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Workshop on Single-Cell Protein

Summary Report

Jakarta, Indonesia
February 1-5, 1983

Jointly sponsored by

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PREFACE

In 1982, Indonesia's Agency for the Assessment and Application of Technology (BPPT) invited the Board on Science and Technology for International Development (BOSTID) of the U.S. National Research Council to join it in sponsoring a workshop on single-cell protein (SCP). This workshop, held in Jakarta, Indonesia, February 1-5, 1983, reviewed Indonesian government plans to establish a rural pilot plant to produce single-cell protein from root crops, primarily cassava. Workshop participants included both Indonesian and U.S. scientists, engineers, economists, and administrators (see Appendix A to this report for a list of participants).

These discussions were one activity in a larger program of cooperation between BOSTID and the Indonesian government. Begun in 1968, the program has featured a series of workshops on food policy, industrial and technological research, natural resources, rural productivity, and manpower planning. BOSTID's participation has been supported in the context of a science and technology loan from the U.S. Agency for International Development (USAID) to the government of Indonesia. The program with BOSTID calls for two to three activities (panel discussions, workshops, seminars, or small advisory groups) to be organized each year.

ORGANIZATION OF THE WORKSHOP

Workshop chairman Suleman Wiriadijaja of BPPT stressed the importance of a visit by BOSTID and Indonesian participants to the site of the SCP demonstration project in Tulang Bawang, Sumatra. The group could thus gain a sharp perspective of both the human and geographical factors involved.

Accordingly, the 5-day workshop schedule began with a day in Jakarta in which Indonesian participants described the background and objectives of the SCP demonstration project, and BOSTID participants outlined briefly the state of the art in SCP production and utilization in the United States. Three days of field visits followed, including a 1-day trip to Sulusuban to see the Ethanol Biomass Energy Center, constructed with Japanese assistance, and a visit to Kabupaten Bantul near Yogyakarta to see food preparation and consumption practices

utilizing cornmeal and cassava. Key Indonesian participants in the workshop joined the field trips, creating a valuable opportunity for informal exchanges between the two groups.

The group had planned to visit the Tulang Bawang transmigration area in the province of Lampung, southern Sumatra, the site of the demonstration plant. Because of flooding, however, the group was only able to proceed as far as Sulusuban.

After the field trip, a half day of meetings was held in Jakarta. Participants discussed recommendations on SCP production in four working groups: (1) the production process, (2) engineering, (3) the use of SCP as animal feed, and (4) the use of SCP as human food. A brief summary of the recommendations are provided in Part Three of this report.

At the end of the site visits, the Minister of State for Research and Technology, B. J. Habibie, met with representatives of the BOSTID and Indonesian participants for a briefing on the findings of the workshop.

The workshop report was prepared by Rose Bannigan of the BOSTID staff using the papers written by the Indonesian and NRC workshop participants. The reports have been edited to eliminate duplication, but they accurately reflect the discussions. The final draft was reviewed and approved by the members of the NRC panel and the Indonesian steering committee. Sabra Bissette Ledent, BOSTID consultant, edited the report.

This workshop was jointly organized and sponsored by the Indonesian Agency for the Assessment and Application of Technology (BPPT) and the U.S. National Research Council.

Participants would like to acknowledge the valuable contribution of the workshop's steering committee to the fine arrangements for the workshop as well as the site visits to Sumatra and Yogyakarta. The steering committee was chaired by Ir. Wardiman Djojonegoro, Deputy Chairman for Administration, BPPT, and included the following:

Prof. Dr. R. Anggorodi
Dr. Indrawati Gandjar
Prof. Dr. K. H. Kho
Ir. H. D. Pusponegoro
Ir. Saraswati
Dr. Untung Iskandar

INTRODUCTION

BACKGROUND

Inadequate consumption of protein and Vitamin A has plagued Indonesia for many years. As of 1976, the average protein consumption of the Indonesian people was 43.7 grams per capita per day as compared to the 55 grams per day recommended by the food workshop sponsored by the NRC and LIPI in 1968. The total protein deficiency is growing each year because of Indonesia's high rate of population growth, estimated at 2.34 percent annually. Because increased production of conventional protein from agriculture, animals, or marine sources may not be able to satisfy the consumption needs of the growing population, protein produced from nonconventional sources such as agricultural wastes must be further developed. Examples of this protein include concentrated fish protein, leaf protein, or single-cell protein (SCP).

Among these nonconventional protein sources, SCP has a high potential to assist in the alleviation of protein deficiency. Compared to conventional protein sources, SCP has two advantages:

- A high protein content, 40-80 percent depending on the microbial species used in its preparation
- A high rate of production per unit time and the fact that it can be produced from agricultural wastes, cellulose, or starches.

Furthermore, SCP can be produced from renewable resources (e.g., root crops such as cassava, sweet potatoes), or other biomass or nonrenewable resources (e.g., hydrocarbon, petrochemical and industrial wastes).

SCP PILOT PROJECT

The Indonesian government is now making efforts to increase the incomes of farmers living in transmigration areas and add value to tubers grown in these areas. Accordingly, the Agency for the Assessment and Application of Technology (BPPT), in cooperation with other Indonesian institutions and at the request of Indonesia's Ministry of State for Research and Technology, is constructing a demonstration unit in a transmigration area in Tulang Bawang, North Lampung, Sumatra, that will produce SCP as a supplement for both animal and human food. This unit

is expected to be in operation by the middle of 1984, and will have the capacity to produce 1 ton of SCP per day with a content of 92 percent dry material and 40-50 percent protein. The Indonesian government is also constructing a microbiology laboratory in Serpong (near Jakarta) which is expected to be operational in 1983 will conduct R&D related to SCP production. It is anticipated that cassava will be the primary source of SCP, but the use of other raw materials is not excluded.

OBJECTIVES OF THE WORKSHOP

The considerable experience of the United States with fermentation processes led Indonesian colleagues to request BOSTID assistance in convening this workshop on single-cell protein to:

- Exchange knowledge and experience on the production and utilization of SCP, including its impacts on users' health
- Discuss the technical aspects of production at the SCP pilot plant in Lampung, as well as research and development to be conducted on SCP in Serpong.

The workshop also provided an exceptional opportunity to involve in the discussions BPPT staff and representatives of other ministries and agencies who have an interest in the project or play some role in its implementation. BOSTID's participation was intended not only to provide American experience but also to inject the perspective of outside views.

Workshop participants sought to identify steps that must be taken in order to introduce SCP as a protein source. Matters that require further study include:

- Research on microbes
  - -- Select microbes that can be utilized to develop SCP.
  - -- Determine isolation technology in tropical conditions.
- Production processes
  - -- Find technology suitable for remote or transmigration areas.
  - -- Develop optimal condition processes such as aeration, temperature, pH, agitation, and substrate concentration.
- Utilization of SCP
  - -- Determine level of SCP utilization for animal feeding and human consumption.
  - -- Define marketing of SCP as a supplement for animal feed and human food.

PART ONE

OPENING ADDRESS AND PRESENTATIONS

OPENING ADDRESS

B. J. Habibie
Minister of State for Research and Technology
Chairman, Agency for the Assessment and
Application of Technology (BPPT)

It is my pleasure to attend the opening of the Workshop on Single-Cell Protein and to share with you my thinking on this subject.

I would like to welcome the scientists from the National Research Council of the United States. BPPT has closely cooperated with the NRC on examining and solving problems related to the implementation of technology, as part of efforts to maximize the utilization of natural resources. This endeavor falls within an agreement signed on December 11, 1978, between the governments of the United States of America and the Republic of Indonesia to cooperate in the field of science and technology development.

INADEQUATE PROTEIN

This workshop is concerned with the very important problem of improving the diet of the Indonesian people for whom inadequate protein consumption has long been a serious problem. Lack of protein causes a serious imbalance in food intake which results in a weakened physical condition and impaired productivity. Because existing prolonged conditions of protein deficiency cannot even maintain the already low levels of productivity, an increase in protein consumption is essential to increase productivity.

The application of conventional technology to protein production--namely, production of protein from agriculture, animal husbandry, and fisheries--cannot satisfy the increasing demand for protein, let alone supplement the already inadequate level of protein consumption, and this problem is aggravated by the growing population. Protein can, however, also be produced by applying a relatively advanced technology based on several selected raw materials. One of these methods is the production of single-cell protein.

Although the technology for producing single-cell protein is relatively new and advanced, we must use this technology as a short-cut solution to increasing the supply of protein--both for human and animal consumption. Raw materials for single-cell protein production can be selected from renewable resources such as cassava and sweet potatoes or from nonrenewable resources such as industrial wastes (for example,

pulp and paper wastes, molasses, and whey). The use of a relatively advanced technology to improve standards of living of the rural poor should not pose a problem. Such technologies are not incompatible with standards of advanced knowledge.

Research and development on single-cell protein involves several sciences: microbiology, fermentation processes, engineering processes, and nutrition. Studies in these sciences should be conducted simultaneously because of their interdependency. In cooperation with other institutions, BPPT is constructing a microbiology laboratory at the PUSPIPTEK Science Center in Serpong and a production unit in Tulang Bawang, Lampung. Studies on microbiology and fermentation processes will be conducted in Serpong, and they will concentrate on the selection of microorganisms and on conditions for maximum production of protein by the selected microorganism.

The results of these studies will form the basis for engineering studies of a production unit in Tulang Bawang, which will utilize sweet potatoes and cassava produced by transmigrants in the area. The operations of this unit will produce the much-needed protein and will increase the incomes of transmigrant farmers by increasing the value added to raw materials they produce. Because a continuous supply of raw materials is crucial when the production unit is operational, an analysis of this subject, involving local farmers, should be started soon.

CONSUMPTION OF SINGLE-CELL PROTEIN

Another aspect of this subject is the consumption of single-cell protein, and several alternative consumption patterns will be considered. This manufactured protein can be consumed directly or indirectly, through cattle, poultry, livestock, or fish. In this respect, I wish to urge you to consider the best method of consumption that will maximize the value added to protein and at the same time not pose any harm to consumers.

Clearly, the production of single-cell protein involves an integrated investigation--from the study of a continuous supply of raw materials to research on selection of the best production process and studies of the consumption pattern. With an integrated approach, these studies will result in an optimal system of single-cell protein production.

I hope the deliberations by participants in this workshop will produce a concrete proposal on steps that should be taken in single-cell protein production in Indonesia, particularly in the next 5 years. I hope this workshop will be fruitful, and I declare the workshop open.

PRESENTATION

Utilization of Microorganisms for the Production of Foods, with Special Reference to Indonesia

Indrawati Gandjar*

INTRODUCTION

In the Oriental world--particularly China, Japan, and Southeast Asian countries--the art of applying microorganisms to convert unpalatable raw agricultural products into palatable foods and beverages dates back many ages. Even the use of molds to ferment wastes from agricultural products into food was practiced by the Javanese. The interaction of microorganisms with the physical environment resulted in spontaneously fermented products which were accepted for human consumption. Trial and error was then used to improve the fermentation process and to obtain better products (Hesseltine 1981).

In Indonesia, organic substrates are easily overgrown with microorganisms because of the relatively high ambient temperature, high humidity, and rich microbial flora of the environment. While the Japanese and Chinese developed soy sauces, miso, and natto, the Indonesians discovered another fungal fermentation of soybean that is of great potential importance in feeding the world of the future--"tempe kedele" or soybean tempe (Steinkraus 1979). It has been proven that many fermented products have a better nutritional quality than the raw material used as substrate. Many complex compounds are broken down or degraded into simpler ones which can be easily absorbed when consumed.

TRADITIONAL FERMENTED FOODS OF INDONESIA

Tempe from Legumes

Tempe (soybean tempe) is a product of the cotyledons of soybeans overgrown with mycelium of the mold Rhizopus. It forms a firm cake that can be cut in thin slices.

On the island of Java, however, other kinds of tempe are made from legume seeds other than soybeans, but they have the same consistency as soybeans (see Table 1). Studies related to nonsoybean tempe have been carried out on the fermentation processes, changes occurring in the

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substrate, analysis of the nutrients and amino acids used, and biological value (Gandjar et al. 1977, 1979, 1981). The results revealed that these kinds of tempe can be used as a substitute for soybean tempe, especially in less fertile areas where those legume seeds are abundantly available.

Before consumption, the tempe products are cooked, deep-fried, or roasted and used as side dishes with rice or other staple foods.

TABLE 1 Kinds of Tempe Made in Indonesia

Substrate	Local Name
Soybean (Glycine max)	Tempe kedelai
Velvet bean (Mucuna prueiens)	Tempe benguk
Jack bean (Canavalia ensiformis)	Tempe koro pedang
Winged bean (Psophocarpus tetragonolobus)	Tempe kecipir
Pigeon pea (Cajanus cajan)	Tempe gude
Wild tamarind (Leucaena leucocephala)	Tempe lamtoro
Peanut presscake	Tempe bungkil
Coconut presscake	Tempe bongkrek
Residue of the tahoo factory	Tempe gembus

SOURCE: Gandjar (1981).

Tempe from Agro-industrial Wastes

The mold Rhizopus also plays an important role in the following fermented products:

Tempe Gembus This tempe is made from the solid refuse of the tahoo factories and has a rather soft consistency with a specific taste. Rhizopus oligosporus and R. oryzae are the molds used for this tempe, which is relatively cheaper than soybean tempe.

Tempe Bungkil Kacang This product is prepared from peanut presscake fermented by R. oryzae. In Western Java, it is often known as "oncom hitam" or black oncom.

Tempe Bongkrek Coconut presscake is used to prepare this kind of tempe. A good tempe bongkrek is completely overgrown with mycelia of the mold Rhizopus oryzae. Since 1975, the Indonesian government has prohibited the manufacture of tempe bongkrek because of the high risk of poisoning from a toxin produced by the bacterium Pseudomonas cocovenenans. Efforts have been made to prevent bongkrek poisoning, but the method has not been popularized. This tempe is still manufactured illegally, not only because of its low price but also for its taste and flavor (Ko 1981).

Oncom

"Oncom" refers to fermented products made with molds from the genus Neurospora. The substrate is a residue. "Oncom kacang" is made from peanut presscake and "oncom tahoo" from the solid refuse of the tahoo factories. Oncom is the only fermented product known where Neurospora is the main organism. Both kinds of oncom are very popular in Western Java.

Tape

Starchy materials such as steamed rice or cassava are easily attacked by microorganisms. In the Southeast Asian countries, tape is made of rice or glutinous rice or cassava tubers. The steamed substrate is inoculated with powder ragi and fermented at room temperature (25°-30°C) for 2-3 days. The final product has a soft texture, sweet-acid taste, and mild alcoholic flavor. Tape from glutinous rice is similar to the Chinese "chin-niang" or "laochao" (Ko 1972, 1981).

The microorganisms in ragi tape (an inoculum) are: Amylomyces rouxii, formerly Clamydomucor oryzae (Ellis et al. 1976); Endomycopsis burtonii or Endomycopsis fibuliger (Ko 1972); Mucor indicus and Candida parapsilosis; and Saccharomycopsis fibuligera (Gandjar 1982). Cronk, et al. (1977) found that the mold Amylomyces rouxii can both hydrolyze starch and produce ethanol from glucose and maltose. At least 50 percent of the total starch is hydrolyzed. Because of the loss of total solids, the protein content may reach 16 percent on a dry solid basis. In one Indonesian study, Saccharomycopsis fibuligera with a strong amlolytic activity was found in ragi tape.

Tape is consumed without cooking or further processing. The microorganisms involved in the fermentation process are consumed "raw" or still alive without harming the consumer.

Brem Bali

This traditional beverage of the island of Bali is made daily by the village people. Brem Bali is used in holy ceremonies and has become a welcome drink for guests in many hotels.

If the fermentation time of glutinous rice tape is prolonged more than 3 days, the substrate becomes largely liquefied and the product obtained is an alcoholic juice. Generally, 5 days after inoculation the tape juice is separated from the substrate and placed in flasks or jars for clearing and aging. The aging period varies from 3 months to 1 year. The residue left after separation of the juice from the substrate is a mixture of carbohydrate and microbial cells. It is used without cooking as a snack.

One kilogram of steamed glutinous rice gives approximately 1 liter of tape juice, with an alcohol content of 6-14 percent. The kind of ragi tape used as inoculum determines the amount of juice produced. The inoculum contains the mold Mucor indicus and the yeast Candida parapsilosis (Gandjar et al. 1982).

Dadih

This fermented product, which is made from buffalo milk, is very popular in Western Sumatra (Padang and surroundings). To produce dadih, buffalo milk is poured into the cavity of bamboo stalks, covered with banana leaves, and kept for 2-3 days in a cool place. The product is white with the consistency of soft cheese, and has an aroma similar to that of yogurt.

In a study of dadih, all samples contained the yeast Geotrichum candidum. Other yeasts found were Candida tropicalis and C. parapsilosis, as well as lactic acid bacteria, but these identifications are not yet complete. Many of the isolates possess a strong lipolytic and proteolytic activity. Their role in clotting the milk is still under study.

The product is consumed shortly after the fermentation process is stopped. It is consumed raw with palm sugar syrup and steamed glutinous rice or mixed with ingredients and prepared as a side dish to be consumed with rice. Dadih is considered a delicacy and healthy food by those living in Western Sumatra. Similarly in this case, microorganisms, yeasts, and bacteria are consumed by man without harm.

SINGLE-CELL PROTEIN

Malnutrition, particularly protein malnutrition, exists in many regions of the world. Although microbiology may not be able to contribute much to the food crisis on a short-term basis, it can on a long-term basis make a significant contribution.

The term "single-cell protein" (SCP) was introduced at the Massachusetts Institute of Technology in May 1966 to avoid the terms "bacterial" and "microbial" which have unpleasant connotations. Although the term is a slight misnomer because all protein comes from single cells, it is now widely accepted (Porter 1974).

Substrates for SCP Production

Humphrey (1975) has distinguished three categories of the many possible substrates that can be used for SCP production (see Table 2):

1. Materials that have a high value as a source of energy or are derived from such material.
2. Materials that are essentially waste and should be recycled back into the ecosystem by some minimal, nonpolluting means.
3. Materials derived from plants and hence are a renewable resource.

Resources as well as financial economy must be considered in selecting a substrate for SCP production.

TABLE 2 Possible Substrates for SCP Production

Substrate	Classification
Natural gas	Energy source material
N-alkanes	" " "
Gas-oil	" " "
Methanol	" " "
Ethanol	" " "
Acetic acid material	Energy source or waste material
Bagasse	Waste material
Citrus waste	" "
Whey	" "
Sulfite waste liquor	" "
Molasses	" "
Animal manure	" "
Sewage	" "
Carbon dioxide	Waste or renewable source material
Starch	Renewable resource material
Sugar	" " "
Cellulose	" " "

SOURCE: Humphrey (1975).

Microorganisms for SCP

Since 1966, intensive research has been conducted to identify microorganisms--mycelial fungi, yeasts, bacteria, and algae--that can be selected for SCP production.

Among these, the yeasts have been known for a long time. They are used as a leavening and flavoring agent in breadmaking, as a dietary supplement in wartime, and as a research tool in the basic studies of enzymology, nutrition, and molecular biology (National Research Council 1979, Peppler 1967). Louis Pasteur unraveled the biological and biochemical nature of yeasts from 1855 to 1875, and discovered that aerobic conditions stimulate yeast growth and repress alcohol formation. In 1868, the first factory to produce bakers' yeast as a primary product was established in Ohio (USA). Eventually, strain improvement and artful processing techniques led to compressed yeast with a long shelf life.

SCP processes are now utilized on a commercial scale, largely employing cane molasses or starchy material, especially cassava. The microorganisms used are: Candida utilis, C. tropicalis, Rhodotorula gracilis, R. pilimanae, R. rubra, Saccharomycopsis fibuligera, Kluyveromyces fragilis, and Saccharomyces cerevisiae. Azoulay, et al. (1981) discovered a strain of Candida tropicalis that possesses the enzyme alpha-amylase and can be grown directly on corn or cassava powder so that the resulting mixture of biomass and residual corn or cassava contains about 20 percent protein. This leads directly to a balanced diet for either animal fodder or human food.

The fermentation process produces heat, which must be removed to maintain growth of the yeast. The cooling water temperature should be at least 10°C lower than the growth temperature of the microorganism. The power cost can be a significant factor in the total energy costs (National Research Council 1979).

PROSPECTS FOR SCP IN INDONESIA

The Indonesian government is aware of the need for a novel protein source, and the BPPT is responsible for initiating the production of SCP within a short period of time. The microorganism used will be "imported" from abroad (BPPT-LIPI 1981).

Maintenance of the strain is very important, since the supplier is not responsible for changes in its characteristics after the microorganism leaves the place of origin. For that reason, among others, BPPT must provide a team of microbiologists to check the characteristics of the strain continuously even if the plant is not in operation.

Studies on new local isolates, especially thermophilic yeasts, should be conducted in the meantime. Until now, the use of microorganisms for human food application has been limited to Saccharomyces cerevisiae and Candida utilis. If they are to be consumed as a significant portion of the protein in the diet, however, they should be processed further to reduce nucleic acid content to below the levels that could lead to kidney stone formation or gout (National Research Council 1979).

Another protist that should receive attention is the group of procaryotic algae that grows well in open ponds and carries out photosynthesis. Spirulina, for example, has been used as food by the

Indians in Mexico for years, while Chlorella and Scenedemus have been used in Japan. Because sunlight is abundantly available in the tropics, the cultivation of algae in Indonesia may be a good prospect.

Since the Indonesian people have used microorganisms for so many years as a part of their daily menu, the introduction of SCP may not be difficult. More important, the cheap raw material is available in large quantities on a year-round basis to suppress the price of the final product.

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PRESENTATION

Production Process for Single-Cell Protein in Indonesia

Ir. Saraswati*

INTRODUCTION

About 60 percent of the populated areas of the world, including Indonesia, suffer from a protein deficiency. According to a workshop on food sponsored by the U.S. National Research Council and the Indonesian Institute of Sciences (LIPI) in 1968, the recommended protein for an Indonesian is 55 grams per day per capita. The average protein available per capita during the last 5 years, however, has been 43.7 g/day, resulting in a deficiency of 11.3 g/day per capita.

Many efforts have been made to fulfill the requirement for protein. The most common method has been increasing the amount of protein available from agriculture and animals, such as soybeans, meat, milk, and eggs. This method, however, faces the problems of the limited and narrowing availability of rich, arable land (soil) for producing protein from agriculture and fodder for feeding animals, and of abnormal climatic conditions.

Because of these problems, a number of nonconventional methods have been tried, including the use of microorganisms to produce single-cell protein (SCP). Some advantages to using SCP as a source of the conventional protein include:

- A faster growth rate than that realized for animal- or plant-produced protein
- High protein content (40-80 percent of cell)
- The capacity to use various carbon compounds for its growth and reproduction
- The capacity to be produced in a small vessel (fermenter). It does not need a large area and is not too dependent on climatic conditions.

Because of these advantages, much attention has been given to the production of single-cell protein, which can be used as a component of animal feed or of food for human consumption.

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UTILIZATION OF SINGLE-CELL PROTEIN

To fulfill the human protein requirement, single-cell protein can be utilized in animal feed to produce animal protein for human consumption or utilized directly in human foods. SCP can be used in animal feed by mixing it with the other components to bring about the necessary protein complement. Other methods include direct protein enrichment of the raw material or the use of solid-state fermentation as a substitute for the traditional complete food commonly used in animal feed. Converting the protein in the animal feed into protein in the produced meat of the animal is extremely inefficient. The ratio of protein intake to protein produced depends on the kind of animal (see Table 1).

The mass doubling times of a variety of microorganisms, plants, and animals when growing at their most rapid rates are shown in Table 2, while Table 3 presents this information in another way (according to Laskin 1977).

TABLE 1 Protein Conversion Efficiency Ratios

Animal	Ratio of Protein Intake to Protein Produced
Chicken (broilers)	4.2 : 1.0
Pig (pork)	6.5 : 1.0
Cattle (beef)	13.0 : 1.0

SOURCE: McLennan (1974).

TABLE 2 Mass Doubling Times of Some Organisms

Organism	Mass Doubling Time
Bacteria and yeast	20-120 minutes
Molds and algae	2-6 hours
Grass and some plants	1-2 weeks
Chickens	2-4 weeks
Hogs	4-6 weeks
Cattle	1-2 months

SOURCE: Laskin (1977).

TABLE 3 Pounds of Protein Produced Per Day by Selected Organisms

Organism (1,000 lbs.)	Protein Produced Per Day (lbs.)
Steer	1
Soybean	100
Yeast	32,000 to 1,000,000
Bacteria	100,000,000,000,000

SOURCE: Laskin (1977).

According to Tables 1-3, the potential protein produced by animals is very low compared to that of microorganisms, leading one to appreciate the direct utilization of single-cell protein for human foods, thereby bypassing animals. As human food, single-cell protein must be considered for its safety, nutrition, and acceptability. Several key safety problems in using single-cell protein for human foods (Litchfield 1979) are:

- High nucleic acid content of many microorganisms which would result in kidney stone formation or gout if they were consumed as a significant portion of a person's protein intake
- Poor digestibility and possible adverse gastrointestinal and skin reactions
- Possible presence of toxic or carcinogenic compounds from the residues of substrates, biosynthesis by the organisms, or chemical reactions during processing and drying.

Calloway (1974) and other scientists (Waslien et al. 1970) concluded that 2 g of nucleic acid per day per capita from microbial protein is the maximum quantity that can be consumed by humans without producing any severe side effects. Thus by reducing the nucleic acid content of single-cell protein, the daily intake of SCP can be increased in quantity. Improving the digestibility of SCP will increase its nutritional value.

The acceptability of SCP for human food also depends on other factors, including psychological, sociological, and organoleptic. In Indonesia, the psychological factor seems to have the greatest influence. Most Indonesians are reluctant to change their traditional foods or to accept unfamiliar, new foods. For example, Indonesia could produce protein from "bekicot" (a kind of snail), but because Indonesians do not like to eat bekicot, they export them. Indonesia is also a good source of worms which have a high protein content, but the acceptability of eating worms is very low.

Thus efforts to make single-cell protein acceptable for human consumption in Indonesia should take into consideration the familiar (traditional) local foods and eating habits. One possibility could

include using a SCP concentrate to enrich noodles, sago, corn and rice flour, cookies, etc. or directly enriching the raw material of food with single-cell protein. An example of the latter might be enriching "tiwul" or "gogik," a staple food of certain parts of Indonesia made of cassava flour, with protein by fermentation.

TECHNOLOGY OF SINGLE-CELL PROTEIN

The production of single-cell protein appears to be a practical solution to the protein deficiency found in Indonesia, and its basic technology is well known. To study the technology most suitable for SCP production in Indonesia, this country is establishing a single-cell protein pilot plant, which will be a side plant of an ethanol pilot plant (the raw material needed by the SCP pilot plant is the intermediate product of the ethanol pilot plant). Matters of concern in SCP production include: (1) raw materials, (2) microorganisms, (3) location, and (4) processing.

Raw Materials

Many kinds of raw material available in Indonesia can be used for SCP production, and they can be divided into two groups:

1. Nonrenewable resources such as hydrocarbons (n-alkane, methane, etc.) and petrochemicals (olefin, natural gas, etc.)
2. Renewable resources, such as materials that contain sugar (fruit juices, molasses, etc.); starch (root crops, sago, corn, rice, etc.); and cellulose (wood, pulp and paper wastes, etc.)

Although Indonesia has both kinds of resources, renewable resources are more suitable for SCP production because of the limited nature and high cost of nonrenewable resources and the great potential of renewable resources in Indonesia.

Of the various renewable raw materials in Indonesia, starch-based materials, particularly cassava, are the most suitable for SCP production. Cassava has the advantages of the ability to withstand drought and grow well in humid air, resistance to insects and diseases, and the capacity to grow well even in low-quality soil. In tropical areas cassava can be planted (it is sowed quite easily) or harvested at any time of the year, and the time of harvesting can be spaced over a few months after planting. With good planting methods, high yields can result (traditional planting: $\pm$ 12 tons per hectare).

The single-cell protein pilot plant in Tulang Bawang will use cassava as the raw material.

Microorganisms

The following microorganisms can be used for SCP production: bacteria (Bacillus, Aerobacter, Pseudomonas, Hydrogenomonas), yeasts (Candida,

Saccharomyces, Torulopsis, and Kluyveromyces), mucelial fungi (Aspergillus, Penicillium, Fusarium), and algae (Chlorella, Spirulina).

Yeasts are usually preferable as a source of nonconventional protein in commercial plants. In the United States, U.S. Food and Drug Administration regulations allow the use of dried yeasts--Saccharomyces cerevisiae, Kluyveromyces fragilis, and Candida utilis--in human foods. The composition of these yeasts is shown in Table 4; Table 5 gives information on their nutritive value.

TABLE 4 Proximate Composition of Three Yeasts Used in Human Food

Yeast	Protein (%)	Fat (%)	Ash (%)
Candida utilis	52	7.0	8.0
Kluyveromyces fragilis	54	1.0	9.0
Saccharomyces cerevisiae	53	6.3	7.3

SOURCE: Adapted from Litchfield (1979).

TABLE 5 Nutritive Value of Two Yeasts

Yeast	Biological Value	Digestibility	Net Protein Utilization
Candida utilis	32-48	85-88	27-42
Saccharomyces cerevisiae	52-87	70-90	36-78

SOURCE: Adapted from Chen and Peppler (1978).

In addition to the above data, the SCP pilot plant in Tulang Bawang will use Saccharomyces cerevisiae for the following reasons:

- The SCP pilot plant is located at the same site as the ethanol pilot plant, and use of the same microorganism for both plants will avoid intercontamination.
- Inoculum for both processes can be prepared in common vessels. This can result in a sizable saving in the purchase of equipment.
- The yeast resulting from the fermentation from ethanol can be harvested and dried together with the harvested yeast from the SCP pilot plant.

Although Saccharomyces cerevisiae will be used initially in the operation of the SCP pilot plant, the equipment in the plant is also designed for study of other yeasts.

Location

Tulang Bawang has been selected as the location of the single-cell protein pilot plant for the following reasons:

- It is easy to obtain the raw material in that area.
- Local farmers will be able to sell their crops (cassava) for a reasonable price.
- The pilot plant will provide new jobs in the transmigration area.
- Because the pilot plant is located at the same site as the ethanol pilot plant, both will use the same raw material and thus the same equipment: pretreatment equipment (peeler, washer, crusher), liquefaction equipment, and utility equipment.

Processing

The process for producing SCP from cassava (sweet potato) can be divided into five steps:

1. Pretreatment
2. Hydrolysis
3. Fermentation
4. Harvesting
5. Drying

Figure 1 shows the engineering flow chart of the SCP pilot plant in Tulang Bawang.

Pretreatment

For this process, fresh cassava is brought by conveyor belt to the peeler. The peeled cassava then goes to the washer, where it is washed with water. The cutter cuts the clean cassava into small pieces, which are then crushed into tiny particles by the rasper. This pretreatment equipment is the same kind commonly used to process starch (tapioca factory). The cassava slurry then flows into a tank where it is prepared for the liquefaction process. The acidity of the slurry is maintained at the optimum acidity level needed for the liquefaction process. The slurry is then mixed with an alpha-amylase enzyme and is now ready for the next process.

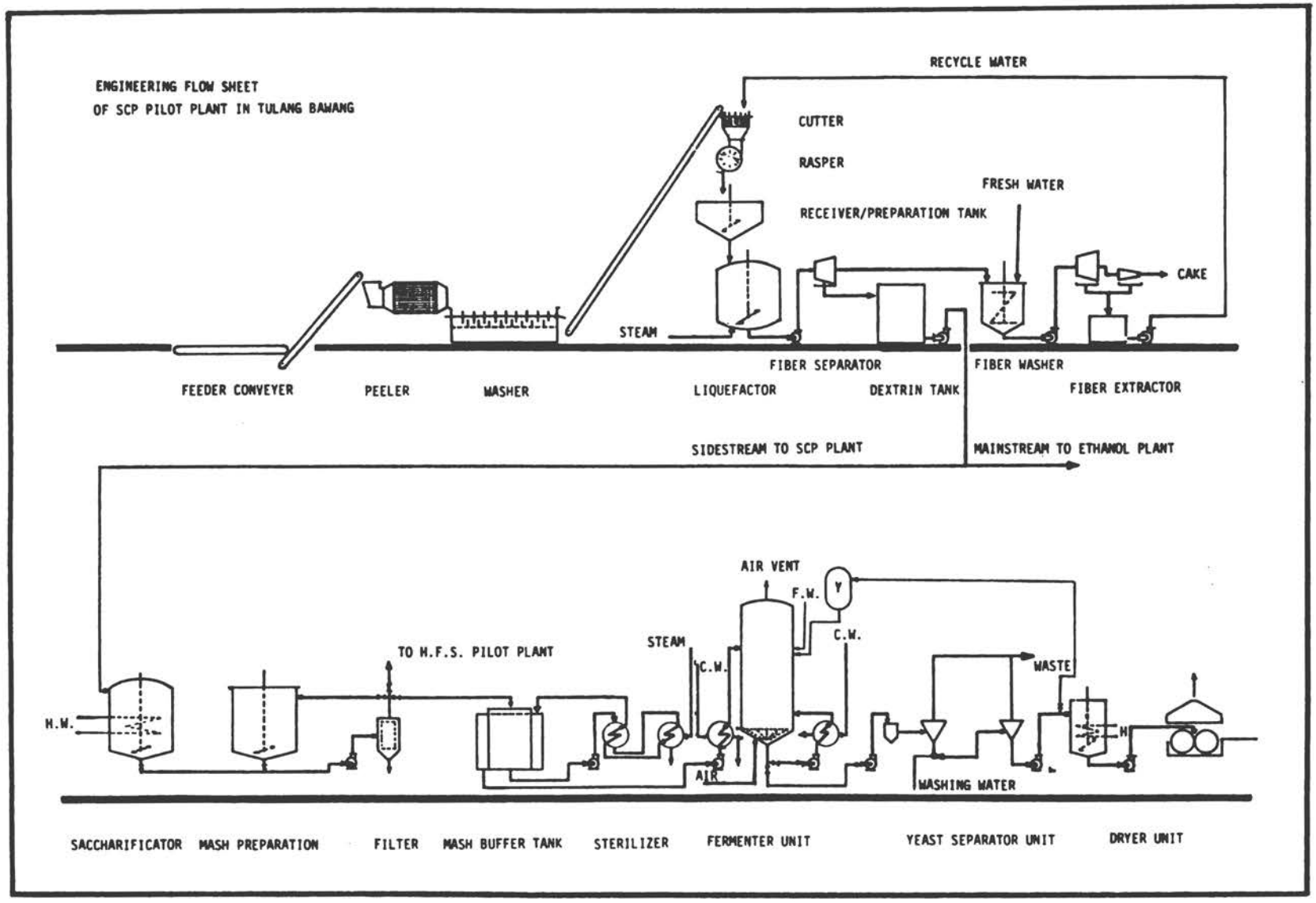

FIGURE 1 Engineering flow chart of SCP plant in Tulang Bawang.

Hydrolysis

The following equipment is used for the hydrolysis process: liquefactor, fiber separator, dextrin tank, fiber washer, fiber extractor, and saccharificator.

The starch slurry in the receiver tank is heated to the optimum temperature for the liquefaction process by using open steam. Starch is then converted into dextrin in the liquefactor. The dextrin slurry is pumped into the fiber separator where the fibers are removed. The resulting liquid runs into the dextrin tank while the fiber cake goes into the fiber washer. Because this fiber cake contains starch/dextrin, the cake is washed and separated in the fiber extractor. The thin slurry produced is recycled into the cutter, and the cake is removed. The dextrin slurry in the dextrin tank is used by the ethanol and SCP pilot plants. Part of the dextrin is pumped into the saccharificator, where the pH is established at 4.5 and the temperature is maintained at 60°C. In this total saccharification process, the enzyme amyloglucosidase is used. The glucose obtained, which is expected to be 95-98 percent DE (dextrose equivalent), is used for the fermentation process.

Fermentation Process

Equipment used for the fermentation process includes a mash preparation tank, filter, mash buffer tank, sterilizer, and fermenter unit.

The glucose from the saccharificator tank is pumped through a filter, and the filtered glucose flows into a mash preparation tank. In this tank, glucose is mixed with the nutrient, and the pH of the mash is established at 4.5, the optimum acidity for the fermentation process. The conditioned mash is then pumped through the filter again, and the mash flows into a mash buffer tank. This tank consists of an inner and outer tank. The mash from the filter first flows into the inner tank; the warmer mash flows into the outer tank.

The mash from the inner tank is pumped into the sterilizer, which consists of two plate heat exchangers. The mash (40°C) enters the first heat exchanger. It is heated by the mash that comes from the second heat exchanger. The outlet temperature of the heated mash is about 90°C. The heated mash then flows into the second heat exchanger, where it is heated again by saturated steam from four bars and its temperature increases to about 130°C. The heated mash is returned to the first heat exchanger, where it heats the remaining mash from the inner tank.

Finally, the sterilized mash is pumped into the heat exchanger for cooling using cold water. The cooled mash is used for the fermentation process. During this process, oxygen is required for growth of the yeast. An air filter sterilizes the air used for the aeration system, which uses perforated pipes. Approximately 3.5 kilocalories per gram of yeast solids (Reed and Peppler 1973) evolve during the aerobic growth of the yeast. Because this heat increases the temperature of the mash, the cooling process is needed to maintain the optimum

temperature for yeast growth. At the end of the fermentation process, the yeast slurry is pumped into the yeast separator unit for harvesting.

Harvesting

The yeast separator unit consists of a strainer and centrifuge. The yeast slurry from the fermenter is pumped into the strainer and then into one of the two centrifuges. The yeast cream obtained from the first centrifuge is injected with water to wash the yeast and is then placed in the second centrifuge. The yeast cream from the second centrifuge is pumped into the dryer unit, while the thin liquid of the first and second centrifuges goes into wastewater treatment.

Drying

The yeast cream first enters the preheater equipment. The heat shock treatment in this equipment reduces the nucleic acid content and improves protein digestibility. Although there are several methods for reducing nucleic acid content, heat shock treatment is the first method studied in this pilot plant. After this treatment, the yeast cream is dried in the drum dryer with the use of steam. The steam condensate can be used for the preheater process. The end product is a dry yeast solid with moisture of 8 percent.

Process for Enriching Cassava with Protein by Fermentation

As mentioned previously, a method has been developed for enriching cassava directly by fermentation. Figure 2 shows the flow chart of a process.

In this process, the crushed cassava from the rasper is divided into two parts. As one part of the cassava is pressed, the pressing water is used for dilution in the other part in the dilution tank. The diluted crushed cassava is then treated with heat to solubilize the starch. Finally, the wort is cooled to fermentation temperature.

The microorganism used for fermentation is Candida tropicalis, which can attack amylose and amylopectin directly. The yeast slurry resulting from the fermentation process is separated and concentrated using a centrifuge. The yeast cream is treated with heat and mixed with the pressed and rasped cassava and dried in the drying unit. The protein content in the final product depends on the ratio of pressed and rasped cassava to the fermented and rasped cassava.

Such an enrichment process could be applied in Indonesia by determining a method suitable for that country and by using microorganisms that are safe for human food (enrichment of tiwul could be considered).

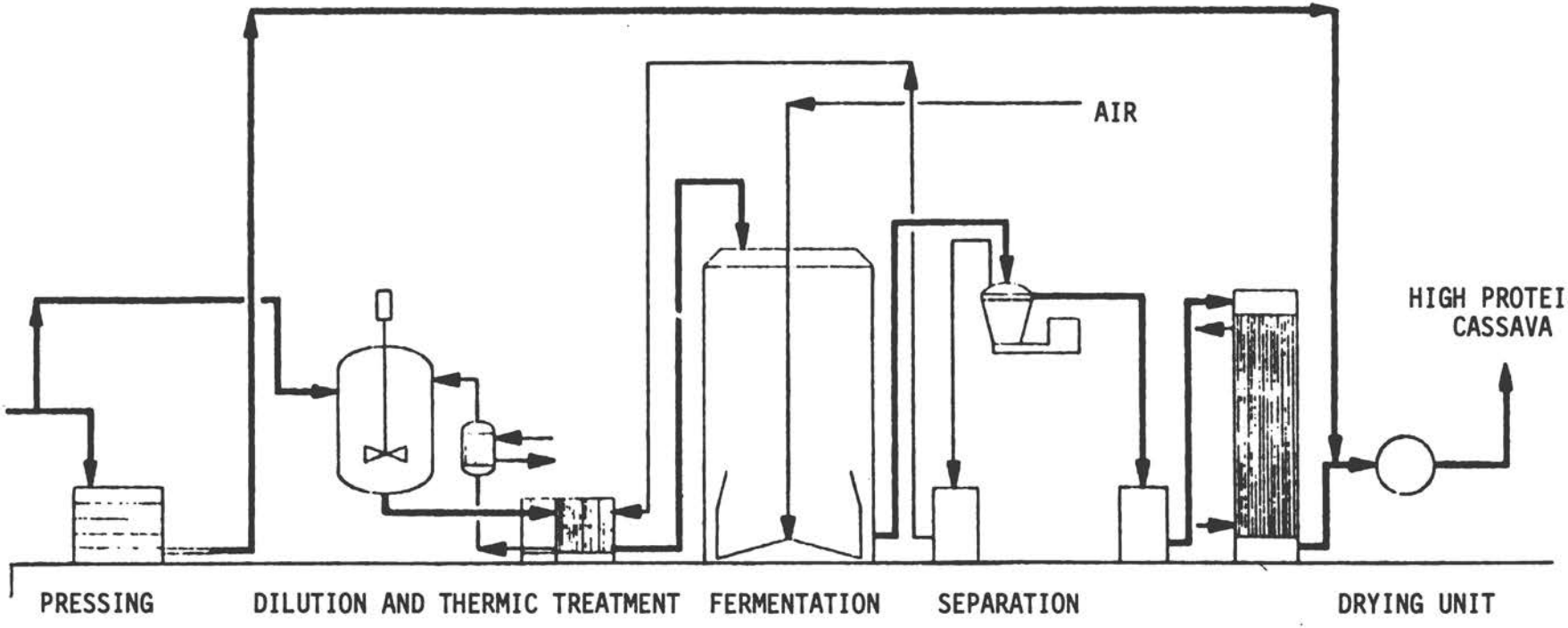

FIGURE 2 Process for enriching cassava with protein by fermentation.

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PART TWO

OBSERVATIONS AND COMMENTS

OF THE NRC PANEL

PRODUCTION OF SINGLE-CELL PROTEIN (SCP)

The NRC panel was asked to comment on the proposed Indonesian SCP project, and this report addresses this subject specifically. However, since the raw material for the alcohol demonstration plant being established in Tulang Bawang by the BPPT and the process of sugar formation used are identical with that used by the single-cell protein (SCP) demonstration plant, and since the yeast species utilized is also identical, frequent reference will be made to the alcohol (ethanol) project. This assumes particular importance because the alcohol demonstration plant is in place and in the final stages of completion. In this report the term "demonstration plant" has been substituted for pilot plant because of the considerable size of the alcohol plant and its use as a training center for technical and operating personnel.

RAW MATERIAL

The raw material to be used for both the alcohol plant and the SCP plant is cassava. The process at the cassava starch plant in Bandaragung, Central Lampung, Sumatra, consists basically of peeling, washing, rasping, and grinding the cassava, followed by centrifugal separation of the starch (continuous discharge centrifuges) and drying, first in a drum dryer to 40 percent moisture, followed by spray drying to 12.5 percent moisture. This starch would be an entirely suitable raw material for both ethanol and SCP production. Higher yields of fermentable sugar, however, may be obtained by the process outlined in the previous section by Ir. Saraswati in which the cassava starch is not completely separated from the fiber before saccharification (the formation of glucose sugar by enzymatic action on gelatinized starch). This results in higher yields of fermentable sugar and should be entirely suitable for SCP production, provided residual fiber is removed prior to media preparation.

The production of fuel ethanol from cassava is practiced in Brazil on an industrial scale. Indonesian scientists have visited these installations, and for this reason this report will, in general, not deal in detail with the extraction of cassava starch and its enzymatic conversion to sugar. Specific information on suggested enzyme uses and process variables is, however, given in Appendix B of this report.

In brief, this process consists of liquefaction (thinning of a viscous solution of gelatinized starch) and gelatinization (swelling and solubilization of starch granules at a temperature of 60°-70°C) of the starch followed by saccharification. The final sugar solution should contain at least 18 percent fermentable sugar for ethanol production and 18-25 percent for SCP production. For this purpose, starch slurries of 18-25 percent are used. The theoretical yield of glucose from starch is about 110 percent, and in practice a yield of 100 percent can be achieved (that is, 1 kg of starch will yield 1 kg of glucose). Gelatinization of the starch slurry at about 68°C makes the starch accessible to enzyme action. Since 25 percent solutions of starch are highly viscous, part of the heat-stable liquefying enzyme is added before heating so that the starch liquefies as it gelatinizes. This is followed by partial cooling and addition of a second portion of the liquefying enzyme and the saccharifying enzyme.

The gelatinization temperature of corn starch is quite similar to that of cassava starch. Thus a detailed description of the process of saccharification of corn starch (see MacAllister et al. 1975) should be applicable. A thorough study of the literature and visits to plants carrying out such saccharification are important particularly in connection with the plans of the Agency for the Assessment and Application of Technology (BPPT) for the production of high-fructose syrups.

Although it may be advisable to start the demonstration plants with purchased industrial enzymes, the enzymes could ultimately be produced more cheaply at the individual demonstration plants (for example, the Clinton, Iowa, USA, plant of Standard Brands is operated in this manner for the production of high-fructose syrups). It seems advisable, however, to concentrate at the outset of operations on the essentials of the process and to address subsidiary cost savings as experience is gained. The following companies are reputable producers of industrial amylases (starch liquefying and saccharifying enzymes): Rohm GmbH, Darmstadt, West Germany; Novo Industries, Copenhagen; Miles Laboratories, Elkhart, Indiana, USA; Kyowa Hakko Kogyo, Tokyo; Gist-Brocades, Delft, The Netherlands.

ALTERNATIVES TO CASSAVA

U.S. companies are considering two types of biomass as prime feedstocks: (1) trees and (2) corn stover, the residue after removal of grain. Other materials might offer advantages, but each has associated problems. For example, sugarcane grows in only a few states. Municipal solid waste may seem a vast resource, but it is diffuse and is a poor match to total needs.

Most interest has been in cellulose as a source of fermentable glucose for producing single-cell protein or ethanol. The economics are poor, however, if other components are wasted. The typical composition of wood is: cellulose, 45 percent by weight; hemicellulose, 25 percent; and lignin, 22 percent.

Processing of trees could begin with wood from existing forests. The present cost of wood chips in several areas of the United States is

$28 per U.S. ton (1 ton equals approximately 900 kg). The forests supplying biomass should be replanted with short-rotation coppicing species such as hybrid poplar which can supply about 30 tons per hectare per year on a drywood basis. These high yields and the ability to harvest the entire tree should lower the price. Likely products from refining biomass are shown in Table 1.

TABLE 1 Biomass Products

Product	Use	Approximate Value per ton
Cellulose	Paper pulp or derivatives for chemicals	$400-450
Lignin	Manufacture of adhesives	$500
Mixed sugars from hemicellulose	Cattle feeding	$ 80
Glucose from cellulose	Feeding	$130-150
SCP from glucose	Feeding	$200
Ethanol from glucose	Fuel	$300-350

The theoretical yield of ethanol from glucose is 0.48, so that the profit is only slightly greater when producing it by fermentation. The yield of SCP from glucose is roughly 0.25, resulting again in only a narrow margin for profit. The best value for cellulose is for paper or chemical purposes, but the quality must be high. Lower-quality cellulose could be converted profitably if lignin is a valuable by-product.

Several companies are attempting to commercialize processes for refining trees, and all hopes hinge on developing products from lignin (Bungay 1982, 1983). Various organic solvents such as ethanol, butanol, or phenol will extract lignin from woods. When conditions are acidic or alkaline, hemicellulose is simultaneously hydrolyzed to water-soluble sugars. The solid residue is cellulose for use in paper manufacture, but all of the organic solvent should be removed. A key factor in these extraction processes is economical recovery of the expensive solvents.

An alternative method uses no expensive organic materials. Wood chips are impregnated with steam, and sudden release of the pressure

disintegrates the structure. Hemicellulose hydrolyzes during the steaming and is easily washed from the residue with water.

A portion of the cellulose and lignin is used as a fermentation substrate to produce cellulase enzymes. The remaining material is contacted with these enzymes to yield glucose and a solid that is rich in lignin. The glucose is fully suitable for the production of SCP or ethanol. The main hurdle to commercialization of a steam-explosion process is the high cost of producing the enzyme. Fortunately, better microbial strains have been found that deliver much higher yields of enzymes, and demonstration factories may open in the United States in a few years.

Economics in the United States can, of course, differ greatly from those in Indonesia, where the initial emphasis on existing feedstock such as cassava appears wise. When the refining of cellulosic materials is commercialized, it will be easy to convert to these new feedstocks whenever it is appropriate. Profits should increase and new jobs requiring trained people created.

YEAST STRAINS

Bakers' compressed yeast, as it is produced in the United States and some European countries, is entirely suitable for the production of SCP. An active dry yeast sold by Gist-Brocades of Delft in Indonesia under the trade name "Fermipan" is also satisfactory. Since SCP may ultimately be used as food, however, it is recommended that a strain from a known culture collection be selected so that the strain will carry an identifying number and its origin can be identified. Examples of such culture collections include that of the U.S. Department of Agriculture at the Northern Regional Research Laboratory, Peoria, Illinois, USA (curator, Dr. Kurtzmann) or of the Bureau voor Schimmelcultures in Delft, The Netherlands.

AMYLOLYTIC STRAINS

Yeast strains that hydrolyze starch have been studied extensively and could be used to combine the saccharification and fermentation steps. This research is known in Indonesia and some plans are under way for using such strains in the future. An additional savings would be direct fermentation of cassava. This would be analogous to the EX-FERM process with sugarcane where the extraction steps are omitted (de Cabrera et al. 1982). Future research on cassava should attempt fermentation of crushed or chipped tubers. Initially, however, this should not have a high priority.

CULTURE DEVELOPMENT

Indonesian microbiologists often suffer from inadequate equipment, but, fortunately, it is relatively inexpensive to equip a microbiology

laboratory. Culture improvement is best performed by persons deeply committed to the project, and good culture development cannot be performed elsewhere for an Indonesian project. The potential rewards are great for developing a high-temperature strain or a flocculating strain for easy sedimentation.

It is recommended that a modest investment be made in apparatus and supplies for microbiologists associated with these projects. The equipment should also be used for teaching when not being used for research and development. Compared to other expenditures for this project, these investments have a very high probability of payoff. Furthermore, some very troublesome engineering problems may disappear if the microbiologists develop more suitable strains.

FERMENTATION

The production of bakers' yeast on sugar-containing molasses (beet or cane) has been described in several books, and this material has been made available to BPPT. A list of such books is found in Appendix C of this report. The fed-batch, aerobic process (addition of media to the fermenter throughout the fermentation in increments or continuously) is used worldwide and need not be reiterated in this report.

There are two minor but important differences, however, between the production of bakers' yeast on molasses for leavening bread and growing the same yeast on sugar syrup for use as a food or feed yeast: (1) substrate (raw material), and (2) a difference in the last stages of the fermentation.

Cassava Sugar Syrup for Media Preparation

The major carbon and energy source for yeast growth is sugar--glucose, fructose, sucrose, which can be used interchangeably. Other important nutrients, such as nitrogen in the form of ammonia and phosphorus, can be added in a similar manner. Cassava sugar syrup differs from sugarcane syrup significantly, however, in its content of (or lack of) nutrients such as calcium, magnesium, zinc, Vitamin B_1, pantothenic acid, and biotin, to mention only the most important ones. Thus the requirement for use of these nutrients in media preparation (liquid containing the cassava glucose syrup and added ingredients) must be determined on a laboratory scale using 5- to 15-liter fermenters, not on a larger scale. It was suggested that this work be done in the United States, and it probably could be completed within a 2- to 3-month period. The only alternative to this study is the random and massive addition of all required nutrients, which is wasteful and expensive.

Conduct of the Fermentation

As mentioned above, fed-batch fermentation is not described here because it requires a lengthy exposition, and because it is well

understood by BPPT engineers. The only required deviation is the following. In the production of bakers' yeast, the rate of media addition to the fermenter is reduced at the end of the fermentation to "mature" the yeast by reducing the number of budding cells. For the production of food or feed yeast, this is not necessary and the rate of media addition should be maintained until the end of the fermentation.

One important point concerning scale-up of a bakers' yeast fermentation from the laboratory to the pilot plant scale: such scale-up can be readily and confidently accomplished with regard to yeast strain, media composition, requirement for cooling, and composition of the final yeast and the waste effluent. The requirement for aeration of the fermenter and hence the rate of media addition can, however, only be estimated roughly and must be determined by trial and error (unless this information is already available for fermenters of identical size and configuration and for identical means of aeration).

Trial and error is carried out by using an exponential feed rate (doubling of the feed rate in each succeeding time interval) for the fermentation and by determining the point at which the yeast begins to form alcohol. This is the point at which the supply of oxygen becomes limiting and defines the maximum feed rate for this fermenter-aeration system, that is, the rate beyond which sugar is used for alcohol formation rather than for yeast growth. Thus the rate of feed for the production of food yeast must be somewhat less than at this critical point.

Product Recovery

Figure 1 on page 21 suggests the use of two nozzle centrifuges for preparation of a washed yeast cream. With approximately 5 percent yeast solids in the fermenter at the end of the fermentation period, the yeast cream should have 15-18 percent yeast solids. It is recommended that a pasteurizer (heat to 80°C) be used prior to drum drying. This results in better sheeting of the yeast coming off the drum dryer, and energy spent in preheating the yeast cream will be saved again in the drying operation.

The drum dryer is a very satisfactory method of drying. It results in a flaky end product with a minimum of fine dusty particles, and it imparts a more desirable toasted flavor. Other devices that may be used are spray dryers, belt tunnel dryers, or air lift dryers.

NOTE: One of the NRC panelists felt it would be unwise to select an old technology with a fermentation time of 48-96 hours. About 200 ethanol factories in Brazil complete fermentation in less than 10 hours. Obviously, it is more economical to produce faster because smaller vessels are used. Indonesia and Brazil have similar climates, so it must be possible to solve the cooling water problems. Admittedly, sugarcane and cassava have important differences, but the Brazilians have extensive experience with the former and some limited experience with the latter. Brazilian recipes and rapid fermentation techniques should be tested in Indonesia.

Contamination with pathogenic organisms is extremely rare up to the drying step, but particular care has to be used in sanitation of the dryer and protection of the dried product. Likely sources of contamination are the vent or vents carrying moisture from the dryer to the outside and portions of the dryer that do not dry the product to a maximum 7 percent moisture content (5 percent preferred).

Additionally, the means of conveying the yeast from the dryer to the packaging operation and the packaging operation itself are potential sources of contamination if any moisture is permitted to form in the equipment. The final product should conform to the following microbial specifications.

Total bacterial count	20,000/g
Coliforms	20/g
E. coli	neg.
Salmonella	neg.
Other pathogens (Staphylococcus aureus)	neg.
Molds and yeasts	50/g

The total bacterial count is a good indicator of sanitary operation, but some leeway is permissible since higher counts of lactic acid bacteria are generally harmless. Coliform counts are also a good indicator of sanitary operation, but high counts should not be tolerated. If necessary, the yeast cream can be treated with ClO_2 (chlorine dioxide) at 50 parts per million and at pH 4.5.

Sanitary operation of the entire plant and especially of the processing following fermentation is of utmost importance, since moist yeast adhering to equipment is one of the best growth media for pathogenic and spoilage organisms.

Equipment

The equipment shown in Figure 1, page 21, is entirely suitable for the process and represents the types that this panel would have chosen. (The panel is not familiar with the defibrinator and thus cannot comment on it or specifically on many of the various pieces of equipment shown since details on type of construction, construction materials, etc. are not available.) The equipment used for initial processing of the cassava roots, including the rasper, appears to be identical with that used by the cassava starch plant in Sumatra.

Alternatives to Centrifugation

Centrifuges are costly to purchase and expensive to operate because large motors consume power, and the bowl of a centrifuge must be cleaned frequently. For quite valuable products, centrifuges can be easily justified when the product is relatively high in solids. Alternative methods of collecting solids are filtration and sedimentation. Filtration is also expensive, and because microbial solids are often slimy, filter aid is required. Sedimentation is common in waste treatment plants because the cost is quite modest, but performance is usually much inferior to that of centrifugation and filtration in that the collected solids are very wet and gelatinous. A special method of sedimentation has been found to collect yeast cells quite well--lamellar sedimentation (see Walsh and Bungay 1979). This is not a well-known, established technique, but it could work very well for an ethanol or a SCP factory.

Old-fashioned sedimentation has long settling paths, and most microbial cells are collected only when very long detention times are employed. Lamellar sedimentation uses closely spaced plates so that a particle soon strikes a surface and is retained. A cross-flow design can be very efficient (Bungay and Millspaugh 1981). Furthermore, research has been performed with Saccharomyces cerevisiae, a yeast that does not tend to flocculate. Other yeast strains agglomerate to large particles that settle relatively rapidly. A lamellar settler should perform very well with a flocculated yeast strain.

Although it may not seem wise for Indonesian engineers to assign high priority to lamellar sedimentation now, they should stay alert to new developments. When the step is made from demonstration plants to large factories, lamellar sedimentation should be evaluated. There would probably have to be a thickening step for yeast solids collected by sedimentation.

Versatility of the Plant

Regarding the versatility of the plant, the scheme shown in Figure 1, page 21, may be used for the production of bakers' yeast (Saccharomyces cerevisiae) or Torula yeast (Candida utilis) from any sugary liquid such as molasses or hydrolyzed starches from cassava, sweet potatoes, sweet sorghum, or corn. It may not be entirely adequate, however, for the fermentation of whole corn mashes or crushed cassava as described in Ir. Saraswati's report. Pumping of heavy, viscous slurries may require the use of special positive displacement pumps, piping with a larger diameter, and use of a mechanical stirrer in the fermenter. (The latter may in turn require using a heavier gauge of stainless steel in the construction of the fermenter.) Thus consideration should be given to the degree of flexibility desired in the installation.

While the fermentation process will not be carried out under conditions of sterility, it will require use of stainless steel with the possible exception of the front end of the process up to the fiber extractor.

BY-PRODUCTS AND WASTE

Based on Figure 1, page 21, the by-products and effluents resulting from the SCP production process are:

- Cassava peel and cassava fiber (semisolid)
- Water for washing cassava
- Water for fiber extraction
- Fermenter effluent and washing water for yeast cream
- Water for cleaning equipment
- Waste from toilets and other facilities for operating personnel (all liquid)
- Carbon dioxide (gas).

It is assumed that the cassava peel and fiber can be used as cattle feed either as is or after drying. Disposal of the liquid waste streams will add a cost to the operation regardless of whether the waste is treated in a biological waste treatment facility or concentrated and used as cattle feed. It is important to choose the "least-cost" option based on a knowledge of the total volume of liquid effluent, its BOD (Biological Oxygen Demand), and its chemical composition and suitability as cattle feed or fertilizer.

A common mistake in the United States has been underdesigning the waste treatment section. For example, some fermentation plants found that a spate of contamination necessitated dumping entire fermenters, thus greatly overloading waste treatment. Objectional conditions persisted for prolonged periods, and the factory had to shut down or operate at reduced capacity because the environment could not accept so much pollution. The Indonesians should not repeat U.S. errors.

Some measures may be taken to minimize effluent volume. For instance, the scheme in Figure 1, page 21, includes recycling of the waste used for fiber extraction. It is recommended that consideration also be given to partial recycling of the liquid effluent from the yeast centrifuges to the fermenter.

A recent trend for both ethanol and single-cell protein production is recycling spent broths after removing the products. Although approximately 92-94 percent of these broths can be recycled, some must be bled to waste so that inhibitory products do not accumulate in the fermentation (Maiorella et al. 1983). The Indonesians appreciate the value of recycling in reducing the costs of waste treatment and the need for make-up water, and in savings by supplying nutrients and growth factors. This is, however, seen as a lower priority effort. Thus it is recommended that the Indonesians assign a higher priority to recycling. The economics are important, and the characteristics of the process can be affected drastically. If a process is developed with no recycling, it may not be easy to switch over. Efforts directed at incorporating recycling from the start would mean no extra time-consuming work to switch over.

USE OF SINGLE-CELL PROTEIN AS FEED FOR ANIMALS

Although a major portion of animal rations consists of conventional feedstuffs such as pasture herbage, hay, silage, grain, and protein supplements, animals also consume considerable amounts of unconventional feeds. Indeed, the feed manufacturing industry in the United States is based on utilization of by-product feeds based on protein oil meals (soybean, cottonseed, peanut meal, etc.), wheat bran, meat meal, corn gluten meal, molasses, distillers' dried grains, feather meal, blood meal, etc. Extensive use of these products has resulted in lower-cost feeds.

Large amounts of by-products which have valuable feedstuffs remain grossly underutilized. The intrinsic value and potential of crop residues (Klopfenstein 1980), animal waste (Fontenot 1980), and food processing residues (Satter et al. 1980) have been documented, and increased use is being made of these for feeding animals. Clearly, safety aspects must be considered when using these wastes and residues (Olson 1980), but with appropriate processing and precautions, these materials can be utilized without compromising animal and human health. Utilization of microbial or single-cell protein by animals is normal. For example, microbial yield from microbial protein synthesized in the rumen is sufficient for growth (Maynard et al. 1979). Furthermore, it is normal for animals to practice coprophagy (Fontenot 1980). The fecal excreta contains substantial amounts of microbial protein. Silage, which is preserved by fermentation, has been used as feed for centuries. Other feedstuff such as distillers' grains and brewers' grains contain the microbial residues from ethanol production.

SCP can be used for feeding all classes of economically important animals, including ruminants (cattle, water buffalo, sheep, goats) poultry, and fish. SCP would have the most value for poultry and fish, since protein quality is more critical for them than ruminants (Maynard et al. 1979).

SCP should be used chiefly as a protein supplement. It can be used to supplement low-protein forage for meat production from cattle, to supplement rations for dairy cows, and to replace conventional protein supplements for poultry and fish.

NUTRITIONAL VALUE

SCP is rich in protein but contains substantial levels of other nutrients. Crude protein in yeast has varied from 41 percent (Yoshida and Hoshii 1980) to 72 percent (Daghir and Abdul-Baki 1977), but there is no explanation for these large variations. The amino acid composition of the protein is fairly constant among samples of yeast protein. SCP is generally low in methionine, but values are comparable to those for soybean meal (Schacklady 1975). A comparison of the amino acid patterns of two kinds of yeasts, fish meal, and soybean meal is given in Table 2. As shown, the total sulfur amino acids are lower for yeast protein than for fish meal, but comparable to the levels for soybean meal.

The protein quality of SCP has been evaluated by feeding it to rats, poultry, and pigs. True digestibility of nitrogen was similar for rats fed diets supplemented with yeast or dried whole egg (Schacklady 1975). The net protein utilization (NPU) and biological value (BV), however, were lower for yeast than egg, but supplementation with 0.3 percent methionine increased the NPU and BV to levels comparable for dried whole egg. In young pigs, apparent digestibility of nitrogen was higher for a SCP diet than for a soybean meal diet (Slagle and Zimmerman 1979). In chicks, it was shown that methionine was the first and arginine the second most limiting amino acid when SCP was used as the sole source of dietary protein (Daghir and Sell 1982).

TABLE 2 Amino Acid Content of Yeasts, Fish Meal, and Soybean Meal (Grams per 16 g Nitrogen)

Amino Acid	Yeast G	Yeast L	Fish meal	Soybean Meal
Isoleucine	5.1	5.3	4.6	5.4
Leucine	7.4	7.8	7.3	7.7
Phenylalanine	4.3	4.8	4.0	5.1
Tyrosine	3.6	4.0	2.9	2.7
Threonine	4.9	5.4	4.2	4.0
Tryptophan	1.4	1.3	1.2	1.5
Valine	5.9	5.8	5.2	5.0
Arginine	5.1	5.0	5.0	7.7
Histidine	2.1	2.1	2.3	2.4
Lysine	7.4	7.8	7.0	6.5
Cystine	1.1	0.9	1.0	1.4
Methionine	1.8	1.6	2.6	1.4
Total S-acids	2.9	2.5	3.6	2.8

SOURCE: Adapted from Schacklady (1975).

SCP also has energy value. Digestible energy in young pigs was similar for diets containing SCP and those containing fish meal (Whittemore et al. 1976). Digestible, metabolizable, and net energy for a yeast SCP product in young pigs are 3.91, 3.65, and 1.79 kilocalories per gram of feed, respectively (Pearson et al. 1978). The values for digestible and metabolizable energy for soybean meal are 3.48 and 2.99 kcal/g, respectively (National Research Council 1979).

The amount of calcium in yeast has been shown to be relatively low, compared to phosphorus (Daghir and Abdul-Baki 1977). The calcium to phosphorus ratio is usually below 1:1, which may affect normal bone calcification. Selenium content may be low in SCP (Stagle and Zimmerman 1979).

ANIMAL PERFORMANCE

Because ruminants depend on microbial protein for a major portion of absorbed nitrogen, it is logical to assume that they could utilize substantial amounts of SCP. Yeast SCP has, in fact, been used as the sole source of protein supplement for young beef cattle (Schacklady 1975). Gross efficiency of growth of milk-fed calves was not altered by equal quantities of SCP and whey (Hinks 1978).

Rate of growth of chicks fed SCP as the only source of nitrogen was lower than for soybean meal controls even when methionine and arginine were supplemented (Daghir and Sell 1982). The difference appeared to be due to difference in feed intake since no difference was noted if the animals were par-fed. In research with natural diets, the powdery consistency of SCP tended to lower the rate and efficiency of gain in poultry (White and Balloun 1977). Pelleting the diet eliminated the effect, showing that no difference existed when the palatability factor was removed.

Replacement of up to 50 percent of soybean meal in pig diet with yeast SCP has not consistently altered performance (Tegbe and Zimmerman 1977, Zimmerman and Tegbe 1977). Response for daily gain and feed intake was maximized when the SCP level was 53 percent of the diet (Tegbe and Zimmerman 1977). There was a linear improvement in feed efficiency with replacement of SCP for soybean meal, indicating that palatability, not toxicity or nutrient imbalance, caused the reduced feed intake and rate of gain.

SAFETY

No deleterious effects have been recorded from feeding SCP to animals (Schacklady 1975). Feeding these materials at certain levels increases susceptibility to gout and urinary stones in man (Scrimshaw 1975), but no such effects have been noted in animals. Feeding of yeast grown in highly purified N-paraffins increases the N-paraffin in adipose tissue (Schacklady 1979). Levels higher than these, however, have been reported in grazing cattle.

POTENTIAL USE

Yeast SCP grown on cassava can be used for all classes of animals. SCP can supplement rice straw and low-protein forages for cattle, water buffaloes, sheep, and goats. If the animals are not fed supplemental concentrates, a vehicle for feeding SCP would be a problem. It could perhaps be mixed with a small amount of palatable feed such as molasses. SCP can also be substituted for conventional protein supplements in concentrate mixtures fed to finishing cattle and dairy cows and in rations for poultry and swine. Special preparation of the feed may be necessary to assure optimum consumption. SCP is also suitable for use in fish production.

USE OF SINGLE-CELL PROTEIN AS FOOD FOR HUMANS

Food production by using the currently available agricultural facilities may not adequately supply the earth's inhabitants. The scarcity of food may become even more acute if the present population growth rate is maintained, and it has been estimated that by the year 2011, the earth's population will have doubled (Kharatyan 1978). Single-cell organisms such as yeast, algae, bacteria, and fungi are rich in protein, vitamins, and minerals and may be used as nutrients to supply the expanding world's population. There are several advantages to the use of single-cell organisms as food (Kihlberg 1972):

1. Microorganisms have a short generation time and thus can provide large amounts of protein and other nutrients over a short period of time.
2. These microbes can be modified genetically by chemical and physical means, resulting in organisms with the required qualities.
3. The protein content of microorganisms is high. Even when a correction for nonprotein nitrogen-containing substances such as purines and pyrimidines is made, the protein content of single-cell organisms is higher than that of most common foods.
4. Single-cell organisms can be grown on raw materials which are available locally in large quantities, such as petroleum products or cassava.
5. SCP production can be carried out in continuous culture, independent of climatic changes. Relatively little land and water are required for the process.

These are strong arguments for supporting the development of facilities to produce single-cell protein for human consumption. There are, however, a number of potential problems when considering the use of microorganisms as food (Lipinsky and Litchfield 1974, Litchfield 1983).

SAFETY CONSIDERATIONS

Nucleic Acid Content

Single-cell organisms have a high nucleic acid content. Nucleic acids are metabolized to uric acid by humans, and this metabolite is excreted

unchanged in the urine. Ingestion of the large amounts of nucleic acids present in SCP elevates the blood concentration of uric acid because it accumulates more rapidly than the kidneys can excrete it. Although increased levels of blood uric acid over a short time may not be detrimental, elevated serum uric acid may result in uric acid crystalization and deposition of urate crystals in joints and kidneys (Wacker and Thorn 1966). The current recommendation for nucleic acid intake is 2.0 g or less per day (Protein Advisory Group 1972, Edozien et al. 1970). When this level is not exceeded, serum uric acid levels remain within normal limits.

Carcinogens

Concern has been voiced about the possible presence of carcinogens in single-cell proteins, particularly when the microorganisms have been grown on hydrocarbons. This possibility was considered at the single-cell protein symposium held in Milan, Italy in 1977 (Scrimshaw 1979). That workshop concluded that

> no protein source for animal feeding or for human consumption has been subjected to such thorough testing as has SCP. Feeding studies in many thousands of mice, rats, quail, dogs, chickens, swine, calves, and even monkeys in many different laboratories in Europe, Japan and the United States have been performed with no indication of carcinogenicity or toxicity. Multi-generation studies have reached 20 generations in rats and 30 generations in quail and there has been no evidence of any increase in the incidence of tumors, change in reproductive capacity or other abnormality.

Finally, studies have been performed to establish whether SCP could act as a cocarcinogen in animals by potentiating the effects of known carcinogens. In no case has any SCP tested indicated that there is a carcinogen, cocarcinogen, or toxin present in the material (Scrimshaw 1979).

Single-Cell Lipids

When compared with common dietary fats, the lipid from single-cell organisms grown on paraffins shows two abnormalities (Tomassi and Serlupi-Crescenzi 1979). First, there is a very high percentage of phospholipids with negligible amounts of triglycerides. Second, more than 50 percent of the fat is in the form of odd-chain fatty acids and not the normally occurring even-chain fatty acids. These odd-chain fatty acids are mainly of the 17-carbon atom series such as is found in Toprina^{R} SCP from Candida lipolytica grown on N-alkanes (Tomassi and Serlupi-Crescenzi 1979).

A U.N. Protein Advisory Group (PAG) symposium was convened in Brussels in 1974 to consider the safety of these fats found in microorganisms used as food. It concluded that odd-chain fatty acids were utilized through normal metabolic pathways and mobilized in the same way as even-carbon atom chain fatty acids, even when fed under conditions of fasting or stress (Scrimshaw 1979). Data presented at the Milan conference in 1977 confirmed that rat liver mitochondria utilized even- and odd-numbered carbon chain fatty acids at about the same rate. Moreover, liver and heart mitochondrion of animals fed Liquipron^{R}, a Candida maltosa yeast which contains odd-numbered carbon chain fatty acids, responded to a number of chemicals that affect metabolism in the same manner as do mitochondrion of control animals (Scrimshaw 1979). That symposium concluded that the increased amounts of odd-numbered carbon chain fatty acids found in some hydrocarbon-grown single-cell organisms do not appear to be associated with adverse side effects or toxicity when fed to experimental animals (Scrimshaw 1979, Tomassi and Serlupi-Crescenzi 1979, Bizzi et al. 1979).

Paraffins

Another concern of nutritionists and food scientists studying the use of SCP as an animal and human food has been the presence of paraffins found in some microorganisms grown on hydrocarbons. Paraffins are not found in large amounts in normal animal tissues. Studies of animals ingesting yeast containing paraffins have demonstrated that there was no adverse effect on the animals (Scrimshaw 1979, Schacklady 1979, Valfre et al. 1979). It has been noted, however, that paraffins accumulate in the adipose tissue of animals fed microorganisms grown on some hydrocarbons, but that this condition gradually clears when the feeding of single-cell protein contaminated with paraffin is stopped (Schacklady 1979, Valfre et al. 1979).

Paraffins are apparently used as energy in several microorganisms as indicated by recoveries of up to 65 percent of the paraffin carbons in respiratory carbon dioxide. Furthermore, it should be noted that these hydrocarbons are present in a variety of substances used in feeding animals including fish meal, hay, alfalfa, fermented milk, tomato peels, and clover. Commercially available foods have also been shown to contain paraffins: lard has 2-20 parts per million (ppm) and fat from cattle products, 2-200 ppm (Scrimshaw 1979).

Allergies

Studies at the Massachusetts Institute of Technology have found that 10-20 percent of human subjects ingesting between 10-20 g of SCP per day have adverse reactions. The most common are intestinal disturbances and skin rashes. Symptoms of gastrointestinal dysfunction include indigestion, increased gas, nausea, occasional vomiting, and diarrhea. The skin rash which occurs in other subjects generally involves the palms of the hands and soles of the feet. These surfaces

become slightly reddened and covered with small "papules" which clear when the SCP is stopped. These reactions have been noted to occur secondary to ingestion of a number of different single-cell proteins from a variety of organisms including Candida utilis grown on beet molasses, Candida utilis grown on sulfite liquor, Candida tropicalis grown on gas oil, and Candida lipolytica grown on N-alkanes. Fewer gastrointestinal and skin reactions were noted in subjects ingesting SCP from the microfungi Fusarium graminearum and Paedilomyces varioti.

NUTRITIVE VALUE

Several factors affect the nutritive value of SCP (Young and Scrimshaw 1975), including protein concentration, amino acid content and profile, and protein digestibility and availability. In addition, other factors may modify the clinical or nutritional response to the ingestion of a given food protein source.

The protein content of microbial cells has been compared with that of selected foods of animal and plant origin (Kihlberg 1972, Young and Scrimshaw 1975, Bressani and Elias 1968). Table 3 shows that the crude protein content of microbial cells is quite high in comparison to that of some of the usual foods.

The amino acid composition of a protein determines the protein's biological value as a source of nitrogen for growth and maintenance. Thus knowledge of a protein's amino acid content provides valuable information about its potential nutritional quality. Microorganisms have a well-balanced amino acid pattern (see Table 2, page 42) except for a low content of sulfur-amino acids (methionine). Metabolic studies at MIT have found that methionine supplementation of food-grade Torula yeast improves the protein nutritional value of the yeast (Young and Scrimshaw 1975). Young and Scrimshaw have also pointed out that wheat and other cereals are low in lysine, threonine, and tryptophan; the relatively high concentration of these amino acids in microorganisms suggests the value of SCP as a potential supplement to cereal-based diets.

Knowledge of the amino acid composition does not always accurately reflect the pattern of the physiologically available amino acids. The cell wall of microorganisms may partly prevent the utilization of cytoplasmic proteins and thus lower the overall availability of nitrogen for tissue protein metabolism. Furthermore, wall-bound amino acids are present in amounts quite different from those of the overall amino acid composition and may have low availability.

Determination of the biologic availability of a protein can help health and nutrition experts assess uses of a novel food. For example, it would not be appropriate to replace a protein in a food of excellent biologic availability such as milk or eggs with a SCP of poor nutritive value. On the other hand, a SCP of comparable nutritive value to milk or eggs may be considered as a substitute for these commonly accepted foods.

Evaluation of the nutritive value of a protein is commonly performed using nitrogen balance studies. The principle of these

TABLE 3 Nitrogen and Protein Content of Microbial Cells Compared with That of Selected Foods of Animal and Plant Origin

Source	Nitrogen (%)	Crude Protein[a] (%)
Filamentous fungi	5 - 8	31 - 50
Algae	7.5 - 10	47 - 63
Yeast	7.5 - 8.5	47 - 53
Bacteria	11.5 - 12.5	72 - 78
Milk	3.5 - 4.0	22 - 25
Beef	13 - 14.4	81 - 90
Egg	5.6	35
Rice	1.2 - 1.4	7.5 - 9.0
Wheat flour	1.6 - 2.2	9.8 - 13.5
Cornmeal	1.1 - 1.5	7.0 - 9.4

[a]N x 6.25.

SOURCE: Adapted from Kihlberg (1972), Young and Scrimshaw (1975), Bressani and Elias (1968).

studies is that proteins of high biological value are digested easily to amino acids and peptides and are absorbed efficiently by the intestinal tract. The proteins are balanced in essential and nonessential amino acids and are therefore retained by the body for synthetic processes. A protein of poor nutritive value will have decreased digestibility, absorption, and utilization. This results in an increase in wasted nitrogen with a fixed protein intake.

Nitrogen balance studies are performed by measuring the dietary nitrogen intake and output in urine and feces by direct analysis of suitable aliquots. Both the amount and quality of protein nitrogen absorbed are taken into account. In clinical trials, nitrogen balance data are used to calculate the digestibility and biologic value of a protein. A number of studies have been performed to assess the nutritive value of SCP, especially that of algal origin, and two conclusions have been drawn from these studies (Young and Scrimshaw 1975): (1) nitrogen balance can be maintained in human subjects using algae as the sole source of dietary protein, and (2) a low digestibility of the algae species is obtained when it is given in a form in which the cell walls have not been subjected to significant disruption. Table 4 is taken from a study by Waslien, et al. (1970) and reported by Young and Scrimshaw (1975). This table shows that the true digestibility of the two algal single-cell proteins was less than that of the control protein (casein supplemented with RNA). The biological value of the two algal proteins was, however, comparable to the casein control.

Litchfield (1983) notes that "the future prospects for large-scale SCP production for human food appear to be limited to use as protein supplements and functional protein ingredients rather than as primary sources of protein in human diets."

TABLE 4 Biological Value of Algae and Yeast at Two Levels of Nitrogen Intake Compared with That of Casein in Young Men

Protein Source	Nitrogen Intake (g/day)	True Digestibility (%)	Biological Value
Casein + RNA	4.25	95 ± 8	66 ± 4
	7.84	99 ± 1	52 ± 6
Chlorella sorokiniana	4.83	89 ± 4	79 ± 12
	7.81	82 ± 6	60 ± 6
Torulopsis utilis	4.51	83 ± 7	70 ± 5
	8.20	87 ± 3	58 ± 6

SOURCE: Adapted from Young and Scrimshaw (1975) and Waslien, et al. (1970).

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PART THREE

WORKING GROUP RECOMMENDATIONS

PRODUCTION PROCESSING

PREPARATION OF FERMENTABLE SUGARS FROM CASSAVA

It is recommended that

- Enzymes for liquefaction and saccharification of cassava starch be purchased but that plant space be assigned for eventual production of enzymes, liquefying alpha-amylase from Bacillus subtilis and saccharifying glucoamylase from Aspergillus niger.

- The National Research Council panelists supply specific recommendations for use of enzymes--pH levels, working cycle, temperatures, etc.--and that these recommendations be checked against those furnished by enzyme suppliers. Suitable enzyme suppliers include Rohm GmbH, Darmstadt, West Germany; Novo Industries, Copenhagen; Gist-Brocades, Delft, The Netherlands; Miles Laboratories, Elkhart, Indiana, USA; and Kyowa Hakko Kogyo, Tokyo.

- All plans for enzymatic cassava starch conversion be coordinated among the working teams for (1) alcohol production, (2) fructose syrup production, and (3) single-cell protein (SCP) production. This is important since this conversion step is common to all three processes (with very minor deviations).

MEDIA PREPARATION

It is recommended that

- The nutrient supplementation of cassava glucose syrup for SCP production--NH_3, P_2O_5, K, Ca, Mg, trace minerals, vitamins--be determined.

- Laboratory work (5- to 15-liter scale, no larger) be carried out on the required supplemental nutrients because the nutrient content of the above-mentioned substrates differs. The production of baker's yeast from molasses is practiced worldwide, and

the carbon and energy source--namely, fermentable sugars--is the same whether cassava or molasses is used. Work could be carried out in the United States if this seems desirable to the appropriate official of the Agency for the Assessment and Application of Technology (BPPT).

YEAST STRAIN

It is recommended that

- Desirable strains be obtained from a very reliable culture collection such as the National Regional Research Laboratory, Peoria, Illinois; Bureau voor Schimmelcultures, Delft, The Netherlands; or others. The National Research Council panelists can provide specific strain number recommendations.

- A strain from a commercial bakers' yeast or a strain isolated from local sources not be used. If SCP is to be used for food, a well-characterized strain with a definite assigned strain number should be used. If BPPT officials decide to use a commercial bakers' yeast strain, however, an active dry yeast, "Fermipan," manufactured by Gist-Brocades in Delft and sold in Indonesia, should be used. Most important, other commercial active dry yeasts may or may <u>not</u> be suitable.

FERMENTATION PROCESS

An aerobic fed-batch fermentation process should be used. Detailed descriptions of such processes for the production of bakers' yeast are available in the literature and have already been given to the BPPT staff. Thus a recommendation is required only when production of bakers' yeast SCP differs from production of bakers' yeast for leavening, and this is needed just for the last stage of fermentation. The production of a metabolically active and stable bakers' yeast requires reducing the feed rate toward the end of the process; however, this is not required for the production of SCP.

ENGINEERING

This working group made the following recommendations:

- The BPPT must have a strong team of microbiologists and a center for cultures.
- For animal feed, many kinds of microorganisms can be used (molds, yeasts, and bacteria).
- Aside from the "imported" strains, new strains from the Indonesian environment should be sought.
- Engineering considerations for the SCP project include:
  - -- Lamellar sedimentation to avoid expensive centrifuges is very promising for the future but should not be assigned high priority now.
  - -- A continuous culture fermentation is desirable.
  - -- Recycling of the used medium should be taken into consideration from the beginning.

USE OF SINGLE-CELL PROTEIN AS ANIMAL FEED

Past research has shown that SCP can be used in the diets of food-producing animals, but its use will be largely determined by the cost of producing SCP relative to other high-protein feeds. Initially, it is visualized that SCP will be dehydrated and used in the dry form. Research may show that for some animals, however, especially ruminants, it is feasible to use SCP fresh or preserved by ensiling.

Before SCP can be recommended for commercial use, research should be conducted in Indonesia using different kinds of animals. The following research is recommended.

NUTRITIONAL VALUE

The nutrient content of SCP produced by the demonstration plant should be determined in samples obtained from different batches over time. The SCP should be analyzed for crude and true protein, amino acids, gross energy, major and minor minerals, and vitamins. In addition, studies should be conducted with animals to measure the biological availability of the nutrients.

SAFETY

Although safety has not been shown to be a problem in previous research with SCP, studies should be conducted of the Indonesian product using laboratory animals and poultry. Such studies may also shed light on the potential use of SCP in humans.

ANIMAL PERFORMANCE

Experiments should be initiated to determine the effect of feeding SCP on performance (production of meat, milk, and eggs). Such research should include studies of the level of SCP in the diet. The highest level would be complete replacement of conventional protein supplements. Amino acid supplementation should be studied. Experiments should be directed toward optimizing the palatability of SCP-containing rations.

PROCESSING

Although the initial research should be conducted employing dry SCP, use of the wet product should be explored to lower cost. Experiments should be conducted, initially with ruminants, to determine the effect of feeding the material fresh or preserved by ensiling. In the ensiling, the product will need to be mixed with other ingredients, since the moisture content of SCP and lack of soluble carbohydrate will preclude ensiling it alone. Ensiling the product with rice straw and molasses, for example, should be feasible.

It is recommended that planning of the research be initiated immediately so that the experiments can begin as soon as the demonstration plant is operational.

USE OF SINGLE-CELL PROTEIN AS HUMAN FOOD

Novel foods are those that are new to the population in question, or those that have not been eaten in significant amounts. Because single-cell proteins are considered novel foods, studies must be undertaken to ensure their acceptability for human use. It is important to document that there are no toxic constituents present in novel foods and that they are of acceptable nutritional value. Studies using animals should be performed initially, followed by closely supervised human studies. The Protein Advisory Group (PAG) of the United Nations System has formulated guidelines* to assist nutritionists and food scientists in evaluating novel foods for human consumption.

Preliminary testing should include a complete chemical analysis of the single-cell protein, including quantitative and qualitative information regarding the protein, lipid, carbohydrate, vitamin, and mineral composition. Thereafter, animal tests should be performed to determine the available energy content of the food, quality of protein, digestibility, and availability of minerals.

Animal feeding experiments must be performed to assure that there are no adverse side effects or toxicity associated with the use of single-cell protein. Toxicity studies should include evaluation of the animal's blood and major organs after feeding SCP for extended periods as outlined by the PAG recommendations.

Experiments with animals ingesting SCP should show that the SCP is of good nutritional value and free of adverse side effects before human testing is begun. It is also important to determine the intended use of SCP in humans before testing it using humans, that is, whether SCP will be used as a food additive or as a substitute for more traditional foods.

It is probably wise to restrict the initial human testing of single-cell protein to adult populations. Infants and children should not be exposed to novel foods until it has been firmly established that they are of good nutritional value and free of toxicity. Recommendations for human testing of single-cell protein are found in PAG

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*Protein Advisory Group (PAG) of the United Nations System. 1972. PAG Guideline No. 6, Preclinical Testing; PAG Guideline No. 7, Human Testing Procedures; PAG Guideline No. 12, Single Cell Protein. United Nations, New York, New York, USA.

guideline number 7, which should be used for any human testing conducted in Indonesia. Recommendations are being reformulated to include the following approach:

- Tolerance studies: These studies document whether SCP can be safely ingested by defined groups of human subjects within a controlled setting. Initially, 25-50 subjects would be appropriate. Medical and dietary histories should be obtained from study participants and pre-testing physical examinations performed to assure that subjects are free of chronic debilitating diseases or not at increased risk for allergic reactions, that is, one may be severely allergic to a number of traditional foods. Informed consent should be obtained according to the norms and regulations in Indonesia so that subjects are completely aware of the purpose, intent, and possible side effects of the human testing situation. The appropriate study design and study interval should be established. In addition, the method of administration, and level of SCP feeding should be explained fully to all subjects participating in the studies. When the tolerance tests are completed and an acceptable level of side effects established, nutritive value studies can then be initiated.

- Nutritive value studies: These studies are controlled more closely, conducted on smaller groups of individuals (6-10 subjects), and demand much more cooperation from participants. They can be completed in 10 days. During that time, the subjects are placed on approximately .30 grams of SCP per kilogram body weight per day and the appropriate urine, stool, and blood samples are collected and tested. The diet should contain the recommended daily supply of calories, vitamins, and minerals. Thereafter, nitrogen balance, digestibility, and biologic value should be determined.

  When tolerance and nutritive value studies in humans have been performed and use of SCP has been shown to be associated with minimal side effects and to be of good nutritional value, it may slowly and carefully be introduced into the diet of Indonesians.

APPENDIX A

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APPENDIX B

Conversion of Cassava Starch to Fermentable Sugars

PRODUCTION OF GLUCOSE SYRUPS FROM CASSAVA STARCH

Production processes for ethanol, yeast, and glucose/fructose syrups are now in various stages of development. All of these processes require as a first step the efficient and complete conversion of the starch to glucose. The enzymatic conversion of starch to glucose is basically a two-step process consisting of (1) gelatinization and dextrinization of the starch, and (2) saccharification to glucose.

For the production of glucose/fructose syrups, complete conversion of the starch to 95-96 DE (dextrose equivalent) is required, and is also highly desirable for the production of yeast. To produce ethanol in a batch process, complete conversion (prior to the start of the fermentation) is not required; that is, saccharification can be completed during the long period of alcoholic fermentation. The following only concerns the best means of completely converting starch to glucose.

STARCH GELATINIZATION AND LIQUEFACTION

Starch cannot be converted to fermentable sugars unless it is first gelatinized (dextrinized) and liquefied, using either an acid or an enzyme process. The enzyme process is recommended for the demonstration plant in Indonesia. Suitable enzymes are the bacterial heat-stable alpha-amylases produced by Bacillus subtilis or Bacillus licheniformis. A suitable starting material is a slurry of cassava starch containing 30 percent cassava starch solids. A typical process is described below.

Adjust the pH (if necessary) to a range of from 6 to 7. Add 0.5 kg of a liquid enzyme preparation (Novo "Termanyl 120-L")* for each ton of

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*The trade names of enzyme manufacturers are given only as examples. Similar enzymes are produced by other manufacturers whose names are given in the body of our report. The authors of this report have no connection (financial or otherwise) with any of the various enzyme manufacturers.

starch solids. Heat with live steam in a so-called jet cooker to 100°-105°C and hold for 5 minutes at that temperature. Then hold at a slightly lower temperature of 90°-100°C for 1-2 hours until the starch is fully liquefied. At that point the DE of the starch is 8-12. Hard water should be used to help the starch slurry supply enough calcium (Ca) ions to stabilize the amylase. Otherwise, about 70 ppm of Ca ions should be added to the slurry.

A similar process may be used with the enzyme from B. subtilis (Rohm "Rohalase AT") using an enzyme dosage of 0.5 liter per ton of dry starch and the same pH values as above.

SACCHARIFICATION OF LIQUEFIED STARCH

Saccharification (or conversion) is carried out with fungal amylases generally derived from Aspergillus niger or Aspergillus oryzae. The common name of the enzyme is glucoamylase or amyloglucosidase. The process requires a longer time period, a lower pH, and a lower temperature than that described above for the liquefaction of starch. A suitable process would be as follows.

Use the liquefied starch with a DE of 8-12 and a starch content of 30 percent. Adjust the pH to 4.5.* Adjust the temperature of the slurry to 55°-60°C. Add 1 liter of a liquid enzyme preparation Novo "AMG 200-L", or Rohm "Rohalase HT liquid" per ton of dry starch solids. The reaction time is 48-60 hours.

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*A pH of 4.5 is suggested for the conversion step because it is close to the optimum for enzyme action and because the subsequent growth of yeast on this substrate is also carried out at a pH of 4.5.

APPENDIX C

Reference Materials Made Available to BPPT

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*The books marked with an asterisk are those with more practical information on the production of bakers' yeast.