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# Use of Agro-Industrial Wastes in Solid-State Fermentation Processes

Solange I. Mussatto, Lina F. Ballesteros, Silvia Martins and José A. Teixeira  
*IBB – Institute for Biotechnology and Bioengineering,  
Centre of Biological Engineering, University of Minho,  
Portugal*

## 1. Introduction

Large amount of wastes is generated every year from the industrial processing of agricultural raw materials. Most of these wastes are used as animal feed or burned as alternative for elimination. However, such wastes usually have a composition rich in sugars, minerals and proteins, and therefore, they should not be considered “wastes” but raw materials for other industrial processes. The presence of carbon sources, nutrients and moisture in these wastes provides conditions suitable for the development of microorganisms, and this open up great possibilities for their reuse in solid-state fermentation (SSF) processes, for example. Agro-industrial wastes can be used as solid support, carbon and/or nutrient source in SSF processes for the production of a variety of value-added compounds.

SSF is a technology lesser explored than submerged fermentation systems, but that has been proved to be able to give higher product yields and productivities, which is of great interest for industrial activities. In addition, costs are much lower due to the efficient utilization and value-addition of wastes. The reuse of agro-industrial wastes in SSF processes is of particular interest due to their availability and low cost, besides being an environment friendly alternative for their disposal. The present chapter deals with the use of agro-industrial wastes in SSF processes. Initially, an overview about the generation of these wastes and their chemical composition is presented. In the sequence, the characteristics of SSF systems and variables that affect the product formation by this process are reviewed and discussed. Finally, potential applications of agro-industrial wastes in SSF processes for the obtainment of value-added compounds are described.

## 2. Agro-industrial wastes: generation and chemical composition

Agro-industrial wastes are generated during the industrial processing of agricultural or animal products. Those derived from agricultural activities include materials such as straw, stem, stalk, leaves, husk, shell, peel, lint, seed/stones, pulp or stubble from fruits, legumes or cereals (rice, wheat, corn, sorghum, barley...), bagasses generated from sugarcane or sweet sorghum milling, spent coffee grounds, brewer's spent grains, and many others. These wastes are generated in large amounts throughout the year, and are the most

abundant renewable resources on earth. They are mainly composed by sugars, fibres, proteins, and minerals, which are compounds of industrial interest. Due to the large availability and composition rich in compounds that could be used in other processes, there is a great interest on the reuse of these wastes, both from economical and environmental view points. The economical aspect is based on the fact that such wastes may be used as low-cost raw materials for the production of other value-added compounds, with the expectancy of reducing the production costs. The environmental concern is because most of the agro-industrial wastes contain phenolic compounds and/or other compounds of toxic potential; which may cause deterioration of the environment when the waste is discharged to the nature.

Large amount of the agro-industrial wastes are mainly composed by cellulose, hemicellulose and lignin, being called "lignocellulosic materials". In the lignocellulosic materials, these three fractions are closely associated with each other constituting the cellular complex of the vegetal biomass, and forming a complex structure that act as a protective barrier to cell destruction by bacteria and fungi. Basically, cellulose forms a skeleton which is surrounded by hemicellulose and lignin (Fig. 1).

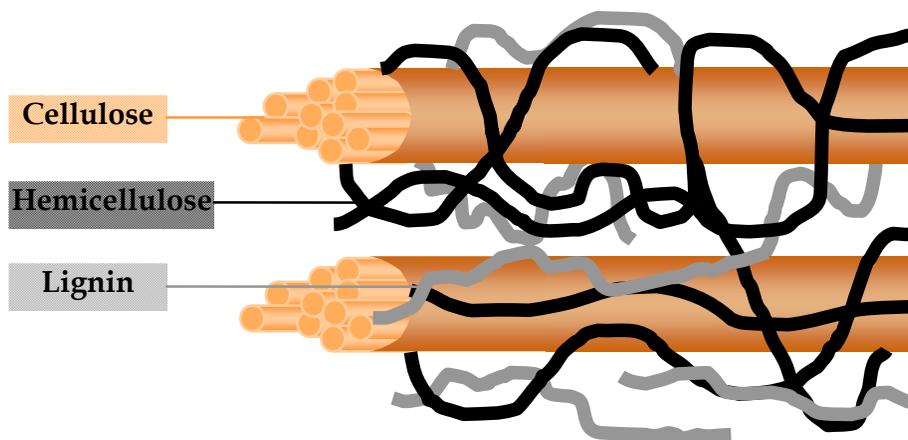


Fig. 1. Schematic representation of the cellulose, hemicelluloses and lignin fractions in the lignocellulosic materials

The cellulose structure is composed only by glucose units, i.e., it is a homopolymer where units of cellobiose (two anhydrous glucose rings joined via a  $\beta$ -1,4 glycosidic linkage) are sequentially repeated (Fig. 2) (Klemm et al., 1998). The long-chain of cellulose polymers, which may have until 10,000 glucose units, are linked together by hydrogen and van der Waals bonds, which cause the cellulose to be packed into microfibrils (Ha et al., 1998). By forming these hydrogen bonds, the chains tend to arrange in parallel and form a crystalline structure (as represented in Fig. 1). 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, being strongly resistant to chemicals (Taherzadeh & Karimi, 2008).

On the contrary of the cellulose, hemicellulose is a heterogeneous polymer usually composed by five different sugars (L-arabinose, D-galactose, D-glucose, D-mannose, and D-xylose) and some organic acids (acetic and glucuronic acids, among others). The structure of the hemicellulose is linear and branched. The backbone of the hemicellulose chain can be formed by repeated units of the same sugar (homopolymer) or by a mixture of different

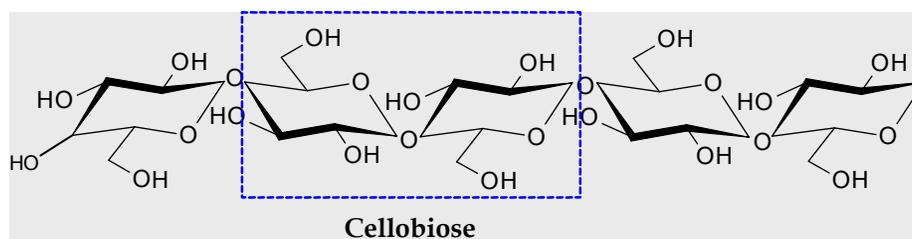


Fig. 2. Representation of the cellulose structure

sugars (heteropolymer). According to the main sugar in the backbone, hemicellulose has different classifications e.g., xylans, glucans, mannans, arabinans, xyloglucans, arabinoxylans, glucuronoxylans, glucomannans, galactomannans, galactoglucomannans and  $\beta$ -glucans. Fig. 3 shows an example of hemicellulose structure formed by a xylan backbone (repeated units of xylose sugar). Besides the differences in the chemical composition, hemicellulose also differs from cellulose structure in other aspects, including: 1) the size of the chain, which is much smaller (it contains approximately 50-300 sugar units); 2) the presence of branching in the main chain molecules, and 3) to be amorphous, being less resistant to chemicals (Fengel & Wegener, 1989).

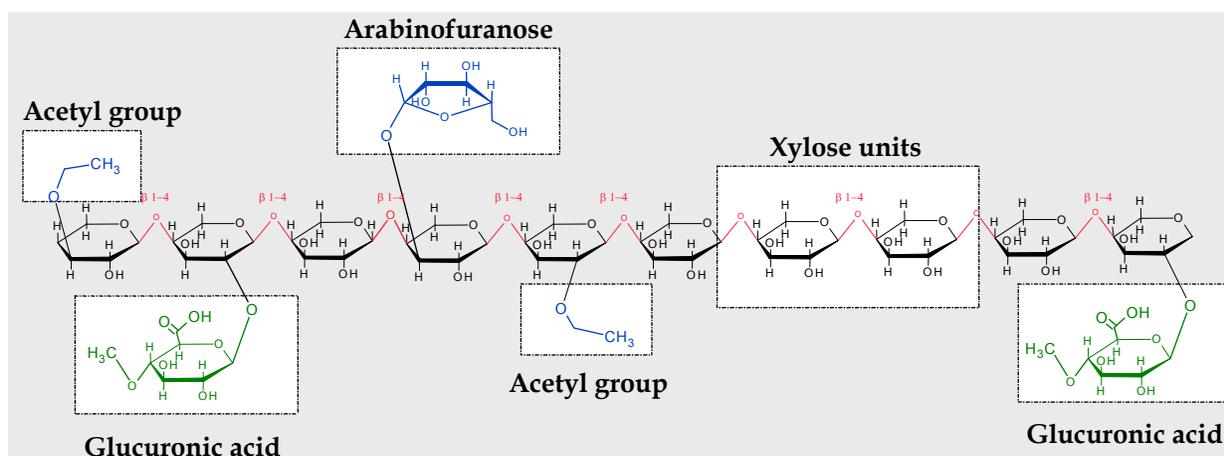


Fig. 3. Representation of a hemicellulose structure formed by a xylan backbone

The lignin structure is not formed by sugar units, but by phenylpropane units linked in a large and very complex three-dimensional structure. Three phenyl propionic alcohols are usually found as monomers of lignin, which include the alcohols *p*-coumaryl, coniferyl, and sinapyl. 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 physico-chemical barrier against microbial attack (Fengel & Wegener, 1989). An example of structure proposed for the lignin is given in Fig. 4. Due to its molecular configuration, lignin is extremely resistant to chemical and enzymatic degradation.

The percentage of cellulose, hemicellulose and lignin is different to each waste since it varies from one plant species to another, and also according to the process that the agricultural material was submitted. In addition, the ratios between various constituents in a single plant may also vary with age, stage of growth, and other conditions. Usually, cellulose is the dominant fraction in the plant cell wall (35-50%), followed by hemicellulose (20-35%) and lignin (10-25%). Average values of the main components in some lignocellulosic wastes are shown in Table 1.

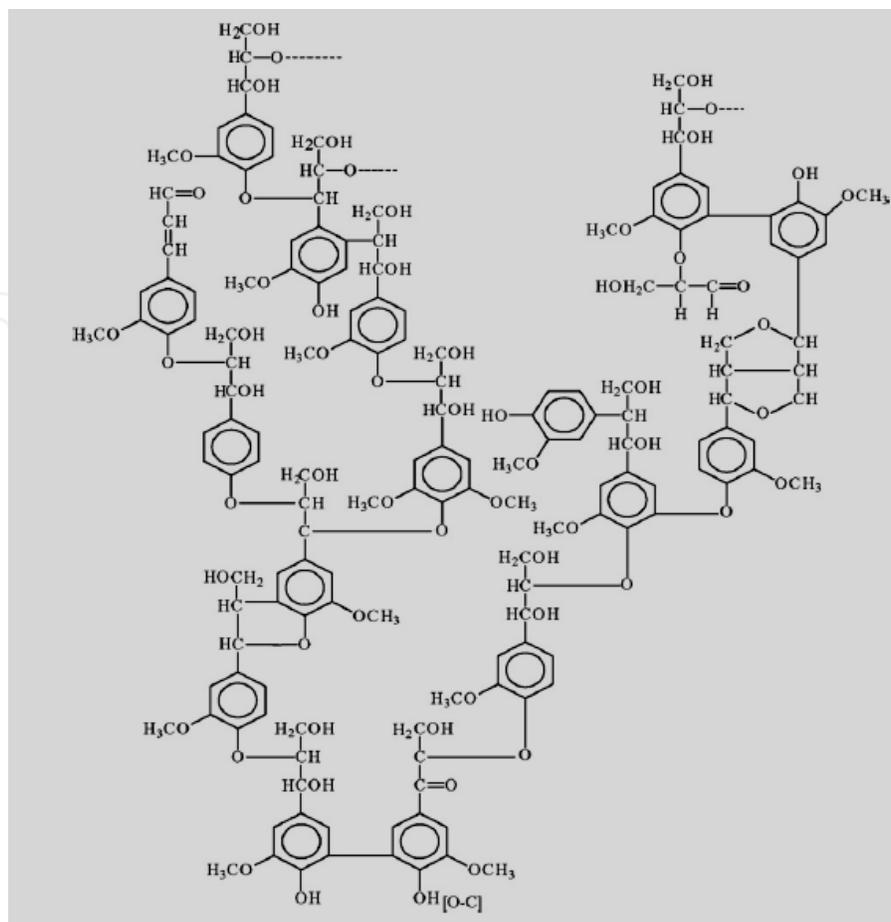


Fig. 4. Structure proposed for the lignin of conifer (Adler, 1977).

The presence of sugars, proteins, minerals and water make the agro-industrial wastes a suitable environment for the development of microorganisms, mainly fungal strains, which are able to quickly grow in these wastes. If the cultivation conditions are controlled, different products of industrial interest may be produced, avoiding the loss of potential energy sources.

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

Table 1. Main components of some lignocellulosic wastes (Nigam et al., 2009)

### 3. Solid-State Fermentation

Solid-state fermentation (SSF) consists of the microbial growth and product formation on solid particles in the absence (or near absence) of water; however, the substrate contains sufficient moisture to allow the microorganism growth and metabolism (Pandey, 2003). This bioprocess has been subject of several studies and it has been proved that SSF has the important advantage of leading to higher yields and productivities or better product characteristics than submerged fermentation (SmF), which is characterized by the cultivation of the microorganisms in a liquid medium. Another great advantage of SSF compared to SmF is the lower capital and operating costs due to the utilization of low cost agricultural and agro-industrial wastes as substrates. The low water volume used in SSF has also a large impact on the economy of the process mainly because of the smaller fermenter-size, the reduced downstream processing, the reduced stirring and lower sterilization costs (Hölker & Lenz, 2005; Nigam, 2009). Several advantages and disadvantages of SSF over SmF are summarized in Table 2.

#### 3.1 Important technical aspects

Despite the numerous processing and biological advantages that SSF has over SmF, the scaling-up of this bioprocess is not well developed and has a drawback associated to control of operations (heat transfer and culture homogeneity problems) and fermentation variables (mainly pH and temperature). Three types of bioreactors are commonly used in SSF processes: packed-bed, horizontal drum and fluidized bed. However, these bioreactors have their own advantages and disadvantages, and there is a necessity to develop novel bioreactors with better design. In order to overcome all these difficulties, research attention has been directed towards the development and implementation of effective control strategies of bioreactors operating in SSF conditions in large-scale and their design (Durand, 2003; von Meien et al., 2004; Mitchell et al., 2000). Also an economical evaluation of the overall process is needed in order to determine its feasibility to a specific purpose.

Separation of biomass represents another challenge in SSF, being essential for the kinetic studies. Several indirect methods have been employed including glucosamine estimation, ergosterol estimation, protein (Kjeldahl) estimation, DNA estimation, dry weight changes and CO<sub>2</sub> evolution; however, all of these methodologies have their own weaknesses. The estimation of oxygen intake and carbon dioxide evolution rate are considered to be most accurate for the determination of the microorganism growth (Pandey et al., 2007). Digital image processing has also been developed for measuring biomass in SSF (Couri et al., 2006).

There are various important factors to be considered for the development of a successful bioprocess under SSF conditions. Some of the most important include the selection of a suitable microorganism and solid support to be used. A diversity of microorganisms, including fungi, yeasts and bacteria can be used in SSF processes. Due to the low moisture content in the SSF media, fungi are the most commonly used microorganisms because of their ability to grow in these environments. Table 3 summarizes some of the most recent studies in SSF, the microorganisms and solid supports employed. Note that a large variety of solid supports have been used in these processes, and fungi are effectively the most used microorganisms. The selection of the microorganism to be used in a SSF process also depends on the desired end product. For example, filamentous fungi have great potential to produce bioactive compounds and thermostable enzymes of high scientific and commercial value by SSF, and therefore they are the most used microorganisms for this purpose.

<b>Advantages</b>	<b>Disadvantages</b>
Similar or higher yields than those obtained in the correspondent submerged cultures	Biomass determination is very difficult
The low availability of water reduces the possibilities of contamination by bacteria and yeast. This allows working in aseptic conditions in some cases	Usually the substrates require pre-treatment (reduction of size by grinding, rasping or chopping, homogenization, physical, chemical or enzymatic hydrolysis, steam treatment)
The environmental conditions are similar to those of the natural habitats of fungi, which constitute the main group of microorganisms used in SSF	Only microorganisms able to grow at low moisture levels can be used
Higher levels of aeration, especially adequate in processes that demand an intensive oxidative metabolism	The solid nature of the substrate causes problems in the monitoring of the process parameters (pH, moisture content, oxygen and biomass concentration)
The inoculation with spores (in those processes that involve fungi) facilitates their uniform dispersion through the medium	Agitation may be very difficult. For this reason static conditions are preferred
Culture media are often quite simple. The substrate usually provides all the nutrients necessary for growth	Frequent need of high inoculum volumes
Simple design reactors with few spatial requirements can be used due to the concentrated nature of the substrates	Many important basic scientific and engineering aspects are yet poor characterized. Information about the design and operation of reactors on a large scale is scarce
Low energetic requirements (in some cases autoclaving or steam treatment, mechanical agitation and aeration are not necessary)	Possibility of contamination by undesirable fungi
Small volumes of polluting effluents are generated. Fewer requirements of solvents are necessary for the product extraction due to its high concentration	The removal of metabolic heat generated during growth may be very difficult
The low moisture availability may favour the production of specific compounds that may not be produced or may be poorly produced in SmF	Extracts containing products obtained by leaching of fermented solids are often of viscous nature
In some cases, the products obtained have slightly different properties (e.g. more thermotolerance) when produced in SSF in comparison to SmF	Mass transfer limited to diffusion In some SSF, aeration can be difficult due to the high solid concentration
Due to the concentrated nature of the substrate, smaller reactors in SSF with respect to SmF can be used to hold the same amounts of substrate	Spores have longer lag times due to the need for germination Cultivation times are longer than in SmF

Table 2. Advantages and disadvantages of SSF compared to SmF (Pérez-Guerra et al., 2003)

Microorganism	Solid support	Reference
<b>Fungi</b>		
<i>Aspergillus niger</i>	Lemon peel, orange peel, apple pomace, pistachio shell, wheat bran, coconut husk, pecan nutshell, creosote bush leaves, bean residues	Orzua et al. (2009)
<i>Aspergillus niveus</i>	Sugar cane bagasse	Guimarães et al. (2009)
<i>Aspergillus oryzae</i>	Red gram plant waste	Shankar & Mulimani (2007)
<i>Aspergillus sojae</i>	Crushed maize, maize meal, corncob	Ustok et al. (2007)
<i>Bjerkandera adusta</i>	Wheat straw	Dinis et al. (2009)
<i>Ganoderma applanatum</i>		
<i>Phlebia rufa</i>		
<i>Trametes versicolor</i>		
<i>Phanerochaete chrysosporium</i>	Rice straw	Yu et al. (2009)
<i>Penicillium sp.</i>	Soybean bran	Wolski et al. (2009)
<i>Sporotrichum thermophile</i>	Sesame oil cake	Singh & Satyanarayana (2008)
<i>Trichosporon fermentans</i>	Rice straw	Huang et al. (2009)
<b>Yeast</b>		
<i>Baker yeast AF37X</i>	Sweet sorghum	Yu et al. (2008)
<i>Saccharomyces cerevisiae</i>	Mahula flowers Corn stover	Mohanty et al. (2009) Zhao & Xia (2009)
<b>Bacteria</b>		
<i>Nocardia lactamdurans</i>	Wheat bran, rice, soybean oil cake, soybean flour	Kagliwal et al. (2009)
<i>Bacillus sphaericus</i>	Wheat bran	El-Bendary et al. (2008)
<i>Pseudomonas aeruginosa</i>	Jatropha curcas seed cake	Mahanta et al. (2008)
<i>Streptomyces</i>	Coffee pulp	Orozco et al. (2008)

Table 3. Recent studies on SSF (Martins et al., 2011)

The process variables such as pre-treatment and particle-size of substrates, medium ingredients, supplementation of growth medium, sterilization of SSF medium, moisture content, water activity (aw), inoculum density, temperature, pH, agitation and aeration, have also to be considered for an efficient SSF process (Nigam & Pandey, 2009). Generally the production yields can be improved with a suitable choice of the substrate or mixture of substrates with appropriate nutrients. As a whole, the support material must present

characteristic favourable for the microorganism development, be of low cost, and present a good availability, which might involve the screening of several agro-industrial wastes. The moisture content and  $a_w$  play also an important role in SSF. In general, the substrates have a water content oscillating between 30 and 85%. Low values might induce the sporulation of the microorganism, while more elevated levels may reduce the porosity of the system, which can produce oxygen transfer limitation. In fact, the water requirements of the microorganism have been considered to be better defined in terms of  $a_w$  rather than water content in the solid substrate (Raimbault, 1998), since the  $a_w$  is the water available or accessible for the growth of the microorganism, and affects the biomass development, metabolic reactions, and the mass transfer processes.

Finally, the selection of the most appropriate downstream process for the final product obtained is also crucial when SSF processes are performed. The product obtained by SSF may be recovered from the solid fermented mass by extraction with solvents (aqueous or other solvents mixtures). The type of solvent and its concentration, as well as the ratio of solvent to the solid and pH are important variables that influence the product extraction. In addition, since the metabolites diffuse throughout the solid mass during the culturing, long extraction-times may be required for complete product recovery. The cost of purification depends on the quality of the obtained extract. For example, the presence and concentration of inert compounds in the extract increase the cost of purification and therefore the cost of recovery is increased. Particularly those secondary metabolites which are used in bulk in the pharmaceutical and health industry and whose purity is governed by stringent regulations need to go through specific purification strategy (Nigam, 2009).

### 3.2 Modelling in SSF

Modelling is an important tool to improve the chances of successfully transforms an SSF process from laboratory to a scaled-up commercial level. Basically, the emergence of modelling in SSF underlie on the need of searching mathematical expressions that represent the system under consideration, focusing the following main problems: (i) the representation of the microbial activity (kinetic patterns and thermodynamic concerns), (ii) studies of the problems of heat accumulation and heterogeneous distribution in complex gas-liquid-solid multiphase bioreactor (or fermenter) systems, (iii) the connection between the two above systems and (iv) the selection of the best type of the fermenter (Koncsag & Kirwan, 2010; Singhanian et al., 2009). The ability to predict the behaviour of a SSF process can ascertain the validity of the system, while establishing different parameters that characterize the process, and appropriate mechanisms for its development and control. The modelling of bioreactors employed in SSF processes can play a crucial role in the analysis, design and development of bioprocesses for the treatment of agro-industrial wastes. The development of models can also significantly reduce the number of wet experiments, and in consequence will have a strong impact on time saving and costs of the process.

Several research studies have been performed in order to develop mathematical models capable of characterizing SSF bioreactors. For example, a mathematical model has been developed for a packed bed SSF bioreactor employing the N-tanks in series approach to analyze the production of protease by *Aspergillus niger* (Sahir et al., 2007). Another simple mathematical model was studied in order to quantify the performance of continuous solid-

state bioreactors having two different solid substrate flow patterns, plug flow and completely mixed flow, where plug flow shown to have superior performance when high product concentration was needed (Khanahmadia et al., 2006). The modelling and optimization of simultaneous influence of temperature and moisture on microbial growth through a quantitative description has been also investigated, as well as the effect of microbial biomass on the isotherm of the fermenting solids in SSF. More recent studies evaluated the modelling of different phases of the bacterial growth curve and the production of  $\alpha$ -amylase by *Bacillus* sp. KR-8104 in a solid-state fermentation process based on variation in dry weight using wheat bran (Hashemi et al., 2011). Another recent subject of research was the determination of the kinetics of microbial growth related to pectinase and xylanase synthesis during the growth of *Aspergillus niger* F3 in a horizontal SSF drum bioreactor charged with 2 kg of dried citrus peel (Rodríguez-Fernández et al., 2011).

It is clear that all the results obtained by these studies are of great importance in the scale-up and optimization of SSF bioreactors configuration for the production of a desired end product using agro-industrial wastes as solid support. Nevertheless, several issues still need to be analyzed and solved for improved modelling of SSF. Information regarding modelling in SSF has been limited because of the unavailability of suitable methods for direct measurement of the microorganism growth. This is due to the difficulty to separate the microorganism from the substrate, and problems related to the determination of the substrate utilization rate. An alternative to overcome these problems is the determination of the microorganism cell growth by measuring the change in gaseous compositions inside the bioreactor, since it is well known that the fermentation kinetics is very sensitive to the variation in ambient and internal gas compositions (Singhania et al., 2009).

#### **4. Potential applications of agro-industrial wastes in SSF processes for the obtainment of value-added compounds**

SSF has two potential areas of application. One of them is for environmental control such as for the production of compost, ensiling and animal feed from solid wastes, bioremediation and biodegradation of hazardous compounds, and biological detoxification of agro-industrial wastes. On the other hand, SSF may be utilized to obtain value-added compounds such as enzymes, mushrooms, amino acids, biopesticides, biofuels, biosurfactants, organic acids, flavours, colorants, aromatic compounds, biologically active secondary metabolites, and other substances of interest to the food industry. The following sections describe some of the most important applications of agro-industrial wastes in SSF processes, the microorganisms used and products obtained.

##### **4.1 Organic acids**

Organic acids are widely used in food and beverages industries because of their excellent characteristic to prevent deterioration and extending the shelf life of food. SSF has been employed for many years to produce citric acid and lactic acid to large-scale production being a successful process. The production of oxalic acid, gluconic acid and gallic acid by SSF has also been reported. Table 4 shows some examples of organic acids produced by SSF, the microorganisms and solid supports utilized.

Product	Microorganism	Solid support
Citric acid	<i>Aspergillus niger</i>	corn cob, sugarcane bagasse, coffee husk, kiwi fruit peel, wheat bran, rice bran, pineapple waste, mixed fruit waste, apple pomace, sawdust with rice hulls, cassava fibrous residue, potato starch residue
	<i>Aspergillus foetidus</i>	pineapple waste
Lactic acid	<i>Lactobacillus delbrueckii</i>	cassava bagasse and sugarcane bagasse
	<i>Rhizopus oryzae</i>	sugarcane bagasse
	<i>Lactobacillus paracasei</i>	sweet sorghum
	<i>Rhizopus oryzae</i>	carrot-processing waste
Oxalic acid	<i>Aspergillus oryzae</i>	wheat kernels
Gluconic acid	<i>Aspergillus niger</i>	tea waste with sugarcane molasses
Gallic Acid	<i>Rhizopus oryzae</i>	gallo seeds cover

Table 4. Organic acids production by SSF

#### 4.1.1 Citric acid

Citric acid is the most important organic acid produced at industrial level, and is extensively used in foods, beverages, cosmetics, pharmaceuticals and chemical products. In the food industry, citric acid has been applied as an additive, being utilized as preservative, flavour enhancer, antifoam, or antioxidant. In the chemical industry, it is exploited as plasticizer, softener, and for the treatment of textiles. This acid has also been widely used in the detergent industry for replacement of polyphosphates, decreasing the production costs.

SmF has been substantially studied to produce citric acid. *Aspergillus niger* is one of the microorganisms commercially used to produce citric acid under SSF conditions, being cultivated on agro-industrial wastes such as corn cob, sugarcane bagasse, coffee husk, kiwi fruit peel, wheat bran, rice bran, pineapple waste, mixed fruit waste, maosmi waste sugar beet molasses, sawdust with rice hulls, cassava fibrous residue, apple pomace, and potato starch residue. In a study on the production of citric acid by *A. niger* LPB 21 cultivated on three cellulosic supports (cassava bagasse, sugarcane bagasse, and vegetable sponge), cassava bagasse was the best substrate, giving 27 g citric acid per 100 g dry substrate under optimum fermentation conditions, which corresponded to 70% yield (based on sugars consumed). When *A. niger* NRRL 2001 was cultivated on sugarcane bagasse, coffee husk and cassava bagasse, cassava bagasse was also the best support for SSF, giving the highest yield of citric acid (88 g/kg dry matter) (Pandey et al., 2000a).

It merits emphasizing that the acid production by SSF is directly influenced by the nitrogen source used in the fermentation, and ammonium salts such as urea, ammonium chloride,

and ammonium sulphate are the most used to obtain high yields. Potassium dihydrogen phosphate has been found to be the most suitable phosphorus source and low levels of phosphorus were found to favour citric acid production. It has been generally found that addition of methanol, ethanol, isopropanol or methyl acetate, copper and zinc enhance the production of citric acid, while magnesium result essential for growth as well as for citric acid production (Krishna, 2005).

#### 4.1.2 Lactic acid

Lactic acid plays an important role in various biochemical processes. It is used, for example, as acidulant and preservative of many food products such as cheese, meat, beer and jellies. Besides the applications in the food industry, lactic acid has also wide uses in pharmaceutical, leather and textile industries. One of the most important applications of this acid is in the synthesis of biodegradable plastics and coatings, but it is also used in the manufacture of cellophane, resins, some herbicides and pesticides.

Lactic acid production by SSF has been carried out using fungal as well as bacterial strains. *Rhizopus* sp. and *Lactobacillus* sp. strains are the most utilized microorganisms to produce this acid; and sugarcane bagasse, sugarcane press mud and carrot-processing wastes have been used as substrates in these processes. The production of L(+)-lactic acid by *R. oryzae* in SSF conditions using sugarcane bagasse as support material was demonstrated to promote slightly higher productivity than the production in SmF conditions (Couto & Sanromán, 2006). *L. Paracasei* and *L. delbrueckii* bacteria have also been reported to efficiently produce this acid by SSF.

#### 4.1.3 Other acids

Oxalic acid and gluconic acid are other examples of organic acids produced by SSF. Some studies report the production of oxalic acid by *Aspergillus oryzae* using wheat kernels as support (Biesebeke et al., 2002), and the production of gluconic acid by *Aspergillus niger* ARNU-) using tea waste as support and sugarcane molasses as carbon source (Sharma et al., 2008). Both compounds have great industrial applications. The main application of the oxalic acid, for example, includes cleaning or bleaching, especially for the removal of rust. It is also used in the restoration of old wood and is an important reagent in lanthanide chemistry. Gluconic acid is utilized as a food additive, acting as acidity regulator. It is also used in cleaning products where it dissolves mineral deposits especially in alkaline solution.

#### 4.2 Production of flavour and aroma compounds

Aroma compounds can be found in food, wine, spices, perfumes and essential oils, but over a quarter of them are used in the food industry. These compounds play an important role in the production of flavours, which are used to improve food quality and give it value-added. Most of the flavouring compounds are presently produced via chemical synthesis or extraction from natural materials. However, since the consumers prefer food free of chemical substances, the microbial biosynthesis or bioconversion systems result as a promising substitute to produce aroma compounds.

Both fungi and bacteria have ability to synthesise aroma compounds by SSF. Fungi from the genus *Ceratocystis* produce a large range of fruit-like or flower-like aromas such as peach,

pineapple, banana, citrus, and rose, depending on the strain and the culture conditions. Another interesting fungal strain for use on the production of aroma compounds is *C. fimbriata*. This strain has an enormous potential for ester synthesis since it grows rapidly, has a great sporulation capacity and produces a wide variety of aromas. Fruity aroma production by *C. fimbriata* in solid cultures have been reported using different agro-industrial wastes such as cassava bagasse (used in combination with soya bean or apple pomace), apple pomace, amaranth and soya bean. Production of a strong pineapple aroma is reported when this fungus is cultivated on SSF using coffee husk as substrate. It has also been demonstrated that the addition of glucose to the solid medium (wheat bran, cassava bagasse and sugarcane bagasse) results in the production of a fruity aroma by *C. fimbriata*, while the addition of leucine or valine causes a strong banana aroma (Christen et al., 1997).

Production of aroma compounds by SSF has also been reported using *Kluyveromyces marxianus* cultivated on different solid substrates such as cassava bagasse, giant palm bran, apple pomace, sugarcane bagasse and sunflower seeds. This strain was able to produce nine and eleven compounds (alcohols, esters and aldehydes) from palm bran and cassava bagasse, respectively; among of which, the esters were responsible for the fruity aroma (Medeiros et al., 2000). In fact, esters are considered the source of the aromas. Pyrazine, for example, is a heterocyclic aromatic organic compound found in a wide variety of food, which possess nutty and roasty flavour.

Other fungal strains reported to produce aroma compounds are the edible fungus *Rhizopus oryzae*, *Neurospora* sp., *Zygosaccharomyces rouxii*, and *Aspergillus* sp. Among the bacterial strains, *Bacillus natto* and *B. subtilis* are reported to produce aroma compounds by SSF.

#### 4.3 Production of enzymes

The production of enzymes has evolved rapidly and nowadays, enzymes are the most important products obtained for human needs through microbial sources. Enzymes have application in a variety of areas including food biotechnology, environment, animal feed, pharmaceutical, textile, paper and others technical and chemical industries. Due to the large industrial application and significant cost, there is a necessity to develop processes able to minimize the production costs. In this sense, the production of enzymes by SSF has been greatly explored and it has been proved that this technology is able to promote higher yields than the production by SmF, for the same microorganism strain. Additionally, a variety of agro-industrial wastes may be used as support material, carbon and nitrogen sources for the production of different enzymes by SSF. This is an important aspect since allows the reuse of a variety of low cost wastes for the production of this value-added product, which contribute for the wastes reuse and to decrease the production costs. Some of the most relevant enzymes obtained by SSF, the microorganisms and solid supports used are shown in the Table 5.

#### 4.4 Fructooligosaccharides production

Fructooligosaccharides (FOS), also called oligofructose or oligofructan, are oligosaccharides that, when ingested, promote enormous benefits to the human health. They can be used as artificial or alternative sweetener and are considered a small dietary fibre with low caloric value. Additionally, FOS has important functional properties due to their capacity of serving

as a substrate for microflora in the large intestine, increasing the overall gastrointestinal tract health. FOS promotes also the calcium and magnesium absorption in animals and human gut, and increases the levels of phospholipids, triglycerides and cholesterol.

Product	Microorganism	Solid support	
Glucoamylase	<i>Aspergillus niger</i>	Wheat bran	
Cellulases	<i>Bacillus subtilis</i>	Banana fruit stalk	
	<i>Aspergillus ustus</i> , <i>Sporotrichum pulverulentum</i> , <i>Trichoderma sp.</i>	Wheat bran, rice straw	
	<i>Botrytis sp.</i>		
	<i>Trichoderma aureoviride</i>	Leached beet pulp	
	<i>Penicillium citrinum</i>	Rice husks	
	<i>Trichoderma reesi</i>	Sweet sorghum silage	
	<i>Bacillus subtilis</i>	Banana fruit stalk	
	<i>Trichoderma viride</i>	Coconut pith	
	Lipases	<i>Candida rugosa</i>	Rice bran, wheat bran, peanut
		<i>Candida sp.</i>	Press cake and coconut oil cake
<i>Monascus fuliginosus</i>			
<i>Neurospora sitophila</i> <i>A. niger</i>			
Proteases	<i>Bacillus licheniformis</i>	Rice straw	
	<i>Penicillium sp.</i>	Defatted soybean cake	
Xylanase	<i>Thermoascus aurantiacus</i>	Corn silage	
	<i>Penicillium decumbens</i>	Corn straw	
$\alpha$ -galactosidase and $\beta$ -galactosidase	<i>Aspergillus niger</i>	Wheat bran, soybean cake waste	
	<i>A. oryzae</i>		
	<i>Candida sp.</i>		
	<i>Neurospora sitophila</i>		
	<i>P. candidum</i>		
	<i>Mucor sp.</i>		
$\alpha$ -amylase and $\beta$ -amylase	<i>Kluyveromyces lactis</i>	Rice husk, coconut cake, tea waste, cassava, cassava bagasse, sugarcane bagasse, banana waste, corn flour	
	<i>Aspergillus sp.</i>		
	<i>Rhizopus sp.</i>		
	<i>Mucor sp.</i>		
	<i>Bacillus sp.</i>		
	<i>Saccharomyces sp.</i>		
	<i>Bacillus subtilis</i>		
<i>Aeromonas caviae</i>			
Ligninase	<i>Pleurotus sp.</i>	Wheat straw and bagasse	
	<i>Phanerochaete chrysosporium</i>		
Tannase	<i>A. niger</i>	Palm kernel cake	
Phytase	<i>A. ficuum</i> , <i>A. carbonarius</i>	Wheat bran	

Table 5. Enzymes produced by SSF

Currently, FOS is mainly produced on industrial scale by SmF from the disaccharide sucrose by microbial enzymes with transfructosylating activity ( $\beta$ -fructofuranosidases, EC.3.2.1.26, also designed as fructosyltransferases EC.2.4.1.9). However, the interest to produce FOS using different fungus such as *Aspergillus*, *Aureobasidium*, and *Penicillium*, in which these enzymes have been found, has increased in the last years. *Aspergillus japonicus* has been considered a potential strain for industrial production of FOS by SSF (Chien et al., 2001; Mussatto et al., 2009), and the development of a viable and economic process that permits to obtain high volumetric productivity is one of the main challenges to be overcome.

In a recent study, some agro-industrial wastes including corn cobs, coffee silverskin, and cork oak were used as support and nutrient source during the FOS production by *Aspergillus japonicus* under SSF conditions. Among the wastes, coffee silverskin was the most suitable support for FOS production. Furthermore, the highest enzymatic activity results were also achieved when using coffee silverskin as solid support (Mussatto & Teixeira, 2010). These results were considered of great importance for the development of an efficient strategy to produce FOS on industrial scale with higher yield and productivity than that currently obtained.

#### 4.5 Bioactive compounds

Bioactive compounds are extra nutritional constituents used as ingredients in food and cosmetic industries. Most common bioactive compounds include secondary metabolites such as mycotoxins, bacterial endotoxins, alkaloids, plant growth factors, antibiotics, immuno-suppressive drugs, food grade pigments, and phenolic compounds. In the last decades, there has been an increasing trend towards the utilization of the SSF to produce bioactive compounds, since this process has been shown more efficient than SmF. Table 6 shows some examples of bioactive compounds produced with significantly higher yields by SSF than by SmF. Besides the higher yields, SSF has also been reported to produce secondary metabolites in shorter times than SmF, with capital costs significantly lesser.

Product	Microorganism
6-pentyl-alpha-pyrone	<i>Trichoderma harzianum</i>
Bafilomycin B1 + C1	<i>Streptomyces halstedii</i> K122
Benzoic acid	<i>Bjerkandera adusta</i>
Benzyl alcohol	<i>Bjerkandera adusta</i>
Cephameycin C	<i>Streptomyces clavuligerus</i>
Coconut aroma	<i>Trichoderma</i> sp.
Ergot alkaloids	<i>Claviceps fusiformis</i>
Giberellic acid	<i>Giberella fujikuroi</i>
Iturin	<i>Bacillus subtilis</i>
Ochratoxin	<i>Aspergillus ochraceus</i>
Oxytetracycline	<i>Streptomyces rimossus</i>
Penicillin	<i>Penicillium chrysogenum</i>
Rifamycin-B	<i>Amycolatopsis mediterranei</i>
Tetracycline	<i>Streptomyces viridifaciens</i>

Table 6. Examples of secondary metabolites produced with higher yield by SSF than by SmF (Hölker et al., 2004)

Alkaloids are secondary metabolites synthesized usually from amino acids. The production of total ergot alkaloids by *Claviceps fusiformis* in SSF was reported to be 3.9 times higher than that in SmF. The production of antibiotics by SSF has also been reported to occur with higher yields and in shorter times when compared to SmF. Among the antibiotics produced by SSF are penicillin, chlorotetracyclines, cephalosprin, tetracyclines, oxytetracyclines, iturin, surfactin, actinorhodin, methylenomycin and monorden. The important factors in antibiotic production by SSF include the type of strain used, the fermenter design, the general methodology, and control of parameters (Krishna, 2005; Pandey et al., 2000b).

Phenolic compounds, which present a large diversity of biological effects, including anti-inflammatory, antimicrobial and antioxidant activities, have also been efficiently produced by SSF. Many researchers have been focused on finding natural sources, including fruits, vegetables, plants, and agro-industrial wastes, for the production of these compounds. Among these sources, SSF of soy flour-supplemented guava residue by *Rhizopus oligosporus* was considered a good strategy to obtain phenolic compounds. *R. oligosporus* was also demonstrated to be an important food-grade fungus for use in SSF systems with others fruit wastes (cranberry pomace and pineapple waste) as carbon sources (Correia et al., 2004).

Most of the bioactive compounds are produced by SSF using agro-industrial wastes as substrate, even though some studies report the use of sugarcane bagasse or agar as inert solid supports (Table 7). Iturin, for instance, known as a potent antifungal peptide antibiotic, effective in suppressing phytopathogens, has been produced by bacterial strains from okara (soybean waste) and wheat bran (Balakrishnan & Pandey, 1996), resulting in a six to eight times more efficient process by SSF compared to SmF. Antifungal and antibacterial metabolites from a sclerotium-colonizing isolate of *Mortierella vinacea* grown under SSF conditions has also been reported.

Microorganism	Solid support	Product/Function
<i>Gibberella fujikuroi</i> , <i>Fusarium moniliforme</i>	Corn cob, sugarcane bagasse <sup>a</sup> , Cassava flour	Gibberellic acid/ Plant growth hormone
<i>B.subtilis</i>	Impregnated loam based compost	Antifungal/ Antifungal compounds
<i>Bacillus thuringiensis</i>	Coconut waste	Bacterial endotoxins/ Insecticide
<i>Penicillium chrysogenum</i>	Sugarcane bagasse <sup>a</sup>	Penicillin/ Antibiotic
<i>S. rimosus</i>	Corn cob	Oxytetracycline/ Antibiotic
<i>S. viridifaciens</i>	Sweet potato waste	Tetracycline chlorotetracycline/ Antibiotic
<i>Monascus purpureus</i>	Sugarcane bagasse	Pigments
<i>R. oligosporus</i>	Pineapple waste, cranberry pomace, guava, soy flour	Phenolic antioxidant compound
<i>Streptomyces</i> sp	Coffee pulp waste	Polyphenols, tannins, chlorogenic acids
<i>B. subtilis</i> Antibiotic	Soybean waste Okara	Surfactin/ Antibiotic

Table 7. Bioactive compounds produced by SSF. <sup>a</sup> Inert solid support

#### 4.6 Bioinsecticides

Biological pest control agents have received considerable attention as a potential alternative to develop eco-friendly pesticides and provide a sustainable agriculture. The identification of a suitable fungal strain that possess pesticide activity is the most important aspect to take into account when developing pesticides. In the last years, bio-pesticides agents for controlling insects and pests have been produced with *entomopathogenic* and *mycoparasitic* fungi. Besides the microorganism, the understanding of the molecular aspects of fungus-fungus, and fungus-insect interactions, the role of hydrolytic enzymes, especially chitinases in killing processes, and the possible use of chitin synthesis inhibitors are crucial aspects to be taken into consideration while making fungi, either singly or in combination, as an effective biopesticide agent.

Several agro-industrial wastes (refused potatoes, coffee husks and sugarcane bagasse) have been used in SSF to produce spores from *Beauveria bassiana* to obtain biopesticides for biocontrol of pests of banana, sugarcane, soybean, and coffee (Santa et al., 2005). *Colletotrichum truncatum* is another fungus that has been studied under SSF and that possesses characteristics to be used as mycoherbicide against the difficult weed *Sesbania exaltata* (Pandey et al., 2000b).

#### 4.7 Bioethanol

Bioethanol production by SSF using agro-industrial wastes have been considered as an excellent alternative for reusing these wastes with additional technological and economic advantages, since this process is of easy operation and save energy. The production of bioethanol by SSF is a field that has not been much explored yet; however, recent studies reveal that this technology merits to be better explored.

Ethanol production by SSF using grape and sugar beet pomaces (Rodríguez et al., 2010), and apple pomace (Hang et al., 2006; Joshi & Devrajan, 2008) as solid substrates, has been recently evaluated. When grape pomace and sugar beet pomace were used for cultivation of the yeast *Saccharomyces cerevisiae*, the obtained ethanol production yields were greater than that obtained by SmF (Rodríguez et al., 2010). Therefore, and considering the importance of the ethanol production in the actual world economy, it is expected to observe an increase in the researches for the development of a suitable process for ethanol production by SSF.

### 5. Conclusion

Agro-industrial wastes are generated in large amounts every year. Finding alternatives for the reuse of these wastes is an objective that has been strongly taken into account by countries around the world, considering environmental and economical aspects. The agro-industrial wastes reuse in SSF processes is of particular interest due to their availability, low cost, and characteristics that allow obtaining different value-added compounds, besides being an environment friendly alternative for their disposal. Additionally, the agro-industrial wastes may be used in these processes as solid support, carbon, nitrogen and/or mineral sources, which would allow obtaining more economical fermentation processes avoiding the use of expensive chemical components in the media formulation. As a consequence, more economical processes could be established for implementation on industrial scale.

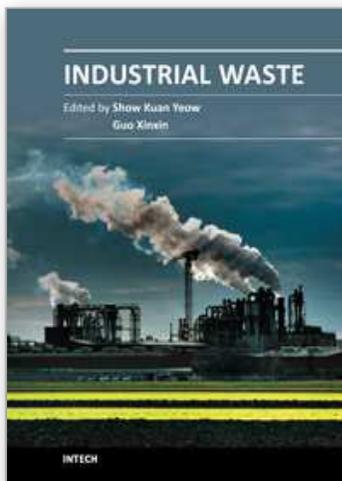
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## **Industrial Waste**

Edited by Prof. Kuan-Yeow Show

ISBN 978-953-51-0253-3

Hard cover, 274 pages

**Publisher** InTech

**Published online** 07, March, 2012

**Published in print edition** March, 2012

This book is intended to fulfil the need for state-of-the-art development on the industrial wastes from different types of industries. Most of the chapters are based upon the ongoing research, how the different types of wastes are most efficiently treated and minimized, technologies of wastes control and abatement, and how they are released to the environment and their associated impact. A few chapters provide updated review summarizing the status and prospects of industrial waste problems from different perspectives. The book is comprehensive and not limited to a partial discussion of industrial waste, so the readers are acquainted with the latest information and development in the area, where different aspects are considered. The user can find both introductory material and more specific material based on interests and problems. For additional questions or comments, the users are encouraged to contact the authors.

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Solange I. Mussatto, Lina F. Ballesteros, Silvia Martins and José A. Teixeira (2012). Use of Agro-Industrial Wastes in Solid-State Fermentation Processes, *Industrial Waste*, Prof. Kuan-Yeow Show (Ed.), ISBN: 978-953-51-0253-3, InTech, Available from: <http://www.intechopen.com/books/industrial-waste/use-of-agro-industrial-wastes-in-solid-state-fermentation-processes>

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51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

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