Review Article

Solid State Fermentation: Substrates Uses and Applications in Biomass and Metabolites Production - A Review

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Abstract: Solid-state fermentation (SSF) is the growth of microorganisms without free flowing liquid phase. SSF has been recently considered as the most cheapest and more environmentally friendly relative to submerged liquid fermentation (SLF) in the production of value added industrial based products such as enzymes, bio fuels and the likes. The comparison of SSF and liquid State Fermentation (LSF) has been summarized in a tabular form. The main microorganisms that occupied a pivotal position in achieving absolute SSF processes have been highlighted. A typical bioreactor has been addressed within the concept of SSF. The applications of the process in various economic sectors such as industrial fermentation, agro food industry and environmental control have been reported. Biomass measurement formula is shown, as well as environmental factors, both essential for studying and optimising solid substrate fermentations. 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. This review aimed at gathering the disperse literature on the current state of art on SSF as it concerns biomass and metabolites formation.

Keywords: Biomass, fermentation, metabolites, microbes, solid state fermentation

INTRODUCTION

Solid-state fermentation (SSF) is a 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 bio-processes 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 faecal matter i.e. low in moisture. In SSF, the moisture necessary for microbial growth exists in an absorbed state or in complex with solid matrix.

General considerations

Aerobic microbial transformation of solid materials or "Solid Substrate/State Fermentation" (SSF) can be defined in terms of the following properties:

A solid porous matrix which can be biodegradable or not, but with a large surface area per unit volume, in the range of 103 to 106 m2/ cm3, for a ready microbial growth on the solid/gas interface

The matrix should absorb water amounting one or several times its dry weight with a relatively high water activity on the solid/gas interface in order to allow high rates of biochemical processes.

- Air mixture of oxygen with other gases and aerosols should flow under a relatively low pressure and mix the fermenting mash.

- The solid/gas interface should be a good habitat for the fast development of specific cultures of moulds, yeasts or bacteria, either in pure or mixed cultures.
- The mechanical properties of the solid matrix should stand compression or gentle stirring, as required for a given fermentation process. This requires small granular or fibrous particles, which do not tend to break or stick to each other. The solid matrix should not be contaminated by inhibitors of microbial activities and should be able to absorb or contain available microbial foodstuffs such as carbohydrates (cellulose, starch, sugars) nitrogen sources (ammonia, urea, peptides) and mineral salts.

Typical examples of SSF are traditional fermentations such as

- Japanese *koji* which uses steamed rice as solid substrate inoculated with solid strains of the mould *Aspergillus oryzae.*
- Indonesian *tempeh* or Indian *ragi* which use steamed and cracked legume seeds as solid substrate and a variety of non-toxic moulds as microbial seed.
- French "blue cheese" which uses perforated fresh cheese as substrate and selected moulds, such as *Penicillium roquefortii*as inoculum.
- Composting of lingo-cellulosic fibres, naturally contaminated by a large variety of organisms including cellulolytic bacteria, moulds and *Streptomyces* spp*.*
- In addition to traditional fermentations, new versions of SSF have been invented. For example, it is estimated that nearly a third of industrial SSF and *koji* processes in Japan has been modernised for large scale production of citric acids.

Furthermore, new applications of SSF have been suggested for the production of antibiotics [2], secondary metabolites [3, 4] or enriched foodstuffs [5]. Presently, SSF has been applied to large-scale industrial processes mainly in Japan. Traditional *koji*, manufactured in small wooden and bamboo trays, has changed gradually to more sophisticated processes: fixed bed room fermentations, rotating drum processes and automated stainless steel chambers or trays with microprocessors, electronic sensors and servo mechanical stirring, loading and discharging.

Outside Japan, Kumar [6] has reported medium scale production of enzymes, such as pectinases, in India. *Koji* type processes are widely used in small factories of the Far East [7] and *koji* fermentation has been adapted to local conditions in United States (USA) and other Western countries, including Cuba.

Advantages and disadvantages of SSF and SLF

Comparative studies between submerged liquid fermentation (SLF) and SSF have proved higher yields and other advantages for products made by SSF (1-6):

- The low availability of water reduces the possibilities of contamination by bacteria and yeast. This allows working in aseptic conditions in some cases.
- Higher levels of aeration, especially adequate in those processes demanding an intensive oxidative metabolism.
- Similar environmental conditions to those of the natural habitats for fungi, which constitute the main group of microorganisms used in SSF.
- The inoculation with spores (in those processes that involve fungi) facilitates its uniform dispersion through the medium.
- The substrate usually provides all the nutrients necessary for growth. Therefore culture medium composition is often quite simple.
- Reactors with simple design and few spatial requirements can be used due to the concentrated nature of the substrates.
- SSF is in most cases characterized with low energy requirement which may likely reduces the production cost at industrial level as autoclaving or vapour treatment, mechanical agitation and aeration are not often necessary in some cases.
- Polluting effluents volumes are generally small. Fewer requirements of dissolvent is evident for product extraction due to their high concentration.
- The peculiar feature of low moisture availability may facilitate the production of specific compounds that may not likely be produced or poorly produced in SLF
- The product obtained in SSF has slightly different desired properties i.e. more thermo tolerance relative to their counterparts obtained in SLF.
- Some SSF bioreactors have easier downstream processing despite the aforementioned advantages of SSF over SLF, SSF is beset with following disadvantages as reported by (1-6): The substrate in most cases requires pre-treatment which include size reduction by grinding, physical or chemical land enzymatic hydrolysis and cooking or vapour treatment.
- Microorganisms like bacteria which may require high moisture levels can perform poorly in SSF. Therefore fungi perform better than bacteria, because of its low moisture requirement.
- Difficulties are usually encountered in biomass determination.
- Monitoring of process parameter such as pH, moisture content, substrate, oxygen and biomass concentration becomes a problem because of solid nature of the substrate.
- Static condition is mostly preferred as agitation most often proved to be very difficult.

- The engineering and some scientific characterization of SSF bioreactors is not yet fully matured as such there are scarcity of information about the design and operation of reactors on a large scale.
- There is possibility of contamination with unwanted fungal species.
- Aeration may be difficult sometimes due to high solid concentrations
- Spores needs to be germinated as they usually have longer lag phases, so cultivation times are longer than in SLF.

SSF has several limitations. Table 1 shows advantages and disadvantages of SSF compared to Liquid substrate fermentation.

Fators	Liquid Substrate Fermentation	Solid Substrate Fermentation
	Soluble Substrates (sugars)	Polymer Insoluble Substrates:
Substrates		Starch Cellulose Pectines Lignin
Aseptic conditions	Heat sterilisation and aseptic Control	Vapour treatment, non- sterile conditions
Water	High volumes ofwater consumed and effluents discarded	Limited Consumption of Water; low Aw. No effluent
Metabolic Heating	Easy control of temperature	Low heat transfer capacity Easy aeration and high surface exchange air/substrate
Aeration	Limitation obey soluble oxygen High level of air required	
pH control	Easy pH control	Buffered solid substrates
Mechanical agitation	Good homogenization	Static conditions preferred
Scale up	Industrial equipments Available	Need for Engineering & New design Equipment
Inoculation	Easy inoculation, continuous process	Spore inoculation, batch
Contamination	Risks of contamination for single strain bacteria	Risk of contamination for low rate growth fungi
Energetic consideration	High energy consuming	Low energy consuming
Volume of Equipment	High volumes and high cost Technology	Low volumes and low costs of equipments

Source: [8]

BIOREACTORS IN SSF

A bioreactor is a vessel in which a chemical process is carried out which involves organisms or biochemically active substances derived from such organisms. This process can either be aerobic and anaerobic. 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 [9].

Various researchers have classified the SSF bioreactors broadly [10]but most bioreactors can be distinguished by a factor whether they are used at small scale and large scale. Despite the disadvantages of SSF, scientist still believes that is going to solve many of the present industrial production and some of the environmental plights.

Microorganisms and substrates involved in ssf processes

Bacteria, yeasts and fungi can grow on solid substrates, and find application in SSF processes. Filamentous fungi are the best adapted for SSF and dominate in research works. Some examples of SSF processes for each category of microorganisms are reported in Table 2. Bacteria are mainly involved in composting, ensiling and some food processes. Yeasts can be used for ethanol and food or feed production. But filamentous fungi are the most important group of microorganisms used in SSF process owing to their physiological, enzymological and biochemical properties. The hyphal mode of fungal growth and their good tolerance to low water activity (A_w) and high osmotic pressure conditions make fungi efficient and competitive in natural micro flora for bioconversion of solid substrates.

Koji and *Tempeh* are the two most important applications of SSF with filamentous fungi. *Aspergillus oryzae* is grown on wheat bran and soybean for "*Koji*" production, which is the first step of soy sauce or citric acid fermentation. *Koji* is a concentrated hydrolytic enzyme medium required in further steps of the fermentation process. "*Tempeh*" is an Indonesian fermented food produced

by the growth of *Rhizopus oligosporus* on soybeans. People consume the fermented product after cooking or toasting. The fungal fermentation allows better nutritive quality and degrades some anti-nutritional compounds contained in the crude soybean.

The hyphal mode of growth gives a major advantage to filamentous fungi over unicellular microorganisms in the colonisation of solid substrates and for the utilisation of available nutrients. The basic mode of fungal growth is a combination of apical extension of hyphal tips and the generation of new hyphal tips through branching. An important feature is that, although extension occurs only at the tip at a linear and constant rate, the frequency of branching makes the kinetic growth pattern of biomass exponential, mainly in the first steps of the vegetative stage. The hyphal mode of growth gives the filamentous fungi the power to penetrate into the solid substrates. The cell wall structure attached to the tip and the branching of the mycelium ensures a firm and solid structure. The hydrolytic enzymes are excreted at the hyphal tip, without large dilution like in the case of LSF, what makes the action of hydrolytic enzymes very efficient and allows penetration into most solid substrates. Penetration increases the accessibility of all available nutrients within particles [11].

Table-2: Main groups of microorganisms involved in SSF

MICROFLORA	SSF PROCESS
BACTERIA	
Clostridium spp	Ensiling, Food
Lactobacillus spp	Ensiling, Food
Streptococcus spp	Composting
Pseudomonas spp	Composting
Serratia spp	Composting
Bacillus spp	Composting, Natto, amylase
FUNGI	
Altemaria spp	Composting
Penicilium notatum, roquefortii	Penicillin, Cheese
Lentinus edodes	Shii-take mushroom
Pleurotus oestreatus, sajor-caju	Mushroom
Aspergillus niger	Feed, Proteins, Amylase, ctric acid
Rhizopus oligosporus	*Tempeh*, soybean, amylase, lipase
Aspergillus oryzae	*Koji*, Food, citric acid
Amylomyces rouxii	Tape cassava, rice
Beauveria spp*, Metharizium* spp	Biological control, Bioinsecticide
Trichoderma spp	Composting Biological control, Bioinsecticide
Phanerochaete Schrysosporium	Composting, lignin degradation
Rhizopus spp	Composting. Food, enzymes, organic acids
Mucor spp	Composting, Food; enzyme
Monilia spp	Composting
Fusarium spp	Composting. Gibberellins
Aspergillus spp	Composting, Industrial, Food
Yeast	
Endomicopsis burtonii	Tape, cassava, rice
Schwanniomyces castelli	Ethanol, Amylase
Saccharomyces cerevisiae	Food, Ethanol

Source: [8]

SUBSTRATES

All solid substrates have a common feature: their basic macromolecular structure. In general, substrates for SSF are composite and heterogeneous products from agriculture or by-products of agro-industry. This basic macromolecular structure (e.g. cellulose, starch, pectin, lignocellulose, fibres etc) confers the properties of a solid to the substrate. The structural macromolecule may simply provide an inert matrix (sugarcane bagasse, inert fibres, resins) within which the carbon and energy source (sugars, lipids, organic acids) are adsorbed. But generally, the macromolecular matrix represents the substrate and provides also the carbon and energy source. Preparation and pre-treatment represent the necessary steps to convert the raw substrate into a form suitable for use include:

- Size reduction by grinding, rasping or chopping;
- Physical, chemical or enzymatic hydrolysis of polymers to increase substrate availability by the fungus.
- Supplementation with nutrients (phosphorus, nitrogen, salts) and setting the pH and moisture content, through a mineral solution.
- Cooking or vapour treatment for macromolecular structure pre-degradation and elimination of major contaminants. Pre-treatments will be discussed under individual applications.

The most significant problem of SSF is the high heterogeneity, which makes difficult to focus one category of hydrolytic processes, and leads to poor trials of modelling. This heterogeneity is of different nature:
- non-uniform substrate structure (mixture of starch, lignocellulose, pectin)
- Variability between batches of substrates, limiting there producibility

Difficulty of mixing solid mass in fermentation, in order to avoid compaction, which causes non uniform growth, gradients of temperature, pH and moisture, that makes representative samples almost impossible to obtain. Examples of substrates Ligno-cellulose, Pectins, Lignin and Starch.

LIGNOCELLULOSE
Lignocellulose occurs within plant cell walls, which consist of cellulose microfibrils embedded in lignin, hemicellulose and pectin. Each category of plant material contains variable proportion of each chemical compound. Two major problems can limit lignocellulose breakdown:
- Cellulose exists in four recognised crystal structures known as celluloses I, II, III and IV. Various chemical or thermal treatments can change the structure from crystalline to amorphous.
- Different enzymes are necessary in order degrade cellulose, e.g. endo and exo-cellulases plus cellobiase.

PECTINS
Pectins are polymers of galacturonic acid with different ratio of methylation and branching. Exo and endo pectinases and demethylases that hydrolyse pectin into galacturonic acid and methanol. Hemicelluloses are divided in major three groups: xylans, mannans and galactans. Most of hemicelluloses are heteropolymers containing two to four different types of sugar residues.

LIGNIN
Lignin represents between 26 to 29% of lignocellulose, and is strongly bounded to cellulose and hemicellulose, hiding them and protecting them from the hydrolase attack. Lignin peroxidase is the major enzyme involved in lignin degradation. Phanerochaete chrysosporium is the most recognised fungi for lignin degradation. So; lignocellulose hydrolysis is a very complex process. Effective cellulose hydrolysis requires the synergetic action of several cellulases, hemicellulases and lignin peroxidases. Despite this, lignocellulose is a very abundant and cheap natural renewable material, so a lot of work has been conducted on its microbial breakdown, especially with fungal species.

STARCH
Starch is another very important and abundant natural solid substrate. Many microorganisms are capable to hydrolyse starch, but generally its efficient hydrolysis requires previous gelatinization. Some recent works concern the hydrolysis of the raw (crude or native) starch as it occurs naturally. The chemical structure of starch is relatively simple compared to lignocellulose substrates. Essentially starch is composed of two related polymers in different proportions according to its source: amylose (16-30%) and amylopectin (65-85%). Amylose is a polymer of glucose linked by α 1, 4 bonds, mainly in linear chains. Amylopectin is a large highly branched polymer of glucose including also α-1, 6 bonds at the branch points. Within the plant, cell starch is stored in the form of granules located in amyloplasts, intracellular organelles surrounded by a lipoprotein membrane. Starch granules are highly variable in size and shape depending on the plant material. Granules contain both amorphous and crystalline internal regions in respective proportions of about 30/70.

During the process of gelatinization, starch granules swell when heated in the presence of water, which involves the breaking of hydrogen bonds, especially in the crystalline regions. Many microorganisms can hydrolyse starch, especially fungi which are then suitable for SSF application involving starchy substrates. Glucoamylase, α-amylase, β-amylase, pullulanase and isoamylase are involved in the processes of starch degradation. Mainly α-amylase and glucoamylase are of importance for SSF. Microorganisms generally prefer gelatinised starch. But large quantity of energy is required for gelatinization so it would be attractive to use organisms growing well on raw (ungelatinised) starch. Different works are dedicated to isolate fungi producing enzymes able to degrade raw starch, as has been done by Soccol *et al.* [12], Bergmann *et al.* [13] and Abe *et al.* [14]. Many studies concerning SSF of cassava, a very common tropical starchy crop, have been conducted with the purpose of upgrading protein content, both for animal feeding using Aspergillus spp and for direct human consumption, using Rhizopus.

Various economic applications of solid state fermentation processess
The various economic applications of SSF offer the potential of significantly improving and raising living standards with only a low technology input requirement. Several authors have reviewed the different applications of Solid State Fermentation. SSF is briefly associated with the production of traditional fermented foods such as "koji", Indonesian tempeh or Indian "ragi". SSF has also been used for the production of high added value compounds (such as enzymes, organic acids, biopesticides, biofuel and flavours). In the last years, new applications of SSF in the environmental control have been developed including bioremediation and biodegradation of hazardous compounds and the detoxification of agro industrial residues. Table 3 shows some examples of SSF

processes in economic sectors of continuous research to scale-up the process to industrial level for efficient realizations of fermentation industry, agro industry and environmental control.

Table-3: Main applications of SSF in various economic sectors

Economic Sector	Application	Examples
Industrial fermentation	Enzymes production	Amylases, amyloglucosidase, cellulases, proteases, pectinases, xylanases, glucoamylases
	Bioactive products	Mycotoxins, gibberellins, alkaloids, antibiotics, hormones
	Organic acid production	Citric acid, fumaric acid, itaconic acid, lactic acid
	Biofuel	Ethanol production
	Miscellaneous compounds	Pigments, biosurfactants, vitamins, Xantham
Agro-Food Industry	Biotransformation of crop residues	Traditional food fermented (*Koji*, sake, ragi, *tempeh*), protein enrichment and single cell protein production, mushrooms production.
	Food additives	Aroma compounds, dyestuffs, essential fat and organic acids
Environmental control	Bioremediation and biodegradation of hazardous compounds	Caffeinated residues, pesticides, polychlorinated biphenyls (PCBs)
	Biological detoxification of agroindustrial wastes	Coffee pulp, cassava peels, canola meal, coffee husk

Source: [15]

SSF plays a significant role at laboratory level than SLF and it is often considered a cost effective process than its counterpart for the production of wide arrays of bio products. SSF, as reported by various authors' in terms of bio productions, possess many fold-higher than those obtained in SLF. Despite its advantages it has some major drawbacks such as control of process parameters, adequate scale-up from laboratory bench to industrial level are some of its key plight. The future trend of this phenomenon may improve as a result of desired product in an eco-efficient and sustainable manner.

Biomass measurement of microbial growth on solid state fermentation

Biomass is a fundamental parameter in the characterisation of microbial growth. Its measurement is essential for kinetic studies on SSF. Direct determination of biomass in SSF is very difficult due to the problem of separating the microbial biomass from the substrate. This is especially true for SSF processes involving fungi, because the fungal hyphae penetrate into and bind tightly to the substrate. On the other hand, for the calculation of growth rates and yields, it is the absolute amount of biomass which is important. Methods that have been used for biomass estimation in SSF belong to one of the following categories [16].

DIRECT EVALUATION OF BIOMASS

Complete recovery of fungal biomass is possible only under artificial circumstances in membrane filter culture, because the membrane filter prevents the penetration of the fungal hyphae into the substrate [16]. The whole of the fungal mycelium can be recovered simply by peeling it off the membrane and weighing it directly or after drying. Obviously, this method cannot be used in actual SSF. However, it could find application in the calibration of indirect methods of biomass determination. Indirect biomass estimation methods should be calibrated under conditions as similar as possible to the actual situation in SSF. The global mycelium composition could be estimated through analysis of the mycelium cultivated in LSF in conditions as close as possible to SSF cultivation.

Microscopic observations can also represent a good way to estimate fungal growth in SSF. Naturally, optic examination is not possible at high magnitude but only at stereo microscope. Scanning Electron Microscope (SEM) is a useful tool to observe the pattern of growth in SSF. New approaches and researches are developed for image analysis by computer software in order to evaluate the total length or volume of mycelium on SEM photography. Another new very promising approach is the Confocal Microscopy, based on specific reaction of fungal biomass with specific fluorochrome probes. Resulting 3D images of biomass can open new ways to appreciate and measure biomass *in situ* in a near future.

Since direct measurement of exact biomass in SSF is a very difficult task, then other approaches have been preferred. That for, the global stoichiometric equation of microbial growth can be considered:

Carbon source + Water + Oxygen + Phosphorus + Nitrogen
Biomass + CO2 + Metabolites + Heat

Variation in each component is strictly related to the variation of others when all coefficients are maintained constants. Then, measuring one of them allows determining the evolution of the others.

Environmentalfactors that affect microbial growth during ssf

Environmental factors such as temperature, pH, water activity, oxygen levels and concentrations of nutrients and products significantly affect microbial growth and product formation. In submerged stirred cultures, environmental control is relatively simple because of the homogeneity of the suspension of microbial cells and of the solution of nutrients and products in the liquid phase.

The low moisture content of SSF enables a smaller reactor volume per substrate mass than LSF and also simplifies product recovery [17]. However, serious problems arise with respect to mixing, heat exchange, oxygen transfer, moisture control and gradients of pH, nutrient and product as a consequence of the heterogeneity of the culture.

The latter characteristics of SSF render the measurement and control of the above mentioned parameters difficult, laborious and often inaccurate, thereby limiting the industrial potential of this technology [18]. Due to these problems, the micro-organisms that have been selected for SSF are the more tolerant to a wide range of cultivation conditions [19].

Moisture content and water activity (aw)

SSF process can be defined as microbial growth on solid particles without the presence of free water. The water present in SSF systems exists in a complexed form within the solid matrix or as a thin layer either absorbed to the surface of the particles or less tightly bound within the capillary regions of the solid. Free water will only occur once the saturation capacity of the solid matrix is exceeded. However, the moisture level at which free moisture becomes apparent varies considerably between substrates and is dependent upon their water binding characteristics. For example, free water is observed when the moisture content exceeds 40% in maple bark and 50-55% in rice and cassava [20]. With most lingo-cellulosic substrates free water becomes apparent before the 80 % moisture level is reached [17].

The moisture levels in SSF processes, which vary between 30 and 85%, has a marked effect on growth kinetics [21]. The optimum moisture level for the cultivation of *Aspergillus niger* on rice was 40%, whereas on coffee pulp the level was 80%, which illustrates the unreliability of moisture level as a parameter for predicting microbial growth. It is now generally accepted that the water requirements of microorganisms should be defined in terms of the water activity (Aw) rather than the water content of the solid substrate. Aw is a thermodynamic parameter defined in relation to the chemical potential of water. Aw is related to the condensed phase of absorbed water, but it is well correlated (less than 0.2 % error) to the relative humidity (RH). Therefore: Aw = RH/100 = p/po, where p is the vapour pressure of the water in the substrate and po is the vapour pressure of pure water at the corresponding temperature, R being the ideal gas constant [22]. Aw represents the availability of water for reaction in the solid substrate.

The optimum moisture content for growth and substrate utilisation is between 40 and 70% but depends upon the organism and the substrate used for cultivation. For example, cultivation of *Aspergillus niger* on starchy substrates, such as cassava [15] and wheat bran [23], was optimal at moisture levels considerably lower than on coffee pulp [24] or sugarcane bagasse [25] This is probably because of the greater water holding capacity of the latter substrate [21]. The optimum Aw for growth of a limited number of fungi used in SSF processes were at least 0.96, whereas the minimum Aw required for growth was generally greater than 0 9. This suggests that fungi used in SSF processes are not especially xerophilic. The optimum Aw values for sporulation in *Trichoderma viride* and *Penicillium roqueforti* were lower than those for growth [26]. Maintenance of the Aw at the growth optimum would allow fungal biomass to be produced without sporulation.

Temperature and heat transfer

Stoichiometric global equation of respiration is highly exothermic and heat generation by high levels of fungal activity within the solids lead to thermal gradients because of the limited heat transfer capacity of solid substrates. In aerobic processes, heat generation may be approximated from the rate or CO_2 evolution or O_2 consumption. Each mole of CO_2 produced during the oxidation of carbohydrates releases 673 Kcal. Therefore, it is important to measure CO_2 evolution during SSF because it is directly related to the risk of temperature increase. Detailed calculations of the relation between respiration, metabolic heat and temperature were discussed in early works on SSF with *Aspergillus niger* growing on cassava or potato starch [27]. The overall rate or heat transfer may be limited by the rates of intra- and inter-particle heat transfer and by the rate at which heat is transferred from the particle surface to the gas phase.

Heat removal is probably the most crucial factor in large scale SSF processes. Conventional convection or conductive cooling devices are inadequate for dissipating metabolic heat due to the poor thermal conductivity of most solid substrates and

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results in unacceptable temperature gradients. Only evaporative cooling devices provide sufficient heat elimination capacity. Although the primary function of aeration during aerobic solid state cultivations was to supply oxygen for cell growth and to flush out the produced carbon dioxide, it also serves a critical function in heat and moisture transfer between the solids and the gas phase. The most efficient process for temperature control is water evaporation.

Maintaining constant temperature and moisture content simultaneously in large scale SSF is generally difficult, but using the proper ancillary equipment can do this. The reactor type can have a large influence on the quality of temperature control achieved. It depends highly of the type of SSF: static on clay or vertical exchangers, drums or mechanically agitated.

Control of ph and risks of contamination

The pH of a culture may change in response to metabolic activities. The most obvious reason is the secretion of organic acids such as citric, acetic or lactic, which will cause the pH to decrease, in the same way than ammonium salts consumption. On the other hand, the assimilation of organic acids which may be present in certain media will lead to an increase in pH, and urea hydrolysis will result in alkalinisation. The kinetics of pH variation depends highly on the microorganism. With *Aspergillus sp.*, *Penicillium sp.,* and Rhizopus *sp.* the pH can drop very quickly below 3.0; for other types of fungi, like *Trichoderma*, *Sporotrichum, Pleurotus sp.* the pH is more stable between 4 and 5.

Besides, the nature of the substrate has a strong influence on pH kinetics, due to the buffering effect of lingo-cellulosic materials.

We have used a mixture of ammonium salt and urea to control the pH decrease during growth of *A. niger on* starchy substrates [27]. A degree of pH control maybe obtained by using different ratios of ammonium salts and urea in the substrate. Hydrolysis of urea liberates ammonia, which counteracts the rapid acidification resulting from uptake of the ammonium ion [15]. In this manner, we obtained optimal growth of *Aspergillus niger* on granulated cassava meal when using a 3:2 ratio (on a nitrogen basis) of ammonium to urea. We observed that during the first stage of cultivation the pH increased as the urea was hydrolysed. During the subsequent rapid growth stage, ammonium assimilation exceeded the rate of urea hydrolysis and the pH decreased, but increased again in the stationary phase. During cultivation, the pH remained within the limits of pH 5 to pH 6.2, whereas a lower urea concentration resulted in a rapid decrease in pH.

In a similar way, pH adjustment during pilot plant cultivation of *Trichoderma viride* on sugar-beet pulp was effective by spraying with urea solutions due to the urease activity of the micro-organism that caused an increase in pH by producing ammonia [28].

Finally, in fungal or yeast SSF, bacterial contamination may be minimised or prevented by employing a suitably low pH.

OXYGEN UPTAKE

Aeration fulfils four main functions in SSF, namely (i) to maintain aerobic conditions, (ii) to desorb carbon dioxide, (iii) to regulate the substrate temperature and (iv) to regulate the moisture level. The gas environment may significantly affect the relative levels of biomass and enzyme production. In aerobic LSF oxygen supply is often the growth limiting factor due to the low solubility of oxygen in water. In contrast, a solid state process allows free access of atmospheric oxygen to the substrate. Therefore, aeration may be easier than in submerged cultivations because of the rapid rate of oxygen diffusion into the water film surrounding the insoluble substrate particles, and also because of the very high surface of contact between gas phase, substrate and aerial mycelium. The control of the gas phase and air flow is a simple and practical mean to regulate gas transfer and generally no oxygen limitation is observed in SSF when the solid substrate is particular. It is important to maintain a good balance between the three phases in SSF [29, 30]. By this very simple aeration process, it is also possible to induce metabolic reactions, either by water stress, heat stress or temperature changes, all processes that can drastically change biochemical or metabolic behaviour.

CONCLUSION

SSF is a well-adapted process for cultivation of fungi on vegetal materials which are breakdown by excreted hydrolytic enzymes. In contrast with Liquid State Fermentation (LSF) where water is in large excess, water activity is a limiting factor in SSF. On the other hand, oxygen is a limiting factor in LSF but not in SSF, where aeration is promoted by the porous and particular structure and by the high surface are of contact which facilitate mass transfer between gas and liquid phases. Liquid and solid state fermentation are age-old techniques used for the preservation and manufacturing of foods. During the second half of the twentieth century, liquid state fermentation developed on an industrial scale to manufacture vital metabolites such as antibiotics.

Economic changes and growing environmental awareness generate new perspectives for solid state fermentation. SSF adds value to insoluble agricultural by-products thanks to its higher energy efficiency and reduced water consumption. The renewal of SSF is now possible thanks to engineering firms, mainly from Asia, that have developed a new generation of equipment. Fujiwara makes vessels able to transform substrate areas up to 400 square metres (4,300 sq ft) for the production of soy sauce or sake. Other

companies use solid state fermentation for enzyme complexes. In France Lyven has manufactured Pectinases and Hemicellulases on beet pulp and wheat bran since 1980. The company (now part of Soufflet Group) is now involved in a global R&D programme focusing on SSF technology.

Solid State Fermentation (SSF) are aerobic processes where respiration is fundamental for energy supply but, because respiratory metabolism is highly exothermic, severe limitation of growth can occur when heat transfer is not efficient enough to avoid temperature increase. 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 liquid 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 modelling.

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