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Solid state fermentation in the development of agro-food by-products

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All human activities give rise to the formation of by-products. As long as the population remained sufficiently dispersed, the waste generated was insufficient to overload the

the culture of different strains of fungi or yeasts in liquid mediums. Harvesting and conditioning synthesized cellular material generally produces a dry end-product with

Table 1 The principal Asiatic fermented products (from Hesseltine & Wang)[5]

Name of Product	Substrate	Micro-organism
Shoyu	Wheat and soya	*Aspergillus oryzae Lactobacillus*
Miso	Rice and soya	*Aspergillus oryzae* *Saccharomyces rouxii*
Natto	Soya	*Bacillus subtilis*
Tempeh	Soya	*Rhizopus oligosporus*
Sufu	Soya	*Actinomucor elegans*
Hamanatto	Soya	*Aspergillus oryzae*
Koji	Wheat and rice	*Aspergillus oryzae*
Ontjom	Ground nuts	*Neurospora sitophila*
Katsuobushi	Fish	*Aspergillus glaucus*
Bagoong	Fish	Undetermined
Nuoc-mam	Fish	Halophilous bacteria

fermentation practices, most often carried out by artisans, is not to increase the amount of protein in food products but to furnish the product with particular organoleptic qualities (Attieke, Miso), improve the digestibility of protein (Tempeh), or to enhance enzyme activity (Koji). Solid state fermentation is likewise used in enzyme production processes: for example in producing amylases,[6] cellulases,[7] or metabolites such as citric acid[8] or gallic acid.[9]

Among solid fermentation products, the production of cheese should be mentioned, as for example in France where the particular "blue" type cheeses resulting from the cultivation of a strain of *Penicillium roqueforti* in the cavities of curd, is produced.

It should be stressed that, among micro-organisms, filamentous fungi are the only ones which, for thousands of years have been an integral part of human nutrients. It would be premature to deduce that all species are high in food value and non-toxic, but the observation should mitigate some anxiety concerning the utilization of micro-organisms.

Several laboratories have worked on solid state fermentation techniques for preparing PEFF, some using cellulosic products,[10] others amylolytic substrates: cassava[11] or cereals.[12] Results have not been conclusive because it has not been possible to maintain the conditions of aeration, pH and temperature necessary for good aerobic growth over sufficient time periods.

In order that an enrichment procedure, should give significant results it is indispensable that the growth of micro-organisms take place under aerobic conditions at all times. However, substrates, particularly when they contain starch, tend to form pasty products that inhibit the transfer of oxygen. It is thus necessary that optimal conditions for fungal growth be maintained so that the sugar released by the hydrolysis of starch can be rapidly consumed.

ORSTOM-IRCHA process of producing PEFF

A new solid state fermentation process which resolves the above problems has been developed in France and is described below. It is principally based on the use of higher fungi which develops easily in humid but not liquid mediums under static conditions.[13] The aim is:

enriched by-products based on a technology that can be applied at the farm level; the mediums utilized should be as concentrated as possible.

— to base the fermentation process on a single-operation technique without requiring aseptic conditions.

— to simplify the technique as far as possible while incorporating the means of controlling the parameters essential to growth i.e. temperature, aeration, and pH.

Laboratory studies

Technique for culture on a solid medium

Our technique is based on the homogeneous distribution of micro-organic spores (such as *Aspergillus niger* and many strains common in Asia and Africa) and of salts in a suitable volume of amylolytic substrate. The preparation of a porous and granulated raw material with appropriate pH and moisture is essential to ensure good aeration and the rapid growth of mycelium. This method engenders the selective growth of the strain owing to the acid-level pH, low relative humidity, and the massive inoculation of spores. Consequently, it does not require aseptic conditions.

After the germination phase, the filaments develop and surround the starch grains, binding them to form a solid mass. Thus aeration is not inhibited and the conditions for aerobic development are maintained at all times. Microscopic examination has revealed that all the spores germinate after six to eight hours of fermentation. When fermentation is complete (twenty to twenty-four hours) all the spores have disappeared; very slight sporulation is observed only after more than forty hours at a very high rate of aeration.

Kinetic studies of development

According to physiological data, the best conditions for studying the kinetics of development are the following: initial humidity 50 per cent, pH 4.5, the source of nitrogen

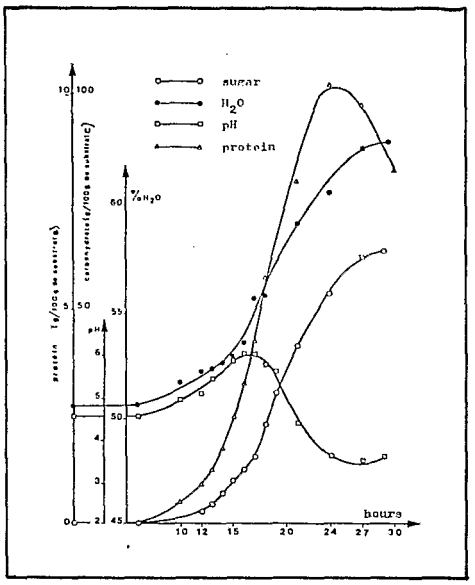

Figure 1 Kinetics of the growth of *aspergillus hennebergii (niger* variety) on cassava flour

being a mixture of ammonium sulphate and urea.

The results of this data in the fermentation of cassava are shown in Figure 1.

Three successive phases are distinguishable:

— a latency phase of six to eight hours corresponding to the period of spore germination.

— an exponential type of incubation phase which lasts about twenty hours. In the course of this phase a direct relationship between the production of protein and the consumption of carbohydrates is observed. The pH value, which increases at the beginning of growth then tends to fall. The total moisture rises from 50 to 65 per cent. It increases in proportion to the consumption of sugar which seems to confirm that this rise is due to the respiratory activity of the micro-organism.

— a slowing down phase characterized by the culmination of protein production, while from 30 to 40 per cent of the carbohydrates remain unconsumed.

Such trials have been carried out on different amylolytic substrates and the results presented in Table 2 show that after thirty hours of incubation, it is possible to obtain a product containing an average of 18 to 20 per cent protein and 30 per cent residual sugar. The rate of overall conversion of carbohydrates into protein is of the order of 20 per cent. Similar results have been obtained with numerous other strains of amylolytic filamentous fungi, especially those utilized in preparations for use as human food.

The method does not require aseptic precautions, the selective growth of the micro-

Table 2 Growth of *A. hennebergii* on different amylolytic substrates. Results expressed as a percentage of the dry weight of the sample

	Initial Composition		Final Composition	
Substrate	Protein	Assimilable Sugar	Protein	Assimilable Sugar
Cassava	2.5	90	18	30
Banana	6.4	80	20	25
Banana waste	6.5	72	17	33

of acid-level pH and a massive inoculation of spores. The size of the inoculum is 2×10^7 spores/gramme of dry flour. Under these conditions, all the spores germinate after eight to ten hours of incubation and no conidia appear in the course of growth. The results of detailed studies in the microbiology, the respiratory metabolism, and the physiology of the growth of mycelium on a solid amylolytic substrate have been reported elsewhere.[14]

Technological study of solid state fermentation

From the laboratory results it has been possible to proceed to a pilot study of material, which while capable of generating solid state fermentation, at the same time enabled the regulation of essential parameters of the process: temperature, aeration, pH, and humidity.

Direct extrapolation from column culture technique is complicated by the difficulties encountered in eliminating the calories produced in the course of growth. Research

in proportion to the duration of these cycles. Complete opening up to 60 litres/minute is attained after three hours of real time functioning of the cooling system.

Necessary pH corrections are made by introducing a corrective solution, at a determined rate, in the water at surface level. In the event, when the medium shows a tendency towards acidification, the correction is made by adding a solution of urea which serves at the same time as a source of nitrogen.

Moisture control is achieved by modifying the output of water sprayers during each cooling cycle.

The action of the different regulatory systems makes it possible to control the growth of micro-organisms in a satisfactory manner. It permits the reproduction of laboratory results at the level of small pilot units. Temperature regulation by means of intermittent cycles represents an appreciable gain since the agitation motors only function for a

(Institut International de la Recherche Chimique Appliquée) in close collaboration with SPEICHIM (Société pour L'Equipement des Industries Chimiques) in order to scale up the process to a fermenter with a production capacity of the order of a metric tonne per day. The basic equipment, which should be operational in the very near future, will be used for nutritional and toxicological experiments on a large scale, for optimizing substrate preparations and development conditions as well as, finally, for the study of investment and operating costs. The experimental stage should be completed by demonstration units in tropical countries in order to adapt the process to local climatic and agro-economic conditions.

Technico-economic aspects[16]

This protein enrichment process appears to be a simplified system compared to fermentation in liquid mediums. Figure 2 shows the essential steps of these two techniques. The simplicity of the operations in solid

Table 3 Optimal productivity of protein-rich nutrients

countries. However, other substrates with

derived from filamentous fungi' in *Progress in*