CHAPTER 16

CONVERSION OF LIGNOCELLULOSICS INTO ANIMAL FEED WITH WHITE ROT FUNGI

Frantisek Zadrazil

Institut für Bodenbiologie, Bundesforschungsanstalt für Landwirtschaft
Bundesallee 50, 3300 Braunschweig, FRG.

1. INTRODUCTION

1.1. Biological Upgrading of Lignocellulosics

The application of lignocellulosic agricultural wastes, particularly cereal straws, as animal feed has received considerable attention in past years. The high content of polysaccharides in lignocellulose is a potential source of dietary energy for ruminants. However, their nutritive value is limited because of the poor digestion of complex polysaccharide in the rumen. The restricted digestibility of these energy rich substances is due to the presence of a recalcitrant component, lignin, which acts as a physical barrier depriving the hydrolytic enzymes access to the polysaccharides.

In order to increase the digestibility of lignocellulose, biological methods of delignification can be used (Han, 1978). The principle of these methods is the splitting of the cellulose-lignin complex by extraction or decomposition of lignin. The main problem of biological upgrading of lignocelluloses into feed is to find suitable microorganisms, with metabolic patterns different from those of the rumen flora and fauna, and a cheap large scale process. "Ideal" microorganisms for upgrading lignocellulosics into animal feed should have a strong lignin metabolism with a low degradation of cellulose and hemicellulose. In this review, fungal activities on lignocelluloses are briefly summarized.

1.2. Fungal Delignification of Lignocellulose

Fungal delignification of wood has been observed in South Chile under natural conditions. The final product of delignification, known as "Palo podrido", is a white decomposed wood which is used as animal feed (Tangol, 1976). The wood degraded to Palo podrido is usually from the logs of *Drymis winteri* Forst, *Eucryphia cordifolia* Cav., *Laurelia philippiana* and *Nothofagus dombeyi* (Mirb.) and the fungi responsible for the degradation of the wood belong either to *Ganoderma applantum* or *Armillariella* spp. The *in vitro* digestibility of wood increased from a bare 3% to 77% in some cases. However, the process of degradation is very long and slow, and it is not possible to calculate how

much carbohydrate is lost before such a highly digestible product occurs in nature (Zadrazil *et al.*, 1982). Zadrazil and Brunnert (1981) confirmed that *G. applanatum* degrades lignin, in the case of wheat straw preferentially at low temperature, and helps to increase the digestibility of the fermented product. The cool weather of South Chile (2-18°C) seems to facilitate the growth of this fungus on wood.

Phanerochaete chrysosporium degrades hemicellulose, cellulose and lignin non-selectively, which adversely affects the digestion rate of cell walls in the rumen (Agosin *et al.*, 1986). In 15 days of fermentation, 32% of dry matter can be degraded, accompanied by only 11% increase in digestbility. Similar results have also been reported by Zadrazil and Brunnert (1982) and Zadrazil (1985). But Reade and McQueen (1983) reported that dry matter loss during fermentation of wood shavings was not as high as reported in the above studies. This might be due to the different physical structures of the two substrates used. Reid (1985) reported that aspen wood was degraded efficiently by *Merulius tremellosus* in solid state fermentation. The fungus degraded 52% of lignin accompanied by only 12% loss of dry matter and increased the cellulase digestibility of the fermented wood from 18 to 52%. More than 200 strains of white rot fungi have been screened by Zadrazil (1985) for substrate degradation, lignin decomposition and increase in digestibility of wheat straw at different temperatures (22, 25 and 30°C) and periods of fermentation.

2. ECOLOGICAL BACKGROUND OF CONVERSION OF LIGNOCELLULOSICS INTO ENRICHED ANIMAL FEED

2.1. Factors Determining Lignin Degradation and *in vitro* Digestibility

Figure 1 shows the factors determining the course of solid state fermentation of lignocelluloses. The number of possible variations between two, three or more factors is 256. However, during large-scale production the controlling key-factors of fermentation are limited.

2.2. Type of Fungal Strains

The influence of fungal species on the decomposition of wheat straw and the *in vitro* digestibility and decomposition of lignin is comprehensively discussed by Zadrazil (1985). In Figure 2 differences between physiologically different fungi are shown.

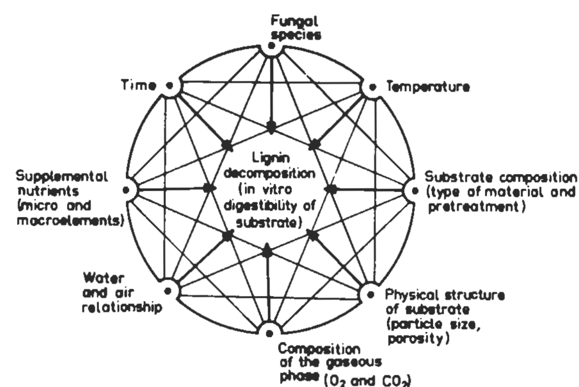

FIGURE 1. Interrelation between factors determining lignin decomposition and *in vitro* digestibility of lignocelluloses during solid state fermentation with white rot fungi.

Fungi in group (a) decompose the substrate without lignin degradation (brown rot fungi). *In vitro* digestibility is negative in comparison with untreated straw. Examples are *Agrocybe aegerita* and *Flammulina velutipes*. Similar results can be obtained by the cultivation of lower fungi, bacteria or yeasts on cereal straw.

2.3. Substrate Composition and Quality

Five different substrates, including beech sawdust, rape-, sunflower- and reed straw, rice husks, were tested with 4 different fungi for speed of decomposition of organic matter, lignin decomposition and *in vitro* digestibility. Each substrate was subjected to 60 days of solid state fermentation by each fungus. All these factors are strongly dependent on fungal species and the kind of plant waste substrate (Zadrazil, 1980). *Pleurotus* sp. *florida*, *Pleurotus cornucopiae* and *Stropharia rugosoannulata* showed good lignin decomposition and increase the *in vitro* digestibility of all substrates except the rice husks. *Agrocybe aegerita* decomposed lignin only to a small extent and decreased the *in vitro* digestibility (Zadrazil, 1980). This effect is probably caused by the high incrustation of rice husks with SiO_2.

2.4. Substrate Pretreatment

Interactions between indigenous substrate microorganisms and the consumption of liberated carbohydrates by the fungus influence the digestibility of substrate. Kamra and Zadrazil (1986) reported that pasteurization of wheat straw at 90°C or sterilization was required for a significant

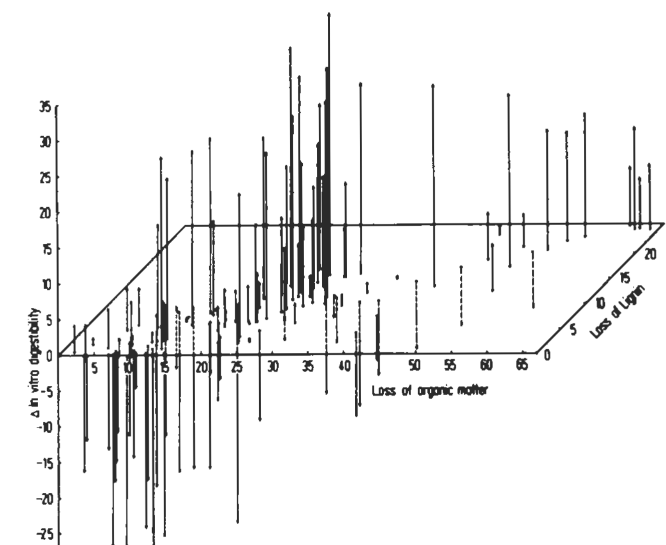

FIGURE 2. Relationship between the loss of organic matter, loss of lignin and differences in the *in vitro* digestibility of wheat straw after fungal treatment (60 days at22,25 and 30°C; *in vitro* digestibility of untreated straw = 0; increased digestibility +; decreased -). (a b c d - see text for explanation).

increase in *in vitro* digestibility following fermentation with *Pleurotus sajor-caju*. Zadrazil and Peerally (1986) reported that anaerobic fermentation of wheat straw at 50°C for 48 h before inoculation with the desired fungus is as effective as sterilization of substrate. It shows that to achieve good digestibility of lignocellulose, the natural microflora must be suppressed and the inoculum must be given the maximum opportunity to colonize the substrate quickly.

2.5. Temperature

The temperature of fermentation not only influenced the speed of decomposition of the organic matter, it also influenced the sequence of decomposition of the substrate components. With all fungi tested, increases in temperature between 22 and 30°C, increased the speed of decomposition of the organic matter (Zadrazil, 1977; 1985). Large differences in the rates of straw decomposition caused by temperature were observed for *Pleurotus cornucopiae* and *Stropharia rugosoannulata*. A positive correlation between increases in temperature and lignin decomposition or *in vitro* digestibility was only visible for *Stropharia rugosoannulata*.

2.6. Duration of Fermentation

In the fungal life cycle, the following stages are distinguished:

1. Colonization of the substrate
2. Maturation of fungus
3. Induction of fruiting bodies
4. Autolysis.

During the first stage of fungal growth, the content of digestible substances for ruminants decreases (Zadrazil, 1977). After colonization was complete, the *in vitro* digestibility of the fungal substrate increases, but it decreases thereafter in old substrates which have a relatively high content of accumulated minerals. Under favourable conditions, some fungi can totally mineralize cereal straw during a 80 - 100 day period of fermentation (Zadrazil, 1985).

2.7. Nitrogen Supplementation

Amendment of the substrate with NH_4NO_3 also changed the decomposition rate and also the sequence of decomposition of substrate components. *Stropharia rugosoannulata*, *Agrocybe aegerita* and *Pleurotus* sp. *florida* were stimulated during decomposition of the substrate by the addition of low NH_4NO_3 concentrations, while *Pleurotus eryngii* was inhibited by these treatments. The lignin decompositon rate was relatively unchanged by NH_4NO_3 amendment. A decrease in the *in vitro* digestibility of the substrate mixture was observed with all fungi when the substrate received higher concentrations of NH_4NO_3 (Zadrazil & Brunnert, 1980, 1982).

2.8. Ratio of Liquid to Gaseous Phase in the Substrate

With increasing water content and with a constant substrate volume, the air content of the substrate decreased. This resulted in increased water tension and an increased swelling of the substrate.

All fungi investigated showed good growth on substrates with varying water contents (from 25 to 150 ml water/25g straw). With both the lowest and the highest water contents, the decomposition rate of the total organic matter decreased as did decomposition of lignin and accumulation of

digestible substances. The fungi tested showed specific growth optima for various air and water contents of the substrate (Zadrazil & Brunnert, 1981, 1982).

2.9. Composition of Gaseous Phase

The losses in organic matter and lignin after fermentation of wheat straw with *Pleurotus sajor-caju* were found to be highest in a 100% oxygen atmosphere (Kamra & Zadrazil, 1986), followed by those in air in the case of *P. eryngii*. Carbon dioxide at 1-20% in the atmosphere influenced neither organic matter loss nor lignin degradation, but at 30% concentrations the organic matter loss was slightly increased and lignin degradation was considerably decreased. Lignin was degraded at a much lower rate with less than 20% oxygen in the atmosphere. The increase in *in vitro* digestibility was highest in pure oxygen, followed by that in an air atmosphere. Carbon dioxide at 1-10% positively influenced the increase in digestibility, but at higher CO_2 concentrations the digestibility was reduced. The process efficiency with respect to increase in digestibility of wheat straw was highest in the presence of oxygen (Kamra & Zadrazil, 1986).

Gaseous metabolites have a strong influence on the mineralization of organic matter, loss of lignin and *in vitro* digestibility following the fungal degradation of straw (Zadrazil *et al.*, 1991). For large scale processes, the composition of gaseous phase is proposed to be the key-factor. The effects on the composition of the substrate of different gaseous treatments have been summarized by Buta *et al.* (1989) and by Chiavari *et al.* (1989). The above investigations can be summarized as follows:

1. *In vitro* digestibility of fungal substrates decreases at the beginning of colonization by white rot fungi and increases afterwards (Zadrazil, 1977; Zadrazil & Brunnert, 1982). During incubation, the content of soluble substances (partly sugars) increases (Zadrazil, 1976; Lindenfelser *et al.*, 1979).
2. The increase in digestibility depends on the fungal species (Zadrazil, 1979), cultivation time, temperature, water/air ratio in the substrate and on the preparation, bulk density and composition of the substrate (Zadrazil & Brunnert, 1981, 1982).
3. The *in vitro* digestibility of lignocelluloses by white rot fungi is decreased by addition of inorganic nitrogen (Zadrazil & Brunnert, 1980).

3. SCALE UP OF SOLID STATE FERMENTATION

The above examples and the analysis of "Palo podrido" samples (Zadrazil *et al.*, 1982) clearly show that use of white rot fungi for upgrading lignocelluloses into feed is possible, at least on a laboratory scale and in natural processes. On the other hand, only a little is known about the large scale process. Solid state fermentation of lignocelluloses in deep layers have been proposed (Schuchardt & Zadrazil, 1982, 1988).

3.1. Definition of Solid State Fermentation

Solid state fermentation can be defined as a process in which solid substrates are decomposed by known mono- or mixed cultures of microorganisms (mainly fungi, which can grow on and through this substrate) under controlled environmental conditions with the aim of producing a high quality standardized product (different to composting). The substrate (mixture of different particles) is characterized by a relatively low water content. Since much of the water is chemically or physically bound to the substrate, physical properties, e.g. porosity, density, are uniform. The substrate is not

mixed or moved during the process (Zadrazil *et al.*, 1990a).

Prepared substrates can also be used in environmental control as biofilters (Hüttermann *et al.*, 1988), or for decontamination of xenobiotics in soil and waste materials (Hüttermann *et al.*, 1988, 1989; Martens & Zadrazil, 1992).

3.2. The Principle of the Process

Many factors influence the course of solid state fermentation, but only some factors such as temperature, humidity and composition of gas phase can be controlled and changed.

The pretreated substrate is filled into the reactor in 1.5-2.0 m deep layers and incubated by percolation of the gas phase through the substrate. The temperature in the substrate is indirectly controlled by conditioned, percolated gas. In the cultivation of *Agaricus* spp., fresh air is added in order to cool the substrate. In contrast, in the production of wood decaying fungi, high carbon dioxide and low oxygen concentrations are required during the period of colonization (Zadrazil, 1975).

3.3. Description of SSF- Reactor

The principal design of a small scale reactor and its control were described by Schuchardt and Zadrazil (1982, 1988). The reactor used in the Institute for Soil Biology, FAL, Braunschweig, Germany, was based on previous experience. The reactor is constructed of polyurethane foam sandwich panels which are covered on both sides with polyester board. The filling height is approximately 2.0 m, the internal width 2.3 m and the depth 2.0 m. This results in a net filling volume of 9.2 m^3 equivalent to 1.5 t of straw or 3.0 t wood chip substrate.

Two similarly insulated swing doors are situated at the front of the reactor, the width of the doors being the width of the reactor itself. Inside the reactor there is a raised slatted floor covered firstly with a gliding net and then with a drag net.

Two reactors of the same shape and construction are used (Fig 3). One reactor is used for substrate pretreatment and the second one for substrate colonisation (Zadrazil *et al.*, 1990a, 1990b). On moving the substrate from one reactor to the other reactor, the substrate is inoculated.

3.4. Conditioning of Gas Phase

Fungal growth and heat exchange from the substrate is controlled by re-circulating the air within the reactor. The total quantity of circulated air can be varied by changing the speed of electronically controlled fans.

In order to humidify the air and raise the substrate temperature for pretreatment of substrate (e.g. pasteurisation), a steam injection pipe is installed under the slatted floor.

Fresh air, oxygen, or carbon dioxide can be added after gas analysis by computer-controlled valves (Fig. 4). Exhaust gases leave the fermentor through an over-pressure valve located above the substrate.

3.5. Control of Gas Humidity

The humidity of gaseous phase was controlled by hygrometers situated (Hygrotest 720) in different parts of the reactor. The air humidity fluctuates between 95 and 100%. Commercially available hygrometers are not sufficiently sensitive enough in this region to give more precise control.

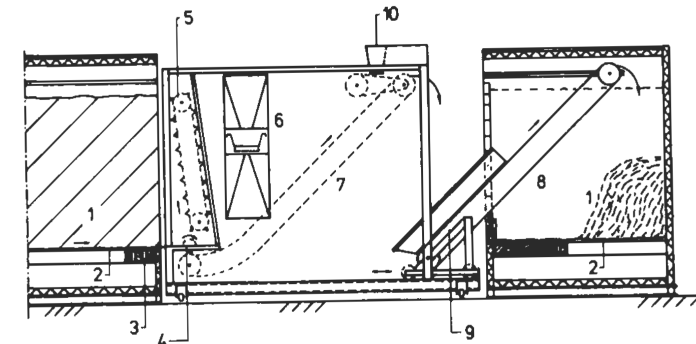

Figure 3. Horizontal cross -section of reactors for SSF and spawning-emptying machine. 1. SSF reactor; 2. Net for emptying the substrate; 3. Panels for aerating the substrate; 4. Equipment for for emptying the substrate; 5. Equipment for fragmenting the substrate; 6. Equipment for filling the substrate; 7. Conveyor to bring substrate to spawning machinery; 8. Conveyor for filling the reactor with substrate; 9. Carrier for moving the conveyor; 10. Spawning machine (Zadrazil *et al.*, 1990).

3.6. Control of Water Evaporation

Saturation deficit of water in the gaseous phase increases during the penetration of gas through the substrate. The gaseous phase has a lower temperature than the substrate and water evaporates. From the circulating gases, water condenses on the cooling equipment and within cold areas of substrate and reaches 100 % relative humidity again. Evaporation of water in circulating gases was

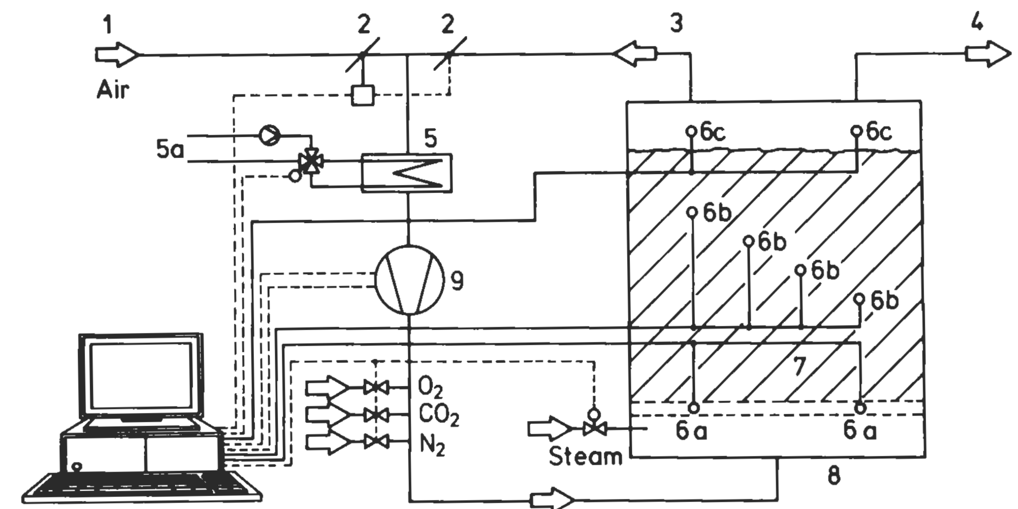

FIGURE 4. Scheme of control system of solid state reactor (Zadrazil *et al.*, 1990). 1. Fresh air supply; 2. Air damper; 3. Recirculation of gas phase; 4. Emptying of gas; 5. Gas-cooling system; a. Liquid-cooling system; 6. Sensors for temperature control; a. Gas-input b. Temperature of substrate c. Gas-output 7. Substrate; 8. Body of SSF reactor; 9. Fan.

measured by water loss from a 20 cm^2 ceramic disc (Czeratzki, 1968) placed in the space above the substrate.

3.7. Water Translocation

Evaporation of water from the substrate and condensation on the cold parts of reactors and cooling units is undesirable but cannot be eliminated. The translocation of water could be a measure of the technical standard of the reactor and the efficacy of the control system. All condensed water is led into the container at the base of the reactor and the quantity is periodically measured.

3.8. Metabolic Heat

For scientific studies the ducts must be insulated to eliminate the uncontrolled heat loss from the reactor. The rate of heat liberation during the fermentation process was measured by a flow meter installed in the cooling system of the reactor.

3.9. Temperature Control

The temperature of the gas phase was monitored by placing 4 PT100 resistance thermometer sensors at the inlet and outlet ducts (Fig. 4: Position No.: a; b; c; d in different layers of substrate). Temperature of substrate is measured in 4 different layers with 8 thermometer sensors and registered in 2 independent computers.

Minimum and maximum limits for temperature can be adjusted. If the temperature of the air rises above or falls below the temperature limit, the heating or cooling system is activated and an alarm could be calibrated for critical temperature areas. By operating temperature controls, temperature differences in different substrate layers can be estimated. Different possibilities and mathematical and physical models for temperature control are discussed by Teifke and Bohnet (1990).

3.10. Air Supply and Control of Circulated Air

The amount of air circulated in the reactor through the substrate is measured on the supply side of the fan by measuring the difference in pressure across a gauge ring. The required fresh air is added with an air pump and measured with a flowmeter.

3.11. Digestibility and Homogenity of Product

The digestibility of substrate (Tilley & Terry, 1963) on fermentation increased on average by 13.8 digestibility units (Table 1). The highest increases (18.7 and 18.3 units) were found in the two layers near the substrate surface and the lowest (7.0 units) on the bottom layer. The observed increases in the digestibility of cereal straw after fungal treatment on a large-scale were comparable with results obtained by sodium hydroxide or ammonia treatment (Sundstol & Owen, 1984). The final product (fungal substrate) differs in water content and digestibility. One may assume that differences between the water contents of different layers have an influence on the digestibility of substrate. We hope that this phenomenon can be eliminated by better control of the gas phase.

3.12. Utilisation of Substrate

After 14 days of incubation, the substrate was also used for production of edible fungi, or for other technologies (soil sanitation, air pollution control, production of chemical feedstocks). Colonized substrate was placed into a container for fructification . The yield of fruit bodies was satisfactory and was comparable to that obtained with other cultivation systems.

3.13. Growth of Competitive Microorganisms

The proposed system of SSF is based on the use of non-sterile culture conditions. Selective propagation of thermophilic and mesophilic microorganisms during substrate pretreatment supports the saprophytic colonization by the cultivated fungus. Infection of substrate was not observed during colonization of substrate under these conditions.

During the production of animal feed, colonies of *Trichoderma* sp. were observed on the surface of the substrate at the end of fermentation. This infection correlates with high substrate digestibility. Infection was frequently observed when conditions for the growth of *Pleurotus* spp. were suboptimal (e.g. temperature too high).

4. CONCLUSIONS

The described SSF-pilot reactor and process, utilized at the Institute of Soil Biology, FAL Braunschweig since 1985, can be used after some technical modifications as a model for large-scale technology. Insufficient knowledge exists about solid state fermentaion and about suitable reactors for this process. More basic and applied research must be done before this "mushroom" technology can be transferred to other areas of biotechnology. For each organism, special strategies for the design

TABLE 1. Increase in *in vitro* digestibility (Tilley & Terry, 1963) of wheat straw (indigestible = 0; totally digestible = 100) in different layers and positions (two replicate samples from each layer) in a large-scale reactor (1500 kg substrate) after 38 days of SSF with *Pleurotus* sp. (digestibility of untreated straw = 40).

Layer	Position in the reactor						Average
	Front		Middle		Back		
(cm)	Left	Right	Left	Right	Left	Right	
0	19.4	11.8	16.0	20.6	21.6	22.8	18.7 ± 4.12
20	18.8	12.8	17.9	16.0	23.7	20.2	18.3 ± 3.71
40	11.9	12.1	16.9	15.7	23.3	14.3	15.7 ± 4.22
60	14.8	15.0	5.7	16.6	14.4	18.5	14.1 ± 4.41
80	8.3	9.5	10.9	11.9	7.8	10.2	9.8 ± 1.57
100	0.0	7.1	7.0	10.2	10.3	7.5	7.0 ± 3.73
Av	12.2 ± 7.30	11.4 ± 2.73	10.7 ± 5.29	15.2 ± 3.70	16.8 ± 6.98	15.6 ± 5.95	13.8 ± 3.59 13.7 ± 3.49

of the reactor and for the control of the process must be developed. For application in developing countries, cheap and easily controlled reactors must therefore be developed.

Based on the studies reported here and elsewhere, one may conclude that there is a need for further research on several aspects of the SSF process. These include the following:

1. Development of new designs and constructions of SSF reactors.
   1.1 Requirements: Homogenous conditions during processing.
   1.2 Minimal differences between process parameters (growth conditions) in different layers of substrates.
2. Development of equipment and sensors for the control of the SSF process.
   2.1 Control of the air speed in different parts of reactors.
   2.2 Control of air humidity (95-100% relative humidity).
   2.3 Control of water evaporation from substrate.
   2.4 Control of water translocation.
3. Development of a strategy for process control.
4. Development of mathematical models of the SSF process.
5. Verification of results in laboratory and pilot-scale reactors.
6. Comparative economic studies with other lignocellulose-upgrading processes.

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