ELSEVIER

Biochemical Engineering Journal 13 (2003) 81–84

Biochemical Engineering Journal

www.elsevier.com/locate/bej

Solid-state fermentation

Ashok Pandey*

Biotechnology Division, Regional Research Laboratory, Council of Scientific and Industrial Research, Trivandrum-695 019, India

Received 15 November 2001; accepted after revision 24 July 2002

Abstract

Solid-state fermentation has emerged as a potential technology for the production of microbial products such as feed, fuel, food, industrial chemicals and pharmaceutical products. Its application in bioprocesses such as bioleaching, biobeneficiation, bioremediation, biopulping, etc. has offered several advantages. Utilisation of agro-industrial residues as substrates in SSF processes provides an alternative avenue and value-addition to these otherwise under- or non-utilised residues. Today with better understanding of biochemical engineering aspects, particularly on mathematical modelling and design of bioreactors (fermenters), it is possible to scale up SSF processes and some designs have been developed for commercialisation. It is hoped that with continuity in current trends, SSF technology would be well developed at par with submerged fermentation technology in times to come.

Keywords: Solid-state fermentation; General aspects; Biochemical engineering aspects; Modelling; Design of bioreactor

1. Introduction

Solid-state fermentation (SSF) is defined as the fermentation involving solids in absence (or near absence) of free water; however, substrate must possess enough moisture to support growth and metabolism of micro-organism [1–4]. SSF stimulates the growth of micro-organisms in nature on moist solids and has been credited to be responsible for the beginning of fermentation technique in ancient time [5]. It is, therefore, not surprising that almost all the fermentation processes used in ancient time were based on the principles of SSF. SSF offers numerous opportunities in processing of agro-industrial residues. This is partly because solid-state processes have lower energy requirements, produce lesser wastewater and are environmental-friendly as they resolve the problem of solid wastes disposal.

A glance at the history of fermentation technology indicates that the SSF processes were nearly completely ignored in western countries after 1940 due to adaptation of submerged fermentation (SmF) technology. However, perhaps there was no logical reasoning for this at that time. Since the development of penicillin took place in SmF and due to enormous importance of penicillin during the world war, SmF became a role model technology for production of any compound by fermentation. Subsequently, researchers of that time put their entire attention on SmF and probably unknowingly SSF was neglected. Still in the isolated pockets research continued on SSF systems and during 1950–1960, steroid transformation was reported using fungal cultures. The trend continued, although slowly and SSF attained another milestone during 1960–1970 when reports appeared on mycotoxins production by SSF. Production of protein enriched cattle feed was the next major activity reported, which involved utilisation of agro-industrial residues, thus offering a unique process development for value-addition of these otherwise low cost residues (and to some extent environment pollutants). In fact, this was one of the areas, which generated interest of researchers globally on SSF. Since then, there has been continuous increase in the extension of SSF arena, which picked up strongly during the last one decade. A large numbers of patents and publications have appeared on fundamental aspects of SSF, development of bioreactors (fermenters), modelling and on production of various microbial products such as food, feed, various primary and secondary metabolites, and bioprocesses such as bioleaching, biopulping, bioremediation, biobeneficiation, etc. [6–12].

2. General aspects of SSF

There are several important aspects, which should be considered in general for the development of any bioprocess in

---

* Tel.: +91-471-515-279; fax: +91-471-491-712.
E-mail addresses: pandey@csrrltrd.ren.nic.in, ashokp_56@hotmail.com (A. Pandey).

1369-703X/02/$ – see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S1369-703X(02)00121-3

SSF. These include selection of suitable micro-organism and substrate, optimisation of process parameters and isolation and purification of the product. Going by theoretical classification based on water activity, only fungi and yeast were termed as suitable micro-organisms for SSF. It was thought that due to high water activity requirement, bacterial cultures might not be suitable for SSF. However, experience has shown that bacterial cultures can be well managed and manipulated for SSF processes [1,3,13–15]. It has been generally claimed that product yields are mostly higher in SSF in comparison to SmF. However, so far there is not any established scale or method to compare product yields in SSF and SmF in true terms. The exact reasoning for higher product titres in SSF is not well known currently. The logical reasoning given is that in SSF microbial cultures are closer to their natural habitat and probably hence their activity is increased.

Selection of a proper substrate is another key aspect of SSF. In SSF, solid material is non-soluble that acts both as physical support and source of nutrients. Solid material could be a naturally occurring solid substrate such as agricultural crops, agro-industrial residues or inert support [1,3,4,16–22]. However, it is not necessary to combine the role of support and substrate but rather reproduce the conditions of low water activity and high oxygen transference by using a nutritionally inert material soaked with a nutrient solution. In relation to selection of substrate, there could be two major considerations; one that there is a specific substrate, which requires suitable value-addition and/or disposal. The second could be related with the goal of producing a specific product from a suitable substrate. In the latter case, it would be necessary to screen various substrates and select the most suitable one. Similarly it would be important to screen suitable micro-organisms and select the most suitable one. If inert materials such as polyurethane foam are used, product isolation could be relatively simpler and cheaper than using naturally occurring raw materials such as wheat bran because while extracting the product after fermentation, along with the product, several other water-soluble components from the substrate also leach out and may pose difficulties in purification process. Inert materials have been often used for studying modelling or other fundamental aspects of SSF.

Other relevant issues here could be the selection of process parameters and their optimisation. These include physicochemical and biochemical parameters such as particle size, initial moisture, pH and pre-treatment of the substrate, relative humidity, temperature of incubation, agitation and aeration, age and size of the inoculum, supplementation of nutrients such as N, P and trace elements, supplementation of additional carbon source and inducers, extraction of product and its purification, etc. Depending upon the kind, level and application of experimentation, single and/or multiple variable parameters optimisation method could be used for these. A brief detail of some of these is provided in the next section.

3. Biochemical engineering aspects of SSF

In recent years, several excellent reports have appeared providing a great deal of knowledge and understanding of the fundamental aspects of SSF and today we know much better information about the heat and mass transfer effects in SSF processes, which have been considered as the main difficulties in handling SSF systems. However, there still remains much to be done in this regard. During SSF, a large amount of heat is generated, which is directly proportional to the metabolic activities of the micro-organism. The solid materials/matrices used for SSF have low thermal conductivities, hence heat removal from the process could be very slow. Sometimes accumulation of heat is high, which denatures the product formed and accumulated in the bed. Temperature in some locations of the bed could be 20 °C higher than the incubation temperature. In the early phases of SSF, temperature and concentration of oxygen remain uniform throughout the substrate but as the fermentation progresses, oxygen transfer takes place resulting in the generation of heat. The transfer of heat into or out of SSF system is closely related with the aeration of fermentation system. The temperature of the substrate is also very critical in SSF as it ultimately affects the growth of the micro-organism, spore formation and germination, and product formation. High moistures results in decreased substrate porosity, which in turn prevents oxygen penetration. This may help bacterial contamination. On the other hand, low moisture content may lead to poor accessibility of nutrients resulting in poor microbial growth.

Water relations in SSF must be critically evaluated. Water activity (a_w) of the substrate has determinant influence on microbial activity. In general, the type of micro-organism that can grow in SSF systems are determined by a_w. The importance of a_w has widely been studied by various authors. The a_w of the medium has been attributed as a fundamental parameter for mass transfer of the water and solutes across the microbial cells. The control of this parameter could be used to modify the metabolic production or excretion of a micro-organism.

3.1. Modelling in SSF

Modelling in SSF system is another important aspect, which needs to be studied in detail. Not enough information is available on kinetics of reactions in SSF systems. This is mainly because of difficulties involved in the measurements of growth parameters, analysis of cellular growth and determination of substrate consumption, etc., which is caused due to heterogeneous nature of the substrate, which are structurally and nutritionally complex. Among the several approaches to tackle this problem, an important one has been to use a synthetic model substrate. It is well known that the fermentation kinetics are sensitive to the variation in ambient and internal gas compositions. The cellular growth of the micro-organisms can be determined by measuring the change in gaseous compositions inside the bioreactor. This can also be determined by substrate digestion, heating and centrifuging substrate, using light reflectance, DNA measurement by glucosamine level, protein content, oxygen uptake rate and carbon dioxide evolution rate.

3.2. Design of bioreactor in SSF

Over the last decade, there has been a significant improvement in understanding of how to design, operate and scale up SSF bioreactors. The key to these advances has been the application of mathematical modelling techniques to describe various physicochemical and biochemical phenomena within the system [23–26]. The basic principle of SSF is the "solid substrate bed". This bed contains the moist solids and an inter particle voids phase. SSF has been conventionally more applicable for filamentous fungi, which grow on the surface of the particle and penetrate through the inter particle spaces into the depth of the bed. The process in most of the cases is aerobic in nature. The suitable bioreactor design to overcome the heat and mass transfer effects, and easy diffusion and extraction of metabolites has become the topic of hot pursuit. While tray and drum type fermenters have been studied and used since long, much focus has been paid in last few years on developing packed bed fermenters as they could provide better process economics and a great deal of handling ease [27–31]. A tray bioreactor could have unmixed beds without forced aeration of (manually) mixed bed without forced aeration. However, there has been no significant advances in tray design. Packed beds could be unmixed beds with forced aeration and rotating drums could have intermittent agitation without forced aeration, operating on continuous or semi-continuous mode. The bed could be agitated intermittently or continuously with forced aeration [23].

4. Applications of SSF

Current trends on SSF have focused on application of SSF for the development of bioprocess 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, enzymes, organic acids, biopesticides, including mycopesticides and bioherbicides, biosurfactants, biofuel, aroma compounds, etc. SSF systems, which during the previous two decades were termed as a 'low-technology' system appear to be a promising ones 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 [1,3,12,32–40].

5. Conclusions

There have been significant developments in SSF technology over past few years. Several approaches have been applied to resolve the issues related with the biochemical engineering aspects of SSF, which include kinetics, mathematical modelling, design of bioreactors, advanced control systems to SSF processes, etc. Modelling could be a good tool for scale-up studies but such results need to be validated by experimental findings. Thus, continuos efforts would be needed to develop SSF as feasible technology for production of microbial products on commercial scale in equivalent terms to liquid fermentation technique.

References

[1] A. Pandey, Recent process developments in solid-state fermentation, Process Biochem. 27 (2) (1992) 109–117.

[2] A. Pandey, Solid-state fermentation: an overview, in: A. Pandey (Ed.), Solid State Fermentation, Wiley, New Delhi, India, 1994, pp. 3–10.

[3] A. Pandey, C.R. Soccol, D.A. Mitchell, New developments in solid-state fermentation. I. Bioprocesses and products, Process Biochem. 35 (10) (2000) 1153–1169.

[4] A. Pandey, C.R. Soccol, J.A.R. Leo, P. Nigam, Solid-state Fermentation in Biotechnology, Asiatech Publishers, Inc., New Delhi, 2001, p. 221.

[5] D.A. Mitchell, B.K. Lonsane, Definition, characterization and economic evaluation, in: H.W. Doelle, C. Rolz (Eds.), General Principles of Solid Substrate Fermentation, Rapid Publications of Oxford Ltd., UK, 1990.

[6] D.N. Ogbonna, T.G. Sokari, S.C. Achinewhu, Development of an owoh-type product from African yam beans (*Sphenostylis stenocarpa*) (Hoechst (ex. A. Rich.) Harms.) seeds by solid substrate fermentation, Plant Foods Human Nutr. 56 (2) (2001) 183–194.

[7] B.Z. Han, F.M. Rombouts, M.J.R. Nout, A Chinese fermented soybean food, Int. J. Food Microbiol. 65 (1–2) (2001) 1–10.

[8] M.S.Y. Haddadin, I.M. Abu-Reesh, F.A.S. Haddadin, R.K. Robinson, Utilisation of tomato pomace as a substrate for the production of vitamin B-12—a preliminary appraisal, Bioresource Technol. 78 (3) (2001) 225–230.

[9] X.X. Yang, H.Z. Chen, H.L. Gao, Z.H. Li, Bioconversion of corn straw by coupling ensiling and solid-state fermentation, Bioresource Technol. 78 (3) (2001) 277–280.

[10] A. Pandey, P. Selvakumar, C.R. Soccol, P. Nigam, Solid-state fermentation for the production of industrial enzymes, Curr. Sci. 77 (1) (1999) 149–162.

[11] J.J. Classen, C.R. Engler, C.M. Kenerley, A.D. Whittaker, A logistic model of subsurface fungal growth with application to bioremediation, J. Environ. Sci. Health A 35 (4) (2000) 465–488.

[12] A.B.P. Medeiros, A. Pandey, R.J.S. Freitas, P. Christen, C.R. Soccol, Optimization of production of aroma compounds by *Kluyveromyces marxianus* in solid-state fermentation using factorial design and response surface methodology, Biochem. Eng. J. 6 (1) (2000) 33–39.

[13] K.M. Nampoothiri, A. Pandey, Solid state fermentation for L-glutamic acid production using *Brevibacterium* sp., Biotechnol. Lett. 16 (2) (1996) 199–204.

[14] P. Selvakumar, A. Pandey, Solid-state fermentation for the synthesis of inulinase from the strains of *Staphylococcus* sp. and *Kluyveromyces marxianus*, Process Biochem. 34 (8) (1999) 851–855.

[15] A. Pandey, P. Kavita, P. Selvakumar, Culture conditions for production of 2-1-β-D-fructan-fructanohydrolase in solid culturing on chicory (*Cichorium intybus*) roots, Braz. Arch. Biol. Technol. 41 (2) (1998) 231–236.

[16] A. Pandey, C.R. Soccol, P. Nigam, D. Brand, R. Mohan, S. Roussos, Biotechnological potential of coffee pulp and coffee husk for bioprocesses, Biochem. Eng. J. 6 (2) (2000) 153–162.

[17] A. Pandey, C.R. Soccol, P. Nigam, V.T. Soccol, L.P.S. Vandenberghe, R. Mohan, Biotechnological potential of agro-industrial residues. II. *Cassava* bagasse, Bioresource Technol. 74 (1) (2000) 81–87.

[18] A. Pandey, C.R. Soccol, P. Nigam, V.T. Soccol, Biotechnological potential of agro-industrial residues. I. Sugarcane bagasse, Bioresource Technol. 74 (1) (2000) 69–80.

[19] A. Pandey, C.R. Soccol, Economic utilization of crop residues for value addition—a futuristic approach, J. Sci. Ind. Res. 59 (1) (2000) 12–22.

[20] A. Pandey, G. Szakacs, C.R. Soccol, J.A.R. Leon, V.T. Soccol, Production, purification and properties of microbial phytases, Bioresource Technol. 77 (3) (2001) 203–214.

[21] M.R. Peralta-Perez, G. Saucedo-Castaneda, M. Gutierrez-Rojas, A. Campero, SiO_2 xerogel: a suitable inert support for microbial growth, J. Sol–Gel Sci. Technol. 20 (1) (2001) 105–110.

[22] M. Hoogschagen, Y. Zhu, H. van As, J. Tramper, A. Rinzema, Influence of wheat type and pretreatment on fungal growth in solid-state fermentation, Biotechnol. Lett. 23 (14) (2001) 1183–1187.

[23] D.A. Mitchell, N. Krieger, D.M. Stuart, A. Pandey, New developments in solid-state fermentation. II. Rational approaches for bioreactor design and operation, Process Biochem. 35 (10) (2000) 1211–1225.

[24] A. Pandey, Aspects for design of fermenter in solid-state fermentation, Process Biochem. 26 (3) (1991) 355–361.

[25] J.P. Smits, H.M. van Sonsbeek, J. Tramper, W. Knol, W.W. Geelhoed, M. Peeters, A. Rinzema, Modelling fungal solid-state fermentation: the role of inactivation kinetics, Bioprocess Eng. 20 (5) (1999) 391–404.

[26] J.P. Smits, A. Rinzema, J. Tramper, H.M. van Sonsbeek, J.C. Hage, A. Kaynak, W. Knol, The influence of temperature on kinetics in solid-state fermentation, Enzyme Microb. Technol. 22 (1998) 50–57.

[27] M.T. Hardin, T. Howes, D.A. Mitchell, Residence time distributions of gas flowing through rotating drum bioreactors, Biotechnol. Bioeng. 74 (2001) 145–153.

[28] M.Y. Lu, I.S. Maddox, J.D. Brooks, Application of a multi-layer packed-bed reactor to citric acid production in solid-state fermentation using *Aspergillus niger*, Process Biochem. 33 (1998) 117–123.

[29] T. Carrasco, E. Valino, I. Medina, D. Ravelo, Design and evaluation of a bioreactor for solid state fermentation, Cuban J. Agric. Sci. 33 (4) (1999) 409–414.

[30] M.Y.P. Lillo, R. Perez-Correa, E. Agosin, E. Latrille, Indirect measurement of water content in an aseptic solid substrate cultivation pilot-scale bioreactor, Biotechnol. Bioeng. 76 (1) (2001) 44–51.

[31] D.A. Mitchell, A. Pandey, P. Sangsurasak, N. Krieger, Scale-up strategies for packed-bed bioreactors for solid-state fermentation, Process Biochem. 35 (1–2) (2000) 167–178.

[32] J. Schmidt, G. Szakacs, E. Cenkvari, J. Sipocz, K. Urbanszki, R.P. Tengerdy, Enzyme assisted ensiling of alfalfa with enzymes by solid substrate fermentation, Bioresource Technol. 76 (2001) 207–212.

[33] P. Nigam, D. Singh, Processing of agricultural wastes in solid-state fermentation for microbial protein production, J. Sci. Ind. Res. 55 (1996) 373–380.

[34] M. Moo Young, A.R. Moreira, R.P. Tengerdy, Principles of solid substrate fermentation, in: J.E. Smith, D.R. Berry, B. Kristiansen (Eds.), Fungal Technology, Filamentous Fungi, vol. 4, Arnold, London, 1983, pp. 117–144.

[35] D. Brand, A. Pandey, J.A.R. Leon, S. Roussos, I. Brand, C.R. Soccol, Packed bed column fermenter and kinetic modeling for upgrading the nutritional quality of coffee husk in solid-state fermentation, Biotechnol. Prog. 17 (6) (2001) 1065–1070.

[36] P. Gautam, A. Sabu, A. Pandey, G. Szakacs, C.R. Soccol, Microbial production of extra-cellular phytase using polystyrene as inert solid support, Bioresource Technol. 83 (3) (2002) 229–233.

[37] A.B.P. Medeiros, A. Pandey, P. Christen, P.S.G. Fontoura, R.J.S. de Freitas, C.R. Soccol, Aroma compounds by *Kluyveromyces marxianus* in solid-state fermentation on a packed bed column bioreactor, World J. Microbiol. Biotechnol. 17 (2001) 767–771.

[38] P. Kashyap, A. Sabu, A. Pandey, G. Szakacs, C.R. Soccol, Extra-cellular L-glutaminase production by *Zygosaccharomyces rouxii* under solid-state fermentation, Process Biochem. 38 (2002) 307–312.

[39] D. Brand, A. Pandey, S. Roussos, C.R. Soccol, Biological detoxification of coffee husk by filamentous fungi using a solid state fermentation system, Enzyme Microb. Technol. 27 (1–2) (2000) 127–133.

[40] M. Soares, P. Christen, A. Pandey, C.R. Soccol, Fruity flavour production by *Ceratocystis fimbriata* grown on coffee husk in solid-state fermentation, Process Biochem. 35 (8) (2000) 857–861.