~~Pilot~~ Plant for Single-Cell Protein Production

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A process for microbial protein production, using cassava as the energy substrate, was developed and tested at a laboratory scale at the University of Guelph. The microorganism used was the fungus *Aspergillus fumigatus* I-21A an asporogenous mutant that could grow under very selective conditions of temperature (45 °C) and pH (3.5). A pilot plant has been built at CIAT to test the technology developed, at a laboratory scale, and to produce a sufficient quantity of biomass for practical evaluation in animal feeding, notably in swine. Preliminary results obtained at the pilot plant are reported, suggesting a potential of the process once completely safe operational procedures can be established. A feeding trial with fungal biomass obtained at the pilot plant indicates that the product has a good nutritive quality if methionine is adequately supplemented.

Root crops including cassava (*Manihot esculenta*) are commonly grown throughout the tropics for food and contribute a considerable proportion of the total caloric intake of the human population (FAO 1973). Cassava has become the staple food of more than 200 million people throughout the tropics (Coursey and Haynes 1970).

The prospects for increasing cassava production in tropical areas are very promising, not only as a consequence of an increase in the area planted in cassava but notably as a result of improved technology, which suggests that drastic improvements in crop yield could be readily obtained by appropriate genetic selection and cultural practices (CIAT 1975, 1976).

Because pigs are efficient converters of the high energy content of cassava roots, the greatest possible increase in cassava utilization as an animal feed is most likely to occur in swine feeding. Extensive experimental information is available on the use of cassava roots in swine feeding.

The most important factor for determining the use of cassava as an animal feed is its price in relation to alternate energy sources and its dependence on the price of supplementary protein sources (Phillips 1974). Because of its low protein content as compared with cereals, any substitution of cassava (fresh, ensiled, or dried) for cereals in mixed feeds would be accompanied by an increased requirement of supplementary protein. Experimental data indicate that a life-cycle feeding program for swine based on the use of cassava meal or flour requires approximately 60–65% more protein supplement (soybean meal) than a similar feeding program based on common maize (Gómez et al. 1976). Therefore, the potential of cassava as an animal feed in the tropics will depend to a great extent on the availability of conventional protein or on the development of new protein sources.

Conventional protein sources such as fish meal and soybean meal, although being used increasingly for human nutrition, are becoming so high in price that their use in swine feeding will be restricted in the future. Other protein sources such as cottonseed meal are of limited use because of their toxic nature. In addition, in many cassava-producing areas it is difficult to grow other crops (i.e. soybeans) that will provide the protein required to balance the animal feeding programs adequately. The need to find alternate nonconventional feed proteins is becoming increasingly important.

The process for converting cassava into microbial protein is an attractive area of research for those cassava-producing areas where animal production — notably swine — could be significantly increased. The production of microbial protein from cassava would substantially upgrade the value of the feed and result in a nutritious product.

The existence of both a cassava program and a swine production unit at CIAT makes it especially advantageous to undertake a project for the production of a fungal protein on a pilot plant scale. CIAT has completed the construction of this pilot plant to study the different aspects involved in the production of fungal protein using cassava as a substrate. This work is being done in cooperation with the University of Guelph under the auspices of the International Development Research Centre of Canada.

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Fig. 1. The pilot plant used to produce microbial protein using cassava roots as the energy source at the CIAT swine unit.

The Pilot Plant Process

The pilot plant at CIAT was built during 1976 and began operating by early 1977 (Fig. 1). The following equipment has already been installed: a washer, a rasper, two self-aspirating fermentors (the starter and main fermentors with working capacities of 200 and 3000 litres, respectively) and a roller-press harvester. The first two machines, built in Colombia, are normally used in the starch factories found around the Cauca Valley. The two fermentors and the biomass harvester were designed and built at the University of Guelph. The characteristics of the fermentors have been described elsewhere (Azi et al. 1975). A single-cell protein (SCP) laboratory has also been allocated and equipped in a locale adjacent to the pilot plant. A Microferm, 10-litre bench-scale fermentor (New Brunswick Scientific Co., New Brunswick, N.J.), designed for batch fermentations and continuous culture of microorganisms, was installed in the SCP laboratory. In addition, accessory facilities consisting of racks and wooden trays for sun and air drying of the biomass are located in an area adjacent to the pilot plant.

A detailed description of the basic aspects of the process was given by Reade and Gregory (1975). The process was designed to operate with a minimum of instrumentation. The parameters for monitoring culture growth are temperature, pH, and dissolved oxygen. Although these parameters would not necessarily be required in practical production units, they facilitate research in that they confirm experimental information obtained on a laboratory scale at the University of Guelph. Both fermentors were provided with side openings for the insertion of instrument probes, which are controlled by means of a master switch box. The composition and preparation of the medium for the laboratory, the 200, and 3000 litre fermentors are basically the same as previously described (Reade and Gregory 1975).

The pilot plant process starts with either fresh cassava roots or cassava meal or flour. When fresh roots are used, they are washed to remove the soil and sand clinging to the outside. Next, the whole roots including the peel

are rasped to break open the cell walls to facilitate the suspension of the starch granules in the fermentation medium. The rasped cassava is then transferred to the fermentor, which is then half filled with water previously heated to about 70 °C by the passage of steam through a heat exchanger; in the case of the large (main) fermentor, a hoist and bucket arrangement is used to lift the rasped cassava. The high temperature of 70 °C needs to be maintained for about 10 min to gelatinize the starch and prevent the development of fungistatic activity in the mash (Reade and Gregory 1975; Gregory et al. 1976). More water is added to the tank to bring the fermentor almost to its full operating volume, as well as to lower the temperature of the fermentation medium to about 46–47 °C. The remaining ingredients necessary to complete the adequate nutrient supply for optimal growth of the microorganisms are urea and monopotassium phosphate, which are added to the medium while stirring. Sulfuric acid (9 *N*) is then used to bring the initial pH of the medium to 3.5. The fermentor is now ready for inoculation of the microorganism. Fermentation is usually completed within 20 h; temperature is maintained throughout the fermentation period by means of a temperature controller, which actuates a solenoid-controlled water valve to regulate the flow of cooling water at ambient temperature. At the end of the fermentation period, the biomass is harvested and can be fed fresh or sun/air dried to be subsequently incorporated into composite diets for animal feeding (Fig. 2).

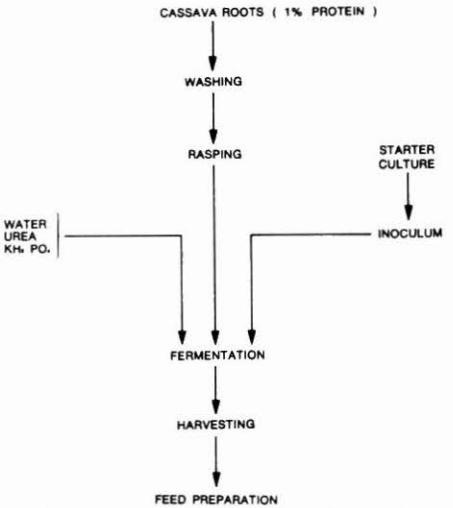

Fig. 2. Flow diagram of cassava single-cell protein fermentation.

Standardization of the process was done with the 200-litre fermentor using either fresh cassava roots or cassava meal. Because people working in the pilot plant might be allergic to or infected by spores from revertants of the asporogenous *Aspergillus fumigatus* I-21A or by hyphal fragments (Sidransky 1975), special safety precautions have been taken so preliminary observations, as well as the work under way, are being obtained with the 200-litre fermentor. Use of the 3000-litre fermentor awaits better defined safety precautions. from a microbiological aspect (Gregory 1977), as well as from experimental results at CIAT's pilot plant.

Preliminary Results

The microorganism used was *Aspergillus fumigatus* I-21A (ATCC 32722) (Reade and Gregory 1975). This fungus is an asporogenous mutant; therefore, the problem of aspergillosis (inhalation of spores) is practically eliminated or significantly reduced. Although a biomass harvester is now installed in the pilot plant, the information presented herein was obtained without the use of this machine; the harvesting of the final biomass was performed by emptying the contents of the fermentation tank into burlap sacks and squeezing it to remove the water, first manually and then with a wine press to obtain a partially dried product, which was placed on wooden-framed trays for further drying by exposure to sun and air.

Average data from fermentations with the 200-litre tank, using either fresh chopped cassava roots or cassava meal or flour as the substrates, are shown in Table 1. The amount of either fresh roots or cassava meal used in each fermentation was determined by the content of total carbohydrates of the substrate so as to obtain an initial carbohydrate concentration in the fermentation medium of approximately 4% (w/v). The yield of the dried biomass was similar for both substrates when expressed on a dry matter basis. The crude protein content of the final dried product was about 28%, which is lower than that reported for laboratory results (Reade and Gregory 1975; Gregory et al.

Table 1. Results of fungal protein (*Aspergillus fumigatus* I-21A) production in a 200-litre fermentor using fresh roots or cassava meal as substrates..

Fresh cassava roots[1]	
Amt. of cassava mash (kg)	25.3
Amt. of sun-dried biomass obtained (kg)	4.4
Product yield (g/litre)	22.2
Yield: weight of dried biomass in relation to	
Fresh cassava (%)	16.9
Cassava, dry matter basis (%)	48.5
Crude protein content in dried biomass (%)	28.6
Cassava meal[2]	
Amt. of cassava meal (kg)	11.5
Amt. of sun-dried biomass obtained (kg)	5.4
Product yield (g/litre)	27.0
Yield: weight of dried biomass to cassava meal (%)	47.0
Crude protein content in dried biomass	28.2

[1]Mean of 10 fermentations.
[2]Mean of 5 fermentations.

1976). The biomass, when water was partially extracted with a wine press, was dried easily when exposed to sun and air; the material became dark and hard when dried in an oven.

A biological evaluation with growing rats was performed to ascertain the nutritive quality of the total or crude protein content of the dried biomass resulting from fermentations with either fresh roots or cassava meal as substrates. Since this fungal protein has been reported (Gregory et al. 1977) to be deficient in sulfur-containing amino acids — notably methionine — the effect of the addition of this amino acid was also studied. Table 2 presents the experimental results obtained with growing rats. Total weight gains over a 28-day experimental period were very poor for the diets based on the unsupplemented biomass; methionine supplementation significantly improved the protein quality of the fungal protein, resulting in body weight gains similar to those obtained with casein and superior to soybean meal protein. PERs (protein efficiency ratio: g body gain/g protein consumed) were adjusted so that standard casein was used as a reference with a value of 2.5; methionine-supplemented microbial protein exhibited adjusted PER values similar to those for casein.

Because of the biohazard for the personnel working at the pilot plant, with regard to aspergillosis derived either from inhalation of revertants producing spores or from hyphal fragments (Sydransky 1975) carried in the aerosols formed at harvesting (Gregory 1977), special safety precautions were taken to reduce risks to a minimum. For these reasons and until completely safe conditions can be assured for the personnel, the fermentation will be carried out in the 200-litre fermentor. There are several aspects that need to be studied with the starter fermentor before progress can be obtained to the extent of using the 3000-litre fermentor. However, despite the present uncertainties, especially as regards safety aspects, the process seems to be very promising for practical application in cassava-producing areas to solve partially the increasing demand for protein supplements for cassava feeding programs, notably for swine.

Table 2. Effect of methionine supplementation on the protein quality of fungal biomass grown on a cassava medium and fed to rats (avg. for 10 male rats per group; 28-day experimental period; avg. initial weight 41.2 ± 2.1 g)

			Biomass produced on			
			Fresh cassava		Cassava meal	
	Control casein	Soybean meal	+ 0.3% methionine	without methionine	+ 0.3% methionine	without methionine
Total feed intake (g)	302.6[a]	308.8[a]	296.0[a]	195.6[b]	323.7[a]	198.8[b]
Total weight gain (g)	78.2[a]	68.2[b]	74.8[a]	24.2[c]	85.0[a]	29.7[c]
Feed/gain	3.9[c]	4.5[c]	4.0[c]	8.5[a]	3.8[c]	6.9[b]
Absolute PER	2.6	2.3	2.5	1.2	2.6	1.5
Adjusted PER (for standard casein 2.5)	2.5[a]	2.2[b]	2.5[a]	1.2[c]	2.5[a]	1.5[c]

NOTE: values with a common superscript are not significantly different.

BIBLIOGRAPHY

Azi, F.A., A. G. Meiering, C.L. Duitschaever and A.E. Reade. 1975. Air injection by self-aspirating impeller in aerobic fermentation. J. Milk Food Technol. 38,94-99.

CIAT. 1975. Cassava production systems. Annual Report. CIAT, Cali, Colombia.

CIAT. 1976. Cassava production systems. Annual Report. CIAT, Cali, Colombia.

Coursey, D.G. and P.H. Haynes. 1970. Root crops and their potentital as food in the tropics. World Crops, 22,261-265.

FAO 1973. Production Yearbook, Vol. 27. Rome, Italy, FAO.

Gómez, G., C. Camacho and J.H. Maner. 1977. Utilization of cassava-based diets in swine feeding. In Cock, J., R. MacIntyre and M. Graham eds., Proceedings of the fourth symposium of the international society for tropical root crops; CIAT, Cali, Colombia, 1-7 August 1976. International Development Research Centre, Ottawa, IDRC-080e, 262-266.

Gregory, K.F. 1977. Cassava as a substrate for single-cell protein production: microbiological aspects. In Nestel, B., and M. Graham, eds., Cassava as animal feed: Proceedings of a workshop held at Guelph, 18-20 April 1977. International Development Research Centre, Ottawa. IDRC-095e, 72-78.

Gregory, K.F., A.E. Reade, G.L. Khor, J.C. Alexander, J.H. Lumsden and G. Losos. 1976. Conversion of carbohydrates to protein by high-temperature fungi. Food Technol. 30,30-35.

Gregory, K.F., A.E. Reade, J. Santos-Núñez, J.C. Alexander, R.E. Smith and S.J. MacLean. 1977. Further thermotolerant fungi for the conversion of cassava starch to protein. Anim. Feed. Sci. Technol. 2,7-19.

Phillips, T.P. 1974. Cassava utilization and potential markets. International Development Research Centre, Ottawa. IDRC-020e, 1-182.

Reade, A.E. and K.F. Gregory. 1975. High-temperature production of protein enriched feed from cassava by fungi. Appl. Microbiol. 30,897-904.

Sidransky, H. 1975. Experimental studies with aspergillosis. In Chick, E.W., A. Balows and M.L. Furcolow, eds. Opportunistic Fungal Infections. Proceedings of the 2nd International Conference. C.C. Thomas Publisher, Springfield, Illinois, 165-176.