodríguez-Chong et al. 2004). Chen et al. (2012) found that the pretreatment using dilute sulfuric acid has been considered as one of the most cost-effective methods. The biomass into dilute acid solution is submitted to controlled and moderate temperature by means of conventional heating that hydrolyzes the sugars from the hemicelluloses and causes the softening of the lignin, facilitating its alkaline removal from the residual material,

generating relatively pure cellulose. Microwave-assisted heating can also be used to pretreat biomass. The microwave electromagnetic field may create nonthermal effects which accelerate the destruction of crystal structures. Binod et al. (2012) developed a process using biomass microwave treatment in alkali solution (1 % NaOH) followed by acid pretreatment (1 % H_2SO_4) and enzymatic hydrolysis, achieving an overall reduction of sugar yield of 0.83 g/g dry sugarcane bagasse.

Hot acid pretreatment has been used to hydrolyze the simple sugars derived from the biomass of polysaccharides, mostly hemicelluloses. The resulting sugars can degrade to furfural (from pentoses) and to 5-hydroxy-methyl-furfural or HMF (from hexoses). These compounds are inhibitory for microorganisms, and their production means loss of fermentable sugars. Organic acids, such as maleic and fumaric, have been suggested as alternatives to avoid HMF formation (Kootstra et al. 2009), but mild thermal conditions can prevent formation of furfural and HMF.

2.4.3.2 Steam Explosion

Steam explosion is a promising method for the pretreatment of lignocellulosic biomass (Soccol et al. 2011) and can be performed in the presence or absence of an acid or alkali catalyst. The grinded biomass is treated with high-pressure saturated steam, at temperatures varying from 160 to 260 °C, and then the pressurized reactor is quickly decompressed, releasing the material to the normal pressure, undergoing an explosion. The process causes the disrupting of the material's structure, degradation of hemicellulose, and lignin partial disruption due to the high temperature, thus facilitating the subsequent obtention of cellulose for further hydrolysis (Öhgren et al. 2007).

Rocha et al. (2012) used steam explosion process to treat sugarcane bagasse (50 % of moisture) at a pressure of almost 1.3 MPa (190 °C) during 15 min, depressurizing suddenly the reactor after this time. The treatment recovered an average of 82.7 ± 4.3 % of the hemicelluloses, and cellulose was hydrolyzed at the ratio of 11.8 ± 3.7 %, probably from the polymer amorphous region. Lignin was solubilized at the proportion of 7.9 ± 9.1 %. Furfural and HMF production is more pronounced at the steam explosion process due to the hard thermal conditions used.

2.4.3.3 Alkaline Pretreatment

The biomass alkaline pretreatment has being studied a lot because it can remove and modify the lignin from the biomass crystalline structure, improving hydrolysis of the remaining polysaccharides and removing acetyl groups and various uronic acid substitutions on hemicellulose. Depending on the temperature and alkali concentration, most of the hemicellulose sugars are degraded; thus, treatment conditions must be carefully studied. Alkaline pretreatment probably causes saponification and hydrolysis of intermolecular ester bonds cross-linking of xylan hemicelluloses and lignin or lignin and other hemicelluloses. Dilute NaOH treatment of lignocellulosic material

causes rupture of structural linkages between lignin and carbohydrates of hemicelluloses and disruption of the lignin structure, leading to a decrease in the lignin polymerization degree, a decrease in cellulose crystallinity, and a separation of the hemicellulose sugars (Soccol et al. 2011; Fan et al. 1987).

Rocha et al. (2012) reported a process where sugarcane bagasse was submitted to the steam explosion and then reacted with a NaOH solution 1.0 % (w/v), for 1 h at 100 °C, using a solid–liquid ratio of 1:10 (w/v). They got 92.7 ± 3.9 % of lignin removal from the biomass, hydrolyzed 31.1 ± 3.5 % of the cellulose, and achieved 94.7 ± 0.9 % of the hemicellulose hydrolysis.

2.4.3.4 Ammonia Fiber Expansion

During ammonia fiber expansion (AFEX) process, biomass is treated with liquid ammonia under pressure (100–400 psi) and temperature (70–200 °C) before rapidly releasing to the atmospheric pressure (Bals et al. 2010). This process causes the decrease in the crystalline cellulose, increasing the rate of enzymatic hydrolysis; hydrolyzes hemicellulose; solubilizes and depolymerizes lignin; and increases the size and number of micropores in the plant cell wall (Mosier et al. 2005).

Krishnan et al. (2010) working with sugarcane bagasse and cane leaf residues reported that AFEX pretreatment improved the accessibility of cellulose and hemicelluloses to the enzymatic hydrolysis by breaking ester linkages and other lignin–carbohydrate complex bonds. The enzymatic hydrolysis efficiency of the AFEX pretreated bagasse and cane leaf residue by cellulases was approximately 85 %, and the use of hemicellulases during enzymatic hydrolysis promoted the xylan hydrolysis to 95–98 %.

2.4.3.5 Organosolv

Organosolv is the biomass pretreatment with an aqueous solution of an organic solvent with or without an acid or alkali catalyst. The process efficiently solubilizes lignin from lignocellulosic materials promoting the partial hydrolysis of lignin bonds and solubilization of the most of the hemicelluloses sugars, resulting in a liquid phase containing the lignin and hemicellulosic sugars and a solid phase composed by a cellulose-enriched pulp. The addition of a catalyst can enhance the lignin removal efficiency (Mesa et al. 2011; Sun and Cheng 2002). The possibility of lignin and polyoses recovering from the liquid phase by distillation with the simultaneous recycling of solvents is an important advantage of this technique when compared with other aqueous-based processes (Novo et al. 2011).

Novo et al. (2011) developed a process using glycerol–water mixtures and obtained a pulp with a residual lignin amount lower than 8 %, extent of delignification close to 80 %, and residual cellulose content higher than 80 %.

Mesa et al. (2011) processed sugarcane bagasse with the combination of a dilute acid pretreatment followed by the organosolv pretreatment with ethanol and NaOH

under optimized conditions (60 min, 195 °C, 30 % (v/v) ethanol), yielding a residual solid material containing 67.3 % (w/w) glucose, which was easily recovered by enzymatic hydrolysis.

2.4.4 Enzymatic Hydrolysis

Enzymes can be used to hydrolyze lignin, cellulose, and hemicellulose which are the main components of lignocellulosic agro-industrial wastes. The advantages of enzymatic hydrolysis over the previous methods may include mild reaction conditions, higher product yields and fewer side reactions, less energy demand, and less reactor resistance to pressure and corrosion (Lee 1997).

White-rot basidiomycetes produce several ligninolytic enzymes that catalyze one-electron oxidation of lignin units, producing aromatic radicals (Giardina et al. 2000). Lignin is normally not degraded as sole carbon and energy sources, requiring additional co-substrates such as cellulose, hemicellulose, or glucose (Silva et al. 2010). There are four major groups of ligninolytic enzymes produced by the white-rot fungi: lignin peroxidase (LiP; 1,2-bis(3,4-dimethoxyphenyl)propane-1,3-diol:hydrogen-peroxide oxidoreductase; EC 1.11.1.14), manganese-dependent peroxidase (MnP; Mn(II):hydrogen-peroxide oxidoreductase or manganese peroxidase; EC 1.11.1.13), versatile peroxidase (VP; EC 1.11.1.16), and laccase (benzenediol: oxygen oxidoreductase; EC 1.10.3.2). However, the process of lignin biodegradation can be further enhanced by the action of other enzymes, such as glyoxal oxidase (EC 1.2.3.5), aryl-alcohol oxidase (veratryl alcohol oxidase; EC 1.1.3.7), pyranose 2-oxidase (glucose 1-oxidase; EC 1.1.3.4), cellobiose/quinone oxidoreductase (EC 1.1.5.1), and cellobiose dehydrogenase (EC 1.1.99.18) (Wong 2009).

Both LiP and MnP belong to the class of peroxidases that oxidize their substrates by two consecutive one-electron oxidation steps with intermediate cation radical formation. Due to its high redox potential, the preferred substrates for LiP are non-phenolicmethoxyl-substituted lignin subunits, and the oxidation occurs in the presence of H_2O_2 (Tuor et al. 1995; Wong 2009), whereas MnP acts exclusively as a phenoloxidase on phenolic substrates using Mn^{2+}/Mn^{3+} as an intermediate redox couple (Tuor et al. 1995). Versatile peroxidases are a group of enzymes, primarily recognized as manganese peroxidases, which exhibit activities on aromatic substrates similar to that of LiP. These enzymes not only are specific for Mn (II) but also oxidize phenolic and non-phenolic substrates that are typical for LiP, including veratryl alcohol, methoxybenzenes, and lignin model compounds in the absence of manganese (Wong 2009).

Laccases are blue multicopper oxidases able to oxidize a variety of phenolic compounds including polyphenols, methoxy-substituted phenols, diamines, and a considerable range of other compounds, with concomitant reduction of molecular oxygen to water (Autore et al. 2009; Dwivedi et al. 2011). They oxidize phenols and phenolic lignin substructures by one-electron abstraction with formation of radicals

that can repolymerize or lead to depolymerization (Higushi 1989). These enzymes have been found to oxidize also non-phenolic compounds in the presence of a mediator (e.g., 2,2′-azinobis-3-ethylbenzthiazoline-6-sulfonate or ABTS) (Wong 2009). Laccases are more readily available and easier to manipulate than both LiP and MnP. Moreover, these enzymes find many industrial applications in the areas of food products, pulp and paper, textiles, nanobiotechnology, soil bioremediation, synthetic chemistry, and cosmetics (Couto and Herrera 2006).

Enzymatic hydrolysis can also be employed to produce reducing sugars from cellulose and hemicellulose. Utility cost of enzymatic hydrolysis is low compared to acid or alkaline hydrolysis because it is usually conducted at mild conditions (pH 4.8 and temperature 45–50 °C) and does not cause corrosion problems mainly for cellulose hydrolysis (Duff and Murray 1996). Both bacteria and fungi can produce cellulases and hemicellulases for hydrolysis of lignocellulosic materials.

The factors that affect the enzymatic hydrolysis of cellulose and hemicellulose include substrates, enzymatic activity, and reaction conditions (Sun and Cheng 2002). Substrate concentration is one of the main factors that affect the yield and initial rate of enzymatic hydrolysis. At low substrate levels, an increase of substrate concentration normally results in an increase of yield and reaction rate of the hydrolysis (Cheung and Anderson 1997).

When cellulose is used as raw material, the cellulase complex is responsible for enzymatic hydrolysis of pretreated cellulosic biomass. There are three major types of cellulases involved in the hydrolysis of cellulose: endo-β-1,4-glucanase (EG), which acts randomically at the molecule producing reducing and nonreducing ends in the cellulose polymer; cellobiohydrolase (CBH) or exoglucanase, which liberates cellooligosaccharides and cellobiose from these reducing and nonreducing ends; and β-glucosidase (BGL) that cleaves cellobiose and liberates glucose (Mathew et al. 2008).

The enzymes of the cellulase complex are strongly inhibited by their hydrolysis products: glucose and short cellulose chains. Several methods have been developed to reduce the inhibition of hydrolysis, including the use of high concentrations of enzymes, the supplementation of β-glucosidases during hydrolysis, and the removal of sugars during hydrolysis by ultrafiltration or simultaneous saccharification and fermentation (Lin and Tanaka 2006).

2.5 Conclusion

The search of economical and more efficient industrial processes is the objective of many research groups around the world, some of them supported by important companies and some from researcher's institutes. Moreover, the development of environmentally friendly processes, with fewer losses, less wastage, generating fewer residues, or reusing the residues in a new process, appears as an alternative to a sustainable world. In addition, use of the energy of the green carbon, phytobiomass produced by photosynthesis will make the world independent of the fossil carbon energy, seems to be the

reasonable way to reach this goal. Many steps and various technologies must be developed to harness the phytobiomass energy. It requires the establishment of some operations in sequence to set a proper and particular industrial process.

References

Achargee TC, Coronella CJ, Vasquez VR (2011) Effect of thermal pretreatment on equilibrium moisture content of lignocellulosic biomass. Bioresour Technol 102(7):4849–4854

Al-Kassir A, Gañan J, Tinaut FV (2005) Theoretical and experimental study of a direct contact thermal screw dryer for biomass residues. Appl Therm Eng 25(17–18):2816–2826

Autore F, Del Vecchio C, Fraternali F, Giardina P, Sannia G, Faraco V (2009) Molecular determinants of peculiar properties of a *Pleurotus ostreatus* laccase: analysis by site-directed mutagenesis. Enzym Microb Tech 45:507–513

Arabhosseini A, Huisman W, Müller J (2010) Modeling of the equilibrium moisture content (EMC) of Miscanthus (*Miscanthus* × *giganteus*). Biomass Bioenergy 34(4):411–416

Balat M, Balat H, Cahide OZ (2008) Progress in bioethanol processing. Progr Energ Combust Sci 34:551–573

Bals B, Rogers C, Jin M, Balan V, Dale B (2010) Evaluation of ammonia fiber expansion (AFEX) pretreatment for enzymatic hydrolysis of switchgrass harvested in different seasons and locations. Biotechnol Biofuels. doi:10.1186/1754-6834-3-1

Binod P, Satyanagalakshmi K, Sindhu R, Janu KU, Sukumaran RK, Pandey A (2012) Short duration microwave assisted pretreatment enhances the enzymatic saccharification and fermentable sugar yield from sugarcane bagasse. Renew Energy 37:109–116

Bapiraju KVSN, Sujatha P, Ellaiah P, Ramana T (2004) Mutation induced enhanced biosynthesis of lipase. Afr J Biotechnol 3(11):618–621

Carioca JOB, Arora HL (1984) Biomassa: fundamento e aplicações tecnológicas. UFC, Fortaleza

Chen WH, Ye SC, Sheen HK (2012) Hydrolysis characteristics of sugarcane bagasse pretreated by dilute acid solution in a microwave irradiation environment. Appl Energ 93:237–244. doi:10.1016/j.apenergy.2011.12.014

Cheung SW, Anderson BC (1997) Laboratory investigation of ethanol production from municipal primary wastewater. Bioresour Technol 59:81–96

Coulson JM, Richardson JF (1991) Chemical engineering, vol II. Oxford, Pergamon, Londres

Couto SR, Herrera JLT (2006) Industrial and biotechnological applications of laccases: a review. Biotechnol Adv 24:500–513

Del Bianchi VL, Moraes IO, Capalbo DMF (2001) Fermentação em estado sólido. In: Schmidell W, Lima UA, Aquarone E, Borzani W (eds) Biotecnologia industrial: engenharia bioquímica. Edgard Blücher Ltda, São Paulo, pp 247–276

Duff SJB, Murray WD (1996) Bioconversion of forest products industry waste cellulosics to fuel ethanol: a review. Bioresour Technnol 55:1–33

Dwivedi P, Vivekanand V, Pareek N, Sharma A, Singh RP (2011) Co-cultivation of mutant *Penicillium oxalicum* SAU_E-3.510 and *Pleurotus ostreatus* for simultaneous biosynthesis of xylanase and laccase under solid-state. N Biotechnol 28:616–626. doi:10.1016/j.nbt.2011.05.006

Fan LT, Gharpuray MM, Lee YH (1987) Cellulose hydrolysis, 1st edn. Springer, New York

Fioretin LD, Menon BT, Barros STD, Pereira NC, Lima OC, Modenes AO (2010) Isotermas de sorção do resíduo agroindustrial do bagaço de laranja. Rev Brasileira de engenharia agrícola e ambiental 14(6):653–659

Foust AS et al (1960) Principles of unit operations. Wiley, New York

Gámez S, González JJ, Ramírez JA, Garrote G, Vázquez M (2006) Study of the sugarcane bagasse hydrolysis by using phosphoric acid. J Food Eng 74:78–88

Gauto MA, Rosa GR (2011) Processos e operações unitárias da indústria Química. Ciência Moderna Ltda, Rio de Janeiro

Giardina P, Palmieri G, Fontanella B, Rivieccio V, Sannia G (2000) Manganese peroxidase isoenzymes produced by Pleurotusostreatus grown on wood sawdust. Arch Biochem Biophys 376(1):171–179

Glasser WG, Wright RS (1997) Steam-assisted biomass fractionation. II. Fractionation behavior of various biomass resources. Biomass Bioenergy 14:219–235

Gomide R (1983) Operações unitárias: operações com sistemas sólidos granulares (1). Cempro, São Paulo

Hendriks ATWM, Zeeman G (2009) Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresour Technol 100:10–18

Hernández-Salas JM, Villa-Ramírez MS, Veloz-Rendón JS, Rivera-Hernández KN, González-César RA, Plascencia-Espinosa MA, Trejo-Estrada SR (2009) Comparative hydrolysis and fermentation of sugarcane and agave bagasse. Bioresour Technol 100:1238–1245

Higushi T (1989) Mechanisms of lignin degradation by lignin peroxidase and laccase of white-rot fungi. In: Lewis NG, Paice MG (eds) Plant cell wall polymers, biogenesis and biodegradation, vol 399. ACS Symposium Series, Washington, pp 482–502

Izumi K, Okishio Y, Nagao N, Niwa C, Yamamoto S, Toda T (2010) Effects of particle size on anaerobic digestion of food waste. Int Biodeter Biodegr 64(7):601–608

Kallemullah S, Kailappan R (2004) Moisture sorption isotherm of red chillies. Biosystems Eng 88(1):95–104

Kartikaa IA, Yulianib S, Kailakub SI, Rigalc L (2012) Moisture sorption behaviour of jatropha seed (*Jatropha curcas*) as a source of vegetable oil for biodiesel production. Biomass Bioenergy 36:226–233

Kootstra AMJ, Beeftink HH, Scott EL, Sanders JPM (2010) Comparison of dilute mineral and organic acid pretreatment for enzymatic hydrolysis of wheat straw. Biochem Eng J 46:126–131

Krishnan C, Sousa LC, Jin M, Chang L, Dale BE, Balan V (2010) Alkali-based AFEX pretreatment for the conversion of sugarcane bagasse and cane leaf residues to ethanol. Biotechnol Bioeng 107(3):441–450

Kumar D, Jain VK, Shanker G, Srivastava A (2003) Citric acid production by solid state fermentation using sugarcane bagasse. Process Biochem 38(12):1731–1738

Laopoolkit P, Suwannaporn P (2011) Effect of pretreatments and vacuum drying on instant dried pork process optimization. Meat Sci 88:553–558

Lavarack BP, Griffin GJ (2002) The acid hydrolysis of sugarcane bagasse hemicellulose to produce xylose, arabinose, glucose and other products. Biomass Bioenergy 23:367–380

Lee J (1997) Biological conversion of lignocellulosic biomass to ethanol. J Biotechnol 56:1–24

Lin Y, Tanaka S (2006) Ethanol fermentation from biomass resources: current state and prospects. Appl Microbiol Biotechnol 69:627–642

Marrison CI, Larson ED (1995) Cost versus scale for advanced plantation-based biomass energy systems in the US. EPA symposium on greenhouse emissions and mitigation research, Washington

Mathew GM, Sukumaran RK, Singhania RR, Pandey A (2008) Progress in research on fungal cellulases for lignocellulose degradation. J Sci Ind Res 67:898–907

Mc Cabe WL, Smith JC, Harriot P (1993) Unit operations in chemical engineering, 5th edn. Book Company, New York

Mesa L, González E, Cara C, González M, Castro E, Mussatto SI (2011) The effect of organosolv pretreatment variables on enzymatic hydrolysis of sugarcane bagasse. Chem Eng J 168:1157–1162

Montross M, Crofcheck C (2010) Energy crops for the production of biofuels. In: Thermochemical conversion of biomass to liquid fuels and chemicals. Crocker M (ed). RSC, London pp 26–45

Mosier N, Wyman C, Dale BE, Elander R, Lee YY, Holtzapple M, Ladisch M (2005) Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour Technol 96:673–686

Novo LP, Gurgel LVA, Marabezi K, Aprigio A, Curvelo S (2011) Delignification of sugarcane bagasse using glycerol-water mixtures to produce pulps for saccharification. Bioresour Technol 102:10040–10046

Öhgren K, Vehmaanperä J, Siika-Aho M, Galbe M, Viikari L, Zacchi G (2007) High temperature enzymatic prehydrolysis prior to simultaneous saccharification and fermentation of steam pretreated corn stover for ethanol production. Enzym Microb Tech 40(4):607–613

Pandey A (1991) Effect of particle size of substrate on enzyme production in solid-state fermentation. Bioresour Technol 37(2):169–172

Perry RH, Green DV, Maloney JO (1997) Chemical engineers' handbook, 7th edn. McGraw-Hill, Malasia

Pessoa A Jr, Kilikian BV (2005) Purificação de Produtos Biotecnológicos. Manole, São Paulo

Ramos LP, Breuil C, Kushner DJ, Saddler JN (1992) Steam pretreatment conditions for effective enzymatic hydrolysis and recovery yields of *Eucalyptus viminalis* wood chips. Holzforschung 46:149–154

Ramos LP, Carpes ST, Silva FT, Ganter JLMS (2000) Comparison of the susceptibility of two hardwood species, *Mimosa scabrella*Benth and *Eucalyptus viminalis*Labill, to steam explosion and enzymatic hydrolysis. Braz Arch Biol Tech 43:185–206

Rocha GJM, Gonçalves AR, Oliveira BR, Olivares EG, Rossell CEV (2012) Steam explosion pretreatment reproduction and alkaline delignification reactions performed on a pilot scale with sugarcane bagasse for bioethanol production. Ind Crop Prod 35:274–279

Rodríguez-Chong A, Ramírez JA, Garrote G, Vázquez M (2004) Hydrolysis of sugarcane bagasse using nitric acid: a kinetic assessment. J Food Eng 61:143–152

Ruiz HA, Rodríguez-Jasso RM, Rodríguez R, Contreras-Esquivel JC, Aguilar CN (2012) Pectinase production from lemon peel pomace as support and carbon source in solid-state fermentation column-tray bioreactor. Biochem Eng J 65:90–95

Santos SFM, Wanderley LR, Souza RLA, Pinto GAS, Silva FLH, Macedo GR (2005) Caracterização físico-química do pedúnculo de caju in natura e do resíduo seco. In: 1th Simpósio Brasileiro de Pós-colheita de Frutos Tropicais. SBF, João Pessoa-PB, 29 November – 02 December 2005 (CD Rom)

Searcy E, Flynn P, Ghafoori E, Kumar A (2007) The relative cost of biomass energy transport. Appl Biochem Biotechnol 140:639–652

Silva I.S, Menezes CR, Franciscon E, Santos EC, Durrant LR (2010) Degradation of lignosulfonic and tannic acids by ligninolytic soil fungi cultivated under icroaerobic conditions. Brazilian Archives of Biology and Tech 53(3) http://dx.doi.org/10.1590/S1516-89132010000300026

Soccol CR, Faraco V, Karp S, Vandenberghe LPS, Thomaz-Soccol V, Woiciechowski AL, Pandey A (2011) Lignocellulosic bioethanol: current status and future perspectives. In: Pandey A, Larroche C, Ricke SC, Dussap CG, Gnansounou E (eds) Biofuels: alternative feedstocks and conversion processes. Academic, San Diego, pp 101–122

Souza RA, Amorim BC, Silva FLH, Conrado L (2007) Caracterização do resíduo seco do maracujá para utilização em fermentação semi-sólida. In: 16th Simpósio Nacional de Bioprocessos. Federal University of Paraná, Curitiba, 1–5 August 2007 (CD Rom)

Sun Y, Cheng J (2002) Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresour Technol 83:1–11

Tuor U, Winterhalter K, Fiechter A (1995) Enzymes of white-rot fungi involved in lignin degradation and ecological determinants for wood decay. J Biotechnol 41:1–17

Wong DWS (2009) Structure and action mechanism of ligninolytic enzymes. Appl Biochem Biotechnol 157:174–209

Yuan X, Shi X, Zhang P, Wei Y, Guo R, Wang L (2011) Anaerobic biohydrogen production from wheat stalk by mixed microflora: kinetic model and particle size influence. Bioresour Technol 102(19):9007–9012

Zhang YP, Ding S, Mielenz JR, Cui J (2007) Fractionating recalcitrant lignocellulose at modest reaction conditions. Biotechnol Bioeng 97:214–223