Solid State Fermentation for the Production of Industrial Enzymes

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Chapter 10
Solid-State Fermentation Technology for Bioconversion of Biomass and Agricultural Residues

Poonam Singh nee' Nigam and Ashok Pandey

Contents

Abstract Solid-state fermentations (SSF) have attracted a renewed interest and attention from researchers due to recent developments in the field of microbial-biotechnology. Hence, for the practical, economical and environmentally-friendly

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bioconversion of agro-industrial wastes, solid state or substrate fermentation has been researched globally and proved to be the ideal technology for this purpose. In this chapter some important aspects of solid-state cultivation system have been discussed, including the variety of substrates and microorganisms used in SSF for the production of various end products; and the performance control of system by regulation of important factors.

Keywords Solid substrates · Agricultural residues · Solid state fermentation · Water activity · Moisture · Bioreactor

10.1 Agro-Residue Bioconversion in SSF

Commonly used substrates in SSF are natural agricultural products, as well as agro-industrial waste residues and by-products serve as a source of carbon in SSF (Table 10.1). Lignocellulosic materials of agriculture origin compose more than 60% of plant biomass produced annually through the process of photosynthesis. This vast resource is the potential and renewable source of biofuels, biofertilizers, animal feed and chemical feedstocks. Lignocellulose may be a substrate for the production of value-added products (Table 10.2), such as biofuels, biochemicals, biopesticides, biopromoters, or may even 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

Table 10.1 Diverse range of agro-residues utilization in SSF technology

Substrates for SSF	Microorganisms used in SSF	Reference
Starchy raw materials	*Aspergillus spp*	Czajkowska and Ilnicka 1988
Bannana waste	*A. niger*	Baldensperger et al. 1985
Barley Husk	*Bjkendra adusta*	Robinson and Nigam 2008
Corn cob	*A. niger*	Singh et al. 1989
Citrus peel	*A. niger*	Rodriquez et al. 1985
Sugarcan by-products	*A. terreus*	Blanko et al. 1990
Cassava	*Rhizopus oryzae*	Daubresse et al. 1987
Sugarbeet pulp	*Trichoderma viride*	Durand 1998
Cassava	*T. resei & yeast*	Opoku and Adoga 1980
Wheat straw	*T. reesei & Endomycopsis fibuleger*	Laukevics et al. 1984
Wheat straw	*T. reesei, Chaeotominum*	Abdullah et al. 1985
Sugarbeet pulp	*T. reesei and Fusarium oxysporum*	Nigam and Vogel 1988, 1990
Sugarcane bagasse	*Polyporus spp*	Nigam 1990
Saccharum munja-	*Pleurotus spp.*	Gujral et al. 1987
Residues Wheat straw	*Coprinus spp.*	Yadav 1989, 1988
Cassava	*Sporotrichum pulverulentum*	Smith et al. 1986
Straw	*Candida utilis*	Han 1987
Sweet potato	*Pichia bartonii*	Yang 1988
Fodder beets	*Saccharomyces cerevisiae*	Gibbon et al. 1984

Table 10.2 Agro-industrial residues used for addcd-value products

Substrate	Microorganisms	Product	Reference
Oat cereal	*Rhizopus oryzae*	Lactic acid; various value added products	Koutinas et al. (2007)
Agro-residues	*Aspergillus niger*	Citric acid	Prado et al. (2004)
Distillers grain	*Aspergillus niger*	Citric acid	Xie and West (2006)
Wheat bran	*Mucor meihei*	Rennet	Thakur et al. 1990
Wheat bran	*Rhizopus oligosporus, Mucor meihei*	Rennet	Karanth (1988)
Agar	*Trichoderma viride*	Cheese aroma	Gervais (1988)
Agro-residues	*Fungal cultures*	Cheese flavour	Revah and Lebeault (1988)
Polished rice	*Neurospora spp*	Aroma	Yamauchi et al. (1989)
Barley	*Strptomyces Cephcdosportum aermonium*	Cephalosporin	Jermini and Demain (1989)
Sweet potato	*Aspergillus*	Tetracyclines	Yang and Ling (1984)
Rice grains	*Streptomyces*	Cephalosporin	Wang et al. (1984)
Bagasse	*Pencillium chrysogenum*	Penicillin	Barrios-G et al. (1990)
Cassava	*Aspergillus niger*	Aflatoxins	Barrios-G et al. (1990)
Corn	*A. flavus*	Mycotoxin	Hesseltine (1972)
Soya	Various moulds	Mycotoxin	Bhumiratna et al. 1980
Oat straw	*Pofyporovs spp*	Lignin degradation	Bone and Munoz (1984)
Birch lignin	*Phanerochaete chrysosporhun*	Lignin conversion	Mudagett and Paradis (1985)
Maple Wood	*Polyporus anceps*	Lignin conversion	Matteau and Bone (1980)
Bagasse	*2 Polyporous spp*	Lignin conversion	Nigam (1990)
Aspen Wood	*Phubia tremelloasa*	Delignification	Reid (1989)
Wheat bran	*Fusarium monoliforme, Gibberrela fujikuroi*	Gibberellic acid	Kumar and Lonsane (1987a, b, c)
Wheat bran	*Fusarium monoliforme, Gibberrela fujikuroi*	Gibberellic acid	Prema et al. (1988)
Wheat straw	*Poms tignium*	Hydroenperoxi de	Maltseva et al. (1989)
Soya bean Cassava	*R. oligsoporus*	Tempeh and Koji	Hesseltine (1972)
Koji-type SSF	*Filamentous fungi*	Fungal spores	Vezina and Singh (1975)
Soya bean	*Filamentous fungi*	Fungal spores	Lotong and Suwarnarit (1983)

the structure of lignocellulose. Economical and effective lignocellulolytic enzyme complexes, containing cellulases, hemicellulases, pectinases and ligninases may be prepared by SSF (Table 10.3). 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 (Pandey et al. 2001).

Lignocellulose in wood may be transformed into good quality paper products with the help of SSF biopulping and biobleaching. Agricultural residues may be converted into animal feed enriched with microbial biomass, enzymes, biopromot-

Table 10.3 Agro-residues used in SSF for enzyme production

Substrates	Microorganisms	Enzymes	Reference
Bagasse, sawdust,corn cobs	*A. niger*	cellulose, beta glucosidase	Madamwar et al. (1989)
Corn cobs	*A. niger*	cellulose	Singh et al. (1989)
Wheat bran	*A. niger*	glucoamylase	Pandey (1990)
Wheat bran	*A. niger*	glucoamylase	Ramakrishnana et al. (1982)
Sugarbeet pulp	*A. phoenicis*	beta glucosidase	Deschamps and Huet (1984)
Wheat bran	*A. flavus*	protease	Malathi and Chakrabarty (1991)
Wheat bran	*A. carbonarius*	pectinase	Karanth (1988)
Wheat bran	*A. niveus*	catalase	Karanth (1988)
Sugarbeet pulp	*T. viride and A. niger*	cellulase and amylase	Desgrenges and Durand (1990)
Wheat bran and rice straw	*Trichoderma spp. A. ustus, Botritis spp., S. pulverulentum*	Cellulose, beta-glucosidase, Xylanse	Shamala and Sreekantiah (1986)
Wheat bran	*Pencillium spp. Geotrichwn Candidum, Mucor meihei & 2 Rhizopus spp.*	lipase	Munoz et al. (1991)
Sugarbeet pulp	*P. capsulatum*	enzymes	Considine et al. (1988)
Citrus pulp-pellets	*P. charlesii, Talaromyces flavus, Tubercularia vulgaris*	Pectic enzymes	Siessere and Said (1989)
Citrus pulp	*T. vulgaris*	pectic enzymes	Vieira et al. (1991)
Bagasse	*Polyporous spp.*	Cellulase & ligninase	Nigam et al. (1987)
Lignocellulo sis	*Lentinula edodus*	enzymes	Mishra and Leatham (1990)
Wheat bran	*Bacillus licheniformis*	alpha amylase	Ramesh and Lonsane (1987a, b, 1990)
Wheat bran	*Bacillus subtilis*	protease	Jermini and Demain (1989)
Straw	*Neurospora crasse*	Carboymethyl cellulase, beta glucosidase	Macris et al. (1987)

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ers, 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.

10.1.1 Nature of Substrates

The major organic material available in nature are polymeric in nature e.g. polysaccharides (cellulose, hemicellulose, pectins, and starch etc.), lignin and protein,

Table 10.4 Agro-residues used for microbial growth studies

Substrate	Microorganism	Reference
Cassava	*A. niger*	Raimbault and Alazard, 1980, Casteada et al., 1990, Oriol 1988
Barley Husk and Barley straw	*Bjkendra adusta*	Robinson and Nigam 2008
Citrus peel	*A. niger*	Rodriguez et al. 1985,
Cassva	*Rhizosporus oligosporus*	Mitchell et al. (1988, 1990)
Soya bean	*R. oligosporus*	Rathbun and Shuler 1983
Sugarbeet pulp	*T. viride, A. niger*	Desgrenges and Durand 1990
Sugarrbeet pulp	*T. viride, Sporotrichum Pulverulentum and Thermoascus auranticus*	Grajek 1988
Buckwheat seeds	*Penicillium roqueforti*	Desfarges et al. 1987
Wheat straw	*Corinus fimetarius*	Singh et al. 1990

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which can be metabolized by different microorganisms as a source of energy. These substrates that are insoluble in water, absorb water onto their matrix, which provides required moisture in SSF system for the growth and metabolic activities of microorganisms. Bacterial and yeast cultures grow on the surface of substrate fibrils and particles while fungal mycelia penetrate into the particles of substrate for nutrition.

The solid phase in SSF provides a rich and complex source of nutrients that may be sufficient or sometimes insufficient and incomplete with respect to the overall nutritional requirements of that particular microorganism that is cultivated on that substrate. The constituents in the agricultural solids are approximately analysed in terms of total carbohydrates, proteins, lipids, various elements and ash content. The solid substrates generally contain some small carbon compounds whereas the bulk of total dry weight is a complex polymer. The polymeric forms require enzymatic hydrolysis for their mineralisation as carbon-energy sources in microbialmetabolism. In comparison with liquid-state fermentation, which generally use less complex carbon energy sources, solid insoluble substrates provide mixed ingredients of high molecular weight carbon compounds. Such complex carbon compounds may contribute inhibition, induction, or repression mechanism in microbial metabolism during solid state cultivation.

10.2 A Bio-Technology Solid State Fermentation

Solid substrate systems have been defined in several ways:

1. Solid substrate fermentation (SSF) is the microbial transformation of biological materials in their natural state, in contrast with liquid or submerged fermentation that is carried out in dilute solutions or slurries (Pandey et al. 2001, 2004).
2. Solid substrate fermentation is generally defined as the growth of microorganisms on solid substrates or sometimes referred to as solid-state fermentation since the process taking place is in the absence or near-absence of *free* water in the system (Nigam and Singh 1994). The substrate however, must contain

enough moisture, which exists in absorbed form within the solid substrate matrix and simulates the fermentation reaction occurring in nature. These moist solid-substrates are insoluble in water and polymeric in nature, are a source of carbon and energy, vitamins, minerals, nutrients and also provide their absorbed water for microbial growth as well as anchorage.

3. Solid-state or solid-substrate fermentation means that the substrate is moistened, often with a thin layer of water on the surface of the particles, but there is not enough water present to make fluid mixture. Weight ratios of water to substrate in SSF are usually between 1:1 and 1:10.
4. SSF can be defined as a system with solid matrix particles, a liquid phase bound to them and a gaseous phase entrapped within the particles. The physical properties of this system such as the water potential and water holding capacity, (can be used as an index of aeration) and bulk density (which predicates the volume of pore space) help to define the conditions of solid-state fermentation.

10.3 Advantages of SSF Over Conventional Liquid Fermentation

Traditional SSF came about for two primary reasons:

1. The desire for more tasty food, as with Oriental fermented foods and mould-ripened cheese; and
2. The need to dispose of agricultural and farm waste materials (as in composting).

A closer examination of SSF processes in recent years in several research centres throughout the world has led to the realisation of its numerous economical and practical advantages (Lonsane et al. 1985; Steinkraus 1984). The attraction of SSF comes from its simplicity and its closeness to the natural way of life for many microorganisms. Since large amount of water are not added to the biological systems, fermenter volumes remain small, necessary manipulations become less expensive and the cost of water removal at the end of fermentation in minimised. This type of fermentation is especially suitable for growing mixed cultures of microorganisms where symbiosis stimulates better growth and productivity (Bushell and Slater 1981). Solid-state fermentations are clearly distinguished from submerged cultures by the fact that microbial colonisaton occur at or near the surfaces of solid substrate, or in few cases the soluble substrate supported on the solid insoluble-matrix in the environment of low-moisture contents. In contrast to liquid fermentation, the substrates traditionally fermented in the solid-state are renewable agricultural products, such as wheat, rice, millet, barley, corn and soybeans. The non-traditional substrates, which can be used in industrial process development, include an abundant availability of agricultural, forest and food-processing wastes.

¿From an engineering point of view, SSF offers many attractive features in comparison to conventional stirred tank reactors or aerated liquid medium fermentations because no free water is present, this leads to many benefits.

Solid-state fermentations can be used to provide low-shear environments for the cultivation of shear-sensitive mycelial organisms. Solid state cultivations can be and have been used for mass production of spores, which can than be used for the transformation of organic compounds such as steroids, antibiotics, fatty acids, and carbohydrates. Fungal spores have applications in the production of food-flavours and insecticides. The advantage of solid state fermentation includes simplicity, yields and the homogeneity of spore preparations. The expected advantages of SSF over submerged fermentations are:

a. Smaller fermenter volume, relative to the yield of the product, as there is no excess water taking space in the fermenter,
b. Lower sterilisation energy costs, as less volume of water needs to be heated,
c. Seed tanks are not necessary in all cases, as the spore inocula can be successfully used to inoculate the solid medium.
d. Easier aeration, as air can circulate easily and freely between the substrate particles, and also because the liquid film covering the substrate has a large surface area compared to its volume. Aeration is facilitated by spaces between substrate particles and particle mixing.
e. Reduced or eliminated capital and operating costs for stirring, since occasional stirring is sufficient.
f. Lower costs of product recovery and drying; in many cases the product is concentrated in the substrate and can be used directly e.g. Oriental foods and cheeses, or the products can be directly incorporated into animal feeds.
g. If the product is to be extracted from the substrate e.g. enzymes and other metabolites, then much less solvent is needed. The fermented solids may be extracted immediately by direct addition of solvents or maintained in frozen storage before extraction.
h. Reduced or eliminated capital and operating costs for effluent treatment due to lower water content in the system.

The other benefits are:

1. The media are relatively simple; a natural, as opposed to a synthetic, medium is used;
2. A more natural environment for microorganisms, e.g. agricultural wastes degrading organisms: many of these fungi grow and perform better under SSF than submerged conditions;
3. A less favourable environment for many bacteria, which require a high moisture level to survive, lowering the risk of contamination, therefore many SSF processes need no sterilisation;
4. SSF is adaptable to either continuous or batch process and the complexity of equipment is no greater than that required for submerged reactors.

Above described advantages are so attractive for the biological processing of agricultural by products that most of the work has used SSF process. These

advantages can outweigh the disadvantages of SSF, which are the slowness of fermentation and the difficulty of controlling the process precisely.

10.4 Performance Control of SSF Process

The difference in process control between SSF and SmF is mainly due to the use of solid substrates with a very low moisture content in system. The disadvantages of large-scale solid cultures are due to the problems of process-control, process scale-up and the major problem of heat build-up. Despite these drawbacks, large-scale SSF processes have been developed successfully in Japan for the manufacture of a variety of products, including fermented foods and food-products, enzymes, and organic acids. The drawbacks have been overcome by carrying these fermentations in stationary and rotary tray processes, where the temperature and humidity-controlled air is circulated through the stacked beds of fermenting solid substrate particles. These tray methods of cultivation have been used for centuries in the manufacture of traditional food products and the cultures experience the shear-sensitivity in some of these processes. These are main reasons of less frequent use of rotary drum-type fermenters.

Little information is available in the West on the details of modern control systems in large-scale solid-state cultivations. The control of temperature and humidity within practical limits is exercised through water temperatures, which is used to humidify the circulating air. The humidified air is circulated at flow-rates to meet the requirements of heat and mass transfer. The gas environment has been found to significantly affect the rate and extent of culture colonisation and product formation in SSF. In the commercial production of amylase using rice substrate in SSF, oxygen pressures above atmospheric have been found to significantly stimulate the enzyme productivity, suggesting oxygen limitation at normal atmospheric pressure. The DNA measurements revealed that this only caused a little effect on biomass formation, but the carbon dioxide pressures above 0.01 atm severely affected the process through the inhibition in amylase productivity.

In a protein production process by *Aspergillus* species using alfalfa residues, cellulase and pectinase activities have been found stimulated by oxygen and carbon dioxide pressures above atmospheric levels, and with no effect on biomass formation. These studies have been conducted in controlled gas environments at constant partial pressures, which is maintained by admitting pure oxygen on demand at pressures below a set point and purging carbon dioxide in 30% KOH at pressures above a set point in a closed aeration system. In another type of SSF performed for the degradation of natural birch lignin employing *Phanerochaete chrysosporium*, high oxygen pressures have been found to be stimulating, whereas the high carbon dioxide pressures have been found inhibiting the process. The stimulatory effect of oxygen on breakdown of lignins has been confirmed in laboratory studies by using labeled synthetic lignins and natural wood lignins.

Given the present state of the art, the most promising approach in solid state fermentation processes development happens to be the measurements and control of

various parameters and process variables, similarly as in any liquid fermentation. In SSF processes, various methods are selected to analyse the temperature, pH, humidity, oxygen and carbon dioxide concentrations in gas phases, biochemical analysis of fermented and unfermented solids and their extracts. The manufacturing productivities of some industrial scale submerged liquid fermentations have increased significantly over years, e.g. antibiotic production. This development has been possible due to applied and basic research in microbial-biochemistry, microbial-physiology, and genetics. To some extent the contribution also goes to engineering research based on concepts of stoichiometry, kinetics, thermodynamics, and heat and mass transfer in control of the microbial fermentation process and its environment.

Direct economic comparisons of solid-state and liquid-state fermentations are not possible, it is apparent that the large-scale solid-state fermentations (known as Koji in Orient) have been developed in Japan on an economic basis. Potential economic advantages of such processes to employ suitable microbe-substrate system include:

1. reduced thermal processing requirements, since many processes are not aseptic;
2. reduced energy requirements for agitation, since surface-to-volume ratios for gas transfer are high and many processes do not require agitation due to their shear-sensitivity;
3. high extracellular product concentrations, that can be efficiently recovered by superficial-extraction or leaching methods.

10.4.1 Performance Control by Particle Size of Agro Residues

SSF processes performance can be varied and controlled by changing physical and chemical factors. It has been reported that substrates with finer particles showed improved degradation due to an increase in surface area for enzymatic action (Moloney et al. 1984). The greater growth of fungal cultures has been found stimulated by smaller particle size substrates. Higher enzyme productivity in SSF has been achieved with substrates, which contained particles of mixed sizes from 180 μm to 1.4 mm.

Particles and kernels of grain must be of suitable size, but not be too small in order to avoid particle agglomeration. The particle size must be in a limited size range to be maintained at relatively low moisture content to prevent contamination. The smaller particle size provides a larger surface area which facilitates heat transfer and gas exchange. Smaller particle sizes also distribute equivalent moisture concentrations in thinner films on external surfaces exposed to the gas environments, given the same void volume fraction (porosity) and pore size distribution. Internal pores maintain the same surface-to-volume ratios with respect to solid surfaces, based on geometric considerations of spherical particles. This results in higher surface nutrient concentrations and the diffusion of nutrients takes place via shorter pathways at the surfaces as well as in the pores of those substrates which have same tortuosity.

Too small a particle size may result in closer packing densities of the substrates and the void space between particles becomes considerable reduced. The reduced space between particles tends to reduce the available area for heat transfer and

gas-exchange with the surrounding environment. If such condition arises, densely packed particles in a cultivation system have to be sufficiently agitated to provide a better separation of particles for the exchanges of gases and heat transfer. There may be a lower limit in particle size at which the heat transfer or gas exchange becomes rate limiting and there may be an upper limit at which the nutrient transfer becomes limiting. Conclusively under any condition, the particle size of the substrate to be used is one of the major variables in the SSF-process development. Various methods are available to obtain particle sizes such as milling, grinding, chopping and sieving to obtain substrates of particular particle-sizes. In the case of lignocellulosic substrates, smaller particle size substrate is usually obtained through ball-milling.

10.4.2 Performance Control by Medium Preparation of Agro-Residues

Some SSF systems do not require any nutritional supplements as do most of the traditional food fermentations. Medium supplementation is necessary in non-traditional SSF fermentations, as it induces enzyme-synthesis, provides balanced growth conditions for mycelial-colonisation and biomass formation, as well as prolonging the production of secondary metabolites. SSF employing brown-rot fungi, require an additional carbon source for the induction of enzymes for the cellulose-utilisation. Certain fungi including *Lentinus lapidus, Poria monticola*, and *Lezites trabea* can be cultivated on lignin-containing natural wood substrates from aspen, pine and spruce, when the SSF medium is supplemented with glucose or cellobiose in smaller quantities of 0.5%, w/v, and an even smaller amount of peptone, asparagine and yeast extract. In unsupplemented media, growth of these fungi was very slow as negligible. A co-metabolite, such as glucose or cellulose, stimulates the lignin-degrading system in white-rot fungi such as *Phaenerochaete chrysosporium* and *Coriolus versicolor* when these organisms are cultivated on spruce lignin. Other supplementations of cellobiose, mannose, xylose, glycerol or succinate have been found less effective.

Studies for the nutritional requirements for a developmental microbe-substrate system to be used on a large-scale SSF, can be done in preliminary experiments in small-scale liquid or SSF on laboratory scale. There is a procedure for evaluating the effects of nutritional supplements on culture-growth and product formation, in which microbial-cultures and the solid substrate are contained in separate compartments divided by a membrane with a molecular-weight-cut-off. The membrane permits the passage of enzymes and small molecular weight compounds but restricts microbial and substrate solids. One of the major difficulties in the development of solid state fermentations has been the problem in separating microbial biomass from the solid substrate particles after the mycelial growth has covered the substrate surfaces. In solid culture cultivation the microorganism and substrate are intimately associated making the analytical methods of limited value in stoichiometric analysis of SSF. The analysis of biomass yield and growth rate by the measurement of

glucosamine, protein, RNA, DNA, oxygen consumption, and carbon dioxide or heat evolution, can not be accurately used in samples of SSF.

Solid cultures for the production of secondary metabolites may have another problem in that the nutrient, whose deficiency triggers the pathway leading to formation of secondary metabolite, may be available in excess when the microbial growth becomes limited by other nutrient. Therefore, the selection of a solid substrate and required-supplements is more critical for a SSF process for antibiotic production that for a SSF designed for enzyme and organic acid biosynthesis.

10.4.3 Performance Control by Moisture Content of Agro Residues

Solid-state or solid-substrate fermentation means that the substrate is moistened, often with a thin layer of water on the surface of the particles, although there is not enough water present to make a fluid mixture. Weight ratios of water to substrate in SSF are usually between 1:1 and 1:10 (Reid 1989a, b). Since biological activity ceases below a moisture content of about 12%, this establishes the lower limit at which SSF can take place. The upper limit is a function of absorbency and hence, moisture content varies with the substrate material type.

Solid substrates may be viewed as gas-liquid-solid mixtures. The aqueous phase in such mixtures is intimately associated with solid surfaces in various states of sorption. The aqueous phase in a cultivation system is in contact with the gas phase continuous with the external gas environment. Different types of solid substrates can absorb different amounts of water. Depending on the moisture content of the solid; some of the water is tightly bound to solid surfaces, some amount of water is less tightly bound and remaining water may exist in a free state inside the capillary regions of the solid substrates. The gas-liquid interface provides a boundary for gaseous exchange between carbon dioxide and oxygen as well as for heat exchanges.

Water in biological materials exists in three states. The moisture isotherm measurements determines that the solids sorb or desorb water vapour in equilibrium with relative humidities in a gas phase (water activities), which can be maintained by saturated salt solutions at a constant temperature. Water is tightly bound to solid surfaces at the surface in a monolayer region. In case of agricultural residues, monolayer binding is generally 5 to 10 g per 100 g of dry solids. Beyond the surface monolayer in a multilayer region, water is less tightly bound in additional layers at progressively decreasing energy levels. Then beyond the multilayer region, free water exists in a region of capillary condensation. In terms of relationships between water activity and moisture content, the distinction between the multilayer and capillary regions is ambiguous. The electric measurements of an agricultural residue containing high starch content has been used to determine the dividing line between multilayer and capillary regions. The dividing line was defined by a moisture content of about 25 to 30% by weight at a water activity of 80 to 85%, which is the lower limit for microbial growth except for some halophilic or osmophilic microbes.

The sorption isotherm may vary from one type of product to another, the hysteresis is seen in sorption and desorption isotherms. Water may exist in free state at moisture levels of interest in solid state fermentation, which is in contrast with general perception about SSF that the free water does not exist in such systems. Moisture is a critical factor in SSF of aflatoxin production on rice; the yields of aflatoxins have been found decreasing rapidly at moistures above 40%. The rice particles become sticky at moistures above 30 to 35%. Moisture content plays an important role on the growth of lactic acid bacteria on feedlot wastes liquids mixed with cracked corn; growth and acid production was limited at moisture level less than 35%, whereas the higher level above 42% in SSF-mixtures caused the contents to become gummy and aggregate. One of the secrets of a successful SSF-process is to keep the fermenting substrate moist enough for fungal-growth and colonisation and to avoid higher moisture level not to promote the unwanted bacterial growth. Therefore, the optimum moisture content for a particular type of SSF for its microbe-substrate system should be determined for a particular end-product and cultivation conditions of that SSF.

The level of moisture content affects the process productivity significantly in any SSF system, when available in lower or higher quantities than the optimum value (Lonsane et al. 1985). Hence, it should be in limited and required amounts in system. The presence of an optimum moisture content in SSF medium has been emphasised also for the cultivation of bacterial cultures (Ramesh and Lonsane 1990). The process productivities are affected by water content because the physiochemical properties of the solids depend and vary with moisture available to them. Therefore, the major key factors determining the outcome of the SSF-process are the moisture content and the relative humidity levels (Lonsane et al. 1985).

Heat removal during fermentation is mostly achieved by evaporative cooling. This leads to an uneven distribution of water in system due to large quantities of water evaporation. Workers have practised various ways to maintain the moisture content of the solids (Lonsane et al. 1985; Ahmed et al. 1987).

10.4.3.1 Control of Water Activity Factor in SSF

Water activity of the substrate has been proposed as the condition of growth and viability of the microbes and hence, the importance of a_w in SSF has widely been studied (Nishio et al. 1979; Raimbault and Alazard 1980; Kim et al. 1985). Water activity is defined as the relative humidity of the gaseous atmosphere in equilibrium with the substrate and the water activity factor, a_w of the substrate quantitatively expresses the water requirement for microbial activity (Smith et al. 1985).

$a_w = -Vm\,\phi/55.5$ where,
V = number of ions formed,
m = Molar concentration of solute
ϕ = Molar osmotic coefficient, and
55.5 = molar concentration of a solution of pure water.

Pure water has an $a_w = 1.00$ and it will decrease with the presence of solutes.

The types of the microorganisms that can grow in SSF systems are determined by the water activity factor, a_w. Bacteria mainly grow at higher a_w values while filamentous fungi and some yeasts can grow at lower a_w values (0.6–0.7). The microorganisms capable of carrying out their metabolic activities at lower a_w values are suitable for SSF processes. High a_w favours sporulation in the course of growth in SSF, but low a_w favours spore germination and mycelial growth.

Numerous experiments have demonstrated the influence of a_w on microbial-metabolism (Gervais and Buttut 1988), such as, on growth rate and sporogenesis of filamentous fungi (Gervais et al. 1988), on enzyme biosynthesis by fungi (Grajek and Gervais 1987), and on cheese aroma production (Gervais et al. 1988).

The a_w of the medium is a fundamental parameter for mass transfer of the water and solutes across the cell membrane (Gervais and Sarrette 1990). The control of this parameter could be used to modify the metabolic production or excretion of a microorganism (Gervais 1989, 1990). A theoretical calculation based on the Ross equation showed that a_w decreased towards the end of the SSF-culture (Oriol 1988). A kinetic model which relates the rate constant of the death of the microbial cells to a_w and temperature has been proposed by Moser (1988), using the equation

$$k = k_\alpha \, a_w \, \exp -E_A \, a_w / RT$$

Constants k_α and E_A are calculated from the experimental value of a_w. Regulation of the a_w can be controlled by the relative humidity of the air. Gervais and Bazelin (1986) reported a SSF process allowing the control of a_w and Gervais (1989) developed a new sensor for the continuous a_w measurement in SSF.

10.5 Microorganisms Used For Agro-Residues Bioconversion

Selection of a suitable microorganism is one of the most important criteria in SSF. The vast majority of wild type microorganisms are incapable of producing commercially acceptable yields of the desired products. The unique characteristics of solid-state cultivations are their ability to provide a selective environment at lower concentrations of moisture ideal for mycelial organisms. The mycelial organisms are capable of producing a range of extracellular enzymes required for the hydrolysis of complex, polymeric solid substrates. Such microorganisms are able to colonise at high nutrient concentrations near solid surfaces. The mycelial organisms include a large number of filamentous fungi and a few bacteria of actinomycetes. The importance of microorganisms can be seen from the fact that a culture of *Aspergillus niger* can produce as many as 19 types of enzymes, while enzyme alpha amylase can be produced by some 28 different types of cultures (Fogarty and Kelly 1979; Pandey 1992). SSF processes can be placed in two main classes based on the type of microorganism involved:

1. *Natural (Indigenous) SSF*: Ensiling and composting are SSF processes, that utilise natural microflora. In nature, SSF is often carried out by mixed cultures in which several microorganisms show symbiotic cooperation.

2. *Pure culture SSF*: Known purified microorganisms are used in such processes either singly or in mixed culture. SSF using a pure culture is known since antiquity e.g. the Koji process with *Aspergillus oryzae*. A pure culture is necessary in industrial SSF process for improved rate of substrate utilisation and controlled product formation. A typical example of pure mixed culture SSF is the bioconversion of agricultural residues to fungal biomass (protein) using two pure cultures of *Chaetomium cellulolyticum* and *Candida utilis*.

Several microorganisms have been employed in a wide range of SSF processes for various objectives. The cultivation of filamentous fungi on solid substrates has been widely used for different purposes at laboratory scale e.g. for Koji fermentation, for lignocellulose fermentation (Matteau and Bone 1980), for fungal spores (Lotong and Suwarnarit 1983), and for mycotoxin production (Hesseltine 1972; Bhumiratna et al. 1980). For various purposes, among the filamentous fungi three classes, viz. Phycomycetes (*Mucor* and *Rhizopus*), Ascomycetes (*Aspergillus* and *Penicillium*) and Basidiomycetes (Nigam and Prabhu 1985), have been most widely used.

SSF has been most commonly used mploying *Aspergillus niger* for protein enrichment (Rodriquez et al. 1985; Baldensperger et al. 1985; Czajkowska and Ilnicka 1988) as well as for enzymes production, such as, cellulase (Singh et al. 1989; Madamwar et al. 1989), amylase, glucoamylase (Ramakrishna et al. 1982; Pandey 1990), beta glucosidase, and protease (Malathi and Chakrabarty 1991). Production of alcohols, ketones and aldehyde in rice fermentation was achieved by the use of *A. oryzae* (Ito et al. 1990). For protein enrichment and kinetic studies related to SSF process *Rhizopus oligosporus* has been employed (Rathbun and Shuler 1983; Mitchell et al. 1988, 1990).

Fungal rennet has been produced by *R. oligosporus* and *Mucor meihei* (Karanth 1988). For enzyme production and protein enrichment cultures of *Trichoderma* spp. have been employed in pure, single and mixed SSF (Daubresses et al. 1987; Grajek 1988). Lipase enzyme production has been reported (Munoz et al. 1991) using six species of *Penicillium*, two species of *Rhizopus, Geotrichum candidum* and *Mucor meihei*, whereas the maximum lipase activity was obtained with *P. candidum, P. camembertii* and *M. meihei*. For the production of several other enzymes e.g. hydrolases and pectic enzymes (Siesser and Said 1989) several other species of *Penicillium* have been employed in SSF.

Production of the antibiotic penicillin was achieved in a non-sterile SSF process on sugar cane bagasse impregnated with culture medium using *Penicillium chrysogenum*. Protein enrichment of lignocellulosic substrates for animal feed production (Nigam 1990; Nigam and Vogel 1990a, b), lignin degradation (Bone and Munoz 1984), and cellulase and ligninase enzyme production (Nigam et al. 1987a, b) have been obtained by white-rot cultures in SSF.

Production of gibberellic acid has been reported using *Fusarium monoliforme* and *Gibberella fugikuroi* (Kumar and Lonsane 1987a, b). Bacterial alpha amylase production is reported using *Bacillus licheniformis* in SSF (Ramesh and Lonsane 1987, 1990). Several yeasts have been used for protein enrichment and ethanol

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fermentation in SSF. For protein enrichment of straw (Han 1987) *Candida utilis* was used whereas *Saccharomyces cerevisiae* has most commonly been employed for ethanol production (Gibbons et al. 1984; Kargi et al. 1985).

10.6 Designing And Types of SSF

10.6.1 Fermenter Design for SSF

Several miscellaneous types of fermenters have been used in batch or continuous mode in SSF processes (Hardin 2004). Process parameters are very important factors and they have to be considered in a bioreactor design for any SSF. Design considerations in types of SS-fermenters used by various researchers are described by Aidoo et al. (1982). The engineering aspects, with major types of fermenters describing their advantages and drawbacks has been reviewed by Fernandez et al. (2004). Solid state cultivations are not as well characterised on a fundamental scientific or engineering basis, as are the liquid fermentation systems that are used in the West for the industrial production of microbial-metabolites. Solid-state fermentations are, however, widely used in the Orient and therefore, the old traditional methods of cultivation systems which have been used in food-processing for more than 2,000 years, have now been modernised and well characterised for their extended application to non-traditional products. Mitchell et al. (2004) have described in detail the modelling aspects of SSF.

The physical state of the substrate and the products to be produced in the system characterise the design-type of solid state cultivation process:

a. Low-moisture solids are fermented

   1. without any agitation for the production of Tempeh and Natto;
   2. by occasional stirring for the production of Miso and Soy sauce;
   3. with continuous stirring for the production of Aflatoxin.

b. Suspended solids are fermented in packed bed columns

   1. through which the liquid is circulated, as for the production of rice-wine;
   2. which contain stationary or agitated liquid media, for the production of Kaffir beer.

10.6.2 Types of SSF Systems

There are two types based on process design:

Type one- Fermentation in static reactor
e.g. Tray fermentations (Lonsane et al. 1985; Viesturs et al. 1987)

Type two- Fermentation with occasional or continuous agitation
e.g. Production of aflatoxin, ochtratoxin and enzymes (Lindenfelser and Ciegler 1975; Hesseltine 1977; Silman 1980).

Type two has 4 variations according to the need of process:

1. Occasional agitation, without forced aeration
2. Slow continuous agitation, without forced aeration
3. Occasional agitation with forced aeration
4. Continuous agitation with forced aeration.

10.6.3 SSF Bioreactors

Three basic groups of reactor exist for SSF, and these may be distinguished by the type of mixing and aeration used. In laboratory scale, SSF occurs mainly in flasks whereas following reactors are used for large-scale product-formation.

10.6.3.1 Tray Bioreactors

Tray bioreactors tend to be very simple in design, with no forced aeration or mixing of the solid substrate. Such reactors are restrictive in the amount of substrate that can be fermented, as only thin layers can be used, so as to avoid overheating and maintain aerobic conditions. Tray undersides are perforated to allow aeration of the solid substrate, each arranged above each other. In such reactors, temperature and relative humidity are the only controllable external parameters (Durand 1998). Wooden trays were initially used for soy sauce production in Koji fermentations by *Aspergillus oryzae*. The use of tray fermenters in large-scale production is limited as they require a large operational area and tend to be labour intensive. The lack of adaptability of this type of fermenter makes it an unattractive design for any large-scale production.

10.6.3.2 Drum Bioreactors

Drum bioreactors are designed to allow adequate aeration and mixing of the solid, whilst limiting the damage to the inoculum or product. As previously mentioned, mixing and aeration of the medium has been explored in two ways: by rotating the entire vessel or through the use of various agitation devices. Rotation or the use of agitation can be carried out on a continuous or periodic basis. In contrast to tray reactors, growth of the inoculum in drum bioreactors is considered to be better and more uniform. Increased sheer forces through mixing, can however, have a detrimental affect on the ultimate product yield.

Although the mass heat transfer, aeration and mixing of the substrate is increased, damage to inoculum and heat build up through sheer forces may affect the final product yield. Application of drum reactors for large-scale fermentations also poses handling difficulties.

10.6.3.3 Packed Bed Bioreactors

Columns are usually constructed from glass or plastic with the solid substrate supported on a perforated base through which forced aeration is applied. They have been successfully used for the production of enzymes, organic acids and secondary metabolites. Forced aeration is generally applied at the bottom of the column, with the humidity of the air kept high to avoid desiccation of the substrate. Disadvantages associated with packed bed column bioreactors for SSF include difficulties in retrieving the product, non-uniform growth, poor heat removal and scale-up problems.

10.7 Scale-Up Stages of SSF

Scale-up of SSF has been defined in many ways. There are mainly four stages:

10.7.1 Flask Level

This is smallest scale using 50–1000 g substrate working capacity, and used for the selection of the organism, optimisation of the process and experimental variables in a short time and at low cost. The vessels used are conical flasks and beakers (Mitchell et al. 1986; Nigam et al. 1987a, b), Roux bottles (Gervais et al. 1988; Nigam 1990), jars (Hang et al. 1986), and glass tubes (Raimbault and Alazard 1980).

10.7.2 Laboratory Fermenter Level

This is next to flask scale using a 5–20 kg substrate working capacity. It is used for a selection of procedures such as, inoculum development, medium sterilisation, aeration, agitation and downstream processing. Standardisation of various parameters, selection of control strategies and instruments, evaluation of economics of the process and its commercial feasibility are also examined at this level. The fermenters used are glass incubators (Deschamps and Huet 1984; Oriol et al. 1988; Smith et al. 1986), column fermenters (Oriol et al. 1988); polypropylene bags (Yadav 1988), and miscellaneous types of fermenters (Raimbault and Alazard 1980; Viesturs et al. 1981).

10.7.3 Pilot Fermenter Level

This scale is a stage before the commercial scale using 50–5000 kg of substrate. This level is necessary for the confirmation of laboratory data and selection of optimised procedures. It facilitates market trials of the product, physicochemical characterisation and determination of viability of the process. Most large scale SSFs employ tray type fermenters as in the oldest soy sauce Koji process (Daubresse et al. 1987), rotating drum type (Lindenfelser and Ciegler 1975; Han and Anderson 1975; Hesseltine 1977), horizontal paddle fermenters and mixed layer

pilot plant fermenters (Laukevics et al. 1984). Durand and Chereau (1988) reported the use of a pilot reactor having a one ton working capacity.

10.7.4 Production Fermenter Level

The commercial scale fermenter utilises 25–1000 tonnes of substrate and is performed for streamlining of the developed process. Yokotsuka (1985) described deep trough methods and mechanical continuous equipment for Koji production generating 50–100 tonnes of Koji per day.

10.8 Factors Affecting SSF

Each microbe-substrate system is unique and the process variables must be considered in terms of the physical properties and chemical composition of its substrate, growth characteristics and physiological properties of the microorganisms to be cultivated in SSF. The nature of the product, if the process involves the synthesis of primary or secondary metabolite may be based on the synthesis of extracellular enzymes in growth-associated metabolism. The process variables affecting a solid state cultivation include, pretreatment of substrates, particle-size of substrates, medium-ingredients, supplementation of growth medium, sterilisation of SSF-medium, moisture-content, inoculum-density, temperature, pH, agitation and aeration. These variables should be considered in process-development of a SSF to be carried out for different purposes. Some of these variables have been discussed in some sections as above, the rest are discussed below.

10.8.1 Significance of Aeration and Mixing in SSF

In any SSF-process an adequate supply of oxygen is required to maintain the aerobic conditions and for the transfer of excess carbon dioxide produced during metabolism. This requirement can be achieved through the process of aeration and mixing of the fermenting solids. In certain cases, the mixture can not be agitated vigorously or in some cases, at all, if the microorganism used in SSF is shear sensitive. The shear sensitivity is attributed to disruption of mycelial-substrate contact; this is particularly concerned to those organisms which possess mycelial-bound enzymes required for the hydrolysis of solid substrate-polymers. Most Koji processes in Japan performed for the commercial production of enzymes do not involve great agitation. The fermenting substrate is gently turned periodically just to bring the bottom of Koji to the top. These processes have been developed in highly controlled environments, using automated systems for inoculum mixing, and turning of the fermenting substrate.

Most of the traditional food-fermentation in Japan use the rotary-tray method for SSF with the circulation of humidified air to create the conditions suitable for gas-exchange and heat-transfer. In the SSF for the production of certain secondary

metabolites such as aflatoxin and ochratoxin, and in some processes for the enzyme production, mixing and particle separation are achieved by agitation on shakers or in rotating vessels with circulating conditioned air. Maximum rotation rates generally decrease with the size of the fermentation-vessel. Therefore, solid-state fermentations are ideal for the cultivation of those microorganisms that are extremely sensitive to the shear rates of the impeller speeds required for stringent oxygen demand rates in liquid fermentaton. Such microorganisms colonise the solid substrates by microbe-substrate attachment and there is no pellet formation in solid-state cultivation, which is added advantage to SSF.

Aeration plays an important role in solid state fermentations as compared to liquid fermentation where it only helps in gas transfer. Aeration facilitates in heat, gas and moisture transfer between the fermenting solid particles and the gas environment of the system. The temperature of the gas phase serves by supplying or removing heat, in maintaining the relative humidity in equilibrium with the liquid phase. In liquid fermentations the substrates are dissolved in at low substrate concentrations in large volumes of fluid, but in solid cultures with respect to moisture transfer, the loss or gain of moisture during SSF is extremely sensitive to the water activity of the gas-phase. Therefore, small changes in the relative humidity of the gas phase in equilibrium with the solids may cause the large changes of moisture content in the solid state, depending on the sorption-desorption characteristics of the solid substrate.

There are two main functions of the gas phase in SSF, the primary function is to supply oxygen and remove the carbon dioxide from the system. The secondary function of aeration is in heat and moisture transfer that is more important, when the rates of oxygen and carbon dioxide are not limiting. The gas phase can facilitate in the ontrol of solid cultures, due to the fact that direct measurements can not be performed to estimate dissolved oxygen or carbon dioxide concentrations in low-moisture solids during the course of the fermentation on either a continuous or sampling basis. The methods of aeration may cause the conditions of gas transfer being relatively stagnant. This condition may be responsible for the oxygen limitation at small penetration depths or may lead to inhibitory carbon dioxide concentrations in normal atmospheric environments. The gas phase in the SSF during the course of microbial metabolism, can be analysed for oxygen, and carbon dioxide pressures using analysers which function on thermal-conductivity, paramagnetism, or infrared absorption. The technique of gas chromatography can also be used for gas-analysis of the gas phase of a SSF.

10.8.2 Significance of Control of Temperature and pH in SSF

Two significant variables affecting any SSF are the incubation temperature and the pH of SSF-medium. Both variables are specific for each SSF process depending on the microoganisms to be cultivated and the product to be formed. Unlike submerged fermentation, these factors are difficult to control in SSF. These variables can not be directly measured in the liquid phase, as these are associated with the solids at

lower moisture content without any free liquid in the fermenting medium. The other difficult situation arises when the growth temperature of cultivated microorganism is different than the optimal temperature for the product formation. Such systems require a possible need for temperature profiling or shift in the later stages of fermentation. The thermal gradients may be induced within SSF-mixture due to the rate of heat generation in SSF-system at high levels of biological activity. This gradient may limit the heat transfer and may lead to sub-optimal conditions for microbial-biomass and product formation.

The local pH levels at solid surfaces near which the biological activity occurs, may be considerable different than the bulk pH of the liquid phase. This difference in pH levels happens due to surface charge effects and ionic equilibria modified by solute transport effects. There is no suitable method to measure the precise pH of fermenting solid residues in SSF. A general method used for measuring pH of solid agricultural residues involves mixing one part of fermented solids (dry weight) and three parts of freshly boiled and cooled water, and measuring the pH of the resultant liquid after five minutes using a glass electrode. This procedure can be used to monitor pH changes during fermentation on intervals using minimum one gram of the SSF-mixture.

It is easier to measure temperature of the fermenting SSF-mixture, in comparison to pH measurement. Temperature can be measured using thermistor or thermocouple probes at various depths of the SSF-mixture below the medium-surface. In various SSF-processes for the production of enzymes, mycelial-biomass or organic acids, total heat generation of up to 600 kcal per kilogram of fermenting solids has been observed. A study of composting of animal wastes and agricultural residue has revealed that such heat generations may lead to rapid temperature rise of the fermenting mass in the system limited by heat transfer. The study also revealed that the biological activity was considerably higher near the surface of the compost pile than in the depth of pile that was at lower oxygen pressure. This phenomenon happens due to a decrease in interior oxygen concentrations inside the SSF-mixture pile of compost. Thus the heat generation in such fermentations is coupled to conditions for heat as well as mass transfer.

10.9 Processes and Products of SSF

Various processes and products from bioconversion of agro-residues of industrial, pharmaceutical, and environmental importance have been discussed in detail in further chapters 11–24 under sections II, III.

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