Current Developments in Solid-state Fermentation

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Preface

God did not create filamentous fungi to grow in fermenter.

–A.P.J. Trinci

Submerged fermentation is an artificial condition (artefact) for filamentous fungi because they live in Nature in solid state.

There could not be any better view than the above about the application of solid-state fermentation for developing bioprocesses involving micro-organisms, especially filamentous fungi. Over the period of last two decades, there has been significant resurgent in solid-state fermentation due to numerous benefits it offers, especially on engineering and environmental aspects. SSF has shown much promise in the development of several bioprocesses and products, which include high volumetric productivity, relatively higher concentration of the products, less effluent generation, and simple fermentation equipments, etc. This resurgent gained further momentum during the last 5-10 years with the development of knowledge-base in the fundamental and applied aspects. A good deal of information has been generated in the published literature and patented information. Several commercial ventures have come up based on SSF in different parts of the world. It was, thus, thought crucial to publish a document allowing to get state-of-the art information in this area, in order to demonstrate that the well-established liquid stirred tank is not always the best technical solution.

During 2001, a book was published by Asiatech Publishers, Inc. on SSF. The book was well received globally. The present book is based on the previous work, although that was an authored volume while this one is an edited work. Also, the coverage in the present volume is much wider and comprehensive.

The book covers a wide range of topics in the field of solid-state fermentation. The contents of the book have been distributed in four parts. The Part 1 deals with the General and Fundamentals aspects of SSF and comprises eight chapters. The Chapter 1 is Introductory and describes the history, development and scientific elaboration of SSF. The Chapter 2 discusses various general issues related with SSF. The Chapter 3 describes the factors that influence SSF, which include physio-chemical and biological factors. The Chapters 4 and 5 are on kinetics and water relations in SSF, respectively. The Chapter 6 describes the designs of different bioreactors (fermenters) developed and used for carrying out SSF in laboratory, or at commercial scale. The Chapter 7 is on instrumentation and controls in SSF and the chapter 8 describes the use of informatics in SSF.

The Part 2 of the book comprises four chapters, which are on the production of bulk chemicals and products in SSF. These include industrial enzymes, organic acids, spores and mushrooms. The Part 3 of the book has also four chapters, which are on the use of SSF for the specialty chemicals such as gibberellic acid, antibiotics and other commercially valuable secondary metabolites, pigments and aroma compounds. The fourth and last part of the book (Part 4) deals with the use of SSF for miscellaneous application such as SSF for the food and feed applications, agro-industrial residues as substrates in the SSF and the production of silage and vermicompost.

All the chapters have incorporated the most significant developments taken place during the last 5-10 years with state-of-art information.

We are hopeful that the book would be useful to the students, teachers, researchers and professionals interested in the area of the industrial biotechnology and microbiology.

Ashok Pandey
Carlos Ricardo Soccol
Christian Larroche

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PART 1

General and Fundamentals Aspects of SSF

1

Introduction

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1.1 BIOPROCESS DEVELOPMENT

Solid-state (substrate) fermentation (SSF) is generally defined as the growth of the micro-organisms on (moist) solid material in the absence or near-absence of free water. In recent years, SSF has shown much promise in the development of several bioprocesses and products. It seems that two terms, solid-state fermentation and solid substrate fermentation have often been ambiguously used. It would be logical only to distinguish these two terms. Solid substrate fermentation should be used to define only those processes in which the substrate itself acts as carbon/energy source, occurring in the absence or near-absence of free water; solid-state fermentation should define any fermentation process occurring in the absence or near-absence of free water, employing a natural substrate as above, or an inert substrate used as solid support.

The history, development and scientific elaboration of SSF have been reviewed by several authors from time to time. Evidently, food fermentation and production of enzymes were the areas where SSF originated. The recorded history of SSF was described in Asia before the birth of Christ on cheese making by *Penicillium rouquefortii*. Egyptians were reported to make bread using SSF process in 2000 BC. The use of soy sauce *koji* in China was reported in the years 1000 BP and probably in the years 3000 BP (*koji* process involves fermentation of steamed rice as solid substrate by the fungal strain of *Aspergillus oryzae*). The *koji* process migrated from China to Japan by the Buddhist priests in seventh century. During this period, there were mentions of several fermented foods such as tempeh, miso etc., in many South-East Asian countries. Tempeh and miso use steamed and cracked legume seeds as solid substrate and fermentation is carried out using non-pathogenic fungal strains such as *Rhizopus* sp.

During the 18th century, for the first time, SSF was used for the production of vinegar from the apple pomace. The period also saw the development of leather tanning process using gallic acid. The period of late 19th century saw the development of composting and solid waste treatment using SSF processes. The beginning of 20th century witnessed for the first time the production of primary metabolites such as enzymes and organic acids using micro-organisms in SSF. In these processes, mostly fungal cultures were used as producing organisms. It was this period exactly when maiden concepts appeared to develop fermenters (bioreactors) suitable for SSF processes, which led to the development of drum-type of fermenters.

The period of 1940's has been termed as the 'Golden Era' of fermentation industry, which saw the dramatic discovery and development of the wonder drug, penicillin. Penicillin was produced using liquid as well as solid culturing techniques. However, this was the period, when, for the reasons best known to the researchers of that period, much attention was paid on the development of liquid fermentation processes and some how or other, knowingly or unknowingly, SSF got totally neglected. This was typically the case with the penicillin, which continued for all other fermentation products. Consequently, there were no serious efforts by the researchers to develop SSF systems, except a few isolated studies, which still continued focusing SSF. Even with such low profiles, during the period of 1950-60, reports were published describing steroid transformations in SSF using fungal cultures, which was yet another milestone achievement in the history of SSF. This was again followed by the period of 1970's when fungal cultures were successfully cultivated in SSF for the production of mycotoxins, which resulted a significant impact on cancer research. During this period, yet another important application oriented finding of SSF research was on the production of protein enriched cattle feed (single-cell protein). Enormous work, since then has been carried out on this process using a large number of substrates and micro-organisms, and various processes with techno-economic feasibility have been successfully developed.

Thus, though historically known since centuries, SSF gained a fresh attention from researchers and industries all over the world since recent few years, mainly due to few major advantages which it offers over liquid (submerged) fermentation (SmF), particularly in the areas of solid waste management, biomass energy conservation and its application to produce high value – low volume products such as biologically active secondary metabolites, etc., apart from the production of food, feed, fuel and traditional bulk chemicals.

Attempts were made to trace the history of SSF describing the general features and also the aspects of fermenter design in SSF (Pandey 1991, 1992, 2003, Pandey et al., 2001, Durand 2003). These reviews discussed various developments since historical time. During 1991-2006, more than 1400 publications have appeared in various journals, proceedings and books, apart from several important publications in book form, or special issue of journals. A few reviews have also been presented discussing some particular features of SSF from time to time. A special review by CW Hesseltine was on the *Thom Award Address,* which reprinted his work on SSF. This very well signified the biotechnological potential of SSF globally. Significantly, it has been CW Hesseltine who first consolidated the scientific information on SSF in 1977 (Pandey 2003, Pandey et al., 2000, 2001, Hoelker 2004, Robinson et al., 2001, Mitchell et al., 2002, Tengerdy and Szakacs 2003, Weinberg and Ashbell 2003, Gervais and Molin 2003, Pandey and Ramachandran 2005).

Current decade has witnessed an unprecedented spurt in SSF for the development of bioprocesses such as bioremediation and biodegradation of hazardous compounds, biological detoxification of agro-industrial residues, biotransformation of crops and crop-residues for nutritional enrichment, biopulping, and production of value-added products such as biologically active secondary metabolites, including antibiotics, alkaloids, plant growth factors, etc., enzymes, organic acids, biopesticides, including mycopesticides and bioherbicides, biosurfactants, biofuel, aroma compounds, etc. in SSF system. During the past time, most of such processes were eventually termed as 'low-technology' systems but presently seen to be a promising one for the production of value-added 'low volume-high cost' products such as biopharmaceuticals. SSF processes offer potential advantages in bioremediation and biological detoxification of hazardous and toxic compounds. With the advent of biotechnological innovations, mainly in the area of enzyme and fermentation technology, many new avenues have opened for the application of SSF. Over the past few years, the increasing demand for the natural products in the food industry has encouraged remarkable efforts towards the development of biotechnological processes for the production of flavour compounds. The use of SSF as a means to improve economical feasibility of these processes would be of potential benefit (Longo and Sanroman 2006).

Holker et al., (2004) opined that despite the increasing number of publications dealing with solid-state (substrate) fermentation (SSF), it was very difficult to draw general conclusion from the data presented. The authors remarked that this was due to the lack of proper standardisation that would allow objective comparison with other processes. Research work has so far focused on the general applicability of SSF for the production of enzymes, metabolites and spores, in that many different solid substrates (agricultural waste) have been combined with many different fungi and the productivity of each fermentation reported. They further commented that on a gram bench-scale SSF appeared to be superior to submerged fermentation technology (SmF) in several aspects. However, SSF up-scaling, necessary for use on an industrial scale, raises severe engineering problems due to the build-up of temperature, pH, O_2, substrate and moisture gradients. Recently, Viniegra-Gonzalez and Favela-Torres (2006) made a critical review of the phenomenon of resistance to catabolite repression of enzyme synthesis by SSF, commenting the practical and theoretical importance of such phenomenon, together with the current ideas to explain it.

Mathematical models have been considered as important tools for optimizing the design and operation of SSF bioreactors. Such models must describe the transport phenomena within the substrate bed and mass and energy exchanges between the bed and the other subsystems of the bioreactor, such as the bioreactor wall and headspace gases. The sophistication with which this has been done for SSF, has improved markedly over the last decade, or so (Mitchell et al., 2003). Mathematical models also must describe the kinetics of microbial growth, how this is affected by the environmental conditions and how this growth affects the environmental conditions. This is done at two levels of sophistication. In many bioreactor models the kinetics are described by the simple empirical equations. However, other models that address the interaction of growth with intraparticle diffusion of enzymes, hydrolysis products and O_2 with the use of mechanistic equations have also been proposed, and give insights into how these microscale processes can potentially limit the overall performance of a bioreactor (Mitchell et al., 2004).

An important development has been in developing sensors and measurements in SSF processes. In a review, Bellon-Maurel et al., (2003) discussed current on-line methods and innovative applications of methods with a potential to measure parameters in SSF. Given the complexity and heterogeneity of the solid medium, process variables are not easily accessible and measurable. Direct measurements of temperature, pH, and water content are considered employing classical sensors, and indirect measurements of the biomass by respirometry or pressure drop (PD). More recent methods such as: aroma sensing, infrared spectrometry, artificial vision, and tomographic techniques (X-rays, Magnetic Resonance Imaging or MRI) should be explored.

1.2 ASPECTS OF DESIGN OF FERMENTER FOR SSF

Design of fermenter for SSF processes is an important aspect. However, in spite of strong resurgent of SSF in last ten years, bioreactor design aspects have not been given enough attention by the researchers, although there are certainly path-breaking developments. Present knowledge, however, does not provide state-of-art information about an ideal fermenter for SSF processes.

Table 2 gives an overview of different types of bioreactors used in solid-state fermentation as fermenters. As is evident, most of the designs are based on two models: tray type or drum type with or without mixing devices and modifications. *Koji* process for soy sauce is considered as a representative of SSF processes. Traditionally this has been carried out in wooden trays. Attempts were also made to operate *koji* process in drum type of bioreactors. However,

Table 1. History and development of solid-state fermentation

Period	***Development***
2000 BC	Bread making by Egyptians
Before birth of Christ in Asia (Recorded history 1000 BP)	Cheese making by *Penicilium roquefortii*
2500 BP	Fish fermentation/preservation with sugar, starch, salts, etc.
2500 BP	*koji* process
7th Century	*koji* process from China to Japan
18th Century	Vinegar from pomace
18th Century	Use of gallic acid in tanning, printing, etc.
1860-1900	Sewage treatment
1900-1920	Production of fungal enzymes, kojic acid
1920-1940	Production of fungal enzymes, gluconic acid, citric acid, development of drum-type fermenter
1940-1950	Fantastic development in fermentation industry, penicillin production
1950-1960	Steriod transformation
1960-1980	Production of mycotoxins, protein enriched feed
1980-1990	Production of various primary and secondary metabolites, development of column type of fermenter, work on kinetics and modelling aspects of SSF
1990-present	Developments on fundamental aspects of SSF, bioprocesses/ products developments:
A. Bioprocess	bioremediation and biodegradation of hazardous compounds, biological detoxification of agro-industrial residues, biotransformations, biopulping, etc.,
B. Products	*Bioactive compounds*: Aflatoxin, ochratoxin, bacterial endotoxins, gibberellic acid, zearalenone, ergot alkaloids, penicillin, cephalosporin, cephamycin C, tetracycline, chlorotetracycline, oxytetracycline, iturin, actinorhodin, methylenomycin, surfactin, monorden, cyclosporin A, ustiloxins, antifungal volatiles, destrucxins A & B, clavulanic acid, mycophenolic acid *Enzymes*: Cellulase, β-glucosidase, CMCase, laccase, xylanase, polygalacturonase, ligninase, xylanases, β-xylosidase, α-arabinofuranosidase, laccase, Li-peroxidase, Mn-peroxidase, aryl-alcohol oxidase, catalase, phenol oxidase, proteases (acidic, neutral and alkaline), lipases, α-galactosidase, β-galactosidase, α-amylase, β-amylase,

Period	*Development*
	glucoamylase, glutaminase, inulinase, phytase, tannase, feruloyl para-coumaroyl esterase *Organic acids*: Citric acid, fumaric acid, lactic acid, oxalic acid, gallic acid *Other products*: L-glutamic acid, pigments, carotenoid, xanthan gum, succinoglycan, ethanol, aroma compounds, vitamins B-12,B-6, riboflavin, thiamine, nicotinic acid, nicotinamide, *g*amma-linolenic acid, biosurfactants, biopesticides/bioherbicides.

Table 2. Developments in design of fermenter for solid-state fermentation

Type of fermenter	*Process/product*	*Micro-organism*
Wooden trays [1923]	Koji fermentation	*Aspergillus oryzae*
Rotating drum [1962]	Cpmposting	mixed cultures
Rotating drum [1964]	Koji fermentation	*A. oryzae*
Rotating drum [1969]	Enzymes	*A. oryzae*
Fermbach flask [1966, 1968, 1983]	Aflatoxins, spores	*A. oryzae*
Aluminium pot [1943]	Enzymes	*A. oryzae*
Horizontal vessel [1975]	Ochratoxins	*A. ochraces*
Horizontal drum [1976]	Continuous process feedlot	fugal strains
Cement mixer [1977]	Corn fermentation	fungal and yeast strains
Cement mixer [1975]	Straw fermentation	*Trichoderma, Candia*
Bread making blender [1979]	Protein enrichment	*A. niger*
Corn storage bin [1979]	Aflatoxins	*A. flavus*
Horizontal vessel [1979]	Koji	*A. oryzae*
Aluminium trays [1980]	Ligno-cellulose fermentation	fungal strains
Valmic bags (porous films) [1985]	Mycotoxins	*A. flavus*
Fixed bed differential reactor [1989]	Spore production	*Penicillium rouqefortii*
Glass columns [1980, 1991, 1992]	Enzyme production	*A. niger*
Glass columns [1999, 2000]	Citric acid, gibberellic acid	fungal strains
Solid-state chamber [2006]	Conidia production	fungal strains
Packed bed [1992, 2006]	Modeling	*G. fujikuori*
Air pulsation dynamic reactor [2005]	Cellulase	*Penicillium decumbens*
Immersion [2006]	Laccase	*Trametes hirsute*
Packed bed	Modeling	fungal strains

Source: Pandey 1991, Pandey et al., 2001, Meien et al., 2004, Chen et al., 2005,Ye et al., 2006, Fernández-Fernández & Pérez-Correa, 2006, Rodríguez-Couto et al., 2006.

not much successes were achieved. As early as in 1964, a 5-gallons rotating drum type of bioreactor was used for the production of fungal enzymes. Some researchers proposed some modifications such as dividing the drum by baffles (three- or four-chambered) to achieve improved mixing and better product formation. While initially drum-type of bioreactors were thought to be useful for processes such as protein enrichment of cattle feed, several attempts were made to use them for production of other products such as enzymes and mycotoxins. For the ease of handing and cost-effectiveness, cement mixers of various sizes have also been tried as bioreactor for SSF processes. A bread-making blender was modified to suit the process requirements of cattle feed production using agricultural residues.

Drum-type of bioreactors have often been used for the bioprocesses where mixing of the substrate during fermentation was recommended useful, which could be achieved by the rotation. There has not been any clear principle behind the extent of mixing of the substrate. While most of the researchers have used a low rotation speed (1-15 rpm), some have used high rates of rotation. One disadvantage, in particular with high rates of rotation, was the damage to fungal mycelia. However, there are contradicting reports concerning the damage per purporated to be cast by mixing. For example, in some reports the mixing (hand or mechanical) of a 3-day duration tray *Koji* for soya sauce production was considered to be essential while other reports stressed the serious check to enzyme production and heat generation due to the mixing. In case of the composting processes, mixing in long drums mounted on a gentle incline position on continuous mode has been considered to produce excellent effect.

During the last 20 years, the concept of column and deep trough type of bioreactor design emerged. These were reported to be useful for the product developments with efficient process controls, particularly for heat removal. However, much is still to be done to realize an optimally suitable size of column for multiple purposes. Moreover, scale-up of column bioreactors poses difficulties.

Durand (2003) reviewed the various reactor designs for SSF, focusing on the differences between lab-scale and industrial-scale designs. He highlighted the main designs that have emerged over the last 10 years and the potential for scaling-up for each category of reactor. Robinson and Nigam (2003) reviewed the bioreactor designs and their use for protein production under SSF conditions using various agricultural by-products. The advantages and disadvantages of various bioreactors and their potential for scale-up are described. SSF was proposed as a suitable low-tech strategy for protein enrichment for animal feed by converting a previously low value substance into a more nutritionally valuable one. Recently, Khanahmadi et al., (2006) described the performance of continuous solid-state bioreactors having two different solid substrates flow patterns, namely plug flow and completely mixed flow. The performance was quantified for both steady-state and transient operation using a simple mathematical model.

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2

General Considerations about Solid-state Fermentation Processes

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2.1 INTRODUCTION

SSF offers numerous advantages over submerged fermentation (SmF). These include high volumetric productivity, relatively higher concentration of the products, less effluent generation, and simple fermentation equipments, etc. The major factors, which affect microbial growth and activity in SSF include the selection of a suitable micro-organism and substrate, pre-treatment of the substrate, particle size (inter-particle space and surface area) of the substrate, moisture content and water activity (a_w) of the substrate, relative humidity, type and size of inoculum, temperature of fermenting matter, removal of metabolic heat generated during respiration, period of cultivation, maintenance of uniformity in environment of SSF, and the gaseous atmosphere, i.e. oxygen consumption rate and carbon dioxide evolution rate.

Based upon the type of micro-organisms involved, SSF processes can be classified into two main groups, natural (indigenous) SSF, and pure culture SSF using individual strains, or a mixed-culture. Composting and ensiling are the two best examples of SSF processes, which involve natural micro-flora. Bioprocesses involving pure culture SSF are also known since long. One such unique example is *Koji* process utilizing a culture of *Aspergillus oryzae*. In industrial SSF processes, however, generally pure cultures are used as it helps in optimum substrate utilization for the targeted product. Examples are the production of enzymes, organic acids, bioactive secondary metabolites, etc. Examples of SSF processes involving mixed (pure) cultures are bioconversion of agro-industrial residues such as wheat straw for protein enrichment in which a cellulolytic organism (e.g. a fungal strain of *Chaetomium cellulolyticum*) and a yeast strain (e.g. *Candida utilis*) are used. In nature, SSF is mostly carried out by the mixed cultures in which several micro-organisms show development and symbiotic co-operation.

Selection of a suitable strain is one of the most important criteria in SSF. The vast majority of wild type micro-organisms are incapable of producing commercially acceptable yields of the desired strains product. The importance of the micro-organism can be seen from the fact that culture of *Aspergillus niger* can produce as many as 21 types of enzymes (Table 1) while one enzyme alone can be produced by several microorganisms in varying quantities. For example, alpha amylase can be produced by as many as 64 different micro-organisms (Table 2). It is also important to ensure that the selected microbial strain is best suited for SSF as some times the same strain's potential could be not best exploited in SSF but in other systems. Such an example is illustrated in Table 3. As is evident, the strain of *Rhizopus formosaensis* produced glucoamylase enzyme in varying concentrations when grown as surface culture in submerged, or solid-state fermentation, or as submerged culture. Yet another crucial aspect involves the screening of the strain for its efficiency to produce the desired end-product as the sole product, although there could be other associated activities but these should be in trace amounts only. This could be exemplified from the results described in Table 4, which showed a case study involving the production of alpha amylase by a strain of *Trichoderma viride*. Indeed this study involved screening of various carbons sources as well. With all the substrates, there was production of another enzyme, namely protease in varying concentrations. These results showed that although corn starch gave the best yields of alpha amylase, the organism also produced simultaneously protease in significant quantities, which, in fact was more than the targeted end-product, i.e. alpha amylase. Thus, corn starch was not a suitable substrate for this process using the selected strain. When lactose was used as the substrate, alpha amylase production was almost negligible and protease production was dominant. However, yield of protease in this case was almost half to that produced by corn starch, which showed that although strain produced only protease as major end-product, this was not suitable for that (protease production) also, as with other substrates, enzyme yields were higher than this case. The results demonstrated that the strain was neither a suitable choice for the production of alpha amylase, nor for protease from the substrates used in the experiment. From these examples, it could be concluded that the selection of the strain for any process development, and so for SSF as well, is an extremely significant step and must be studied with utmost care. While doing so, it would be necessary to keep in mind all the necessary associated factors such as process technique, substrate, associated products formation, etc.

The selection of a substrate for SSF process depends upon several factors, mainly related with the cost and availability and, thus, may involve their screening. Agro-industrial residues, particularly of tropical origin offer potential advantages for their application as substrates. In SSF process, the solid substrate not only

Table 1. Spectrum of enzymes obtainable from the strains of *Aspergillus niger*

Sl. No.	*Name of enzyme*
1.	Alpha amylase
2.	Beta amylase
3.	Glucoamylase
4.	Lactase
5.	Catalase
6.	Protease
7.	Trehalase
8.	Tannase
9.	Dipetalase
10.	Polypetalase
11.	Lipase
12.	Xylanase
13.	Polygalactouronase
14.	Amidase
15.	Cellulases
16.	Glucose oxidase
17.	Glucose dehydrogenase
18.	Urease
19.	Insulase
20.	Melibase
21.	Zymase

Table 2. Micro-organisms reported to produce alpha amylase

1.	*Aeromonas caviae*
2.	*Alicyclobacillus acidocaldarius*
3.	*Alteromonas haloplanetis*
4.	*Anaerobic bacterium*
5.	*Archaeobacterium pyrococcus woesei*
6.	*Aspergillus* sp.
7.	*A awamori*
8.	*A. flavus*
9.	*A. fumigatus*
10.	*A. kawachi*
11.	*A. niger*
12.	*A. oryzae*
13.	*A. usanii*
14.	*Bacillus* sp.
15.	*B. acidocoldarius*
16.	*B. amyloliquefaciens*
17.	*B. brevis*
18.	*B. circulans*
19.	*B. coagulans*

20.	*B. flavothermus*
21.	*B. globisporus*
22.	*B. licheniformis*
23.	*B. megaterium*
24.	*B. stearothermophilus*
25.	*B. subtilis*
26.	*Chloroflexus aurantiacus*
27.	*Clostridium acetobutylicum*
28.	*C. butricum*
29.	*C. thermohydrosulfuricum*
30.	*C. thermosulfurogenes*
31.	*Eubacterium* sp.
32.	*Filobasidium capsuligenum*
33.	*Halobacterium halobium*
34.	*H. salinarium*
35.	*Humicola insolens*
36.	*H. lanuginosa*
37.	*H. stellata*
38.	*Lactobacillus brevis*
39.	*L. cellobiosus*
40.	*Malbrachea pulchella var. sulfurea*
41.	*Micrococcus luteus*
42.	*M. varians*
43.	*Micromonospora vulgaris*
44.	*Mucor pusillus*
45.	*Myceliophthora thermophila*
46.	*Myxococcus coralloides*
47.	*Nocardia asteroides*
48.	*Penicillium brunneum*
49.	*Pseudomonsa stutzeri*
50.	*Pycnoporus sanguineus*
51.	*Pyrococcus woesei*
52.	*Rhizopus* sp.
53.	*Scytalidium* sp.
54.	*Schizophyllum commune*
55.	*Talaromyces thermophilus*
56.	*Thermus* sp.
57.	*Thermoactinomyces* sp.
58.	*T. vulgaricus*
59.	*Thermococcus profundus*
60.	*Thermomonospora viridis*
61.	*Thermonospora curvata*
62.	*T. vulgaris*
63.	*Thermomyces lanuginosus*
64.	*Thermotoga maritima*

Table 3. Glucoamylase production by a strain of *Rhizopus formosaensis*

Fermentation method	*Enzyme yield*
Submerged fermentation	44.32 U/ml
Surface culture in submerged fermentation	74.25 U/ml
Surface culture in Solid-state fermentation	5120 U/g

Table 4. Screening of alpha amylase production by a strain of *Trichoderma viride*

Carbon source in the medium	*Alpha amylase U/ml*	*Protease U/ml*
Corn starch	235	351
Malotose	179	175
Glucose	52	243
Sucrose	17	350
Lactose	3	175

supplies the nutrients to the microbial culture growing in it, but also serves as an anchorage for the cells. The substrate that provides all the needed nutrients to the micro-organisms growing in it should be considered as the ideal substrate. However, some of the nutrients may be available in sub-optimal concentrations, or even not present in the substrates. In such cases, it would be necessary to supplement them externally. It has also been a practice to pre-treat (chemically, or mechanically) some substrates before use in SSF processes (e.g. ligno-cellulosics), which makes them more easily accessible for microbial growth.

Among the several other factors, which are important for the microbial growth and activity in a particular substrate, particle size and moisture level/water activity are the most critical. Generally, smaller substrate particles would provide larger surface area for the microbial attack and, thus, should be considered as a desirable factor. However, too small substrate particles may result substrate agglomeration in most of the cases, which may interfere with microbial respiration/ aeration, and, thus, may result poor cellular growth. At the same time, larger particles provide better respiration/aeration efficiency (due to increased inter-particle space) but provide limited surface for the microbial attack. Thus, it would be necessary to arrive at a compromised particle size for a particular process. Table 5 shows a typical example of particle size distribution in three different commercial samples of wheat bran, which is the most frequently used substrate in SSF processes. Sample A and B were obtained from a local market in India while sample C was prepared by mixing A and B in 1:1 ratio (w/w). Sample A contained 76% particle smaller than 500 microns while sample B has only 11% particles smaller than 500 microns. Sample C showed a wide

Table 5. Particle size distribution in a commercial samples of wheat bran

Particle size	*Percent distribution*		
	Sample A	*B*	*C*
Bigger than 1.4.mm	1.06	41.72	21.39
1.0-1.4 mm	1.10	27.57	14.35
850 µm-1.00 mm	2.38	11.69	7.04
600-850 µm	10.04	4.90	7.47
500-600 µm	8.41	3.04	5.73
425-400 µm	17.03	3.35	10.19
300-425 µm	13.83	2.76	8.30
180-300 µm	21.98	2.02	13.00
Smaller than 180 µm	21.45	2.82	12.14

Source: Pandey A, 1991, Bioresource Technololgy, 37, 169-172

distribution of particles of various sizes containing approximately 44% smaller and 56% large than 500 microns. When SSF was carried out using these samples by taking individual sized particles prepared from these samples by sieving and individual samples as well, best results on enzyme production were obtained by the substrate containing particles of 425-500 microns, followed by 500-600 microns. Substrates with lower particles resulted in lower enzyme activity and the same was true for bigger particles substrates. The lowest enzyme activity was obtained with the substrate containing particles bigger than 1.4 mm. The specific enzyme activity of sample A was much better than sample B but this was best in sample C, which showed a unique distribution of particles of different sizes. Although, enzyme titres obtained from sample C was not the same as from the best individual sized particles substrate, from the techno-economic feasibility point of view, it was the most appropriate to use the mixed substrate.

Substrate moisture and water activity play very important role in SSF. The water activity of the medium is considered as a fundamental parameter for the mass transfer of the water and solutes across the cell membrane. In fact, the control of this parameter could be used to control and modify the metabolic activity of the micro-organism. In general the types of micro-organisms that can grow in SSF system are determined by the water activity factor, a_w. Water activity is defined as the relative humidity of the gaseous atmosphere in equilibrium with the substrate. Pure water has $a_w = 1.00$ and a_w decreases with the addition of solutes. Bacteria generally grow at higher a_w values while filamentous fungi and some yeast can grow relatively at lower a_w values. Due to this fact, filamentous fungi have often been preferred over the other groups of micro-organisms for SSF processes. In case of fungal growth in SSF, higher water activity generally results sporulation, while low water activity with suitable range favours spore germination and mycelial growth.

Research on the selection of a suitable substrate has mainly centred around tropical agro-industrial crops and residues. These include crops such as cassava, soybean, sugar beet, sweet potato, potato, and sweet sorghum, crop residues such as bran and straw of wheat and rice, hull of soy, corn and rice, bagasse of sugarcane and cassava, residues of coffee processing industry such as coffee pulp, coffee husk, coffee spent-ground, residues of fruit-processing industries such as pomace of apple and grape, wastes of pine-apple and carrot processing, banana waste, waste of oil-processing mills such as coconut cake, soybean cake, peanut cake, canola meal and palm oil mill waste, and others such as saw-dust, corn cobs, carob pods, tea waste, chicory roots, etc. Wheat bran has been the prime among all. Many processes have been developed that utilize these as raw material for the production of bulk chemicals and value-added fine products. We would not go into details of these aspects here, as there is a separate chapter exclusively devoted to this in the next section.

In relation to SSF processes using inert substrates, two approaches have been adopted; one, in which synthetic materials such as Amberlite, or polyurethane are used, and other which utilises natural materials such as sugarcane bagasse as the inert solid support. Since natural substrates create problems in the fermentation kinetics studies (due to their heterogeneous nature), such studies could better be performed using synthetic inert solid substrates.

2.2 ESTIMATION OF GROWTH IN SSF

In SSF, estimation of biomass, which is essential for the kinetic studies, poses difficulties as generally the microbial cells remain attached with the substrate and it is difficult to separate them. In case of the fungal cultures, it is more difficult because fungal mycelia penetrate into the substrate particles. Generally the estimation of growth of microorganism in SSF is carried out by the indirect methods, although for model studies, some methods are used, which provide direct growth estimation. For example, gelatin could be used to grow microbial cells, e.g. *Saccharomyces cerevisae* in solid cultures and after the growth, gelatin could be melted at about 37°C and subsequently the cells could be recovered from this melted medium by centrifugation. For yeast cells, one another method has been applied which involved the washing of fermented matter with water and then counting the cells in the washed medium. However, the efficiency of method largely depends upon the efficiency of washing out the cells from the fermented matter.

Among the indirect or the other methods used for the estimation of microbial growth in SSF, assay of biomass components such as DNA, glucosamine, ergosterol and protein (Kjeldahl) or assay of metabolic activity (respirometry) have been considered useful. Estimation of glucosamine for fungal biomass assay has been considered as the most suitable and frequently used method.

In recent times, particularly while using column type of bioreactors, estimation of oxygen utilization rate (OUR) and carbon dioxide evolution rate (CER) are considered to be most accurate for the determination of growth of the microorganisms in SSF. Some more details about growth estimation methods have been provided in another chapter in the next section.

2.3 CONCEPT OF STERILITY

Sterility is very often required in any SmF process because many contaminants could out-compete the process organism under the conditions of high water availability provided. Often SSF processes involve an organism, which grows quite rapidly under the low water conditions, and if an active inoculum is added to a (cooked) substrate, the process organism is able to out-compete the contaminating organisms. Thus, strict aseptic operation of the bioreactor may not be essential in SSF, although, of course, operation should be carried out in as clean a manner as possible. The less stringent design requirements for such bioreactors, and correspondingly lower costs could be considered as favourable point for the SSF process, providing an economic advantage over the SmF process. However, there are a number of products for which SSF has good potential but for which the process organisms grow generally relatively slowly, which could be overtaken by contaminants. For example, this is the case in the production of gibberellic acid by *Giberella* sp. In such cases, it would be essential to use a bioreactor, which can be operated under aseptic conditions. In such a case, the bioreactor costs would be expected to be similar to those for SmF bioreactors, and SSF would only be chosen if it could provide any specific advantage, such as higher product titres, or lower downstream processing costs, which finally could lead to better economic performance.

2.4 ACADEMIC RESEARCH VS. INDUSTRIAL APPLICATIONS

In order to understand this, let's look into some fundamental questions. There is a lot of research going on into SSF - why? There are relatively few commercial applications (at least compared to SmF) - why? A general opinion about the choice of fermentation method for the production of any microbial product would normally be SmF, unless there appears a particular reason why SSF should be chosen. There is no question that SmF is intrinsically less problematic-heat transfer is better, and homogeneity is much-much better. SSF would be chosen if (a) particular economic conditions favored it (therefore, in some parts of the world some enzymes are produced by SSF, whereas in other parts, the same enzymes are produced by SmF), (b) the product is only produced in SSF - or if produced in both systems, the SSF product is far superior (for example, fungal spores for use as biopesticides tend to be much more robust when they are produced in SSF compared to when they are produced in SmF, and some fungi simply don't sporulate well in SLF), (c) use of solids becomes an imperative (government regulations in response to environmental pressures caused by dumping of organic solids).

Thus, although SSF has potential mainly in specific areas, under the conditions that it is "off the main track", it has received relatively little attention. Its potential to operate reliably at large-scale simply has not been investigated to the same degree as the SmF method. It is possible that SSF processes could be routinely operated at large-scale, following rational design rules - but we currently don't know enough to really be sure if this is the case or not. Certainly, there are a few quite successful SSF processes - such as enzyme production processes, various biopesticide production processes, etc, even if many of these are at relatively small scales. There is also the case with the *koji* industry.

SSF should not be seen as a technology, which can simply replace SmF. In fact, SmF has many features, which would make it the preferred method in a case where SmF and SSF had similar economic performances. The greater homogeneity in a SmF system simply makes the process less problematic. The relatively few commercial SSF processes compared to the range of products, which have been investigated in the laboratory, could probably be a confirmation of this. However, there are a number of products for which SSF is the superior production technology, and a number of large-scale SSF processes have begun operating commercially. There is a continued need to develop SSF technology to allow such processes to operate at their full potential.

2.5 COMPARISON OF CHARACTERISTICS OF SSF AND SUBMERGED FERMENTATION

Table 6 highlights the major differences in the characteristics of solid and submerged fermentation. As is evident, due to static nature of substrate and microorganism, the system poses difficulties for heat and mass transfer effects. Due to respiration, carbon dioxide evolves and being heavier than oxygen, remains accumulated in the substrate bed, which in turn results in increase in the temperature of the fermenting bed. This needs to be controlled. Two other characteristics of SSF, mainly low physical energy requirements and high human