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REVIEW ARTICLE

Influence of Process Parameters on the Production of Metabolites in Solid-State Fermentation

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ABSTRACT

Solid-state fermentation (SSF) involves the growth of microorganisms on moist solid substrates in the absence of free water. This low moisture content makes the SSF different from submerged fermentation. Unlike the situation in submerged fermentation there is no systematic study guiding the design and operation of large scale SSF with proper controls. The understanding and modeling of microbial growth kinetics and transport phenomena play important roles in the SSF. The design of bioreactors from tray type to stirred tank is discussed. The packed bed, rotating drum, rocking drum, fluidized bed and stirred tank reactors are used in SSF with and without modifications. The parameters like pH, temperature, agitation and aeration also need to be controlled. There is a large gradient of temperature throughout the trays. By manipulating the nitrogen source requirement, the pH of the system is generally controlled. The different factors that control the agitation and aeration in the SSF are discussed. Finally the advantages and disadvantages of SSF compared to submerged fermentation were mentioned. Moreover, such understanding is very much required in the design, scale up and process control in SSF. This paper deals with the influence of environmental parameters such as airflow rate, temperature, pH, substrate concentration and other physico-chemical parameters on the production of specific metabolites.

Keywords: Solid-state fermentation, submerged fermentation, bioreactors

INTRODUCTION

During the 20th century, the great advances in chemistry and chemical engineering led to the commercial production of different products. However, with the latest advancements in the field of biotechnology and biochemical engineering, the production of chemicals, bioactive compounds and food materials using biological entities like microorganisms, cells and enzymes is considered as a superior alternative. These biological agents are far better than their chemical counterparts because of their higher selectivity, mild operating conditions and easy availability of the substrates mainly from the agriculture wastes. Although most of the biotechnology industries relied on submerged liquid fermentation process (SLF), where the microorganisms are grown in liquid media, some of the biotransformation reactions can be carried out in a different fermentation process that is known as solid-state fermentation (SSF). SSF proves to be a better option than the SLF. Interest in SSF has been increasing because of its important applications in producing enzymes, biopesticides, aroma compounds, biopharmaceuticals, organic acids and other bioactive compounds. Agro-industrial residues are processed using SSF because it has lower energy requirement, produce lesser waste water and are environment-friendly. The major difference between SSF and SLF is of the free water content in the substrate. As suggested by Moo-Young, the term solid-substrate fermentation is used in a more general sense to describe any process in which solid particles of substrates were involved, regardless of the amount of free water (Moo-Young *et al.* 1983). Therefore, SSF includes the processes in a solid matrix with an aqueous phase leaching through it, slurries of solid particles and solids suspended in an aqueous phase. In the case of SLF, substrate is freely accessible to the microorganisms while in SSF the availability of substrate to the microorganisms may increase, decrease, or remain relatively constant during the fermentation (Knapp and Howell 1985). SSF stimulates the growth of microorganisms in nature on the moist solids and has been credited to be responsible for the beginning of fermentation technique in ancient time (Cannel and Moo-Young 1980).

The first milestone in the development of SSF was achieved during 1950-1960, when steroid transformation was reported using fungal cultures. The trend continued with the production of mycotoxins by SSF. Then production of protein rich feed was the next major activity reported, which involved utilization of agro-industrial residues. This was the time when SSF was actively

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researched, followed by numerous patents and publications on basic aspects of SSF, development of bioreactors, production of microbial products using SSF such as food and feed (Han *et al.*, 2001), various primary and secondary metabolites, bioprocess such as bioleaching, biopulping, biobenefication, bioremediation (Classen *et al.*, 2000) and industrial enzymes (Pandey *et al.*, 1999). Several authors had reviewed the history and development of SSF. Aidoo *et al.,* (1982) made an attempt to trace the history of growth of microorganisms on solid substrates and the term solid-state fermentation came after this. Cannel and Moo-Young in 1980 reviewed the developments of SSF in food industry and composting. They compared SSF with SLF and also described the advantages and disadvantages. Some differences between SSF and SLF are discussed in Table 1

Table 1: Differences between Solid-state and Submerged liquid Fermentation

Solid-state fermentation (SSF)	Submerged liquid fermentation (SLF)
• Organisms requiring less water for growth are preferred such as filamentous fungi.	• Media concentration is very much lower as compared to water content.
• Inert support (natural or artificial), containing all components for growth in the form of solution.	• Required processed ingredients are expensive.
• Less chances of contamination because of low availability of water.	• Higher water activity becomes the major cause of contamination in SLF.
• Small size bioreactors can be used.	• Large-scale bioreactors are required because media is very much diluted.
• Less consumption of energy for aeration and gas transfer.	• High air pressure consumes more power and there is poor transfer of gas in SLF.
• Limiting factor for growth is diffusion of nutrients.	• Vigorous mixing makes diffusion easy.
• Lots of difficulties in measuring the quantity of biomass present and other online processes.	• Online sensors are available and sampling is easy for biomass measurement.
• Downstream processing is easy, cheaper and less time consuming.	• Water makes downstream process difficult and very expensive.
• Liquid waste is not produced	• High quantity of liquid waste is produced, causes difficulties in dumping

GENERAL ASPECTS OF SSF PROCESS

The important aspects which should be considered before starting a solid-state fermentation include the selection of suitable microorganism and substrate, optimization of process parameters and isolation and purification of the product. SSF follows the same steps as in the SLF, namely substrate preparation, inoculum preparation, cultivation within a bioreactor and finally the downstream processing and recovery of the product. Fungi and yeast seem to be the favorable one than bacteria because of their ability to grow on the substrates, which are low in water content. Due to high water activity requirement, bacterial cultures might not be suitable for SSF. However, Pandey in 1992 reported that bacterial culture can be well managed and manipulated for SSF process. It seems that the high yield in SSF as compared to SLF is due to the growth of the microorganisms similar to their natural habitat, resulting in higher metabolic activities.

Another aspect that affects the production is the selection of proper substrate. Substrate in SSF is non-soluble material that acts both as physical support and source of nutrients. Byproducts of agricultural activities or the products obtained after the processing of agricultural materials can be used as substrates. Some of the substrate may require pretreatment. Chopping or grinding may be necessary to reduce particle size, cracking may be necessary to increase the accessibility of the interior of the particle to the microorganism, thermal pretreatment or even chemical hydrolysis might be necessary to increase the susceptibility of macromolecules within the solid to attack by microbial enzymes (Mitchell *et al.,* 2000). Selection could be done by considering suitable value-addition and/or disposal of specific substrate (Moo-Young *et al.,* 1983, Pandey *el al*., 2000). The second thing one has to consider is the production of a specific product from a suitable substrate and for this, different substrates have to be screened for a specific product. Similarly, it would be important to screen different microorganisms and select the most suitable one. Product isolation could be relatively tougher and more costly when using naturally occurring raw materials such as wheat bran as the substrate

because while extracting the product after fermentation, along with the product, several other water-soluble components from the substrate also leach out and may pose difficulties in the purification process.

Selection of process parameters and their optimization are other key aspects of SSF. This part includes physiochemical and biochemical parameters such as particle size, initial moisture content, initial pH, relative humidity, incubation temperature, agitation, aeration, age and size of inoculum, supplementation of nutrients and trace elements, supplementation of additional carbon source and inducers, extraction of product and its purification.

MICROBIAL TYPES USED IN SSF

The low amount of free liquid in the substrate affects the whole process of SSF and therefore it becomes the most important feature of SSF. All the factors in SSF depend upon the amount of water present in the substrate. Care should be taken while selecting the microorganism for fermentation on solid-substrates. Due to the less availability of free water in SSF process than the majority of liquid fermentations, most of the SSF processes involve fungi, although there are number of reports involving bacteria and yeast. Bacteria are mainly involved in processes like composting, ensiling and yeasts have been used for ethanol and food or feed production (Doelle *et al.,* 1992).

The yeast grows on solid-substrate as minor member and the dominant species are bacteria for example *Lactobacilli* in ensiling and in tape manufacturing (Moo Young *et al.,* 1983). *Saccharomyces cerevisiae* has been employed in semi-solid fermentation of grape pomace and for SSF of sweet sorghum, sugar beets (Kirby and Mardon 1980) and fodder beets (Gibbons *et al.,* 1984, Kargi and Curme 1985). A number of bacterial species have been employed for solid-substrate fermentation using many substrates. *Lactobacilli* are used for ensilage and thermophilic bacteria predominate in composting. *Bacillus subtilis* is the major organism used for production of Japanese food Natto (Beuchat *et al.,* 1985). *Lactobacillus plantarum* and *Propionibacterium shermanii* are used to ferment and preserve high moisture grain (Flores *et al.,* 1985).

Out of all the groups of fungi, the filamentous fungi are the major group of microorganisms, which predominate in the SSF process. Different species of fungi used in SSF process include many species of *Aspergillus, Rhizopus, Alternaria, Fusarium, Monilia, Mucor, Trichoderma* and some species of *Penicillium*. Most of the species belong to filamentous fungi, as these are best suited because of their ability to spread over and to penetrate inside the solid-substrate. The other advantage of using filamentous fungi is that the fungal mycelia synthesize and release large quantity of extra-cellular hydrolytic enzymes.

SUBSTRATES USED IN SSF

Substrates used in SSF are generally insoluble in water. In practice, water is absorbed onto the substrate particles, which can then be used by microorganisms for growth and metabolic activities. Bacteria and yeast grow on the surface of the substrate while fungal mycelium penetrates into the particles of the substrates (Pandey, 1992).

Substrate for SSF can be divided into three groups, starchy substrates, cellulose or lignocellulose and those with soluble sugar. Example of starchy substrate includes rice, cassava, wheat bran, rice bran, buckwheat seeds, corn meal, and sweet potato residue and banana meal. Lignocellulosic solid-substrate includes wheat straw, corn, rice stover, wheat bran, sugar-beet pulp and wood. Substrates containing soluble sugars include grape pomace, sweet sorghum, sugar-beet, pineapple waste, carob pods and coffee pulp. Another strategy is to impregnate inert solid material such as bagasse or hemp with soluble sugars in order to provide a SSF environment for the growth (Weber *et al.,* 1999).

Natural substrates are easily available and are cheaper than synthetic substrates. But, they generally require pretreatment to make their chemical constituents more accessible and their physical structure more susceptible to mycelial penetration. Physical treatment includes chopping or grinding to reduce size and cracking to make the interior of the particle more accessible. Chemical treatment includes high temperature cooking and acid or alkali treatment. Supplementation of additional nutrients may be required in order to stimulate growth, induce enzyme synthesis or to prolong secondary metabolite production such as the supplementation of 0.5% of glucose or cellobiose, 0.5% peptone, asparagine or yeast extract are in use. Many references can be cited where synthetic substrates have also been used for the growth of microbes such as synthetic polymers or substances like agar and gelatin which provide homogeneous solid-substrate (Georgiou and Shuler 1986). Gelatin and kappa-carrageenan have also been used. However, these substrates are not exploited commercially because of their high cost and low availability. The use of these substrates is restricted for research purpose only.

Physical factors affecting utilization of solid-substrate include accessibility of substrate to the microbes (porosity and particle sizes affect the accessible surface area to the organisms), film effect and mass effect. The size of substrate particles affects the extent and rate of microbial colonization, air penetration, CO_2 removal and downstream extraction. The optimum particle size often represents a compromise between the accessibility of nutrients and the availability of oxygen. Particle size from 1 mm to1cm is often used in SSF. Knapp and Howell (1985) reviewed the effect of alteration of substrate particle size on SSF. Pandey (1992) has reported that productivities were higher with the substrate that contains particles of mixed sizes varying from 180 µm to 1.4 mm. Chemical factors affecting utilization of solid-substrate includes degree of polymerization and crystallinity. The

selection of substrates and optimization of its concentration plays an important role in yielding the higher growth rates of microbes. At higher growth rate, the ratio between heat losses to heat production is low and therefore helps to approach the behavior of large scale bioreactor.

TEMPERATURE

Microbial growth in SSF generates significant amount of metabolic heat. It has been reported that 100-300 kJ of heat per kg of cell mass is generated in a SSF process (Prior *et al.*, 1992). Establishment of temperature gradients and localized overheating of the substrate occurs because of inefficient removal of heat from the substrate. Heat transfer problem in SSF includes temperature gradients that may cause belated microbial activity, dehydration of the medium and undesirable metabolic deviations (Rathbun and Shuler 1983, Saucedo-Castaneda *et al.,* 1990). Heterogeneous materials make heat removal difficult; this is due to low transfer coefficient and low thermal conductivity (Narahara 1984). Temperature can rise rapidly, because there is little water to absorb the heat or in other words mean specific heat capacity of the fermenting mass is much lower than that of water. Therefore, heat generated must be dissipated immediately as most of the microorganisms used in SSF are mesophilic, having optimal temperature for growth between 20 and 40^{o}C and maximum growth below 50ºC.

Different methods are used for dissipation of heat such as cooling of metal trays using circulating coolant and intermittent agitation. Other methods include forced aeration, water circulation through a jacket surrounding the fermenter, agitation of the solids and covering the external surface of fermenter with water soaked burlaps. However, forced aeration is generally the technique of choice for heat removal. This has been reported that most of the heat generated (up to 80%) in the SSF process is removed by using forced aeration. Heat generation rates indicate that conduction heat transfer was the least efficient mechanism (8.65%) when compared with convective (26.65%) and evaporative (64.7%) mechanism (Barstow *et al.*, 1988).

Effect of temperature on both specific growth rate and specific death rate has been well described by Arrhenius type of equation (Szewezyk and Myszka 1994).

$$\mu_{m} = \mu_{mo} \exp(-E_g/RT) - k_{do} \exp(-E_d/RT)$$

Where R is universal gas constant, T is the absolute temperature, E_g and E_d are the activation energies for growth and death and μ_{mo} and k_{do} are specific growth rate and specific death rate, respectively.

The deleterious effect of high temperature on spore germination, cell growth, product formation, sporulation and consequently on the overall productivity of the fermentation process are well described by Moreaux (Moreaux, 1980). A temperature of 47°C at the centre of the fermentating substrate in tempeh production, 67°C in a rectangular fermenter with an 80 mm bed depth and 60-70°C in the centre of a composting bed have been reported (Rathbun and Shuler 1983, Hayes 1977). Peak heat generation rates in Koji processes lies in the range 71-159 kJ/Kg.h but average rates are more moderate, at 25-67 kJ/Kg.h. An increase of about 2-5°C in the temperature of the solids in a fermenter of 40 mm depth resulted in a doubling of the fermentation time required to attain similar enzyme titer to those in the temperature gradient free fermenter of 20 mm depth.

EFFECT OF pH AND ITS CONTROL

There are remarkable changes, which occur in the pH of the substrates. These are mainly for the production of acids due to incomplete oxidation of the substrate or uptake of ammonium ions, which will cause the pH to fall, while the release of ammonia by deamination of urea, or other amines will increase the pH. As we cannot monitor pH in the SSF it is very difficult to control the pH. So, it is desirable to use microorganisms which can grow over a wide range of pH and which have broad pH optima. By using different ratios of ammonium salts and urea in the substrate, pH control in both natural and model SSF system can be obtained (Raimbault and Alazard 1980).

The effect of increasing buffer concentration, effect of pH on the maximum specific growth rate and the optimization of different buffers for the growth and enzyme parameter were investigated by Nagel *et al.,* (1999). They have also calculated the required buffer concentration by utilizing expected biomass production, initial pH and pH change. Exact control of pH is very difficult in SSF process, but one can maintain pH during the process by using pH-correcting solutions (Mitchell *et al.,* 1991). Many substrates are effective buffers. This is particularly true for protein rich substrate, especially if deamination of protein is minimal. Model has been proposed which relate growth rate to pH based on empherical equations describing experimental data for the effect of pH on growth (Muck *et al.,* 1991, Pitt, 1993).

WATER ACTIVITY

Water activity (a_w) of the moist solid substrate is the ratio of vapour pressure of water above the substrate in a closed system to the vapour pressure of the pure water at same temperature. It is measured as relative humidity divided by 100. Pure water has a_w = 1.00 and a_w decrease with the addition of solutes. Bacteria mainly grow at higher a_w values, while filamentous fungi and some yeasts can grow at lower a_w value (0.6-0.7). The microorganisms, which can grow and are capable of carrying out their metabolic activities at lower a_w values, are good for SSF processes. For fungi, the optimal moisture requirement varies between 40 and 80% (w/w), but for the same organism growing on different substrate, the optimum moisture level may differ widely, hence, the amount of moisture by itself is unreliable for predicting growth (Prior *et al.*, 1992). The fermentation process itself leads to a

change in a_w as products are formed and the substrate is substrate particle and hence aeration is made difficult. Water activity also affects product formation and the spectrum of product produced and characteristics such as aroma are also affected by water activity. Since the water activity depends on the concentration of dissolved solutes, salts, sugars or other solutes can be added to alter the activity. The optimal a_w also depends on the factors such as agitation rate and cultivation temperature. During fermentation a_w is calculated by aeration with humidified air and sometimes by intermittent water spray. It has been shown that in the course of fungal growth in SSF, higher water activities favour sporulation while low water activities favour spore generation or mycelia growth. Gervais (Gervais *et al*., 1988) studied the influence of a_w on enzyme biosynthesis and enzyme activities produced by fungi and developed a new sensor which allowed continuous a_w measurement of submerged and SSF. A kinetic model, which relates the rate constant of death of the microbial cells to water activity and temperature, has been proposed by Moser in 1988. Prosser in 1982 reported that the width of the peripheral growth zone of the colonies is constant despite variation in water activity and radial extension rate is directly proportional to the specific growth rate.

APPLICATIONS OF SSF

From centuries human beings have been producing their food by employing different microorganisms but they did not know the theory behind it. It is the case with SSF, it was a traditional process, but the exact potential of SSF was only known during 21st century. The traditional Koji process may be considered the archetype of SSF started in 7th century in Japan. Its importance is because of the high content of amylolytic and proteolytic enzymes, which catalyze the degradation of starches and proteins. Koji production is done using strains of *Aspergillus oryzae* on steamed rice and incubated under controlled temperature and humidity. A Japanese biochemist and industrialist leader Dr Jokichi Takamine began commercial production of koji from the fungus *Aspergillus oryzae* and called it "takadiastase" introduced koji in USA in the year 1891 using wheat bran. Most of the cheese such as blue-veined cheese using *Penicillium roqueforti,* Camembert and Brie cheese using *Penicillium camemberti* and *Penicillium caseicolum,* are produced by SSF (Mial, 1975). SSF is also widely employed in processes like ensiling, composting using thermophilic bacteria, mesophiles, actinomycetes and fungi (Stentiford and Dodds 1992). Nowadays, lots of byproducts such as enzymes, organic acids, ethanol, biogas, antibiotics, surfactants, toxins, bioremediation agents, mushrooms, compost, microbial polysaccharides, biopesticides, protein-enriched fermented foods, predigested feeds for ruminants are produced by SSF process. The different applications of SSF are given in Table 2 and Table 3.

Table 2: Application of SSF in the production of pharmaceuticals

COMPOUND (FUNCTION)	SOURCE	SUBSTRATE	REFERENCE
Pharmaceuticals			
Gibberellic acid (Plant growth harmone)	*Fusarium moniliforme*	Sugarcane bagasse	(Tomasini *et al*., 1997)
Zearalenone (Growth promoter)	*Fusarium moniliforme*	Corn	(Balakrishnan and Pandey 1996)
Bacterial endotoxin (Insecticide)	*Bacillus thuringensis*	Coconut waste	(Balakrishnan and Pandey 1996)
Ergot alkaloids (Migrane)	*Cleviceps purpurea, C. fusiformis*	Sugarcane bagasse	(Hernandez *et al.*, 1993.
Penicillin (Antibiotic)	*Penicillium chrysogenum*	Sugarcane bagasse	(Balakrishnan and Pandey 1996)
Cephalosporin (Antibiotic)	*Cephalosporium armonium*	Barley	(Balakrishnan and Pandey 1996
Cephamycin C (Antibiotic)	*Streptomyces clamuligerus*	Wheat rawa with cottonseed cake and sunflower cake	(Kota and Sridhar 1999)
Tetracycline (Antibiotic)	*S. viridifaciers*	Sweet potato residue	(Balakrishnan and Pandey 1996)
Chlortetracycline (Antibiotic)	*S. viridifaciers*	Sweet potato residue	(Balakrishnan and Pandey 1996)
Oxytetracycline (Antibiotic)	*S. rimosus*	Corn cob	(Yang and Swei 1996)
Acitinorhodin Methylenomycin (Antibiotic)	*S. coelicolor*	Agar medium	(Balakrishnan and Pandey 1996)
Surfactin (Antibiotic)	*Bacillus subtilis*	Soyabean residue, okara	(Balakrishnan and Pandey 1996)
Cyclosporin A (Immuno suppressive drug)	*Tolypocladium inflautum*	Wheat bran	(Sekar and Balaraman 1998)
Clavulanic acid [β- lactamase inhibitor (antibacterial)]	*S. clavulingerus*	Wheat rawa with soy flour and sunflower cake	(Sircar *et al.*, 1998)

Table 3: Other applications of SSF

PRODUCT OR PROCESS	ORGANISM	SUBSTRATE	REFERENCE
Enzymes			
Glucoamylase	*Aspergillus sp.*	Tea waste, rice bran, wheat bran.	(Selvakumar *et al.*, 1998) (Arasaratnam *et al.*, 1997)
Lipase	*Aspergillus niger,* *Candida rugosa,* *Penicillium restrictum.*	Gingelly oil cake, coconut cake, babassu oil cake,	(Kamini *et al.* 1998) (Benjamin and Pandey 1997) (Gombert *et al.*, 1999)
Cellulases	*Bacillus sutilis,* *Aspergillus sp.,*	Banana fruit stalk wastes, soyabean meal,	(Krishna 1999) (Gutierrez *et al.*, 1999)
Pectinases	*Talaromyces flavus,* *Aspergillus niger*	Citrus wastes, soy bran and wheat bran, apple pectin.	(Crotti *et al.*, 1999) (Castilho *et al.*, 2000) (Berovic and Ostroversnikj 1997)
Inulinase	*Staphylococcus sp. Or* *Kluyveromyces marxianus*	Wheat bran, rice bran, coconut oil cake and corn flour.	(Selvakumar and Pandey 1999)
Xylanases	*Aspergillus tamari,* *Trichoderma longibrachiatum,* *Trichoderma reesei, A. niger,* *Bacillus sp.*	Corn cob, wheat bran, sugar-cane bagasse, soymeal, wheat bran.	(Ferrreira *et al.* 1999) (Gutierrez and Tengerdy 1998) (Gessesse and Memo 1999)
Biopesticides			
Bioinsecticide	*Coniothyrium minitans*	Impregnated hemp	(Weber *et al.*, 1999)
Bioremediation	*Phanerochaete chrysosporium,* *Lentinula edodes*	Sugarcane bagasse pith, pentachlorophenol in soil	(Rodriguez *et al.*, 1999) (Okeke *et al.*, 1997)
Biofilter	Various	Peat + volatile organics	(Wu *et al.*, 1998)
Food and Feed			
Fermentated food	*Aspergillus orzyae or* *A. sojae*	Various fruit peel	(Sardjono and Knol. 1998)
Caffine removal	*Aspergillus tamarii*	Impregnated sugarcane bagasse	(Hakil *et al.*, 1999)
Delignification	*White rot fungi*	Wheat straw	(Dorado *et al.*, 1999)
Improved nutrition	*Penicilium sp.*	Bergamot fruit peel	(Scerra *et al.*, 1999)
Protein enrichment	*Neurospora sitophila*	Sugar beet pulp or citrus waste	(Shojaosadati *et al.*, 1999)
Aroma substances			
Aroma compounds	*Bjerkandera adusta*	Wheat bran	(Lapadatescu and Bonnarme 1999)
Pyrazines	*Bacillus subtilis*	Ground soybeans	(Larroche *et al.*, 1999)
Organic compounds			
Citric acid	*Aspergillus niger*	Dry coffee husk, sweet potato, carob pod, pineapple waste, corncobs.	(Roukas 1999) (Tran *et al.*, 1998) (Hang and Woodams 1998)
Kojic acid	*Aspergillus oryzae*	Steamed rice	(Mial 1975)
Ethanol	*Saccharomyces cerevisiae*	Sweet potato or sweet sorghum	(Sree *et al.*, 1999)
Polymers			
Succinoglycan	*Agrobacterium tumefaciens,* *Rhizobium hedysaris.*	Spent malt grain or ivory nutshaving or grated carrots, impregnated spent malt grains	(Stredansky and Conti 1999a) (Stredansky and Conti 1999b)
Xanthan gum	*Xanthomonas campestris.*	Spent malt grains, citrus peels, apple pomace, or grape pomace, Impregnated spent malt grains.	(Stredansky *et al.*, 1999b])

CONCLUSION

SSF is a well-adapted process for fermentation of filamentous fungi on solid biomass which are broken down by excreted hydrolytic enzymes. Better knowledge is required on how to design and optimize the various process parameters in order to allow successful SSF to be carried out. Development of adequate process control strategies is a major challenge. Auxiliary processing steps such as substrate preparation, inoculum preparation and downstream processing need more attention. It is necessary to evaluate rigorously the economic performance of SSF processes, and compare this with similar processes carried out by SLF. Only with such a concerted effort, solid state bioprocessing will be developed sufficiently to fulfill its potential.

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