THE MORPHOLOGICAL DEVELOPMENT OF *ASPERGILLUS NIGER* IN TOWER FERMENTER CULTURE

BY

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A thesis submitted in part fulfilment of the requirements for the degree of Doctor of Philosophy.

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DECLARATION

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

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CERTIFICATE

I hereby certify that the work embodied in this thesis has not already been submitted in substance for any degree and is not being concurrently submitted in candidature for any degree.

Roland Cocks

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SUMMARY

This study deals with the role played by the morphological development of Aspergillus niger M.1 during submerged fermentation in tower fermenters. A necessary part of the preliminary work was the improvement of basic tower fermenter designs at laboratory and pilot scale.

The fermentation conditions in a tower fermenter constitute a complex multivariate system, and therefore account has had to be taken of a wide variety of variables, each of which may affect or be affected by morphological changes. Features scrutinised include the relationships between morphological form, column design, inoculum characteristics, surface tension effects, mycelial density, mass transfer, suspension rheology, gas flow rate, and medium characteristics. The results are incorporated into a preliminary systems model, an understanding of which allows control of the morphological development of this strain in tower fermenters of various sizes.

CONTENTS

SECTION	TITLE	PAGE
PART ONE	INTRODUCTION	1
1:0	Initial Perspectives	1
1:2	The Choice of Reactors for Aerobic Fermentation	5
1:3	Tubular Reactors in Chemical Process Technology	5
1:4	Early Application of Tubular Reactors to Fermentation	6
1:5	Factors Underlying Reactor Choices	6
1:6	Recent Applications of Tower Fermenters	8
PART TWO	SYSTEMS ANALYSIS APPROACH	10
2:0	The Origins of Systems Analysis	10
2:1	Complex Systems	10
2:2	Definition of the System	10
2:3	Information Survey	10
2:4	Systems Diagrams and Other Models	11
2:5	Application to this Study	11
PART THREE	ORGANISM-RELATED PARAMETERS	13
3:0	Introduction	13
3:1	Genetically-Determined Characteristics	14
3:2	Description and Quantitation	16
3:3	Hyphal Branching	20
3:4	Hyphal Interactions	21
3:5	Mechanical Properties of Hyphae	22
3:6	Growth Kinetics	22
3:7	Fundamental Considerations of Growth	25

PART FOUR	A SURVEY OF THE INFLUENCE OF MEDIUM COMPOSITION ON THE GROWTH OF A. NIGER STRAINS	28
4:1	Trace Metals	28
4:2	Toxicity of Heavy Metals	33
4:3	Chelating Agents	35
4:4	Nitrogen and Phosphorus	36
4:5	Amino Acids	36
4:6	Purine and Pyrimidine Concentrations	37
4:7	Lipid Content	37
4:8	Alcohols	38
4:9	Available Sulphur	39
4:10	Carbohydrates	41
4:11	Surfactants	44
4:12	Contaminants	44
4:13	Plant Growth Factors and Alkaloids	46
4:14	Polymers	46
PART FIVE	CHEMICAL ENGINEERING ASPECTS	49
5:1	Gas Distributors	49
5:2	Column geometry	51
5:3	Baffle Systems	53
5:4	Expansion Sections	54
5:5	Superficial Gas Velocity	54
5:6	Bubble Size	55
5:7	Gas-Mixture	55
5:8	Viscosity	55
5:9	Hold-up	56
5:10	Shear-Forces	56
5:11	Heterogeneity	58
5:12	Temperature	58
5:13	Surfactants	58

PART SIX	METHODS	59
6:1	Organism	59
6:2	Culture Maintenance	59
6:3	Inoculum Preparation	59
6:4	Media	59
6:5	Antifoam	61
6:6	Sampling Methods	61
6:7	Direct Examination	62
6:8	Staining	62
6:9	Microscopy	62
6:10	Photography	64
6:11	Colony-sizing	64
6:12	Spore-Counts	65
6:13	Mycelial Dry Weight Estimation	65
6:14	Viscometry	65
6:15	Fluorescence Brightener Adsorption	70
6:16	Agar Culture	73
6:17	Equipment Development	76
PART SEVEN	EXPERIMENTAL RESULTS AND DISCUSSION	95
7:1	Rheological Studies	95
7:2	The Nature and Extent of Morphological Variation in Batch Culture	119
7:3	Dependence of Morphology on Medium Characteristics	126
7:3:1	Detergents	126
7:3:2	Antifoam	126
7:3:3	Suspended Solids	126
7:3:4	pH	129
7:3:5	Temperature	129
7:3:6	Carbon/Nitrogen Ratio	129
7:3:7	Zinc Concentration	129
7:3:8	Chelating Agents	129

7:3:9	Plant Growth Regulators	130
7:4	Mechanisms of Colony-Formation	134
7:4:1	The Role of Agglutination and Flocculation	134
7:4:2	The Role of the Spore-Concentration at Germination	137
7:5	Relationship of Morphological Type to Growth-Kinetics	139
7:6	Final Conclusions	158
	References	
	Published Work	

ILLUSTRATIONS

FIGURE	TITLE	PAGE
3:1	Types of Sample-Distribution	17
3:2	Growth of a Germ-Tube of Rhizopus stolonifer at at 25 °C (after Trinci)	19
5:1	General Relationship of Average Gas Hold-up to Superficial Gas Velocity	50
5:2	Effect of A. niger Morphology on suspension Rheology	57
6:1	Ferranti Model VL Rotational Viscometer	66
6:2	Viscometer Head with Inner Cylinder Withdrawn to reveal Internal Vanes and Vent-holes	67
6:3	Tinopal Absorbance vs. Tinopal Concentration and Tinopal Adsorption	69
6:4	Membrane Filtration Unit used for Determination of Dry Weight and Tinopal Adsorption	71
6:5	Three-Day-Old Petri-Dish Culture Showing Inoculation-Pattern	72
6:6	Diagram of Ten-Litre Fermenter Column	74
6:7	Ten-Litre Fermenter Columns	76
6:8	Quickfit-Jointed Fermenter-Column with Expansion-Chamber	77
6:9	Details of Column-Components	78
6:10	General View of Ten-Litre Column with Support Framework and Instrumentation	81
6:11	Probe Designs	83
6:11a	Circuit-Diagram of Thermistor Relay Circuit used to Switch Mains Power-Relay of Thermocirculator	85

6:12 Gas-Distributor 87

6:13 Fifty-Litre Fermenter 88

6:14 Pilot-Scale Fermenter 90

6:15 Pilot-Scale Fermenter During Installation Showing Attemperation Jackets 91

6:16 Details of Probe-Seal for Pilot-Scale Fermenter 93

7:1 Comparison of Axial Variation in Apparent Viscosity with Time for type I and type III colonies 96

7:2 Comparative Rheological Behaviour and Dry Weight gains for type I and type III Colonies (450 l. scale) 97

7:3 Relationship of Apparent Viscosity to Dry Weight Changes during Fermentation 98

7:4 Suspension Rheology of type I Colonies 99

7:4a Characteristics of various Types of Rheological Behaviour 100

7:5 Apparent Viscosity vs. Time for Type I Colonies at each Shear Rate 101

7:6 Dry Weight Profile During a 450l. Fermentation Showing axial Distribution of Mycelium (Type I) in Column 102

7:7 Three-Dimensional Relatioship between Shear-Stress, Shear-Rate and Time, for a 450l. Fermentation involving Type I Growth (Isometric Projection) 103

7:8 Three-Dimensional Relationship between Apparent Viscosity , Shear-Rate and Time for a 450l. Fermentation involving Type I Growth (Isometric Projection) 104

7:9 Axial-Distribution-Profile of Apparent Viscosity, During a 450l. Fermentation with Type III Colonies 105

7:10 Dry Weight Profile During a 450l. Fermentation showing Axial Distribution of Mycelium in Column 106

7:11 Relationship of Suspension Viscosity to Predicted Viscosity for A. niger M1. 108

7:12 Interlacing of Hyphae from Type II Colonies to form a Transient Matrix 109

7:13 Free-Flowing Behaviour of Type II Colonies 110

7:14 Free-Flowing Behaviour of Type II Colonies 111

7:15 Scatter-Diagram Showing Morphological Groupings in Relationship to Final Biomass Dry Weight Yields and Time of Fermentation to Maximum Biomass Value 113

7:16 Morphological Classification 115

7:17 Morphological Classification 116

7:18 Morphological Classification 117

7:19 Morphological Classification 118

7:20 Heterogeneity of Morphology 120

7:21 Heterogeneity of Morphology caused by Hyphal Fragments from Type VI Colonies which appear as aCloudiness in the Medium 121

7:22 Compound, Irregularly-Shaped Colonies formed by the Entanglement of Embryo Colonies 123

7:23 Large, Multiple-Centred Colonies 124

7:24 Type VI Colonies Showing Large Fragments Being Released from the Original Colonies 125

7:25 Effect of Antifoam on Dissolved Oxygen Tension 127

7:26 The Adsorption of Suspended Solids from Carob Medium onto Embryo Colonies 128

7:28 The Entanglement of Freshly-Germinated Spores 131

7:29 The Adsorption of Spores onto a Germ-Tube 132

7:30 Entrainment of freshly-germinated Spores by Larger Colonies or Aggregates 133

7:31 Diagrammatic Representation of the Agglutination and Growth Mechanisms which Participate in the Formation of Embryo Colonies 134

7:32 Entanglement of Unicells and Pseudomycelium of a Sewage Yeast in a Tower Fermenter , illustating the Formation of irregular Colonies by Aggregation 136

7:33 Examples of Radial Growth-Curves with Corresponding Dry Weight Increases 140

7:34 Stereoscan View of type VI Mycelium and type VII Mycelium Showing interstitial Spacing 141

7:35 Appearance of Culture 36 Hours after Inoculation ($d=0.022 hr^{-1}$) 143

7:36 Appearance of Culture 60 Hours after Inoculation ($d=0.022 hr^{-1}$) 144

7:37 Appearance of Culture after 130 Hours ($d= 0.022 hr^{-1}$) 145

7:38 Appearance of Mycelium in effluent after 130hr 146

7:39 Appearance of Mycelium from Fermenter after 780 hr ($d=0.022 hr^{-1}$) 147

7:40 Appearance of Culture Twenty- three days after Inoculation ($d=0.022 hr^{-1}$) 148

7:41 Tinopal Fluorescence Micrograph of Mycelium at a constant Dilution-Rate of $0.022 hr^{-1}$ 150

7:42 Tinopal Fluorescence Micrograph of Mycelium five hours after Step-up from d= 0.022 to d= 0. $1hr^{-1}$ 151

7:43 Appearance of Mycelium by Tinopal Fluorescence six hours after Step-up from d=0.1 to d=0.9 hr^{-1} 152

7:44 Tinopal Fluorescence Micrograph of Mycelium seventeen hours after Step-up from d= 0.1 to d= 0.9 hr^{-1} 153

7:45 Tinopal Micrograph of Hypha showing Fluorescence of Tips, Septa, Buds, and developing bud-points 154

7:46 Adsorption of Tinopal BOPT for Batch Fermentations 155

7:47 Variations in Tinopal Adsorption caused by Step-Changes in Dilution-Rate 156

7:48 Examples of Growth-Curves for Colonial Mycelium on Synthetic Media 157

7:49 Block-Diagram of the Systems-Relationship Between Parameters 160

TABLES

TABLE NO.	TITLE	PAGE
3:1	Length of the Apical Cell and Hyphal Compartments of Surface Hyphae at the Periphery of Fungal Colonies	15
4:1	Effect of Concentration of Manganese on the Growth of *Aspergillus niger*	31
4:2	The Influence of Methionine on Growth and Molybdenum-Uptake by *A. niger* when Molybdenum is at Toxic Levels	32
4:3	Effect of Adding Potassium Chloride on the Dry Weight of Mycelium by *Aspergillus niger*	34
4:4	TheEffect of Glucose and Galactose Singly and in combination on the Mycelial Yield of *Aspergillus niger* in liquid at 20°C after 7 days	40
4:5	Final Dry Weight, Growth-rate, and Cellular Sugar content of *A. niger* vs. presence of Single Carbon Sources and Concentration of Sugars in the Medium,	42
4:6	Effect of Plant Growth regulators on Growth Rate	47
6:1	Details of Equipment for Fluorescence Microscopy	63
6:2	Operating Data for Ferranti VLC Viscometer Head	68
6:3	Fermenter Design Problems	79
6:4	Inventory of Parts for Ten-Litre Fermenter	80

6:5 Details of Side-Arms and Range of Fittings for Ten-Litre Fermenter..... 82

6:6 List of Fittings (QVF) for 50 Litre Fermenter.....89

6:7 Internal Dimensions of Columns Used.....89

7:1 Reduction of Spore-Concentration on Carob Extract Medium using the Standard Method..... 137

PART ONE: INTRODUCTION

1:0. Initial Perspectives

In recent years, a great deal of effort has been devoted to the task of increasing foodstuff yields, particularly with respect to high-protein foodstuffs. The main reasons for the size of this effort have been the concern over the discrepancy between the expansion of food production and the expansion of the human population, together with the realisation by commercial organisations that the present shortage of high-protein concentrates will provide a large and expanding market for novel protein sources. For example, the most up-to-date study of microbial protein production (Wolfson-Interplan, 1974) predicts that by 1980, European calf, pig and poultry breeders could be buying £50 million worth of microbial protein products, and that a decade later, the market would be worth £300 - £400 million. Undoubtedly the companies now involved in biomass projects have for some time had the benefit of confidential analyses and projections on which they have based their investments. It is interesting to note that at about £110 /ton, yeast from sulphite liquor is already ousting skim milk (£300/ton) and whey (£200) in calf foods, and I.C.I. report that the cost of their S.C.P. grown from methanol is £100/ton. In more general terms, microbial protein has to compete with soya meal at £80/ton and fishmeal at £100/ton, though it will undoubtedly benefit from any shortages of these materials.

Many of the biomass projects which are in the development stage involve complex technological approaches, viz. the growth of microbes on n-alkanes and methanol. These projects are characteristically large in scale, involve centralised production operations and require massive capital investment. It is not surprising that such operations are at present restricted to the

"advanced" countries of the world.

One of the more useful attributes of microorganisms is that they provide a means of converting low-grade materials with high nuisance values such as sulphite liquor, scrap paper and factory effluents, to products which have positive value, such as protein, fat, alcohols, solvents, organic acids. (An important feature of such conversions is that they must not produce more troublesome effluents than those used as substrate!). Suitable raw materials for such conversions (sugar cane juice, carob beans, pineapple waste) are available in countries of the world which require the agricultural and economic boost that such fermentation processes would provide , but many of these countries do not have ready access to the technological resources or skilled manpower appropriate to the sophisticated fermentation processes previously mentioned. It is also becoming apparent that there are many industries in Europe and the U.S.A. which produce effluents particularly suited to microbiological upgrading, but which do not wish to involve themselves in expensive or complex fermentation plant.

There is therefore a requirement for fermentation processes which do not require the constant attention of skilled scientists, and do not involve plant which is constructed from expensive, sophisticated components and materials.

There are many traditional examples of fermentation processes which have been successful when operated by relatively unskilled personnel and which do not require complex plant, e.g. brewing, wine-production, yeast manufacture, vinegar acetification, alcohol production, soya sauce manufacture, silaging. It therefore seems feasible that with careful application, additional processes can be developed which will fulfil the gap in fermentation technology,

even though they may involve the initial application of a sophisticated understanding of selective or mixed culture techniques, biochemical engineering etc. to provide them with the required system-stability and consequent simplicity of operation. The work of which this project was a part is based largely on the above approach, and involves the exploration and exploitation of the complex systems-behaviour of simple vertical tube-fermenters. Research is progressing on topics such as the production of biomass or alcohol from low grade carbohydrates such as maltsters and brewery effluents, food processing wastes and agricultural byproducts. More specifically, the work detailed in this thesis was undertaken in cooperation with Tate and Lyle Ltd. as part of a feasibility study of the production of fungal biomass from low-grade carbohydrates such as carob bean pods. The production stage of such processes is likely to take place in less developed countries where the raw materials and the market for the product are available.

This research was also undertaken as part of a joint programme by members of the Departments of Chemical Engineering (Dr. E.L.Smith) and Biological Sciences (Dr. R.N.Greenshields) of the University of Aston in Birmingham, to explore tower fermenter technology.

The biologists have been concerned with the following topics:-

1. Continuous tower fermentation of beer, wine and alcoholic washes (Dr. S.D.J.Coote).
2. Initial evaluation of operating data required to design and build tower fermenters for growth of A. niger M1 on carbohydrate wastes (Dr. G.G. Morris).
3. Nutritional and toxicological studies of filamentous fungi for biomass production (Dr. B. Daunter).
4. Development of a continuous aerobic tower fermenter for fungal biomass production (Mr. S.D. Pannell).

5. Application of aerobic and anaerobic tower fermentation to the utilisation of low-grade carbohydrate wastes (Mr. R.A. Spensley).

The chemical engineers were concerned with the following topics:-

1. Properties of microbial suspensions and fluidisation studies (Dr. A. James).
2. Gas hold-up and mixing in tower reactors (Dr. J. Shayegan Salek).
3. Mass-transfer studies of gas-liquid-solid systems in tower reactors (Mr. A. Dowen).
4. Development of mathematical models to aid in design, scale-up and operation of tower reactors (Dr. M. Fidgett).

Some of the goals of this research were associated with the informational requirements of this multidisciplinary team, and thus it was felt important to prepare descriptions of the following:-

1. maximum possible extremes of morphological variation and all forms observed within these extremes.
2. Microscopic and macroscopic details of the various morphologies.
3. Response of morphological development to environmental influences, particularly those likely to be most important in fungal biomass production on a commercial basis.
4. Effect of morphology on environmental parameters important to fermenter performance.

It was also hoped to throw more light on the fundamental rules governing morphological development of filamentous fungi during submerged culture. Species variations with respect to morphology, physiology etc. indicated that the most practical approach was to build up a comprehensive picture of a single species. Aspergillus niger M.1 was chosen because of the large amount of information available on the physiology of A.niger strains, and also because of information available on the characteristics of this particular strain (Morris, 1972; Daunter, 1972).

1:2 The Choice of Reactors for Aerobic Fermentations

For the most part, the stirred tank reactor (S.T.R.) is identified most strongly amongst possible reactor configurations as that most suitable for aerobic , microbially-mediated reactions . Ever since the rapid conscription of stirred tank reactors for the production of penicillin and food yeast during World War II, manufacturers of other enzymes, antibiotics, etc., have for pragmatic reasons adopted the established technology . A large proportion of laboratory fermenters have followed suit in pursuit of the established goals of ideal mixing, homogeneity, and consequent ease of measurement and control. However, by far the largest-scale application of aerobic fermentation, that of sewage treatment, employs reactors which depend on heterogeneity and differ radically from the S.T.R. concept, and there is at the present time an upsurge of interest in alternative types of reactor, among them various types of overtly tubular reactor such as the pressure cycle fermenter and various types of tower-fermenter.

1:3 Tubular Reactors in chemical process technology

The tubular reactor is well-established in the chemical process industries and is used for gas-phase reactions such as the oxidation of nitric oxide, the halogenation of olefins, and for fixed- bed catalytic reactions such as ammonia synthesis, methanol synthesis, and thermal cracking reactions. The essential feature of a tubular reactor is an axial heterogeneity which results in a spatial separation of reaction-stages which would otherwise form a time- dependent sequence. The use of cylindrical tubular reactors also confers cost advantages such as the availability of mass-produced piping systems, standardised production - systems such as extrusion, helical welding, and seam-welding, and lower land - requirements in the case of vertically-erected pipes. Compound assemblies provide a way of exploiting further the advantages of tubular reactors for certain processes, for example, the calandria (a bundle of tubes joined together at their ends to form

ends to form one vessel) similar to that used in the CANDU and SGHWR nuclear reactors because of the ease of heat-transfer and pressurisation. However, the variants which most closely resemble tower fermenters of the type investigated here are those known as bubble-columns, and these are also widely used in the chemical industries (Fair, 1967; Mashelkar, 1968). Such systems were reviewed by Oestergaard (1968), who classified two types which approximate to tower fermenters, namely "gas/liquid fluidised reactors" (where the solid phase is suspended by the upward movement of gas and liquid) and "bubble-column slurry reactors" (where the solid phase is suspended by the upward flow of gas-bubbles).

1:4 Early Applications of Tubular Reactors to Fermentation

In view of the widespread role of tubular reactors in chemical process industries, it is perhaps surprising that they have been only slowly adopted by the fermentation industry. Patents and publications describing various designs of tubular fermenter in beer production(Victorero, 1968; De Mattos, 1948), vinegar (Shimwell, 1955; Simonin and Bernard, 1958), activated sludge treatment (Chipperfield, 1967) and citric acid production (Martin and Waters, 1952; Steel, Lentz, 1959; Clark and Lentz, 1963) did not stimulate much parallel activity by the fermentation industry in general.

1;5 Factors underlying Reactor Choices

There are some historical clues as to why S.T.R's have predominated to such an extent. A brief survey of these clues shows that there is now no strong reason why tubular reactors, and tower fermenters in particular, are not precluded as feasible, competitive alternatives to stirred fermenters in many cases.

A fundamental reason seems to have been the initial commitment to S.T.R's during World War II. A lack of adequate process control abilities resulted in a ready acceptance of the "ideal" mixing

characteristics seemingly offered by this option. Subsequent chemical engineering studies were specifically orientated towards this type of reactor, and not towards tubular reactors. Knowledge relevant to S.T.R's was therefore misleading when harnessed to allow assessment of tower-fermenters. Such assessments were most frequently carried out with respect to oxygen-transfer capability. For example, studies utilising the sulphite oxidation test showed that oxygen-transfer was most efficient in S.T.R's when the impeller was working and was proportional to the power input up to an optimum level. It had also been demonstrated that for maximum yield the power input had to exceed a minimum value corresponding to the limiting value of K_La, the "oxygen-transfer efficiency" (Cooper, Fernstrom and Miller, 1944; Homer and Blakeborough, 1963; Richard, 1963). Crude extrapolation of these results suggested that sparged columns were not competitive. There was also by this time a considerable investment of capital in commercial S.T.R's and this was a powerful force for conservatism. Profound commercial secrecy, together with notable isolation between chemical, petrochemical, brewing and antibiotic industries further reinforced this conservative tendency.

In recent years, and with the benefit of lengthy experience in actual fermentations, there has been a realisation that stirred fermenters have drawbacks. Large stirred fermenters require expensive pressure vessels and moving parts and absorb considerable quantities of power (Finn, 1967). They also require very large quantities of cooling water in consequence of the need to dissipate frictional heat. Where filamentous fungi are concerned, the problems are compounded by the typical suspension rheology. Not only does the viscosity become very high (Solomons, 1962, reports 20,000 cp. for A. niger) increasing power requirements, but the rheology becomes

increasingly non-Newtonian (Deindörfer & Gaden, 1955; Solomons, 1962; Takahashi, 1969). This has the effect of restricting the agitated zone to the immediate vicinity of the impeller and creating stagnant zones at the periphery of the vessel. Filamentous fungi are also notorious for their habit of growing on probes, turbine impellers, baffles etc. This becomes a serious problem at the laboratory scale, especially during continuous culture work, where this is often the reason for terminating long fermentations (Finn & Wilson, 1954; Maxon, 1955; Evans, 1965). As will later be apparent, tower fermenters are remarkably free from such problems because they provide a fundamentally different environment.

1:6 Recent Applications of Tubular Fermenters

There therefore seems to be a considerable number of reasons why tubular fermenters in general are being more extensively scrutinised for their possible advantages in certain situations. Hybrid tubular/stirred reactors, some utilising internal air-lift devices such as draft tubes, have begun to appear on the scene. Projects for the growth of biomass on hydrocarbons provide examples of this type, where the high oxidation demand for the hydrocarbons requires the improved gas transfer capabilities of the airlift system (Humphreys, 1968). The I.C.I. pressurised-loop fermenter for the growth of bacterial biomass on methanol is an example of a more overtly tubular system. The advantages claimed for its design (I.C.I. Ltd., 1974) are lack of moving parts, easier maintenance during continuous fermentation, thinner walls, easier heat-transfer, lower construction costs, and a heterogeneity of reaction. In this case, the heterogeneity was used to allow a subtle control of the oxygen partial pressure and the rate of carbon dioxide desorption for maximum biomass yield. Elsewhere, the heterogeneity obtainable in tubular fermenters has been of

particular use where secondary metabolites are the end product of fermentation. In tower fermenters, the desired degree of heterogeneity is often obtained by the use of baffles, assisted by sedimentation effects. Thus, tower fermenters have been used commercially for ale and lager production (Greenshields, 1972), "charging wort" used in vinegar manufacture (Greenshields and Smith, 1971), wine (Peynaud, 1963), novobiocin (Ross and Wilkin, 1968), neomycin (Lumb, Macey, Wright & Petchell, 1970), citric acid (Martin & Waters, 1952; Steel, Lentz & Martin, 1955; Murphy, Clark & Lentz, 1963; Horitsu, 1971) and also in vinegar acetification (Greenshields, 1974). Tower fermenters have also been used for biomass production using yeast (Rosen, 1968; Bennett, Hondermack & Todd, 1969), bacteria (Pannell, 1972) and filamentous fungi (Daunter, 1971; Morris, 1973).

PART TWO :SYSTEMS ANALYSIS APPROACH

The overall appraoch to this thesis embodies many of the conceptual and practical approaches of systems analysis. A brief description of the aims , methods , and relevance of the systems approach follows.

2:0 The origins of Systems Analysis

Many of the techniques of systems analysis were originally gathered together during the second World War by economists , industrial managers, and military strategists under the title of " Operational Analysis ".The techniques were used to analyse and manage complex systems such as the supply and demand logistics of food , ordnance, and materials. Systems Analysis has been widely used in the fields of social behaviour , psychology ecology, traffic theory , design analysis, etc.

2:1 Complex Systems

Complex sytems are multivariate in character and usually involve an intricate interlocking relationship between component parts. Such systems often have to be viewed as a whole because it is either impossible to dissect them into their component parts or because it would leave out essential links and possibly mislead if a purely analytical view were taken.

2:2 Definition of the system

The first major step in the analysis - procedure is to recognise and define the system it is desired to understand. The system is defined according to the objectives of the investigator rather than by practical limitations of knowledge and technique . Consequently the analysis may (A) prevent the formation of conclusions based on inadequate information, (B) bring into focus extra variables which need examination , (C) highlight the need for information on areas previously ignored. Put more simply, it is useful to know exactly what is not understood about the system in addition to knowing that which has been found out about the system already.

2:3 Information Survey

The information necessary for the delineation of the system - boundaries and for the construction of models (or theoretical understandings) of the system is gathered together from as wide a variety of sources as possible. Intuition, literature - survey, pilot experiments, etc. are used to provide an initial impression of the system.

2:4 Systems Diagrams and Other models

One of the more useful ways of recording the inital impression of the system is the use of block- diagrams , also known as systems- diagrams. The very act of constructing such simple models clarifies the understanding of the interactions between variables and allows the convenient inclusion of apparently less plausible, contradictory or alternative theories concerning details of thesystem . The process may itself actively stimulate additional ideas and theories about the system. A useful example of the value of such diagrams is that of the metabolic chart. In addition to providing a simple clear record of our knowledge of metabolic systems , metabolic charts may be the vehicle for postulating newpathways or links between pathways; experimental findings which do not accord with the chart are easily recognised and lead to modification of the chart.

Once sufficient information has been accumulated, a dynamic analogue can be made. The process of experimental feedback, problem-solving,etc. can then be carried out with the aid of a "live " model. Such models may be physico - mechanical, as in the case of clocks, or gyrocompasses, or in more complex situations, computer-based, as in the case of metabolic systems or fermentation-systems.

2:5 Application to This Study

The first important conclusion reached in this work was that the growth and development of A. niger M. 1. in tower fermenters represents a complex system within the above definition(2:1). It therefore has characteristics which require a comprehensive , systems approach, rather than a concentration on specific aspects .

The system includes the behaviour of aerobic tower fermenters as one of its components, necessitating description ofoperating characteristics of tower fermenters. The difference in environment provided by tower fermenters from that provided by stirred fermenters and shake- flasks isof considerable interest from a fundamental viewpoint and has been highlighted as far as possible.Much of the relevant information is diverse and ill-defined, so that the information - stage in this case is an important goal in itself. Parts three , four , and five of this thesis are therefore devoted to collating information . to give a broad description suitable for consultation by any of the disciplines involved.

Many gaps, inconsistencies, and contradictions were apparent, and therefore the experimental programme was devoted largely to qualitative improvements in knowledge. For example, a considerable number of theories are available in the literature which relate to the formation of rounded colonies known as "pellets" and which relate their morphology to their growth kinetics, physiology, etc. These include the suggestion that spore-clumping is a prerequisite for "pellet" formation, or that spherical colonies form as a consequence of abrasion, or that such colonies are incapable of exponential growth, etc. (see the review by Whitaker & Long, 1974)

All the time , there was a need to discover the role of morphological development in determining fermentation behaviour, coupled with the need to discover how , and to what extent, fermentation behaviour could be controlled in order to promote the desired fermentation behaviour. From the point of view of fundamental mycology, such an ability to control hyphal and colonial differentiation would open the way to further study of the biochemical and physiological organisation of the fungus in a variety of colonial types. From a commercial point of view, it seems likely that this ability would be useful in providing additional opportunities for process control and for controlling directly the separation qualities of the mycelium for post-fermentation work, for example, the more compacted colonial forms filter readily under gravity , whereas the more filamentous forms require the skilled application of rotary vacuum filters etc.

PART THREE: ORGANISM-RELATED PARAMETERS

3:0 Introduction

Certain features of the organism may be decided *a priori* to be important features in the system-behaviour . The following sections introduce these factors together with critical appraisals of the experimental theoretical approach to these topics.

For any organism in any environmental system, the interaction of the genotype with the environment determines the outward response of the organism. For reasons previously specified (2:5) , genetic variation has as far as possible been excluded from this work, and section 3:1 briefly discusses the possible role of genetic variation.

The reciprocal interaction of the genotype with the environment provided by tower fermenters elicits a phenotypic response which is expressed in two main ways: the metabolic or biochemical response, and the physiological response. Work carried out using stirred fermenters has highlighted the dynamic, reciprocal interaction in biochemical respects, but perhaps because of the dominance of the shear-forces imposed by the impeller system, has tended to minimise the effect of physiological interaction with the system. In tower fermenters , the physiological response, in the shape of morphological development is seen to play a greater overall role in the system-behaviour. For example, the morphology of the organism may affect $K_L a$ much more than in a stirred tank reactor. The effects of the presence of the organism in this system are here interpreted from the point of view of the morphological interactions within the system. For example, cytochemical variations are examined in the light of their dependence on morphology. (3:8).

3:1. Genetically Determined Characteristics

The intrinsic suitability of any particular mould strain to the fermentation is determined ultimately by its genotype. It is well-known that the morphology of fungal hyphae varies from class to class and from species to species, indeed, this has often been the basis for their taxonomic distinction. Even between distinct strains and mutants of the same species, differences in hyphal structure or dimensions may occur, and therefore one would expect that differences will also occur with respect to the morphological development of various species and strains in submerged culture.

One of the earliest and most comprehensive impressions of species variation with respect to colonial morphology in submerged cultures is given by the series of photographs by Burkohlder and Sinnott (1945). It is important to note that the morphological forms they obtained were all the result of shake-flask culture under very similar conditions and therefore indicate the varied response of a range of species to a particular environment. A similarly broad-based study using simple tower fermenters was carried out by Daunter (1971) as part of a biomass screening survey. Using beet molasses and carob media similar to those used in this study (Methods, 6:4), he observed a considerable variation in the appearance of the colonies formed by filamentous fungi drawn from the classes; Zygomycetes, Ascomycetes and Basidiomycetes and from the Fungi Imperfecti. Particularly notable was the formation of large, hollow spheres or hoops with diameters approaching 30 mm.

Whilst this study has excluded species and strains other than A. niger M.1 in order to reduce complexity, there are strong indications that even with this restriction, the usual stringent precautions must be taken to prevent contamination with other

Lengths of the apical cell (length from hyphal tip to first observable septum) and hyphal compartments of surface hyphae at the periphery of fungal colonies

Organism	Strain no.	Temperature (°)	Glucose concn. (g./l.)	Apical cell (μm.)	First hyphal compartment (μm.)	Second hyphal compartment (μm.)	Third hyphal compartment (μm.)	Fourth hyphal compartment (μm.)	Mean of all hyphal compartments (μm.)	No. of septa: In peripheral growth zone (w) of leading hyphae	No. of septa: Formed/h.
Geotrichum lactis	F I	25	10	290 (±99)*	81 (±7)	76 (±5)	75 (±4)	−†	77	2	2·6
Aspergillus niger	A I	25	10	426 (±181)	65 (±22)	59 (±30)	75 (±20)	−	66	11	2·0
A. wentii	A 9	25	10	449 (±99)	66 (±22)	60 (±30)	60 (±20)	−	62	14	2·5
Penicillium chrysogenum	WIS-54 1255	25	0·2	136 (±16)	29 (±11)	28 (±11)	32 (±8)	28 (±5)	29	13	·2·6
A. nidulans	BWB 224	25	10	299 (±66)‡	52 (±19)‡	69 (±19)	(43 ±10)	53 (±14)	54	6	2·7
A. nidulans	BWB 224	30	10	300 (±64)‡	55 (±22)‡	61 (±14)	−	−	58	7	3·7
A. nidulans	BWB 224	37	10	327 (±54)‡	48 (±13)‡	51 (±17)	46 (±7)	−	48	7	5·6
A. nidulans	BWB 224	37	0·2	270 (±38)§	43 (±18)	46 (±25)	−	−	45	16	6·7
A. nidulans	BWB 480	37	0·2	81 (±31)§	25 (±9)	26 (±8)	23 (±7)	24 (±8)	25	16	8·4

* Standard deviation.
† Not determined.
‡ $P > 0{\cdot}5$. Therefore there is no significant difference between either the length of the apical cells or the length of the first hyphal compartments.
§ $P < 0{\cdot}001$. Therefore there is a significant difference between the lengths of these two apical cells.

Table3:1 Data of Trinci,(1971b)

A. niger strains and to prevent mutation. This is reflected in the precautions taken with stock-cultures (Methods, 6:2, 6:3) and with equipment design (Methods, 6:17).

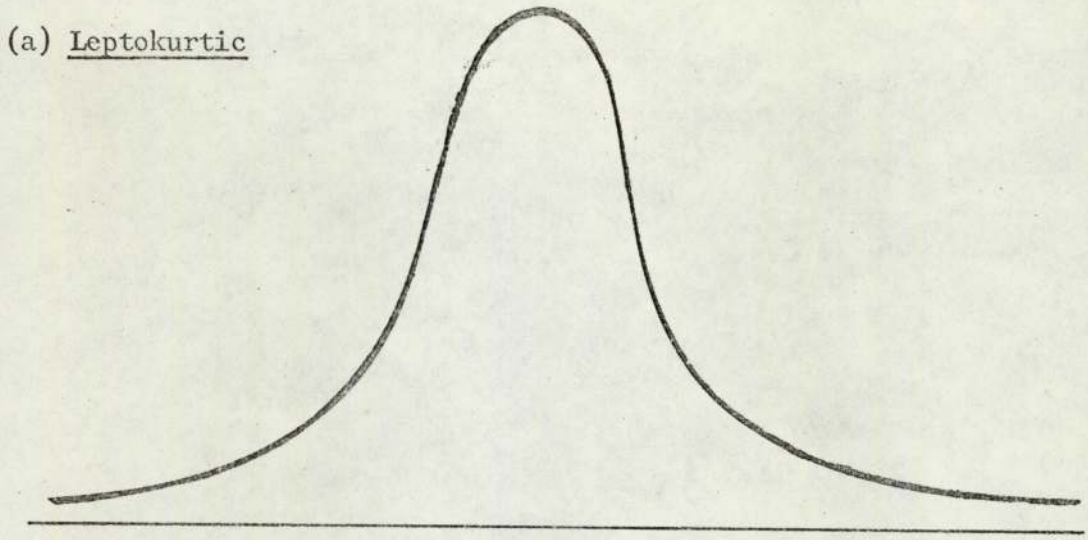
3:2. Description and Quantitation

The detailed study of fungal morphology during submerged culture is subject to considerable difficulties of description and quantitation. Mainly because of a lack of adequate methods, many workers have contented themselves with subjective verbal descriptions such as "flocculent", "pelleted","granular", "gelatinous", "loose pellets" (Whitaker and Long, 1973).

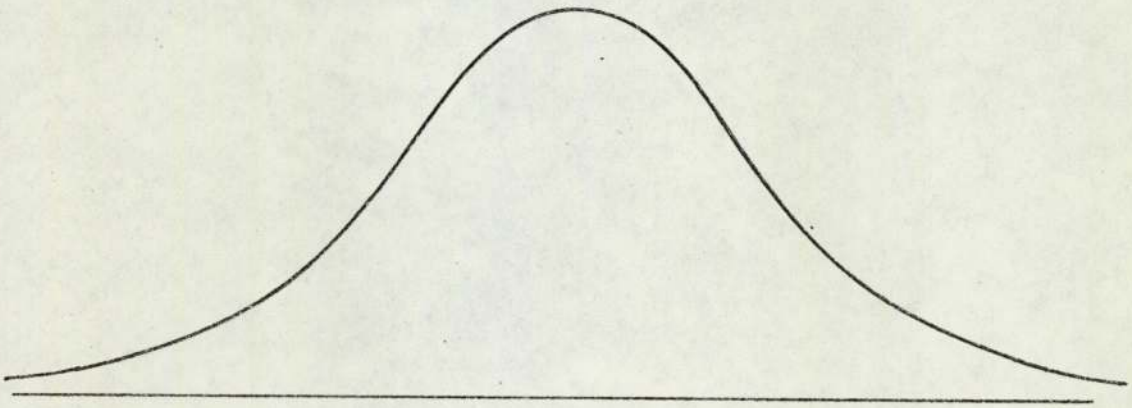
For certain morphologies, notably the purely filamentous form on the one hand, and the essentially spherical form on the other, measurements of various hyphal and colonial dimensions are possible, including mean internode length (Trinci, 1969), hyphal growth unit (Trinci, 1973), radial growth-rate and peripheral hyphal density (Trinci, 1969). However, there are certain problems associated with direct measurements of this type. Even when applied to colonies grown on solid media, where growth is largely restricted to two dimensions, a wide statistical range (or large standard deviation) is apparent (Table 3:1). It can be seen that many of the measurements vary by one or even two orders of magnitude, e.g. the lengths of the apical cells in cultures of A.niger M 1.vary from - 200 μm to approximately 970 μm and those of the second hyphal compartment from 0 μm to 150 μm, based on the assumption that for a normal distribution the approximate range is given by $\bar{x} \pm 3s$, where $\bar{x}$ is the mean and s is the standard deviation. We know, a priori, that compartments rarely approach 0 μm in length; so the distribution of the data is platykurtic (Fig.3) and skewed toward the higher values. In such situations, it is advisable to have very large samples of data, for

Fig.3. TYPES OF SAMPLE DISTRIBUTION

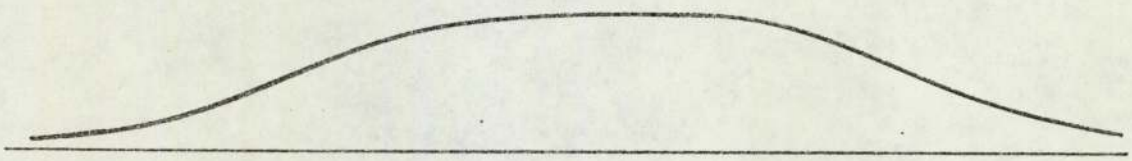
(a) Leptokurtic



(b) Mesokurtic*



(c) Platykurtic



* Normal Distributions are Mesokurtic

reliable comparison between samples. In practical terms, this may mean an unmanageable quantity of measurements, depending on the variable under consideration. There is also a tendency toward diminishing returns, so that the only descriptions possible are vague approximations. It is also obvious from the lack of results for the fourth hyphal compartments (Table 3:1) that even for surface cultures the problem of unambiguous observation is increased considerably as the hyphal density increases. Because of the three-dimensional freedom of growth in submerged culture, such experimental difficulties, and hence statistical variation, can be expected to increase considerably. Many other variables, such as overall hyphal packing, assume a new importance, though it is obviously quite difficult to quantitate by direct observation the packing density of hyphae in certain types of colony.

An alternative approach to the problem of describing morphology is that of recording pictorially the colonial forms which arise. This approach may have advantages in that it is indiscriminate and records details of the morphological development which may not be considered important until later. In other words, photography, autoradiography or even line-drawings can provide comprehensive and faithful methods of description and communication. It is interesting to note that workers using such methods (e.g. Burkholder and Sinnott, 1945; Duckworth and Harris, 1949; Clark, 1962; Bent and Morton, 1963; Yanagita and Kogane, 1963) often avoid the descriptive and conceptual limitations of verbal descriptions and give equal emphasis to forms of growth other than "filamentous" and "pelleted". The problem with pictorial representations is that they are not in themselves quantitative. However, photographs in particular do lend themselves to qualitative distinctions between individuals and in the case of

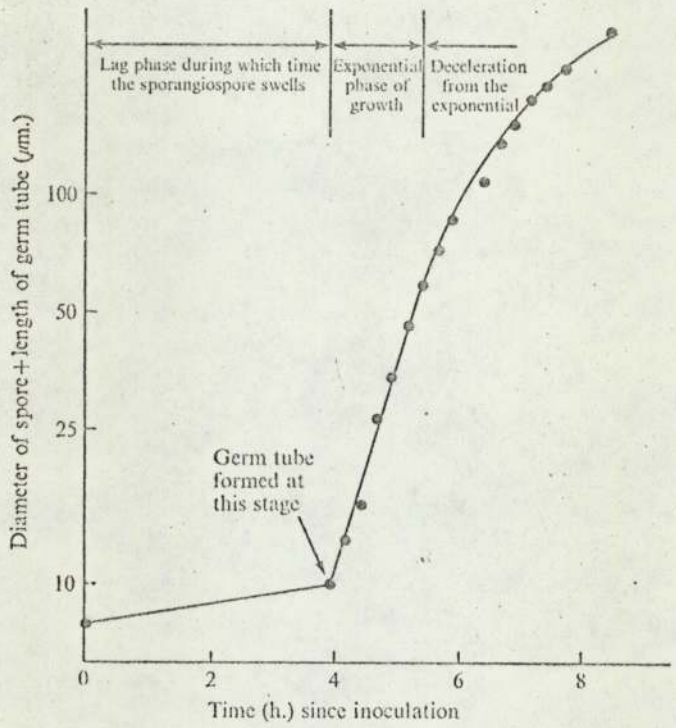


FIG.3:2 Growth of a germ tube of *Rhizopus stolonifer* at 25°. The medium was inoculated with a freshly prepared suspension of sporangiospores.

Data of Trinci (1971b)

parameters which have a platykurtic distribution, such arbitrary distinctions may initially provide as much information as painstaking measurements. This is especially so as the number of separate parameters required to describe each individual increases, so that it becomes increasingly difficult to give data from which the individual could be completely reconstructed. A useful analogy is the task of describing human individuals. There are easily definable, absolute characteristics such as height and weight, and characteristics which initially appear definite, e.g. colour of hair or eyes, but subsequently are seen to form a continuous range, for which an arbitrary system such as a colour-chart must be used. Overall, however, photographs are very useful when such descriptive problems are encountered.

3:3. Hyphal Branching

Butler (1966) has speculated that the gross features of colonies formed during submerged culture of filamentous fungi are a direct consequence of the branching characteristics of the constituent hyphae. It seems reasonable to expect that the degree of branching is also largely responsible for the packing density of hyphae in colonies. The interaction of such features as agitation, mass-transfer and mixing with various morphologies is also likely to differ in important ways, as suggested by Greenshields et al. (1972). Methods of controlling and characterising hyphal branching are therefore of interest. Robertson (1959, 1965) reports success in controlling branching of Hyphomyces species by varying the concentrations of trace-metals, griseofulvin and succinic acid in the culture medium, and many other workers have reported factors which have been found to control branching. A particularly well-documented phenomenon is the production of abnormally short, highly-branched, swollen hyphae under adverse conditions such as lack of oxygen,

high acidity, high ferricyanide activities, toxic metals etc. (e.g. Foster, 1949; Dion, Carilli, Sermonti and Chain, 1954).

3.4. Hyphal Interactions

Any adhesive interaction between hyphae is likely to affect the response of the mycelium to agitation effects. Work devoted to the flocculation of yeast cells has indicated the importance of a phosphate-mannan-protein complex, the formation of which depends on the protein and carbohydrate constituents of the cell-wall (Eddy and Rudin, 1958; Rainbow, 1966). It may be that mould cell walls have an analagous adhesive mechanism, or that they are able to entwine without the help of such adhesive properties. On the other hand, fungal hyphae of many species are known to "repel" each other, as in the case of well-established colonies on agar plates, which form straight edges at boundaries between colonies with a gap between. It is not known what happens to this interaction during submerged culture. The nature of this interaction raises interesting questions about the extent to which individual hyphae are sensitive to the presence of other hyphae. The dominant radial symmetry of fungal colonies which are allowed to develop freely is remarkable. On solid media, hyphae only rarely grow back toward the colony centre, and the distance between hyphae is controlled by some mechanism, forming a regular, neat, fringe to the colony. Interesting exceptions to such rules are provided by the spirally-developing colonies of certain strains of A.niger (Yuill and Yuill, 1954), by the ability of "young" colonies to grow together, and by the tendency of certain combinations of species to grow into each other.

Mechanical interactions between hyphae can be expected to influence the response of colonies to shear forces or the rheological properties of the suspension as a whole.

3.5. Mechanical Properties of Hyphae

Much of the behaviour of a mycelial suspension will depend on the mechanical properties of the hyphae: to what extent are they able to flex? How strong are they? Whilst little information exists regarding such properties, (but see under 5:0) it is important to note that species other than A. niger may have different hyphal dimensions, rigidities and strengths. Biological variation between strains of A. niger, or even for A. niger M. I samples from different environments may be a significant source of variation in engineering terms.

3.6. Growth Kinetics

Most workers have found that the growth of various fungi in submerged culture may obey either a cube-root relationship:-

$$M = K_c.t.M_o^{\frac{1}{3}}$$

where M = mass of mycelium per unit volume t minutes after the mass was M_o, per unit volume and K_c is an empirical constant (Emerson, 1950; Machlis, 1957; Marshall & Alexander, 1960) or that it may follow an exponential relationship :-

$$M = M_o.e^{-\alpha t}$$

where α is an experimental constant (Zalokar, 1959; Pirt and Callow, 1960; Borrow et al, 1964; Choudhary and Pirt, 1965). It is important to remember, however, that these results are empirical results based on total dry weight or cell nitrogen, and therefore include non-viable cells in the estimation. Pirt (1966) argued that exponential growth was associated mainly with filamentous growth and cube-root growth with "pellets", where mass-transfer into cells in the core of the colonies was restricted. He also envisaged a more loosely-packed

type of colony in order to explain his earlier observation that the dry weight values for A. niger colonies could follow an exponential law (Choudhary and Pirt, 1965).

Several workers (e.g. Emerson, 1950; Trinci and Banbury, 1967) have reported that on solid medium, individual hyphae extend according to linear kinetics, and Katz, Goldstein and Rosenberger (1972) have proposed that for an exponentially growing mycelium, the rate of extension reaches a maximum characteristic of the strain. In order to explain the observed exponential growth of fungi in submerged cultures, it has been proposed that linear hyphal extension, coupled with the formation of new branch points, can account for exponential growth (Plomley, 1959; Kubitschek, 1970; Caldwell and Trinci, 1973). However, under certain conditions, individual hyphae may elongate according to exponential kinetics. Trinci (1969, 1971a) and Plomley (1959) have both reported that germ-tubes of a total of nine different species (including an A.niger strain) elongate exponentially. It has also been shown (Trinci, 1969; 1971a; Trinci and Gull, 1970) that the estimated specific growth rate in such cases was higher than that of submerged cultures. It has been pointed out by Mitchison (1973) that an exponential increase in length or volume of cells does not necessarily mean that the dry weight will increase at the same rate, but nevertheless, the increase in dry weight is probably exponential. Trinci (1971a) reported that for the strains he tested, the initial high specific growth rate entered a decceleration phase when the germ-tube reached a certain length. He does not show whether the deceleration resulted in a lower doubling time or in linear kinetics (Fig.3:2) Machek and Fencl (1973) used a special growth cell to observe the elongation of hyphae in a liquid medium flowing past the hypha under invest-

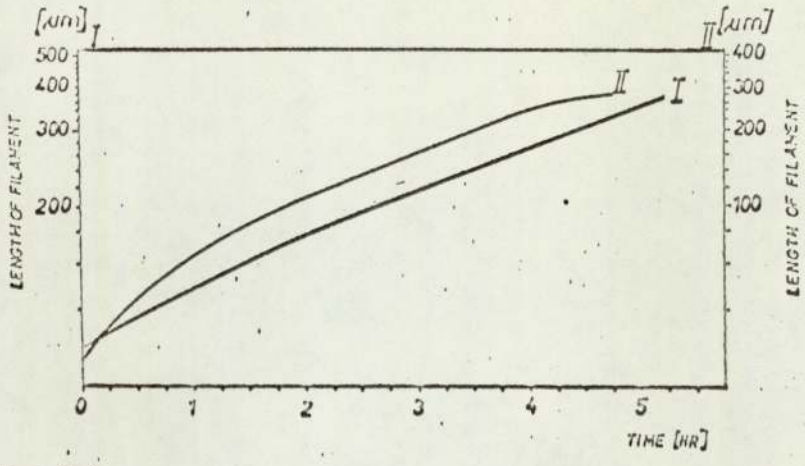


Fig. 3:3 *Aspergillus niger* filament elongation measured in submerged culture. (After Machek & Fencl)

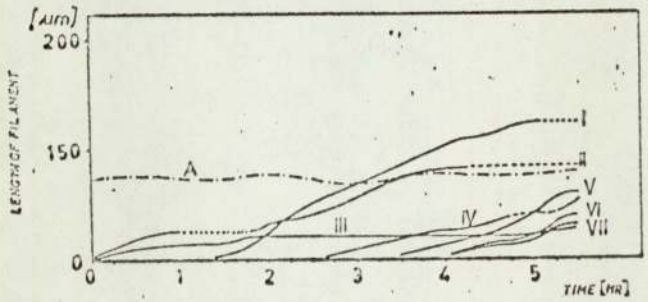


Fig33 Length of nongrowing portion of filament and of branches. Curves I-VII indicate the length of branches; curve A the length of the nongrowing portion of the filament. (After Machek & Fencl)

igation. They found that in this situation , hyphae could elongate according to either linear or exponential rules, but that there were many transient,intermediate types of growth (Figs.3:3 and 3:4). From the graph (Fig.3;3) it can be calculated that the doubling-time is approximately 1.7 hr, whereas Trinci (1971a) calculated a mean doubling time of 2.54 hr, standard deviation 0.42 hr (Sample-size > 10). The apparent contradiction may be a result of strain differences or of differences in the conditions, and it is interesting to note that in Machek and Fencl's system (Fig.3:3 , curve I and II) the initial growth within fifteen minutes of the start must be at a higher logarithmic rate than subsequently, and that (curve II) the decceleration phase certainly starts after about 20 - 30 μm of hypha have been formed. Fig.3:4 shows that the sum of elongation of all the branches forming from a single length of hypha probably follows a logarithmic relationship. Smith (1924) also reported that on solid media individual hyphae longer than about 200 μm were able to extend exponentially.

These results suggest that the model for exponential growth based solely on the influence of branching on linearly-extending apices may be too simple. The comparison of a fungal hypha with a series of single cells (Kubitschek, 1970) neglects the functional differentiation within a hypha which allows several cells to contribute to the growth of an apical cell (see "Functional Considerations of Growth" 3:7). In other words, the cells in this case are able to interact and this interaction has previously been neglected in theoretical treatments.

The only inference which is consistent with all the above results is that individual hyphae may elongate according to linear or exponential rules, depending on the favourability of their immediate environment, and on the origin and length of the individual hypha. Thus, germ tubes and long hyphae may achieve exponential growth, but long hyphae have a lower specific growth rate than germ tubes, possibly because of competition and other interactions between hyphae which may occur at this more advanced phase of growth. Short hyphae which originate as branches probably grow according to linear kinetics until they are about 100 μm in length (Machek and Fencl, 1973) after which they may, under favourable conditions, achieve exponential growth. Such a view of the kinetics of fungal growth is consistent with the idea that fungi maintain a subtle dynamic relationship with their environment as mentioned earlier, and implies that fungal hyphae may adjust their rates of elongation and branching to match the environmental conditions.

3.7. Fundamental Considerations of Growth

As mentioned in part 3.6, there has been a tendency where growth kinetics are concerned to regard fungal hyphae as chains of functionally discrete cells. This does not accord with the present understanding of hyphal growth. It has been established that the growth of each hyphal apex is physiologically dependent on a length of subapical hypha which has ceased to extend, but which contributes its biosynthetic activities to the extending apical region, (e.g. Zalokar, 1959; Yanagita and Kogane, 1962; McClure, Park & Robinson, 1968; Nagasaki, 1968; Fencl & Machek, 1970; Magee et al., 1970; Gooday, 1971; Trinci, 1971b). The approximate length of this functionally active zone may be determined in the case of colonies growing on solid medium by determining the minimum distance from

the apex at which the hypha may be severed without reducing the extension rate (Ryan, Beadle & Tatum, 1943; Clutterbuck & Roper, 1966; Trinci, 1971b). In the case of side branches from such hyphae, there is no such information available, but we are led to consider what happens when branches are too short to have their own "support zone". Plomley (1959) has proposed the concept of a "growth unit", implying that each branch is supported by a specific portion of the main hypha. Measurements for A. niger strains by the peripheral growth zone method indicate that at the periphery, the approximate length of hypha involved is 1200 μm (Lhoas, 1968; Trinci, 1971b). Branches are usually formed much closer together than 1200 μm, and therefore probably share the same "growth unit". This might restrict the rate of growth of branches until they reach a certain length, as observed by Machel and Fencl (1973).

In attempting any kind of extrapolation of the available data to the study of submerged cultures, there is a need to consider the effect of the extra degrees of freedom present in this type of system. It is possible to speculate, for instance, that if the peripheral growth zone of an approximately spherical colony 5 mm in diameter were 1200 μm, then this zone would occupy 13 - 14 mm^3 of a total colony volume of 66 mm^3, i.e. approximately one-fifth of the colony volume. However, it may be erroneous to assume that the peripheral growth zone will not change in character and dimensions in such a different environment. Pirt (1966) envisaged that some very compact spherical colony-types have a peripheral zone to which growth is restricted because it is the only part of the colony in which mass transfer is adequate, resulting in "cube root" growth. However, his observation that a less compact type of colony could grow exponentially (in terms of dry weight) suggests

that radical differences in functional organisation may occur between different colony types. It seems probable that colonies in submerged culture will develop according to more varied patterns than those on solid media. The extra spatial dimension places a much greater importance on the role played by hyphal branches in mycelial proliferation. Even the concept of a peripheral growth zone may become redundant if mass transfer occurs freely between all parts of the mycelium and the medium. This is the situation envisaged by many authors for filamentous growth in submerged cultures, but the intermediate types of growth between the filamentous form and the compact spherical form may also come into this category. A. niger strains grown in stirred fermenters as the filamentous form often produce filaments considerably less than 1200 μm in length and the question arises of whether the growth zones are reduced pro rata or the filaments are undergoing physiological damage caused by rupture within "growth zones". There is an obvious need to understand more fully the interaction of the inherent physiological organisation of fungi with the environments provided by the various forms of submerged culture. To what extent is the metabolic and morphological development of the mould controlled by limiting environmental conditions rather than by its own control systems and/or physiological capacities?

PART FOUR : A SURVEY OF THE INFLUENCE OF MEDIUM COMPOSITION ON THE GROWTH OF A. NIGER STRAINS

A great number of medium ingredients have been shown to influence the morphology of fungi under a variety of conditions, and this part of the thesis consists of ageneral survey designed to put in context those factors which were studied experimentally. As previously stated, this study was centred almost exclusively on a single A. niger strain because of the extensive literature describing the response of A. niger strains to environmental changes. The basis of the experimental approach was to survey widely the variables involved, then to select from these the variables which could be conveniently exploited, especially under industrial conditions.

Thus, it was possible to restrict the number of variables to be considered and to search for putative common denominators which might be useful in a wider sense. Interpretation of the published evidence is complicated by the existence of genetic variation within the species, even to the point where results appear contradictory, but it is still possible to use the information if care is exercised.

4:1 Trace Metals

Trace metals are known to have a profound effect on the growth of A. niger strains, particularly in the production of organic acids, and the use of A. niger strains for the bioassay of the levels of potassium, mineral nitrogen, mineral phosphorus, molybdenum, magnesium, and manganese in agricultural soils indicates the extent to which the species is affected (Mehlich, et al , 1933; Zodrow, 1966; Walczyna, 1966; Nicholas and Fielding, 1950; Sulochana and Laksmanan, 1968).

The roles played by these cations in tower fermenters may be complicated by the fact that several of them are known to act as flocculating agents and they will also form chelates which can affect the physico - chemical balance of the medium.

Magnesium and Calcium

Salts of magnesium and calcium are very often used as precipitating or flocculating agents in chemical processes and are also known to assist flocculation of antibody/antigen mixtures and of suspended yeast-cells. Nowosielski (1960) used the A. niger method of Nicholas and Fielding (1950) to measure the magnesium content of soil-extracts made in 10% ammonium acetate or hydrochloric acid and found good agreement with results from flame-photometry. This colinearity held until a threshold point is reached at around 0.1% magnesium when abnormal growth was found (Zodrow et al.,1966). At higher concentrations, particularly above 0.306% magnesium, the medium probably becomes toxic to A. niger (Kauffmann-cosla et al., 1946). Measurement of biomass yields should be carefully conceived, since magnesium content has been shown to alter the water content of A. niger tissues. In particular, using Rawlin's medium Kauffmann- cosla and Tudor (1946) found that a drop in magnesium from 0.34% to 0.0002% was accompanied by a drop of 89.4 to 78.7 g water per 100g tissues at 37°C after 96 hours. In addition, a triple interaction has been found between the action of different concentrations of zinc, cadmium, and magnesium salts, with magnesium acting as an antagonist to the toxic effects of the other two (Laborey and Lavollay, 1967). These findings should, however, be subject to experimental verification in the light of the findings of Zodrow et al. (1966), who tested 17 A. niger strains over the range 0 - 1000 µg/10 ml. Six strains did not respond to magnesium, eight responded at about the same rate, and three responded very markedly.

Calcium seems to have little effect on A. niger except when other essential trace-elements are missing (Garnier and Kientizler, 1968).

Molybdenum

Aspergillus niger strains have been used as indicators of molybdenum content in soil (Nicholas and Fielding, 1950) and in chemical solutions using molybdenum-starved spores (Bussler, 1970). Molybdenum-deficient cultures were found to have shortened, irregularly thickened, malformed hyphae. More specifically, Raman et al. (1962) found that molybdate was inhibitory to growth of A. niger in the range 2 - 10 mg/10 ml, but that methionine prevented both inhibition by, and uptake of, molybdenum ions. This antagonistic effect between molybdenum and methionine is paralleled in the case of Neurospora crassa (Sastry et al.,1958) except that only 50 mg methionine were sufficient to produce a similar antagonism with the latter.

Manganese

Solochana and Lakshmanan (1968) tested five strains of A. niger for their quantitative responses (mycelial dry weight) to graded concentrations of manganese in otherwise optimal media as regards trace-elements. No significant increase in weight occurred above 0.006 mg/ml. This corresponds with earlier results by Steinberg (1939).

Copper, Iron and Zinc

The essential importance of these three heavy metals to the nutrition of fungi in general has been recognised since the very first studies. Copper has been determined biologically using A. niger (Upitis et al., 1966) and whilst all three are essential in trace amounts, they are very easily found in inhibitory concentrations (Fiedler, 1959; Laborey and Lavollay, 1967; and Upitis et al., 1966). Their presence in either excess or deficit produces morphological abberations and growth-inhibition.

TABLE 4:1 EFFECT OF CONCENTRATION OF MANGANESE ON THE GROWTH OF ASPERGILLUS NIGER

Concentrations of manganese (µg/ml)	NRRL 346	NRRL 334	NRRL 323	MUBL 1	M
	Mycelial dry wt. (mg)				
1. 0.000	146	150	140	87	153
2. 0.001	216*	211	183	177	252
3. 0.002	229	202	266	264	278
4. 0.004	259	273	300	345	295
5. 0.006	275	310	311	371	307
6. 0.008	292	303	314	375	292
7. 0.010	291	307	309	381	303
8. 0.020	298	301	309	387	310

* differences between responses joined by same bar are not significant at the 5% level by the F. Test.

Data adapted from Sulochana and Lakshmanan (1968).

TABLE 4:2 THE INFLUENCE OF METHIONINE ON GROWTH AND MOLYBDENUM UPTAKE BY A. NIGER, WHEN MOLYBDENUM IS AT TOXIC LEVELS.

Supplements to 10 ml medium	Dry wt. mycelium mg.	Mo uptake (µg/100 mg dry wt. mycelium)
None	189.0	
5 mg Mo	46.5	4119
5 mg Mo + 200mg Methionine	172.4	160
10 mg Mo	24.1	4350
10 mg Mo + 200 mg Methionine	162.0	807

Adapted from Raman et al. (1962)

Lithium, Sodium, Potassium and Rubidium

The presence of these ions stimulates sporulation in the order Li>Na>K>Rb, and potassium has a marked growth promoting effect which none of the others completely replaces (Fiedler, 1959; Garnier and Kientizler, 1968). A sodium/potassium relationship similar to that found in most flora and fauna has been demonstrated. The growth response to potassium is particularly marked, and this is another metal whose bioassay in soils has been carried out using A. niger (Mehlich et al., 1933; Walczyna, 1966).

Aluminium

Bertrand (1963) cites aluminium as a "Dynamic trace element for Aspergillus niger". There may be other trace elements of nutritional importance to A. niger but it seems highly unlikely that any of these would be lacking.

4.2. Toxicity of Heavy Metals.

The toxicity of zinc, copper and cadmium has already been discussed, but several other heavy metals are likely to be present in fermentation media. Zlochevskaya and Rabotnova (1966) showed that a concentration of 296 mg/free lead ions per litre only slightly affected mycelium production and did not suppress sporulation. Mercurial compounds of various kinds may also accidentally occur in culture fluids; from agricultural fungicides, for example. A. niger will tolerate relatively high concentrations of mercury and organomercurials, probably by a mechanism involving the protective over-production of non-proteinaceous thiol compounds, as noted by Ashworth and Amin (1964). The toxicity of silver and mercury does, however, double for each threefold increase in

TABLE 4:3 EFFECT OF ADDING POTASSIUM CHLORIDE ON THE DRY WEIGHT OF MYCELIUM BY A. NIGER

% K_2O equivalent of KCl added	Mycelial dry wt. gm.
0.001	1.64
0.002	2.39
0.003	2.85
0.004	3.68
0.005	4.35
0.006	4.70
0.007	4.64
0.008	4.60

Adapted from Mehlich et al. (1933)

Effects of the salts of these metals via their influence on osmotic pressure are discussed later.

exposure time (McCallan and Miller, 1957). It seems that the chief concern with lead, mercury and silver may be their toxicity to animals in the end-product of biomass fermentations. The rare-earth elements, whilst inhibitory, can be detoxified by the formation of rare earth oxalates (Talburt and Johnson, 1967). Borax has been found toxic to A. niger at levels around 1.3 g/l (Bowen and Gauch, 1966).

4.3. Chelating Agents

The presence of chelating agents has a profound effect on both nutritious and toxic concentrations of free trace-metal ions and hence on the metabolism, morphology and sporulation of A. niger. The production of organic acids by Aspergillus species is heavily dependent on the nutritional control of metabolism, using chelating agents to prevent the functioning of enzymes which need metallic cofactors. Specific blocking of metabolic pathways allows the accumulation of kojic, itaconic, citric, gluconic or oxalic acids. Examples of chelating treatments for citric acid production using ferrocyanide salts are given by Gold and Kieber, 1967; Shoedler, 1968; Perlman, 1943; Gerhardt et al., 1966; Bernhauer et al., 1949 and Martin and Waters, 1952).

Ferrocyanide salts are also potent inhibitors of respiration in their own right, and obviously, metabolic inhibition such as this seriously retards growth rates, but work using EDTA has shown that the final maximum weight under otherwise constant conditions was the same with or without EDTA (Bertrand and de Wolf, 1960). Many naturally occurring media constituents, e.g. organic acids such as citric, gluconic and humic acid, are chelate-formers and it is interesting to note that these may act as buffering agents for the metals in a manner analagous to that of pH buffers (Choudhary and Pirt, 1965a, 1965b).

4.4. Nitrogen and Phosphorus

An adequate supply of nitrogen and phosphorus compounds is essential for this system to function efficiently. A. niger is heavily dependent on phosphorus compounds (Marchesini, 1966) which are known to be incorporated into the RNA of the highly active peripheral extending zone of A. niger colonies and pellets (Yanagita and Kogane, 1962, 1963a, 1963b). A common inorganic source of nitrogen for moulds is as the nitrate or ammonium salts. Nitrate is reduced via nitrite to ammonium ion both intra and extracellularly (Kostychev and Tsvetkova, 1920; Iteryott, 1936; Ivanov et al., 1936) and converted to amino acids. Whilst both nitrate and ammonium ion may be absorbed by the hyphae, fumarate, succinate and malate assist the absorption of ammonium more than that of nitrate; citrate, α-ketoglutarate and oxaloacetate vice-versa (Peter, 1956). Amino acids, purine or pyrimidine compounds may be utilised instead (see later) and it has also been shown that A. niger can utilise betaine, nicotine, quinine, pyridine and even cyanides by adaptive enzyme formation when starved of nitrogen sources (Ivanov et al., 1936).

4.5. Amino Acids

The presence of individual amino acids in addition to inorganic nitrogen sources has a stimulatory effect of growth rate (Delic-Koch, 1957; Kasatkina, 1961; Liczuk and Galeziewska, 1968). Mixtures of amino acids have an even greater stimulatory effect (Ibid, idem). Low nitrogen to carbon ratios in the medium increase the likelihood of sporulation taking place (Galbraith and Smith, 1969b) and synthesis of fats becomes a prominent metabolic function (Prescott and Dunn, 1959).

4.6. Purine and Pyrimidine Concentrations

Most pyrimidines and purines other than vitamins seem to help growth, since they play an important part in metabolism. However, derivatives and analogues of nucleic-acid bases, for example, caffeine, theobromine, 5-bromouracil etc. can be strongly inhibitory by prevention of nucleotide base synthesis, or by base-substitution and consequent mutagenic effects. (Hayes, 1968)

4.7. Lipid Content

Of the various lipid classes the fatty acids are probably the most likely class to be found in fermentation media of vegetable origin. They are important for A. niger as inhibitors of growth, spore-swelling and spore-germination, particularly the short chain fatty acids, including formic, acetic, butyric, propionic, capric, caprylic, valeric, isovaleric, levulenic, undecyclic, 2,3-nonenoic, 2,3-decenoic, 10-undecenoic, 2-3 dodecenoic and 2,3-tridecenoic acids (Fencl and Leopold, 1956; Philip et al., 1963; Ozaki and Baba, 1943; Millis et al., 1963; Romano and Kornberg, 1969).

The acids are thought to act at the cell surface by preventing uptake of nutrients and they are known to prevent phosphate and sulphate absorption in A. niger. This effect can be partly reversed by excess phosphate but not by excess potassium, sodium or ammonium ions. The dissociated and associated forms show very different levels of inhibitory effect (Fencl and Leopold, 1957). Higher fatty acids, including oleic and linoleic, but not palmitic acid, found in plants can be utilised as sole carbon source by A. niger (Waheed Khan and Chughtai, 1960). Peanut oil and tributyrin have also been found to inhibit spore germination for one A. niger strain (Millis et al., 1963). However, although it was found that peanut oil and maize oil could be utilised by A. niger during growth as

sole carbon source, they could not replace sucrose for citric acid production and thus they did not form an alternative supply of acetyl coenzyme A. in this case (Millis et al., ibid, idem.). Lewis, 1970, reports the formation of methylketones from fatty acids and his paper is a useful source of references on fatty acid utilisation by Aspergillus niger and other fungi. He concludes that fatty acids may play an important part in the regulation of metabolism in A. niger. The effect of sterols on growth of A. niger is interesting. In certain cases, 0.5 to 1.0% cholesterol additions were found to increase the dry weight of shake cultures of A. niger by 122 to 160% (Matkovics, 1957; Matkovic and Pulay, 1958) and Hanifa, Moursi and Refai (1968) found the following effects of hormones on A. niger (Table 4.6) Obviously, the results on steroid hormones are not sufficiently quantitative but they did seem to merit further investigation. It is well known that moulds can transform steroids, (as a means of detoxification probably) and A. niger is also known to produce ergosterol independently of a steroid source (Izuka et al., 1962; 1965), in amounts ranging from 1.10% to 0.31% of dry weight (Data from 60 standard strains).

The effect of lipids as surfactants may also be significant, depending on the amount in the medium.

4.8. Alcohols

The low molecular weight alcohols seem to have a similar inhibitory effect on Aspergillus niger. Methanol in particular inhibits sporulation, sugar-consumption, growth-rate and stimulates citric acid production. The most effective concentrations were 3% (Uchi et al., 1965; Qadeer et al.,1968) and 49%(Echevarria and Varela, 1959). Ethanol and propan-2-ol were found to have a similar but less marked effect at an optimum concentration of 2% (Qadeer et al., 1968) and

methanol, ethanol, propan-2-ol, butan-1-ol and 2-methyl-propan-1-ol were all found to affect the morphology of A. niger (Qadeer et al., 1968; Puente and Regueiro, 1962).

Alcohols can have a strong antifoam effect, especially at chain lengths of about eight carbon atoms, and they can be expected to be detrimental to aeration efficiency in tower fermenters (see later). However, at higher chain lengths colloidal properties take effect and seem to promote an overall beneficial action on growth of A. niger (see under "polymers".)

4.9. Available sulphur

Sulphur is yet another element for which Aspergillus niger has been used as a bioassay agent to indicate the amount of this element actually available to plants (Katta and Lynd, 1965; Naik and Das, 1964). Maximum growth (final dry weight) was obtained with three different strains at 2 mg sulphur as Na_2SO_4/50 ml culture at 34°C. Sulphate, sulphite, thiosulphate or preferably organic sulphur compounds such as cystine, cysteine and methionine can be utilised by A. niger but thiocyanate and sulphide ions cannot. Growth rate is depressed by sulphide ion concentration. (Doubly important, because the concentration of this ion may be increased by bacterial activity in stored media: see under 'Parasites and Contaminants').

Tracer experiments (Robson, 1957, 1958, 1963c) have shown that radioactive sulphur is accumulated largely in the spores and Takebe (1960) found that choline sulphate was stored to the extent of 1.5% w/w dry spores compared with a mycelial content of 0.2 - 0.3% (Woolley and Peterson, 1937). This compound seems to act as a reservoir for energy and sulphur, since spores can germinate on sulphur free media and the compound is broken down during germination.

TABLE 4:4 THE EFFECT OF GLUCOSE AND GALACTOSE, SINGLY AND IN COMBINATION ON THE MYCELIAL YIELD OF ASPERGILLUS NIGER IN LIQUID CULTURE AT 20°C after 7 days. (from Horr, 1936)

Carbon Source		Yield (mg dry wt)
Galactose	10	45.1
	18	42.4
	20	44.3
Glucose	2	145.6
	10	411.0
Glucose + Galactose	2 + 18	577.0
	10 + 10	1151.6

4.10. Carbohydrates

A knowledge of both the types of sugar and the amounts of each is necessary for fermentations using A. niger. Not very much information is available, but that which is available indicates interactions between utilisation of sugars: D-xylose stimulates galactose utilisation (Lindberg, 1967), fructose is utilised in preference to glucose (Brannon, 1923) and a galactose/glucose mixture is utilised far better than either sugar singly (Horr, 1936 - see Table 4:4

With a variety of single sugars as sole carbon source, Hasija and Wolf (1970) found that the growth rate and final mycelial dry weight were not the same in all cases. Hexoses were generally as good as pentoses, with certain exceptions which seem to depend on the strain. Disaccharides and starch were also satisfactory with the exception of lactose on which growth was poor. Lactose has been found in washed mycelium and this has been taken to show that it is used directly (Lilly and Barnett, 1953). Note particularly the high growth rates and final dry weights on maltose in Table 4:5

Takami (1968) found that vitamin production by A. niger was affected by the type of sugar and that maltose, sucrose and glucose gave better yields of vitamins than other sugars tested.

A. niger also produces polysaccharides, some of which may escape into the medium and alter its characteristics, for example, polyols and glucosans such as nigeran (Kawamura and Baba, 1964; van Sumere and Shu, 1957; Barker et al. 1953a, 1953b, 1954, 1955, 1956, 1957, 1958). A.niger is not as tolerant of high osmotic pressure as other Aspergillus species such as Aspergillus flavus and 50% glucose has been cited as being "toxic" (Kaufmann-cosla and Tudor, 1946). The speed with which A. niger uses up or excretes sugars at high sugar concentrations could therefore have a bearing on its growth rate through an osmotic effect.

TABLE 4:5 FINAL DRY WEIGHT, GROWTH RATE AND CELLULAR SUGAR CONTENT OF *A. niger* V.S. PRESENCE OF SINGLE CARBON SOURCES AND CONCENTRATION OF SUGARS IN THE MEDIUM (From Hasija and Wolf, 1970).

Sugars	Days of incubation	Dry wt. in mg.	Growth rate in mg.	Presence/ absence of sugar in the med.	Concentration of sugar in the mycelium (mg/10 mg.mycelium)
D. Glucose	5	14.1	14.1	+	6.5
	10	39.2	25.1	+	4.0
	15	40.5	10.3	traces	6.0
D. Fructose	5	14.5	14.5	+	7.5
	10	39.3	24.8	+	5.5
	15	55.8	16.5	-	0.1
D. Galactose	5	18.1	18.1	+	2.0
	10	40.3	22.2	+	2.0
	15	49.2	8.9	+	1.75
D. Mannose	5	44.9	44.9	+	negligible
	10	79.1	34.2	+	negligible
	15	110.9	31.8	+	3.0
L. Sorbose	5	traces	0.0	+	-
	10	6.4	6.4	+	3.5
	15	21.1	14.7	+	6.5
D Xylose	5	38.5	38.5	+	negligible
	10	78.7	40.2	+	negligible
	15	112.3	33.6	+	0.01
L. Rhamnose	5	14.4	14.4	+	0.75
	10	37.5	23.1	+	0.5
	15	40.9	3.4	+	0.1
D. Ribose	5	Nil	0.0	+	-
	10	traces	0.0	+	-
	15	traces	0.0	+	-
Sucrose	5	16.7	16.7	hydrolysed fructose +	4.0
	10	32.5	15.8	- do -	8.0
	15	58.3	25.8	- do -	5.0
Maltose	5	73.6	73.6	hydrolysed glucose +	0.01
	10	144.3	70.7	-	0.1
	15	146.5	2.2	-	0.75
Lactose	5	traces	0.0	+	-
	10	8.0	8.0	+	3.0
	15	13.3	5.3	+	4.5
Cellibiose	5	41.8	41.8	+	2.0
	10	97.3	55.5	hydrolysis starts. cellibiose & glucose present	1.75

TABLE 45 (continued)

Sugars	Days of incubation	Dry wt. in mg.	Growth rate in mg.	Presence/ absence of sugar in the med.	Concentration of sugar in the mycelium (mg/10 mg mycelium)
Cellibiose	15	120.2	22.9	Glucose present & one Oligo-saccharide Rf.0.08 appears	0.5
Starch	5	53.9	53.9	Hydrolysed maltose & glucose present.	0.09
	10	119.0	65.1	- do -	0.08
	15	108.5	-10.5	-do- also one oligo Rf.0.07 appears	0.75

Another very important carbohydrate parameter is the level of various sugar acids and TCA cycle acids in the medium. Aspergillus niger is famous as an industrial provider of citric and gluconic acid and it has been noted that besides having a drastic depressant effect on pH, these acids may act as energy-storage compounds in a manner analagous to that in higher plants (Ranson, 1965). In the citric acid fermentation, the concentration of citric acid begins to decline after a peak value because it is used as a substrate. It is interesting to note the competitive advantages of acid production; both in an industrial fermenter and in a natural environment, carbohydrates of value to other organisms are removed and the pH lowered to a level inhibitory to many micro-organisms.

4.11. Surfactants

Antifoam and detergent compounds both have the effect of reducing oxygen-transfer in tower fermenters (Murphy et al., 1959; Jones, 1972). The addition of antifoam increases the bubble size and detergents can cause serious foaming problems. Some antifoams may inhibit the mould, or be used as a carbon source. An interesting feature of detergents is that they may affect the morphology. Takahashi et al., (1960a, 1960b, 1965) found that 0.05 - 0.1% non-ionic detergents encouraged filamentous growth of A. niger e.g. sucrose monosteareate, sorbitan monolaurate, sorbitan trioleate and Span 20 increased dry weight and prevented pellet formation. Tween 20 was inhibitory, i.e. it encouraged formation of large pellets. This effect was also measured using mycelial inocula, so the effect was not that of controlling spore clumping (see Morphology).

4.12. Contaminants

In spite of the above, and other competitive advantages, Aspergillus niger does have a small number of potentially trouble-

some microbial antagonists and parasites, and these fall into two distinct groups where this project is concerned. The first group are persistent parasites which affect the stock cultures and seed cultures during germentation work with A. niger strains. Penicillium purpurogenum and P. rugulosum parasitise living or dead mycelium of A. niger, giving rise to a red "bloom" on surface cultures. Such growth is made possible by a battery of lytic enzymes (Leopold, 1953; Leopold, Valtr and Seichertova, 1958). Elimination is very difficult, although possible (Leopold, 1953). It seems the best defence is painstaking care over the manipulation of culture collections and seed cultures. A complicating factor in the handling of stock and seed cultures is the contamination hazard presented by mites, which usually carry Cephalosporium species. Once established or introduced to a culture collection, they are notoriously pervasive. A more complete description including eradication measures is given by Smith (1969).

The second group are possible contaminants which may be transient or persistent but which distort fermentation results or produce "morphogens". Various bacteria of the Enterobacteriaceae and Bacillaceae produce hydrogen sulphide which inhibits growth of A. niger. These are commonly found in ingredients such as beet molasses (Seichert, L., 1962; Karklisn and Berkholde, 1965). They may also produce substances which alter the growth pattern and modify morphology (Koltin, Y.L., & Chorin-Kirsch, I , 1971; Moore-Landecker, E. & Stotzky, G , 1972). Whilst such microbial interactions may provide an interesting subject for future study, for this work, stringent precautions were used to prevent such effects from taking place.

Certain yeasts and bacteria, especially spore formers are also persistent contaminants if allowed to enter fermentation equipment where A. niger is being cultured, even at pH values of 2.0 or less (Milsom, 1969; Morris, 1972).

4.13. Plant Growth Factors and Alkaloids

Plant growth factors are undoubtedly present in most industrial media because of the predominance of vegetable extracts. For instance, the carob extract medium used in this study (METHODS 6:4) contains gibberellic acid (Corcoran & West, 1968) and has a high tannin content (Most, 1971). Plant hormones increase dry weight yields of A. niger at low concentrations but are inhibitory to A. niger at higher concentrations, e.g. 2,4-D is an effective stimulant at concentrations as low as 0.5 - 1 ppm or 10^{-6}M (Smith & Shennan, 1966; Ito & Surano, 1952; Arnold et al, 1966; Sankhla & Sharma, 1969). Other plant substances which are known to affect A. niger strains include coumarins and these have been shown to be effective inhibitors at 100 - 1000 ppm (Knypl, 1963). Certain halogen and alkyl derivatives of coumarins have even been proposed as fungicides (Stauffer Chemical Co., 1966). Tannins at 15 - 20% w/v stimulate "luxuriant" growth (Neithammer, 1956).

4.14. Polymers

Synthetic polymers including polyvinyl acetate, polyacrylate, polymethacrylate and carboxypolymethylene have been observed to stimulate growth of A. niger strains (Botri, Cieri and Giodani, 1964; Elmayergi and Moo-Young, 1973; Elmayergi, Scharer & Moo-Young, 1973). It may be certain that certain detergents and other polymers increase mass transfer across the cell wall either by enhancing hydrodynamic slip (Oldroyd, 1949; Oldroyd and Toms Davies,

TABLE 4:6 EFFECT OF PLANT GROWTH REGULATORS ON GROWTH RATE (dry wt. in mg after 6 days at 28°C) of Aspergillus niger

Growth Regulators	Concentrations in ppm					
	0.0	0.5	1.0	5.0	25.0	50.0
CONTROL (mean of five)	185	-	-	-	-	-
1-Naphthylacetic acid (NAA)	-	232*	202	196	179	166
2-Naphthoxyacetic (NOAA)	-	233	264	237	208	197
Indole-3-yl-propionic acetic acid (IPA)	-	203	225	212	176	134
2,4,5-trichloro-phenoxyacetic acid	-	202	226		187	126
Colchicine	-	281	309	287	282	227

Adapted from Sankhla and Sharma (1969)

*Each weight is the mean of three

1949; Shaver & Merrill, 1959; Hoyt & Fabula, 1965; Moo- oung, Hirose & Ali, 1970), or by facilitating more rapid diffusion at the cell wall/medium interface (Elmayergi & Moo-Young, 1973).

PART FIVE: CHEMICAL ENGINEERING ASPECTS

5.1. Gas Distributors

The various investigators who have studied the efficacy of various designs of gas distributor in tower fermenters and bubble columns have concentrated mainly on the criterion of gas transfer efficiency. Morris (1972) however, discussed the problem of blockage by mould growth and the effect of this on gas transfer efficiency. Where glass or stainless steel sinters were used, mycelium adhered to the sinter, effectively reducing sinter area. The drilled alloy plates he used were less susceptible to surface growth and he attributed this to a greater shearing action of gas passing through the holes. Apart from this problem, the literature concerning the efficiency of various designs of gas distributor in terms solely of gas transfer performance is apparently contradictory. Some authors claim that gas distributor design does not affect gas transfer, whilst others report instances where the opposite is true. Freedman and Davidson (1969) found that for air-water systems, the greater the portion of the cross sectional area aerated, the greater the gas hold-up.*

Murphy et al. (1969) and Morris (1972) reached similar conclusions on gas transfer using sulphite oxidation tests with sintered glass air-distributors. They also found that finer porosity discs increased K_La, which confirms the observations of Kato and Nishikawa (1972) who found that a decrease in hole size was accompanied by an increase in hold up. On the other hand, investigations in air-water

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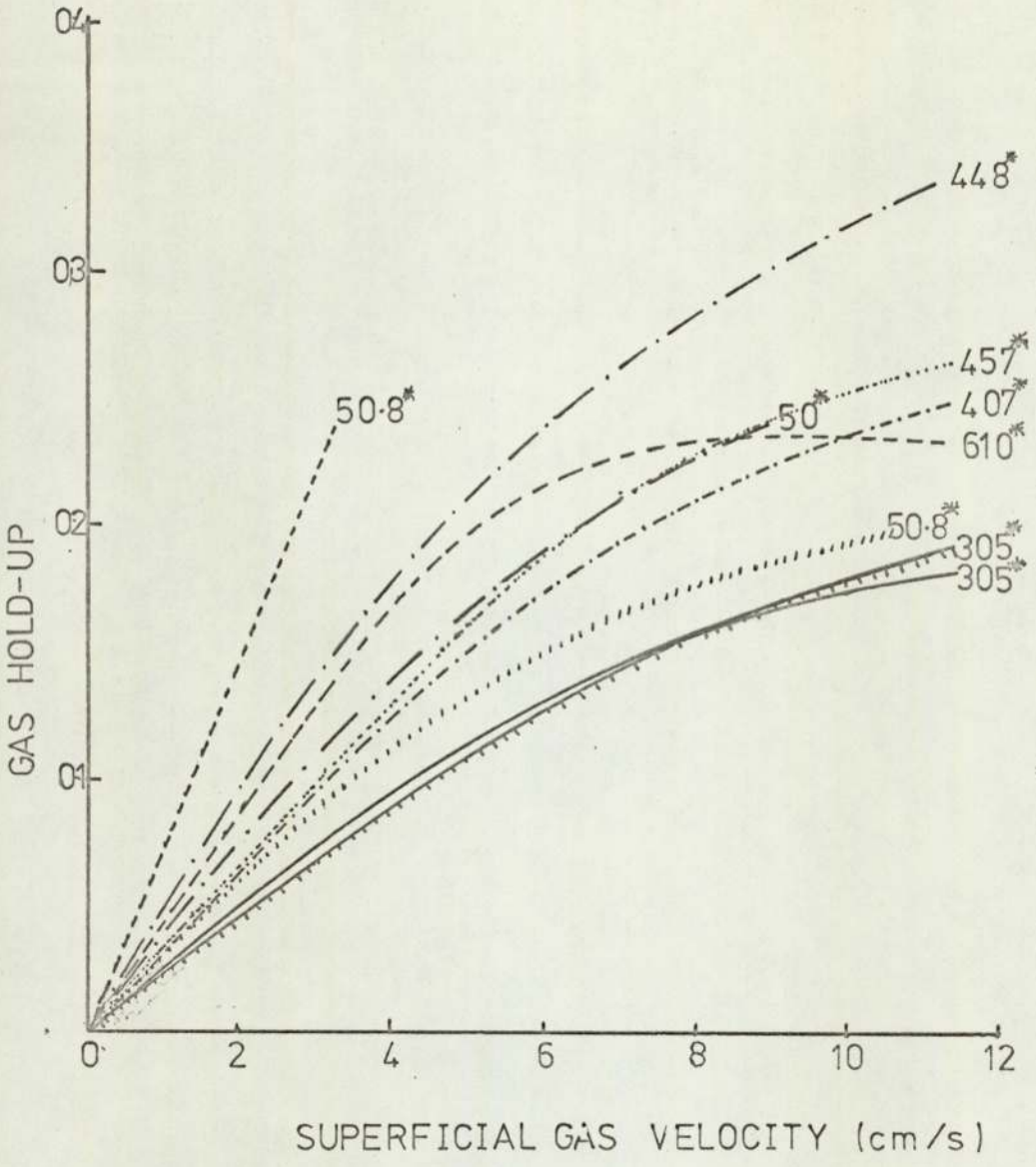
* Chemical engineering studies have concentrated on measurements of gas hold-up. These may be taken as a very crude indicator of gas transfer potential. (Fig. 5:1)

FIG.5:1GENERAL RELATIONSHIP OF AVERAGE GAS-HOLD-UP TO SUPERFICIAL GAS VELOCITY



systems using single and multiple nozzles, fine gauzes, gas rings and perforated plates with varied hole sizes and numbers of holes, desmonstrated little change in hold up values (Yoshida & Akita, 1965; Towell, Strand & Ackerman, 1965; Reith, Ranken & Israel, 1968; Shayegan-Salek, 1974). However, there are indications of a basic difference in behaviour between sinters and the other types of gas distributor. Thus, sintered discs have been shown to increase both $K_L a$ and hold-up values in a linear fashion until a point is reached where bubble coalescence occurs and these values decrease (Aoyama, Ogushi & Kubota, 1968; Murphy et al., 1969; Morris, 1972). This does not happen with perforated plates, gauzes etc (Aoyama, Ogushi & Kubota, 1968; Shayegan-Salek, 1974) and there is instead a more gradual decrease in hold-up relative to increases in gas flow rates.

Other work on mass transfer of oxygen in fermentation equipment deals with relatively squat, mechanically agitated vessels rather than unstirred columns or towers (Cooper et al., 1944; Maxon & Johnson, 1953). This information can be very misleading if extrapolated to the case of tower fermenters (see "Column Geometry")

5.2. Column Geometry

Most tower fermenters at present used for aerobic fermentations consist of upright cylindrical columns ranging from 6 cm to 600 cm in diameter and with an aspect ratio of between 10:1 and 15:1. Air or other gases are supplied by various types of sparging system situated at the base of the column. Some designs also incorporate an expansion chamber situated on top of the column to deal with the effect of foaming. The information which is available on column geometry and its effect on performance at present relates to work with air-water systems only, and so it is only a guide where fermentation work is concerned.

The most immediate consequence of the relatively high aspect ratio of these fermenters is its effect on the superficial gas velocity (S.G.V) for a given volumetric gas input (V.V.M) where

$$\text{S.G.V. } (U_L) = \frac{\text{liquid height (cm) x V.V.M}}{60} \quad \text{cm sec}^{-1}$$

Obviously a squat column will have a lower superficial gas velocity than a tall, slender one for a given V.V.M. It has been found with air-water systems that in the absence of mechanical agitation systems the S.G.V. is directly proportional to the gas transfer and mixing chracteristics of the reactor (see later) and therefore relatively high aspect ratios aredesirable for improved mixing performance in the absence of impellors.

In general, most workers have found that for air-water systems, column diameter is inversely proportional to gas hold-up, this effect being more significant for columns below 15 cm (6 in) in diameter (Fair, Lambright & Andersen, 1962; Yoshida & Akita, 1965; Reith et al., 1968; Ellis & Jones, 1965; Freedman & Davidson, 1969). This finding suggests that a wall-effect exists which appears to be useful. Possibly it may be better to scale-up by bundling together columns of a relatively small diameter into a calandria, rather than by increasing diameter and height. A further consequence of the relatively small diameter of these columns is the reduction in capital cost.

Column height is generally related to the desired height of the liquid column except that a head space may be required to accommodate a limited amount of foam. In general, liquid height does not have a significant effect on gas hold-up in air-water systems (Yoshida & Akita, 1965; Towell et al., 1965; Fair et al., 1962; Bhaga & Weber, 1972), except for heights below 100 cm where end-effects

become significant (Yoshida & Akita, 1965). Murphy et al. (1969) found that these trends also applied to K_La measurements in sulphite oxidation work. The upper limit of height or the optimum height are still unknown quantities, though presumably the optimum would be related to the magnitude of the wall-effects and therefore to the column diameter and the suspension rheology.

5.3. Baffle Systems

Although this study, together with other work by the Aston team did not cover baffled systems, several studies on tower fermenters with various types of baffles have been reported. Sieve plates have been shown to increase hold-up in air-water systems (Ellis & Jones, 1965; Fair, 1967). As might be expected, baffles of this type make the suspension characteristics more heterogeneous. Ross and Wilkin (1968) used sieve plates to enhance heterogeneity in their tower with a view to improving the production of secondary metabolites. This was followed by Lumb et al. (1970) . who used bubble-cap baffles similar to those used in distillation columns, also to improve secondary metabolite production. In this configuration, the fermenter approximates very well to a series of well-mixed tanks with multistage performance characteristics (Kitou, Okamoto and Ozaki, 1972). The same authors also claimed that such a reactor compared favourably in oxygen transfer tests with a stirred fermenter of the same volume (2 V.V.M. and 500 r.p.m). However, Morris (1972) found that a tower fermenter without baffles gave K_La values (measured by the sulphite oxidation method) which were comparable with those in a stirred fermenter (>500 r.p.m., 1 - 3 V.V.M).

5.4. Expansion Sections

Tower fermenters as originally developed for brewing applications (Greenshields, 1972) utilised an upper expansion chamber. This was included in the design to prevent foaming. The expansion chamber provides a relatively quiescent zone where the overall S.G.V. is lower than in the column, thus reducing the foaming tendency. For aerobic systems, expansion sections are obviously undesirable, since a relatively large volume of medium is kept poorly mixed and aerated and the overall efficiency of the reactor significantly reduced.

Expansion sections also complicate the overall structural design and increase costs.

5.5. Superficial Gas Velocity (S.G.V)

In recent years, many chemical engineers have studied the effect of S.G.V. on gas-holdups and although conditions varied considerably, the general trend is shown in Figure 5:1 It can be seen that for superficial gas velocities below about 6 $cm.s^{-1}$, the hold-up increases linearly with increased S.G.V. Shayegan (1974) observed that above this range the coalescence of bubbles caused uneven "slug-flow". The hold-up in this range is difficult to measure because of the wild fluctuations caused by the uneven gas-flow. It seems likely that the discrepancies in the published results (fig. 5:1) are a result of differences in gas-distributor design (see 5:1). Mixing studies have also revealed that mixing coefficients increase linearly with increases in S.G.V. up to the onset of slug-flow behaviour. High superficial gas velocities (>6 cm/s) have other detrimental effects including excessive foaming and carbon dioxide desorption (Morris, 1972).

5:6. Bubble-size

The gas bubbles in a tower reactor have been shown to effect mixing during their passage up the column by the displacement of water and by the formation of wakes. The magnitude of this effect increases with increased bubble size (Shayegan, 1974). On the other hand, it is generally recognised that smaller bubbles have a larger surface area for gas-transfer, although very tiny "ionic bubbles" which form in electrolyte solutions including fermentation media may have very long residence times (Fair et al., 1962; Yoshida & Akita, 1965). The interaction of bubbles of different sizes with microbial particles is also likely to affect gas-transfer and mixing. Morris (1972) observed that hard spherical colonies increased hold-up, but filamentous growth encouraged bubble coalescence.

5:7. Gas-mixture

Although the mass-transfer of carbon-dioxide in tower fermenters has been largely neglected, Clark and Lentz (1963) have looked in detail at the economics of increasing the oxygen partial pressure. They found that increases in oxygen partial pressure or total gas pressure were effective in improving gas transfer. Thus, low superficial gas velocities could be used without decreasing oxygen transfer efficiency.

5:8. Viscosity

The suspension viscosity during mould fermentations has been shown to have profound effects on mixing and gas-transfer in stirred reactors (Deindorfer & Gaden, 1955; Solomons, 1962; Steel & Maxon, 1962; Takahashi, 1969). Undoubtedly, the problems of realistic measurement of the rheological behaviour of gassed and non-gassed mould suspensions are severe, in fact, it seems that no reliable technique is available for the study of gassed mould suspensions. Most of the measurements carried out on non-gassed suspensions have

related to filamentous growth, but Takahashi (1969) compared filamentous suspensions with "pelleted" suspensions and observed considerably higher viscosities for the filamentous form (Fig.5:2) Work by Morris (1972) and Daunter (1972) indicated that it might be possible in tower fermenters to modify the colony-type and thus lower the viscosity of the suspension with beneficial results, although it was also found that the tower fermenter was very sensitive to increases in viscosity. Bubble-coalescence, together with low K_La values in sulphite oxidation tests was a consequence of filamentous growth.

5:9. Hold-up

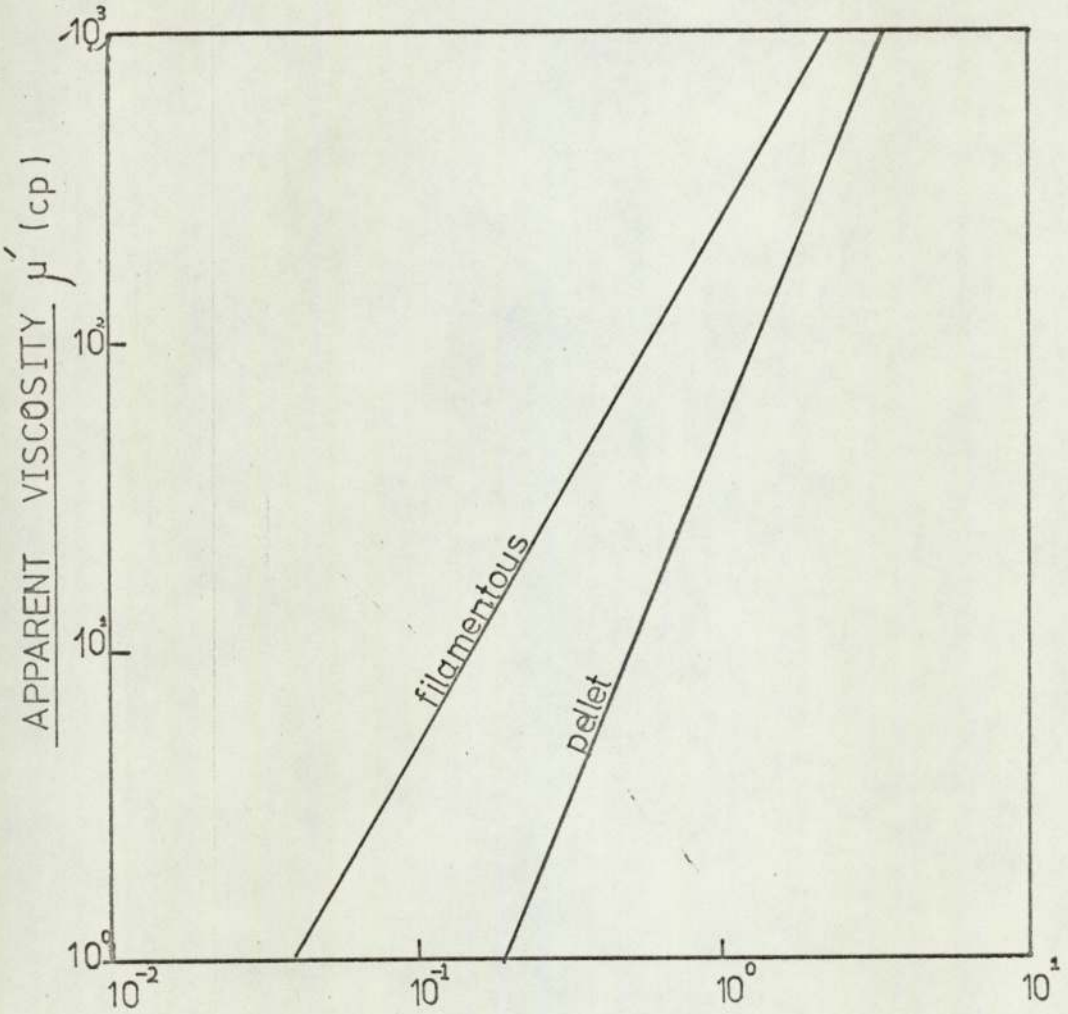
The hold-up or fractional gas retention volume, certainly plays a part in reducing the overall fluid density and viscosity of the mould suspension. This has the effect of reducing the effective buoyancy of mycelial particles and improving gas-transfer and mixing. Oldshue (1966) showed that hold-up in viscous polymer solutions was in turn affected by the fluid viscosity because the rise-velocity of gas-bubbles is reduced at higher viscosities. Thus, for a carboxy-methylcellullose solution of 1000 centipoise viscosity, bubbles less than 0.76 mm in diameter remain in the system and only those 2.03 mm or larger will pass upwards with any appreciable velocity. It seems possible that the long residence times of "ionic"bubbles may be imitated by much larger bubbles in viscous fermentation broths.

5:10. Shear Forces

The shear-forces effective in a fermentation-broth may cause disruption of the mycelium, depending on the tensile strength of the hyphae. Taguchi and Miyamoto (1968) studied the effect of agitation in a stirred tank on A. niger "pellets" and found that for their "pellets," the tensile strength (0.278 $Kg.cm^{-2}$) was higher than the maximum value of Reynolds stress, but this value of tensile strength

FIG. 5:2 EFFECT OF A. NIGER MORPHOLOGY ON SUSPENSION RHEOLOGY

(Takahashi, 1969)



was diminished by repeated loadings, allowing some disruption of the colonies. The evidence is that these colonies were very hard, smooth spheres, since the above authors mention the abrasive removal of pellicles from the pellet surface. There is no other information on other types of mycelial growth.

In general, it has been assumed that shear-forces in tower fermenters are less than those in stirred reactors.

5:11. Heterogeneity

The degree of heterogeneity in a tower fermentation is likely to vary as a function of many variables, including hold-up, viscosity, particle size, particle buoyancy, superficial gas velocity etc. In common with most of the variables in this section, heterogeneity results from the interaction of a complex system of variables. Axial heterogeneity may be the basis of improved continuous secondary metabolite production as previously discussed (1:3)

5:12. Temperature

The effect of temperature changes on performance of the reactor are not considered in detail in this study because temperature was controlled at the optimum for A. niger M.1. that is 30°C. Temperature variations will cause complex effects on the system itself which at the present stages of knowledge are impossible to predict. Precise temperature-control is therefor crucial.

5:13. Surfactants

The effect of antifoam agents in increasing bubble-size and decreasing $K_L a$ have been described previously, (Morris, 1972) and it seems that tower fermenters are very sensitive to antifoam additions. Detergents obviously increase foaming tendencies, though they do decrease the bubble-size. This has been shown to result in increased hold-up values (Chakravarty, et al , 1963)

PART SIX. METHODS

6.1. Organism

The organism used was a strain of Aspergillus niger designated M.1. It was originally isolated by workers at Tate & Lyle Ltd. from kibbled carob (granulated bean pods from the tree, Ceratonia siliqua,L) which had been allowed to become mouldy. Master cultures obtained from Tate & Lyle research laboratories were recultured on carob extract agar and the most vigorous cultures used to provide the silica gel masters which were used throughout this work.

6.2. Culture Maintenance

Silica gel spore-cultures prepared by the method of Roberts (Onions, 1971) were maintained in triplicate at room temperature in desiccators.

6.3. Inoculum Preparation

Subcultures were made by spore-transfer from a silica gel master into wide-necked Carré flasks containing carob medium agar which were incubated at 30°C. Spores were then washed from five- to ten-day old cultures using an 0.01% v/v solution of octylphenoxy-polyethoxyethanol (Triton X-100, Sigma Chemicals Ltd.) The suspensions thus obtained were shaken to disperse the spores, the suspension being checked by microscopic observation until dispersion was complete.

6.4. Media

Several basic medium formulations were used to study a wide range of conditions.

Most of the work at the ten-litre scale was carried out using a fully-defined medium, designated COR: sucrose 60g, $(NH_4)_2SO_4$ 13.25g, $NaHPO_4$ 1.0g, EDTA 0.6g, $MgSO_4.7H_2O$ 0.25g, $CaCl_2$ 0.1g, $ZnSO_4.7H_2O$ 0.2g, $MnSO_4.4H_2O$ 0.02g, KCl 0.5g, $CaSO_4.5H_2O$ 0.05g, $FeSO_4.7H_2O$ 0.1g,

$NaMoO_4.2H_2O$ 0.05g. Distilled water to one litre (In practice, a twentyfold concentration of EDTA and all the mineral salts was prepared and an aseptic addition of one litre to the other components was used to make twenty litres of medium in a glass aspirator which was then autoclaved for 45 minutes at 120°C.)

Larger volumes of semi-synthetic medium for pilot scale work were prepared using SPA medium:- Sucrose 5.5%,w/v $(NH_4)_2SO_4$ 0.1%, w/v NaH_2PO_4 0.1%, w/v KCl 0.05%, w/v $MgSO_4 7H_2O$ 0.02%, w/v yeast extract 0.1%, $CaCl_2$ 0.01% in Birmingham town water, sterilised as before.

Beet molasses medium for fifty-litre and five-hundred litre fermentations was prepared in town water as follows:
Beet molasses (ex B.S.C., Kidderminster) 10%, $(NH_4)_2SO_4$ 0.5%, NaH_2PO_4 0.1%. It was sterilised by passage through a continuous steriliser (Junior Paraflow, A.P.V.Ltd., Manor Royal, Crawley, Sussex).

Carob medium was used in each size of fermenter, and although its preparation for the pilot scale work differed with respect to the ten and fifty litre work in that a specialised percolation extractor was used for the former, the basic method was as follows - approximately 0.25 Kg/l of kibbled carob was steeped in tap water at 80°C for thirty minutes. After filtering off the extracted carob, the liquid was diluted to give 5% w/v total sugar (method of Lane and Eynon, 1923); 0.1% w/v NaH_2PO_4 and 1.0% w/v $(NH_4)_2SO_4$ were added and the pH adjusted to 3.0 using concentrated hydrochloric acid. In pilot scale work, the medium was sterilised continuously as described above. For the ten litre scale the medium was autoclaved as for COR medium. Solid carob medium for inoculum preparation was prepared simply by adding agar (15 g/l) to liquid carob medium and autoclaving.

6.5. Antifoam

The antifoam used in this work was a 10% w/v emulsion in water of methyl polysiloxane which was prepared by diluting Silcolapse 437 (I.C.I.Ltd) in the ratio 1:2 with water and autoclaving. This antifoam was never used for the synthetic and semi-synthetic media.

6.6. Sampling Methods

For all the fermentations, sampling volumes had to be carefully measured to minimise reductions in fermenter working volume. Where possible, portions of the sample were used in more than one test, for example, viscosity determination was carried out on the whole sample before it was used for the various "destructive" testing procedures. Similarly, Markham microkjeldahl estimations were carried out on the dried remnants of the membrane filter and mycelium after dry weight determinations. Even so, the large sample volumes required for suspension viscometry limited the application of this technique mainly to the 450 litre scale and even here, a reduction in sampling frequency was necessary. To provide continuity of measurement, samples were withdrawn from the upper middle port every hour and every fourth hour after eight hours fermentation time from the other three ports. It should be noted that for a fermentation lasting for forty hours, sixty-two litres of suspension would be removed even when restrictions such as these are maintained.

At the ten and fifty litre scale, a sample size of 30 ml was used together with microscale estimations as detailed elsewhere. It was recognised that this could lead to sampling error, particularly when most samples were withdrawn from the lower part of the tower.

6.7. Direct Examination

In most cases, it was helpful to dilute the sample in order to allow individual colonies to be distinguished and observed closely. For photography, approximately 5 ml. of the sample was diluted with 3% v/v formalin and the specific gravity of the solution adjusted with glycerol until the mycelium was just buoyant. The suspension was then stirred with a glass rod to obtain an even dispersion and poured into a 20 x 100 x 40 mm perspex viewing cell. To remove air bubbles clinging to the inner wall of the cells, a drop of dilute detergent can be added and a rubber policeman used to wipe off the bubbles which remain.

6.8. Staining

Wet film preparations of the mycelium were stained with fluorescent material by drawing Tinopal BOPT solution (200 mg/l) across the preparation using capillary absorption. The specimen can be viewed immediately. The fluorescence proved very stable to U.V. light during this work, allowing ample preparation time for photography.

6.9. Microscopy

For fluorescence microscopy a Vickers M32 microscope was obtained. The choice of equipment and conditions had a considerable bearing on the quality of the results and those detailed in table 6:1 were found to give good results with this microscope (Vickers Ltd., Vickers Instruments, Haxby Road, York).

Although the conventional light microscopy in the later stages of the work was carried out using the M32 microscope, most of this work was carried out using an Olympus EHB microscope. The corresponding Olympus camera and exposure meter were used. Optics were chosen

TABLE 6:1 DETAILS OF EQUIPMENT FOR FLUORESCENCE MICROSCOPY

Item.	Magnification	Numerical Aperture	Light Transmission
Vickers Mo25111 objective	x 10	.25	optics with the highest N.A. for a given magnification give best light transmission for fluorescence work
Mo23911 objective	x 20	.65	
Mo23611 objective	x 50	.95	
Mo22611 objective	x100	1.20 imm.	
M320940 bright field condenser (Abbe type)	-	1.20 imm.	
Mo41312 eyepieces + phototube lens	x 10 (wide field)	-	(x8 wide field eyepieces for dim fluorescence
M151530 primary visible light filter (2 mm thick U.G.2 glass)	-	-	300 - 400 mμ
O.G.1/1.5 mm U.V.Barrier filter	-	-	470 mμ upwards
BG 38/4 mm infra-red and visible red barrier filter			
OSRAM HBO 200 Mercury vapour lamp*			
low fluorescence immersion oil M322245			

* Blue light fluorescence equipment using quartz halogen illumination gives poor results

to allow adequate low magnification studies, e.g. x4, x10, x20, x100 objectives and x7 eyepieces. Even the relatively low magnification of x28 was not appropriate for some large colonies and objectives around x2 magnification might be useful in future work.

Specimens for stereoscan electron microscopy (SEM) were prepared by decanting off the excess medium from a 5 ml aliquot and washing twice with 95% methylated spirits for fifteen minutes to dehydrate the cells and remove dissolved solids. Samples with the minimum volume of liquid were transferred to mirror polished aluminium SEM stubs using an 8 mm diameter glass dropping tube. The stubs were allowed to dry for at least three days in a desiccator before carbon coating and examination using a Cambridge S2A stereoscan microscope.

A less laborious alternative to polishing the stubs was adopted in the later stages of the project: pieces of perspex sheet 1 cm x 1 cm square were fixed to the stubs using contact adhesive and the samples placed on the perspex surface. After use, the perspex was replaced by a fresh piece.

6.10. Photography

The viewing cells were photographed against a matt black background using side illumination to obtain a crude dark-field effect. Photographs were also taken by optical and electron microscopy of the corresponding hyphal and colonial appearance. Choice of film is very wide but in this work Ilford FP4 exposed at 200 A.S.A. together with Microphen developer (7 min at 20°C) proved satisfactory.

6.11. Colony Sizing

Where colonies were approximately spherical in shape, and given a size distribution range not greater than 30%, mean colony diameters of thirty colonies were estimated using either an eyepiece micrometer or the viewing-cell scales for colonies above 1 mm diameter.

These measurements were also used to produce size distribution curves as the fermentations progressed.

6.12. Spore Counts

The concentration of spores in suspensions was estimated using a Thoma haemacytometer cell.

6.13. Mycelial Dry Weight Estimations

(a) 450 litre scale

100 ml aliquots of mycelial suspension taken after viscosity determinations were vacuum filtered on dry, tared, Whatman No.1 filter papers using a Buchner funnel. The filter papers were then dried to constant weight in a drying oven maintained at 80°C.

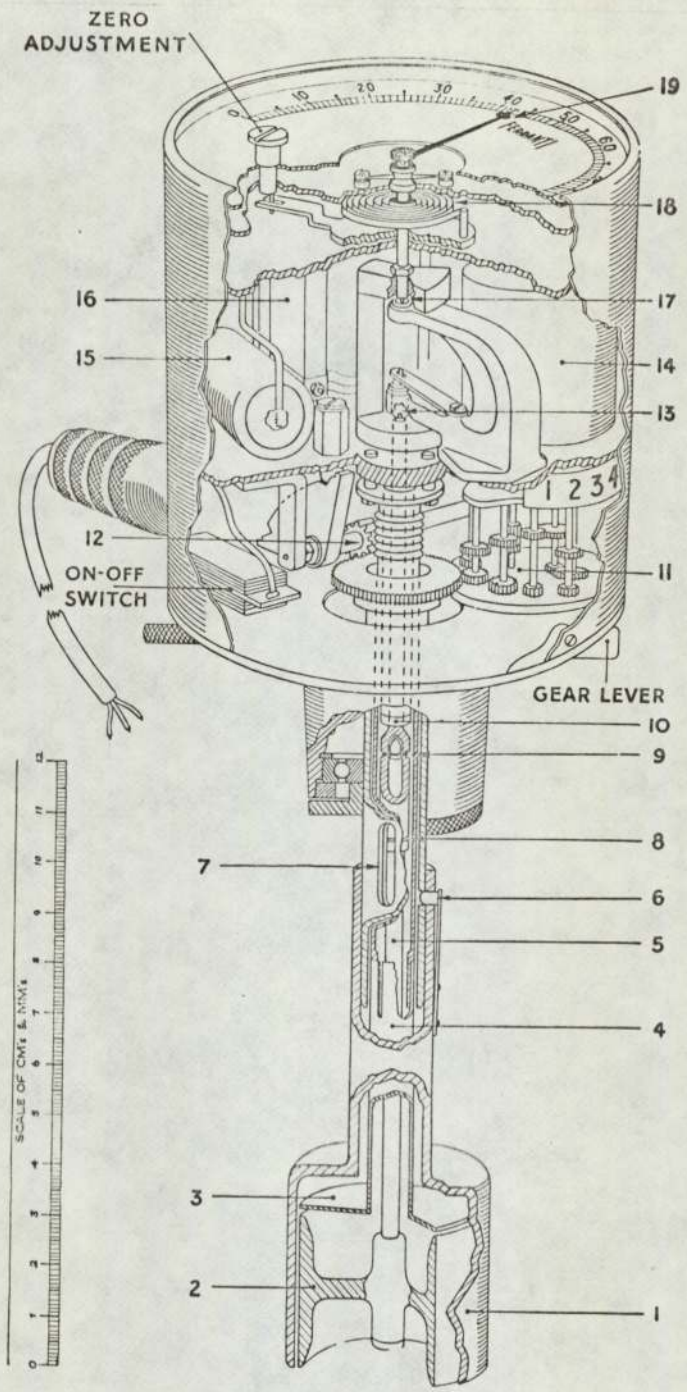
(b) 50 and 10 litre scale

10 ml aliquots of mycelial suspension were vacuum filtered on Oxoid 25 mm cellulose acetate membranes (cat.no.MF25), until the membrane was dry to the touch. (This was necessary to prevent membranes sticking to the dish during drying). They were then spread in batches of five in the base or lid of a glass petri dish and dried to constant weight in a drying oven maintained at 80°C. These membranes were chosen in the first place because they were found to have a very narrow weight distribution when dried (Sample size = 18; range = 0.0007 g, mean wt. = 0.0265 g, median wt. = 0.0263 g). Thus the only weighing carried out was that of the total weight of the membrane plus the mycelium when dry. To obtain the true dry weight figure, the median membrane weight (0.0263 g) was subtracted from the total weight. The error introduced by this method is negligible compared with the magnitude of the total weight.

6.14. Viscometry

One litre aliquots of suspension from the pilot scale fermenter were withdrawn in one litre beakers 12 cm in diameter every hour

FIG. 6:1 FERRANTI MODEL VL ROTATIONAL VISCOMETER



KEY			
No.	ITEM	10	STEADY BEARING
1	OUTER CYLINDER	11	TURRET GEAR BOX
2	INNER CYLINDER	12	INNER CYLINDER LOCKING MECHANISM
3	GUARD RING	13	SPRING LOADED SECONDARY BEARING
4	GUARD RING SUPPORT TUBE (DETACHABLE)	14	SYNCHRONOUS MOTOR
5	INNER CYLINDER SPINDLE	15	PHASING CONDENSER
6	OUTER CYLINDER QUICK RELEASE ATTACHMENT	16	TRANSFORMER
7	INNER CYLINDER RELEASE APERTURE	17	MAIN JEWELLED BEARING
8, 9	INNER CYLINDER QUICK RELEASE SYSTEM	18	TORQUE SPRING
		19	VISCOSITY INDICATING POINTER

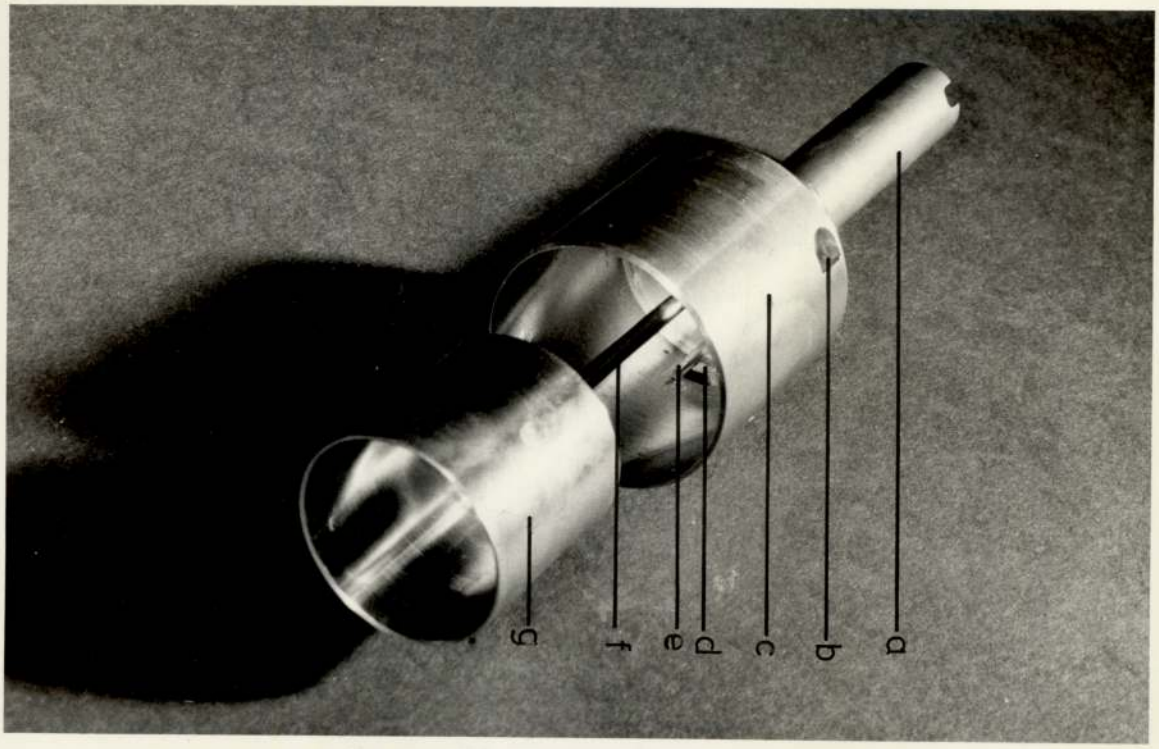


FIG.6:2 VISCOMETER HEAD WITH INNER CYLINDER WITHDRAWN TO REVEAL INTERNAL VANES AND VENT-HOLES

a. Drive shaft

b. Vent hole

c. Outer cylinder

d. Vent hole

e. Vane

f. Inner cylinder spindle

g. Inner cylinder

from the upper middle port and every fourth hour from the other three. The apparent viscosity of each sample was immediately measured using a Ferranti model VL rotational viscometer (Fig.6.1). This viscometer measures the angular deflection against a calibrated spring of an immersed hollow cylinder caused by the viscous drag generated by an annular coaxially mounted cylinder. The outer cylinder can be rotated at a range of speeds which allows a corresponding range of shear forces to be imposed on the suspension in the annular space. To prevent blockage of the cylinders by mycelium the outer cylinder has a system of vanes and perforations to remove mycelium (Fig.6.2). It was also found necessary to use the largest cylinders (VLC types, fig.6.2) to prevent clogging at high suspension densities. The operating data for these cylinders is given in table 6.2 . Smaller vessels or samples are to be avoided with this instrument in order to prevent wall effects when using high viscosity solutions.

TABLE 6.2. OPERATING DATA FOR FERRANTI VLC VISCOMETER HEAD

Speed	Shear Rate of Inner Cylinder Surface (sec^{-1})
1	68
2	102
3	135
4	170
5	204

Measurements were also carried out on the supernatant viscosity using a standard U-tube viscometer. Filtrate from mycelial suspensions were allowed ten minutes at 30°C for equilibration before carrying out duplicate measurement on each sample.

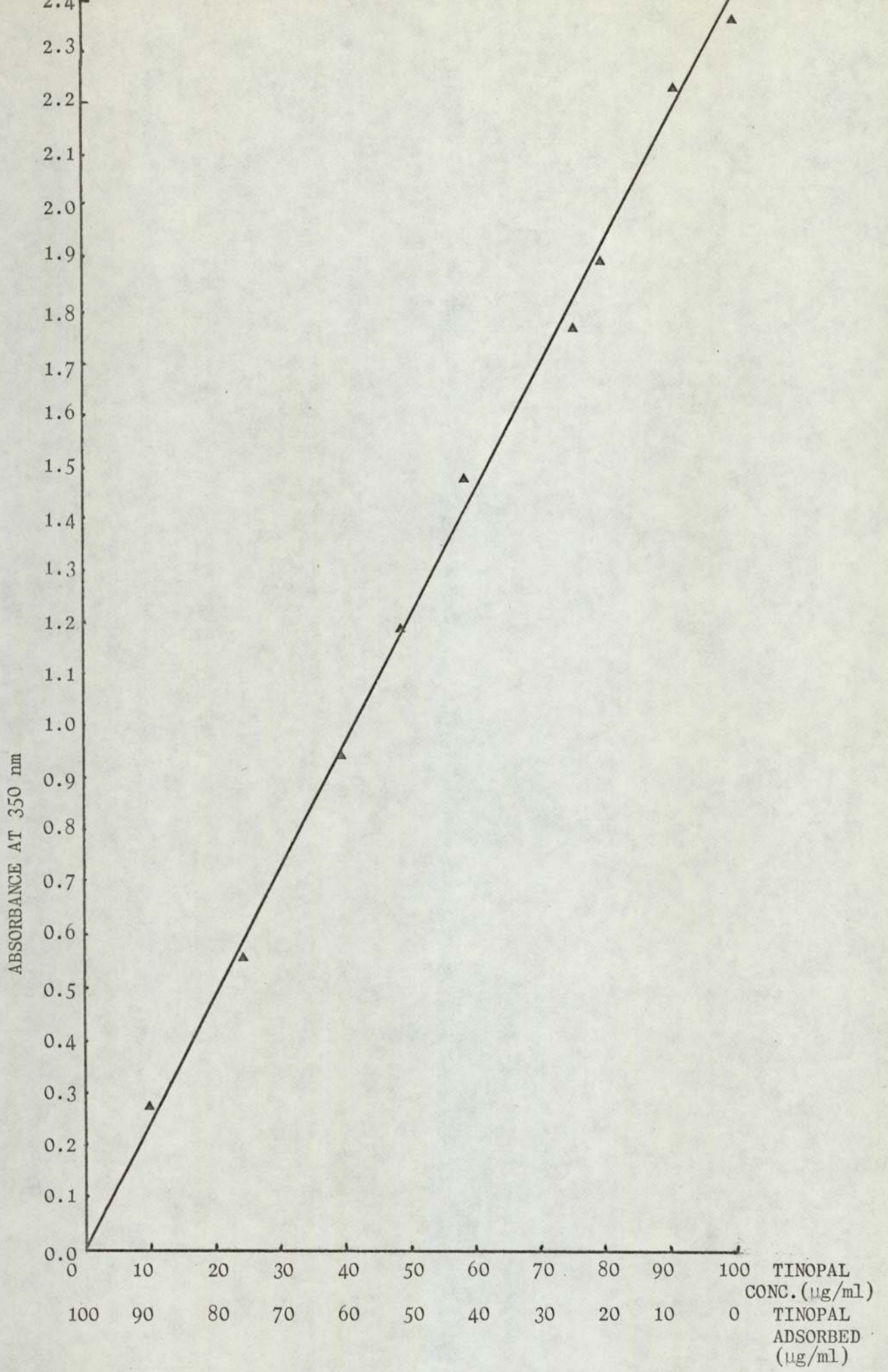


FIG 6:3 TINOPAL ABSORBANCE vs CONCENTRATION AND TINOPAL ADSORPTION

6.15. Fluorescent Brightener Adsorption

The adsorption of Tinopal BOPT (Ciba-Geigy Ltd., Leicester) by the mycelium at different times during the fermentation was estimated by a modification of the method used for fungal protoplast studies by Peberdy and Buckley (1973). These authors had found their sample of Tinopal BOPT to give solutions unstable in daylight and therefore precautions to exclude daylight from the sample were observed. A solution (200 mg/l) of Tinopal BOPT in distilled water was made up in a black-painted one litre graduated flask. A 5 mm wide vertical strip of glass had been left unpainted on the neck of the flask to allow the meniscus to be seen. This flask was emptied into a storage bottle made of actinic glass and the bottle stored in the dark. This standard solution was used to refill a 250 ml black painted bottle for bench use. Dilutions to 10, 25, 40, 50, 60, 75, 80, 90 and 100 µg/ml were made and a calibration curve (Fig.6.3) prepared by measuring their optical density at 350 nm in a 1 cm light path. A Unicam SP spectrophotometer was used for these measurements.

The adsorption of Tinopal BOPT was determined as follows: A specially calibrated wide- mouthed 10 ml pipette was used to transfer 10 ml. of suspension from the sample into a glass membrane filtration unit (Fig.6.4). The medium was removed by suction at outlet 'B' with outlet 'A' closed and the mycelial cake washed with 30 ml distilled water using the same outlet. The stopcock at outlet 'B' was then removed momentarily to allow the equilisation of pressure across the membrane and was replaced in the closed position. 5 ml. of distilled water were pipetted on to the mycelial cake and the mycelium re-suspended by stirring with a glass rod, taking care not to damage the membrane. 5 ml of the

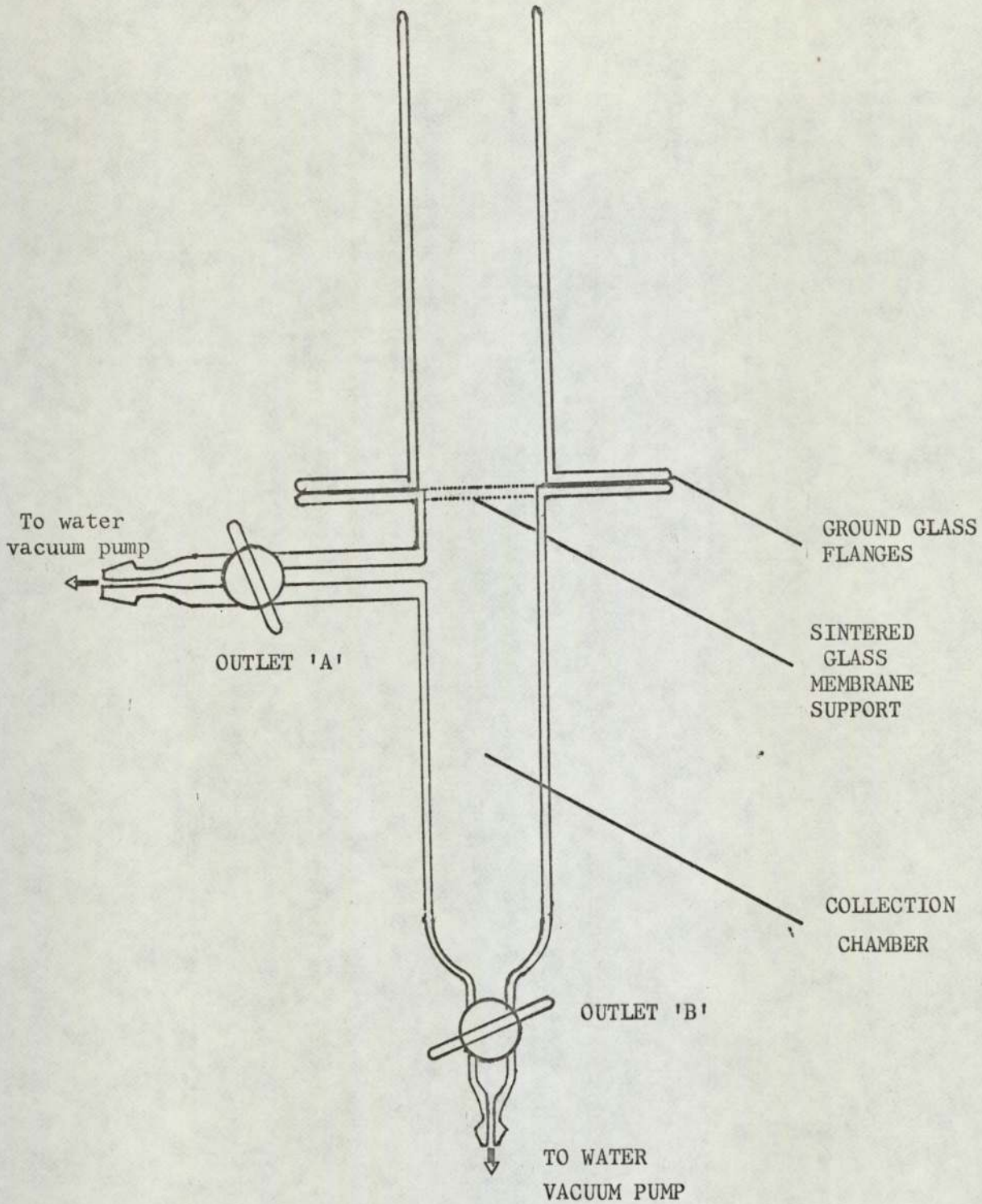
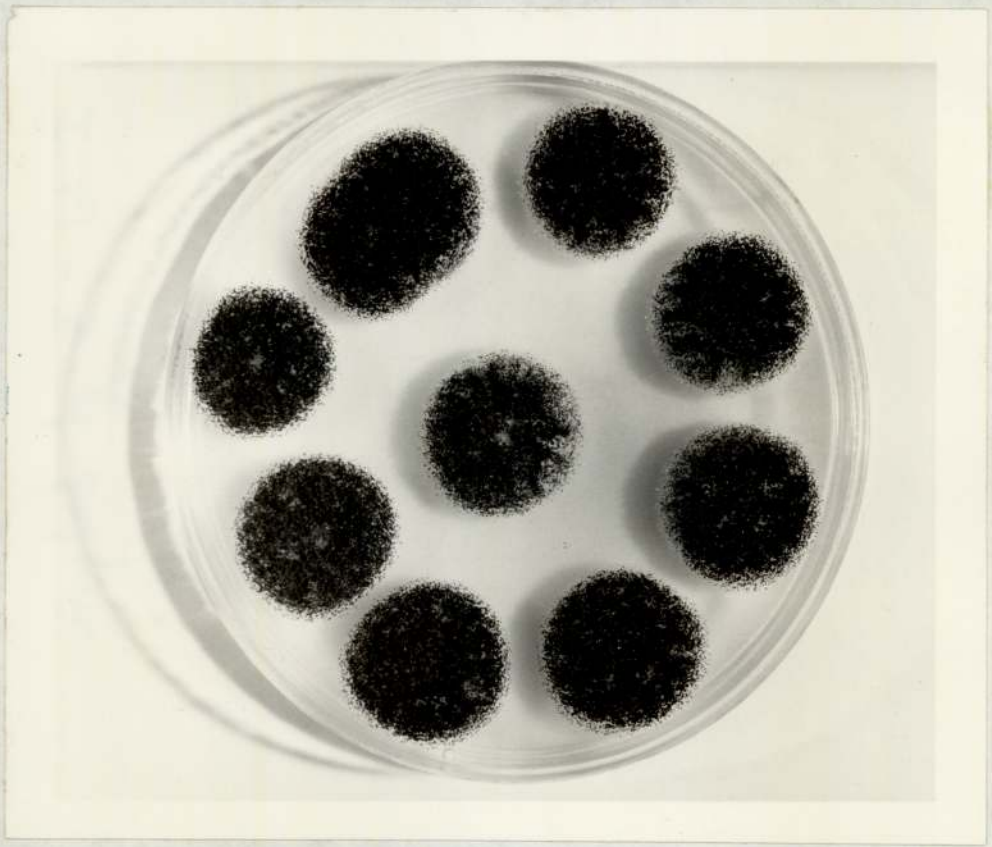


FIG.6:4 MEMBRANE FILTRATION UNIT USED FOR DETERMINATION OF DRY WEIGHT AND TINOPAL ADSORPTION

FIG.6:5 THREE DAY OLD PETRI DISH CULTURE SHOWING INOCULUM PATTERN



standard Tinopal BOPT solution were pipetted into this suspension and the unit covered with a black polythene bag. After one minute, the optical brightener solution was drawn into the collection chamber by opening outlet 'A'. When sufficient Tinopal solution had been collected, it was decanted into a 1 ml glass cuvette by closing outlet 'A' and opening outlet 'B'. The optical density at 350 nm was then measured against a distilled water blank sample. From the reading the amount of Tinopal adsorbed on to the mycelium in the sample was read off on the calibration curve (Fig.6.3).

6.16. Agar culture

Petri dish cultures were carried out for the rapid comparison of stimulatory or inhibitory medium parameters. A sterile suspension of each growth factor was added to 100 ml of sterile COR medium agar at 40°C and the agar poured into four plates. The plates were then inoculated using a suspension of M1 in liquid paraffin such that each dish had nine point inoculum sites (Fig.6.5) and incubated at 30°C. The effect of the additive was expressed as the ratio of mean colony diameter in the experiment to the mean diameter in the control after three days.

i.e.

$$\frac{\sum_{1}^{36} \text{experimental colony diameter}}{\sum_{1}^{36} \text{control colony diameter}} = \text{"growth ratio"}$$

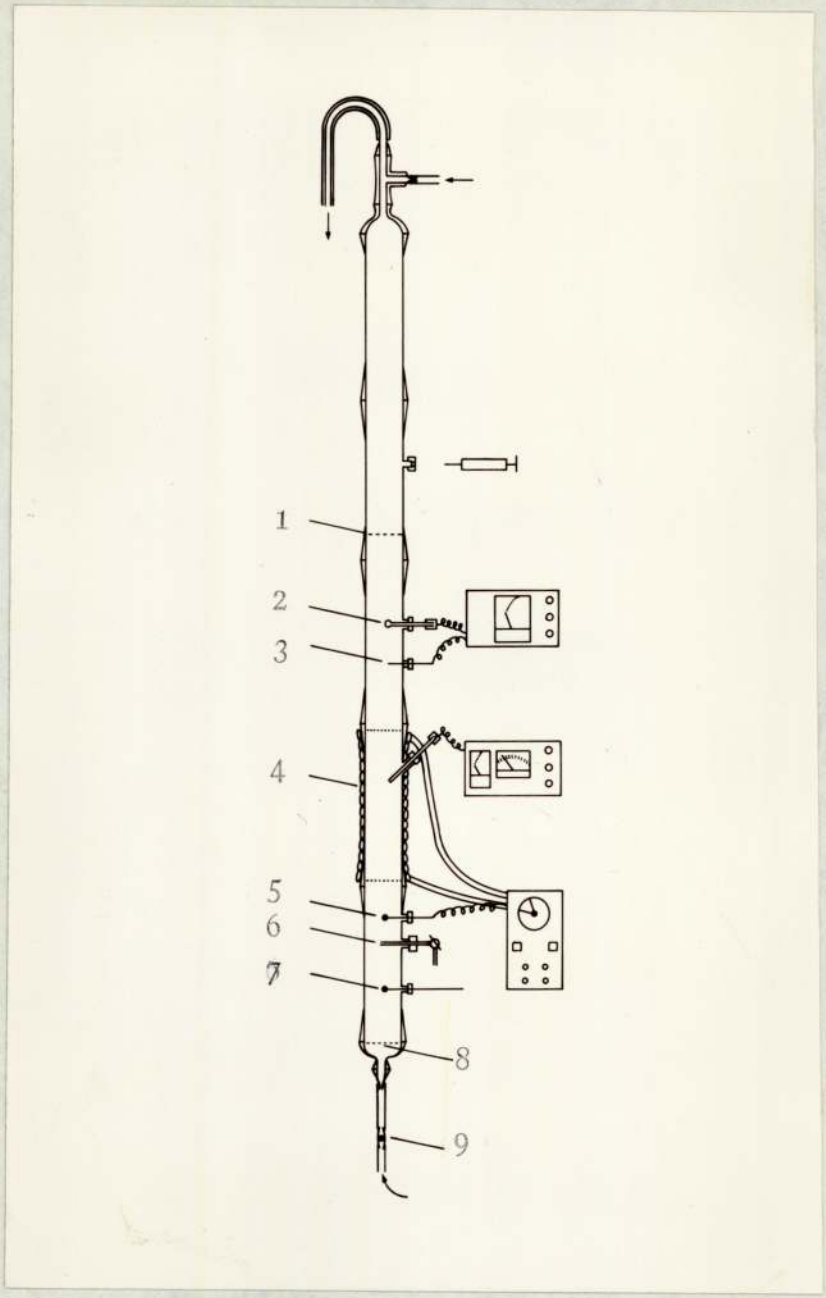


FIG.6:6. DIAGRAM OF TEN LITRE FERMENTER COLUMN DESIGN

1. Liquid level (ten litres)
2. pH probe
3. Remote reference electrode
4. Attemperation jacket
5. Thermistor probe
6. Sampling port
7. Thermometer
8. Gas distributor
9. Non-return valve

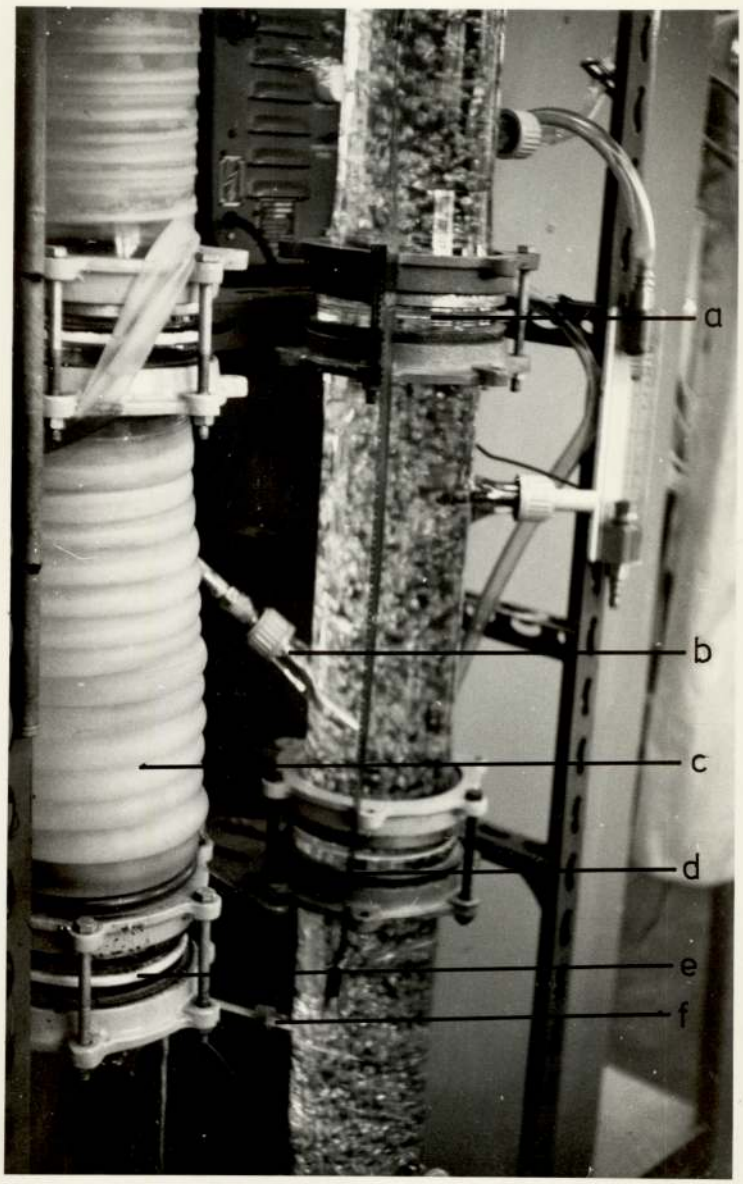


FIG.6:7. TEN-LITRE FERMENTER COLUMNS

a. Silicone rubber gasket

b. Angled screw cap port

c. Water jacket

d. Support bracket

e. P.T.F.E. gasket

f. S.Q.13 horizontal side arm

6.17. EQUIPMENT DEVELOPMENT

Tower fermenters available at the outset of this project were subject to various design limitations, many of which had become obvious from preliminary studies (Morris, 1972). Table 6.3 summarises these drawbacks with respect to each fermenter size.

There were therefore a number of design problems to be solved before the fermentation system could be considered adequate for detailed work. A fermenter with a working volume between ten and fifteen litres was developed during preliminary fermentation work and has proved itself more than adequate. The following description includes several alternative solutions to specific problems as mentioned above. Less successful solutions are also discussed to avoid repetition.

(a) Ten-litre column

Ten-litre column

The fermenter column (Fig.6.6;Fig.6.7; table 6.4) was built from standard glass pipeline components (Q.V.F.Quick-fit, Jobling Laboratory Division, Stone, Staffs) providing a robust, modular design. Fig. 6.8 also gives an example of the more fragile groundglass jointed system.

Ports for sampling, aseptic transfers and insertion of sensory probes were added by fusing Quickfit screw-cap fittings (SQ13 or SQ28) to the walls of the pipe-sections or by using the appropriate QVF side-arm pipe section (PTU4/1.0). The SQ side-arms accept inserts of between six and nineteen millimeters in diameter. Thus, thermistor, thermometer and reference electrode pass through SQ13 parts and other inserts through the larger size, using the appropriate rings and gaskets (Table 6.5,Fig.6.9) Although the range of acceptable sizes is shown to be discontinuous, the rings can in fact be adapted for a continuous range.

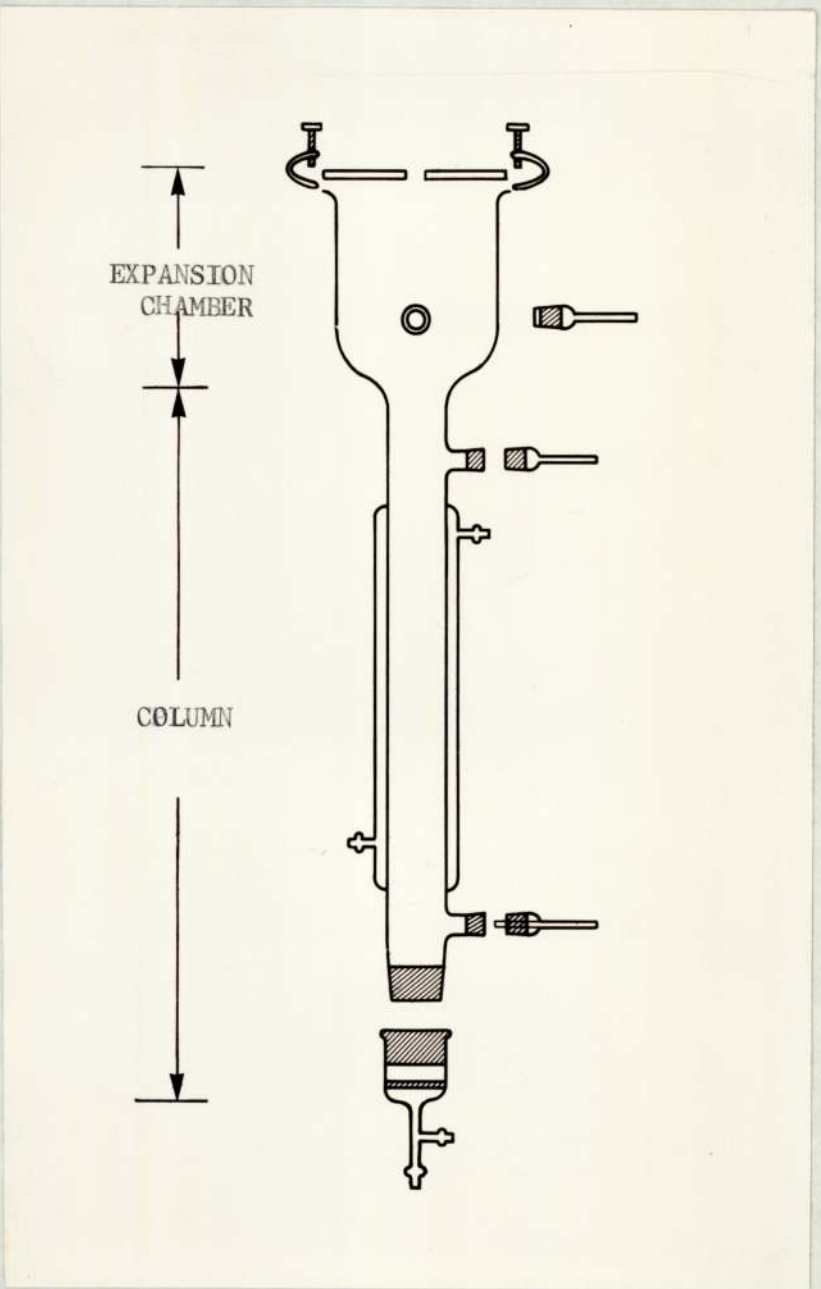


FIG.6:8 QUICKFIT JOINTED FERMENTER COLUMN WITH EXPANSION CHAMBER

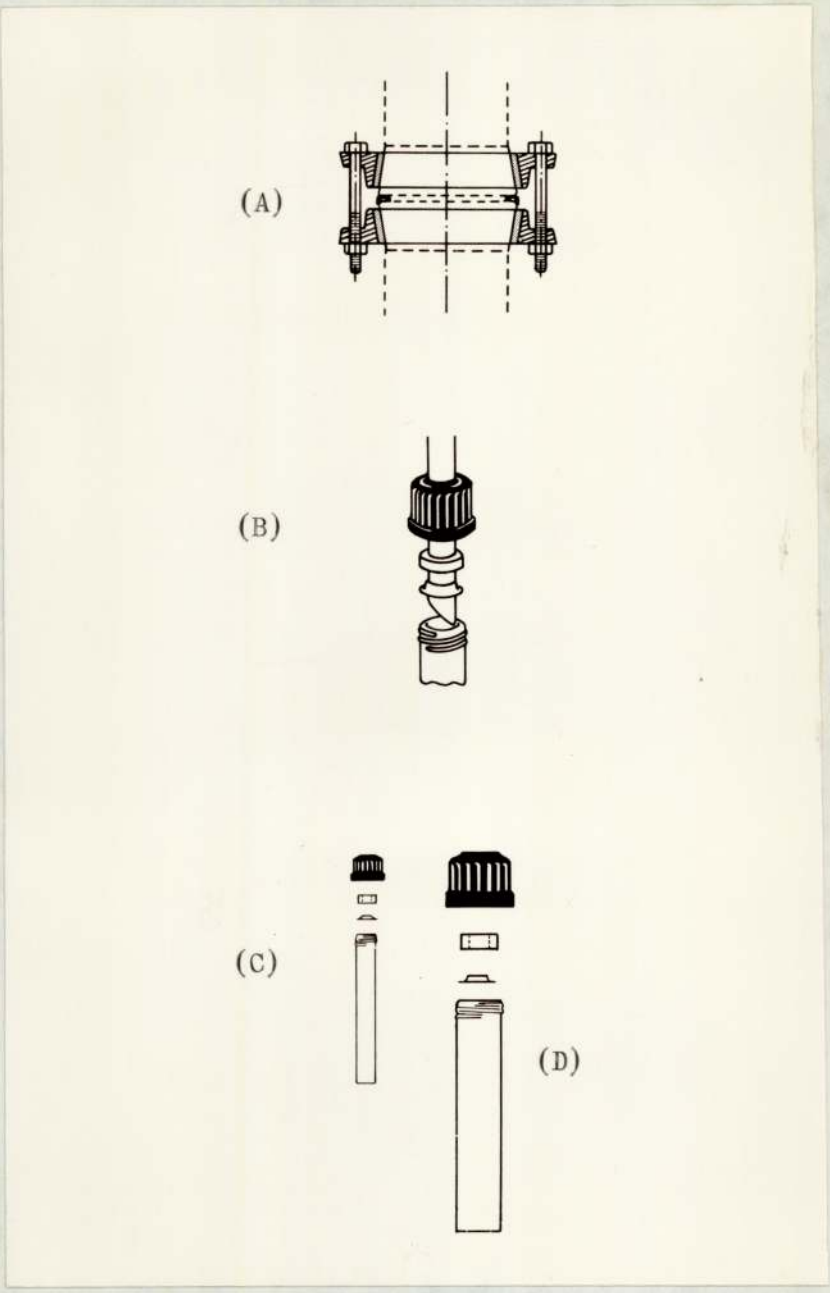


FIG.6:9 DETAILS OF COLUMN COMPONENTS

(A) Column buttress joint with P.T.F.E. gasket

(B) Quickfit screw cap side arm with insert

(C) SQ13 side arm

(D) SQ28 side arm

TABLE 6.3. FERMENTER DESIGN PROBLEMS

1 - 3 litre capacity	10 - 15 litre capacity	50 litre capacity	500 litre capacity
1 sample port only no probe inserts	1 sample port only possible to add ports	1 sample port no probe ports	1 sample port no probe inserts
very fragile, joints easily broken	very fragile, joints easily broken	robust (glass) Q.V.F.	robust polypropylene
ground glass, joints subject to leakage	ground glass, joints subject to leakage.	pressure limits defined by manufacturer	5 - 10 p.s.i. pressure limit
sinters only	sinters only, diameters less than column diameter	choice of material and design, may be same diameter as column	choice of material and design, may be same diam. as column
water jacket thermostat, feedback control of water in jacket only	water jacket thermostat, feedback control of water in jacket only	no jacket manual control of heating tapes, tapes not waterproof	no jacket no control of temperature
rubber bungs at port insecure and leaky, stick to glass, encourage surface growth	rubber bungs at ports insecure and leaky, stick to glass, encourage surface growth	rubber bungs at ports insecure and leaky, stick to glass, encourage surface growth	rubber bungs at ports insecure and leaky, stick to glass, encourage surface growth
overflow problems internal diameter varies by ±10%	overflow problems internal diameter varies by ±5%	overflow problems. I.D. variation ± 1%	overflow problems. I.D. variation caused by distortion when full
-	-	surface growth at gaskets	-
cotton wool air filter	cotton wool air filter	cotton wool air filter	purpose made filter unit
air supply contaminated with oil, dust, water.	air supply contaminated with oil, dust, water	air supply contaminated with oil, dust, water.	purpose made purifying unit
fluctuating air pressure	fluctuating air pressure	fluctuating air pressure	pressure control valve

TABLE 6.4. INVENTORY OF PARTS FOR TEN-LITRE FERMENTER

Quantity	Description	Part No.
5	pipe sections	PS4/400
7	4" couplings	C4T
7	4" gaskets	CEN4 or TR4
2	pipe reducers	PR4/1
4	* 1" gaskets	CGN1 or TRI or TRB1
1	tee-piece	PT 1
2	hose-connectors	PHC1/0.5
1	hose-connector	PHC1/0.75

*The gaskets were replaced in the later stages of the work by a silicone rubber compound, (Dow Corning Bath Caulk or I.C.I. "Silcoset") eliminating surface growth problems by providing a smooth, clean joint. (Fig.6.7)

This system is cheaper and eliminates the need for providing an assortment of gasket sizes.

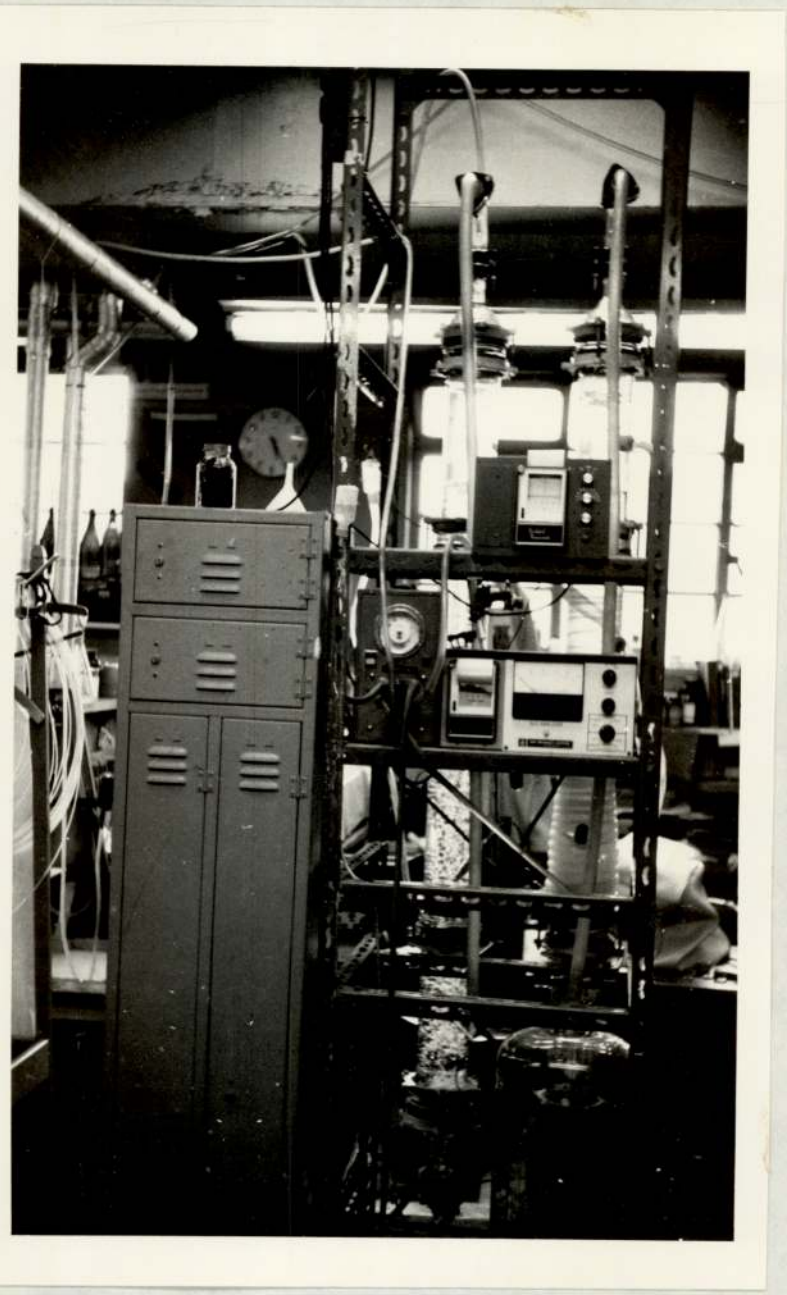


FIG.6:10 GENERAL VIEW OF TEN LITRE COLUMN WITH SUPPORT FRAME-WORK AND INSTRUMENTATION.

TABLE 6.5. DETAILS OF SIDE ARMS AND RANGE OF FITTINGS FOR FERMENTER (10 litre)

Thread Size (QVF No)	Acceptable Diameter of probe (minimum)	Catalogue Numbers Plastic cap	Rubber ring	PTFE Gasket
SQ 13	6.0 - 7.0	QC 13/7	QR 13/6	QW 13/6
SQ 28	6.0 - 7.0	QC 28/9	QR 28/6	QW 28/6
SQ 28	10.5 -11.5	QC 28/13	QR 28/11	QW 28/11
SQ 28	18.0 -19.0	QC 28/21	QR 28/18	QW 28/18

The fermenter can be sterilised by steam heat with or without pressure or by the use of chemical sterilants.

On the 150 mm diameter tubing used for air-water studies (Shayegan-Salek, 1974), brass bulkhead fittings were used. These were prone to leakage and difficult to seal against the curved wall of the column, restricting the maximum probe diameter to 5 - 8 mm. As stainless steel versions were very expensive, 1" B.S.P.polyproplene fittings were tried on the 100 mm tubing. These were also unsatisfactory, being difficult to seal and prone to leakage and wall-growth. However, because of the technical difficulty of fitting glass screw-cap side arms to glass columns in excess of 150 mm diameter, bulkhead fittings may be the best answer for large glass columns.

Ancillaries

The reactor was supported on a metal framework (Handy Angle). Instrumentation and control equipment was supported on platforms resting on this framework (Fig.6.10). For pH measurements, a meter-recorder (Analytical Measurements, Spring Corner, Feltham, Middlesex) fitted with a $\frac{1}{2}$ r.p.m. motor was used in conjunction with a

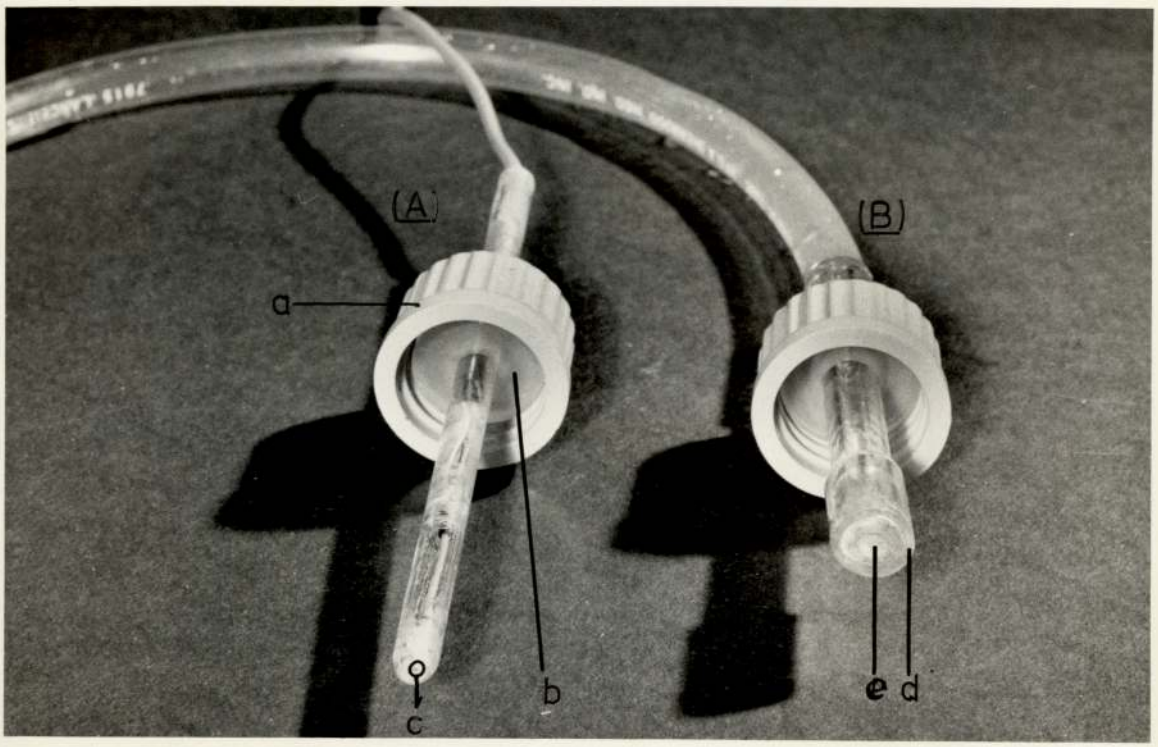


FIG.6:11 PROBE DESIGNS

(A) THERMISTOR

a. Screw cap

b. Silicone rubber seal

c. Thermistor (circled)

(B) REMOTE REFERENCE

d. Silicone rubber retaining tubing

e. Dialysis membrane

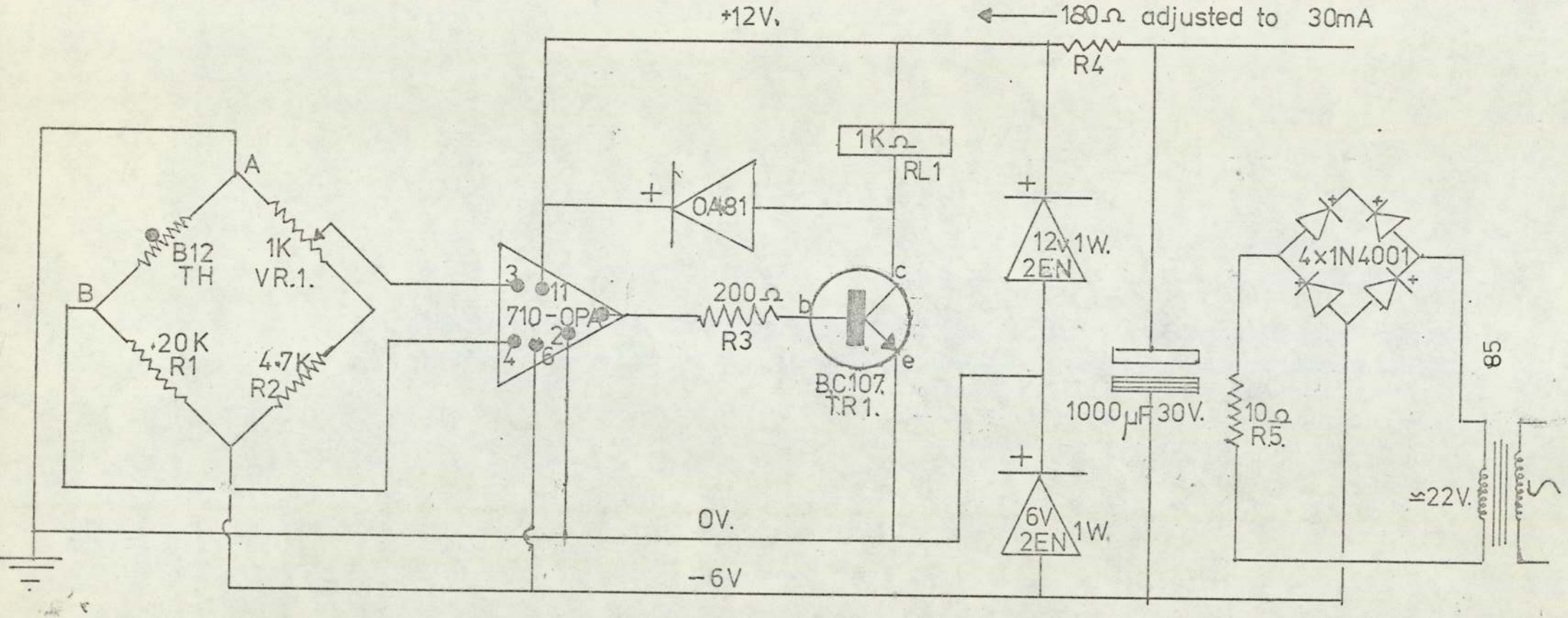
toughened glass electrode containing a gel electrolyte, allowing it to be used in the horizontal position (E.I.L.33-1070-030). The remote reference probe for this arrangement was constructed by using dialysis tubing stretched over the tip of a four-inch, 8 mm diameter glass tube, the membrane being held in place by 8 mm silicone rubber tubing (Fig.6:11) This was linked by 8 mm P.V.C. tubing to an electrolyte reservoir where the calomel electrode (E.I.L.Ltd) was situated.

Dissolved oxygen measurements were carried out using a dissolved oxygen analyser and probe (New Brunswick type DO-50 fitted with $\frac{1}{2}$ r.p.m. motor). The probe used had to be positioned at least 30^{o} to the horizontal, so that an angled port was provided, but electrodes can of course be purchased or constructed which will perform satisfactorily when positioned horizontally.

The temperature of the medium was controlled to within $\pm 0.2^{o}C$ of the set-point by an external jacket of water in a helically-coiled jacket of Paul's tubing (Portex Ltd., Hythe, Kent). The Paul's tubing is one inch in diameter, thin-walled, and is made from flexible PVC. The water was heated and cooled using a modified thermocirculator (Churchill Instruments, Walmgate road, Perivale, Middx.) controlled by a thermistor circuit developed at Aston. (Fig. 6:11a) However, where cooling is not required, attemperation can be more easily achieved by using helically wound waterproof heating tapes controlled by a similar thermistor mains relay circuit (Hotfoil type GW50-70, Hotfoil Ltd., Heath Mill Road, Wombourne, Wolverhampton WV5 8AG)

Additions of spore-suspensions, detergents, antifoam, tracer dyes and filter-sterilised components were made using a syringe/ diaphragm system at the upper port. Samples were withdrawn from the lower port. For this work, no elaborate sampling device was used.

FIG. 6:11a CIRCUIT DIAGRAM OF THERMISTOR RELAY CIRCUIT USED TO SWITCH MAINS POWER-RELAY OF THERMOCIRCULATOR



KEY

R1-R5 FIXED RESISTORS
VR1 VARIABLE RESISTOR (ADJUSTS SET POINT)
TH RS. COMPONENTS B12 THYRISTOR

NOTES

* RL1 SWITCHES POWER RELAY OF THERMOCIRCULATOR
* CIRCUIT CONTROLLED BY B12 THERMISTOR
* VR1 ADJUSTS SET-POINT
* ALL RESISTORS 0·5W

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The gas distributor was of stainless steel woven mesh, pore size 0.3 mm (Fig.6.12). Alternatives include woven glass fibre or plastic, sintered stainless steel, plastic or glass (porosity 03) or perforated plastic or steel plates. Perforated P.T.F.E. plate has proved very resistant to surface growth in continuous fermentation (S.D.Pannell, Personal Communication). Gases were supplied from a continuously-rated compressor, although pressurised bottles may be convenient for gases other than air or in the absence of a compressor. Air from the compressor contained oil, dust and water and this was dealt with by fitting automatic in-line equipment (Norgren, F40 series, Norgren, Shipston-on-Stour, Warwickshire) before passing the air through a flowmeter (Gapmeter Ltd.) and thence through a sterilising filter (Whatman Gamma Twelve, H. Reeve Angel & Co Ltd., 14 New Bridge Street, London, E.C.4). It was found useful to insert a non-return valve (X-lon Plastics) immediately before the column inlet to prevent accidental wetting of the air-filter in the event of compressor or pipework failure.

(b) Fifty-litre Fermenter

The fermenter used was as described by Morris (1972) except for the provision of attemperation, overflow piping and air treatment as with the ten-litre fermenter (Fig.6.13). An improved design has been produced and brief details are given in table 6.6. The expansion chamber should be omitted from the design if possible as it reduces gas mixing and increases capital cost.

(c) Pilot Scale Fermenter

The 500 litre fermenter was constructed of 300 mm diameter food grade polypropylene tubing of 8 mm wall thickness. Attemperation was provided by three water jackets (Figs.6.14,6.15) and a heavy duty cooler/thermocirculator. As there was no thyristor

FIG.6:12. GAS DISTRIBUTOR

FIG.6:13 FIFTY LITRE FERMENTER

1. Effluent gas line
2. Upper sample port
3. Attemperation jacket
4. Lower sample port

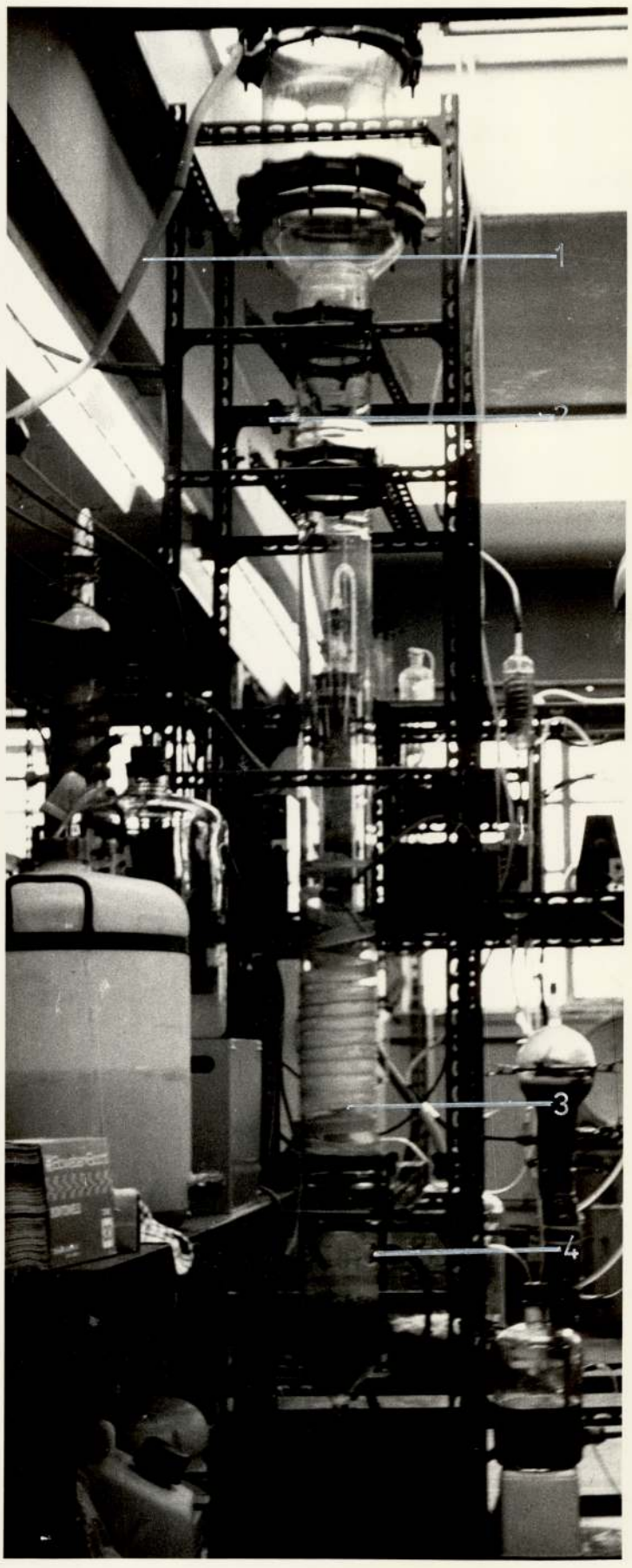


TABLE 6.6. LIST OF FITTINGS (QVF) FOR 50 LITRE TOWER FERMENTER

(6" diameter)

With Expansion Chamber	Without Expansion Chamber
5 x P.S. 6/500	7 x PS 6/500
1 x P.R. 6/1	2 x PR 6/1
2 x P.H.C. 1/0.75	2 x P.H.C. 1/0.75
1 x P.T.1	1 x P.T.1
1 x P.H.C.1/1	1 x P.H.C. 1/1
1 x P.R. 12/6	-
1 x P.S. 12/400	-
1 x P.R. 12/1	-

or with QVF side arms PS 6/500 should be substituted by PTU6 series, e.g. PTU 6/1 where required.

Appropriate couplings and gaskets or silicone sealant are also necessary.

TABLE 6.7. INTERNAL DIMENSIONS OF COLUMNS USED

Normal Working Volume	Diameter (long section)	Length (long section	Expansion Section Diameter	Expansion Section Length
10 litre	10 cm	160 cm	-	-
50 litre	15 cm	210 cm	30 cm	40 cm
450 litre	30 cm	460 cm	200 cm	150 cm

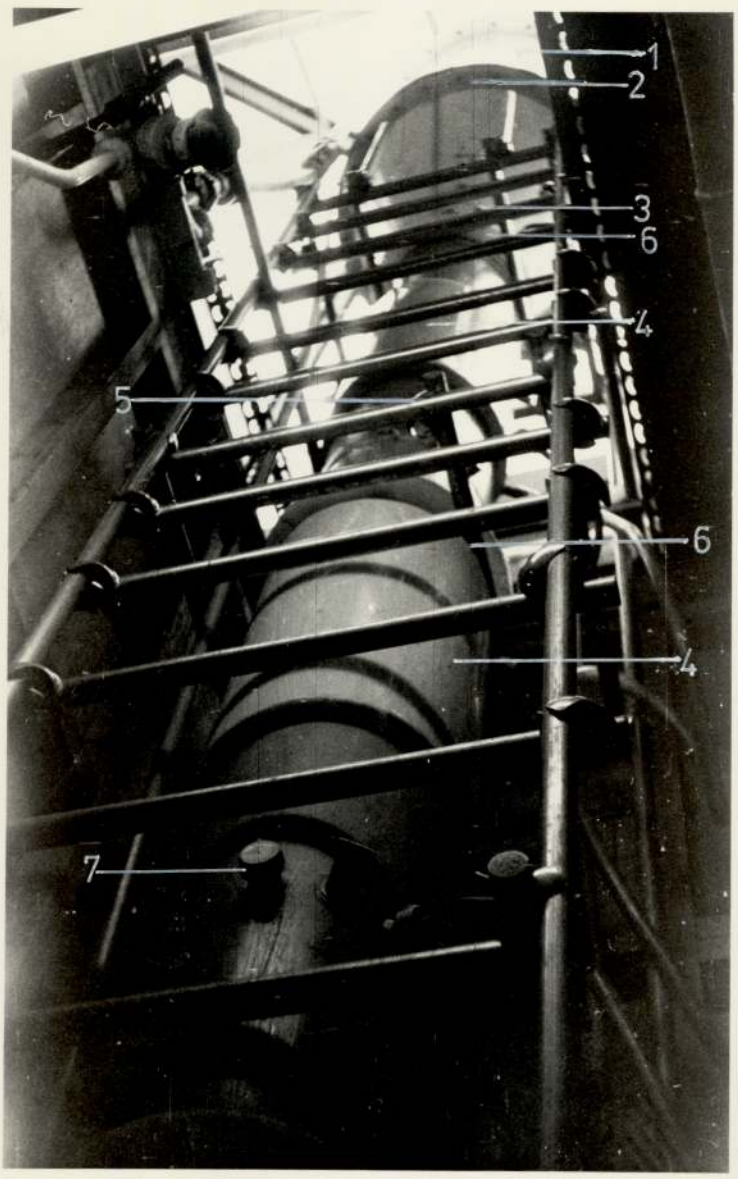


FIG.6:14. PILOT SCALE FERMENTER

1. Expansion section
2. Upper flange
3. Upper ports
4. Attemperation jackets
5. Upper middle ports
6. Support points
7. Lower middle ports

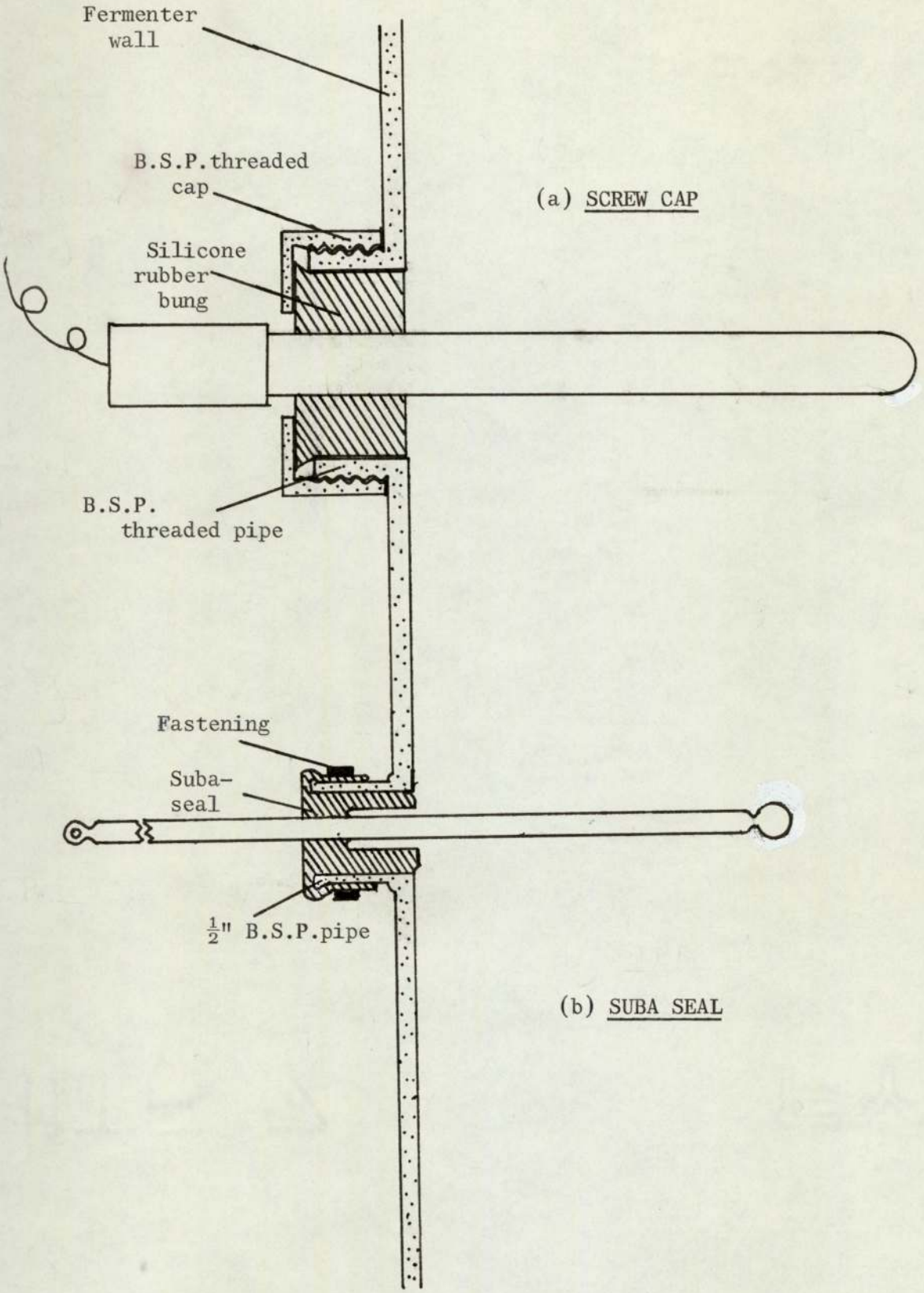
6:15. PILOT SCALE FERMENTER DURING INSTALLATION SHOWING ATTEMPERATION JACKETS

relay control for this system, the internal temperature of the fermenter was controlled manually throughout the fermentations by altering the external thermociculator thermostat.

Ports were added to the column as required by drilling holes of appropriate diameter and welding on $\frac{1}{2}$", 1" and $1\frac{1}{2}$" B.S.P. food-grade polypropylene tubing. This 1" and $1\frac{1}{2}$" tubing had previously been given the corresponding B.S.P. thread. The tube was then cut off within one to two inches of the fermenter wall and an appropriate size blanking cap screwed on to the pipe. In the case of the $\frac{1}{2}$" B.S.P.tubing, a rubber seal was used (Suba-Seal, Gallenkamp Ltd) and held in place by a Jubilee clip or plastic fastener (Schuco Ltd.). Probes and sampling inserts were sealed in place with rubber bungs and the screw caps used to hold them in place against the pressure (Fig. 6.16) Angled ports were of necessity two inches longer to allow room for the shoulders of the cap, illustrating the desirability of probes which will operate in the horizontal position.

Overall dimensions of the fermenters used in the study are given in Table 6.7.

FIG.6:16 DETAILS OF PROBE SEAL FOR PILOT SCALE FERMENTER



Flanges between the bottom pipe of the column and the column proper, between the column and the expansion cone and the expansion tank and lid were sealed with white rubber (food grade) gaskets using stainless steel bolts. The perforated steel air distributor was held in place at the lower flange. In the absence of a suitable non-return valve, the air supply tube was looped up to the top of the expansion chamber then down to the air filter (Gelman paper type, model 12505-L) The air was supplied via an in-line prefilter, pressure reducer and condensate trap (Norgren, typeFZ) from a continuously-rated industrial compressor. Air flow and pressure were also monitored (Gapmeter type I.G.U./$1\frac{1}{2}$"B)

The lid of the fermenter incorporated a port for addition of antifoam and spore suspension, a viewing window (TPX plastic, ICI Ltd.) and an exit pipe for overflows and effluent gases.

All modifications and repairs to this column were conveniently carried out in situ using hot weld techniques (British Celanese Ltd., Plastics Group Sales, Spondon, Derby) and because of its lightweight construction, it was easily installed by members of the Tower Fermentation Group.

Ancillaries

The column was supported at its flanges (Fig. 6.14) by a framework of $1\frac{1}{4}$" diameter tubing (Triclamp, W.C.B. Containers Ltd., Stalybridge, Cheshire). The instrumentation was transferred without modification from the ten-litre tower. Medium preparation was carried out in the purpose-built pilot plant situated in the adjacent Tate & Lyle laboratory.

PART SEVEN : EXPERIMENTAL RESULTS AND DISCUSSION

7:1 ; Rheological studies

For reasons of practical limitation, namely the problem of sample size, (see 6:14) rheological studies were carried out only on fermentations involving the pilot scale fermenter. In effect , this limited the study to only two morphological types .

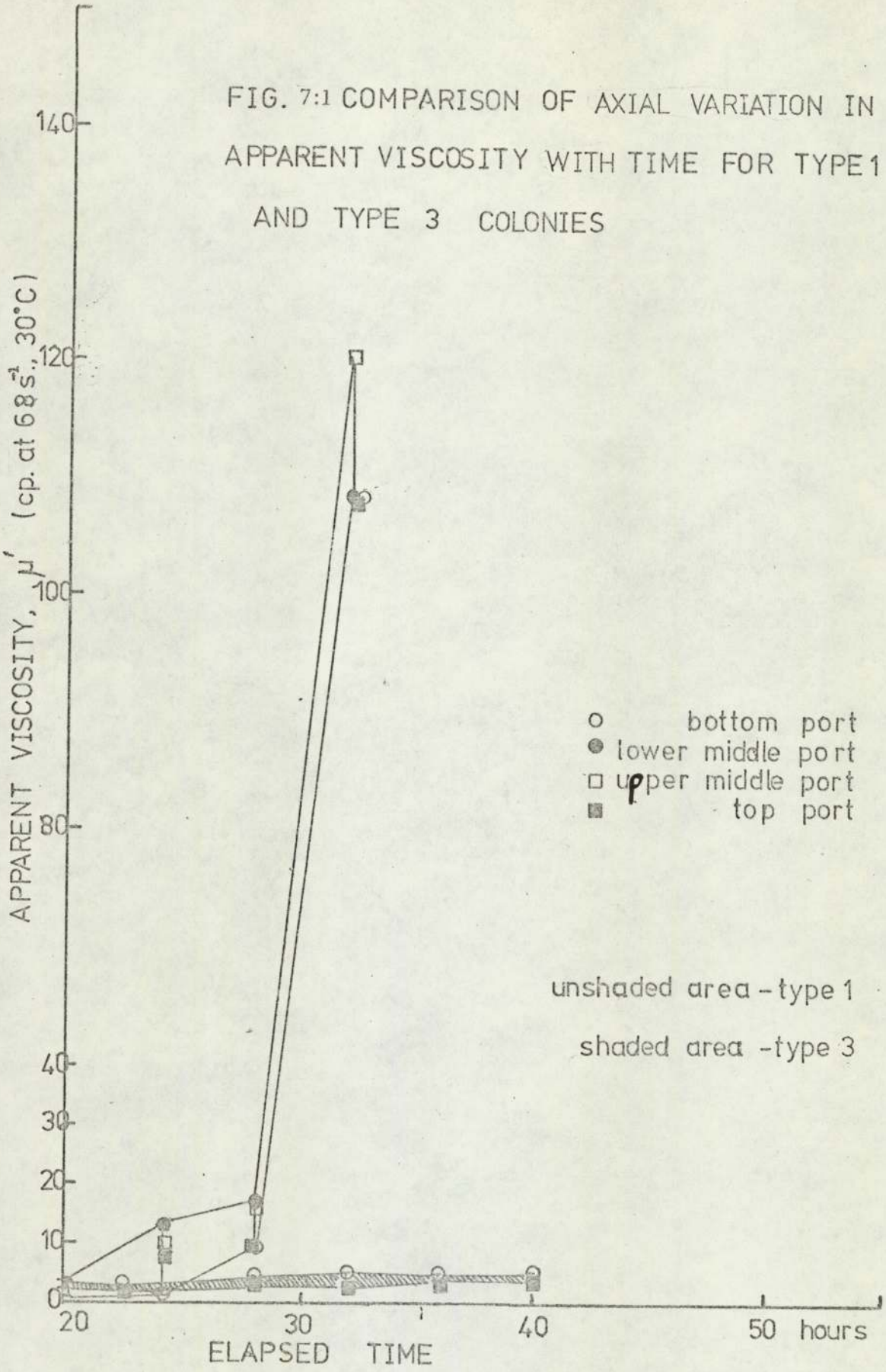
The results are summarised in figures 7:1 - 7:10. Certain relationships, for example , between apparent viscosity and dry weight and time , were difficult to illustrate using conventional projections and therefore several of the graphs are isometric projections (giving an apparent three - dimensional image). Such projections are also used in metallurgy to deal with three - dimensional subjects such as the composition of three - component alloys. A comparison of figs. 7:4 & 7:7 illustrates the difference.

The apparent viscosity of the broth was found to be more dependent on the morphological type than on the mycelial dry weight, as shown by differences between type I* and type III* for apparent viscosity measurements (fig. 7:1) and more emphatically by the radical difference between the curves for apparent viscosity vs. dry weight(FIG.7:2). Note particularly the large increase in apparent viscosity as a function of dry weight for type I mycelium at a time when there was no corresponding increase in dry weight (FIG. 7:3 , 29 - 30 hrs) The apparent viscosity / dry weight ratio can be seen to be far higher for type I mycelium than for type III.

Attempts to characterise the shear - stress / shear rate relationship (FIG. 7:11) were found impractible in this case because of the inability of the Ferranti instrument to operate in the critical range of 0 - 68 s^{-1}. Thus , it was impossible to describe the behaviour of type I mycelial suspensions as either Bingham plastic or Pseudoplastic (FIG. 7:4a). The fluctuations in the shapes of the curves determined for succesive samples (FIG. 7:4, FIG. 7:7) suggests a behaviour which is not fully explained by either the Bingham plastic or the Pseudoplastic model.

(*See section 7:2 for details of this classification .)

FIG. 7:1 COMPARISON OF AXIAL VARIATION IN APPARENT VISCOSITY WITH TIME FOR TYPE 1 AND TYPE 3 COLONIES



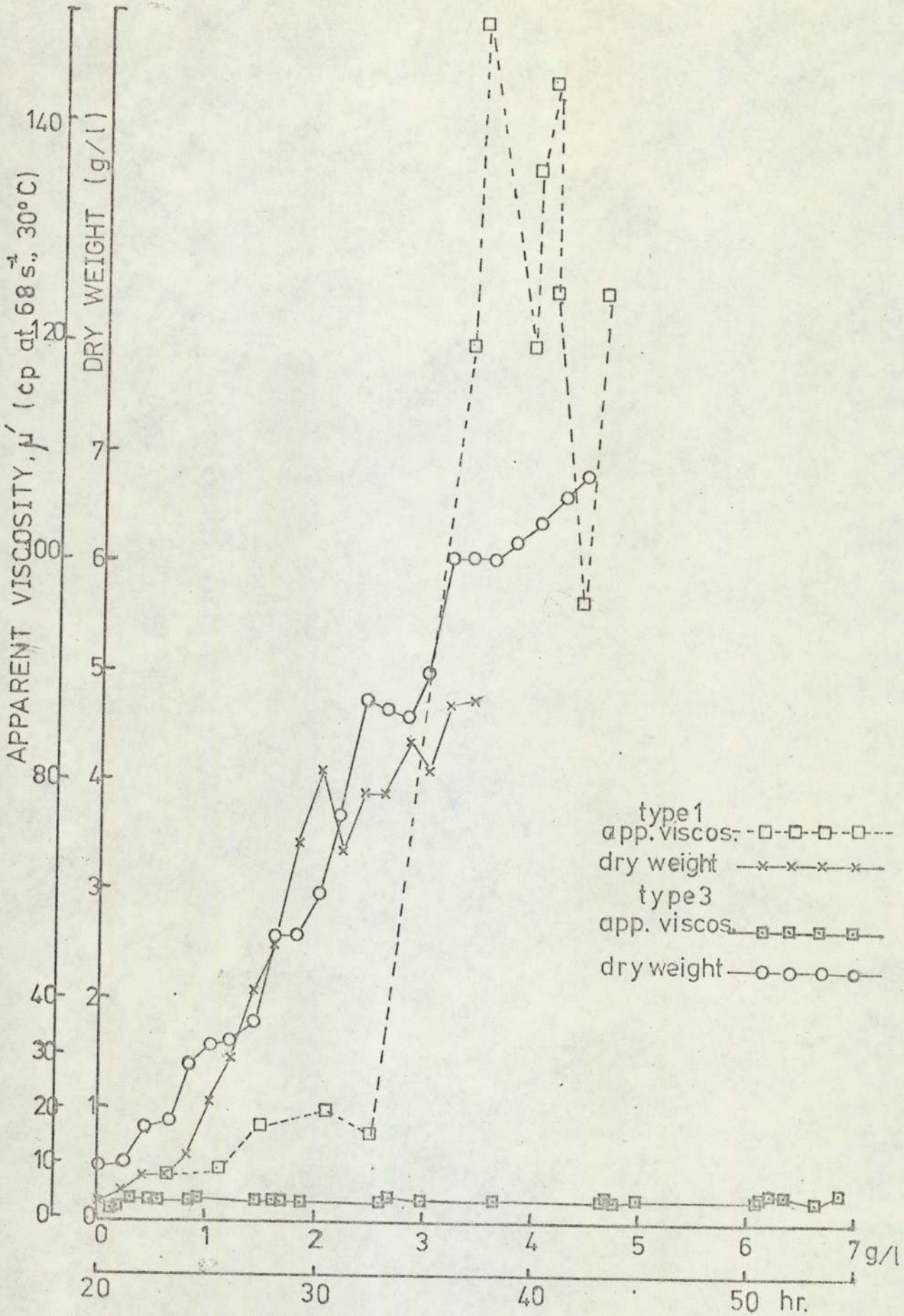


FIG. 7:2 COMPARATIVE RHEOLOGICAL BEHAVIOUR AND DRY WEIGHT GAINS FOR TYPE 1 AND TYPE 2 COLONIES (450 L. SCALE)

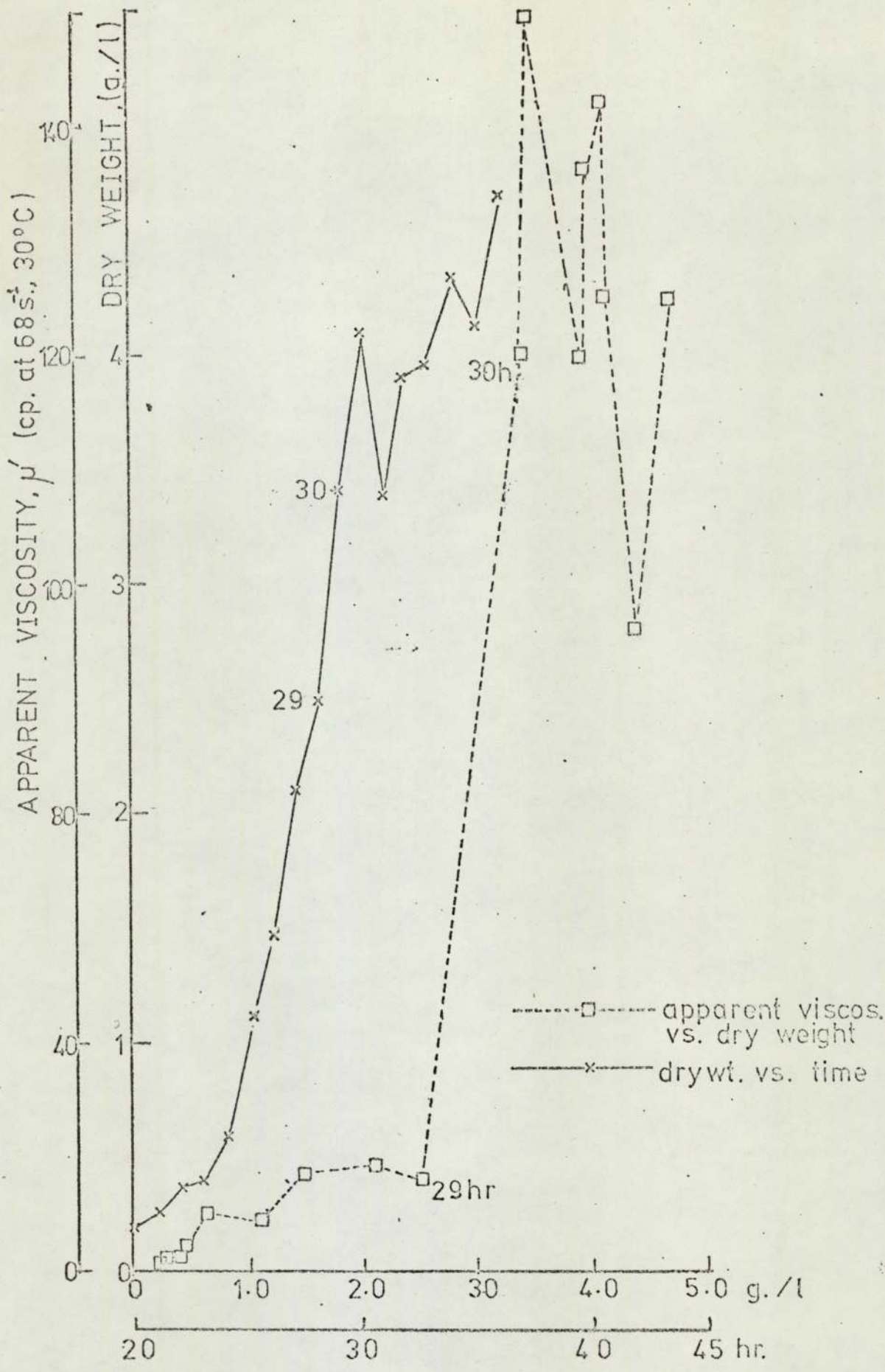


FIG. 7:3 RELATIONSHIP OF APPARENT VISCOSITY, μ'. TO DRY WEIGHT CHANGES DURING FERMENTATION

FIG. 7:4 SUSPENSION RHEOLOGY OF TYPE I COLONIES (samples withdrawn at hourly intervals from pilot-scale fermenter at the upper middle level)

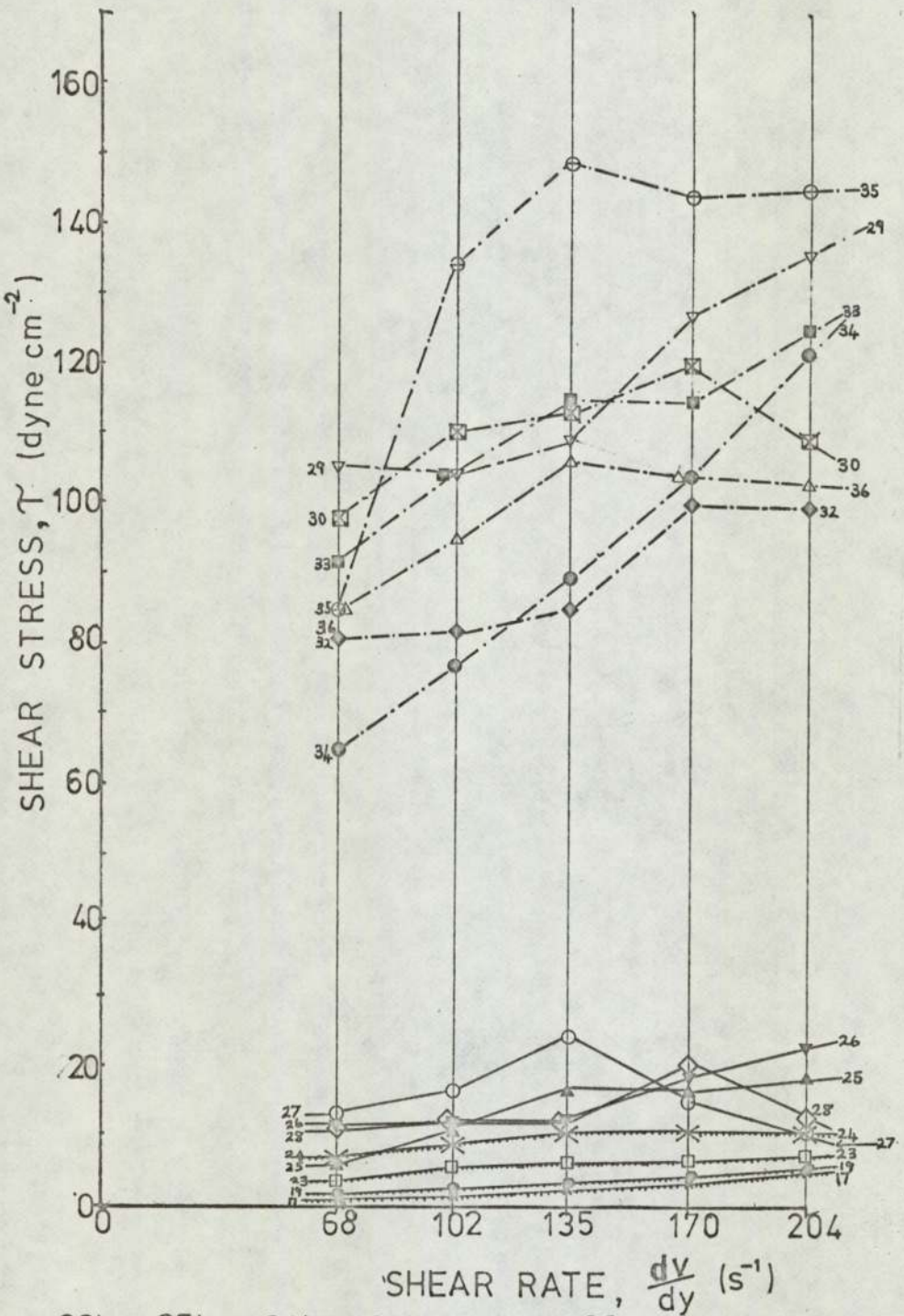


FIG. 7:4a ; CHARACTERISTICS OF VARIOUS TYPES OF RHEOLOGICAL BEHAVIOUR

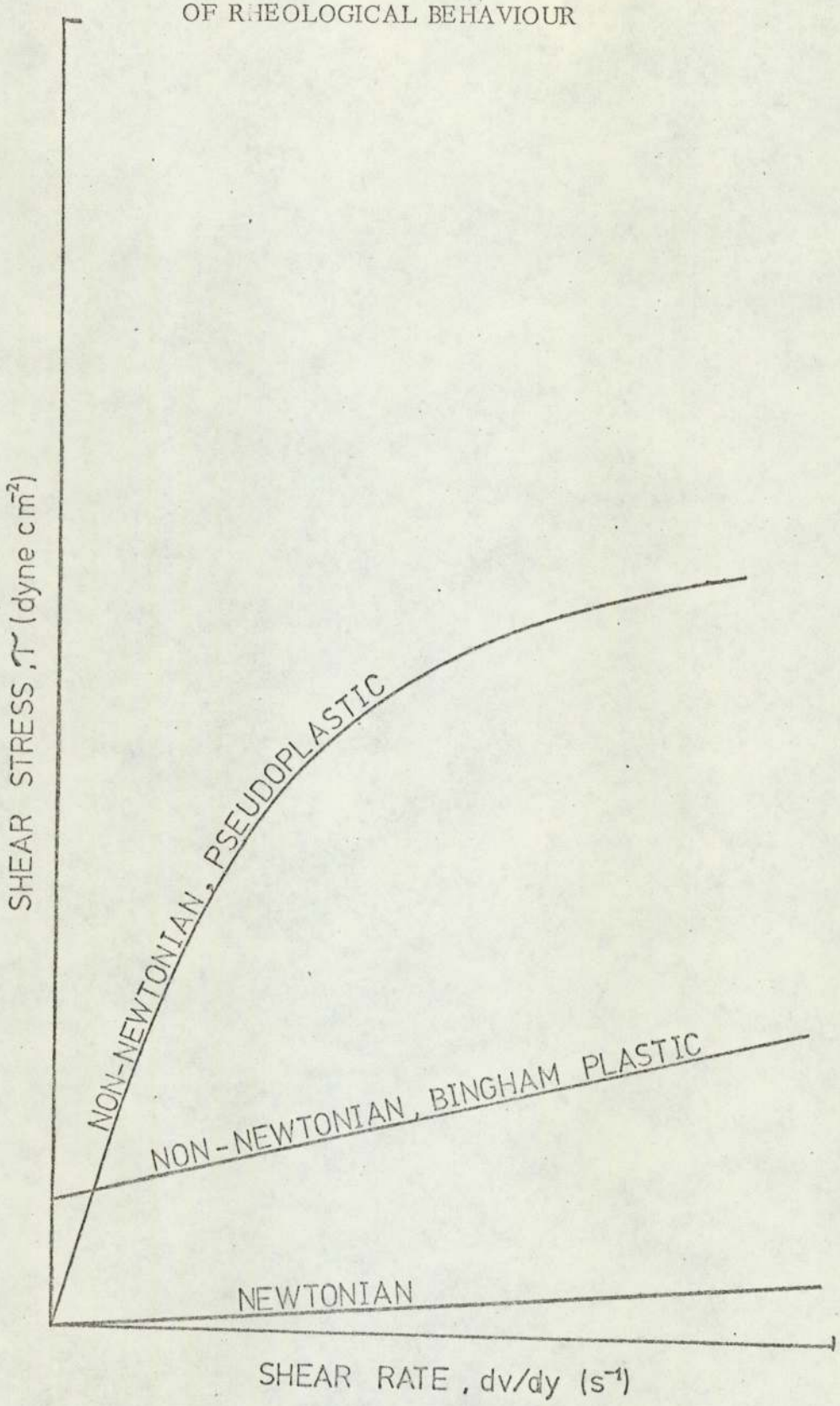


FIG. 7:5 APPARENT VISCOSITY vs. TIME FOR TYPE I COLONIES AT EACH SHEAR -RATE

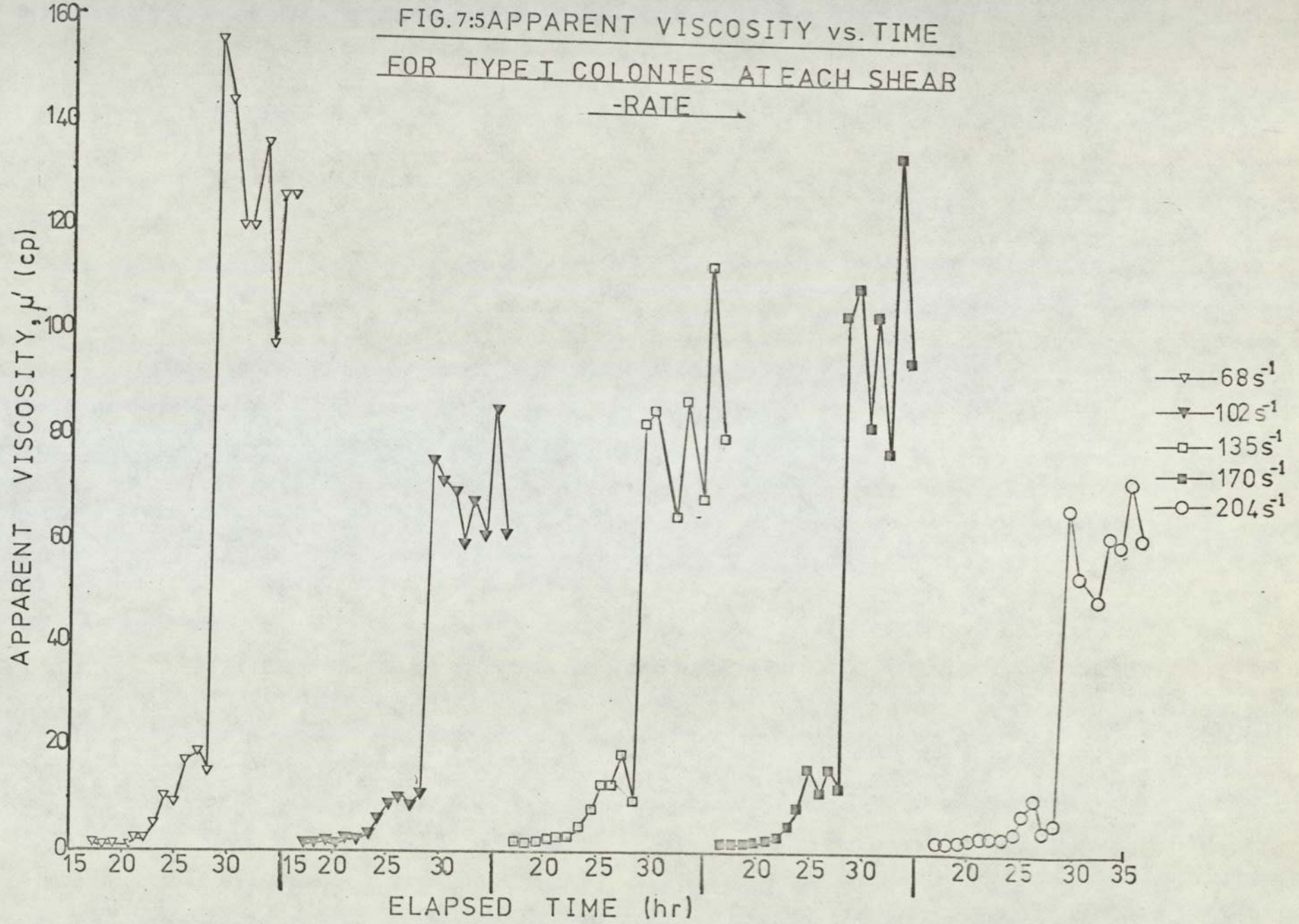


FIG. 7:6 DRY WEIGHT PROFILE DURING A 450L FERMENTATION SHOWING AXIAL DISTRIBUTION OF MYCELIUM (TYPE I) IN COLUMN

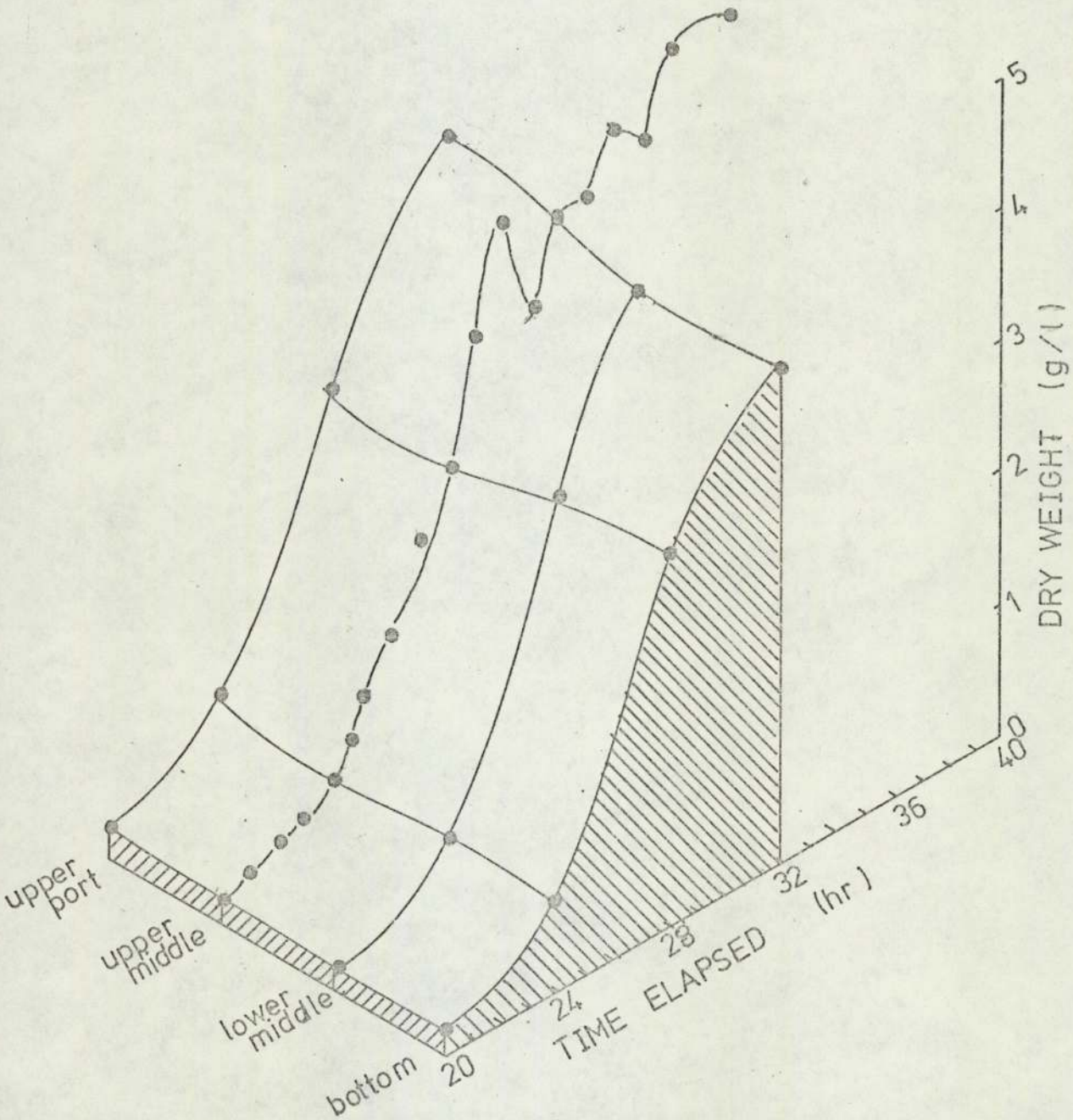


FIG. 7:7 THREE-DIMENSIONAL RELATIONSHIP BETWEEN SHEAR STRESS, SHEAR-RATE, AND TIME, FOR A 450L. FERMENTATION INVOLVING TYPE I GROWTH
[ISOMETRIC PROJECTION]

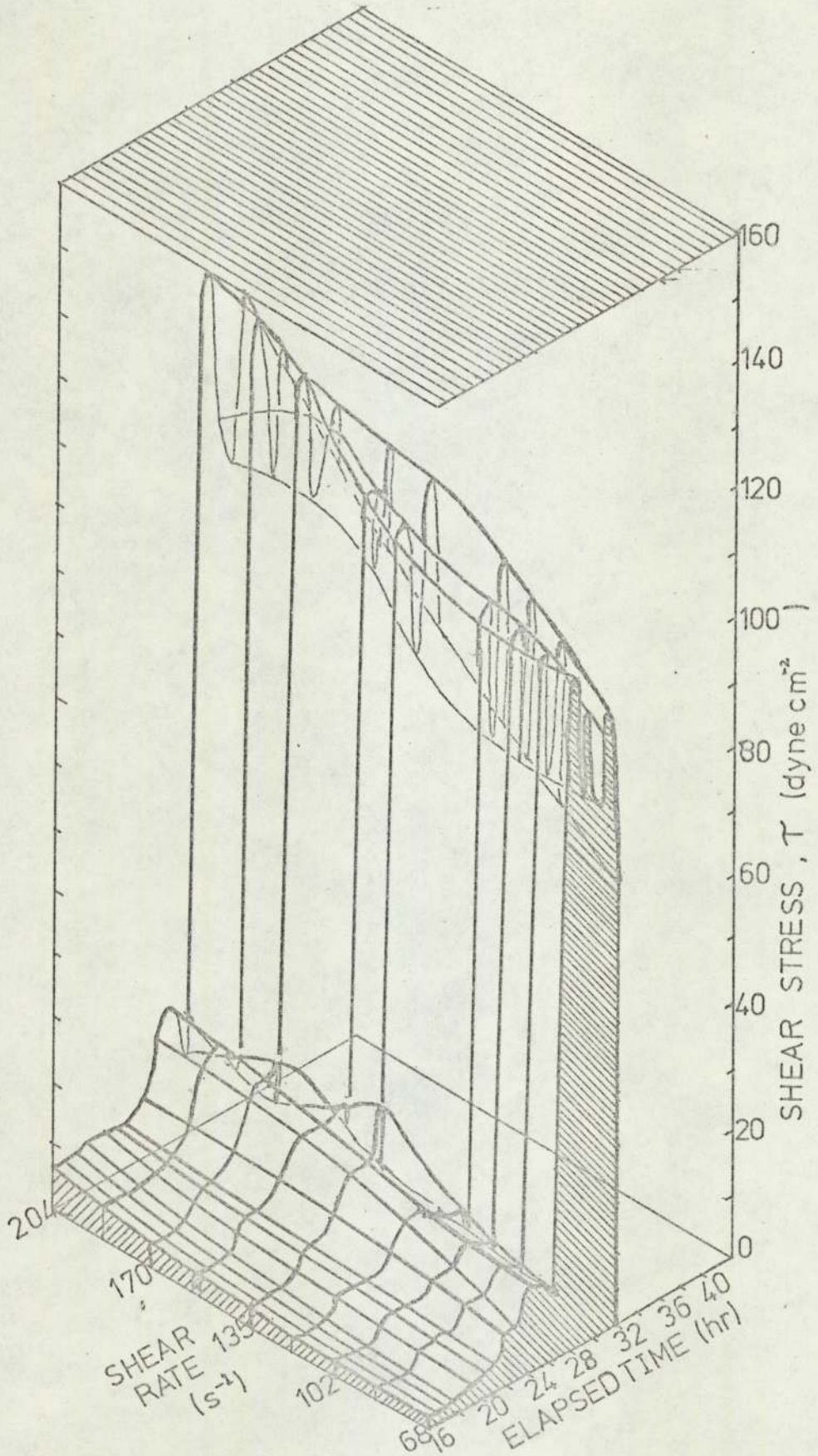


FIG. 7:8 THREE-DIMENSIONAL RELATIONSHIP BETWEEN APPARENT VISCOSITY, SHEAR-RATE, AND TIME, FOR A 450 L. FERMENTATION INVOLVING TYPE I GROWTH

[ISOMETRIC PROJECTION]

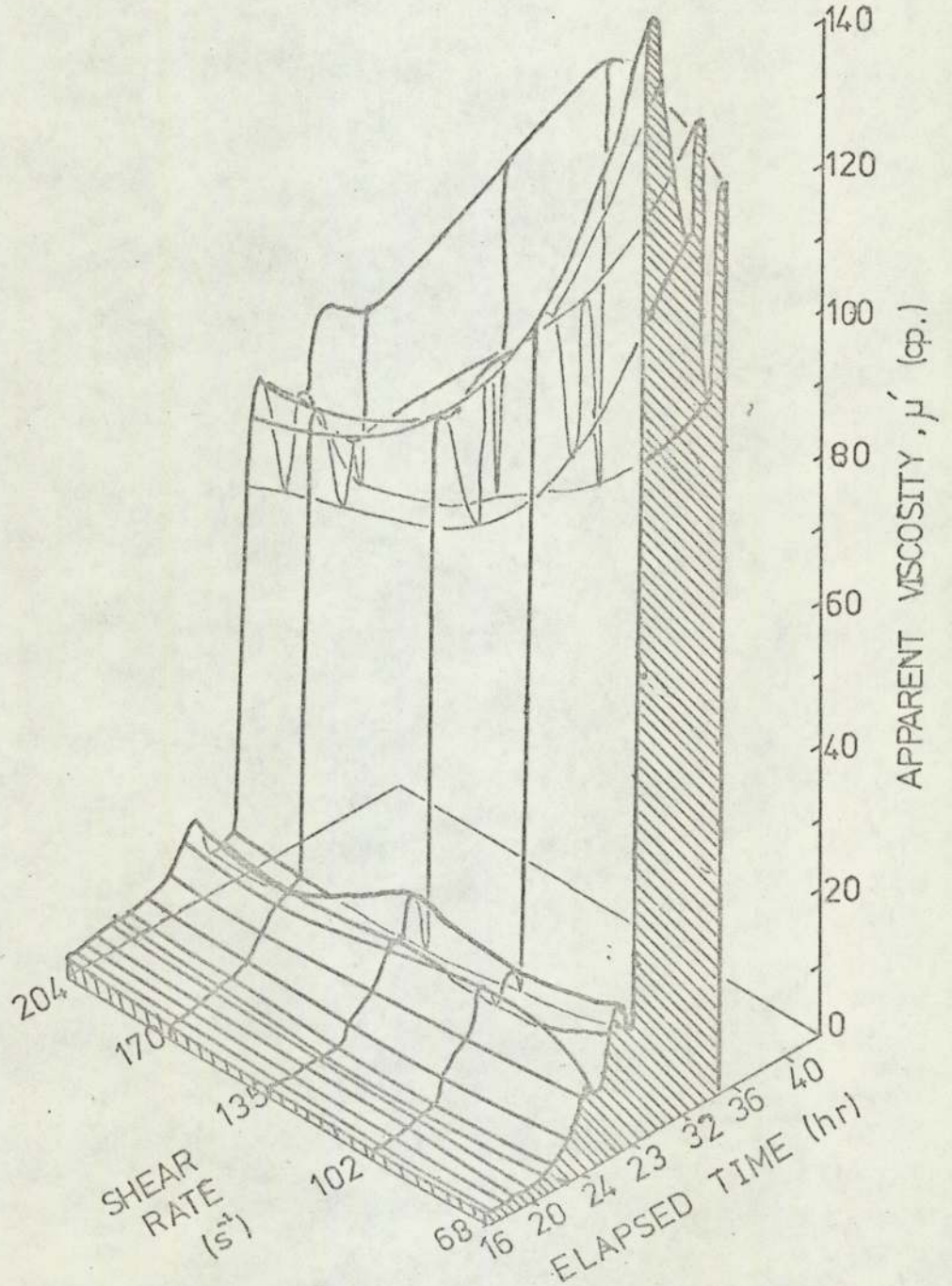


FIG.7:9 AXIAL DISTRIBUTION - PROFILE OF APPARENT VISCOSITY DURING A 450 L. FERMENTATION WITH TYPE III COLONIES

*scale for vertical axis is twice that of fig.

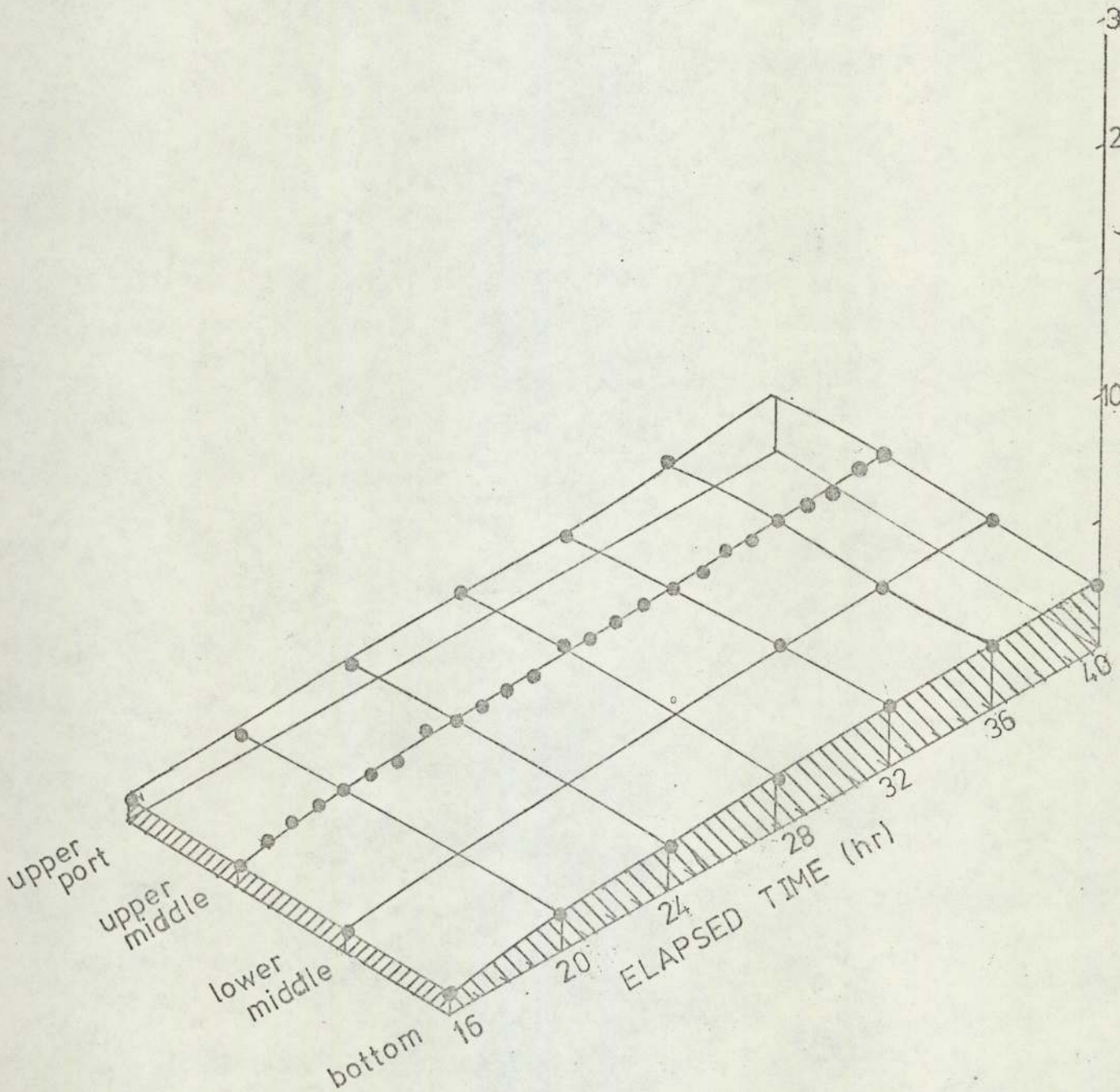
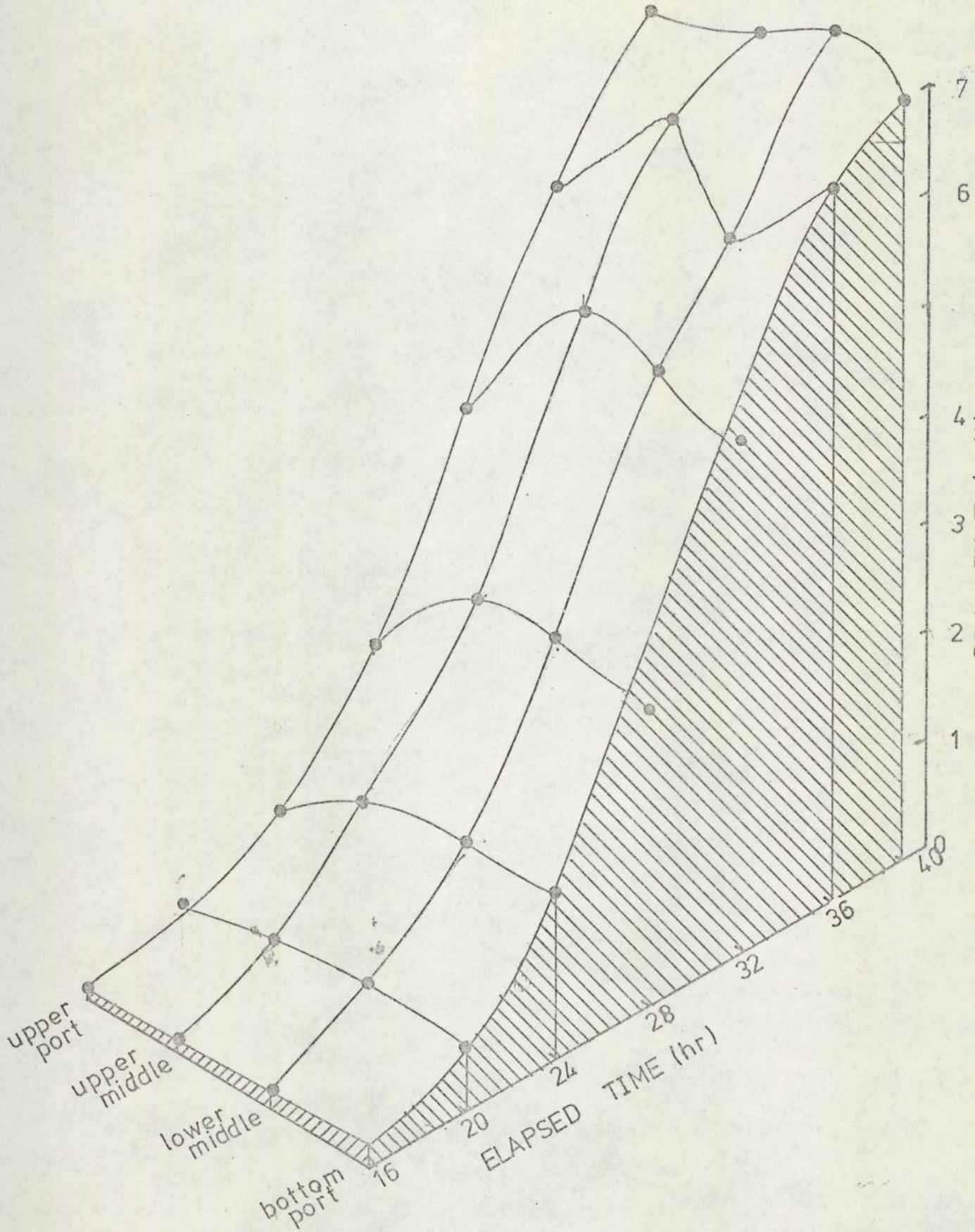


FIG. 7:10 DRY WEIGHT PROFILE DURING A 450L. FERMENTATION SHOWING AXIAL DISTRIBUTION OF MYCELIUM (TYPE III) IN COLUMN



The observed tailing-off in the shear rate/shear- stress curves at some points suggests the influence of flow- effects during the viscometric tests (FIG. 7:4, 27 , 28, 30, 36 & 35- hour test samples.)

The fundamental mechanisms by which mould-colonies affect the viscosity of their suspensions have not been considered in detail by many workers, but some work has been carried out for simpler particulate suspensions, and it is pertinent toexamine the present results in the light of existing theories. The first theoretical consideration of the rheology of particulate suspensions was that by Einstein (1911). He noted that the presence of small, rigid spheres in a liquid deformed flow-patterns. The suspension therefore has a higher viscosity than the supernatant liquid. He therefore determined that

$$\mu_r = \mu_s/\mu_o = 1+2.5\ \emptyset$$

where μ_r = viscosity ratio

μ_s = suspension viscosity

μ_o = liquid viscosity

$\emptyset$ = volume-fraction of solids plus any immobilised liquid.

This relationship applied only to dilute suspensions ($\emptyset < 0.05$) and was later modified by Guth, Sinha, & Kolloid (1956) toaccount for higher particle concentrations, who found that for $\emptyset < 0.08$:

$$\mu_r = \frac{(1+0.5\emptyset - 0.5\emptyset^2)}{(1-2\emptyset - 9.6\emptyset^2)}$$

Microbial particles , cells , or conglomerates often bear little resemblance to rigid spheres, for example, Aiba et al (1962) found that for dilute yeast- suspensions, the viscosity was less than that predicted by the above formulae, and they advanced the theory that the flexibility of the biological material reduced their viscous drag such that

$$\mu_r = 1 + \emptyset\ (<2.5)$$

Fig.7:11 Relationship of Suspension Viscosity to Predicted Viscosity for A.niger M.1.

(data of James, 1973)

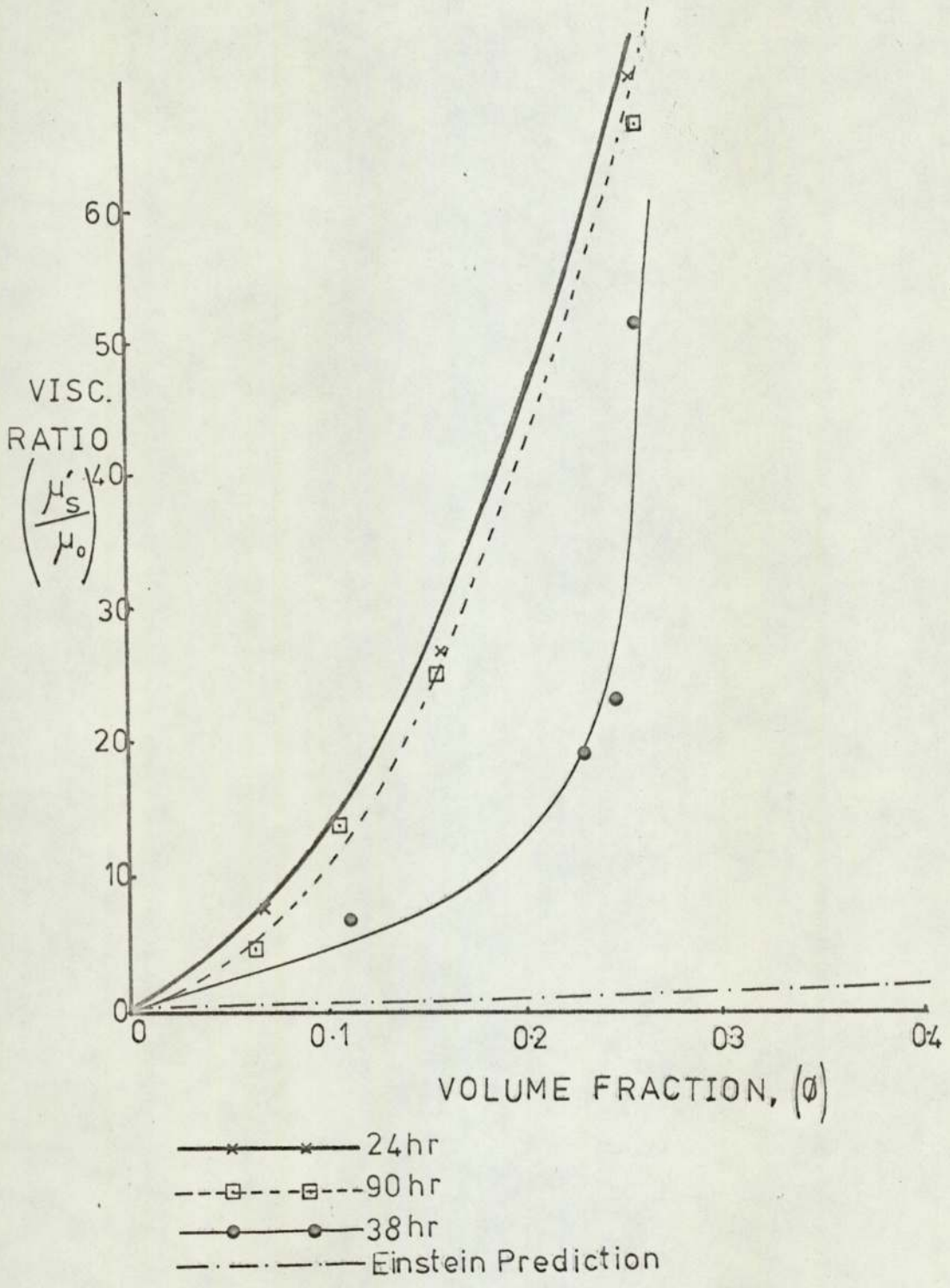


FIG. 7:12 INTERLACING OF HYPHAE FROM TYPE II COLONIES TO FORM A TRANSIENT FIBROUS NETWORK

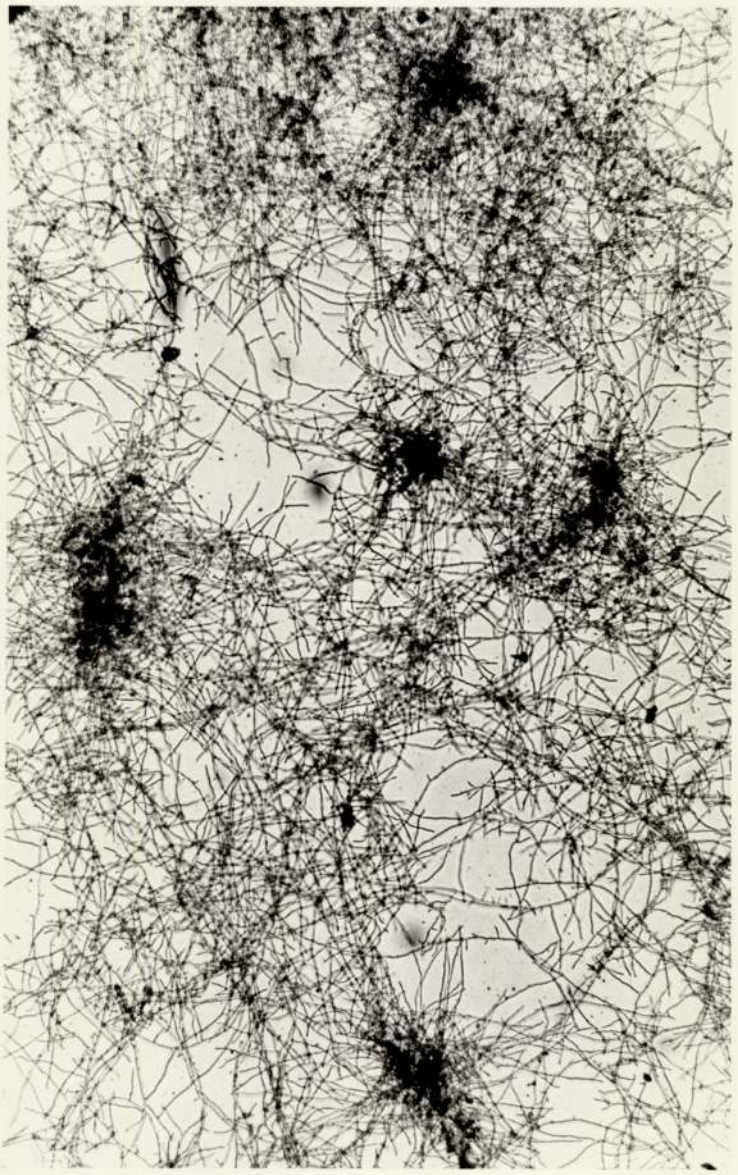


FIGURE 7:13 FREE-FLOWING BEHAVIOUR OF HYPHAE IN TYPE II COLONIES

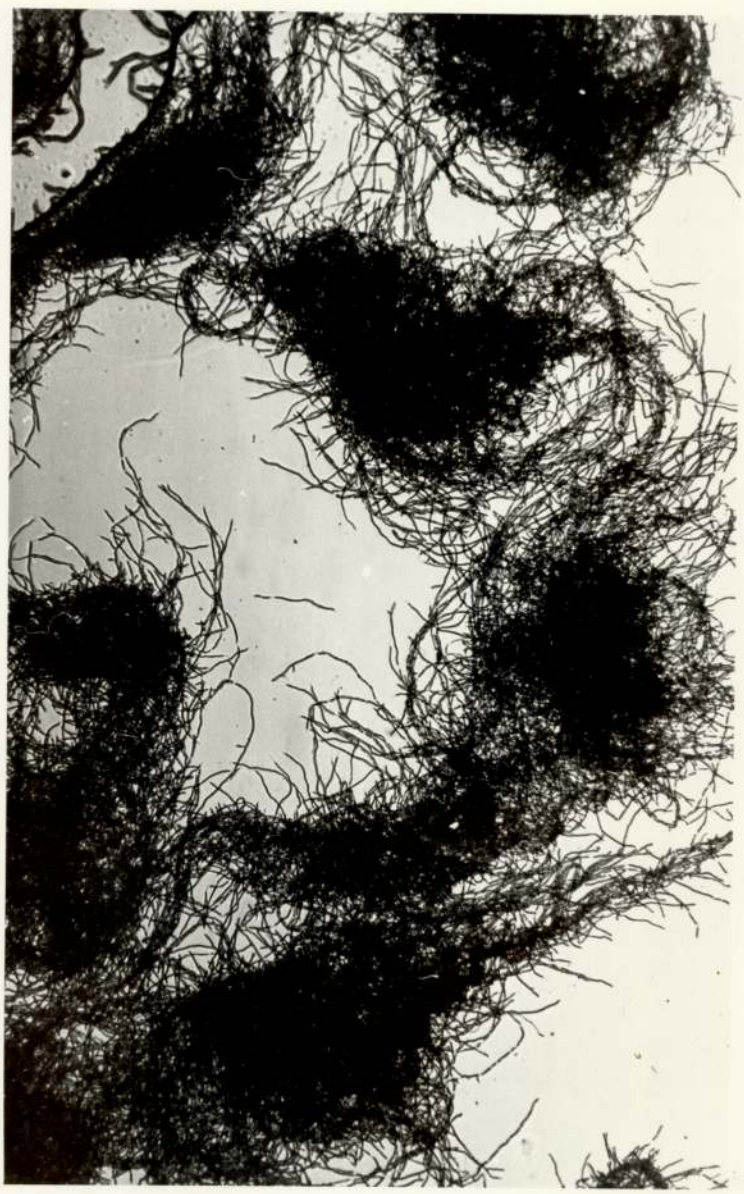
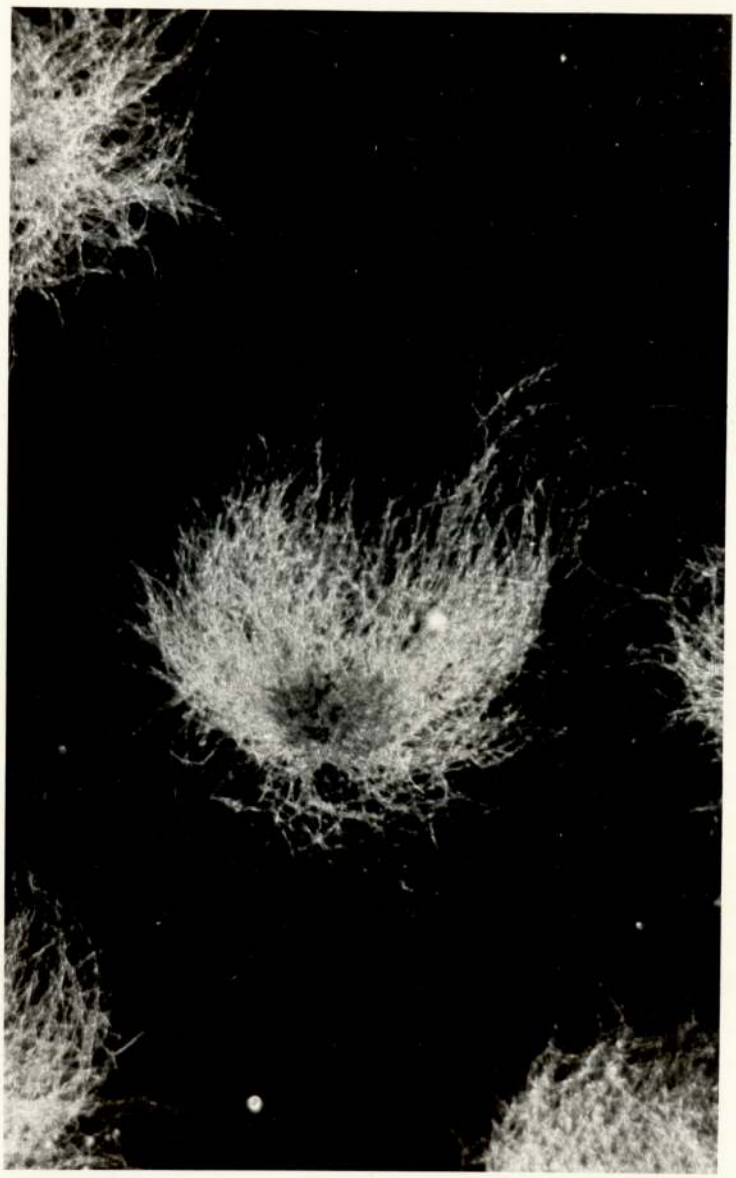


FIGURE 7:14 FREE-FLOWING BEHAVIOUR OF HYPHAE IN TYPE II COLONIES.



Measurements by James (1973) on A. niger M. 1 colonies of type III from tower fermenters gave results that differed widely from Einstein's for smooth spheres, the viscosity being considerably higher (FIG. 7:11). This suggests that the open structure of the spheres increases viscous drag between colonies and between liquid and colonies .

The difficulty of developing theoretical models for such large particles is illustrated in this case by the sharp fourfold increase in apparent viscosity and shear-stress (FIGS. 7:7; 7:8), followed by a random fluctuating behaviour. It was originally thought that this complex behaviour was a result of the diameter of the mould colonies being approximately the same size as the width of the viscometer annulus(FIG. 6:2) but since the width of the viscometer annulus was found to be approximately three times the colony-size, this explanation is discounted. The sudden increase in viscosity, followed by irregular fluctuations, suggests rather the presence of transient mechanical effects such as the interlacing of hyphae from adjacent colonies to form bridges or networks in the suspension (FIG. 7:12). Further evidence is provided by the sudden transition to a porridge-like consistency and the simultaneous onset in the fermenter of gas-slugging and inhomogeneity of solids(FIG. 7:3; 7:6); indicating that this is not merely an artifact of the viscometric test. Microscopic examination has on many occasions revealed the ability of type I and II colonies especially to "flow" under the stimulus of gentle pressure on the cover-slip of wet- film preparations . They may also "flip- flop" in the manner of a mop-head , squeezing out liquid in the process (FIGS. 7:13; 7:14). This behaviour may also be observed directly in a diluted sample, for instance by agitating the suspension in a viewing-cell. This combination of particle flexibity with the ability to form transient matrices within the suspension may account for the unusual rheology of such broths, if the gel-like structures repeatedly collapse in the face of excess shear to be replaced by streamlined particles offering minimal resistance to flow.

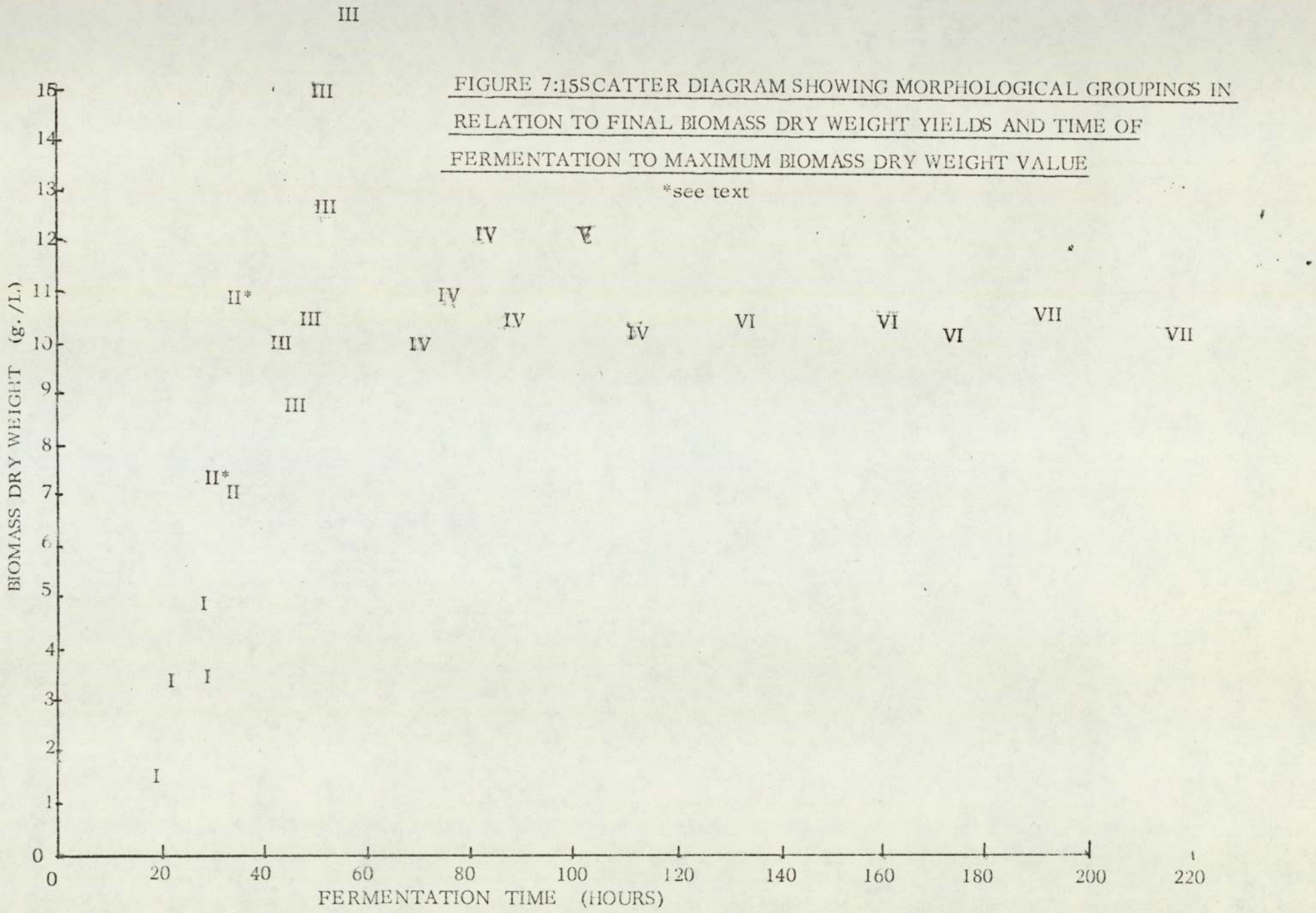


FIGURE 7:15 SCATTER DIAGRAM SHOWING MORPHOLOGICAL GROUPINGS IN RELATION TO FINAL BIOMASS DRY WEIGHT YIELDS AND TIME OF FERMENTATION TO MAXIMUM BIOMASS DRY WEIGHT VALUE

*see text

Suspensions with this behaviour would be expected to display time-dependency in their response to shear (thixotropy), and it was found during this work that short-term fluctuations in apparent viscosity meant that readings had to be made instantaneous. This difficulty has also been recently reported by Metz (1975), when using a turbine-type viscometer.

The implications of these findings for fermenter performance are important: it will be noted that until the transition to high viscosity levels, the growth of typeI colonies was more rapid than for type III colonies (compare figs 7:6 &7:10). This correlates with a more general trend for type I colonies to grow faster than typeII and for type II in turn to grow faster than type III, even though the final biomass-yields are in the reverse order of ascendancy(FIG. 7:15). In earlier work, it was found that the dilution of the broth with ordinary tap-water was sufficient to restore the growth-rate at this stage in the fermentation(FIG. 7:15). At the time this strongly suggested that the growth of the organism was self-inhibitory, either because of the production of toxic metabolites , or some type of "crowding" effect. The rheological studies reported here suggest that the suspension viscosity is the underlying component governing this change.

Interpreting these results in a wider context, the highest apparent viscosity measured in this work (200cp.) contrasts markedly with those values associated with stirred fermenters. The tower-fermenters as used at present do not operate well above a threshold viscosity value, at which mixing and mass-transfer behaviour deteriorate seriously. However, the operation of S.T.R's is not without its problems in dealing with non-Newtonian, highly viscous broths, but they generally cope more successfully with the problem , characteristically working at higher biomass-levels than tower-fermenters.

FIG. 7:16 MORPHOLOGICAL CLASSIFICATION

TOP LEFT : Type I colonies , viewing cell. (scale division = 1mm)

MID LEFT : Type I colonies , bright field microscopy (bar = 100 mμ)

BOT LEFT : Type I colonies , stereoscan (bar = 100 mμ)

TOP RIGHT: Type II colonies, viewing-cell (scale division =1mm)

MID RIGHT: Type II colonies , dark ground microscopy (bar = 100mμ)

BOT RIGHT: Type II colonies , hyphal detail (bar = 100 mμ)

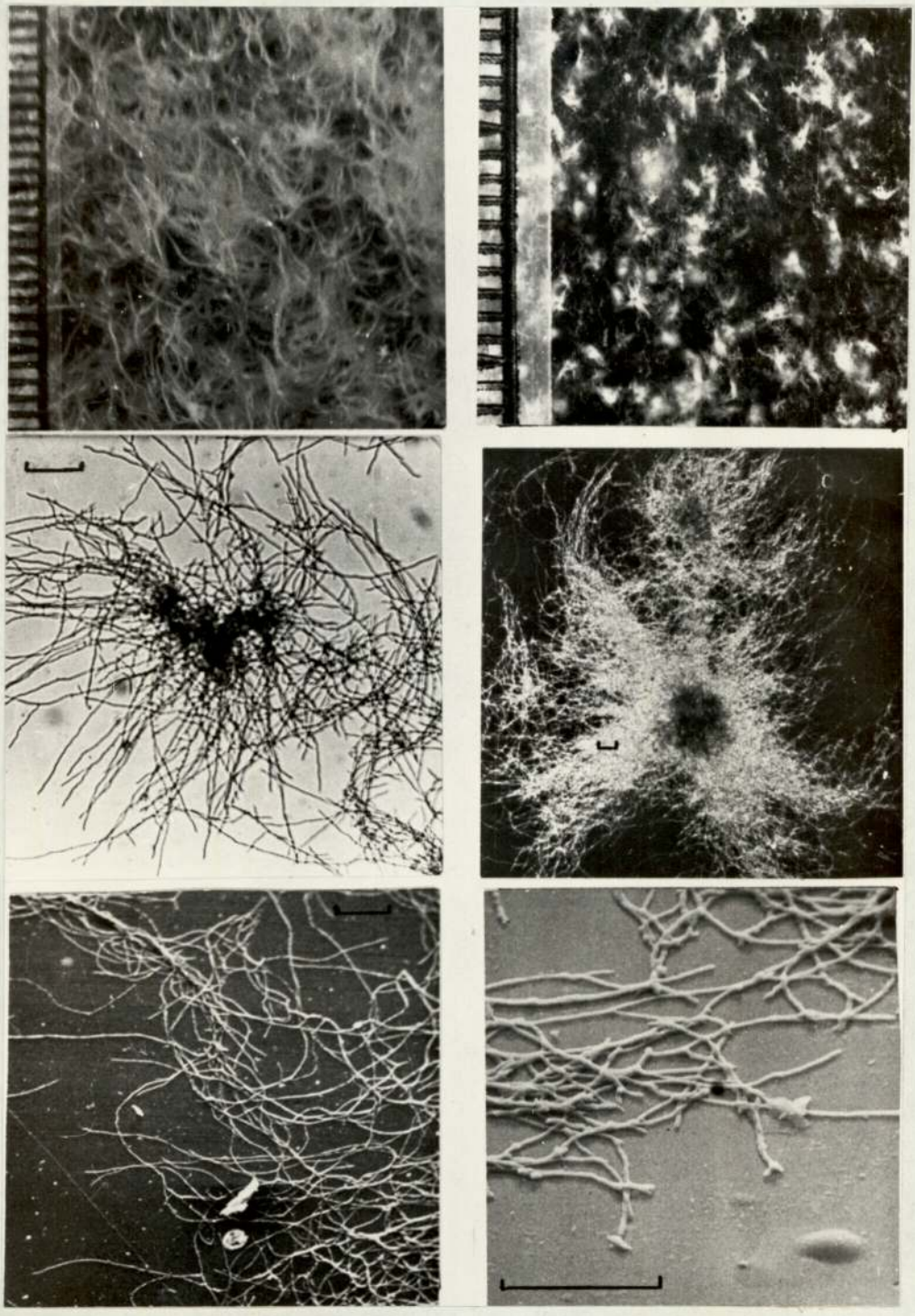


FIG. 7:17 MORPHOLOGICAL CLASSIFICATION

TOP LEFT : Type III colonies in viewing cell. (scale division = 1mm)

MID LEFT: Type III colonies by dark ground microscopy (bar = 100mμ)

BOT LEFT : Type III colony detail, stereoscan (bar = 100mμ)

TOP RIGHT : Type IV colonies in viewing cell (scale division = 1mm)

MID RIGHT : Type IV colonies bright field (bar = 100 mμ)

BOT RIGHT : Type IV colonies by stereoscans (bar = 100mμ)

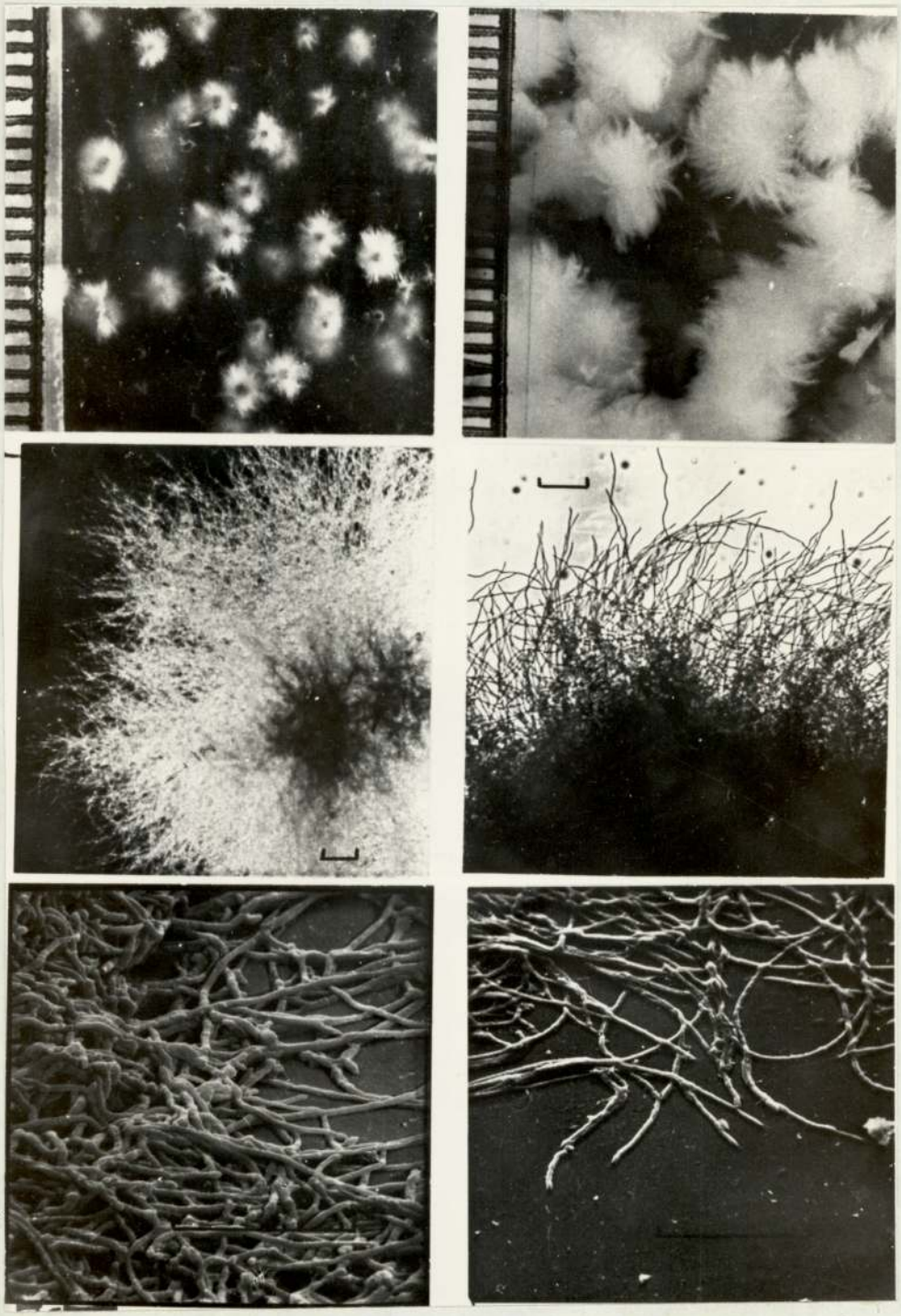


FIG. 7:18 MORPHOLOGICAL CLASSIFICATION

TOP LEFT Type V colonies in viewing cell (scale division = 1mm)

MID LEFT Type V colonies showing edge detail (bar = 100 mμ)

BOT LEFT Type V colonies showing detail (stereoscan , bar = 100mμ)

TOP RIGHT Type VI colonies in viewing cell (scale division = 1mm)

MID RIGHT Type VI colonies , bright field (bar = 100 mμ)

BOT RIGHT TYPE VI colonies , stereoscan (bar = 100 mμ)

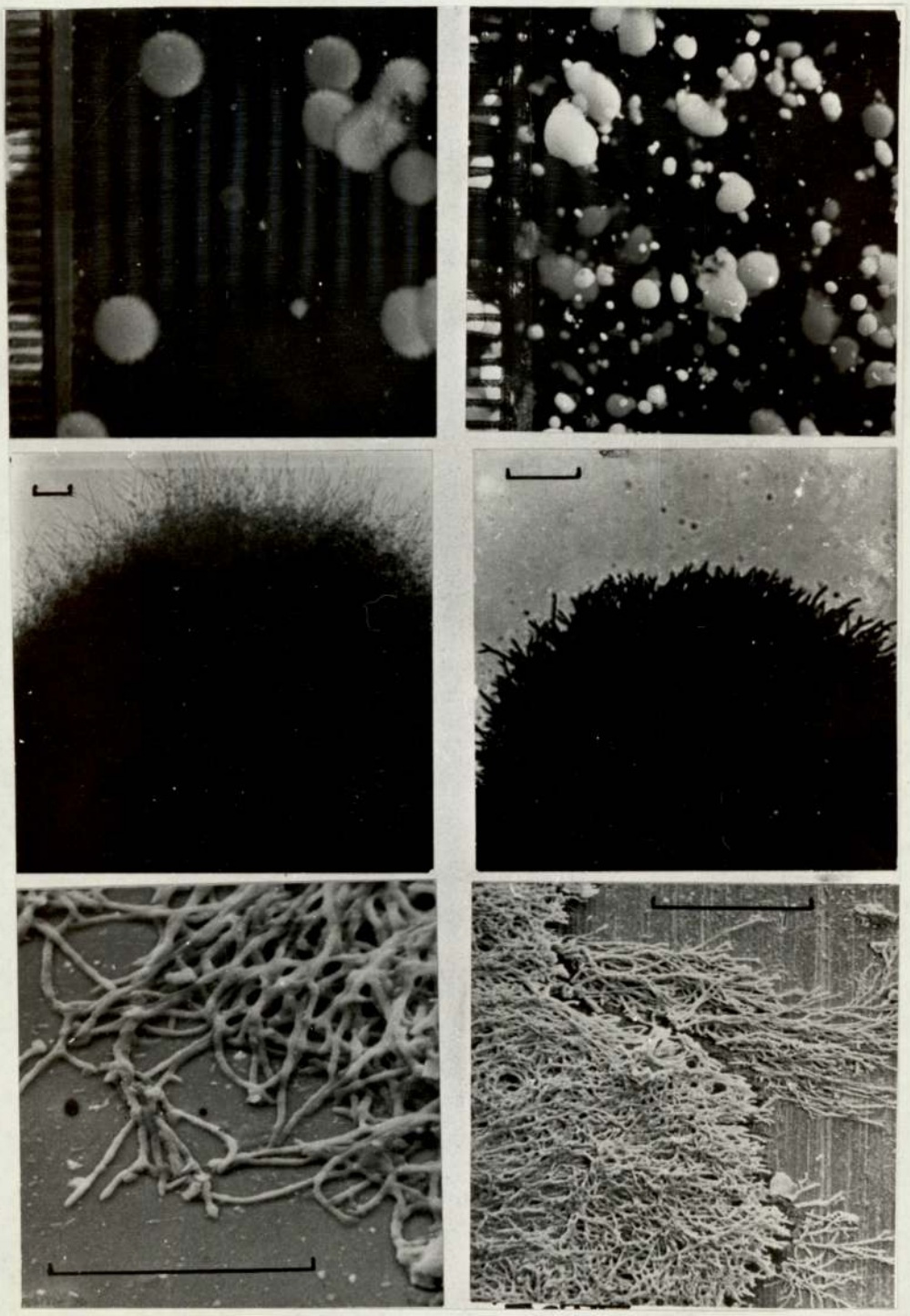
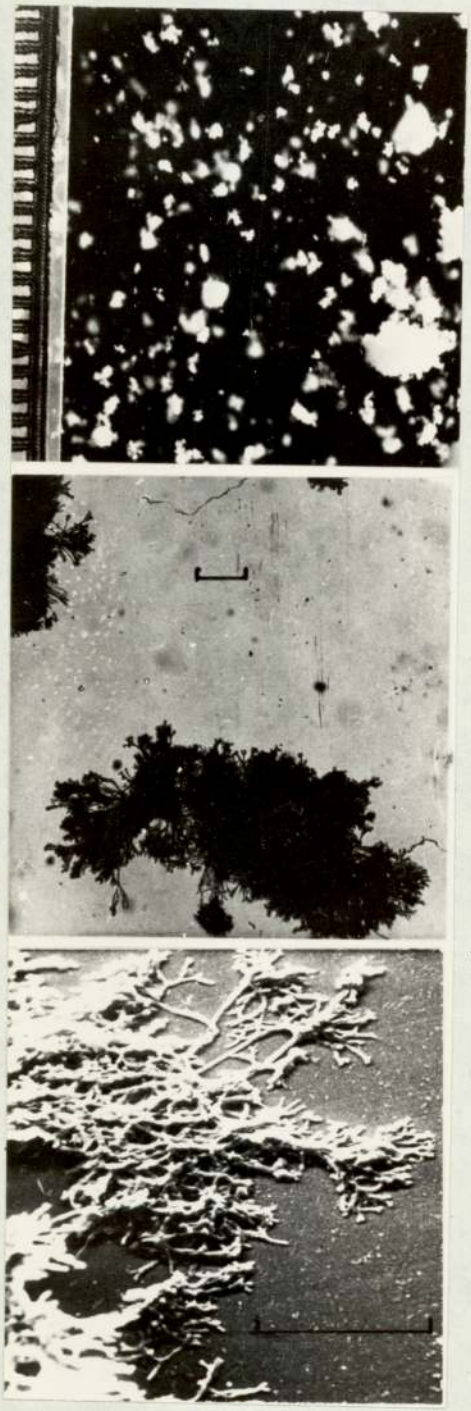


FIG. 7:19 MORPHOLOGICAL CLASSIFICATION

TOP : Type VII colonies in viewing cell. (scale division = 1mm)

MIDDLE : Type VII colonies by bright field (bar = 100 mμ)

BOTTOM : Type VII colonies (edge detail, stereoscan, bar = 100 mμ)



7:2 The Nature and Extent of Morphological Variation In Batch Culture

In contrast to the stirred fermenter, the tower fermenters used in this work did not seem capable of producing short fragmented filaments in any quantity, certainly not without also producing a much larger quantity of colonies. Under a wide range of conditions(see 7:3) what at first seemed a bewildering array of morphological variety manifested itself. After considerable fermentation experience, however, patterns began to emerge, and to allow description of the results, seven colony-types were chosen as reference points in the continuous range, and these are defined by the photographs.

At one extreme, already encountered, is type I, then type II, both of which are loose, irregular in shape, and feathery. These are followed by type III, which is less irregular. For types V, VI, VII, the hyphae are more densely-packed and the colonies smoother and more spherical in shape. At the other extreme, radial symmetry disappears and the hyphae appear stunted and highly branched.

Many workers have not submitted photographs when describing morphologies and have instead been content with verbal descriptions such as "flocculent", "filamentous pellets", "loose pellets" or "hard pellets". The use of the term "pellet" has been avoided as far as possible in this study because it was felt that no definition had ever been firmly and universally accepted. For example, Martin and Waters (1952) appear to regard only type VI as "pellets", whereas the review of Whitaker and Long (1973) indicates that other authors extend this definition by degrees to include types V, IV, III, and II. The use of photographs in this study was intended to avoid ambiguity, in the absence of an adequate numerical classification.

Methods available for the numerical characterisation of morphological features have been developed for surface cultures in the main, and include colony radius, mean internode length (Trinci, 1969), hyphal growth unit (Trinci, 1973), peripheral hyphal density (Trinci, 1969)

FIG. 7:20 HETEROGENEITY OF MORPHOLOGY CAUSED BY FRAGMENTS OF MYCELIUM BEING RELEASED FROM TYPE III COLONIES

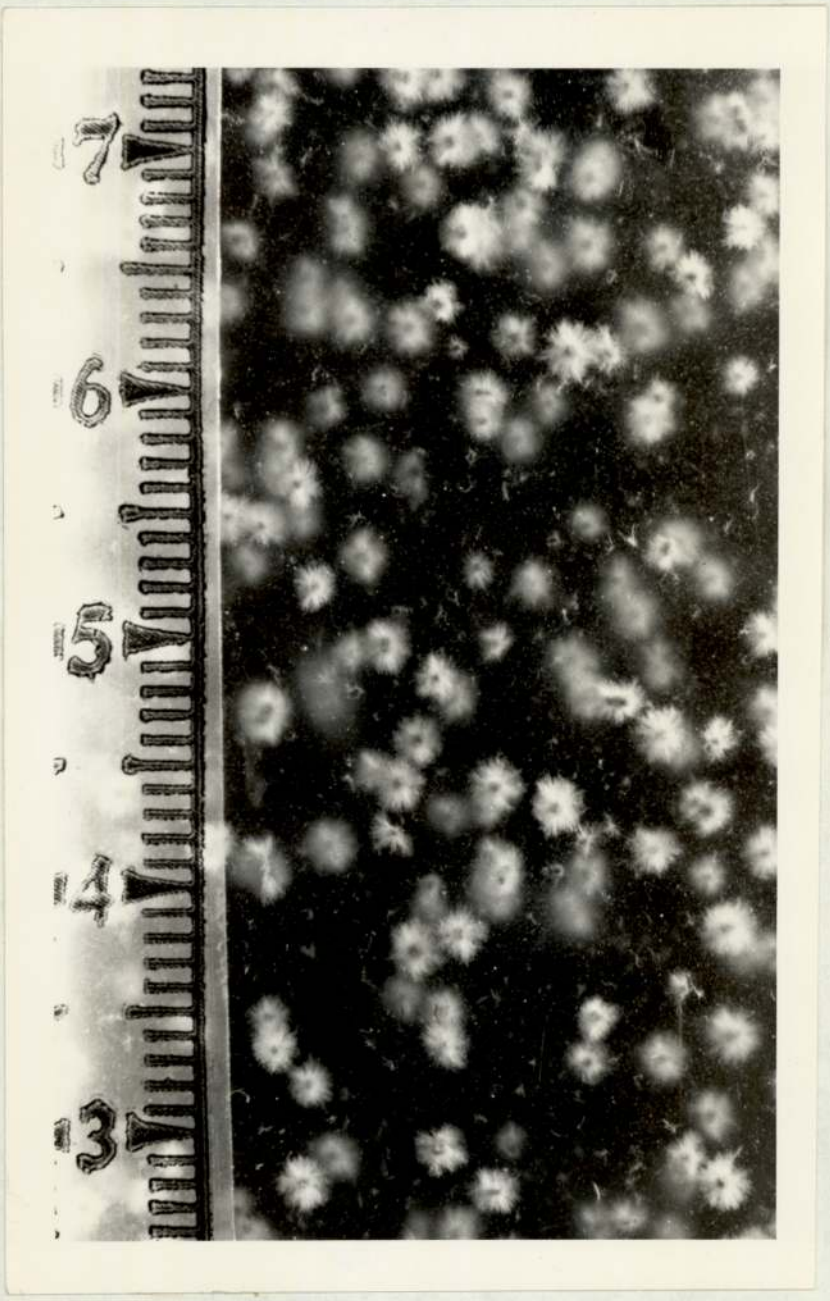
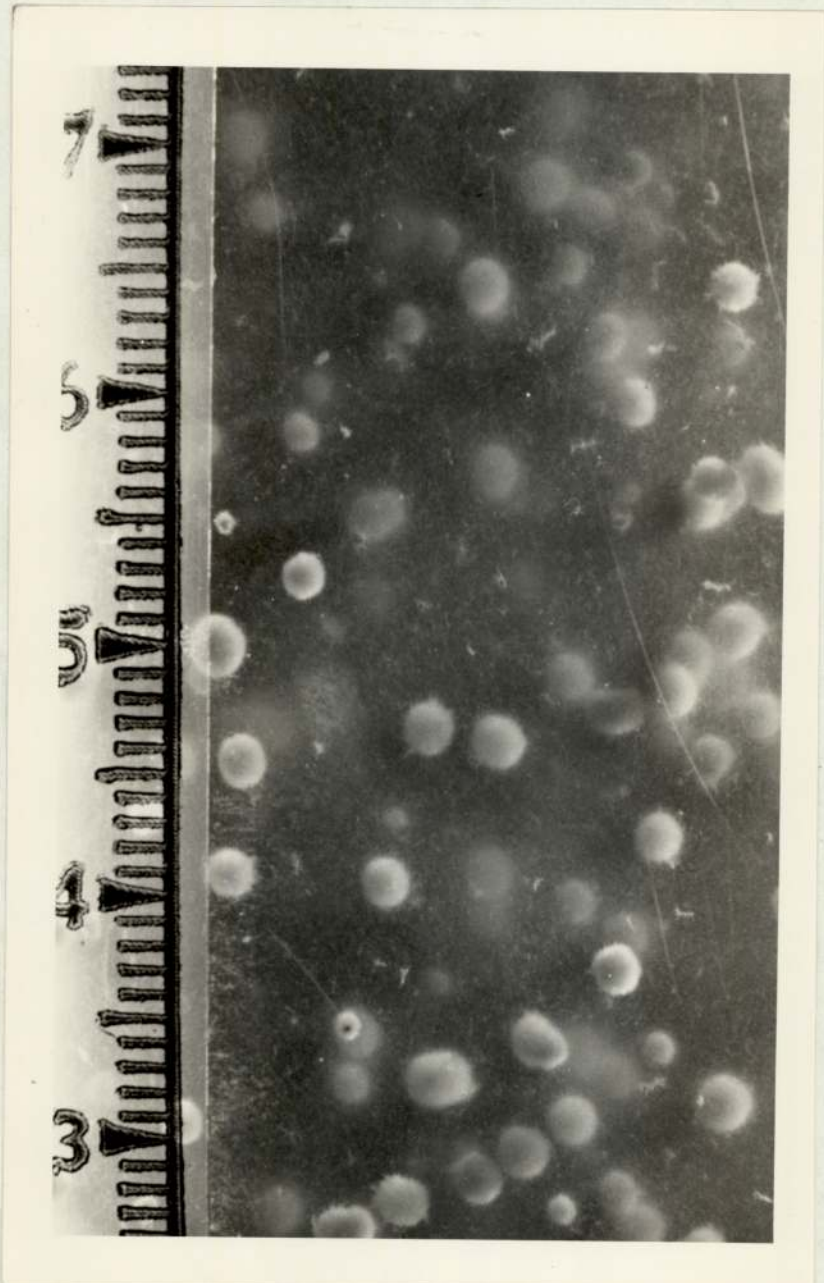


FIG. 7:21 HETEROGENEITY OF MORPHOLOGY CAUSED BY HYPHAL FRAGMENTS FROM TYPE VI COLONIES WHICH APPEAR AS A CLOUDINESS IN THE MEDIUM



and peripheral growth-zone width(Pirt, 1966). Some of the problems associated with such measurements (when applied to colonies from submerged cultures) have been discussed (see 3:2), but even cursory examination of the morphological range produced(FIGS. 7:16 to 7:19) shows that these functions are not universally applicable, may be difficult to estimate reliably, (Morrison and Righelato, 1974), and may involve unacceptable degrees of subjectivity, except with restricted ranges of morphology. More painstaking examination often reveals an easily-overlooked heterogeneity of morphology which has been found to be nonetheless important in the capabilities of the culture.(FIGS. 7:20 7:21). For example, heterogeneous cultures of this type can be induced to grow exponentially, and it becomes obvious during sequential observations that the ability to perform in this way depends on the presence of the minute hyphal fragments (visible as a cloudy background in fig. 7:21) There is therefore a real source of experimental error in basing measurements on selected, "convenient" colonies. The derivation of mathematical relationships from such measurements is obviously liable to error, and it goes without saying that the detailed mathematical modelling of such systems should be based upon scrupulous observation of the morphologies involved.

In addition to heterogeneity with respect to colony-types, there were additional sources of morphological variation which superimpose additional features on the basic morphological types. colonies which entangle early in their development form compound, irregularly-shaped colonies which have a recognisable "multiple-centered" appearance(FIG. 7:22) This type of agglomeration may even take the form of large, hollow, multiple-centred colonies(FIG. 7:23). Autolytic activity may affect the basic types in several ways:small fragments may be released into the medium, especially with types IV-VI (FIG. 7:24); colonies may become hollow; or large fragments may break away either partially or totally from the main colony.(FIG. 7:24)

FIG. 7:22 COMPOUND, IRREGULARLY-SHAPED COLONIES FORMED BY THE ENTANGLEMENT OF EMBRYO COLONIES

FIG. 7:23 LARGE, MULTIPLE - CENTRED COLONIES

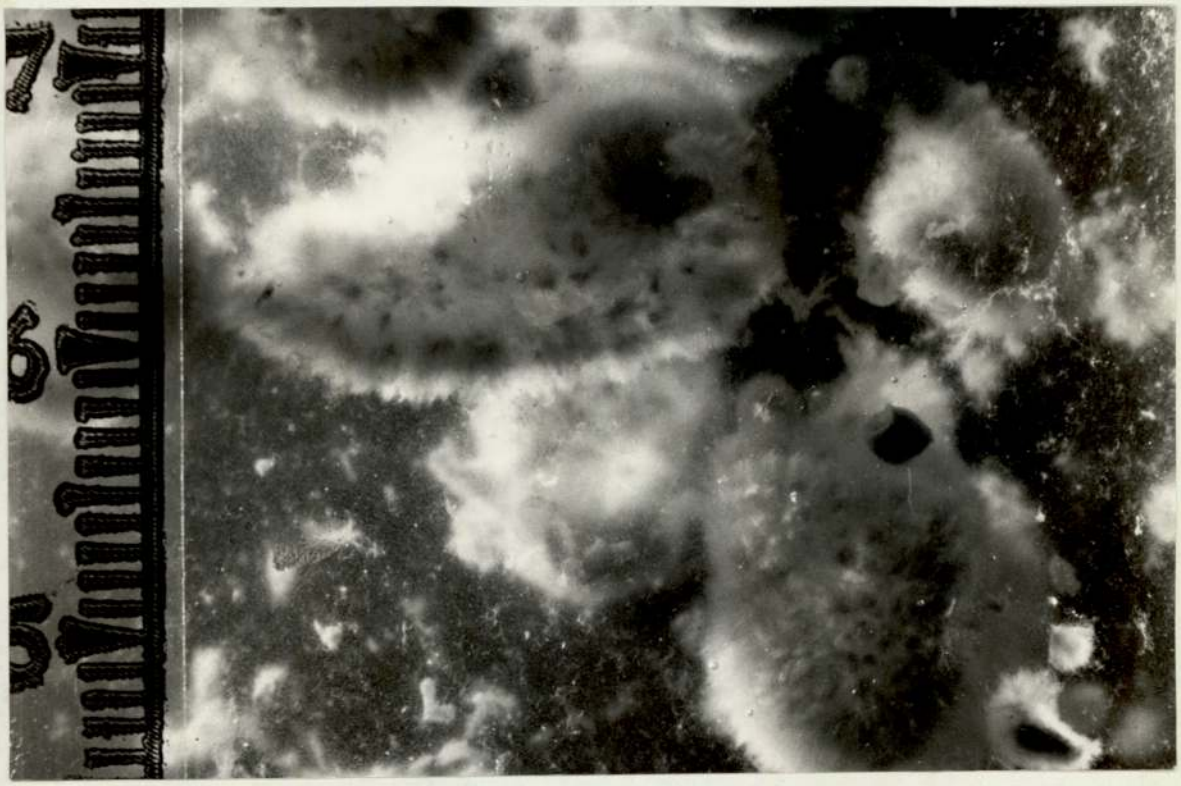
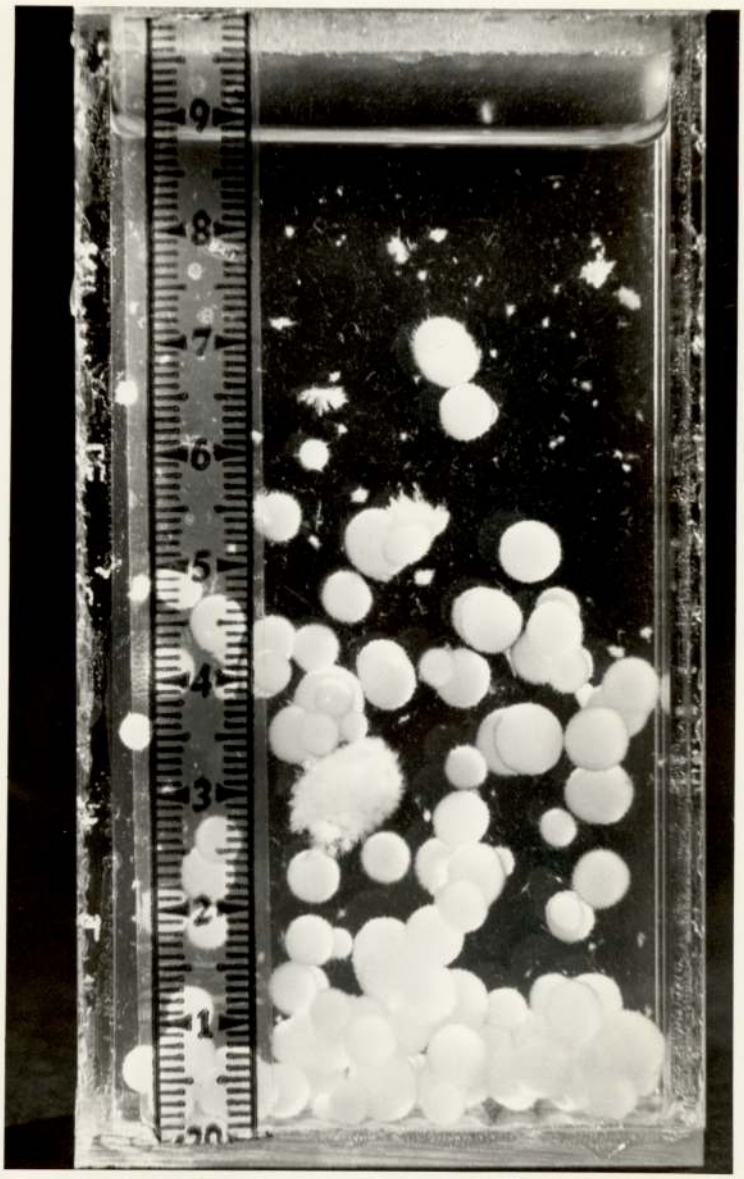


FIG. 724 TYPE VI COLONIES SHOWING LARGE FRAGMENTS BEING RELEASED FROM THE ORIGINAL COLONIES



7:3 Dependence of morphology on Medium Characteristics

7:3:1 detergents

Early in the experimental work, it was discovered that when 0.1% aqueous Teepol (Shell Chemicals U.K. LTD.) was used as a wetting - agent for the preparation spore-suspensions, it severely inhibited germination. In the author's experience, using a large number of A. niger strains, this was unique amongst A. niger strains. Trials with petri-plate cultures(see 6:16, also FIG. 6:5) showed that on media containing 0.01% v/v Tween 80, Tween 60, Tween 40, and Triton X-100, no observable difference in growth or appearance was detected. However, in deep culture, fermentations involving the use of Triton X-100 gave colonies which were half the size of those where any of the Tweens was used, given otherwise identical conditions.

7:3:2 antifoam

Silicone antifoam was also found during early work to have several effects deleterious to biomass-oriented work. The ten-litre fermenter had been designed to accomodate a substantial head-space of foam(total volume fifteen litres), however, it was occasionally necessary to add antifoam(in 5ml aliquots , until foam reduced), and these additions resulted in a substantial reduction in dissolved oxygen-tension(FIG. 7:25) and growth-rate. Where spores for example during the germination phase of growth, spores and/or small colonies agglomerated and subsequently floated to the surface by froth-flotation. The effect of this flotation is discussed under 7:4 .

7:3:3 suspended solids

Complex industrial media may contain up to 2% w/v suspended solids. The carob medium prepared as specified (Methods, 6;4) contained 1.5 to 2.0%w/v of suspended solids in the form of fine, silt - like particles, most of which were less than 10mμ in diameter. These particles were adsorbed by germinating spores and small colonies (FIG.7:25), resulting in the clarification of the medium. Assuming total assimilation of the silt, calculations based on the original concentration of silt and on the final dry weight of mycelium plus silt yield estimates of between 1.5% w/v and 2.0% w/v for the proportion of dry silt in the final dry mycelium. Although the particles led to the formation of an optically-opaque nucleus, (FIG7:26)no distinctive morphological effect was attributable to their presence.

FIG. 7:25 EFFECT OF ADDITION OF ANTIFOAM ON DISSOLVED OXYGEN TENSION (TYPE III)

(ANTIFOAM ADDED AT INOCULATION TIME PLUS 43 HR)

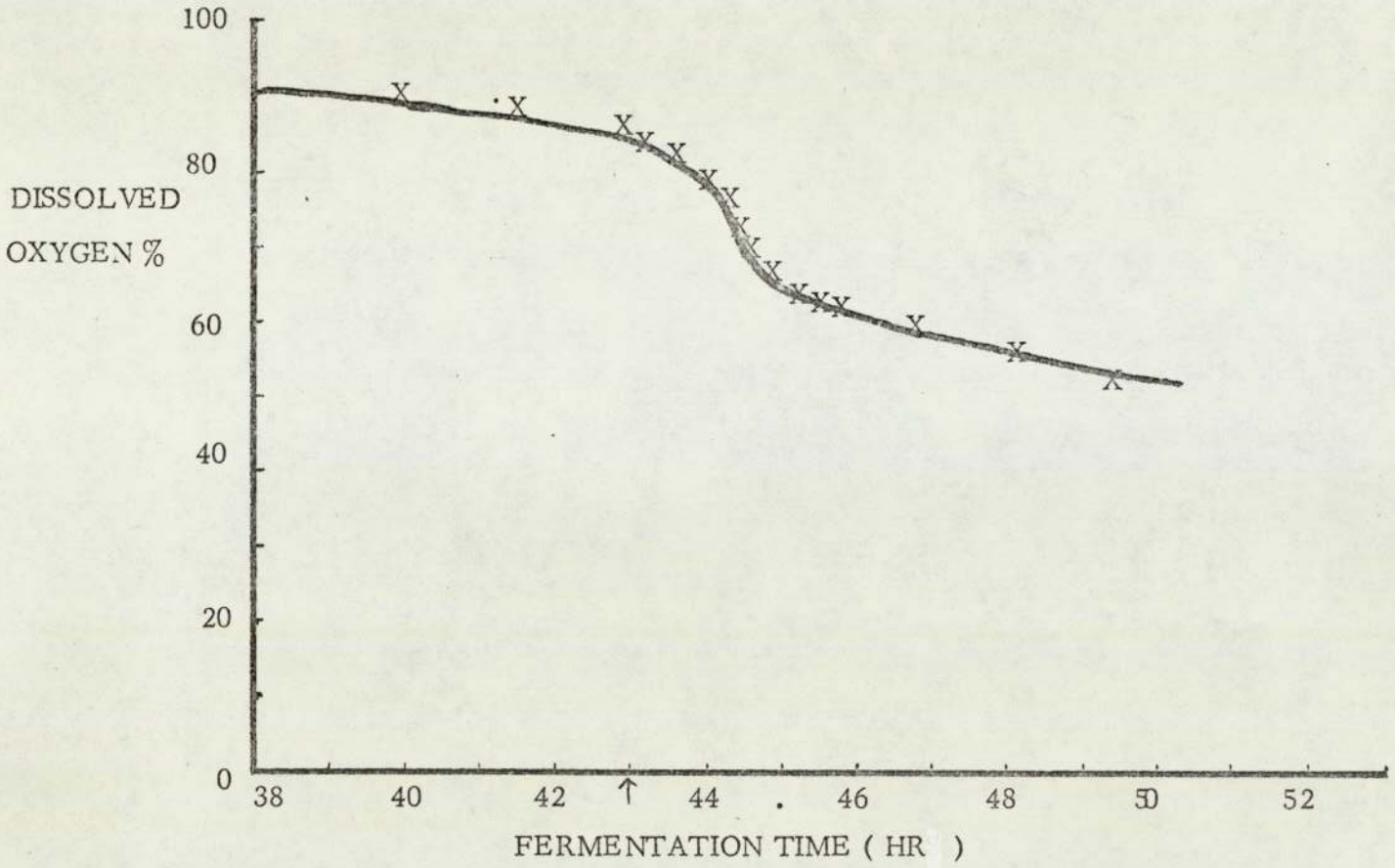


FIG. 7:26 THE ADSORPTION OF SUSPENDED SILT-LIKE PARTICLES FROM CAROB MEDIUM ONTO EMBRYO COLONIES

7:3:4 pH

This was varied in the range pH1.6 to pH 7.0 by direct addition of filter-sterilised acid or alkali to the carob extract medium or the molasses medium. There was a tendency to form loose, filamentous colonies at low pH values (< pH 2.0).

7:3:5 Temperature

Fermentations at 25°C produced colonies which were of types IV or V in conditions which otherwise gave type III colonies. Only one fermentation was carried out at 35°C, and this produced type I-II colonies.

7:3:6 Carbon / Nitrogen Ratio

At constant carbohydrate concentrations, a C/N ratio of 9:1 gave fast growth and produced colonies which were typeIII and 0.5 mm to 5.0 mm in diameter. At a C/N ratio of 15:1 (effectively nitrogen-starvation) type VI colonies up to 7mm in diameter formed and at ratios higher than 15:1 type VII colonies were formed, with very long fermentation times (FIG. 7:15)

7:3:7 Zinc concentration

Fermentation times to maximum dry weight were of the order of one week when the zinc concentration in Carob extract medium and COR medium was above 0.01% w/v., producing colonies of type VI. At 0.15% and above, type VII colonies as large as 11-20mm in diameter were produced. The possible reason for this increased size is discussed under 7:4 .

7:3:8 Chelating Agents

An increase in the concentration of EDTA in the COR medium was used to study the effect of a general increase in the concentration of trace metals. It was found that an increase of 0.2 g. /l or more gave type VI and VII colonies against controls which produced type III colonies. This effect was subsequently found to be reproducible in the carob extract medium. Reductions of the amount of EDTA from 0.6 g. $l.^{-1}$ to 0.5 g. $l.^{-1}$ was sufficient to produce type VII colonies, and at 0.4 g. $l.^{-1}$ the appearance was identical with that of the cultures with high zinc concentrations.

7:3:9 ; Plant Growth Regulators

The incorporation of 10^{-9} molar 2, 4, 5, -trichlorophenoxyacetic acid into COR medium stimulated growth in terms of final dry weight yield. Colonies of type II-III, 1mm. in diameter were produced after forty hours in batch. 4-(3-indolyl)-butanoate was also stimulatory at 0.1%w/v, but 2-(3-indolyl)-ethanoate and 3-(3-indolyl)-propanoate at 0.1% w/v were inhibitory, producing dense, type VI colonies.

The overall strategy behind the above experiments with medium-composition was to use essentially inhibitory or stimulatory modifications of the various media, having previously monitored the growth on these media in their unadulterated forms. As far as possible, the use of agents which might have complex, multiple effects on the system was avoided. For example, although the effect of temperature was studied, it is recognised that the results may depend on a complex pattern of interactions between variables such as bubble-size, gas solubility, viscosity, fluidisation, etc.,

The general trends which were observed are summarised in figure 7:15, i.e. type I-II were encouraged by "stimulatory" conditions, types II-IV by conditions less so, and types V-VI by conditions of progressively more severe metabolic inhibition.

The precise action of the detergents in submerged culture is probably dependent on more than one phenomenon. It is well-established that detergents help prevent spore-agglutination and that this is the basis of their routine application in the formulation of spore-suspensions. It may be that some detergents such as Triton X100 (octylphenoxy-polyethoxyethanol) tend to produce more disperse forms because they have a greater ability to prevent agglutination subsequent to inoculation. However, the use of type I inoculum from stirred fermenters has confirmed the results of Takahashi, Abekawa, and Yamada (1960) and Takahashi, Hidaka and Yamada (1965) who found that detergents such as sorbitan monostearate, sorbitan monolaurate and sorbitan trioleate induced a more filamentous type of growth with both vegetative and spore - inocula. In view of the lack of any observed parallel effects of detergents on A. niger M1 grown by the petri-culture method, it seems

that certain detergent-types may have a boundary-layer effect at the cell-wall / medium interface. Similar effects have been reported when synthetic polymers such as polyvinyl acetate, polyacrylate, polymethacrylate, and carboxypolymethylene were incorporated in the medium for deep culture of A. niger strains (Botri, Cieri, and Giodani, 1964; Elmayergi and Moo-Young, 1973; Elmayergi, Scharer and Moo-Young, 1973). In view of the similarity in the physical behaviour and the structure of the polymers and the detergents which have the above effect, it seems that they either enhance hydrodynamic slip at the hyphal surface (Oldroyd, 1949; Oldroyd and Toms Davies, 1949; Shaver and Merrill , 1959; Hoyt and Fabula, 1965; Moo-Young, Hirose and Alt, 1970), or they facilitate more rapid diffusion across the cell-wall / medium interface(Elmayergi and Moo-Young, 1973). Either effect would be expected to improve mass-transfer and therefore increase the growth-rate and militate towards type I-III.

FIG. 7:28 ; THE ENTANGLEMENT OF FRESHLY-GERMINATED SPORES

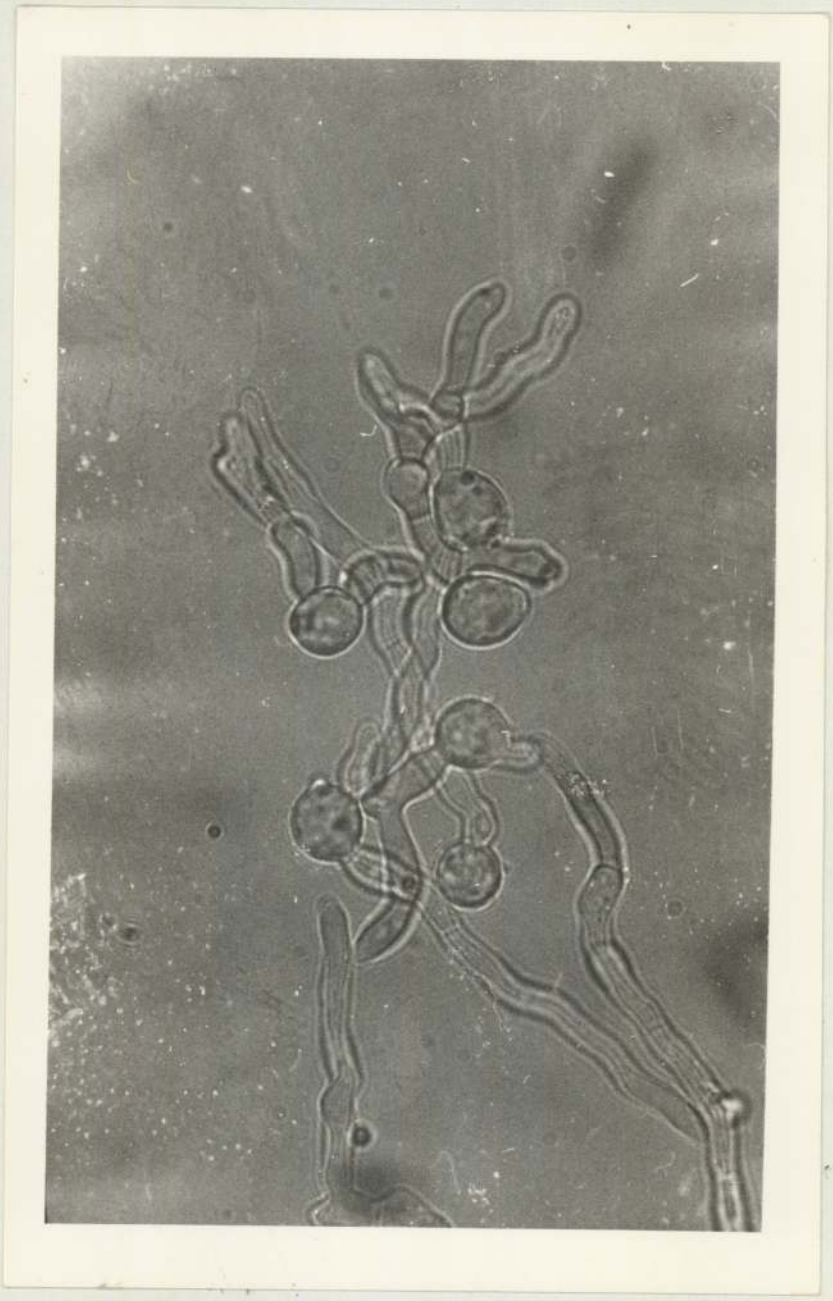
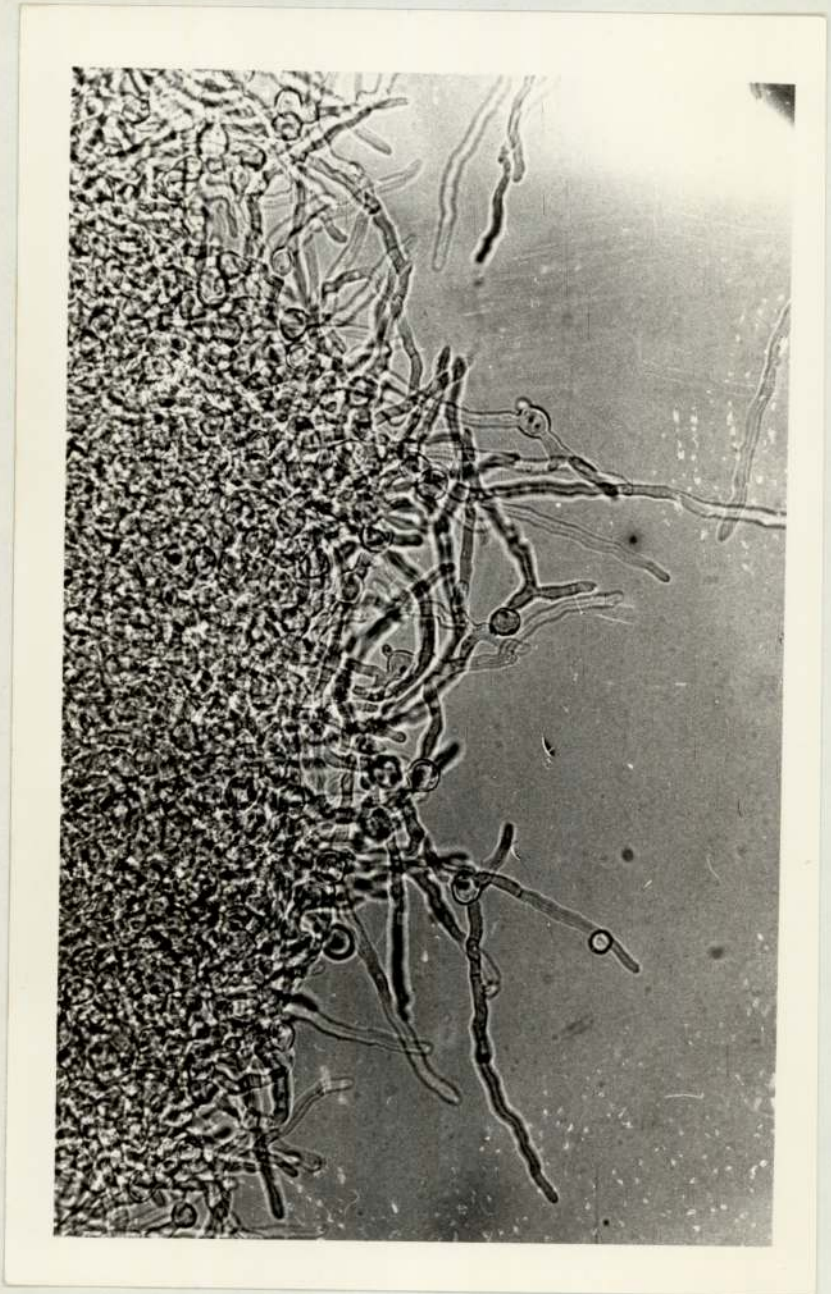


FIG. 7: 29 THE ADSORPTION OF SPORES ONTO A GERM - TUBE

FIG. 7:30 ENTRAINMENT OF NEWLY-GERMINATED SPORES BY LARGER COLONIES OR AGGREGATES



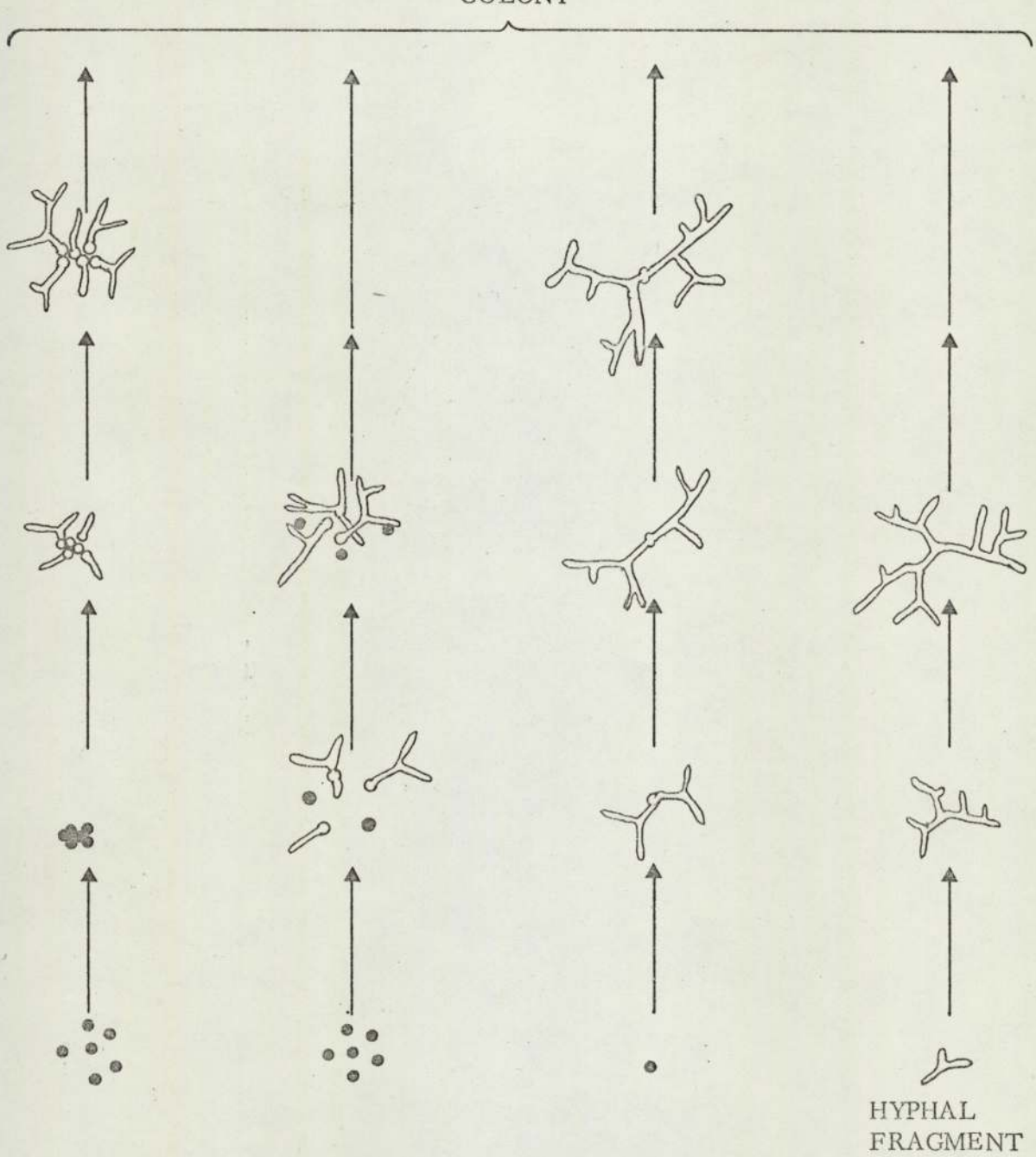
7:4 Mechanisms of colony-formation

7:4:1 The Role of Agglutination and Flocculation

Under the conditions present in the tower fermenters as operated in these experiments, colonies were observed to form in a variety of ways. the aggregation of swollen spores (FIG.7:27) or of newly-germinated spores (FIG7:28) would give rise to relatively large colony-nuclei. Adhesion of ungerminated spores to germ-tubes may also take place (FIG.7:29) and the overall result is a tendency to reduce the overall concentration of potential colonies in the medium . However, spore-agglutination was not a prerequisite for colony-formation under these conditions, and colonies were observed to form from single spores and filaments used as an inoculum. Filaments which have fragmented from larger colonies (FIG. 7:21) may also have the ability to form new colonies, for instance in continuous culture . Less frequently observed was the tendency for larger aggregates to entrain hyphae at the germ-tube stage(FIG.7:30). The above processes are represented diagrammatically in figure 7:31.

These results appear to be at variance with the situation found by other workers (Burkholder and Sinnot, 1945; Takahashi and Yamada 1960a;1960b; Takahashi , Hidaka and Yamada, 1965), where pellet fomation was apparently encouraged by the initial clumping of spores so that the formation of pellet nuclei results . Galbraith and Smith (1969a) demonstrated that spore-agglutination was not related to surface charge, and to repeat their analysis of the mechanisms of adhesion involved: "Electron microscopy of the conidial walls of A. niger has revealed a structureless outer surface sculptured into warty projections surrounding an electron- dense and an innermost electron-transparent layer. Hawker (1966) has shown that the wall becomes stretched and consequently thinner during swelling, the protruberances are flaked off and a thin electron transparent new inner wall is formed. Electrophoretic mobility indicates starch is one of the suface components in A. niger (Hannah, 1961) ..... therefore metabolic events leading to spore-agglutination may be initiated at a very early stage following inoculation, although the fact that clumping increases with the percentage of swollen spores suggests the immediate cause is swelling itself."

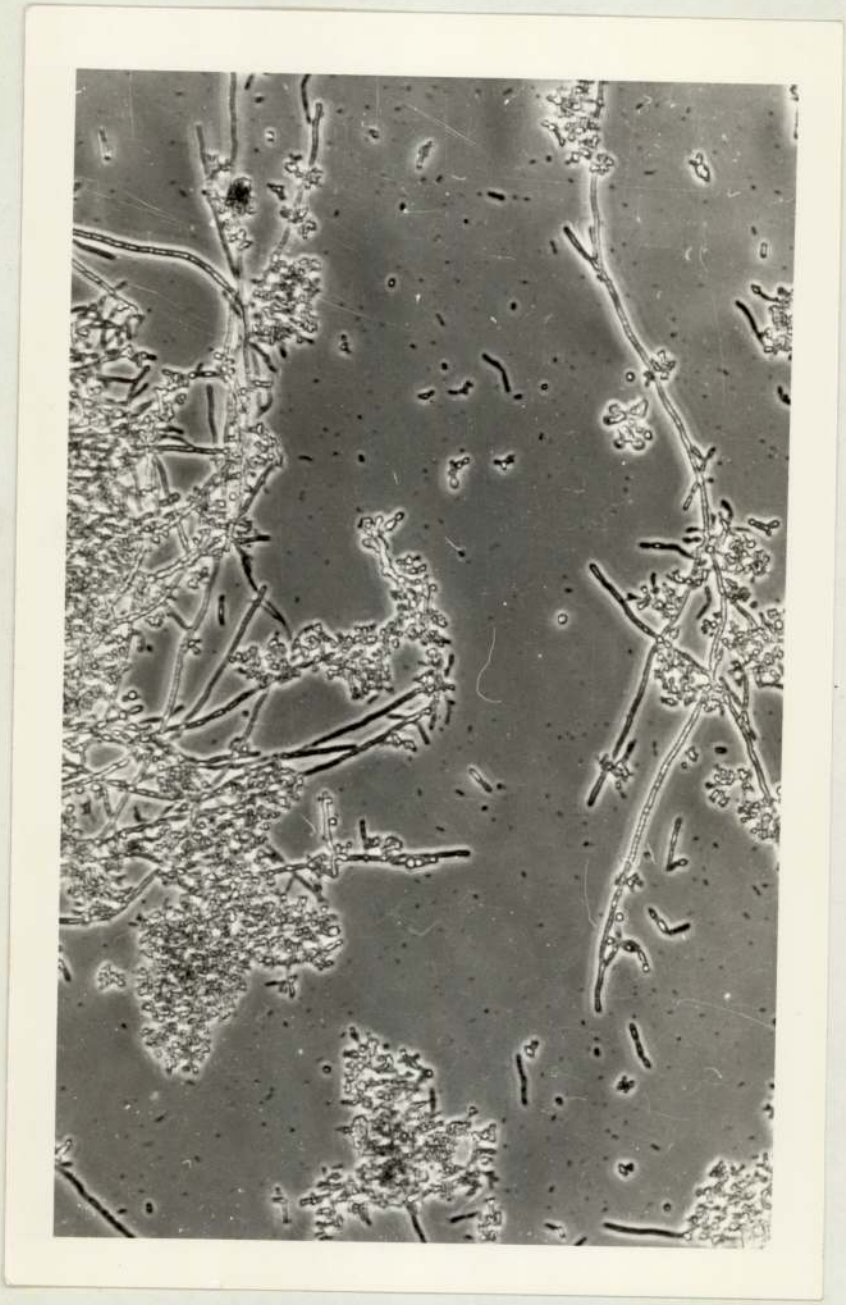
FIG. 7:31; DIAGRAMMATIC REPRESENTATION OF THE AGGLUTINATION AND GROWTH MECHANISMS WHICH PARTICIPATE IN THE FORMATION OF COLONIES



There is also some evidence that the tendency of spores to clump together at the stage where they are swollen is a function of the frequency of collision between spores since the superficial gas velocity in tower fermenters can be used to modify the degree of clumping (Daunter, 1971). Increasing the inoculum-size also encourages clumping (Morris, 1973), and thus it seems that the more random agglomeration-patterns found in this study are the result of conditions which minimise spore-collisions. The observation that single spores or hyphal fragments can form colonies demonstrates that clumping is not necessary for colony- formation in tower-fermenters . The introduction of spores into a static environment, for example a broth-culture in a test-tube, allows the formation of perfectly spherical colonies, and it would seem that the contrast between the above behaviour and that found for cultures in stirred fermenters depends on the difference in the level of agitation.

It has been found (Yanagita, 1957; Bhatnagar and Krishnan, 1960a; 1960b;) that the processes of spore- swelling and germination are quite complex, and depend on both physical and biochemical reactions Factors which inhibit metabolism at the germination-stage allow a greater time for mixed patterns of agglutination to take place, resulting in a more random distribution of colony-size, colony-shape, and in a smaller overall number of colonies. An inverse relationship between colony-size and colony-concentration was easily demonstrated by replacing aliquots of culture with fresh medium under otherwise standard conditions . Colonies up to 25mm in diameter were produced using this technique, but a reduction in the size of the spore-inoculum was not succesful in increasing colony-size, apparently because the concentration of embryo colonies is governed more by the extent of agglutination than by the concentration of spores in the inoculum. However , no attempt was made to alter the agglutination-pattern for example by altering the concentration of detergent used as a wetting-agent in the inoculum, and it might be possible that a reduction in the inoculum- size, together with a reduction in the concentration of wetting-agent, would reduce the concentration

FIG.7:32 ENTANGLEMENT OF UNICELLS AND PSEUDOMYCELIAL FORMS OF A SEWAGE YEAST IN A TOWER FERMENTER ILLUSTRATING THE FORMATION OF IRREGULAR COLONIES BY AGGLOMERATION MECHANISMS



of embryo colonies and thus increase the colony- size. The alternative approach of varying the superficial gas velocity in order to vary the degree of agitation, and hence the agglutination rate is likely to be complicated because this procedure would strip out the dissolved carbon dioxide, which is necessary for germination to take place (Yanagita, ibid idem.)

Aggregation to form colonies may also be seen to occur when organisms other than filamentous fungi are cultured in tower fermenters, but these do not possess the distinctive radial symmetry of the fungal types (FIG. 7:32 , micrographs taken in collaboration with Mr. A. Chesson, undergraduate student.) The mechanism by which this symmetry is maintained is elusive, but it probably depends on interactions between hyphae which are designed to prevent undue competition for nutrients between hyphae. The fact that types III-VI display this symmetry, whilst types I-II do not could be explained by the tighter, more rigid structure of the former which would allow such interactions to operate, whereas the flexibility of the latter would prevent any lasting symmetrical effects. (see 3:4).

7:4:2 The Role of the Spore-Concentration at Germination

The spore-concentration at germination has been found to be the most reliable parameter of spore-concentration for this work because it was found that the inoculum-concentration was a poor indicator of subsequent spore-concentration, except under standard conditions. The spore-concentration tends to be reduced by flotation of the spores to the upper surface of the liquid in the fermenter (Table7:1)

TABLE 7:1

REDUCTION OF SPORE-CONCENTRATION ON CAROB EXTRACT MEDIUM USING THE STANDARD METHOD

elapsed time (hr)	spore-concentration (ml^{-1})
0	$1;96 \times 10^{-7}$
1.0	1.96×10^{-7}
3.5	1.20×10^{-7}
4.0	0.72×10^{-7}
7.0	0.70×10^{-7}

At higher initial aeration-rates than that used in the standard method, especially in the presence of emulsified oils, such as the antifoam used in this work, the concentration of spores at the instant of germination can be as little as one thousandth of that at the time of inoculation.

At spore-concentrations (at the time of germination) less than 10^2 ml^{-1}, type I-III colonies developed. It was observed that type III colonies were formed when aggregation was more pronounced. At between 10^2 and 10^6 spores ml^{-1}, the initial growth was characterised by the agglutination of germinating spores (FIG. 7:28) to form colony-nuclei and in this case, the nuclei formed colonies of type II-V. Above 10^6 - 10^7 spores ml^{-1}, the initial growth was of type I-II (under the mild agitation conditions obtaining in these experiments). Filamentous inocula from a stirred fermenter were introduced at a relatively low concentration of individual fragments, and usually gave type III-V growth on the carob extract and beet molasses media. Alteration of the morphological type subsequent to the above events was always possible by manipulation of the other conditions, for example, by addition of inhibitory concentrations of zinc, dilution of the culture, or by stimulation of the growth-rate using plant growth hormones.

7:5 Relationship of Morphological Type to Growth-Kinetics

It was found that the simplest way of characterising differences between the growth of the various morphological types from the results of batch fermentations, using variations of COR and SPA media, was to plot maximum biomass dry weight versus the time taken to acheive this maximum (FIG. 7:15). It can be seen that fermentations with type IV-VII all acheive approximately the same dry weight figure, merely varying in the time taken to acheive this. Type III colonies, however, attained the maximum level, and these were in excess of those for any other morphological type (FIG. 7:15). As previously discussed, in section 7:1, type I had the fastest growth-rate, followed by type II, then type III, but the fermentations with type I and II ceased much earlier than for type III, with a consequent low yield. However, the two readings marked II* show the "improvement" in yield, based on the original volume of medium, obtainable by dilution with tap-water. This suggests that the packing-density of the colonies in the fermenter is inversely related to their potential diameter. This correlates well with findings of Trinci (1970), using A. nidulans He found that the radius of colonies grown singly in shake-flasks increased as a linear function of time, but as the number of colonies per flask was increased the radial growth rate began to decrease as a function of time. This latter pattern is similar to the radial growth-curves found in this study. (FIG. 7:33). The overall relationship between growth in terms of biomass and in terms of colony-diameter was less clear-cut than that between morphological type and growth-rate(FIG.7:15). It has been suggested (Pirt, 1966), that growth of colonies is restricted above a certain diameter by the increased resistance to mass-transfer within the colonies, but for types I-V this does not seem likely in view of the flow of liquid over the hyphae generated by their flexibility(7:1). It may be that types V-VII, with their more densely-packed hyphae, are more susceptible to this problem, but insufficient data were available to test this theory.

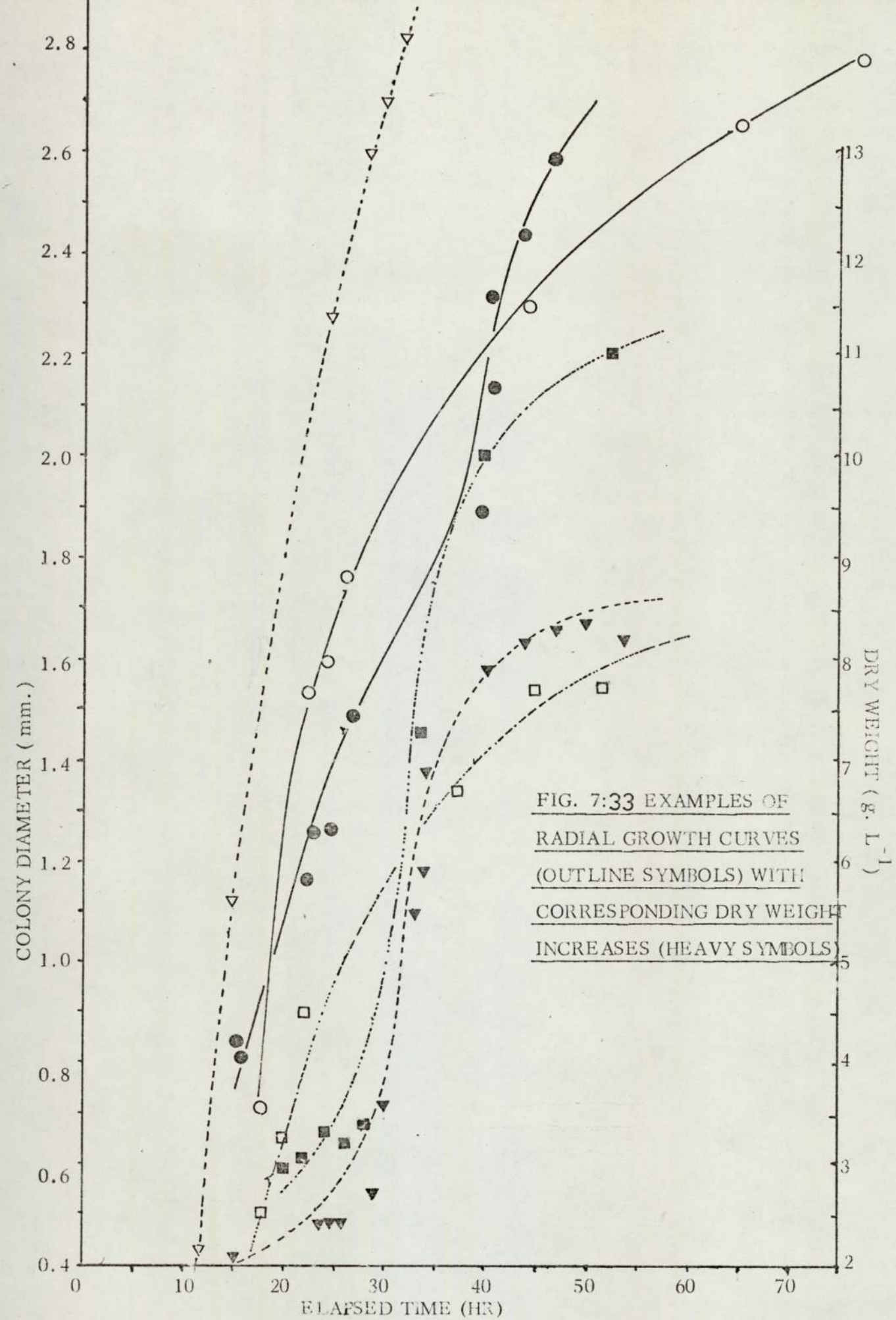
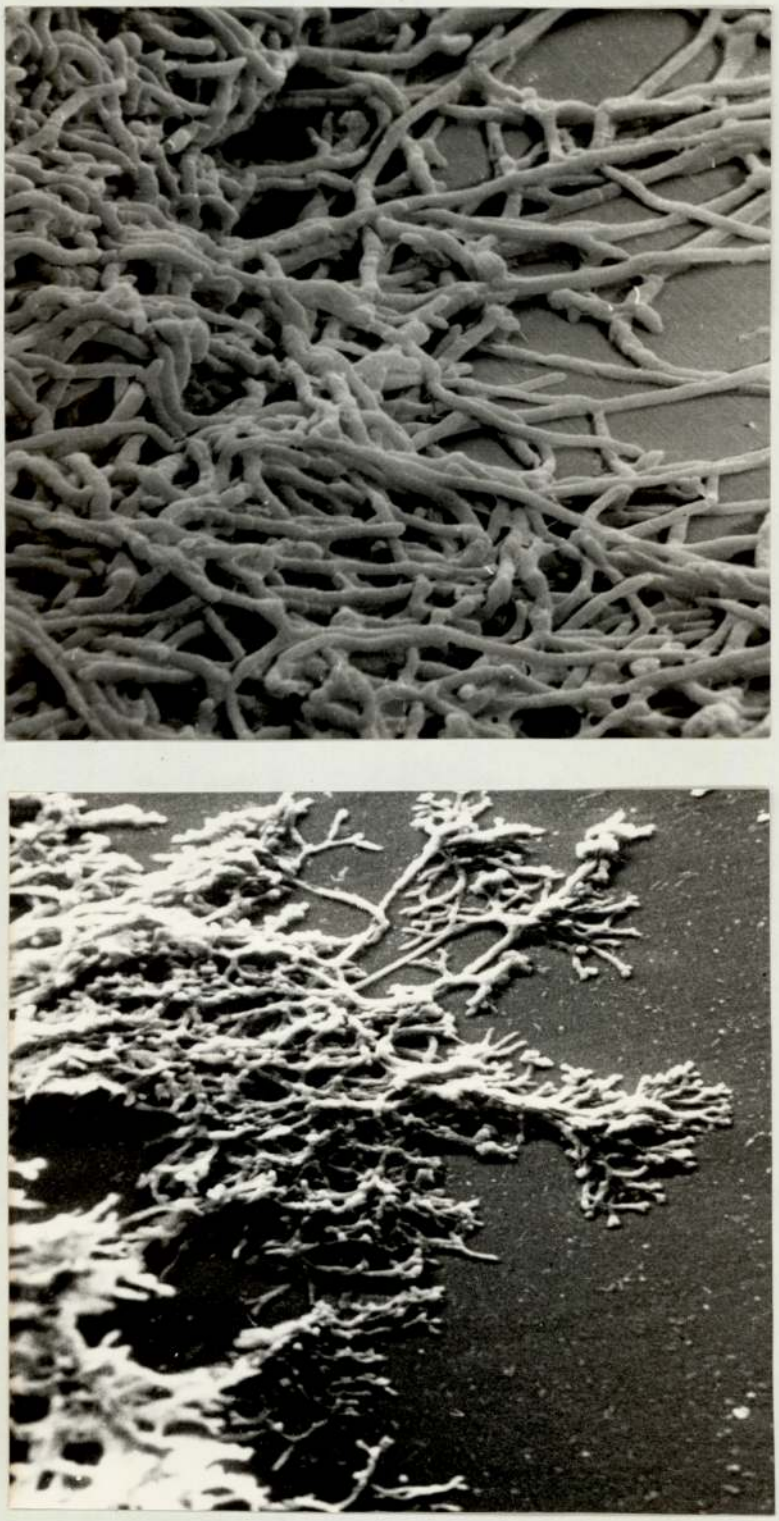


FIG. 7:33 EXAMPLES OF RADIAL GROWTH CURVES (OUTLINE SYMBOLS) WITH CORRESPONDING DRY WEIGHT INCREASES (HEAVY SYMBOLS)

FIG. 7:34 STEREOSCAN VIEW OF TYPE VI MYCELIUM (TOP) AND TYPE VII MYCELIUM SHOWING INTERSTITIAL SPACING



Stereoscan micrographs reveal a surprisingly open three-dimensional structure for types VI and VII colonies (FIG. 7;34) which contrasts with the densely-packed appearance under optical microscopy and to the naked eye (FIGS. 7:18; 7:19). James (1973) has suggested that these relatively rigid colonies behave as smooth, solid spheres, in that liquid is immobilised in the mycelial interstices, producing "stagnant" conditions in which diffusion is main mechanism of mass-transfer. Translocation of substances within the hyphae is well-known, however, and thus the mycelium may actively assist in mass-transfer. Thus it may be misleading to consider the colonies as inert particles which are entirely passive with respect to mass-transfer.

Continuous culture work represents a special case, in that the new colonies have to be formed from vegetative fragments of older colonies, and,because of the unique configuration of the tower fermenter, fluidisation, flotation, and sedimentation mechanisms begin to select for the retention of specific morphological types within the column, whilst other types are removed by washout. This process can be seen in the sequence depicted in figures 7:35-7:40 . Continuous feeding was commenced at a dilution-rate of 0.022 hr^{-1} after 36 hours of batch growth, and after sixty hours , the type IV colonies had reached their maximum diameter and were beginning to fragment and autolyse. After 130 hours, (FIG. 7:37), the mycelium in the fermenter was very heterogeneous in appearance, and sedimentation mechanisms were favouring the retention of colonies which were more sedimentary in their behaviour. The appearance of the effluent at this stage (FIG. 7:38) was consequently distinctly different, there being more small particles. After 780 hours (FIG. 7:39), type II colonies had been formed, and after twenty-three days, the growth was type I .

At dilution-rates above 0.022 hr^{-1}, the fluidisation behaviour of the fermentation fluids dominated the morphological development, and there was a powerful wash-out effect which selected for the retention of sedimentary colonies. The sparsely-branched type I mycelium in the fermenter branched more and more profusely as the dilution-rate was increased. This is

FIGURE 7:35 ; APPEARANCE OF CULTURE IN VIEWING-CELL THIRTY-SIX HOURS AFTER INOCULATION ($d=0.022hr^{-1}$)

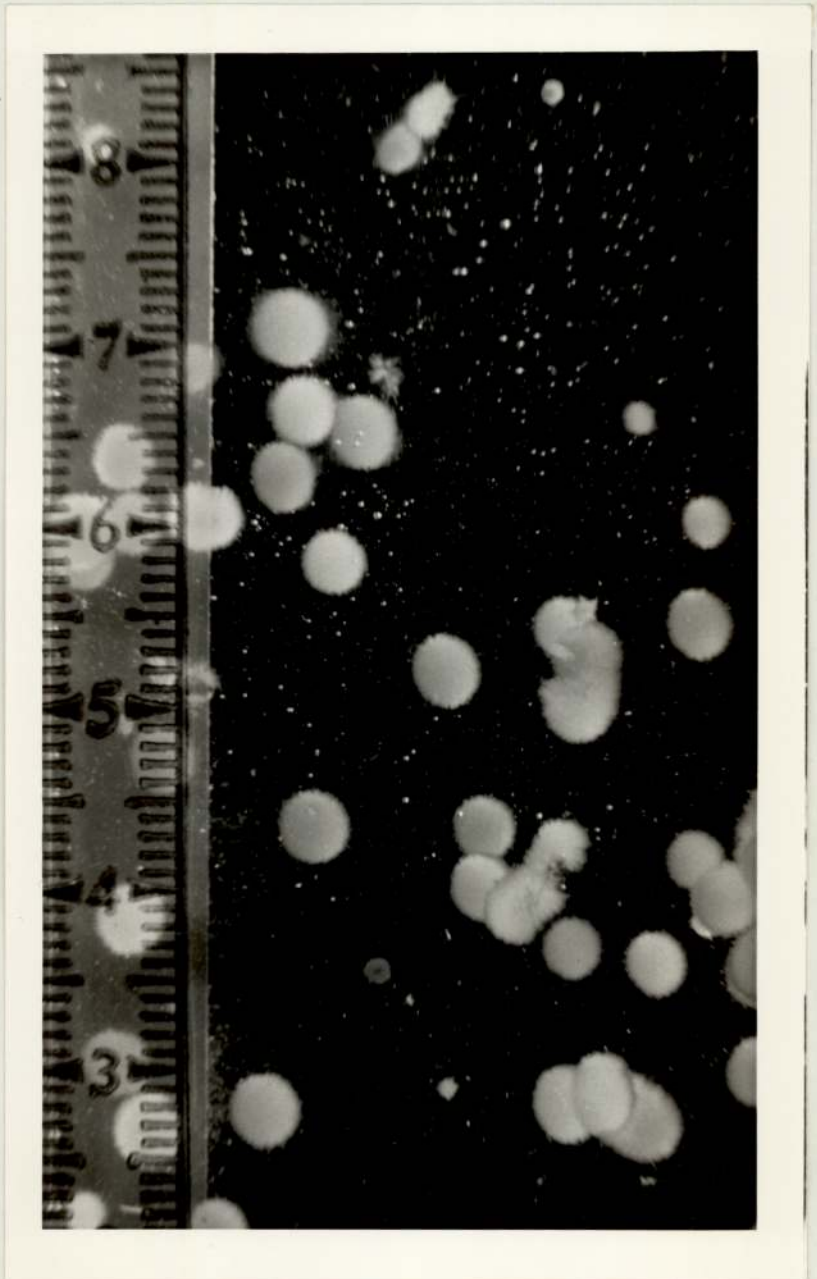


FIGURE 7:36 ; APPEARANCE OF CULTURE SIXTY HOURS AFTER INOCULATION ($d=0.022\ hr^{-1}$)

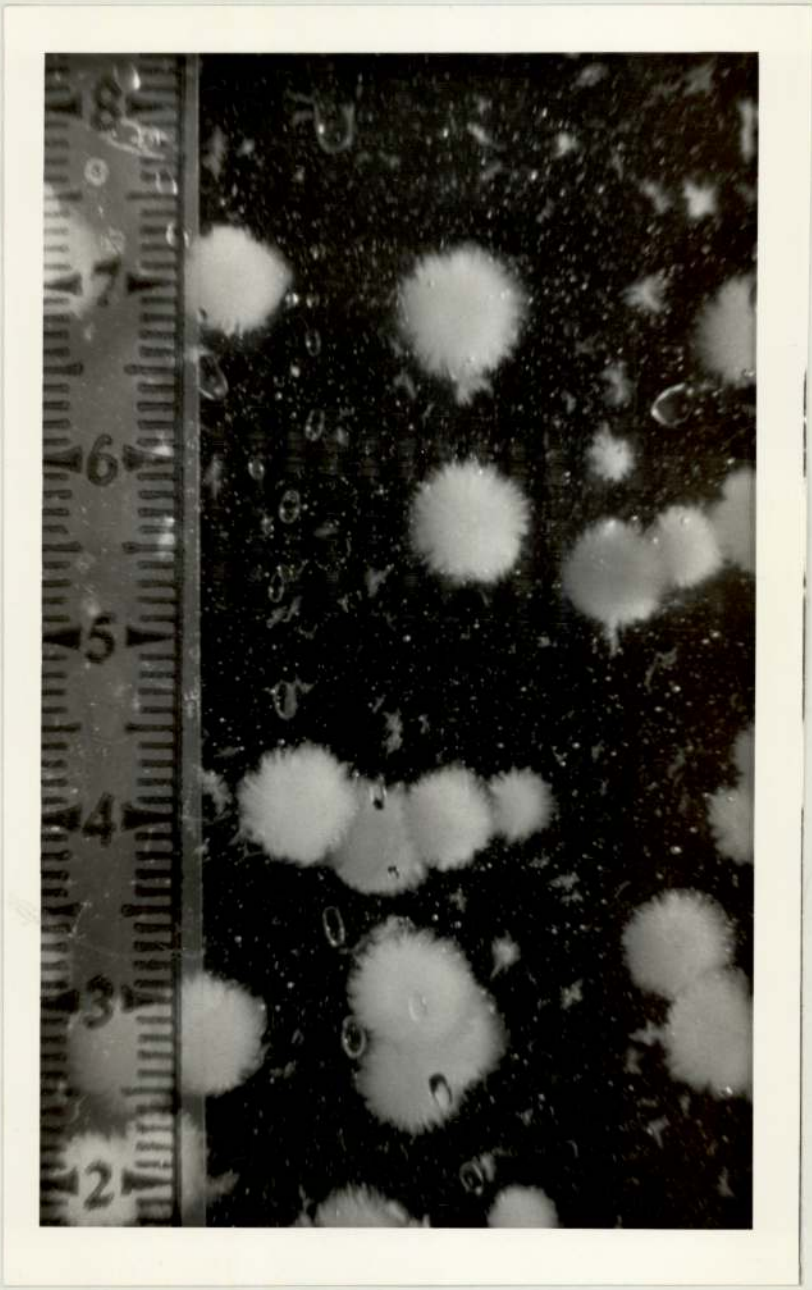


FIGURE 7:37 ; APPEARANCE OF MYCELIUM IN FERMENTER AFTER 130hr. AT A DILUTION-RATE OF 0.022 hr^{-1}.

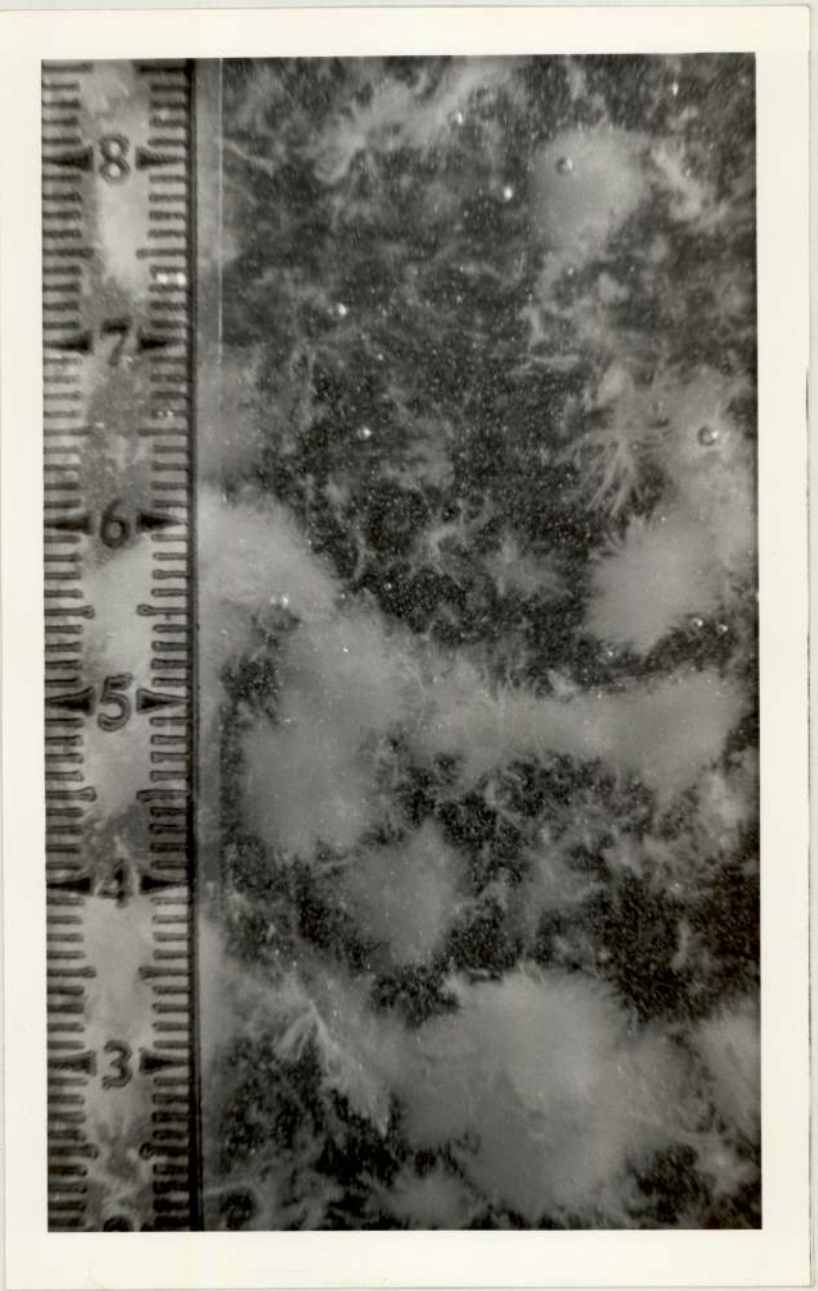


FIGURE 7:38 ; APPEARANCE OF MYCELIUM IN EFFLUENT AFTER 130 hr. AT A DILUTION-RATE OF 0.022 hr^{-1}.

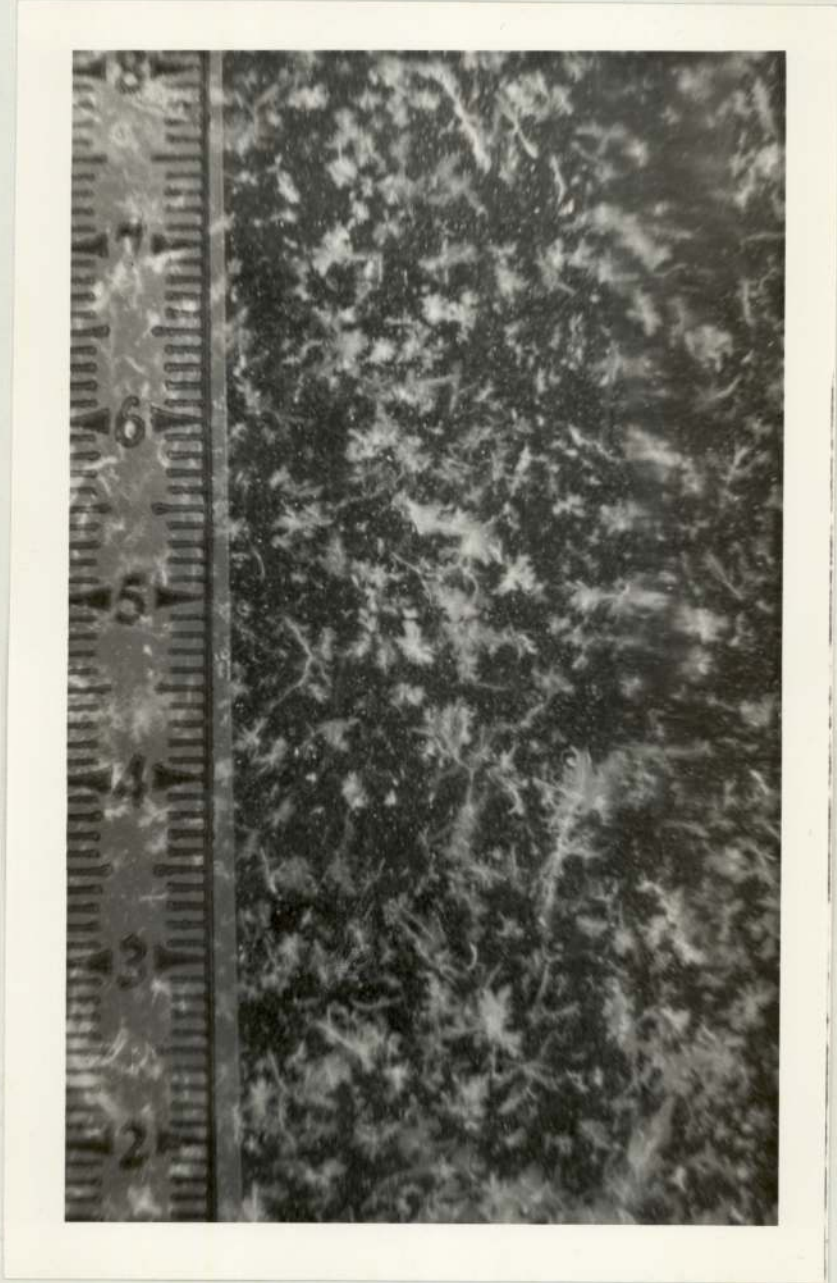


FIGURE 7:39 ; APPEARANCE OF MYCELIUM FROM FERMENTER 780 hr. AFTER INOCULATION ($d=0.022\ hr^{-1}$)

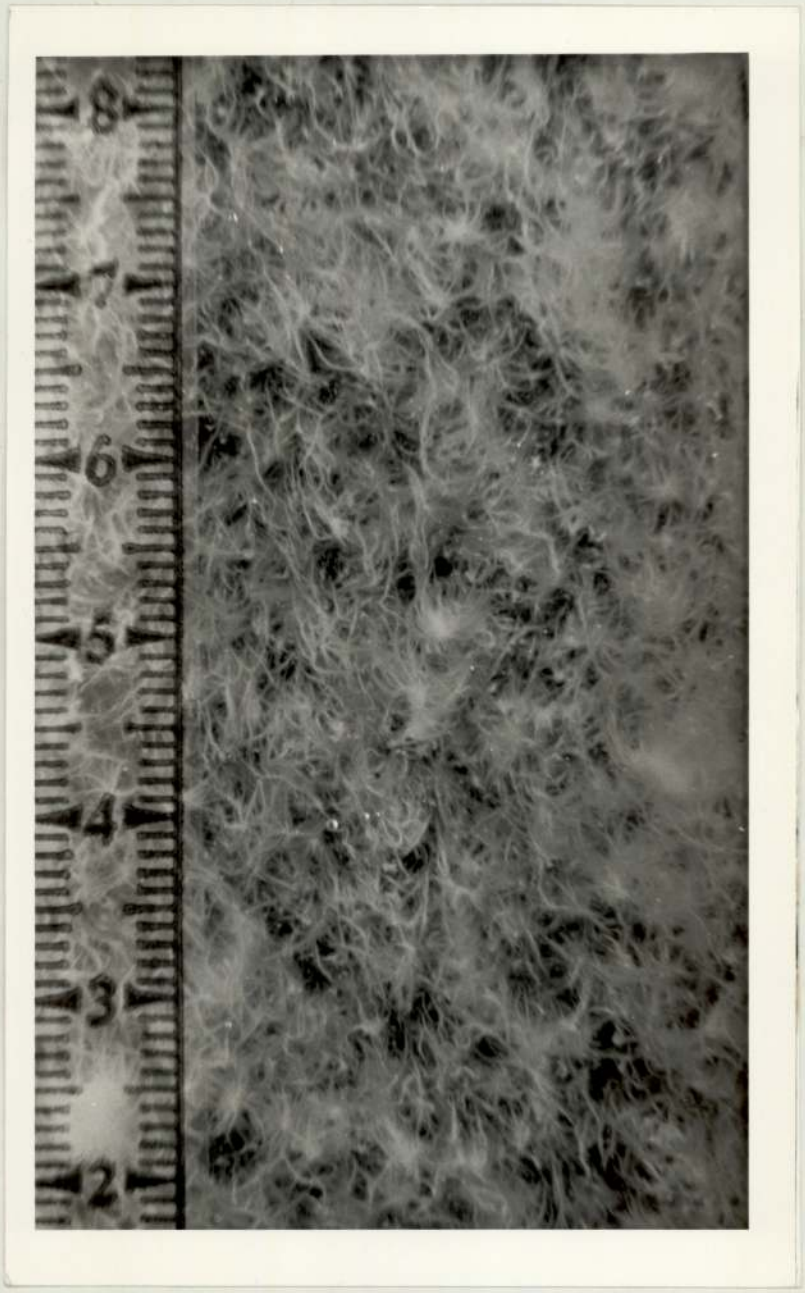


FIGURE 7;40 ; APPEARANCE OF CULTURE TWENTY-THREE DAYS AFTER INOCULATION ($d = 0.022\ hr^{-1}$)

particularly well-highlighted under fluorescence microscopy (FIGS. 7:41- 7:44). In order to monitor the extent of differentiation-oriented growth during the above step-changes in dilution-rate, the preferential adsorption of fluorescent brightener by the tips, buds, bud-points and septa (FIG. 7:45) was followed quantitatively. The increased branching activity visible in figures 7:41-7:44) is reflected by a sharp increase in the adsorption of the fluorescent compound(FIG. 7:47) but then the adsorption-level reduces, to establish itself at a new level. At a dilution-rate of 0.9 hr^{-1}, the adsorption-rate decreases to a level lower than before. This was taken to indicate that the (by then) heavily-sedimentary forms were governed in their activity by the indirect effects of their concentration in the fermenter (for example, viscosity or reduced oxygen-transfer), rather than by the limiting effect of a nutrient whose concentration was a function of the dilution-rate. In batch culture, the adsorption of fluorescent brightener varied markedly with the morphological type. Type I exhibited the steepest rise in adsorption as the fermentation progressed, whilst type VII adsorbed the compound at a relatively slow rate. These results were the exact opposite of those expected, i.e. colonies which were densely-branched did not adsorb Tinopal BOPT at as high a level as Type I colonies. If the adsorption-level at any instant during the fermentation is indicative of the quantity of new wall material and septa being formed, then it would seem that in a batch-culture, densely-branched colonies have a lower hyphal proliferation-rate than less densely-branched forms. The contrast between this situation and that encountered on step-up of the dilution-rate in continuous fermentations may seem paradoxical, but then the environmental conditions of the individual colonies are governed by different sets of rules.

FIG.7:41 TINOPAL FLUORESCENCE MICROGRAPH OF MYCELIUM AT A CONSTANT DILUTION RATE OF 0.022hr^{-1}

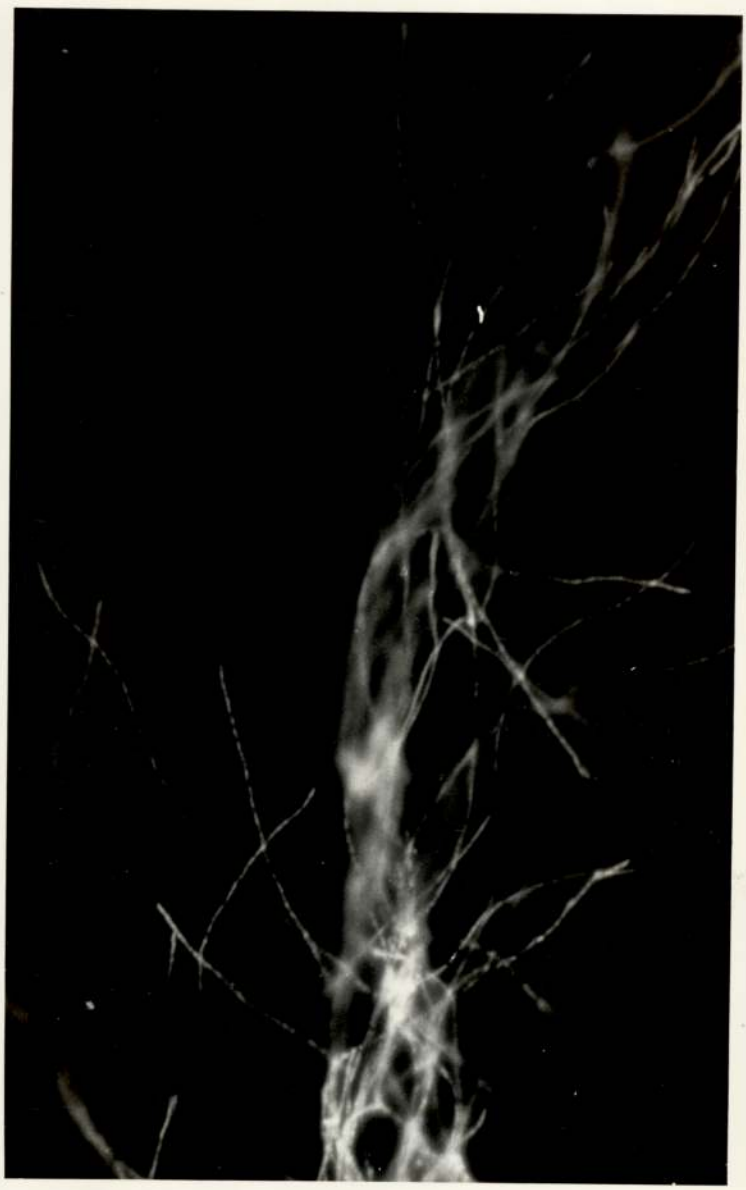


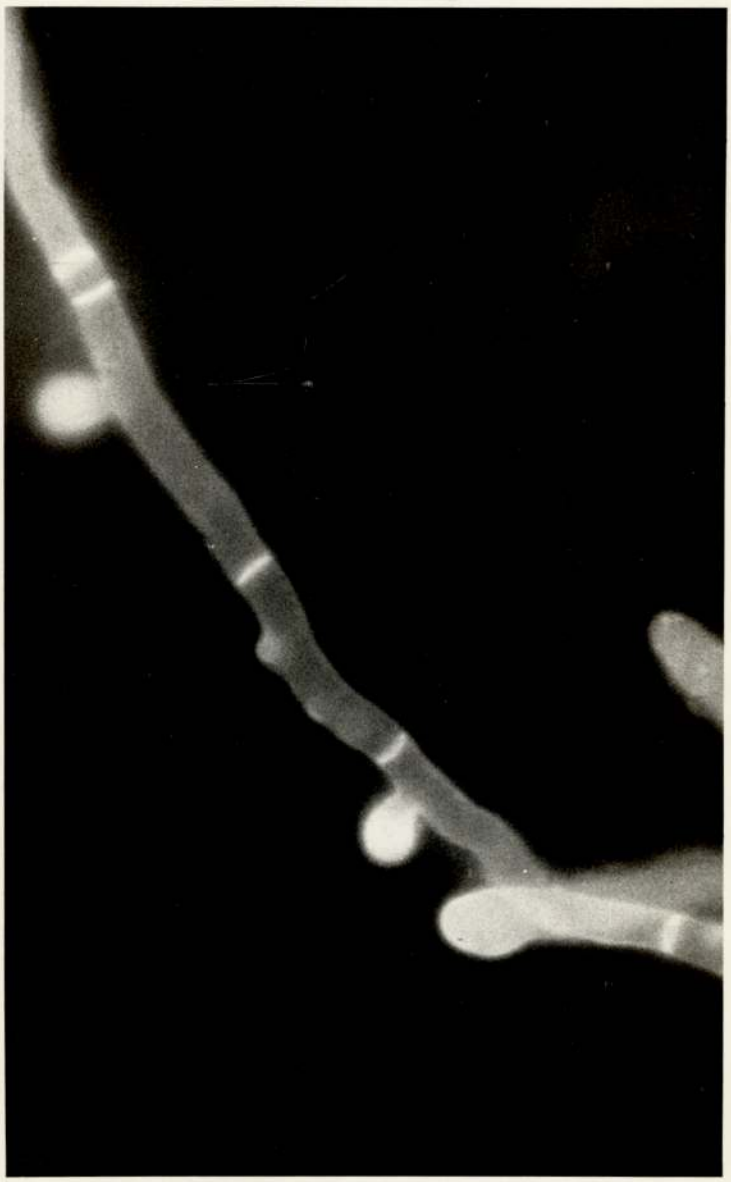
FIG. 7:42 TINOPAL FLUORESCENCE MICROGRAPH OF MYCELIUM FIVE HOURS AFTER STEP-UP FROM 0.022hr^{-1} TO 0.1hr^{-1}

FIG. 7:43 APPEARANCE OF MYCELIUM BY TINOPAL FLUORESCENCE SIX HOURS AFTER THE DILUTION RATE HAD BEEN INCREASED FROM 0.1 hr^{-1} TO 0.9hr^{-1}

FIG. 7.44 TINOPAL FLUORESCENCE MICROGRAPH OF MYCELIUM SEVENTEEN HOURS AFTER STEP-UP FROM 0.1hr^{-1} TO 0.9hr^{-1}

FIG.7:45 TINOPAL MICROGRAPH OF HYPHA SHOWING FLUORESCENCE OF TIPS, SEPTA, BUDS, AND DEVELOPING BUD-POINTS

(note the double septum, top left-hand)



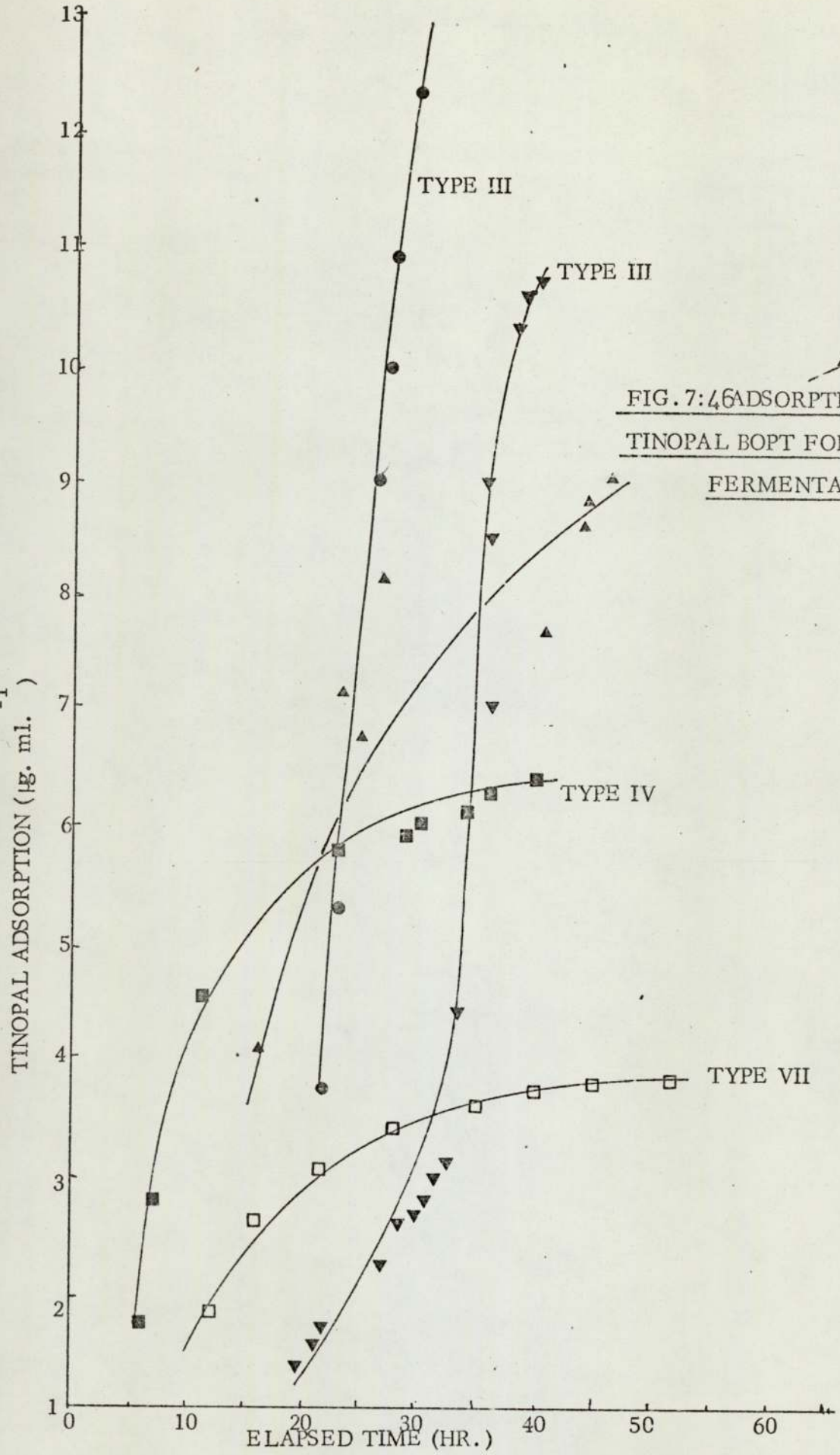
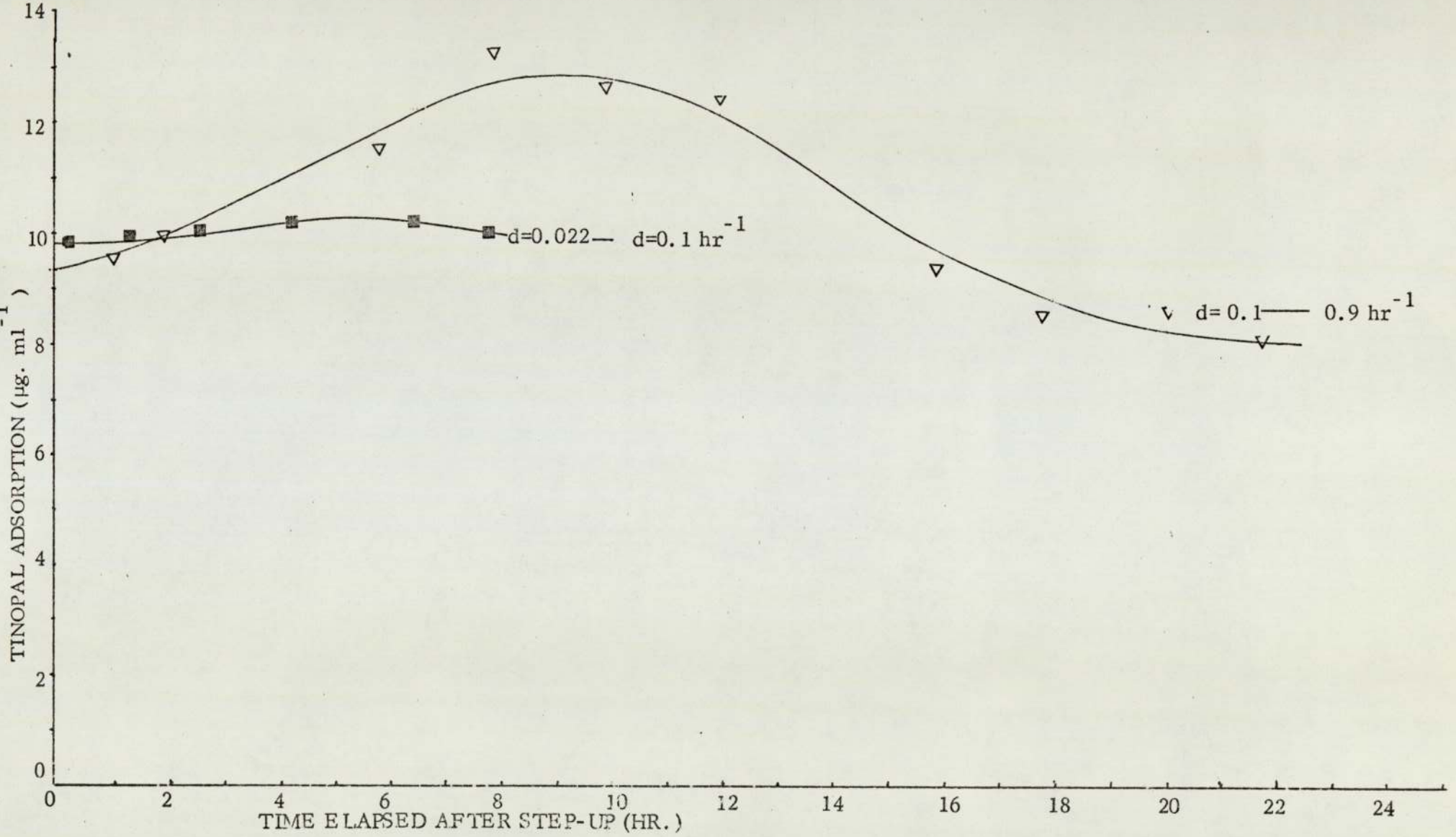


FIG. 7:46 ADSORPTION OF TINOPAL BOPT FOR BATCH FERMENTATIONS

FIG. 7:47 VARIATION IN TINOPAL ADSORPTION CAUSED BY STEP-CHANGES IN DILUTION-RATE



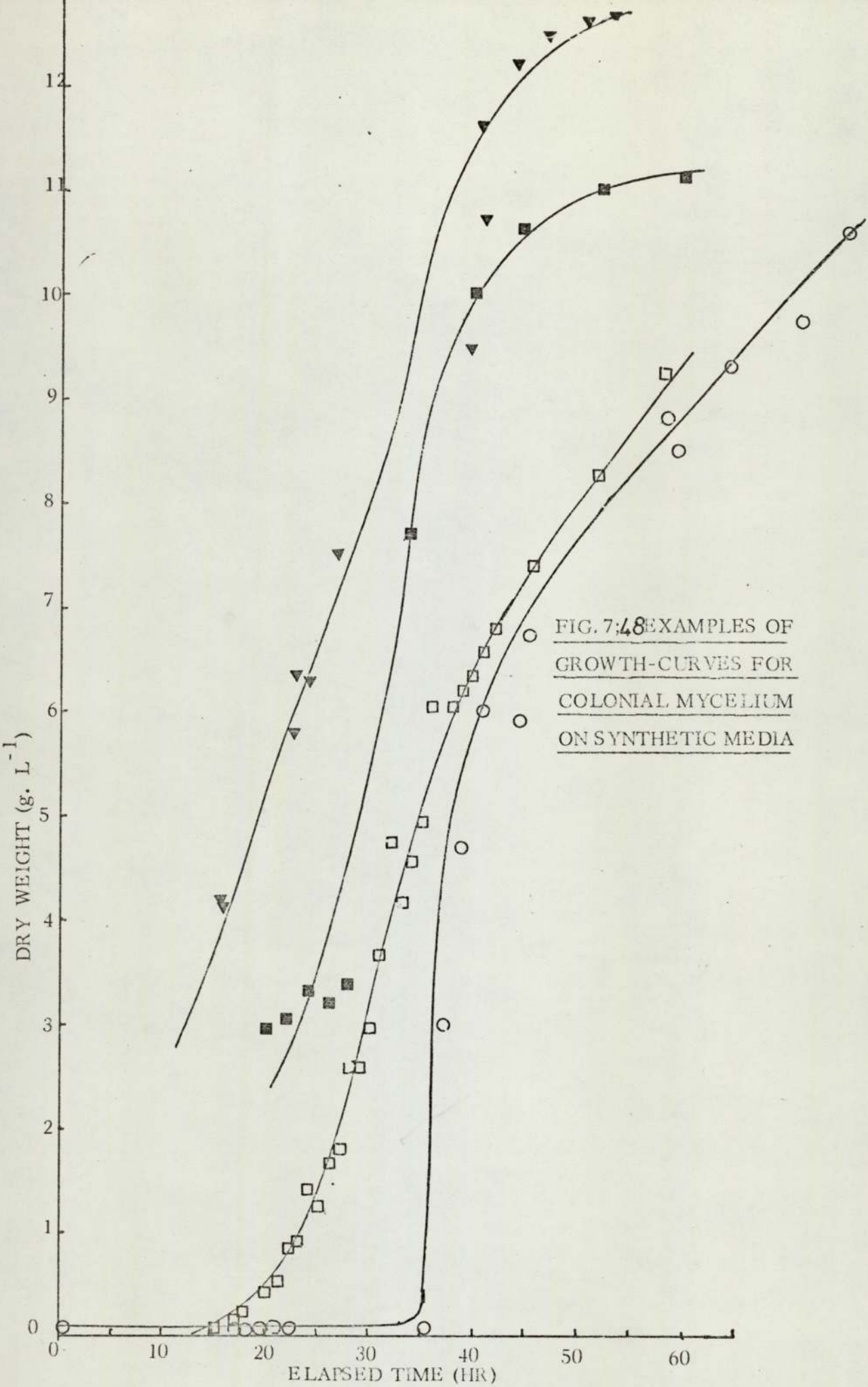


FIG. 7;48 EXAMPLES OF GROWTH-CURVES FOR COLONIAL MYCELIUM ON SYNTHETIC MEDIA

7:6 Final Conclusions

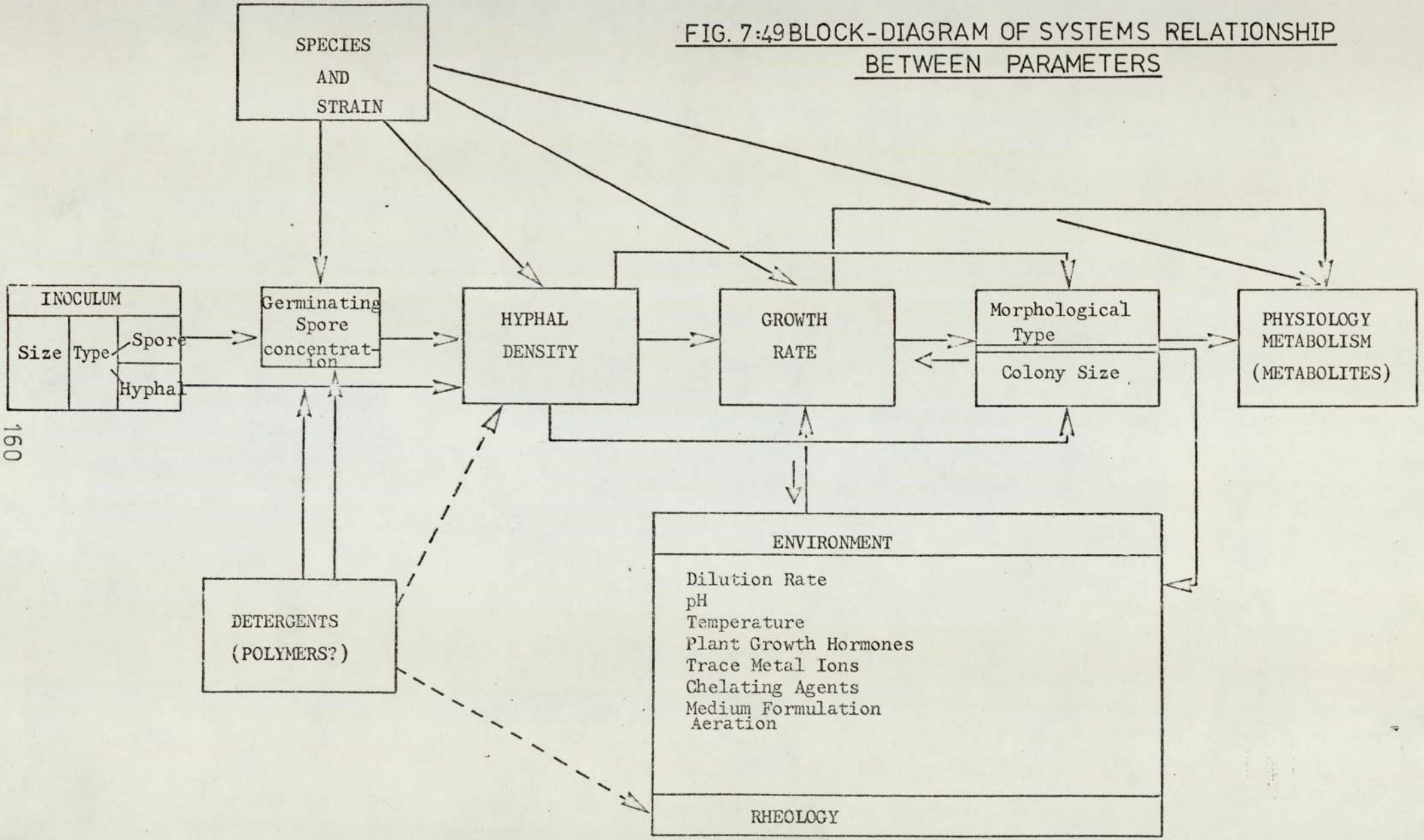
Whilst it would be extremely difficult to completely summarise the complex system represented by the morphology of A niger M1 in tower fermenters, the purpose of this study has been to provide a broadly -based approach to the problem. As such it represents the preliminary stages of a more detailed programme of study. The overall pattern of relationships was used to formulate a block-diagram (FIG. 7:49) which summarises as far as possible the functional relationships which were inferred from this study. The existence and direction of influences between variables is denoted by the arrows. As can be seen, the most important feature of the model at this stage is the dominance of growth-rate. It is notable that an understanding of the relationships embodied in the model has on many occasions enabled successful predictions of morphological development.

Many of the variables which are of interest from a commercial and academic viewpoint are dependent in a complex fashion on several other variables; for example, growth kinetics can be seen to be dependent on morphology, medium characteristics, suspension viscosity, inoculum - size, gassing rates, etc. It is felt most important that this complexity is appreciated.

The ability to control the morphology of colonies in submerged culture should open the way to further studies (and possibly exploitation) of the various cytochemical activities of colonial forms. Given that the individual hyphae in a culture exhibit an axial cytoplasmic differentiation then it might be expected that considerable overall metabolic differences

might exist between colonies characterised by differing branching-densities. Variations in colony-structure may also indirectly affect cytoplasmic activity via mass-transfer inhibition. It has been shown using radioactive tracers that for hard, spherical colonies, metabolic activity is ordered into annular zones, (Yanagita and Kogane, 1962; Nagasaki, 1968; Shinmyo and Terui, 1970) whereas in filamentous cultures from stirred fermenters, biosynthetic activity is relatively homogeneous(Shinmyo and Terui, 1970) The more open structure of colony found in tower fermenters probably allows novel types of growth with respect to cytochemical differentiation and kinetics, for instance, the concept of a discrete peripheral growth-zone should be replaced in favour of one which allows for gradual gradations between "growing " and 'hon-growing" mycelium, as suggested in the model of Koch (1975). The nature and extent of cellular interdependence has important consequences for the overall expression of the phenotype. Thus Novak and Fencl (1973) comment " the differentiation of cells in the filament impedes elaboration of a universal (kinetic) model". The growth of A niger species in nature is almost always of a colonial, differentiated form, itself the product of natural selection. The question arises as to what extent the colonial form is a direct geometric result of growth in the form of filaments or of innate physiological and genetic determinants. If either case is true, the "filamentous"forms which are found in stirred fermenters are likely to be unique in their metabolic and physiological properties, and whilst a homogeneous system results, and is relatively easy to monitor, the information thus obtained is unlikely to apply generally.

FIG. 7:49 BLOCK-DIAGRAM OF SYSTEMS RELATIONSHIP BETWEEN PARAMETERS



The Tower Fermenter thus offers a valuable tool for the manipulation of the morphology of filamentous fungi and allows a fresh insight into the physiological and organisational features of filamentous fungi. The ability to control the colonial morphology is of itself an important mechanism for the control of the mixing characteristics of the fermentation broth, and hence for the control of the energy costs of this type of fermenter. Perhaps not so obvious is the usefulness of colonial forms in post-fermentation processing at the commercial scale, because the colonial forms produced in the tower fermenters are easily filtered from the supernatant broth using simple mesh filters (0.4mm. mesh size), whereas filamentous cultures from S.T.R. fermentations invariably require the employment of expensive rotary vacuum filtration. In countries which have relatively unsophisticated engineering support, the facility with which separation may be accomplished with broths from Tower Fermenters is a distinct advantage, allowing the development of fermentation plants which are simple to construct and operate, with a commensurate reduction in capital cost. In our own country, the reduced cost of filtration has to be balanced against possible reductions in titre or of biomass-yield and the associated costs which may be incurred. Even so, the Tower Fermenter must be viewed as a viable and useful addition to our range of fermentation equipment, providing a novel combination of characteristics which may be exploited at both the experimental and commercial level of fermentation practice. It is the author's belief that the design of fermenters will diversify considerably as fermentation technology

advances, so that just as the chemical engineer can choose the most appropriate reactor for a given chemical reaction, so will the fermentation engineer and the research microbiologist.

More particularly, the study of the physiology and vegetative differentiation of filamentous fungi would benefit from the further use of Tower Fermenters and of other alternatives to the S.T.R., for example the tube fermentation system of Machek & Fencl (1973).

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Design for laboratory scale tower fermenters

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FERMENTATION system design has only recently begun to realize the possibilities provided by the extensive knowledge of the various types of reactor as used in chemical engineering technology. Stirred tank reactors (STR) have initially been predominant in the fermentation industry and therefore it is not surprising that the laboratory-scale systems available are based on small-scale versions of this type of reactor (Postgate, 1965). However, specialist parts, materials and manufacture dictate a relatively high price.

Extensive research into the use of the tubular reactor as a fermenter for processes as varied as the production of alcohol, beer, wine, cider, vinegar, citric acid, yeast, mould and bacterial biomass has firmly established in our minds the adaptability, cheapness and usefulness of tubular systems in teaching and research work. As such we feel that they provide a useful alternative or complement to the conventional STR laboratory fermenter.

The ten-litre fermentation system described has proved itself in terms of reliability, operational ease, and production of research data. Variations in capacity, shape and construction materials are also briefly described.

Equipment

The fermenter column (Figure 1) is constructed using the items of standard glass pipeline indicated in Table I. (QVF Quickfit, Jobling Laboratory Division, Stone, Staffs ST15 0BG).

Ports for sampling, aseptic transfers and insertion of sensory probes are added by fusing Quickfit screw-cap fittings (SQ13 or SQ28) to the walls of the pipe-sections or by using the appropriate QVF side-arm pipe section (PTU4/1.0). The SQ side-arms accept inserts of between six and nineteen millimeters in diameter. Thus, thermistor, thermometer and reference electrode pass through SQ13 parts and other inserts through the larger size, using the appropriate rings and gaskets (see Table II). Although the range of acceptable sizes is shown to be discontinuous, the rings can in fact be adapted for a continuous range.

The fermenter can be sterilized by steam heat with or without pressure or by the use of chemical sterilants.

Ancillaries

The reactor can be supported on a metal framework (Dexion, Handy Angle, or Tri-clamp, WCB Containers, Stalybridge, Cheshire). Instrumentation and control equipment can be supported on platforms resting on this framework. For pH measurements, a meter-recorder (Analytical Measurements, Spring Corner, Feltham, Middlesex) was used in conjunction with toughened glass electrode containing a gel electrolyte, allowing it to be used in a horizontal position. A remote reference electrode is necessary with this arrangement. (pH electrode, EIL 33-1070-030, remote reference electrode, E.I.L. Ltd., Hanworth Lane, Chertsey, Surrey). Dissolved oxygen measurements were carried out using a dissolved oxygen analyzer and probe (New

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Brunswick type DO-50). The probe used has to be positioned at least 30° to the horizontal, so that an angled port was provided, but electrodes can of course be purchased or constructed which will perform satisfactorily when positioned horizontally.

The temperature of the medium is controlled to within ±0.2°C of the set-point by an external jacket of water in a helically-coiled jacket of Paul's tubing (Portex Ltd., Hythe, Kent). The Paul's tubing is one inch in diameter, thin-walled, and is made from flexible PVC. The water is heated and cooled using a modified thermocirculator controlled by a thyristor mains relay circuit (Churchill Instruments, Walmgate Road, Perivale, Middlesex). However, where cooling is not required, attemperation can be more easily achieved by using helically-wound waterproof heating tapes controlled by a similar thermistor mains relay circuit (Hotfoil type GW50-70, Hotfoil Ltd., Heath Mill Road, Wombourne, Wolverhampton WV5 8AG).

Samples may be introduced or withdrawn using either a syringe-diaphragm system at the upper port or by using an aseptic sampling arrangement at the lowest port.

The gas-distributor may be of stainless steel, glass-fibre or plastic woven mesh; sintered stainless steel, plastic or glass (porosity 03) or of perforated plastic or steel. Gases may be supplied using pressurized bottles or air from a suitable continuously-rated compressor. Air from a compressor contains oil, dust and water. This can be removed by installing automatic in-line equipment (Norgren F40 series, Norgren, Shipston-on-Stour, Warwickshire) before passing the air through a flowmeter and thence through a sterilizing filter (e.g. Whatman Gamma Twelve, H. E. Reeve Angel & Co. Ltd., 14 New Bridge Street, London, EC4). It is useful to insert a non-return valve (X-lon Plastics) immediately before the inlet to prevent accidental wetting of the air-filter if the air-supply is interrupted.

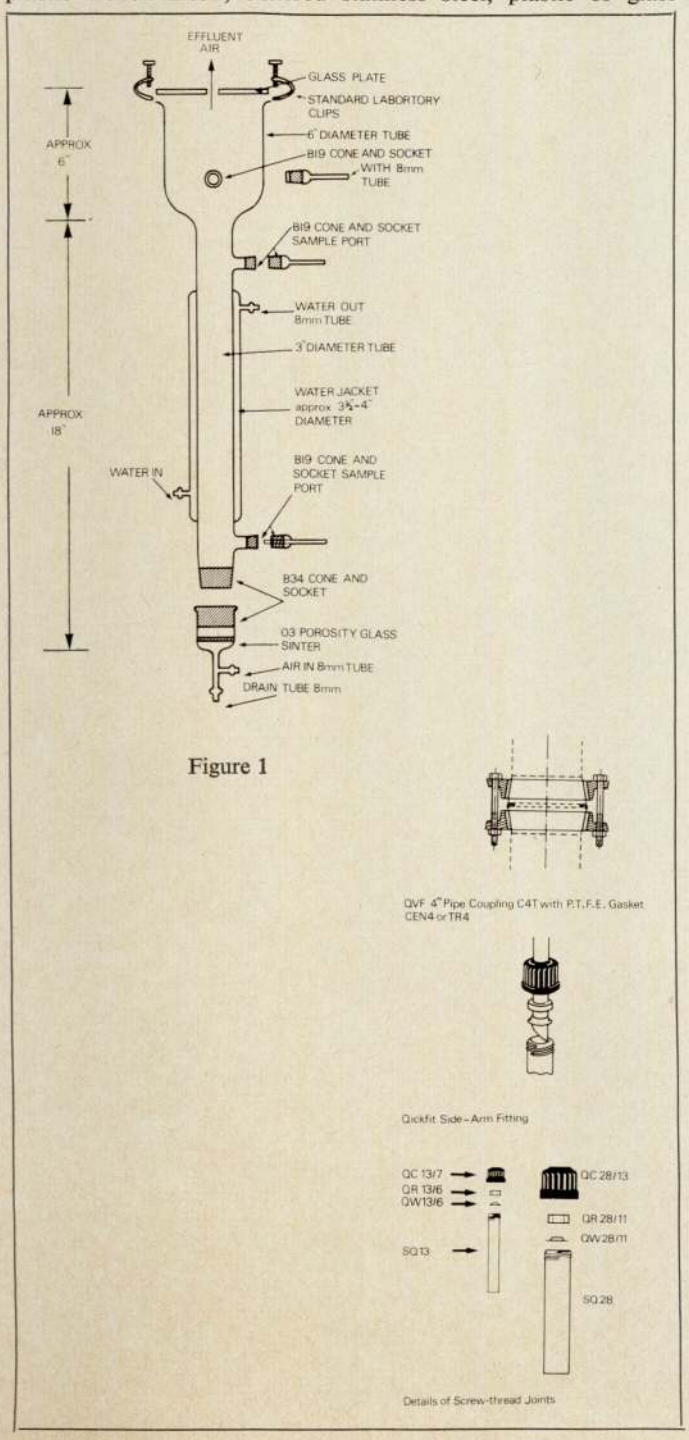


Figure 1

Variations

(a) *Size.* Fermenters of one to five litre capacity can be readily constructed from 3 in. glass tubing by most glassblowers or purchased commercially (Frances Harmon Ltd., 54 Brookhouse Road, Barnt Green, Birmingham B45 8JS). A suggested structure is given in Figure 2. Above ten litres, QVF is more suitable. For instance, 6 in. diameter QVF sections can easily be used to construct a fifty-litre capacity tower. The fusion of Quickfit side-arms to this size tubing is more difficult however, and it is easier to order the modified sections from the manufacturers or to use sections with QVF side-arms (PTU series, e.g. PTU6/1.0). Table III gives a list of suitable parts but complete assemblies may be purchased (through Frances Harmon Ltd.).

(b) *Alternative materials to glass*

Although not having the advantage of transparency, polypropylene or high density polythene tubing and sheeting can be welded into suitable structures. Certainly the fabrication of much larger vessels of up to 500 and 1,000 litres is a relatively simple proposition. With a little practice and correct instructions it is possible

Table I *Details of fittings (QVF) for fermenter* (10 *l*)

Quantity	*Description*	*Part No.*
5	pipe sections	PS4/400
7	4″ couplings	C4T
7	4″ gaskets	CEN4 or TR4
2	pipe reducers	PR4/1
4	1″ gaskets	CGN1 or TR1 or TRB1
1	tee-piece	PT1
2	hose-connectors	PHC1/0.5
1	hose-connector	PHC1/0.75

Table II

Details of side arms and range of fittings for fermenter (10 l)

Thread Size (QVF No.)	*Acceptable Diameter of probe (minimum)*	*Catalogue Numbers*		
		Plastic Cap	*Rubber ring*	*PTFE Gasket*
SQ 13	6.0– 7.0	QC 13/7	QR 13/6	QW 13/6
SQ 28	6.0– 7.0	QC 28/9	QR 28/6	QW 28/6
SQ 28	10.5–11.5	QC 28/13	QR 28/11	QW 28/11
SQ 28	18.0–19.0	QC 28/21	QR 28/18	QW 28/18

Table III

List of fittings (QVF) for 50 litre tower fermenter (6″ *diameter*)

With expansion chamber	*Without expansion chamber*
5 x P.S. 6/500	7 x PS 6/500
1 x P.R. 6/1	2 x P.R. 6/1
2 x P.H.C. 1/0.75	2 x P.H.C. 1/0.75
1 x P.T.1	1 x P.T.1
1 x P.H.C. 1/1	1 x P.H.C. 1/1
1 x P.R. 12/6	—
1 x P.S. 12/400	—
1 x P.R. 12/1	

or with side arms PS 6/500 should be substituted by PTU6 Series, e.g. PTU 6/1 where required. Appropriate couplings and gaskets are also necessary.

carry out modifications and repairs *in situ* by a hot-air welding chnique (British Celanese Ltd., Plastics Group Sales, Spondon, erby). Further details of the design and construction of large-ale vessels will be given in another article.

) *Shape*

ne ten-litre fermenter described lacks the expansion chamber ssessed by the one-litre design. This feature is required for rtain fermentations particularly alcoholic fermentations where provides an area of reduced turbulence, thus gases, organisms or foam can be separated from the fermenting liquid. It may also incorporate a weir-type overflow designed to separate cells more efficiently (Greenshields & Smith, 1971).

Discussion

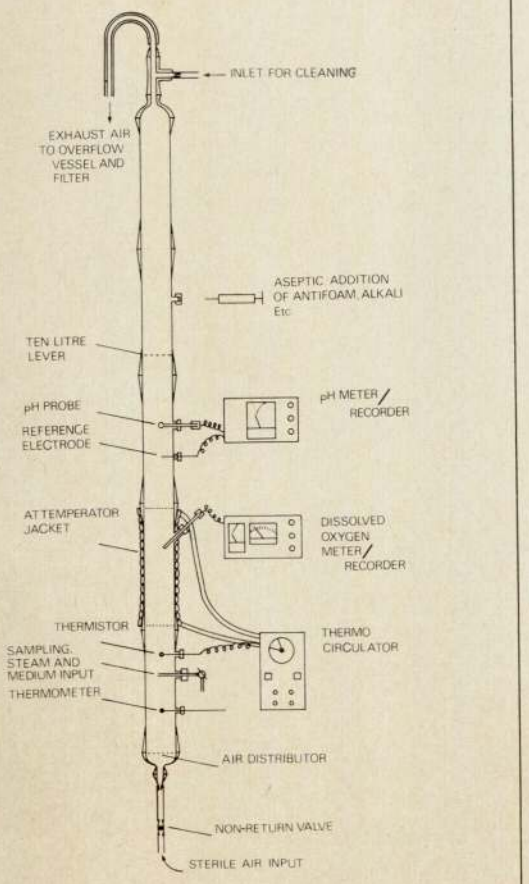
The type of tubular reactor described has been successfully used in teaching and research at all levels by chemical engineers and biologists to study a variety of fermentations. For the experimenter they provide relatively cheap, flexible units and are particularly valuable where a multiple series of fermentations are required, for example, screening for the expression of various characteristics in conditions of submerged fermentations, to produce results which are directly comparable to those in larger scale fermenters.

Specific examples from work in our laboratory include the creening of yeasts for the continuous production of beer, lager, vine and high-alcohol washes; production in quantity of small amples of individual fungal species for nutritional evaluation; n examination of the parameters involved in aerobic fermentation systems which are of importance from the viewpoint of the chemical engineer; an evaluation of the morphology of microbes in submerged culture, batch and continuous aerobic production of fungal, bacterial and yeast biomass; and the development of a new acetification process for vinegar manufacture.

The last example of the use of this type of fermenter involved the scaling-up of the process from the laboratory to commercial production. It was found that scale-up problems were relatively slight, indeed efficiency in economic terms improved at each increase in scale. Fermentations of one, ten, fifty and one thousand litres in capacity were easily and cheaply carried out in the laboratory with extension to three thousand litres for pilot and production scale in a commercial environment. A list of references are given which describe these examples of the uses of this fermenter.

Our strongest motive in concentrating on the development of tower fermenters is to establish them as a means for the rapid and cheap evaluation and exploitation of new fermentation possibilities. An anticipated upsurge in demand for intermediate technology and flexible methods of waste recycling is likely in our eyes to include a requirement for fermenters with the qualities described. There is also a lack of fermentation equipment on the market at a price which teaching and research establishments can readily afford, and indeed, our experience is that industrial organizations can also benefit from the availability of this system to complement existing facilities.

Figure 2



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