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SOLID-STATE FERMENTATION IN ANIMAL NUTRITION

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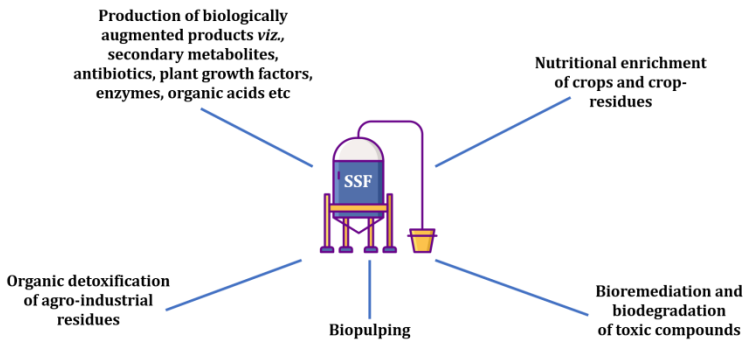
Solid-state fermentation (SSF) is a fermentation process occurring in a solid matrix in the presence of a little or negligible amount of free water, provided that the substrate must have sufficient moisture to support the growth and metabolism of microbes. It can be delineated as a process of the growth of micro-organisms on a solid substrate without a free-flowing aqueous phase.The solid matrix under consideration might be a reservoir of nutrients or merely a support infused with nutrients allowing microorganisms to grow.It is a fermentation method used by industries like the pharmaceuticals, food, textile etc., to produce biologically active secondary metabolites from microbes; and is emerging as a potential alternative to submerged/liquid fermentation(Nigam and Pandey, 2009).The advantage of SSF is accountable to the fact that it brings the microorganisms under cultivation in close proximity of the substrate and hence maximal substrate concentration is achieved. It mimics natural microbiological processes of fermentation like composting and ensiling. SSF provides a favourable habitat to microbes, replicating their natural environment they grow in, hence is highly preferred to grow and produce useful value added products in biotechnological industries. Processes where SSF finds application are:



Research in the field of SSF dates back to 1960–1970, when production of protein enriched cattle feed by SSF was done utilizing agro-industrial residues, thus offering a perfect solution for the development of nutritionally enriched roughages for livestock, and simultaneously reduction of air pollution. Recently, it is gaining more popularity in the solid waste management, biomass energy conservation and biotechnology industries (Pandey, 2007).

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Rice straw, sugarcane bagasse, wheat straw, rice hulls, and corn cobs are among the agricultural wastes utilised as SSF substrate (Gonzalez et al., 1993).

Steps in Solid State Fermentation

SSF is normally multistep processes involving the following steps:

i. The process is initiated with the pre-treatment of substrate raw material by mechanical, chemical or biochemical processing to increase the surface area and increase nutrient availability.

ii. Deposition of a solid culture substrate, such as rice or wheat bran, on flatbeds after seeding it with microbes for fermentation

iii. The substrate is then left in a room with temperature-control system for some days.

iv. Hydrolysis of primarily polymeric substrates, e.g., polysaccharides and proteins.

v. Utilization (fermentation) of hydrolysis products.

vi. Separation and purification of end products.

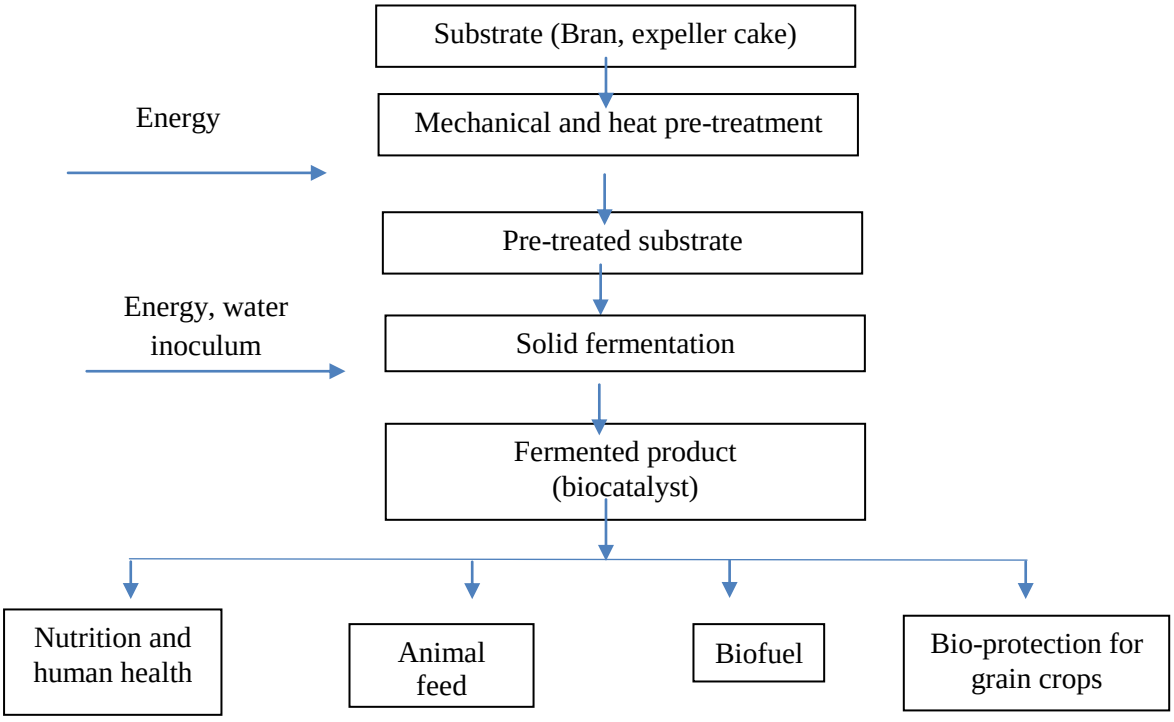

Fig.1: Solid-state fermentation process for production biomolecules and value-added products (Srivastava *et al.*, 2019)

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Applications of SSF

Solid-state fermentation has emerged as a potential methodology for the production of microbial products such as feed, fuel, industrial chemicals, and pharmaceutical products.

It is widely employed to manufacture several enzymes, organic acids, which are subjected to extraction and purification, employed to produce different products.

It can also be used in bioremediation and bio-leaching.

Application of SSF in production of certain active secondary metabolites are listed below.

Product	**Use**	**Source**	**Substrate**
Pharmaceuticals			
Zearalenone	Growth promoter	*Fusariummoniliforme*	Corn
Bacterial endotoxin	Insecticide	*Bacillus thuringensis*	Coconut waste
Penicillin	Antibiotic	*Penicilliumchrysogenum*	Sugarcane bagasse
Cephalosporin	Antibiotic	*Cephalosporiumarmonium*	Barley
Oxytetracycline	Antibiotic	*S. rimosus*	Corn cob
Cyclosporin A	Immuno suppressive drug	*Tolypocladiuminflautum*	Wheat bran
Enzymes			
Lipase	*Aspergillus niger, Candida rugosa, Penicilliumrestrictum*	Gingelly oil cake, coconut cake, babassu oil cake,	
Cellulases	*Bacillus subtilis, Aspergillus sp*	Banana fruit stalk wastes, soyabean meal,	
Pectinases	*Talaromycesflavus, Aspergillus niger*	Citrus wastes, soy bran and wheat bran, apple pectin.	
Polymers			
Succinoglycan	Agrobacterium tumefaciens, Rhizobium hedysaris	Spent malt grain or ivory nutshaving or grated carrots, impregnated spent malt grains	
Xanthan gum	Xanthomonascampestris	Spent malt grains, citrus peels, apple pomace, or grape pomace, Impregnated spent malt grains.	

Conditions for SSF

The technical and economic success of SSF is determined by a number of factors, some of which are listed below:

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i) **The choice of substrate**: It determines the fermentation by-products and biomolecules.

Material used for substate: For production of enzymes like amylase, starch-based substrates are used as material for SSF, while for the production of cellulase enzymes cellulosic or lignocellulosic substrates are used.The morphology and chemical composition of the substrate play crucial roles for enzyme production. (Soccol et al.,2017). Based on the type of substrate under consideration, SSF can be of two types: SSF with non-reactive (inert) materials acting as mere support and;

Noninert materials, such as biomass which act as support, and serve as carbon and nutrient sources to promote microbial growth (Carbou et al., 2018)

***Particle size of substrate*:** Substrates with finer particle sizes provide a fixed geometry and a greater surface area: volume ratio, show better enzyme production than substrates with larger particle sizes. But if the particle is too small, it causes agglomeration and interferes with microbial respiration, decreasing the microbial growth (Oriol et al., 1988).

***Moisture*:** Substrate moisture influences the SSF process significantly. Low substrate moisture is unfavourable for microbes, resulting in their poor growth, while high moisture content serves as an obstacle to oxygen penetration and hence slow down the process. Since the substrate is the fundamental parameter for microbial growth,kineticsdepend on it (Thomas *et al.,* 2013).

ii) **The choice of microorganisms**:

The choice of the microbes is apparently based on the selection of the substrate and desired product.Fungi and yeast are the most preferred microbes for SSF, as they thrive on solid medium and are able penetrate it with their hyphae and rhizoids, and their water activity is suited for SSF.Bacterial contamination can be avoided in fungal SSF by increasing the substrate: moisture ratio.The microbiological components of SSF can occur as single pure cultures, mixed identifiable cultures or totally mixed indigenous microorganisms. In bacterial species, Bacillus and Clostridium are the potential bacteria, while *Aspergillus, Trichoderma, and Mucor* are well-known fungal species for the SSF process (Sangsurasak et al.,1996). Additionally, filamentous fungi are best suited to produce industrially important enzymes by solid state fermentation.

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iii) Physio-chemical characteristics:

Volume of inoculum volume, viability, and vegetative cells' ability are the first physiochemical parameters that determine the growth of mycelium and microbes and their interactions with the substrate in medium.

Moisture: The moisture level is another important parameter. A moisture level in range of 60-70% is generally suited for both fungi and bacteria under the SSF condition. The requirements for water by microorganisms is expressed as the water activity (A_w) of the microorganisms and not the amount of water present in the solid substrate (Desgranges et al., 1991). Bacteria mainly grow at higher a_w values, while filamentous fungi and some yeasts can grow at lower a_w value (0.6-0.7)

The optimum pH for action of fungal metabolic activity is 4-5. pH: It is a significant parameter which makes the SSF process efficient. However, the accumulation of organic acids as fermentation by-products causes a decline in pH, it can be maintained by other salts present in the medium.

Temperature:In general, SSF is carried outat optimum levels by mesophilic microorganisms. Particularly, fungi can survive in the wide range of temperature of 20°C-55°C. The maximum product output is obtained as microbes function best at their optimum temperature (Penaloza et al., 1991).

Nutrient requirements: The fulfilment of nutritional demands of the microorganism during the SSF is a strong determinant of the output. Macro- and microelements are a pre-requisite and improve the metabolic activities of microorganisms during the fermentation (Krishna et al., 2001). A Carbon/Nitrogen ratio of 16 is best suited for the composition of the substrate and is used for fermentation processes (Krishna et al., 2005).

SSF Treatment of Crop Residues for Ruminant Feeding

Crop residues are commonly known as "lignocellulosics" because they have high content of cellulose and are associated with the biopolymer lignin. Even with the assistance of hydrolytic enzymes, the rumen microbiota (bacteria, protozoa, and fungus) are not capable of efficiently cleaving these bonds. White rot fungi (WRF) can break the ligno-cellulose

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complexes in such crop residues, releasing free cellulose and thus increasing their feeding value for ruminants. Compared to untreated roughages, biologically treated roughages exhibit greater digestibility for most nutrients (both cell walls and cell solubles) and a greater crude protein content and more fermentable carbohydrates.

Furthermore, recent findings have shown that feedstuffs exposed to solid state fermentation (SSF) using fungi result in lower methanogenesis as a result of enhanced digestion and nutrient absorption, and a decrease in structural carbohydrates. Kamra and Zadrazil (1988) elucidated that when the product is meant for ruminant feeding, the bioconversion procedure should increase lignocellulose digestibility. The biological upgradation of crop residues into animal feed should be characterised by considerable lignin degradation and nutrient liberation from the matrix and the accumulation of digestible components (Zadrazil et al., 1999) as enhancing the nutritional status of the finished product using microbial protein. Silva et al. (2002) reduced substrate fibre and CP levels treated with *Pleurotuspulmonaris*. However, all fungi do not improve the digestibility of straw. Jalc et al. (1994) reported that during bioconversion of wheat straw with *Polyporus ciliates*, digestibility was improved whereas with *Lentinustigrinus*, it was reduced.

A major proportion of animal trials on the application of fungal-treated agro wastes elucidated favourable nutrient utilisation response, nitrogen (N) balance and gain in body weight (Walli et al., 1988; Mahesh 2012; Shrivastava et al., 2012) although it is not consistent with all types of fungi. It is an established fact that good quality forage NDF in lactating cow diet is needed to maintain rumen functioning and optimum milk yield (Robinson and McQueen, 1992), so fungal treatment of fibrous feed improves digestible NDF and hence improves milk yield. Ward and Perry (1982) reported an improved digestibility of DM and NFE of corn cobs treated with *Trichodermaviride* in lambs. Fazaeli et al. (2004) observed that inclusion of fungal treated straw upto 30% of the total mixed ration in lactating Holstein cows improved the digestibility and an increase in fat corrected milk yield by 13% and average body weight gain. Ruminants release more CH_4 on fibrous diets. Mahesh (2012) observed a linear reduction in CH_4 (%) from fungal treated wheat straws which contained lesser fibre fractions (NDF and ADF) than untreated straw. Abo-Donia et al. (2005) and Omer et al. (2012) reported a substantial improvement in ruminal pH and the NH3-N concentration of the rumen liquor in biologically treated groundnut hulls and sugarcane bagasse, and *Trichodermaressi* treated corn stalks (Omer et al., 2012).

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Differences between Solid State Fermentation (SSF) and Submerged Liquid Fermentation (SLF)

Solid State Fermentation (SSF)	*Submerged Liquid Fermentation (SLF)*
Preferred organisms need less water for growth. Eg: filamentous fungi.	Water content is much higher than the media concentration
The inert support (natural or artificial), containing all components for growth are in the form of solution.	The essential processed ingredients are expensive.
Less probability of contamination as there is lesser availability of water	Higher water activity is the major cause of contamination in SLF.
Small size bioreactors can be used.	Large-scale bioreactors are required as the volume of the media is more
Less consumption of energy is needed for aeration and gas transfer.	Power consumption is more due to high air pressure
The limiting factor for growth is diffusion of nutri	Vigorous mixing makes diffusion easy
Downstream processing is easier, cheaper and less time consuming.	The aqueous form makes downstream process difficult and expensive
Liquid waste is not produced	High quantity of liquid waste is produced

(Manpreet *et al.*, 2005)

Limitations of SSF

- Microorganisms that can only withstand low moisture levels can be employed.
- Precise monitoring of SSF conditions (e.g., O_2 and CO_2 levels, moisture content) is not possible.
- As a result of the sluggish growth of the organisms, product creation is limited.
- Heat generation causes issues, and controlling the growing environment is quite challenging.

Conclusions

SSF is a potential clean technology for producing microbial metabolites from solid substrates like agro-industrial waste. SSF is a cost-effective green technology for the production of high-efficiency metabolites. Many value-added compounds are produced using SSF at the industrial level. To make the process more efficient, this technology must be improved in the areas of mass transfer, aeration, agitation, and maximal substrate conversion into products. This field has enormous potential, with the ability to expand to a bigger scale

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in additional sectors in the near future to generate more industrially relevant metabolites and produce cost effective animal feed.

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