2.4 SOLID STATE FERMENTATION

There are many biotechnological processes that involve the growth of organisms on solid substrates in the absence or near absence of free water (Table 2.1). Solid state fermentation (SSF) deals with substrates that are solid and contain low moisture levels. The most regularly used solid substrates are cereal grains (rice, wheat, barley and corn), legume seeds, wheat bran, lignocellulose materials such as straws, sawdust or wood shavings, and a wide range of plant and animal materials. Most of these compounds are polymeric molecules – insoluble or sparingly soluble in water – but most are cheap and easily obtainable and represent a concentrated source of nutrients for microbial growth.

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 103 to 106 m2/ cm3, for a ready microbial growth on the solid/gas interface.
- The matrix should absorb water amounting to 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.

Table 2.1: Examples of SSF

EXAMPLE	SUBSTRATE	MICROORGANISM(S) INVOLVED
Mushroom production (European & Oriental)	Straw, manure	*Agaricus bisporus*, *Lentinus edodes*, *Volvariella volvacea*
Sauerkraut	Cabbage	Lactic acid bacteria
Soy sauce	Soya beans and wheat	*Aspergillus oryzae*
Tempeh	Soya beans	*Rhizopus oligosporus*
Ontjom	Peanut press cake	*Neurospora sitophila*
Cheeses	Milk curd	*Penicillium roquefortii*
Leaching of metals	Low-grade ores	*Thiobacillus* sp.
Organic acids	Cane sugar, molasses	*Aspergillus niger*
Enzymes	Wheat, bran etc.	*Aspergillus niger*
Composting	Mixed organic material	Fungi, bacteria, actinomycetes
Sewage treatment	Components of sewage	Bacteria, fungi, protozoa

The microbiological components of SSF can occur as single pure cultures, mixed identifiable cultures or totally mixed indigenous microorganisms. Some SSF processes e.g., tempeh and ontjom production, requires selective growth of organisms such as moulds that need low moisture levels to carry out fermentation with the help of extracellular enzymes secreted by fermenting microorganisms. However, bacteria and yeasts, which require higher moisture content for efficient fermentation can also be used for SSF, but with a lower yield.

SSF are normally multistep processes involving the following steps:

1. Pre-treatment of substrate raw materials either by mechanical, chemical or biochemical processing to enhance the availability of the bound nutrients and also to reduce the size of the components, e.g., pulverizing straw and shredding vegetable materials to optimize the physical aspects of the process. However, the cost of pre-treatment must be balanced with eventual product value.
2. Hydrolysis of primarily polymeric substrates, e.g., polysaccharides and proteins.
3. Utilization (fermentation) of hydrolysis products.
4. Separation and purification of end products.

The low moisture content of SSF enables a smaller reactor volume per substrate mass than LSF and also simplifies product recovery. However, serious problems arise with respect to mixing, heat exchange, oxygen transfer, moisture control and gradients of pH, nutrient and product as a consequence of the heterogeneity of the culture. The latter characteristic of SSF renders the measurement and control of the above mentioned parameters difficult, laborious and often inaccurate, thereby limiting the industrial potential of this technology. Due to these problems, the micro-organisms that have been selected for SSF are the more tolerant to a wide range of cultivation conditions.

2.6.1 ENVIRONMENTAL PARAMETERS THAT INFLUENCE SSF

Water activity: Water lost during fermentation through the processes of evaporation and metabolic activity is normally replaced by humidification or periodic addition of water. When moisture levels drop too low, the substrate becomes less accessible and when moisture levels are too high, the porosity of the substrate is reduced resulting in lower diffusion rates and decreased gas exchange. This would result in a decreased rate of substrate degradation and may lead to an increased risk of contamination.

Temperature: Is controlled by aeration and/or agitation of the substrate. Heat generation in this system is more problematic than in liquid fermentations and has a big impact on relative humidity.

Aeration: Depends on the microorganisms in the fermentation. Most systems are aerobic. Aeration rates are also closely related to the need to dissipate heat, CO_2 and other volatile substances that may be inhibitory. The rate of oxygen transfer in SSF is related to the size of the particles which determines void space. Oxygen transfer in the void space is related to moisture level as the oxygen dissolves in the film of moisture around the substrate particles.

2.6.2 BIOREACTORS USED IN SSF

Most SSF processes are batch fermentations. Some processes do not require vessels and require spreading of substrate on a suitable floor surface. Bioreactor designs (Fig. 2.6) for SSF are inherently simpler than for liquid cultivations. They are classified into fermentations without agitation (tray systems and air flow systems), with occasional agitation and with continuous agitation (slow rotating drums).

Rotating drum: usually a cylindrical drum mounted on its side onto rollers that support and rotate the vessel. They are equipped with an inlet and outlet for circulation of humidified air and often contain baffles or sections to agitate the contents. They are used in enzyme and microbial biomass production. Their main disadvantage is that the drum is not filled to capacity (only 30% capacity or mixing is inefficient). Rotary movements of the drum need to be controlled so as to minimize damage to the mycelial growth due to shear.

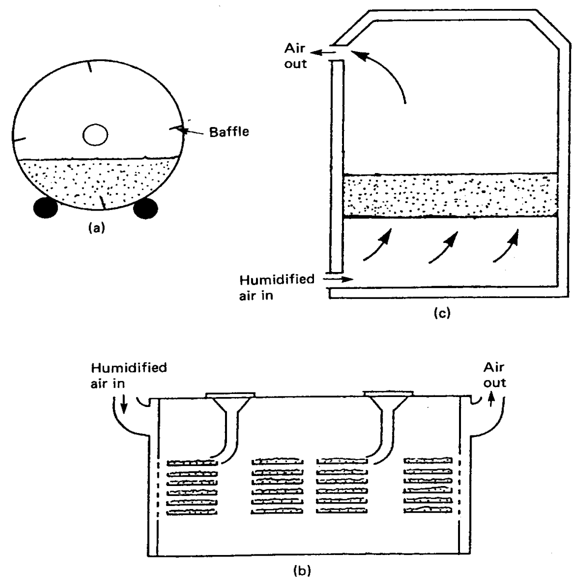

Fig.2.6: Solid substrate fermenter designs. (a) Rotating drum, (b) tray system, (c) forced air-flow system (Ward, 1992).

Tray fermenters: used extensively for the production of fermented oriental foods and enzymes. Trays hold layers of substrate 2.5-5 cm deep and are stacked in chambers usually force aerated with humidified air. In a forced-air cultivation chamber, bed temperature is monitored and appropriate temperature adjustments are made to the recycling air flow. There is less shear in these systems.

Bed systems: used in commercial koji production. Consist of a bed of substrate (1 m deep) through which humidified air is forced from below, continuously.

Column bioreactors: a column, usually glass or plastic that contain loosely packed solid substrate. Temperature control is achieved by a jacket surrounding the column. These systems are used for organic acids, ethanol and biomass production.

Fluidized bed reactors: used for biomass production for animal feeds. To prevent adhesion and aggregation in these systems, they are continuously agitated with forced air.

2.6.3 ADVANTAGES OF SSF

1 Low moisture content of the substrates allow for minimal contamination.

2 Can be carried out with small volumes of substrate, lowering the operational cost of the reactors.
3 More energy economical.
4 Product separation is easy and less cumbersome.
5 Low cost media.
6 No problems with foaming.
7 Low waste water output.

2.6.4 DISADVANTAGES OF SSF

1 Media are heterogenous, hence the mash is not properly mixed.
2 Substrate moisture level is difficult to control.
3 Reactor parameters such as pH, temperature and dissolved oxygen need precise control.
4 Continuous mixing or agitation of the medium is required to overcome control parameters.
5 Continuous agitation of the medium often damages the mycelia, retarding their growth and resulting in poor growth of the organisms.