EJB Electronic Journal of Biotechnology ISSN: 0717-3458
Vol.1 No.3, Issue of August 15, 1998.

Invited review paper / Received 5 September 1998
REVIEW ARTICLE

General and microbiological aspects of solid substrate fermentation

Maurice Raimbault
Laboratoire de Biotechnologie Microbienne Tropicale, Centre ORSTOM-LBMT 911 av. Agropolis - B.P.:5045 - 34032 Montpellier (France)
E-mail: maurice.raimbault@mpl.orstom.fr

http://www.mpl.orstom.fr

At first some general considerations about specificity and characteristics of SSF, their advantages and disadvantages as compared to LSF, are presented. Microorganisms involved in solid substrate fermentations are identified, considering the better performances of filamentous fungi. The solid substrates and their basic macromolecular compounds are detailed in relation to this complex and heterogeneous system. Biomass measurement is examined in detail, as well as environmental factors, both essential for studying and optimising solid substrate fermentations.

General considerations

Aerobic microbial transformation of solid materials or "Solid Substrate Fermentation" (SSF) can be defined in terms of the following properties:
- A solid porous matrix which can be biodegradable or not, but with a large surface area per unit volume, in the range of 10^3 to 10^6 m^2/cm^3, for a ready microbial growth on the solid/gas interface.
- The matrix should absorb water amounting one or several times its dry weight with a relatively high water activity on the solid/gas interface in order to allow high rates of biochemical processes.
- Air mixture of oxygen with other gases and aerosols should flow under a relatively low pressure and mix the fermenting mash.
- The solid/gas interface should be a good habitat for the fast development of specific cultures of moulds, yeasts or bacteria, either in pure or mixed cultures.
- The mechanical properties of the solid matrix should stand compression or gentle stirring, as required for a given fermentation process. This requires small granular or fibrous particles, which do not tend to break or stick to each other.
- The solid matrix should not be contaminated by inhibitors of microbial activities and should be able to absorb or contain available microbial foodstuffs such as carbohydrates (cellulose, starch, sugars) nitrogen sources (ammonia, urea, peptides) and mineral salts.

Typical examples of SSF are traditional fermentations such as:
- Japanese "koji" which uses steamed rice as solid substrate inoculated with solid strains of the mould *Aspergillus oryzae.*
- Indonesian "tempeh" or Indian "ragi" which use steamed and cracked legume seeds as solid substrate and a variety of non toxic moulds as microbial seed.
- French "blue cheese" which uses perforated fresh cheese as substrate and selected moulds, such as *Penicillium roquefortii* as inoculum.
- Composting of lignocellulosic fibres, naturally contaminated by a large variety of organisms including cellulolytic bacteria, moulds and *Streptomyces sp.*
- In addition to traditional fermentations, new versions of SSF have been invented. For example, it is estimated that nearly a third of industrial SSF and koji processes in Japan has been modernised for large scale production of citric and itaconic acids.

Furthermore, new applications of SSF have been suggested for the production of antibiotics (Barrios et al., 1988), secondaries metabolites (Trejo-Hernandez et al., 1992, 1993) or enriched foodstuffs (Senez et al., 1980).

Presently SSF has been applied to large-scale industrial processes mainly in Japan. Traditional *koji*, manufactured in small wooden and bamboo trays, has changed gradually to more sophisticated processes: fixed bed room fermentations, rotating drum processes and automated stainless steel chambers or trays with microprocessors, electronics sensors and servomechanical stirring, loading and discharging. The usual scale in *sake* or *miso* factories is around 1 or 2 metric tons per batch, but reactors can be made and delivered by engineering firms to a capacity as large as 20 tons (Fujiwara, Ltd.).

Outside Japan, Kumar (1987) has reported medium scale production of enzymes, such as pectinases, in India. *Koji* type processes are widely used in small factories of the Far East (Hesseltine, 1972) and *koji* fermentation has been adapted to local conditions in United States (USA) and other Western countries, including Cuba (III A). In France, a new firm (Lyven S.A.) was recently created to commercialise a process for pectinase production from sugarbeet pulp. Blue cheese production in France is being modernised with improvements on the mechanical conditioning of cheeses, production of mould spores and control of environmental conditions.

Composting, which was developed for small-scale production of mushrooms, has been modernised and scaled up in Europe and USA. Also, various firms in Europe and USA produce mushroom spawn by cultivating Agaricus, Pleurotus or Shii-Take aseptically on sterile grains in static conditions.

New versions for SSF reactors have been developed in France (Durand et al., 1988; Roussos et al., 1993, Durand et al., 1997), Cuba (Cabello, 1985; Enríquez, 1983 and Rodríguez, 1986), Chile (Fernández et al., 1996) and fundamental studies on process engineering are being conducted in Mexico (Saucedo-Castañeda, 1990).

SSF is a batch process using natural heterogeneous materials (Raimbault, 1981 and Tengerdy, 1985), containing complex polymers like lignin (Agosin et al, 1989), pectin (Kumar, 1987; Oriol, 1988a), lignocellulose (Roussos, 1985). SSF has been focused mainly to the production of feed, hydrolytic enzymes, organic acids, gibberelins, flavours and biopesticides.

Most of the recent research activity on SSF is being done in developing nations as a possible alternative for conventional submerged fermentations, which are the main process in pharmaceutical and food industries in industrialised nations.

SSF seems to have theoretical advantages over liquid substrate fermentation (LSF). Nevertheless, SSF has several important limitations. Table 1 shows advantages and disadvantages of SSF compared to LSF.

Table 2 presents a list of SSF processes in economical sectors of agro-industry, agriculture and fermentation industry. Most of the processes are commercialised in South-East Asian, African, and Latin American countries. Nevertheless, a resurgence of interest has occurred in Western and European countries over the last 10 years. The future potentials and applications of SSF for specific processes are discussed later.

Table 1. Comparison between Liquid and Solid Substrate Fermentations.

FACTOR	**Liquid Substrate Fermentation**	**Solid Substrate Fermentation**
	Soluble Substrates (sugars)	Polymer Insoluble Substrates:
Sub strates		Starch Cellulose Pectines Lignin
Aseptic conditions	Heat sterilisation and aseptic Control	Vapor treatment, non sterile conditions
Water	High volumes ofwaterconsu-med and effluents discarded	Limited Consumption of Wa-ter; low Aw. No effluent
Metabolic Heating	Easy control of temperature	Low heat transfer capacity Easy aeration and high surfa ce exchange air/substrate
Aeration	Limitation oby soluble oxygen High level of air required	
pH control	Easy pH control	Buffered solid substrates
Mecanical agitation	Good homogeneization	Static conditions prefered
Scale up	Industrial equipments Available	Need for Engineering & New design Equipment
Inoculation	Easy inoculation , continuous process	Spore inoculation, batch
Contamination	Risks of contamination for single strain bacteria	Risk of contamination for low rate growth fungi
Energetic consideration	High energy consuming	Low energy consuming
Volume of Equipment	High volumes and high cost technology	Low volumes &low costs of equipments

Effluent & pollution	High volumes of polluting effluents	No effluents, less pollution
Concentration S /Products	30-80 g/1	100/300g/1

The following considerations summarise the present status of SSF:

- Potentially many high value products, as enzymes, primary and secondary metabolites, could be produced in SSF. But improvements in engineering and socio-economic aspects are required because processes must use cheap substrates locally available, low technology applicable in rural areas, and processes therefore must be simplified.
- Potential exists in developed countries, but close co-operation and exchange between developing and industrialised countries are required for further application of SSF.
- The greatest socio-economical potential of SSF is the raising of living standards through the production of protein rich foods for human consumption. Protein deficiency is a major cause of malnutrition and the problem will become worse with further increases in world population. Two alternatives can be explored to tackle this problem:
- Production of protein-enriched fermented foods for direct human consumption. This alternative involves starchy substrates for its initial nutritional caloric value. Successful production of such foods will require demonstration of economical feasibility, safety, significant nutritional improvement, and cultural acceptability.
- Production of fermented materials for animal feeding. Starchy substrates protein-enriched by SSF could be fed to monogastric animals or poultry. Fermented lignocellulosic substrates, by increasing its fibre digestibility, could be fed to ruminants. In this case, the economical feasibility should be favourable in comparison to the common model using protein of soybean cake, a by-product of soybean oil. product of soybean oil.

Table 2. Main applications of SSF processes in various economical sectors

Economical Sector	Application	Examples
Agro-Food Industry	Traditional Food Fermentations	Koji, Tcznpch, Rae, Attickc, Fermented cheeses
	Mushroom Production & spawn	Agaricus, Pleurotus, Shn-take
	Bioconversion By-products	Sugar pulp Bagass Coffee pulp' Silage Composting, Detoxication
	Food Additives	Flavours. Dyestuffs. Essential Fat and organic acids
Agriculture	Biocontrol , Bioinsecticide	Beauveria Metarhizium, Tricho derma
	PlantGrowth, Hormones	Gioberellins, Rhizobium, In-choderma
	Mycorhization, Wild Mushroom	Plant inoctiation,
Industrial Fermentation	Enzymes production	Amylases, Cellulases Proteases, Pectinases, Xylanases
	Artibiotic prduction	Penecillin, feed & Probiotics
	Organic acid Production	Ciric acid Fumaric acid Gallic acic Lactic acid
	Ethanol Prodixtion	Schwanniomyces sp. Sbrch Malting and Brewing
	Fungal Metabolites	Hormones Alcaloides,

For the last 15 years, the Orstom group has been working on solid fermentation process for improving protein content of cassava and other tropical starchy substrates using fungi, specially from *Aspergillus* group, in order to transform starch and mineral salts into fungal proteins (Raimbault, 1981).