Solid state fermentation for the production of industrial enzymes

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Enzymes are among the most important products obtained for human needs through microbial sources. A large number of industrial processes in the areas of industrial, environmental and food biotechnology utilize enzymes at some stage or the other. Current developments in biotechnology are yielding new applications for enzymes. Solid state fermentation (SSF) holds tremendous potential for the production of enzymes. It can be of special interest in those processes where the crude fermented products may be used directly as enzyme sources. This review focuses on the production of various industrial enzymes by SSF processes. Following a brief discussion of the microorganisms and the substrates used in SSF systems, and aspects of the design of fermenter and the factors affecting production of enzymes, an illustrative survey is presented on various individual groups of enzymes such as cellulolytic, pectinolytic, ligninolytic, amylolytic and lipolytic enzymes, etc.

SOLID state fermentation (SSF) holds tremendous potential for the production of enzymes. It can be of special interest in those processes where the crude fermented product may be used directly as the enzyme source[1]. In addition to the conventional applications in food and fermentation industries, microbial enzymes have attained significant role in biotransformations involving organic solvent media, mainly for bioactive compounds. Table 1 lists some of the possible applications of the enzymes produced in SSF systems. This system offers numerous advantages over submerged fermentation (SmF) system, including high volumetric productivity, relatively higher concentration of the products, less effluent generation, requirement for simple fermentation equipments, etc.[2–9].

Microorganisms used for the production of enzymes in solid state fermentation systems

A large number of microorganisms, including bacteria, yeast and fungi produce different groups of enzymes. Table 2 enumerates the spectrum of microbial cultures employed for enzyme production in SSF systems. Selection of a particular strain, however, remains a tedious task, especially when commercially competent enzyme yields are to be achieved. For example, it has been reported that while a strain of *Aspergillus niger* produced 19 types of enzymes, α-amylase was being produced by as many as 28 microbial cultures[3]. Thus, the selection of a suitable strain for the required purpose depends upon a number of factors, in particular upon the nature of the substrate and environmental conditions. Generally, hydrolytic enzymes, e.g. cellulases, xylanases, pectinases, etc. are produced by fungal cultures, since such enzymes are used in nature by fungi for their growth. *Trichoderma* spp. and *Aspergillus* spp. have most widely been used for these enzymes. Amylolytic enzymes too are commonly produced by filamentous fungi and the preferred strains belong to the species of *Aspergillus* and *Rhizopus*. Although commercial production of amylases is carried out using both fungal and bacterial cultures, bacterial α-amylase is generally preferred for starch liquefaction due to its high temperature stability. In order to achieve high productivity with less production cost, apparently, genetically modified strains would hold the key to enzyme production.

Substrates used for the production of enzymes in SSF systems

Agro-industrial residues are generally considered the best substrates for the SSF processes, and use of SSF for the production of enzymes is no exception to that. A number of such substrates have been employed for the cultivation of microorganisms to produce host of enzymes (cf. Table 2). Some of the substrates that have been used included sugar cane bagasse, wheat bran, rice bran, maize bran, gram bran, wheat straw, rice straw, rice husk, soyhull, sago hampas, grapevine trimmings dust, saw dust, corncobs, coconut coir pith, banana waste, tea waste, cassava waste, palm oil mill waste, aspen pulp, sugar beet pulp, sweet sorghum pulp, apple pomace, peanut meal, rapeseed cake, coconut oil cake, mustard oil cake, cassava flour, wheat flour, corn flour, steamed rice, steam pre-treated willow, starch, etc.[10–19]. Wheat bran however holds the key, and has most commonly been used, in various processes.

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Table 1. Industrial applications of enzymes produced by solid state fermentation processes[1]

Process	Enzyme
Enzyme-assisted ensiling	Fungal cellulases and hemicellulases
Bioprocessing of crops and crop residues	Fungal cellulases and hemicellulases
Fibre processing (retting)	Fungal pectinases, cellulases, hemicellulases
Feed supplement	Amylases, proteases, lipases, cellulases, hemicellulases
Biopulping	Xylanases
Directed composting	Hydrolytic enzymes
Soil bioremediation	Laccases, ligninases
Post-harvest residue decomposition	*Trichoderma harzianum* cellulases
Biopesticide	*T. harzianum* cellulase for helper function

Table 2. Spectrum of microbial cultures employed for producton of various enzymes in solid state fermentation systems

Substrate	Microorganisms	Enzyme	References
Bagasse	*Trichoderma reesei* + *Aspergillus phoenicis*	Cellulases	22
Coconut coir pith	*A. niger*	Cellulases, β-glucosidase	23
Grapevine trimming dust	*Cerrena unicolor*	Cellulase, xylanase, ligninases	24
Rice husk	*Penicillium citrinum*	Cellulases	25
Rice husk	*Mesophilic fungi* (10 species)	Cellulase (FP)	26
Tea production waste	*Cerrena unicolor, Coriolus hirsutus, Pleurotus ostreatus*	CMCase, xylanase, laccase	27
Wheat bran	*A. niger*	Cellulase, xylanase, polygalacturonase	28
Cellulose, starch	*T. viride, A. niger*	Cellulase, amylase	29
Ligno-cellulosic materials	*Lentinula edodus*	Various enzymes	30
Bagasse	Strains of Basidiomycetes	Cellulase, ligninase	31
Cellulosics	*T. reesei*	Cellulase	32
Sweet sorghum silage, wheat straw	*Gliocladium* sp., *Trichoderma* sp., *Penicillium* sp.	Cellulase, xylanase	33
Agro-wastes	*A. niger*	Cellulase, β-glucosidase	34
Agro-wastes	*T. reesei*	Cellulase	35
Sugar beet pulp	*P. capsulatum*	Polysaccharide degrading enzymes	36
Wheat bran + rice straw, spent wheat bran	*Trichoderma* sp., *Botritis* sp., *A. ustus, Sporotrichum pulverulentum*	Cellulase, β-glucosidase, xylanase	37
Bagasse	*Polyporus* sp.	Cellulase, ligninase	38
Wheat straw	*Neurospora crassa*	CMCase, β-glucosidase	39
Rice straw, spent wheat bran	*Botritis* sp., *A. ustus, S. pulverulentum*	β-glucosidase, xylanase	40
Wheat bran	*T. reesei, S. pulverulentum*	Cellulase	41
Wheat straw + wheat bran	*T. harzianum*	Cellulase	42
Cellulosic wastes	*T. reesei*	Cellulase	43
Agro-wastes	*Spiecellum roseum*	CMCase	44
Bagasse, wheat bran, rice bran	*Aspergillus* sp.	Cellulase, β-glucosidase	45
8 ligno-cellulosic substrates	*Streptomyces* sp.	Cellulase	46
Agro-wastes	*Pestalotiopsis versicolor*	Cellulase	47
Wheat bran	*T. reesei*	Cellulase	48
Cellulosic wastes	*T. viride*	Cellulase	49
Agro-wastes	*Trichoderma* sp.	Cellulase	50
Wheat bran	*T. reesei*	Cellulase	51
Saw dust + wheat bran	*T. koningii*	Cellulase	52
Grapevine cutting waste	*C. unicolor*	Cellulases, xylanase, laccase	53
Palm oil mill waste	*A. niger*	Cellulases, xylanase	54
Cellulosics	*T. viride*	Cellulases	55
Bagasse	*A. ellipticus, A. fumigatus*	Cellulases, β-glucosidase	56, 57
Wheat bran	*T. reesei*	Cellulases	58
Sago hampas	*P. sajor-caju*	Cellulases, xylanase, laccase	59
Sweet sorghum pulp, wheat straw	Ligninolytic fungal cultures	Cellulases, xylanase	60
Cassava waste	*T. harzianum*	Cellulases, xylanase	61
Steam pre-treated willow	*T. reesei*	Cellulases	62
Wheat bran	*T. reesei*	CMCase	63
Sweet sorghum	*Gliocladium* sp.	Cellulases, xylanase	64
Wheat straw	*T. reesei*	Cellulases	65, 66
Soyhull	*Phanerochaete chrysosporium*	Cellulases	67
Paddy straw	*T. reesei*	Cellulases	68
Wheat straw	*Lentinus edodus*	Cellulases	69
Sweet sorghum silage	*T. reesei, A. niger*	Cellulases, xylanase	70
Cellulosic wastes	*A. oryzae*	Cellulases, β-glucosidase	71

Table 2. (*Contd*)

Substrate	Microorganisms	Enzyme	References
Bagasse	*T. reesei, A. niger, A. phoenicis*	Xylanase	72
Wheat straw	*Phlebia radiata, P. eryngii*	Xylanase, laccase, aryl-alcohol oxidase	73
Wheat bran	*Bacillus licheniformis*	Xylanase	74
Wheat bran + bagasse	*A. niger*	Xylanase	75
Wheat bran, defined media	*P. chrysosporium*	Xylanase, β-xylosidase	76
Wheat straw	*P. sajor-caju*	Xylanase, cellulase, β-glucosidase	77
Rice straw + cotton stalks + kenaf	*L. edodus*	Xylanase, laccase, β-xylosidase	79
Ligno-cellulosics	Bacterial isolate B698	Xylanase	80
Rice straw, soybean hull, wheat bran, birchwood, oat spelt, kraft pulp, beet pulp	*A. sojae*	Xylanase, α-arabinofuranosidase	81
Wheat/rice straw, bagasse, rice husk	*Melanocarpus albomyces*	Xylanase, acetoesterase	82
Wheat bran, apple pomace, sugar beet pulp, wheat straw	*Chaetomium globosum, A. niger*	Xylanase	83
Rice straw, corn hull, corncobs, bagasse, wheat bran	*A. fumigatus*	Xylanase, xylosidase	84
Wheat straw	*P. sanguineous*	Xylanase, CMCase, catechol-oxidase,	85
Wheat bran	*Thermomyces lanuginosus (Humicola lanuginosa), Thermascus aurantiacus*	Xylanase	86
Wheat bran	*Humicola* sp.	Xylanase	87
Apple pomace	*A. niger, A. fumigatus, T. viride*	Xylanase, CMCase	88
Coffee processing plant waste	*Thermomonospora* sp.	Xylanase, cellulase, α-arabinofuranosidase, β-xylosidase	89
Solka floc, wheat bran, barley straw, oat straw, wheat straw	*Talaromyces emersonii*	Xylanase	90
Wheat bran	*A. awamori*	β-Xylosidase	91
Wheat bran	*T. harzianum*	β-Xylosidase	92
Wheat bran	*A. terreus*	β-Xylosidase	93
Wheat bran	*A. fumigatus*	β-Xylosidase, β-glucosidase	94
^{14}C-lignin (perlite)	*P. ostreatus*	Laccase	96
Wheat straw	*Pleurotus* sp.	Laccase, Mn-peroxidase	97
Bagasse	*P. chrysosporium* and 44 species of Basidiomycetes	Laccase, Li-peroxidase, Mn-peroxidase	98
Synthetic media	*P. floridae*	Laccase, Li-peroxidase, Mn-peroxidase	99
^{14}C-lignin-labelled wheat straw	*P. chrysosporium* and four species of *Pleurotus*	Mn-peroxidase, laccase, aryl-alcohol oxidase	100
Synthetic media	*P. ostreatus*	Mn-peroxidase, laccase, catalase	101
Wheat straw	*Phlebia radiata*	Laccase, Li-peroxidase, Mn-peroxidase	102
Bagasse	*Trametes versicolor*	Laccase, Mn-peroxidase	103
Bagasse	*Flammulina velutipes*	Phenol oxidase	103
Wheat straw	*Pleurotus* sp.	Laccase, aryl-alcohol oxidase	104
Wood chips	*P. chrysosporium*	Laccase, Li- and Mn-peroxidase	105
Wheat straw	*Panus tigrinus, Phlebia radiata*	Laccase, Li-peroxidase, Mn-peroxidase	106,
Cotton stalks	*P. chrysosporium, P. ostreatus*	Laccase	108
Saw dust	*Rigidoporus lignosus*	Mn-peroxidase	109
Wheat straw	*Panus tigrinus*	Laccase, Mn-peroxidase	110
Wheat straw, kraft lignin, cellulose powder	*T. versicolor, P. ostreatus*	Laccase, Li-peroxidase, Mn-peroxidase	111
Bagasse	*Polyporus* sp.	Laccase	112
Soybean flour, sunflower flour, coffee husk	*Penicillium citrinum*	Protease	113
Soybean meal	*Bacillus amyloliquefaciens*	Alkaline protease	114
Wheat bran	*A. niger*	Acid protease	115
Wheat bran	*Bacillus licheniformis*	Neutral protease, α-amylase	116
Rice bran	*Rhizopus oligosporus*	Acid protease	117
Rice bran	*R. oligosporus*	Protease	118
Wheat bran	*B. amyloliquefaciens*	Protease	119

Table 2. (*Contd*)

Substrate	Microorganisms	Enzyme	References
Wheat bran	*Pseudomonas* sp.	Alkaline protease	120
Polyurethane foam	*A. oryzae*	Alkaline protease	121
Wheat bran + bean cake	*A. niger*	Acid protease	122
Wheat bran, soybean, rice, rice bran	*R. oligosporus*	Protease	123
Wheat bran	*A. niger*	Acid protease	124
Wheat bran + rice bran	*T. koningii*	Alkaline protease	125
Aspen wood	*P. chrysosporium*	Acid protease	126
Wheat bran, rice bran, corn bran, rice hull	*A. oryzae*	Protease	127
Wheat bran	*A. flavus*	Alkaline protease	128
Sweet potato residue	*A. niger*	Acid protease	129
Wheat bran	*A. flavus*	Alkaline protease	130
Wheat bran	Actinomycetes strains	Protease	131
Waste hair	*Streptomyces* sp.	Protease	132
Peanut press cake	*Neurospora sitophila*, *R. oligosporus*	Lipase	135
Wheat bran	Several filamentous fungi	Lipase	136
Coconut oil cake	*Candida rugosa*	Lipase	18
GYP medium	*A. oryzae*	Lipase	141
Amberlite	*R. delemer*	Lipase	142
Rice bran, wheat bran	*Candida* sp.	Lipase	143
Wheat bran	*P. candidum*	Lipase	144, 145
Rice bran	*C. rugosa*	Lipase	146, 147
Wheat bran	*A. niger*	Polygalacturonase	150, 151
Coffee pulp	*A. niger*	Polygalacturonase	152, 153
Citrus waste	*A. foetidus*	Polygalacturonase	154
Apple pomace	*A. foetidus*	Polygalacturonase	155, 156
Wheat bran	*A. oryzae*	α-galactosidase	160
Wheat bran	*A. niger*	α-galactosidase	161
Soybean cake residue	*A. oryzae*	β-galactosidase	164
Wheat bran	*A. fonsecaeus*	β-galactosidase	165
Wheat bran	*Rhizomucor* sp.	β-galactosidase	166
Wheat bran	*Kluyveromyces lactis*	β-galactosidase	168, 169
Polystyrene	*Vibrio costicola*	L-glutaminase	170, 172, 173
Wheat bran, rice husk, saw dust, coconut oil cake	*V. costicola*	L-glutaminase	171
Starch waste	*B. megatarium*	β-amylase	175
Wheat bran	*Pycnoporus sanguineus*	α-amylase	176
Banana waste	*Aeromonas caviae*	α-amylase	177
Polyurethane foam	*A. oryzae*	α-amylase	178, 188
Wheat bran	*A. kawachii*	α-amylase	179
Banana waste	*B. subtilis*	α-amylase	182
Corn flour, wheat flour, potato, sweet potato	*Saccharomycopsis capsularis*	α-amylase, glucoamylase	184
Wheat bran	*B. coagulans*	α-amylase	185
Wheat bran	*B. licheniformis*	α-amylase	189, 190, 192–194
Rice bran	*A. oryzae*, *A. niger*	α-amylase, glucoamylase	191
Wheat bran	*A. niger*	Glucoamylase	195, 197–203, 209, 216–218
Urethane foam	*A. oryzae*	Glucoamylase	220
Wheat bran	*A. awamori*	Glucoamylase	222
Rice bran, soybean meal	*A. niger*	Glucoamylase	196
Rice bran, defatted soybean meal	*A. niger*	Glucoamylase	221
Copra waste	*A. niger*	Glucoamylase	16
Wheat bran	*Rhizopus* sp.	Glucoamylase	207
Rice bran +cassava starch +rice hulls	*Aspergillus* sp.	Glucoamylase	210
Rice	*Amylomyces rouxii*	Glucoamylase	211
Cassava	*Rhizopus* sp.	Glucoamylase	215
Rye meal + beet pulp	*A. oryzae*, *A. awamori*	Glucoamylase, α-amylase	223
Cassava starch/kappa carrageenan	*R. oligosporus*	Glucoamylase	224
Chicory roots, wheat bran	*Staphylococcus* sp., *Kluyveromyces marxianus*	Inulinase	225–227
Canola meal	*A. ficuum*, *A.carbonarius*	Phytase	228–230
Wheat bran + tannic acid	*R. oryzae*	Tannase	231
Sugar beet pulp	*T. reesei*	α-L-arabinofuranosidase	232
Wheat bran	*Acremonium strictum*	Glucooligosaccharide oxidase	233
Wheat straw	*Penicillium pinophilum*	Feruloyl-para-coumaroyl esterase	234

The selection of a substrate for enzyme production in a SSF process depends upon several factors, mainly related with cost and availability of the substrate, and thus may involve screening of several agro-industrial residues. In a SSF process, the solid substrate not only supplies the nutrients to the microbial culture growing in it but also serves as an anchorage for the cells. The substrate that provides all the needed nutrients to the microorganisms growing in it should be considered as the ideal substrate. However, some of the nutrients may be available in sub-optimal concentrations, or even absent in the substrates. In such cases, it would become necessary to supplement them externally with these. It has also been a practice to pre-treat (chemically or mechanically) some of the substrates before using in SSF processes (e.g. lignocellulose), thereby making them more easily accessible for microbial growth.

Among the several factors that are important for microbial growth and enzyme production using a particular substrate, particle size and moisture level/water activity are the most critical[3,4,6,20,21]. Generally, smaller substrate particles provide larger surface area for microbial attack and, thus, are a desirable factor. However, too small a substrate particle may result in substrate agumulation, which may interfere with microbial respiration/aeration, and therefore result in poor growth. In contrast, larger particles provide better respiration/aeration efficiency (due to increased inter-particle space), but provide limited surface for microbial attack. This necessitates a compromised particle size for a particular process.

SSF processes are distinct from submerged fermentation (SmF) culturing, since microbial growth and product formation occurs at or near the surface of the solid substrate particle having low moisture contents. Thus, it is crucial to provide an optimized water content, and control the water activity (a_w) of the fermenting substrate—for, the availability of water in lower or higher concentrations affects microbial activity adversely. Moreover, water has profound impact on the physico-chemical properties of the solids and this, in turn, affects the overall process productivity.

Aspects of design of fermenter for enzyme production in solid state fermentation systems

Over the years, different types of fermenters (bioreactors) have been employed for various purposes in SSF systems. Pandey[8] reviewed the aspects of design of fermenter in SSF processes. Laboratory studies are generally carried out in Erlenmeyer flasks, beakers, petri dishes, roux bottles, jars and glass tubes (as column fermenter). Large-scale fermentation has been carried out in tray-, drum- or deep-trough type fermenters. The development of a simple and practical fermenter with automation, is yet to be achieved for the SSF processes.

Factors affecting enzyme production in solid state fermentation systems

The major factors that affect microbial synthesis of enzymes in a SSF system include: selection of a suitable substrate and microorganism; pre-treatment of the substrate; particle size (inter-particle space and surface area) of the substrate; water content and a_w of the substrate; relative humidity; type and size of the inoculum; control of temperature of fermenting matter/removal of metabolic heat; period of cultivation; maintenance of uniformity in the environment of SSF system, and the gaseous atmosphere, i.e. oxygen consumption rate and carbon dioxide evolution rate.

Enzymes produced by solid state fermentation processes

Ideally, almost all the known microbial enzymes can be produced under SSF systems. Literature survey reveals that much work has been carried out on the production of enzymes of industrial importance, like proteases, cellulases, ligninases, xylanases, pectinases, amylases, glucoamylases, etc.; and attempts are also being made to study SSF processes for the production of inulinases, phytases, tannases, phenolic acid esterases, microbial rennets, aryl-alcohol oxidases, oligosaccharide oxidases, tannin acyl hydrolase, α-L-arabinofuranosidase, etc. using SSF systems (cf. Table 2). In the following sections, a brief account of production on various enzymes in SSF systems is discussed.

Cellulases, Xylanases and Xylosidases

Cellulases are a complex enzyme system, comprising endo-1,4-β-D-glucanase (EC-3.2.1.4), exo-1,4-β-glucanase (exocellobiohydrolase, EC-3.2.1.91) and β-D-glucosidase (β-D-glucoside glucanhydrolase, EC-3.2.1.21). These enzymes, together with other related enzymes, viz. hemicellulases and pectinases, are among the most important group of enzymes that are employed in the processing of ligno-cellulosic materials for the production of feed, fuel, and chemical feedstocks. Cellulases and xylanases (endo-1,4-β-D-xylanase, EC-3.2.1.8) however find applications in several other areas, like in textile industry for fibre treatment and in retting process. Xylanases find specific application in jute fibre upgradation also.

Currently, industrial demand for cellulases is being met by production methods using submerged fermentation (SmF) processes, employing generally genetically modified strains of *Trichoderma*. The cost of production in SmF systems is however high and it is uneconomical to use them in many of the aforesaid processes. This there-

fore necessitate reduction in production cost by deploying alternative methods, for example the SSF systems.

Tengerdy[19] compared cellulase production in SmF and SSF systems. While the production cost in the crude fermentation by SmF was about $ 20/kg, by SSF it was only $ 0.2/kg if *in situ* fermentation was used. The enzyme in SSF crude product was concentrated; thus it could be used directly in such agro-biotechnological applications as silage or feed additive, ligno-cellulosic hydrolysis, and natural fibre (e.g. jute) processing. A number of reports have appeared on microbial cellulase production in recent years (cf. Table 2)[22–71]. Nigam and Singh[13] have reviewed processing of agricultural wastes in SSF systems for cellulolytic enzyme production. They argued that with the appropriate technology, improved bioreactor design, and operation controls; SSF may become a competitive method for the production of cellulases. They also enumerated advantages of cellulase production together with the factors affecting the cellulase production in SSF systems.

In a recent study on the ligninolytic system of *Cerrena unicolor* 062 – a higher basidiomycete – upon supplementation of the medium with carbon sources and phenolic compounds in SSF system, it was observed that the growth of *C. unicolor* 062 could be regulated by the exogenous addition of these compounds. The efficiencies of the degradation of cellulose and lignin were dependent on the nature and concentration of the compounds added[53]. Sun *et al.*[55] developed a novel fed-batch SSF process for cellulase production which could overcome the problems associated with high initial nutrients concentration while retaining advantages from the high total effective salt concentration.

There are several reports describing co-culturing of two cultures for enhanced enzyme production. Gupte and Madamwar[56,57] cultivated two strains of *Aspergillus ellipticus* and *A. fumigatus* and reported improved hydrolytic and β-glucosidase activities compared to when they were used separately using SSF system, improved enzyme titres were achieved by Kanotra and Mathur[68] when a mutant of *Trichoderma reesei* was co-cultured with a strain of *Pleurotus sajor-caju* with wheat straw as the substrate. However, the media constituents too play an important role in mixed culturing. Gutierrez-Correa and Tengerdy[72] reported that single culture of *T. reesei* and *Aspergillus phoenicus*, when supplemented with inorganic nitrogen source, produced similar xylanase levels as mixed cultures. However, when the fermentation medium was supplemented with soy meal, 35–45% more xylanase (than the single culture) was produced by these cultures.

In a significant finding, Smits *et al.*[58] reported that glucosamine level of the fungi in liquid culture could not be used to estimate the biomass contents in SSF. They studied the SSF of wheat bran by *T. reesei* and reported that using glucosamine, correlation between the fungal growth and respiration kinetics could only partly be described with the linear growth model of Pirt. A decline in O_2 consumption rate (OCR) and CO_2 evolution rate (CER) started the moment glucosamine was 50% of its maximum value. After the glucosamine level reached its maximum, OCR and CER still continued to decrease.

A pan bioreactor, requiring a small capital investment, was developed for SSF of wheat straw[65,66]. High yields of complete cellulase system were obtained in comparison to those in the SmF. A complete cellulase system is defined as one in which the ratio of the β-glucosidase activity to filter paper activity in the enzyme solution is close to 1.0. The prototype pan bioreactor however required further improvements so that optimum quantity of the substrate could be fermented to obtain high yields of complete cellulase system per unit space.

Although xylanases produced by fungi, yeast and bacteria, filamentous fungi are preferred for commercial production as the levels of the enzyme produced by fungal cultures are higher than those obtained from yeast or bacteria. In many microorganisms, xylanase activity has generally been found in association with cellulases, β-glucosidase or other enzymes, although there are many reports that have described in SSF systems, production of cellulase-free and other enzymes-free xylanase (cf. Table 2)[72–90]. Haltrich *et al.*[78] reviewed the different factors that influence xylanase production by fungi. In view of the considerable commercial importance of enzymes, it was emphasized that efforts should be directed towards enhanced enzyme production with reduced associated costs.

Archana and Satyanarayana[74] described a SSF process for the production of thermostable xylanase by thermophilic *Bacillus licheniformis*. Enzyme production was 22-fold higher in SSF system than in SmF system. Cai *et al.*[75] also reported production of a thermostable xylanase in SSF system. Enzyme produced in SSF system was more thermostable than in SmF system. Dunlop *et al.*[80] described a bacterium, isolated from wood compost, producing xylanase that was active at 80°C. Jain[82] too described a SSF process for the production of xylanase by thermostable *Melanocarpus albomyces*.

Alam *et al.*[86] using SSF process, isolated a thermostable cellulase-free xylanase produced by *T. lanuginosa*. Addition of 0.7% xylan induced enzyme production to an extent of 28%. The enzyme was stable at 70°C. A thermostable xylanase preparation from *Humicola* sp. showed the temperature optima at 75°C (ref. 87). Srivastava[89] reported a xylanase from *Thermomonospora* sp., which was stable at 80°C. Tuohy and Coughlan[90] compared thermostable xylanase production on various substrates by a strain of *Talaromyces emersonii* in liquid culture and SSF systems. The latter showed higher enzyme activity compared to former, but liquid culture resulted in greater yields (U/g substrate).

Several authors have compared the performance of various microbial strains, grown on different substrates

(individual or in combination) and reported varying results. Wiacek-Zychlinska *et al.*[83] compared xylanase production by *C. globosum* and *A. niger* on four different substrates. Although activities obtained by *A. niger* were higher than those from the other microbial cultures, but high-spore production by the *A. niger* strain could result in problems for a pilot plant or large-scale process.

In order to achieve improved enzymes titre, it is generally a common practice to pre-treat cellulosic or ligno-cellulosic substrates before using them in SSF systems. Pre-treatment may be by physical processes or chemical processes[22,57,61,62,65,72,82]. Pre-treatment of palm oil mill waste, however, did not affect xylanase production[54].

β-xylosidase is another important enzyme used in textile industry. A β-xylosidase (EC-3.2.1.37) was produced by *A. awamori* K4 in SSF system on wheat bran, which was used for transxylosylation reactions[91]. There are other reports as well describing the production of β-xylosidase in SSF systems[92–94].

Ligninases

Lignin is a three-dimensional phenylpropanoid polymer which is considerably resistant to microbial degradation in comparison to polysaccharides and other naturally occurring biopolymers. Biological delignification by SSF processes using microbial cultures producing ligninolytic enzymes – the ligninases – can have applications in delignification of ligno-cellulosic materials[95], which can be used as the feedstock for the production of biofuels or in paper industry or as animal feedstuff. These may also be used in pulp bleaching, paper mill wastewater detoxification, pollutant degradation, or conversion of lignin into valuable chemicals.

Lignin peroxidase (LiP, EC-1.11.1.7), manganese peroxidase (MnP, EC-1.11.1.13) and laccase (EC-1.10.3.2) are the most important lignin-modifying enzymes. LiP and MnP are heme-containing glycoproteins requiring hydrogen peroxide as an oxidant. LiP oxidizes nonphenolic lignin structures by abstracting one electron and generating cation radicals, which are then decomposed chemically. MnP oxidizes Mn(II) to Mn(III), which then oxidizes phenolic compounds to phenoxy radicals. This leads to the decomposition of the lignin substructure. Laccase, a copper containing oxidase, utilizes molecular oxygen as the oxidant and oxidizes phenolic components to phenoxy radicals.

Literature survey shows that a number of micro-organisms produce ligninases[96–112], but white-rot fungi generally show the most desirable qualities, in particular *Pleurotus* species and *Phanerochaete chrysosporium* are the most widely studied (cf. Table 2).

Wheat straw was used for cultivating several fungal strains to produce laccase, Li-peroxidase, and Mn-peroxidase[97,102,104,106,107,110,111]. Several authors have used bagasse also[98,103,112]. Homolka *et al.*[96] studied laccase production from three strains of *Pleurotus* sp. (obtained after protoplast regeneration of the control strain). While two strains showed significantly higher laccase activity, one strain showed lower activity. The rate of mineralization of ^{14}C-lignin in SSF system by the latter and the control strain were almost the same, but it was higher than that of the other two strains. ^{14}C-lignin in SSF of wheat straw was also used by Camarero *et al.*[100] for studying Mn-mediated lignin degradation by four strains of *Pleurotus* sp., and comparing with by *P. chryso-sporium*. At the end of the incubation period, strains of *Pleurotus* sp. acquired higher delignification values than *P. chrysosporium*. All the species of genus *Pleurotus*, studied so far, produce Mn-peroxidase, laccase, and aryl-alcohol-oxidase (EC-1.1.3.13).

Dombrovskaya and Kostyshin[99] studied the effects of different ionic nature surfactants on ligninolytic enzyme complexes of the white-rot fungi in SSF processes. The cationic surfactant, ethonium, enhanced the laccase and Mn-peroxidase activity by 1.8 fold and 1.6 fold, respectively for *P. floridae*. Kerem and Hadar[101] studied the effects of Mn on the production of ligninolytic enzyme complexes of *P. ostreatus* in a chemically defined SSF system. Laccase, Mn-peroxidase, and catalase (EC-1.11.1.6) activities, and H_2O_2 production were all affected by Mn levels.

Laplante and Chahal[105] compared ligninase production in SmF system and SSF system using a culture of *P. chrysosporium* ATCC 24725. Higher yields of ligninases, especially laccase and Mn-peroxidase, were obtained in SSF system. Kerem *et al.*[108] compared the ligninolytic activity of a strain of *P. chrsosporium* BKM with *P. ostreatus* Florida f16. The former grew vigorously resulting in rapid, non-selective degradation of 55% of the organic components of the cotton stalks within 15 days. *P. ostreatus* grew more slowly with obvious selectivity for lignin degradation, resulting in the degradation of only 20% or the organic matter in 30 days.

Proteases

Proteolytic enzymes account for nearly 60% of the industrial market in the world. They find application in a number of biotechnological processes, viz. in food processing and pharmaceuticals, leather industry, detergent industry, etc. Recently, Mitra *et al.*[10] reviewed production of proteolytic enzymes in SSF systems. From their viewpoint, proteases produced by SSF processes have greater economic feasibility.

In recent years, there have been increasing attempts to produce different types of proteases (acid, neutral, alkaline) through SSF route, using agro-industrial residues (cf. Table 2)[113–132]. It is interesting to note that although a

number of substrates have been employed for cultivating different microorganisms, wheat bran has been the preferred choice in most of the studies. Malathi and Chakraborty[128] evaluated a number of carbon sources (brans) for alkaline protease production and reported wheat bran to be the best for cultivation of *A. flavus* IMI 327634. Studies were carried out to compare alkaline protease production in SmF systems and SSF systems[114]. The total protease activity present in one-gram bran (SSF) was equivalent to 100-ml broth (SmF). A repeated batch mode SSF process was described for alkaline protease production in which polyurethane was used as the inert solid support[121]. A thermostable alkaline protease was reported to be produced by a novel *Pseudomonas* sp. in SSF system[120]. A process has been developed at CLRI, Chennai (India), for the commercial production of an alkaline protease (Clarizyme) which was produced by SSF of wheat bran using a strain of *A. flavus*[130].

A new strain of *A. niger* Tieghem 331221 produced large quantities of an extra-cellular acid protease when grown in SSF system using wheat bran as the sole substrate[115]. Various C-sources inhibited protease synthesis, indicating the presence of catabolic repression of protease biosynthesis. The enzyme showed potential for usage as a bating agent. Ikasari and Mitchell[117] used rice bran for acid protease synthesis by a strain of *R. oligospora*. They observed that although the enzyme showed optimum activity at pH 4, a leaching solution of pH 7 gave the optimum recovery of the enzyme from the fermented matter. They made stepwise changes in the gas environment and temperature during SSF process to mimic those changes which arose during SSF due to mass and heat transfer limitations. It was observed that a decrease of O_2 concentration from 21% to 0.5% did not alter protease production[118]. Yaoxing *et al.*[122] carried out SSF of wheat bran with a strain of *A. niger* QX 1066 for acid-resistant protease. High enzyme activities were obtained in a medium containing high carbon and low nitrogen content. Addition of a suitable phosphate in the medium further improved the enzyme titres. Villegas *et al.*[124] studied the effects of O_2 and CO_2 partial pressure on acid protease production by a strain of *A. niger* ANH-15 in SSF of wheat barn. Results showed a direct relationship between pressure drop, production of CO_2, and temperature increase. Acid protease production increased when the gas had 4% CO_2 (v/v), and it was directly related with the fungus metabolic activity as represented by the total CO_2 evolved.

Germano *et al.*[113] used a strain of *P. citrinum* for serine protease production using agro-industrial residues. The strain also exhibited lipase activity. Datta[126] used aspen wood for the production of protease from the fungal strain of *P. chrysosporium* BKM-F-1767. Study of this enzyme's characteristics showed that this protease had properties of aspartate-type protease as well as of thiol-type protease.

Lipases

Fat splitting has been completely revolutionized by the introduction of lipases (EC-3.1.1.3) into the industrial arena. The conventional physico-chemical means of lipolysis have now been undershadowed by the biocatalysis using microbial lipases. Lipases have a wide array of industrial applications in the production and processing of detergents, oils, fats and dairy-products. In addition, they are also used in the preparation of therapeutic agents[133,134].

Until recently, SmF was in vogue for microbial lipase production. However, in recent years the shift has been towards the study and development of lipase production in SSF system[135–147]. Beuchat[135] investigated SSF of peanut press-cake using *Neurospora sitophila* and *Rhizopus oligosporus*. Rivera-Munoz *et al.*[136] compared SmF systems and SSF systems for lipase production using several filamentous fungi. Enzyme titres by SSF processes were higher and stable. Among the tested microbial strains, *P. candidum*, *P. camembertii*, and *M. miehei* proved the best for lipase production.

Benjamin and Pandey[18,137–139] and Benjamin[140] cultivated *Candida rugosa* on coconut oil cake for lipase production using SSF and SmF systems. Enzyme yields were higher in the former. Several carbon sources – individually and in combinations – were tested for their efficiency to produce lipases. Raw cake supported the growth and lipase synthesis by the yeast culture. However, supplementation with additional C- and N-sources increased enzyme titres. In contrast to this, however, Ohnishi *et al.*[141] reported less lipase production from *A. oryzae* using SSF compared to SmF where high enzyme yields were obtained. Yet, in another comparative study on lipase production in SmF and SSF systems, Christen *et al.*[142] observed a 5-fold increase in lipase productivity in SSF system.

Bhusan *et al.*[143] reported lipase production in SSF system from an alkalophilic yeast strain belonging to *Candida* sp. Rice bran and wheat bran, oiled with different concentrations of rice bran oil were used as the substrate. Rice bran supplemented with oil gave higher lipase yields. Ortiz-Vazquez *et al.*[144] and Granados-Baeza *et al.*[145] used wheat bran for cultivating the strains of *P. candidum*. They designed an enzyme-recovery procedure and reported that 0.01 M NaCl was adequate to recover enzyme from the fermented matter.

Pectinases

Studies have been conducted on comparative production of pectinases in systems of SmF and SSF[148,149]. When the fermentation medium was supplemented with different carbon sources, like glucose, sucrose and galacturonic acid, polygalacturoanase (PG, EC-3.2.1.15) production by

A. niger CH4 increased in SSF system but decreased in SmF system. Overall productivity by SSF was 18.8 and 4.9-fold higher for endo-PG and exo-PG, respectively, than those obtained by SmF[148]. Minjares-Carranco *et al.*[149] made physiological comparisons between pectinase-producing mutants of *A. niger* C28B25, adapted either to SmF or SSF. *A. niger* produced isozymes with difference in PG properties depending on the culture technique and strain used. The results also suggested that pleiotropic mutations of different kinds simultaneously affect the sporulation and enzymological patterns of each class of mutants.

Media acidity plays a significant role on pectinases' production by SSF processes. Cavalitto *et al.*[150] and Hours *et al.*[151] studied growth and pectinase production by *A. foetidus* and *A. awamori*, respectively in SSF systems at different media acidities. Both used wheat bran as the substrate. Results showed that higher the HCl concentration used, higher was the total pectolytic activity achieved. The low pH of the culture condition maintained asepsis during fermentation.

Apart from wheat bran, several other substrates have also been used for pectinase production in SSF system. These include coffee pulp[152,153], citrus waste[154], and apple pomace[155,156]. Huerta *et al.*[157] used bagasse as the inert substrate to produce PG in a 130 litres-packed bed fermenter by *A. niger* CH4 (they referred it as 'absorbed substrate fermentation'). They claimed that the process was an efficient one for PG production as well as an interesting model since the culture medium, water, nutrients and specific inducers could be varied depending on the concentrations required. Acuna-Arguelles *et al.*[158] studied effect of water activity (a_w) on exo-pectinase production by *A. niger* CH4 in SSF system. Sugar cane bagasse was used as the (inert) substrate and ethylene glycol was used as the water activity depressor. Results showed that although PG production decreased at low a_w values, the activity was present even at as low as 0.90 a_w values. The specific activity increased up to 4.5 fold by reducing the a_w from 0.98 to 0.90.

Galactosidases

There has been considerable interest to produce α-galactosidase (EC-3.2.1.22) and β-galactosidase (EC-3.2.1.23) in SSF processes. Both these enzymes have applications in the pharmaceutical and food industries.

Cruz and Park[159] reported production of α-galactosidase in SSF system and its application in the hydrolysis of galactooligosaccharides in soybean milk. Addition of soybean carbohydrate in the fermenting medium, using *A. oryzae*, was shown to induce enzyme production. Annunzaiato *et al.*[160] carried out SSF of wheat bran for α-galactosidase production using a strain of *A. oryzae* QM 6737 with the aim of improving enzyme yields and lowering production costs. Enzyme yield increased 3 fold when soy flour or soybeans were used as the substrate, but no enzyme was produced using rice. Somiari and Balogh[161] used a strain of *A. niger* for α-galactosidase production on wheat bran or rice bran. Srinivas *et al.*[162] described the use of Plackett–Burman design for rapid screening of several nitrogen sources, growth/product promoters, minerals and enzyme inducers for the production of α-galactosidase by *A. niger* MRSS 234 in SSF.

In 1990, Wakamoto Pharma patented (two patents) the production of β-galactosidase in SSF systems[163,164]. Strains of *Aspergillus* sp. and *Penicillium* sp. were used[163]. Details have been provided in these patents by giving an example of the cultivation conditions and yields using a strain of *A. oryzae*. Enzyme preparation from *A. fonsecaeus*, which was cultivated on wheat bran[165], showed superior qualities than the other commercial preparation using a strain of *A. oryzae* and the enzyme was more suitable for biotechnological applications. Gonzalez and Monsan[165] also used a strain of *A. fonsecaeus* for β-galactosidase production by SSF of wheat bran.

A thermostable β-galactosidase was reported from a thermophilic *Rhizomucor* sp[166]. Enzyme activities by SSF were 9-fold more than by SmF processes. Strains of *Kluyveromyces* sp. have also been employed for β-galactosidase synthesis in SSF systems[167–169]. Becherra and Siso[168] cultivated *K. lactis* NRRL T-1140 on corn grits and wheat bran in SmF and SSF systems. They observed that change from liquid to solid state culturing did not promote β-galactosidase secretion by the yeast strain, though there were problems of drying of medium etc. in SSF. However, studies on production of β-galactosidase in SSF systems had already been published in 1995 (ref. 169).

Glutaminases

L-glutaminase is considered a potent anti-leukamic drug and has found application as a flavour-enhancing agent in food industry. In a maiden report, Prabhu and Chandrasekaran[170] reported L-glutaminase production by SSF using marine *Vibrio costicola*. Polystyrene was used as the inert substrate. They also evaluated several organic substrates for their ability to produce glutaminases by SSF using the same strain. Among the tested materials, wheat bran and rice bran were found superior in comparison to saw dust, coconut oil cake, and groundnut cake[171]. However, use of polystyrene as the substrate offered several advantages over organic substrtes[172,173]. For example, leachate from polystyrene-SSF system was not only less viscous but also showed high specific activity of the enzyme.

Amylases

The amylase family of enzymes has been well characterized through the study of various microorganisms. Presence of two major classes of starch-degrading enzymes have been identified in the microorganisms, viz. α-amylase (endo-1,4-α-D-glucan glucohydrolase, EC-3.2.1.1) which randomly cleaves the 1,4-α-D-glucosidic linkages between the adjacent glucose units in linear amylose chain, and glucoamylase (synonym amyloglucosidase – also referred to as glucogenic enzyme, starch glucogenase, gamma amylase; exo-1,4-α-D-glucan glucanohydrolase, EC-3.2.1.3) which hydrolyses single glucose units from the nonreducing ends of amylose and amylopectin in a stepwise manner. Unlike α-amylase, most glucoamylases are also able to hydrolyse the 1,6-α-linkages at the branching points of amylopectin, although at a slower rate than 1,4-linkages.

Amylases and glucoamylases are produced by various microorganisms, including bacteria; fungi and yeast, but a single strain can produce both these enzymes as well. These enzymes have found applications in processed-food industry, fermentation technology, textile and paper industries, etc. Selvakumar *et al.*[174] reviewed microbial synthesis of starch-saccharifying enzymes in solid cultures.

SSF has been employed to produce amylases. In a recent study, Ray *et al.*[175] compared the production of β-amylase (EC-3.2.1.2) from starch waste by a hyper-amylolytic strain of *Bacillus megaterium* B6 mutant UN12 by SmF and SSF processes. The starchy wastes used as substrates were from arrowroot, arum, maize, potato, pulse, rice, rice husk, tamarind, kernel, cassava, water chestnut, wheat and wheat bran. Arum and wheat bran gave the highest yields.

Comparative studies on α-amylase production using different substrates have been studied as well[176–181]. A new source of α-amylase was identified in *Pycnoporus sanguineus*. Cultivation of it in SSF system resulted in 4-fold higher enzyme production than in SmF system. Krishna and Chandrasekaran[177,182] cultivated *Aeromonas caviae* (CBTK 185) on banana waste. The results indicated excellent scope for utilizing this strain and banana waste for commercial production of α-amylase by SSF. Sudo *et al.*[179] compared acid-stable α-amylase production in SmF and SSF systems to ascertain as to why *A. kawachii* IFO 4308 produced larger amounts of acid-stable α-amylase in SSF system than in SmF system. Some of the attributes of SSF system were reported as the major reasons for higher enzyme production by SSF. A comparative study on SmF and SSF of inert substrate using a strain of *A. oryzae* CBS 125-59 also showed superiority of SSF system[178].

Lonsane and Ramesh[183] reviewed the production of bacterial thermostable α-amylases by SSF, which they referred to as the potential tool for achieving economy in enzyme production and starch hydrolysis. Various methods to reduce the cost of production were discussed, taking into consideration enzyme production by *B. amyloliquefaciens* and *B. licheniformis*.

Numerous other microorganisms like *Saccharomycopsis capsularia*[184], *B. coagulans*[185], *Bacillus* sp. HOP-40[186], and *B. megatarium* 16 M (ref. 187) have also been used for α-amylase production by SSF using agro-industrial residues.

Recovery of the enzymes from the fermented matter is an important factor that affects the cost-effectiveness of the overall process. In a significant finding, Padmanabhan *et al.*[190] reported that the recovery of α-amylase from the solid fermented matter depended on the temperature of extraction. When enzyme was extracted and recovered at 50°C, the quantum of recovery was 2.2 fold higher than at 30°C. A further increase of about 19% in leaching efficiency was observed when contact time was extended from 60 to 120 min.

The other important enzyme of the amylase family is glucoamylase (GA). Traditionally, glucoamylase has been produced by SmF and one-way process in solution has been well developed. In recent years, however, the SSF processes have been increasingly applied for the production of this enzyme.

A strain of *A. niger* was used for the production of glucoamylase in solid cultures[11,14–17,20,195–206]. The study included screening of a number of agro-industrial residues including wheat bran, rice bran, rice husk, gram flour, wheat flour, corn flour, tea waste, copra waste, etc., individually and in various combinations[14,17,195,196,204]. Apart from the substrate's particle size, which showed profound impact on fungal growth and activity, substrate-moisture content and water activity also significantly influenced the enzyme's yield[15,20,199]. Different types of bioreactors were used to evaluate their performances. These included flasks, aluminium trays, and glass columns (vertical and horizontal)[195,200,201]. Enzyme production in trays occurred optimally in 36 h in comparison to typically required 96 h in flasks[195]. In a significant study on the effect of yeast extract on glucoamylase synthesis by *A. niger* NCIM 1248 in SSF system, it was observed that supplementation with 0.5% yeast extract resulted in about 20% increase in enzyme yields[203]. GA was purified 32.4 fold with the final specific activity of 49.25 U/mg protein. Four different forms (GA-I, GA-I', GA-II, and GA-II'), having different characteristics were reported. This was the first report on the four forms of GA produced by *A. niger* by SSF[202].

There are reports describing a comparative profile of glucoamylase production in SmF and SSF systems[207–210]. Interestingly, contrary to the general findings, Fujio and Morita[207] reported a 4.6-fold lower glucoamylase yield by *Rhizopus* sp. A-11 in a conventional SSF process using wheat bran medium than by SmF which used metal-ion supplemented medium. Solid and liquid cultures yielded 150 and 189 mg of protein, respectively. Hata *et al.*[208]

compared the two glucoamylases produced in SmF and SSF systems using *A. oryzae*. Enzyme produced by SSF could digest raw starch but that by SmF could not. GA obtained by the two systems exhibited different characteristics. Tani *et al.*[210] too compared characteristics of GA produced by either SmF and SSF processes. Solid culture was more efficient than liquid culture for GA production.

Rajgopalan *et al.*[212] used a bacterial strain of *B. coagulans* for modelling of substrate-particle degradation in SSF system of GA. Enzyme diffusion was found to be a critical factor in degradation of the substrate particle. Mitchell *et al.*[213] studied an empirical model of growth of *R. oligosporus* in SSF system. An equation was developed to describe glucoamylase activity on the substrate, which was then used to predict the growth. Apart from an early discrepancy, the growth rate correlated reasonably with the GA activity. Elegado and Fujio[214] screened 39 *Rhizopus* isolates and 9 authentic *Rhizopus* strains (grown on wheat bran in a SSF system) for their soluble starch digestive GA (SSGA) and raw starch digestive GA (RSGA) activities. Results showed that these strains could be classified into four groups, based on their SSGA and RSGA production and ratio of SSGA to RSGA. Soccol *et al.*[215] also screened 19 *Rhizopus* strains for their ability to grow on raw cassava. Only three strains grew significantly, and GA production was higher on raw cassava than on cooked cassava.

A patent was granted to Snow Brand Milk Prod in 1990 for a process for GA production on multi-stage culture medium[219]. An effective method for GA production in SSF was also described by Kobayashi *et al.*[220]. There are many other reports on GA production in SSF systems using different strains on various substrates[221–224].

Misclleneous enzymes

There are some reports describing SSF processes for the production of various other enzymes also, viz. inulinase[225–227], phytase[228–230], tannase[231], α-L-arabinofuranosidase[232], oligosaccharide oxidase[233], and phenolic acid esterase[234], etc. (cf. Table 2).

Conclusion

Critical analysis of the literature shows that production of industrial enzymes by SSF offers several advantages. It has been well established that enzyme titres produced in SSF systems are many-fold more than in SmF systems. Although the reasons for this are not clear, this fact is kept in mind while developing novel bioreactors for enzyme production in SSF systems. It is hoped that enzyme production processes based on SSF systems will be the technologies of the future. Genetically improved strains, suitable for SSF processes, would play an important role in this.

1. Tengerdy, R. P., in *Advances in Biotechnology* (ed. Pandey, A.) Educational Publishers and Distributors, New Delhi, 1998, pp. 13–16.
2. Hesseltine, C. W., *Process Biochem.*, 1977, **12**, 24–27.
3. Pandey, Ashok, *Process Biochem.*, 1992, **27**, 109–117.
4. Pandey, Ashok, in *Solid State Fermentation* (ed. Pandey, A.) Wiley Eastern Publishers, New Delhi, 1994, pp. 3–10.
5. Chahal, D. S. and Moo-Young, M., *Dev. Ind. Microbiol.*, 1981 **22**, 143.
6. Nigam, P. and Singh, D., *J. Basic Microbiol.*, 1994, **34**, 405–422.
7. Aidoo, K. E., Hendry, R. and Wood, J. B., *Adv. Appl. Microbiol.* 1982, **28**, 201–237.
8. Pandey, Ashok, *Process Biochem.*, 1991, **26**, 355–361.
9. Doelle, H.W., Mitchell, D. A. and Rolz, C. E. (eds.), *Solid State Fermentation*, Elsevier, London, 1992.
10. Mitra, P., Chakraverty, R. and Chandra, A. L., *J. Sci. Ind. Res.* 1994, **55**, 439–442.
11. Selvakumar, P., Ashakumary, L. and Pandey, Ashok, *J. Sci. Ind. Res.*, 1994, **55**, 443–449.
12. Babu, K. R. and Satyanarayana, T., *J. Sci. Ind. Res.*, 1994, **55**. 464–467.
13. Nigam, P. and Singh, D., *J. Sci. Ind. Res.*, 1994, **55**, 457–463.
14. Pandey, Ashok and Radhakrishnan, S., *Process Biochem.*, 1993, **28**, 305–309.
15. Pandey, Ashok, Ashakumary, L., Selvakumar, P. and Vijayalakshmi, K. S., *World J. Microbiol. Biotechnol.*, 1994, **10**, 485–486.
16. Pandey, Ashok, Ashakumary, L. and Selvakumar, P., *Biores. Technol.*, 1995, **51**, 217–220.
17. Selvakumar, P., Ashakumary, L. and Pandey, Ashok, *Biores. Technol.*, 1998, **65**, 83–85.
18. Benjamin, S. and Pandey, Ashok, *Acta Biotechnol.*, 1997, **17**, 241–251.
19. Tengerdy, R. P., *J. Sci. Ind. Res.*, 1996, **55**, 313–316.
20. Pandey, Ashok, *Biores. Technol.*, 1991, **37**, 169–172.
21. Zadrazil, F. and Puniya, A. K., *Biores. Technol.*, 1995, **54**, 85–87.
22. Duenas, R., Tengerdy, R. P. and Gutierrez-Correa, M., *World J. Microbiol. Biotechnol.*, 1995, **11**, 333–337.
23. Muniswaran, P. K. A., Selvakumar, P. and Charyulu, N. C. L. N., *J. Chem. Technol. Biotechnol.*, 1994, **60**, 147–151.
24. Zakariasvili, N. G. and Elisashvili, V. I., *Mikrobiologia*, 1993, **62**, 876.
25. Kuhad, R. C. and Singh, A., *World J. Microbiol. Biotechnol.*, 1993, **9**, 100.
26. Begum, A. A., Chaudhury, N. and Sardar, A. H., *Bangladesh J. Microbiol.*, 1993, **10**, 21–25.
27. Kokhreidze, N. G. and Elisashvili, V. I., *Prikladnaya Biokhimiya Mikrobiol.*, 1993, **29**, 227–231.
28. Talukdar, S. H., Mohiudin, G., Ahmed, K., Ara, C. and Rahman, M. S., *Bangladesh J. Microbiol.*, 1992, **9**, 37–42.
29. Desgranges, C. and Durand, A., *Enzyme Microb. Technol.*, 1990, **12**, 546–548.
30. Mishra, C. and Leatham, G., *J. Ferment. Bioeng.*, 1990, **69**, 8–15.
31. Nigam, P., Pandey, Ashok and Prabhu, K. A., *Biol. Chem. Hoppe Seyler*, Suppl., 1986, **367**, 210.
32. Chahal, D. S., *ACS Symp. Ser.*, 1991, **460**, 111–122.
33. Szakacs, G. and Tengerdy, R. P., *Abstr. Pap. Am. Chem. Soc.*, 1996, Pt. 1, CELL 168.
34. Madamwar, D., Patel, S. and Parekh, S., *J. Ferment. Technol.*, 1989, **67**, 424–426.
35. Allen, A. L. and Roche, C. D., *Biotechnol. Bioeng*, 1989, **33**, 650.
36. Considine, P. J., O'Rorke, A., Hackett, T. J. and Coughlan, M. P., *Biotechnol. Bioeng.*, 1988, **31**, 433.
37. Shamla, T. R. and Sreekantiah, K. R., *Enzyme Microb. Technol.*, 1987, **9**, 197–200.
38. Nigam, P., Pandey, Ashok and Prabhu, K. A., *Biol. Waste*, 1987, **20**, 1–9.
39. Maeris, B. J., Kekos, D., Evangelidou, X., Panayotou, M. G. and Rodis, P., *Biotechnol. Lett.*, 1987, **9**, 661–664.

40. Shamla, T. R. and Sreekantiah, K. R., *Enzyme Microb. Technol.*, 1987, **8**, 178–180.
41. Kim, J. H., Hosobuchi, M., Kishimoto, M., Seki, T., Yoshida, T., Taguchi, H., Ryu, D. and Doo, Y., *Biotechnol. Bioeng.*, 1985, **27**, 1445–1451.
42. Deschamps, F., Giuliano, C., Asther, M., Huet, M. C. and Roussos, S., *Biotechnol. Bioeng.*, 1985, **27**, 1385–1389.
43. Chahal, D. S., *Appl. Environ. Microbiol.*, 1985, **49**, 205–208.
44. Silman, R. W., McGhee, J. E. and Bothast, R. J., *Biotechnol. Lett.*, 1984, **6**, 115.
45. Gupte, A. and Madamwar, D., in *Solid State Fermentation* (ed. Pandey, A.), Wiley Eastern Publishers, New Delhi, 1994, pp. 130–133.
46. Modi, H. A., Patel, K. C. and Ray, R. M., in *Solid State Fermentation* (ed. Pandey, A.), Wiley Eastern Publishers, New Delhi, 1994, pp. 137–141.
47. Rao, M. N. A., Mithal, B. M., Thakkar, R. N. and Sastry, K. S. M., *Biotechnol. Bioeng.*, 1983, **26**, 869–876.
48. Mudgett, R. E., Nash, J. and Ruffner, R., *Dev. Ind. Microbiol.*, 1982, **23**, 397.
49. Toyama, N. and Ogawa, K., in Proceedings of the Symposium on Bioconversion of Cellulosic Substrates for Energy Chemicals and Microbial Protein (ed. Ghosh, T. K.), IIT, New Delhi, 1978, pp. 305.
50. Sternberg, D., *Biotechnol. Bioeng. Symp.*, 1976, **6**, 35.
51. Toyama, N., *Biotechnol. Bioeng. Symp.*, 1976, **6**, 207.
52. Arima, K., in *Global Impacts of Applied Microbiology* (ed. Starr, M. P.), J Wiley, NewYork, 1964, p. 277.
53. Elisashvili, V. I., Daushvili, L. P., Zakariashvili, N. G. and Kachlishvili, E. T., Kiknadeze, M. O. and Tusishvili, K. A., *Microbiology*, 1998, **67**, 33–37.
54. Prasertsan, P., Kittikul, A. H., Kunghae, A., Maneesri, J. and Oi, S., *World J. Microbiol. Biotechnol.*, 1997, **13**, 555–559.
55. Sun, T., Liu, B. H. and Li, Z. H., *J. Chem. Technol. Biotechnol.*, 1997, **69**, 429–432.
56. Gupte, A. and Madawar, D., *Appl. Biochem. Biotechnol.*, 1997, **62**, 267–274.
57. Gupte, A. and Madawar, D., *Biotechnol. Prog.*, 1997, **13**, 166–169.
58. Smits, J. P., Rinzema, A., Tramper, J., VanSonsbeck, H. M. and Knol, W., *Appl. Microbiol. Biotechnol.*, 1996, **46**, 489–496.
59. Kumaran, S., Sastry, C. A. and Vikineswary, S., *World J. Microbiol. Biotechnol.*, 1997, **13**, 43–49.
60. Szakacs, G. and Tengerdy, R. P., *ACS Symp. Ser.*, 1996, **655**, 175–182.
61. Onilude, A. A., *J. Basic Microbiol.*, 1996, **36**, 421–431.
62. Reczey, K., Szengyel, Z., Eklund, R. and Zacchi, G., *Biores. Technol.*, 1996, **57**, 25–30.
63. Smits, J. P., Rinzema, A., Tramper, J., VanSonsbeck, H. M. and Knol, W., *Process Biochem.*, 1996, **31**, 669–678.
64. Tengerdy, R. P., Szakacs, G. and Sipocz, J., *Appl. Biochem. Biotechnol.*, 1996, **57**, 563–569.
65. Chahal, P. S., Chahal, D. S. and Le, G. B. B., *Appl. Biochem. Biotechnol.*, 1996, **57/58**, 433–442.
66. Awafo, V. A., Chahal, D. S., Simpson, B. K. and Le, G. B. B., *Appl. Biochem. Biotechnol.*, 1996, **57/58**, 461–470.
67. Jha, K., Khare, S. K. and Gandhi, A. P., *Biores. Technol.*, 1995, **54**, 321–322.
68. Kanotra, S. and Mathur, M. S., *J. Environ. Sci. Health*, 1995, **A30**, 1339–1360.
69. Giovannozzisermanni, G., Dannibale, A., Perani, C., Porri, A., Pastin, F., Minelli, V., Vitale, N. and Gelsomino, A., *Tappi J.*, 1994, **77**, 151–156.
70. Castillo, M. R., Gutierrez-Correa, M., Linden, J. C. and Tengerdy, R. P., *Biotechnol. Lett*, 1994, **16**, 967–972.
71. Ray, L. and Majumdar, S. K., *Res. Ind*, 1994, **39**, 105–108.
72. Gutieera-Correa, M. and Tengerdy, R. P., *Biotechnol. Lett.*, 1998, **20**, 45–47.
73. Guadalix, M. E., Almendros, G., Martinez, A. T., Gonzalez-Vila, F. J. and Lankes, U., *Biores. Technol.*, 1997, **60**, 245–249.
74. Archana, A. and Satyanarayana, T., *Enzyme Microb. Technol.*, 1997, **21**, 12–17.
75. Cai, J., Wu, K., Zhang, J., He, X. and Pang, R., *Ind. Microbiol.*, 1997, **27**, 1–4.
76. Brana, C. B. E. and Berry, D. R., *Prog Biotechnol.*, 1992, **7**, 523–528.
77. Bisaria, R., Madan, M. and Vasudevan, P., *Biores. Technol.*, 1997, **59**, 5–8.
78. Haltrich, D., Nidetzky, B., Kulbe, K. D., Steiner, W. and Zupancic, S., *Biores. Technol.*, 1996, **58**, 137–161.
79. Giovannozzi-Sermanni, G., D'-Annibale, A., Crestini, C. and Cappelletto, P. L., *Meded Fac Landbouwwet Rijksuniv Gent*, 1996, **61**, 2129–2132.
80. Dunlop, R. W., Wang, B. and Ball, D., PN-WO 9527779, 1995.
81. Kimura, I., Sasahara, H. and Tajima, S., *J. Ferment. Bioeng.*, 1995, **80**, 334–339.
82. Jain, A., *Process Biochem.*, 1995, **30**, 705–709.
83. Wiacek-Zychlinska, A., Czakaj, J. and Sawicka-Zukowska, R., *Biores. Technol.*, 1994, **49**, 13–16.
84. Kitpreechavanich, V., Srisuk, W. and Lotong, N., *Prog. Biotechnol.*, 1992, **7**, 519–522.
85. Singh, R., Kamra, D. N., Kewalramani, N. and Pancholi, R., *Int J. Anim. Sci.*, 1994, **9**, 105–107.
86. Alam, M., Gomes, I., Mohiuddin, G. and Hoq, M. M., *Enzyme Microb. Technol.*, .1994, **16**, 298–302.
87. Da-Silva, R., Yim, D. K. and Park, Y. K., *J. Ferment. Bioeng.*, 1994, **77**, 109–111.
88. Bhalla, T. C. and Joshi, M., *Indian J. Microbiol.*, 1993, **33**, 253–255.
89. Srivastava, K. C., *Biotechnol. Adv.*, 1993, **11**, 441–465.
90. Tuohy, M. G. and Coughlan, M. P., *Biores. Technol.*, 1991, **39**, 131–137.
91. Kurakake, M., Osada, S. and Komaki, T., *Biosci. Biotechnol. Biochem.*, 1997, **61**, 2010–2014.
92. Ximenes, FdeA, Silveria, FqdeP and Filho, E. X. F., *Curr. Microbiol.*, 1995, **33**, 71–77.
93. Chakrabarti, S. K. and Ranu, R. S., *J. Plant Biochem. Biotechnol.*, 1995, **4**, 117–120.
94. Kitpreechavanich, V., Hayashi, M. and Nagai, S., *Agric. Biol. Chem.*, 1986, **50**, 1703–1711.
95. Kondo, R., *J. Sci. Ind. Res.*, 1994, **55**, 394–399.
96. Homolka, L., Paltiel, J., Volakova, I., Nerud, F. and Hadar, Y., *Folia Microbiol.*, 1997, **42**, 527–529.
97. Lang, E., Nerud, F., Novotna, E. and Martens, R., *Folia Microbiol.*, 1996, **41**, 489–493.
98. Machado, K. M. G., Silva, W. R. F. and Bononi, V. L. R., *Int. Biodeterior. Biodegrad.*, 1996, **37**, 130.
99. Dombrovskaya, E. N. and Kostyshin, S. S., *Biokhimiya*, 1996, **61**, 288–295.
100. Camarero, S., Boeckle, B., Martinez, M. J. and Martinez, A. T., *Appl. Environ. Microbiol.*, 1996, **62**, 1070–1072.
101. Kerem, Z. and Hadar, Y., *J. Cell Biochem.*, 1995, **A21**, 48.
102. Vares, T., Kalsi, M. and Hatakka, A., *Appl. Environ. Microbiol.*, 1995, **61**, 3515–3520.
103. Pal, M., Calvo, A. M., Terror, M. C. and Gonzalez, A. E., *World J. Microbiol. Biotechnol.*, 1995, **11**, 541–545.
104. Martinez, A. T., Camarero, S., Guillen, F., Gutierrez, A., Munoz, C., Varela, E., Martinez, M. J., Barrasa, J. M., Ruel, K. and Pelayo, J. M., *FEMS Microbiol. Rev.*, 1994, **13**, 265–273.
105. Laplante, S. and Chahal, D. S., *Abstr. Gen. Meet. Am. Soc. Microbiol.*, 1993, 327.
106. Maltseva, O. V., Niku-Paavola, M. L., Myasoedova, N. M., Baskunov, B. P. and Golovleva, L. A., *VTT Symp.*, 1991, **122**, 60–69.
107. Golovleva, L. A., Leontievsky, A. A., Maltseva, O. V. and Myasoedova, N. M., *VTT Symp.*, 1991, **122**, 93–109.

108. Kerem, Z., Friesem, D. and Hadar, Y., *Appl. Environ. Microbiol.*, 1992, **58**, 1121–1127.
109. Galliano, H., Gas, G., Seris, J. L. and Boudet, A. M., *Enzyme Microb. Technol.*, 1991, **13**, 478–482.
110. Maltseva, O. V., Niku-Paavola, M. L., Myasoedova, N. M., Baskunov, B. P. and Golovleva, L. A., *Biotechnol. Appl. Biochem.*, 1991, **13**, 291–302.
111. Gupte, A., Huttermann, A., Majcheroczyk, A. and Madamwar, D., in *Advances in Biotechnology* (ed. Pandey, A.), Educational Publishers and Distributors, New Delhi, 1998, pp. 40–49.
112. Nigam, P., Pandey, Ashok and Prabhu, K. A., *Biol. Wastes*, 1987, **21**, 1–10.
113. Germano, S., Lima, V. M. G., Soccol, C. R., Pontarolo, R., Fontana, J. D. and Krieger, N., in *Advances in Biotechnology* (ed. Pandey, A.), Educational Publishers and Distributors, New Delhi, 1998, pp. 59–66.
114. George, S., Raju, V., Subramanian, T. V. and Jayaraman, K., *Bioprocess Eng.*, 1997, **16**, 381–382.
115. Chakraborty, R., Srinivasan, M., Sarkar, S. K. and Raghavan, K. V., *J. Microbiol. Biotechnol.*, 1995, **10**, 17–30.
116. Ramesh, M. V., Charyulu, N. C. L. N., Chand, N. and Lonsane, B. K., *Bioprocess Eng.*, 1996, **15**, 289–294.
117. Ikasari, L. and Mitchell, D. A., *Enzyme Microb. Technol.*, 1996, **19**, 171–175.
118. Ikasari, L. and Mitchell, D. A., *Biotechnol. Lett.*, 1998, **20**, 349–353.
119. George, S., Raju, V., Krishnan, M. R., Subramanian, T. V. and Jayaraman, K., *Process Biochem.*, 1995, **30**, 457–462.
120. Chakraborty, R. and Srinivasan, M., *J. Microbiol. Biotechnol.*, 1993, **8**, 7–16.
121. Ozawa, S., Sato, K. and Endo, I., *Bioprocess Eng.*, 1996, **14**, 63–68.
122. Yaoxing, X., Linlin, Y., Yuying, Q., Guanqian, F. and Dibo, F. *Ind. Microbiol.*, 1993, **23**, 16–20.
123. Ikasari, L. and Mitchell, D. A., *World J. Microbiol. Biotechnol.*, 1994, **10**, 320–324.
124. Villegas, E., Aubague, S., Alcantara, L., Auria, R. and Revah, S. *Biotechnol. Adv.*, 1993, **11**, 387–397.
125. Mnonmani, H. K. and Joseph, R., *Enzyme Microb. Technol.*, 1993, **15**, 624–628.
126. Datta, A., *J. Biol. Chem.*, 1992, **267**, 728–736.
127. Battaglino, R. A., Huergo, M., Pilosof, A. M. R. and Bartholomai, G. B., *Appl. Microbiol. Biotechnol.*, 1991, **35**, 292–296.
128. Malathi, S. and Chakraborty, R., *Appl. Environ. Microbiol.*, 1991, **57**, 712–716.
129. Yang, S. S. and Chiu, W. F., Int. Congr. Microbiol., 14 Meet, 1987, p. 283.
130. Purushotham, H., Rao, P. V., Rai, C. L., Immunuel, M. M. and Raghavan, K. V., in *Solid State Fermentation* (ed. Pandey, A.), Wiley Eastern Publishers, New Delhi, 1994, pp. 114–121.
131. Mitra, P. and Chandra, A. L., in *Solid State Fermentation* (ed. Pandey, A.), Wiley Eastern Publishers, New Delhi, 1994, pp. 134–136.
132. Mukhopadhyay, R. P. and Chandra, A. L., in *Solid State Fermentation* (ed. Pandey, A.), Wiley Eastern Publishers, New Delhi, 1994, pp. 142–144.
133. Benjamin, S. and Pandey, Ashok, *J. Sci. Ind. Res.*, 1998, **57**, 1–9.
134. Benjamin, S. and Pandey, Ashok, *Yeast*, 1999, **14**, 1069–1087.
135. Beuchat, L. R., *Ind. Eng. Chem. Prod. Res. Dev.*, 1982, **21**, 533–536.
136. Rivera-Munoz, G., Tinoco-Valencia, J. R., Sanchez, S. and Farres, A., *Biotechnol. Lett.*, 1991, **13**, 277–280.
137. Benjamin, S. and Pandey, Ashok, *Indian J. Microbiol.*, 1996, **36**, 201–204.
138. Benjamin, S. and Pandey, Ashok, *Biores. Technol.*, 1996, **55**, 167–170.
139. Benjamin, S. and Pandey, Ashok, *Process Biochem.*, 1997, **32**, 437–440.
140. Benjamin, S., Ph D thesis, Regional Research Laboratory, Trivandrum, India, 1997.
141. Ohnishi, K., Yoshida, Y. and Sekiguchi, J., *J. Ferment. Bioeng.*, 1994, **77**, 490–495.
142. Christen, P., Angeles, N., Corzo, G., Farres, A. and Revah, S., *Biotechnol. Tech.*, 1995, **9**, 597–600.
143. Bhusan, B., Dosanjih, N. S., Kumar, K. and Hoondal, G. S., *Biotechnol. Lett.*, 1994, **16**, 841–842.
144. Ortiz-Vazquez, E., Granados-Baeza, M. and Rivera-Munoz, G., *Biotechnol. Adv.*, 1993, **11**, 409–416.
145. Granados-Baeza, M. J. and Rivera-Munoz, G., *Abstr. Gen. Meet. Am. Soc. Microbiol.*, 1997, 428.
146. Rao, P. V., Jayaraman, K. and Lakshmanan, C. M., *Process Biochem.*, 1993, **28**, 391–395.
147. Rao, P. V., Jayaraman, K. and Lakshmanan, C. M., *Process Biochem.*, 1993, **28**, 385–389.
148. Solis-Pereira, S., Favela-Torres, E., Viniengra-Gonzalez, G. and Gutierrez-Rojas, M., *Appl. Microbiol. Biotechnol.*, 1993, **39**, 36–41.
149. Minjares-Carranco, A., Trejo-Aguilar, B. A., Agulilar, G. and Viniegra-Gonzalez, G., *Enzyme Microbiol. Technol.*, 1997, **21**, 23–31.
150. Cavalitto, S. F., Arcas, J. A. and Hours, R. A., *Biotechnol. Lett.*, 1996, **18**, 251–256.
151. Hours, R. A., Katsuragi, T. and Sakai, T., *J. Ferment. Bioeng.*, 1994, **78**, 426–430.
152. Antier, P., Minijares, A., Roussos, S. and Viningra-Gonzalez, G., *Biotechnol. Tech.*, 1993, **11**, 429–440.
153. Antier, P., Minijares, A., Roussos, S., Raimbault, M. and Viningra-Gonzalez, G., *Enzyme Microb. Technol.*, 1993, **15**, 254–260.
154. Garzon, C. G. and Hours, R. A., *Biores. Technol.*, 1991, **39**, 93–95.
155. Hours, R. A., Voget, C. E. and Ertola, R. J., *Biol. Wastes*, 1988, **24**, 147–157.
156. Hours, R. A., Voget, C. E. and Ertola, R. J., *Biol. Wastes*, 1988, **23**, 221–228.
157. Huerta, S., Favela, E., Lopez-Ulibarri, R., Fonseca, A., Viniegra-Gonzalez, G. and Gutierrez-Rojas, M., *Biotechnol. Tech.*, 1994, **8**, 837–842.
158. Acuna-Arguelles, M., Gutierrez-Rojas, M., Viniengra-Gonzalez, G. and Favela-Torres, E., *Biotechnol. Lett.*, 1994, **16**, 23–28.
159. Cruz, R. and Park, Y. K., *J. Food Sci.*, 1982, **47**, 1973–1975.
160. Annunziato, M. E., Mahoney, R. R. and Mudgett, R. E., *J. Food Sci.*, 1986, **51**, 1370–1371.
161. Somiari, R. I. and Balogh, E., *Enzyme Microbiol. Technol.*, 1995, **17**, 311–316.
162. Srinivas, M. R. S, Chand, N. and Lonsane, B. K., *Bioprocess Eng.*, 1994, **10**, 13–144.
163. Wakamoto Pharm., PN: JP J02203782, 1990.
164. Wakamoto Pharm., PN: JP J02203784, 1990.
165. Gonzalez, R. R. and Monsan, P., *Enzyme Microb. Technol.*, 1991, **13**, 349–352.
166. Shaikh, S. A., Khire, J. M. and Khan, M. I., *J. Ind. Microbiol. Biotechnol.*, 1997, **19**, 239–245.
167. Univ La Coruna, PN: ES ES 2102975, 1997.
168. Becerra, M. and Siso, M. I. G., *Enzyme Microb. Technol.*, 1996, **19**, 39–44.
169. Siso, M. I. G., Becerra, M. and Cerdan, E., *Yeast*, 1995, **11**, S529.
170. Prabhu, N. G. and Chandrasekaran, M., *World J. Microbiol. Biotechnol.*, 1995, **11**, 683–684.
171. Prabhu, N. G. and Chandrasekaran, M., *J. Mar. Biotechnol.*, 1996, **4**, 176–179.
172. Prabhu, N. G. and Chandrasekaran, M., *Process Biochem.*, 1997, **32**, 285–289.
173. Prabhu, N. G. and Chandrasekaran, M., in *Advances in Biotechnology* (ed. Pandey, A.), Educational Publishers and Distributors, New Delhi, 1998, pp. 51–58.

174. Selvakumar, P., Ashakumary, L. and Pandey, Ashok, *J. Sci. Ind. Res.*, 1996, **55**, 443–449.
175. Ray, R. R., Jana, S. C. and Nanda, G., *Indian J. Expt. Biol.*, 1997, **35**, 285–288.
176. DcAlmeida, S. E. M., Mizuta, K. and Giglio, R., *Mycol. Res.*, 1997, **101**, 188–190.
177. Krishna, C. and Chandrasekaran, M., *J. Cell Biochem.*, 1995, **A21**, 48.
178. Torrado, A., Gonzalez, P., Pintado, J., Montemayor, I., Miron, J. and Murado, M., *Meded Fac Landbouwwet Rijksuniv Gent*, 1994, **59**, 2471–2473.
179. Sudo, S., Ishikawa, T., Sato, T. and Oba, T., *J. Ferment. Bioeng.*, 1994, **77**, 483–489.
180. Shankaranand, V. S., Ramesh, M. V. and Lonsane, B. K., *Process Biochem.*, 1992, **27**, 33–36.
181. Shah, N. K., Ramamurthy, V., Kothari, R. M., *Biotechnol. Lett.*, 1991, **13**, 361–364.
182. Krishna, C. and Chandrasekaran, M., *Appl. Microbiol. Biotechnol.*, 1996, **46**, 106–111.
183. Lonsane, B. K. and Ramesh, M. V., *Adv. Appl. Biochem.*, 1990, **35**, 1–56.
184. Soni, S. K., Bath, K. S. and Soni, R., *Indian J. Microbiol.*, 1996, **36**, 157–159.
185. Babu, K. R. and Satyanarayana, T., *Process Biochem.*, 1995, **30**, 305–309.
186. Ramesh, M. V. and Lonsane, B. K., *Biotechnol. Lett.*, 1987, **9**, 501–504.
187. Ramesh, M. V. and Lonsane, B. K., *Biotechnol. Lett.*, 1987, **9**, 323–328.
188. Murado, M. A., Gonzalez, M. P., Torrado, A. and Pastrana, L. M., *Process Biochem.*, 1997, **32**, 35–42.
189. Ramesh, M. V. and Lonsane, B. K., *Appl. Microbiol. Biotechnol.*, 1991, **35**, 591–593.
190. Padmanabhan, S., Ramakrishna, M., Lonsane, B. K. and Krishnaiah, M. M., *Lett. Appl. Microbiol.*, 1992, **15**, 235–238.
191. Thenawidjaja, M., Hartanto, T. M., Damardjati, J. S. and Suliantari, *Aust. J. Biotechnol.*, 1990, **4**, 26–33.
192. Ramesh, M. V. and Lonsane, B. K., *Appl. Microbiol. Biotechnol.*, 1990, **33**, 501–505.
193. Ramesh, M. V. and Lonsane, B. K., *Process Biochem.*, 1989, **24**, 176–178.
194. Ramesh, M. V. and Lonsane, B. K., *Biotechnol. Lett.*, 1989, **11**, 49–52.
195. Pandey, Ashok, *Biol. Wastes*, 1990, **34**, 11–19.
196. Pandey, Ashok, Ashakumary, L. and Selvakumar, P., *World J. Microbiol. Biotechnol.*, 1994, **10**, 348–349.
197. Pandey, Ashok, in *Industrial Biotechnology* (eds Malik, V. S. and Padma Sridhar), IBH and Oxford Publishing Co, New Delhi, 1992, pp. 525–537.
198. Pandey, Ashok, *Starch/Starke*, 1992, **39**, 75–77.
199. Pandey, Ashok and Radhakrishnan, S., *Enzyme Microb. Technol.*, 1992, **14**, 486–488.
200. Pandey, Ashok, Selvakumar, P. and Ashakumary, L., *Process Biochem.*, 1996, **31**, 43–46.
201. Ashakumary, L., Selvakumar, P. and Pandey, Ashok, in *Solid State Fermentation* (ed. Pandey, A.), Wiley Eastern Publishers, New Delhi, 1994, pp. 33–37.
202. Selvakumar, P., Ashakumary, L., Helen, A. and Pandey, Ashok, *Lett. Appl. Microbiol.*, 1996, **23**, 403–406.
203. Pandey, Ashok, Ashakumary, L., Selvakumar, P. and Vijayalakshmi, K. S., *Indian J. Microbiol.*, 1995, **35**, 335–338.
204. Pandey, Ashok and Radhakrishnan, S., *Proc. Kerala Acad. Sci.*, 1990, **1**, 69–79.
205. Pandey, Ashok, *Starch/Starke*, 1995, **47**, 439–445.
206. Mukherjee, P. S., Pandey, Ashok, Selvakumar, P., Ashakumary, L. and Gurusamy, P., *J. Sci. Ind. Res.*, 1998, **57**, 583–586.
207. Fujio, Y. and Morita, H., *J. Ferment. Bioeng.*, 1996, **82**, 554–557.
208. Hata, Y., Ishida, H., Kojima, Y., Ichikawa, E. K. A., Suginami, K. and Imayasu, S., *J. Ferment. Bioeng.*, 1997, **84**, 532–537.
209. Ramadas, M., Holst, O. and Mattiasson, B., *World J. Microbiol. Biotechnol.*, 1996, **12**, 267–271.
210. Tani, Y., Vongsuvanlert, V. and Kumnuanta, J., *J. Ferment. Technol.*, 1986, **64**, 210.
211. Wnag, H. L., Swain, E. W. and Hesseltine, C. W., *J. Food Sci.*, 1984, **49**, 1210–1211.
212. Rajagopalan, S., Rockstraw, D. A. and Munson-McGee, S. H., *Biores. Technol.*, 1997, **61**, 175–183.
213. Mitchell, D. A., Do, D. D., Greenfield, P. F. and Doelle, H. W., *Biotechnol. Bioeng*, **38**, 1991, 353–362.
214. Elegado, F. B. and Fujio, Y., *J. Gen. Appl. Microbiol.*, 1993, **39**, 541–546.
215. Soccol, C. R., Marin, B., Raimbault, M. and Lebeault, J. M., *Appl. Microbiol. Biotechnol.*, 1994, **41**, 330–336.
216. Ghildyal, N. P., Ramakrishna, M., Lonsane, B. K., Karanth, N. G. and Krishnaiah, M. M., *Chem. Eng. J.*, 1993, **51**, B17–B23.
217. Jaleel, S. A., Srikanta, S. and Karanth, N. G., *J. Microbiol. Biotechnol.*, 1992, **7**, 1–8.
218. Ramadas, M., Holst, O. and Mattiasson, B., *Biotechnol. Tech.*, 1995, **9**, 901–906.
219. Snow Brand Milk Products, PN: JP J02053478, 1990.
220. Kobayashi, T., Nagamune, T. and Endo, I., *Ann. NY Acad. Sci.*, 1990, **613**, 846–855.
221. Bhumibhamon, O., *MIRCEN J. Appl. Microbiol. Biotechnol.*, 1986, **2**, 473–482.
222. Witkowska, G. A., Ilnicka, O. O. and Czajkowska, D., *Acta Biotechnol.*, 1988, **8**, 495–502.
223. Czajkowska, D. and Ilnicka, O. O., *Acta Biotechnol.*, 1988, **8**, 407–413.
224. Mitchell, D. A., Greenfield, P. F. and Doelle, H. W., *World J. Microbiol. Biotechnol.*, 1990, **6**, 201–208.
225. Joseph, Simon, M. Sc. thesis, Regional Research Laboratory, Trivandrum, India, 1996.
226. Kavita, P., M. Sc. thesis, Regional Research Laboratory, Trivandrum, India, 1998.
227. Pandey, Ashok, Internal Report to the Department of Biotechnology, New Delhi, 1998.
228. Alasheh, S. and Duvnjak, Z., *Biotechnol. Prog.*, 1994, **10**, 353–359.
229. Ebune, A., Alasheh, S. and Duvnjak, Z., *Biores. Technol.*, 1995, **54**, 241–247.
230. Alasheh, S. and Duvnjak, Z., *Acta Biotechnol.*, 1994, **14**, 223–233.
231. Chatterjee, R., Dutta, A., Banerjee, R. and Bhattacharyya, B. C., *Bioprocess Eng.*, 1996, **14**, 159–162.
232. Roche, N., Berna, P., Desgranges, C. and Durand, A., *Enzyme Microb. Technol.*, 1995, **17**, 935–941.
233. Lin, S. F., Hu, H. M., Inukai, T. and Tsai, Y. C., *Biotechnol. Adv.*, 1993, **11**, 417–427.
234. Castanares, A., Mccrae, S. I. and Wood, T. M., *Enzyme Microb. Technol.*, 1992, **14**, 875–884.