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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 surrounding environment, and was degraded naturally. But population growth, the aggregation of people and industrial development have made it necessary to utilize such by-products. The treatment of waste has become indispensable as much to avoid pollution as to recover whatever organic material is available in the waste. Today, residue should be considered as a vast and poorly exploited resource which can and ought to be re-utilized.

The use and transformation of organic compounds produced by agricultural crops, leaves a certain quantity of residual material at each stage either in the form of solid waste or in solution in effluents. Cellulose and starch are the most abundant substrates available for conversion into foodstuffs. Cellulosic residue constitutes the non-food part of crops. It is the essential constituent of straw which is left in the field after harvesting cereals or collected and used as fuel or as litter for animals. Dried sugar beet roots, fruit peelings, sugar cane bagasse, etc., can also be recovered after extraction or from the processing industry.

Numerous experiments are underway in the use of cellulosic material. However the separation of cellulose and lignin requires difficult pretreatment operations; thus the hydrolysis of the B 1.4 cellulose bond is a costly chemical process, and slow and difficult by biological means. However, the results of recent research indicate the possibility of significant improvements in procedures for utilizing cellulose.

The conversion of starchy residues, on the other hand is comparatively easy to achieve. These include discarded food products, surplus production, or by-products from food processing industries. Cassava and bananas in tropical regions, and potatoes in temperate climates provide substrates with high potential because of their productivity and their excellent rate of conversion to biomass by a great number of fast-growing micro-organisms.

SCP production in liquid mediums

Various studies of the use of starch in the production of protein have concentrated on 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 high protein content. Two types of process must however be distinguished: those aimed at the total microbial transformation of starch into protein,[1 2] and those aimed at a partial transformation of starch into a protein-enriched product.[3 4]

In spite of efforts to simplify the technology, cultures in an agitated liquid state still involve relatively high capital investment and operating costs. They also require a semi-industrial scale of production, often poorly adapted to dispersed substrates from agricultural production and even less able to deal with agro-industrial residues. The low added value of products and the considerable cost of raw materials and their transportation are indicative of the need for low-cost, simple, or even rustic transformation procedures.

Solid state fermentation

Given these considerations, we were led to experiment with a totally different approach. Rather than producing microbial cells, we developed a procedure for protein enrichment of glucocidic material, to be used as such in feed. The process consists of fostering the development of selected aerobic micro-organisms on a solid substrate. The result is protein enriched fermented foods (PEFF).

This concept of solid state fermentation is based uniquely on filamentous fungi. A solid state can be defined as one in which the substrate possesses a granulometry and an organized structure. It is in fact a type of medium which is closest to the natural environment of fungi. Fungi are most often found in a medium such as aerated soil, cereal grains, vegetal products or granulated minerals in which water does not occupy the totality of interstices and cannot pass freely into crevices. Such media are conducive to the growth of filamentous fungi which spread by apical lengthening and ramification, rapidly exploring the environment over large areas.

Fermentation processes based on the development of filamentous fungi on a solid substrate have long existed and are the basis of numerous traditional foods.

Table 1 shows the principal Asiatic fermented products. The object of such

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

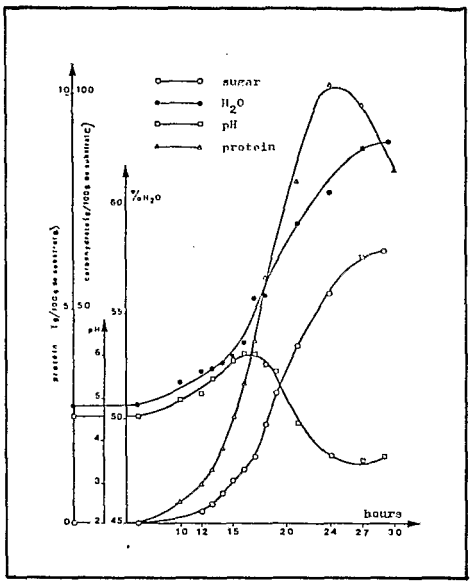

Figure 1 Kinetics of the growth of *aspergillus hennebergii (niger* variety) on cassava flour

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

Organisms being isolated by the [illegible] 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 has thus been oriented towards the most simplified equipment possible which allows both the homogenization of the medium and the regulation of temperature.

After various trials, a machine was chosen which resembles the baker's dough mixer with a tank turning freely on its axis and fitted with an agitation rod. This apparatus was modified by an inner lining pierced with holes for aeration of the product and the installation of a small motor to regulate the speed of rotation. Around this basic apparatus it has been possible to install different regulatory systems, the functioning of which can be appreciated in the following description of the fermentation cycle.[15]

Six to eight kilogrammes of dry amylolytic substrate, reduced to 30 per cent moisture is placed in a 65 litre fermenter. It is steamed at 80°C for ten minutes by the introduction of hot vapour. The material is then cooled and reduced, according to the substrates to between 45 and 55 per cent moisture with the introduction of mineral salts containing the spores of the chosen micro-organism.

When fermentation is at time zero, a probe to monitor temperature and another for pH are placed in the medium; the aeration rate is 10 litres/minute. As with column fermentation, a latency phase of ten to twelve hours is observed during which the material is regularly homogenized for one minute every two hours. The rest of the time the equipment is stationary.

When growth begins and metabolic calories start to emerge, the fermentation process is controlled by regulating the temperature. When the temperature of the material exceeds the optimum level, a cooling cycle is automatically activated. This cooling action is obtained by the simultaneous action of agitation and the introduction of a spray of water on the surface of the material. It continues until the temperature falls to the desired level.

Other regulatory operations take place during the course of the cooling cycles: [illegible] is opened 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 total duration of approximately four hours, for an overall fermentation time of thirty hours.

Studies of solid state fermentation are currently being pursued in France by ORSTOM (Office de la Recherche Scientif[illegible]ique et Technique Outre-Mer) and IRCHA (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 fermentation is clear in this comparison in so far as the final drying can be avoided if the product is introduced directly into animal feed.

Table 3 also makes it possible to compare

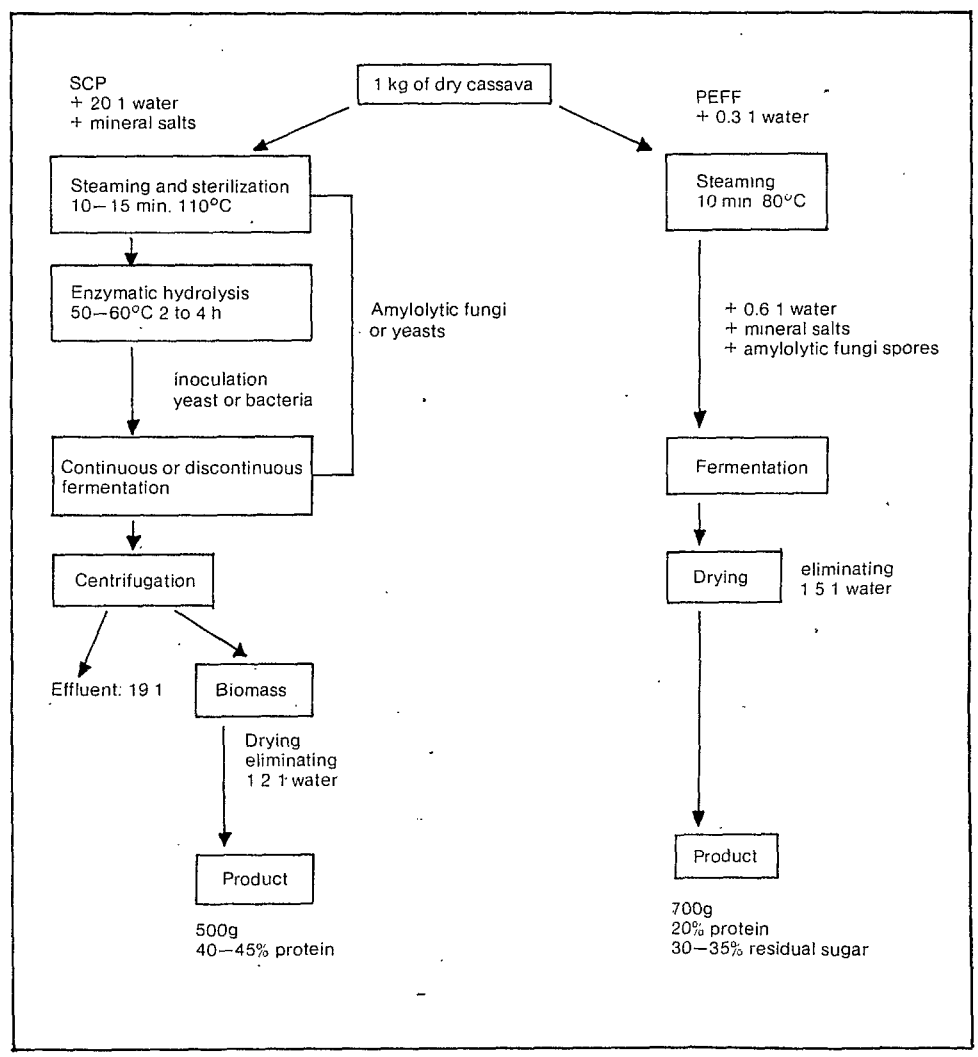

Figure 2 Fermentation processes

Table 3 Optimal productivity of protein-rich nutrients

	Total Yield (t/ha)	Protein Content %	Protein Yield t/ha
Soya	1.8	34	0.6
Colza	3.0	23.3	0.7
Sunflower	2.5	22	0.6
Horse bean	3.2	28	0.9
Peas	3.0	25	0.75
Protein-enriched cassava	9.0*	20	1.8

* Cassava: 40 t/ha; water content: 70%
Loss of dry weight in fermentation: 25%

the productivity per hectare of various protein-rich plants with cassava cultivated for the purpose of enrichment. It seems clear that enriched cassava is the winner in this comparison.

For technical reasons, experiments on protein-enriched nutrients have not advanced as much as technological studies. However, preliminary experiments carried out on rats and chickens make it possible to affirm:

— total absence of acute toxicity of the fermented products;

— good assimilation of synthesized proteins with total absence of anti-nutritional factors.

Conclusion

As we have pointed out, the principal sources of potentially available starch are cassava in tropical regions and potatoes in temperate countries. However, other substrates with high development potential also exist in large quantities: for example fecula pulp, banana refuse.

It is evident that the competitive viability of producing PEFF depends on investment and operating costs. It also depends on how products are introduced, given that their introduction in human nutrition would not be a real innovation in a large number of countries.

It would be premature to estimate the results of experiments which can only be evaluated realistically when pilot projects have been carried out in the field. However, the results obtained thus far indicate that the preparation of PEFF has a promising future.

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The Waterloo SCP process: direct conversion of cellulosic materials into proteinaceous foods

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Process rationale and outline

Waste lignocellulosic materials can be upgraded into proteinaceous animal or human foodstuff by the mass cultivation of micro-organisms on them. Previous technologies used indirect conversion by growing yeasts on liquid hydrolysates prepared from the solids. The Waterloo SCP Process uses a fungal organism, *Chaetomium cellulolyticum,* for the direct conversion of the cellulosic components of agricultural and forestry residues by solid-substrate fermentation. The non-carbon nutrient supplements are commercial fertilizer-grade chemicals and/or animal manure, another agricultural residue. Optimal fermentation conditions are in the raw [illegible] with a [illegible] and [illegible] rates of up to 0.25 per hour can be obtained, the highest for any known direct conversion SCP process. The solid-substrate basis coupled with low pH conditions allow contamination-free operation. An outline of the process is shown in Figure 1. The core as well as the optinal stages are identified.

Depending on the inherent recalicitrance of the raw material, a mild caustic pretreatment may be required and the pretreatment liquor is concurrently fermented. Typically, grain crop residues, such as straw and cornstover, require a 0.25 — 0.5 per cent weight per volume NaOH treatment at 121°C for 15 minutes. Certain preprocessed materials such as Kraft paper pulp mill sludge and conditions, typical particle size conditions are: up to 0.5 cm average diameter for dilute (1—3 per cent weight per volume) slurry systems and up to 5 cm for dense slurry (solid-state) systems.

Economics and marketing

The process has been successfully tested on a 1.3 cubic metre fermenter pilot unit under batch, repeated fed-batch, and continuous (chemostat) conditions. Preliminary feeding trials on mice, rats, poultry and sheep of products made from wheat straw, cornstover and paper-mill pulp sludge, have indicated good protein nutritional value comparable with soymeal, and no toxic or teratogenic effects. Sensitivity analyses of the process indicate that it is economically feasible for a wide range of industrial and semi-industrial scenarios in several countries, both developed and developing. To date, licensing rights for the use and sale of the Waterloo process have been contracted out to three organizations: Envivocon Ltd., Vancouver, Canada; Innotech Inc., Becanson, France and the Provincial government of Novi Sad, Yugoslavia. Other contracts are under negotiation.

Tables 1, 2 and 3 summarize the amino acid profile of the protein product and the profitability as DCFR (discount cash flow based on the selling price of soybean meal and normal industrial financing) for minimum