Solid state fermentation at ORSTOM: Evolution and perspectives

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SUMMARY

An attempt is made to formulate the general global history of solid state fermentation right from ancient times to the present day, with emphasis on the resurgence of interest in the technique throughout the world. ORSTOM, Institut Français de Recherche Scientifique pour le Développement en Coopération, France, understood the utility value of solid state fermentation as early as 1975 and has put intensive efforts from different angles. The history and evolution of solid state fermentation research at ORSTOM are described, along with the perspectives. The ORSTOM activities on cassava protein enrichment, lignocellulosics waste upgradation, enzymes production, spores for inoculation, physiological aspects, support cultures, bioremediation, and biopesticides are reviewed critically, along with the perspective for next few years.

Keywords: Solid state fermentation, global history, history in ORSTOM, evolution, cassava, sugar cane, protein enrichment, filamentous fungi, mushrooms, physiology, enzymes, spores, support cultures, bioremediation, biopesticides, perspectives.

RESUME

Fermentation en milieu solide à l'ORSTOM: Evolution et perspectives.

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L'historique du programme de recherche mené à l'ORSTOM depuis vingt ans sur les fermentations en milieu solide a été brossé dans le but de faire une synthèse de nos travaux de recherche, au moment même où apparait un regain d'interêt international sur cette technique originale de culture de microorganismes. L'ORSTOM, Institut Français de Recherche Scientifique pour le Développement en Coopération, avait compris dès 1975 l'intérêt scientifique et les applications potentielles des fermentations en milieu solide (FMS). L'historique et l'évolution des feremententations en milieu solide ont été développées tout au long de cette présentation. Les activités de l'ORSTOM dans le domaine de l'enrichissement en protéines du manioc, du tourteau de coprah, de la valorisation des substrats lignocellulosiques, la production d'enzymes, des spores pour l'inoculation, les aspects physiologiques, les cultures sur support, la bioremédiation, les biopesticides, les métabolites secondaires, ont été analysées d'une manière critique afin de présenter les perpectives de cette technique de culture dans le domaine de la recherche pour les années à venir.

Mots clés: Fermentation en milieu solide, historique global, historique à l'ORSTOM, évolution, manioc, bagasse de canne à sucre, enichissement protéique, champignons filamenteux, champignons comestibles, physiologie, enzymes, spores, culture sur support, bioremédiation, biopesticides, perspectives.

INTRODUCTION

It is really interesting to derive the general global history of solid state fermentation, right from ancient times to the present period. One has to let the imagination loose and even assume that a number of things or events might have occurred, with a view to come to a stage from where the history is documented. The present exercise for global history is no exception and some wild guesses might not have taken place at all.

Obviously, the first such natural activity of micro-organisms, observed by the man in ancient time, was the process of solid state fermentation. Hence, it is not surprising that the initial biotechnological processes exploited by man from ancient times are those based on solid state fermentation. A plenty of examples can be and have been cited on this aspect by many researchers interested in solid state fermentation.

Similar phenomena in liquid state fermentation was also evident. Probably, it was more visible, amenable to mixing and resulted in more homogenous product. Consequently, some groups of our ancestors preferred this technique over the solid state fermentation processes. Such tribes increased day-by-day and then started neglecting solid state fermentation processes.

Luckily, both techniques were intensively studied up to 1940, but then the leaders of science from many countries took decision to use only liquid state fermentation and to completely ignore solid state fermentation. The decision was more or less arbitrary, as it was neither taken on scientific grounds nor were the two techniques compared effectively. The reasons considered were probably the amenability of liquid state culture for manipulation, monitoring and control. The validity of this decision was recently debated by Ramesh and Lonsane (1991), as they observed that the production of alpha-amylase by *Bacillus licheniformis* in liquid state culture is lower, unless the medium is agitated, as against no need of agitation when solid state fermentation is employed. Similarly, Viniegra-Gonzalez (1996) has commented that the disadvantage, which were attributed to solid state fermentation, have, in fact, been recognised as its advantages in recent years. It has been even suggested that this neglect of SSF in Western and European countries is responsible for the slow growth of fermentation industries, as compared to those in Japan (Lonsane *et al* 1985).

Fortunately, a few groups, scattered in different parts of the world, have not subscribed to the view that liquid state fermentation is superior to solid state fermentation and continued their R&D efforts in the latter technique, even after 1940. They were in a very small minority, but slowly and steadily they proved that solid state fermentation cannot be ignored. It took 40-45 years for them to prove this point and to convince the others. These efforts have ultimately borne fruit and a resurgence of interest in solid state fermentation was generated throughout the world. Ultimately, even those who considered solid state fermentation as a crude or inferior technique, started researching it with an intense vigour. Consequently, the star was reborn and this is of industrial and economic advantage.

The biotechnology group in ORSTOM, France, is one such group which realised the value of solid state fermentation as early as 1975 and invested intensive efforts

on the technique. The present paper describes the history, evolution and perspectives of solid state fermentation in ORSTOM, France.

HISTORICAL HIGHLIGHTS OF SSF IN ORSTOM

The work on SSF in ORSTOM was initiated in 1975 at its Senegal centre by Raimbault (1980), in collaboration with Jacques SENEZ, reverently referred to as the father of industrial fermentation in France (Senez, 1979; Senez *et al.* 1979). The work involved upgradation of cassava for its protein content by SSF, using a column fermentor at laboratory scale and *Aspergillus niger* 10 as the filamentous fungus (Raimbault and Germon, 1976). During 1978-1982, the work mainly focused on development of agitated pilot and industrial scale reactors and initiation of the work on cellulases production (Deschamps *et al.* 1979; Senez *et al.* 1980; Deschamps *et al* 1985. The need for inoculum in large quantity for pilot scale work led to the development of an efficient sporulator (Raimbault and Roussos, 1985), and to the design and fabrication of 50 kg capacity fermentor, Zymotis (Prebois *et al.* 1985). The importance of physiology of the culture during fermentation was also realised and culminated in the development of efficient methods and analysis systems for respirometric studies on column fermentors (Raimbault and Alazard, 1980; Aufeuvre and Raimbault, 1982).

The period of 1982-1985 witnessed expansion of the activities on tropical waste upgradation, cellulases production on an inert support (sugar cane pith bagasse), development of efficient methods for recovery of the product from fermented solids and formulation of a simpler strategy for disposal of spent solids by ensiling (Roussos, 1985). The conversion of waste banana for its upgradation and use as animal feed was also realised (Baldensperger *et al.* 1985). Teaching of SSF at Universidad Autonoma Metropolitana (UAM-Iztapalapa), Mexico was also initiated by ORSTOM during this period and this activity was extended to many universities (Viniegra-Gonzalez *et al.* 1991).

During 1981, collaboration with UAM-I, Mexico, was established for upgradation of different tropical agro-industrial wastes (cassava, bagasse and coffee pulp). It continues to the present day and has yielded many useful results (Table 1). Engineering studies, along with designs of static/agitated reactors, were initiated and studies were made on heat and mass transfer, energy conservation, water activity, isolation of about 500 tropical fungi and evaluation of these fungi for production of different enzyme as well as microbial metabolites (Oriol *et al.* 1988; Aquiahuatl *et al.* 1988, Saucedo-Castañeda *et al.* 1990). Different fermentation systems were

successfully computerised for efficient monitoring as well as control of different parameters, in addition to the development of appropriate sensors (Gutierrez-Rojas *et al.* 1988).

From 1990, the attention under the above collaborative project was also focused on genetic improvements of the cultures and molecular approaches were extended to explain different behaviour of the micro-organism in liquid and solid state cultures (Antier *et al.* 1993a and b; Augur and Viniegra-Gonzalez, 1995). Efficient mathematical models were developed and physiological studies were extended to the processes involving cell growth and metabolism (Viniegra-Gonzalez, 1988; Saucedo-Castañeda *et al.* 1992; Gutierrez-Rojas *et al.* 1995). Recently, bioremediation of solid waste and air purification were conducted through SSF (Perraud-Gaime, 1995; Morales *et al.* 1994; Revah *et al.* 1995).

Collaboration was subsequently developed with a number of institutions in France, other European countries and also with a wide spectrum institutions from around the globe (Deschamps *et al.* 1982; Lonsane *et al.* 1991; Marakis *et al.* 1995; Roussos *et al.* 1994; Viniegra-Gonzalez *et al.* 1991).

At ORSTOM, Montpellier, intensive efforts have been devoted, right from 1987, on the physiology, metabolism and respirometry of fungi in SSF processes (Dufour, 1990; Saucedo-Castañeda, 1991; Soccol, 1992; Trejo-Hernandez, 1992; Perraud-Gaime, 1995; Denis, 1996). New areas, such as biopesticides, ectomycorrhiza and mushrooms mycelium physiology as well as secondary metabolites (alkaloids and aroma) were also initiated for intensive R&D efforts (Montero *et al.* 1989; Roussos *et al.* 1989; Roussos *et al.* 1996; Trejo-Hernandez *et al.* 1992 and 1993). As it stands now, ORSTOM has achieved an international recognition for its efforts on SSF and it has a number of planned prospects for intensive inputs on this fermentation technique of industrial importance (Raimbault, 1988).

SSF BASED ON NATURAL ORGANIC SUBSTRATES

As mentioned earlier, natural organic substrates were the first on which work was initiated in 1975 in Dakar, Senegal (Raimbault *et al.* 1979). The efforts were initially taken up with a view to obtain upgraded agro-industrial products for use as human food (Senez, 1979). However, the later work was for developing upgraded agro-industrial products/residues/wastes for animal feeding (Senez *et al.* 1980). The details of the efforts on the natural organic substrates are presented in Table 1, with respect to the substrates, micro-organisms used in fermentation and the end products. A glance at Table 1 indicates that the efforts were studies on diverse natural organic

substrates as well as fungal/yeast species for production of a large number of primary and secondary metabolites.

FERMENTATION BASED ON INERT SUPPORT

The efforts on natural organic substrates indicated a severe problem of variation in the composition of substrates and consequent difficulties in obtaining end-product of uniform quality. Moreover, many of these natural substrates are available in a limited period in the year and may not be not be available in many countries. Their import from other countries is impracticable from the economic point of view (Raimbault *et al.* 1989). The efforts on inert supports are illustrated in Table 2.

Table 1. Solid State Fermentation based on natural organic substrates.

Substrates	End products	Microorganisms studied	References
Cassava	Biomass,	*A. niger 10*	Raimbault and Alazard (1980) Raimbault *et al.* (1985)
	Amylases,	*A. niger 10*	Alazard and Baldensperger, 1982
		R. arrhizus	Soccol (1992)
	Biopesticides,	*B. bassiana*	Roussos *et al*, (1989)
	Conidiospores	*T. harzianum*	Roussos *et al*, (1991)
	Lactic acid	*R. oryzae*	Soccol *et al*, (1994)
	Ethanol	*Sw. castellii*	Saucedo-Castañeda *et al*, (1993)
	Alkaloids	*C. purpurea*	Trejo *et al*, (1993)
Sugar cane bagasse	Biomass	*A. terreus*	Gonzalez-Blanco *et al* (1990)
	Cellulases, Animal feed, Biopesticides, Conidiospore	*T. harzianum* *B.bassiana*	Montero et al. (1989); Roussos *et al*, (1991a, 1992, 1993); Tapia *et al.* (1988)
Carob waste	Tannase	*A. carbonarius*	Lambraki *et al*, (1994)
Wheat straw	Cellulases	*T. harzianum*	Deschamps *et al*, (1985)
Sugar beet cosset	Biomass, cellulases	*T. harzianum*	Roussos *et al*, (1982)
Wheat straw+ wheat bran	Cellulases, Biomass,	*T. harzianum*	Roussos *et al*, (1991b)
Coprah cake	Biomass, Probiotics	*A. niger*	Roussos *et al*, (1994); Ramirez-Islas *et al*, (1996)
Coffee pulp	Pectinase,	*Aspergillus*	Antier *et al.* (1993); Boccas *et*
	Decaffinated	*sp.Penicillium*	*al*, (1994) Roussos *et al*, (1994)
	Substrate, Silage	*sp.*	Perraud and Roussos (1996)
Banana	Biomass	*A. niger 10*	Baldensperger *et al*, (1982)
Potato waste	Biomass	*A. niger 10*	Raimbault *et al*, (1979)

Table 2. Solid state fermentation based on inert support.

Inert supports	End products	Microorganisms studied	References
Sugar cane pith bagasse	Amylases	*A. niger 10*	Oriol *et al*, (1988)
	Pectinases	*A. niger CH4*	Trejo *et al*, (1991)
	Ethanol	*S. castellii*	Saucedo *et al*, (1992)
	Lactic acid	*R. oryzae*	Soccol *et al*, (1994)
	Alkaloïdes	*C. purpurea*	Trejo *et al*, (1993)
	Aroma	*T. harzianum*	Sarhy-Bagnon *et al*, (1996)
		C. fimbriata	Christen and Raimbault, (1991)
		M. esculenta	Kabbaj *et al*, (1996)
	Biomass	*S. castellii*	Saucedo *et al*, (1992)
	Conidia	*T. harzianum*	Roussos *et al*, (1991)
		A. niger	Roussos *et al*, (1991)
	Citric acid	*A. niger*	Gutierrez-Rojas *et al*, (1995)
	Penicillin	*P. chrysogenum*	Barrios-Gonzalez *et al*, (1988)
	Gibberellins	*G. funjikuroi*	Barrios-Gonzalez *et al*, (1989)
	Lipases	*R. oligosporus*	Christen *et al*, (1993, 1994)
	Ectomycorrhizes	*Suillus collinitus*	Roussos *et al*, (1995)
		Lactarius deliciosus	Roussos *et al*, (1995)
	Aflatoxin	*A. flavus*	Barrios-Gonzalez *et al*, (1988)
	Probiotics	*A. niger*	Tapia *et al*, (1988 and1989)
	Mushroom	*P. cornucopiae*	Roussos *et al*, (1996)
	Biomass	*A. niger 10*	Oriol *et al*, (1990)
Polyurethan	Penicillin	*P. chrysogenum*	Barrios-Gonzalez *et al*, (1988)
Vermiculite	Biomass	*Suillus collinitus*	Roussos *et al*, (1995)
		Pisolithus tinctorius	Roussos *et al*, (1995)
Amberlite	Ethanol	*C. utilis*	Christen *et al*. (1994)
	Biomass	*A. niger*	Auria *et al*, (1990, 1992, 1993)

Sugar cane pith bagasse has been extensively used in ORSTOM, because it offers many advantages (Dufour, 1990, Raimbault *et al*. 1989; Roussos, 1985; Saucedo-Castañeda, 1991 and Trejo-Hernandez, 1992). It does not find any worth-while use and is available in plenty in sugar cane processing countries. A method was also developed for making it inert and also store over a long duration (Oriol *et al*, 1987; Raimbault *et al*, 1989; Saucedo-Castañeda *et al*, 1992).

A number of chemical or mineral compounds can serve as inert support in solid state fermentation (Auria *et al.* 1992; 1993; 1994; 1995; Barrios-Gonzalez *et al*, 1988; Christen *et al.* 1995). These compounds, such as polyurethane, alginates, amberlite and vermiculite, were also investigated (Table 2).

DESIGN AND DEVELOPMENT OF BIOREACTORS

Researchers have always faced difficulties in obtaining bioreactors from commercial sources, as laboratory scale bioreactors for solid state fermentation are not produced industrially. Consequently, a number of different types of bioreactors have been used by various workers (Lonsane *et al,* 1985, Gutierrez-Rojas *et al,* 1988) and the choice is determined mainly by the infrastructure available in the laboratory. The efficiency of the fermentation also depends on the type of the bioreactor, parameters control and reliability of parameter control strategies. Consequently, comparison of literature data is not possible.

With the above in view, ORSTOM has developed a number of bioreactors for laboratory, pilot plant and industrial scale operation (Raimbault and Alazard, 1980; Prebois et al. 1985; Raimbault and Roussos, 1985 and Lepilleur *et al,* 1997). These are described in Tables 3a, b and c, along with details of their specific features and uses in different projects in ORSTOM. Different reactor designs have also been developed for static and agitated fermentation as well as for pilot scale inoculum production (Gutierrez-Rojas *et al.* 1988; Roussos *et al.* 1993, Auria *et al.* 1992).

Table 3a: Design and development of laboratory solid state fermentation bioreactors for study fungal physiology, respirometry, sporulation and large scale cultivation.

Bioreactor	Agitation status	Working capacity, kg DM	Distinct features	Uses	References
Column fermenter (20 columns	static	0.01-0.1 /column	Control of temperature, moisture, aeration ; on-line respirometry; sterility maintenance ; suitable or all kinds of microorganisms	Screening of strains, optimization, kinetics, metabolic patterns, respirometry	Raimbault and Alazard (1980) ; Saucedo *et al*, (1993)
Column fermenter (16 columns)	static	0.01-0.1	Available commercially from Gauthier, Montpellier		
Modulated, multiple-column verticle fermenter with double jacket	static	0.1-2	Control of temperature, moisture, aeration, on-line respirometry;Double jacket ;Increase or devrease in bed height ; Sterility maintenance comparative of different bed height ; recycle of air; Suitable for all kinds of microorganims	Scale-up fo process; Collection of engineering data	Auria et al (1992) Saucedo *et al*, (1992, 1993)

Table 3b: Design and development of disk fermentor for study fungal physiology, respirometry, sporulation and large scale inoculum production.

Bioreactor	Agitation status	Working capacity, kg DM	Distinct features	Uses	References
Disk fermenter	Agitated or static	0.2-2.0	Control of temperature, aeration and moisture; on-line respirometry; sterility maintenance; adjustible distance between two plates; Uniform conidiation; Overcoming of fungal competition during growth and sporulation; suitable for filamentous fungi	Pilot scale spore inoculum production in absolutely sterile conditions; Optimization of sporulation; Physiological studies of fungal sporulation; In situ inoculation and recovery of spore inoculum in liquid in concentrated form and without agar piece	Raimbault and Roussos (1985) Montero *et al* (1989) Roussos *et al* (1991)

Table 3c: Design and development of pilot solid state fermentation bioreactors for study fungal physiology, respirometry, sporulation and large scale cultivation.

Bioreactor	Agitation status	Working capacity, kg DM	Distinct features	Uses	References
Zymotis	Static	10-100	Control of temperature, one-line respirometry, moisture, aeration, and weight, recycle air, In pith driying of medium; modulated compartments; Sterility maintenance; Suitable for all kinds of microorganism	Scale-up of process; Pilot plant production; Industrial conidia production; Data for design of industrial bioreactor;	Prebois *et al*, (1985); Roussos *et al*, (1994); Gonzalez-Blanco *et al*, (1990); Roussos *et al*, (1991)
IRCHA-ORSTOM Pilot scale reactor	Agitated	5-20	Control of temperature, agitation, pH, aeration, nitrogen and weight; Efficient heat and mass transfers; In situ drying of medium, inoculation, homogénization, and substrate treatment	Agitation effect on mycelial growth; Pilot scale studies; Engineering data collection; protein enrichment of starchy materials	Senez *et al*, (1980) Deschamps *et al* (1979, 1985) Raimbault (1980)
IRCHA-ORSTOM industrial reactor	Agitated	100-500	" "	Feed production	Deschamps *et al* (1982)

COMPUTERISATION OF BIOREACTORS

Is it well known that the heat and mass transfer are poor in many types of bioreactors for solid state fermentation (Lonsane *et al*, 1992; Saucedo-Castañeda *et al*, 1993, Roussos *et al*, 1993). Consequently, it is difficult to control temperature and maintain desired moisture levels in most bioreactors (Gonzalez-Blanco *et al.* 1990). Similarly, carbon dioxide level in the fermenting mass is of critical importance in many processes (Lonsane *et al*, 1985, ; Saucedo-Castañeda *et al*, 1992). In order to have accurate control of these parameters, bioreactors have been coupled to computer for efficient control. The details are presented in Table 4.

Table 4. : Computerisation of reactors for solid state fermentation.

Reactors	Computerised regulation for	References
Lab. scale column fermentor	Temperature, oxygen carbon dioxide, pressure drop, humidity, aeration rate	Saucedo-Castañeda *et al*, (1990, 1992, 1993, 1994); Auria *et al*, (1993, 1994, 1995)
Double jacketed multi-column vertical fermentor	Temperature, carbon dioxide, aeration rate	Saucedo-Castañeda *et al*, (1992) Auria *et al*, (1990, 1992)
IRCHA-ORSTOM Pilot agitated reactor	Temperature, pH, weight	Deschamps *et al*, (1979, 1981, 1985)
Zymotis, static pilot reactor	Temperature, aeration rate	Gutierrez-Rojas *et al*, (1988) Gonzalez-Blanco *et al.* (1990)

PHYSIOLOGICAL STUDIES

Physiology of filamentous fungi during growth in solid state fermentation has been a speciality of ORSTOM group. A number of improvements in process productivity were possible through the physiological studies.

Table 5. Physiological studies on filamentous fungi.

Physiological attribute	Microorganisms studied	End products	References
Spore germination	*T. harzianum*	Cellulases	Roussos and Raimbault (1982)
	A. niger	Biomass	Barrios-Gonzalez *et al*, (1989)
	Penicillium sp. *Aspergillus* sp.	Detoxified coffee pulp	Perraud and Roussos (1996), Denis (1996)
	A. carbonarius	Tannase	Gaitis and Marakis (1994)
	C. purpurea	Alkaloid	Trejo *et al*, (1993)
	R. oryzae	lactic acid	Soccol *et al*, (1994)
	A. niger	Amylases	Roussos *et al*, (1989)
Spore viability during preservation	*T. harzianum*	Cellulases	Roussos *et al*, (1989)
	B. bassiana	Biopesticide	Montero *et al*, (1989)

The contributions of ORSTOM in this specific area of vital importance are presented in Table 5. It is emphasised that physiological aspects of filamentous fungi in solid state fermentation have been largely neglected by most researchers studies (Oriol, 1987; Perraud-Gaime, 1995; Roussos, 1985; Saucedo-Castañeda, 1991; Soccol, 1992, Trejo-Hernandez, 1992).

MATHEMATICAL MODELS

A number of mathematical models have been developed for every aspect of submerged fermentation and these proved highly useful in understanding the intrinsic details of the process, optimisation of parameters, scale-up of the process and designing industrial technology. In contrast, mathematical models for solid state fermentation were rare before 1990 and these started appearing in the literature subsequently. Formulating efficient mathematical model for solid state fermentation is difficult because of the non-homogeneity of the system (Gutierrez-Rojas, 1995).

ORSTOM and its team of collaborators from Mexico and Cuba have put intensive efforts into developing mathematical models for solid state fermentation. The types of the mathematical models developed are given in Table 6.

Table 6. : Mathematical models for solid state fermentation.

- Growth of *A. niger* 10 on cassava for protein enrichment in static column fermentor (Raimbault, 1980).
- Solid State Fermentation limitation and monitoring Viniegra-Gonzalez (1996).
- CO_2 balance in solid state fermentation of cassava for protein enrichment (Rodriguez-Leon *et al*, 1991).
- Mycelia fungal growth on inert support (Gutierrez *et al*, 1996)
- Energy activation in cassava silage (Saucedo-Castañeda *et al*, 1990).
- Heat transfers in solid state fermentation (Saucedo-Castañeda *et al*, 1990).
- Biomass formation in solid state fermentation involving alginate as inert support (Auria *et al*, 1994)

UPGRADATION OF AGRO-INDUSTRIAL MATERIALS

Protein-rich food for humans was the goal of the ORSTOM team in initiating work on solid state fermentation in 1975. The philosophy behind this approach was the fact that plants make carbohydrates easily and abundantly, but not proteins. These plant materials, therefore, can be made nutritionally balanced by upgrading their protein content, via the fermentation route (Senez, 1997). The initial efforts were concentrated on cassava to a large extent and on banana, potato waste as well as sugar beet waste to a limited extent (Table 7).

Table 7. Upgradation of agro-industrial products/wastes/residues.

- Banana (Baldensperger *et al*, 1982)
- Carob waste (Lambraki *et al*, 1994)
- Cassava (Raimbault and Alazard 1980; Raimbault *et al.* 1985)
- Coffee pulp (Perraud-Gaime and Roussos 1995; Roussos *et al*, 1989)
- Copra cake (Ramirez-Islas *et al*, 1995; Roussos *et al*, 1994)
- Potato waste (Raimbault *et al*, 1979, 1981)
- Sugar beet waste (Roussos *et al*, 1982)
- Sugar cane bagasse (Gonzalez-Blanco *et al*, 1990; Roussos *et al*, 1991)
- Wheat straw + wheat bran (Roussos *et al*, 1991b; Deschamps *et al*, 1985)

Attention was subsequently focused from 1980, on upgrading the agro-industrial residues/wastes with a view to produce efficient animal feed (Senez, 1979; Senez et al. 1981; Deschamps et al. 1982). A number of residues/wastes were investigated as specified in Table 7.

ENZYME PRODUCTION

Among the microbial products in international and national markets, enzymes occupy a prominent place and large monetary turn-over. A number of enzymes have been economically manufactured in Japan and other Oriental countries by using solid state fermentation, though submerged fermentation is relied on in European and Western countries. This was mainly due to the neglect of solid state fermentation in these latter countries.

Table 8. : Studies on enzyme production in solid state fermentation system.

Enzymes	References
Amylases	Alazard and Baldensperger (1981) ; Raimbault and Alazard (1980) ; Soccol *et al*, (1993a and b) ; Raimbault (1989)
Glucoamylases	Oriol *et al* (1988) ; Soccol *et al*, (1993)
Cellulases	Roussos *et al* (1991) ; Soccol *et al*, (1994)
Pectinases	Boccas *et al*, (1994) ; Favela *et al*, (1989) ; Antier *et al*, 1(993a, b) ; Solis *et al*, (1994) ; Cordova *et al*, (1996)
Decaffeinases	Denis, (1996)
Tannase	Lambraki *et al*, (1994; 1996)
Lipase	Martinez-Cruz *et al.* (1993); Christen *et al*, (1994; 1995)
Protease	Villegas *et al*, (1993)

Intensive efforts have been made at ORSTOM and by its collaborators to investigate solid state fermentation processes for the production of different enzymes as specified in Table 8.

PRODUCTION OF METABOLITES OTHER THAN ENZYME

In addition to the production of fermented foods and enzymes by solid state fermentation, a number of other primary and secondary metabolites can be produced, with a number of advantages (Lonsane *et al*, 1985, 1992). It is of interest to note that penicillin was produced industrially in USA during 1940s by solid state fermentation (Zeifer et al. 1988).

Table 9. : Studies on production of metabolites other than enzymes by solid state fermentation.

Metabolites	References
Alkaloids	Trejo-Hernandez *et al*, (1992, 1993a, 1993b)
Aflatoxin	Barrios-Gonzalez *et al*, (1990)
Penicillin	Barrios-Gonzalez *et al*, (1988, 1990, 1996)
Gibberellic acid	Barrios-Gonzalez *et al*, (1988)
Ethanol	Saucedo-Castañeda *et al*, (1991, 1992, 1993, 1994); Christen *et al*. (1994)
Lactic acid	Soccol *et al*, (1994)
Fumaric acid	Soccol (1992)
Citric acid	Gutierrez-Rojas *et al*, (1995)
Alpha-pentyl pyrone	Lozano *et al*, (1995), Sarhy-Bagnon *et al*, (1996)
Octane-1-ol	Kabbaj *et al*, (1996)

A number of industrially important metabolites were identified by ORSTOM and efforts were put to study their production by solid state fermentation (Table 9). In all the cases, the process was optimised and a number of interesting facts were discovered. The metabolites studied are diverse in nature and include organic acids, antibiotics, plants growth hormones, alkaloids, toxins and aroma compounds (Table 9).

BIOMASS WITH SPECIFIC CHARACTERISTICS

It is well known that chemically synthesised pesticides are problematic, mainly because of their difficult biodegradation and persistent residues in foods and even in human bodies. Biopesticides offer efficient alternatives and are being used increasingly throughout the world. Mainly filamentous fungi have antagonistic properties against a number of plant pathogens, in addition to specificity of action. ORSTOM has invested efforts on production of the biomass of such fungi for their use as biopesticides (Montero *et al*, 1989). The fungi studied include *Trichoderma harzianum* and *Beauveria bassiana*. (Table 10).

Table 10. Biomass production in solid state fermentation with specific characteristics.

Products	References
Probiotics	Tapia *et al*, (1989) ; Ramirez-Islas *et al*, (1996); Herrera-Saltaña *et al*, (1990) ; Campos *et al*, (1994)
Biopesticides	Roussos *et al*, (1989) ; Montero *et al*, (1989)
Mushroom spawn	Roussos *et al*, (1993; 1996)
Ectomycorrhiza mycelial cells	Roussos *et al*, (1995)

Mushroom production is one of the major examples of the exploitation of solid state fermentation and the process involves SSF at three stages, i.e. composting of substrate for use in mushroom production, spawn production and the cultivation process of mushrooms itself (Lonsane *et al.* 1985). Cereal grains are mainly used in spawn production but these are either costly, not sufficiently available for spawn production or not produced in many countries. The efforts in ORSTOM were, therefore, directed to studies on production of spawn on sugar cane pith bagasse as an inert support, after impregnation with nutritive solution (Roussos *et al.* 1996).

Studies have also been initiated on production of ectomycorrhiza mycelial cells by solid state fermentation (Table 10) and economically useful results have been obtained (Roussos *et al*, 1995).

BIOREMEDIATON

The chemical methods for remediation are either elaborate, cost-intensive or are non efficient. Bioremediation overcomes these problems to a large extent. Hence, efforts were put in ORSTOM to develop solid state fermentation processes for bioremediation of commodities, such as coffee pulp and carob pod for detoxification, decaffeination, and degradation of polyphenol as well as tannin (Table 11). Solid state fermentation process was also relied up on for degradation of volatile contaminants in air for its purification (Morales *et al*, 1994; Revah, 1995).

Table 11. Bioremediation through solid state fermentation.

Bioremediation	References
Detoxification of coffee pulp through polyphenol degradation	Gaime -Perraud *et al.* 1991; Perraud-Gaime (1995); Perraud-Gaime *et al.* (1996)
Decaffeination of coffee pulp for its use in animal feed	Roussos *et al*, (1989, 1991, 1993, 1994, 1995) ; Perraud -Gaime and Roussos, (1996)
Polyphenol and tannin degradation in carob waste for detoxification	Lambraki *et al,* (1992, 1993, 1994, 1995, 1996)
Degradation of volatile contaminants in air for its purification	Morales *et al*, (1994); Revah *et al*, (1995)

NATIONAL AND INTERNATIONAL COLLABORATIONS

Collaboration with developing and developed countries is one of the goals of ORSTOM and it has strived hard to achieve it. The example of collaboration with Mexico can be cited as a best collaboration that could be established between two countries. Similar collaboration exists with a number of other developing and developed countries in most part of the world (Table 12). The output from these collaborative efforts is commendable and found recognition internationally.

Table 12. National and International collaborations.

Institution	Country	Area of co-operation
UAM-Iztapalapa	Mexico	Physiology and metabolism of fungi, proteins enrichment of cassava and sugar cane bagasse, coffee pulp detoxification, collection of micro-organisms, bioreactors, pilot scale, mathematical models, solid state fermentation, genetic of filamentous fungi, molecular biochemistry and academic formation.
UASP	Mexico	Solid state fermentation of agricultural wastes.
UNAM	Mexico	Pectinases, academic formation.
INMECAFE	Mexico	Coffee pulp upgradation.
ICIDCA	Cuba	Solid state fermentation, sugar cane bagasse upgradation, mathematical models, physiology of filamentous fungi sporulation.
UNIVALLE	Columbia	Cassava rot protein enrichment, solid state fermentation, sugar cane bagasse upgradation, lactic acid bacteria, academic formation.
CFTRI	India	Sugar cane bagasse upgradation, cellulases and amylases production, coffee pulp detoxification, solid state fermentation and new bioreactors.
Athens University	Greece	Carob pod protein enrichment, polyphenols degradation, solid state fermentation, physiology of *Aspergillus carbonarius*.
Montpellier university	France	Academic formation, solid state fermentation.
Provence university	France	Academic formation, solid state fermentation.
CIRAD	France	Cassava and copra cake, protein enrichment, Coffee pulp upgradation, lactic acid bacteria.
UTC	France	Academic formation, solid state fermentation.
ENSBANA	France	Academic formation, aroma production by fungi.
CNRS	France	Biodegradation of new plastics.
INRA	France	Mushrooms spawn physiology; aroma production, ectomycorhyzal mycelium, biopesticides.

PATENTS

The efforts of ORSTOM on solid state fermentation were always centred on topics of economic and industrial importance. In many cases, the resulting technology was either novel or with larger economic benefits. It is, therefore, not surprising that ORSTOM obtained 9 patents on its solid state fermentation processes or bioreactor design (Table 13).

Table 13 : Solid State Fermentation patents developed by ORSTOM

Patent	Authors	References
Protein enrichment process	Raimbault and Germont	Fr 76.06.67
Spores production process	Raimbault and Roussos	Fr 85.08.555 Eur 0223.809
Zymotis, static bioreactor	Prebois *et al*, 1985	Fr 85.17.934
Coconut aroma production	Lozano, Pioch and Roussos	Fr 95.01.713
Moulds control system	Roussos and Bossis	Fr 95.02.204
Fungal metabolites production	Barrios-Gonzalez *et al*, 1988	Mex. Secofi n° 17.184
Fungal enzymes production	Roussos *et al*, 1988	Mex. Secofi n° 17.185
Fungal spores production	Gutierrez-Rojas *et al*, 1988	Mex. Secofi n° 17.187

Table 14: Doctoral theses presented to different universities in the aera of SSF.

Autors	Aerea	University	Year	Page
Raimbault Maurice	*Aspergillus* growth in starchy substrates	Toulouse (Fr)	1980	291
Huerta-Ochoa Sergio	Heat and mass transfert on cassava SSF	UAM-I (Mex)	1984	147
Roussos Sevastianos	*Trichoderma* growth on cellulosic materials	Marseille-I (Fr)	1985	193
Oriol Eric	*A. niger* growth on natural support	Toulouse (Fr)	1987	133
Trejo-Hernandez Maria	Pectinase production in natural support	UNAM-I (Mex)	1986	106
Saucedo-Castaneda Gerardo	Cassava protein enrichment and silage	UAM-I (Mex)	1987	169
Dufour Dominique	Pectinase production on natural support	UTC (Fr)	1990	262
Saucedo-Castaneda Gerardo	Yeast growth and metabolism on support	Montpellier II (Fr)	1991	197
Soccol Carlos	Cassava protein enrichment by *Rhizopus*	UTC (Fr)	1992	218
Trejo-Hernandez Maria	Alcaloïds production by *Claviceps*	Marseille-I (Fr)	1992	163
Aquiahuatl Maria	Isolation of caffeine degrading fungi	UNAM- (Mex)	1992	72
Perraud-Gaime Isabelle	Coffee pulp silage and decaffeination	Montpellier II (Fr)	1995	209
Gutierrez-Rojas Mariano	Mathematical models for SSF on inert support	UAM-I (Mex)	1995	

DOCTORAL THESES

A number of students from the collaborating countries have realised their doctoral theses at ORSTOM, under the fellowship from France government. The staff of ORSTOM also worked for their doctoral thesis. A total of 14 doctoral theses have emerged from these efforts during 1980-1995 and these were accepted for doctoral degree by a number of different universities in France (Table 14).

PUBLICATIONS

The research and development efforts on solid state fermentation at ORSTOM found place in a number of national and international, peer-reviewed standard journals. The information on publication of research as well as review papers and papers presented at various national and international symposia/seminar/convention/meetings is given in Table 15.

Table 15: Publications and papers presented at national and international symposium.

Publications	Number
Research papers	102
papers	8
Papers present at symposium	148

TECHNOLOGY TRANSFER

Some of the technologies developed at ORSTOM have been successfully transferred to industries for commercial exploitation. For example, the process for conidiospore production of *Beauveria bassiana* and *T. harzianum*, for use as biopesticide, has been utilised by two French companies Calliope and Letellier. In addition, the technology for production of *Pleurotus* spawn by solid state fermentation based on the use of inert support, after impregnation with nutritive solution, has been commercially exploited by Ayusa, Mexico. The laboratory scale column fermentor unit, consisting of 16 columns, has been successfully manufactured by Gauthier, Montpellier, France, for international marketing.

SYMPOSIA / SEMINARS ORGANISED

ORSTOM has successfully organised a number of national and international symposia/seminars on solid state fermentation (Table 16). The organisation of these events have been commended by the scientific community involved in solid state fermentation. Proceedings of all these seminars/symposia have been published, except for FMS-89 in Mexico.

Table 16. : Symposia/Seminars organised.

Year	Evenment	References
1988	FMS-88	Montpellier, France
1989	FMS-89	Mexico D.F., Mexico
1989	SIBAC-I	Xalapa, Ver., Mexico
1991	SIBAC-II	Manizales, Columbia
1995	FMS-95	Montpellier, France
1995	Vème Entretiens Agropolis	Montpellier, France

TEACHING AND TRAINING

As a part of co-operation, in Mexico ORSTOM participated in teaching of biotechnology, with specific emphasis on solid state fermentation, at graduate, post-graduate and post-doctoral levels in the Universidad Autonoma Metropolitana - Iztapalapa, Mexico. A course of 4 weeks duration, entitled "Ecole Chercheur Orstom en Fermentation en milieu Solide, ECO-FMS" is conducted once in a year at ORSTOM, Montpellier, France. It involves theory, practical and research in solid state fermentation, along with computer application.

LITERATURE BANK

ORSTOM, at its Montpellier centre, has developed a large literature bank on solid state fermentation. It includes 90 theses and 7.000 reprints on filamentous fungi and solid state fermentation.

STAFF STRENGTH

The staff strength at ORSTOM, Montpellier is around 10 and their specialisation is in microbiology, biochemistry, physics and engineering. In addition, about 10 graduate students from various universities are trained for a period of 2-6 months, at any given time. The work on solid state fermentation is also carried out by 6 doctoral students and 2-3 post-doctoral students/annum at any given time.

PERSPECTIVES

The experience on different aspects of solid state fermentation and its exploitation for production of biomass and primary as well as secondary metabolites over the past 20 years have provided an efficient in-sight in this fermentation technique to ORSTOM and its collaborating teams. This formed an efficient base for chalking out the perspectives in solid state fermentation at ORSTOM for the next years:

- Isolation of new fungal strains adapted in SSF
- Molecular Biology and genetic engineering of fungi involved in SSF
- Mixed Cultures with bacteria, yeast and fungi
- Production of fungal biomass and metabolites
- Engineering of bioreactors and sensors
- Solid state fermentation in continuous system
- Bioremediation of commodities and soil detoxification
- Degradation of volatile contaminants in air for its purification

As can be seen, the perspectives are diverse and more focused on specific areas of economical and industrial aspects. These perspectives may also lead to extensive commercial exploitation of solid state fermentation processes.

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