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REVIEW ARTICLE

An overview of Engineering Aspects of Solid State Fermentation

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Abstract

Solid substrate cultivation (SSC) or solid state fermentation (SSF) is envisioned as a prominent bio conversion technique to transform natural raw materials into a wide variety of chemical as well as bio-chemical products. This process involves the fermentation of solid substrate medium with microorganism in the absence of free flowing water. Recent developments and concerted focus on SSF enabled it to evolve as a potential bio- technology as an alternative to the traditional chemical synthesis. SSF is being successfully exploited for food production, fuels, enzymes, antibiotics, animal feeds and also for dye degradation. This paper discusses the various micro and macro level engineering problems associated with SSF and some possible solutions for its full commercial realization.

Keywords: Solid-state fermentation, substrate, diffusivity, nutrients, bio-reactor, mass transfer, heat transfer.

INTRODUCTION

Modern chemical synthesis aims at three E's. Energy, Economy and Environment. Any new chemical product today must be produced with minimum energy requirement at optimal cost with zero environmental pollution. As the bio-chemical processes are environment friendly, cost effective and carried out at ambient conditions, satisfy the above constraints of three E's for the production of a wide variety of chemical and biochemical products to serve the modern society. Hence, bio syntheses have emerged as the potential alternative to traditional chemical syntheses. There are two types of bioconversion methods in operation, one is the submerged fermentation (SmF) which is well established and the other is solid state fermentation (SSF), which is still in evolutionary state and under intensive research. There are several recent publications describing the solid state fermentation of agro-industrial residues such as rice bran, rice husk, potato wastes, cassava husk, wheat bran, sugar cane bagasse, sugar beet pulp, palm kernel cake, rice straw, cocoa pod, fruit wastes etc. into bulk chemicals and value added fine products such as ethanol, enzymes, antibiotics, biofuel, mushrooms, organic acids, amino acids, biologically active secondary metabolites (Moo-Young *at al*., 1983; Doelle *at al*., 1992; Pandey and Soccol, 1998; Pandey *at al*., 1999; Soccol and Vandenberghe, 2003; Prasertsans, 1996; Villas-Boas *et al*., 2002). More recently the focus has been the conversion of natural wastes into animal, poultry and fish meals through SSF (Villas-Boas *et al*., 2002; Nigam, 1998; Leonowicz *et al*., 1999). In principle SSF refers to the microbial growth on moist solid substrates or within the pores with out free flow of water. The required moisture for SSF exists in the solid as absorbed or complex form is more helpful for oxygen availability to the microbial population. In SSF the microbe is in contact with atmospheric oxygen unlike in submerged fermentation (SmF). SSF is simpler and requires less processing energy. The basic differences between SSF and SmF are presented in Table 1 for better understanding. Some of the SSF processes developed are published in the literature are also presented in Table 2 (Pandey and Soccol, 1998). The low moisture content means that fermentation can only be carried out by a limited number of microorganisms, mainly yeasts and fungi. Therefore, because of low moisture levels spoilage or contamination by unwanted bacteria is reduced, and more concentrated product is produced.

There have been reviews on some of the aspects of SSF in the literature. However, this paper discusses an overview of the engineering problems with an emphasis on bio reactor design and operation on commercial scale. The paper also highlights some of the commercial applications of SSF.

SSF PROCESS METHODOLOGY

In any SSF process the basic steps carried out are:

1. The preparation of a solid substrate (d_p, pH, Cn, Cs).
2. Sterilization of the substrate.
3. Rising of suitable inoculum. Traditional or pure culture technique.
4. The inoculation of the moist substrate
5. The incubation in appropriate culture of vessels or reactors.

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6. Maintenance of optimal conditions. (pH, T, Hm, mixing, aeration, flow pattern, Q, N_A)
7. Harvesting of solids
8. Drying / Extraction of product
9. Further downstream processing if necessary.

The major problems encountered in the above sequential steps are mainly design of appropriate reactor which can maintain required moisture, temperature and microbe concentration on the solid substrate with no inter and intraparticle oxygen gradients.

Table 1: Basic differences in solid state fermentation and submerged

Solid state fermentation	Submerged fermentation
Medium is not free-flowing	Medium free flowing
Shallow depth	Greater
Single solid substrate provides C, N_2, minerals and energy	Employed
Medium absorbs water, up-takes nutrients.	Disolved in water
Gradients of T, pH, C_s, Cn	Uniform
Minimum water,(lessVolume).	More water, more volume
3 – phase system	2 – phases system
T, O_2, H_2o control (H_2o critical)	T, O_2 control
Inoculum ratio large	Low
Intra particle resistances	No such resistances
Bacterial and yeast cells adhere to solid and grow	Uniformly distributed
Highly concentrated product	Low concentration product

MASS TRANSFER PROBLEMS

The efficiency, productivity and economy of SSF are affected by various factors like mass and heat transport phenomena at micro and macroscopic levels in the reactor. At micro level the mass transfer depends on the nature and growth pattern of the micro organism and their response to local environment change. The growth of microbe depend on inter and intra particle diffusion of gases like O_2, CO_2 and enzymes, nutrients and products of metabolism in the substrate ((Mitchell *et al*., 2003).

At the macro level, the bulk flow of air into and out of the reactor which affects the sensible heat and compositions of O_2, CO_2 and moisture. Problems in SSF occur in industrial reactors where, the problem of the lack of free water and generation of metabolic heat is greatly exaggerated as the system struggles to provide adequate agitation, aeration and cooling. As a result high temperature gradients may result. During forced aeration, natural or forced convection, diffusion and conduction of heat and mass take place in a direction normal to the flow of air. To ensure good heat and mass transfer in the reactor, proper flow rates, contact patters be adopted between the phases in the reactor. Excessive shear forces within the bioreactor due to mixing may affect the functions of the microbe. But reasonable shear will keep it hale and active during the process (Brauer and Sucker, 1979).

The particle size and shape of substrate may affect the flow pattern and porosity in the bed. Mixing and aeration provide good transfer of nutrients and product gases in the bed. To maintain adequate moisture in the bed, continuous monitoring of moisture in the inlet and outlet air is essential. In order to transfer O_2 to the

Microorganism growing on and in the particle it has to cross hydrodynamic boundary layers, interparticle space and diffusion within the particle. Overall seven resistance steps are involved in O_2 transfer to reach the microbe. These resistances will decide specially the highest resistance step, the overall rate of conversion in SSF reactor. The hydrodynamic conditions of air and a moisture across the bed will either increase or decrease the overall rate of transfer of oxygen as well as moisture in the bed (Smith, 1970). In dealing with intraparticle transfer of O_2, nutrients and enzymes secreted by growing microorganisms, the effectiveness factor (η) is a very useful concept. It is the ratio of the observed reaction rate (R_{ob}) to that in the absence of any substrate concentration gradients. Though it is used for quantifying the diffusional limitations in catalysis also applicable to SSF as it is visualized as an heterogeneous system. An important parameter required for the evaluation of the 'η' is the Theile modulus (Φ)

which is the ratio of biochemical reaction rate to that of diffusional mass transfer rate within the solid. By making use of this concept, a criterion can be developed to evaluate intraparticle mass and heat transfer limitations to provide greater insight and understanding into the heat and mass transfer mechanisms in SSF reactors. Mitchel *et al.,* (1990), studied the diffusional limitations of glucoamylase in a gel substrate to convert to glucose. Its production was as low as 20% because they did not consider the O_2 diffusion and consumption at the intraparticle level. Mitchel *et al*., (1990), Rajagopalan *et al* (1995), and Moo-Young *et al*., (1983) studied the diffusional limitations on SSF of different substrates, which hindered the growth rate of microorganisms and product yields.

In systems with forced aeration, O_2 transfer is less likely to be rate limiting, but some nutrients transfer might affect the growth of the microorganism. In the case of filamental fungi, the layer on the substrate depends on the intra particle oxygen transfer and the moisture content of the particle (Durand *et al*., 1998).

DIFFUSION OF ENZYMES

In majority of SSF processes the carbon and energy substrate is macromolecular hence microorganisms cannot transport macromolecular substrate across the cell membrane, so the action of extracellular enzymes in degrading the solid state substrate into soluble fragments is a very important step. Sometimes enzyme diffusion in the SSF reactor could be the rate controlling step (Knapp and Howell, 1980). Thus exoenzymes may experience diffusion and steric limitations depending on the porosity of the macromolecular structure (Suga *et al*., 1975). The diffusion of enzymes is caused by the open pore geometry

of the substrate, but when the porosity of substrate is less the degradation occurs at the outer surface. So, the particle size, shape, porosity, consistency and strength are some of the important parameters to be considered in industrial solid state fermentation as they affect profoundly the heat and mass transfer rates and maintenance of adequate moisture in the bed. (Suga *et al*., 1975; Mitchell *et al*., 1988; Zadrazil and Punia, 1995). However for optimum particle size and structure modifications before the fermentation are yet to be found experimentally though pretreatment techniques like steaming, puffing, extrusion etc can be adapted to enhance the interfacial area and accessibility of O_2, nutrients and enzymes to the microbes in the solid substrate. These mass transfer studies indicate forced circulation of air through bed can enhance O_2 transfer and CO_2 dissipation in the bed if fluidized bed is used as SSF.

HEAT TRANSFER PROBLEMS

The heat generation is directly proportional to the metabolic activity in the SSF reactor (Chahal, 1983; Robinson and Nigam, 2003). As fermentation progresses O_2 diffuses and triggers the bioreactions, liberating heat which accumulates in the reactor due to poor transport property of substrate. Hot spots may develop with a temperature rise of as high as 70^{o}C. This may affect porosity of the bed. The heat removal and regulation depends on the aeration of the fermentation system. High temperatures affect spore germination, growth, product-formation and sporulation, where low temperatures affect adversely (Moreira *et al*., 1981).

Low moisture, poor thermal conductivity of the substrate result in poor heat transfer in SSF. Hence it is very difficult to maintain favourable temperature in the reactor. Thus water addition with continuous mixing is favourable in SSF, which can be achieved in a properly designed fluidized bed bioreactor. The importance of evaporative cooling and moisture content of the substrate on the performance of SSF bioreactor has been highlighted in the literature to control the rising temperature (Nagel *et al*., 2001; Lonsane *et al*., 1985). But this type of cooling may affect the reactor performance (Smits *et al.,* 1999).

Water activity could also drop due to build-up of solutes such as glucose, amino acids, etc. This could be prevented by spraying water on to the solid substrate coupled with mixing. Sufficient water supply must be made available to the growing spores or fungi, and for water activity of the substrate (Raghava Rao *et al.,* 2003). In industrial SSF reactors forced moist air or dry air circulation, cooling the external surface or with internal surface of the reactor with chilled water or by covering it with water soaked burlap are worth trying to control the fermentation temperature.

BIOREACTOR DESIGN

SSF processes could be operated in batches, fed batches or continuous modes. Shear sensitivity of the substrate and the microorganism must be taken into account during reactor design. Over the period a good understanding of SSF led to design, operate and scale up SSF bioreactors. The process is aerobic in nature and contains a solid substrate bed with moisture and porosity. The SSF bioreactor system should fulfill the following requirements.

1. A suitable vessel for holding the solid substrate. The material of construction should be mechanically strong, non-toxic, corrosion resistant and less cost.
2. Environmental friendly. Should be able to control the bio-emissions during its operation.
3. Should be equipped with controls and regulators for effective aeration, mixing, heat removal and moisture control.
4. Sterilization mechanisms (in-situ or off-line)
5. Safe loading and unloading and product recovery systems.

In contrast to submerged fermentation systems, SSF bioreactor systems are yet to reach sophistication and perfection (Nigam, 1998). To overcome the problems of heat and mass transfer phenomena and easy diffusion of O_2, CO_2 and other metabolites, a suitable bioreactor design is yet to be achieved (Pandey, 1994). Temperature and conducive moisture maintenance in the reactor have become the main topics for research. Many bioreactor designs like tray type, packed bed, rotating, rocking drum, stirred type have been proposed recently (Durand, 2003). Mass diffusion mechanisms and limitations, in the interstices and inter particle transfer of by product gasses and O_2 are to be understood clearly for efficient design (Raghava Rao *et al*., 2003). Recently, a product specific bioreactor called PLAFRACTOR (Kumar, 2001) has been reported in literature for pharmaceutical production. Another modern SSF bioreactor is being manufactured by M/S Fujiwara, Japan (2000). All of the above reactors suffer from proper control of design parameters like humidity, oxygen transfer, temperature etc. To address these problems a good computer controlled fluidized bed bioreactor is proposed and is under construction to study the SSF of PKC to animal and poultry feed at UMS, Sabah.

MEASUREMENT AND CONTROL OF SSF PARAMETERS

Measurement and control of state or operating variables is crucial for better performance of the reactors. Moisture, temperature, oxygen and gaseous products like CO_2 concentrations are to be accurately measured on-line and controlled for good fermentation and product yield at optimum levels (Fernandez *et al*., 1997). The operating variables which can be manipulated to achieve optimum conditions depend on the type of reactors and the operating variables like flow rates, humidity of inlet air, frequency and intensity of agitation. Thermocouples can be used for online measurement of temperatures. Sensors will be effective to minimize the error in measurements so

that control becomes easy and accurate. On-line sensors to measure relative humidity, pH, and pressure and concentration gradients across the SSF reactors are the best bet for the accuracy and consistency (Durand, 1998). Smoothing algorithms may be used to account for noise in the measurement of reactor variables. Bio mass estimation can be carried out based on oxygen consumption or CO_2 evolution during the fermentation process. Applications of off-line measurement techniques for water activity, pH and biomass provide a check on on-line measurements as they suffer from less noise (Nagel *et al*., 2000; 2001).

SCALE-UP PROBLEMS OF SSF BIOREACTORS

Scale-up of bioreactors can be done based on chemical engineering fundamental principles of mass and energy balances on moisture, temperature, aeration and oxygen transfer and dynamic conditions of the substrate in the reactor (Le Kanda and Perez – Correa, 2003; Nagel *et al*., 2000; 2001; Saucedo-Castenada *et al*., 1992). The important factor here is to find operating conditions for the bioreactor that will allow the water and the energy balances to remain at a constant value as scale increases. Theory without experiment is dry and experiment without theory is sterile. So, both theory and formulating the model and experiment in verifying their validity is essential. The models proposed should be simple and easily able to incorporate the complexities of SSF processes into the model equation to get better insight into the understanding of the growth kinetics and transport phenomena of heat and mass transfer. Most of the models proposed in the literature to date need improvement and experimental validation (Mitchell *et al*., 2003; Raghava Rao *et al*., 2003). An ideal model of SSF bioreactors should represent the following features.

1. Distribution of substrate particles in the reactor and their dynamics under agitation.
2. Heat generation, transfer and its effect on growth of microbes.
3. O_2 and CO_2 diffusion and their effect on growth.
4. Exoenzyme production and its diffusion.
5. Substrate degradation and uptake.
6. Biomass production and its dynamics.
7. pH and water activity changes.
8. Change in physiology of the biomass.

Such an ideal model may never be achieved but some of the critical parameters can be embedded in the model to describe the true characteristics of SSF processes thereby maximizing their economic performance. The kinetic and transport models of SSF system will help in the design, development and operation of commercial reactors (Doelle *et al*., 1992). Geometric and dynamic similarity approach will be a rationale and realistic one for scale-up of SSF reactors. Dimensional analysis of the parameters affecting the overall performance of the rector must be done critically for a reliable scale-up. The dimension less numbers like Reynolds, Nusselts, Dam Koehler, Weber, Prandtle etc. must be adapted to describe the simulation of the conditions in industrial reactors through laboratory reactor data to ensure adaptability in the designing of commercial reactors. Pressure drop criterion is one of the possible scale-up factors to be explored. There is no information on characterization of heat and mass transfer coefficient in SSF reactors up to date. There is need for experimental work to formulate the models on pressure drop, mass and heat transfer coefficients in SSF labscale bioreactors to adopt in commercial units.

Applications of SSF

Following the global trends on SSF research the potential applications of SSF are classified as follows.

1. Agro-industrial residues conversion into value added and protein enriched end products for poultry and cattle feed. Residues like coffee pulp and husk, soybeans, cassava husk and bagasse, sugarcane bagasse, sugarbeet pulp, fruit wastes, palm tree wastes etc bioconverted into ethanol, single cell protein, organic acids like citric and lactic acids, aminoacids, pigments, antibiotics, mushrooms, biopesticides, gibberellic acid, flavor and aroma compounds, etc. The fungus culture on coffee husk produced a strong alcoholic aroma with fruity flavour compounds such as acetal dehyde, ethanol, etyl acetate, were the major compounds produced. The head space of the cultures composed of the compounds given in Figure 1. Citric acid is probably the only product-produced on large tonnage 500,000 t/yr by fermentation which is used in food and pharmaceutical industry (Soccol *et al*., 2003). Many bacteria, yeast and fungi are capable of growth on solid substrates, but filamentous fungi are the best adapted for SSF process and dominates the research presently due to their physiological capabilities and hyphal mode of growth under low moisture. Filamentous fungi is extensively used for protein enrichment of starch substrates such as cassava, sago and banana wastes as well as of cellulosic substrates such as wheat straw, corn straw and sugar beet pulp (Oriol *et al*., 1988; Gumbira Said *et al*., 1991; Yadav, 1998; Nigam and Vogel, 1988). All these SSF products are aimed for animal feed and animal feed supplementation.

Table 2: Solid state fermentation processes developed

Process/ product	Substrate	Microbe	Reference
Protein enrichment	Cassava bagasse Cassava crude	*Rhizopus sp.*	Soccol *et al*., 1994
Citric acid	Cassava bagasse	*Aspergillus niger*	Vandenberghe *et al*., 2000
Lactic acid	Sugarcane bagasse	*Rhizopus oryzae*	Soccol *et al*., 1994
Mushrooms	Cassava bagasse	*Pleurotus ostrreatus*	Barbosa *et al*., 1997
	Coffee residues	*Lentinus edodes* *Flamulina velutipes*	Leifa *et al*., 2000
Aroma production	Cassava bagasse	*Ceratocystis*	Soares *et al*., 2000
	Coffee husks	*Rhizopus sp.*	
Detoxification	Coffee husks	*Aspergillus sp.*	Brand *et al*., 2000
Bio pesticide	Potato waste	*Bauveria bassiana*	Soccol *et al.,* 1997
Hormones	Coffee husks	*Gibberella fugikuroi*	Machado *et al*.,2000
Xanthan gum	Sugarcane bagasse	*Xanthomonas campestri*	Woiciechowski, 2001
Plant cell culture	Sugarcane bagasse	*Molus prunifolia Borkh*	Radjiskumar, 2001
Amylase	Casava bagasse	*Rhizopus arrhizus*	Pandey *et al*., 2000
Protease	Soybean defatted cake	*Penicillum sp.*	Germana *et al*.,1998

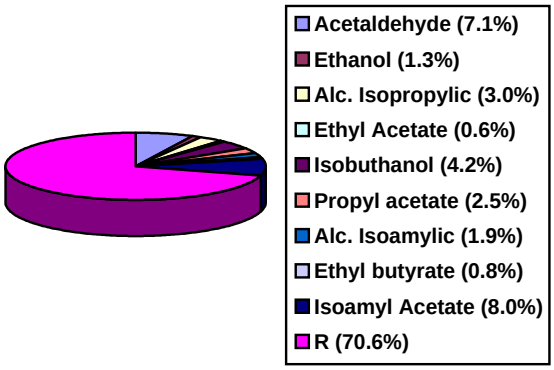

Figure 1: Volatile proportion in headspace of 26% glucose medium with the addition of leucine

2. SSF is increasingly applied in environmental control and monitoring. Bioremediation and biodegradation of hazardous compounds. Biological detoxification of industrial wastes are the latest. Bio insecticides for pest control in crops are looked at with promise by SSF (Pandey *et al*., 2000).

3. Enzyme production. Enzymes like amylase, protease, xylaxase, lipase etc were produced by SSF using cheap carbon sources like cassava bagasse and soybean defatted cake etc. Development of a lab-scale reactor for tannase production from coffee industrial waste is reported by some of the researchers in recent literature. Laccase and Manganese-peroxidase are produced from malted barley waste using *Lentinus edodes*. Amyloglucosidase and lignin peroxidase were also produced from SSF process (Pandey *et al*., 2002; Lagemaat and Pyle, 2001; Halvani and Mecs, 2001).

4. Biopulping of wheat straw using *Phanerochaete chrysosporium* was reported by Chen *et al* (1998). Work is also being carried out-on enzyme inhibitors and bio molecules production through SSF (Chen *et al*., 2002).

CONCLUSIONS

A country like Malaysia whose biotechnological potential abilities depend not only on the reuse of agro-industrial residues but also value addition to the agriculture commodities like palm kernel cake, palm tree wastes, forest wastes, tea wastes, tapioca and sago wastes, fruit waste, municipal solid waste etc. are produced in huge quantities annually, which have low value in the market. SSF could be perfected for value-addition and utilization of these products and their residues to boost the economy of the nation. Hence the research on SSF to develop commercial processes with techno-economic feasibility is worth continuing. The intricacies in solid state fermentation technology is to be understood clearly through modeling, kinetics of growth of microbes, control of parameters etc, and finally scale up and commercialization of SSF processes are essential for establishing the SSF technologies to apply in divergent areas.

NOMENCLATURE:

SSF	Solid State Fermentation
SmF	Submerged fermentation
T	Temperature (^oC)
pH	Hydrogen ion potential
C_s	Substrate concentration
C_n	Nutrients concentration
d_p	Diameter of the particle
Hm	Humidity of the air
Q	Heat transfer rate
N_A	Mass transfer rate

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