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ENRICHMENT OF FEED INGREDIENTS THROUGH SOLID STATE FERMENTATION

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Introduction

Many process in fermentation technology have been practiced for thousands of years, like: production of cheese, fermented milk products, beer, wine and vinegar. These processes were developed from an art of 'taste' and only recently have been evolved to more technically sophisticated processes.

Solid State fermentation (SSF) refers to the controlled growth of microorganisms on moist solid substrate in the absence of free water. The free water indispensable to microbial growth is absorbed in a solid or complexed support inside a solid material. SSF can be better defined as the method of culturing microorganisms on and or within particles of a solid matrix. The liquid content, bound with them is at the level corresponding to the water activity (aw) assuring correct growth and metabolism of cells but not exceeding the maximal water holding capacity of the solid matrix. SSF, established since centuries has gained global attention, as potential biotechnological tool for the production of various substances. Some major advantages that SSF offers over submerged fermentation are in the areas of solid waste management and biomass energy conservation. Other advantages are:

- Natural habitat of the microbe can be minimized
- Lower expenditure
- Production of enzymes with higher specific activities.
- Improved enzyme stability
- Easier down stream processing and
- Generation of a protein enriched byproduct for possible use as an animal feed

The wide range of solid substrate/matrix used in SSF can be classified in three broad categories.

i) Organic materials: Organic materials are invariably polymeric molecules insoluble or sparingly soluble in water. The particles of solid substrate play a part at the same time, of support and substrates. *E.g.*: Lignocellulosic and starchy products such as sugar beet pulps, sugar cane begasses, wheat bran, wood chips, straw and so on.

ii) Mineral materials. These materials form only as support and they have to be moistened with nutrient solutions. *E.g.*: Clay granules, puzzola, perlite *etc.*

iii) Synthetic materials: Act only as support and need to be moistened. *E.g.*: Polyurethane foams, sponges *etc.*

To define the fermenter (reactor), the nature of the solid substrate used must be considered in terms of not only nutrient source for the microorganisms when it is an organic material, but also physical parameters, geometric configuration of the solid matrix, granulometry, porosity, maximum water holding capacity, resistance to compression and agitation.

The fermenter design is dependent on type of microorganism used, structure of the solid substrate, the environment conditions needed for the process and the type of use aimed at (*i.e.* research or industrial application).

Microbial growth in SSF

The solid substrate is inoculated with the desired microorganism at desired level (10^4 to 10^7 cells ml^{-1} or spores g^{-1} substrate). In case of fungi the spores germinate to form micro colonies. As it expand, the fungi quickly cover the whole substrate surface to form a mycelial layer, which increases in height and density. Fungal hyphae may penetrate into the solid particle that typically has a complex structure and complex nutritional composition. The major carbon sources are often carbohydrate, which may be soluble or macromolecular. In this case the mycelium releases enzymes, which diffuse into the substrate and hydrolyze the polysaccharide. The soluble sugars released then diffuse back to the mycelium. At the same time, oxygen is diffusing from the inter particle spaces through a stagnant gas film into the mycelial layer on the particle surface. The mycelium simultaneously consumes the soluble sugars and oxygen. As the fungal hyphae penetrate into substrate, it softens the substrate by pushing cells apart and degrading structural polymers. The degree to which this causes transformation of the substrate depends on the substrate structure and the capability of the fungi to penetrate into intracellular and intracellular spaces.

Application of SSF

1. Nutritional enrichment of agricultural byproducts/wastes

Proteins are the most important components of the food. Among the processes that can be used to supply proteins, the most important and processing are those, which are based on the microbial growth and production of microbial biomass.

The bioconversion of plant polymers (cellulose, hemicelluloses, lignin *etc.*) to protein has an increasing economic importance in many countries. Lignocelluloses can be transformed into high quality human food by feeding it to rumens. Lignification limits rumen digestibility of polysaccharides, presumably by blocking access to rumen bacteria and their enzymes to the polysaccharides. Through SSF, enrichment of protein of agricultural wastes and sub products is made possible. The SSF technology has the advantage of direct utilization of none or very few pre-treated solid substrates under aerobic conditions to produce microbial biomass products (MBP), which contain a mixture of unused substrates, cell substances of the microorganisms and external metabolites.

The nutritive value of any ingredient depends not only on the total nutrient content, but depends also on the nutrient availability and digestibility. For example, the use of bran in

human nutrition is limited by its low digestibility. The cellulose- hemicellulose matrix of the aleurone cell walls often act as a barrier to the attack on nutrients by human digestive enzymes. Moreover, anti nutritive factors originally present in the cereal grain, may limit the availability of nutrients or act as enzyme inhibitors. In particular phytic acid, the main storage form of phosphorous by interacting with cations or protonated basic residues of proteins, reduces the bioavailability of minerals and proteins. Significant reduction in phytic acid by SSF has been well documented in wheat bran by fungal phytase an enzyme that breaks down phytic acid.

The use of SSF for protein production from starchy substrates has been shown to be a feasible alternative for animal feeding. A process has been successfully developed to increase the protein content of cassava upto 20% in 30h with a suitable strain of *Aspergillus niger*. *Rhizopus olegosporus* and *Aspergillus oryzae* have also been used for the protein enrichment of cassava. *A. niger* has been reported to utilize dried citrus peel in a controlled SSF process in such a manner that the simple sugars are converted to protein fractions. Apple pomace (residue left after extraction of juice) has high acidity and though it is rich in soluble sugars, it has very small amount of proteins. It is reported that growth of yeast an apple pomace increases its protein and vitamin contents. The co-culture of *Candida utilis* and *A. niger* were found the best among several combinations which increased the protein content of dried and pectin extracted apple pomace by 20% and 17%, respectively, under SSF conditions.

The amino acid profiles of fungal protein show variations among groups of microorganisms (yeasts and filamentous fungi). On studying the application of microscopic fungi for protein biosynthesis other favourable properties of these organisms are also considered. These properties are:

i) Their ability to form an enzymic complex permitting transformation into microbial protein of various raw materials.

ii) The low content of nucleic acids in fungal biomass

Fungal culture in SSF medium affords products with protein content more than 100% higher than that in the raw material.

2. SSF of fishery wastes and agro industrial wastes

Any suitable strains of bacteria or fungi can be effectively utilized for fermentation to obtain bioenriched products. The low nutrient wastes are dried to <10% moisture content and pulverized to about 200μ size or less. The proximate composition analyses are carried out and if necessary, a combination of ingredients can be used. Moisture is adjusted to about 60-65% and pH to neutral. The aseptic substrate is then inoculated with the desired bacteria or fungi or a consortium of either (10^7 to 10^9 cell ml^{-1} for bacteria or 3-9 x 10^6 spores ml^{-1} of fungi for $10g^{-1}$ substrate) and the contents are mixed thoroughly and incubated under controlled static conditions preferably with maximum surface area for varying periods. Depending on the purpose of SSF, after desired duration, either, enzymes can be extracted from the fermented biomass or it is dried to a constant weight and used directly as a feed

ingredient. At Central Marine Fisheries Research Institute, we have undertaken fermentation of soybean flour, mixed oil cakes, soy flakes, cabbage waste and water hyacinth with both bacteria and fungi (industrial as well as local isolates). It has been observed that digestibility and feed conversion ratio has been considerably improved while using fermented products in shrimp post larval diet as fish meal substitute at 10-15% levels of incorporation. A bioenriched ornamental fish feed developed by CMFRI has been proved to be a successful alternative for imported ornamental fish feed for multispecies aquaria.

In a developing country like India, where import of costly raw materials as protein supplement for animal and aquafeed production is quite expensive, bioenrichment of cheaper ingredients through SSF would be a profitable option. Since the technology is cheaper and simple, it could be taken as a small-scale industry with a positive impact on aquafeed production.

3. Enzyme production for the food industries

A large number of microorganisms, including bacteria, yeast and fungi produce different groups of enzyme in SSF systems. It has been reported that a strain of *Aspergillus niger* produced 19 types of enzymes, -amylase was being produced by as many as 28 microbial cultures. 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. Agro industrial residues are generally considered the best substrates for the SSF processes. Some of the substrates that have been used include sugar cane bagasse, wheat bran, rice bran, maize bran, wheat straw, banana waste, apple pomace, peanut meal, coconut oil cake, wheat flour, cassava flour, *etc.* The selection of substrate depends on various factors mainly related with cost and availability of the substrate. Other factors to be considered in enzyme production are particle size and moisture level/water activity of the substrate.

The major factors that affect microbial synthesis of enzymes in 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 water activity (aw) of the substrate
- Relative humidity
- Type and size of 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.

Almost all known microbial enzymes can be produced under SSF systems. Production of industrial enzymes like: proteases, cellulases, ligninases, xylanases, pectinases, amylases,

glucoamylases *etc.* and inulinases, phytases tannases *etc.* are also reported by SSF processes. The enzyme titres produced in SSF systems are many fold more than in submerged fermentations, it generates less effluents and require only simple equipments. SSF holds tremendous potential for enzyme production. The crude fermented products may be used directly as enzyme sources. In addition to the conventional applications in food and fermentation industries, microbial enzymes have significant role in biotransformation involving organic solvent media, mainly for bioactive compounds. It is hoped that enzyme production processes based on SSF systems will be the technologies of the future. Genetically improved strains would play an important role in this.

4. Production of biologically active secondary metabolites

Antibiotics: Higher yield of penicillin in a relatively shorter duration is reported. The production of penicillin ranged from 0.6 to 16.7 times relative to submerged fermentation. SSF system for production of cephalosporins with *Streptomyces clavuligerus* is well advanced. Moist barley could be effectively used for this purpose. The antibiotics, tetracycline produced by SSF was more stable than that produced by submerged fermentation and the product is able to be stored temporarily without losing activity significantly.

Other metabolites that could be produced by SSf include cyclosporin A, Iturin, ergot alkaloids, gibberellic acid (GA3) and mycotoxins.

The biosynthesis of secondary metabolites is triggered by the limitation of growth caused by the exhaustion of a key nutrient (nitrogen or phosphorous). The bosynthesis starts when the growth starts decreasing. There is no doubt that SSf offers better opportunity for the biosynthesis of low volume high cost products, *viz.* biologically active secondary metabolites. With optimization of the process and proper bioreactor, such products could be commercialized from microorganisms of different origin.

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