Biochemical Engineering Journal 81 (2013) 146–161

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Review

Current developments in solid-state fermentation

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ARTICLE INFO

Article history:
Received 22 July 2013
Received in revised form
14 September 2013
Accepted 15 October 2013
Available online xxx

Keywords:
Solid-state fermentation
Bioprocess design
Enzyme production
Modeling production kinetics
Agro-industrial residues
Other industrial products

ABSTRACT

Solid-state fermentation (SSF) is a three-phase heterogeneous process, comprising solid, liquid and gaseous phases, which offers potential benefits for the microbial cultivation for bioprocesses and products development. Over the last two decades, SSF has gained significant attention for the development of industrial bioprocesses, particularly due to lower energy requirement associated with higher product yields and less wastewater production with lesser risk of bacterial contamination. In addition, it is eco-friendly, as mostly utilizes solid agro-industrial wastes (resides) as the substrate (source of carbon). This article aims to present and analyze the current development on SSF taken place mainly during the last five years, linking the developments with earlier two papers published in this journal in 2003 (Pandey, 2003 [1]) and in 2009 (Singhania et al., 2009 [2]). The article reviews the current state-of-art scenario and perspectives on the development of bioprocesses and products in SSF and also discusses microbes employed in these processes, the types of bioreactors used for these and also presents the modeling and kinetics aspects.

Contents

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http://dx.doi.org/10.1016/j.bej.2013.10.013

1. Introduction

Solid-state fermentation (SSF) has continued to built up credibility in recent years in biotech industries due to its potential applications in the production of biologically active secondary metabolites, apart from feed, fuel, food, industrial chemicals and pharmaceutical products and has emerged as an attractive alternative to submerged fermentation [1,2]. Bioremediation, bioleaching, biopulping, biobeneficiation, biological delignification, etc. are the major applications of SSF in bioprocesses, which have set another milestone. The human quest for eco-friendly and green processes in place of chemical processes for the production of industrial products has turned the industrial manufacturing strongly 'bio-based'. SSF has attained much relevance in this context during the last one decade as SSF processes offer potential environmental benefits [3]. Yet another very relevant concern, though of generic nature, in this regard is the economic feasibility and sustainability of the bioprocesses, where also SSF offers potential benefits, as it utilizes low-cost agro-industrial residues as the substrate, which is very attractive for bioprocessing.

SSF has been defined as the bioprocess carried out in the absence, or near-absence of free water; however, the substrate must possess enough moisture to support the growth and metabolic activity of the microorganism. The solid matrix could be either the source of carbon (and other nutrients), or it could be an inert material to support the growth of the microorganisms on it (with impregnated growth solution).

As has been advocated, the potential of SSF is to provide the cultivated microorganism an environment as close and in vicinity as possible to natural environment where usually they exist and from where they are isolated. This apparently is the main factor why microbes perform well and give higher products yields in SSF when compared with the liquid fermentation carried out in a closed bioreactor, even if with optimal conditions for growth and activity. As mentioned above, the use of agro-industrial residues and by-products as feedstock in SSF processes on one hand adds economic value to these wastes, or by-products, and on other hand it solves the problem of their disposal, which otherwise would cause pollution [3].

There has been substantial improvement in the fundamental understanding the biochemical engineering aspects, particularly on mathematical modeling and design of bioreactors (fermenters) during the last decade, which has helped in developing several designs for the SSF bioreactors. These have also helped in better understanding of heat and mass transfer effects, leading to better design of process and product developments.

We had earlier reviewed the development of SSF and attributed its growth and developments as the timely need due to environmental and economic benefits it offered. The paper presented the detailed perspectives in 2003 [1], which was followed by another in 2009, describing the developments taken place chiefly in five years since the publication of 2009 [2]. The present article focuses on SSF process and product developments mainly from the last five years since the publication of 2009 article and provides an update to our previous reviews.

2. Critical aspects of SSF

SSF is governed by a large number of factors, each of which is critical for the technical and economic feasibility of the process development. While several of these are of generic nature, they still hold a significant impact and need to be considered in a holistic manner. These included the selection of microorganism and substrate, optimum physical–chemical and biological process parameters and also purification of the desired products, which have been a challenge for SSF. In general, fungal and yeast cultures have been considered as the most suitable microorganisms for SSF processes. This has been essentially based on the theoretical concept of water activity, as fungi and yeast have lower water activity requirements, typically around 0.5–06 a_w. Bacterial cultures have higher water activity requirement (around 0.8–09 a_w), which tend them not suitable for SSF processes. However, it is now well established that this theoretical concept was not correct as a large number of bioprocesses have been described, which are bacterial-based. The choice of the microbe should apparently be linked with the selection of the substrate and product aimed at.

The identification of the physiology of the microorganisms and the physico-chemical factors where it grows leads to the development of process parameters, which are required for its optimal growth and activity. These factors include temperature, pH, aeration, water activity and moisture, bed properties, nature of solid substrate employed, including the particle size, etc. These must be optimized based on factorial design experiments and response surface methodology so as to identify the critical factors and their interactions. Modern biotechnological tools involving artificial neural network (ANN) and genetic algorithm offer potential advantage for the optimization of bioprocesses.

Understanding of heat and mass transfer effects are among the most critical aspects of SSF, which need attention. These pose challenge for the design and operation of bioreactors and their scale-up for the commercialization of SSF processes. The heterogeneous nature of the substrate (agro-industrial residues) poses problem in kinetics and modeling studies, which are mandatory information for the development of design of the bioreactors and its operation [1,2,4].

The substrates used in SSF differ greatly in composition, chemical nature, mechanical properties, particle size (including inter- and intra-particle spaces), water retention capacity, surface area, etc. These factors affect the overall process design and product development. During the last five years, there have been significant developments on these aspects, which would be discussed later in this review.

3. Industrial products developed by SSF

3.1. Enzymes

The field of industrial enzymes is now experiencing major research and development initiatives, resulting in the development of a number of new products and an improvement in the process and performance of several existing products. With environmental and cost issues in conventional chemical processes being subjected to considerable scrutiny, biotechnology is gaining rapid ground as it offers several advantages over conventional technologies. Industrial enzymes represent the heart of biotechnology. The global market for industrial enzymes is estimated at $3.3 billion in 2010. This market is expected to reach $4.4 billion by 2015, a compound annual growth rate (CAGR) of 6% over the 5-year forecast period. Technical enzymes are valued at just over $1 billion in 2010. This sector will increase at a 6.6% compound annual growth rate (CAGR) to reach $1.5 billion in 2015. The highest sales of technical enzymes occurred in the leather market, followed by the bioethanol market. The food and beverage enzymes segment is expected to reach about $1.3 billion by 2015, from a value of $975 million in 2010, rising at a compound annual growth rate (CAGR) of 5.1% [5].

Industrial enzymes have been among the various products produced most successfully at commercial level by SSF. Efforts have continued to study the production of different enzymes in SSF with the ultimate aims to obtain high production of the enzyme at lesser cost from new microbial sources, improved media

Table 1
Solid-state fermentation for the production of industrial enzyme.

Microorganism	Substrate	Product	Reference
Aspergillus niger strains	AFEX-treated rice rust and rice bran, whey and sugarcane bagasse	Cellulolytic enzymes	[13]
Neurospora sitophila	Steam exploded wheat straw	Cellulases, including beta xylosidases and endo-glucanase	[15]
Rhizopus stolonifer JS-1008	Corn cob	Xylanase	[38]
Bacillus subtilis NRC1aza	Starch	Levansucrase	[24]
Aspergillus heteromorphus MTCC 8818	Rosewood saw dust	Tannase	[33]
Kluyveromyces marxianus var. *marxianus*	Press mud	Inulinase	[21]
Trichoderma harzianum	Castor oil cake and sugarcane bagasse	Lipase	[25]
Aspergillus fumigatus	Wheat straw	Exo-glucanase	[18]
Bacillus subtilis GXA-28	Soybean residue	Fibrinolytic enzymes	[19]
Aspergillus niger GS1	Corn pericarp	Beta xylosidase	[37]
Natural microbial fauna from raw sludge	Soy fibers residue	Alkaline protease	[31]
Aspergillus oryzae MTCC 5341	Wheat bran	Acid protease	[32]
Bacillus sp. KR-8104	Wheat bran	α-Amylase	[7,8]
Bacillus sp. UEB-S	Millet	Lichenase	[30]
Cladosporium sp.	Wheat bran	L-Asparaginase L-Glutaminase	[10] [20]
Aspergillus foetidus MTCC 4898	Wheat bran	Xylanase	[40]
Thermomyces lanuginosus 195	Wheat bran	Xylanase	[141]
Aspergillus niger	Citrus peel	Phytase	[29]
Aspergillus niger	Apple pomace	β-Mannanase	[27]
Pleurotus ostreatus	Sugarcane bagasse	Laccase	[23]
Colletotrichum lindemuthianum	Shrimp shell chitin waste and wheat bran	Chitin deacetylase	[16]
Aspergillus caespitosus	Wheat bran	Invertase	[22]
Trichoderma koningii	Wheat bran and chitosan	Chitosanase	[17]
Oerskovia xanthineolytica		Chitinase	[139]
Aspergillus oryzae	Red gram plant waste-wheat bran based medium	α-Galactosidase, invertase	[138]
Thielavia terrestris NRRL 8126	Chick pea seed	α-Galactosidase	[48]
Streptomyces griseoloalbus	Soybean flour	α-Galactosidase	[47]
Streptomyces sp. NRC 13S	Chicken feather	Keratinase	[140]
Penicillium viridicatum RFC3	Orange bagasse and wheat bran	Pectate lyase	[28]
Aspergillus fumigatus ASH	Agro-residues	Homocysteine γ-lyase	[26]

engineering, strain improvement to get hyper-producer, or with desirable and/or improved properties, etc. These include α-amylase [6–9], L-asparaginase [10], cellulases, including β-glucosidase [11–15], chitin deacetylase [16], chitosanase [17], exo- and endo-glucanases [18], fibrinolytic enzymes [19], L-glutaminase [20], inulinase [21], invertase [22], laccase [23], levansucrase [24], lipase [25], homocysteine γ-lyase [26], β-mannanase [27], pectate lyase [28], phytase [29], lichenase [30], alkaline protease [31], acid protease [32], tannase [33], xylanases and xylosidase [34–38], etc. (Table 1).

3.1.1. Enzymes for biofuels applications

With the resurgent of biofuels research globally, considerable attention is being paid currently on the production of biomass saccharifying enzymes, chiefly cellulases for lignocellulose hydrolysis. The critical stage of lignocellulose pulp bioconversion, which hampers its commercial use, is the enzymatic conversion of cellulose to glucose. Efforts are being made to isolate new microbes for new and efficient cellulases and hemicellulase, and also to develop desirable properties in the existing ones. Studies are being carried out on the improvement of existing microbial strains to increase the productivities in order to reduce their cost of production [5]. SSF has been a method of choice by the researchers for the production of cellulases. Rocha et al. [13] investigated the production of cellulase enzyme complexes by several strains of *Aspergillus niger* strains SSF on AFEX-treated rice rust and rice bran, whey and sugarcane bagasse and obtained a maximum of 40 U/g of dry substrate by *A. niger* ATCC 16404. The enzyme complex produced by this culture was 2.25 times better than that produced by *Trichoderma reesei* CCT 2768 for the hydrolysis and ethanol fermentation. Xin et al. [14] also produced cellulases enzyme complex for the hydrolysis of organosolv-pretreated horticultural waste (HW) for ethanol production. The crude cellulases showed better reducing sugar yield using filter paper than the commercial enzyme blend.

It is generally believed that the microbial behavior (growth and metabolic profile) in SSF and SmF differ at times. When Li et al. [15] compared the cellulase production by *Neurospora sitophila* in SSF and SmF using steam exploded wheat straw as carbon source, highly interesting results were obtained. Not only the CMCase, FPA and β-glucosidase activities in SSF were far higher (53–181 times) compared to SmF. The culture also produced β-xylosidase exclusively in SSF. Authors concluded that SSF was more likely served as a natural habitat for the fungus to facilitate the enzyme secretion. Barrios-Gonzalez [39] reviewed the physiology of the medium, its molecular basis and applications for SSF and mentioned that the higher secondary metabolites production has been related to higher transcription of the biosynthetic genes. Several studies on enzymes production in SSF have identified SSF-specific genes and provided deeper insight into their genetic expression and regulation. Studies on basic aspects have led to the description of solid

culture environmental stimuli (signals). Such basis knowledge is being applied for the genetic improvement methods, novel culture systems, and other technological advances, which is expected to bring much better understanding about the fundamental aspects of SSF.

Recently, Singhania et al. [12] presented an overview on the role and significance of beta-glucosidase (BGL) for the hydrolysis of cellulose and its relevance for biofuels program. It is known that one of the major challenges in the bioconversion of lignocellulosic biomass into liquid biofuels includes the search for a glucose tolerant beta-glucosidase. Beta-glucosidase is the key enzyme component present in cellulases and completes the final step during cellulose hydrolysis by converting the cellobiose to glucose. This reaction is always under control as BGL is inhibited by the product of hydrolysis, i.e., glucose. It is a major bottleneck in the efficient biomass conversion by cellulase. To circumvent this problem several strategies have been adopted which we have discussed in the article along with its production strategies and general properties. It plays a very significant role in bioethanol production from biomass through enzymatic route. Hence several amendments took place in the commercial preparation of cellulase for biomass hydrolysis, which contains higher and improved beta-glucosidase for efficient biomass conversion.

Xylanolytic enzymes are another important group of enzymes for the biofuels program, although they find several other applications [35,36]. Xylanases have been produced by several fungal, bacterial and actinomycetes cultures cultivated in SSF using different substrates (cf. Table 1). For efficient economic gain, it is necessary to optimally hydrolyze the C-5 sugars from the hemicellulose fraction of the biomass, which can be effectively done by xylanolytic enzymes, including xylanase chiefly. Chapla et al. [40] reported the production of xylanase by *Aspergillus foetidus* MTCC 4898 in SSF using wheat bran and anaerobically treated distillery spent wash, employing the response surface methodology involving Box–Behnken design. The predicted and validated xylanase activities under the optimized conditions were 8200–8400 and 8450 U/g, respectively. Recently, Pirota et al. [41] reported an instrumented lab-scale SSF bioreactor equipped with an on-line automated monitoring and control system and achieved 2830 IU/g xylanases. However, yields as high as 115,000 U/gds of xylanase have also been reported by a strain of *Aspergillus terreus* MTCC 8661 in SSF on a palm fibers' based medium [42].

As is known, the properties of the enzymes produced in SSF and SmF often vary. Kar et al. [43] investigated the production and properties of xylanase produced by *T. reesei* SAF3 in SSF and SmF. SSF was carried out employing different agro-residues and among them wheat bran was found to be the best substrate that gave maximum xylanase production of 299.7 U/gds. Purified xylanase from SSF showed better stability in salt and pH, was catalytically and thermodynamically more efficient over enzyme from SmF, though the molecular weight of both enzymes was identical (53.8 kDa). Microwave alkali pretreated sugarcane bagasse was used as a substrate for production of cellulolytic enzymes by *Aspergillus flavus*, which produced a mixture of enzymes, including xylanase and beta xylosidases, which were effectively used for biomass hydrolysis [44]. Diaz-Malvaez et al. [37] reported the production of β-xylosidase from an *A. niger* GS1 in SSF using corn pericarp (CP) with innovative alkaline electrolyzed water (AEW) pretreatment at room temperature. According to authors, the pretreatment of CP using AEW was an ecologically friendly alternative to chemical and heat treatments for the production of relatively high levels of β-xylosidase.

Laccases are oxidative enzymes linked to biological degradation of lignin. Karp et al. [23] evaluated the production of laccase in SSF by *Pleurotus ostreatus* in a sugarcane bagasse medium. Under the optimized conditions, the highest enzymatic activity obtained was 167 U/gds. Results of protein identification by mass spectrometry confirmed the presence of POXC and POXA3 as the main isoenzymes, and also identified a glyoxal oxidase and three galactose oxidases.

3.1.2. Enzymes for food and feed

Enzymes can be used to improve the nutritional quality of food for humans and animals. A large number of enzymes used in the food and feed industries have been reportedly produced in SSF at large-scale. These include alpha amylase, glucoamylase, pectinase, protease, lipase, phytase, etc. Since the inception of SSF, food enzymes have been produced and used for different applications. An analysis of the current trend, as evidenced by the number of papers published in last five years and patents filed showed that the trend is even stringer at present. Within the food and beverage enzymes segment, the milk and dairy market had the highest sales, with $401.8 million in 2009. The market segmentation for various areas of application shows that 34% of market is for food and animal feed [5].

As stated above, the growth and metabolic profiles of the microorganisms may differ when cultivated in SSF or in SmF. Several studies compared the production of food and feed enzymes in SSF and SmF. Hashemi et al. [7,8] studied the production of α-amylase by *Bacillus* sp. KR-8104 in SSF and SmF and observed difference characteristics in the enzymes produced. For example, increasing the temperature to 45 °C reduced the enzyme activity significantly (~20.5%) compared to that observed at 37 °C in SmF. However, the reduction of α-amylase activity between 45 and 37 °C was significantly higher in SSF. There were differences in pH and temperature optima for maximizing the crude enzyme activity. While the activity of α-amylase produced in SSF was maximum at 70 °C and pH 4.0, the activity of α-amylase produced in a SmF was highest at 60 °C and pH 3.38. The lowest activities were obtained at 45.86 °C and pH 5.5, and 74.14 °C and pH 5.5 for the enzyme from SSF and SmF, respectively. These results could be very interesting as they provide an avenue to select the production method according to the profile of the enzyme required for the specific application such as low or high pH optima, temperature, etc.

SSF has been extensively used for the production of several enzymes used in the food industries. These include alpha amylase, inulinase, α-galactosidase, acid protease, levansucrase, invertase, etc. Sharma and Satyanarayana [9] did extensive studies on the production of alpha amylase for food application. They used an immobilized *Bacillus acidicola* cells for α-amylase production in SmF and SSF, which showed 3.8-fold higher production in SSF. The bread made by supplementing the dough with α-amylase scored better than those with the xylanase of *Bacillus halodurans* and thermostable α-amylase of *Geobacillus thermoleovorans*. Roses and Guerra [6] used response surface methodology and empirical modeling for the production of alpha amylase by *A. niger* strain UO-01 in SSF using with sugarcane bagasse and obtained 457.82 EU/g of dry support. Authors corroborated the effectiveness and reliability of the empirical models for such studies.

There are several reports on the production of inulinase in SSF using different microbes such as *Kluyveromyces marxianus* var. *marxianus*, *Geotrichum candidum*, etc., different substrate such as press mud, leek, etc., and different experimental approaches, including kinetics, modeling and process parameters optimization. Under the optimized conditions, the production of inulinase was 535.2, 300.5 unit/gram of dry substrate (U/gds) by *G. candidum, K. marxianus*, respectively [21,45].

α-Galactosidase is an important enzyme used for various biotechnological and medicinal applications. A comparative study was made on the production of α-galactosidase by *A. niger* and its de-repressed mutant in SSF and SmF using basal Vogel's medium, or corn steep liquor as nitrogen source and observe the response of

latter source under both cultural techniques under different temperature regimes, and determine if SSF could be exploited in a wide range of temperature expected to vary in this fermentation system. Results of higher melting temperature and negative values of entropy of activation in SSF indicated that the genetic system of both the cultures was thermodynamically resistant in the presence of corn steep liquor but sensitive to inactivation in the presence of Vogel's nitrogen sources in SmF. This was reflected as the cultures required higher magnitudes of energy for product formation in the presence of ammonium salts. Further studies on the effects of corn steep liquor showed stabilizing effect too SSF as well as SmF and that the mutant strain was more stable. Thus, it was concluded that the mutant strain could offer benefits for the production of α-galactosidase in SSF [46].

α-Galactosidase production has also been reported in SSF by actinomycetes (*Streptomyces griseoloalbus*) on soybean flour medium using a packed-bed bioreactor, which gave the highest yield of 197.2 ± 0.02 U/gds with a forced aeration of 2 vvm. This yield was approximately 2-fold higher than the yield obtained in the flasks. The enzyme production was growth-associated. This was claimed as the first report on α-galactosidase production by a filamentous bacterium in SSF using packed-bed bioreactor [47]. Saad and Fawzi [48] evaluated several seeds and husks of some plants belonging to leguminosae, Graminae, Compositae and Palmae as carbon substrates to produce α-galactosidase by the thermophilic fungus, *Thielavia terrestris* NRRL 8126 in SSF. The results showed that *Cicer arietinum* (chick pea seed) was the best substrate for the enzyme production. Gu et al. [49] produced α-galactosidase by *A. niger* zju-Y1 in SSF and obtained the maximum yield of 230 U/gds on a wheat bran and soybean meal based medium.

Vishwanatha et al. [32] reported the production of several acid proteases by *Aspergillus oryzae* MTCC 5341, when grown on wheat bran as substrate. A major acid protease production under optimized conditions gave an yield of 8.93×10^5 U/gds, which authors claimed as the highest reported so far. A strain of *Bacillus subtilis* NRC1aza produced levansucrase under SSF using starch as support. A sequential optimization strategy, based on statistical experimental designs and fractional factorial design resulted a maximum enzyme productivity of 170 U/gds [24].

The filamentous fungus *Aspergillus caespitosus* has been reported as a good producer of intracellular and extracellular invertases under SmF and SSF on wheat bran as carbon source. The production of extracellular enzyme in SSF was around 5.5-fold higher than that obtained in SmF. The production of enzyme in SSF was further increased about 2.2-folds when oat-meal was supplemented in the medium [22].

In a first report, Suresh et al. [16] reported the production of extracellular chitin deacetylase by *Colletotrichum lindemuthianum* ATCC 56676 in SSF using shrimp shell chitin waste (SSCW) and wheat bran (WB). The WB medium supplemented with chitosan resulted 460.4 ± 14.7 U enzyme/gds at 96 h as compared to 392.0 ± 6.4 U/gds at 192 h in SSCW medium. The culture produced maximum endo-chitinase (0.28 ± 0.03 U/gds) and β-N-acetylhexosaminidase (0.79 ± 0.009 U/gds) in WB medium and 0.49 ± 0.05 U/gds of endo-chitinase and 0.38 ± 0.04 U/gds of β-N-acetylhexosaminidase in SSCW medium.

3.1.3. Enzymes for detergent and laundry

The market segmentation for detergent and laundry application shows that 29% of the global market is for these enzymes. Research focus have directed on the production of different enzymes such as alkaline protease, xylanase, alkaline α-amylase, lipase, etc. in SSF. Vijayaraghavan and Vincent [50] reported the production of a halo-tolerant-alkaline protease by *Halomonas* sp. PV1 in SSF using cow dung and obtained 1351 U/g enzyme when compared with wheat bran (1013 U/g). The enzyme was extremely stable on sodium dodecyl sulfate and on various other commercial detergents. Coradi et al. [25] compared the production of lipase by *Trichoderma harzianum* in submerged fermentation and SSF using several agro-industrial residues. A mixed substrate comprising castor oil cake and sugarcane bagasse (1:2) supplemented with 1% (v/w) olive oil showed the maximum enzyme production as 4 U/gds, which was far higher than the yields obtained in submerged fermentation. Authors claimed this as the first report on the production of lipase production by *T. harzianum* in SSF. Maktouf et al. [30] reported a lichenase produced by *Bacillus* sp. UEB-S in SSF on millet and obtained 503 U/g of enzyme activity. The enzyme was stable toward non-ionic surfactants and oxidizing agents with a good stability and compatibility with a wide range of commercial solid detergents, which showed its possible commercial use in detergent formulation.

A study was made on the production of thermophilic alkaline protease by *Streptomyces* sp. CN902 in SSF using different agro-industrial residues individually or in combination as the substrate. Wheat bran (WB) with chopped date stones (CDS) (5:5) proved to be the best as it gave the highest enzyme activity (90.50 U g^{-1}) when compared to individual WB (74.5 U g^{-1}), or CDS (69.5 U g^{-1}) substrates. With further optimized conditions, the protease production was 245.5 U g^{-1} [51]. Abraham et al. [31] used raw sludge from a wastewater treatment plant as the source of microbial fauna for the production of alkaline proteases in SSF using coffee husk, hair waste from the tanning industry and soy fiber residues. Highest enzyme was produced by soy fiber residues.

3.1.4. Miscellaneous

SSF has been used for the production of various other enzymes. Most of the studies have involved on the optimization of the bioprocess for the production of enzyme focusing on the selection of the microorganisms and substrate, followed by the evaluation of process parameters in single factor mode (one parameter at a time) and/or factorial design studies following response surface methodology. Efforts have also been made on comparing the production in SSF and SmF and also to characterize the enzyme for application. A fibrinolytic enzyme production was reported by *B. subtilis* GXA-28 (CCTCC M 2012347) on soybean residue SSF containing cane molasses and monosodium glutamate waste liquor under sterilized and non-sterilized condition. Under optimum conditions, 986 U/g-substrates enzyme was produced at 24 h under non-sterilized condition [19]. L-Asparaginase was produced in SSF by *Cladosporium* sp. using several agro-industrial residues. Wheat bran supported maximum enzyme production followed by rice bran and bagasse, giving 3.74 U enzyme/gds [10]. Vuddaraju et al. [52] also studied the L-asparaginase production in SSF *Serratia marcescens* NCIM 2919 using sesame oil cake (SOC) as the sole substrate. The maximum enzyme produced was 110.795 U/gds.

Abrunhosa et al. [53] reported the production of a chratoxin A (OTA) degrading enzyme by *A. niger* MUM 03.58 in SSF. OTA is a mycotoxin present in several food and feed products and is acute toxic. Hence, efforts have been to develop the biological methods for its degradation. Under the optimized conditions, a final productivity of 154 U/gds was achieved, which represented an approximately 3.7-fold increase in enzyme yield when compared with the starting point conditions. A SSF process was developed for the production of a thermostable chitinase by *Oerskovia xanthineolytica* NCIM 2839 on wheat bran, which was used for fungal protoplasts formation. The culture produced 148 U enzyme/gds. Ferreira et al. [28] compared the production of pectate lyase (PL) by the filamentous fungus *Penicillium viridicatum* RFC3 in SSF using a mixture of orange bagasse and wheat bran (1:1, w/w), or orange bagasse, wheat bran and sugarcane bagasse (1:1:0.5, w/w), and SmF with orange bagasse and wheat bran (3%) as the carbon source. The enzyme production was highest (1500 U ml^{-1} or 300 U g^{-1} of

substrate) in SSF on wheat bran and orange bagasse. The enzymes' profiles and iso-forms were different in SSF and SmF.

3.2. Biopolymers

SSF has been explored for the production of biopolymers such as exopolysaccharides (EPS), polyhydroxyalkanoates (PHA), and others such as dextran, etc. EPS produced by the microorganisms are of great industrial significance. They can be produced by a number of fungi, especially mushrooms, bacteria, such as lactic acid bacteria (LAB), etc. These polysaccharides possess show several biological activities such as antitumor, hypoglycemic, and immunostimulating activities. Several studies have been made to produce these EPS in SmF and high yields have been achieved but they involved expensive media components. In view of this, SSF has been recently explored for the production of EPS, especially employing mushrooms and LAB.

Seesuriyachan et al. [54] compared the production of EPS production by *Lactobacillus confusus* in SSF and SmF using coconut water and sugarcane juice as renewable wastes. High concentrations of EPS (62 and 18 g/l of sugarcane juice and coconut water, respectively) were obtained in SSF. It is believed that high concentrations of sodium chloride (NaCl) suppress the biosynthesis of EPS in LAB. Seesuriyachan et al. [55] demonstrated the overproduction of EPS due to high salinity stress in SSF carried out on an agar medium. Under the optimized conditions with NaCl 4.97% and sucrose 136.5 g/l, the EPS yield was 86.36 g/l, which was 259%, higher than the maximum yield produced with the modified MRS medium containing only 120 g/l of sucrose without NaCl. It was interesting to note there was no relationship between the cell growth (biomass production) and EPS production. These results offered promising perspectives for EPS production in SSF.

Polyhydroxyalkanoates (PHA) are biodegradable polymers produced by prokaryotic microorganisms. The production of PHA by submerged fermentation processes has been extensively studied with very good results on cell biomass as well as PHA productivities. However, the process has been unfavorable economically, forcing search for alternative strategies. SSF could be considered as one such potential option as the use of the low-cost agro-industrial residues as substrate may reduce the cost of production of PHA. Castilho et al. [56] reviewed the production of PHA from the waste materials and by-products in SSF and SmF. They stated that due to the large impact of the carbon source price on the costs, one of the most important approaches to reduce costs is to use wastes and by-products as raw material for the fermentation process.

Moussa and Khalil [57] used SSF for the production of dextran by a culture of *Saccharomyces cerevisiae* on a ground date seeds medium. Different concentrations of date seeds were applied and the highest dextran production was achieved at 6 g/flask. The purified dextran showed good properties.

3.3. Biosurfactants

There has been considerable attention in recent times on producing the biosurfactants due to their wide and increasing application in the areas of healthcare, environment, etc. It is widely known that their production in submerged conditions poses problems associated with severe foaming and at times increase in the viscosity of the medium due to the associated formation of exopolysaccharides. Hence, researches have been directed toward their production in SSF. One of these, sophorolipids (SLs), a kind of extracellular biosurfactant have been known to be produced by the yeasts. They comprise one sophorose molecule, hydrophilic part, linked to one hydroxyl fatty acid, lipophilic part, by one or two crosslines. Sophorolipids show good properties and could be used to inhibit the growth of some microorganisms, in cosmetic as a high value skin moisturizer, in the petroleum industry, in food areas as emulsifiers, and in pharmacological field as anticancer drugs. There are several reports on the production of biosurfactants such as sophorolipids, rhamnolipids, surfactins and lipopeptide biosurfactants in SSF (Table 2).

A SSF process was developed for the production of sophorolipids by *Starmerella bombicola* NRRL Y-17069 to produce sophorolipids on a blend of carbon and lipids source and wheat bran, isabgol husk, soya seed powder, and peanut seed powder. Maximum yield of sophorolipids obtained was 18 g per 100 g of the substrate (18% conversion) using glucose and oleic acid (blended with wheat bran [58]). Rhamnolipids are another group of biosurfactants, which have drawn attention for their production in SSF due to their low toxicity and high biodegradability. A SSF process was developed for the production of rhamnolipids by *Pseudomonas aeruginosa* UFPEDA 614 on sugarcane bagasse and corn bran (1:1), which resulted the highest production (45 g/l of impregnating solution used), when the impregnating medium contained 6% (v/v) of each of glycerol and soybean oil. The cheaper medium component made the process attractive economically [59]. Slivinski et al. [60] studied the production of surfactin by *Bacillus pumilus* UFPEDA 448 in SSF using a okara mixed with sugarcane bagasse (1:1) as a bulking agent. The optimum temperature for surfactin production was 37 °C, but the incubation temperature affected the ratios of the various surfactin homologues produced. SSF in column bioreactor with forced aeration under optimized conditions resulted 809 mg L^{-1} surfactin of impregnating solution, which corresponded to 3.3 g kg-dry-solids^{-1}. Authors claimed this as the highest surfactin level produced to-date in SSF with a non-recombinant microorganism. Zhu et al. [61] reported the production of surfactin by *Bacillus amyloliquefaciens* in SSF using rice straw and soybean flour. The bacterial culture grew best and produced surfactin in a substrate with a moisture content of 62.8% (v/w) at 26.9 °C for 48 h, with an yield of 15.03 mg/gds.

The biosurfactant belonging to lipopeptide group have been often used for the microbial enhanced oil recovery processes in the marine environments. Recently, a two-temperature-stage process was developed for the production of lipopeptides in SSF by Zhu et al. [61,62], which showed the 30 and 37 °C as the best temperature for the growth of the strain for the biosynthesis of lipopeptides, respectively. The yield of lipopeptides increased by 8.4% in flasks and by 13.11% in the fermenter, with a 4 h decrease of fermentation time in the fermenter. Authors could scale-up the process to 1000-fold successfully [62]. Mnif et al. [63] also reported the production of a lipopeptide biosurfactant by *B. subtilis* SPB1 on a mixed solid substrate comprising tuna fish flour and potato waste flour with a moisture content of 76%. Under the optimized conditions, the culture produced 28 mg of crude lipopeptide preparation per gram of wet solid material. A new lipopeptide has been produced by a marine actinobacterium *Brevibacterium aureum* MSA13 was optimized using pre-treated molasses as substrate and olive oil along with other nutrients. Under optimized conditions, the culture produced three-times more lipopeptide biosurfactant with a hydrophobic moiety of octadecanoic acid methyl ester and a peptide part predicted as a short sequence of four amino acids, including pro-leu-gly-gly [64].

3.4. Organic acids

Citric acid is an important organic acid with multiple industrial applications, including in food industry, which is its largest consumer. There has been continuous increase in its demand over the years. With the technological developments, efforts have continued to improve the process of its production through biotechnological interventions. Efforts have also continued to produce it through fermentative route with an aim to reduce the cost of production.

Table 2
Solid-state fermentation for the production of biosurfactants.

Microorganism	Substrate	Biosurfactant	Reference
Starmerella bombicola NRRL Y-17069	Wheat bran, isabgol husk	Sophorolipids	[58]
Bacillus amyloliquefaciens	Rice straw and soybean flour	Surfactins	[61]
Bacillus amyloliquefaciens XZ-173	Rice straw and soybean flour	Lipopeptides	[62]
Brevibacterium aureum MSA13	Pretreated molasses-based medium	Lipopeptides	[64]
Bacillus pumilus UFPEDA 448	Okara and sugarcane bagasse based medium	Surfactins	[60]
Pseudomonas aeruginosa UFPEDA 614	Sugarcane bagasse and corn bran based medium	Rhamnolipids	[59]

Several studies have been made in SSF for its production using agro-industrial residues as the substrate such as apple pomace [65,66], peat moss [67], banana peel [68], fruit wastes [69], etc. Citric acid production using *A. niger* NRRL 567 grown on peat moss was studied in a column bioreactor using a statistically based method in SSF, which showed aeration rate and fermentation temperature as the most critical parameters. Maximum citric acid production (123.9 g/kg) was achieved with the aeration rate of 0.84 vvm, bed depth of 22 cm and fermentation temperature of 32 °C [67]. Dhillon et al. [65,66] carried out SSF for the production of citric acid using different agro-industrial residues. Best results were obtained when apple pomace was used as the substrate. When SSF was carried out in a 12-L rotating drum type bioreactor by *A. niger* NRRL 567 cultivated on apple pomace. Maximum citric acid (220.6 ± 13.9 g/kg dry solids) was produced with the supplementation of 3% (v/v) methanol, intermittent agitation of 1 h after every 12 h at 2 rpm, 1 vvm of aeration rate and 120 h incubation time.

Succinic acid is another important organic acid, which has drawn attention for its production by fermentation. Du et al. [70] worked on a novel generic feedstock production strategy based on SSF for the fermentative production of succinic acid. Wheat was fractionated into bran, gluten and gluten-free flour by milling and gluten extraction processes. The bran was used to produce glucoamylase and protease enzymes via SSF using *Aspergillus awamori* and *A. oryzae*, respectively. The resulting solutions were separately used for preparing the hydrolyzates from gluten-free flour and gluten, which were combined and used for succinic acid production by *Actinobacillus succinogenes* and around 22 g/l succinic acid was obtained. By further optimization, the yield could be enhanced to about 64 g/l. Dorado et al. [71] extended this work further and achieved an yield of 50.6 g/l succinic acid. Leung et al. [72] adopted almost the similar biorefinery concept for the production of succinic acid by using waste bread as a sole nutrient by *Actinobacillus succinogenes*. The waste bread was used first fermented by *A. awamori* and *Aspergillus oryzae* that produced amylolytic and proteolytic enzymes, respectively in SSF. Then the whole fermented matter was mixed directly with the bread suspension to generate a hydrolyzate, which was used to produce succinic acid by *A. succinogenes*. An overall yield of 0.55 g succinic acid per g bread was achieved, which authors claimed as the highest succinic acid yield compared from other food waste-derived media reported to-date. From these results, it could be concluded that SSF-based biorefinery concept could be a promising approach for the production of succinic acid.

Lactic acid fermentation has been well defined in SSF and SmF. Current efforts on its production in SSF have focused to reduce the cost of production by employing various approaches such as the use of cheaper feedstock as substrate, media engineering, bioprocess optimization by different techniques, etc. In this direction, Ghosh and Ghosh [73] used pine needles as bed material impregnated with a glucose and nutrients solution for lactic acid production in SSF by several strains of Lactobacilli, such as *L. delbrueckii* NCIM 2025, *L. pentosus* NCIM 2912, *Lactobacillus* sp. NCIM 2734, *Lactobacillus* sp. NCIM 2084 and a co-culture of the first two strains. Maximum lactic acid production achieved was 45.1 g/l by the co-culture, followed by *L. delbrueckii* NCIM 2025 (43.87 g/l) and *Lactobacillus* sp. NCIM 2084 (26.15 g/l) from the impregnating solution containing 80 g/l glucose. In another study, the potential use of tea waste for the production of lactic acid using *Lactobacillus plantarum* MTCC 6161 was explored. The tea waste comprise of tannin, which acts as a carbon source, which upon microbial activity gets converted to glucose. Based on a fractional factorial design (FFD), followed by the response surface methodology using a central composite design, 13.82 g lactic acid was produced from the tea waste [74]. Ray et al. [75] also used a strain of *Lactobacillus plantarum* MTCC 1407 for the production of lactic acid in SSF but they employed cassava fibrous residue as solid substrate, which was impregnated by Mann Rogassa Sharpe medium. The culture produced 29.86 g of lactic acid from 60 g of starch present in 100 g of cassava residue, which corresponded to 49.76% conversion efficiency.

Li et al. [76] developed a SSF-based bioprocess for the production of oxalic acid by *Phanerochaete chrysosporium* grown on straw. Authors paid specific attention on the effect of Pb on the production. Results showed a maximum of 22.84 mM oxylate at the initial Pb^{2+} concentration of 200 mg kg^{-1} dry straw, while the minimum (15.89 mM) at the concentration of 600 mg Pb^{2+} kg^{-1} dry straw, and at moderate concentration of Pb^{2+} the oxalic acid production was increased. The results could improve the understandings of the interactions of heavy metals with white-rot fungi. Recently, Certk et al. [77] reviewed the microbial production of γ-linolenic acid in SSF and SmF. γ-Linolenic acid (C18:3 n-6; GLA) is the key intermediate in the n-6 fatty acid family and is involved to maintain the proper mammalian cell functions.

3.5. Pigments

Monascus purpureus is a versatile fungus, which produces secondary metabolites of polyketide structure, which are synthesized by the polymerization of acetyl and propionyl subunits in a similar process to fatty acid synthesis. *Monascus* sp. has been known to produce at least six molecular structures of pigments, which are classified into three groups depending on their color (i) yellow pigments, including monascin and ankaflavin, (ii) orange pigments, including monascorubrin and rubropunctatin, and (iii) red pigments, including monascorubramine and rubropuntamine. The pigment produced by the culture largely depends upon the medium composition, including the source of carbon and cultivation conditions. Aldohexoses such as glucose and dextrose are better carbon sources for growth of *M. purpureus* than sugar alcohols such as sorbitol and mannitol, while sucrose reduce the growth of the fungus [78].

The pigments produced by *Monascus* sp. have been used as pharmaceuticals, or food additives. SSF has continued to be preferred method of fermentation for producing and studying the profiles of pigment production by *Monascus* sp. and other microorganisms (Table 3). Several studies have been made on evaluating different strains, carbon sources and process parameters to optimize the production process. *M. purpureus* CMU001 produced maximum pigment (19.4 U/gds) when corn meal was the substrate when compared to peanut meal, coconut residue, and soybean meal. The highest pigment yield (129.63 U/gds) was obtained when corn meal was supplemented with 8% (w/w) glucose, followed by

Table 3
Production of pigments in SSF.

Microorganism	Substrate	Pigment	Reference
Monascus *purpureus* CMU001	Corn meal	Red	[79]
M. sanguineus and *M. purpureus* MTCC410	Unpolished rice	Red	[80]
Monascus purpureus KACC 42430	Corn cobs	Red	[81]
Monasus ruber	Rice	Orange, yellow and red pigments	[82]
Monascus purpureus	Rice	Lovastatin	[83]
Penicillium sp. NIOM-02	Wheat	Red	[84]
Serratia sakuensis subsp. nov strain KRED	Agar medium	Biochrome-red	[85]
Rhodotorula glutinis DM 28	Rice bran	β-Carotene	[86]
Monascus pilosus NBRC4520	Rice	Red-lovastatin	[142]
Pantoea agglomerans	Agar medium	Blue	[143]

coconut residue (63.50 U/gds), peanut meal (52.50 U/gds), and soybean meal (22.5 U/gds) [79]. Dixit and Tallapragada [80] compared the red pigment production by two strains of *Monascus* sp., *M. sanguineus* and *M. purpureus* MTCC410. Both the strains produced maximum red pigment on the 16th day of incubation (21.9 CVU/ml for *M. sanguineus* and 16.9 CVU/ml for *M. purpureus*) using local unpolished rice as the solid substrate. Supplementation of the substrate with glucose resulted a multi-fold increase in the pigment yield by *M. sanguineus*, but there was no positive impact on *M. purpureus*. For variable N sources, *M. sanguineus* showed maximum pigment with 1% peptone whereas *M. purpureus* showed similar results with substrate supplemented with 5% yeast extract and MSG.

Velumurugan et al. [81] investigated the feasibility of corncob powder as a substrate for the production of pigments by *Monascus purpureus* KACC 42430 in SSF. A pigment yield of 25.42 OD Units/gram of dry fermented substrate was achieved with corn cob powder and optimized process parameters, and the yield using corn cobs was higher than those of most other agricultural waste substrates. Vidyalakshmi et al. [82] compared the production of pigment by a strain of *Monascus ruber* in SSF and SmF using rice as a substrate. Studies on the regulation of pigments production by nitrogen sources showed the formation of pigments by the yeast extract as the sole nitrogen source, giving 0.524 (370 nm), 1.084 (400 nm) and 0.616 (500 nm) Units/g for orange, yellow and red pigments, respectively. The highest red yeast rice yield of 21% was recorded in yeast extract treatment. Supplementation of the medium with the inorganic nitrogen sources showed better results.

A strain of *Monascus purpureus* MTCC 369 has been reported to produce lovastatin (3.422 mg/g) under optimized conditions on a rice-based medium in SSF [83]. Lovastatin is an anti-cholesterol drug and inhibitors of 3-hydroxy-3-methylglutaryl-CoA reductase (HMG CoA reductase) that catalyses the conversion of HMG CoA to mevalonate involved in cholesterol biosynthesis. Lovastatin does not only find a role as anti-cholesterol agent but is known to plays a significant role as an anti-inflammatory agent, cancer cell apoptosis, renal function restoration, treatment for bone disorders, etc.

SSF has been reported for the production of red pigment by another fungal culture, *Penicillium* sp. NIOM-02. The culture gave higher pigments production (9.232 OD Units) when cultivated on wheat in comparison to sugarcane bagasse. The pigment scavenged 2,2-diphenyl-1-pycrylhydrazyl (DPPH) radical. The culture also produced amylase. These properties of the culture could make it a versatile agent for its applications in food, pharmaceuticals and nutraceuticals industries [84]. It is interesting to note that usually the pigments produced by *Penicillium* and *Monascus* are structurally similar. Lovastatins, or monacolins produced by *Penicillium*, *Monascus*, *Aspergillus* and *Rihzopus* inhibit cholesterol biosynthesis. Vaidyanathan et al. [85] described a novel red biochrome, 514 Da in size, produced by a bacterial isolate, *Serratia sakuensis* subsp. nov strain KRED on a agar medium. Chemical characteristics of the biochrome produced showed it to be useful in dying of silk, wool, and cotton fabrics. SSF was carried out to produce β-carotene by *Rhodotorula glutinis* DM 28 on rice bran as a substrate. The biomass and β-carotene content produced were 54 g/kg rice bran and 1.65 mg kg^{-1} rice bran, respectively, which were increased by 60 and 30%, respectively after factorial design experimental optimization of the process, using the central composite design for the optimization of its cultivation conditions [86].

3.6. Other products

SSF has been used for the production of various other products such as bioactive compounds/secondary metabolites, biofertilizers, biopesticides, flavoring compounds, etc.

3.6.1. Secondary metabolites

Hypocrellins and hypericins, structurally related plant pigments isolated from *Hypocrella bambuase* and *Hypericum* respectively, are known photodynamic agents. Due to their specific properties, these pigments have been considered useful for therapeutic and diagnostic applications. Studies have been made to produce hypocrellin A in SSF by *Shiraia* sp. SUPER-H168 using eight different agro-industrial crops and residues, which showed corn as the best substrate and under optimized conditions resulted 4.7 mg/gds hypocrellin A was produced [87]. Lopez-Calleja et al. [88] compared the production of cephalosporin c in SSF and SmF. Authors stated that despite the importance of *Acremonium chrysogenum* as the only cephalosporin C (CPC) producer, there has been still a limited understanding about the molecular mechanisms regulating the antibiotic biosynthesis in this fungus. In view of this, studies were undertaken on the expression of genes related to CPC production in *A. chrysogenum*, finding out for the first time similarities and differences in SSF and SmF under different process parameters. Results showed interesting differences in intermediate (Pen N) and certain biosynthetic gene expression levels and also that there was some relationships between the physiological features and gene expression.

3.6.2. Biofertilizers and biopesticides

A bioprocess was developed using agro-industrial wastes of cattle dung, vinegar-production residue and rice straw in SSF employing *Trichoderma harzianum* SQR-T037 (SQR-T037) for the production of bioorganic fertilizers containing SQR-T037 and 6-pentyl-α-pyrone (6PAP) to control *Fusarium* wilt of cucumber. Process optimization through fractional factorial design experiments and central composite design resulted a biofertilizer containing 8.46 $\log_{10}$ ITS copies g^{-1} dry weight of SQR-T037 and 1291.73 mg kg^{-1} dry weight of 6PAP, and having the highest ($p < 0.05$) biocontrol efficacy [89]. Similarly, a SSF process was developed for the production of *Bacillus thuringiensis*-based biopesticide using kitchen waste as the substrate. The culture medium contents were optimized by an orthogonal test, which showed 55.21% kitchen waste, 22.08% wheat bran, 11.04% soybean cake power, 11.04% grain hulls, and 0.63% mixed ions as the optimal medium components, resulting in a spore count of

5.01×10^{10} CFU/g and entomotoxicity of 15,200 IU/mg. The process was successfully scaled-up to 35 kg level and offered promising economic benefits [90].

3.6.3. Flavors and aroma

SSF has been applied for the production of vanillic acid, which is a flavoring agent and also serves as precursor for vanillin production. For this, process optimization was carried out using a culture of *Phanerochaete chrysosporium* and lignocellulosic waste as substrate by response surface methodology, which resulted 73.69 mg vanillic acid/gds. The yield obtained was slightly more than the predicted value of 73.58 mg/gds under the optimum conditions [91].

4. Industrial processes developed by SSF

SSF has been used developing bioprocesses with varied applications. One of these, which has attained significance in view of the increasing interest on biofuels from biomass program is biological delignification, as biological deconstruction of plant biomass represents an attractive alternative and eco-friendly bioprocess [92]. White-rot fungi have been potentially used for this application. However, there are reports about actinomycetes being effective for delignification [93–95]. Saritha et al. [95] studied the biological delignification of paddy straw using *Streptomyces griseorubens* ssr38 in SSF with ultimate aim for improved hydrolysis. The culture was highly effective for the depolymerize/solubilize of lignin to a high extent, which was evident from the increase in cellulose content of the straw after 10 and 21 days of SSF. This, in turn, improved the hydrolysis efficiency upon enzymatic hydrolysis. It is known that the biological delignification process is effective depending up on the amount and properties of the enzymes such as MnP, LiP, etc. secreted by the microorganism [96]. Knezevic et al. [97] studied the dynamics of laccase, Mn-dependent peroxidase, and Mn-independent peroxidase activity and levels of wheat straw lignin degradation in seven wood-rotting fungi, which showed *Pleurotus ostreatus* and *P. eryngii* as the best laccase producers; *Lenzites betulinus and Fomitopsis pinicola* were the best Mn-dependent peroxidase producers, and *P. ostreatus* showed the lowest efficiency.

SSF has also been used for the bioremediation of textile industries dyes effluent [98–100]. There are large numbers of physico-chemical and biological methods for the remediation of dyes from industrial effluent. However, these methods have several drawbacks such as high cost and release of toxic and hazardous secondary products (wastes). Biological processes offer potential benefits here also being eco-friendly and could provide economic feasibility as well. Kadam et al. [101] used pretreated sugarcane bagasse and a strain of *Providensia staurti* E*bt*SPG for the removal of textile dyes. Using tray bioreactor and reported 86% dye removal in 72 h under un-sterilized conditions. This result appeared very interesting and could be explored at large-scale.

Another important application of SSF has been in developing the bioprocess for the detoxification of Jatropha cake. *Jatropha curcas* seed cake is a by-product obtained after the extraction of oil from the seeds. It contains a high amount of protein, small quantities of phorbol esters and anti-nutritional factors such as phytate, trypsin inhibitor, lectin and saponin. Due to the presence of these anti-butrional factors, the cake finds no application in food or feed industries. However, if this could be detoxified, it offers potential usage. With this view, efforts have been made to detoxify the toxic and anti-nutritional compounds present in the cake. Phengnuam and Suntornsuk [101] cultivated *B. subtilis* and *B. licheniformis* in SSF and SmF, which showed that *B. licheniformis* cultivation in SmF was the most effective method to degrade toxic and anti-nutritional compounds in the seed cake. Joshi et al. [102] were able to completely degrade the phorbol esters present in the cake by *Pseudomonas aeruginos*a PseA strain in SSF. In a related study, Neifar et al. [103] studied the nutritional improvement of another oil cake – olive cake with the ultimate aim to use the fermented cake as animal feed by cultivating the medicinal mushroom, *Fomes fomentarius* in SSF. The results showed an increase in the crude protein from 6% (control) to 22% along with significant decreases in neutral detergent fiber (hemicelluloses, cellulose and lignin), acid detergent fiber (lignin and cellulose) and acid detergent lignin.

5. Design and operation of bioreactors in SSF

Design and operation of bioreactors have remained a challenge in the commercialization of SSF processes. In one of the earliest reviews, Pandey [104] had traced the design aspects of fermenters (bioreactors) in SSF, which was further discussed in earlier two reviews [1,2]. As mentioned above, the substrate (carbon and energy source) is usually natural materials such as agro-industrial residues, which while being in organic nature offer benefits for the microbial culture growing in it, it also poses serious problem in parametric studies, in particular for kinetics and modeling due to heterogeneous nature. It is known that for the development of design of the bioreactors and its proper operation, quantitative information about the kinetics and stoichiometry of the process reaction is required. However, the structural and nutritional complexity of the substrates hampers it. A bioreactor in the fermentation process should provide the environment for the growth and activity for the microbial culture growing in it [1,2,4].

The substrates used in SSF differ greatly in composition and nature. The bed metrics also behaves differently due to variation in composition, mechanical properties, porosity (including inter- and intra-particle spaces), water retention capacity, surface area, etc. These factors must be taken into consideration while developing or using a bioreactor in SSF process. Another important related issue is the type of the microorganism to be used as they differ in their morphology (such as single cells or mycelia), which in turn would also impact the performance of the SSF process. For example, while mixing of the substrate may help in heat and mass transfer effects; it could damage the mycelia of fungal culture, affecting the growth and product formation. Substrate loading and discharge and maintenance of sterile operation are other critical issues for the bioreactors in SSF. During recent years, the research and technological development have continued on the design and operation of bioreactors in SSF processes. These, however, have resolved around tray, packed bed and rotary drum type bioreactors chiefly (Table 4).

Tray bioreactors (TB) have been conventionally used in the laboratory studies for SSF. Interestingly, most of the commercial processes being currently employed by various industries too use the TB for different applications [3,5]. TB are simple and lost cost and offer ease of operation. The major disadvantages TB pose is high space requirement and lower substrate loading. However, TB still continues to be most preferred SSF system for the production of industrial enzymes such as phytase, pectinase, cellulases, etc. Vaseghi et al. [105] used a TB for cultivating *Rhizopus oryzae* on sugarcane bagasse and evaluated the SSF process parameters such as the influence of temperature and humidity of the cabinet, depth of solid bed, particle size, initial moisture content and supplementary substrate (olive oil) as carbon source. It was interesting to note that there was difference in the maximum enzyme produced in top, middle and bottom trays, which was 215.16, 199.36 and 52.64 U gds^{-1}, respectively after 72 h. Ruiz et al. [106] developed a SSF bioreactor comprising a vertical column, which had eight perforated trays to support the solid substrate (lemon peel pomace) for the production of pectinase by *A. niger*. Forced aeration helped

Table 4
SSF bioreactors and products.

Type of bioreactor	Substrate	Microorganism	Product	Reference
Packed-bed	Pearl barley	*Penicillium brevicompactum*	Mycophenolic acid	[119]
Packed-bed	Sugarcane bagasse and soybean bran	*Kluyveromyces marxianus*	Inulinase	[118]
Packed-bed	Press mud	*Kluyveromyces marxianus var. marxianus*	Inulinase	[21]
Packed-bed	Polyurethane as inert support	*A. niger*	Tannase	[115]
Fixed-bed			Inulinase	[117]
Laterally aerated moving bed	Palm kernel cake	*A. flavus*	Enzymes	[121]
Gas double-dynamic	Wheat bran	*Fungal cultures*	Enzymes	[122]
Counter-current	Sugarcane bagasse	*A. niger*	Enzymes	[123]
Deep-bed	Sorghum stalk	*Issatchenkia orientalis*	Ethanol	[125]
Tray	Sugarcane bagasse	*Rhizopus oryzae*	Enzymes	[105]
Tray with vertical columns	Lemon peel pomace	*A. niger*	Pectinase	[106]
Tray		*A. niger*	Pectinase	[107]
Tray	Rice	*Beauveria bassiana*	Conidia	[109]
Modified tray		*A oryzae*	Alpha amylase	[110]
Tray		*Trichoderma* sp.	Fungal products	[111]
Rotary drum	Seaweed	*A. niger*	Fucoidanase	[112]
Rotary drum	Apple pomace	*A. niger*	Citric acid	[65]
Modular	Pine wood chips and orange peel	*Trametes hirsuta*	Enzymes	[114]

the removal of process heat and improved the performance of the process, which resulted the maximum pectinase activity. Alcantara and Da Silva [107] used a tray bioreactor for the production of pectinase enzyme by a fungal culture *A. niger* CCT0916. A model was developed based on the factorial design experiments, which showed temperature as the most critical factor for the process.

In order to better understand the effect of internal air circulation by forced convection on heat and water transfer, Figueroa-Montero et al. [108] developed a stainless steel tray bioreactor to cultivate *A. niger* C28B25 on an inert support, and temperature, moisture content, biomass and substrate concentrations were measured. The heat and mass transfer coefficients were determined by the water and energy integral balances, which were used to develop a mathematical model for the predictions of the temperature and moisture content of the fermentation bed. Results showed a high goodness-of-fit with the experimental results. Authors claimed this as the first report describing the effect of N_{Re} of air in the headspace of a SSF tray bioreactor on the heat and mass transfer coefficients and temperature regulation in SSF.

Xie et al. [109] used a TB for the production of conidia of *Beauveria bassiana* Bb-202 in SSF for the control of the coleopteran pests using rice as the substrate. Results showed the limitation of bed thickness for increasing the productivity (higher the solid substrate thickness, the production of conidia decreased). When the substrate bed was cut into smaller pieces, it helped in metabolic heat and gas transfer in the center of substrate. Best results were obtained when the substrate bed thickness was 2.0 cm and each piece was as small as 6 cm × 4 cm × 2 cm, which produced the highest yield of 3.94×10^{12} conidia kg^{-1} rice. Dey and Banerjee [110] modified the TB, which they claimed as the new bioreactor to study the α-amylase production, which comprised a tray in a vessel. The moistened substrate was placed on the upper tray in the vessel and water (liquid medium) was filled in the lower vessel. The bioreactor was supplied the compressed air that was claimed to lift the liquid medium into the upper vessel and touched the substrate bed. This condition probably facilitated the heat transfer to liquid medium, reduced water loss and catabolic repression. The bioreactor was claimed as novel, which could overcome some of the major problems associated with SSF process, resulting higher productivities.

Heat and mass transfer in static tray fermentation using *Trichoderma* sp. were studied by Jou and Lo [111] who evaluated the air velocity, air temperature, illumination, pH, carbon dioxide (CO_2) concentration, and substrate temperature, and the effects of bed height, moisture of substrate, and relative humidity of air and reported 1.0 cm bed thickness as the optimal for fungal growth and activity.

Laboratory-scale rotary drum bioreactors (RDB) have been used for the production of enzymes, etc. in SSF. RDB offer advantages in the sense that it allows the mixing of fermenting solid bed with control of rotation speed (typically very low). Rodriguez-Jasso et al. [112] used drum bioreactor for the production of fucoidanase enzymes in SSF using *A. niger* PSH and *Mucor* sp. 3P and three algal substrates (untreated, autohydrolyzed, and microwave processed seaweed *Fucus vesiculosus*). SSF with moving bed (10 rpm) showed advantages in the induction of the enzyme when compared to the static ones. When the bioreactor was scaled-up (10 times), with control of moisture resulted 2.5 times higher enzyme activities. In another study, a 12-L RDB was used for the production of citric acid in SSF using apple pomace as the substrate by *A. niger* NRRL 567. The optimized process parameters such as the effect of aeration, inducers, etc. improved the product yield. However, the agitation rate was 200 rpm, which was much higher than reported by others [65].

Modular bioreactor could be a promising bioreactor system as it helps in maintaining the homogeneity of the bed at optimal levels. Cunha et al. [113] studied the optimum geometry of elementary modules of a hexahedral bioreactor subjected to constant volume. The bioreactors had a square section with no external cooling. The geometric optimization followed the Constructal principle of minimum heat resistance. The numerical simulations were studied for the inlet air temperature and velocity, and module volume. Results showed that this hexahedral modular bioreactor was effective for SSF processes. Bohmer et al. [114] used a novel modular bioreactor for the cultivation of *Trametes hirsuta* on a mixture of pine wood chips and orange peel. Evidently the speed of rotation of the bioreactor had a significant impact on the enzyme production.

Packed-bed bioreactors (PBR) or column-type bioreactors (CB) have often been used in SSF for different application. They offer potential benefits as higher substrate loading per bioreactor volume (higher packing density) could be achieved in these. One essential element for smooth SSF process using PBR is the requirement of adequate inter-particle size of the substrate so as to allow the passage of air through it (forced aeration), which helps in heat and mass transfer effects as well.

Several studies have been made employing the PBR for the production of industrial enzymes and process parameters have been optimized using response surface methodology, or factorial design experiments to maximize the productivity [21,115–117]. The bioreactors were packed using the natural substrate as the source of carbon, or using the inert material as the solid support only, which was impregnated with the growth and/or production media. Dilipkumar et al. [21] used a PBR for the production of

inulinase enzyme in SSF by *Kluyveromyces marxianus* var. *marxianus* using press-mud as the substrate. Authors employed the response surface methodology to optimize the process parameters such as air-flow rate, packing density and particle size were optimized using response surface methodology (RSM) and reported higher inulinase production. Mazutti et al. [118] also used a PBR (3-kg, dry basis) for inulinase production using sugarcane bagasse and soybean bran by *Kluyveromyces marxianus* NRRL Y-7571 and response surface methodology. Results showed that CO_2 evolution and the metabolic heat generation were directly associated with the consumption of total reducing sugars present in the medium.

A PBR was used for the production of tannase by *A. niger* using polyurethane foam as an inert support impregnated with defined culture media. The process parameters influencing the enzyme production were evaluated using a Plackett–Burman design, followed by Box–Behnken design to optimize the substrate concentration, initial pH, and incubation temperature, which led to achieve 1.97-fold increase in the enzyme production [115]. Alani et al. [119] used a PBR for the production of mycophenolic acid (MPA) by *Penicillium brevicompactum* using pearl barley, which was far superior in SSF to liquid production.

Yet another major advantage PBR, or CB offer is the simple and accurate measurement of the gaseous environment (CO_2 and O_2) in the bioreactor, which could be used to determine the growth (respiratory quotient) of the culture and also to determine the kinetics. CB has another advantage in the sense that a number of columns could be used in a single set of experiment in which each column could be used to study a set of parameters and would behave like a bioreactor. Each of these columns could be connected with the sensors for monitoring the parameters such as temperature, humidity, gaseous composition, and could also be sampled through a sampling port. Pliego-Sandoval et al. [120] devised a multiplex gas sampler for monitoring the CO_2 and O_2 for CB. The sampler was attached mechanically to the columns (bioreactors) and was connected to centralized CO_2 and O_2 sensors. It was small in size and portable, and was claimed to be economical. It was user-friendly and offered benefits such as to set the fermentation time, the number of bioreactors to be sampled by the sampling port, the sampling rate and the delay time between sampling, etc.

Another CB was used for the production of inulinase, in which the bioreactor was a fixed-bed reactor (34 cm diameter and 50 cm height) with working capacity of 2-kg of dry substrate [117]. The bioreactor was operated in batch and fed-batch modes with different strategies for feeding the inlet air (saturated and unsaturated air). Batch mode operation of the bioreactor was efficient for the removal of the metabolic heat generated during the fermentation fed-batch was more effective, which resulted higher enzyme productivities.

Wong et al. [121] developed a novel laterally aerated moving bed (LAMB) bioreactor. The LAMB consisted of a perforated acrylic packed bed column with a free area of 15%, 95 mm internal diameter and 855 mm in height. A perforated distributor pipe with a free area of 15% passed through the center of the column for the distribution of humidified air introduced from the top of the bioreactor. SSF was carried out using the palm kernel cake as the substrate and *A. flavus*, which resulted a 5.6-folds increase in enzyme production, which was due to better heat and mass transfer due to humidified air flow passing radially across the substrate PKC bed.

With an intention to develop a new design for SSF processes, He and Chen [122] used the gas double-dynamic solid-state fermentation (GDD-SSF) technology for the production of different enzymes at 800-L scale. This technology has been used for the large-scale production of biopesticide and some other products. Results on the liquid–solid ratio and air pressure on enzyme production in different fermentation modes showed that GDD-SSF offered several advantages in comparison to the static SSF for the production of pectinase, glucoamylase, protease, and cellulase by improving the enzyme activities (up to a maximum of 2.84 times) and shortening the fermentation period. Authors also reported decreased temperature gradient, which could be crucial for online controlling at large-scale production.

Attempts have also been made to develop continuous processes for SSF as this could be result higher productivity. With this view, Varzakas et al. [123], developed a counter-current bioreactor using a pre-germinated conidia of *A. niger* as inoculum and sugarcane bagasse as the solid (inert) support, which was fed in the bioreactor (as compartments) in blue color with 20 h solids residence time distribution (RTD) time. Results showed a marked increase in the biomass in the progressive compartments from 1 to 9 and possibilities of continuous production of enzymes by SSF under un-sterile conditions.

As discussed often, one of the major difficulties in scale-up of the SSF bioreactors is heat accumulation in the solid fermenting bed, which consequently limits the thickness of the solid bed [1,2]. It is known that there could be substantial temperature variation, depending upon the thickness of the bed, which adversely affects the growth and metabolic activities of the microorganism growing in it. However, for economic efficiency and technological gain, efforts have continued to study the deep-bed SSF processes adopting various strategies to control the heat and mass transfer effects efficiently. One of the approaches toward this has been to better understand and characterize the solid matrix structure, which significantly determines the transfer properties. Duan and Chen [124] studied the change of water retention, permeability and thermal conductivity of substrate with the variation of its three-phase structure. Based on the results that showed that liquid phase dominated the phase structure variation, a three-phase structural index (TPSI) was proposed based on weighed variation coefficient. Water evaporation rate of substrate showed exponential increase with the rise of TPSI, which showed that there were some other factors influencing the water retention of the substrate. The effect of particle size on substrates' permeability weakened gradually with increase of water content, and its variation with TPSI accorded with Bidoseresp function. The thermal property of substrate, such as volumetric specific heat, thermal resistivity and thermal conductivity, varied with the increase of TPSI according to power exponent functions. Authors concluded that the TPSI could be effectively applied to characterize the effect of substrate structure on its transfer properties, which could be highly beneficially for SSF process design.

Kwon et al. [125] used a deep-bed SSF bioreactor using sorghum stalk and observed temperature gradient in the substrate bed (due to heat accumulation in the bioreactor), which was dependent on the depth of the substrate bed and temperature of fermentation. The use of a thermo-tolerant yeast strain was beneficial for the process as it could tolerate the rise in the temperature in the bed up to 15–20 cm bed thickness. Hendges et al. [126] used a cylindrical double surface bioreactor for cultivating *A. niger* T0005/007-2 in solid medium with 170 mm of height and forced aeration. Forced aeration and pressure pulse showed no positive effect on the production of enzyme; also, none of the conditions evaluated could control the temperature of the fermenting bed. Chen and Li [127] recently developed an industrial level system with non-isothermal simultaneous solid-state saccharification, fermentation and separation for ethanol production (NSSSFS), in which the enzymatic saccharification and fermentation proceeded at around 50 °C and 37 °C, respectively, and were coupled together by the hydrolyzate loop. The glucose produced from the enzymatic saccharification was timely consumed by yeast, and the resulting ethanol was separated online by CO_2 gas stripping, coupled with the adsorption of activated carbon. The system offered higher solids substrate loading and increased ethanol yields. The NSSSFS could be a novel and

feasible engineering solution to the inherent problems of simultaneous saccharification and fermentation, which would be used in large scale and in industrial production of ethanol.

From the above studies, it could be concluded that while these have mostly been on using the known bioreactors designs such as packed-bed, fixed-bed, tray and rotary drum (as these remain the basic designs for practically any chemical or biochemical processes), but have added-up data-based fundamental knowledge on the use and operation of these bioreactors at lab and at larger scales. These have also explored newer substrate such as olive waste or lemon peel waste, or modified the design such as vertical column with perforated trays, GDD-SSF technology, or bioreactor for continuous process. Efforts have also been made to modify the known design such as TB to determine the beneficial impact on the product yields. New information have been generated on the impact of process parameters such as aeration to better define the heat and mass transfer effects. Thus, while the basic designs of the bioreactors have remained the same, a lot new information has been generated on their modification, or on other processing aspects.

6. Process and product modeling and kinetics

As discussed above, the major challenges to overcome in scale-up SSF processes include the heat accumulation and heterogeneous nature of the substrate, comprising a three-phase gas–liquid–solid multiphase system during the fermentation. In order to overcome these, it is necessary to understand and estimate the heat and mass transfer parameters, which would be helpful in developing the mathematical models, which are considered as the key for scale-up data.

Numerous efforts have been made in recent years to study and understand these aspects. A kinetic model was proposed for the microbial growth in the SSF bioreactors using *A. niger* on wheat bran. In this model, it was assumed that the growth rate not only depended on the current temperature of bioreactor but also on the past temperatures that the microorganism had previously undergone. Model predictions agreed reasonably with experimental data. Results on modeling the temperature gradients in the PBR two dynamic heat transfer models (lumped and distributed) showed that the predictions of the distributed model were better agreed with the literature experimental data than the lumped model [128]. Wang et al. [129] developed a mathematical model of a rotating drum bioreactor considering the radial temperature distribution in the substrate bed (sorghum stalk) for the production of ethanol. The model fit well with the experimental data, which showed that this mathematical model was a powerful tool to investigate the design and scale-up of SSF processes.

Mitchell et al. [130] used a mathematical model, based on the N-tanks-in-series approach, to evaluate the potential advantages that could be obtained in SSF processes by operating the packed-bed bioreactors as multi-layer beds. In a classical operation, the air was blown uni-directionally through a static substrate bed with the movement of two layers. In the batch operation, the positions of the layers were changed at 1 h intervals, in a cycling motion. Alternatively, in a continuous plug-flow system, the layers were added regularly at the air outlet and removal of spent layers at the air inlet. Under the conditions of the simulation, the rate of metabolic heat generation during the steady state of the continuous plug-flow process was only 60% of the peak value predicted for the classical operation. As a result, the maximum bed temperature in the continuous plug-flow process was 4.5 °C lower than that predicted for the classical operation. Thus, the operation of multi-layer packed beds in the continuous plug-flow mode could improve the performance of the bioreactor.

Gonzalez-Figueredo et al. [131] described a mathematical model that predicted the non-isothermal dynamical behavior of a pilot-plant SSF fed-batch reactor, employed in the preparation of compost for the *Agaricus bisporus* mushroom cultivation. The biomass was categorized into three different macro-kinetics phases: mesophilic bacteria and fungi that inhibited the *A. bisporus* growth, thermophilic fungi associated to the growth selectivity of *A. bisporus* and thermophilic actinobacteria degrading the lignocellulose. Free-convection and evaporative heat transfer was included in the energy balance and the kinetic parameters were adjusted using a sensitivity analysis. The resulting model reproduced accurately the temperature profiles of the pilot-plant SSF reactor for different initial conditions and predicted final microora concentration ratios were within the expected values.

Mazutti et al. [132] studied the kinetics of the cell growth and inulinase production by *Kluyveromyces marxianus* NRRL Y-7571 were investigated in a packed-bed bioreactor using seven experimental runs to evaluate the influence of the inlet air temperature and volumetric air flow rate on the process dynamics. The results showed that the manipulated variables affected significantly the process performance. The results obtained were evidently useful in the scale-up and optimization of packed-bed bioreactors configuration for inulinase production. Rodriguez-Fernandez et al. [133] studied the behavior of kinetic parameters in production of pectinase and xylanases in SSF using citrus peel by *A. niger* F3 was in a 2 kg bioreactor. A mathematical model was developed to determine the different kinetic parameters related to SSF. The specific growth rate and biomass oxygen yield decreased during the fermentation, whereas an increase in the maintenance coefficient for the different employed carbon sources was concurrently observed.

A mathematical modeling study was undertaken for the growth of *Grifola frondosa* (maitake, an edible and medicinal mushroom) in SSF. Considering its increasing popularity, there are limited references for its cultivation. The fungal growth (mycelial content) was determined by measuring N-acetyl-D-glucosamine (NAGA). Two mathematical models were selected measuring the reducing sugars consumption and NAGA synthesis, as an indirect assessment of fungal growth. Both models showed a good fit between the predicted and experimental data, although, the logistic model required a minor number of adjustment parameters [134].

Erdogan and Ermurat [135] studied the modeling kinetics of lipolysis and proteolytic activities of *Penicillium roqueforti* in SSF and the effects of different strains and temperatures on the cheese ripening. The results of the ripening bioprocesses carried out in batch-packed solid-state bioreactors at 5 °C and 12 °C during five months were used to determine the lipolytic and proteolytic kinetic equations and coefficients of *P. roqueforti* by using Michaelis–Menten kinetic model. The results of the kinetic activities and variance analysis indicated that the toxic strain of *P. roqueforti* showed low level of lipolysis and proteolysis effects at 5 °C and high level at 12 °C. Mahanama et al. [136] used statistical modeling of SSF to build a framework for quantitative and mechanistic fermentation design for the microbial production of Vitamin K_2 using *B. subtilis* in static bed tray fermentation. It was interesting to note that the yield reductions due to increased bed heights were reduced considerably with the corresponding increase in particle sizes by improved substrate porosity. The polynomial model fitted the experimental data well. Farinas et al. [137] modeled the effects of SSF operative conditions on endo-glucanase production by *A. niger* cultivated using an instrumented lab-scale bioreactor equipped with an on-line automated monitoring and control system. The effects of air-flow rate, inlet air relative humidity and substrate initial moisture on endo-glucanase production using a statistical design methodology allowed the modeling of the production under different process conditions, which ultimately resulted higher enzyme production. It was interesting to note that the total

amount of CO_2 produced was linearly correlated with enzyme production as revealed by the respirometric analysis.

7. Conclusions

Solid-state fermentation (SSF) is a promising key for bioprocess and products development for industrial applications. Recent developments on the production of industrial enzymes, biopolymers, pigments, secondary metabolites, etc. have shown interesting results, which seem offering potential for large-scale production with techno-economic feasibility. An interesting development in this regard has been successful utilization of citrus peels as substrates for the cultivation of fungi, paving the way for their utilization, which has not been possible due to presence of toxic components in them. While much has been done on the use of different configurations of bioreactors, the best results still apparently have been achieved with tray bioreactor for scale-up. Data generated on heat and mass transfers effects in this regard have been helpful in design development with mathematical modeling. It is envisaged that SSF would continue to be studied more with continued focus on exploitation of agro-industrial residues as substrate and cultivating various microbes on them. Also, engineering parameters should be exploited more for developing large-scale processes.

Acknowledgements

Leya Thomas acknowledges the award of a research fellowship to her by the University Grants Commission, New Delhi. Authors gratefully acknowledge the support by the French government research program Investissements d'avenir through the IMobS3 Laboratory of Excellence (ANR-10-LABX-16-01) by the European Union through the program Regional competitiveness and employment 2007–2013 (ERDF – Auvergne region) and by the Auvergne region.

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