THE UTILIZATION OF LOW-GRADE CARBOHYDRATES.

by

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SUMMARY.

This thesis is concerned with the application of fermentation technology to the production of useful materials from a class of carbohydrate resources presently under-utilized and even wasted.

The use of both carbohydrate-containing crops and processing wastes as fermentation substrates was extensively reviewed. It was concluded that a simple cheap fermentation process for the production of "biomass" directly from low-grade starch containing materials could have widespread application.

Experimental work was carried out using a Continuous Tower Fermenter System, cultures of Aspergillus niger M1 and three types of media. These were based on potato starch/salts (a semi-synthetic medium), raw potatoes and a waste from a "dry caustic" potato peeling process.

In all the fermentations, mould grew in the form of colonies, which were responsible in part, for the radical differences in behaviour which distinguished the tower fermenter from other continuous fermentation systems. The morphology of the mould both governed and was governed by fermenter conditions such as the oxygen transfer rate.

The concentration of mould in the fermenter was determined largely by a balance of the fluidisation and sedimentation forces acting on the colony morphology. In contrast the concentration in the effluent stream depended on the growth conditions and was usually significantly lower.

Experiments were carried out to determine the responses to

extremes of temperature and substrate limitation; in the latter, submerged sporulation occurred. When using alkaline media, steady states occurred in which the specific growth rate of the mould exceeded the culture dilution rate, attributed to a "froth flotation" effect.

The disappointingly low productivity rates and yield coefficients observed were the direct result of oxygen transfer limitations imposed by the mould morphology.

In the author's opinion the system is best suited to the production of "biomass" from effluents containing low carbohydrate concentrations.

CERTIFICATE.

I hereby certify that the work which constitutes this thesis has neither been, nor is being submitted in application for any other degree.

Signed .....

Date 20/3/77

DECLARATION.

I hereby declare that the work submitted in this thesis is the result of my own investigations except where reference is made to published literature or assistance acknowledged.

Candidate .....

Supervisor.....

Date ..20.3.77.....

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INDEX.

PREFACE.

The voracious demands of an increasingly industrialized and populated world are rapidly leading to the exhaustion of the Earth's finite resources. The food "crisis", about which so much has been written in recent years, is but one aspect of this problem, another is the rapid depletion of the oil and gas reserves that we rely on for energy and chemical feedstocks. The situation has been aggravated by our toleration of processes in which too high a proportion of the raw material input is destined for the waste stream. It is morally wrong to discard material which can be reclaimed or recycled (not necessarily in its original form), especially when this quite often results in serious pollution problems. Similarly the recent application of so much research effort to the production of (admittedly, much needed) microbial protein from such a finite source as petroleum seems equally shortsighted.

The longterm future of mankind will depend upon the adoption of a complete materials recycling system with the only inputs being resources derived from solar energy, which is most easily captured on a large scale by photosynthesis and therefore agriculture. The object of this research has been to furthur investigate the role of fermentation in this context.

SECTION I.

INTRODUCTION.

1.1 FERMENTATION - A BRIEF HISTORY.

Vital activities of microorganisms have wittingly or unwittingly been exploited by mankind probably since its earliest days. The fermentations involved in the production of cheese, yoghurt, soy sauce, wine, beer, vinegar and bread have been practised in various parts of the world for thousands of years. The art of breadmaking was well known, in Egypt certainly, at least 6000 years ago while the fermentation of fruit juices to wine had been used for so long that Ancient Greeks attributed its invention to a god, Dionysus.

However it was not until 1857 that Pasteur proved that alcoholic fermentation was brought about by living cells, yeasts, a discovery which sprang from his involvement with the problems of a small French alcohol manufacturer using sugar beet mashes. Subsequently, during the late 19th and early 20th centuries the increasing use of microorganisms in the laboratory was paralleled by the development of large-scale industrial processes such as the growth of Bakers' Yeast on molasses-salts media in deep aerated tanks and the anaerobic production of industrial alcohol.

The successful industrialization of the glycerol, acetone-butanol and citric acid fermentations in particular stimulated a rapid expansion of the field of fermentation technology during the 1920's and 1930's, resulting in methods for the production of a wide range of chemicals such as gluconic acid, itaconic acid, sorbose and 2,3-Butanediol. The expertise gained with these plants enabled the establishment during World War II of a large-scale antibiotic industry within 4 years of the first successful laboratory scale fermentations. During the postwar period the

market for bulk chemicals has been dominated by the more economic oil industry, resulting in the use of fermentation being restricted to specialized high value products such as antibiotics, vitamins, organic acids, enzymes, amino acids, sterols and modified steroids.

Traditionally the fermentation industry has relied on carbohydrate substrates, but in recent years microorganisms have been isolated which are able to metabolize hydrocarbons enabling processes to be developed for the production of metabolites and microbial protein from these resources. Unfortunately the advanced technology involved necessitates very expensive large-scale plants which limits their application.

In recent years considerable attention has been focussed on the problem of a global shortage of protein. Today a number of practicable processes exist, of which fermentation is but one, that together could totally eliminate this need if given the wholehearted support that, say, recent space programmes have enjoyed. Growth of microorganisms as a source of food is not a new idea. Quite apart from the numerous species of mushrooms that have been cultivated throughout the world for thousands of years, yeast, in various forms, has been an important dietary supplement for at least 100 years. Fodder yeast (Candida utilis) in particular has been grown on numerous waste substrates and used successfully as an animal feed throughout the world during the last 50 years.

The production of microbial protein from wastes using simple fermentation techniques could make a significant contribution to improving the diet of developing nations. The technology exists, the problems are merely with the application.

1.2 CARBOHYDRATE RESOURCES.

Increased exploitation of photosynthesis as a means of solar energy capture will result in plant materials and therefore carbohydrates assuming greater importance not only as foodstuffs but as alternatives to oil in the production of the range of chemicals that have become essential to our present lifestyle - the situation prior to the mid-20th century boom. More attention must be given to the use of a class of carbohydrate sources we term "Low-Grade", which can be considered as mainly materials which cannot easily be used in human or animal diets. The problems may be of low protein content, indigestibility or even toxicity, although in the case of effluents it is usually the high dilution which precludes their use.

1.2.1 LOW-GRADE CARBOHYDRATES.

These resources can usually be considered in the following categories:-

(1) Liquid Wastes and Effluents. Usually containing mono- and disaccharides such as Glucose, Sucrose and Maltose, though some have the polysaccharide starch present in a gelatinised state. Examples are effluents from canneries, dairies, breweries, distilleries and wood and paper processing plants.

(2) Solid Processing Residues. The polysaccharides starch and/or cellulose usually predominate in these wastes, though often lower concentrations of monosaccharides are also present. Examples include Bagasse, Potato, Cassava, Wood, Coffee and Olive Processing Wastes.

(3) Waste Portions of Crops and Uncropped Plant Materials. These are materials that our present technology is unable to utilize directly but constitute a vast source of organic matter which could be fermented. Some of the periodic crop surpluses which seem to plague the world's agricultural and marketing system could also be included in this category of presently wasted fermentation substrates. Examples are Bananas, Potatoes, Cassava, Water Hyacinths, Asphodelus tubers, Cacti, Jute, Apples and Tomatoes.

In addition we must promote the concept of growing crops specifically as substrates for fermentation. These will be selected on the basis of high yield of fermentable carbohydrate (in effect, a measure of efficiency of capture of solar energy), ease of harvesting and processing and (if necessary) ability to withstand harsh environmental conditions. Agro-industrial complexes based on cassava have been advocated by Maclennan (1) and McCann and Saddler (2) for tropical areas particularly Northern Australia. The potential yields are enormous, Inkson et al. (3) quoted an estimate that in the tropics the light energy incident upon one square metre is sufficient for the photosynthetic production of 72 $g.day^{-1}$ (i.e. 72 $tonne.hectare^{-1}.day^{-1}$).

1.3 SOME ECONOMIC COMMENTS.

It has often been argued that it is uneconomic to use Low-grade carbohydrates as substrates for the production of chemicals and microbial protein by fermentation, for example by Solomons(4).

Generally condemnations are made by comparison with the cost of synthesis from oil - an easy but somewhat shortsighted approach when dealing with such a finite reserve, especially one whose costs and availability are so vulnerable to political manipulation.

The economics of the transformation of a carbohydrate-containing crop or product from the state of a discarded waste to a useful byproduct is governed not only by substrate availability, price and processing costs, but also by LOCAL needs and even legislation, rather than on a worldwide basis. The importance of the fermentation of these materials as an antipollution and waste disposal process must be stressed. At present the community as a whole invests heavily in plant for the disposal of wastes and effluents, so any alternative process yielding a useful product must be welcome. In fact, in the extreme, a process could be costed at or below the break-even point, taking into account the present cost of disposal.

However, probably the most important economic requirement is the minimisation of capital investment, which necessitates the adoption of a simple technology using equipment fabricated with cheap materials and methods. This approach ideally should result in plants which are capable of running for long periods with little maintenance and with a low energy input - all factors which contribute to the low processing costs which are essential to this type of operation. Quite simply, in the words of Gaden (5) "process designs must reflect the realities of the situations in which they are to be employed".

SECTION 2.

LOW-GRADE CARBOHYDRATES AS FERMENTATION SUBSTRATES.

2.1 INTRODUCTION.

This review is intended to:-

(1) Include as many such carbohydrate sources as possible, on a world-wide basis, giving details of their approximate composition, seasonal availability and present use or means of disposal.

(2) Describe the work carried out so far on the fermentation of these substrates, particularly with respect to the production of microbial biomass as a source of protein.

(3) Provide information on the yields and/or economics of the processes described.

(4) Discuss other treatment systems which could be used as alternatives to, or in conjunction with, fermentation in a total utilization scheme.

For convenience, substrates will be considered in three categories:-

(1) As liquid wastes and effluents.

(2) As solid residues and wastes.

(3) As crops grown specifically for fermentation.

though, of course, the applications of the processes described frequently overlap these divisions.

2.2 UTILIZATION OF LIQUID WASTES AND EFFLUENTS.

2.2.1 MOLASSES.

During the first half of the 20th century molasses was the principal raw material for the production of ethyl alcohol. Throughout this period it was available for very little more than the cost of transportation and according to Thaysen (6) and

Jackson (7), was even dumped at sea. In fact a U.S. Treasury Report (8) indicated that even in 1956, 25.54% of the total U.S. production of ethyl alcohol was derived from this source, amounting to 575 million proof litres. However today the industry survives mainly in situations where necessity is a more important factor than economics, such as India. Continuous systems are particularly suited to this process, examples being those developed by Bilford et al. (9), Owen (10), Borzani (11) and Bose and Ghose (12). Such systems were reviewed by Coote (13) who also described the considerable increases in efficiency and rate of fermentation achieved using the Tower Fermenter.

Molasses, supplemented with ammonium salts is an important substrate for the aerobic production of Bakers' Yeast. For optimum yields the level of biotin, pantothenate and inositol are important, so complementary blends of Beet, Blackstrap and Cane varieties are usually used, typical ratios being given by White (14). By incremental nutrient addition (as in the Zulauf Method), the sugar concentration in the fermenter can be kept below 5 g/l., increasing conversion efficiency and lessening the problems of alcohol formation (Dawson, 15). Total fermentation time, including a short maturation period for the yeast, is usually about 10 - 12 hours in batch systems. In continuous systems the Specific Growth Rate is 0.15 - 0.25h^{-1}. Many descriptions of the processes used have been published, including those by Balls (16), Walter (17), White (14), Olsen (18), Plevako (19), Harrison (20), Rosen (21), Beran and Zemanova (22) and the excellent review of Reed and Peppler (23).

As a substrate for microbial protein production, molasses

has probably received more attention than any other material, but surprisingly few production scale plants have been built. The Colonial Food Yeast Co. Ltd. (24) described the plant erected and operated at Frome, Jamaica, growing a polyploid yeast, Torulopsis utilis var major, which had been selected by Thaysen and Morris (25). Tower type fermenters, intensely aerated via ceramic candles, gave the plant a capacity of 12 tonnes of dry yeast per day using either molasses, raw cane sugar or raw sugar juice as a substrate. According to Thaysen (6), the fermenters were operated continuously at a Dilution Rate of $0.25h^{-1}$ maintaining the cell population at 2×10^9 cells/ml. An unexpected benefit of continuous operation of these fermenters proved to be a reduction in infection problems.

Other contributions have been made by Agarwal et al. (26), who compared the yields and vitamin contents of Candida utilis, C. arborea, Geotrichum candidum and Saccharomyces cerevisiae grown on various beet and cane molasses samples, Lewis et al. (27), Vincenty (28), Singh et al. (29), Wiley et al. (30) and Chiao and Peterson (31).

More recently, Allen et al. (32) described work carried out at both the laboratory and pilot scale growing Candida arborea, C. utilis, Rhodotorula gracilis and Zygosaccharomyces lactis on a molasses/urea/phosphate medium in stirred tank fermenters, both batch and semicontinuously. Yeast extracts were prepared, feeding trials carried out and secondary feed products produced, all successfully. The problem was simply the uneconomically high price of the substrate.

Mannan and Ahmad (33) studied the growth of Saccharomyces

cerevisiae and Torula utilis on Indian molasses (and other carbohydrate containing media) supplemented with corn steep liquor. Yields were increased to 60% and 52.6% with protein contents of 50% and 42% respectively.

Shukla and Dutta (34) described their work on the growth of fungal protein, a strain of Rhizopus sp. with a high methionine content, on a molasses/ammonium chloride/calcium phosphate medium. The batch fermentation occupied 12 days, yielding only 9.6 $g.l^{-1}$ mycelium (28% of sugar supplied) with a protein content of 34.3%. Spicer (35) also indicated the intention of Rank, Hovis, McDougall Ltd. to use molasses as a substrate for production of their fungal protein, a Fusarium sp. where economics permit.

Estevez and Almazan (36) cultivated Candida utilis on clarified cane juice and molasses at sugar concentrations of 30, 60 and 100 $g.l^{-1}$. Yields declined with increasing sugar content which was attributed to the formation of secondary metabolites which suppressed growth, insufficient nonsugar media constituents and population density.

Waste carbohydrate materials in general, but molasses especially, have been used for the synthesis of fat by microorganisms under conditions of National emergency. A yeast, Rhodotorula glutinis, is of particular interest because of its high fat content, up to 63% (on a dry basis); high fat coefficient (can be around 0.17 based on sugar used)ability to grow readily in submerged culture; high rate of fat formation and ease of recovery from the propagation medium. Ordinarily sugar concentrations of 40 - 80 $g.l^{-1}$ are used but according to Steinberg and Ordal (37) concentrations of up to 200 $g.l^{-1}$ have no effect on yeast fat

content. Nitrogen must be deficient, a concentration of 1 $g.l^{-1}$ of ammonium sulphate was found to be optimum by Enebo et al. (38) and was confirmed by Pan et al. (39). Mention must also be made of the publications of Balls (40), White and Werkman (41), Nielson and Nilsson (42) as well as the review of Dankwerts and Sellars (43).

Many organic chemicals can be produced by the fermentation of molasses substrates. Glycerol has been manufactured by a number of processes which usually depend on the addition of high concentrations of sulphite to a medium inoculated with yeast. This fixes the acetaldehyde intermediate in the anaerobic glucose to ethanol metabolic pathway, leading to the accumulation of the alternative, glycerol, in equimolar quantities. Examples include The Eoff Process described by Eoff, Linder and Beyer (44), The Schade - Farber Process (45) and the Fulmer - Underkofler - Hickey Process (46). Further information has been given by Duchenne (47), Underkofler and Hickey (48) and Freeman and Donald (49).

Molasses has also been used for the production of acetone, butanol, isopropanol, 2.3-butanediol, lactic, citric, gluconic and acetic acids, riboflavin and vitamin B12, though in some cases maize or wheat mashes are preferred. These processes have been reviewed in great detail by Underkofler and Hickey (48) and Prescott and Dunn (50).

Citrus molasses, the product of the concentration of citrus-peel press liquor has been used both as a substrate for industrial alcohol production and the growth of food yeast (Van Loesecke, 51; Hendrickson and Kesterson, 52, 53). More recently, citrus wastes, particularly molasses, have been used for the production

of β-carotene by Blakeslea trispora mycelium. The rate of production and final yield are stimulated by a compound β-Ionone, occurring in the citrus waste (Ciegler et al, 54). It is usual, however, to omit the concentration step to molasses and to use peel juice or pressed liquor directly for fermentation.

2.2.2 CANNERY LIQUID WASTES.

Effluents produced by fruit and vegetable canneries arise largely from the necessity to wash and blanch the product and particularly as a result of leaching of cell "sap" from cut surfaces. In their review of the industries wastes, Ben-Gera and Kramer (55) quote that even fresh peas can lose up to 22% and 14% of their total sugars and protein respectively during a 3 minute blanching. Bough (56) carried out a study of the wastes from the canning of 5 types of leafy green vegetables to determine the contribution of each unit operation to the composite waste load, finding that washing and blanching accounted for 77% of the composite C.O.D. load and 90% of the flow. These wastes, together with those due to excessive peeling and trimming, make ideal fermentation substrates and have been widely used as such in the past though usually they are disposed of by an activated sludge process after mixing with city effluent (Goodson and Smith, 57). Other reviews of the sources and quantities of these wastes include those of Anderson (58) and Jones (59).

Effluents arising from the vegetable processing industry have been used by numerous workers as substrates for the culture of fungi. Humfeld (60) grew mushroom mycelium, Agaricus bisporus, in submerged culture on asparagus butt juice and the press juice

from pear waste. Litchfield and Overbeck (61) described the growth of another mushroom, Morchella hortensis on maize and pumpkin canning wastewaters, yielding 8.65 $g.l^{-1}$ over a 4 - 6 day period at 25 - 30°C.

Church et al. (62) described a process for the continuous treatment of maize and pea cannery wastewaters using Fungi Imperfecti. After mixing with nutrients, the wastewater was pumped into an open pool equipped with a 2 H.P. aerator at a Dilution Rate of 0.05 h^{-1}. The initial inoculum was of Trichoderma viride though in time the culture became mixed. A B.O.D. removal of up to 95% proved possible with mycelial yields of up to 0.646 and 0.42 $g.l^{-1}$ on maize and pea waste respectively, which was harvested for use as an animal feed. Aspergillus oryzae has also been used successfully for the treatment of maize and soya food processing waste waters (North Star Research and Development Institute, 63).

Wastewaters from citrus fruit processing and canning plants have been widely used as substrates for both biomass and chemical production. They are usually very acid (mainly citric) though the total solids are mainly sugars, but it is usually necessary to add nitrogen and/or phosphorus compounds for optimum treatment by biological processes.

The production of Food Yeast (Candida utilis) on diluted citrus peel juice supplemented with salts was studied as a batch and continuous process by Nolte et al. (64) and Veldhuis and Gordon (65) respectively. Yields as high as 60% dry weight (of the sugar supplied) were obtained, but the methionine content of the product was low. Block et al. (66) cultured mycelium of a

mushroom A. bisporus on various media containing citrus press water, orange juice and corn steep liquor. Nolte et al. (64) also demonstrated the feasibility of the production of industrial alcohol from citrus peel juice.

Vinegar has also been made by the double fermentation of orange peel juice but McNary and Dougherty (67) found that elimination of peel oils from the medium was necessary for a satisfactory product. Long and Patrick (68) were able to successfully ferment citrus peel juice with two Aerobacter aerogenes strains to 2,3-butylene glycol with a yield of 4.8 - 5.3%.

The disposal of Pineapple processing wastes has received a great deal of attention. According to Collins (69) sugar syrups for canning, cattle feed, alcohol and organic acid production are derived on a commercial basis from waste skins and cores. The first product is a mill juice containing c. 130 $g.l^{-1}$ soluble solids of which 75 - 80% are sugars, 7 - 9% citric acid, 2% malic acid and 2.5 - 4% protein. About one third of this can be clarified (by the method of Gould and Ash, 70), fortified with more sugar and used in canning. The remainder, if not fermented, is concentrated and fed to live-stock.

Utilization of this pineapple mill juice for vinegar production has been studied by Spurgin (71) and Richardson (72). The juice contains between 90 and 130 $g.l^{-1}$ sugar (as sucrose, glucose and fructose) in winter and summer respectively whereas the optimum for vinegar base production is 90 $g.l^{-1}$. Dilutions are made accordingly and the medium fermented with Saccharomyces ellipsoideus (a high alcohol tolerant "wine" yeast).

Richardson produced vinegars of up to 70 $g.l^{-1}$ acetic acid in less than 24 hours with a 90% conversion efficiency. The addition of bacterial nutrients did not improve either the yield or the rate of production of acetic acid.

2.2.3 WHEY.

To appreciate the suitability of whey as a fermentation medium, it is necessary first to consider its gross composition. According to data cited by Van Slyke and Price (73) and Olling (74) a typical whey contains:-

Water	931 $g.l^{-1}$	Lactose	49 $g.l^{-1}$
Fat	3 $g.l^{-1}$	Protein	9 $g.l^{-1}$
Ash	6 $g.l^{-1}$	Lactic Acid	2 $g.l^{-1}$

The ash fraction, which imposes certain limitations on the use of whey in fermentations, consists mainly of potassium and phosphorus compounds. The composition of whey is variable because cheese making procedures and milk composition are not constant, whey resulting from the manufacture of cottage or cream cheese contains more lactic acid and correspondingly less lactose than does whey from, say, Cheddar cheese manufacture. Although whey contains a variety of salts, it is deficient in inorganic nitrogen compounds which must be added if certain fermentations are to proceed satisfactorily.

Lactic acid was first produced commercially from whey in 1936 by a batch process described by Olive (75) and Burton (76), though the fermentation had been carried out continuously on a semi plant basis by Whittier and Rogers (77). A similar process using cheese whey was also described by Cambell (78).

In each case pasteurized whey is inoculated with a culture of Lactobacillus bulgaricus and held at 43°C for between 24 and 42 h., in the continuous system the Dilution Rate being about 0.05 h^{-1}. Yields of 85 - 90% are possible based on the weight of lactose used. Swaby (79) however advocated the use of a mixed culture of L. bulgaricus and Mycoderma sp.

A successful method for producing ethyl alcohol from whey was also described by Browne (80) and Rogosa et al. (81). The process consisted of two stages, Protein was precipitated by acidifying the whey to pH 5.0 and boiling. After cooling the resulting liquor to 34°C, a culture of Candida pseudotropicalis was added and the fermentation allowed to proceed at this temperature for 48 - 72 hours. The yield of alcohol was between 84 and 91% with byproducts of course being whey protein and surplus yeast. Wilharm and Sack (82) supplemented whey with lactose and though the yield of alcohol increased, the efficiency of conversion of the fermentation was reduced.

Of all the fermentations carried out with whey, most interest in recent years has centred around the production of food yeast, of which Saccharomyces fragilis is undoubtedly the organism of choice. A variety of procedures have been suggested for growth of yeast in whey, but the most detailed recently reported studies on the maximum yield of yeast cells (and protein) are those of Wasserman and his associates (Wasserman, 83, 84, 85; Wasserman and Hampson, 86; Wasserman et al, 87, 88,89).

Supplementation of whey with phosphorus and ammonium sulphate at the rate of 2.25 and 8.5 $g.l^{-1}$ respectively is necessary for maximum yields. The optimum pH is in the range of 5.0 to 5.7

and temperature, 33°C. However good yields have been obtained at temperatures of up to 43°C, such wide tolerances reducing the need for precise control of the fermenter conditions. Heavy yeast inocula are used (about 24 $g.l^{-1}$ wet equivalent in dry weight to approximately 30% of the weight of lactose in the medium) which permits a 4 h. incubation period for maximum yield. Harvesting of the product is by centrifugation.

Excess oxygen is necessary if growth rate and yield coefficient are not to suffer. Initially the demand is about 0.03 $l.l^{-1}min^{-1}$. rising steadily to about 0.11 $l.l^{-1}.min^{-1}$ after 2 hours and declining sharply to less than 0.02 $l.l^{-1}.min^{-1}$ at the end of the 4 hours fermentation. The yield is about 0.42 kg. of dry yeast per kg. of lactose.

In his review Oborn (90) described two commercial processes for the production of substantial amounts of dried yeast from whey. The "Wheast" Process is based on the findings of Wasserman and associates, described above, whereas the "S.A.V." Process is a two stage system which can be operated in either the batch or continuous mode. In the first stage lactose is fermented, whilst in the second, a major portion of the lactic acid is utilized. In this case the product is recovered by evaporation and spray drying.

Whey has also been used as a substrate for the production of the enzyme lactase which has numerous uses in the preparation of milk-based food products. Myers and Stimpson (91) patented one of the first processes using S. fragilis, S. lactis, C. spherica, C. pseudotropicalis and C. utilis. More recently, Wendorff et al. (92) reported that for maximum lactase

production, S.fragilis required whey containing 10 - 15% lactose, a pH of 4.0 - 4.5, the addition of corn steep liquor or casein digest and incubation at 27°C.

Whey has also been used as a substrate for vitamin production. Lutskova (93) grew Proprionibacterium shermanii in batch culture with incremental addition of whey/ammonium sulphate/cobalt chloride medium for 4 - 5 days at 30°C and pH 7.0. The yield was 5.6g.l^{-1} Vitamin B12 in 13.4g.l^{-1} biomass.

Several investigators have suggested the use of microorganisms in the genus Geotrichum to increase the protein and/or fat content of whey. The "Biosyn" method described by Fabel (94) uses Geotrichum candidum which can have a protein content of about 50%. According to Balls (95), during World War II, a process was employed to grow this organism for incorporation in sausage for human consumption. More recently Atkin et al. (96) used Geotrichum rotundatum to produce protein from whey in both batch and continuous culture. This organism assimilates but does not ferment lactose and proved to have a much higher yield factor than S. fragilis grown under comparable conditions.

Tomisek et al. (97) grew Torulopsis utilis, T. casei and T. cremoris on whey with yields of 16 - 21g.l^{-1}, whilst Zalashka and Samtsevich (98) used 113 yeast strains, the most productive being Candida humicola and C. curvata. Biomass yield was in the range of 18 - 30g.l^{-1}, but the addition of nitrogen to the medium increased only the protein content, not the yield.

Delaney and Donnelly (99) advocated fractionation of whey

using such processes as Reverse Osmosis, Ultrafiltration and/or Gel Filtration (which have now become feasible on a large scale), followed by fermentation with S. fragilis or Fusarium sp. According to Wasserman (84) whey discarded in the U.S. contains 204,000 tonnes of sugar and 36,000 tonnes of protein, representing a potential yield of 90 - 100,000 tonnes of protein in about 150,000 tonnes of animal fodder - probably about 10% of U.S. concentrate requirements.

2.2.4 SULPHITE LIQUOR.

In the manufacture of paper pulp, softwoods such as spruce are fractionated by cooking with sulphites at about 140°C. This results in a waste liquor, sulphite liquor, which contains 100 - 120 $g.l^{-1}$. total solids of which 15 - 20 $g.l^{-1}$ are sugars. For each tonne of pulp produced there are 8 - 10 tonnes of sulphite liquor (c. 9000 l.) but the composition varies widely with the source, type of wood pulped and the nature of the process. Eweson (100) gave a typical analysis for liquor obtained from Swedish Spruce, consisting of (for each 1000 kg. of cellulose produced):-

Lignin	644 kg.	Carbohydrates	311 kg.
Proteins	15 kg.	Resin and fats	73 kg.
Sulphur dioxide combined with lignin			235 kg.
Calcium oxide combined with lignosulphonic acid			102 kg.

The carbohydrates consisted of 49.4% glucose, 15.6% mannose, 8.1% galactose and 26.9% pentosans (arabinose).

Eweson (100) gave this analysis as part of his description of the "Heijkenskjold Process" for the manufacture of Bakers'

Yeast, Saccharomyces cerevisiae from sulphite liquor mixed with a small quantity of molasses. However, for food yeast production on this waste, Candida utilis is a more suitable choice on account of its ability to assimilate a wide variety of carbon and nitrogen sources including hexoses, pentoses, organic acids and other constituents that are characteristic of acid hydrolysates of wood and other cellulosic materials.

Laboratory studies on food yeast production from waste sulphite liquors have been carried out by Walker and Morgen (101), Harris et al. (102) and Markham et al (103). According to Saeman et al. (104) and Locke (105) food yeast was produced on a large scale from sulphite liquors in Germany during World War II, principally by the "Waldhof Process", but Geotrichum candidum was also grown (Frazier, 106). A continuous commercial process using a Waldhof fermenter was also described by Inskeep et al. (107), and in 1958, Mead (108) reported that 25% of all sulphite liquor solids were made into yeast, representing about 50,000 tonnes/year production.

However, Barta (109) used another yeast, Cryptococcus diffluens on a production scale on account of its superior amino acid content, Kosaric et al. (110) even grew mushrooms, a Morchella sp. in submerged culture on this substrate. Recently two new processes have been announced (111) the "Attisholz" and "Pekilo" Processes in which filamentous fungi are grown on sulphite wastes as a source of protein, the latter apparently being easily adapted to other wastes.

Ethyl alcohol has been produced commercially from sulphite liquor in the United States, Canada, Sweden and other countries.

Ericsson (112) described the process operated at the Bellingham, Washington, plant which produced about 300 tonnes of pulp per day. The waste liquor was steam stripped to remove sulphur dioxide, flash cooled to 30°C (concentrating it by 10 - 12%) and limed to pH 4.5, before being fed to a seven stage continuous agitated fermenter system with a residence time of about 20 h. The yield of alcohol was about 100 $l.tonne^{-1}$ of pulp, giving the plant a capacity of at least 30,000 $l.day^{-1}$. Reuse of yeast was essential for a rapid and efficient fermentation.

Leonard et al. (113) thoroughly studied the production of lactic acid from sulphite waste liquor using a number of strains of Lactobacillus pentosus in a batch fermentation lasting 40 - 48 hours. From 9,000 litres of liquor (resulting from the production of 1 tonne of pulp) they were able to produce 130 kg. of lactic and acetic acids respectively, at a recovery efficiency of 95%.

2.2.5 STARCH AND SUCROSE PROCESSING WASTES.

Starch is prepared commercially from three main sources - cereal grains especially maize, cull potatoes and low-grade cassava, the effluent from these plants constituting a major pollution problem. Treatment is usually at a conventional sewage works with no attempt being made to recover a useful product. In the manufacture of potato starch all the solubles are dumped, which can amount to 27% of the dry weight of the potato according to White (114).

However protein recovery from potato effluents is becoming more common. Most methods rely upon coagulation of the protein from the acidified liquor using steam injection heating (to about

85°C) as in the "Avebe Process" (115) and the method described by Strolle et al. (116). Coagulated protein can be used as an animal feed and the residual liquor subjected to further treatment. Hausler and Malcher (117) advocated methane fermentation. Milanovich (118) used yeasts to assimilate the organic acids in the waste, finding 0.45 g. organic acids yielded 0.158 g. of yeast of 90% dry matter. In their economic analysis of methods of treating potato waste liquors, Stabile et al. (119) found that recovery of free amino acids from the waste was also a viable proposition.

Weaver et al. (120) used a mixed culture of Torulopsis utilis and Aspergillus niger to treat potato waste liquor continuously in a Waldhof fermenter, achieving appreciable reductions in the B.O.D. Reiser (121) grew Candida utilis on similar wastes, again with no added nutrients and recovered 50% of the solids as yeast, reducing the B.O.D. by at least 60%. He experimented with both batch and continuous culture with a stirred tank fermenter run at 30 - 32°C and pH 5.0.

According to Stanton (122), Torula yeast has been grown successfully on Tapioca starch waste liquors in Thailand at pilot plant scale. C. utilis and Lentinus edodes were used by Takakawa and Furukawa (123) for the treatment of the waste juice from sweet potato starch manufacture.

Effluent liquors from Beet and Cane sugar processing plants have been largely neglected as fermentation substrates, being at present treated in conventional sewage works (Fischer and Hungerford, 124). However Blackwood et al. (125) used these, corn processing and cheese factory (whey) wastes as substrates

for the growth of Rhodotorula glutinis as a source of lipids. They used a stirred chemostat with ammonium sulphate as the limiting nitrogen source. Yields could be increased by using oxygen instead of air and with a two stage system in which conditions were optimum for growth and fat production respectively. Fat contents of 40 - 45% of the dry weight were usual with an analysis typical of vegetable oils.

Mention must also be made of a waste from the preparation of maize starch, corn steep liquor, which is used widely as a medium constituent by the fermentation industry particularly for antibiotic production, on account of its proven stimulatory effects on microbial growth.

2.2.6 BREWERY AND DISTILLERY EFFLUENTS.

Brewery effluents arise from such sources as tank washing, beer destruction, keg and bottle washing and even the occasional practice of resuspending spent grains and hops to remove them from vessels. They have been considered in detail by many authors including Quittenton (126), Walker (127) and Isaac and Anderson (128). Because of their extremely high Carbon : Nitrogen ratio these effluents are rarely treated in isolation, usually after mixing with city sewage. Liquors can contain as much as 2% total solids of which 50% are Proteins, 25% Fats and 20% soluble carbohydrates (some pentoses and hexoses, but mainly dextrins).

Middlekrauf et al. (129) worked on the biological degradation of spent grain effluent in a 122 x 15.25 cm. plastic

tower packed with ceramic material, using an "enriched" culture of garden soil. Effluent C.O.D. could be reduced by 90% in continuous culture at the rate of 0.5 $l.h^{-1}$, but no attempt was made to use pure culture.

Distillery effluents have received rather more attention. The B.O.D. of waste pot ale is so high, often 25 - 30,000 $mg.l^{-1}$, that the best means of treatment is evaporation to a syrup and incorporation into animal feed either in this form or as a spray dried powder. These processes were described by Rae (130) and Lines (131). According to Lines (131), spent wash solids from molasses-alcohol fermentations can be recovered in a similar manner. The low protein and high mineral content of the product make it uneconomic to manufacture in Britain but some is made in South Africa.

In Russia, Chastukin (133) cultured mould mycelia on residual liquids (spent washes) from "treacle" (molasses) "hydrolytic" (grain?) and sulphite alcohol manufacturing plants. The surface culture method used was not practicable in view of the large areas and labour required, especially as the growth rate was comparatively slow. Later, Chastukin et al. (134) described their work with the growth of 22 different fungi on "treacle liquor" (most likely the concentrated stillage from a blackstrap or beet molasses - alcohol plant). The four most promising species were Aspergillus niger, A. oryzae, Oidium lactis and a Fusarium sp.

Falanghe (135) attempted to produce mushroom mycelium in submerged culture on a medium prepared from vinasse, a waste product from the distillation of fermented sugar cane juice.

Agaricus bisporus had the highest protein content but Boletus indecisus yielded far more mycelium and had a greater total weight of protein.

2.2.7 COCONUT PROCESSING EFFLUENTS.

Coconut wastewaters constitute a serious environmental pollution in many tropical countries, containing typically about 4.7% total sugars present as glucose, fructose, sucrose and sorbitol. Smith and Bull (136) grew Saccharomyces fragilis on this waste supplemented with 4 $g.l^{-1}$ urea. The batch cultivation was characterised by two consecutive exponential phases. During Glucose, Fructose and Sucrose utilization the growth rate was high (0.72 h^{-1}) falling to 0.15 h^{-1} when the sorbitol remaining was being used. Single stage continuous cultivation resulted in sequential utilization of the carbon sources with increasing dilution rate, it was only at dilution rates of less than 0.09 h^{-1} that all four components were assimilated.

These diauxic effects of mixed sugar substrates on the growth phases of microorganisms have been discussed in detail by Monod (137), Baidya et al. (138) and Harte and Webb (139)

Hipolito et al. (140, 141), Aliwalas et al. (142) and Baens-Arcega (143) described work carried out in the Philippines growing a number of food yeasts, particularly Rhodotorula pilmanae on these wastes using a simple 100 l. Tower Fermenter. With an initial sugar concentration of 20 $g.l^{-1}$ the fermentation occupied 60 hours, the yeast yield being 51% (based on sugar used) with a protein content of 51.8%. Aliwalas et al. (142)

supplemented the medium with 0.3 g. Ammonium sulphate, 0.05 g. disodium hydrogen phosphate and 0.006 g. magnesium sulphate per gram. of reducing sugars, obtaining a yield of 5.93 $g.l^{-1}$. after 12 h.

According to Stanton (144) economically viable operations have been developed in Ceylon and the Philippines for the production of a potable alcohol and a high-quality vinegar, respectively, from the waste water from copra extraction from coconuts. Another process unique to the Philippines is the production of a jelly-like material by a continuous Leuconostoc sp. fermentation of acidified fruit juice, coconut water or similar wastes. This material, "Nata di coco" is a cellulosic polysaccharide which acquires the flavour of the raw juice from which it is derived.

2.2.8 SOYBEAN WHEY.

This is a waste product of the rapidly expanding soybean protein isolation and concentration industry. Falanghe et al. (145) cultured a number of mushroom species on this medium, the most suitable being Boletus indecisus which grew in the form of spherical colonies. The yield of protein was almost doubled if 30 $g.l^{-1}$ glucose was added to the medium. The highest yield of protein was 6.3 $g.l^{-1}$ in 12 days, produced by Tricholoma nudum, but the mycelium was diffuse and not readily separated from the spent liquor. Soybean whey has also been mentioned as a suitable substrate for the growth of Fusarium sp. (35).

2.1.9 SAUER KRAUT WASTES.

Hang et al. (147, 148, 149) have investigated the use of waste brine solutions from Sauerkraut processing. During the process 29% of the initial weight of cabbage is discarded as brine, which has a typical analysis of:-

B.O.D.	12,400 $mg.l^{-1}$
Kjeldahl Nitrogen	620 $mg.l^{-1}$
Sodium Chloride	18,600 $mg.l^{-1}$
Total acid (as lactic)	7,400 $mg.l^{-1}$
Total Phosphate	81 $mg.l^{-1}$

Of the yeasts cultivated, C. utilis yielded 6 - 7 $g.l^{-1}$ dry weight in 24 hours, S. fragilis and S. cerevisiae only 4 - 5 $g.l^{-1}$. During the fermentation the pH rose from 3.4 to just over 7, no residual lactic acid being found. The B.O.D. was reduced to about 1,550 $mg.l^{-1}$.

These workers also found that the specific yield of invertase with this medium was over twice as much as on peptone, sucrose and yeast extract medium. Yields of 62 g. of Geotrichum candidum per 100 g. B.O.D. utilised were also obtained in later experiments when the reduction in B.O.D. was 88%.

2.2.10 MISCELLANEOUS WASTES.

Stanton (144) briefly mentioned work carried out by McGarry (150) on the utilization of the effluent from rubber processing, a moderately clear, grey liquid, with high concentrations of nitrogen compounds which can be used as a culture medium for algae of the Chlorella sp. The advantage over domestic sewage (often used as a substrate for algal growth) is the considerably lower

chance of pathogenic bacteria being present.

Food production systems in which the combined harvest consists of the conventional crop, leaf protein, single cell protein grown on leaf juices and leaf fibre have been advocated for a number of years. Worgan (151) was able to increase the yields of protein from maize and pea crops sixfold by this method, another example being the work of Parades-Lopez and Camargo-Rubio (152) with alfalfa residual juice.

McLoughlin (153 and 154) used Peat extract medium for the growth of C. utilis showing that the yield was a direct function of its carbohydrate concentration. Fogarty et al. (155) used similar extracts for biomass and enzyme production. Extracts alone supported growth, but greatly improved yields of A. oryzae were obtained if as little as 2 $g.l^{-1}$ starch was added. Roberts and Murphy (156) reported that the growth of A. niger was stimulated by peat extracts, an effect which applies to a wide range of microorganisms and probably accounts for the quality of the product of the malt whisky industry of Scotland.

Yeasts have also been grown on byproducts of chemical processing. Howard and Wiken (157,158) mentioned the use of oxanone-water, a mixture of lower fatty and dicarboxylic acids resulting from the manufacture of Nylon, and Shimizer et al. (159) treated phenol-containing waste waters continuously with C. tropicalis. The maximum dilution rate was 0.25 $hr.^{-1}$ for treating an inlet concentration of 0.001 $g.l^{-1}$. The use of chemical effluents as fermentation media was reviewed by Edwards and Finn (160).

2.3 UTILIZATION OF SOLID RESIDUES AND WASTES.

This category consists mainly of wastes containing celluloses, hemicelluloses and lignin though some crop residues and food processing wastes containing starch are also included. Three general schemes can be proposed for the microbial utilization of these polymers:-

(1) Chemical hydrolysis to liberate the constituent sugar molecules which can be utilized by microorganisms.

(2) Enzymic degradation to yield the component sugars which can be metabolized by microorganisms.

(3) Use of a microorganism which can metabolize the polymer directly.

Options (1) and (2) have been most widely exploited in the past, largely because they permit greater flexibility in the choice of organism and fermentation. Option (3) necessarily reduces the choice of organism and fermentation and almost invariably involves a limitation of reaction rate.

2.3.1 WASTES CONTAINING STARCH.

These substrates, which include the wastes resulting from the processing of potatoes, sweet potatoes, cassava or tapioca and cereal grains will be dealt with in section 2.4 in conjunction with their respective parent materials.

2.3.2 WASTES CONTAINING CELLULOSES, HEMICELLULOSES AND LIGNIN.

According to Hall and Slessor (161), of the total weight of carbon photosynthetically fixed in one year (estimated at 2×10^{11} tonnes), approximately one half is in the form of cellulose,

making it probably the most abundant organic material on Earth. However, on account of its relative inertness, the difficult and often uneconomic degradation necessary in the preparation of a suitable medium for fermentation has precluded the use of cellulose in most processes. If the recent industrialisation of the amylase and glucose isomerase processes can be successfully repeated with cellulases then its use in the future seems assured.

2.3.2.1 STRAW AND CEREAL WASTES.

As long ago as 1920, Pringsheim and Lichenstein (162) described a method used to upgrade the protein content of straw in Germany as a wartime emergency measure to provide cattle food. Ammonium salts (mainly the sulphate) were mixed with the straw which was then inoculated with spores of an Aspergillus sp. During an incubation period the crude protein content increased from 0.9% to about 8.0%.

These agricultural wastes have been subjected to many saccharification processes to yield fermentation media (see also sections 2.3.2.2 - 2.3.2.4). Dunning and Lathrop (163) described one of the more successful continuous methods operated with corncobs, bagasse, flax shives, oat hulls and cottonseed hulls. A two stage hydrolysis was involved, the first using dilute sulphuric acid (1.9%) to separate the pentosans as xylose and furfural without saccharifying the cellulose. The second stage used concentrated sulphuric (8%) acid at 120 - 130°C for 7 - 10 min. to release hexose sugars from the cellulose, which were fermented to ethyl alcohol by Saccharomyces cerevisiae. With five products, xylose, furfural, ethyl alcohol, lignin and yeast,

it was claimed the process could be operated economically. The xylose solution could also be fermented to acetone and butanol by Clostridium acetobutylicum (Langlykke, Van Lanen and Fraser (164) or to 2,3-butanediol by Aerobacter aerogenes (Perlman, 165).

Straw was used by Bes et al. (166) as a substrate for the growth of Candida utilis after partial acid hydrolysis. The maximum concentration of reducing sugar was obtained by digesting 10 kg. of chopped straw with 40 litres of 1% sulphuric acid for 1 hour at 3 atm. steam pressure (137 - 138°C), the sugar yield being about 20% of the weight of oven-dry straw used. The composition of the extract was approximately 85% pentose (practically xylose only) because of the low acid concentration used, 14% hexose (practically glucose only) and 11% acetic acid. This extract was diluted to a concentration of 2% reducing sugars and placed in the culture vessel, a simple glass tower fermenter, 84 cm. long and 7.4 cm. in diameter with a porous stone aerator. The yield of yeast was as high as 9.52 $g.l^{-1}$ dry weight in 10 hours.

Processes involving the enzymic degradation of these wastes are few. Woo and Lee (167) used enzymes from Aspergillus niger to break down Rice Hulls and Straw. Peitersen (168)investigated the growth and cellulase production of Trichoderma viride on barley straw (pretreated with sodium hydroxide under high pressure) in a 5 l. stirred fermenter. Increasing the initial straw concentration from 10 - 20 $g.l^{-1}$ caused an increase in the initial lag phase from 0 to 2 days and the time of cessation of growth from 2 to 6 days. The protein content of the product was in the range of 21 - 26% with yield coefficients of 0.4 - 0.56

and up to 70% utilization of the straw.

2.3.2.2 CELLULOSE AND PAPER PULPS.

Grosser and Bernhauer (169) produced mycelium of 25% crude protein content by inoculation of a vigorously aerated suspension of cellulose with cultures of various species of Penicillium.

Ghose (170) obtained a solution containing more than 5% glucose by incubation of Trichoderma viride with waste sulphite-processed cellulose pulp at 50°C and pH 5.0 for 40 hours. However by using membrane concentrated cellulase and 300 $g.l^{-1}$ ground cellulose suspension, a 125 $g.l^{-1}$ reducing sugar solution could be obtained in 72 hours with a 99% conversion efficiency. This limitation of about 40% conversion of the available cellulose is at the same level that Bomar and Schmid (171) noted in their work with Cellulomonas sp. and attributed to the cellulose being present in a non-hydrolysable form.

Crawford et al. (172) used waste cellulose as a substrate for the growth of Thermonospora fusca, a thermophilic actinomycete common in soil, compost and manure, as a source of microbial protein. Updegraff (173) described work carried out on the growth of Myrothecium verrucaria on waste paper. However the maximum protein synthesis rates and cell yields were very low compared to those achieved using glucose. Rogers and Spino (174) and Bellamy (175) have reviewed the production of microbial protein from cellulosic wastes, stressing the necessity for a pretreatment process (such as that described by Han and Callihan, 176, and Callihan and Irwin, 177) to increase yields and accelerate conversion rates.

Porteous (178, 179) has focussed attention on the large percentage of cellulose found in domestic refuse (due to the paper content) and proposed hydrolysis as a recovery process. Using a very small continuous flow reactor, with hydrolysis by 4 $g.l^{-1}$ sulphuric acid solution at 230°C for 1 - 2 min. the conversion rates could be high with very little destruction of the sugar formed. It was claimed the process could be operated economically for the production of alcohol, whilst the results indicated that a large change in refuse composition had little effect on the rate of fermentation or yield of alcohol.

2.3.2.3 BAGASSE.

Bagasse, the residual fibrous material remaining after the extraction of raw juice from sugar cane, has always caused problems to sugar processers on account of the sheer volume of production. Even with all the research effort so far put into finding a profitable use, most of the world's bagasse is burned (its calorific value is equivalent to 380 $l.tonne^{-1}$ fuel oil). Of the uses which have been most successful, the wallboard and paper and pulp industries have taken the greatest tonnages (Hansen, 180).

The approximate composition of bagasse is as follows (Dunning and Lathrop, 181):-

Celluloses	42.2%
Hemicelluloses	17.8%
Lignin	14.0%
Total Nitrogen	1.43%
Ash (Mainly Silicon dioxide and Ferric Oxide).	1.42%

Bagasse has attracted much attention as a possible substrate for microbial protein production, degradation of the cellulose and hemicellulose portions having been carried out by both acid hydrolysis and enzymic means. Much of the early work on the acid hydrolysis of bagasse was reviewed by Cid (182), including the work of Dunning and Lathrop (163).

Recent work has been carried out by El Nawawy (183, 184) on the growth of Candida pelliculosa on bagasse hydrolysed with 1%, 5% and 10% sulphuric acid. Yields were as high as 84 g. of yeast protein from 1 kg. of pith. The yeast was mixed with the bagasse residue to constitute a cattle feed.

Dunlap et al. (185) and Callihan and Dunlap (186) gave details of the process and described the construction and operation of a pilot plant for single cell protein production by the growth of Cellulomonas sp. on cellulosic waste matter, particularly bagasse. Further work, described by Han et al. (187) was concerned with the growth of this and other species symbiotically to yield a cost-competitive product with a favourable amino acid profile.

Toyama and Ogawa (188) attempted to saccharify cellulosic wastes including lignin, such as bagasse, straw, sawdust, corrugated cardboard and newspaper. Highly active commercial cellulase preparations derived from Trichoderma viride and Aspergillus niger were used, though proved to be too expensive. Cellulase production by Trichoderma viride on a solid medium was also investigated and Candida utilis found to be suitable for submerged production from sulphite pulp waste.

2.3.2.4 WOOD.

Dry and bark-free wood wastes such as shavings and sawdust contain 50 - 70% of carbohydrate material, which is of two types - cellulose and hemicelluloses. The cellulose portion, comprising about 50% of the wood is the more resistant to chemical action, whereas the hemicelluloses (comprising about 20% of the wood) are a mixture of chemically bound sugars which can be readily decomposed by boiling for a short time with dilute acid.

The hemicelluloses from hardwoods - such as beech - yield a high percentage of Xylose, a 5-carbon sugar, whereas those from softwoods - such as pine and fir - yield a mixture of sugars composed of about one half 6-carbon sugars such as glucose, mannose and galactose.

Processes for the hydrolysis of wood to its constituent sugars have been known for more than a century, as early as 1819 Braconnot (189) reported the production of sugar from wood, and in 1898 Simonsen (190) described a process that yielded a 6% sugar solution. Two commercial plants were built in the U.S.A. during the early years of the 20th century, employing a 45 minute hydrolysis with dilute acid at 170°C. A yield of 20 - 25% sugar was obtained, which was used to produce high-quality industrial alcohol - about 100 litres of 95% alcohol from 1 tonne of dry wood waste. The plants closed in 1920 because of competition from alcohol made from blackstrap molasses (which was available at give-away prices). Other hydrolysis processes used successfully include those of Scholler (191), Bergius (192) and Giordani (193). Saeman et al. (194) described plants operated

in Germany between 1939 and 1945 producing yeast and ethanol from wood hydrolysed by the "Scholler" and "Bergius Processes".

In 1947 a new process was developed at the U.S. Forest Products Laboratory, known as the "Madison Wood-Sugar Process", described by Harris and Beglinger (195). In this a spray of hot 0.5 - 0.6% acid was pumped onto wood chips heated in a stationary digester to 150°C at the start and gradually increasing to 185°C. The resulting solution contained 5 - 6% of a mixture of simple sugars. This solution was used as a substrate for Candida utilis production in a continuous Waldhof fermenter. The residence time of the liquor was between 2½ and 3 hours with a yield of up to 50% dry weight yeast on wood sugars. It was also suggested that wood sugar plants could be built alongside other seasonally operating plants such as beet plants or sugar refineries so that steam generation, power, water and effluent disposal facilities could be shared. A number of operational difficulties were identified and resolved by Gilbert et al. (196). Based on results obtained with pilot plant procedures, a yield of 668 kg. of 50% molasses could be obtained from 1 tonne of hardwood chips.

Kobayashi (197) briefly reviewed recent work on wood hydrolysis and fermentation by various organisms. As well as using acid hydrolysis, he also mentioned the use of enzymes.

Of course, certain species of wood have been used for centuries as substrates for mushroom production, particularly of the "Shiitake" mushroom (Lentinus shiitake), L. edodes and Volvaria spp. in the Far East. These processes were well described by Block et al. (198) and Gray (199) and constitute

a major industry in Japan, the Philippines and Malaysia. However, up to the present time, little use is made of the small-section trunk and branches, sawmill and trimming waste for the culture of these fungi.

2.3.2.5 MISCELLANEOUS.

In the Far East there are a number of processes, mostly operated at the cottage level, which make use of fungi to produce palatable human food from such wastes as groundnut press-cake, coconut press-cake and defatted soybean grits. These classical processes are known collectively as the "Tempe" processes, and have been fully reviewed by Hesseltine (200) and Stanton (201). In Japan, the residual soybean presscake from oil extraction is used in the manufacture of the lower grades of soya sauce.

Bhattacharyya and Tamhane (202) described a process for the enrichment of groundnut cake with microbial protein. A screening programme of soil samples was carried out using Czapeck's medium with groundnut cake cellulose as the sole carbon source, resulting in the isolation of a strain of Streptomyces albus. By growing this organism on the cake, an appreciable increase in the protein content could be achieved.

Brewers' and distillers' spent grains have been a constant embarrassment to their producers, as their sole means of disposal is as a cattle food. They contain typically about 22% dry matter of which approximately half is carbohydrate material (limit dextrins) and one fifth protein. The high cost of haulage or drying can make them uneconomic as animal feed, so other processes for their utilization have been investigated.

Pan et al. (203) used cultures of Aspergillus niger to saccharify distillers' corn mashes, upon which they then grew food yeast. Pool and Pollock (204) treated spent grains to recover amino acids by maintaining an aqueous slurry with a bacterial or fungal protease, though the economics of this process are perhaps a little dubious in view of the low concentrations of amino acids produced.

The residues from olive-packing plants have also attracted attention as substrates for microbial protein production. Both Tomiyasu and Zenitani (205) and Tsilensis and Hedrick (206) grew food yeast on acid hydrolysates of the residues. In the latter case the solid was pulverized and treated twice with 3.5% sulphuric acid (in the liquid : solid ratio of 5 : 1) at 120°C for 3 hours. After neutralization, yeast or malt extract was added and the medium fermented. Candida utilis proved to be the most suitable organism, yielding 50 - 65% dry yeast based on sugar utilized; 4.5 - 5.5% of the olive residue could be converted to protein by this method.

Updegraff et al. (207) and Rolz (208) gave details of work carried out in Central America on wastes from coffee processing. The principal byproducts are the hulls, composed mainly of cellulose and related polysaccharides, which are all burnt (as fuel for the coffee dryers) and the pulp which is used as a mulch in the coffee fields. This pulp is a problematic substrate for microbial conversions due to its alkaloid and polyphenolic contents. The waters used for depulping and washing the beans are mainly discarded and constitute a grave pollution problem.

Rolz used these waters as substrates for the growth of Aspergillus oryzae, as well as growing yeast on molasses, and attempting enzymatic degradation of bagasses. Detailed costings of the plants and processes were also presented.

In recent years wastes from cattle and pig fattening units have attracted considerable attention as substrates for microbial growth. Griffin et al. (209) investigated cellulase production by Trichoderma viride in the batch fermentations of cattle feedlot waste, finding that the enzyme was elaborated in quantities comparable to commercial preparative fermentations; Weiner and Rhodes (210) grew T. viride, Fusarium oxysporum and F. aquaeductum directly on filtrates of the same waste. Chemical, microbial and enzymatic hydrolysates of swine waste were prepared by Savage et al. (211) and used for growth of Candida utilis. The best medium resulted from waste delignified with peracetic acid then hydrolysed with crude T. viride cellulase solution.

Brown and Reddington (212) proposed a recycling scheme using the waste from a pig fattening unit. This was to be supplemented with sucrose and used to grow Aspergillus niger (M1) which could be added to the pig feed. The maximum specific growth rate for this organism under these conditions was quoted as 0.072 h^{-1}, considerably lower than a value of 0.263 h^{-1} established by Pannell (213) growing this organism on a sucrose-salts medium in a tower fermenter.

Summinoe and Ketsu (214) obtained a cattle feed supplement by growing Aspergillus oryzae on a mixture of 10 parts dried tree leaves and 3 parts human, cow or pig faeces.

2.4 UTILIZATION OF WHOLE OR SUBSTANDARD FRACTIONS OF CROPS.

Because crops containing high carbohydrate levels are almost invariably more productive than "high-protein" crops (i.e. they are more efficient capturers of solar energy, as measured in terms of weight of carbon fixed per unit ground area) it seems likely that at some time in the future they will be grown specifically as substrates for fermentation either for microbial protein or chemical production. The particular choice of crop for a given plant location will of course be dictated by the following points:-

(1) Suitability of the crop to local conditions of climate, soil type etc.

(2) Total yield of crop and fermentable carbohydrate.

(3) Economics and ease of cultivation, harvest, processing etc.

(4) Year-round availability or ease of storage.

(5) Alternative uses or ease of disposal of byproducts (if any).

Considerable knowledge was gained in this field during the first half of the 20th century particularly in the U.S.A. when alternative substrates were being sought for the manufacture of industrial alcohol by fermentation.

Jacobs and Newton (215) produced tables of yields of alcohol from various crops based on their average fermentable sugar content. Whilst cereal grains have the highest fermentable carbohydrate content, in terms of carbohydrate synthesised per unit area crops such as sugar cane, sugar beet, cassava and potatoes are between 4 and 10 times more efficient. However

Jacobs and Newton neglected any contribution that fermentation of a hydrolysate of the cellulose portion of the crops could make to the total yield. Reference must also be made to the publications of Jacobs (216), Jackson (217) and Miller (218) for information regarding the potential use of agricultural products as fermentation substrates.

2.4.1 SUGAR CANE.

Sugar cane is grown as a cash crop in many tropical areas for the production of raw sugar for export, upon which the whole economy of a number of countries is based. At present the world potential for the production of sugar is far greater than the actual production, which is about 4.2×10^8 tonnes (F.A.O., 219), regulated in the interests of stability of price and supply. It is significant to note that in most producing areas protein deficiency is present.

Sugar cane contains 12 - 18% sucrose, and yields are usually about 15 tonnes (sucrose) per hectare. Gray and Paugh (220) grew a Cladosporium sp. (designated I - 75) on raw cane juice diluted to 2% sugar in shake flasks. When ammonium nitrate was added to the medium, the mycelial yield was 10.71 $g.l^{-1}$ and the protein content 13.7%. With the addition of corn steep liquor the figures were 15.36 $g.l^{-1}$ and 17.6% protein.

Even though these protein contents seem low by comparison with other members of the Fungi Imperfecti and Yeasts, Gray and Paugh concluded that up to 1455 kg. of protein (in approx. 7.5 tonnes of fungal mycelium) could be produced from a typical 100

tonne.hectare^{-1} cane crop. If the protein content of the cane itself were extracted directly, the total yield could be 1910 kg. of protein/hectare - not taking into account the possibility of fermentation of the bagasse (see section 2.3.2.3). Whilst the mycelial yield seems reasonable (approx. 50% based on sugar utilized) the protein content quoted is very low and in this case the production of Food Yeast would be a more suitable choice of process - as carried out commercially by Thaysen and Morris (25).

According to Spicer (35) cane juice has been used successfully as an alternative to molasses by Rank, Hovis, McDougall Ltd. for the production of a Fusarium sp. with a protein content of 55 - 60%.

2.4.2 SUGAR BEET.

The sugar beet crop is essentially one of temperate regions, particularly of Western Europe and, as in the case of sugar cane, cultivation is strictly controlled so as to achieve market stability, even though there is great potential for increase. Crops of up to 50 tonnes/hectare with a sucrose content of 16 - 22% are usual.

Gray and Abou El-Seoud (221) attempted to use minced whole sugar beets and beet shreds as a substrate for the growth of a Cladosporium sp. (I-75). Highest values of both total crude protein synthesized (per 100 g. of beet roots) and mycelial protein content, were obtained in a medium containing 85 g.l^{-1} of minced beet, 2 ml.l^{-1} of corn steep liquor and 2 g.l^{-1} of ammonium chloride. It was found that higher yields (measured

as protein synthesized per unit weight of beet roots) were obtained in a medium of lower beet content. With a sugar beet concentration of 140 $g.l^{-1}$ the total mycelial yield was 20.8 $g.l^{-1}$ with a crude protein content of 20.32%.

It seems certain that with the use of a higher yielding organism with a higher protein content, an economic process for the production of microbial protein could be devised. This process could be based on a slurry system as used by Gray and Abou El-Seoud, or use a medium resulting from more conventional techniques such as are used at present in sucrose recovery factories. A hot water extraction could take place to yield a medium containing sucrose and other beet solubles which could be fermented. Spent beet pulp is already in great demand by farmers as an animal food and would be even more so if its protein content were higher.

Willer and Sowada (222) were granted an East German Patent (No. 67,024) for the production of animal feeds with a high protein content by the fermentation of sugar beet slurries with yeast.

2.4.3 SWEET POTATO. (Ipomoea batatas).

Throughout the world, sweet potatoes are used primarily for human food. Cull and other unmarketable subgrade potatoes are fed to livestock, but such use is essentially a salvage measure. Well over 90% of the sweet potatoes used for food are purchased as the fresh raw product, the majority of the remainder being canned, though large quantities are also dehydrated to form sweet

potato flour and meal.

Freshly dug sweet potatoes contain 1.5 - 3.0% mono- and disaccharides, mostly sucrose, and have a starch content of 24 - 26% (Dawson, Greathouse and Gordon, 223). After curing and storage the sugar level increases, at the expense of the starch content, to between 5 and 6%. Certain varieties, for example the "Puerto Rico", have particularly high levels of the enzyme β- amylase, so high in fact that if a mash is made and held at 60 - 70°C for 1 - 2 hours, almost all the starch can be converted to reducing sugars.

Ethyl alcohol has been produced from dehydrated sweet potatoes using exactly the same techniques and equipment as used for grain substrates. Jump, Zarow and Stark (224) gave details of the process which involved cooking and mashing the potatoes followed by saccharification with malt, yields were approximately 27 proof litres of alcohol per bushel (approx. 25 kg.). No use was made of the potatoes' capabilities of auto-saccharification.

Both methods of saccharification have been used widely in the U.S.A. for the production of Glucose syrups (e.g. Delamarre, 225. Gore, 226), but this work has not been applied to the production of microbial protein.

Gray and Abou El-Seoud (227) used minced whole sweet potatoes as a substrate for the growth of a Cladosporium sp. (1 - 75). The highest yield was 17.54 $g.l^{-1}$ dry weight of which the protein content was 5.87 g. From 100 g. of sweet potato it was possible to produce 81.2 g. of dried product (mycelium and unused potato tissue) containing 31.6 g. protein. Since the initial protein content of the potato was only 6.9 g.%, the

total protein content was increased by a factor of 4.5. This would be of great value in the many sub-tropical areas which the sweet potato constitutes a significant part of the diet.

Otsuka et al. (228) used the sweet potato as a substrate for the production of L - Glutamic acid by the growth of a strain of Micrococcus sp.. Takakuwa et al. (229) and Takakuwa and Furukawa (123) cultured Saccharomyces cerevisiae, Candida utilis, Lentinus edodes and L. shiitake on waste juice resulting from the separation of sweet potato starch. This proved to be a very good medium, containing a growth promotant which could be extracted and assayed.

2.4.4 JERUSALEM ARTICHOKE (Helianthus tuberosus).

This plant is cultivated on a large scale in some parts of Europe and North America, where it has been widely used as a substrate for industrial alcohol production. The principal storage product is a polysaccharide, inulin, which is readily hydrolysed to fructose. A 6-year mean analysis of 20 varieties investigated by Boswell et al. (230) showed 13.33% fructose and 16.38% total sugars. The mean crop yield at that time was 26.75 tonnes.hectare^{-1}, the maximum yield noted being 40.5 tonnes.hectare^{-1}.

An inulin/fructose syrup can be extracted with hot water and fermented to alcohol directly by yeasts such as Saccharomyces cerevisiae, S. anamensis and especially Schizosaccharomyces pombe without the need for a preliminary hydrolysis or the addition of nutrients. The yeasts used have shown adaptation to the unhydrolysed syrup

according to Underkofler et al. (231).

The production of acetone and butanol from Jerusalem Artichokes was studied by Wendland et al. (232) using a mild acid hydrolysate of the tubers. It was found necessary to add corn or soybean meal to obtain maximum yields with Clostridium acetobutylicum. A similar hydrolysate was used by Andersen and Greaves (233) for the production of lactic acid using an aerobic Bacillus dextrolacticus fermentation at 47 - 50°C.

2.4.5 CASSAVA. (also known as manioc, mandioca, aipum, yuca, cassada, tapioca etc.).

Cassava is the starchy root of two plants, Manihot esculenta (the bitter cassava) and Manihot dulcis (the sweet cassava), both of which are important sources of food starch and major root crops of the tropics although they may also be grown in temperate regions such as the Southern U.S.A.

The cassava plants are semi-shrubby perennials with large fleshy roots up to 1 m. long and 15 - 20 cm. diameter containing a milky juice. The sap of the bitter cassava contains hydrogen cyanide, but this is dissipated on heating rendering the material edible. Sweet cassava does not contain this poison and can be used safely fresh. The tubers contain up to 33% carbohydrates (based on fresh weight) but are very low in protein content (approx. 1%). The crop constitutes the major portion of the diet in many developing countries e.g. Brazil, West Africa and Indonesia. World production is of the order of 100 million tonnes per year.

Cassava has many important advantages over other tropical food plants. Chief of these is the high yield, which can be up to 75 tonnes.hectare^{-1}.year^{-1} of fresh roots. This productivity is maintained even in poor soils and because of the plants semi-perennial nature, it can be harvested at any time during the year. Cassava is also resistant to drought, insect damage (in particular locusts) and diseases. Other virtues are the ease of planting and harvesting, and the use of both the roots and leaves as food.

Unfortunately mechanization in harvesting and processing of cassava is still in the earliest stages of development. The only sizeable plants are in Brazil, Thailand and the Dominican Republic - these produce cassava starch mainly for export. (Whistler and Paschall, 234). The properties and present uses of cassava were reviewed by Ayres (235) and (including fermentation) Grace (236). Use of starch-containing agricultural products, including cassava, as substrates for microbial protein synthesis was also reviewed by Nordhelm (237) and Brook et al. (238).

Much of the work carried out on the fermentation of cassava has been carried out in the solid state. Stanton (201) and Stanton and Wallbridge (239) used fungi such as Rhizopus sp., Mucor sp., Actinomucor sp. or Monilia sp. growing on cassava in the form of a paste, with an assimilable non-proteinaceous nitrogen source such as urea or ammonium salts.

The "Amylo Process", described by Grove (240) and Owen (241) was applied to a number of starch substrates as a means of producing alcohol. Rhizopus delamar and Saccharomyces cerevisiae were inoculated at a temperature of 38°C, so that saccharification and fermentation took place simultaneously in the same vessel.

With cassava and potatoes it was necessary to add a small quantity of malt to keep the mash in the desired state.

Brudzynski and Munyanganizi (242) produced alcohol by a similar process involving saccharification and simultaneous fermentation of a cassava mash using commercial enzymes and yeast. Cassava was used as a substrate for acetone-butanol production by Banzon (243), though best results were obtained with mixture containing at least 20% maize.

Gray and Abou El-Seoud (244) grew Cladosporium cladosporoides on a cassava substrate, obtaining a 5.7 fold increase in protein content in 4 days. Strasser et al. (245) described the operation and economics of starting and running a plant producing 10 tonnes. day^{-1} of a dried yeast containing meal by growing a yeast on cassava. Adegbolu et al. (246) reported on the growth and protein content of Rhizopus oligosporus, an organism used widely in Eastern (native) fermented food processes, on cassava starch media.

2.4.6 CEREAL GRAINS.

Cereal grains such as wheat, maize, barley and rice have starch and protein contents in the ranges 65 - 75% and 8 - 14% respectively. Whilst in many parts of the world there are shortages of cereals, in the U.S.A., Canada and Australia, particularly, the area planted has at times been restricted to maintain stability in price and supply.

Apart from the use throughout the world in the brewing of alcoholic beverages, during the early part of the 20th century maize and (in times of national emergency or unusual price

situation) wheat were the main substrates for ethanol production by processes which have been fully described by numerous authors (e.g. Jacobs, 247, Corman and Tsuchiya, 248, Underkofler and Hickey, 48, Prescott and Dunn, 50). The theoretical yield of alcohol on starch is 56.8% (by weight) i.e. 0.71 $l.kg^{-1}$ Data derived from these sources relating to many years production at numerous plants indicates that the theoretical yield of alcohol on maize is 511 $l.tonne^{-1}$, 460 $l.tonne^{-1}$ being the usual yield at the industrial scale. For wheat the values are 490 and 440 $l.tonne^{-1}$ respectively, whilst as a comparison, they are 128 and 115 $l.tonne^{-1}$ in the case of potatoes.

The decline in grain alcohol production was the direct result of competition from alcohol derived by petroleum and natural gas based processes. However with the recent price rises in these resources, Miller (218, 249) among others, has argued that it is now time for a complete reappraisal of the economics of production and use of fermentation alcohol.

Few processes have been developed for upgrading the protein content of cereals by fermentation. Gray and Kane (250) grew Trichoderma sp. and Dactylium dendroides in liquid culture on a rice medium. The former organism gave the greater increase in protein content by a factor of 2.85, but this was not sufficient to justify further development of the process. Smith and Reade (251) described a simple farm process growing Aspergillus oryzae on barley so as to increase the protein content sufficiently for use directly as a pig feed.

2.4.7 POTATOES AND THEIR WASTES.

Historically, the cultivation of potatoes appears to have begun around 200 A.D. in the Andes Mountains of Peru, where they were used as food in both their fresh and dehydrated state. Spanish and English explorers recognised their excellent food value and carried them as provisions on their ships, introducing them to Europe certainly before 1573 (Hatfield, 252). Until the late 19th century their sole use was in human and animal diets.

The composition of whole, fresh potatoes typically falls within the following ranges:-

Dry Matter	18 - 28%
Total Carbohydrates	15 - 23%
Starch	12 - 20%
Protein	1.3 - 2.7%
Fat	Trace - 0.5%
Crude Fibre	0.33 - 1.0%
Ash	0.64 - 1.3%

Organic acids are also present including oxylic, citric, succinic, malic and tartaric acids as well as enzymes, pectin and vitamins.

Potatoes, because of their low dry matter and protein contents have only limited use as animal food, but in certain parts of the world have been and are important industrial raw materials. Until 1945, they were the most important raw material for the extraction of starch. However, competition particularly from grain-derived supplies has led to a decline in the production and use of potato starch except where special qualities are required (Adler, 253) or where such processing is

used as a regulator of the fresh potato market. The waste pulp after pressing is in great demand as a cheap cattle food.

The alcohol industry is also used as a means of disposal of surplus and substandard potato crops, particularly in Central Europe where the distilleries are small and usually part of a large farm (Adler, 253). Larger plants have been built and operated in the U.S.A. using the processes described by Jacobs and Newton (215) and Beresford and Christensen (254). In the latter, saccharification of cooked potatoes was carried out using mold bran (made by growing Aspergillus oryzae on wheat bran, containing amylolytic enzymes) rather than malt. The yields were about 115 $l.tonne^{-1}$ with 22% Dry Matter potatoes.

Recently, however, the use of enzymes has replaced these products in saccharification of potato and cereal mashes for industrial alcohol manufacture, a topic which was reviewed by Aschengreen (255). Papers by Kreipe (256) and Yarovenko and Nakhmanovich (257) have described experimental work and kinetics of simultaneous saccharification and fermentation in batch and continuous alcohol producing systems respectively. Yarovenko et al. (258) also described how pasteurization of the mash could be avoided, by the addition of lactomycin, without significantly affecting alcohol yield.

Potatoes have received little attention as substrates for microbial protein production. Janicki et al. (259) grew yeasts on malt and mould saccharified mashes enriched with nitrogen and phosphorus salts. Mashes of up to 20% Dry Matter could support growth, but the optimum was 9% using Torula cremoris and

T. utilis in both batch and continuous processes. Using temperatures in the range 30 - 32°C, pH 3.0 and aeration rates of 1.0 - 1.6 V.V.M. in stirred tank reactors, the yields were 450 - 530 $g.kg^{-1}$ of sugar, with a protein content of 50%.

According to Janicki et al. (259) potatoes and potato wastes are important fermentation substrates in the U.S.S.R. and Poland. They quote papers by Zelanka (260) on the production of antibiotics, Melcher (261) on the production of acetone, butanol and ethanol and Dietrich (262) on the synthesis of Vit. B12, all from potato juice. In the production of biomass, mixed yeast and mould cultures are often used to give greater yields, such combinations as Trichothecium roseum and Rhodotorula aurentiaca and T. roseum and Aspergillus oryzae.

A number of other workers have used potato mashes and waste juices as substrates for the production of yeasts and filamentous fungi. Tong et al. (263, 264) grew Rhodotorula rubra and Torula utilis on acid hydrolysed potato tissue in a stirred tank fermenter, achieving protein contents of up to 57%. Simard et al. (265) treated potato chip waste water with a strain of Rhodotorula glutinis, which reduced the C.O.D. of the liquor by 85% and provided biomass with a protein content of 53%. Reiser (121) gave design figures and detailed costings for batch and continuous plants producing 5 $tonne.day^{-1}$ of dry Candida utilis from potato starch factory wastes without the addition of nutrients. Culture conditions in the continuous stirred tank fermenter were a Dilution Rate of 0.25 $h.^{-1}$, pH 5.0 and temperature 30 - 32°C. Weaver et al. (266) treated potato starch factory wastes successfully with various "activated sludge"

and soil cultures. However their experiments with a mixed culture of Torulopsis utilis and Aspergillus niger in a Waldhof fermenter gave erratic results. Their inference was that, had the process control been better, use of this mixed culture would have given the greatest C.O.D. reduction (about 85%).

Mixed culture techniques are also employed in the "Symba Process", described by Tviet (267), Jarl and Tviet (268) and Jarl (269, 270), which is a process designed for utilizing waste starch (from any source) for the production of yeast, primarily a strain of Candida utilis. This organism is propagated in continuous stirred tank fermenters in mixed culture with another yeast an Endomycopsis fibuliger strain with a high amylase producing capacity. With potato waste liquor containing about 25 $g.l^{-1}$ dry matter and a fermenter being operated at a Dilution Rate of 0.1 $h.^{-1}$, the output of yeast is about 1.05 $g.l^{-1}.h^{-1}$, a yield of about 43% of dry matter supplied. The protein content of the yeast is about 43% and the B.O.D. removal from the liquor about 85% under these conditions.

Extraction of protein directly from potato waste liquors has received a great deal of attention particularly when used in conjunction with processes for extracting other organic molecules. Most processes depend on heating the acidified liquor to coagulate and precipitate the protein - high temperatures (preferably 100+^{o}C) and low pH's (as low as pH 4) give the most efficient extractions and an easily filterable product. Examples of processes are the "Avebe Process" (115) and those used by Hausler and Malcher (271) prior to methane fermentation of the liquor and Strolle et al. (116) before an ion exchange process

for the recovery of Potassium and free amino and organic acids.

Potatoes have been used as a substrate for the production of Lactic Acid by the method described by Cordon et al. (272). Ground potatoes are saccharified with fungal amylase, an excess of calcium carbonate added and then fermented with a strain of Lactobacillus pentosus for about 5 days at 30°C. Yields are about 80 - 90% based on sugar consumed. Acid and enzyme hydrolysates of potatoes were also used by Szejtli (273) as a cheap substrate for the production of Vit. B12 by Proprionobacterium sp. and Joseph and Rao (274) to produce glutamic acid by fermentation with Micrococcus glutamicus. In the latter case it was necessary to first reduce the biotin content of the mash by absorption on activated charcoal to obtain best yields.

The Infra-Red Dry Caustic Peeling Process now used widely by the potato industry produces a peel waste of high solids content (about 15 - 25% solids) which is easily handled and collected but has a high residual alkalinity (about 1 - 2% expressed as Sodium Hydroxide). When neutralized with, preferably, hydrochloric acid the waste has a limited use as animal feed. Gee et al. (275) used a semicontinuous lactic acid fermentation as a low-cost method of neutralizing the waste to enable it to be used as a cattle feed.

However Bloch et al. (276) used the peel as a substrate for the production of alcohol. Firstly Aspergillus foetidus was cultured on hydrochloric acid neutralized waste with added nutrients. This was the same strain, N.R.R.L. 337, which gave the highest yield of Amylase of the 350 fungal strains tested by

Le Mense et al. (277) and which was, at that time, classified as an Aspergillus niger strain. The filtrate from this culture was used to saccharify more neutralized peel waste which was then fermented by yeast to ethanol. The yield varied considerably with the pretreatment process applied to the peel, but was as high as 45 $l.tonne^{-1}$ of 15.25% solid waste. The byproducts of the process when combined, were also an excellent cattle feed.

2.4.8 MISCELLANEOUS CROPS.

Recent research, described by Wolverton and McDonald (278), has shown that a tropical weed, the water hyacinth (Eichhornia crasspipes) could have tremendous value in pollution control and as a source of energy, food and animal feed. This water plant, when grown in warm sewage nutrients, can yield 17.8 tonnes of wet biomass. $hectare^{-1}$. day^{-1} (approx. 1.8 tonnes of dry plant material) and annual production rates of 212 tonnes of dried plant material per hectare are possible - unrivalled by terrestrial plants in terms of conversion of solar energy to organic molecules. The protein content of this plant, being relatively high (17 - 22% of the dry weight), can be easily and efficiently extracted directly.

Mannan and Ahmad (33) grew Saccharomyces cerevisiae and Torula utilis on acid hydrolysates of water hyacinths but the yields were poor - hardly surprising though, considering the maximum hydrolysis temperature used was 30°C. The crop is certainly worthy of further attention.

Plants which are able to thrive in semi-arid conditions are

likely to be extremely valuable in the future. The Carob tree (Ceratonia siliqua), a native of Mediterranean regions which bears pods containing about 55% sugars, has been considered a potential source of microbial protein. Imrie and Phillips (279), Imrie and Vlitos (280) and Sekeri-Pataryas et al. (281) have described work carried out on the growth of a mould Aspergillus niger M1 on hot water extracts of these pods, obtaining yields of about 43% (based on sugar supplied) with true protein contents of about 22%.

Paredes - Lopez et al. (282) grew Candida utilis on juice from the Prickly Pear or Nopal Fruit, a shrub found in arid zones. In Mexico it is found in more than 50% of the territory, but human consumption of the fruit is negligible. When operating a stirred tank fermenter at 30°C with a juice of 10 $g.l^{-1}$ sugar content, at a Dilution Rate of 0.55 h^{-1} the yeast productivity was 2.38 $g.l^{-1}.h^{-1}$ with a yield coefficient of 0.47 and Protein Content of 43%.

Citrus Fruit crop surpluses have been used widely for the production of ethanol, but, in general fruit crops have tended to be neglected as fermentation substrates. Dako et al. (283) published the results of a survey of the carbohydrate composition of 25 fruits grown in Ghana. Samples were taken at different times of the year and average total sugar values usually fell in the range of 8 - 15%, sufficient for most fermentation processes.

Benon (284) patented a process for the production of Banana Wine and Vinegar. Very ripe bananas, no longer containing starch, were mashed at 65 - 70°C for 1 hour, cooled and treated with pectinases at 40°C for 24 hours. The decanted

juice was fermented with Saccharomyces cerevisiae to produce a wine which could be used directly, distilled, or acetified to vinegar. Patel (285) found that Mango fruits (which contain 10 - 18% total sugars) were particularly susceptable to rotting as a result of growth of Aspergillus niger during storage and transport.

Arrazola (286) grew Candida utilis on hydrolysates of several plants native to Spain, particularly tubers of Asphodelus albus and A. microcarpus. On acid hydrolysis these gave juices of up to 125 $g.l^{-1}$ reducing sugars, almost exclusively fructose, which only required supplementation with ammonium salts. C. utilis and C. liquefaciens were also grown successfully on a pectin-rich must resulting from a 110°C water extraction of the pulp remaining from the extraction of essences from oranges, lemons and grapefruit.

2.5 CONCLUSIONS.

It is obvious that carbohydrate containing materials which could be used as fermentation substrates are available, or could be easily cultivated throughout the world. Similarly reliable fermentation processes have been developed which can provide food and most of the chemical "necessities" of life, or their precursors. These processes are ideal for dealing with raw plant materials and are also, in general, well suited to small-scale operations.

Processes based on materials containing mono- and disaccharides are the most versatile. With optimisation and

the use of simple, cheap fermentation plant, many of these could, almost certainly, be operated economically in the underdeveloped parts of the world.

Polymeric substrates, cellulosic materials particularly, present a much greater problem. Conventially, this has been overcome by acidic or enzymic hydrolysis to yield a fermentable solution, but this necessity often renders processes uneconomic. Use must be made of organisms which are able to ferment these polymers directly. In the case of cellulose this technology is in its infancy, but microorganisms which can grow on starch directly are well known, particularly for "biomass" production. Fermentations using these polymers, the commonest plant structural and storage materials respectively, will be of tremendous value in the total utilization of agricultural products.

SECTION 3.

THE EXPERIMENTAL WORK UNDERTAKEN.

3.1 BACKGROUND INFORMATION.

Whilst a research student at the University of Aston the author has been a member of the Tower Fermentation Research Group, a joint group of Biological Scientists working under the direction of Dr. R.N. Greenshields and Chemical Engineers under Dr. E.L. Smith. Such an inter-disciplinary approach has been found to be of great value in the Theoretical and Practical research carried out by Group Members in the development of Tower Fermentation Systems.

Prior to the author joining the group, research topics involving this fermenter had included:-

(1) Metabolite production by moulds in batch culture.

(2) Growth of moulds as "biomass" in batch culture.

(3) The morphology of mould colonies in batch culture.

(4) Fluidization/Sedimentation of Microbial Aggregates.

(5) Oxygen Transfer.

(6) Continuous Vinegar Fermentation.

(7) Continuous Alcohol Fermentation.

Thus considerable expertise had been acquired in the design, construction and operational characteristics of tower fermentation systems, particularly when applied to mould culture.

3.2 CONSIDERATIONS IN THE PROJECT PLANNING.

3.2.1 CHOICE OF SUBSTRATE.

An extensive literature survey (particularly Sections 2.2.5, 2.4.5, 2.4.6 and 2.4.7) indicated that a process for the

production of "biomass" directly from starch-containing crops and wastes could have widespread application throughout the world and probably require only minor modifications to suit individual circumstances.

The source of starch chosen for this study was the Potato, for the following reasons:-

(1) The crop is available throughout the year.

(2) Several types of industrial wastes are also available.

(3) Separated potato starch could be obtained for defined media work.

(4) With carbohydrate and total solids contents of about 20 and 25% respectively, potatoes are typical of many crops which are suitable for microbial upgrading.

3.2.2 CHOICE OF ORGANISM.

For some years, previous members of the Tower Fermentation Research Group had successfully cultured a strain of Aspergillus niger designated M1, which had been isolated by Tate and Lyle Ltd. from rotting carob beans. This Company had also carried out extensive toxicity and feeding trials with this strain, which had proved acceptable as a source of microbial protein.

This organism grows well at low pH values (pH 2 - 3) reducing the problems of contamination and because of its filamentous form, is readily harvested from the spent medium. It seemed, therefore, an ideal choice for this study.

However, prior to this work A. niger M1 had not been cultured on starch-containing media, but the ability of other strains to do so is well documented. Hasija and Wolf (287) grew

an unspecified strain of A. niger on 13 different sugar-containing media, maltose and starch giving by far the highest mycelial yields. Drews et al. (288) noted that the amylolytic activity of A. niger in initially unfavourable starchy industrial waste media would approach that of optimal media if the opportunity for adaption was provided. This proved to be the case in preliminary experiments carried out with A. niger M1.

3.2.3 CHOICE OF FERMENTATION SYSTEM.

Preliminary experiments with shake flask culture indicated that the viscosity of gelatinised starch media was a major problem, effectively limiting the initial starch concentration to a maximum of 25 $g.l^{-1}$. This, at best, would yield only 12 $g.l^{-1}$ mycelium, well below the highest concentrations achieved by Morris (289) of 22 $g.l^{-1}$ growing A. niger M1 on molasses media in a 10 l. batch tower fermenter. With a continuous system this problem of viscosity would not arise as the substrate in the fermenter would be mainly in the form of starch hydrolysis products.

At that time a colleague, Mr. S.D. Pannell, was working on the development of a 10 l. continuous tower fermenter, a new system which appeared to be ideally suited to this project and which was therefore adopted. It was also felt that the additional data generated by such a practical application would lead to a better understanding of the operating characteristics of the new fermenter.

3.3. FERMENTATION EQUIPMENT USED FOR THE PROJECT.

3.3.1 THE FERMENTER. (Fig. 3.1 - Plate 3.1).

The fermenter was built up of standard Q.V.F. Glass Pipeline Components (J.A. Jobling Ltd., Stone, Staffs.) modified by the addition of SQ-fitting, glass, screw-capped parts where necessary by the University glassblowers. This method of construction was described in detail by Cocker (290).

The main portion of the fermenter consisted of three, 400 mm. long, 100 mm. diameter pipe sections which had a total of 8 ports fitted. The upper and lower ends were closed by two 100/25 mm. pipe reducers and 25/10 mm. hose connectors. One of these was modified by the addition of a length of 12 mm. bore glass tubing, which allowed the passage of air and fermenter contents to a 20 l. aspirator bottle, the product receiver.

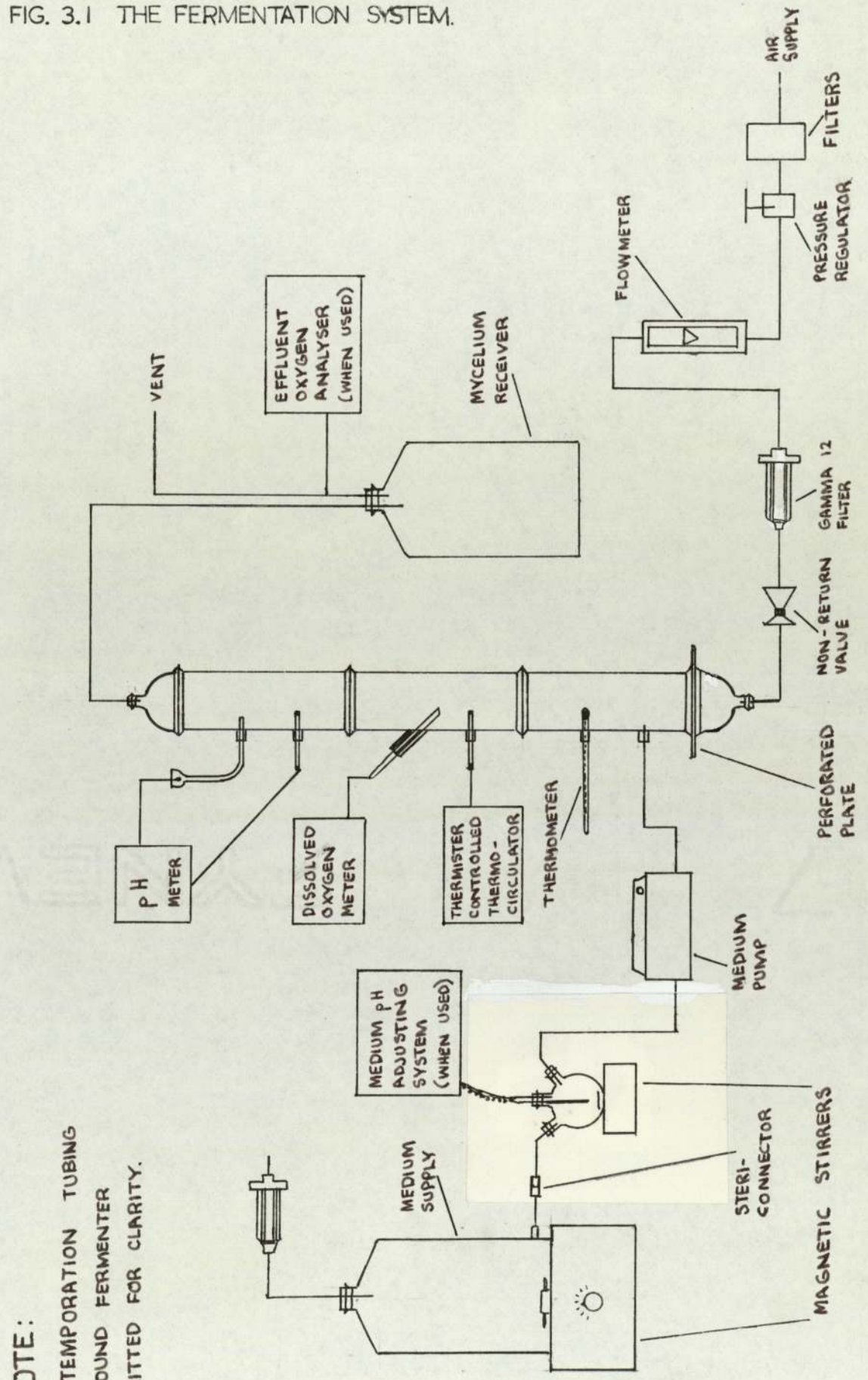
The air distributor, at the base of the 100 mm. diameter column, was a P.T.F.E. plate with 1 mm. diameter holes drilled in a regular pattern, 10 mm. apart. Gaskets between all the glass sections were also made of P.T.F.E. and all tubing in contact with medium or fermenter contents was of silicone rubber.

3.3.2 THE TEMPERATURE CONTROL SYSTEM.

Fermenter temperature was sensed by the thermistor probe of a thermocirculator (Churchill Instruments) which controlled the temperature of water circulated through "Portex" (Hythe, Sussex) 25 mm. diameter vinyl tubing wrapping the fermenter. A thermometer ($-10^{\circ}C$ - $110^{\circ}C$) was also provided to enable the temperature of the fermenter contents to be checked.

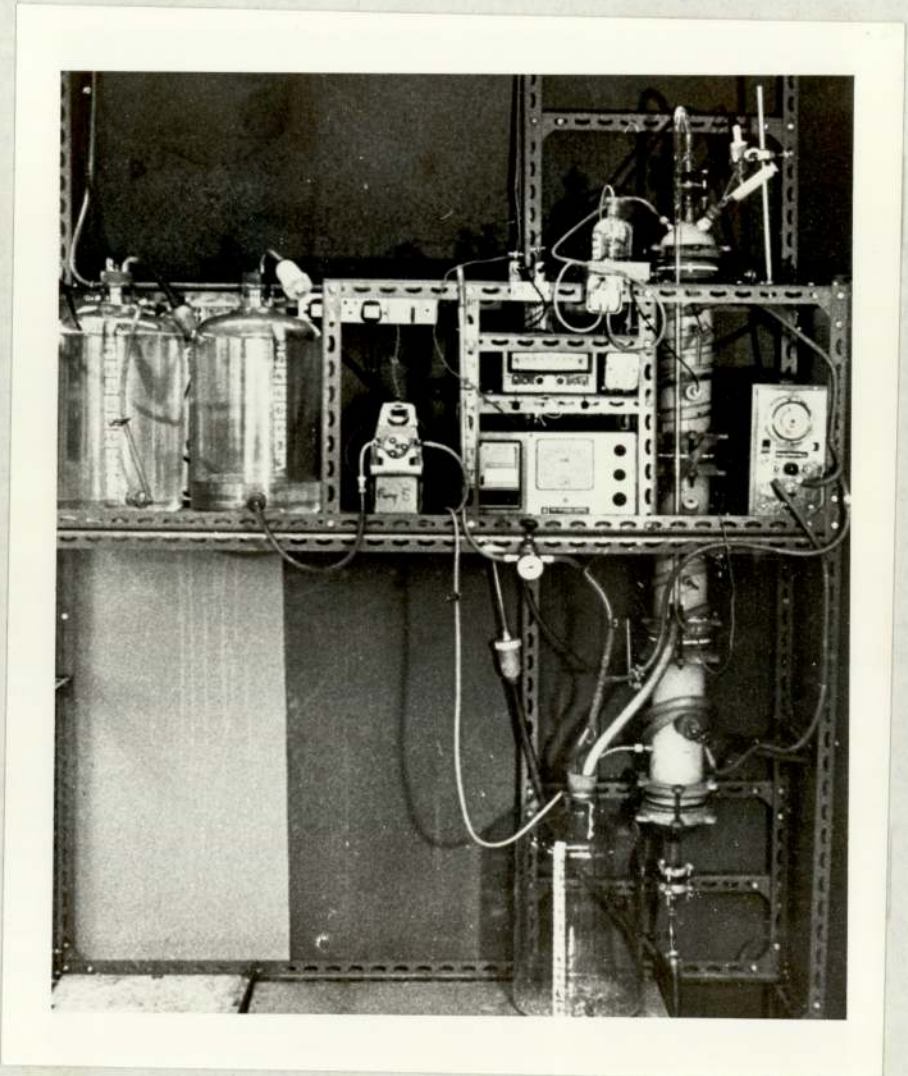
This equipment could maintain the fermenter temperature within $0.5^{\circ}C$ of the required value over the range investigated,

FIG. 3.1 THE FERMENTATION SYSTEM.



NOTE:
ATTEMPORATION TUBING AROUND FERMENTER OMITTED FOR CLARITY.

Plate 3.1 The Fermentation System.



though in theory was capable of greater accuracy.

3.3.3 THE AIR SUPPLY.

Double-filtered, oil-free compressed air was supplied via a pressure (and therefore, flow) controller and a "Rotameter" flow measuring device to a Whatman "Gamma 12" (Gallenkamp Ltd.) filter unit fitted with a Grade 03 filter element. The sterile air flow was piped to the fermenter via a non-return valve, which was intended to prevent filter wetting in the event of an air supply failure.

Calibration of the air flow measuring tube (Rotameter Ltd., Croyden) was achieved by a water displacement technique using the exit air from the fermenter in its usual operating condition. The air flow rate used throughout this study was 10.2 $l.min^{-1}$, obtained at a gauge pressure of 0.2 $kg.cm^{-2}$. This aeration rate corresponds to a superficial gas velocity of 2 $cm.sec^{-1}$ in the 10 cm. diameter sections.

3.3.4 THE MEDIUM SUPPLY.

20 l. "Pyrex" glass aspirators equipped with "Gamma 12" inlet air filters and magnetic stirrer followers were used as medium supply vessels, being constantly mixed by means of a magnetic stirrer (L.H. Engineering, Stoke Poges, Bucks.). Medium was pumped through 5 mm. bore silicone rubber tubing to the fermenter by a peristaltic pump. For the early work, a Watson Marlow MHRE pump (Watson Marlow, Marlow, Bucks.) was used but this was later replaced with a Baron Yemm BYO 800 (Baron Yemm

Developments, Watford, Herts.) which had a more constant pumping rate. "Steri-connectors" (L.H. Engineering) were used to reduce the chances of contamination when changing aspirators.

To avoid blockages by suspended solids 5 mm. bore tubing was necessary, resulting in minimum medium flow rates considerably greater than required. A simple electric timer device was used so that power was only supplied to the pump for 15 sec. in each minute, thus reducing the flow rate to a more suitable range.

For some later work, a small magnetically stirred chamber, equipped with a pH probe and an acid/alkalie injection system was inserted in the medium input line between the pump and the fermenter. This enabled adjustment and control of the medium input pH.

3.3.5 pH EQUIPMENT.

pH was monitored by an Analytical Measurements meter-recorder, using an E.I.L. 1050 series toughened, gel-type probe with a separate reference probe (connected by a KCl bridge to the fermenter contents). This very reliable probe could be steam-sterilized and functioned perfectly in a horizontal position in a fermenter port.

In later work, where pH control was required, an E.I.L. 91B meter-controller was used (with the same probes) acting via 2 "Delta" pumps (Watson Marlow) with 2 mm. bore silicone rubber tubing, pumping hydrochloric acid or sodium hydroxide as necessary.

3.3.6 OXYGEN ANALYSIS EQUIPMENT.

Dissolved oxygen concentration in the fermenter was monitored with a New Brunswick (New Brunswick, New Jersey, U.S.A.) meter-recorder and probe. Though this probe was steam-sterilizable, it had to be mounted in a vertical position, necessitating an angled port in the fermenter wall.

The concentration of oxygen in the input and exit air lines could be monitored by a Servomex (OA101) oxygen analyser (Servomex Ltd., Crowborough, Sussex) but in practice the difference was so small that measurement was of little value.

3.4 ANALYTICAL METHODS AND EQUIPMENT.

3.4.1 SAMPLING TECHNIQUES.

FERMENTER. To obtain representative samples of the fermenter contents, a completely unobstructed sample pipe was essential. The first 0.05 l. was disgarded and then 0.120 l. run into a measuring cylinder. The volume was then adjusted to 0.100 l., the usual sample size.

PRODUCT RECEIVER. This vessel was removed from the fermenter and shaken vigorously to ensure the contents were well mixed before sampling. The volume taken was at least 0.5 l., usually 1.0 l. and when the mycelial concentration was very low, 2.0 or 4.0 l.

FREQUENCY OF SAMPLING. The fermenter contents could not be sampled as frequently as was desired. Though each sample only removed about 2% of the fermenter contents, at the lowest growth

rates investigated this could represent 6 hours productivity. Therefore, when operating at the lowest growth rates, sampling was restricted to once per day. For most of the experimental work, samples were taken twice per day but at the highest growth rates they were taken at 4 hour intervals. Samples of the contents of the product receiver were usually taken at the same time.

3.4.2 DRY WEIGHT DETERMINATION.

FERMENTER SAMPLES. 0.100 l. samples of the fermenter contents were filtered on Greens Hyduro 904 filter paper on a 7 cm. diameter Buchner funnel. The mycelial pad was dried at 105°C to constant weight (at least 24 h.) and a 0.03 l. sample of the filtrate stored in a McCartney bottle at 4°C until analysed.

PRODUCT RECEIVER SAMPLES. These samples were filtered on the same grade of paper on a 21 cm. diameter Buchner funnel and the mycelial pad dried under the same conditions.

3.4.3 REDUCING SUGAR DETERMINATIONS.

Initially these determinations were carried out using the Ferricyanide Method described by Somogyi (291) and could be carried out routinely to an accuracy of ± 2%. However the method was not particularly suitable for large numbers of samples or those containing gelatinized starch.

After comparing numerous other methods, the 3,5 - Dinitro-salicylic acid method described by Sumner (292), Klemme and Poe (293), Edson and Poe (294) and Lindsay (295) was chosen. Using an E.E.L. "Spectra" Colourimeter (E.E.L. Ltd., Halstead, Essex)

routine analysis of samples with 0 - 2 $g.l^{-1}$ reducing sugar content could be carried out to an accuracy of ± 1%.

Details of these methods are given in Appendix I.

3.4.4 TOTAL SUGAR DETERMINATION.

Samples were acid hydrolysed in a boiling water bath for 10 minutes, neutralized and the reducing sugars formed estimated by the 3, 5 - Dinitrosalicylic acid Method. Further details are to be found in Appendix I.

3.4.5 AMMONIA NITROGEN DETERMINATION.

The standard micro-kjeldahl Method described by Markham (296) was used. Ammonia, liberated from the sample by the addition of sodium hydroxide solution (400 $g.l^{-1}$), was steam-distilled off in a Markham Apparatus and collected in saturated (40 $g.l^{-1}$) Boric acid solution. This was titrated with 0.01N Hydrochloric acid using a Methylene Blue/Methyl Red indicator. Blank and standard checks were made regularly, the method usually being accurate to within ± 1%.

3.4.6 TOTAL NITROGEN DETERMINATION.

Samples were digested with concentrated sulphuric acid and a catalyst containing selenium, copper sulphate and potassium sulphate in the ratio 1 : 5 : 32 parts by weight. The ammonia formed was estimated as in section 3.4.5.

3.4.7 RATE OF REDUCING SUGAR FORMATION FROM STARCH.

The combined effects of the enzymes α- and β-amylase and maltase in the culture filtrate were measured by the technique described in more detail in Appendix II. Reducing sugars produced by the filtrate from a starch solution buffered at pH 4.6 and maintained at 30°C were assayed by the Dinitrosalicylic acid Method.

Whilst yielding a value for the rate of production of reducing sugars under standard conditions, it was found that the result obtained from the calculation of Mass Balances (described in Section 3.6) was a better indication of the experimental situation, so the test was only used infrequently.

3.5. FERMENTATION TECHNIQUES.

3.5.1 SELECTION AND MAINTENANCE OF CULTURES.

The parent culture of Aspergillus niger M1 was obtained in the form of a silica gel master. Spores from this were subcultured at 30°C on Potato Agar (twice) then Starch Agar (twice) in Petri dishes, in each case the inoculum being spores from the fastest - growing colonies on the previous plate. Finally spores from this last plate were used to inoculate McCartney bottle starch agar slopes, the new master and submaster cultures, which were stored at 4°C until required.

POTATO AGAR. (Booth, 297).

Potatoes (Peeled)	250 g.
Agar	25 g.
Water	1 l.

Chopped potatoes were gently boiled in the water for 30 min. then allowed to cool and settle. The liquid was decanted off, made up to 1 l. and agar added. After heating on a water bath to dissolve the agar, the medium was autoclaved at 1.05 kg./cm^2 for 15 min.

STARCH AGAR.

Starch	20 g.
Ammonium Sulphate	4.15 g.
Sodium Dihydrogen Orthophosphate	0.4 g.
Yeast Extract	0.4 g.
Potassium Chloride	0.2 g.
Magnesium Sulphate	0.08 g.
Calcium Chloride	0.04 g.
Agar	20 g.
Distilled Water	1 l.

The starch, salts and yeast extract were creamed in about 100 cm^3 of the distilled water while the agar was being dissolved in the remainder as it was being gently brought to the boil. The two liquids were mixed whilst being stirred vigorously and then autoclaved at 1.05 kg./cm^3 for 15 min.

3.5.2 PREPARATION OF INOCULA.

250 cm^3 conical flasks containing about 50 cm^3 of starch agar medium were inoculated with spores from a submaster culture and incubated at 30°C until mycelial growth had covered the medium surface and sporulated (about 5 - 7 days). These spore-containing flasks could be used immediately or stored at 4°C for

up to 3 months with no apparent deletarious effects.

About 3 h. before use, 0.04 l. of sterile "Tween 80" solution (of concentration 1 $g.l^{-1}$) were added, the flask vigorously shaken and incubated at 30°C to allow the spores to begin germinating prior to inoculation into the fermenter.

3.5.3 MEDIA COMPOSITION.

Three different types of medium were used in this investigation, though most of the fermentations were carried out with semisynthetic starch-based media. This was a result of the difficulty experienced in achieving steady states with aspirators of potato homogenate medium having varying composition - a result of the comparatively small scale of the experimentation.

All the media used had ammonium sulphate added in such quantity that the carbon : nitrogen ratio was 12 : 1, the semisynthetic type containing other salts in the ratio originally suggested by Cocker (290). Their compositions are detailed in Table 3.1 and Table 3.2.

The potato starch used was donated by Golden Wonder Ltd. (Corby, Northants.), being derived from the effluent stream of their potato processing factory. It was approximately 95% pure, the remainder being accounted for as water and small soil particles. Most of the potatoes were also donated by this company, being of the variety "Golden Wonder", which had a very high dry matter and low free reducing sugar content.

The caustic peel waste was obtained from Witch Chips Ltd. (Boroughbridge, N. Yorks.) and resulted from a process in which

TABLE 3.1 COMPOSITIONS OF THE SEMISYNTHETIC MEDIA USED.

(in $g.l^{-1}$)

	"$20g.l^{-1}$" Medium	"$13.2g.l^{-1}$" Medium	"$3.3g.l^{-1}$" Medium
Potato Starch.	21.0	12.5	3.125
Ammonium sulphate.	3.5	2.075	0.52
Sodium dihydrogen phosphate	0.675	0.4	0.1
Potassium chloride.	0.3375	0.2	0.05
Magnesium sulphate.	0.135	0.08	0.02
Calcium chloride.	0.0675	0.04	0.01
Yeast Extract.	0.675	0.4	0.1

TABLE 3.2 COMPOSITIONS OF THE POTATO MEDIA USED.

(in $g.l^{-1}$ or $ml.l^{-1}$)

POTATO MEDIUM.	"$20g.l^{-1}$" Medium	"$10g.l^{-1}$" Medium	"$5g.l^{-1}$" Medium
Whole,Raw Potato.(g.)	100	50	25
Ammonium sulphate.(g.)	3.7	1.85	0.925

PEEL WASTE MEDIUM.	"$20g.l^{-1}$" Medium	"$10g.l^{-1}$" Medium
Peel Waste.(g.)	100	50
Ammonium sulphate.(g.)	2.5	1.25
Concentrated Hydrochloric Acid.(ml.)	4 approx.	2 approx.

potatoes were dipped in a caustic bath (200 - 370 $g.l^{-1}$ sodium hydroxide depending on skin thickness) and subjected to infra-red heating to enable the skin to be brushed off. The peel waste was produced as a gelatinous mass at a temperature of 75 - 85°C and contained about 19% solids. It was extremely alkaline, being equivalent to about 2% sodium hydroxide on a wet basis (i.e. about 10% of the dry matter), had a pH of about 13.0 and a strong ammoniacal odour.

All the other medium constituents were of Laboratory Reagent Grade and, with the exception of the ammonium sulphate (B.D.H. Chemicals, Atherstone, Warks.) and yeast extract (Bovril Ltd., Burton on Trent), were supplied by Hopkin and Williams Ltd., Cradley Heath, Essex.

3.5.4 PREPARATION OF MEDIA.

1. SEMI SYNTHETIC STARCH MEDIUM.

Small batches of 40 - 50 g. starch were slurried in about 0.5 l. cold water and 2 l. boiling water quickly added whilst being vigorously agitated. This ensured that the starch was gelatinized and so solubilized. The batches were mixed in a 20 l. aspirator, salts and 1 cm^3 polyethylene glycol P-2000 antifoam added and the medium made up to volume with boiling water. The aspirator and contents were then autoclaved at 1.05 $kg.cm^{-2}$ for 45 min.

2. POTATO MEDIUM.

Whole potatoes were cooked in the autoclave at 1.05 $kg.cm^{-2}$ for 15 min. and then thoroughly homogenised in about 10 l. water

with 1 cm^3 polyethylene glycol P-2000 antifoam added using a mixer/disintegrator (Silverson Equipment, London S.E.1). Ammonium sulphate was added, the volume made up to 20 l. and the aspirator and contents autoclaved at 1.05 $kg.cm^{-2}$ for 45 min.

3. CAUSTIC PEEL WASTE.

The heating process which resulted in this waste gelatinised the starch and reduced contaminants to a negligible level. The peel was diluted and homogenised then neutralized with concentrated hydrochloric acid to pH10.0 -10.5 and ammonium sulphate and P-2000 antifoam added. Providing this medium was used within 24 h. further pretreatment proved unnecessary.

3.5.5 PREPARATION OF THE FERMENTER.

The fermenter was set up as in Fig. 3.1, the air inlet line, filter and medium inlet line removed and autoclaved separately. The fermenter was washed with "Pyroneg" detergent (Diversey Chemicals Ltd.) and rinsed thoroughly with tap water before being connected to a steam supply via the sample port. Steam sterilization of the fermenter, assisted in later fermentations by the injection of 10 cm^3 formalin solution, was carried out for at least 24 h. prior to commencement of a fermentation.

3.5.6 START-UP AND RUNNING OF FERMENTATIONS.

Air and medium inlet line connections were made whilst the fermenter was being steamed, with all possible precautions being taken to prevent ingress of contaminants. The air and steam supplies were slowly increased and decreased respectively so as to

maintain a positive pressure in the fermenter and especially to avoid sudden cooling which would create a partial vacuum.

The temperature controller was set to the required value and about 3 l. of medium quickly pumped into the fermenter. The germinating spore suspension was injected through a port into the fermenter from a sterile disposable syringe. With continuous fermentations, the culture was left to grow up for 24 h. before medium pumping began at the Dilution Rate to be investigated. (In the case of batch fermentations, the culture volume was immediately made up to 10 l. and antifoam added if necessary).

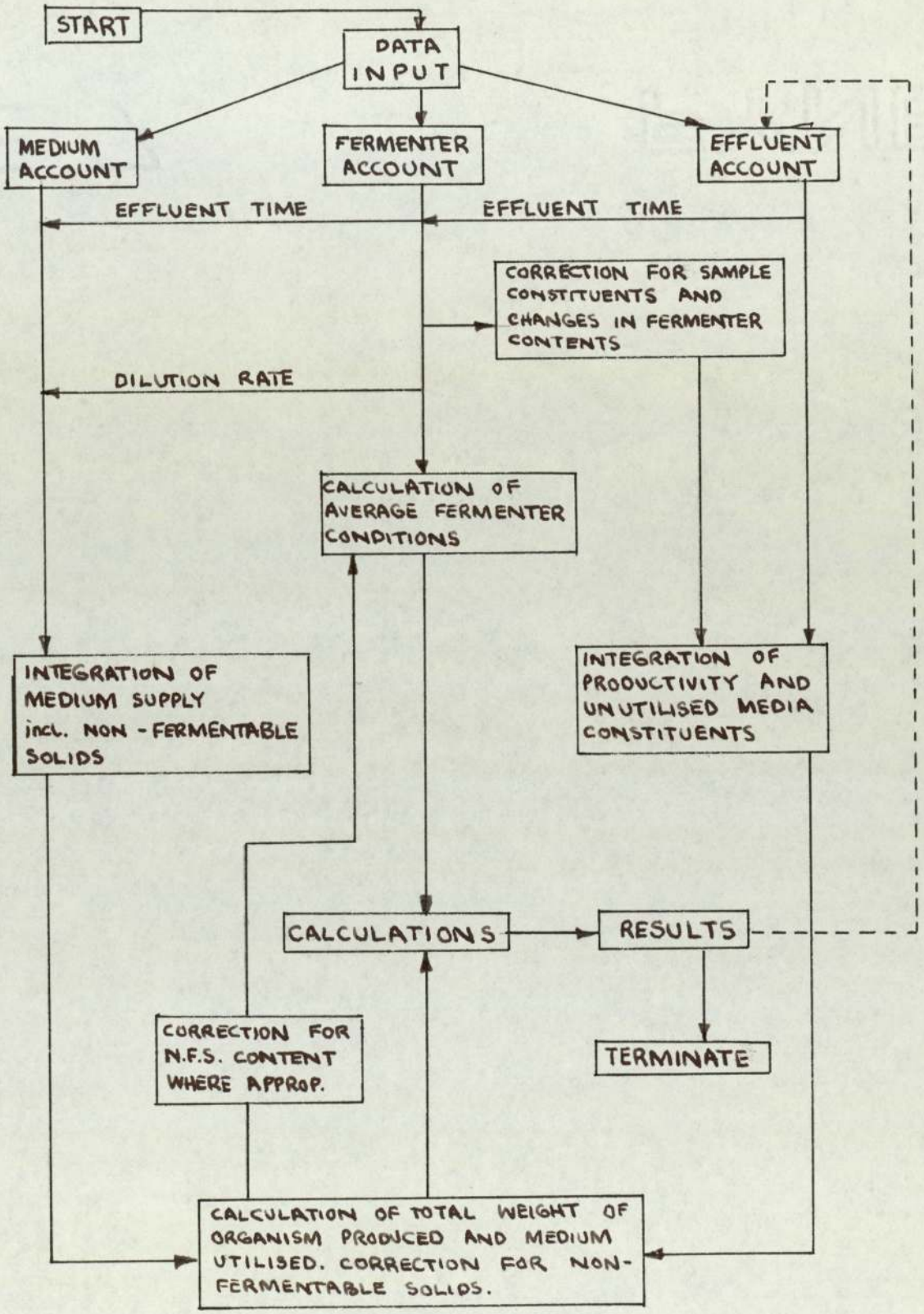
Sampling and analyses were carried out as detailed in Section 3.4 until it was considered that the fermentation had reached a steady state, when parameters such as dilution rate, temperature or medium concentration were altered. The longest fermentations carried out were in excess of 2500 h. duration.

3.6 DATA HANDLING AND TREATMENT.

3.6.1 INTRODUCTION.

The large volume of data generated from approximately 12,000 hours of continuous culture necessitated the development of a Mass Balance Programme for use with the University Computer. Whilst this programme was originally conceived and its capabilities specified by the author it was actually written and developed to suit these experimental results by Dr. M. Fidgett (Dept. of Chemical Engineering) with the author rendering assistance wherever possible.

FIG 3.2 SCHEMATIC DIAGRAM OF THE MASS BALANCE PROGRAMME



3.6.2 AN OUTLINE OF THE MASS BALANCE PROGRAMME.

This programme is shown schematically in Fig. 3.2 and reproduced in full as Appendix III.

Data is supplied as groups of Fermenter, Medium and Effluent values prefixed and chronologically arranged by the Elapsed Time (from the beginning of the fermentation) at which each was recorded. Calculations are performed whenever Effluent Data is supplied and the results refer to the preceding time period to which that data relates.

Briefly, the programme:-

(A) Calculates the total weights of media constituents supplied to the fermenter during the time period under consideration.

(B) Calculates the total weights of organism and residual media constituents present in the effluent stream from the fermenter during this time period. Corrections aremade to take account of samples removed from the system and for any changes in the fermenter contents.

(C) Combines these results (from A and B) to arrive at the actual weights of medium constituents utilized and organism produced (corrected for non-fermentable solids, where appropriate).

(D) Calculates the average fermenter conditions during this period. Where necessary, the dry weight of the fermenter contents is corrected for non-fermentable solids, based on the assumption that at steady state, the ratio of organism/NFS is the same as in the effluent stream.

(E) Calculates such factors as growth rate (MU), yield coefficient (YXS) and reducing sugar formation rate (RSRATE) from the results of calculations detailed in points C and D.

(F) Prints the results as part of a standard format, which includes all the fermenter conditions (both real and averaged), medium supply data and any comment statements which have been included in the data input.

3.6.3 LOGGING OF RAW DATA.

Input data is divided into four categories - Comments, Medium, Fermenter and Effluent Data. It is essential that data is:

supplied exactly in the order given below.

in the correct units.

in the correct chronological order.

prefixed by a character (1, 2, 3 or 4) indicating its type.

COMMENTS. Prefix Character 1.

Time of Comment.	T	h.
Comment (written and inserted verbatim into the results printout).		

MEDIUM DATA. Prefix Character 2.

Time of Medium Change	MT	h.
Ammonia Nitrogen Content	NH3M	$g.l^{-1}$
Total Sugar Concentration	TSM	$g.l^{-1}$
Non-Fermentable Solids Content	NFS	$g.l^{-1}$

FERMENTER DATA. Prefix Character 3.

Time of Sampling	FT	h.
pH	PH	Units
Temperature	TEMP	^{o}C
Dilution Rate	D	h^{-1}
Organism Concentration	XF	$g.l^{-1}$
Reducing Sugar Concentration	RSF	$g.l^{-1}$
Total Sugar Concentration	TSF	$g.l^{-1}$
Ammonia Nitrogen Concentration	NH3F	$g.l^{-1}$
Nitrogen Content of Organism	NBUG	$g.g^{-1}$

EFFLUENT DATA. (i.e. Product Receiver Data) Prefix Character 4.

Time Receiver Emptied	ET	h.
Volume of Effluent	VE	l.
Organism Content	XE	$g.l^{-1}$

Fig. 3.3 is a typical extract from a data file showing this arrangement of experimental results.

3.6.4 OUTPUT OF RESULTS.

The results printout includes Raw Data, Averaged Fermenter Data and the Calculated Results. The latter are presented in the following order:-

Growth Rate	MU	h^{-1}
Yield Coefficient (organism on sugar)	YXS	$g.organism.\ g^{-1}_{sugar}$
Productivity	PROD	$g.l^{-1}.h^{-1}$
Reducing Sugar Production Rate	RSRATE	$g.l^{-1}.h^{-1}$

Reciprocal of Growth Rate	RMU	h.
Reciprocal of Yield Coefficient	RYXS	$g.sugar.g^{-1}$ organism
Nitrogen Taken up in Period	DN	g.
Nitrogen in Organism Produced in period	NINBUG	g.
Yield Coefficient (organism on nitrogen)	YN	$g.organism.g^{-1}$ nitrogen

Fig. 3.4 shows the results print-out equivalent to the data presented in Fig. 3.3.

3.6.5 ADDITIONAL INFORMATION.

Linear averaging of fermenter data was used, being the only practicable method. This was acceptable where small variations occurred, but was not applicable to pH data and so averages of this parameter were disregarded.

A sample volume of 0.180 l. was assumed but multiple samples in a given time period could be accounted for. The sample contents were significant under certain conditions where the growth rate was very low.

When a data value was not known, -1.0 was substituted. This was an instruction to assume the last known value of that parameter in the calculation.

With fermentations containing Non-fermentable Solids (which passed through the system unchanged) the ratio of organism to Non-fermentable Solids in the fermenter was assumed to be the same as in the effluent stream.

Step changes in parameters such as dilution rate could be

Fig. 3.3 An Extract from a typical Data File (from run SR 5).

```
 3
264.75  3.0  30.0  0.0708  3.65  2.97  2.99  0.148  0.06397
 4
264.75  17.0  3.58
 2
265.0  0.418  13.2
 3
288.5  3.05  30.5  0.0710  3.2  3.66  3.685  0.156  0.06255
 4
288.5  17.0  3.08
 2
289.5  0.432  13.2
 3
317.0  3.05  30.0  0.0712  3.5  4.17  4.795  0.162  0.06112
 4
317.0  19.1  2.56
 2
317.0  0.422  13.2
 3
332.75  3.05  30.5  0.0705  3.65  4.47  4.67  0.162  0.06184
 4
332.75  11.0  2.60
 2
339.75  0.422  13.2
 3
362.5  3.05  30.0  0.0762  3.6  4.65  4.782  0.163  0.06221
 4
362.5  21.5  2.89
 2
363.25  0.420  13.2
 3
377.5  3.05  30.5  0.0740  3.5  3.74  3.95  0.166  0.06106
 4
377.5  11.0  3.01
```

Fig. 3.4 Results Printout for the Data in Fig. 3.3 (from run SR 5).

FERMENTER	264.75	3.000	30.000	0.071	3.65	2.97	2.99	0.148	0.064
EFFLUENT	264.75	17.000	3.580						
FERMENTAV	250.50	3.000	30.000	0.070	3.975	3.10	3.185	0.144	0.064
RESULTS	0.049	0.259	0.194	0.923	20.516	3.857	6.022	3.519	9.169
FERMENTER	288.50	3.050	30.500	0.071	3.200	3.660	3.685	0.156	0.062
EFFLUENT	288.50	17.000	3.080						
FERMENTAV	276.625	3.025	30.250	0.071	3.425	3.315	3.337	0.152	0.063
RESULTS	0.060	0.308	0.205	0.931	16.741	3.251	4.346	3.069	11.180
FERMENTER	317.000	3.050	30.000	0.071	3.500	4.170	4.795	0.162	0.061
EFFLUENT	317.000	19.100	2.56						
FERMENTAV	302.750	3.050	30.250	0.071	3.350	3.915	4.240	0.159	0.062
RESULTS	0.055	0.301	0.184	0.893	18.173	3.324	5.615	3.243	9.356
FERMENTER	332.750	3.050	30.500	0.070	3.650	4.470	4.670	0.162	0.062
EFFLUENT	332.750	11.000	2.600						
FERMENTAV	324.875	3.050	30.250	0.071	3.575	4.320	4.732	0.162	0.061
RESULTS	0.055	0.322	0.196	0.927	18.281	3.102	2.895	1.894	10.640
FERMENTER	362.500	3.050	30.000	0.076	3.600	4.650	4.782	0.163	0.062
EFFLUENT	362.500	21.500	2.890						
FERMENTAV	347.625	3.050	30.250	0.073	3.625	4.560	4.726	0.163	0.062
RESULTS	0.058	0.343	0.210	0.948	17.292	2.919	5.599	3.868	11.139
FERMENTER	377.500	3.050	30.500	0.074	3.500	3.740	3.950	0.166	0.061
EFFLUENT	377.500	11.000	3.010						
FERMENTAV	370.000	3.050	30.250	0.075	3.550	4.195	4.366	0.165	0.062
RESULTS	0.062	0.304	0.219	0.966	16.220	3.285	2.855	2.023	11.500

accomplished by submitting Fermenter Data twice (with the appropriate change) with a very small Elapsed Time difference (say, 0.001 h.), otherwise the averaging procedure led to errors.

Under conditions of extreme limitation, negative growth rates occurred, indicative of more material being lost from the fermenter than was accounted for in the effluent stream. This could have been due to a small experimental error in the determination of fermenter organism concentration - the results are extremely sensitive to such an error under these conditions - or the value obtained could be interpreted as representing a Specific Rate of Autolysis.

SECTION 4.

RESULTS AND DISCUSSION.

Page No.

4.1 INTRODUCTION.

The experimental work carried out was in two related areas. Determination of the kinetic characteristics of the fermenter and the fermentation process was accomplished using semisynthetic starch media. These media could be made up with constant substrate concentrations and did not contain non-fermentable solids. They were also most suitable for investigations into the effects of temperature and pH on the system.

The other work was of a more applied nature, being concerned with the fermentation of potatoes and alkaline peel waste. The presence of non-fermentable solids in these media led to problems not only in the experimental work but also in the interpretation of the data, particularly in the estimation of such factors as growth rates and yield coefficients.

In each Section, information concerning that type of fermentation is presented and described, with all the experimental results being collated and their implications discussed in Section 4.5, together with suggestions for further work.

4.2 SEMISYNTHETIC STARCH MEDIA : FERMENTATIONS CARRIED OUT AT 30°C.

These fermentations yielded information regarding the operating characteristics of the system under "normal" conditions and provide a basis for comparison with all the other results obtained.

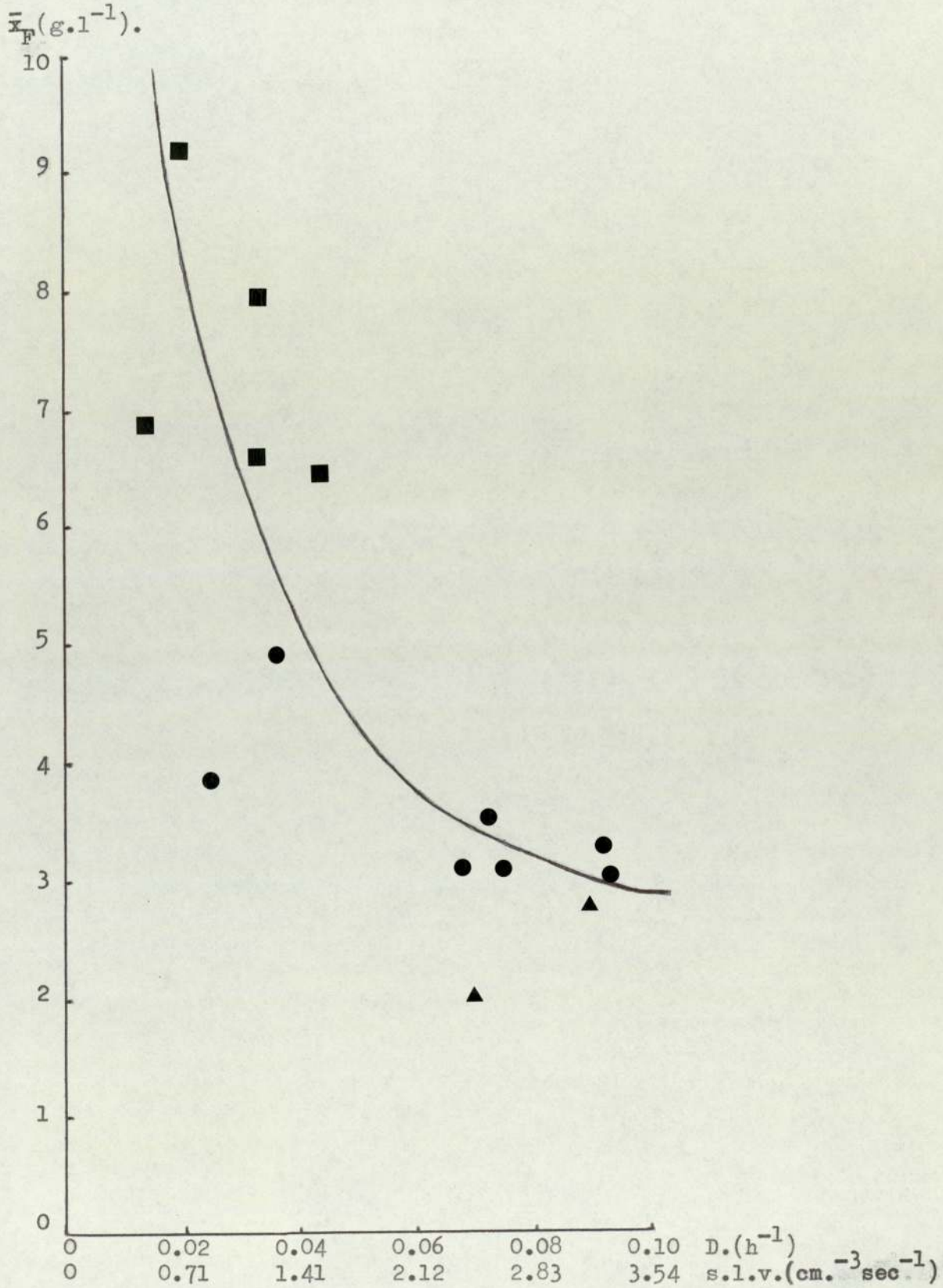
4.2.1 CONCENTRATION OF ORGANISM IN THE FERMENTER (x_F).

This is the fundamental parameter which affects the fermentation. For a given medium it is determined by a complex interplay of both physical and physiological factors - at any one time the dry weight of organism present and its morphology are both governing and being governed by the fermenter conditions. Such a "control" mechanism tends to make this parameter relatively unstable, both within and between fermentations, particularly at low dilution rates.

The Medium Flow Rate, usually expressed as a Dilution Rate, must be considered as representing both a nutrient supply rate and a liquid flow rate - a superficial liquid velocity (s.l.v.) - which is responsible for the fluidisation effects on the microbial colonies. Fig. 4.1 shows the effect of medium flow rate on the steady-state fermenter organism concentration ($\bar{x}_F$) at three substrate levels in fermentations carried out at 30°C. The use of a dual-scale abscissa allows the flow rate to be expressed as either a Dilution Rate (h^{-1}) or a Superficial Liquid Velocity ($cm.10^{-3}.\ sec.^{-1}$) which of course depends upon the fermenter configuration.

At low Dilution Rates (below 0.05 h^{-1}) values of the organism concentration at steady state ($\bar{x}_F$) of up to 9 $g.l^{-1}$ and 5 $g.l^{-1}$ were obtained using 20 and 13.2 $g.l^{-1}$ medium respectively. With higher Dilution Rates (0.05 - 0.1 h^{-1}) substrate concentration had considerably less effect on $\bar{x}_F$, within this range values of 3 - 3.5 $g.l^{-1}$ being typical. Unfortunately the gelatinous nature of starch media at these concentrations precludes their use

Fig. 4.1 The Effect of Medium Flow Rate(D. & s.l.v.) on the Mould Concentration in the Fermenter($\bar{x}_F$).
(■ 20, ● 13.2, ▲ 3.3g.l^{-1} glucose equivalent medium)



with this organism at Dilution Rates of greater than 0.10 - 0.12 h^{-1} i.e. starch supply rates of greater than 2 $g.l^{-1}.\ h^{-1}$.

The position of this response curve is dependent upon growth conditions such as the carbon source and other medium constituents, temperature and aeration rate, which act upon the organism morphology. This was shown by comparison with results obtained by Pannell (213) who grew the same strain of A. niger (M1) in an identical fermenter on sucrose - salts media. The form of the curve was similar over this range of dilution rates but the organism concentrations were significantly higher.

Briefly, the factors determining Dry Weight in the fermenter can be summarized by the following points:-

(1) Low superficial liquid velocities decrease the fluidisation effect (which is responsible for washout of organism from the fermenter) permitting high organism concentrations.

(2) Low nutrient supply rates limit the amount of organism which can be maintained in the fermenter.

(3) Low nutrient supply rates lead to more "inhibited" morphologies which have a higher mycelial packing density and therefore sedimentation rate, both factors which lead to an increase in $\bar{x}_F$.

(4) When conditions are changed in a continuous culture, the new steady-state morphology and organism concentration are influenced by those prior to the change.

As will be seen in the following sections, the non-linear response of dry weight to medium flow rate influences all the

characteristics of the operation of this fermentation system.

4.2.2 CONCENTRATION OF ORGANISM IN THE EFFLUENT STREAM (x_E).

Although $\bar{x}_F$ is determined primarily by physical means (Section 4.2.1), $\bar{x}_E$ is related to the growth rate (μ) of the fermenter contents and is capable of varying independently.

For a given $\bar{x}_F$, steady states can exist where $\bar{x}_E$ is within a range from almost zero (under conditions of extreme limitation) to being equal to $\bar{x}_F$. Steady states of over 200 hours duration have even been achieved in which x_E has exceeded x_F (see Sections 4.3 and 4.4) though this situation usually only exists transiently when organism is being "washed out" of the fermenter.

Fig. 4.2 shows the lack of correlation between $\bar{x}_F$ and $\bar{x}_E$ with regard to the medium flow rate.

4.2.3 SPECIFIC GROWTH RATE AND PRODUCTIVITY OF THE ORGANISM.

In the derivation of continuous culture kinetics, the rate of growth of a microorganism and the productivity of a fermenter are represented by the expressions

$$\text{Rate of Growth} \quad (dx/dt) = \mu x_F$$

$$\text{Productivity} \quad (dx/dt) = Dx_E$$

where x_F and x_E are the concentrations of organism in the fermenter and effluent stream respectively. Therefore at steady state

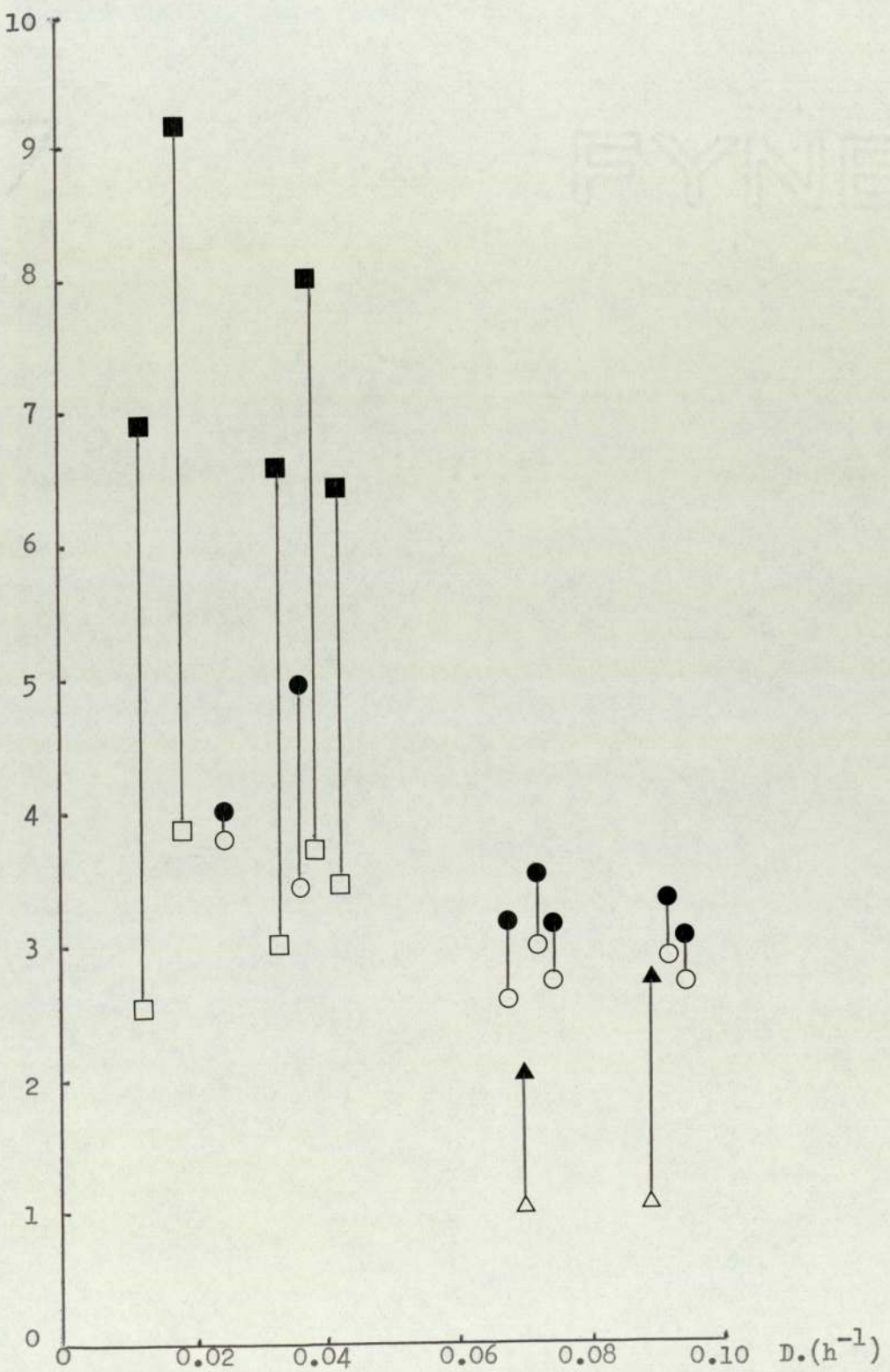
$$\mu \bar{x}_F = D\bar{x}_E \qquad \ldots\ldots\ldots \text{Eqn. 4.1}$$

and rearranging

$$\mu = \frac{D\ \bar{x}_E}{\bar{x}_F} \qquad \ldots\ldots\ldots \text{Eqn. 4.2}$$

Fig. 4.2 The Effect of Medium Flow Rate(D) on the Mould Concentration in the Effluent Stream($\bar{x}_E$).

(■ 20, ● 13.2, ▲ 3.3g.l^{-1} glucose equivalent medium - x_F)
(□ 20, ○ 13.2, △ 3.3g.l^{-1} glucose equivalent medium - x_E)



When considering homogeneous fermentation systems where $\bar{x}_F = \bar{x}_E$ (such as the continuous stirred tank fermenter) Eqn. 4.1 reduces to $\mu = D$. However, in the case of continuous tower fermenters, this simplification cannot be made and the growth rate must be calculated using Eqn. 4.2. This value is in fact the average growth rate of all the fermenter contents and assumes that the whole of the culture contributes to the production of new cellular material - a point which will be discussed further in Section 4.5.4.

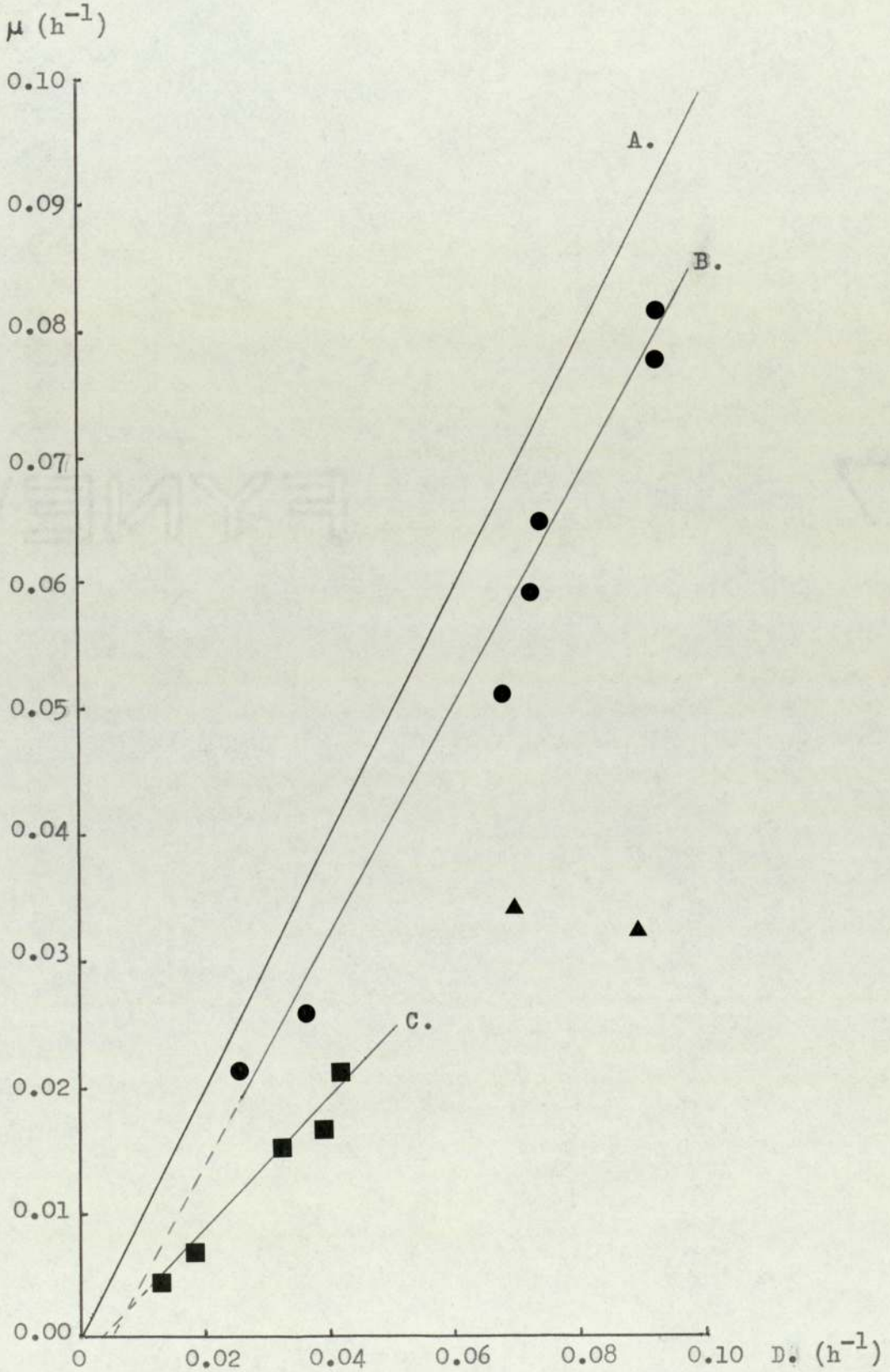
4.2.3.1 SPECIFIC GROWTH RATE.

Fig. 4.3 illustrates the relationship between the specific growth rate (μ) and the dilution rate (D) in this series of experiments. Line A represents the case of $\mu = D$, whereas the regression lines B (122 readings; correlation coefficient r = 0.9703) and C (37 readings; r = 0.9562) are derived from experimental results. The significance of line B is increased even further when additional data using this medium at different temperatures is included (in Section 4.5.2). Results obtained with 3.3 $g.l^{-1}$ medium exemplify the effects of carbon limitation on the growth rate.

Extrapolation of lines B and C indicates that when $\mu = 0$, D = 0.00594 and 0.00397 h^{-1} respectively. These values represent glucose supply rates of 0.0792 and 0.0784 $g.l^{-1},h^{-1}$, which can be interpreted as the carbohydrate requirement for maintenance of the culture (discussed in more detail in Section 4.5.2). At these dilution rates, Pannell (213) found that $\bar{x}_F \approx$ 19.0 $g.l^{-1}$, which suggests that the Maintenance Coefficient, m,

Fig. 4.3 The Effect of Medium Flow Rate(D) on the Specific Growth Rate of the Fermenter Contents(μ).

(■ 20, ● 13.2, ▲ 3.3g.l^{-1} glucose equivalent medium)



of the organism growing under these conditions is approximately 0.004 g.glucose - g^{-1} organism - h^{-1}.

4.2.3.2 PRODUCTIVITY.

The effect of dilution rate on the productivity of organism of the system is shown in Fig. 4.4. The results form a smooth curve which intercepts the abscissa when $\mu = 0$, i.e. at a dilution rate of 0.004 - 0.006 h^{-1}. The form and position of this curve under conditions of substrate excess are determined by the interaction of characteristics of the system such as the effects of D on x_F and x_F morphology and viscosity of the culture on $K_L a$ (the oxygen transfer coefficient - see also Section 4.5.3). This interaction is compounded by the effect of culture broth pH on the growth rate of the organism.

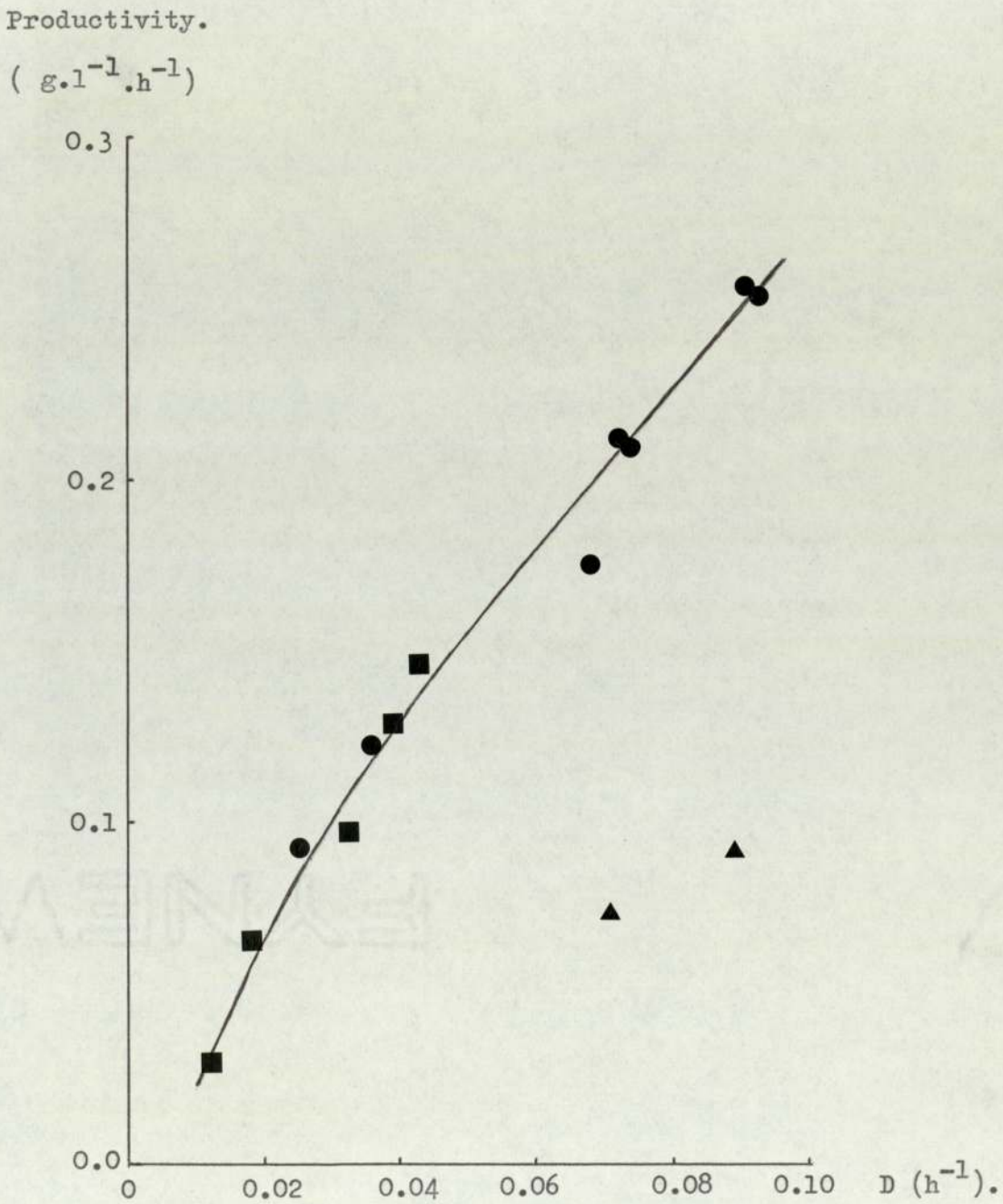
Assuming that the True Growth Yield of Organism on carbohydrate (Yg) and the Maintenance Coefficient (m) are constants, the productivity of the system can be represented by the equation

$$\mu.\bar{x}_F = D.\bar{x}_E = Yg\ (D.s_R - D.\bar{s} - m.\bar{x}_F) \quad \ldots . \text{ Eqn. 4.3.}$$

Under conditions of carbohydrate limitation, diminution of $D.s_R$ caused $D.\bar{s} \rightarrow 0$, increasing the significance of the proportion of the carbohydrate supply used for maintenance ($m.\bar{x}_F$). This resulted in restriction of the productivity and, therefore, the specific growth rate (μ), accounting for the steady state values obtained with 3.3 $g.l^{-1}$ glucose equivalent media.

This situation can be exploited as another means of estimating the maintenance coefficient of the organism in the Tower

Fig. 4.4 The Effect of Dilution Rate(D) on the Organism Productivity.
(■ 20, ● 13.2, ▲ 3.3g.l^{-1} glucose equivalent medium)



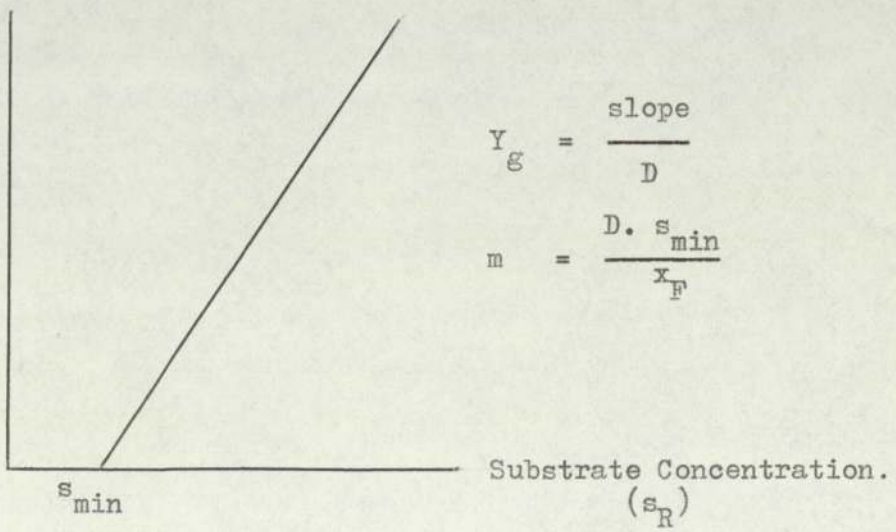
Fermenter. Under conditions of carbon limitation, when the Dilution Rate (D) is kept constant, $\bar{x}_F$ tends to remain so even if s_R is varied. Assuming that Y_g and m are constants, in this situation reductions in s_R result in such steady states that productivity ($D.\bar{x}_E$) decreases linearly and can even be zero (when $\mu = 0$) as in Fig. 4.5. Therefore, when $\mu = 0$ and $s_R = s_{min}$, Eqn. 4.3 can be reduced and rearranged to

$$m = \frac{D.\ s_{min}}{x_F} \quad (\text{units} - g.g^{-1}.\ h^{-1}) \ldots\ldots \text{Eqn. 4.4.}$$

giving the value of the maintenance coefficient of the mould at zero growth rate in this system.

Fig. 4.5 Theoretical Effect of reducing the Substrate Concentration on the Productivity of a Carbon-limited Fermentation.

(when D, $\bar{x}_F$, Y_g and m are constants.)



4.2.3.3 SUBMERGED SPORULATION OF THE CULTURE.

When $D.s_R$ was reduced to a level approximately equal to or less than $m.\bar{x}_F$, sporulation of the mould took place in submerged culture in the fermenter. Typical conditions were a Dilution Rate of 0.022 h^{-1} when starch medium of 3.3 $g.l^{-1}$ glucose equivalent (s_R) was being used. The conditions which led up to this phenomenon were better monitored in a potato medium fermentation and will therefore be discussed more fully in Section 4.4.6.

4.2.4 HYDROLYSIS AND UTILIZATION OF THE STARCH SUBSTRATE.

4.2.4.1 REDUCING SUGAR FORMATION BY HYDROLYSIS OF STARCH.

In common with most other microorganisms, Aspergillus niger M1 is unable to absorb and utilize polymeric carbohydrates directly; it secretes enzymes into the culture medium which hydrolyse these substrates to their constituent monomeric sugar molecules.

In the case of starch substrates the enzymes concerned are maltase and a group known collectively as amylases. Acting together, these result in the formation of glucose which can be assimilated by A. niger M1 hyphae. Pazur and Ando (298), Pazur and Kleppe (299), Kearney (300) and Allam and Khalil (301, 302) have all attempted to estimate the relative inportance of these enzymes in cultures of A. niger. However, in this study it was more important to show that the rate of starch hydrolysis and, therefore, glucose formation, was not a rate limiting step in the fermentation.

The rate of glucose production in samples of culture filtrate was ascertained by the method described in Appendix II, though this estimation was not used routinely. Typical values were in the range 1.6 - 2.0 $g.l^{-1}.\ h^{-1}$ for samples taken from 20.0 and 13.2 $g.l^{-1}$ glucose equivalent medium fermentations. However, as the method measured the glucose production rate at pH 4.6 - the usual optimum for amylases - it was not necessarily indicative of the true rate of hydrolysis in the fermenter.

The reducing sugar production rate in the fermenter was easily derived from mass balance data. It proved to be the most constant and reliable fermentation parameter probably because the source data, the reducing and total sugar contents of culture liquid filtrates, could be very accurately determined.

Figs. 4.6 and 4.7 relate the Reducing Sugar Production Rate and the Effectiveness of Substrate Conversion, respectively, to the Rate of Substrate Supply to the fermenter, $D.s_R$ (expressed as glucose equivalent). In both cases, lines (designated A) have been drawn to represent the situation in which complete conversion of starch to glucose takes place.

In fermentations with 20.0 and 13.2 $g.l^{-1}$ glucose equivalent medium the maximum reducing sugar production rates were approximately 0.65 and 1.2 $g.l^{-1}.\ h^{-1}$ respectively. These values are confirmed by data from other periods of fermentation in which conditions could not be kept constant for sufficient time to ensure a steady state had been achieved. The discrepancies are almost certainly due to the pH of the culture - over the range of conditions investigated, fermentations at higher medium concentrations had lower pH's (see Section 4.2.7).

Fig. 4.6 The Relationship between the Substrate Supply and Reducing Sugar Production Rates in 30°C Fermentations.
(■ 20, ● 13.2, ▲ 3.3g.l^{-1} glucose equivalent medium)

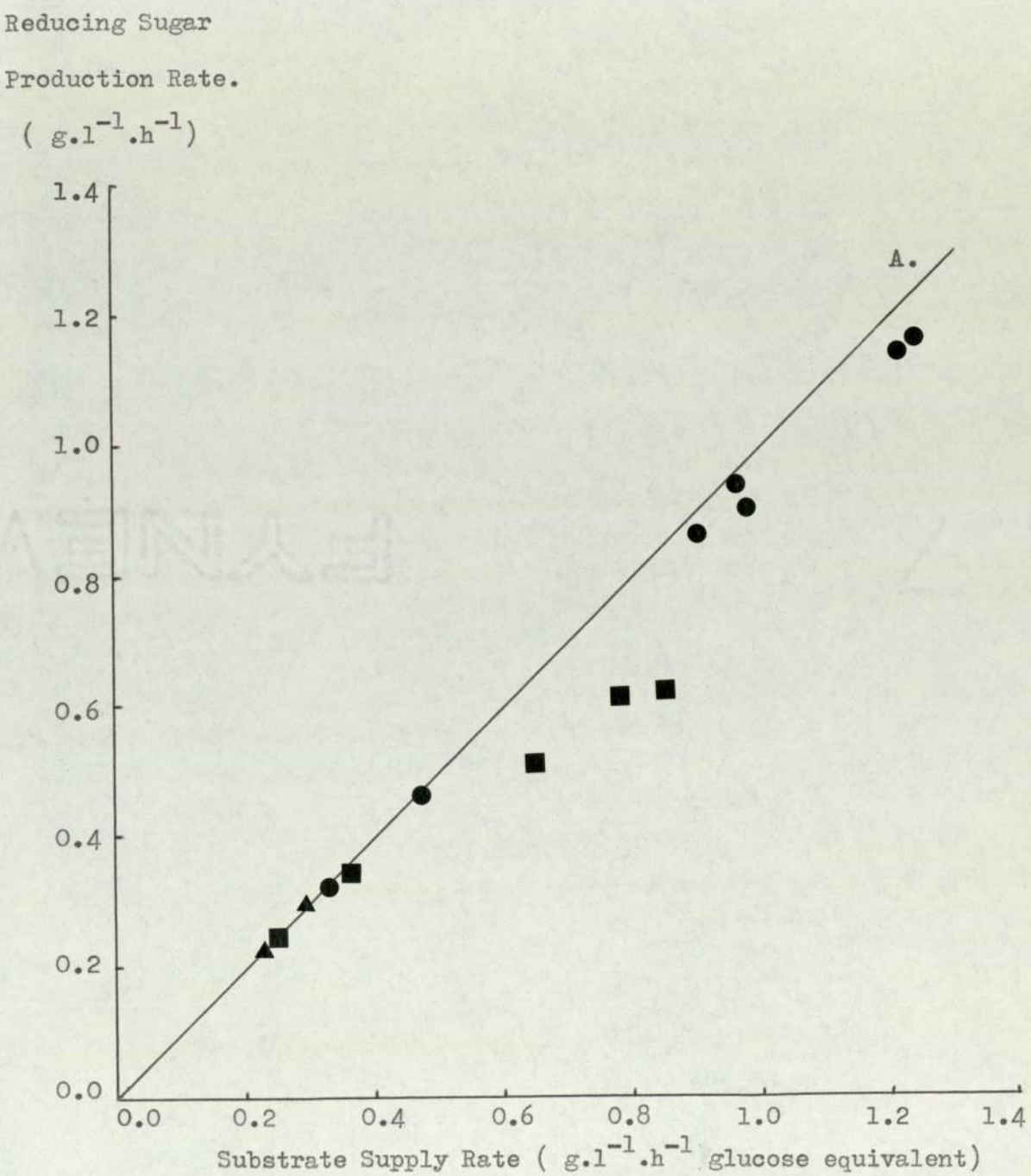


Fig. 4.7 The Relationship between the Substrate Supply Rate and the Effectiveness of its Conversion to Glucose.
(■ 20, ● 13.2, ▲ 3.3g.l^{-1} glucose equivalent medium)

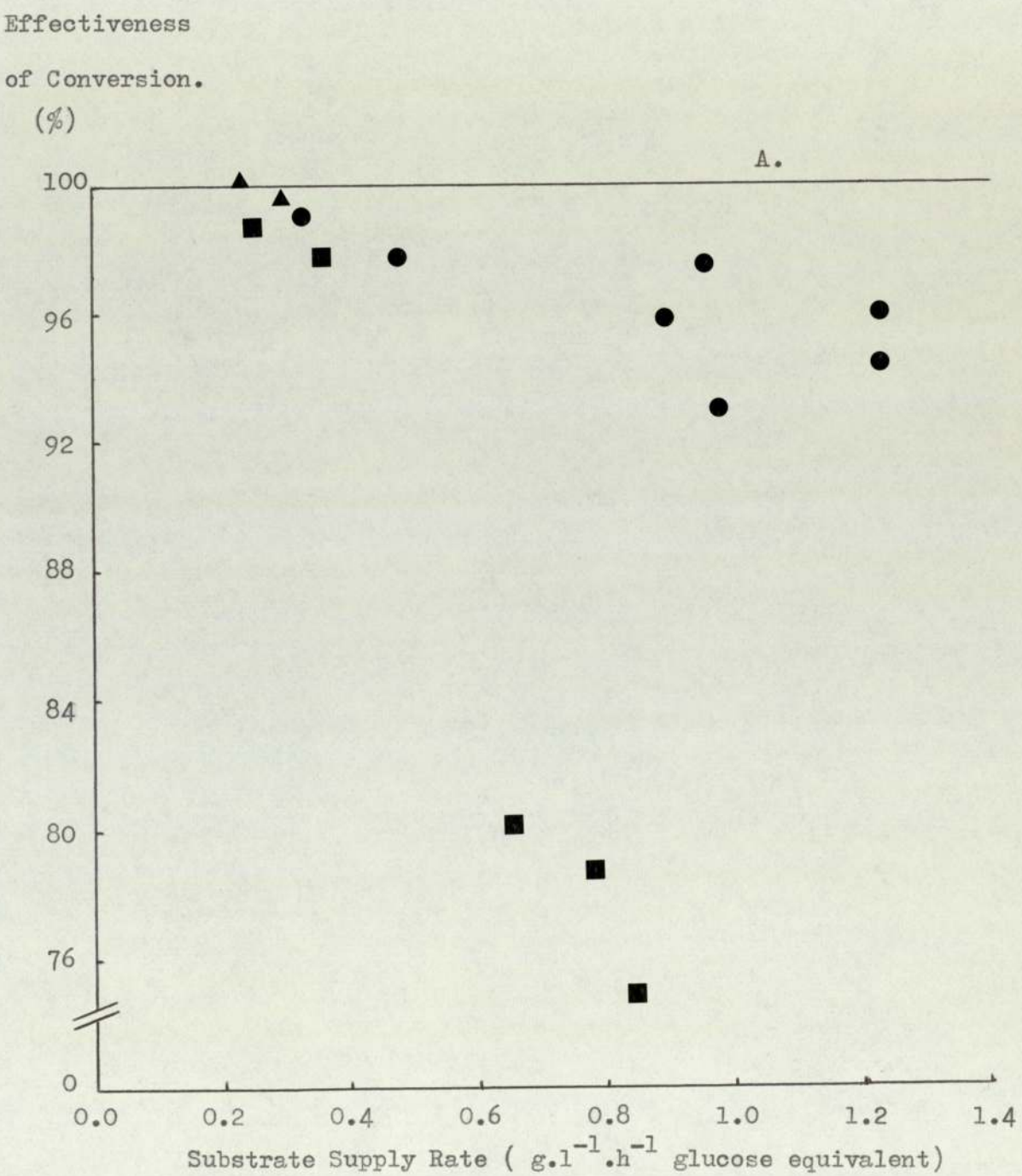


Table 4.1 Summary of Data relating to the effect of pH on the Reducing Sugar Production Rate.

Source of Data.	pH Units.	Highest R.S. Rate $g.l^{-1}.h^{-1}$.
20.0 $g.l^{-1}$ Medium Contin. Ferms.	2.13 (Av.)	0.65
13.2 $g.l^{-1}$ Medium Contin. Ferms.	2.64 (Av.)	1.254
In vitro determinations (Appendix II)	4.6 (Const.)	2.04
Jarl (270) Batch Cultures Endomycopsis fibuliger		
Strain NRRL Y - 1062	Not Quoted Probably c. 5.0.	0.983
Strain a : 3		2.70

Values quoted by Jarl (270) for batch cultures of strains of Endomycopsis fibuliger used in the Symba Process are included in Table 4.1 for comparison. In these yeast fermentations there are indications that the rate of saccharification becomes limiting after about 8 hours, when the reducing sugar production rate, though increasing, is about 0.8 $g.l^{-1}.h^{-1}$.

Throughout the range of medium supply rates used in this experimental work even though the maximum starch hydrolysis rates were lower, the conversion was almost always in excess of 90% complete. Except, of course, under carbon limited conditions, the availability of glucose was always in excess of the requirement of the mould for growth and maintenance; it did not, therefore, limit the fermentation.

4.2.4.2 CARBOHYDRATE UTILIZATION RATES.

Carbohydrate substrates, in the form of glucose, are utilized by A. niger M1 both for maintenance of the culture and for the growth of new "biomass". Taking both these factors into account, the overall rate of substrate consumption can be represented by the equation:-

$$\frac{ds}{dt} = \frac{D.\ x_E}{Y_g} + m\ x_F \quad \ldots\ldots \text{Eqn. 4.4.}$$

Fig. 4.8 illustrates the relationship between the sugar utilization rate and the dilution rate (D) in 30°C, starch medium fermentations. The results form 2 straight lines designated A and B with correlation coefficients (r) of 0.9484 (37 readings) and 0.9789 (122 readings) respectively. The highest steady state utilization rate noted in this series of fermentations was 0.84 $g.l^{-1}.\ h^{-1}$.

Though a steady state cannot exist when D = 0 h^{-1}, extrapolation of lines A and B gives an indication of the rate of consumption when μ = 0 h^{-1} i.e. the factor m x_F in Eqn. 4.4; the values obtained are 0.14 and 0.12 $g.l^{-1}.\ h^{-1}$ respectively. Assuming as in Section 4.2.3.1, that when $D \approx 0\ h^{-1}$, $x_F \gg$ 19.0 $g.l^{-1}$, these values suggest that the maintenance coefficient (m) is less than 0.0065 $g.g^{-1}.\ h^{-1}$. This figure is in agreement with values obtained by other plots.

When considering the specific substrate utilization rate (q), Eqn. 4.4 reduces to :-

$$q = \frac{\mu}{Y_g} + m \quad \ldots\ldots \text{Eqn. 4.5.}$$

Fig. 4.8 The Relationship between the Substrate Utilization and Dilution Rates in 30°C Fermentations.

(■ 20, ● 13.2, ▲ 3.3g.l^{-1} glucose equivalent medium)

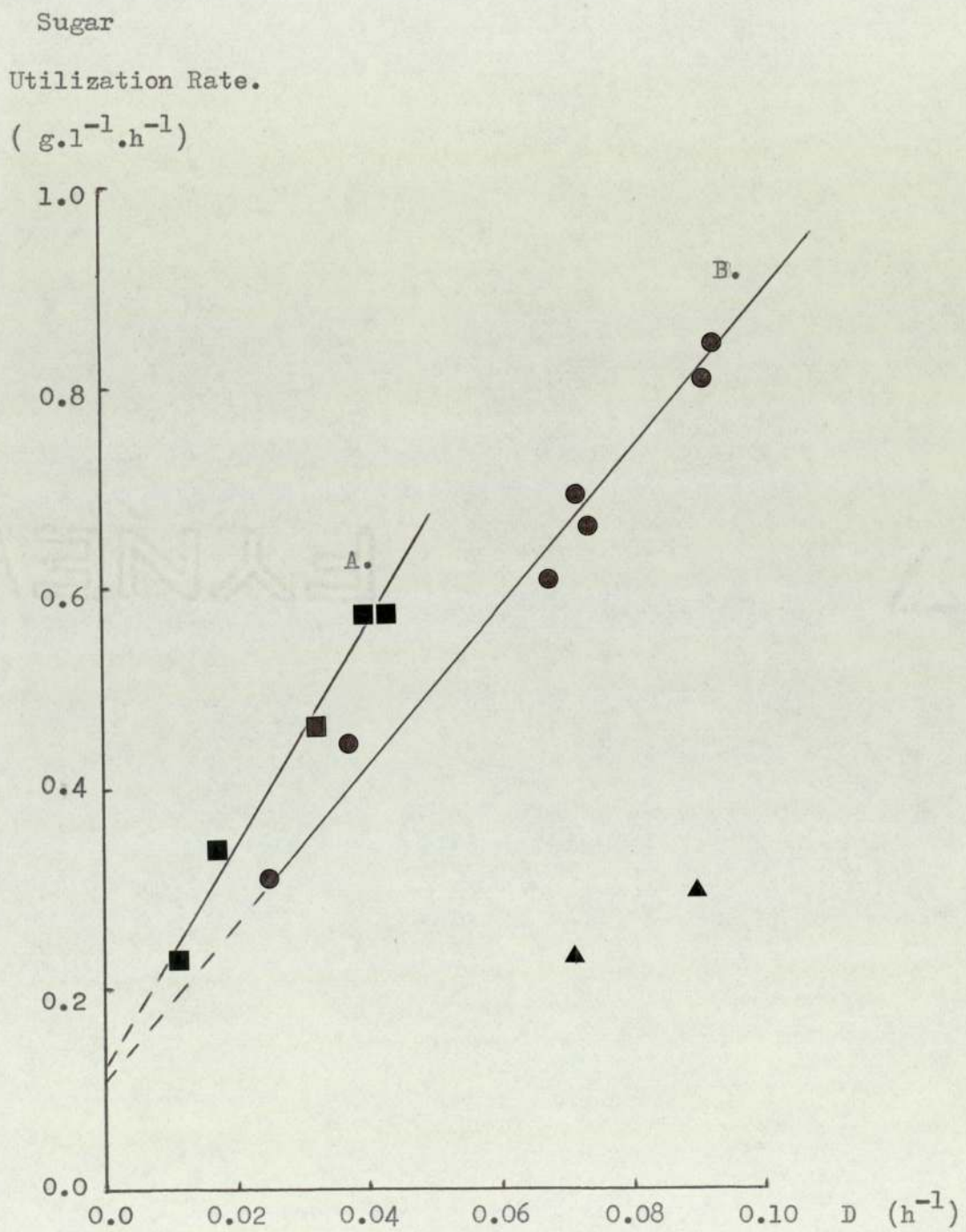
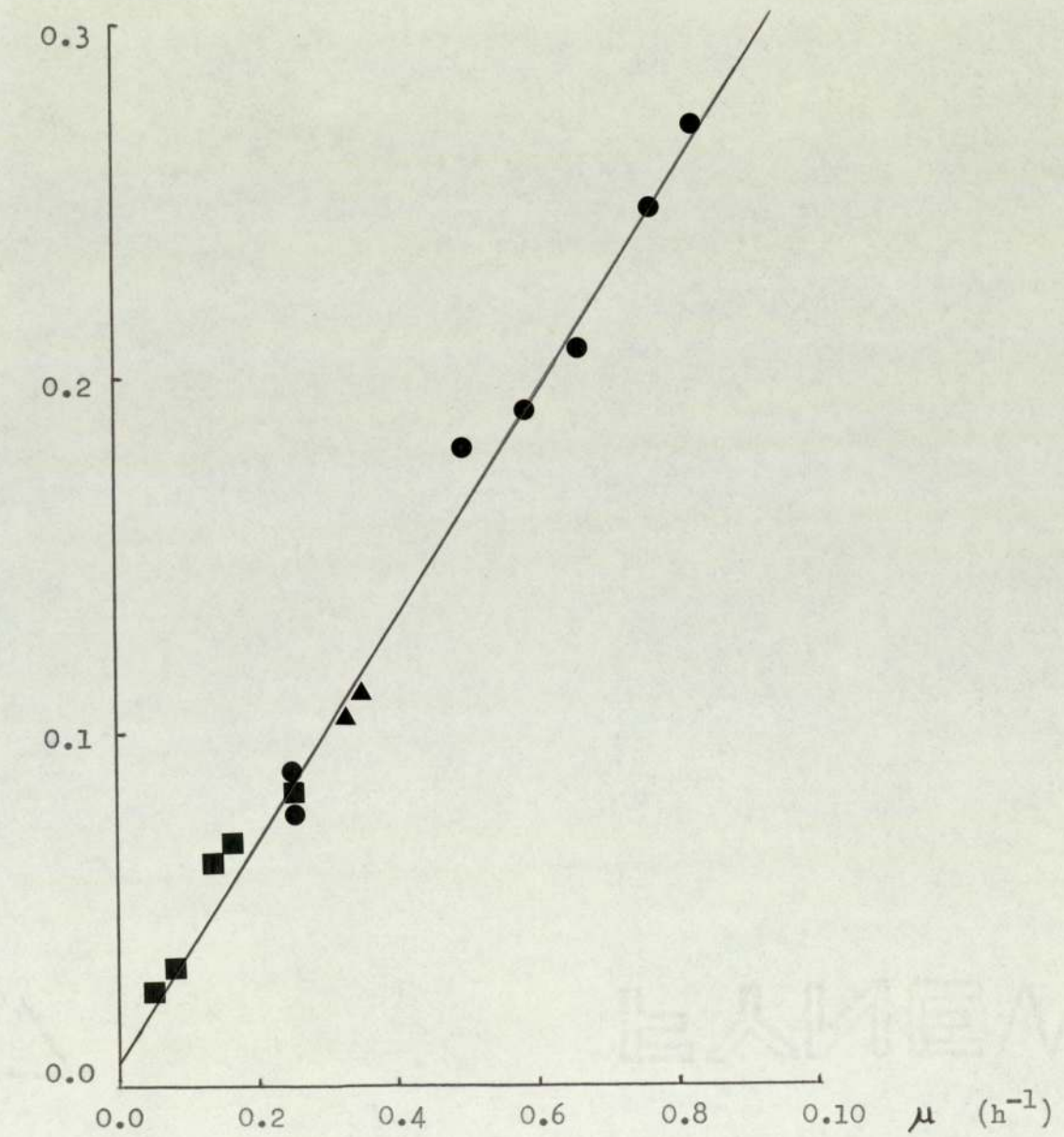


Fig. 4.9 The Relationship between the Specific Rates of Growth and Substrate Utilisation in 30°C Fermentations.

(■ 20, ● 13.2, ▲ 3.3g.l^{-1} glucose equivalent medium)



This equation is the basis of the graphical method used by Tempest and Herbert (303), Righelato et al. (304) and Von Meyenberg (305) for the determination of the maintenance coefficient (m) of microorganisms (discussed further in Section 4.5.2).

Data from this series of fermentations at 30°C is plotted in such a manner in Fig. 4.9. The results form a straight line (Correlation Coefficient 0.9917 ; 195 readings) which, if extrapolated to $\mu = 0$, yields a value $m = 0.00424$ $g.g^{-1}.h^{-1}$. The significance of this line is also increased when data obtained at higher temperatures is included.

The slope of this line is $^1/Y_g$; using the data presented in Fig. 4.9 a value of $Y_g = 0.3158$ g organism. g glucose^{-1}. h^{-1} was obtained for these fermentation conditions. The significance of this low figure will be discussed in Section 4.5.2

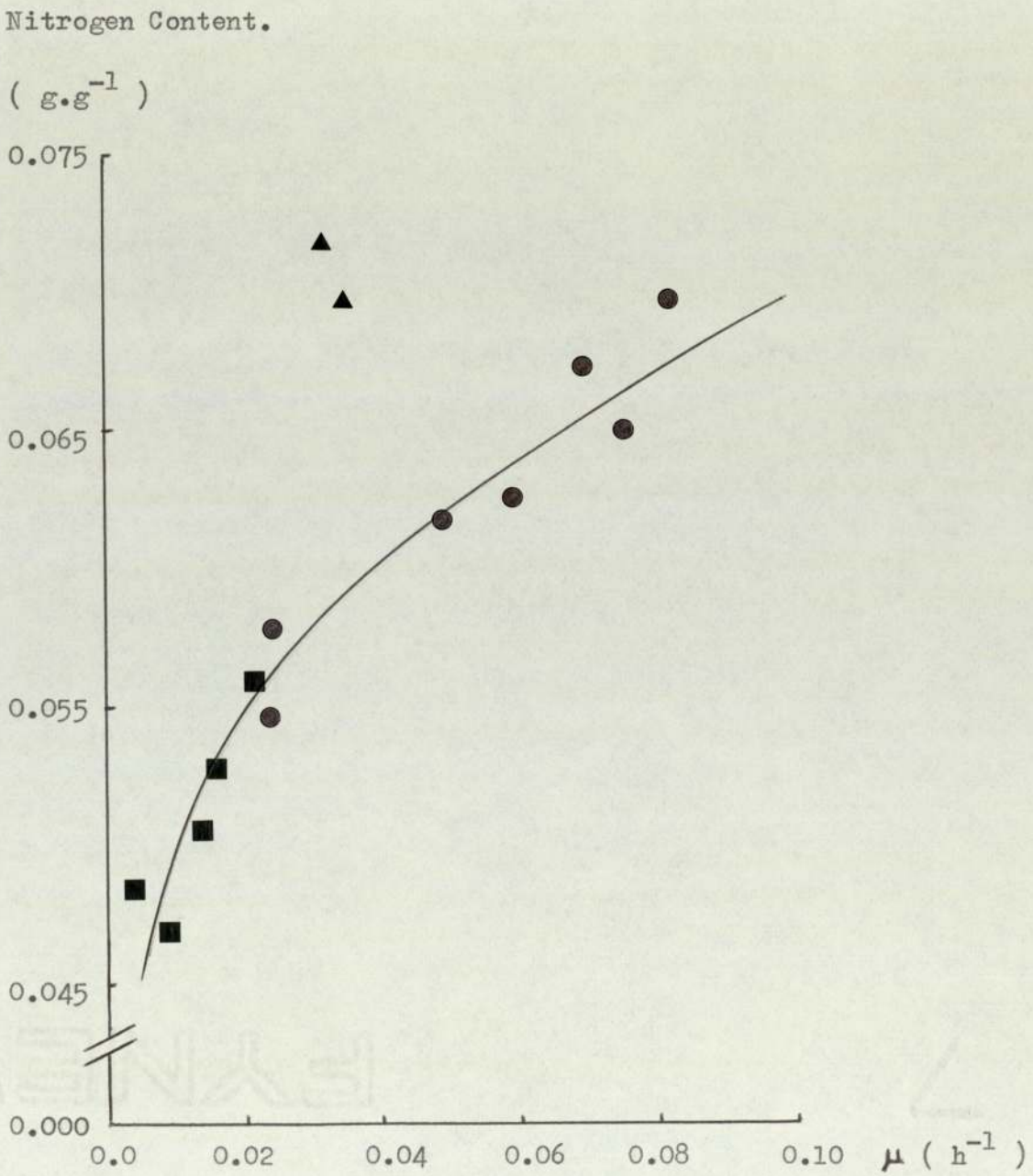
4.2.5 NITROGEN CONTENT OF THE ORGANISM.

The nitrogen content of the mould, though being influenced by the dilution rate, showed little direct relationship. However, as illustrated in Fig. 4.10, this parameter increased linearly with the specific growth rate of the organism. The highest steady state nitrogen content recorded was 0.0719 $g.g^{-1}$ organism (actually, under carbon limited conditions) though values in the range 0.0525 - 0.0675 $g.g^{-1}$ were more usual in this series of fermentations. These correspond to a crude protein content of 32.5 - 42.5% on a (N x 6.25) basis.

Pannell (213) estimated the true protein content of A. niger M1 by summation of amino acids and found that the ratio of actual/crude protein varied from 0.57 - 0.84 depending on the growth rate.

Fig. 4.10 The Effect of Specific Growth Rate (μ) on the Nitrogen Content of the Organism in 30°C Fermentations.

(■ 20, ● 13.2, ▲ 3.3g.l^{-1} glucose equivalent medium)



This he compared to a range of 0.45 - 0.75 reported for a strain of Fusarium sp.

The factors affecting the nitrogen content of the mould can be summarized thus:-

(A) Both true protein and nucleic acid contents increase as the specific growth rate is raised.

(B) At low specific growth rates, non-protein nitrogen (such as in chitin, a cell wall component) makes up a significant proportion of the total nitrogen content.

(C) The nitrogen content is "diluted" by non-nitrogen containing metabolites which may have either a structural or storage function and themselves vary with the fermenter conditions. This accounts for significantly higher biomass nitrogen contents under carbon limited conditions.

(D) Depending so closely upon the physiology of the organism, the total nitrogen content is particularly susceptible to changes in fermenter conditions. As a result, it is usually the last parameter to reach steady state in a fermentation.

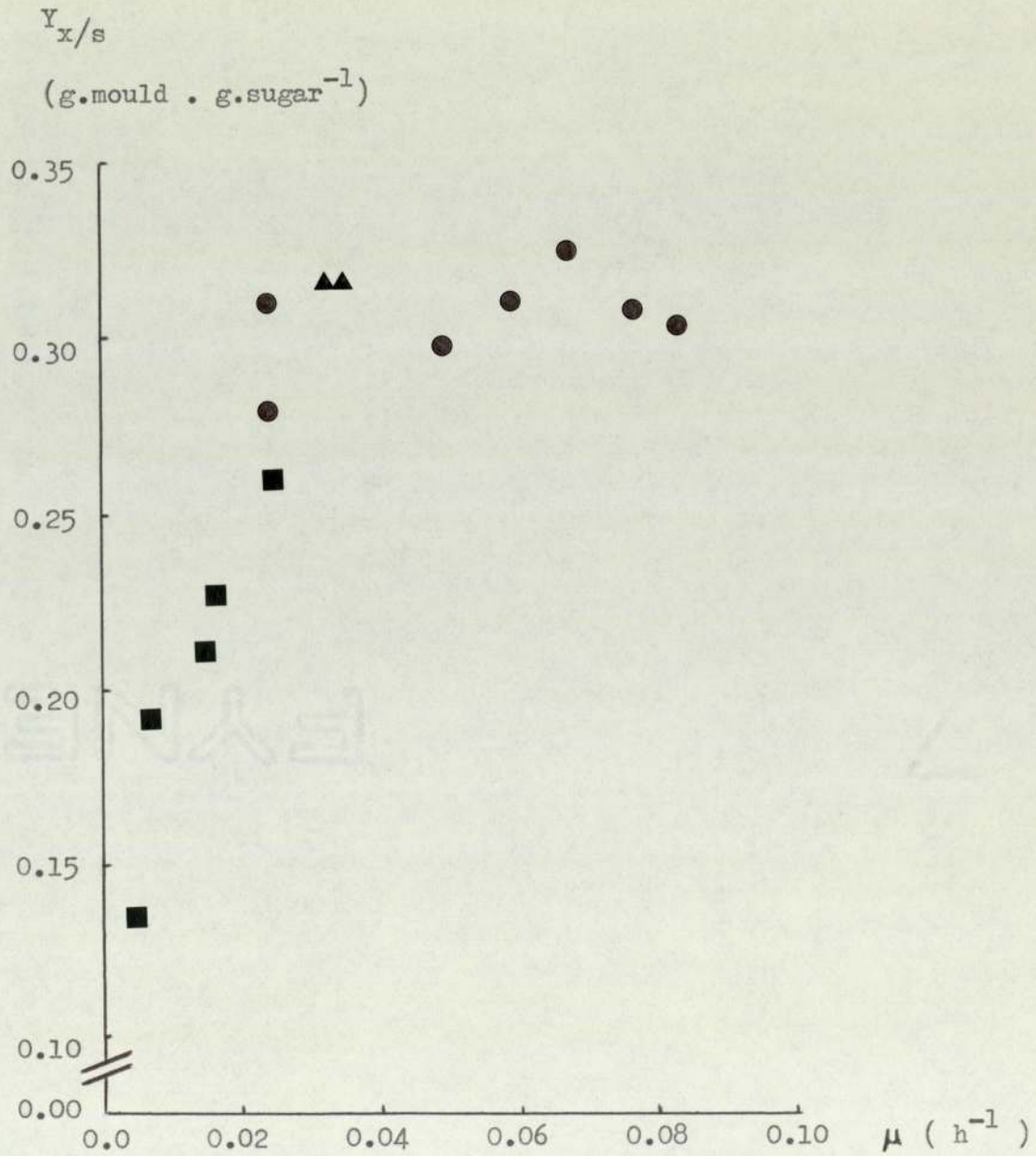
4.2.6 YIELD COEFFICIENT ($Y_{x/s}$) OF THE MOULD.

The Yield Coefficient was easily derived using the Mass Balance Programme and in a given time period is simply

$$Y_{x/s} = \frac{dx}{ds}$$

which is equivalent to

Fig. 4.11 The Effect of Specific Growth Rate (μ) on the Yield Coefficient of Mould on Substrate ($Y_{x/s}$).
(■ 20, ● 13.2, ▲ 3.3g.l^{-1} glucose equivalent medium)



$$Y_{x/s} = \frac{\mu \bar{x}_F}{D(s_R - \bar{s})} \quad \ldots\ldots \text{Eqn. 4.6.}$$

Fig. 4.11 shows the relationship between $Y_{x/s}$ and the specific growth rate (μ). Except when using 20.0 $g.l^{-1}$ glucose equivalent medium, the steady state results fall within a very small range, 0.28 - 0.33 g organism. g^{-1} substrate. These yield coefficients are very low in comparison to other "biomass" production processes, where values in the range 0.4 - 0.5 $g.g^{-1}$ are usual. These discrepancies are almost certainly due to oxygen limitation (see section 4.5.3).

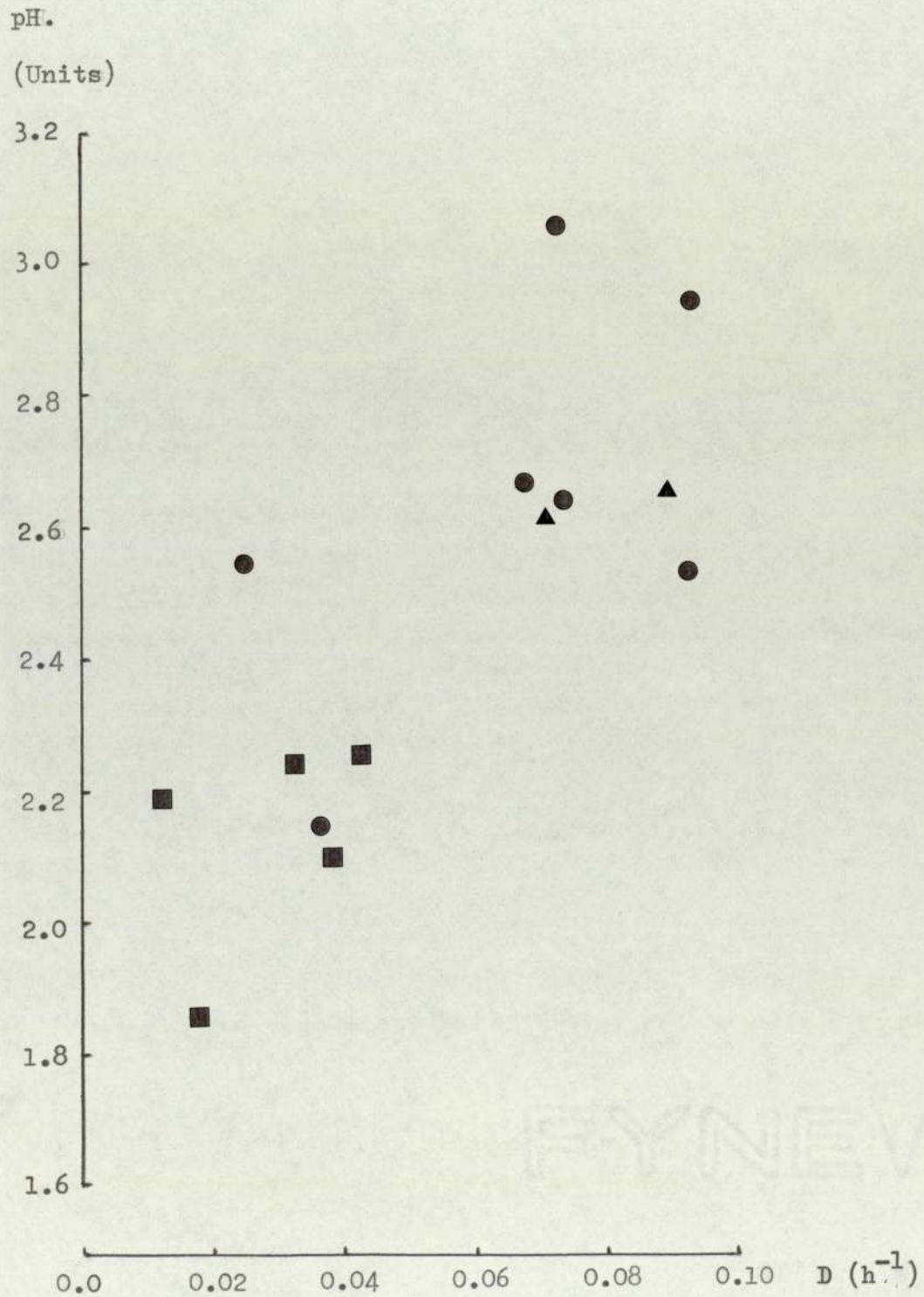
Fermentations in which the specific growth rate was very low were carried out using 20 $g.l^{-1}$ medium and low dilution rates. In these, $\bar{x}_F$ was much higher (see section 4.2.1), so the quantity of substrate consumed for maintenance ($m.\bar{x}_F$) was larger and consequently $Y_{x/s}$ decreased. This effect is compounded by the fact that high values of $\bar{x}_F$ result in low oxygen transfer coefficients ($K_L a$). As oxygen uptake becomes limiting, the mould curtails its respiratory pathways and produces more organic acids. This causes $Y_{x/s}$ to decrease further and lowers the pH of the fermenter contents.

4.2.7 pH OF THE CULTURE.

Mould growth was always accompanied by a reduction in the pH of the medium supply. In general this reduction was greatest at the lowest dilution rates (Fig. 4.12) but the pH value at steady state could not be accurately predicted for any given fermentation. This is because it depends upon a complex balance of factors:-

Fig. 4.12 The Effect of Dilution Rate (D) on the Culture pH in 30°C Fermentations.

(■ 20, ● 13.2, ▲ 3.3g.l^{-1} glucose equivalent medium)



(A) Uptake of nutrient molecules from medium salts can result in pH changes e.g. the uptake of nitrogen from ammonium sulphate gives sulphuric acid which decreases the pH.

(B) Organic acid production by the mould both as an energy reserve and in response to oxygen limitation decreases the pH.

(C) Use of these organic acids as substrates for growth under conditions of acute carbon limitation increases the pH.

(D) Increase in dilution rate lowers the concentration of acids formed by the mould and increases the pH.

(E) The buffering capacity of the medium, though low and probably variable, results in higher pH values.

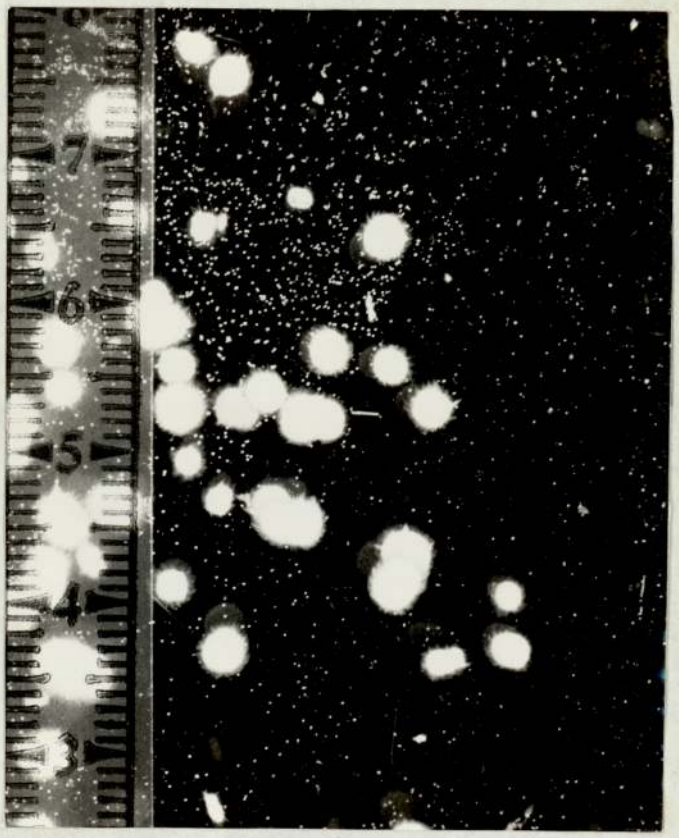
Whilst the range of pH values noted under widely varying fermentation conditions appears small, being on a logarithmic scale, these represent considerable differences in acid concentration. The lowest value noted was pH 1.7 ($D = 0.0179\ h^{-1}$; $s_R = 20.0\ g.l^{-1}$) which represents an acid concentration 10x higher than does pH 2.7 ($D = 0.0679\ h^{-1}$; $s_R = 13.2\ g.l^{-1}$), assuming the dissociation constants remain unchanged.

4.2.8 THE MORPHOLOGY OF THE MOULD.

Samples of mycelium were photographed in a flat-sided perspex viewing cell after being diluted approximately 50x with warm water containing C. 0.05% "Tween 80" detergent solution. In spite of these precautions it proved impossible to prevent the formation of

Plate 4.1 Aspergillus niger M1 : Typical Morphology 46 h. after Spore Inoculation.

(Fermentation SR 2; D= 0.025 h^{-1}; s_R = 20g.l^{-1}gluc. eq.)



1 cm.

minute air bubbles on the walls of the cell when illuminated by "photoflood" lamps.

In all the fermentations carried out Aspergillus niger M1 mycelium developed in the form of colonies. The use of this description is deliberate to avoid implications of structure and packing density or mechanism of formation which are inherent in such terms as "pellet", "floc" and "aggregate".

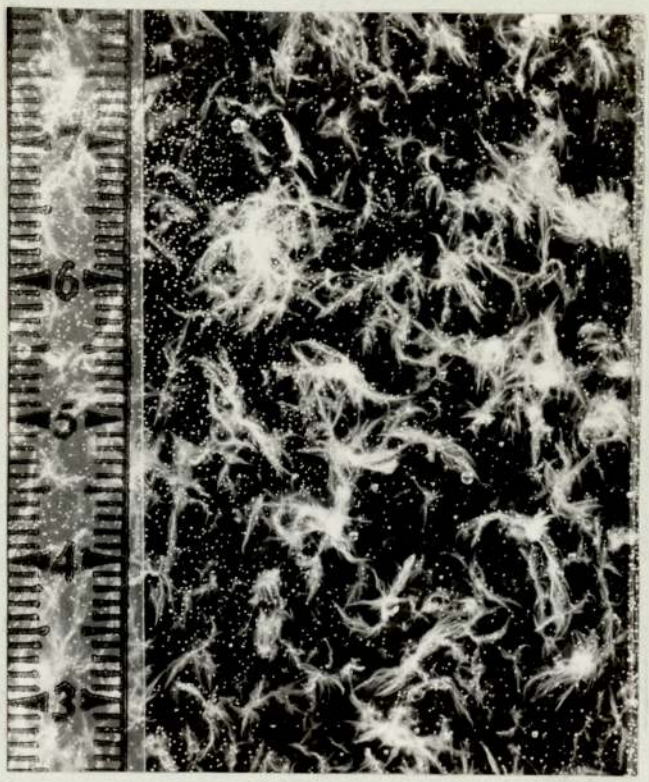
The initial morphology of the organism and its changes could not be accurately predicted for a given fermentation. With the size of spore inoculum and start-up conditions used in this work, colonies such as those in Plate 4.1 usually developed. Typically these were about 2 - 4 mm diameter "hairy" spheres of mycelium conforming to the usual descriptions of "pellets" (Cocker, 290).

Stable morphologies of the types illustrated in Plates 4.2 and 4.3 gradually evolved from these colonies over a period of continuous culture. At the lowest dilution rates investigated this process often occupied 200 - 300 h., the result being comparatively large ($>$ 8mm) tangled bundles of mycelium. In general, colonies from a 20 $g.l^{-1}$ medium fermentation were larger than those from a 13.2 $g.l^{-1}$ medium situation and in such samples the proportion of small colonies was reduced.

In one steady state ($D = 0.025\ h^{-1}$; $s_R = 13.2\ g.l^{-1}$) $\bar{x}_F$ was much lower than expected and this was reflected in all the other fermentation parameters. The colonies appeared more "feathery" loosely packed and filamentous (see Plate 4.4). Such a steady state results from a combination of the filamentous morphology

Plate 4.2 Aspergillus niger M1 : Steady State Morphology after 443 h. Continuous Fermentation.

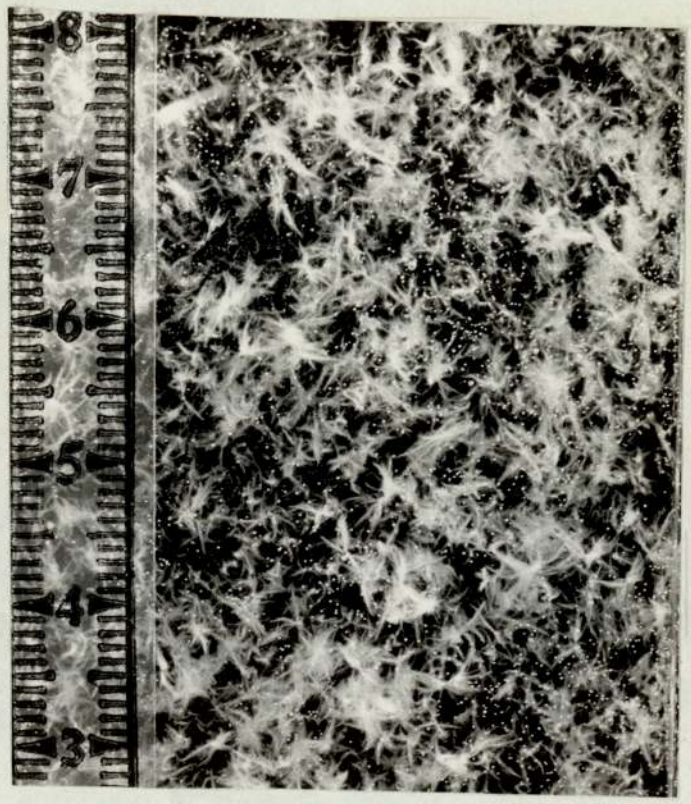
(Fermentation SR 1; D=0.042 h^{-1}; s_R = 20g.l^{-1} gluc. eq.)



1 cm.

Plate 4.3 Aspergillus niger M1 : Steady State Morphology after 543 h. Continuous Fermentation.

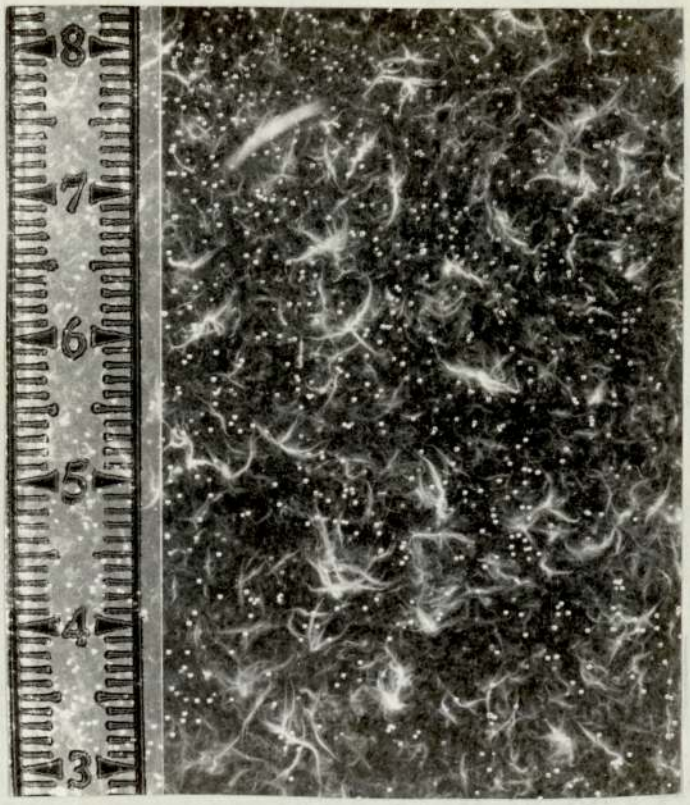
(Fermentation SR 5; D=0.073 h^{-1}; s_R = 13.2g.l^{-1} gluc. eq.)



1 cm.

Plate 4.4 Aspergillus niger M1 : A More Filamentous Morphology.

(Fermentation PN 4B; $D=0.025\ h^{-1}$; $s_R = 13.2 g.l^{-1}$ gluc. eq.)



1 cm.

causing a low $\bar{x}_F$ and vice versa. In other words the mould morphology and concentration simultaneously influence and are influenced by the fermenter conditions.

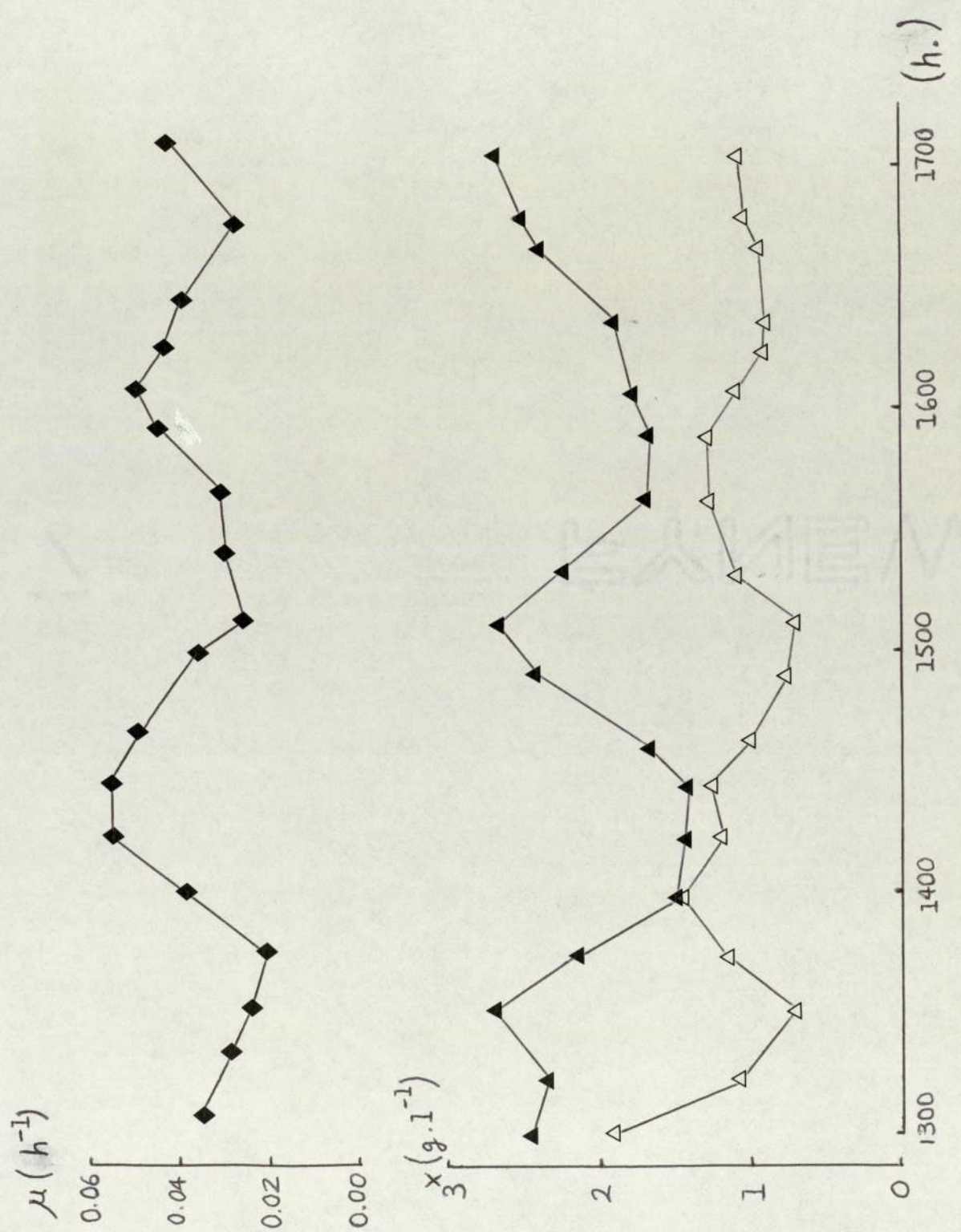
4.2.9 OSCILLATIONS IN THE MOULD CULTURES.

In most fermentations, particularly those at low dilution rates, the measured values of parameters tended to fluctuate even though D, s_R and T remained constant. This was almost certainly due in part to errors in measurement - the error in determining x_F could have been as great as $\pm$ 0.1 $g.l^{-1}$ with samples in the range 3.0 - 4.0 $g.l^{-1}$.

However, particularly in substrate limited conditions there were periods in which, with x_F constant, some degree of synchrony of growth and productivity was apparent (as in the results of Pannell, 213). In other cases x_F varied considerably in a cyclical manner (see Fig. 4.13). Usually the periods of these oscillations did not appear to bear any simple relationship to the specific growth rate of the mould, or correlate with any environmental change.

Fig. 4.13 illustrates data collected over a period of 400 h. fermentation at a dilution rate of 0.0704 $\pm$ 0.0007 h^{-1} (21 readings) using 3.3 $g.l^{-1}$ glucose equivalent medium. The concentration of mould in the fermenter (x_F) varied from 1.43 $g.l^{-1}$ to 2.75 $g.l^{-1}$, changes which were reflected in x_E. Even with corrections for dx_F (in the Mass Balance Programme), the specific growth rate (μ) of the mould cycled between 0.023 h^{-1} and 0.57 h^{-1} with the same period as x_F and x_E, 180 h.

Fig. 4.13 An Unsteady State : Variations in the Specific Growth Rate (μ) and the Mould Concentrations in the Fermenter (x_F) and Effluent Stream (x_E). (D= 0.0704 h^{-1}. (◆ μ ; ▲ x_F; △ x_E) s_R= 13.2 $g.l^{-1}$gluc. eq.)



Theoretically, at steady state,

$$\mu = \frac{(D\,(s_R - \bar{s}) - m.\bar{x}_F)\,Y_g}{\bar{x}_F} \quad \ldots \text{ Eqn. 4.7.}$$

Substituting: $Y_g = 0.3158\ g.g^{-1}$. $m = 0.00424\ g.g^{-1}.h^{-1}$.

$x_F = 2.08\ g.l^{-1}$ (Av.) $D = 0.0704\ h^{-1}$.

$s_R = 3.3\ g.l^{-1}$

$(s_R - \bar{s}) \approx s_R$ (as the culture was substrate limited).

$\underline{\mu = 0.03393\ h^{-1}}$.

This theoretical value of μ is in agreement with the average value of all the experimental results under these conditions, $0.0348\ h^{-1}$. The 400 h. period of fermentation was equivalent to approx. 28 fermenter throughputs of medium and 20 "generation times" of the organism. This data emphasizes the point that, when growing moulds in tower fermenter systems, steady state conditions are achieved only after considerable time periods and in some cases are not necessarily ever reached.

4.3 SEMI-SYNTHETIC STARCH MEDIA : EFFECTS OF TEMPERATURE AND MEDIUM pH ON THE FERMENTATIONS.

4.3.1 EFFECTS OF TEMPERATURE.

4.3.1.1 INTRODUCTION.

To enable direct comparison, the effects of temperature on Aspergillus niger M1 were investigated in two fermentations using $13.2\ g.l^{-1}$ glucose equivalent medium and a constant dilution rate

of 0.067 h^{-1}. The temperatures chosen initially were 30, 35, 40 and 45°C, though later, 42°C was used in an attempt to establish the thermal death point of the mould more accurately. Results obtained at 35°C using a dilution rate of 0.075 h^{-1} have not been included in this comparison.

Steady states were achieved at all temperatures, except 45°C, at which almost complete "washout" of the mould took place over 94 h. However, the most significant feature of these results was the change in morphology of the organism around 37 - 38°C, effects of which could be seen in all the fermentation parameters.

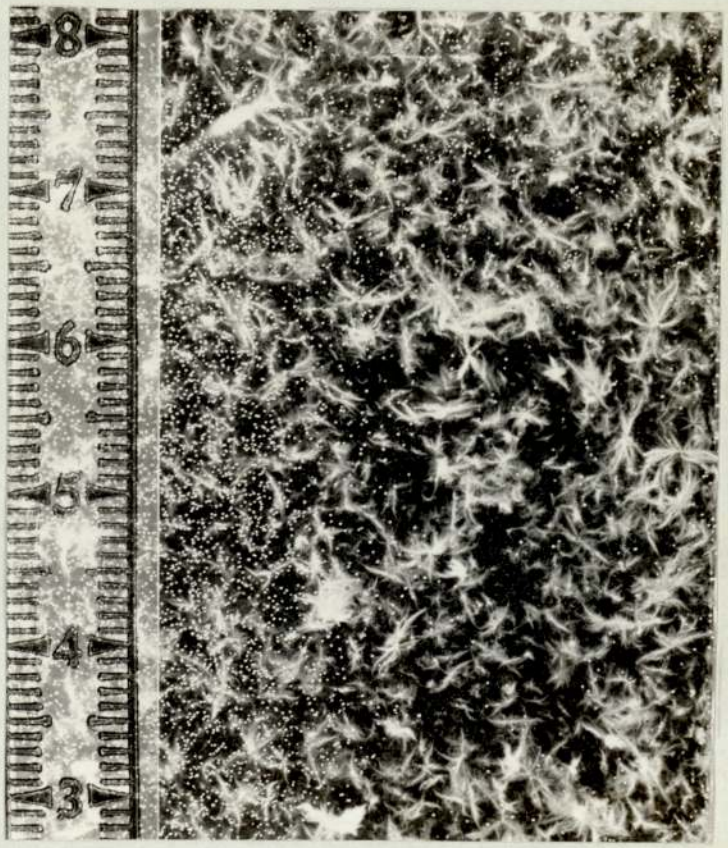
4.3.1.2 THE EFFECT OF TEMPERATURE ON THE MOULD MORPHOLOGY.

The progressive changes in mould morphology with increase in temperature are shown in a series of photographs taken at 35, 40, 42 and 45°C (Plates 4.5, 4.6, 4.7 and 4.8 respectively). These can also be compared to Plate 4.3 which shows the morphology at 30°C though at a slightly higher dilution rate, 0.073 h^{-1}.

The colonies at 30 and 35°C were indistinguishable to the naked eye (Plates 4.3, 4.5). They consisted of a number of radially arranged feather-like, tangled bundles of mycelium, up to approximately 6 mm diameter.

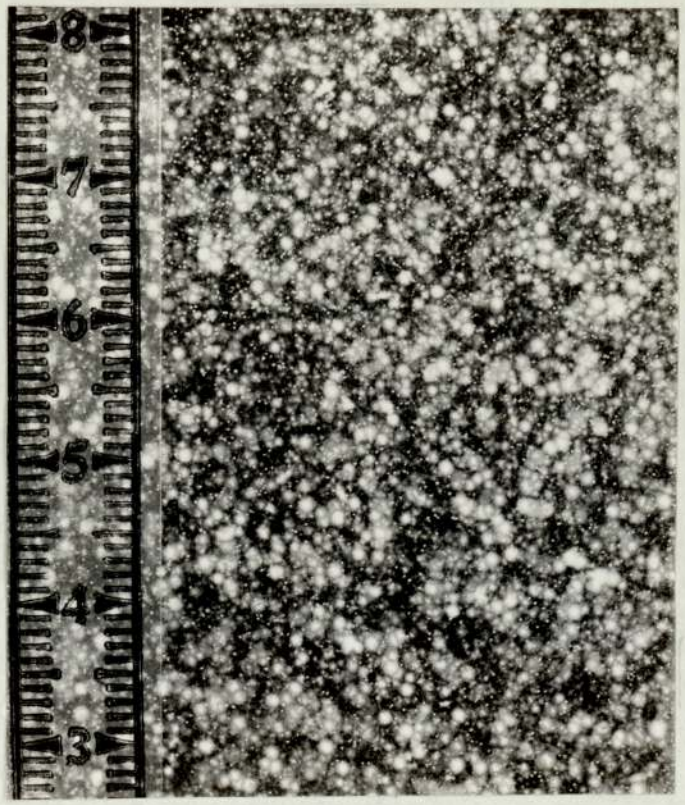
Increasing the temperature above 38°C resulted in the formation of small ($\approx$ 1 mm diameter) densely packed, "inhibited" mycelial colonies (Plate 4.6). At 42°C these were larger ($>$ 2 mm diameter) but fewer in number, with the presence of very small filamentous colonies becoming more obvious. The colour of

Plate 4.5 Aspergillus niger M1 : The Morphology after 167 h.
Continuous Cultivation at 35°C.
(Fermentation SR 7; D= 0.067 h^{-1}; s_R =13.2g.l^{-1} gluc. eq.)



1 cm.

Plate 4.6 Aspergillus niger M1 : The Morphology after 259 h.
Continuous Cultivation at 40°C.
(Fermentation SR 6; D= 0.067 h^{-1}; s_R =13.2g.l^{-1} gluc. eq.)

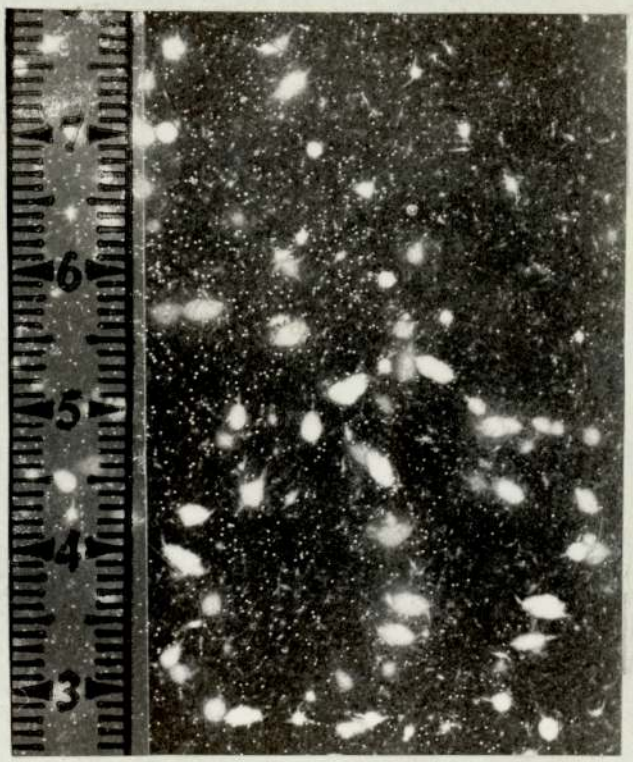


1 cm.

Plate 4.7 Aspergillus niger M1 : The Morphology after 217 h.

Continuous Culture at 42°C.

(Fermentation SR 6; D= 0.067 h^{-1}; s_R =13.2g.l^{-1} gluc. eq.)



1 cm.

Plate 4.8 Aspergillus niger M1 : The Morphology after 76 h.
Continuous Cultivation at 45°C.
(Fermentation SR 6; D= 0.067 h^{-1}; s_R=13.2g.l^{-1} gluc. eq.)

1 cm.

the culture as a whole changed from cream to light grey.

After 76 h. at 45°C little mycelium remained in the fermenter. It was in the form of very small "wisp-like" colonies which were grey in colour.

The changes are those expected in response to the progressive increase in temperature from being optimum for growth, to being inhibitory and finally to causing death of the mould.

4.3.1.3. CONCENTRATIONS OF ORGANISM IN THE FERMENTER (x_F) AND EFFLUENT STREAM (x_E).

The use of a constant dilution rate (and, therefore, s.l.v.) ensured that changes in $\bar{x}_F$ and $\bar{x}_E$ were due solely to temperature related factors. Changes in the morphology of the mould (section 4.3.1.2), both of the packing density and resistance to fluidisation, were in effect quantified as changes in $\bar{x}_F$ (illustrated in Fig. 4.14).

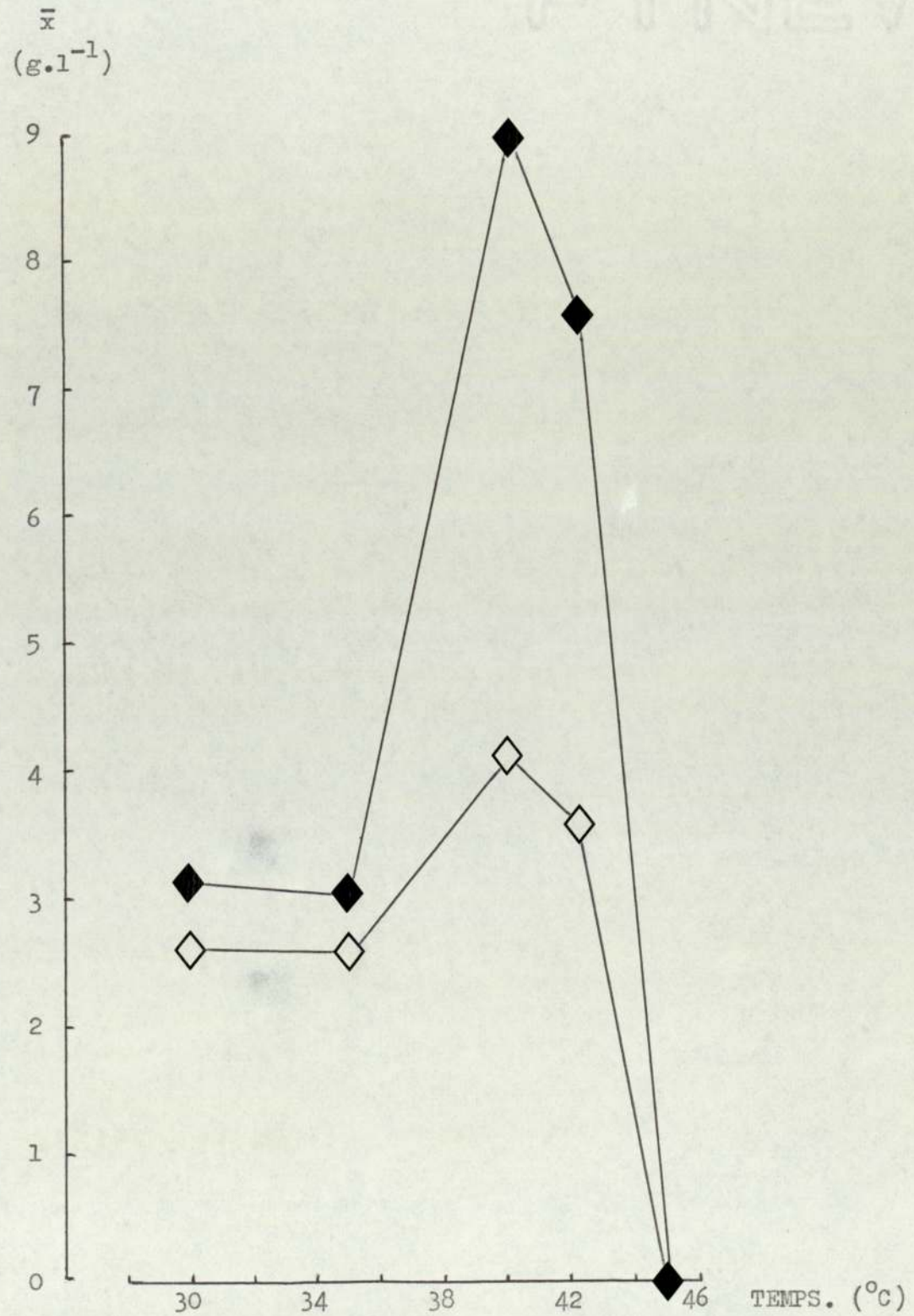
In the case of the 30°C and 35°C fermentations, when the colonies were indistinguishable, the respective values of $\bar{x}_F$ and $\bar{x}_E$ were almost the same. However, the change in morphology at approximately 37 - 38°C resulted in an increase in $\bar{x}_F$ from 3 $g.l^{-1}$ to 9 $g.l^{-1}$.

At 45°C both $\bar{x}_F$ and x_E declined to less than 0.1 $g.l^{-1}$. After 94 h., this temperature was reduced to 38°C but the culture could only be re-established by re-inoculation with spores. This temperature, therefore, is believed to be above the thermal death point of A. niger M1 and so $\bar{x}_F$ and $\bar{x}_E$ assumed to be zero.

Fig. 4.14 The Effect of Temperature on the Mould Concentration in the Fermenter (x_F) and Effluent Stream (x_E).

($D = 0.067\ h^{-1}$; $s_R = 13.2\ g.l^{-1}$ gluc. eq.)

(◆ x_F ; ◇ x_E)



4.3.1.4 THE SPECIFIC GROWTH RATE (μ) AND PRODUCTIVITY OF THE ORGANISM.

The effects of temperature on the Productivity and Specific Growth Rate (μ) of cultures of A. niger M1 are shown in Figs. 4.15 and 4.16 respectively.

The productivities at 40 and 42°C (0.26 - 0.27 $g.l^{-1}.\ h^{-1}$) were approximately 50% higher than those at 30 and 35°C and were in fact higher than when D = 0.093 h^{-1} at 30°C. However, this increase was counteracted by the increase in $\bar{x}_F$ by a factor of 3x, so that μ decreased considerably at temperatures above 37 - 38°C.

As the mould was presumed to be dead in the 45°C fermentation, both these parameters were assumed to be zero at steady state at this temperature. As x_F declined, two negative values of productivity and, therefore, μ were noted (μ = - 0.010 h^{-1}). These could have been due to small errors in the determination of x_F and x_E (which were both $< 0.5\ g.l^{-1}$) or could have represented a specific rate of autolysis (as explained in section 3.6.5).

4.3.1.5 STARCH HYDROLYSIS AND UTILIZATION RATES.

The rate of production of reducing sugars increased gradually up to 40°C and then declined (Fig. 4.17). The maximum rate was 0.8607 $g.l^{-1}.\ h^{-1}$ compared to a substrate supply rate of 0.89 $g.l^{-1}.\ h^{-1}$ (glucose equivalent) throughout this series of experiments.

Point A represents the reducing sugar production rate (0.648 $g.l^{-1}.\ h^{-1}$) after 94 h. continuous fermentation at 45°C. However, had the fermentation been continued, this rate would have decreased to zero as washout of enzymes from the system took place.

Fig. 4.15 The Effect of Temperature on the Mould Productivity.

($D = 0.067\ h^{-1}$; $s_R = 13.2\ g.l^{-1}$ gluc. eq.)

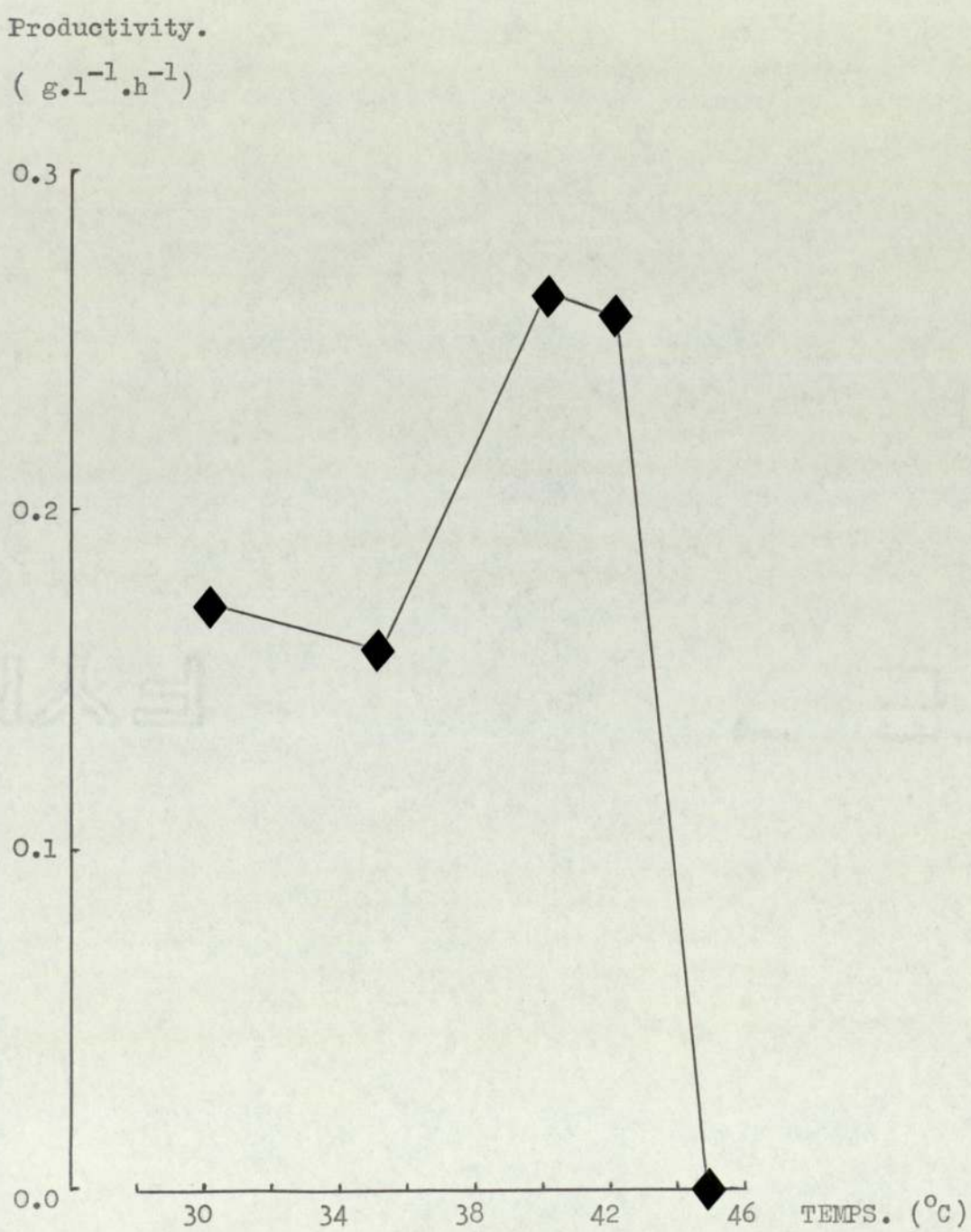


Fig. 4.16 The Effect of Temperature on the Specific Growth Rate (μ) of the Mould.

($D = 0.067\ h^{-1}$; $s_R = 13.2\ g.l^{-1}$ gluc. eq.)

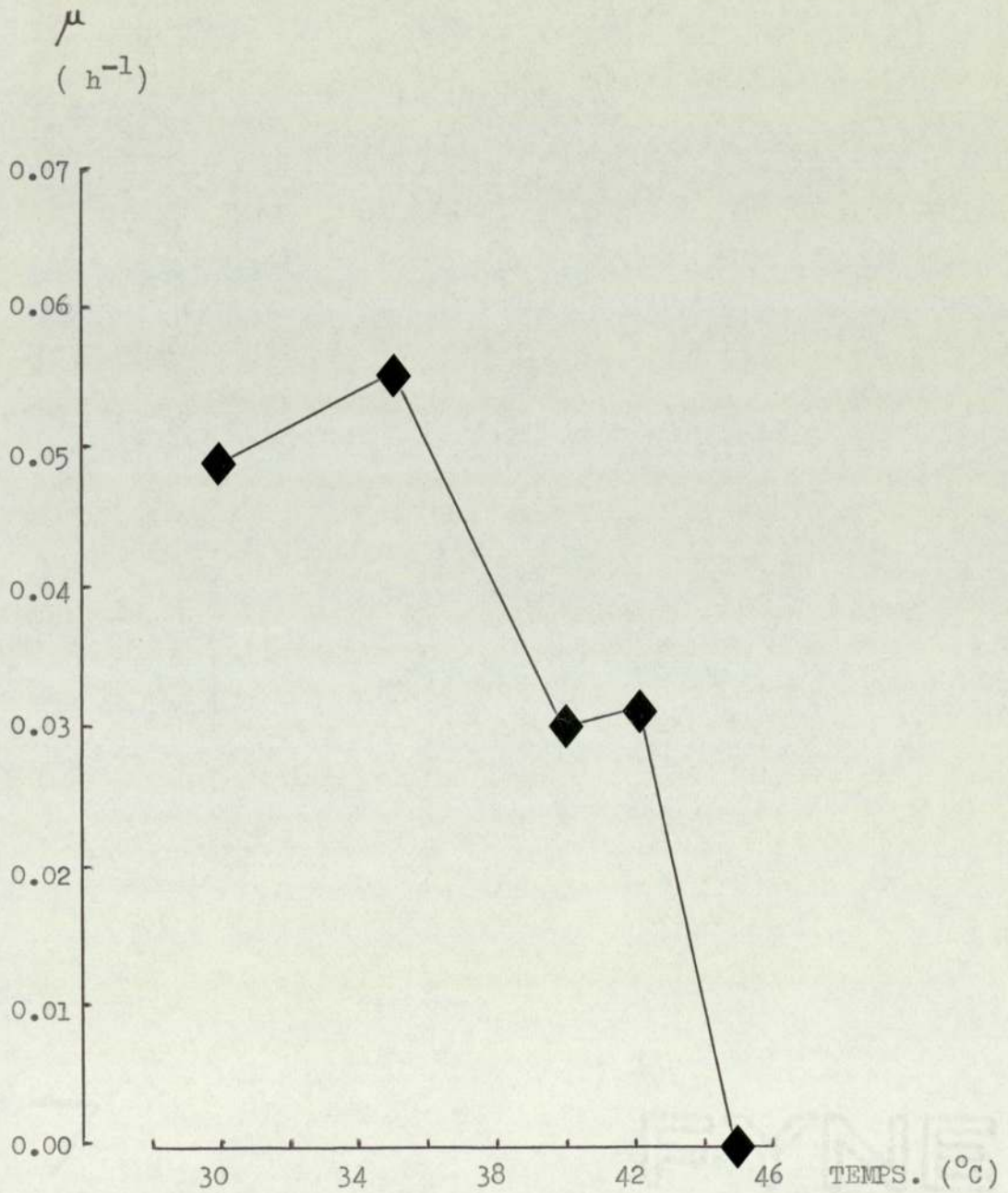
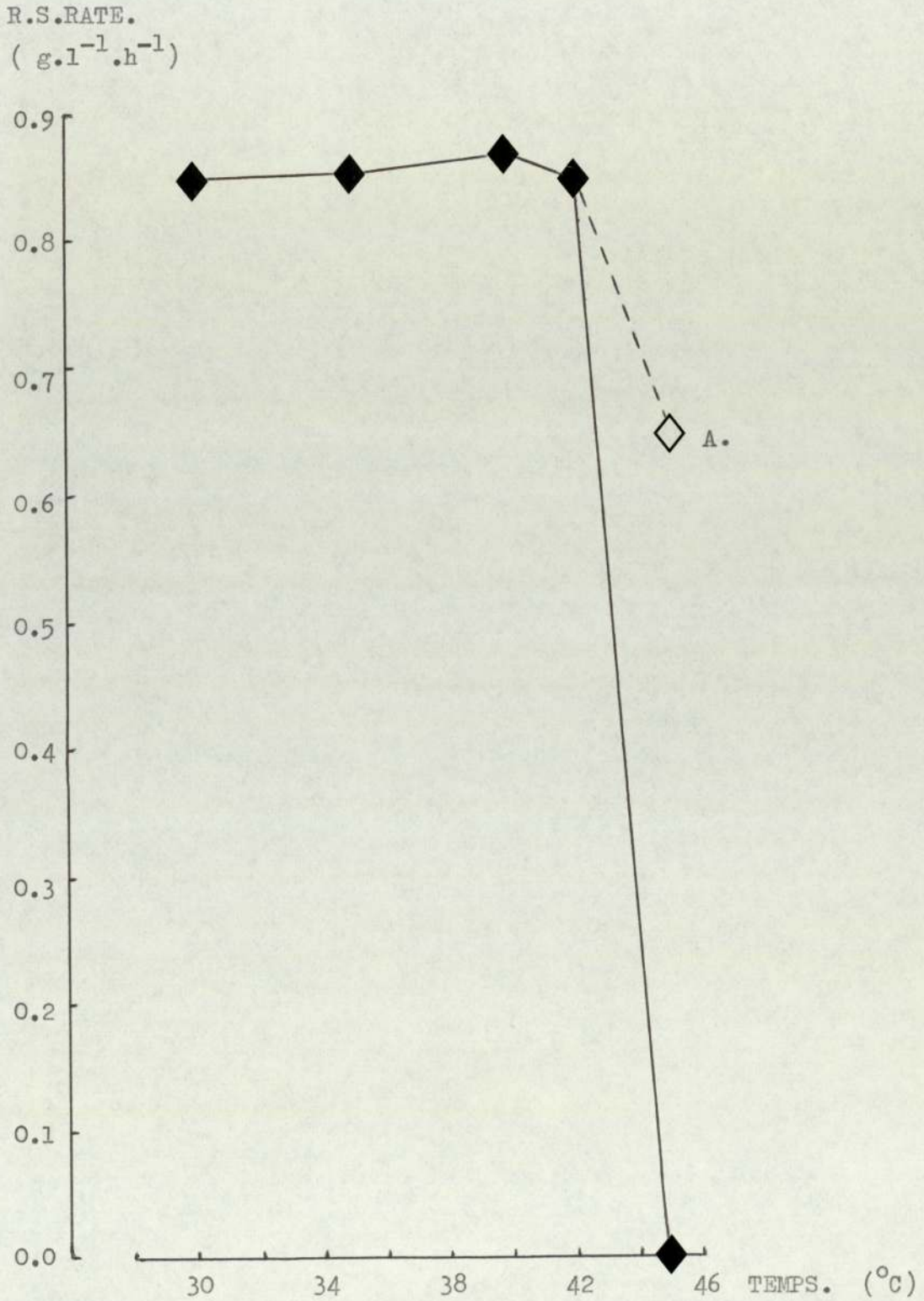


Fig. 4.17 The Effect of Temperature on the Reducing Sugar Production Rate.

($D = 0.067\ h^{-1}$; $s_R = 13.2\ g.l^{-1}$ gluc. eq.)



The substrate utilization rates of the culture both totally ($g.l^{-1}.h^{-1}$) and specifically ($g.g^{-1}.\ h^{-1}$) show the same response to temperature as mould productivity and μ respectively (see Fig. 4.18). The total rates of mould productivity and substrate utilization were considerably higher at 40 and 42°C than at 30 - 35°C. However, as a result of the much higher values of $\bar{x}_F$, both μ and the specific substrate utilization rate (q) were much lower at elevated temperatures.

4.3.1.6 THE NITROGEN CONTENT OF THE MOULD.

When considering the effect of temperature on the mould nitrogen content it is essential to distinguish two possibilities:

(A) That temperature affects the macromolecular composition and therefore the nitrogen content of the organism directly.

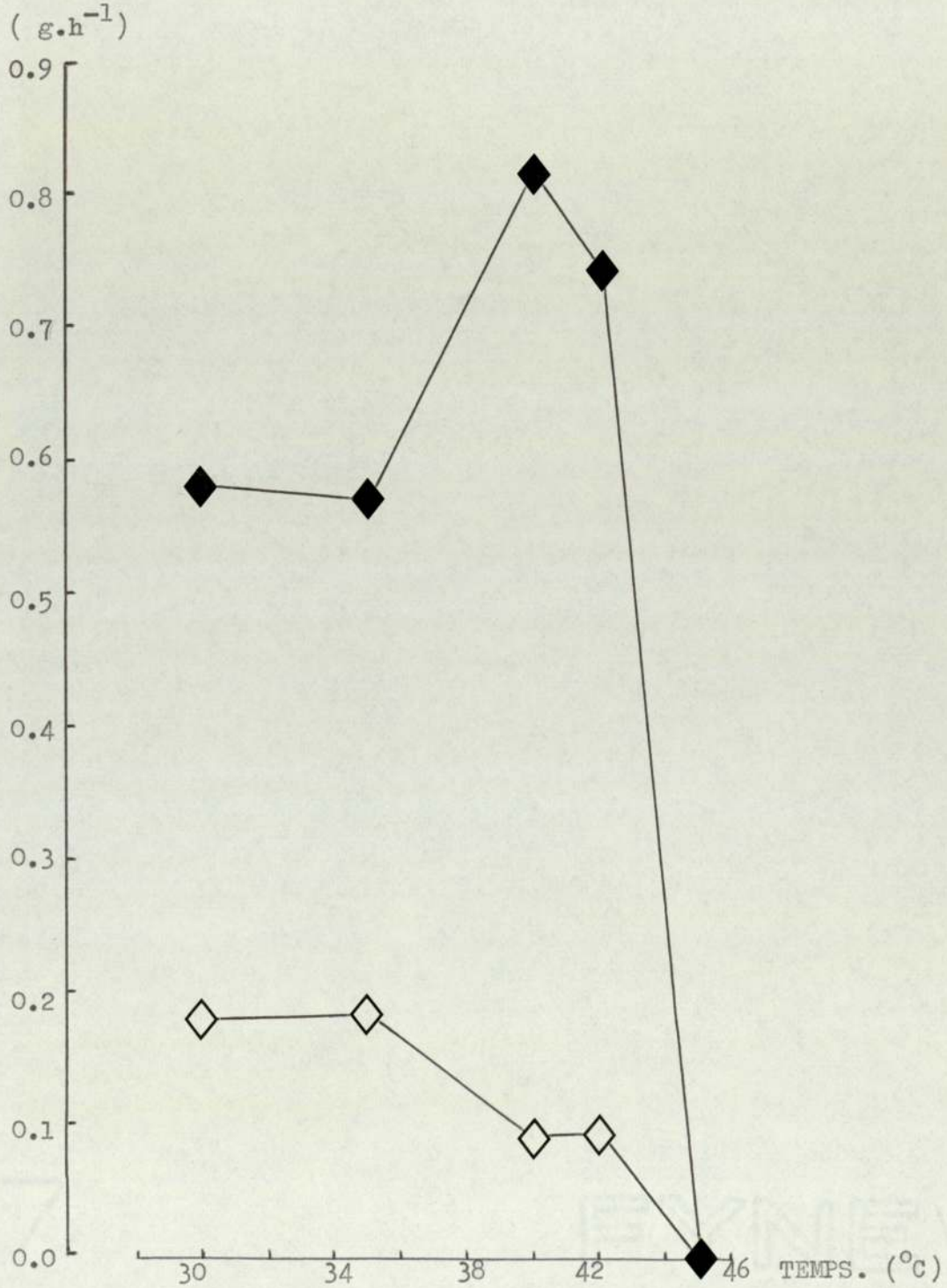
(B) That temperature acts indirectly on nitrogen content via the relationship of both these parameters with μ (see section 4.2.5, Fig. 4.10).

Fig. 4.19 shows the effect of temperature on this parameter. Points joined by a solid line (designated A) are the steady state experimental values. Each is compared with a point (on the line designated B) which represents the equivalent nitrogen content at the same μ in a 30°C fermentation (derived from Fig. 4.10).

In all cases, increasing the temperature resulted in a lower "biomass" nitrogen content; in each steady state the value was significantly lower than would have been expected at the same specific growth rate at 30°C. This indicates that both

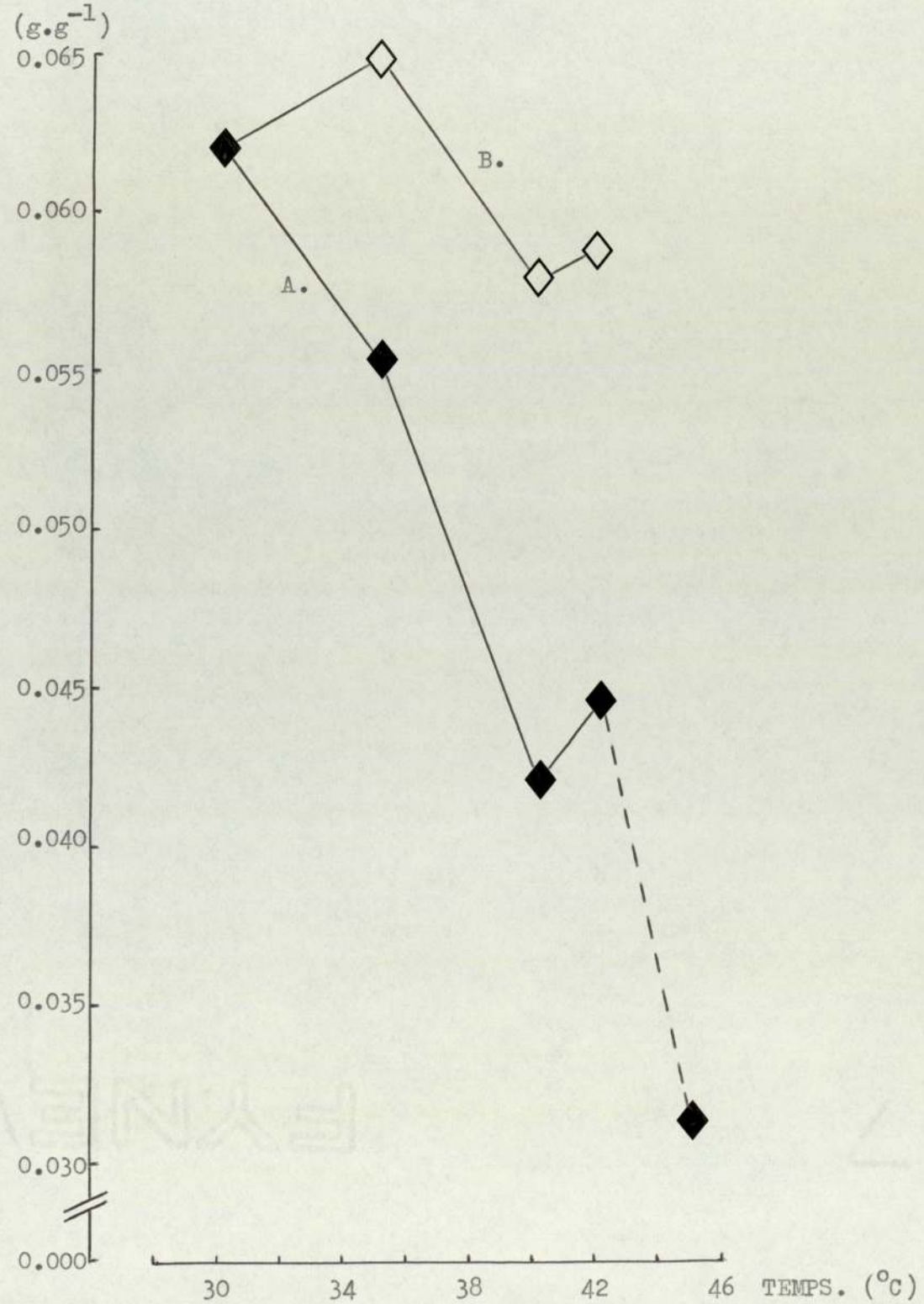
4.18 The Effect of Temperature on the Total and Specific Substrate Utilization Rates.

($D = 0.067\ h^{-1}$; $s_R = 13.2\ g.l^{-1}$ gluc. eq.)
(◆ Total, $g.l^{-1}.h^{-1}$; ◇ Specific, $g.g^{-1}.h^{-1}$.)



4.19 The Effect of Temperature on the Nitrogen Content of the Mould.

($D = 0.067\ h^{-1}$; $s_R = 13.2\ g.l^{-1}$ gluc. eq.)
(◆ Readings; ◇ 30°C equivalents.)



possibilities, A and B, contributed to the diminution of this factor.

The minimum steady state nitrogen content was 0.04235 $g.g^{-1}$ at 40°C, but the lowest individual reading was that of mould "washed out" of the 45°C fermentation, 0.03159 $g.g^{-1}$.

4.3.1.7 THE YIELD COEFFICIENT ($Y_{x/s}$).

This factor showed the same discontinuity in response to temperature as the other parameters directly related to the mould physiology (Fig. 4.20). From 30 - 35°C $Y_{x/s}$ declined, but as a result of the morphology change at 37 - 38°C increased at the higher temperatures. The values at 40 and 42°C ($\approx$ 0.34 $g.g^{-1}$) were also higher than their equivalents at the same μ at 30°C.

These low values could also be explained by the premise that the culture was oxygen limited. The more "pelleted" form of the mycelium at higher temperatures would have permitted a greater oxygen transfer rate and therefore an increased value of $Y_{x/s}$. However this effect would have been counteracted by the higher mould concentration (x_F) and the direct effect of temperature on the oxygen transfer rate. This situation is yet another aspect of the complex interaction of the mould and the environmental conditions.

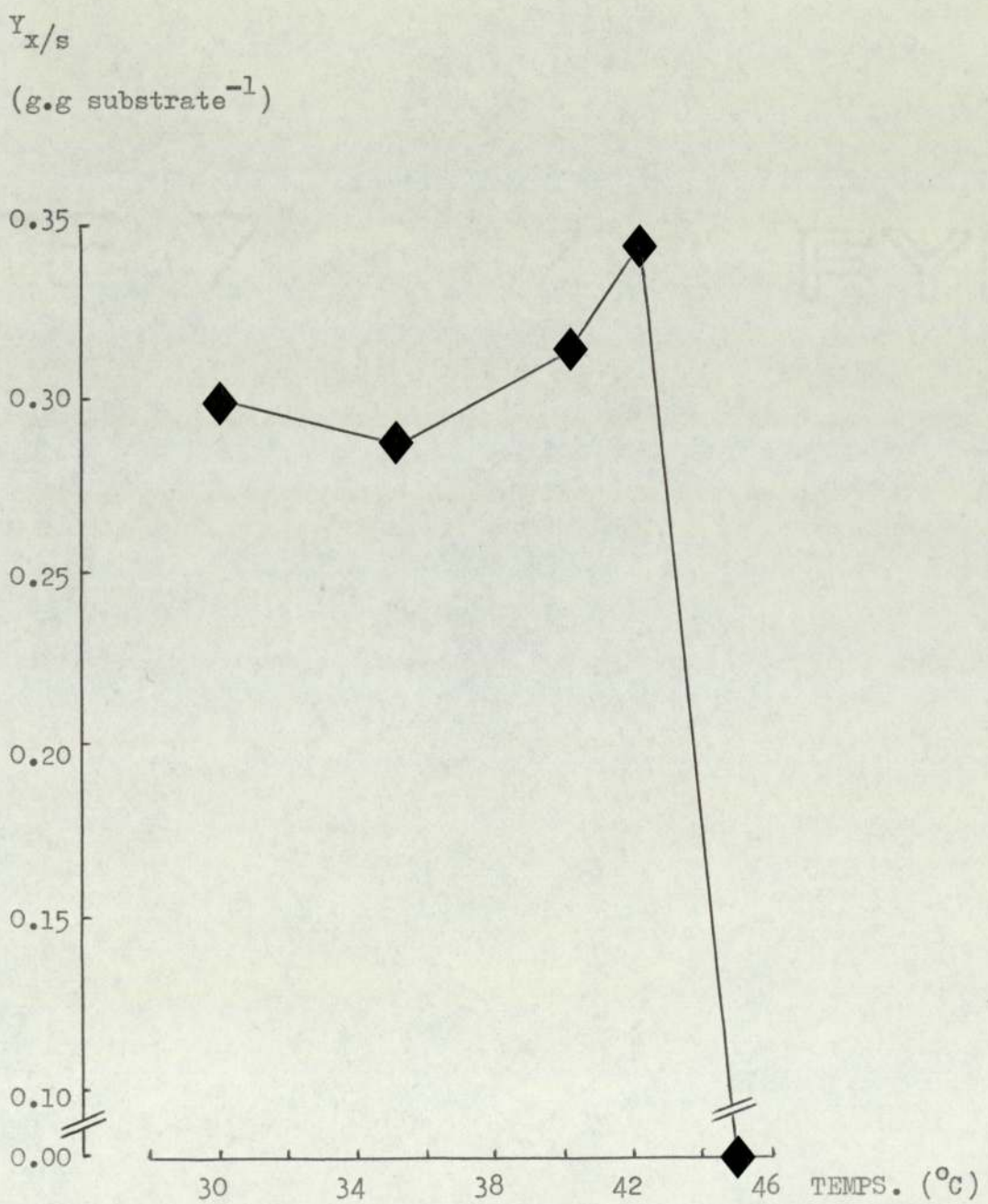
4.3.2 EFFECTS OF MEDIUM pH.

4.3.2.1 INTRODUCTION.

Three fermentations were carried out (two at 30°C, one at 35°C) to investigate the effect of alkaline medium on continuous

4.20 The Effect of Temperature on the Yield Coefficient of the Mould ($Y_{x/s}$).

($D = 0.067\ h^{-1}$; $s_R = 13.2\ g.l^{-1}$ gluc. eq.)



cultures of A. niger M1, prior to the alkaline potato peel waste experiments. The anticipated pH of a medium made up from such a waste was 10.5, so these investigations were carried out at pH 11.0 to allow a reasonable margin for error. The same medium was used throughout, having a substrate concentration of 13.2 $g.l^{-1}$(glucose equivalent).

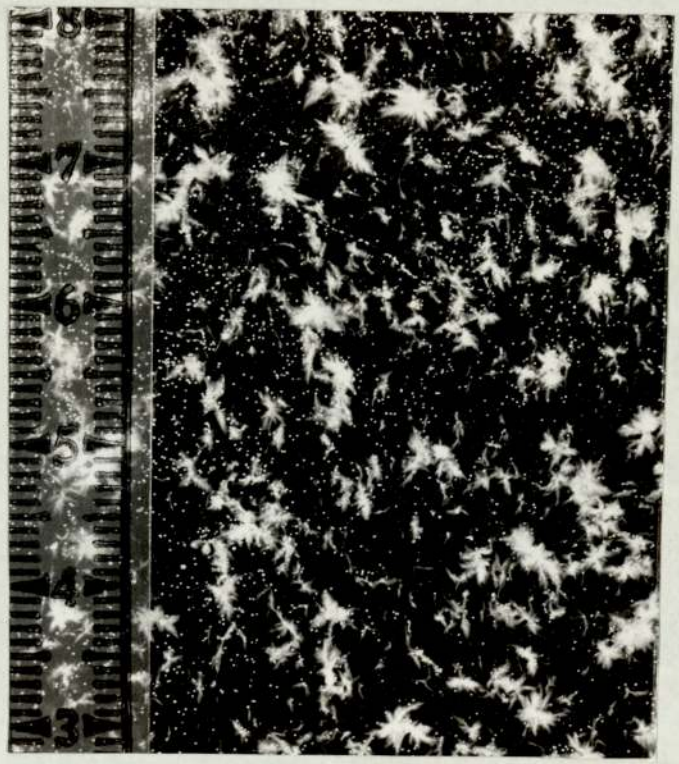
The actual pH adjustment was carried out in a small chamber (c. 600 ml. capacity) inserted in the medium supply line between the medium pump and the fermenter. This chamber, whose contents were magnetically stirred, was equipped with a pH probe and an alkalie injection needle to enable a pH meter-controller/alkalie pump system to be used. Carrying out this adjustment within a closed system obviated problems of contamination and losses of ammonia from the ammonium sulphate in the medium (which occurs above pH 8.0)

With only three sets of steady state data, the conclusions which could be drawn directly were limited. But in each case an identical fermentation was carried out using pH 7.0 medium. Comparison of all six sets of data yielded valuable information on the effect of this factor on the mould cultures.

4.3.2.2 THE MORPHOLOGY OF THE MOULD.

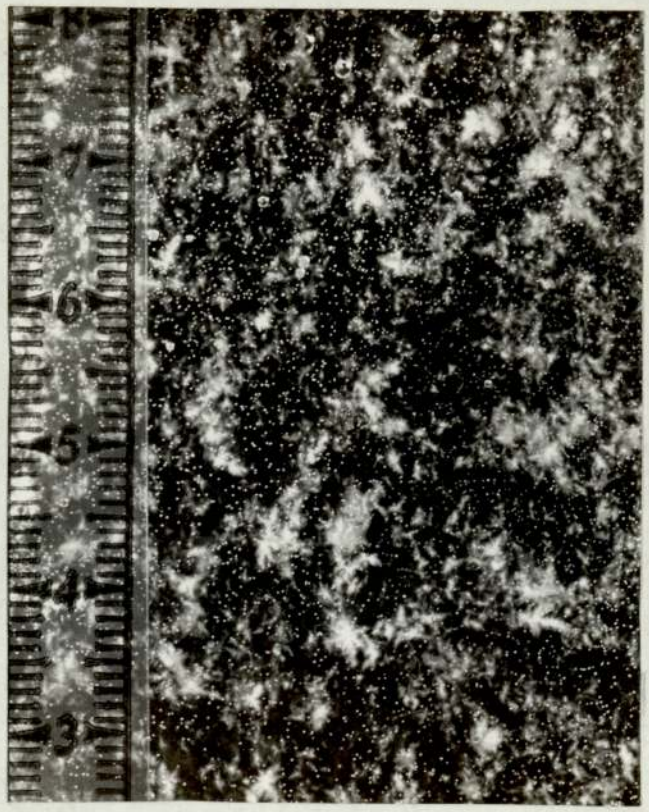
The colonies retained the same overall structure as in 30 and 35°C, pH 7.0 medium fermentations, that of a number of tangled bundles of mycelium radiating from a central point. However, within each bundle the hyphae appeared to be more numerous and less tightly packed. To the naked eye, this gave the colonies

Plate 4.9 Aspergillus niger M1 : The Morphology after 211 h.
Continuous Cultivation at 30°C on pH 11.0 Medium.
(Fermentation SR 5; D=0.0724 h^{-1}; s_R=13.2g.l^{-1} gluc. eq.)



1 cm.

Plate 4.10 Aspergillus niger M1 : The Morphology after 64 h. Continuous Cultivation at 35°C on pH 11.0 Medium. (Fermentation SR 7; D=0.0745 h^{-1}; s_R=13.2 $g.l^{-1}$ gluc. eq.)



1 cm.

a "fuller" appearance, without increasing their overall dimensions. A simple analogy would be the difference in appearance of a well nourished and an emaciated animal.

The steady state morphologies of the mould growing on 13.2 $g.l^{-1}$ (glucose equivalent), pH 11.0 starch medium at 30 and 35°C are shown in Plates 4.9 and 4.10 respectively.

4.3.2.3 CONCENTRATIONS OF MOULD IN THE FERMENTER (x_F) AND EFFLUENT STREAM (x_E).

The effects of the two media on the concentration of mould in the fermenter and effluent are compared in Fig. 4.21. The fermenter concentration (x_F) was little affected, in two fermentations it declined by 0.3 - 0.4 $g.l^{-1}$ and in the third remained constant.

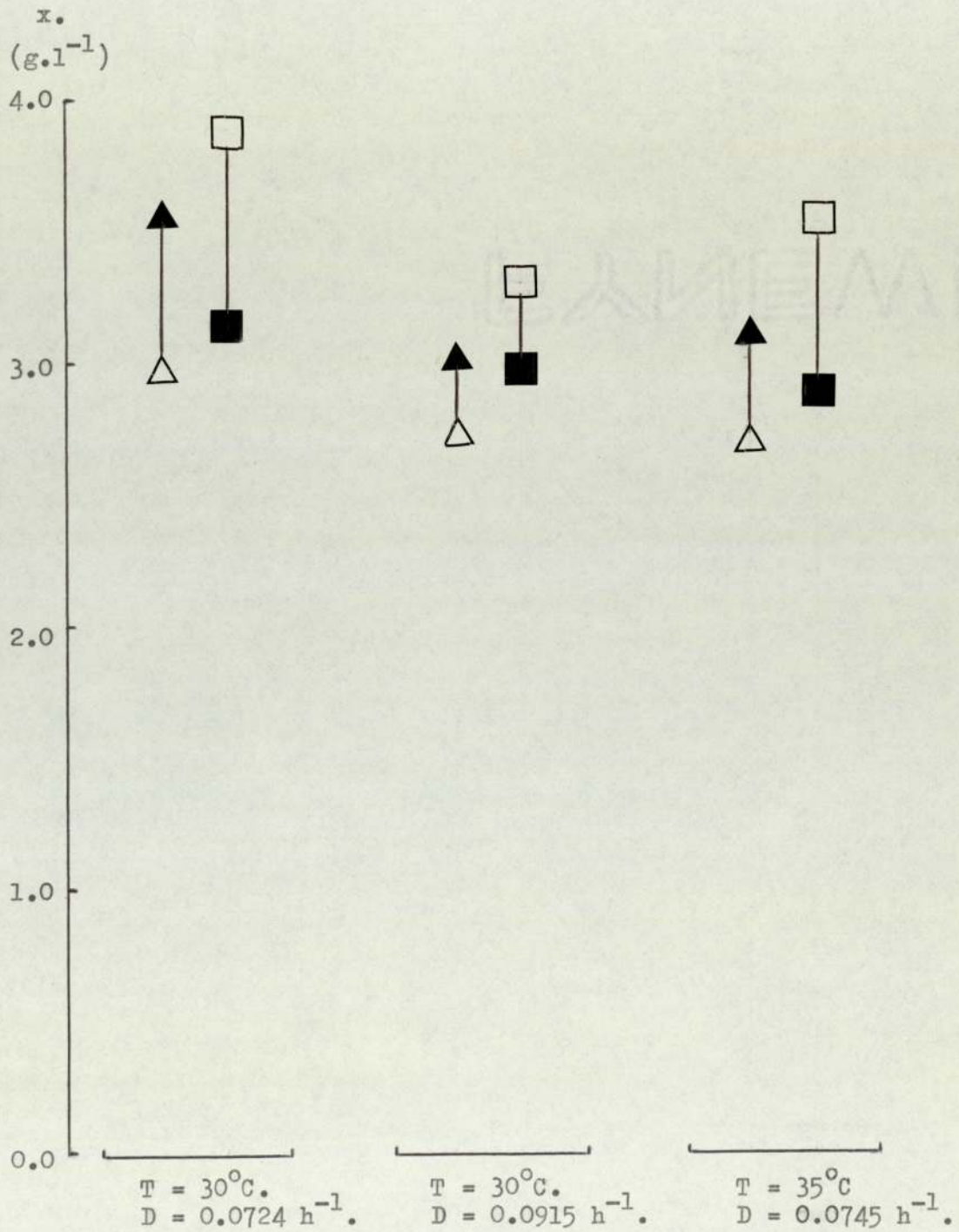
In contrast, $\bar{x}_E$ increased from being consistantly lower than $\bar{x}_F$ (usually about 0.85. $\bar{x}_F$) to being greater than $\bar{x}_F$ (about 1.2. $\bar{x}_F$) in these fermentations. This implied that a concentration mechanism was operating in the fermenter, which is difficult to visualise as a steady state situation in a well mixed culture.

The mould concentration in a tower fermenter is determined by a balance between the superficial liquid velocity (s.l.v.) and the colony fluidisation index - a factor which depends upon the overall density of the colonies and their configuration. One explanation of values of $\bar{x}_E$ exceeding those of $\bar{x}_F$ was that colonies grown on alkaline medium had a lower overall density. This could have been due to minute ($\approx$ 0.5 mm) air bubbles which tended to become adsorbed onto (and possibly entrapped within)

Fig. 4.21 Comparison of the Mould Concentrations in the Fermenter (x_F) and Effluent Stream (x_E) in pH 7.0 and 11.0 Media Fermentations.

(pH 7.0 Medium: ▲x_F; △x_E.)

(pH 11.0 Medium: ■ x_F; □ x_E.)



their structure.

Adsorption of air bubbles in this manner is the basis of "froth flotation" methods widely used in the mining industry for separation of ores. The efficiency varies inversely with particle size but concentration factors of 100 x are not unusual. A foam separation phenomenon occurred in batch cultures of Serratia marcesens in tower fermenters at Aston (306), but had not been noted previously with cultures of A. niger M1. In view of the colony sizes involved, concentration factors of 1.2x could have been the result of this effect.

The phenomenon of $\bar{x}_E > \bar{x}_F$ could also have been due to the existence of a concentration gradient in the fermenter. Additional sampling was therefore carried out from a second port in the upper quarter of the fermenter. Although there were differences in the values of x_F between the upper and lower sample ports ($\pm$ 0.1 $g.l^{-1}$ in samples 3.0 - 3.5 $g.l^{-1}$), these appeared to be random and were probably due to sampling errors. There was no evidence to support the hypothesis that a concentration gradient existed in the fermenter under these conditions.

4.3.2.4 PRODUCTIVITY AND SPECIFIC GROWTH RATE.

A direct consequence of the change in $\bar{x}_E$ was an increase in the mould productivity (by approximately 1.35x) in these fermentations (Fig. 4.22). Use of alkaline medium (which caused the pH of the fermenter contents to rise to 6.0 - 7.0) resulted in the highest steady state productivity on starch medium being recorded, 0.317 $g.l^{-1}.h^{-1}$ with D = 0.0915 h^{-1}.

Fig. 4.22 Comparison of the Mould Productivity in pH 7.0 and 11.0 Media Fermentations. (● 7.0; ○ 11.0.)

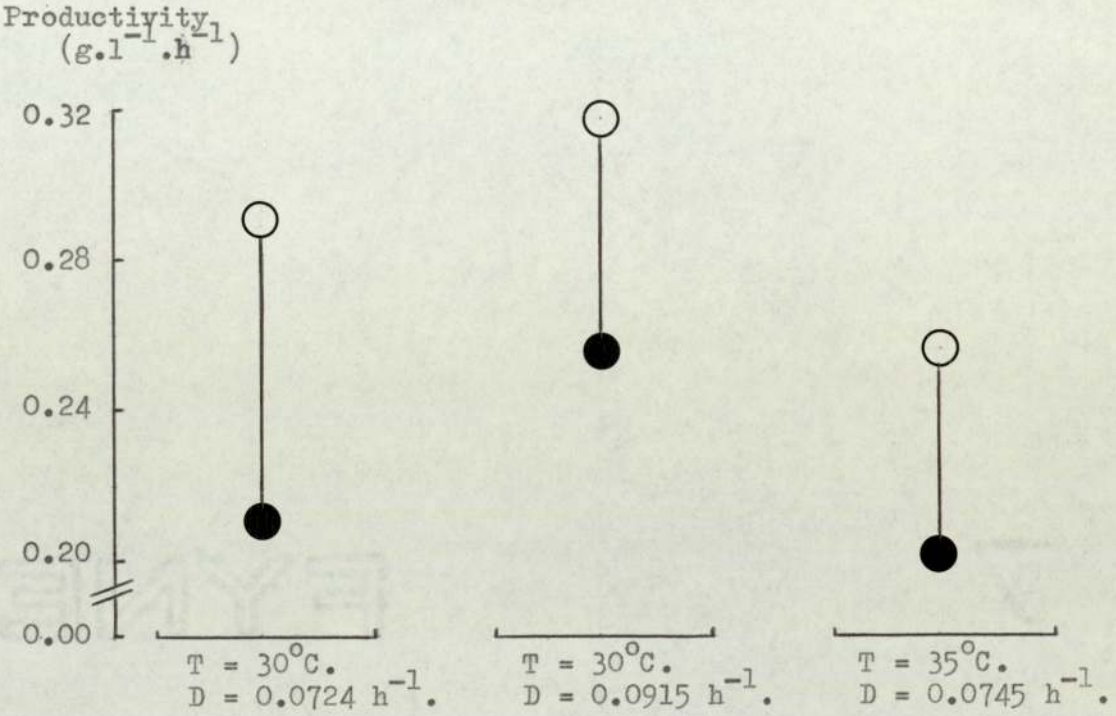
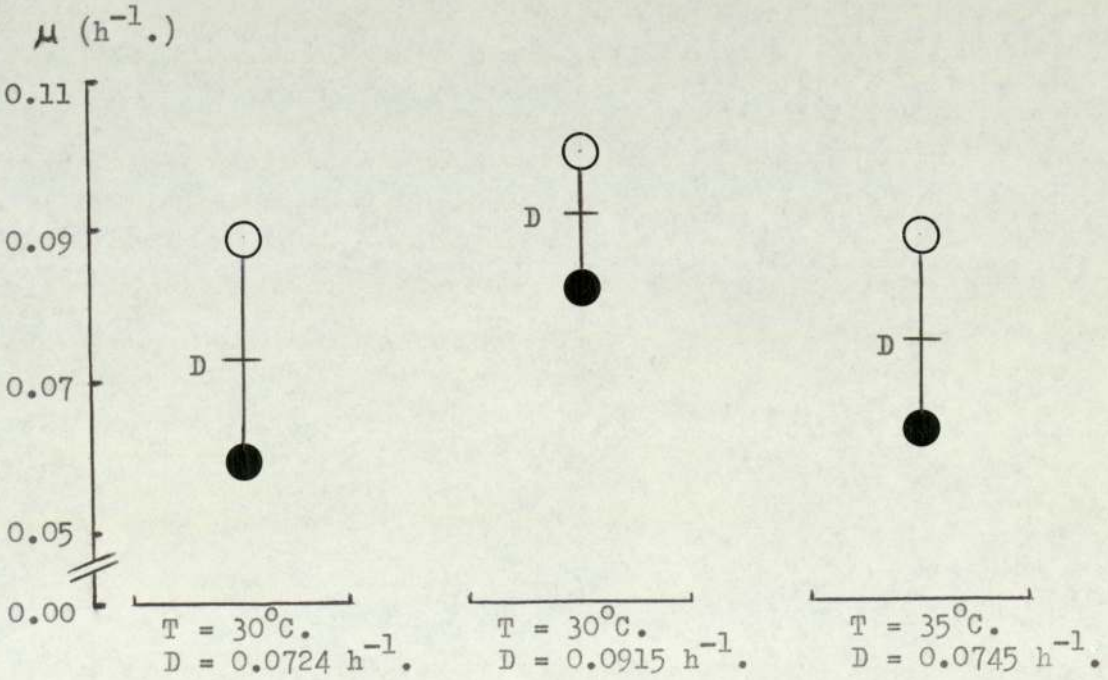


Fig. 4.23 Comparison of the Mould Specific Growth Rate in pH 7.0 and 11.0 Media Fermentations. (● 7.0; ○ 11.0.)



With the increase in productivity as a result of $\bar{x}_E > \bar{x}_F$, the specific growth rate rose. This was expected, as the existence of pH effects and pH optima for the growth of most microorganisms are well known. But, surprisingly, steady states occurred in which μ exceeded D by a considerable margin, usually 10 - 25% (Fig. 4.23).

In the tower fermenter, where x_E is determined by physical forces, there can be no theoretical objections to $\mu > D$ providing nutrients are in excess. Such a situation cannot exist in the majority of fermentation systems, such as the continuous stirred tank fermenter, because vessel design and intense mixing negate the fluidisation and sedimentation effects so that $\bar{x}_F \approx \bar{x}_E$ and $\mu \approx D$.

4.3.2.5 SUBSTRATE UTILIZATION - RELATED FACTORS.

As expected, the rate of conversion of starch to reducing sugars was little affected by the change in medium and, therefore, fermenter pH (Fig. 4.24).

In the 30°C fermentations both total and specific rates of substrate utilization also showed little change (Fig. 4.24), a consequence of the improved yield coefficient (Fig. 4.25). But in the 35°C fermentation $Y_{x/s}$ remained constant and both substrate utilization rates increased considerably.

The differences in response of the fermentations in these characteristics are in contrast to their consistancy with respect to all the other parameters. An explanation of such a difference could lie in the critical effect of mould morphology on the oxygen

Fig. 4.24 Comparison of the Rates of Substrate Hydrolysis and Utilization in pH 7.0 and 11.0 Media Fermentations.

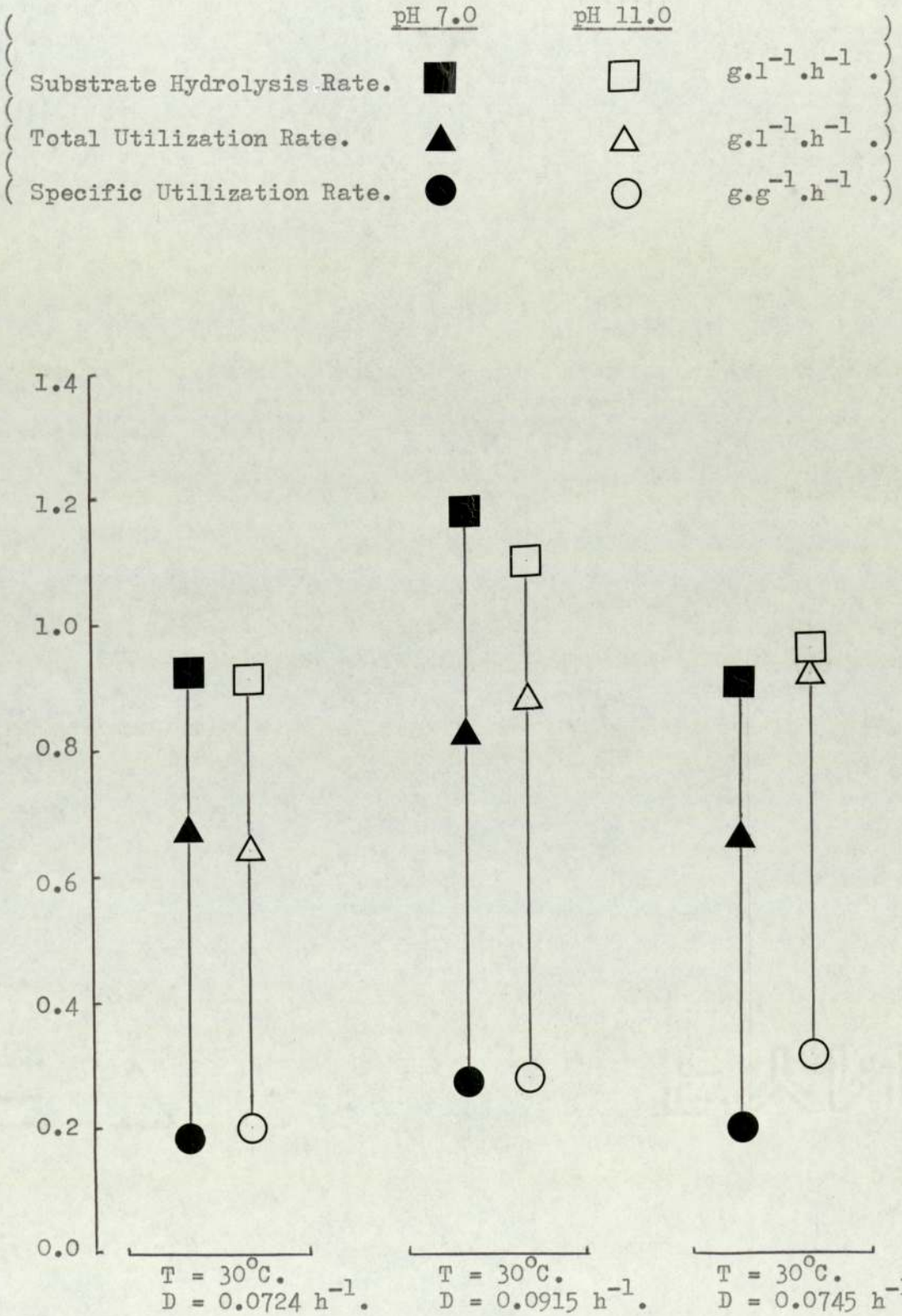


Fig. 4.25 Comparison of the Organism Yield Coefficients in pH 7.0 and 11.0 Media Fermentations. (● 7.0; ○ 11.0.)

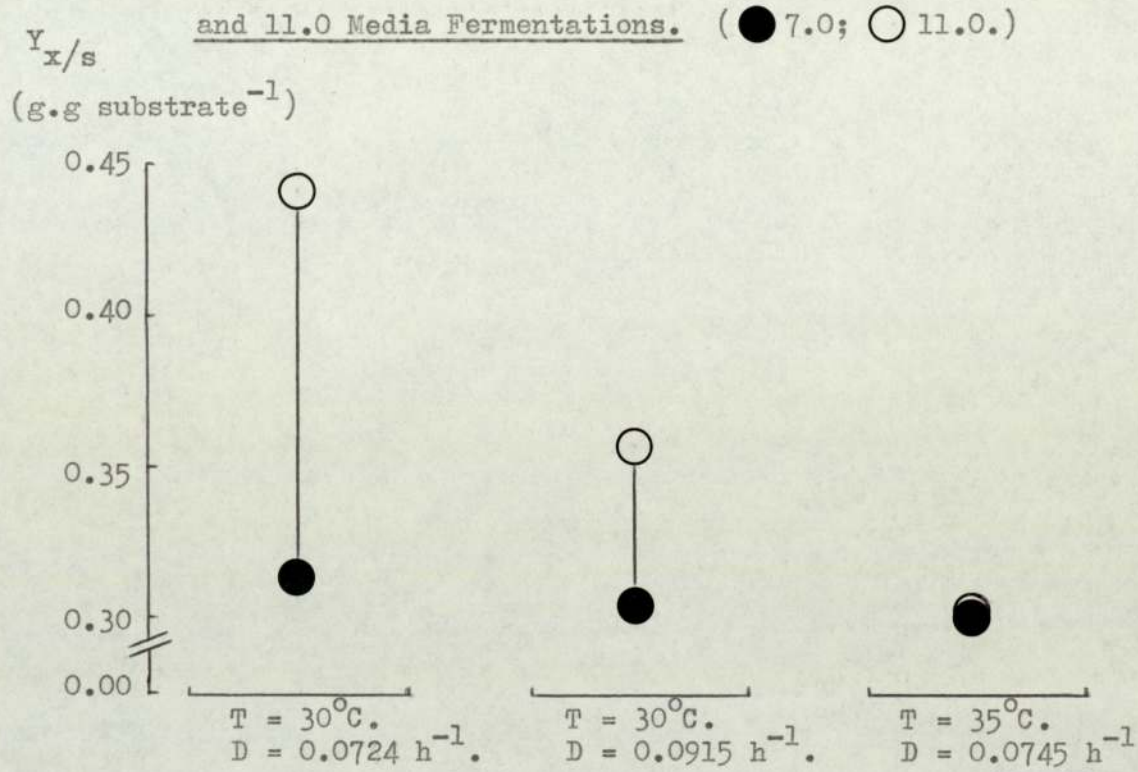
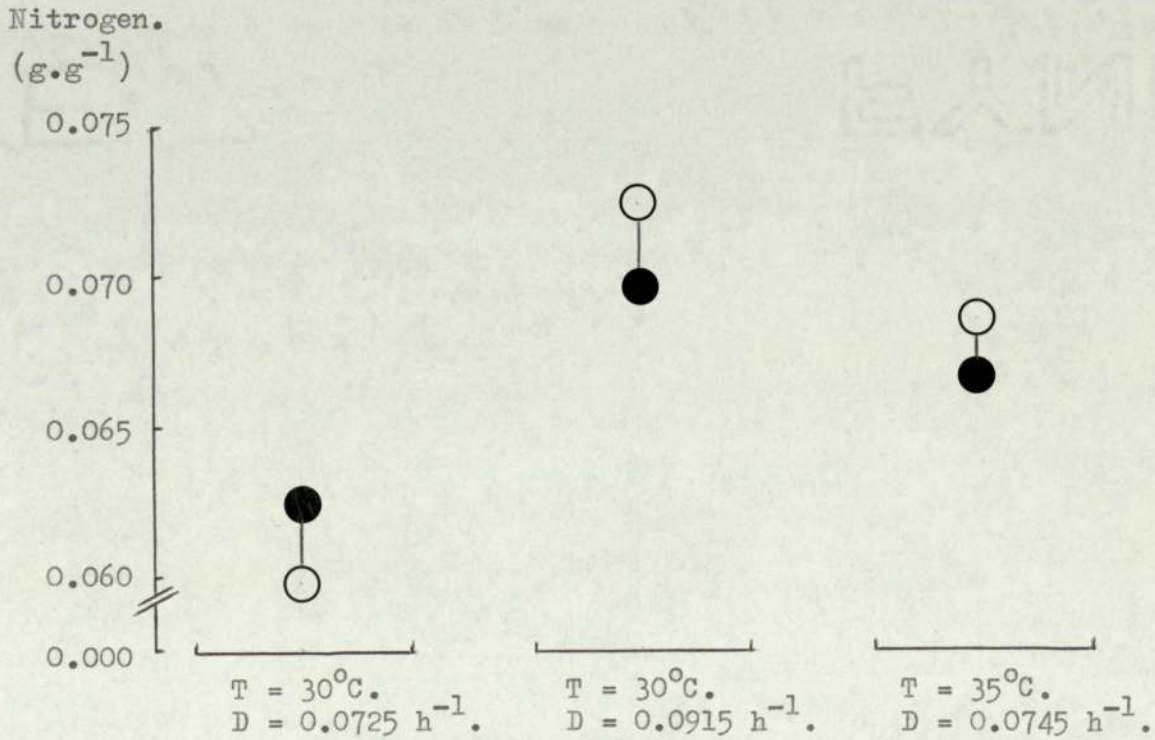


Fig. 4.26 Comparison of the Mould Nitrogen Contents in pH 7.0 and 11.0 Media Fermentations. (● 7.0; ○ 11.0.)



transfer rate of the system.

4.3.2.6 NITROGEN CONTENT OF THE MOULD.

Had the nitrogen content of the mould been determined solely by μ , then the values in each of these fermentations would have risen in proportion to their respective increases in this parameter. However this was not the case, the nitrogen content increased in only two of the fermentations and actually decreased in the other ($D = 0.0725\ h^{-1}$ at $30^{o}C$) as shown in Fig. 4.26.

This response confirms the results of the temperature experiments (section 4.3.1.6) which also indicated that the change in the macromolecular composition of the mould was not due to μ alone.

4.3.2.7 pH OF THE CULTURE.

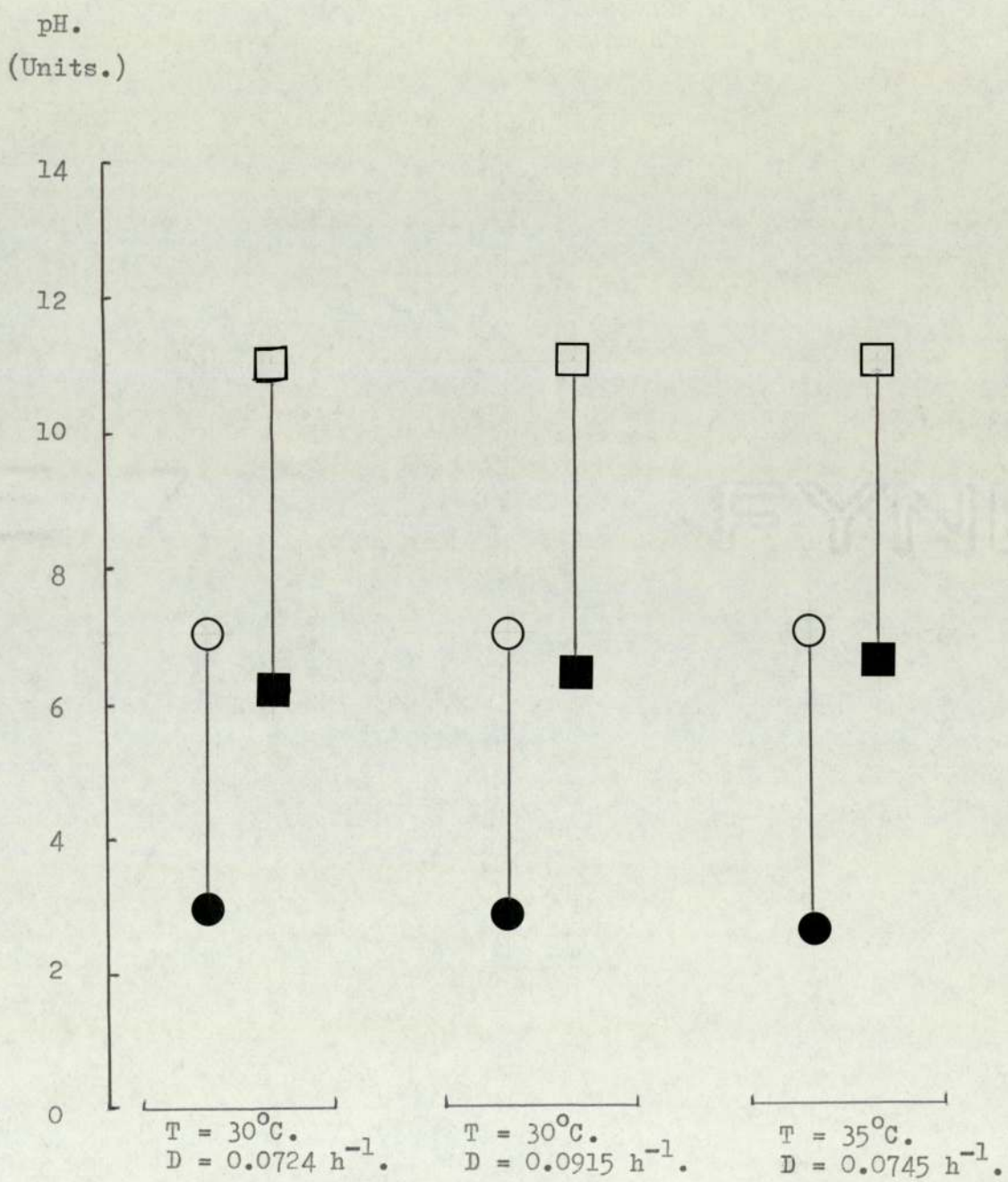
In all three fermentations pH 11.0 medium was neutralized. Stable situations resulted in which the pH of the cultures was between 6.0 and 7.0 instead of 2.0 - 3.0 in the case of pH 7.0 medium (Fig. 4.27). In both cases the pH of the medium was being reduced by 4.0 - 5.0 units, which suggested that acid production probably continued at a similar level.

As the pH of the culture remained below 8.0 there were no problems of loss of the nitrogen nutrient source as free ammonia. However fermentations in this pH range were particularly susceptible to bacterial contamination, especially at dilution rates of less than $0.08\ h^{-1}$ (an s.l.v. of 2.83×10^{-3} cm. sec^{-1}). Above this dilution rate,"wash out" of non-flocculent contaminants took place as a result of froth flotation and fluidisation.

Fig. 4.27 Comparison of the Culture pH values in pH 7.0 and 11.0 Media Fermentations.

(pH 7.0 Medium : ○ Medium ; ● Culture.)

(pH 11.0 Medium: □ Medium ; ■ Culture.)



4.4 POTATO AND PEEL WASTE FERMENTATIONS.

4.4.1 INTRODUCTION.

In total, nearly 3500 hours fermentation were carried out using potato-based media. These proved particularly troublesome as a result of the comparatively small scale of the equipment. Experimentally, the problems were almost entirely in making up batches of medium of constant composition, which resulted in difficulties in maintaining steady states for long periods. Under substrate limited conditions especially, "steady state" values tended to fluctuate far more than in starch based, semi-defined media fermentations.

The other major problem with this series of fermentations was in the interpretation of the experimental results. The "total filterable solids" content of the fermenter consisted of both mould and non-fermentable solids (NFS) such as particles of soil and cellulosic debris (from the potato skin, etc.). The latter materials "diluted" the true organism content of the fermenter and to a certain extent contributed to the colony morphology. This relationship is analagous to that of non-viable cells or hyphae being present in, but not contributing to the growth of a culture.

In the treatment of the results, therefore, attempts were made to express parameters in terms relative to both the organism fraction and to the whole fermenter contents. This necessitated two assumptions regarding the relationship between the mould and NFS concentrations:

(A) That, at steady state, the concentration of NFS in the

effluent receiver was equal to that in the medium i.e. NFS did not accumulate in the fermenter.

(B) That the ratio of mould : NFS in the fermenter was the same as that in the effluent i.e. neither was selectively "washed out" from, or retained in the fermenter.

These assumptions were justified by the experimental observations. As the mould grew, NFS became entrapped and immobilized in the colony structure, while material harvested from the fermenter and the product receiver was indistinguishable to the naked eye. Steady states were maintained for periods of 200 - 300 h. during which changes due to a build-up of NFS would have been noted.

In this section, results calculated using these assumptions are presented in both forms, relating to the fermenter contents as a whole and to the mould fraction alone.

4.4.2 TOTAL SOLIDS CONCENTRATION IN THE FERMENTER AND EFFLUENT STREAM.

4.4.2.1 UNDER CONDITIONS OF SUBSTRATE EXCESS (Figs. 4.28 and 4.29).

At low dilution rates ($< 0.04\ h^{-1}$) the total solids concentrations in the fermenter were within the same range as those obtained with semi-synthetic media under the same conditions. This was expected as with similar colony morphologies and therefore packing densities and fluidisation indices, the tower could not support higher concentrations of mould/NFS. So increases in productivity could only be shown as changes in the effluent stream concentrations, which in fact rose considerably (by approx. 50%).

Fig. 4.28 Comparison of the Filterable Solids and Assumed Mould Concentrations in the Fermenter with Excess Substrate.

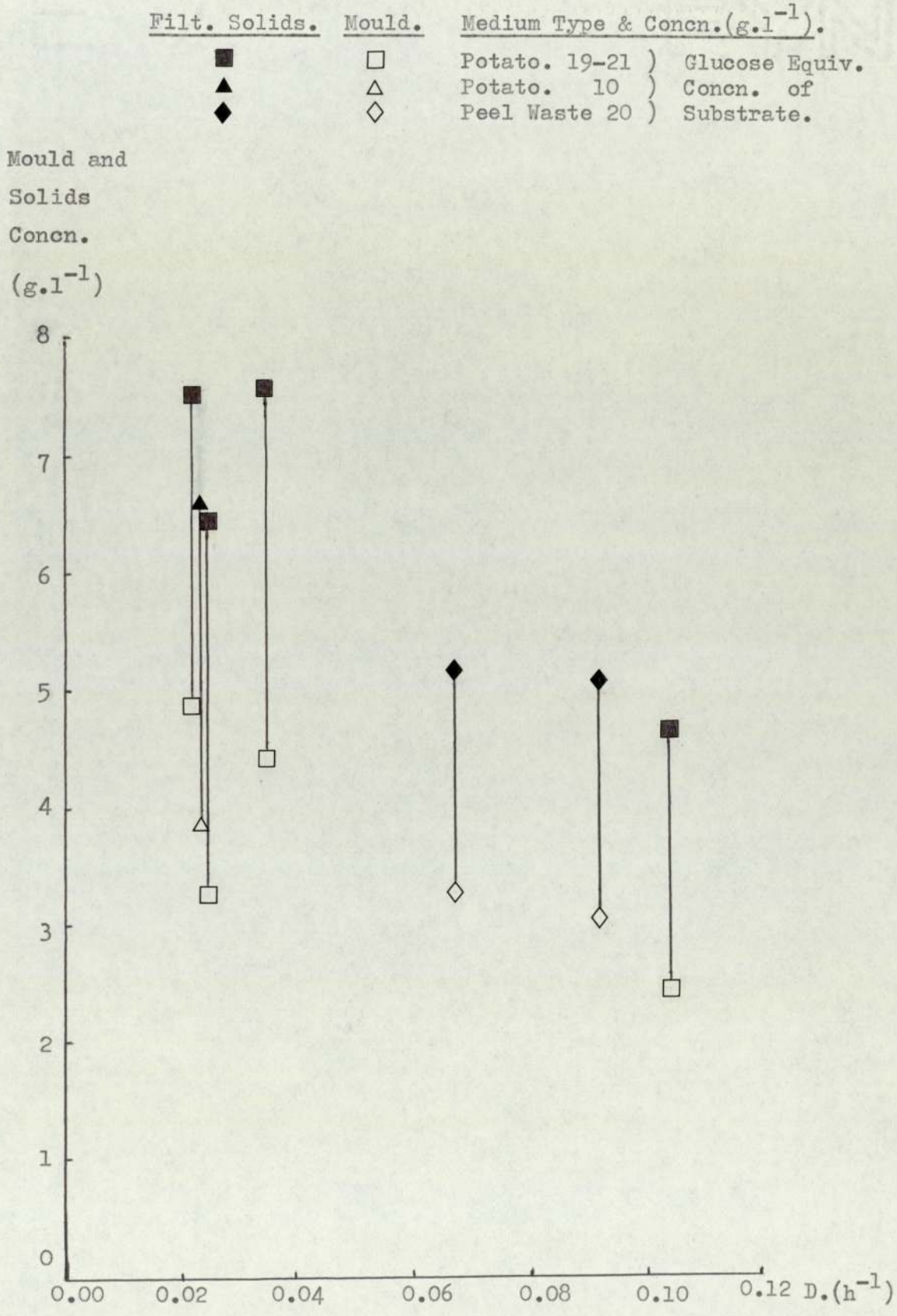
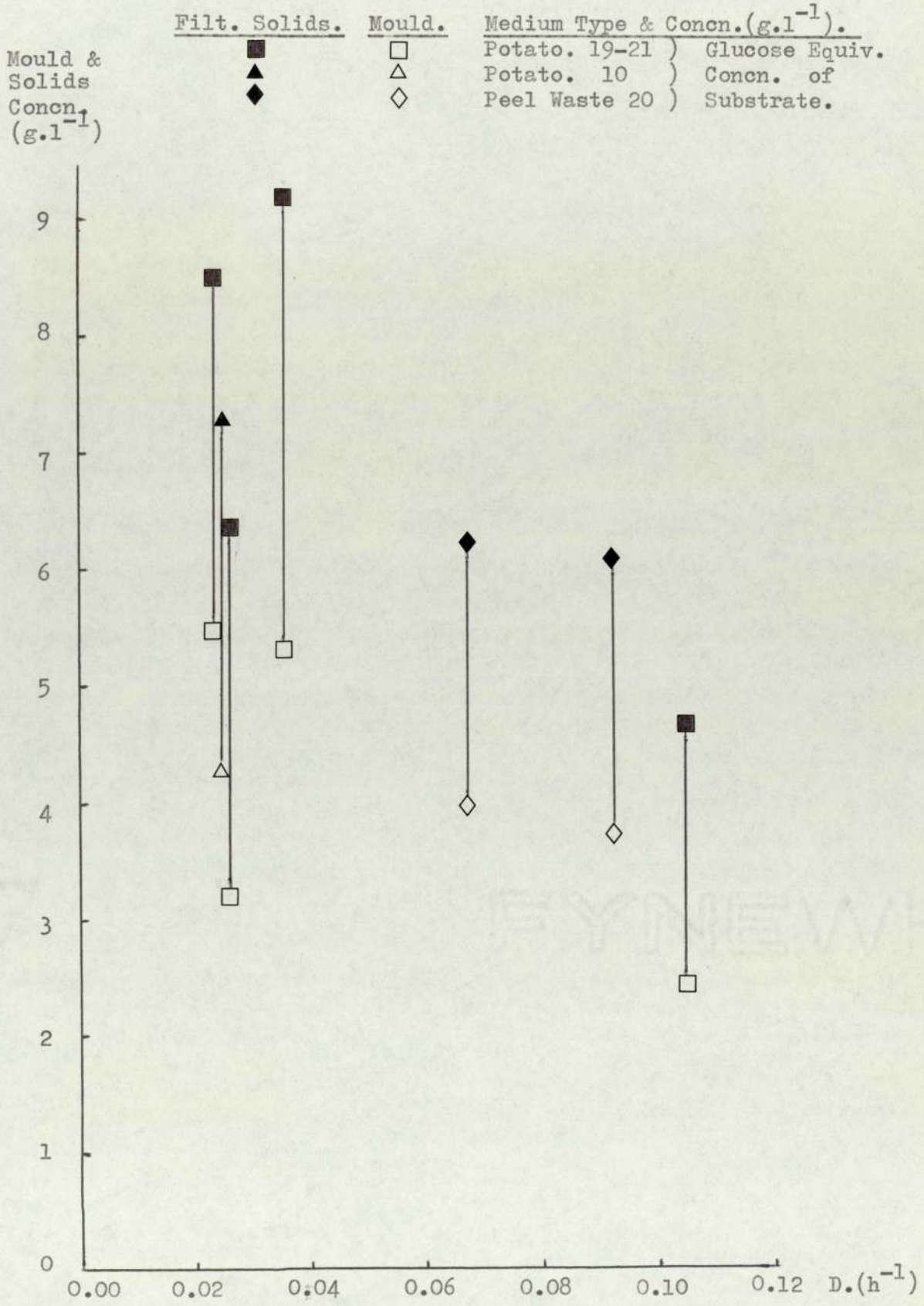


Fig. 4.29 Comparison of the Filterable Solids and Assumed Mould Concentrations in the Effluent Stream with Excess Substrate.



At higher dilution rates, although the total solids concentrations in the fermenter and effluent stream were much higher than with semi-synthetic media, the mould concentrations were similar.

It was noted that $\bar{x}_E > \bar{x}_F$ in five of the seven fermentations in this series, only two of which were with alkaline media. In the other two, $\bar{x}_F \approx \bar{x}_E$. Possible explanations for this phenomenon, which otherwise had only occurred with alkaline semi-synthetic media, were outlined in Section 4.3.2.3.

4.4.2.2 UNDER SUBSTRATE-LIMITED CONDITIONS.(Figs. 4.30 and 4.31).

The concentrations of filterable solids in the fermenter and effluent streams showed the same response to substrate limitation as in the case of semi-synthetic media. The concentration in the fermenter decreased and varied with the NFS and fermentable substrate contents of the medium. The calculated organism concentration was usually within the range 2.0 - 3.0 $g.l^{-1}$, comparing well with the two values obtained with 3.3 $g.l^{-1}$ semi-synthetic medium (2.83 and 2.077 $g.l^{-1}$).

The concentration in the effluent stream was also governed by both the NFS and fermentable substrate contents of the medium and at steady state could in theory be predicted by a modification of Eqn. 4.3 :-

$$(\text{Filt. Solids})_E = \frac{Y_g (D.s_R - D.\bar{s} - m.\bar{x}_F)}{D} + \text{NFS Medium} \quad \ldots\ldots \text{Eqn. 4.8.}$$

Fig. 4.30 Comparison of the Filterable Solids and Assumed Mould Concentrations in Substrate Limited Fermenters.

Filt. Solids.	Mould.	Medium Type & Concn.($g.l^{-1}$)	
●	○	Potato. 5.27	) Glucose Equiv.
▲	△	Potato. 4.0-4.4	) Concn. of
▼	▽	Potato. 3.0-3.3	) Substrate.
■	□	Potato. 2.0	)

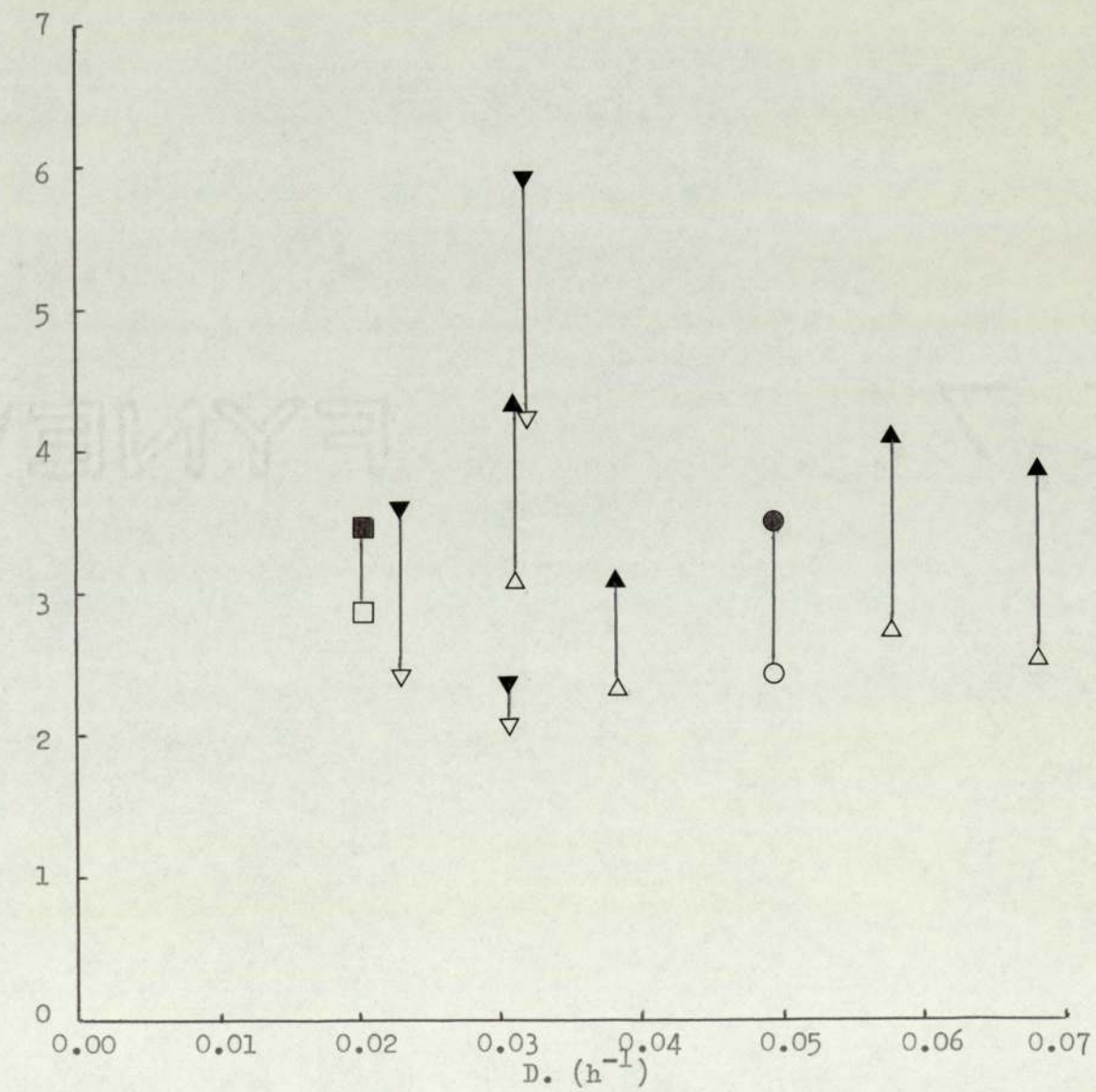
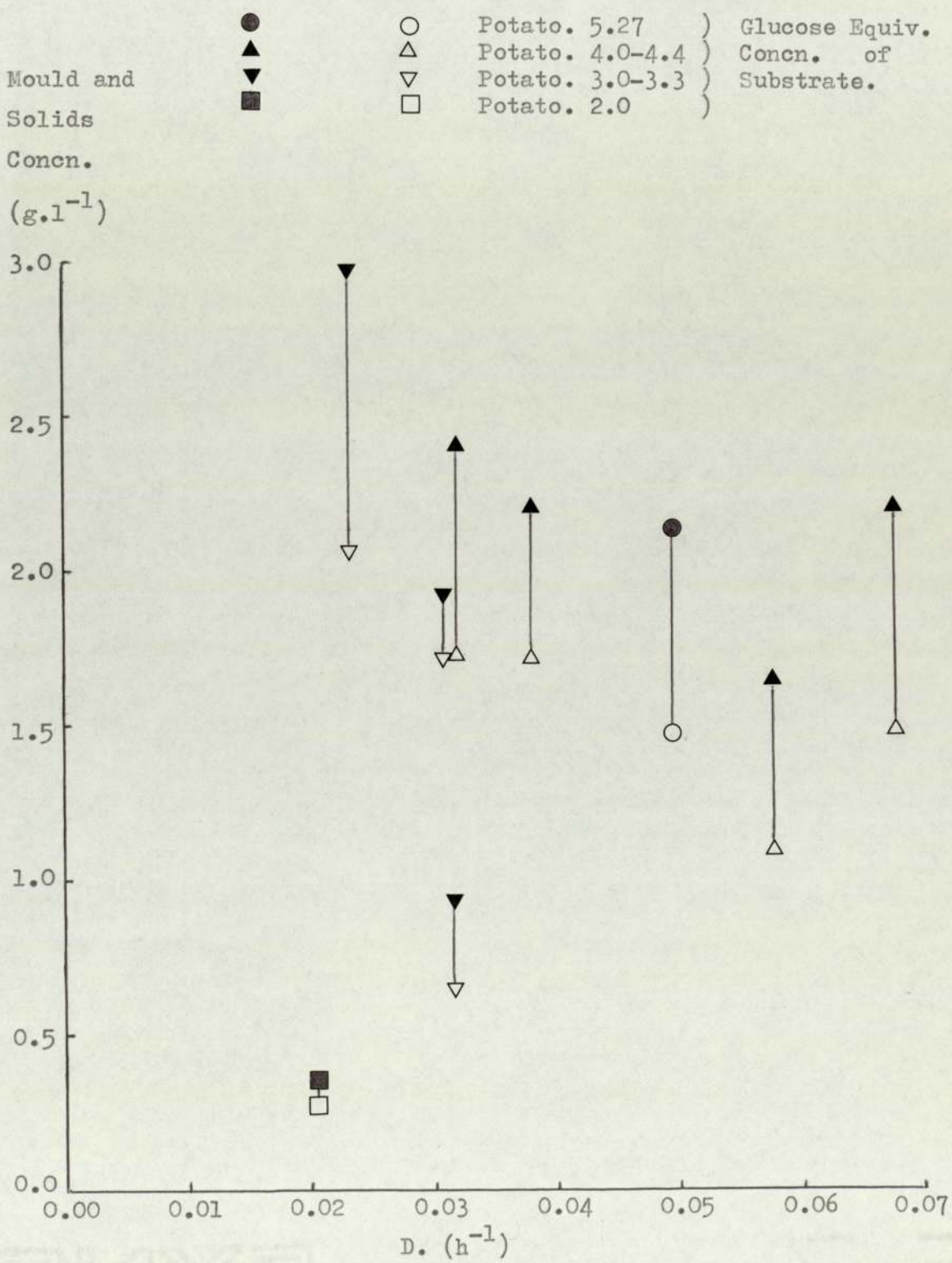


Fig. 4.31 Comparison of the Filterable Solids and Assumed Mould Concentrations in the Effluent Stream from Substrate Limited Fermenters.



Also, theoretically, as $D \longrightarrow \mu_{max} \longrightarrow \infty h^{-1}$, the filterable solids content of the effluent stream would approach NFS_{medium}.

4.4.2.3 CONCLUSIONS.

These parameters were determined by a complex balance between the non-fermentable solids present in the medium supply and the mould which grew in the fermenter. The major points could be briefly summarized:-

(A) The Tower Fermenter was able to physically support a certain concentration of solids, which depended upon their morphology and sedimentation characteristics.

(B) Change in the mould : NFS ratio altered the morphology and therefore the total solids concentration in the fermenter.

(C) The mould : NFS ratio in the fermenter contents and product depended upon the relationship between the rates of NFS and substrate supply to the fermenter and growth of the mould.

(D) At steady state the concentration of NFS in the fermenter was not necessarily the same as that in the medium, as the fermenter could exert a concentrating effect.

4.4.3 PRODUCTIVITY AND SPECIFIC GROWTH RATE OF THE FERMENTER CONTENTS.

4.4.3.1 PRODUCTIVITY.

In these cases, again, a distinction had to be made between

total values (direct measurements) and those of the mould fraction alone (based on steady state assumptions). These could in theory be predicted from Eqn. 4.8, by multiplication of all three parts of the equation by D.

When substrate was in excess the total productivity was considerably higher than with semi-defined starch media (Fig.4.32). In contrast, the calculated value for the mould fraction alone was significantly lower (by approx. 30%).

With peel waste medium, the increase in productivity was of the same order of magnitude as in earlier experiments with alkaline media (section 4.3.2). The maximum steady state value noted was 0.563 $g.l^{-1}.h^{-1}$, of which the mould content was calculated to be 0.3403 $g.l^{-1}.h^{-1}$.

Under substrate limited conditions the total productivity increased linearly with dilution rate (Fig. 4.33). However, the mould fraction showed the same form of response curve as in semi-synthetic media fermentations (compared with Fig. 4.4), so that considerable changes in the composition of the product occurred. At higher dilution rates (c. 0.07 h^{-1}) mould constituted a smaller proportion of the total, which was illustrated by the decline in nitrogen content at higher specific growth rates (section 4.4.5).

4.4.3.2 SPECIFIC GROWTH RATE.

A consequence of the assumption that the mould : NFS ratios in fermenter and effluent stream were the same, was that the specific growth rates calculated for the total solids content

Fig. 4.32 The Productivity of Potato Medium Fermentations with Excess Substrate.

Filt. Solids.	Mould.	Medium Type & Concn.($g.l^{-1}$).	
■	□	Potato. 19-21	) Glucose Equiv.
▲	△	Potato. 10	) Concn. of
◆	◇	Peel Waste 20	) Substrate.

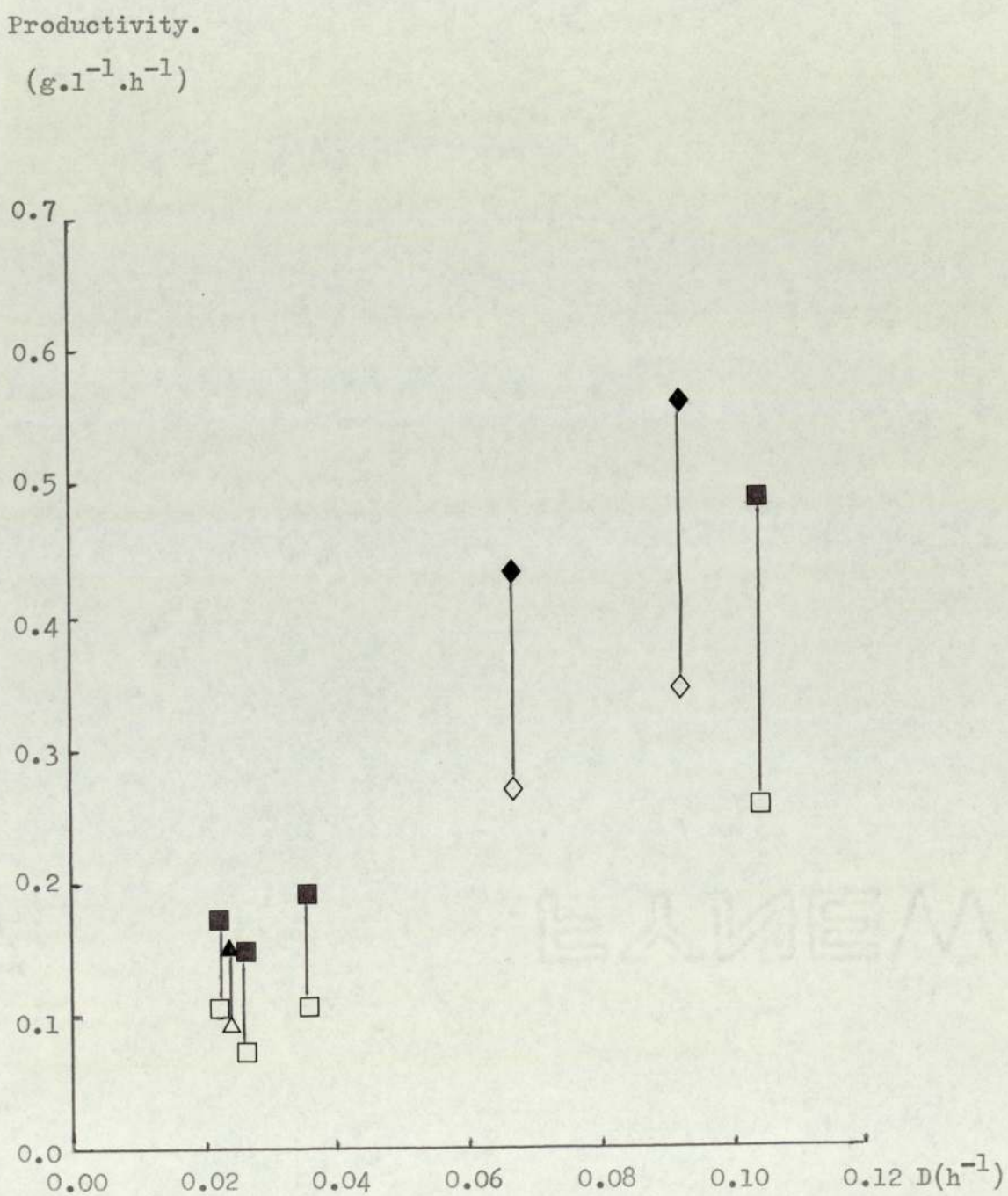
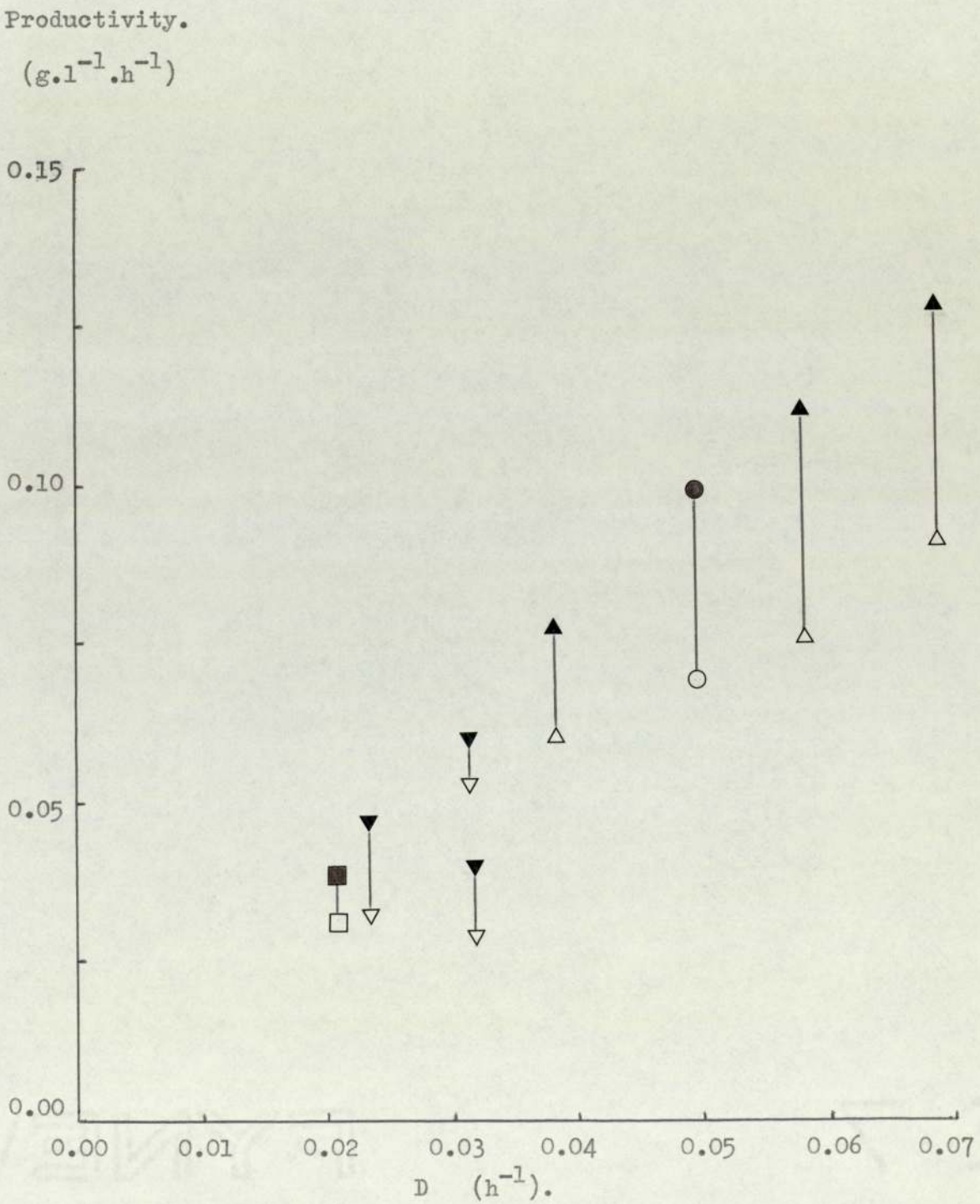


Fig. 4.33 The Productivity of Substrate Limited Potato Media Fermentations.

Filt. Solids.	Mould.	Medium Type & Concn.($g.l^{-1}$)	
●	○	Potato. 5.27	Glucose Equiv.
▲	△	Potato. 4.0-4.4	Concn. of
▼	▽	Potato. 3.0-3.3	Substrate.
■	□	Potato. 2.0	



and mould fraction were equal.

As in the case of semi-defined media, μ was almost always considerably less than D (Fig. 4.34). Under substrate limited conditions the results confirmed that the primary determinant of μ was the rate of substrate supply to the culture.

Values of μ only exceeded those of D under exceptional conditions, as in previous fermentations (section 4.3.2.4) these were with alkaline medium.

4.4.4 YIELD COEFFICIENTS.

In terms of the product as a whole, values of this parameter had little meaning as they could range from $0 \rightarrow \infty$, depending on the mould : NFS ratio of the contents of the effluent stream.

However when expressed in terms of the mould fraction alone, the yield coefficients showed some relationship to the degree of substrate limitation of the culture and therefore, to μ (Fig. 4.35). In general, low values of s_R resulted in increases in $Y_{x/s}$, often to the levels reported for moulds in other continuous culture systems.

The results of these fermentations suggested that, except when the substrate supply rate was very low, oxygen limitation was a significant factor. In most cases, incomplete oxidation of the substrate resulted in organic acid production, which represented a diminution of $Y_{x/s}$.

This theory was supported by the steady state conditions in the two cases in which $Y_{x/s}$ was c. 0.55 $g.g^{-1}$. The dissolved oxygen level in these cultures ranged from 20 - 40% of the

Fig. 4.34 The Variation of Specific Growth Rate with Dilution Rate in Potato Media Fermentations.

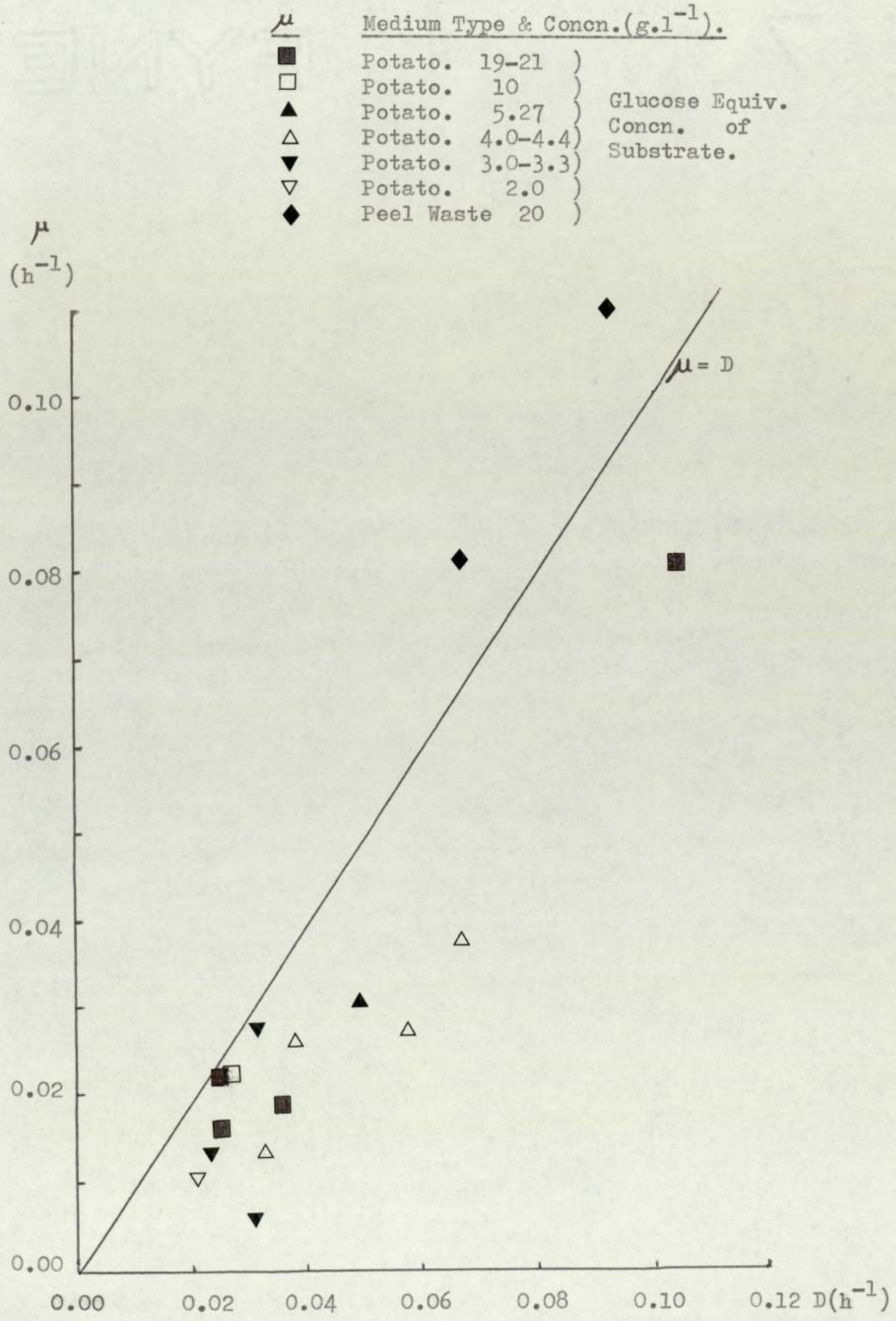
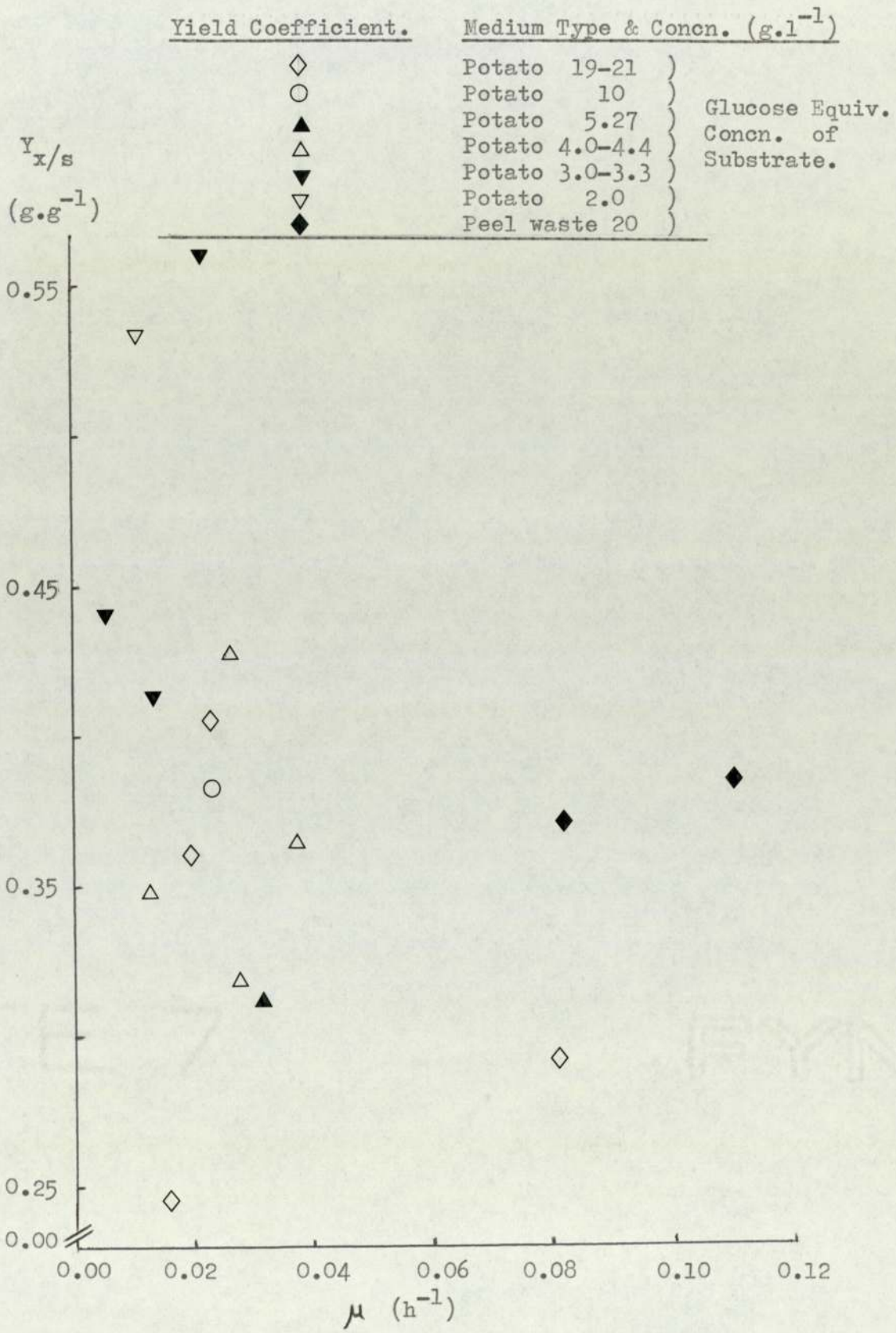


Fig. 4.35 The Yield Coefficient of the Mould Fraction in Potato Media Fermentations.



saturation value (for the medium concerned at 30°C) and the pH readings varied between 3.5 and 4.5. Similar increases in these parameters were also noted when the substrate supply to a limited culture was interrupted. Response times of c. 2 min. from 20 → 80% dissolved oxygen saturation were not unusual.

4.4.5 THE NITROGEN CONTENT OF THE PRODUCT. (Fig. 4.36).

The nitrogen content of the product was determined by the balance between 2 factors:-

(A) The relative nitrogen contents of the NFS (always less than 0.015 $g.g^{-1}$) and the mould (usually 0.045+ $g.g^{-1}$, but increasing with μ - section 4.2.5).

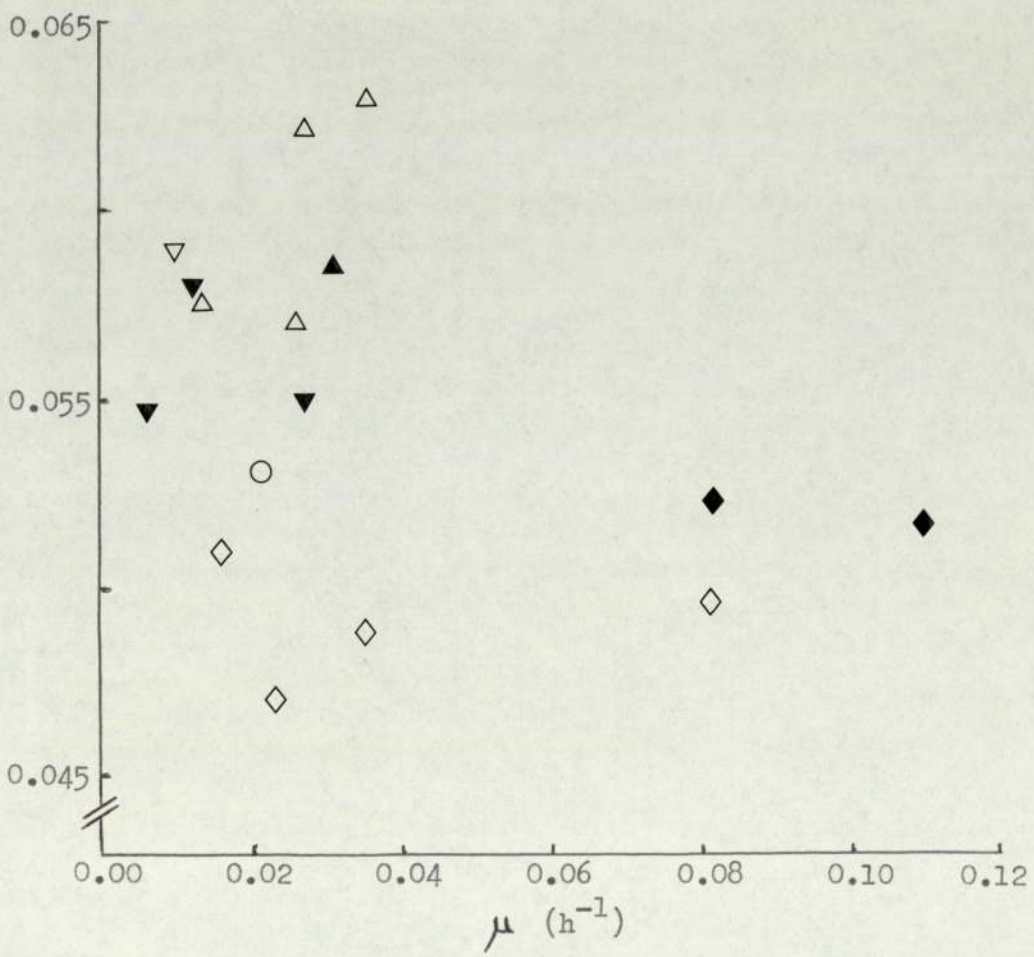
(B) Changes in the proportions of mould and NFS in the product (section 4.4.3), which resulted in a tendency for its nitrogen content to decline at higher dilution rates.

As expected, this content was diminished when compared to that of the product from semi-synthetic medium fermentations (mould alone). Values were usually in the range 0.05 - 0.06 $g.g^{-1}$, corresponding to a crude protein content of 31.25 - 37.5% (on a N x 6.25 basis).

In the case of the bland, fibrous product grown on alkaline peel the crude protein content was c. 33%, which represented a considerable "upgrading" of a totally discarded waste.

Fig. 4.36 The Variation in Nitrogen Content of the Product from Potato Medium Fermentations.

Nitrogen Content.	Medium Type & Concn.($g.l^{-1}$)	
◇	Potato 19-21	Glucose Equiv. Concn. of Substrate.
○	Potato 10	
▲	Potato 5.27	
△	Potato 4.0-4.4	
▼	Potato 3.0-3.3	
▽	Potato 2.0	
◆	Peel Waste 20	



4.4.6 THE EFFECTS OF EXTREME SUBSTRATE LIMITATION.

These effects were investigated in three fermentations altogether, but were best monitored in a potato medium fermentation which had previously been run for over 400 h. under substrate limited conditions ($s_R \approx 3.3$ g.l^{-1}).

The responses were most easily summarized by means of a flow diagram (Page 175), commencing when s_R was reduced to 1.985 g.l^{-1} at a time designated T = 0 h. The dilution rate throughout was in the range 0.0197 - 0.0210 h^{-1}.

Sporulation occurred, in this case between 66 and 74 h. later, being preceded in each fermentation by the same series of colour changes, some of which were obviously due to the formation of black spores.

The maximum duration of sporulation could not be ascertained, as between 52 and 58 h. after its onset, germination (as a result of an increased level of s_R) was noted.

4.5 GENERAL DISCUSSION.

4.5.1 THE MOULD CONTENT OF THE FERMENTER.

This was the most crucial factor controlling the fermentations, depending totally upon the interaction between fluidisation and sedimentation forces and the colonial structure of the mould. Although measurement of x_F is easy, quantification of the other factors involved, especially the morphology, is particularly difficult. The chief problems are that:-

(A) A fermentation broth is a 3-phase situation, containing mould, culture medium and air bubbles.

FLOW DIAGRAM - Conditions during the Sporulation Phase.

TIME (h.)	Colour	Morphology
0	Cream	Colonies
	Yellow	becoming smaller,
26	Pink	more densely
	Red	packed and
		"inhibited" in
46	Brown	character.
	Black	
74	Presence of Conidiophores and spores in the fermenter first noticed.	
82		Mainly spores present in effluent stream, very little
94		mycelium.
126	Germination of spores noted. Mould became more "mycelial"in character and lighter in colour.	

s_R = 1.985 $g.l^{-1}$; pH = 3.45
x_F = 2.8 $g.l^{-1}$; D.O. = 28%
Mould nitrogen content 5.736 $g.g^{-1}$.

PLATE 4.11

s_R = 1.97 $g.l^{-1}$; pH = 5.2
x_F = 2.57 $g.l^{-1}$; D.O. = 66%

x_F = 2.45 $g.l^{-1}$; pH = 5.5
D.O. = 76% Mould Nitrogen Content 5.942 $g.g^{-1}$.

PLATE 4.12

s_R = 2.41 $g.l^{-1}$; x_F = 2.3 $g.l^{-1}$.

x_F = 2.77 $g.l^{-1}$; pH = 4.6
D.O. = 52% saturation.
Mould Nitrogen Content 6.288 $g.g^{-1}$.

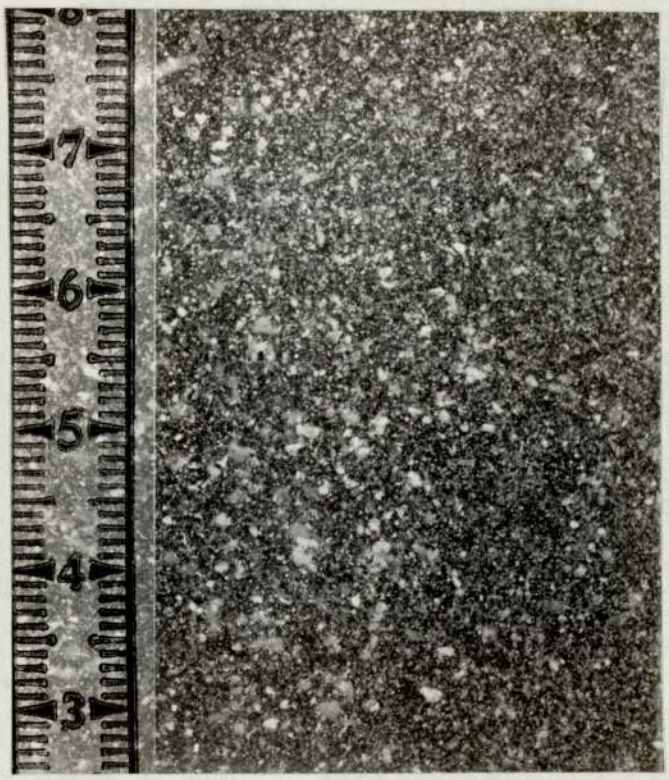
Plate 4.11 Aspergillus niger M1 : Mould Morphology as a Result of Acute Substrate Limitation, 58 h. prior to Sporulation.

(Fermentation PN 4A; $D=0.0202h^{-1}$; $s_R=1.985\ g.l^{-1}$ gluc. eq.)

1 cm.

Plate 4.12 Aspergillus niger M1 : Macro-Morphology of the Sporulating Mould.

(Fermentation PN 4A; $D=0.0205h^{-1}$; $s_R=1.97\ g.l^{-1}$ gluc.eq.)



1 cm.

(B) The characteristics of the colonies are not uniform throughout the culture - within a given sample there are variations in size, form and packing density.

(C) Being composed of hyphae, the colonies are not rigid structures with clearly defined margins.

(D) The characteristics of the colonies are variable in response to the environmental conditions in the culture, changes in form which probably influence such factors as the substrate requirement of the mould and possibly even its viable fraction.

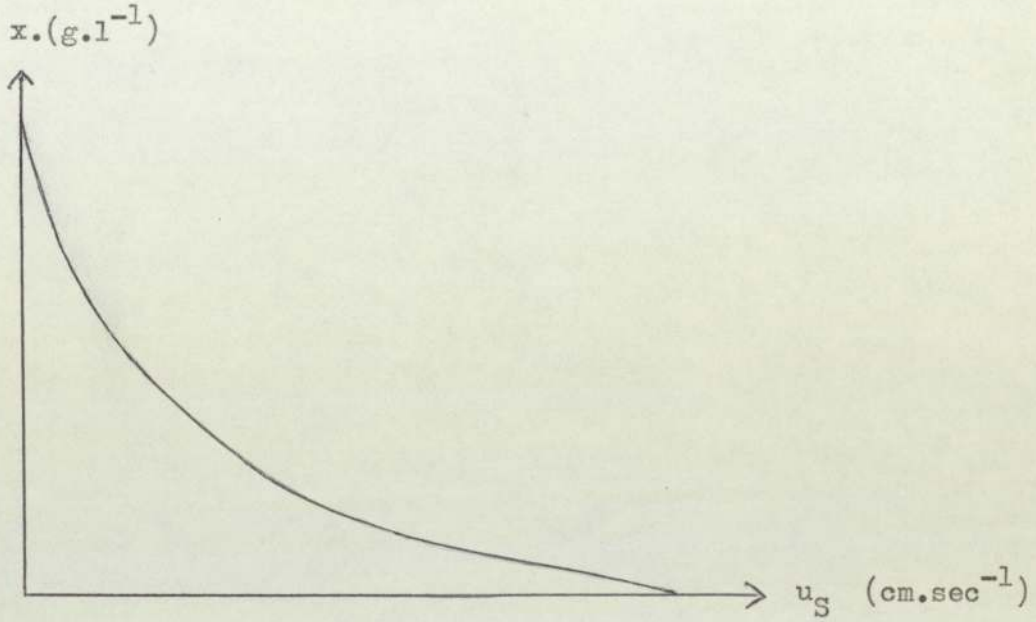
Attempts to quantify the morphology of the mould in all respects but that of overall dimension, have not proved meaningful, being considered in detail by Cocker (290). Even in recent reviews, such as that by Whitaker and Long (307), the possibility of describing such a crucial factor in anything but purely subjective terms such as "loose" or "hard" or "hairy" "pellets" is not considered.

James (308) and Fidgett (309) attempted to account for the effect of fluidisation on colonies of A. niger M1 mathematically and suggested that in a 2 phase non-growing situation, the relationship could be represented by an adaptation of the generalized fluidization equation:-

$$x = x_m \left[1 - \left(\frac{u_S}{u_T} \right) \frac{1}{n} \right] \quad \text{. . . . . Eqn. 4.9}$$

where u_S = superficial liquid velocity (cm. sec^{-1}) u_T = terminal velocity of the colony; n = an effectiveness factor and x_m = the maximum biomass concentration ($g.l^{-1}$). This equation gives a

Fig 4.37 Graphical Representation of the 2-phase Fluidisation Equation. (Fidgett, 309)



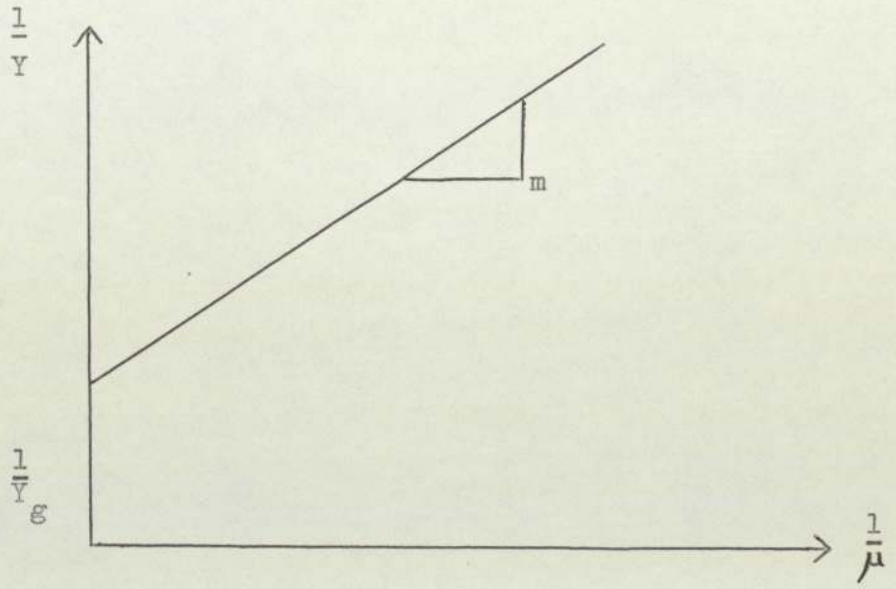
form of response curve for a particular colony form, shown in Fig. 4.37. For obvious reasons different morphologies, having different values of u_T, n and x_m, will modify this form, but it is perhaps a sound basis for future work.

The ability to adapt its morphology to a more sedimentary form is so great that the organism has been able to remain within the fermenter and grow at dilution rates far in excess of μ_{max}. Certainly fermentations at dilution rates of up to 7.0 h^{-1} were possible in this system using sucrose salts media (Pannell, 213) albeit with very low mould concentrations.

4.5.2 GROWTH RATES AND YIELDS OF MOULD.

The consistantly low values of the yield coefficient in this work could have been due to the substrate requirement for maintenance representing a substantial proportion of the total utilization.

Fig. 4.38 Reciprocal Plot used by Pirt for the Determination of the Maintenance Energy of Microorganisms.



Attempts were therefore made to determine the value of the maintenance coefficient (m) of A. niger M1 when growing semi-defined starch medium in the tower fermenter.

Two methods were used, both of which relied on the assumptions that m and Y_g (the true growth yield) were constants. The first was a method described by Pirt (310) which utilizes a reciprocal plot (illustrated in Fig. 4.38) based on the equation:-

$$\frac{1}{Y_g} = \frac{m}{\mu} + \frac{1}{Y_g} \quad \ldots\ldots\ldots\ldots \text{Eqn. 4.10}$$

In practice however, this method proved unsatisfactory; when applied to all the steady state readings, the maintenance coefficient indicated was - 0.0027 $g.g^{-1}.h^{-1}$, an obvious fallacy. This error was due, in part, to the method itself. In the reciprocal plot the least reliable data, that relating to very low

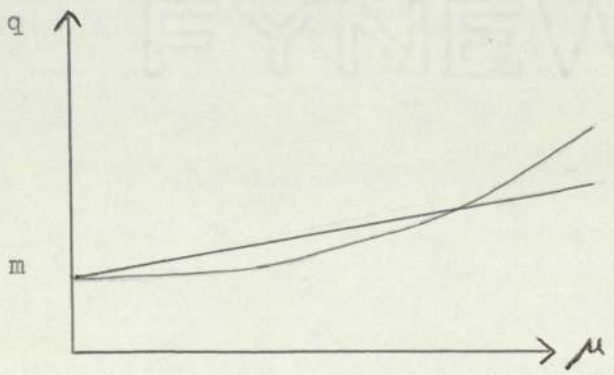
growth rates, has the greatest influence in the regression equation and therefore on the slope of the line (m).

A better method proved to be that used by Tempest and Herbert (303) and Righelato et al. (304), which is based on the assumption that the specific substrate utilization rate (q) can be related to the specific growth rate (μ) by the equation:-

$$q = \frac{\mu}{Y_g} + m \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{Eqn. 4.5}$$

Details of its application to the experimental data are in Section 4.2.4.2 and Fig. 4.9. But when μ was very low, observed values of Y exceeded that of Y_g predicted by the method which suggested that Eqn. 4.5 was not valid in this instance.

An explanation could lie in the fact that when $\mu >$ c.0.02 h^{-1} the culture probably became increasingly subject to oxygen limitation. In spite of the extremely high correlation coefficient (r = 0.9917; 195 readings) this method probably attempted to linearize data which actually formed part of a curve, i.e. schematically:-



This would enable 1/slope, the value of Y corrected for m (not necessarily Y_g), to decline with increase in μ and oxygen limitation.

However the prediction of a value of m of approx. 0.004 $g.g^{-1}.h^{-1}$ was in agreement with information derived from other aspects of the work. In general, the experimental results suggested that in these fermentations, the value of the maintenance coefficient was low compared to those usually reported, almost certainly less than 0.006 $g.g^{-1}.h^{-1}$ and not necessarily constant.

4.5.3 OXYGEN TRANSFER LIMITATIONS.

This point is best illustrated by an "order of magnitude" calculation of the oxygen demand of the culture and the oxygen transfer rate (K_La) necessary.

Values were assumed for the oxygen requirements for mould growth (1.56 g.g cells^{-1}) and maintenance (0.024 g.g cells$^{-1}.h^{-1}$), those quoted by Righelato (304) for Penicillium chrysogenum. Calculations were based on data from a starch medium fermentation in which, at a D of 0.088 h^{-1}, x_F = 3.05 $g.l^{-1}$ and Productivity = 0.256 $g.l^{-1}.h^{-1}$.

The oxygen demand (Q) was :-

$$Q = Y_O \cdot \text{Productivity} + m_O \cdot x_F$$
$$= 0.3994 + 0.0732$$
$$= \underline{0.4726 \ g.l^{-1}.h^{-1}.}$$

The oxygen supply rate is given by:-

$$Q = K_La \ (C^* - C)$$

where C^* and C are the saturation and actual concentrations of oxygen in the culture broth, which were assumed to be 0.008 $g.l^{-1}$ and zero respectively. So to satisfy the culture oxygen demand:-

$$K_La = \frac{0.4726}{0.008} = \underline{59.07 \ h^{-1}}$$

Using polarographic techniques, Dowen (311) measured the oxygen transfer rate in the fermenters used for the author's work. With sucrose-salts medium and the same aeration rate (10 l. min^{-1} - a superficial gas velocity of 2 cm. sec^{-1}), he found that $K_La \approx 60\ h^{-1}$. This was significantly lower than previous estimates using the sulphite oxidation technique in tower fermenters (Morris, 289).

It should be noted that this was achieved in the absence of mould which, because its filamentous nature leads to high culture viscosities, has a particularly deletarious effect on K_La. Reductions by up to 85% are not unusual in fermenters containing up to 13.5 $g.l^{-1}$ dry weight of mould (312).

4.5.4 MOULD VIABILITY AND SPORULATION.

Even in actively growing cultures, a proportion of the total population consists of dead cells; this proportion increases at low growth rates. With unicellular microorganisms viable and non-viable cells can be readily distinguished and accounted for but with moulds it is far more difficult. According to Trinci and Righelato (313) both apparently viable and apparently dead cells are often found in the same hyphae.

So measured values of specific ($g.g^{-1}$) rates such as μ and m are in fact underestimates of the true values, which could in part account for some of the discrepancy between the level of m in this work and in the literature. Similarly, the colonial form of the mould, especially the classical "pellet", leads to diffusional limitations on the supply of substrates to a

proportion of the culture. Prediction therefore, of the concentration of mould actually taking part in the growth process becomes impossible.

The most widely used condition for the induction of submerged sporulation has been the absence of available nitrogen in the presence of an assimilable carbon source (Morton, 314; Vezina et al., 315 and Galbraith and Smith, 316). In this study, however, nitrogen was present in excess. Sporulation occurred when the carbon substrate supply decreased below approx. 0.025 $g.g^{-1}$ h^{-1}(the value of m often quoted for moulds). Above this, the culture grew normally as was the case in the chemostat studies of Righelato et al. (304). In their experiments too, the culture (P. chrysogenum) required a low concentration of glucose to spore in submerged culture, but remained vegetative above a critical glucose concentration.

4.5.5 SUGGESTIONS FOR FURTHER WORK.

Preliminary work with any novel fermentation system raises many points worthy of further investigation, especially with one whose behaviour is radical in so many respects. Seven major areas needing considerably more work are listed below, including in each case, the most important requirements of the investigation:-

(A) Morphology of the Organism. The relationship between physiological and physical factors and their control by (and of) the fermenter conditions.

(B) Oxygen Transfer in the Culture. Improvements through design of equipment and modification of the morphology of the organism.

(C) Control of the Specific Growth Rate. Mechanisms which lead to a limited independence of μ and D, especially in situations where $\mu > D$.

(D) Behaviour at low substrate supply rates. High and low dilution rates could be important in effluent treatment and sporulation studies respectively.

(E) Fermentation of Media containing Solids. Effects of both fermentable and non-fermentable solids on the process and their relationship to the mould morphology. Accounting for their presence when estimating organism related parameters.

(F) Use of other organisms, especially Yeasts and Bacteria whose morphology may be better suited to the system.

(G) Development of practical applications, especially those exploiting the simplicity of construction of the fermenter and the use of high dilution rates with nonaseptic media.

SECTION V.

CONCLUSIONS.

Throughout this work, the intention has been to determine the operating characteristics of a novel fermentation system and to assess its suitability for one possible application, the production of "biomass" from presently under-utilized carbohydrate resources.

Observations made during the course of this research have given a more detailed insight into the problems of mould physiology and behaviour in a Tower Fermenter. The large amount of data collected necessitated the development of a mass balance programme and in certain aspects a complete reassessment of conventional continuous culture theory.

The Continuous Fermenter used in this project was the ultimate in simplicity in terms of design and construction, an aerated tube containing the mould culture. In practice however, difficulties arose from the colonial growth form of the organism selected, Aspergillus niger M1. The form of the colonies both determined and was determined by fermenter conditions such as viscosity. As a research tool, the Continuous Tower Fermenter proved ideal for the study of the colonial forms and physiological responses of the organism without the "unnatural" effects of high shear forces.

An understanding of the operation of the system hinged upon the dual nature of the dilution rate, both as a fluidising force and as a means of nutrient supply to the culture. The concentration of organism in the fermenter was determined primarily by the former, acting on its morphology. In contrast, material in the effluent stream represented surplus growth and

was governed to some extent by the latter. This situation resulted in a considerable degree of independence of the specific growth rate relative to the dilution rate. This characteristic can normally only be achieved in single stage fermenters by recourse to an external "biomass" recycle system.

As a "biomass" production process the Continuous Tower Fermenter suffered from a low productivity/unit capacity and poor yield coefficient. Both were the direct results of oxygen transfer limitation aggravated by the morphology of the mould. Nevertheless the possibility of efficiently utilizing much lower substrate concentrations at higher dilution rates than conventional systems could be an advantage in certain situations; similarly the ability to ferment non-aseptic media, though one of the protective effects (that of pH) is only achieved at the expense of the yield coefficient.

Ideally, such a system might best be utilized as the final stage of an integrated process in which the initial production of useful materials from the low grade carbohydrate source was performed by a more sophisticated method, be it physical, chemical, or biological.

APPENDICES.

APPENDIX I.

METHODS FOR THE ESTIMATION OF SUGAR CONCENTRATION.

THE FERRICYANIDE METHOD.

REAGENTS.

Ferricyanide Solution, made up by dissolving 10 g. Potassium ferricyanide, 200 g. Sodium hydroxide and 0.1 g. Methyl Orange in 1 l. distilled water.

Methylene Blue Solution (10 $g.l^{-1}$).

Glucose Standard Solution (2 $g.l^{-1}$).

PROCEDURE.

A 25 cm^3 burette was filled with the test sample (or standard) containing 1 - 3 $g.l^{-1}$ reducing sugars. 20 cm^3 Potassium ferricyanide solution and 5 cm^3 indicating Sodium hydroxide solution were pipetted into a 250 cm^3 conical flask containing a few glass beads and brought to the boil within 90 sec. 7 cm^3 of the sample were pipetted into this flask, 0.1 cm^3 Methylene Blue added and the titration continued. The dark green/blue solution lightened to pink within 0.2 cm^3 of the endpoint, at which it became colourless.

For greater accuracy, the titration was repeated, adding the methylene blue approx. 0.5 cm^3 before the expected endpoint and ensuring that it was completed within 3 min. of the solution first boiling. The titration value must fall within the range 7 - 17 cm^3, a 2 $g.l^{-1}$ Glucose standard gave a value of 10.66 cm^3. Unfortunately the relationship is not linear, so the equivalent reducing sugar concentration was read from a graph constructed from a table of standards. (Fig. A1.1).

Fig.A1.1 SUGAR EQUIVALENTS FOR THE FERRICYANIDE METHOD.

TITRATION VALUE (cm^3)	EQUIVALENT GLUCOSE CONCN. ($g.l^{-1}$)
5	4.171
6	3.494
7	3.010
8	2.644
9	2.360
10	2.130
11	1.943
12	1.785
13	1.652
14	1.538
15	1.438
16	1.351
17	1.275
18	1.205
19	1.144
20	1.088
21	1.039
22	0.992
23	0.951
24	0.912
25	0.877

A Titration Value of 10.66 cm^3 is equivalent to 2$g.l^{-1}$ Invert Sugar.

THE 3,5-DINITROSALICYLIC ACID METHOD.

REAGENT.

1 g. of 3,5-dinitrosalicylic acid was dissolved in 20 cm^3 of 2M Sodium hydroxide solution and 50 cm^3 of distilled water. 30 g. of Rochelle salt (Sodium Potassium tartrate) were added and the solution made up to 100 cm^3 with distilled water. This solution is stable for at least 4 months if protected from carbon dioxide in well-filled bottles.

STANDARDS.

Solutions containing up to 2 $g.l^{-1}$ d-glucose (from an anhydrous stock) were prepared both separately and by dilution. Distilled water was used as the zero standard.

PROCEDURE.

Sample volumes of 1 cm^3 were pipetted into tubes containing 2 cm^3 of the dinitrosalicylic acid reagent and heated in a boiling water bath for 10 minutes. The tubes were cooled to room temperature, 2 cm^3 distilled water added and the optical density read at 570 nm with an E.E.L. "Spectra" Colourmeter equipped with a flow-through cell. The meter was first set to zero with the tubes containing distilled water standards.

The concentration of reducing sugars in the samples could then be estimated from the graph of Optical Density v. Glucose concentration obtained with the standards.

DISCUSSION.

This method is particularly suitable for estimating large numbers of samples, the author routinely estimating batches of 90 tubes. These were 15 cm. long x 1 cm. i.d., so that they

could be immersed to a depth of 5 cm. with little risk of accidental ingress of water.

At 95°C the reaction is almost instantaneous and for an individual tube, 4 min. immersion in boiling water is adequate. When dealing with large batches of tubes, a 10 min. immersion was used to ensure the adequate heating of all samples. Boiling for 20 min. produced no detectable hydrolysis of starch and sucrose samples.

The bandwidth of the E.E.L. instrument used was only 30 nm, considerably less than the 90 nm of the Ilford 626 filter used in some meters. For this reason, with the method described above, the optical density produced by the reaction obeys Beer's Law with samples in the range 0 - 2 $g.l^{-1}$ reducing sugars and the method is accurate to $\pm$ 1%. By diluting the solution to 10 cm^3 samples containing up to 5 $g.l^{-1}$ can be estimated, though with some loss of accuracy.

TOTAL SUGAR ESTIMATION.

PROCEDURE.

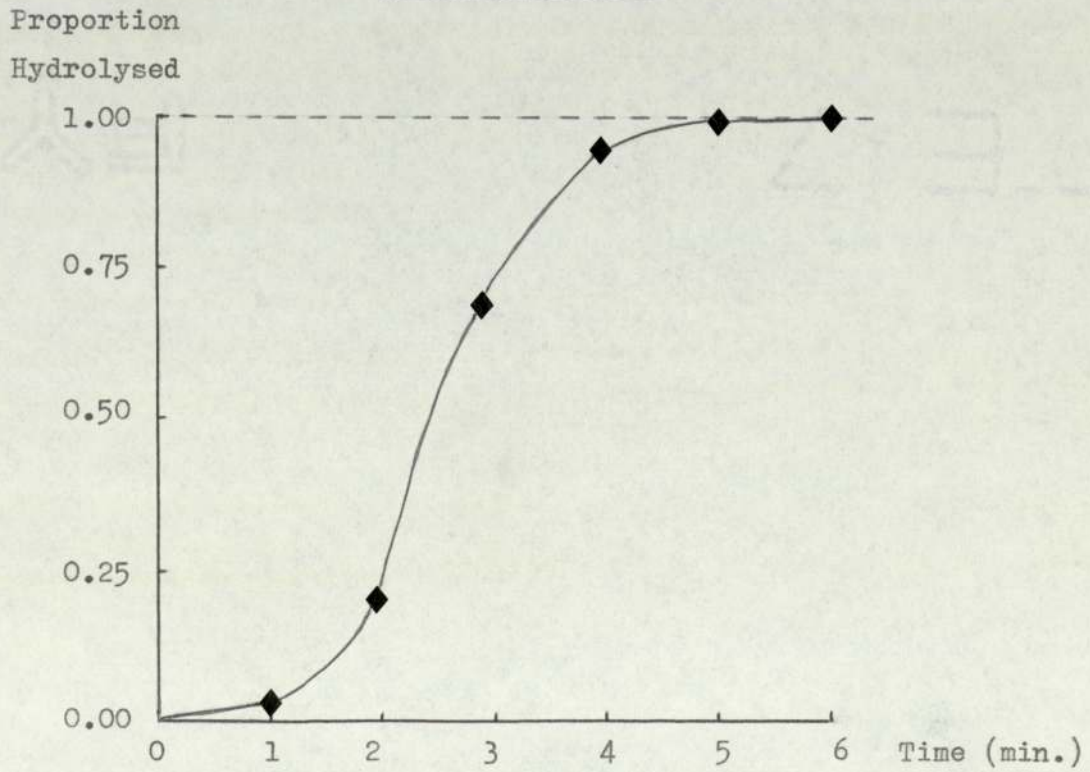
Sample volumes of 10 cm^3 were pipetted into boiling tubes together with 1 cm^3 of concentrated hydrchloric acid and boiled on a water bath for 10 min. The samples were cooled and neutralized with 1.2 cm^3 of 10.M sodium hydroxide before being made up to a volume such that the concentration of reducing sugars was less than 2 $g.l^{-1}$. This solution was then estimated by the dinitrosalicylic acid method and a correction factor applied to account for the dilution of the original sample. Results were expresses as $g.l^{-1}$ Glucose Equivalent.

DISCUSSION.

Under these conditions, hydrolysis of a 10 $g.l^{-1}$ starch solution was found to be complete within 6 minutes (see Fig. A1.2) so the use of a 10 min. hydrolysis time allows a considerable reserve but it is not so long as to cause significant caramelization.

Acid hydrolysis and neutralization (and the resultant sodium chloride present) of the sample was found not to affect the sugar estimation providing the final sample/reagent mixture was alkaline, though, of course the accuracy was slightly reduced because of the need for a dilution multiplication factor.

Fig. A1.2 Hydrolysis of Starch (10$g.l^{-1}$soln.) by Concentrated Hydrochloric acid.



APPENDIX II.

ESTIMATION OF RATE OF REDUCING SUGAR FORMATION FROM STARCH.

THE BUFFER STOCK.

34 g. sodium acetate ($C H_3 COONa. 3H_2O$) were dissolved in 250 cm^3 1.0N Acetic acid (i.e. 14.325 cm^3 glacial acid diluted to 250 cm^3) and made up to 500 cm^3 with distilled water. When a 20 cm^3 sample of this stock was diluted to 1 l. the resulting pH was 4.6.

THE STARCH SUBSTRATE.

2 g. Soluble starch were creamed with 20 cm^3 cold distilled water and poured into approximately 400 cm^3 boiling distilled water whilst being vigorously stirred. This solution was boiled for 2 min. and cooled under cover to 20°C. A 10 cm^3 portion of the Acetate Buffer was added and the volume made up to 500 ml.

The substrate had, therefore, a starch concentration of 4 $g.l^{-1}$ and was buffered at pH 4.6.

THE PROCEDURE.

A 100 cm^3 portion of the filtrate from a sample of the fermenter broth was added to 200 cm^3 of starch substrate at 30°C, shaken and incubated at this temperature for 3 h. 2 cm^3 samples were removed at 15 min. intervals and the reducing sugar content assayed by the Dinitrosalicylic acid Method (Appendix I).

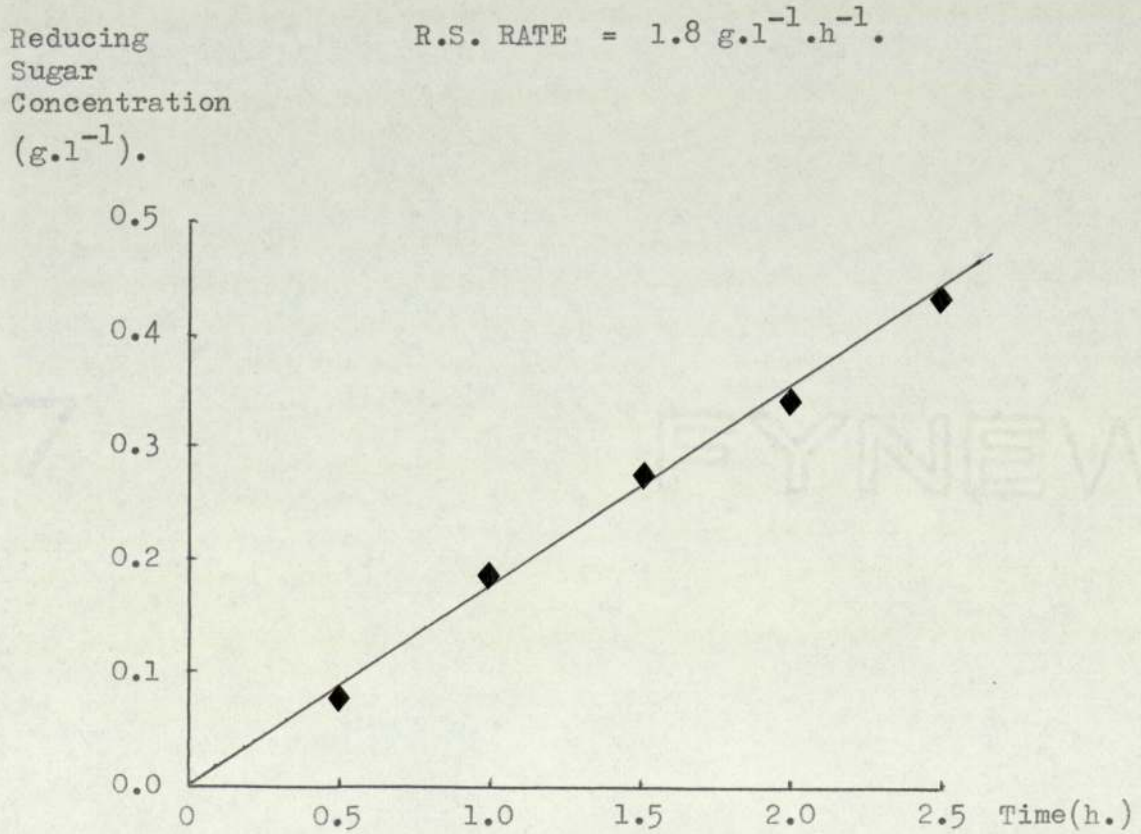
The total weight of reducing sugar present in the reaction mixture is plotted against incubation time, the slope of the straight line portion of this graph being the required reaction rate. Multiplication of the numerical value of the slope by 10

(because the sample volume is 100 ml.) enables the reaction rate to be expressed in the units $g.l^{-1}.h^{-1}$.

COMMENTS.

The dinitrosalicylic acid reagent is strongly alkaline and on mixing with the sample, terminates these enzyme reactions immediately. Providing this part of the estimation is carried out without delay, the procedure can be continued with all the samples and standards "en masse", making this a very simple and straightforward assay. Fig. A.2.1 shows the results of a typical estimation.

Fig. A2.1 Results of an Estimation of Reducing Sugar Formation Rate.



APPENDIX III.

THE MASS BALANCE PROGRAMME.

```
0         MASTER FERMENTER
1         REAL M(4),MT,MU,NX,MT1,MT2,NFS
2         INTEGER START,ST(100),COM(72)
3         DIMENSION AJF(9),DIF(9),E(3),F(9),F1(9),
          ISUMF(9),Z(9),F2(9)
4       100 FORMAT(I0)
5       101 FORMAT(12F0.0)
6       102 FORMAT(72A1)
7       200 FORMAT(10H FERMENTER,5X,9(F8.3,2X),//)
8       201 FORMAT(7H MEDIUM,8X,7(F8.3,2X),//)
9       202 FORMAT(18H FLAG INCORRECT,K=,I2)
10      203 FORMAT(8H RESULTS,7X, 9(F8.3,2X))
11      204 FORMAT(9H EFFLUENT,6X,4(F8.3,2X),//)
12      205 FORMAT(////)
13      240 FORMAT(23H ZERO EFFLUENT AT TIME=,F7.2)
14      241 FORMAT(25H MEDIUM:TIME,ANM,TSM,NFS=,
          4(F9.4,2X))
15      250 FORMAT(46H FERMENTER:TIME,PH,TEMP,D,XF,
          RSF,TSF,NH3F,NBUG,//)
16      251 FORMAT(43H MEDIUM:TIME,NH3M,TSM,MT,
          VUSED,SUMTSM,SUMNM,//)
17      252 FORMAT(32H EFFLUENT:TIME,VE,XE,SUMVE,
          SUMXE,//)
18      253 FORMAT(49H RESULTS:MU,YXS,PROD,RSRATE,
          RMU,RYXS,DN,NINBUG,YN,////)
19      260 FORMAT(29H DATA TIME SEQUENCE INCORRECT)
20      261 FORMAT(18H END OF FAIRY TALE,////)
21      262 FORMAT(1X,72A1)
22          READ(3,102) COM
23          WRITE(2,205)
24          WRITE(2,262) COM
25          WRITE(2,205)
26          WRITE(2,250)
27          WRITE(2,251)
28          WRITE(2,252)
29          WRITE(2,253)
```

```
C
C *** DATA INPUT
C *** K=1 FOR COMMENT CARD
C *** K=2 FOR MEDIUM ACCOUNT
C *** K=3 FOR FERMENTER ACCOUNT
C *** K=4 FOR EFFLUENT ACCOUNT
C *** K=9 FOR TERMINATOR
C
    2 READ(3,100) K
      IF(K.EQ.1) GOTO 10
      IF(K.EQ.2) GOTO 20
      IF(K.EQ.3) GOTO 30
      IF(K.EQ.4) GOTO 40
      IF(K.EQ.9) GOTO 99
      WRITE(2,202) K
      GOTO 99
   10 READ(3,102) COM
      WRITE(2,262) COM
      GOTO 2
C
C *** MEDIUM ACCOUNT
C
   20 READ(3,101) (M(I),I=1,4)
      WRITE(2,241) M
      IF(M(1).LT.T-0.01) GOTO 98
      T=M(1)
      MT2=M(1)
      MT=MT2-MT1
      VUSED=VF*D*MT
      SUMTSM=SUMTSM+TSM*VUSED
      SUMANM=SUMANM+ANM*VUSED
      SUMNFS=SUMNFS+NFS*VUSED
      MT1=M(1)
      ANM=M(2)
      NFS=M(4)
      TSM=M(3)
      GOTO 2
```

```
C
C *** FERMENTER ACCOUNT
C
   30 READ(3,101) (F(I),I=1,9)
      WRITE(2,200) F
      IF(F(1).LT.T-0.01) GOTO 98
      T=F(1)
      TSSAMP=TSSAMP+VSAMP*F1(7)
      ANSAMP=ANSAMP+VSAMP*F1(8)
      XSAMP=XSAMP+VSAMP*F1(5)
      DO 31 I=1,9
      IF(F(I).GT.-0.1) GOTO 37
      F(I)=F1(I)
   37 DIF(I)=F(I)-F1(I)
   31 CONTINUE
      IF(ET1.LT.F1(1)-0.01) GOTO 33
      FRAC=(ET1-F1(1))/DIF(1)
      DO 32 I=1,9
      F1(I)=F1(I)+DIF(I)*FRAC
   32 CONTINUE
      DIF(1)=F(1)-F1(1)
   33 DO 34 I=1,9
      AVF(I)=(F(I)+F1(I))/2.0
      TSUMF(I)=TSUMF(I)+AVF(I)*DIF(I)
   34 CONTINUE
      DO 36 I=1,9
      F2(I)=F1(I)
      F1(I)=F(I)
   36 CONTINUE
      ET1=F(1)
      GOTO 2
C
C *** EFFLUENT ACCOUNT
C
   40 READ(3,101) (E(I),I=1,3)
      IF(E(1).LT.T-0.01) GOTO 98
      IF(E(3).GT.-0.1) GOTO 41
      E(3)=XFDUM
   41 T=E(1)
      ET2=E(1)
      VE=E(2)
      XE=E(3)
      SUMXE=XE*VE
      WRITE(2,204) E,SUMXE
      XFDUM=E(3)
      ET=ET2-ET1
C
```

```
C *** FERMENTER DATA MANIPULATION
C
      FT=ET2-FT1
      DO 50 I=1,9
      TSUMF(I)=TSUMF(I)+F1(I)*FT
      F(I)=TSUMF(I)/ET
   50 CONTINUE
      WRITE(2,200) F
      D=F(4)
      XF=F(5)*(SUMXE-SUMNFS)/SUMXE
      RSF=F(6)
      TSF=F(7)
      ANF=F(8)
      NX=F(9)
      DXF=F1(5)-F2(5)
      DRSF=F1(6)-F2(6)
      DTSF=F1(7)-F2(7)
      DANF=F1(8)-F2(8)
C
C *** MEDIUM DATA MANIPULATION
C
      MT=ET2-MT1
      VUSED=VF*D*MT
      SUMTSM=SUMTSM+TSM*VUSED
      SUMANM=SUMANM+ANM*VUSED
      SUMNFS=SUMNFS+NFS*VUSED
      SUMXE=SUMXE-SUMNFS
C
C *** CALCULATIONS
C
      DS=SUMTSM-VE*TSF-DTSF*VF-TSSAMP
      DN=SUMANM-VE*ANF-DANF*VF-ANSAMP
      DX=SUMXE+DXF*VF+XSAMP
      SUMNX=DX*NX
      YXS=DX/DS
      RYXS=1.0/YXS
      MU=DX/ET/XF/VF
      RMU=1.0/MU
      PROD=MU*XF
      RSRATE=RSF*VE/VF/ET+PROD/YXS+DRSF/ET
      YXN=DX/DN
```

```
C
C *** PRINT ROUTINE
C
      Z(1)=MU
      Z(2)=YXS
      Z(3)=PROD
      Z(4)=RSRATE
      Z(5)=RMU
      Z(6)=RYXS
      Z(7)=DN
      Z(8)=SUMNX
      Z(9)=YXN
      WRITE(2,203) Z
      GOTO 3
   98 WRITE(2,260)
   99 WRITE(2,261)
      STOP
      END
      FINISH
****
```

APPENDIX IV.

STEADY STATE DATA.

Medium Type	Units	Starch	Starch	Starch
s_R	$g.l^{-1}$	19.6	19.94	19.76
Medium pH	Units	7.0	7.0	7.0
Temperature	^oC	30.0	30.0	30.0
D	h^{-1}	0.0424	0.0387	0.0324
Non-fermentable Solids	$g.l^{-1}$	-	-	-
$\bar{x}_F$	$g.l^{-1}$	6.6	8.03	6.5
$\bar{x}_E$	$g.l^{-1}$	3.53	3.7	2.996
μ	h^{-1}	0.0251	0.01613	0.0149
$Y_{x/s}$	$\frac{gmould}{gsubs}^{-1}$	0.2854	0.2273	0.211
Productivity	$g.l^{-1}.h^{-1}$	0.147	0.1295	0.09708
Reducing Sugar Production Rate	$g.l^{-1}.h^{-1}$	0.6234	0.608	0.5121
Substrate Conversion Effectiveness	$g.g\ supplied^{-1}$	0.7502	0.7878	0.8015
Substrate Utilization Rate	$g.l^{-1}.h^{-1}$	0.5681	0.5697	0.4602
Specific Substrate Utilization Rate	$g.g^{-1}.h^{-1}$	0.08619	0.07086	0.07075
Culture pH	Units	2.26	2.105	2.24
Mould Nitrogen Content	$g.g^{-1}$	0.05575	0.0531	0.05091

Medium Type	Starch	Starch	Starch	Starch
s_R	19.71	19.8	13.2	13.2
Medium pH	7.0	7.0	7.0	7.0
Temperature	30.0	30.0	30.0	30.0
D	0.0179	0.0123	0.093	0.0923
Non-fermentable Solids	-	-	-	-
$\bar{x}_F$	9.2	6.84	3.086	3.352
$\bar{x}_E$	3.88	2.59	2.7799	2.916
μ	0.00717	0.00455	0.08186	0.0763
$Y_{x/s}$	0.1919	0.1368	0.3044	0.3083
Productivity	0.066	0.03112	0.2545	0.256
Reducing Sugar Production Rate	0.3487	0.2372	1.193	1.164
Substrate Conversion Effectiveness	0.9782	0.9842	0.9718	0.9553
Substrate Utilization Rate	0.3452	0.2273	0.8361	0.7955
Specific Substrate Utilization Rate	0.0375	0.0333	0.2709	0.2477
Culture pH	1.86	2.15	2.94	2.53
Mould Nitrogen Content	0.04732	0.0482	0.0702	0.06475

Medium Type	Starch	Starch	Starch	Starch
S_R	13.2	13.2	13.2	13.2
Medium pH	7.0	7.0	7.0	7.0
Temperature	30.0	30.0	30.0	30.0
D	0.074	0.0725	0.0679	0.0364
Non-fermentable Solids	-	-	-	-
$\bar{x}_F$	3.158	3.575	3.155	4.981
$\bar{x}_E$	2.76	3.012	2.603	3.481
μ	0.0669	0.0588	0.04896	0.0244
$Y_{x/s}$	0.3259	0.3115	0.2987	0.2793
Productivity	0.210	0.2124	0.1733	0.1244
Reducing Sugar Production Rate	0.908	0.934	0.858	0.4707
Substrate Conversion Effectiveness	0.9295	0.9759	0.9573	0.9796
Substrate Utilization Rate	0.6444	0.6819	0.5802	0.4454
Specific Substrate Utilization Rate	0.204	0.1907	0.1839	0.08942
Culture pH	2.64	3.06	2.66	2.15
Mould Nitrogen Content	0.06739	0.06257	0.06194	0.0584

Medium Type	Starch	Starch	Starch	Starch
s_R	13.2	3.3	3.3	13.2
Medium pH	7.0	7.0	7.0	7.0
Temperature	30.0	30.0	30.0	35.0
D	0.025	0.0893	0.0706	0.075
Non-fermentable Solids	-	-	-	-
$\bar{x}_F$	3.942	2.826	2.077	3.244
$\bar{x}_E$	3.85	1.052	1.048	2.732
μ	0.0238	0.0325	0.0348	0.0632
$Y_{x/s}$	0.3110	0.3172	0.3186	0.3059
Productivity	0.0944	0.09326	0.0744	0.2038
Reducing Sugar Production Rate	0.3218	0.2934	0.2334	0.929
Substrate Conversion Effectiveness	0.9882	0.9956	1.0013?	0.9384
Substrate Utilization Rate	0.3035	0.2941	0.2335	0.6662
Specific Substrate Utilization Rate	0.077	0.1048	0.1124	0.2054
Culture pH	2.55	2.65	2.62	2.67
Mould Nitrogen Content	0.05475	0.07191	0.06969	0.06686

Medium Type	Starch	Starch	Starch	Starch
s_R	13.2	13.2	13.2	13.2
Medium pH	7.0	7.0	7.0	11.0
Temperature	35	40	42	30
D	0.0666	0.0671	0.0671	0.0915
Non-fermentable Solids	-	-	-	-
$\bar{x}_F$	3.011	8.99	7.596	3.087
$\bar{x}_E$	2.584	3.966	3.551	3.315
μ	0.0555	0.0293	0.035	0.09986
$Y_{x/s}$	0.288	0.3175	0.3439	0.3573
Productivity	0.161	0.257	0.259	0.317
Reducing Sugar Production Rate	0.849	0.8607	0.8469	1.107
Substrate Conversion Effectiveness	0.9657	0.9717	0.9562	0.9165
Substrate Utilization Rate	0.559	0.8185	0.7531	0.8872
Specific Substrate Utilization Rate	0.1857	0.09284	0.09915	0.2821
Culture pH	2.85	2.9	2.49	6.5
Mould Nitrogen Content	0.05541	0.04235	0.04486	0.07228

Medium Type	Starch	Starch	Potato	Potato
s_R	13.2	13.2	20.2	21.0
Medium pH	11.0	11.0	7.0	7.0
Temperature	30	35	30.0	30.0
D	0.0725	0.0745	0.104	0.0351
Non-fermentable Solids	-	-	2.23	2.2
$\bar{x}_F$	3.165	2.9125	4.677 (2.476)	7.64 (4.424)
$\bar{x}_E$	3.953	3.542	4.675 (2.475)	9.2 (5.327)
μ	0.08835	0.0885	0.08096	0.0197
$Y_{x/s}$	0.44365	0.301	0.5502 (0.2913)	0.6225 (0.3604)
Productivity	0.2866	0.256	0.4864 (0.2575)	0.1829 (0.1059)
Reducing Sugar Production Rate	0.9179	0.9635	1.050	0.4728
Substrate Conversion Effectiveness	0.9604	0.9797	0.4998	0.6414
Substrate Utilization Rate	0.6459	0.9242	0.884	0.2938
Specific Substrate Utilization Rate	0.2041	0.3173	0.1890 (0.357)	0.03846 (0.0664)
Culture pH	6.31	6.65	-	-
Mould Nitrogen Content	0.05974	0.06845	0.04993	0.04897

Figures in parentheses relate to the mould content alone.

Medium Type	Potato	Potato	Potato	Potato
s_R	21.03	19.0	10.13	5.27
Medium pH	7.0	7.0	7.0	7.0
Temperature	30.0	30.0	30.0	30.0
D	0.0248	0.0237	0.024	0.0491
Non-fermentable Solids	2.8	2.665	1.984	0.818
$\bar{x}_F$	6.45 (3.271)	7.53 (4.836)	6.62 (3.827)	3.487 (2.424)
$\bar{x}_E$	6.38 (3.235)	8.517 (5.4702)	7.314 (4.228)	2.112 (1.468)
μ	0.01653	0.0228	0.0227	0.031
$Y_{x/s}$	0.4836 (0.245)	0.695 (0.406)	0.685 (0.383)	0.4527 (0.3123)
Productivity	0.1408 (0.0714)	0.1708 (0.1097)	0.1498 (0.0866)	0.0997 (0.0693)
Reducing Sugar Production Rate	0.3706	0.403	0.232	0.2377
Substrate Conversion Effectiveness	0.7106	0.8949	0.9542	0.9186
Substrate Utilization Rate	0.2912	0.2702	0.2261	0.2219
Specific Substrate Utilization Rate	0.04514 (0.089)	0.0359 (0.0559)	0.03415 (0.0591)	0.0636 (0.0915)
Culture pH	-	-	-	-
Mould Nitrogen Content	0.05109	0.0471	0.053	0.0585

Figures in parentheses relate to the mould content alone.

Medium Type	Potato	Potato	Potato	Potato
s_R	4.46	4.40	4.237	4.01
Medium pH	7.0	7.0	7.0	7.0
Temperature	30.0	30.0	30.0	30.0
D	0.0576	0.0671	0.03145	0.0378
Non-fermentable Solids	0.614	0.65	0.355	0.458
$\bar{x}_F$	4.136 (2.765)	3.802 (2.56)	4.315 (3.039)	3.021 (2.342)
$\bar{x}_E$	1.64 (1.096)	2.205 (1.485)	2.416 (1.702)	2.211 (1.714)
μ	0.0273	0.0375	0.0137	0.02616
$Y_{x/s}$	0.476 (0.3195)	0.498 (0.3649)	0.489 (0.3495)	0.5707 (0.4458)
Productivity	0.1125 (0.0752)	0.128 (0.0915)	0.0575 (0.0405)	0.0778 (0.0603)
Reducing Sugar Production Rate	0.2527	0.282	0.1286	0.150
Substrate Conversion Effectiveness	0.9836	0.9551	0.9666	0.9896
Substrate Utilization Rate	0.2354	0.2508	0.1159	0.1353
Specific Substrate Utilization Rate	0.0569 (0.0851)	0.0659 (0.09795)	0.02685 (0.0381)	0.04477 (0.05775)
Culture pH	-	-	-	-
Mould Nitrogen Content	0.0623	0.063	0.0578	0.0571

Figures in parentheses relate to the mould content alone.

Medium Type	Potato	Potato	Potato	Potato
s_R	3.296	3.187	2.906	1.97
Medium pH	7.0	7.0	7.0	7.0
Temperature	30.0	30.0	30.0	30.0
D	0.0232	0.03115	0.0315	0.0205
Non-fermentable Solids	0.511	0.354	0.528	0.29
$\bar{x}_F$	3.576 (2.477)	2.355 (2.087)	5.945 (4.182)	3.472 (2.924)
$\bar{x}_E$	2.98 (2.064)	1.918 (1.70)	0.936 (0.658)	0.337 (0.2834)
μ	0.0131	0.0272	0.00617	0.0105
$Y_{x/s}$	0.555 (0.4138)	0.672 (0.579)	0.6593 (0.427)	0.833 (0.533)
Productivity	0.0462 (0.032)	0.0598 (0.053)	0.0398 (0.028)	0.038 (0.032)
Reducing Sugar Production Rate	0.0839	0.0973	0.0721	0.0405
Substrate Conversion Effectiveness	1.097?	0.9817	0.7876	1.0028?
Substrate Utilization Rate	0.0773	0.0955	0.06557	0.06004
Specific Substrate Utilization Rate	0.02163 (0.0312)	0.04056 (0.0458)	0.01103 (0.0157)	0.01729 (0.0205)
Culture pH	-	-	-	-
Mould Nitrogen Content	0.0583	0.0551	0.0548	0.059

Figures in parentheses relate to the mould content alone.

Medium Type	Peel Waste	Peel Waste
s_R	20.4	19.8
Medium pH	10.8	10.4
Temperature	30.0	30.0
D	0.067	0.092
Non-fermentable Solids	2.38	2.42
$\bar{x}_F$	5.23 (3.276)	5.1 (3.083)
$\bar{x}_E$	6.29 (3.996)	6.12 (3.7)
μ	0.0817	0.1104
$Y_{x/s}$	0.601 (0.3768)	0.655 (0.3958)
Productivity	0.427 (0.2675)	0.563 (0.3403)
Reducing Sugar Production Rate	0.97	1.16
Substrate Conversion Effectiveness	0.7097	0.6368
Substrate Utilization Rate	0.71	0.86
Specific Substrate Utilization Rate	0.1357 (0.2167)	0.1686 (0.2789)
Culture pH	6.4	6.7
Mould Nitrogen Content	0.0523	0.0517

Figures in parentheses relate to the mould content alone.

APPENDIX V.

LIST OF ABBREVIATIONS AND SYMBOLS.

approx.	approximately.
atm.	atmosphere.
av.	average.
B.O.D.	Biological Oxygen Demand.
c.	circa.
cm.	centimetre.
$^{o}C.$	Degrees Centigrade.
C.O.D.	Chemical Oxygen Demand.
concn.	concentration.
const.	constant.
D.	Dilution Rate.
d.	diameter.
Eqn.	Equation.
equiv.	equivalent.
Ferms.	Fermentations.
g.	gram.
gluc.	glucose.
h.	hour.
H.P.	Horse Power.
i.d.	internal diameter.
Kg.	Kilogram.
$K_La.$	Oxygen Transfer Coefficient.
l.	litre.
m.	metre.
max.	maximum.
mg.	milligram.
ml.	millilitre.

min.	minute.
min.	minimum.
mm.	millimetre.
μ.	specific growth rate.
N.	Normality.
NFS.	Non-fermentable Solids.
nm.	nanometre.
Prod.	Productivity.
q.	Specific Substrate Utilization Rate.
r.	regression coefficient.
R.S.	Reducing Sugars.
s.	substrate concentration.
s_R	medium substrate concentration.
sec.	second.
soln.	solution.
s.l.v.	superficial liquid velocity.
sp.	species.
T.	Temperature.
v.v.m.	volumes per volume per minute.
x.	organism.
x_F.	fermenter organism concentration.
x_E.	effluent stream organism concentration.
Y.	Yield Coefficient.
Y_g.	True growth yield coefficient.
$Y_{x/s}$.	Yield of organism on substrate.
$\pm$.	plus or minus.

$>x.$	greater than x.
$<x.$	less than x.
$\gg x.$	very much greater than x.
$\longrightarrow x.$	value approaches that of x.
$\approx x.$	approximately equal to x.
$\infty .$	infinity.
$\bar{x}.$	denotes steady state value of x.
$x_F.$	denotes fermenter value of x.
$x_E.$	denotes effluent stream value of x.

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THE UTILIZATION OF LOW-GRADE CARBOHYDRATES.

by

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A thesis submitted to the University of Aston in Birmingham in support of an application for the degree of Doctor of Philosophy.

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SUMMARY.

This thesis is concerned with the application of fermentation technology to the production of useful materials from a class of carbohydrate resources presently under-utilized and even wasted.

The use of both carbohydrate-containing crops and processing wastes as fermentation substrates was extensively reviewed. It was concluded that a simple cheap fermentation process for the production of "biomass" directly from low-grade starch containing materials could have widespread application.

Experimental work was carried out using a Continuous Tower Fermenter System, cultures of Aspergillus niger M1 and three types of media. These were based on potato starch/salts (a semi-synthetic medium), raw potatoes and a waste from a "dry caustic" potato peeling process.

In all the fermentations, mould grew in the form of colonies, which were responsible in part, for the radical differences in behaviour which distinguished the tower fermenter from other continuous fermentation systems. The morphology of the mould both governed and was governed by fermenter conditions such as the oxygen transfer rate.

The concentration of mould in the fermenter was determined largely by a balance of the fluidisation and sedimentation forces acting on the colony morphology. In contrast the concentration in the effluent stream depended on the growth conditions and was usually significantly lower.

Experiments were carried out to determine the responses to

extremes of temperature and substrate limitation; in the latter, submerged sporulation occurred. When using alkaline media, steady states occurred in which the specific growth rate of the mould exceeded the culture dilution rate, attributed to a "froth flotation" effect.

The disappointingly low productivity rates and yield coefficients observed were the direct result of oxygen transfer limitations imposed by the mould morphology.

In the author's opinion the system is best suited to the production of "biomass" from effluents containing low carbohydrate concentrations.

CERTIFICATE.

I hereby certify that the work which constitutes this thesis has neither been, nor is being submitted in application for any other degree.

Signed .....

Date 20/3/77

DECLARATION.

I hereby declare that the work submitted in this thesis is the result of my own investigations except where reference is made to published literature or assistance acknowledged.

Candidate .....

Supervisor.....

Date .....20.3.77.....

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INDEX.

PREFACE.

The voracious demands of an increasingly industrialized and populated world are rapidly leading to the exhaustion of the Earth's finite resources. The food "crisis", about which so much has been written in recent years, is but one aspect of this problem, another is the rapid depletion of the oil and gas reserves that we rely on for energy and chemical feedstocks. The situation has been aggravated by our toleration of processes in which too high a proportion of the raw material input is destined for the waste stream. It is morally wrong to discard material which can be reclaimed or recycled (not necessarily in its original form), especially when this quite often results in serious pollution problems. Similarly the recent application of so much research effort to the production of (admittedly, much needed) microbial protein from such a finite source as petroleum seems equally shortsighted.

The longterm future of mankind will depend upon the adoption of a complete materials recycling system with the only inputs being resources derived from solar energy, which is most easily captured on a large scale by photosynthesis and therefore agriculture. The object of this research has been to furthur investigate the role of fermentation in this context.

SECTION I.

INTRODUCTION.

1.1 FERMENTATION - A BRIEF HISTORY.

Vital activities of microorganisms have wittingly or unwittingly been exploited by mankind probably since its earliest days. The fermentations involved in the production of cheese, yoghurt, soy sauce, wine, beer, vinegar and bread have been practised in various parts of the world for thousands of years. The art of breadmaking was well known, in Egypt certainly, at least 6000 years ago while the fermentation of fruit juices to wine had been used for so long that Ancient Greeks attributed its invention to a god, Dionysus.

However it was not until 1857 that Pasteur proved that alcoholic fermentation was brought about by living cells, yeasts, a discovery which sprang from his involvement with the problems of a small French alcohol manufacturer using sugar beet mashes. Subsequently, during the late 19th and early 20th centuries the increasing use of microorganisms in the laboratory was paralleled by the development of large-scale industrial processes such as the growth of Bakers' Yeast on molasses-salts media in deep aerated tanks and the anaerobic production of industrial alcohol.

The successful industrialization of the glycerol, acetone-butanol and citric acid fermentations in particular stimulated a rapid expansion of the field of fermentation technology during the 1920's and 1930's, resulting in methods for the production of a wide range of chemicals such as gluconic acid, itaconic acid, sorbose and 2,3-Butanediol. The expertise gained with these plants enabled the establishment during World War II of a large-scale antibiotic industry within 4 years of the first successful laboratory scale fermentations. During the postwar period the

market for bulk chemicals has been dominated by the more economic oil industry, resulting in the use of fermentation being restricted to specialized high value products such as antibiotics, vitamins, organic acids, enzymes, amino acids, sterols and modified steroids.

Traditionally the fermentation industry has relied on carbohydrate substrates, but in recent years microorganisms have been isolated which are able to metabolize hydrocarbons enabling processes to be developed for the production of metabolites and microbial protein from these resources. Unfortunately the advanced technology involved necessitates very expensive large-scale plants which limits their application.

In recent years considerable attention has been focussed on the problem of a global shortage of protein. Today a number of practicable processes exist, of which fermentation is but one, that together could totally eliminate this need if given the wholehearted support that, say, recent space programmes have enjoyed. Growth of microorganisms as a source of food is not a new idea. Quite apart from the numerous species of mushrooms that have been cultivated throughout the world for thousands of years, yeast, in various forms, has been an important dietary supplement for at least 100 years. Fodder yeast (Candida utilis) in particular has been grown on numerous waste substrates and used successfully as an animal feed throughout the world during the last 50 years.

The production of microbial protein from wastes using simple fermentation techniques could make a significant contribution to improving the diet of developing nations. The technology exists, the problems are merely with the application.

1.2 CARBOHYDRATE RESOURCES.

Increased exploitation of photosynthesis as a means of solar energy capture will result in plant materials and therefore carbohydrates assuming greater importance not only as foodstuffs but as alternatives to oil in the production of the range of chemicals that have become essential to our present lifestyle - the situation prior to the mid-20th century boom. More attention must be given to the use of a class of carbohydrate sources we term "Low-Grade", which can be considered as mainly materials which cannot easily be used in human or animal diets. The problems may be of low protein content, indigestibility or even toxicity, although in the case of effluents it is usually the high dilution which precludes their use.

1.2.1 LOW-GRADE CARBOHYDRATES.

These resources can usually be considered in the following categories:-

(1) Liquid Wastes and Effluents. Usually containing mono- and disaccharides such as Glucose, Sucrose and Maltose, though some have the polysaccharide starch present in a gelatinised state. Examples are effluents from canneries, dairies, breweries, distilleries and wood and paper processing plants.

(2) Solid Processing Residues. The polysaccharides starch and/or cellulose usually predominate in these wastes, though often lower concentrations of monosaccharides are also present. Examples include Bagasse, Potato, Cassava, Wood, Coffee and Olive Processing Wastes.

(3) Waste Portions of Crops and Uncropped Plant Materials. These are materials that our present technology is unable to utilize directly but constitute a vast source of organic matter which could be fermented. Some of the periodic crop surpluses which seem to plague the world's agricultural and marketing system could also be included in this category of presently wasted fermentation substrates. Examples are Bananas, Potatoes, Cassava, Water Hyacinths, Asphodelus tubers, Cacti, Jute, Apples and Tomatoes.

In addition we must promote the concept of growing crops specifically as substrates for fermentation. These will be selected on the basis of high yield of fermentable carbohydrate (in effect, a measure of efficiency of capture of solar energy), ease of harvesting and processing and (if necessary) ability to withstand harsh environmental conditions. Agro-industrial complexes based on cassava have been advocated by Maclennan (1) and McCann and Saddler (2) for tropical areas particularly Northern Australia. The potential yields are enormous, Inkson et al. (3) quoted an estimate that in the tropics the light energy incident upon one square metre is sufficient for the photosynthetic production of 72 $g.day^{-1}$ (i.e. 72 $tonne.hectare^{-1}.day^{-1}$).

1.3 SOME ECONOMIC COMMENTS.

It has often been argued that it is uneconomic to use Low-grade carbohydrates as substrates for the production of chemicals and microbial protein by fermentation, for example by Solomons(4).

Generally condemnations are made by comparison with the cost of synthesis from oil - an easy but somewhat shortsighted approach when dealing with such a finite reserve, especially one whose costs and availability are so vulnerable to political manipulation.

The economics of the transformation of a carbohydrate-containing crop or product from the state of a discarded waste to a useful byproduct is governed not only by substrate availability, price and processing costs, but also by LOCAL needs and even legislation, rather than on a worldwide basis. The importance of the fermentation of these materials as an antipollution and waste disposal process must be stressed. At present the community as a whole invests heavily in plant for the disposal of wastes and effluents, so any alternative process yielding a useful product must be welcome. In fact, in the extreme, a process could be costed at or below the break-even point, taking into account the present cost of disposal.

However, probably the most important economic requirement is the minimisation of capital investment, which necessitates the adoption of a simple technology using equipment fabricated with cheap materials and methods. This approach ideally should result in plants which are capable of running for long periods with little maintenance and with a low energy input - all factors which contribute to the low processing costs which are essential to this type of operation. Quite simply, in the words of Gaden (5) "process designs must reflect the realities of the situations in which they are to be employed".

SECTION 2.

LOW-GRADE CARBOHYDRATES AS FERMENTATION SUBSTRATES.

2.1 INTRODUCTION.

This review is intended to:-

(1) Include as many such carbohydrate sources as possible, on a world-wide basis, giving details of their approximate composition, seasonal availability and present use or means of disposal.

(2) Describe the work carried out so far on the fermentation of these substrates, particularly with respect to the production of microbial biomass as a source of protein.

(3) Provide information on the yields and/or economics of the processes described.

(4) Discuss other treatment systems which could be used as alternatives to, or in conjunction with, fermentation in a total utilization scheme.

For convenience, substrates will be considered in three categories:-

(1) As liquid wastes and effluents.

(2) As solid residues and wastes.

(3) As crops grown specifically for fermentation.

though, of course, the applications of the processes described frequently overlap these divisions.

2.2 UTILIZATION OF LIQUID WASTES AND EFFLUENTS.

2.2.1 MOLASSES.

During the first half of the 20th century molasses was the principal raw material for the production of ethyl alcohol. Throughout this period it was available for very little more than the cost of transportation and according to Thaysen (6) and

Jackson (7), was even dumped at sea. In fact a U.S. Treasury Report (8) indicated that even in 1956, 25.54% of the total U.S. production of ethyl alcohol was derived from this source, amounting to 575 million proof litres. However today the industry survives mainly in situations where necessity is a more important factor than economics, such as India. Continuous systems are particularly suited to this process, examples being those developed by Bilford et al. (9), Owen (10), Borzani (11) and Bose and Ghose (12). Such systems were reviewed by Coote (13) who also described the considerable increases in efficiency and rate of fermentation achieved using the Tower Fermenter.

Molasses, supplemented with ammonium salts is an important substrate for the aerobic production of Bakers' Yeast. For optimum yields the level of biotin, pantothenate and inositol are important, so complementary blends of Beet, Blackstrap and Cane varieties are usually used, typical ratios being given by White (14). By incremental nutrient addition (as in the Zulauf Method), the sugar concentration in the fermenter can be kept below 5 g/l., increasing conversion efficiency and lessening the problems of alcohol formation (Dawson, 15). Total fermentation time, including a short maturation period for the yeast, is usually about 10 - 12 hours in batch systems. In continuous systems the Specific Growth Rate is 0.15 - 0.25h^{-1}. Many descriptions of the processes used have been published, including those by Balls (16), Walter (17), White (14), Olsen (18), Plevako (19), Harrison (20), Rosen (21), Beran and Zemanova (22) and the excellent review of Reed and Peppler (23).

As a substrate for microbial protein production, molasses

has probably received more attention than any other material, but surprisingly few production scale plants have been built. The Colonial Food Yeast Co. Ltd. (24) described the plant erected and operated at Frome, Jamaica, growing a polyploid yeast, Torulopsis utilis var major, which had been selected by Thaysen and Morris (25). Tower type fermenters, intensely aerated via ceramic candles, gave the plant a capacity of 12 tonnes of dry yeast per day using either molasses, raw cane sugar or raw sugar juice as a substrate. According to Thaysen (6), the fermenters were operated continuously at a Dilution Rate of $0.25h^{-1}$ maintaining the cell population at 2×10^9 cells/ml. An unexpected benefit of continuous operation of these fermenters proved to be a reduction in infection problems.

Other contributions have been made by Agarwal et al. (26), who compared the yields and vitamin contents of Candida utilis, C. arborea, Geotrichum candidum and Saccharomyces cerevisiae grown on various beet and cane molasses samples, Lewis et al. (27), Vincenty (28), Singh et al. (29), Wiley et al. (30) and Chiao and Peterson (31).

More recently, Allen et al. (32) described work carried out at both the laboratory and pilot scale growing Candida arborea, C. utilis, Rhodotorula gracilis and Zygosaccharomyces lactis on a molasses/urea/phosphate medium in stirred tank fermenters, both batch and semicontinuously. Yeast extracts were prepared, feeding trials carried out and secondary feed products produced, all successfully. The problem was simply the uneconomically high price of the substrate.

Mannan and Ahmad (33) studied the growth of Saccharomyces

cerevisiae and Torula utilis on Indian molasses (and other carbohydrate containing media) supplemented with corn steep liquor. Yields were increased to 60% and 52.6% with protein contents of 50% and 42% respectively.

Shukla and Dutta (34) described their work on the growth of fungal protein, a strain of Rhizopus sp. with a high methionine content, on a molasses/ammonium chloride/calcium phosphate medium. The batch fermentation occupied 12 days, yielding only 9.6 $g.l^{-1}$ mycelium (28% of sugar supplied) with a protein content of 34.3%. Spicer (35) also indicated the intention of Rank, Hovis, McDougall Ltd. to use molasses as a substrate for production of their fungal protein, a Fusarium sp. where economics permit.

Estevez and Almazan (36) cultivated Candida utilis on clarified cane juice and molasses at sugar concentrations of 30, 60 and 100 $g.l^{-1}$. Yields declined with increasing sugar content which was attributed to the formation of secondary metabolites which suppressed growth, insufficient nonsugar media constituents and population density.

Waste carbohydrate materials in general, but molasses especially, have been used for the synthesis of fat by microorganisms under conditions of National emergency. A yeast, Rhodotorula glutinis, is of particular interest because of its high fat content, up to 63% (on a dry basis); high fat coefficient (can be around 0.17 based on sugar used)ability to grow readily in submerged culture; high rate of fat formation and ease of recovery from the propagation medium. Ordinarily sugar concentrations of 40 - 80 $g.l^{-1}$ are used but according to Steinberg and Ordal (37) concentrations of up to 200 $g.l^{-1}$ have no effect on yeast fat

content. Nitrogen must be deficient, a concentration of 1 $g.l^{-1}$ of ammonium sulphate was found to be optimum by Enebo et al. (38) and was confirmed by Pan et al. (39). Mention must also be made of the publications of Balls (40), White and Werkman (41), Nielson and Nilsson (42) as well as the review of Dankwerts and Sellars (43).

Many organic chemicals can be produced by the fermentation of molasses substrates. Glycerol has been manufactured by a number of processes which usually depend on the addition of high concentrations of sulphite to a medium inoculated with yeast. This fixes the acetaldehyde intermediate in the anaerobic glucose to ethanol metabolic pathway, leading to the accumulation of the alternative, glycerol, in equimolar quantities. Examples include The Eoff Process described by Eoff, Linder and Beyer (44), The Schade - Farber Process (45) and the Fulmer - Underkofler - Hickey Process (46). Further information has been given by Duchenne (47), Underkofler and Hickey (48) and Freeman and Donald (49).

Molasses has also been used for the production of acetone, butanol, isopropanol, 2.3-butanediol, lactic, citric, gluconic and acetic acids, riboflavin and vitamin B12, though in some cases maize or wheat mashes are preferred. These processes have been reviewed in great detail by Underkofler and Hickey (48) and Prescott and Dunn (50).

Citrus molasses, the product of the concentration of citrus-peel press liquor has been used both as a substrate for industrial alcohol production and the growth of food yeast (Van Loesecke, 51; Hendrickson and Kesterson, 52, 53). More recently, citrus wastes, particularly molasses, have been used for the production

of β-carotene by Blakeslea trispora mycelium. The rate of production and final yield are stimulated by a compound β-Ionone, occurring in the citrus waste (Ciegler et al, 54). It is usual, however, to omit the concentration step to molasses and to use peel juice or pressed liquor directly for fermentation.

2.2.2 CANNERY LIQUID WASTES.

Effluents produced by fruit and vegetable canneries arise largely from the necessity to wash and blanch the product and particularly as a result of leaching of cell "sap" from cut surfaces. In their review of the industries wastes, Ben-Gera and Kramer (55) quote that even fresh peas can lose up to 22% and 14% of their total sugars and protein respectively during a 3 minute blanching. Bough (56) carried out a study of the wastes from the canning of 5 types of leafy green vegetables to determine the contribution of each unit operation to the composite waste load, finding that washing and blanching accounted for 77% of the composite C.O.D. load and 90% of the flow. These wastes, together with those due to excessive peeling and trimming, make ideal fermentation substrates and have been widely used as such in the past though usually they are disposed of by an activated sludge process after mixing with city effluent (Goodson and Smith, 57). Other reviews of the sources and quantities of these wastes include those of Anderson (58) and Jones (59).

Effluents arising from the vegetable processing industry have been used by numerous workers as substrates for the culture of fungi. Humfeld (60) grew mushroom mycelium, Agaricus bisporus, in submerged culture on asparagus butt juice and the press juice

from pear waste. Litchfield and Overbeck (61) described the growth of another mushroom, Morchella hortensis on maize and pumpkin canning wastewaters, yielding 8.65 $g.l^{-1}$ over a 4 - 6 day period at 25 - 30°C.

Church et al. (62) described a process for the continuous treatment of maize and pea cannery wastewaters using Fungi Imperfecti. After mixing with nutrients, the wastewater was pumped into an open pool equipped with a 2 H.P. aerator at a Dilution Rate of 0.05 h^{-1}. The initial inoculum was of Trichoderma viride though in time the culture became mixed. A B.O.D. removal of up to 95% proved possible with mycelial yields of up to 0.646 and 0.42 $g.l^{-1}$ on maize and pea waste respectively, which was harvested for use as an animal feed. Aspergillus oryzae has also been used successfully for the treatment of maize and soya food processing waste waters (North Star Research and Development Institute, 63).

Wastewaters from citrus fruit processing and canning plants have been widely used as substrates for both biomass and chemical production. They are usually very acid (mainly citric) though the total solids are mainly sugars, but it is usually necessary to add nitrogen and/or phosphorus compounds for optimum treatment by biological processes.

The production of Food Yeast (Candida utilis) on diluted citrus peel juice supplemented with salts was studied as a batch and continuous process by Nolte et al. (64) and Veldhuis and Gordon (65) respectively. Yields as high as 60% dry weight (of the sugar supplied) were obtained, but the methionine content of the product was low. Block et al. (66) cultured mycelium of a

mushroom A. bisporus on various media containing citrus press water, orange juice and corn steep liquor. Nolte et al. (64) also demonstrated the feasibility of the production of industrial alcohol from citrus peel juice.

Vinegar has also been made by the double fermentation of orange peel juice but McNary and Dougherty (67) found that elimination of peel oils from the medium was necessary for a satisfactory product. Long and Patrick (68) were able to successfully ferment citrus peel juice with two Aerobacter aerogenes strains to 2,3-butylene glycol with a yield of 4.8 - 5.3%.

The disposal of Pineapple processing wastes has received a great deal of attention. According to Collins (69) sugar syrups for canning, cattle feed, alcohol and organic acid production are derived on a commercial basis from waste skins and cores. The first product is a mill juice containing c. 130 $g.l^{-1}$ soluble solids of which 75 - 80% are sugars, 7 - 9% citric acid, 2% malic acid and 2.5 - 4% protein. About one third of this can be clarified (by the method of Gould and Ash, 70), fortified with more sugar and used in canning. The remainder, if not fermented, is concentrated and fed to live-stock.

Utilization of this pineapple mill juice for vinegar production has been studied by Spurgin (71) and Richardson (72). The juice contains between 90 and 130 $g.l^{-1}$ sugar (as sucrose, glucose and fructose) in winter and summer respectively whereas the optimum for vinegar base production is 90 $g.l^{-1}$. Dilutions are made accordingly and the medium fermented with Saccharomyces ellipsoideus (a high alcohol tolerant "wine" yeast).

Richardson produced vinegars of up to 70 $g.l^{-1}$ acetic acid in less than 24 hours with a 90% conversion efficiency. The addition of bacterial nutrients did not improve either the yield or the rate of production of acetic acid.

2.2.3 WHEY.

To appreciate the suitability of whey as a fermentation medium, it is necessary first to consider its gross composition. According to data cited by Van Slyke and Price (73) and Olling (74) a typical whey contains:-

Water	931 $g.l^{-1}$	Lactose	49 $g.l^{-1}$
Fat	3 $g.l^{-1}$	Protein	9 $g.l^{-1}$
Ash	6 $g.l^{-1}$	Lactic Acid	2 $g.l^{-1}$

The ash fraction, which imposes certain limitations on the use of whey in fermentations, consists mainly of potassium and phosphorus compounds. The composition of whey is variable because cheese making procedures and milk composition are not constant, whey resulting from the manufacture of cottage or cream cheese contains more lactic acid and correspondingly less lactose than does whey from, say, Cheddar cheese manufacture. Although whey contains a variety of salts, it is deficient in inorganic nitrogen compounds which must be added if certain fermentations are to proceed satisfactorily.

Lactic acid was first produced commercially from whey in 1936 by a batch process described by Olive (75) and Burton (76), though the fermentation had been carried out continuously on a semi plant basis by Whittier and Rogers (77). A similar process using cheese whey was also described by Cambell (78).

In each case pasteurized whey is inoculated with a culture of Lactobacillus bulgaricus and held at 43°C for between 24 and 42 h., in the continuous system the Dilution Rate being about 0.05 h^{-1}. Yields of 85 - 90% are possible based on the weight of lactose used. Swaby (79) however advocated the use of a mixed culture of L. bulgaricus and Mycoderma sp.

A successful method for producing ethyl alcohol from whey was also described by Browne (80) and Rogosa et al. (81). The process consisted of two stages, Protein was precipitated by acidifying the whey to pH 5.0 and boiling. After cooling the resulting liquor to 34°C, a culture of Candida pseudotropicalis was added and the fermentation allowed to proceed at this temperature for 48 - 72 hours. The yield of alcohol was between 84 and 91% with byproducts of course being whey protein and surplus yeast. Wilharm and Sack (82) supplemented whey with lactose and though the yield of alcohol increased, the efficiency of conversion of the fermentation was reduced.

Of all the fermentations carried out with whey, most interest in recent years has centred around the production of food yeast, of which Saccharomyces fragilis is undoubtedly the organism of choice. A variety of procedures have been suggested for growth of yeast in whey, but the most detailed recently reported studies on the maximum yield of yeast cells (and protein) are those of Wasserman and his associates (Wasserman, 83, 84, 85; Wasserman and Hampson, 86; Wasserman et al, 87, 88,89).

Supplementation of whey with phosphorus and ammonium sulphate at the rate of 2.25 and 8.5 $g.l^{-1}$ respectively is necessary for maximum yields. The optimum pH is in the range of 5.0 to 5.7

and temperature, 33°C. However good yields have been obtained at temperatures of up to 43°C, such wide tolerances reducing the need for precise control of the fermenter conditions. Heavy yeast inocula are used (about 24 $g.l^{-1}$ wet equivalent in dry weight to approximately 30% of the weight of lactose in the medium) which permits a 4 h. incubation period for maximum yield. Harvesting of the product is by centrifugation.

Excess oxygen is necessary if growth rate and yield coefficient are not to suffer. Initially the demand is about 0.03 $l.l^{-1}min^{-1}$. rising steadily to about 0.11 $l.l^{-1}.min^{-1}$ after 2 hours and declining sharply to less than 0.02 $l.l^{-1}.min^{-1}$ at the end of the 4 hours fermentation. The yield is about 0.42 kg. of dry yeast per kg. of lactose.

In his review Oborn (90) described two commercial processes for the production of substantial amounts of dried yeast from whey. The "Wheast" Process is based on the findings of Wasserman and associates, described above, whereas the "S.A.V." Process is a two stage system which can be operated in either the batch or continuous mode. In the first stage lactose is fermented, whilst in the second, a major portion of the lactic acid is utilized. In this case the product is recovered by evaporation and spray drying.

Whey has also been used as a substrate for the production of the enzyme lactase which has numerous uses in the preparation of milk-based food products. Myers and Stimpson (91) patented one of the first processes using S. fragilis, S. lactis, C. spherica, C. pseudotropicalis and C. utilis. More recently, Wendorff et al. (92) reported that for maximum lactase

production, S.fragilis required whey containing 10 - 15% lactose, a pH of 4.0 - 4.5, the addition of corn steep liquor or casein digest and incubation at 27°C.

Whey has also been used as a substrate for vitamin production. Lutskova (93) grew Proprionibacterium shermanii in batch culture with incremental addition of whey/ammonium sulphate/cobalt chloride medium for 4 - 5 days at 30°C and pH 7.0. The yield was 5.6g.l^{-1} Vitamin B12 in 13.4g.l^{-1} biomass.

Several investigators have suggested the use of microorganisms in the genus Geotrichum to increase the protein and/or fat content of whey. The "Biosyn" method described by Fabel (94) uses Geotrichum candidum which can have a protein content of about 50%. According to Balls (95), during World War II, a process was employed to grow this organism for incorporation in sausage for human consumption. More recently Atkin et al. (96) used Geotrichum rotundatum to produce protein from whey in both batch and continuous culture. This organism assimilates but does not ferment lactose and proved to have a much higher yield factor than S. fragilis grown under comparable conditions.

Tomisek et al. (97) grew Torulopsis utilis, T. casei and T. cremoris on whey with yields of 16 - 21g.l^{-1}, whilst Zalashka and Samtsevich (98) used 113 yeast strains, the most productive being Candida humicola and C. curvata. Biomass yield was in the range of 18 - 30g.l^{-1}, but the addition of nitrogen to the medium increased only the protein content, not the yield.

Delaney and Donnelly (99) advocated fractionation of whey

using such processes as Reverse Osmosis, Ultrafiltration and/or Gel Filtration (which have now become feasible on a large scale), followed by fermentation with S. fragilis or Fusarium sp. According to Wasserman (84) whey discarded in the U.S. contains 204,000 tonnes of sugar and 36,000 tonnes of protein, representing a potential yield of 90 - 100,000 tonnes of protein in about 150,000 tonnes of animal fodder - probably about 10% of U.S. concentrate requirements.

2.2.4 SULPHITE LIQUOR.

In the manufacture of paper pulp, softwoods such as spruce are fractionated by cooking with sulphites at about 140°C. This results in a waste liquor, sulphite liquor, which contains 100 - 120 $g.l^{-1}$. total solids of which 15 - 20 $g.l^{-1}$ are sugars. For each tonne of pulp produced there are 8 - 10 tonnes of sulphite liquor (c. 9000 l.) but the composition varies widely with the source, type of wood pulped and the nature of the process. Eweson (100) gave a typical analysis for liquor obtained from Swedish Spruce, consisting of (for each 1000 kg. of cellulose produced):-

Lignin	644 kg.	Carbohydrates	311 kg.
Proteins	15 kg.	Resin and fats	73 kg.
Sulphur dioxide combined with lignin			235 kg.
Calcium oxide combined with lignosulphonic acid			102 kg.

The carbohydrates consisted of 49.4% glucose, 15.6% mannose, 8.1% galactose and 26.9% pentosans (arabinose).

Eweson (100) gave this analysis as part of his description of the "Heijkenskjold Process" for the manufacture of Bakers'

Yeast, Saccharomyces cerevisiae from sulphite liquor mixed with a small quantity of molasses. However, for food yeast production on this waste, Candida utilis is a more suitable choice on account of its ability to assimilate a wide variety of carbon and nitrogen sources including hexoses, pentoses, organic acids and other constituents that are characteristic of acid hydrolysates of wood and other cellulosic materials.

Laboratory studies on food yeast production from waste sulphite liquors have been carried out by Walker and Morgen (101), Harris et al. (102) and Markham et al (103). According to Saeman et al. (104) and Locke (105) food yeast was produced on a large scale from sulphite liquors in Germany during World War II, principally by the "Waldhof Process", but Geotrichum candidum was also grown (Frazier, 106). A continuous commercial process using a Waldhof fermenter was also described by Inskeep et al. (107), and in 1958, Mead (108) reported that 25% of all sulphite liquor solids were made into yeast, representing about 50,000 tonnes/year production.

However, Barta (109) used another yeast, Cryptococcus diffluens on a production scale on account of its superior amino acid content, Kosaric et al. (110) even grew mushrooms, a Morchella sp. in submerged culture on this substrate. Recently two new processes have been announced (111) the "Attisholz" and "Pekilo" Processes in which filamentous fungi are grown on sulphite wastes as a source of protein, the latter apparently being easily adapted to other wastes.

Ethyl alcohol has been produced commercially from sulphite liquor in the United States, Canada, Sweden and other countries.

Ericsson (112) described the process operated at the Bellingham, Washington, plant which produced about 300 tonnes of pulp per day. The waste liquor was steam stripped to remove sulphur dioxide, flash cooled to 30°C (concentrating it by 10 - 12%) and limed to pH 4.5, before being fed to a seven stage continuous agitated fermenter system with a residence time of about 20 h. The yield of alcohol was about 100 $l.tonne^{-1}$ of pulp, giving the plant a capacity of at least 30,000 $l.day^{-1}$. Reuse of yeast was essential for a rapid and efficient fermentation.

Leonard et al. (113) thoroughly studied the production of lactic acid from sulphite waste liquor using a number of strains of Lactobacillus pentosus in a batch fermentation lasting 40 - 48 hours. From 9,000 litres of liquor (resulting from the production of 1 tonne of pulp) they were able to produce 130 kg. of lactic and acetic acids respectively, at a recovery efficiency of 95%.

2.2.5 STARCH AND SUCROSE PROCESSING WASTES.

Starch is prepared commercially from three main sources - cereal grains especially maize, cull potatoes and low-grade cassava, the effluent from these plants constituting a major pollution problem. Treatment is usually at a conventional sewage works with no attempt being made to recover a useful product. In the manufacture of potato starch all the solubles are dumped, which can amount to 27% of the dry weight of the potato according to White (114).

However protein recovery from potato effluents is becoming more common. Most methods rely upon coagulation of the protein from the acidified liquor using steam injection heating (to about

85°C) as in the "Avebe Process" (115) and the method described by Strolle et al. (116). Coagulated protein can be used as an animal feed and the residual liquor subjected to further treatment. Hausler and Malcher (117) advocated methane fermentation. Milanovich (118) used yeasts to assimilate the organic acids in the waste, finding 0.45 g. organic acids yielded 0.158 g. of yeast of 90% dry matter. In their economic analysis of methods of treating potato waste liquors, Stabile et al. (119) found that recovery of free amino acids from the waste was also a viable proposition.

Weaver et al. (120) used a mixed culture of Torulopsis utilis and Aspergillus niger to treat potato waste liquor continuously in a Waldhof fermenter, achieving appreciable reductions in the B.O.D. Reiser (121) grew Candida utilis on similar wastes, again with no added nutrients and recovered 50% of the solids as yeast, reducing the B.O.D. by at least 60%. He experimented with both batch and continuous culture with a stirred tank fermenter run at 30 - 32°C and pH 5.0.

According to Stanton (122), Torula yeast has been grown successfully on Tapioca starch waste liquors in Thailand at pilot plant scale. C. utilis and Lentinus edodes were used by Takakawa and Furukawa (123) for the treatment of the waste juice from sweet potato starch manufacture.

Effluent liquors from Beet and Cane sugar processing plants have been largely neglected as fermentation substrates, being at present treated in conventional sewage works (Fischer and Hungerford, 124). However Blackwood et al. (125) used these, corn processing and cheese factory (whey) wastes as substrates

for the growth of Rhodotorula glutinis as a source of lipids. They used a stirred chemostat with ammonium sulphate as the limiting nitrogen source. Yields could be increased by using oxygen instead of air and with a two stage system in which conditions were optimum for growth and fat production respectively. Fat contents of 40 - 45% of the dry weight were usual with an analysis typical of vegetable oils.

Mention must also be made of a waste from the preparation of maize starch, corn steep liquor, which is used widely as a medium constituent by the fermentation industry particularly for antibiotic production, on account of its proven stimulatory effects on microbial growth.

2.2.6 BREWERY AND DISTILLERY EFFLUENTS.

Brewery effluents arise from such sources as tank washing, beer destruction, keg and bottle washing and even the occasional practice of resuspending spent grains and hops to remove them from vessels. They have been considered in detail by many authors including Quittenton (126), Walker (127) and Isaac and Anderson (128). Because of their extremely high Carbon : Nitrogen ratio these effluents are rarely treated in isolation, usually after mixing with city sewage. Liquors can contain as much as 2% total solids of which 50% are Proteins, 25% Fats and 20% soluble carbohydrates (some pentoses and hexoses, but mainly dextrins).

Middlekrauf et al. (129) worked on the biological degradation of spent grain effluent in a 122 x 15.25 cm. plastic

tower packed with ceramic material, using an "enriched" culture of garden soil. Effluent C.O.D. could be reduced by 90% in continuous culture at the rate of 0.5 $l.h^{-1}$, but no attempt was made to use pure culture.

Distillery effluents have received rather more attention. The B.O.D. of waste pot ale is so high, often 25 - 30,000 $mg.l^{-1}$, that the best means of treatment is evaporation to a syrup and incorporation into animal feed either in this form or as a spray dried powder. These processes were described by Rae (130) and Lines (131). According to Lines (131), spent wash solids from molasses-alcohol fermentations can be recovered in a similar manner. The low protein and high mineral content of the product make it uneconomic to manufacture in Britain but some is made in South Africa.

In Russia, Chastukin (133) cultured mould mycelia on residual liquids (spent washes) from "treacle" (molasses) "hydrolytic" (grain?) and sulphite alcohol manufacturing plants. The surface culture method used was not practicable in view of the large areas and labour required, especially as the growth rate was comparatively slow. Later, Chastukin et al. (134) described their work with the growth of 22 different fungi on "treacle liquor" (most likely the concentrated stillage from a blackstrap or beet molasses - alcohol plant). The four most promising species were Aspergillus niger, A. oryzae, Oidium lactis and a Fusarium sp.

Falanghe (135) attempted to produce mushroom mycelium in submerged culture on a medium prepared from vinasse, a waste product from the distillation of fermented sugar cane juice.

Agaricus bisporus had the highest protein content but Boletus indecisus yielded far more mycelium and had a greater total weight of protein.

2.2.7 COCONUT PROCESSING EFFLUENTS.

Coconut wastewaters constitute a serious environmental pollution in many tropical countries, containing typically about 4.7% total sugars present as glucose, fructose, sucrose and sorbitol. Smith and Bull (136) grew Saccharomyces fragilis on this waste supplemented with 4 $g.l^{-1}$ urea. The batch cultivation was characterised by two consecutive exponential phases. During Glucose, Fructose and Sucrose utilization the growth rate was high (0.72 h^{-1}) falling to 0.15 h^{-1} when the sorbitol remaining was being used. Single stage continuous cultivation resulted in sequential utilization of the carbon sources with increasing dilution rate, it was only at dilution rates of less than 0.09 h^{-1} that all four components were assimilated.

These diauxic effects of mixed sugar substrates on the growth phases of microorganisms have been discussed in detail by Monod (137), Baidya et al. (138) and Harte and Webb (139)

Hipolito et al. (140, 141), Aliwalas et al. (142) and Baens-Arcega (143) described work carried out in the Philippines growing a number of food yeasts, particularly Rhodotorula pilmanae on these wastes using a simple 100 l. Tower Fermenter. With an initial sugar concentration of 20 $g.l^{-1}$ the fermentation occupied 60 hours, the yeast yield being 51% (based on sugar used) with a protein content of 51.8%. Aliwalas et al. (142)

supplemented the medium with 0.3 g. Ammonium sulphate, 0.05 g. disodium hydrogen phosphate and 0.006 g. magnesium sulphate per gram. of reducing sugars, obtaining a yield of 5.93 $g.l^{-1}$. after 12 h.

According to Stanton (144) economically viable operations have been developed in Ceylon and the Philippines for the production of a potable alcohol and a high-quality vinegar, respectively, from the waste water from copra extraction from coconuts. Another process unique to the Philippines is the production of a jelly-like material by a continuous Leuconostoc sp. fermentation of acidified fruit juice, coconut water or similar wastes. This material, "Nata di coco" is a cellulosic polysaccharide which acquires the flavour of the raw juice from which it is derived.

2.2.8 SOYBEAN WHEY.

This is a waste product of the rapidly expanding soybean protein isolation and concentration industry. Falanghe et al. (145) cultured a number of mushroom species on this medium, the most suitable being Boletus indecisus which grew in the form of spherical colonies. The yield of protein was almost doubled if 30 $g.l^{-1}$ glucose was added to the medium. The highest yield of protein was 6.3 $g.l^{-1}$ in 12 days, produced by Tricholoma nudum, but the mycelium was diffuse and not readily separated from the spent liquor. Soybean whey has also been mentioned as a suitable substrate for the growth of Fusarium sp. (35).

2.1.9 SAUER KRAUT WASTES.

Hang et al. (147, 148, 149) have investigated the use of waste brine solutions from Sauerkraut processing. During the process 29% of the initial weight of cabbage is discarded as brine, which has a typical analysis of:-

B.O.D.	12,400 $mg.l^{-1}$
Kjeldahl Nitrogen	620 $mg.l^{-1}$
Sodium Chloride	18,600 $mg.l^{-1}$
Total acid (as lactic)	7,400 $mg.l^{-1}$
Total Phosphate	81 $mg.l^{-1}$

Of the yeasts cultivated, C. utilis yielded 6 - 7 $g.l^{-1}$ dry weight in 24 hours, S. fragilis and S. cerevisiae only 4 - 5 $g.l^{-1}$. During the fermentation the pH rose from 3.4 to just over 7, no residual lactic acid being found. The B.O.D. was reduced to about 1,550 $mg.l^{-1}$.

These workers also found that the specific yield of invertase with this medium was over twice as much as on peptone, sucrose and yeast extract medium. Yields of 62 g. of Geotrichum candidum per 100 g. B.O.D. utilised were also obtained in later experiments when the reduction in B.O.D. was 88%.

2.2.10 MISCELLANEOUS WASTES.

Stanton (144) briefly mentioned work carried out by McGarry (150) on the utilization of the effluent from rubber processing, a moderately clear, grey liquid, with high concentrations of nitrogen compounds which can be used as a culture medium for algae of the Chlorella sp. The advantage over domestic sewage (often used as a substrate for algal growth) is the considerably lower

chance of pathogenic bacteria being present.

Food production systems in which the combined harvest consists of the conventional crop, leaf protein, single cell protein grown on leaf juices and leaf fibre have been advocated for a number of years. Worgan (151) was able to increase the yields of protein from maize and pea crops sixfold by this method, another example being the work of Parades-Lopez and Camargo-Rubio (152) with alfalfa residual juice.

McLoughlin (153 and 154) used Peat extract medium for the growth of C. utilis showing that the yield was a direct function of its carbohydrate concentration. Fogarty et al. (155) used similar extracts for biomass and enzyme production. Extracts alone supported growth, but greatly improved yields of A. oryzae were obtained if as little as 2 $g.l^{-1}$ starch was added. Roberts and Murphy (156) reported that the growth of A. niger was stimulated by peat extracts, an effect which applies to a wide range of microorganisms and probably accounts for the quality of the product of the malt whisky industry of Scotland.

Yeasts have also been grown on byproducts of chemical processing. Howard and Wiken (157,158) mentioned the use of oxanone-water, a mixture of lower fatty and dicarboxylic acids resulting from the manufacture of Nylon, and Shimizer et al. (159) treated phenol-containing waste waters continuously with C. tropicalis. The maximum dilution rate was 0.25 $hr.^{-1}$ for treating an inlet concentration of 0.001 $g.l^{-1}$. The use of chemical effluents as fermentation media was reviewed by Edwards and Finn (160).

2.3 UTILIZATION OF SOLID RESIDUES AND WASTES.

This category consists mainly of wastes containing celluloses, hemicelluloses and lignin though some crop residues and food processing wastes containing starch are also included. Three general schemes can be proposed for the microbial utilization of these polymers:-

(1) Chemical hydrolysis to liberate the constituent sugar molecules which can be utilized by microorganisms.

(2) Enzymic degradation to yield the component sugars which can be metabolized by microorganisms.

(3) Use of a microorganism which can metabolize the polymer directly.

Options (1) and (2) have been most widely exploited in the past, largely because they permit greater flexibility in the choice of organism and fermentation. Option (3) necessarily reduces the choice of organism and fermentation and almost invariably involves a limitation of reaction rate.

2.3.1 WASTES CONTAINING STARCH.

These substrates, which include the wastes resulting from the processing of potatoes, sweet potatoes, cassava or tapioca and cereal grains will be dealt with in section 2.4 in conjunction with their respective parent materials.

2.3.2 WASTES CONTAINING CELLULOSES, HEMICELLULOSES AND LIGNIN.

According to Hall and Slessor (161), of the total weight of carbon photosynthetically fixed in one year (estimated at 2×10^{11} tonnes), approximately one half is in the form of cellulose,

making it probably the most abundant organic material on Earth. However, on account of its relative inertness, the difficult and often uneconomic degradation necessary in the preparation of a suitable medium for fermentation has precluded the use of cellulose in most processes. If the recent industrialisation of the amylase and glucose isomerase processes can be successfully repeated with cellulases then its use in the future seems assured.

2.3.2.1 STRAW AND CEREAL WASTES.

As long ago as 1920, Pringsheim and Lichenstein (162) described a method used to upgrade the protein content of straw in Germany as a wartime emergency measure to provide cattle food. Ammonium salts (mainly the sulphate) were mixed with the straw which was then inoculated with spores of an Aspergillus sp. During an incubation period the crude protein content increased from 0.9% to about 8.0%.

These agricultural wastes have been subjected to many saccharification processes to yield fermentation media (see also sections 2.3.2.2 - 2.3.2.4). Dunning and Lathrop (163) described one of the more successful continuous methods operated with corncobs, bagasse, flax shives, oat hulls and cottonseed hulls. A two stage hydrolysis was involved, the first using dilute sulphuric acid (1.9%) to separate the pentosans as xylose and furfural without saccharifying the cellulose. The second stage used concentrated sulphuric (8%) acid at 120 - 130°C for 7 - 10 min. to release hexose sugars from the cellulose, which were fermented to ethyl alcohol by Saccharomyces cerevisiae. With five products, xylose, furfural, ethyl alcohol, lignin and yeast,

it was claimed the process could be operated economically. The xylose solution could also be fermented to acetone and butanol by Clostridium acetobutylicum (Langlykke, Van Lanen and Fraser (164) or to 2,3-butanediol by Aerobacter aerogenes (Perlman, 165).

Straw was used by Bes et al. (166) as a substrate for the growth of Candida utilis after partial acid hydrolysis. The maximum concentration of reducing sugar was obtained by digesting 10 kg. of chopped straw with 40 litres of 1% sulphuric acid for 1 hour at 3 atm. steam pressure (137 - 138°C), the sugar yield being about 20% of the weight of oven-dry straw used. The composition of the extract was approximately 85% pentose (practically xylose only) because of the low acid concentration used, 14% hexose (practically glucose only) and 11% acetic acid. This extract was diluted to a concentration of 2% reducing sugars and placed in the culture vessel, a simple glass tower fermenter, 84 cm. long and 7.4 cm. in diameter with a porous stone aerator. The yield of yeast was as high as 9.52 $g.l^{-1}$ dry weight in 10 hours.

Processes involving the enzymic degradation of these wastes are few. Woo and Lee (167) used enzymes from Aspergillus niger to break down Rice Hulls and Straw. Peitersen (168)investigated the growth and cellulase production of Trichoderma viride on barley straw (pretreated with sodium hydroxide under high pressure) in a 5 l. stirred fermenter. Increasing the initial straw concentration from 10 - 20 $g.l^{-1}$ caused an increase in the initial lag phase from 0 to 2 days and the time of cessation of growth from 2 to 6 days. The protein content of the product was in the range of 21 - 26% with yield coefficients of 0.4 - 0.56

and up to 70% utilization of the straw.

2.3.2.2 CELLULOSE AND PAPER PULPS.

Grosser and Bernhauer (169) produced mycelium of 25% crude protein content by inoculation of a vigorously aerated suspension of cellulose with cultures of various species of Penicillium.

Ghose (170) obtained a solution containing more than 5% glucose by incubation of Trichoderma viride with waste sulphite-processed cellulose pulp at 50°C and pH 5.0 for 40 hours. However by using membrane concentrated cellulase and 300 $g.l^{-1}$ ground cellulose suspension, a 125 $g.l^{-1}$ reducing sugar solution could be obtained in 72 hours with a 99% conversion efficiency. This limitation of about 40% conversion of the available cellulose is at the same level that Bomar and Schmid (171) noted in their work with Cellulomonas sp. and attributed to the cellulose being present in a non-hydrolysable form.

Crawford et al. (172) used waste cellulose as a substrate for the growth of Thermonospora fusca, a thermophilic actinomycete common in soil, compost and manure, as a source of microbial protein. Updegraff (173) described work carried out on the growth of Myrothecium verrucaria on waste paper. However the maximum protein synthesis rates and cell yields were very low compared to those achieved using glucose. Rogers and Spino (174) and Bellamy (175) have reviewed the production of microbial protein from cellulosic wastes, stressing the necessity for a pretreatment process (such as that described by Han and Callihan, 176, and Callihan and Irwin, 177) to increase yields and accelerate conversion rates.

Porteous (178, 179) has focussed attention on the large percentage of cellulose found in domestic refuse (due to the paper content) and proposed hydrolysis as a recovery process. Using a very small continuous flow reactor, with hydrolysis by 4 $g.l^{-1}$ sulphuric acid solution at 230^{o}C for 1 - 2 min. the conversion rates could be high with very little destruction of the sugar formed. It was claimed the process could be operated economically for the production of alcohol, whilst the results indicated that a large change in refuse composition had little effect on the rate of fermentation or yield of alcohol.

2.3.2.3 BAGASSE.

Bagasse, the residual fibrous material remaining after the extraction of raw juice from sugar cane, has always caused problems to sugar processers on account of the sheer volume of production. Even with all the research effort so far put into finding a profitable use, most of the world's bagasse is burned (its calorific value is equivalent to 380 $l.tonne^{-1}$ fuel oil). Of the uses which have been most successful, the wallboard and paper and pulp industries have taken the greatest tonnages (Hansen, 180).

The approximate composition of bagasse is as follows (Dunning and Lathrop, 181):-

Celluloses	42.2%
Hemicelluloses	17.8%
Lignin	14.0%
Total Nitrogen	1.43%
Ash (Mainly Silicon dioxide and Ferric Oxide).	1.42%

Bagasse has attracted much attention as a possible substrate for microbial protein production, degradation of the cellulose and hemicellulose portions having been carried out by both acid hydrolysis and enzymic means. Much of the early work on the acid hydrolysis of bagasse was reviewed by Cid (182), including the work of Dunning and Lathrop (163).

Recent work has been carried out by El Nawawy (183, 184) on the growth of Candida pelliculosa on bagasse hydrolysed with 1%, 5% and 10% sulphuric acid. Yields were as high as 84 g. of yeast protein from 1 kg. of pith. The yeast was mixed with the bagasse residue to constitute a cattle feed.

Dunlap et al. (185) and Callihan and Dunlap (186) gave details of the process and described the construction and operation of a pilot plant for single cell protein production by the growth of Cellulomonas sp. on cellulosic waste matter, particularly bagasse. Further work, described by Han et al. (187) was concerned with the growth of this and other species symbiotically to yield a cost-competitive product with a favourable amino acid profile.

Toyama and Ogawa (188) attempted to saccharify cellulosic wastes including lignin, such as bagasse, straw, sawdust, corrugated cardboard and newspaper. Highly active commercial cellulase preparations derived from Trichoderma viride and Aspergillus niger were used, though proved to be too expensive. Cellulase production by Trichoderma viride on a solid medium was also investigated and Candida utilis found to be suitable for submerged production from sulphite pulp waste.

2.3.2.4 WOOD.

Dry and bark-free wood wastes such as shavings and sawdust contain 50 - 70% of carbohydrate material, which is of two types - cellulose and hemicelluloses. The cellulose portion, comprising about 50% of the wood is the more resistant to chemical action, whereas the hemicelluloses (comprising about 20% of the wood) are a mixture of chemically bound sugars which can be readily decomposed by boiling for a short time with dilute acid.

The hemicelluloses from hardwoods - such as beech - yield a high percentage of Xylose, a 5-carbon sugar, whereas those from softwoods - such as pine and fir - yield a mixture of sugars composed of about one half 6-carbon sugars such as glucose, mannose and galactose.

Processes for the hydrolysis of wood to its constituent sugars have been known for more than a century, as early as 1819 Braconnot (189) reported the production of sugar from wood, and in 1898 Simonsen (190) described a process that yielded a 6% sugar solution. Two commercial plants were built in the U.S.A. during the early years of the 20th century, employing a 45 minute hydrolysis with dilute acid at 170°C. A yield of 20 - 25% sugar was obtained, which was used to produce high-quality industrial alcohol - about 100 litres of 95% alcohol from 1 tonne of dry wood waste. The plants closed in 1920 because of competition from alcohol made from blackstrap molasses (which was available at give-away prices). Other hydrolysis processes used successfully include those of Scholler (191), Bergius (192) and Giordani (193). Saeman et al. (194) described plants operated

in Germany between 1939 and 1945 producing yeast and ethanol from wood hydrolysed by the "Scholler" and "Bergius Processes".

In 1947 a new process was developed at the U.S. Forest Products Laboratory, known as the "Madison Wood-Sugar Process", described by Harris and Beglinger (195). In this a spray of hot 0.5 - 0.6% acid was pumped onto wood chips heated in a stationary digester to 150°C at the start and gradually increasing to 185°C. The resulting solution contained 5 - 6% of a mixture of simple sugars. This solution was used as a substrate for Candida utilis production in a continuous Waldhof fermenter. The residence time of the liquor was between 2½ and 3 hours with a yield of up to 50% dry weight yeast on wood sugars. It was also suggested that wood sugar plants could be built alongside other seasonally operating plants such as beet plants or sugar refineries so that steam generation, power, water and effluent disposal facilities could be shared. A number of operational difficulties were identified and resolved by Gilbert et al. (196). Based on results obtained with pilot plant procedures, a yield of 668 kg. of 50% molasses could be obtained from 1 tonne of hardwood chips.

Kobayashi (197) briefly reviewed recent work on wood hydrolysis and fermentation by various organisms. As well as using acid hydrolysis, he also mentioned the use of enzymes.

Of course, certain species of wood have been used for centuries as substrates for mushroom production, particularly of the "Shiitake" mushroom (Lentinus shiitake), L. edodes and Volvaria spp. in the Far East. These processes were well described by Block et al. (198) and Gray (199) and constitute

a major industry in Japan, the Philippines and Malaysia. However, up to the present time, little use is made of the small-section trunk and branches, sawmill and trimming waste for the culture of these fungi.

2.3.2.5 MISCELLANEOUS.

In the Far East there are a number of processes, mostly operated at the cottage level, which make use of fungi to produce palatable human food from such wastes as groundnut press-cake, coconut press-cake and defatted soybean grits. These classical processes are known collectively as the "Tempe" processes, and have been fully reviewed by Hesseltine (200) and Stanton (201). In Japan, the residual soybean presscake from oil extraction is used in the manufacture of the lower grades of soya sauce.

Bhattacharyya and Tamhane (202) described a process for the enrichment of groundnut cake with microbial protein. A screening programme of soil samples was carried out using Czapeck's medium with groundnut cake cellulose as the sole carbon source, resulting in the isolation of a strain of Streptomyces albus. By growing this organism on the cake, an appreciable increase in the protein content could be achieved.

Brewers' and distillers' spent grains have been a constant embarrassment to their producers, as their sole means of disposal is as a cattle food. They contain typically about 22% dry matter of which approximately half is carbohydrate material (limit dextrins) and one fifth protein. The high cost of haulage or drying can make them uneconomic as animal feed, so other processes for their utilization have been investigated.

Pan et al. (203) used cultures of Aspergillus niger to saccharify distillers' corn mashes, upon which they then grew food yeast. Pool and Pollock (204) treated spent grains to recover amino acids by maintaining an aqueous slurry with a bacterial or fungal protease, though the economics of this process are perhaps a little dubious in view of the low concentrations of amino acids produced.

The residues from olive-packing plants have also attracted attention as substrates for microbial protein production. Both Tomiyasu and Zenitani (205) and Tsilensis and Hedrick (206) grew food yeast on acid hydrolysates of the residues. In the latter case the solid was pulverized and treated twice with 3.5% sulphuric acid (in the liquid : solid ratio of 5 : 1) at 120°C for 3 hours. After neutralization, yeast or malt extract was added and the medium fermented. Candida utilis proved to be the most suitable organism, yielding 50 - 65% dry yeast based on sugar utilized; 4.5 - 5.5% of the olive residue could be converted to protein by this method.

Updegraff et al. (207) and Rolz (208) gave details of work carried out in Central America on wastes from coffee processing. The principal byproducts are the hulls, composed mainly of cellulose and related polysaccharides, which are all burnt (as fuel for the coffee dryers) and the pulp which is used as a mulch in the coffee fields. This pulp is a problematic substrate for microbial conversions due to its alkaloid and polyphenolic contents. The waters used for depulping and washing the beans are mainly discarded and constitute a grave pollution problem.

Rolz used these waters as substrates for the growth of Aspergillus oryzae, as well as growing yeast on molasses, and attempting enzymatic degradation of bagasses. Detailed costings of the plants and processes were also presented.

In recent years wastes from cattle and pig fattening units have attracted considerable attention as substrates for microbial growth. Griffin et al. (209) investigated cellulase production by Trichoderma viride in the batch fermentations of cattle feedlot waste, finding that the enzyme was elaborated in quantities comparable to commercial preparative fermentations; Weiner and Rhodes (210) grew T. viride, Fusarium oxysporum and F. aquaeductum directly on filtrates of the same waste. Chemical, microbial and enzymatic hydrolysates of swine waste were prepared by Savage et al. (211) and used for growth of Candida utilis. The best medium resulted from waste delignified with peracetic acid then hydrolysed with crude T. viride cellulase solution.

Brown and Reddington (212) proposed a recycling scheme using the waste from a pig fattening unit. This was to be supplemented with sucrose and used to grow Aspergillus niger (M1) which could be added to the pig feed. The maximum specific growth rate for this organism under these conditions was quoted as 0.072 h^{-1}, considerably lower than a value of 0.263 h^{-1} established by Pannell (213) growing this organism on a sucrose-salts medium in a tower fermenter.

Summinoe and Ketsu (214) obtained a cattle feed supplement by growing Aspergillus oryzae on a mixture of 10 parts dried tree leaves and 3 parts human, cow or pig faeces.

2.4 UTILIZATION OF WHOLE OR SUBSTANDARD FRACTIONS OF CROPS.

Because crops containing high carbohydrate levels are almost invariably more productive than "high-protein" crops (i.e. they are more efficient capturers of solar energy, as measured in terms of weight of carbon fixed per unit ground area) it seems likely that at some time in the future they will be grown specifically as substrates for fermentation either for microbial protein or chemical production. The particular choice of crop for a given plant location will of course be dictated by the following points:-

(1) Suitability of the crop to local conditions of climate, soil type etc.

(2) Total yield of crop and fermentable carbohydrate.

(3) Economics and ease of cultivation, harvest, processing etc.

(4) Year-round availability or ease of storage.

(5) Alternative uses or ease of disposal of byproducts (if any).

Considerable knowledge was gained in this field during the first half of the 20th century particularly in the U.S.A. when alternative substrates were being sought for the manufacture of industrial alcohol by fermentation.

Jacobs and Newton (215) produced tables of yields of alcohol from various crops based on their average fermentable sugar content. Whilst cereal grains have the highest fermentable carbohydrate content, in terms of carbohydrate synthesised per unit area crops such as sugar cane, sugar beet, cassava and potatoes are between 4 and 10 times more efficient. However

Jacobs and Newton neglected any contribution that fermentation of a hydrolysate of the cellulose portion of the crops could make to the total yield. Reference must also be made to the publications of Jacobs (216), Jackson (217) and Miller (218) for information regarding the potential use of agricultural products as fermentation substrates.

2.4.1 SUGAR CANE.

Sugar cane is grown as a cash crop in many tropical areas for the production of raw sugar for export, upon which the whole economy of a number of countries is based. At present the world potential for the production of sugar is far greater than the actual production, which is about 4.2×10^8 tonnes (F.A.O., 219), regulated in the interests of stability of price and supply. It is significant to note that in most producing areas protein deficiency is present.

Sugar cane contains 12 - 18% sucrose, and yields are usually about 15 tonnes (sucrose) per hectare. Gray and Paugh (220) grew a Cladosporium sp. (designated I - 75) on raw cane juice diluted to 2% sugar in shake flasks. When ammonium nitrate was added to the medium, the mycelial yield was 10.71 $g.l^{-1}$ and the protein content 13.7%. With the addition of corn steep liquor the figures were 15.36 $g.l^{-1}$ and 17.6% protein.

Even though these protein contents seem low by comparison with other members of the Fungi Imperfecti and Yeasts, Gray and Paugh concluded that up to 1455 kg. of protein (in approx. 7.5 tonnes of fungal mycelium) could be produced from a typical 100

tonne.hectare^{-1} cane crop. If the protein content of the cane itself were extracted directly, the total yield could be 1910 kg. of protein/hectare - not taking into account the possibility of fermentation of the bagasse (see section 2.3.2.3). Whilst the mycelial yield seems reasonable (approx. 50% based on sugar utilized) the protein content quoted is very low and in this case the production of Food Yeast would be a more suitable choice of process - as carried out commercially by Thaysen and Morris (25).

According to Spicer (35) cane juice has been used successfully as an alternative to molasses by Rank, Hovis, McDougall Ltd. for the production of a Fusarium sp. with a protein content of 55 - 60%.

2.4.2 SUGAR BEET.

The sugar beet crop is essentially one of temperate regions, particularly of Western Europe and, as in the case of sugar cane, cultivation is strictly controlled so as to achieve market stability, even though there is great potential for increase. Crops of up to 50 tonnes/hectare with a sucrose content of 16 - 22% are usual.

Gray and Abou El-Seoud (221) attempted to use minced whole sugar beets and beet shreds as a substrate for the growth of a Cladosporium sp. (I-75). Highest values of both total crude protein synthesized (per 100 g. of beet roots) and mycelial protein content, were obtained in a medium containing 85 g.l^{-1} of minced beet, 2 ml.l^{-1} of corn steep liquor and 2 g.l^{-1} of ammonium chloride. It was found that higher yields (measured

as protein synthesized per unit weight of beet roots) were obtained in a medium of lower beet content. With a sugar beet concentration of 140 $g.l^{-1}$ the total mycelial yield was 20.8 $g.l^{-1}$ with a crude protein content of 20.32%.

It seems certain that with the use of a higher yielding organism with a higher protein content, an economic process for the production of microbial protein could be devised. This process could be based on a slurry system as used by Gray and Abou El-Seoud, or use a medium resulting from more conventional techniques such as are used at present in sucrose recovery factories. A hot water extraction could take place to yield a medium containing sucrose and other beet solubles which could be fermented. Spent beet pulp is already in great demand by farmers as an animal food and would be even more so if its protein content were higher.

Willer and Sowada (222) were granted an East German Patent (No. 67,024) for the production of animal feeds with a high protein content by the fermentation of sugar beet slurries with yeast.

2.4.3 SWEET POTATO. (Ipomoea batatas).

Throughout the world, sweet potatoes are used primarily for human food. Cull and other unmarketable subgrade potatoes are fed to livestock, but such use is essentially a salvage measure. Well over 90% of the sweet potatoes used for food are purchased as the fresh raw product, the majority of the remainder being canned, though large quantities are also dehydrated to form sweet

potato flour and meal.

Freshly dug sweet potatoes contain 1.5 - 3.0% mono- and disaccharides, mostly sucrose, and have a starch content of 24 - 26% (Dawson, Greathouse and Gordon, 223). After curing and storage the sugar level increases, at the expense of the starch content, to between 5 and 6%. Certain varieties, for example the "Puerto Rico", have particularly high levels of the enzyme β- amylase, so high in fact that if a mash is made and held at 60 - 70°C for 1 - 2 hours, almost all the starch can be converted to reducing sugars.

Ethyl alcohol has been produced from dehydrated sweet potatoes using exactly the same techniques and equipment as used for grain substrates. Jump, Zarow and Stark (224) gave details of the process which involved cooking and mashing the potatoes followed by saccharification with malt, yields were approximately 27 proof litres of alcohol per bushel (approx. 25 kg.). No use was made of the potatoes' capabilities of auto-saccharification.

Both methods of saccharification have been used widely in the U.S.A. for the production of Glucose syrups (e.g. Delamarre, 225. Gore, 226), but this work has not been applied to the production of microbial protein.

Gray and Abou El-Seoud (227) used minced whole sweet potatoes as a substrate for the growth of a Cladosporium sp. (1 - 75). The highest yield was 17.54 $g.l^{-1}$ dry weight of which the protein content was 5.87 g. From 100 g. of sweet potato it was possible to produce 81.2 g. of dried product (mycelium and unused potato tissue) containing 31.6 g. protein. Since the initial protein content of the potato was only 6.9 g.%, the

total protein content was increased by a factor of 4.5. This would be of great value in the many sub-tropical areas which the sweet potato constitutes a significant part of the diet.

Otsuka et al. (228) used the sweet potato as a substrate for the production of L - Glutamic acid by the growth of a strain of Micrococcus sp.. Takakuwa et al. (229) and Takakuwa and Furukawa (123) cultured Saccharomyces cerevisiae, Candida utilis, Lentinus edodes and L. shiitake on waste juice resulting from the separation of sweet potato starch. This proved to be a very good medium, containing a growth promotant which could be extracted and assayed.

2.4.4 JERUSALEM ARTICHOKE (Helianthus tuberosus).

This plant is cultivated on a large scale in some parts of Europe and North America, where it has been widely used as a substrate for industrial alcohol production. The principal storage product is a polysaccharide, inulin, which is readily hydrolysed to fructose. A 6-year mean analysis of 20 varieties investigated by Boswell et al. (230) showed 13.33% fructose and 16.38% total sugars. The mean crop yield at that time was 26.75 tonnes.hectare^{-1}, the maximum yield noted being 40.5 tonnes.hectare^{-1}.

An inulin/fructose syrup can be extracted with hot water and fermented to alcohol directly by yeasts such as Saccharomyces cerevisiae, S. anamensis and especially Schizosaccharomyces pombe without the need for a preliminary hydrolysis or the addition of nutrients. The yeasts used have shown adaptation to the unhydrolysed syrup

according to Underkofler et al. (231).

The production of acetone and butanol from Jerusalem Artichokes was studied by Wendland et al. (232) using a mild acid hydrolysate of the tubers. It was found necessary to add corn or soybean meal to obtain maximum yields with Clostridium acetobutylicum. A similar hydrolysate was used by Andersen and Greaves (233) for the production of lactic acid using an aerobic Bacillus dextrolacticus fermentation at 47 - 50°C.

2.4.5 CASSAVA. (also known as manioc, mandioca, aipum, yuca, cassada, tapioca etc.).

Cassava is the starchy root of two plants, Manihot esculenta (the bitter cassava) and Manihot dulcis (the sweet cassava), both of which are important sources of food starch and major root crops of the tropics although they may also be grown in temperate regions such as the Southern U.S.A.

The cassava plants are semi-shrubby perennials with large fleshy roots up to 1 m. long and 15 - 20 cm. diameter containing a milky juice. The sap of the bitter cassava contains hydrogen cyanide, but this is dissipated on heating rendering the material edible. Sweet cassava does not contain this poison and can be used safely fresh. The tubers contain up to 33% carbohydrates (based on fresh weight) but are very low in protein content (approx. 1%). The crop constitutes the major portion of the diet in many developing countries e.g. Brazil, West Africa and Indonesia. World production is of the order of 100 million tonnes per year.

Cassava has many important advantages over other tropical food plants. Chief of these is the high yield, which can be up to 75 tonnes.hectare^{-1}.year^{-1} of fresh roots. This productivity is maintained even in poor soils and because of the plants semi-perennial nature, it can be harvested at any time during the year. Cassava is also resistant to drought, insect damage (in particular locusts) and diseases. Other virtues are the ease of planting and harvesting, and the use of both the roots and leaves as food.

Unfortunately mechanization in harvesting and processing of cassava is still in the earliest stages of development. The only sizeable plants are in Brazil, Thailand and the Dominican Republic - these produce cassava starch mainly for export. (Whistler and Paschall, 234). The properties and present uses of cassava were reviewed by Ayres (235) and (including fermentation) Grace (236). Use of starch-containing agricultural products, including cassava, as substrates for microbial protein synthesis was also reviewed by Nordhelm (237) and Brook et al. (238).

Much of the work carried out on the fermentation of cassava has been carried out in the solid state. Stanton (201) and Stanton and Wallbridge (239) used fungi such as Rhizopus sp., Mucor sp., Actinomucor sp. or Monilia sp. growing on cassava in the form of a paste, with an assimilable non-proteinaceous nitrogen source such as urea or ammonium salts.

The "Amylo Process", described by Grove (240) and Owen (241) was applied to a number of starch substrates as a means of producing alcohol. Rhizopus delamar and Saccharomyces cerevisiae were inoculated at a temperature of 38°C, so that saccharification and fermentation took place simultaneously in the same vessel.

With cassava and potatoes it was necessary to add a small quantity of malt to keep the mash in the desired state.

Brudzynski and Munyanganizi (242) produced alcohol by a similar process involving saccharification and simultaneous fermentation of a cassava mash using commercial enzymes and yeast. Cassava was used as a substrate for acetone-butanol production by Banzon (243), though best results were obtained with mixture containing at least 20% maize.

Gray and Abou El-Seoud (244) grew Cladosporium cladosporoides on a cassava substrate, obtaining a 5.7 fold increase in protein content in 4 days. Strasser et al. (245) described the operation and economics of starting and running a plant producing 10 tonnes. day^{-1} of a dried yeast containing meal by growing a yeast on cassava. Adegbolu et al. (246) reported on the growth and protein content of Rhizopus oligosporus, an organism used widely in Eastern (native) fermented food processes, on cassava starch media.

2.4.6 CEREAL GRAINS.

Cereal grains such as wheat, maize, barley and rice have starch and protein contents in the ranges 65 - 75% and 8 - 14% respectively. Whilst in many parts of the world there are shortages of cereals, in the U.S.A., Canada and Australia, particularly, the area planted has at times been restricted to maintain stability in price and supply.

Apart from the use throughout the world in the brewing of alcoholic beverages, during the early part of the 20th century maize and (in times of national emergency or unusual price

situation) wheat were the main substrates for ethanol production by processes which have been fully described by numerous authors (e.g. Jacobs, 247, Corman and Tsuchiya, 248, Underkofler and Hickey, 48, Prescott and Dunn, 50). The theoretical yield of alcohol on starch is 56.8% (by weight) i.e. 0.71 $l.kg^{-1}$ Data derived from these sources relating to many years production at numerous plants indicates that the theoretical yield of alcohol on maize is 511 $l.tonne^{-1}$, 460 $l.tonne^{-1}$ being the usual yield at the industrial scale. For wheat the values are 490 and 440 $l.tonne^{-1}$ respectively, whilst as a comparison, they are 128 and 115 $l.tonne^{-1}$ in the case of potatoes.

The decline in grain alcohol production was the direct result of competition from alcohol derived by petroleum and natural gas based processes. However with the recent price rises in these resources, Miller (218, 249) among others, has argued that it is now time for a complete reappraisal of the economics of production and use of fermentation alcohol.

Few processes have been developed for upgrading the protein content of cereals by fermentation. Gray and Kane (250) grew Trichoderma sp. and Dactylium dendroides in liquid culture on a rice medium. The former organism gave the greater increase in protein content by a factor of 2.85, but this was not sufficient to justify further development of the process. Smith and Reade (251) described a simple farm process growing Aspergillus oryzae on barley so as to increase the protein content sufficiently for use directly as a pig feed.

2.4.7 POTATOES AND THEIR WASTES.

Historically, the cultivation of potatoes appears to have begun around 200 A.D. in the Andes Mountains of Peru, where they were used as food in both their fresh and dehydrated state. Spanish and English explorers recognised their excellent food value and carried them as provisions on their ships, introducing them to Europe certainly before 1573 (Hatfield, 252). Until the late 19th century their sole use was in human and animal diets.

The composition of whole, fresh potatoes typically falls within the following ranges:-

Dry Matter	18 - 28%
Total Carbohydrates	15 - 23%
Starch	12 - 20%
Protein	1.3 - 2.7%
Fat	Trace - 0.5%
Crude Fibre	0.33 - 1.0%
Ash	0.64 - 1.3%

Organic acids are also present including oxylic, citric, succinic, malic and tartaric acids as well as enzymes, pectin and vitamins.

Potatoes, because of their low dry matter and protein contents have only limited use as animal food, but in certain parts of the world have been and are important industrial raw materials. Until 1945, they were the most important raw material for the extraction of starch. However, competition particularly from grain-derived supplies has led to a decline in the production and use of potato starch except where special qualities are required (Adler, 253) or where such processing is

used as a regulator of the fresh potato market. The waste pulp after pressing is in great demand as a cheap cattle food.

The alcohol industry is also used as a means of disposal of surplus and substandard potato crops, particularly in Central Europe where the distilleries are small and usually part of a large farm (Adler, 253). Larger plants have been built and operated in the U.S.A. using the processes described by Jacobs and Newton (215) and Beresford and Christensen (254). In the latter, saccharification of cooked potatoes was carried out using mold bran (made by growing Aspergillus oryzae on wheat bran, containing amylolytic enzymes) rather than malt. The yields were about 115 $l.tonne^{-1}$ with 22% Dry Matter potatoes.

Recently, however, the use of enzymes has replaced these products in saccharification of potato and cereal mashes for industrial alcohol manufacture, a topic which was reviewed by Aschengreen (255). Papers by Kreipe (256) and Yarovenko and Nakhmanovich (257) have described experimental work and kinetics of simultaneous saccharification and fermentation in batch and continuous alcohol producing systems respectively. Yarovenko et al. (258) also described how pasteurization of the mash could be avoided, by the addition of lactomycin, without significantly affecting alcohol yield.

Potatoes have received little attention as substrates for microbial protein production. Janicki et al. (259) grew yeasts on malt and mould saccharified mashes enriched with nitrogen and phosphorus salts. Mashes of up to 20% Dry Matter could support growth, but the optimum was 9% using Torula cremoris and

T. utilis in both batch and continuous processes. Using temperatures in the range 30 - 32°C, pH 3.0 and aeration rates of 1.0 - 1.6 V.V.M. in stirred tank reactors, the yields were 450 - 530 $g.kg^{-1}$ of sugar, with a protein content of 50%.

According to Janicki et al. (259) potatoes and potato wastes are important fermentation substrates in the U.S.S.R. and Poland. They quote papers by Zelanka (260) on the production of antibiotics, Melcher (261) on the production of acetone, butanol and ethanol and Dietrich (262) on the synthesis of Vit. B12, all from potato juice. In the production of biomass, mixed yeast and mould cultures are often used to give greater yields, such combinations as Trichothecium roseum and Rhodotorula aurentiaca and T. roseum and Aspergillus oryzae.

A number of other workers have used potato mashes and waste juices as substrates for the production of yeasts and filamentous fungi. Tong et al. (263, 264) grew Rhodotorula rubra and Torula utilis on acid hydrolysed potato tissue in a stirred tank fermenter, achieving protein contents of up to 57%. Simard et al. (265) treated potato chip waste water with a strain of Rhodotorula glutinis, which reduced the C.O.D. of the liquor by 85% and provided biomass with a protein content of 53%. Reiser (121) gave design figures and detailed costings for batch and continuous plants producing 5 $tonne.day^{-1}$ of dry Candida utilis from potato starch factory wastes without the addition of nutrients. Culture conditions in the continuous stirred tank fermenter were a Dilution Rate of 0.25 $h.^{-1}$, pH 5.0 and temperature 30 - 32°C. Weaver et al. (266) treated potato starch factory wastes successfully with various "activated sludge"

and soil cultures. However their experiments with a mixed culture of Torulopsis utilis and Aspergillus niger in a Waldhof fermenter gave erratic results. Their inference was that, had the process control been better, use of this mixed culture would have given the greatest C.O.D. reduction (about 85%).

Mixed culture techniques are also employed in the "Symba Process", described by Tviet (267), Jarl and Tviet (268) and Jarl (269, 270), which is a process designed for utilizing waste starch (from any source) for the production of yeast, primarily a strain of Candida utilis. This organism is propagated in continuous stirred tank fermenters in mixed culture with another yeast an Endomycopsis fibuliger strain with a high amylase producing capacity. With potato waste liquor containing about 25 $g.l^{-1}$ dry matter and a fermenter being operated at a Dilution Rate of 0.1 $h.^{-1}$, the output of yeast is about 1.05 $g.l^{-1}.h^{-1}$, a yield of about 43% of dry matter supplied. The protein content of the yeast is about 43% and the B.O.D. removal from the liquor about 85% under these conditions.

Extraction of protein directly from potato waste liquors has received a great deal of attention particularly when used in conjunction with processes for extracting other organic molecules. Most processes depend on heating the acidified liquor to coagulate and precipitate the protein - high temperatures (preferably 100+^{o}C) and low pH's (as low as pH 4) give the most efficient extractions and an easily filterable product. Examples of processes are the "Avebe Process" (115) and those used by Hausler and Malcher (271) prior to methane fermentation of the liquor and Strolle et al. (116) before an ion exchange process

for the recovery of Potassium and free amino and organic acids.

Potatoes have been used as a substrate for the production of Lactic Acid by the method described by Cordon et al. (272). Ground potatoes are saccharified with fungal amylase, an excess of calcium carbonate added and then fermented with a strain of Lactobacillus pentosus for about 5 days at 30°C. Yields are about 80 - 90% based on sugar consumed. Acid and enzyme hydrolysates of potatoes were also used by Szejtli (273) as a cheap substrate for the production of Vit. B12 by Proprionobacterium sp. and Joseph and Rao (274) to produce glutamic acid by fermentation with Micrococcus glutamicus. In the latter case it was necessary to first reduce the biotin content of the mash by absorption on activated charcoal to obtain best yields.

The Infra-Red Dry Caustic Peeling Process now used widely by the potato industry produces a peel waste of high solids content (about 15 - 25% solids) which is easily handled and collected but has a high residual alkalinity (about 1 - 2% expressed as Sodium Hydroxide). When neutralized with, preferably, hydrochloric acid the waste has a limited use as animal feed. Gee et al. (275) used a semicontinuous lactic acid fermentation as a low-cost method of neutralizing the waste to enable it to be used as a cattle feed.

However Bloch et al. (276) used the peel as a substrate for the production of alcohol. Firstly Aspergillus foetidus was cultured on hydrochloric acid neutralized waste with added nutrients. This was the same strain, N.R.R.L. 337, which gave the highest yield of Amylase of the 350 fungal strains tested by

Le Mense et al. (277) and which was, at that time, classified as an Aspergillus niger strain. The filtrate from this culture was used to saccharify more neutralized peel waste which was then fermented by yeast to ethanol. The yield varied considerably with the pretreatment process applied to the peel, but was as high as 45 $l.tonne^{-1}$ of 15.25% solid waste. The byproducts of the process when combined, were also an excellent cattle feed.

2.4.8 MISCELLANEOUS CROPS.

Recent research, described by Wolverton and McDonald (278), has shown that a tropical weed, the water hyacinth (Eichhornia crasspipes) could have tremendous value in pollution control and as a source of energy, food and animal feed. This water plant, when grown in warm sewage nutrients, can yield 17.8 tonnes of wet biomass. $hectare^{-1}$. day^{-1} (approx. 1.8 tonnes of dry plant material) and annual production rates of 212 tonnes of dried plant material per hectare are possible - unrivalled by terrestrial plants in terms of conversion of solar energy to organic molecules. The protein content of this plant, being relatively high (17 - 22% of the dry weight), can be easily and efficiently extracted directly.

Mannan and Ahmad (33) grew Saccharomyces cerevisiae and Torula utilis on acid hydrolysates of water hyacinths but the yields were poor - hardly surprising though, considering the maximum hydrolysis temperature used was 30°C. The crop is certainly worthy of further attention.

Plants which are able to thrive in semi-arid conditions are

likely to be extremely valuable in the future. The Carob tree (Ceratonia siliqua), a native of Mediterranean regions which bears pods containing about 55% sugars, has been considered a potential source of microbial protein. Imrie and Phillips (279), Imrie and Vlitos (280) and Sekeri-Pataryas et al. (281) have described work carried out on the growth of a mould Aspergillus niger M1 on hot water extracts of these pods, obtaining yields of about 43% (based on sugar supplied) with true protein contents of about 22%.

Paredes - Lopez et al. (282) grew Candida utilis on juice from the Prickly Pear or Nopal Fruit, a shrub found in arid zones. In Mexico it is found in more than 50% of the territory, but human consumption of the fruit is negligible. When operating a stirred tank fermenter at 30°C with a juice of 10 $g.l^{-1}$ sugar content, at a Dilution Rate of 0.55 h^{-1} the yeast productivity was 2.38 $g.l^{-1}.h^{-1}$ with a yield coefficient of 0.47 and Protein Content of 43%.

Citrus Fruit crop surpluses have been used widely for the production of ethanol, but, in general fruit crops have tended to be neglected as fermentation substrates. Dako et al. (283) published the results of a survey of the carbohydrate composition of 25 fruits grown in Ghana. Samples were taken at different times of the year and average total sugar values usually fell in the range of 8 - 15%, sufficient for most fermentation processes.

Benon (284) patented a process for the production of Banana Wine and Vinegar. Very ripe bananas, no longer containing starch, were mashed at 65 - 70°C for 1 hour, cooled and treated with pectinases at 40°C for 24 hours. The decanted

juice was fermented with Saccharomyces cerevisiae to produce a wine which could be used directly, distilled, or acetified to vinegar. Patel (285) found that Mango fruits (which contain 10 - 18% total sugars) were particularly susceptable to rotting as a result of growth of Aspergillus niger during storage and transport.

Arrazola (286) grew Candida utilis on hydrolysates of several plants native to Spain, particularly tubers of Asphodelus albus and A. microcarpus. On acid hydrolysis these gave juices of up to 125 $g.l^{-1}$ reducing sugars, almost exclusively fructose, which only required supplementation with ammonium salts. C. utilis and C. liquefaciens were also grown successfully on a pectin-rich must resulting from a 110°C water extraction of the pulp remaining from the extraction of essences from oranges, lemons and grapefruit.

2.5 CONCLUSIONS.

It is obvious that carbohydrate containing materials which could be used as fermentation substrates are available, or could be easily cultivated throughout the world. Similarly reliable fermentation processes have been developed which can provide food and most of the chemical "necessities" of life, or their precursors. These processes are ideal for dealing with raw plant materials and are also, in general, well suited to small-scale operations.

Processes based on materials containing mono- and disaccharides are the most versatile. With optimisation and

the use of simple, cheap fermentation plant, many of these could, almost certainly, be operated economically in the underdeveloped parts of the world.

Polymeric substrates, cellulosic materials particularly, present a much greater problem. Conventially, this has been overcome by acidic or enzymic hydrolysis to yield a fermentable solution, but this necessity often renders processes uneconomic. Use must be made of organisms which are able to ferment these polymers directly. In the case of cellulose this technology is in its infancy, but microorganisms which can grow on starch directly are well known, particularly for "biomass" production. Fermentations using these polymers, the commonest plant structural and storage materials respectively, will be of tremendous value in the total utilization of agricultural products.

SECTION 3.

THE EXPERIMENTAL WORK UNDERTAKEN.

3.1 BACKGROUND INFORMATION.

Whilst a research student at the University of Aston the author has been a member of the Tower Fermentation Research Group, a joint group of Biological Scientists working under the direction of Dr. R.N. Greenshields and Chemical Engineers under Dr. E.L. Smith. Such an inter-disciplinary approach has been found to be of great value in the Theoretical and Practical research carried out by Group Members in the development of Tower Fermentation Systems.

Prior to the author joining the group, research topics involving this fermenter had included:-

(1) Metabolite production by moulds in batch culture.

(2) Growth of moulds as "biomass" in batch culture.

(3) The morphology of mould colonies in batch culture.

(4) Fluidization/Sedimentation of Microbial Aggregates.

(5) Oxygen Transfer.

(6) Continuous Vinegar Fermentation.

(7) Continuous Alcohol Fermentation.

Thus considerable expertise had been acquired in the design, construction and operational characteristics of tower fermentation systems, particularly when applied to mould culture.

3.2 CONSIDERATIONS IN THE PROJECT PLANNING.

3.2.1 CHOICE OF SUBSTRATE.

An extensive literature survey (particularly Sections 2.2.5, 2.4.5, 2.4.6 and 2.4.7) indicated that a process for the

production of "biomass" directly from starch-containing crops and wastes could have widespread application throughout the world and probably require only minor modifications to suit individual circumstances.

The source of starch chosen for this study was the Potato, for the following reasons:-

(1) The crop is available throughout the year.

(2) Several types of industrial wastes are also available.

(3) Separated potato starch could be obtained for defined media work.

(4) With carbohydrate and total solids contents of about 20 and 25% respectively, potatoes are typical of many crops which are suitable for microbial upgrading.

3.2.2 CHOICE OF ORGANISM.

For some years, previous members of the Tower Fermentation Research Group had successfully cultured a strain of Aspergillus niger designated M1, which had been isolated by Tate and Lyle Ltd. from rotting carob beans. This Company had also carried out extensive toxicity and feeding trials with this strain, which had proved acceptable as a source of microbial protein.

This organism grows well at low pH values (pH 2 - 3) reducing the problems of contamination and because of its filamentous form, is readily harvested from the spent medium. It seemed, therefore, an ideal choice for this study.

However, prior to this work A. niger M1 had not been cultured on starch-containing media, but the ability of other strains to do so is well documented. Hasija and Wolf (287) grew

an unspecified strain of A. niger on 13 different sugar-containing media, maltose and starch giving by far the highest mycelial yields. Drews et al. (288) noted that the amylolytic activity of A. niger in initially unfavourable starchy industrial waste media would approach that of optimal media if the opportunity for adaption was provided. This proved to be the case in preliminary experiments carried out with A. niger M1.

3.2.3 CHOICE OF FERMENTATION SYSTEM.

Preliminary experiments with shake flask culture indicated that the viscosity of gelatinised starch media was a major problem, effectively limiting the initial starch concentration to a maximum of 25 $g.l^{-1}$. This, at best, would yield only 12 $g.l^{-1}$ mycelium, well below the highest concentrations achieved by Morris (289) of 22 $g.l^{-1}$ growing A. niger M1 on molasses media in a 10 l. batch tower fermenter. With a continuous system this problem of viscosity would not arise as the substrate in the fermenter would be mainly in the form of starch hydrolysis products.

At that time a colleague, Mr. S.D. Pannell, was working on the development of a 10 l. continuous tower fermenter, a new system which appeared to be ideally suited to this project and which was therefore adopted. It was also felt that the additional data generated by such a practical application would lead to a better understanding of the operating characteristics of the new fermenter.

3.3. FERMENTATION EQUIPMENT USED FOR THE PROJECT.

3.3.1 THE FERMENTER. (Fig. 3.1 - Plate 3.1).

The fermenter was built up of standard Q.V.F. Glass Pipeline Components (J.A. Jobling Ltd., Stone, Staffs.) modified by the addition of SQ-fitting, glass, screw-capped parts where necessary by the University glassblowers. This method of construction was described in detail by Cocker (290).

The main portion of the fermenter consisted of three, 400 mm. long, 100 mm. diameter pipe sections which had a total of 8 ports fitted. The upper and lower ends were closed by two 100/25 mm. pipe reducers and 25/10 mm. hose connectors. One of these was modified by the addition of a length of 12 mm. bore glass tubing, which allowed the passage of air and fermenter contents to a 20 l. aspirator bottle, the product receiver.

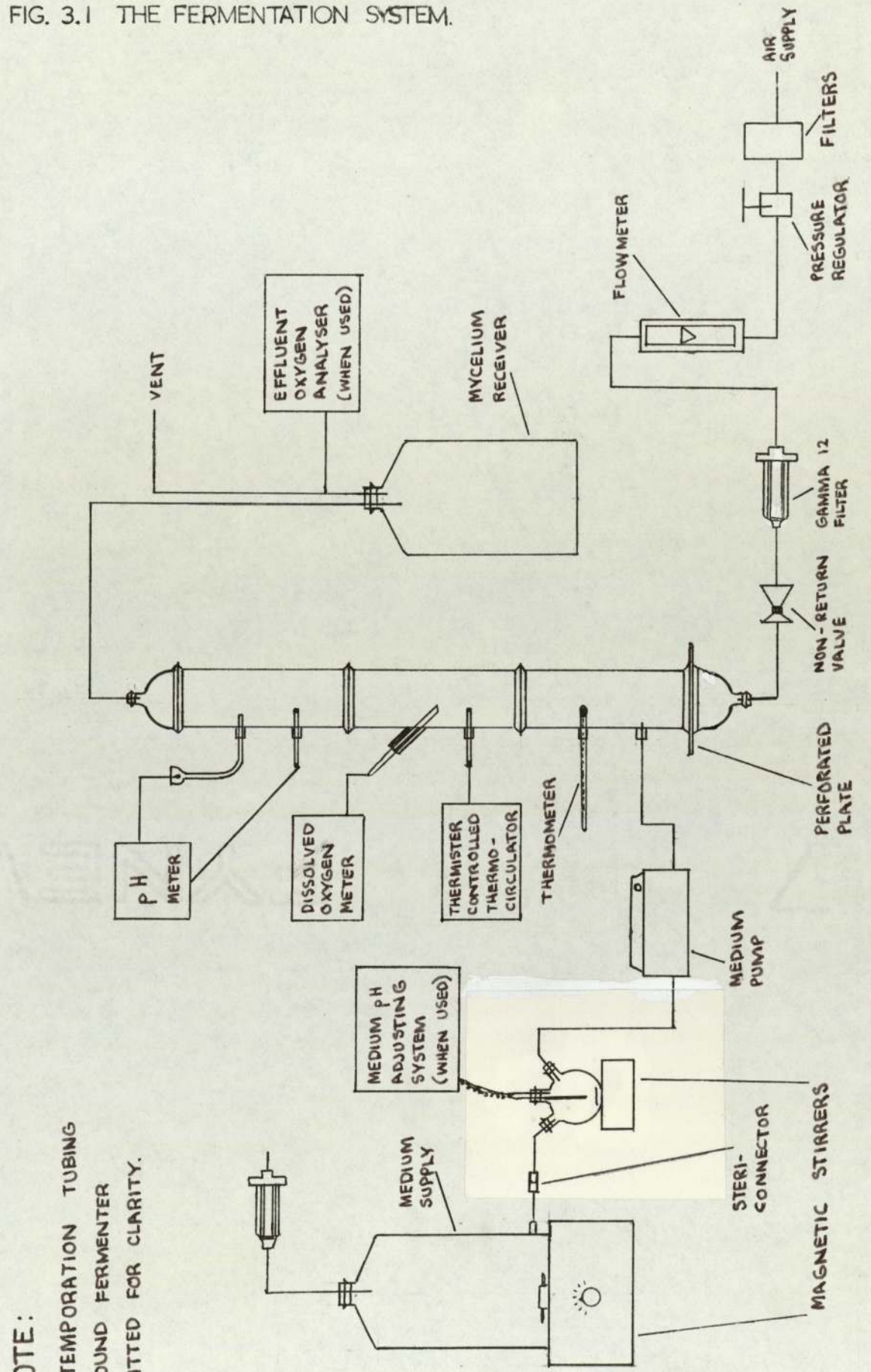
The air distributor, at the base of the 100 mm. diameter column, was a P.T.F.E. plate with 1 mm. diameter holes drilled in a regular pattern, 10 mm. apart. Gaskets between all the glass sections were also made of P.T.F.E. and all tubing in contact with medium or fermenter contents was of silicone rubber.

3.3.2 THE TEMPERATURE CONTROL SYSTEM.

Fermenter temperature was sensed by the thermistor probe of a thermocirculator (Churchill Instruments) which controlled the temperature of water circulated through "Portex" (Hythe, Sussex) 25 mm. diameter vinyl tubing wrapping the fermenter. A thermometer ($-10^{\circ}C$ - $110^{\circ}C$) was also provided to enable the temperature of the fermenter contents to be checked.

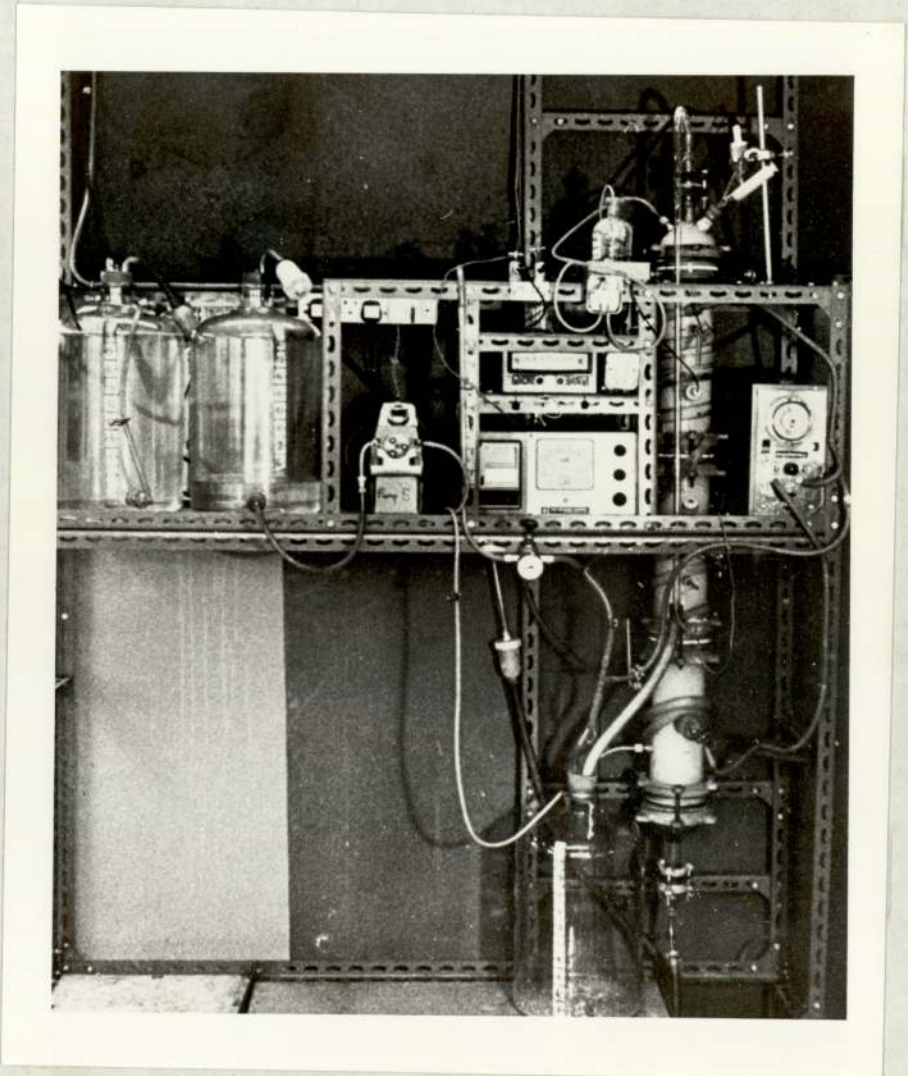
This equipment could maintain the fermenter temperature within $0.5^{\circ}C$ of the required value over the range investigated,

FIG. 3.1 THE FERMENTATION SYSTEM.



NOTE:
ATTEMPORATION TUBING
AROUND FERMENTER
OMITTED FOR CLARITY.

Plate 3.1 The Fermentation System.



though in theory was capable of greater accuracy.

3.3.3 THE AIR SUPPLY.

Double-filtered, oil-free compressed air was supplied via a pressure (and therefore, flow) controller and a "Rotameter" flow measuring device to a Whatman "Gamma 12" (Gallenkamp Ltd.) filter unit fitted with a Grade 03 filter element. The sterile air flow was piped to the fermenter via a non-return valve, which was intended to prevent filter wetting in the event of an air supply failure.

Calibration of the air flow measuring tube (Rotameter Ltd., Croyden) was achieved by a water displacement technique using the exit air from the fermenter in its usual operating condition. The air flow rate used throughout this study was 10.2 $l.min^{-1}$, obtained at a gauge pressure of 0.2 $kg.cm^{-2}$. This aeration rate corresponds to a superficial gas velocity of 2 $cm.sec^{-1}$ in the 10 cm. diameter sections.

3.3.4 THE MEDIUM SUPPLY.

20 l. "Pyrex" glass aspirators equipped with "Gamma 12" inlet air filters and magnetic stirrer followers were used as medium supply vessels, being constantly mixed by means of a magnetic stirrer (L.H. Engineering, Stoke Poges, Bucks.). Medium was pumped through 5 mm. bore silicone rubber tubing to the fermenter by a peristaltic pump. For the early work, a Watson Marlow MHRE pump (Watson Marlow, Marlow, Bucks.) was used but this was later replaced with a Baron Yemm BYO 800 (Baron Yemm

Developments, Watford, Herts.) which had a more constant pumping rate. "Steri-connectors" (L.H. Engineering) were used to reduce the chances of contamination when changing aspirators.

To avoid blockages by suspended solids 5 mm. bore tubing was necessary, resulting in minimum medium flow rates considerably greater than required. A simple electric timer device was used so that power was only supplied to the pump for 15 sec. in each minute, thus reducing the flow rate to a more suitable range.

For some later work, a small magnetically stirred chamber, equipped with a pH probe and an acid/alkalie injection system was inserted in the medium input line between the pump and the fermenter. This enabled adjustment and control of the medium input pH.

3.3.5 pH EQUIPMENT.

pH was monitored by an Analytical Measurements meter-recorder, using an E.I.L. 1050 series toughened, gel-type probe with a separate reference probe (connected by a KCl bridge to the fermenter contents). This very reliable probe could be steam-sterilized and functioned perfectly in a horizontal position in a fermenter port.

In later work, where pH control was required, an E.I.L. 91B meter-controller was used (with the same probes) acting via 2 "Delta" pumps (Watson Marlow) with 2 mm. bore silicone rubber tubing, pumping hydrochloric acid or sodium hydroxide as necessary.

3.3.6 OXYGEN ANALYSIS EQUIPMENT.

Dissolved oxygen concentration in the fermenter was monitored with a New Brunswick (New Brunswick, New Jersey, U.S.A.) meter-recorder and probe. Though this probe was steam-sterilizable, it had to be mounted in a vertical position, necessitating an angled port in the fermenter wall.

The concentration of oxygen in the input and exit air lines could be monitored by a Servomex (OA101) oxygen analyser (Servomex Ltd., Crowborough, Sussex) but in practice the difference was so small that measurement was of little value.

3.4 ANALYTICAL METHODS AND EQUIPMENT.

3.4.1 SAMPLING TECHNIQUES.

FERMENTER. To obtain representative samples of the fermenter contents, a completely unobstructed sample pipe was essential. The first 0.05 l. was disgarded and then 0.120 l. run into a measuring cylinder. The volume was then adjusted to 0.100 l., the usual sample size.

PRODUCT RECEIVER. This vessel was removed from the fermenter and shaken vigorously to ensure the contents were well mixed before sampling. The volume taken was at least 0.5 l., usually 1.0 l. and when the mycelial concentration was very low, 2.0 or 4.0 l.

FREQUENCY OF SAMPLING. The fermenter contents could not be sampled as frequently as was desired. Though each sample only removed about 2% of the fermenter contents, at the lowest growth

rates investigated this could represent 6 hours productivity. Therefore, when operating at the lowest growth rates, sampling was restricted to once per day. For most of the experimental work, samples were taken twice per day but at the highest growth rates they were taken at 4 hour intervals. Samples of the contents of the product receiver were usually taken at the same time.

3.4.2 DRY WEIGHT DETERMINATION.

FERMENTER SAMPLES. 0.100 l. samples of the fermenter contents were filtered on Greens Hyduro 904 filter paper on a 7 cm. diameter Buchner funnel. The mycelial pad was dried at 105°C to constant weight (at least 24 h.) and a 0.03 l. sample of the filtrate stored in a McCartney bottle at 4°C until analysed.

PRODUCT RECEIVER SAMPLES. These samples were filtered on the same grade of paper on a 21 cm. diameter Buchner funnel and the mycelial pad dried under the same conditions.

3.4.3 REDUCING SUGAR DETERMINATIONS.

Initially these determinations were carried out using the Ferricyanide Method described by Somogyi (291) and could be carried out routinely to an accuracy of ± 2%. However the method was not particularly suitable for large numbers of samples or those containing gelatinized starch.

After comparing numerous other methods, the 3,5 - Dinitro-salicylic acid method described by Sumner (292), Klemme and Poe (293), Edson and Poe (294) and Lindsay (295) was chosen. Using an E.E.L. "Spectra" Colourimeter (E.E.L. Ltd., Halstead, Essex)

routine analysis of samples with 0 - 2 $g.l^{-1}$ reducing sugar content could be carried out to an accuracy of ± 1%.

Details of these methods are given in Appendix I.

3.4.4 TOTAL SUGAR DETERMINATION.

Samples were acid hydrolysed in a boiling water bath for 10 minutes, neutralized and the reducing sugars formed estimated by the 3, 5 - Dinitrosalicylic acid Method. Further details are to be found in Appendix I.

3.4.5 AMMONIA NITROGEN DETERMINATION.

The standard micro-kjeldahl Method described by Markham (296) was used. Ammonia, liberated from the sample by the addition of sodium hydroxide solution (400 $g.l^{-1}$), was steam-distilled off in a Markham Apparatus and collected in saturated (40 $g.l^{-1}$) Boric acid solution. This was titrated with 0.01N Hydrochloric acid using a Methylene Blue/Methyl Red indicator. Blank and standard checks were made regularly, the method usually being accurate to within ± 1%.

3.4.6 TOTAL NITROGEN DETERMINATION.

Samples were digested with concentrated sulphuric acid and a catalyst containing selenium, copper sulphate and potassium sulphate in the ratio 1 : 5 : 32 parts by weight. The ammonia formed was estimated as in section 3.4.5.

3.4.7 RATE OF REDUCING SUGAR FORMATION FROM STARCH.

The combined effects of the enzymes α- and β-amylase and maltase in the culture filtrate were measured by the technique described in more detail in Appendix II. Reducing sugars produced by the filtrate from a starch solution buffered at pH 4.6 and maintained at 30°C were assayed by the Dinitrosalicylic acid Method.

Whilst yielding a value for the rate of production of reducing sugars under standard conditions, it was found that the result obtained from the calculation of Mass Balances (described in Section 3.6) was a better indication of the experimental situation, so the test was only used infrequently.

3.5. FERMENTATION TECHNIQUES.

3.5.1 SELECTION AND MAINTENANCE OF CULTURES.

The parent culture of Aspergillus niger M1 was obtained in the form of a silica gel master. Spores from this were subcultured at 30°C on Potato Agar (twice) then Starch Agar (twice) in Petri dishes, in each case the inoculum being spores from the fastest - growing colonies on the previous plate. Finally spores from this last plate were used to inoculate McCartney bottle starch agar slopes, the new master and submaster cultures, which were stored at 4°C until required.

POTATO AGAR. (Booth, 297).

Potatoes (Peeled)	250 g.
Agar	25 g.
Water	1 l.

Chopped potatoes were gently boiled in the water for 30 min. then allowed to cool and settle. The liquid was decanted off, made up to 1 l. and agar added. After heating on a water bath to dissolve the agar, the medium was autoclaved at 1.05 kg./cm^2 for 15 min.

STARCH AGAR.

Starch	20 g.
Ammonium Sulphate	4.15 g.
Sodium Dihydrogen Orthophosphate	0.4 g.
Yeast Extract	0.4 g.
Potassium Chloride	0.2 g.
Magnesium Sulphate	0.08 g.
Calcium Chloride	0.04 g.
Agar	20 g.
Distilled Water	1 l.

The starch, salts and yeast extract were creamed in about 100 cm^3 of the distilled water while the agar was being dissolved in the remainder as it was being gently brought to the boil. The two liquids were mixed whilst being stirred vigorously and then autoclaved at 1.05 kg./cm^3 for 15 min.

3.5.2 PREPARATION OF INOCULA.

250 cm^3 conical flasks containing about 50 cm^3 of starch agar medium were inoculated with spores from a submaster culture and incubated at 30°C until mycelial growth had covered the medium surface and sporulated (about 5 - 7 days). These spore-containing flasks could be used immediately or stored at 4°C for

up to 3 months with no apparent deletarious effects.

About 3 h. before use, 0.04 l. of sterile "Tween 80" solution (of concentration 1 $g.l^{-1}$) were added, the flask vigorously shaken and incubated at 30°C to allow the spores to begin germinating prior to inoculation into the fermenter.

3.5.3 MEDIA COMPOSITION.

Three different types of medium were used in this investigation, though most of the fermentations were carried out with semisynthetic starch-based media. This was a result of the difficulty experienced in achieving steady states with aspirators of potato homogenate medium having varying composition - a result of the comparatively small scale of the experimentation.

All the media used had ammonium sulphate added in such quantity that the carbon : nitrogen ratio was 12 : 1, the semisynthetic type containing other salts in the ratio originally suggested by Cocker (290). Their compositions are detailed in Table 3.1 and Table 3.2.

The potato starch used was donated by Golden Wonder Ltd. (Corby, Northants.), being derived from the effluent stream of their potato processing factory. It was approximately 95% pure, the remainder being accounted for as water and small soil particles. Most of the potatoes were also donated by this company, being of the variety "Golden Wonder", which had a very high dry matter and low free reducing sugar content.

The caustic peel waste was obtained from Witch Chips Ltd. (Boroughbridge, N. Yorks.) and resulted from a process in which

TABLE 3.1 COMPOSITIONS OF THE SEMISYNTHETIC MEDIA USED.

(in $g.l^{-1}$)

	"20$g.l^{-1}$" Medium	"13.2$g.l^{-1}$" Medium	"3.3$g.l^{-1}$" Medium
Potato Starch.	21.0	12.5	3.125
Ammonium sulphate.	3.5	2.075	0.52
Sodium dihydrogen phosphate	0.675	0.4	0.1
Potassium chloride.	0.3375	0.2	0.05
Magnesium sulphate.	0.135	0.08	0.02
Calcium chloride.	0.0675	0.04	0.01
Yeast Extract.	0.675	0.4	0.1

TABLE 3.2 COMPOSITIONS OF THE POTATO MEDIA USED.

(in $g.l^{-1}$ or $ml.l^{-1}$)

POTATO MEDIUM.	"20$g.l^{-1}$" Medium	"10$g.l^{-1}$" Medium	"5$g.l^{-1}$" Medium
Whole,Raw Potato.(g.)	100	50	25
Ammonium sulphate.(g.)	3.7	1.85	0.925

PEEL WASTE MEDIUM.	"20$g.l^{-1}$" Medium	"10$g.l^{-1}$" Medium
Peel Waste.(g.)	100	50
Ammonium sulphate.(g.)	2.5	1.25
Concentrated Hydrochloric Acid.(ml.)	4 approx.	2 approx.

potatoes were dipped in a caustic bath (200 - 370 $g.l^{-1}$ sodium hydroxide depending on skin thickness) and subjected to infra-red heating to enable the skin to be brushed off. The peel waste was produced as a gelatinous mass at a temperature of 75 - 85°C and contained about 19% solids. It was extremely alkaline, being equivalent to about 2% sodium hydroxide on a wet basis (i.e. about 10% of the dry matter), had a pH of about 13.0 and a strong ammoniacal odour.

All the other medium constituents were of Laboratory Reagent Grade and, with the exception of the ammonium sulphate (B.D.H. Chemicals, Atherstone, Warks.) and yeast extract (Bovril Ltd., Burton on Trent), were supplied by Hopkin and Williams Ltd., Cradley Heath, Essex.

3.5.4 PREPARATION OF MEDIA.

1. SEMI SYNTHETIC STARCH MEDIUM.

Small batches of 40 - 50 g. starch were slurried in about 0.5 l. cold water and 2 l. boiling water quickly added whilst being vigorously agitated. This ensured that the starch was gelatinized and so solubilized. The batches were mixed in a 20 l. aspirator, salts and 1 cm^3 polyethylene glycol P-2000 antifoam added and the medium made up to volume with boiling water. The aspirator and contents were then autoclaved at 1.05 $kg.cm^{-2}$ for 45 min.

2. POTATO MEDIUM.

Whole potatoes were cooked in the autoclave at 1.05 $kg.cm^{-2}$ for 15 min. and then thoroughly homogenised in about 10 l. water

with 1 cm^3 polyethylene glycol P-2000 antifoam added using a mixer/disintegrator (Silverson Equipment, London S.E.1). Ammonium sulphate was added, the volume made up to 20 l. and the aspirator and contents autoclaved at 1.05 $kg.cm^{-2}$ for 45 min.

3. CAUSTIC PEEL WASTE.

The heating process which resulted in this waste gelatinised the starch and reduced contaminants to a negligible level. The peel was diluted and homogenised then neutralized with concentrated hydrochloric acid to pH10.0 -10.5 and ammonium sulphate and P-2000 antifoam added. Providing this medium was used within 24 h. further pretreatment proved unnecessary.

3.5.5 PREPARATION OF THE FERMENTER.

The fermenter was set up as in Fig. 3.1, the air inlet line, filter and medium inlet line removed and autoclaved separately. The fermenter was washed with "Pyroneg" detergent (Diversey Chemicals Ltd.) and rinsed thoroughly with tap water before being connected to a steam supply via the sample port. Steam sterilization of the fermenter, assisted in later fermentations by the injection of 10 cm^3 formalin solution, was carried out for at least 24 h. prior to commencement of a fermentation.

3.5.6 START-UP AND RUNNING OF FERMENTATIONS.

Air and medium inlet line connections were made whilst the fermenter was being steamed, with all possible precautions being taken to prevent ingress of contaminants. The air and steam supplies were slowly increased and decreased respectively so as to

maintain a positive pressure in the fermenter and especially to avoid sudden cooling which would create a partial vacuum.

The temperature controller was set to the required value and about 3 l. of medium quickly pumped into the fermenter. The germinating spore suspension was injected through a port into the fermenter from a sterile disposable syringe. With continuous fermentations, the culture was left to grow up for 24 h. before medium pumping began at the Dilution Rate to be investigated. (In the case of batch fermentations, the culture volume was immediately made up to 10 l. and antifoam added if necessary).

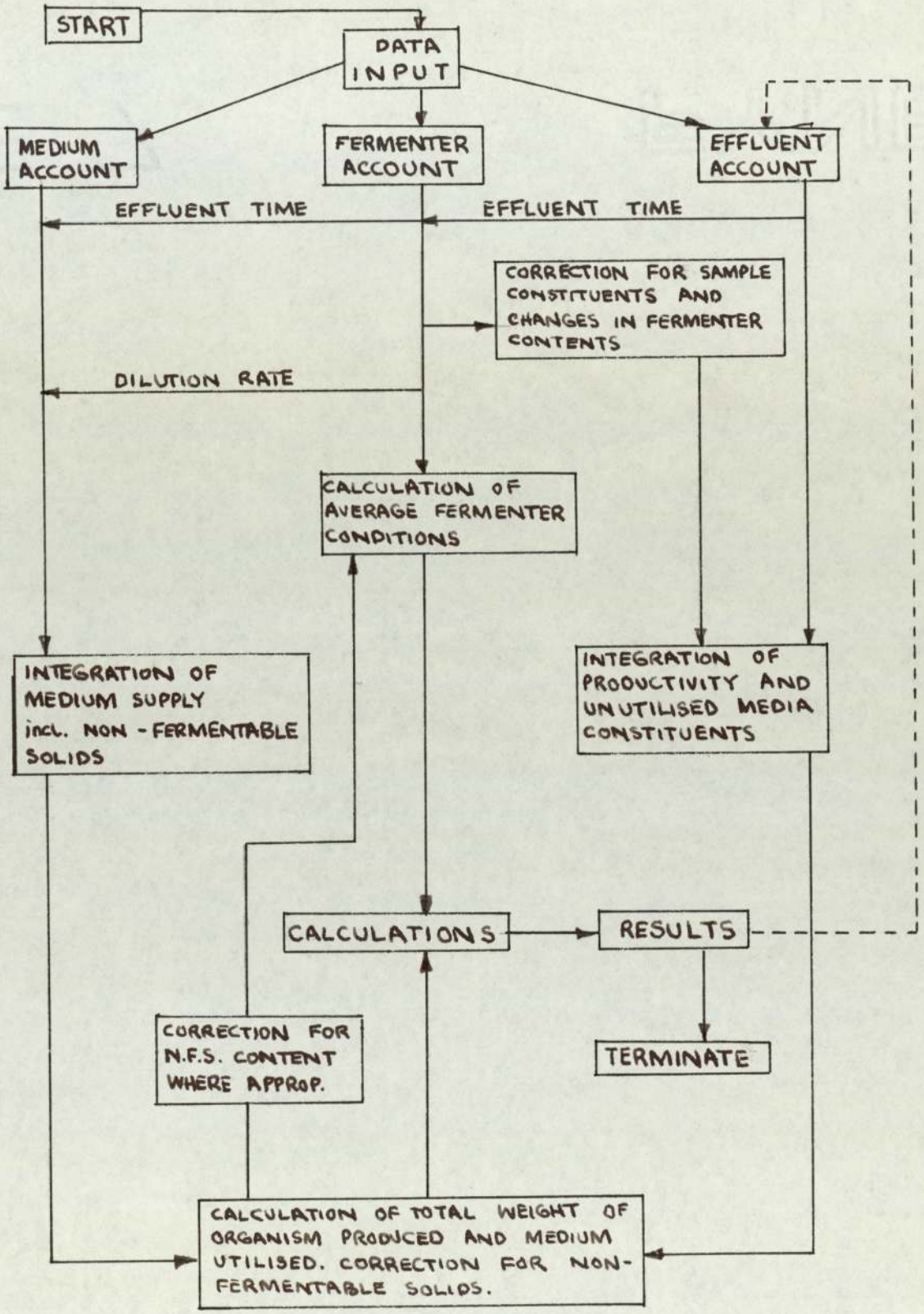
Sampling and analyses were carried out as detailed in Section 3.4 until it was considered that the fermentation had reached a steady state, when parameters such as dilution rate, temperature or medium concentration were altered. The longest fermentations carried out were in excess of 2500 h. duration.

3.6 DATA HANDLING AND TREATMENT.

3.6.1 INTRODUCTION.

The large volume of data generated from approximately 12,000 hours of continuous culture necessitated the development of a Mass Balance Programme for use with the University Computer. Whilst this programme was originally conceived and its capabilities specified by the author it was actually written and developed to suit these experimental results by Dr. M. Fidgett (Dept. of Chemical Engineering) with the author rendering assistance wherever possible.

FIG 3.2 SCHEMATIC DIAGRAM OF THE MASS BALANCE PROGRAMME



3.6.2 AN OUTLINE OF THE MASS BALANCE PROGRAMME.

This programme is shown schematically in Fig. 3.2 and reproduced in full as Appendix III.

Data is supplied as groups of Fermenter, Medium and Effluent values prefixed and chronologically arranged by the Elapsed Time (from the beginning of the fermentation) at which each was recorded. Calculations are performed whenever Effluent Data is supplied and the results refer to the preceding time period to which that data relates.

Briefly, the programme:-

(A) Calculates the total weights of media constituents supplied to the fermenter during the time period under consideration.

(B) Calculates the total weights of organism and residual media constituents present in the effluent stream from the fermenter during this time period. Corrections aremade to take account of samples removed from the system and for any changes in the fermenter contents.

(C) Combines these results (from A and B) to arrive at the actual weights of medium constituents utilized and organism produced (corrected for non-fermentable solids, where appropriate).

(D) Calculates the average fermenter conditions during this period. Where necessary, the dry weight of the fermenter contents is corrected for non-fermentable solids, based on the assumption that at steady state, the ratio of organism/NFS is the same as in the effluent stream.

(E) Calculates such factors as growth rate (MU), yield coefficient (YXS) and reducing sugar formation rate (RSRATE) from the results of calculations detailed in points C and D.

(F) Prints the results as part of a standard format, which includes all the fermenter conditions (both real and averaged), medium supply data and any comment statements which have been included in the data input.

3.6.3 LOGGING OF RAW DATA.

Input data is divided into four categories - Comments, Medium, Fermenter and Effluent Data. It is essential that data is:

supplied exactly in the order given below.

in the correct units.

in the correct chronological order.

prefixed by a character (1, 2, 3 or 4) indicating its type.

COMMENTS. Prefix Character 1.

Time of Comment.	T	h.
Comment (written and inserted verbatim into the results printout).		

MEDIUM DATA. Prefix Character 2.

Time of Medium Change	MT	h.
Ammonia Nitrogen Content	NH3M	$g.l^{-1}$
Total Sugar Concentration	TSM	$g.l^{-1}$
Non-Fermentable Solids Content	NFS	$g.l^{-1}$

FERMENTER DATA. Prefix Character 3.

Time of Sampling	FT	h.
pH	PH	Units
Temperature	TEMP	^{o}C
Dilution Rate	D	h^{-1}
Organism Concentration	XF	$g.l^{-1}$
Reducing Sugar Concentration	RSF	$g.l^{-1}$
Total Sugar Concentration	TSF	$g.l^{-1}$
Ammonia Nitrogen Concentration	NH3F	$g.l^{-1}$
Nitrogen Content of Organism	NBUG	$g.g^{-1}$

EFFLUENT DATA. (i.e. Product Receiver Data) Prefix Character 4.

Time Receiver Emptied	ET	h.
Volume of Effluent	VE	l.
Organism Content	XE	$g.l^{-1}$

Fig. 3.3 is a typical extract from a data file showing this arrangement of experimental results.

3.6.4 OUTPUT OF RESULTS.

The results printout includes Raw Data, Averaged Fermenter Data and the Calculated Results. The latter are presented in the following order:-

Growth Rate	MU	h^{-1}
Yield Coefficient (organism on sugar)	YXS	g.organism. g^{-1} sugar
Productivity	PROD	$g.l^{-1}.h^{-1}$
Reducing Sugar Production Rate	RSRATE	$g.l^{-1}.h^{-1}$

Reciprocal of Growth Rate	RMU	h.
Reciprocal of Yield Coefficient	RYXS	g.sugar.g^{-1} organism
Nitrogen Taken up in Period	DN	g.
Nitrogen in Organism Produced in period	NINBUG	g.
Yield Coefficient (organism on nitrogen)	YN	g.organism.g^{-1} nitrogen

Fig. 3.4 shows the results print-out equivalent to the data presented in Fig. 3.3.

3.6.5 ADDITIONAL INFORMATION.

Linear averaging of fermenter data was used, being the only practicable method. This was acceptable where small variations occurred, but was not applicable to pH data and so averages of this parameter were disregarded.

A sample volume of 0.180 l. was assumed but multiple samples in a given time period could be accounted for. The sample contents were significant under certain conditions where the growth rate was very low.

When a data value was not known, -1.0 was substituted. This was an instruction to assume the last known value of that parameter in the calculation.

With fermentations containing Non-fermentable Solids (which passed through the system unchanged) the ratio of organism to Non-fermentable Solids in the fermenter was assumed to be the same as in the effluent stream.

Step changes in parameters such as dilution rate could be

Fig. 3.3 An Extract from a typical Data File (from run SR 5).

```
 16  3
 17  264.75  3.0   30.0   0.0708  3.65  2.97  2.99  0.148  0.06397
 18  4
 19  264.75  17.0  3.58
 20  2
 21  265.0   0.418 13.2
 22  3
 23  288.5   3.05  30.5   0.0710  3.2   3.66  3.685 0.156  0.06255
 24  4
 25  288.5   17.0  3.08
 26  2
 27  289.5   0.432 13.2
 28  3
 29  317.0   3.05  30.0   0.0712  3.5   4.17  4.795 0.162  0.06112
 30  4
 31  317.0   19.1  2.56
 32  2
 33  317.0   0.422 13.2
 34  3
 35  332.75  3.05  30.5   0.0705  3.65  4.47  4.67  0.162  0.06184
 36  4
 37  332.75  11.0  2.60
 38  2
 39  339.75  0.422 13.2
 40  3
 41  362.5   3.05  30.0   0.0762  3.6   4.65  4.782 0.163  0.06221
 42  4
 43  362.5   21.5  2.89
 44  2
 45  363.25  0.420 13.2
 46  3
 47  377.5   3.05  30.5   0.0740  3.5   3.74  3.95  0.166  0.06106
 48  4
 49  377.5   11.0  3.01
```

Fig. 3.4 Results Printout for the Data in Fig. 3.3 (from run SR 5).

FERMENTER	264.75	3.000	30.000	0.071	3.65	2.97	2.99	0.148	0.064
EFFLUENT	264.75	17.000	3.580						
FERMENTAV	250.50	3.000	30.000	0.070	3.975	3.10	3.185	0.144	0.064
RESULTS	0.049	0.259	0.194	0.923	20.516	3.857	6.022	3.519	9.169
FERMENTER	288.50	3.050	30.500	0.071	3.200	3.660	3.685	0.156	0.062
EFFLUENT	288.50	17.000	3.080						
FERMENTAV	276.625	3.025	30.250	0.071	3.425	3.315	3.337	0.152	0.063
RESULTS	0.060	0.308	0.205	0.931	16.741	3.251	4.346	3.069	11.180
FERMENTER	317.000	3.050	30.000	0.071	3.500	4.170	4.795	0.162	0.061
EFFLUENT	317.000	19.100	2.56						
FERMENTAV	302.750	3.050	30.250	0.071	3.350	3.915	4.240	0.159	0.062
RESULTS	0.055	0.301	0.184	0.893	18.173	3.324	5.615	3.243	9.356
FERMENTER	332.750	3.050	30.500	0.070	3.650	4.470	4.670	0.162	0.062
EFFLUENT	332.750	11.000	2.600						
FERMENTAV	324.875	3.050	30.250	0.071	3.575	4.320	4.732	0.162	0.061
RESULTS	0.055	0.322	0.196	0.927	18.281	3.102	2.895	1.894	10.640
FERMENTER	362.500	3.050	30.000	0.076	3.600	4.650	4.782	0.163	0.062
EFFLUENT	362.500	21.500	2.890						
FERMENTAV	347.625	3.050	30.250	0.073	3.625	4.560	4.726	0.163	0.062
RESULTS	0.058	0.343	0.210	0.948	17.292	2.919	5.599	3.868	11.139
FERMENTER	377.500	3.050	30.500	0.074	3.500	3.740	3.950	0.166	0.061
EFFLUENT	377.500	11.000	3.010						
FERMENTAV	370.000	3.050	30.250	0.075	3.550	4.195	4.366	0.165	0.062
RESULTS	0.062	0.304	0.219	0.966	16.220	3.285	2.855	2.023	11.500

accomplished by submitting Fermenter Data twice (with the appropriate change) with a very small Elapsed Time difference (say, 0.001 h.), otherwise the averaging procedure led to errors.

Under conditions of extreme limitation, negative growth rates occurred, indicative of more material being lost from the fermenter than was accounted for in the effluent stream. This could have been due to a small experimental error in the determination of fermenter organism concentration - the results are extremely sensitive to such an error under these conditions - or the value obtained could be interpreted as representing a Specific Rate of Autolysis.

SECTION 4.

RESULTS AND DISCUSSION.

Page No.

4.1 INTRODUCTION.

The experimental work carried out was in two related areas. Determination of the kinetic characteristics of the fermenter and the fermentation process was accomplished using semisynthetic starch media. These media could be made up with constant substrate concentrations and did not contain non-fermentable solids. They were also most suitable for investigations into the effects of temperature and pH on the system.

The other work was of a more applied nature, being concerned with the fermentation of potatoes and alkaline peel waste. The presence of non-fermentable solids in these media led to problems not only in the experimental work but also in the interpretation of the data, particularly in the estimation of such factors as growth rates and yield coefficients.

In each Section, information concerning that type of fermentation is presented and described, with all the experimental results being collated and their implications discussed in Section 4.5, together with suggestions for further work.

4.2 SEMISYNTHETIC STARCH MEDIA : FERMENTATIONS CARRIED OUT AT 30°C.

These fermentations yielded information regarding the operating characteristics of the system under "normal" conditions and provide a basis for comparison with all the other results obtained.

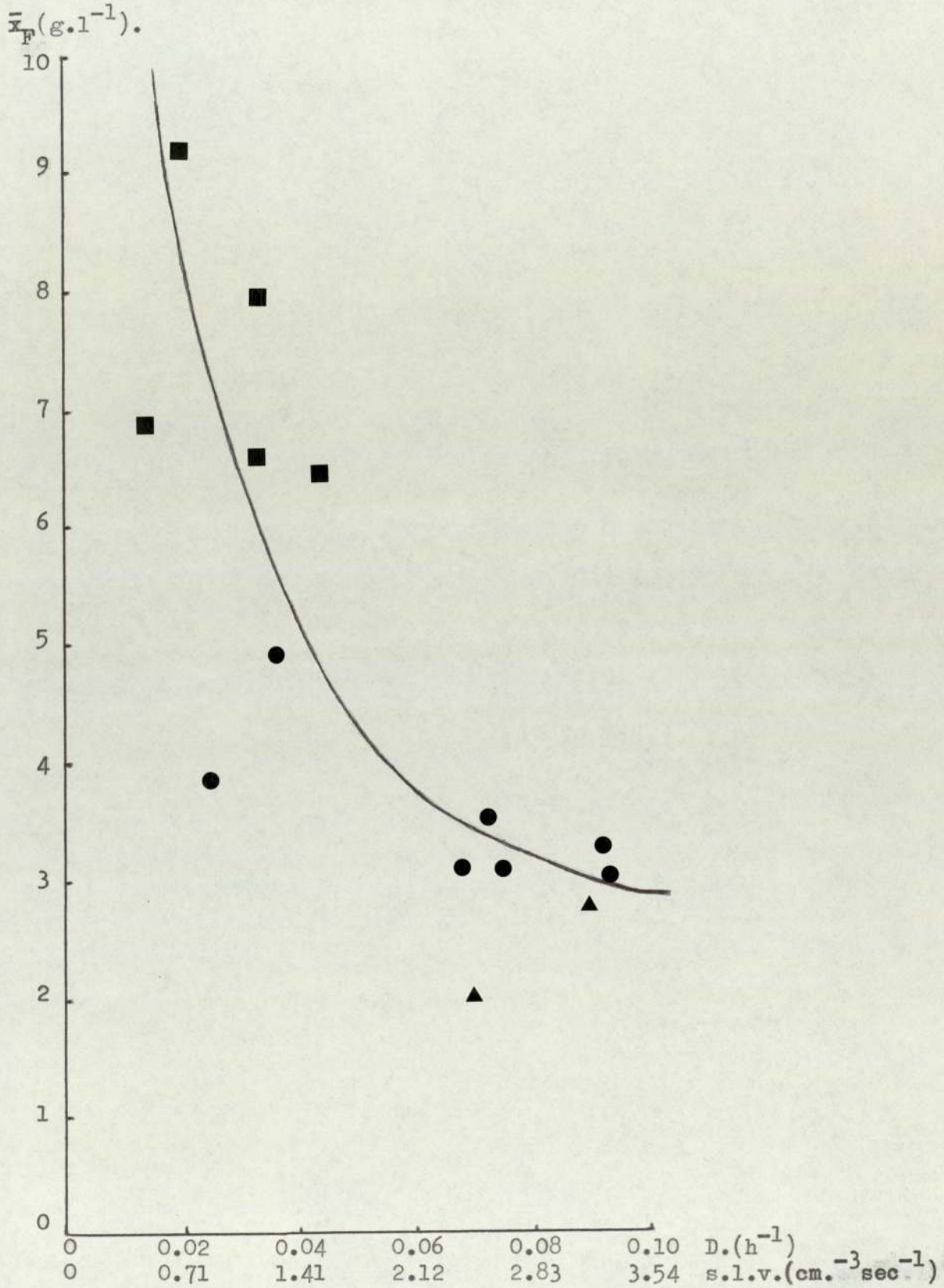
4.2.1 CONCENTRATION OF ORGANISM IN THE FERMENTER (x_F).

This is the fundamental parameter which affects the fermentation. For a given medium it is determined by a complex interplay of both physical and physiological factors - at any one time the dry weight of organism present and its morphology are both governing and being governed by the fermenter conditions. Such a "control" mechanism tends to make this parameter relatively unstable, both within and between fermentations, particularly at low dilution rates.

The Medium Flow Rate, usually expressed as a Dilution Rate, must be considered as representing both a nutrient supply rate and a liquid flow rate - a superficial liquid velocity (s.l.v.) - which is responsible for the fluidisation effects on the microbial colonies. Fig. 4.1 shows the effect of medium flow rate on the steady-state fermenter organism concentration ($\bar{x}_F$) at three substrate levels in fermentations carried out at 30°C. The use of a dual-scale abscissa allows the flow rate to be expressed as either a Dilution Rate (h^{-1}) or a Superficial Liquid Velocity ($cm.10^{-3}.\ sec.^{-1}$) which of course depends upon the fermenter configuration.

At low Dilution Rates (below 0.05 h^{-1}) values of the organism concentration at steady state ($\bar{x}_F$) of up to 9 $g.l^{-1}$ and 5 $g.l^{-1}$ were obtained using 20 and 13.2 $g.l^{-1}$ medium respectively. With higher Dilution Rates (0.05 - 0.1 h^{-1}) substrate concentration had considerably less effect on $\bar{x}_F$, within this range values of 3 - 3.5 $g.l^{-1}$ being typical. Unfortunately the gelatinous nature of starch media at these concentrations precludes their use

Fig. 4.1 The Effect of Medium Flow Rate(D. & s.l.v.) on the Mould Concentration in the Fermenter($\bar{x}_F$).
(■ 20, ● 13.2, ▲ 3.3g.l^{-1} glucose equivalent medium)



with this organism at Dilution Rates of greater than 0.10 - 0.12 h^{-1} i.e. starch supply rates of greater than 2 $g.l^{-1}.\ h^{-1}$.

The position of this response curve is dependent upon growth conditions such as the carbon source and other medium constituents, temperature and aeration rate, which act upon the organism morphology. This was shown by comparison with results obtained by Pannell (213) who grew the same strain of A. niger (M1) in an identical fermenter on sucrose - salts media. The form of the curve was similar over this range of dilution rates but the organism concentrations were significantly higher.

Briefly, the factors determining Dry Weight in the fermenter can be summarized by the following points:-

(1) Low superficial liquid velocities decrease the fluidisation effect (which is responsible for washout of organism from the fermenter) permitting high organism concentrations.

(2) Low nutrient supply rates limit the amount of organism which can be maintained in the fermenter.

(3) Low nutrient supply rates lead to more "inhibited" morphologies which have a higher mycelial packing density and therefore sedimentation rate, both factors which lead to an increase in $\bar{x}_F$.

(4) When conditions are changed in a continuous culture, the new steady-state morphology and organism concentration are influenced by those prior to the change.

As will be seen in the following sections, the non-linear response of dry weight to medium flow rate influences all the

characteristics of the operation of this fermentation system.

4.2.2 CONCENTRATION OF ORGANISM IN THE EFFLUENT STREAM (x_E).

Although $\bar{x}_F$ is determined primarily by physical means (Section 4.2.1), $\bar{x}_E$ is related to the growth rate (μ) of the fermenter contents and is capable of varying independently.

For a given $\bar{x}_F$, steady states can exist where $\bar{x}_E$ is within a range from almost zero (under conditions of extreme limitation) to being equal to $\bar{x}_F$. Steady states of over 200 hours duration have even been achieved in which x_E has exceeded x_F (see Sections 4.3 and 4.4) though this situation usually only exists transiently when organism is being "washed out" of the fermenter.

Fig. 4.2 shows the lack of correlation between $\bar{x}_F$ and $\bar{x}_E$ with regard to the medium flow rate.

4.2.3 SPECIFIC GROWTH RATE AND PRODUCTIVITY OF THE ORGANISM.

In the derivation of continuous culture kinetics, the rate of growth of a microorganism and the productivity of a fermenter are represented by the expressions

$$\text{Rate of Growth} \quad (dx/dt) = \mu x_F$$

$$\text{Productivity} \quad (dx/dt) = Dx_E$$

where x_F and x_E are the concentrations of organism in the fermenter and effluent stream respectively. Therefore at steady state

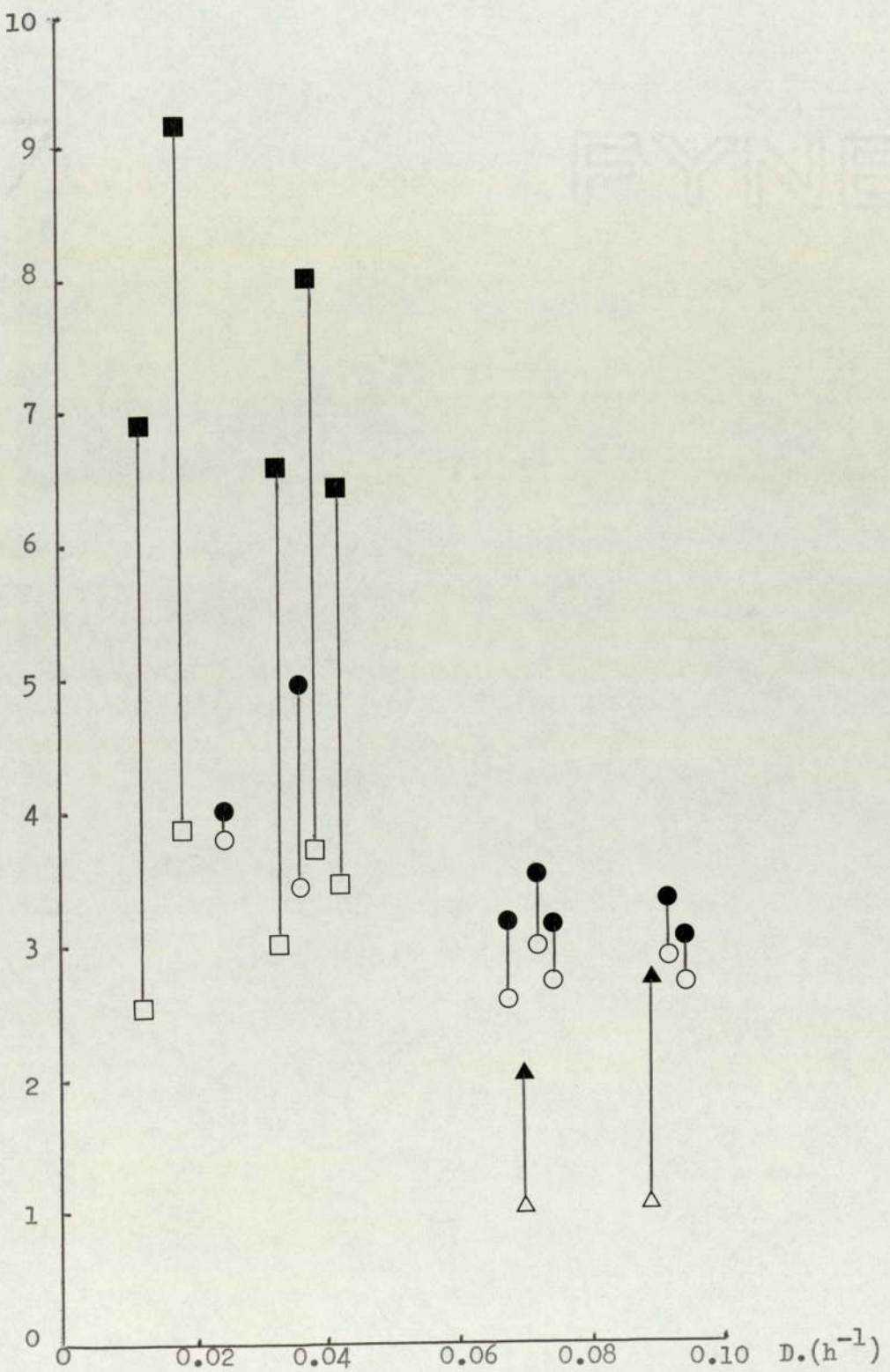
$$\mu \bar{x}_F = D\bar{x}_E \qquad \ldots\ldots\ldots \text{Eqn. 4.1}$$

and rearranging

$$\mu = \frac{D\ \bar{x}_E}{\bar{x}_F} \qquad \ldots\ldots\ldots \text{Eqn. 4.2}$$

Fig. 4.2 The Effect of Medium Flow Rate(D) on the Mould Concentration in the Effluent Stream($\bar{x}_E$).

(■ 20, ● 13.2, ▲ 3.3g.l^{-1} glucose equivalent medium - x_F)
(□ 20, ○ 13.2, △ 3.3g.l^{-1} glucose equivalent medium - x_E)



When considering homogeneous fermentation systems where $\bar{x}_F = \bar{x}_E$ (such as the continuous stirred tank fermenter) Eqn. 4.1 reduces to $\mu = D$. However, in the case of continuous tower fermenters, this simplification cannot be made and the growth rate must be calculated using Eqn. 4.2. This value is in fact the average growth rate of all the fermenter contents and assumes that the whole of the culture contributes to the production of new cellular material - a point which will be discussed further in Section 4.5.4.

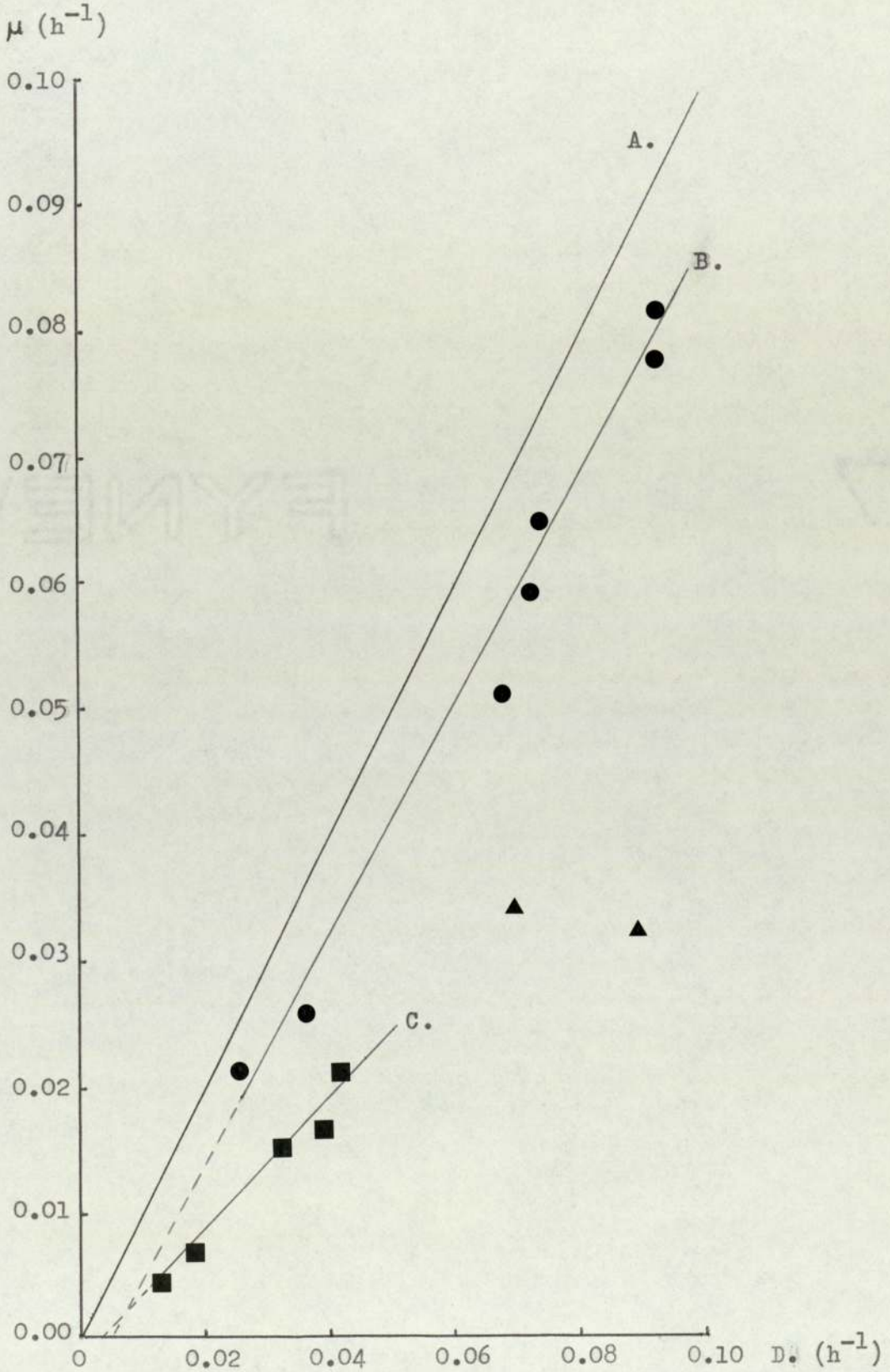
4.2.3.1 SPECIFIC GROWTH RATE.

Fig. 4.3 illustrates the relationship between the specific growth rate (μ) and the dilution rate (D) in this series of experiments. Line A represents the case of $\mu = D$, whereas the regression lines B (122 readings; correlation coefficient r = 0.9703) and C (37 readings; r = 0.9562) are derived from experimental results. The significance of line B is increased even further when additional data using this medium at different temperatures is included (in Section 4.5.2). Results obtained with 3.3 $g.l^{-1}$ medium exemplify the effects of carbon limitation on the growth rate.

Extrapolation of lines B and C indicates that when $\mu = 0$, D = 0.00594 and 0.00397 h^{-1} respectively. These values represent glucose supply rates of 0.0792 and 0.0784 $g.l^{-1},h^{-1}$, which can be interpreted as the carbohydrate requirement for maintenance of the culture (discussed in more detail in Section 4.5.2). At these dilution rates, Pannell (213) found that $\bar{x}_F \approx$ 19.0 $g.l^{-1}$, which suggests that the Maintenance Coefficient, m,

Fig. 4.3 The Effect of Medium Flow Rate(D) on the Specific Growth Rate of the Fermenter Contents(μ).

(■ 20, ● 13.2, ▲ 3.3g.l^{-1} glucose equivalent medium)



of the organism growing under these conditions is approximately 0.004 g.glucose - g^{-1} organism - h^{-1}.

4.2.3.2 PRODUCTIVITY.

The effect of dilution rate on the productivity of organism of the system is shown in Fig. 4.4. The results form a smooth curve which intercepts the abscissa when $\mu = 0$, i.e. at a dilution rate of 0.004 - 0.006 h^{-1}. The form and position of this curve under conditions of substrate excess are determined by the interaction of characteristics of the system such as the effects of D on x_F and x_F morphology and viscosity of the culture on K_La (the oxygen transfer coefficient - see also Section 4.5.3). This interaction is compounded by the effect of culture broth pH on the growth rate of the organism.

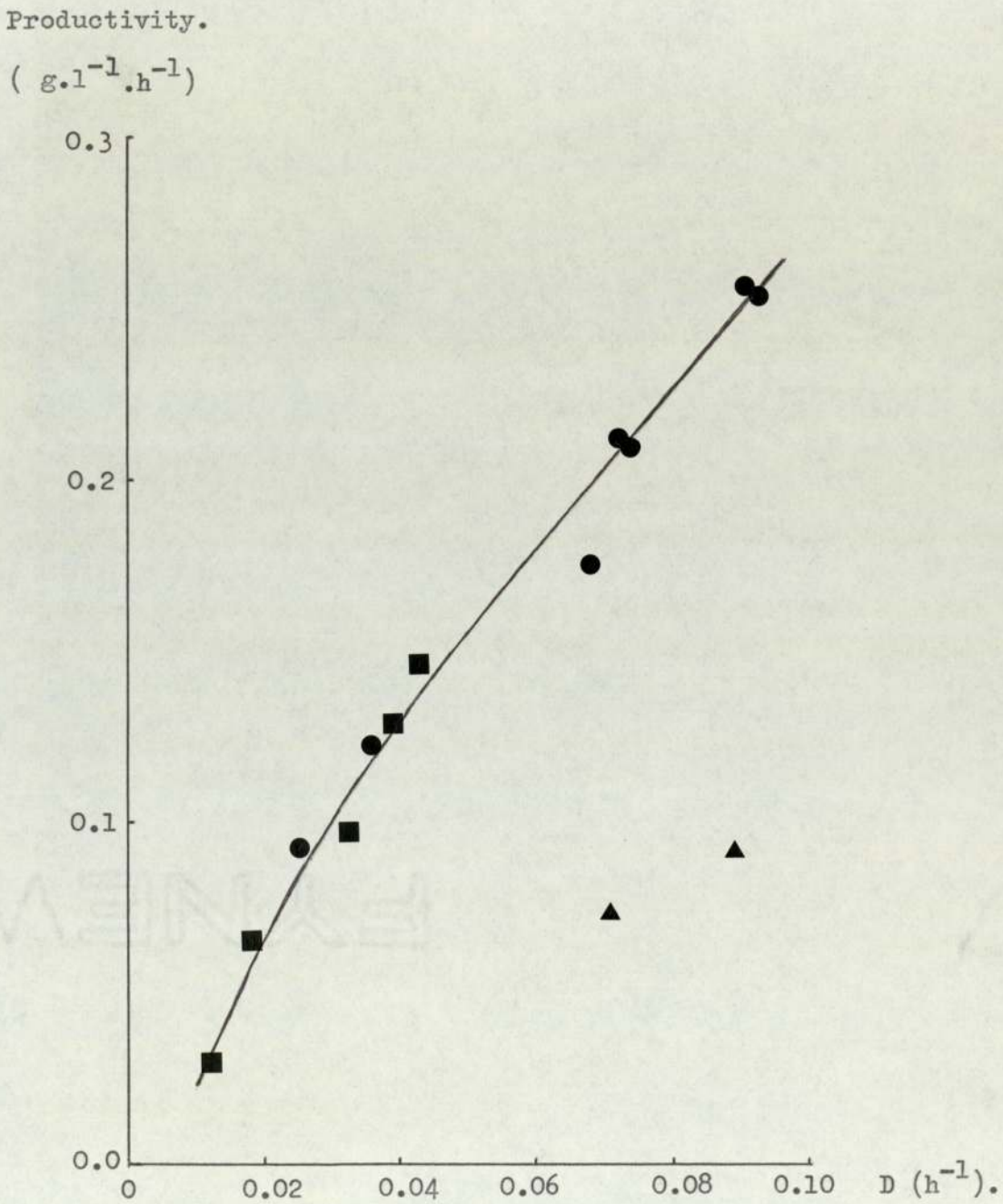
Assuming that the True Growth Yield of Organism on carbohydrate (Yg) and the Maintenance Coefficient (m) are constants, the productivity of the system can be represented by the equation

$$\mu.\bar{x}_F = D.\bar{x}_E = Yg\ (D.s_R - D.\bar{s} - m.\bar{x}_F) \quad \ldots . \text{ Eqn. 4.3.}$$

Under conditions of carbohydrate limitation, diminution of $D.s_R$ caused $D.\bar{s} \rightarrow 0$, increasing the significance of the proportion of the carbohydrate supply used for maintenance ($m.\bar{x}_F$). This resulted in restriction of the productivity and, therefore, the specific growth rate (μ), accounting for the steady state values obtained with 3.3 $g.l^{-1}$ glucose equivalent media.

This situation can be exploited as another means of estimating the maintenance coefficient of the organism in the Tower

Fig. 4.4 The Effect of Dilution Rate(D) on the Organism Productivity.
(■ 20, ● 13.2, ▲ 3.3g.l^{-1} glucose equivalent medium)



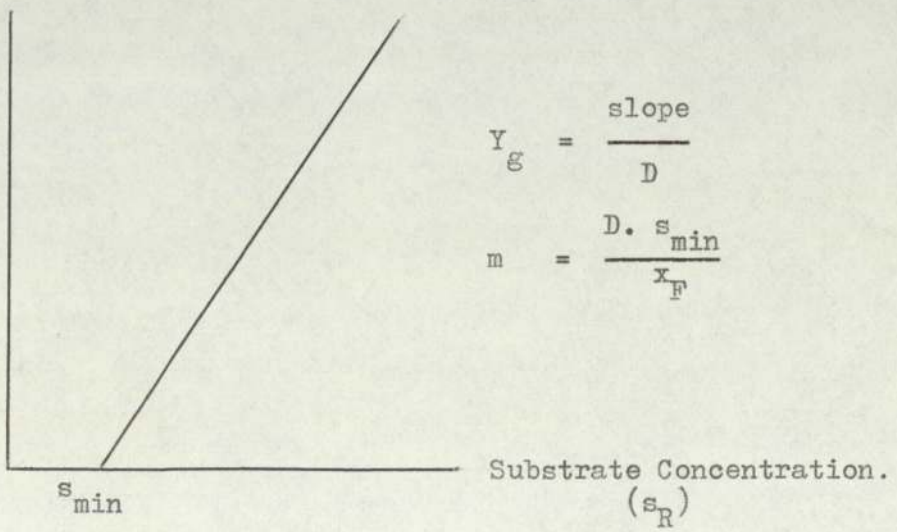
Fermenter. Under conditions of carbon limitation, when the Dilution Rate (D) is kept constant, $\bar{x}_F$ tends to remain so even if s_R is varied. Assuming that Y_g and m are constants, in this situation reductions in s_R result in such steady states that productivity ($D.\bar{x}_E$) decreases linearly and can even be zero (when $\mu = 0$) as in Fig. 4.5. Therefore, when $\mu = 0$ and $s_R = s_{min}$, Eqn. 4.3 can be reduced and rearranged to

$$m = \frac{D.\ s_{min}}{x_F} \quad (\text{units} - g.g^{-1}.\ h^{-1}) \ldots\ldots \text{Eqn. 4.4.}$$

giving the value of the maintenance coefficient of the mould at zero growth rate in this system.

Fig. 4.5 Theoretical Effect of reducing the Substrate Concentration on the Productivity of a Carbon-limited Fermentation.

(when D, $\bar{x}_F$, Y_g and m are constants.)



4.2.3.3 SUBMERGED SPORULATION OF THE CULTURE.

When $D.s_R$ was reduced to a level approximately equal to or less than $m.\bar{x}_F$, sporulation of the mould took place in submerged culture in the fermenter. Typical conditions were a Dilution Rate of 0.022 h^{-1} when starch medium of 3.3 $g.l^{-1}$ glucose equivalent (s_R) was being used. The conditions which led up to this phenomenon were better monitored in a potato medium fermentation and will therefore be discussed more fully in Section 4.4.6.

4.2.4 HYDROLYSIS AND UTILIZATION OF THE STARCH SUBSTRATE.

4.2.4.1 REDUCING SUGAR FORMATION BY HYDROLYSIS OF STARCH.

In common with most other microorganisms, Aspergillus niger M1 is unable to absorb and utilize polymeric carbohydrates directly; it secretes enzymes into the culture medium which hydrolyse these substrates to their constituent monomeric sugar molecules.

In the case of starch substrates the enzymes concerned are maltase and a group known collectively as amylases. Acting together, these result in the formation of glucose which can be assimilated by A. niger M1 hyphae. Pazur and Ando (298), Pazur and Kleppe (299), Kearney (300) and Allam and Khalil (301, 302) have all attempted to estimate the relative inportance of these enzymes in cultures of A. niger. However, in this study it was more important to show that the rate of starch hydrolysis and, therefore, glucose formation, was not a rate limiting step in the fermentation.

The rate of glucose production in samples of culture filtrate was ascertained by the method described in Appendix II, though this estimation was not used routinely. Typical values were in the range 1.6 - 2.0 $g.l^{-1}.\ h^{-1}$ for samples taken from 20.0 and 13.2 $g.l^{-1}$ glucose equivalent medium fermentations. However, as the method measured the glucose production rate at pH 4.6 - the usual optimum for amylases - it was not necessarily indicative of the true rate of hydrolysis in the fermenter.

The reducing sugar production rate in the fermenter was easily derived from mass balance data. It proved to be the most constant and reliable fermentation parameter probably because the source data, the reducing and total sugar contents of culture liquid filtrates, could be very accurately determined.

Figs. 4.6 and 4.7 relate the Reducing Sugar Production Rate and the Effectiveness of Substrate Conversion, respectively, to the Rate of Substrate Supply to the fermenter, $D.s_R$ (expressed as glucose equivalent). In both cases, lines (designated A) have been drawn to represent the situation in which complete conversion of starch to glucose takes place.

In fermentations with 20.0 and 13.2 $g.l^{-1}$ glucose equivalent medium the maximum reducing sugar production rates were approximately 0.65 and 1.2 $g.l^{-1}.\ h^{-1}$ respectively. These values are confirmed by data from other periods of fermentation in which conditions could not be kept constant for sufficient time to ensure a steady state had been achieved. The discrepancies are almost certainly due to the pH of the culture - over the range of conditions investigated, fermentations at higher medium concentrations had lower pH's (see Section 4.2.7).

Fig. 4.6 The Relationship between the Substrate Supply and Reducing Sugar Production Rates in 30°C Fermentations.
(■ 20, ● 13.2, ▲ 3.3g.l^{-1} glucose equivalent medium)

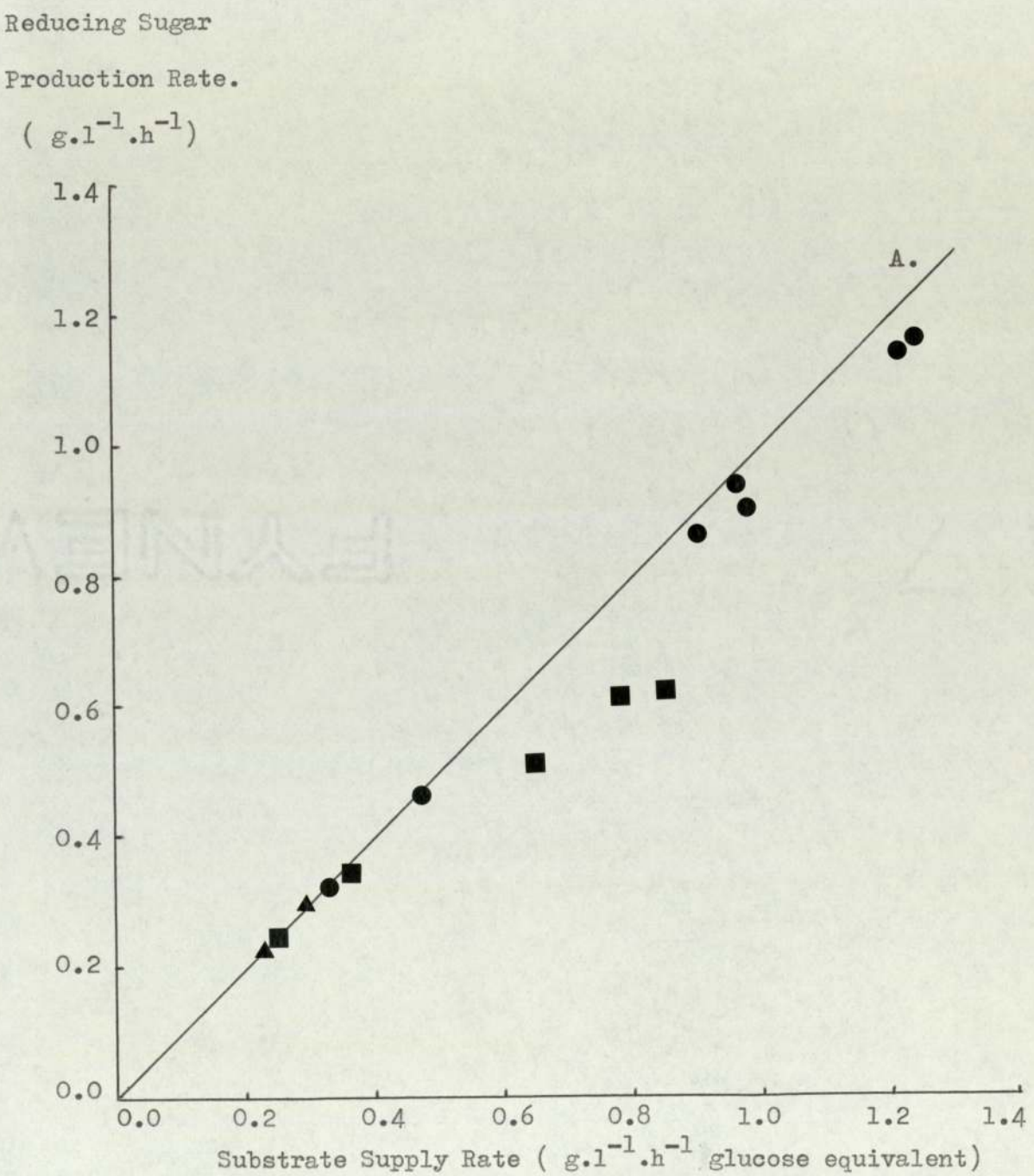


Fig. 4.7 The Relationship between the Substrate Supply Rate and the Effectiveness of its Conversion to Glucose.
(■ 20, ● 13.2, ▲ 3.3g.l^{-1} glucose equivalent medium)

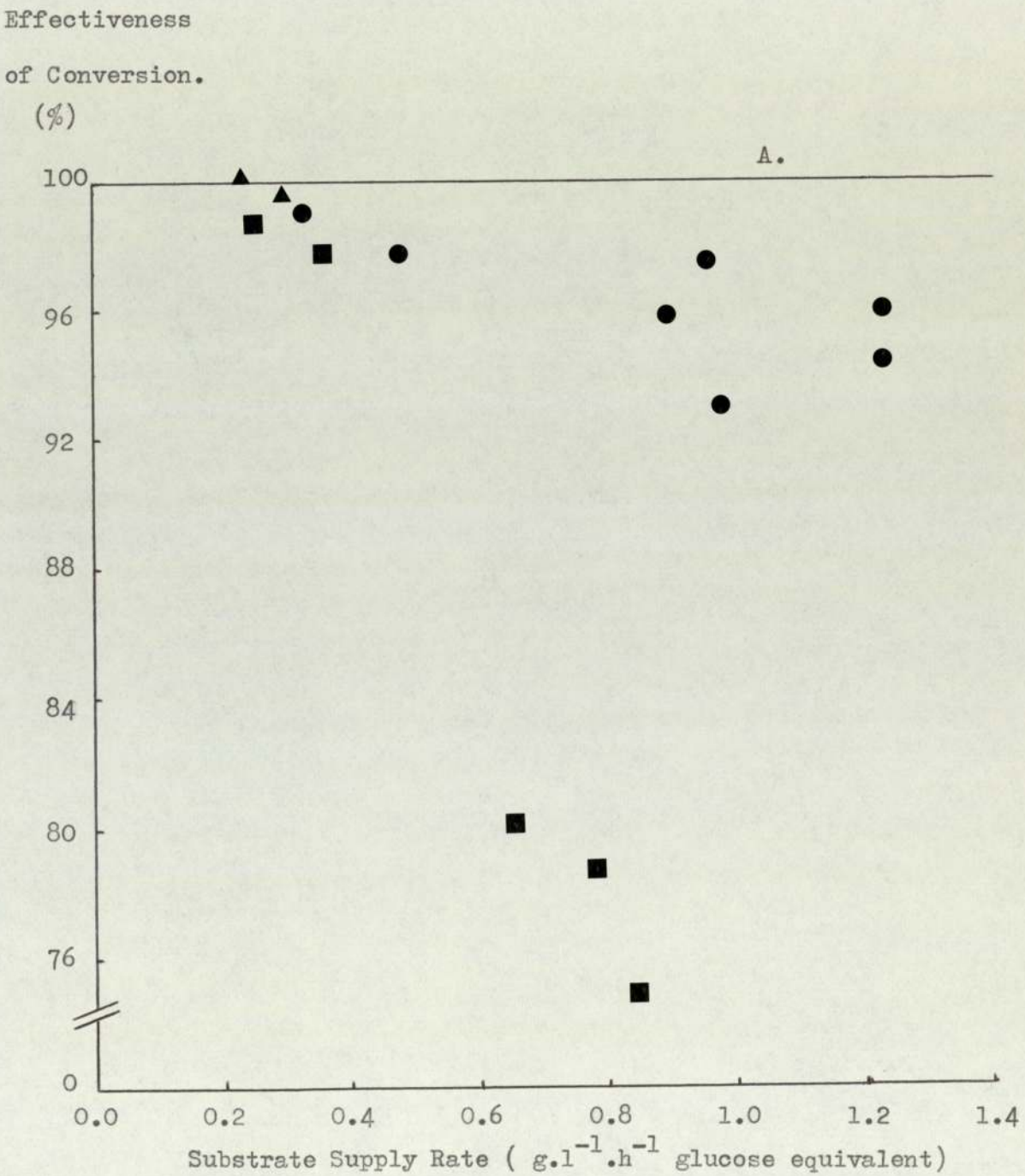


Table 4.1 Summary of Data relating to the effect of pH on the Reducing Sugar Production Rate.

Source of Data.	pH Units.	Highest R.S. Rate $g.l^{-1}.h^{-1}$.
20.0 $g.l^{-1}$ Medium Contin. Ferms.	2.13 (Av.)	0.65
13.2 $g.l^{-1}$ Medium Contin. Ferms.	2.64 (Av.)	1.254
In vitro determinations (Appendix II)	4.6 (Const.)	2.04
Jarl (270) Batch Cultures Endomycopsis fibuliger		
Strain NRRL Y - 1062	Not Quoted Probably	0.983
Strain a : 3	c. 5.0.	2.70

Values quoted by Jarl (270) for batch cultures of strains of Endomycopsis fibuliger used in the Symba Process are included in Table 4.1 for comparison. In these yeast fermentations there are indications that the rate of saccharification becomes limiting after about 8 hours, when the reducing sugar production rate, though increasing, is about 0.8 $g.l^{-1}.h^{-1}$.

Throughout the range of medium supply rates used in this experimental work even though the maximum starch hydrolysis rates were lower, the conversion was almost always in excess of 90% complete. Except, of course, under carbon limited conditions, the availability of glucose was always in excess of the requirement of the mould for growth and maintenance; it did not, therefore, limit the fermentation.

4.2.4.2 CARBOHYDRATE UTILIZATION RATES.

Carbohydrate substrates, in the form of glucose, are utilized by A. niger M1 both for maintenance of the culture and for the growth of new "biomass". Taking both these factors into account, the overall rate of substrate consumption can be represented by the equation:-

$$\frac{ds}{dt} = \frac{D.\ x_E}{Y_g} + m\ x_F \quad \ldots\ldots \text{Eqn. 4.4.}$$

Fig. 4.8 illustrates the relationship between the sugar utilization rate and the dilution rate (D) in 30°C, starch medium fermentations. The results form 2 straight lines designated A and B with correlation coefficients (r) of 0.9484 (37 readings) and 0.9789 (122 readings) respectively. The highest steady state utilization rate noted in this series of fermentations was 0.84 $g.l^{-1}.\ h^{-1}$.

Though a steady state cannot exist when D = 0 h^{-1}, extrapolation of lines A and B gives an indication of the rate of consumption when μ = 0 h^{-1} i.e. the factor m x_F in Eqn. 4.4; the values obtained are 0.14 and 0.12 $g.l^{-1}.\ h^{-1}$ respectively. Assuming as in Section 4.2.3.1, that when $D \approx 0\ h^{-1}$, $x_F \gg$ 19.0 $g.l^{-1}$, these values suggest that the maintenance coefficient (m) is less than 0.0065 $g.g^{-1}.\ h^{-1}$. This figure is in agreement with values obtained by other plots.

When considering the specific substrate utilization rate (q), Eqn. 4.4 reduces to :-

$$q = \frac{\mu}{Y_g} + m \quad \ldots\ldots \text{Eqn. 4.5.}$$

Fig. 4.8 The Relationship between the Substrate Utilization and Dilution Rates in 30°C Fermentations.

(■ 20, ● 13.2, ▲ 3.3g.l^{-1} glucose equivalent medium)

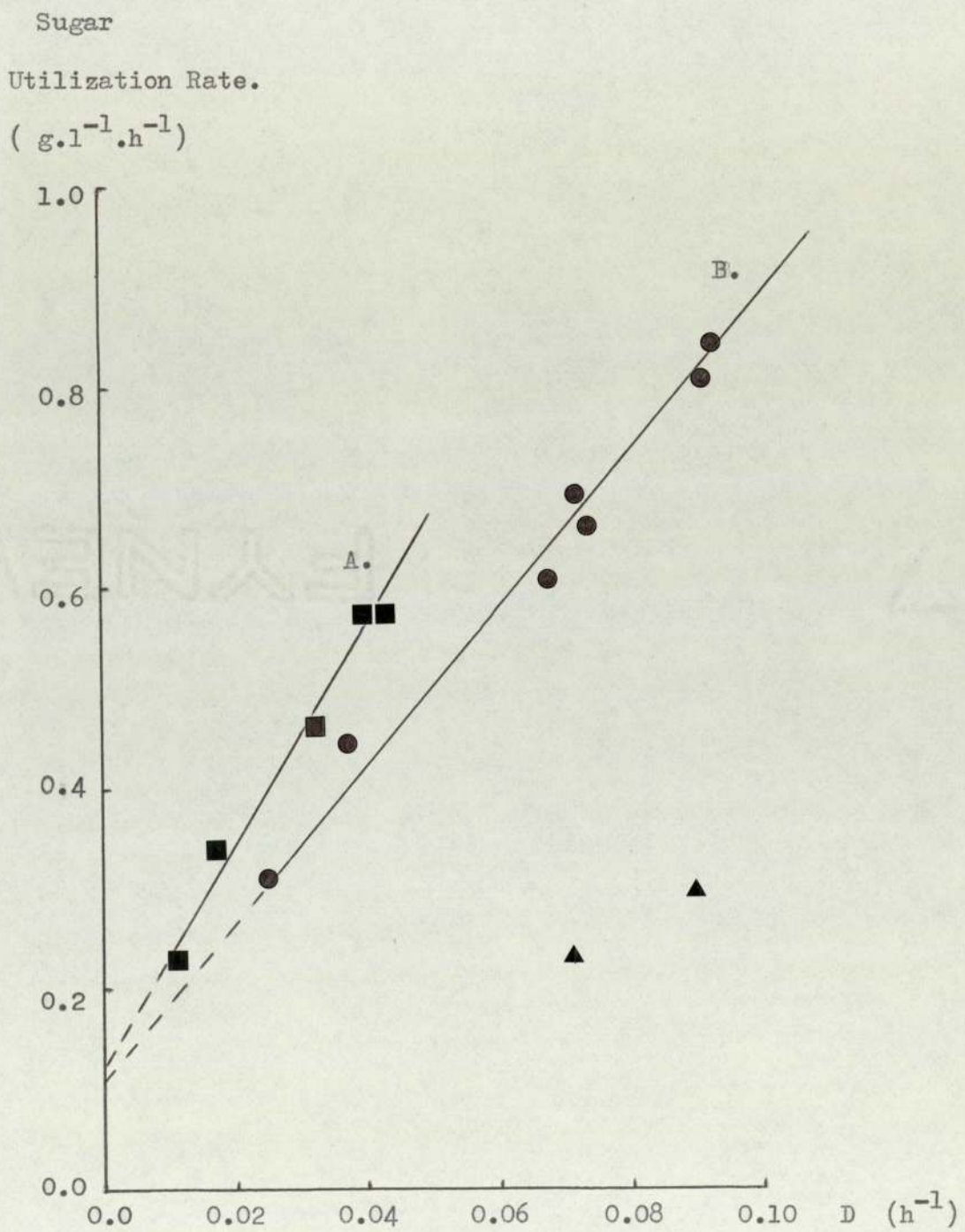
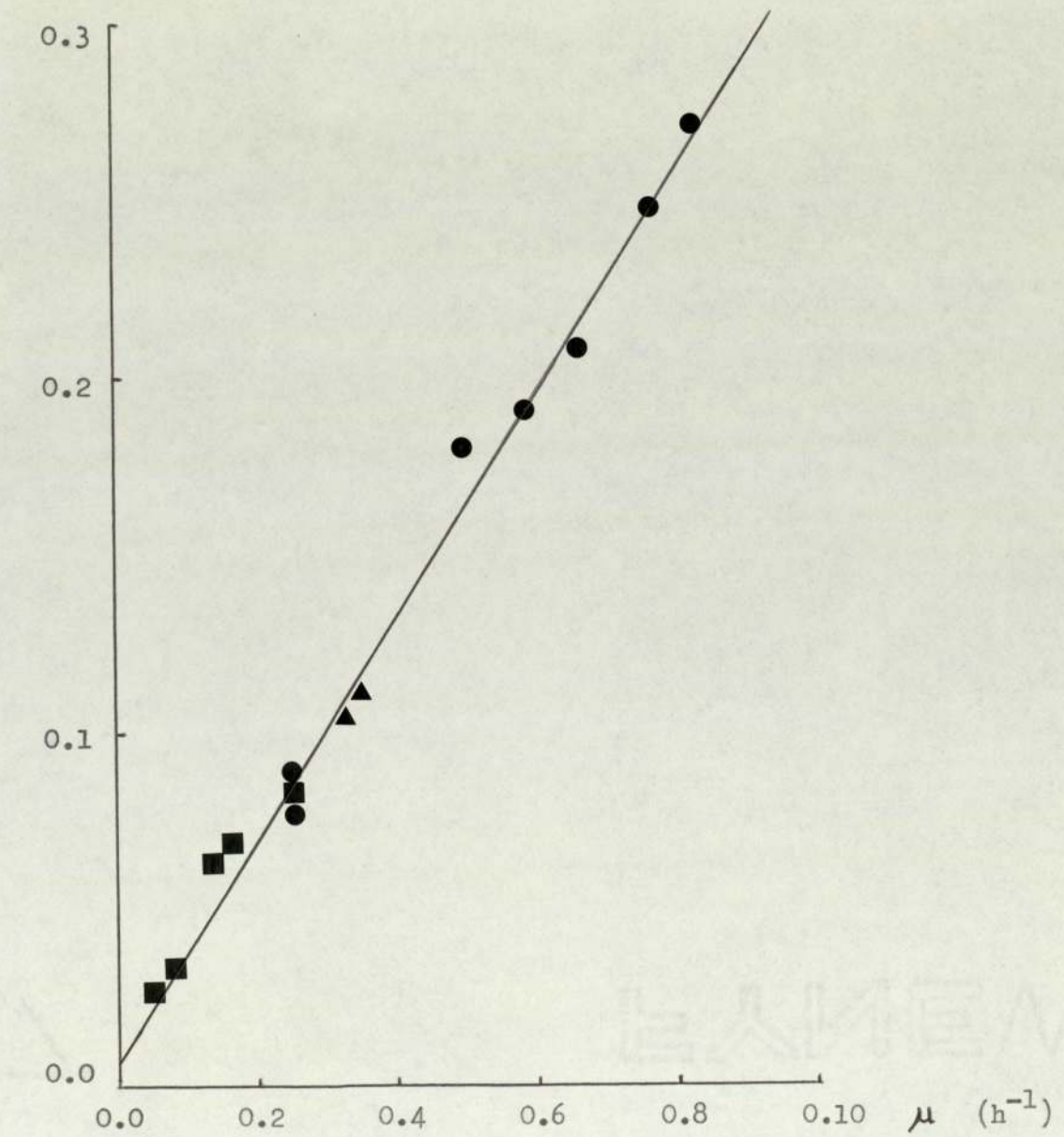


Fig. 4.9 The Relationship between the Specific Rates of Growth and Substrate Utilisation in 30°C Fermentations.

(■ 20, ● 13.2, ▲ 3.3g.l^{-1} glucose equivalent medium)



This equation is the basis of the graphical method used by Tempest and Herbert (303), Righelato et al. (304) and Von Meyenberg (305) for the determination of the maintenance coefficient (m) of microorganisms (discussed further in Section 4.5.2).

Data from this series of fermentations at 30°C is plotted in such a manner in Fig. 4.9. The results form a straight line (Correlation Coefficient 0.9917 ; 195 readings) which, if extrapolated to $\mu = 0$, yields a value $m = 0.00424$ $g.g^{-1}.h^{-1}$. The significance of this line is also increased when data obtained at higher temperatures is included.

The slope of this line is $^{1}/Y_{g}$; using the data presented in Fig. 4.9 a value of $Y_{g} = 0.3158$ g organism. g glucose^{-1}. h^{-1} was obtained for these fermentation conditions. The significance of this low figure will be discussed in Section 4.5.2

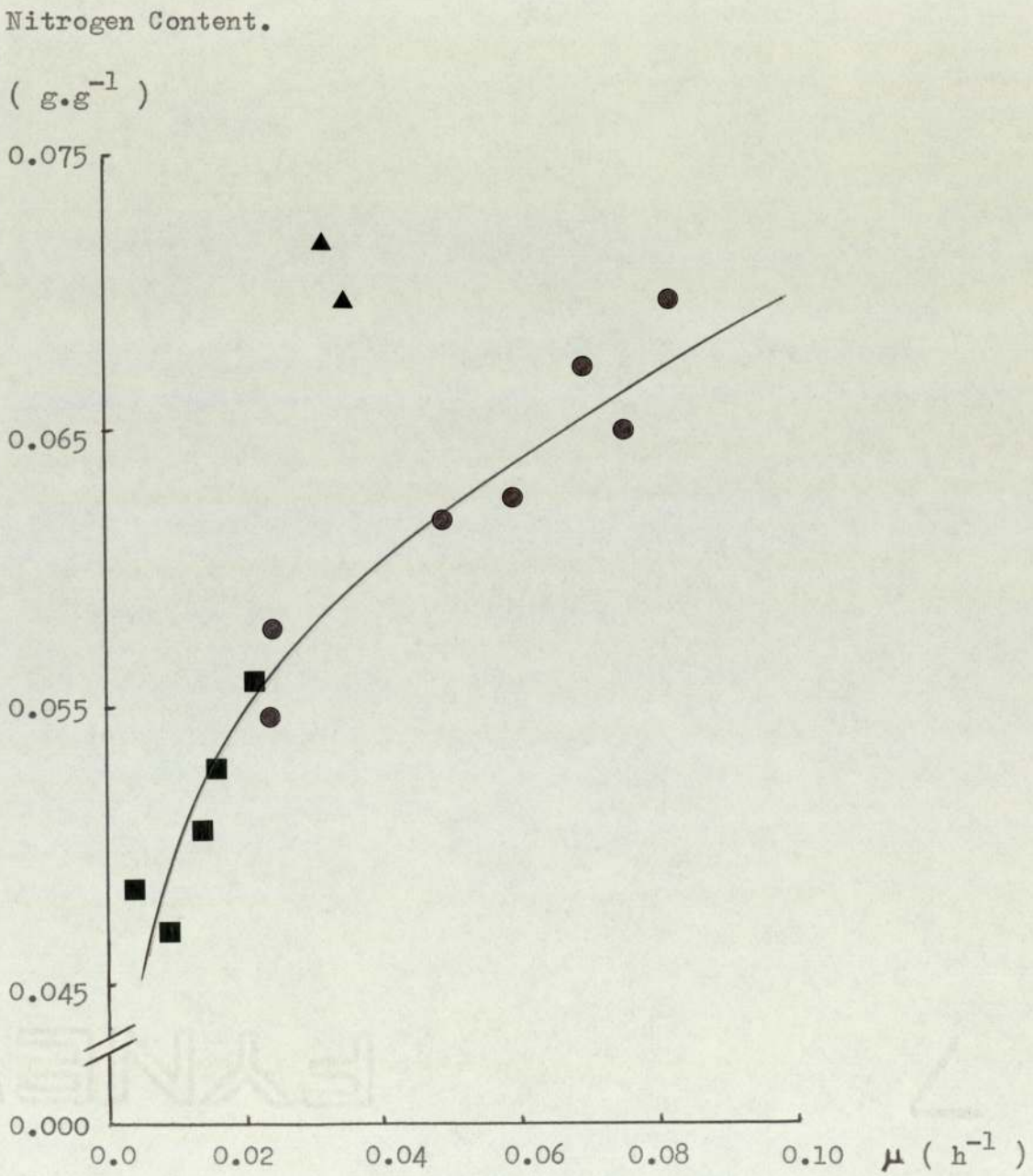
4.2.5 NITROGEN CONTENT OF THE ORGANISM.

The nitrogen content of the mould, though being influenced by the dilution rate, showed little direct relationship. However, as illustrated in Fig. 4.10, this parameter increased linearly with the specific growth rate of the organism. The highest steady state nitrogen content recorded was 0.0719 $g.g^{-1}$ organism (actually, under carbon limited conditions) though values in the range 0.0525 - 0.0675 $g.g^{-1}$ were more usual in this series of fermentations. These correspond to a crude protein content of 32.5 - 42.5% on a (N x 6.25) basis.

Pannell (213) estimated the true protein content of A. niger M1 by summation of amino acids and found that the ratio of actual/crude protein varied from 0.57 - 0.84 depending on the growth rate.

Fig. 4.10 The Effect of Specific Growth Rate (μ) on the Nitrogen Content of the Organism in 30°C Fermentations.

(■ 20, ● 13.2, ▲ 3.3g.l^{-1} glucose equivalent medium)



This he compared to a range of 0.45 - 0.75 reported for a strain of Fusarium sp.

The factors affecting the nitrogen content of the mould can be summarized thus:-

(A) Both true protein and nucleic acid contents increase as the specific growth rate is raised.

(B) At low specific growth rates, non-protein nitrogen (such as in chitin, a cell wall component) makes up a significant proportion of the total nitrogen content.

(C) The nitrogen content is "diluted" by non-nitrogen containing metabolites which may have either a structural or storage function and themselves vary with the fermenter conditions. This accounts for significantly higher biomass nitrogen contents under carbon limited conditions.

(D) Depending so closely upon the physiology of the organism, the total nitrogen content is particularly susceptible to changes in fermenter conditions. As a result, it is usually the last parameter to reach steady state in a fermentation.

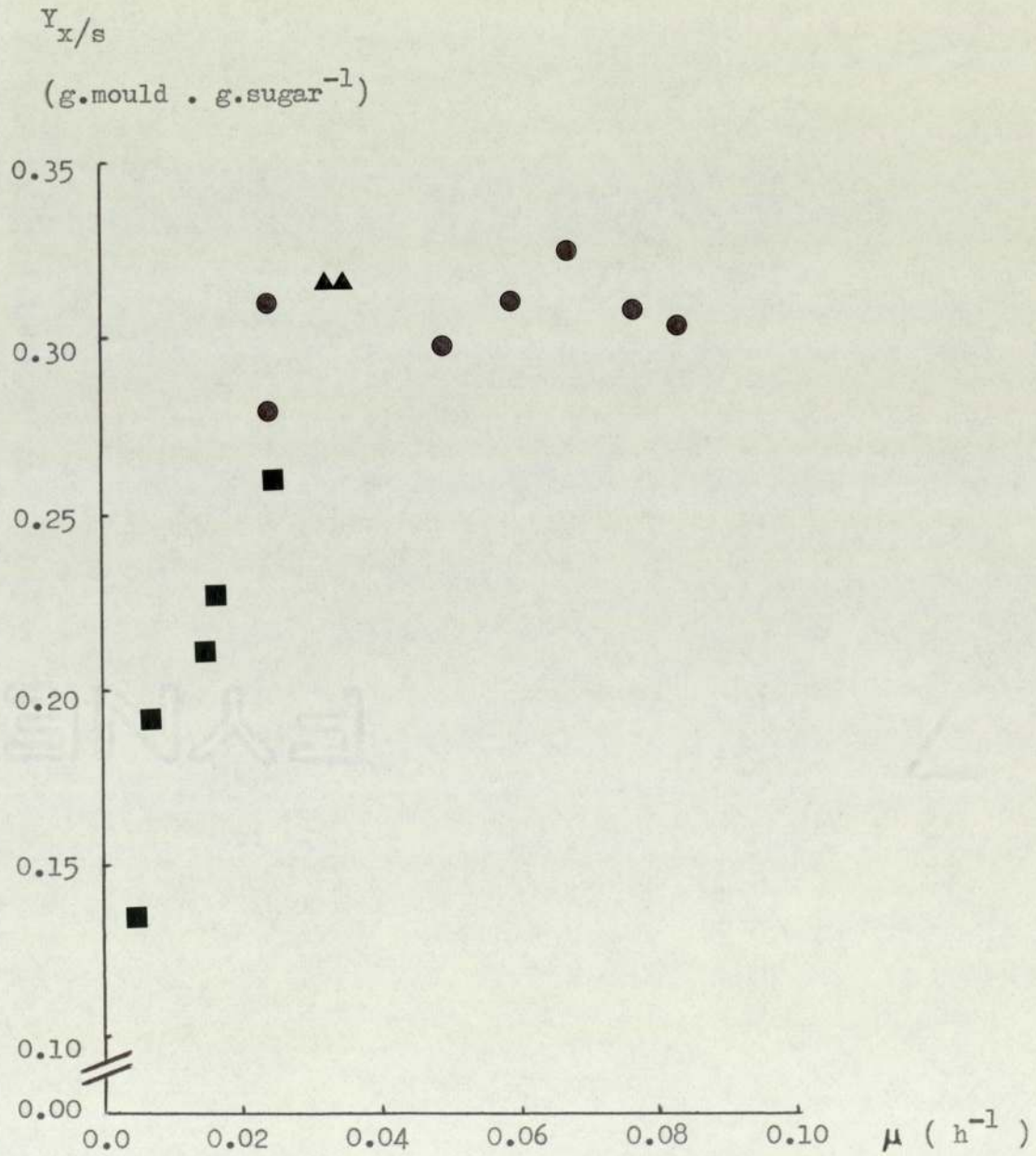
4.2.6 YIELD COEFFICIENT ($Y_{x/s}$) OF THE MOULD.

The Yield Coefficient was easily derived using the Mass Balance Programme and in a given time period is simply

$$Y_{x/s} = \frac{dx}{ds}$$

which is equivalent to

Fig. 4.11 The Effect of Specific Growth Rate (μ) on the Yield Coefficient of Mould on Substrate ($Y_{x/s}$).
(■ 20, ● 13.2, ▲ 3.3g.l^{-1} glucose equivalent medium)



$$Y_{x/s} = \frac{\mu \bar{x}_F}{D(s_R - \bar{s})} \quad \ldots\ldots \text{Eqn. 4.6.}$$

Fig. 4.11 shows the relationship between $Y_{x/s}$ and the specific growth rate (μ). Except when using 20.0 $g.l^{-1}$ glucose equivalent medium, the steady state results fall within a very small range, 0.28 - 0.33 g organism. g^{-1} substrate. These yield coefficients are very low in comparison to other "biomass" production processes, where values in the range 0.4 - 0.5 $g.g^{-1}$ are usual. These discrepancies are almost certainly due to oxygen limitation (see section 4.5.3).

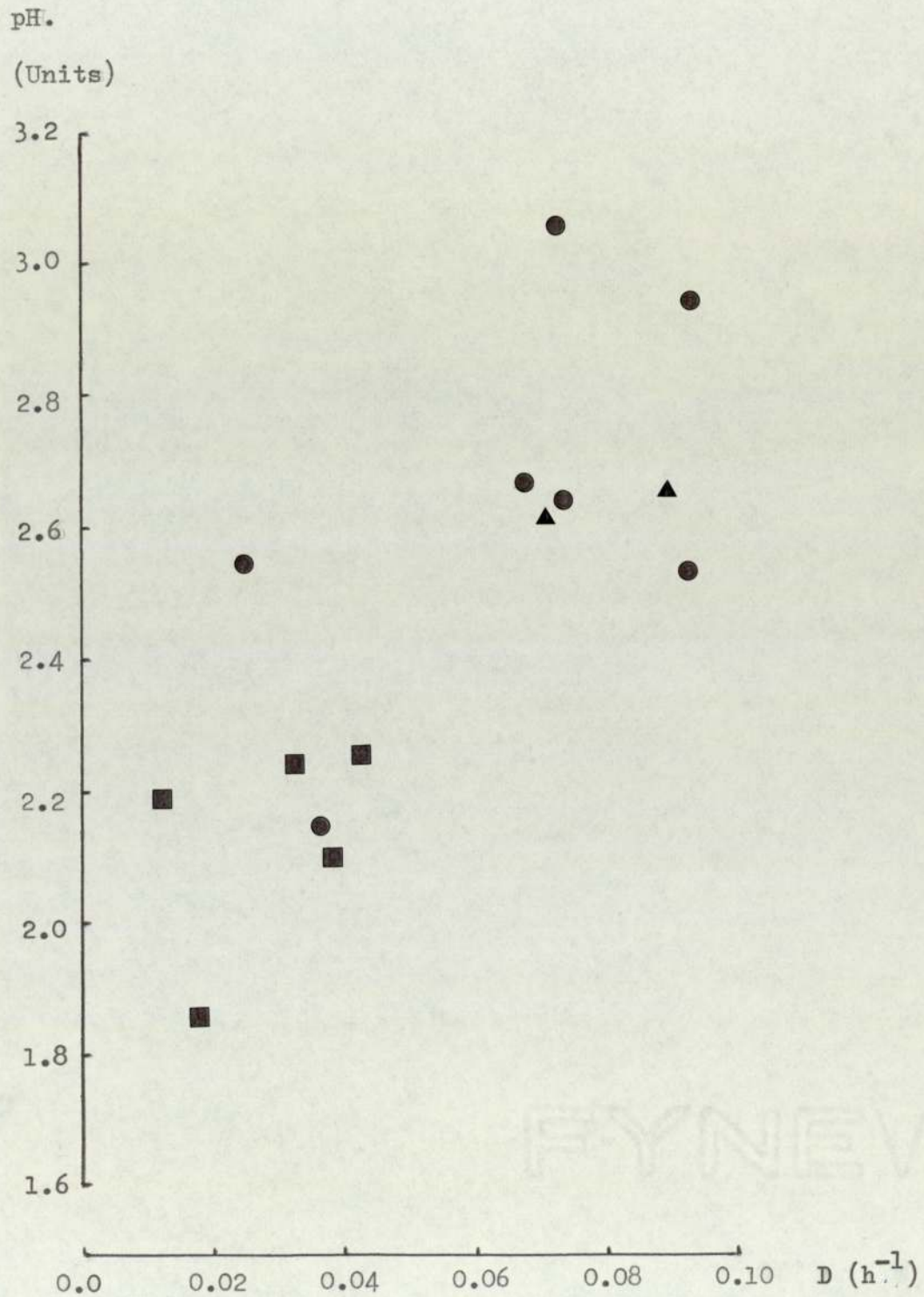
Fermentations in which the specific growth rate was very low were carried out using 20 $g.l^{-1}$ medium and low dilution rates. In these, $\bar{x}_F$ was much higher (see section 4.2.1), so the quantity of substrate consumed for maintenance ($m.\bar{x}_F$) was larger and consequently $Y_{x/s}$ decreased. This effect is compounded by the fact that high values of $\bar{x}_F$ result in low oxygen transfer coefficients ($K_L a$). As oxygen uptake becomes limiting, the mould curtails its respiratory pathways and produces more organic acids. This causes $Y_{x/s}$ to decrease further and lowers the pH of the fermenter contents.

4.2.7 pH OF THE CULTURE.

Mould growth was always accompanied by a reduction in the pH of the medium supply. In general this reduction was greatest at the lowest dilution rates (Fig. 4.12) but the pH value at steady state could not be accurately predicted for any given fermentation. This is because it depends upon a complex balance of factors:-

Fig. 4.12 The Effect of Dilution Rate (D) on the Culture pH in 30°C Fermentations.

(■ 20, ● 13.2, ▲ 3.3g.l^{-1} glucose equivalent medium)



(A) Uptake of nutrient molecules from medium salts can result in pH changes e.g. the uptake of nitrogen from ammonium sulphate gives sulphuric acid which decreases the pH.

(B) Organic acid production by the mould both as an energy reserve and in response to oxygen limitation decreases the pH.

(C) Use of these organic acids as substrates for growth under conditions of acute carbon limitation increases the pH.

(D) Increase in dilution rate lowers the concentration of acids formed by the mould and increases the pH.

(E) The buffering capacity of the medium, though low and probably variable, results in higher pH values.

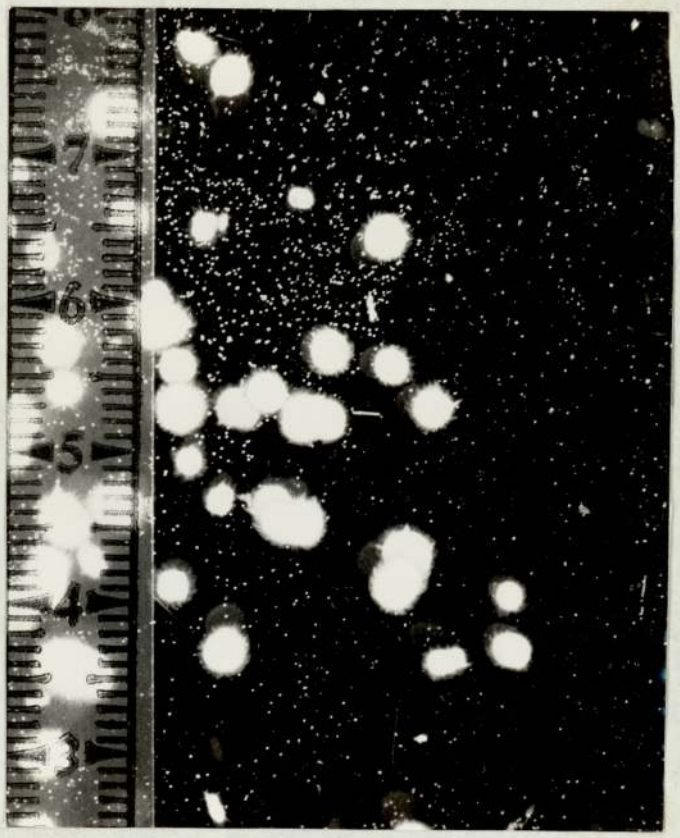
Whilst the range of pH values noted under widely varying fermentation conditions appears small, being on a logarithmic scale, these represent considerable differences in acid concentration. The lowest value noted was pH 1.7 ($D = 0.0179\ h^{-1}$; $s_R = 20.0\ g.l^{-1}$) which represents an acid concentration 10x higher than does pH 2.7 ($D = 0.0679\ h^{-1}$; $s_R = 13.2\ g.l^{-1}$), assuming the dissociation constants remain unchanged.

4.2.8 THE MORPHOLOGY OF THE MOULD.

Samples of mycelium were photographed in a flat-sided perspex viewing cell after being diluted approximately 50x with warm water containing C. 0.05% "Tween 80" detergent solution. In spite of these precautions it proved impossible to prevent the formation of

Plate 4.1 Aspergillus niger M1 : Typical Morphology 46 h. after Spore Inoculation.

(Fermentation SR 2; D= 0.025 h^{-1}; s_R = 20g.l^{-1}gluc. eq.)



1 cm.

minute air bubbles on the walls of the cell when illuminated by "photoflood" lamps.

In all the fermentations carried out Aspergillus niger M1 mycelium developed in the form of colonies. The use of this description is deliberate to avoid implications of structure and packing density or mechanism of formation which are inherent in such terms as "pellet", "floc" and "aggregate".

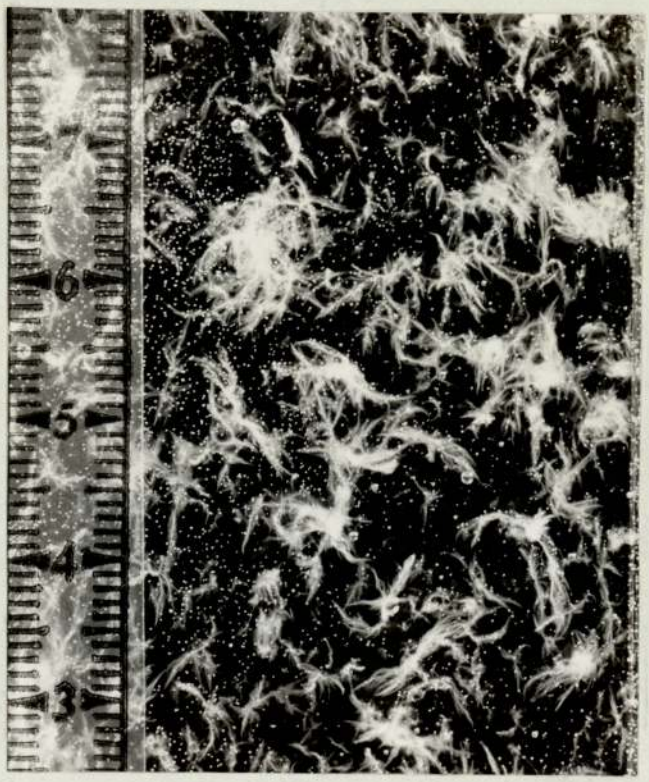
The initial morphology of the organism and its changes could not be accurately predicted for a given fermentation. With the size of spore inoculum and start-up conditions used in this work, colonies such as those in Plate 4.1 usually developed. Typically these were about 2 - 4 mm diameter "hairy" spheres of mycelium conforming to the usual descriptions of "pellets" (Cocker, 290).

Stable morphologies of the types illustrated in Plates 4.2 and 4.3 gradually evolved from these colonies over a period of continuous culture. At the lowest dilution rates investigated this process often occupied 200 - 300 h., the result being comparatively large ($>$ 8mm) tangled bundles of mycelium. In general, colonies from a 20 $g.l^{-1}$ medium fermentation were larger than those from a 13.2 $g.l^{-1}$ medium situation and in such samples the proportion of small colonies was reduced.

In one steady state ($D = 0.025\ h^{-1}$; $s_R = 13.2\ g.l^{-1}$) $\bar{x}_F$ was much lower than expected and this was reflected in all the other fermentation parameters. The colonies appeared more "feathery" loosely packed and filamentous (see Plate 4.4). Such a steady state results from a combination of the filamentous morphology

Plate 4.2 Aspergillus niger M1 : Steady State Morphology after 443 h. Continuous Fermentation.

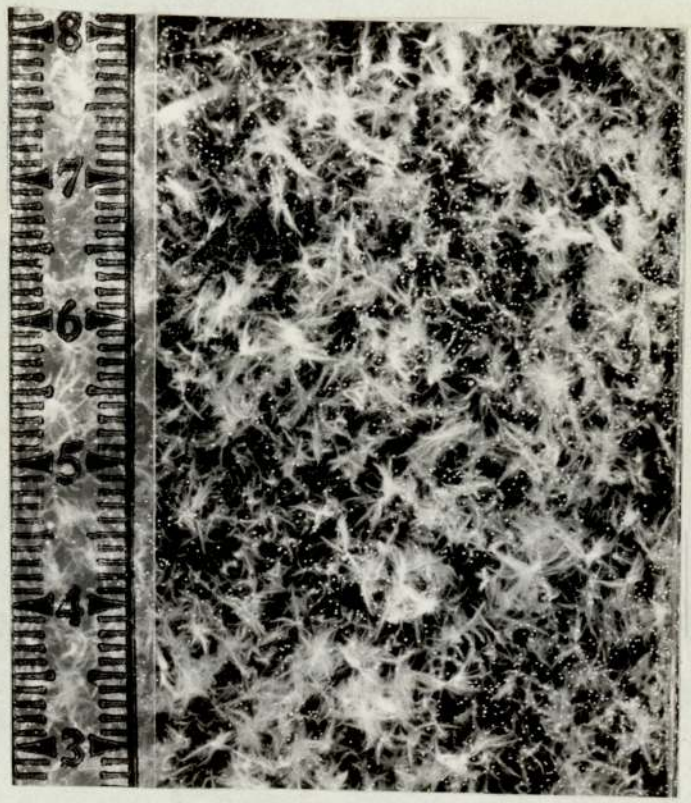
(Fermentation SR 1; D=0.042 h^{-1}; s_R = 20g.l^{-1} gluc. eq.)



1 cm.

Plate 4.3 Aspergillus niger M1 : Steady State Morphology after 543 h. Continuous Fermentation.

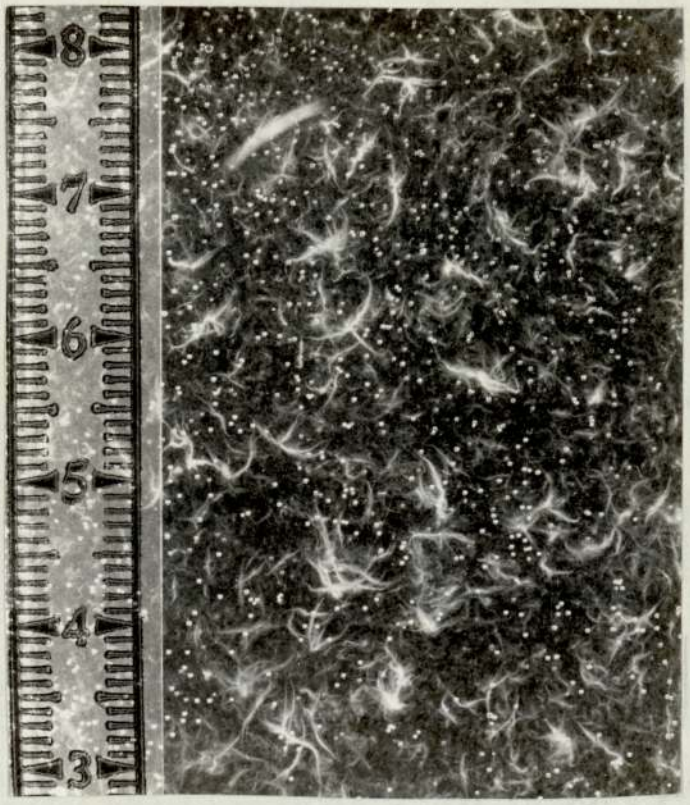
(Fermentation SR 5; D=0.073 h^{-1}; s_R = 13.2g.l^{-1} gluc. eq.)



1 cm.

Plate 4.4 Aspergillus niger M1 : A More Filamentous Morphology.

(Fermentation PN 4B; $D=0.025\ h^{-1}$; $s_R = 13.2 g.l^{-1}$ gluc. eq.)



1 cm.

causing a low $\bar{x}_F$ and vice versa. In other words the mould morphology and concentration simultaneously influence and are influenced by the fermenter conditions.

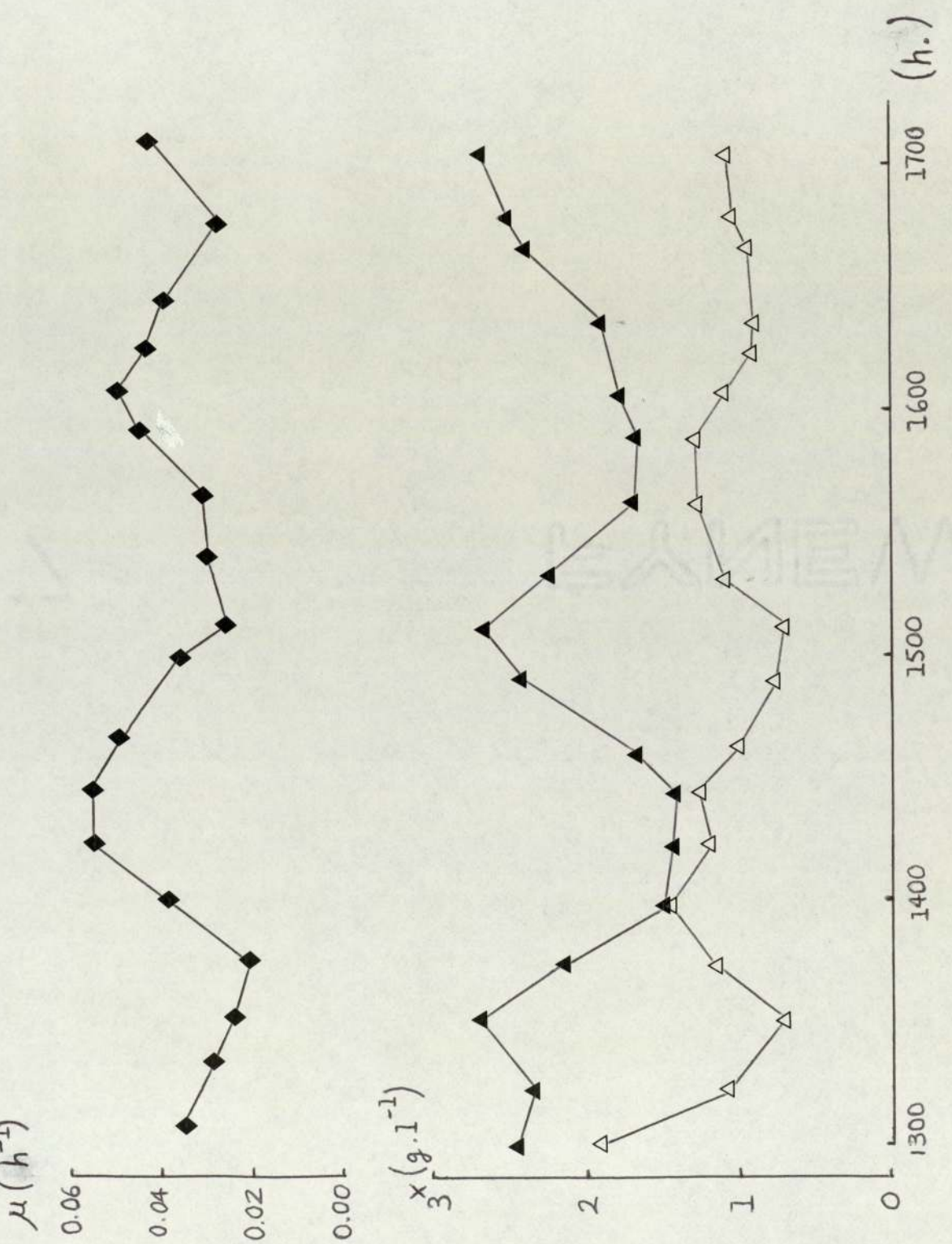
4.2.9 OSCILLATIONS IN THE MOULD CULTURES.

In most fermentations, particularly those at low dilution rates, the measured values of parameters tended to fluctuate even though D, s_R and T remained constant. This was almost certainly due in part to errors in measurement - the error in determining x_F could have been as great as $\pm$ 0.1 $g.l^{-1}$ with samples in the range 3.0 - 4.0 $g.l^{-1}$.

However, particularly in substrate limited conditions there were periods in which, with x_F constant, some degree of synchrony of growth and productivity was apparent (as in the results of Pannell, 213). In other cases x_F varied considerably in a cyclical manner (see Fig. 4.13). Usually the periods of these oscillations did not appear to bear any simple relationship to the specific growth rate of the mould, or correlate with any environmental change.

Fig. 4.13 illustrates data collected over a period of 400 h. fermentation at a dilution rate of 0.0704 $\pm$ 0.0007 h^{-1} (21 readings) using 3.3 $g.l^{-1}$ glucose equivalent medium. The concentration of mould in the fermenter (x_F) varied from 1.43 $g.l^{-1}$ to 2.75 $g.l^{-1}$, changes which were reflected in x_E. Even with corrections for dx_F (in the Mass Balance Programme), the specific growth rate (μ) of the mould cycled between 0.023 h^{-1} and 0.57 h^{-1} with the same period as x_F and x_E, 180 h.

Fig. 4.13 An Unsteady State : Variations in the Specific Growth Rate (μ) and the Mould Concentrations in the Fermenter (x_F) and Effluent Stream (x_E). (D= 0.0704 h^{-1}. (◆ μ ; ▲ x_F; △ x_E) s_R= 13.2 $g.l^{-1}$gluc. eq.)



Theoretically, at steady state,

$$\mu = \frac{(D\,(s_R - \bar{s}) - m.\bar{x}_F)\,Y_g}{\bar{x}_F} \quad \ldots \text{ Eqn. 4.7.}$$

Substituting: $Y_g = 0.3158\ g.g^{-1}$. $m = 0.00424\ g.g^{-1}.h^{-1}$.

$x_F = 2.08\ g.l^{-1}$ (Av.) $D = 0.0704\ h^{-1}$.

$s_R = 3.3\ g.l^{-1}$

$(s_R - \bar{s}) \approx s_R$ (as the culture was substrate limited).

$\underline{\mu = 0.03393\ h^{-1}}$.

This theoretical value of μ is in agreement with the average value of all the experimental results under these conditions, $0.0348\ h^{-1}$. The 400 h. period of fermentation was equivalent to approx. 28 fermenter throughputs of medium and 20 "generation times" of the organism. This data emphasizes the point that, when growing moulds in tower fermenter systems, steady state conditions are achieved only after considerable time periods and in some cases are not necessarily ever reached.

4.3 SEMI-SYNTHETIC STARCH MEDIA : EFFECTS OF TEMPERATURE AND MEDIUM pH ON THE FERMENTATIONS.

4.3.1 EFFECTS OF TEMPERATURE.

4.3.1.1 INTRODUCTION.

To enable direct comparison, the effects of temperature on Aspergillus niger M1 were investigated in two fermentations using 13.2 $g.l^{-1}$ glucose equivalent medium and a constant dilution rate

of 0.067 h^{-1}. The temperatures chosen initially were 30, 35, 40 and 45°C, though later, 42°C was used in an attempt to establish the thermal death point of the mould more accurately. Results obtained at 35°C using a dilution rate of 0.075 h^{-1} have not been included in this comparison.

Steady states were achieved at all temperatures, except 45°C, at which almost complete "washout" of the mould took place over 94 h. However, the most significant feature of these results was the change in morphology of the organism around 37 - 38°C, effects of which could be seen in all the fermentation parameters.

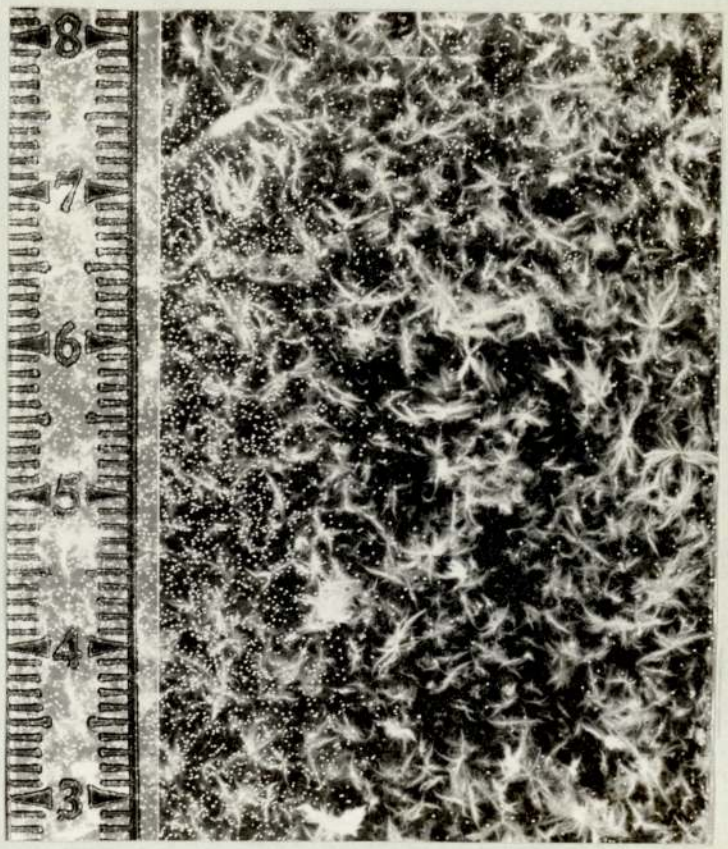
4.3.1.2 THE EFFECT OF TEMPERATURE ON THE MOULD MORPHOLOGY.

The progressive changes in mould morphology with increase in temperature are shown in a series of photographs taken at 35, 40, 42 and 45°C (Plates 4.5, 4.6, 4.7 and 4.8 respectively). These can also be compared to Plate 4.3 which shows the morphology at 30°C though at a slightly higher dilution rate, 0.073 h^{-1}.

The colonies at 30 and 35°C were indistinguishable to the naked eye (Plates 4.3, 4.5). They consisted of a number of radially arranged feather-like, tangled bundles of mycelium, up to approximately 6 mm diameter.

Increasing the temperature above 38°C resulted in the formation of small ($\approx$ 1 mm diameter) densely packed, "inhibited" mycelial colonies (Plate 4.6). At 42°C these were larger ($>$ 2 mm diameter) but fewer in number, with the presence of very small filamentous colonies becoming more obvious. The colour of

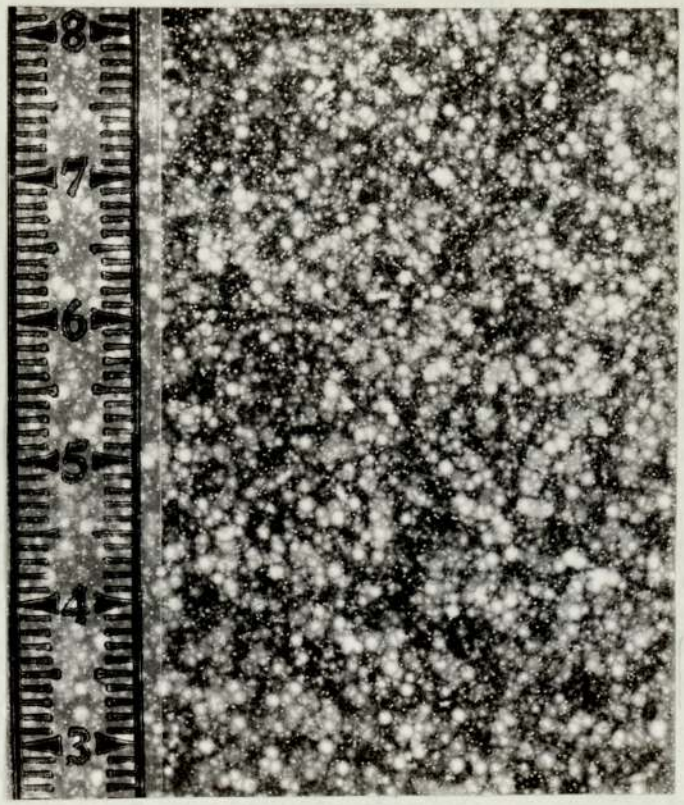
Plate 4.5 Aspergillus niger M1 : The Morphology after 167 h.
Continuous Cultivation at 35°C.
(Fermentation SR 7; D= 0.067 h^{-1}; s_R =13.2g.l^{-1} gluc. eq.)



1 cm.

Plate 4.6 Aspergillus niger M1 : The Morphology after 259 h. Continuous Cultivation at 40°C.

(Fermentation SR 6; D= 0.067 h^{-1}; s_R =13.2g.l^{-1} gluc. eq.)

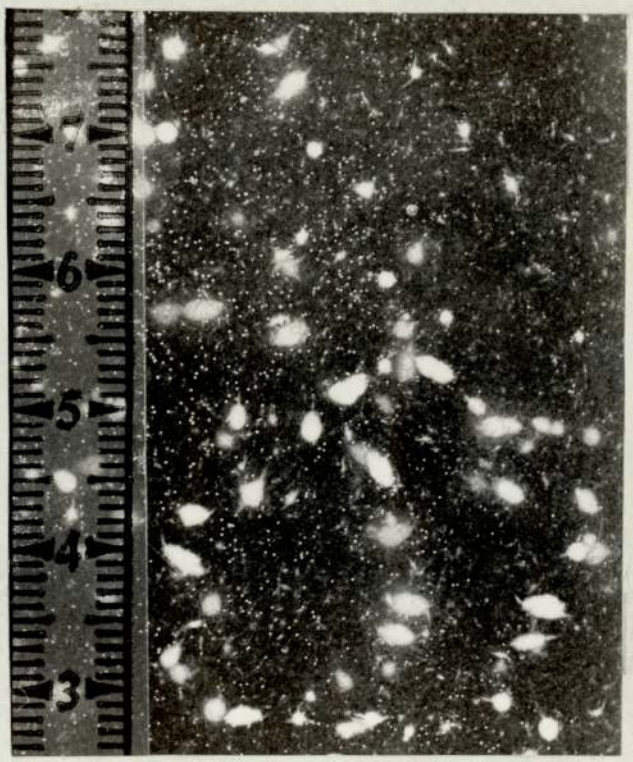


1 cm.

Plate 4.7 Aspergillus niger M1 : The Morphology after 217 h.

Continuous Culture at 42°C.

(Fermentation SR 6; D= 0.067 h^{-1}; s_R =13.2g.l^{-1} gluc. eq.)



1 cm.

Plate 4.8 Aspergillus niger M1 : The Morphology after 76 h.
Continuous Cultivation at 45°C.
(Fermentation SR 6; D= 0.067 h^{-1}; s_R=13.2g.l^{-1} gluc. eq.)

1 cm.

the culture as a whole changed from cream to light grey.

After 76 h. at 45°C little mycelium remained in the fermenter. It was in the form of very small "wisp-like" colonies which were grey in colour.

The changes are those expected in response to the progressive increase in temperature from being optimum for growth, to being inhibitory and finally to causing death of the mould.

4.3.1.3. CONCENTRATIONS OF ORGANISM IN THE FERMENTER (x_F) AND EFFLUENT STREAM (x_E).

The use of a constant dilution rate (and, therefore, s.l.v.) ensured that changes in $\bar{x}_F$ and $\bar{x}_E$ were due solely to temperature related factors. Changes in the morphology of the mould (section 4.3.1.2), both of the packing density and resistance to fluidisation, were in effect quantified as changes in $\bar{x}_F$ (illustrated in Fig. 4.14).

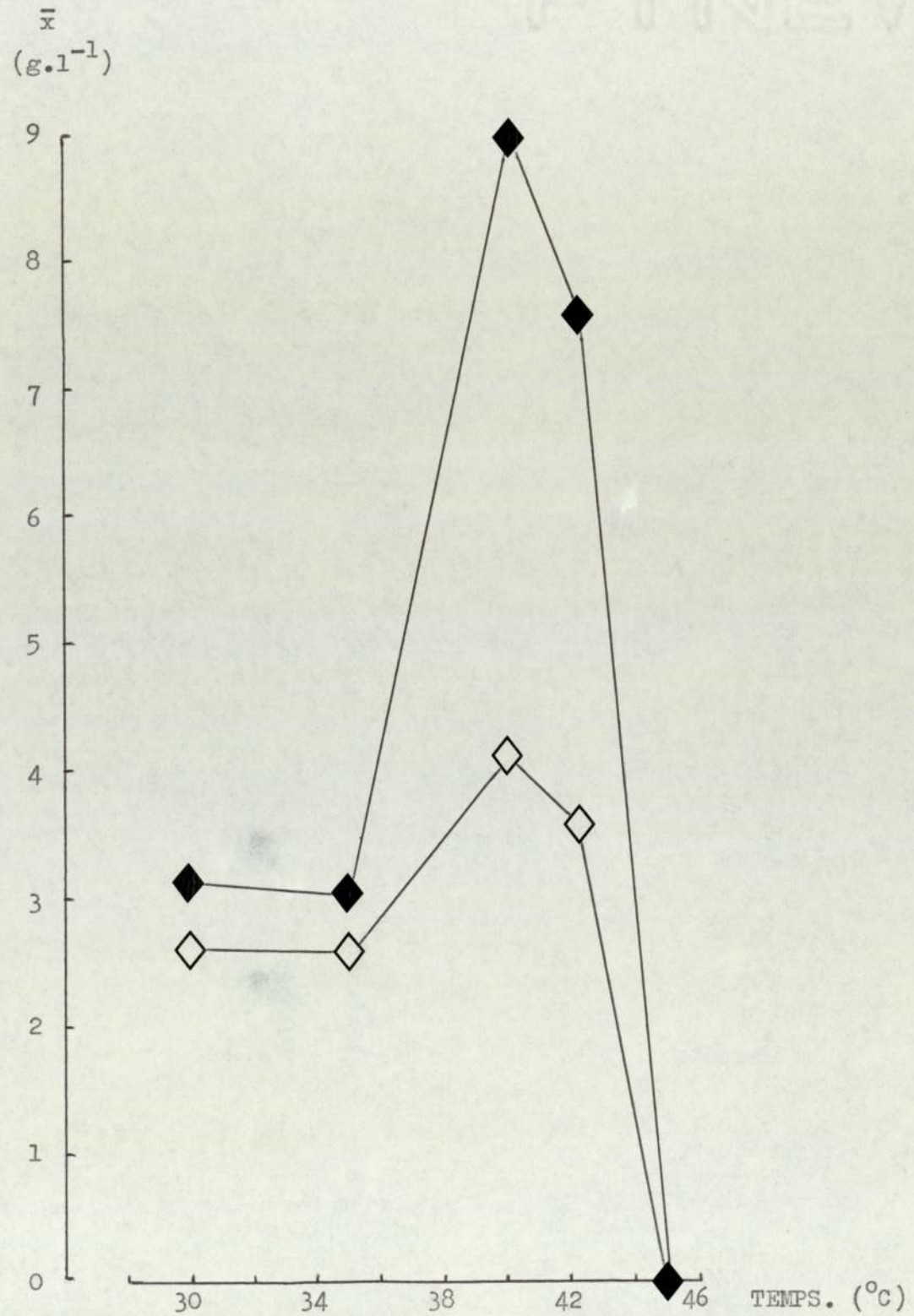
In the case of the 30°C and 35°C fermentations, when the colonies were indistinguishable, the respective values of $\bar{x}_F$ and $\bar{x}_E$ were almost the same. However, the change in morphology at approximately 37 - 38°C resulted in an increase in $\bar{x}_F$ from 3 $g.l^{-1}$ to 9 $g.l^{-1}$.

At 45°C both $\bar{x}_F$ and x_E declined to less than 0.1 $g.l^{-1}$. After 94 h., this temperature was reduced to 38°C but the culture could only be re-established by re-inoculation with spores. This temperature, therefore, is believed to be above the thermal death point of A. niger M1 and so $\bar{x}_F$ and $\bar{x}_E$ assumed to be zero.

Fig. 4.14 The Effect of Temperature on the Mould Concentration in the Fermenter (x_F) and Effluent Stream (x_E).

($D = 0.067\ h^{-1}$; $s_R = 13.2\ g.l^{-1}$ gluc. eq.)

(◆ x_F ; ◇ x_E)



4.3.1.4 THE SPECIFIC GROWTH RATE (μ) AND PRODUCTIVITY OF THE ORGANISM.

The effects of temperature on the Productivity and Specific Growth Rate (μ) of cultures of A. niger M1 are shown in Figs. 4.15 and 4.16 respectively.

The productivities at 40 and 42°C (0.26 - 0.27 $g.l^{-1}.h^{-1}$) were approximately 50% higher than those at 30 and 35°C and were in fact higher than when D = 0.093 h^{-1} at 30°C. However, this increase was counteracted by the increase in $\bar{x}_F$ by a factor of 3x, so that μ decreased considerably at temperatures above 37 - 38°C.

As the mould was presumed to be dead in the 45°C fermentation, both these parameters were assumed to be zero at steady state at this temperature. As x_F declined, two negative values of productivity and, therefore, μ were noted (μ = - 0.010 h^{-1}). These could have been due to small errors in the determination of x_F and x_E (which were both < 0.5 $g.l^{-1}$) or could have represented a specific rate of autolysis (as explained in section 3.6.5).

4.3.1.5 STARCH HYDROLYSIS AND UTILIZATION RATES.

The rate of production of reducing sugars increased gradually up to 40°C and then declined (Fig. 4.17). The maximum rate was 0.8607 $g.l^{-1}.h^{-1}$ compared to a substrate supply rate of 0.89 $g.l^{-1}.h^{-1}$ (glucose equivalent) throughout this series of experiments.

Point A represents the reducing sugar production rate (0.648 $g.l^{-1}.h^{-1}$) after 94 h. continuous fermentation at 45°C. However, had the fermentation been continued, this rate would have decreased to zero as washout of enzymes from the system took place.

Fig. 4.15 The Effect of Temperature on the Mould Productivity.

($D = 0.067\ h^{-1}$; $s_R = 13.2\ g.l^{-1}$ gluc. eq.)

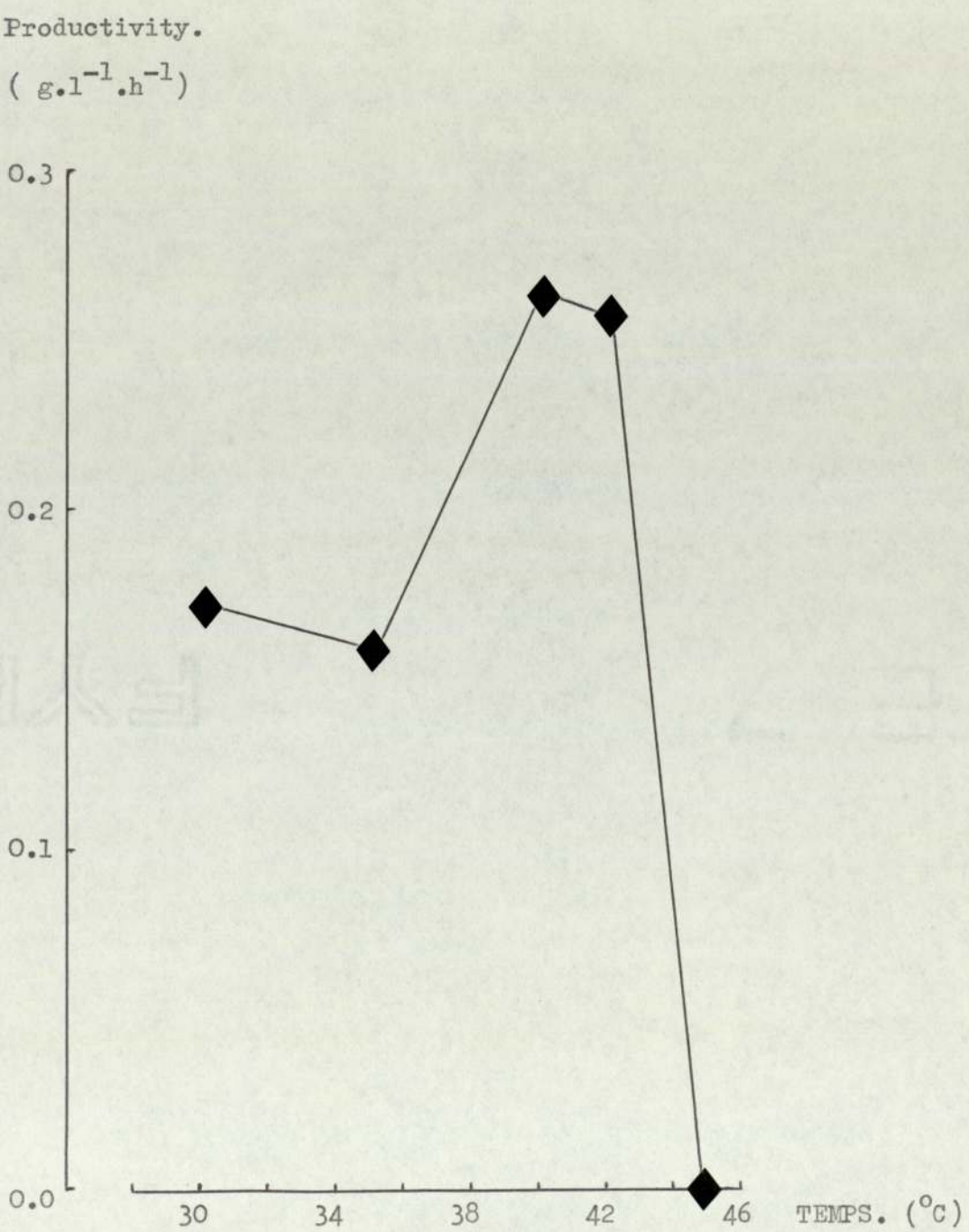


Fig. 4.16 The Effect of Temperature on the Specific Growth Rate (μ) of the Mould.

($D = 0.067\ h^{-1}$; $s_R = 13.2\ g.l^{-1}$ gluc. eq.)

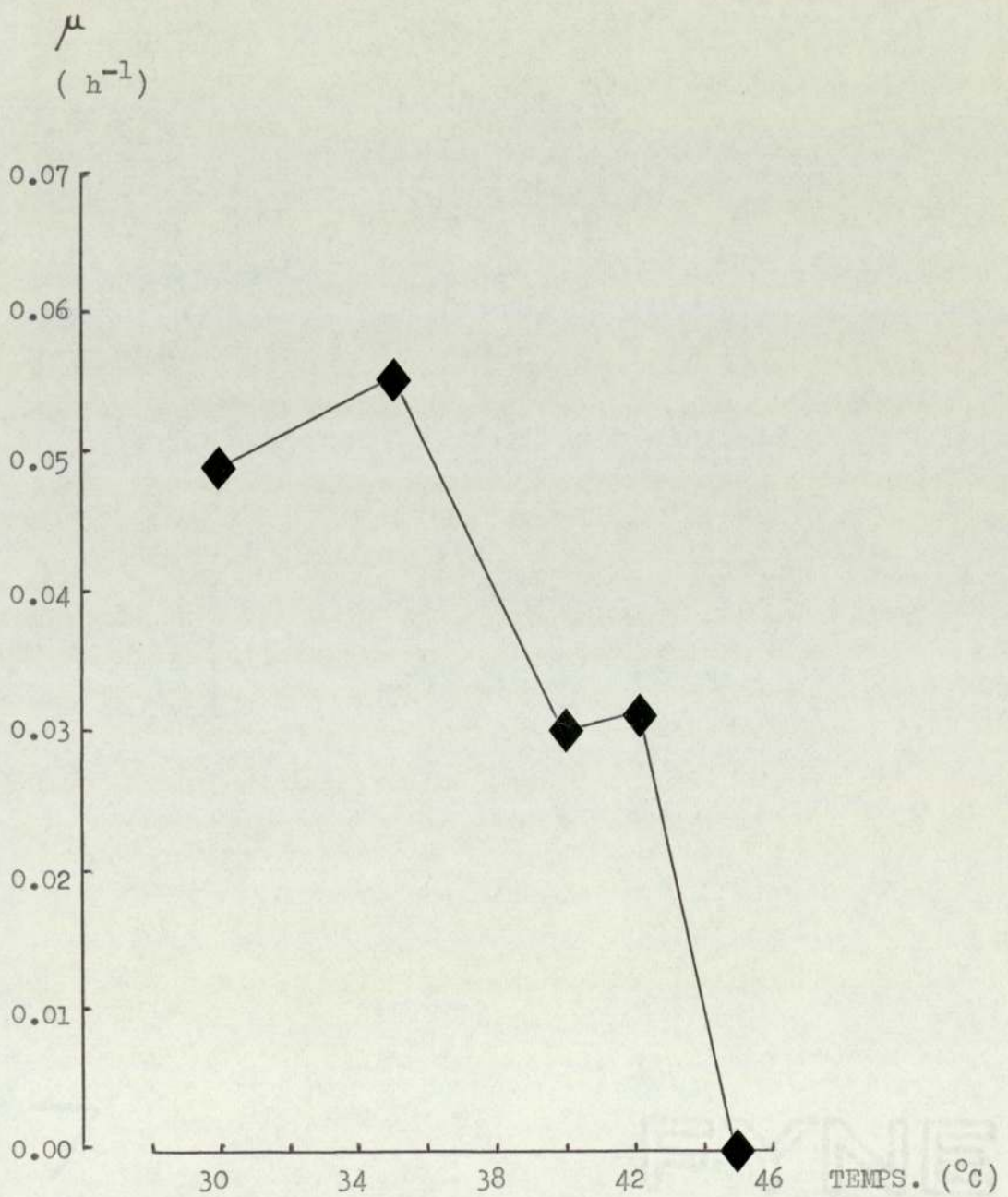
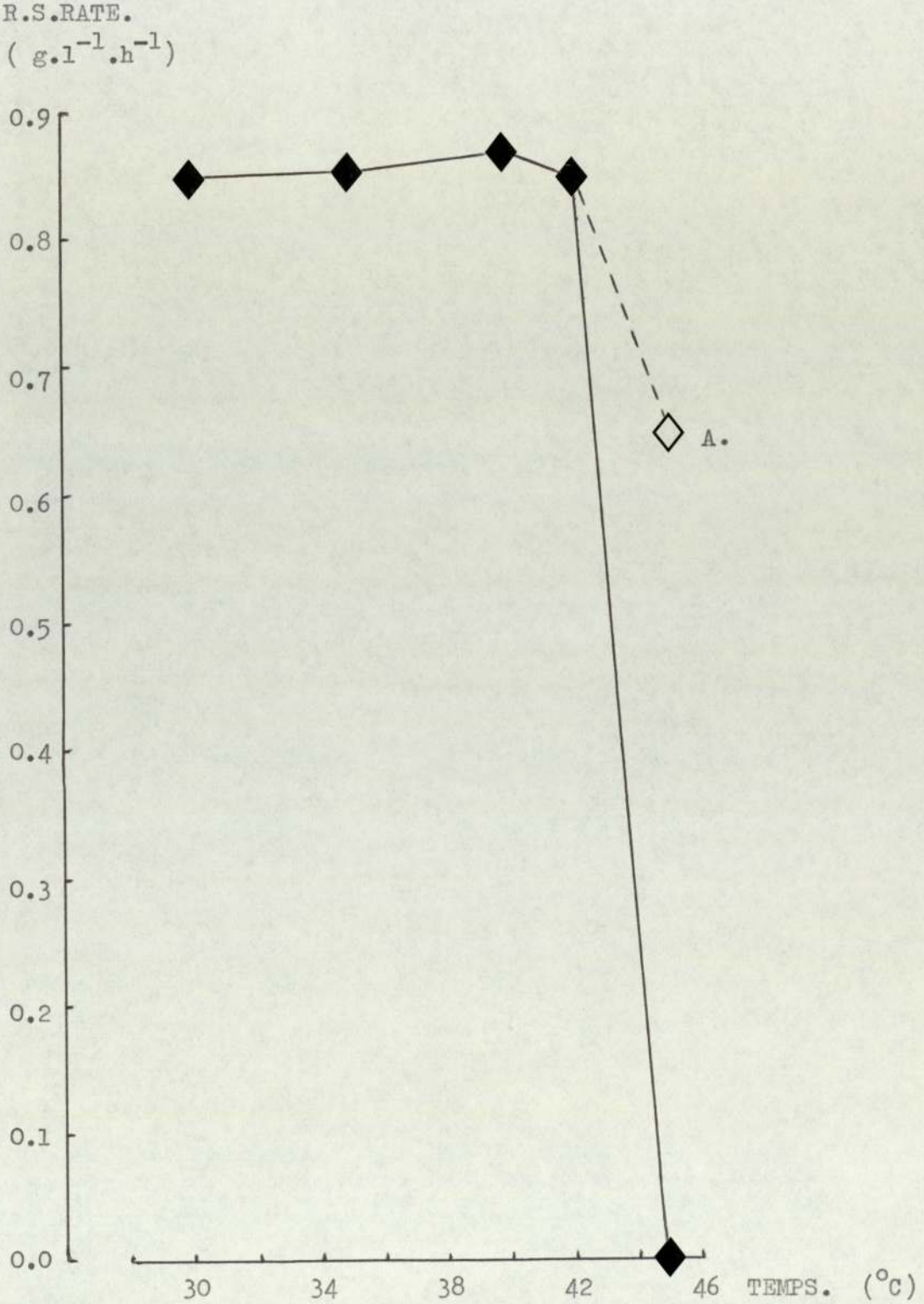


Fig. 4.17 The Effect of Temperature on the Reducing Sugar Production Rate.

($D = 0.067\ h^{-1}$; $s_R = 13.2\ g.l^{-1}$ gluc. eq.)



The substrate utilization rates of the culture both totally ($g.l^{-1}.h^{-1}$) and specifically ($g.g^{-1}.\ h^{-1}$) show the same response to temperature as mould productivity and μ respectively (see Fig. 4.18). The total rates of mould productivity and substrate utilization were considerably higher at 40 and 42°C than at 30 - 35°C. However, as a result of the much higher values of $\bar{x}_F$, both μ and the specific substrate utilization rate (q) were much lower at elevated temperatures.

4.3.1.6 THE NITROGEN CONTENT OF THE MOULD.

When considering the effect of temperature on the mould nitrogen content it is essential to distinguish two possibilities:

(A) That temperature affects the macromolecular composition and therefore the nitrogen content of the organism directly.

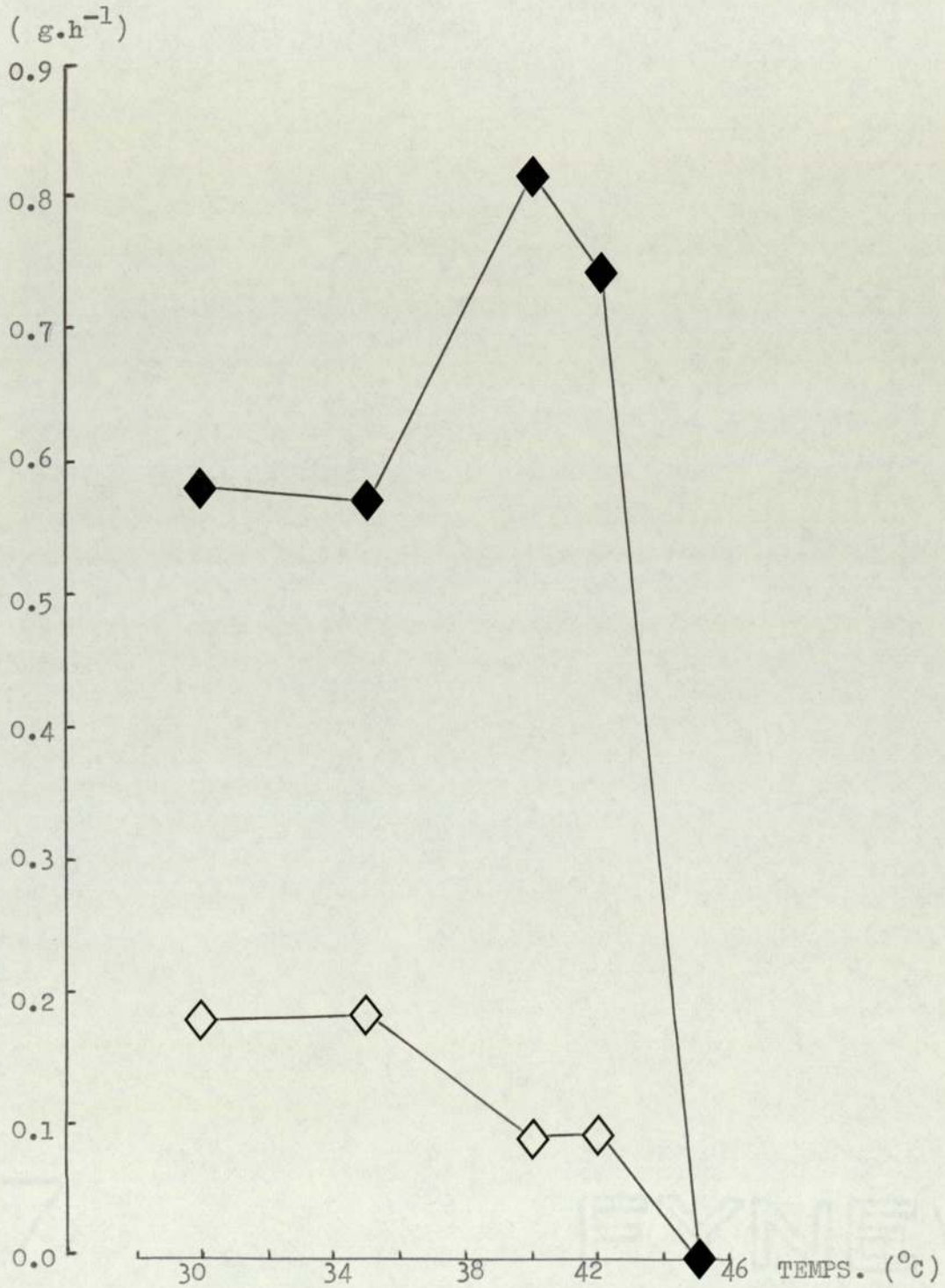
(B) That temperature acts indirectly on nitrogen content via the relationship of both these parameters with μ (see section 4.2.5, Fig. 4.10).

Fig. 4.19 shows the effect of temperature on this parameter. Points joined by a solid line (designated A) are the steady state experimental values. Each is compared with a point (on the line designated B) which represents the equivalent nitrogen content at the same μ in a 30°C fermentation (derived from Fig. 4.10).

In all cases, increasing the temperature resulted in a lower "biomass" nitrogen content; in each steady state the value was significantly lower than would have been expected at the same specific growth rate at 30°C. This indicates that both

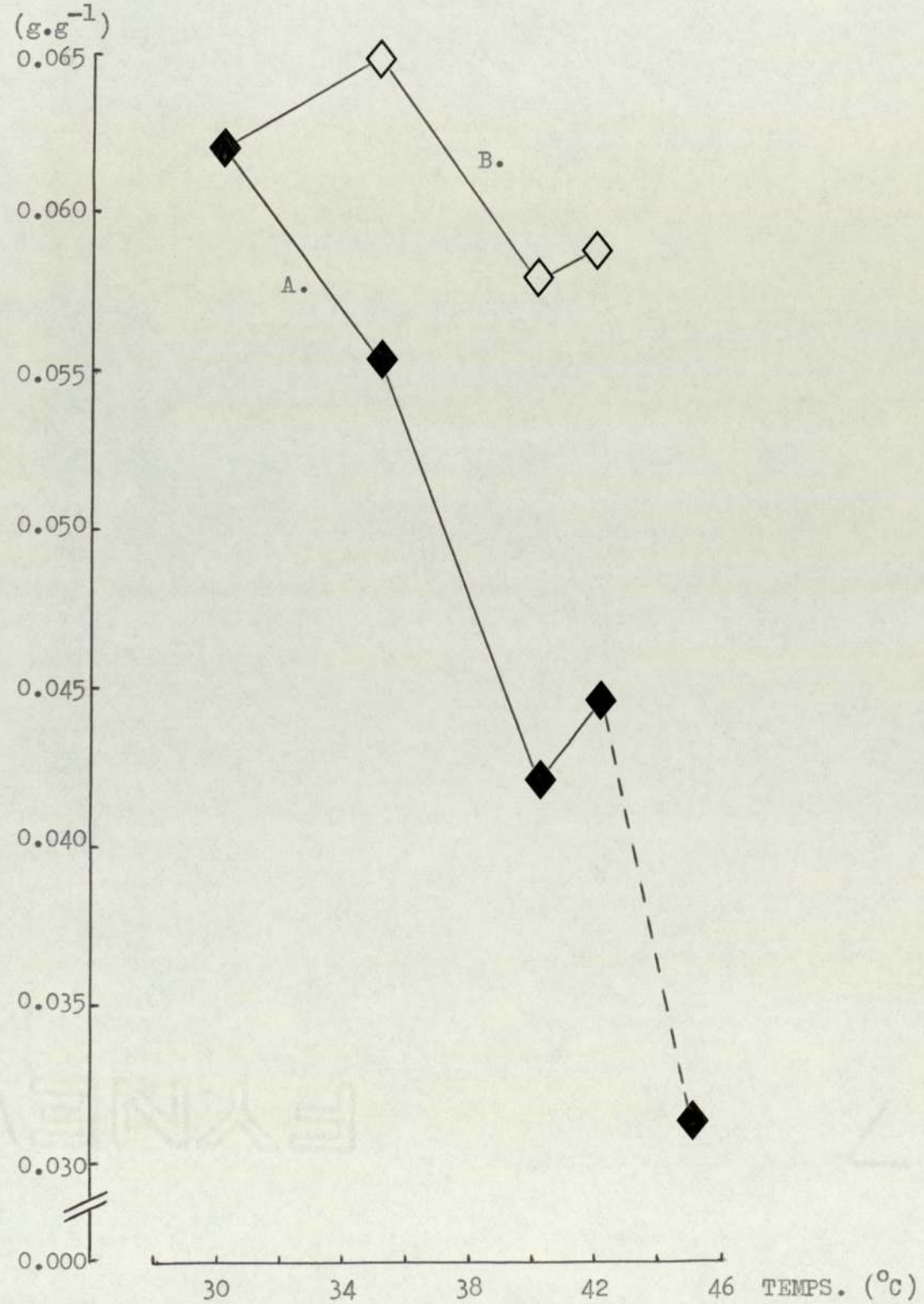
4.18 The Effect of Temperature on the Total and Specific Substrate Utilization Rates.

($D = 0.067\ h^{-1}$; $s_R = 13.2\ g.l^{-1}$ gluc. eq.)
(◆ Total, $g.l^{-1}.h^{-1}$; ◇ Specific, $g.g^{-1}.h^{-1}$.)



4.19 The Effect of Temperature on the Nitrogen Content of the Mould.

($D = 0.067\ h^{-1}$; $s_R = 13.2\ g.l^{-1}$ gluc. eq.)
(◆ Readings; ◇ 30°C equivalents.)



possibilities, A and B, contributed to the diminution of this factor.

The minimum steady state nitrogen content was 0.04235 $g.g^{-1}$ at 40°C, but the lowest individual reading was that of mould "washed out" of the 45°C fermentation, 0.03159 $g.g^{-1}$.

4.3.1.7 THE YIELD COEFFICIENT ($Y_{x/s}$).

This factor showed the same discontinuity in response to temperature as the other parameters directly related to the mould physiology (Fig. 4.20). From 30 - 35°C $Y_{x/s}$ declined, but as a result of the morphology change at 37 - 38°C increased at the higher temperatures. The values at 40 and 42°C ($\approx$ 0.34 $g.g^{-1}$) were also higher than their equivalents at the same μ at 30°C.

These low values could also be explained by the premise that the culture was oxygen limited. The more "pelleted" form of the mycelium at higher temperatures would have permitted a greater oxygen transfer rate and therefore an increased value of $Y_{x/s}$. However this effect would have been counteracted by the higher mould concentration (x_F) and the direct effect of temperature on the oxygen transfer rate. This situation is yet another aspect of the complex interaction of the mould and the environmental conditions.

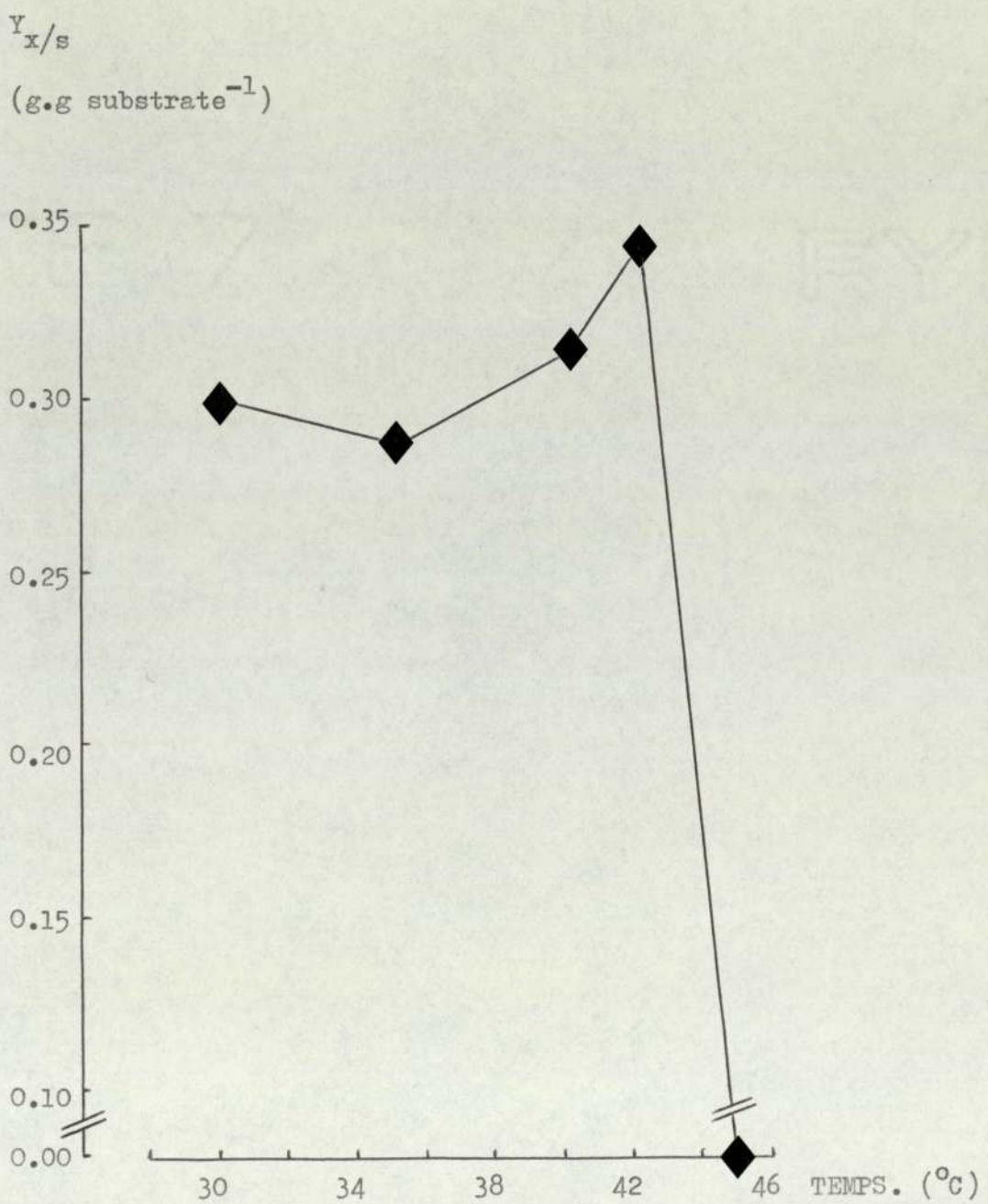
4.3.2 EFFECTS OF MEDIUM pH.

4.3.2.1 INTRODUCTION.

Three fermentations were carried out (two at 30°C, one at 35°C) to investigate the effect of alkaline medium on continuous

4.20 The Effect of Temperature on the Yield Coefficient of the Mould ($Y_{x/s}$).

($D = 0.067\ h^{-1}$; $s_R = 13.2\ g.l^{-1}$ gluc. eq.)



cultures of A. niger M1, prior to the alkaline potato peel waste experiments. The anticipated pH of a medium made up from such a waste was 10.5, so these investigations were carried out at pH 11.0 to allow a reasonable margin for error. The same medium was used throughout, having a substrate concentration of 13.2 $g.l^{-1}$(glucose equivalent).

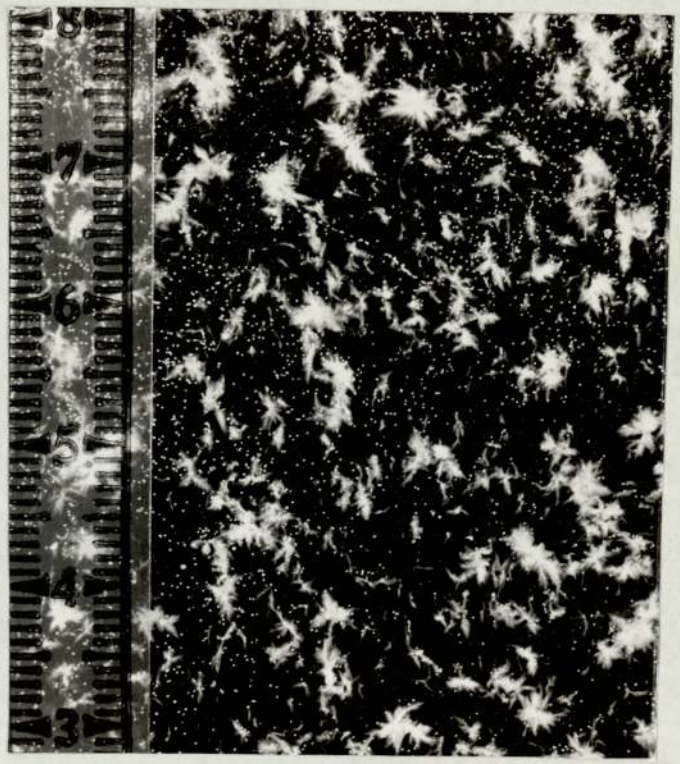
The actual pH adjustment was carried out in a small chamber (c. 600 ml. capacity) inserted in the medium supply line between the medium pump and the fermenter. This chamber, whose contents were magnetically stirred, was equipped with a pH probe and an alkalie injection needle to enable a pH meter-controller/alkalie pump system to be used. Carrying out this adjustment within a closed system obviated problems of contamination and losses of ammonia from the ammonium sulphate in the medium (which occurs above pH 8.0)

With only three sets of steady state data, the conclusions which could be drawn directly were limited. But in each case an identical fermentation was carried out using pH 7.0 medium. Comparison of all six sets of data yielded valuable information on the effect of this factor on the mould cultures.

4.3.2.2 THE MORPHOLOGY OF THE MOULD.

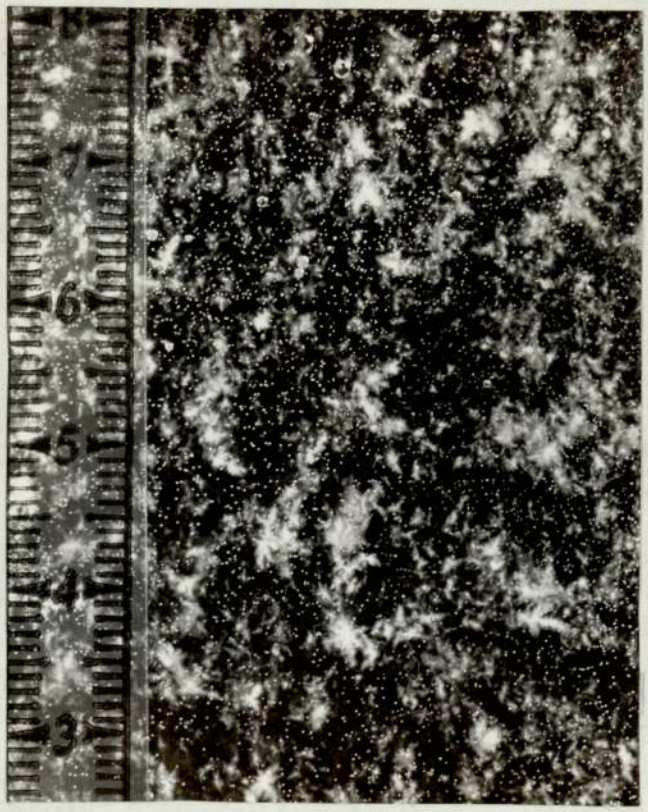
The colonies retained the same overall structure as in 30 and 35°C, pH 7.0 medium fermentations, that of a number of tangled bundles of mycelium radiating from a central point. However, within each bundle the hyphae appeared to be more numerous and less tightly packed. To the naked eye, this gave the colonies

Plate 4.9 Aspergillus niger M1 : The Morphology after 211 h.
Continuous Cultivation at 30°C on pH 11.0 Medium.
(Fermentation SR 5; D=0.0724 h^{-1}; s_R=13.2g.l^{-1} gluc. eq.)



1 cm.

Plate 4.10 Aspergillus niger M1 : The Morphology after 64 h. Continuous Cultivation at 35°C on pH 11.0 Medium. (Fermentation SR 7; D=0.0745 h^{-1}; s_R=13.2 $g.l^{-1}$ gluc. eq.)



1 cm.

a "fuller" appearance, without increasing their overall dimensions. A simple analogy would be the difference in appearance of a well nourished and an emaciated animal.

The steady state morphologies of the mould growing on 13.2 $g.l^{-1}$ (glucose equivalent), pH 11.0 starch medium at 30 and 35°C are shown in Plates 4.9 and 4.10 respectively.

4.3.2.3 CONCENTRATIONS OF MOULD IN THE FERMENTER (x_F) AND EFFLUENT STREAM (x_E).

The effects of the two media on the concentration of mould in the fermenter and effluent are compared in Fig. 4.21. The fermenter concentration (x_F) was little affected, in two fermentations it declined by 0.3 - 0.4 $g.l^{-1}$ and in the third remained constant.

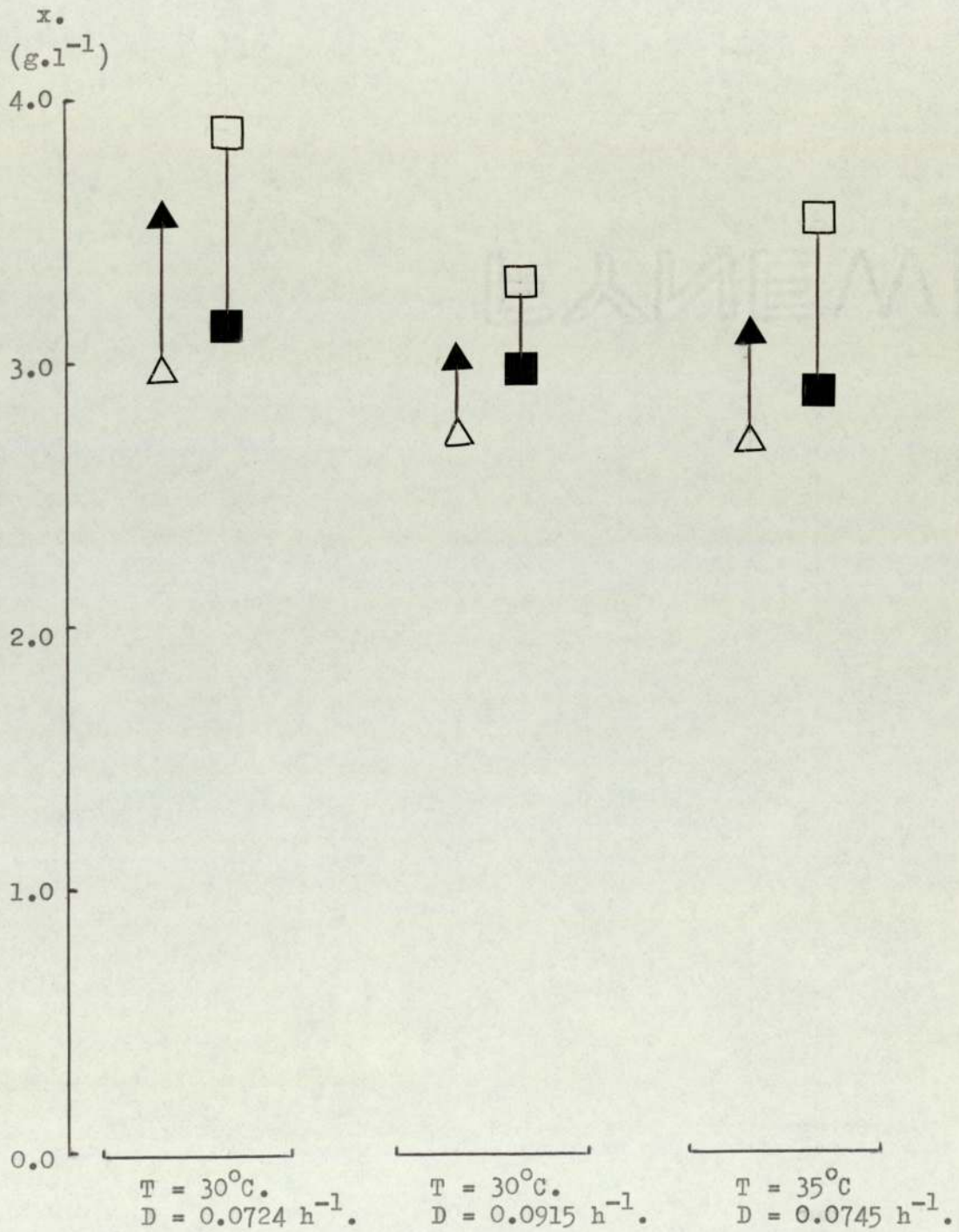
In contrast, $\bar{x}_E$ increased from being consistantly lower than $\bar{x}_F$ (usually about 0.85. $\bar{x}_F$) to being greater than $\bar{x}_F$ (about 1.2. $\bar{x}_F$) in these fermentations. This implied that a concentration mechanism was operating in the fermenter, which is difficult to visualise as a steady state situation in a well mixed culture.

The mould concentration in a tower fermenter is determined by a balance between the superficial liquid velocity (s.l.v.) and the colony fluidisation index - a factor which depends upon the overall density of the colonies and their configuration. One explanation of values of $\bar{x}_E$ exceeding those of $\bar{x}_F$ was that colonies grown on alkaline medium had a lower overall density. This could have been due to minute ($\approx$ 0.5 mm) air bubbles which tended to become adsorbed onto (and possibly entrapped within)

Fig. 4.21 Comparison of the Mould Concentrations in the Fermenter (x_F) and Effluent Stream (x_E) in pH 7.0 and 11.0 Media Fermentations.

(pH 7.0 Medium: ▲x_F; △x_E.)

(pH 11.0 Medium: ■ x_F; □ x_E.)



their structure.

Adsorption of air bubbles in this manner is the basis of "froth flotation" methods widely used in the mining industry for separation of ores. The efficiency varies inversely with particle size but concentration factors of 100 x are not unusual. A foam separation phenomenon occurred in batch cultures of Serratia marcesens in tower fermenters at Aston (306), but had not been noted previously with cultures of A. niger M1. In view of the colony sizes involved, concentration factors of 1.2x could have been the result of this effect.

The phenomenon of $\bar{x}_E > \bar{x}_F$ could also have been due to the existence of a concentration gradient in the fermenter. Additional sampling was therefore carried out from a second port in the upper quarter of the fermenter. Although there were differences in the values of x_F between the upper and lower sample ports ($\pm$ 0.1 $g.l^{-1}$ in samples 3.0 - 3.5 $g.l^{-1}$), these appeared to be random and were probably due to sampling errors. There was no evidence to support the hypothesis that a concentration gradient existed in the fermenter under these conditions.

4.3.2.4 PRODUCTIVITY AND SPECIFIC GROWTH RATE.

A direct consequence of the change in $\bar{x}_E$ was an increase in the mould productivity (by approximately 1.35x) in these fermentations (Fig. 4.22). Use of alkaline medium (which caused the pH of the fermenter contents to rise to 6.0 - 7.0) resulted in the highest steady state productivity on starch medium being recorded, 0.317 $g.l^{-1}.h^{-1}$ with D = 0.0915 h^{-1}.

Fig. 4.22 Comparison of the Mould Productivity in pH 7.0 and 11.0 Media Fermentations. (● 7.0; ○ 11.0.)

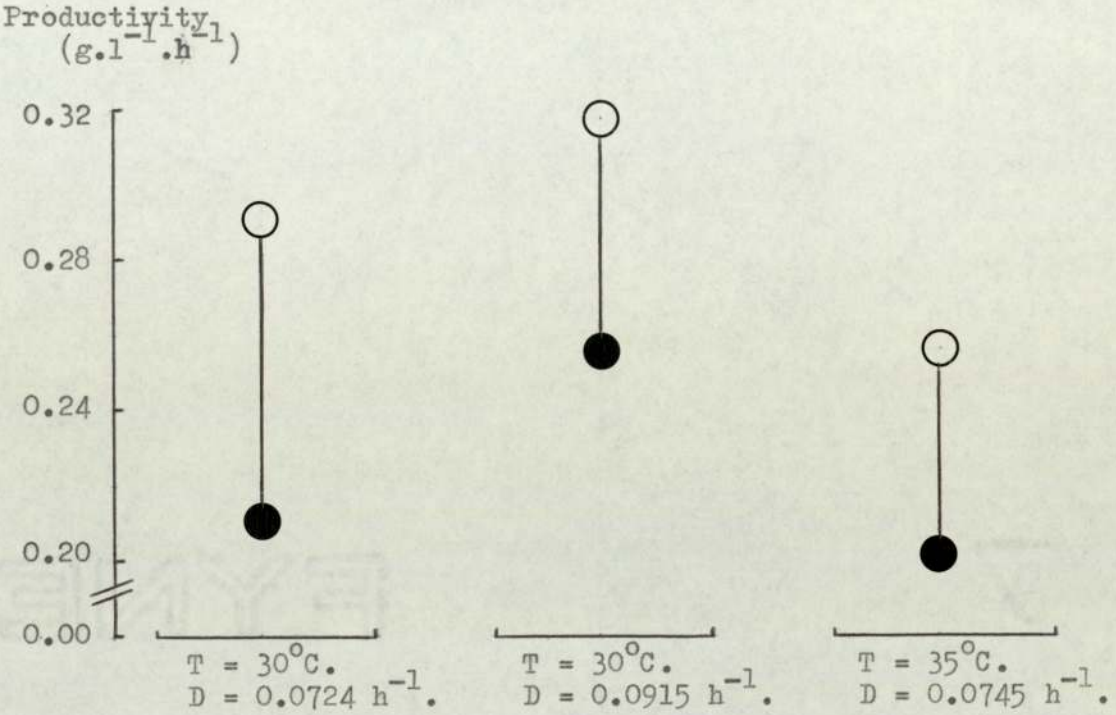
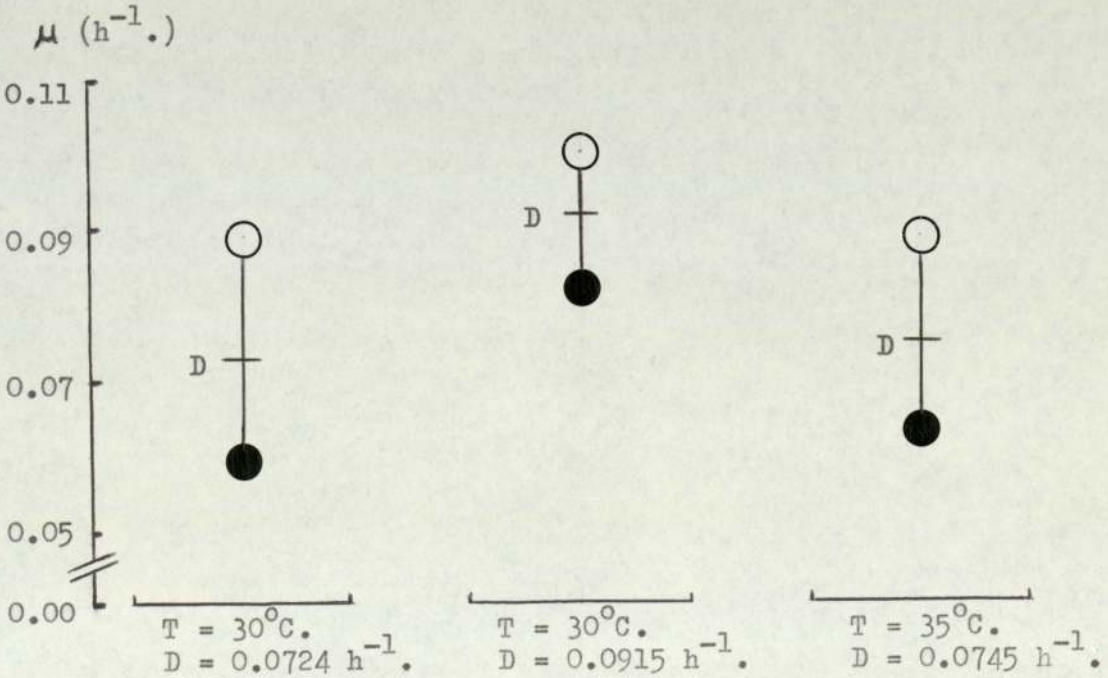


Fig. 4.23 Comparison of the Mould Specific Growth Rate in pH 7.0 and 11.0 Media Fermentations. (● 7.0; ○ 11.0.)



With the increase in productivity as a result of $\bar{x}_E > \bar{x}_F$, the specific growth rate rose. This was expected, as the existence of pH effects and pH optima for the growth of most microorganisms are well known. But, surprisingly, steady states occurred in which μ exceeded D by a considerable margin, usually 10 - 25% (Fig. 4.23).

In the tower fermenter, where x_E is determined by physical forces, there can be no theoretical objections to $\mu > D$ providing nutrients are in excess. Such a situation cannot exist in the majority of fermentation systems, such as the continuous stirred tank fermenter, because vessel design and intense mixing negate the fluidisation and sedimentation effects so that $\bar{x}_F \approx \bar{x}_E$ and $\mu \approx D$.

4.3.2.5 SUBSTRATE UTILIZATION - RELATED FACTORS.

As expected, the rate of conversion of starch to reducing sugars was little affected by the change in medium and, therefore, fermenter pH (Fig. 4.24).

In the 30°C fermentations both total and specific rates of substrate utilization also showed little change (Fig. 4.24), a consequence of the improved yield coefficient (Fig. 4.25). But in the 35°C fermentation $Y_{x/s}$ remained constant and both substrate utilization rates increased considerably.

The differences in response of the fermentations in these characteristics are in contrast to their consistancy with respect to all the other parameters. An explanation of such a difference could lie in the critical effect of mould morphology on the oxygen

Fig. 4.24 Comparison of the Rates of Substrate Hydrolysis and Utilization in pH 7.0 and 11.0 Media Fermentations.

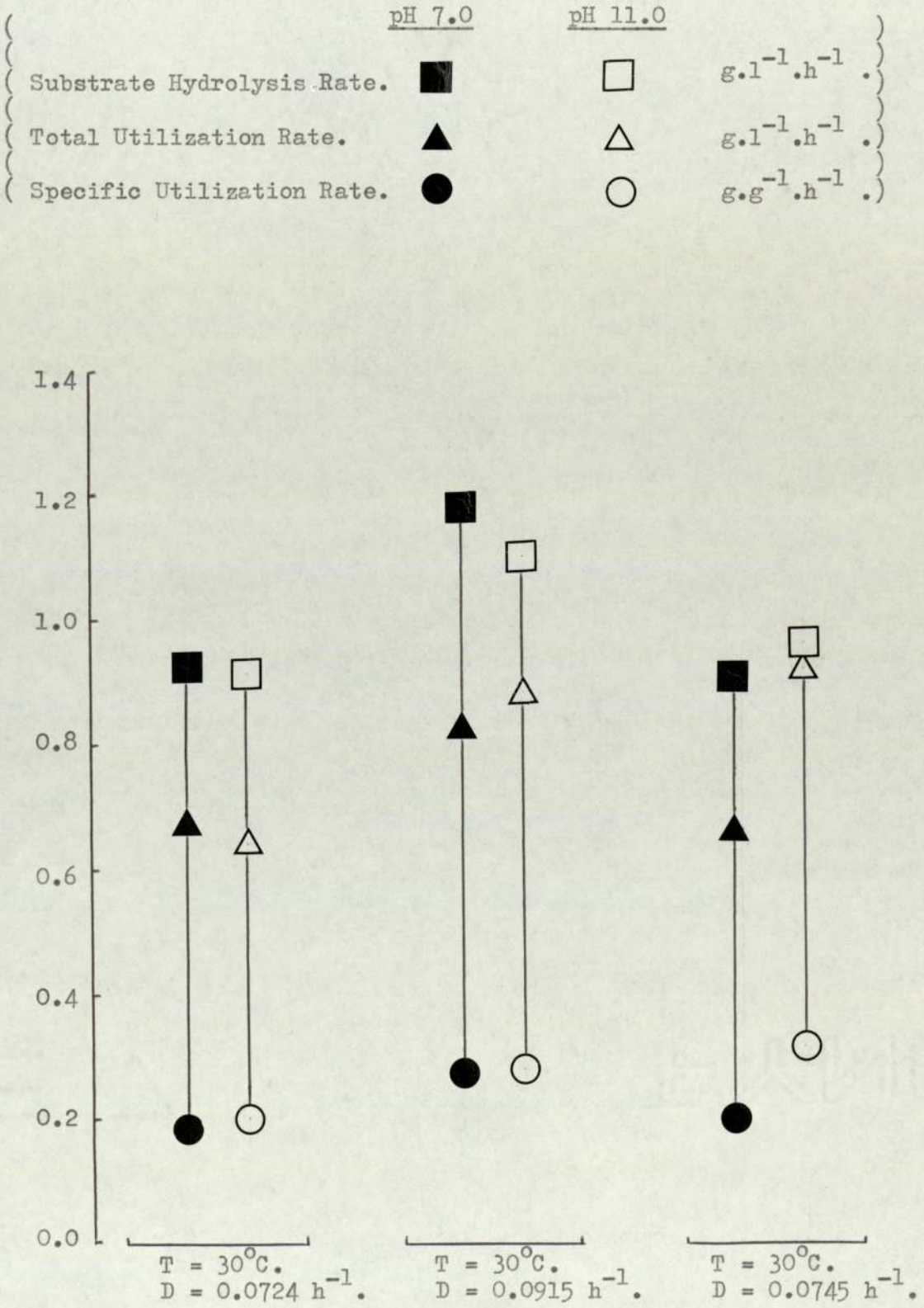


Fig. 4.25 Comparison of the Organism Yield Coefficients in pH 7.0 and 11.0 Media Fermentations. (● 7.0; ○ 11.0.)

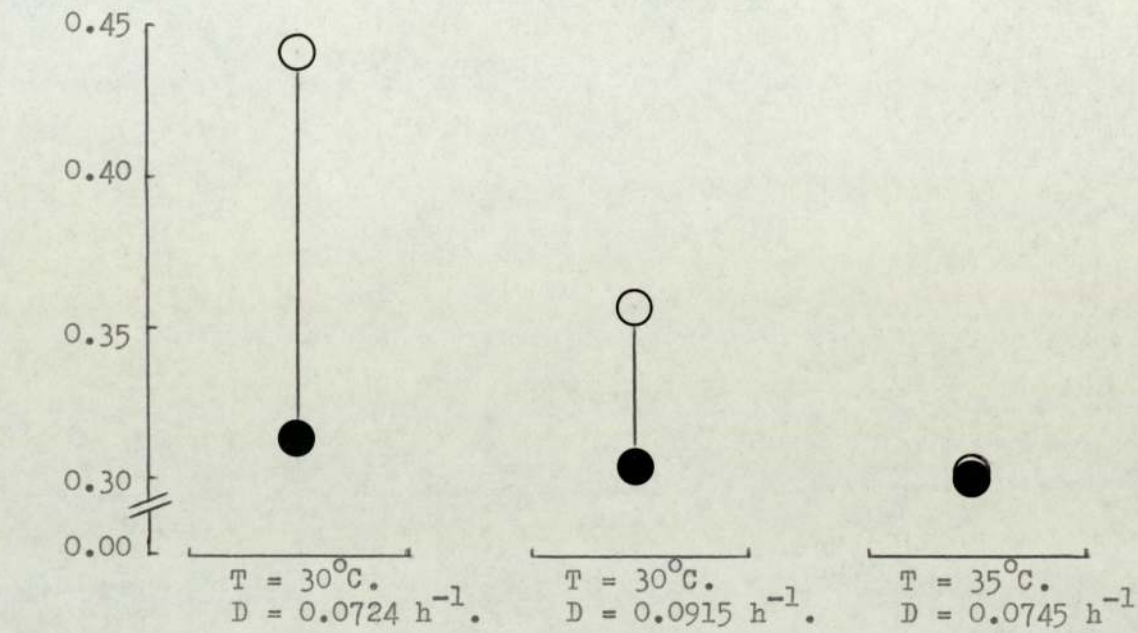
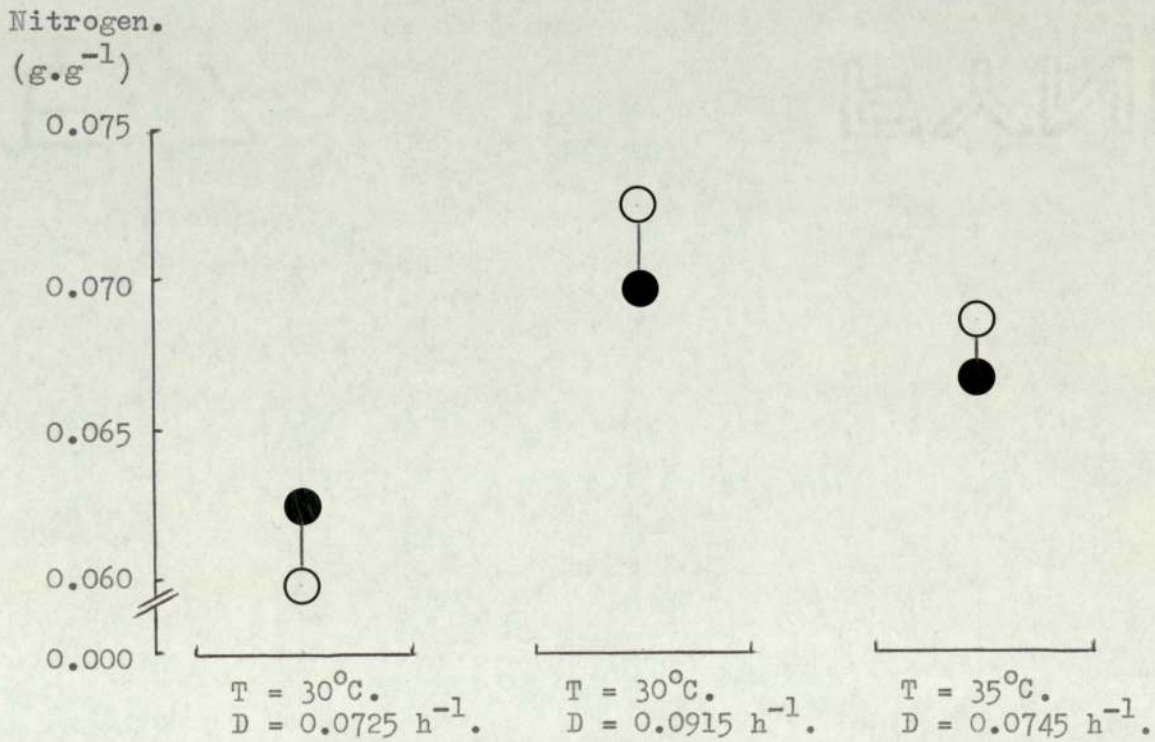


Fig. 4.26 Comparison of the Mould Nitrogen Contents in pH 7.0 and 11.0 Media Fermentations. (● 7.0; ○ 11.0.)



transfer rate of the system.

4.3.2.6 NITROGEN CONTENT OF THE MOULD.

Had the nitrogen content of the mould been determined solely by μ , then the values in each of these fermentations would have risen in proportion to their respective increases in this parameter. However this was not the case, the nitrogen content increased in only two of the fermentations and actually decreased in the other ($D = 0.0725\ h^{-1}$ at $30^{o}C$) as shown in Fig. 4.26.

This response confirms the results of the temperature experiments (section 4.3.1.6) which also indicated that the change in the macromolecular composition of the mould was not due to μ alone.

4.3.2.7 pH OF THE CULTURE.

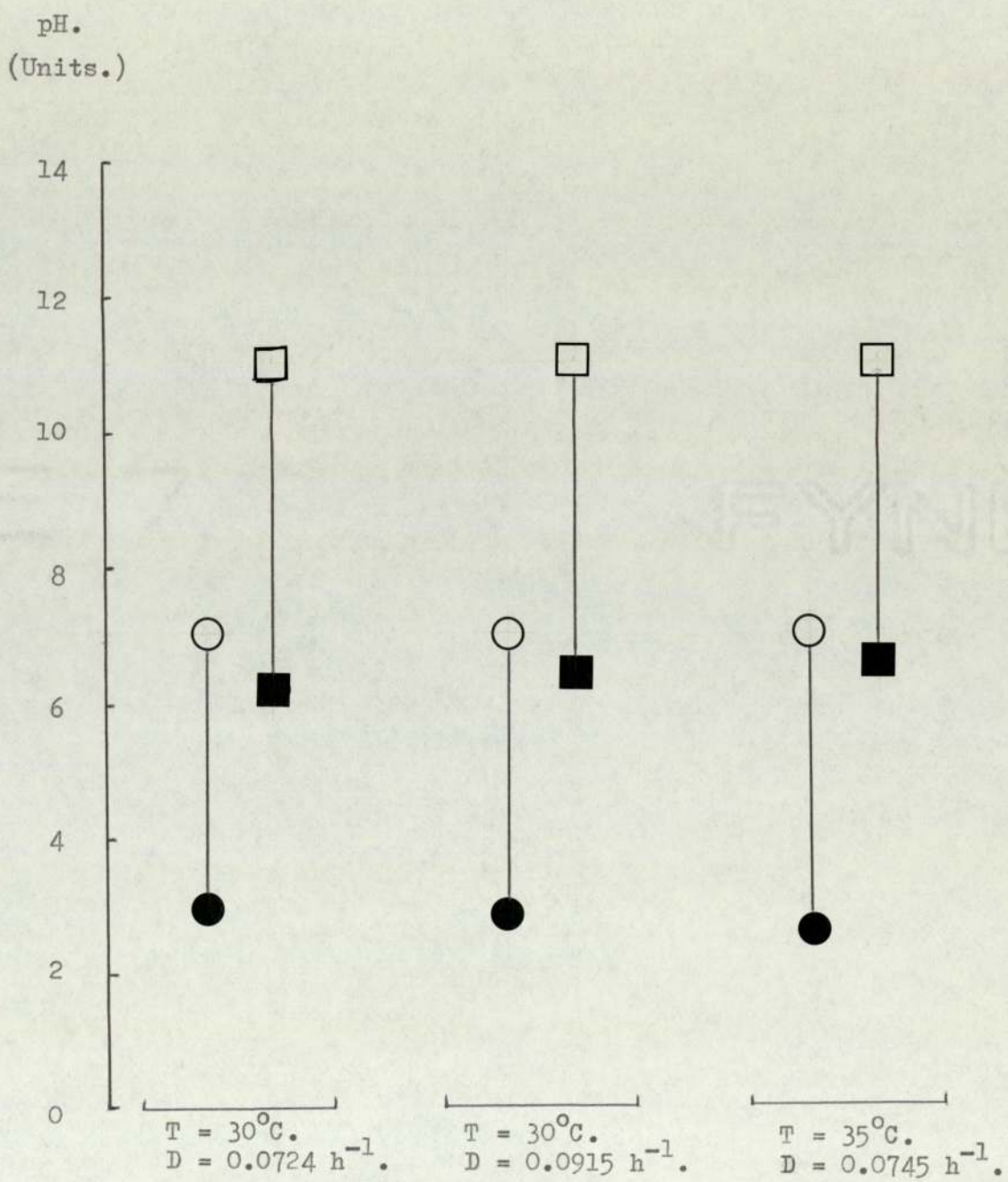
In all three fermentations pH 11.0 medium was neutralized. Stable situations resulted in which the pH of the cultures was between 6.0 and 7.0 instead of 2.0 - 3.0 in the case of pH 7.0 medium (Fig. 4.27). In both cases the pH of the medium was being reduced by 4.0 - 5.0 units, which suggested that acid production probably continued at a similar level.

As the pH of the culture remained below 8.0 there were no problems of loss of the nitrogen nutrient source as free ammonia. However fermentations in this pH range were particularly susceptible to bacterial contamination, especially at dilution rates of less than $0.08\ h^{-1}$ (an s.l.v. of 2.83×10^{-3} cm. sec^{-1}). Above this dilution rate,"wash out" of non-flocculent contaminants took place as a result of froth flotation and fluidisation.

Fig. 4.27 Comparison of the Culture pH values in pH 7.0 and 11.0 Media Fermentations.

(pH 7.0 Medium : ○ Medium ; ● Culture.)

(pH 11.0 Medium: □ Medium ; ■ Culture.)



4.4 POTATO AND PEEL WASTE FERMENTATIONS.

4.4.1 INTRODUCTION.

In total, nearly 3500 hours fermentation were carried out using potato-based media. These proved particularly troublesome as a result of the comparatively small scale of the equipment. Experimentally, the problems were almost entirely in making up batches of medium of constant composition, which resulted in difficulties in maintaining steady states for long periods. Under substrate limited conditions especially, "steady state" values tended to fluctuate far more than in starch based, semi-defined media fermentations.

The other major problem with this series of fermentations was in the interpretation of the experimental results. The "total filterable solids" content of the fermenter consisted of both mould and non-fermentable solids (NFS) such as particles of soil and cellulosic debris (from the potato skin, etc.). The latter materials "diluted" the true organism content of the fermenter and to a certain extent contributed to the colony morphology. This relationship is analagous to that of non-viable cells or hyphae being present in, but not contributing to the growth of a culture.

In the treatment of the results, therefore, attempts were made to express parameters in terms relative to both the organism fraction and to the whole fermenter contents. This necessitated two assumptions regarding the relationship between the mould and NFS concentrations:

(A) That, at steady state, the concentration of NFS in the

effluent receiver was equal to that in the medium i.e. NFS did not accumulate in the fermenter.

(B) That the ratio of mould : NFS in the fermenter was the same as that in the effluent i.e. neither was selectively "washed out" from, or retained in the fermenter.

These assumptions were justified by the experimental observations. As the mould grew, NFS became entrapped and immobilized in the colony structure, while material harvested from the fermenter and the product receiver was indistinguishable to the naked eye. Steady states were maintained for periods of 200 - 300 h. during which changes due to a build-up of NFS would have been noted.

In this section, results calculated using these assumptions are presented in both forms, relating to the fermenter contents as a whole and to the mould fraction alone.

4.4.2 TOTAL SOLIDS CONCENTRATION IN THE FERMENTER AND EFFLUENT STREAM.

4.4.2.1 UNDER CONDITIONS OF SUBSTRATE EXCESS (Figs. 4.28 and 4.29).

At low dilution rates ($< 0.04\ h^{-1}$) the total solids concentrations in the fermenter were within the same range as those obtained with semi-synthetic media under the same conditions. This was expected as with similar colony morphologies and therefore packing densities and fluidisation indices, the tower could not support higher concentrations of mould/NFS. So increases in productivity could only be shown as changes in the effluent stream concentrations, which in fact rose considerably (by approx. 50%).

Fig. 4.28 Comparison of the Filterable Solids and Assumed Mould Concentrations in the Fermenter with Excess Substrate.

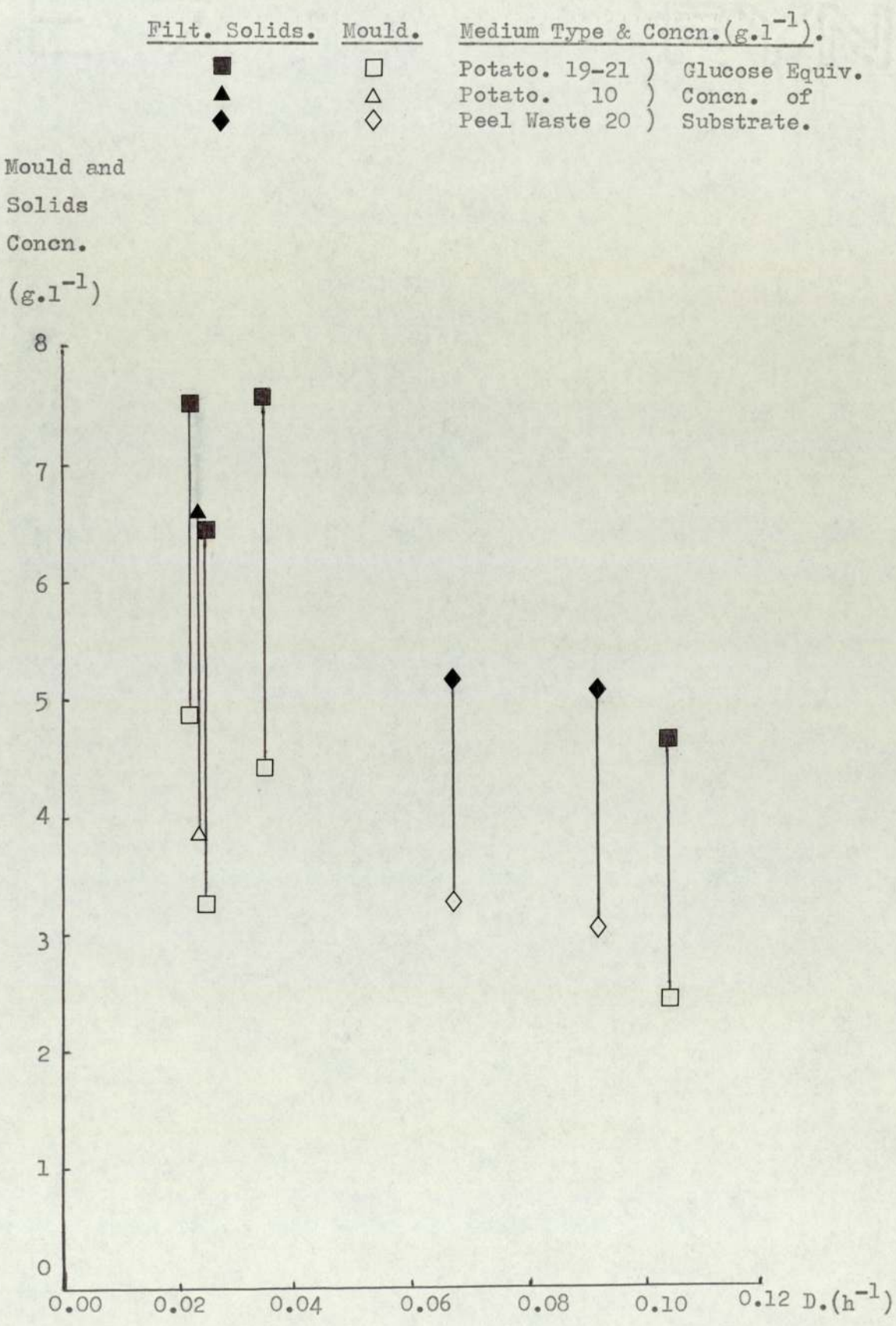
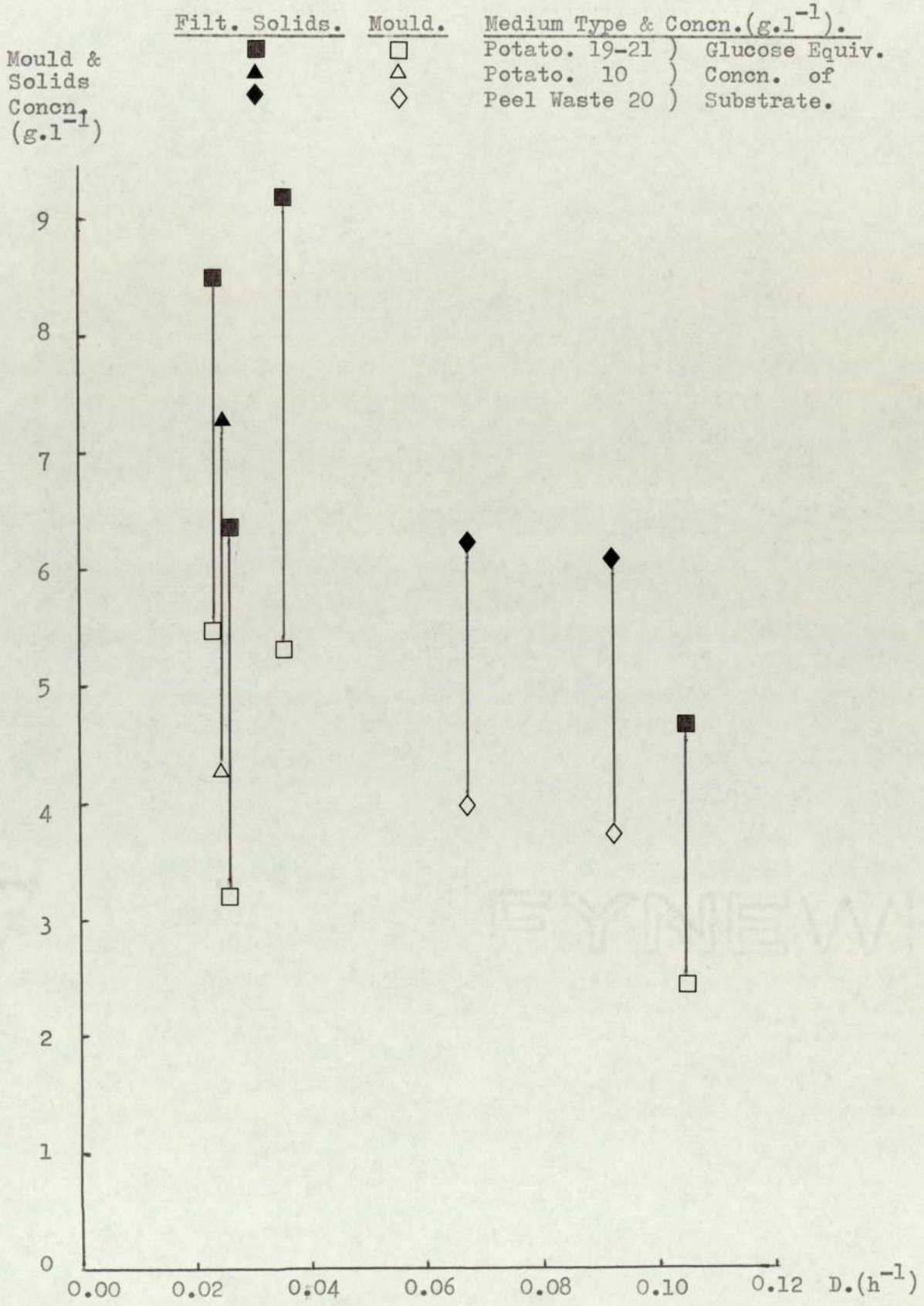


Fig. 4.29 Comparison of the Filterable Solids and Assumed Mould Concentrations in the Effluent Stream with Excess Substrate.



At higher dilution rates, although the total solids concentrations in the fermenter and effluent stream were much higher than with semi-synthetic media, the mould concentrations were similar.

It was noted that $\bar{x}_E > \bar{x}_F$ in five of the seven fermentations in this series, only two of which were with alkaline media. In the other two, $\bar{x}_F \approx \bar{x}_E$. Possible explanations for this phenomenon, which otherwise had only occurred with alkaline semi-synthetic media, were outlined in Section 4.3.2.3.

4.4.2.2 UNDER SUBSTRATE-LIMITED CONDITIONS.(Figs. 4.30 and 4.31).

The concentrations of filterable solids in the fermenter and effluent streams showed the same response to substrate limitation as in the case of semi-synthetic media. The concentration in the fermenter decreased and varied with the NFS and fermentable substrate contents of the medium. The calculated organism concentration was usually within the range 2.0 - 3.0 $g.l^{-1}$, comparing well with the two values obtained with 3.3 $g.l^{-1}$ semi-synthetic medium (2.83 and 2.077 $g.l^{-1}$).

The concentration in the effluent stream was also governed by both the NFS and fermentable substrate contents of the medium and at steady state could in theory be predicted by a modification of Eqn. 4.3 :-

$$(\text{Filt. Solids})_E = \frac{Y_g (D \cdot s_R - D.\bar{s} - m.\bar{x}_F)}{D} + \text{NFS Medium} \quad \ldots\ldots \text{Eqn. 4.8.}$$

Fig. 4.30 Comparison of the Filterable Solids and Assumed Mould Concentrations in Substrate Limited Fermenters.

Filt. Solids.	Mould.	Medium Type & Concn.($g.l^{-1}$)	
●	○	Potato. 5.27	) Glucose Equiv.
▲	△	Potato. 4.0-4.4	) Concn. of
▼	▽	Potato. 3.0-3.3	) Substrate.
■	□	Potato. 2.0	)

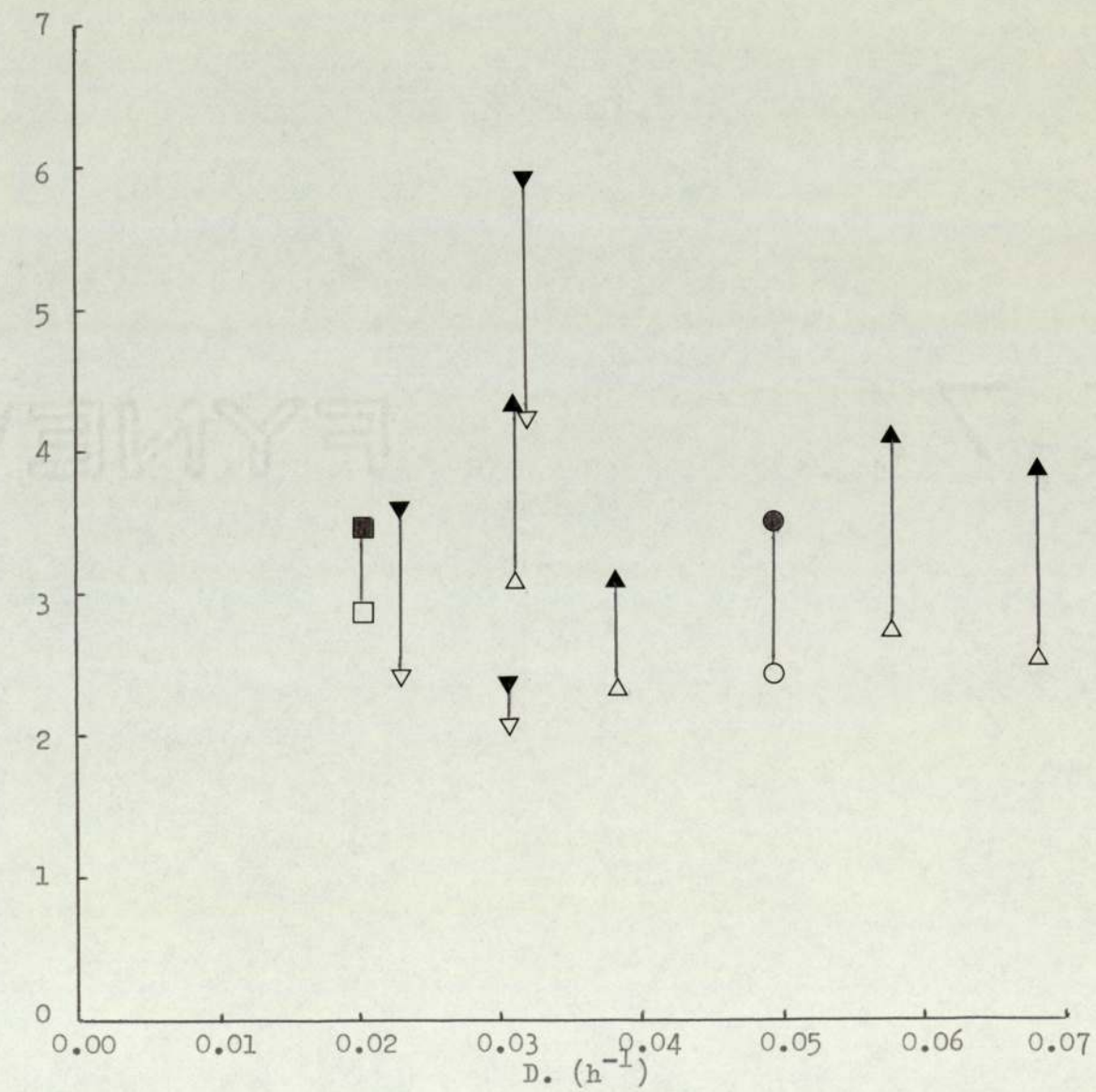
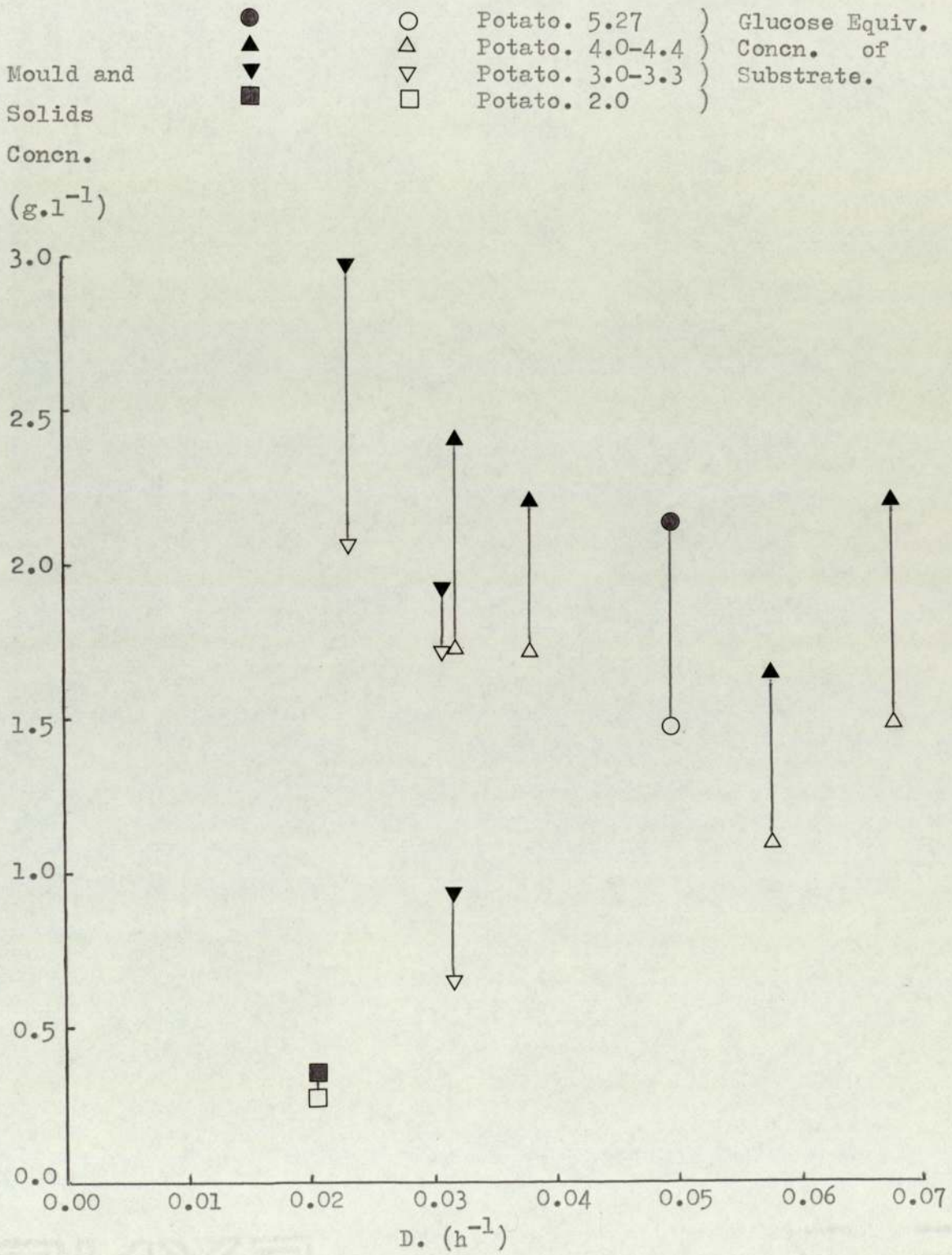


Fig. 4.31 Comparison of the Filterable Solids and Assumed Mould Concentrations in the Effluent Stream from Substrate Limited Fermenters.



Also, theoretically, as $D \longrightarrow \mu_{max} \longrightarrow \infty h^{-1}$, the filterable solids content of the effluent stream would approach NFS_{medium}.

4.4.2.3 CONCLUSIONS.

These parameters were determined by a complex balance between the non-fermentable solids present in the medium supply and the mould which grew in the fermenter. The major points could be briefly summarized:-

(A) The Tower Fermenter was able to physically support a certain concentration of solids, which depended upon their morphology and sedimentation characteristics.

(B) Change in the mould : NFS ratio altered the morphology and therefore the total solids concentration in the fermenter.

(C) The mould : NFS ratio in the fermenter contents and product depended upon the relationship between the rates of NFS and substrate supply to the fermenter and growth of the mould.

(D) At steady state the concentration of NFS in the fermenter was not necessarily the same as that in the medium, as the fermenter could exert a concentrating effect.

4.4.3 PRODUCTIVITY AND SPECIFIC GROWTH RATE OF THE FERMENTER CONTENTS.

4.4.3.1 PRODUCTIVITY.

In these cases, again, a distinction had to be made between

total values (direct measurements) and those of the mould fraction alone (based on steady state assumptions). These could in theory be predicted from Eqn. 4.8, by multiplication of all three parts of the equation by D.

When substrate was in excess the total productivity was considerably higher than with semi-defined starch media (Fig.4.32). In contrast, the calculated value for the mould fraction alone was significantly lower (by approx. 30%).

With peel waste medium, the increase in productivity was of the same order of magnitude as in earlier experiments with alkaline media (section 4.3.2). The maximum steady state value noted was 0.563 $g.l^{-1}.h^{-1}$, of which the mould content was calculated to be 0.3403 $g.l^{-1}.h^{-1}$.

Under substrate limited conditions the total productivity increased linearly with dilution rate (Fig. 4.33). However, the mould fraction showed the same form of response curve as in semi-synthetic media fermentations (compared with Fig. 4.4), so that considerable changes in the composition of the product occurred. At higher dilution rates (c. 0.07 h^{-1}) mould constituted a smaller proportion of the total, which was illustrated by the decline in nitrogen content at higher specific growth rates (section 4.4.5).

4.4.3.2 SPECIFIC GROWTH RATE.

A consequence of the assumption that the mould : NFS ratios in fermenter and effluent stream were the same, was that the specific growth rates calculated for the total solids content

Fig. 4.32 The Productivity of Potato Medium Fermentations with Excess Substrate.

Filt. Solids.	Mould.	Medium Type & Concn.($g.l^{-1}$).	
■	□	Potato. 19-21	) Glucose Equiv.
▲	△	Potato. 10	) Concn. of
◆	◇	Peel Waste 20	) Substrate.

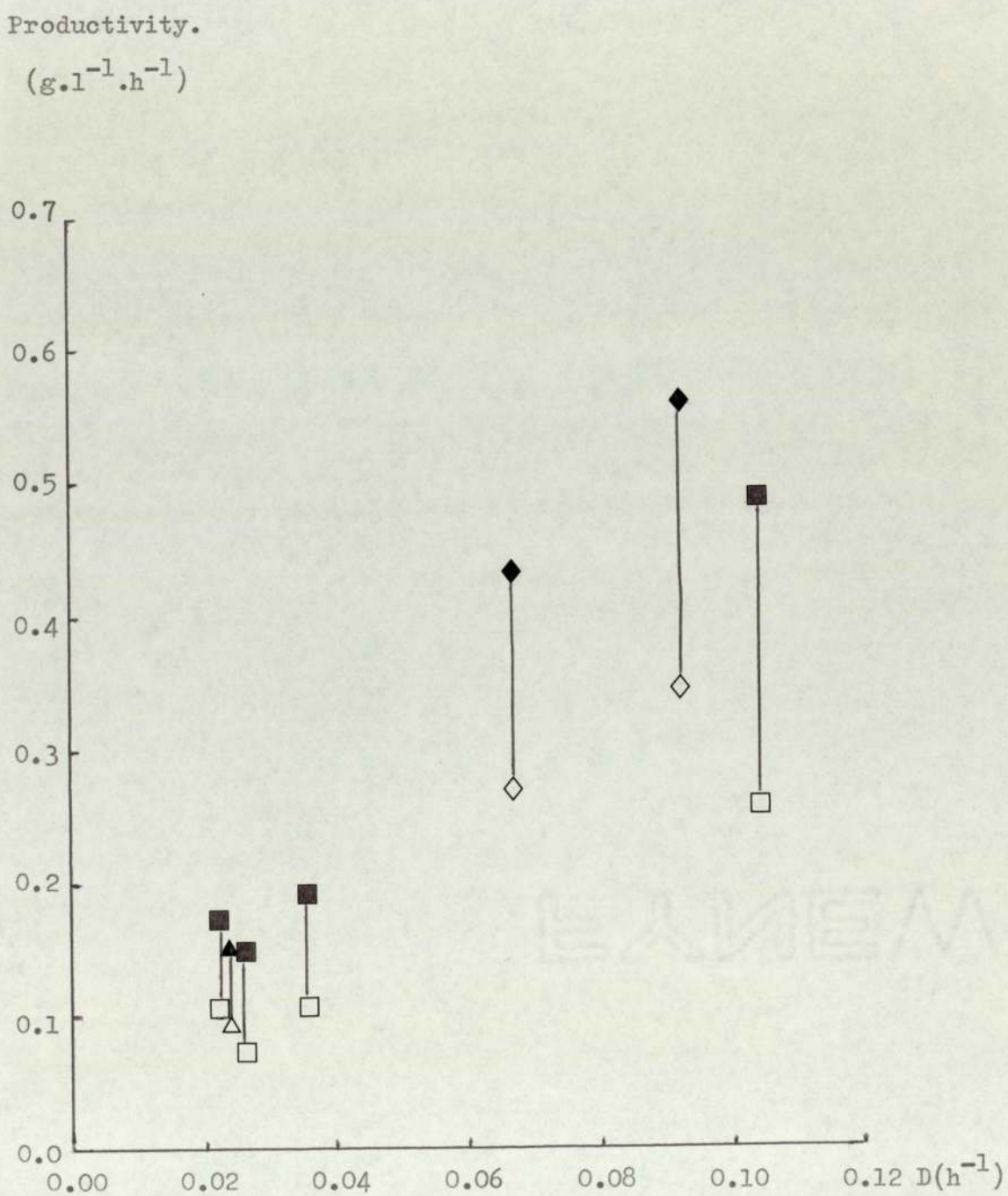
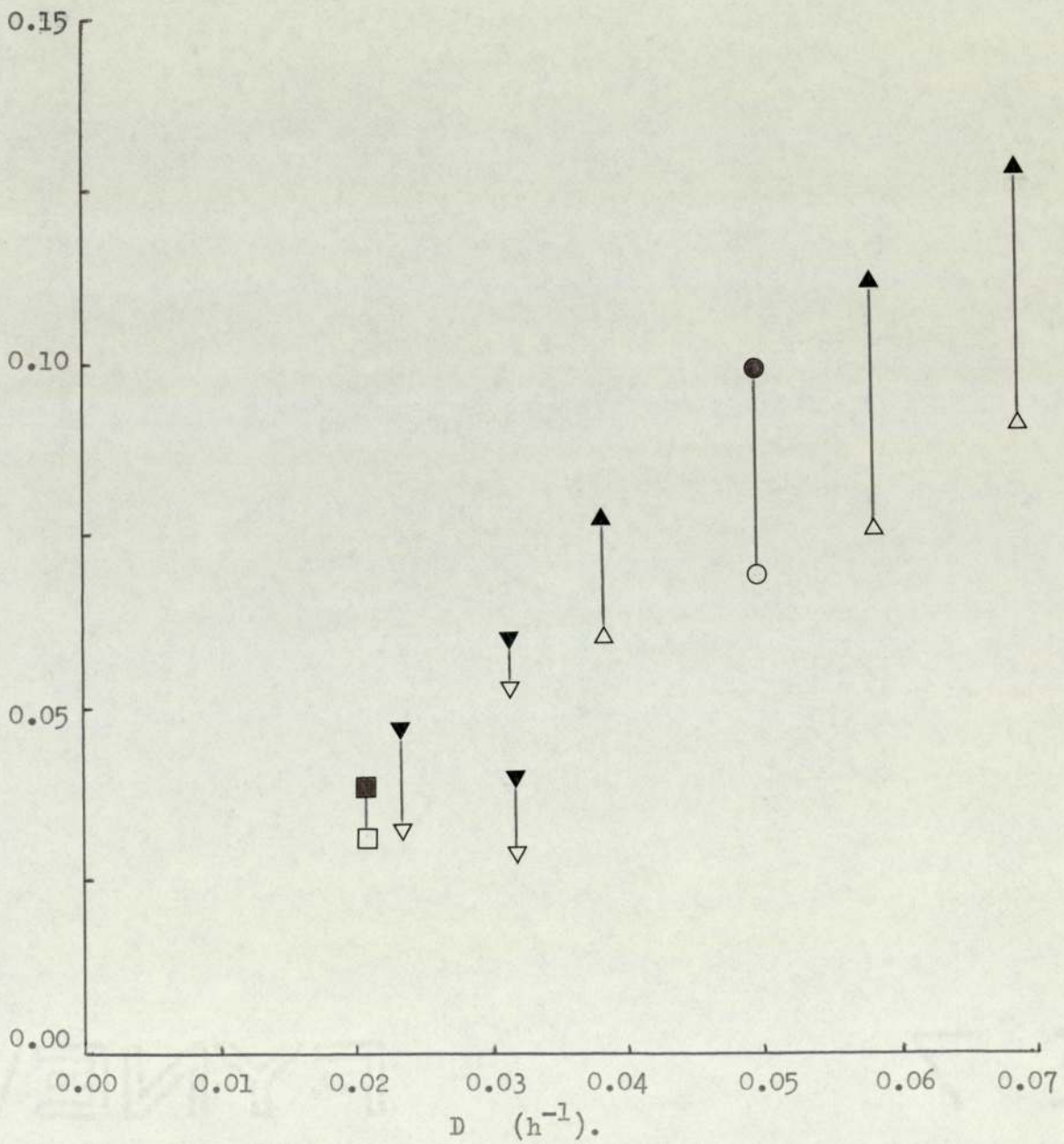


Fig. 4.33 The Productivity of Substrate Limited Potato Media Fermentations.

Filt. Solids.	Mould.	Medium Type & Concn.($g.l^{-1}$)	
●	○	Potato. 5.27	Glucose Equiv.
▲	△	Potato. 4.0-4.4	Concn. of
▼	▽	Potato. 3.0-3.3	Substrate.
■	□	Potato. 2.0	



and mould fraction were equal.

As in the case of semi-defined media, μ was almost always considerably less than D (Fig. 4.34). Under substrate limited conditions the results confirmed that the primary determinant of μ was the rate of substrate supply to the culture.

Values of μ only exceeded those of D under exceptional conditions, as in previous fermentations (section 4.3.2.4) these were with alkaline medium.

4.4.4 YIELD COEFFICIENTS.

In terms of the product as a whole, values of this parameter had little meaning as they could range from $0 \rightarrow \infty$, depending on the mould : NFS ratio of the contents of the effluent stream.

However when expressed in terms of the mould fraction alone, the yield coefficients showed some relationship to the degree of substrate limitation of the culture and therefore, to μ (Fig. 4.35). In general, low values of s_R resulted in increases in $Y_{x/s}$, often to the levels reported for moulds in other continuous culture systems.

The results of these fermentations suggested that, except when the substrate supply rate was very low, oxygen limitation was a significant factor. In most cases, incomplete oxidation of the substrate resulted in organic acid production, which represented a diminution of $Y_{x/s}$.

This theory was supported by the steady state conditions in the two cases in which $Y_{x/s}$ was c. 0.55 $g.g^{-1}$. The dissolved oxygen level in these cultures ranged from 20 - 40% of the

Fig. 4.34 The Variation of Specific Growth Rate with Dilution Rate in Potato Media Fermentations.

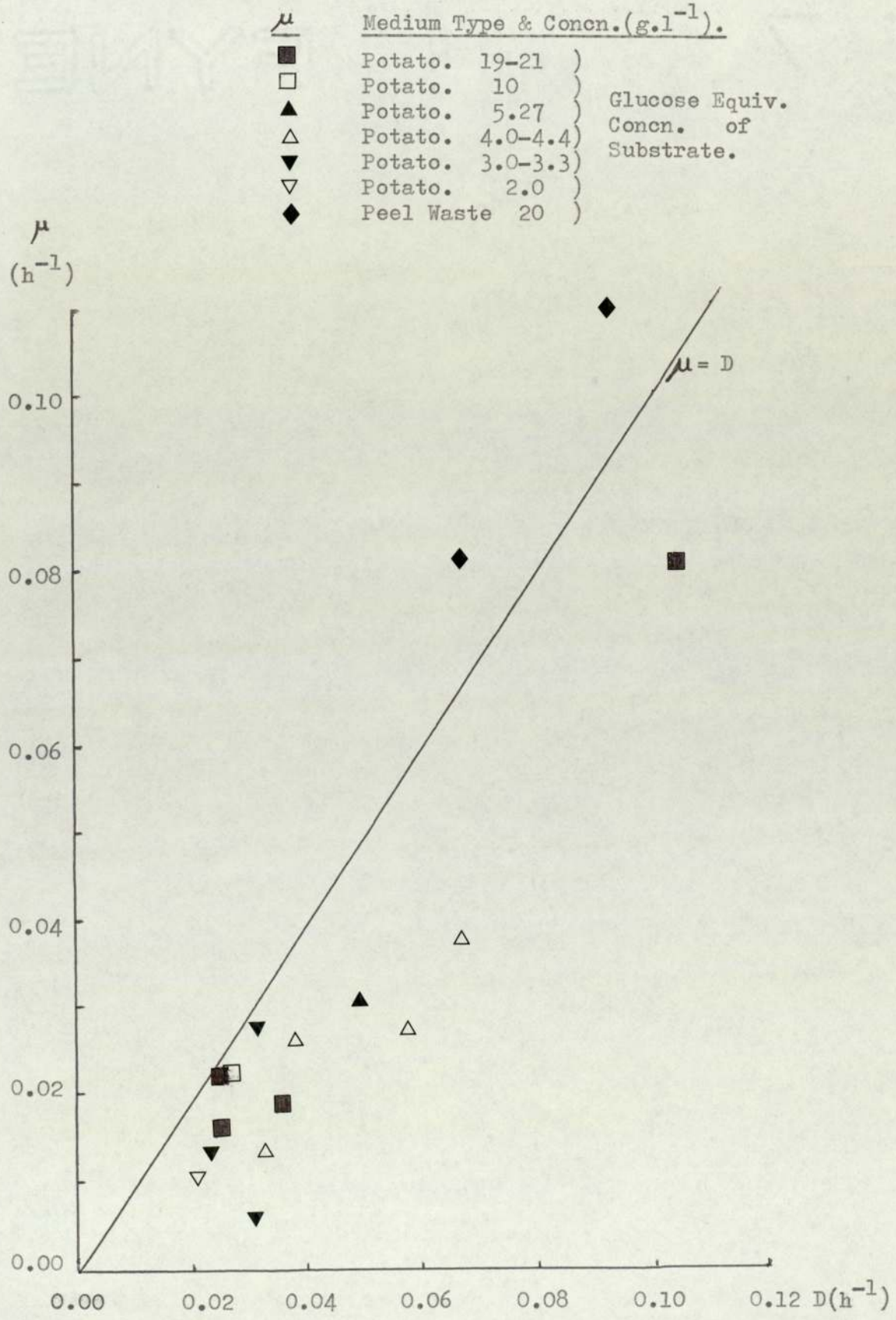
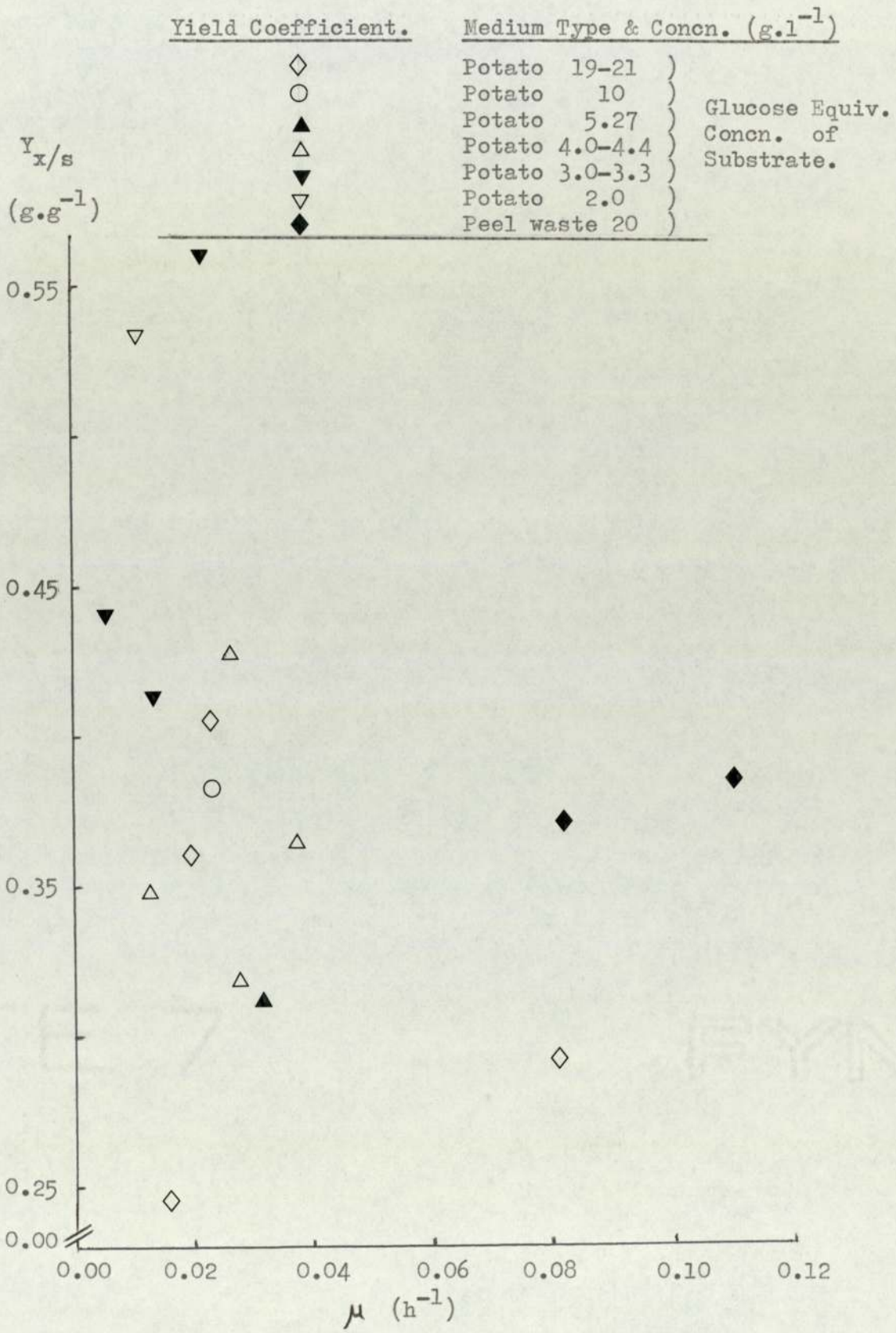


Fig. 4.35 The Yield Coefficient of the Mould Fraction in Potato Media Fermentations.



saturation value (for the medium concerned at 30°C) and the pH readings varied between 3.5 and 4.5. Similar increases in these parameters were also noted when the substrate supply to a limited culture was interrupted. Response times of c. 2 min. from 20 → 80% dissolved oxygen saturation were not unusual.

4.4.5 THE NITROGEN CONTENT OF THE PRODUCT. (Fig. 4.36).

The nitrogen content of the product was determined by the balance between 2 factors:-

(A) The relative nitrogen contents of the NFS (always less than 0.015 $g.g^{-1}$) and the mould (usually 0.045+ $g.g^{-1}$, but increasing with μ - section 4.2.5).

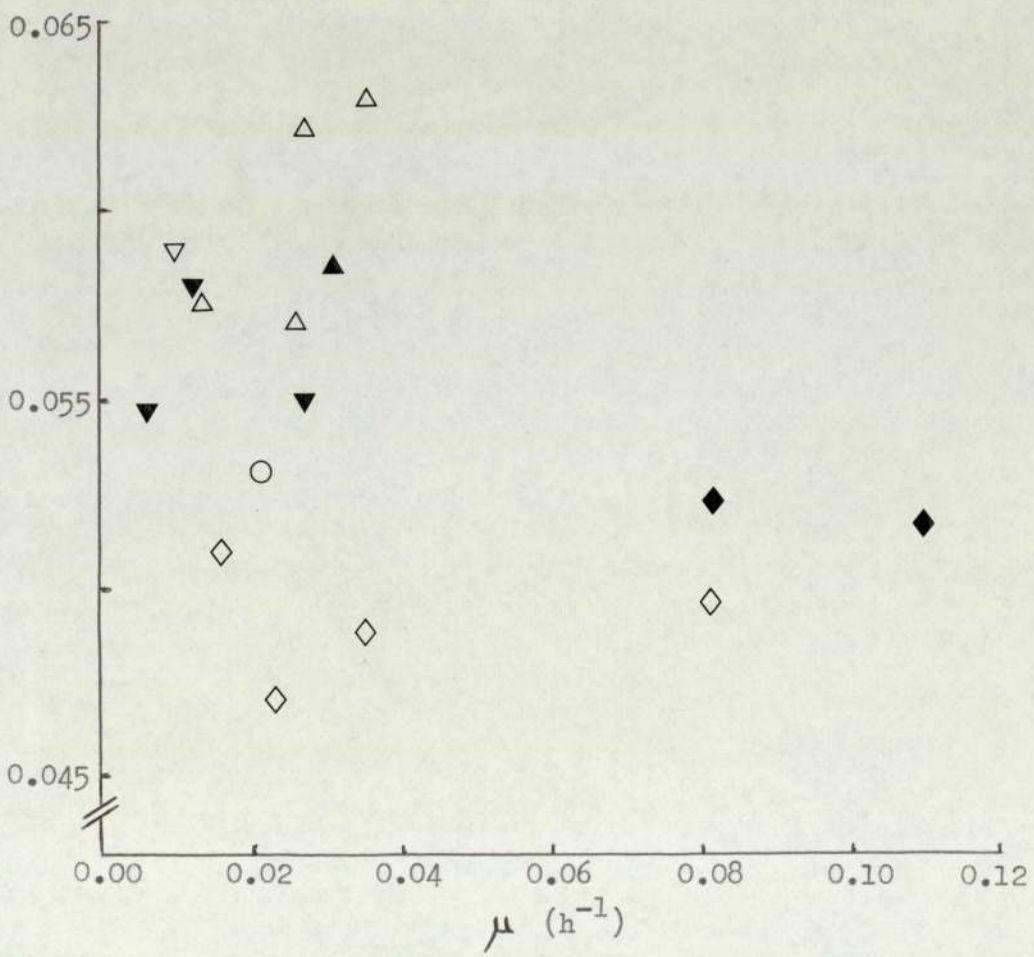
(B) Changes in the proportions of mould and NFS in the product (section 4.4.3), which resulted in a tendency for its nitrogen content to decline at higher dilution rates.

As expected, this content was diminished when compared to that of the product from semi-synthetic medium fermentations (mould alone). Values were usually in the range 0.05 - 0.06 $g.g^{-1}$, corresponding to a crude protein content of 31.25 - 37.5% (on a N x 6.25 basis).

In the case of the bland, fibrous product grown on alkaline peel the crude protein content was c. 33%, which represented a considerable "upgrading" of a totally discarded waste.

Fig. 4.36 The Variation in Nitrogen Content of the Product from Potato Medium Fermentations.

Nitrogen Content.	Medium Type & Concn.($g.l^{-1}$)	
◇	Potato 19-21	Glucose Equiv. Concn. of Substrate.
○	Potato 10	
▲	Potato 5.27	
△	Potato 4.0-4.4	
▼	Potato 3.0-3.3	
▽	Potato 2.0	
◆	Peel Waste 20	



4.4.6 THE EFFECTS OF EXTREME SUBSTRATE LIMITATION.

These effects were investigated in three fermentations altogether, but were best monitored in a potato medium fermentation which had previously been run for over 400 h. under substrate limited conditions ($s_R \approx 3.3$ g.l^{-1}).

The responses were most easily summarized by means of a flow diagram (Page 175), commencing when s_R was reduced to 1.985 g.l^{-1} at a time designated T = 0 h. The dilution rate throughout was in the range 0.0197 - 0.0210 h^{-1}.

Sporulation occurred, in this case between 66 and 74 h. later, being preceded in each fermentation by the same series of colour changes, some of which were obviously due to the formation of black spores.

The maximum duration of sporulation could not be ascertained, as between 52 and 58 h. after its onset, germination (as a result of an increased level of s_R) was noted.

4.5 GENERAL DISCUSSION.

4.5.1 THE MOULD CONTENT OF THE FERMENTER.

This was the most crucial factor controlling the fermentations, depending totally upon the interaction between fluidisation and sedimentation forces and the colonial structure of the mould. Although measurement of x_F is easy, quantification of the other factors involved, especially the morphology, is particularly difficult. The chief problems are that:-

(A) A fermentation broth is a 3-phase situation, containing mould, culture medium and air bubbles.

FLOW DIAGRAM - Conditions during the Sporulation Phase.

TIME (h.)	Colour	Morphology
0	Cream	Colonies
	Yellow	becoming smaller,
26	Pink	more densely
	Red	packed and
		"inhibited" in
46	Brown	character.
	Black	
74	Presence of Conidiophores and spores in the fermenter first noticed.	
		Mainly spores
82		present in
		effluent stream,
		very little
94		mycelium.
126	Germination of spores noted. Mould became more "mycelial"in character and lighter in colour.	

s_R = 1.985 $g.l^{-1}$; pH = 3.45
x_F = 2.8 $g.l^{-1}$; D.O. = 28%
Mould nitrogen content 5.736 $g.g^{-1}$.

PLATE 4.11

s_R = 1.97 $g.l^{-1}$; pH = 5.2
x_F = 2.57 $g.l^{-1}$; D.O. = 66%

x_F = 2.45 $g.l^{-1}$; pH = 5.5
D.O. = 76% Mould Nitrogen Content 5.942 $g.g^{-1}$.

PLATE 4.12

s_R = 2.41 $g.l^{-1}$; x_F = 2.3 $g.l^{-1}$.

x_F = 2.77 $g.l^{-1}$; pH = 4.6
D.O. = 52% saturation.
Mould Nitrogen Content 6.288 $g.g^{-1}$.

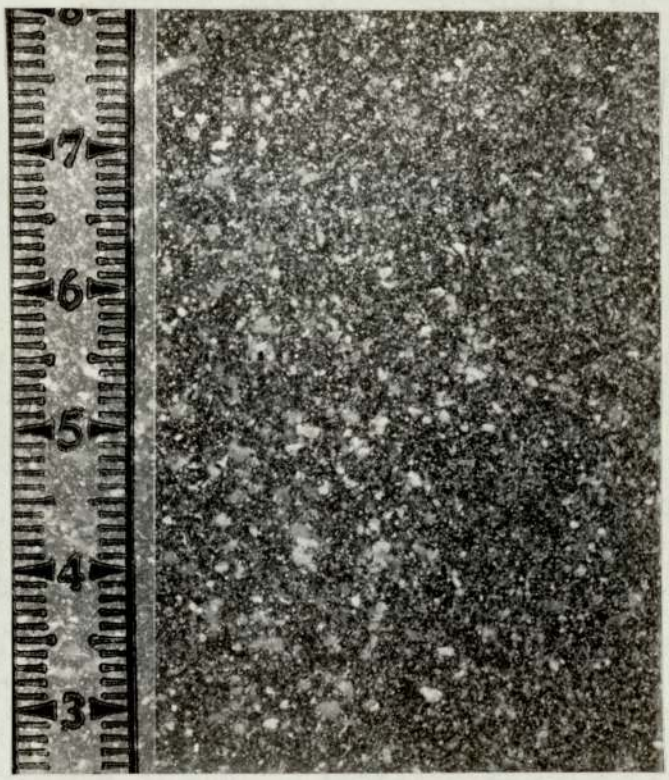
Plate 4.11 Aspergillus niger M1 : Mould Morphology as a Result of Acute Substrate Limitation, 58 h. prior to Sporulation.

(Fermentation PN 4A; $D=0.0202h^{-1}$; $s_R=1.985\ g.l^{-1}$ gluc. eq.)

1 cm.

Plate 4.12 Aspergillus niger M1 : Macro-Morphology of the Sporulating Mould.

(Fermentation PN 4A; $D=0.0205h^{-1}$; $s_R=1.97\ g.l^{-1}$ gluc.eq.)



1 cm.

(B) The characteristics of the colonies are not uniform throughout the culture - within a given sample there are variations in size, form and packing density.

(C) Being composed of hyphae, the colonies are not rigid structures with clearly defined margins.

(D) The characteristics of the colonies are variable in response to the environmental conditions in the culture, changes in form which probably influence such factors as the substrate requirement of the mould and possibly even its viable fraction.

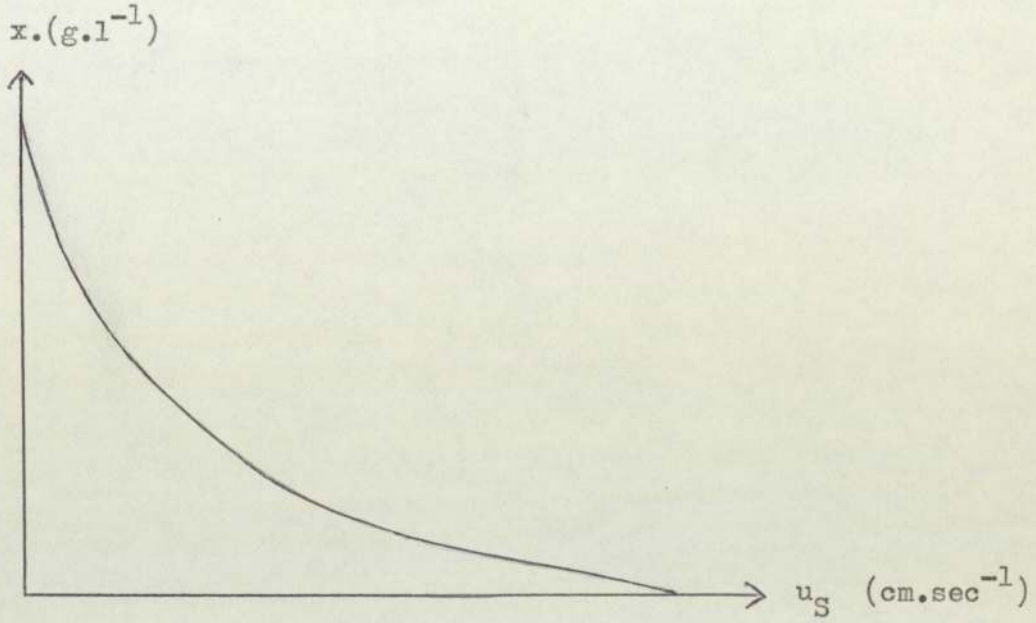
Attempts to quantify the morphology of the mould in all respects but that of overall dimension, have not proved meaningful, being considered in detail by Cocker (290). Even in recent reviews, such as that by Whitaker and Long (307), the possibility of describing such a crucial factor in anything but purely subjective terms such as "loose" or "hard" or "hairy" "pellets" is not considered.

James (308) and Fidgett (309) attempted to account for the effect of fluidisation on colonies of A. niger M1 mathematically and suggested that in a 2 phase non-growing situation, the relationship could be represented by an adaptation of the generalized fluidization equation:-

$$x = x_m \left[1 - \left(\frac{u_S}{u_T} \right) \frac{1}{n} \right] \quad \text{. . . . . Eqn. 4.9}$$

where u_S = superficial liquid velocity (cm. sec^{-1}) u_T = terminal velocity of the colony; n = an effectiveness factor and x_m = the maximum biomass concentration ($g.l^{-1}$). This equation gives a

Fig 4.37 Graphical Representation of the 2-phase Fluidisation Equation. (Fidgett, 309)



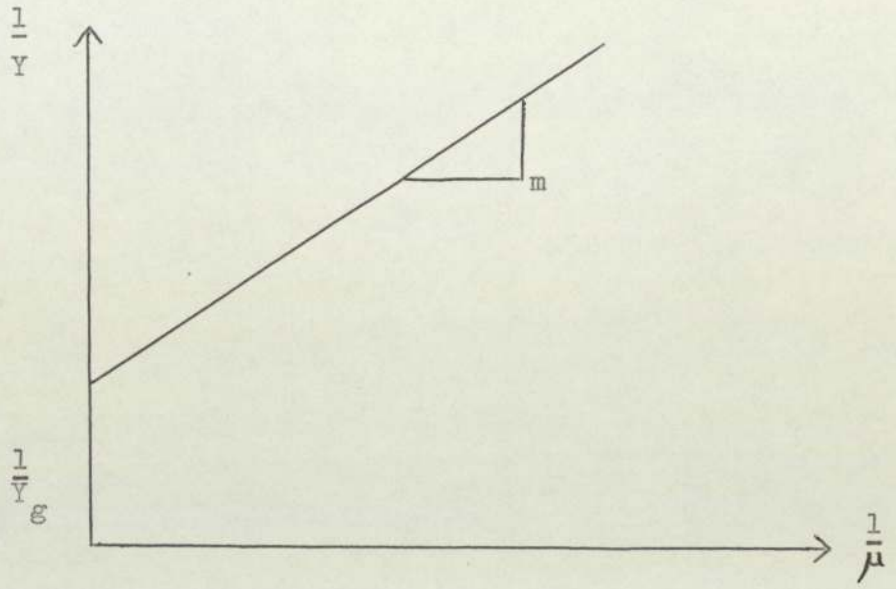
form of response curve for a particular colony form, shown in Fig. 4.37. For obvious reasons different morphologies, having different values of u_T, n and x_m, will modify this form, but it is perhaps a sound basis for future work.

The ability to adapt its morphology to a more sedimentary form is so great that the organism has been able to remain within the fermenter and grow at dilution rates far in excess of μ_{max}. Certainly fermentations at dilution rates of up to 7.0 h^{-1} were possible in this system using sucrose salts media (Pannell, 213) albeit with very low mould concentrations.

4.5.2 GROWTH RATES AND YIELDS OF MOULD.

The consistantly low values of the yield coefficient in this work could have been due to the substrate requirement for maintenance representing a substantial proportion of the total utilization.

Fig. 4.38 Reciprocal Plot used by Pirt for the Determination of the Maintenance Energy of Microorganisms.



Attempts were therefore made to determine the value of the maintenance coefficient (m) of A. niger M1 when growing semi-defined starch medium in the tower fermenter.

Two methods were used, both of which relied on the assumptions that m and Y_g (the true growth yield) were constants. The first was a method described by Pirt (310) which utilizes a reciprocal plot (illustrated in Fig. 4.38) based on the equation:-

$$\frac{1}{Y_g} = \frac{m}{\mu} + \frac{1}{Y_g} \quad \ldots\ldots\ldots\ldots \text{Eqn. 4.10}$$

In practice however, this method proved unsatisfactory; when applied to all the steady state readings, the maintenance coefficient indicated was - 0.0027 $g.g^{-1}.h^{-1}$, an obvious fallacy. This error was due, in part, to the method itself. In the reciprocal plot the least reliable data, that relating to very low

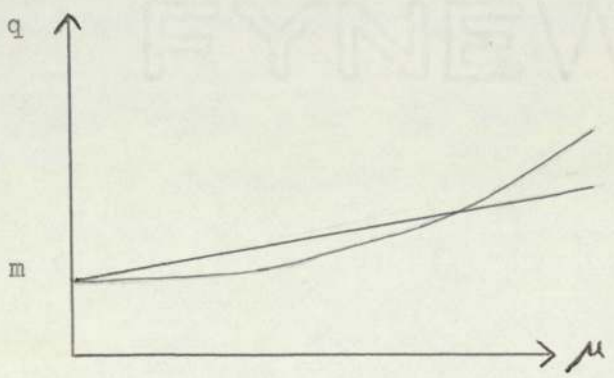
growth rates, has the greatest influence in the regression equation and therefore on the slope of the line (m).

A better method proved to be that used by Tempest and Herbert (303) and Righelato et al. (304), which is based on the assumption that the specific substrate utilization rate (q) can be related to the specific growth rate (μ) by the equation:-

$$q = \frac{\mu}{Y_g} + m \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{Eqn. 4.5}$$

Details of its application to the experimental data are in Section 4.2.4.2 and Fig. 4.9. But when μ was very low, observed values of Y exceeded that of Y_g predicted by the method which suggested that Eqn. 4.5 was not valid in this instance.

An explanation could lie in the fact that when $\mu >$ c.0.02 h^{-1} the culture probably became increasingly subject to oxygen limitation. In spite of the extremely high correlation coefficient (r = 0.9917; 195 readings) this method probably attempted to linearize data which actually formed part of a curve, i.e. schematically:-



This would enable 1/slope, the value of Y corrected for m (not necessarily Y_g), to decline with increase in μ and oxygen limitation.

However the prediction of a value of m of approx. 0.004 $g.g^{-1}.h^{-1}$ was in agreement with information derived from other aspects of the work. In general, the experimental results suggested that in these fermentations, the value of the maintenance coefficient was low compared to those usually reported, almost certainly less than 0.006 $g.g^{-1}.h^{-1}$ and not necessarily constant.

4.5.3 OXYGEN TRANSFER LIMITATIONS.

This point is best illustrated by an "order of magnitude" calculation of the oxygen demand of the culture and the oxygen transfer rate ($K_L a$) necessary.

Values were assumed for the oxygen requirements for mould growth (1.56 g.g cells^{-1}) and maintenance (0.024 g.g cells$^{-1}.h^{-1}$), those quoted by Righelato (304) for Penicillium chrysogenum. Calculations were based on data from a starch medium fermentation in which, at a D of 0.088 h^{-1}, x_F = 3.05 $g.l^{-1}$ and Productivity = 0.256 $g.l^{-1}.h^{-1}$.

The oxygen demand (Q) was :-

$$Q = Y_O \cdot \text{Productivity} + m_O \cdot x_F$$
$$= 0.3994 + 0.0732$$
$$= \underline{0.4726 \ g.l^{-1}.h^{-1}.}$$

The oxygen supply rate is given by:-

$$Q = K_L a \ (C^* - C)$$

where C^* and C are the saturation and actual concentrations of oxygen in the culture broth, which were assumed to be 0.008 $g.l^{-1}$ and zero respectively. So to satisfy the culture oxygen demand:-

$$K_L a = \frac{0.4726}{0.008} = \underline{59.07 \ h^{-1}}$$

Using polarographic techniques, Dowen (311) measured the oxygen transfer rate in the fermenters used for the author's work. With sucrose-salts medium and the same aeration rate (10 l. min^{-1} - a superficial gas velocity of 2 cm. sec^{-1}), he found that $K_La \approx 60\ h^{-1}$. This was significantly lower than previous estimates using the sulphite oxidation technique in tower fermenters (Morris, 289).

It should be noted that this was achieved in the absence of mould which, because its filamentous nature leads to high culture viscosities, has a particularly deletarious effect on K_La. Reductions by up to 85% are not unusual in fermenters containing up to 13.5 $g.l^{-1}$ dry weight of mould (312).

4.5.4 MOULD VIABILITY AND SPORULATION.

Even in actively growing cultures, a proportion of the total population consists of dead cells; this proportion increases at low growth rates. With unicellular microorganisms viable and non-viable cells can be readily distinguished and accounted for but with moulds it is far more difficult. According to Trinci and Righelato (313) both apparently viable and apparently dead cells are often found in the same hyphae.

So measured values of specific ($g.g^{-1}$) rates such as μ and m are in fact underestimates of the true values, which could in part account for some of the discrepancy between the level of m in this work and in the literature. Similarly, the colonial form of the mould, especially the classical "pellet", leads to diffusional limitations on the supply of substrates to a

proportion of the culture. Prediction therefore, of the concentration of mould actually taking part in the growth process becomes impossible.

The most widely used condition for the induction of submerged sporulation has been the absence of available nitrogen in the presence of an assimilable carbon source (Morton, 314; Vezina et al., 315 and Galbraith and Smith, 316). In this study, however, nitrogen was present in excess. Sporulation occurred when the carbon substrate supply decreased below approx. 0.025 $g.g^{-1}$ h^{-1}(the value of m often quoted for moulds). Above this, the culture grew normally as was the case in the chemostat studies of Righelato et al. (304). In their experiments too, the culture (P. chrysogenum) required a low concentration of glucose to spore in submerged culture, but remained vegetative above a critical glucose concentration.

4.5.5 SUGGESTIONS FOR FURTHER WORK.

Preliminary work with any novel fermentation system raises many points worthy of further investigation, especially with one whose behaviour is radical in so many respects. Seven major areas needing considerably more work are listed below, including in each case, the most important requirements of the investigation:-

(A) Morphology of the Organism. The relationship between physiological and physical factors and their control by (and of) the fermenter conditions.

(B) Oxygen Transfer in the Culture. Improvements through design of equipment and modification of the morphology of the organism.

(C) Control of the Specific Growth Rate. Mechanisms which lead to a limited independence of μ and D, especially in situations where $\mu > D$.

(D) Behaviour at low substrate supply rates. High and low dilution rates could be important in effluent treatment and sporulation studies respectively.

(E) Fermentation of Media containing Solids. Effects of both fermentable and non-fermentable solids on the process and their relationship to the mould morphology. Accounting for their presence when estimating organism related parameters.

(F) Use of other organisms, especially Yeasts and Bacteria whose morphology may be better suited to the system.

(G) Development of practical applications, especially those exploiting the simplicity of construction of the fermenter and the use of high dilution rates with nonaseptic media.

SECTION V.

CONCLUSIONS.

Throughout this work, the intention has been to determine the operating characteristics of a novel fermentation system and to assess its suitability for one possible application, the production of "biomass" from presently under-utilized carbohydrate resources.

Observations made during the course of this research have given a more detailed insight into the problems of mould physiology and behaviour in a Tower Fermenter. The large amount of data collected necessitated the development of a mass balance programme and in certain aspects a complete reassessment of conventional continuous culture theory.

The Continuous Fermenter used in this project was the ultimate in simplicity in terms of design and construction, an aerated tube containing the mould culture. In practice however, difficulties arose from the colonial growth form of the organism selected, Aspergillus niger M1. The form of the colonies both determined and was determined by fermenter conditions such as viscosity. As a research tool, the Continuous Tower Fermenter proved ideal for the study of the colonial forms and physiological responses of the organism without the "unnatural" effects of high shear forces.

An understanding of the operation of the system hinged upon the dual nature of the dilution rate, both as a fluidising force and as a means of nutrient supply to the culture. The concentration of organism in the fermenter was determined primarily by the former, acting on its morphology. In contrast, material in the effluent stream represented surplus growth and

was governed to some extent by the latter. This situation resulted in a considerable degree of independence of the specific growth rate relative to the dilution rate. This characteristic can normally only be achieved in single stage fermenters by recourse to an external "biomass" recycle system.

As a "biomass" production process the Continuous Tower Fermenter suffered from a low productivity/unit capacity and poor yield coefficient. Both were the direct results of oxygen transfer limitation aggravated by the morphology of the mould. Nevertheless the possibility of efficiently utilizing much lower substrate concentrations at higher dilution rates than conventional systems could be an advantage in certain situations; similarly the ability to ferment non-aseptic media, though one of the protective effects (that of pH) is only achieved at the expense of the yield coefficient.

Ideally, such a system might best be utilized as the final stage of an integrated process in which the initial production of useful materials from the low grade carbohydrate source was performed by a more sophisticated method, be it physical, chemical, or biological.

APPENDICES.

APPENDIX I.

METHODS FOR THE ESTIMATION OF SUGAR CONCENTRATION.

THE FERRICYANIDE METHOD.

REAGENTS.

Ferricyanide Solution, made up by dissolving 10 g. Potassium ferricyanide, 200 g. Sodium hydroxide and 0.1 g. Methyl Orange in 1 l. distilled water.

Methylene Blue Solution (10 $g.l^{-1}$).

Glucose Standard Solution (2 $g.l^{-1}$).

PROCEDURE.

A 25 cm^3 burette was filled with the test sample (or standard) containing 1 - 3 $g.l^{-1}$ reducing sugars. 20 cm^3 Potassium ferricyanide solution and 5 cm^3 indicating Sodium hydroxide solution were pipetted into a 250 cm^3 conical flask containing a few glass beads and brought to the boil within 90 sec. 7 cm^3 of the sample were pipetted into this flask, 0.1 cm^3 Methylene Blue added and the titration continued. The dark green/blue solution lightened to pink within 0.2 cm^3 of the endpoint, at which it became colourless.

For greater accuracy, the titration was repeated, adding the methylene blue approx. 0.5 cm^3 before the expected endpoint and ensuring that it was completed within 3 min. of the solution first boiling. The titration value must fall within the range 7 - 17 cm^3, a 2 $g.l^{-1}$ Glucose standard gave a value of 10.66 cm^3. Unfortunately the relationship is not linear, so the equivalent reducing sugar concentration was read from a graph constructed from a table of standards. (Fig. A1.1).

Fig.A1.1 SUGAR EQUIVALENTS FOR THE FERRICYANIDE METHOD.

TITRATION VALUE (cm^3)	EQUIVALENT GLUCOSE CONCN. ($g.l^{-1}$)
5	4.171
6	3.494
7	3.010
8	2.644
9	2.360
10	2.130
11	1.943
12	1.785
13	1.652
14	1.538
15	1.438
16	1.351
17	1.275
18	1.205
19	1.144
20	1.088
21	1.039
22	0.992
23	0.951
24	0.912
25	0.877

A Titration Value of 10.66 cm^3 is equivalent to 2$g.l^{-1}$ Invert Sugar.

THE 3,5-DINITROSALICYLIC ACID METHOD.

REAGENT.

1 g. of 3,5-dinitrosalicylic acid was dissolved in 20 cm^3 of 2M Sodium hydroxide solution and 50 cm^3 of distilled water. 30 g. of Rochelle salt (Sodium Potassium tartrate) were added and the solution made up to 100 cm^3 with distilled water. This solution is stable for at least 4 months if protected from carbon dioxide in well-filled bottles.

STANDARDS.

Solutions containing up to 2 $g.l^{-1}$ d-glucose (from an anhydrous stock) were prepared both separately and by dilution. Distilled water was used as the zero standard.

PROCEDURE.

Sample volumes of 1 cm^3 were pipetted into tubes containing 2 cm^3 of the dinitrosalicylic acid reagent and heated in a boiling water bath for 10 minutes. The tubes were cooled to room temperature, 2 cm^3 distilled water added and the optical density read at 570 nm with an E.E.L. "Spectra" Colourmeter equipped with a flow-through cell. The meter was first set to zero with the tubes containing distilled water standards.

The concentration of reducing sugars in the samples could then be estimated from the graph of Optical Density v. Glucose concentration obtained with the standards.

DISCUSSION.

This method is particularly suitable for estimating large numbers of samples, the author routinely estimating batches of 90 tubes. These were 15 cm. long x 1 cm. i.d., so that they

could be immersed to a depth of 5 cm. with little risk of accidental ingress of water.

At 95°C the reaction is almost instantaneous and for an individual tube, 4 min. immersion in boiling water is adequate. When dealing with large batches of tubes, a 10 min. immersion was used to ensure the adequate heating of all samples. Boiling for 20 min. produced no detectable hydrolysis of starch and sucrose samples.

The bandwidth of the E.E.L. instrument used was only 30 nm, considerably less than the 90 nm of the Ilford 626 filter used in some meters. For this reason, with the method described above, the optical density produced by the reaction obeys Beer's Law with samples in the range 0 - 2 $g.l^{-1}$ reducing sugars and the method is accurate to $\pm$ 1%. By diluting the solution to 10 cm^3 samples containing up to 5 $g.l^{-1}$ can be estimated, though with some loss of accuracy.

TOTAL SUGAR ESTIMATION.

PROCEDURE.

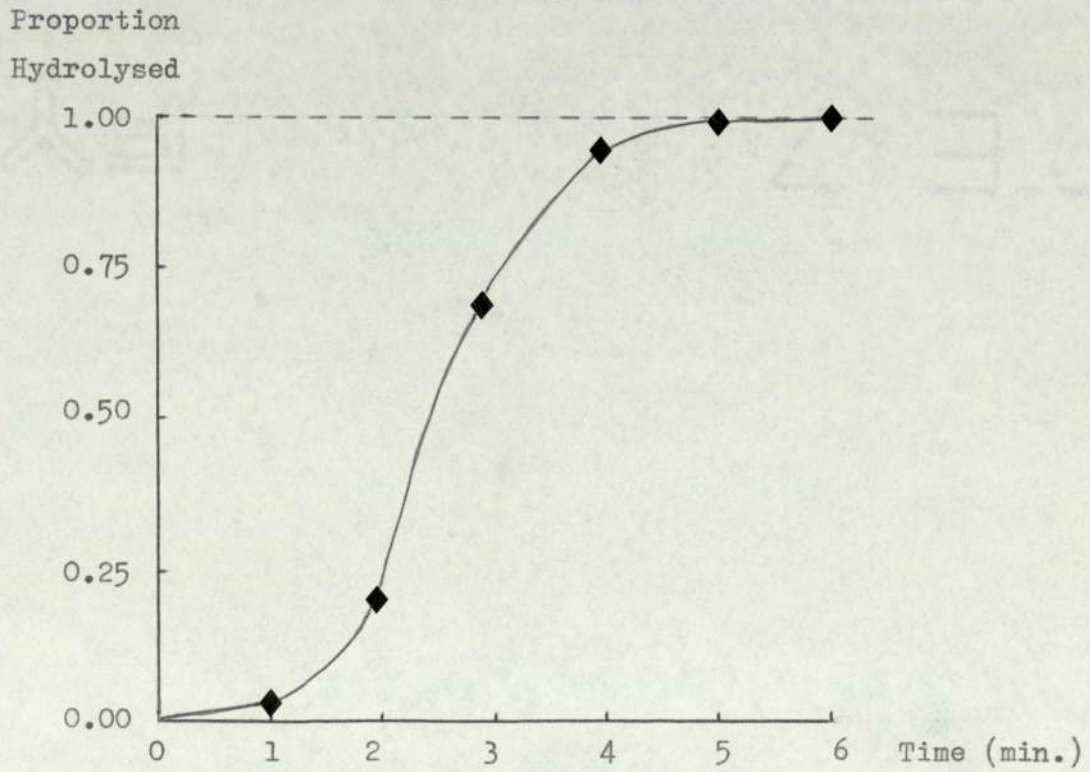
Sample volumes of 10 cm^3 were pipetted into boiling tubes together with 1 cm^3 of concentrated hydrchloric acid and boiled on a water bath for 10 min. The samples were cooled and neutralized with 1.2 cm^3 of 10.M sodium hydroxide before being made up to a volume such that the concentration of reducing sugars was less than 2 $g.l^{-1}$. This solution was then estimated by the dinitrosalicylic acid method and a correction factor applied to account for the dilution of the original sample. Results were expresses as $g.l^{-1}$ Glucose Equivalent.

DISCUSSION.

Under these conditions, hydrolysis of a 10 $g.l^{-1}$ starch solution was found to be complete within 6 minutes (see Fig. A1.2) so the use of a 10 min. hydrolysis time allows a considerable reserve but it is not so long as to cause significant caramelization.

Acid hydrolysis and neutralization (and the resultant sodium chloride present) of the sample was found not to affect the sugar estimation providing the final sample/reagent mixture was alkaline, though, of course the accuracy was slightly reduced because of the need for a dilution multiplication factor.

Fig. A1.2 Hydrolysis of Starch (10$g.l^{-1}$soln.) by Concentrated Hydrochloric acid.



APPENDIX II.

ESTIMATION OF RATE OF REDUCING SUGAR FORMATION FROM STARCH.

THE BUFFER STOCK.

34 g. sodium acetate ($C H_3 COONa. 3H_2O$) were dissolved in 250 cm^3 1.0N Acetic acid (i.e. 14.325 cm^3 glacial acid diluted to 250 cm^3) and made up to 500 cm^3 with distilled water. When a 20 cm^3 sample of this stock was diluted to 1 l. the resulting pH was 4.6.

THE STARCH SUBSTRATE.

2 g. Soluble starch were creamed with 20 cm^3 cold distilled water and poured into approximately 400 cm^3 boiling distilled water whilst being vigorously stirred. This solution was boiled for 2 min. and cooled under cover to 20°C. A 10 cm^3 portion of the Acetate Buffer was added and the volume made up to 500 ml.

The substrate had, therefore, a starch concentration of 4 $g.l^{-1}$ and was buffered at pH 4.6.

THE PROCEDURE.

A 100 cm^3 portion of the filtrate from a sample of the fermenter broth was added to 200 cm^3 of starch substrate at 30°C, shaken and incubated at this temperature for 3 h. 2 cm^3 samples were removed at 15 min. intervals and the reducing sugar content assayed by the Dinitrosalicylic acid Method (Appendix I).

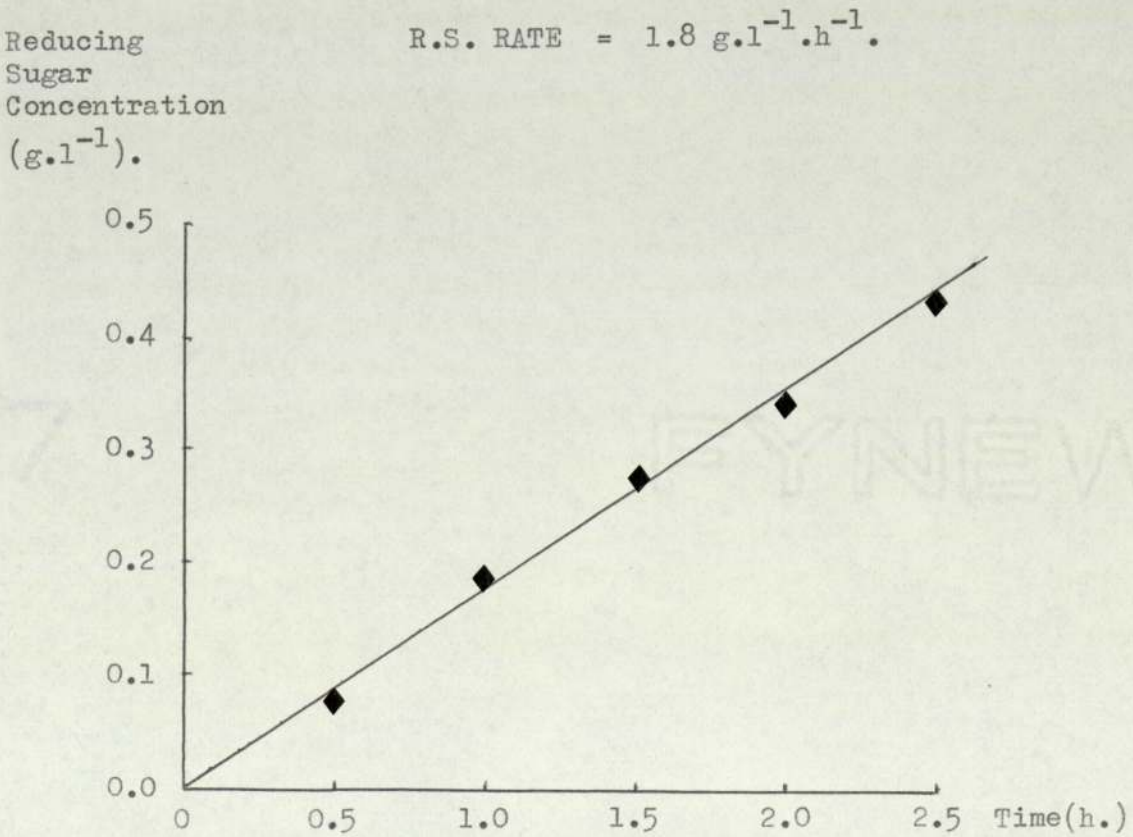
The total weight of reducing sugar present in the reaction mixture is plotted against incubation time, the slope of the straight line portion of this graph being the required reaction rate. Multiplication of the numerical value of the slope by 10

(because the sample volume is 100 ml.) enables the reaction rate to be expressed in the units $g.l^{-1}.h^{-1}$.

COMMENTS.

The dinitrosalicylic acid reagent is strongly alkaline and on mixing with the sample, terminates these enzyme reactions immediately. Providing this part of the estimation is carried out without delay, the procedure can be continued with all the samples and standards "en masse", making this a very simple and straightforward assay. Fig. A.2.1 shows the results of a typical estimation.

Fig. A2.1 Results of an Estimation of Reducing Sugar Formation Rate.



APPENDIX III.

THE MASS BALANCE PROGRAMME.

```
0         MASTER FERMENTER
1         REAL M(4),MT,MU,NX,MT1,MT2,NFS
2         INTEGER STAR,ST(100),COM(72)
3         DIMENSION AJF(9),DIF(9),E(3),F(9),F1(9),
          ISUMF(9),Z(9),F2(9)
4       100 FORMAT(I0)
5       101 FORMAT(12F0.0)
6       102 FORMAT(72A1)
7       200 FORMAT(10H FERMENTER,5X,9(F8.3,2X),//)
8       201 FORMAT(7H MEDIUM,8X,7(F8.3,2X),//)
9       202 FORMAT(18H FLAG INCORRECT,K=,I2)
10      203 FORMAT(8H RESULTS,7X, 9(F8.3,2X))
11      204 FORMAT(9H EFFLUENT,6X,4(F8.3,2X),//)
12      205 FORMAT(////)
13      240 FORMAT(23H ZERO EFFLUENT AT TIME=,F7.2)
14      241 FORMAT(25H MEDIUM:TIME,ANM,TSM,NFS=,
          4(F9.4,2X))
15      250 FORMAT(46H FERMENTER:TIME,PH,TEMP,D,XF,
          RSF,TSF,NH3F,NBUG,//)
16      251 FORMAT(43H MEDIUM:TIME,NH3M,TSM,MT,
          VUSED,SUMTSM,SUMNM,//)
17      252 FORMAT(32H EFFLUENT:TIME,VE,XE,SUMVE,
          SUMXE,//)
18      253 FORMAT(49H RESULTS:MU,YXS,PROD,RSRATE,
          RMU,RYXS,DN,NINBUG,YN,////)
19      260 FORMAT(29H DATA TIME SEQUENCE INCORRECT)
20      261 FORMAT(18H END OF FAIRY TALE,////)
21      262 FORMAT(1X,72A1)
22          READ(3,102) COM
23          WRITE(2,205)
24          WRITE(2,262) COM
25          WRITE(2,205)
26          WRITE(2,250)
27          WRITE(2,251)
28          WRITE(2,252)
29          WRITE(2,253)
```

```
C
C *** DATA INPUT
C *** K=1 FOR COMMENT CARD
C *** K=2 FOR MEDIUM ACCOUNT
C *** K=3 FOR FERMENTER ACCOUNT
C *** K=4 FOR EFFLUENT ACCOUNT
C *** K=9 FOR TERMINATOR
C
    2 READ(3,100) K
      IF(K.EQ.1) GOTO 10
      IF(K.EQ.2) GOTO 20
      IF(K.EQ.3) GOTO 30
      IF(K.EQ.4) GOTO 40
      IF(K.EQ.9) GOTO 99
      WRITE(2,202) K
      GOTO 99
   10 READ(3,102) COM
      WRITE(2,262) COM
      GOTO 2
C
C *** MEDIUM ACCOUNT
C
   20 READ(3,101) (M(I),I=1,4)
      WRITE(2,241) M
      IF(M(1).LT.T-0.01) GOTO 98
      T=M(1)
      MT2=M(1)
      MT=MT2-MT1
      VUSED=VF*D*MT
      SUMTSM=SUMTSM+TSM*VUSED
      SUMANM=SUMANM+ANM*VUSED
      SUMNFS=SUMNFS+NFS*VUSED
      MT1=M(1)
      ANM=M(2)
      NFS=M(4)
      TSM=M(3)
      GOTO 2
```

```
C
C *** FERMENTER ACCOUNT
C
   30 READ(3,101) (F(I),I=1,9)
      WRITE(2,200) F
      IF(F(1).LT.T-0.01) GOTO 98
      T=F(1)
      TSSAMP=TSSAMP+VSAMP*F1(7)
      ANSAMP=ANSAMP+VSAMP*F1(8)
      XSAMP=XSAMP+VSAMP*F1(5)
      DO 31 I=1,9
      IF(F(I).GT.-0.1) GOTO 37
      F(I)=F1(I)
   37 DIF(I)=F(I)-F1(I)
   31 CONTINUE
      IF(ET1.LT.F1(1)-0.01) GOTO 33
      FRAC=(ET1-F1(1))/DIF(1)
      DO 32 I=1,9
      F1(I)=F1(I)+DIF(I)*FRAC
   32 CONTINUE
      DIF(1)=F(1)-F1(1)
   33 DO 34 I=1,9
      AVF(I)=(F(I)+F1(I))/2.0
      TSUMF(I)=TSUMF(I)+AVF(I)*DIF(I)
   34 CONTINUE
      DO 36 I=1,9
      F2(I)=F1(I)
      F1(I)=F(I)
   36 CONTINUE
      ET1=F(1)
      GOTO 2
C
C *** EFFLUENT ACCOUNT
C
   40 READ(3,101) (E(I),I=1,3)
      IF(E(1).LT.T-0.01) GOTO 98
      IF(E(3).GT.-0.1) GOTO 41
      E(3)=XFDUM
   41 T=E(1)
      ET2=E(1)
      VE=E(2)
      XE=E(3)
      SUMXE=XE*VE
      WRITE(2,204) E,SUMXE
      XFDUM=E(3)
      ET=ET2-ET1
C
```

```
C *** FERMENTER DATA MANIPULATION
C
      FT=ET2-FT1
      DO 50 I=1,9
      TSUMF(I)=TSUMF(I)+F1(I)*FT
      F(I)=TSUMF(I)/ET
   50 CONTINUE
      WRITE(2,200) F
      D=F(4)
      XF=F(5)*(SUMXE-SUMNFS)/SUMXE
      RSF=F(6)
      TSF=F(7)
      ANF=F(8)
      NX=F(9)
      DXF=F1(5)-F2(5)
      DRSF=F1(6)-F2(6)
      DTSF=F1(7)-F2(7)
      DANF=F1(8)-F2(8)
C
C *** MEDIUM DATA MANIPULATION
C
      MT=ET2-MT1
      VUSED=VF*D*MT
      SUMTSM=SUMTSM+TSM*VUSED
      SUMANM=SUMANM+ANM*VUSED
      SUMNFS=SUMNFS+NFS*VUSED
      SUMXE=SUMXE-SUMNFS
C
C *** CALCULATIONS
C
      DS=SUMTSM-VE*TSF-DTSF*VF-TSSAMP
      DN=SUMANM-VE*ANF-DANF*VF-ANSAMP
      DX=SUMXE+DXF*VF+XSAMP
      SUMNX=DX*NX
      YXS=DX/DS
      RYXS=1.0/YXS
      MU=DX/ET/XF/VF
      RMU=1.0/MU
      PROD=MU*XF
      RSRATE=RSF*VE/VF/ET+PROD/YXS+DRSF/ET
      YXN=DX/DN
```

```
C
C *** PRINT ROUTINE
C
        Z(1)=MU
        Z(2)=YXS
        Z(3)=PROD
        Z(4)=RSRATE
        Z(5)=RMU
        Z(6)=RYXS
        Z(7)=DN
        Z(8)=SUMNX
        Z(9)=YXN
        WRITE(2,203) Z
        GOTO 3
    98  WRITE(2,260)
    99  WRITE(2,261)
        STOP
        END
        FINISH
****
```

APPENDIX IV.

STEADY STATE DATA.

Medium Type	Units	Starch	Starch	Starch
s_R	$g.l^{-1}$	19.6	19.94	19.76
Medium pH	Units	7.0	7.0	7.0
Temperature	^oC	30.0	30.0	30.0
D	h^{-1}	0.0424	0.0387	0.0324
Non-fermentable Solids	$g.l^{-1}$	-	-	-
$\bar{x}_F$	$g.l^{-1}$	6.6	8.03	6.5
$\bar{x}_E$	$g.l^{-1}$	3.53	3.7	2.996
μ	h^{-1}	0.0251	0.01613	0.0149
$Y_{x/s}$	$\frac{gmould}{gsubs}^{-1}$	0.2854	0.2273	0.211
Productivity	$g.l^{-1}.h^{-1}$	0.147	0.1295	0.09708
Reducing Sugar Production Rate	$g.l^{-1}.h^{-1}$	0.6234	0.608	0.5121
Substrate Conversion Effectiveness	$g.g\ supplied^{-1}$	0.7502	0.7878	0.8015
Substrate Utilization Rate	$g.l^{-1}.h^{-1}$	0.5681	0.5697	0.4602
Specific Substrate Utilization Rate	$g.g^{-1}.h^{-1}$	0.08619	0.07086	0.07075
Culture pH	Units	2.26	2.105	2.24
Mould Nitrogen Content	$g.g^{-1}$	0.05575	0.0531	0.05091

Medium Type	Starch	Starch	Starch	Starch
s_R	19.71	19.8	13.2	13.2
Medium pH	7.0	7.0	7.0	7.0
Temperature	30.0	30.0	30.0	30.0
D	0.0179	0.0123	0.093	0.0923
Non-fermentable Solids	-	-	-	-
$\bar{x}_F$	9.2	6.84	3.086	3.352
$\bar{x}_E$	3.88	2.59	2.7799	2.916
μ	0.00717	0.00455	0.08186	0.0763
$Y_{x/s}$	0.1919	0.1368	0.3044	0.3083
Productivity	0.066	0.03112	0.2545	0.256
Reducing Sugar Production Rate	0.3487	0.2372	1.193	1.164
Substrate Conversion Effectiveness	0.9782	0.9842	0.9718	0.9553
Substrate Utilization Rate	0.3452	0.2273	0.8361	0.7955
Specific Substrate Utilization Rate	0.0375	0.0333	0.2709	0.2477
Culture pH	1.86	2.15	2.94	2.53
Mould Nitrogen Content	0.04732	0.0482	0.0702	0.06475

Medium Type	Starch	Starch	Starch	Starch
S_R	13.2	13.2	13.2	13.2
Medium pH	7.0	7.0	7.0	7.0
Temperature	30.0	30.0	30.0	30.0
D	0.074	0.0725	0.0679	0.0364
Non-fermentable Solids	-	-	-	-
$\bar{x}_F$	3.158	3.575	3.155	4.981
$\bar{x}_E$	2.76	3.012	2.603	3.481
μ	0.0669	0.0588	0.04896	0.0244
$Y_{x/s}$	0.3259	0.3115	0.2987	0.2793
Productivity	0.210	0.2124	0.1733	0.1244
Reducing Sugar Production Rate	0.908	0.934	0.858	0.4707
Substrate Conversion Effectiveness	0.9295	0.9759	0.9573	0.9796
Substrate Utilization Rate	0.6444	0.6819	0.5802	0.4454
Specific Substrate Utilization Rate	0.204	0.1907	0.1839	0.08942
Culture pH	2.64	3.06	2.66	2.15
Mould Nitrogen Content	0.06739	0.06257	0.06194	0.0584

Medium Type	Starch	Starch	Starch	Starch
s_R	13.2	3.3	3.3	13.2
Medium pH	7.0	7.0	7.0	7.0
Temperature	30.0	30.0	30.0	35.0
D	0.025	0.0893	0.0706	0.075
Non-fermentable Solids	-	-	-	-
$\bar{x}_F$	3.942	2.826	2.077	3.244
$\bar{x}_E$	3.85	1.052	1.048	2.732
μ	0.0238	0.0325	0.0348	0.0632
$Y_{x/s}$	0.3110	0.3172	0.3186	0.3059
Productivity	0.0944	0.09326	0.0744	0.2038
Reducing Sugar Production Rate	0.3218	0.2934	0.2334	0.929
Substrate Conversion Effectiveness	0.9882	0.9956	1.0013?	0.9384
Substrate Utilization Rate	0.3035	0.2941	0.2335	0.6662
Specific Substrate Utilization Rate	0.077	0.1048	0.1124	0.2054
Culture pH	2.55	2.65	2.62	2.67
Mould Nitrogen Content	0.05475	0.07191	0.06969	0.06686

Medium Type	Starch	Starch	Starch	Starch
s_R	13.2	13.2	13.2	13.2
Medium pH	7.0	7.0	7.0	11.0
Temperature	35	40	42	30
D	0.0666	0.0671	0.0671	0.0915
Non-fermentable Solids	-	-	-	-
$\bar{x}_F$	3.011	8.99	7.596	3.087
$\bar{x}_E$	2.584	3.966	3.551	3.315
μ	0.0555	0.0293	0.035	0.09986
$Y_{x/s}$	0.288	0.3175	0.3439	0.3573
Productivity	0.161	0.257	0.259	0.317
Reducing Sugar Production Rate	0.849	0.8607	0.8469	1.107
Substrate Conversion Effectiveness	0.9657	0.9717	0.9562	0.9165
Substrate Utilization Rate	0.559	0.8185	0.7531	0.8872
Specific Substrate Utilization Rate	0.1857	0.09284	0.09915	0.2821
Culture pH	2.85	2.9	2.49	6.5
Mould Nitrogen Content	0.05541	0.04235	0.04486	0.07228

Medium Type	Starch	Starch	Potato	Potato
s_R	13.2	13.2	20.2	21.0
Medium pH	11.0	11.0	7.0	7.0
Temperature	30	35	30.0	30.0
D	0.0725	0.0745	0.104	0.0351
Non-fermentable Solids	-	-	2.23	2.2
$\bar{x}_F$	3.165	2.9125	4.677 (2.476)	7.64 (4.424)
$\bar{x}_E$	3.953	3.542	4.675 (2.475)	9.2 (5.327)
μ	0.08835	0.0885	0.08096	0.0197
$Y_{x/s}$	0.44365	0.301	0.5502 (0.2913)	0.6225 (0.3604)
Productivity	0.2866	0.256	0.4864 (0.2575)	0.1829 (0.1059)
Reducing Sugar Production Rate	0.9179	0.9635	1.050	0.4728
Substrate Conversion Effectiveness	0.9604	0.9797	0.4998	0.6414
Substrate Utilization Rate	0.6459	0.9242	0.884	0.2938
Specific Substrate Utilization Rate	0.2041	0.3173	0.1890 (0.357)	0.03846 (0.0664)
Culture pH	6.31	6.65	-	-
Mould Nitrogen Content	0.05974	0.06845	0.04993	0.04897

Figures in parentheses relate to the mould content alone.

Medium Type	Potato	Potato	Potato	Potato
s_R	21.03	19.0	10.13	5.27
Medium pH	7.0	7.0	7.0	7.0
Temperature	30.0	30.0	30.0	30.0
D	0.0248	0.0237	0.024	0.0491
Non-fermentable Solids	2.8	2.665	1.984	0.818
$\bar{x}_F$	6.45 (3.271)	7.53 (4.836)	6.62 (3.827)	3.487 (2.424)
$\bar{x}_E$	6.38 (3.235)	8.517 (5.4702)	7.314 (4.228)	2.112 (1.468)
μ	0.01653	0.0228	0.0227	0.031
$Y_{x/s}$	0.4836 (0.245)	0.695 (0.406)	0.685 (0.383)	0.4527 (0.3123)
Productivity	0.1408 (0.0714)	0.1708 (0.1097)	0.1498 (0.0866)	0.0997 (0.0693)
Reducing Sugar Production Rate	0.3706	0.403	0.232	0.2377
Substrate Conversion Effectiveness	0.7106	0.8949	0.9542	0.9186
Substrate Utilization Rate	0.2912	0.2702	0.2261	0.2219
Specific Substrate Utilization Rate	0.04514 (0.089)	0.0359 (0.0559)	0.03415 (0.0591)	0.0636 (0.0915)
Culture pH	-	-	-	-
Mould Nitrogen Content	0.05109	0.0471	0.053	0.0585

Figures in parentheses relate to the mould content alone.

Medium Type	Potato	Potato	Potato	Potato
s_R	4.46	4.40	4.237	4.01
Medium pH	7.0	7.0	7.0	7.0
Temperature	30.0	30.0	30.0	30.0
D	0.0576	0.0671	0.03145	0.0378
Non-fermentable Solids	0.614	0.65	0.355	0.458
$\bar{x}_F$	4.136 (2.765)	3.802 (2.56)	4.315 (3.039)	3.021 (2.342)
$\bar{x}_E$	1.64 (1.096)	2.205 (1.485)	2.416 (1.702)	2.211 (1.714)
μ	0.0273	0.0375	0.0137	0.02616
$Y_{x/s}$	0.476 (0.3195)	0.498 (0.3649)	0.489 (0.3495)	0.5707 (0.4458)
Productivity	0.1125 (0.0752)	0.128 (0.0915)	0.0575 (0.0405)	0.0778 (0.0603)
Reducing Sugar Production Rate	0.2527	0.282	0.1286	0.150
Substrate Conversion Effectiveness	0.9836	0.9551	0.9666	0.9896
Substrate Utilization Rate	0.2354	0.2508	0.1159	0.1353
Specific Substrate Utilization Rate	0.0569 (0.0851)	0.0659 (0.09795)	0.02685 (0.0381)	0.04477 (0.05775)
Culture pH	-	-	-	-
Mould Nitrogen Content	0.0623	0.063	0.0578	0.0571

Figures in parentheses relate to the mould content alone.

Medium Type	Potato	Potato	Potato	Potato
s_R	3.296	3.187	2.906	1.97
Medium pH	7.0	7.0	7.0	7.0
Temperature	30.0	30.0	30.0	30.0
D	0.0232	0.03115	0.0315	0.0205
Non-fermentable Solids	0.511	0.354	0.528	0.29
$\bar{x}_F$	3.576 (2.477)	2.355 (2.087)	5.945 (4.182)	3.472 (2.924)
$\bar{x}_E$	2.98 (2.064)	1.918 (1.70)	0.936 (0.658)	0.337 (0.2834)
μ	0.0131	0.0272	0.00617	0.0105
$Y_{x/s}$	0.555 (0.4138)	0.672 (0.579)	0.6593 (0.427)	0.833 (0.533)
Productivity	0.0462 (0.032)	0.0598 (0.053)	0.0398 (0.028)	0.038 (0.032)
Reducing Sugar Production Rate	0.0839	0.0973	0.0721	0.0405
Substrate Conversion Effectiveness	1.097?	0.9817	0.7876	1.0028?
Substrate Utilization Rate	0.0773	0.0955	0.06557	0.06004
Specific Substrate Utilization Rate	0.02163 (0.0312)	0.04056 (0.0458)	0.01103 (0.0157)	0.01729 (0.0205)
Culture pH	-	-	-	-
Mould Nitrogen Content	0.0583	0.0551	0.0548	0.059

Figures in parentheses relate to the mould content alone.

Medium Type	Peel Waste	Peel Waste
s_R	20.4	19.8
Medium pH	10.8	10.4
Temperature	30.0	30.0
D	0.067	0.092
Non-fermentable Solids	2.38	2.42
$\bar{x}_F$	5.23 (3.276)	5.1 (3.083)
$\bar{x}_E$	6.29 (3.996)	6.12 (3.7)
μ	0.0817	0.1104
$Y_{x/s}$	0.601 (0.3768)	0.655 (0.3958)
Productivity	0.427 (0.2675)	0.563 (0.3403)
Reducing Sugar Production Rate	0.97	1.16
Substrate Conversion Effectiveness	0.7097	0.6368
Substrate Utilization Rate	0.71	0.86
Specific Substrate Utilization Rate	0.1357 (0.2167)	0.1686 (0.2789)
Culture pH	6.4	6.7
Mould Nitrogen Content	0.0523	0.0517

Figures in parentheses relate to the mould content alone.

APPENDIX V.

LIST OF ABBREVIATIONS AND SYMBOLS.

approx.	approximately.
atm.	atmosphere.
av.	average.
B.O.D.	Biological Oxygen Demand.
c.	circa.
cm.	centimetre.
$^{o}C.$	Degrees Centigrade.
C.O.D.	Chemical Oxygen Demand.
concn.	concentration.
const.	constant.
D.	Dilution Rate.
d.	diameter.
Eqn.	Equation.
equiv.	equivalent.
Ferms.	Fermentations.
g.	gram.
gluc.	glucose.
h.	hour.
H.P.	Horse Power.
i.d.	internal diameter.
Kg.	Kilogram.
$K_La.$	Oxygen Transfer Coefficient.
l.	litre.
m.	metre.
max.	maximum.
mg.	milligram.
ml.	millilitre.

min.	minute.
min.	minimum.
mm.	millimetre.
μ.	specific growth rate.
N.	Normality.
NFS.	Non-fermentable Solids.
nm.	nanometre.
Prod.	Productivity.
q.	Specific Substrate Utilization Rate.
r.	regression coefficient.
R.S.	Reducing Sugars.
s.	substrate concentration.
s_R	medium substrate concentration.
sec.	second.
soln.	solution.
s.l.v.	superficial liquid velocity.
sp.	species.
T.	Temperature.
v.v.m.	volumes per volume per minute.
x.	organism.
x_F.	fermenter organism concentration.
x_E.	effluent stream organism concentration.
Y.	Yield Coefficient.
Y_g.	True growth yield coefficient.
$Y_{x/s}$.	Yield of organism on substrate.
$\pm$.	plus or minus.

$>x.$	greater than x.
$<x.$	less than x.
$\gg x.$	very much greater than x.
$\rightarrow x.$	value approaches that of x.
$\approx x.$	approximately equal to x.
$\infty .$	infinity.
$\bar{x}.$	denotes steady state value of x.
$x_F.$	denotes fermenter value of x.
$x_E.$	denotes effluent stream value of x.

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