Microbial Upgrading of Cellulose-Based Materials

By

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SUMMARY OF THESIS

Title: MICROBIAL UPGRADING OF CELLULOSE-BASED MATERIALS

The main objective of the research was to develop and design a process for the microbial upgrading of cellulosic waste materials by solid substrate fermentation to produce a protein-rich animal feed-stuff.

A large number of fungi were tested for their cellulolytic activity and their ability to produce protein when grown on the selected raw material: barley-straw. Both mono and mixed cultures were used either in single-or multi-stage fermentation systems. As a result of this work a single-stage, mixed culture (cellulolytic and non cellulolytic) system operating in the thermophilic range was selected for more detailed work.

The optimum carbon to nitrogen ratio for growth and protein production was established. The effect of different raw material treatments (Ball milling) on fungal growth was also examined but no improvements were achieved. Preliminary feeding trials indicated that the end-product was acceptable and non-toxic to mice.

The detailed design of a plant to treat 1,000 tons of straw per year was carried out. This design work highlighted a number of biological and process engineering problems, including:-

1. maintenance of sterile conditions,
2. maintenance of uniform aeration,
3. temperature control,
4. recirculation and usage of nutrient salt solution,
5. removal and transport of fungal material by the salt solutions, and
6. process start up.

An attempt was made to develop preliminary models for microbial growth on solid substrates under thermophilic, isothermal conditions. Finally an economic feasibility study was carried out. This involved estimation of capital costs, operating costs, cost of end-product, and a cost comparison with existing, competing products.

C O N T E N T S

1. INTRODUCTION

1.1. A General Introduction

The project was initiated by Lucas Furnace Developments Ltd. and Dr. H. O. W. Eggins of the Biodeterioration Information Centre, The University of Aston in Birmingham, in 1969. Lucas Furnace Developments Ltd. have developed and are developing plants to handle such waste materials as sewage, chicken manure, plastics, liquid waste and town waste mostly by incineration. They were one of the first firms to develop a fully automated sewage-sludge furnace and equipment is now being built for customers in the U.K., U.S.A., Australia and Japan. The sewage furnace was followed by the scrap type furnace of which the first two are now operating in London and Wales. Lucas Furnace Ltd. have also been interested in developing processes and equipment required for the treatment of agricultural waste - materials, either to produce protein-rich animal feed-stuffs or humus-rich compost.

The work described in this thesis forms part of their overall programme. It involves a feasibility study of a process to produce protein-rich animal feed-stuff by solid-substrate fermentation using cellulose-based (agricultural) raw materials. Most of the solid substrate fermentation processes involving protein upgrading of food have been reviewed. Possible fermenter designs have also been outlined. However, the choice of raw materials, screening of microbes along with process design and economics form the major part of the thesis.

Because expertise from several fields of science and technology were required, the project was set up within the Inter-disciplinary Higher Degrees Scheme [1] of the University of Aston in Birmingham.

Dr. E. L. Smith from the Department of Chemical Engineering was appointed the main supervisor. The process design and development and technical costing were carried out under his guidance. Dr. H. O. W. Eggins, the Head of the Biodeterioration Information Centre, supervised problems related to process biology. Dr. Eggins has carried out basic research with mesophilic and thermophilic fungi for a number of years, and this provided the starting point for the microbiological studies. Mr. A. J. Bennett from the Department of Industrial Administration helped in simplifying problems involving process economics. Mr. J. B. Stribling of Lucas Furnace Developments Ltd. was appointed as the industrial supervisor under the I. H. D. Scheme.

1.2 Overall Project Plan:

1.2.1. The Objective: The overall objective of the research was to develop and design a process for the microbial upgrading of cellulose-based raw materials. The restrictions made were that:-

1. the processes should involve solid-substrate fermentation,
2. the substrate should be cellulose based, and
3. the end product should be a protein enriched animal-feed.

To meet the objective it was decided to:

1. review existing methods of upgrading materials in the form of solid substrates (e. g. composting of towns' waste, agricultural wastes, and production of fermented foods);

2. examine sources and costs of cellulose-based raw materials (e. g. paper, wood wastes, and agricultural wastes);

3. outline the design of a process for upgrading such raw materials on a large scale, particular emphasis being given to the design of the fermentation stage;

4. design, build and operate a pilot scale fermentation unit, and

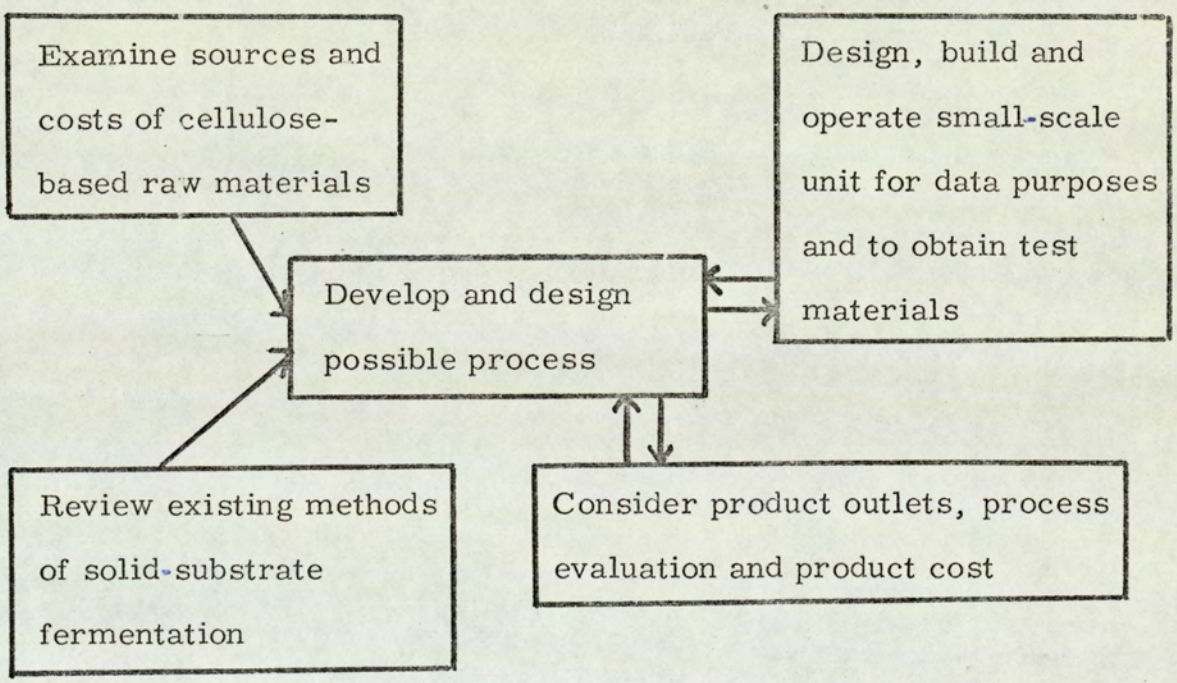
5. study the economic feasibility of such a process, as well as consider outlets for product.

The various activities mentioned above were obviously interconnected and this made it very difficult to breakdown research work initially. This inter-relationship between activities is illustrated in figure 1.

FIGURE 1

Overall Project Scheme

(Broad Outline)



1.2.2. Choice of Raw Material

The selection of substrate as a carbon source is influenced by the process and the type of micro-organisms to be used. The main factors to be considered are as follows.

It should be

1. easily available,
2. easy to transport,
3. cheap,
4. easy to handle,
5. contain the maximum amount of desired carbon source, and
6. not contain any toxic contaminant.

Some substances are very resistant to microbial attack such as lignin in comparison with say simple sugars. In the present research work the restriction was made that the raw material should be cellulose-based.

Cellulose occurs abundantly in nature mainly as the principal constituent of the cell walls of plants. Natural sources contain varying amounts of cellulose[2] as shown in table 1. Wood, in addition to cellulose (50%), contains lignin (20 - 30%), hemicellulose and other polysaccharides (10 - 30%) and of all cellulosic materials it is the most widely used. Straw is another material and barley straw was selected for bench scale experiments. Straw consists of[3] cellulose (46%), hemicellulose (36%), lignin (10%), the remainder being sugars,

TABLE 1

Cellulose Content of Typical Raw Materials[2]

Raw Materials	% Cellulose Content
Wood	40 - 50
Cotton	91
Flax	82
Ramie	85
Jute	65 - 75
Kapok	55 - 65

resinious materials, oils and waxes etc.

1. 2. 3. Screening of Micro-organisms

The biological literature contains numerous studies on pure cultures of cellulolytic organisms. Their isolation from wheat straw has been reported by Chang and Hudson[4]. Previous research in the Biodeterioration Information Centre at Aston has also been concerned with cellulolytic (thermophilic and mesophilic) organisms. Consideration has also been given to the use of mixed populations in single and multi-stage fermentations. Since the main object of the research is to produce protein-rich animal-feed in the shortest possible time it is very essential to select micro-organisms which are very cellulolytic in nature and build up high protein level. The end product must be non toxic and palatable.

1. 2. 4. Process Design and Economics

Microbial protein systems have both a short- and a long-term ptoential for supplying protein for animal-feed supplements, human food supplements and protein isolates for food analogues. However, detailed microbiological, nutritional and biochemical engineering studies are needed in order to achieve economic production of microbial protein. More development work must be carried out on designing fermenters where the problems of maintaining temperature, required rates of oxygen transfer from gas to solid phase, desired rates of substrate transfer into cell wall and optimal mixing must be solved. Techniques for scale up must also be developed.

The market value of the end-product will determine how high the production cost can go. At the moment very few microbial proteins can compete with soybean protein. Some of the factors most important to microbial protein economics may be outlined as follows[5]:-

1. Raw Materials

   (a) Ease of collection to a central area.
   (b) Availability to a given site.
   (c) Bulk handling properties.
   (d) Seasonal fluctuations in availability.
   (e) Pre-treatment of raw materials.
   (f) Cost of raw materials.

2. Sterility Requirements

   (a) Microbial encroachments. Inhibiting the growth of undesired organisms at minimum cost.

3. Fermentation

   (a) Residence time in reactor.
   (b) Cell concentration attainable.
   (c) Operating temperature and its maintenance.
   (d) Total O_2 requirements.
   (e) Power requirements.
   (f) Cell yield per pound of substrate consumed.
   (g) Capital cost for equipment.

4. Purification (if necessary)

(a) Residual substrate.

(b) Raw material impurities.

(c) Metabolic by-products.

5. Product Value

(a) Percentage protein.

(b) Limiting amino acids.

(c) Digestibility.

(d) Texture and flavour.

For the development and design of a process involving a solid substrate the important aspects from the engineering point of view are as follows:-

1. Treatment of Raw Materials

(a) Collection and storage.

(b) Mechanical handling involving size reduction, separation, preforming.

2. Fermentation stage

(a) Addition of nutrient salt solutions.

(b) Sterilization.

(c) Inoculation.

(d) Mixing.

(e) Control of temperature, pH, moisture and aeration.

3. Treatment of Crude Product

(a) Washing or removal of unwanted solubles and insolubles.

(b) Drying to control the desired moisture content.

(c) Size reduction and separation.

(d) Pelleting or compacting into a desired shape.

(e) Storage and distribution.

This scheme pre-supposes no final extraction process for protein if the end product is an animal-feed. This is not likely to be necessary. In case of human consumption cleaning up stages must be considered.

The important aspects from a microbiological point of view may be outlined as follows:-

1. Treatment of Raw Materials

(a) Storage of raw materials to avoid deterioration.

(b) Microflora present prior to fermentation.

2. Fermentation Stage

(a) Choice of micro-organism[6] and environment.

(b) Use of monoculture or mixed cultures in either a single or multi-stage process.

3. Treatment of Crude Product

(a) Removal and treatment of nutrients, salts etc. from the product.
(b) Storage problems.
(c) Nutritional evaluation of final product.

From the economic view point the problems to be considered are as follows:-

1. Treatment of Raw Materials

(a) Cost of collection, storage and pre-treatment.
(b) Alternative uses for raw materials - and their costs.

2. Fermentation Stage(s)

(a) Technical economics which involve capital investment for setting up plant and operating costs.

3. Treatment of Crude Product

(a) Overall product costs (following costs of synthesis from previous stages).

(b) Outlets for products and cost structure of markets.

(c) Rival products or materials.

N. B. The feasibility of operating the process in technologicallly advanced or developing countries may be decided by the economic studies, and this could have considerable effect on the choice of process.

1.3 Background Information and Literature Survey

1.3.1. Biodeterioration and Biodegradation

Biodeterioration has been defined by Hueck[7] (1963) as "any undesirable change in the properties of any material caused by the normal vital activities of living organisms". This definition deliniates biodeterioration from "corrosion" and "wear" of materials, which comprise the undesirable changes brought about respectively by chemical agents and mechanical (physical) influences. At first glance, the biodeterioration of materials has much in common in its methodology with phytopathology and more remotely with human pathology and biologists have studied if biodeterioration has something in common with diseases of animals or plants.

Although micro-organisms are frequently involved in biodeterioration processes, they also operate in a similar way in useful degradation processes. This is clearly seen in the decay

processes involved in the carbon cycle. As a result of increased knowledge of biodeterioration and biodegradation, it is now possible to consider microbial processes for upgrading certain raw materials to useful products. However, it is also true that man has been successfully using what are in fact natural degradation processes without fully understanding the detailed microbiology. The best example is the process of composting, by which cellulosic and other organic waste materials may be treated to produce humus-rich organic fertiliser. In agriculture it is necessary to maintain the health of soil by returning to it organic matter which will provide humus and plant nutrients. It will be apparent then that two problems may be overcome by the use of composting to produce fertiliser, viz the feeding of impoverished soils and the disposal of large amounts of cellulosic waste.

In composting[9,6] the organic matter is broken down by bacteria and fungi under aerobic conditions. The process can be mechanized and made continuous as in the treatment of town waste. Schulze[10] showed that complete mixing could be provided by the use of a rotating drum-type fermenter and that the aerobic decomposition of organic waste materials could be achieved between 50°C and 70°C in a continuous manner. The importance of maintaining the correct balance of aeration, moisture, temperature and pH in order to achieve a very rapid breakdown of the waste have been discussed by Jeris and Regan[11]. Composting has also been recently reviewed by Gray and Sherman[12].

Initially, at temperatures up to 30°C the biodegradation is carried out by mesophilic organisms. During the course of fermentation, heat is liberated and the temperature rises very rapidly. Beyond 40°C the thermophiles take over and break down carbohydrate, protein and cellulose very rapidly. The thermophilic fungi grow in the range from 45°C to 55°C and the major degradation of cellulose takes place in this range. The thermophilic actinomycetes begin to breakdown the cellulose in the 50 to 65°C region. Above this the microbial activity slows down. As the system cools down to 30° to 40°C the thermophilic micro-organisms become less active and a rapid rise is observed in the number of bacteria and fungi capable of growing. Under these temperature conditions their high number is maintained until the material cools down to ambient temperature, after which a very slow decline in number takes place.

During all stages of a composting cycle many important antagonistic relationships develop. Antagonism in a compost heap is not confined solely, or even mainly, to the production of chemical substances toxic to other organisms and usually referred to as antibiotics. There is a very complex relationship between the mixed microflora and fauna in which numerous species are involved in preying on each other. This sequential development of different micro-organisms is very characteristic of the composting process. Composting is also a potential process for the production of fungal feed stuff. This topic is introducted in detail in section 1.3.3.

1.3.2. Microbiological Degradation of Cellulose-based Materials

The large amount of cellulose required for modern industrial processes is obtained from woody matter, straw, stalks and hulls etc.[2]. Wood is the most abundantly available cellulosic raw material. Wood contains a wide range of chemical compounds amongst which cellulose, hemicelluloses, lignin, water-soluble carbohydrates and terpenes all have a bearing on the type of attack to be expected from biological agents.

Three main types of fungal attack on plant tissues are possible[2]:-

1. The decompostion of soluble sugars and starch by members of the Uredineae and Ustilaginaceae (rusts and smuts respectively).

2. The breakdown of sugars, starch, hemicellulose and cellulose by saprophytic fungi (e.g. Penicillia, Aspergilli).

3. Attack on cellulose, hemicellulose and lignin by degrading Basidiomycetes and Ascomycetes.

The microbial degradation of cellulosic materials, whether by fungi or bacteria, is caused by the attack of enzymes (cellulase), produced by the micro-organism for the purpose of obtaining food. The first step in the degradation of insoluble cellulosic substrates

to soluble sugars, which can then be metabolized inside the bacterial or fungal cell, is effected by extra-cellular-enzymes. Reese and his co-workers (1950, 1952)[13,14] suggested that there must be at least two components in the cellulase system. They also suggested that organisms which degrade highly ordered (crystalline) forms of cellulose must contain a component, C_1, whose function is to render the substrate accessible to attack by another component or components, C_x, which alone can do little to such forms of cellulose. The combination of C_1 and C_x displays powerful synergistic action. When micro-organisms capable of attacking both insoluble and soluble cellulosic substrates yield cell-free filtrates active on the latter but not the former, the inference is that the C_x-component has been isolated, but not the C_1 component. Inspite of many attempts to prepare filtrates active against native cotton very little success had been achieved by 1961. Mandels and Reese[15] (1964) showed that a cell-free filtrate from their strain of Trichoderma viride was capable of producing 70 - 80% solubilization of cotton in a single treatment. Finally the existence of these two components was confirmed by Selby and Maitland[16](1965) by gel filtration studies.

The major portion of the agricultural cellulosic waste is woody material. This has a protective shield of lignin to prevent microbial attack. The two well-known types of wood decaying fungi are the Brown rots and White rots. The former type is known to attack cellulose but not lignin whereas white rots are known to attack lignin but not cellulose.

Another type of decay in which the fungal hyphae tunnel in

the cell walls of the timber is termed soft rot. Such decay occurs in timber in contact with soil. This decay is brought about by micro-fungi of the class Ascomycetes and Fungi Imperfecti and often decay is not as visible in cell lumens as is the case with brown and white rots.

Chang and Hudson[4] have recorded a large number of species of fungi from wheat straw-compost. In samples taken from the centre of the compost heap the fungi usually appeared in a particular order. The fungi have been grouped according to this sequence of occurrence[4]. The most important group of fungi is the one enlisting thermophilic fungi. These thermophiles were also isolated by Cooney and Emerson[17](1964) from various self-heating materials. These fungi were studied in detail for their ability to degrade barley straw as well as to upgrade the protein content of the straw in the experimental part of the research. Chang[3] (1967) analysed wheat-straw compost biochemically for the ethanol and diastase soluble fractions, hemi-cellulose, cellulose, lignin and total nitrogen. The fungi isolated were studied for their ability to utilize a variety of carbohydrates at a temperature (45, 35 or 25°C) as well as their ability to utilize various carbon sources.

Thermophilic fungi were reported by Mulinge and Apinis[18] in moist stored barley grain. Absidia spp., Aspergillus candidus, A. flavus, A. fumigatus, A. terreus, Dactylomyces crustaceus, Eurotium amstelodami, Monascus spp and Mucor pusillus were frequently isolated from both grain husks and dehusked surface sterilized grain. Mycelium of thermophilic fungi was found to be mostly confined to husk tissue of the grain. However, after prolonged

storage a number of these species, such as A. candidus, A. terreus and Dactylomyces crustaceus invaded the grain tissues after self-heating of grain took place.

Fergus[19](1969) investigated the ability of some thermophilic fungi and actinomycetes to produce C_x enzyme. The influence of time on the production of enzyme and the need for inducing compounds in the medium were also investigated and he concluded that the thermophilic mould showed greater cellulolytic ability than did the thermophilic actinomycetes.

1.3.3. Solid Substrate Fermentation Processes

1.3.3.1. Introduction: Little work has been done to develop the technique of solid substrate fermentation as a continuous process on a large scale. Indeed composting of towns' waste [10] seems to be the only process in which this method is widely used.

On small-scale, using traditional batch methods, the principle is used for the production of protein-rich foods in the far east and other parts of the world. Cheese making and mushroom growing are other well known processes involving solid substrates.

Protein Resources: Reports describing various raw materials[20] which might be used for the production of edible protein or protein-rich food have already been published. These materials may be classified into two different groups, viz. conventional and unconventional proteins.

Cereal grains are the primary suppliers of calories and protein in the human diet. Wheat, rice, corn, sorghum, millet and certain other cereals provide half of the world protein needs and when other plant foods are added, such as legumes and tubers, plant sources account for almost three quarters of the world food supply. The rest of the protein is of animal origin which includes meat, eggs, milk and fish.

The unconventional proteins may be sub-divided into two classes. The first includes those which are available in vast quantities but are not used as food. New processes would have to be developed to make them into palatable and sanitary human foods. Fish protein and oilseed protein concentrates may be included in this group. Cotton, sesame and sunflower seeds, peanuts, coconuts, soybeans, palm kernals, leaf, grass and others could also be used for the production of protein concentrate. In addition the presently available residues from oilseed processing operations are comparable with the bulk of animal food produced; and yet only a fraction of this huge resource is being used for animal feeding, and virtually none for human consumption.

The other major group of unconventional proteins include those produced by micro-organisms such as yeast, algae, bacteria, fungi growing on varieties of substrates. Single cell protein is the most common and familiar form of protein obtained from micro-organisms.

Potential of Microbiological Proteins: The crude protein contents of microbial cells vary widely[21] e. g. bacteria 47 - 87%, fungi 19 - 57%, algae 24 - 80%, which is very high when compared

with agricultural protein. Micro-organisms offer many advantages over green plants. Most of these are associated with their cultivation, compared with conventional agriculture[22]. These may be outlined as follows:-

1. Micro-organisms have a very high protein content and their culture offers a concentrated source of protein. There is no differentiation of the cells into various tissues due to the unicellular nature of the biomass. Hence it is treated as a whole although protein may have to be extracted from the single cells.

2. Owing to the high density of cropping, cells can be easily cultivated in compact units whereas plants need vast areas of land. The rates of growth of micro-organisms are also much faster than those of plants and animals. For example, rates of synthesis of protein by microbes are faster by a factor of 10^2 to 10^4 than by cattle.

3. Since micro-organisms are grown in industry under controlled conditions their growth is predictable and harvesting becomes a routine job. This is not true for agricultural crops everywhere in the world (with some exceptions).

4. Micro-organisms grow continuously twentyfour hours a day.

There are some disadvantages in producing microbial protein. Since plants designed to produce biomass or edible fungi can be sophisticated, the initial capital investment may be very high. Production of microbial protein on large scale may also need technical supervision thus adding to cost of production. The biggest problem facing microbial food is that it is relatively less acceptable to consumers in comparison with the conventional protein-rich foods.

1.3.3.2. Some Traditional Batch Solid-Substrate Fermentation Processes

Production of Fermented Foods - Asia. Although the production of fermented food has been practiced for centuries, the biochemical and microbiological aspects of various processes were not understood until very recently. Countries like Indonesia, Japan, China, U.S.S.R. and India and some of the South East Asian countries produce many types of fermented foods which are very popular locally[23, 24, 25].

One of the most important functions of such fermentations is the synthesis of one enzyme (or more) which, depending on its characteristics, may enhance the breakdown of protein, starch, carbohydrates, etc. For example, in miso-fermentation, both the

protein of soybean and the starch in rice are hydrolyzed by the enzymes of the strains of Aspergillus oryzae employed. This mould digestion of the plant consitiuents in turn enhances the digestibility of the food consumed.

Fermentation also changes the flavour, which makes food more acceptable. The flavouring materials produced by micro-organisms may be such as glutamic-acid (in miso and shoyu) or a small amount of alcohol (found in products made from Chinese yeast).

Fermented foods are also produced to act as colouring agents. In "Augkak" the white colour of rice turned red and then this product is used to colour rice, wine, meat , etc.

In some instances fermentation may increase the vitamin content of foods. Sometimes fermentation also causes a change in the physical state of the food product; for example, in "tempeh fermentation", a solid product is made from loose particles of soybeans.

A few of the most common fermented foods from Eastern countries, the types of organisms and the raw materials used are summarized briefly in table 2.

The actual production of food may be divided into several stages, viz. pretreatment of raw materials, fermentation stage (one or two), and final treatment which may involve ripening of the fermented food, addition of certain materials or transformation of final product into a desired physical form.

TABLE 2.

Summary of Fermented Foods - Eastern Countries (25)

Name of the Product	Organisms Used	Substrate	Nature of Product	Area where produced
		Of Fungal Origin		
Tempeh	Rhizopus (R. oligosporus)	Soybeans	Solid	Indonesia and vicinity
Sufu	Principally Actino mucor elegans and Mucor sp.	Soybeans	Solid	China
Ragi	Mucor, Rhizopus and Yeast	Rice	Solid	Indonesia China
Miso	Aspergillus oryzae, Saccharomyces rouxii	Rice and other cereals plus soybeans	Paste	Japan China
Shoyu	Aspergillus oryzae Lactobacillus, Hensenula, Saccharomyces	Soybeans Wheat	Liquid	China Japan Philipines
		Of Bacterial Origin		
Natto	Bacillus-subtilis	Soybeans	Solid	Japan

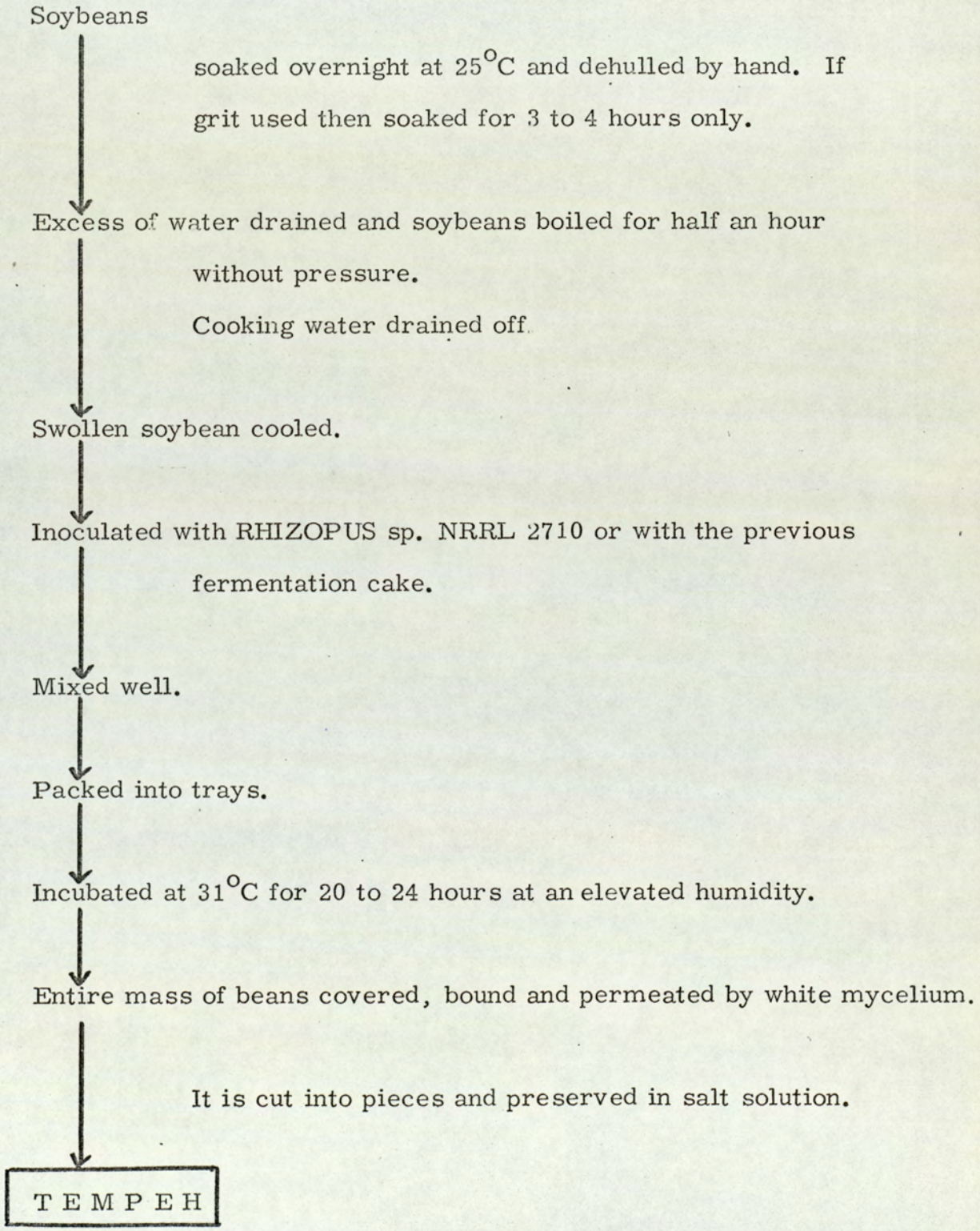
Pretreatment of the raw material is frequently an important stage before materials may be passed over to the fermentation stage. This may involve mechanical handling of the raw materials, i.e. size reduction and screening (if necessary), cleaning and washing, draining of excess water and so on. In the case of an inoculum of fungal origin the substrate should be moist enough (approximately 60%) for mould growth but not so moist that bacterial growth is promoted.

After inoculation the moulded mass of the solid substrates is held for thirty to one hundred hours at a known temperature suitable for the growth of micro-organisms until it is covered by the microbial mycelium. This stage is mainly devoted to the production of various enzymes (e.g. lipase, protease, amylase, pectinase) depending on the type of inoculum used. In many processes the use of a pure culture is avoided and instead a mixed culture, from previous food samples, is used. In conventional processes this stage is usually carried out either in a deep container, or in perforated trays or with substrate in the form of cubes, rectangles or any other desired shape.

The second stage of the fermentation involves the production of the desired product. Here either a fresh culture is used to inoculate the product (degradation) of the first stage fermentation or due to the use of mixed culture in the first stage, the desired culture takes over the fermentation if optimal conditions for its growth are provided. This stage may involve either dry or wet conditions, depending on the form of the desired end product. Two to seven days are usually required for this stage.

FIGURE 2

The following flow diagram outlines (26)
the process of tempeh fermentation



After the second stage of fermentation is over, it may be necessary to allow the product to ripen or it may also be necessary to introduce additives to make the product commercially acceptable.

Finally, it may be necessary to transform the product into some physical form depending on the marketing requirements.

Flow diagrams describing the production of some conventional foods are given by Hesseltine[25](1965). The tempeh process[26] is summarized below by way of example in figure 2.

Production of Cheeses

Cheese has been prepared in Asia and Europe for many centuries. At present many varieties of cheese are available which is evident from the fact that it can be described in terms of hardness, the principal ripening agents (mould or bacterial cheeses), the kind of milk from which it is manufactured, the country of origin, the method of coagulation, added substances (such as sage) and the fat content of the milk. The following steps indicate the general method used in the production of cheese[27].

Formation of Curd:

Milk contains fat, lactose, protein, mineral salts and water. Fat is present as an emulsion, but the sugar, minerals and some of the proteins are soluble in the water of the milk. Casein (the principal protein) is combined with calcium and exists in a colloidal condition. If acid is produced in milk as a result of the fermentation by lactic acid bacteria the casein is freed from calcium and accumulates

in large lumps of curd. An enzyme, rennet, has the ability to alter the colloidal condition of casein causing curdling. Commercial pepsin may also be used as a subsititute for rennet. The watery portion of milk separated from curd is known as whey.

Treatment of Curd:

After the curd has settled into a fairly compact jelly-like mass, it is usually cut into pieces to facilitate the removal of occluded whey and, if necessary, some pressure may also be applied. The curd of soft and semi-soft cheeses are pressed just sufficiently to remove the excess of whey, while those of the hard cheeses may be heated and pressed until curd forms a firm mat. The curd is treated with varying quantities of salt by various methods. Finally the treated curd is molded into the desired shape and is ready for ripening.

Ripening:

Ripening has three main aspects which, in practical order of importance, are as follows[28]:-

1. Flavour which has two components, aroma and taste.

2. Body which can be defined as the feel, and texture as the appearance of the cheese.

3. Protein degradation which is intimately related to flavour production and to body and texture. The nature of the changes depends on the method used in treating the curds, the method and quantity of salting, the micro-organisms present or added and control of temperature and relative humidity in the curing chambers.

Roquefort Cheese: An Example[29]

Cubes of sterile whole wheat bread are inoculated with Penicilium roqueforti. After extensive growth of the mold on the bread cubes, the cubes are removed, dried and powdered and used as inoculum for making cheese. The addition of a lactic acid culture and rennet curdles the milk. The curd is cut when it becomes firm. The curd particles are then removed and placed in metal hoops. The addition of the spores of Penicillium roqueforti may take place in either of these steps. The hoops are then placed on a draining board to facilitate drainage of whey and matting of the curd, after which the curd is removed, salted periodically and eventually placed in an area of high humidity (95 - 98%) and low temperature (9 to 12^{o}C), where the ripening process occurs over a period of several months.

Mushroom Production

Production of edible mushrooms is one of the classic examples of utilization of the technique of solid-substrate fermentation to produce high protein food. Although submerged fermentation has

been tried in the past, it has not been proved to be feasible. The history of mushroom production[30, 31, 32] as well as some modern technological developments in understanding its growth has been outlined by many scientists. The cultivation of edible fungi in the western world is confined almost exclusively to Agricus bisporus and to the truffle, whilst in the east the Shiitake*, a fungus which grows on dead wood and Padi-straw, are cultivated.

In general the growth of mushrooms may be divided into two stages, the first one being the vegetative stage where mycelium grows uniformly throughout the compost and in the second stage the growth of fruiting bodies takes place.

Spawn (normally grain spawn) is mechanically mixed through the compost. If this inoculum is well distributed, mycelium grows quickly through the entire compost and ensures a more thorough and uniform utilisation of compost constituents. 150 - 200 gm. of spawn are used per 60 lb. compost. The spawn boxes are stacked in such a way that an approximate gap of nine inches is left between the top of one box and the bottom of another. The spawn boxes are incubated at 20 - 24°C in a relative humidity of about 95% with air circulating for two weeks. When the spawn is well run through the compost the temperature is dropped to 15°C and relative humidity to 90%, and the compost covered or cased with 3 - 4 cm. of a soil mixture (often equal parts peat and limestone). Without the covering of soil the production of mushrooms is greatly decreased. The casing soil is the medium in which the mushrooms are formed. The quality of the soil must be

* Cortinellus Berkeleyanus

exact, since it must be water absorbent at the same time as being freely pervious to air to obtain good ventilation of the crop. Casing soil is normally sterilised by treatment with 2 - 3 litre formalin per cubic meter of soil.

Silage Production

Silage is the product formed when grass, legumes or other crops are stored anaerobically. It is a palatable animal-feed in which the majority of nutrients present in the original herbage are effectively preserved.

It is always easier to make good silage of crops that have been chopped short or thoroughly bruised. When short-cropped material is ensiled it is practicable to make use of deep silos and subsequently to use a front loader and forage box for mechanized feeding. As a general rule, dry matter content of crops ensiled in horizontal silos should not exceed about 35%, otherwise it is difficult to avoid waste due to over-heating. With tower silos, silage can be made without excessive in-silo losses at considerably higher dry matter content; but there is little advantage in very dry silage, since field losses may be excessive, and there is more danger of appreciable secondary fermentation just before the silage is used.

To produce high protein silage, additives such as three gallons of molasses[33] or 8 - 10 lb of sodium metabisulphite per ton of green fodder need be uniformly applied by spraying a warm concentrated solution. Preservation is achieved by a combination of

anaerobiosis (inhibiting aerobic organisms) and a low pH (inhibiting clostridial activity). The most important condition for adequate preservation is an anaerobic environment. Losses of material, up to 30% of original dry matter, will occur if air gains access to the silage during storage. The low pH can be achieved by adding acid but the normal way is to allow a lactic acid fermentation to occur. This can lower the pH from an initial pH 6.5 to pH 4 in two days. In the early stages streptococci and leuconostocs are dominant but as the pH falls they are replaced by Lactobacilli and Pediococci.

If the pH falls to about pH4 the material will be well preserved. If, however, the pH does not fall this low, due either to access of air or to insufficient fermentable carbohydrate in the herbage, the clostridia present in the silage will not be inhibited and may grow. Two types are normally encountered, Clostridium-butyricum, which by fermenting lactate to produce butyrate will cause a rise in pH so making the situation worse, and varieties of Cl. sporogenes, which cause proteolysis and amino acid fermentation and make the silage objectionable.

In addition to the lactic acid bacterial fermentation and the clostridial fermentations there are organic acid fermentations. These organic acid fermentations cause a marked increase in the buffering capacity of the silage. The higher the buffering capacity the greater the acid production needed to preserve the material. The main acids present are malic, citric and glyceric; malic and citric acids are subject to breakdown by lactic acid bacteria.

1.3.4. Types of Solid-Substrate Fermenters

Although the production of food by solid-substrate fermentation has been in practice for many centuries, it has usually been carried out in small-scale, batch operated non-mechanized plant. More sophisticated equipment has been used in waste treatment processing, the most common example of which is the composting of town waste (Figure 3). With some modification in the design of fermenters used for such composting, it may be possible to adopt them for food processing. Some composting processes[34] are described very briefly in this section.

The Simon Lawden Refuse Digester (Figure 4)

This is in the form of a circular tower with many floors. There is a central shaft with sweeping arms. As the central shaft rotates the sweeping arms propel the contents of the fermenter from the top to the bottom floor of the digestor. The number of floors used and the speed of rotation of the central shaft are adjusted to achieve the desired residence time in the fermenter. Air is fed through the arms and its supply may be automated. Probes may also be inserted into the vessel to detect various gases evolved. The temperature of the fermenter may also be maintained at the required level by thermostat arrangements. The incoming substrate may be inoculated by recycling a part of the composted material.

Fermentation in Vertically Arranged Trays (Figures 5 and 6)

A number of this type of composting plant are in use and

one of the best known is The John Thomson Jersey Process.

This type of fermenter has many floors. The number of floors is chosen to to achieve the required residence time. Each floor has two rows of troughs and there is a narrow footway between these rows. The size of each trough may be varied, depending on the required total capacity of the fermenter. The most common size of each trough is four feet wide at the top, ten feet long and of trapezoidal cross-section. Each trough is perforated for aeration purposes and there is also a two inch gap between troughs for the same purpose. Nozzles may also be provided if extra air is required. The substances are fed by belt to each of the troughs evenly. The material is allowed to ferment at each floor for a known period as decided beforehand. The mixture is passed to successive floors by inverting these troughs by an electromagnetically controlled system. The fermenting particles absorb oxygen while moving from one floor to another.

There is a temperature gradient set up inside the fermenter, the minimum being at the top and the maximum at the lower floor. The product obtained at the outlet may be sent for further processing whereas part of the product may be recycled for inoculation of the substrate at the inlet point.

Fermascreen Type Fermenter (Figure 8)

The Fermascreen manufactured by John Thompson Ltd. is essentially a hexagonal drum. Three alternate sides are perforated

and each is provided with a hinged door to completely seal the aperture. The other three sides are of plain steel and the drum as a whole is mounted on circular tracks which are supported by wheels to allow rotation of the drum. The whole unit is driven by suitable gearing, either by electric motor or diesel engine. An arrangement is also made for charging of substances through one of the plain sides. Inlets for water and nutrients are also provided through various pipes whilst the unit is rotating.

Firstly substrates, nutrients and water are added when it is stationary. The unit is then shut and allowed to rotate for some time so that mixing of all components added may take place. The unit is then stopped and the doors covering the perforated sides are opened, thus allowing aeration necessary for aerobic fermentation to take place. The doors are then closed and the unit is rotated so that new surface comes into contact with the micro-organisms. Thus the Fermascreen is alternatively stationary for aeration and rotated with doors closed, to provide fresh substrate to the growing micro-organisms.

At the end of the retention time the doors covering the perforated side are removed and the equipment is rotated and the product discharged. This equipment is useful for batch type processes only.

Dano Rotating Drum Type Fermenter (Figure 9)

This is one of the most efficient types and may be of great use in continuous solid-substrate fermentation processes. It is

a long, slowly-rotating drum charged with raw materials and nutrients through a feeding device fixed at one end. Air is blown in counter-currently into the whole mass of substrates through nozzles to allow aerobic fermentation to take place along its length. The fermentation temperature can be maintained by regulating the airflow and by lagging its surface. As the drum rotates its contents are mixed well, and at the same time some size reduction of particles also takes place by internal friction. There is continuous movement of solid to the discharge end.

Some Traditional Fermenter Systems

Stack Maturing. Finely ground raw materials mixed with nutrients and inoculum may be stacked in tanks through which air may be passed. This is obviously a batch method for fermenting materials. It may be used for the production of animal feed which may need the addition of other materials before it is acceptable to the farmer.

Tray Fermentation (Figure 10). This is a very widely accepted technique for small-scale batch fermentations. Trays act as containers and have perforated bottoms and tops so that aerobic fermentation can proceed rapidly. The depth of tray[26] plays a very important part. For example, in tempeh fermentation the depth of trays should not exceed 2.5 cm. if a satisfactory amount of oxygen is to be supplied for the growth of micro-organisms. Fermentation in such trays is carried out for the production of tempeh, sufu and shoyu, etc.

Fermentation of Preformed Shapes. Substrate is converted into the shape of rectangles, cubes, pellets, tablets or spheres, etc. It is then inoculated and incubated under desired conditions. For example, this technique is used for the production of sufu or Chinese cheese from soybeans. Kovac and Ziemba[35] have suggested various types of equipment which may be used for producing products in different shapes depending on whether a wet or dry process is to be followed.

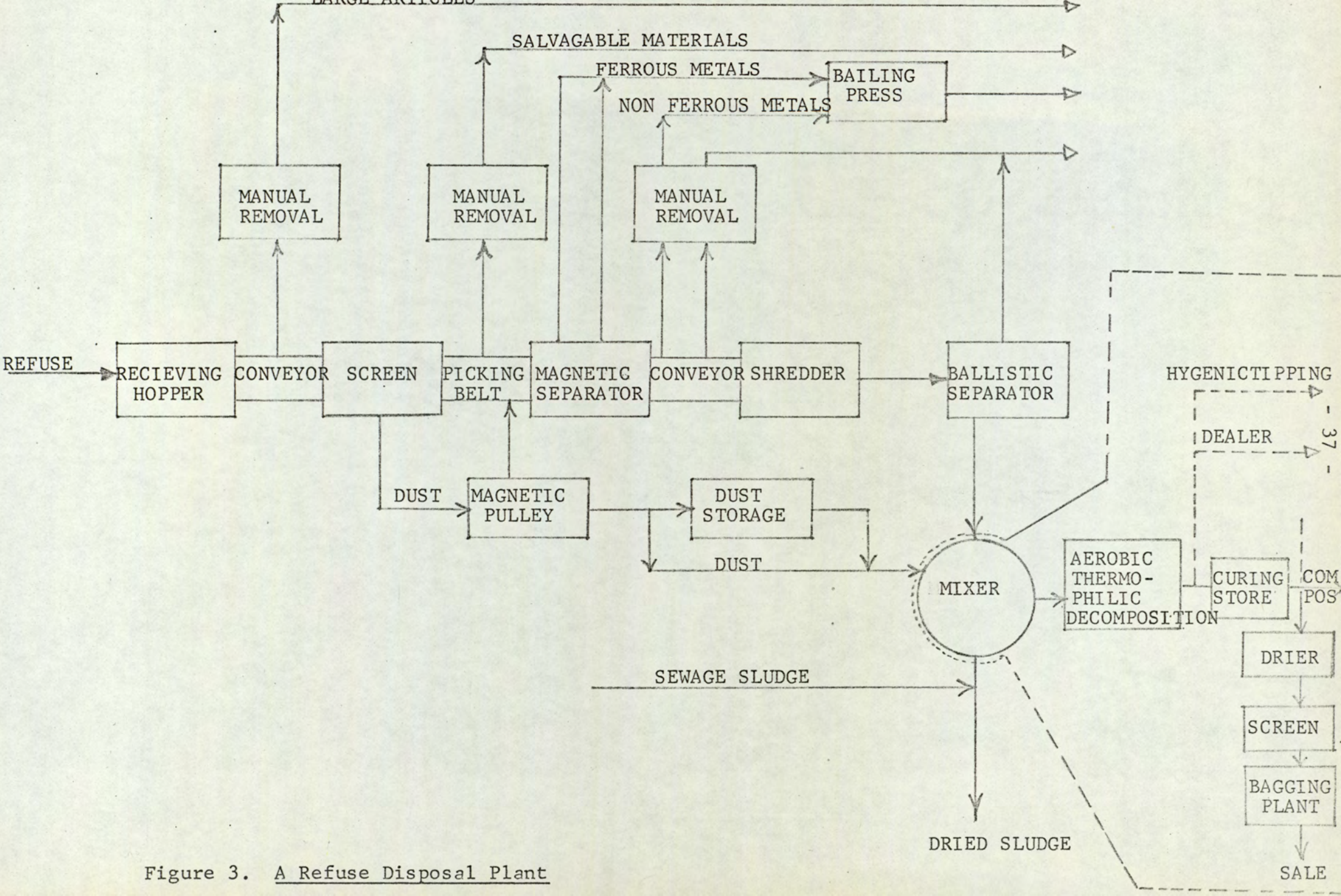


Figure 3. A Refuse Disposal Plant

SIMON LAWDEN REFUSE DIGESTOR TYPE

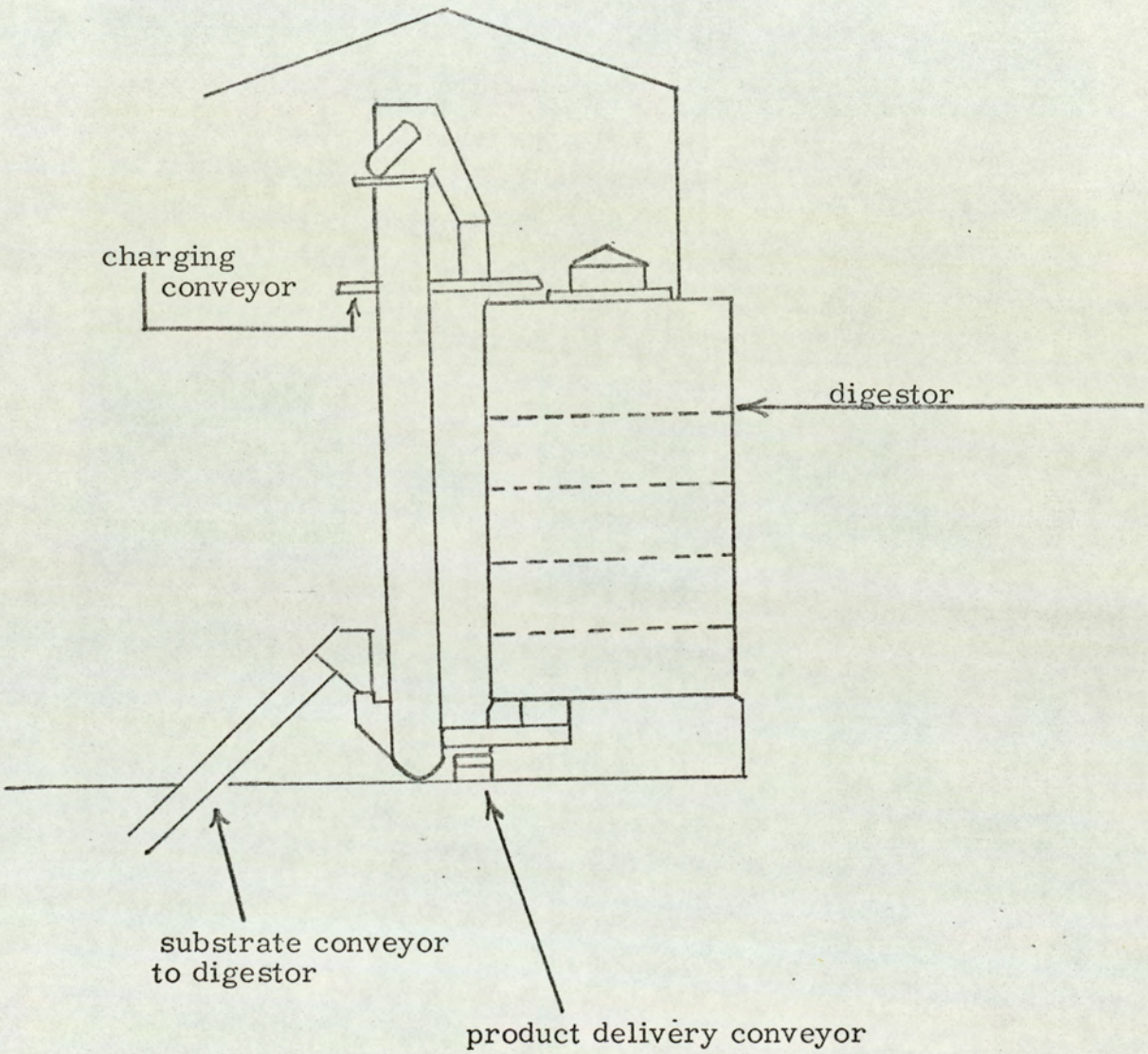


FIGURE 4

SINGLE TRAY - JOHN THOMPSON "JERSEY" PROCESS

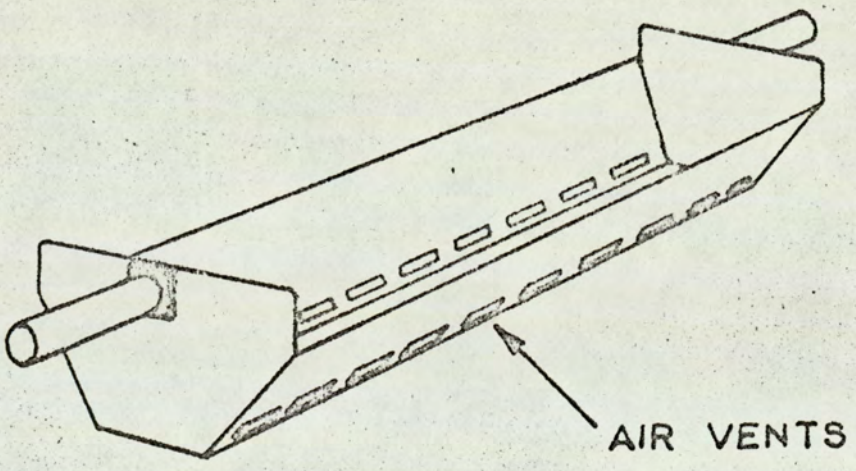


FIGURE 5

COMPOST HOUSE JOHN THOMPSON "JERSEY" PROCESS

(OUTER WALLS REMOVED)

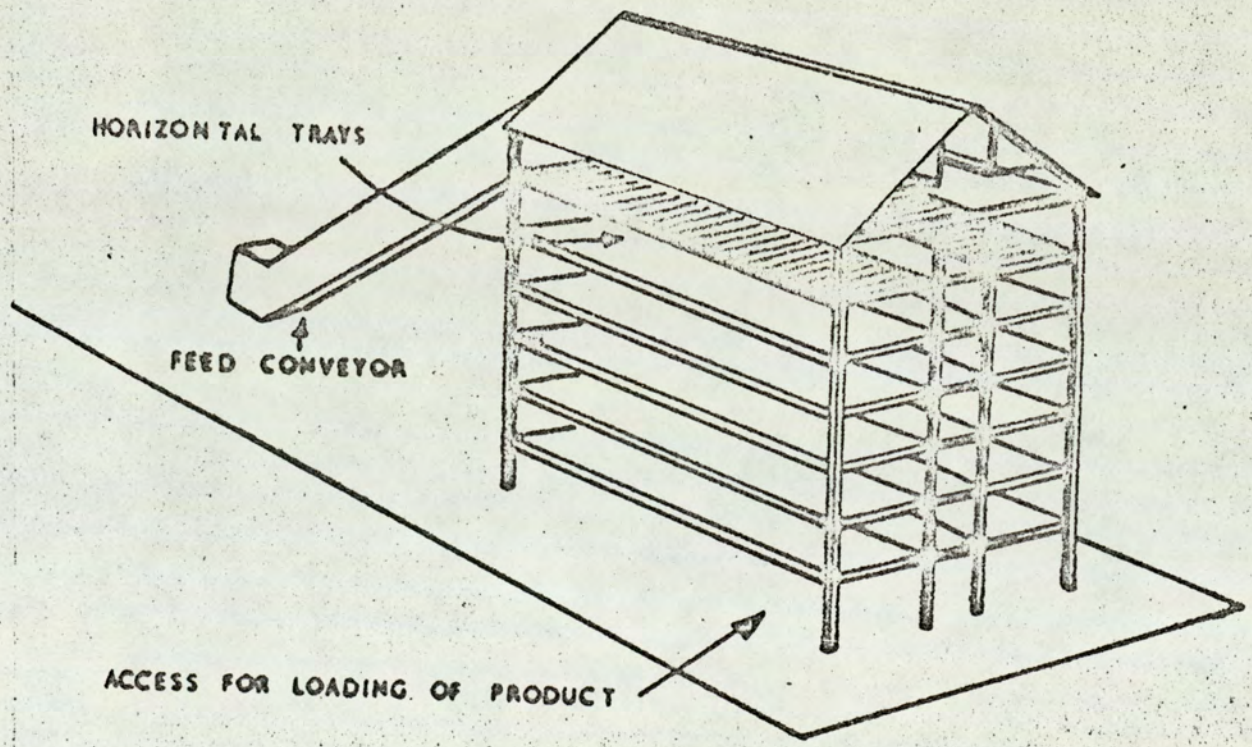
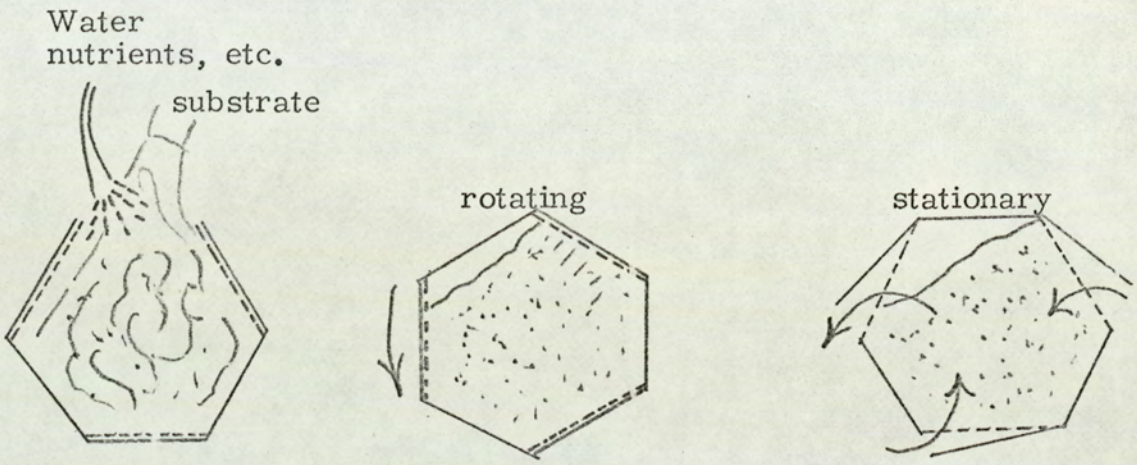
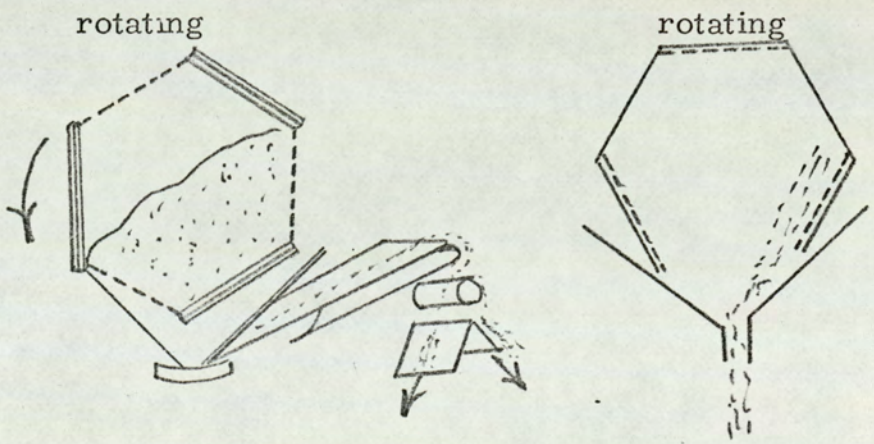


FIGURE 6

OPERATION OF FERMASCREEN UNIT



1. Charging
2. Mixing
3. Fermentation



4. Discharging product
5. Discharging oversize

FIGURE 8

DANO BIOSTABILIZER TYPE FERMENTER

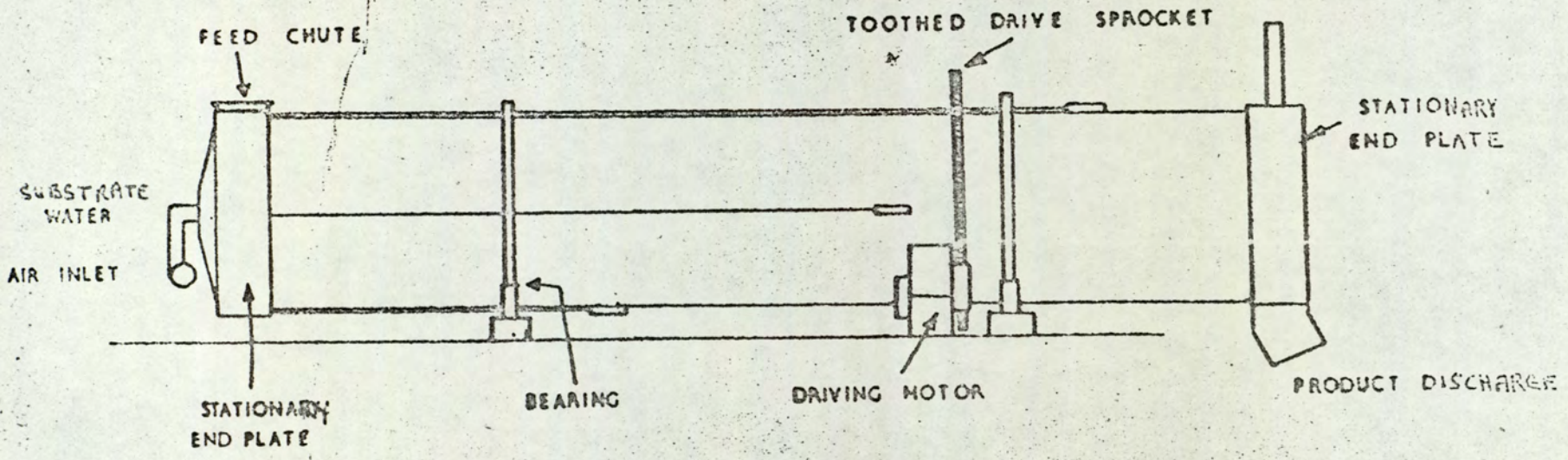


FIGURE 9

(26)

PERFORATED TRAY FOR FERMENTATION

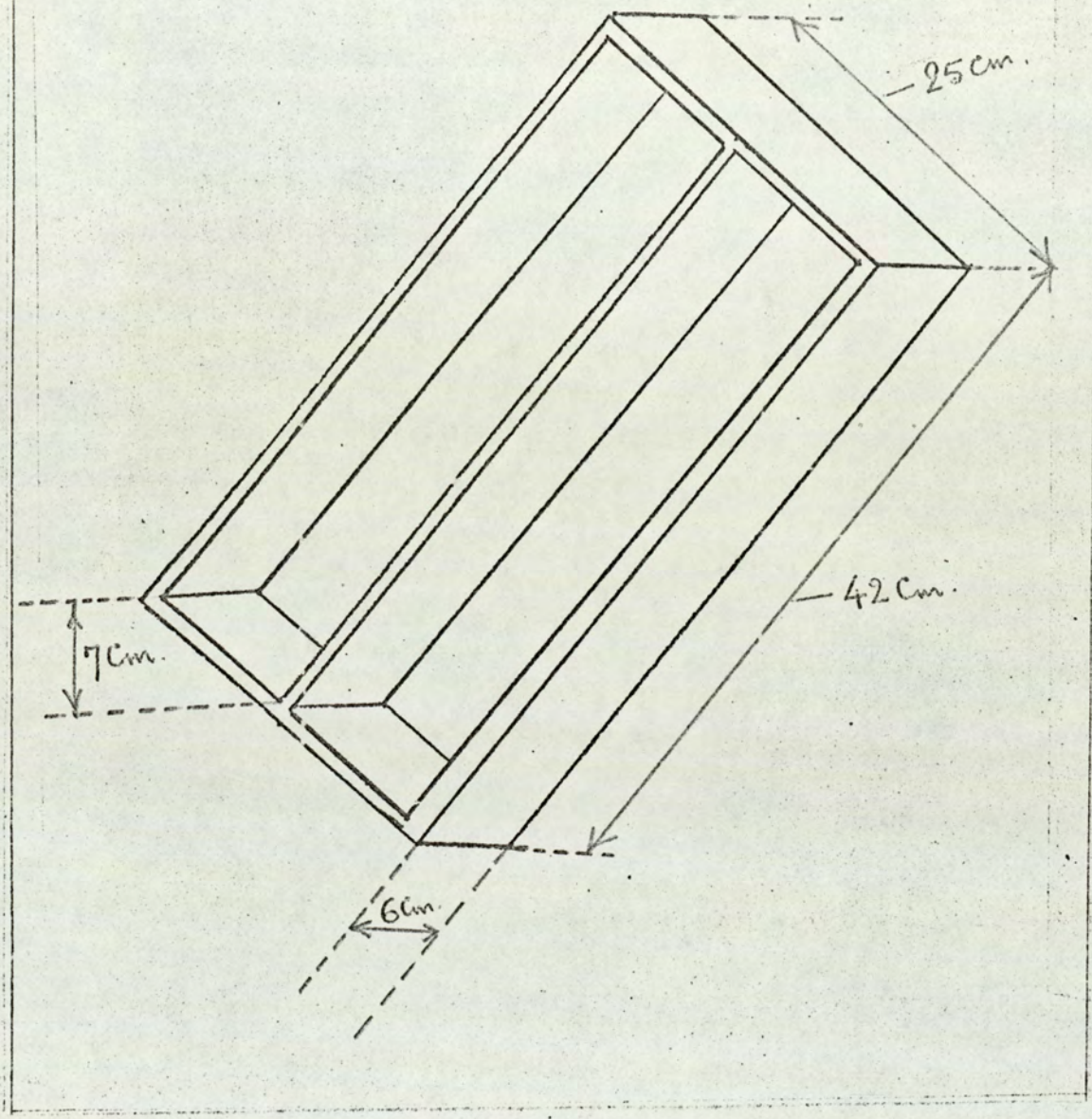


FIGURE 10

2. Selection of Microbial System

2.1. Reasons For Choice of Micro-organisms

For the microbial upgrading of cellulosic waste materials to produce protein rich animal feed the organisms used should have the following properties:-

1. The organism must be as highly cellulolytic as possible with the ability of rapidly degrading the raw material.

2. It must have a very high protein content with a wide spectrum of most of the essential amino-acids. These are necessary to produce a rich and balanced animal feed.

3. The organism must not produce toxic metabolites.

4. It must be able to grow rapidly on cellulose and simple, inexpensive salts.

A lot of research work on cellulolytic fungi has been carried out at the Biodeterioration Information Centre of the University of Aston. All the micro-organisms tested were either recommended by biologists based on previous experimental work in the Department or were picked from the literature because of their desirable properties. Particular consideration was given to thermophilic cellulolytic fungi

since they are known to degrade cellulose into simple sugars more rapidly than mesophiles. The thermophilic micro-organisms can grow well within the temperature range of 45°C to 55°C where 50°C is the acceptable temperature for many thermophiles. These thermophilic micro-organisms have an ecological advantage over the mesophilic organisms, since when cellulosic materials are degraded at 50°C, the chances of the system getting infected by mesophilic-cellulolytic micro-organisms are very remote. Cooney and Emerson[17] have reviewed the occurence, the general biology and other important properties of thermophilic micro-organisms. Some of the fungi are non-cellulolytic (e. g. Humicola lanuginosa, Thermoascus aurantiacus, Talaromyces emersonii, Talaromyces dupontii etc.), some mildly cellulolytic (e. g. Myriococcum albomyces, Malbranchea pulchella etc.) and a few are highly cellulolytic (e. g. Chaetomium thermophile, Humicola insolens, Humicola grisea, Torula thermophile, Sporotrichum thermophile, Cephalosporium). Although the slightly cellulolytic fungi do not grow very well on their own, they have, with non-cellulolytic fungi, been found in abundance with highly cellulolytic fungi in compost heaps. This suggests that mixtures of cellulolytic and non-cellulolytic microbes could grow very well together.

Malik[36] did a considerable amount of work on mesophilic cellulolytic fungi. He performed soil enrichment and colonisation studies of mesophiles and thermophiles. According to his results the most frequently occuring fungi at 25°C were Fusarium solani, Trichoderma viride. Chaetomium globosum was fairly common in the beginning but tended to decrease in frequency later. Dicoccum sp.

and Streptomyces sp. were also isolated throughout the period of incubation, whereas Papulaspora sp., Gliocladium roseum, Penicillium sp. and Aspergillus fumigatus appeared in the later stages of the biodeterioration.

Sharpe[37] (1970) studied various fungi on different substrates. He found that Chaetomium globosum, Dicoccum sp., Paecilomyces elegans and Aspergillus niger tended to be found on wood only. There was a higher incidence of some species on wood and they were Humicola grisea, Fusarium sp., Gliocladium roseum and Penicillium sp. Arthrobotrys sp. and Zygorhynchus moelleri tended to favour paper. Generally those fungal species that were most prevalent colonized the wood more frequently than the paper.

Sharpe[37] (1970) also studied the interaction of species. Of the isolates analysed it was found that if two inoculated species did not mix the normal level of deterioration observed with each fungus occurred. If the two species mixed then without exception the amount of decay in the area of mixing was equal to that normally produced by the most cellulolytic species of the two. No distinct antagonism (whereby the decay activity of a species was completely prevented) occurred and similarly no one fungus enhanced or inhibited all the fungi encountered. Penicillium sp., Fusarium sp., and Trichoderma viride gave no ordered pattern and probably reacted amphoterically according to the prevailing conditions of substrate and environment. Torula sp. tended to mix with the weakly or non-cellulolytic group and not with the strongly cellulolytic fungi. Papulaspora sp. and Streptomycetes sp. were capable of mixing but

microscopical examination revealed that they grew respectively under and over other species. A desirable feature of thermophiles is that since they grow at high temperatures the chance of the system getting infected is minimized. This is because many of the pathogenic organisms and most mesophiles cannot grow within the thermophilic temperature range.

Some fungi were selected because of their ability to produce antibiotic substances. This characteristic of a fungus could be very desirable for minimising infection when solid substrate fermentation has to be carried out under natural conditions or even under sterile conditions when an infecting organism's growth must be supressed. Bilai[38] has mentioned some antibiotic producing fungi which are also known to be cellulolytic. Some of these are listed in Table 3[38]. In some cases the antibiotic concentration is higher in the mycelium than in the culture fluid; e. g. the antibiotic Chaethomin is isolated from mycelium where it is almost twenty times as abundant as in the culture fluid. The biological significance of antibiotic production by different species of micro-organisms in natural associations has not yet been adequately explained. Their significance even for the producing micro-organisms is not clear. Under certain growth conditions in the soil many species of microfungi produce the same antibiotics as when they are cultured. Various species exhibit different antibiotic activity against phyto-pathogenic bacteria and fungi. They also inhibit the germination of spores of several species of rust fungi.

TABLE 3[38]

Fungi	Antibiotics
Penicillium chrysogenum Penicillium funiculosum	Penicillins, notatin Helenin
Aspergillus fumigatus Aspergillus humicola Aspergillus terreus	Fumigatin, helvolic acid, fumagillin Humicolin Geodin
Cephalosporium-acremonium	Cephalosporins P: P_1, P_2, P_3, P_4, P_5 Cephalosporin C.
Trichoderma lignorum	Gliotoxin, Viridin, Volatile antibiotics
Alternaria terreus Alternaria solani	Alternariol Alternaric acid, Alternarin
Fusarium lateritium Fusarium chlamydosporium	Lateritiins Chlamydosporin
Gliocladium roseum	Gliorosein
Chaetomium cochlioides	Chaethomin

2.2 Screening Tests With Barley Straw

In the screening of micro-organisms it was necessary

1. to observe their cellulolytic activity and
2. to estimate the amount of fungal protein produced using barley straw as the cellulose-based substrate.

Many techniques[2] to study cellulolytic activity are available and these are considered below. They include assessment of:

1. the increase in reducing sugar value
2. the effect on viscosity of soluble derivatives
3. the loss in weight of substrate
4. turbidimetry with a cellulose suspension
5. the oxygen uptake during the enzymic oxidation of glucose produced by hydrolysis
6. the changes in mechanical properties of the substrate and
7. the changes in bi-refringence of films.

In solid substrate fermentation only techniques 1, 3, 5, and 6 are applicable. Techniques 2, 4 and 7 are not feasible.

TABLE 4 (39)

Nutrient Solution

Rose Bengal	15 mls
Ammonium sulphate	0.5 gm.
L.-Aspergine	0.5 gm.
Potassium dihydrogen phosphate	1.0 gm.
Potassium chloride	0.5 gm.
Magnesium sulphate (hydrate)	0.2 gm.
Calcium chloride	0.1 gm
Yeast extract	0.5 gm.
(For plating Oxoid Agar	20.0 gms.)
Total volume [made up with distilled water]	1 litre

In the method involving analysis of reducing sugars the liquor should ideally be uniformly distributed throughout the mass. In solid substrate fermentation under thermophilic conditions the thorough mixing of solid mass and nutrient solution is very difficult to achieve. Hence this method was not used.

In order to apply method 6 one has to have a number of identical samples some for the initial strength tests and others to observe the decrease in mechanical strength due to microbial growth. From practical experience it was observed that two straw pieces rarely matched and so this technique for measuring cellulolytic activity was also discarded.

Thus the loss in weight method was the technique which seemed feasible. During the biodegradation of cellulosic raw material (i. e. straw) there is a gradual decrease of cellulose present in the raw material with the growth of micro-organisms. Hence the loss in weight of substrate may be regarded as an acceptable way of monitoring the cellulolytic activity of micro-organisms.

2. 2. 1. Initial Weight Loss Measurements

A simple screening test to study the cellulolytic activity of fungi by weight loss measurement was devised as explained below.

A known weight of straw (in triplicate) soaked in a nutrient solution (see table 4) was steam sterilized. The substrate was then allowed to cool down and transferred aseptically to an agar

TABLE 5

Coding of Thermophiles

Micro-organisms	Reference Code
Chaetomium thermophile	T_1
Sporotrichum thermophile	T_2
Torula thermophile	T_3
Humicola grisea	T_4
Humicola insolens	T_5
Cephalosporium thermophile	T_6
Malbranchea pulchella	T_7
Myriococcum albomyces	T_8
Thermoascus aurantiacus	T_9
Humicola lanuginosa	T_{10}
Talaromyces dupontii	T_{11}
Mucor pusillus	T_{12}
Talaromyces emersonii	T_{13}
Mucor meihei	T_{14}

plate. A standardised inoculum of test organism was cut using a cork-borer from a pure culture plate and was then placed on the substrate in three petri dishes. The inoculated petri dishes (in triplicate) were then incubated at the required temperature (48°C). After a number of days samples were removed and dried in an oven at 105°C for 24 hours. After the mycelium grown on the surface had been brushed off very gently, the straw was weighed and the percent loss in weight calculated separately for three samples. The average of percent loss of three samples was used for all purposes.

Experimentally it was found that the average moisture content of raw straw used was 7.9% (w/w). Another set of experiments was also carried out to measure the average increase in weight of straw due to the agar and nutrients in the solution. A figure of 3.7% (w/w) was obtained.

If A = initial weight of the sample (straw) and

B = final weight of the sample (after brushing off mycelium),

then

$$\text{percent loss in weight of straw} = \frac{0.921A - 0.963B}{0.921A} \times 100$$

As previously mentioned research work done at the Biodeterioration Information Centre indicated that thermophilic micro-organisms are more cellulolytic as well as faster in degrading cellulose than mesophiles. Hence the cellulolytic activity of a number of thermophiles were studied first. The species along with their codings are shown in table 5. Only T_1 to T_9 (inclusive) were tested.

TABLE 6

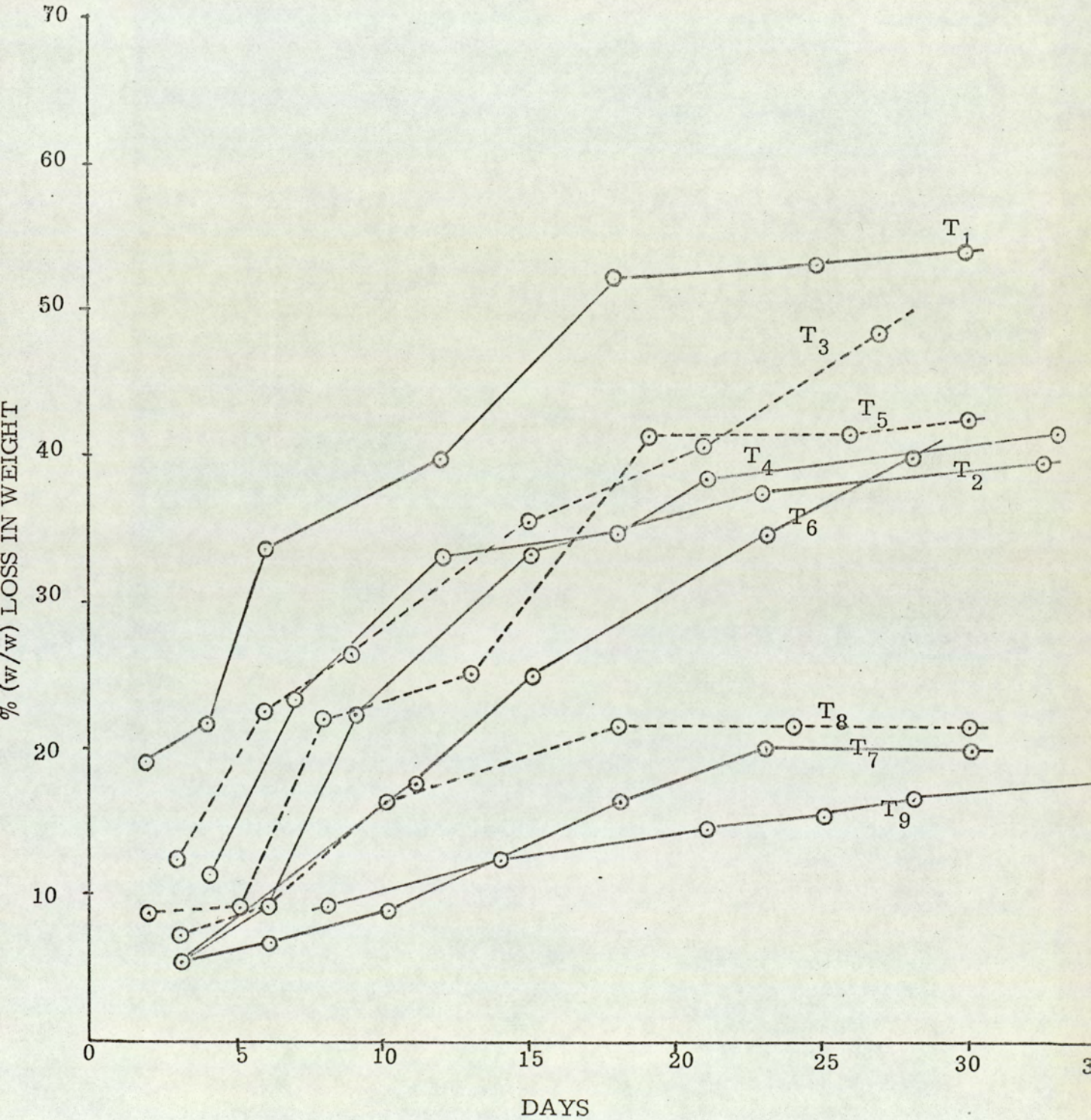
Percent Loss in Weight of Straw

Time	Fungi / Percent loss in weight								
days	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9
2	19.04				9.01				
3		5.51	12.44			5.42	5.99	7.27	
4	21.80			11.73					
5					9.19				
6	33.86	9.17	22.81				7.07	9.44	
7				23.95					
8					22.06				9.30
9		22.75	26.51						
10							9.02	16.23	
11						17.70			
12	39.63			33.10					
13					25.21				
14									12.17
15		33.51	35.46			25.20			
17							16.61	21.84	
18	52.10			34.90					
19					41.50				
21			40.80	38.36					14.65
23		37.20				34.73	20.09		
24								21.60	
25	53.00								15.47
26					41.68				
27			48.43						
28						40.07			16.86
30	54.00				42.70		19.97	21.40	
33		39.83		41.60					

FIGURE 12

Percent loss in weight of straw

in known duration of time



Of the others listed T_{12} and T_{14} are known to be pathogenic and the rest are non-cellulolytic. Plots of the percent loss in weight of straw against time (days) for every test organism are shown in figure 12. From the plots it is easy to assess the cellulolytic activity of the fungi i. e. the greater the loss in weight of substrate the more cellulolytic the test fungi are. From figure 12 it may be concluded that Chaetomium thermophile is the most cellulolytic followed, in decreasing order, by Torula thermophile, Humicola insolens, Humicola grisea, Sporotrichum and Cephalosporium.

Previous work carried out in the Biodeterioration Information Centre on the cellulolytic activity of fungi on an ideal substrate (3MM Whatman Chromatography paper) made use of the perfusion technique. The results obtained were comparable with those obtained with the weight loss method.

Mesophiles being many in number and inferior to thermophiles in their cellulolytic activity were not tested using this technique. Their screening was mainly based on the comparative ability to upgrade the protein content of straw (see section 2. 2. 3).

2. 2. 2. Selection and Development of a Protein Estimation Method

Proteins consist of discrete molecules of indeterminate length. These molecules contain a large, fixed number of the twenty or so amino acids, linked mostly "head to tail" in a chemically random, but genetically predetermined, sequence. One protein will differ from another not only in the arrangement of the individual amino-acids,

but also in their relative proportions. These individual amino-acids differ considerably one from another. Thus some contain integral ring structures (proline and hydroxyproline), which cause them to react differently to certain colorimetric tests. Some contain aromatic side chains (phenylalanine, tyrosine) and will give positive reactions for aromatic groups, as well as the more general reactions. In addition some contain aliphatic side chains (alanine, leucine) and are therefore hydrophobic, whilst others contain groups which may ionise to give positive (lysine, arginine and histidine) or negative (glutamic, aspartic acids, tyrosine) charges. These positive and negative charges per molecule depend on the pH of the solution. These differing properties tend to make the estimation of protein very difficult.

During the initial planning of the experimental work it was decided to test many fungi either as pure mono-cultures or as mixed cultures. Little is known about the proteins formed by individual micro-organisms and the problem is even more difficult when mixed cultures are used. Hence a standard curve representing the concentration of protein could not be prepared, thus making colorimetric analytical techniques unsuitable. The most commonly used techniques, for estimating protein are outlined below.

2. 2. 2. 1. Biuret Reaction. This reaction is given by protein with $CuSO_4$ in the presence of NaOH, and is caused by the complexing of Cu with CO - NH_2 peptide bond. Consequently it is stoichiometric with respect to the number of amino acid residues in the protein but not, of course, with respect to the molecular weight of the protein, because

of the differing molecular weights of the component amino acids. The sensitivity of the reaction is low too, requiring protein quantities of the order of 1 mg or more. Also, being a colorimetric estimation, a standard curve is required for each fungus or a combination of fungi; consequently it was considered unsuitable.

2. 2. 2. 2. Folin Ciocaltue Method. This sensitive method based on the Folin-ciocaltue phenol reagent[40] is very widely used for the estimation of particular proteins. This technique was made more reliable by Lowery[41]. Folin-Ciocaltue phenol reagent contains phosphomolybdic and phosphotungstic acids which react with the aromatic amino acids to give blue complexes. This is an extremely sensitive method but being colorimetric was not used.

2. 2. 2. 3. Ultra violet absorption at 280 mμ. Aromatic compounds absorb strongly in the ultraviolet region (tryptophan, tyrosine and to a minor extent phenylalanine). The method has the disadvantage that it measures only a few components of the system, and the tryptophan or tyrosine content can vary considerable from protein to protein. Thus protamine contains no tyrosine or tryptophan, and 1% chymotrypsin gives about seven times the reading of 1% tropomyosin. Finally being a colorimetric technique it was rejected for the reasons given above.

2. 2. 2. 4. Micro Kjeldahl Method[42]. This is the most convenient method for the determination of total organically bound nitrogen. This method involves incinerating the sample of protein with concentrated sulphuric acid and various catalysts. During the period of incineration

the protein is hydrated to amino acids which are then broken down, their nitrogen content being converted to ammonium sulphate. The ammonia is then distilled off from the reaction mixture under the influence of 40% NaOH, and is then estimated either:

1. by collection in standard acid, the remainder being back titrated with standard alkali,

or 2. by collection in boric acid when a complex is formed which may be titrated directly with standard acid,

or 3. by using the Nessler colorimetric reagent and referring the optical density of the yellow ammonium-mercuric complex to a standard curve.

The method is accurate with regard to nitrogen values, fairly rapid, cheap and easy. The main disadvantage is that all cell-nitrogen-compounds are included in the analysis.

Most proteins contain 16% nitrogen hence the amount of nitrogen estimated by this technique may be multiplied by a factor of 6.25 to get an estimate of the protein value of the sample. This is the only approximation involved in the technique. It was considered to be the only method suitable for estimation of protein. Details of this technique are given in Appendix 1.

2.2.3. Simplified Screening Test

Before carrying out screening tests by observing the increase in protein content of the system due to fungal growth it was

essential to select a simple fermenter. Some initial experiments were conducted using water jacketed, fixed-bed batch fermenters as well as a design in which mixing of substrate and inoculum was caused by air fluidisation. The advantages and disadvantages of these systems will be discussed in a later section. Eventually simple conical flasks (250ml) fitted with cotton plugs and placed in an incubator, where temperature, humidity, and air supply could be controlled, were selected. The main object of the series of experiments was to select those organisms which can grow rapidly on straw and at the same time yield high protein levels.

The experiments carried out are summarised below.

2.2.3.1. Experimental Programme

a. Single stage monoculture systems. Thermophiles and mesophiles.

b. Single stage mixed culture systems. Combinations of cellulolytic and non-cellulolytic micro-organisms under thermophilic conditions.

c. Two stage systems: a single thermophile in the first stage and a mesophilic pure culture in the second stage.

d. Two stage systems: a mixed culture of (cellulolytic and non-cellulolytic) thermophiles in the first stage

with a single mesophile active in the second stage.

e. Two stage systems: a single culture (thermophile) in the first stage and mixed cultures of (cellulolytic and non-cellulolytic) mesophiles in the second stage.

f. Two stage systems: mixed cultures active at every stage.

2. 2. 3. 2. Experimental Method

250 ml conical flasks were used as the fermenters. About 10 gm. barley straw, cut into small lengths ($\frac{1}{2}$ to 1 mm), were placed in each fermenter and then the straw was soaked in nutrient and salt solution. The composition of this solution is given in table 3. Then the flasks with their contents were steam sterilized and allowed to cool down. After draining off the excess solution aseptically, the straw was then inoculated with the desired strain of micro-organism from a slope culture. The flasks were then placed in an incubator at a pre-decided temperature: 48^{o} to 50^{o}C was the range suggested by the microbiologists at the Biodeterioration Information Centre. The optimum temperature for mesophilic growth was recommended as 26^{o}C. In previous research done at the Centre it was observed that variations of pH between 5. 5 to 6. 2 had little effect on the growth of fungi. Hence the pH of nutrient salt solution used was 5. 8.

Samples of straw were then removed at intervals over a period of 4 to 5 weeks and analysed for protein (Appendix 2).

TABLE 7

Coding of Mesophiles

Micro-organisms	Reference Code
Chaetomium globosum	M_1
Graphium sp.	M_2
Trichoderma viride	M_3
Fusarium sp.	M_5
Dicoccum sp.	M_6
Penicillium sp.	M_7
Chaetomium subbafine	M_8
Poria placenta	M_9
Polyporus tuliferus	M_{10}
Poria xantha	M_{11}
Polyporus anceps	M_{12}
Lenzite trabea	M_{13}
Poria rigria	M_{14}
Epicoccum nigrum	M_{16}
Xylaria polymorpha	M_{17}
Chaetomium cochliodes	M_{18}
Polyporus versicolor	M_{19}
Porialenmaquiata	M_{20}
Chaetomium aureum	M_{21}
Chaetomium angustipirale	M_{22}
Chaetomium affine	M_{23}
Chaetomium olivacium	M_{24}
Chaetomium trilaterale	M_{25}
Chaetomium crispatum	M_{26}
Humicola grisea mesophilae	M_{27}
Chaetomium funiculum	M_{28}
Aspergillus niger	M_{29}

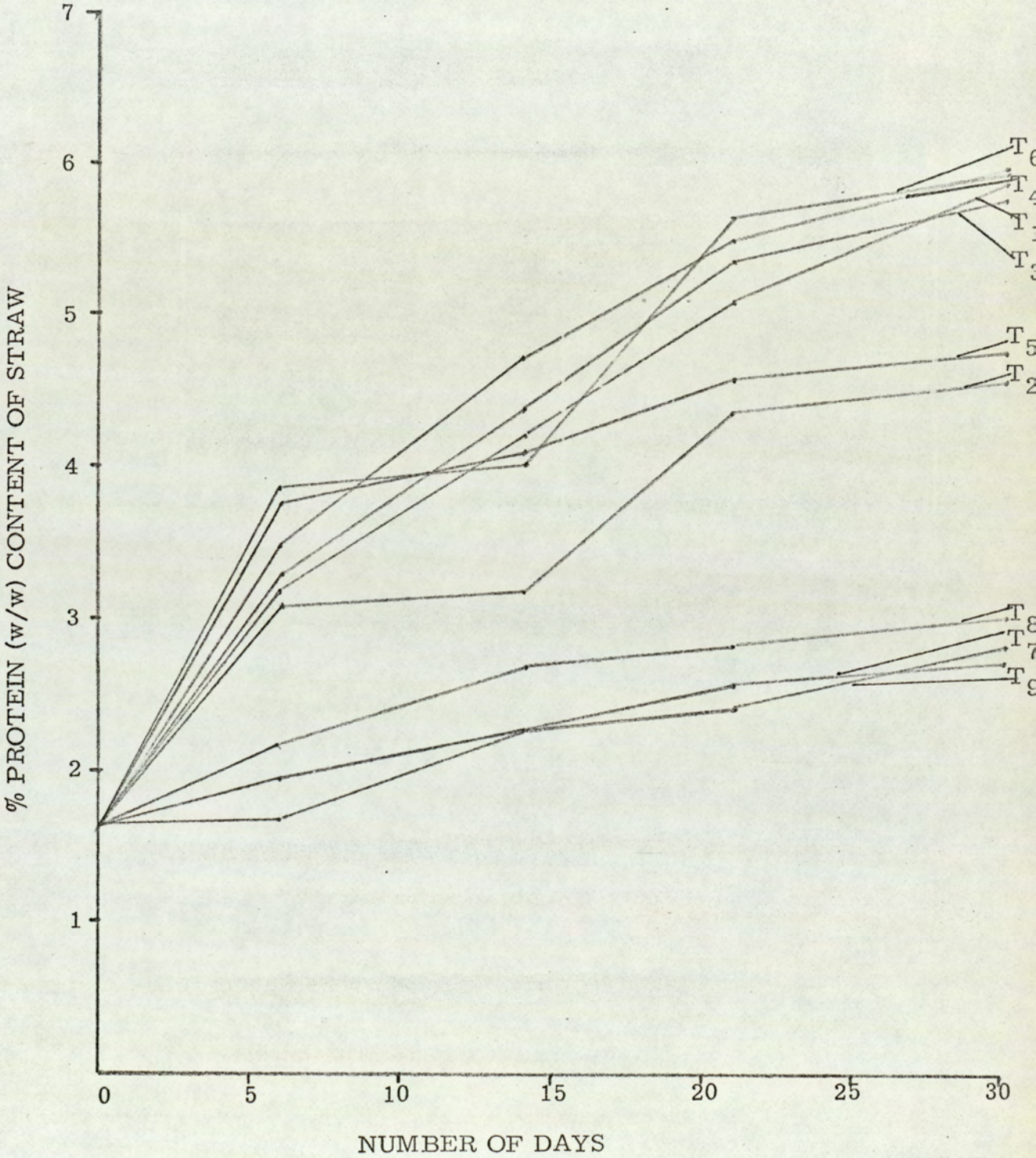
TABLE 8

Single stage thermophilic mono culture system

Fungus	% Protein in following days			
	6	14	21	30
T_1	3.17	4.20	5.05	5.85
T_2	3.08	3.17	4.33	4.59
T_3	3.26	4.35	5.33	5.73
T_4	3.45	4.70	5.49	5.98
T_5	3.75	4.07	4.59	4.72
T_6	3.85	4.01	5.61	5.93
T_7	1.95	2.28	2.57	2.70
T_8	2.14	2.65	2.80	3.00
T_9	1.68	2.25	2.40	2.80

FIGURE 13

Protein upgrading of straw by thermophilic fungi in single stage system



2. 2. 3. 1. a. Single stage monoculture systems

Thermophiles

Nine thermophiles were studied over a period of thirty days for their ability to upgrade the protein content of straw. The organisms used are shown in table 5 (T_1 to T_9). In this system cellulose is broken down into soluble simple sugars which are then converted into fungal mycelia. At the same time nitrogen compounds are converted into protein and in this way the protein level of the straw/mycelia mixture increases. Results obtained are tabulated in table 8 and plotted in figure 13.

Mesophiles

Twenty-six mesophiles (M_1 to M_{26} in table 7) were used to inoculate straw. The increase in protein content was observed over a period of about five weeks. The results obtained are presented in table 9 and plotted in figure 14.

2. 2. 3. 1. b Single stage mixed culture systems

In a monoculture system, whether it be mesophilic or thermophilic, the growth of the micro-organisms on straw depends on their cellulolytic activity i. e. their ability to break down cellulose into simple sugars and then to use these as a carbon source for growth. Now it is known that micro-organisms which cannot degrade cellulose grow well in systems containing cellulolytic micro-organisms. This may be because some of the simple sugar molecules produced extra-cellularly by the cellulolytic microbes diffuse into the cells of the

TABLE 9

Single stage mesophilic monoculture system

Fungus	% Protein in following days			
	6	14	21	30
M_1	2.85	3.50	3.80	4.50 -
M_2	2.24	2.45	3.60	4.13
M_3	2.52	2.57	2.90	3.20
M_4	2.86	3.32	3.50	3.70
M_5	2.01	2.80	3.00	3.15
M_6	2.81	3.46	3.50	3.82
M_7	2.40	2.58	2.80	3.10
M_8	2.95	3.63	3.90	4.10
M_9	2.30	2.80	3.44	3.50 -
M_{10}	1.95	2.40	2.70	3.00 -
M_{11}	1.80	2.25	2.84	2.85
M_{12}	1.90	2.30	2.70	3.20
M_{13}	2.10	2.65	2.87	3.60
M_{14}	1.70	2.10	2.49	3.50
M_{15}	2.18	2.25	2.32	3.80
M_{16}	3.24	3.36	3.85	4.20
M_{17}	3.06	3.17	3.40	4.17
M_{18}	2.97	3.40	3.80	4.42
M_{19}	2.26	2.56	2.70	3.06
M_{20}	2.91	3.05	3.20	3.47
M_{21}	2.54	3.84	4.50	6.46 -
M_{22}	1.96	2.88	5.10	6.37
M_{23}	2.07	2.83	5.62	6.16 -
M_{24}	2.89	3.14	4.45	5.60 +
M_{25}	2.53	3.64	4.60	6.24
M_{26}	3.12	3.36	4.78	5.16 -

FIGURE 14

Protein upgrading of straw using mesophilic fungus in single stage system

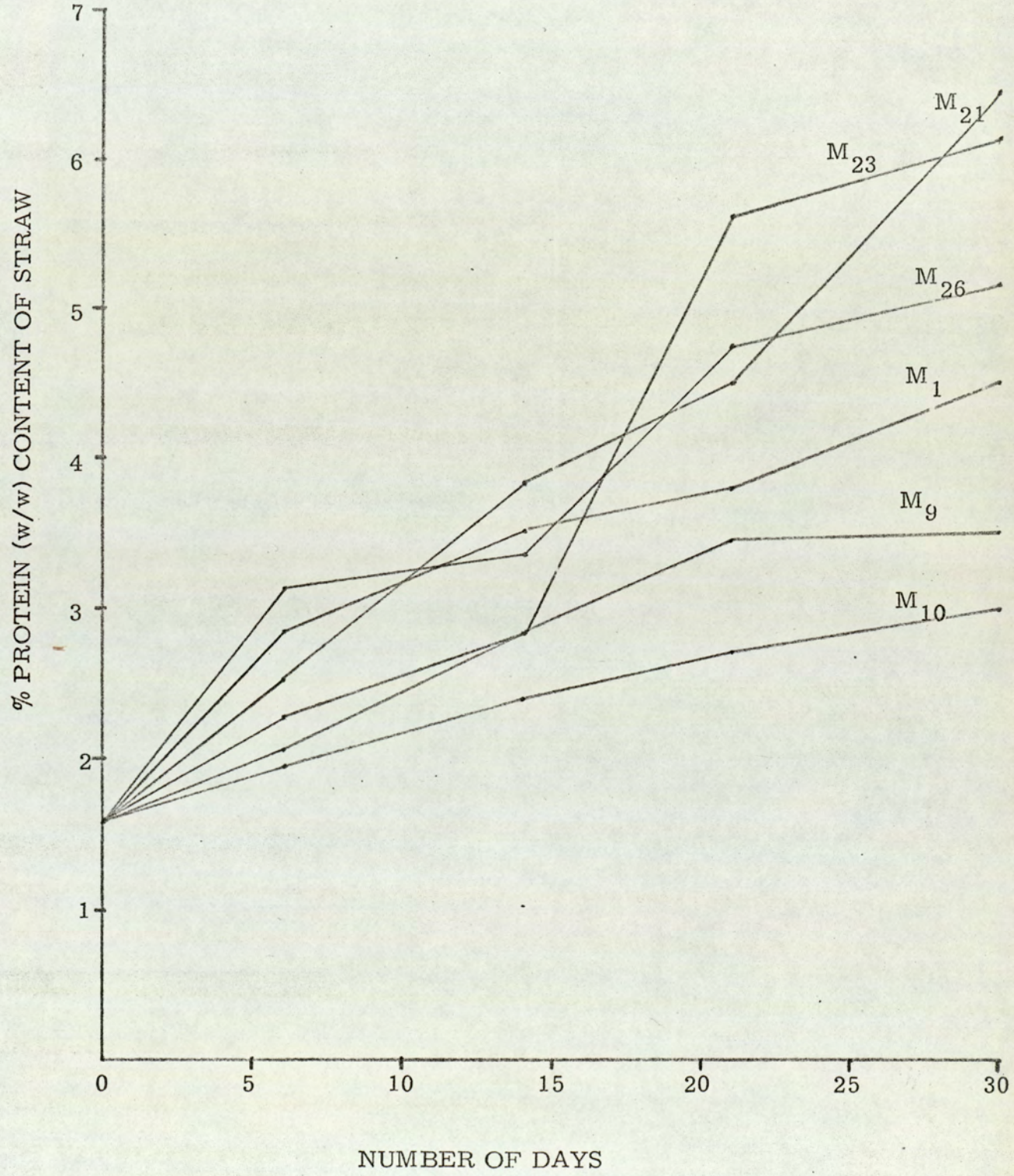


TABLE 10

Single stage thermophilic mixed culture systems

Micro-organisms	% Protein in following days			
	6	14	21	30
$T_1 + T_{10}$	2.18	5.15	6.21	6.01
$T_1 + T_{11}$	2.40	3.26	4.25	5.59
$T_1 + T_{13}$	3.70	4.30	5.90	7.95
$T_1 + T_{14}$	3.80	5.40	6.10	7.55
$T_1 + T_{12}$	4.36	5.60	6.67	8.50
$T_4 + T_{11}$	3.14	5.10	5.72	6.28
$T_2 + T_{12}$	3.80	4.18	4.81	5.28
$T_3 + T_{13}$	3.15	3.75	4.45	4.81
$T_6 + T_{14}$	2.80	3.96	4.80	6.04

FIGURE 15

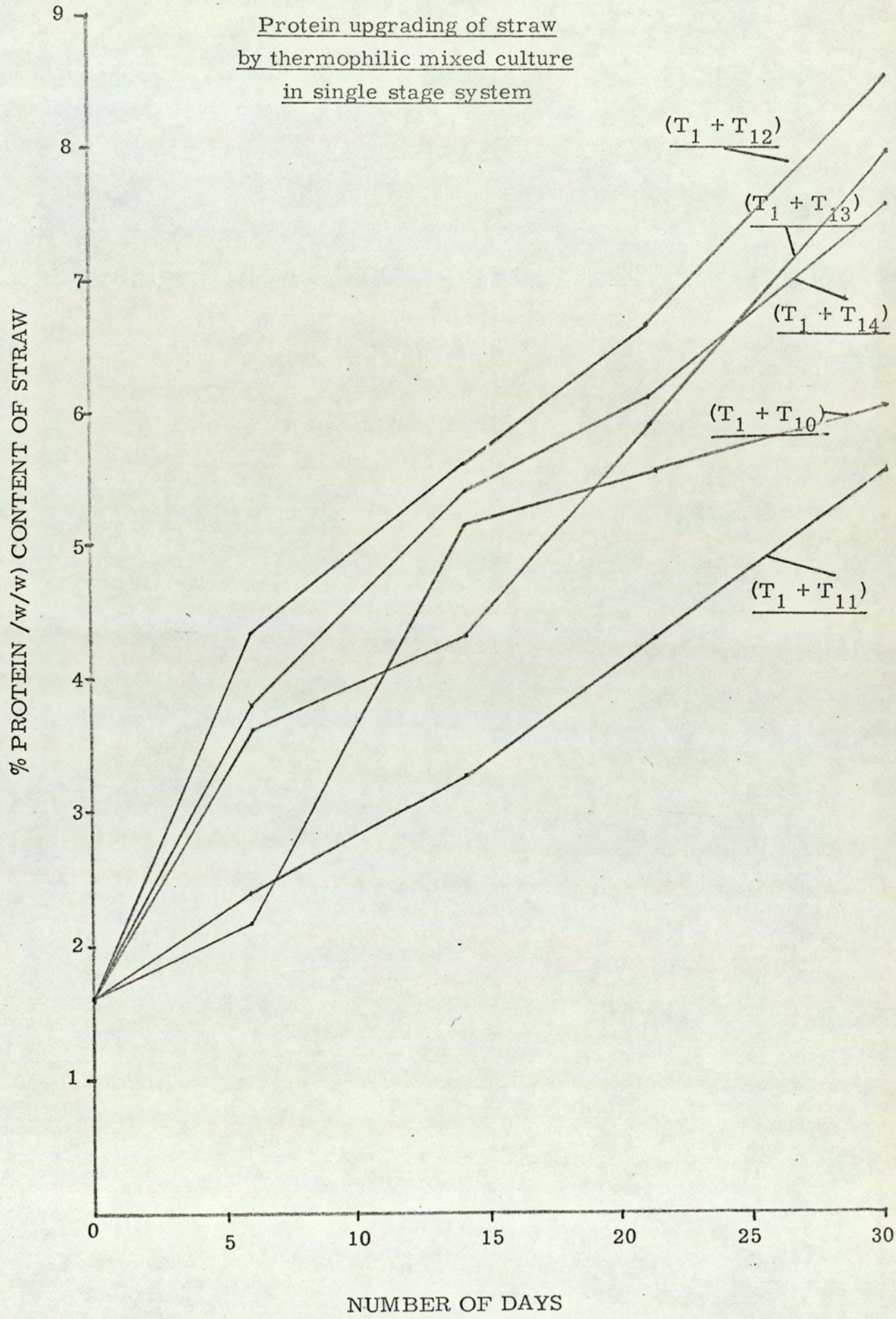

Protein upgrading of straw
by thermophilic mixed culture
in single stage system
(T1 + T12)
(T1 + T13)
(T1 + T14)
(T1 + T10)
(T1 + T11)
% PROTEIN /w/w) CONTENT OF STRAW
9
8
7
6
5
4
3
2
1
0
5
10
15
20
25
30
NUMBER OF DAYS

TABLE 11

Two stage systems with single culture active at each stage

Micro-organism in first stage 48 - 49°C	%Protein in following days		Micro-organism in second stage 26°C	% Protein in following days	
	6	14		21	30
T_4	3.20	4.19	$T_4 + M_{13}$	5.19	7.28
T_2	3.70	4.12	$T_2 + M_{23}$	4.72	6.31
T_1	3.25	4.37	$T_1 + M_{27}$	5.20	5.51
T_1	3.28	4.20	$T_1 + M_8$	4.39	6.34
T_3	3.34	3.93	$T_3 + M_{28}$	4.93	6.26
T_6	3.05	3.17	$T_6 + M_{26}$	4.59	5.15
T_1	2.76	3.17	$T_1 + M_1$	4.05	5.20
T_1	2.46	3.35	$T_1 + M_3$	4.31	5.37

FIGURE 16

Protein upgrading of straw in two stage system with single culture active in each stage

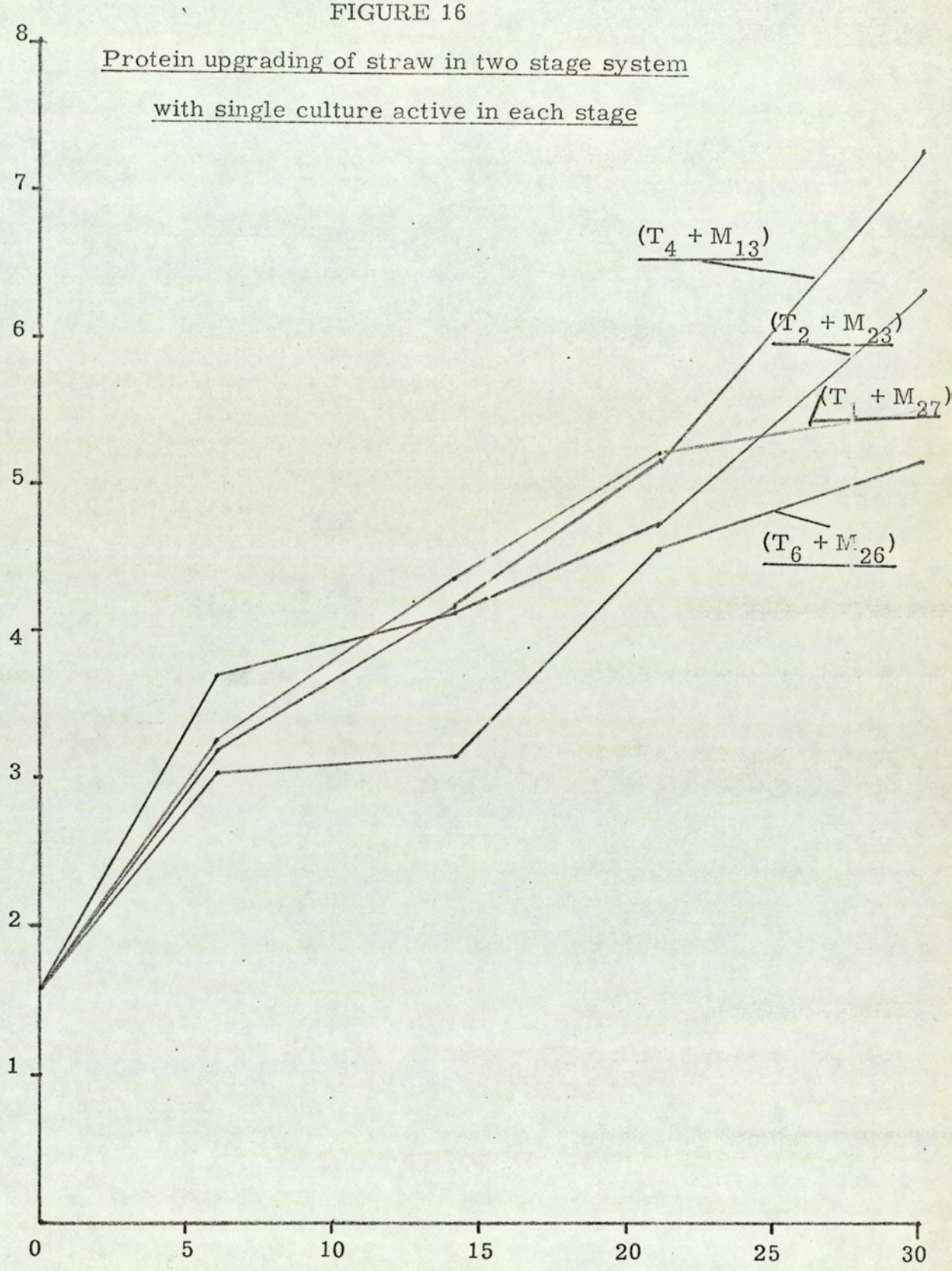


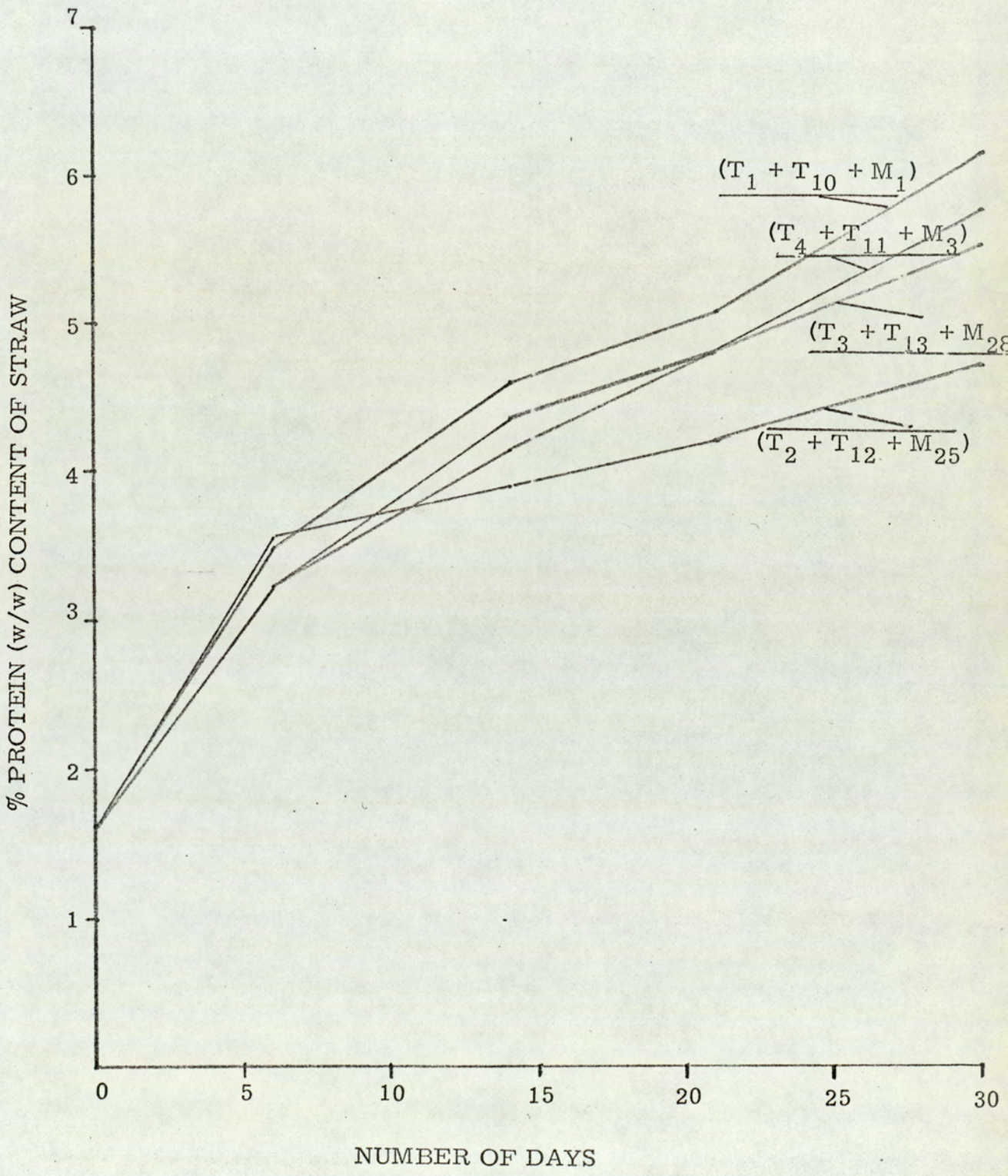
TABLE 12

Two stage system with thermophilic mixed culture active in first stage and mesophilic mono culture in second stage

Micro-organisms in first stage 48 - 49°C	% Protein in following days		Micro-organisms in second stage 26°C	% Protein in following days	
	6	14		21	30
$T_1 + T_{10}$	3.50	4.60	$T_1 + T_{10} + M_1$	5.08	6.15
$T_3 + T_{13}$	3.26	4.16	$T_3 + T_{13} + M_{28}$	4.80	5.53
$T_2 + T_{12}$	3.54	3.90	$T_2 + T_{12} + M_{25}$	4.20	4.70
$T_4 + T_{11}$	3.27	4.35	$T_4 + T_{11} + M_3$	4.80	5.76

FIGURE 17

Protein upgrading of straw in two stage system with mixed culture active in first stage and single culture in second stage



non-cellulolytic fungi. Consequently it may be possible to break down cellulose and convert it into fungal protein more efficiently using a mixed culture system. This set of experiments was planned to test the above approach. Nine experiments involving combinations of cellulolytic and non-cellulolytic micro-organisms under thermophilic conditions were carried out. The results obtained are shown in table 10 and plotted in figure 15.

2. 2. 3. 1. c. Two stage systems with single culture active at each stage

It is known from previous experimental work that thermophilic fungi can breakdown cellulose at a very fast rate. Hence once the substrate is degraded into simple saccharides mesophilic organisms could be used to inoculate the system to convert the simple sugars into fungal protein. In this series of experiments one thermophilic cellulolytic fungus was active for 14 days in the first stage and a mesophilic cellulolytic fungus was active in the second stage for the remainder of the fermentation. Eight different combinations were tried and results obtained are given in the table 11 and plotted in figure 16.

2. 2. 3. 1. d. Two stage system with mixed culture in the first stage and pure culture in the second stage.

A mixture containing a cellulolytic and a non-cellulolytic micro-organism in the thermophilic (first stage) and a single mesophilic culture was used in the second stage. Four different combinations were tried. The results are given in table 12 and plotted in figure 17.

2. 2. 3. 1. e.

Two stage systems with single culture active in the first

(thermophilic) stage and mixed culture (cellulolytic and non-cellulolytic) active in the second (mesophilic) stage. This series was a modified form of previous ones. Aspergillus niger (M_{29}) was used as the non-cellulolytic mesophile throughout, six different combinations were used and the results are shown in the table 13 and plotted in figure 18.

2. 2. 3. 1. f.

Two stage systems with mixed cultures active in both stages. This is the most complex series in which a non-cellulolytic culture was made to grow with a cellulolytic culture in the thermophilic stage (first) and also in the mesophilic (second) stage. Four different combinations were tried and the results are summarised in table 14 and plotted in figure 19.

TABLE 13

Two stage systems with thermophilic pure culture in the first stage and mixed culture in the second stage

Micro-organisms in first stage 48 - 49°C	% Protein in following days		Micro-organisms in second stage 26°C	% Protein in following days	
	6	14		21	30
T_4	3.26	4.06	$T_4+M_{13}+M_{29}$	5.03	7.65
T_2	3.27	4.36	$T_2+M_{23}+M_{29}$	4.58	6.64
T_4	3.15	4.20	$T_4+M_{28}+M_{29}$	5.67	7.62
T_6	3.80	4.60	$T_6+M_{21}+M_{29}$	5.56	7.66
T_1	3.34	4.65	$T_1+M_1+M_{29}$	5.85	6.55
T_1	3.45	4.72	$T_1+M_3+M_{29}$	5.17	5.66

FIGURE 18

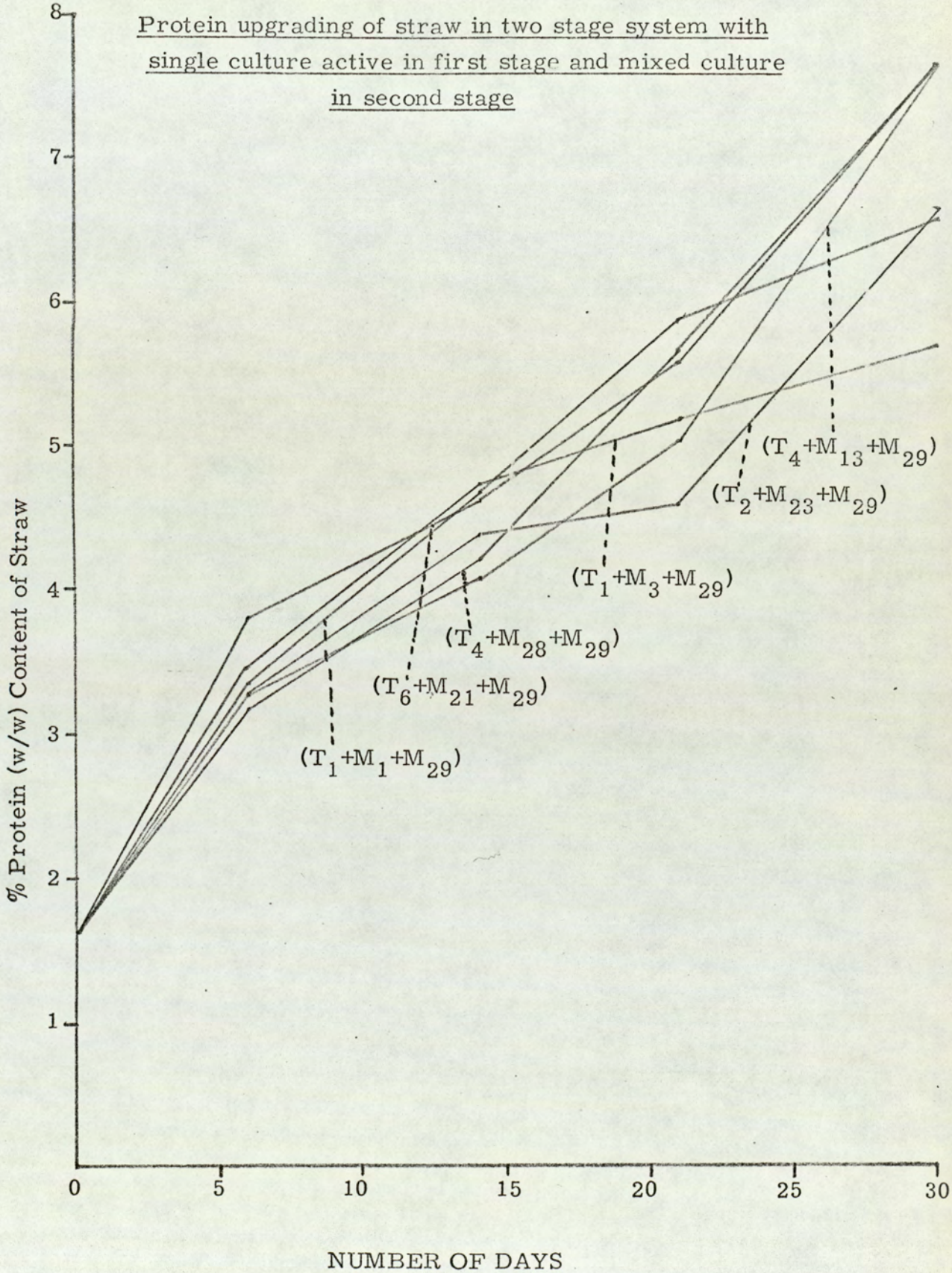

Protein upgrading of straw in two stage system with single culture active in first stage and mixed culture in second stage
% Protein (w/w) Content of Straw
NUMBER OF DAYS
0
5
10
15
20
25
30
1
2
3
4
5
6
7
8
(T1+M1+M29)
(T6+M21+M29)
(T4+M28+M29)
(T1+M3+M29)
(T2+M23+M29)
(T4+M13+M29)

TABLE 14

Two stage system with mixed culture in both

Micro-organisms in first stage 48 - 49°C	% Protein in following days		Micro-organisms in second stage 26°C	% Protein in following days	
	6	14		21	30
$T_1 + T_{10}$	3.29	4.25	$T_1+T_{10}+M_1+M_{29}$	6.69	7.94
$T_3 + T_{13}$	3.98	4.41	$T_3+T_{13}+M_{28}+M_{29}$	5.36	5.62
$T_2 + T_{12}$	3.37	3.81	$T_2+T_{12}+M_{25}+M_{29}$	4.20	4.31
$T_4 + T_{11}$	3.76	4.48	$T_4+T_{11}+M_3+M_{29}$	5.47	6.30

FIGURE 19

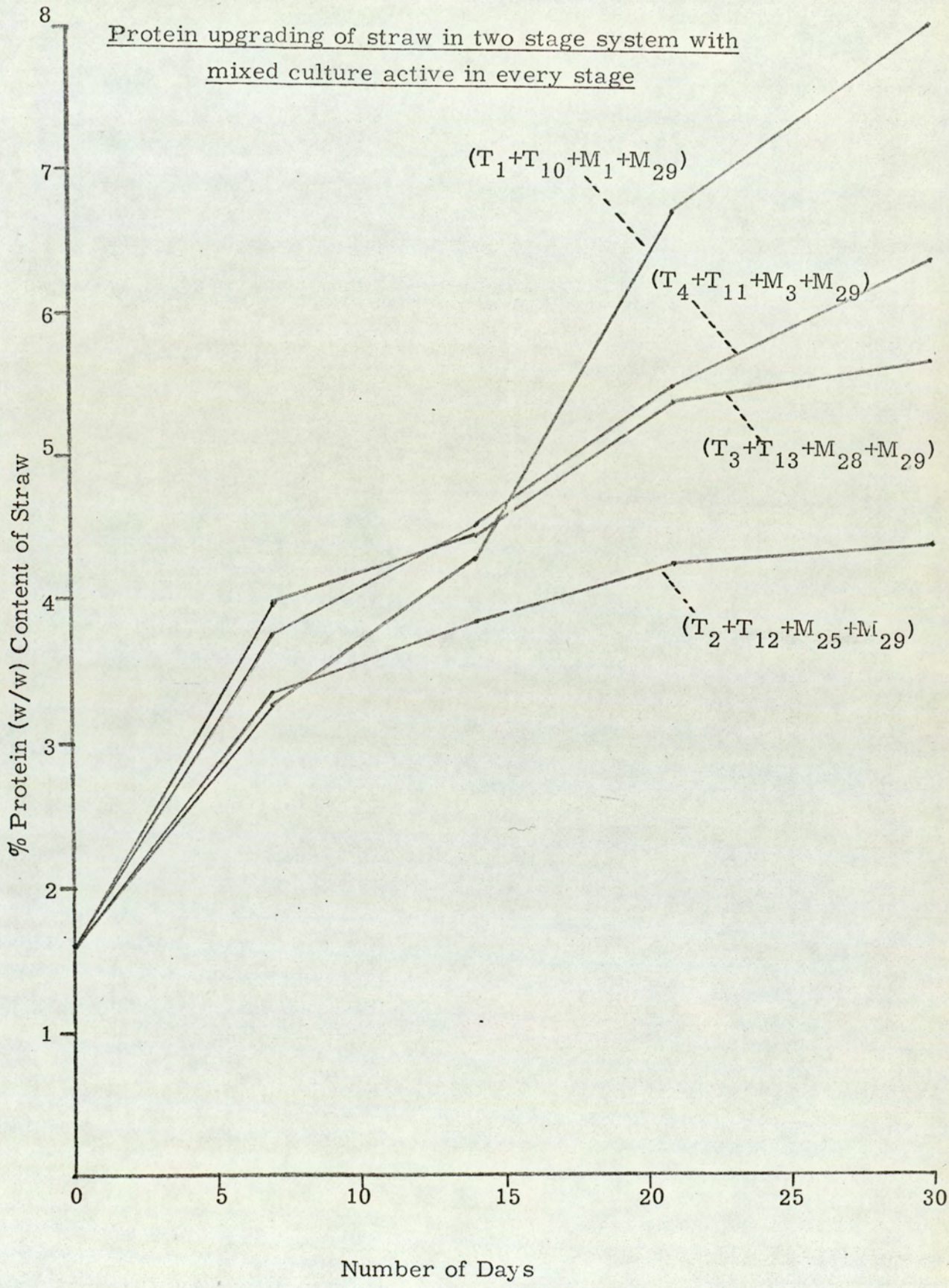

Protein upgrading of straw in two stage system with mixed culture active in every stage
(T1+T10+M1+M29)
(T4+T11+M3+M29)
(T3+T13+M28+M29)
(T2+T12+M25+M29)
% Protein (w/w) Content of Straw
Number of Days
0
5
10
15
20
25
30
1
2
3
4
5
6
7
8

TABLE 15

Comparison between cellulolytic breakdown and protein level

Thermophilic fungus	Protein content after 30 days %(w/w)	Weight loss of straw after 30 days % (w/w)
T_1	5.85	54
T_2	4.59	39
T_3	5.73	51
T_4	5.98	41
T_5	4.72	42
T_6	5.93	42
T_7	2.70	20
T_8	3.00	22
T_9	2.80	17

2.2.4 Discussion and Design Implications

Before analysing the experimental results in depth it is worth carrying out a theoretical material balance on the process as shown in the Figure 20. The maximum protein content of dried solid at the completion of such a process amounts to about 10% (w/w). In some cases the protein figures obtained were very low compared to this (10%) theoretical maxima. This may be due to either:-

1. the low cellulolytic nature of the fungi used

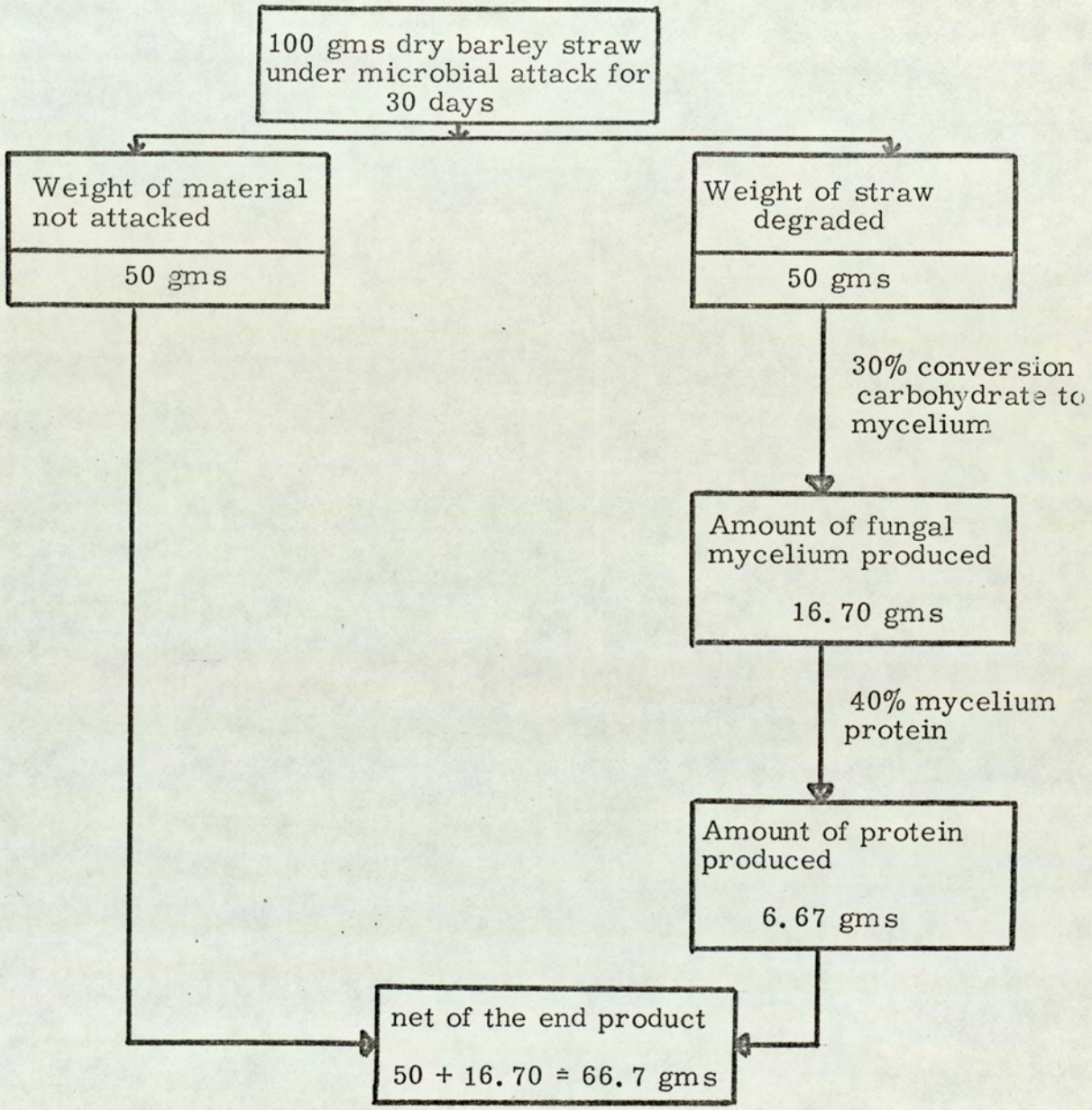
or 2. the use of sub-optimal conditions

or 3. some of the spores and mycelia being lost in preparing the sample for analytical purpose.

In the single stage thermophilic monoculture system nine micro-organisms were tested. It was observed that by the end of three weeks the rate of protein upgrading was slowing down and, thereafter, there was only a slight change in protein content. Chaetomium thermophila, Torula thermophila, Humicola grisea and Cephalo sporium produced the maximum amount of protein as shown in the table 8. Now if these results are compared with their cellulolytic nature (table 15), a very interesting trend may be observed. From table 15 it may be noted that the most cellulolytic fungus, Chaetomium thermophile, also increases the protein content of straw to a maximum level. In the same way other fungi which are very highly cellulolytic in nature also increase the protein content of straw to a high level.

FIGURE 20

Theoretical Material Balance



$\therefore$ % Protein of the end product (w/w dry wt. basis) $= \frac{6.67}{66.7} \times 100$

$= 10$

$\therefore$ Theoretically the amount of protein in the end product on dry weight basis will be $= 10\%$ (w/w)

In single stage monoculture systems mesophiles behaved in a very interesting way. Some of these, Chaetomium sp., produced protein figures between 6% to 7% (w/w) [M_{21}, M_{22}, M_{23}, M_{25}]. These levels are higher than those obtained with the thermophiles. But mesophiles are slower in breaking down cellulose during the first two weeks. The rate of breakdown in fact is almost linear with time. From the results (table 9) and graphs (Figure 14) for some of the mesophiles it is clear that the process of biodegradation could have been carried out for up to five weeks.

In the case of the single stage mixed culture thermophilic system nine combinations of cellulolytic and non cellulolytic fungi were tested. It was found that this system produced the maximum amount of protein at a relatively rapid rate Some of the results obtained (table 10) have been plotted in figure 15.

Four different types of two stage systems were tested:

a. where a single culture is active in every stage,
b. where a mixed culture is active in the first stage and a single culture in the second stage,
c. where a single culture is active in the first stage and a mixed culture in the second , and
d. where a mixed culture is active in both stages.

Results obtained are shown in tables 11, 12, 13 and 14. These are also expressed graphically in the figures 16, 17, 18, and 19.

Although results obtained with the two stage systems are as good as those with the single stage mixed culture thermophilic systems, the latter type of system is preferred for the following reasons:

In the two stage system each stage has to be maintained at a different temperature, hence to use both the stages efficiently the residence time of the substrate may increase considerably. The temperature change also makes the job of the process engineer more complicated since the humidity, heat losses and moisture of the fermenting mass will have to be controlled accordingly whereas in the single stage system the steady state may be achieved relatively easily. On the other hand in the case of single stage thermophilic mixed culture systems an end product with maximum amount of protei may be produced. Since this kind of system is maintained under thermophilic conditions biodegradation of cellulosic materials is more rapid and the chances of the fermenting mass becoming infected are relatively less. Hence a single stage mixed culture thermophilic system was found to be more desirable.

2. 2. 5 Comparative Effect of Sugar and Starch on the Growth of Fungi

The main object of this series of experiment was to observe any changes in the rate of growth of micro-organisms in the initial stage of fermentation following the addition of various carbon sources to the system. It was hoped that such additions might reduce the fermentation time. It was suggested by the microbiologists in the project team that initially some fungi might grow very well on glucose whereas others might prefer to grow on starch. Thus the fungi would be expected to attack firstly glucose and/or starch and secondly the cellulose present in the straw. It was expected that in a very short period of time the additional carbon source would be consumed thus providing a large amount of well distributed inoculum for cellulolytic action. Growth of micro-organisms when grown under these conditions and in the presence of nutrients may be compared with these when grown only on straw in the presence of the usual salt solution by estimating the protein upgrading of straw.

2. 2. 5. 1. Experimental Method

It was decided to grow six mesophilic chaetomium species under identical conditions but in the presence of two different nutrient salt solutions. The first set was grown using ordinary nutrient salt solution and, in the case of the second set, 0. 1% (w/v) of soluble starch and 0. 1% w/v glucose were added to the nutrient salt solution. These experiments were set up in conical flasks (250 ml) as in the previous experiments (section 2. 2. 3. 1.)and incubated at 25^{o}C. The samples were removed at known intervals of time and analysed for

TABLE 16

Comparative growth of micro-organisms on straw using E & P medium with and without glucose and starch

Days / %Protein		6	14	21	30
Chaetomium aureum	-	2.54	3.84	4.50	6.46
	G + S*	2.05	3.92	5.42	6.38
Chaetomium angustipirale	-	1.96	2.88	4.10	6.37
	G + S	2.11	2.90	4.66	5.89
Chaetomium affine	-	2.07	2.83	5.62	6.16
	G + S	2.24	3.11	4.98	5.80
Chaetomium olivaceum	-	2.89	3.14	4.45	5.60
	G + S	3.24	3.84	4.68	5.25
Chaetomium trilaterale	-	2.53	3.64	4.60	6.24
	G + S	3.19	3.69	5.27	6.28
Chaetomium crispatium	-	3.12	3.36	4.78	5.16
	G + S	3.35	4.15	5.20	5.80

* G + S = 0.1% w/v glucose + 0.1% w/v starch

FIGURE 21

CHAETOMIUM AUREUM

Comparative effect of the presence of glucose and starch in growth medium on fungal protein in the end product

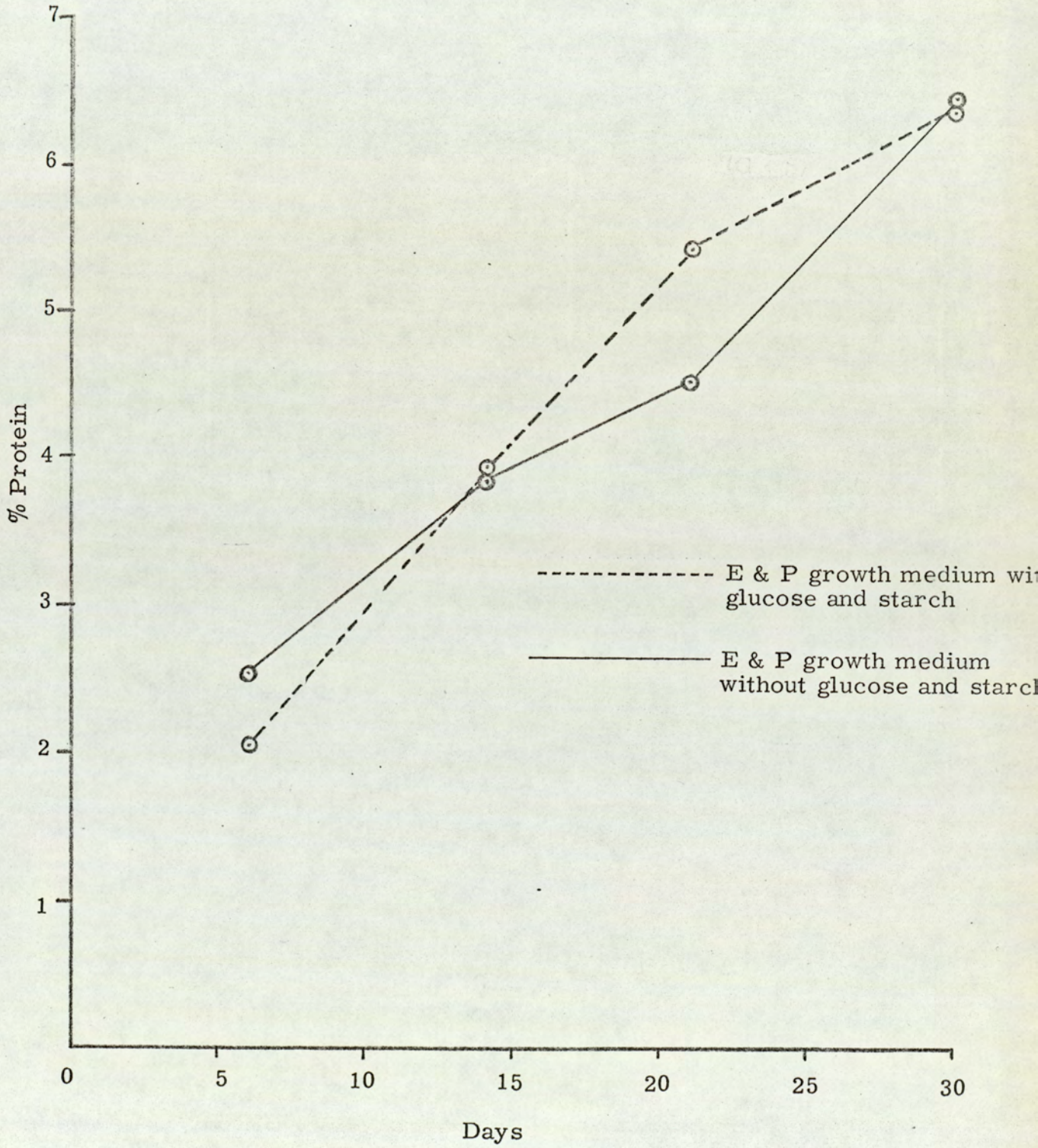


FIGURE 22

CHAETOMIUM ANGUSTIPIRALE

Comparative effect of the presence of glucose and starch in growth medium on fungal protein in the end product

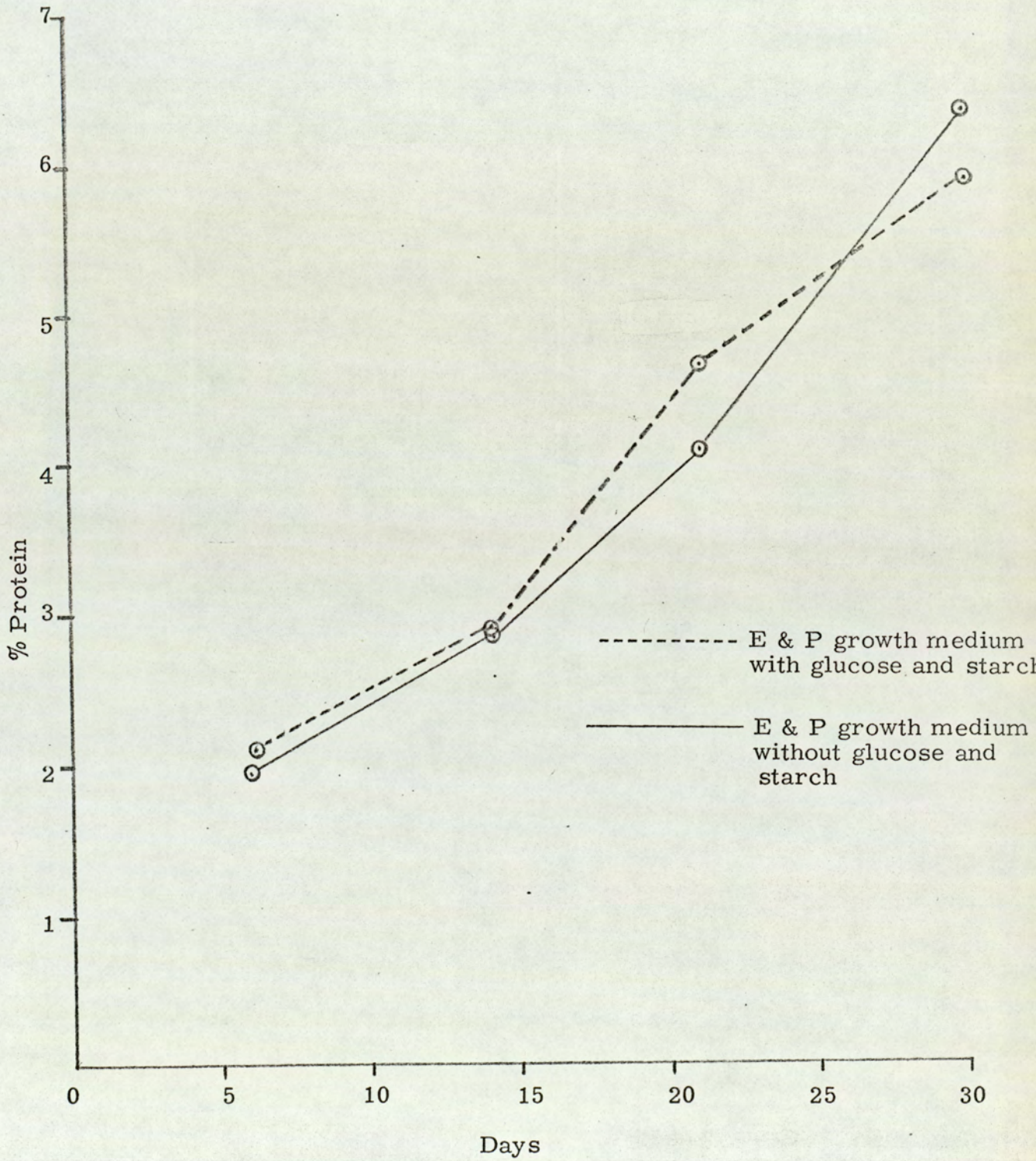


FIGURE 23

CHAETOMIUM AFFINE

Comparative effect of the presence of glucose and starch in growth medium on fungal protein in the end product

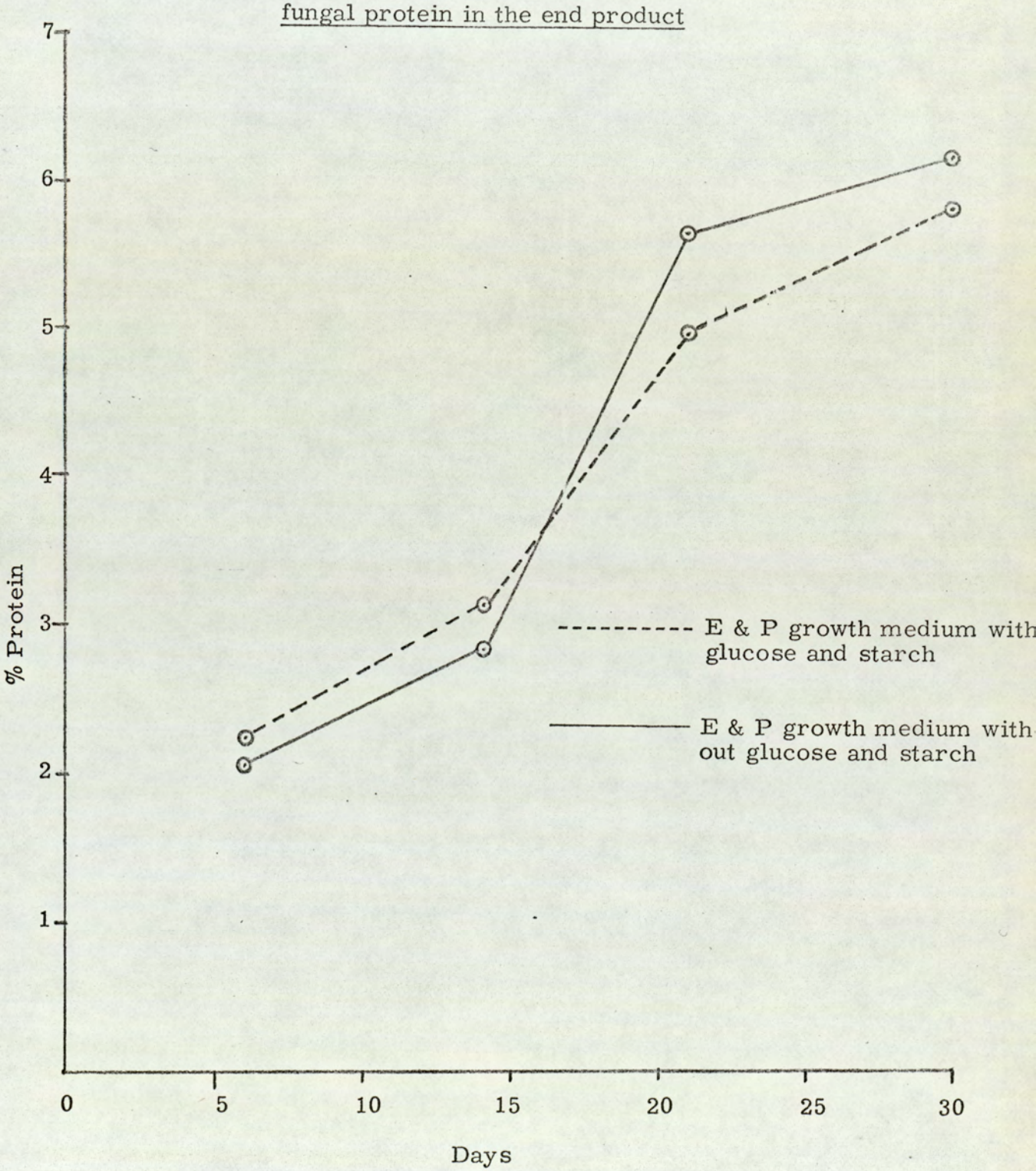


FIGURE 24

CHAETOMIUM OLIVACIUM

Comparative effect of the presence of glucose and starch in growth medium on fungal protein in the end product

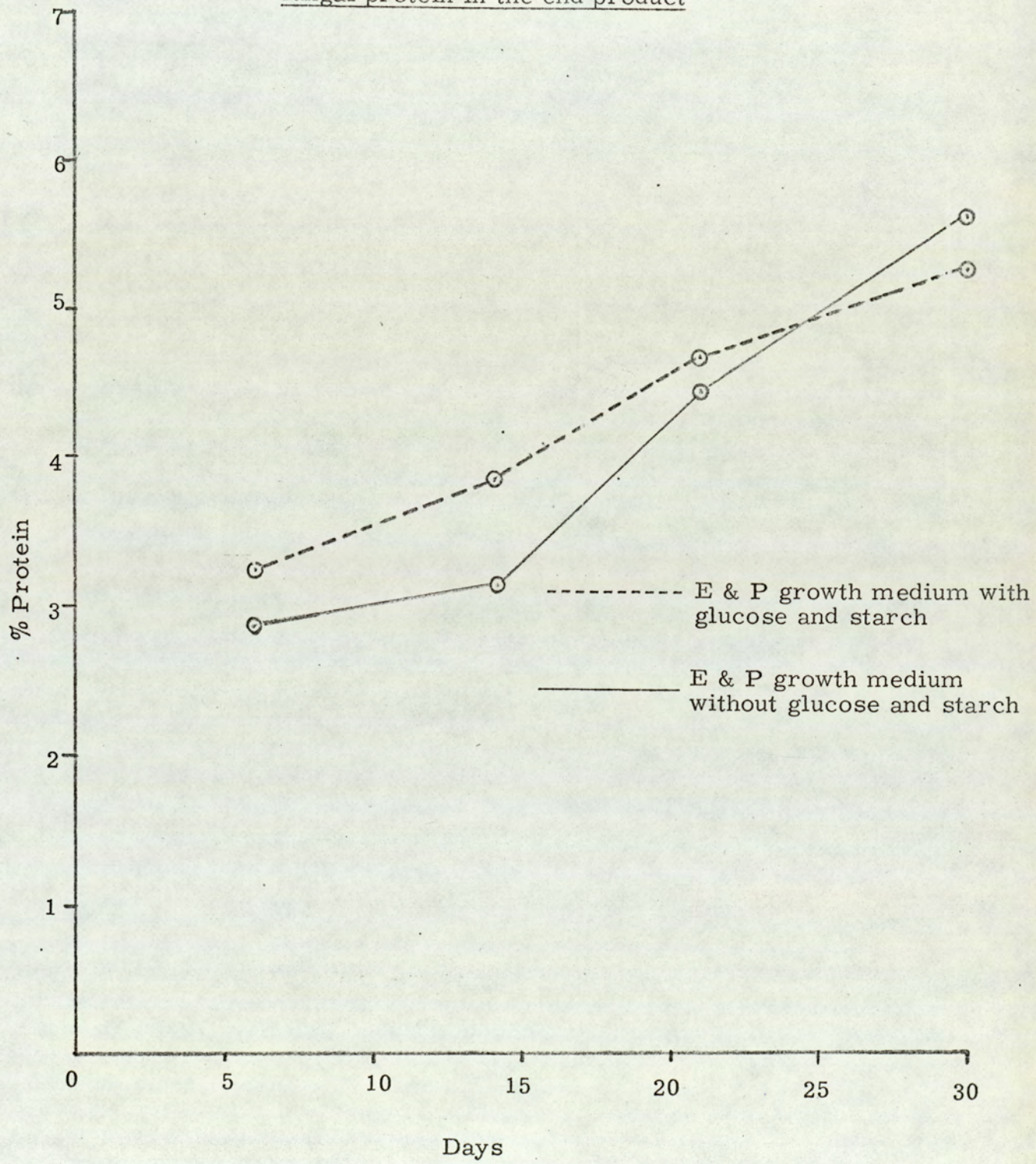


FIGURE 25

CHAETOMIUM TRILATERALE

Comparative effect of the presence of glucose and starch in growth medium on fungal protein in the end product

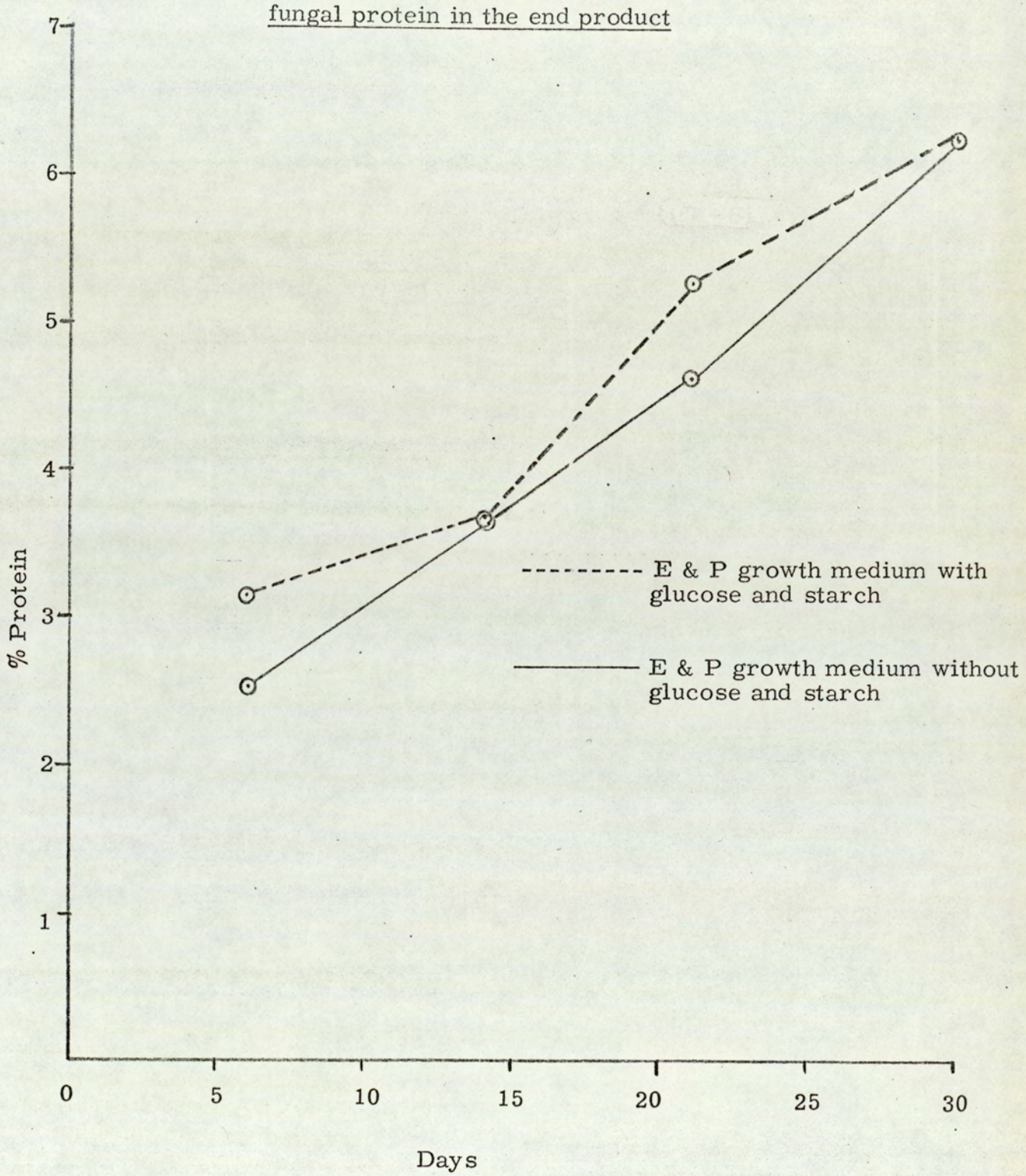
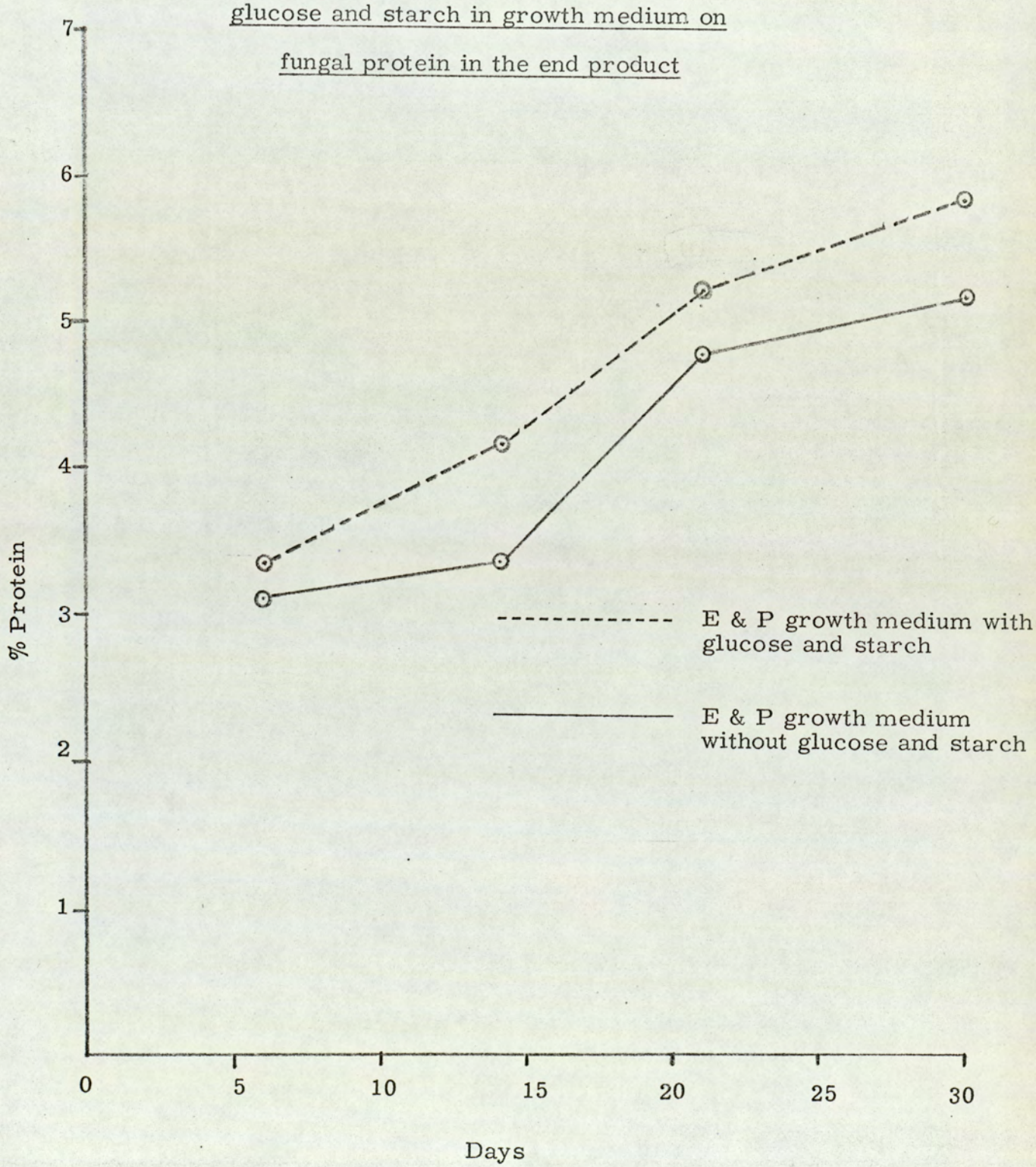


FIGURE 26

CHAETOMIUM CRISPATUM

Comparative effect of the presence of glucose and starch in growth medium on fungal protein in the end product



protein content. Results obtained are shown in table 16.

2. 2. 5. 2. Discussion (Figures 21 to 26)

In the initial stages (0 - 6 days), the microbes grew faster in the fermenters provided with small quantities of starch and sugar. As soon as the starch and glucose were depleted the growth of fungi tended to slow down possibly because the fungi had to adapt to the new carbon source (i.e. cellulose in straw). Those growing in the fermenters not containing any added glucose and starch produced an end product containing a similar amount of protein. The addition of starch and glucose although resulting in quicker early growth did not reduce the overall fermentation time. Higher concentrations of these additives would possibly have a greater effect but the cost would be increased.

2. 2. 6 Fungal Growth on Ground Straw

The object of this set of experiments was to study whether microbial attack on ground straw was faster than that on large pieces of untreated straw.

2. 2. 6. 1. Experimental Method

Straw was cut into small pieces and then ground using a high speed blender. On the whole straw tended to maintain its basic physical structure and the effect of treatment was to peel off concentric layers of material resulting in a reduction of

TABLE 17

The comparative study of upgrading of ground and unground straw using the combination of Chaetomium thermophile + Mucor pusillus

Days	% Protein	
	ground straw	unground straw
6		4.36
8	3.15	
14	4.36	5.60
20	5.85	
21		6.67
28	7.1	
30		8.50

FIGURE 27

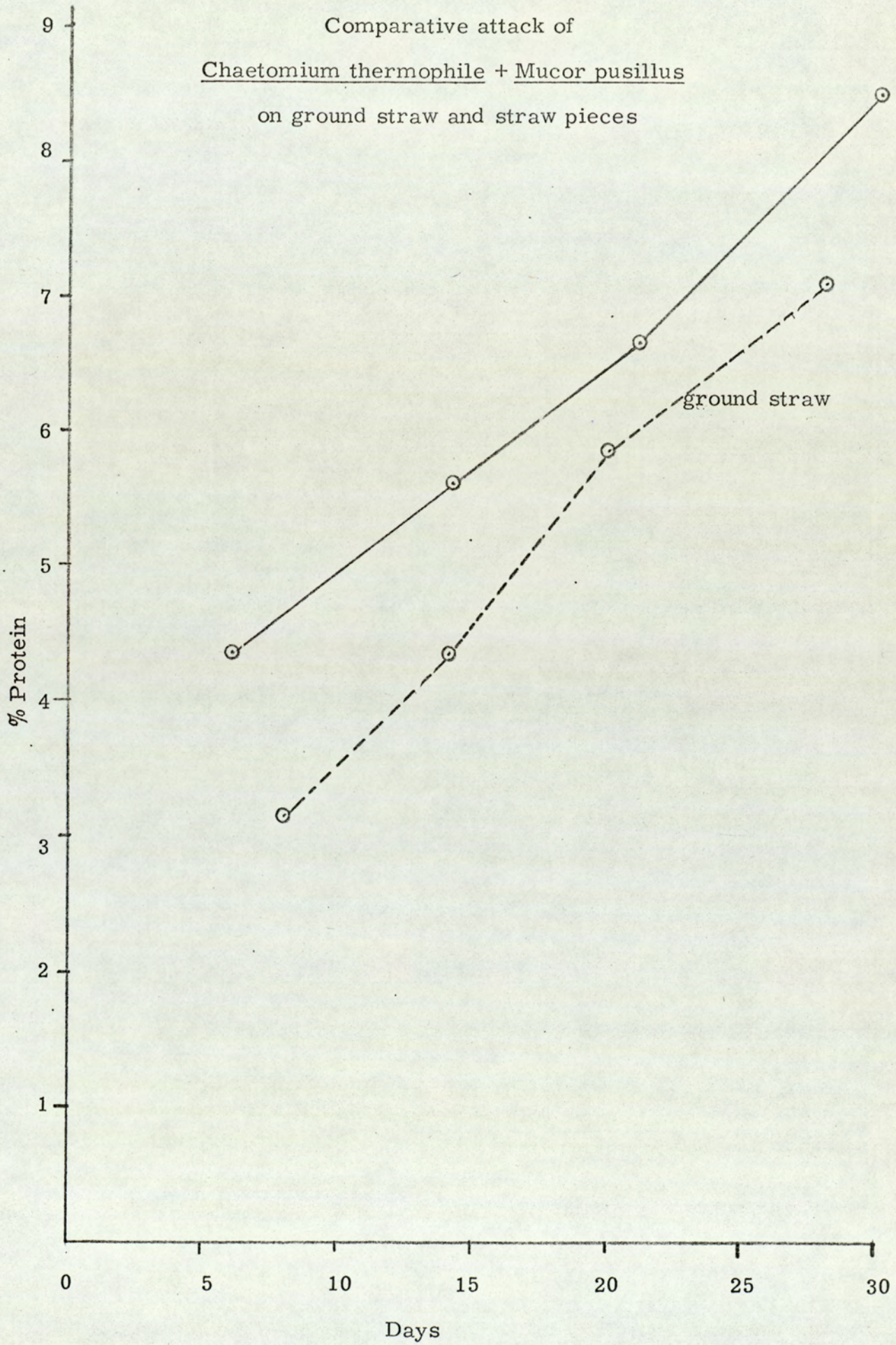

Comparative attack of
Chaetomium thermophile + Mucor pusillus
on ground straw and straw pieces
ground straw
% Protein
Days
9
8
7
6
5
4
3
2
1
0
5
10
15
20
25
30

TABLE 18

The comparative study of upgrading of ground and unground straw using the combination of Chaetomium thermophile + Humicola lanuginosa

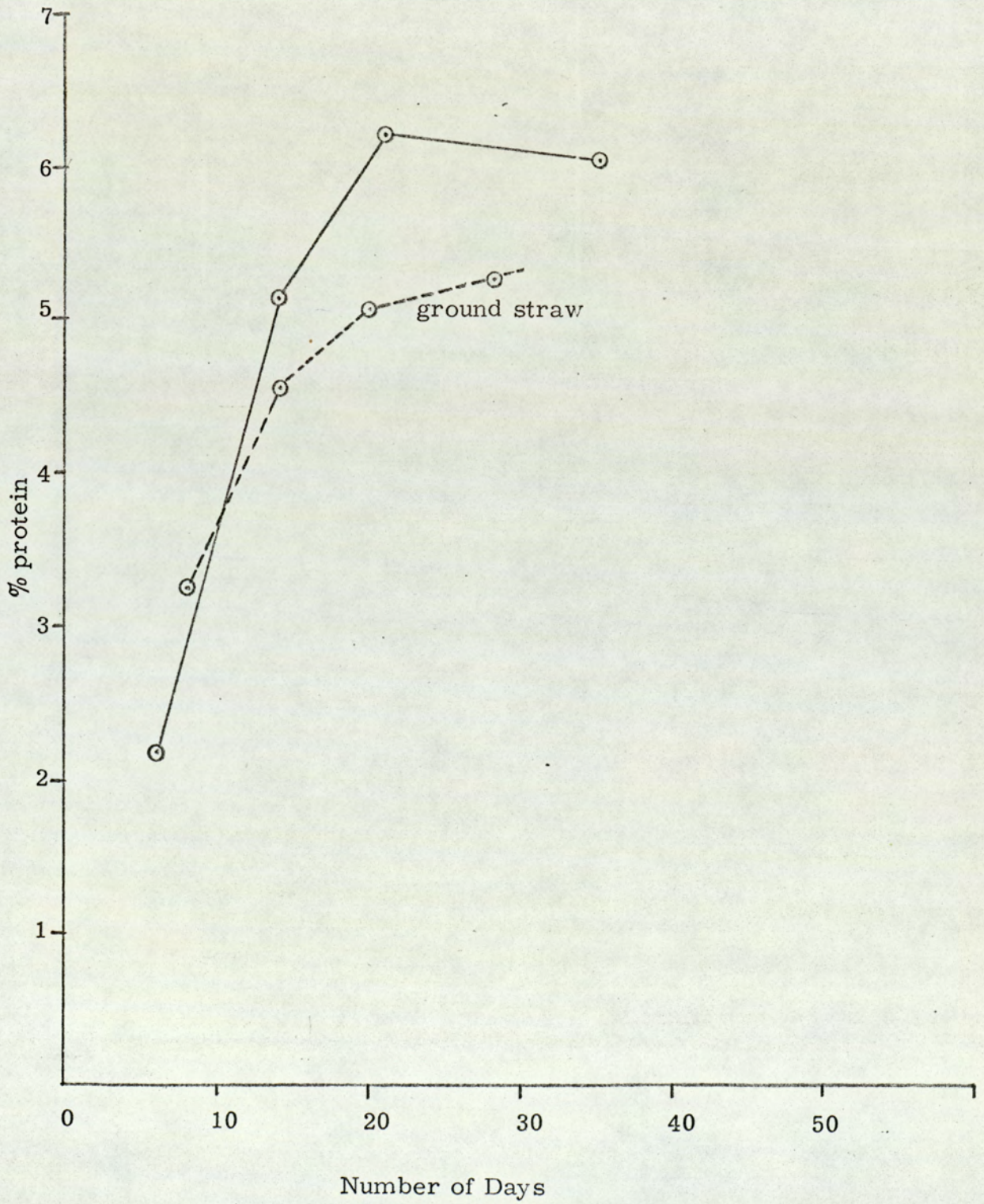
Days	% Protein	
	ground straw	unground straw
6		2.18
8	3.23	
14	4.55	5.15
20	5.09	
21		6.21
28	5.24	
35		6.01

FIGURE 28

Comparative attack of

Chaetomium thermophile + Humicola lanuginosa

on ground straw and straw pieces



diameter. Most of the straw pieces maintained their length but fines were also present. Two experiments using mixed culture, single stage, thermophilic systems were set up. The same E & P salt solution (pH5.8) as described in the section (2.2.3) was used. After sterilizing, the microbial systems used were (a) Chaetomium thermophile + Mucor pusillus (b) Chaetomium thermophile + Humicola lanuginosa incubated at 48°C. Samples were removed after a predecided period and analysed for protein content. The results obtained are shown in tables 17 and 18. Graphs of % protein against time (figures 27 and 28) were plotted and results compared with those for fungi grown on unground straw.

2.2.6.2. Discussion

Experimental results indicated that the protein content of the end-product obtained from straw (cut into pieces) was higher than that obtained from ground straw. During the course of fermentation it was observed that the ground straw formed a very compact substrate. Under these conditions uniform microbial attack might not have been achieved. With straw cut into small pieces a very coarse structure was maintained throughout the fermentation. Consequently aeration, mixing and nutrient supply were more suitable for fungal attack. Although a smaller particle size results in a potentially greater surface area per unit mass, the aggregation of particles minimizes this advantage. Hence grinding of straw did not prove to be advantageous.

2.2.7. Optimising C:N ratio for a selected system

Introduction

The object of this set of experiments was to choose the range

of C:N ratios within which a selected microbial system can be made to grow most efficiently. There should always be a sufficient amount of nitrogen in the system for the fungal growth to take place. In the case of very low C:N ratios the salt concentration of the nutrient solution may be very high resulting in high osmotic pressures which may inhibit the growth of the micro-organism. On the other hand, if the nitrogen concentration of the nutrient salt solution is depleted in the initial stages of the fermentation, this may retard fungal growth. It is well known that for the town-waste solid-substrate fermentation a C:N ratio of 15:1 is a very acceptable one [9]. In some submerged fermentations (e.g. Aspergillus niger) a figure around 8:1 is very desirable [43]. By experiment it was found that when straw was soaked in the nutrient-salt solution it could absorb up to five times its own weight of moisture. The percentage of usable carbon in cellulose was calculated to be 40% (see appendix 3). All the calculations involving C:N ratios in the system were based on these figures and an assumed cellulose content in the straw of 50%.

Theoretical calculations for the optimum range of C:N ratio.

Let the end product contain 7% (w/w) protein on a dry weight basis

$\therefore$ Amount of protein in 0.20 gm of sample = (0.20 x 0.07)
= 0.014

$\therefore$ Total nitrogen in the sample = $\left(\frac{0.014}{6.25}\right)$
= 0.00224 gms.

Since the nitrogen content of straw = 0.23% (w/w)

$\therefore$ weight of nitrogen in raw sample (0.20gm) = $\left(\frac{0.23 \times 0.20}{100}\right)$
= 0.00046 gm

$\therefore$ excess of nitrogen to be supplied = (0.00224 - 0.00046)
= 0.00178

TABLE 19

Theoretical requirement of C:N ratio

C:N ratio	N concentration in medium gm/l	N concentration gm/ml x	Theoretical amount of N required gm/ml (0.00224-x)	If C:N ratio is acceptable
175:1	0.233	0.000233	0.00201	NO
80:1	0.509	0.000509	0.00173	NO
40:1	1.018	0.001018	0.00122	NO
20:1	2.036	0.00236	-0.00012	YES
15:1	2.730	0.00273	-0.00049	YES
10:1	4.072	0.004072	-0.00183	YES
8:1	5.090	0.00509	-0.00285	YES

Since straw absorbs liquid equivalent to five times its weight, therefore 0.2 gm of sample will absorb 1 ml of solution. From table 19 it can clearly be seen that the theoretical requirements of nitrogen (0.00178 gm per ml) as calculated above is only available when the C:N ratio of the system is maintained at 20:1 or less. The nitrogen concentration of the medium is more than the theoretical requirement to produce 7% protein by an amount indicated by the negative sign. A number of experiments were carried out to select the optimum carbon to nitrogen ratio within the limits of 20:1 to 8:1, thus avoiding both excessively low and excessively high concentrations of nitrogen in the growth medium.

Following on from these calculations three combinations viz (1) Chaetomium thermophile + Talaromyces emersonii, (2) Sporotrichum thermophile + Talaromyces emersonii and, (3) Torula thermophile + Talaromyces emersonii) of cellulolytic (former) and non-cellulolytic (latter) thermophilic micro-organisms were grown under identical conditions to those described above.

TABLE 20

Growth media used

C:N / Salts gms	20:1	15:1	10:1	8:1
Potassium dihydrogen phosphate	10	10	10	10
Ammonium sulphate	9.6	12.85	19.20	24.00
Potassium chloride	5	5	5	5
Yeast extract	0.5	0.5	0.5	0.5
Magnesium sulphate	0.5	0.5	0.5	0.5
Calcium chloride	1	1	1	1

Final volume made up to 1 litre using distilled water

TABLE 21

% Protein (w/w) dry weight basis

C:N ratio	Number of days				Micro-organisms
	7	16	20	27	
8:1	3.01	3.15	4.19	5.44	Chaetomium thermophile and Talaromyces emersonii
10:1	3.96	4.39	4.40	4.75	
15:1	3.90	4.19	4.94	5.56	
20:1	3.76	4.09	5.53	5.64	
8:1	3.57	4.28	4.35	6.71	Sporotrichum thermophile and Talaromyces emersonii
10:1	3.41	4.76	4.50	6.55	
15:1	3.52	3.78	3.95	4.28	
20:1	3.74	3.90	4.25	4.60	
8:1	3.64	4.70	5.05	5.56	Torula thermophile and Talaromyces emersonii
10:1	3.99	4.79	5.95	6.02	
15:1	3.45	4.55	5.34	6.07	
20:1	3.75	3.82	4.41	4.57	

FIGURE 29

Growth of Chaetomium thermophile and Talaromyces emersonii at different C:N ratios

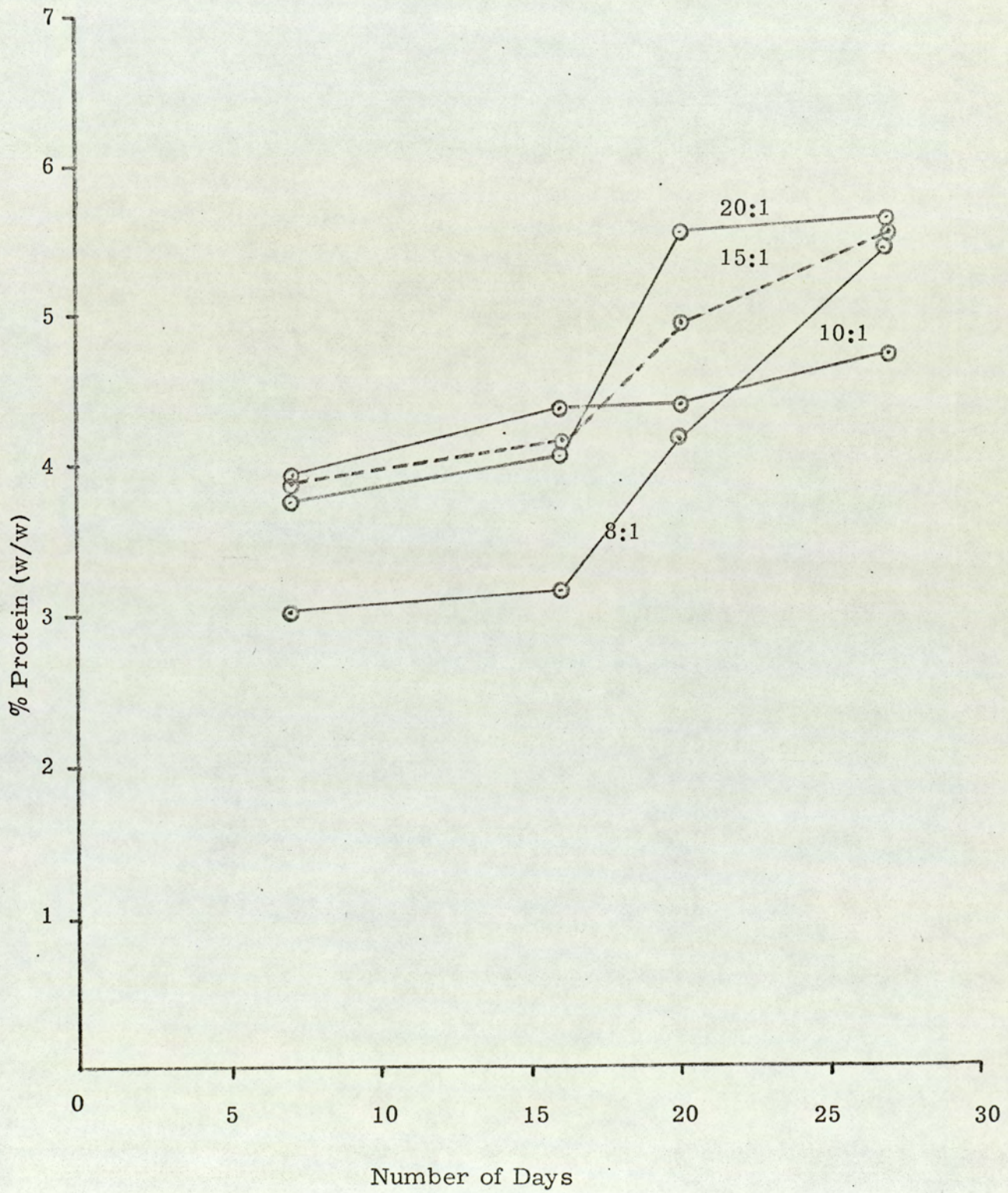


FIGURE 30.

Growth of Sporotrichum and Talaromyces emersonii

at different C:N ratios

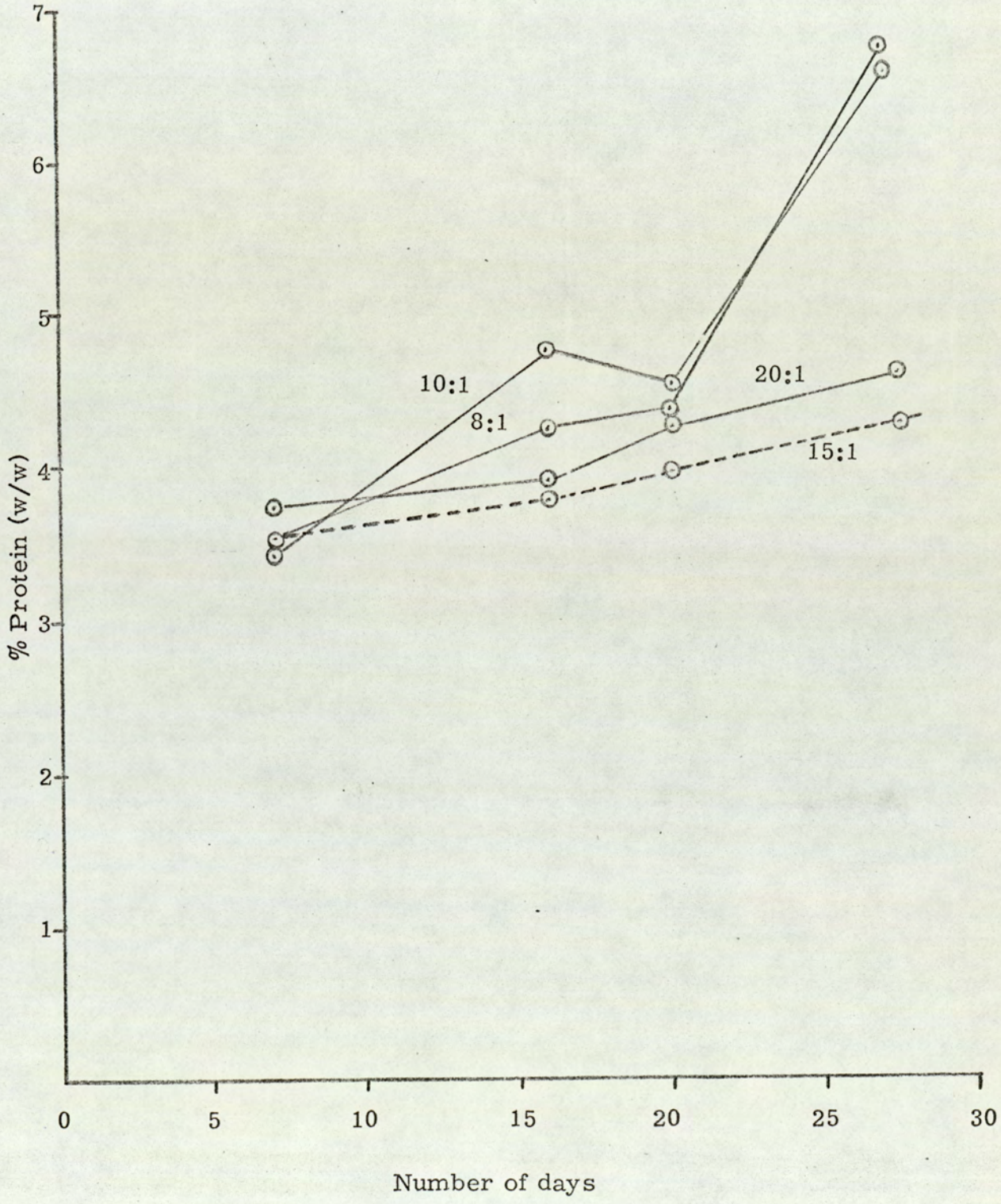
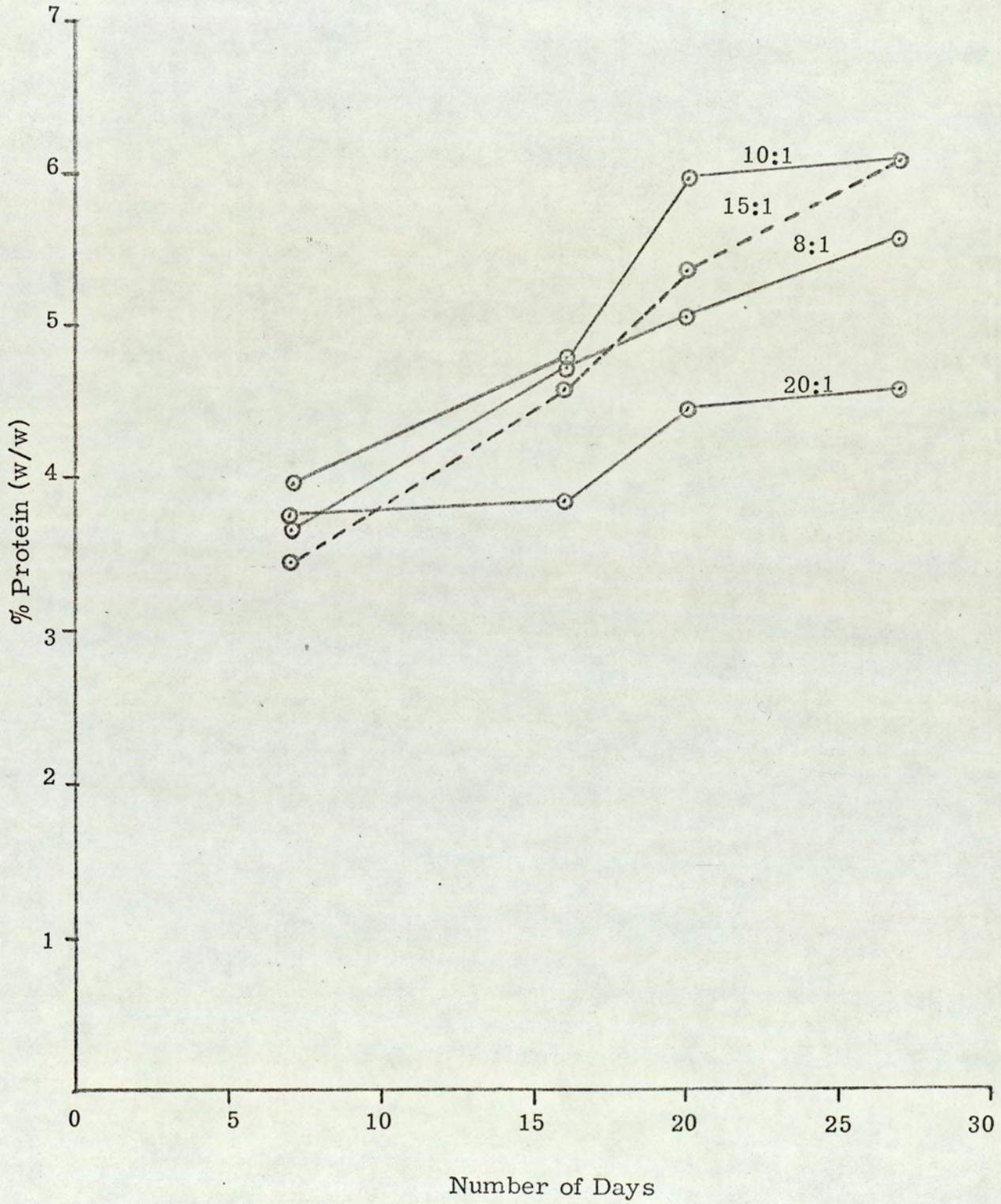


FIGURE 31

Growth of Torula thermophile and

Talaromyces emersonii at different C:N ratios



The experimental results are summarised in Table 21 and plotted in figures 29, 30 and 31.

Comments

With the above combinations of fungi grown at different C:N ratios the following conclusions may be drawn. In the case of Chaetomium thermophile and Talaromyces emersonii it was found that they grow best within the C:N range of 15:1 to 20:1; this can be seen from figure 29. By contrast Sporotrichum thermophile and T. emersonii grow well at low C:N ratios and 10:1 is found to be the best value (see figure 30). Finally Torula thermophile and T. emersonii grow best at C:N ratios between 15:1 to 10:1 as shown in figure 31. Therefore for every combination there is a different C:N ratio for maximum protein production.

2.2.8 pH Optimization

From the literature survey[36] it is known that thermophilic fungi can grow within the pH range 4.5 to 8.0. Hence it was thought to be desirable to find an optimum pH or a range of pH's which enable fungi to grow satisfactorily. Although it is impossible to study this for a number of combinations of fungi it was decided to investigate it for Torula thermophile and T. emersonii.

Since this combination is supposed to grow well at C:N ratio of 15:1, the range of pH (i.e. 5.5, 6, 6.5, 7 and 7.5) at C:N ratio of 15:1 were studied by growing these similarly as in the previous experiments.

TABLE 22

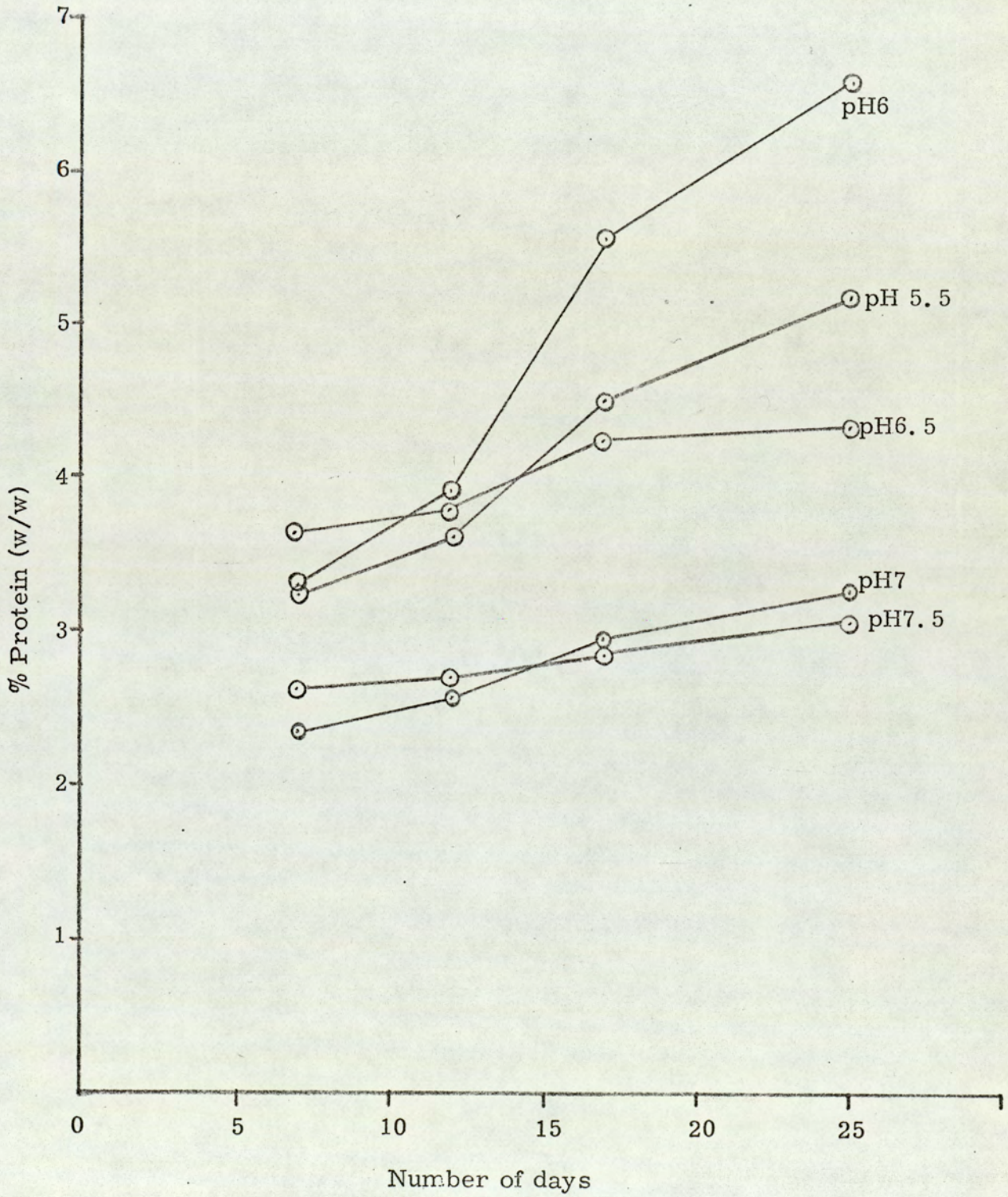
The growth of Torula thermophile and Talaromyces emersonii at C:N - 15:1 and various pH s

pH	% Protein in number of days			
	7	12	17	25
5.5	3.26	3.60	4.50	5.18
6.0	3.30	3.90	5.56	6.59
6.5	3.64	3.80	4.26	4.32
7.0	2.34	2.55	2.96	3.28
7.5	2.63	2.70	2.85	3.08

FIGURE 32

Growth of Torula thermophile and Tal. emersonii

at C:N of 15:1 and different pHs



The results obtained are shown in table 22 and graphs of % protein against number of days are plotted in figure 31. From figure 31 it is clearly observed that the combination of Torula thermophile and Talaromyces emersonii grows best under the conditions of C:N - 15:1 and pH6.

2.3 Preliminary Feeding Trials

When a new feedstuff is produced it is essential to investigate its acceptability, toxicity, nutritional value and side effects both in the short and long term. Such investigation calls for considerable knowledge of animal physiology, nutrition and involves considerable experimentation. In the present project it was decided that only a very crude feeding trial was justified.

The main objectives of the trial were to assess (1) the acceptability of the fermented straw as a food and (2) its toxicity.

Clearly the information obtained from this preliminary feeding trial may be used as a guide line for embarking on a more detailed feeding programme.

2.3.1. Materials

Mice: Twenty-one freshly weaned (21 day old) white mice, strain T.O. were obtained from the animal house of the University of Aston in Birmingham.

Feed: The feed used was obtained from the Bugbrook Mills of Heygate and Sons. Ltd. This was classified as diet 41B (normal breeding diet) containing 15% protein, 2.5% oil and 5.5% fibre.

2.3.2 Preparation of Fungal Feed Stuff

Two types of fungal foods were produced using different combinations of fungi. In this series the much larger volumes of straw required were inoculated in 2 litre conical flasks. The microbial combinations used were Chaetomium thermophile with Talaromyces emersonii and Chaetomium thermophile with Talaromyces dupontii. In both pairs the former fungus is cellulolytic and the latter non-cellulolytic. Since both systems are thermophilic they were inoculated at 48°C. These two combinations were chosen because:-

1. They have been found to grow together under natural conditions.

2. They are thought to be non toxic.

3. They have been found to produce high protein feeds.

After four weeks the fungal mixtures were removed from the fermenters, dried and analysed for their protein content. The fungal food produced using combination of Chaetomium thermophile and Tal. emersonii had a protein content of 6% (dry weight basis) whereas the other combination produced a food with a protein content of 6.16%. 20% (w/w) of the fungal food was added to the ground, normal breeding diet. This mixture was once again ground to reduce the straw size in a high-speed blender. Sufficient water was then added to wet the powder so that it could be extruded through a 30 m.l.

syringe. The pellets so formed were oven dried. Three different types of feed were available for test purposes:

1. F_1 Normal breeding food as control (protein content 15%)

2. F_2 Normal breeding food (diet 41B) and fungal food (Chaetomium thermophile + Talaromyces emersonii) mixed in the ratio of 80:20 (w/w) (protein content 13.2% w/w)

3. F_3 A mixture of normal food and fungal food (Chaetomium thermophile + Tal. dupontii) in the ratio of 80:20 (w/w) (Protein content 13.22% w/w).

2.3.3. Experimental Method

Three groups of mice, each caged separately, were fed on the three feeds. The mice were identified by colour-code markings on their tails. The cages used were 41cm x 25cm x 14 cm with about three to four cms of sawdust at the bottom. Each cage was supplied with water bottle. The temperature of the room was fairly constant at $22^{o}C \pm 2^{o}C$. A weighed amount of food was served every day and the amount consumed per day per mouse was observed. The mice were weighed periodically but not every day in order to minimise changes in their feeding and sleeping habits. The cages were cleaned twice every week. The results obtained are listed in tables 23, 24, 25,

and 26. Two graphs, one of the average weight of food consumed per mouse per day against time, and the other of average weight per mouse against time were plotted (figures 33 and 34).

2.3.4 Discussion

During the course of the experiment the fungal foods did not prove to be toxic. Most of the mice in the three cages were quite active, and none had died after three weeks as a result of fungal food. These tentative conclusions need to be tested further using a much greater number of mice. Also, in future tests, the mice would need to be dissected for physiological examination.

Initially it was observed that both fungal foods were eaten in greater quantity than the normal feed. As time passed by the consumption of the (F_2) fungal food per mouse per day increased over that of the normal feed, which followed a similar trend, whilst that of the (F_1) fungal feed decreased. Variations in the amount of food consumed per day is partly explained by the fact that any kind of disturbance can scare and upset the mice, resulting in the variations in the amount of food eaten.

The fungal food (F_3), which was eaten least, may have had either an unacceptable flavour or texture or both. There is no clear explanation why the amount of the other fungal food eaten was greater than that of the normal food.

From figure 34 it can be seen that mice on the normal food

showed a steady increase in weight. Of the fungal foods the combination of Chaetomium thermophile and Talaromyces emersonii proved to be better than the combination of Chaetomium thermophile and Talaromyces dupontii. The mice on the former feed (F_2) showed a slight gain in weight, those on the latter feed (F_3) decreased in weight.

TABLE 23

15% Protein control diet

Changes in weights of mice when living on F_1

Time (days)	0	2	5	7	14	20	23
1st weight gms	17.60	17.50	18.30	18.30	24.40	23.60	24.10
2nd weight gms	18.10	19.10	18.80	17.80	17.70	20.70	20.70
3rd weight gms	12.40	17.70	19.70	20.60	22.30	24.60	23.80
4th weight gms	20.60	19.30	19.90	19.30	22.60	26.20	26.20
5th weight gms	20.70	21.20	23.70	24.50	26.80	28.70	30.00
6th weight gms	16.50	16.80	17.80	18.00	12.40	Dead	Dead
mean weight	17.65	19.10	19.53	19.73	22.36	24.76	24.96

TABLE 24

20% fungal food containing 13.23% protein

(Chaetomium thermophile + Talaromyces dupontii)

Changes in weights of mice when living on F_3

Time (days)	0	2	5	7	14	20	23
1st wt. gms	20.70	21.00	21.90	20.70	21.00	21.30	20.70
2nd wt. gms	15.70	15.20	15.90	15.50	12.70	12.70	12.20
3rd wt. gms	15.50	16.20	15.40	15.60	13.20	12.60	12.90
4th wt. gms	21.20	-	20.60	20.00	17.70	17.00	17.90
5th wt. gms	14.10	12.60	14.20	13.40	10.80	10.60	9.90
6th wt. gms	19.60	18.20	20.40	20.50	18.80	19.30	18.00
7th wt. gms	15.30	15.20	15.20	15.00	14.10	14.10	13.60
Mean weight	17.40	16.40	17.75	16.95	15.47	15.22	14.87

TABLE 25

20% fungal food containing 13.20% protein

(Chaetomium thermophile + Tal. emersonii)

Changes in weights of mice when living on F_2

Time (days)	0	2	5	7	14	20	23
1st wt. gms	18.80	17.70	19.60	19.60	19.30	20.30	20.00
2nd wt. gms	18.60	17.90	20.10	20.50	21.70	22.60	22.40
3rd wt. gms	17.80	15.50	16.80	17.40	16.70	18.80	15.50
4th wt. gms	17.70	15.20	16.70	16.20	14.70	15.10	16.20
5th wt. gms	20.50	19.40	21.00	21.50	22.00	22.60	22.60
6th wt. gms	20.00	18.50	19.90	21.20	21.01	22.50	23.30
7th wt. gms	17.60	16.00	17.50	18.00	15.70	16.80	17.10
Mean weight	18.71	16.71	18.80	19.20	18.70	19.81	19.58

TABLE 26

Comparative study of each food consumed

Time (days)	Normal food consumed per mouse per day F_1 gms	Fungal food (Ch. thermophile + Tal. dupontii) consumed per mouse per day F_3 gms	Fungal food (Ch. thermophile + Tal. emersonii) consumed per mouse per day F_2 gms
5	2.03	6.24	6.05
6	3.80	-	-
7	4.16	7.64	4.78
8	4.05	6.27	5.35
9	3.36	3.71	4.50
10	4.58	2.80	5.48
11	3.66	4.00	5.42
13	5.16	3.78	8.83
14	4.12	4.28	7.21
15	4.36	4.71	8.42
17	4.60	4.64	7.60
19	4.48	2.55	7.14
22	5.39	3.42	5.55

FIGURE 33

Feeding Trial I

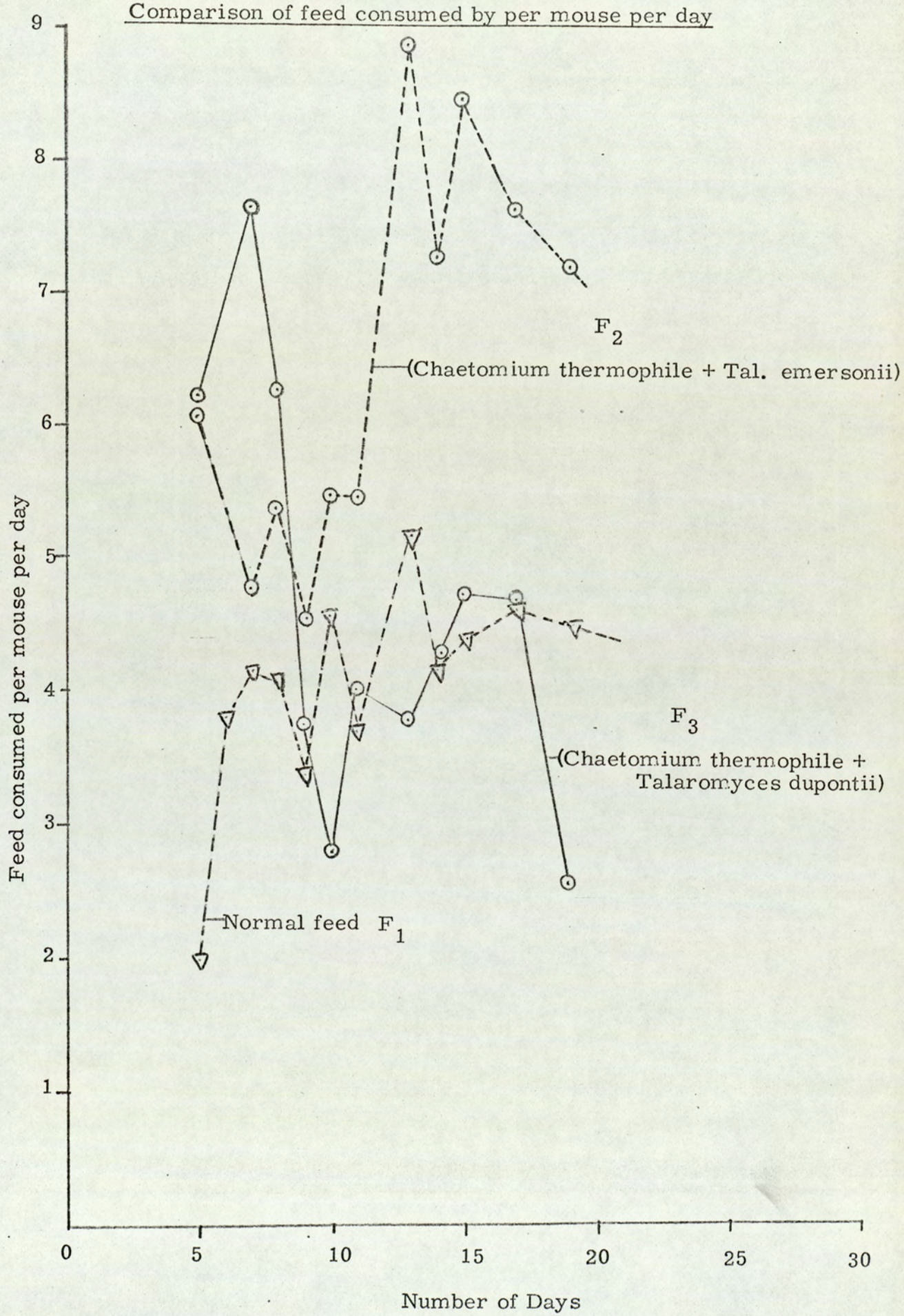
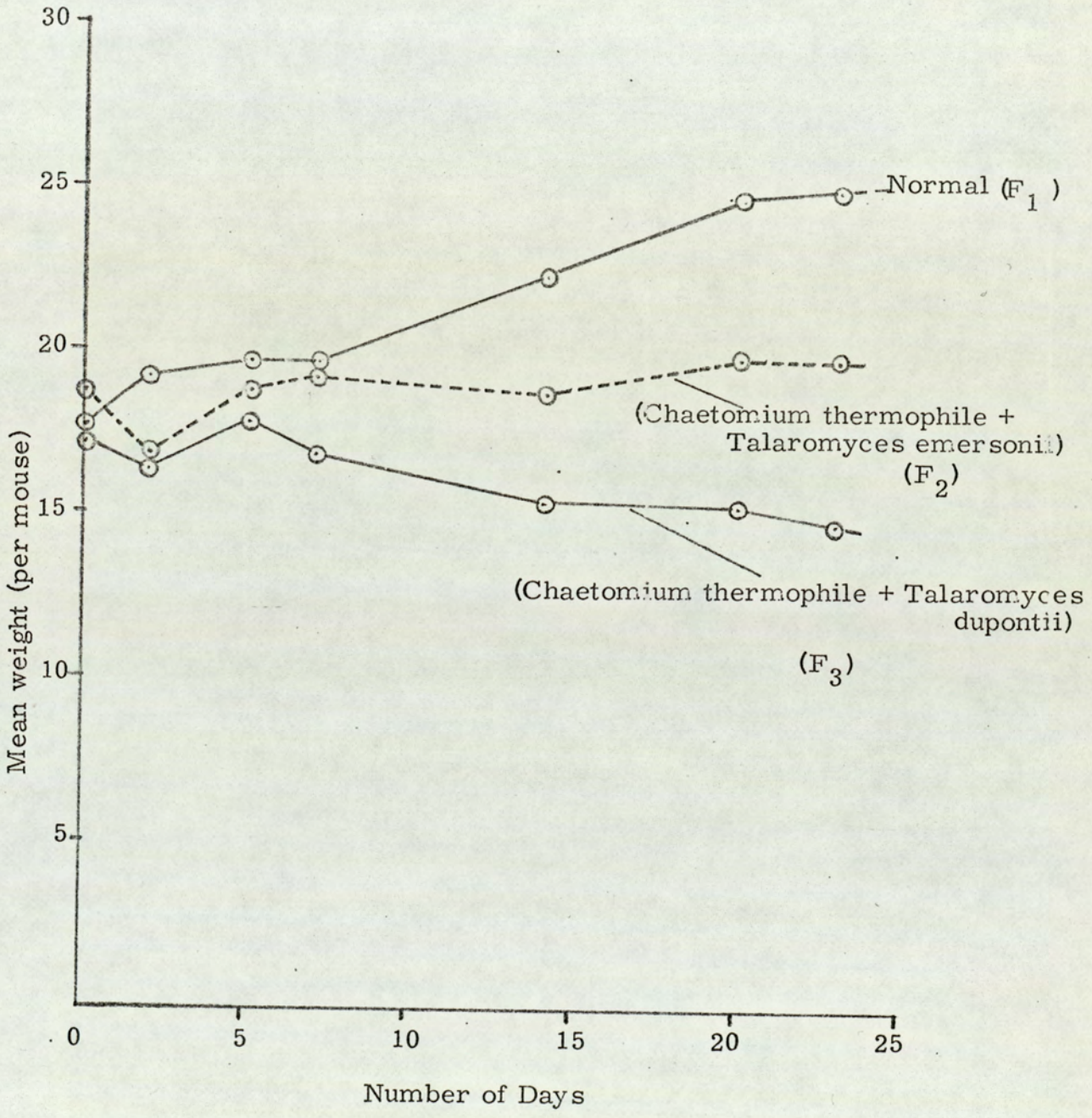


FIGURE 34

Average weight changes when different foods were fed

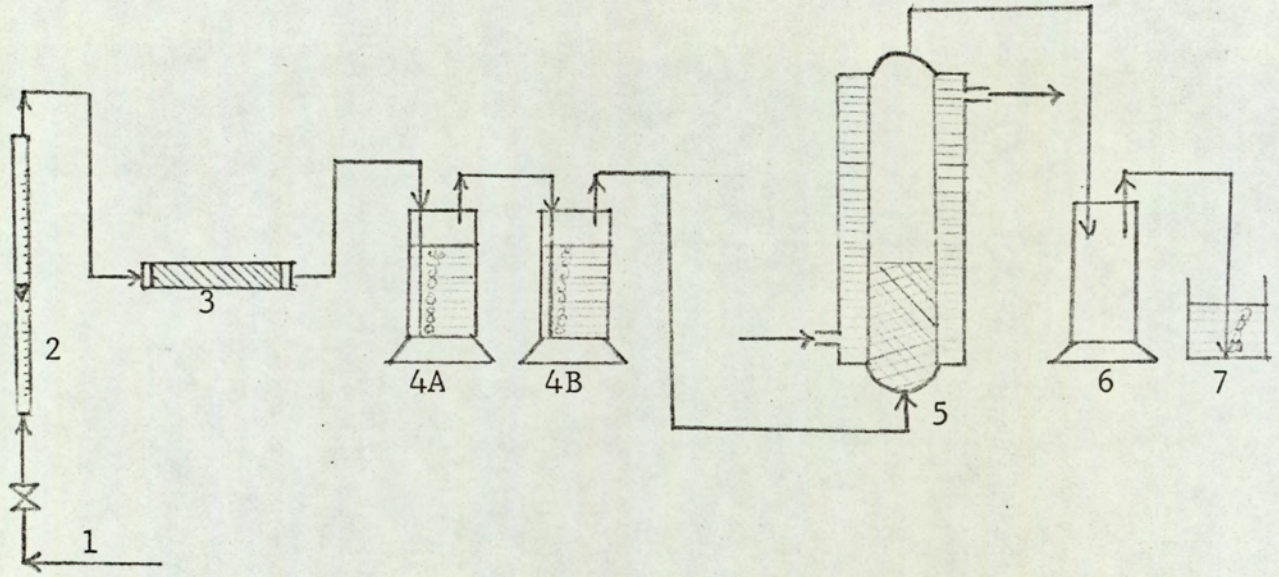


3. Process Design and Economic Evaluation

3.1. Qualitative Approach: Preliminary Design Considerations

A bench-scale fluidized type fermenter was built to observe the physical form of end product, to assess the degree of mixing attained, to determine the amount of aeration required and finally also to investigate the effect of the loss of heat and moisture on the design of the fermenter. The arrangement of the apparatus is shown in the flow diagram (Fig.35).

After a series of experiments it was concluded that a very high air flow-rate was not desirable, since the loss of moisture and heat with the exit air would be detrimental to the growth of fungi and result in an uneconomic process. Due to the very high porosity of straw particles, air passed through very easily without causing any mixing by fluidisation; consequently this mode of operation has not been studied any further. The physical form of the end-product remained unchanged during microbial attack and so it may be assumed that physical handling of the fermented mass will not be a problem during all stages of processing. These preliminary experiments highlighted a number of important problems which influenced the design of laboratory systems as well as of the large-scale plant (for treating 1,000 tons of barley straw per year).



1. Compressed air
2. Rotameter
3. Air filter
4. 4A and 4B Humidifying chambers.
5. Fermenter
6. Water trap
7. Water reservoir

Fig. 35 FLOW DIAGRAM REPRESENTING BENCH-SCALE FLUIDIZED TYPE FERMENTER

3.2. General Approach to Fermenter Design

3.2.1. Introduction

The technique of solid-substrate fermentation as a continuous process has not been widely used in the food industry and consequently there is not much information available for the purpose of engineering design. However, data obtained for submerged fermentations are plentiful and have been used below as a basis for some preliminary design work.

The process of solid-substrate fermentation may be carried out under isothermal or adiabatic conditions or under some intermediate condition. In adiabatic operation the temperature of the system rises rapidly and eventually results in inactivation of the enzyme system of the microbes. The cycle may be repeated when the system cools down. This method of operation cannot be relied upon for the production of fungal food of standard quality.

In the case of operation with heat losses the temperature will vary with time and growth-rate will not be optimal. At low temperatures growth will be slow; at higher temperatures, the growth rate will also fall off.

In isothermal operations the heat is removed or added at a controlled rate from the fermenter so that temperature can be maintained at a figure corresponding to the maximum value of the rate constant. Under these conditions the substrate can be utilized very efficiently in the shortest possible time. In other words the operation

is optimal, and the quality of end-product can be standardised. In this respect this system is preferable to all others. On the other hand an isothermal process can be more expensive to operate since extra care has to be taken to keep the temperature constant. On an industrial scale isothermal operation would generally be most acceptable.

3.2.2. Background

In the calculation procedure the following assumptions were made:-

1) a single organism is present in the system,
2) the fermenter is well mixed, so that temperature and composition are uniform throughout,
3) the growth rate is limited by the availability of cellulose and is zero-order,
4) the effect of temperature on rate constant increases to a maximum and then decreases rapidly as the enzyme-system of the microorganism is inactivated,
5) the substrate is in the form of a cube and heat can be transferred to the surroundings by convection and radiation.

3.2.3. Basic Equations

Mass Balance

If N = number of microbes per unit volume of fermenter,

V = effective volume of fermenter (assumed constant),

t = time in seconds,

$+\hbar_N$ = rate of appearance of microbes in unit volume of the fermenter, then

$$(+\hbar_N)\, V = V \cdot \frac{dN}{dt} \qquad \text{(1)}$$

If the fermentation is growth associated, then

$$-(+r_N)\,V = \gamma \cdot \frac{dM}{dt} \qquad \text{——————————(2)}$$

where γ = number of microorganisms per unit mass of substrate consumed, and

M = mass of solid in the system. From equations (1) and (2).

$$\gamma = -\frac{dN}{d\left(\frac{M}{V}\right)} = -\frac{dN}{dc_M} \qquad \text{——————————(3),}$$

where c_M = mass of substrate per unit volume of fermenter.

Energy Balance:

ΔH_R = heat evolved during formation of one micro-organism,

L = length of one side of the cubic fermenter,

U = heat transfer coefficient,

T = temperature of system,

T_E = temperature of surroundings (assumed constant),

W = total mass of system (assumed constant),

and cp = thermal heat capacity per unit mass of the system.

$$(+r_N)\,V\,(\Delta H_R) - (6L^2)\,U\,(T - T_E) = W\,cp\,\frac{dT}{dt} \qquad \text{———(4)}$$

and dividing throughout by V,

$$(+r_N)\,(\Delta H_R) - \left(\frac{6L^2}{V}\right)\,U\,(T - T_E) = \left(\frac{W}{V}\right)\,cp\,\frac{dT}{dt} \qquad \text{———(5)}$$

It should be noted that $\left(\frac{6L^2}{V}\right)$ is the surface area per unit volume of the fermenter and infact equals (6/L): Scale-up has a significant effect on the value of this parameter.

Growth-Rate Equation

Experimental data suggests that the rate is approximately constant,

$$(+r_N) = K \qquad (6)$$

where K is the rate constant and is f(T). Integration of equation (3) leads to

$$(N - N_o) = -Y(C_M - C_{M_o}) \qquad (7)$$

or

$$C_M = C_{M_o} + \frac{No}{Y} - \frac{N}{Y} \qquad (8)$$

and for equation (6)

$$(N - No) = Kt \qquad (9)$$

Growth of microorganisms in the system is also dependant on the availability of substrate as well as the number of growth points in the system.

3.2.4. Isothermal Operation

Growth of Torula thermophile on barley straw for a period of 30 days has been studied (see figure 12). The percent loss in weight of straw indicated that the growth rate could be approximated by two zero-order equations; the first accounting for the relatively fast rate upto the 5th day and the second for the rest of the test period. Two separate rate constants were therefore calculated and then used to determine the number of microorganisms at any time. The following results were obtained with Torula thermophile when grown on straw in a petri dish at 50^{o}c

Time (days) t	Percent loss in weight of substrate $1-\frac{C_M}{C_{M_O}}$
0	0
5	20
10	28
15	36
20	40
25	46
30	52

Where C_M = mass of substrate at time t per unit volume of fermenter

C_{M_O} = initial mass of substrate at time t per unit volume of fermenter

From equations (7) and (9)

$$Kt = \gamma \cdot (C_{M_O} - C_M)$$

or

$$\frac{K}{\gamma} = \frac{1}{t} (C_{M_O} - C_M)$$

Dividing throughout by C_{M_O}

$$\frac{K}{\gamma \cdot C_{M_O}} = \frac{1}{t} \left(\frac{C_{M_O} - C_M}{C_{M_O}} \right)$$

After simplifying

$$K = \frac{\gamma \cdot C_{M_O}}{t} \left(1 - \frac{C_M}{C_{M_O}} \right) \qquad (10)$$

Equation (10) is first used to calculate the two rate-constants, for the periods 0 to 5 day and 5 to 30 days. Then equation (9) can be used to get the value of N at a known time t.

Calculation of K_1 from t = 0 to 5 days

$t = 5 \times 24 \times 3600 = 0.432 \times 10^6$

$C_{M_0} = 0.1\ g/cm^3$,

and

$\frac{1-C_M}{C_{M_0}} = 0.20$, $\gamma = 0.5 \times 10^{12}$ microbes/g

$\therefore$ using equation (10) i.e. $K_1 = \left(\frac{\gamma C_{M_0}}{t}\right)\left(\frac{1-C_M}{C_{M_0}}\right)$

$$= \frac{.5 \times 10^{12} \times .1 \times 0.20}{.432 \times 10^6}$$

$\therefore K_1 = 0.231 \times 10^5$ microbes/$\delta\ cm^3$

Calculation of K_2 for the second period when t = 5 to 30 days

$\therefore t = 25 \times 24 \times 3600$

$C_{M_0}^1 = 0.080 g/cm^3$

$C_{M_F} = 0.048\ g/cm^3$

$\therefore K_2 = \frac{\gamma}{t}\left(C_{M_0}^1 - C_{M_F}\right)$

$= \frac{0.50 \times 10^{12}}{25 \times 24 \times 3600}(0.080 - 0.048)$

$= 0.741 \times 10^4$ microbes/$\delta.cm^3$

Using K_1 for 0 to 5 days and K_2 for the calculations from the 5th day onward the value of N, i.e. the number of microbes per unit volume, was calculated from the equation $(N-N_0) = Kt$.

The following results were obtained when $K_1 = 0.231 \times 10^5$ microbes/$\delta.cm^3$

$K_2 = 0.741 \times 10^4$ microbes/$\delta.cm^3$

Y = 0.5×10^{12} microbes per g,

and N_0 = 10^6 microbes / cm^3

Time (days)	number of microbes/cm^3
5	0.996×10^{10}
10	1.316×10^{10}
15	1.636×10^{10}
20	1.957×10^{10}
25	2.276×10^{10}
30	2.596×10^{10}

3.2.5. Adiabatic operation

From equation (5): $\frac{dN}{dt} \cdot (\Delta H_R) = (\frac{W}{V} \cdot cp) \frac{dT}{dt}$

or

$$\left(\frac{\Delta H_R}{\frac{W}{V}.cp}\right) = \frac{dT}{dt} \cdot \frac{dt}{dN} = \frac{dT}{dN} \qquad \text{——————————(11)}$$

For numerical solution equation (11) can be written as

$$\Delta T = \frac{\Delta H_R}{\frac{W}{V}.cp} \Delta N \qquad \text{——————————(12)}$$

Information about the effect of T on K is also required

i.e. $K = f(T)$ ——————————(13)

From equation (1) and (6)

$$\Delta t = \frac{\Delta N}{K} \qquad \text{——————————(14)}$$

Using equations (12), (13) and (14) it is possible to calculate changes in T and N with time. These computations are not included for the reasons given in section 3.2.1.

3.3. Design of System for Treating 1000 tons of Barley Straw per year

3.3.1. Philosophy of Design

The objective was to design a plant for treating 1000 tons of straw per year by solid-substrate fermentation to produce a protein-rich animal-feed. It was also decided that the plant should be suitable for individual farms as well as for large-scale co-operative units compounding animal feed. The figure of 1000 tons per year was arrived at after considering raw material supply and end-product use. On average one ton of straw is produced per acre of agricultural land. The proposed plant could thus utilize raw-material obtained from either ten small agricultural farms (each of 100 acres) or one large farm of 1000 acres. The output would be sufficient to supplement the feed for 2000 cattle or 15000 sheep or 9000 pigs or 250,000 poultry, as shown in table 27 These figures indicate that the animal-feed produced would be enough either for ten small farms or a large co-operative farm. Finally the design of a plant of the size proposed was expected to:

(1) highlight the problems involved in designing and operating plant of almost any size,

(2) provide basic information for capital cost and operating cost estimation.

There are a number of basic problems involved in designing such a plant. Firstly, there are problems arising from the very low bulk-density of straw. This fact and the long process-time required make it necessary to use big fermenters. In addition the storage areas for raw materials

TABLE 27

Feed requirement and supply

	Cattle	Sheep	Pigs	Poultry
Daily feed requirement (lb dry-matter)	30	4	7	0.25
Fungal feed at 10% of daily feed (lb dry-matter)	3	0.4	0.70	0.025
Annual requirement per animal (lb dry-matter)	1100	145	250	9
Number of animals fed by the plant	2000	15000	9000	250,000

as well as the end-products have to be large. The low bulk-density also make transportation of raw materials and distribution of final product relatively expensive. Equipment for the mechanical handling of materials adds considerably to the final cost of production as well.

Secondly, a decision must be taken regarding the degree of sterility required. Since thermophilic, cellulolytic microorganisms are utilized in the process the chance of the system getting infected is not high. Indeed only certain thermophilic bacteria or Aspergillus fumigatus are likely to cause difficulties. The growth of these can be inhibited without fully sterilizing the system. One way of doing this is to utilize a large inoculum and maintain the temperature of the fermenter at 50°c. This procedure involves heating the fermenter upto 50°c initially and then controlling it at this figure throughout the fermentation. The alternative procedure is based on complete sterilization of the substrate and growth medium at about 110°c followed by inoculation when the system cools down to 50°c. Again it is necessary to control the temperature of the system at about 50°c throughout the system for the growth of thermophiles. To be safe it was decided that the system should be designed with the latter procedure in mind.

Thirdly, there is a mixing problem arising from the need to control the temperature of the system and to ensure uniform distribution of water and soluble nutrients (mainly mineral salts). It was decided that this could be best solved by recirculating the liquid phase through the system.

Fourthly, there is the need to aerate the system to provide oxygen. Because the process is relatively slow, a continuous supply may not be essential. However, it was decided to make provision for a continuous system. Because of the high operating temperature (viz 50°c) the loss of moisture with the exit air cannot be ignored.

Finally, because of the need to simplify the process it was decided to carry out all of the following process-stages in the fermenter.

(1) soaking of the substrate (straw) in nutrient salt solution,
(2) steam-sterilizing of the fermenter contents,
(3) draining of excess liquor and cooling down to 50°c,
(4) addition of inoculum,
(5) fermentation,
(6) leaching of salts from fermenter contents using water as solvent, and
(7) drying of fermenter contents by blowing hot air through the fermenter.

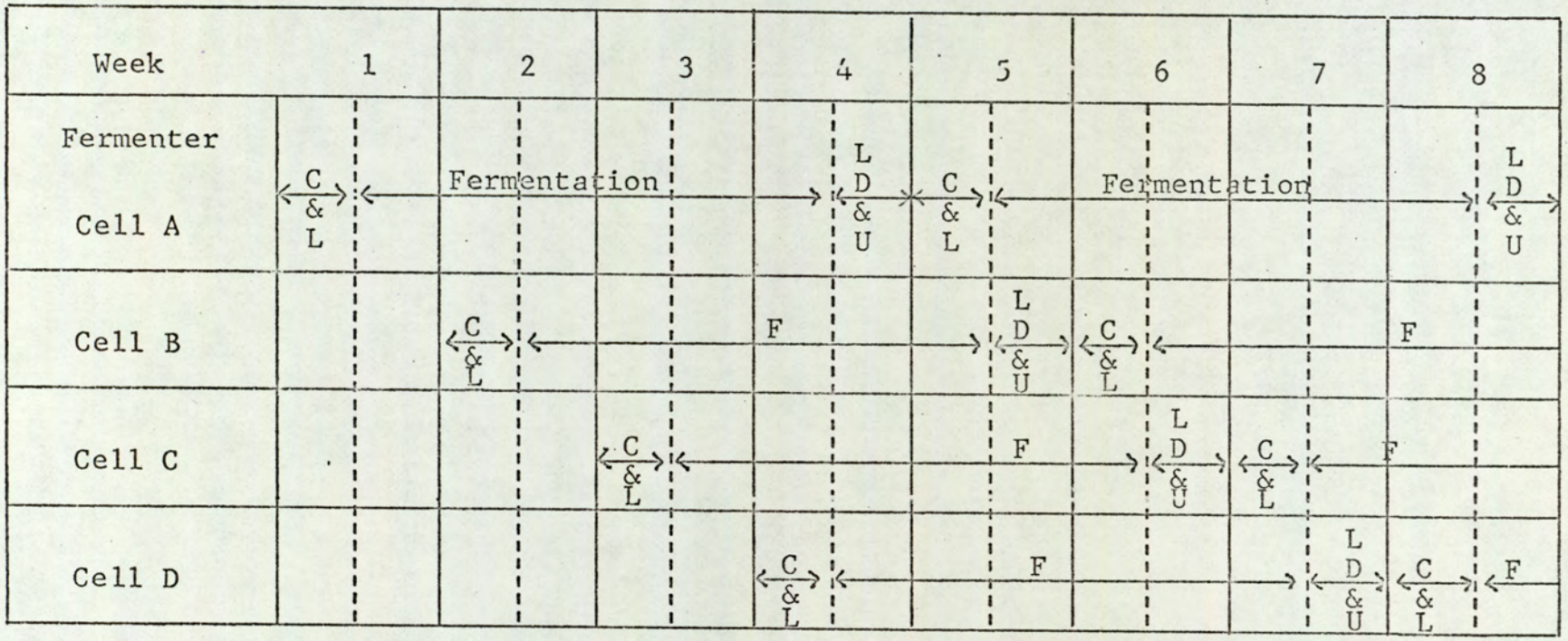
Carrying out these stages in the same vessel also minimizes problems of material handling and of maintenance of sterility.

3.3.2. Production Scheduling

Since the duration of the fermentation is between 3 to 3½ weeks the total time required to treat each batch was taken as 4 weeks. The extra time is required for initial mechanical handling of materials, cleaning of fermenter, drying and removal of product and transportation to store. Using 4 fermenter cells, the production programme is shown

FIG. 36.

Start-up and production scheduling



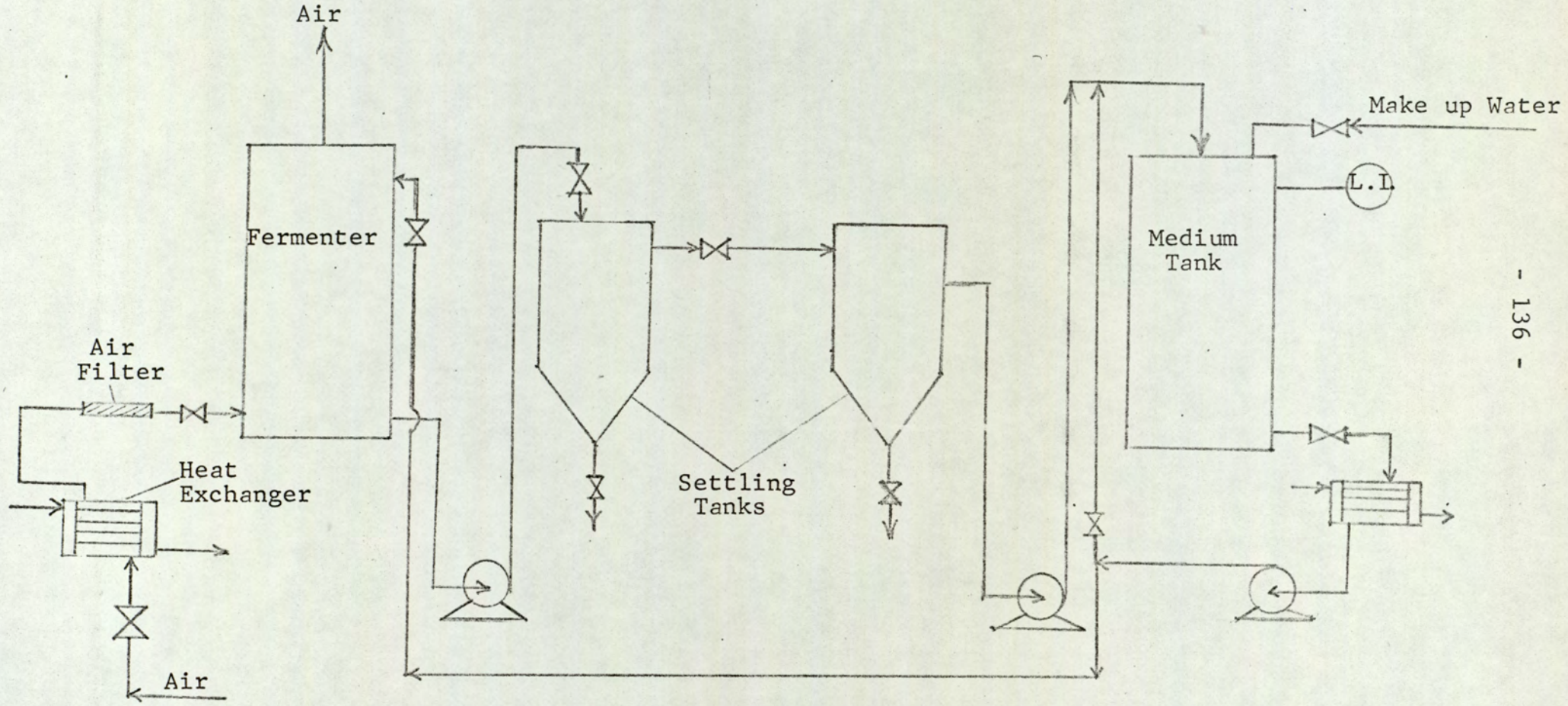
C and L = clean, load and sterilise a fermenter

F = fermentation

L,D & U = leach dry and unload fermenter contents

Fig. 57

FLOW DIAGRAM OF THE PROCESS



in fig. 36. Since the duration of each run is four weeks, the total number of runs to be carried out in each fermenter per year will be 13. Therefore total runs for four fermenters will be 52 (say 50). The amount of straw fermented in each cell per run = $\frac{1000}{50}$

= 20 tons

= 20,000 kgs.

3.3.3. Process Description (Fig 37)

The flow diagram of the process is self explanatory. The philosophy of design is explained in the previous section (3.3.1.). The sterile air is blown through the fermenting mass and medium is sprayed intermittently on the substrate. The position of nozzles may be varied. The sprayed liquid is removed at the bottom of the fermenter and pumped to settling tanks. Two settling tanks are used to clarify the medium thus avoiding the use of an expensive centrifuge. From settling tanks, the medium is pumped to the medium tank where its temperature is brought back to $50^{o}c$ by circulating through a heat exchanger. At the end of fermentation the product is leached with water to extract the excess of salts and then the end-product can be dried by blowing with hot air. Finally the product is removed pneumatically and stored with usual precautions.

3.3.4. Material Balances

Design Calculations and Equipment Specification

(1) Straw Requirement per Batch

Amount of straw required = 20 tons
or 20,000 kg.

(2) Nitrogen Requirement per Batch

Assumptions:

1) loss in weight of materials at the end of biodegradation is 20% (w/w),
2) nitrogen is used to produce fungal protein only,
3) protein content of end-product is 7% (w/w), and
4) nitrogen content of protein is 16% (w/w).

Total weight of dried end-product per batch = 16000 kg.

Total fungal protein produced = 1120 kg.

Total nitrogen used to produce 1120 kg. of protein = 179.2 kg.

(3) Oxygen Requirement per Batch

The assumptions made were:

1) the composition of the fungal cell is similar to that for yeast; and
2) the relationship of 67.2 gm of oxygen per 100 gm of yeast (44) also holds for cellulolytic fungi used in solid-substrate fermentation.

Since nitrogen content of yeast (45) is 8.5% (w/w),

amount of fungal mycelium produced = 2110 kg.,

and amount of O_2 required = 1418 kg.

$\therefore$ minimum (theoretical) quantity of air required per batch. $= \frac{1418 \times 10^3 \times 23.7}{32 \times .21}$

$= 5000\ m^3$ at 1 atm. & 15^oc

Assuming an aeration efficiency of 10%

amount of air required per hour per batch $= \frac{5000 \times 10}{23 \times 24}$

$= 90.6 m^3$

(4) Inorganic Salts Required per Batch

The growth medium to be used has to contain the ingredients shown in table (28). Straw must first be soaked in this solution, then sterilized and excess solution drained off. The solution can be used from batch to batch and loss of water with the exit air can be made up in the medium storage tank. The usable carbon to nitrogen for the process was selected as 15.1 on the basis of laboratory work (see section 2.27). It was found experimentally that straw could absorb five times as much water as its own weight. Hence it was assumed that the amount of medium required would be 10 times the weight of straw per batch.

Amount of straw treated per batch = 20,000 kg,

and so the amount of solution to be prepared per batch = 200,000 kg.

Since the specific gravity of solution is only = 1.03

the volume of solution required = 200,000 l.

The weights of salts needed are shown in table 28

Table 28:

Composition of Growth Medium and Batch Weights

Salt	Concentration (g/l)	Weight per Batch (kg)
KH_2PO4	10	2000
$(NH_4)_2SO_4$	13	2600
Yeast Extract	.50	100
KCl	5	1000
$Mg\ SO_4\ .10H_2O$	2	400
$CaCl_2$	1	200

The amount of salts unused at the end of each batch was calculated by assuming that (1) the composition of the mycelium was the same at that of yeast, viz. N=8.5%, S=0.6% P=1.1%, K=2.2% and (2) 20% of yeast extract and 10% of calcium chloride was used in every batch fermentation.

The calculations are summarised below:

Table 29:

Salt	Amount added to solution (kg)	Amount utilised (kg)	Amount unused (kg)
KH_2PO_4	2000	100	1900
$(NH_4)_2SO_4$	2600	845	1755
Yeast Extract	100	20	80
KCl	1000	90	910
$MgSO_4 . 10H_2O$	400	60	340
$CaCl_2$	200	10	190

Assuming that at all times the moisture absorbed by straw is 5 times its own weight, 100,000 l will be held up in the fermenter with the remainder in circulation. Thus at the end of the batch cycle, 100,000 l of solution will be available for subsequent batches. If the fermented straw is not subsequently washed then the loss of salts is considerable. It was assumed, therefore, that 100,000 l of fresh water would be used for washing with an extraction efficiency of 90%.

The situation at the end of each batch fermentation is as summarised below:

Salt	Salt present in fermenter with 100,000 l of Solution (kg)	Salt Extracted with Wash liquor (kg)	Total Amount of salt in Medium Tank (200,000 l.sol.) (kg)
KH_2PO4	950	855	1805
$(NH_4)_2SO4$	877	790	1667
Yeast Extract	40	36	76
KCl	455	410	865
$MgSO_4.10H_2$	170	150	320
$CaCl_2$	95	85	180

Hence, total make up of salts added per batch is as given below:

Salt	Make-up Required per Batch (kg)
KH_2 PO4	195
$(NH_4)_2SO4$	933
Yeast Extract	24
KCl	135
$MgSO_4 10H_2O$	80
$CaCl_2$	20

From the view-point of production scheduling it would seem best to have a medium storage/recirculation system associated with each fermenter cell. It would then be necessary to prepare four batches of chemicals at the start up stage with make-up between batches. Assuming that there is no need to completely change the solution except very infrequently, the annual requirements

would be as below:

Salt	Annual Requirement (kg)
KH_2PO4	(4 x 2000 + 46 x 195) = 16,960
$(NH_4)_2SO_4$	(4 x 2600 + 46 x 933) = 53,400
Yeast Extract	(4 x 100 + 46 x 24) = 1,500
KCl	(4 x 1000 + 46 x 135) = 10,200
$MgSO_4.10H_2O$	(4 x 400 + 46 x 80) = 5,280
$CaCl_2$	(4 x 200 + 46 x 20) = 1,720

3.3.5. Thermal Energy Balances

The basic assumptions made were:

(1) ambient temperature is 15^oc,

(2) heat losses from the fermenter due to radiation and convection are 1% of heat of fermentation,

(3) the fermenter temperature is maintained by adjusting the flow-rate of recirculating solution and/or exit air;

(4) fungal growth-rate is constant, so that the heat of fermentation is liberated at a constant rate,

(5) 3.44 K.cal of heat are produced per gm of $^O2$ consumed (46),

(6) fermentation continues for 23 days.

The specific heat of straw can be estimated from the formula (47)

$$Cp = \frac{U + 0.324}{1 + U},$$

where U is % moisture content based on oven-dried weight. This is applicable to straw containing moisture upto 8%. The moisture content of straw before treatment is

7-8% and so

$$Cp = \frac{7 + 0.324}{1 + 7} = 0.915 \quad cal/g\ ^{o}c$$

In the fermenting mass there is one part of straw to five parts water (w/w) and so the average specific heat (C_{P_A}) of the fermenting mass:

$$= \frac{1.C_{P_S} + 5\ C_{P_{H_2O}}}{1 + 5}$$

$$= 0.985 \quad cal/g\ ^{o}c$$

At steady state, the total

(heat of the fermenting mass at $50^{o}c$ relative to $15^{o}c$) = (120,000x1000)(0.985)(50-15)

$= 4.14 \times 10^{6}$ K.cal.

Amount of oxygen consumed per batch = 1418 Kg and so the total heat produced per hour $= \frac{1418 \times 10^{3} \times 3.44}{23 \times 24}$

$= 8.84 \times 10^{3}$ K.cal.

To estimate the heat losses with the exit air use can be made of enthalpy data for saturated air.

Enthalpy of saturated air at 1 atm. and $15^{o}c$ ($59^{o}F$)

= 18 B.T.U. per lb dry air.

Enthalpy of saturated air at 1 atm. and $50^{o}c$ ($122^{o}F$)

= 118 B.T.U. per lb.

Heat loss in K.cal/h = (118-18) x0.252 x 90.6 x 1.2 x 2.2

Heat loss with exit air = 6040 K.cal/h.

Heat loss due to radiation and convection from the fermenter = 88.4 K.cal/h

The heat balance over the fermenter is given as below:

heat produced during fermentation (K.cal.)	=	heat loss with exit air (K.cal.)	+	heat loss from fermenter (K.cal.)	+	heat loss with circulating solution (K.cal.)

or

$$8840 = 6040 + 88 + H.$$

Consequently, H, the heat to be removed with the circulating solution = 2712 K.cal/h.

Amount of Liquid Sprayed into the Fermenter

The liquid circulation rate could be determined in various ways. For packed columns the minimum wetting rate is about 1 $ft^3/h\ ft$. From experiments the surface area of the straw per unit volume of packing was found to be 5.18cm^{-1} (158 ft^{-1}): Consequently on this basis the minimum liquid flow-rate is about 158 $ft^3/h\ ft^2$ (48.2m^3/hm^2). Preliminary calculations show that the fermenter cross-sectional area is likely to be about 40m^2. So that the required flow-rate is about 2000m^3/h. This figure for the liquid flow-rate is high and is unnecessary because

(1) the fermenter contents are fully wetted during sterilization and loss of water with the exit air is not great,

(2) the solid substrate fermentation is a slow process and there is an adequate supply of nutrients in the solution taken up by the straw,

(3) the fungal mycelium may be washed off the straw.

By contrast, in a trickling bed filter used for sewage treatment the flow rate is of the order of only 1 $ft^3/h\ ft^2$. From the microbiological view-point, a similar

flow would probably be satisfactory in the straw fermenter: the corresponding flow rate is $12m^3/h$. The circulation rate finally selected was $20m^3/h$. Hence the temperature change (T) in the liquid as a result of removing 2712 K.cal/h is

$$T = \frac{2712}{20 \times 10^3} = 0.1356^{o}c$$

There is thus no problem in controlling the system temperature at $50^{o}c$ by this method. On the other hand, care must be taken to ensure that the temperature of the liquid phase in the system does not fall below $50^{o}c$, since the thermal energy of the circulating liquid above datum of $15^{o}c$

$$= 20 \times 10^3 \times 1 \times (50-15) \text{ K.cal/h}$$

$$= 7 \times 10^5 \text{ K.cal/h}$$

As in the trickling-bed filter, the liquid could be sprayed intermittently over each section the the bed.

Amount of Make-up Water: The water removed with the exit air has to be replaced to keep a constant level in the medium tank. The loss is calculated as follows:

Humidity of saturated air at $50^{o}c$ = 0.088 Kg H_2O per Kg dry air,

Humidity of saturated air at $15^{o}c$ = 0.011 Kg H_2O per Kg dry air,

Air supplied per hour = 111 Kg.

Amount of water lost = 111 (0.088 - 0.011)

= 8.55 Kg/h.

Amount of water to be added to medium tank = 8.55 1/h

= 4720 1/batch.

This part of the balance is not strictly correct since (1) water is produced as a result of the fermentation and (2) the loss in weight of the solid during fermentation

may reduce the holdup of liquid inside the fermenter. If the final end-product, on a dry-weight basis, is 16000 Kg then the volume of water present would be about 80,000 Kg. This is a decrease of 20,000 Kg on the original figure, and so it would seem that make-up water is not essential.

A Summary of the Calculations presented in Section 3.3.5.

Under steady-state conditions:

(1) total heat content of fermenting mass at $50^{o}c$ relative to $15^{o}c$ = 4.14×10^{6} K.cal;

(2) heat loss with exit air at $50^{o}c$ = 6040 K.cal/h;

(3) heat loss due to radiation and convection from the fermenter = 88.4 K.cal/h;

(4) heat removed with growth medium at $50^{o}c$ = 2712 K.cal/h;

(5) amount of growth medium sprayed = 20 m^{3}/h;

(6) amount of make up water to be added to medium tank = 8.55 l/h or 4720 l/batch

3.3.6. Equipment Specification

Fermenters

Amount of straw treated per batch = 20,000 Kg.

Since the bulk-density of straw equals 0.08 g/cm^3, the volume occupied by 20,000 Kg of straw = $\frac{20000}{.08 \times 10^3}$

$= 250\ m^3$

Since the amount of solution absorbed by the straw is five times its weight (ie 100,000 Kg), the 100,000 Kg of solution occupies $= \frac{100,000}{1 \times 10^3} = 100\ m^3$

Because of the voidage and hollow structure of the straw, 100,000 Kg. of solution can easily be absorbed without there being any need to increase volume of the vessel.

Let the diameter of the fermenter equal 6m,

then the height of the fermenter, h, is given by

$$h = \frac{4 \times 250}{3.14 \times 6 \times 6} = 8.85\ m$$

Four fermenters are required of these dimensions.

Settling Tanks: This stage is used to clarify the solution by allowing any mycelium and other suspended solid to settle out. Two tanks are used with each system and so eight settling tanks are required in all. If the capacity of each settling tank must be sufficient to contain the liquid flow for five hours, then the volume = 100 m^3.

If the diameter of the tank is chosen as 3.5 m,

then height $= \frac{4 \times 100}{3.14 \times 3.5 \times 3.5} = 10.4$ m.

Medium Tanks: Four medium tanks are required, the capacity of each tank should be 10% more than the volume of medium = 220 m^3.

With a diameter of 5.5 m, the height = $\frac{4 \times 220}{3.14 \times 5.5 \times 5.5}$ = 9.27m.

Pumps (see fig.37)

Pump P_1. This is the pump situated at the bottom of each fermenter to pump away the liquid to the settling tanks. The pump supplies 20,000 Kg/h (44000 lbs/h) of liquid. Assuming a maximum pumping height of 35ft and pipe-line losses equivalent to a 20ft static head, the total height to be pumped = 55ft. Let the efficiency of the pump be 70%, then the H.P. required = $\frac{44000 \times 55}{0.70 \times 3600 \times 550}$ = 1.75.

Four pumps each of 1.75 H.P. are required.

Pump P_2. This pump delivers liquid from the settling tank to the medium tank. The capacity of this pump is similar to that of P_1, consequently, four pumps each of 1.75 H.P., are required.

Pump P_3 This pump delivers liquid from the medium tank to the fermenter at a rate of 20,000 Kg/h, and it will be assumed that the same amount is recirculated to the medium tank to aid mixing. Hence the desired capacity of P_3 is double that of pump P_1.

In all, four pumps, each of 3.5 H.P., are required.

Steam Boiler The steam produced is to be used to (1) sterilize the straw and medium, (2) maintain the temperature of the liquid phase and (3) heat the air for drying the end-product. Although the boiler will not be used to provide steam for sterilizing and drying at the same time,

it was nevertheless designed with simultaneous operations in mind.

Let the weight of the dried end-product per batch be 16000 Kg, then the amount of moisture present would be about 80,000 Kg. Assuming that if the fermenter is left over-night, 50% of the moisture drains off under gravity, then the amount of moisture to be removed = 40,000 Kg. Let the air be heated by steam using a finned tube heat exchanger from $15^{o}c$ ($59^{o}F$) to $95^{o}c$ ($203^{o}F$).

Humidity of saturated air at $95^{o}c$ ($203^{o}F$) = 2.9 Kg/Kg.

Humidity of saturated air at $15^{o}c$ ($59^{o}F$) = 0.011 Kg/Kg.

Consequently the amount of moisture removed by 1.0 Kg of saturated air at $95^{o}c$ ($203^{o}F$) = 2.9 Kg.

Total air required for drying the product of one fermenter $= \frac{40,000}{2.9}$

$= 13,800$ Kg.

Let the drying time be 10h, then the amount of air required per hour = 1380 Kg.

Let Q_A be the amount of heat required to heat the air from $15^{o}c$ to $95^{o}c$, then

$Q_A = 1380 \times 0.25 \times (95-15)$

$= 27,600$ K.cal/h or 109,000 B.T.U./h.

Amount of heat required to sterilize the fermenter contents and growth medium at start-up of system, assuming a heating-up period of 10h, is

$Q_S = \frac{220,000}{10} \times 0.985 \times (115-15)$

$= 87.1 \times 10^{5}$ B.T.U./h

$\therefore$ Total thermal energy required = 88.19×10^{5} B.T.U./h

For steam at 150 p.s.i.a. and 358.4°F the latent heat is 864.5 B.T.U. per lb of steam: consequently the amount of steam required $= \frac{88.19 \times 10^5}{864.5}$

$= 10,090$ lb/h

Finned tube heat-exchanger

This is used to heat air from 15°c (59°F) to 95°c (203°F) by steam (counter-currently). If the temperature of the steam is taken as 249°F (30 p.s.i.a.) then the log mean temperature difference $= \frac{190 - 46}{2.303 \log_{10} (\frac{190}{46})}$

$= 102°F.$

(48)

Taking the overall heat-transfer coefficent to be 50 B.T.U./h ft^2 °F, then the heat transfer area

$= \frac{109 \times 10^3}{50 \times 102}$

$= 21.5$ ft^2.

∴ the heat-transfer area of the finned tube heat exchanger is to be 21.5 ft^2

Heat exchanger to heat circulating medium

This heat exchanger is used to maintain the solution at 50°c. The amount of solution to be heated is 20,000 l/h or 20,000 Kg./h. The maximum amount of heat to be transferred is 7×10^5 K.cal/h or 27.72×10^5 B.T.U./h. Let the condensing steam be 212°F and the overall heat-transfer coefficient be 500 B.T.U./h ft^2 °F then the log mean temperature difference

$= 123°F$

Thus the heat-transfer area required

$= \frac{2772 \times 10^3}{500 \times 123}$

$= 45.0$ ft^2.

Four heat-exchangers each with a heat-transfer area of 45 ft^2 are required.

3.3.7. Costing

Although it is very difficult to estimate the capital and operating costs of the plant, an attempt to do this has been made. The figures obtained can then be compared with those available for other plants of similar size manufacturing competing products. The feasibility of the process can also be evaluated.

3.3.7.1. Capital Cost Estimation

In this section the cost of all equipment, including buildings, is estimated as accurately as possible by up-dating price indices.

Settling Tanks 8 settling tanks are required, each of capacity 100 m^3 (22000 gallons). Cost per tank (mild steel) $= \frac{4.3 \times (22000)^{0.57} \times 128}{105}$

$= £1,580$

$\therefore$ Total cost of eight tanks (mild steel) = £12,640

Total cost of eight tanks (stainless steel) = £34,100

Medium Tanks (49) These are cylindrical tanks. The cost of each tank (mild steel) with a capacity of 48500 gallons

$= \frac{4.3 \times (48500)^{0.57} \times 128}{150}$

$= £2,440$

$\therefore$ Total cost of four medium tanks (mild steel) = £9,760

Total cost of four medium tanks (stainless steel) = £24,400

Fermenters (49). Four fermenters each of volume 250 m^3 (54500 gallons) are required. Each fermenter consists of a closed, cylindrical tank with manhole and cooling jacket.

The cost of other essential parts, such as perforated tubes for aeration, maybe added separately as 10 per cent of the total cost.

Cost of fermenter = cost of tank + $\frac{\text{cost of tank}}{10}$

Cost of fermenter (mild steel) = 5960 + 596 = £6,556

∴ Total cost of four fermenters (mild steel) = £26,224

Total cost of four fermenters (stainless steel) = £65,560

Pumps.(59) Since pumps P_1 and P_2 are of the same size they will be considered together. With a capacity of 4410g/h the cost per pump = £695

Total cost of eight pumps (P_1 and P_2) = £5,560

Cost of pump (P_3) with a capacity of 8000g/h is assumed to be 1.5 times that of a 2000 gal/h pump.

Total cost of four such pumps = £3,800

∴ Total cost of pumps =£9,360

Compressor.(56) Air supplied per hour to four fermenters = 362.4 m^3; air supplied per minute = $\frac{362.4 \times 35.25}{60}$

= 212 ft^3

Let the maximum amount of air required be 250 ft^3/min. The cost for a single-stage, double acting, heavy duty, water cooled, electric driven, 125 p.s.i.g. compressor, including motor, air filter, after-cooler and receiver = £5,230

Total cost including pneumatic conveying system = £6,000

Conveying Equipment.(56) Belt conveyors and rotary feeders can be used for transporting bags of chemicals up to tanks and moving bales of straw. A 50 feet length (16" wide) of conveyor belt is considered adequate for most of these operations. The price of the belt conveyor includes steel

welded framing, head and tail pulleys, 5" diameter troughing and return idlers, roller bearings, screw take up, duct belt and cold rolled shafts. By variation in speed, between 150 and 350 tons per hour of material could be handled.

Cost of belt conveyor = £1,490

Cost of 5 H.P. drive motor = £ 124

Total cost of the system = £1,614

Two such systems for handling materials in the warehouses will probably be required.

Total cost of mechanical conveying units = £3,228

Chopper Blower This is used for pretreating the straw before it is fed into the fermenter. A typical unit is the 7P7 chopper-blower manufactured by Coleman and Company (Agricultural) Ltd.

Standard equipment includes long, wide folding trough, transport wheels, feed auger, adjustable feed-roll, safety release with forward, reverse and idling positions, heavy motor disc - with positions for optional 1,2,3 and 6 knives or counterbalance weight, 2 knives, 3 pairs of exchange gears for selecting intake speeds, guard etc.

Total cost = £1,191

Pneumatic Conveyor System This is used to feed the fermenter as well as to blow out the dried end-product.

Cost of 100 ft length of 4" i.d. piping with fittings

= £209

Total cost of chopper/blowing system = 1191 + 209

= £1,400

It would be advisable to have two such systems available at a

cost of = £2,800

Warehouses (51)

Building and Construction Cost: One warehouse of 10m x 15m x 20m is required to store the straw bales. The cost of wall construction is £2.46 per m^2 and so the approximate cost of walls = £1,720

Cost of corrugated roofing is £1.39 per m^2 to give a total of £417.

Cost of 4" thick concrete flooring (50) $= \frac{15 \times 20 \times 2.10}{2.45} \times \frac{128}{105}$

$= £314$

∴ Total cost of warehouse = 1720 + 417 + 314

= £2,451

Store for Production. A similar building would be required ∴ cost = £2,451

Instrumentation Cost Since the process does not involve any complicated equipment nor any automation, the cost of instrumentation will not be very high. An attempt is made to estimate the cost below.

Pressure reducing valves (P.R.V.): The main steam and air-lines have 1 P.R.V. and with another one on each service-line to a fermenter, the total number is 10.

Total cost = 11 x 10 = £110

Pressure Guages: Including air-lines to all fermenters and steam-lines to the fermenters, storage tanks, and heat exchangers, the total number is about 20.

Total cost = 2.5 x 20 = £50

Temperature Gauges:

(1) one for each fermenter,

(2) one for each medium tank,

(3) one for inlet and one for outlet temperature measurement of liquid when passed through the heat exchanger,

(4) one to measure inlet and one to measure the outlet temperature of compressed air when passed through a fermenter,

(5) one to measure the temperature of the heated air used to dry the product.

Total number of temperature indicators = 25

$\therefore$ Total cost = 25 x 5 = £125

Flow Meaurement - Requirements:

(1) a rotameter to measure the air flow-rate through the fermenter,

(2) a rotameter to measure the input liquid flow-rate to the fermenter,

(3) a rotameter before and after each settling system,

(4) a rotameter to measure the inlet flow-rate to the medium tank,

(5) a rotameter to meter the make-up water,

(6) a rotameter to measure the outlet liquid flow-rate from the medium tank.

$\therefore$ Total number of rotameters required = 28

$\therefore$ Total cost = 28 x 25 = £700

Steam Traps 8 condensate traps are required on heat-exchange systems at a total cost of £46

Overall total cost of instruments (as purchased) = £1,031

Let the installation cost be 30% of the total purchase cost

$\therefore$ Total cost of instrumentation = £1031 + 309

= £1,340

Pipe-work and fittings

At this stage of costing it is impossible to estimate the total purchase-cost as well as the cost of installation of pipe-lines. In the plant a lot of piping is not involved compared with say a conventional chemical plant. Hence, the cost of piping is assumed to be low and figures of £2,000 for mild steel and £5,000 for stainless steel are assumed.

Boiler (52)

For a gas-or oil-fired, water-tube boiler with economiser and field erected;

$$\text{Total cost} = 9.5 \times (10)^{.68} \times \frac{128}{105} \times 10^3$$

$$= £22,800$$

Cost of Heat Exchangers

Since the heat-transfer area of both the heaters is less than 50 sq.ft. the cost of each can be fixed at £300

∴ Total cost of five heaters (mild steel) = £1,500

Total cost of five heaters (stainless steel) = £3,500

Cost of Land

Assuming 5 acres are required, the total cost = £1,500

Approximate Capital Cost Summary

	£ Mild Steel	£ Stainless Steel
Settling tanks	12,640	34,100
Medium tanks	9,760	24,400
Fermenters	26,224	65,560
Pumps	9,360	
Compressors	5,230	
Conveying equipment	3,228	
Chopper/blower system	2,800	

Ware house	2,451	
Store for product	2,451	
Instrumentation	1,340	
Piping	2,000	5,000
Boiler	22,800	
Heaters	1,500	3,500
Land	1,500	

∴ Approximate capital cost when mild steel is material of construction = £93,284

Approximate capital cost when some of the equipment is made of stainless steel = £162,412

Let 7% of the approximate capital cost (with mild steel) cover the cost of clearing site, foundation costs, structural costs, structural steel-work, bagging equipment and bagging

∴ Total capital cost when mild steel is used

= 93284 + 6529

= £99,813

∴ Total capital cost when stainless steel is used

= 162412 + 6529

= £168,941

From the above capital cost calculations, it is clear that if stainless steel is used as material of construction for various equipment the overall capital cost is considerably increased.

Since the process conditions are not very extreme in terms of temperature, pressure and pH, the use of stainless steel as a material of construction is not that essential. Therefore cheaper material of construction i.e. mild steel, is probably acceptable.

3.3.7.2 Operating Costs

Annual Cost of Chemicals

Salt	Total requirement (Kg)	Cost per Kg (£)	Total cost (£)
KH_2PO_4	16,940	0.25	4,235
$(NH_4)_2SO_4$	53,400	0.0143	764
Yeast Extract	1,500	0.35	525
K.Cl	10,200	0.034	354
$MgSO_4.10H_2O$	5,280	0.044	232
$Cacl_2$	1,720	0.025	43
		Total cost of chemicals	£6,153

Water Basis: 1 year

Total volume of make up-water required = 4720 x 50

= 236,0001

Total volume of water required for preparing solutions, including that used for leaching = 800,000 + 4600,000

= 5,400,0001.

∴ Total volume of water required = 5,636,0001.

= 1.24×10^6 gallons

or 1.5×10^6 gallons overall

∴ Cost of water at the rate of £0.175 per 1000 gallons = $\frac{1.5 \times 10^6}{1000} \times 0.175$ = £262

Electricity

On average the amount of electricity used = 20 H.P. on a continuous basis; thus the number of units (KWH) used per year = $20 \times 50 \times 23 \times 24 \times 0.746$

$= 4.12 \times 10^5$

Total cost of electricity used per year (at the rate of 0.75 pence per unit) $= \frac{4.12 \times 10^5 \times .75}{100}$

$= £3,085$

Gas (for Boiler)

Thermal energy required = 8.75×10^6 B.T.U./hr.

Total thermal energy required per annum = $(8.75 \times 10^6 \times 50 \times 7 \times 24)$ B.T.U.

1 therm = 10^5 B.T.U. and considering Boiler to be 70% efficient the total cost of gas at the rate of 5 pence per Therm is given by $= \frac{8.75 \times 10^6 \times 50 \times 7 \times 24 \times 5}{10^5 \times 0.70 \times 100}$

$= £5,700$

Labour

Assuming that two process operators are used to run the plant, the labour costs amount to about £4,000 per annum.

Depreciation

Assuming a 20 year plant life, the cost due to depreciation per year $= \frac{99813}{20}$

$= £4,990.65$

or £5,000

Raw Material

Although there are seasonal fluctuations in the price of straw, for the purpose of costing, an average price of £3.5 per ton of barley straw can be used.

Cost of raw material used = £3,500 per annum.

Transport costs

Assuming that the product is to be delivered upto 100 miles, then the cost of transport will be approximately £2.70 per ton.

The total cost of 800 tons of product = £2,160

Maintenance Cost for Equipment

The maintenance cost for equipment is taken to be about 2% of the capital cost = £1,996 per annum.

Packaging Costs

Let the product be delivered in 10Kg bags and the cost of packaging be 5 pence per bag; total cost of packaging 800 tons = $\frac{800 \times 100}{10} \times \frac{5}{100}$ = £4,000

Summary of Operating Costs

Cost of chemicals	£6,153
Cost of water	262
Cost of electricity	3,085
Cost of gas	5,700
Cost of labour	4,000
Depreciation	5,000
Cost of raw material	3,500
Transport cost	2,160
Maintenance cost	1,996
Packaging cost	4,000
Total operating cost	£35,856

Product cost before profit = $\frac{35856}{800}$ = £44.84 per ton.

3.3.7-3 Final Cost of Product

Let there be a gross return of 15% of the fixed capital investment (before tax).

Total cost of product = 35856 + $\frac{99813 \times 15}{100}$ = £50,826

Final cost per ton of product = $\frac{50,826}{100}$

= £63.53

Results of Costing Exercise

Initial capital investment required when mild steel equipment is used	= £ 99,813
Capital investment required when stainless steel equipment is used	= £168,941
Operating cost of plant per annum	= £ 31,856
Cost of product per ton before any profit	= £ 44.84
Final cost of product per ton with 15% return (before tax).	= £ 63.53

3.3.8. Competing Products

Many types of animal feed are available with which the proposed fungal feed will have to compete. Bulky, fibrous materials known as roughages are suitable for feeding ruminant animals. On the other hand non-fibrous starch or proteinaceous concentrates are suitable for every type of animal. Supplements such as minerals, vitamins, antibiotics and amino acids may also be added to keep the animal healthy. Concentrates used for animal feed compounding may be classified as below[53]:-

1. Ceareals and their by-products (such as wheat, barley, oats, rye, maize, sorghum, millet and rice).

2. Pulses (peas and beans).

3. Residues from oil seeds after oil extraction (including soya, ground nuts, linseed, cotton seed, rape seed, mustard seed, kapok seed, palm kernals, coconuts, sesame and sunflower seed).

4. Dried starchy roots and tubers (such as cassava and potatoes).

5. Animal and fish products and by-products (such as meat, bone and blood meals, fish meal, dried fish solubles and dried milk products).

TABLE 30

Cost comparison of compounded feeding stuffs [53]

produced by plants of various sizes

September 1971	Model I	Model II	Model III	Model IV
Type of Compound	Dairy Compound	Dairy Compound	Poultry Products	Poultry Products
Approximate Protein Content (w/w%)	14 to 17	14 to 17	15 to 18	15 to 18
Animal Feed Output (tons p. a.)	2,400	6,000	10,500	16,800
Total Capital Invested (£)	41,231	102,816	168,206	254,518
Total Operating Costs (£ p. a.)	87,447	210,544	382,729	601,923
Cost per Ton of Output (£)	37.30	36.00	37.20	36.50

6. Miscellaneous materials (including grass and lucerne meals, dried sugar beet pulp, molasses and carobs).

Jones and Halliday [53] constructed four cost models to illustrate in detail the economics of small-scale production of compounded animal feeds. The information regarding these cost models is summarised in the table (30).

Costs of some animal feeding stuffs published by the Ministry of Agriculture, Fisheries and Food for December 1970 are shown below:

Feeding Stuff	Cost (£ per ton)
Wheat by-products (bran)	36.33
Barley Meal	36.99
Maize Meal	38.54
Cottonseed Cake and Meal (expellers Sudanese)	58.72
Cottonseed Meal (extracted Indian)	47.00
Cottonseed Cake	46.00
Groundnut Cake and Meal (expellers)	
Burmese	58.16
Gambian	55.51
Nigerian	58.21
Groundnut Meal (extracted Indian)	55.42
Linseed Cake and Meal	50.50
Soybean Meal (extracted Canadian)	59.00
Sunflower Seed Meal (extracted Argentine)	47.06
Palm-kernel Meal (extracted home products)	37.50
White-fish Meal (home produced)Canadian	99.04
Canadian	94.00
Icelandic	93.62
Fish Meal (South African)	98.67

TABLE 31

Price list of protein foods

Food Product	Wholesale price for Commercial sales		Price of Protein
	Price of product £ per ton	Percentage Protein	£ per ton
Less Conventional Protein Foods			
Cotton Seed Flour	70	55	120
Soy Protein Flour (Israel)	70	52	130
Toasted Soy Protein	80	50	160
Groundnut Cake (India)	70	42	170
Fish Protein Concentrate	270	85	320
Protein Isolate (Provine)	350	97	360
Multipurpose Food (India)	180	42	420
Multipurpose Food (Brazil)	240	50	480
Arlac	210	42	500
Lypro	350	65	540
Incaparina (Guatemala)	190	28	680
Pronutro	150	22	680
Unconventional Protein Foods			
Lysine	-	-	70
Chlorella	130	60	210
Yeast (Petroleum)	70	50	140
Yeast (Vegetable)	140	50	280
Spirulina	180	65	280
Leaf Protein	470	50	940
Algae (scenedesmus)	30	50	60

Date: 1969

(Appx) conversion factor used: 10 U.S. cents per pound = £ per ton

TABLE 32

Price list of various protein foods

Food Product	Price £ per ton	Percentage Protein	Price of Protein £ per ton
Cottonseed Meal	40	41	100
Soybean Meal	40	44	88
Defatted Soy Flour (edible)	70	50	140
Soy Protein Concentrate	180	70	260
Cottonseed Flour (air classified	97	57.5	170
Soy Protein Isolate	350	95	370
Peanut Flour (U.S. price)	250	60	420
Torula Yeast Food Grade	170	48	360
Skim Milk Powder (Grade A)	200	37	540
Casein (Food-grade)	400	100	400
Fish Protein concentrate	180	80	225
Spun Soy Tow	400	70	570
Texturized Vegetable Protein	25	50	500
Egg Albumin	750	88	850
Lacto-albumin	650	80	820
Wheat Gluten	300	80	380
Wheat Germ	80	30	270

Date: 1969

This table suggests that economies due to scale are not highly significant but it does indicate the sort of selling prices a fungal feed will have to compete with.

The cost of protein in some less conventional and unconventional human feeds have been given by Abbott[54] and are listed in table (31).

Vix and Decossas[55] have also summarised the cost of protein in various foods available for either human or animal consumption. Their figures are quoted in table (32).

Although the cost figures given are not completely up to date they indicate that the selling price of £63.53 per ton of product or £907.5 per ton of protein for the fungal feed stuff produced by solid substrate fermentation is comparable with that of leaf protein.

Considerable cost reductions are required to make the process viable.

3.3.9 Discussion

The process design calculations given in sections (3.3.) have highlighted a number of problems in solid-substrate fermentation. From an economic viewpoint it is the long residence time (over three weeks) which makes solid-substrate fermentation relatively so

expensive. Although there is little prospect of reducing process time, this does not mean that the process will never be feasible. More detailed examination of the cost components is very illuminating, since this indicates where savings must be made if the price is to be reduced.

Looking at the operating costs, one of the most expensive items is the cost of chemicals (£6,153 per annum). There is clearly need for further research to minimize the amount of chemicals used. Only more experimental work can show to what extent each chemical can be reduced in quantity and which chemicals can be replaced by cheaper ones.

The next most expensive items are electricity (£3,085 per annum) and gas (£5,700 per annum). These figures presuppose that all parts of the system work to full capacity throughout. This is not strictly the case and these figures need further study in depth.

Other expensive items are the cost of transporting the end-product as well as the packaging cost. These two items can be reduced if the plant supplies local farm units.

Complete initial sterilization of the system (including raw materials) is the second processing feature which adds to the overall cost. A slightly different approach to this problem seems justified. If the system is maintained at 50°C and a large amount of inoculum is used, then complete steam sterilization may not be necessary. This approach is supported by the microbiologists working within the project group.

4. Conclusions

(1) Technologically the process of solid substrate-fermentation for microbial upgrading of cellulose based materials to produce animal feed is possible.

(2) A thermophilic, mixed culture, single stage system is to be preferred. The mixed culture should contain cellulolytic and non-cellulolytic fungi.

(3) The long residence time of substrate in the fermentation stage means that the process equipment is bulky and relatively expensive if sterilisation is necessary.

(4) An economic feasibility study has indicated that the cost of the product is greater than that of equivalent products used in animal-feed compounding. More work is required to see whether or not costs can be significantly reduced.

5. Suggestions For Future Work

It is believed that this project has opened up a number of new research areas in the field of solid-substrate fermentation.

Firstly, preliminary studies with mixed culture systems have been carried out with straw as a substrate. These studies need to be extended particularly in the mesophilic range using combinations of cellulolytic and non-cellulolytic microorganisms. From the processing viewpoint a mesophilic system has the advantage that the thermal energy content is lower than that of a thermophilic system. However, there are some draw backs, viz. that mesophilic systems can be easily infected and that they grow relatively slowly.

Secondly, the most promising systems encountered have involved a single stage fermentation. That this is generally true needs to be explored experimentally. Only a limited number of microbial combinations and processing procedures have been examined to date.

Thirdly, although the design of a large-scale plant has been considered, there is clearly scope for ingenuity in developing plant for solid-substrate fermentations. It is evident that the degree of sterility necessary is a problem requiring very careful study if the cost of production is to be cut significantly. It is now thought that with a thermophilic system operating at say 50°c the addition of a large inoculum may be sufficient to keep the system sterile. This idea needs to be thoroughly investigated. The design calculations presented in this

thesis also reveal that the concentration of salts in the growth medium adds considerably to the processing costs. Further effort is required to determine to what extent such costs can be minimised.

Finally, more mathematical work is required to develop satisfactory equations for the design of solid-substrate fermenters, whether operated isothermally or adiabatically. The kinetics of growth of filamentons fungi is a topic that has received little attention to date: it is one directly relevant to the development of processes involving solid-substrate fermentation.

APPENDIX 1

The Micro-Kjeldahl Method For Determining Nitrogen and Protein

Prinicple

Between 0.1 to 0.25 mg of dried solid is digested with concentrated sulphuric acid (nitrogen free) for three hours in the presence of a catalyst. Ammonia is then liberated from the mixture by adding sodium hydroxide and is then collected in boric acid solution. The amount of ammonia absorbed is then estimated by titration with standard hydrochloric acid. The amount of nitrogen in the original sample can then be computed (1 ml of $\frac{N}{100}$ HCl = 0.14 mg of nitrogen). Since the average nitrogen content of proteins is about 16% w/w, an estimate of the protein-content can be obtained by multiplying the % N by a factor of 6.25.

Reagents

1. Catalyst:- the following chemicals are ground into a fine powder:-

K_2SO_4	= 80 gm
$CuSO_4 . 5H_2O$	= 20 gm
$Na_2SeO_4 . 10H_2O$	= 0.34 g.

2. Conc. H_2SO_4 (nitrogen free)

3. H_3BO_3 (A. R.)

A 4% (w/v) solution is made up in distilled water.

4. Indicator Equal volumes of 0.2% methyl red and 0.1% methylene blue solution made up in absolute alcohol are mixed together.

5. $\frac{N}{100}$ HCl solution (standardised)

6. NaOH (A. R.) A 40% (w/v) solution is made up in distilled water.

Preparation of sample:-

A few grains of biodegraded substrate were removed from the fermenters aseptically at the predecided time. It was then washed very gently with distilled water. The washing technique was standardised very carefully. A small beaker (50 ml) containing the sample (1 to 2 gms. approx.) was filled with distilled water. It was left to stand for fifteen minutes with occasional stirring, and then the excess liquor was drained off. This was repeated five times. It was hoped in this way to remove inorganic nitrogen from the sample with the minimum loss of fungal mycelium grown on the surface of the substrate. After drying the sample was ready for analysis.

Method

. In a 30 ml incineration flask the following substances were

introduced in the given order: 0.5 gm catalyst mixture, 0.2 to 0.25 mg. of test sample and 2ml Conc H_2SO_4. The mixture was heated for three hours (until it became clear). It was allowed to cool and was transferred quantitatively to the distillation apparatus. For steam distillation of the digested liquor a Markham Apparatus[56] was used. In addition to the digest and distilled water used to wash the flask and apparatus inlet, 20 mls of 40% NaOH were added. The liberated ammonia was collected in 10 mls of 4% boric acid solution (containing indicator) to a final volume of about 30 ml. It was then titrated against $\frac{N}{100}$ HCl to a colour change of green to purple grey. The final calculation to estimate the protein content of sample was based on the relationship: 1ml of $\frac{N}{100}$ HCl = 0.14 mg. of nitrogen (N). The protein content of the sample is then found by multiplying the N% (w/w) by a factor of 6.25.

APPENDIX 2

Reproducibility of Results

Ten samples of barley straw were analysed to estimate % protein (w/w) by the Micro Kjeldahl Method.

The results obtained are tabulated below.

Weight of Sample gms	% Protein (w/w)
0.3051	1.12
0.2328	1.57
0.3027	1.51
0.1724	1.27.
0.2426	1.77
0.2171	1.41
0.2009	1.75
0.3553	1.04
0.1761	1.05
0.1359	1.35

Assuming these results have a fairly symmetrical, unimodal distribution the standard deviation of these results was calculated by the formula:

$$S = \sqrt{\frac{\Sigma(X - \overline{X})^2}{n - 1}}$$

where S = standard deviation

X = result

$\overline{X}$ = arithmatic mean of various values of X

n = number of results

By calculation

$$\bar{X} = 1.384$$

$$\Sigma(X - \bar{X})^2 = 0.6480$$

$$\frac{\Sigma(X - \bar{X})^2}{n - 1} = 0.072$$

$$S = \sqrt{0.072} = 0.2684$$

For any distribution which is reasonably symmetrical about its average and is unimodal one can safely assume that two thirds of the distribution lies less than one standard deviation away from the mean and 95% of the distribution lies less than two standard deviations away from the mean.

This is confirmed below by examining the range

$\bar{X} + S = 1.652$ and $\bar{X} - S = 1.116$

One can see that two results (i. e. 1.04 and 1.05) are less than the lower limit 1.116 and two results (1.77 and 1.75) are outside the upper limit of 1.652 i. e. just less than two thirds of the results lie within the limits of $\bar{X} + S$ to $\bar{X} - S$.

Similarly $\bar{X} + S = 1.910$ and $\bar{X} - 2S = 0.758$

From this it can be seen that all the results lie between the limits of 0.758 to 1.910.

Hence one can say that the analytical technique used is fairly reliable .

Appendix 3

Percentage of usable carbon in cellulose

The native cellulose molecule[2] contains 3100 ± 100 (say 3200) anhydroglucose units.

The approximate molucular weight of native cotton cellulose = 570,000.

Molecular weight of one glucose molecule = 180

= 72 of carbon in glucose.

∴ Amount of carbon present in one native cellulose molecule = 72 x 3200.

% of carbon present in native cellulose molecule = $\frac{72 \times 3200}{57,000} \times 100$

= 40.5%

(say 40%)

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