BIOCONVERSION OF STARCH INTO PROTEIN

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STARCHY SUBSTRATES

Starchy materials, more specifically cassava and sago in tropical regions, are of great interest owing to both their high productivity per unit area and excellent rate of conversion to microbial protein by a large number of fast growing micro-organisms.

For food production, the question arises if it is better to cultivate a protein-rich plant of relatively low productivity or a highly productive starch-rich plant, being settled that starch can be transformed into protein at an average rate of 25%. Table 1 clearly demonstrates that it is more promising, from the aspect of food supply, to cultivate starchy plants for supplying both calorie and protein.

LIQUID FERMENTATION

The problem is to ascertain which are the processes available for transforming starch into protein by microorganisms. Much work was carried out recently in this field, but available processes are essentially based on liquid fermentation technology. They can be classified into three groups:

1) Two-step processes - starch hydrolysis using amylolytic enzymes, amylase or amyloglucosidase, then yeast culture on hydrolysate; these processes require sterilisation and aseptic conditions.
2) One-step processes with two organisms - one amylolytic fungus and one yeast, known as the Symba Process; sterilisation and aseptic conditions are required too.
3) Direct growth of filamentous fungus to produce biomass; some processes are described that do not require

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and fermentation will also require slight modifications in terms of retention times.

As the fuel alcohol concept becomes more widespread, technological breakthroughs are bound to reduce the overall costs, hence making fuel alcohol an even more viable alternative to imported petroleum.

Appendix: Theoretical Yield Calculations for the Conversion of Starch into Ethanol

The stoichiometry of the conversion is as follows:

Starch polymer $\xrightarrow[\text{Enzymatic hydrolysis}]{+H_2O}$ n $C_6H_{12}O_6$ Glucose

$C_6H_{12}O_6 \xrightarrow{\text{Yeast fermentation}}$ 2 C_2H_5OH Ethanol + 2 CO_2

It can be seen that the hydrolysis of starch involves a nett weight gain due to the addition of one water molecule per glucose molecule released.

Theoretically therefore:

1) 1,000 kg starch give 1,111 kg glucose;
2) From stoichiometric relationships 180 g glucose is converted to 92 g of ethanol plus 88 g of carbon dioxide;
3) The specific density of ethanol is 0.79, hence 92 g of ethanol fills a volume of 116 ml;
4) From combining (1), (2) and (3) we obtain the following:

$$1{,}111 \text{ kg} \times \frac{92}{180} \times \frac{116}{92}$$

$$1{,}111 \text{ kg} \times \frac{116}{180} = 715.9 \text{ litres}$$

Theoretically, 715.9 litres of ethanol can be produced from a tonne of starch.

← le code barre doit être mis sur la 1ère page des articles

Table 1. Productivity of Some Foodcrops

Crop	Yield t/ha	Protein t/ha
Protein-rich plants		
Soybean	1.8	0.6
Sunflower	2.5	0.6
Horse bean	3.2	0.9
Pea	3.0	0.75
Rapeseed	3.0	0.7
Cereals		
Corn	6.0	1.9
Wheat	5.0	1.7
Starch plants		
Cassava[a]	12.0	3.0
Sago[b]	14.5	3.5

[a] Cassava potentiality is calculated on the basis of 40 t/ha of fresh roots

[b] Sago production is calculated on the basis of extensive cultivation, with spacing of 6 m between palms and a yield of 500 kg of crude meal per trunk after 8 years.

sterilisation and aseptic conditions. The most interesting feature is the facility of harvesting the biomass; on the other hand, filamentous fungi increase the viscosity of the liquid.

Technically, all these processes exist and are quite feasible. They provide biomass with high content in protein of excellent nutritional quality. However the protein cost is not competitive with conventional protein production because of the high capital investment and energy demand, and the low price of conventional protein; but this last point could be changed in the future if the protein shortage happens, or if palm oil competes with soya oil.

The liquid processes are mainly used in starch factories when it is important to treat the effluent in order to avoid too much pollution of the environment; in this case, the price of the substrate is negative.

SOLID FERMENTATION

Another promising technology is solid fermentation, so called in contrast with liquid fermentation. Solid fermentation in Southeast Asia is exemplified by many traditional fermentations, e.g. tempeh, ragi, koji. However, these fermentations do not increase the protein content, but rather improve acceptability, digestibility or flavour of the food.

Knowledge of solid fermentation is not as advanced as the liquid fermentation. Nevertheless, Hesseltine for tempeh processing and Stanton for cassava fermentation carried out very interesting and important researches in this field of solid fermentation. Since 1974 I have investigated at ORSTOM the possibility of low technology processes to enrich the protein content in starchy materials by direct growth of fungi in solid fermentation, in order to obtain a product containing not necessarily a very high protein content but enough to make it usable for animal feeding, viz. 15-20%.

At first, I fitted up a solid fermentation method of culture for studying microbial, physiological and biochemical aspects of the growth of fungi in such a solid fermentation. The principle of the technique is based on the homogeneous distribution of spores and mineral salts in the mass of the starchy material put in suitable form. The preparation of a porous granulated material with adequate pH, temperature and moisture content is essential to ensure good aeration and fast growth of mycelium all in the mass.

The coarsely ground raw material with 30-35% moisture is maintained at 70-80^{o}C for 10 minutes by gentle steaming in order to gelatinise starch granules; after cooling to 40^{o}C, this steamed substrate is mixed with water containing the inoculum of spores and mineral salts to 55% moisture content. For laboratory purposes we adopted a very simple incubator (Figure 1). This method has already been worked out with a variety of starchy materials, namely cassava, whole potatoe, potato waste from industrial fecula works and banana refuse. We have not tried sago yet, but I think it would be interesting to test this starchy material. The results are reported in Table 2.

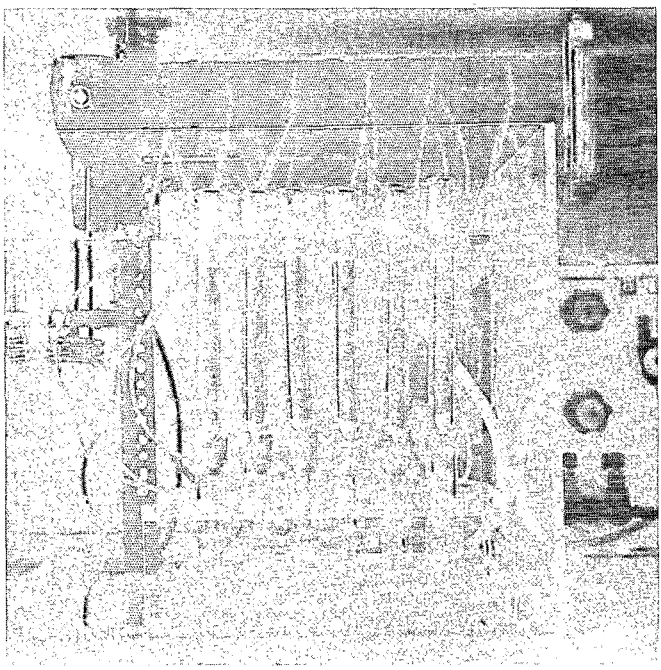

Figure 1. Laboratory Design for Solid State Fermentation

Experiments were performed with a selected strain of Aspergillus niger, having high amylolytic activity and suitable amino-acid composition. Many amylolytic fungi, particularly among strains used in Asian traditional fermentations for human consumption, were successfully tested by this technique with comparable results. This method does not require aseptic

Table 2. Protein Enrichment from Several Starchy Substrates

Substrate	Initial Product		Final Product	
	Protein	Carbohydrate	Protein	Carbohydrate
	g/100 g DM			
Cassava	2.5	90	18	30
Banana	6.4	80	20	25
Banana waste	6.5	72	17	33
Potato	5	90	20	35
Potato waste	5	65	18	28

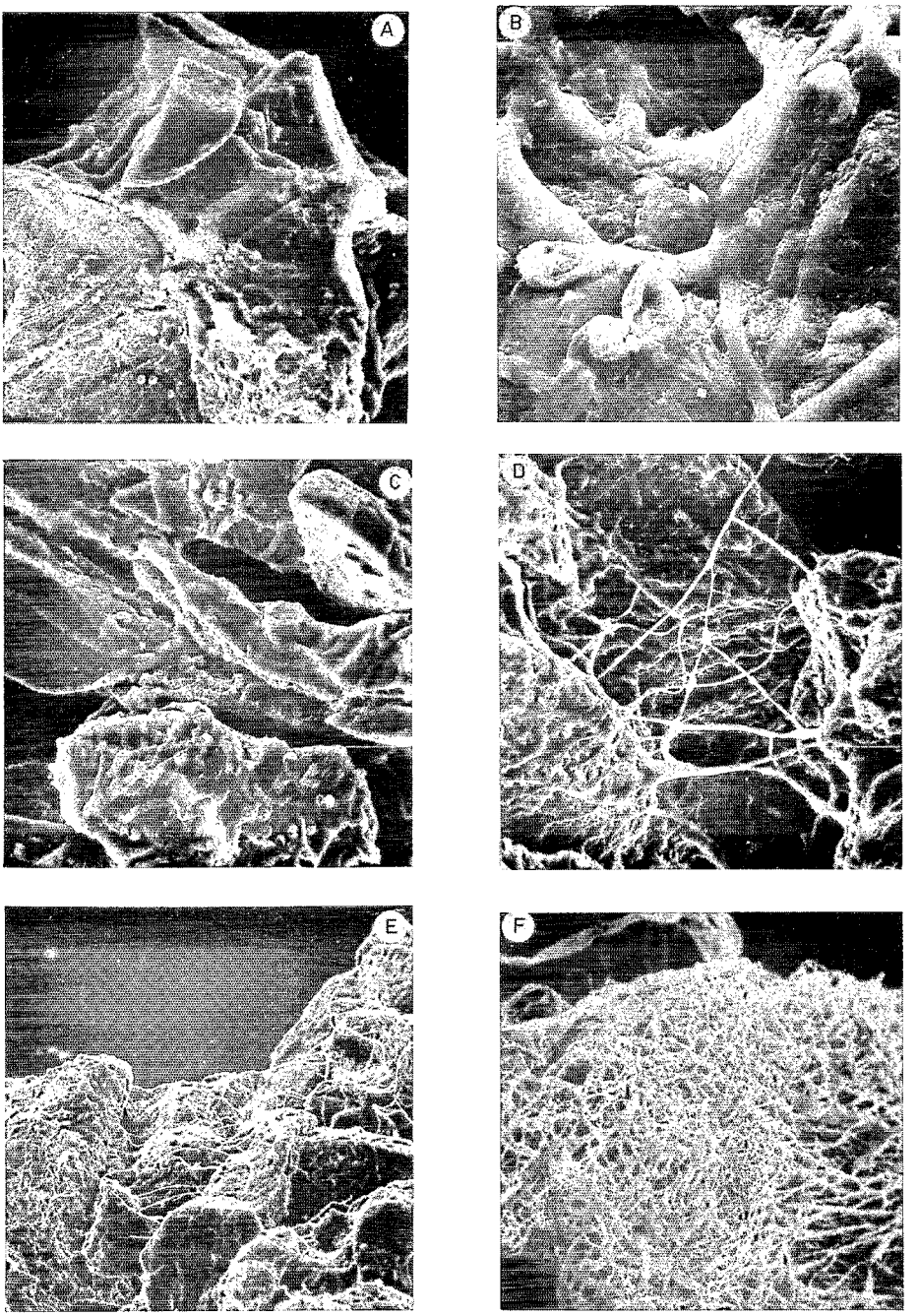

Figure 2. Growth of the Fungus in Starchy Substrate during Solid State Fermentation

A : t_o; B : t = 8 h; C : t = 12 h; D : t = 16 h; E : t = 20 h; F : t = 24 h.

conditions, selective growth of the fungus resulting from acidic pH, low moisture content and heavy spore inoculation.

I want to show you a series of photographs demonstrating the development of the fungus inside the starch substrate, with the aid of scanning electron microscopy (Figure 2).

From laboratory experimentation, equipment was designed for the solid state enrichment process at the pilot scale (Figure 3).

Figure 3. Pilot Scale Fermentor for Protein Enrichment by Solid Culture (200 kg DM capacity)

All the operations are conducted in a commercial bread-making blender modified for the purpose. Steaming and aeration are performed by passing steam or air through the perforated bottom of the tank. A simple control system using conventional probes was designed to keep suitable pH, moisture and temperature; this control system is activated by the temperature sensor as soon as temperature reaches the set point.

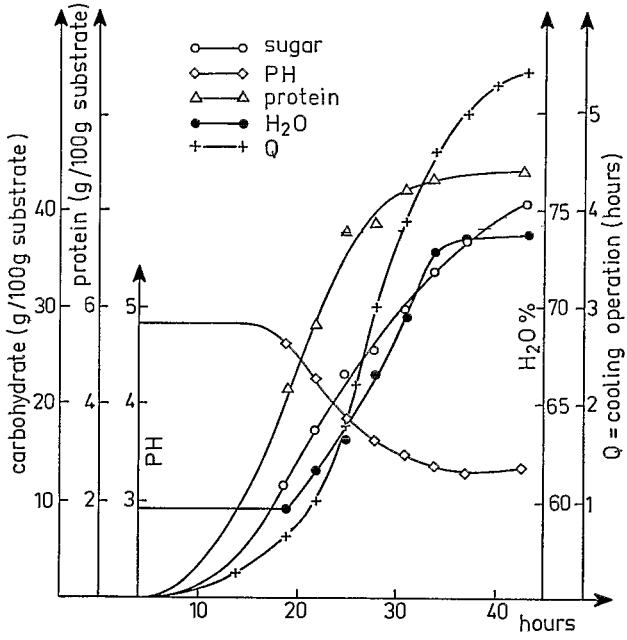

Figure 4. Kinetics Evolution during Solid State Fermentation of Potato Waste in Pilot Experimentation.

Figure 4 represents the kinetics of a fermentation on potato waste, with protein production, residual sugars and moisture. The curve marked by crosses is of special interest; it shows that during a fermentation of 30 hours, the mechanical mixing and spraying had to operate for five hours only, indicating a low expenditure of power, a fact of obvious importance in regard to production cost by solid state fermentation and to its economical feasibility at a low-level unit operation.

Presently a fermentor unit of one cubic metre, capacity 250 kg dry matter, is tested in a potato factory for protein enrichment of wastes. This equipment will be used for large scale nutritional and toxicological testing on target animals (pig, poultry) and for the determination of the actual investment and operation costs. It is intended that the experimentation will be extended to tropical countries, in order to adapt the procedure to local climatic and agroeconomic conditions and also to different potential starchy substrates like cassava, banana and sago for animal feeding purposes.

VALUE OF STARCH FERMENTATION

When we compare biomass production by liquid fermentation and protein enrichment by solid fermentation, we are surprised that the rate of conversion of starch into protein and the growth rate of the fungus are quite similar; the solid technology being very simple would have to be more competitive. Finally, starch is a very promising substrate for the future and starchy products will certainly become essential from the aspect of solar energy storage and fuel or food energy supply.

ORSTOM: Office de la Réchèrche Scientifique et Technique Outre-Mer (Overseas Scientific and Technical Research Bureau)

THE ROLE OF LACTOBACILLI IN STARCH ASSISTED FERMENTATION

YEOH Quee Lan

MICROORGANISMS

Microorganisms have long been employed by man for the preservation of raw materials for food. Especially in the tropics where the warm ambient conditions favour the rapid growth of microorganisms, man has to wage a constant battle to preserve his food supply against spoilage organisms. On the other hand, by manipulating the conditions of growth, it is possible to eliminate spoilage organisms and encourage the proliferation of the desired microflora. Food fermentation is not without its hazards and unless careful control of the fermentation conditions are maintained, problems of microbial food poisoning may result (Stanton and Yeoh 1978).

Lactic acid bacteria

Only a few species of microorganisms are important in food fermentations. The lactic acid bacteria are a group which plays a major role in many food fermentations. They may be found in the commensal flora of many habitats (Table 1), but their common characteristic is that they are able to produce lactic acid as the major end product.

The lactic acid bacteria are generally recognised as consisting of "Gram positive, non-sporing, carbohydrate fermenting lactic acid producers, acid tolerant, of non-aerobic habit and catalase negative, typically they are non-motile and do not reduce nitrate" (Ingram 1973).

Physiologically, the lactic acid bacteria can be divided into two subgroups defined by the products of glucose fermentation. All strains which produce 1.8 moles of lactic acid per mole of

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