T H E S I S

"BIOSYNTHESIS OF UTILIZABLE MICROBIAL BIOMASS IN THE TREATMENT OF MILK WASTES IN HIGH-RATE FILTERS."

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A C K N O W L E D G E M E N T S

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S U M M A R Y

The feasibility of producing utilizable microbial biomass while treating dairy wastes in a pilot-scale high-rate Flocor tower was examined.

The literature on different methods of utilizing organic wastes and on the growth and control of fungi in bacteria beds was reviewed.

A survey of dairies using Flocor high-rate filters to treat their waste was carried out and samples of bios were removed from the filters for microbial analysis. Fungi dominated all the film samples and three species were selected from twenty-nine for further studies based on their frequency of isolation. Laboratory studies established the optimum growth conditions for these fungi in batch culture. The effects of temperature, pH and feed strength on fungal growth were determined using agar plates, flask cultures and horizontal plastic screens. The method of culture turbidity as a measure of mould growth was assessed.

One of the selected fungi, *Fusarium aquaeductuum*, was excluded from further studies because of its potential toxicity. A continuous culture apparatus was devised and developed to produce sufficient fungal biomass for analysis and assessment of the nutritive value of two fungi. The results of the analysis compared favourably with commercially-based fungal products and pilot plant studies commenced.

The pilot plant was sited at a dairy, which already possessed a full-scale Flocor tower, and was commissioned in October, 1978. The temperature and strength of the effluent from the dairy were governed by the products being manufactured. The feed was acidified to encourage the growth of the selected fungi. Equipment failures prevented the plant from operating consistently and reliably but results obtained have shown that the production of fungal biomass is feasible. Analysis of pilot plant biomass revealed amino acid levels lower than those obtained from the fungi grown in the laboratory. The need for continued investigations was discussed.

Key words: BIOSYNTHESIS, EFFLUENT TREATMENT, HIGH-RATE FILTERS,

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C H A P T E R   O N E

INTRODUCTION

Waste waters produced by dairies and dairy-products factories generally consist of milk residues, milk derivatives and detergents diluted with water in varying quantities. The volume of waste waters discharged from a particular dairy depends on the type of process as more water is used in milk-products manufacture than in milk-bottling and distribution. Dairy effluents are not normally toxic but they are capable of rapid decomposition and the released organic matter provides a very good nutritional basis for the growth of micro-organisms. In the past many streams and rivers have been polluted by dairy effluents as a result of bacteria flourishing in the discharged organic matter and de-oxygenating the water. Legislation has been gradually introduced since the late nineteenth century to prevent such pollution, and many dairies now discharge their waste waters directly to the public sewers for treatment at the local sewage works. Each dairy must obtain the consent of the local water authority before a new or altered effluent is discharged to the sewers. Details of the nature and composition of the effluent, the maximum temperature, maximum daily volume and the highest discharge rate must be given to the water authority prior to consent. Permission to discharge effluent to the sewers may be granted with certain conditions imposed and charges can be made for receiving, conveying and treating the effluent.

A report published by the Ministry of Agriculture, Fisheries and Food (1969) gave substantial information on all aspects of dairy operations with particular emphasis placed on standards and charges imposed by the authorities. The information was gained

as a result of a questionnaire sent to one thousand and seventy-five dairies. Three hundred and eighty-four dairies responded to the questionnaire and the majority (314) discharged their effluent into the sewers. The remaining dairies discharged effluent to a river (57), a ditch(10), the sea (two) and one to open land. Fifty four of these dairies had their own effluent treatment plant, out of which 36 had to meet the Royal Commission 'standard' for sewage effluents. This 'standard' specifies limits of no more than 30 mg/l. suspended solids and 20 mg/l. BOD (see section 4.2, Chapter 4 for explanation of BOD) for discharge to a water-course offering at least an eight-fold dilution of clean water. The water authority may set a higher 'standard' if the water-course receiving the discharge is used for potable water. There is often an additional requirement that the effluent pH lies within the range pH 6 to 9. The volume and strength of an effluent are used to determine the charge levied by the water authority applicable when the discharge is made to a public sewer. Dairies discharging effluent to tidal waters generally have to comply with less stringent standards than those discharging to fresh water or public sewers, and may need only partial treatment of their waste waters. A biological process is generally used to treat dairy effluents because a simple chemical or physical process cannot remove the sugars in the waste waters at an economic price. There are several biological treatment processes available for dairy effluents, most of which use settlement followed by biological filtration or a system based on the activated sludge process.

The biological filtration process is based on circular or

rectangular beds of inert, specially graded media such as blast furnace slag, hard clinker, fragmented rock, gravel or plastic. The liquid to be treated is distributed evenly over the bed surface and percolates over the media on which develops a film of bacteria, fungi, protozoa and other micro-organisms. Eventually a balanced community develops with oligochaete worms and insect larvae feeding directly or indirectly on the main decomposers the majority of which are bacteria, fungi and certain protozoa, mainly flagellates. The developing film of micro-organisms utilize the organic matter in the percolating liquid by partial oxidation to carbon dioxide, water and various stable end-products and by synthesis of new cell material. Solid matter excreted by the grazing worms and insect larvae together with portions of dislodged film are removed in the effluent, collected at the base of the bed, and settled out as humus sludge. An increase in the concentration of the waste waters results in increased film growth which can lead to blockage of the interstitial spaces in the bed. Further applications of liquid cannot percolate through the media, (a condition known as ponding) air circulation is affected and the film causing the blockage undergoes decomposition resulting in putrid odours. It is, therefore, important to maintain a balance between the waste concentration and film growth in order to achieve efficient purification. The filter beds described in the biological filtration process may be used singly, in pairs in series (double filtration), or in pairs in series with the order changed at regular intervals (alternating double filtration).

In the activated sludge process the waste waters are treated in large tanks where they are aerated by compressed air or by surface aeration. The sludge is a mixed culture of micro-

organisms dominated by bacteria which feed on the organic matter in the waste waters. Intially the bacteria are dispersed but they multiply rapidly and flocculate which enables the activated sludge to be settled and prevents it being washed away in the effluent. The structure and biological activity of the individual flocs determine the efficiency of the activated sludge process. Flocs grow by increasing the numbers of bacteria by synthesis and accumulation of organic matter. Nematodes, protozoa and rotifers penetrate the flocs and feed on the bacteria resulting in an increasing proportion of dead cells and inert material as the flocs age. There is a decrease in the oxidative capacity of the flocs but adsorption remains efficient. The concept of sludge age (age of the flocs) is used in part to operate activated sludge plants and determine the aeration period. In a manner similar to the biological filtration process it is important to balance the waste concentration (BOD) entering the system with the biomass of organisms feeding on it. Eventually the effluent is transferred to sedimentation tanks where some of the sludge is recycled to inoculate the incoming waste and the purified water is discharged into a river.

The majority of dairies with effluent treatment plants have probably chosen to use biological filtration instead of the activated sludge process as a result of research. In the 1930's the Water Pollution Research Board conducted a series of laboratory and large-scale experiments to compare the treatment of milk washings and whey washings using biological filtration and the activated sludge process. (Water Pollution Research Technical Paper No. 8, 1941). The Board had previously concluded that large scale treatment of dairy waste waters by anaerobic fermentation or chemical coagulation would be unsuitable because of

bad odours and excessive sludge. The activated sludge process was generally found to require more control than biological filtration, and a sudden influx of very strong liquid tended to upset the settleability of the sludge. Temperature had a profound effect on the efficiency of treatment by the activated sludge process with better quality effluents produced in the warmer weather. However, the overall conclusion was that effluents obtained by the treatment of similar liquids were of a poorer quality from the activated sludge process than from the double filtration process.

The single filtration process was found to be unsuccessful for treating milk washings due to excessive accumulation of biological film and fat unless pre-treatment to remove fat and protein was employed. Milk wastes have a high carbon to nitrogen ratio which favours the growth of fungi. The fungi are able to utilize the organic matter in the waste with greater efficiency of assimilation than the bacteria resulting in a greater film accumulation and its attendant problems. Recirculation of purified effluent to dilute the incoming waste can be used in single or double filtration. This method of dilution tends to diminish the surface film but does not necessarily allow an increase in the BOD loading of the filter.

Double filtration (two filters in series) was found to be unsuitable for treating dairy wastes unless the problems of single filtration could be overcome. The primary filter can be used as a coarse roughing filter with the effluent recycled at a high-rate, this removes most of the organic strength of the waste and prevents excessive film accumulation on the secondary filter. Unless this type of design is used there is no advantage in

using two filters in series.

The alternating double filtration process surmounted the problems of excess film and ponding of filters treating milk washings. The process was developed by the Water Pollution Research Board and uses two filters in series with settlement after each stage. The order of the filters is alternated periodically which allows any film accumulation on the primary filter to be washed off when it assumes a secondary role treating weaker waste. This system allows a much greater volume of polluting matter to be removed per cubic metre of medium than the other filtration methods. The initial cost of an alternating double filtration plant is high but the running costs are lower than those of an activated sludge plant hence the adoption of this method by many dairies.

There are some dairies which are able to discharge their effluent to tidal waters such as estuaries or the sea. In many cases the water authority does not require such stringent standards for the effluent and high-rate filtration may be employed as a method of treating the wastes. High-rate filters use coarse media with large voids to prevent blockages caused by film accumulation. Purified effluent can be recirculated at high volumetric loadings enabling strong organic wastes such as dairy wastes to be reduced in strength prior to discharge to a public sewer, tidal waters or another biological treatment process.

In 1960 Jenkins and Hawkes reported that the use of high-rate filtration as a method of treating sewage in Great Britain was virtually unknown. Since that date the situation has changed substantially with the advent of specially fabricated plastics media. Several types of plastics media have been

developed in Great Britain and the U.S.A. In 1962 the Water Pollution Research Laboratory began conducting experiments with a fabricated polystyrene medium. A pilot-scale filter was constructed and comparative tests were made with conventional media of similar specific surface also in pilot-scale filters all treating Stevenage settled sewage. The plastic medium was slightly inferior in performance judging by BOD removal and the degree of nitrification. Later reports by the Board in 1964 indicated that the difference in performance of the plastic medium was related to the retention time of the liquid in the filter. However, in 1967 Chipperfield stated that polystyrene and blended polystyrene were found to be unsuitable for production packings of plastic media. The reasons for rejection were low brittle strength which reduced further when exposed to weather and poor chemical resistance. Polyvinyl chloride was chosen for plastic media construction for use in high-rate biofiltration systems. The fabricated plastic media were designed to remove a large weight of BOD per unit volume at high organic and hydraulic loadings. Strong organic wastes such as dairy wastes could be treated by plastic media which would not become blocked by biological film accumulation because of the high percentage of voids. The concept of a 'roughing' filter became established as plastic media filters were employed in existing treatment plants which were overloaded or wanted to expand with limited land available. A 'roughing' filter removes a considerable amount of the overall BOD load applied to the plant without producing an effluent of Royal Commission standard. Chipperfield (1967) has outlined the basic requirements for an ideal roughing packing. The light

weight of the plastic media enables tall towers to be constructed without the need for large areas of land used by conventional media filter beds and their inherent high costs for civil engineering work. The plastic media filters can thus be installed at plants which intend to expand production and require a large surface area of filter media in a compact space. This has been the situation at many dairies located in rural areas and facing the prospect of high charges if their effluent is treated by the regional water authority. The economics of plastics packings are least favourable if the waste water strength is below 300 mg/l. BOD but dairy effluents tend to be much higher than this. The majority of full-scale plants treating dairy effluents by plastic media high-rate biofiltration in Britain have used 'Flocor' developed by I.C.I. Ltd. Each 'Flocor' module is constructed of alternate flat and corrugated sheets of polyvinyl chloride bonded to form units of 1200 mm. by 600 mm. by 600 mm. Each sheet is doubly corrugated to prevent the applied liquid from travelling straight downwards. A polyvinyl chloride-based adhesive holds the sheets together and the modules are placed in the filter with each layer at right-angles to its neighbour. The weight of each module is seventeen kilograms, surface area is equivalent to a minimum of 90 m^2/m^3 and there is a minimum void space of 96%. (Plates 1 and 2).

This thesis describes a three and a half year investigation into the possibility of producing utilizable biomass from the treatment of milk wastes in high-rate filters. It has been mentioned that the problems of treating milk wastes on biological filters arose from the large accumulations of predominantly fungal film. It was thought that by taking advantage of and

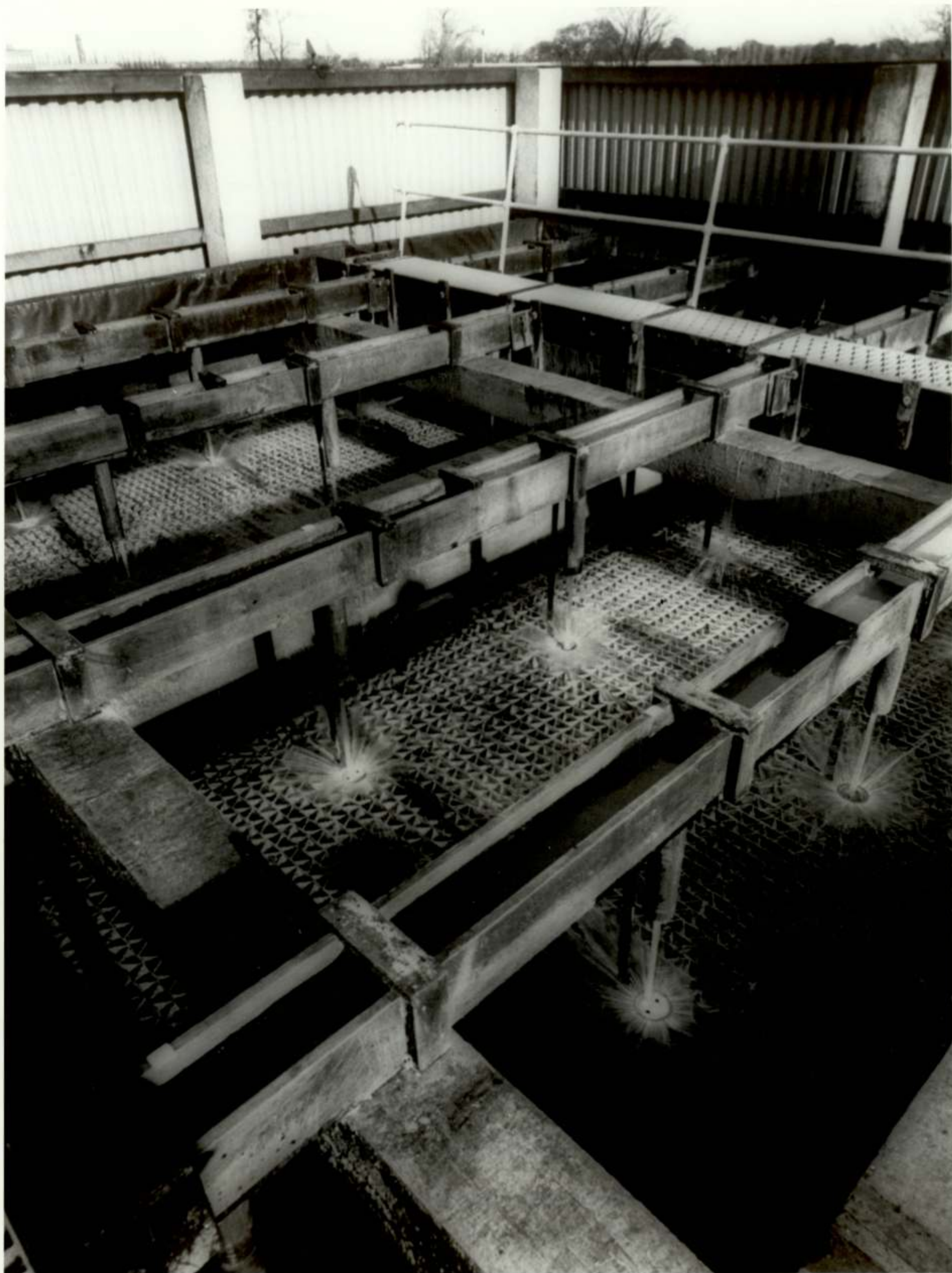


PLATE 1

View of the top of a 'Flocor' filter showing the positioning of the circular splash-plates directing the liquid over the upper surface of the plastic media.

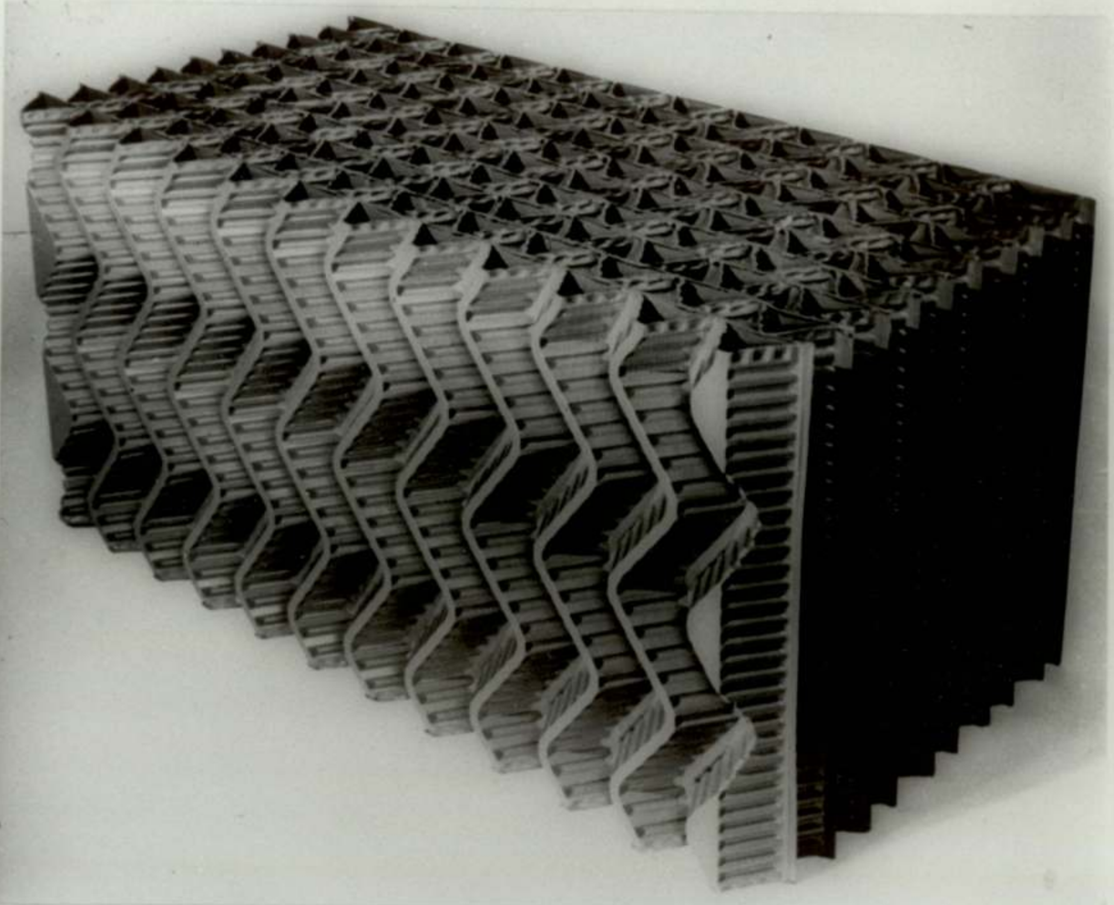


PLATE 2

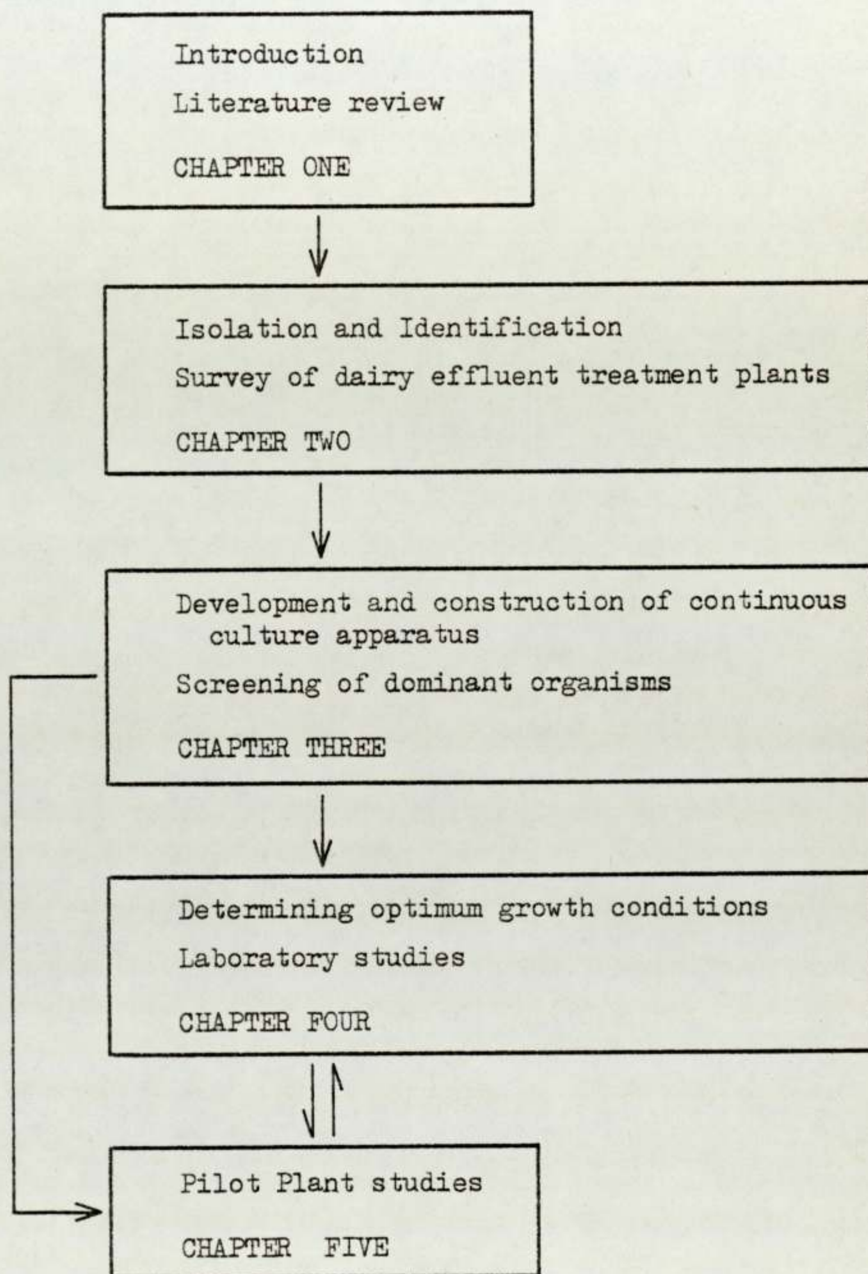
A single module of Flocor E, 1200 mm. long, 600 mm. wide and 600 mm. high.

encouraging the growth of fungi in (initially) a pilot scale high-rate plastic media filter that the harvested biomass could be used as animal feed. All the filters used the plastic media 'Flocor' manufactured by I.C.I. Ltd., who sponsored the investigation. The initial studies involved visiting a number of dairies using plastic media high-rate biofiltration to treat or partially treat their effluent. Samples of bios were removed from the media and examined for the presence and relative abundance of fungi (see Chapter 2). The high carbon to nitrogen ratio of dairy effluents favours the growth of fungi and many species were ultimately isolated. Having isolated and identified the fungi producing considerable bios in the filters, the dominant organisms were selected for further study (see Chapters 3 and 4). Batch-culture experiments were carried out to investigate the optimum temperature, pH and feed strength of the chosen fungi. Continuous-culture apparatus was devised to produce sufficient biomass for biochemical analysis to assess the nutritional status of the organisms. The work was planned to terminate if the species selected and examined were unsuitable, otherwise the laboratory investigations were to be expanded by using a pilot-plant (see Chapter 5). The pilot-plant studies were planned to maximize biomass production by adjusting parameters such as the pH, loading and dosing frequency of the milk wastes based on the findings of the laboratory studies. The fungal biomass would be harvested at prescribed intervals and analysed for its amino acid content and suitability for feed. The reasons for basing the study on the use of suitable fungi as opposed to bacteria will be discussed in the literature review. It was intended that if there were no organisms isolated considered

suitable for nutritional purposes then it might be possible to devise a method of minimizing sludge production. The cost of treating and removing sludge can be extremely high depending on the nature of the waste and the siting of the plant for disposal of the sludge to farm land or land-fill.

Figure 1.1 shows the relationship of separate stages involved in this project together with the relevant chapters in the thesis.

FIG. 1.1 Development of the thesis - related investigations and associated chapters.



LITERATURE REVIEW

The rapid expansion of the human population has resulted in many serious problems throughout the world. The production of sufficient nutritionally adequate, inexpensive, high quality protein to satisfy the needs of an increasing number of people is one of the main difficulties. In recent years it has become clear that the world shortage of protein cannot be fulfilled by conventional means alone. Cultivating more land and improving agricultural methods to produce more food grains and vegetables is one answer. Available land, however, is becoming more restricted and the protein obtained from cereals is of a lower quality for human nutrition than animal protein. Feeding agricultural products to animals subsequently used as protein sources is more inefficient than using them directly as food. Grazing animals require large areas of land which, even if available, is also an inefficient method of producing protein. An alternative, which is gradually gaining acceptance, is the use of microbial (single-cell) protein. Single-cell protein (SCP) can be produced on a large-scale independent of agriculture and the inherent problems of climate. Large amounts of single-cell protein can be produced by the fermentation of a wide variety of substrates such as grains, molasses, waste sulphite liquor and whey. In recent years the cost of effluent treatment and the rapid increase in protein and energy prices have encouraged research into the feasibility of different methods of utilizing organic waste waters. These methods have been based on one of three techniques for producing protein. The first approach involves directly extracting protein from a protein-rich waste using methods such as precipitation, ultrafiltration and reverse osmosis, flotation and ion exchange. The second approach

produces SCP from wastes rich in carbohydrate by a process of microbial conversion and the third approach is to utilize the waste directly.

The direct extraction of protein from animal and vegetable processing using selective chemical precipitation has been described by Tønseth and Berridge (1968). Pure lignin sulphonic acid is added to the waste from slaughter-houses, bone processing plants and fish meal factories (as a few examples) in a ratio related to the soluble protein content, The modified waste is subjected to dissolved air flotation where the liquid is separated from the precipitated proteinaceous sludge. Jørgensen (1971) examined the possibility of using other chemicals for the precipitation of proteins in waste water. He used glucose trisulphate, azoprotein, sulphite liquor containing lignin sulphonic acid, aluminium sulphate, polyacrylamide and acidified blood albumin. The economics of the precipitation process demanded that the resultant sludge be used for feed and fodder production. Protein precipitated with lignin sulphonic acid had a very low biological value and the rats which fed on it suffered from diarrhoea and malnutrition. However, an amino acid analysis of proteins precipitated by glucose trisulphate and azoprotein proved favourable in comparison with blood albumin and bone meal. Hopwood and Rosen (1972) also described a protein precipitation process, (the Alwatech process) using purified sodium lignosulphonate, developed for the treatment of slaughterhouse and poultry packing waste waters. The flocs produced by precipitation have a specific gravity similar to water and therefore have to be separated using dissolved air flotation. The low pH used in the process enables fat to be incorporated in the flocs resulting in BOD removals of 70-90% and an effluent low in suspended solids, fat and organic nitrogen. The high cost of the purified chemicals can be offset against the sale of the protein-fat precipitant mixture as animal feed.

Developments in membrane separation technology have resulted in the use of the related reverse osmosis/ultrafiltration processes for concentrating and fractionating valuable components of waste materials such as whey. Both reverse osmosis and ultrafiltration use molecular screens of specially made polymeric films to separate the solute and solvent molecules on the basis of size, shape and chemical structure. Reverse osmosis is used to separate low molecular weight solutes (salts, sugars, simple acids) using high hydraulic pressures to exceed the osmotic pressure of the solution. Virtually all molecules, except the solvent, are retained by the membrane, thus reverse osmosis is a concentration process. Nielsen et al. (1972) reported on the installation of the first industrial scale reverse osmosis plants for the dairy industry by the Danish Sugar Corporation Limited. They indicated that the treatment of whey by reverse osmosis could result in more economical use of the nutritive value of whey and reduce the problems caused by pollution.

Ultrafiltration is a separation or fractionation process, in which the membrane allows both solvent and smaller solute molecules, of low molecular weight (lactose and salts from whey for example), to pass through while retaining the larger, higher molecular weight solutes (proteins). Ultrafiltration of whey, for example, results in two fractions - a deproteinized whey (permeate) and a protein concentrate. Delaney et al. (1972) and Hargrove et al. (1974) have described the use of ultrafiltration to obtain protein concentrates from cheese whey. The former (Irish) paper was concerned with the production of concentrates suitable for human nutrition which was desirable for nutritional and economic reasons. The latter (American) paper was more concerned with the utilization of the large volumes of permeate resulting from ultrafiltration. The permeate contains

most of the lactose, non-protein nitrogen, salts, vitamins and free amino acids normally present in whey with only a slight decrease in BOD compared to whey itself. A process had been developed for converting the liquid permeate into solid blocks which were then fed to cattle.

Delaney and Donnelly (1973) experimented with gel filtration for recovering whey proteins. This is a chromatographic process which separates the whey into a high molecular weight protein fraction and a low molecular weight lactose and salts fraction. The separation occurs when the gel makes contact with the whey; the low molecular weight compounds enter the gel pores which retain them while the proteins are eluted. There is usually a very good recovery of protein (approximately ninety-five per cent) and the lack of heat treatment leaves an undenatured concentrate with potential as a valuable food ingredient.

Lewin and Forster (1974) described a process for the removal of a proportion of fat and protein from dairy wastes using acid coagulation and electroflotation. Sodium lignosulphate at pH4 induced the flocculation of the fats and proteins in the effluent which facilitated their separation using electrolysis. Microbubbles, produced by adjusting the current between the electrodes in the electroflotation chamber, transport the flocculated solids to the surface of the liquid for removal by scraping. Further research into suitable drying techniques and acceptability of the products using animal feeding trials was necessary before a full evaluation of the process could be determined.

Jones (1974) has described the use of a pilot-scale, three-stage, ion exchange process for recovering protein from effluents. The pilot plant was sited at an abattoir and the ion exchange medium (Vistec) was regenerated cellulose subjected to the viscose process.

The porous nature of the cellulose absorbs high molecular weight proteins from the effluent under treatment. Competing ions from a salt solution or a change in pH desorbs the protein from the media in the second stage of the process. The protein concentrate remains unchanged in nature from that in the feed and can be spray or freeze dried to retain its undenatured state. The final stage involves washing the media to remove excess salt or carry out pH adjustment prior to the return to the first stage for recirculation. Palmer (1977) has expanded on the previous description by Jones (1974) of the Vistec Protein Recovery Process. High quality undenatured protein was obtained from cheese whey using a redesigned process which can be used alone or supplement other treatment methods. Full details of the protein obtained from cheese whey are given by Palmer.

The direct extraction of protein from protein-rich wastes, by methods outlined above, can still leave an effluent with a high BOD requiring further treatment. Over the years many workers have researched into ways of converting wastes of low value into single-cell protein using various micro-organisms. Cheese whey can cause serious pollution problems if disposed of as a dairy waste. The composition of the whey is variable, depending on the choice of the cheese-making process and the composition of the original milk, Storage of whey results in rapid acidification as the lactose decomposes and organic acids including lactic acid are formed. Wasserman et al. (1961) described a large-scale process using whey as a substrate for yeast production. Saccharomyces fragilis was propagated in 2840 litres (750 U.S. gallons) of cheese whey and growth supplements for maximum yeast yield in four hours at 31-33°C and pH5.5 - 5.7. Oxygen was supplied in sufficient quantities to

ensure maximum combustion and assimilation of the lactose by the yeast. It was feasible to use raw unpasteurized whey as the substrate owing to the rapid growth rate of *S.fragilis* which reduced the cost of yeast production. The amino acid and vitamin composition of *S.fragilis* was given by Wasserman (1960). Later work by Wasserman (1961) reported that neither the strain of yeast nor the growth medium had any influence on the amino acid composition of the protein. In a comparison with other microorganisms and yeast protein *S.fragilis* compared favourably, however, the vitamin concentration varied and enrichment with thiamine would improve the yeast protein.

In 1966 Gray suggested that members of the Fungi Imperfecti were suitable for converting carbohydrate and inorganic nitrogen to protein. He outlined the basic requirements that a fungus had to fulfil such as good growth in submerged culture, ability to grow quickly and efficient conversion of substrate material. Fungi Imperfecti were chosen because of their ubiquity and rapid growth. Experimental results showed that white and sweet potatoes, sugar beets, manioc, corn, rice, cassava flour and cane molasses all provided a satisfactory carbohydrate source for fungal protein biosynthesis. The mycelia were harvested in pellet form, dried and made into flour flakes or tablets. Shukla and Dutta (1967) determined the optimum conditions for growth, protein and methionine synthesis of a strain of *Rhizopus* using cane waste molasses as substrate. Ammonium chloride and calcium diphosphate were added to the basic medium to obtain maximum yield of fungal protein. The authors intended to use the methionine-rich *Rhizopus* protein to supplement yeast protein to provide a more complete food from molasses.

The use of yeast species as animal feed has been well established over many years, (Braude, 1942), their food value has been

determined and they are capable of degrading a wide range of substrates to produce good quality protein. One process which uses two species of yeast in symbiosis to convert waste starch materials or effluents containing starch into protein has been described by Jarl (1969). This process is known as the Symba yeast process in which the starch is hydrolyzed by amylase produced by Endomycopsis fibuliger. Candida utilis assimilates the low molecular weight sugars as soon as they are produced and growth proceeds rapidly until this species dominates. The resulting yeast cream is washed, concentrated and dried. The raw protein content varies according to the raw material and the product has so far been fed to chickens and pigs. The process can be used to treat effluents containing starch particularly when there is a high starch concentration. Application of the yeast process to potato processing waste-waters resulted in a removal of 90-95% applied BOD and a valuable end-product which does not happen with biological filters or activated sludge.

Spent sulphite liquor has been used world-wide as a fermentation substrate for the production of food yeast. (Mueller and Walden 1970, Moo-Young 1976). Pulp mills using the sulphite process to delignify wood solubilise the lignin with acid bisulphite which results in undegraded wood cellulose and monosaccharides from hemicellulose hydrolysis. Spent sulphite liquor contains almost half the raw soluble wood and the carbohydrate fraction provides the substrate for yeast growth.

In 1976 Moo-Young briefly reviewed the facilities for single-cell protein production throughout the world. The most frequently used organisms utilizing a wide variety of substrates in both pilot and large-scale processes were yeasts. However, many processes have been developed which result in fungal protein production and

utilization possessing a number of advantages when compared with yeasts. In many parts of the world fungi form animportant component of the daily diet which is useful when introducing new fungal species cultured from carbohydrate waste (Janardhanan et al.,1970). Fungi possess a better protein profile than single cell organisms, their filamentous structure facilitates recovery from the culture fluid and aids the manufacture of textured food products (Spicer 1971). Yeasts tend to have a fairly high nucleic acid content which can result in increasing the level of uric acid in the blood if too much is ingested by man. A surfeit of uric acid in the blood leads to a condition known as gout. Dietary yeasts also tend to need supplementing with amino acids particularly cystine and methionine. Many workers have investigated the ability of fungi to utilize various wastes and produce an economical end-product. Church and Nash (1970) listed the conditions that a fungus must satisfy if it is to be used successfully in waste control. The aim of any waste treatment process is a reduction of BOD to a low level which must be fulfilled by the fungi used in waste control. The ability to grow quickly in an unsterile medium and remain dominant over organisms even when conditions change is also desirable. Fungal mycelium as a food product must be non-toxic, digestible and have a good protein content. Church and Nash (1970) screened forty-eight species of eighteen genera of Fungi Imperfecti for their ability to convert soluble and suspended organic matter found in corn and soy food-processing waste to fungal protein. _Trichoderma viride_ _Gliocladium deliquescens_ and _Aspergillus oryzae_ gave the best results on the wastes chosen for experimental treatment. Further work by Church et al.,(1973) showed that the natural contaminants of corn and pea wastes _T.viride_, _G.deliquescens_ and a species of _Geotrichum_

were the most effective at treating these wastes. They suggested that the suitability of a fungus for a particular waste must be determined initially on a small-scale system. Church et al. (1973) reported a change in the dominant fungus in their pilot-scale oxidation ditch when a species of Geotrichum out-numbered the previously dominant Trichoderma viride and a species of Fusarium dominated the pea waste. Changes in the composition of the wastes were believed to have affected the fungal flora but they were not considered to be undesirable. The effects of small changes in the manufacturing process together with seasonal variations can have a profound effect on the fungal flora associated with a particular waste. The result may lead to difficulties during harvesting of the mycelium and too much variation from the specified SCP product. Alternatively some processes have been developed which favour the growth of one species of fungus against that of other micro-organisms. Righelato et al. (1976) described a fermentation process developed to treat carbohydrate wastes using either a species of Fusarium or Aspergillus niger. Both fungi efficiently converted numerous waste carbohydrates into single cell protein at a low pH and high temperature, eliminating the need for expensive aseptic conditions. The amino acid profiles of both fungi were similar to soya bean meal. Neither fungus was able to use cellulose as a source of carbon because they lacked the necessary enzymes. Spano et al. (1976) described the use of enzyme complexes derived from mutant strains of Trichoderma viride which effectively convert cellulose wastes to glucose syrups. It is possible to use acid hydrolysis to convert cellulose to glucose but the equipment must be corrosion-resistant and the conditions needed for the hydrolysis tend to decompose the end-products. T.viride was grown in a medium containing shredded cellulose and nutrients, the cellulase enzyme complex was collected as the filtrate

after separation from the fungus and solids. Two groups of enzymes form the main components of the complex, one group is common and the other is quite rare. The best source of these rare enzymes necessary for effective saccharification is T.viride and the optimum conditions for their production have been defined. The glucose produced as a result of enzymatice hydrolysis of cellulose can be converted to food products, chemical feedstocks and fuel.

A further example of using a selected fungus and highly selective growth conditions was described by Gregory et al., (1976). They used a mutant strain of Aspergillus fumigatus in a non-aseptic fermentation process designed to convert carbohydrate wastes (cassava) to fungal protein at pH3.5 and high temperatures of 45°C to 50°C. The process was designed to be inexpensive and easy to maintain and the final product was aimed at animal feed either moist or dried. The fungus was chosen for its amylolytic and thermotolerant properties and its filamentous growth facilitated harvesting.

Fruit and vegetable processing operations producing alkaline effluents cause disposal problems in the food industry. Beuchat et al.,(1978) studied the growth of Neurospora sitophila on alkaline effluents as a means of reducing the COD and producing single-cell protein as an end-product of fermentation.

The utilization of fish oil as a substrate for conversion to single-cell protein has been described by Hottinger et al.,(1974a, 1974b). Candida lipolytica and Geotrichum candidum were selected because of their known lipolytic activity and the optimum conditions for maximum biomass production were determined using shake flasks. The feasibility of using fish oil stemmed from the conversion of hydrocarbons to microbial protein (Vilenchich and Akhtar, 1971). Shacklady (1974) reviewed the development of single-cell protein using bacteria grown on methane and methanol and yeasts grown on

alkanes. The acceptability of the protein as animal feed depends on rigorous toxicity tests but the economics of the processes depend entirely on the ever-increasing price of crude oil. Micro-organisms can also be encouraged to become sources of oils and fats by exhausting the supply of a non-carbonaceous nutrient during the growth period. Suitable oleagenous micro-organisms continue to consume the carbon in the medium and convert it into an oil or fat. (Whitworth and Ratledge, 1974). Moulds and yeasts were considered suitable for bio-oil production because of their long association with man's diet. The wide variety of fats and oils produced during the fermentation processes using different species is encouraging but the economics depend on the choice of substrate.

The use of an unusual substrate for microbial growth has been described by LeDuy (1979) proposing fermentation of the world's peat resources by _Candida utilis_. Peat hydrolysate is rich in carbohydrates and minerals resulting in high yields of yeast when cultured to produce single-cell protein.

The use of mutant strains of various species of micro-organisms has already been mentioned in connection with obtaining maximum yield of SCP from particular substrates. Genetic manipulation used to enhance a desirable property of a certain organism can be successfully applied to a waste control process if stability can be maintained (Calam, 1972). Moo-Young et al. (1979) described the use of a new cellulolytic fungus, _Chaetomium cellulolyticum_, in a new fermentation process (the Waterloo Process) for converting agricultural and forestry wastes into SCP. The proteinaceous products of mass fungal cultivation have successfully been used in animal feeding trials.

Traditional methods of treating sewage and other organic waste waters using either bacteria beds (biological filters) or the activated sludge process result in the production of large quantities of sludge.

Direct utilization of the sludge by application to agricultural land is used extensively in this country. The sludge may be dried, partially dry or in liquid form after anaerobic digestion at the works. The cost of treating sludge, which often requires the addition of chemical conditioners to improve filtration, was given by Gale (1968) as thirty to forty per cent of the total cost of sewage treatment. There are many problems associated with the treatment of sludge, digesters are expensive, transport for disposal to land or sea is becoming increasingly costly and drying beds occupy large areas of land with the disadvantage of unpleasant odours. The need to develop methods of either decreasing the amount of sludge or cheaper methods of utilization are very important.

Bacteria beds were an accepted means of sewage treatment in 1908 and the activated sludge process was invented soon afterwards in 1914. Both treatment methods rely on micro-organisms to oxidize the organic matter in the waste and subsequently purify it while synthesising material for their own growth. Saprophytic bacteria usually predominate bacteria bed film and activated sludge flocs when sewage is treated, but under certain conditions such as lower temperatures or a change in the carbon: nitrogen ratio of the waste fungi tend to become dominant. Fungi are equal in efficiency at removing organic matter from wastes but their synthesising capacity is much greater than the bacteria and thick fungal films cause many problems (Hawkes, 1965). The problems caused by fungi in bacteria beds arise because the thick film blocks the downward seepage of the waste and reduces aeration. Anaerobic areas develop within the bed and the final effluent is reduced in quality. Fly problems also arise from fungal film accumulation because of the additional supply of nutrients. Fungi predominating in activated sludge tend to affect the settling properties of the sludge which results in poor quality final effluent.

Fungi have been regarded as a nuisance in bacteria beds since the development of this method of treating sewage. In 1906 Rettger reported the appearance of "a slimy vegetable growth" which he discovered to be fungal hyphae at an experimental sewage filtration plant in Connecticut. The fungal growth reduced the filtration rate and was removed by resting the beds for at least one day, raking the surface and flushing out the "parasite" with a strong water jet. In 1921 Cox reviewed the literature regarding sewage trickling filters and concluded that fungoid growths should be regarded as nuisances and were more harmful than helpful. These early reports stimulated investigations concerning the seasonal distribution of fungi (Haenseler et al. 1923; Lackey, 1925) and control of film accumulation. In England Bell (1926) showed that fungal film could be controlled by the grazing activity of an insect Hypogastrura viatica (Achorutes subviaticus). In the United States Cooke carried out extensive surveys on fungi isolated from polluted water and sewage (Cooke, 1954; Cooke and Hirsch, 1958; Cooke, 1958). A list of fungal species was compiled as a result of some of the surveys (Cooke, 1957a). In 1959 Cooke stated that at some future date fungi may be used as producers of chemical and food by-products. Sewage treatment plants offered fungi a wide range of nutrients and by controlling their growth they could have an additional role to that of stablizing waste organic compounds.

Sewage treatment works had to be able to cope with an increasing population and the solution was not simply an increase in the number of works. Bacteria beds were used initially as single units but it became clear that increasing the rate of application of sewage to each bed resulted in heavy film growth and all the attendant problems. A number of methods were tried to solve the problem of increased loading without causing operational difficulties. In 1941 Tomlinson

described a new treatment process called "alternating double filtration" (described previously) which, briefly, consisted of two filter beds in series with a change in the order at regular intervals. The results were encouraging and the new process was shown to cope with a much larger volume of sewage than the single filtration process without excessive film accumulation. Mills (1945a) expanded on Tomlinson's work and found that alternating double filtration was capable of treating sewage at four times the rate of single filtration and producing good quality effluent. Fibrous growths were present in both systems and limited the rate of treatment in the single filtration plant. The omission of settlement of the primary effluent in the alternating double filtration process (ADF) was considered by Mills (1945a) to increase fungal accumulation in the winter. Tomlinson and Hall (1953) qualified Mills' claim with two experiments but decided that the capital cost would be reduced dramatically if the intermediate settlement tank was omitted. Constructing new bacteria beds was expensive and it was necessary to ensure that all parts of the system were needed for successful operation. Meanwhile other experiments were being performed to determine whether methods other than ADF could treat sewage at an increased rate. Many works would have problems with layout and design if the ADF process was used and this led to experiments with recirculation of the effluent. Mills (1945b) compared single filtration with recirculation of part of the filter effluent with alternating double filtration. He concluded that the process of recirculation was comparable in efficiency with ADF provided that care was taken in the winter to remove obstructive subsurface growth. The growth causing these problems in the winter was mainly due to the fungus Sepedonium sp. identified by Tomlinson. In 1958 Lumb and Eastwood carried out more detailed experiments with

recirculation by comparing different methods of returning the purified effluent to the beds. They also varied the ratio of the recirculation rate with the inflowing rate of the sewage. The results showed that the beds coped successfully with more than double the sewage loading when the recirculation process used a mixture of diluted settled sewage and purified aeration plant effluent. The maintenance of a constant ratio of recirculated effluent to sewage treated at all flow rates was found to be the best. The modified recirculation process was successful in virtually eliminating ponding due to the dilution of the feed resulting in film distribution extending more than two-thirds down the depth of the bed. These results confirmed the findings of Tomlinson (1941) who stated that the average amount of film per unit volume of medium was less than that found in the single filter bed. Studying ways of improving sewage treatment was not limited to engineering, biological investigations were also carried out as part of the experiments (Tomlinson, 1941; Reynoldson, 1942; Hesseltine, 1953; Hawkes, 1957). Tomlinson (1941) made detailed observations on the flora and fauna in a single filter and those operated on ADF at Minworth in Birmingham. Reynoldson (1942) gave detailed descriptions of filter conditions related to the flora and fauna in a double filtration plant at Huddersfield. Reynoldson studied the growth curve of fungi and defined four stages related to his studies of the macro-fauna and temperature. The relationship between the rate of accumulation of growths and solids and the rate of destruction by the macro-fauna was basic to the problem of ponding. An increase in temperature, such as in the summer, could encourage fungal growth to such an extent that serious ponding could result without the presence of the scouring macro-fauna.

A different approach to controlling film growth at the surface

of filter beds was described by Lumb and Barnes (1948) and Hawkes (1955) as changing the periodicity of dosing. Lumb and Barnes recorded observations that slowly-rotating distributors on filter beds resulted in a cleaner condition and lessened the tendency for blockage by film growth. They determined the optimum dosing interval to be between four and nine minutes, good quality effluents were obtained from heavily-dosed beds and the success of the method was attributed to dispersal of the surface growths by the periodic downward flushes of the sewage. Hawkes (1955) explained the results of changing the periodicity of dosing on alternating double filters. The film growth was checked as a result of starvation and increased flushing of the sewage applied in sudden and regular intervals. The decreased amount of film resulted in the virtual elimination of flies which was another advantage of this method. The flushing action of the sewage was thought to have more of an adverse effect on the fly larvae than the film, but film control was effective as the comparison with the control filters showed. In 1964 Hawkes and Jenkins compared the processes of double filtration and alternating double filtration both using controlled frequency of dosing of an industrial sewage. They found that the controlled frequency of dosing was insufficient to prevent solids accumulating within the primary bed of the double filters at the highest treatment rate but the upper layers supported less film than beds dosed continuously. The ADF beds did not suffer from an excess of fungal film at any time even though the nature of the sewage favoured fungal growth. The ADF process has been shown to be effective in controlling film accumulation and this was enhanced by low frequency dosing which resulted in partial starvation of the fungi and gave a more uniform film distribution throughout the beds. In the ADF process the organic load is shared by both beds but it is

the primary bed in the double filtration process which removes the greater organic load. It is important, therefore, to control film accumulation on the primary bed in order to obtain efficient organic removal and low frequency dosing provided the solution.

Investigations concerning the basis of all bacteria beds, the filter media, were performed in both the United States and Britain (Schroepfer, 1951; Hawkes and Jenkins, 1955, 1958), to determine the effect of media size on film accumulation. Schroepfer experimented with size and shape ratio related to surface area and the effect of shape ratio on the voids. Hawkes and Jenkins compared four grades of media composed of crushed gravel, round gravel and cracked granite. They concluded that the smallest medium produced the best effluent but the larger medium had a higher percentage of voids and thus better aeration. The choice of medium ultimately depends on the nature of the sewage, the rate of application and the operation of the filters as single or double units, the periodicity of dosing is also a factor to be considered. Sewage likely to encourage fungal growth is best treated using large media which are able to cope with excessive winter film growths because of the greater voids. It was not feasible to recommend one type of medium for all filters and the choice would probably be limited to local material capable of producing a good quality effluent without encouraging excessive film accumulation in the winter. The problems caused by the accumulation of mainly fungal film, the methods of operating filters and the related ecology have all been discussed in an extensive review by Hawkes (1961).

The development of plastic media in modular form with a high percentage of uniform void spaces provided an alternative to the existing mineral media. Filters using plastic media did not require expensive construction methods because of the lightness of the new

material and high towers with a large surface area could be built in works with restricted room for expansion. The large voids were designed to enable wastes to be treated at a higher rate than before, since the media could cope with thick films without becoming blocked. At first there was little interest shown in the new media but intensive marketing by one manufacturer resulted in a number of filter towers being constructed. The Water Pollution Research Laboratory performed a number of investigations comparing plastic and mineral media in high rate filters treating domestic sewage (Bruce and Merkens, 1970, 1973; Bruce and Boon, 1971; Joslin et al., 1971). Four types of modular plastic media from three manufacturers were compared with slag and basalt of large grade. In most cases there was a definite seasonal variation in the efficiency of BOD removal closely related to changes in sewage temperature. None of the filters became blocked by film in the winter months. The performance of a high-rate filter depended on the specific surface area of the medium providing all the surfaces were adequately wetted and the plastic media were found to be comparable in efficiency with the mineral media of the same specific surface area. It was found that high-rate filtration was best used for partial treatment ("roughing") of waste waters with a high BOD. Increasing the feed strength and decreasing the hydraulic loading improved the efficiency of BOD removal at a given loading, but the effluent required further treatment. Once a decision has been taken to use high-rate filtration to treat a particular waste the choice of medium depends on the capital cost, land availability and pumping costs (Joslin et al., 1971). Chipperfield (1967) stated that a loading of less than 590 g/m^3d was generally unsuitable for plastic media on technical and economic grounds. A description of the plastic media "Flocor", marketed by I.C.I.Limited, with a list of requirements for the ideal roughing packing, performance

and treatment of various industrial effluents has been given by Chipperfield (1967). Many dairies have installed high-rate plastic media filters to treat variable, strong wastes and the advantages of using one particular medium have been described by Hemming (1971). Earlier work at the Water Pollution Research Laboratory by Eden et al. (1966) compared the performances of plastic and granite media fiters treating simulated milk waste which was known to cause excessive film accumulation in conventional media. The granite filter had an unpleasant smell resulting from the anaerobic conditions caused by heavy film accumulation and part of the surface had ponded. The plastic filter medium remained well aerated due to a lesser accumulation of film, there was no ponding and no smell. One problem with dairy wastes arises from the sludge which rapidly becomes anaerobic and has a strong smell. Ford (1977) recommended the use of a centrifuge which still leaves a disposal problem although the sludge is reduced in volume.

The literature describes many ways of utilizing organic waste waters from direct extraction of protein from protein-rich wastes, microbial conversion of carbohydrate-rich waste into more valuable single-cell protein to direct utilization of waste as fertilisers and animal feed supplements. The nature of the waste, its strength and composition determine the type of utilization from new technology to the old traditional methods. The present work is concerned with the controlled growth and potential utilization of fungal biomass which is known to accumulate in filters treating dairy waste of high organic strength. High-rate filters using plastic media have been installed at a number of dairies throughout this country and it may be possible to provide them with an alternative to disposing of their sludge on to agricultural land.

C H A P T E R   T W O

SURVEY OF DAIRY EFFLUENT TREATMENT PLANTS

CHAPTER 2

SURVEY OF DAIRY EFFLUENT TREATMENT PLANTS

2. 1 CHARACTERISTIC PROBLEMS ASSOCIATED WITH DAIRY EFFLUENTS

Dairy effluents differ in composition depending on current production of items such as cheese, butter, milk and yogurt. Yogurt, in particular, has seasonal production peaks related to consumer demand. However, one major factor which nearly all dairy effluents share, is their strength in terms of the organic content; they are often much stronger than domestic sewage. (Table 2.1)

TABLE 2.1 (M.A.F.F. 1969)

Comparison of the BOD (Biochemical Oxygen Demand) of milk, milk by-products and typical dairy effluents, with that of domestic sewage

	Typical BOD (mg/l)*
Milk	110,000
Skimmed milk	72,000
Separated buttermilk	72,000
Separated Whey	44,000
Waste waters from:	
Milk reception depot	300
Milk bottling plant	500
Butter making	500
Cheese making	2,000
Drying and condensing milk	1,500
Milk products factory	1,500
Domestic sewage	300

*(milligrams per litre)

Dairy effluents do not usually contain substances with directly toxic properties, but the organic matter present can cause problems when discharged into a water-course. The organic matter in these effluents undergoes rapid decomposition since it is soluble and easily oxidized. Unless the volume of the receiving stream or river is large enough to cope with such oxygen depletion, aquatic life suffers. This has been a serious problem in the past when dairy effluents were discharged directly into water-courses. Production at the plants tended to be higher in the warmer months of the year, coinciding with reduced water levels in streams and rivers. The increased polluting load of the effluents caused serious de-oxygenation and odour problems when discharged.

Dairy effluents, being strong in nutrients, can also encourage the development of filamentous bacteria such as Sphaerotilus natans, which is also known as "sewage fungus." Growth of such organisms leads to turbid waters, which decreases the light available to aquatic plants for photosynthesis and creates the deposition of solids. Water-courses, which are affected in this way, become unsuitable for abstraction for either public use or industry. Other problems attributable to dairy effluents are high pH and high chloride content. Large amounts of caustic soda are used to cleanse milk churns and other equipment and the effluent from these operations tends to be highly alkaline even when mixed with other waste waters in the plant. Cheese effluents can have a high chloride content since sodium chloride is added to nearly all types of

cheese. The salt enhances the flavour of the cheese, withdraws whey from the curd and thereby aids control over acidity and moisture.

At any time of the year, dairy effluents are likely to be quite warm and this can lead to anaerobic conditions and odours of hydrogen sulphide in the sewers. Formation of the latter can result in sulphuric acid formation, which is known for its destructive powers in pipes and fittings.

Dairy effluents contain fat and it is desirable to remove this before discharge because it poses a potential threat of blockage in the pipework.

All these problems have become more concentrated since the move to larger establishments for manufacturing dairy produce.

2. 2 SITING AND GENERAL DESCRIPTION OF THE TREATMENT PLANTS

In the past, the farmers distributed most of the milk directly to the consumers, with a fraction handled by other distributing depots. Butter and cheese were manufactured mainly on the farms where the milk was produced.

Nowadays, there are basically three types of dairies: reception depots, manufacturing plants and milk distribution depots. The depots are usually sited in urban areas, whereas the manufacturing plants tend to be found in rural areas where the milk is produced.

The siting of a dairy is important because of the cost of treating the effluent. Legislation has been in operation for over a century to protect water supplies against pollution. Dairy effluents have to receive treatment to a certain degree

even if they are discharged into the sea which offers a considerable amount of dilution.

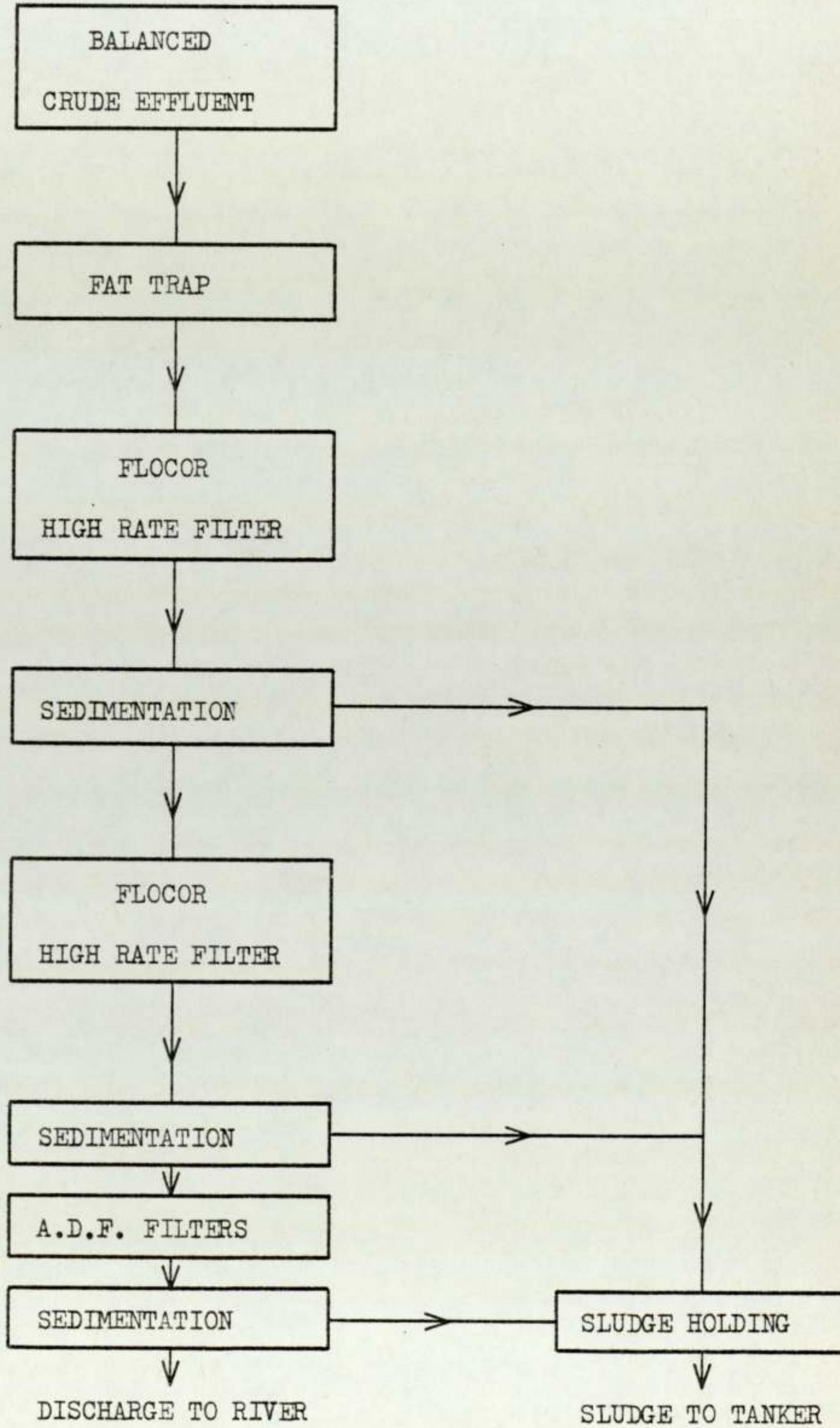
Dairies sited in urban areas usually find it cheaper to pre-treat their effluents before discharge to the local authority sewer rather than provide complete effluent treatment. Pre-treatment can involve screening, fat separation, pH adjustment, flow balancing and settlement, usually the first two procedures are the only ones necessary. Milk-bottling depots tend to have an alkaline effluent from the cleansing processes and may need to adjust the pH of the effluent before discharge.

Dairies in rural areas may be sited at a considerable distance from the local sewage works, which, even if nearby, are often quite small with insufficient capacity to cope with large volumes of strong effluent. Complete effluent treatment systems have been installed at many dairies to overcome these problems.

The charges for effluent treatment, levied by the local water authority, usually depend on the volume and strength of the effluent (as determined by the BOD test). Charges are also imposed if the receiving sewage works needs to be expanded in any way to cater for the extra discharge.

In some cases, dairies have to conform with other standards, apart from the strength of their effluent, these can include pH value, suspended solids and temperature. However, those dairies with complete effluent treatment systems generally have to satisfy or improve on the required standard for sewage effluent as laid down by the Royal Commission in 1868. This

FIG. 2.1 Flow diagram of a typical dairy effluent treatment plant with provision for recycling high-rate filter effluent.



standard required effluents, discharged to non-tidal streams, to be treated so that the suspended solids content and BOD should not exceed 30mg/l and 20 mg/l respectively, where there is at least an eight-fold dilution factor of clean water.

Fig. 2.1 shows a generalised plan of a complete effluent treatment system. The crude effluent is retained in a balancing tank for not more than twenty-four hours as it can become anoxic if left for too long. The effluent can then be released at an even rate throughout the day, enabling full utilization of the treatment plant under steady conditions. Settlement and skimming to remove fat may also be combined with the balancing tank feature to even the composition of the effluent.

The pH value of the effluent may require adjustment prior to being applied to the filters. The plan shows two high-rate biofilters, containing the modular Flocor media, arranged in series with settlement after both towers. High-rate biofilters have been designed to remove large quantities of BOD and have been successful in treating dairy effluents with their high content of soluble organic matter. In order to maintain the efficiency of these biofilters by utilising all the active surfaces of the media, it is necessary to have a minimum irrigation rate to the top of the towers, and this is the main reason for the use of a balancing tank. The settlement stage after each biofilter also enables some of the effluent to be recycled through the system if necessary.

Pre-treatment through high-rate biofilters can be employed by urban dairies, if necessary, since the towers require

considerably less land than conventional filters and can appreciably reduce the organic strength of an effluent prior to treatment at the local sewage works.

In the complete treatment system, the effluent passes through the high-rate filters and then through a conventional stone filter, or filters, or sometimes the activated sludge process is used. The most common arrangement for filters treating dairy waste is alternating double filtration (ADF). In this process the high-rate filter effluent is diluted, after settlement, with recirculated final effluent thus reducing the organic strength. The waste waters are then passed through two filters in series, with settlement after each filter; at regular intervals, the order of the filters, but not the settlement tanks, is reversed. The Water Pollution Research Laboratory developed the ADF process after receiving reports of filters becoming ponded during the treatment of milk washings. (Water Pollution Research Technical Paper No. 8.1941). Ponding occurs at the surface of filter beds because of excessive film growth which prevents the downward flow of the waste waters. The ADF system changes the conditions of each filter at regular intervals, film accumulates on the surface of the primary filter, but is soon removed by the weaker feed when it becomes the secondary filter. In this way the surfaces of both filters continually accumulate and lose film and do not persistently suffer from ponding. The final effluent is then settled to remove the suspended solids and the supernatant is discharged into a nearby river or stream. The sludge from the high-rate filters

and the stone-bed ADF filters is collected in a holding tank. This sludge can then be removed in tankers and used on agricultural land in its wet state, processed and dried for use as a fertiliser or dumped in a land-fill site or at sea.

2.3 DETAILS OF DAIRIES VISITED

All the dairies visited were sited in rural areas in the South West of England, Cheshire, Yorkshire and the Midlands. Five of the effluent treatment plants were constructed as shown in the generalised plan, the other six had similar arrangements except that a single high-rate biofilter was used instead of two units.

Table 2.2 gives the location of the dairies and the volume of Flocor media employed to treat the effluent. There is quite a variation in the volume of the high-rate filters from the smallest at $29m^3$ at Cannington to the largest, $710m^3$ at Minsterley.

Table 2.3 gives details of the different types of dairy effluent together with the volume, strength and pH of each waste when applied to the high-rate filters. The figures for the volume and BOD concentration will obviously differ slightly throughout the year but the BOD load, or pollution load as it is sometimes referred to, determines the size of the actual Flocor biofilters. The volume of Flocor media employed is related to the BOD load and the calculated efficiency of BOD removal shows the percentage remaining for further treatment by percolating filters. The design loadings of the filters in

TABLE 2.2

Location of Flocor towers sampled and their capacity

Location of Dairy	Volume of Flocor (m^3)	
	Primary Stage	Secondary Stage
Honiton, Devon	458	
Chard, Somerset	692	
Cannington, Somerset	29	
Frome, Somerset	573	229
Ilminster, Somerset	280	
Cuddington, Cheshire	295	295
Tarvin, Cheshire	206	103
Coverham, Yorkshire	33	
West Marton, Yorkshire	36	36
Uttoxeter, Staffordshire	380	380
Minsterley, Shropshire	710	

TABLE 2.3

Operational details of the effluent treatment plants visited and sampled

Location of Dairy	Type of effluent	Volume m^3/day	BOD conc. mg/l	BOD load kg/day	pH of tower feed	Special features
Honiton	Butter and cream	910	1500	1365		FLOW BALANCING FAT TRAP
Chard	Butter, cream milk	1589	860	1362	8.4	FLOW BALANCING FAT TRAP NUTRIENT ADDITION pH CONTROL
Cannington	Cheese	59	960		7.6	FLOW BALANCING
Frome	Processed cheese and cream	1136	1500	1698	6.9	FLOW BALANCING FAT TRAP
Ilminster	"Horlicks" and domstic sewage	1365	800	872		GRIT REMOVAL COARSE SCREENING FINE SCREENING
Cuddington	Yogurt	550	1250	690		FLOW BALANCING FAT TRAP pH CONTROL
Tarvin	Milk bottling	820	750	615		FLOW BALANCING FAT TRAP
Coverham	Milk and Cheese	100	750	75	5.5	FLOW BALANCING FAT TRAP
West Marton	Milk and Cheese	64	1500	96	7.0	FLOW BALANCING FAT TRAP
Uttoxeter	Milk and creamery	1136	1000	1133		FLOW BALANCING FAT TRAP pH CONTROL
Minsterley	Cheese, Yogurt	1360	1500	2040	6.2	FLOW BALANCING FAT TRAP

Table 2.3 were all expected to have a BOD removal efficiency of between sixty and seventy per cent. The special features column in Table 2.3 refers to the pre-treatment stages used at each plant.

The purpose of visiting as many dairies as possible, using Flocor biofilters to treat their effluent, was to examine each effluent for fungi and determine any organisms common to each plant. Eleven dairies were visited altogether, three effluent treatment plants were sited at the local sewage works in order to use their percolating filters to polish the effluent but the rest had their own complete systems as shown in Fig. 2.1. Dairies in the South West, Cheshire and Yorkshire were all visited and sampled once, in the colder months of the year, (November, March and October respectively). Dairy effluents have a high carbon to nitrogen ratio which favours fungi, and the colder temperatures in winter also encourage their growth, hence the time of sampling. Obviously, there are problems involved in drawing conclusions from the results of sampling any effluent once, so it was necessary to examine as many effluents as possible for common fungi. The problems involved are related to the different products manufactured by the dairies during the year and also the seasonal aspect of the market. Yogurt production tends to have two peaks in the year, related to consumer demand, the larger of the two is in the spring. Factors such as changing products inevitably affect the composition of the effluent which could change the ratio of fungal flora in the film. Numerous film and sludge samples were taken at each

dairy but only two dairies were visited more than once. Sampling on this basis means that the organisms isolated as a result of one visit, cannot be taken as being representative of the ecology of each biofilter throughout the year. However, after sampling a number of dairy effluents once, it was hoped to monitor any effects of seasonal changes by regular sampling of one dairy, preferably at a nearby location.

2.4 MATERIALS AND METHODS

In order to obtain as much information as possible, concerning the ecology of high-rate filters treating dairy waste, it was necessary to collect numerous samples of bios and sludge at each dairy. The top surface, of all the filters visited, was accessible for sampling and, by carefully studying the film colour and thickness, many samples were taken from all over the surface in order to obtain a good representation of the growth. It was not possible to take samples at intermediate depths between top and bottom due to the cladding which protected the sides of the towers. At some of the plants samples could not be taken from the bottom of the high-rate filter towers because of the mode of construction. In many instances, samples from the bottom of the towers could only be obtained from close to the edges and never from any of the central areas. All the samples of bios, obtained in this study, were collected in universal-bottles, labelled and stored at approximately 4°C, where necessary, and examined in the laboratory as soon as possible.

The contents of each universal-bottle were placed in a petri-dish and washed with distilled water. The colours

of the component film fractions were compared with notes made during the sampling visits, with a view to relating certain colours with particular fungi. The samples were teased apart and fifty sub-samples examined microscopically to assess the components and their significance. Notes were made on the flora and fauna found in all the samples with particular emphasis on the fungi. Most fungi remain in a vegetative condition when nutrients are plentiful (Hawker 1957) and their hyphal dimensions and presence or lack of septa are the principal distinguishing features. In order to obtain pure cultures of the dominant fungi observed in each film sample, it was decided to adopt the ring-plate method of isolation. This method was described by Raper (1937), who used it to free water molds from bacterial contamination and subsequently obtained pure fungal cultures. Sections of glass tubing, sixteen millimetres internal diameter and six millimetres deep were cut to form the rings. Fused to one end of each ring, at equal spacings, were three small rounded pieces of glass to act as a stand. Each ring was sterilised before use and placed in the centre of a sterile petri-dish.

Molten agar was carefully poured into each petri-dish (ring-plate) to a level approximately half-way up the ring. The upper surface of the agar, within the ring, was thus separated from the main agar mass, but retained continuity lower down by virtue of the spaces inbetween the fused glass feet. The dominant hyphae observed in each film sample were carefully teased and washed thoroughly in distilled water. The washing process was necessary in order to remove as many bacteria and other organisms, such as nematodes, as possible. Having thus

cleansed the sample, the hyphae were transferred to ring plates of different agars, a small sample being placed in the centre of each ring. The plates were labelled, incubated at 20°C and examined daily. The fungal hyphae inside the ring gradually spread downwards through the agar and outside the ring by growing in the spaces between the feet of the ring. Bacteria and other contaminants remained within the enclosures of each ring being unable to spread in the same mode as the fungi. When the hyphae had grown clear of the ring and spread into the surrounding agar small pieces of the growth were removed and transferred to fresh agar plates for identification.

It was decided to use the ring-plate method first in the isolation procedure, because it was the most direct and by choosing a range of agar media, with different nutrient status, and by daily examinations, it was easy to ascertain whether the intended fungi had in fact been isolated and were growing successfully.

All the agar media employed in isolating and obtaining pure fungal cultures were obtained from Oxoid Limited. (Oxoid Manual, 1969). The following media were used to encourage the growth of the isolated fungi: water agar, Czapek-Dox agar, potato dextrose agar, nutrient agar, corn meal agar and S.F. agar. (The latter medium, S.F. agar containing glucose, peptone and yeast extract, was formulated by Hawkes, 1965 and is reproduced in the Appendix). The range of media was necessary in the initial ring-plates to determine which was favoured by the individual fungi. The appropriate agars were then used for subculturing and maintaining stock cultures of the isolated

organisms.

The ring-plate method of isolating fungi was successful with the first film samples from the South-Western dairies (Devon and Somerset) and so it was decided to continue with this method. It is possible to use antibiotics, incorporated into agar media, to suppress bacterial growth, but some fungi have been recorded as being adversely affected by them (Cooke 1963) and therefore none were used in this study. The fungi which were isolated from the eleven dairies were producing appreciable amounts of biomass in all the film samples. In agreement with Sladka and Ottova (1968) only those fungi which make extensive vegetative growth in filter beds even though sporulation is sparse, can be considered as playing a significant role in the ecosystem. Hence the isolation technique employed was not required to recover all the species of fungi, present in each film sample, but only those which flourished in the purification process.

In the next section (2.5) the film samples from the effluent treatment plants are described and their components identified as far as possible. The fungi could not be identified in their vegetative state, hence the need to obtain pure cultures, and species lists of the principal fungi (together with their arbitrary culture number), found in this study are given in Tables 2.4 to 2.12. When full identification was not possible the fungi concerned were carefully drawn and documented and maintained in stock culture. Features of the fungal hyphae such as size, septation, branching mode and colony morphology

were used in conjunction with any sexual stages produced to identify each organism.

Numerous books, monographs and papers were used to identify the fungi in this study. In the following lists of literature only the names of the authors and the year are given, further details are to be found in the reference list.

The general mycology books referred to were:

Ainsworth and Bisby	1966
Alexopoulos	1962
Barnett	1960
Bessey	1968
Cooke	1963
Gilman	1957
Smith	1969
Tomlinson and Williams	1975

The monographs and important papers concerned with particular groups and genera of fungi were:

Ascoidea:	Walker 1931
Fusarium:	Booth 1971
	Joffe 1974
	Toussoun and Nelson 1968
	Wollenweber et al. 1925
Geotrichum:	Carmichael 1957
Penicillium:	Raper & Thom 1949
Subbaromyces:	Hesseltine 1953
Yeasts:	Lodder 1970
Saprolegniaceae:	Coker 1923
	Fitzpatrick 1930
	Harvey 1930
	Sparrow 1960

2.5 DESCRIPTION AND MICROSCOPIC EXAMINATION OF THE BIOS.

This section is divided into two parts; part one concerns all the dairies which were sampled once, and part two concerns the two treatment plants which were sampled on more than one occasion at Uttoxeter and Minsterley.

Uttoxeter promised to be a very useful location with a new two-stage high-rate Flocor filtration plant designed to cope with a production expansion at the local dairy. The treatment plant was commissioned in March 1976 and bios samples were taken regularly until February, 1977. It was hoped to follow the maturation of the filters, but this was not possible, and the explanation is contained in the description of the plant at Uttoxeter.

PART 1 - DAIRIES SAMPLED ON ONE OCCASION

Honiton, Devon (Express Dairies Ltd.)

Thick, cream-coloured mats of growth were removed from the surface of the filter. These mats were composed of numerous broad aseptate fungal hyphae of varying diameters. Nematodes and zoogloeal bacteria were closely associated with the hyphae. Interspersed with the cream-coloured mats were patches of orange-grey film. These darker areas were composed of vacuolated septate hyphae with a diameter of 8 µm.

Samples of film taken from the base of the filter were reminiscent of sludge. There were no thick mats of growth and the only fungal hyphae present were the septate type closely resembling those at the top of the filter. Zoogloeal bacteria and enchytraeid worms dominated the sludge.

A sample of sludge was withdrawn from the post-Flocor settlement tank; this proved to be very fragmented, being composed of bacteria, dead nematodes and a few septate fungal hyphae.

Chard, Somerset (Unigate Foods)

Samples of the bios from the top of the filters were matted and creamy-white in colour. Numerous zoogloeal bacteria and nematode worms covered many of the broad aseptate fungal hyphae which constituted the matted growth.

Film taken from the base of the filter was creamy-grey in colour with a sludge-like consistency. Fine septate hyphae interlaced with broader septate hyphae of 8 µm diameter were removed from the creamy-grey humus. Zoogloeal bacteria dominated the bios together with abundant nematodes.

Samples were also taken from the outlet channel at the base of the tower. This channel conveyed treated effluent, not destined for recycling, to the post-Flocor settlement tank which was also sampled.

Both sludge samples from the channel and the tank were very similar in colour and composition. They were light-brown with bacteria forming the dominant component covering a minority of fine septate fungal hyphae.

Cannington, Somerset (Milk Marketing Board)

Extremely thick mats of film were removed from the top of the filter. There was evidence of ponding in some of the interstices of the media which may have been caused by dislodged film blocking the lower levels of the filter. The mats were creamy-white in colour with grey and yellow patches which were difficult to separate. Broad aseptate hyphae were removed from the creamy-white and yellow areas, very fine septate hyphae were also found in the yellow parts. Septate hyphae with a diameter of 8 μm were observed in the grey areas. Associated with the dense mats of film were abundant zoogloeal bacteria and a minor component of a filamentous bacteria which was identified as a species of Beggiatoa. Nematodes were present but not abundant and a few holotrichous protozoa were observed.

Samples from the base of the filter were grey in colour and of a similar density to the surface film. Fine septate hyphae seen in the surface samples together with the broader septate hyphae of 8 μm diameter were removed from the grey film. Psychoda larvae and pupae, enchytraeid worms and nematodes were very numerous in the bottom samples. Zoogloeal bacteria covered many of the hyphae and worms.

Sludge withdrawn from the post-Flocor settlement tank was dark grey, and anaerobic. Bacteria and sparse fragments of fungal hyphae were observed.

Frome, Somerset (Express Dairies Ltd.)

Thick mats of cream and yellow film were removed from the surface of the primary filter. Tightly interwoven septate hyphae of coarse and fine dimensions were the dominant components of these mats. The coarse hyphae were particularly abundant and were associated with masses of zoogloeal bacteria and a minority of filamentous bacteria. Psychoda larvae and pupae were also abundant.

The surface of the secondary filter also possessed thick cream-coloured mats but these were frequently interspersed with green patches. The mats were composed of similar hyphae to those found on the primary filter with the same ratio of abundance. The green patches were due to the presence of algae identified as members of the two genera Chlorella and Stigeoclonium. Large numbers of Psychoda larvae and pupae were recorded.

Samples from the bottom of the primary filter were greyish-brown and closely resembled sludge. Masses of zoogloeal bacteria and Psychoda larvae were present. Two types of fungal hyphae were noted, both septate, one was fine and in the minority, the other was broader with a diameter of 8 μm in the film.

The film from the base of the secondary filter was orange-brown in colour and also resembled sludge. The main components of the film were zoogloeal bacteria covering a minority of septate hyphae of the same dimensions as the broader type from the base of the primary filter.

Ilminster, Somerset (Wessex Water Authority)

This was the only tower in the whole sampling programme which treated domestic sewage admixed with effluent from the manufacture of milk beverages.

There was a negligible amount of film on the surface of the filter and numerous scrapings were needed to obtain a reasonable sample. Unicellular algae were distributed fairly evenly over the top of the filter; their presence probably achieved through lack of competition from fungi. The only hyphae from this tower were removed from the greyish- green film scraped off the top surface. The hyphae were very similar in appearance and size to the two types recorded from the base of the primary filter at Frome.

The base of the tower yielded a dense grey sludge which was easily separated and composed of bacteria and a few enchytraeid worms. No fungal hyphae were observed in the sludge.

Cuddington, Cheshire (Express Foods)

At the time of sampling the two Flocor towers were temporarily operating "in parallel" instead of "in series" and this was reflected in the similarity of the bios. The filters were designated 'A' and 'B' from their previous mode of operation as primary and secondary units.

Surface samples from filter A were light creamy-brown with green patches due to algal growth. The film was moderately thick and spread uniformly over the surface of the media. The creamy-brown film was primarily composed of fungal hyphae both septate and aseptate. The septate hyphae were of two widths, one being approximately half the width of the other. The aseptate hyphae were of similar dimensions to the broader septate strands and these two components were in the minority. Zoogloeal bacteria were abundant, holotrichous protozoa were common and the algal growth was confined to the two genera Chlorella and Ulothrix.

Bios from the top of filter B was similar in composition. The film was not quite as homogeneous in colour, there was a larger component of algae and the only fungal hyphae were fine septate filaments. The algae belonged to three genera: Chlorella, Ulothrix and Stigeoclonium, with the latter forming the dominant component. Protozoa were both holotrichous and peritrichous and several filamentous bacteria of the genus Beggiatoa were observed. Enchytraeid worms and zoogloeal bacteria were abundant.

The base of filter A yielded large clumps of film with a brown-cream-grey colouration. Enchytraeid worms, both red and white, were very numerous, together with Psychoda larvae,

zoogloeal bacteria and holotrichous protozoa. The fungal hyphae which composed the clumps of film were all of similar thickness both septate and aseptate tightly inter-woven.

Film from the bottom of filter B was rather more grey in colour than that from A. Enchytraeid worms, _Psychoda_ larvae, holotrichous and peritrichous protozoa, zoogloeal and filamentous bacteria were all very abundant. Fungal hyphae removed from the greyish clumps were mainly medium course and aseptate with a minor component of fine septate filaments similar to those from the top of filter A.

Sludge, from the post-Flocor settlement tank, from filter A resembled the film samples in appearance and odour, it was not anaerobic. The creamy-brown lumps of film were composed chiefly of septate and aseptate hyphae with diameters of 8 μm. The hyphae were encrusted with bacteria and the remainder of the sludge consisted of enchytraeid worms, _Psychoda_ larvae a few peritrichous protozoa and filamentous bacteria.

Settled sludge from filter B was more grey in colour reflecting the difference in film colour between it and filter A. There were fewer clumps of material but they revealed similar septate hyphae to those found in the sludge from A. Bacteria and protozoa composed the rest of the sludge.

Tarvin, Cheshire (Express Dairies Ltd.)

The primary and secondary filters were combined to form one tower, the primary stage being twice the size of the secondary. Algal growth was common in the splash zones between the distribution jets of both stages and was predominantly of the genus Stigeoclonium.

Clumps of grey-brown film were removed from the surface of the primary filter. The clumps were composed mainly of aseptate hyphae of varying widths with a minority of septate hyphae, all were heavily encrusted with bacteria. Enchytraeid worms and Psychoda larvae were fairly numerous.

Film from the top of the secondary stage was grey and consisted of masses of zoogloeal bacteria, enchytraeid worms, nematodes and peritrichous protozoa. Fungal hyphae were similar in appearance and dimensions to those from the primary filter.

The bottom of the primary stage revealed light-grey-brownish cream film composed of aseptate hyphae with septate hyphae in the grey-brown areas, all similar to the hyphae from the surface. Most of the bios was composed of zoogloeal bacteria and nematodes, with a few holotrichous protozoa.

Bios from the base of the secondary stage was similar in appearance to the base of the primary stage. Very few fungal hyphae were found and they were all aseptate, the main components of this sample were the masses of zoogloeal bacteria.

Settled sludge from both primary and secondary stages was dark grey and anaerobic, no hyphae were visible amongst the bacteria.

Coverham Creamery, Middleham, Yorkshire (Unigate Foods)

The surface of the filter supported a creamy-grey film of apparently uniform thickness. Fungal hyphae were tightly interwoven to form this dense growth. The majority of hyphae were fine septate with a minor component of a coarse septate type and medium-coarse septate filaments dominated the grey areas. The remainder of the bios was composed of masses of zoogloeal bacteria and fairly numerous Psychoda larvae.

Film from the base of the filter was very similar in appearance to the surface growth. Fine septate and medium-coarse septate hyphae were revealed on examination. Bacterial masses and fly larvae were numerous.

An effluent sample was removed from the collecting-tray underneath the filter. This latter contained numerous Psychoda larvae and pupae zoogloeal bacteria and fine septate fungal hyphae.

The freshly settled sludge was light-brown in colour and possessed abundant fly larvae and Lumbricillid worms. The older sludge was dark grey and anaerobic, there were no fungal hyphae.

West Marton, Yorkshire (Associated Dairies)

Film from the top of the primary stage was thick and creamy-grey in colour with clumps of growth similar to those seen at Cannington in Somerset. The clumps were composed of broad septate hyphae tightly interwoven and difficult to separate. Fine septate hyphae formed the dominant component in the creamy coloured parts of the film and broader septate hyphae dominated the grey areas. Masses of zoogloeal bacteria composed the rest of the bios. Psychoda flies were numerous at the filter-surface .

The top of the secondary stage supported a darker thinner film with patches of green due to the presence of the alga Stigeoclonium sp. Zoogloeal bacteria and Psychoda larvae were numerous, nematode worms were present but not abundant. Fungal hyphae observed in the film were all of the coarse septate type.

Samples from the base of the primary stage were also creamy-grey in colour with similar hyphae to the top samples, zoogloeal bacteria seemed to be even more abundant than at the surface.

Film from the base of the secondary stage was dark creamy-grey in colour and composed of numerous bacteria, fly larvae and holotrichous protozoa. Coarse septate hyphae were the only type observed.

PART 2 - DAIRIES SAMPLED ON MORE THAN ONE OCCASION

Uttoxeter, Staffordshire (Unigate Foods)

Week one (April)

A thin coating of film had developed on the surface and the base of the primary filter.

The surface sample was creamy-coloured and easily separated to reveal zoogloeal bacteria and a few holotrichous protozoa.

The bottom sample was darker, pinkish-brown in colour with the same composition as above.

No fungal hyphae were present in either sample.

Week two

Samples were taken from the top and base of the primary filter at the end of the second week in operation.

The surface sample was yellowish-brown and composed entirely of zoogloeal bacteria.

The bottom sample was greyish-brown with orange areas and this too was entirely bacterial.

Neither sample possessed fungal hyphae.

Week four

Bios from the surface of the primary filter was pinkish-brown with grey areas. The latter were composed of zoogloeal bacteria, while the former revealed fine septate hyphae with crescentic conidia indicative of a species of *Fusarium*. A minority of coarse aseptate hyphae were found in part of the grey film.

The bottom samples were grey with orange-brown, pinkish-brown and cream-coloured components. The grey-cream areas were

composed of medium coarse septate hyphae and coarser aseptate hyphae similar to the top. The orange-brown areas were also dominated by the latter hyphae, whereas the pinkish-brown parts were composed almost entirely of fine septate hyphae similar to the surface.

Zoogloeal bacteria and holotrichous protozoa completed the bios under examination.

Sufficient film had developed on the secondary filter to allow sampling for the first time. The surface samples were yellow-orange-brown with pink and cream factions. Fine septate hyphae dominated the film with minor components of broader septate and aseptate hyphae, zoogloeal bacteria were abundant.

Samples from the base of the secondary filter were similar to the top but revealed only fine septate hyphae and masses of zoogloeal bacteria.

Week six (May)

Surface samples from the primary filter were predominantly dark grey with cream areas and consisted mainly of zoogloeal bacteria with a few nematodes and holotrichous protozoa. Fungal hyphae were in the minority compared to the bacteria and were all fine septate filaments.

Fine septate hyphae were also observed in the brownish-grey film from the base of the primary filter. Numerous bacteria, protozoa and nematodes constituted the bulk of the bios.

Samples from the surface of the secondary filter were pinkish-brown with green patches due to unicellular algae. Fine septate hyphae were removed from the pinkish-brown areas, and associated with them were masses of zoogloeal bacteria, a

few nematodes and holotrichous protozoa.

The base of the secondary filter possessed film which was lighter in colour, more pink than brown, which had a similar composition to the surface samples.

Week seven

Film from the top of the primary filter was dark grey-brown with isolated green patches due to unicellular algae. Coarse septate, coarse aseptate and fine septate hyphae were the chief-components of the film. Filamentous bacteria were observed amongst the gelatinous masses of zoogloeal bacteria which constituted the remainder of the film.

Samples from the base were similar in colour and composition to the surface, apart from the absence of coarse septate hyphae. Bios from the surface of the secondary stage was pinkish-brown with greyish-green patches. Coarse septate hyphae dominated the darker areas, whilst the lighter areas were composed of fine septate filaments, the remaining bulk of the film was purely bacterial.

Associated bottom samples were pinkish-brown in colour with numerous fine septate hyphae and zoogloeal bacteria.

Week eight

These samples were taken towards the end of May. There were no lumps of film removed from either of the filters probably due to the overall decrease in fungal hyphae which tend to bind the film together.

Zoogloeal bacteria and enchytraeid worms dominated the dark-grey fragmented sample from the primary stage surface.

A minority of fine septate hyphae were observed amongst the bacteria.

Bottom samples were thicker in composition but had essentially the same components with the addition of nematode worms.

Pinkish-brown film composed of fine septate hyphae smothered with bacteria was removed from the top of the secondary stage.

Week thirteen (June)

Samples of bios were dominated by Psychoda larvae and pupae with associated humus, the film had been removed from the media by these agents.

Week twenty-eight (October)

Unfortunately, due to pH control problems at the plant, there was a four-month break in the sampling programme which was resumed in the first week of October.

Top of the primary stage film was grey with numerous Psychoda larvae and coarse septate hyphae covered in bacteria. There were several green patches due to unicellular algae associated with the grey film.

The bottom samples were brown and fragmented, of similar composition to the top but with fewer hyphae. A few fine hyphal filaments were observed with the coarse type.

Film from the top of the secondary stage was basically brown with green and grey areas. The green colour was due to the alga Stigeoclonium and the grey to the latter when smothered with bacteria. Fine septate hyphae were removed from the brown areas but they were also covered with bacteria.

Week twenty-nine

The film samples were becoming thicker as the temperature dropped. Numerous *Psychoda* larvae and zoogloeal bacteria were removed from the creamy-grey film of the primary filter. Coarse septate hyphal filaments were equally dominant. Samples from the base were fragmented, being composed of *Psychoda* larvae and pupae with egg cases and associated debris, bacteria and short pieces of fungal hyphae. The hyphae were in a minority and all were very fine and septate.

The top of the secondary stage also possessed few fungal hyphae, the majority of those found were fine septate, some were very fine septate and there was a small component of broader aseptate hyphae in a cream-coloured patch. The remainder of the film was brown and composed of zoogloeal bacteria with dark-amber Naviculoid diatoms. Several fragments of the green alga *Stigeoclonium* were removed with the film.

Week thirty

Film from the primary stage surface was thick and grey-cream in colour. The dark grey areas were dominated by coarse septate hyphae whereas the lighter grey and cream areas , although dominated by the latter, also revealed fine septate filaments. *Psychoda* larvae and pupae were present but not abundant and the remainder of the film sample was composed of zoogloeal bacteria.

The bottom of the primary stage was similar to the previous week being highly fragmented and composed almost entirely of *Psychoda* in all stages of its life-cycle with associated humus.

Stigeoclonium and *Ulothrix* were found on the surface of the secondary stage. The film was pinkish-brown with yellow areas and composed of numerous bacteria, nematodes, Naviculoid diatoms and a few fine septate hyphae.

Week thirty-one

Thick creamy-grey film with abundant bacteria a few fly larvae and numerous coarse septate hyphae was removed from the primary stage surface.

Bottom samples were pinkish-brown with numerous bacteria and fine septate hyphae, there was much less fly debris than the previous week.

Fairly thick brownish-grey film was removed from the secondary stage surface. Algae were again prominent and there were numerous bacteria, nematodes, boat-shaped diatoms and fine septate hyphae observed.

Week thirty-three (November)

The surface film was predominantly grey with numerous algal filaments, masses of *Psychoda* larvae and bacterial zoogloeae, a few colonies of peritrichous protozoa and coarse septate hyphae with filamentous bacteria in apparent association.

Samples from the base of the primary filter were also grey but fragmented. There were no fungal hyphae. The chief constituents were the masses of zoogloeal bacteria and nematode worms, there were very few fly larvae.

The top of the secondary stage film had also reverted to the form of a slurry composed of algal filaments, bacteria, nematodes and a few coarse aseptate hyphae covered with

filamentous bacteria. A minor component of fine aseptate and broader septate hyphae were recorded.

Week thirty-five

A relatively small proportion of the creamy-grey film was composed of coarse septate hyphae; the rest of the surface sample consisted of algal filaments, Psychoda larvae and pupae, numerous nematodes, a few peritrichous protozoa and zoogloeal bacteria.

Once again, the bottom primary samples were easily separated into their components. A few short pieces of coarse septate hyphae were removed from the fly debris, bacteria and nematodes which contributed to the grey-brown humus.

Film from the top of the secondary stage was composed of Stigeoclonium fragments, fly larvae and pupae, enchytraeid worms, diatoms of the genera Navicula and Nitzschia, a few holotrichous protozoa, bacterial zoogloeae and fine septate and aseptate hyphae. There were a few coarse aseptate hyphae observed in the creamy areas of the brownish-cream film.

No samples were received from the base of the secondary stage since the middle of May. The reason for this failure was the lack of film related to the operation of the filters.

Week thirty-six (December)

Thick, matted creamy-grey film was removed from the primary-stage surface. It was composed of masses of zoogloeal bacteria, a few filamentous bacteria and peritrichous protozoa, algal fragments, a few Psychoda pupae and co-dominant coarse septate and aseptate fungal hyphae. Other hyphae were also

observed and these were mostly fine septate filaments with broader septate hyphae in the darker areas of the film.

Bottom samples were of a very different nature, being very easily separated out. The creamy-brown colouration was due to the numerous Psychoda pupae, bacteria including filaments of Sphaerotilus, nematodes and septate hyphae.

Film from the top of the secondary stage was cream-brown-yellow with algal fragments, numerous enchytraeid worms and bacteria and coarse aseptate and septate hyphae. The film was easier to separate than that of the top primary stage and, again, diatoms of the genus Navicula were observed.

Week thirty-nine

Unusually, the film from the primary stage surface was fairly easy to separate, the pink-grey-brown colour revealing Psychoda pupae and associated debris, numerous bacteria and five different types of fungal hyphae. A medium-coarse septate mycelium dominated the other fungi which were also septate apart from one. The other three septate hyphae were of varying hyphal diameters, one coarse, one very fine and the third of intermediate dimensions.

The bottom sample was of similar colour and composition to the top, the darker areas seemed to possess more of the coarser hyphae than the lighter pink-brown parts.

The film from the secondary stage was creamy-brown with green patches due to species of the algal genera of Stigeoclonium and Ulothrix, Naviculoid diatoms were also present. Three different types of fungal hyphae were observed, two were fairly broad and co-dominant one being septate, the other aseptate.

The third possessed fine septate filaments and formed a minor component of the film.

Week forty-two (January)

Film from the top of the primary stage remained pinkish-brown in colour, it was fairly easy to disperse the constituents in water. Psychoda larvae were present but not numerous, nematodes and peritrichous protozoa were also observed but not in abundance. There were masses of zoogloeal bacteria and several strands of filamentous bacteria covering the three types of septate fungal hyphae in the film. Dominant hyphae were coarse septate with equally subdominant medium-coarse and fine septate filaments.

Bottom samples were of the same colour but the components were more numerous and the protozoa were of the holotrichous species. Two of the coarser septate fungi were co-dominant in the bios and were also observed at the surface. Medium-coarse aseptate and very fine septate hyphae were in the minority.

Top of the secondary stage samples were composed chiefly of enchytraeid worms, nematodes, peritrichous protozoa, filamentous bacteria and numerous masses of zoogloeal bacteria. The only fungal hyphae were of the medium-coarse septate and very fine septate types and all those observed were in short pieces.

Week forty-four

The surface film from the primary stage was creamy-grey and similar to that of eight weeks ago. Numerous Psychoda larvae and pupae and white enchytraeid worms were covered with brown masses of zoogloeal bacteria and fairly numerous filamentous bacteria. The hyphae of three different fungi were removed from the film, all were septate and varied in diameter.

Hyphae of similar dimensions were observed in the bottom samples, which were brownish-grey and composed of zoogloeal bacteria, fly pupae and associated debris.

Very numerous enchytraeid worms were found on the surface of the secondary stage. The remainder of the bios was composed of fragments of filamentous algae and zoogloeal bacteria. There was very little fungal material the only hyphae observed were very fine and septate and covered with bacteria.

Week forty-six (February)

Primary-stage surface film was pinkish-grey-brown in colour, the pink hue resulting from a fairly large number of enchytraeid worms. Filamentous bacteria, unicellular algae, holotrichous protozoa, fly larvae and nematodes composed the remaining bulk of the film. Four types of fungal hyphae were observed in the darker areas of the film, all were septate and ranged from very fine to coarse in diameter, with the two coarser types being equally dominant.

Similar hyphae were observed in the bottom samples with the coarser ones being dominant. Fungi by no means dominated the samples though. The brown bios was mainly composed of *Psychoda* larvae and pupae, numerous nematodes and masses of zoogloeal bacteria.

Pinkish-brown film was removed from the secondary stage surface. Again, the pink colouration was due to abundant enchytraeid worms. Naviculoid diatoms, numerous nematodes, algal filaments and masses of zoogloeal bacteria composed the brown areas. The only hyphae were fairly fine septate filaments found in the brown parts of the film and these formed a minor component.

For the first time since May, of the previous year, a sample from the bottom of the secondary stage was taken. Fungal hyphae were scarce, those found being septate and of moderately thick diameter. Most of the bios was composed of masses of zoogloeal bacteria, pinkish coloured enchytraeid worms and a number of filamentous bacteria.

The samples taken in February were the last for the purposes of this project. Unfortunately, an intended expansion in production, at the associated dairy, was not realised and this meant that the Flocor towers would not be operated to their full extent as high-rate filters. The records over the past months have shown that although the fungal flora was varied, and sometimes dominated the bios, there were frequent periods when bacteria and worms became the chief components. Another important reason for the termination of sampling at the Uttoxeter site was the need for a suitable location for the pilot plant. Several criteria had to be satisfied:-

1. The dairy should produce a strong effluent in terms of its organic content.
2. The effluent receives pre-treatment by means of high-rate filtration preferably using Flocor media.
3. The location should be at a reasonable distance to allow frequent sampling and regular maintenance.

The dairy which appeared to be the most suitable was situated in Shropshire.

Minsterley, Shropshire (Express Dairy Foods Ltd.)

The first samples were taken in March, one month after

the cessation of sampling at Uttoxeter.

The single-stage Flocor towers received intermittent doses of effluent, which impinged on splash plates and spread out, over the surface of the media, according to the accuracy of positioning of the plates. Several samples of bios were removed from all over the surface of the tower, including the splash plates.

Filaments of the green alga Stigeoclonium were removed from the sides of the tower. A brownish-yellow sample revealed a few nematode worms, Psychoda larvae, numerous zoogloeal bacteria and abundant fungal hyphae, some were coarse and septate and an equal number of finer septate filaments were found. Another film sample was thick in consistency and creamy-grey in colour. Numerous Psychoda pupae were removed from this growth and five different types of hyphae were found in the paler grey areas. Vacuolated septate hyphae were teased out of pink-orange-yellow areas of the film, these hyphae were very common in most of the samples taken. Zoogloeal bacteria were present in great abundance.

Vacuolated aseptate hyphae were removed from thick creamy-yellow film taken from a number of splash-plates. Vacuolated septate hyphae, described above, were present but in a minority; there were numerous holotrichous protozoa, bacterial zoogloeal and nematodes were few in comparison with the other samples. Fine septate hyphae were removed from pinkish-brown areas of film and Naviculoid diatoms were also observed. Film removed from poorly-irrigated media was composed of zoogloeal bacteria.

Samples from the bottom of the filter looked uniform

in colour when viewed from underneath. It was only possible to remove film from around the edges of the base. The samples were brownish-grey in colour and the thick lumps were due to the numerous fungal hyphae which were tightly interwoven. The dominant hyphae were of a coarse septate type with a lesser component of a finer septate type. Psychoda larvae, pupae and associated humus constituted most of the main bulk of the film, holotrichous and peritrichous protozoa were present but not common, nematodes were fairly numerous and zoogloeal bacteria were abundant. Filamentous bacteria were also recorded.

There was a strong fungal component in the film samples from Minsterley and, because it satisfied the previously described criteria, it was proposed to site the pilot plant at this dairy. The next visit to Minsterley concerned the siting of the pilot plant and was the next opportunity to take more samples; this took place in October of the same year.

There were numerous flies on the top of the filter especially at the sides. The film was creamy-yellow in colour with numerous green patches due to the presence of the algae Stigeoclonium and Ulothrix. Microscopic examination revealed an almost pure stand of coarse septate hyphae. Finer septate hyphae were observed in many of the sub-samples but they were all subdominant to the coarse filaments.

Zoogloeal bacteria were very numerous but there were no filamentous species, protozoa and nematodes were present but not common.

Some of the surface film was in the form of large clumps of yellow-grey-brown material. They were difficult to tease apart and consisted of coarse granular septate hyphae with associated Psychoda larvae and pupae and zoogloeal bacteria.

Bottom samples were grey-brown in colour and composed of fly larvae and pupae, numerous zoogloeal bacteria and two types of septate coarse; the latter was dominant in the film.

2.6 RESULTS WITH DISCUSSION

From the descriptions of the film samples taken from all the Flocor filters visited, it becomes clear that the general ecology does not differ greatly when different dairy wastes are treated. Fungi tend to dominate the filter film especially in the cooler months and, in some cases, have been found as components of thick mats of growth. Bacteria have been predominantly found in the zoogloeal condition, occasionally filamentous types such as Sphaerotilus and Beggiatoa have also been observed. Nematode worms were recorded from nearly all the samples, whereas enchytraeid worms, although common, were not always of the same type. Unless specifically mentioned, most of the enchytraeid worms were white, being identified as Enchytraeus albidus, the red enchytraeids, Lumbricillus lineatus, whose colour is derived from haemaglobin, were less commonly found.

Larvae, pupae and adults of the dipteran fly Psychoda were frequently observed at most of the treatment plants and sometimes were the dominant components of the film.

Stigeoclonium was the most frequently recorded alga often found at the surface of the secondary stage filters. The reduced concentration of organic nutrients allows such autotrophs to compete with the heterotrophs for the inorganic nutrients remaining in the effluent.

The protozoa were identified as far as the genus level (Curds 1969), the holotrichs were confined to members of the genera Litonotus, Colpoda, Colpidium and Paramoecium

and the peritrichs belonged to the genera Vorticella, Carchesium, Opercularia and Epistylis. The differences in the protozoan fauna of the eleven dairy wastes appeared to be quantitative rather than qualitative.

The principal fungi in the film samples described were cultured and identified using the method and literature listed in section 2.4. Species lists of the fungi are given in Tables 2.4 to 2.6, for the ten mature Flocor filters, and Tables 2.7 to 2.12 for the recently commissioned Uttoxeter filters. Fig. 2.2 summarises the findings of Tables 2.4 to 2.6 in the form of a bar chart and similarly Fig. 2.3 summarises the findings of Table 2.13.

The treatment plant at Uttoxeter was considered separately for two reasons; first, the filters were new and sampling began after only one week in operation, and secondly the filters were sampled on fifteen occasions during the first year in commission. The species lists of principal fungi, isolated from the Uttoxeter filters, are considered separately for the primary and secondary stages. Table 2.12, summarises the total number of occurences of each fungus during the year, regardless of the position of each organism in the stages of filtration.

Considering Fig. 2.2 it becomes apparent that three fungal species were isolated from fifty per cent or more of the ten dairies. These three species were: Geotrichum candidum Link, Fusarium aquaeductuum Lagh., and Fusarium tabacinum (Beyma)W.Gams, and their identity was confirmed by the Commonwealth Mycological Institute at Kew. The same three species were also isolated after at least fifty per cent of

the sampling visits to the Uttoxeter plant, as well as *Subbaromyces splendens* and *Mucor racemosus* (fig. 2.3.)

However, although the two latter fungi were frequently found as principal film components of the Uttoxeter fitlers, they were rarely isolated from the ten mature plants (as illustrated in Fig. 2.2) and it was, therefore, decided to omit *Subbaromyces* and *Mucor* from futher examination. From these results it was decided to carry out comprehensive investigations concerning the utilization of biomass produced by *G.candidum*, *F.aquaeductuum* and *F.tabacinum*.

TABLE 2.4

Principal fungi isolated after one sampling visit, in November 1975, to each treatment plant named below.

	HONITON		CHARD		CANNINGTON		FROME		ILMINSTER	
	T	B	T	B	T	B	T	B	T	B
1 Isoachlya sp.	X									
2 Saprolegnia sp.	X									
3 Achlya sp.	X									
4 Thraustotheca sp.			X							
5 Brevilegnia sp.			X	X						
6 Dictyuchus sp.			X			X				
7 vegetative					X					
8 Penicillium sp.				X	X					
9 Alternaria tenuis						X	X	X	X	
10 Sepedonium sp.							X			
11 Geotrichum candidum		X		X	X			X	X	
12 Pythium sp.										
14 Fusarium aquaeductuum					X		X			
15 Pythium sp.										
16 Fusarium tabacinum			X		X					
17 vegetative										
19 Trichosporon sp.										
20 Subbaromyces splendens										
21 Cephalosporium sp.										
22 Mucor racemosus										
23 Candida sp.										
24 Mortierella jenkini										
25 Aureobasidium pullulans										
26 Verticillium elodeae										
27 Penicillium sp.										
28 Pyrenochaeta sp.										
29 Ascoidea rubescens										

T = TOP OF FILTER

B = BOTTOM OF FILTER

TABLE 2.5

Principal fungi isolated after one sampling visit to each treatment plant named below. (1975)

No.	Fungus	CUDDINGTON (MARCH)		TARVIN (MARCH)		COVERHAM (OCTOBER)		WESTMARTON (OCTOBER)	
		T	B	T	B	T	B	T	B
1	Isoachlya sp.								
2	Saprolegnia sp.								
3	Achlya sp.								
4	Thraustotheca sp.								
5	Brevilegnia sp.								
6	Dictyuchus sp.			X	X				
7	vegetative								
8	Penicillium sp.								
9	Alternaria tenuis								
10	Sepedonium sp.								
11	Geotrichum candidum	X	X			X	X	X	X
12	Pythium sp.	X	X	X					
14	Fusarium aquaeductuum	X	X			X		X	
15	Pythium sp.		X						
16	Fusarium tabacinum	X				X			
17	vegetative			X					
19	Trichosporon sp.					X	X	X	X
20	Subbaromyces splendens					X		X	X
21	Cephalosporium sp.					X			
22	Mucor racemosus								
23	Candida sp.								
24	Mortierella jenkini								
25	Aureobasidium pullulans								
26	Verticillium elodeae								
27	Penicillium sp.								
28	Pyrenochaeta sp.								
29	Ascoidea rubescens								

T = TOP OF FILTER

B = BOTTOM OF FILTER

TABLE 2.6

Principal fungi isolated from Minsterley on two sampling visits in 1977.

		OCCURRENCE OF FUNGI			
		IN MARCH		AND OCTOBER	
		TOP	BOTTOM	TOP	BOTTOM
1	Isoachlya sp.				
2	Saprolegnia sp.				
3	Achlya sp.				
4	Thraustotheca sp.				
5	Brevilegnia sp.				
6	Dictyuchus sp.				
7	vegetative				
8	Penicillium sp.				
9	Alternaria tenuis				
10	Sepedonium sp.				
11	Geotrichum candidum	X	X	X	X
12	Pythium sp.				
14	Fusarium aquaeductuum				
15	Pythium sp.	X	X		
16	Fusarium tabacinum	X		X	
17	vegetative				
19	Trichosporon sp.	X			
20	Subbaromyces splendens	X	X	X	X
21	Cephalosporium sp.				
22	Mucor racemosus				
23	Candida sp.		X		
24	Mortierella jenkini				
25	Aureobasidium pullulans				
26	Verticillium elodeae				
27	Pencillium sp.			X	
28	Pyrenochaeta sp.	X			
29	Ascoidea rubescens			X	

TOP = TOP OF FILTER

BOTTOM = BOTTOM OF FILTER

FIG. 2.2

Bar chart summarising the frequency of occurrence of principal fungi isolated from the samples taken from the dairies named in TABLES 2.4 to 2.6

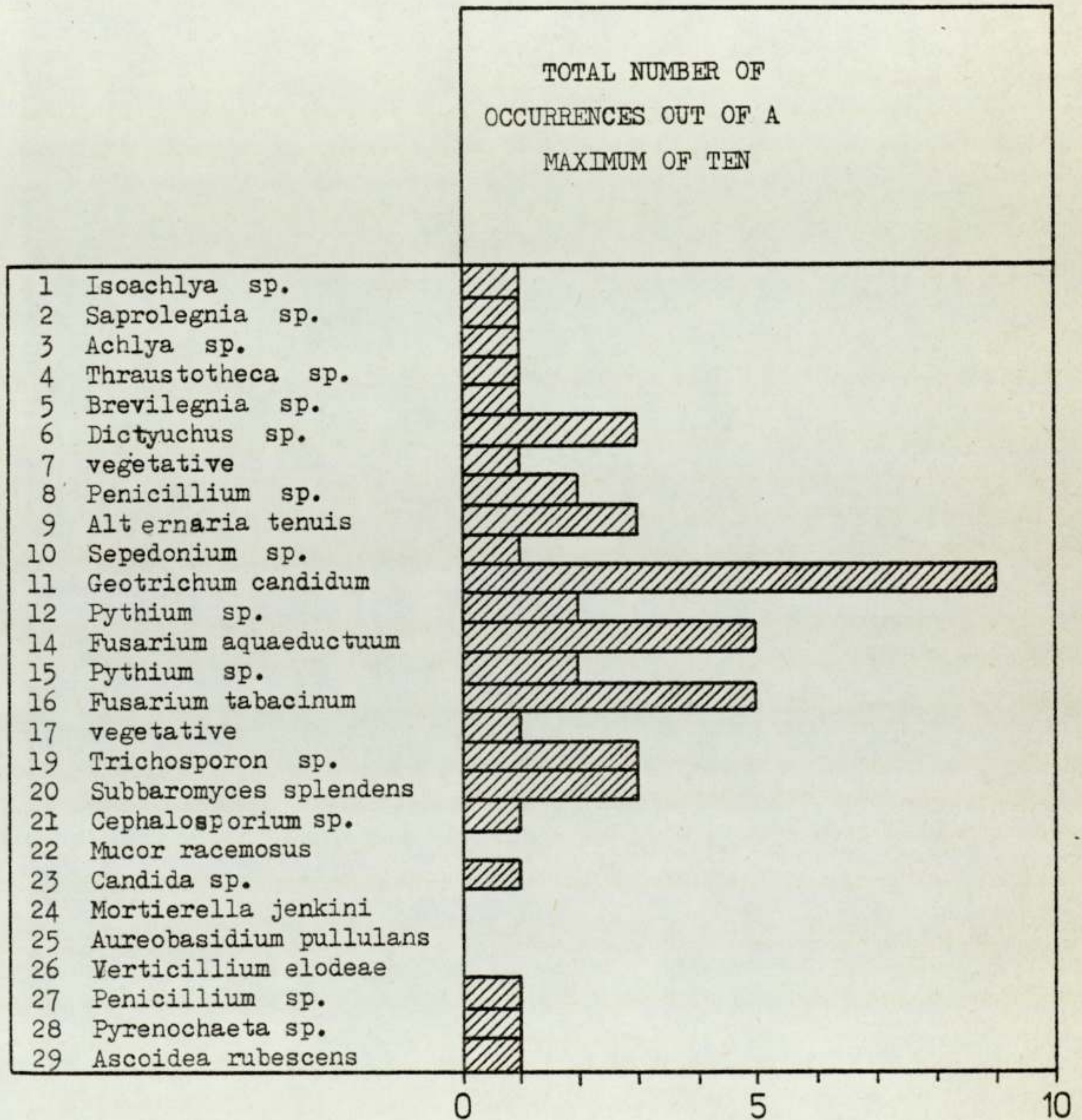


TABLE 2.7

Principal fungi isolated from the primary stage Flocor tower at Uttoxeter in the spring and autumn of the first year in operation. (1976)

		WEEK FOUR (APRIL)		WEEK SIX (MAY)		WEEK SEVEN (MAY)		WEEK EIGHT (MAY)		WEEK TWENTY-EIGHT (OCTOBER)	
		T	B	T	B	T	B	T	B	T	B
1	Isoachlya sp.										
2	Saprolegnia sp.										
3	Achlya sp.										
4	Thraustotheca sp.										
5	Brevilegnia sp.										
6	Dictyuchus sp.										
7	vegetative										
8	Penicillium sp.										
9	Alternaria tenius										
10	Sepedonium sp.										
11	Geotrichum candidum		X								
12	Pythium sp.										
14	Fusarium aquaeductuum	X	X	X	X	X	X				
15	Pythium sp.										
16	Fusarium tabacinum										
17	vegetative										
19	Trichosporon sp.		X	X	X	X	X	X	X		X
20	Subbaromyces splendens					X				X	
21	Cephalosporium sp.										
22	Mucor racemosus	X	X			X	X				
23	Candida sp.										
24	Mortierella jenkini										
25	Aureobasidium pullulans										
26	Verticillium elodeae										
27	Penicillium sp.										
28	Pyrenochaeta sp.										
29	Ascoidea rubescens										

T = TOP OF FILTER

B = BOTTOM OF FILTER

TABLE 2.8

Principal fungi isolated from the secondary stage Flocor tower at Uttoxeter during spring and autumn of the first year in operation. (1976)

		WEEK FOUR (APRIL)		WEEK SIX (MAY)		WEEK SEVEN (MAY)		WEEK EIGHT (MAY)		WEEK TWENTY-EIGHT (OCTOBER)	
		T	B	T	B	T	B	T	B	T	B
1	Isoachlya sp.										
2	Saprolegnia sp.										
3	Achlya sp.										
4	Thraustotheca sp.										
5	Brevilegnia sp.										
6	Dictyuchus sp.										
7	vegetative										
8	Penicillium sp.										
9	Alternaria tenuis										
10	Sepedonium sp.										
11	Geotrichum candidum	X		X	X						
12	Pythium sp.										
14	Fusarium aquaeductuum		X	X		X					
15	Pythium sp.										
16	Fusarium tabacinum										
17	vegetative										
19	Trichosporon sp.	X	X	X	X	X	X	X		X	
20	Subbaromyces splendens					X					
21	Cephalosporium sp.										
22	Mucor racemosus	X			X						
23	Candida sp.										
24	Mortierella jenkini										
25	Verticillium elodeae										
27	Penicillium sp.										
28	Pyrenochaeta sp.										
29	Ascoidea rubescens										

T = TOP OF FILTER

B = BOTTOM OF FILTER

TABLE 2.9

Principal fungi isolated from Uttoxeter (primary stage) in the autumn of 1976.

		WEEK TWENTY-NINE (OCTOBER)		WEEK THIRTY (OCTOBER)		WEEK THIRTY-ONE (OCTOBER)		WEEK THIRTY-THREE (NOVEMBER)		WEEK THIRTY-FIVE (NOVEMBER)	
		T	B	T	B	T	B	T	B	T	B
1	Isoachlya sp.										
2	Saprolegnia sp.										
3	Achlya sp.										
4	Thraustotheca sp.										
5	Brevilegnia sp.										
6	Dictyuchus sp.										
7	vegetative										
8	Penicillium sp.										
9	Alt e rnaria tenuis										
10	Sepedonium sp.										
11	Geotrichum candidum			X							
12	Pythium sp.										
14	Fusarium aquaeductuum		X	X							
15	Pythium sp.										
16	Fusarium tabacinum			X		X		X			
17	vegetative										
19	Trichosporon sp.										
20	Subbaromyces splendens	X		X		X		X		X	X
21	Cephalosporium sp.										
22	Mucor racemosus										
23	Candida sp.						X				
24	Mortierella jenkini										
25	Aurebasidium pullulans										
26	Verticillium elodeae										
27	Penicillium sp.		X								
28	Pyrenochaeta sp.										
29	Ascoidea rubescens										

T = TOP OF FILTER

B = BOTTOM OF FILTER

TABLE 2.10

Principal fungi isolated from Uttoxeter (secondary stage) in the autumn of 1976.

		WEEK TWENTY-NINE (OCTOBER)		WEEK THIRTY (OCTOBER)		WEEK THIRTY-ONE (OCTOBER)		WEEK THIRTY-THREE (NOVEMBER)		WEEK THIRTY-FIVE (NOVEMBER)	
		T	B	T	B	T	B	T	B	T	B
1	Isoachlya sp.										
2	Saprolegnia sp.										
3	Achlya sp.										
4	Thraustotheca sp.										
5	Brevilegnia sp.										
6	Dictyuchus sp.									X	
7	vegetative										
8	Penicillium sp.										
9	Alternaria tenuis										
10	Sepedonium sp.										
11	Geotrichum candidum	X						X			
12	Pythium sp.										
14	Fusarium aquaeductuum	X		X						X	
15	Pythium sp.									X	
16	Fusarium tabacinum			X		X		X			
17	vegetative										
19	Trichosporon sp.										
20	Subbaromyces splendens										
21	Cephalosporium sp.										
22	Mucor racemosus	X						X			
23	Candida sp.					X					
24	Mortierella jenkini							X			
25	Aureobasidium pullulans										
26	Verticillium elodeae										
27	Penicillium sp.										
28	Pyrenochaeta sp.										
29	Ascoidea rubescens										

T = TOP OF FILTER

B = BOTTOM OF FILTER

TABLE 2.11

Principal fungi isolated from Uttoxeter (primary stage) at the end of 1976 and early 1977.

		WEEK THIRTY-SIX (DECEMBER)		WEEK THIRTY-NINE (DECEMBER)		WEEK FORTY-TWO (JANUARY)		WEEK FORTY-FOUR (JANUARY)		WEEK FORTY-SIX (FEBRUARY)	
		T	B	T	B	T	B	T	B	T	B
1	Isoachlya sp.										
2	Saprolegnia sp.										
3	Achlya sp.										
4	Thraustotheca sp.										
5	Brevilegnia sp.										
6	Dictyuchus sp.										
7	vegetative										
8	Penicillium sp.										
9	Alternaria tenuis										
10	Sepedonium sp.										
11	Geotrichum candidum	X	X	X		X	X	X	X	X	X
12	Pythium sp.										
14	Fusarium aquaeductuum	X		X			X				
15	Pythium sp.								X		
16	Fusarium tabacinum	X		X		X		X		X	X
17	vegetative										
19	Trichosporon sp.	X									
20	Subbaromyces splendens	X	X	X		X	X	X	X	X	X
21	Cephalosporium sp.										
22	Mucor racemosus			X			X				
23	Candida sp.			X		X		X		X	
24	Mortierella jenkini										
25	Aureobasidium pullulans	X									
26	Verticillium elodeae										
27	Penicillium sp.							X	X	X	
28	Pyrenochaeta sp.										
29	Ascoidea rubescens										

T = TOP OF FILTER

B = BOTTOM OF FILTER

TABLE 2.12

Principal fungi isolated from Uttoxeter (secondary stage) at the end of 1976 and early 1977.

		WEEK THIRTY-SIX (DECEMBER)		WEEK THIRTY-NINE (DECEMBER)		WEEK FORTY-TWO (JANUARY)		WEEK FORTY-FOUR (JANUARY)		WEEK FORTY-SIX (FEBRUARY)	
		T	B	T	B	T	B	T	B	T	B
1	Isoachlya sp.										
2	Saprolegnia sp.										
3	Achlya sp.										
4	Thraustotheca sp.										
5	Brevilegnia sp.										
6	Dictyuchus sp.										
7	vegetative										
8	Penicillium sp.										
9	Alternaria tenuis										
10	Sepedonium sp.										
11	Geotrichum candidum	X		X		X				X	X
12	Pythium sp.										
14	Fusarium aquaeductuum					X		X			
15	Pythium sp.										
16	Fusarium tabacinum	X		X		X		X		X	
17	vegetative										
19	Trichosporon sp.	X									
20	Subbaromyces splendens										
21	Cephalosporium sp.										
22	Mucor racemosus	X		X							
23	Candida sp.										
24	Mortierella jenkini										
25	Aureobasidium pullulans										
26	Verticillium elodeae			X							
27	Penicillium sp.										
28	Pyrenochaeta sp.										
29	Ascoidea rubescens										

T = TOP OF FILTER

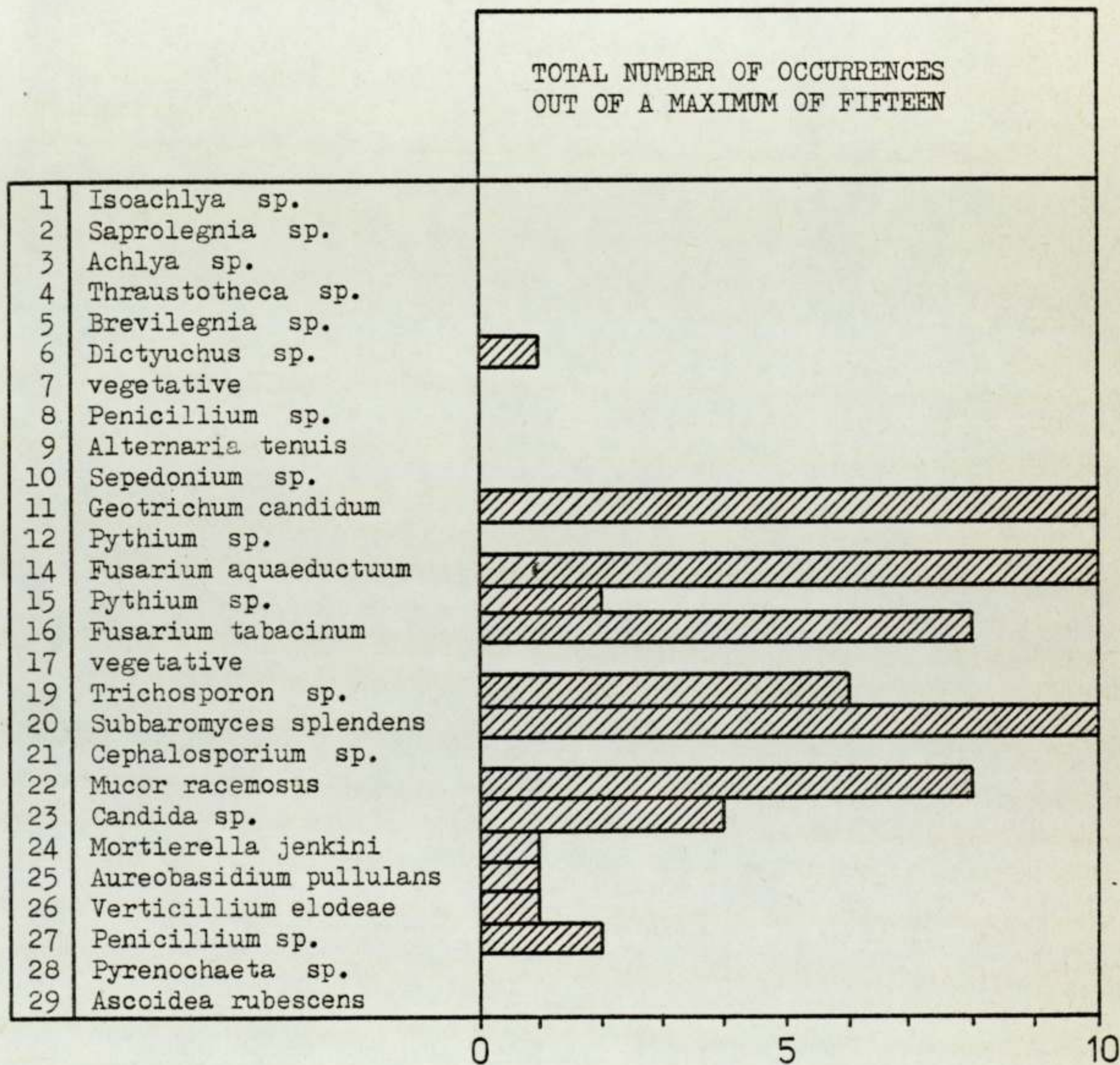
B = BOTTOM OF FILTER

TABLE 2.13 Summary of the total number of isolations of principal fungi in both filtration stages at Uttoxeter in the first year of its commission. (shown as the month and week number, taken from TABLES 2.7 to 2.12)

		APR.	MAY			OCTOBER				NOV.		DEC.		JAN.		FEB.	TOTAL
		4	6	7	8	28	29	30	31	33	35	36	39	42	44	46	
1	Isoachlya sp.																
2	Saprolegnia sp.																
3	Achlya sp.																
4	Thraustotheca sp.																
5	Brevilegnia sp.																
6	Dictyuchus										X						1
7	vegetative																
8	Penicillium sp.																
9	Alternaria tenuis																
10	Sepedonium sp.																
11	Geotrichum candidum	X	X				X	X		X		X	X	X	X	X	10
12	Pythium sp.																
14	Fusarium aquaeductuum	X	X	X			X	X			X	X	X	X	X		10
15	Pythium sp.										X				X		2
16	Fusarium tabacinum							X	X	X		X	X	X	X	X	8
17	vegetative																
19	Trichosporon sp.	X	X	X	X	X						X					6
20	Subbaromyces splendens			X				X	X	X	X	X	X	X	X	X	10
21	Cephalosporium sp.																
22	Mucor racemosus	X	X	X			X			X		X	X	X			8
23	Candida sp.								X				X	X	X		4
24	Mortierella jenkini									X							1
25	Aureobasidium pullulans											X					1
26	Verticillium elodeae												X				1
27	Penicillium sp.						X								X		2
28	Pyrenochaeta sp.																
29	Ascoidea rubescens																

FIG. 2.3

Bar chart, taken from TABLE 2.13 to illustrate the total number of isolations of principal fungi.



C H A P T E R T H R E E

SCREENING OF DOMINANT ORGANISMS

CHAPTER 3

SCREENING OF DOMINANT ORGANISMS

3.1 INTRODUCTION

Three fungi, Geotrichum candidum, Fusarium aquaeductuum and Fusarium tabacinum, were chosen from a total of twenty-seven organisms producing appreciable growths in Flocor towers treating dairy wastes at eleven treatment plants. The reasons for the choice of the three organisms were given at the end of Chapter 2.

In order to assess the nutritive value of the fungi it was necessary to obtain sufficient biomass for the various analyses and biochemical tests. A continuous culture apparatus was developed to produce the required biomass and the same apparatus was used in further studies for determining optimum growth conditions (Chapter 4).

3.2 LITERATURE REVIEW OF THE THREE DOMINANT FUNGI

3.2.1 Geotrichum candidum (Link) (Isolate No. 11)

Link described and named this fungus from soil in 1809. Subsequent isolations from a wide variety of sources have determined the ubiquity of this organism.

In 1906 Rettger was probably the first to record the presence of fungal growth as coloured slime on sewage percolating filters. Cox (1921) reviewed the literature of the interposing years concerned with non-bacterial growths on filter beds, and he also recorded fungoid growths on the upper surface of the stones. Cooke's literature review (1954) clarified the records of the earlier workers by studying their descriptions and illustrations, and concluded that the dark coloured slime (Rettger) and the grey fungoid growth (Cox) were the

same organism, namely Geotrichum candidum. This species was also recorded by Cooke (1954) among six important fungi isolated from sewage and polluted waters by workers in Britain, Europe and America from 1901 to 1953. In 1955 Becker and Shaw took samples from two sewage treatment plants, one of which received a small amount of dairy waste. They found G.candidum occurred in abundance throughout the study and was most frequently isolated from raw sewage.

In Britain Bell (1926) isolated and identified Geotrichum candidum (reported as Oidium or Oospora lactis) from sewage filter film at Stratford-on-Avon (1915-16) and in 1941 Tomlinson also reported this fungus (as Oospora) in similar film at Minworth. In 1942 Reynoldson found the fungal flora of a double filtration plant at Huddersfield was restricted to one mold, G.candidum, reported as Oospora. Acidic chemicals, discharged into the sewers by textile firms, were believed to be responsible for the toxic nature of the Huddersfield sewage and reduction in fungal flora.

Geotrichum candidum was known and reported for many years as Oospora lactis (Fresenius) Sacc., but, in 1957, Carmichael wrote a definitive paper concerning the colony morphology, occurrence and taxonomy of this fungus, also providing a long list of synonyms which rendered the old names obsolete.

Carmichael's work was extended by Van Uden and Do Carmo-Sousa (1959) in their studies of the physiological properties in twenty-three strains of G.candidum.

Brancato and Golding (1953), Painter (1954) and Cooke (1957b) all investigated growth determining parameters and nutritional requirements of G.candidum. Jones continued these studies in 1964 having mistaken G.candidum for the filamentous bacterium, Sphaerotilus

natans (Pipes and Jones 1963), but in this instance, the mold was isolated from activated sludge. Filamentous organisms have been reported as causing sludge bulking, a condition which prevents adequate settlement of the sludge, hence Jones's study of these organisms and their requirements.

Other workers have concentrated on the pathogenicity of *Geotrichum candidum* on plant and animal tissue. Butler (1960, 1965) studied the ability of numerous isolates to incite fruit-rot. Partially ripened or green fruit were not normally attacked but chilled green tomatoes lost their resistance to the mold. Isolates affecting citrus fruits were recorded from California, Honduras, Rhodesia, Nigeria, Egypt and Japan. El-Tobshy and Sinclair (1965) discovered a range of pathogenicity on fruit and vegetables when the fungus was inoculated deep into the plant tissue. Lewis and Sinclair (1966) used three plant and one human isolate of *G.candidum* to test the relationship between pathogenicity and tissue pH. They discovered that the host plant tissue was susceptible to attack below pH 5·3.

In Nigeria, this fungus was recorded as one of several species causing tomato rot, gaining entry to the fruit when damaged by insects (Onesirosan and Fatunla 1976). However, Bussel and Shavit (1975) suggested that the incidence of fruit-rot could be considerably reduced by careful handling and reducing the time between picking and washing.

G.candidum has also been reported as the cause of rubbery rot of potatoes in Wales (Humphreys-Jones 1969) and as a normal contaminant of silage (Woolford, 1975, Britt et al, 1975) and corn wastes (Church et al. 1973).

G.candidum is regarded as a member of the normal flora in

man, it is a feeble pathogen which occasionally exploits local or general weakness of its human host. (Anon 1976, Restrepo and De Uribe 1976). This mold has also been isolated from the droppings of pet pigeons in China (Volz and Yeh 1976) and from dermal lesions of flamingos (Spanoghe et al. 1976), but the findings suggest that predisposing factors are responsible for altering the host tissue to allow the fungal invasion.

Geotrichum candidum occurs naturally in a variety of dairy products such as cream and cottage cheese, milk and butter. The mold can cause spoilage of cottage cheese and surface discoloration of butter but it can be useful in the initial softening of Camembert cheese and also help to develop the flavour. (Foster et al. 1958, Prescott and Dunn 1959). The importance of G.candidum in milk and dairy products is due to its oxidative and lipolytic (fat-splitting) properties. The mold can grow on coagulated milk by oxidizing the lactic acid to carbon dioxide and water. Nelson (1952) investigated the characteristics of G.candidum lipase, suggesting that it was primarily an extracellular enzyme which attacked natural fats more readily than synthetic fatty compounds. The lipase was shown to have a remarkable specificity related to the double-bond position in unsaturated fatty acids (Jensen et al. 1972), and the molecular weight was found to be between fifty-three thousand and fifty-five thousand (Sugihara et al. 1975). G.candidum secretes lipase (glycerolester hydrolase) even in static culture, calcium ions are important for its reaction but phosphate has been found to inhibit this enzyme. (Dooijewaard-Kloosterziel and Wouters 1976).

Geotrichum candidum has been frequently used in studies of the physiological and morphological aspects of fungal differentiation. This mold possesses complete septa which divide the hyphae into many compartments or cells. Two cell types have been determined,

apical cells containing numerous vesicles and sub-apical cells whose vesicles are associated with developing septa. Each septum possesses characteristic plasmodesmata which vary in number up to a maximum of approximately fifty (Steele and Fraser 1973a). The plasmodesmata are clearly demonstrated in negatively stained or shadowed preparations of isolated septa studied with an electron microscope (Hashimoto et al. 1973).

G.candidum is a member of the Fungi Imperfecti or Deuteromycotina, a perfect stage has been described and named Endomyces geotrichum (Butler and Petersen 1972) but the imperfect state, reproducing by means of arthrospore formation, is more frequently found. Arthrospores are formed by simple fragmentation of fertile hyphae whose tips cease to grow prior to septation in an acropetal order (youngest cells at the apex). The initial diameter of the arthrospores is dependent on that of the fertile hyphae but their length is slightly variable (Cole 1975). Arthrospore development has been followed using time-lapse photomicrography by Cole and Kendrick (1969) whilst ultrastructural studies have been conducted by Steele (1973), Steele and Fraser (1973b) and Cole (1975). Park and Robinson (1970) discovered that the arthrospores of G.candidum could germinate in the absence of exogenous nutrients at low spore concentrations but were self-inhibited at high spore concentrations. They also revealed a relationship between spore concentration and the nutrient level necessary to overcome the inhibition. Robinson (1973) presented evidence which suggested that tropisms related to the point of arthrospore germ-tube emergence were probably a positive response to oxygen rather than a negative response to accumulating metabolites. Kier et al. (1976) has shown that it is possible to obtain two morphologically different types of arthrospores by culturing the fungus

in the same medium with different glucose concentrations.

Geotrichum candidum readily produces arthrospores in percolating filter film and, together with the frequently observed dichotomous terminal branching of the hyphae, form useful diagnostic features which assist in identifying the fungus in film samples and plate cultures. (Plate 3).

3.2.2 Fusarium aquaeductuum (Lagh.) (Isolate No. 14)

This fungus is the imperfect stage of Nectria purtonii (Grev.) Berk.

Link initiated the taxonomy of the genus Fusarium by describing it in 1809. Since then, numerous workers have attempted to clarify the classication of the genus and a review of the problems has been given by Snyder and Toussoun (1965). Fusarium isolates cultured in the light have been shown to produce longer conidia with more septa, than those grown in darkness (Harter 1941). A further example of cultural inconsistency is the variability of colony morphology on agar media exhibited by the genus Fusarium. These problems have been dealt with by Booth (1971) who has clarified the taxonomy and given full details of each Fusarium species. (Plate 4)

Almost a century after Link's description of the genus Fusarium, one species in particular became prominent on sewage filter beds - Fusarium aquaeductuum. Early workers described and attempted to identify the fungal growth but Rettger (1906) was probably the first to record the presence of pale or salmon pink coloured slime on experimental sewage percolating filters at Waterbury, Connecticut, The slimy growth was sufficient to reduce the rate of filtration and microscopic examination revealed the presence of a mold whose colour, Rettger believed, was derived from iron.

In 1921, Cox reported a pink to brown coloured gelatinous

(a)

(b)

PLATE 3 *Geotrichum candidum*

(a) mycelium

(b) mycelium and arthrospores

as seen in plate culture (hyphal diameter 6 µm)

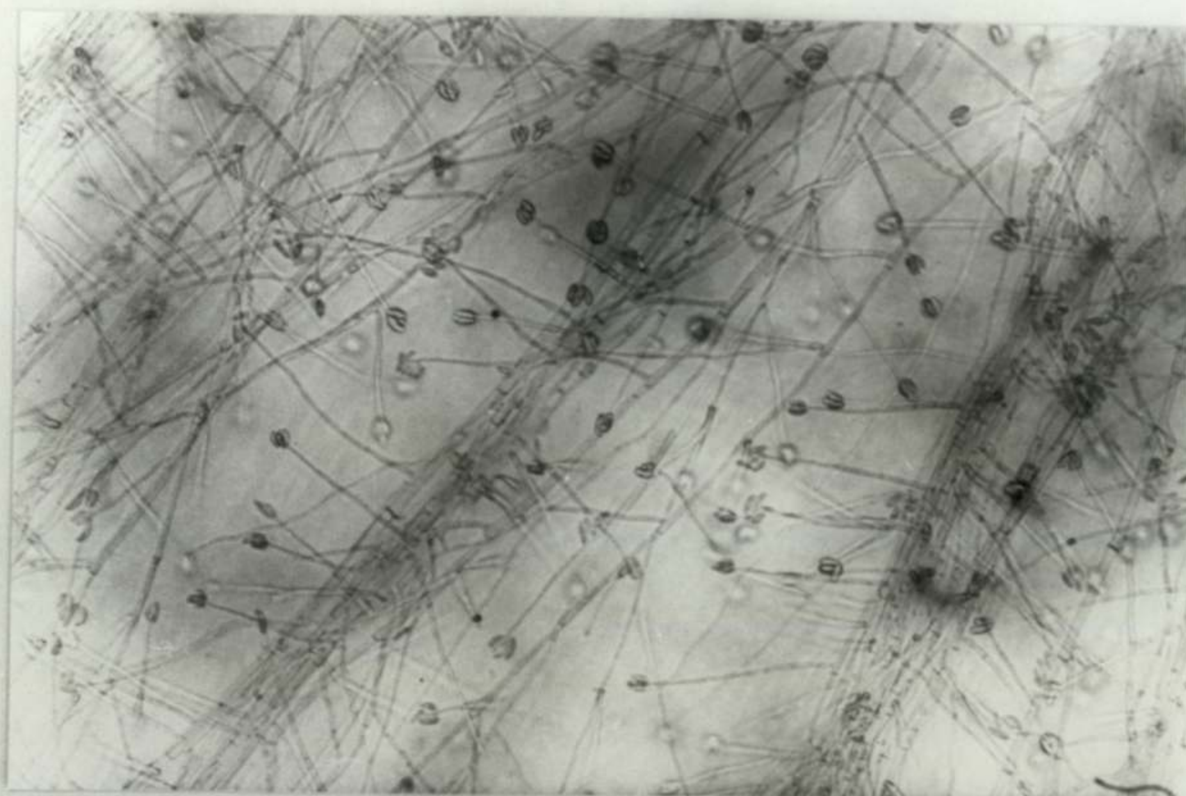


PLATE 4

Fusarium aquaeductuum

mycelium and condidia as seen in plate culture

(hyphal diameter 3 - 3.5 µm)

growth on sewage filter beds at Worcester, Massachusetts, which appeared in the cooler months and disappeared during the summer. From the descriptions given by these early workers Cooke (1954) deduced that the pink growths were due to the mold Fusarium aquaeductuum which seemed to be associated with Geotrichum candidum recorded as grey slimy growth by Rettger and Cox (see 3.2.1)

F.aquaeductuum has since been frequently recorded from the surface of sewage filter beds where it can withstand the full force of liquid from the distributor jets by means of a prostrate system of hyphae clinging firmly to the underlying media. (Tomlinson 1941). This mode of growth allows the mold to compete with algae on the filter surface.

In 1953, Hesseltine made microscopic examinations of the fungal flora isolated from percolating filters treating a mixture of antibiotic wastes and industrial wastes from a chemical laboratory. Over a period of two years, only three species of fungi were reported regularly as Fusarium episphaeria (≡ aquaeductuum), Oospora lactis (Geotrichum candidum) and Subbaromyces splendens. Hesseltine believed that Fusarium was strictly aerobic since it was mainly confined to the upper surface of the filter beds. The mold was abundant in the warmer months when it imparted a dull red colour to the filter surface and was often associated with the alga Chlorella.

Cooke recorded Fusarium aquaeductuum as one of the most frequently isolated fungi from sewage and polluted water in his own extensive surveys and when summarising the relevant literature of this field (Cooke 1954 , 1957a, 1958)

Hawkes (1957) reported considerable fungal film accumulation during the winter months on the Minworth filters (Birmingham, England) treating an industrial sewage, F.aquaeductuum was one of

the dominant fungal components of the film.

F.aquaeductuum was found to be the most abundant mold on the surface of a high-rate stone filter and a member of the dominant flora of a standard-rate filter in an investigation of microbial populations executed by Cooke and Hirsch (1958).

Fusarium aquaeductuum has been frequently recorded from filter beds (as above) often causing impaired efficiency because of its extensive growth which supports the growth of other fungi whose biomass obstructs the downward passage of the settled sewage. The nutritional requirements of this mold have been investigated in the hope of being able to utilize the information to control its growth (Painter 1954, Cooke 1957b). Painter's isolate was able to utilize ammonium salts and nitrate without the need of an external vitamin supply, whereas Cooke's isolate was deficient for the vitamin thiamine. Further studies of F.aquaeductuum were performed by Steensland (1973) who isolated it from attached growth in a Norwegian river, heavily polluted with spent sulphite liquor, and cultured it in a chemostat.

The importance of Fusarium aquaeductuum in percolating filter film and the frequency of its occurence, both in this study and those previously reviewed, confirmed the decision to continue cultural experiments with this mold (Chapter 2). Temperature and pH were the only variables investigated (Chapter 4) before a further literature survey revealed the unsuitable nature of this organism for the ultimate aims of this project.

Species of the genus Fusarium are well-distributed throughout nature occuring saprophytically in soil and decaying vegetation, as plant parasites and as etiologic agents of plant disease especially in cereals (Colhoun 1972, Lacey 1975, Wyllie and Morehouse 1977).

Consumption of affected grain by animals and man often leads to mycotoxicosis as a result of toxins produced by fungal metabolism (Ueno et al. 1974, Moss 1975). In 1971(a) Ueno et al. screened several strains of Fusaria and conducted bioassays and toxicity tests on various animals. Fusarium episphaeris (=aquaeductuum) produced fusarenon - X (isolated by Ueno et al. 1971(b)), irrespective of the culture medium, which showed marked toxicity to mice, rabbit reticulocytes (immature erythrocytes) and caused cellular degeneration and karryorrhexis (fragmentation of the cell nucleus). This mold also produced nivalenol which can be formed from fusarenon - X by enzymatic deacetylation.

Nivalenol and fusarenon - X are trichothecene toxins produced as secondary metabolites by several species of Fusarium. They are not carcinogenic but are capable of inhibiting DNA and protein synthesis and at present their incidence in nature is uncertain. Details of the trichothecene toxins including their structure, chemistry, spectral properties and biosynthesis are fully described by Wyllie and Morehouse (1977).

Since it was intended to use the biomass of the chosen fungal isolates for use in animal feed and the records of Fusarium aquaeductuum, as a producer of substances toxic to animals, eliminate it as a useful biproduct, it was decided not to continue with further studies of this organism.

3.2.3 Fusarium tabacinum (Beyma) W.Gams (Isolate No. 16)

Fusarium tabacinum was known for a long time as Fusarium affine (Fautrey and Lambotte) or Hymenula affinis (Fautrey and Lambotte) Wollenweber until it was renamed by Gams, it is the imperfect stage of Plectosphaerella cucumeris (Klebahn).

F.tabacinum has been isolated from the dry stems of potatoes by Fautrey and Lambotte (1896), from garden and wheat-field soil in Manitoba by Bisby et al.(1933) and from arable soil by Gams and Gerlagh (1968). Booth (1971) has produced a list of several sources of isolates of *F.tabacinum* which include air, water, dung, potato root eelworm, animal feed and numerous plants. This species has been frequently found in arable soil associated with decaying vegetation and has been recorded as a frequent component in the gut of the wheat-bulb saw fly at Rothamstead, England.

Pathogenicity of *F.tabacinum* has been reported on tobacco and pansies (Booth 1971). In 1968 Gams and Gerlagh attempted to demonstrate pathogenicity of *F.tabacinum* towards potatoes, cucumbers, wheat and rape but were unsuccessful. More recently, this species has been reported as the cause of a serious outbreak of wilt in experimental sugar beet fields in Wageningen, Holland, by Van derSpek (1973).

The nutritional requirements of *Fusarium tabacinum* do not appear to have been studied although full cultural details have been given by Booth (1971). (See Plate 5)

3.3 DEVELOPMENT OF A CONTINUOUS CULTURE APPARATUS

In 1952 Gloyna et al. developed a series of inclined rotating plastic tubes for use as an experimental equivalent to trickling filters. Settled domestic sewage was passed through the tube system and a microbial film grew on the inner walls. Various parameters such as the effects of different loading rates and recirculation of effluent on B.O.D. removal were investigated successfully.

Schulze (1957) used a number of wire grids (screens)

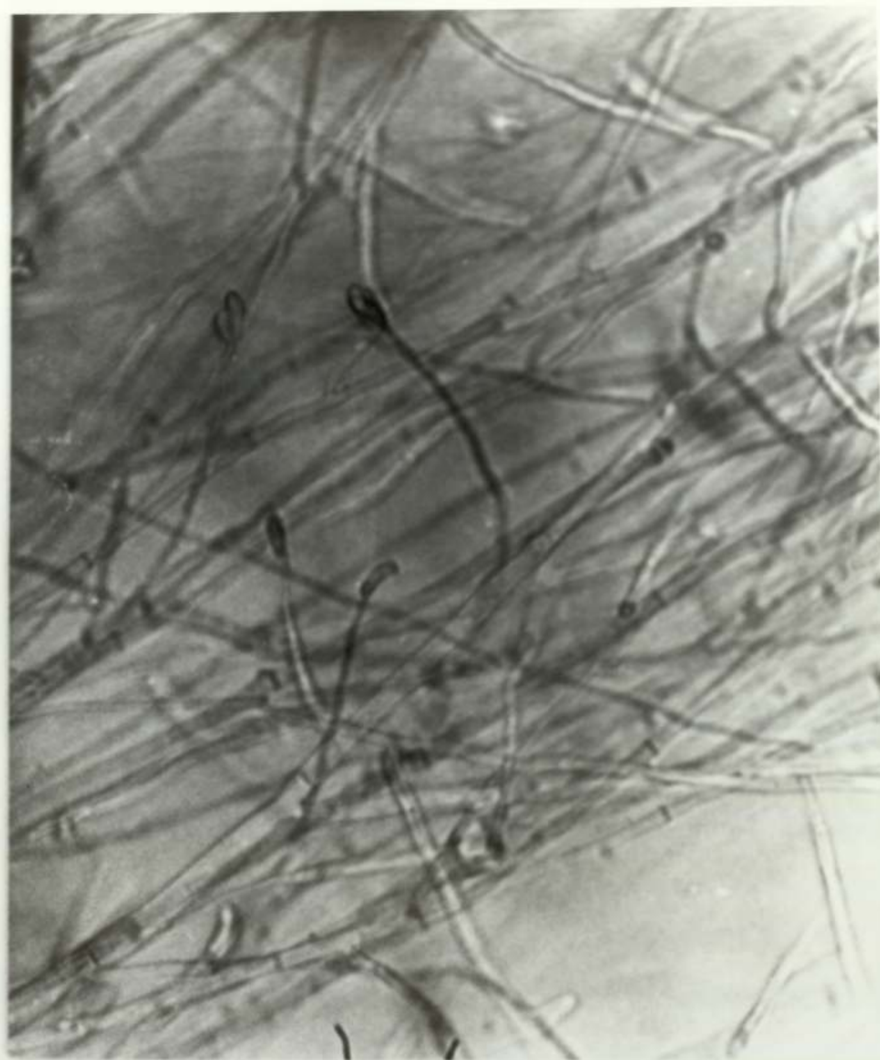


PLATE 5

Fusarium tabacinum

mycelium and conidia as seen in plate culture.

(hyphal diameter 3 - 4 µm.)

suspended vertically down which sewage or whey (or both) were trickled to simulate a conventional stone filter in operation. He used the apparatus to investigate the effects of different volumetric and organic loads on the thickness of the microbial film related to the efficiency of B.O.D. removal and contact time.

Experimental apparatus incorporating the use of vertical screens to investigate the effects of temperature, nutritional requirements (provided by the feed liquor) and dosing frequency on the growth rate of film in percolating filters was described by Green et al. in 1965. The apparatus, which was designed by Hawkes, was housed in four temperature-controlled cabinets and consisted of a set of six stainless-steel screens suspended vertically from a supporting framework by long stainless-steel springs. Each screen was provided with a ten-litre polythene bottle which contained enough sewage for a twenty-four hour supply. The sewage from the header tank was metered to the screens through pipettes which were successively filled and emptied by a system of kinking-tube valves activated by a reciprocating electric motor operating on a timed cycle. (later referred to as a multiple dosing unit). Full details of the apparatus have been given by Green et al. (1965).

Initially the experimental continuous culture apparatus used in this study was based on that used by Green et al. (1965). The apparatus was constructed in a temperature-controlled room with thermograph charts employed to continually monitor the room temperature. A metal framework supported a multiple dosing unit, six horizontal plastic screens and a small plastic tank (34 litre capacity) containing a float-switch. A large plastic tank (205

litre capacity), fitted with a plastic tap near the base for easy drainage, was placed to one side below the small header tank with a peristaltic pump between the two tanks. Silicone rubber tubing (8mm. diameter) was used to connect the large tank with the small one via the peristaltic pump. The dosing unit was designed to deliver approximately 9 ml. of liquid to each screen every 2½ minutes. As the liquid in the header tank was used it was replenished from the large storage tank below by means of a peristaltic pump activated by a float-switch in the header tank.

In the first experimental trials with this apparatus the six screens were made of black plastic-mesh squares (Netlon type STR 7001), 100 cm^2 in area of 4 mesh/cm. The screens were each suspended from the framework, supporting the dosing unit, by metal clips attached to centrally-placed metal rings which, in turn, were tied by nylon thread from each corner of the screen. The dosing tubes were positioned in the centre of each metal ring so that the liquid could drip in the centre. The liquid used throughout this study was skimmed milk reconstituted from spray-dried skimmed milk powder (Unigate Foods Ltd.)

TABLE 3.1

Comparison of the nutritional composition of reconstituted skimmed milk with fresh milk. (Figures in grammes per litre)

	Skimmed milk	Fresh milk
Fat	1.1	39.1
Protein	35.9	34.0
Carbohydrate	52.8	49.4
Calories (approx)	352	669

100 g/l. skimmed milk = concentration of fresh milk

It can be seen from Table 3.1 that skimmed milk contains considerably less fat than fresh milk, but it is realistic to use

skimmed milk because the majority of dairy effluent treatment plants use fat traps before the filtration stage resulting in a fat-reduction in the waste-water to be treated. The instructions for reconstituting the skimmed milk powder indicated that 100g. /litre was equivalent to the concentration of fresh milk. It was necessary to use realistic milk strengths in these experiments to simulate the waste waters from dairies, such as those described in Chapter 2, and the chemical oxygen demand (C.O.D.) test was employed for this purpose, (see methods in Chapter 4). The results are obtained in a few hours making the test considerably faster than the five days needed for measuring the biochemical oxygen demand (B.O.D.). It was found that a concentration of 5g/l. of milk powder in water resulted in a C.O.D. of approximately 5000, this figure was used as the upper limit liable to occur at a typical dairy and was used initially to encourage the growth of the fungi chosen for screening.

The fungi assayed in this screening procedure were Geotrichum candidum and Fusarium tabacinum, both cultured on potato dextrose agar at 20°C. When both fungi had produced sufficiently large colonies, discs of agar and fungus were cut aseptically with a cork-borer (7mm. diameter) and transferred to flasks containing 100ml. of skimmed milk at a concentration of 5g/l. The flasks were placed on an orbital shaker at 132 r.p.m. for five days at 15°C, in a temperature-controlled cabinet, to acclimatize the fungi to the milk medium and the lower temperature.

After five days the fungal discs were harvested and three discs of each fungus were placed centrally on the horizontal square plastic screens. The screens were dosed with skimmed milk (5g./l.) and the excess liquid was collected in a section of plastic gutter which drained into a sink. The temperature-controlled room which

housed the apparatus was set at 15°C. The skimmed milk was not sterilized (by autoclaving) in this study because the continuous culture apparatus was supposed to simulate a full-scale treatment plant and aseptic procedures on a large-scale are too expensive. It was hoped that bacterial contamination could be considerably reduced by lowering the pH of the milk and a pH of 5 was used initially, achieved by the addition of a predetermined amount of 0.5M sulphuric acid to the milk in the large tank, The apparatus was in operation for six weeks, during which time several problems were encountered which eventually caused the apparatus to be re-designed. The screens, although small, were not rigid enough to resist tilting when milk solids accumulated and, as a result, certain areas dried out. Stiff plastic-coated wire was threaded through the peripheral mesh of each screen to prevent the tilting but this was to no avail. The other major problem involved the multiple dosing unit and its associated pipes and tubes which became blocked with milk solids. Bacteria in the milk lowered the pH sufficiently to cause precipitation of the casein fraction which ultimately spread through the system (as the doser functioned each day). The tanks and dosing unit tubes and pipes were cleaned on alternate days but the latter took so long to clean and re-assemble that it was decided to abandon this dosing system in favour of a multi-channel peristaltic pump.

The final version of the continuous culture apparatus can be seen in Plate 6 and Fig. 3.1. The multiple dosing unit was replaced by a multi-channel peristaltic pump (Watson-Marlow MHRE 22 Delta) fitted with an extra module to accomodate a total of six 5mm. diameter tubes. The tubes were silicone rubber, which reached the bottom of the large tank on one side of the pump, and were held in place above each screen, on the other side of the

PLATE 6

PLATE 6

Continuous culture apparatus and six vertical screens at 15°C with fungal biomass after 65 days growth.

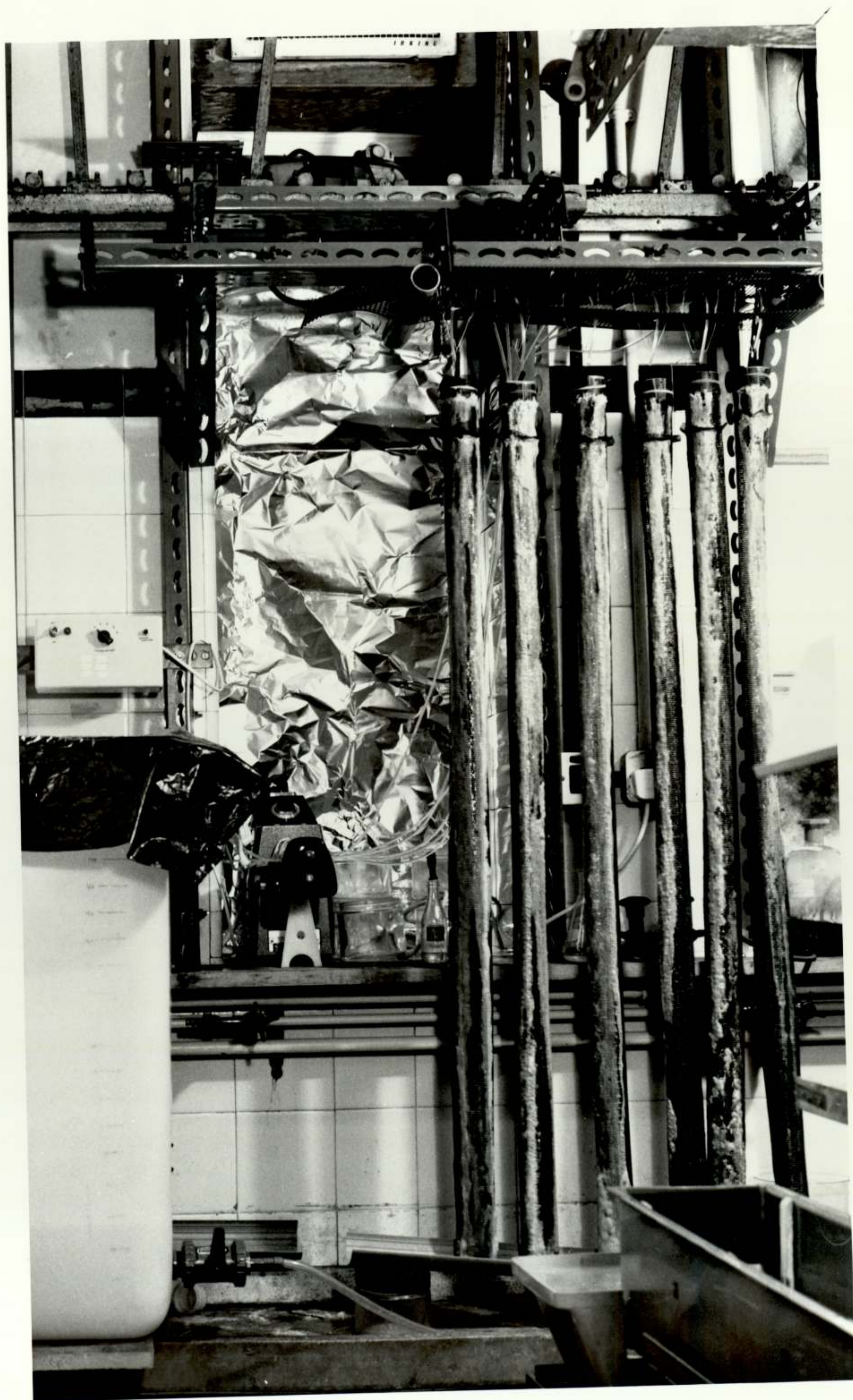
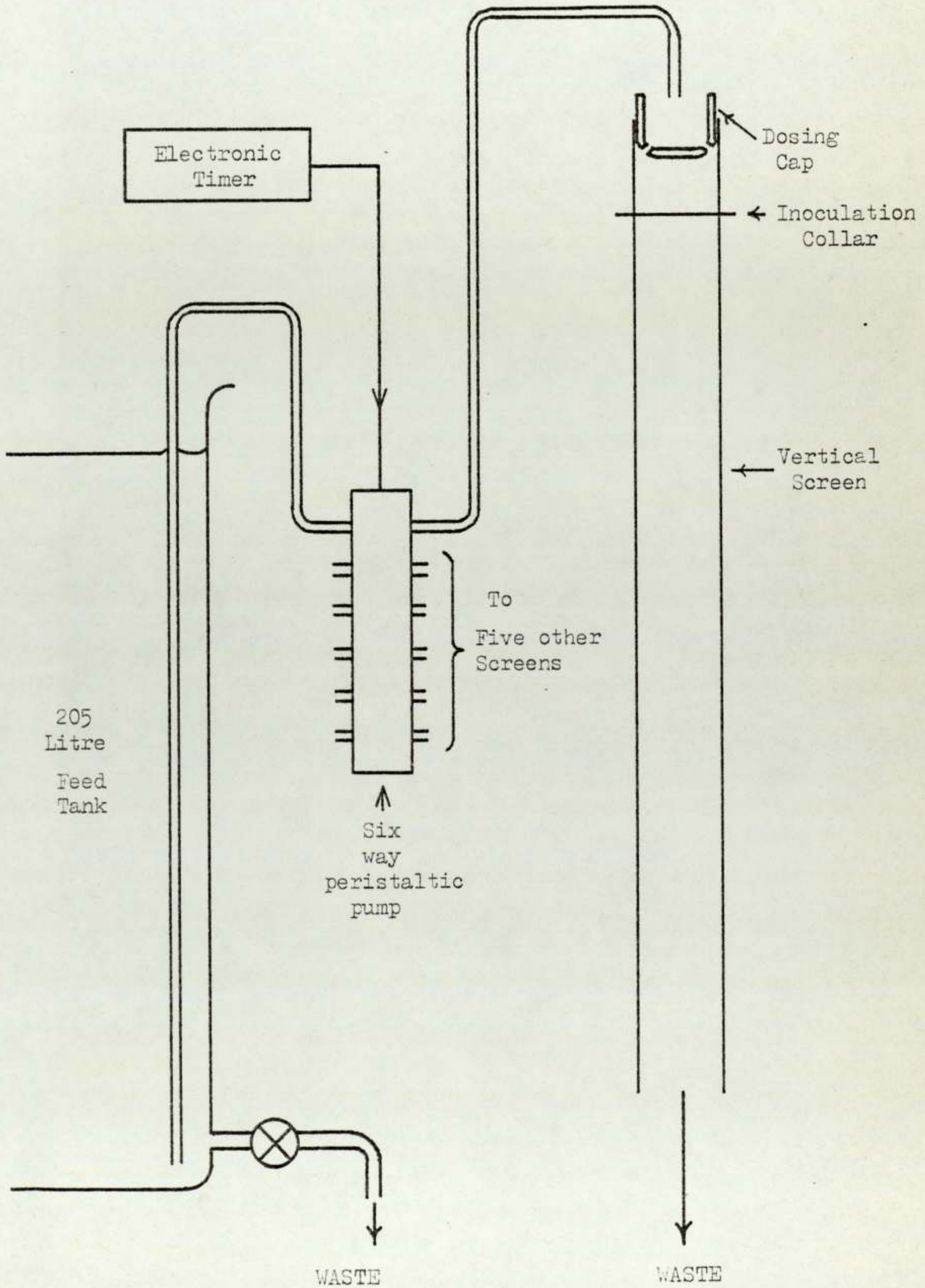


FIG. 3.1 Diagram to show the layout of the continuous culture apparatus as seen in Plate 6 (not to scale)



pump, by being pushed through a double layer of coarse plastic-mesh. The screens themselves were made from the same material as the horizontal screens, 4 mesh/cm., made into cylinders 1.2m. long with a total surface area of $0.4m^2$. The flat mesh rectangles were sewn to form the cylinders with nylon fishing line (6lb. breaking strain) except for each top which housed a grey plastic cap of 3.2 cm. diameter, held in place by an elastic band. Each grey cap was drilled with two rows of holes, 3mm. in diameter and 7mm. apart, to allow an even distribution of milk down each vertical screen. A pair of holes were drilled on the opposite sides of each cap, near to the top, and fishing-line threaded through to form a loop. The loops were held clear of the supporting frame-work by metal clips and each screen could be removed from the clips and hung by its loop on to a spring balance for weighing purposes. A length of plastic gutter was placed below the screens to transport excess liquid to the sink. The small plastic tank and float switch were not needed in this re-designed apparatus but in order to obtain a controllable flow of liquid over the screens the peristaltic pump was operated in a timed cyclical mode. This was achieved using a National Semiconductor Integrated Circuit LM 555 (Electronics Today International 1975) in the astable mode to control a relay which, in turn, operated the peristaltic pump. The flow-rate was adjusted using a potentiometer so that the screens were maintained in a moist condition and allowed the feeder tank to be re-filled at a sensible time interval, (usually on alternate days). Skimmed milk was made up with a concentration of 5g./l. in a clean plastic bucket, the contents were poured into the large tank and tap water was added until the required volume graduation on the tank was reached. The tank was then covered with a sheet of black polythene to prevent dust and other contaminants from

entering.

In the initial trials with this new apparatus, the screens were inoculated with flask-cultured agar discs of fungus tied to the top of each screen below the dosing cap. This method was difficult to implement and so collars were made of the same mesh as the screens. These collars were circles of plastic mesh with a central aperture through which the screens were pushed until each collar was positioned below the dosing caps. The collars tended to fold upwards and gave additional support to the agar discs of fungus which were placed at intervals between the collars and the screens. Three screens were inoculated with Geotrichum candidum and three with Fusarium tabacinum, When the pump and timer were switched on and the milk delivered to each screen the fungal discs were thoroughly wetted and eventually the hyphae grew out of the discs and down the screens as seen in Plate 6.

The apparatus worked successfully and the cleaning procedure was much easier because the dosing system was simple. The tubes were cleaned by removing them from the screens and passing 5% ($^{w}/v$) sodium hydroxide through them to remove any milk solids. Water was then passed through the tubes to remove all traces of alkali and the tubes were returned to each screen and a fresh supply of skimmed milk was delivered from the clean tank. The addition of 0.5M sulphuric acid was used to adjust the pH of the milk to 5.5 which was found by experience to allow a few days operation before curdling occurred.

3.4 NUTRITIONAL ANALYSIS OF THE SCREENED FUNGI

The apparatus described in section 3.3 was used continuously for a period of nearly seventy days, during which time frequent wet weight measurements were taken to monitor the progress of the

fungi in the hope that there would be sufficient biomass for a detailed analysis. It became clear that there would not be enough fungal material to perform all the analytical tests, hence the harvesting after almost ten weeks. The growth was removed from each screen initially by scraping with a rod and finally by soaking in a large plastic cylinder of water. The wet fungal growth was bulked into two large samples of the two fungi, placed in open petri-dishes covered with gauze and freeze-dried. The wet fungal growth weighed approximately 1400 gm. in each case but after freeze-drying this was reduced to less than 50 gm. The freeze-dried fungi were parcelled and dispatched to I.C.I. Agricultural Division at Billingham where analyses of the nucleic acid content, fatty acid content and the important amino acid profile of both fungi were carried out. The results of the amino acid composition of Geotrichum candidum (Isolate No. 11) and Fusarium tabacinum (Isolate No. 16) are given in Table 3.2, together with the amino acid profiles of other fungi and protein concentrates assembled from the literature.

Table 3.2 shows that although Isolates 11 and 16 have less of each amino acid than the hydrocarbon-grown yeast, fishmeal and soya bean meal, they compare favourably with the other profiles quoted. Wasserman (1960), Robe (1964) and Shacklady (1969) all claim that their isolates have been successful in animal feeding trials. Olsen et al. (1977) states that their isolate of Geotrichum candidum produced mycelium with a high protein content and good amino acid composition when cultivated on sugar factory waste-water. Olsen et al. (1977) listed seven essential amino acids of G.candidum and their figures are very similar to those of G.candidum (Isolate No. 11) of this study.

TABLE 3.2 The amino acid composition of *Saccharomyces fragilis*, protein concentrates, fishmeal, soya bean meal and an isolate of *Geotrichum candidum* compared with that of Isolate No. 11 (*Geotrichum candidum*) and No. 16 (*Fusarium tabacinum*) screened in this study. (Each amino acid is expressed as a percentage of the total amino acids analysed).

	S. fragilis in whey Wasserman (1960)	"Wheast" Whey-grown yeast and milk albumin Robe (1964)	Hydrocarbon-grown yeast	Fishmeal	Soya bean meal	Geotrichum candidum grown in Sugar Factory waste-water Olsen et al. (1977)	Geotrichum candidum (Isolate 11) grown in skimmed milk	Fusarium tabacinum (Isolate 16) grown in skimmed milk
			Shacklady - (1969)					
Alanine	8.78						7.93	7.87
Arginine	7.61	2.63	9.65	10.57	15.16		4.96	5.07
Aspartic acid							10.12	10.85
Cystine			1.74	2.11	2.75			
Glutamic acid	14.25						15.69	15.32
Glycine	4.98	2.49					4.39	4.40
Histidine	1.01	1.34	4.05	4.86	4.72		2.59	2.52
Isoleucine	6.45	2.99	10.23	9.72	10.63	5.0	4.82	5.28
Leucine	10.32	4.41	15.06	15.43	15.16	6.9	8.88	8.45
Lysine	10.96	4.26	15.06	14.79	12.79	8.3	7.55	7.65
Methionine	1.34	0.85	4.83	5.49	2.75	2.0	2.25	1.50
Phenylalanine	5.79	2.11	9.27	8.46	10.04	*	4.74	4.84
Proline	4.63						5.20	5.13
Serine	7.48	2.23					4.91	4.91
Threonine	6.94	3.00	10.42	8.88	7.87		5.23	5.21
Tyrosine	3.68	1.80	7.72	6.13	5.31	*	4.70	4.72
Valine	8.36	3.59	11.19	10.99	9.83	6.0	6.04	6.30

* combined value = 8.7

The conclusion was reached that Geotrichum candidum (Isolate No. 11) and Fusarium tabacinum (Isolate No. 16) screened for their nutritional value, as judged by their amino acid profiles, were suitable for use as animal feed components, probably requiring a supplement of the sulphur amino acid methionine to increase their biological value in feeding tests. Further investigations of the growth requirements of the two fungi are performed in Chapter 4.

CHAPTER FOUR

LABORATORY STUDIES

CHAPTER FOUR

LABORATORY STUDIES

4.1 INTRODUCTION

Geotrichum candidum Link (Isolate No.11) and Fusarium tabacinum (Beyma) W.Gams. (Isolate No. 16) were screened for their nutritive value (Chapter 3) and found to have amino acid profiles comparable with several sources of protein (Table 3.2) including a number of commercial preparations. The results of the screening procedure were sufficiently encouraging to proceed to laboratory investigations to determine the optimum growth conditions for G. candidum and F.tabacinum.

4.2 MATERIALS AND METHODS

The laboratory studies involved the use of several different methods of fungal culture, the general methods used in the pure culture studies are described in this section with further details given in the relevant sections dealing with the different investigations.

Microorganisms

The fungi used in these studies were: Geotrichum candidum Link (Isolate No. 11), Fusarium aquaeductuum Lagh. (Isolate No. 14) and Fusarium tabacinum (Beyma) W. Gams (Isolate No. 16).

Stock culture maintenance

Stock culture of G.candidum, F.aquaeductuum and F.tabacinum were maintained on corn meal agar slants at 4°C and transferred to fresh slants at four-month intervals.

Preparation for culture

An inoculum was prepared by aseptically transferring loops of fungal growth from the agar slants to 9 cm. Petri dishes containing 20 cm^3 of agar. G.candidum was transferred to plates of Potato

Dextrose Agar (P.D.A.) and S.F. agar (S.F.), formulated by Hawkes (1965) and composed of 1% peptone, 1% glucose, 0.1% yeast extract and 1.5% agar (Oxoid No. 3) with the pH adjusted to 6.8 prior to autoclaving at 15 p.s.i. for 15 minutes. F.aquaeductuum and F.tabacinum were transferred to plates of Potato Dextrose agar (P.D.A.) and Czapek-Dox agar (C.Z.) which were both obtained from Oxoid Ltd. Each Petri dish was inoculated at its centre and incubated at 20°C, inocula for the various investigations were obtained from these initial cultures by cutting 7mm. diameter discs from the vegetative margin after four to six days. The discs were cut aseptically with a cork-borer to provide inocula of as similar size as possible.

Analytical methods

Biochemical Oxygen Demand

The biochemical oxygen demand test (BOD test) was performed using the Dilution Method which is fully described in "Analysis of Raw, Potable and Waste Waters", 1972. The BOD test was devised by a member of the Royal Commission on Sewage Disposal as a measure of the oxygen absorbed by a sample of river water containing a polluting effluent during five days at 20°C. Biochemical oxidation of the polluting organic matter by microorganisms in the watercourse eventually results in purification and the BOD test gives an indication of the "strength" of the polluting substance as follows. The initial dissolved oxygen content of the sample is determined prior to incubation in the dark, to avoid complications due to algal photosynthesis, and repeated after five days. The difference between the dissolved oxygen measurements enables the BOD to be calculated. Samples often have to be diluted before performing the test and the dilution factor and the oxygen demand of the diluent must be allowed for in the calculations, the results being expressed as mg. oxygen per litre of sample.

Chemical Oxygen Demand

The Chemical Oxygen Demand (COD) test is faster to perform than the BOD test since the results are obtained in hours rather than days. This test was carried out as described in "Analysis of Raw, Potable and Waste Waters", 1972. The sample under test is boiled under reflux with potassium dichromate and a silver sulphate catalyst in concentrated sulphuric acid which results in a much greater degree of oxidation of organic matter than with the BOD test. The organic matter in the sample reduces part of the dichromate and the residual dichromate is titrated with ferrous sulphate with the addition of one drop of ferrous 1:10 phenanthroline indicator. The COD can then be calculated from the results of the blank and sample titrations, making allowances for the dilution factor. The final result is given as milligrams of oxygen absorbed from standard dichromate per litre of sample.

4.3 SPECIFIC INVESTIGATIONS AND RESULTS

4.3.1 The effect of temperature on the growth of *Geotrichum candidum*, *Fusarium aquaeductuum* and *Fusarium tabacinum* in pure culture.

Geotrichum candidum

The direct effect of temperature on growth was initially investigated using *G.candidum*. Sterile Petri dishes of 9 cm. diameter, each containing 20 ml. of S.F. agar were inoculated centrally with discs of fungus as described in section 4.2. Plates were incubated at different temperatures from 5°C to 35°C, achieved by using a refrigerator for 5°Cand incubators for the other temperatures. As part of these tests a comparison was made between growth in incubators and water-baths at 25°C, 30°C and 35°C. For this purpose inoculated plates were placed in cylindrical brass canisters (Astell Ltd.) used for

bacteriological studies, fitted with a screwed lid housing a ventilation tube. The top of the tube was plugged with cotton-wool and the canisters were placed in stirred water-baths (Grant Instruments Ltd.) immersed up to the base of their screwed lids. The incubators and water-baths were checked for their accuracy and reliability in maintaining a set temperature by taking thermometer readings daily for a week preceding and during the investigation.

Three replicate plates of G.candidum were used for each temperature and the colony diameters, measured at twenty-four hour intervals, were taken as the mean of three diameters recorded to the nearest half-millimetre. These measurements were recorded in Table 6.1 and expressed graphically in Fig. 4.1.

RESULTS

The results of this study showed that G.candidum was capable of growth over a wide range of temperatures with an optimum between 20°C and 30°C. Growth of the colonies in the water-baths indicated a higher growth-rate than achieved by the incubated colonies and this was analysed statistically using the students "t" test. (Bishop 1971) in Table 4.1.

The "t" test is used to compare the means of two small samples, "t" is not normally distributed since its variance depends upon sample size and a special "t" table has been formulated.

$$t = \frac{\text{deviation from a mean}}{\text{standard deviation}}$$

or

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\left(\frac{\Sigma (x-\bar{x}_1)^2 + \Sigma (x-\bar{x}_2)^2}{n_1 + n_2 - 2}\right)\left(\frac{n_1+n_2}{n_1 n_2}\right)}}$$

where $\Sigma(x-\bar{x})^2 = \Sigma x^2 - \frac{(\Sigma x)^2}{n}$

entering the t table at n_1+n_2-2 degrees of freedom

(n = number in sample)

FIG. 4.1 Geotrichum candidum:-

Effect of ambient temperature on growth (5°C - 35°C).

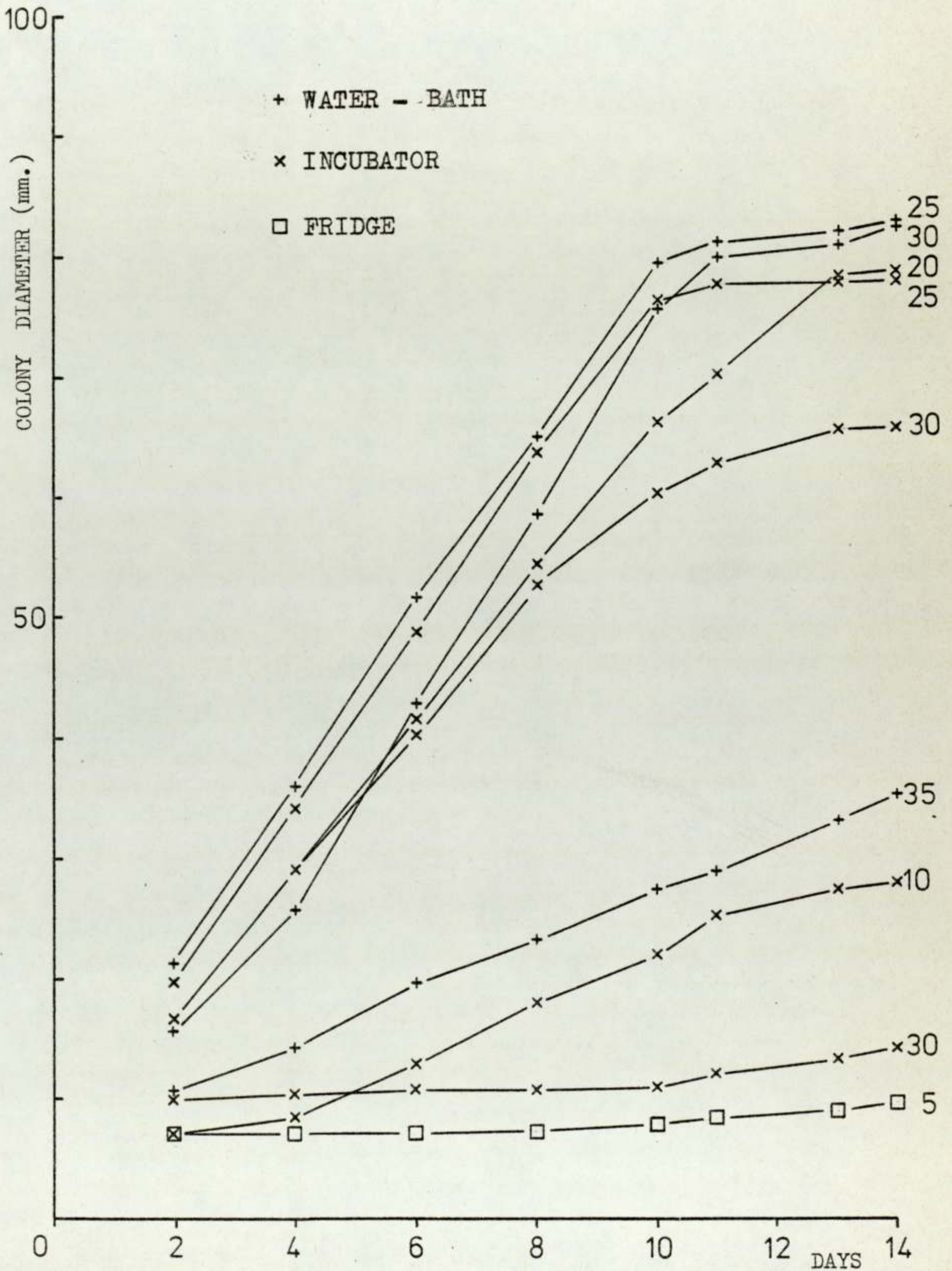


TABLE 4.1

Statistical analysis of data taken from TABLE 6.1

(Temperature-growth relationships of *Geotrichum candidum* in incubators (INC.) and water-baths (W.B.))

	20°C INC.	25°C INC.	30°C INC.	35°C INC.	25°C W.B.	30°C W.B.	35°C W.B.
Day 2	19.0	20.0	18.0	11.0	18.0	15.0	11.0
	15.0	19.0	18.5	10.0	16.5	14.0	11.0
	16.0	20.0	12.5	9.0	29.0	18.0	10.0
Mean	16.66	19.66	16.33	10.00	21.16	15.66	10.66
Std-deviation	2.08	0.57	3.33	1.00	6.83	2.08	0.57
Variance	4.33	0.33	11.08	1.00	46.58	4.33	0.33
Degrees of freedom		4	4			4	
't' value		2.41	1.71			1.34	
Degrees of freedom				4	4	4	
't' value				0.38	0.29	1.00	
Day 8	56.0	65.0	56.0	12.5	63.5	59.0	27.5
	51.5	64.5	53.0	10.5	62.5	57.5	24.0
	56.0	62.0	49.0	9.0	69.0	59.5	1.85
Mean	54.50	63.83	52.66	10.66	65.00	58.66	23.33
Std-deviation	2.60	1.61	3.51	1.76	3.50	1.04	4.54
Variance	6.75	2.58	12.33	3.08	12.25	1.08	20.58
Degrees of freedom		4	4			4	
't' value		5.29	5.00			3.00	
Degrees of freedom				4	4	4	
't' value				0.52	2.84	4.51	
Day 14	79.0	77.5	63.0	23.0	82.0	83.0	34.5
	79.0	78.5	67.0	11.0	83.0	82.0	40.0
	79.0	78.5	68.5	9.0	84.0	83.0	31.5
Mean	79.0	78.16	66.16	14.33	83.00	82.66	35.33
Std-deviation	0.00	0.58	2.84	7.57	1.00	0.58	4.31
Variance	0.00	0.33	8.08	57.33	1.00	0.33	18.58
Degrees of freedom		4	4			4	
't' value		2.50	7.16			0.50	
Degrees of freedom				4	4	4	
't' value				7.25	9.85	4.17	

When $p = 0.05$ $t = 2.78$ with 4 degrees of freedom

TABLE 4.2

Extract from Table 4.1 showing 't' values and their related significance (SIG.) in comparing fungal growth in incubators (INC.) and water-baths (W.B.)

COMPARISON OF CONDITIONS	't' VALUES		
	2 DAYS	8 DAYS	14 DAYS
20°C INC. v. 25°C INC.	2.41 NOT SIG.	5.29 SIG.	2.50 NOT SIG.
25°C INC. v. 30°C INC.	1.71 NOT SIG.	5.00 SIG.	7.16 SIG.
25°C INC. v. 25°C W.B.	0.38 NOT SIG.	0.52 NOT SIG.	7.25 SIG.
30°C INC. v. 30°C W.B.	0.29 NOT SIG.	2.84 SIG.	9.85 SIG.
35°C INC. v. 35°C W.B.	1.00 NOT SIG.	4.51 SIG.	4.17 SIG.
25°C W.B. v. 30°C W.B.	1.34 NOT SIG.	3.00 SIG.	0.50 NOT SIG.
When $p = 0.05$ $t = 2.78$ with 4 degrees of freedom			

In Table 4.1 the "t" test was applied to colony diameter data taken from Table 6.1 for Day 2, 8 and 14 of the investigation. The term p=0.05 refers to the chosen level of probability which implies that there is a 5% chance of the findings of a significant difference between the means of being untrue. (The level of 5% is generally regarded as sufficiently reliable for most biological purposes.) Table 4.2 shows that there was a significant difference between the colony diameter means in incubators and water-baths at 30°C and 35°C on Day 8 and 14, but this was not apparent until Day 14 at 25°C even though the difference was visible in Fig. 4.1. The colonies grown in the incubators and water-baths were examined both with the naked eye and microscopically (x150) to assess any morphological differences. The hyphal density did not appear to be affected by temperature when viewed at several different planes under the microscope. However, it was observed that the plates at the higher temperatures in the incubators were drying out and the difference between growths in the incubators and water-baths was probably due to humidity. This observation was further investigated.

Comparison of the effects of normal (humid) and dry conditions in water-baths

Geotrichum candidum inocula were prepared as described in section 4.2, 20ml. of S.F. agar was dispensed into 9 cm. Petri dishes and inoculated centrally with 7 mm. fungal discs. Three replicates were used for each temperature placed in the brass canisters in water-baths set at 25°C, 30°C and 35°C. The normal (humid) conditions were those of the last experiment, that is, plates placed in brass canisters with the cotton-wool plug inserted in the ventilation tube to prevent contaminants from entering. The dry conditions were

achieved by placing a dessicator between the top-most plate and cotton-wool plug thus drying out the incoming air. Each dessicator was made from the base of a 9 cm. Petri dish, filled with silica gel crystals and covered with muslin. The crystals changed colour from deep blue to white as they absorbed moisture and it was found necessary to replace the crystals daily at 30°C and 35°C and on alternate days at 25°C. The colony diameters were measured, at the same time (within one hour) each day, as the mean of three diameters recorded to the nearest half-millimetre. The results were recorded in Table 6.2 and expressed graphically in Fig. 4.2 which showed little difference between the humid and dry conditions. The "t" test was applied to the data from Table 6.2 from Day 2, 7 and 14 and recorded in Table 4.3. There was found to be no difference at the 5% level of significance between the normal (humid) and dry conditions imposed on the fungal colonies. Colony morphology and hyphal density were not affected by the different conditions of humidity, any differences were related to temperature, both sets of plates at 35°C having much smaller colony diameters than at 25°C and 30°C. There was a noticeable lack of condensation in the "dry" plates when compared to the "humid" ones so it can be assumed that the dessicators were having some effect on the incoming air. However, the lack of difference between the two conditions may have been due to the dessicators being too small and requiring more frequent crystal changes than given. The agar depth in the "dry" plates was unchanged after fourteen days in marked contrast to that of the incubated plates of the previous study (section 4.3.1). It was decided to use water-baths for all future temperature studies because they maintained a set temperature with minimum fluctuation, the agar depth was unchanged after fourteen days and the humid

FIG. 4.2 Geotrichum candidum:-

Comparison of the effects on growth of normal (humid) (×) and dry conditions (+) in water baths.

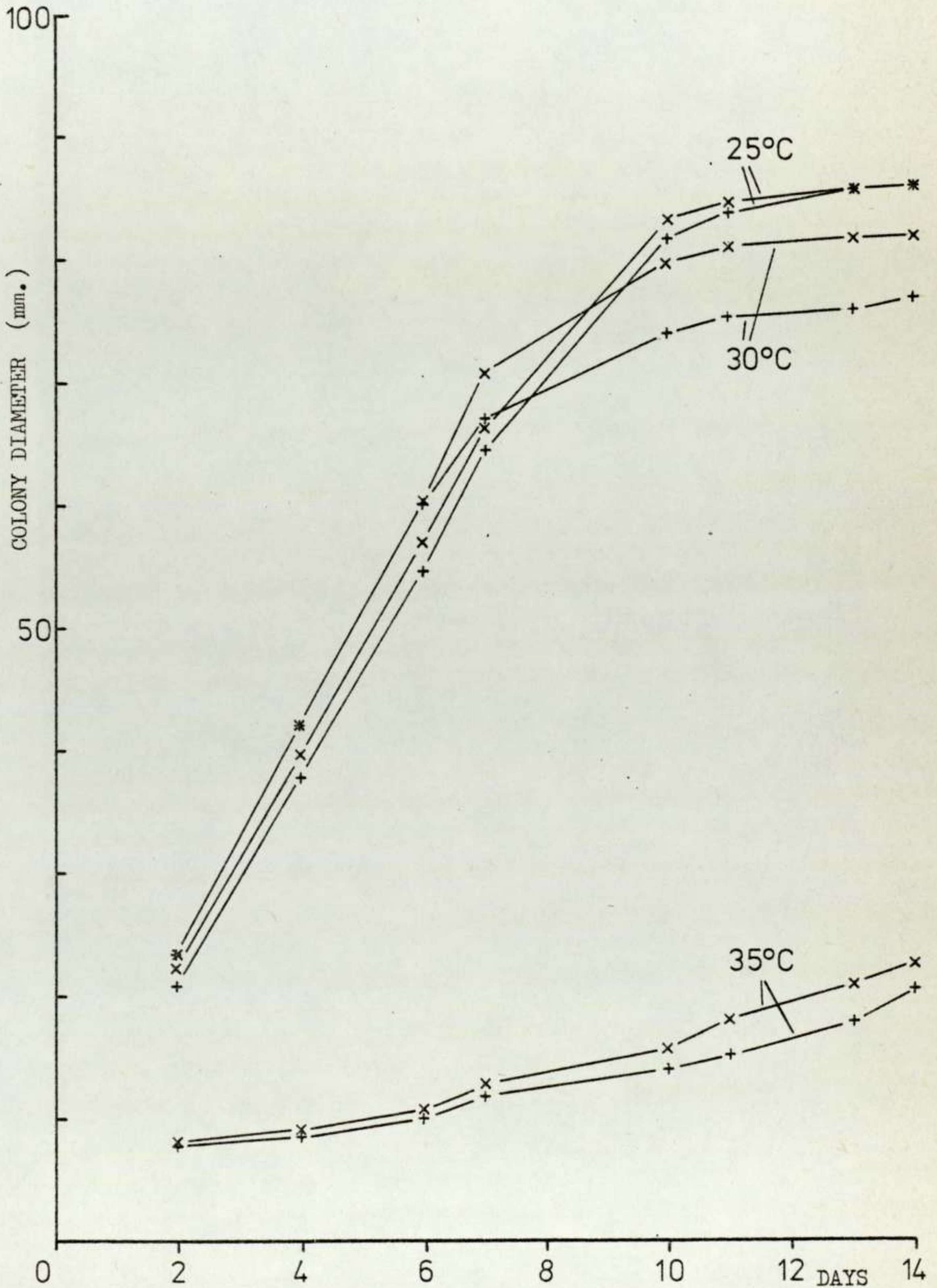


TABLE 4.3

Statistical analysis of data taken from TABLE 6.2

(Temperature - growth relationships of <u>Geotrichum candidum</u> under normal and dry conditions in water-baths)

	25°C NORMAL	25°C DRY	30°C NORMAL	30°C DRY	35°C NORMAL	35°C DRY
Day 2	21.5	20.0	24.0	25.5	8.0	8.0
	24.5	21.0	22.0	23.5	8.0	8.0
	21.0	21.5	24.5	21.5	8.0	7.5
Mean	22.33	20.83	23.50	23.50	8.00	7.83
Std-deviation	1.89	0.76	1.32	2.00	0.00	0.29
Variance	3.58	0.58	1.75	4.00	0.00	0.08
Degrees of freedom	4		4		4	
't' value	1.27		0.00		1.00	
Day 7	66.0	64.5	72.0	72.5	13.5	13.5
	68.0	64.0	71.0	61.0	13.0	11.5
	65.0	65.0	69.0	67.5	12.0	10.5
Mean	66.33	64.50	70.66	67.00	12.83	11.83
Std-deviation	1.53	0.50	1.53	5.77	0.76	1.53
Variance	2.33	0.25	2.33	33.25	0.58	2.33
Degrees of freedom	4		4		4	
't' value	1.98		1.06		1.00	
Day 14	86.0	86.0	77.5	85.0	26.0	23.0
	86.0	86.0	85.0	62.5	22.0	22.0
	86.0	86.0	83.0	81.5	20.0	16.5
Mean	86.0	86.0	81.83	76.33	22.66	20.50
Std-deviation	0.00	0.00	3.88	12.11	3.06	3.50
Variance	0.00	0.00	15.08	146.56	9.33	12.25
Degrees of freedom	4		4		4	
't' value			0.75		0.81	

When p = 0.05 t = 2.78 with 4 degrees of freedom

conditions were more realistic considering that the fungi under investigation were isolated from an aquatic environment.

Fig. 4.1 of the initial investigation, involving incubators and water-baths, showed that the optimum temperature for G.candidum lay between 10°C and 35°C. Using 9cm. Petri dishes, containing 20ml. S.F. agar inoculated centrally with 7mm. discs of fungus, G.candidum was subjected to three further investigations of the effect of temperatures from 15°C to 33°C on growth. An upper limit of 36°C was also used for this fungus since some pathogenic strains are capable of good growth at 37°C (human body temperature). The results were recorded in triplicate in Tables 6.3 to 6.5 and expressed graphically in Figs. 4.3 to 4.5 as the mean of three colony diameters measured to the nearest halfmillimetre every twenty-four hours. The graphs showed the optimum temperature for growth of G.candidum to be 29°C but this was only marginally better than 28°C and 31°C. As far as Figs. 4.3 to 4.5 are concerned, the optimum temperature for the growth of G.candidum lies within the range of 27.5°C to 31°C.

From Figs. 4.1 to 4.5 it was apparent that the linear phase of extension of the growth of G.candidum occurred between Day 1 and Day 8. It was decided to calculate the actual growth rate of G.candidum from 5°C to 36°C using the colony diameter data from Tables 6.1 to 6.5 applied to the method of regression analysis. Linear regression analysis is used to find the equation of the closest line to the points on the graph representing an estimated linear distribution. The best straight line through a set of points always passes through $\bar{x},\bar{y}$ (called the mean centre of the distribution).

The equation for the best straight line is:

$$y = \bar{y} + b\,(x - \bar{x})$$ (Bishop, 1971)

FIG. 4.3 Geotrichum candidum:-

Effect of ambient temperature on growth ($15^{o}C$ - $27.5^{o}C$).

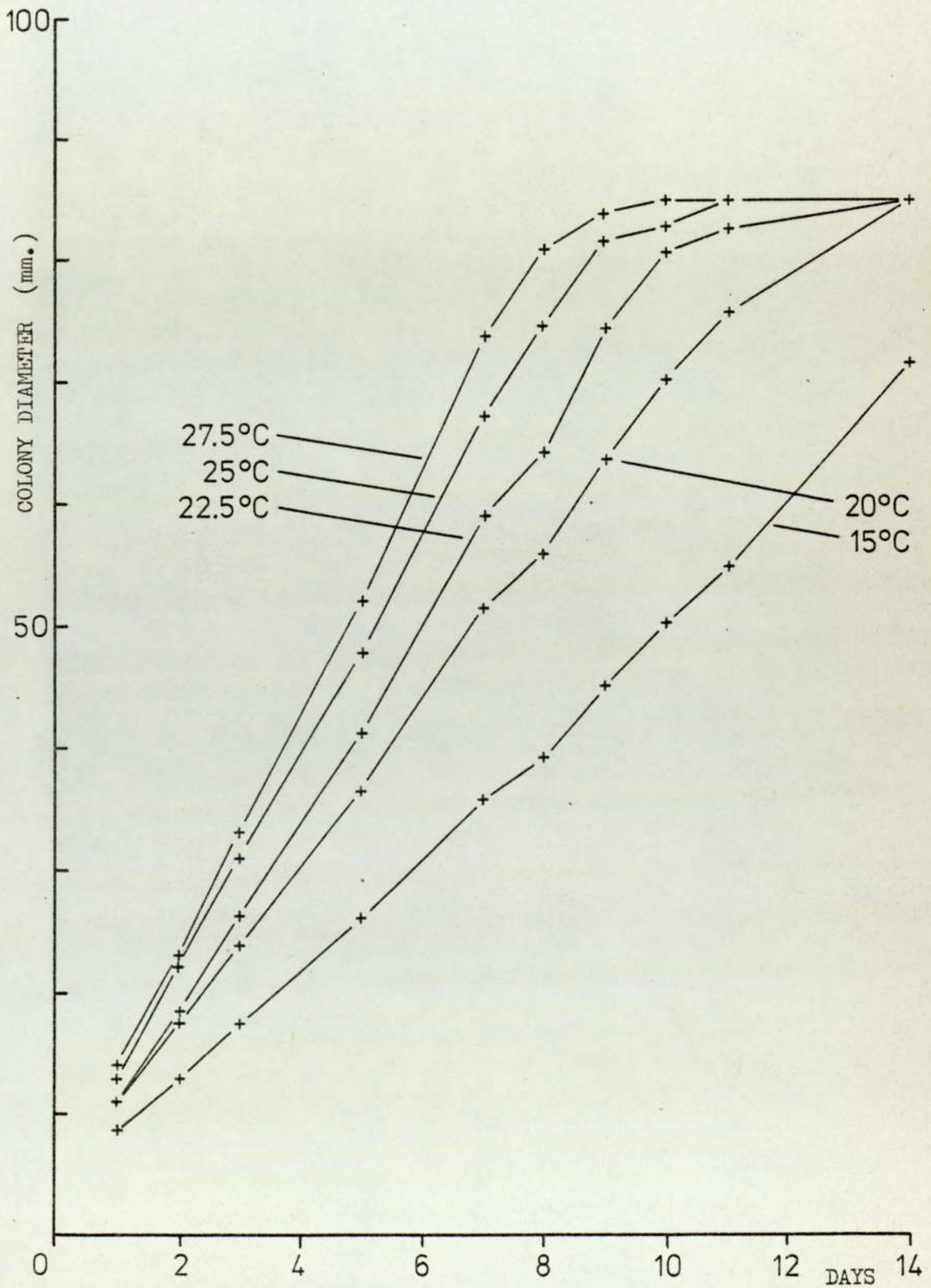


FIG. 4.4 Geotrichum candidum:-

Effect of ambient temperature on growth (25°C - 36°C).

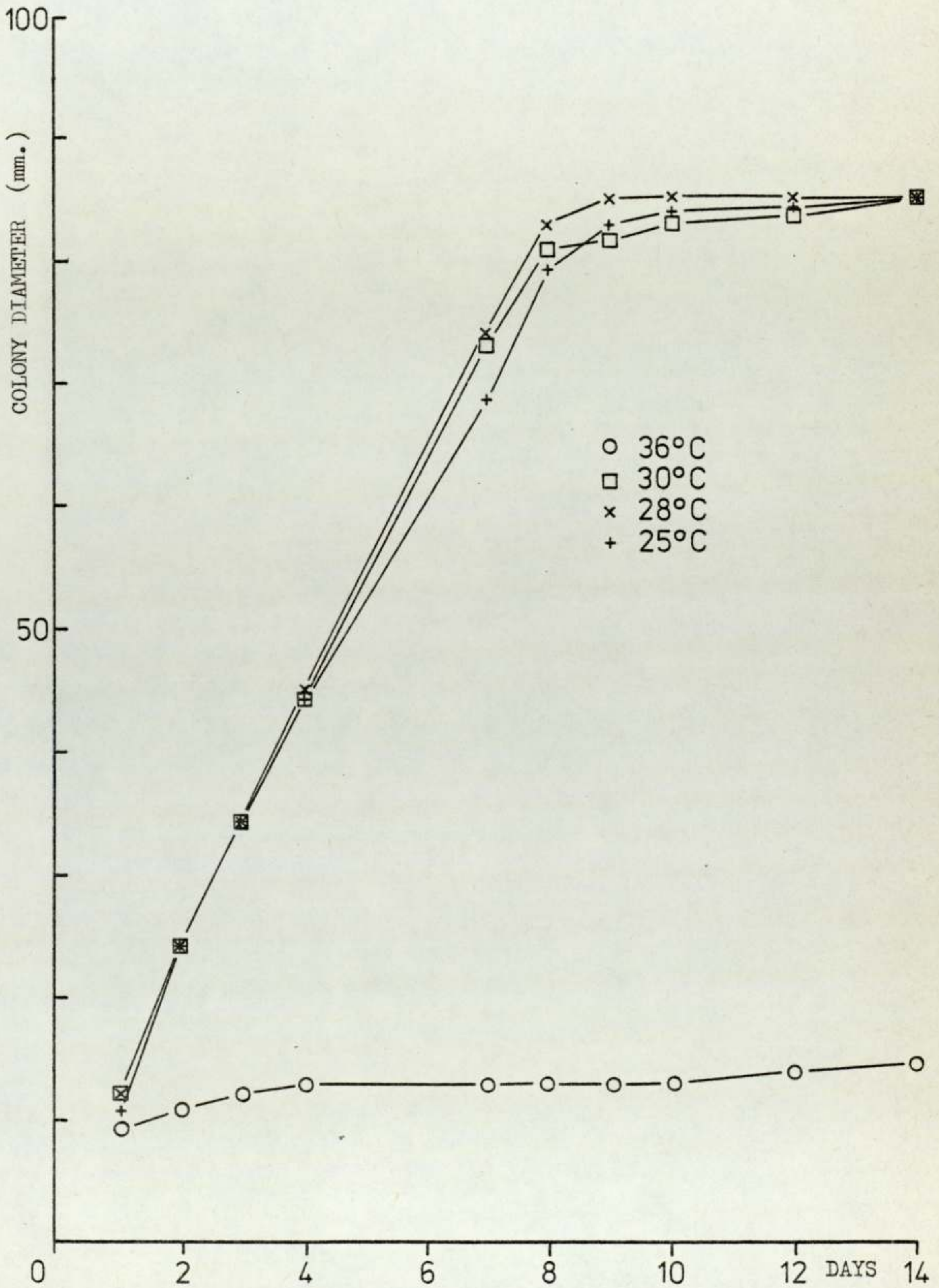
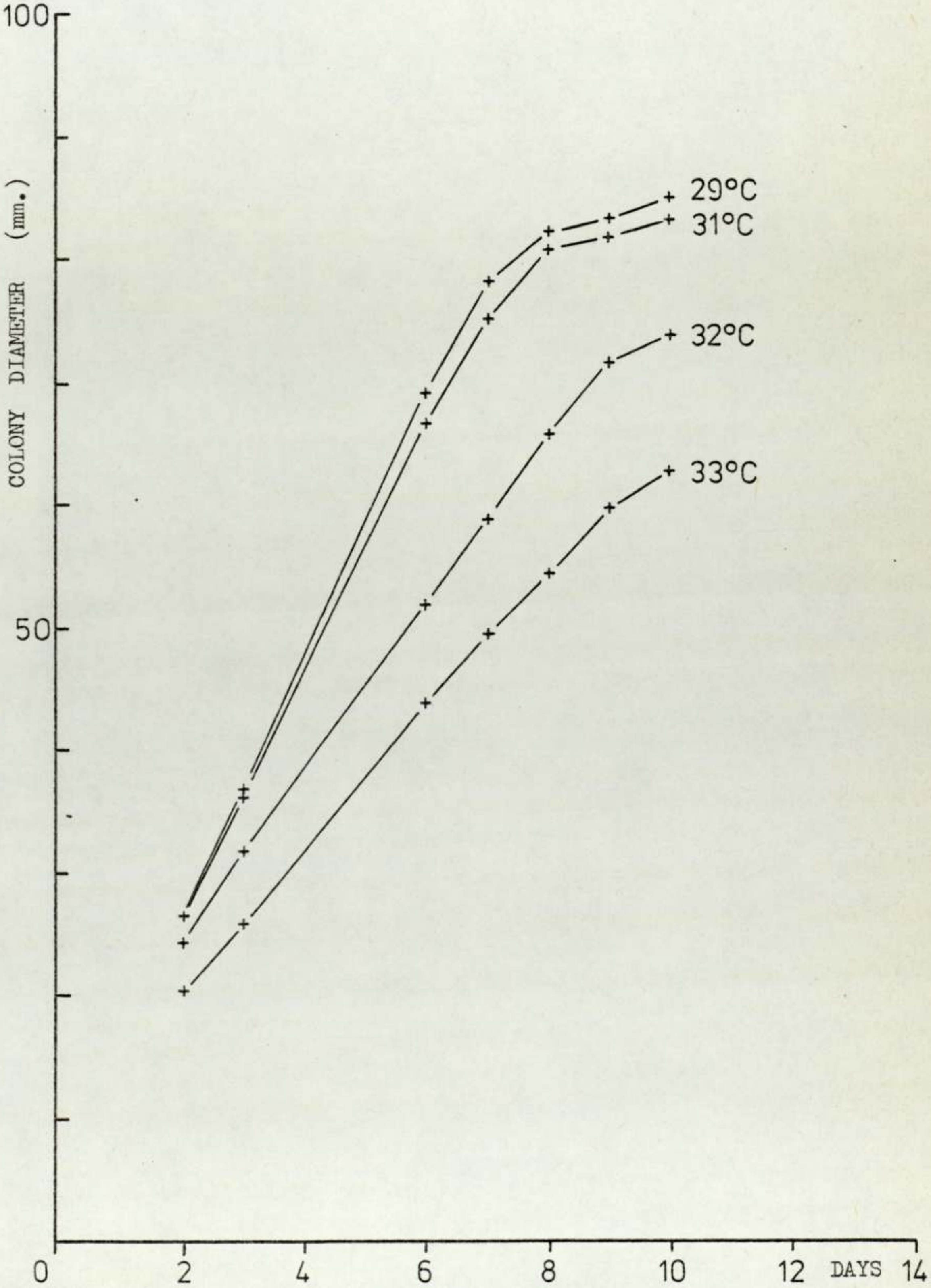


FIG. 4.5 Geotrichum candidum:-

Effect of ambient temperature on growth (29°C - 33°C).



The regression coefficient b is the slope of the line and is given by:

$$b = \frac{\sum (x-\bar{x})\ (y-\bar{y})}{\sum (x-\bar{x})^2} \qquad \text{(Bishop, 1971)}$$

The slope or gradient, b, was calulated, from the formula, for each temperature-growth curve of G.candidum recorded in Table 4.4 and expressed graphically in Fig. 4.6.

Certain temperatures were used more than once in this study hence a range of slopes was obtained and plotted in Fig. 4.6. However, the poorest growth rates as determined by the slope of the line were obtained in incubators at 25°C, 30°C and 35°C, the remaining data were obtained in water-baths with much less variation. Fig. 4.6 confirms mathematically the visual findings of Figs. 4.3 to 4.5 that G.candidum is capable of growth from 10°C to 36°C with an optimum temperature for growth at 28°C.

Fusarium aquaeductuum

The same procedure for determining the temperature range and optimum temperature was adopted for F.aquaeductuum as for Geotrichum candidum 20 cm^3 of Potato Dextrose Agar (PDA) were dispensed into 9 cm. Petri dishes and each dish was inoculated at its centre with 7 mm. discs cut from the vegetative margin of a seven-day colony growing on PDA. Three replicate plates were used for each temperature, the plates were placed in brass canisters in stirred water-baths using a temperature range from 5°C to 35°C. Growth was assessed by measuring the colony diameters, at twenty-four hour intervals, taken as the mean of three diameters to the nearest half-millimetre. The results of these daily measurements are recorded in Tables 6.6 and 6.7 and expressed graphically in Figs. 4.7 and 4.8

Fig. 4.7 showed that F.aquaeductuum was capable of growth between

TABLE 4.4

Geotrichum candidum:- Colony diameters measured during the first eight days of growth (taken from TABLES 6.1 - 6.5) and the corresponding growth rates (SLOPES) calculated from these diameters

TEMP. °C	COLONY DIAMETER (mm.) DAYS FROM START OF EXPERIMENT 1	2	3	4	5	6	7	8	SLOPE mm/day
5		7.0		7.0		7.0		7.3	0.05
10		7.0		8.5		13.0		18.0	1.88
15	8.5	12.8	17.4		26.0		35.9	39.1	4.45
20		16.7		28.8		41.5		54.5	6.31
	10.9	17.3	23.8		36.5		51.5	56.0	6.57
22.5	11.0	18.3	26.1		41.1		59.0	64.3	7.79
25		19.7		34.2		48.8		63.8	7.35
		21.2		36.0		51.7		65.0	7.36
	12.9	22.0	31.0		47.9		67.4	74.6	8.89
	10.6	24.1	34.4	44.3			68.5	79.1	9.37
27.5	13.8	22.9	33.0		52.0		74.3	80.8	9.80
28	11.9	24.3	34.4	44.9			73.6	82.4	9.95
29		26.5	36.8			69.3	78.3	82.5	9.76
30		16.3		29.0		40.3		52.7	6.03
		15.7		25.7		42.8		58.7	7.31
	11.9	24.0	34.8	44.1			73.0	80.6	9.74
31		26.5	36.1			66.6	75.1	80.9	9.36
32		24.3	31.9			51.9	58.9	65.9	6.87
33		20.3	25.8			43.9	49.5	54.5	5.79
35		10.0		10.3		10.7		10.7	0.13
		10.7		14.3		19.7		23.3	2.16
36	9.3	10.9	12.3	12.6			12.6	12.6	0.37

FIG. 4.6 <u>Geotrichum</u> <u>candidum</u>:-

Growth rate v. temperature illustrating the data of TABLE 4.4.

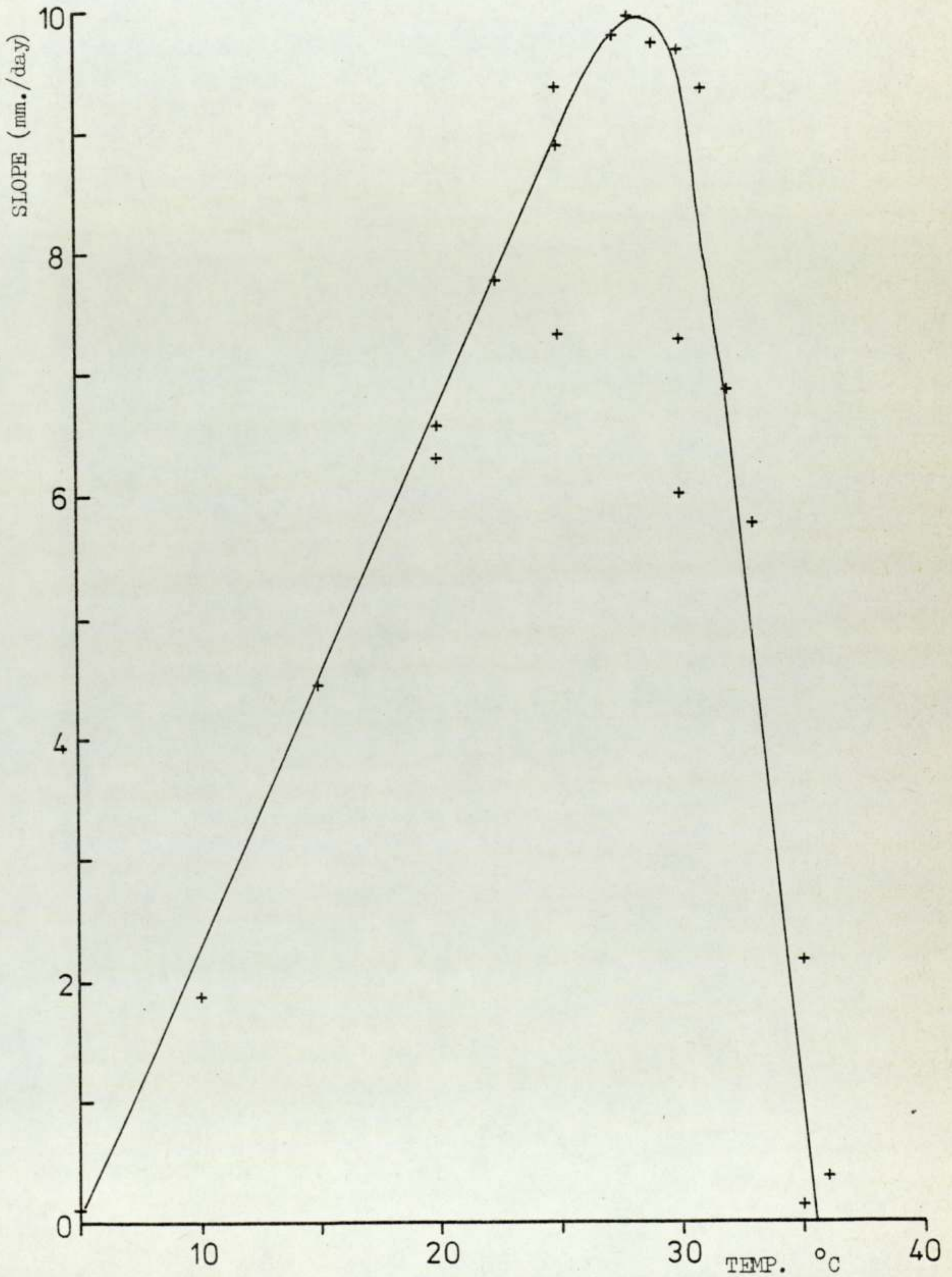


FIG. 4.7 <u>Fusarium aquaeductuum</u>:-

Effect of ambient temperature on growth (5°C - 35°C).

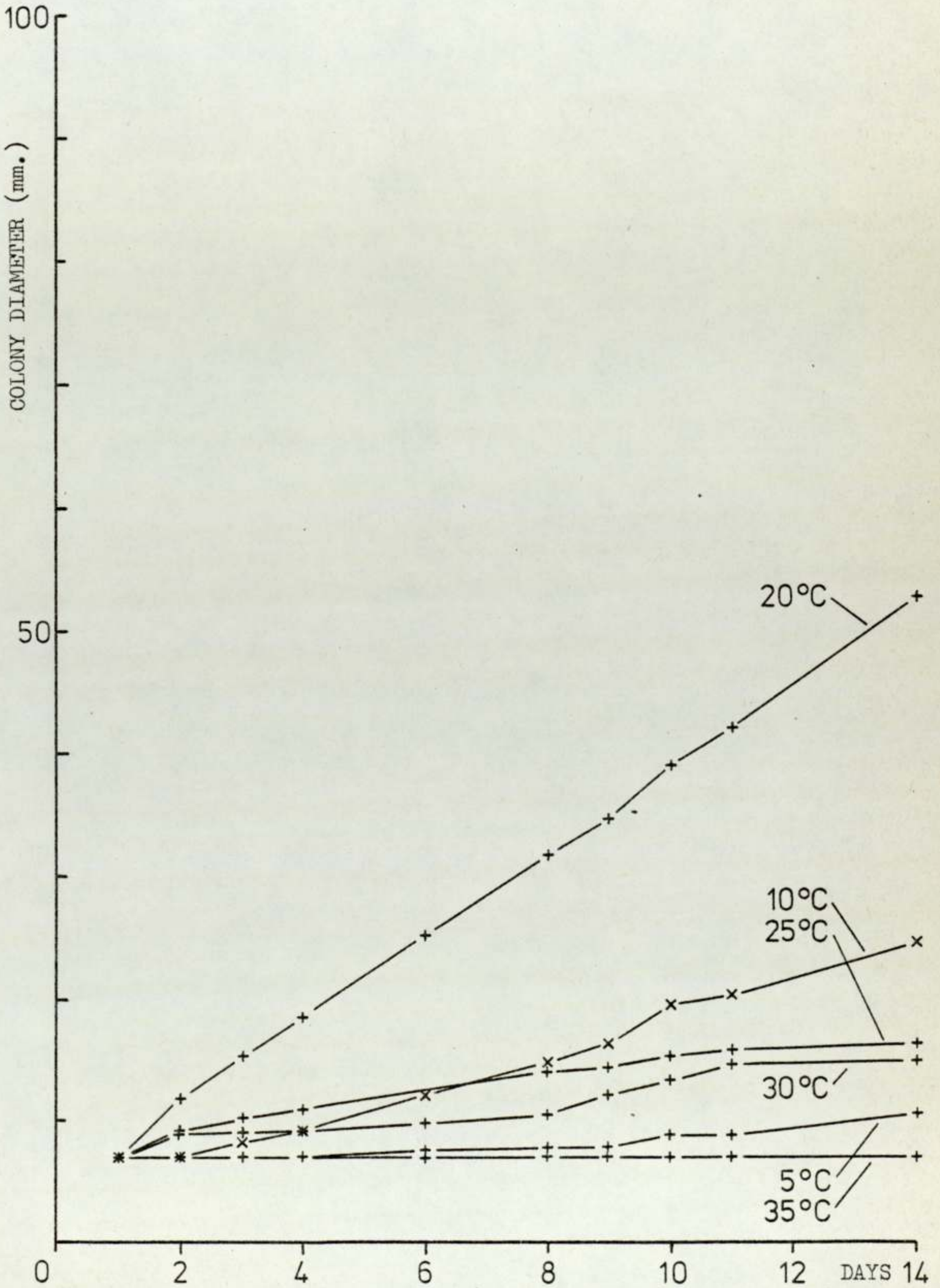
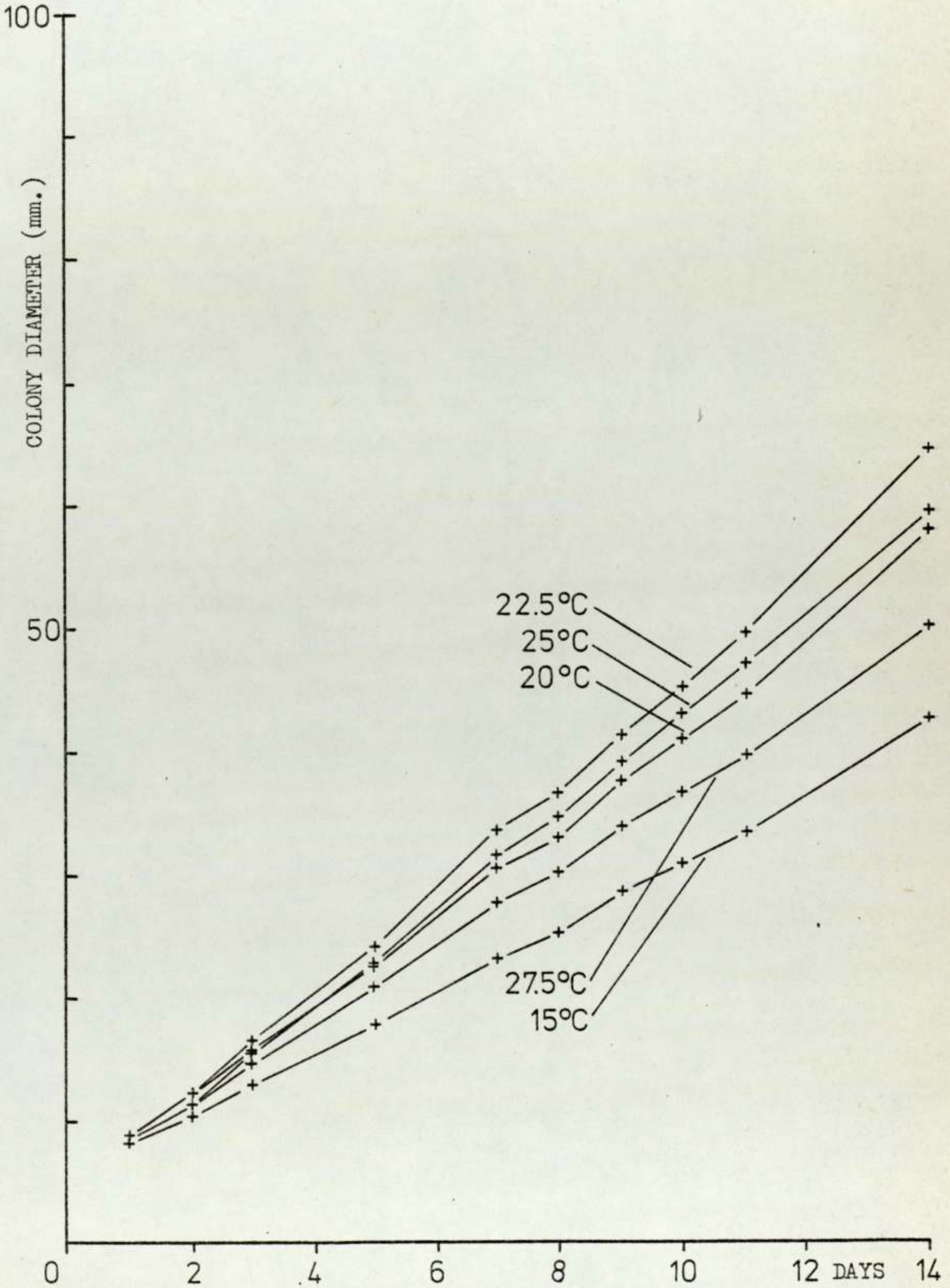


FIG. 4.8 <u>Fusarium aquaeductuum</u>:-

Effect of ambient temperature on growth (15°C - 27.5°C).



$5^{\circ}C$ and $30^{\circ}C$ with no growth at $35^{\circ}C$ and an optimum temperature between $10^{\circ}C$ and $25^{\circ}C$.

Fig. 4.8 showed that the optimum temperature in the range of $15^{\circ}C$ to $27.5^{\circ}C$ was $22.5^{\circ}C$. The temperature-growth curves were subjected to linear regression analysis to determine the growth rate of F.aquaeductuum from $5^{\circ}C$ to $35^{\circ}C$.

The colony diameter data from Tables 6.6 and 6.7 were used to calculate the regression coefficient (or slope) of each curve and the results were recorded in Table 4.5 and graphically in Fig. 4.9.

Fig. 4.9 confirmed the initial results obtained by examining Figs. 4.7 and 4.8 visually, the temperature range for F.aquaeductuum extended from $5^{\circ}C$ to $30^{\circ}C$ with the optimum temperature for growth being $22.5^{\circ}C$.

Fusarium tabacinum

The procedures for determining the range of temperatures over which the fungus can grow, and the optimum temperature for growth, were performed in exactly the same manner as for Fusarium aquaeductuum. Colony diameter data were recorded in Tables 6.8 to 6.11 and expressed graphically in Figs. 4.10 to 4.13.

Visual examination of Figs. 4.10 to 4.13 showed that F.tabacinum was capable of growth between $10^{\circ}C$ and $36^{\circ}C$, with an optimum temperature for growth at $29^{\circ}C$. This fungus resembled Geotrichum candidum in the pattern of its temperature-growth curves sharing the lack of a clearly-defined optimum temperature. Growth of F.tabacinum seemed to be equally good between $27.5^{\circ}C$ and $31^{\circ}C$. The gradient of the line of best fit for each graph was calculated from the data taken from Tables 6.8 to 6.11, collated in Tables 4.6 and expressed graphically in Fig. 4.14. The gradient of each line, in this case, was a measure of the growth rate at each temperature, and as with G.candidum and

TABLE 4.5

Fusarium aquaeductuum:- Colony diameters measured during the first eight days of growth (taken from TABLES 6.6 and 6.7) and the corresponding growth rates (SLOPES) calculated from these diameters

TEMP. °C	COLONY DIAMETER (mm.) DAYS FROM START OF EXPERIMENT 1	2	3	4	5	6	7	8	SLOPE mm./day
5	7.0	7.0	7.0	7.0		7.5		7.7	0.11
10	7.0	7.0	8.2	9.2		12.0		14.8	1.18
15	8.1	10.4	13.0		17.9		23.3	25.3	2.50
20	7.0	11.8	15.2	18.5		25.0		31.7	3.44
	8.9	12.0	15.6		22.6		30.5	33.1	3.54
22.5	8.8	12.1	16.5		24.3		33.8	36.9	4.12
25	7.0	9.3	10.2	11.0		12.5		14.0	0.92
	8.5	11.5	15.4		22.9		31.6	34.8	3.85
27.5	8.5	11.4	14.6		21.0		27.6	30.3	3.16
30	7.0	9.0	9.0	9.0		9.8		10.5	0.40
35	7.0	7.0	7.0	7.0		7.0		7.0	0.00

FIG. 4.9 Fusarium aquaeductuum:-

Growth rate v. temperature illustrating the data of TABLE 4.5.

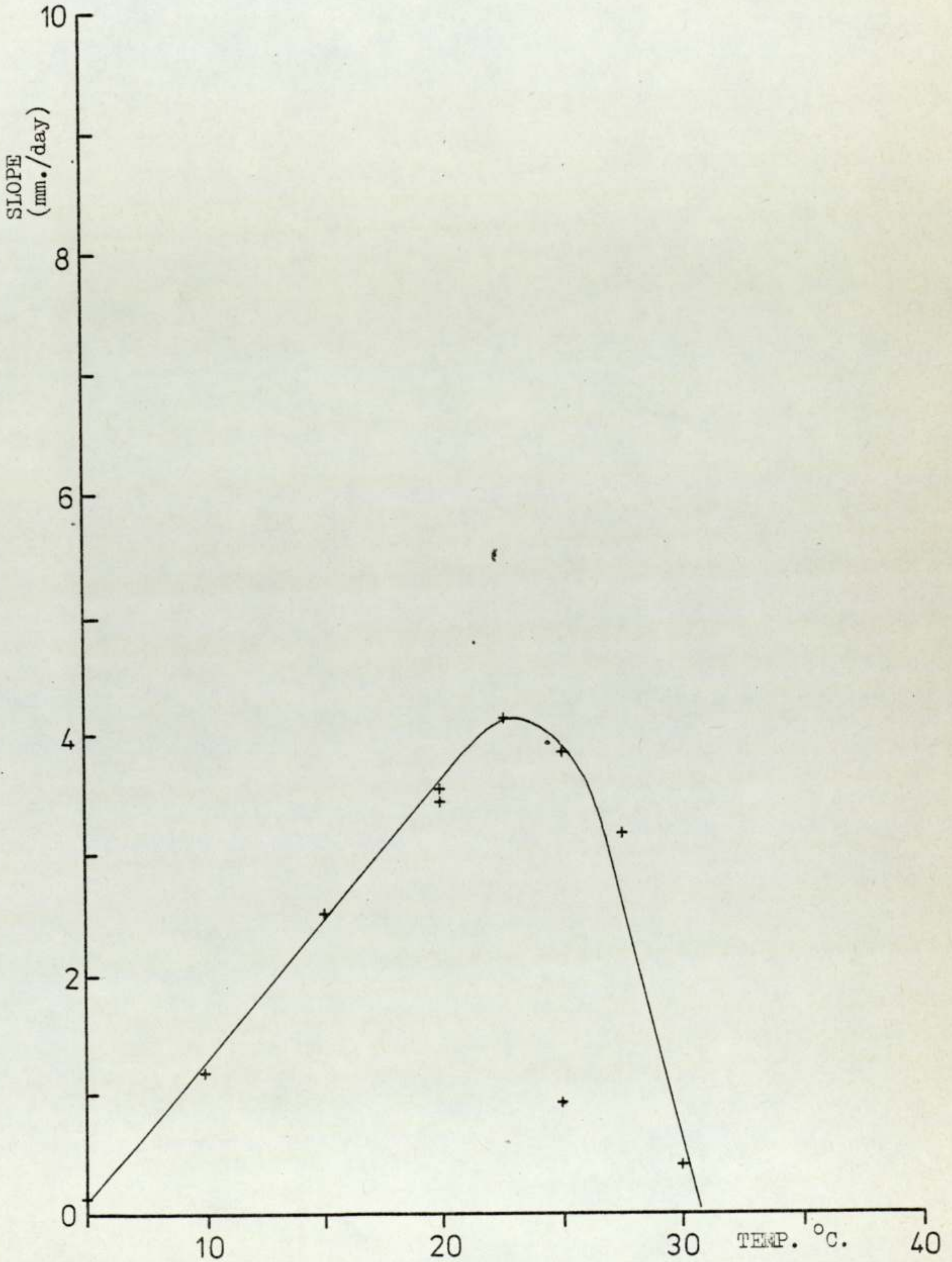


FIG. 4.10 Fusarium tabacinum:-

Effect of ambient temperature on growth ($5^{\circ}C$ - $35^{\circ}C$).

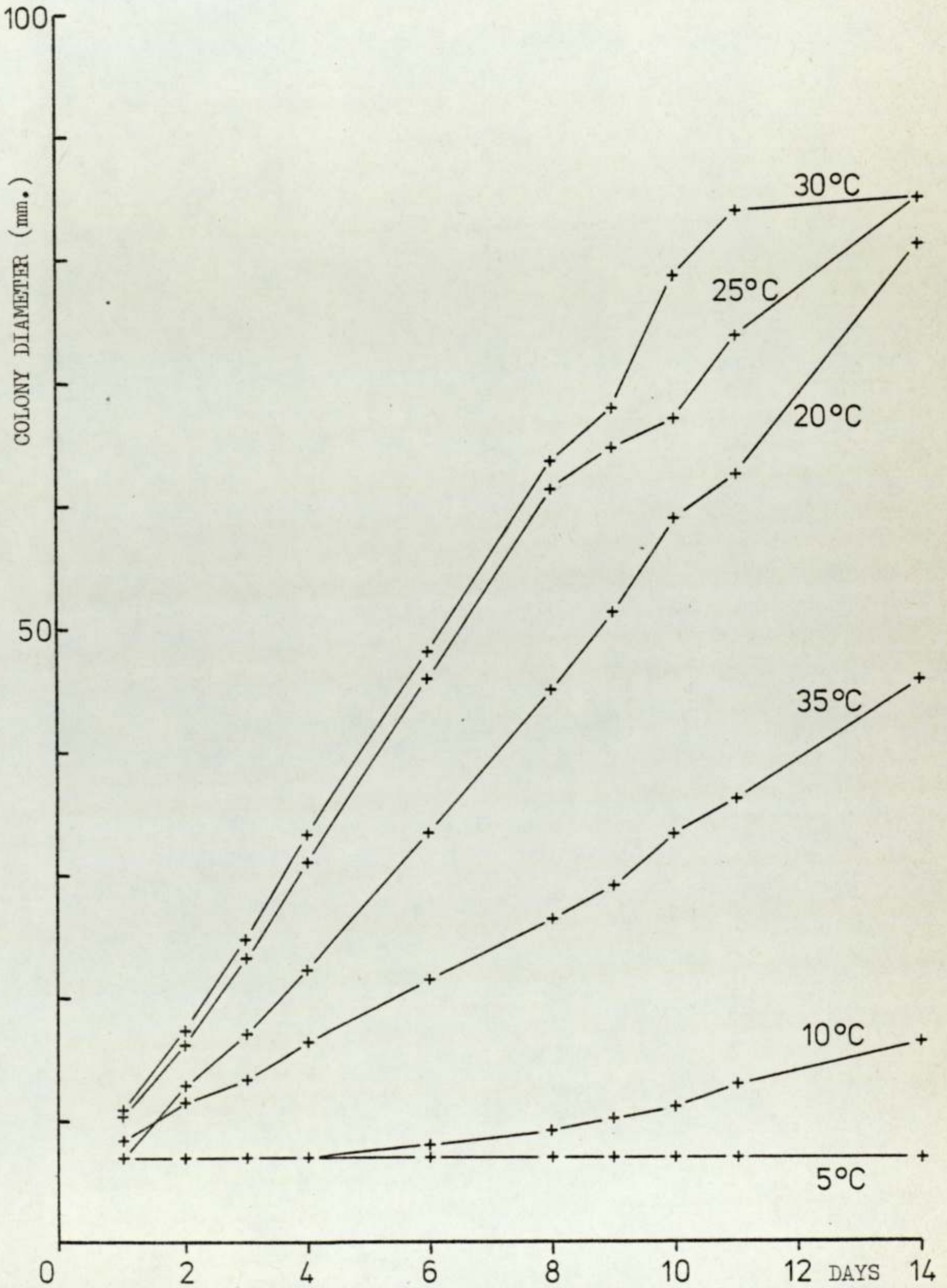


FIG. 4.11 Fusarium tabacinum:-

Effect of ambient temperature on growth ($15^{\circ}C$ - $27.5^{\circ}C$).

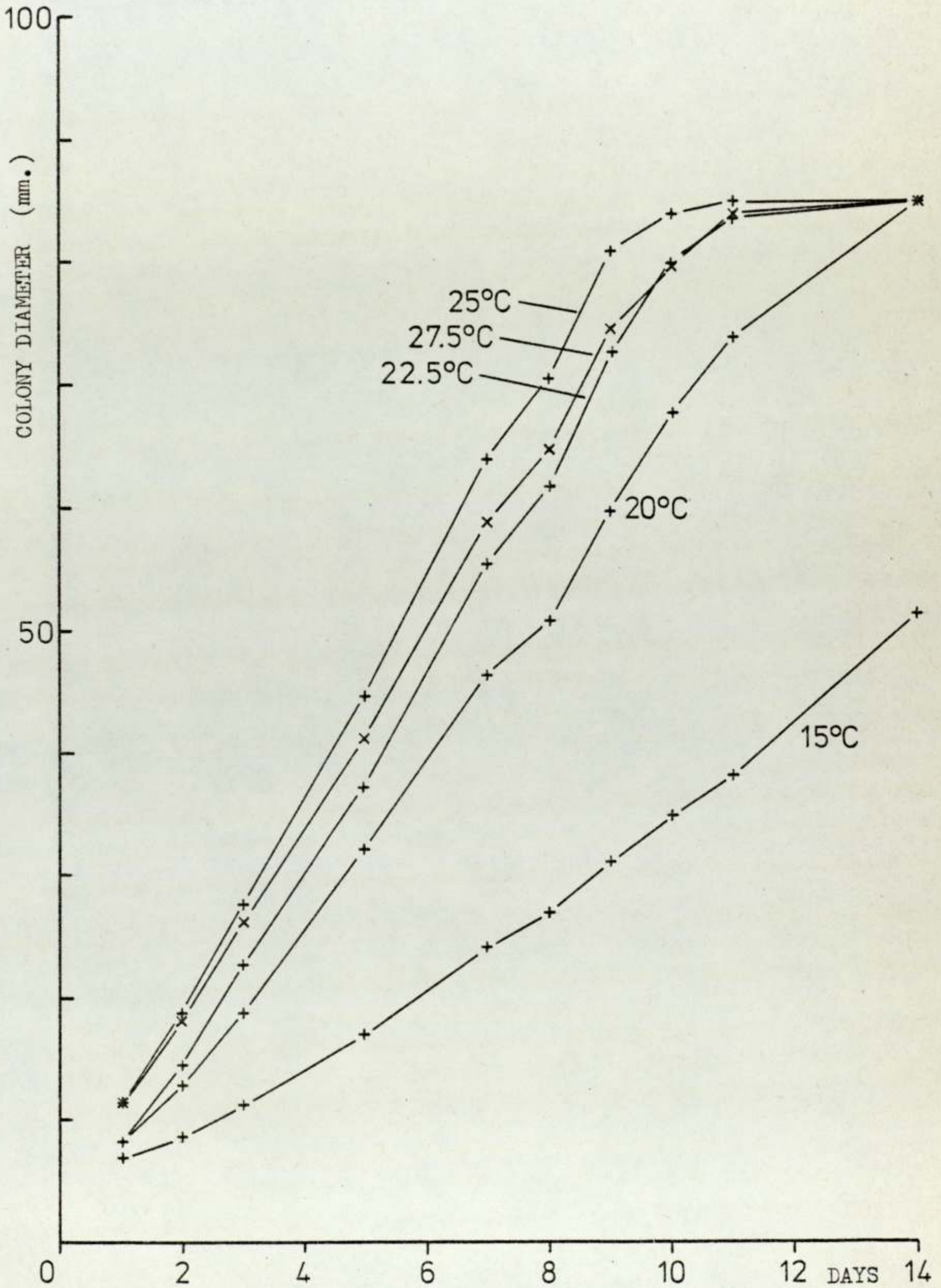


FIG. 4.12 _Fusarium_ _tabacinum_:-

Effect of ambient temperature on growth (25°C - 36°C).

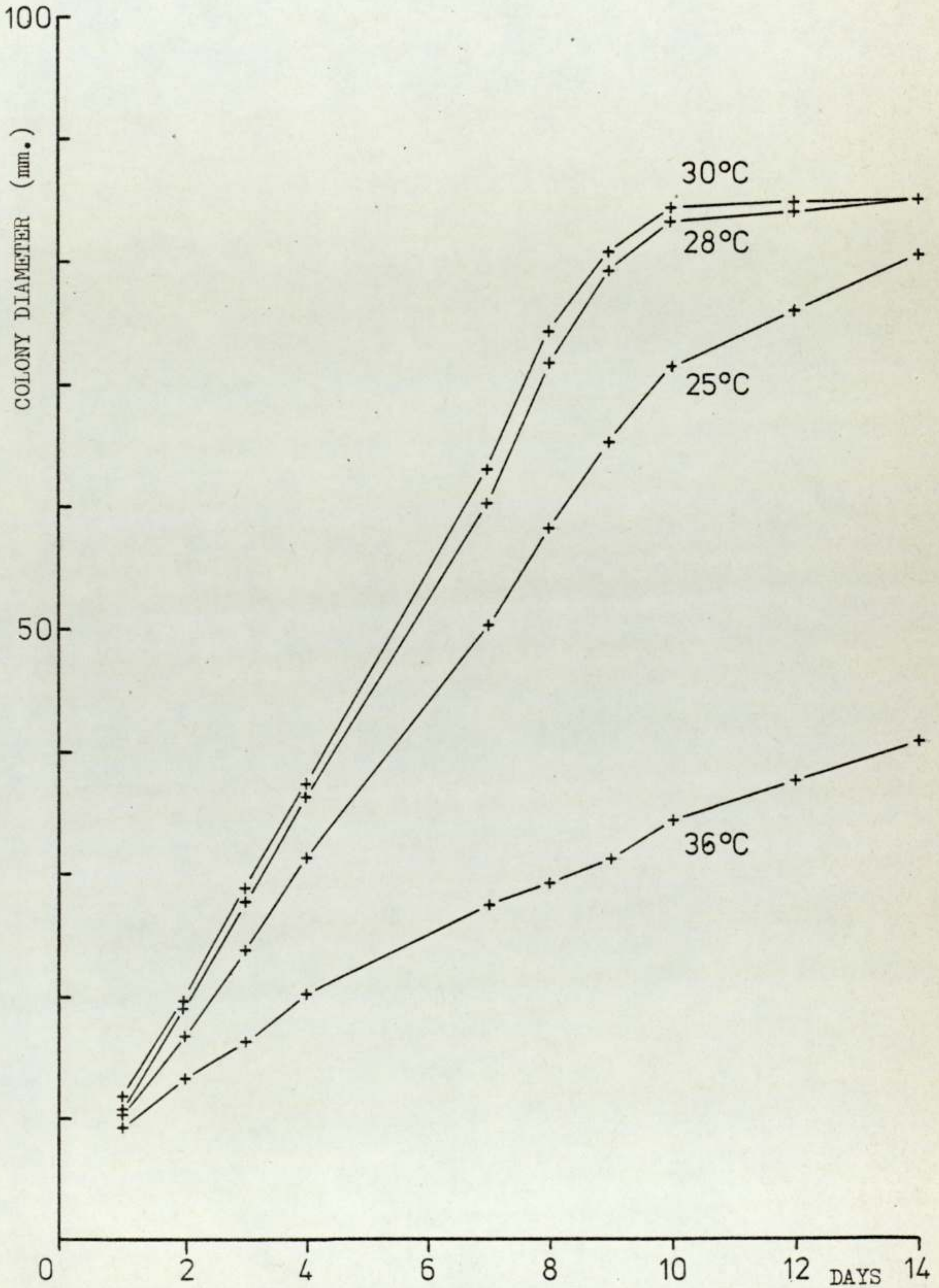


FIG. 4.13 Fusarium tabacinum:-

Effect of ambient temperature on growth (29°C - 33°C).

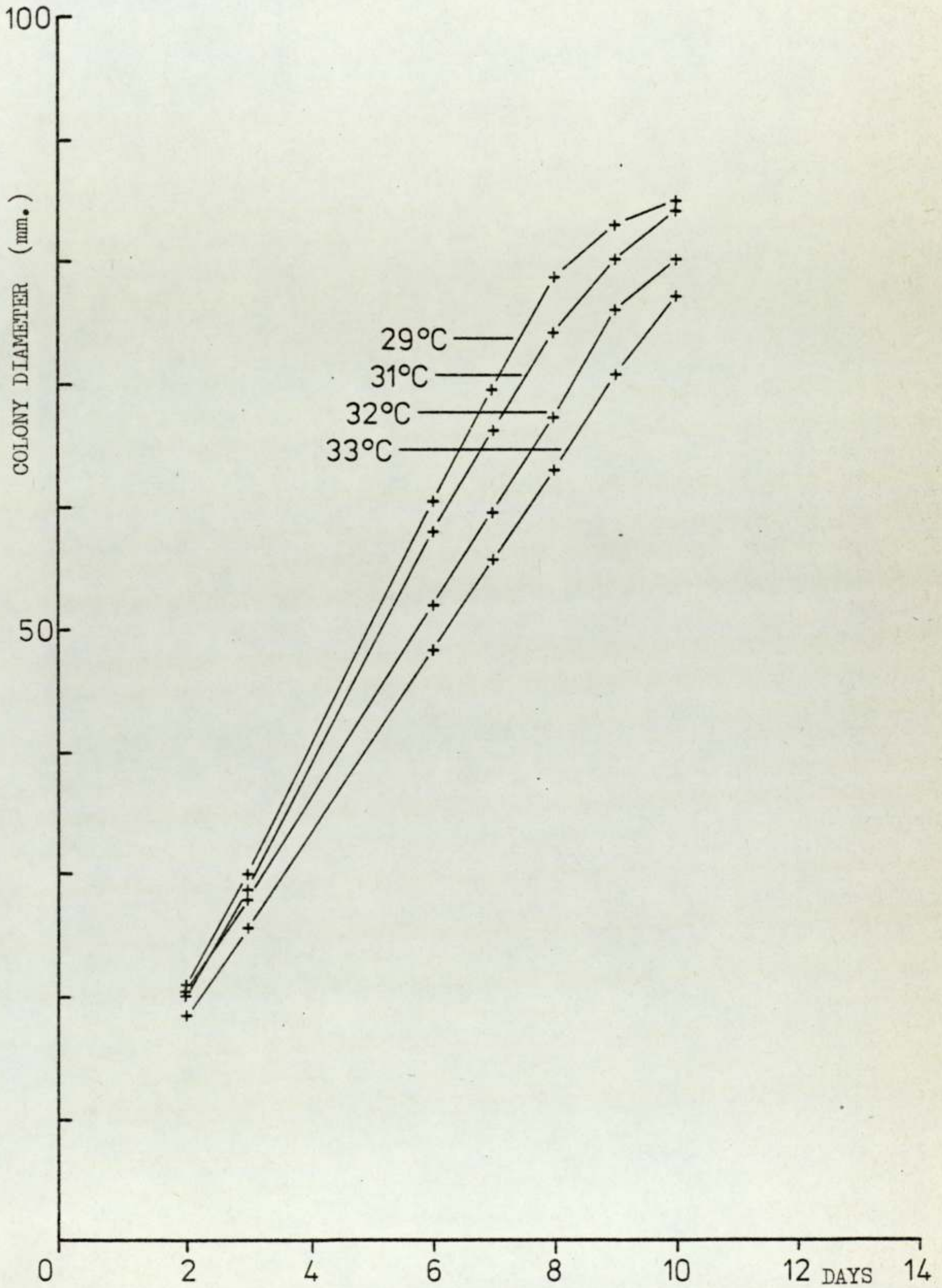


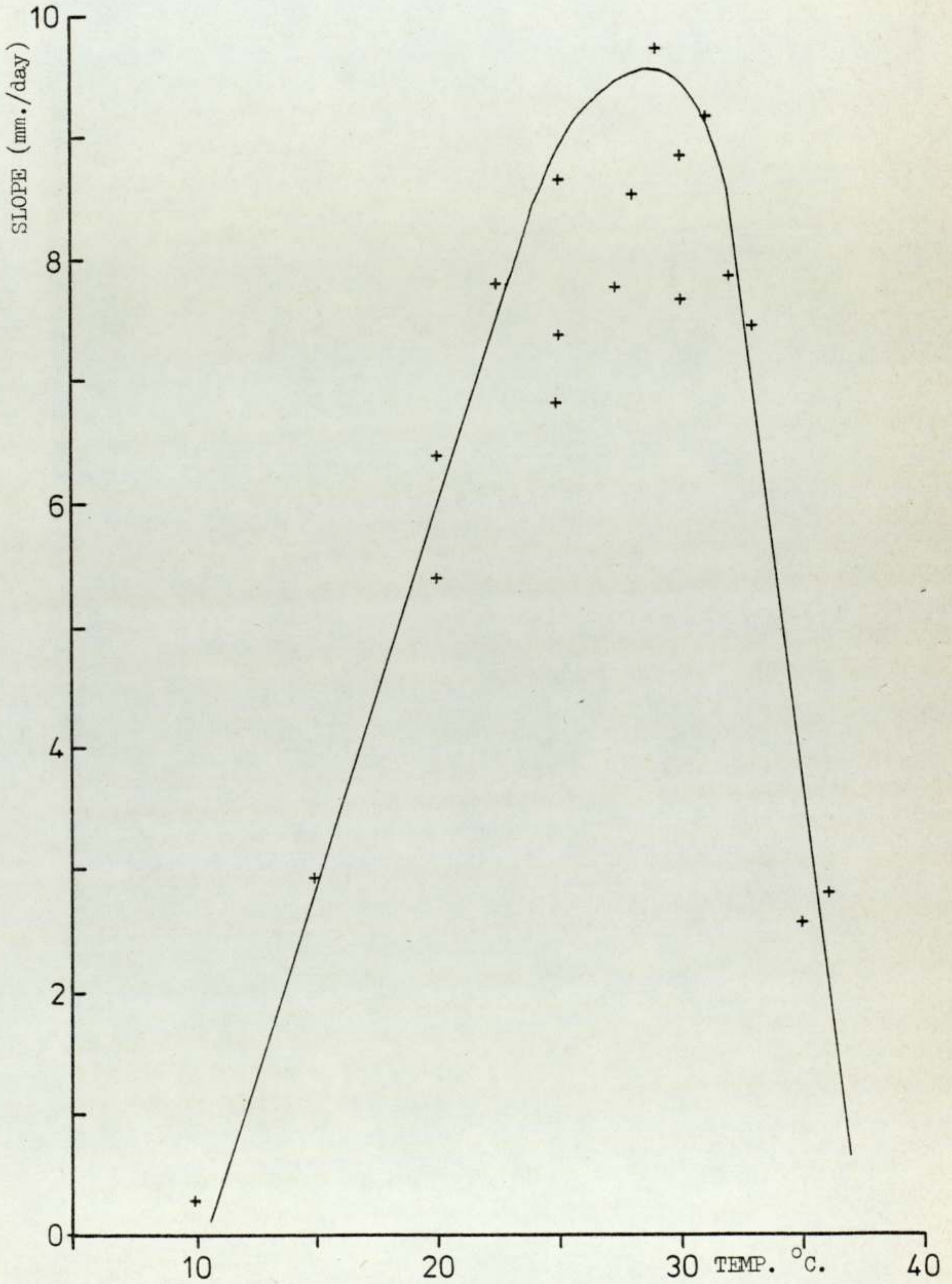
TABLE 4.6

Fusarium tabacinum:- Colony diameters measured during the first eight days of growth(taken from TABLES 6.8 - 6.11) and the corresponding growth rates (SLOPES) calculated from these diameters

TEMP, °C	COLONY DIAMETER (mm.) DAYS FROM START OF EXPERIMENT								SLOPES mm./day
	1	2	3	4	5	6	7	8	
5	7.0	7.0	7.0	7.0		7.0		7.0	0.00
10	7.0	7.0	7.0	7.0		7.8		9.0	0.28
15	7.0	8.8	11.3		17.0		24.1	27.0	2.94
20	7.0	12.8	17.2	22.2		33.5		45.0	5.39
	8.8	12.9	18.9		32.3		46.5	51.8	6.38
22.5	8.9	14.5	22.8		37.4		55.5	61.9	7.78
25	10.1	16.8	23.6	31.4			50.4	58.3	6.83
	10.3	16.2	23.3	31.0		46.0		61.3	7.37
	11.3	18.6	27.6		44.6		64.0	70.5	8.67
27.5	11.4	18.1	26.1		41.3		58.9	64.9	7.82
28	10.9	19.0	27.6	36.4			60.1	71.6	8.52
29		21.0	30.0			60.5	69.6	78.8	9.74
30	10.5	17.3	24.5	33.3		48.0		63.7	7.65
	11.6	19.5	28.6	37.4			63.0	74.1	8.86
31		20.1	28.8			58.0	66.4	74.3	9.19
32		20.4	28.0			52.0	59.5	67.4	7.86
33		18.5	25.6			48.3	55.6	63.0	7.45
35	8.3	11.5	13.3	16.3		21.5		26.3	2.56
36	9.4	13.3	16.3	20.1			27.5	29.3	2.81

FIG. 4.14 Fusarium tabacinum:-

Growth rate v. temperature illustrating the data of TABLE 4.6.



F.aquaeductuum the calculations were based on data from Day 1 to Day 8 during the linear phase of extension.

Fig. 4.14 comparing the growth rate at different temperatures clearly showed the optimum temperature, producing the highest growth rate, to be 29°C.

4.3.2 The effect of hydrogen ion concentration on the growth of Geotrichum candidum, Fusarium tabacinum and Fusarium aquaeductuum in pure culture.

Experiments described by Painter (1954) were used as the basis for this study because two of the four fungi studied by him were Geotrichum sp. and Fusarium aquaeductuum and their pH requirements were available for comparison using a defined medium. The medium used was Painter's Medium A which consisted of (g./l.) : glucose (Oxoid),5.0; vitamin-free Casamino acids (Difco),5.0; KH_2PO_4,0.5; $MgSO_4.7H_2O$,0.5; KCl,0.25, solidified with 15 g./l. Oxoid agar (No.3). The pH of this medium was 5.7 and a range of initial pH values from pH2 to 10 was obtained by adding 0.05M H_2SO_4 for acidic values and 0.05M NaOH for alkaline values. Difficulty was experienced with agar plates at pH2 and 3 because the agar remained molten, double strength agar was tried (30 g./l.) and was successful in setting pH3 plates but not pH2. It was decided to use the agar plate method initially to obtain a range of pH values which the fungi could tolerate and then continue the study using a liquified form of medium A.

Known volumes of medium A agar were adjusted with H_2SO_4 or NaOH and autoclaved, 20 cm^3. of adjusted medium were dispensed into 9 cm. Petri dishes and each dish was inoculated centrally with

a 7 mm. disc of Geotrichum candidum cut from the vegetative margin of six-day old cultures growing on SF agar. Three replicate plates were used for each pH, the plates were placed in brass canisters in a stirred water-bath at 20°C. Growth was measured by taking the mean of three colony diameters to the nearest half-millimetre every twenty-four hours. The results were recorded in Table 6.12 and expressed graphically in Figs. 4.15 and 4.16.

Fig. 4.15 showed that after ten days there was negligible growth at pH2, whereas pH3 to 10 had encouraged very similar growth with the best result at pH4. Fig. 4.16 showed the similarity between the curves of growth at pH4 and pH10 and it was decided that the initial pH of the medium may have been adjusted, by autoclaving, to a value below pH10, since this result was in marked contrast to that of Painter (1954). Small portions of agar were removed from the pH10 plates and a few drops of B.T.L. Universal Indicator Solution were added to test the pH. The resultant colour change showed that the pH10 plates had altered to pH7. This may have been as a result of the fungal growth adjusting the pH of the agar but it was decided to repeat the pH study using liquid media to allow daily pH checks as the fungi grew.

The effect of hydrogen ion concentration on the growth of the three fungi was studied further using liquified Painter's Medium A. The medium contained the same ingredients as before but, instead of agar, double-distilled water was used.

Known volumes of 100 cm^3 Painter's Medium A, previously adjusted to one of eight pH values with H_2SO_4 or NaOH, were dispensed into 250 cm^3 rimless conical flasks and sterilised by autoclaving. The flasks were fitted with metal caps which were pushed on and gripped the flask necks with protruding metal strips fitted internally to each

FIG. 4.15 Geotrichum candidum:- Effect of initial pH of medium on colony diameter after ten days.

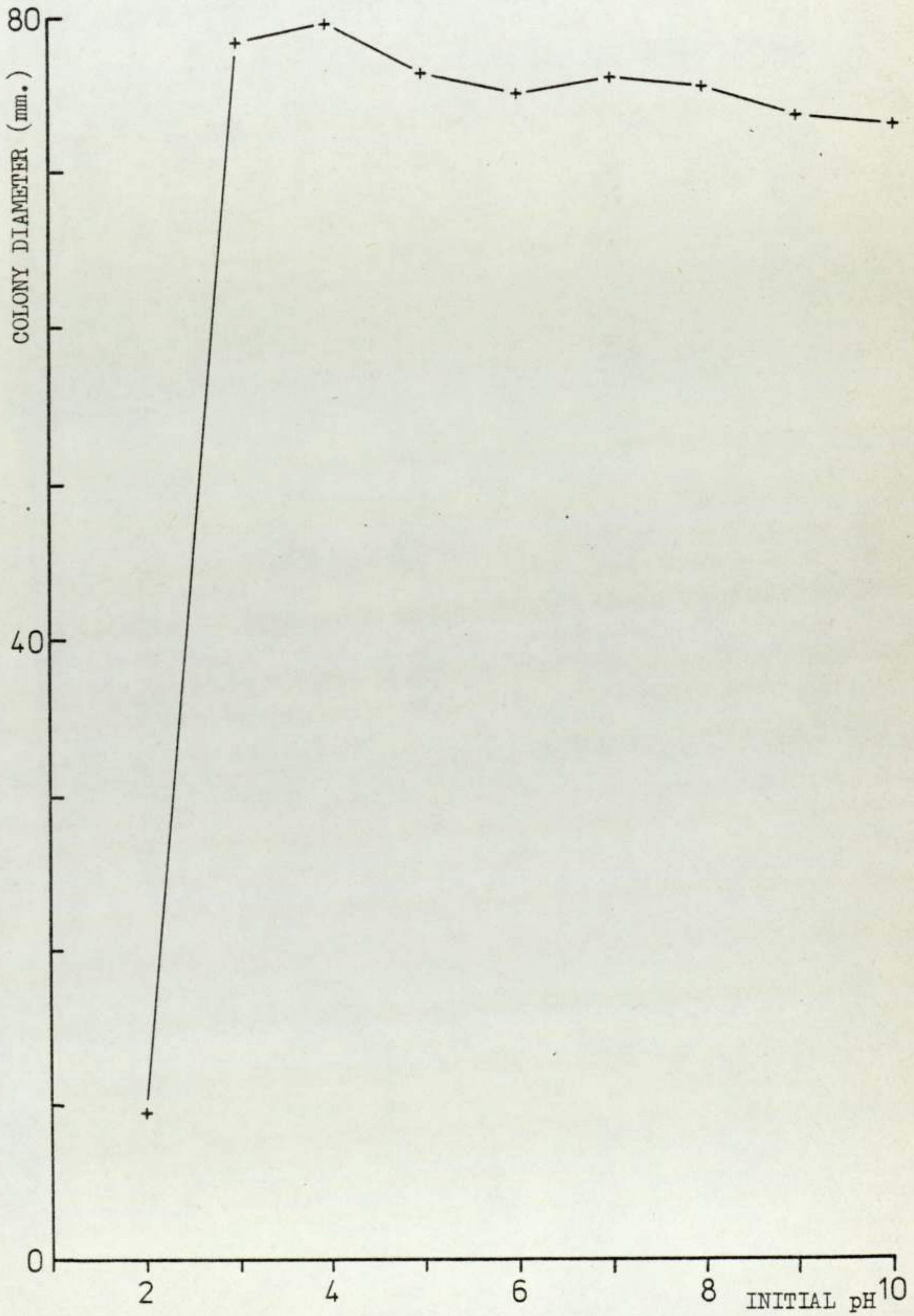
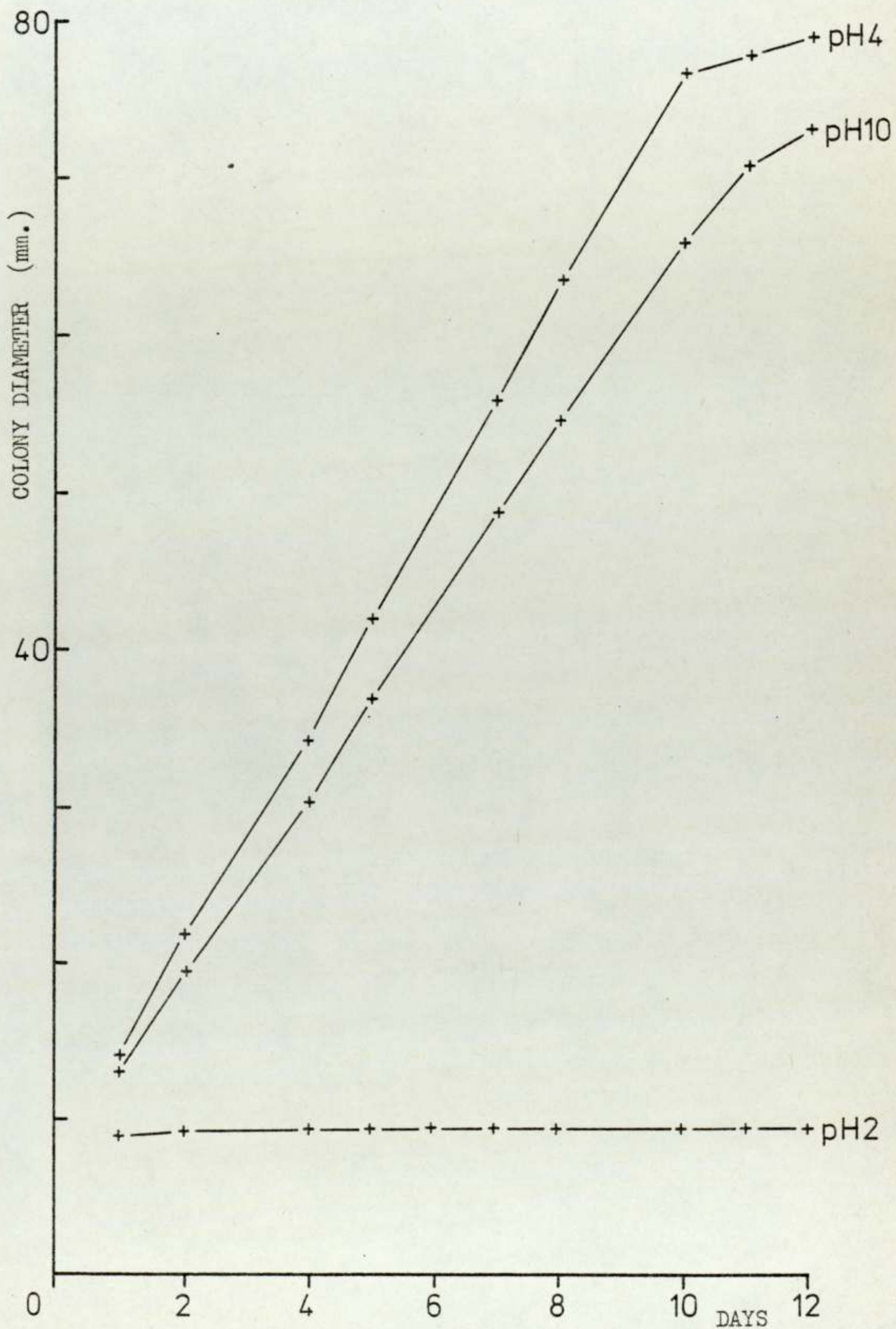


FIG. 4.16 Geotrichum candidum :-

Effect of initial pH of medium (pH2, 4, 10) on colony diameter over a period of ten days.



cap. Triplicate flasks were used for each pH from 2 to 9 and each flask was inoculated with a 7mm. disc of Geotrichum candidum cut from the vegetative margin of a six-day old colony growing on SF agar. The flasks were installed on an orbital shaker set at 130 r.p.m. which was itself housed in a constant-temperature cabinet maintained at 20°C.

The flasks were removed from the shaker after eleven days and the pH was measured using a pH meter (Corning-Eel Digital 110) prior to harvesting. The contents of each flask were filtered, under vacuum, through previously weighed glass-fibre filter paper (Whatman GF/C). Each flask was thoroughly rinsed with distilled water and the filter papers were dried at 105°C for four hours. The papers were cooled in a dessicator and then weighed to constant weight. The results were recorded in Table 6.13 and expressed graphically in Fig. 4.17.

Harvesting was carried out after eleven days to serve as a comparison with the results obtained by Painter (1954) who originally chose this period. Fig. 4.17 showed that G.candidum was capable of growth over a wide range of pH2 to 9, with good growth between pH4 and 8. The pH values at the time of harvesting had risen between pH4 and 7 and fallen slightly outside this range. The implication of these results was that the hydrogen ion concentrations promoting the best growth were those which the fungus was able to alter within the range of 6.4 to 7.9 (Table 6.13).

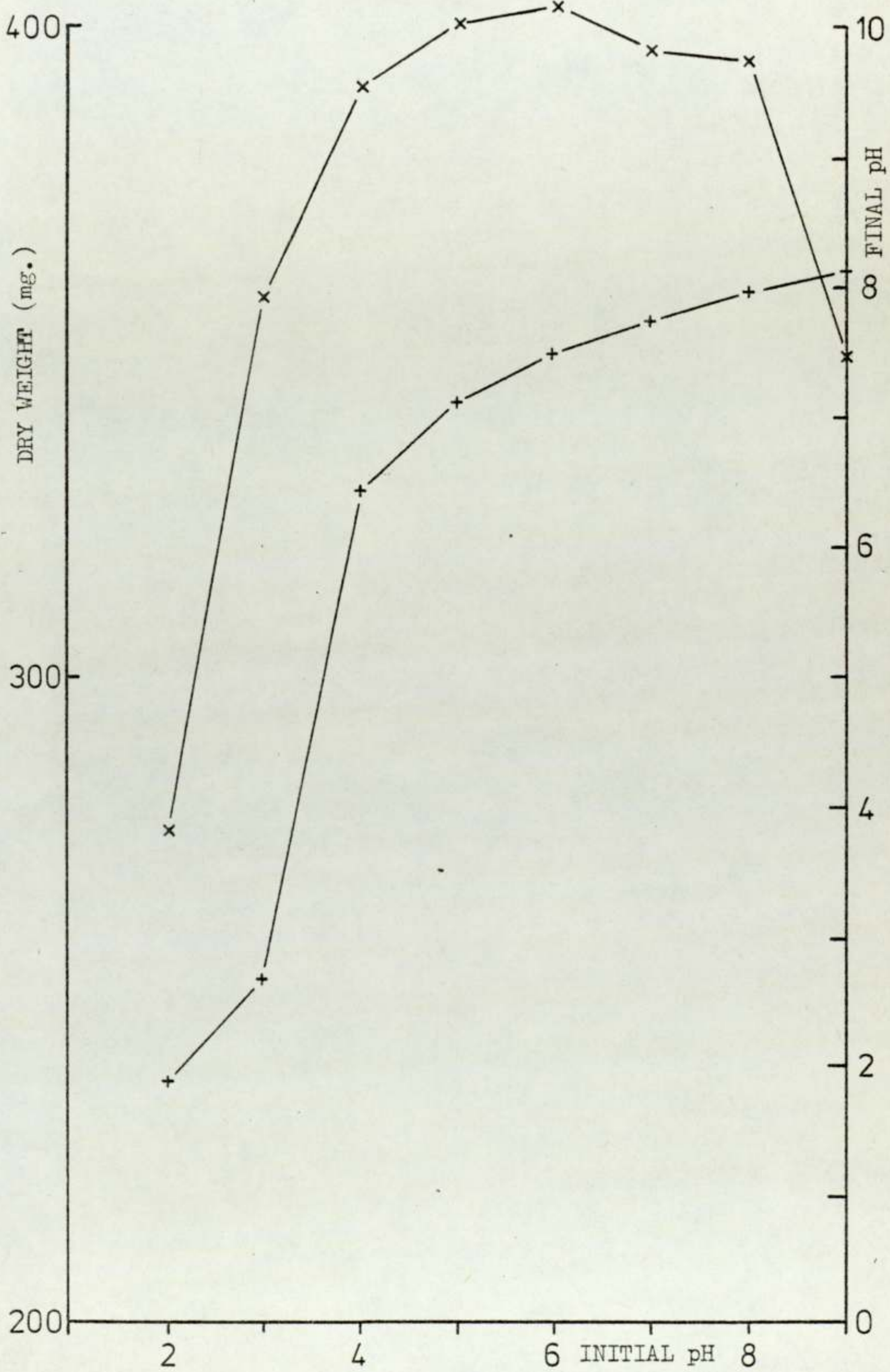
Fusarium tabacinum

The experimental procedure for determining the pH range of this fungus using flask cultures of Medium A was performed in exactly the same manner as previously described for G.candidum.

The flasks were harvested after eleven days at 20°C, their pH

FIG. 4.17 <u>Geotrichum</u> <u>candidum</u>:-

Relationship of initial pH to final pH (+) and dry weight (×) of fungus after eleven days in shake flasks at 20°C.



values were measured and the contents were vacuum-filtered through glass-fibre filter papers, dried and weighed to constant weight. The results were recorded in Table 6.14 and expressed graphically in Fig. 4.18.

F.tabacinum was capable of growth between pH3 and 9 with good growth from pH5 to pH8. The final pH values at harvesting revealed considerable changes from the initial pH in the range pH4 to 8, they were all adjusted upwards to approximately pH8 indicating a preference for a slightly alkaline medium.

Fusarium aquaeductuum

The relationship between initial pH, final pH and the dry weight of the fungus after eleven days in shake-flasks of Medium A at 20°C was investigated in the same manner as for G.candidum and F.tabacinum. The results were recorded in Table 6.15 and expressed graphically in Fig. 4.19.

F.aquaeductuum was capable of growth between pH4 and 9 with a marginal preference for growth at pH4. The depression in the curve at pH5 was unaccountable since the fungus grew well beyond this value. The final pH values again revealed an adjustment towards slightly alkaline values from pH4 to 7.

A comparison of the relationship between the initial pH and the dry weight of the three fungi after eleven days in shake-flasks was collated in Table 6.16 and expressed graphically in Fig. 4.20. Geotrichum candidum produced the best growth of the three over the whole range from pH2 to 9. The two Fusarium species exhibited a similar trend with no growth at pH2, good growth around pH7 declining at pH9.

FIG. 4.18 *Fusarium tabacinum*:-

Relationship of initial pH to final pH (+) and dry weight (×) of fungus after eleven days in shake flasks at 20°C.

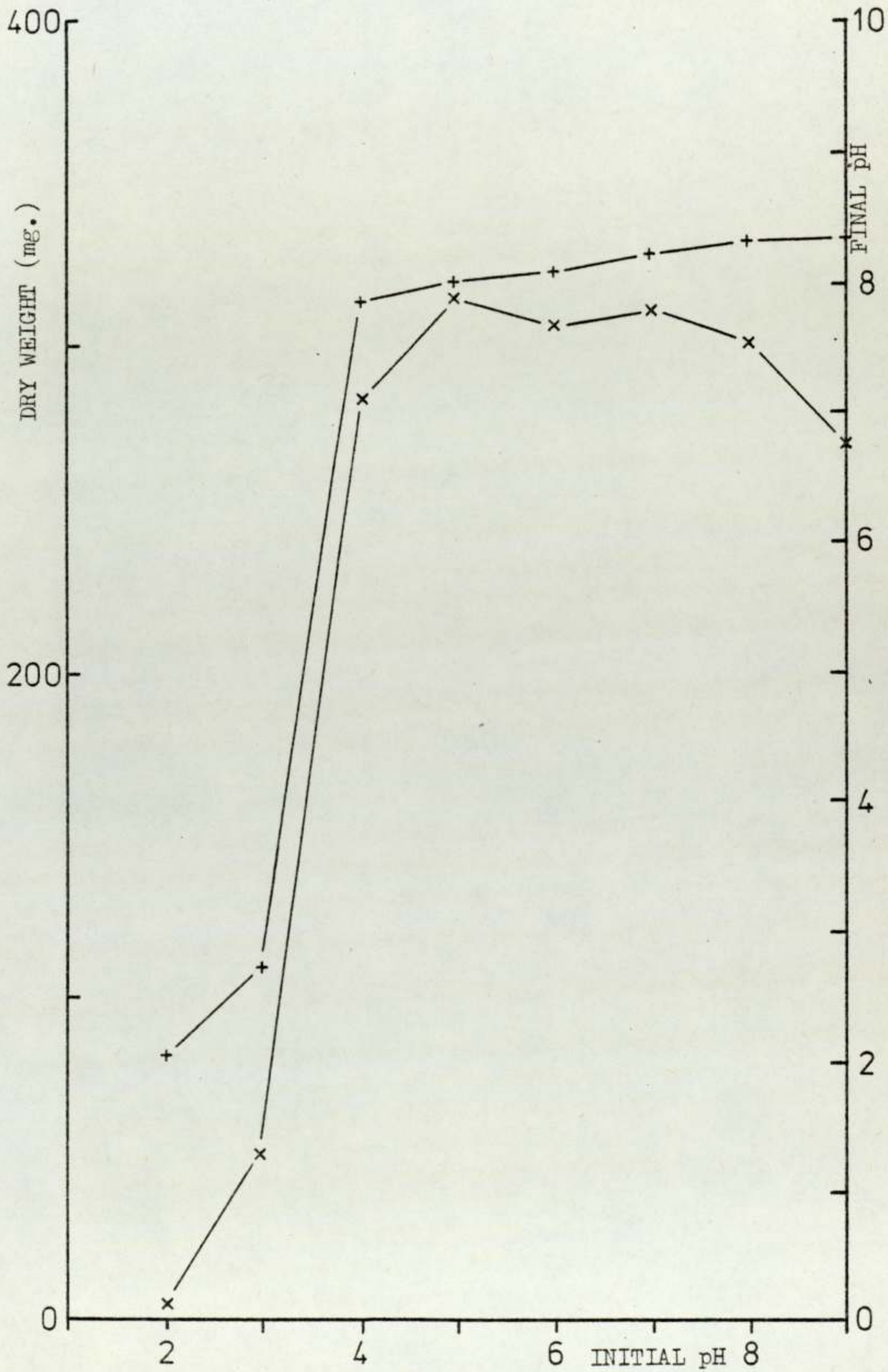


FIG. 4.19 Fusarium aquaeductuum:-

Relationship of initial pH to final pH (+) and dry weight (x) of fungus after eleven days in shake flasks at 20°C.

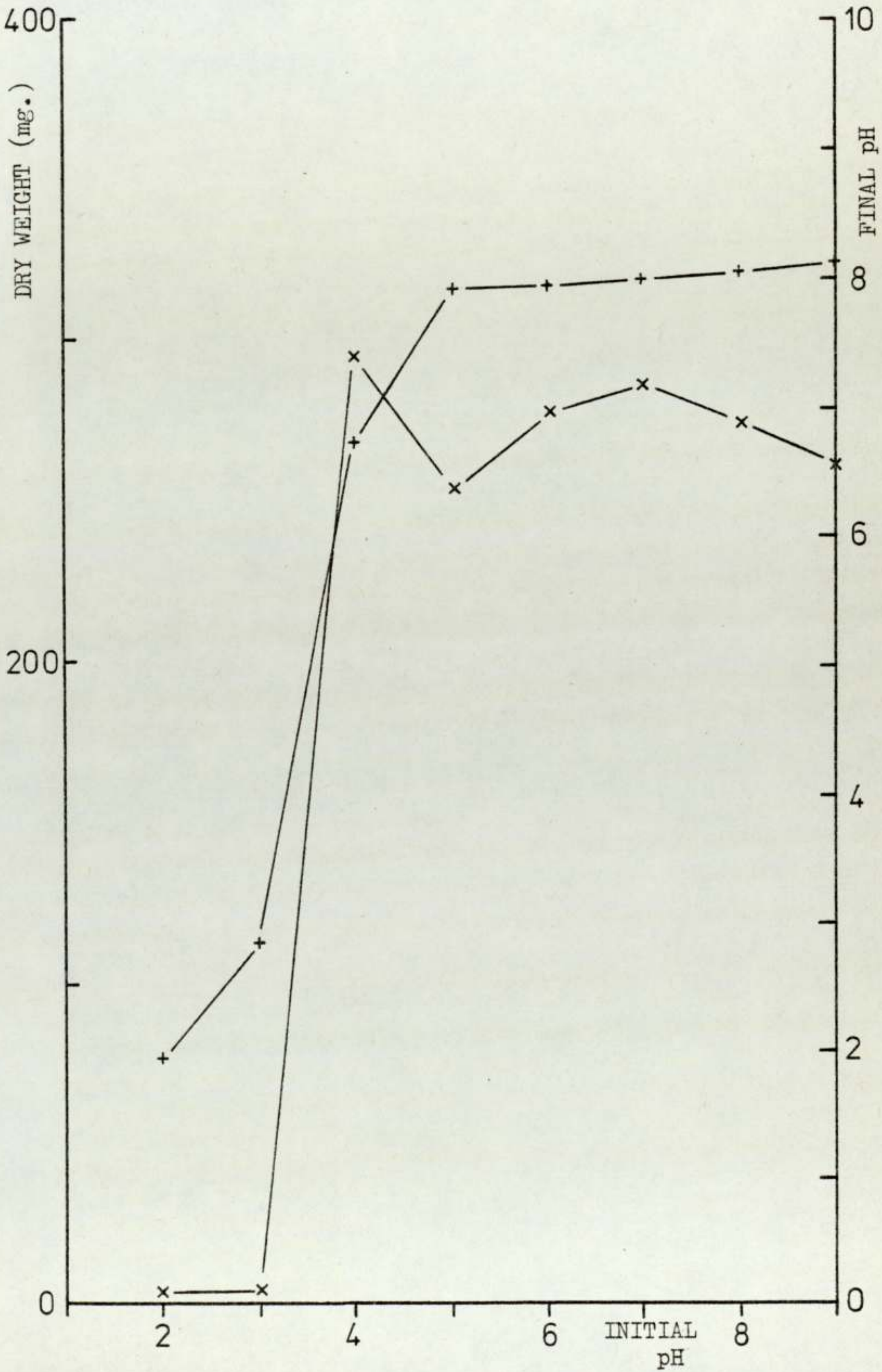
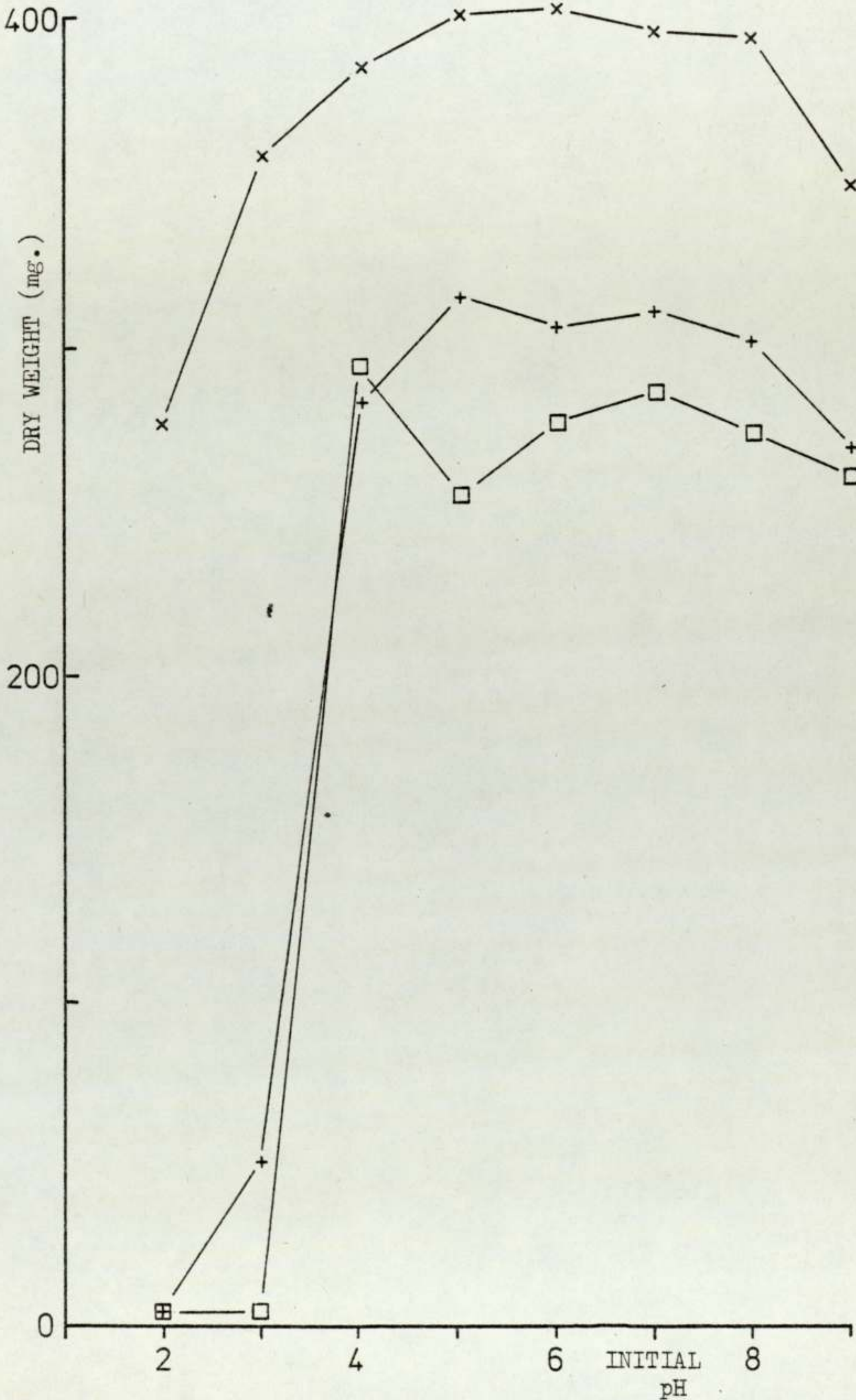


FIG. 4.20 Comparison of the relationship between initial pH and dry weight of Geotrichum candidum (×), Fusarium tabacinum (+) and Fusarium aquaeductuum (□) after eleven days in shake flasks of 20^{0}C.



4.3.3 Detailed study of the effect of hydrogen ion concentration on the growth of G.candidum and F.tabacinum

The second set of experiments in this investigation, concerning the optimum pH for growth, was more successful with liquid Medium A than with the solid form. The preliminary trial with Medium A agar plates showed the general trend in the relationship between pH and growth which was confirmed with the results of the shake-flask experiment. However, the final pH and dry weights were only obtained at the completion of the experiment with no knowledge of the changes during the eleven days. It was decided to repeat the experiment with two fungi using four initial pH values which had promoted the best growth in the previous study.

Fusarium aquaeductuum was no longer included in experimental work for the reasons given in Chapter 3, section 3.2.2.

Geotrichum candidum.

Known volumes of Painter's Medium A were adjusted to pH4,5,6 & 7 and dispensed in 100 cm^3 aliquots to 250 cm^3 rimless conical flasks fitted with metal caps. Twenty-seven flasks were needed for each pH to allow harvesting of three flasks each day over a period of nine days.

It was decided to try a different preparation for the inocula (see Section 4.2) in an attempt to minimize any lag produced by using fungal discs and to minimize the weight difference of replicate flasks, 7 mm. cork-bored discs, cut from the vegetative margin of six-day old cultures of G.candidum, were introduced to flasks of 100cm^3 Medium A adjusted to pH5. The particular pH value was chosen as a result of the previous study (4.3.2). The flasks were placed on an

orbital shaker at 130 r.p.m. for three days at 20°C, when the flask contents were considered to be sufficiently turbid for further use. Sterile Pasteur pipettes were used to transfer two drops of fungal culture to each of the twenty-seven flasks, using one pipette to every three replicate flasks.

The inoculated flasks were then placed on the orbital shaker set at 130 r.p.m. and three flasks were removed every twenty-four hours to measure the pH and harvest the contents as described in 4.3.2. The temperature was 20°C.

The results for the twenty-seven flasks with an initial pH4 were recorded as the mean of three values in Table 6.17 and expressed graphically in Fig. 4.21.

Geotrichum candidum showed a sharp increase in weight between the first and second day of growth with a steady increase in weight until Day 7 after which the weight began to decline. As growth increased, the pH decreased as far as Day 5 and then increased slightly to Day 9.

The results for the twenty-seven flasks with an initial pH5 were recorded as the triplicate mean in Table 6.18 and expressed graphically in Fig. 4.22.

A similar pattern of growth at pH5 was exhibited with maximum biomass on Day 8. There was a decrease in growth on Day 5 which was reflected in a slight increase in pH in an apparently downward trend. From Day 7 onwards the pH increased up to Day 9.

The results for the twenty-seven flasks of G.candidum with an initial pH6 were recorded as the mean of triplicate values in Table 6.19 and expressed graphically in Fig. 4.23. The growth curve was similar to that of pH5 with a maximum at Day 8 and a slight decrease

FIG. 4.21 Geotrichum candidum:-

Daily changes in dry weight (×) and pH (+) in shake flasks at 20°C. (Initial pH 4).

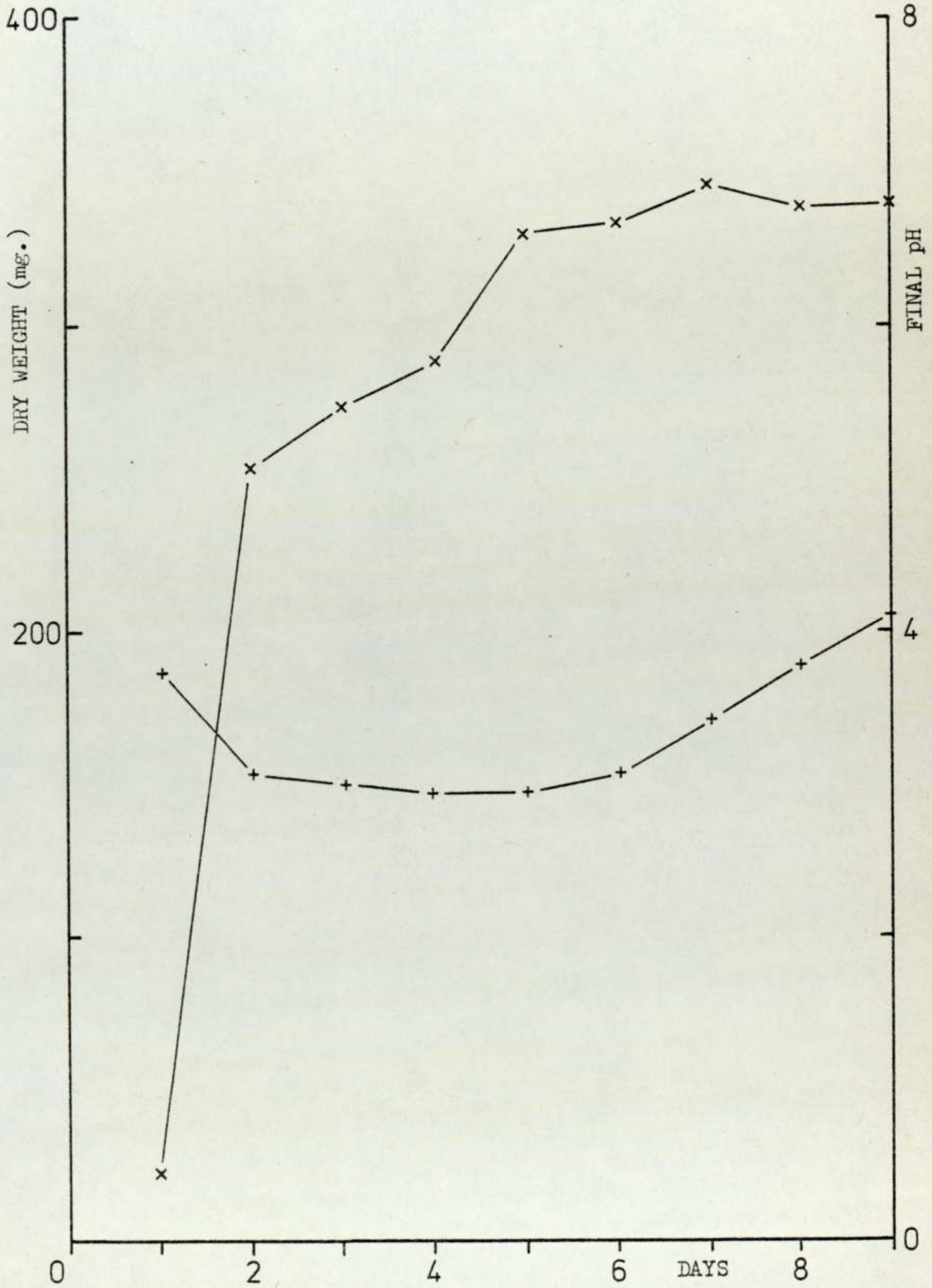


FIG. 4.22 Geotrichum candidum:-

Daily changes in dry weight (×) and pH (+) in shake flasks at 20°C. (Initial pH 5).

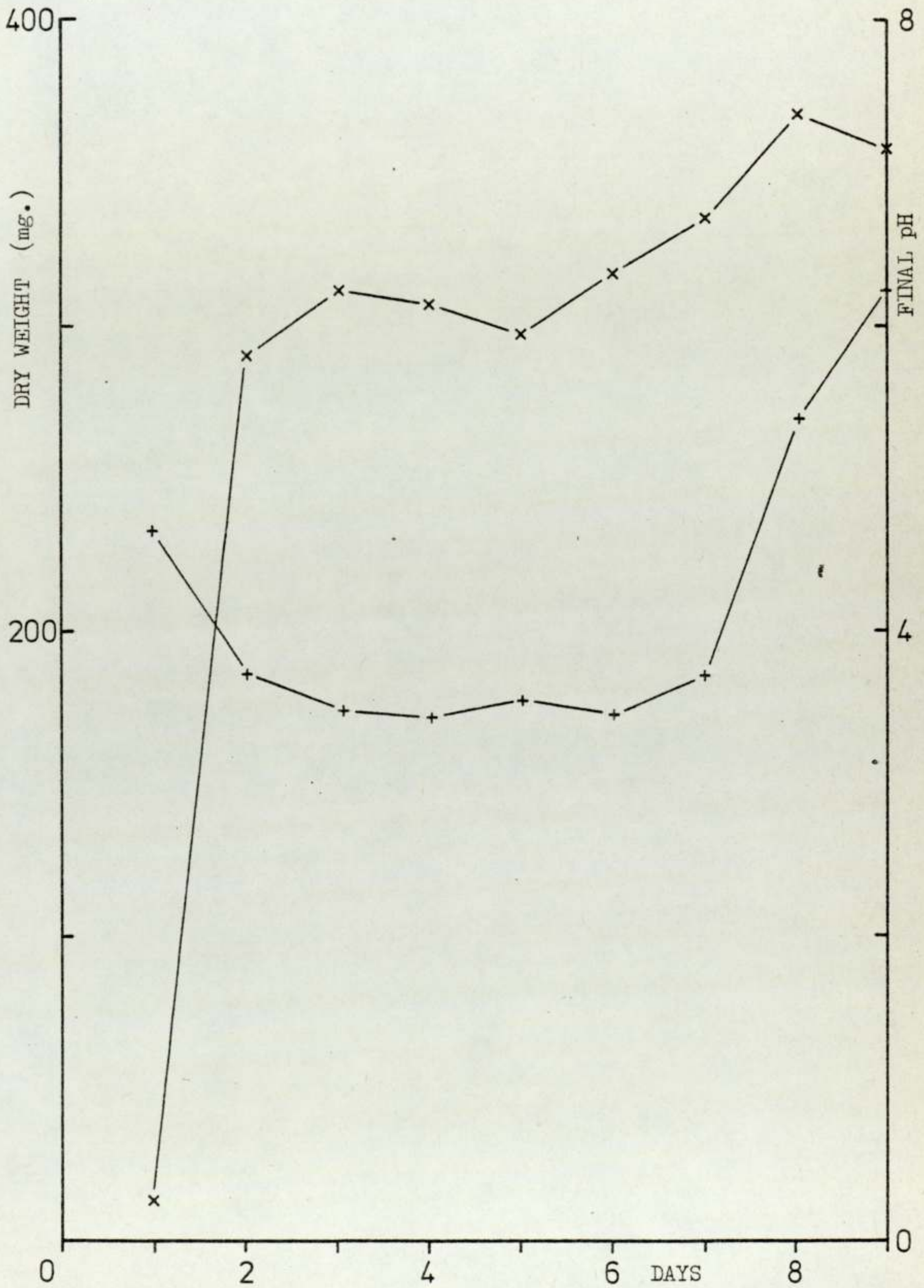
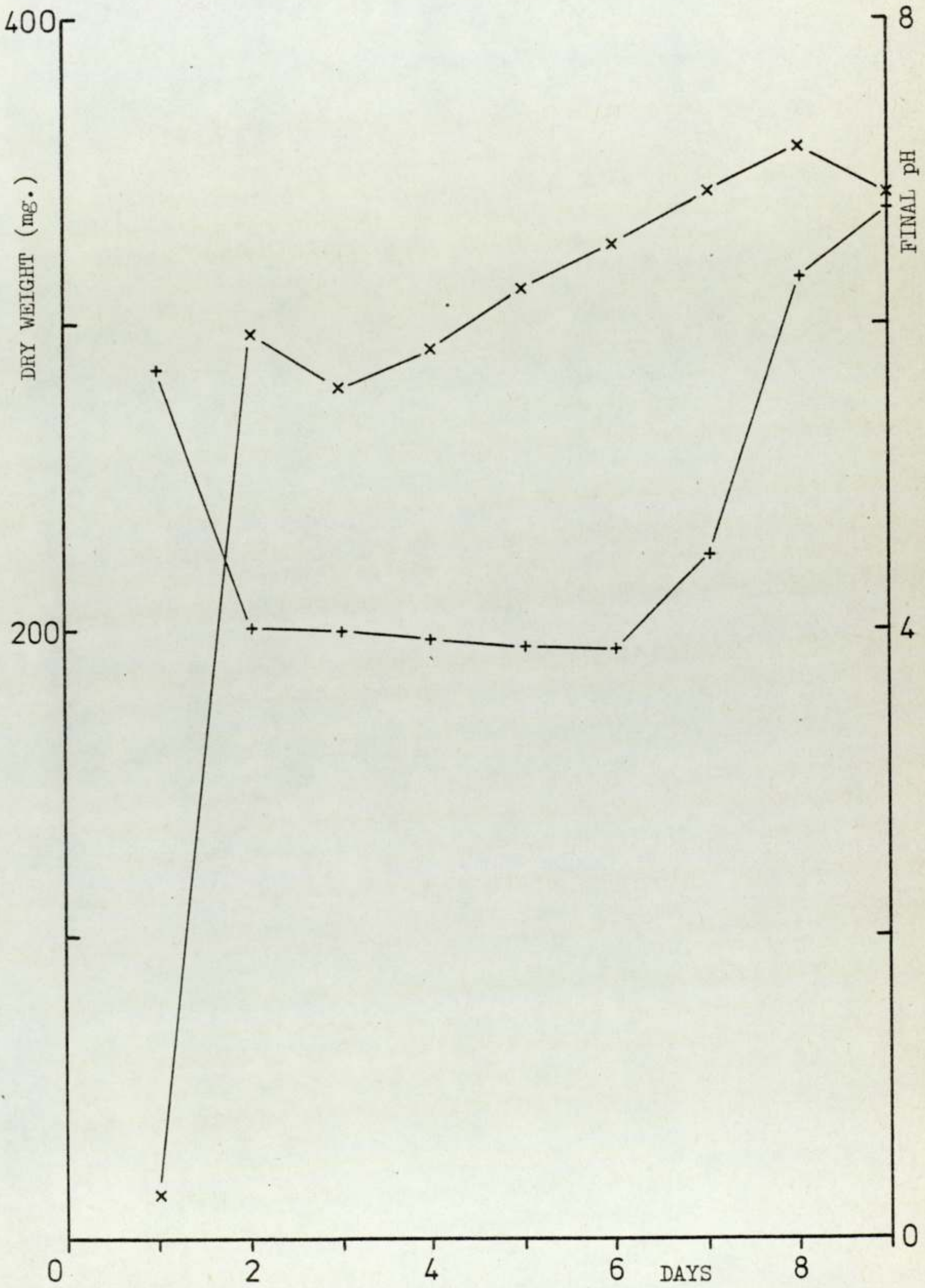


FIG. 4.23 Geotrichum candidum:-

Daily changes in dry weight (×) and pH (+) in shake flasks at 20°C. (Inital pH 6).



in weight on Day 3 which was produced by a low weight in one flask. The pH dropped between Day 1 and Day 2, fell slightly until Day 5 and then rose sharply until the final determinations on Day 9.

The results for pH 7 were recorded in Table 6.20 and expressed graphically in Fig. 4.24. At pH 7 G. candidum reached its maximum mass on Day 8. The pH decreased until Day 4, fluctuated for two days and then increased sharply between Day 6 and 7 until final readings on Day 9.

There appeared to be a generally similar trend in the pH curves of G.candidum regardless of the initial pH. (Figs 4.21 - 4.24). The initial pH decreased sharply at first, levelled for a few days and then climbed steeply as the growth progressed. G.candidum had produced its maximum biomass on Day 7 with an initial pH 4 whereas Day 8 showed the best crop at the other pH values of 5, 6 and 7.

Examining the individual data for each flask in Tables 6.17 to 6.20 revealed a pattern in the relationship between flask-culture pH and dry weight. In the initial stage of growth a low pH was associated with a comparatively high weight for each set of three replicates on a particular day. In the intermediate phase, when the pH was levelling off, the relationship between flask pH and dry weight became untenable. The final phase of a sharp rise in pH signified a change in the relationship when, generally, the highest pH resulted in the highest dry weight.

Fig. 4.25 was drawn from the data recorded in Tables 6.17 to 6.20 showing the daily changes in dry weight of G.candidum over nine days. Fig. 4.25 suggested that the optimum pH for G.candidum over nine days. Fig. 4.25 suggested that the optimum pH for G.candidum was pH7 with the best crop produced between Day 7 and 8, but the other pH curves were similar after the first four days and

FIG. 4.24 Geotrichum candidum:-

Daily changes in dry weight (×) and pH (+) in shake flasks at 20°C. (Initial pH 7).

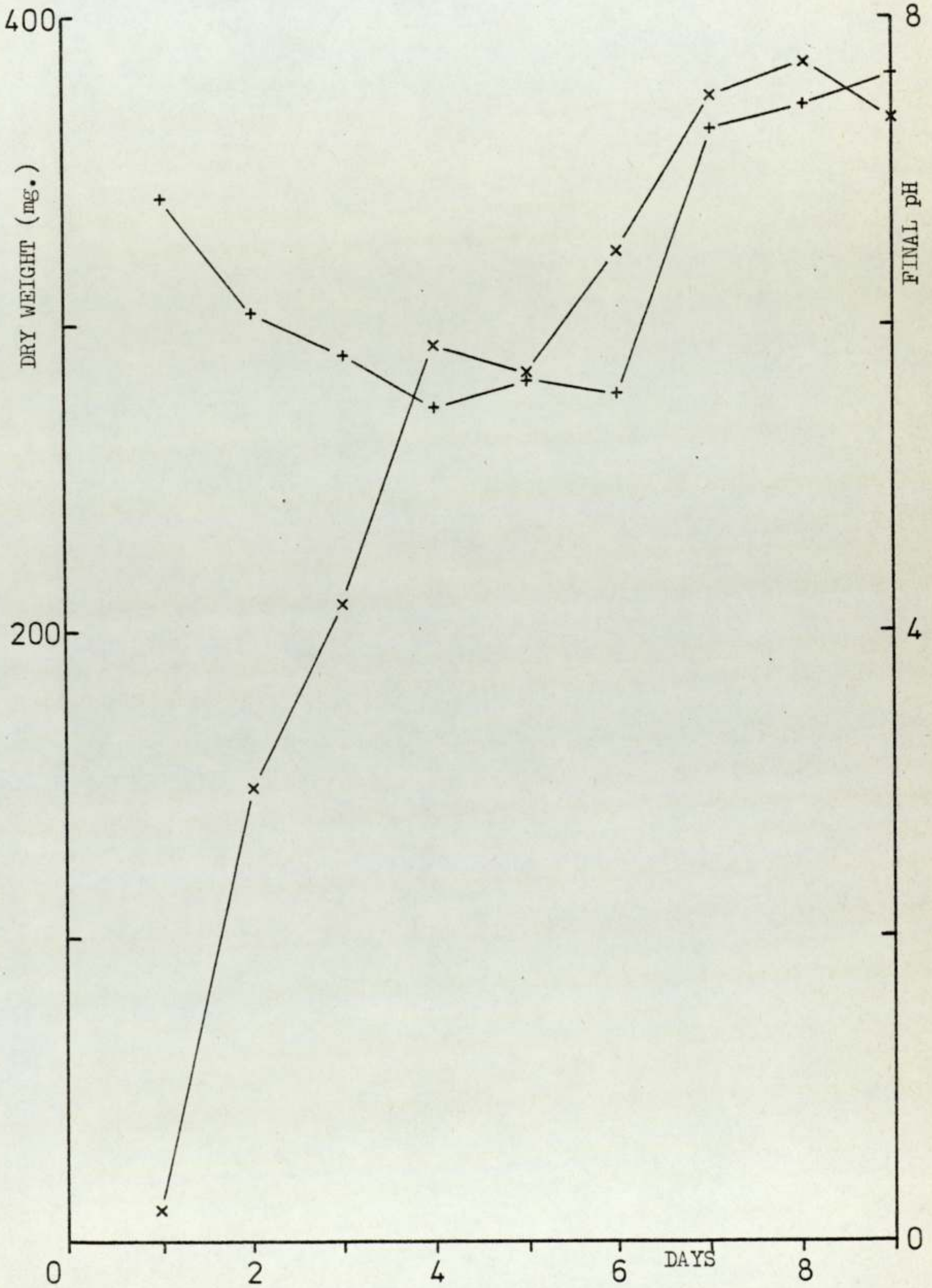
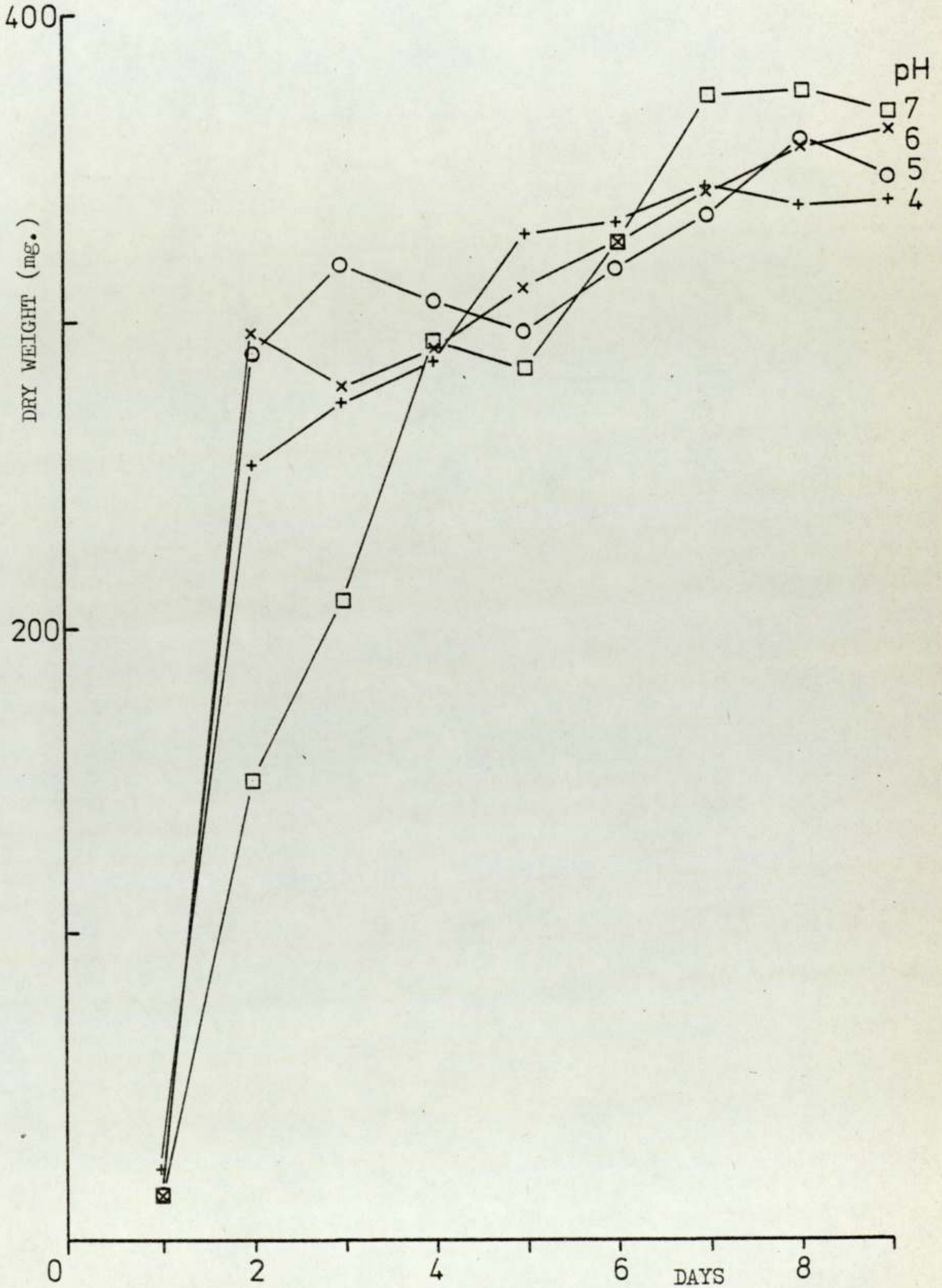


FIG. 4.25 Geotrichum candidum:-

Extract from TABLES6.17 to 6.20 showing daily changes in dry weight of the fungus in shake flasks at 20°C. (Initial pH4, 5, 6, 7).



even had a shorter lag phase than pH 7.

A microscopical examination of the contents of four flasks of Geotrichum candidum was performed to monitor the effects of four different initial pH values (4, 5, 6 and 7). The flask cultures were all similar in colour, on Day 9 the arthrospores in the pH4 flask were swollen in appearance and there seemed to be a higher concentration of them at pH4 and 5 than at pH6 and 7.

Fusarium tabacinum

The relationship between initial pH value of medium and growth, measured as dry weight over a period of nine days, was investigated using a procedure identical to that used for Geotrichum candidum.

Changes in pH and the dry weight of fungus over nine days were recorded in Tables 6.21 to 6.24 for initial pH values of 4, 5, 6 and 7 (respectively) and these results were expressed graphically in Figs 4.26 to 4.29.

The graphs followed a broadly similar pattern regardless of the initial pH value of the medium. There was an initial decrease in pH over the first one to two days, then a sharp but steady increase with a small dip in each pH curve on Day 7. On the ninth day, the mean final pH lay between pH 7 and 8.

The dry weight curves were similar over the first six days regardless of the initial pH. There was a marked increase in weight between Day 1 and Day 2 with a peak on Day 4 (Fig. 4.26 and 4.27), Day 5 (Fig. 4.28) and Day 6 (Fig. 4.29). There was a decrease in weight after the peak on each curve followed by slight fluctuations. Fig. 4.26 and 4.28 showed a decline in weight from Day 6 onwards (initial pH 4 and 6) whereas Fig. 4.27 and 4.29 (initial pH5 and 7) showed an increase from Day 8.

Examination of the individual results for each flask (Tables 6.21 to 6.24) showed that there was a fairly close relationship

FIG. 4.26 Fusarium tabacinum:-

Daily changes in dry weight (×) and pH (+) in shake flasks at 20°C. (Initial pH 4).

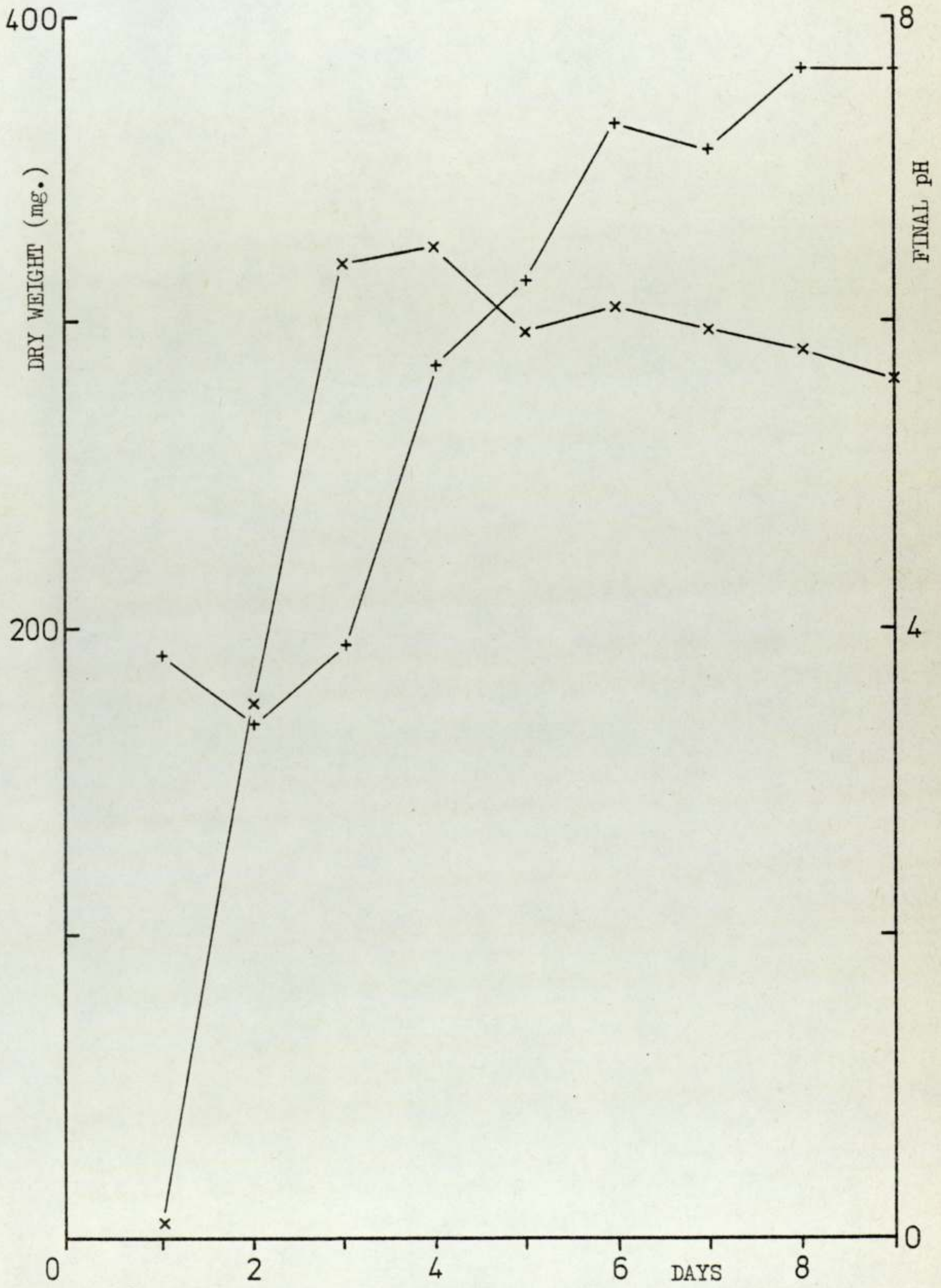


FIG. 4.27 Fusarium tabacinum:-

Daily changes in dry weight (×) and pH (+) in shake flasks at 20°C. (Initial pH 5).

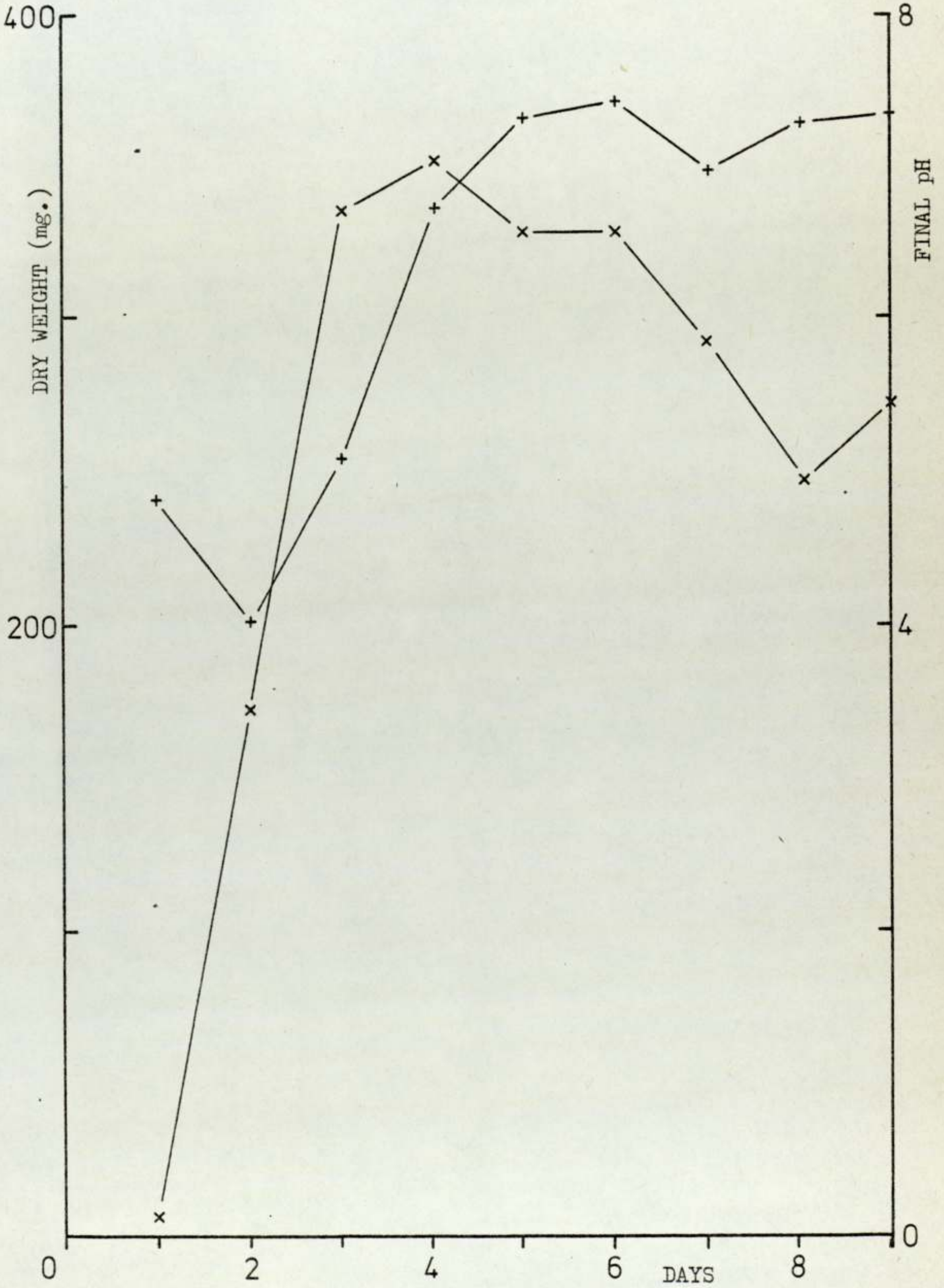


FIG. 4.28 _Fusarium_ _tabacinum_:-

Daily changes in dry weight (×) and pH (+) in shake flasks at 20°C. (Initial pH 6).

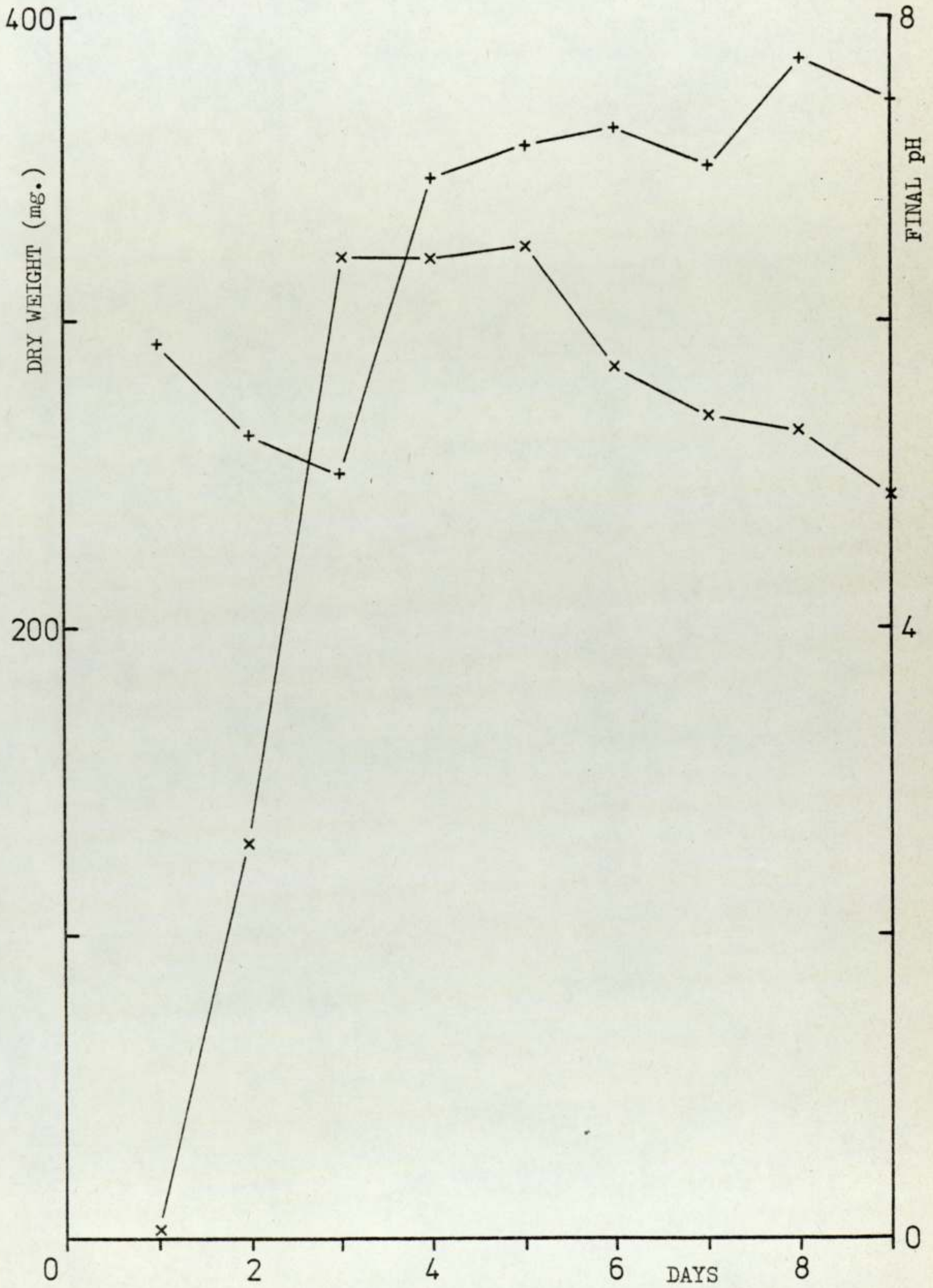
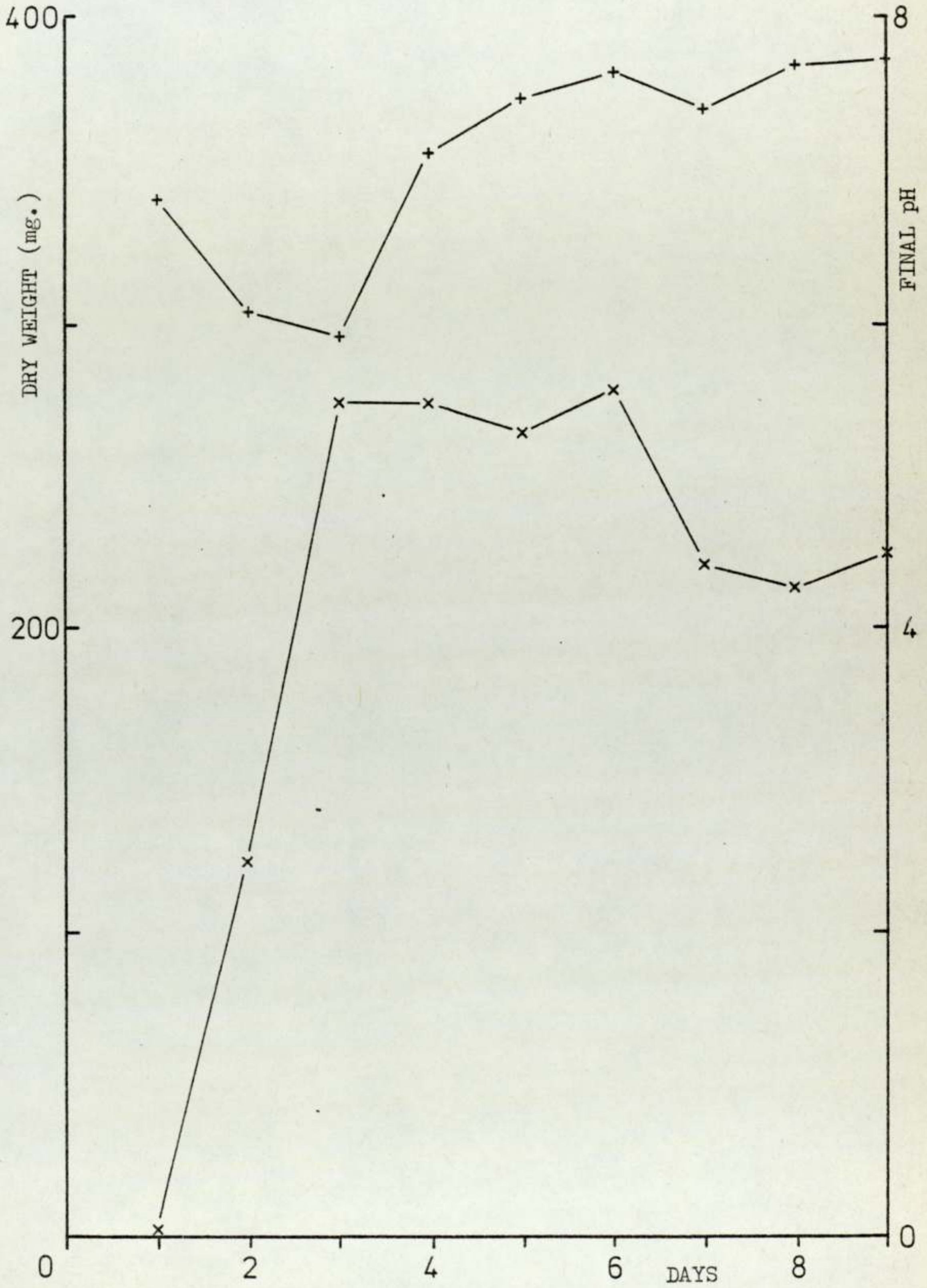


FIG. 4.29 Fusarium tabacinum:-

Daily changes in dry weight (×) and pH (+) in shake flasks at 20°C. (Initial pH 7).



between pH and dry weight especially in the initial phase of a sharp weight increase and pH decrease. Generally, the lowest pH was associated with the highest weight for each group of triplicate flasks.

Fig. 4.30 was drawn from data compiled from Tables 6.21 to 6.24. Comparing the four curves showed that the optimum pH for F.tabacinum was pH5 and the best crop was achieved on Day 4 at this pH.

A microscopical examination of the contents of four flasks of F.tabacinum, at four different initial pH values (4, 5, 6 and 7), was performed using extra flasks that were not harvested but maintained to monitor any effects of the pH range. There were no outstanding differences among the four pH values, in each flask were large numbers of conidia with marginally more mycelium at pH4. The flask cultures did have distinctive colours, pH4 was pink, pH5 was whitish-yellow, pH6 was yellowish-white and pH7 was yellow,

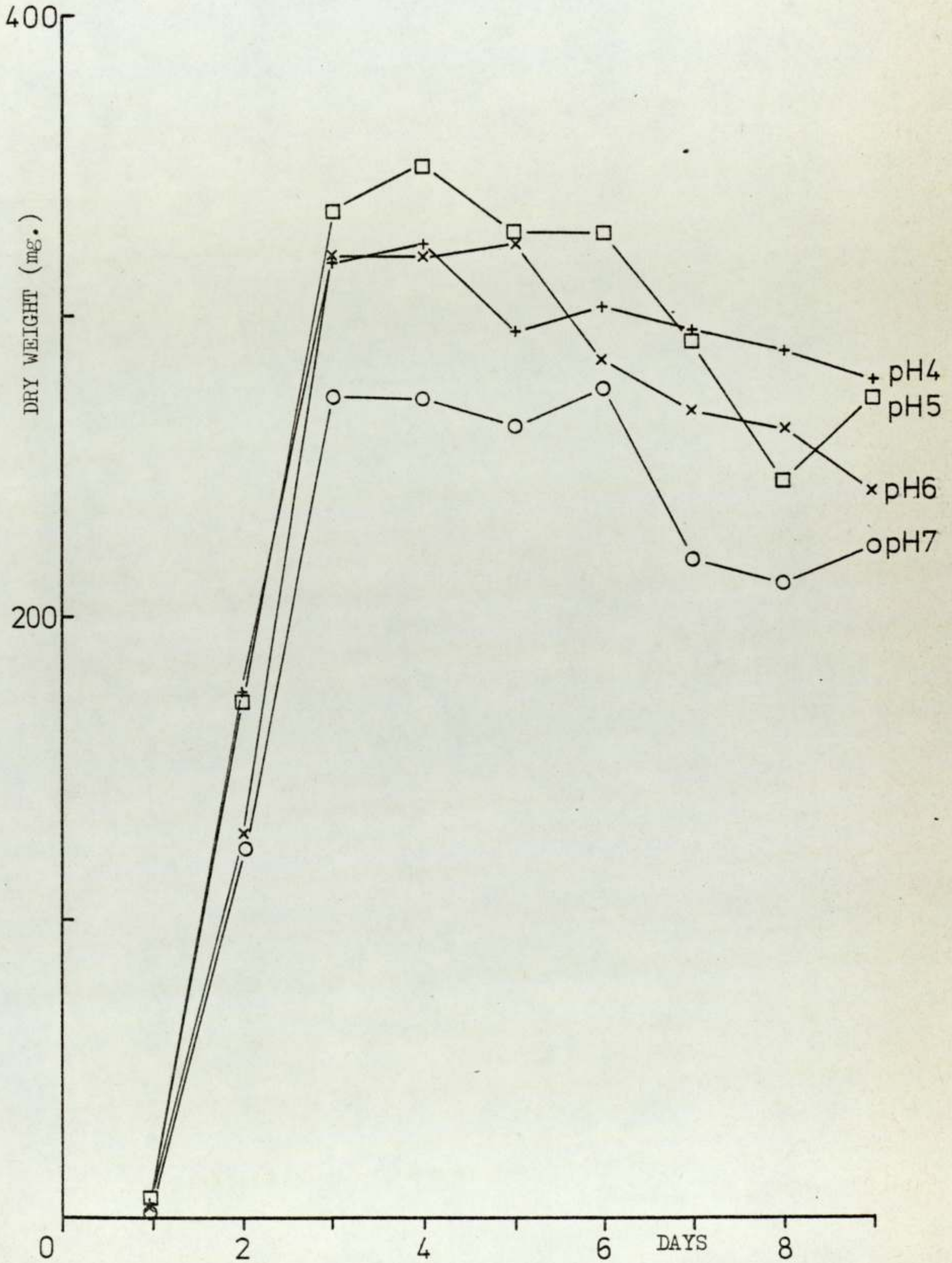
During the investigation from Day 5 onwards it was increasingly difficult to vacuum-filter the flasks at pH5, 6 and 7 and as many as five filter papers were needed for one flask. All the pH4 flasks filtered very easily, probably due to the higher proportion of mycelium to conidia, which may have affected the porous paper by causing blockages.

4.3.4 The effect of hydrogen ion concentration on the growth of Geotrichum candidum and Fusarium tabacinum measured as culture turbidity using a buffered medium.

The experiments performed in section 4.3.3 were based on similar work by Painter (1954) using a medium which he formulated and called medium A. This medium was not buffered which meant that fungi growing in it were able to adjust the pH to values which favoured their growth.

FIG. 4.30 _Fusarium_ _tabacinum_:-

Extract from TABLES 6.21 to 6.24 showing daily changes in dry weight of the fungus in shake flasks at 20°C. (Initial pH4, 5, 6, 7).



The changes in pH of shake-flask cultures of G.candidum and F.tabacinum were monitored and expressed graphically in Figs 4.21 to 4.24 and 4.26 to 4.29, proving that both fungi were able to alter the pH of the medium supporting their growth. The graphs confirmed the suspicions of Tomlinson and Williams (1975) concerning the probable influence of an unbuffered medium on the results reported by Painter (1954). Taking these facts into consideration, it was decided to pursue the pH investigations using buffered Painter's medium A and a different method for monitoring the growth based on work done by Trinci (1972).

Geotrichum candidum was one of the four species of fungus studied by Trinci in 1972 and used to evaluate the measurement of growth in submerged culture by changes in culture turbidity. The method was successful in following the growth of the fungi and it was possible to monitor the different phases. The reason for the success was due to the homogeneous, filamentous nature of the growth of the chosen fungi in submerged culture. The majority of moulds produce pellets of mycelium, when grown in submerged shake-flask culture, which have a tendency to settle during measurements of the turbidity and produce inaccurate readings.

In the third stage of these investigations of pH and growth, the first buffer to be chosen was the BDH Universal Buffer Mixture. This buffer was a mixed salt formulated according to Prideaux and Ward (1924) and was used in conjunction with Painter's Medium A (4.3.2). The buffer solution contained a mixture of phosphoric, acetic and boric acids made up so that the final solution was 0.02N with respect to each hydrion and the whole was 0.1N. The buffer was supplied in tubes, the contents of one tube being sufficient for one litre of solution and the constituents of Medium A were added accordingly. The addition of 0.2N HCl or NaOH to the buffered medium enabled

a range of pH values from 2.7 to 11.4 to be achieved. The buffered solution was dispensed in 100 ml. aliquots to 250 ml. conical flasks fitted with metal caps over rimless necks. Two flasks were used for each pH from 3 to 10, one flask of each pair being inoculated with Geotrichum candidum and the other with Fusarium tabacinum. The inocula were 7 mm. agar discs cut from the vegetative margin of a seven-day old colony of both fungi. The flasks were placed on an orbital shaker set at 132 r.p.m. in a constant-temperature cabinet at 15°C for a trial period of seven days.

After seven days the flasks were removed from the shaker and examined for fungal growth. Neither G.candidum nor F.tabacinum had produced any more than minimal growth at any pH from 3 to 10. Boric acid and Borax were two components of the mixed buffer salt which operated in the range of pH7 to 9, it was decided that these and the other components of the mixed salt were toxic to the two fungi and an alternative buffer complex was sought.

The choice of buffer mixture was dependent on the range of pH values attainable so Britton and Robinson's Straight-Line Buffer Mixture was chosen for the second trial. The buffer mixture in solution was capable of producing any pH value between 2.5 and 12.0 by adding 0.2N HCl or NaOH. Britton and Robinson described the buffer mixture in 1931. (J. Chem. Soc., 1456, 1931), the "Straight-Line" of the name arose because the buffer solution changed pH between 4.4 and 8.35 in exact proportion to the quantity of alkali added. The buffer mixture was composed of four acids 0.2N with respect to the total replaceable hydrogen giving rise to seven stages of dissociation which are successively neutralised with sodium hydroxide. The solution contained potassium dihydrogen phosphate, citric acid, veronal, boric acid and hydrochloric acid dissolved

in water and made up to a litre. One tube of the buffer mixture was sufficient to make one litre of solution of pH 3.85, values above this were achieved by the addition of 0.2N NaOH according to the equation:

$$pH = 3.91 = 0.0853\ V$$

$$\text{or} \quad V = 11.723\ (pH-3.91)$$

where V = ml. 0.2N NaOH added to 100ml. of the buffer solution. pH values below 3.85 were achieved by the addition of 0.2N HCl.

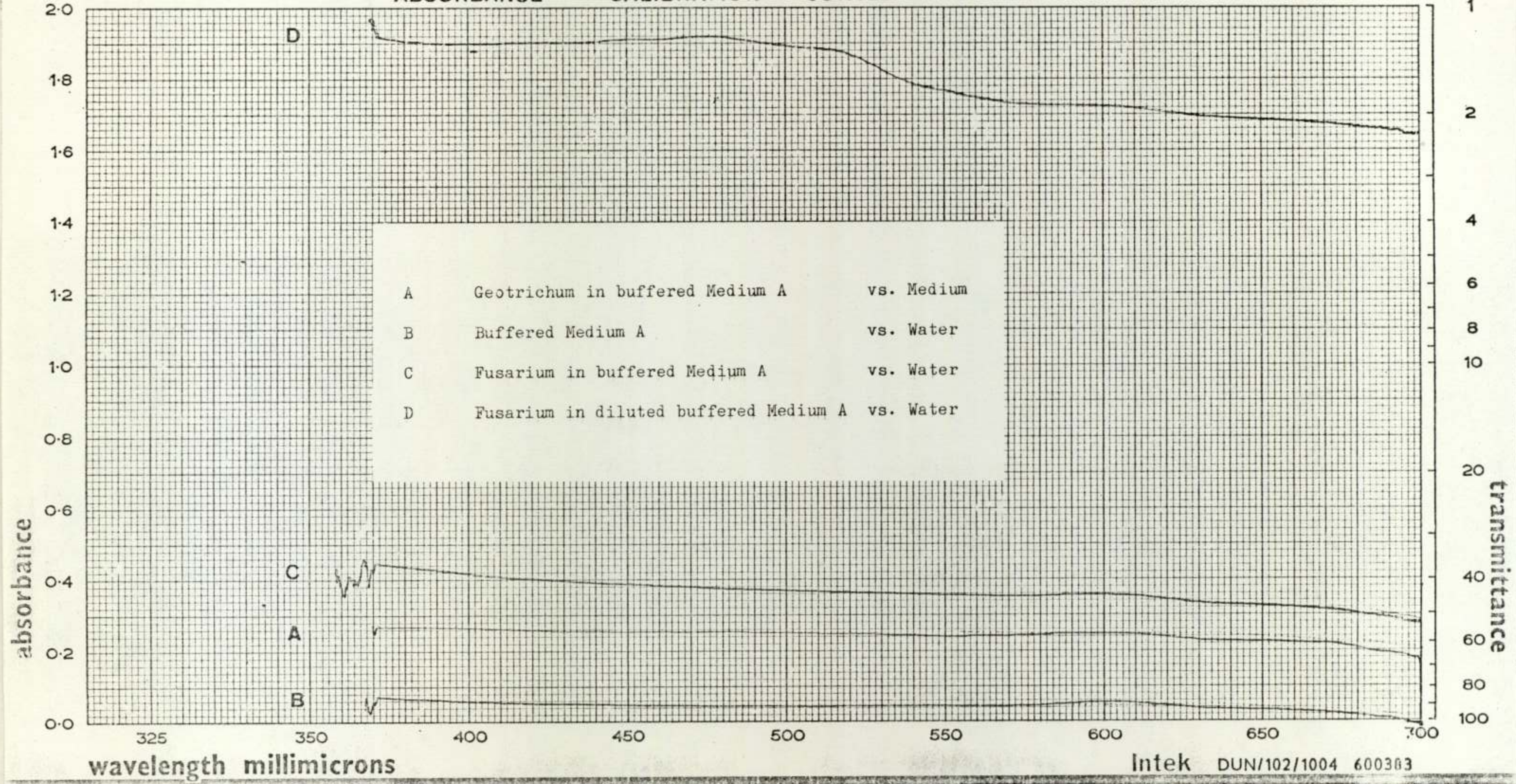
The constituents of Painter's Medium A were mixed with Britton and Robinson's buffer in litre quantities, the glucose used was dispensed and autoclaved separately to prevent caramelisation. Two flasks were used for each pH ranging from 2 to 10, one flask was for Geotrichum candidum and the other for Fusarium tabacinum inoculated in the form of agar discs cut from the vegetative margin of seven-day old colonies. The buffered medium A mixture was dispensed in 100 ml. aliquots to each 250ml. rimless conical flask, after inoculation the flasks were placed on an orbital shaker set at 132 r.p.m. in a constant-temperature cabinet kept at 15°C. The flasks were left for seven days with daily visual checks on the progess of fungal growth.

At the end of seven days the contents of each flask was examined. The results were encouraging, G.candidum produced plenty of hyphae and arthrospores between pH3 and 6 but very little growth at pH2, 8, 9 and 10.

F.tabacinum produced plenty of hyphae and conidia between pH4 and 7 with no growth at pH2 and minimal growth at pH3, 8, 9 and 10.

These results indicated that both fungi were able to grow in the buffered medium and it was decided to carry out further studies on the pH-growth relationship related to harvest times.

In the previous section (4.3.3) the pH-growth relationship


SP 800
ABSORBANCE CALIBRATION CURVES
A Geotrichum in buffered Medium A vs. Medium
B Buffered Medium A vs. Water
C Fusarium in buffered Medium A vs. Water
D Fusarium in diluted buffered Medium A vs. Water
D
C
A
B
absorbance
2·0
1·8
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
0·0
325
350
400
450
500
550
600
650
700
wavelength millimicrons
transmittance
1
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Intek DUN/102/1004 600383
ALIGN WITH INDEX ON THE RECORDER
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PATH LENGTH
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SCAN SPEED FAST
SLOW
DATE 20-2-78
OPERATOR
RLF NO.

was studied using shake-flasks but each flask was harvested directly after the pH had been measured. It was decided that a system which enabled continuous pH monitoring until the time chosen for harvesting would reveal more information than the previous discrete system. The method of culture turbidity measurement as adopted by Trinci (1972) was chosen for continuous monitoring of growth. The initial step in this study was to determine the wavelength of light suitable for use in measuring the growth of G.candidum and F.tabacinum. The instrument used for calibrating the optical density of a sample at different wavelengths was an Ultraviolet Spectrophotometer S.P.800. Both fungi were cultured from 7 mm. discs in shake-flasks of 100ml. of medium A buffered with Britton and Robinson's Straight-Line Buffer with the pH unaltered at 5.7. After six days in this medium two drops of culture fluid and fungus were transferred aseptically to fresh flasks of the same medium. The flasks were examined daily but there was no appreciable growth until four days had passed. On the fifth day samples were withdrawn from the flasks, transferred to glass cuvettes and placed in the spectrophotometer. Distilled water and the buffered medium were used as references for the samples under test. Both fungi maintained a steady absorbance of light in the wavelength range from four-hundred to six-hundred millimicrons so it was decided to carry out further experiments using a wavelength of five-hundred and eighty millimicrons. **(See facing page)**

The inocula for these experiments were obtained in a similar manner to the last pH studies using shake-flasks. Geotrichum candidum and Fusarium tabacinum were cultured on 20 ml. plates of SF agar and PD agar respectively. After six days 7 mm. cork-bored discs were removed from the vegetative margin of the colonies and transferred aseptically to shake-flasks containing 100 ml. of

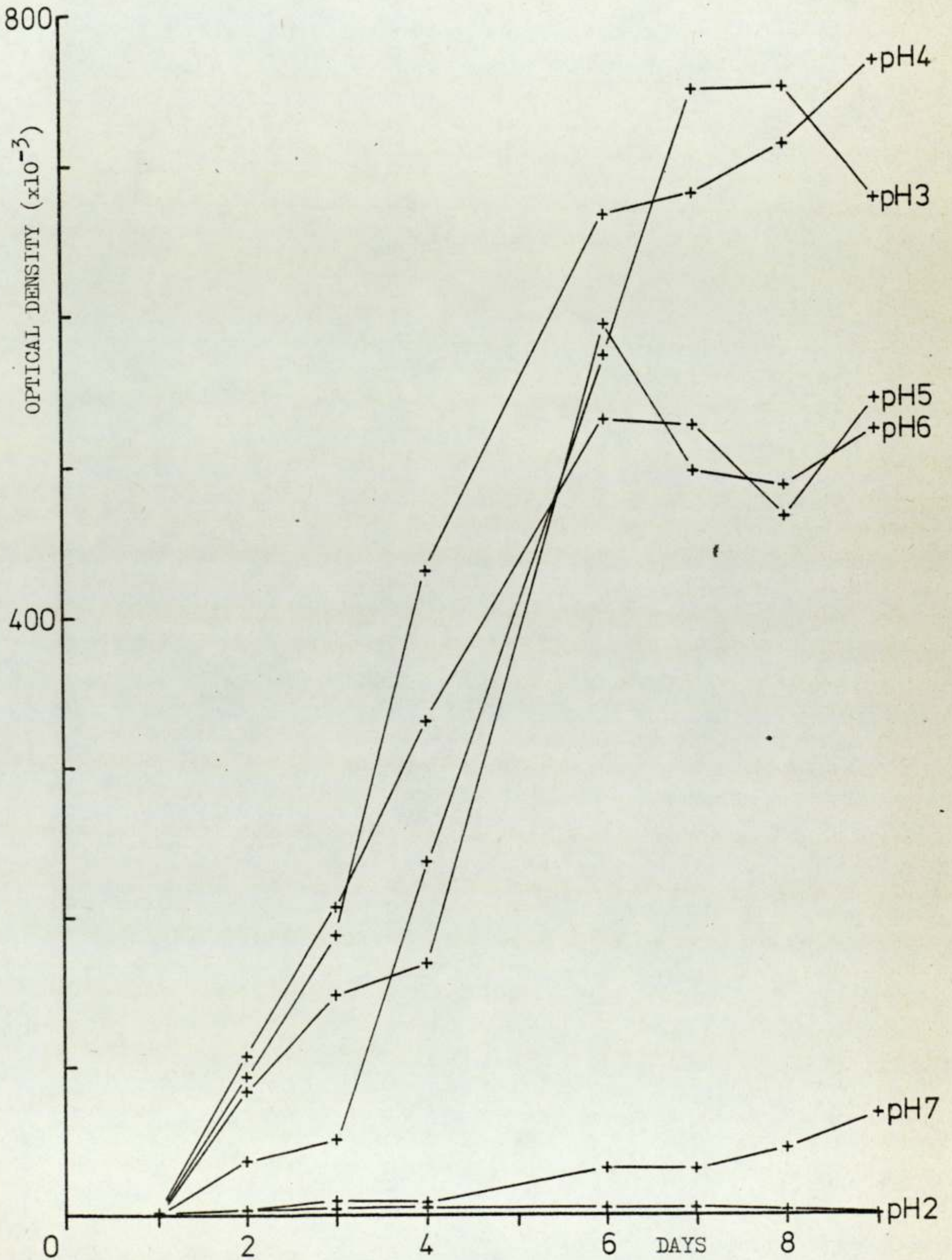
Painter's medium A/Britton and Robinson's buffer. Three discs of fungus were used per flask, these were placed on an orbital shaker at 100 r.p.m. in a constant-temperature cabinet set at 15°C and left for six days. The experimental flasks with different pH values were inoculated from the shake-flask cultures using sterile Pasteur pipettes to deliver two drops of culture fluid to each batch of three flasks for each pH.

The first trial was performed using G.candidum inoculated, as described, into triplicate flasks of buffered medium A with a range of pH values from 2 to 7, based on the initial experiment with this medium. Samples of culture fluid were removed from the 100ml. shake-flask cultures using sterile Pasteur pipettes, a dilution of 1 in 10 with distilled water was used initially to facilitate the spectrophotometer readings. The diluted samples were placed in glass cuvettes and their absorbance of light compared with distilled water used as a reference. The readings were taken, using a Beckman DB Spectrophotometer, daily for nine days and the results were recorded in Table 6.25 and expressed graphically in Fig. 4.31. On the ninth day the flasks were harvested after withdrawing the samples for the optical density measurements. The final pH of each flask and the dry weight were recorded with the final optical density readings in Table 6.26 and the results were expressed graphically in Figs. 4.32 and 4.33.

Fig. 4.31 showed the daily changes in optical density of G.candidum and revealed the difference between growth in medium A when buffered. The results in the previous section showed G.candidum to have a broad range of pH which supported good growth but the buffered medium reduces the range from pH3 to 6. The graph showed that this fungus is capable of growth at acidic pH values with a possible optimum harvest time of

FIG. 4.31 Geotrichum candidum:-

Effect of initial pH(2-7) on optical density (absorbance at 580 nm.) over nine days (First trial)



six days within the limitations of flask-culture.

Fig. 4.32 showed that even in a buffered medium the final pH had been raised by G.candidum above the initial pH values ranging from pH3 to 6, and lowered from the initial values ranging from pH7 to 10. There was very little difference in the final dry weight of the fungus between pH3 and 6 through there was a marginal increase in weight above the other readings at pH3.

Fig. 4.33 was drawn to see if it was feasible to relate the optical density readings with dry weight as an alternative measure of growth. The two curves were very similar in shape except at pH4 which had the highest optical density, recorded as the mean of three determinations, but a lower dry weight than pH3. Examining the individual results in Table 6.26 revealed that the flask with the highest optical density reading produced the heaviest dry weight, for each group of three flasks at each pH.

It appeared from the results with G.candidum that the optimum harvest time was on Day 6. The second trial was carried out in the same manner as before only this time it was completed on the sixth day. The results of the daily optical density readings, the final pH values and the dry weights after harvesting were recorded in Table 6.27 and graphs plotted using the means of the triplicate readings in Figs. 4.34 to 4.36.

Fig. 4.34 showed pH4 to be the optimum in the range pH3 to 7, the results for pH3 were similar to pH4 apart from a sharp decrease in the sixth day which was unexpected.

Fig. 4.35 compared the initial pH to the final pH and dry weight of G.candidum. The final pH values were adjusted upwards with pH4 and 5 and downwards with the other values, the optimum pH was pH4 which had a marginal lead over pH3 and 5, confirming the findings

FIG. 4.32 Geotrichum candidum:-

Relationship of initial pH to final pH (+) and dry weight (×) when harvesting on Day 9 (First trial).

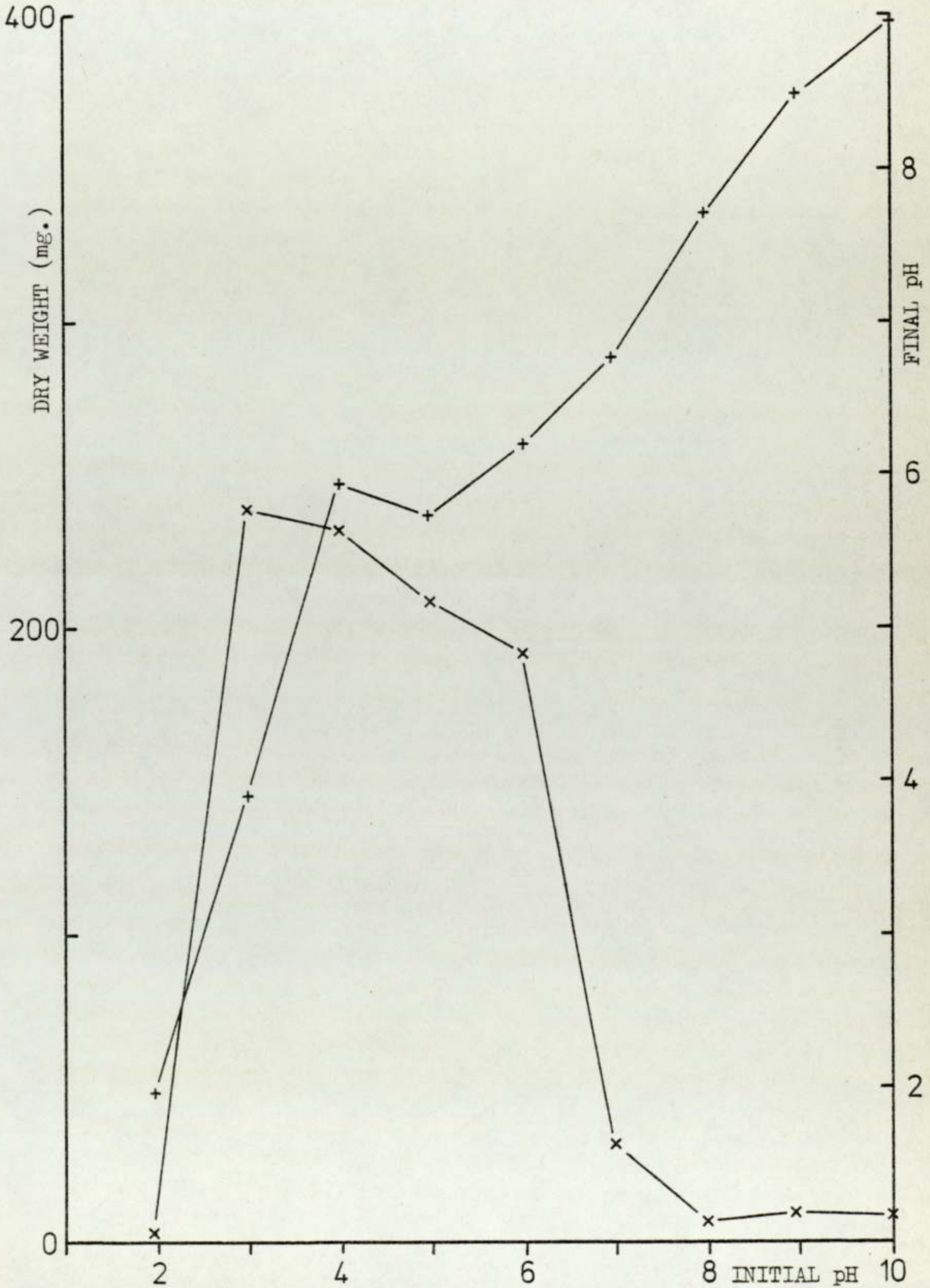


FIG. 4.33 Geotrichum candidum:-

Relationship of initial pH to dry weight (×) and optical density at 580 nm. (+) when harvesting on Day 9. (First trial).

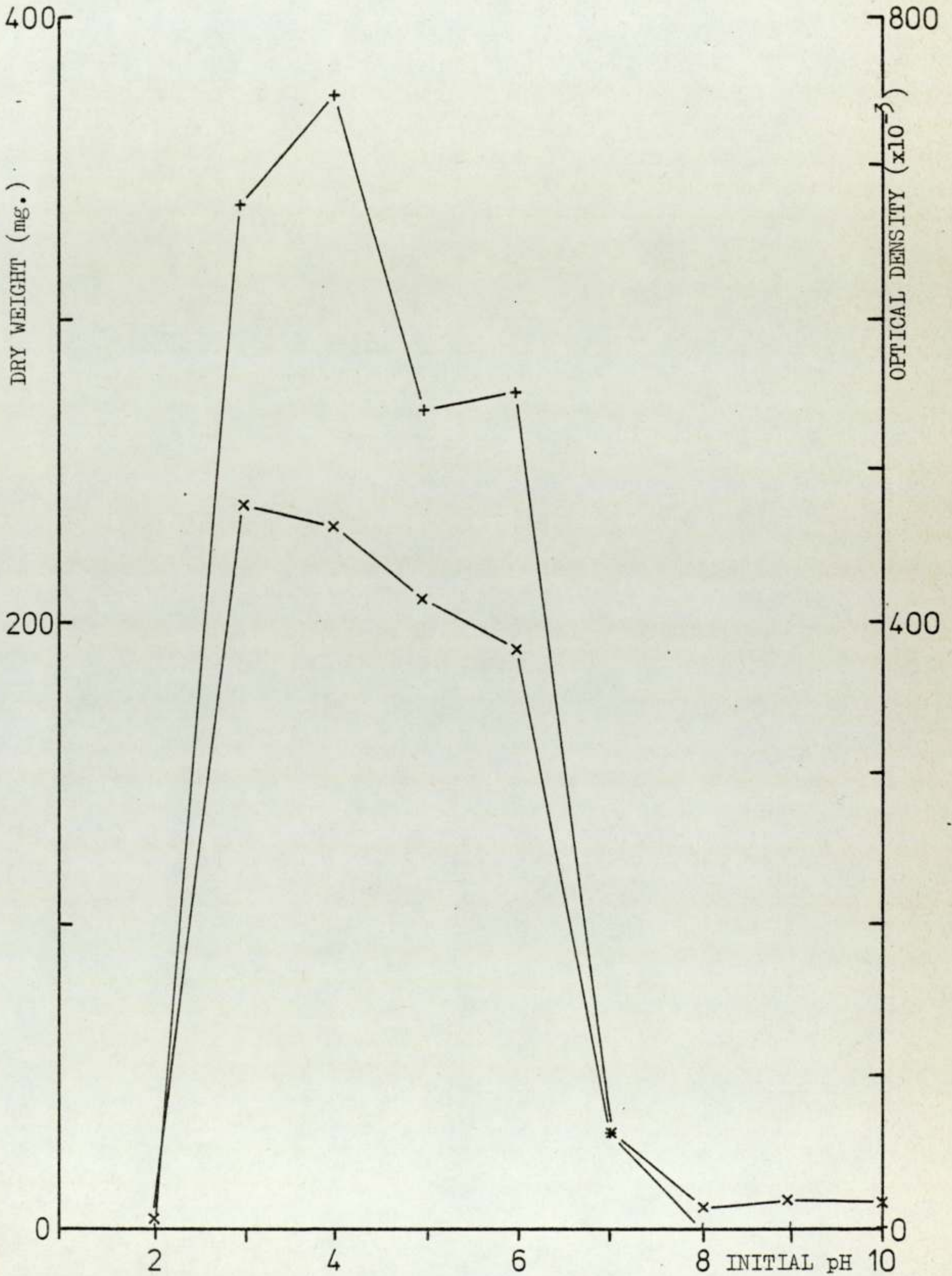


FIG. 4.34 Geotrichum candidum:-

Effect of initial pH (3-7) on optical density (absorbance at 580 nm.) over six days. (Second trial).

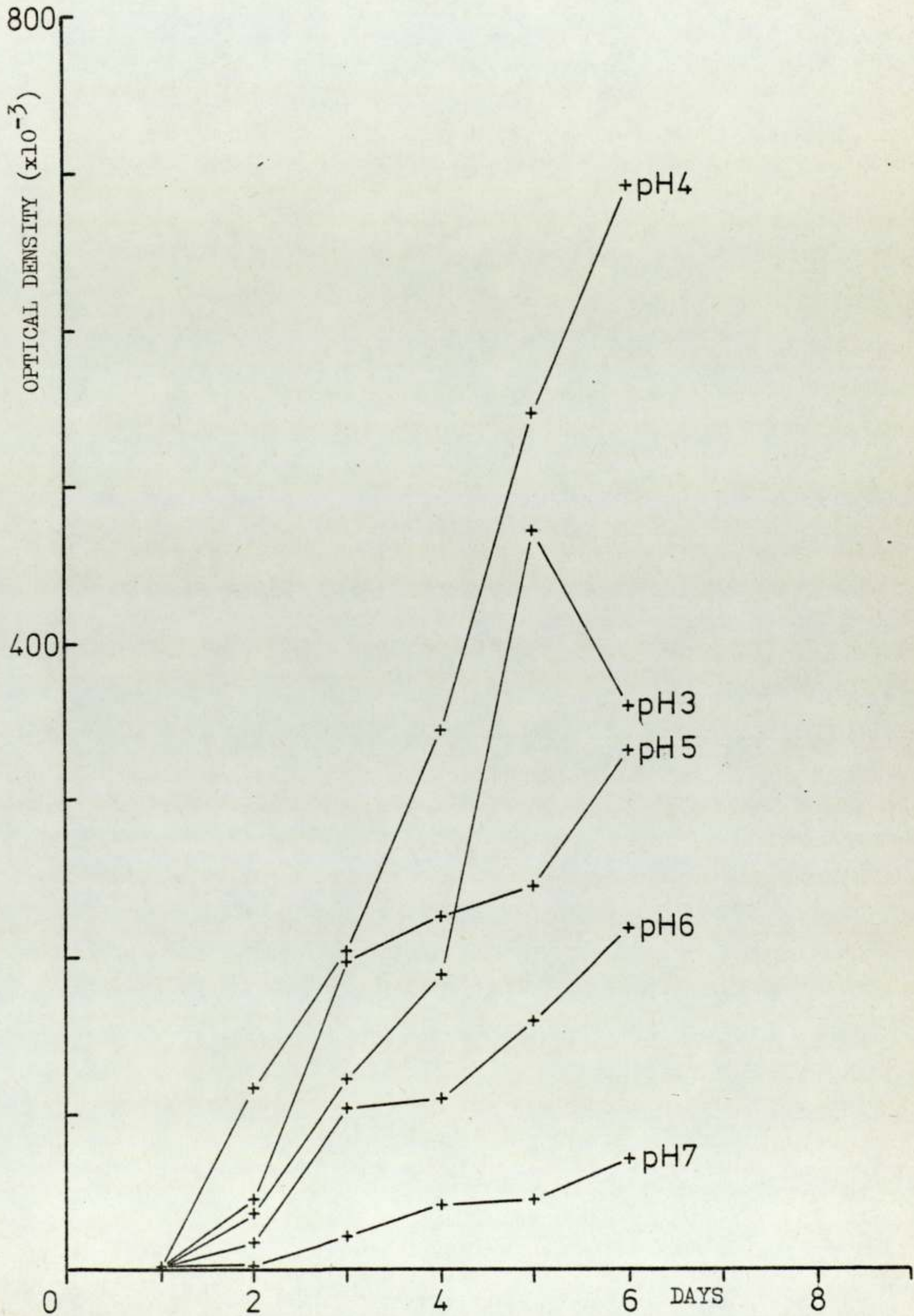


FIG. 4.35 Geotrichum candidum:-

Relationship of initial pH to final pH (+) and dry weight (×) when harvesting on Day 6 (Second trial).

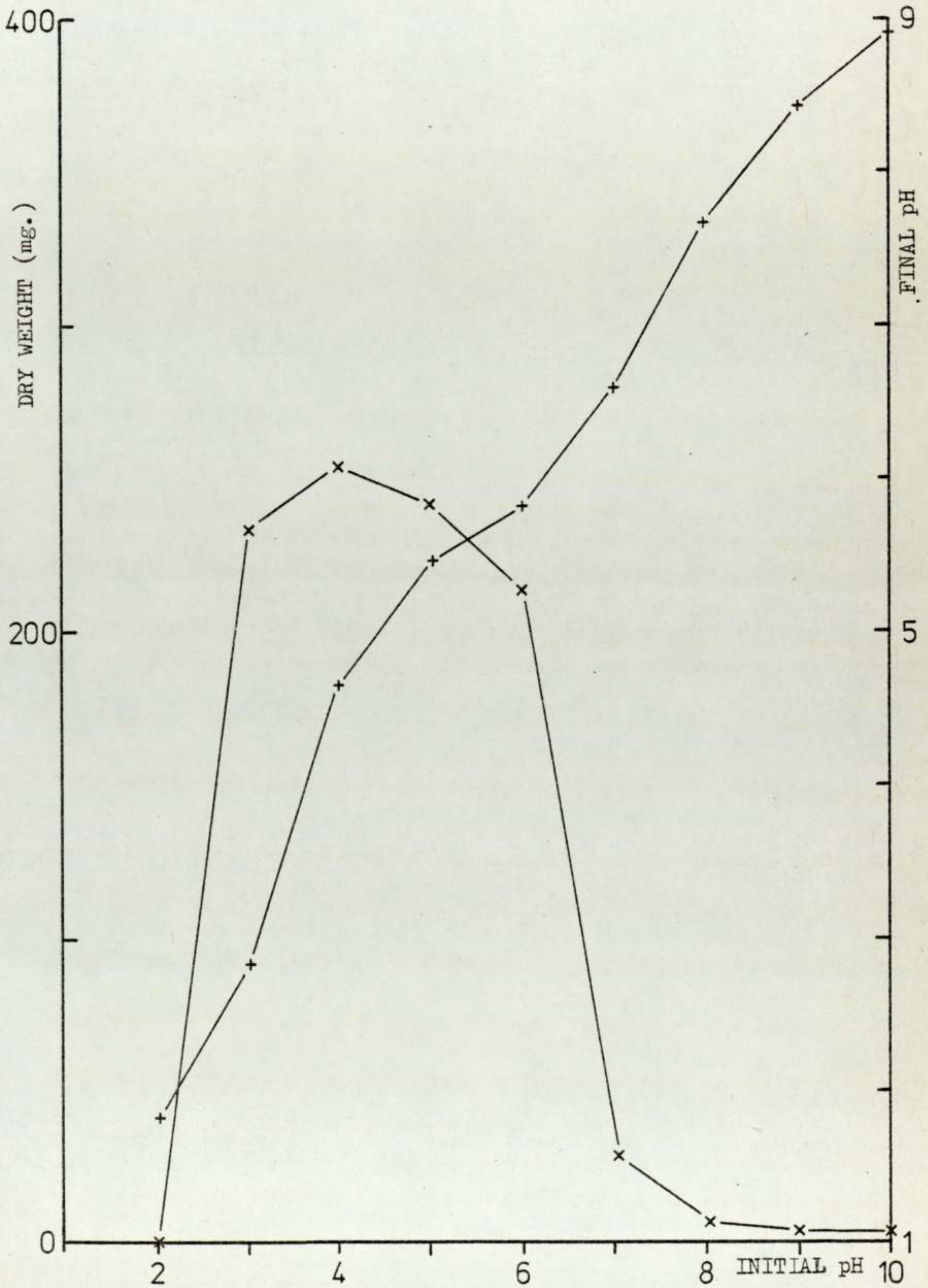
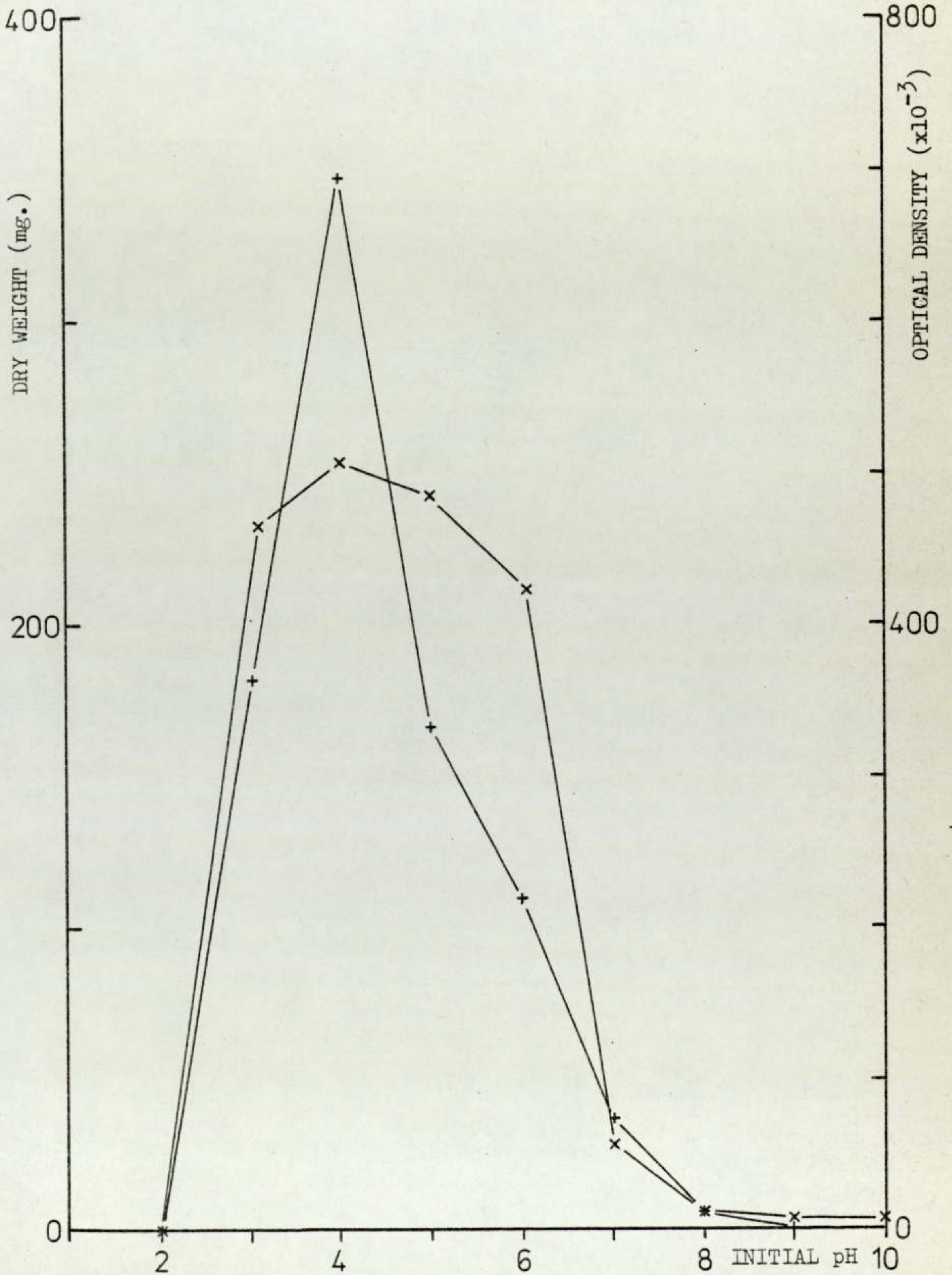


FIG. 4.36 Geotrichum candidum:-

Relationship of initial pH to dry weight (×) and optical density at 580 nm. (+) when harvesting on Day 6 (Second trial).



of the daily optical density readings.

Fig. 4.36 compared the final dry weight and optical densities for each pH. The pattern of the two curves was again broadly similar with the optical density readings giving a much clearer maximum at pH4.

The method of using optical density readings as a measure of fungal growth in a buffered medium appeared to be successful with Geotrichum candidum and the experiment was repeated using Fusarium tabacinum.

Triplicate flasks of buffered medium A at pH values ranging from 2 to 10 were inoculated with F.tabacinum as described. The flasks were placed on an orbital shaker set at 110 r.p.m. in a constant-temperature cabinet maintained at 15°C. Optical density measurements were taken daily over nine days with harvesting and dry weight measurement on Day 9. The results were recorded in Table 6.28 and graphs plotted of the means of the triplicate data in Figs. 4.37 to 4.39.

Fig. 4.37 showed the daily changes in optical density over the nine days. The optimum pH was pH5, closely followed by growth at pH4 and 6, growth at pH3 and 8 only became apparent on the seventh day but increased noticeably until harvesting on the ninth day.

Fig. 4.38 showed that the optimum pH was not as clearly defined when the measurement of growth was based on dry weight. In a buffered medium F.tabacinum also showed that its optimum pH lay in a narrow range from pH4 to 6.

Fig. 4.39 compared the two curves of optical density and dry weight against initial pH. These curves followed a very similar pattern and showed that optical density measurements were a reliable means of measuring growth of F.tabacinum as well as G.candidum.

FIG. 4.37 Fusarium tabacinum :-

Effect of initial pH (3-8) on optical density (absorbance at 580 nm.) over nine days. (First trial).

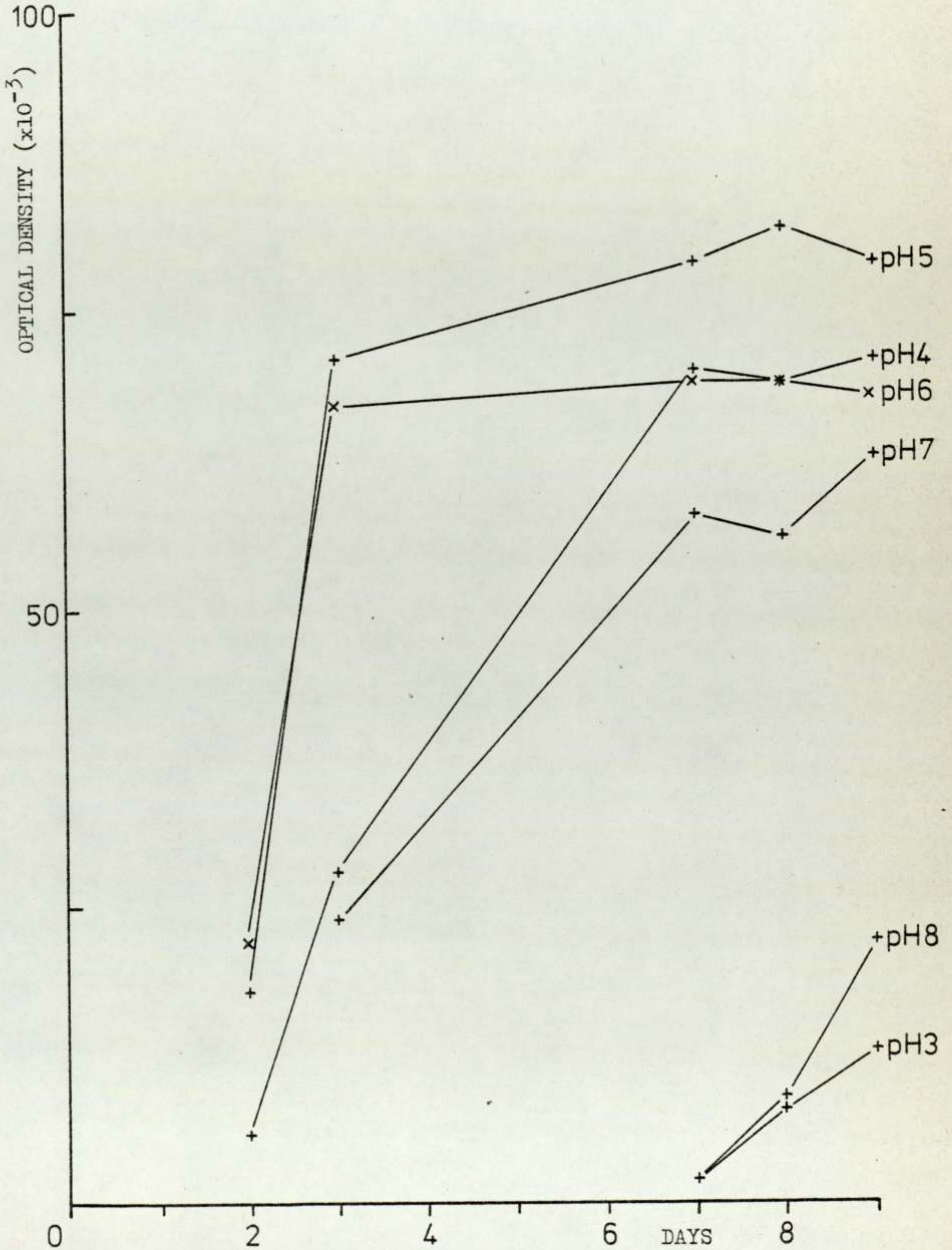


FIG.4.38 Fusarium tabacinum:-

Relationship of initial pH to dry weight when harvesting on Day 9. (First trial).

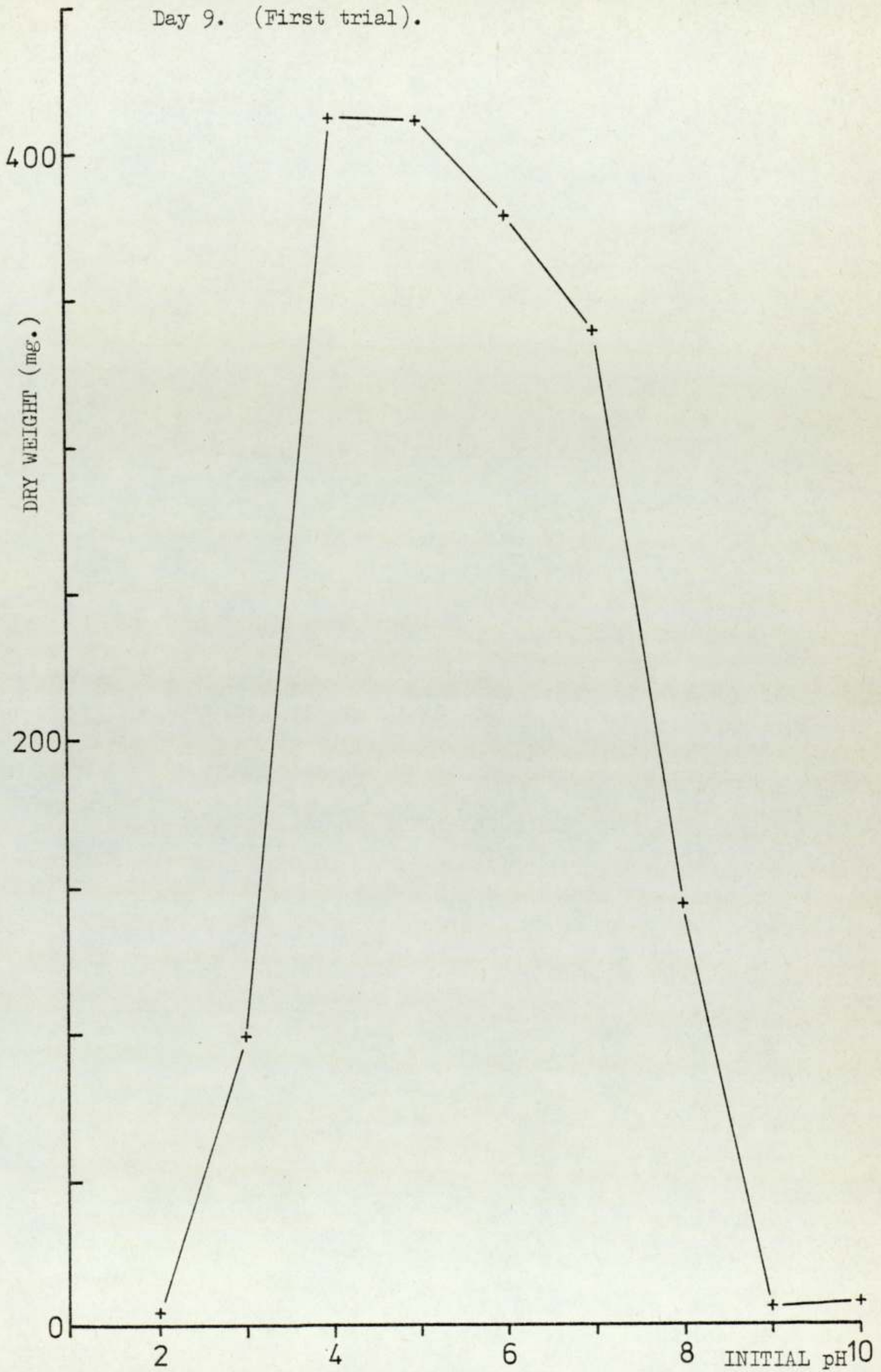
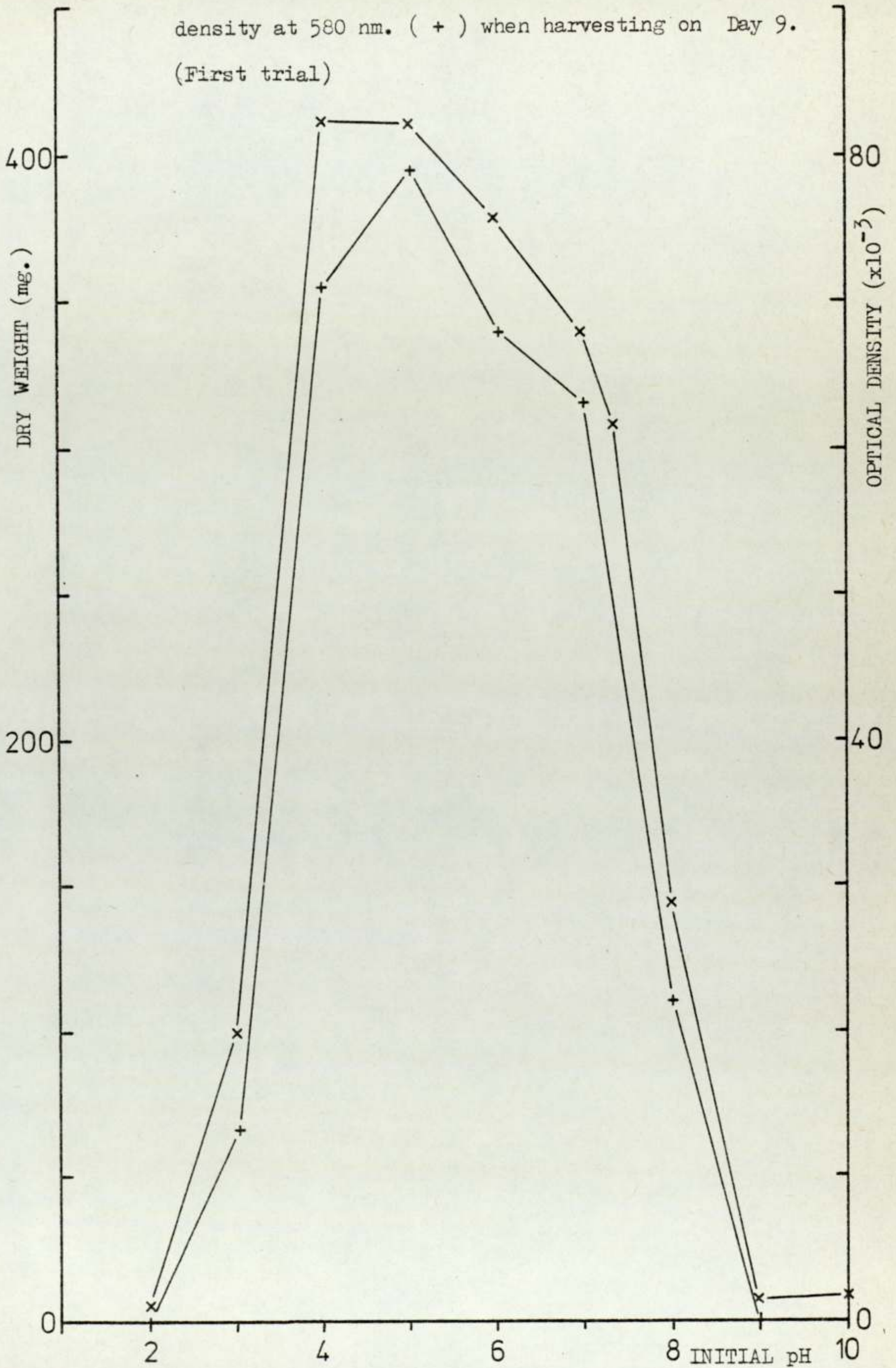


FIG. 4.39 Fusarium tabacinum:-

Relationship of initial pH to dry weight (×) and optical density at 580 nm. (+) when harvesting on Day 9. (First trial)



The study was continued with Fusarium tabacinum with harvesting on Day 6 to compare the results with G.candidum since they would be harvested together if grown in mixed culture. The procedure was carried out as before and the results were recorded in triplicate in Table 6.29 and the means of the data plotted in Figs. 4.40 to 4.42.

Fig. 4.40 revealed an increase in optical density and, therefore, growth up to the fifth day with an optimum at pH5. On the sixth day these readings had all decreased with pH 5 still supporting the best growth.

Fig. 4.41 showed that the pH values at the time of harvesting had increased above the initial values from pH 3 to 7 with marked increases at pH 5 and 6. The increases in pH were reflected in the shape of the dry weight curve with the highest values achieved at pH5 and 6 indicating a much narrower range of favourable pH than previously determined in an unbuffered medium.

Fig. 4.42 compared the curves of dry weight and optical density against initial pH, which apart from the mean optical density at pH 6 were very similar. There was no apparent reason for the discrepancy at pH 6.

The results of the second trial indicated that harvesting on the fifth day would have produced results while the fungus, F.tabacinum, was still growing and not in the decline phase. A third and final trial was performed as before with daily optical density measurements taken over five days with final pH and dry weights determined after harvesting on the fifth day. The results were recorded in Table 6.30 and expressed graphically as the means of triplicate readings in Figs. 4.43 to 4.45.

Fig. 4.43 showed the daily changes in optical density readings with the optimum still at pH 5. However, in this trial there was growth at the higher pH values from pH8 to 10 which was not experienced

FIG. 4.40 Fusarium tabacinum :-

Effect of initial pH (4-7) on optical density (absorbance at 580 nm.) over six days (Second trial).

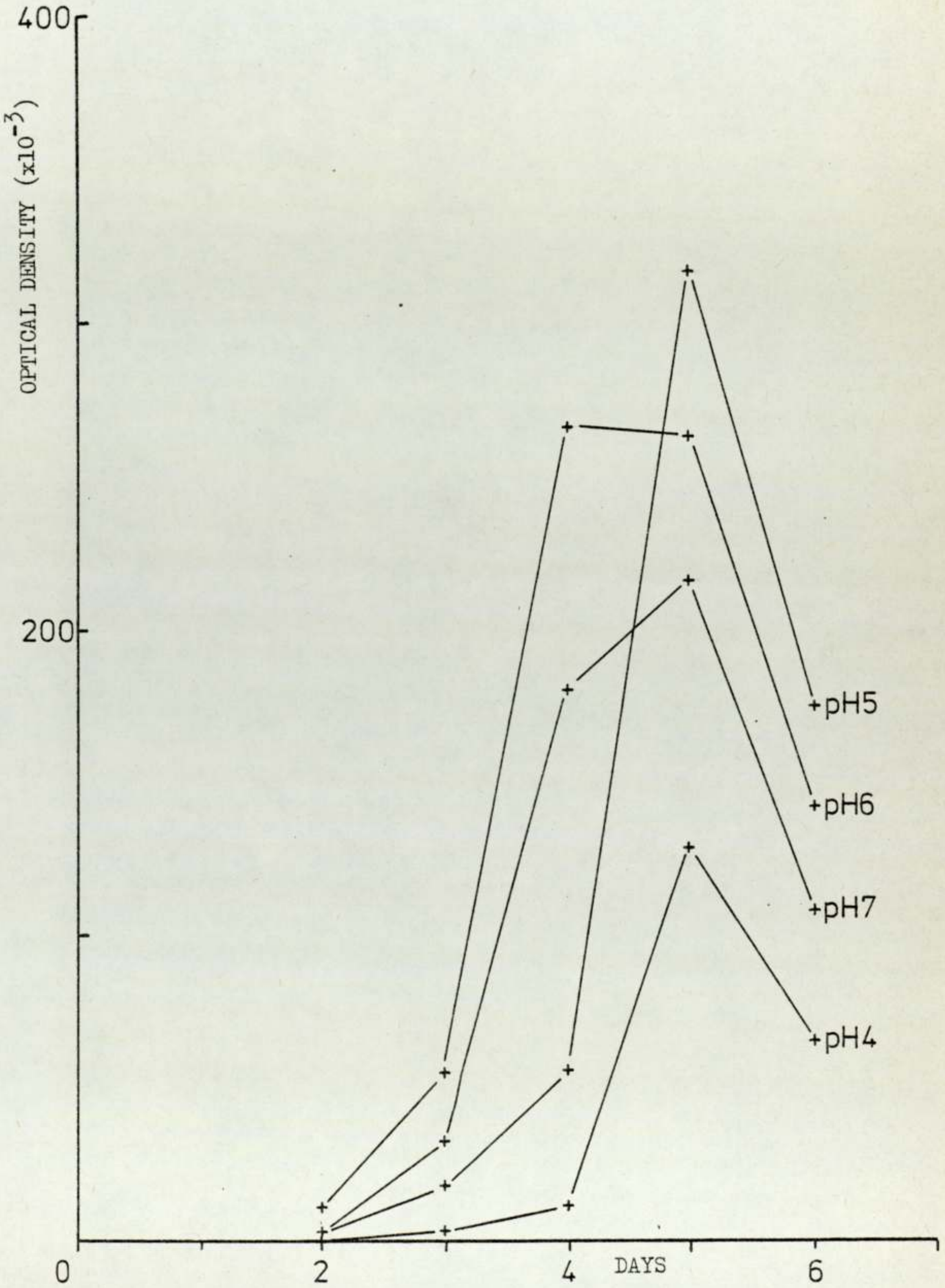


FIG. 4.41 Fusarium tabacinum:-

Relationship of initial pH to final pH (+) and dry weight (×) when harvesting on Day 6. (Second trial).

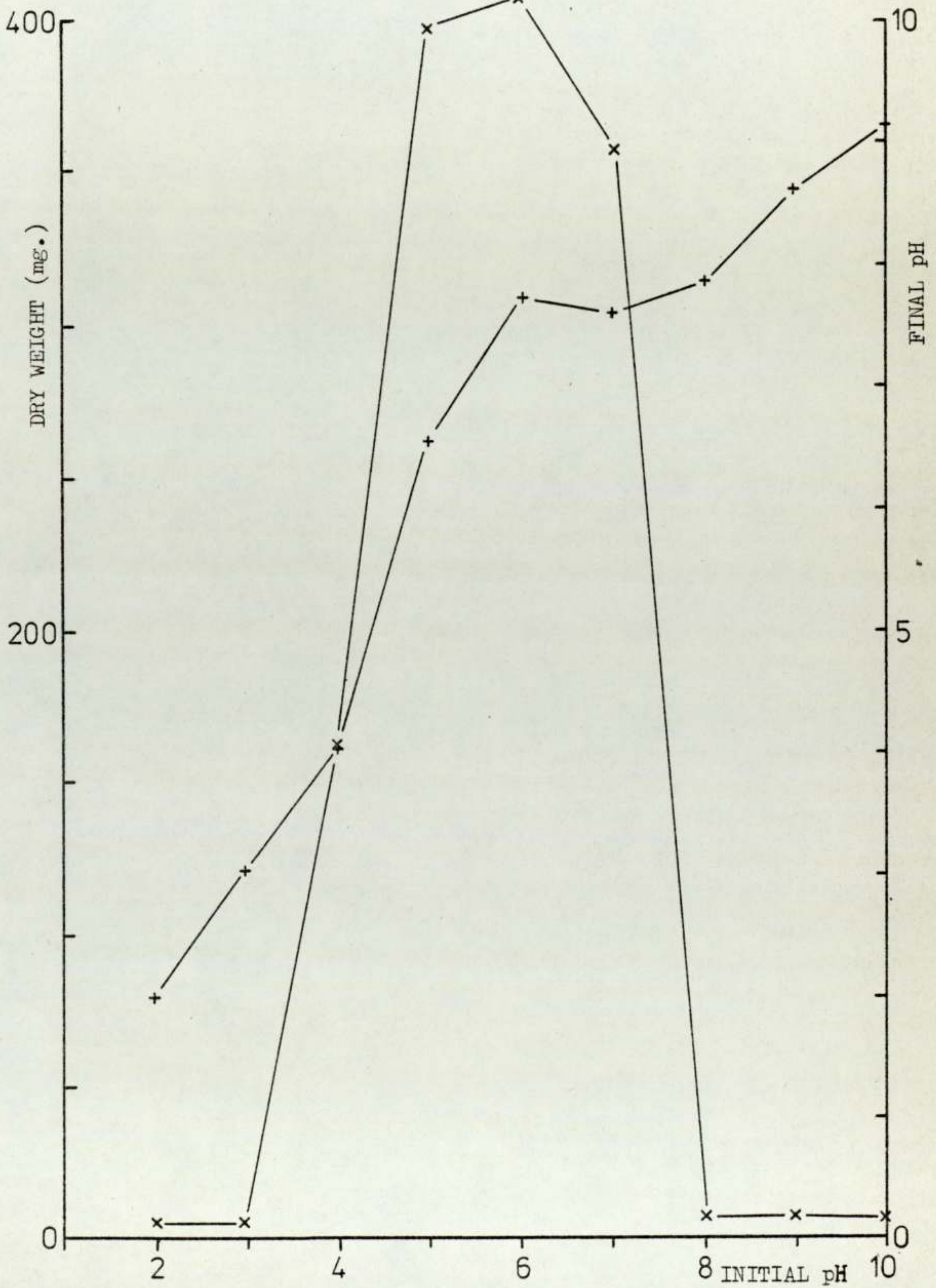


FIG. 4.42 Fusarium tabacinum:-

Relationship of initial pH to dry weight (×) and optical density at 580nm. (+) when harvesting on Day 6. (Second trial).

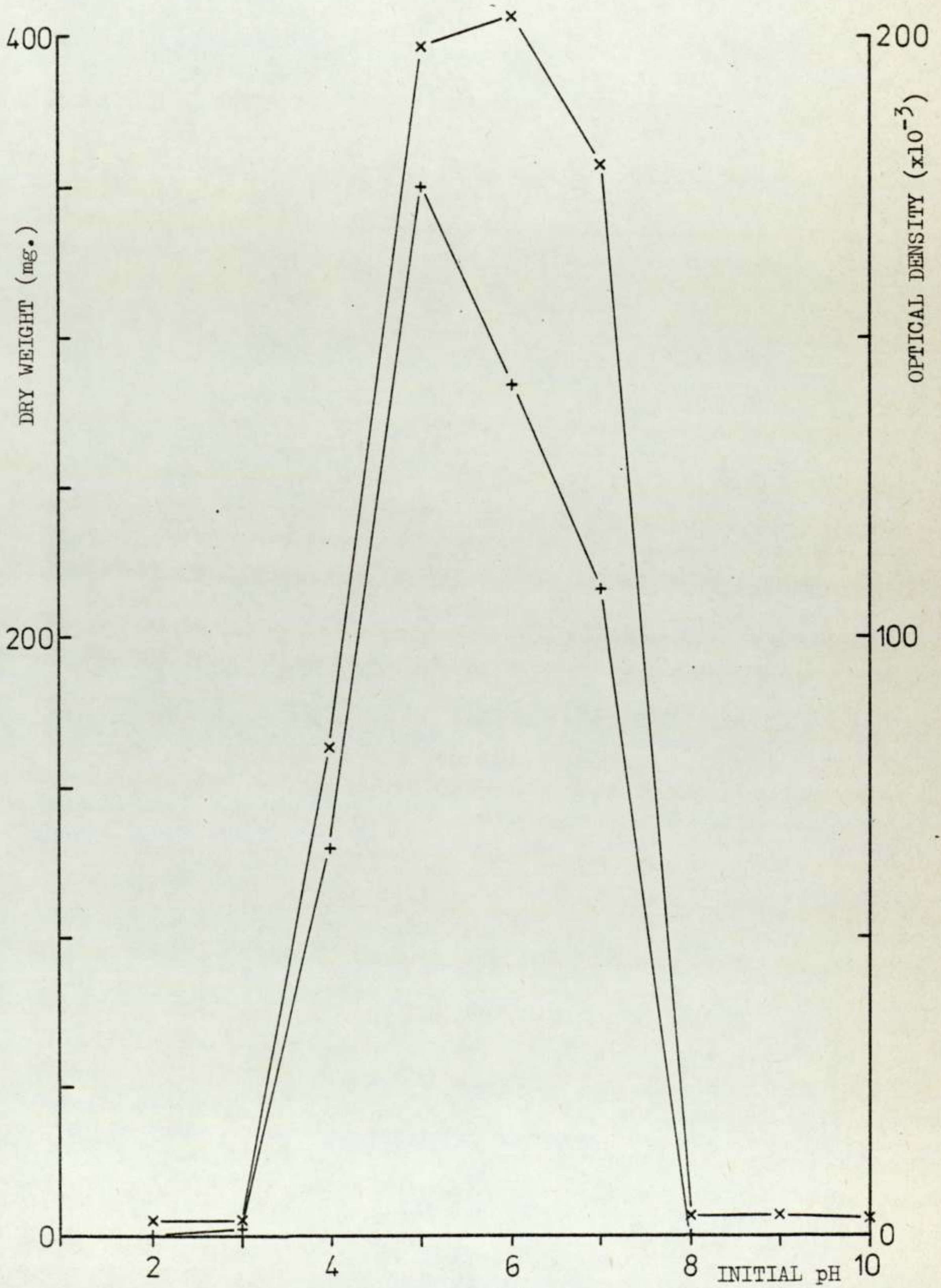


FIG. 4.43 Fusarium tabacinum :-

Effect of initial pH (4-10) on optical density (absorbance at 580 nm.) over five days. (Third trial).

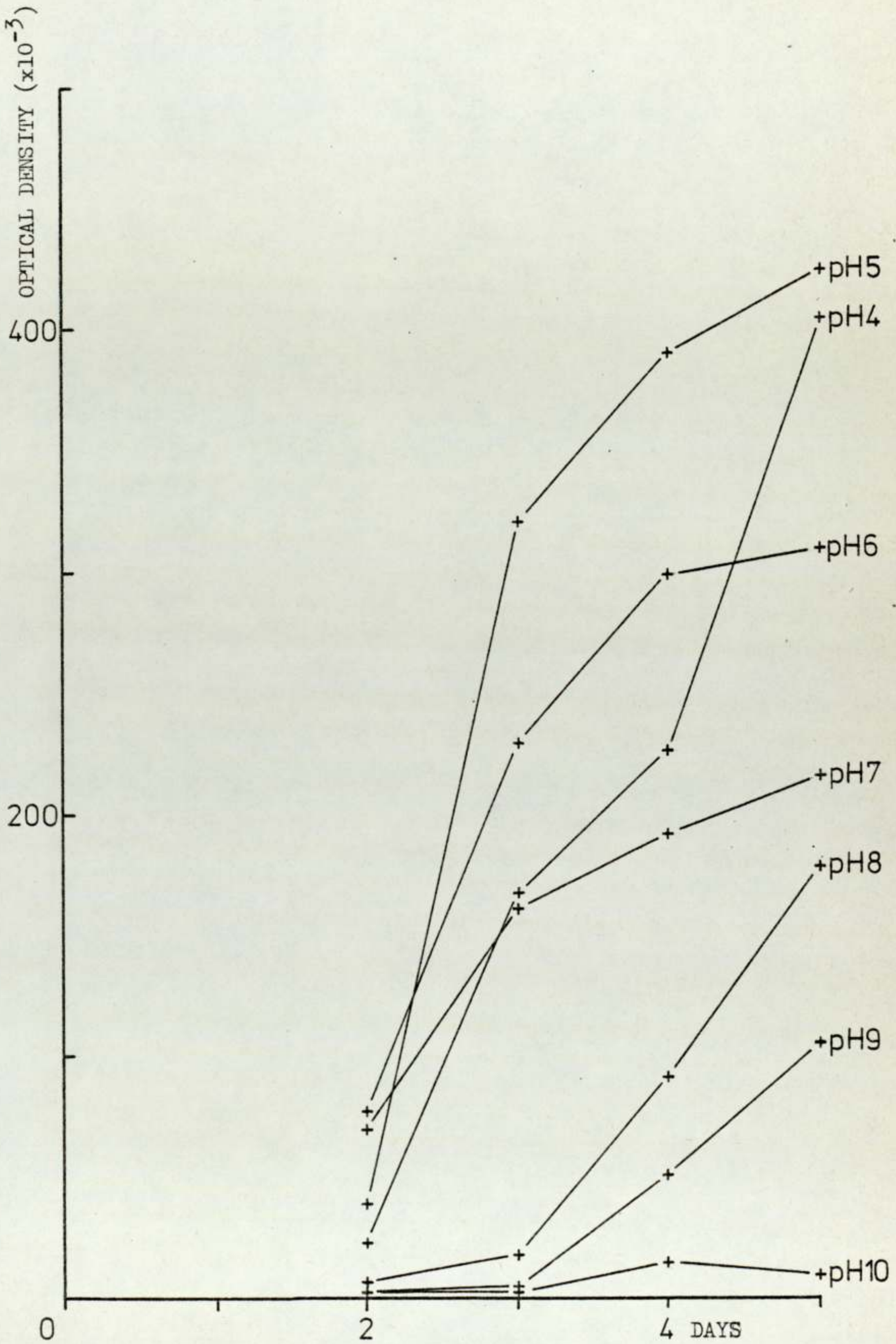


FIG. 4.44 Fusarium tabacinum:- Relationship of initial pH to final pH (×) and dry weight (+) when harvesting on Day 5. (Third trial).

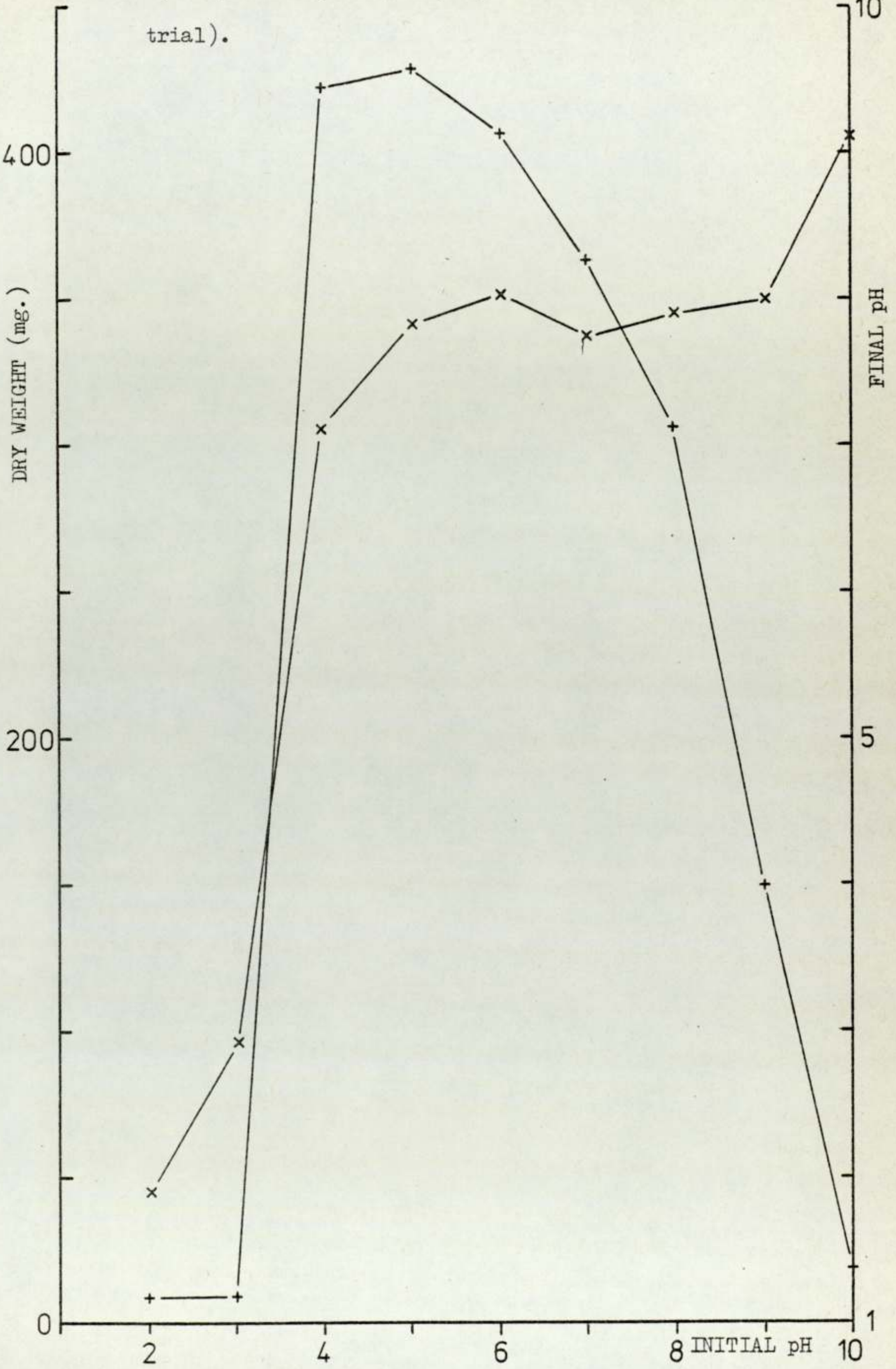
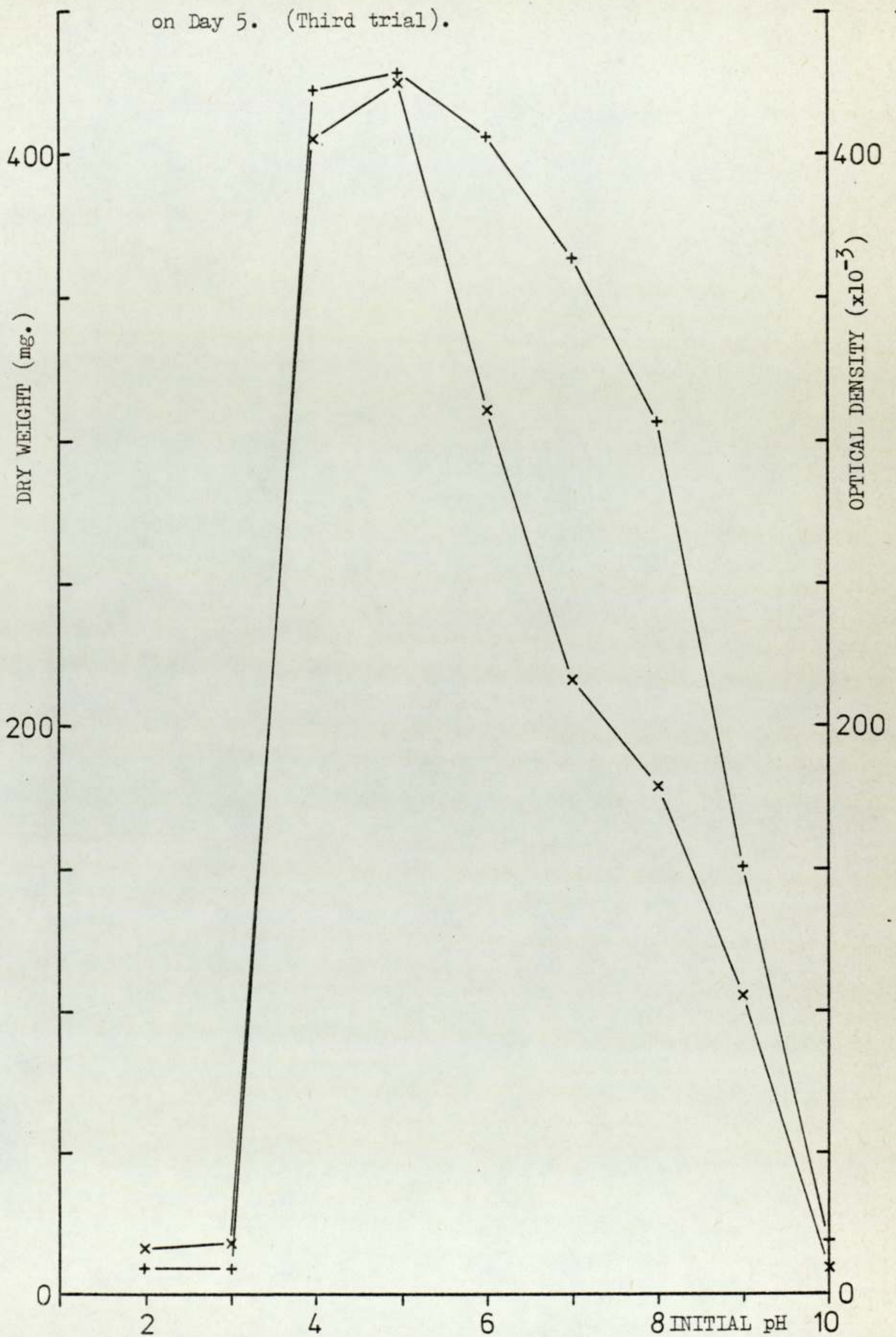


FIG. 4.45 Fusarium tabacinum:- Relationship of initial pH to dry weight (+) and optical density at 580nm. (×) when harvesting on Day 5. (Third trial).



in the first two trials.

Fig. 4.44 revealed dramatic increases in the final pH values from pH4 to 7 with slight decreases over the remainder of the range outside this band. The increase in final pH values over the initial values was again reflected in the shape of the dry weight curve which indicated a broader range of favourable pH from 4 to 8 than in the preceding trials.

Fig. 4.45 showed the similarity in the curves of optical density and dry weight against initial pH.

The results showed that growth of Fusarium tabacinum at different pH values could be monitored successfully using optical density measurements. The use of a buffered medium clarified the previous results with an unbuffered medium and showed that the optimum pH was the same in both cases but growth at the extremes of the pH range was reduced in a buffered medium.

4.3.5 Growth of fungi on vertical and horizontal screens using different concentrations of skimmed milk.

Introduction

A laboratory-scale continuous-culture apparatus was developed as described in Chapter 3, section 3.3, and initially used to produce as much fungal biomass as possible for various analyses performed by ICI at Billingham. A photograph and explanation of the apparatus can be seen in Chapter 3, plate 6 and Fig. 3.1 respectively.

Spray-dried skimmed milk powder was reconstituted to a concentration of 5g./litre which had previously been shown to give a C.O.D. value of 5000. This concentration was thought to be realistic for the effluent of a large dairy producing cheese and was likely to

be encountered at one of the plants visited. It was also necessary to produce as much fungal biomass as possible for the analyses and a strong feed was also favoured for this reason. The skimmed milk was delivered to six vertical screens by a peristaltic pump, fitted with 5 mm. diameter tubing, at a rate of 10 ml. for 48 seconds in every 2 minutes. Each vertical screen was made from a rectangular piece of black plastic mesh formed into a cylinder and sewn with nylon fishing line. The total surface area of each screen available for fungal growth was 0.4 m^2. Three screens were inoculated with Geotrichum candidum and three with Fusarium tabacinum in the form of 7mm. agar discs cut from the vegetative margin of six-day old colonies. The agar discs were placed at the top of each screen, held in place by a circular collar of plastic mesh which was fitted just below the dosing caps. The skimmed milk entered the dosing caps and emerged through an arrangement of holes to wet the agar discs and eventually the fungi grew downwards on both the internal and external mesh of the screens.

The first experiment continued for sixty-eight days, the screens were weighed regularly with a spring balance and the results of the total weight of fungus and screen were recorded in Table 6.31 and plotted graphically in Figs. 4.46 and 4.47. During the sixty-eight days the mean flow rate of skimmed milk to each screen was calculated, recorded in Table 6.32 and plotted with the total wet weights of fungi and screens in Figs. 4.46 and 4.47.

Figs. 4.46 showed that Geotrichum candidum maintained a reasonably steady increase in wet weight during the first fifty days on all the screens. Screen numbers one and three reached their maximum weights for the period after sixty-one days, they then decreased in weight with screen number one recovering slightly. These decreases in wet

FIG. 4.46 Geotrichum candidum:- Variation with time of total wet weight of fungus (×) grown on three vertical screens at 15°C and variation in the mean flow rate of feed of 5% $^{w}/_{v}$ skimmed milk. (+).

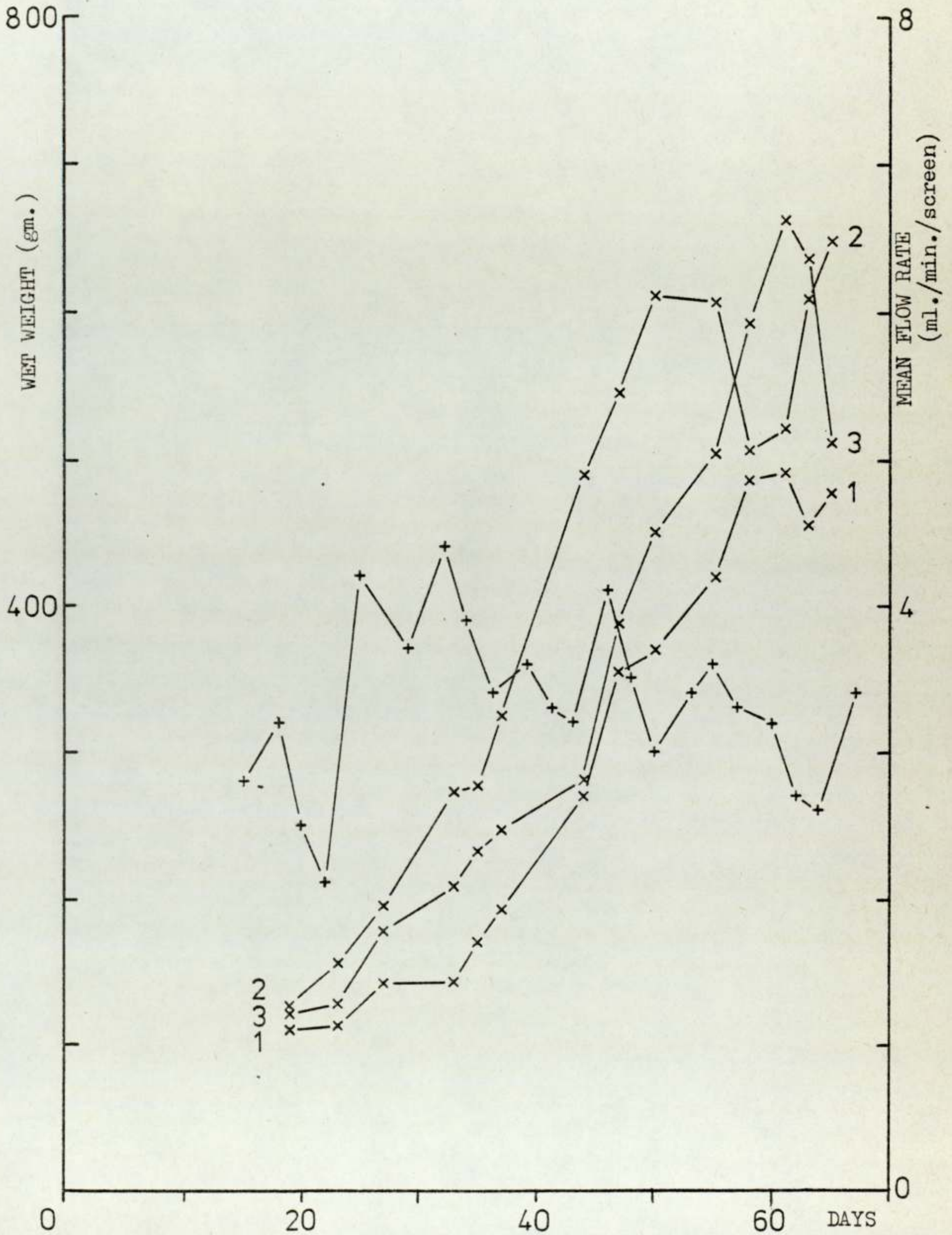
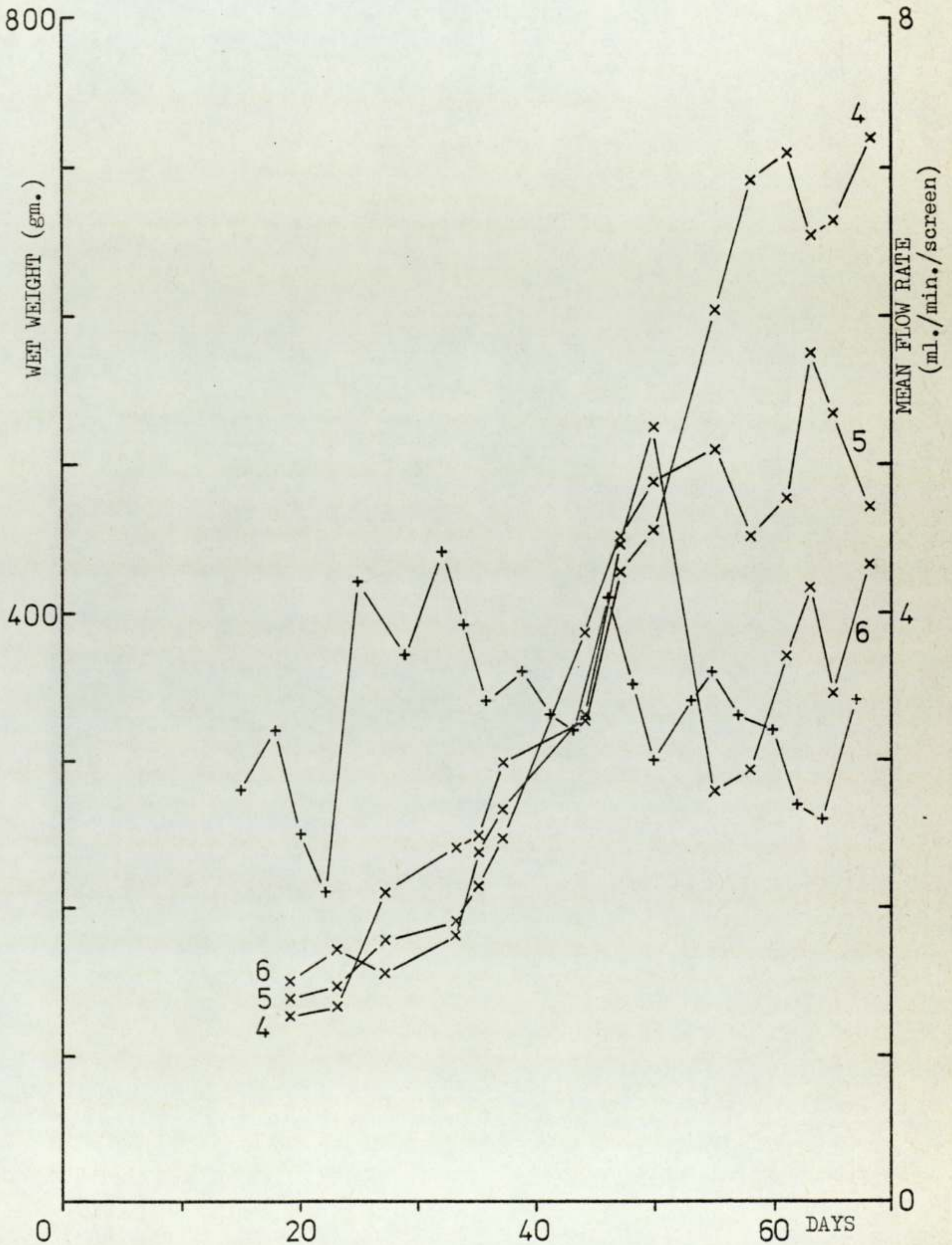


FIG. 4.47 Fusarium tabacinum:- Variation with time of total wet weight of fungus (×) grown on three vertical screens at 15°C and variation in the mean flow rate of feed of 5% $^{w}/_{v}$ skimmed milk. (+).



weight appeared to be related to the mean flow-rate which was low at that time, screen number two increased in weight up to the fiftieth day, stabilised, dropped sharply, then increased to its maximum weight on the day of harvesting.

The three screens bearing the weight of Geotrichum candidum were harvested on the sixty-fifth day. The original dry weights of the screens were 85 gm., 80gm., and 77 gm., for screens one, two and three respectively. The final wet weights of the screens including fungal film were 476 gm., 648 gm., and 511 gm., which by subtraction of the dry screen weights gave a total wet weight of fungus of 1393gm. The total surface area available to the fungus was 0.4 m^2 per screen, for three screens the surface area was 1.2 m^2. From Table 6.32 the mean of the mean flow rates were calculated from the addition of the mean flow-rates divided by the number of readings, (73.1÷ 22), resulting in an overall mean flow rate of 3.3 ml./min./screen. The hydraulic loading was calculated as follows:-

$$\text{dosage per three screens} = 9.9 \text{ ml./min./}1.2\text{m}^2$$

$$= \frac{9.9}{1.2} \times 1 \times 10^{-6} \times 60 \text{ m}^3/\text{m}^2/\text{hr.}$$

$$= 4.95 \times 10^{-4} \text{ m}^3/\text{m}^2/\text{hr.}$$

The total wet weight of fungal film of 1393 gm. (1393÷1.2=1161 gm./m^2) was obtained with a hydraulic loading of 4.95×10^{-4} m^3/m^2/hr.

Fig. 4.47 showed a similar pattern for the growth of Fusarium tabacinum which also increased on all three screens (numbers four, five and six) up to the fiftieth day. After this period the growth on screens four and five continued to increase with fluctuations in the case of the latter. The film on screen six sloughed off after the fiftieth day and lost more than half its weight. After this heavy decline the film weight increased, with a slight fluctuation, until

it was harvested. Subtracting the dry weights of the screens from the final wet weights on the day of harvesting gave the following results:-

screen number four 720 gm. - 75 gm. = 645 gm.

screen number five 470 gm. - 77 gm. = 393 gm.

screen number six 432 gm. - 82 gm. = 350 gm.

Total wet weight of fungal film = 1388 gm. ($1388 \div 1.2 = 1157$ gm./m^2)

The hydraulic loading was identical to that used for the screens of G.candidum which was 4.95×10^{-4} $m^3/m^2/hr$.

During the experiment described above C.O.D. values were obtained on the liquid collected from the base of the vertical screens after growth had continued for forty-seven days. The results were variable, ranging from a 6 to 16% reduction in the feed which had a COD of 5000. The retention time of the liquid was very small due to the smooth vertical face of the film on all the screens.

The experiment was continued after the screens had been cleaned and all traces of the previous growth removed. The feed strength was reduced to a concentration of 2g./litre skimmed milk which gave a COD of 2000 and was frequently the strength of the effluent treated by high-rate filters at the dairies visited. The screens were inoculated as before with Geotrichum candidum on numbers one to three and Fusarium tabacinum on numbers four to six. The total wet weights were recorded over a period of one hundred and twenty-three days for G.candidum and one hundred and eight days for F.tabacinum in Table 6.33. The mean flow-rates were calculated and recorded in Table 6.34 and the graphs incorporating the data from these two tables were plotted as Figs. 4.48 to 4.51.

Studying Figs. 4.48 and 4.49 showed that the wet weight of G.candidum was much more variable with the weaker feed. After the eighty-fourth day the screens were supplied with water only and this

FIG. 4.48 Geotrichum candidum:- Variation with time of total wet weight of fungus (×) grown on three vertical screens at 15°C and variation in the mean flow rate of feed of 2%w/v skimmed milk (+) from Day 7 to Day 42.

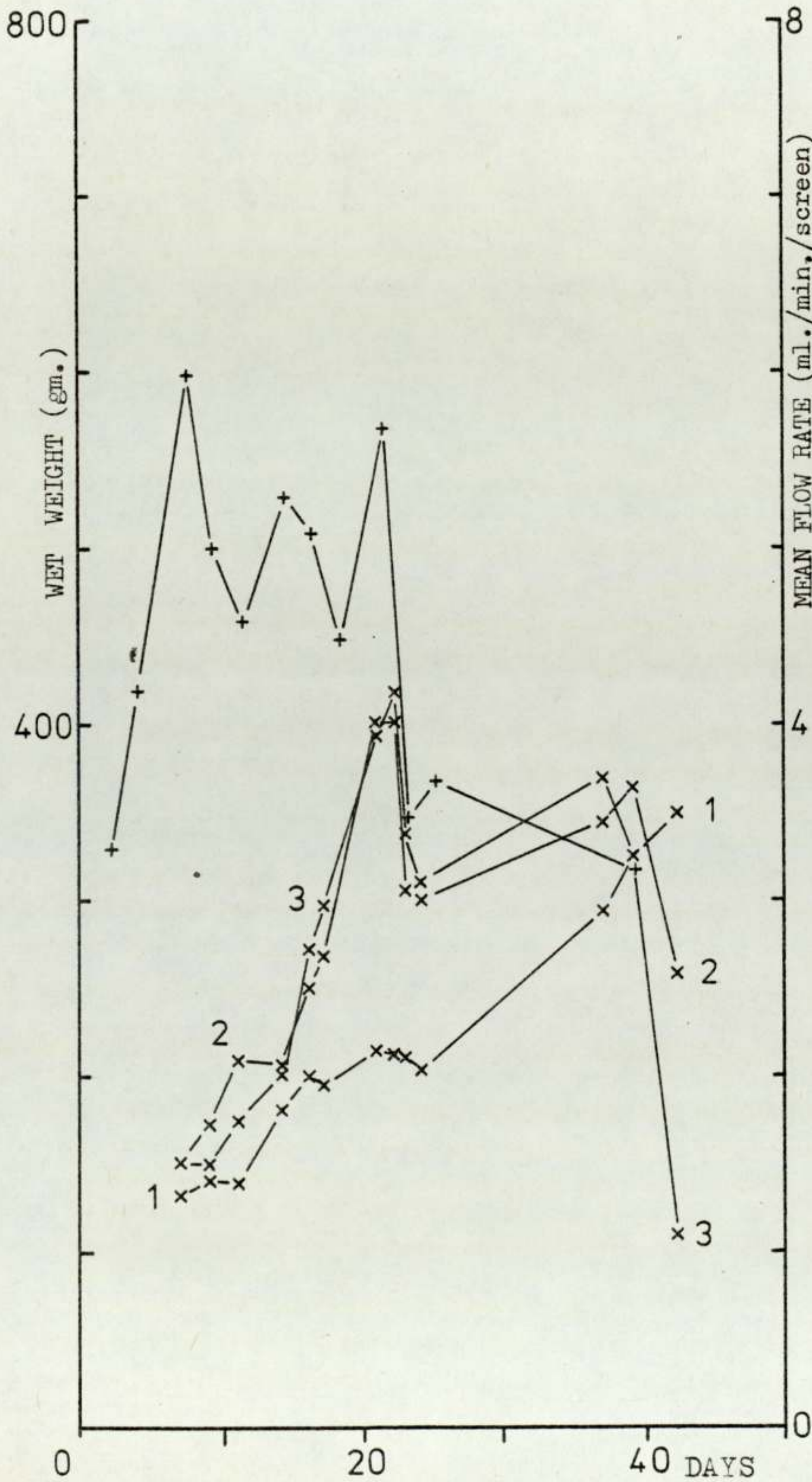


FIG. 4.49 Geotrichum candidum:- Variation with time of total wet weight of fungus (×) grown on three vertical screens at 15°C and variation in the mean flow rate of feed of 2% $^w/_v$ skimmed milk. (+) from Day 77 to Day 123.

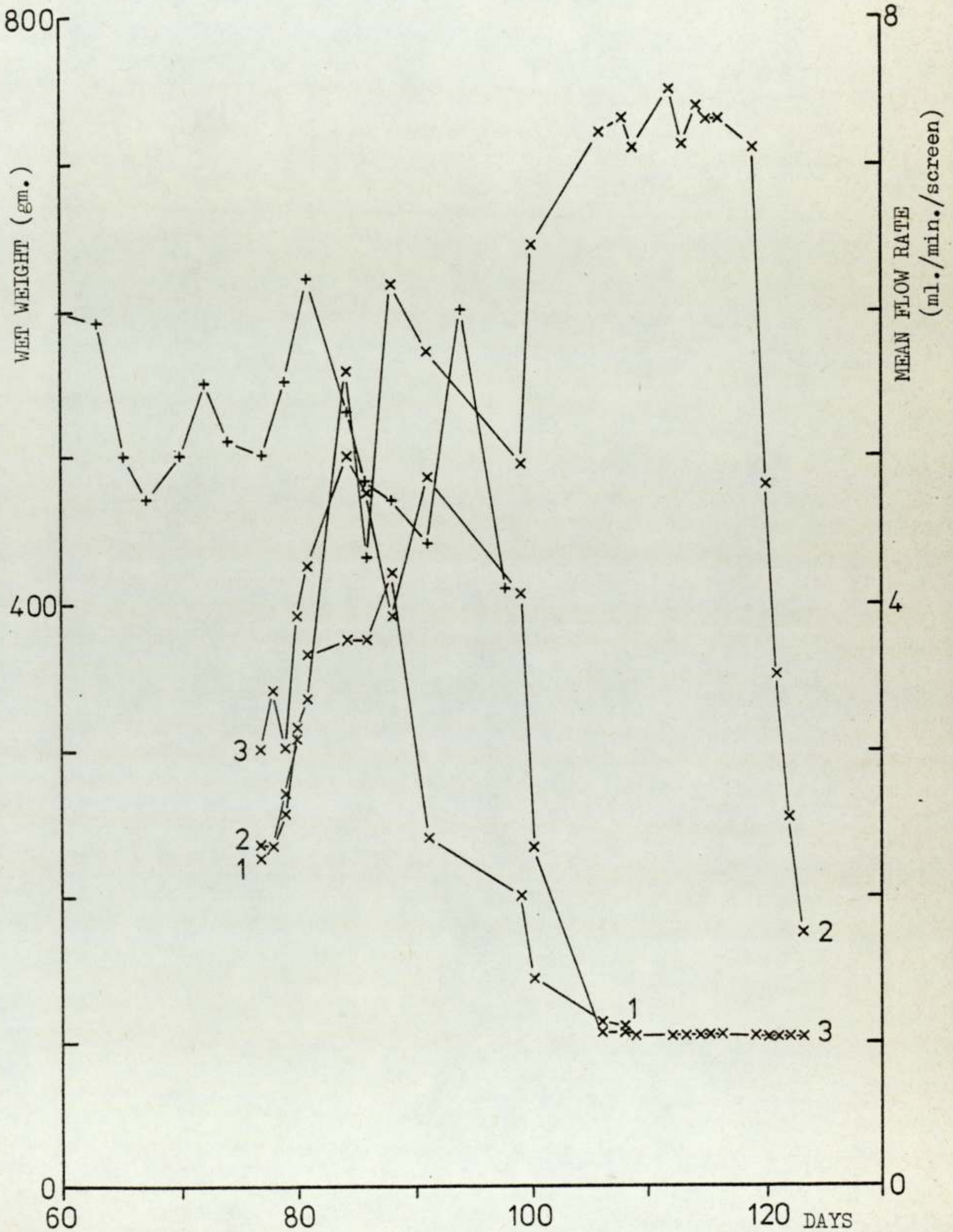


FIG. 4.50 Fusarium tabacinum:- Variation with time of total wet weight of fungus (×) grown on three vertical screens at 15°C and variation in the mean flow rate of feed of 2% w/v skimmed milk (+) from Day 7 to Day 42.

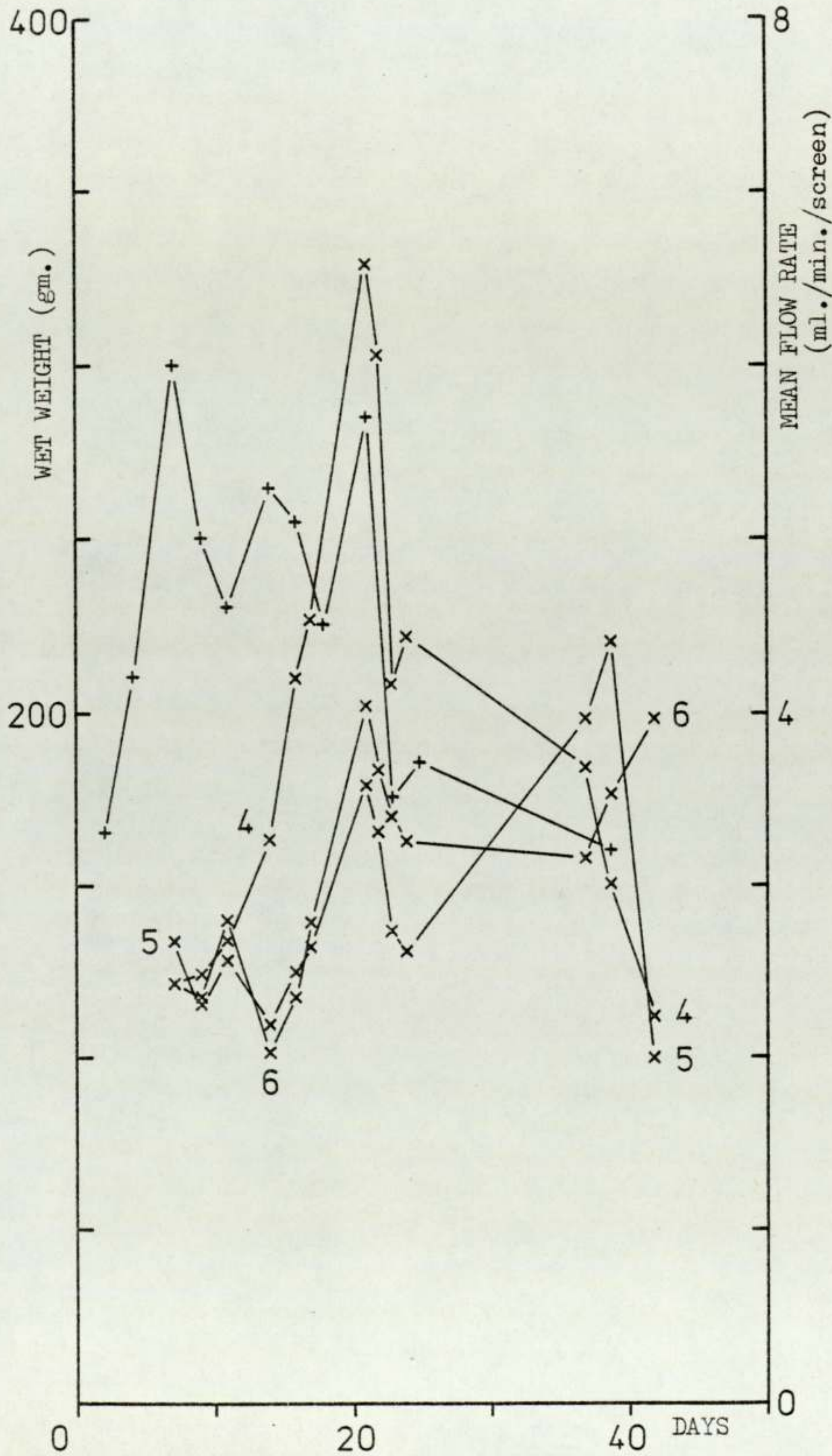
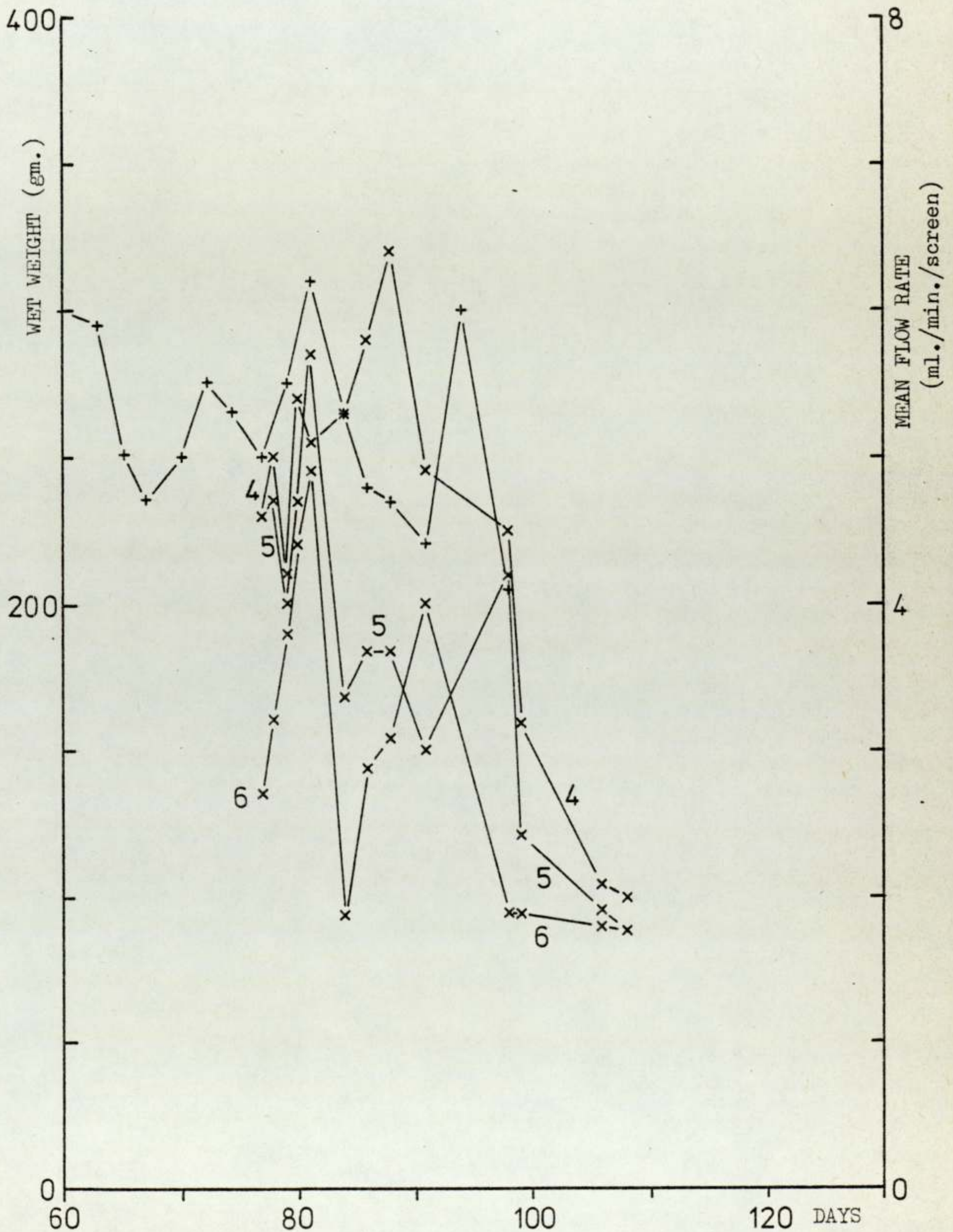


FIG. 4.51 Fusarium tabacinum:- Variation with time of total wet weight of fungus (×) grown on three vertical screens at 15°C and variation in the mean flow rate of feed of 2% w/v skimmed milk (+) from Day 77 to Day 108).



resulted in a steady loss of weight until the hundredth day. The feed was then reverted to 2g./litre skimmed milk but only screen two recovered its capacity for growth.

Figs. 4.50 and 4.51 reflected a similar pattern in the growth of Fusarium tabacinum on 2g./litre skimmed milk. Growth fluctuated much more with the weaker feed. Some of the peaks and troughs of the graphs coincided with those of the mean flow-rate curves, which was similar to G.candidum, but the weights were much lower than before.

During the two experiments the apparatus was housed in a temperature-regulated room at 15°C. The growth on all the screens was monitored regularly, there was a temporary growth of Dictyuchus sp. and Trichosporon sp., but apart from these fungi the screens supported the growth of the inoculated species, bacteria and milk solids. The pH of the milk was adjusted with sulphuric acid to pH5.6 which was previously found to be in the range of good growth for both fungi and allowed growth to proceed without curdling of the milk.

The experiments showed that changing the strength of the feed resulted in lower weights of fungus but it was hoped that a larger scale operation, such as a pilot plant, would produce more reliable results with a more constant flow-rate and fewer interruptions for cleaning the apparatus.

The hydraulic loading was calculated for the first forty-two days in operation using the weaker feed of 2g./litre from the figures in Table 6.34. For the two groups of three screens the hydraulic loading was found to be 6.76×10^{-4} $m^3/m^2/hr$. which may have been related to the greater fluctuations in wet weights with the weaker feed.

The fungal growth in both experiments grew upwards and downwards

from the inocula causing blockages in the dosing caps and diverting the fluid down the screens. Once growth had established itself downwards the feed ran down over the same path thus building up thick layers of film in restricted channels. There was a limited amount of lateral growth but most of the film was concentrated in thick vertical strips. Sloughing of the film occurred when the film had accumulated to such an extent that the underlying biomass in contact with the mesh screen could no longer support the weight. Anaerobic areas were not found in this system, probably because of the construction of the screens which allowed air to circulate around and inside them. It was possible to induce sloughing in this system by directing a jet of water, under a fairly high pressure, on to the screens and this eventually dislodged the growth from the mesh. A smoother supporting structure such as the Flocor modules may be easier to wash clean of fungal growth since the hyphae cannot penetrate the media, this was another possibility for the pilot-plant studies.

The experiments with the horizontal screens used similar apparatus to that of the vertical screens. Plates 7 and 8 show the apparatus used for the horizontal-screen studies.

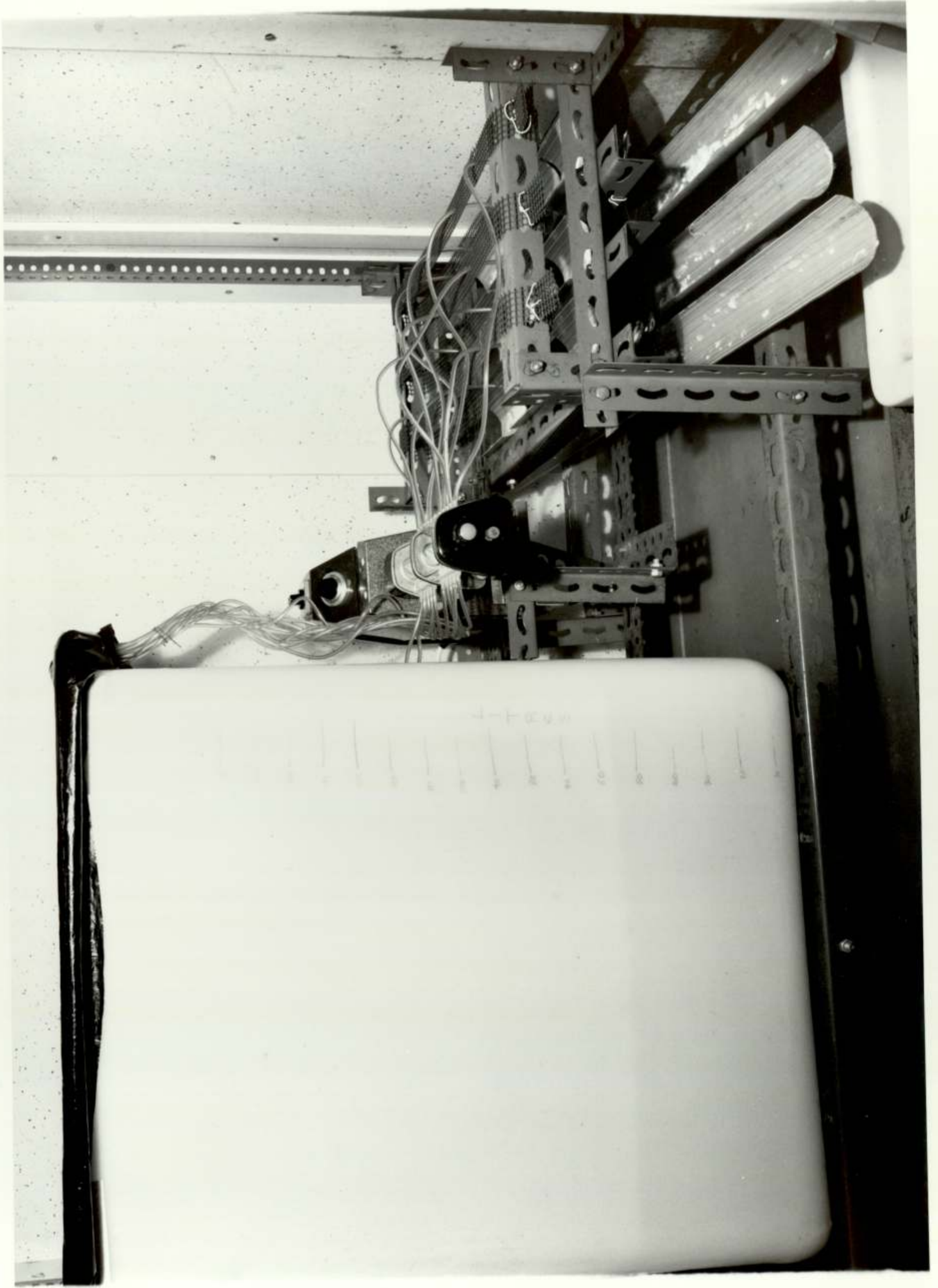
The apparatus consisted of a 205 litre tank to contain the skimmed milk, a peristaltic pump (Watson-Marlow MHRE 22 Delta) fitted with an additional module to accomodate ten 5mm. diameter tubes, ten plastic screens and three drainage gutters. A close-up photograph of the screens and pump can be seen in Plate 8. Each screen was a piece of grey ridged plastic gutter, perforated with fifteen small holes for drainage, 10cm. in length and 110 cm^2 internal surface area. 7mm. cork-bored agar discs of <u>G.candidum</u> and <u>F.tabacinum</u> were inoculated centrally on each screen, five screens for each fungus. Over each two-week period, the feed to the ten screens was

P L A T E 7

CONTINUOUS CULTURE APPARATUS

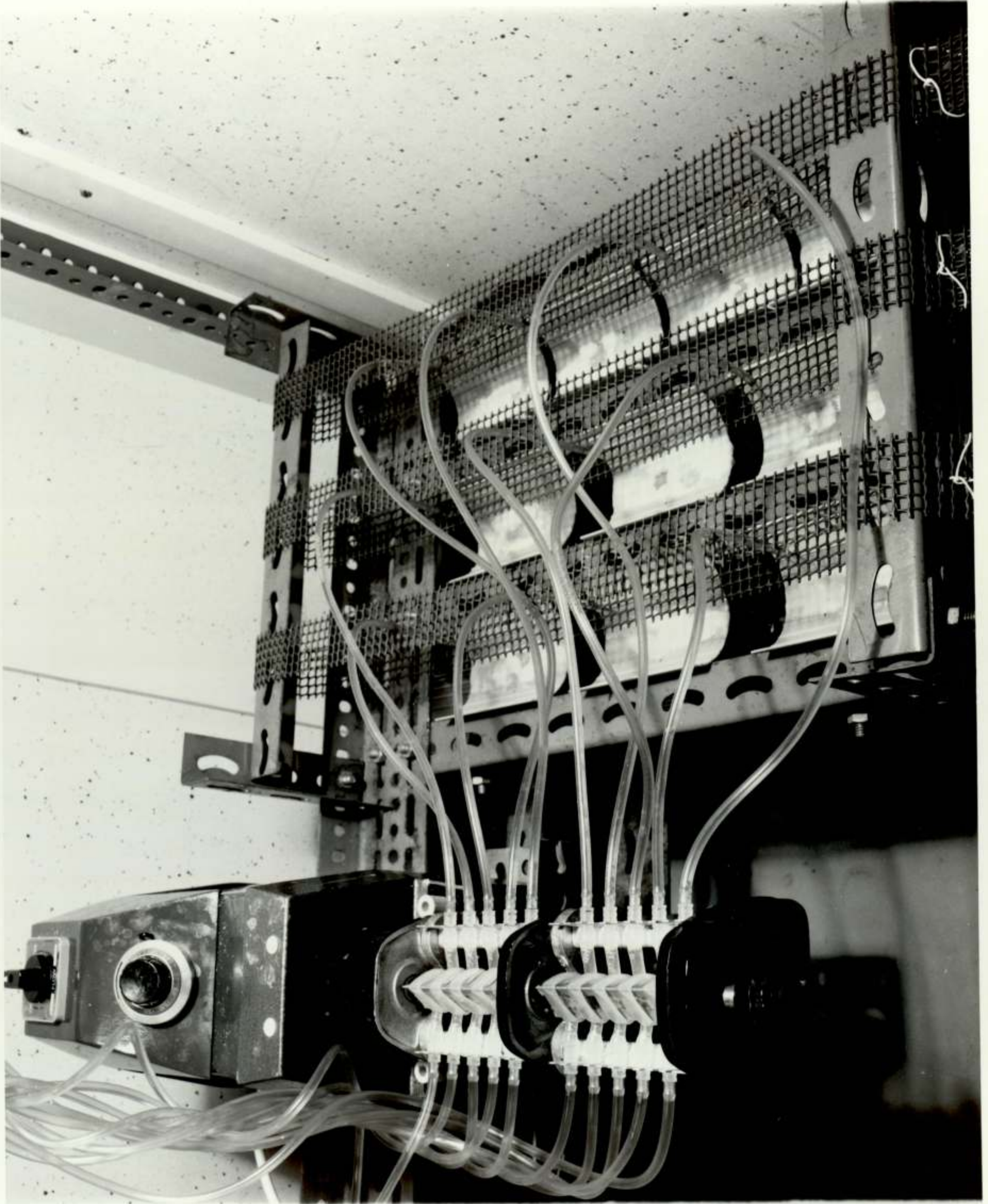
SHOWING THE OVERALL LAYOUT OF

THE EQUIPMENT.



P L A T E 8

A DETAILED VIEW OF THE CONTINUOUS CULTURE APPARATUS SHOWING MULTI-CHANNEL PERISTALIC PUMP DOSING TEN HORIZONTAL SCREENS BEARING FUNGAL GROWTH.



changed using a range decreasing from 5g./litre to 0.5g./litre and the wet and dry weights of the two fungi were recorded after harvesting. The screens were dosed for 13 seconds with a 20 second quiescent period in every minute. The apparatus was housed in a temperature-controlled cabinet at 15°C and thermograph charts were employed to monitor the temperature during each fourteen-day period. The results for the wet and dry weights of the two fungi were recorded in Table 6.35 and expressed graphically in Figs. 4.52 and 4.53. The dry weights were obtained by washing the fungal material off each screen into a glass vacuum filtration apparatus fitted with Whatman glass fibre filter papers. The papers were dried in an oven at 105°C for four hours, placed in a dessicator for five minutes and weighed. The hydraulic loading for the two groups of five screens was calculated from the data in Table 6.36. The mean of the mean flow-rates were 3.41 ml./min./screen, for five screens with a total surface area of 5.5 m^2 the hydraulic loading was:

$$\frac{3.41x5}{5.5} \times 1x10^{-6} \text{ a } 60 = 1.86x10^{-4} \ m^3/m^2/hr.$$

The loading was lower than with the vertical screens but the results showed a clear trend of an increase in both wet and dry weights of both fungi related to an increase in the strength of the feed.

It was hoped that the availability of a pilot-plant, sited at a dairy with a treatable effluent with at least a COD of 2000, would confirm the results obtained with the laboratory-scale apparatus and enable more studies to be made, particularly those associated with frequency of dosing, contact time and composition of the feed.

FIG. 4.52 Effect of increasing the concentration of skimmed milk on the wet weight of Geotrichum candidum (×) and Fusarium tabacinum (+) harvested after fourteen days at 15°C. (Each point is the mean of five determinations).

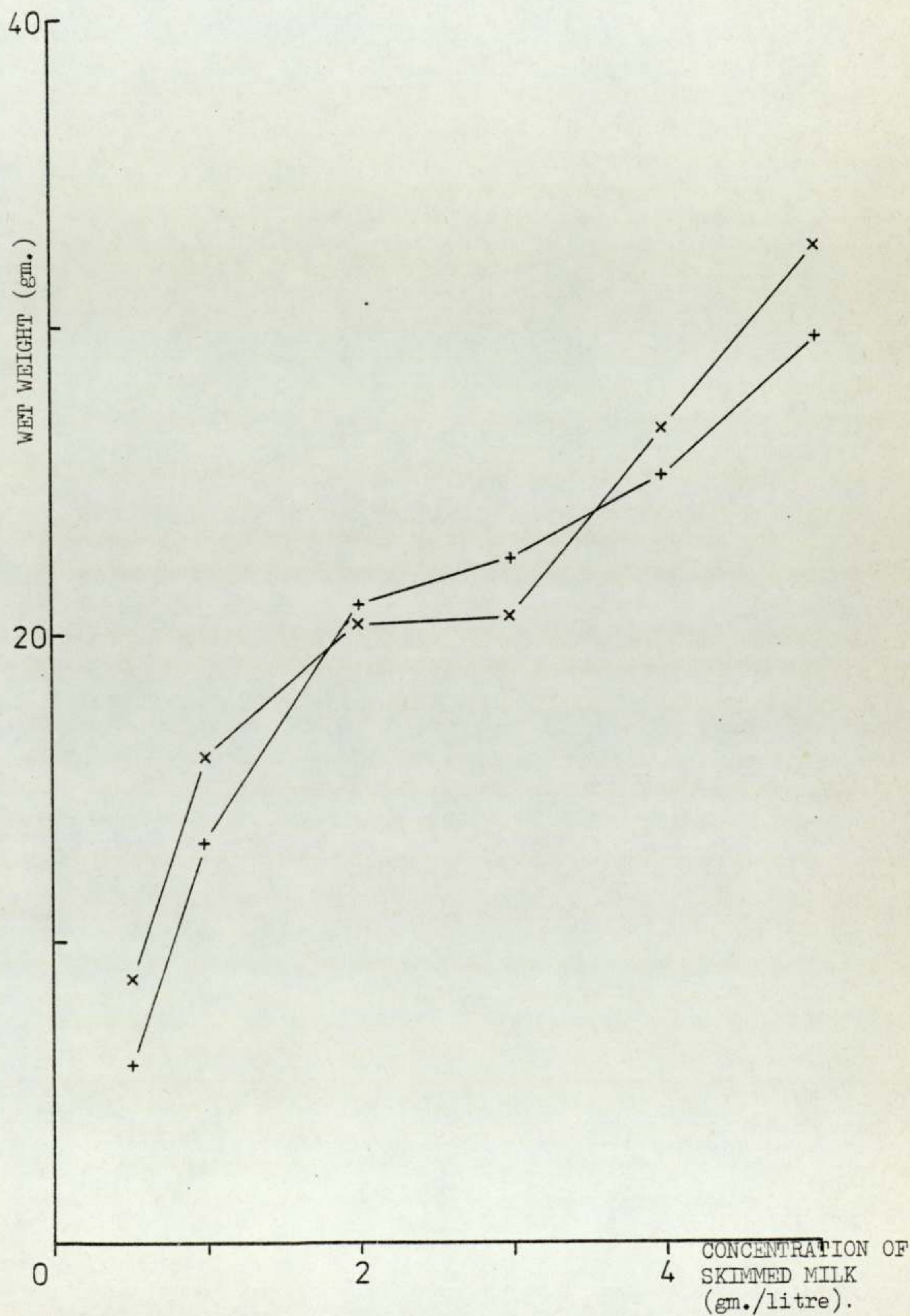
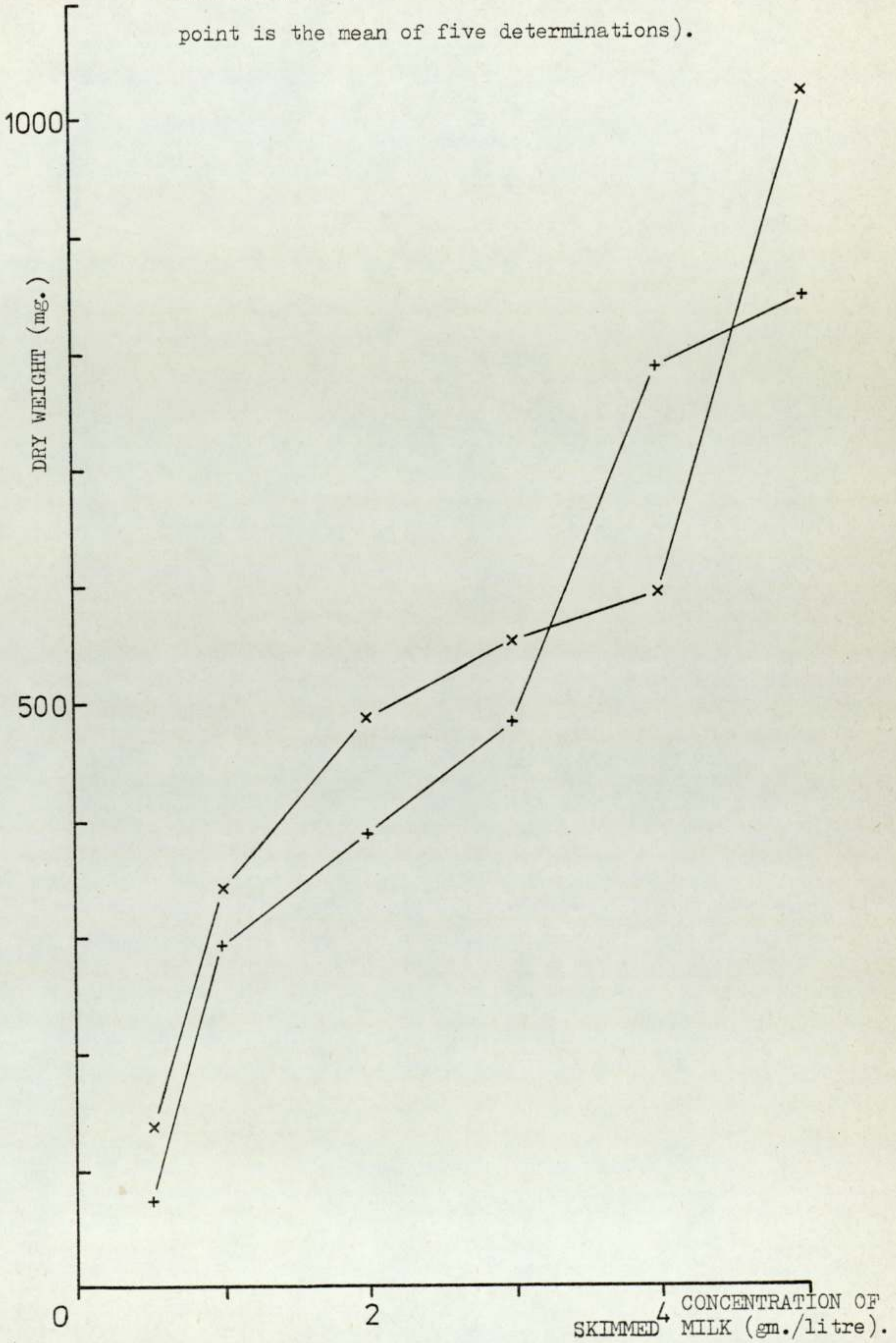


FIG. 4.53 Effect of increasing the concentration of skimmed milk on the dry weights of Geotrichum candidum (×) and Fusarium tabacinum (+) harvested after fourteen days at 15°C. (Each point is the mean of five determinations).



4.4 SUMMARY OF RESULTS

Laboratory studies using plate, flask and continuous culture apparatus were performed to establish the optimum conditions for biomass production of three species of fungi: Geotrichum candidum, Fusarium aquaeductuum and Fusarium tabacinum.

In pure culture the growth rate of each species increased with an increase in temperature, up to a maximum at 28°C for G.candidum, 22.5°C for F.aquaeductuum and 29°C for F.tabacinum, above these values there was a decrease in growth rate.

In an unbuffered medium (Painter's Medium A) all three fungi tolerated a wide pH rangefrom pH2 to 9 for G.candidum, pH3 to 9 for F. tabacinum and pH4 to 9 for F.aquaeductuum. The optimum pH in terms of maximum biomass was pH6 for G.candidum, pH5 for F.tabacinum and pH4 for F.aquaeductuum.

In buffered Medium A G.candidum and F.tabacinum were restricted to growth within a narrower pH range from pH3 to 7. G.candidum produced maximum biomass at pH3 whereas F.tabacinum gave equally good yields over a pH range of 4 to 6.

Continuous culture of G.candidum and F.tabacinum on vertical plastic screens resulted in an overall increase in wet weight, during the first fifty days, when fed intermittently with 5g./l. skimmed milk at a hydraulic loading of 4.95×10^{-4} m^3/m^2/hr. When the feed strength was lowered to 2g./l. at a hydraulic loading of 6.76×10^{-4} m^3/m^2/hr. the wet weights of both fungi were much lower and showed greater fluctuations over forty days duration.

When skimmed milk was intermittently fed at a hydraulic loading of 1.86×10^{-4} m^3/m^2/hr. on to horizontal plastic screens there was an increase in both wet and dry weights of G.candidum and F.tabacinum corresponding to an increase in feed strength. Above the value

of 0.5 g./l. skimmed milk, (corresponding to a COD of 500), there was little difference in the biomass production of either fungus.

4.5 DISCUSSION OF LABORATORY RESULTS

The effects of temperature, pH and feed strength on the growth of Geotrichum candidum, Fusarium tabacinum and Fusarium aquaeductuum were measured as described in section 4.3. Initially the optimum growth conditions were determined using laboratory-scale apparatus and pure cultures of the fungi prior to larger scale pilot plant studies (Chapter 5).

The optimum temperature for growth was determined by taking colony diameter measurements of pure cultures of the three selected fungi grown on agar plates. Synthetic media of a standardized depth was used in these experiments as recommended by Cochrane (1958). G.candidum and F.tabacinum were both capable of growth over a wide range of temperatures from 10°C to 35°C (Figs. 4.1 and 4.10 respectively). F.aquaeductuum was capable of growth between 10°C and 30°C (Fig. 4.7). All three fungi had an initial lag phase at all temperatures while the inocula became acclimatized to the imposed conditions. The lag phase was noticeably prolonged at the extremes of the temperature range compared with that at more favourable temperatures. Growth rates were calculated during the approximately linear extension phase. The temperatures which encouraged the highest rates of growth were 28°C for G.candidum, 29°C for F.tabacinum and 22.5°C for F.aquaeductuum. The diameter of the agar plates limited growth at temperatures favourable to the fungi as the leading hyphae exhausted the food supply.

The determination of an optimum temperature for growth should be qualified by the medium used, method of measurement and the duration of the experiment. The temperature which encourages the highest growth rate may not coincide with maximum biomass production. Hawkes (1965) grew Sepedonium sp. (Fungi Imperfecti)

in nutrient solution and obtained a steady increase in growth rate up to 25°C with a rapid decline above this temperature. Under conditions of nutrient limitation , however, Sepedonium sp. suffered an increasing rate of lysis with increasing temperature between 10°C and 25°C. In a pilot plant situation the temperature of the feed is dependent on the functions of the main plant. The laboratory studies have shown that the selected fungi can grow over a wide temperature range with adverse effects only at very low or very high temperatures.

Temperature affects growth, spore germination, reproduction and all activities of organisms. Changes in temperature cause changes in respiration rate and medium dissolved-oxygen tension. The respiration rate usually reaches a maximum at the optimum temperature for mycelial growth (Hawker, 1950). The medium dissolved-oxygen tension varies inversely with increasing temperature (Bull and Bushell, 1976). The temperature effects combine with changes in the composition of the medium as the fungal colonies develop. The hyphae absorb nutrients from the medium and return their metabolic products as growth proceeds. The slow process of diffusion can result in different conditions in the same colony as gradients of acidity and nutrients are established. Agar plate cultures are thus seen to have their disadvantages which are shared by other batch culture techniques.

Colony diameter measurements as a method of monitoring fungal growth under various conditions also has its disadvantages. The measurements are incapable of including the depth of the mycelium as they are only concerned with the spread of the colony. Cochrane (1958) and Caldwell and Trinci (1973) agreed that linear colony measurements were unsuitable for nutritional studies such

as growth on different carbon sources.

Brancato and Golding (1953) and Trinci (1971) concluded that colony measurements were sufficiently reliable for determining fungal growth rates at different temperatures on the same medium. Brancato and Golding (1953) investigated the effects of several environmental factors on the colony diameters of six moulds including Geotrichum candidum. They found that colony diameter measurements were a reliable measure of mould growth but careful control was needed when investigating the effects of temperature and concentration of the medium.

The central area of fungal colonies, growing on solid media, is older than the periphery. The younger hyphae of the peripheral growth zone supply protoplasm to the tips of the leading hyphae resulting in apical extension and increase in colony diameter. Fungal hyphae increase in length only at their apices and their rate of extension depends on the length of the terminal contributory cells. Trinci (1971) severed hyphae at varying distances from their apices to determine the limits of the contributory peripheral growth zone. He found the growth rate of a colony was dependent on the width of its peripheral growth zone and on the specific hyphal growth rate internal to that zone. The width of the peripheral growth zone varied with changes in glucose concentration but was not influenced by temperature or the addition of an inhibitor to the medium, Fungi are capable of spreading rapidly across solid surfaces such as agar plates and bacteria bed media and their mode of growth, concentrated at the hyphal apices, makes this possible. (Trinci, 1978).

The initial investigations to determine the influence of pH on the growth of Geotrichum candidum, Fusarium tabacinum and Fusarium aquaeductuum were based on a paper by Painter (1954).

The formulation of Painter's Medium A was used in flask cultures of the three fungi and harvesting was carried out after eleven days. G.candidum tolerated a wide pH range from pH2 to 9 with maximum biomass produced at pH6 (Fig. 4.17). Painter found that Geotrichum sp. reached its maximum dry weight at pH3 and produced similar weights between pH4 and 9, showing a decrease in weight at pH10. There was a decrease in dry weight at pH9 with the isolate of G.candidum used in the present investigations (Fig. 4.17). Brancato and Golding (1953) used colony diameter measurements to monitor the effect of pH on several moulds including Geotrichum candidum. The isolate of G.candidum used by Brancato and Golding achieved similar colony diameters between pH4.4 and pH9.1 with the largest diameter at pH6.4.

The effect of pH on the growth of Fusarium aquaeductuum (Fig. 4.19 was broadly similar to that of Painter (1954). Growth was poor at pH2 and 3 with a maximum at pH4 and a decline in growth above pH7.

Fusarium tabacinum tolerated a wide pH range from pH3 to 9 with maximum biomass at pH5. (Fig. 4.18).

The initial pH values between pH4 and pH7 had risen to values between pH7.8 and 8.3 by the end of the eleven day experiment. Painter's Medium A was, therefore, lacking in buffer capacity and this was corrected in a separate study (4.3.4).

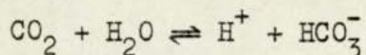
A detailed study of the daily changes in culture pH and the relationship with the dry weight of the three selected fungi was described in section 4.3.3. Initial pH values ranging from pH4 to 7 were chosen because they were found to promote the most biomass in the previous study. Painter's Medium A was used as the medium to maintain continuity with the previous study.

Figs. 4.21 to 4.24 showed that G.candidum increased in weight considerably between the first and second day between pH4 and pH7. The weight increase corresponded to a sharp decrease in the initial pH value. The culture pH values levelled off from the second day until the sixth day when they began to rise steadily until the time of harvesting.

Figs. 4.26 to 4.29 showed that F.tabacinum had a different growth pattern from G.candidum apart from the same sharp increase in weight between the first and second days. The pH curves all followed a similar pattern with a marked decrease, corresponding to fungal weight increase, then a steady increase in pH until harvesting. The dry weight curves were all similar, regardless of the initial pH, with maximum biomass produced on Day 4 at initial pH4 and 5 on Day 5 at initial pH6 and on Day 6 at initial pH7. The dry weights all decreased noticeably once the maximum had been reached. (Fig. 4.30)

Painter's Medium A is composed of glucose, vitamin-free Casamino acids and three salts. The initial sharp decrease in pH associated with increase in dry weight of both G.candidum and F.tabacinum can be explained by the breakdown of glucose and the formation of gluconic acid by the fungi (Dimond and Peltier, 1945; Lilly and Barnett, 1947; Allen et al., 1954). When the supply of glucose was exhausted the pH began to rise and continued rising as the organic acids from the glucose and Casamino acids were utilized by the fungi.

pH is the abbreviation of "potential of hydrogen" which was the name given to the logarithmic scale devised by Sørensen to express the concentration of hydrogen ions in solutions. Since the scale is logarithmic a difference of one pH unit indicates a ten-fold difference in the hydrogen ion concentration.



$$CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^-$$

The concentration of dissolved bicarbonate (HCO_3^-) formed from gaseous CO_2 is influenced by the culture pH. Increasing the culture pH tends to produce more carbon dioxide and problems arise when determining the tolerance of fungi to alkaline media. If a fungus is capable of growing in an alkaline medium, the carbon dioxide produced lowers the pH, organic acids may be produced thus enabling further growth to take place. Alkaline media also absorb carbon dioxide from the air. Culture pH and composition of the media influence the rate of diffusion of oxygen into the media. Submerged mycelia have more oxygen available to them in acidic rather than alkaline media (Lilly and Barnett, 1951). Allen et al . (1954) found that not only the rate of air supply but the method of aeration, shape and size of the container and the depth of the liquid influenced chemical changes resulting from microbial growth. Violent aeration of a simple medium was found to delay growth or even suppressed it. In the liquid shake cultures used in this study the flasks were not agitated vigorously so it is unlikely that lack of growth at the extremes of the pH range was caused by aeration. Macauley and Griffin (1969) found that dry weight production of some soil fungi was most noticeably affected at high pH values in liquid shake cultures. They attributed the difference in growth to the bicarbonate ion concentration rather than the direct effect of too much carbon dioxide.

The effect of pH on fungal growth had been investigated initially using an unbuffered medium. The results have shown that the fungi. were able to alter the culture pH to suit their needs. A buffer was found which was non-toxic and this was used in conjunction with Painter's Medium A.

The composition of a particular medium affects the ease with which the pH is modified. Buffers are used to maintain the pH of a solution at a relatively constant level when an acid or base is added or when the solution is diluted. Buffers are generally mixtures of weak acids or bases and their salts and in culturing fungi it is important to choose buffers which maintain the pH of the medium in the desired range (Child et al., 1973). The phosphate buffer is frequently used in fungal cultures but its usefulness is limited by tolerance of the fungus to the ion (Cochrane, 1958). The high levels of phosphate required to provide adequate buffering may also prove toxic to some fungi. (Lilly and Barnett, 1951). Citrate used as a buffer system may become a source of carbon for the fungus resulting in a misleading pH optimum (Cochrane, 1958).

Britton and Robinson's buffer mixture was composed of potassium dihydrogen phosphate, citric acid, veronal, boric acid and hydrochloric acid. Geotrichum candidum and Fusarium tabacinum were cultured in shake flasks of buffered Medium A as described in Chapter 4 (4.3.4). The method of culture turbidity to measure mould growth, as described by Trinci (1972), was used instead of relying on dry weight determinations alone. Culture turbidity was measured daily and dry weights and final dry readings were obtained at the end of each experimental trial. The constant-temperature cabinet housing the orbital-shaker apparatus was set at 15°C which was lower than during the experiments with unbuffered Medium A. The first trial for both G.candidum and F.tabacinum continued for nine days and the results enabled a time limit to be established for the next trial.

Comparing Fig. 4.17 (unbuffered medium) with Fig. 4.32 the maximum dry weight of G.candidum was higher in the unbuffered

medium than in the buffered medium (first trial). The final culture pH values had been raised by the fungus in the buffered medium between pH3 and pH6 but these values were lower than those in the unbuffered medium. In the second trial (Fig. 4.35), which lasted for six days, the final culture pH was raised at initial pH4 and pH5 and the dry weights were higher at these pH values compared to the first trial. Maximum biomass was produced at pH3 in the first trial and pH4 in the second trial compared with pH6 in the unbuffered medium.

Comparing Fig. 4.38, 4.41 and 4.44 with Fig. 4.18 (unbuffered medium) maximum biomass of F.tabacinum was achieved with much higher dry weights in the buffered medium in all three trials compared with the unbuffered medium. These results contrast with the findings described for the isolate of G.candidum used in the present investigations and recorded in Figs. 4.17 and 4.32. The lower temperature of 15°C (in the experiments with buffered Medium A) should have had more effect on the growth of F.tabacinum judging by the curves of the growth rates of the two fungi (Fig. 4.6 for G.candidum and Fig. 4.11 for F.tabacinum). The difference in growth of the two fungi in the buffered and unbuffered media must, therefore, be attributable to the buffer.

Maximum biomass was achieved at pH5 with F.tabacinum in all three trials which supported the results with the unbuffered medium. The final culture pH values in the second and third trials were raised above the initial values between pH4 and pH7 but these were lower than those reached in the unbuffered medium.

Comparing the effect of pH on G.candidum and F.tabacinum in unbuffered and buffered Medium A showed that growth was restricted at values above pH7 using the buffer system. Maximum biomass of F.tabacinum was achieved at pH5 in both media but was achieved at

a lower pH in buffered medium with G.candidum. The effect of pH depends on the composition of the medium in the case of G.candidum. Dimond and Peltier (1945) showed that the pH of Penicillium notatum cultures could be controlled by using different carbon and nitrogen sources. Lilly and Barnett (1947) showed that Sordaria fimicola was capable of growth between pH3.4 and 3.8 provided that thiamin was added to the medium at the correct moment. These authors also showed that perithecial formation by S.fimicola required biotin in conjunction with the necessary pH. Tomlinson and Williams (1975) described the effect of pH on six fungi using a basic medium and substituting sodium butyrate for glycerol. The substitution was found to limit the growth of all the fungi at the lower pH values.

Dry weight determinations could only be obtained at the end of each experiment so the daily monitoring of growth was achieved by measuring the optical density of samples from each flask. This method can only be used for fungi which grow in a homogeneous, filamentous form such as G. candidum (Trinci, 1972) and species of the genus Fusarium (Burkholder and Sinnott, 1945). Optical density measurements were made using a spectrophotometer. The importance of preparing a standard curve on a particular instrument was emphasised by Koch (1970). Culturing the same organisms under different conditions involves preparing new standard curves so the fungi used were maintained under the same conditions throughout the trials.

G.candidum was capable of growth between pH2 and 9 in unbuffered medium but Fig. 4.31 and 4.34 only show growth between pH3 and 7 with the highest optical density at pH4 in both trials using the buffered medium. Figs. 4.33 and 4.36 showed that the dry weight and optical density curves were compatible.

F.tabacinum was capable of growth between pH3 and 9 in unbuffered medium but Figs. 4.37, 4.40 and 4.43 showed most growth between pH4 and 7. In all three trials in buffered medium the maximum growth of *F.tabacinum* as indicated by the highest optical density was at pH5. Fig. 4.43 showed that *F.tabacinum* was capable of growth at pH8 and 9 with a trace of growth at pH10. The final culture pH readings do not offer any explanation for these results which were obtained in the third trial. Fig. 4.37 (first trial) showed a small amount of growth of *F.tabacinum* at pH3 and pH8 but the final readings at pH8 were much lower than those of the third trial (Fig. 4.43).

Comparing the optical density curves of *F.tabacinum* with the dry weight curves (Figs. 4.39, 4.42 and 4.45) showed that there was good agreement with the two methods of measuring growth. Maximum growth as indicated by optical density readings was recorded at pH5 in all three trials. The maximum dry weight of *F.tabacinum* was recorded at pH4 in the first trial (dry weight at pH5 was only one milligram lower), at pH6 in the second trial and at pH5 in the third trial.

The results obtained with the use of optical density as a measure of mould growth have been favourable with the two fungi used in these trials. Culture turbidity was measured regularly and enabled fungal growth to be monitored so that harvesting could be carried out at suitable time. Using a buffered medium demonstrated the inablility of the fungi to grow at the higher pH values previously achieved in the unbuffered medium. Fungi tend to change the pH of the media in which they grow as a result of their metabolism. Failure to adjust the culture pH to a value favourable for growth, as in a buffered

medium, restricts the growth as described. A strong correlation between the optimum pH range for growth and the optimum pH range for most enzymes has been recorded (Lilly and Barnett, 1951). The rate of certain enzyme reactions is probably modified by pH changes which affect the fungal growth rate.

Fungi utilizing carbohydrates producing inorganic acids such as carbonic acid and organic acids such as pyruvic, citric and succinic acids. Carbonic acid is unstable in the presence of stronger acids and decomposes liberating carbon dioxide. Carbonic acid reacts with bases to form bicarbonates in alkaline solutions which can affect fungal growth at these higher pH values. Pyruvic acid can cause the early decrease in pH of nutrient solutions supporting fungal growth by accumulating in the medium. The pyruvic acid is eventually utilized by the fungi and the pH of the solution is raised.

Magnesium and phosphate ions, both present in Medium A, are soluble in acidic solutions but combine to form an insoluble compound as the pH rises and the hydrogen ion concentration falls. These ions become unavailable to fungi cultured in alkaline media which may affect their growth. In this way ions which are essential for growth or toxic may become more or less easy to absorb with changing pH of the medium. The protoplasmic membrane of the fungal cells may become saturated with hydrogen ions preventing the entry of necessary cations at low pH values. At alkaline pH values the hydroxyl ions may prevent the entry of anions into the fungal cells. Changes in external pH may affect the internal pH of cells but the effect on permeability is probably the most important (Cochrane, 1958).

The investigations of the effects of temperature and pH

on the growth of the selected fungi were performed in batch cultures. Both fungi tolerated a wide range of temperatures and were able to produce maximum biomass at acidic pH values of below pH5. However, the conditions experienced in shake-flasks or on agar-plates differ markedly from cultural conditions in a pilot-plant. Batch cultures have initially high levels of nutrients which are utilized by the fungi but are not replenished. The metabolites produced as a result of fungal growth will gradually accumulate and may become toxic since they cannot be removed from the system. Continuous culture of fungi would provide conditions for growth closer to those found in a bacteria bed and enable further experiments with variables such as dosing frequency. The experiments using continuous culture conditions were a preliminary to the pilot plant studies.

The continuous culture apparatus was developed as described (Chapter 3, section 3.3). It was used in experiments described in Chapter 4, section 4.3.5, and was initially based on apparatus used by Green et al., (1965).

Geotrichum candidum was grown for sixty-five days on vertical, cylindrical, plastic-mesh screens at 15°C. The feed was 5% $^{w}/_{v}$ skimmed milk delivered to the screens at a hydraulic loading of 4.95×10^{-4} $m^3/m^2/hr$. The wet weight of all three screens increased progressively until day 50 for screen number two and day 61 for screen numbers one and three (Fig. 4.46). Fluctuations in the wet weights of the screens did not appear to be related to the changes in the mean flow-rate to each screen.

Fusarium tabacinum was cultured on the same apparatus under the same conditions of feed strength, flow-rate and temperature (Fig. 4.47). The wet weight increased on all three screens until day 61 with screen number four, day 55 with screen

number five and day 50 with screen number six. Between day 50 and day 55 screen number six lost almost half its wet weight and this was not attributable to the changes in the flow-rate of the feed. The weight loss from screen number six was the greatest recorded at this feed strength and was probably due to excessive film accumulation concentrated on one strip of the screen.

Reducing the strength of the skimmed milk to 2% $^{w}/_{v}$ resulted in greater fluctuations of the wet weights of the screens inoculated with Geotrichum candidum (Figs. 4.48 and 4.49). The wet weights of screen numbers two and three increased steadily between day seven and day 22. The decrease in the mean flow-rate to the screens coincided with loss of weight from screens two and three. After losing weight these two screens supported a further increase in weight and then exhibited a dramatic loss of biomass. During the period between day 39 and day 42 screen three lost more than 200 gm. in wet weight. Growth on screen one did not increase as much as on the other screens but the weight gain was steady throughout the period from day seven to day 42.

From day 77 to day 123 the growth of G.candidum on all three screens fluctuated dramatically. Between day 84 and day 100 the screens were dosed with tap water which resulted in overall weight loss from screens one and three from which there was no recovery. The wet weight of screen two increased during the period of dosing with tap water then decreased as the flow-rate decreased. Reverting from tap water to 2% $^{w}/_{v}$ skimmed milk, after day 100, resulted in a rapid weight gain on screen two until day 114 after which the weight rapidly decreased.

Growth of F.tabacinum on vertical screens dosed with 2% $^w/_v$ skimmed milk fluctuated in weight in a manner similar to that described for G.candidum. Changes in wet weight coincided with the mean flow-rate of feed to a noticeable extent at this feed strength (Figs. 4.50 and 4.51) compared to 5% $^w/_v$ skimmed milk.

When dosed with tap water the wet weights of the three screens increased over the first four days (day 84 to day 88) and then decreased. All three screens continued to lose weight until the experiment reached completion.

The maximum wet weight of G.candidum was 648 gm. after 65 days dosed with 5% $^w/_v$ skimmed milk. The weaker feed of 2% $^w/_v$ skimmed milk did not generally encourage high wet weights and although the maximum was 750 gm. it took 112 days to achieve.

The maximum wet weight of F.tabacinum was 720 gm. after 68 days on 5% $^w/_v$ skimmed milk and 320 gm. after 88 days on 2% $^w/_v$ skimmed milk.

Observations of the fungal growth on the vertical screens revealed that the pattern of growth was initially determined by the distribution of the feed. Skimmed milk was distributed as evenly as possible over the upper area of each screen. Fungal growth was then limited to those areas which received an initial wetting. The successive build up of film eventually became too heavy when confined to a narrow vertical strip of screen and sloughed off. Examination of the film after sloughing did not reveal any anaerobic areas and fungi were successfully cultured from all depths. Tomlinson and Snaddon (1966) found that fungal mycelium was capable of attaining and maintaining itself at considerable thickness due to protoplasmic streaming. Sanders (1966) found that dense slimes sloughed off after approximately fourteen days had elapsed since inoculation but the sparsely

populated slimes continuously sloughed off. The results described here have supported Sanders findings to the extent that there were fewer fluctuations in wet weight of film at the stronger feed (5% $^{w}/_{v}$) than there were with the weaker feed (2% $^{w}/_{v}$) of skimmed milk.

Atkinson and Fowler (1974) discussed the significance of "penetration depth" which represents the distance through which nutrients can penetrate to maintain film organisms in an active state. These authors considered that there was likely to be an optimum film thickness, closely related to the penetration depth, leading to a maximum rate of substrate uptake at the film and nutrient interface at a particular substrate concentration. When the thickness of the film surpasses the penetration depth the organisms furthest from the nutrient supply begin to autolyse . The adhesion of the film to the support surface deteriorates and the film eventually sloughs off under its own weight (Atkinson and Fowler, 1974).

Hawkes (1957) described the findings of investigations concerning the relationship between film accumulation and grazing activity in Birmingham sewage filters. The film of the Minworth filters was dominated by fungi. When excessive accumulations of fungal film occurred the results were anaerobic conditions in the filters. The fungal film broke down under these conditions producing a watery sludge which was discharged from the filters.

Tomlinson and Snaddon (1966) used inclined rotating tubes to determine the effects of the thickness and weight of the film on the treatment of organic matter in sewage. These authors found that film could be prevented from detaching from the tube walls by increasing the oxygen content of the atmosphere inside the tube. The increased supply of oxygen to the underlying layers

prevented anaerobiosis and sloughing.

In the present investigation the cause of sloughing seemed to be solely due to too much film concentrated on a small area of the screens. The screens themselves were hollow cylinders with a mesh construction to allow air to circulate freely. The situation in the pilot plant filter was different from the laboratory apparatus because the media was not perforated. Film in the pilot filter cannot penetrate the media, therefore, it should be easier to induce sloughing in this system than in the laboratory apparatus. The laboratory screens were washed with a strong jet of water which removed some of the heavy growths but there was a considerable amount of film still attached. The importance of being able to control sloughing stems from the need to obtain fungal biomass in a healthy condition for use as a feed and not when the cells have entered a decline phase. It may be possible to wash the fungal film off the pilot plant media as a means of harvesting the biomass. This method has not been tried because insufficient biomass has been produced by the pilot plant.

Figs. 4.52 and 4.53 show that the wet and dry weights of both Geotrichum candidum and Fusarium tabacinum were much higher at the most concentrated feed strength (5g./litre) than at the weakest feed strength (0.5 g./litre) of skimmed milk. These results confirm the findings of Tomlinson (1946) and Hawkes (1965) who have both shown that the growth rate of film was related to the strength of the sewage, quite apart from the rate of application. Fungal growth is, therefore, encouraged by strong organic feeds such as dairy wastes. The creamery at Minsterley was known to produce a sufficiently strong effluent and was chosen to site the pilot plant. In the pilot plant studies it was intended to use the creamery effluent as feed and control the pH, loading

and dosing frequency to encourage fungal biomass production. Harvesting the biomass in a healthy condition will depend on the control of the variables mentioned and the ability to induce sloughing.

CHAPTER FIVE

T H E   P I L O T   P L A N T

CHAPTER 5

THE PILOT PLANT

5.1 INTRODUCTION

The main objective of the investigations was the production of utilizable fungal biomass in high-rate Flocor filters treating dairy waste. The laboratory studies (Chapter 4) were intended to establish the optimum growth conditions of the selected fungi chosen for their occurence and suitablity for biomass production (Chapters 2 and 3). The pilot plant was intended to operate under the conditions determined in the laboratory and to ascertain the feasibility of using a full-scale plant.

5.2 SITING THE PILOT PLANT

The initial site for the pilot plant was at Uttoxeter in Staffordshire where Unigate Limited used a Flocor high-rate filter to treat milk and creamery effluent. Unfortunately, proposed plans for yoghurt production at the dairy were shelved and consequently the effluent strength did not increase. The strength of the waste was considered to be too weak to support fungal growth (BOD values of 100 mg./l.). After an inspection of the plant and assessment of the fungal growths the site was considered to be unsuitable for further experimental work.

Express Dairies creamery at Minsterley in Shropshire was chosen as the nearest suitable site to Birmingham. The effluent was known to be strong with BOD values of approximately 1500mg./l. and an acidic pH. Production at the creamery had expanded with a greater intake of milk and the addition of a cottage-cheese factory was proposed. A single-stage Flocor high-rate filter was

constructed to relieve the overloaded percolating filters at the creamery's treatment plant. The Flocor filter contained 710 m^3 of media and was designed to cope with a flow of 1360m^3/day with a BOD of 1500 mg./l. and a daily BOD load of 3 kg. BOD/m^3d. There was sufficient land at the base of the Flocor filter to construct the pilot plant and permission to build was granted by Express Dairies in the summer of 1978.

5.3 DESCRIPTION OF THE PILOT PLANT

The pilot plant was constructed at one corner of the large high-rate Flocor tower installed at the Minsterley creamery (Plate 9). The pilot scale filter tower was constructed of four half-modules of Flocor media 0.6m x 0.6m x 0.6m (see Plate 2, Chapter 1) stacked vertically. Each half-module was arranged at right-angles to the layer below to allow sufficient retention of the applied liquid. To enable incremental weights to be determined the Flocor modules were suspended from a steel tower set in a concrete base.

Fig. 5.1 shows the method used for supporting and weighing the Flocor modules (using rods "A" and "B"). Each module rests on a supporting frame which is suspended from either the main supporting tower on rods "A" or a weighing system on rods "B". In order to weigh a Flocor module it is necessary to detach its supporting frame from rods "A" and attach the supporting frame to rods "B". The module to be weighed can then be raised by tightening the wire strainer and the weight read from the spring balance. When a module other than the top one is raised all higher modules will be raised thus giving a cumulative weight reading. Weights of individual modules being calculated by

FIG. 5.1 Diagram showing method of supporting and weighing modules used in pilot filter tower (not to scale)

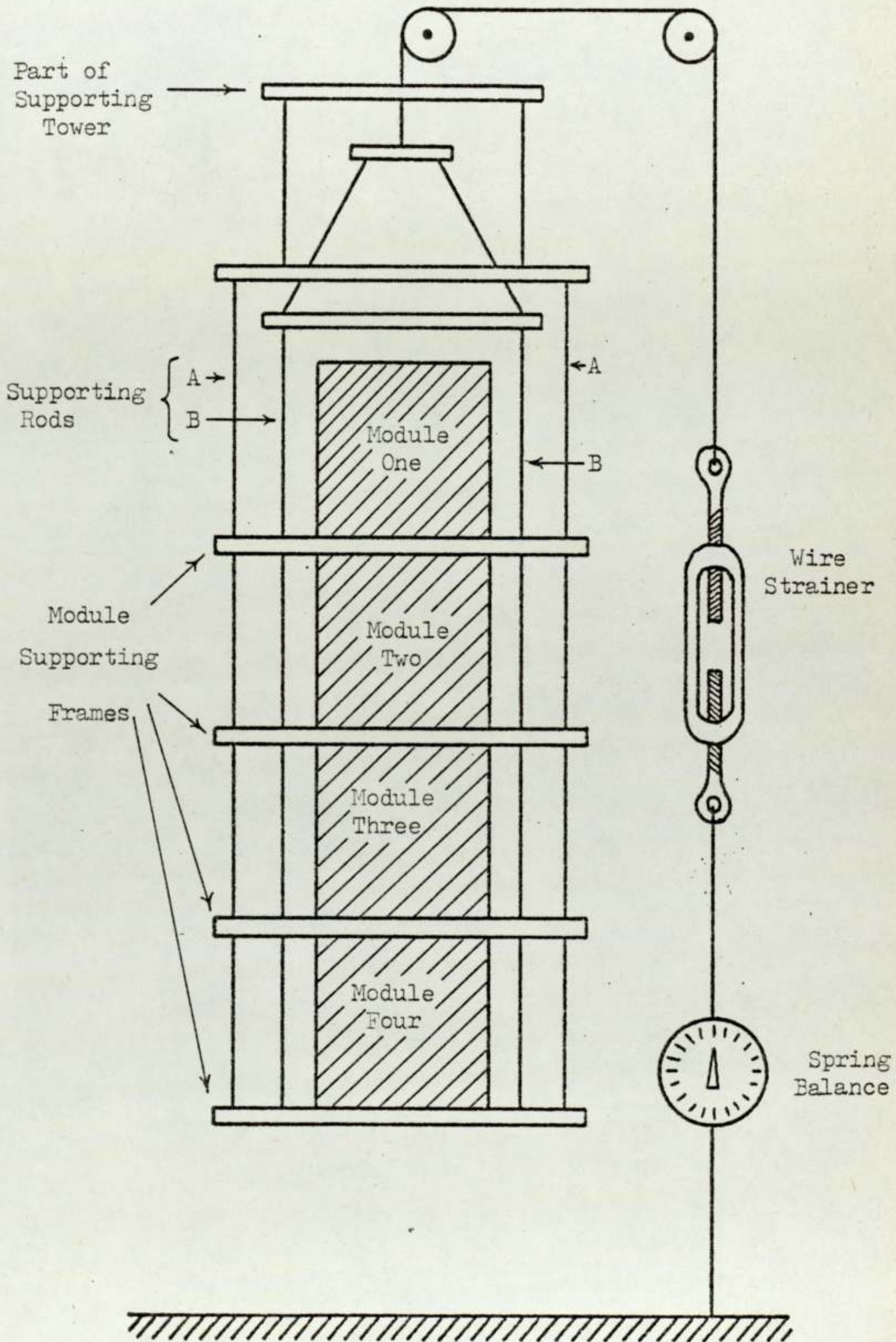
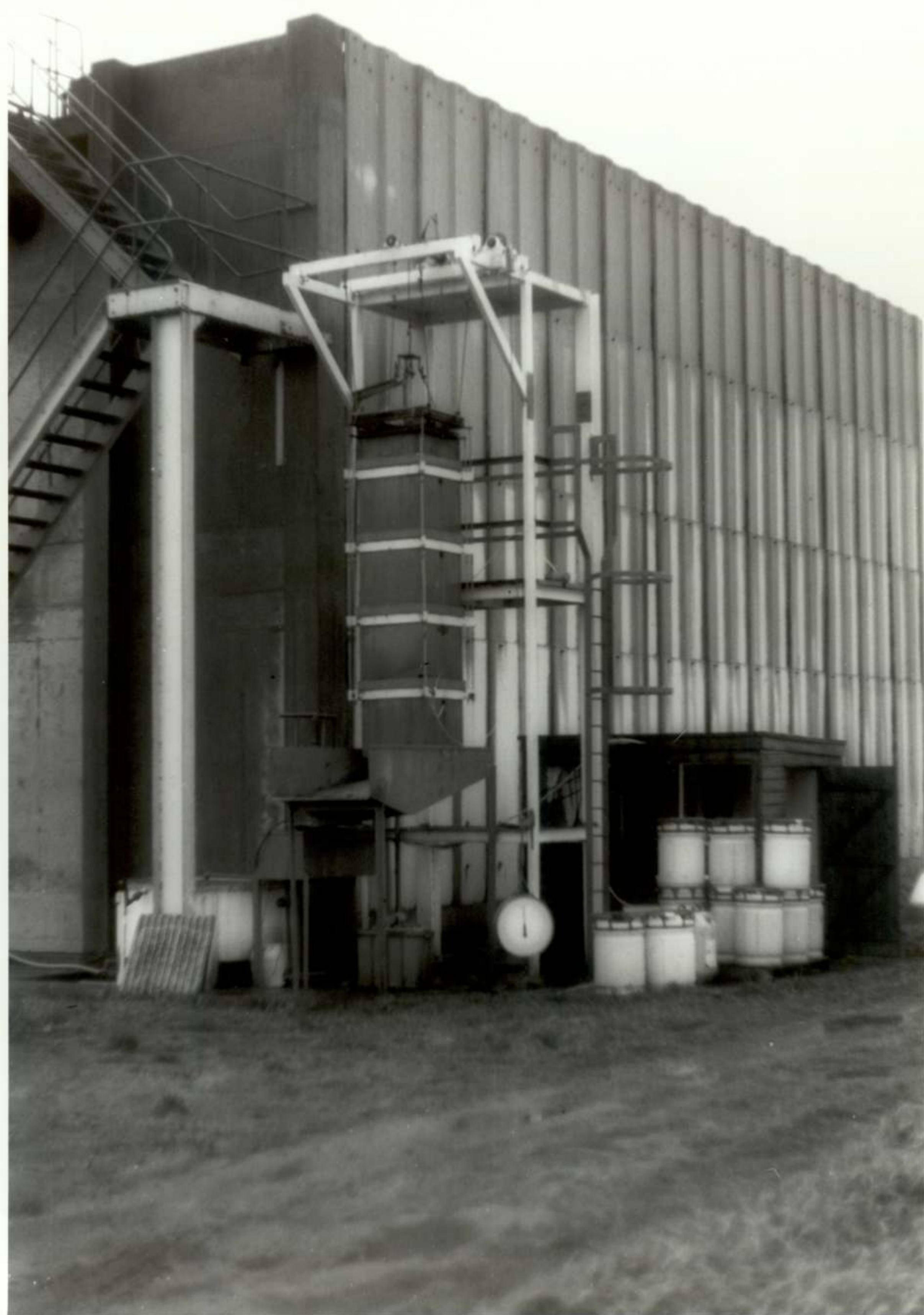


PLATE 9

VIEW OF THE PILOT PLANT SITED IN
FRONT OF THE LARGE FLOCOR FILTER
AT MINSTERLEY



subtraction. The distribution of film throughout the filter was to have been determined from the calculated weights of the modules giving an insight into internal conditions. This knowledge could have been beneficial when harvesting the biomass from the filter as a build-up of film in one section may have indicated the likelihood of sloughing. Unfortunately during construction of the pilot filter the rods were misaligned thus only the total weight of the four modules could be measured.

Part of the effluent from the creamery was fed to the main Flocor tower, and part to the pilot plant where it entered a blending tank fitted with a pH probe (Fig. 5.2). The pH of the feed was adjusted to 3.5 by the addition of sulphuric acid as required. A wooden shed located behind the pilot filter (Plate 10) housed the automatic pH control and recording equipment, the influent and effluent temperature recorders, timing equipment and a refridgerated sampling unit. Isolation switches were provided for the electrical equipment in the shed and an over-riding isolation switch was provided to disconnect the mains electricity from the whole pilot plant if necessary.

The feed was acidified to provide the optimum conditions for the growth of selected fungi and to minimise bacterial contamination in non-aseptic conditions (Tomlinson, 1976b). The intention was to provide the optimum conditions for the growth of Geotrichum and Fusarium and try to culture them selectively if possible in the pilot plant. The laboratory studies had shown that an acidic pH was favourable for the growth of these fungi hence the pH control system.

The feed was pumped to the top of the pilot filter and distributed over the surface of the media by impinging on a single central splash-plate (Plate 11). The effluent from

Fig. 5.2

Diagram to show the functional layout of the pilot plant.

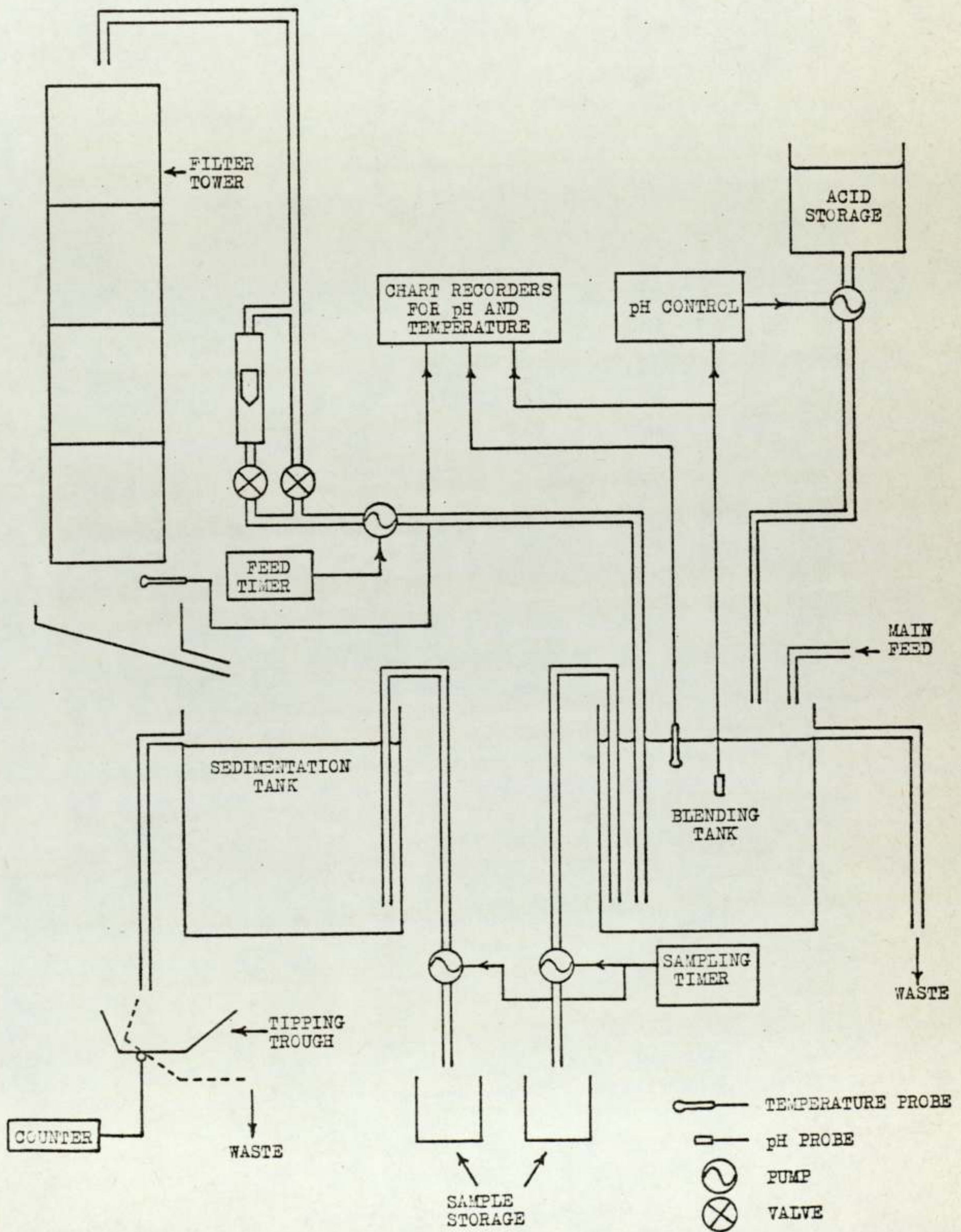
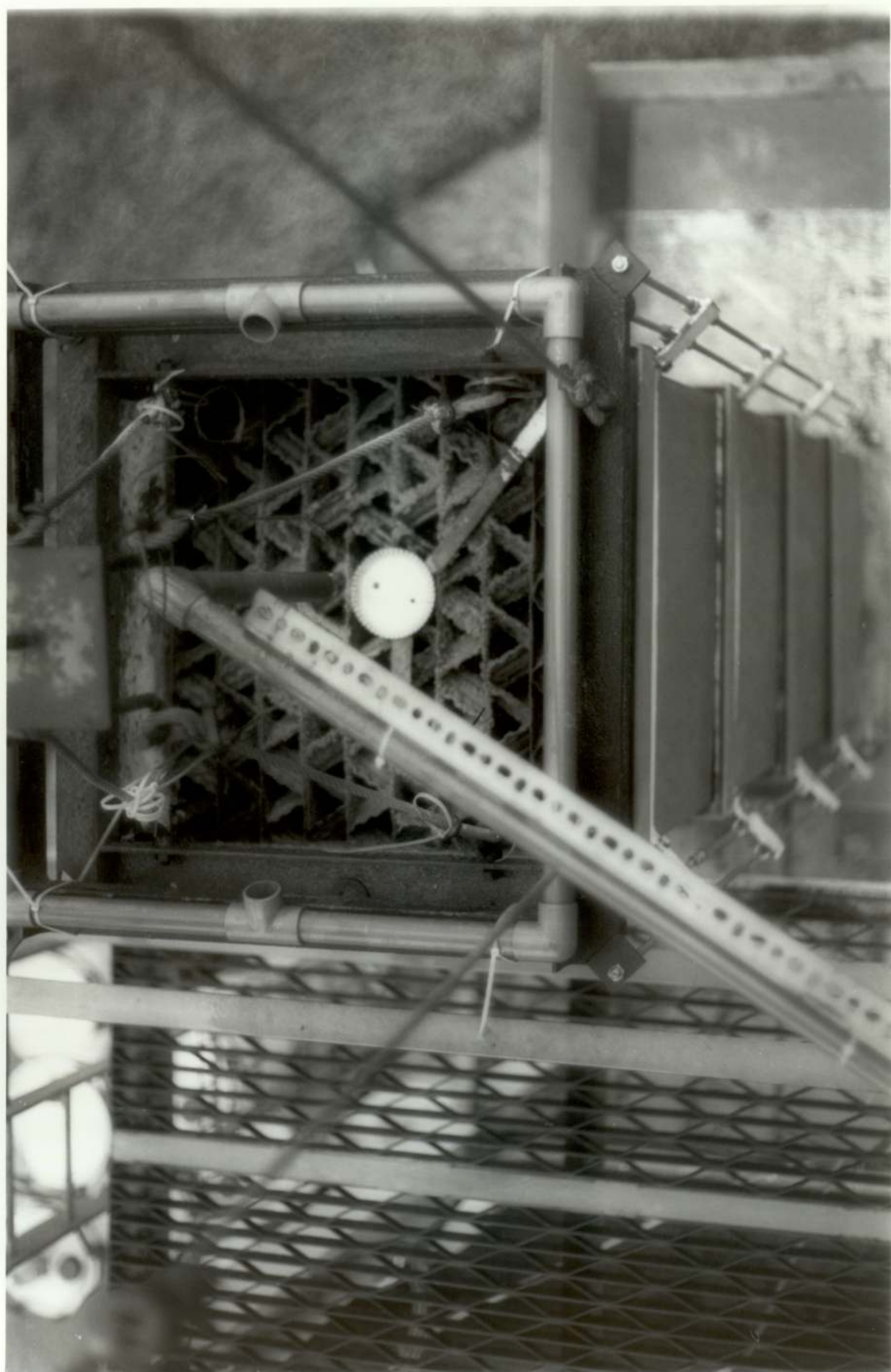


PLATE 10

SIDE VIEW OF THE PILOT PLANT
SHOWING THE WOODEN SHED HOUSING
SAMPLING AND TIMING EQUIPMENT

PLATE 11

VIEW OF THE TOP OF THE PILOT FILTER SHOWING
SINGLE CENTRAL CIRCULAR SPLASH-PLATE AND ROD
SYSTEM SUPPORTING TOWER



the filter passed through an inclined screen, to collect bios, mounted under the base of the tower (Plate 12). Inclined screens of different porosity could be fitted in accordance with the nature of the bios being discharged. The effluent then passed through a separate compartment of the mixing chamber below the inclined screen, and was gravity fed to a sedimentation tank (bottom of Plate 13). The overflow from this tank fed into a 20 litre tipping trough fitted with a counter which recorded the number of times the trough emptied and thus the flow-rate through the system could be calculated. The effluent from the pilot plant was channelled to meet the effluent from the main Flocor tower and received further treatment in the stone bacteria beds operating on alternating double filtration.

The pilot filter influent temperature was measured by a probe in the blending tank below the inclined screen and the effluent temperature was measured by a probe entering the base of the lowest module in the tower. Samples of influent and effluent were withdrawn in 100 ml. aliquots from the blending tank and sedimentation tank (respectively) every four hours. The samples were stored in 10 litre polythene bottles in the refridgeration unit until they were collected and taken to the laboratory for analysis. BOD and COD determinations were made on the collected samples, the results of these analyses together with the temperature and pH readings have been recorded in Table 5.1. It has not been possible to obtain accurate measurements of the flow rate through the pilot plant because of malfunctions of the tipping trough counter.

P L A T E 1 2

VIEW OF THE BASE OF THE PILOT FILTER

SHOWING THE INCLINED SCREEN

PLATE 13

SIDE VIEW OF THE PILOT PLANT SHOWING

THE SEDIMENTATION TANK

TABLE 5.1

Pilot plant results from November, 1978 to November, 1979.

INF = INFLUENT

EFF = EFFLUENT

% R = PERCENTAGE REMOVAL

		TEMP. °C		SETTLED COD			SETTLED BOD			FILM (kg.)
MTH.	pH	INF	EFF	INF	EFF	% R	INF	EFF	% R	WET. WT.
NOV				2640	2420	8	1750	1350	23	
DEC				2372	1548	35	1475	1100	25	
FEB				2820	3120		1550	1400	10	
FEB				1730	1450	16	850	850	0	
MAR				2280	2040	10	1400	1250	11	90.8
MAR				2580	1600	38	1325	725	45	181.5
APR	3.0	11	6	2500	1800	28	1212	222	82	113.5
APR	4.5	20	17	1200	840	30	1175	950	19	181.5
APR	4.0	24	10	2580	2000	22	1300	632	51	142.9
MAY	4.5						1425	1150	19	34.1
JUN	3.5			1320	1040	21				56.8
JUN	3.5			2540	1480	42	1200	912	24	
JUN	6.0	26	22	2080	1520	27	3975	1400	65	63.6
JUL	6.0	23	23	1140	920	19	1000	862	14	45.4
JUL		21	26	1720	1060	38	1412	1375	3	59.0
JUL	4.0	20	22	1960	1540	21	1412	1310	7	47.7
JUL	6.0	18	25				1115	925	17	45.4
JUL		27	27	1320	620	53	850	675	21	50.9
AUG	4.5	23	28	680	560	18	987	512	48	15.0
SEP	4.0	24	17	1200	940	22				
SEP	4.6			1020	540	47				52.2
OCT	4.8	23	18	980	750	23	1150	950	17	54.5
OCT	4.5	23	16	660	300	54	812	637	21	25.0
OCT	4.5	23	13	1240	760	39	962	750	22	27.3
NOV	4.5	24	14	1140	880	23	1112	737	34	40.9
NOV	4.3	23	15	1440	1140	21				57.7

5.4 OPERATING THE PILOT PLANT

The pilot plant was commissioned early in October 1978. Problems with the feed occurred almost immediately due to the presence of solids from the cottage cheese and butter production. The solids accumulated and blocked the down-pipe of the gravity feed system. This pipe was changed for one with a larger diameter which increased the load to an unacceptable level of 10 kg./m^3d. The loading was reduced by using intermittent dosing to 5 kg./m^3d. Serious problems with pumps, recorders, samplers and sump blockages were encountered later on in October. The equipment received attention and the plant continued to operate, however, there were more problems with solids. The inclined screen was unable to cope with the biomass discharged from the filter and different mesh sizes were tried. These difficulties were eventually dealt with by installing a larger inclined screen, a new pumping sump and feed pump. Facilities for recycling the effluent through the filter and for intermittent dosing were provided.

The modified pilot plant began operating in May 1979 with the filter media dosed intermittently (10 minutes every 15 minutes) at a loading of 2.5 kg. BOD/m^3d. Unfortunately there were further problems with the pumps and the timer which persisted through out the summer months. During June and July the dosing system, controlled by the timer, was only delivering the feed for twenty seconds in every fifteen minutes. Both the feed pump and the acid pump failed and consequently very little bios developed on the filter media. The pumps were repaired and the timer was adjusted to deliver feed for five minutes in every fifteen minutes. Bios was collected from the inclined

screen in September but was virtually absent in October when the population of Psychoda fly larvae increased dramatically. Very little bios was collected throughout November, the timer was adjusted to deliver feed for ten minutes in every fifteen minutes and then reduced to deliver for six minutes in fifteen. Increasing the dosing frequency was intended to encourage fungal growth but this attempt proved to be unsuccessful. At the end of November problems were encountered with the weighing machine, these were rectified but the pilot plant stopped running in early December 1979 due to pump failures.

During the period from November 1978 to November 1979 the pilot plant was operated for as long as possible unless the main feed pump failed. Problems encountered during the periods of operation unfortunately make it impossible for any conclusions to be drawn from the results. The influent and effluent temperatures, COD and BOD analyses, pH and weight of the tower were all measured when possible.

5.5 RESULTS

The results of the analyses, and parameters measured during the operation of the pilot plant from November 1978 to November 1979, are recorded in Table 5.1. The wet weights of the film in the pilot filter were obtained by subtracting the weight of the clean dry tower (204 kg.) from those obtained during operation.

The COD and BOD analyses generally confirmed that the effluent from the creamery was of a sufficient strength to support fungal growth. The influent pH was adjusted, if necessary, to pH 4.5. Failure of the acid pump occasionally resulted in the pH rising to pH 6. The highest film wet weights were

obtained in April 1979 but this coincided with excess solids in the creamery effluent and may not have been due to film micro-organisms alone.

Bios was collected from the pilot filter tower upper and lower surfaces and from the inclined screen. The samples were examined in the laboratory with the aid of a microscope. Any fungal hyphae present were measured, assessed for their relative abundance, washed and plated out on ring-plates of agar media (Chapter 2, section 2.4). Pure cultures were obtained and identified at irregular intervals due to the operation of the pilot plant. Equipment failures resulted in poor biomass production reflected in the low wet weights of film recorded in Tables 5.1. Four species of fungi were isolated from the film samples on all occasions. Geotrichum candidum and Mucor racemosus were equal in abundance and both were dominant over Fusarium sp. and Trichosporon sp.

Biomass was collected from the inclined screen from the end of May 1979 to the end of November 1979. The samples were dried with an infra-red heat source and sent to I.C.I. at Billingham for analysis. The results of the analysis are recorded in Table 5.2.

5.6 DISCUSSION

The pilot plant studies were intended to extend the laboratory studies and expand on the results obtained. The choice of site for the plant was suitable because of the strength of the effluent and an existing large-scale filter containing Flocor media to compare the ecology. The distance of the pilot plant from the University was a disadvantage in terms of maintainance but was unavoidable. The pilot filter itself had

previously been employed in investigations on basic film accumulation studies in high rate filters. The filter had been housed inside a laboratory and therefore not exposed to external weathering. Dairy wastes tend to include solids depending on the nature of the product, there was a fat trap installed at the works which should have overcome the problem of solids in the pilot plant. Intermittent dosing of the feed was introduced to alleviate the problem of increased loading. It was intended to investigate the relationship between dosing frequency and fungal growth as a means of controlling biomass production. Low frequency dosing has been developed as a successful method of controlling fungal film in filters and the theory has been discussed by Hawkes (1961). The use of too low a dosing frequency on the pilot plant would result in limiting the growth of fungi which was not desirable in this investigation. Equipment failures involving the pumps, and the timers which controlled the dosing frequency, have not allowed sufficient trouble-free operation to obtain results regarding changing dosing frequencies. It was also intended to assess the effects of changing the organic loading on the amount of biomass produced. Pump failures and other faults have precluded obtaining any meaningful results which could be attributable to changing the organic loading.

Percolating filters treating industrial effluents have film growth dominated by fungi in the winter months. The reasons for the seasonal fluctuations in film levels are related to temperature, the strength of the feed and the loading of the filter. Fungi have less competition for food during the winter and their ability to survive at low temperatures enables them to dominate the film. In the warmer summer temperatures the fungal

growth rate is higher but the insects which graze on them increase in number and the feed usually weakens in strength. The result of this combination of conditions is that fungi do not tend to dominate film in the warmer months of the year. (Tomlinson, 1946; Hawkes, 1965). Heukelekian (1945) considered temperature to be an important factor influencing the removal of BOD and applied solids in high-rate filters. He compared conditions between a high-rate filter and a standard filter and arrived at a number of conclusions. He found the volumetric application rate had a limited influence on film accumulation whereas the applied load of BOD and suspended solids had a direct influence on the quantity of film. In the high-rate filter the film decreased in the summer but there was no evidence of seasonal unloading. Tomlinson (1946) stated that the growth rate of film was related to the strength of the sewage apart from the rate of application. He found that fungi became vulnerable to bacterial attack in effluents with poor nutrient content.

The results obtained from the pilot plant, recorded in Table 5.1, have confirmed the findings of Tomlinson (1946) and Hawkes (1965). The highest temperatures were recorded in July and August which coincided with the weakest feed (measured as COD and BOD) and the lowest wet weights of biomass. The pH values of the pilot plant feed varied between pH3 and pH6, the higher values occurred when the acid supply diminished or the pump failed. It is difficult to assess the effect of such low pH values on the selectivity and growth of fungi in the pilot plant because of the effects of equipment failures on the operation of the plant. It took seven months to harvest sufficient biomass for amino acid analysis which is an indication of the

time lost due to equipment breakdowns. Comparing the amino acid analysis of the pilot plant biomass with that of Geotrichum candidum and Fusarium tabacinum grown in the laboratory (Table 5.2) shows much lower levels of amino acids than is desirable. G.candidum and F.tabacinum both had amino acid profiles which compared favourably with commercial fungal feed products. The fungi were cultured in the laboratory on vertical plastic screens and fed with skimmed milk with a COD of 5000. Biomass harvested from the pilot plant between May and November 1979 had been fed with dairy effluent with COD values ranging from 660-2540. Weak feeds do not encourage fungal growth particularly in the warmer months of the year and there was probably an insufficient proportion of fungal material in the biomass harvested hence the poor analysis results.

The highest weights of biomass in the pilot filter were obtained in March and April, 1979 coinciding with low pH values and strong feed. Geotrichum candidum has been cultured at low pH values, at pH3.4 in acid brine (Hang et al., 1974) and at pH 3.5 in potato processing waste (Tomlinson, 1976a) and corn waste (Church et al., 1973). Fusarium species have been found to grow successfully at pH4.6 in pea waste (Church et al., 1973) and on a wide range of carbohydrate-based substrates between pH3 and 6 (Righelato et al., 1976). The ability to grow at low pH is a valuable feature of Geotrichum and Fusarium because most contaminating organisms such as saprophytic bacteria grow poorly or not at all at acid pH. Aseptic precautions are rendered unnecessary and this reduces the cost of large-scale production.

The objective of the pilot plant studies was to produce

TABLE 5.2

Amino acid analysis (g./100g.) and ion analysis of biomass collected from a pilot scale high-rate filter compared with *Geotrichum candidum* and *Fusarium tabacinum* grown on laboratory scale vertical screens.

		Pilot plant	Geotrichum candidum	Fusarium tabacinum
Amino acids (g./100g.)	Alanine	1.65	3.33	3.33
	Arginine	1.30	1.85	1.91
	Aspartic acid	2.35	3.92	4.24
	Glutamic acid	2.90	5.99	5.90
	Glycine	1.07	1.93	1.96
	Histidine	0.41	0.98	0.96
	Isoleucine	1.10	1.87	2.07
	Leucine	2.50	3.45	3.31
	Lysine	1.45	2.88	2.95
	Methionine	0.39	0.86	0.57
	Phenylalanine	1.05	1.78	1.83
	Proline	0.86	2.07	2.05
	Serine	1.15	1.98	2.00
	Threonine	1.30	2.06	2.07
	Tyrosine	0.93	1.75	1.77
	Valine	1.30	2.39	2.51
	% Mg	0.11	0.19	0.20
	% K	0.36	1.3	1.4
	% P	0.97	1.7	1.7
	% S	0.71	0.82	0.49
	% C	48.4		
	% H	7.7		
	% N(total)	6.1	7.8	8.0

utilizable fungal biomass while treating dairy effluent to an acceptable degree of purification. The effluent was sufficiently strong in organic matter to support fungal growth as established in the laboratory studies. The temperature of the pilot plant feed was high enough to promote good fungal growth rates and the pH was lowered to encourage Geotrichum and Fusarium. Film samples taken from the pilot filter had revealed the presence of Geotrichum and Fusarium throughout the period between November 1978 and November 1979. However, until it is possible to operate the pilot plant continuously (without interruption from equipment malfunctions) it is difficult to assess the effects of imposed conditions on fungal growth. Establishing Geotrichum and Fusarium as the most abundant species in the pilot plant film and maintaining their growth at low pH should produce fungal biomass with minimal bacterial contamination. Harvesting the biomass in sufficient quantity in a healthy condition will be important from the commercial aspect. The cultural conditions imposed on the fungi should result in adequate effluent purification in conjunction with biomass production. From the limited results obtained from the pilot plant (Table 5.1) the highest percentage removals of COD and BOD were 54% and 82% respectively. However, until the plant functions reliably and consistently it is not possible to predict the performance of effluent purification in relation to production.

The film in the pilot filter and the harvested biomass will need regular and frequent examination for contaminants. A contaminant may have a suitable amino acid profile for use as feed but it must not be toxic. The growth of undesirable organisms must be controlled or the final products, if contam-

inated, will be useless. The production of large quantities of fungal biomass will necessitate toxicity testing on a regular basis. The results of the pilot plant operations indicate that it is feasible to produce fungal biomass but the amino acid levels were lower than those obtained from the laboratory cultured fungi (Table 5.2).

CONCLUSIONS

1. High-rate Flocor filters treating dairy wastes support the growth of numerous species of fungi. Certain of these species occur more frequently than others regardless of the strength of the waste.

2. Geotrichum candidum, Fusarium tabacinum and Fusarium aquaeductuum produce appreciable growth in milk wastes ranging in strength from BOD values of 750 to 1500 mg./l.

3. In pure culture both G.candidum and F.tabacinum are capable of growth between 10°C and 36°C. A maximum growth rate is achieved by G.candidum at 28°C and by F.tabacinum at 29°C. F.aquaeductuum is capable of growth between 5°C and 30°C and has a maximum rate of growth at 22.5°C.

4. F.aquaeductuum has been shown to produce compounds which are toxic to animals irrespective of the culture medium and has been discontinued from further studies because of this undesirable property.

5. The amino acid profiles of G.candidum and F.tabacinum, grown in continuous culture in the laboratory, compare favourably with those of fungal and yeast products indicating suitability for use as animal feed.

6. Both G.candidum and F.tabacinum are capable of growth in buffered Painter's Medium A between pH3 and pH7. G.candidum

produces maximum biomass at pH3 whereas F.tabacinum produces a high yield between pH4 and pH6. In the same medium without the buffer both fungi are able to grow between pH3 and pH9 with maximum biomass at pH6 for G.candidum and at pH5 for F.tabacinum. These results show that the fungi can alter the pH of an unbuffered medium to facilitate their growth. If the buffer capacity of a medium changes for any reason this could affect fungal biomass yields, a factor that is particularly important in a pilot plant.

7. Monitoring the progress of fungal growth can be achieved successfully on a daily basis by measuring the optical density of the fungal culture. This has been illustrated by the similarity between final dry weight and final optical density readings.

8. Continuous culture of G.candidum and F.tabacinum on vertical plastic screens revealed greater fluctuations in wet weights and lower overall weights when dosed with skimmed milk at 2g./l. compared to 5 g./l.

9. Increasing the organic strength of the feed (up to a COD of 5000) to G.candidum and F.tabacinum encouraged a corresponding increase in biomass production in batch culture on horizontal plastic screens.

10. Pilot plant studies show that it is feasible to encourage the growth of certain specified fungi by adjusting cultural conditions. The resultant biomass can be harvested using an

inclined screen.

11. Amino acid analysis of film from the pilot plant indicates that it contains a lower concentration of each amino acid than the laboratory cultured film. The amino acid content should increase when the plant is operating reliably.

RECOMMENDATIONS FOR FURTHER WORK

1. The pilot plant should continue to be operated to overcome previous problems with equipment failures and obtain consistent and reliable data.

2. Experimenting with different dosing frequencies and changing the loading to try and maximize biomass production can proceed when the plant is functioning satisfactorily.

3. The potential productivity of the system needs to be assessed. Maximum biomass production must be related to an acceptable degree of purification of the effluent under treatment.

4. A method of inducing sloughing of the fungal film needs to be developed so that the harvested material is in a healthy condition for use as feed. Encouraging high growth rates and the build up of thick film may result in sloughing as the bios becomes too heavy to remain attached to the underlying media.

5. When sufficient biomass can be produced regularly it will need to be thoroughly tested. Toxicity tests will have to be performed to ensure the safety of the product before full-scale feeding trials begin. Regular amino acid analyses will indicate the usefulness of each harvest and determine any need for supplements to maintain the nutritional status.

6. Full-scale feeding trials will be necessary if the biomass

is accepted as a potential feed. It may also prove necessary to dry the harvested biomass to improve acceptability to animals or facilitate transport and storage.

A P P E N D I X

MEDIA

S. F. agar formulated by Hawkes (1965)

10 gm. peptone	(Oxoid Mycological)
10 gm. dextrose	(Oxoid L.71)
15 gm. agar	(Oxoid No. 3)
1 gm. yeast extract	(Oxoid L.21)
1 litre distilled water	

The ingredients were steamed for 30 minutes before adjusting the pH to 6.8 with sodium hydroxide solution. The medium was then sterilised by autoclaving for 15 minutes at 121°C.

Agar tables (for water agar) (Oxoid CM 49)

Corn meal agar (Oxoid CM 104)

Czapek-Dox agar (Oxoid CM 98)

Nutrient agar (Oxoid CM 4)

Potato dextrose agar (Oxoid CM 140)

Details of these media are given in the Oxoid manual (Oxoid Ltd., 1965)

TABLE 6.1

Geotrichum candidum :-

Effect of temperature variation on colony diameter ($5^{o}C$ - $35^{o}C$)

	TEMP. °C	COLONY DIAMETER (mm.)							
		DAYS FROM START OF EXPERIMENT							
		2	4	6	8	10	11	13	14
REFRIDGERATOR	5	7.0	7.0	7.0	7.0	7.0	7.5	8.5	8.5
		7.0	7.0	7.0	7.0	7.0	7.5	8.0	9.0
		7.0	7.0	7.0	8.0	9.5	10.0	10.5	11.5
	MEAN	7.0	7.0	7,0	7.3	7.8	8.3	9.0	9.7
INCUBATORS	10	7.0	9.0	13.0	19.5	22.5	26.5	28.5	29.0
		7.0	8.5	13.0	18.0	22.5	26.5	28.0	28.5
		7.0	8.0	13.0	16.5	21.0	23.5	26.0	26.5
	MEAN	7.0	8.5	13.0	18.0	22.0	25.3	27.5	28.0
	20	19.0	31.5	43.5	56.0	68.0	73.5	79.0	79.0
		15.0	27.0	39.5	51.5	63.5	67.0	77.5	79.0
		16.0	28.0	41.5	56.0	67.5	70.5	79.0	79.0
	MEAN	16.7	28.8	41.5	54.5	66.3	70.3	78.5	79.0
	25	20.0	34.0	50.0	65.0	76.5	77.0	77.5	77.5
		19.0	34.0	49.0	64.5	76.5	78.0	78.5	78.5
		20.0	34.5	47.5	62.0	76.0	78.5	78.5	78.5
	MEAN	19.7	34.2	48.8	63.8	76.3	77.8	78.2	78.2
	30	18.0	31.0	42.0	56.0	62.0	62.0	63.0	63.0
		18.5	31.0	42.0	53.0	61.0	64.5	66.5	67.0
		12.5	25.0	37.0	49.0	58.5	61.5	68.0	68.5
	MEAN	16.3	29.0	40.3	52.7	60.5	62.7	65.8	66.2
	35	11.0	12.0	12.5	12.5	13.5	16.5	20.0	23.0
		10.0	10.0	10.5	10.5	10.5	10.5	10.5	11.0
		9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
	MEAN	10.0	10.3	10.7	10.7	11.0	12.0	13.2	14.3
WATER - BATHS	25	18.0	33.0	48.5	63.5	78.0	80.0	81.0	82.0
		16.5	31.0	47.5	62.5	77.5	80.5	82.0	83.0
		29.0	44.0	59.0	69.0	83.0	83.5	84.0	84.0
	MEAN	21.2	36.0	51.7	65.0	79.5	81.3	82.3	83.0
	30	15.0	26.0	43.0	59.0	76.0	81.0	82.0	83.0
		14.0	25.0	42.0	57.5	74.5	80.0	81.0	82.0
		18.0	26.0	43.5	59.5	76.5	79.0	80.0	83.0
	MEAN	15.7	25.7	42.8	58.7	75.7	80.0	81.0	82.7
	35	11.0	16.0	22.5	27.5	32.5	34.0	34.5	34.5
		11.0	15.0	20.5	24.0	28.5	30.0	36.0	40.0
		10.0	12.0	16.0	18.5	21.5	23.0	29.5	31.5
	MEAN	10.7	14.3	19.7	23.3	27.5	29.0	33.3	35.3

TABLE 6.2

Geotrichum candidum :-

Comparison of the effects of normal (humid) and dry conditions, in water-baths, on colony diameter

	COLONY DIAMETER (mm.)							
	DAYS FROM START OF EXPERIMENT							
	2	4	6	7	10	11	13	14
25°C NORMAL	21.5 24.5 21.0	39.5 41.5 38.0	56.5 59.0 55.5	66.0 68.0 65.0	83.0 83.0 83.5	84.0 85.0 85.0	85.0 86.0 86.0	86.0 86.0 86.0
MEAN	22.3	39.7	57.0	66.3	83.2	84.7	85.7	86.0
25°C DRY	20.0 21.0 21.5	37.5 38.0 38.0	54.5 54.5 55.0	64.5 64.0 65.0	83.0 80.0 82.5	84.0 83.0 84.0	86.0 85.0 86.0	86.0 86.0 86.0
MEAN	20.8	37.8	54.7	64.5	81.8	83.7	85.7	86.0
30°C NORMAL	24.0 22.0 24.5	44.0 42.5 40.5	62.0 59.5 59.5	72.0 71.0 69.0	74.5 83.0 81.5	76.5 84.0 82.5	77.0 85.0 83.0	77.5 85.0 83.0
MEAN	23.5	42.3	60.3	70.7	79.7	81.0	81.7	81.8
30°C DRY	25.5 23.5 21.5	43.5 41.0 42.0	62.0 60.0 58.0	72.5 61.0 67.5	81.0 61.0 80.0	83.5 61.0 81.0	84.0 62.5 81.0	85.0 62.5 81.5
MEAN	23.5	42.2	60.0	67.0	74.0	75.2	75.8	76.3
35°C NORMAL	8.0 8.0 8.0	9.5 9.0 8.5	11.5 10.5 10.0	13.5 13.0 12.0	19.0 15.0 13.0	21.5 18.0 14.5	24.5 19.5 19.0	26.0 22.0 20.0
MEAN	8.0	9.0	10.7	12.8	15.7	18.0	21.0	22.7
35°C DRY	8.0 8.0 7.5	8.5 8.5 8.5	10.0 10.0 10.0	13.5 11.5 10.5	15.0 14.5 12.5	16.0 16.0 13.0	19.5 19.0 15.0	23.0 22.0 16.5
MEAN	7.8	8.5	10.0	11.8	14.0	15.0	17.8	20.5

TABLE 6.3

Geotrichum candidum :-

Effects of temperature variation on colony diameter.

(15°C - 27.5°C)

	COLONY DIAMETER (mm.)									
	DAYS FROM START OF EXPERIMENT									
	1	2	3	5	7	8	9	10	11	14
15°C	8.5	13.0	17.5	26.5	36.5	40.0	46.0	51.0	56.0	73.5
	8.5	12.5	17.0	25.5	36.0	39.0	45.0	50.0	55.0	72.0
	8.5	13.0	17.5	25.5	35.5	38.5	45.0	50.0	55.0	71.5
	8.5	12.5	17.5	26.5	35.5	39.0	45.0	50.0	54.0	70.0
MEAN	8.5	12.8	17.4	26.0	35.9	39.1	45.3	50.3	55.0	71.8
20°C	11.0	17.0	23.5	36.0	51.5	56.0	63.5	70.5	76.5	85.0
	11.0	18.5	25.0	38.0	52.5	57.0	65.0	70.5	76.0	85.0
	11.0	17.0	23.5	36.5	51.5	56.0	64.0	70.5	76.0	85.0
	10.5	16.5	23.0	35.5	50.5	55.0	63.0	69.0	74.5	85.0
MEAN	10.9	17.3	23.8	36.5	51.5	56.0	63.9	70.1	75.8	85.0
22.5°C	11.5	18.5	26.5	41.5	59.0	65.0	75.0	81.0	83.0	85.0
	11.0	18.0	26.0	41.0	59.0	64.0	75.0	80.0	82.0	85.0
	10.5	18.0	26.0	41.0	59.0	64.0	74.0	80.0	82.0	85.0
	11.0	18.5	26.0	41.0	59.0	64.0	74.0	81.5	83.5	85.0
MEAN	11.0	18.3	26.1	41.1	59.0	64.3	74.5	80,6	82.6	85.0
25°C	13.0	21.5	30.5	47.0	67.0	75.0	81.0	83.0	85.0	85.0
	13.0	23.0	32.0	49.0	68.5	75.0	82.5	83.0	85.0	85.0
	13.0	22.0	31.5	48.0	68.0	75.0	81.5	83.0	85.0	85.0
	12.5	21.5	30.0	47.5	66.0	73.5	82.0	82.5	85.0	85.0
MEAN	12.9	22.0	31.0	47.9	67.4	74.6	81.8	82.9	85.0	85.0
27.5°C	14.0	23.5	34.0	53.0	75.0	81.5	84.0	85.0	85.0	85.0
	14.0	23.5	33.5	53.0	76.0	81.5	84.0	85.0	85.0	85.0
	13.5	22.5	32.0	51.0	73.0	80.0	84.0	85.0	85.0	85.0
	13.5	22.0	32.5	51.0	73.0	80.0	84.0	85.0	85.0	85.0
MEAN	13.8	22.9	33.0	52.0	74.3	80.8	84.0	85.0	85.0	85.0

TABLE 6.4

Geotrichum candidum :-

Effect of temperature variation on colony diameter.
(25°C - 36°C)

	COLONY DIAMETER (mm.)									
	DAYS FROM START OF EXPERIMENT									
	1	2	3	4	7	8	9	10	12	14
25°C	11.0	21.5	30.5	40.0	66.0	77.0	82.5	83.0	84.0	85.0
	9.5	26.5	41.0	51.5	72.0	81.5	83.0	84.0	84.5	85.0
	11.0	22.5	31.0	41.0	67.0	78.0	83.0	84.0	84.5	85.0
	11.0	26.0	35.0	44.5	69.0	80.0	83.0	84.0	84.5	85.0
MEAN	10.6	24.1	34.4	44.3	68.5	79.1	82.9	83.8	84.4	85.0
28°C	12.5	24.5	35.0	46.0	74.5	82.5	85.0	85.0	85,0	85.0
	13.0	24.5	35.5	45.0	73.0	82.0	85.0	85.0	85.0	85.0
	11.0	24.0	33.5	44.5	73.5	82.0	85.0	85.0	85.0	85.0
	11.0	24.0	33.5	44.0	73.5	83.0	85.0	85.0	85.0	85.0
MEAN	11.9	24.3	34.4	44.9	73.6	82.4	85.0	85.0	85.0	85.0
30°C	12.0	25.0	36.0	41.5	74.5	81.5	82.5	83.5	84.0	85.0
	11.5	23.0	34.0	44.5	72.0	80.5	81.5	82.5	83.0	85.0
	11.5	23.5	34.0	44.5	72.0	80.0	82.0	82.5	83.0	85,0
	12.5	24.5	35.0	46.0	73.5	80.5	81.5	83.0	84.0	85.0
MEAN	11.9	24.0	34.8	44.1	73.0	80.6	81.9	82.9	83.5	85.0
36°C	10.5	17.0	19.0	20.0	20.0	20.0	20.0	20.0	21.0	22.0
	10.5	10.5	11.5	12.0	12.0	12.0	12.0	12.0	12.5	13.0
	8.0	8.0	9.5	9.5	9.5	9.5	9.5	9.5	11.5	12.0
	8.0	8.0	9.0	9.0	9.0	9.0	9.0	9.0	9.5	10.5
MEAN	9,3	10.9	12.3	12.6	12.6	12.6	12,6	12.6	13.6	14.4

TABLE 6.5

Geotrichum candidum ;-

Effect of temperature variation on colony diameter.
(29°C - 33°C).

	COLONY DIAMETER (mm.)						
	DAYS FROM START OF EXPERIMENT						
	2	3	6	7	8	9	10
29°C	27.0 26.5 27.0 25.5	37.5 37.0 37.0 35.5	70.0 69.5 69.5 68.0	79.0 79.0 78.5 76.5	82.0 83.0 83.0 82.0	83.0 84.0 84.0 83.0	85.0 85.0 85.0 85.0
MEAN	26.5	36.8	69.3	78.3	82.5	83.5	85.0
31°C	28.0 26.5 26.5 25.0	37.5 36.0 36.5 34.5	68.5 66.5 67.0 64.5	76.0 76.0 75.0 73.5	81.0 81.0 81.0 80.5	82.0 82.0 82.0 81.5	83.0 83.0 84.0 82.5
MEAN	26.5	36.1	66.6	75.1	80.9	81.9	83.1
32°C	26.5 24.5 23.5 22.5	34.0 32.5 31.5 29.5	55.5 53.0 51.0 48.0	63.0 60.0 57.5 55.0	69.5 67.5 65.0 61.5	75.0 73.5 71.5 66.5	77.0 76.5 74.0 68.0
MEAN	24.3	31.9	51.9	58.9	65.9	71.6	73.9
33°C	22.0 20.0 20.0 19.0	27.5 25.0 25.5 25.0	46.0 43.0 42.5 44.0	51.0 49.0 49.0 49.0	56.0 53.5 53.5 55.0	60.5 60.0 59.0 59.5	64.0 62.0 61.5 63.0
MEAN	20.3	25.8	43.9	49.5	54.4	59.8	62.6

TABLE 6.6.

Fusarium aquaeductuum :-

Effect of temperature variation on
Colony diameter.
(5^oC - 35^oC)

	COLONY DIAMETER (mm.)									
	DAYS FROM START OF EXPERIMENT									
	1	2	3	4	6	8	9	10	11	14
5^oC	7.0	7.0	7.0	7.0	7.5	8.0	8.0	9.0	9.0	10.5
	7.0	7.0	7.0	7.0	7.5	8.0	8.0	9.0	9.0	11.0
	7.0	7.0	7.0	7.0	7.5	7.0	7.0	8.5	8.5	10.5
MEAN	7.0	7.0	7.0	7.0	7.5	7.7	7.7	8.8	8.8	10.7
10^oC	7.0	7.0	8.5	10.5	13.0	16.0	17.0	21.0	21.0	26.5
	7.0	7.0	8.0	8.5	11.5	14.5	16.0	18.5	20.0	24.0
	7.0	7.0	8.0	8.5	11.5	14.0	16.0	19.0	20.0	23.5
MEAN	7.0	7.0	8.2	9.2	12.0	14.8	16.3	19.5	20.3	24.7
20^oC	7.0	11.0	15.0	18.0	23.5	31.0	34.0	38.5	41.5	52.5
	7.0	12.0	15.0	18.5	25.0	31.0	34.0	38.5	42.0	52.5
	7.0	12.5	15.5	19.0	26.5	33.0	36.0	40.0	43.0	53.5
MEAN	7.0	11.8	15.2	18.5	25.0	31.7	34.7	39.0	42.2	52.8
25^oC	7.0	9.0	9.5	10.5	12.5	15.0	15.0	17.0	17.5	18.0
	7.0	9.5	11.0	12.0	13.5	15.0	16.0	16.5	17.0	17.5
	7.0	9.5	10.0	10.5	11.5	12.0	12.0	12.5	13.0	13.5
MEAN	7.0	9.3	10.2	11.0	12.5	14.0	14.3	15.3	15.8	16.3
30^oC	7.0	9.0	9.0	9.0	9.5	10.0	11.0	12.0	14.0	14.5
	7.0	9.0	9.0	9.0	10.0	11.0	13.0	14.0	15.5	16.5
	7.0	9.0	9.0	9.0	10.0	10.5	12.0	13.5	14.5	16.0
MEAN	7.0	9.0	9.0	9.0	9.8	10.5	12.0	13.2	14.7	15.0
35^oC	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
MEAN	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0

TABLE 6.7

Fusarium aquaeductuum :-

Effect of temperature variation on colony diameter.
(15°C - 27.5°C)

	COLONY DIAMETER (mm.)									
	DAYS FROM START OF EXPERIMENT									
	1	2	3	5	7	8	9	10	11	14
15°C	8.5	10.5	13.0	18.0	23.5	25.5	28.5	31.0	33.0	43.0
	8.0	10.0	13.0	17.5	23.0	25.0	28.5	31.0	33.5	43.0
	8.0	10.5	13.0	18.0	23.0	25.0	28.5	31.0	33.5	42.5
	8.0	10.5	13.0	18.0	23.5	25.5	29.0	31.0	34.0	43.0
MEAN	8.1	10.4	13.0	17.9	23.3	25.3	28.6	31.0	33.5	42.9
20°C	9.5	12.5	16.0	23.5	31.0	34.0	38.0	42.0	46.0	59.0
	8.5	12.0	16.0	23.0	31.0	34.0	38.5	42.0	45.5	59.5
	9.0	12.0	15.5	22.5	30.5	33.0	38.0	41.0	45.0	59.0
	8.5	11.5	15.0	21.5	29.5	31.5	36.0	39.5	43.0	55.5
MEAN	8.9	12.0	15.6	22.6	30.5	33.1	37.6	41.1	44.9	58.3
22.5°C	9.0	12.0	16.5	24.5	33.5	37.0	41.0	45.0	49.0	65,0
	8.5	12.0	16.5	24.0	34.0	37.0	41.5	46.0	49.0	65.0
	8.5	12.0	16.5	24.0	33.5	36.5	41.5	45.5	50.0	64.5
	9.0	12.5	16.5	24.5	34.0	37.0	42.0	46.5	50.5	65.0
MEAN	8.8	12.1	16.5	24.3	33.8	36.9	41.5	45.8	49.6	64.9
25°C	8.5	11.0	15.0	22.5	31.5	35.0	39.0	43.5	47.0	60.5
	8.5	11.5	15.5	23.5	31.0	34.5	39.5	43.5	47.5	60.5
	8.5	11.5	15.5	22.5	32.0	35.0	39.5	43.5	47.0	60.0
	8.5	12.0	15.5	23.0	32.0	34.5	39.5	42.0	47.0	57.5
MEAN	8.5	11.5	15.4	22.9	31.6	34.8	39.4	43.1	47.1	59.6
27.5°C	8.5	11.5	15.0	21.5	28.0	31.0	35.0	38.0	41.0	52.5
	9.0	11.5	14.5	21.0	27.5	30.5	34.0	36.5	39.5	49.5
	8.0	11.0	14.5	20.5	27.5	29.5	33.5	36.5	39.5	50.0
	8.5	11.5	14.5	21.0	27.5	30.0	33.5	36.5	39.5	49.5
MEAN	8.5	11.4	14.6	21.0	27.6	30.25	34.0	36.4	39.4	50.4

TABLE 6.8

Fusarium tabacinum :-

Effect of temperature variation on colony diameter.
(5°C - 35°C)

	COLONY DIAMETER (mm.)									
	DAYS FROM START OF EXPERIMENT									
	1	2	3	4	6	8	9	10	11	14
5°C	7.0 7.0 7.0	7.0 7.0 7.0	7.0 7.0 7.0	7.0 7.0 7.0	7.0 7.0 7.0	7.0 7.0 7.0	7.0 7.0 7.0	7.0 7.0 7.0	7.0 7.0 7.0	7.0 7.0 7.0
MEAN	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
10°C	7.0 7.0 7.0	7.0 7.0 7.0	7.0 7.0 7.0	7.0 7.0 7.0	8.5 7.5 7.5	10.0 9.0 8.0	11.0 10.0 9.0	11.5 11.0 10.5	12.5 13.5 12.0	15.5 16.5 17.0
MEAN	7.0	7.0	7.0	7.0	7.8	9.0	10.0	11.0	12.7	16.3
20°C	7.0 7.0 7.0	13.0 12.5 13.0	17.5 17.0 17.0	22.0 22.5 22.0	34.5 34.5 31.5	45.0 45.0 45.0	52,0 51.0 51.0	59.0 59.5 58.5	65.0 65.0 65.0	81.0 82.0 81.0
Mean	7.0	12.8	17.2	22.2	33.5	45.0	51.3	59.0	65.0	81.3
25°C	10.0 10.0 11.0	16.5 16.0 16.0	23.5 23.0 23.5	31.5 30.0 31.5	46.5 45.0 46.5	62.0 59.0 63.0	65.0 62.0 67.0	68.0 65.0 68.0	75.0 72.0 74.0	85.0 85.0 85.0
MEAN	10.3	16.2	23.3	31.0	46.0	61.3	64.7	67.0	73.7	85.0
30°C	10.6 10.5 10.5	17.5 17.5 17.0	25.0 24.5 24.0	34.0 33.5 32.5	50.0 47.5 46.5	67.0 64.0 60.0	71.0 68.0 65.0	83.0 79.0 74.0	84.0 84.0 84.0	85.0 85.0 85.0
MEAN	10.5	17.3	24.5	33.3	48.0	63.7	68.0	78.7	84.0	85.0
35°C	8.0 8.5 8.5	13.0 10.5 11.0	15.5 12.0 12.5	20.0 14.5 14.5	26.5 19.0 19.0	31.0 24.0 24.0	34.0 27.0 26.0	39.0 31.0 29.5	42.0 34.0 42.0	53.5 45.0 39.0
MEAN	8.3	11.5	13.3	16.3	21.5	26.3	29.0	33.2	36.0	45.8

TABLE 6.9

Fusarium tabacinum : -

Effect of temperature variation on colony diameter.

(15°C - 27.5°C)

	COLONY DIAMETER (mm.)									
	DAYS FROM START OF EXPERIMENT									
	1	2	3	5	7	8	9	10	11	14
15°C	7.0	9.0	11.0	17.0	25.5	27.5	32.5	36.5	40.0	56.0
	7.0	8.0	12.0	17.0	23.5	26.5	31.0	33.5	37.0	48.0
	7.0	9.0	11.0	17.0	23.5	27.0	31.0	35.0	38.0	51.0
	7.0	9.0	11.0	17.0	24.0	27.0	31.0	35.0	38.0	51.0
MEAN	7.0	8.8	11.3	17.0	24.1	27.0	31.4	35.0	38.3	51.5
20°C	8.0	13.0	19.0	32.0	47.0	52.0	60.0	68.0	74.0	85.0
	9.0	13.0	19.5	32.5	46.0	52.0	60.0	68.0	73.0	85.0
	9.5	12.5	18.0	32.0	46.0	51.0	59.0	67.0	74.0	85.0
	8.5	13.0	19.0	32.5	47.0	52.0	60.0	68.0	75.0	85.0
MEAN	8.8	12.9	18.9	32.3	46.5	51.8	59.8	67.8	74.0	85.0
22.5°C	9.0	14.0	21.0	34.0	50.0	56.0	65.0	73.5	80.0	85.0
	9.0	14.5	23.5	39.0	58.0	65.0	76.0	83.0	85.0	85.0
	8.5	15.0	23.5	38.5	57.0	63.5	75.0	83.0	85.0	85.0
	9.0	14.5	23.0	38.0	57.0	63.0	74.5	82.0	85.0	85.0
MEAN	8.9	14.5	22.8	37.4	55.5	61.9	72.6	80.4	83.8	85.0
25°C	11.0	18.5	28.0	45.0	65.0	71.0	82.0	84.0	85.0	85.0
	11.0	18.0	27.0	44.5	63.5	70.0	80.0	84.0	85.0	85.0
	11.5	19.0	27.5	44.0	63.0	70.0	80.0	84.0	85.0	85.0
	11.5	19.0	28.0	45.0	64.5	71.0	82.0	84.0	85.0	85.0
MEAN	11.3	18.6	27.6	44.6	64.0	70.5	81.0	84.0	85.0	85.0
27.5°C	11.5	19.0	27.0	42.5	61.5	68.0	78.0	82.0	85.0	85.0
	11.0	18.0	25.5	40.5	56.5	62.5	72.5	78.0	82.0	85.0
	11.0	16.5	24.0	38.0	54.0	60.0	70.0	77.0	84.0	85.0
	12.0	19.0	28.0	44.0	63.5	69.0	77.5	82.0	85.0	85.0
MEAN	11.4	18.1	26.1	41.3	58.9	64.9	74.5	79.8	84.0	85.0

TABLE 6.10

Fusarium tabacinum :-

Effects of temperature variation on colony diameter.
($25^{o}C$ - $36^{o}C$)

	COLONY DIAMETER (mm.)									
	DAYS FROM START OF EXPERIMENT									
	1	2	3	4	7	8	9	10	12	14
$25^{o}C$	10.0	16.5	22.5	30.0	48.5	56.5	63.5	70.0	76.0	82.0
	11.0	18.0	26.0	34.0	54.5	63.0	71.0	77.0	80.0	83.0
	9.5	15.5	22.5	30.0	48.5	55.5	61.5	68.0	73.0	78.0
	10.0	17.0	23.5	31.5	50.0	58.0	64.5	71.0	75.0	79.0
MEAN	10.1	16.8	23.6	31.4	50.4	58.3	65.1	71.5	76.0	80.5
$28^{o}C$	11.0	18.5	26.5	34.5	57.0	68.5	77.0	83.0	83.5	85.0
	11.0	18.5	27.0	34.5	57.0	68.5	76.5	83.0	83.5	85.0
	10.5	19.0	27.5	37.0	61.5	72.0	81.0	83.0	84.5	85.0
	11.0	20.0	29.5	39.5	65.0	77.5	83.0	84.0	84.5	85.0
MEAN	10.9	19.0	27.6	36.4	60.1	71.6	79.4	83.3	84.0	85.0
$30^{o}C$	11.5	19.0	28.0	36.5	61.0	72.0	80.0	84.0	84.5	85.0
	12.5	19.0	28.0	36.0	60.0	72.0	80.0	84.5	85.0	85.0
	12.0	21.0	30.5	40.5	69.5	79.0	82.5	84.5	85.0	85.0
	10.5	19.0	28.0	36.5	61.5	73.5	81.0	84.0	84.5	85.0
MEAN	11.6	19.5	28.5	37.4	63.0	74.1	80.8	84.3	84.8	85.0
$36^{o}C$	10.0	14.5	18.0	23.0	32.5	35.5	38.0	42.0	46.5	51.0
	10.0	15.0	18.0	23.0	32.0	34.0	36.0	40.0	43.5	47.0
	8.5	12.0	14.5	17.5	24.5	25.5	27.5	30.0	33.0	36.0
	9.0	11.5	14.5	17.0	21.0	22.0	23.5	25.5	27.0	29.5
MEAN	9.4	13.3	16.3	20.1	27.5	29.3	31.3	34.4	37.5	40.9

TABLE 6.11

Fusarium tabacinum :-

Effect of temperature variation on colony diameter.
(29°C - 33°C)

	COLONY DIAMETER (mm.)						
	DAYS FROM START OF EXPERIMENT						
	2	3	6	7	8	9	10
29°C	21.5	31.0	62.0	71.0	80.0	83.0	85.0
	19.5	28.0	57.0	65.5	75.0	82.5	85.0
	22.0	31.0	62.0	71.5	80.5	83.5	85.0
	21.0	30.0	61.0	70.5	79.5	83.0	85.0
MEAN	21.0	30.0	60.5	69.6	78.8	83.0	85.0
31°C	21.0	30.5	61.5	70.0	75.0	78.0	85.0
	21.0	30.0	60.0	68.0	77.0	83.0	85.0
	18.5	26.0	53.0	61.0	70.0	78.0	82.0
	20.0	28.5	57.5	66.5	75.0	82.0	85.0
MEAN	20.1	28.8	58.0	66.4	74.3	80.3	84.3
32°C	21.0	28.5	52.0	59.5	67.0	76.0	79.0
	21.0	28.5	53.0	60.5	68.5	77.0	81.0
	19.5	27.0	51.0	58.0	66.0	75.0	79.0
	20.0	28.0	52.0	60.0	68.0	76.0	82.0
MEAN	20.4	28.0	52.0	59.5	67.4	76.0	80.3
33°C	19.0	26.5	50.0	57.0	64.0	72.0	78.0
	19.0	27.0	50.0	57.5	64.5	72.5	78.5
	17.0	23.0	43.0	50.0	58.0	65.0	74.0
	19.0	26.0	50.0	58.0	65.5	74.0	79.0
MEAN	18.5	25.6	48.3	55.6	63.0	70.9	77.4

TABLE 6.12

Geotrichum candidum :-

Effect of initial pH of medium on growth.

pH	COLONY DIAMETER (mm.)									
	DAYS FROM START OF EXPERIMENT									
	1	2	4	5	6	7	8	10	11	12
2	8.5	9.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
	9.0	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5
	9.0	9.0	9.0	9.0	9.5	9.5	9.5	9.5	9.5	9.5
MEAN	8.8	9.2	9.5	9.5	9.7	9.7	9.7	9.7	9.7	9.7
3	11.0	20.0	32.0	40.0	46.5	54.5	61.0	73.5	77.0	78.0
	13.0	19.5	31.5	39.0	46.5	53.0	60.5	73.5	78.0	78.5
	11.0	18.5	31.0	38.0	45.0	53.0	60.0	74.0	77.5	78.5
MEAN	11.7	19.3	31.5	39.0	46.0	53.5	60.5	73.7	77.5	78.2
4	13.5	22.5	34.0	42.0	49.5	56.5	63.5	77.0	78.0	79.0
	13.5	21.0	33.5	41.0	48.5	55.5	62.5	77.0	78.0	79.5
	15.0	22.0	35.0	43.0	50.0	56.0	64.5	76.5	78.0	79.0
MEAN	14.0	21.8	34.2	42.0	49.3	56.0	63.8	76.8	78.0	79.2
5	13.0	20.0	30.5	37.0	44.0	51.0	58.0	71.0	74.5	75.0
	13.0	20.5	31.5	37.5	44.0	51.0	57.5	72.0	76.0	77.0
	12.5	19.5	30.5	37.0	43.5	50.5	57.0	71.5	75.5	76.5
MEAN	12.8	20.0	30.8	37.2	43.8	50.8	57.5	71.5	75.3	76.2
6	13.0	21.0	31.5	38.0	44.5	50.5	57.5	71.5	74.0	74.0
	14.5	21.5	32.0	38.0	45.5	52.0	59.0	72.5	76.0	76.0
	13.0	20.0	30.0	36.5	43.5	49.5	56.5	70.0	75.0	75.0
MEAN	13.5	20.8	31.2	37.5	44.5	50.7	57.7	71.3	75.0	75.0
7	12.5	19.5	30.5	38.0	44.0	51.0	58.0	70.5	75.0	76.0
	11.5	18.0	29.0	37.0	43.0	49.0	56.5	70.0	74.0	76.0
	12.5	19.5	30.5	37.5	43.5	51.0	57.5	70.5	75.5	76.5
MEAN	12.2	19.0	30.0	37.5	43.5	50.3	57.3	70.3	74.8	76.2
8	12.5	20.0	30.0	37.5	43.0	49.5	56.0	68.5	74.0	76.5
	15.0	21.5	32.0	38.5	44.5	51.0	57.0	69.5	75.0	75.5
	13.0	20.0	30.5	36.5	42.5	51.0	56.0	68.5	73.5	75.0
MEAN	13.5	20.5	30.8	37.5	43.3	50.5	56.3	68.8	74.2	75.7
9	13.0	20.0	31.0	37.5	44.5	50.0	56.5	67.5	71.0	73.5
	14.0	21.0	32.0	38.0	44.5	51.0	56.0	68.0	72.5	73.5
	12.5	19.5	30.5	37.0	43.5	50.0	55.5	67.5	71.5	74.0
MEAN	13.2	20.2	31.2	37.5	44.2	50.3	56.0	67.7	71.7	73.7
10	14.5	21.0	32.0	38.0	43.0	49.0	55.0	67.0	71.0	72.0
	12.0	18.5	29.0	36.0	42.5	48.5	54.5	65.5	70.0	73.5
	12.5	19.5	30.0	36.5	42.5	48.5	54.5	65.5	71.0	74.5
MEAN	13.0	19.7	30.3	36.8	42.7	48.7	54.7	66.0	70.7	73.3

TABLE 6.13

Geotrichum candidum :-

Relationship between pH value of medium and dry weight of fungus after 11 days in shake flasks at 20°C.

	pH AT START OF EXPERIMENT							
	2	3	4	5	6	7	8	9
DRY	276	356	403	414	409	412	399	350
WT.	276	358	363	404	392	390	393	350
(mg)	277	360	391	383	409	386	392	347
MEAN	276.3	358.0	385.7	400.3	403.0	396.0	394.7	349.0
	pH AT TIME OF HARVESTING							
	1.85	2.80	6.20	7.11	7.40	7.80	7.96	8.10
	1.87	2.65	6.48	7.18	7.49	7.72	7.93	8.18
	1.89	2.52	6.65	7.05	7.50	7.63	7.95	8.13
MEAN	1.87	2.66	6.44	7.11	7.46	7.72	7.95	8.14

TABLE 6.14

Fusarium tabacinum :-

Relationship bewteen pH value of medium and dry weight of fungus after 11 days in shake flasks at 20°C.

	pH AT START OF EXPERIMENT							
	2	3	4	5	6	7	8	9
DRY	6	32	294	312	304	304	310	269
WT.	6	58	281	316	299	330	298	266
(mg)	5	62	272	318	317	300	295	274
MEAN	5.7	50.7	282.3	315.3	306.7	311.3	301.0	269.7
	pH AT TIME OF HARVESTING							
	2.05	2.78	7.80	8.00	8.08	8.18	8.33	8.30
	2.04	2.70	7.86	8.00	8.10	8.21	8.28	8.39
	2.05	2.70	7.82	8.00	8.00	8.20	8.30	8.30
MEAN	2.05	2.73	7.83	8.00	8.06	8.20	8.30	8.33

TABLE 6.15

Fusarium aquaeductuum :-

Relationship between pH value of medium and dry weight of fungus after 11 days in shake flasks at 20°C.

	pH AT START OF EXPERIMENT							
	2	3	4	5	6	7	8	9
DRY	6	7	297	269	278	291	272	262
WT.	5	5	310	245	272	286	276	261
(mg)	6	6	277	246	283	282	274	258
MEAN	5.7	5.7	294.7	253.3	277.7	286.3	274.0	260.3
	pH AT TIME OF HARVESTING							
	1.92	2.83	7.00	7.84	7.91	7.98	8.03	8.10
	1.92	2.83	7.40	7.95	7.91	8.00	8.02	8.09
	1.92	2.83	5.73	7.90	7.91	8.00	8.02	8.11
MEAN	1.92	2.83	6.71	7.90	7.91	7.99	8.02	8.10

TABLE 6.16

Comparison of the relationship between initial pH and dry weight of the three fungi after 11 days in shake flasks at 20°C.

pH AT START OF EXPERIMENT	MEAN DRY WEIGHT (mg)		
	Geotrichum candidum	Fusarium tabacinum	Fusarium aquaeductuum
2	276.3	5.7	5.7
3	358.0	50.7	5.7
4	385.7	282.3	294.7
5	400.3	315.3	253.3
6	403.0	306.7	277.7
7	396.0	311.3	286.3
8	394.7	301.0	274.0
9	349.0	296.7	260.3

TABLE 6.17

Geotrichum candidum :-

Daily changes in dry weight and pH (initial pH 4) in shake flasks at 20°C.

	pH AT TIME OF HARVESTING	DRY WEIGHT (mg.)
DAY 1	3.78 3.68 3.73	19.0 29.0 22.0
MEAN	3.73	23.3
DAY 2	3.04 3.05 3.08	270.0 251.0 238.0
MEAN	3.06	253.0
DAY 3	3.00 3.01 2.99	273.0 264.0 284.0
MEAN	3.00	273.7
DAY 4	2.85 3.04 2.91	316.0 242.0 304.0
MEAN	2.93	287.3
DAY 5	3.00 2.90 2.91	316.0 326.0 345.0
MEAN	2.94	329.0
DAY 6	3.01 3.12 3.01	327.0 335.0 337.0
MEAN	3.05	333.0
DAY 7	3.38 3.43 3.45	350.0 339.0 346.0
MEAN	3.42	345.0
DAY 8	3.90 3.72 3.71	344.0 328.0 342.0
MEAN	3.78	338.0
DAY 9	4.53 3.92 3.89	353.0 334.0 333.0
MEAN	4.11	340.0

TABLE 6.18

Geotrichum candidum :-

Daily changes in dry weight and pH (initial pH 5) in shake flasks at 20°C.

	pH AT TIME OF HARVESTING	DRY WEIGHT (mg.)
DAY 1	4.70 4.52 4.71	12.0 23.0 12.0
MEAN	4.64	15.7
DAY 2	4.05 3.50 3.62	267.0 320.0 283.0
MEAN	3.72	290.0
DAY 3	3.45 3.52 3.44	320.0 292.0 343.0
MEAN	3.47	318.3
DAY 4	3.50 3.35 3.48	303.0 332.0 287.0
MEAN	3.44	307.3
DAY 5	3.55 3.55 3.58	321.0 288.0 284.0
MEAN	3.56	297.7
DAY 6	3.40 3.63 3.35	325.0 304.0 323.0
MEAN	3.46	317.3
DAY 7	4.10 3.31 3.72	349.0 313.0 345.0
MEAN	3.71	335.7
DAY 8	4.42 5.99 5.80	344.0 375.0 358.0
MEAN	5.40	359.0
DAY 9	6.70 5.71 6.45	353.0 347.0 342.0
MEAN	6.29	347.3

TABLE 6.19

Geotrichum candidum :-

Daily changes in dry weight and pH (initial pH 6) in shake flasks at 20°C.

	pH AT TIME OF HARVESTING	DRY WEIGHT (mg.)
DAY 1	5.67 5.70 5.75	18.0 15.0 14.0
MEAN	5.71	15.7
DAY 2	4.04 3.93 4.03	275.0 321.0 294.0
MEAN	4.00	296.7
DAY 3	4.03 3.92 4.01	244.0 305.0 290.0
MEAN	3.99	279.7
DAY 4	4.11 3.81 3.80	254.0 295.0 325.0
MEAN	3.91	291.3
DAY 5	3.92 3.82 3.92	304.0 322.0 308.0
MEAN	3.89	311.3
DAY 6	3.79 3.89 3.89	345.0 319.0 316.0
MEAN	3.86	325.7
DAY 7	4.21 4.99 4.18	342.0 342.0 346.0
MEAN	4.46	343.3
DAY 8	6.42 6.32 6.11	357.0 363.0 354.0
MEAN	6.28	358.0
DAY 9	6.04 7.00 7.34	333.0 366.0 361.0
MEAN	6.79	353.3

TABLE 6.20

Geotrichum candidum :-

Daily changes in dry weight and pH (initial pH 7) in shake flasks at 20°C.

	pH AT TIME OF HARVESTING	DRY WEIGHT (mg.)
DAY 1	6.79 6.81 6.82	14.0 11.0 11.0
MEAN	6,81	12.0
DAY 2	6.21 6.71 5.59	141.0 101.0 208.0
MEAN	6.17	150.0
DAY 3	5.70 5.80 5.89	240.0 184.0 205.0
MEAN	5.80	209.7
DAY 4	5.52 5.51 5.36	283.0 292.0 304.0
MEAN	5.46	293.0
DAY 5	5.82 5.62 5.48	255.0 296.0 304.0
MEAN	5.64	285.0
DAY 6	5.70 5.60 5.34	344.0 303.0 330.0
MEAN	5.55	325.7
DAY 7	7.34 7.10 7.38	379.0 370.0 375.0
MEAN	7.27	374.7
DAY 8	7.50 7.40 7.39	377.0 378.0 373.0
MEAN	7.43	376.0
DAY 9	7.65 7.64 7.65	369.0 374.0 361.0
MEAN	7.65	368.0

TABLE 6.21

Fusarium tabacinum :-

Daily changes in dry weight and pH (initial pH 4) in shake flasks at 20°C.

	pH AT TIME OF HARVESTING	DRY WEIGHT (mg.)
DAY 1	3.82	6.0
	3.81	6.0
	3.82	5.0
MEAN	3.82	5.7
DAY 2	3.30	206.0
	3.52	117.0
	3.28	205.0
MEAN	3.37	176.0
DAY 3	3.82	317.0
	3.95	329.0
	3.82	311.0
MEAN	3.86	319.0
DAY 4	6.19	344.0
	6.13	331.0
	4.80	300.0
MEAN	5.71	325.0
DAY 5	6.25	288.0
	5.62	277.0
	6.90	323.0
MEAN	6.26	296.0
DAY 6	7.20	307.0
	7.40	293.0
	7.25	312.0
MEAN	7.28	304.0
DAY 7	6.93	299.0
	7.22	296.0
	7.15	296.0
MEAN	7.10	297.0
DAY 8	7.68	280.0
	7.52	296.0
	7.70	294.0
MEAN	7.63	290.0
DAY 9	7.65	285.0
	7.62	281.0
	7.60	274.0
MEAN	7.62	280.0

TABLE 6.22

Fusarium tabacinum :-

Daily changes in dry weight and pH (initial pH 5) in shake flasks at 20°C.

	pH AT TIME OF HARVESTING	DRY WEIGHT (mg.)
DAY 1	4.80 4.80 4.84	8.0 7.0 4.0
MEAN	4.81	6.3
DAY 2	4.05 4.00 4.04	166.0 167.0 184.0
MEAN	4.03	172.3
DAY 3	4.80 5.45 5.03	333.0 339.0 334.0
MEAN	5.09	335.3
DAY 4	6.70 6.82 6.65	350.3 351.0 352.0
MEAN	6.72	351.0
DAY 5	7.28 7.20 7.32	329.0 322.0 335.0
MEAN	7.27	328.7
DAY 6	7.30 7.48 7.45	341.0 314.0 330.0
MEAN	7.41	328.3
DAY 7	6.90 7.08 6.91	289.0 292.0 297.0
MEAN	6.96	292.7
DAY 8	7.48 6.73 7.56	284.0 219.0 236.0
MEAN	7.26	246.3
DAY 9	7.38 7.38 7.32	269.0 280.0 273.0
MEAN	7.36	274.0

TABLE 6.23

Fusarium tabacinum :-

Daily changes in dry weight and pH (initial pH6) in shake flasks at 20°C.

	pH AT TIME OF HARVESTING	DRY WEIGHT (mg.)
DAY 1	5.85 5.85 5.83	3.0 3.0 5.0
MEAN	5.84	3.7
DAY 2	5.33 5.19 5.18	94.0 153.0 140.0
MEAN	5.23	129.0
DAY 3	5.01 5.13 4.81	325.0 327.0 312.0
MEAN	4.98	321.3
DAY 4	6.88 6.92 6.92	308.0 329.0 324.0
MEAN	6.91	320.3
DAY 5	7.35 6.80 7.29	330.0 315.0 330.0
MEAN	7.15	325.0
DAY 6	7.10 7.50 7.18	269.0 312.0 278.0
MEAN	7.26	286.3
DAY 7	7.01 7.08 7.11	265.0 272.0 273.0
MEAN	7.01	270.0
DAY 8	7.75 7.75 7.70	269.0 262.0 263.0
MEAN	7.73	264.7
DAY 9	7.55 7.38 7.36	266.0 233.0 230.0
MEAN	7.43	243.0

TABLE 6.24

Fusarium tabacinum :-
Daily changes in dry weight and pH (initial pH7) in shake flasks at 20°C.

	pH AT TIME OF HARVESTING	DRY WEIGHT (mg)
DAY 1	6.80 6.80 6.80	3.0 3.0 2.0
MEAN	6.80	2.7
DAY 2	6.05 6.08 6.08	132.0 112.0 128.0
MEAN	6.07	124.0
DAY3	5.97 5.86 5.86	279.0 261.0 282.0
MEAN	5.90	274.0
DAY 4	7.15 7.15 7.00	307.0 272.0 242.0
MEAN	7.10	273.7
Day 5	7.33 7.48 7.50	283.0 270.0 240.0
MEAN	7.44	264.3
DAY 6	7.55 7.65 7.65	283.0 296.0 255.0
MEAN	7.62	278.0
DAY 7	7.40 7.40 7.39	224.0 221.0 217.0
MEAN	7.40	220.7
DAY 8	7.70 7.68 7.65	218.0 207.0 214.0
MEAN	7.68	213.0
DAY 9	7.80 7.59 7.80	226.0 226.0 224.0
MEAN	7.73	225.3

TABLE 6.25

Geotrichum candidum :-

Effect of initial pH on optical density readings ($x10^{-3}$) (absorbance at 580 nm.) taken over 9 days (FIRST TRIAL)

	DAYS FROM START OF EXPERIMENT							
pH	1	2	3	4	6	7	8	9
2	4.0	5.0	5.0	3.0	3.0	3.0	0.0	0.0
	0.0	1.0	0.0	3.0	3.0	3.0	0.0	0.0
	2.0	4.0	5.0	1.0	0.0	0.0	0.0	0.0
MEAN	2.0	3.0	3.0	2.0	2.0	2.0	0.0	0.0
3	1.0	35.0	51.0	400.0	580.0	740.0	740.0	688.0
	0.0	34.0	54.0	210.0	590.0	780.0	780.0	690.0
	4.0	39.0	45.0	75.0	550.0	730.0	730.0	650.0
MEAN	2.0	36.0	50.0	228.0	573.0	750.0	750.0	676.0
4	1.0	98.0	241.0	615.0	780.0	800.0	800.0	800.0
	4.0	96.0	182.0	479.0	700.0	660.0	739.0	785.0
	0.0	83.0	138.0	190.0	520.0	580.0	600.0	660.0
MEAN	2.0	92.0	187.0	428.0	666.0	680.0	713.0	748.0
5	3.0	118.0	212.0	340.0	500.0	530.0	480.0	524.0
	1.0	115.0	190.0	337.0	560.0	520.0	450.0	559.0
	1.0	85.0	213.0	308.0	530.0	525.0	465.0	540.0
MEAN	2.0	106.0	205.0	328.0	530.0	525.0	465.0	541.0
6	0.0	90.0	130.0	150.0	600.0	500.0	490.0	545.0
	4.0	110.0	176.0	190.0	580.0	520.0	470.0	460.0
	1.0	50.0	135.0	165.0	600.0	470.0	500.0	560.0
MEAN	2.0	83.0	147.0	168.0	593.0	496.0	486.0	522.0
7	0.0	5.0	13.0	6.0	45.0	37.0	55.0	65.0
	0.0	4.0	4.0	5.0	27.0	35.0	40.0	71.0
	0.0	1.0	6.0	5.0	17.0	20.0	35.0	58.0
MEAN	0.0	3.0	8.0	5.0	29.0	31.0	43.0	65.0
8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
MEAN	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
MEAN	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
MEAN	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

TABLE 6.26

Geotrichum candidum :-

Relationship of initial pH to final pH, optical density (absorbance) and dry weight when harvesting on DAY 9 (FIRST TRIAL)

INITIAL pH	FINAL pH	OPTICAL DENSITY($x10^{-3}$)	DRY WEIGHT (mg.)
2	1.99 1.98 1.98	0.0 0.0 0.0	4.0 3.0 4.0
MEAN	1.98	0.0	3.7
3	3.53 4.52 3.69	688.0 690.0 650.0	217.0 274.0 227.0
MEAN	3.92	676.0	239.3
4	5.90 6.30 5.62	800.0 785.0 660.0	261.0 226.0 210.0
MEAN	5.94	748.0	232.3
5	6.17 6.02 5.05	524.0 559.0 540.0	190.0 227.0 209.0
MEAN	5.75	541.0	208.7
6	6.26 6.20 6.15	545.0 460.0 560.0	192.0 190.0 195.0
MEAN	6.20	541.0	192.3
7	6.77 6.76 6.75	65.0 71.0 58.0	31.0 35.0 31.0
MEAN	6.76	65.0	32.3
8	7.70 7.70 7.69	0.0 0.0 0.0	5.0 8.0 6.0
MEAN	7.70	0.0	6.3
9	8.49 8.49 8.50	0.0 0.0 0.0	8.0 12.0 9.0
MEAN	8.49	0.0	9.7
10	8.98 8.99 8.99	0.0 0.0 0.0	8.0 7.0 9.0
MEAN	8.99	0.0	8.0

TABLE 6.27

Geotrichum candidum :-

Effect of initial pH on optical density readings (x10^{-3}) (absorbance at 580 nm.) over 6 days and on final pH and dry weight on DAY 6 (SECOND TRIAL)

	DAYS FROM START OF EXPERIMENT						DAY 6 FINAL	DAY 6 DRY
pH	1	2	3	4	5	6	pH	WT. (mg)
2	2.0	2.0	1.0	1.0	1.0	1.0	1.85	1.0
	0.0	0.0	0.0	0.0	0.0	0.0	1.82	1.0
	1.0	1.0	1.0	0.0	0.0	0.0	1.82	1.0
MEAN	1.0	1.0	0.0	0.0	0.0	0.0	1.83	1.0
3	1.0	6.0	9.0	14.0	41.0	125.0	2.70	181.0
	1.0	48.0	114.0	235.0	580.0	540.0	2.85	250.0
	1.0	60.0	250.0	320.0	800.0	420.0	2.88	272.0
MEAN	1.0	38.0	124.0	189.0	474.0	362.0	2.81	234.3
4	1.0	49.0	175.0	249.0	460.0	480.0	3.90	231.0
	1.0	149.0	170.0	384.0	431.0	798.0	4.91	269.0
	5.0	155.0	270.0	405.0	760.0	800.0	5.15	259.0
MEAN	2.0	117.0	205.0	346.0	550.0	693.0	4.65	253.0
5	1.0	28.0	165.0	193.0	201.0	350.0	5.06	212.0
	0.0	68.0	170.0	175.0	210.0	285.0	5.51	254.0
	3.0	42.0	260.0	315.0	329.0	360.0	5.75	260.0
MEAN	1.0	46.0	198.0	228.0	247.0	332.0	5.44	242.0
6	1.0	8.0	43.0	51.0	53.0	127.0	5.70	197.0
	1.0	26.0	111.0	114.0	197.0	260.0	5.85	233.0
	0.0	24.0	160.0	165.0	230.0	270.0	5.91	212.0
MEAN	0.0	19.0	105.0	110.0	160.0	219.0	5.82	214.0
7	0.0	3.0	8.0	12.0	15.0	37.0	6.68	12.0
	0.0	3.0	5.0	10.0	14.0	85.0	6.67	27.0
	0.0	1.0	20.0	41.0	45.0	104.0	6.40	46.0
MEAN	0.0	2.0	11.0	21.0	25.0	75.0	6.58	28.3
8	0.0	0.0	0.0	1.0	6.0	17.0	7.68	4.0
	0.0	0.0	0.0	1.0	4.0	7.0	7.66	4.0
	0.0	0.0	0.0	1.0	4.0	5.0	7.62	11.0
MEAN	0.0	0.0	0.0	1.0	5.0	10.0	7.65	6.3
9	0.0	0.0	0.0	0.0	0.0	0.0	8.41	3.0
	0.0	0.0	0.0	0.0	0.0	0.0	8.43	3.0
	0.0	0.0	0.0	0.0	0.0	0.0	8.43	5.0
MEAN	0.0	0.0	0.0	0.0	0.0	0.0	8.42	3.7
10	0.0	0.0	0.0	0.0	0.0	0.0	8.92	4.0
	0.0	0.0	0.0	0.0	0.0	0.0	8.92	3.0
	0.0	0.0	0.0	0.0	0.0	0.0	8.93	4.0
MEAN	0.0	0.0	0.0	0.0	0.0	0.0	8.92	3.7

TABLE 6.28

Fusarium tabacinum :-

Effect of initial pH on optical density readings (10^{-3}) (absorbance at 580 nm.) taken over 9 days and dry weight on DAY 9 (FIRST TRIAL)

	DAYS FROM START OF EXPERIMENT					FINAL DRY
pH	2	3	7	8	9	WEIGHT (mg)
2	0.0	0.0	0.0	0.0	0.0	4.0
	0.0	0.0	0.0	0.0	0.0	5.0
	0.0	0.0	0.0	0.0	0.0	5.0
MEAN	0.0	0.0	0.0	0.0	0.0	4.7
3	0.0	0.0	3.0	14.0	23.0	197.0
	0.0	0.0	2.0	9.0	15.0	92.0
	0.0	0.0	0.0	1.0	1.0	9.0
MEAN	0.0	0.0	1.6	8.0	13.0	99.3
4	6.0	30.0	66.0	68.0	67.0	408.0
	6.5	29.0	78.0	78.0	76.0	417.0
	5.7	25.5	65.0	60.0	70.0	411.0
MEAN	6.1	28.3	70.0	69.0	71.0	412.0
5	17.5	62.0	69.0	79.0	75.0	398.0
	18.5	73.0	84.0	79.0	76.0	415.0
	18.0	77.0	84.0	89.0	87.0	420.0
MEAN	18.0	71.0	79.0	82.0	79.0	411.0
6	16.0	68.0	73.0	70.0	67.0	382.0
	24.0	68.0	65.0	72.0	70.0	373.0
	25.5	65.0	68.0	66.0	68.0	381.0
MEAN	21.8	67.0	69.0	69.0	68.0	378.7
7	0.0	27.0	64.0	61.0	59.0	340.0
	0.0	18.0	57.0	53.0	67.0	335.0
	0.0	26.0	53.0	55.0	62.0	343.0
MEAN	0.0	24.0	58.0	56.0	63.0	339.0
8	0.0	0.0	6.0	23.0	45.0	255.0
	0.0	0.0	0.0	2.0	12.0	84.0
	0.0	0.0	1.0	3.0	8.0	93.0
MEAN	0.0	0.0	2.3	9.0	22.0	144.0
9	0.0	0.0	0.0	0.0	1.0	9.0
	0.0	0.0	0.0	0.0	0.0	7.0
	0.0	0.0	0.0	0.0	0.0	6.0
MEAN	0.0	0.0	0.0	0.0	0.0	7.3
10	0.0	0.0	0.0	0.0	2.0	9.0
	0.0	0.0	0.0	0.0	0.0	6.0
	0.0	0.0	0.0	0.0	0.0	10.0
MEAN	0.0	0.0	0.0	0.0	0.1	8.3

TABLE 6.29

Fusarium tabacinum :-

Effect of initial pH on optical density readings (10^{-3}) (absorbance at 580nm.) over 6 days and on final pH and dry weight on DAY 6. (SECOND TRIAL)

pH	DAYS FROM START OF EXPERIMENT					DAY 6	
	2	3	4	5	6	FINAL pH	DRY WT.(mg)
2	0.0	0.0	0.0	0.0	0.0	1.99	5.0
	0.0	0.0	0.0	0.0	0.0	1.98	6.0
	0.0	0.0	0.0	0.0	0.0	1.97	6.0
MEAN	0.0	0.0	0.0	0.0	0.0	1.98	5.7
3	0.0	0.0	0.0	0.0	0.0	3.01	5.0
	0.0	0.0	0.0	0.0	0.0	3.01	6.0
	0.0	0.0	0.0	0.0	0.0	3.01	6.0
MEAN	0.0	0.0	0.0	0.0	0.0	3.01	5.7
4	0.0	0.0	7.0	111.0	65.0	4.01	187.0
	0.0	0.0	12.0	122.0	60.0	4.02	171.0
	0.0	9.0	14.0	154.0	70.0	4.12	132.0
MEAN	0.0	3.0	11.0	129.0	65.0	4.05	163.3
5	1.0	17.0	35.0	280.0	135.0	5.48	336.0
	4.0	24.0	85.0	282.0	170.0	6.85	415.0
	3.0	13.0	50.0	390.0	220.0	7.31	440.0
MEAN	2.7	18.0	56.7	317.0	175.0	6.55	397.0
6	18.0	46.0	230.0	223.0	129.0	7.74	401.0
	8.0	68.0	300.0	318.0	160.0	7.65	411.0
	9.0	54.0	270.0	248.0	136.0	7.72	409.0
MEAN	11.7	56.0	266.7	263.0	141.7	7.70	407.0
7	2.0	20.0	175.0	155.0	105.0	7.58	354.0
	5.0	49.0	190.0	250.0	112.0	7.61	363.0
	4.0	32.0	175.0	242.0	106.0	7.55	356.0
MEAN	3.7	33.7	180.0	215.7	107.7	7.58	357.7
8	0.0	0.0	0.0	0.0	0.0	7.86	7.0
	0.0	0.0	0.0	0.0	0.0	7.86	6.0
	0.0	0.0	0.0	0.0	0.0	7.85	8.0
MEAN	0.0	0.0	0.0	0.0	0.0	7.86	7.0
9	0.0	0.0	0.0	0.0	0.0	8.61	7.0
	0.0	0.0	0.0	0.0	0.0	8.62	8.0
	0.0	0.0	0.0	0.0	0.0	8.62	6.0
MEAN	0.0	0.0	0.0	0.0	0.0	8.62	7.0
10	0.0	0.0	0.0	0.0	0.0	9.18	7.0
	0.0	0.0	0.0	0.0	0.0	9.16	6.0
	0.0	0.0	0.0	0.0	0.0	9.16	6.0
MEAN	0.0	0.0	0.0	0.0	0.0	9.17	6.3

TABLE 6.30

Fusarium tabacinum :-

Effect of initial pH on optical density readings (10^{-3}) (absorbance at 580 nm.) over 5 days and on final pH and dry weight on DAY 5 (THIRD TRIAL)

pH	DAYS FROM START OF EXPERIMENT 2	3	4	5	DAY 5 FINAL pH	DAY 5 DRY WT.(mg)
2				18.0	1.91	11.0
				15.0	1.90	8.0
				15.0	1.92	8.0
MEAN				16.0	1.91	9.0
3				22.0	2.90	11.0
				19.0	2.92	9.0
				13.0	2.97	7.0
MEAN				18.0	2.93	9.0
4	21.0	185.0	230.0	410.0	7.10	423.0
	25.0	220.0	250.0	415.0	7.09	427.0
	19.0	95.0	200.0	390.0	7.15	416.0
MEAN	21.7	166.7	226.7	405.0	7.11	422.0
5	27.0	290.0	385.0	380.0	7.88	419.0
	39.0	320.0	390.0	425.0	7.83	429.0
	51.0	350.0	395.0	470.0	7.75	438.0
MEAN	39.0	320.0	390.0	425.0	7.82	428.7
6	85.0	300.0	340.0	400.0	7.98	430.0
	78.0	193.0	335.0	275.0	8.00	414.0
	64.0	190.0	220.0	255.0	8.09	376.0
MEAN	75.7	227.7	298.3	310.0	8.02	406.6
7	61.0	160.0	191.0	205.0	7.79	351.0
	68.0	160.0	185.0	215.0	7.74	363.0
	78.0	160.0	200.0	228.0	7.69	375.0
MEAN	69.0	160.0	192.0	216.0	7.74	363.0
8	6.0	18.0	97.0	192.0	7.72	327.0
	4.0	13.0	84.0	159.0	8.09	289.0
	5.0	17.0	89.0	183.0	7.86	302.0
MEAN	5.0	16.0	90.0	178.0	7.89	306.0
9	1.0	4.0	52.0	113.0	7.85	155.0
	1.0	3.0	49.0	103.0	7.95	148.0
	1.0	2.0	46.0	99.0	8.20	147.0
MEAN	1.0	3.0	49.0	105.0	8.00	150.0
10	1.0	2.0	15.0	10.0	9.06	20.0
	1.0	1.0	12.0	7.0	9.24	16.0
	1.0	3.0	15.0	10.0	9.06	21.0
MEAN	1.0	2.0	14.0	9.0	9.12	19.0

TABLE 6.31

Variation, with time, of total wet weight (gm) of two fungi on six vertical screens dosed with 5% skimmed milk at 15°C.

	Geotrichum candidum			*Fusarium tabacinum*		
	SCREEN NUMBER			SCREEN NUMBER		
DAY	1	2	3	4	5	6
19	110	125	121	127	139	149
23	112	156	128	132	146	172
27	142	195	177	210	178	157
33	144	272	208	240	190	183
35	169	276	232	248	215	236
37	193	324	247	298	246	265
44	269	488	280	328	387	329
47	355	544	387	427	450	446
50	370	611	449	455	488	524
55	418	607	502	604	510	279
58	485	505	592	690	451	293
61	490	520	662	710	477	370
63	454	608	635	655	574	416
65	476	648	511	664	535	345
68				720	470	432

TABLE 6.32

Variation, with time, of mean flow rate to six vertical screens dosed with 5% skimmed milk, over the period covered by TABLE 6.31.

DAYS OVER WHICH FLOW-RATE WAS MEASURED	MEAN FLOW-RATE ml./min./screen
13 - 15	2.8
15 - 18	3.2
18 - 20	2.5
20 - 22	2.1
22 - 25	4.2
27 - 29	3.7
30 - 32	4.4
32 - 34	3.9
34 - 36	3.4
37 - 39	3.6
39 - 41	3.3
41 - 43	3.2
43 - 46	4.1
46 - 48	3.5
48 - 50	3.0
50 - 53	3.4
53 - 55	3.6
55 - 57	3.3
58 - 60	3.2
60 - 62	2.7
62 - 64	2.6
64 - 67	3.4

TABLE 6.33

Variation, with time, of total wet weight (gm) of two fungi on six vertical screens dosed with 2% skimmed milk at 15°C.

	Geotrichum candidum SCREEN NUMBER			Fusarium tabacinum SCREEN NUMBER		
DAY	1	2	3	4	5	6
7	134	152	152	122	134	122
9	142	174	151	124	116	118
11	140	210	175	134	128	140
14	181	205	202	163	110	102
16	201	250	273	210	125	118
17	197	270	298	227	132	139
21	216	403	397	329	179	202
22	215	403	417	303	165	183
23	212	306	339	208	137	170
24	205	301	312	222	131	163
37	295	345	370	184	198	158
39	325	365	325	150	220	176
42	350	260	110	112	100	198
77	225	235	300	230	230	135
78	235	235	340	250	235	160
79	270	255	300	210	200	190
80	310	315	390	270	235	220
81	365	335	425	255	285	245
84	375	558	500	265	168	94
86	375	430	475	290	184	144
88	420	616	390	320	184	154
91	240	571	485	245	150	200
99	200	495	405	225	210	95
100	143	644	255	159	121	94
106	112	720	107	104	95	90
108	109	730	107	100	88	88
109		710	103			
112		750	103			
113		713	103			
114		740	103			
115		730	103			
116		730	102			
119		710	102			
120		480	102			
121		350	102			
122		252	102			
123		175	102			

TABLE 6.34

Variation with time of mean flow-rate to six vertical screens dosed with 2% skimmed milk over the period covered by TABLE 6.33.

DAYS OVER WHICH FLOW-RATE WAS MEASURED	MEAN FLOW-RATE ml./min./screen
0 - 2	3.3
2 - 4	4.2
4 - 7	6.0
7 - 9	5.0
9 - 11	4.6
11 - 14	5.3
14 - 16	5.1
16 - 18	4.5
18 - 21	5.7
21 - 23	3.5
23 - 25	3.7
37 - 39	3.2
49 - 51	3.0
51 - 53	3.7
57 - 60	6.0
61 - 63	5.9
63 - 65	5.0
65 - 67	4.7
67 - 70	5.0
70 - 72	5.5
72 - 74	5.1
74 - 77	5.0
77 - 79	5.5
79 - 81	6.2
81 - 84	5.3
84 - 86	4.8
86 - 88	4.7
88 - 91	4.4
91 - 94	6.0
95 - 98	4.1

TABLE 6.35

Effect of increasing strength of skimmed milk on wet and dry weights of two fungi, each grown on 5 horizontal screens at 15°C for 14 days.

STRENGTH OF SKIMMED MILK (g/l)	*Geotrichum candidum*		*Fusarium tabacinum*	
	WET WT. (gm)	DRY WT. (mg)	WET WT. (gm)	DRY WT. (mg)
0.5	8.95	152	3.70	54
	7.61	81	6.30	118
	5.47	79	4.99	87
	12.66	229	7.71	70
	9.18	144	6.68	37
MEAN	8.77	137.0	5.88	73.2
1	14.28	215	15.15	321
	15.08	233	10.83	228
	14.00	252	11.34	249
	12.65	197	12.45	216
	23.90	816	15.87	451
MEAN	15.98	342.6	13.13	293.0
2	18.11	400	27.81	532
	21.20	460	20.50	459
	15.97	527	19.97	259
	21.66	491	17.78	282
	24.10	565	18.85	400
MEAN	20.21	488.6	20.98	386.4
3	11.12	267	14.02	336
	21.09	443	23.95	511
	23.67	853	27.54	859
	26.18	554	24.19	217
	20.54	652	22.45	496
MEAN	20.52	553.8	22.43	483.8
4	20.96	448	27.23	880
	28.56	814	25.28	759
	26.57	462	24.36	848
	29.23	555	23.54	735
	27.67	683	24.67	707
MEAN	26.60	592.4	25.02	785.8
5	31.46	776	31.23	937
	32.81	790	23.23	860
	43.09	667	26.58	529
	24.89	1602	33.92	768
	30.66	1272	32.98	1141
MEAN	32.58	1021.4	29.59	847.0

TABLE 6.36

Variation, with time, of mean flow-rate (ml./min./screen) to 10 horizontal screens dosed with six strengths of skimmed milk over fourteen day growth periods at 15°C.

DAY	STRENGTH OF SKIMMED MILK (g/1) 0.5	1	2	3	4	5
1	3.6	3.5	3.1	2.9	3.3*	3.1
2	3.6*	3.5*	3.1*	2.9*	3.8	3.1*
3	3.4	3.5	3.9	2.1*	3.8	3.05
4	3.4	3.5	3.9	2.5	3.8*	3.05
5	3.4*	3.5*	3.9*	2.5	3.6	3.05*
6	3.0	3.9	3.7	2.5*	3.6*	2.9
7	3.0*	3.9*	3.7*	3.2	3.3	2.9*
8	3.3	3.6	3.9	3.2*	3,3*	2.7
9	3.3*	3.6*	3.9*	3.6	3.5	2.7*
10	3.4	3.6	4.1	3.6*	3.5	4.3
11	3.4	3.6	4.1	3.6	3.5*	4.3
12	3.4*	3.6*	4.1*	3.6	3.3	4.3*
13			3.7	3.6*	3.3*	2.7
14			3.7*			2.7*
MEAN	3.35	3.61	3.77	3.06	3.51	3.20
STD. DEVIATION	0.19	0.14	0.32	0.53	0.20	0.61

* Day on which flow-rate was calculated
Mean of the Mean 3.415

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