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Biochemical Engineering Journal 13 (2003) 169–179

Engineering Journal

www.elsevier.com/locate/bej

Bioconversion of lignocellulose in solid substrate fermentation

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Received 15 November 2001; accepted after revision 24 July 2002

Abstract

In this review the state of the art of lignocellulose bioconversion by solid substrate fermentation (SSF) is presented. The most important lignocellulolytic fungi and their properties are described, and their application in novel solid state bioreactors with on-line process control is discussed. The most important bioconversion products, biofuels, enzymes, animal feeds, biofertilizers, biopesticides, biopromoters, secondary metabolites, and the economy of their production by SSF is discussed. The use of SSF in the pulp and paper industry and in integrated crop management is illustrated.

Keywords: Lignocellulose; Bioconversion; Solid substrate fermentation

1. Introduction

Lignocellulose composes more than 60% of plant biomass produced on earth. This vast resource is the potential source of biofuels, biofertilizers, animal feed and chemical feedstocks. Lignocellulose is also the raw material of the paper industry. To fully utilize the potential of lignocellulose, it has to be converted by chemical and/or biological processes. Solid substrate fermentation (SSF) plays an important role, and has a great perspective for the bioconversion of plant biomass. Lignocellulose may be a good feedstock for the production of biofuels, enzymes and other biochemical products by SSF. Crop residues (straw, corn by-products, bagasse, etc.) are particularly suitable for this purpose, since they are available in large quantities in processing facilities.

Lignocellulose in wood may be transformed into paper products with the help of SSF biopulping and biobleaching. Agricultural residues may be converted into animal feed enriched with microbial biomass, enzymes, biopromoters, and made more digestible by SSF. Lignocellulosic waste may be composted to targeted biofertilizer, biopesticide and biopromoter products. Post-harvest residue may be decomposed on site by filamentous fungi and recycled to the soil with improved biofertilizer and bioprotective properties. In this review the state of the art of lignocellulose bioconversion by SSF is presented, the microbes used in the process, the fermentation technology with its engineering aspects, the main products of the bioconversion, and future trends in practical applications.

2. Lignocellulolytic fungi

2.1. Characteristics of lignocellulose degrading fungi

In nature, there are many microorganisms, bacteria as well as fungi that live on natural lignocellulose. The most efficient decomposers of wood and other natural lignocellulose are the white-rot fungi and some mushrooms. Recent reviews and books summarize existing knowledge on lignocellulose biodegradation [9,25,34,43,83,91,94,100].

Lignocellulose degrading fungi are used now in industrial scale, mostly for the production of cellulases, xylanases, and for biopulping. Most investigated, used and genetically improved are fungi of *Trichoderma* spp. [34,59,95]. Very efficient mutants of *Trichoderma reesei* have been developed and used in biofuel production [21,34,35,62]. These mutants were designed for use in submerged fermentation (SF), and may not work equally well in SSF. The very different conditions of SF and SSF would require strain improvement specifically targeted for SSF application. Such efforts are needed in improving the efficacy of SSF operations.

SSF closely resembles the natural way of life of filamentous fungi. Fungi are adapted to life on specific natural substrates, thus host specificity is a primary consideration in SSF. The life of fungi on natural solid substrates requires different conditions, different cellular structures,

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1369-703X/02/$ – see front matter
PII: S1369-703X(02)00129-8

enzymes, metabolites than in liquid cultures [27,37,45,49,68,84,89,95]. Nutrient availability is more restricted in natural solid substrates than in liquid cultures, therefore, it is likely that fungi have to develop more efficient enzyme systems for host cell degradation than in liquid cultures. This may translate into more efficient hydrolysis of the substrate in a bioreactor.

Another feature of fungal life on natural substrates is coexistence with other microorganisms in commensal or symbiotic associations [68]. This coexistence may be approximated in SSF by mixed culturing of different fungi, e.g. a hypercellulolytic mutant and a host-specific "helper" fungus. Since the current hypercellulolytic mutants have been developed for SF processes, in SSF they may benefit from the help of host-specific fungi, promoting better colonization, host penetration, and possibly metabolic enhancement from co-metabolites. In the future, host-specific hypercellulolytic or ligninolytic fungi may be developed specially targeted for SSF by genetic engineering.

The main enzymes produced by lignocellulolytic fungi are cellulases, hemicellulases, pectinases and ligninases. A detailed review of these enzymes and their mode of action is given elsewhere in this volume.

2.2. Fungal growth on lignocellulose

Bacteria and yeasts grow in the surface film of solid substrates much like in free liquid. Filamentous fungi, however, can grow in the absence of free water, utilizing the bound water of the substrate [55,93]. On lignocellulosic substrates, fungi grow with a linear rather than logarithmic rate, limited by steric hindrance and substrate accessibility. The fungus (spore or hypha) must first colonize the substrate by adhesion, then spread from one substrate particle to another by branching. Some fungi have special appendages for anchoring to surfaces, others rely on secreted adhesive polysaccharides, or both. Substrate accessibility is initially provided through cracks and holes in the plant cell wall.

Host-specificity is critical in SSF due to the complexity of the substrate and hence the need for a special enzyme complex for its degradation [45,84,90,95,105]. The composition of the enzyme complex depends on the fungus as well as its host.

There is a growing awareness that the physiology of fungi is different on solid substrates than in liquid cultures [27,45,53]. Limited nutrient availability, complex growth requirements for colonization, substrate penetration and metabolism require special cellular structures, special enzyme systems, special regulatory mechanisms. There is evidence that the hydrolyzing capacity of SSF enzymes is different from SF enzymes and highly host dependent [37,89].

Fungi may satisfy such complex requirements by symbiotic associations on natural substrates [68], and may well do this in SSF too by mixed culturing. Mixed culturing has been successfully employed in this laboratory [11,12,19,28–31] and other laboratories [4,10,20,50,58,72], mainly with the limited goal of improving the enzyme production by a hypercellulolytic mutant with a helper fungus. The co-culturing of *T. reesei* mutants with *Aspergillus* spp. improved cellulase production by 50% and improved the cellulase beta-glucosidase ratio, thereby partially removing product inhibition both for cellulase production and for hydrolysis [11,19]. Co-culturing also resulted in greater overall growth and higher specific enzyme yields based on both biomass and secreted protein. Other symbiotic associations, such as successive colonization and substrate penetration, co-metabolite production, metabolite inducers need to be scrutinized in future studies [72,108].

To assure reliable, reproducible fungal growth in SSF, good quality, readily available, packaged fungal cultures are necessary, much like in the mushroom industry. In contrast to submerged liquid cultures where skilled microbiologists handle sophisticated sterile cultures from test tube to final product, SSF must be viable as a low technology, semi-aseptic or non-sterile cultivation technique, conducted by unskilled farmhands. Even though pretreatment (steaming, cooking, alkali or acid treatment) may reduce the indigenous microflora, the effect of residual microflora and non-sterile handling must be overcome by using sufficiently high numbers of fungal cells with the optimal physiological activity in starter cultures. This is particularly true for lignocellulose SSF where key enzymes must be pre-induced for a quick start of lignocellulose breakdown and fungal growth [93].

Traditionally, spore suspensions are employed in fungal SSF. The drawback of the spore inoculum is the long lag period, resulting from slow spore germination and enzyme induction. This may be overcome by using carrier attached germinated spores as starter culture.

An experimental technology is illustrated in Fig. 1. The advantage of the fluidized bed reactor is that the spores are rapidly germinated on the carrier without forming mycelia, making possible easy dosing and fast start in the bioreactor.

T. reesei or *Trichoderma harzianum* spores were initially coated on corncob particles (10^8 spores/g) and fluidized in the liquid fluidized bed reactor for 30 h at 28 °C with increasing fluidization velocity, 0.15–0.25 cm/s, keeping pace with the increasing thickness of fungal biofilm. After biofilm thickness reached 75% of the maximum possible, the loaded particles were drained and used as starters, or stored after sterile air drying [1,96]. The finished starter contained 30–40 mg/g (wet/wet) fungal biomass.

3. Process control and bioreactor design for lignocellulose SSF

From the engineering point of view, the structure of the lignocellulosic substrate, its change during fermentation, its heat conductivity and moisture content and O_2 mass transfer are the main points of consideration in reactor design. Steric

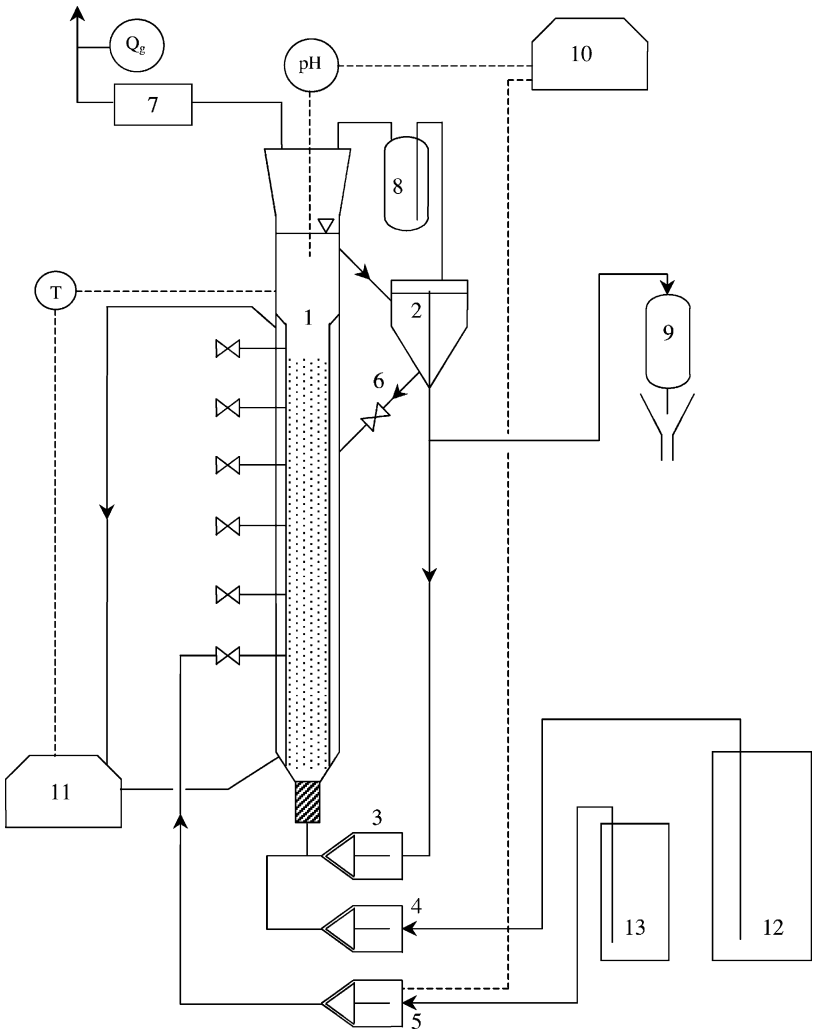

Fig. 1. Experimental liquid fluidized bed reactor. (1) reactor with six sample ports; (2) settler; (3) recirculation pump; (4) feed pump; (5) alkali pump; (6) sediment return; (7) sterile gas filter; (8) gas trap for level control; (9) outflow trap; (10) pH control device; (11) temperature control device; (12) feed reservoir; (13) alkali reservoir. Instrumentation: Q_g, gas flow meter; pH and T designate pH meter and thermocouples.

hindrance is an important limitation of fungal growth in SSF bioreactor. The growing fungal hyphae must find accessible attack points on the substrate, depending on the geometric position and proximity of substrate particles and the space requirement of branching mycelia [44]. The space utilization of filamentous fungi may be expressed as the packing density (Y_x):

$$Y_x = \frac{V_m}{V_x}$$

where V_m is the volume of mycelial population and V_x the volume available for fungal growth. Theoretically, uniform cylindrical hyphae in close contact may reach a Y_x of approximately 0.9, but in a finely chopped typical lignocellulose substrate, such as wheat straw, the packing density is only 0.05–0.07 [44]. This imposes a severe theoretical limitation on attainable biomass concentration, a maximum of 20–30% of the substrate dry weight. Substrate conversion rarely exceeds 30–40% in most lignocellulose SSF, thus the substrate imposed steric hindrance prevails throughout the culture. In contrast, in SSF of starch the substrate gradually disappears and its place may be taken by more tightly packed hyphae [67].

Fungal growth on lignocellulose substrates is particularly sensitive to morphological and physiological changes leading to sporulation. Nutrient limitation, deviation from optimum moisture and temperature, toxic metabolic products and steric hindrance are among factors that may trigger the onset of sporulation [55].

Spores may be desired end products in some SSF processes such as starter culture production or plant protection by *Trichoderma* spp., but in other processes, sporulation must be avoided (e.g. biomass or enzyme production).

A great concern and hesitation for using SSF technology in the western world is caused by the difficulty of process control and the labor intensity associated with SSF. Precise mathematical models for growth, mass transfer and product formation are being developed for SSF, but are not widely

used as yet [7,18,52,71,73]. Many attempts have been made to improve reactors for SSF [54], using stationary tower reactors, rotating drum or rocking reactors, but variations of traditional Koji type shallow tray reactors with raking and moisture-temperature-O_2 supply control proved to be the most successful so far [18]. The operational process control of SSF is more difficult and less developed than that of SF. The most critical parameters are moisture, temperature and O_2 supply. At Colorado State University a sophisticated computer controlled on-line evaporative moisture and temperature control has been developed in a rocking reactor [7,71]. The temperature is controlled by forced evaporation, using a variable humidity air stream and replenishing evaporated moisture by water spray. Oxygen mass transfer is regulated by packing density of the substrate in the reactor and by gentle agitation (rocking, tumbling, etc.). O_2 mass transfer may also be facilitated by temperature and pressure oscillation in a bioreactor [80,101,113,114].

For large-scale SSF, bioengineering adopted technologies used in composting, ensiling, malting in breweries, and the Koji industry. These are the traditional SSF technologies. A composting type SSF bioreactor is used in biopulping [3,77]. Another recent bioreactor is the Plafractor™ developed at Biocon India [82]. SSF bioreactor design and operation is described in detail elsewhere in this volume.

4. Application of lignocellulose SSF

Lignocellulose may be a substrate for the production of value added products, such as biofuels, biochemicals, biopesticides, biopromoters, or may be a product itself after biotransformation (e.g. compost, biopulp). In all applications the primary requirement is the hydrolysis of lignocellulose into fermentable sugars by lignocellulolytic enzymes, or appropriate modification of the structure of lignocellulose. Economical and effective lignocellulolytic enzyme complexes, containing cellulases, hemicellulases, pectinases and ligninases may be prepared by SSF. In this review lignocellulolytic enzyme production by SSF, and the most important applications achieved with such enzymes are described.

4.1. Lignocellulolytic enzyme production by SSF

Currently lignocellulolytic enzymes are produced mostly by SF, targeting two predominant enzymes, cellulase and xylanase, by using hyperproducing mutants [15,21,32,34,62,81]. Despite increased efforts to develop hyperproducing mutants with higher specific activity and more efficient hydrolytic capacity [22,78], also by using on site production technology integrated into biofuel production (cf. Section 4.2), the cost of SF enzymes remains high. An alternative technology is SSF, using host-specific native filamentous fungi that produce an optimal enzyme complex for the degradation of the host lignocellulose. Such enzyme complexes have been produced by SSF on various agricultural residues, using host-specific fungi for best results [5,13,18,57,60,61,76,84,87,89,95,98,99,102]. There is indication that the hydrolytic potential of SSF enzyme complexes prepared with host-specific enzymes is higher than that of SF enzymes [84,89]. A particular feature of SSF is the possibility of producing the most suitable enzyme complex for a given lignocellulolytic substrate by mixed culturing. Mixed cultivation resulted in better cellulase production and more efficient lignocellulose degradation in various agricultural residues [19,28,29,31]. It is expected that with genetic improvement of existing native strains, specifically targeted for host specificity and SSF application, also with improvement in SSF technology, lignocellulolytic enzyme production by SSF may be the choice for many agrobiotechnological applications, where the crude enzyme source, the fermented substrate, may be directly used in a process (e.g. biofuels, biopulping, biobleaching). In many other applications, e.g. composting and integrated crop management, the necessary enzymes are produced in situ, and exert their effect directly in the soil.

4.2. Biofuels

Bioethanol is currently produced almost exclusively from either sucrose (sugarcane or beets) or starchy feedstocks (principally corn) using *Saccharomyces* yeast [107,110]. Starchy feedstocks, however, are primary food for humans and feed for animals, and their conversion to ethanol is not economical. If biofuels ever become alternatives for fossil fuels, their production must be based on lignocellulolytic feedstocks.

Lignocellulosic biomass (energy crops) and wastes (forest, agricultural, and municipal) represent a vast potential alternative resource for ethanol production [21,47,48,92,106,111]. According to Lynd et al. [46], it is the lack of appreciation of this enormous resource potential as well as of the difficulties in processing technologies that has fostered misconceptions about the potential of lignocellulose for ethanol, and hindered the development of emerging biomass-to-ethanol process technologies.

Lignocellulose may be converted to hexoses and pentoses by dilute acid hydrolysis efficiently at a cost of about US$ 0.20 per gal EtOH [78]. This process, however, requires expensive stainless steel equipment and salt removal, and probably reached its maximal potential.

Enzymatic hydrolysis by lignocellulolytic enzymes is a mild treatment in simple equipment and with great potential for improvement. Although current technology using SF enzymes is still expensive, about US$ 0.4–0.6 per gal EtOH for enzyme cost, modern genetic techniques for improving enzyme specific activity or production efficacy promise an eventual reduction of enzyme cost to US$ 0.07 per gal EtOH [78].

An alternative perspective is SSF technology that promises cost reduction and higher hydrolytic efficiency due

to the presence of an optimal enzyme complex produced by host-specific fungi in single or mixed culturing. Genetic improvement of such host-specific fungi targeted for SSF applications has even greater potential than genetically improved fungi used in SF.

The cost efficacy of currently available enzymes by SF and SSF production is compared below. For illustration, the enzyme cost in a current advanced bioethanol process, in the simultaneous saccharification and co-fermentation process (SSCF) [63,79,112] is compared using currently available SF and SSF enzymes. The SSCF process combines enzymatic lignocellulose hydrolysis with alcoholic fermentation in one step. The reducing sugars produced by enzymatic hydrolysis are immediately converted to ethanol by yeast or a xylose fermenting *Zymomonas mobilis*, thus only very low levels of glucose and cellobiose are in the system, preventing feedback inhibition of the cellulase system. This in turn increases hydrolysis efficiency, sugar production rates, concentrations and yields, and decreases enzyme loading requirements. SSCF uses one bioreactor, whereas in separate hydrolysis and fermentation processes two separate reactors are needed, which doubles capital equipment costs for these steps relative to SSCF.

A preliminary economic analysis of enzyme cost for bioethanol production is based on laboratory scale SSF enzyme production and on National Renewable Energy Laboratory's (NREL) experience with bioethanol production [35,70,109]. NREL estimates the minimal cost of enzyme by a novel *on site* SF production as US$ 0.3 per gal ethanol, at 10 filter paper unit (FPU)/g cellulose loading rate, assuming a US$ 40 per dry ton (DT) feedstock cost, 200 FPU/g cellulose + hemicellulose yield and 75 FPU/l h productivity. The unit cost of enzyme is US$ 0.38/100,000 FPU. Assuming an ethanol production cost of US$ 1.5 per gal, the enzyme cost represents 20% of the production cost. In comparison, a commercial enzyme, Novo-Nordisk (Novozyme) Celluclast 1.5 l would have a unit price of US$ 16/100,000 FPU, prohibitive for this or any other large-scale agrobiotechnological application.

The cost of SSF enzymes produced on corn stover with an average production of 100×10^6 FPU/MT and feedstock plus fermentation cost = *US*$ 150 per MT would be: $150/100 \times 10^6 = 1.5 \times 10^{-6}$ per FPU or US$ 0.15/100,000 FPU with a corresponding cost of US$ 0.118 per gal ethanol, about 8% of total costs. Even from this preliminary estimate it is evident that the SSF enzyme is about half as costly as the most optimistically estimated SF enzyme. At the projected 100 FPU/g yield and 6 FPU/g corn stover (~10 FPU/g cellulose) loading rate, the crude SSF enzyme would represent about a 6% addition to the hydrolyzable mass. This amount could be produced easily with a small capacity SSF reactor *on site*. As a plus, the remaining carbohydrates in the fermented corn stover also would be converted to ethanol in the bioreactor.

Another example for process efficiency via SSF enzymes is the enzyme assisted ensiling of sweet sorghum. SSF enzyme produced on extracted pulp improved sugar yield, partly by breaking down cell walls, thus converting cellulose to glucose, partly by making the cell more permeable for sugar extraction. The microbial protein and the nutrients in the substrate represent bonus values in addition to the value of the SSF enzymes. The process is illustrated in Fig. 2.

The freshly harvested sweet sorghum was ensiled with in situ enzymes, produced on the extracted pulp by SSF. Only 2–3% of the fermented pulp was used as an enzyme source, the rest was used as enzyme enriched animal feed. The cost of the SSF enzyme for this process was estimated as US$ 0.5 per MT sweet sorghum, compared to a cost of commercial enzyme at approximately US$ 9.0 per MT sweet sorghum [97].

4.3. Other enzymes

Xylanase enzymes are used commercially in the pulp and paper, food, and animal feed industries. In the pulp and paper industry, xylanase enzymes enhance the bleaching of pulp, thereby decreasing the amount of chlorine-containing compounds in the process and the subsequent discharge of organochlorines in the effluent [8,42,103,104]. In the food industry, xylanase enzymes are used to accelerate the baking of cookies, cakes, crackers, and other foods by helping to break down polysaccharides in the dough. In animal feeds, xylanase aids in the digestibility of wheat by poultry and swine, by decreasing the viscosity of the feed. Most commercial xylanases are produced by *Trichoderma*, *Bacillus*, *Aspergillus*, *Penicillium*, *Aureobasidium*, *Humicola* and *Talaromyces* spp. In many microorganisms, xylanase activity has generally been found in association with cellulases, beta-glucosidase and other enzymes, although there are many reports that have been described in SSF systems, production of cellulase-free and other enzymes-free xylanases [8,14,24,32,39–41,57,66,86,88].

Beside the lignocellulotic enzyme complex, comprising cellulases, hemicellulases, pectinases and ligninases, lignocellulolytic fungi may also produce other enzymes, such as proteases, lipases and phytases on lignocellulosic substrates, in single or mixed cultures. Frequently, such enzymes are accessory to lignocellulolytic enzymes, and may have application as animal feed supplements [94]. A lignocellulosic agricultural residue, fermented with appropriate lignocellulolytic and other fungi in single or mixed culture SSF may yield a directly applicable feed supplement that improves feed digestibility, phytate degradation, etc.

4.4. Secondary metabolites

Valuable secondary metabolites may be produced by SSF on agro-industrial residues and by-products. Biocon India manufactures three pharmaceuticals of fungal origin (lovastatin, cyclosporin and mycophenolic acid) by SSF on optimized wheat bran media in a new kind of bioreactor called the Plafractor™ [82]. Sweet sorghum fiber, wheat

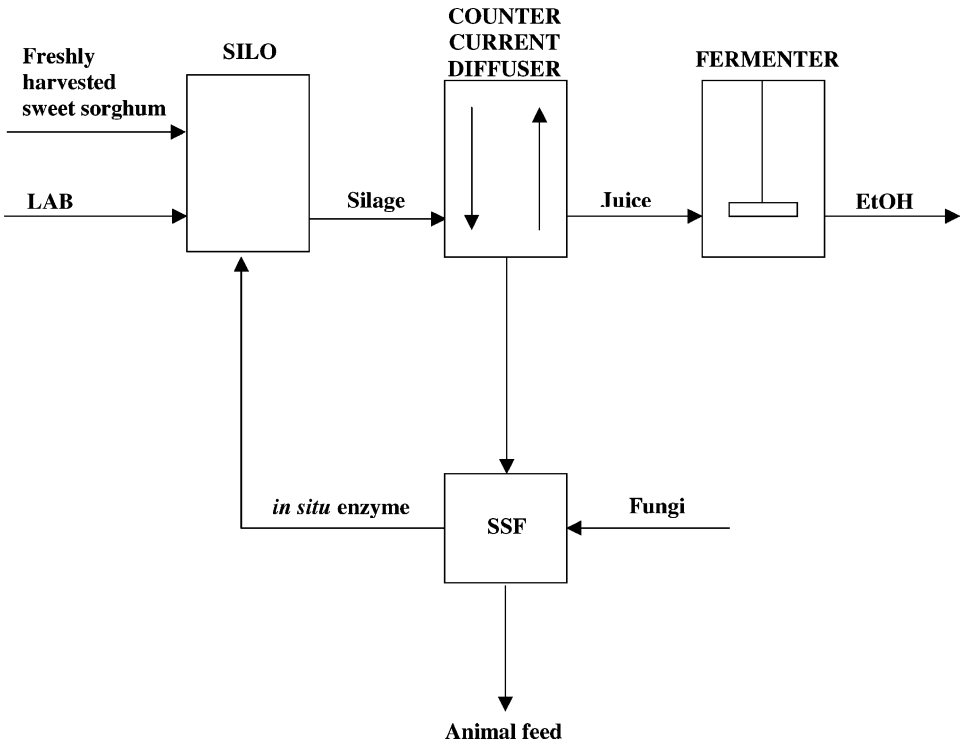

Fig. 2. Scheme of integrated bioprocessing of sweet sorghum for ethanol production.

straw/wheat bran mixture, corn fiber and spent brewing grain supplemented with whey and minerals were found to be also suitable substrate for lovastatin production with *Aspergillus terreus* strains in SSF [85,90]. Other high value metabolites produced on impregnated lignocellulosics (bagasse, corn stalk, wheat bran) by SSF are ergot alkaloids and gibberellic acid [6,26].

4.5. Biopulping, biobleaching

The pulp and paper industry is emerging as one of the potential large markets for enzyme application. The demand for paper increases globally. Microbial enzymes create new technologies for pulp and paper processing. Lignocellulolytic enzymes, cellulases, xylanases and lignases, produced by SF technology have been used successfully in the pulp and paper industry. Xylanases reduce the amount of chemicals required for bleaching, cellulases smooth fibers, enhance drainage, and promote ink removal. Lignin-degrading enzymes remove lignin from pulps [23,42, 103,104]. Enzymes are highly selective in action, their application is environment friendly, but the current prices of commercial enzymes are too high for economical application, and the enzymes are not sufficiently stable in the harsh environment of the paper industry.

There is a potential advantage of producing such enzymes by SSF technology: (1) vastly improved economy, due to the much cheaper fermentation process and the direct applicability of in situ enzymes, improving process efficacy; (2) slow release of SSF enzymes from fungal mycelia during biobleaching, affording protection in the harsh process environment. A novel process have been developed for biobleaching (pre-bleaching), where substrate specific filamentous fungi and actinomycetes were grown on eucalyptus and bagasse pulps by SSF, and the fermented substrates were used for biobleaching [14,86]. The fermented eucalyptus and bagasse pulps contained predominantly xylanases with only traces of cellulases.

SSF technology has been used successfully in biopulping too, to partially remove lignin from wood chips during fermentation with selected basidiomycetes [69]. Biopulping is defined as the treatment of wood chips with lignin-degrading fungi prior to pulping [3,77]. Fungal pretreatment prior to mechanical pulping reduces electrical energy requirements during refining or increases mill throughput, improves paper strength, reduces pitch content, and reduces the environmental impact of pulping. The biopulping process have been scaled up towards the industrial level using SSF technology [3,77]. The large-scale industrial process, resembling to composting, is illustrated in Figs. 3 and 4.

4.6. Integrated crop management

The recycling of crop residues as compost, biofertilizers, biopromoters and biopesticides is a significant part of modern integrated crop management [98]. SSF technology plays an important role here. A special application of SSF is the preparation of microbial soil inocula, biocontrol agent and

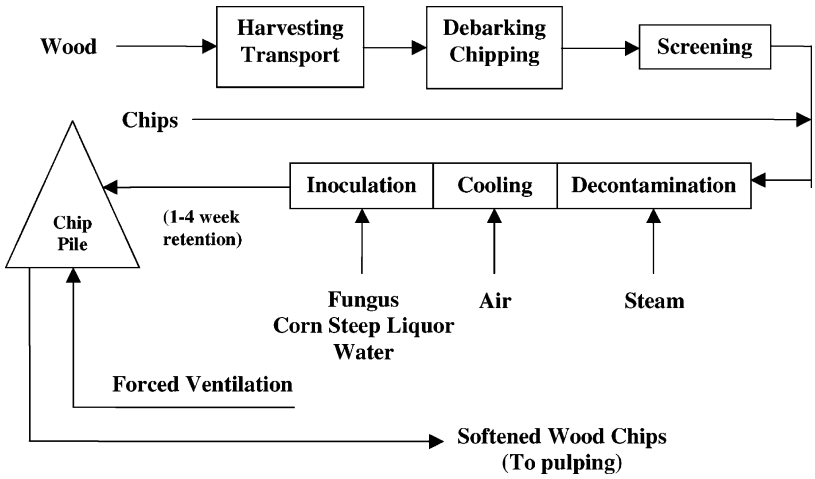

Fig. 3. Overview of the biopulping process showing how the biotreatment process fits into an existing mill's wood-handling system.

directed organic composts, where a special microflora, suitable for a particular crop or a particular soil is developed [2,16,17]. The worldwide need to restore the productivity and humus forming ability of infertile or overburdened soils may make this one of the most profitable and widespread applications of SSF. For directed composting, the desired microorganisms, soil bacteria and fungi, N-fixers, humus formers, lignocellulose decomposers, biocontrol and growth promoting agents, etc. are first propagated as a monoculture to 10^{11}–10^{12} CFU/ml in shake cultures, later in a small SSF bioreactor, then these monocultures are mixed in a specific proportion (dependent on the final use) at about 10^9 CFU/g level in a compost pile of steam-treated organic material, such as fruit and winery processing residues, food processing waste, agricultural and forestry residues, municipal sewage sludge, etc. The C:N ratio is adjusted to 30–50, and the pH, pO_2, temperature are optimized for maximum efficacy of composting [74,75]. The process is illustrated in Fig. 5.

For soil application the directed compost product is preferable (Fig. 5). For seed coating spores are mass produced in a two stage process, then the spores are coated on seeds in the presence of adhesives, and the coated seeds are granulated for easy application in planting. An alternative application, particularly for conifers, is encapsulation of micropropagated plantlets and spores in alginate beads. Spores mixed with fertilizers also may be sprayed on post-harvest residues to accelerate decomposition and provide protection to the next crop (Fig. 5).

The true criterion of the value of soil inocula, compost inocula and similar microbial starter cultures that are added to substrates containing indigenous microflora, is the survival and dominance of the added microorganisms. Sophisticated marker techniques are being developed to follow the fate of added microorganisms in such mixed populations [33,65]. By reducing the initial microbial load (steaming, radiation) and by adding large numbers of the desired microorganisms

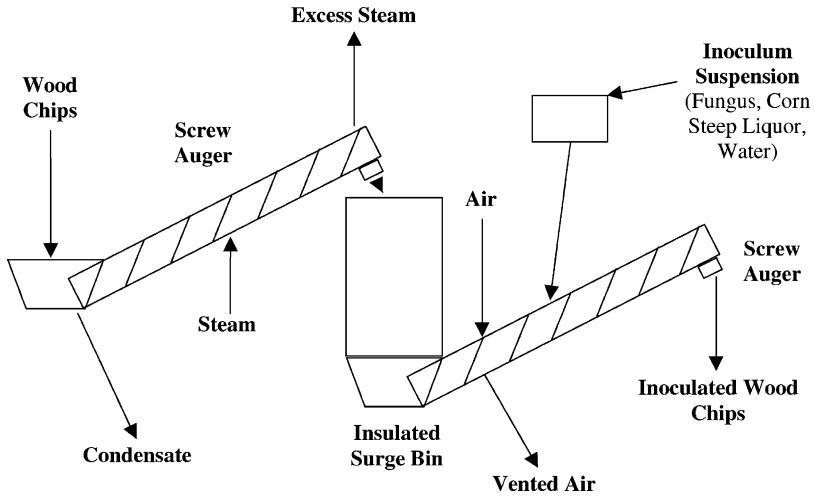

Fig. 4. Continuous treatment system to decontaminate and inoculate wood chips. Wood chips are steamed in the first screw conveyor before being placed into a surge bin. The second screw conveyor then picks up the chips, cools them, and applies the inoculum.

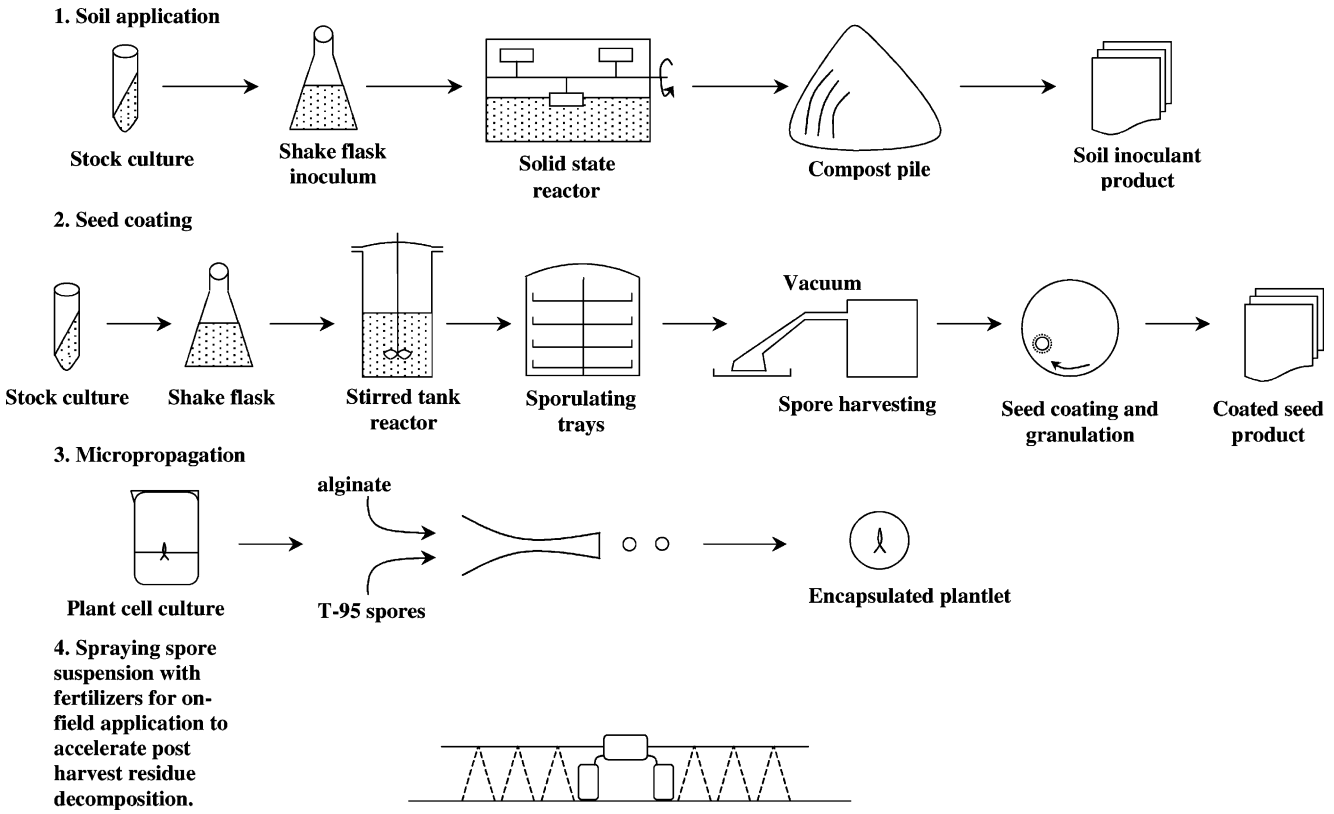

Fig. 5. Delivery systems for *T. harzianum* spores.

(10^5–10^6 CFU/g), the added microorganisms may be favored. At the very least, the controlled composting ensures a large increase in total microbial activity.

There are several commercial soil inocula and directed compost preparations that use SSF for microbial enrichment. Depending on the microbial concentration, the directed compost may be used as a starter culture for field or garden level composting, or the compost may be mixed directly with soil. A side benefit of this process is that burdensome agricultural, industrial and municipal waste may be turned into a valuable commodity.

The directed compost may include humidifiers, N_2-fixers, growth promoters and biopesticide agents. Some options for delivering such agents are also presented in Fig. 5.

Spores of *T. harzianum* may be used as seed coats, may be incorporated into encapsulated tissue culture plantlets or sprayed or dusted directly on field for post-harvest residue decomposition. The partially decomposed residue rich in *Trichoderma* spp. is plowed in to provide soil nutrients and improve soil structure, and promote biocontrol and better plant growth. A summary of tested and proposed delivery systems is given in Fig. 5.

4.7. Animal feed production

Lignocellulosic agricultural residues enriched in microbial protein, enzymes and biofactors by SSF may be used as animal feed [36,38,56]. The large-scale enrichment of lignocellulose in microbial protein by SSF, however, proved to be non-economical, similarly to single cell protein production by traditional SF [69,93]. A more promising avenue is the supplementation of animal rations with feed enzymes produced by SSF [51,64]. For instance, in the integrated bioprocessing of sweet sorghum, shown in Fig. 2, the extracted partially digested and enzyme enriched pulp is a valuable feed ingredient in animal feed rations [97]. SSF enzyme also may be applied in other enzyme assisted processes, e.g. for alfalfa [96]. Here 1–5% of a SSF crude cellulolytic enzyme complex, produced on alfalfa and corn silage mixture (1:1) by SSF improved ensiling efficiency as well as much more expensive commercial enzyme preparations.

5. Future trends

Lignocellulose bioconversion by SSF will have an important role in future biotechnologies, mainly because of its favorable economy, and ease of *on site* operation in agricultural facilities. The focus in SSF application will be on searching for host-specific, SSF targeted fungi, and on their genetic improvement for desired tasks. Those applications will have the greatest perspective, where the transformed lignocellulose is a value added product, biopulp, compost, biofertilizer, biopesticide, biopromoter, or where a

fermented SSF product (enzyme, chemicals, etc.) may be used directly in animal feeds or in biofuel reactors. The engineering aspects of SSF must be further developed, with special attention to mixed culturing and the behavior of lignocellulose during SSF.

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