Solid-state Fermentation: An Overview

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Review
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Solid-state fermentation (SSF) is defined as the growth of microbes without free flowing aqueous phase. The SSF is alternative to submerged fermentation for production of value added products like antibiotics, single cell protein, Poly unsaturated fatty acids, enzymes, organic acids, biopesticides, biofuel and aroma production. However, the advantages of SSF in various processes are found to be greater than in submerged fermentation. This paper reviews the advantages of solid-state fermentation over submerged in production of different value added products, important features of various bioreactor designs, recent developments in utilization of various agro-industrial residues as substrates and the importance of mathematical modeling. With advances through modeling and optimization techniques, production-using SSF is advantageous and appropriate for production of many value added products like enzymes, antibiotics, and organic acids. This technique not only decreases the cost of the process but also makes product cheaper for consumers.

Key words:
Solid-state fermentation, submerged, aroma, biocontrol, biofuel, antibiotics, aroma, bioreactors, substrates, enzymes, acids, biochemical engineering, modeling, PUFA, exopolysaccharides

Introduction

Solid-state fermentation (SSF) is defined as the fermentation process in which microorganisms grow on solid materials without the presence of free liquid.[1] The concept of using solid substrates is probably the oldest method used by man to make microorganisms work for him. In recent years, SSF has shown much promise in development of several bioprocesses and products. However, SSF has also some disadvantages. There are some processes in which solid-state fermentation cannot be used as in bacterial fermentation.

Solid-state offers greatest possibilities when fungi are used. Unlike other microorganisms, fungi typically grow in nature on solid substrates such as pieces of wood, seeds, stems, roots and dried parts of animals such as skin, bones and fecal matter i.e. low in moisture.[2] In SSF, the moisture necessary for microbial growth exists in an absorbed state or in complex with solid matrix. However, SSF differs from solid substrate fermentation. In solid substrate fermentation, the substrate itself acts as a carbon source and occurs in absence or near absence of free water. However, in solid-state fermentation, the process occurs in absence or near absence of free water by employing a natural substrate or inert substrate as solid support.[3] The aim of SSF is to bring cultivated fungi or bacteria in tight contact with the insoluble substrate and to achieve the highest nutrient concentration from the substrate for fermentation. This technology so far is run only on a small scale, but has an advantage over submerged fermentation.

Two types of SSF systems have been distinguished depending on the type of solid phase used. The most commonly used system involves cultivation on a natural material and less frequently on an inert support impregnated with liquid medium.[4] Solid-state fermentation processes can also be classified based on whether the seed culture for fermentation is pure or mixed. In pure culture SSF, individual strains are used for substrate utilization and with mixed culture, different microorganisms are utilized for the bioconversion of agro-industrial residues simultaneously.

Biochemical engineering aspects of solid-state fermentation

A considerable amount of work has been done in recent years to understand the engineering aspects of solid-state fermentation.

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Moisture and water activity in SSF

The concept of water availability in substrate becomes important. Water activity, A_w is defined as the ratio of vapor pressure of an aqueous solution to that of pure water at the same temperature.[5] Scott in 1953 introduced to microbiology the concept of water activity (A_w), which is a different way of describing the water relations in medium from the term "water content". In solid-state, the low moisture within the substrate limits the growth and metabolism of microorganisms when compared to submerged fermentation. A_w is a very useful parameter for measuring water potential, characterizing the energetic state of water.[6]

Water relations in SSF have been studied under quantitative aspects.[7,8,9] Water activity of substrates has a strong influence on microbial activity. A_w determines the type of organisms, that can grow in SSF. A_w of the medium has been attributed as a fundamental parameter for mass transfer of water across the microbial cells. The control of this parameter could be used to modify microbial metabolic production and its excretion.[10]

Grevais and Molin studied the effect of water in solid culture medium on fungal physiology such as cellular mechanisms, radial growth rate and orientation of fungus. They observed that the radial extension rate of mycelia related to the water activity value. The optimum water activity was found to be 0.99 for *T. viride*, and below 0.90 no fungal development occurred, for *Penicillum roquefortii* the optimal water activity was 0.97.[11] Molin *et al.* described the effect of water activity on hyphal and radial growth.[12] Moreover, hyphal growth rate was always greater than radial growth rate, showing that hyphae do not simply extend in radial directions.

Fungal spore production is also influenced by water activity. Maximum sporulation value of *Penicillum roquefortii* was obtained at water activity of 0.96 i.e. at lower water activity maximum sporulation occurs. The influence of low water activity value on fungal fermentation is not well understood; at low water activity there is loss of essential nutrients necessary for fungal growth.[13,14]

Production of secondary metabolites also depends on water activity. It was found that there is a direct relation between the amounts of enzyme produced vs. A_w. In a solid culture medium of *T. viride* biosynthesis of polygalactouronase, D-xylanase and β- galactosidase is affected by water activity of substrate. It was found that maximum polygalactouronase and D-xylanase production occur at A_w of 0.99. However, β-galactosidase formation was optimum between A_w of 0.96 and 0.98.[15] Lipase production by *Candida rugosa* was enhanced by mixed solid substrate with initial A_w value of 0.92.[16] A_w also effects the fungal aroma production, bioconversion of 2-heptanone from octanoic acid by *T. viride* was optimum at water activity value of 0.96.[11]

Moreover, higher A_w also enhanced substrate conversion to fungal biomass as reduced water activity causes lower mass transfer and little water availability for microorganisms. This results in spore production. Increase in A_w of substrate increases specific growth rate and spore germination time of the fungus.[5]

Grajek and Grevais reported that decrease of A_w by 0.01 (equivalent to 1 % of relative air humidity) causes reduction in biomass production and protein content of culture medium.[17] Narahara found that optimum A_w for *Aspergillus* spp. was between 0.970 and 0.990.[18] Fungus was unable to grow below A_w of 0.97. These data prove that fungal growth and their secondary metabolite production during SSF are strongly affected by A_w of substrate.[11,17,18]

Temperature and heat transfer

Fungal growth and secondary metabolite production in SSF are greatly influenced by temperature and heat transfer processes in the substrate bed. During SSF a large amount of heat is generated, which is proportional to the metabolic activities of the microorganism. However, fungus can grow over a wide range of temperatures from range of ϑ = 20 °C to 55 °C. Nevertheless, optimum temperature for fungal growth could be different from that required for product formation.[19] The substrates used for SSF have low thermal conductivities that decrease heat removal and increase its accumulation. Therefore, the key issues in SSF are heat removal, and hence most studies are focused on maximizing heat removal.

The heat transfer in or out of an SSF system is closely associated with microbial metabolic activity and aeration of the fermentation system. High temperature effects fungal germination, metabolites formation and sporulation.[20] The effect of temperature on kinetic parameters of *T. ressei* QM9414 have been estimated by measuring radial growth of fungus, its glucosamine content and specific respiration activity using wheat bran as substrate. The fungal activity declined exponentially when optimum temperature for growth reached above maximum.[21]

Mitchell and Meien used modeling experiments to study the potential of *Zymotis* packed bed bioreactor. The rate of cooling was higher at the top of the bed as compared to the bottom. Vertical temperature gradients were decreased. Bioreactor performance was estimated by determining the time required for volume average biomass concentration to

reach 90 % of maximum biomass concentration. The highest biomass concentration was above the bottom of the bioreactor where inlet air temperature is lower than optimum temperature. The biomass near the top was less than maximum biomass indicating unfavorable temperature.[22]

Coupled control of temperature and moisture is also an important issue for consideration. The low moisture and poor thermal conductivity of the substrate makes heat transfer and temperature control difficult in SSF. Minimizing the substrate bed height can solve heat transfer problems, but it is possible only in small-scale solid-state fermentation. Good mixing of substrate with sparged oxygen can also solve heat transfer problems. Moreover mixing not only aids in the homogeneity of the bed but also ensures an effective heat and mass transfer. Continuous mixing along with addition of water is advantageous for simultaneous control of temperature and moisture in large-scale SSF.[20] Adjusting the aeration rate controls temperature during evaporative cooling in SSF. If the temperature is too low, the aeration rate decreases, which increases temperature in the bed due to respiration of the microorganism. But if temperature is too high, increasing the aeration rate promotes cooling of the substrate.[23,24,25] However, with larger size particles or by forming aggregates of available agro-industrial residues, such as in the case of wheat bran, heat removal can be achieved with forced cold aeration, as larger particles have greater space between them.

Biomass and growth kinetics

Biomass is a fundamental parameter for characterizing the fungal growth and is essential for measuring growth kinetics in SSF. However, culturing fungus over membrane filter can perform direct estimation of biomass. A disadvantage of this method is that the membrane filter prevents penetration of fungal hyphae within the substrate.[26] Fungal biomass in an SSF system can be estimated by different techniques, like scanning electron microscope, reacting fungal biomass with specific flourochrome probes, respiration rate of organism, and reflectance infrared (IR) spectroscopy.[27,28,29]

Determination of fungal biomass in SSF can also be done through some biochemical methods. Changes in three fungal constituents such as Glucosamine, Ergosterol and total sugar represents the effect on fungal biomass. Among these, Glucosamine is considered a good biomass indicator if the fermentation media has the same constituents but not the same carbon-to-nitrogen ratio. The amount of Ergosterol varies throughout development of fungus with different medium composition and appears only when the fungi sporulate. Hence, it does not allow estimation of mycelial growth but it is a good indicator for sporulation. In conclusion, Glucosamine is the most reliable measure for mycelial biomass determination but it can only be used for optimization studies of nutrient concentration. Ergosterol is, however, an unreliable measure of biomass but when determined along with Glucosamine gives useful information regarding spore amount and fungal sporulation ability. Estimation of total sugar is not a good biomass indicator because it depends on fungus age and medium composition. The disadvantage of these manual biochemical methods are that they are time consuming, and require lengthy analytical procedures.[29]

Direct methods such as measurement of CO_2 or O_2 consumed are most powerful when coupled with the use of a correlation model, which correlates biomass with a measurable parameter.[30] Desgranges *et al*. estimated the biomass in solid-state fermentation by Infrared measurement of cell components such as Glucosamine, Ergosterol, medium residues (sucrose, nitrogen) and CO_2 evolution rate. Glucosamine estimation is not used when medium contains insoluble nitrogen. When manual methods for biochemical estimations are not possible, the CO_2 production rate and IR measurement are more beneficial. In SSF system CO_2 production correlates well with other parameters. Moreover, it can indicate even low physiological activity since the method is very sensitive.[31]

Mass transfer

In SSF the fungal hyphae forms a mat on the substrate surface and penetrates by secreting secondary metabolites and enzymes.[32,33] Interparticle concentration gradients due to nutrient consumption in combination with mass transfer limitations can have a strong effect on the rate and efficiency of the process.[34,35,36] Mass transfer in SSF involves micro-scale and macro-scale phenomenon. Micro-scale mass transfer depends on the growth of microorganisms which depends on inter and intra particle O_2 and CO_2 diffusion, enzyme, nutrient absorption and metabolites formation. Macro-scale mass transfer includes airflow into and out of the SSF system, types of substrate, mixing of substrate, bioreactor design, space between particles, variation in particle size and microorganisms within the SSF system.[20,37,38]

Micro-scale mass transfer

Limitation of O_2 affects the aerobic SSF performance. In SSF fungal mycelia develops on a solid surface and in a void area within the substrate. Fungal mycelia are filled with water and oxygen in the space between substrate.[39] Oxygen consumption takes place at interface between the substrate parti-

cles and fungal hyphae. Several researchers have modeled O_2 transport between the water and fungal hyphae by considering fungal hyphae as a biofilm of unicellular organisms. Mitchell *et al*. studied intra-particle oxygen transfer of *Rhizopus oligosporus* in SSF conditions on a nutritionally defined medium and reported that O_2 transfer depends on the interfacial gas-liquid surface area and thickness of the wet fungal layer. These two factors play an important role for O_2 transfer in the SSF system. Similar to O_2 transfer, CO_2 diffusion takes place from fungal hyphae through the void area of the solid substrate.[32]

Apart from O_2, CO_2 diffusion micro-scale mass transfer includes transfer of nutrients, enzymes, secondary metabolites and different metabolic products. Enzymes secreted from the fungal hyphae act upon the complex organic substrate converting it into simplified carbon sources, which are again utilized by fungus. Therefore, in SSF there is mass transfer of enzymes from fungus to substrate and transfer of nutrients from substrate to fungus. When *Rhizopus oryzae* is cultured on a complex tannin substrate, the secreted tannase breaks the substrate into glucose and gallic acid, and the glucose is utilized by the fungus as a carbon source.[37]

Macro-scale mass transfer

Macro-scale mass transfer is directly influenced by type and design of the bioreactors used in SSF: transfer rate of O_2, CO_2 into and out of the bioreactor, transfer of water in the bioreactor, mixing speed of solid substrate, water and microorganisms, thickness of bioreactor wall, and temperature of the cooling agent in the bioreactor. The optimum design and suitable selection of a bioreactor is required for maximum mass transfer process.[20] However, a particle simulation model for optimizing better design of rotating drum bioreactor was developed by Schutyser *et al*. and particle simulation models provide better details about the transport process and optimizes high mass transfer.[40]

Modeling

Mathematical modeling in the SSF system is an important tool for optimum design and operation of bioreactors. The cost procured in predicting fermentation conditions at larger scale eases with modeling. Models in bioreactor can be of two types: Kinetic models and Transport models. A kinetic model describes how the microorganisms are influenced by different process parameters, while the transport model describes the mass and heat transfer within the bioreactor SSF systems. Kinetic modeling depends on particle size, packing density, respiration rate, pore size of substrate particles, depth of fungal mycelium penetration within the substrate, and water content of the substrate bed. Transport modeling depends on the growth rate of fungus, rate of airflow through the bed, width of bioreactor walls, height of the bed, and rate of the heat removal process. Moreover, mathematical models help in finding the relationship between O_2 and the substrate consumption rate, substrate consumption rate and biomass synthesis rate, oxygen uptake rate and biomass synthesis rate, heat evolved and its relation with the biomass synthesis rate.

Modeling and simulation of biological processes provide a basis for economic and ecological evaluation, which enables integrated optimization of the processes. This helps in prediction of the process at larger scale. Ecological assessments are based on material and energy balance of the process to identify the most relevant materials and process steps.[41]

Microbial growth kinetics along with operating variables such as moisture, temperature, product accumulation, heat and wastes accumulation play an important role in continuous SSF processes. Lagemaat and Pyle, attempted to develop an unstructured growth model, to form a basis of continuous tannase production. The model described the uptake and growth kinetics of *Penicillum glabrum* on inert impregnated polyurethane foam. The time delay between biomass production and tannase and spore formation was described using logistic kinetics, since tannase is not produced after stationary phases.[42]

Determination of biomass production through fungal indicators such as Glucosamine, Ergosterol is time-consuming but using sensitive methods like determining respiration saves time and improves accuracy of the process. A kinetic model for monitoring bed conditions in SSF was developed using respiratory gases such as O_2, CO_2. The model was calibrated using *Gibberella fujikoroi* on wheat bran starch in an agitated aseptic SSF bioreactor. The various assumptions were divided into 4 groups: kinetic, evaporation, energy balance, and water balance modules. The CO_2 production rate is a direct indicator for biomass growth. First CO_2 production rate, water content and bed temperature were obtained through water and energy balance models, but due to the complexity of the process these estimations were considered to result in significant errors. Determination of the CO_2 production rate through kinetic models reduced the chances of errors and noise production. Simulations in single step were enabled with help of kinetic models.[43]

A mathematical growth model for converting the absolute mass ratio to relative mass ratio was

developed using logistic equation kinetics. However, by growing *Rhizopus oligosporus* on cassava and gel solid based substrates validated the model. The model was used for determining indirect measurements of growth such as protein estimations.[44] The curvature in graphs between biomass and microbial growth kinetics represents the dependence of biomass on the length of the growth phase. It is better to study biomass produced in lag phase through logistic equations. Logistic equations explain final limitations of biomass production in the stationary phase.[45] Since, data interval particularly representing growth kinetics is required for fitting an equation; hence, interpretation of growth kinetics is important for developing an appropriate bioreactor vessel. Logistic equations are simple and give an approximation of the whole growth curve including the lag phase and stoppage of growth in other stages of fermentation.[46] These logistic equations fail to provide complete change in biomass and production of secondary metabolites during the death phase.[47]

Mitchell *et al.* have reviewed the use of transport modeling optimum operation of bioreactors. Mathematical modeling provides easier way of optimization among different operating variables for maximum productivity. Heat and mass transfer in tray bioreactors is limited due to intraparticle oxygen diffusion, growth rate of fungus and lack of heat exchange processes. Many differential and algebraic equations are used for correlating variables like bed height with oxygen, diffusion in particles and increase in temperature. These studies influence the removal of heat and enhance production of important metabolic compounds. Scale-up in tray bioreactors can be achieved by increasing the number of trays. Oxygen limitation is absent in packed bed reactors due to forced aeration. Packed bed bioreactors are affected by superficial velocity of air and heat removal through convective, evaporative cooling. Decrease in water content of substrate bed affects mass transfer within the bed, causing shrinkage of the bed. The shrinkage directly affects growth kinetics of the inoculated organism. Axial temperature gradients are unavoidable in packed bed due to unidirectional flow of air. The *Zymotis* packed bed reactor minimizes axial temperature gradients due to vertical arrangement of cooling plates. Heat removal through rotating drum bioreactors can be controlled by regulating rotational speed. Mass transfer in such bioreactors depends on particle size of substrates, growth rate of fungal mycelia, rate of inlet air, and bed height. Rate of inlet airflow and particle size have a significant effect on heat and mass transfer in bioreactors with forced aeration such as fluidized bed reactors. These factors directly influence the conductive cooling through the bioreactor walls and exchange of heat between the solid and gaseous phase.[48] Recently, various other reviews stressed the use of micro-scale mathematical modeling in SSF for improving bioreactor performance and providing a better yield of product with improved characteristics.[46,49]

One of the major problems in SSF bioreactors is heat accumulation. Changing design of bioreactors through mathematical modeling can solve the problem, which affects cooling rate by bringing thermodynamic changes in the reactor. Oostra *et al*. evaluated the applicability of mixed and non-mixed systems for producing spores of bio-control agent *Coniothyrium minitans*. Conductive cooling through the walls of the non-mixed reactor was evaluated by simple mathematical models based on parameters such as thermal conductivity of bed and metabolic heat production from fungus (calculated from O_2 consumption rate). Increase in bed diameter with decrease in bed height enhanced the temperature of the core, which decreased the radial growth rate and relative activity of fungus. Hence, this showed requirement of additional evaporative cooling. Additional simulations also showed that large volumes could be cooled via conductive cooling through the walls at low mixing intensities and small temperature driving forces. The effect of mixing depends on the fungal hyphae used in the experiment. Experimental studies showed no detrimental effect of mixing on spore production by *C. minitans*. The spore production yield in a continuously mixed scraped-drum reactor (n_s = 0.2 min^{-1}) was $5 \cdot 10^{12}$ spores per kg of dry oats after t = 450 h.[50]

Heat removal is an important aspect of packed bed reactor. Packed bed reactors are suitable for static SSF processes. Modeling in packed bed systems has been done to study the factors for heat removal. Packed bed systems have a static bed without forced aeration. Ashley *et al.* have evaluated the phenomenon of air reversal and mixing by heat transfer dynamics in packed bed for preventing overheating. In air reversal, air moves from right to left and vice versa. The time in which air flows from one side to the other, the temperature of the cooled area increases due to accumulation of metabolic heat. As a result, significant temperature fluctuations were reported. However, discontinuous mixing at controlled rotational speed and bed height can be highly useful for controlling overheating in packed beds.[51] The design of the *Zymotis* bioreactor with internal cooling plates has solved the problem of heat removal in a packed bed reactor. The cooling plates are arranged at a distance of l = 5 cm vertically. The distance between the plates is increased for fast growing organisms, and for proper loading

and unloading of the reactor. The aim of *Zymotis* is to minimize vertical temperature gradients and maximize the heat transfer through cooling water.[22]

In conclusion, most researchers for bioreactor development have used mathematical modeling at microscopic scale using simple logistic kinetics. Logistic equations are simpler than complex partial differential equations and represent whole data without complexity. Modeling at microscopic level directly affects heat and mass transfer in bioreactors, which affects scale-up of the bioprocess. Continuous operating bioreactors based on agro-industrial residues are to be modeled. These bioreactors should have arrangements for substrate addition, water replacement and air inlet. However, validation of artificial results at smaller scale is necessary in reality at a larger scale for success of the mathematical modeling.

Bioreactors

In fermentation processes, bioreactor systems provide the environment for growth, cultivation of microbes. However, some of the factors affecting the growth of the product in SSF bioreactors are temperature, humidity of substrate bed, type of substrate used, size of the bioreactor, aeration, cooling rate, height of bed and fungal morphology. When compared to submerged fermentation, SSF is carried out in simple bioreactor systems; SSF bioreactors are fitted with a humidifier and with or without an agitator unit. Poor thermal conductivity of the substrate bed presents a great challenge to bioreactor design, but composition, particle size, porosity and water-holding capacity of the substrate used also affects the bioreactor.

Various researchers have classified the SSF bioreactors broadly[20,50,52,53] but most bioreactors can be distinguished by a factor whether they are used at small scale and large scale.

Small-scale bioreactors

Solid-state fermentation at laboratory scale is carried out in Petri dishes, jars, wide mouthed Erlenmeyer flasks, roux bottles, and roller bottles. These systems are simple and experiments are carried out easily.[53] There are several forms of small-scale bioreactors such as column bioreactors. Mauris Raimbault and J. C. Germon in ORSTOM (Institut Français de Recherche Scientifique pour le Developpment en Cooperation, France) laboratory of soil microbiology designed it in 1980, for growing *Aspergillus niger* on cassava meal in solid-state. Column bioreactors consist of small columns (diameter: D = 22 mm, height: H = 210 mm) which can hold 20 g of pre-inoculated solid material. Approximately 24 such columns were put together in thermo-regulated water baths and water-saturated air was passed through each column at a flow rate of $Q = 4 - 6$ L h^{-1}. Column bioreactors are useful for optimization of the medium, and constitute an important part of research. However, difficulty lies in obtaining the product and poor heat removal. Pandey *et al*.[54] determined the performance of the column bioreactor for glucoamylase production. Columns of different diameters were used and arranged with different substrate bed heights of h = 4.5, 9, 18, 22.5 cm. Enhancement in substrate bed height increased the enzyme synthesis. The column with 18 cm of bed height produced maximum enzyme in 48 h with an aeration rate of Q_a = 1 to 1.5 L L^{-1} min^{-1}. However, with further increase in bed height, enzyme synthesis decreased. The decrease may be due to the reduced aeration rate with increased height of the substrate bed.

A few years later the INRA (Institut National de la Recherche Agronomique, Dijon, France) team developed a reactor with one liter working volume fitted with a relative humidity probe, a cooling coil in heating circuit, and a cover for the reactor vessel. These reactors were filled with pre-inoculated solid material, and a computer controlled all parameters of the reactor. Sampling for analysis was easier in INRA bioreactors. This was the advantage over ORSTOM bioreactors. The automatic control of relative humidity and temperature makes these reactors useful in scale-up studies.

Apart from non-agitated column reactors, there are bioreactor systems with agitator systems such as perforated-drum reactor and horizontal paddle mixer (with or without water jacket). Drum bioreactors are designed to mix the solid substrate by rotating horizontal rotation vessels (which are with or without baffles). The rotation or agitation creates tumbling in the matrix of the solid medium by minimizing the heat produced. However, mixing of inoculum with substrate is uniform in drum bioreactors but fungi morphology is readily damaged by high shear, stress is not used in such bioreactors. However, an intermittent mixing strategy is involved in these bioreactors, which gives balance between positive effects of increasing temperature on deleterious effects on fungal hyphae.[52,55] A paddle mixer was developed by Wageningen University of Agriculture. It consists of a number of blades making mixing more efficient than rotating drum.[52]

Various advancements have been made through rotating drum bioreactors. Kalogeris *et al*. have developed a laboratory scale intermittent agitation rotating drum type bioreactor for SSF of thermophillic organisms. The main parts of the apparatus are: perforated (pore size, S = 1 mm^2) cylindrical

drum (diameter, $D = 0.15$ m; length, $L = 0.59$ m; capacity, $V = 10$ L), vessel with water jackets, motor connected to controller, heat exchanger, condenser at air outlet, and humidifier.[56] Kalogeris *et al*. determined the efficiency of the bioreactor by producing hemicellulases and cellulases with a thermophillic fungus, *Thermoascus aurantiacus*, using wheat straw as substrate. He examined the effects of temperature, moisture and aeration. Among the various kinetic models tested, Le Duy model described the relationship between *T. auranticus* growth and enzyme production. However, increase in aeration rate and high moisture levels favored the enzyme and enzyme production. The bioreactor can be used for production of hydrolases from thermophillic organisms due to their superior thermo-stability, optimum activity at elevated temperatures, and high rates of substrate hydrolysis.[57]

A *Zymotis* packed bed reactor was developed at laboratory scale by the ORSTOM team. *Zymotis* bioreactors are a modification of packed bed bioreactors with internal plates in which cold water circulates at an optimum temperature. Variation in optimum temperature retards the initial growth of the culture used. In packed bed bioreactor, large axial temperature gradients arise leading to poor microbial growth at the end of the base near the outlet, with an increase in vertical temperature gradients.[22]

Another bioreactor (Growtek) was developed by a team of scientists at IIT (Indian Institute of Technology) Kharagpur, India, for plant tissue culture but later it was used for solid-state fermentation. This bioreactor has a cylindrical vessel (width $D = 11.3$ cm, height $H = 16$ cm) with a spout at the base, inclined at an angle of $\alpha = 15°$ having a diameter of $d = 2.6$ cm and length of $l = 8.5$ cm. Both vessel and spout have lids. A float of area $A = 72$ cm^2 is provided inside the vessel, which consists of a perforated base. Fermentation of solid substrate is carried out on a float to which the seed culture is added, while liquid salt solution is kept below the float. An advantage of the Growtek bioreactor is that the inoculated solid comes into direct contact with the liquid medium. The products formed during the fermentation process leach in liquid medium. This type of bioreactor was used for production of gallic acid from tannin-rich solid material.[37] Ahamad *et al*. used a Growtek bioreactor for production of mevastatin by *Penicillium citrinum* from wheat bran.[58]

Large scale bioreactors

The successful operation of large-scale bioreactors depends on design features obtained after mathematical modeling, susceptibility of the substrate and fungal morphology to increase in temperature, effect of particle size and voids between them, quantity of the substrate used, and height of the substrate bed. There are several forms of large-scale bioreactors used in SSF.

Koji types of bioreactors are the simplest and without forced aeration. These types of bioreactors are also known as tray bioreactors. The trays are made of wood, plastic, metal. It is not necessary that trays should be perforated. Trays are arranged one above the other with suitable gaps between them and placed in climatic controlled chamber under circulating air, which maintain uniform temperature. An advantage of this technology is that by increasing the number of trays, the scale-up is easier. However, the requirement of sterility, large space and labor makes the process difficult. Rodriguez *et al*. reported the tray bioreactor to be appropriate for producing laccase by *T. versicolor* in solid-state conditions operating with lignocellulosic supports besides their several disadvantages.[59] Rodriguez *et al*. also proved superiority of grape seeds over nylon sponge supports using tray configuration during laccase production (with *Trametes hirsuta* under SSF conditions).[60] A Shallow tray fermentor was used at laboratory scale for cellulase production with *Trichoderma reesei* ZU02 strain on corncob residues.[61]

However, BIOCON India has used this technology for large-scale production of immuno-suppressants. They have simplified problems of sterility by keeping the trays in HEPA (High Efficiency Particulate Air) filtered air, using automated machines for layering the substrate in trays.

Packed bed bioreactors are modifications of laboratory-scale column reactors. A sieve present at the bottom is used for holding the substrate. Passing forced air through the static bed provides aeration. Heat produced during fermentation within the substrate bed is a major limitation of these reactors. However, reduction in water content within the substrate bed increases its hardening. This decreases fungal penetration within the bed, causing reduction in product formation. Hence, it is advantageous to use thermo-stable fungus for producing a thermo--stable product (especially enzymes) in packed bed reactors. Heat produced in the packed column can be reduced by increasing the moisture of air inside it, which is achieved by passing cold saturated air or using heat exchangers as in *Zymotis*. Pressure drop is an important feature of the packed reactor. High-pressure drop reduces airflow from the substrate favoring channeling within the bed.[53] The channeling decreases optimum temperature required for growth of organisms in the substrate bed. Some large-scale bioreactors like PLAFRACTOR[62] use heat exchangers for controlling temperature during fermentation. However, enhancing agitation

in the unmixed static bed solves the problem of heat removal and scale-up. Oostra *et al*.[50] using the model of a scraped drum reactor have proved this.

Another bioreactor used at industrial scale is the rotating drum bioreactor (RDB). The RDB mixes the substrate with the cultivating organism, while rotation can be continuous or intermittent depending on the bed height and speed of rotation. Some critical factors affecting RDB are type of substrate, height of bed, fungal physiology, and size of substrate particles. Incorporation of baffles may improve the operation of RDB. Various researchers have studied the impact of baffles within RDB. Schutyser *et al.* have used discrete particle simulators to predict that curved baffles have better radial mixing characters than other designs. Curved baffles are independent of particle rotation, and continuous rotation of drum is possible with them.[40] RDB are characterized as mixed bioreactors. However, with higher shear rates and rotational speed, the fungal physiology may be destroyed.[20,50,63]

In fluidized bed reactors, the solid substrate is fluidized by upward airflow. The bioreactor operates on the flow of air and velocity with which the substrate moves upwards. Sufficient velocity of air is supplied to fluidize the solid substrate. The column of the bioreactor is high enough for bed expansion. Widening of the column near the top allows disengagement of solids from the gas stream. Maintenance of uniform conditions throughout the substrate and increase in surface area is an advantage of this bioreactor.

After many advances in SSF reactors, heat control and scale-up still require consideration through modeling.

Substrates used in solid-state fermentation

For SSF processes, different agro-industrial wastes are used as solid substrates. Selection of agro-industrial residues for utilization in SSF depends on some physical parameters such as particle size, moisture level, intra-particle spacing and nutrient composition within the substrate. In recent years, some important agro-industrial residues such as cassava bagasse, sugarcane bagasse, sugar beat pulp/husk, orange bagasse, oil cakes, apple pomace, grape juice, grape seed, coffee husk, wheat bran, coir pith etc. have been used as substrates for solid-state fermentation.

Cassava bagasse and sugarcane bagasse offer an advantage over other substrates such as rice straw, wheat straw, because of their slow ash content.[64] One of the factors making cassava an optimum substrate for SSF is its high water retention capacity.[65] Cassava bagasse is highly used for production of citric acid, flavors, mushrooms, and different biotransformation processes. However, in comparison to sugarcane bagasse, cassava offers an advantage as it does not require pretreatment, and can probably be decomposed by most organisms for various purposes.[64] Some experiments have proved that the protein mass fraction of cassava may be improved from $w = 1.67$ % to 12 % by carrying out its biotransformation with tray bioreactors.[54] However, sugarcane bagasse and wheat bran are used for commercial production of most compounds using SSF, but their potential is still to be explored completely. Sugarcane bagasse has many efficient and economical applications, such as production of protein-enriched cattle feed and protein.[64] Pretreatment of sugarcane bagasse is an important aspect since assimilation and accessibility of microorganisms to substrate becomes easy, thus resulting in decomposition of hemi cellulose and lignin.[66]

Another upcoming agro-industrial substrate is coffee pulp/husk due to its rich organic nature and high nutritive value. Obtaining coffee pulp or coffee husk usually depends on the method employed for refining the coffee cherries, i.e. wet or dry method. However, fungi Basidiomycetes are found mostly in husk rather than in pulp.[67]

Okara (soybean curd residue), rich in water-insoluble ingredients, is a useful substrate for microbial fermentation. The main disadvantage of okara is natural spoilage when it is not refrigerated. Dehydration of the soybean curd residue has been reported to improve its utilization. Ohno *et al*. successfully produced an antibiotic iturin from okara.[68]

Substrates such as agro-industrial residues are proved by many researchers to be better for filamentous fungi. The morphology of filamentous fungi supports them to penetrate the hardest surface due to the presence of turgid pressure at the tip of their mycelium. Hence, the raw materials considered as waste are used for production of value added fine products and reducing pollution problems.[69]

Applications of solid-state fermentation

Production of enzymes by SSF

Enzyme production is one of the most important applications of SSF. SSF has advantages over submerged fermentation such as high volumetric productivity, low cost of equipment involved, better yield of product, lesser waste generation and lesser time consuming processes etc.

The type of strain, culture conditions, nature of the substrate and availability of nutrients are the

other important factors affecting yield of enzyme production.[70] It is crucial to provide optimized water content and control the water activity for good enzyme production. Agro-industrial substrates are considered best for enzyme production in SSF. The cost of enzyme production by submerged fermentation is higher compared to SSF. Tangerdy *et al.* have also proved this by comparing cellulase production costs in SSF and SMF.[71]

Protease

Various attempts for producing acid, neutral and alkaline protease have been done using agro-industrial residues with SSF, depending on their catalytic activity and optimum pH. Proteases are produced by fermentation of agro-industrial substrates such as rice bran, wheat bran, soy meal, green gram husk, rice husk, spent brewing grain, coconut oil cake, palm kernel cake, sesame oil cake, jackfruit seed powder, olive oil cake, lentil husk etc.

Ikasari and Mitchell[72] used rice bran for producing acid proteases with *Rhizopus oligosporus,* as no toxin production occurred during SSF. Acid protease production has also been obtained using bacteria *Mucor bacilliformis* in SSF.[73] Tunga *et al*. produced extra-cellular alkaline serine protease using *Aspergillus parasiticus* and *Rhizopus oryzae* NRRL-21498 with wheat bran as substrate.[74] Prakasham *et al*. obtained maximum alkaline protease production from green gram husk.[75] The alkaline proteases obtained using SSF of *Aspergillus parasiticus* was thermo-stable at $\vartheta = 50$ to 60 °C, high pH, in the presence of surfactants, metals and oxidizing agents.[74] However, most researchers have used wheat bran for production of alkaline protease.

Protease production in SSF is higher than with submerged fermentation. Down streaming and extraction of the product is cheaper in SSF due to lesser water content of the substrate. *Bacillus subtillis* obtained from excreta of larvae *Bruckdaole pachymerusnucleorum* was also used for protease production using soy cake as substrate. Maximum protease productivity was $P = 15.4$ U g^{-1} h^{-1} for SSF but it was $P = 1.3$ U g^{-1} h^{-1}. The enzyme productivity was 45 % higher in SSF.[76] Some critical fermentation parameters for obtaining maximum protease production are accumulation density of microbe within the substrate and height of the substrate bed. Sandhya *et al*. studied the effect of parameters such as incubation temperature, pH, inoculum size and incubation time on neutral protease production through submerged fermentation and SSF. In solid-state, the total enzyme yield was 31.2 U enzyme per gram of fermented substrate, but in submerged fermentation the yield was 8.7 U enzyme per gram of fermented substrate. The yield in SSF was 3.5 times higher than in SMF.[77] Similarly, George *et al*. compared the production of proteases by *Bacillus amyloliquefaciens* in solid-state and submerged fermentation conditions. In submerged state, $8 \cdot 10^6$ units of enzyme were obtained in a $V = 20$ L fermentor under optimal conditions. However, in solid-state the enzyme production was $25 \cdot 10^4$ units g^{-1}. They reported SSF to be simpler in operation than submerged fermentation.[78]

Lipase

In recent years, considerable increase in lipase production from microbes and agro-industrial wastes using SSF has gained importance. However, fungi are best lipase producers.[79] Most researchers have used wheat bran for maximum lipase production. Kamini *et al*. used gingelly oil cake for the production of lipase by *Aspergillus niger*. However, supplementation of various nitrogen sources, carbohydrates and inducers to substrates was ineffective.[80] In contrast, SSF of babassu oil cake with *Penicillum restrictum* proved that lipase activity depends on the type of supplementation. Enrichment of high carbon containing substances such as peptone, olive oil, resulted in high lipase activity.[81] Enhancement in lipase activity by olive oil supplementation has also been proved by Cordova *et al*.[82] The fungal spp. *Rhizopus oligosporus* has been found to be the best mould among 10 different fungal cultures for production of lipases without the addition of supplements.[83]

Castilho performed the economic analysis of solid-state and submerged fermentation for production of lipase using *Penicillum restrictum.* He reported that, for the production scale of 100 m^3 lipase concentrate per year, a total capital investment needed for submerged process was 78 % higher than that for SSF. The unitary product cost in submerged processes was 68 % higher than the product selling price.[84]

Mahadik *et al*. compared lipase production in submerged and solid-state fermentation. In SMF a synthetic oil-based medium was used, but SSF used wheat bran supplemented with olive oil, which resulted in increased lipase production.[79] Mateos Diaz *et al*. reported that lipase from solid-state to be more thermo-tolerant than from submerged fermentation (thermal stability, half life at $\vartheta = 50$ °C was $t = 0.44$ h with SMF, and 0.72 h with SSF). The temperature at which maximum activity of lipases occurs was $\vartheta = 30$ °C with SMF, and $\vartheta = 40$ °C with SSF. The lipase produced was from fungus *Rhizopus homothallicus.* Process development requires proper utilization of enzymes. Hence, they should show high functional stability. The enzymes obtained from thermophillic fungus have high ther-

mal stability, and activity at mesophillic temperatures are generally utilized in chemical processes.[85]

Some of the agro-industrial wastes used in SSF for lipase production are babassu oil cake, wheat bran, rice bran, gingelly oil cake, almond meal, mustard meal, coconut meal, rice husk, sugarcane bagasse, cassava bagasse, coconut oil cake, olive oil cake, etc.

Cellulase

Cellulase is an enzyme complex used for the conversion of lignocellulosic residues and used for production of ethanol, single-cell protein, bleaching of pulp, for treatment of waste papers and for fruit juice extraction. In SSF, using lignocellulosic wastes as substrates can reduce the cost of cellulase production.[61] Lignocellulosic materials are cheaper and pretreatment is required to improve their utilization. Pretreatment of lignocellulosic matrix increases the potential of cellulases to act on cellulose, hemicelluloses.

The concerted action of enzymes like endoglucanases, exoglucanases and β- glucosidase is used for hydrolyzing cellulose. The rate-limiting step is the ability of endoglucanases to reach amorphous regions within the crystalline matrix and create new ends with which endoglucanases can act.[86] Ethanol production from lignocellulose biomass requires hydrolysis by cellulase and hemicellulase for converting lignocellulosic biomass to biofuel.[87]

Some factors like moisture content, particle size, pH, incubation temperature, inoculum size, incubation period and enrichment of medium with carbon and nitrogen were considered optimum for cellulase production by bacterial strain *Bacillus subtilis* on banana fruit stalk wastes. However, total enzyme production was 12 times higher in SSF than in submerged fermentation under similar experimental condition.[88] It is necessary to consider these factors for cellulase production from lignocellulosic wastes as their nutrients are already depleted.

Water content of substrate and aeration rate are critical factors in cellulase production using SSF. Corncob residue was used for cellulase production with *Trichoderma reesei* ZU02 in shallow tray fermentors. Xia and Cen used a deep trough fermentor with forced aeration for cellulase production. Forced aeration enhanced the mass transfer to a greater extent, which increased cellulase activity to 305 IU per g of cellulose.[61] It has been reported by Fujian *et al*. that substrates in solid-state with continuous circulation of air and convective diffusion with pressure are better for fungal propagation than static cultures. This periodic air circulation increases the looseness of substrates and enhances cellulase activity. The filter paper activity of cellulase enzyme increased to 20.4 IU g^{-1} at a bed height of $h = 9$ cm in $t = 60$ h, while maximum filter paper enzyme activity was 10.8 IU g^{-1} in 84 h within static cultures. The work was performed using steam-exploded wheat straw as carrier with *Penicillum decumbens* in SSF.[89] However, changes in the amplitude of air pressure increased the oxygen availability to the cultures used and heat removal. The variations enhanced the cellulase production by *Trichoderma viride* in SSF.[90]

Co culturing of two fungi in SSF enhances the enzyme production. Co culturing of *Trichoderma reesei* mutants with *Aspergillus* spp. increased the cellulase production by 50 % and improved the cellulase glucosidase ratio, by partially removing product inhibition and its hydrolysis.[91] Co culturing *Aspergillus ellipticus* and *Aspergillus fumigatus* resulted in improved hydrolytic and β-glucosidaes activity.[61,71] However, some newly developed agro-industrial wastes used for cellulase production are banana wastes, rice straw, corn cob residue, rice husk, wheat straw, banana fruit stalk, and coconut coir pith.

Pectinase

Pectinases are constitutive or inducible enzymes produced by microbes for breaking pectin. Different substrates used for production of pectinase are wheat bran, soy bran, apple pomace, cranberry pomace, strawberry pomace, beet pulp, coffee pulp & husk, cocoa, lemon & orange peel, combination of sugarcane bagasse and orange bagasse, wheat bran etc.

Production of polygalactouronase (PG) and pectinesterase (PE) was 6.4 times higher in SSF compared to submerged fermentation. PE and PG activity was measured to be 500 U L^{-1} and 350 U L^{-1} at 24 h of incubation with pectin as the sole carbon source with SSF, but in submerged fermentation enzyme production was 127 U L^{-1} and 55 U L^{-1} at $t = 48$ h of incubation. Supplementation of glucose decreased the production of enzymes due to catabolite repression in submerged fermentation. However, PE and PG enzymes increased by 30 % and 33 % respectively with addition of glucose.[92] Similarly, exopectinase activity increased from 623 to 7150 (IU L^{-1}) in SSF, but decreased from 1714 to 355 (IU L^{-1}) in submerged fermentation in the presence of sucrose at A_w of 0.995. Similar results were at A_w of 0.96. Increase in water activity increased pectinase activity in SSF.[93] Production using deseeded sunflower head as substrate resulted in step-up of exopectinase and endopectinase enzyme using SSF. In SMF the endopectinase was 18.9 U mL^{-1}, which increased to 19.8 U mL^{-1} in SSF.

Similarly, exopectinase increased from 30.3 U mL^{-1} in SMF to 45.9 U mL^{-1} in SSF, at optimum moisture of $s = 65$ % and particle size of $d = 500$ μm.[94,95] Polyurethane, when used as inert support for exopectinase production using *Aspergillus niger* in Erlenmeyer flasks, resulted in direct measurement of biomass production, substrate uptake and enzyme activity in culture medium.[93] Botella *et al*. used grape pomace for exopectinase production with *Aspergillus awamori* in SSF. Initial substrate moisture content of 65 % and supplementation of 6 % glucose as carbon source enhanced enzyme activity. However, particle size did not influence the increased enzyme production, which is contradictory to earlier reports.[96,97]

Besides moisture, the production of pectinase enzymes also depends on physical parameters of the bioreactor used, amount of substrate used, type and pH of substrate and supplements added during fermentation. A mixture of orange bagasse, wheat bran (1:1) was used for production of endo-polygalactouronase, exo-polygalactouronase and pectinlyase by *Penicillum viridicatum* in Erlenmeyer flasks and polypropylene packs at 2 different moisture $s = 70$ %, 80 %. However higher production of all three enzymes was obtained in polypropylene packs at $s = 70$ % moisture, but lower moisture content was highly significant for Pectinlyase production. Increase in pectinase enzymes using polypropylene packs favored it for scale-up.[98]

Martins *et al*. used the *Thermoascus auranticus* for pectinlyase and polygalactouronase production. The quantity of pectinlyase and polygalactouronase was found to be higher when compared with other pectinolytic strains such as *Aspergillus niger, Penicillum italicum, Aspergillus foetidus.* However, addition of fibrous material such as sugarcane bagasse to the mixture of wheat bran, orange bagasse, resulted in intra-particle spacing, causing increase in aeration, nutrient and enzyme diffusion, which supported pectinlyase production. These results proved that media composition did not affect polygalactouronase production.[99] Pectinase and polygalactouronase using *Moniellia, Penicillum* spp. were produced using orange bagasse, sugarcane bagasse and wheat bran as substrate. Addition of fibrous material such as sugarcane bagasse caused interparticle spacing resulting in increase of aeration and nutrients supply. However, sugarcane bagasse as the sole carbon source, did not allow the growth of fungi, indicating that microorganisms are unable to hydrolyze cellulose, hemicelluloses fibers but they can support mycelia formation. The sugarcane bagasse was used as the inert support for growth & pectinase production by *Aspergillus niger, Penicillum viridicatum, Thermoascus auranticus*.[100]

Solid substrates resulted in higher pectinase production as they supply nutrients to microbial cultures along with anchorage. Nutrients, which are unavailable or are in sub-optimal concentration, are usually supplemented externally. A *Bacillus* sp. DT 7 was used for production of pectinase using wheat bran, rice bran, apple pomace as substrates in Erlenmeyer flasks. Maximal enzyme production was obtained from wheat bran supplemented with polygalactouronic acid and neurobion. Supplementation of 27 μL of neurobion enhanced pectinase production by 65.8 %.[101]

Phytase

Several strains of bacteria, yeasts, fungi such as *Bacillus subtillis, E. coli, Bacillus amyloliquefaciens, Schwanniomyces castellii, Schwanniomyces occidentalis, Hansenula polymorpha, Aspergillus flavipes, Aspergillus fumigatus, Aspergillus oryzae* and *Aspergillus ficcum* are employed for phytase production in SSF systems. Some of the substrates generally used for phytase production are canola meal, coconut oil cake, wheat bran, black bean flour, cowpea meal, mustard cake, cotton cake, palm kernel cake, sesame oil cake, rapeseed meal, olive oil cake, groundnut oil cake etc.

Phytase production increases with fermentation of mixed substrates. The increase may be due to the availability of different nutrients from different substrates in the same reactor simultaneously. This may step-up phytase production. Roopesh *et al*. used SSF for phytase production from various substrate combinations of wheat bran and various oil cakes using *Mucor racemosus* under SSF. Combination of wheat bran and oil cake yielded greater Phytase when compared to their individual production. Optimization of conditions resulted in 44.5 U g^{-1} of phytase with the combination of wheat bran and oil cake, which were 1.5 times and 4 times higher, respectively, when the oil cake and wheat bran were used as substrates. The type of carbon and nitrogen source used is an important factor for consideration in any fermentation process.[102] However, along with imbalance in the carbon-to-nitrogen ratio, many parameters affect phytase production such as incubation time, initial moisture content and incubation temperature. Similarly, Ramachandran *et al*. used *Rhizopus* spp. with coconut oil cake, sesame oil cake, palm kernel cake, groundnut oil cake, cottonseed oil cake and olive oil cake for phytase production. Cottonseed oil cake and olive oil cake poorly supported the phytase production individually, but mixed substrate fermentation of both increased enzyme production to 35 U/gds. Supplementation of glucose further enhanced phytase activity to 52 U/gds. Enhancement was re-

ported due to the combined effects of nutrients in them.[103]

Various other substrates like wheat bran, oil cakes such as groundnut oil cake, canola meal, rapeseed meal and soybean meal when supplemented with surfactants resulted in higher production of phytase. *Aspergillus ficcum* was used for phytase production on canola meal using SSF.[104] Extra cellular phytase produced using thermo-tolerant *Aspergillus niger* was maximum with cowpea meal. Increase in membrane permeability of fungus has been reported to be the optimum cause for phytase enhancement in SSF.[105]

L-glutaminase

L-glutaminase is used as antileukaemic and flavor enhancing agent. Solid-state fermentation was found to be more suitable than submerged fermentation for biosynthesis of L-glutaminase using *Pseudomonas flourescens,* as 25-fold enhancement was obtained.[106] The moisture of substrate highly effects enzyme production. L-glutaminase was produced by yeast *Zygosacharomyces rouxii* using wheat bran and sesame oil as substrate with s = 64.2 % moisture, t = 48 h old inoculum and ϑ = 30 °C incubation temperature. Addition of 10 % NaCl and seawater to wheat bran and sesame oil enhanced enzyme production. The enzyme produced from wheat bran and sesame oil cake in dry state was 2.2 and 2.17 U/gds but with supplementation, the concentration increased to 7.5 and 11.61 U/gds respectively.[107] *Beauveria* spp. an alkalophilic and salt-tolerant fungus isolated from marine sediment, was used for L-glutaminase production using seawater-based medium supplemented with L-glutamine (w = 0.25 %) as substrate.[108] Seawater being a natural reserve for marine organisms can provide them sufficient nutrients when used as supplement in SSF for production of industrially important enzymes.

Amylase

Amylase has potential application in a number of industrial processes such as food, fermentation, textiles and paper industries. The two major classes of starch degrading amylases are glucoamylases and α-amylase. Solid-state fermentation holds tremendous potential for production of these enzymes industrially on a large scale.

Production of α-amylase is not limited to fungal cultures but it is also done by bacterial cultures of genus *Bacillus*. Bacterial cultures reduce the time required for fermentation, reducing a lot of expenditure involved. Wheat bran has been reported to be the best substrate even for bacterial cultures. Babu and Satyanarayana produced α-amylase with *Bacillus coagulans* in SSF using wheat bran as substrate and supplemented with tap water as moistening agent. They compared the production of enzyme in different vessels.[109] Ramesh and Lonsane produced α-amylase with *Bacillus licheniformis* M27 under SSF conditions, using wheat bran as substrate.[38]

An advantage of SSF over submerged fermentation is the inhibition of catabolic repression by regulation of end product synthesis from the product formed. α-amylase production by *Bacillus licheniformis* decreased from 420 to 30 units mL^{-1} with increase in fraction of starch from 0.2 to 1 % in SMF. However, in SSF the enzyme activity enhanced 29-fold with a 42 times increase in concentration of starch. Hence, inhibition of enzyme by product is overruled in solid-state fermentation.[110] Ramachandran *et al*. used coconut oil cake, a dry product obtained from copra for production of α-amylase with *Aspergillus niger* in SSF. Supplementation with starch, peptone and glucose increased the enzyme synthesis.[111]

Glucoamylase is still produced by large-scale submerged fermentation, but the production cost remains high in all respects. There are less reports of glucoamylase production in SSF. However, cost of production is lower in SSF. Cost reduction can be achieved by using cheaper substrates such as agro-industrial residues. Selection of the proper substrate is an important parameter for maximum enzyme activity. Ellaiah *et al*. optimized different substrates like wheat bran, green gram husk, black gram bean, barley flour, jowar flour, maize bran, rice bran, and wheat rawa for maximum glucoamylase production with *Aspergillus* spp. under SSF. However, wheat bran showed maximum enzyme activity to be 247 U g^{-1}. Supplementation of w = 1 % fructose and w = 1 % urea enhanced enzyme activity. A very high moisture content of 80 % favored maximum enzyme production.[112] Tea waste was used as substrate for maximum glucoamylase production using *Aspergillus niger*. Supplementation of tea waste with mineral solution containing salts such as K, Mn, Mg, Zn and Ca resulted in 198.4 IU/gds of enzyme. The results were at t = 96 h with 4 % inoculum, 60 % moisture, and pH of 4.5. Supplementation with w = 1 % sucrose enhanced enzyme activity to 212.6 IU/gds at 96 h. Further addition of w = 1 % malt extract increased enzyme activity to 226 IU/gds at 72 h after inoculation.[113]

Anto *et al*. produced glucoamylase using rice flakes (categorized as coarse, fine and medium), wheat bran and rice powder as substrates with *Aspergillus* spp. HA-2. Supplementation of sucrose as carbon source to wheat bran and glucose to coarse, medium waste enhanced enzyme production. Addition of yeast extract and peptone to the substrates also enhanced enzyme production. Maximum enzyme production was obtained in wheat

bran (264 ± 0.64 U/gds) but glucoamylase production in different categories of rice flakes varied. Maximum enzyme was produced in coarse (211.5 ± 1.44 U/gds) and medium wastes (192.1 ± 1.15 U/gds). The factors affecting enzyme activity with combination of wastes were attributed to the increase in agglomeration of particles, which resulted in reduced aeration and penetration by fungal mycelia.[114]

Nutrient supplementation from organic sources increases enzyme production to a greater extent than inorganic sources. It has also been found that most researchers used wheat bran as substrate for α-amylase production because it contains sufficient nutrients, it remains loose even in moist conditions, and has a larger surface area. Due to these factors, aeration and mycelial penetration are easier in wheat bran.[109]

Ligninase

Lignin is a biopolymer with complex phenylpropanoid structure and contributes to environmental pollution. The most common organisms for lignin degradation are white rot fungi. However, activity of *Phanerochaete chrysosporium* is found to be most suitable for efficient lignin degradation. Enzymes secreted by white rot fungi are ligninases. They are Lignin Peroxidase (LiP), Manganese Peroxidase (MnP) and H_2O_2 generating enzymes. Secondary metabolism of these enzymes is triggered by C, N, S depletion.[115,116]

The respiration rate of an organism is indicator of the organism's activity. Cordova *et al*. determined the relation between the CO_2 evolution rate and enzymatic activity of fungus when grown on sugarcane bagasse pith. There was an increase in CO_2 evolution rate with increase in metabolism. Activity of manganese peroxidase (MnP) was expressed in the idiophase where the residual glucose level was the least in the medium. The MnP activity enhanced and then decreased rapidly due to simultaneous protease activity. Lignin peroxidase activity was reported during the exponential phase of the organism's growth. However, it decreased after fungal secondary metabolism. This indicates that LiP is less sensitive to MnP for proteolytic action.[117] CO_2 generation took place after uptake of O_2 by fungus. However, LiP and MnP activities cannot be studied in an anaerobic media.

Fujian *et al.* compared lignolytic enzyme activities in submerged and solid-state fermentation using steam-exploded wheat straw as substrate. LiP, MnP activities in optimum conditions were 61.67 U L^{-1} and 27.12 U L^{-1} in submerged fermentation, but in solid-state the activities were 365.12 U L^{-1} and 265 U L^{-1}, respectively. However, in SSF the maximum enzymatic activities reached 2600 U L^{-1} (LiP) and 1375 U L^{-1} (MnP).[118] This proves the feasibility of solid-state over submerged fermentation.

Developing semi solid-state conditions within the bioreactor also enhances lignin enzymes. Dominguez *et al*. developed a bioreactor based on rotating drums for producing lignolytic enzymes. The enzyme activities at 1 L L^{-1} min^{-1} of air were found to be 1350 U L^{-1} (LiP) and 364 U L^{-1} (MnP) respectively. Semisolid-state conditions were maintained by using nylon sponges for providing inert support, which is rotated by regularly passing through the nutrient medium. Nylon sponges were used due to its hydrophobicity, porous nature, affinity for fungus and moisture retaining capacity. Higher aeration rate avoided the support clogging and made nutrient availability to organism easier.[116] Similarly, Couto *et al*. used polypropylene sponges as inert support for lignolytic enzyme production.[115] Lignin-degrading enzymes were obtained from mushroom *Bjerkandera adusta* by immobilizing it on polyurethane foams.[119]

Degradation by lignin enzymes is a non-specific reaction on the basis of free radicals resulting in destabilization of bonds and finally breaking of macromolecule. This characteristic increases the potential for chemical industries; environmental industries; coal industries and extracting important metabolites from natural sources.

Xylanase

Different food processing with products such as straws and brans of different cereals, corn, hull and cobs, sugarcane and cassava bagasse, various saw dusts and different fruit processing and oil processing residues have been used for producing xylanase. Xylanase production requires substrates in very high concentration, with prominent water absorbance capacity. Xylanase are produced mainly by *Aspergillus* and *Trichoderma* spp.[120,121] Xylanase production was achieved successfully by *Aspergillus fischeri, Aspergillus niger* using wheat bran and wheat straw as main substrates.[122,123]

Addition of nitrogen source as supplement is an important step for xylanase production.[123] Sodium nitrite played a significant role in production of alkali stable cellulose free xylanase by *Aspergillus fischeri*. Xylanase in conjunction with cellulolytic enzymes is used for bioconversion of lignocellulosic material to produce fuels and other chemicals.[122]

The *Koji* process was used for production of xylanase by *Aspergillus sulphureus* using dry koji.[124] Ghanem *et al*. produced xylanase using *Aspergillus terreus* on wheat straw medium.[55] Topakas *et al*. used the *Sporotrichum thermophile*

for xylanase production in a horizontal bioreactor with wheat straw and destarched wheat bran as substrate.[125]

Pretreatment of lignocellulosic substrates was found to be advantageous in many cases, however in some it proved disadvantageous.[26] Pretreatment for xylanase production using wheat straw by *Aspergillus terreus* resulted in reduction of enzyme activity.[123] Some other molds that can be used for production of xylanase using SSF are *Melanocarpus albomyces*,[126] *Thermomyces lanuginosus*,[127] *Humicola insolens*[128] and *Aureobusidium pullulans*.[129]

Production of organic acids under SSF

Fermentation plays a key role in the production of organic acids. The production of organic acids progressed with development of SSF. However, biotechnological processes for large-scale production of organic acids are still in early phases of development. Organic acids are the most common ingredients of food and beverages because of their three main properties: solubility, hygroscopic quality, and their ability to chelate.[130] Some of the acids produced using SSF are citric acid, lactic acid, gallic acid, fumaric acid, γ-linoleic acid, and kojic acid.

Citric acid

Various agro-industrial residues such as sugarcane press mud, coffee husk, wheat bran, cassava fibrous residue, de-oiled rice bran, carob pods, apple pomace, grape pomace, kiwi fruit peels, okara, pineapple wastes, mausami wastes, kumara etc. are most potential substrates for production of citric acid in SSF.

Solid-state fermentation has been proved advantageous over SMF in many respects. Metal ions such as Fe^{+2}, Mn^{+2} and Zn^{+2} repress biosynthesis of citric acid by *Aspergillus niger* in submerged fermentation, but this does not happen in SSF. It is reported by Kumar *et al*. that addition of these metal ions enhances production of citric acid by 1.4 – 1.9 times in SSF.[3] Kumar *et al*. compared wheat bran and sugarcane bagasse for maximum citric acid production with *Aspergillus niger* DS-1 strain. Sugarcane bagasse proved to be a better carrier when compared with wheat bran. Agglomeration in wheat bran caused non-uniform mixing of the substrate, affected heat and mass transfer along with growth and product formation. Bagasse did not show agglomeration even when moisture level was increased from 65 % to 85 %.[3] Supplementation of methanol to agro-industrial residues enhanced citric acid production. Kumar *et al*. utilized pineapple, mixed fruit and mausami residues for producing citric acid using *Aspergillus niger* DS-1 in SSF. Supplementation of methanol to substrates was done at different moisture levels. Methanol being a dehydrating agent enhanced the moisture requirement of substrates. In the presence of methanol sugar consumption decreased but citric acid production increased. The maximum citric acid yield was $Y = 51.4$, 46.5 and 50 % (based on sugar consumed) from pineapple, mixed fruit and mausami residues, respectively.[131] Similar reports with carob pods and corncobs have been published by Roukas and Hang *et al*., respectively. Addition of methanol to the substrate increased citric acid production.[132,133] Figs were used for production of citric acid due to their high carbohydrate content and yielded 8 % citric acid. However, the addition of methanol increased citric acid production from 64 – 490 g kg^{-1} dry fig.[134] Enhancement in citric acid also depends on other additives such as carbon and nitrogen sources and metal ions.

Researchers have used different bioreactors for production of citric acid. Flasks proved their superiority over tray and rotating drum bioreactors when compared among different bioreactors using pineapple waste as substrate for citric acid production, with strains of *Aspergillus*.[135] Packed bed reactors proved their superiority when compared to flask cultures in production of citric acid**.** Kumara, starch-containing substrate was used for citric acid production using *Aspergillus niger* with a packed bed bioreactor. Kinetic analysis of the packed bed bioreactor showed an overall reactor productivity of $P = 0.82$ g h^{-1} citrate per kg wet mass kumara on 5th day, which in the flask was 0.42 g h^{-1} citrate per kg wet mass kumara on 8th day. Some adverse effects of packed bed such as reactor blockage due to fungal biomass, forced aeration and limited space, were dominated by optimizing particle size of substrate ($d_p = 4 – 8$ mm) and airflow rate ($Q = 0.5 – 1.5$ L min^{-1}). Higher bed height and larger particle size retard the citric acid production. Larger particle size promotes aeration at lower bed height in the reactor, which can destroy filamentous fungi. The high productivity in packed bed was due to initial utilization of starch in the substrates for citric acid production and occurrence of CO_2 throughout the process.[136] However, some of the results above were contraindicated when apple pomace was used as substrate for production of citric acid using *Aspergillus niger* in packed bed bioreactor. Adjustment of the aeration rate provided sufficient O_2 to filamentous fungi, increase in bed height promoted physical stability and better air distribution in the reactor, optimum moisture content provided proper diffusion to fungal cells, and increase in particle size prevented compaction of the substrate promoting aeration in the reactor. Hence, 124 g citric acid was pro-

duced from 1 kg dry apple pomace with a yield of $Y = 80$ % based on total sugar.[137]

Prado *et al*. related the metabolic activity of fungus *Aspergillus niger* to citric acid production on cassava bagasse. The cassava bagasse containing different percentages of gelatinized starch was used during the experiment. Gelatinization of starch in cassava bagasse made it more susceptible to fungal action. In horizontal drum bioreactor and tray bioreactor, it was observed that the lower respiration rate i.e. high O_2 uptake and low CO_2 production, favored the citric acid production in horizontal drum, but the presence of high partial pressure of CO_2 within the cassava bed also favored citric acid due to entrapment of fungal spores. Maximum citric acid production in tray bioreactor was with 80 % gelatinized starch. But in horizontal drum bioreactors, the maximum results were obtained with 100 % gelatinized starch. Increasing the bed height in tray bioreactors enhanced citric acid production.[138] When efficiency of different substrates such as cassava bagasse, sugarcane bagasse and coffee husk were compared for citric acid production with *Aspergillus niger*, cassava bagasse proved to be better since it increased the protein content of fermented matter. The presence of calcium, phosphorus, vitamin B_2, thiamine and niacin in cassava may have enhanced the yield of citric acid.[139]

Aspergillus niger has been found to be the most suitable strain for citric acid production. Most strains are unable to produce citric acid in acceptable yields since it is a metabolite of energy metabolism. Its accumulation rises in appreciable amounts in drastic conditions. The main advantages of using *Aspergillus niger* are its easy handling and its ability to ferment a wide variety of cheap raw materials. Enhancement in citric acid depends on the selection of proper nutrient supplements, organism and substrates to prevent drastic changes in pH.[140] However, significant optimization may make production cheaper in SSF.

Lactic acid

Some of the substrates used for production of lactic acid are wheat bran, wheat straw corncob, cassava, sweet sorghum, sugarcane bagasse, sugarcane press mud and carrot processing wastes. Aeration of moistened medium is an important factor for the SSF. It provides humidity to solid support and oxygen for growth. Soccol *et al*. used *Rhizopus oryzae* NRRL 395 on sugarcane bagasse impregnated with glucose and $CaCO_3$ to produce lactic acid. Lactic acid production in SSF and SMF was $\gamma = 137.0$ and 93.8 g L^{-1} respectively. Thus, productivity was $\gamma = 1.38$ g L^{-1} per hour in liquid medium and $\gamma = 1.43$ g L^{-1} per hour in solid medium, which makes SSF suitable for higher production of lactic acid.[141] Miura *et al*. utilized corncobs for L-lactic acid production using *Acremonium thermophilus* and *Rhizopus* in an airlift bioreactor.[142]

Inert support when used should provide good conditions for fermentation along with the purity of the product. Sugarcane bagasse impregnated with the sugar solution from gelatinized cassava bagasse was used as an inert support for production of lactic acid using *Lactobacillus delbruecki*.[143]

Naveena *et al*. used statistical analysis to optimize medium for lactic acid production from wheat bran using *Lactobacillus amylophilus* GV6 in SSF. Wheat bran not only makes the process economical but also brings the organism closer to its natural habitat. *Lactobacillus amylophilus* has been found to be efficient in direct fermentation of starch to lactic acid, avoiding the multi-step processes of simultaneous saccharification and fermentation.[144] Optimization of nutrients by response surface methodology resulted in production of 36 g of lactic acid per 100 g of wheat bran having 54 g of starch, with the *Lactobacillus amylophilus* strain. The increase in lactic acid production was 100 % (from 0.18 to 0.36). The conversion from rough surface with extensive complex mesh to smooth surface particles after inoculation confirmed alteration of raw starch to glucose, which then converted to lactic acid.[145] Some parameters to be considered for lactic acid production are aeration rate, substrate selection and nutritional supplementation.

However, lactic acid production from cheaper substrates is still a challenge in SSF, which has to be overcome by development of low cost mediums.

Gallic acid

Gallic acid is a phenolic compound. Tannase enzyme is used for converting tannin to gallic acid using *Rhizopus oryzae* on tannin-rich substrate in a Growtek bioreactor.[37] In the Growtek bioreactor, the solid substrate comes into direct contact with the liquid medium, and thus heat removal is easy. This reactor was used for producing gallic acid with mixed cultures of filamentous fungi such as *Rhizopus oryzae* and *Aspergillus foetidus*. Co-culturing of two organisms has advantage of providing internal regulation and product formation.[146]

Secondary metabolites production under SSF condition

Solid-state fermentation can be used for production of secondary metabolites. Most of these are accumulated in later stages of fermentation (idiophase). However, product formation has been found superior in solid-state processes. Problems associated with secondary metabolite production in liquid

fermentation are shear forces, increase in viscosity due to metabolite secretion, fungal morphology, and reduction in metabolite stability.

Gibberellic acids

Gibberellic acid is a fungal secondary metabolite produced in its stationary phase. The SSF system not only minimizes production and extraction costs, but also increases the yield of gibberellic acids. Accumulation of gibberellic acid was 1.626 times higher in SSF than SMF using wheat bran as substrate with *Gibberella fujikuroi* P3.[147] Limitation in nitrogen sources stops the exponential growth of fungus triggering production of secondary metabolites such as gibberellins.[148]

Various bioreactors using different substrates have been used for production of gibberellic acid in SSF. Bendelier *et al*. developed an aseptic pilot scale reactor for production of gibberellic acid using *Gibberella fujikoroi* in fed batch SSF.[149] Gelmi *et al*. used amberlite as inert solid support in glass column reactors under different conditions of temperature and water activity for gibberellic acid production.[148] A model using maize cob particles soaked in an amylaceous effluent was developed for studying factors affecting gibberellic acid production in SSF. The factors were particle diameter, volume of liquid phase and substrate concentration.[150] Tomasini *et al*. produced gibberellic acid on different SSF systems such as cassava flour, sugarcane bagasse, and polyurethane foam. With SSF 250 mg of gibberellin per kg of dry solid medium was produced in 36 h on cassava, but 23 mg of gibberellin per L of the medium was produced in submerged fermentation.[151] Scaling up and optimizing the operation of solid-state cultivation bioreactors will be simplified if there is availability of accurate process models, but problems may still exist due to absence of *online* measuring devices.[47]

Aroma production

Microorganisms play an important role in generation of natural compounds, such as fruity aroma. Although bacteria, yeast and fungi produce aroma compounds only a few spp. of yeast and fungi have been preferred due to their GRSA (Generally Regarded as Safe) status. Solid-state fermentation has been used for production of aromas by cultivating *Neurospora* spp, *Zygosaccharomyces rouxii*, *Aspergillus* spp. and *Trichoderma viride* using pre-gelatinized rice, miso, cellulose fibers, and agar.

Medeiros *et al*. used *Kluyveromyces marxianus* on cassava bagasse, as substrate in packed bed reactor with forced aeration at two different flow rates of $Q = 0.06$ and 0.12 L h^{-1} g^{-1}. However, with lower aeration, the total volatile content increased at $t = 24$ h but with higher aeration the total volatile content was less, and the rate of production decreased. Acetaldehyde, ethyl acetate and ethanol were the three major volatile compounds. Production of acetaldehyde and ethyl acetate decreased with increase in aeration rate. This has been attributed to the decrease in vapor pressure within the fermented medium with the increase of the aeration rate.[152] Strain of *Kluyveromyces marxiamus* was cultivated on five different agro-industrial residues such as cassava bagasse, giant palm bran, apple pomace, sugarcane bagasse, and sunflower seeds. Palm bran and cassava bagasse proved feasible for aroma production. Ethanol and ethyl acetate were in highest concentration with palm bran and cassava bagasse respectively in $t = 72$ h. Difference in composition of fermentation medium highly affected the aroma production.[153]

Ceratocystis fimbriata was used for producing volatile compounds in column and horizontal drum bioreactors over coffee husk as substrate. However, production was higher in the horizontal drum bioreactor. The dominant compounds in the process were ethyl acetate, acetaldehyde and ethanol. These were extracted by using porous absorbents. Resins like (Tenax and Amberlite) showed the best results for volatile compounds. In the case of Tenax, acetaldehyde was recovered in significant amounts ($n = 649.7$ μmol). The type of reactor used and absorbent influences the production.[154] *Ceratocystis fimbriata* when grown on different agro-industrial residues medium containing cassava bagasse, apple pomace, amaranth and soybean resulted in aroma production. The medium containing amaranth produced pineapple aroma. However, the medium containing cassava bagasse, apple pomace and soybean resulted in fruity aroma. The aroma produced was found to depend on the growth rate of fungus and its respiratory rate. The greater the respiratory activity of fungus the greater is its growth.[155] Hot water treated coffee husk was used for production of aroma production by *Ceratocystis fimbriata* in SSF. Supplementation of glucose, leucine increased the aroma production but an opposite effect was seen by addition of saline solution.[156]

Ferron *et al*. reviewed the microbial prospects of aroma production using SSF.[157] Longo and Sanroman reviewed the production of aroma compounds for the food processing industry by microbial cultures. Production form microorganisms has great advantages over traditional methods, such as decrease in production costs, ease of downstreaming processes, and use of cheaper agro-industrial substrates.[158]

A cheap alternative to agro-industrial residues for aroma production is cereal grain. Various spp. of *Aspergillus, Penicillum, Rhizopus* etc, can be cultivated on it for production of various aroma compounds such as esters, aldehydes, alcohols etc.

The development of novel, cheap production processes such as solid-state fermentation may solve some current limitations of microbial flavor production, as well as widen the spectrum of some biotechnologically accessible compounds.

Antibiotics

Many antibiotics such as penicillin**,** cephamycin C, neomycin, iturin, cyclosporin A, cephalosporins are produced by SSF.

Penicillin was produced by using *Penicillum chrysogenum* with substrates such as wheat bran of high moisture content ($s = 70$ %) and sugarcane bagasse.[159,160,161] Cephamycin C is produced by a variety of microorganisms including *Streptomyces cattleya, Streptomyces clavuligerus* and *Nocardia lactamdurans*. Wheat raw supplemented with cottonseed-de-oiled cake and sunflower cake was used for production of cephamycin C using SSF.[162] Wheat raw supplemented with raspberry proved to be optimum for production of neomycin by SSF. Some critical parameters considered to be optimum for production of neomycin are particle size of substrate, initial moisture content, inoculum volume, and incubation temperature.[163]

Iturin, an anti-fungal antibiotic, produced by SSF had stronger antibiotic activity due to its longer side-chain.[164] Dehydrated okara (containing $s = 82$ % moisture) mixed with wheat bran, when treated with *Bacillus subtilis* produced Iturin.[165,68] The amount of iturin produced per unit mass of wet substrate in SSF was found to be 5–6 times higher than in submerged fermentation when wheat bran was treated with *Bacillus subtilis* NB22.[164] Several parameters such as perforation in SSF trays, solid substrate thickness, type and size of inoculum and effect of relative humidity have been optimized for cyclosporin A production with wheat bran by *Tolypocladium inflatum* in SSF.[166,167]

Adinarayana *et al*. optimized the additives and parameters such as incubation temperature, moisture content, and inoculum level etc. for maximum cephalosporin C production using various substrates with *Acremonium chrysogenum***.** However, wheat raw was found to be the best substrate.[168] Strains of *Streptomyces* were assessed for tetracycline production using various agro-industrial residues as substrates. All the strains produced maximum tetracycline with peanut as carbohydrate source.[169]

Production of poly unsaturated fatty acids (PUFA) under SSF

Poly unsaturated fatty acids (PUFA) have to be supplied in diet, as they are not produced in the body. Submerged and solid-state fermentation can be used for PUFA production. Fungus involved in PUFA production decreases anti-nutrient substances, such as phytic acid, within the substrate, and partially hydrolyzes the biopolymers in it making them suitable for food and feed supplement. An important parameter to be considered in SSF is mass transfer during fermentation. Problems involved in scale-up also require solving.[170]

Gamma linoleic acid (GLA) is the most extensively studied PUFA by SSF. In addition, SSF processes have also been used for production of arachidonic acid, eicosapentanoic acid-rich byproducts.[170] *Cunninghamella japonica* when grown on various cereal substrates such as unhurled barley, pearl barley, peeled barley, hulled wheat, hulled millet, and polished rice in SSF produced GLA and lipids. The highest amount of lipid, GLA was obtained during SSF of rice and millet.[171] Similarly, optimization and screening of cereals was done in roller bottles and cultivation bags for maximum GLA production. Pearled barley resulted in highest GLA production. Supplementation of peanut oil further enhanced the production.[172] Among various fungal strains, the highest GLA was obtained from *Thamnidium elegans* in a roller bottle with apple pomace and spent malt grains as substrate.[173] Commercially, GLA is being produced in Japan using the genus *Mortierella* and *Mucorales*.[171,173]

Production of poly gamma glutamate (PGG) under SSF

Poly gamma glutamate (PGG) is an anionic, water-soluble, and highly viscous polypeptide. PGG is used as thickener, humectant, drug carrier, heavy metal absorber and feed additive. Uncontrollable foaming, limitation of O_2 and mass transfer decreases PGG production in submerged fermentation, but control over foaming and cost of substrates is achieved in SSF. A high protein-containing material is a good substrate for PGG production using *Bacillus subtilis*. Hence, wheat bran supplemented with soybean cake powder and additives such as glutamate, citric acid as substrate resulted in maximum PGG production (83.61 g per kg of dry substrate) at $s = 60$–65 % moisture level. However, with optimization a four-fold enhancement was observed.[174]

Production of poly hydroxy alkanoates (PHA) under SSF

Poly hydroxy alkanoates are microbial polyesters accumulated by microbes as energy reserve. Production using SSF decreases environmental problems and costs involved in the process. Substrates such as olive oil, babassu oil cake treated with *Ralstonia eutropha* can be used for PHA production by SSF. Addition of sugarcane molasses to soy cake increases PHA production.[175]

Exo-polysaccharides production under SSF

Exo-polysaccharides such as succinoglycan, xanthan are the future products of SSF.[65] *Agrobacterium* spp. has been used for succinoglycan production on an industrial scale as they are non-pathogenic and produce good yield of polysaccharide. Succinoglycan was produced using *Agrobacterium tumaefaeciens* on agar medium, spent malt grains, ivory nut and grated carrot in a horizontal bioreactor.[176]

Similarly, *Xanthomonas compestris* was grown on a variety of solid substrates such as spent malt grains, apple pomace and citrus peels for xanthan gum production. Fermentation was done in rotating culture bottles.[177]

Production of biocontrol agents under SSF

Development of sustainable agriculture through eco-friendly pesticides is needed due to their stability and reliability. They are used as an alternative to toxic residues. Among the various microbial agents as biocontrol agents, fungal agents are found to have greater potential because of their different modes of action. *Liagenidium giganteum*, a fungal agent used for control of mosquitoes, act by encysting on their larvae. It uses the larvae as a substrate for growing by entering inside it. This fungus acts by producing motile zoospores.[30]

The recognition of the fungal strain with pesticide activity is significant for development of infective propagules such as conidiospores, blastospores, chlamydospores, oospores and zygospores,[65] along with assessment of its biocontrol activity. Similarly, the mechanism of infection is also an important parameter to be considered for large-scale production strategy.[3] Retention of moisture is an important aspect for development of biopesticide formulation. Vrije *et al*.[179] illustrated the various mode of action and requirements of down stream processing for the biocontrol agents.

Spores produced in SSF are more heat resistant and are more stable through SSF.[70] Heat evolution and retention are the big problem in SSF system, but using lignocellulosic substrates, which increase the water retention capacity and forced aeration,[178] can prevent it.

E. nigrum produced on peat, vermiculite and lentil meal is an antagonist of *Monilinia laxa*, responsible for loss in stone fruits. Production using SSF is done in plastic bags because increasing the number of bags can do scale-up.[180] A glass column reactor was used with forced aeration with different substrate bed height and diameter for the production of spores of *Metarhizium anisophilae*. Forced aeration reduces heat transfer.[128] Similarly, a packed column bed reactor has been used to study interactions between *Fusarium culmorum* and its potential biocontrol agent, *Trichoderma harzianum*.

Solid-state fermentation of broiler litter has been done for the production of spores of *B. thuringenesis* and *P. flourescens*.[181] Oostra *et al*. used mixed bioreactors for large-scale production of spores for biocontrol agent *Coniothyrium minitans* and evaluated the heat produced during the process. *C. minitans* has the ability to grow and sporulate in a wide temperature range and is responsible for controlling *Sclerotinia sclerotinium* infections.[50]

Production of biofuel by SSF

Ethanol is the most widely used biofuel today. Although it is easier to produce ethanol using submerged fermentation, SSF is preferred due to its lower water requirement, smaller volumes of fermentation mash, prevention of end product inhibition, and disposal of less liquid water, which decreases pollution problems.

Cellulosic materials are receiving major attention for ethanol production because of their abundant availability.[182] Solid-state fermentation of apple pomace supplemented with ammonium sulfate and controlled fermentation with *Saccharomyces cerevisiae* has been reported to produce ethanol.[183] Kargi *et al*. used the rotating drum fermentor to estimate the influence of rotational speed on the rate of ethanol formation. The rate of ethanol formation decreased with the increase in rotational speed.[184] Various spp. of yeasts that can be used for ethanol production by SSF are *Saccharomyces cerevisiae, Kloeckera apiculate, Candida stellata, Candida pulcherrina* and *Hensenula anomala*. Bacteria like *Zymomonas mobilis* and fungus like *Fusarium oxysporum* also have the ability to produce ethanol. Ethanol production using thermotolerant yeasts has been supported because of lower cooling costs required and faster determination rates.[149,185] Various substrates that can be used for alcohol production are sweet sorghum, sweet potato, wheat flour, rice starch, soluble starch and potato starch using *Saccharomyces cerevisiae*. However, maximum ethanol production is achieved by using mixed substrates.[186,187]

Conclusions

There is a continuous development in SSF technology over the last two decades. The advantages of SSF processes overweigh the obstacles due to engineering problems involved in fermentation processes. Presently, in most SSF systems fungi are more suitable than bacterial strains and yeasts, but genetically improved or genetically modified bacterial and yeast strains may be made to suite SSF processes. Bacterial cultures decrease the time required

for fermentation and hence reduce the capital involved. Many difficulties are involved in SSF, that require extensive attention, such as: difficulty in scale-up, requirement for controlling process variables like heat generation, unavailability of direct analytical procedures to determine the biomass directly in the substrate bed, and heterogeneous fermentation conditions. It has been noted that the use of inert support conditions provides good conditions for fermentation along with the purity of the product.[93,143] Improvement in bioreactors, process control for continuous SSF is required in the biotechnology industry for producing most value added products. Analysis of existing literature has proved that most value added products could be produced in higher amounts by SSF than by submerged fermentation. Optimization of the proper substrate and additives are an important part of the process. Recent developments made by various researchers, show that control of heat transfer, scale-up in SSF should be solved through prior laboratory-scale mathematical modeling.

List of symbols

A – float of area, cm^2
A_w – water activity
D – diameter of column, mm, m
d – diameter, mm
d_p – particle diameter, mm
H – height of column, mm, m
h – height of substrate bed, cm
l – length, m
n – amount of substance, μmol
n_S – stirring speed, min^{-1}
P – productivity, U g^{-1} h^{-1}
Q – volume flow rate, L h^{-1}
s – moisture, %
S – hole surface, mm^2
t – time, h
V – volume, L
w – mass fraction, %
Y – yield, %
α – inclination angle, °
γ – mass concentration, g L^{-1}
ϑ – temperature, °C

List of abbreviations

SSF – solid-state fermentation
SMF – submerged fermentation
GDS – gram of dried substrate
IU/gds – International Unit per gram of dried substrate

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