


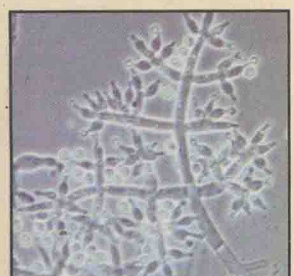
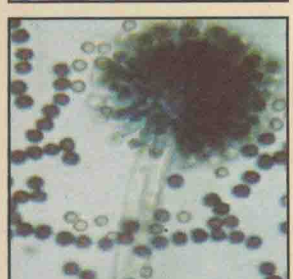
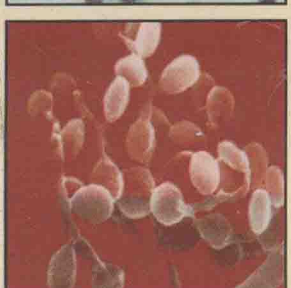

Current Developments in Solid-state Fermentation

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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.

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Contents

Part 1: General and Fundamentals Aspects of SSF

PART 1

General and Fundamentals Aspects of SSF

1 Introduction

Ashok Pandey, Carlos R Soccol & Christian Larroche

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,