THE CONTINUOUS PRODUCTION OF ALCOHOL IN TOWER FERMENTERS

by

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SUMMARY

This research has been concerned with the continuous production of alcohol in tower fermenters and the development of equations to describe such systems. Initially the increasing market for both potable alcohol and ethanol as a fuel have been discussed. Detailed attention was also paid to the design of inlet/outlet ports for reactants/products to ensure efficient operation of a pilot-scale tower fermenter.

A number of batch fermentations, with flocculent yeasts later employed in the continuous fermenter, have been described. The kinetic data from these experiments were used to develop a mathematical model of the batch alcohol fermentation. The variables in the model are easily measured and although the model is empirical in nature it was found to be able to account for wide variations in results from fermentations.

During continuous fermentations it was found that the tower consisted of three distinct reaction zones;

(a) the base of the fermenter where a packed bed of yeast (20% of the tower height) existed,

(b) the top zone of the fermenter (60% of the tower height) which could be considered as a continuous well mixed stirred reactor,

(c) a transition zone between the latter two zones.

Sugar utilisation, nitrogen utilisation, and alcohol production rates were among the variables investigated during experimental work and some suggestions have been made for the stable operation of tower fermenters.

Finally, a mathematical model of the continuous fermenter, which combined the batch kinetic model with the physical characteristics of continuous operation, was developed. Results predicted by the model have been compared with those obtained by experiment; the differences have been discussed although not fully explained.

KEY WORDS: Tower fermenters, continuous alcohol production

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CHAPTER 1

ALCOHOL PRODUCTION

1.1 Introduction

1.1.1 The Tower Fermenter Research Group

A research group at the University of Aston in Birmingham has been concerned with what may be termed "biotechnology" for a number of years. The group is composed of chemical engineers headed by Dr. E. L. Smith and microbiologists supervised by Dr. R. N. Greenshields. This thesis is concerned with the continuous production of alcohol in tower fermenters. This is one of a number of continuous biochemical engineering processes on which the group is working.

1.1.2 The Market for Beer

At conception, the project was mainly concerned with the continuous production of potable alcohol in the form of beer. Traditionally beer has been brewed by a batch process. There are a number of systems of batch brewing, each with their advocates. These systems, and their history, have been comprehensively reviewed by Coote (1974): they will not be discussed further in this dissertation. Interest in continuous brewing has been stimulated during the last decade by;

(1) the rapid expansion in the market for beer, and

(2) rationalisation of the U.K. brewing industry.

Concerning the volume of beer produced worldwide, a recent report (The Daily Telegraph [1977a]) stated that beer production has doubled since 1967 and the demand for beer is likely to

double again in the next ten years. Rose (1977), using data from various sources, has shown that the annual production of beers is approximately 75×10^6 litres; the current value of this beer would be of the order of £19000 million. With the probable aim of capturing some of this lucrative market U.K. brewers were planning to invest £1000 million up to 1980. However, according to further information, (The Daily Telegraph [1977b], this planned investment may be cut due to arguments with the Government over profit margins.

Concerning rationalisation of the U.K. brewing industry, groups have enlarged considerably over the last twenty or so years and seven major breweries now control 80 per cent of the U.K. market. The relatively large amounts of capital controlled by these groups and the quest for greater profit margins has allowed investment in new brewing processes, including continuous fermentation processes. This is a radical change in what can be regarded as the most conservative of U.K. industries. However, the lack of innovation in the industry is probably justified because brewers have been successfully marketing a batch brewed and proven product for hundreds of years. Because of the interest in new brewing processes a brief review of the available options has been undertaken in Section 1.3.

1.2 Industrial Alcohol as a Chemical Intermediate and Fuel

It was previously stated above that the original research programme was to be primarily concerned with the production of potable alcohol. As the project progressed it became clear that the continuous fermentation of sugar-rich media to ethanol destined for industrial use was also of growing significance.

Continuous fermentations using a molasses-based medium were carried out for this reason (Section 5.4).

Because of its use in beverages nearly every country in the world has considered alcohol as a commodity for taxation and government control. The tax on alcohol is usually so high that the cost of pure alcohol for industrial processes would be many times greater than the cost of manufacture. However, ethanol can be rendered "denatured": this involves the addition of various chemicals (compatible with the industrial operation) to render it unfit for human imbibition, and hence untaxable as such. Lange (1956) lists over 120 uses of denatured alcohol: these include antibiotic processing, miscellaneous chemicals production, dye solution, embalming fluids, explosives, perfumes, soaps and shampoos, tobacco sprays and flavours, and yeast processing. Those not quoted are the uses of ethanol as a feedstock for large-scale chemicals manufacture and as a fuel. These are uses that are likely to receive more and more attention in the future.

In 1977 Ramalingham and Finn noted that three-quarters of the world's demand for industrial alcohol was met by fermentation, although in the United States 80 per cent was manufactured from synthetic processes. This situation is probably mirrored in other developed countries with their present virtually unlimited access to supplies of crude oil. However, the world supplies of crude are finite and a world energy crisis has been predicted in the future. The large-scale production of ethanol could go some way towards solving this problem.

Becher (1977) has quoted figures indicating cellulose (from

forests, pulp mills, and municipal waste) could adequately meet U.S. chemical needs. The U.S. growth of wood per year is estimated at 10^9 tons, of which 250×10^6 tons is presently used commercially. The remaining 750×10^6 tons returns to its elements through natural decay. It is estimated that 175×10^6 tons of cellulose would have supplied the total U.S. consumption of ethylene and butadiene in 1974. The first step in producing chemicals in this way involves either hydrolysis of cellulose either by enzymatic degradation, using the organism Trichoderma viride, or by chemical means, utilising high temperatures and acid degradation. The simple sugars produced by the hydrolysis process are then fermented to ethanol. This ethanol can serve as a base for a large number of chemicals. Other authors have also studied cellulose utilisation, mainly in the field of municipal waste treatment. Nyiri (1976) estimated the economic plant output for an end-product of ethanol as being between 7^3 and 20^3 m^3 per annum. The likely capital cost for such plant was estimated at between 6 and 12 million U.S. $.

Ethanol as a direct fuel additive is a subject being taken very seriously by a number of countries. After the oil crisis of October 1973 countries with no indigenous oil supplies were faced with a huge increase in their oil import bills. One way to help reduce these costs is to use ethanol as a direct additive to gasoline. Brazil is among the leaders in this field. Jackman (1976) stated that tests in Brazil showed standard gasoline engines would comfortably handle mixtures of ethanol and gasoline containing 20 per cent ethanol. For diesel engines the alcohol did not need to be anhydrous and

70 - 80 per cent alcohol blend compositions could be employed. Ethanol also has the commendatory advantage of being sulphur free. Thus ethanol blended fuels will reduce the pollutant load on the atmosphere, which is especially desirable in city environments. The feedstock for ethanol production plants in Brazil is envisaged to be molasses, sugar cane, or manioc (cassava). The main advantage of sugar cane is that its fibre (bagasse) can supply the energy required for the ethanol plant. However, manioc has an unusually high photosynthetic potential conversion of solar energy into starch: this results in a higher alcohol yield than from sugar cane on a weight for weight basis. Carioca et al. (1978) have suggested the exploitation of a further crop for alcohol production: this is the Babbasu nut. This crop is currently grown in abundance for its "almonds" which are rich in vegetable oil. These "almonds" only amount to approximately 7 per cent of the crop on a weight basis; the remainder of the coconut, much of which is starch rich, is at present discarded.

Other developed countries interested in alcohol production are New Zealand and Australia. Chapman (1977) has stated that one aspect of New Zealand's forest management strategy is the fermentation of sugars, produced by wood hydrolysis, to yield industrial alcohol. One of the advantages of using wood as the feedstock in an alcohol production programme is that the forest harvest is not a seasonal crop. In 1978 Australia played host to a three-day meeting of the Institution of Chemical Engineers, the sole concern of this conference was alcohol fuels. The United States is not without interest in ethanol-gasoline blended fuels. Scheller (1977) studied the production costs of grain

alcohol in very large plants, (50 - 100 x 10^6 gallons per year of anhydrous ethanol), and estimated grain alcohol could be produced at 60 ¢ per gallon. Grain and its fermentation are less closely coupled to the price of crude oil than gasoline, and so Scheller forecast that grain alcohol would become more attractive in the future as an automotive fuel additive.

A further point which may expedite the production of industrial alcohol is the current world surplus of sugar. The Daily Telegraph (1978) observed that world surplus sugar stocks were heading for 33 x 10^6 tons, with more surpluses likely in future years. The Financial Times (1978) reported that Brazil has the potential to become the worlds largest sugar producer. However the cost of sugar produced in Brazil in 1977 was more than the internationally agreed world price. With Brazil's export quota likely to be cut by the International Sugar Meeting alcohol production becomes even more attractive in Brazil. Nearer home the E.E.C.'s own surplus of sugar is running at approximately 50000 tons per week (2.6 x 10^6 tons per annum). If this sugar could be used for alcohol production then this "sugar mountain", created by the powerful sugar beet lobby in the E.E.C., may be put to good use.

1.3 Continuous Beer Production

1.3.1 Introduction

The concept of semi-continuous and continuous fermentation for the production of beer can be readily dated to the latter part of the 19th century. Early workers in the field recognised that to achieve an accelerated fermentation there are two main

requisites:

i) a high yeast concentration, and

ii) intimate contact between the yeast and substrate to maintain a high yeast viability.

Reviews and descriptions of the early systems for continuous alcohol production have been presented by a number of authors (Green (1962), Parsons (1963), Hospodka (1966), Hough and Button (1972), and Coote (1974)). These early endeavours were not pursued due to a combination of reasons:

i) a natural conservatism on the part of the brewers,

ii) the difficulty of maintaining asceptic conditions and a lack of proper process control, and

iii) the fact that labour costs were low, (and so there was little incentive to improve efficiency in this zone of the operation).

1.3.2 Yeast Stability and Viability

Thorne (1968a, 1968b, and 1970) expressed the fear that due to the unusual conditions associated with continuous beer fermentation spontaneous genetic mutation of the yeast would occur. In experiments with strains of *Saccharomyces carlsbergensis* Thorne (1968b, 1970) found considerable evidence of mutation in a continuous fermentation system: the cell isolates gave mutations which featured loss of flocculence, a reduction in fermentation efficiency, and the production of undesirable flavours. However, Ault et al. (1969) found no evidence of mutation in a commercial fermenter. Coote (1974)

confirmed this latter view when he carried out extensive tests for mutation, using a laboratory scale tower fermenter. Bishop (1970) suspected successful mutations had occurred in the Watney-Mann system. However, as the vast majority of mutations would be disadvantageous, i.e. they would not proliferate, they would tend to wash out of the system. Bishop experienced no mutation interference in continuous runs exceeding twelve months.

Hudson and Button (1965) and Watson and Hough (1966) recorded that cell viability decreased markedly in continuous fermentations containing high concentrations of yeast. However, Portno (1968a) and Ault et al. (1969) have shown this problem can be overcome by correct aeration of the system.

1.3.3 Continuous Stirred Tank Systems

A number of stirred tank systems for the continuous production of beer are in current commercial use and a brief description of the various processes will be given. For a more detailed comparison of these systems the reader is referred to either Stewart and Laufer (1960) or Purcell and Smith (1967). All the current production size stirred tank processes are essentially similar in that they employ two stirred fermentation vessels and equipment for separation of the yeast from the final product.

The Dominion Breweries of New Zealand have employed such a system for a number of years (Fig. 1.1). The plant operation is fully described by Coutts (1966) and was patented by the Dominion Breweries Ltd. in 1957. The two stirred fermentation vessels are preceded by a "yeast propagator" in which aerated wort, recycled yeast, and recycled fermenting beer are mixed.

FIG 1.1 THE DOMINION BREWERIES LTD. SYSTEM OF CONTINUOUS FERMENTATION.

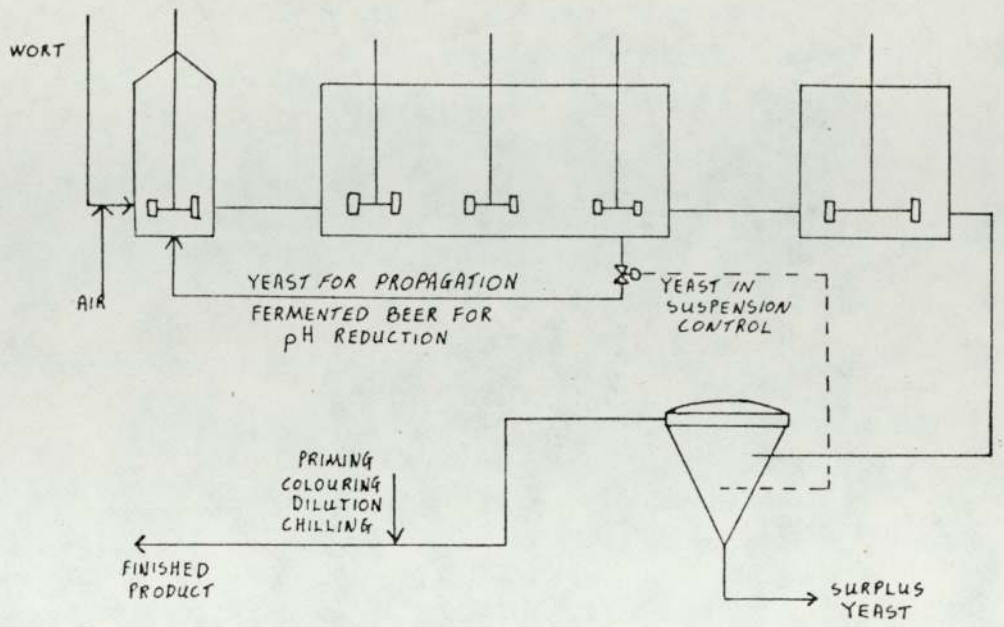
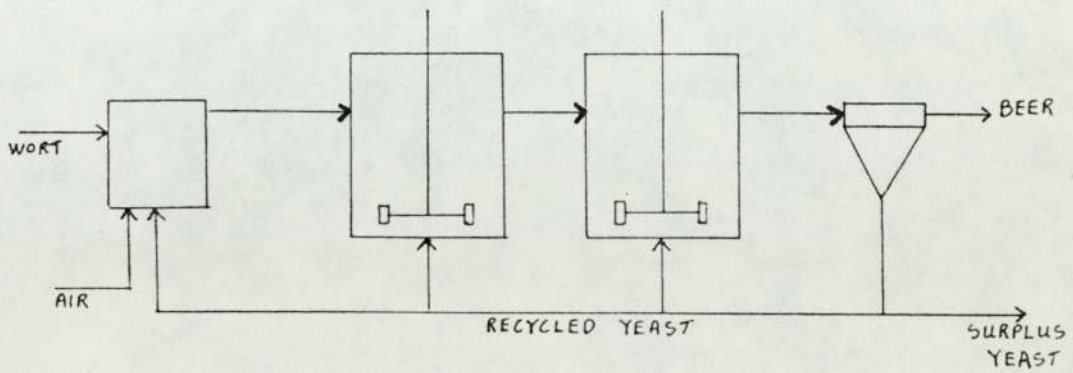


FIG 1.2 THE JOHN LABATT FERMENTATION SYSTEM.



John Labatt Ltd. (1957) patented a system (Fig. 1.2) similar to the plant used by Dominion Breweries. Geiger (1961) described the pilot plant work on this process. A yeast propagation vessel is not employed as such; however, aerobic conditions are maintained in the first stirred fermenter in order to encourage some yeast growth. Again a yeast separation vessel and yeast recycle are employed to maintain high yeast concentrations in the fermentation vessels.

The Fort Worth process (Fig. 1.3) for the continuous production of lager beers was developed by Williams and Brady (1965) for the Carling Brewery Co., U.S.A. In this process the two fermentation vessels are proceeded by an unstirred baffled tank known as the "lag vessel". Wort and recycled yeast are forced to flow relatively slowly through this primary vessel in order to simulate, to some extent, the lag phase of the batch fermentation. However, the process was susceptible to bacterial infection, and Hough and Button (1972) reported that the plant was eventually closed down.

As the above three systems are all essentially similar in their physical aspects it is not surprising to find that the liquor residence time in all three is of the order of 35 hours. The latest continuous stirred system (Fig. 1.4) has the much lower wort residence time of 15 hours. The process was developed for the Watney-Mann group of the U.K. and is described in detail by Bishop (1970) and Maule (1973). Prior to fermentation the wort is held in a chilled condition and is pasteurised as it flows into the process. The two stirred fermenters are preceded by an "oxygenation column" which aerates the wort

FIG 1.3 THE FORT WORTH PROCESS

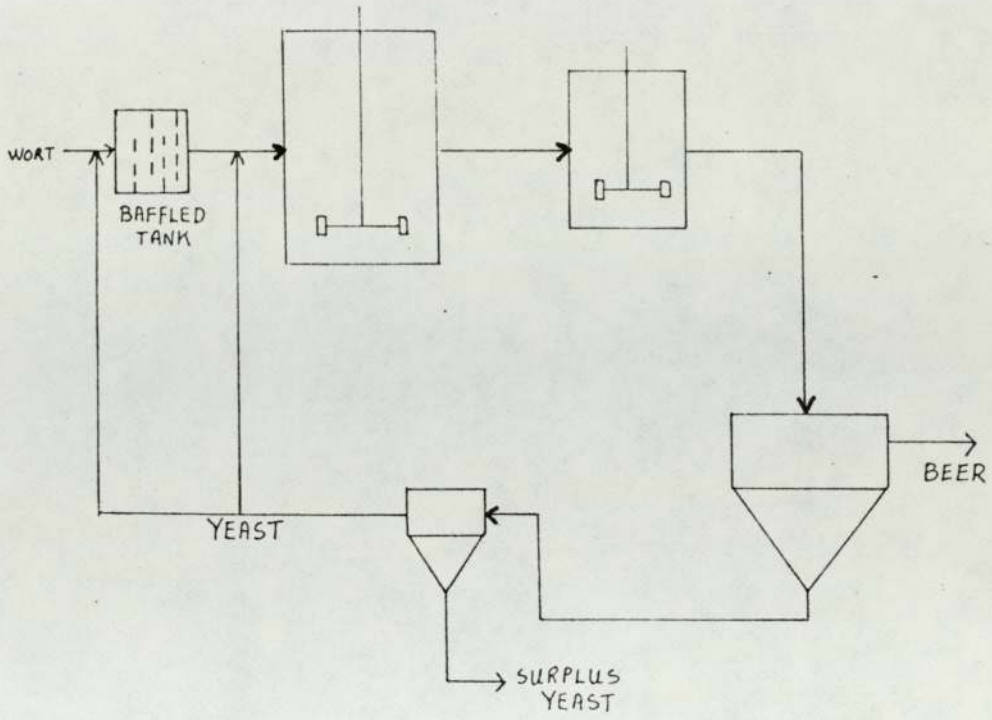
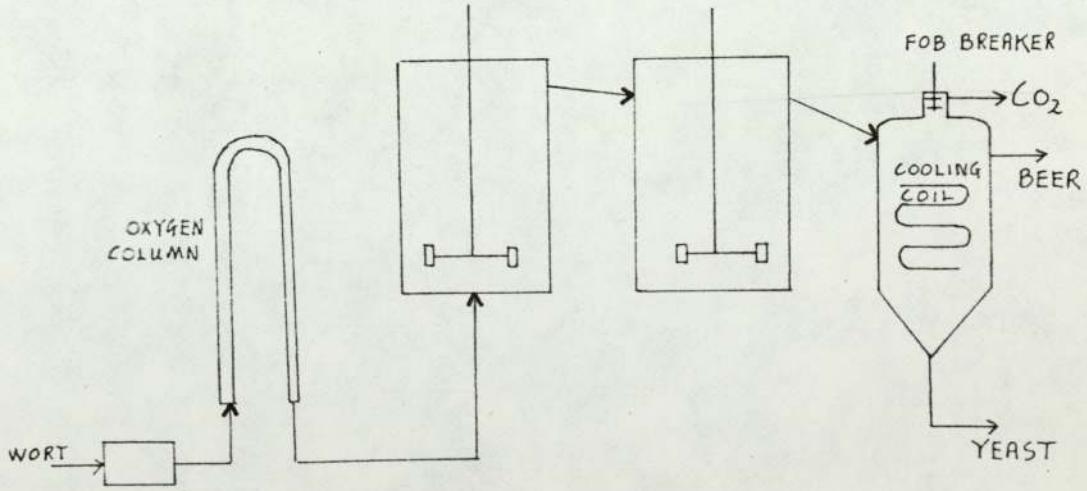


FIG 1.4 THE WATNEY-MANN SYSTEM



to a predetermined value. After fermentation the yeast is separated by sedimentation in a cooled cylindro-conical tank. Carbon dioxide is also collected from this vessel. An interesting feature of this plant, with regard to earlier systems, is that the lower residence time is achieved without a yeast recycle.

1.3.4 Miscellaneous Systems

Schaus (1969) and Portno (1969) have suggested the use of a segmented vessel, with stirring in each compartment, as a means of reducing the cost of building a multi-stage process.

Portno (1967a, 1967b) described a stirred tank for fermentation where the amount of yeast leaving the vessel was controlled by taking off the beer via the arms of a rotor. As the beer left the vessel it was therefore subjected to a centrifugal force which caused the majority of the yeast cells to be returned to the fermenter.

Portno (1967b) also experimented with a heterogeneous system of fermentation in which yeast and wort were fed into a narrow-bore tube: fermentation then proceded along the length of the reactor. By employing a sedimentary strain of yeast clear beer could be drawn off from a settling vessel while the settled yeast slurry was recycled.

Hall and Howard (1968) patented an inclined tube system for continuous fermentation. In this device gas bubbles rose to the upper surface of the tube while yeast sedimented out, thus maintaining a high yeast concentration. The system exhibited many of the characteristics of the tower fermenter.

In particular, it should be noted that;

i) an average yeast concentration of 225 g/l (wet yeast basis) was observed in the fermenter,

ii) a low air flow-rate was used to stimulate fermentation without causing excessive yeast growth,

iii) the system had a low cell overflow rate, (2×10^6 cells/ml, .42%w/w pressed yeast), and

iv) carbon dioxide was used to promote some mixing in the fermenter.

Recently, Wick and Popper (1967) have used this system for the continuous fermentation of grape juice.

Finally, Hough (1961) and Hough and Riketts (1960, 1963) have described the operation of a "V-tube" fermenter. This system may be regarded as a hybrid of the stirred tank system and the tower fermenter. One arm of the "V" was agitated to promote intimate contact between wort and yeast, the second arm was shielded from mixing effects by a baffle. Flocculent yeast strains were used so that cells entering the non-agitated arm sedimented out and returned to the fermentation zone: in this way high yeast concentrations were achieved in the vessel. This is the principle by which high yeast concentrations are maintained in the tower fermenter.

1.3.5 Tower Fermenters

Tower type fermentation systems for fermentation have been put forward by Owen (1948), de Mattos (1948), and Victero (1948). In the system described by Owen (1948) a molasses medium flowed downwards through the tower, across decks on which the yeast

suspension was maintained. The fermenters of de Mattos (1948) and Victero (1948) were fitted with baffles to restrict the rate of cell overflow, thus maintaining a higher than normal yeast concentration in the fermenter. Victero (1948) in particular has drawn attention to many of the operational details which are characteristic of the modern tower fermenter: these include the following:

i) $^{2}/_{3}$ of the fermentation is completed in the lower quarter of the tower,

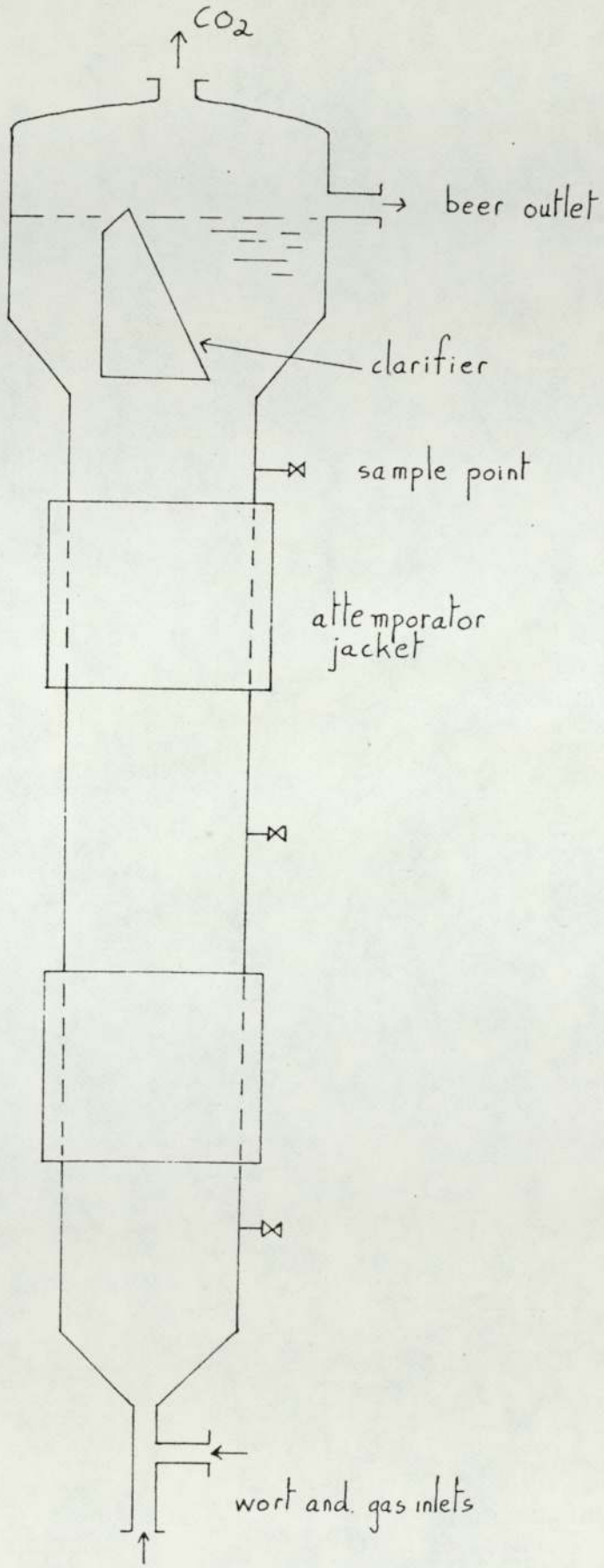
ii) there is no lag phase in the system due to the yeast in the fermenter being acclimatised to fresh wort,

iii) carbon dioxide is kept in solution in the lower sections of the tower fermenter due to the high hydrostatic pressure, and

iv) the system is less vulnerable to bacterial infection than a cascade of stirred tank fermenters.

The tubular reactor design in present commercial use (Fig. 1.5) was developed in the early 1960's by the A.P.V. Co., Crawley. Royston (1961, 1965), Shore and Watson (1961), and Shore et al. (1962) were granted a number of patents pertaining to a tubular reactor for the anaerobic fermentation of sugar-rich media. Various aspect ratios were tested, and the development of the "still zone" concept in the head of the fermenter was an important feature of the design. When reactor diameters above 6 inches (0.152 m) were employed it was found necessary to fit the fermenter with baffles: these helped to maintain an equal yeast distribution across the fermenter. Residence

FIG. 1.5 THE A.P.V. TOWER FERMENTER



times of wort in the fermenter were as short as 4 hours, although when the tower was employed as an homogeneous reactor the residence time of the wort had to be increased to 7-12 hours for the same degree of attenuation.

Klopper et al. (1965), Royston (1966a, 1966b), and Ault et al. (1969) have discussed pilot plant work and commercial experience with the A.P.V. tower fermenter. Klopper et al. (1965) used a pilot scale tower fermenter as a "fermentation accelerator" in a system for the production of lager. The wort was pitched as normal, then fed through the tower fermenter which contained a high concentration of yeast. Klopper and his co-workers also used a slightly larger fermenter for the production of ale-type beers. Initially a fermenter with an aspect ratio, (height: diameter), of 12:1 was employed. However, homogeneous conditions developed in this tower and the aspect ratio had to be increased to 30:1. This is in marked contrast to the commercial fermenter which has an aspect ratio of 7:1.

Klopper et al. (1965), Royston (1966b), and Ault et al. (1969) have all recorded the existence of a definite fermentation gradient in the tower fermenter, the fermentation reaction proceeding in space in the tower as it would in time in a batch fermentation. Klopper et al. (1965) and Royston (1966b) have also noted that a yeast concentration gradient existed in their tower fermenters. This concentration profile was dependent on the wort flow-rate: at low liquor flow-rates Klopper et al. (1965) found there was little evidence of a yeast gradient; as the flow-rate increased the concentration of yeast at the top of the tower decreased to $\frac{1}{2}$-$\frac{1}{3}$ of that at the base. Further increase in wort flow led to the

maximum yeast concentration occurring at some mid-point in the tower, and finally the yeast was washed out of the system. The mean yeast concentration in the tower was said by Royston (1966b) to be around 250 g/l, expressed as centrifuged yeast.

Klopper et al. (1965) found some problems with the flavour of tower-produced beer; however, Ault et al. (1969) found that by aerating the wort prior to fermentation these problems could be overcome. In a detailed analysis Ault et al. (1969) showed batch and continuously fermented beers were both chemically and organoleptically indistinguishable. Den Blanken (1974) worked on the continuous production of lager beers using tower fermenters. He confirmed the finding of Ault. et al. (1969) that to achieve a product consistent with batch beer, yeast growth had to be of the same order of magnitude as that in batch fermentation. However, den Blanken (1974) found that to achieve an organoleptically acceptable product the liquor residence time in the fermenter had to be greater than 20 hours. Experiments carried out by Coote (1974) confirm this result. It is interesting to note here that continuous lager production seems to present problems whether stirred tanks, (Williams and Brady (1965)), or tower fermenters are employed.

Greenshields and Smith (1971) and Smith and Greenshields (1973) have discussed some of the engineering concepts involved in the tower fermentation of beer, and in particular they have examined diffusion of nutrients, both from the bulk of the liquid and within the pores of the flows. Preliminary calculations indicated that bulk diffusion and pore diffusion were unlikely

to be rate controlling and that the rate-controlling step was likely to be chemical reaction, at, or in the yeast cells. Evidence supporting this statement is that the rate of fermentation in the tower remains constant over a wide range of wort flow-rates (Klopper et al. (1965) and Ault et al. (1969)).

1.3.6 Comparison Between Continuous and Batch Fermentations

The advantages and disadvantages of the various systems of fermentation have been summarized by Ricketts (1971) and Hough et al. (1971). The benefits of continuous systems are said to be that;

i) the fermentation vessels are nearly always full

ii) no downtime is required for cleaning, with a relative saving in costs

iii) there is an even demand on brewery services

iv) hop requirements are lower

v) fermentation times may be as low as 6 hours

vi) beer losses are cut to a minimum

(In a traditional batch fermentation up to 5% of the beer may be lost during processing. As brewers pay duty on the wort prior to fermentation a substantial saving results by employing continuous fermentation methods.)

vii) labour costs are lower (see Bishop (1970))

viii) carbon dioxide collection is simple and efficient (see Turvill (1973)).

Hough et al. (1971) calculated that the savings likely to accrue from the adoption of continuous fermentation to be at least 12 n.p. per barrel (7.33 n.p. per hl). Ricketts (1971) quoted the much higher saving of £1.15 per barrel for a brewery converting

from the traditional batch to the tower fermentation process. Since Bishop (1970) has calculated that the saving on hop requirements and duty for the pitching yeast volume alone can be 12½ n.p. per barrel, the higher figure quoted by Ricketts (1971) may not be unreasonable.

The drawbacks have been stated to be that;

i) continuous systems are inflexible in the amount and variety of beer produced
(However, Ault et al. (1969) and Bishop (1970) have demonstrated that continuous systems can be shut down for relatively long periods without deleterious effects. This feature has also been demonstrated by the author (Section 5.4.1).)

ii) continuous fermenters are susceptible to bacterial infection and consequently require expensive wort storage and pasteurisation facilities (see Klopper et al. (1965) and Maule and Thomas (1973))

iii) continuous fermentation vessels and their ancillary equipment are relatively expensive
(In answer to these last two points Royston (1966a) and Bishop (1970) have stated that the capital cost of continuous plant will be lower than the corresponding batch vessels for the same production capacity.)

iv) there are logistic problems involving wort production, storage, weekend working, and continuous reception of beer for post fermentation treatment.
(These points are answered to some extent in (i) above.)

Though the advantages of continuous fermentation appear to outweigh the disadvantages, Macleod (1977) and Hough et al. (1976) state the Nathan (1930) system of batch fermentation is becoming increasingly popular: Harris (1974) and Thompson (1970) have reported the installation of such vessels by two large brewing groups. Hough et al. (1976) have also made the point that the spin-off in technology from continuous fermentation work has increased the efficiency of the more traditional batch fermentation processes.

1.3.7 Specific Advantages of the Tower Fermenter

Beer contains a large number of minor components, often in quantities less than 1 p.p.m, which account for its characteristic flavour. Royston (1966b) stated that these flavour components are more likely to be precisely reproduced in a heterogeneous than a homogeneous system of fermentation. Portno (1976b) has demonstrated that this is the case.

When a highly sedimentary yeast is employed much higher yeast concentrations occur in the tower fermenter than in the stirred tank systems: thus the fermentation rate per unit volume of plant is greater in the tower.

The tower fermenter is a relatively simple piece of plant from an engineering point of view. For example, the complexities of asceptic stirrer glands are avoided. Also, as agitators are not required there is a saving in power costs.

The reaction kinetics of the fermentation may also favour the adoption of the tower fermenter. If the reaction is zero order then calculations show that the volume required for reaction

is the same for both tubular and stirred vessels. If the reaction is first order with respect to substrate concentration, then a plug-flow reactor is more efficient. Royston (1966b) has suggested that a plug-flow reactor could be less than half the volume of a homogeneous reactor for the first-order situation. A more detailed discussion of fermentation kinetics is presented in Chapters 4 and 6.

1.4 Research Objectives

Tower fermenters have been used with varying degrees of success for the production of potable alcohol. This may in part be due to the fact that it is not yet possible to design such systems from first principles. Current plant has been empirically built using laboratory data on suitable yeast strains and by conducting pilot plant tests. The main aim of the work reported in this thesis has been to develop universal design equations for the tower fermenter when used for alcohol production.

As part of the overall research programme it was decided that a small-scale tower fermenter should be built in which to attempt to simulate conditions said to occur in commercial fermenters. As a result of previous experience within the T.F.R.G. it was felt that particular attention should be paid to the design of the inlet/outlet ports for the reactants/products.

To permit rational design and scale-up of continuous fermentation systems kinetic data are required. Because of the lack of published information, it was felt that a detailed study of the rates of fermentation was required. For conveneience work in batch fermenters was planned. Because of the work of Fidgett

(1975), it was anticipated that data analysis would be complex and mathematical modelling techniques would have to be used to determine equations describing the kinetics of the alcohol fermentation.

A series of continuous experiments were planned for the previously mentioned small-scale fermenter. The main purpose of these experiments was to study the yeast concentration profile in the fermenter: information on this topic had previously been lacking. The physical operating characteristics of the tower fermenter were also noted.

Finally the kinetic data and experimental results from the continuous fermenter were combined in an attempt to mathematically define the system.

CHAPTER 2

DISTRIBUTOR PLATE DESIGN

2.1 Introduction

On inspecting the individual components that make up a tower fermenter it was perceived that an efficient distributor, for both air and liquid entering the tower, could be important in two ways. During the start-up phase of the micro-aerophilic alcohol fermenter the yeast requires relatively large quantities of oxygen to promote organism growth. An efficient air distributor could therefore be important in determining the optimum start-up period of the fermenter. During continuous operation both wort and air are pumped into the tower fermenter. A well designed distributor would help prevent channelling of the medium through the biomass. Consequently this would promote intimate contact between the biomass and nutrients and aid in ensuring that the fermenter was performing at its optimum efficiency.

2.2 Literature Survey

A literature survey was undertaken to determine the extent of previous work on the design of perforated plates for fluid distribution. There are perhaps two initial criteria of design when considering a distributor plate. One is that the orifices should be of such a size that leakage of the continuous phase is prevented: i.e. backflow through the plate is minimised. The second criterion is that there should be sufficient pressure drop across the plate to produce equal flow through the orifices. These two will affect one another to some extent.

The conditions of sufficient pressure drop to produce equal

flow through the openings have been summarized by Kunii and Levenspiel (1969). They state that the minimum pressure drop across the distributor plate should be the maximum of the following: -

i) 10% of the pressure drop across a fluidised bed on the plate

ii) 350 mm of water

iii) 100 x (the pressure drop due to expansion of the fluid flowing into the vessel).

Other pressure drop limits have also been put forward. These have been summarized by Perry (1973). They include that: -

i) the pressure drop across the plate should be at least 30% of the pressure drop across any fluidised bed

ii) a minimum of 10 inches (254 mm) of water is required

iii) pressure drops in excess of 2 p.s.i.g. (115 kN m^{-2}) are not normally employed.

The next stage, therefore, is the determination of a suitable method for calculating the pressure drop across the distributor plate. Once the pressure drop has been calculated it must then be ascertained that it lies within certain specified limits. There have been a number of publications dealing with the calculation of pressure drops across perforated plates.

Kolodzie and van Winkle (1957) were interested in the industrial utilization of perforated-plate distillation columns. Here one of the paramount design considerations is the pressure drop through the column. A major portion of this pressure drop is the pressure lost through the plates. Kolodzie and van Winkle (1957)

therefore undertook an investigation to determine and correlate the variables which were thought to affect the pressure drop across dry perforated plates. They decided that the nature and number of the variables involved would make a theoretical analysis virtually impossible. Hence they adopted a semi-empirical approach.

Discharge coefficients were calculated for various plates and correlated against the physical characteristics of the fluid, column, and plate. The basic equation employed is written below,

$$G = C_D A_f Y \left[\frac{2\rho \Delta P}{(1-(A_f/A)^2)} \right]^{1/2} \qquad 2.1$$

Kolodzie and van Winkle (1957) assumed the expansion factor, Y, to be near unity for the small pressure drops encountered. The ratio of total orifice area to column cross-sectional area, (A_f/A), will generally be small, hence

$$1 - (A_f/A)^2 \simeq 1,$$

rearranging eq. 2.1 and writing in terms of volumetric flow-rate,

$$\Delta P = \frac{\rho v_o^2}{2C_D^2} \qquad 2.2$$

From their experimental work Kolodzie and van Winkle (1957) plotted a family of curves of orifice coefficient, C_D, versus orifice Reynolds number, Re_o, for various values of the parameters T/Do and P/Do. It was found that the curves could be represented by:

$$C_D = K \cdot \left(\frac{Do}{P} \right)^{0.10} \qquad 2.3$$

or

$$K = C_D \cdot \left(\frac{Do}{P} \right)^{-0.10} \qquad 2.4$$

Smith and van Winkle (1958) extended the previous work to include low orifice Reynolds' numbers. Again it was found that,

$$K = C_D \left(\frac{P}{Do} \right)^{0.10} \qquad 2.5.$$

K is a constant for a particular ratio of plate thickness to orifice diameter, T/Do. Therefore a plot of K versus T/Do enables the coefficient of discharge, C_D, to be calculated for a particular orifice Reynolds number.

McAllister et al. (1958) were also interested in dry plate pressure drops with regard to distillation columns. They stated that the pressure loss as a gas passes through a perforated plate is made up by three components. These are the pressure drops due to contraction losses, frictional losses through the orifice, and expansion losses. Equation 2.6 was used to determine the overall pressure drop through perforated plates.

$$\Delta P = \frac{K_1 \rho v_o^2}{2} \left[0.4 \left(1.25 - \frac{A_f}{A} \right) + 4f \frac{T}{Do} + \left(1 - \frac{A_f}{A} \right)^2 \right] \qquad 2.6$$

The constant, K_1, was designated by McAllister et al. (1958) as a factor necessary to "take care of" interference between adjacent jets and velocity gradient irregularities within the orifices. This concept was not considered by either Kolodzie and van Winkle (1957) or Smith and van Winkle (1958): they assumed each orifice produced independent and parallel streams.

McAllister et al. (1958) plotted the plate pressure drop, ΔP, against the parameter $\left[0.4(1.25A_f/A) + 4fT/Do + (1-A_f/A)^2\right] . \rho v_o^2 /2$. This resulted in a straight line of slope K_1. Thus once the physical parameters of a system had been fixed the pressure drop could be readily derived.

Yoshitome (1963) was concerned with the pressure drop

through bubble columns. A portion of this pressure drop is that through the dry gas distributor. Yoshitome (1963) calculated the coefficient of discharge for various plates with the equation used by Kolodzie and van Winkle (1957);

$$C_D = \left[\frac{v_o^2 \rho}{2\Delta P} \right]^{\frac{1}{2}} \qquad 2.7.$$

Yoshitome (1963) found C_D for a particular plate to be constant over a wide range of orifice Reynolds' numbers: some typical values are given in Table 2.1.

TABLE 2.1 Experimental Data from Yoshitome (1963)

Do	T	N	C_D	Re_o
mm	mm			
0.5	1.0	37	0.80	103-4412
3.0	1.0	37	0.62	176-3529
1.1	1.0	169	0.77	323-6471
2.0	1.6	60	0.80	706-3529
4.0	1.6	19	0.58	706-9412

However, the calculated values of orifice Reynolds' numbers in the above table are not consistent with other data given by Yoshitome.

In particular a plot of C_D versus Re_o is given in which C_D is only constant for a particular plate when $1000 < Re_o < 5000$. A further plot, analogous to that of Kolodzie and van Winkle (1957), shows C_D to be independent of Re_o. With this plot Yoshitome (1963) showed that for a particular value of the parameter Do/T;

$$C_D \cdot \left(\frac{P}{Do} \right)^{0.05} = K_2 \qquad 2.8$$

and for Do/T>2.5

$$C_D \cdot \left(\frac{P}{Do} \right)^{0.05} = 0.07 \qquad 2.9$$

Rehakova and Kastanek (1957) were interested in pressure drops across plates of extremely low free area, $A_f < 1.0\%$. They considered that pressure drops for such plates could not be estimated accurately using standard published procedures. Combining equations 2.2 and 2.3 implies;

$$\Delta P = \frac{\rho v_o^2}{2} \cdot \frac{1}{K^2} \left[\frac{P}{D_o} \right]^{0.20} \qquad 2.10.$$

Kolodzie and van Winkle (1957) found K to be independent of T/Do in the range $4000 < Re_o < 20000$ and $2 < T/Do < 3$. Rehakova and Kastanek (1957) were "completely at odds" with this. The latter found K to be strongly dependent on Re_o as well as being some function of the free area of the grid, A_f, and the number of orifices, N.

For the distributor plates of low free area $A_f/A \rightarrow 0$ and eq. 2.6 used by McAllister et al. (1958) can be written,

$$\Delta P = \frac{K_1}{2} v_o^2 \rho \left(1.5 + 4f \cdot \frac{T}{Do} \right) \qquad 2.11.$$

Rehakova and Kastanek (1975) rejected eq. 2.11 by stating that it was not possible for the pressure drops for grids of different free areas to be the same for given values of T, Do, and Re_o (assuming identical friction factors, f). However, if the grid free area is altered, then the fluid flow-rate must also be changed to maintain Re_o, and v_o, constant. Thus the statement of Rehakova and Kastanek (1975) is not necessarily correct.

In order to calculate the pressure drop across perforated plates Rehakova and Kastanek (1975) used the basic Venturi equation with two correction parameters introduced; these were to account for mutual interference of the individual streams emerging from the plate. One of these parameters corrected the

discharge coefficient, the other corrected the expansion factor. The pressure drop was calculated from;

$$\Delta P = \frac{K_3 \rho v_o^2}{2Y^2} \qquad 2.12.$$

The expansion factor, Y, is given by an A.S.M.E. (1959) report on fluid meters as;

$$Y = \left[r^{2/x} \left(\frac{x}{x-1} \right) \left(\frac{1 - r^{(x-1)/x}}{1 - r} \right) \left(\frac{1-\beta^4}{1-\beta^4 r^{2/x}} \right) \right]^{\frac{1}{2}} \qquad 2.13.$$

For plates of low free area $\beta \to 0$. Rehakova and Kastanek (1975) then deduced that eq. 2.3 could be approximated to;

$$Y = K_4 \cdot \left[\frac{(1-r)}{x} \right] \qquad 2.14.$$

Equation 2.14 implies that as $r \to 1$ then $Y \to 0$. However Perry (1973) and an A.S.M.E. (1959) report both state that as $r \longrightarrow 1$ then $Y \longrightarrow 1$. This can also readily be proven by the application of L'Hopital's rule (Appendix 2.1) to eq. 2.13.

The constants K_3 and K_4 employed by Rehakova and Kastanek (1975) are defined by equations 2.15 and 2.16.

$$K_3 = \left[3.85 + \frac{48.316}{N^{2.3}} \right] \left(\frac{A_f}{A} \right) \qquad 2.15$$

$$K_4 = 1.446 \text{ for } T/Do \geq 6 \qquad 2.16$$

Values for K_4 for $T/Do < 6$ are not given. By combining equations 2.12 and 2.14-2.16 it is stated that a relationship in the form of a polynominal is obtained for the pressure drop of low free area grids. However, as eq. 2.14 appears to incorrectly describe the dependence of Y on r the overall relationship between ΔP and other variables may not be satisfactory.

2.3 Theoretical Pressure Drop Considerations

2.3.1 Introduction

The published literature concerning pressure drops across perforated plates is conflicting on a number of points. Hence, it was decided to carry out an experimental programme: the aim of this work was to investigate the effects of orifice diameter, number of orifices, and air flow-rate on the pressure drop across perforated plates. The results were to be correlated with existing theory if possible, or a new predictive equation, taking into account the special features of continuous fermentation was to be developed. These special features are -:

i) the relatively low gas flow-rates encountered

ii) the probability of having to use extremely small orifices. (These were envisaged to be necessary to provide sufficient pressure drop across the plate to produce equal flow through the orifices and to prevent backflow of the small particles encountered in fermentations.)

Prior to implementing an experimental programme an idea of "reasonable" air flow-rates and pressure drops was required. A superficial gas velocity of 2 cm/s in the tower fermenter was said by Smith (1975) to result in a volumetric gas flow-rate suitable for the growth of micro-organisms. In a 3 inch (76.2 mm) diameter column this results in a volumetric flow-rate of 5.47 l/min (9.12×10^{-5} $m^3 . s^{-1}$)

2.3.2 Minimum Allowable Pressure Drop

The criteria for determining the minimum pressure drop across a distributor plate have been mentioned previously

(see Section 2.2). These will now be considered invidividually.

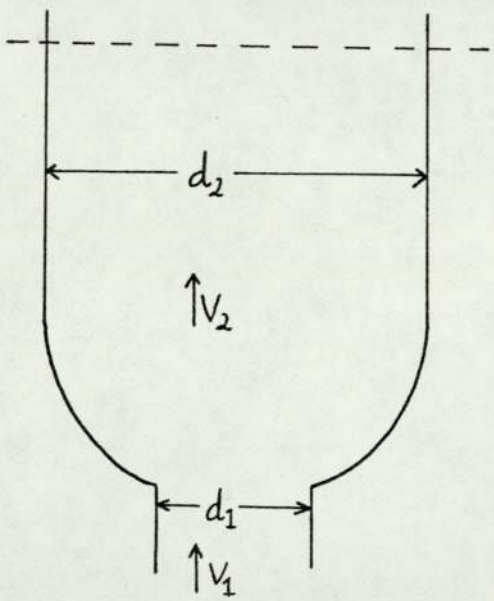
(1) <u>The pressure drop due to expansion of the fluid flowing into the vessel.</u>

A sketch of the base of the tower fermenter is shown in Fig. 2.1. The head lost due to expansion of the fluid flowing into the vessel, he, is given by;

$$he = \frac{(V_1 - V_2)^2}{2g} \qquad Re > 2000 \qquad 2.17$$

$$he = \frac{(V_1 - V_2)^2}{g} \qquad Re < 2000 \qquad 2.18.$$

Fig. 2.1 Sketch of the Base of the Tower Fermenter



Consider V_2 = 2 cm/s

then $Re_2 = \frac{d_2 . V_2 . \rho}{\mu}$

$$= \frac{76.2 \times 10^{-3} \times 2 \times 10^{-2} \times 1.2055}{1.78 \times 10^{-5}}$$

$$= 103.2$$

By the law of continuity,

$$V_1 A_1 = V_2 A_2$$

$\therefore V_1 = 18$ cm/s for $d_1 = 25.4$ mm

and $Re_1 = 310.$

Therefore the loss of head due to expansion (from eq. 2.18) will be 2.61×10^{-3} m of air. The pressure drop across the grid should be at least one hundred times greater than this; i.e. 0.261 m of air or 3.145×10^{-4} m of water.

(2) The minimum head of water

A minimum pressure drop over the plate of 350 mm of water has been stated to be desirable. Obviously this is much greater than the previously calculated "expansion" criterion.

(3) The pressure drop across the fluidised bed above the plate

The minimum desirable pressure drop across the plate has been said to be either 10% or 30% of the pressure drop across a fluidised bed on the plate. The biomass in the tower may be considered analogous to a fluidised bed. However, a fermenting broth is a three phase system and due attention must be paid to the liquid phase. This latter phase exerts a pressure on the plate: it is therefore reasonable to assume that the minimum pressure drop across the plate may be considered as 10% or 30% of the head of liquid above the plate. Consider the liquid head above the grid to be 9 ft. (2.74 m) of water; and taking the 30% criterion the minimum pressure drop over the plate should be 82 cm of water (1.1 p sig).

This final value is less than the maximum of 2 p sig. (115 kNm^{-2}) quoted by Perry (1973), but greater than the previously calculated "expansion" and "minimum head of water" criteria. The

adoption of this value as the minimum allowable pressure drop should ensure efficient gas distribution through the phases above the plate. (This was later verified during experimental work. Plates designed to this minimum pressure drop had all their orifices functioning equally and allowed no backflow of liquid through the plate.)

2.3.3. Initial Pressure Drop Estimations

In order to reduce the experimental work a computer program was written to estimate pressure drops across grids with various numbers of orifices of varying diameters. The aim of writing the program was to highlight areas for experimental work. A program flow sheet is shown in Fig. 2.2 and a detailed listing is given in Appendix 2.2.

A Venturi formula (eq. 2.19) was used to estimate plate pressure drops.

$$Q = C_D A_o \left[\frac{2gH}{(1-\beta^4)} \right]^{\frac{1}{2}} \qquad 2.19$$

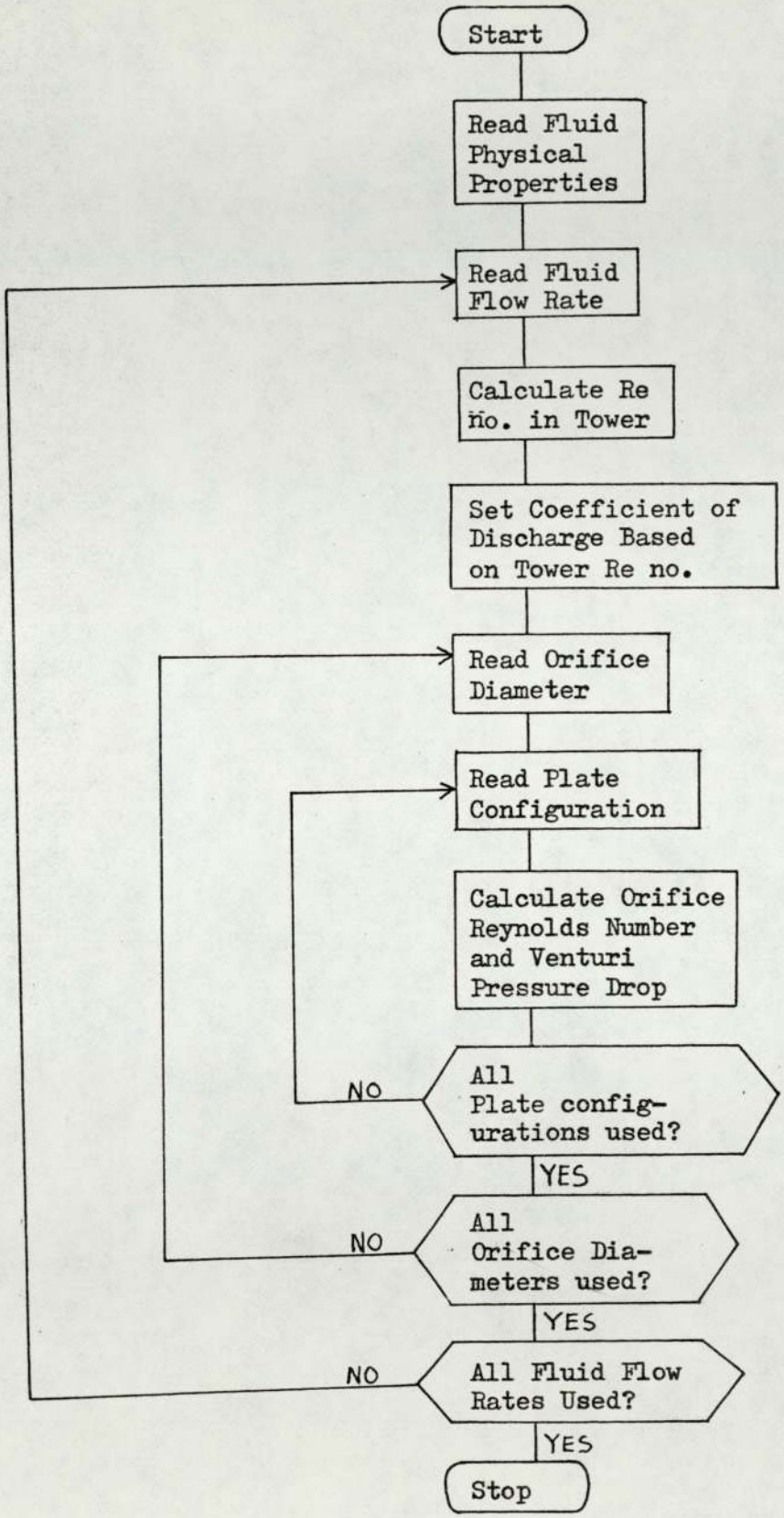
In the calculations carried out in the program β was small and hence rearranging eq. 2.19 gives;

$$H = \frac{1}{2g} \left[\frac{Q}{C_D A_o} \right]^2 \qquad 2.20$$

$$= \frac{V_o^2}{2g.C_D} \qquad 2.21.$$

The coefficient of discharge, C_D, was initially estimated on the basis of the Reynolds number in the tube preceding the grid. This method has been used previously by Kunii and Levenspiel (1969).

Fig. 2.2 Flow Sheet for Calculating Distributer Plate Pressure Drops



2.3.4 Results

(1) Medium

The minimum liquor residence time in the fermenter was anticipated to be 2 hours: this implies a medium flow-rate of 5.6 l/h. With this liquor flow-rate a theoretical pressure drop of 1650 N/m^2 gauge was calculated for a thirteen orifice grid (orifies 1/64 in. (3.968×10^{-4} m) diameter). However, the minimum allowable pressure drop required to produce efficient fluid distribution across the plate was calculated to be 82 cm of water or 8040N/m^2 gauge (see Section 2.3.2). From this result there appears to be no advantage to be gained by designing a distributor plate solely for wort distribution.

Air

With an air flow-rate of 10 l/min ($0.167 \times 10^{-4} m^3/s$) through a thirteen orifice grid, (orifices 1/32 in (7.937×10^{-4} m) diameter), the calculated pressure drop was 690N/m^2 gauge. This flow-rate of 10 l/min was probably an over-estimation of the maximum air flow. Therefore only orifices of 1/64 in diameter were considered. Table 2.2 shows theoretical pressure drops for such plates with differing orifice numbers. At low gas flow-rates the pressure drop is lower than the previously calculated minimum for efficient gas distribution with all the plate configurations used.

2.4 Experimental Work

After the theoretical assessment of efficient fluid distribution in the fermenter a brief experimental programme was undertaken. The aim of these experiments was to measure pressure

TABLE 2.2 Theoretical Distributor Plate Pressure Drop

AIR FLOW-RATE	PRESSURE DROP (CM OF WATER)		
	No. of Orifices		
L/Min	13	29	49
1	2.64	0.53	0.18
2	7.33	1.47	0.51
3	14.1	2.82	1.00
4	25.0	5.02	1.76
5	33.7	6.77	2.37
6	48.5	9.74	3.41
7	66.0	13.3	4.64
8	86.0	17.3	6.06
9	109	21.9	7.68
10	135	27.1	9.48
12	194	39.0	13.7
14	264	53.0	18.6
16	345	69.3	24.3
18	436	87.7	30.7
20	539	108	37.9

All orifices 1/64 in $(3.968 \times 10^{-4}$ m) diameter.

drops across perforated plates with known numbers of orifices and fixed orifice diameter: and then to compare experimental results with theoretical work.

The three distributor plates shown in Fig. 2.3 were manufactured by the author. Pressure drops across these plates were measured with a U-tube manometer; the manometric fluid being water. The experimental results are shown in Table 2.3.

2.5 Discussion

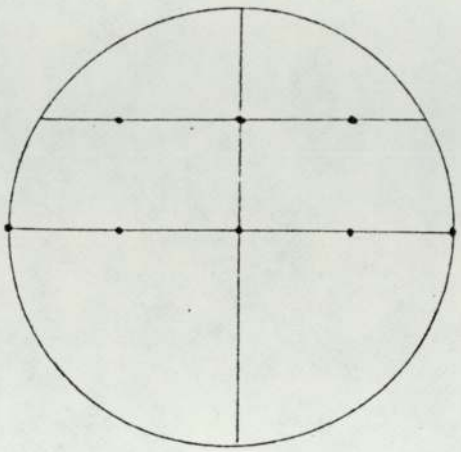
The theoretical and experimental results, Tables 2.2 and 2.3 respectively, were close to each other in a number of cases. The two sets of results are compared on Graphs 2.1-2.3. With the thirteen and twenty-nine orifice plates the results are in relatively close accordance over the whole range of experimental pressure drops: however, for the forty-nine orifice plate the theoretical and experimental results diverge with increasing flow-rate.

It was therefore concluded that the Venturi equation (eq. 2.1) was adequate for estimating grid pressure drops. However, the relatively crude method of estimating the coefficient of discharge (see Section 2.3.3) used in the computer program (Appendix 2.2) was discarded and coefficients of discharge re-calculated from the re-arranged Venturi equation;

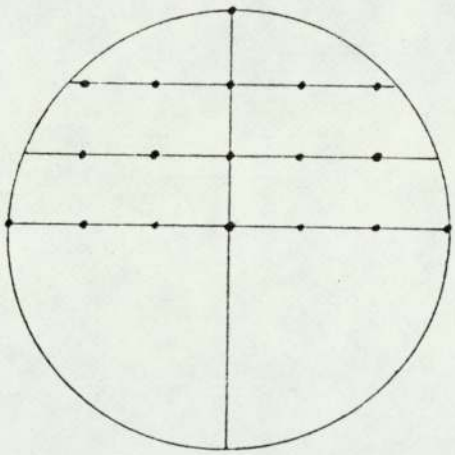
$$C_D = \frac{v_o}{Y} \left(\frac{\rho}{2gH} \right)^{\frac{1}{2}} \qquad 2.22.$$

As the plate free area, A_f, is small the term $\sqrt{1 - (A_f/A)^2}$ does not appear in eq. 2.22. The expansion factor, Y, is defined by 2.13: and as $r \longrightarrow 1$ then $Y \longrightarrow 1$;

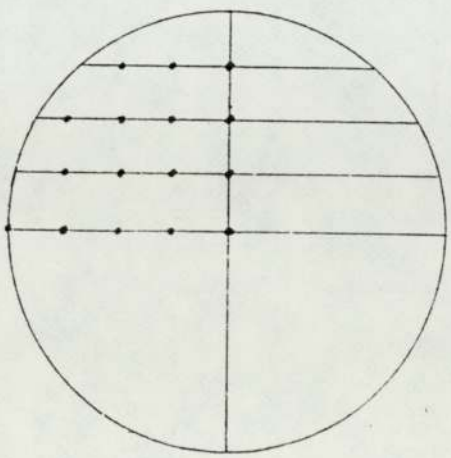
FIG. 2.3 DISTRIBUTOR PLATES USED IN THE EXPERIMENTAL PROGRAMME



13 ORIFICES,
18 MM PITCH



29 ORIFICES,
12 MM PITCH

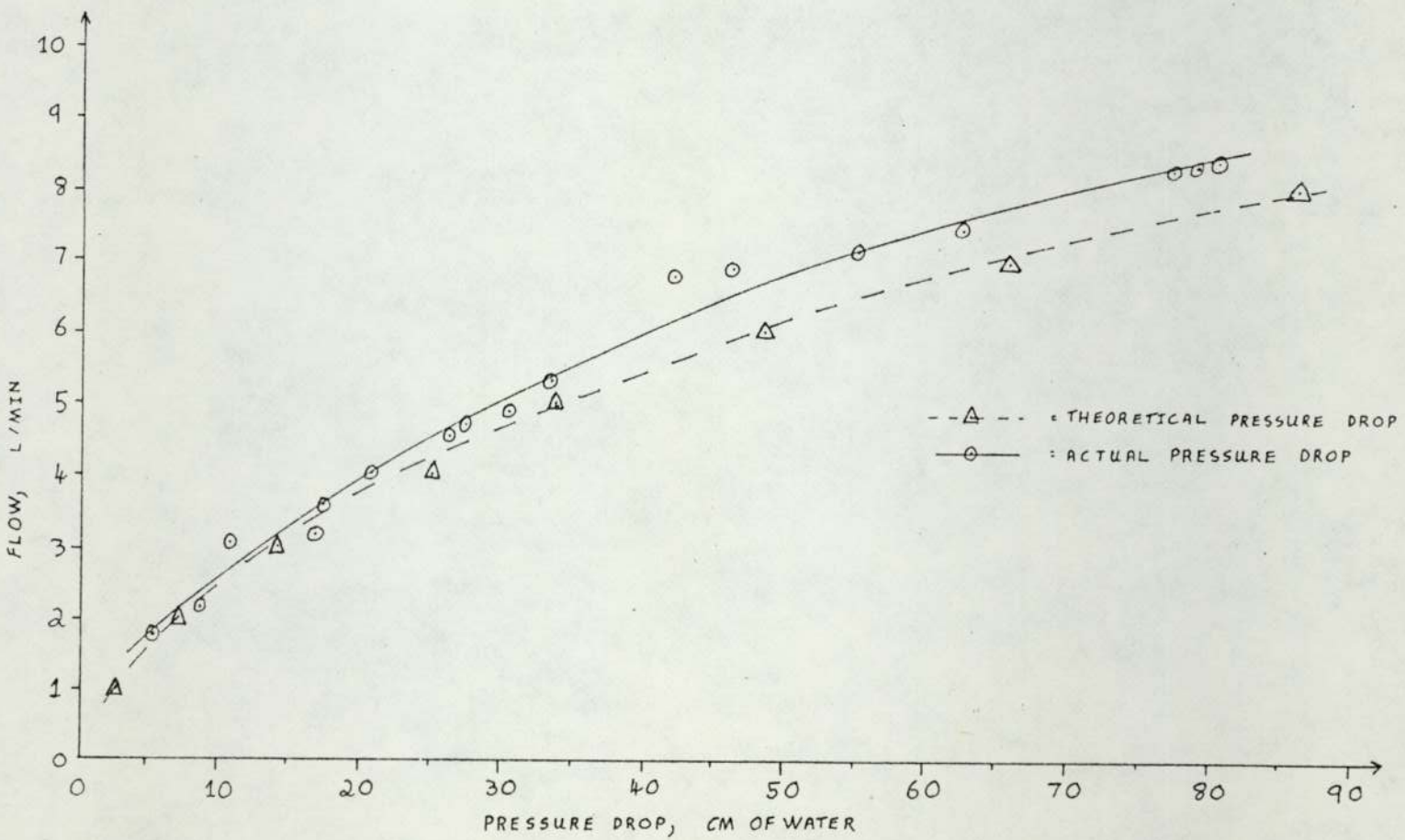


49 ORIFICES,
9 MM PITCH

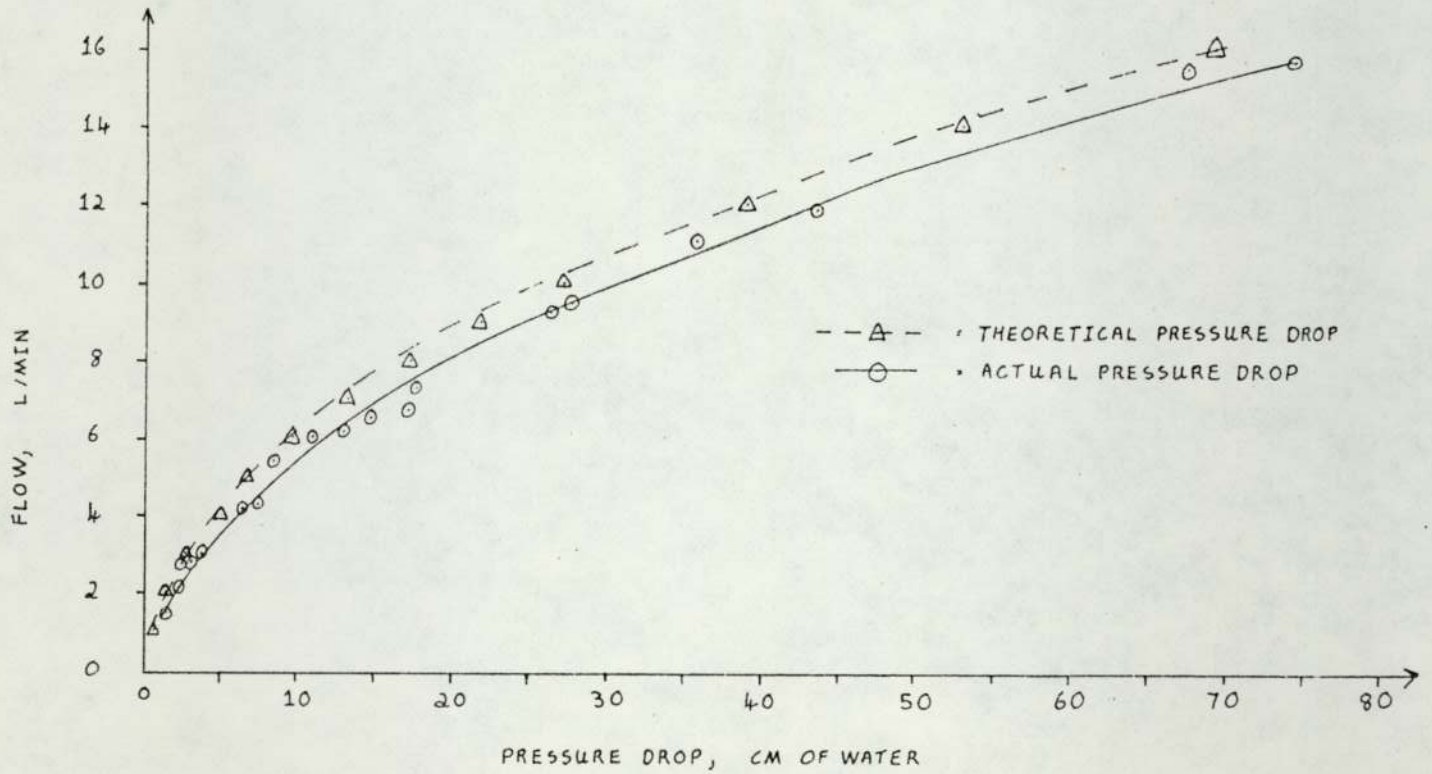
TABLE 2.3 Experimental Distributor Plate Pressure Drops

NUMBER OF ORIFICES					
13		29		49	
Flow	Pressure Drop	Flow	Pressure Drop	Flow	Pressure Drop
L/min	cm of water	l/min	cm of water	l/min	cm of water
1.80	5.3	1.55	1.5	1.95	0.9
2.20	8.5	2.15	2.1	2.20	1.2
3.10	10.8	2.80	2.5	3.10	2.0
3.20	16.7	2.90	3.2	3.35	2.4
3.60	17.4	3.00	3.8	4.10	2.8
4.00	20.8	4.20	6.6	4.55	3.5
4.60	26.2	4.30	7.2	4.60	4.0
4.70	27.2	5.40	8.5	4.80	4.7
4.80	30.4	6.00	10.8	6.75	7.7
5.25	33.2	6.20	12.9	7.25	8.1
6.80	42.0	6.50	14.4	7.35	8.2
6.90	46.2	6.75	17.1	7.40	8.5
7.15	55.0	7.30	17.6	7.75	9.4
7.45	62.2	9.30	26.4	9.45	13.6
8.30	77.4	9.45	27.6	10.00	15.4
8.35	78.8	9.50	27.7	11.10	17.7
8.40	80.2	9.60	28.0	11.20	18.8
-	-	11.10	35.7	13.40	26.9
-	-	11.90	43.5	15.00	33.5
-	-	15.55	67.4	18.20	48.4
-	-	15.65	74.5	19.50	55.6

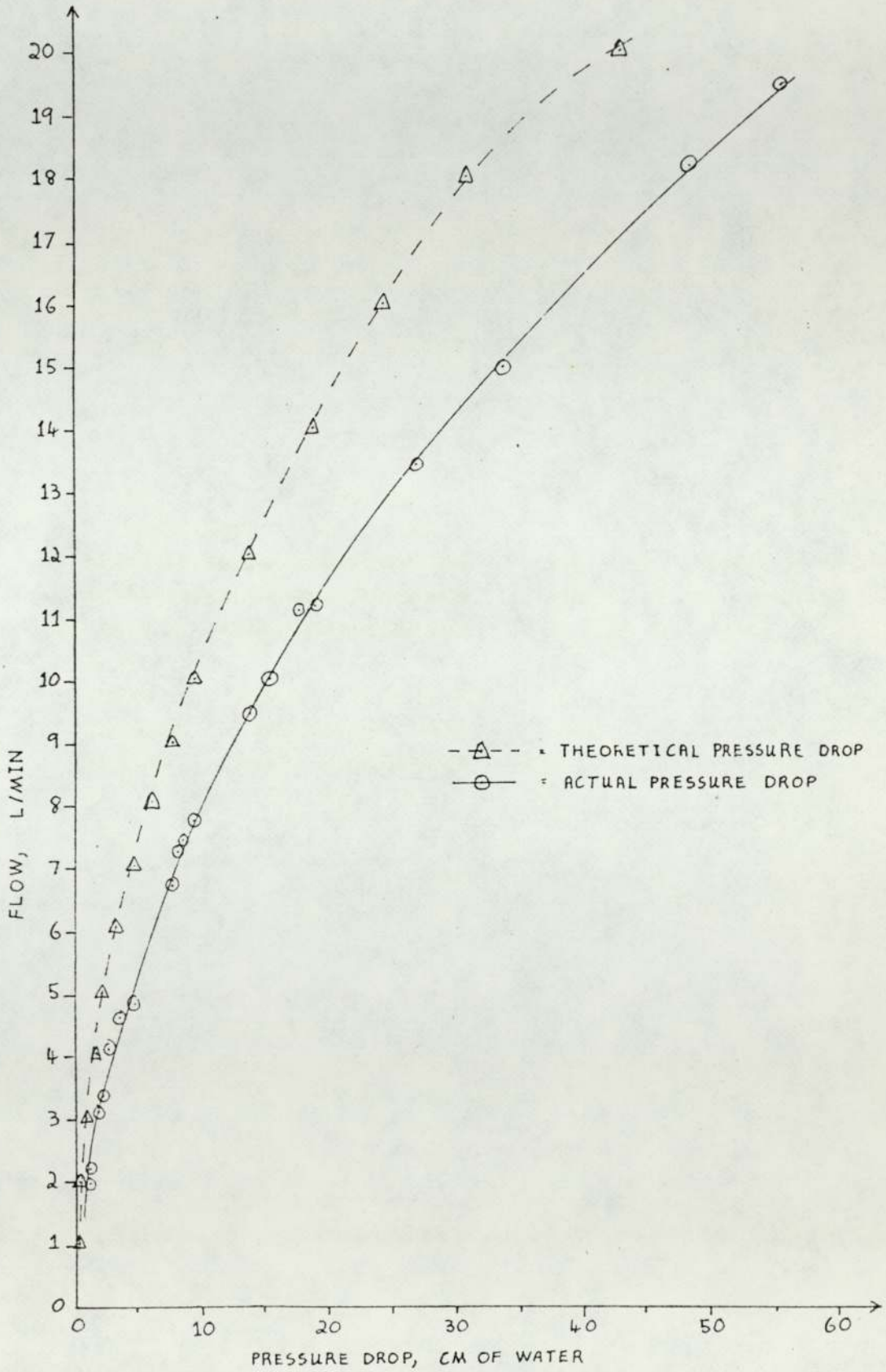
GRAPH 2.1 AIR FLOW-RATE VERSUS PRESSURE DROP,
13 ORIFICE PLATE



GRAPH 2.2 AIR FLOW-RATE VERSUS PRESSURE DROP, 29 ORIFICE PLATE



GRAPH 2.3 AIR FLOW-RATE VERSUS PRESSURE DROP, 49 ORIFICE PLATE



where $r = \frac{\text{pressure above the grid}}{\text{pressure below the grid}} = \frac{P_2}{P_1}$ 2.23.

The minimum value of r encountered here was 0.93, which, from Perry (1973) results in Y = 0.98. Hence as Y was close to 1 it was neglected from eq. 2.22. The coefficients of discharge calculated from the experimental pressure drops are given in Table 2.4 together with their corresponding orifice Reynolds' numbers.

The coefficients of discharge calculated from experimental results were compared with values of C_D predicted by the correlations of other authors. This work was complicated by the fact that the experimental parameters used by the various authors did not correlate with one another and were not in the region of parameters used by the author (see Table 2.5): hence, extrapolations were made where necessary.

Pressure drop results calculated using the correlation developed by Rehakova and Kastanek (1975) are shown in Table 2.6. It was found that as the gas flow-rate increased the calculated pressure drop decreased for a particular grid: hence, this correlation was discarded.

The equation of McAllister et al. (1958) can be written,

$$\Delta P = \frac{\rho v_o^2}{2} \cdot K_1 \cdot (1.5 + 16f) \qquad 2.24$$

when $T/D_o = 4$.

Values of the "constant" K_1 are given in Table 2.7. For the experimental work considered here values of K_1 for a particular grid appear dependent on the fluid orifice velocity to some extent:

TABLE 2.4 Coefficients of Discharge and Orifice Reynolds' Numbers for Various Grids.

NUMBER OF ORIFICES								
13			29			49		
Flow	Coeff. of Discharge	Orifice Re. No.	Flow	Coeff. of Discharge	Orifice Re. No.	Flow	Coeff. of Discharge	Orifice Re. No.
l/min.			L/Min.			L/Min.		
1.80	0.635	501	1.55	0.461	194	1.95	0.443	144
2.20	0.613	613	2.15	0.540	268	2.20	0.433	163
3.10	0.766	863	2.80	0.645	350	3.10	0.472	229
3.20	0.636	891	2.90	0.590	362	3.35	0.466	248
3.60	0.701	1000	3.00	0.561	375	4.10	0.528	303
4.00	0.713	1115	4.20	0.595	525	4.55	0.524	336
4.60	0.730	1280	4.30	0.584	537	4.60	0.496	340
4.70	0.732	1310	5.40	0.675	675	4.80	0.477	355
4.80	0.707	1340	6.00	0.665	750	6.75	0.524	499
5.25	0.740	1460	6.20	0.629	775	7.25	0.549	536
6.80	0.853	1895	6.50	0.624	811	7.35	0.553	543
6.90	0.825	1920	6.75	0.595	843	7.40	0.547	547
7.15	0.783	1990	7.30	0.634	911	7.75	0.545	573
7.45	0.768	2075	9.30	0.659	1160	9.45	0.552	698
8.30	0.767	2310	9.45	0.655	1180	10.00	0.549	739
8.35	0.764	2325	9.50	0.657	1185	11.10	0.569	820
8.40	0.762	2340	9.60	0.661	1200	11.20	0.557	828
			11.10	0.67	1385	13.40	0.557	990
			11.90	0.657	1485	15.00	0.559	1110
			15.55	0.690	1940	18.20	0.564	1345
			15.65	0.660	1955	19.50	0.564	1440

TABLE 2.5 Experimental Parameters Used by Various Authors in Grid Pressure Drop Measurements

Author(s)	Orifice Diameter (Do) mm	P/Do	Plate Thickness (T) mm	Plate Free Area (A_f) %	Orifice Reynolds' Number	T/Do	Pitch (P)	No. of Holes (N)
Kolodzie and Van Winkle (1957) and Smith and Van Winkle (1958)	1.59 - 6.35	2.0 - 5.0	2.08 - 14.93	2.73 -14.93	400 -20000	0.324 -4.00	Triangular	-
McAllister et al. (1958)	2.381 -12.70	2.25 -3.60	0.73 -12.7	3.92 -8.65	1280 -27800	0.093 -5.33	-	77 -2180
Yoshitome (1963)	0.5 -4.5	5.22 -54.5	1.0 -1.6	0.04 -2.88	100 -20000	0.22 -2.0	Triangular and Square	19 - 217
Rehakova and Kastanek (1975)	0.7 -1.0	6.6 -15.71	2.0 -14.4	0.1 -1.0	4000 -11000	2.7 -16	Triangular	4 - 19
Author	0.39	22.68 -45.35	1.59	0.035 -0.13	145 -2230	4.0	Square	13 - 49

TABLE 2.6 Pressure Drops Calculated from the Correlation of Rehakova and Kastanek (1975)

NUMBER OF ORIFICES					
13			29		
Flow	Orifice Velocity	Pressure Drop	Flow	Orifice Velocity	Pressure Drop
L/min.	M/S	N/M^2	L/min	M/S	N/M^2
2.2	22.8	493×10^6	2.15	10.0	1.30×10^7
4.7	48.7	2.27×10^6	6.20	28.8	3.54×10^6
7.8	80.0	8.18×10^6	15.55	72.2	9.03×10^5

TABLE 2.7 Values of K_1, (McAllister et al. (1958)), Calculated from Experimental Results

NUMBER OF ORIFICES								
13			29			49		
Flow	Orifice Velocity	K_1	Flow	Orifice Velocity	K_1	Flow	Orifice Velocity	K_1
L/min.	M/S		L/min.	M/S		L/min.	M/S	
1.80	18.7	1.232	2.15	10.0	1.395	1.95	5.36	1.555
3.20	33.1	1.382	4.20	19.5	1.418	4.10	11.3	1.529
3.60	37.3	1.158	6.75	31.4	1.568	6.75	18.6	1.875
4.70	48.7	1.092	9.60	44.6	1.336	11.10	30.5	1.706
7.45	77.2	1.042	15.55	72.2	1.287	19.50	53.6	1.876

TABLE 2.8 Values of the coefficient of Discharge using the correlation of Kolodzie and van Winkle (1957)

NUMBER OF ORIFICES								
13			29			49		
Re_o	C_D	K	Re_o	C_D	K	Re_o	C_D	K
501	0.526	0.770	375	0.509	0.715	355	0.498	0.680
1000	0.479	0.890	775	0.608	0.855	536	0.563	0.770
1280	0.618	0.905	1160	0.640	0.900	820	0.626	0.855
2075	0.659	0.960	1485	0.654	0.920	1110	0.651	0.890
2340	0.659	0.965	1955	0.679	0.955	1440	0.666	0.910

in one case K_1 decreases with increasing V_o while in another the opposite is the case. Hence, this latter correlation also has to be put aside.

For plates with $D_o/T < 0.5$ the correlation derived by Yoshitome (1963), (eq. 2.8), results in a constant coefficient of discharge for a particular distributor plate,

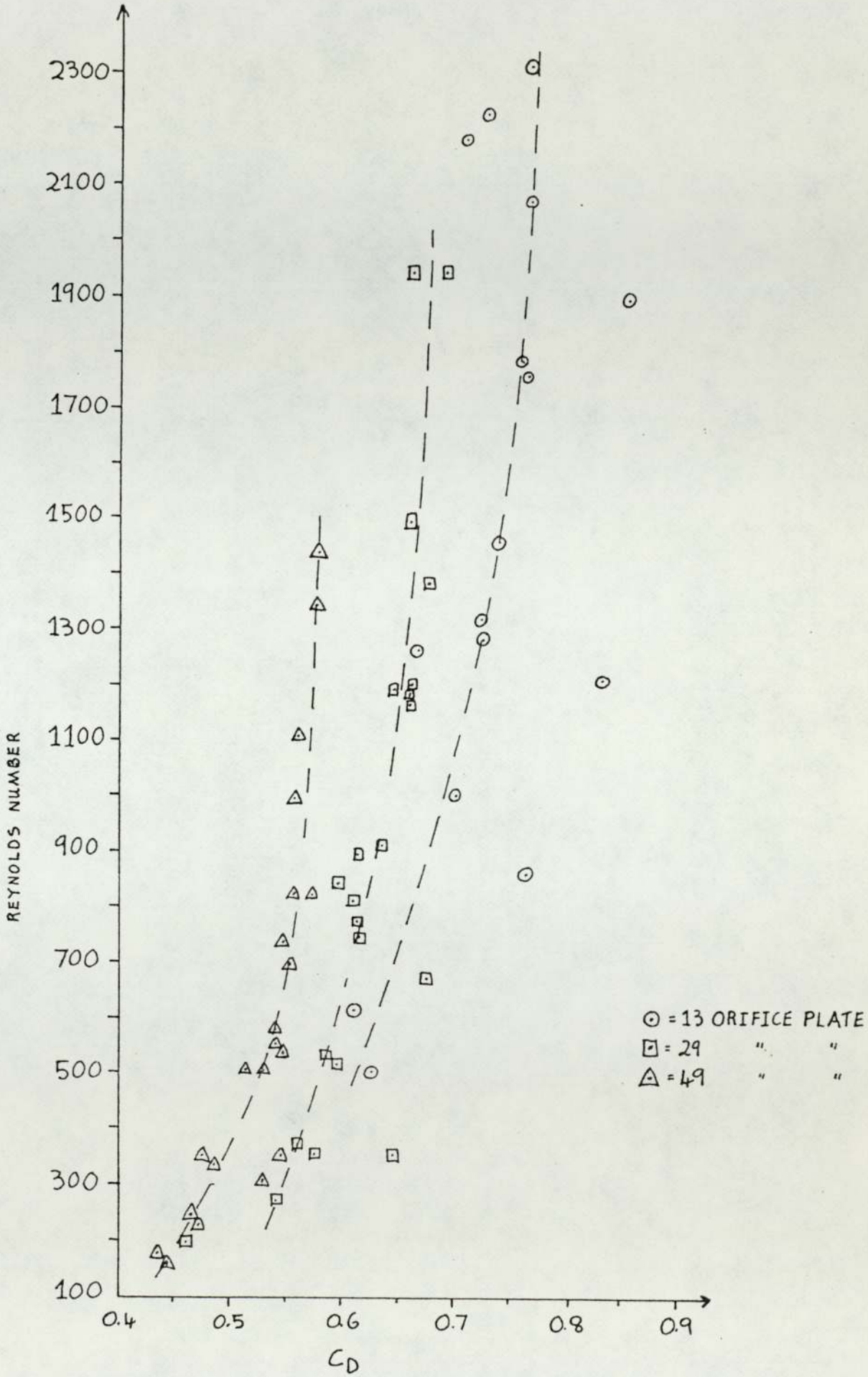
$$C_D \left(\frac{P}{Do} \right)^{0.05} = 0.97 \qquad 2.25.$$

This results in values of C_D of 0.802, 0.818, and 0.830 for the 13, 29, and 49 orifice plates shown in Fig. 2.3. Experimentally deduced values of C_D were variable and much lower than 0.8 (Table 2.4): hence, this correlation must be rejected.

Extrapolating the correlation deduced by Kolodzie and van Winkle (1957) leads to the coefficients of discharge and values of K shown in Table 2.8. In agreement with Rehakova and Kastanek (1975) the "constant" K is seen to be dependent on the orifice Reynolds number. However, the values of the coefficient of discharge vary from experimental values by 3% - 16%, depending on the particular grid considered: hence, in some instances this correlation is satisfactory.

Though the latter considered correlation proved the most suitable of those discussed for estimating grid coefficients of discharge it was not completely satisfactory. Hence a plot (Graph 2.4) was made of coefficient of discharge (from Table 2.4) versus orifice Reynolds number. From this plot it appears as if a family of curves could be built up for plates with differing numbers of orifices. Also the curves are asymtotic, leading to

GRAPH 2.4 ORIFICE REYNOLDS NUMBER VERSUS COEFFICIENT OF DISCHARGE



the conclusion that C_D becomes constant once a certain orifice Reynolds number has been attained. However, the relationship between the coefficient of discharge, number of orifices, and orifice Reynolds number appears complex. Due to this fact, and the necessity of the experimental work being limited, no general correlation relating C_D, Re_o, and N has been put forward.

CHAPTER 3

BATCH FERMENTATIONS

3.1 INTRODUCTION

The fields of microbiology and chemical engineering have come together in recent years, resulting in the relatively new science of biochemical engineering. Chemical engineering principles can be applied with advantage to microbiological processes. In particular the study of fermentation kinetics promises more efficient exploitation of biochemical reaction systems by aiding rational design and scale-up of continuous processes.

The study of batch fermentations is an important initial step in the study of continuous processes. Thus a number of batch beer fermentations were effected with the aim of providing kinetic data.

However, the physical processes which accompany the fermentation must also be considered, as these may have a significant effect on the biochemical rate processes involved in fermentations.

3.2 Traditional Beer Production

A brief précis of the traditional methods of beer production will be given to serve as a background for the"non-brewer". For more detailed information the reader is referred to the two excellent texts by Hough et al. (1971) and Ricketts (1971).

The production of beer traditionally begins with barley. The barley undergoes a process known as malting. Malting involves steeping the barley grain in water until it germinates and then

drying and curing the grain.

The malted barley is then mashed with hot water at about 65°C. The mashing process produces a sweet-wort. This liquor, as the name suggests, contains a mixture of fermentable and non-fermentable carbohydrates, or sugars.

The wort is then boiled with hops. The boiling process, among other things, extracts bitter materials from the hops. The final liquor is then filtered, to remove the hops, and cooled. The liquor is then pitched with a strain of brewers' yeast and fermentation takes place. A typical wort carbohydrate composition is shown in Table 3.1.

However, besides carbohydrates, the yeast requires a host of minor constituents to enable a successful fermentation to take place. Nitrogen, from inorganic salts and amino-acids, is necessary for yeast growth. A detailed breakdown of the amino-acid and protein content of wort is provided by Hough and Button (1972). Phosphorous, sulphur, potassium, magnesium, calcium, copper, iron, and zinc in trace proportions have also been found essential for successful fermentation. For further information on this topic the reader is referred to the excellent review by Suomalainen and Oura (1971).

3.3 The Batch Fermentation

The course of a typical top fermentation is shown in Fig. 3.1. Each aspect of the fermentation is described comprehensively in the following sections.

TABLE 3.1

OLIGOSACCHARIDES OF WORTS AND BEERS. (g/100 ml and expressed in terms of glucose). (HOUGH AND BUTTON (1972)).

Oligosaccharides	Ale Wort S.G.=1.037	Ale S.G.=1.015	Lager S.G.=1.004
Major Fermentables			
Fructose	Trace	0	0
Glucose	0.48	Trace	0.02
Sucrose	3.44	0	0
Maltose	3.55	0.26	0.07
Maltotriose	1.42	0.12	0.17
	8.89	0.38	0.26
Non Fermentables			
Maltotetraose	0.55	0.53	0.30
Higher Oligosaccharides	1.50	1.39	0.70
	2.05	1.92	1.00

% w/w of the major fermentables assimilated by the yeast in ale production = 93.55

3.3.1 Yeast Pitching and Reproduction Rates

Hough and Button (1972) have stated that a typical yeast pitching rate is 0.4 g of dry yeast per litre of wort: Hudson (1967) and Griffin (1970c) give the equivalent figure of 1 lb. of moist yeast per barrel (2.5 g moist yeast/l). Griffin (1970c) found that increasing the pitching rate from 2 g/l to 10 g/l had little effect on the rate of fermentation, whereas Hudson (1967) found an 84 hour fermentation could be reduced by 40 hours by increasing the pitching rate fourfold.

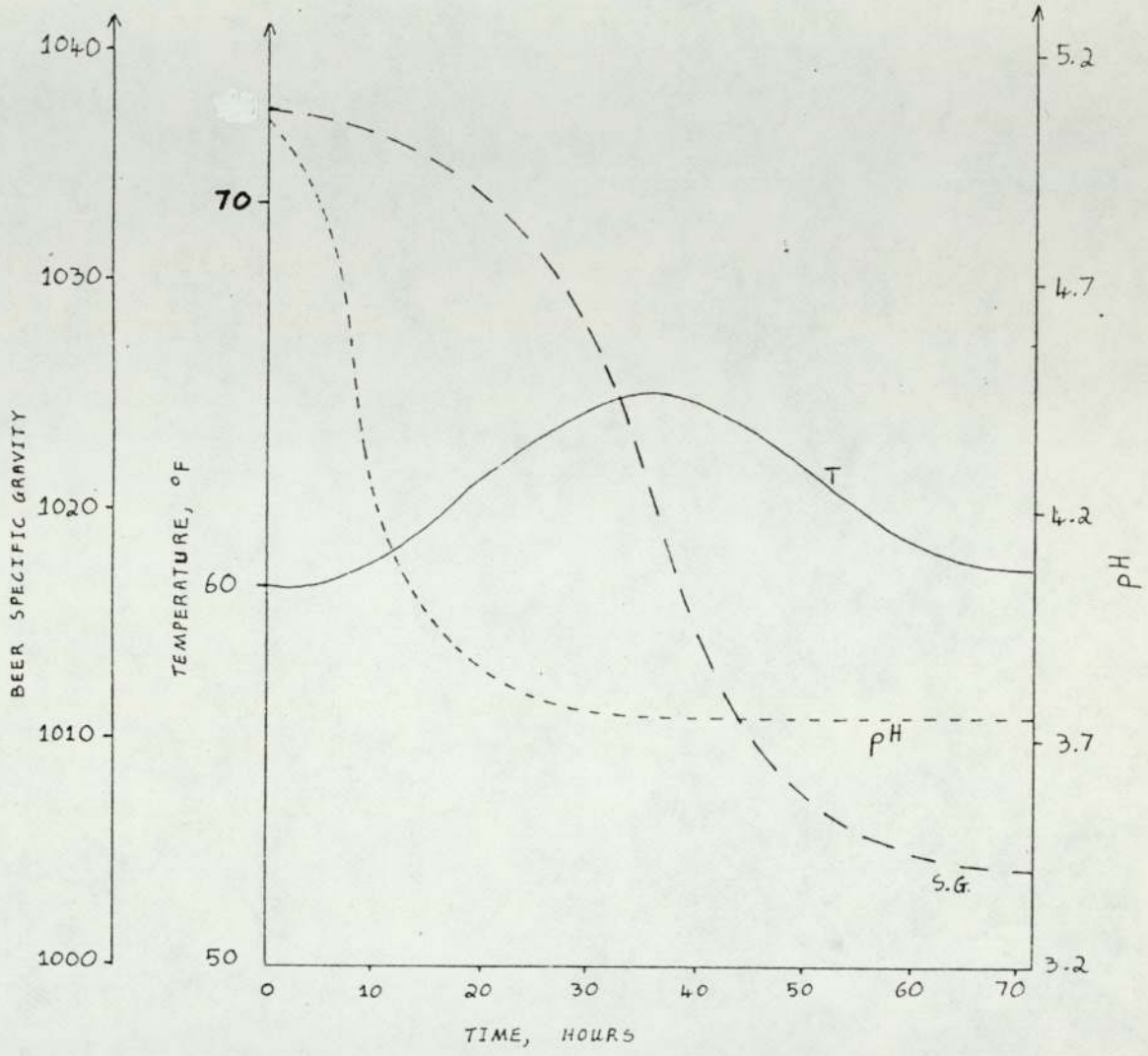
Ricketts (1971) has suggested that a reasonable rate of yeast reproduction in a batch beer fermentation is 3-7 lb. of moist yeast per barrel (8.3-19.4 g/l). Portno (1968a) found yeast growth in a batch fermentation to be 3.10 g of dry yeast per litre.

Brown and Kirsop (1972) experimented with a stirred batch fermentation system. A yeast pitching rate of 0.25 g of dry yeast per litre was employed. Maximum concentrations of up to 7 g of dry yeast per litre were observed at one stage in the fermentation. However, the final yeast concentration was about 4 g/l. This decrease in yeast mass concentration towards the end of the fermentation was attributed to the yeast metabolising internal stored carbohydrates, chiefly for cell maintenance.

3.3.2. Cellular Moisture Content

It is difficult to calculate the precise moisture content of individual yeast cells. In measuring the moisture content of, say, filter cakes of yeast, it is difficult to discriminate between the liquor trapped in the cake pores and the actual

FIG. 3.1 THE COURSE OF A TYPICAL TOP FERMENTATION (HOUGH ET AL. (1971)).



cell moisture content.

James (1973) has computed the moisture content of filtered yeast cakes. For the ratio, filtered yeast wet weight/yeast dry weight, James (1973) found a value of 4.48.

This is in agreement with Thorne (1954), who gave a value of 4.5 for the same ratio. These values give cake moisture contents of 77.7%w/w and 77.8%w/w respectively.

James (1973) also calculated the moisture content of centrifuged yeast cakes: these are given in Table 3.2.

TABLE 3.2

CENTRIFUGED WET WEIGHT TO DRY WEIGHT RATIOS FOR VARIOUS STRAINS OF YEAST. (James (1973))

Culture Collection No.	Yeast	Centrifuged Wet Wt. / Dry Wt.
CFCC 8	Saccharomyces cerevisiae	4.5
CFCC 34	" "	5.9
CFCC 3	" "	5.5
CFCC 83	Saccharomyces carlsbergensis	5.4

Taking the mean value for the centrifuged wet weight to dry weight ratio the moisture content of centrifuged yeast cakes is 81%w/w.

Taking an average moisture content of approximately 80%w/w for wet yeast cakes, Hough and Button's (1972) dry yeast inoculum of 0.4 g/l (section 3.3.1) is equivalent to 2 g of wet yeast per litre. Portno's (1968a) value of 3.10 g of dry yeast per litre for yeast growth is equivalent to a reproduction rate of 15.5 g of wet yeast per litre. This is in agreement with Ricketts' (1971) results. However, Brown and Kirsop's (1972) data fall slightly outside the range given by Ricketts (1971).

3.3.3 Yeast Growth

The life of each individual yeast cell is finite, and so growth of yeast is essential during fermentation. However, yeast is a product of low commercial value to the brewer. Yeast growth also occurs at the expense of alcohol production. Hence it is advantageous to restrict yeast reproduction to a low level in alcohol production.

The growth of micro-organisms is well documented and a typical unicellular growth curve is shown in Fig. 3.2. From this curve a number of phases in the life cycle of the organism are evident. Note that letters on the plot correspond with those for each sub-section below.

a) The Lag Phase

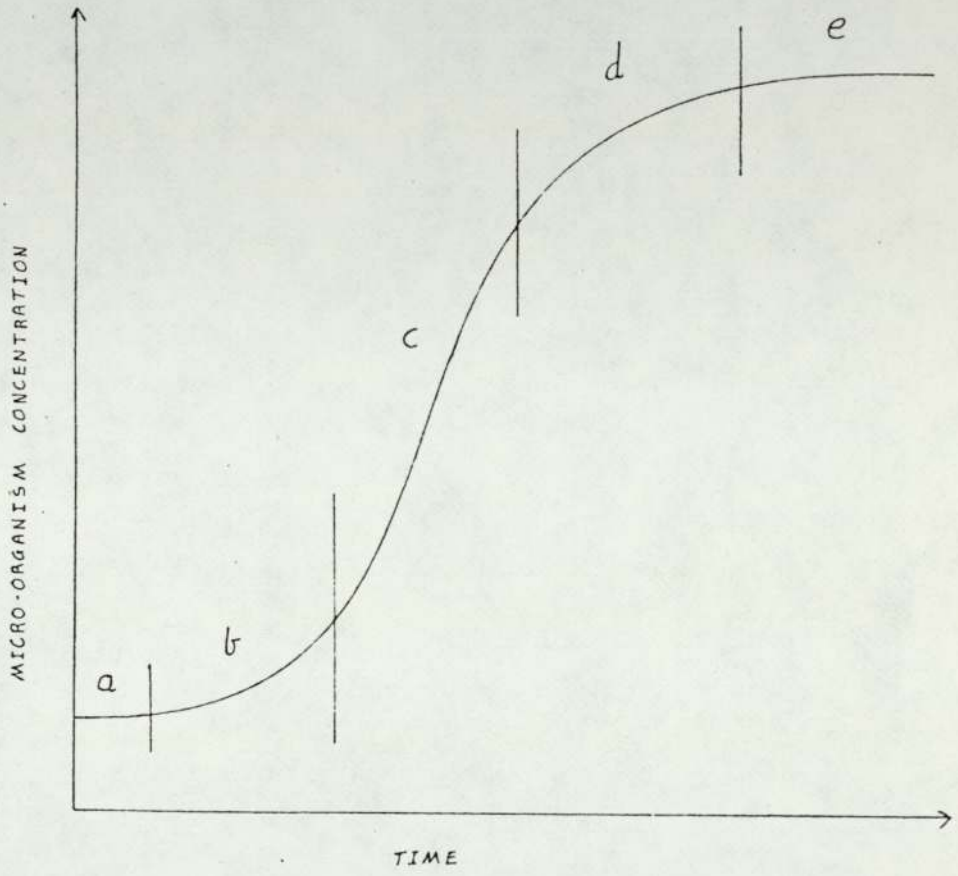
When brewers' wort is inoculated with a living yeast population there is little detectable activity on the part of the yeast. The period that elapses between inoculation and detection of visible growth is known as the lag phase. The period of cell adaption to a new environment is dependent on a range of physical and chemical factors. For further details the reader is referred to Rhodes and Fletcher (1966), Dean and Hinshelwood (1966), and Stanier et al. (1971).

The cells are metabolizing actively in the lag phase and may be regarded as preparing for growth (Hough et al. (1971)). Indeed in a typical beer fermentation the dissolved oxygen falls rapidly during the lag phase (David and Kirsop (1973)).

b) The Acceleration Phase

This is the period during which the growth rate of the

FIG. 3.2 TYPICAL GROWTH CURVE OF A MICRO-ORGANISM



organism increases to a constant value.

c) The Exponential Phase

The exponential phase of growth begins when the growth-rate reaches a constant value per unit mass of cells. The achievement of a constant growth-rate requires all the various nutritional elements in the medium to be in excess of the basic cell requirements. The rate of yeast growth is then proportional to the amount of yeast present, and the cellular matter will double at constant intervals.

With alcohol production there is, out of necessity, a lack of dissolved oxygen. Hence, it could be argued that exponential growth cannot take place due to the lack of this nutrient. However, from experimental results (Appendix 3.1) there does appear to be such a period of growth. Therefore the concept of an "anaerobic maximum specific growth rate" does have meaning.

d) The Retardation Phase

Depletion of nutrients in the medium or the accumulation of toxic metabolic products will eventually limit the exponential growth phase. A decline in the growth-rate of the organism will then occur. In beer production the concentration of ethanol nears its peak as nutrients become depleted: these changes in medium composition are known to have an adverse effect on the yeasts' metabolism.

e) The Stationary Phase

Eventually the culture enters the stationary phase. This

is characterised by a zero growth-rate. However, if cell lysis does not occur there may still be a slight increase in the cell mass in the broth. This is the final stage in a beer fermentation: flocs of yeast will have sedimented out or been froth-flotated and skimmed off from the top of the fermenter.

If the fermentation is allowed to proceed there is an increase in the death-rate of the organism and an exponential death phase may occur (Stanier et al. (1971)).

3.3.4 Carbohydrate Utilisation

The major fermentable sugars present in brewers' wort are shown in Table 3.1. These sugars are nearly all assimilated by the cell by two mechanisms: facilitated diffusion and active transport. Fidgett (1975) and Hough et al. (1971) have discussed these mechanisms in detail. Briefly, facilitated diffusion can be regarded as a catalysed diffusion process. In active transport the yeast concentrates nutrients in the cell against a nutrient concentration gradient.

Philips (1955) found that the sugars present in wort were used sequentially by the yeast. The order of utilisation was found to be: - sucrose, monosaccharides (glucose and fructose), maltose, and maltotriose. A number of workers (Bavisotto et al. (1958), Barton-Wright (1953), and Harris et al. (1951)) noted that the sucrose present in wort was rapidly assimilated by the yeast. This is due to the rapid inversion of sucrose to fructose and glucose, by the enzyme invertase, present on the yeast cell wall. Thus sucrose is actually assimilated into the cell as fructose and glucose.

The order of carbohydrate uptake is due to the suppression of maltose utilisation by glucose, and the suppression of maltotriose utilisation by maltose (Hough et al. (1971)). A substantial number of other workers have recorded that glucose suppresses maltose uptake: these include Monod and Cohen (1952), Sutton and Lampen (1962), Macquillan and Halvorsen (1962), Millin (1963), Harris and Millin (1963), Halvorsen et al. (1966), Harte and Webb (1967), and Griffin (1969, 1970a, 1970b). This effect may in fact be detrimental in some continuous fermentations. Portno (1968b) found that there was frequently a significant degree of fluctuation in the ability of yeast cells to ferment maltose in a single C.S.T.R. system used for beer production. This instability, in part, was attributed to glucose causing maltose repression when introduced into a medium of relatively low maltose concentration. When the single C.S.T.R. was replaced by a two vessel system both Portno (1968b) and Millin (1966) found an improvement in the system stability.

Griffin (1970a, 1970b) found that the concentration level of one carbohydrate which suppresses the uptake of another to be dependent on yeast strain. Griffin (1970a) also found some overlapping in utilisation of the different carbohydrates by certain yeasts. This is supported by earlier work by Amaha (1966). He found the fermentative activity (measured as rate of carbon dioxide evolution) of yeast towards mixtures of glucose, maltose, and maltotriose was 80%-90% of that towards wort. This fact evidently shows that the actual rate of fermentation is an additive rate of fermentation of the individual fermentable sugars in the wort at each stage of the fermentation.

Millin (1963) viewed the order of carbohydrate uptake as a stepwise adaptation by the organism to the changing environment as each carbon source is removed. This on-off concept of sugar utilisation was used by Fidgett (1975) in the development of a model for batch beer fermentation. This model successfully simulated sugar utilisation during a fermentation and has been published by Fidgett and Smith (1975).

As the sugars are utilised by the yeast, and metabolites (mainly ethanol) are excreted, the present gravity of the medium falls (Fig. 3.1). From graphs of reducing sugar concentration versus time (Appendix 3.1) it is seen that these are analogous to the specific gravity curve.

The concentration of reducing sugar falls slowly at first. This is due to the low amount of yeast present and the fact that the yeast is in its lag phase. The sugar concentration then decreases rapidly as the relatively "easy" sugars are assimilated by the yeast. The rate of sugar uptake then decreases, due mainly to two reasons:

(i) from the paper of Bavisotto et al. (1958) it can be seen that the bulk of the remaining fermentable carbohydrate is maltotriose: the maltotriose can be regarded as a difficult sugar for the yeast to ingest;

(ii) at this stage in the fermentation the cells' metabolism is being inhibited by alcohol in the medium. In 1967 Holzberg et al. found, while experimenting with grape juice fermentations, that alcohol completely inhibited the yeasts' metabolism at

alcohol levels greater than 6.85% w/v.

3.3.5 pH

The initial pH of hopped wort for ale production is usually about 5.0. As fermentation takes place the pH drops rapidly (Fig. 3.1). A third of the way through the fermentation the pH of the fermenting medium falls to about 4.0 and then remains constant.

Thorne (1954) and Visuri and Kirsop (1970) have studied the effect of pH on the rate of fermentation. Thorne (1954) found that an increase in pH from 3.2 to 4.4 increased the fermentation velocity by 13%. Increasing the pH from 4.4 to 5.3 (the usual range of a beer fermentation) had no further effect on the fermentation velocity. On the other hand, Visuri and Kirsop (1970) found that the fermentation rates of both maltose and maltotriose were strongly influenced by pH; the optimum pH for maltose utilisation being between 4.0 and 5.5 and that for maltotriose being between 4.5 and 5.5. Visuri and Kirsop (1970) also found that the fermentation of maltose was less affected by low pH than that of maltotriose. It may be concluded that the low pH towards the end of a fermentation may inhibit the uptake of maltotriose to some extent.

The relatively low pH of fermenting wort is beneficial in some ways. Together with the low dissolved oxygen concentration and the presence of mildly antibacterial ethanol and hop antiseptics it helps prevent bacterial infection of the brew.

3.3.6 Temperature

For ale production the yeast is pitched into the wort

at about 16°C (61°F). During fermentation the temperature rises and then falls back to approximately its original value. The temperature rise is of the order of 5°C (see Fig. 3.1). For further information on this topic see Section 3.4.2.

3.3.7 Ethanol Production

The fermentation of brewers' wort by yeast involves an extremely complex set of biochemical reactions. These reactions have been studied in detail by microbiologists and are known as metabolic pathways. The principal pathway involved in the production of ethanol is the Embden-Meyerhof-Parnas route. For further details the reader is referred to Hough et al. (1971), or any other comprehensive text on microbiology.

For simplicity the fermentation cycle can be represented by the equation governing ethanol production from a disaccharide.

$$C_{12}H_{22}O_{11} + H_2O \longrightarrow 2C_6H_{12}O_6 \longrightarrow 4C_2H_5OH + 4CO_2 + 100 \text{ kcal.}$$

The energy released accounts for the temperature rise in a fermentation (see Section 3.3.6). Under anaerobic conditions 51.1% w/w of the sugar is converted to ethanol and 48.9% w/w to carbon dioxide. This theoretical yield of ethanol cannot be achieved in practice because of;

(1) formation of other metabolic by-products by the yeast

(2) entrainment of ethanol in the exit gas stream

(3) the utilization of some sugar by the yeast for growth and cell maintenance. (Hough et al. (1971) have stated that 10 g of dry yeast are produced when 200 g of maltose are fermented anaerobically.)

In practice yields of ethanol amount to 90% - 95% of those expected from the theoretical equation.

3.3.8 Beer

Beer is the potable end-product of the brewers' art, most of the carbohydrates having been converted to ethanol. There are also a host of minor metabolites present: these are extremely important in giving beer its characteristic taste. One of the more important flavour components is a pair of compounds known as vicinal diketones. These are discussed in more detail in section 5.4.6. Typical beer analyses are shown in Tables 3.1 and 3.3.

3.4 The Batch Experimental System

After studying the traditional batch beer fermentation and considering the physical system for effecting continuous fermentations it was decided to carry out batch fermentations in an attemperated stirred tank system. There are two primary reasons for this: these are discussed in the following sub-sections.

3.4.1 Flocculation Effects

Towards the end of a conventional batch beer fermentation the yeast tends to flocculate. Flocculation is the agglomeration of the unicellular yeast into clumps.

In the British system for producing ales, use is made of strains of *Saccharomyces cerevisiae* and the yeast flocs are carried to the surface of the brew by bubbles of carbon dioxide. The yeast mass is periodically skimmed off by the brewer and some yeast may be retained for pitching further brews. On the continent,

TABLE 3.3.

TASTE THRESHOLDS OF SOME BEER CONSTITUTENTS (μg/ml).
(Hough and Button (1972))

Substance	Thresholds		Typical Levels Present	
	In Lager	In Stout	In Lager	In Stout
Methanol	-	100	-	-
Ethanol	-	-	2.8-3.2 *	2.0-8.9 *
Propanol	-	50	5-10	13-60
Isopropanol	2	100	-	-
2-Methylpropanol	-	100	6-11	11-98
2-Methylbutanol	-	50	8-16	9-41
3-Methylbutanol	-	50	32-57	33-169
β-Phenylethanol	47.9	50	25-32	20-55
Ethyl acetate	93.5	5	8-14	11-69
Butyl acetate	2.63	-	-	-
Isobutyl acetate	-	1	-	-
Amyl acetate	3.44	-	-	-
Isoamyl acetate	2.30	1	1.5-2.0	1.0-4.9
Diacetyl	0.162	0.005	0.02-0.08	0.02-0.58

* percentage (w/v)

where lager beers are preferred, strains of S. carlsbergensis are used. The flocs of this yeast tend to sediment out of the beer and a relatively clear product is run off from this sediment.

In the continuous tower fermenter it is necessary to use strains of yeast which are always highly flocculent. The actual mechanism of flocculation is still not fully understood. The factors said to influence flocculation are yeast genetics, yeast metabolism, the cell wall structure, and other external environmental factors. An excellent review of the subject is provided by Rainbow (1970).

The aim of executing batch fermentations was to determine the kinetics of selected systems. It was then intended to use this information in developing a model of the continuous fermenter. Consequently it was considered essential to use the same yeasts in the batch fermentations as were to be employed in the continuous process.

If a strain of highly flocculent yeast is pitched into an unstirred batch vessel then it is obvious it will immediately settle out: thus no fermentation, or fermentation at a reduced rate will occur. To avoid this problem it is clearly necessary to use a stirred vessel for batch experiments.

Stirring provides a further advantage when studying batch fermentation. When flocculation of the yeast is occuring at the end of a batch fermentation the amount of "active" yeast in contact with the wort is decreasing. The system is changing from a relatively homogeneous one, caused by large volumes of carbon dioxide being released, to an unmixed situation. This obviously leads to complications in determining the kinetics

of the system. By employing a stirred vessel the effect of changes in yeast concentration are minimised.

3.4.2 Temperature Effects

In a conventional batch fermentation it has already been noted that there is a considerable variation in temperature with time. It is also well known that small temperature changes in fermentation processes can lead to large changes in the rate of fermentation. Fidgett (1975) showed from the data of White (1966) that an increase in temperature from 15°C to 20°C can cause the rate of a beer fermentation to double.

Merrit (1966) studied the effect of temperature on fermentation rate by measuring the amount of carbon dioxide evolved over various time intervals. These data are shown in Table 3.4 and plotted on Graph 3.1. From this graph it can be seen that there is a linear relationship between temperature and fermentation rate over the range 25°C-35°C. In the range 20°C-25°C, the likely area for beer fermentations, the rate of fermentation increases by 1.5.

Thorne (1954) also examined the effect of temperature on fermentation rate. His data are shown in Table 3.5 and plotted on Graph 3.2: there is roughly a linear relationship between fermentation rate and temperature over the range 15°C-36°C. Between 15°C and 20°C the fermentation rate increases by 1.65, and between 20°C and 25°C the increase is by 1.4. The latter value corresponds closely with Merrit's (1966) value of 1.5 for the same temperature range.

From the above discussion it is obvious that small changes

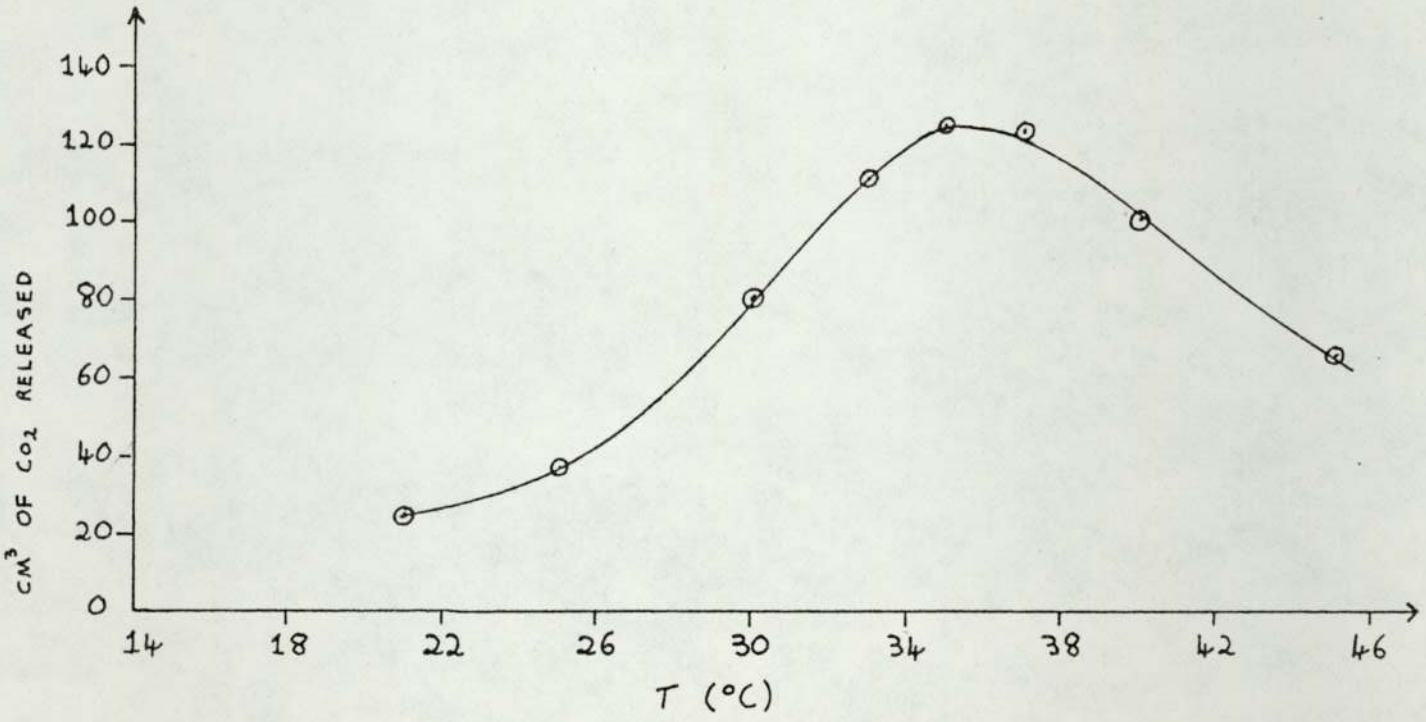
TABLE 3.4 THE INFLUENCE OF TEMPERATURE ON RATE OF PRODUCTION OF CARBON DIOXIDE FROM DISTILLERS' WORT INNOCULATED WITH 2.5% w/v of PRESSED YEAST (Merrit 1966))

Temperature	Co_2 Evolved after 120 minutes
^oC	cm^3
20	24.3
25	37.1
30	79.6
33	111.6
35	126.0
37	124.0
40	100.0
45	66.4

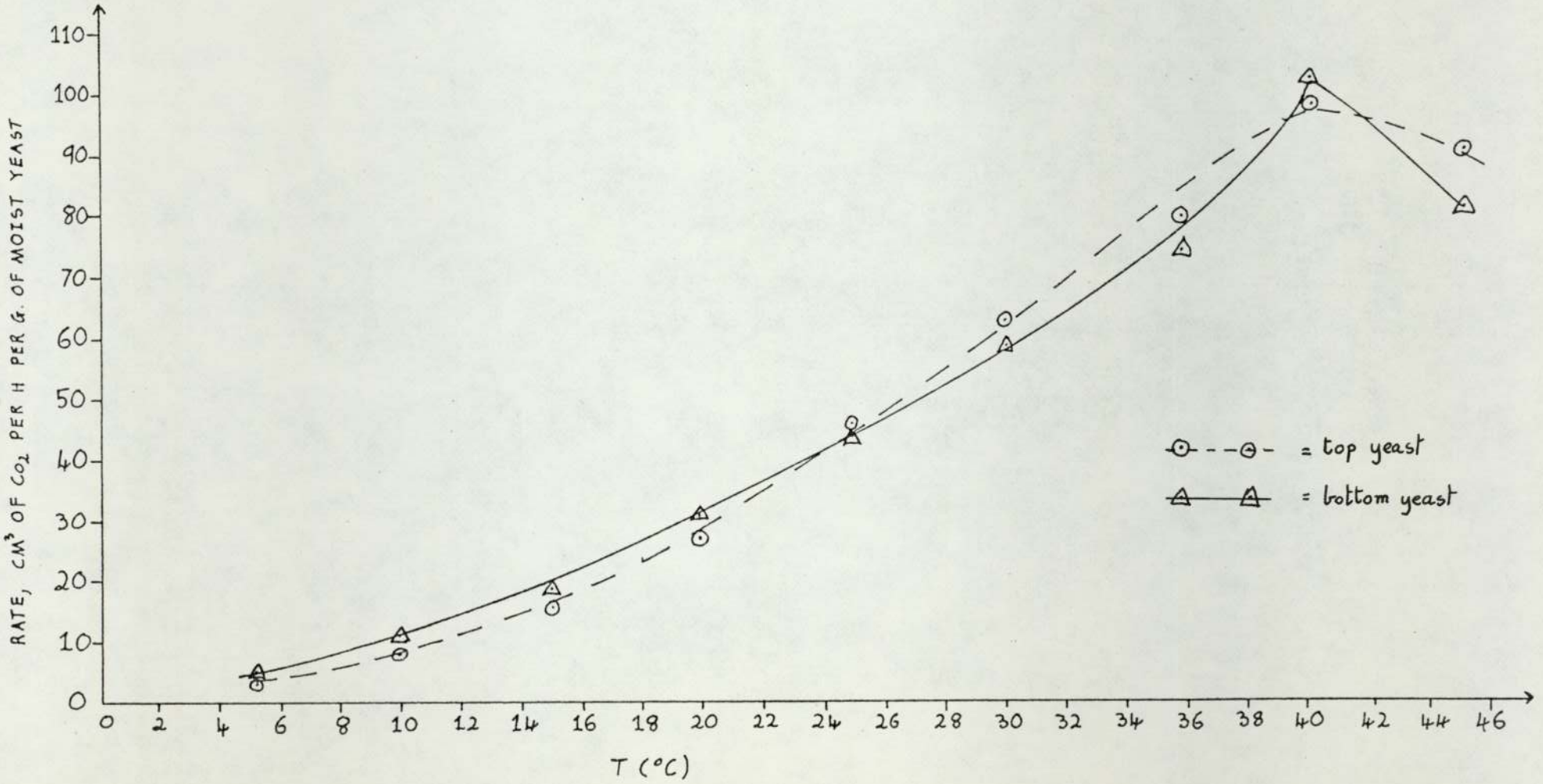
TABLE 3.5 MEAN FERMENTATION VELOCITIES OF TWO YEASTS AT VARIOUS TEMPERATURES (THORNE (1954))

Temperature	Fermentation Rate	
^oC	cm^3 of Co_2/h/g moist yeast	
	Top Yeast	Bottom Yeast
5.1	3.1	4.8
9.9	8.1	10.4
14.9	15.5	18.6
19.8	27.1	30.7
24.9	45.6	43.9
29.9	62.8	58.5
35.7	79.8	74.1
40.1	98.2	102.5
45.0	90.7	80.8

GRAPH 3.1 INFLUENCE OF TEMPERATURE ON THE RATE OF FERMENTATION (MERRIT (1966))



GRAPH 3.2 INFLUENCE OF TEMPERATE ON FERMENTATION RATE (THORNE (1954))



in temperature have a profound effect on fermentation rate and hence on the overall fermentation kinetics. Therefore, it was decided to carry out the experimental fermentations under thermostatic conditions.

Fermentation experiments were carried out in a cylindrical glass vessel of 20 l working volume. Using this volume it was hoped to eliminate any disturbances to the system caused by removing samples.

A combined stirrer and temperature controller (Townson and Mercer Ltd.) was used. This stirrer gave an "underwater" circulatory type turbulence. This was important as it allowed a head of foam to form on the surface of the brew. This prevented excess oxygen being transferred into the system. The effects of aeration on alcohol fermentations have been studied and a summary is given elsewhere (see section 5.4.4).

3.5 The Experimental Programme

3.5.1 Choice of Yeast

Three strains of yeast were chosen for use in the experimental work: these are tabulated below.

TABLE 3.6 FLOCCULENT YEASTS EMPLOYED IN THIS WORK

CULTURE COLLECTION NO.	YEAST STRAIN
CFCC 1	Saccharomyces cerevisiae
CFCC 39	Saccharomyces carlsbergensis
CFCC 54	Saccharomyces diastaticus

The yeasts are designated by the Continuous Fermentation Culture Collection Numbers used in the Department of Biological Sciences at the University of Aston in Birmingham. The main reasons for choosing these yeasts are given below.

1) Yeast Strain and Fermentation Rate.

S. carlsbergensis is said to ferment maltotriose to a greater extent than S. cerevisiae (Table 3.1). S. diastaticus is also able to ferment maltotetraose (Philips (1955)). This leads to what has been termed "super-attenuation" of beer worts. It was felt that some indication of the effect of yeast strain on kinetics would be given by using the three selected strains.

2) Results of Earlier Screening Tests.

Coote (1974) carried out an extensive yeast selection programme for the A.P.V. Co. Ltd. The aim of this survey was to evaluate the performance of various yeasts in a tower fermenter. The three strains above were among those judged to be most

suitable for use in the tower fermenter; that is to say they fermented the wort readily at high liquid flow-rates without being washed out of the system.

3) Comparison with Previous Research

The strain of S. cerevisiae, (CFCC 1), is held in the National Collection of Yeast Cultures as NCYC 1026. This yeast has been used by many other workers in continuous fermentations (Hough (1961), Hough et al. (1962), Watson and Hough (1966), Portno (1967c, 1969), and Ault et al. (1969)).

4) Flavour of Final Product

Coote (1974) found the strain of S. carlsbergensis, (CFCC 39), was the best "lager yeast" for use in the tower fermenter regarding flavour characteristics.

3.5.2 Batch Operating Conditions

Yeast pitching rates of approximately 2 g of wet yeast per litre of wort were aimed at in order to simulate industrial practice (see section 3.3.1). Fermentations were run in a thermostat for reasons outlined in section 3.4.2. The chosen temperature of operation was 20°C as this was thought to be close to industrial practice (see section 3.3.6). Diluted hopped malt extract syrup was used to supply the sugars for fermentation; though this did not give a defined medium it was thought likely to result in a medium similar in composition to a commercial brew. Initial sugar concentrations of approximately 100 g/l were aimed at, which results in a medium present gravity of approximately 50. This is higher than commercial brews with an initial P.G. of 35; however, sugar concentrations of 100 g/l were envisaged

for the media for the continuous tower fermenter and breweries hold down the initial P.G. of their brews partly for taxation reasons.

3.6 Experimental Materials

3.6.1 Wort Preparation

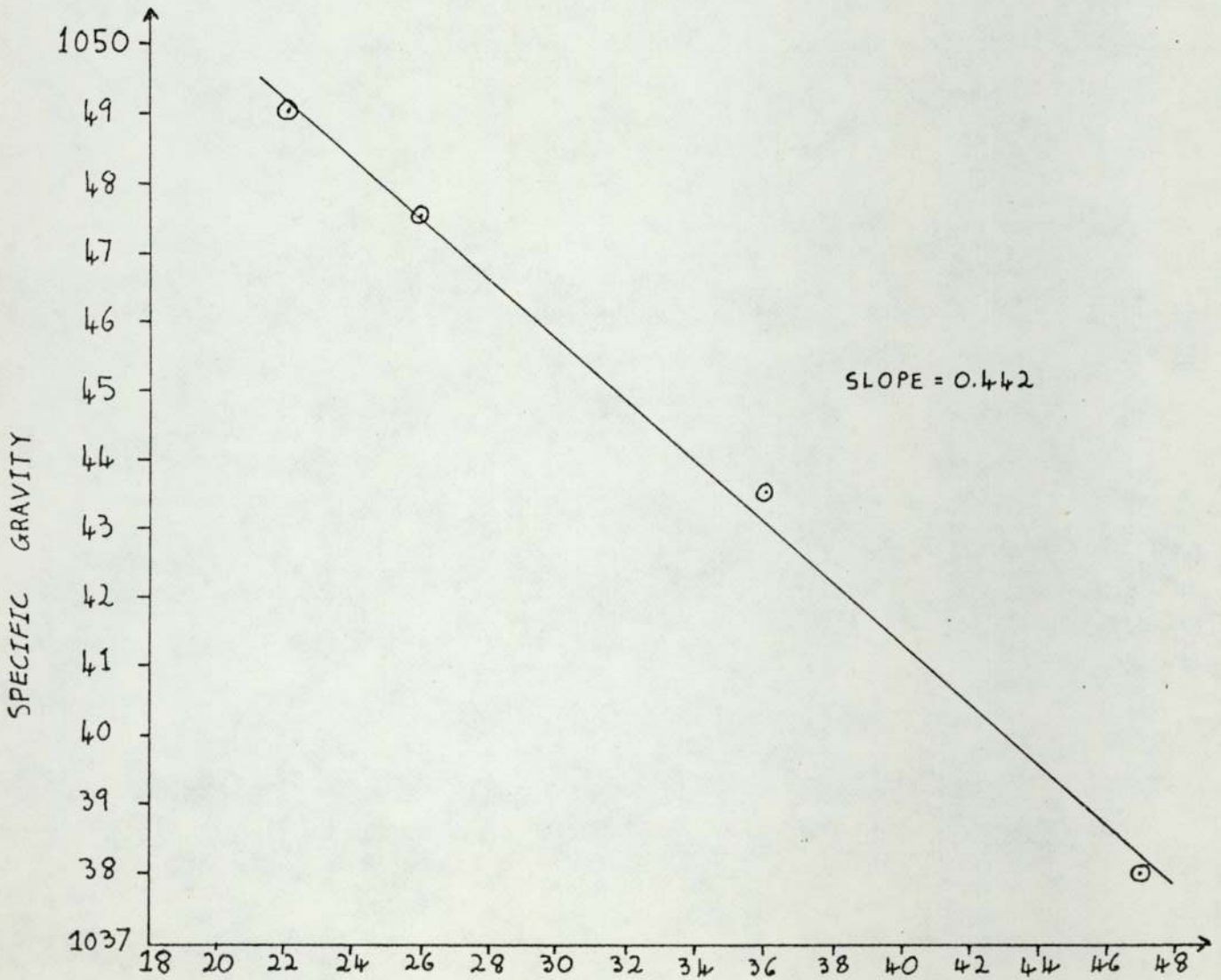
Concentrated hopped malt extract (E.D.M.E. Ltd.) was diluted with hot water to give a wort of specific gravity of approximately 1.050 on cooling. Wort specific gravity versus temperature is given in Graph 3.3. It shows that for every $1^{\circ}C$ rise in temperature the S.G. of the wort falls by 0.442: this confirms the results of Shales (1967). The wort was then autoclaved in 20 l aspirators for 25 minutes at $121^{\circ}C$ and 2.05×10^5 N/m^2 ($250^{\circ}F$, 15 psig.). The aspirators were fitted with Whatman Gamma - 12 in-line filters having grade -03 filter tubes; this was to prevent bacterial infection of the wort after it had been removed from the autoclave. After autoclaving there was normally a layer of proteinaceous matter coagulated in the base of the aspirator.

3.6.2. Storage of Yeast Cultures

Autoclaved hopped wort and agar were boiled to dissolve the agar. McCartney bottles, containing this liquor, were autoclaved for 15 minutes under the conditions mentioned above.

After cooling cultures were innoculated onto these agar slopes and incubated for 3 to 4 days at $30^{\circ}C$. The yeast cultures obtained were then stored in a refrigerator at $4^{\circ}C$. Subcultures were made routinely to ensure the cultures remained pure.

GRAPH 3.3 SPECIFIC GRAVITY OF WORT VERSUS TEMPERATURE



3.6.3 Preparation of Inocula

Autoclaved wort, free from proteinaceous matter, was run into 250 ml shake flasks. These were tightly plugged with non-absorbent cotton wool and autoclaved for 15 minutes as previously mentioned. After cooling the flasks were innoculated with the desired strain of yeast. The flasks were then placed in an orbital incubator at 25°C for 4 to 5 days. The resultant yeast was centrifuged out, weighed, and used to pitch 20 l of sterile wort.

3.7 Experimental Measurements

3.7.1 Yeast Concentration Determinations

100 ml samples of liquor were pipetted from the centre of the fermentation vessel. These samples were centrifuged for 10 minutes at 5000 r.p.m. using an M.S.E. bench centrifuge. The clear liquor was decanted off. The centrifuged yeast was then washed with distilled water and re-centrifuged. After discarding the clear liquor the yeast wet weight was recorded. The dry weight was also noted after drying the sample for 24 hours at 105°C.

In two experiments the concentration of viable and non-viable cells in the medium was estimated using a haemocytometer. The yeast cells were stained with a solution of methylene blue, according to the method of Townsend and Lindegren (1953), before counts were made with the haemocytometer.

3.7.2 Reducing Sugar Determinations

The clear liquor decanted from the centrifuged yeast was titrated against a 1% w/v solution of potassium ferricyanide in order to determine the reducing sugar concentration.

The reducing sugars were recorded as equivalent mg of glucose per 100 ml of solution; this method is attributed to Somogyi (1945). A full description of the method, enabling a high degree of accuracy to be attained, has been given by Spensley (1977). Hodge and Davis (1952) have also stated that the method is an improvement on that based on Fehlings solution and yields results of high precision.

3.7.3 Specific Gravity

The specific gravity of the medium was measured, either by hydrometer (from Reeves and Co.), or by S.G. bottle.

3.8 Summary of Experiments

Table 3.7 shows the batch beer fermentations carried out, the strain of yeast used, the initial yeast concentration (centrifuged wet weight basis), sugar concentration and present gravity, and the final values of these variables.

TABLE 3.7 BATCH BEER FERMENTATION SUMMARY

Experiment No.	Yeast Strain	Yeast conc.		Sugar Conc.		Present Gravity	
		Initial g/l	Final g/l	Initial g/l	Final g/l	Initial	Final
1	CFCC 54	2.55	26.0	106.6	8.3	51.7	1.2
2	CFCC 54	2.13	53.9	110.2	20.2	53.2	7.0
3	CFCC 1	1.89	56.1	110.8	25.4	53.5	10.0
4	CFCC 54	-	-	98.1	22.6	49.8	10.9
5	CFCC 1	2.35	52.6	114.7	18.4	50.0	8.0
6	CFCC 39	3.49	26.9	94.5	28.0	40.0	11.0
7	CFCC 39	1.84	34.1	114.5	29.0	47.0	12.0
8	CFCC 1	2.50	28.7	102.5	16.9	50.5	8.3

The methods of measurement of the variables shown in Table 3.7 are detailed in section 3.7. Measurements of the variables were taken through each fermentation and plotted against time. The tabulated data and plots of S.G., reducing sugar, and yeast wet and dry weights versus time are given in Appendix 3.1 for each experiment.

3.9 Discussion

This discussion of the experimental work is no more than a qualitative introduction. Quantitative analysis will be considered in Chapter 4. In all the experiments (Appendix 3.1) the yeast growth and sugar utilisation curves are of the characteristic "S" type found with beer, and other fermentations. However, in experiment No. 6, in which a relatively high yeast inoculum was used a yeast lag phase was not observed. Consequently, there was a rapid attenuation of the wort. This observation was not unexpected since Hudson (1967) has found that increasing the size of the yeast inoculum leads to increased rates of fermentation.

In a number of experiments, particularly Nos. 1, 5, 7, and 8, the yeast concentrations in the very early stages of fermentation seem high; this is especially true of the centrifuged wet weight figures. As very little sugar had been utilised in the early stages of fermentation the amount of yeast growth could not have been so great. The error in these early yeast determinations could stem from two sources. First, there could have been some proteinaceous solids suspended in the wort, though care was taken to exclude such solid matter; if such solids were taken up in a sample then this would obviously lead to higher sample weights

than expected. Second, in the early stages of fermentation the amount of yeast in each sample was very small. Consequently, any errors caused by weighing or by the drying method would be magnified in these early yeast concentration determinations. The supposition that excess moisture was measured is born out by the fact that these early samples have a higher percentage moisture content than other samples. However, this could partly be due to the physiological state of the cell at this stage in the fermentation: Stanier et al. (1971) and Aiba et al. (1973) have pointed out that the mass of an individual cell may vary, with cell weight increasing rapidly in the early stages of fermentation and then decreasing.

The final yeast concentration in a number of experiments, nos. 2, 3, and 5 (see Table 3.7), appears high when compared with that for conventional batch beer fermentations. There are probably two causes of this. First, the wort used in the experimental work was more concentrated than conventional brewery worts. Wort specific gravities of approximately 1.050 were used in experimental work compared with commercial wort gravities of 1.035-1.040. This higher sugar concentration will lead to some increase in yeast production. Second, a stirred fermenter was used throughout the experimental work, whereas the conventional beer fermenter is unstirred. In the latter system the yeast flocculates at some stage in the fermentation and settles out from the active area of the brew. By contrast, the yeast in a stirred vessel is always in suspension and more likely to be reproducing because of increased contact time with the carbohydrate

source.

The effect of yeast strain on the amount of carbohydrates assimilated from the wort is inconclusive. Figures from Table 3.7 show the three strains of yeast utilised similar amounts of sugars and generally attenuated the wort to the same degree.

In experiment nos. 1 and 2 approximate mass balances were carried out by back calculating the original gravity of the wort from the specific gravities of the distilled alcohol, the distillate residue, and the acidity of the brew. The calculated original gravities are in close agreement with the observed wort original gravity (Appendix 3.2).

In the experiments where yeast viability was measured, nos. 4 and 5, there are two opposing results. Experiment no. 4 shows a high yeast viability throughout the fermentation. This is in agreement with the results of Brown and Kirsop (1972). However, experiment no. 5 shows a marked decrease in viability towards the end of the fermentation. Though Townsend and Lindegrew (1953), and other authors, have been satisfied with the reliability of the methylene blue staining technique for estimating the viability of a yeast population, other workers have lacked faith in the method. Graham (1970) has reviewed other procedures for measuring viability, and has stated the methylene blue stain to be equivocal; e.g. cells which have lost the ability to bud will stain with methylene blue, though they are not necessarily inactive.

In experiment nos. 4 and 5, where the yeast concentration was measured in terms of cell number, the lag phase of the yeast

growth curve can clearly be observed. A wide range of values was found when cell counts were compared with centrifuged wet yeast concentrations. A mean value of 5.4×10^6 cells/cm^3 was found to be equivalent to 1 g of centrifuged yeast per litre of wort: a value of 3.8×10^6 cells/$cm^3 \equiv$ 1 g of yeast/l was found in Section 5.4.5.

A final point to note is that the expected rapid fall in medium pH followed by a constant pH was observed in experiment no. 4 (see Appendix 3.1).

CHAPTER 4

MATHEMATICAL MODELLING OF THE BATCH FERMENTATION

4.1 Introduction

This chapter is concerned with the development of a kinetic model for anaerobic beer fermentation. The necessity for knowledge of the kinetics of a reaction, even if empirical in form, is discussed. The philosophy of modelling is considered, and models of varying complexity which have been used to describe biological systems are reviewed. Finally, a model of the batch beer fermentation is presented and discussed.

4.2 Reaction Kinetics

Generally, the rate of a given chemical reaction depends on the composition and temperature of the system, and not on the size and form of the reactor. Hence, once the kinetics of a particular reaction have been determined, these are applicable to virtually any situation in which the particular reaction occurs. In particular, kinetic data are needed to build up a basic understanding of fermentation processes, and this in turn permits rational design and scale-up of batch and continuous systems.

The utilisation of a substrate by a micro-organism generally follows a complex cycle with many intermediary substances and by-products being formed; these are sometimes re-utilised by the cell. Clearly, reactants in the liquid phase have to be transported to the microbial mass where the reaction takes place; reaction products are similarly transported. Thus, the over-all rate expression for the reaction becomes complicated because of interaction between physical and chemical processes. It has long been recognised by chemical engineers that in a complex reaction

system involving a number of steps one of these is likely to be rate-controlling. Monod (1942) and Ierusalimsky (1967) have discussed the application of this principle to microbial growth.

4.3 Mathematical Modelling

When a reaction mechanism is difficult to formulate an empirical approach is often employed. This technique lends itself readily to mathematical modelling. If a reaction can be described mathematically by a set of differential equations then these may be solved by;

i) simple analytical methods

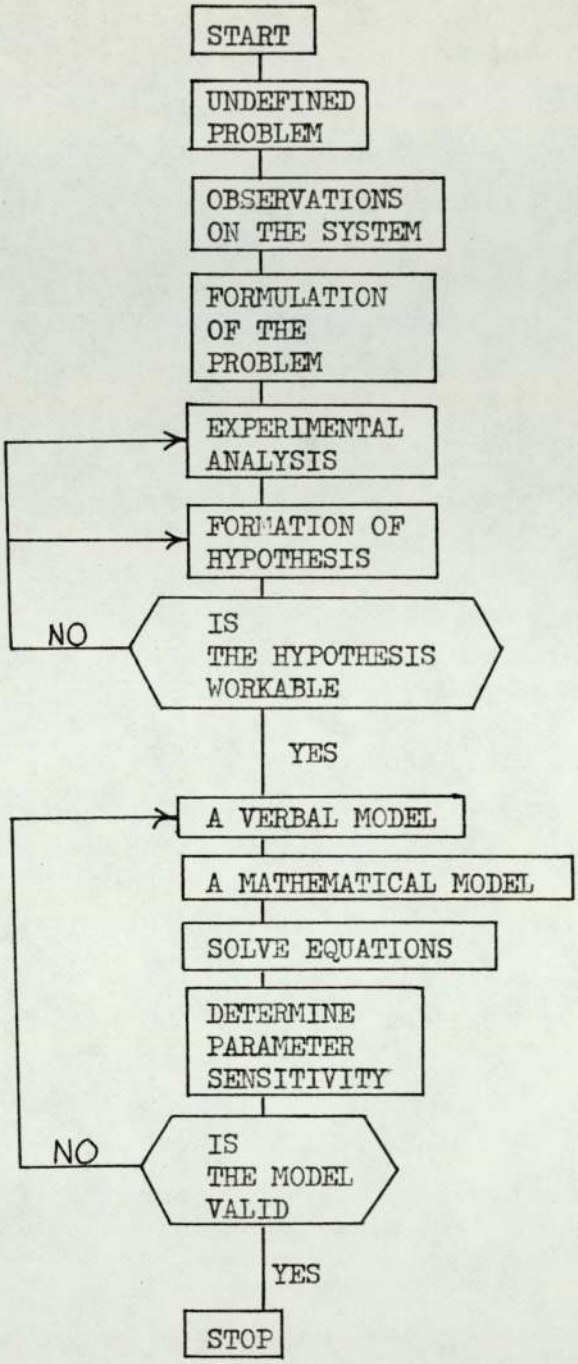
ii) simulation of the model with analogue or digital computers in the case of complex differential equations.

The approach to modelling of a system is summarised by Fig. 4.1.

Milson (1973) stated that a model of any system cannot fulfil all levels of detail and the crucial aspects of concern to the modeller should be concentrated on. Generally the more complex a model is the more difficult it is to use. For instance, a comprehensive model of cell growth which incorporated D.N.A. synthesis, the complex activities of enzymes, and the physiological effects of environmental factors would be mathematically cumbersome and probably more of a hindrance than an aid to research. However, complex models should provide the user with a fuller picture of the process.

Finally, parameter values are obviously very important. These need to be optimised, tested for stability, and examined to ensure that they are realistic.

FIG 4.1 System Modelling



4.4 MODELS OF MICROBIAL GROWTH AND SUBSTRATE UTILISATION

4.4.1 The Monod Model

Monod (1942, 1949) was among the earliest workers to develop mathematical models of microbial growth. Monod suggested that micro-organisms grow in an exponential manner at a maximum specific growth rate, μ_m, until the rate of cell division is limited by some environmental condition. In mathematical terms;

$$\frac{dx}{dt} = \mu_m x \qquad 4.1$$

which on integration gives

$$x = x_o \exp(\mu_m t) \qquad 4.2$$

He also suggested that the specific growth rate, μ, of any microbial culture is proportional to the limiting substrate concentration, S, but approaches a maximum value, (μ_m), when the substrate is not limiting. A hyperbolic form of equation, (e.g. 4.3), was chosen because of the good fit with experimental growth data;

$$\mu = \frac{\mu_m S}{K_s + S} \qquad 4.3$$

K_s is a "saturation constant" and is numerically equal to the substrate concentration at which the growth rate is half the maximum value.

The model is of the same form as the so-called Michaelis-Menten equation, which describes the relationship between substrate concentration and the velocity of a single enzymic reaction. The Michaelis-Menten expression applies to a fixed quantity of enzyme. With organism growth the amount of enzyme present increases with an increase in the cell biomass. Thus, Monod

substituted the reaction velocity per unit of biomass, (μ), for the absolute velocity of the process given by the Michaelis-Menten equation.

Monod (1942, 1949) also observed a constant relationship between rates of growth of organisms and the amount of substrate consumed. This is expressed by equations4.4 and 4.5; Y is known as the yield coefficient.

$$\frac{dx}{dt} = -Y\frac{dS}{dt} \qquad 4.4$$

$$Y = \frac{x - x_o}{S_o - S} \qquad 4.5$$

The concept of a constant yield coefficient has since been challenged by many authors, and it is generally accepted that Y will not be constant due to endogenous metabolism.

4.4.2 Further Empirical Models

A number of authors have developed empirical models to account for cellular growth. Often these are modifications of, or may be reduced to, the Monod equation (eq. 4.3). Examples are given in Table 4.1.

4.4.3 Complex Models

Tsuchiya et al. (1966) have classified microbial growth models according to the scheme shown in Fig. 4.2

Fig. 4.2 Model Classification

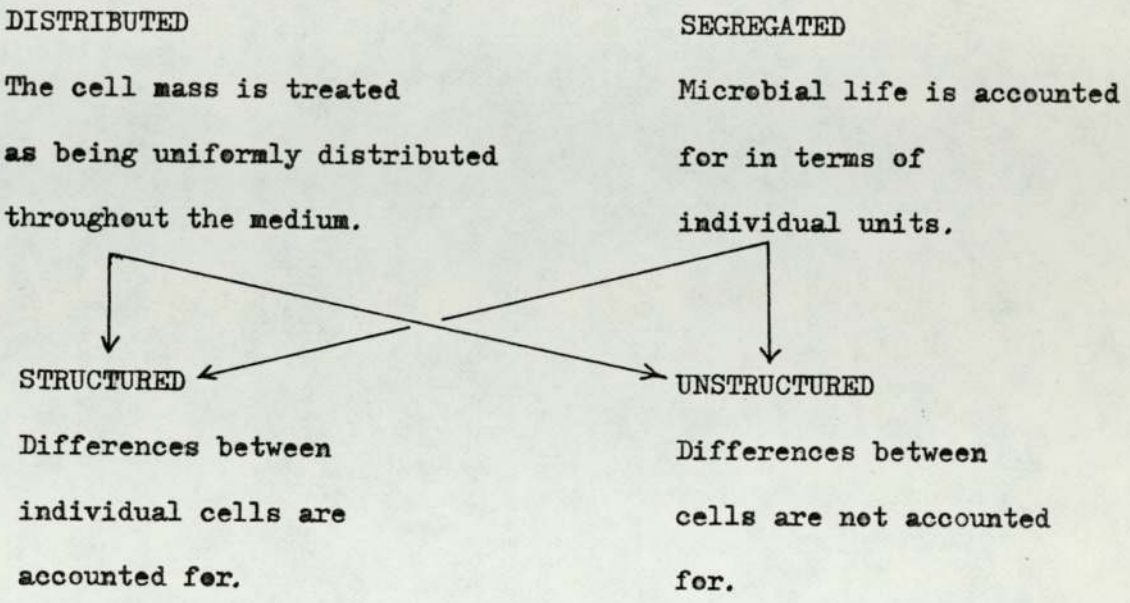


Table 4.1 Empirical Models of Cellular Growth

EQUATION FORM	COMMENTS	AUTHOR(S)
$\mu = \mu_m \left[1 - \exp\left(-\frac{S}{r}\right)\right]$	The curve of this equation is the same overall shape as the rectangular hyperbola of Monod (1942)	Teisser (1942)
$\mu = \frac{\mu_m S^r}{H^r + S^r}$	When r = 1, reduces to the Monod equation	Moser (1958)
$\mu = \frac{\mu_m (K_s + L + S)}{2L} \cdot \left[1 - \left(\frac{1 - 4LS}{(K+L+S)^2}\right)^{1/2}\right]$	Under certain conditions this equation can be approximated to $\mu = \mu_m S/(K+L+S)$, a form of the Monod equation	Powell (1967)
$\mu = \mu_o e^{-k_1 P}\left[\frac{S}{K_s + S}\right]$	P = product concentration, μ_o is the equivalent to μ_m when P = 0	Aiba et al. (1968)
$\mu = \frac{\mu_m S}{(K_s + S + S^2/K_1)}$ $\mu = \frac{aS - bS^2}{(c + S - dS^2 + eS^3)}$	These equations account for the situation where organism growth is suppressed by the nutrient source. When S is small both these equations reduce to the Monod equation.	Jackson and Edwards (1975) Chen et al. (1976)

TABLE 4.1 Empirical Models of Cellular Growth - (Continued)

EQUATION FORM	COMMENTS	AUTHOR(S)
$\mu = \frac{V_{GM} S}{(K_s + S)} \cdot Y' \cdot \left[\frac{1}{1+b'C_L}\right]$	Y', the growth yield, was a complex undefined function of the substrate and biomass present at any one time. C_L = dissolved oxygen as % of saturation.	Peringer et al. (1973)
$\frac{dx}{dt} = \frac{\mu_m S x}{(K_s + S)} - K_2 x - K_3 x$	Inhibition of the growth rate is expressed by using K_2 and K_3 : constants for endogenous metabolism and cell death respectively.	Sinclair and Topiwala (1970) and Topiwala (1973)
$\mu = \frac{Y_m k' S}{(K_m + S)} - d'$	d' = the specific maintenance rate. Y_m = the maximum yield factor when there is no maintenance energy requirement. The model allows for a variable yield factor	Van Uden (1967)
$\frac{dx}{dt} = \frac{\mu_m S x}{(K_s + S)} \quad S < S^*$ $\frac{dx}{dt} = \frac{\mu_m S x}{(K_s + S)} - C x (S - S_o) \quad S \geq S^*$	When the substrate concentration is below a threshold concentration, S*, organism growth is described by Monod kinetics. Above the threshold value growth is inhibited and C = an inhibition constant.	Wayman and Tseng (1976)

Ramkrishna et al. (1967) extended the work of Tsuchiya et al. (1966) and developed and compared unstructured and structured models. It was concluded that structured models could be used to account for a wide variety of situations not described by unstructured models. Fredrickson (1976) has restated his belief in structured, segregated models and advocated the use of mathematical models that contain variables which are related to the quality of the biomass. However, experimental techniques are still inadequate to verify many of the situations predicted by the structured models developed by Ramkrishna et al. (1967).

Kono (1968) and Kono and Asai (1969a, 1969b) have formulated a complex model in which different portions of the growth curve are represented by equations of the form;

$$\frac{dN}{dt} = K_4{}^{h} K_5{}^{j} C_m{}^{h} C_s{}^{j} - K_6 C_r$$

where N = cell concentration

C_s = limiting substrate concentration

C_m = concentration of substance m which reacts with the limiting substrate to produce cell mass

C_R = the concentration of secretion R which accumulates in the broth and represses cell growth

h, j, K_4, K_5, K_6 = constants pertaining to a particular portion of the growth curve.

Many authors have developed two-stage cell models to account for the many facets of microbial behaviour. Williams (1967) considered the cell as comprising two basic parts - one fed from the other. He suggested that cell division could only

occur when the structural portion of the cell had doubled in size. The basic equations of the model are;

$$\frac{dS'}{dt} = K_o(s_o - s) - K_1' S M$$

$$\frac{dS}{dt} = K_1' SM - K_2' C_p J - K_o J$$

$$\frac{dJ}{dt} = K_2' C_p N - K_o J$$

where S = concentration of external nutrient

C_p = synthetic portion of the cell

J = structural/genetic portion of the cell

M = total size of the cell

K_o, K_1', K_2' = constants

Verhoff et al. (1972) have proposed a similar model in which cell growth is divided into two processes - assimilation and ingestion. This type of model was also considered by Ramkrishna et al. (1967). Bijkerk and Hall (1977) have recently derived a model for the aerobic growth of Saccharomyces cerevisiae on glucose. The growth curve was divided into a number of sections following the ideas of Kono (1968), and the cell mass divided into two portions in a similar way to that proposed by Verhoff et al. (1972).

4.4.4 Discussion

The Monod model was developed from equations applying to single enzyme systems. However, it has been used to describe processes of no less complexity than R.N.A. and protein biosynthesis and cellular growth. Ierusalimsky (1967) stated that this approach is permissible because the cellular growth rate may

be proportional to the rate of a single enzymic reaction, (of the multitude involved in growth), which is a "bottle-neck" in metabolism. Monod (1942) was also aware of this fact when he postulated that organism growth could be limited by a "master reaction" in the cells' metabolism. Dabes et al. (1973) have extended this concept with a two stage model of cellular growth.

The Monod model is simple and empirical and, as a result, has certain deficiencies. Powell (1967) observed that owing to loss of cell viability and the physical requirements of endogenous metabolism deviations from equations 4.1 - 4.5 may occur.

Endogenous metabolism may be conveniently regarded as the cellular maintenance energy and is derived from the substrate by the cell without additional biomass or extracellular products being produced. Herbert (1958, 1961) was among the first to apply this concept to continuous culture of micro-organisms. Monod (1942) pointed out that energy was consumed by the cell to maintain its integral structure, but since he found that the yield was constant, he considered it negligibly small.

Bijkerk and Hall (1977) and other workers, who have developed segregated structured models of cell growth, have discarded the Monod model on the grounds that it cannot take into account all the phases of growth of a culture. However, other workers have been satisfied with results from the Monod model. Burrows (1970) stated that Monod kinetics were adequate for describing most cases of microbial growth. Tempest (1970) observed that μ_m was generally constant for a particular organism, although influenced

by the concentration of essential nutrients. Papers by Gaudy et al. (1967), Sinclair and Ryder (1975) and Ziegler et al. (1977) also demonstrate the usefulness of the simple Monod model.

Peringer et al. (1973) have criticised some of the more complex physiological models on the grounds that they require the measurement of variables which are difficult, if not impossible, to evaluate precisely, e.g. R.N.A., D.N.A., enzymes, and intracellular substrates. Ramkrishna et al. (1967) also noted that experimental evidence was required to validate the theoretical and complex structured models.

This discussion leads to the conclusion that the simplest available model should be used to represent a biological system. If the model first selected is inadequate, then more sophisticated models can be tested. The advantages of simple models are summed up by the following points :

i) highly specific mechanisms need not be postulated in the absence of empirical evidence;

ii) modifications to the model in the light of new empirical evidence are simple if the model is simple;

iii) insight may be gained into actual biological mechanisms;

iv) a temporary conceptual framework is created within which predictions may be made and new experiments suggested;

v) non-obvious properties of the biological system may emerge from the simple model without recourse to more complex assumptions.

As long as it is realised that the results of fitting data to a simple model only provide a convenient summary for

engineering purposes, rather than proof of the system kinetics, then it is valid to employ an empirical model. Consequently, an attempt was made initially to model the batch beer fermentation using conventional Monod kinetics. It will be shown that modifications to this model were necessary. The results of this analysis are discussed in the remainder of this chapter.

4.5 Batch Beer Fermentation - Analysis based on the Monod Model

4.5.1 Testing the Monod Model

According to the Monod model, the maximum specific growth rate can be determined from the equation;

$$\mu_m = \frac{1}{t} \cdot \ln\left(\frac{x}{x_o}\right) \qquad 4.6$$

and the yield constant, Y, is given by eq. 4.5;

$$Y = \frac{(x - x_o)}{(s_o - s)} \qquad 4.5$$

When the nutrient supply is in excess, then the biomass increases at an exponential rate (see Section 3.3.3).
In the batch beer fermentation, by measuring both the yeast and substrate concentrations at specified time intervals, the hypotheses of constant values for μ_m and Y can be explored.

From equations 4.3 and 4.4 the following expression for the utilisation of nutrient by the organism can be derived,

$$\frac{ds}{dt} = \frac{\mu_m}{Y} \cdot \frac{s.x}{(K_s + s)} \qquad 4.7$$

This equation covers the more general case where the

nutrient becomes limiting.

Letting $k_4 = \frac{\mu_m}{Y}$ and $r = -\frac{ds}{dt}$

then $$\frac{x}{r} = \frac{K_s}{k_4} \cdot \frac{1}{S} + \frac{1}{k_4} \qquad 4.8$$

Equation 4.8 represents a straight line of slope K_s/k_4 with intercept $1/k_4$ on the x/r axis. Experimental data (Appendix 3.1) can readily be plotted in the form suggested by eq. 4.8.

To summarize, we now have three ways of testing the validity of the Monod model applied to the batch beer fermentation:

i) Check if μ_m is constant when substrate is non-limiting

ii) Check if Y is constant

iii) Check if eq. 4.8 applies over a wide range of substrate concentrations.

4.5.2 Maximum Specific Growth Rate and Yield Constant

If Monod's model can be applied to the batch beer fermentation then the maximum specific growth rate, μ_m, and the yield coefficient, Y, will be constant over the "exponential" growth-phase. Data for yeast and reducing sugars concentration at specific time intervals were obtained from experimental plots (Appendix 3.1). The data were readily and efficiently analysed by computational techniques. A flowsheet for such a program is shown in Fig. 4.3 and a complete program listing is given in Appendix 4.1. Calculations were carried out using yeast concentrations based on both dry and wet weight. Results are summarised in Table 4.2.

A mean value of yield on a dry weight basis calculated from Table 4.2 gives Y = 0.143, which is of the same order as

Fig. 4.3

Flowsheet for the calculation of the maximum specific growth rate and yield coefficient of a micro-organism.

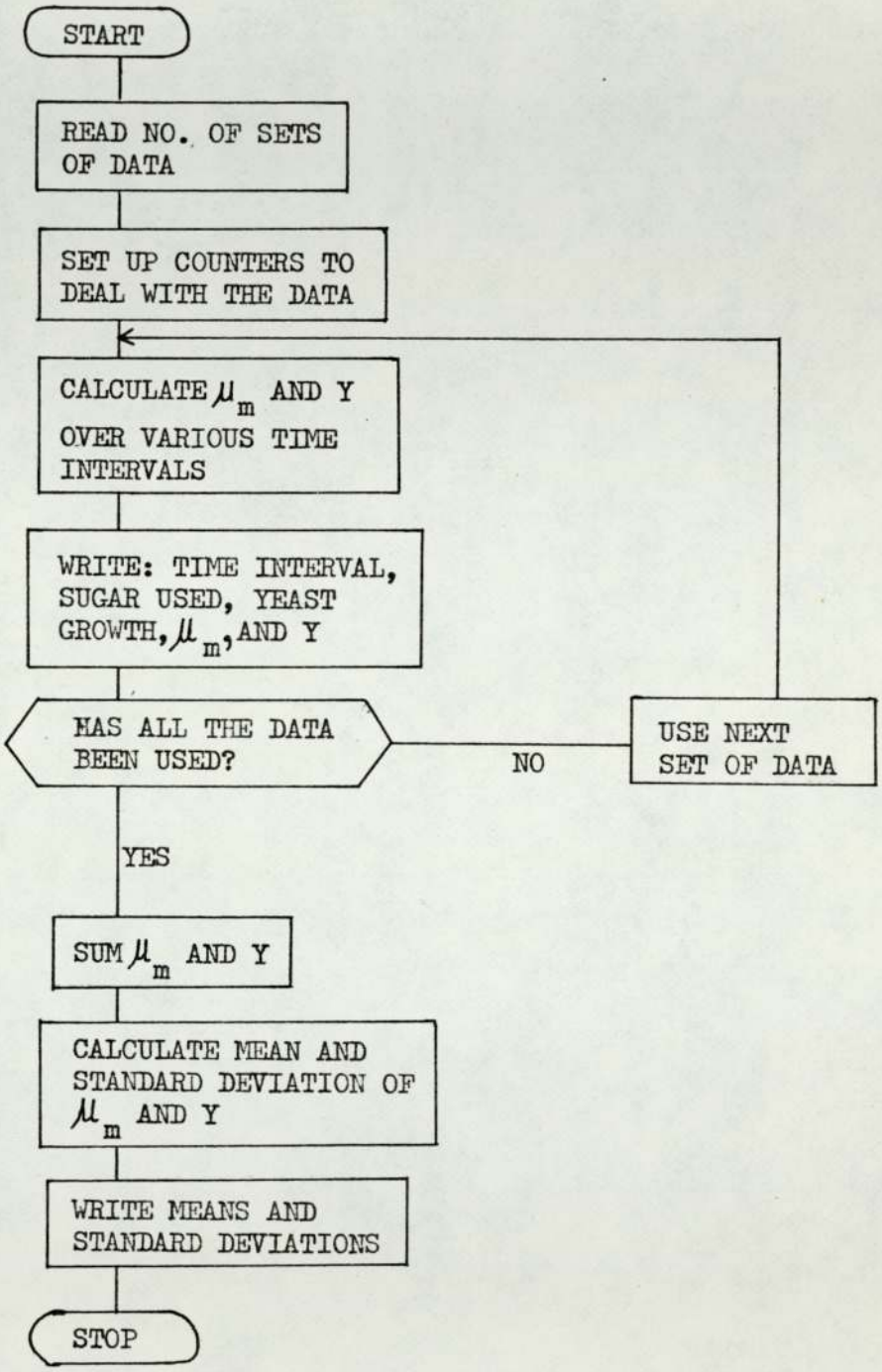


TABLE 4.2

Mean Values of the Yield Constant and Maximum Specific Growth Rate

EXPERIMENT NO.	WET YEAST		DRY YEAST	
	Y	μ_m	Y	μ_m
	g Yeast per g Sugar	H	g Yeast per g Sugar	H
1	0.3928	0.1056	0.0597	0.0888
2	0.4664	0.0626	0.0671	0.0559
3	2.4603	0.1363	0.3594	0.1144
* 4	-	0.1171	-	-
5	0.6528	0.0928	0.1162	0.0875
6	0.6186	0.0923	0.0761	0.0635
7	1.0348	0.1236	0.2134	0.1279
8	1.2164	0.0893	0.1095	0.0594

* μ_m calculated from cell count data

measured by Aiba et al. (1973). Taking a cake moisture content of 82%, the mean yield on a wet weight basis should be 0.794 g of wet yeast per g of substrate. The mean in fact is 0.977, which indicates that the yield coefficient tends to be over-estimated by calculating it on a wet weight basis.

The maximum specific growth rates of various yeasts have been calculated by a number of authors (Table 4.3). A wide range of values are evident, with μ_m for aerobic growth being higher than for anaerobic growth. The range of values found in this work, (Table 4.2), was 0.0626-0.1236 h^{-1} based on centrifuged wet yeast measurements and 0.0559-0.1279 h^{-1} calculated on a dry cell basis. These values are at the lower end of the range reported by other authors.

Though the mean values for μ_m and Y, (Table 4.2), were in agreement with estimates made by other workers, both parameters were very variable over the period of "exponential growth". Graphs 4.1 to 4.11, (Appendix 4.2) show how both μ_m and Y tended to attain a maximum value and then diminish as the fermentation proceeded.

Experiment nos. 6 and 8 in particular, and all experiments in general (Table 4.2) show μ_m estimated on a centrifuged wet yeast basis to be higher than μ_m evaluated on a dry cell basis. It is difficult to explain this difference as the samples were dried to a constant weight and an approximate moisture content of 82% was recorded. This was in close agreement with James (1973).

The variation in the values of Y and μ_m, the fact that differences existed between μ_m calculated on a dry and wet yeast

TABLE 4.3 Maximum Specific Growth Rates of Various Yeasts

μ_m / H^{-1}	Comments	Author(s)
0.40–0.45 0.07–020	Aerobic growth of Saccharomyces cerevisiae on glucose and a secondary phase utilising ethanol	Bijkerk and Hall (1977)
0.37–0.03	Baker's Yeast production	Burrows (1970)
0.32	Torula utilis grown aerobically on a molasses medium	Fencl (1966)
0.155	An anaerobic value for the beer fermentation	Markham (1969)
0.32–0.56	Aerobic measurements with low values for a large inoculum and vice-versa	Mian et al. (1969)
0.29	An anaerobic value for the alcohol fermentation	Ramalingham and Finn (1977)
0.081–0.232	Anaerobic values in a C.S.T.R. at various throughputs	Vairo et al. (1977)
0.34, 0.55	Candida tropicalis limited by hexadecane and glucose respectively	Ziegler et al. (1977)

basis, and the observations of impossibly large values of Y in certain cases indicated that the simple Monod model, (eq. 4.5 and 4.6), was unsuitable for describing the full course of the batch beer fermentation.

4.5.3 The Linearised Monod Model

The full empirical model developed by Monod can be conveniently tested in the form of eq. 4.8;

$$\frac{x}{r} = \frac{K_s}{k_4} \cdot \frac{1}{s} + \frac{1}{k_4} \qquad 4.8$$

Two computer packages available at the University of Aston in Birmingham were used to analyse the experimental data. A statistics package was used to determine the correlation coefficient for the line of best fit to the data; the other program plotted the regression line through the data points. These programs allowed for rapid analysis of the data.

At the commencement of the fermentation and in the latter stages values of r were difficult to obtain due to the slow rate of sugar utilisation at these times. The consequences of reducing the number of data points near the points of inflexion on the sugar utilisation curve are seen in Table 4.4, expt. no. 5. The regression coefficient increased from 0.654 to 0.890 by considering a 45 hour period rather than a 55 hour period of the fermentation. The rates of sugar decrease and yeast increase are relatively constant over the middle range of the fermentation. Consequently, a linear relationship between x and s may be expected in this region. This fact reinforces

TABLE 4.4 Specific Growth Rates and Monod Constants calculated from the Linearised Monod Model

Expt. No.	Yeast Basis	H of Ferm.	Regression Coeff.	Inter-cept	Slope	Specific Growth Rate	Monod Constant
						H^{-1}	g Sugar/L
1	Dry	15-50	0.737	-1.204	1.824	-0.830	-1.515
	Wet	15-50	0.745	-7.887	11.294	-1.268	-1.432
	Dry	20-50	0.808	-2.090	2.101	-0.479	-1.005
	Wet	20-50	0.815	-13.295	12.987	-0.075	-0.977
2	Dry	15-50	0.684	-2.670	3.709	-0.375	-1.389
	Wet	15-50	0.690	-15.425	22.414	-0.065	-1.453
	Dry	20-50	0.723	-4.635	4.236	-0.216	-0.914
	Wet	20-50	0.737	-27.699	25.703	-0.036	-0.928
3	Dry	20-70	0.463	-29.744	22.317	-0.034	-0.750
	Wet	20-70	0.452	-163.393	124.731	-0.006	-0.763
	Dry	20-60	0.791	-6.552	6.972	-0.153	-1.064
	Wet	20-60	0.760	-31.285	37.322	-0.032	-1.193
4	Total Cells	15-40	0.917	-84.178	84.667	-0.012	-1.006
	Viable Cells	15-40	0.920	-57.012	61.084	-0.017	-1.071
5	Dry	10-65	0.654	-6.631	5.946	-0.151	-0.897
	Wet	10-65	0.656	-40.144	35.208	-0.025	-0.877
	Dry	10-60	0.869	-2.773	3.463	-0.361	-1.249
	Wet	10-60	0.873	-17.366	20.547	-0.058	-1.183
	Dry	15-60	0.890	-3.393	3.630	-0.295	-1.070
	Wet	15-60	0.887	-20.528	21.398	-0.049	-1.042
	Total Cells	10-40	0.974	-91.649	121.934	-0.011	-1.330
6	Dry	0-35	0.545	-6.212	5.165	-0.161	-0.832
	Wet	0-35	0.542	-34.267	29.064	-0.029	-0.848
	Dry	0-30	0.778	-1.679	2,038	-0.596	-1.214
	Wet	0-30	0.764	-8.628	11.378	-0.116	-1.319
7	Dry	0-50	0.717	-6.660	7.174	-0.150	-1.077
	Wet	0-50	0.722	-32.593	35.022	-0.031	-1.075
	Dry	0-40	0.884	-0.611	1.608	-1.637	-2.631
	Wet	0-40	0.873	-3.387	8.149	-0.295	-2.406
8	Dry	5-50	0.714	-1.846	2.614	-0.542	-1.416
	Wet	5-50	0.656	-7.752	14.004	-0.129	-1.807
	Dry	5-45	0.774	0.248	1.226	4.040	4.954
	Wet	5-45	0.639	4.952	5.582	0.202	1.127

the view that the Monod model is not suitable for describing the full course of the batch alcohol fermentation.

It was possible to represent the data by a straight line for many of the experiments, as evidenced by graphs 4.12 to 4.17 in Appendix 4.3. However, it is evident particularly from graphs 4.14, 4.16, and 4.17 that a curvilinear function would correlate the data points more accurately.

Though the data in some cases could be approximated by a straight line the intercept of this line on the x/r axis proved to be negative in all but two cases. This leads in turn to negative values for the specific sugar utilisation rate, k_4, and the Monod constant, K_s (Table 4.4). Clearly such figures have no meaning in biological terms.

With these results and the previous observations regarding μ_m and Y, it was concluded that the Monod Model could not be used to represent cellular growth and substrate utilisation in the batch beer fermentation.

4.6 Batch Beer Fermentation - Development of the Monod Model

4.6.1 Basis of the Model

The way in which the Monod model was modified to account for the experimental results is described in this section. First, equations 4.1 and 4.3 were retained to account for yeast growth;

$$\frac{dX}{dt} = \frac{\mu_m . S . X}{(K_s + S)} \qquad 4.9$$

Second, equations of the form of eq. 4.7 were used to describe substrate uptake. However, more than one equation was used for the following reason. Experimental evidence suggested that when

the reducing sugar concentration fell to approximately 25-30 g/l the sugar utilisation rate was greatly reduced. This closely corresponds to the stage in the fermention when the yeast commences uptake of maltotriose, which is a difficult sugar for most yeasts to metabolise. Hence, when the sugar level, S, fell to some limiting concentration, S^*, the rate of substrate utilisation was represented by

$$\frac{dS}{dt} = -\frac{K5.S.X}{K_m + S} \qquad (S \leq S^*) \qquad 4.10$$

in which the rate constant K5, was expected to be much less than K1 ($\equiv k_4$). K5 may be regarded, for convenience as the rate constant for maltotriose uptake.

When the limiting value of sugar concentration, S*, was reached the rate of biomass increase also decreased. This was probably due to the following factors:

i) the alcohol concentration in the medium was nearing its peak - a high alcohol level is known to inhibit the yeasts' metabolism

ii) the available nutrient supply was diminished and difficult to breakdown

iii) the culture was ageing, and as a result of this and the two previous factors the number of non-viable and "resting" cells in the system was increasing.

Hence, the concept of a cell death-rate was introduced. This was assumed to be first order with respect to the yeast concentration. Endogenous metabolism and ethanol inhibition of the cell metabolism were also considered. By assuming that these two effects would also be described by first-order rate equations,

it was possible to incorporate all three rate constants into a single rate constant, K6. As in the case of substrate concentration, the inhibition of yeast growth was assumed to occur at some limiting yeast concentration, X*. The equation used to describe this phase of growth was

$$\frac{dX}{dt} = \frac{\mu_m S.X}{(K_s + S)} - K6.X \quad (X \geq X^*) \qquad 4.11.$$

4.6.2 Fitting of Model Equations to Experimental Data

The four equations representing the new model for the batch beer fermentation are shown in Fig. 4.4. Obviously the four equations could not be integrated and solved as they stood because of their mutual interdependence. However, such sets of equations may be "solved" in a stepwise fashion by taking an initial value for a variable and estimating the new variable value over a short time interval. The new value then serves as the initial value for the next time interval. This process would be tedious to attempt manually, and so use was made of a computer package available in the Chemical Engineering Department of the University of Aston in Birmingham - the Basic Aston Simulation Program (B.A.S.P.). A flow sheet which incorporates B.A.S.P. and utilises the equations in Fig. 4.4 is shown as Fig. 4.5. The time-axis marching procedure selected was the Runge-Kutta fourth order method, detailed in Appendix 4.4. Fig. 4.5 indicates that it was possible to produce plots showing both the predicted model curves and the experimental points. A complete program listing is given in Appendix 4.5. The program was interactive, so that it could be edited at run time and the values of parameters changed as simulation proceeded. The

FIG. 4.4 Equations representing sugar utilisation and yeast growth in the batch beer fermentation

$$\frac{dS}{dt} = -\frac{K1.S.X}{K2 + S} \qquad S>S^* \qquad 4.12$$

$$\frac{dS}{dt} = -\frac{K5.S.X}{K2 + S} \qquad S\leq S^* \qquad 4.13$$

$$\frac{dX}{dt} = \frac{K3.S.X}{K4 + S} \qquad X<X^* \qquad 4.14$$

$$\frac{dX}{dt} = \frac{K3.S.X}{K4 + S} - K6.X \qquad X\geq X^* \qquad 4.15$$

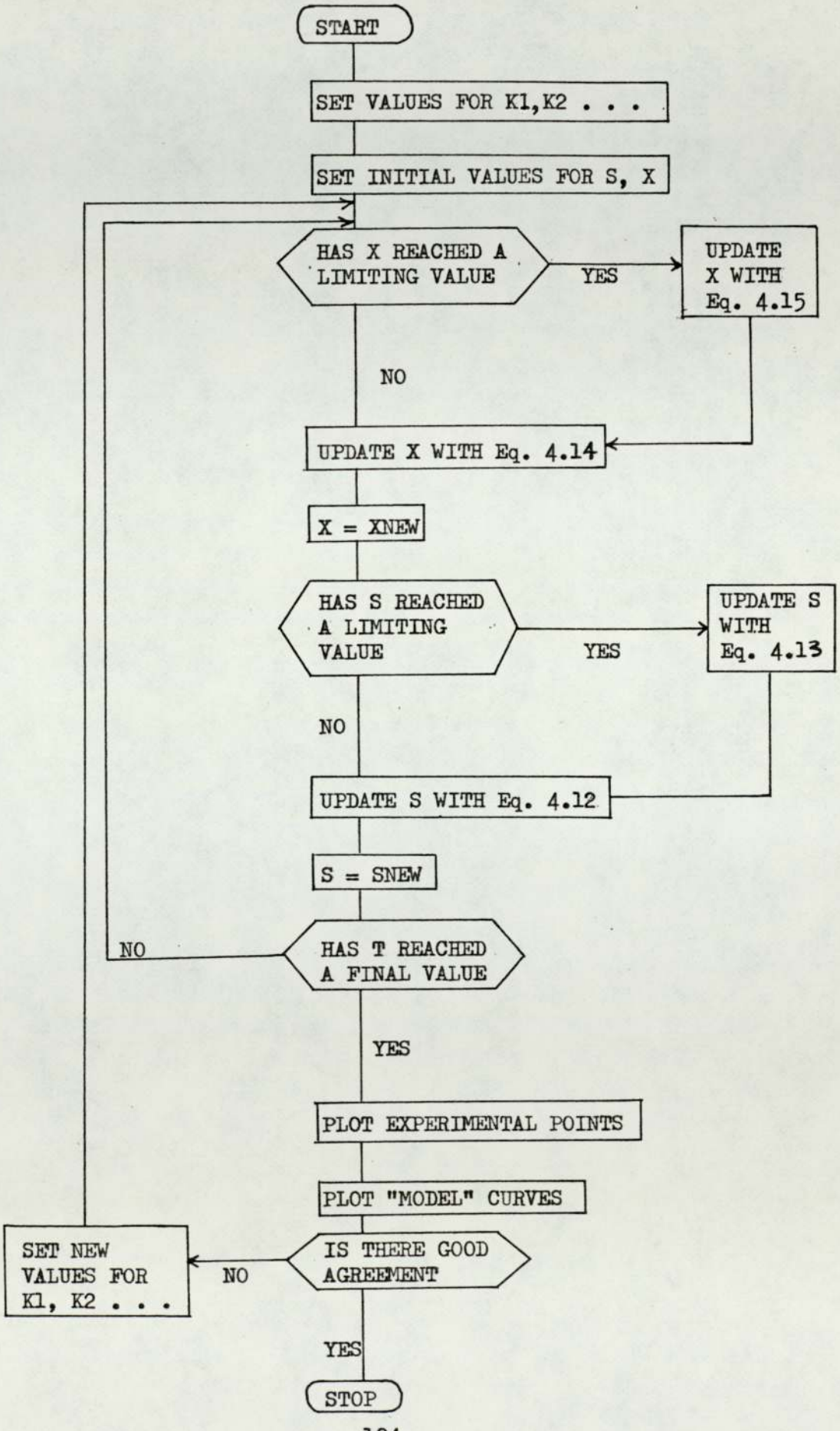
The notation has been altered slightly from that presented in the text for computational reasons so that:

$$K2 \equiv K_m,$$

$$K3 \equiv \mu_m,$$

and $$K4 \equiv K_s.$$

FIG. 4.5 Simulation of the Batch Beer Fermentation and Comparison with Experimental Work



model was tested for numerical stability by varying the step length of integration between 0.01, 0.1, 1, and 5 hours. Only at the 5 hourly interval did the model become unstable.

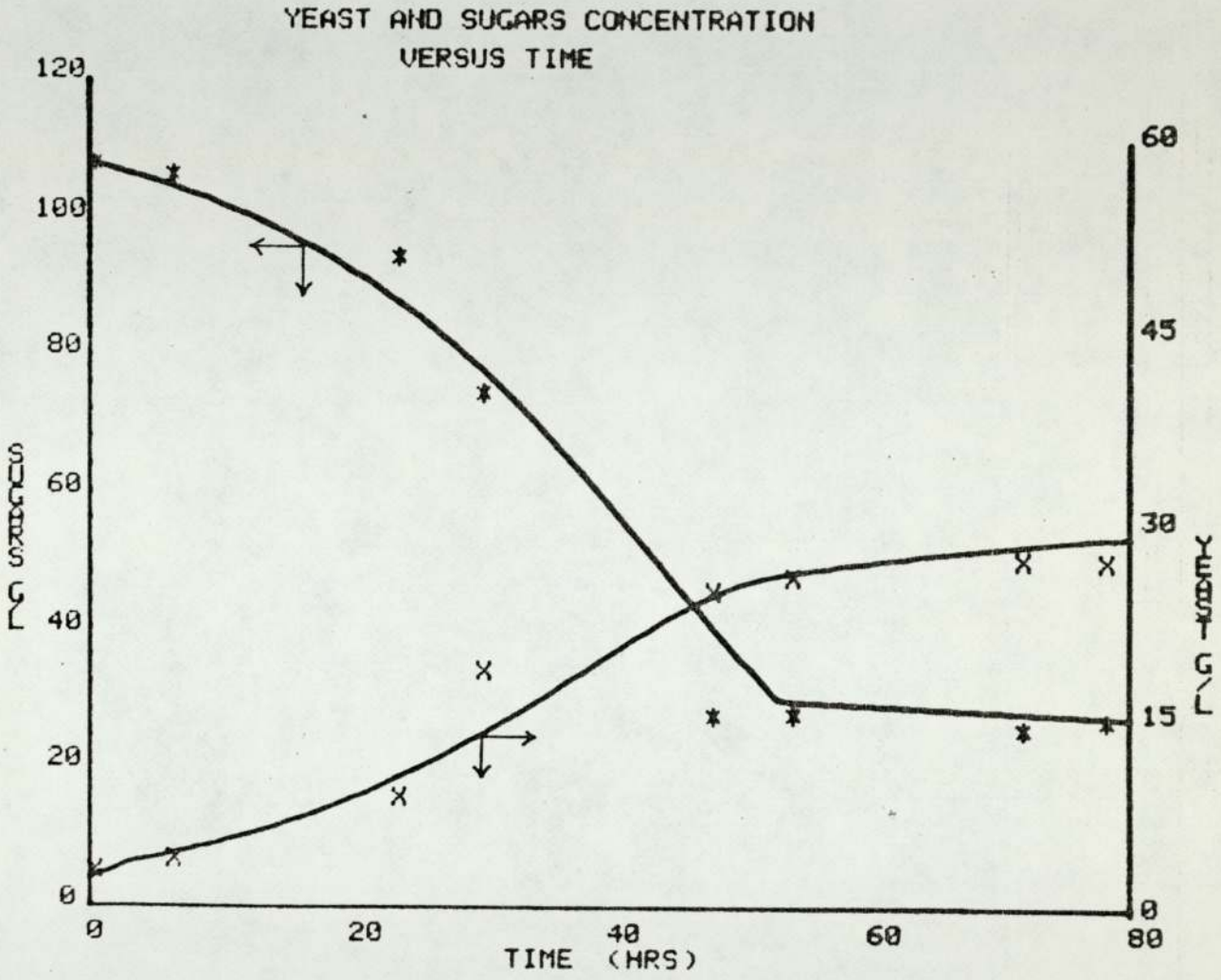
4.6.3 Results and Discussion

The results of employing this model are shown in Figs. 4.6 - 4.14. The predicted model curves are shown as continuous lines: experimental measurements are represented by points on these figures. As can be seen the predicted curves closely fit the experimental points on most of the graphs.

Different patterns of cellular growth are evident. For instance, in experiment nos. 2 and 3, (Figs. 4.7 and 4.8), relatively high concentrations of yeast were produced: this may have been due to excessive oxygen gaining access to the broth. In experiment No. 6, (Fig. 4.10), the initial yeast growth was rapid, probably as a result of the use of a relatively large inoculum. Fig. 4.12 shows a noticeable peak in the biomass concentration. In all these cases the model was sufficiently flexible to account for the variations. The model was also used to describe published data (see Figs. 4.13 and 4.14).

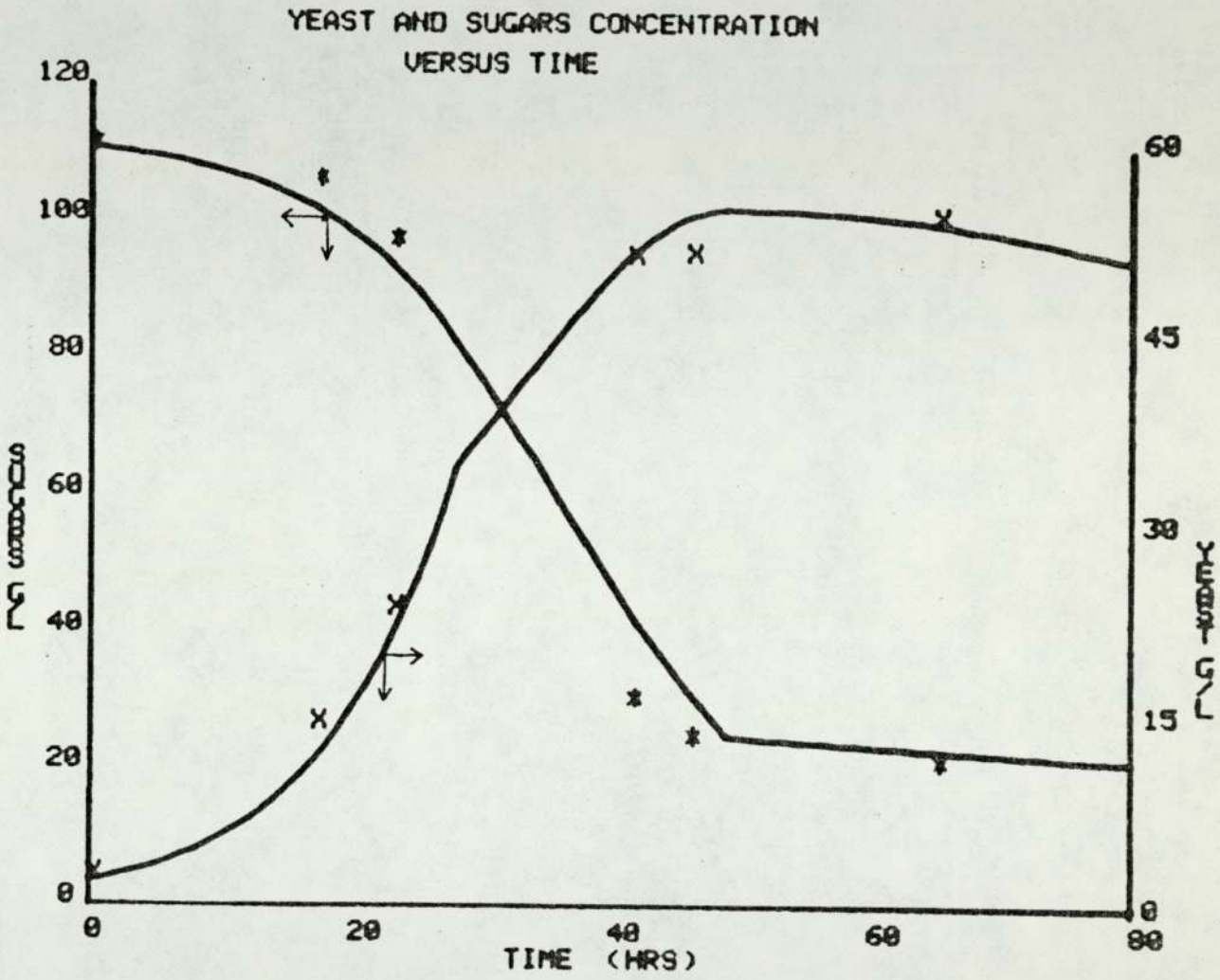
The values of the constants K1 $\longrightarrow$ K6 calculated for each experiment are shown in Table 4.5. The first item of note is that K3 (corresponding to μ_m) has a mean value of $0.129h^{-1}$, which is of the same order of magnitude as figures reported by other authors, (see Table 4.3). The values of the constants K2 and K4, corresponding to K_m and K_s respectively, were found to be much higher than those obtained by previous authors for experiments involving yeast growth. Mean values of 52.9 g/l

FIG 4.6 : EXPT. NO.1



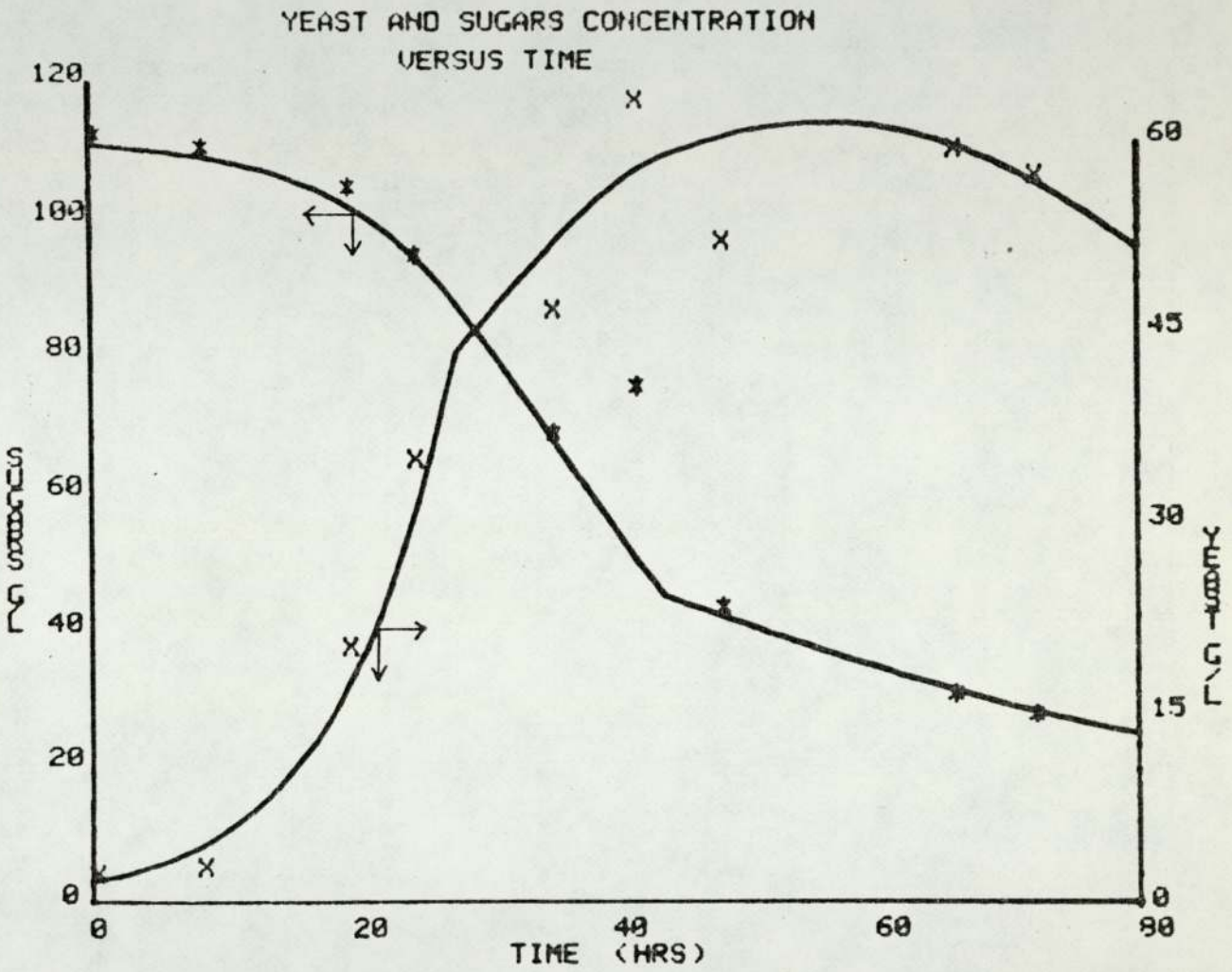
K1 = 0.22
K2 = 60
K3 = 0.17
K4 = 25
K5 = 0.01
K6 = 0.087
$X^* = 3.8$
$S^* = 30$

FIG. 4.7 : EXPT. NO. 2



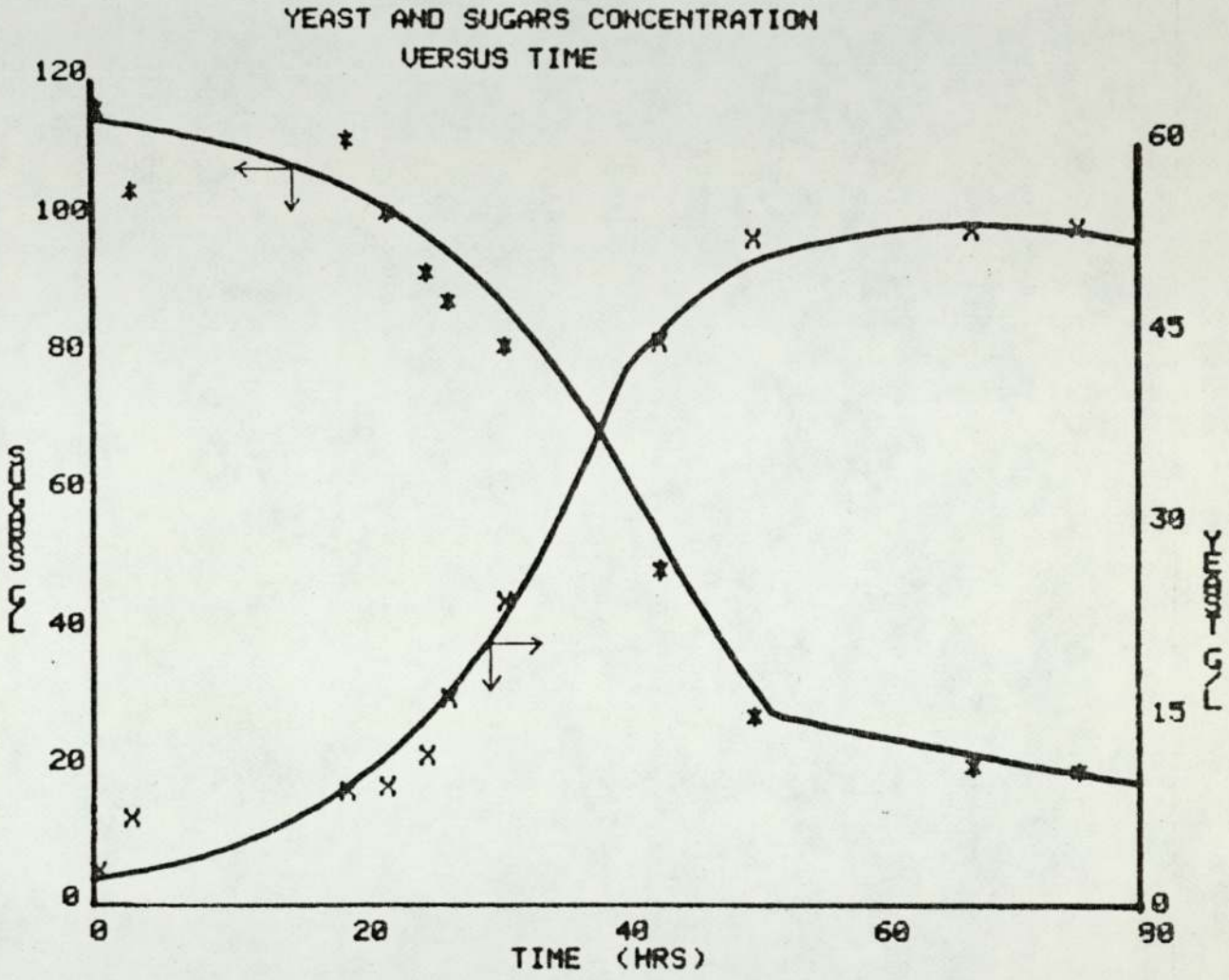
K1 = 0.16
K2 = 85
K3 = 0.13
K4 = 31
K5 = 0.01
K6 = 0.058
$X^* = 35$
$S^* = 25$

FIG. 4.8 : EXPT. NO. 3



K1 = 0.095
K2 = 55
K3 = 0.145
K4 = 30
K5 = 0.025
K6 = 0.08
$X^* = 43$
$S^* = 45$

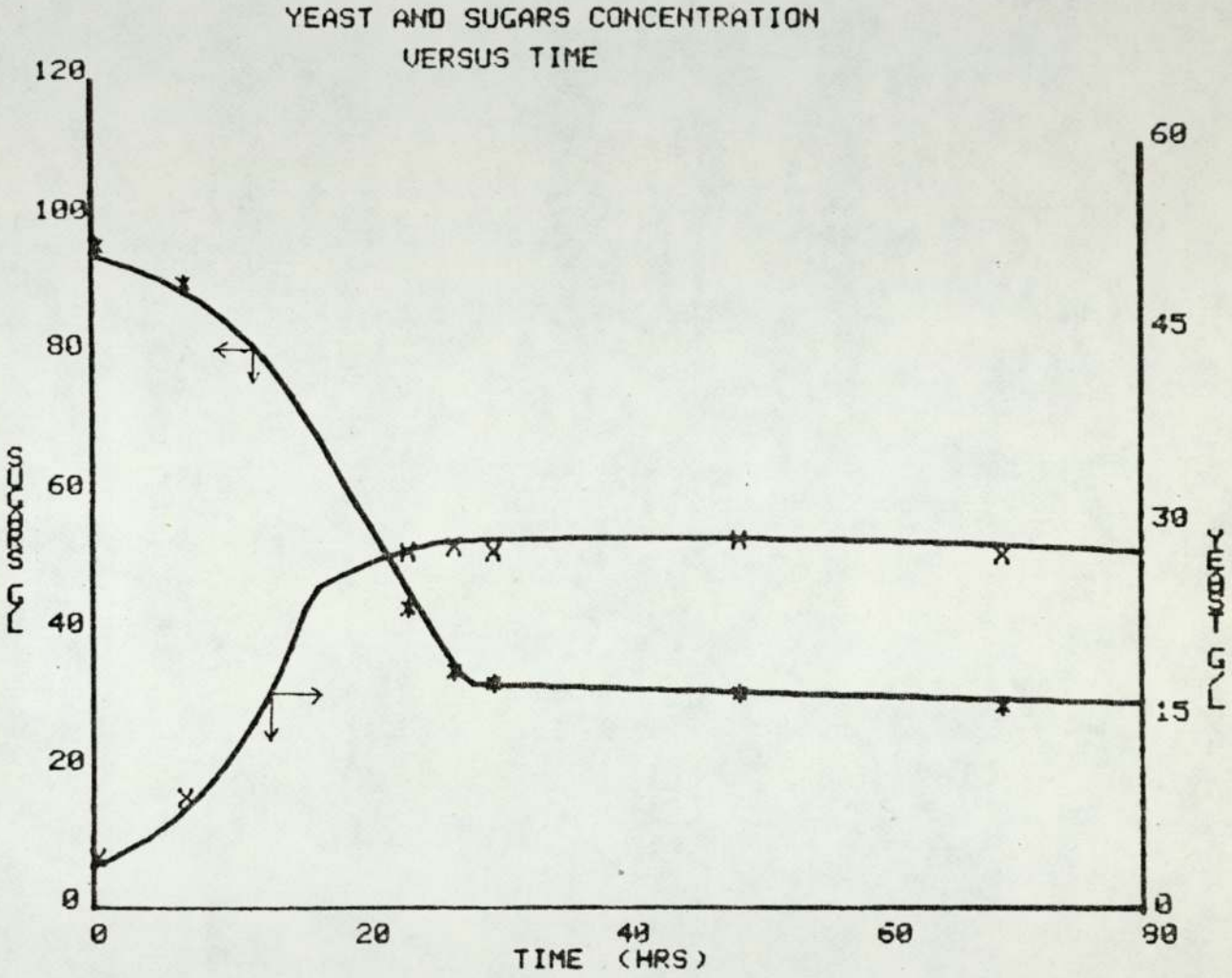
FIG. 4.9 : EXPT. NO. 5



K1 = 0.142
K2 = 52
K3 = 0.102
K4 = 42

K5 = 0.022
K6 = 0.035
X^* = 42
S^* = 28

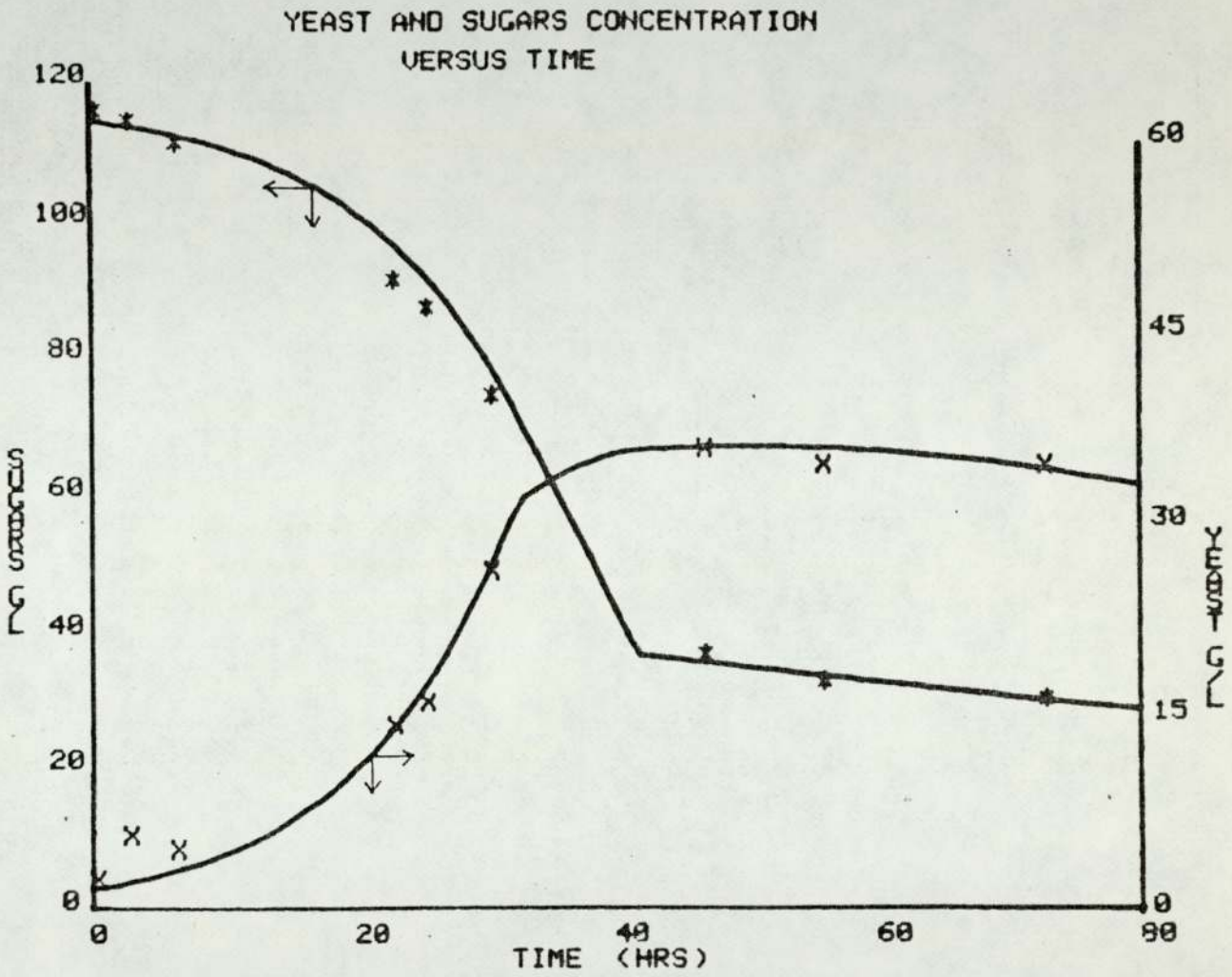
FIG. 4.10 : EXPT. NO. 6



K1 = 0.195
K2 = 35
K3 = 0.14
K4 = 15

K5 = 0.004
K6 = 0.095
X^* = 25
S^* = 32.5

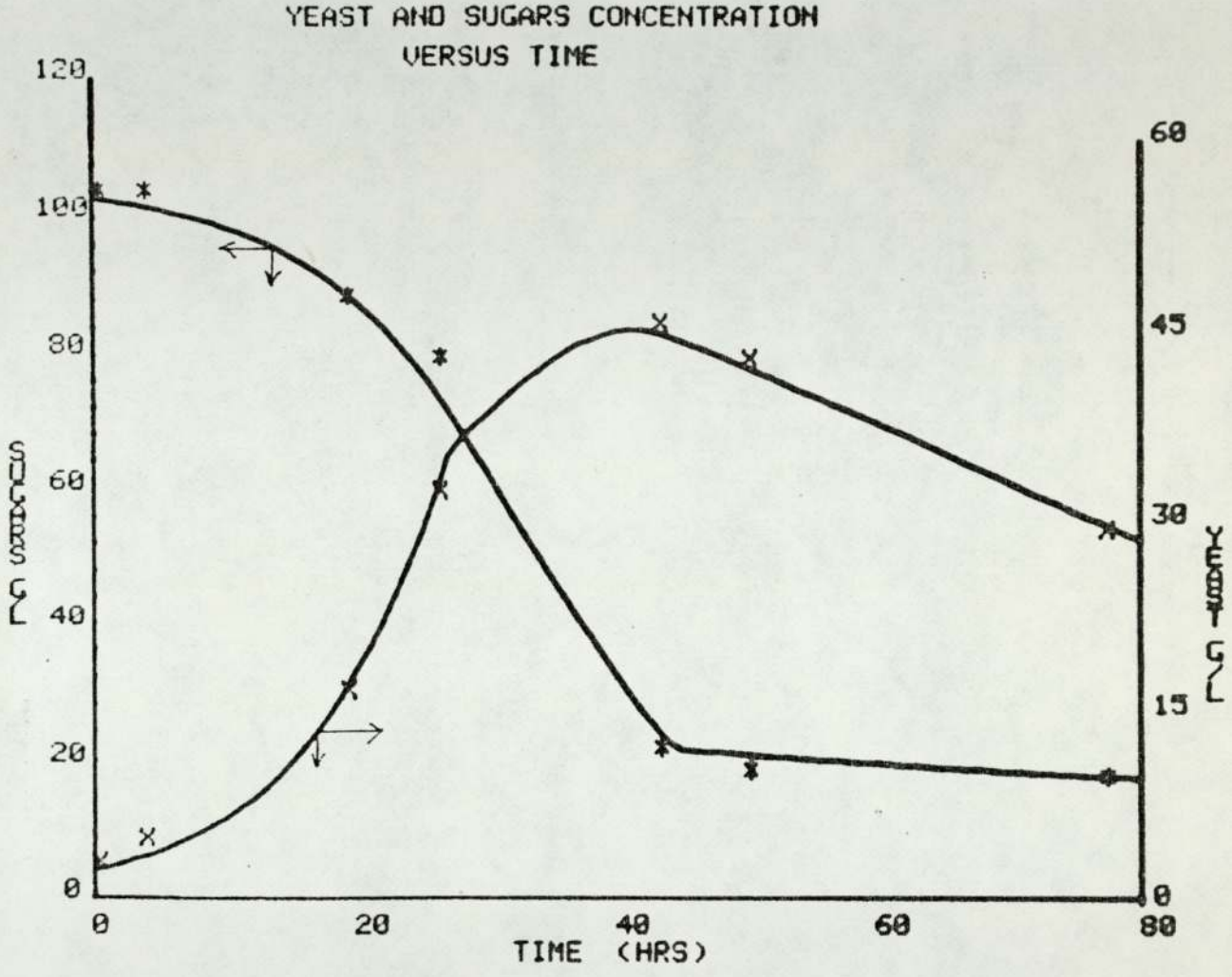
FIG. 4.11 : EXPT. NO. 7



K1 = 0.152
K2 = 22
K3 = 0.125
K4 = 43

K5 = 0.01
K6 = 0.056
X* = 32
S* = 37

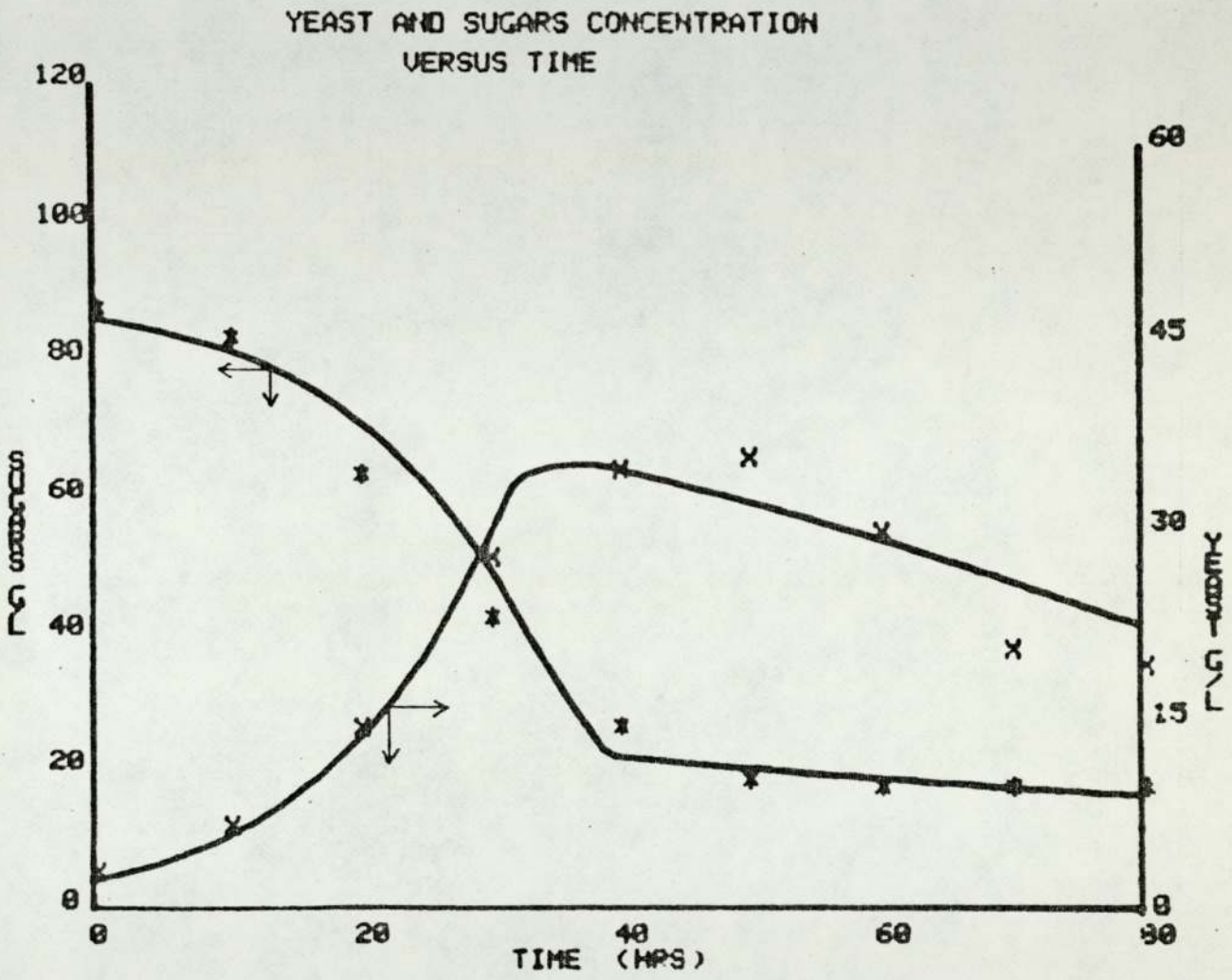
FIG. 4.12 : EXPT. NO. 8



K1 = 0.135
K2 = 42
K3 = 0.135
K4 = 35

K5 = 0.01
K6 = 0.061
X* = 35.5
S* = 22

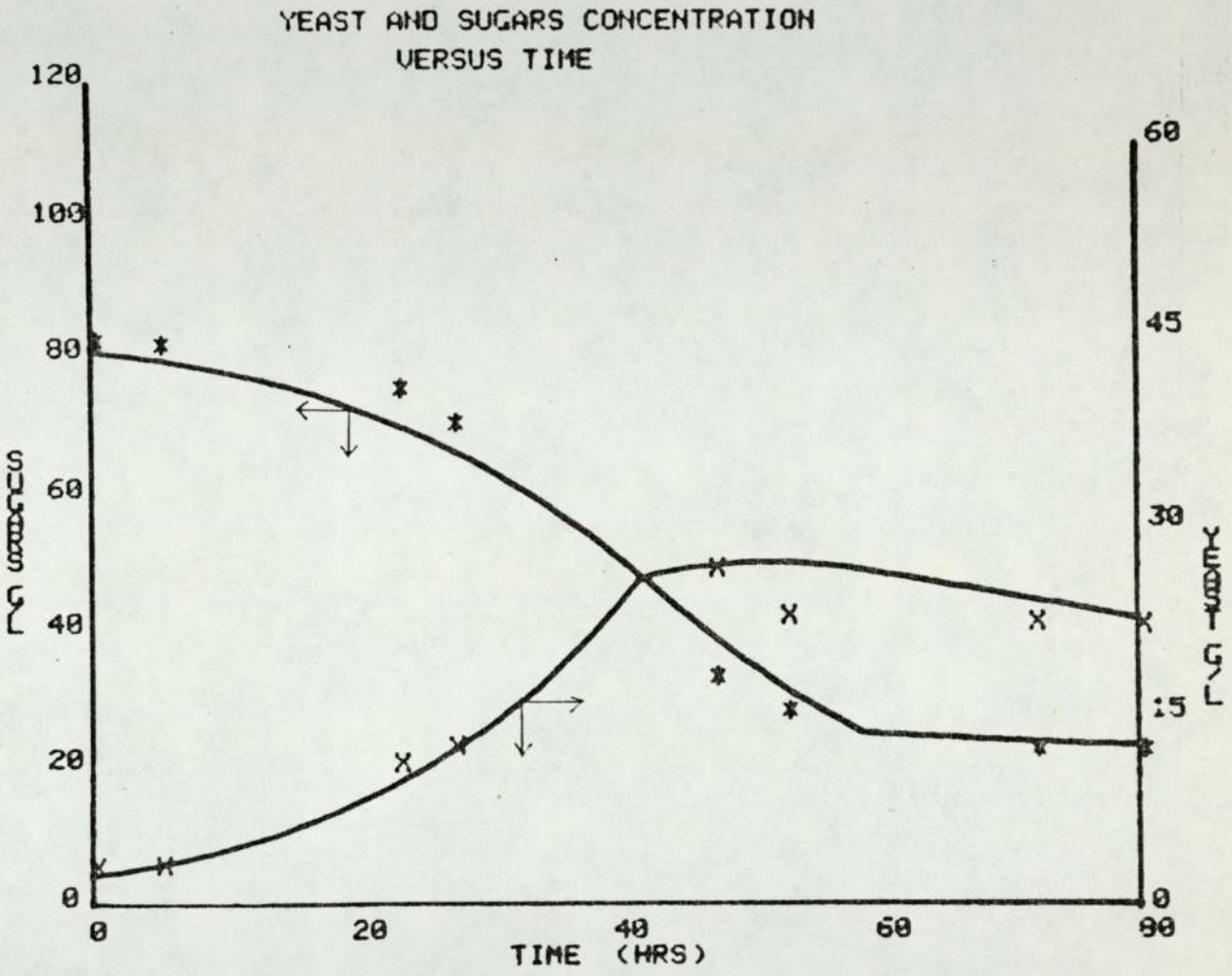
FIG. 4.13 : BROWN AND KIRSOP (1972)



K1 = 0.14
K2 = 20
K3 = 0.115
K4 = 30

K5 = 0.01
K6 = 0.055
X* = 34
S* = 22

FIG. 4.14 : GRIFFIN (1970a)



K1 = 0.2
K2 = 105
K3 = 0.095
K4 = 50

K5 = 0.02
K6 = 0.038
X^* = 26
S^* = 25

TABLE 4.5 Calculated Constants for the Batch Beer Fermentation Model.

Expt. NO.	K1 H^{-1}	K2 G/L	K3 H^{-1}	K4 G/L	K5 H^{-1}	K6 H^{-1}	X^* G/L	S^* G/L
1	.220	60	.170	25	.010	.087	3.8	30
2	.160	85	.130	31	.010	.058	35	25
3	.095	55	.145	30	.025	.080	43	45
4	.142	52	.102	42	.022	.035	42	28
6	.195	35	.140	15	.004	.095	25	32.5
7	.152	22	.125	43	.010	.056	32	37
8	.135	42	.135	35	.010	.061	35.5	22
9*	.140	20	.115	30	.010	.055	34	22
10**	.200	105	.095	50	.020	.038	26	25

* Data from Brown and Kirsop (1972)

** Data from Griffin (1970a)

and 33.4 g/l were found for K_m and K_s respectively with the author's model: published values of K_s for yeasts and most other micro-organisms are of the order of decades of milligrammes per litre of medium. However, such low values for the saturation constant are not always found, and Monod (1949) recorded that exceptionally large values of K_s have occurred in a few instances. A number of such cases are given in Table 4.6. Finally it should be noted that the values of K_s and K_m deduced here are of the same order of magnitude as those found by Fidgett (1975) when using a more complex model of batch beer fermentation.

The model is essentially similar to many of the models derived by other workers to account for endogenous metabolism and organism decay (section 4.4.2). A range of decay constants is given in Table 4.7. The mean value found here for K6 (K_d) was 0.0628, which is of the same order of magnitude as values published by other authors, Table 4.7.

4.7 Conclusions

A model which describes and accounts for the various processes occurring in the batch beer fermentation has been developed. Though the model is empirical in nature it can account for wide variations in the results from the fermentation, such as a relatively high yeast concentration. The model is essentially similar to other kinetic models used to account for microbial growth processes in which endogenous metabolism and organism decay cannot be ignored. However, the values of the saturation constants are generally higher than those found by other workers, though of the same order of magnitude as those deduced by Fidgett (1975) when using a more complex model of the alcohol fermentation. The values of

TABLE 4.6 Values of the Saturation Constant, K_s, Found by various Authors.

K_s g/l	Comments	Author(s)
0.033	Torula utilis grown on molasses	Fencl (1966)
2-28	Range of values for the individual sugars involved in the batch beer fermentation	Fidgett (1975)
0.0013 -0.04	Aerobic growth model for yeast	Mason and Mills (1976)
3.91	Mycobacterium tuberculosis grown on glucose	Moser (1958)
0.004 - 0.015	Aerobic growth of bacteria	Sinclair and Topiwala (1970)
66 and 166	Values for activated sludge kinetics	Yang (1977)
0.65 and 1.2	Candida utilis glucose limited and hexadecane limited respectively	Ziegler (1977)

TABLE 4.7 Decay Constants for Organism Growth

K_d g/l	Comments	Author(s)
0.24-0.60	A model describing the aerobic growth of yeast	Mason and Mills (1976)
0.026 -0.21	A "total" for the combination of an endogenous metabolism constant and death rate constant	Sinclair and Topiwala (1970)
0.0142	A cellular maintenance coefficient for activated sludge	Yang (1977)
0.0147 -0.0189	Values for various models with decay terms added	Chiu et al. (1972)
0.038-0.095	The range of values found in this work	

other constants, such as the maximum specific growth rate, are similar to those found experimentally by other authors. These facts, taken together, support the validity of the model.

CHAPTER 5

CONTINUOUS FERMENTATION EXPERIMENTS

5.1 Introduction

This section of the thesis describes the design and operation of a small-scale tower fermenter. A number of continuous fermentations carried out in this system using both wort and molasses media are discussed. These experiments were conducted in order to gain both physical snd chemical insight into the operation of the fermenter. It was also intended to combine this information with that from the study of batch fermentation kinetics in order to develop a model for the continuous tower fermenter.

The observations made are compared with the limited amount of published data, and some suggestions are made for the continuous stable operation of tower fermenters.

5.2 Fermenter Design, Ancillary Equipment and Operation

The Fermenter

The initial fermenter design was carried out based on the observations of other authors. In early work with tower fermenters Royston (1961) and Shore and Watson (1961) used tower height: diameter (H:D) ratios of 20:1 and 11:1 respectively. For small scale experiments Coote (1974) used a fermenter having a lower section with dimensions of 175 cm x 2.54 cm (H:D = 68.9:1) and an upper section with dimensions of 54 cm x 8 cm. Klopper et al. (1965) experimented with both lager and ale production in pilot scale fermenters. For lager production a tower 4 m high by 150 mm diameter (H:D = 26.7:1) was used. Initially for ale

production a fermenter of dimensions 1.83 m x 152 mm was employed. However, homogeneous conditions developed in this vessel and it was necessary to increase the height to 4.57 m to prevent the tower operating as a stirred reactor: thus a similar configuration as employed with the first fermenter was found necessary. The above H:D ratios are in marked contrast to those for production-scale fermenters described by Klopper et al. (1965), Royston (1966b), Ault et al. (1969), and den Blanken (1974): typical tower dimensions are 7 m high by 0.9 m diameter (H:D = 7.78:1).

In this work a column diameter of 76 mm was selected. This size was used in an attempt to avoid wall effects described by Coote (1974): he observed plugs of yeast, the diameter of the column, rising up the tower on cushions of evolved carbon dioxide. Bearing in mind the work of Klopper et al. (1965) with a pilot scale fermenter, a height:diameter ratio of 30:1 was aimed for : this led to the use of an eventual tower height of 2.48 m (H:D = 32.5:1).

The head of the fermenter or "separation zone" has been designed to different volume ratios relative to the main section of the fermenter. Shore et al. (1962) set the head volume equal to the volume of the tower, whereas Coote (1974) used a seperation zone of volume slightly greater than three times the volume of the tower. den Blanken (1974) has reported that commercial fermenters have 32.5% of the total capacity in the head of the fermenter: this is confirmed by figures given by Ault et al. (1969), who used a fermenter with a tubular section of 27 brls. ($4.419 m^3$) capacity and a head of 13 brls ($2.128 m^3$) capacity.

The diameter of the head of the experimental fermenter used by the author was twice the column diameter, 152 mm, with the liquid outlet 290 mm above the top of the straight section. The volumes of the lower and upper sections of the fermenter were 11.31 l and 4.74 l respectively. The tower fermenter is shown in Fig. 5.1 The yeast separator, shown diagrammatically in Fig. 5.2, was based on a design initially described by Klopper et al. (1965). Other designs were tested but the design shown in Fig. 5.2 was the simplest one that gave trouble-free operation.

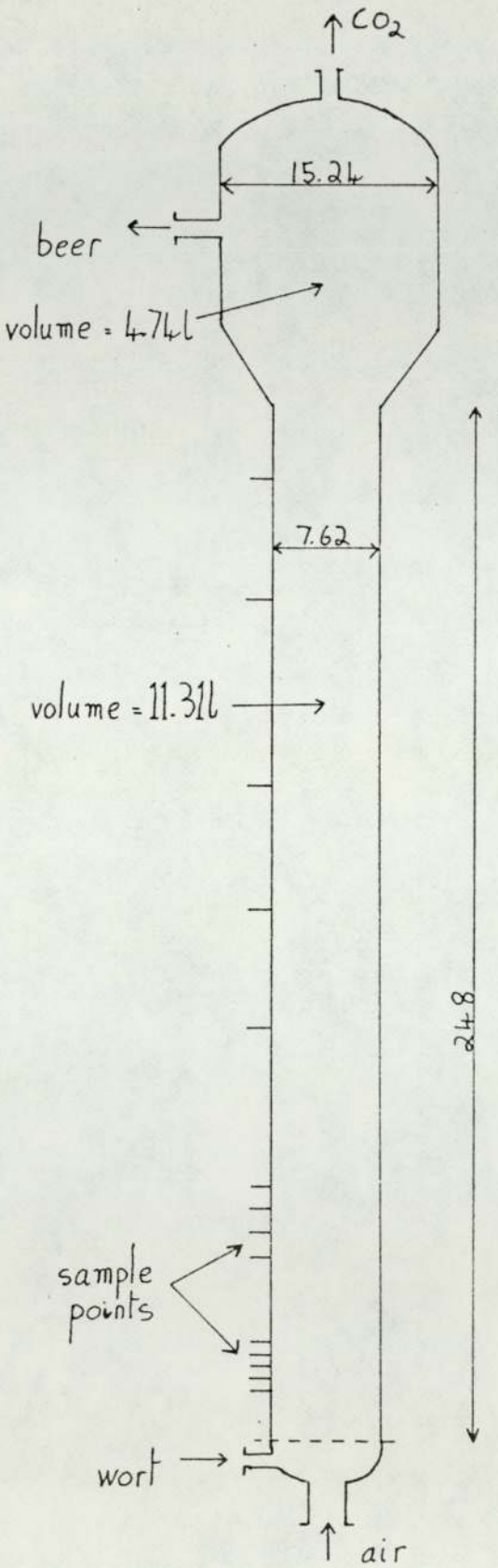
Ancillary Equipment

A flowsheet of the process is shown in Fig. 5.3

Sterile medium was held in 20 l glass aspirators, (1). The aspirators were fitted with expanding bung closures (L. H. Engineering Co. Ltd.) and a Whatman Gamma-12 grade 03 air filter, (2), to prevent ingress of bacteria as the medium was utilised. The medium was pumped from storage via a D.C.L. micro-metering pump, (3), (Metering Pumps Ltd.) through a "surge" vessel, (4). This system provided a constant flow to the rotameters, (5): (G. A. Platon Ltd.), which covered the ranges 0-80cm^3/min. and 20-300 cm^3/min. of water: they were recalibrated for medium prior to being used in the system. The medium entered the tower, (6), below a distributor plate, (7).

The tower was constructed of standard 3 inch N.B. Q.V.F. glassware sections. A separating device, (8), allowed clear, fermented liquor to overflow into a receiver, (9). The temperature in the tower was controlled by a chiller and coils, (10).

FIG 5.1 THE EXPERIMENTAL TOWER FERMENTER.



dimensions in cm

FIG 5.2 THE YEAST SEPARATOR

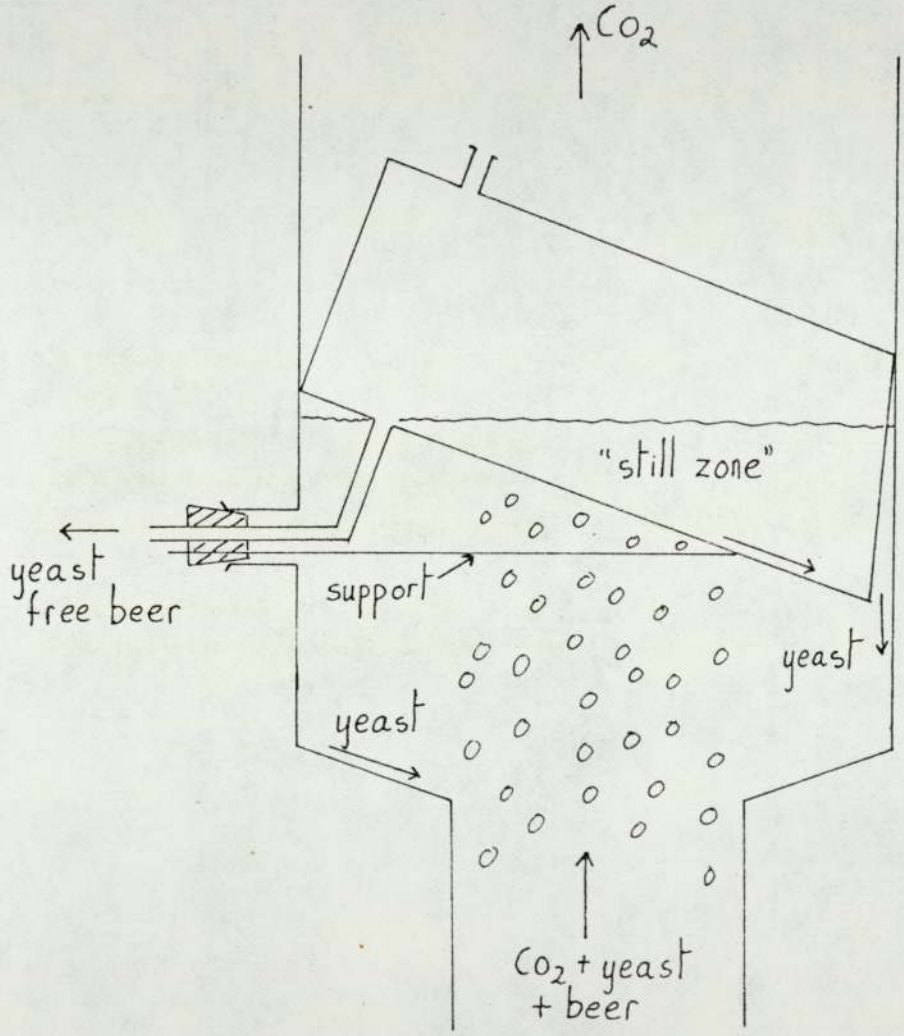
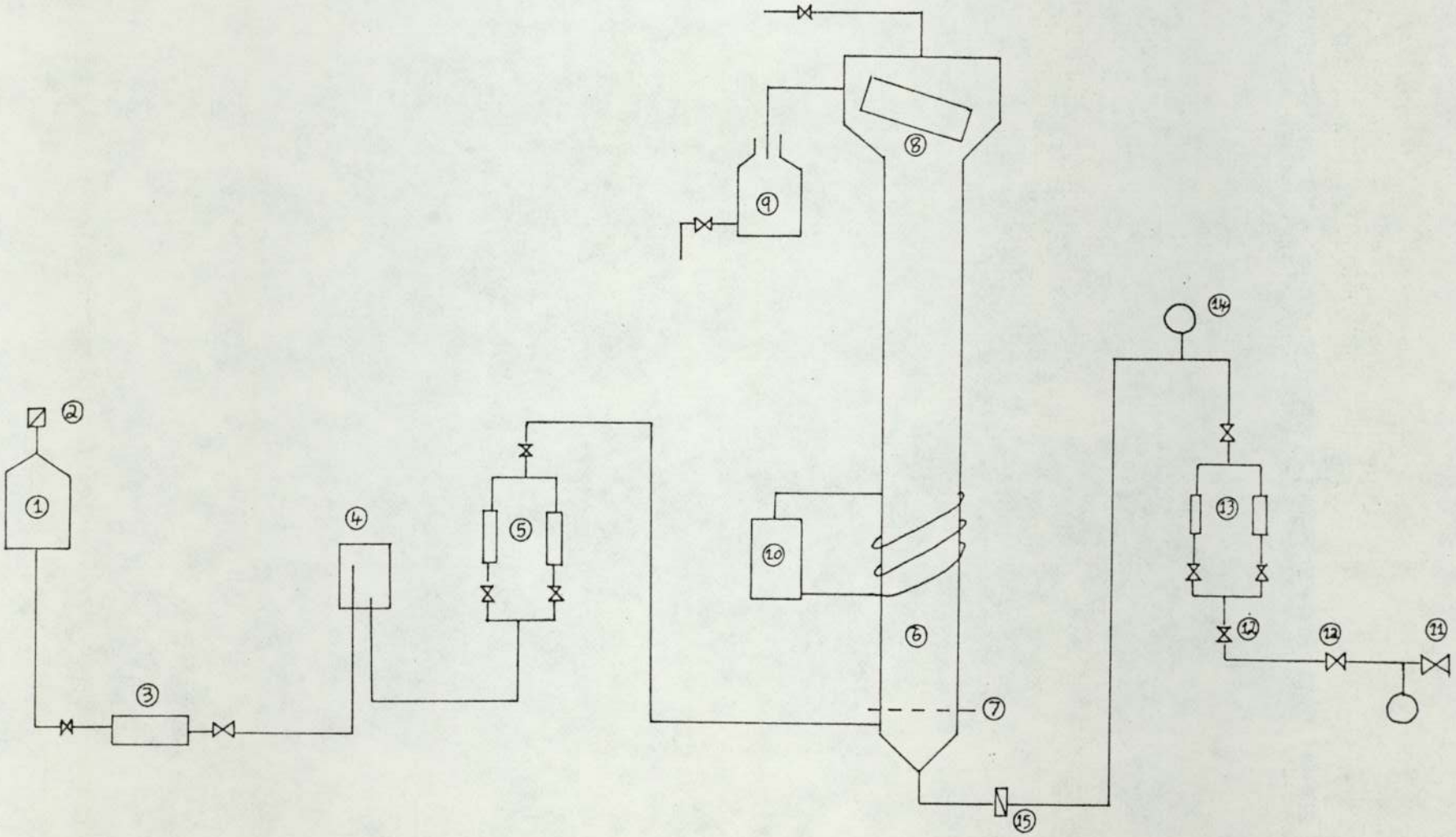


FIG 5.3 EXPERIMENTAL EQUIPMENT FLOWSHEET.



Compressed air was fed to the tower initially through a combined pressure reducer and grade 03 air filter, (11), (Spirax-Monier). The air-flow was controlled by needle valves, (12), (Brooks Instruments) and measured by rotameters, (13), (Glass Precision Eng. Ltd.). These rotameters were calibrated for 0-70cm^3/min. and 50-1300 cm^3/min. of air at S.T.P. The calibration charts were checked and found to be accurate at various pressures up to 103.4 kN/m^2 gauge (15 p.s.i.g.). Air pressure into the tower was monitored by a gauge, (14), and the air passed through a final "sterile" filter, (15), before entering the tower.

Fermenter Operation

Wort and yeast inocula were prepared as detailed in section 3.6

Prior to inoculation the fermenter and associated lines were steamed out for 12 hours with low pressure steam. When the tower was judged ready for innoculation an autoclaved filter and housing were fitted in the air-supply line at the base of the fermenter. The air flow was then started as the steam supply was cut-off; in this way the system was filled with sterile air on condensation of any steam in the system.

A quantity of sterile wort was then pumped into the fermenter, and using a sterile syringe, a 10 cm^3 aliquot was taken from a shake-flask containing asceptically grown yeast. The fermenter was innoculated via one of the sample ports, which was washed thoroughly with industrial methanol before and after innoculation. A relatively high air flow-rate was maintained at innoculation to ensure a positive pressure within the fermenter.

The air and medium flow-rates were then set as required.

5.3 Experimental Programme : Materials and Methods

Five continuous experiments were carried out in the tower fermenter. Three experiments were effected with brewers' wort and the yeasts CFCC1, CFCC 39, and CFCC 54; strains of *Saccharomyces cerevisiae*, *S. carlsbergensis*, and *S. diastaticus* respectively. These experiments were effected in order to compare the performance of the three strains of yeast in the continuous fermenter. Previous batch experiments had shown all three yeasts to give similar batch fermentations (see section 3.9).

Two experiments were carried out with a molasses-based medium, using the strain of *S. cerevisiae*. The first of these experiments used a sterile medium, initially supplemented with ammonium salts, then unsupplemented. The second experiment employed a non-sterile medium after the fermenter had been started up asceptically.

The methods of analysis of the various variables are listed below.

Specific Gravity

Difficulties were experienced in measuring the specific gravity of the wort at various stages in the tower, especially in the lower zones of the column. This was due to the very high yeast concentrations in the lower section of the fermenter. As samples were withdrawn they could be seen to be actively fermenting. To overcome this problem samples were run into standard volumes of formalin solution of known specific gravity. The samples were spun down, the S.G. of the mixture of formalin

and beer measured, and the S.G. of the beer then calculated.

Yeast Concentration

Measurements were effected by withdrawing measured volumes of broth and determining the yeast wet and dry weight as detailed in section 3.7.

Yeast Viability and Cell Counts

Measurements of the yeast population viability were carried out according to the methylene blue staining technique described by Townsend and Lindegren (1953). Cell counts were made using a haemocytometer.

Nitrogen Determinations

The cellular and medium nitrogen concentrations on a % w/w basis were calculated according to a micro-Kjeldahl technique attributed to Markham (1942).

Vicinal Diketones

The method of analysis used was that described by Ault (1968) and recommended by the Institute of Brewing, although Harrison et al. (1965) have commented that this method may lead to slightly higher levels of V.D.K.s than are actually present.

Dissolved Gas Measurements

Determinations of dissolved oxygen, nitrogen, and carbon dioxide were carried out with a mass spectrometer. Catheter probes were fixed permanently in the fermenter at various heights and the spectrometer connected to each of these in turn.

Sugar Analysis

In the author's original work programme, plans were made to measure the concentration of individual sugars in the wort at various heights in the fermenter. Tuning (1971) and Brobst (1972) have described a method of estimating the constituent

sugars present in brewers' wort. The method entails preparing the trimethylsilyl derivatives of the sugars and using gas chromotography to separate the various derivatives. However, with standard solutions of single sugars it proved impossible to obtain reproducible results when following the prescribed method; hence, this means of analysis had to be abandoned.

5.4 Results, Observations and Discussion

5.4.1 Visual Observations

Yeast Floc Behaviour

Observations of the fermenter were made daily, and detailed notes for each experiment can be found in Appendix 5.1. A video film of the fermenter in operation was made during experiment no. 13: the observations briefly described below are typical.

In the base of the fermenter, up to approximately 10 cm in height, there appeared to be individual yeast flocs, albeit very tightly packed. The majority of the flocs were in the size range 0.5-1.0 cm. Gas bubbles were also seen forcing paths through the yeast structure, without actually disturbing it. The floc structure gradually broke down as the yeast took the form of a slurry at a height above about 10 cm.

Between 30 cm and 60 cm the yeast plug was still tightly packed, with gas channelling through the yeast. The yeast plug now had the appearance of a thick, homogeneous slurry. This slurry was continually in motion, though movement was very slow. The yeast plug had an inherent circular motion with yeast tending to be carried up one side of the fermenter and drawn down the

other (see Fig. 5.4). A mean of a number of measurements showed the plug to be moving at approximately 5 cm/min. Gas bubble velocity through channels in the yeast bed varied between 0 and 6 cm/s.

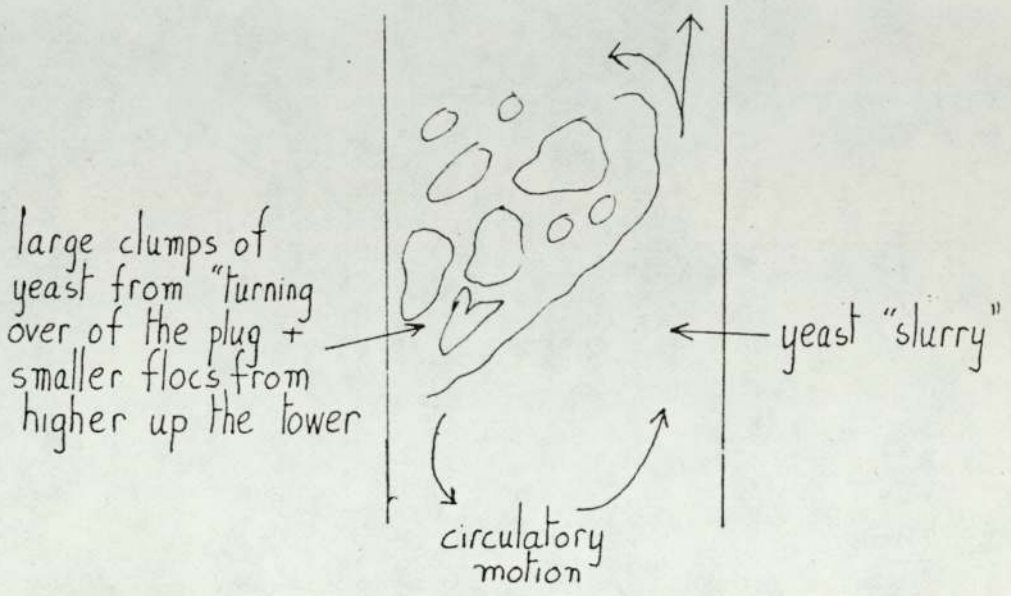
By a height of 90 cm the plug structure had broken down and a "transition zone" existed in the fermenter. Flocs up to 1 cm diameter were occasionally evident, but the majority of the flocs were between 1 mm - 2 mm diameter. The flocs were either being carried upwards or sedimenting rapidly. Occasionally, there would be very little movement and the yeast flocs would almost "waft" to and fro: at other times violent agitation was apparent, obviously due to the sudden release of carbon dioxide gas.

In the upper section of the fermenter the appearance was much as around the 90 cm height, though with a lower yeast concentration and no large yeast flocs. An estimate of gas bubble velocities gave values in the range 3 cm /s - 8 cm /s. This was probably dependent on whether the upward motion of the bubble had been affected by either upward or downward drag from yeast flocs or liquid. The gas bubbles were of a uniform diameter of the order of 1.5 mm.

System Behaviour during Shut-down

During experiment no. 11 the fermenter was closed down for 64 hours, though a low air supply was maintained to attempt to retain some pattern of yeast distribution. However, the yeast settled into a dense plug at the base of the fermenter. When the wort flow was re-started a compact plug of yeast was initially forced up the fermenter. Large, apparently non-porous

FIG. 5.4 YEAST MOTION WITHIN THE YEAST PLUG.



flocs broke off from the base of this plug and settled to the base of the fermenter. "Explosions" of gas from the plug then occurred due to wort penetration of the plug, fermentation, and subsequent carbon dioxide evolution. The compact yeast plug then began to break up into smaller flocs and within 8 hours the fermenter was running smoothly and normally.

Shut-down of the fermenter has been discussed by two authors. den Blanken (1974) operating a tower fermenter for lager production stopped the fermenter at a number of weekends. However, this practice was not recommended as rapid yeast autolysis was said to have occurred. On the other hand, Ricketts (1971) reported that the tower fermenter can be closed down for up to 14 days with no adverse effects other than a slight rise in pH due to limited autolysis.

From the author's limited experience, Ricketts' (1971) view is more acceptable. During shut-down, the yeast, being highly sedimentary, rapidly settles out from the beer in the column. The beer thus remains as if it were barrelled, being virtually free of any yeast cells. A low flow of sterile air, or carbon dioxide, would maintain a positive pressure within the fermenter and prevent ingress of any contaminating micro-organisms.

Effect of Discontinuation of the Air Supply

As described previously, air was admitted directly into the base of the fermenter as a source of oxygen for the yeast. This air was also found to have an important effect on the physical operation of the fermenter. On closing off the air supply the plug of yeast could be made to lift from the base and rise up the tower. The rate of rise of the plug was less than the medium flow-rate and therefore some wort must still

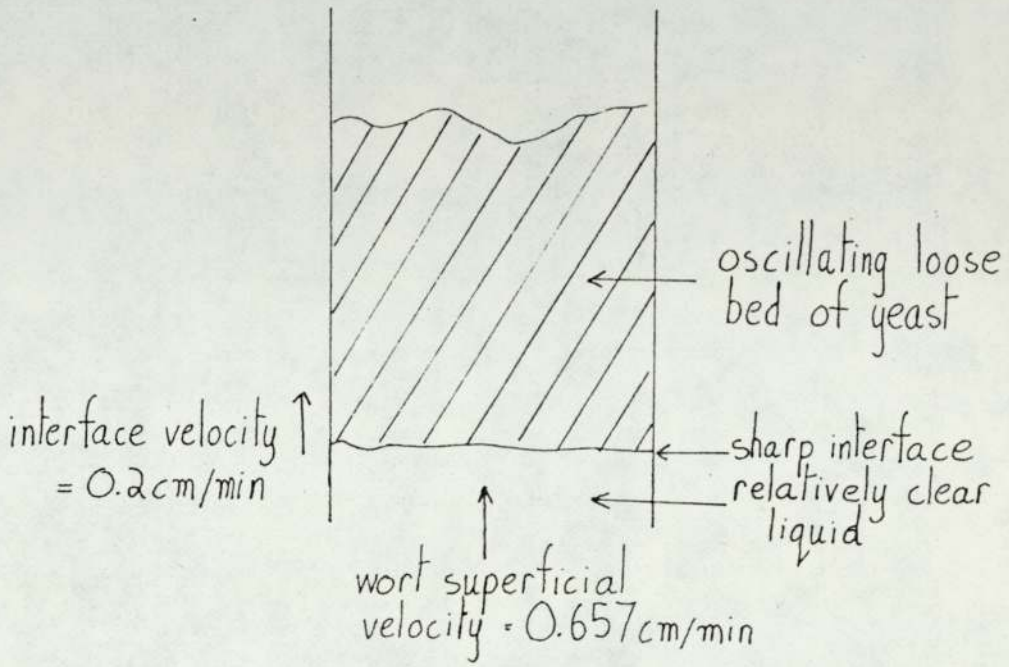
have been passing through the plug, otherwise no fermentation could occur. Fig. 5.5 shows the effect of discontinuing the air supply to the fermenter; this phenomenon was also recorded during video photography of the fermenter.

The air supply to the fermenter was also discontinued completely towards the end of experiment no. 13. Originally the fermenter was running with air and wort flow-rates of 9.3 cm^3/min. and 42 cm^3/min. respectively. On discontinuing the air flow the yeast plug began to rise up the tower as described above. A large amount of yeast was initially washed out of the fermenter.

The wort flow-rate was then reduced to 20.2 cm^3/min. and the wort/yeast interface at the base of the plug broke down somewhat allowing some yeast to re-settle to the base of the fermenter. The fermenter operated in a very unstable manner during this period: the sudden and periodic release of large volumes of carbon dioxide caused large outflows of medium and yeast every few minutes. Within 12 hours the outflow became smoother, although the main yeast plug had risen to 10 cm - 12 cm from the distributor plate. There was also an overall decrease in the yeast concentration within the fermenter, and the effluent present gravity (P.G.) began to increase. Within 48 hours of commencing anaerobic operation the P.G. had risen from 8.0 to 9.5 and within 96 hours to 15.0. This coincided with a change in the yeast morphology from creamy, yellow, and densely flocculent to small, powdery brown flocs which had a very open structure.

The medium flow-rate was then reduced further to 14.7 cm^3/min.

FIG. 5.5 THE EFFECT OF DISCONTINUING THE AIR SUPPLY TO THE TOWER FERMENTER.



This caused an initial fall in the effluent P.G. to 12.5 from 16, but within 24 hours the P.G. had risen to 16.0 again. The condition of the yeast appeared to have deteriorated further and the experiment was therefore terminated. The decrease in the yeast concentration within the fermenter is shown in graph 5.1.

Effect of High Medium Flow-Rate

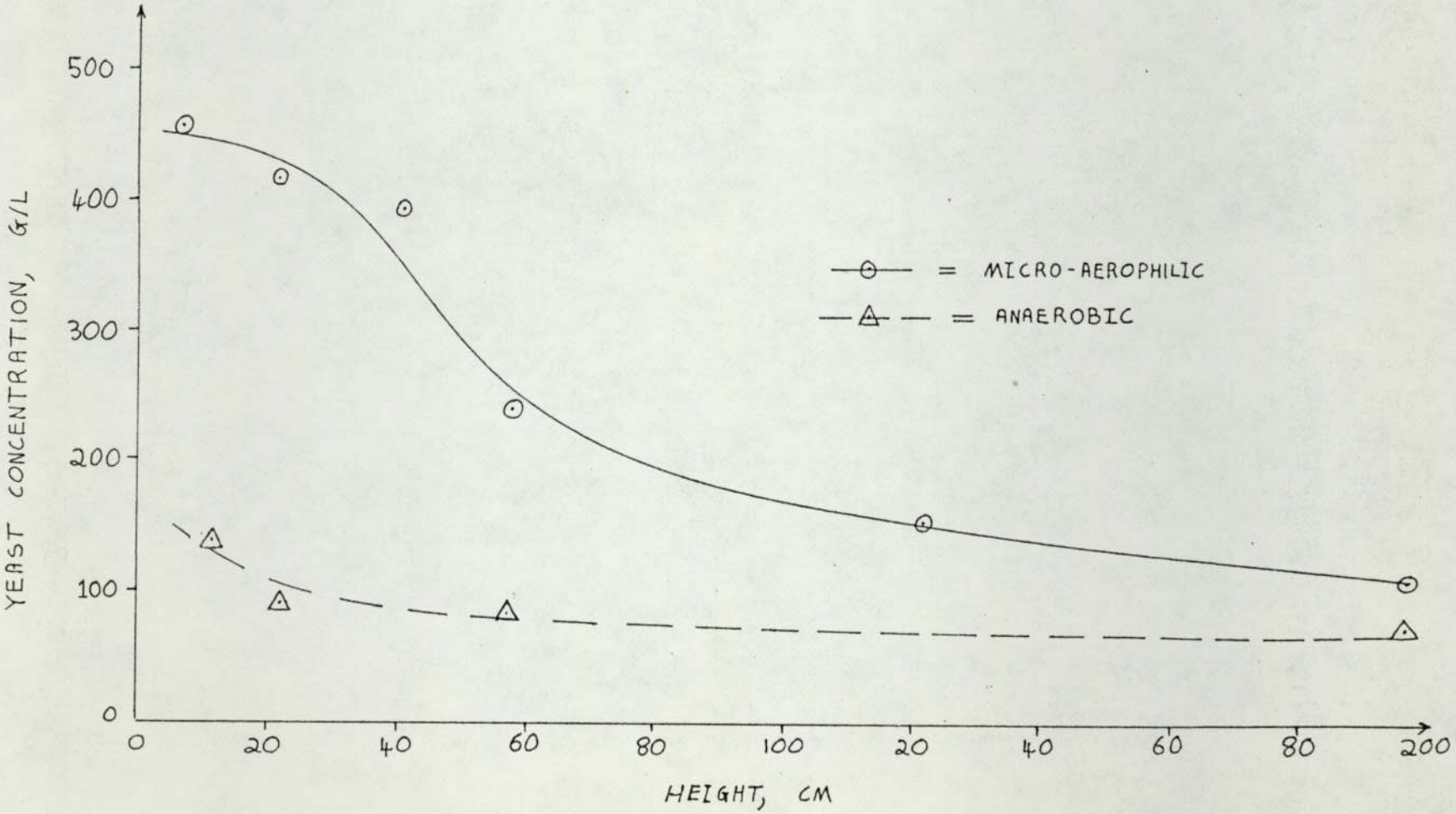
Prior to terminating experiment no. 14 an attempt was made to wash the yeast out of the fermenter, the medium flow-rate being increased from 35.5 cm^3/min. to 75.2 cm^3/min. This change resulted in a reduction in medium apparent residence time from 5.3 hours to 2.5 hours.

Initially, the yeast plug was forced 55 cm up the fermenter at a speed of 0.348 cm/min. (compared with a medium superficial velocity of 1.65 cm/min). The yeast/medium interface then broke down and the yeast re-settled to the base of the fermenter, although the floc structure was much more open and fluidised than at the lower medium flow-rate. Analysis of the yeast concentration profile showed a decrease at the base of the fermenter (graph 5.2), indicating that more homogeneous conditions may have developed at the higher flow-rate.

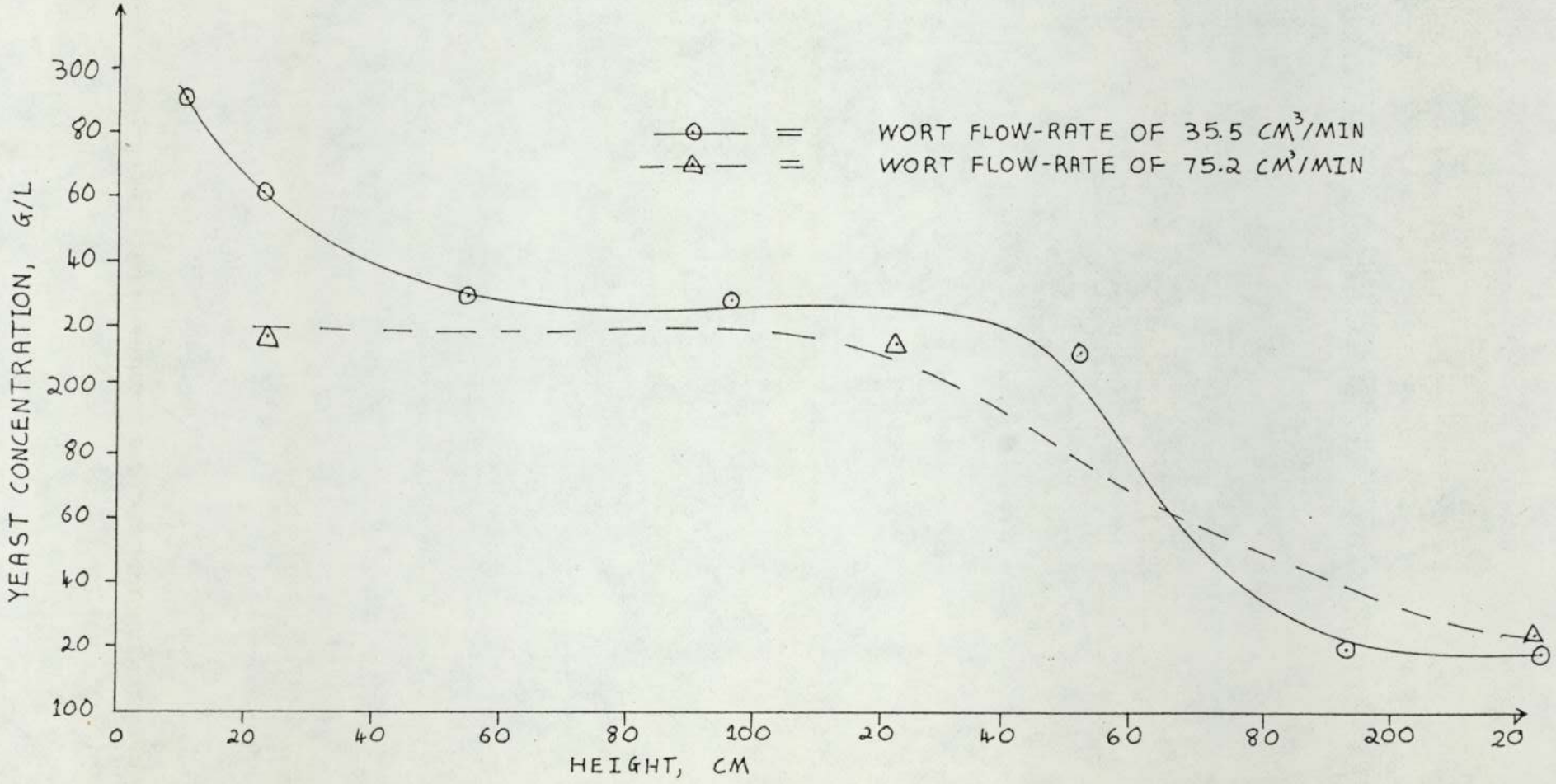
Immediately after increasing the wort flow-rate, the effluent P.G. rose to 19.0 from 14.0 and a quantity of yeast was washed out of the tower. However, within 11 hours the effluent P.G. had fallen back to 14.0 and then remained steady.

Because of the increased fermentation rate there was a marked increase in turbulence in the upper sections of the

GRAPH 5.1 COMPARISON OF FERMENTER YEAST CONCENTRATIONS, MICRO-AEROPHILIC AND ANAEROBIC (EXPT. NO. 13).



GRAPH 5.2 YEAST CONCENTRATION PROFILE VARIANCE WITH WORT FLOW-RATE (EXPT. NO. 14).



column due to increased carbon dioxide evolution.

Unfortunately, because of the high volumetric flow-rate, only a limited period of operation was possible.

Typical liquor superficial velocities in the author's tower fermenter were of the order of 15×10^{-3} cm/s, though a maximum of 27.5×10^{-3} cm/s, corresponding to a 2.5 hour residence time, was found to be possible. Typical medium flow-rates used by other authors, with their corresponding residence times and superficial velocities are given in Table 5.1. Experimentally found values of the latter mentioned variables are of the same order as found with production scale fermenters, though the experimental superficial velocity is lower: this may be expected to some extent because of the more concentrated medium used by the author.

5.4.2 Yeast Concentration Profile

Little work has been recorded concerning the yeast concentration profile in tower fermenters. Royston (1966b) found that at low wort flow-rates and wort densities the yeast formed a quasi-solid plug in the base of the fermenter. At higher wort flow-rates and higher wort densities the yeast concentration in the mid-section of the tower was 2-4 times that at the base, as a result of the yeast plug lifting. The lifting of the yeast plug was attributed to a combination of the de-flocculating effect of fresh wort and increases in the wort flow-rate.

Royston (1966b) reported that the average yeast concentration in the fermenter was 250 g/l, measured as centrifuged wet yeast. Results published by Ault et al. (1969) were in general agreement with those of Royston (1966b), although they found a

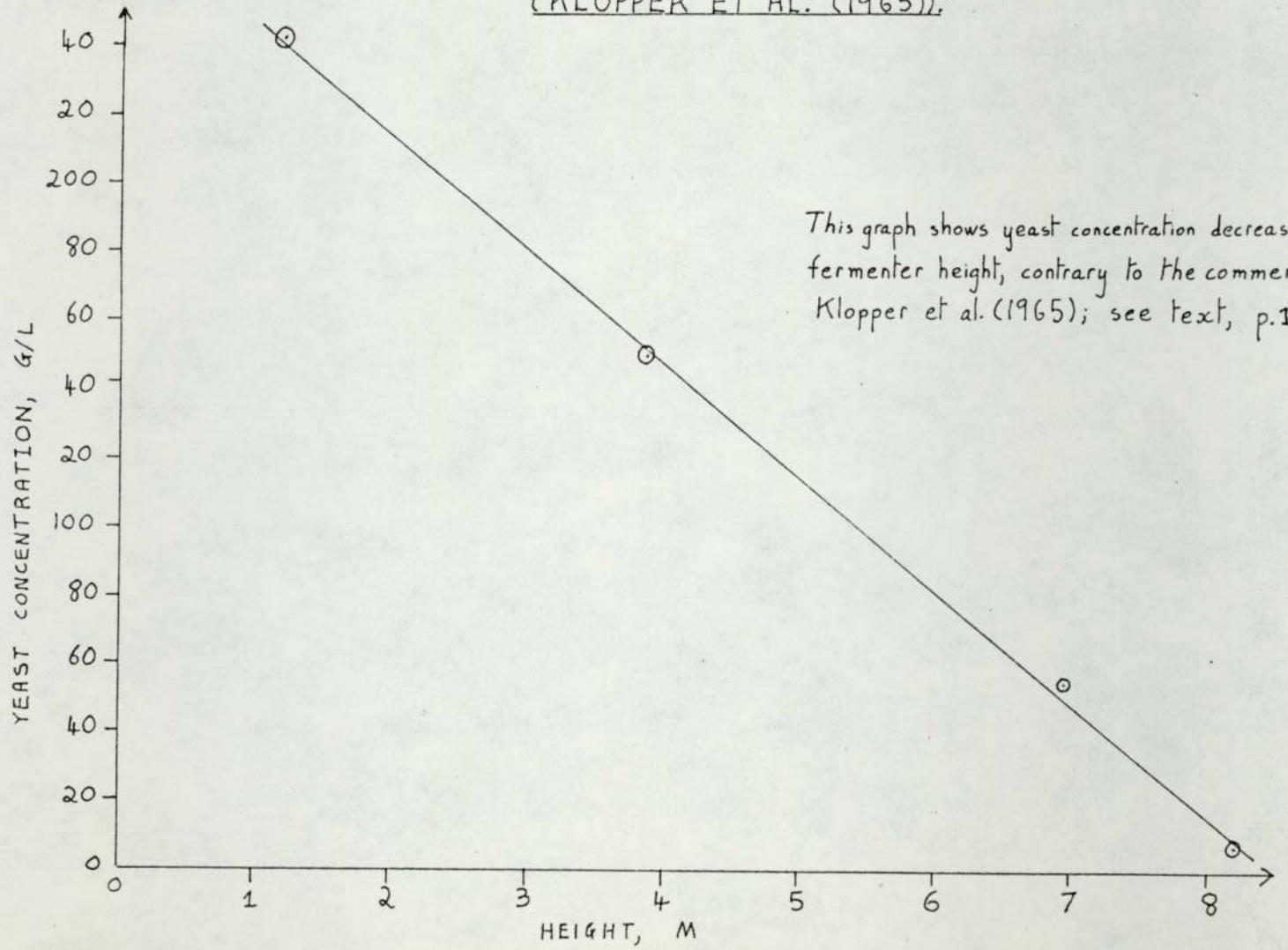
TABLE 5.1 Medium Flow-Rates in Tower Fermenters.

FLOW RATE	LIQUOR RESIDENCE TIME	SUPERFICIAL LIQUID VELOCITY	AUTHOR(S)
CM^3/S	HOURS	CM/S	
1.26	18.35	6.92×10^{-3}	Klopper et al. (1965)
6.31	3.67	34.6×10^{-3}	Klopper et al. (1965)
90.8	13.62	14.3×10^{-3}	Klopper et al. (1965)
250	4.95	39.3×10^{-3}	Klopper et al. (1965)
227	5.44	35.7×10^{-3}	Ault et al. (1969)
455	2.72	71.5×10^{-3}	Ault et al. (1969)
116	11.44	18.2×10^{-3}	den Blanken (1974)

higher average yeast concentration of yeast in the fermenter of 300 g/l. Klopper et al. (1965) also determined an average yeast concentration of 300 g/l in a pilot scale fermenter. However, at low wort flow-rates there was no evidence of a yeast concentration gradient within the fermenter. As the medium flow-rate was increased the yeast concentration in the head of the tower decreased, and as stated by Royston (1966b) and Ault et al. (1969), the zone of maximum yeast concentration moved up the tower. Klopper et al. (1965), when using the equipment later taken over by Ault et al. (1969), observed that at low medium flow-rates the yeast concentration in the tower rapidly decreased between the middle and top sample points. This indicates, in accord with Royston (1966b), that the yeast had formed a plug in the base of the fermenter. Graph 5.3 shows the yeast concentration gradient recorded by Klopper et al. (1965) in a commercial scale fermenter, indicating a mean yeast concentration of 167.5 g/l in the straight section of the fermenter. den Blanken (1974) employed an identical fermenter but for lager production: he found the average yeast concentration in the tower ranged between 180 g/l - 220 g/l. These estimates were made by simply averaging the yeast concentrations at the top and bottom sample points.

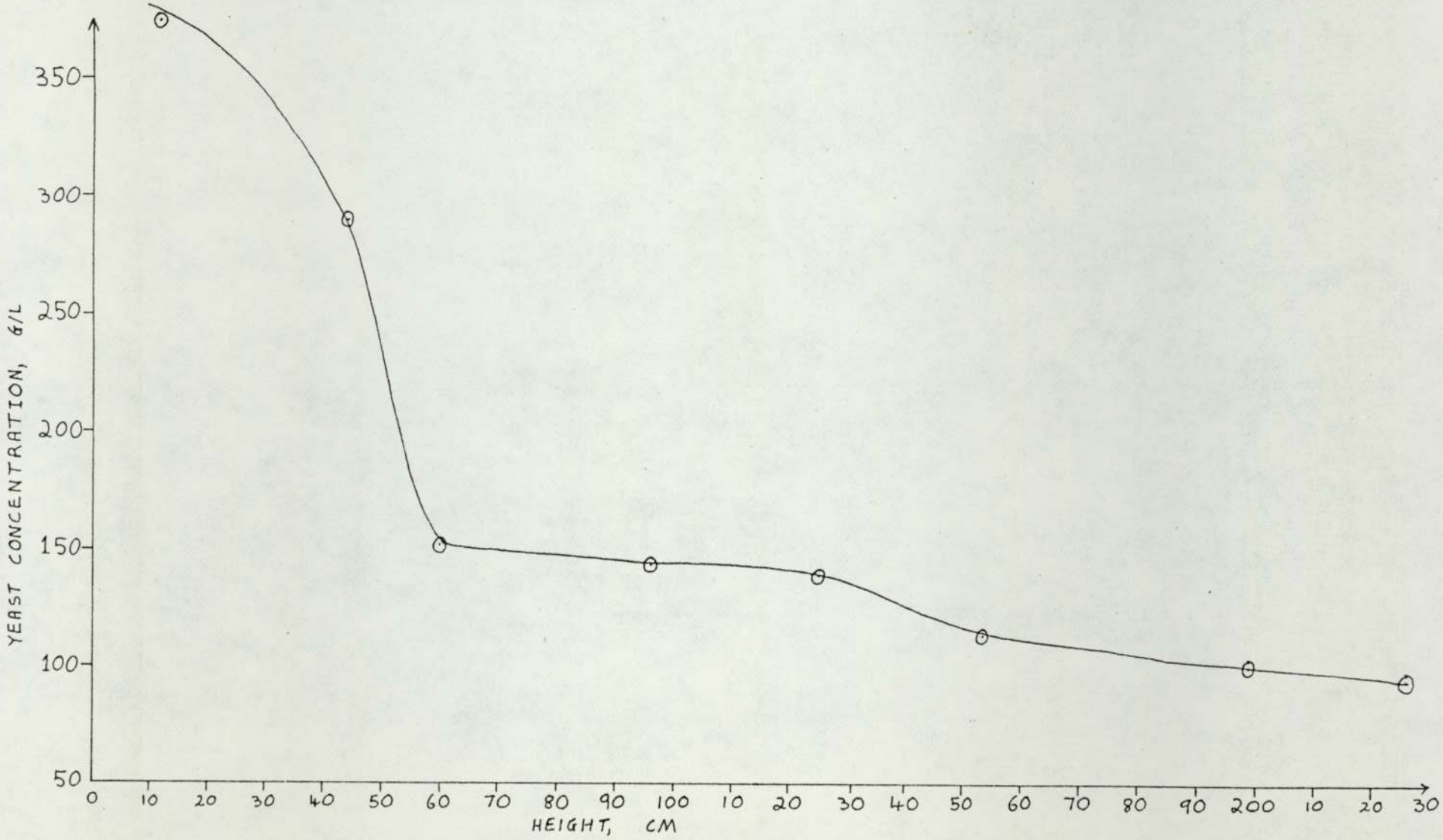
Graphs 5.4-5.8 show the yeast concentration profiles recorded in experiment nos. 11-15 respectively. These graphs were used to estimate the mean yeast concentration in the fermenter for each run (Table 5.2, column 2). The yeast concentration in the base of the fermenter varied enormously from run to run, the range covered being 300 g/l to 700 g/l.

GRAPH 5.3 YEAST CONCENTRATION VERSUS HEIGHT IN FERMENTER (KLOPPER ET AL. (1965)).

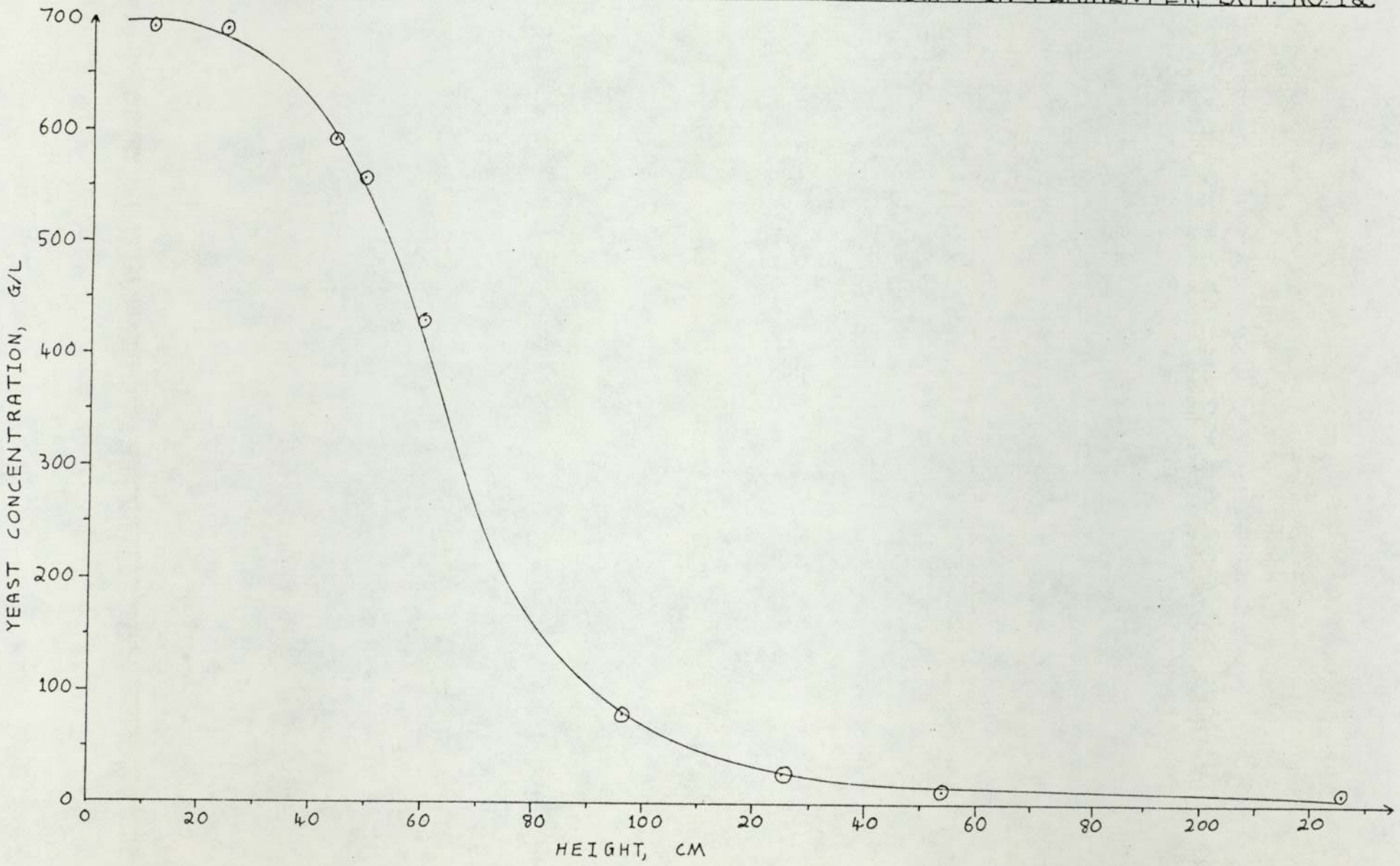


This graph shows yeast concentration decreasing with fermenter height, contrary to the comments of Klopper et al. (1965); see text, p.140.

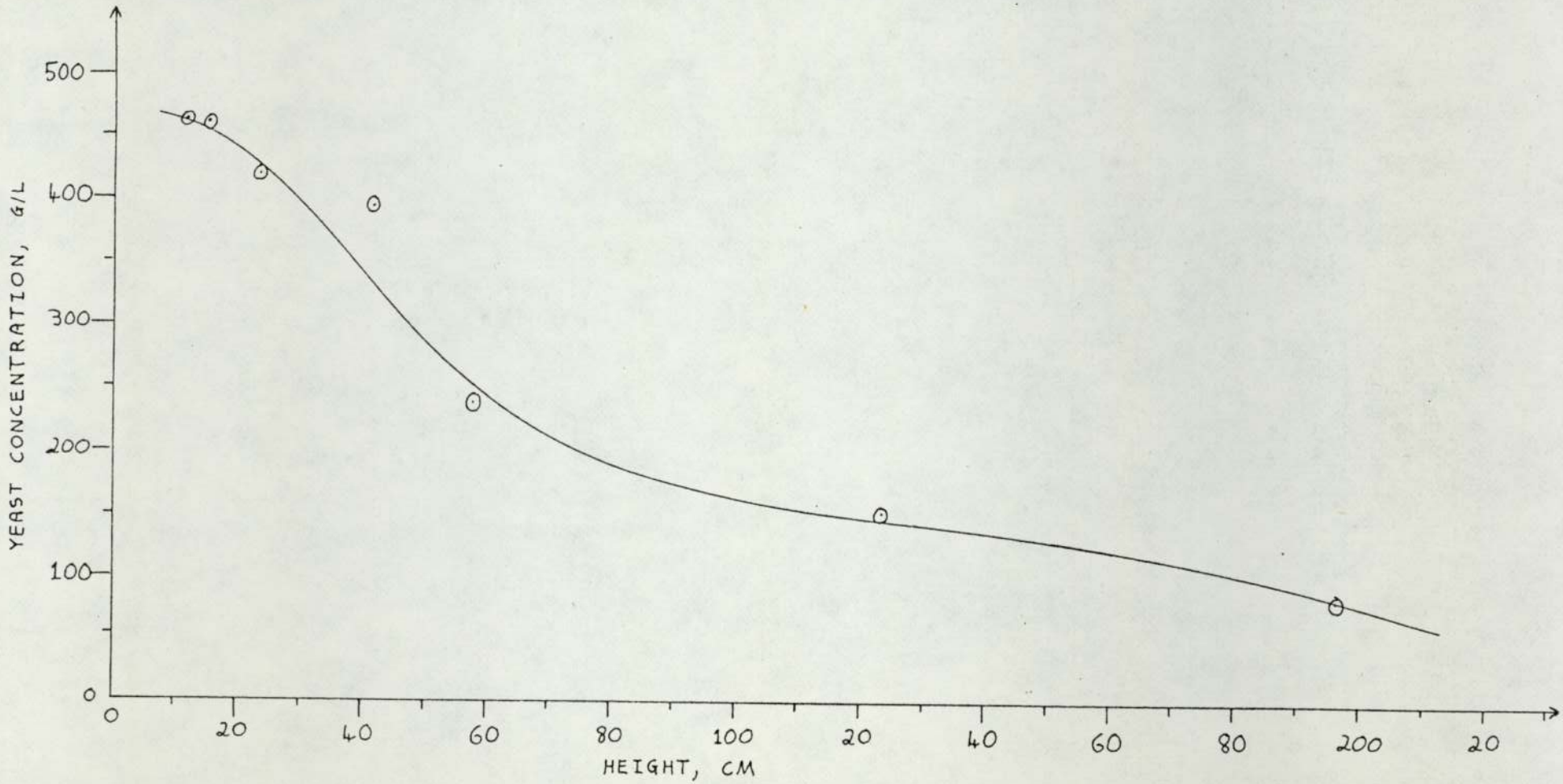
GRAPH 5.4 YEAST CONCENTRATION VERSUS HEIGHT IN FERMENTER, EXPT. NO. 11.



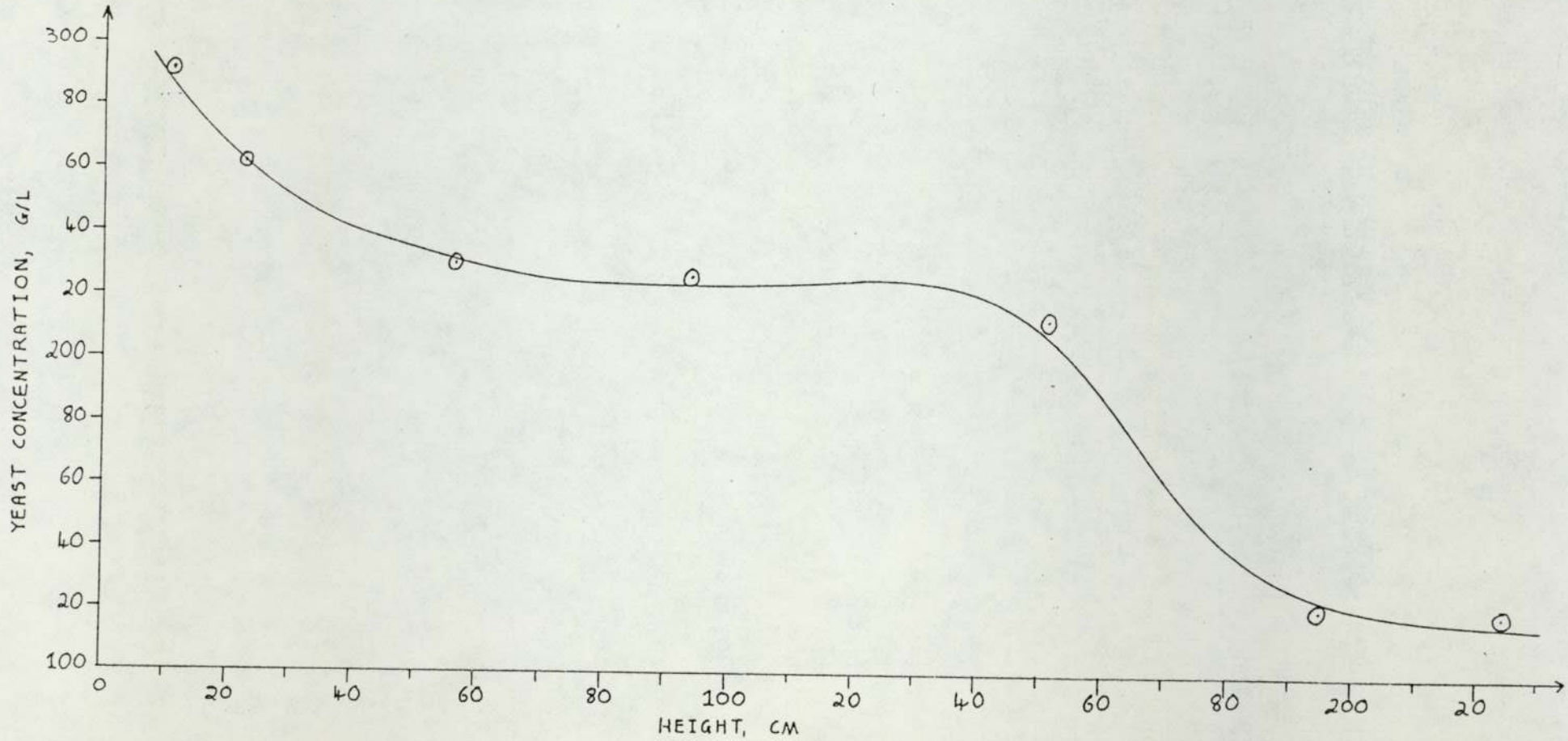
GRAPH 5.5 YEAST CONCENTRATION VERSUS HEIGHT IN FERMENTER, EXPT. NO. 12



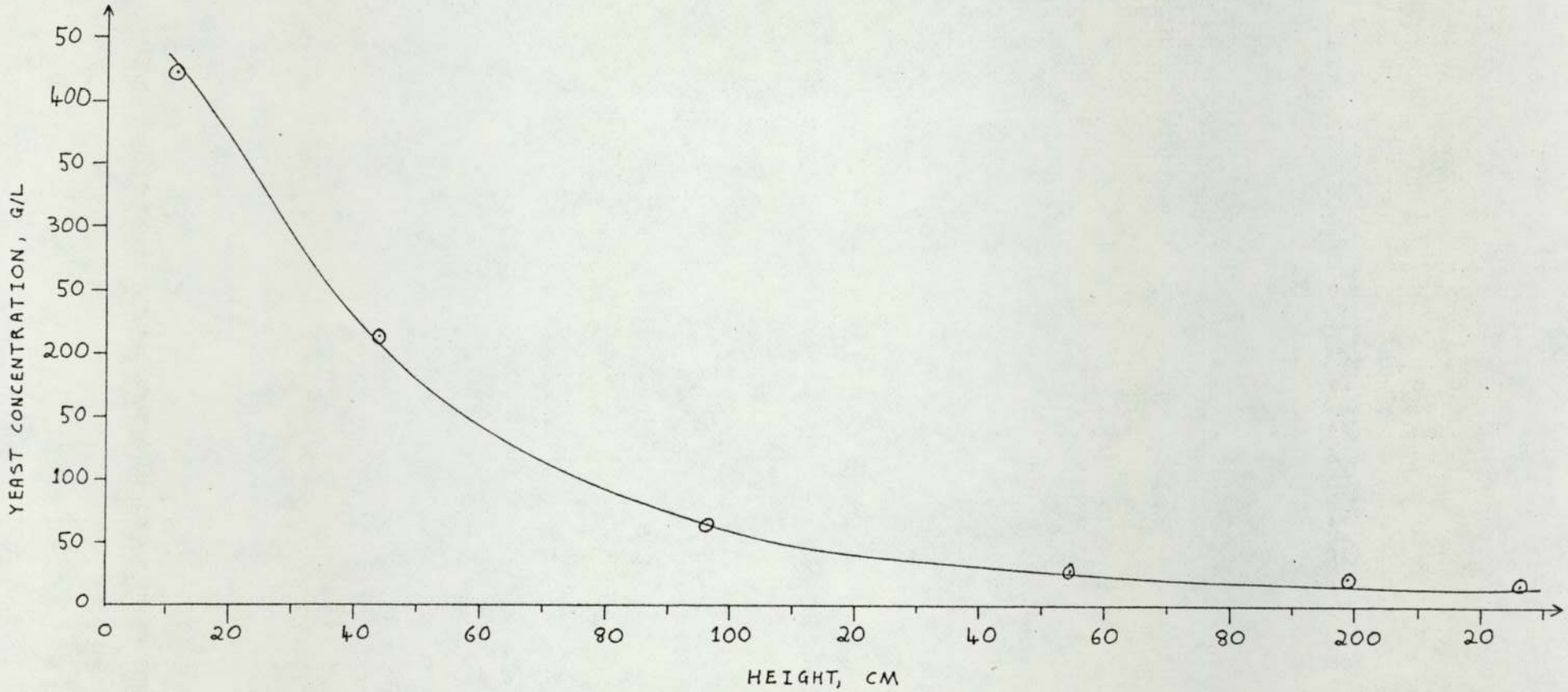
GRAPH 5.6 YEAST CONCENTRATION VERSUS HEIGHT IN FERMENTER, EXPT. NO. 13.



GRAPH 5.7 YEAST CONCENTRATION VERSUS HEIGHT IN FERMENTER, EXPT. NO. 14.



GRAPH 5.8 YEAST CONCENTRATION VERSUS HEIGHT IN FERMENTER, EXPT. NO. 15.



However, in runs where there was a low yeast concentration in the base of the fermenter it was found that relatively high concentrations occurred in the upper sections of the tower, and vice-versa. These facts combined to result in similar average yeast concentrations in four of the runs in the tower fermenter. Table 5.2, column 3, shows the result of estimating the yeast concentration by taking a mean of the yeast concentrations at the top and bottom sample points. These latter figures vary between 225 g/l - 350 g/l and are of the same order of magnitude as those observed by Klopper et al. (1965), Royston (1966b), and Ault et al. (1969): this suggests that the concentration of yeast in the tower fermenter has generally been over-estimated.

5.4.3 Ethanol Production and Sugar Utilisation

Ethanol Yields

Pasteur (1872), cited by Harrison and Graham (1970), noted over a century ago that only approximately 95% of the glucose utilised in an anaerobic fermentation was converted to ethanol (48.4%) and carbon dioxide (46.6%). The remaining carbohydrate is incorporated as yeast material or directed into alternative metabolic pathways to yield the multitude of minor components associated with fermentations. In present distillery practice the yield of ethanol normally attained is 47.1 g per 100 g of fermented monosaccharide; this corresponds to a yield of 92.15% of the theoretical value, though Paturau (1969) states fermentation efficiencies of 95% are possible. Among the factors which affect the final yield of ethanol are the

TABLE 5.2 Mean Yeast Concentrations in the Tower Fermenter

DATA SOURCE	Mean Yeast Concentration * G/L	Mean Yeast Concentration ** G/L
Klopper et al. (1965)	167	167
Expt. No. 11	165	232
" " 12	201	350
" " 13	192	270
" " 14	199	257
" " 15	107	225

* Estimated from graphs 5.4-5.8

** Estimated by taking a mean of the yeast concentrations at the top and bottom sample points.

individual broth constituents. Harrison and Graham (1970) found the available nitrogen source to be particularly important. Table 5.3 gives ethanol yields for media with various nitrogen sources.

TABLE 5.3 Ethanol Yield Related to Media Nitrogen Source (Harrison and Graham (1970))

NITROGEN SOURCE	ETHANOL YIELD %
Amino Acids	96.0
Ammonia	90.5
No Nitrogen Source	93.1

The Gay-Lussac equation has been used to express the formation of ethanol in an anaerobic fermentation.

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2$$

However in beer and molasses-based fermentations most of the assimilable sugar is available as disaccharide;

$$C_{12}H_{22}O_{11} + H_2O \longrightarrow 2C_6H_{12}O_6 \longrightarrow 4C_2H_5OH + 4CO_2.$$

The theoretical yield of ethanol is thus increased to 53.8 g per 100 g of fermented disaccharide compared with the 51.1 g for ethanol formation from 100 g of monosaccharide.

Table 5.4 gives the theoretical ethanol yield and actual yield obtained from the author's tower fermenter in expt. no. 12.

TABLE 5.4 Ethanol Yields (Expt. No. 12).

TIME INTO FERMENTATION	SUGARS FERMENTED	THEORETICAL ETHANOL	ACTUAL ETHANOL	YIELD
H	G /100CM^3	G /100CM^3	G /100CM^3	%
192	7.03	3.78	3.17	83.9
216	7.03	3.78	2.77	73.2
240	6.82	3.77	3.40	92.8
264	-	-	-	-
288	7.45	4.00	3.35	83.6
312	7.45	4.00	3.58	89.3
336	7.45	4.00	3.12	77.8
360	7.39	3.98	3.70	93.0
384	6.53	3.51	3.17	90.4
408	6.53	3.51	3.29	93.5
432	-	-	-	-
456	6.82	3.67	3.23	88.0
480	6.82	3.67	3.40	92.8
504	6.82	3.67	3.63	99.1
528	6.82	3.67	3.23	88.0
552	6.82	3.67	3.40	92.8

Table 5.5 shows similar figures for expt. no. 13 and includes the theoretical and actual yields of carbon dioxide. Figures pertaining to expt. no. 12 show ethanol yields between 73%-99%. Obviously the very high yields may be attributed in part to experimental error: however, many of the measurements are of the order expected in distillery practice. Similar values are shown in Table 5.5. Often the yields of ethanol and carbon dioxide are in disagreement, though generally not greatly so, and again this may be attributed in part to experimental error. Experiments were also conducted with molasses-based media. The ethanol yields from these experiments are shown in Tables 5.6 and 5.7 for expt. nos. 14 and 15 respectively. In expt. no. 14, in which the medium was supplemented with ammonium salts and pH adjusted, the yields of ethanol were between 68%-78%. This result is low when compared with the expected yield of approximately 92% and when compared with the results from the continuous beer experiments. The relatively low yeast concentration within the tower at this time and the higher cell washover rate indicate that yeast growth was occurring at the expense of alcohol production: this provides a partial explanation of the poor yield. When the unsupplemented medium was started the alcohol concentration in the effluent increased, coinciding with an increase in yeast concentration in the fermenter and a decrease in the rate of cell wash-out. However, the yield of ethanol from the molasses fermentations was generally lower than those from the wort fermentations. Coote (1974) observed ethanol yields of 70%-86% with continuous molasses fermentations; this is generally lower than observed here.

Sugar Conversion

The utilisation of the fermentable sugars in the tower

TABLE 5.5 Ethanol and Carbon Dioxide Yields (Expt. No. 13).

TIME INTO FERMENTATION	THEORETICAL ETHANOL	ACTUAL ETHANOL	YIELD	THEORETICAL CARBON DIOXIDE	ACTUAL CARBON DIOXIDE	YIELD
H	G /100CM^3	G /100CM^3	%	l/min	l/min	%
72	-	-	-	-	-	-
96	3.95	3.45	87.2	0.219	0.209	95.4
120	4.20	3.82	91.0	0.233	0.210	90.5
144	-	-	-	-	-	-
168	4.05	4.06	100.2	0.224	0.224	100.0
192	3.99	4.45	112.1	0.331	0.316	95.5
216	4.27	4.23	99.2	0.355	0.329	92.6
240	3.96	3.69	93.2	0.329	0.310	94.3
264	-	-	-	-	-	-
288	3.89	3.34	85.9	0.677	0.662	97.7
312	3.84	3.40	88.8	0.668	0.615	92.0
336	-	-	-	-	-	-
360	3.90	3.69	94.6	0.679	0.643	94.6
384	3.93	3.52	89.6	0.685	0.611	89.2
408	3.93	3.99	101.5	0.863	0.782	90.7
432	3.80	3.75	98.8	0.834	0.835	100.1
456	3.86	3.58	92.8	0.847	0.811	95.7
480	3.90	3.23	82.8	0.857	0.844	98.5
504	3.90	3.82	97.9	0.857	0.840	98.0
528	-	-	-	-	-	-
552	3.86	3.40	88.1	0.408	0.428	104.8
576	-	-	-	-	-	-
600	3.53	3.23	91.5	0.373	0.315	84.4
624	3.54	3.06	86.3	0.272	0.242	89.0
648	3.63	3.47	95.6	0.279	0.296	106.3
672	3.44	3.23	93.8	0.265	0.251	94.9

TABLE 5.6 Ethanol Yields (Expt. No. 14)

TIME INTO FERMENTATION	FERMENTED SUGARS	THEORETICAL ETHANOL	ACTUAL ETHANOL	YIELD
H	G /100CM^3	G /100CM^3	G /100CM^3	%
240	11.4	6.13	4.40	71.8
264	11.4	6.13	4.77	77.8
288	11.6	6.24	4.29	68.8
312	11.4	6.13	4.83	78.8
336	11.4	6.13	4.64	75.7
360	11.4	6.13	4.23	69.0
384	11.6	6.24	4.23	67.8
408	-	-	-	-
432 *	11.6	6.24	4.35	69.7
456	-	-	-	-
480	-	-	-	-
504	11.4	6.13	4.35	70.9
528	11.7	6.29	4.59	72.9
552	11.5	6.19	5.08	82.1
576	11.5	6.19	5.21	84.1
600	11.4	6.13	5.83	95.0
624	-	-	-	-
648	11.8	6.35	5.89	92.8
672	11.8	6.35	5.51	86.9
696	11.8	6.35	5.95	93.8
720	11.8	6.35	5.76	90.8
744	11.8	6.35	5.95	93.8
768	11.8	6.35	5.63	88.8
792	11.8	6.35	5.39	84.9
816	11.8	6.35	5.45	85.9
840	11.8	6.35	5.83	91.8

* Unsupplemented medium started

TABLE 5.7 Ethanol Yields (Expt. No. 15)

TIME INTO FERMENTATION	FERMENTED SUGARS	THEORETICAL ETHANOL	ACTUAL ETHANOL	YIELD
H	G /100CM^3	G /100CM^3	G /100CM^3	%
144	9.56	5.14	4.07	79.1
168	-	-	-	-
192	9.70	5.22	4.20	80.4
216	9.70	5.22	4.35	83.3
240	9.70	5.22	4.41	84.6
264	9.64	5.19	4.23	81.6
288	9.64	5.19	4.53	87.4
312	-	-	-	-
336	10.2	5.48	4.41	80.5
360	9.64	5.19	4.53	87.4
384	9.42	5.07	4.45	88.2
408	9.58	5.15	4.65	90.2
432	9.74	5.24	4.53	86.5
456 *	-	-	-	-
480	9.60	5.16	4.23	81.9
504	-	-	-	-
528	9.97	5.36	4.59	85.5

* Non-sterile medium started

fermenter was generally virtually complete. The analysis of beet molasses, (See Appendix 5.2), contains the following figures:

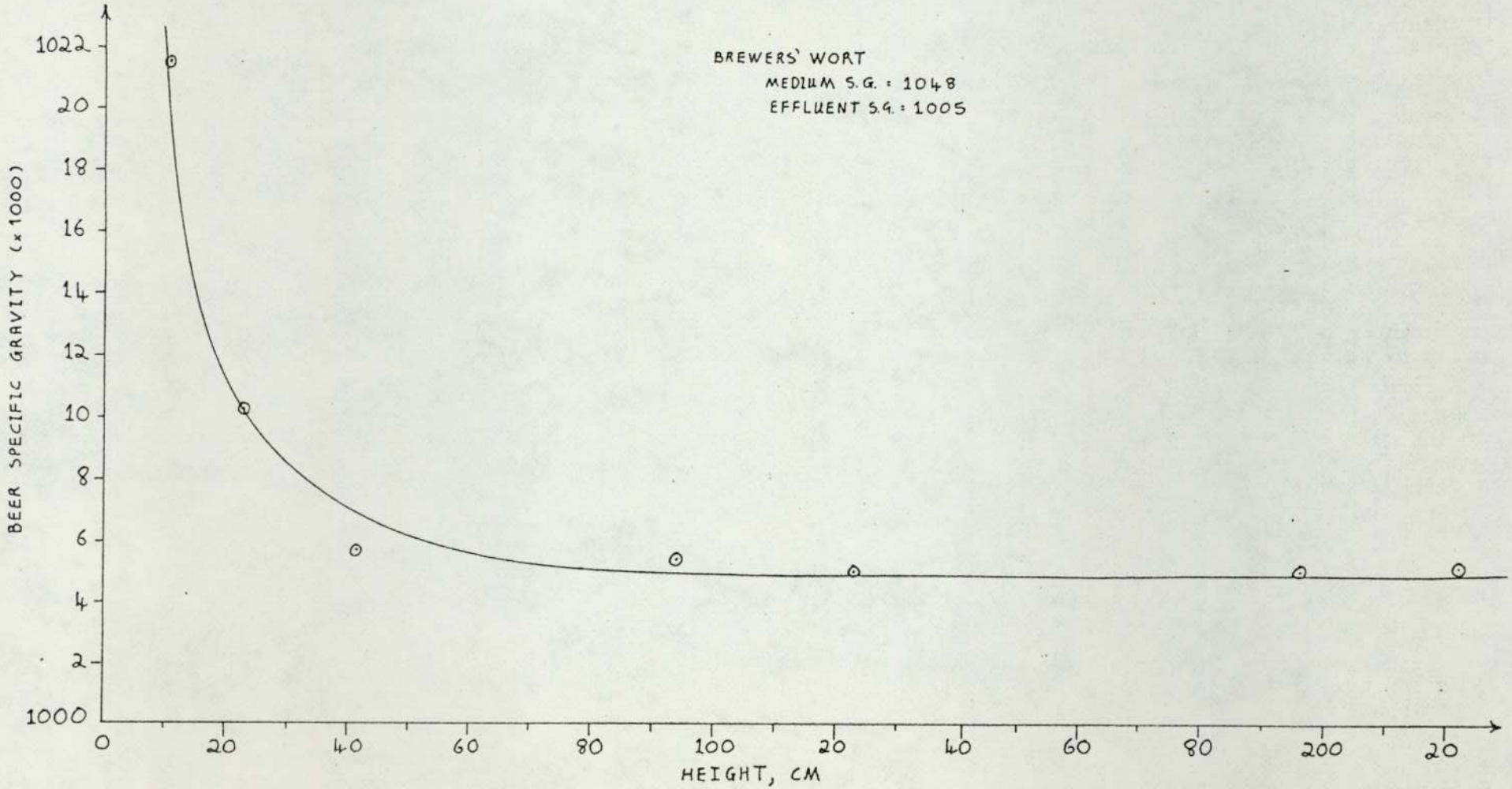
sucrose	49.7%
reducing sugars	1.3%
total sweetening matter	54.7%.

Assuming the sucrose and reducing sugars to be fermentable, then the molasses had a fermentability of 93%. A typical input medium sugar concentration in expt. no. 14 was 125 g/l. At 93% fermentability, then 116 g/l would be available for conversion to ethanol and yeast. Typical effluent sugar concentrations were 11-12 g/l, indicating 2-3 g/l of fermentables in the effluent and 97%-98% utilisation of the fermentable sugars. Slightly lower figures of approximately 93% utilisation of the fermentable sugars were observed in expt. no. 15.

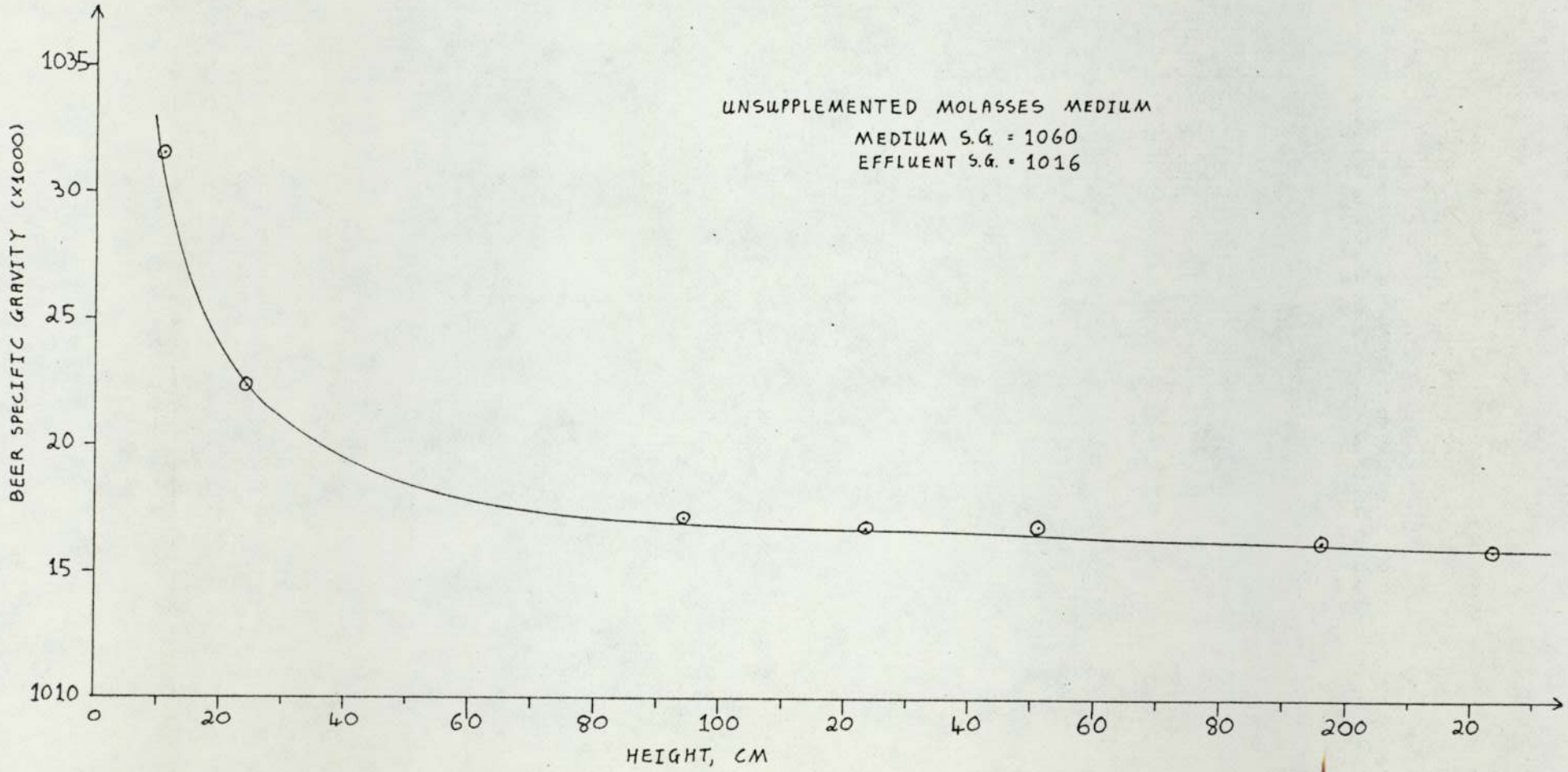
In the experiments involving brewers' wort, (nos. 11-13), the strain of yeast affected the sugar uptake to some extent. The strain of Saccharomyces diastaticus (CFCC 54) used in expt. no. 11 attenuated the wort from a present gravity of 50 to a P.G. of 5. The strains of S. cerevisiae and S. carlsbergensis, CFCC 1 and CFCC 39 respectively, gave similar attenuations to a P.G. = 9.0.

Analysis from expt. nos. 11 and 14 (see graphs 5.9 and 5.10 respectively) show that the majority of the fermentation had taken place within a height of 20-30 cm (9-13%) above the base of the column. Ault et al. (1969) found a slower fall in P.G. with fermenter height, but Klopper et al. (1965) and Royston (1966b) found the fermentation to be virtually complete within the

GRAPH 5.9 BEER SPECIFIC GRAVITY VERSUS FERMENTER HEIGHT, EXPT. NO. 11.



GRAPH 5.10 BEER SPECIFIC GRAVITY VERSUS FERMENTER HEIGHT, EXPT. NO. 14



first 11% of the column height. All three authors noted increases in the beer P.G. (at a fixed height in the column) with increases in the wort flow-rate. This was not detected in the author's work. However, an increase in medium flow-rate from 16.2 cm^3/min to 41.5 cm^3/min in expt. no. 12 caused an increase in the effluent P.G. from 7.5 to 9.0. In other cases, where the wort flow-rate was increased, the effluent P.G. increased then fell back to its former value within 24-48 hours: this indicates that the fermenter can adapt to changes in flow-rate. Similar observations were made by Ault et al. (1969) up to a limiting value of the volumetric flow-rate, when unstable conditions developed in the fermenter.

Klopper et al. (1965), Royston (1966b), and Ault et al. (1969) all observed that the zone of maximum yeast concentration rose up the tower when increases in the wort flow-rate were made. This obviously must account in part for the lower fermentation rates observed in the lower zone of the tower at relatively high medium flow-rates. Such changes were not encountered in the author's work, probably due to the effect of introducing air into the bottom of the fermenter (see sections 5.4.2 and 5.4.4).

Sugar Utilisation Rates

The actual rate of sugar utilisation and the rate expressed in terms of unit volume of the fermenter are shown in Table 5.8.

TABLE 5.8 Sugar Utilisation Rates in the Tower Fermenter

EXPT. NO.	SUGAR UTILISATION RATE	
	G/H	G Sugar/H.L. of Fermenter Vol.
11	194	17.2
12	185	16.4
13	189	16.7
14	242	21.4
	512*	45.3
15	160	14.1

* at a medium apparent residence time of 2.5 hours

The experiments conducted with wort (nos. 11, 12, 13) all show similar rates of sugar utilisation in terms of grams of sugar utilised per hour. These figures can be compared with the data of Klopper et al. (1965): when using a wort of original gravity of 1035.6 they found the outlet beer specific gravity was 1005.9, corresponding to a sugar usage of 62 g/l. Ault et al. (1969) suggested that the fermenter operated in a stable manner at flow-rates up to 8 brl/h (1309.3 l/h). Hence, the maximum rate of sugar utilisation achievable by Klopper et al. (1965) was 8.2×10^4 g/h, or 18 g/h.l of fermenter volume. The mean of the results from expt. nos. 11, 12 and 13 is 16.8 g of sugar/h.l of fermenter volume, which is similar in value.

However results from expt. no. 14, in which a molasses-based medium was used, indicate that 45.3 g of sugar/h.l of

fermenter volume is a readily attainable sugar utilisation rate. The low utilisation rate in expt. no. 15 is attributable to the fact that high medium flow-rates were not used.

5.4.4 Aeration and Fermentation

Introduction

The alcohol fermentation has been classically thought of as an anaerobic fermentation. However, a number of authors have shown that trace amounts of oxygen stimulate the rate of alcohol production (Cowland and Maule (1966), White (1966), Cowland (1967), Hall and Howard (1968), Cysewski and Wilke (1976), and Margaritis and Wilke (1978)).

Trace amounts of oxygen in the broth are rapidly utilised by the yeast. David and Kirsop (1973) found that the dissolved oxygen in wort fell from 100% to 0% of saturation within 1 hour of pitching the yeast. Riemann and Bruecher (1974) added aerated wort containing 7×10^{-6} g/cm^3 dissolved oxygen to "green beer" and within 30 seconds the dissolved oxygen concentration had fallen to $0.2 - 0.8 \times 10^{-6}$ g/cm^3.

The rate of oxygen supply has also been found to influence the fermentation markedly. Ricketts and Hough (1961) aerated the head space of a continuous stirred tank reactor used for beer production: the rates of aeration and subsequent observations are given in Table 5.9. Cowland and Maule (1966) and Cowland (1967), using similar equipment, found that the maximum rate of sugar uptake per unit mass of yeast and the maximum conversion of sugar to ethanol occurred at an oxygen supply rate of 0.16 ml/l of wort/h. This figure corresponds to a dissolved oxygen

TABLE 5.9 The Effect of Aeration on Beer Production in a C.S.T.R. (Ricketts and Hough (1961))

AIR FLOW $CM^3/H.L$ of Vessel contents	OBSERVATIONS
0-30	The rate of beer production increased dramatically over the anaerobic state.
30-500	Yeast reproduction was equivalent to that in the batch beer fermentation. No further effect on the fermentation rate.
500-2500	The rate of beer production and yeast concentration increased at the expense of the alcohol concentration.
2500 →	No further change

concentration of 5.1 μg/ml, the air saturation level of wort. These findings are in agreement with those of Markham (1969) who found the optimum rate of fermentation in a batch reactor occurred at an initial oxygen concentration of 21% of saturation - equivalent to 100% air saturation.

Ricketts and Hough (1961), Cowland and Maule (1966), and Cowland (1967) have run continuous fermentations completely anaerobically. Steady state conditions lasting up to 60 generations of yeast (40 days) were observed, the period being dependent on yeast strain. However, fermentation rates were found to be depressed under these conditions.

Aeration of fermenting wort has been said to affect the concentration of minor components in the final product, although this may be indirectly as a result of affecting yeast growth (see section 5.4.6). However, Portno (1967c), experimenting with a heterogeneous tube reactor and wort oxygen levels up to 95% of oxygen saturation, found only a slight variation in the concentrations of minor components in the finished product: organoleptic evaluation also failed to discriminate between the beers.

In commercial scale fermenters Maule (1973) stated that wort is aerated to 10 ppm in the Watney-Mann stirred system, while Ault et al. (1969) aerated wort to 6 ppm when using a tower fermenter. As commented by Hough et al. (1976), the degree of aeration employed in industry is a matter of some secrecy; one brewery using tower fermenters is said to use air at 0.07 l/l of wort/h, while another is said to use a mixture of carbon dioxide and air.

Dissolved Gas Measurements

Measurements of dissolved gases were carried out at various heights in the fermenter during expt. no. 13. The results are shown in Table 5.10.

The first point of interest is that the concentration of dissolved carbon dioxide decreased with increasing height in the fermenter. A plausible explanation is that nucleation and lower pressures combined to cause transfer of the carbon dioxide from the liquid to the gaseous phase. (These results are in accordance with the view that the majority of the fermentation occurs at the base of the fermenter.)

It is interesting to note that dissolved oxygen levels increased with height whilst dissolved nitrogen concentrations fell. As the contact time of the air bubbles with the wort increases with height in the fermenter the dissolved oxygen and nitrogen concentrations may be expected to increase. However, as Royston (1966b) pointed out, nucleation of carbon dioxide will tend to strip other dissolved gases (air) from the wort. It is postulated, in the absence of other evidence and because of the consistency of the results, that nitrogen is preferentially stripped from the wort by bubbles of carbon dioxide.

Though absolute values of dissolved gases are given in Table 5.10, it must be appreciated that these may be in error. Catheter probes for the mass spectrometer were calibrated by conducting measurements in water saturated either with air or carbon dioxide. As no data for the combined system were available it was assumed that the solubility of one gas was unaffected by another. The values for dissolved oxygen,

TABLE 5.10 Dissolved Gases in the Tower Fermenter (Expt. No.13)

WORT FLOW	AIR FLOW AT S.T.P.	TIME INTO FERMENTATION	DISSOLVED OXYGEN			DISSOLVED NITROGEN			DISSOLVED CARBON DIOXIDE		
			PPM			PPM			PPM		
CM^3/MIN	CM^3/MIN	Hours	17.1*	94.3*	220*	17.1	94.3	220	17.1	94.3	220
15.9	11	240	0.147	0.202	0.239	1.20	1.14	1.11	3860	3200	2550
33.3	11.2	312	0.208	0.234	0.272	2.25	2.06	1.90	3280	2880	2400
33.3	11.2	336	0.274	0.323	0.339	2.39	2.28	2.06	2920	2560	2020
42	9.3	480	0.159	0.182	0.293	1.41	1.21	0.84	4100	2880	2300

* Height in Fermenter (CM)

nitrogen, and carbon dioxide in water at 20°C and atmospheric pressure were taken as 9.09 ppm, 15.4 ppm and 1688 ppm respectively. (Lange's Handbook of Chemistry (1956), International Critical Tables (1928) and Stephen and Stephen (1963)).

Aeration and Viability

Ricketts and Hough (1961) carried out a continuous fermentation in a C.S.T.R. and reported no loss of viability of the yeast cells during anaerobic operation. However, den Blanken (1974) found aeration of the wort was necessary to maintain the dead cell count below 5% and so ensure efficient operation of a tower fermenter. Ault et al. (1969) also operated a tower fermenter with both aerated and unaerated wort. In the anaerobic situation the viability in the tower decreased to 55%: aerated wort resulted in viabilities of 75%–85%.

The viability of the cells in both the tower and effluent beer was measured in a number of experiments carried out by the author. The measured viabilities ranged between 80%–95%. There was no indication that dead cells were preferentially washed out of the fermenter, as was suggested would occur by Royston (1966b).

It is interesting to note that the lowest viabilities (approximately 80%) were observed towards the end of expt. no. 14. This coincided with a low cellular overflow rate and a period of predominately negative nitrogen uptake i.e. medium nitrogen increased indicating cell lysis (see section 5.4.5).

5.4.5 Nitrogen Uptake in the Tower Fermenter

Cellular Nitrogen

Burrows (1970) stated that ammoniacal nitrogen is rapidly converted within the cell into amino acids, nucleic acids, and numerous other constituents. The nitrogen content of the cell may vary within wide limits but is generally in the range 5-10%w/w of the cell. Peppler (1970) has supplied the typical nitrogen fraction of a number of yeasts (see Table 5.11).

Cellular nitrogen determinations were carried out on samples of dried cellular matter drawn from the fermenter at different times and from various sample ports. The results are given in Tables 5.12 and 5.13. From these figures it is obvious that there was no systematic variation of yeast nitrogen content with either height in the fermenter or time into the fermentation. However, the mean cellular nitrogen content of the yeast from the molasses fermentation (expt. no. 14) is slightly lower than the figure from the wort fermentation (expt. no. 12), the respective figures being 6.2%w/w and 6.6%w/w. A strain of Saccharomyces cerevisiae, CFCCl, was used in both experiments.

Table 5.11 Yeast Cell Nitrogen Fractions (Peppler (1970))

YEAST STRAIN	NITROGEN (%w/w)
Saccharomyces cerevisiae	6.5
" "	8.1
Candida utilis	8.0
Brewers' Yeast	7.9
Distillers' Yeast	6.6

TABLE 5.12 Yeast Nitrogen Fraction (Expt. No. 12)

TIME INTO FERMENTATION	HEIGHT IN FERMENTER	CELLULAR NITROGEN
H	CM	%w/w
240	13.7	6.147
	57.5	7.207
312	57.5	5.882
	151.4	7.004
336	46.7	5.019
	151.4	6.102
360	52.1	7.551
	57.5	7.701
	122.9	6.629
384	57.5	7.580
	122.9	5.887
408	17.1	6.271
	122.9	7.883
432	14.3	6.824
	220.0	6.639
456	11.4	6.449
	94.3	5.737
	220.0	6.972

TABLE 5.13 Yeast Nitrogen Fraction (Expt. No. 14)

TIME INTO FERMENTATION	HEIGHT IN FERMENTER	CELLULAR NITROGEN
H	CM	%w/w
240	14.3	5.427
	122.9	7.111
264	11.4	6.338
	151.4	6.210
288	11.4	6.811
	41.3	6.266
384	14.3	7.055
	195.9	6.729
408	11.4	6.883
	151.4	6.967
504	122.9	5.497
	220.0	5.562
528	22.9	5.774
	151.4	5.137
576	11.4	6.118
	195.9	6.861
672	22.9	6.403
	151.4	5.329
744	11.4	6.783
	122.9	5.990
792	94.3	5.227
	195.9	5.700

Nitrogen Utilisation

Nitrogen utilisation in the tower fermenter was examined in a continuous fermentation with a molasses-based medium (Expt. No. 14). The nitrogen content of molasses has often been said to be too low for sufficient yeast growth to occur to ensure efficient alcohol production. In rum production Lehtonen and Suomalainen (1977) mention that ammonium sulphate or urea is added to the medium as yeast nutrient and that the pH is also adjusted to 5.5. Paturau (1969) reports a similar practice occurs in industrial ethanol production with the broth acidified to a pH of 4.5-4.7 to minimise bacterial infection. Coote (1974) supplemented a molasses-based feedstock with 3.57 g/l of ammonium sulphate and 1.0g/l of sodium dihydrogen phosphate to ensure the yeast had sufficient available assimilable nitrogen; the medium pH was also adjusted to 4.5.

B.S.C. Ltd. supplied raw molasses for use in this work: a complete analysis is given in Appendix 5.2. A medium consisting of 2.25 l of raw molasses diluted to 20 l with tap water and supplemented according to the formula of Coote (1974) gave a theoretical nitrogen content of 3.6 g/l; unsupplemented medium would have contained 3.0 g/l of nitrogen. These figures are confirmed by the analyses presented in Tables 5.14 and 5.15.

A continuous fermentation with supplemented medium was run for over 400 hours. This fermentation was characterized by a low yeast concentration in the fermenter, the brown and powdery nature of the yeast, and the lack of any significant yeast concentration profile within the fermenter. The amount

TABLE 5.14 Nitrogen Utilisation in the Tower Fermenter

(Expt. No. 14)

TIME INTO FERMENTATION	NITROGEN			% UTILISATION
	MEDIUM	EFFLUENT	UTILISED	
Hours	G/L	G/L	G/L	
216	3.972	3.742	0.230	5.79
240	3.807	3.544	0.263	6.91
264	3.843	3.596	0.247	6.43
288	3.896	3.655	0.241	6.19
312	3.918	3.682	0.236	6.02
336	-	-	-	-
360	3.843	3.604	0.239	6.22
384	3.785	3.567	0.218	5.76
408	3.797	3.593	0.204	5.37
*432	-	-	-	-
456	-	-	-	-
480	-	-	-	-
504	2.955	2.754	0.201	6.80
528	3.094	2.896	0.198	6.40
552	3.020	2.827	0.193	6.39
576	3.011	2.938	0.073	2.42
600	2.766	2.774	-0.008	-0.29
624	2.986	2.979	0.007	0.23
648	3.000	3.021	-0.021	-0.70
672	2.894	2.912	-0.018	-0.62
696	-	-	-	-
720	3.044	3.067	-0.023	-0.76
744	3.008	3.004	0.004	0.13
768	2.947	2.989	-0.042	-1.43
792	3.017	3.047	-0.030	-0.99
816	2.954	3.011	-0.057	-1.93
840	2.877	2.923	-0.046	-1.60
864	-	-	-	-

* Unsupplemented medium started

TABLE 5.15 Nitrogen Utilisation in the Tower Fermenter

(Expt. No. 15)

TIME INTO FERMENTATION	NITROGEN			% UTILISATION
	MEDIUM	EFFLUENT	UTILISED	
Hours	G/L	G/L	G/L	
240	2.997	2.984	0.013	0.42
288	3.036	3.027	0.009	0.29
384	3.013	3.025	-0.012	-0.39
408	3.006	2.986	0.020	0.69
432	2.957	2.974	-0.017	-0.55
*504	3.014	2.990	0.024	0.82
528	3.009	2.968	0.041	1.37

*Non-Sterile medium started at 456 hours into the fermentation.

of nitrogen utilised, (see Table 5.14), was only approximately 6% of that supplied. Consequently, the salts addition was discontinued and an unsupplemented medium put on line. Initially the nitrogen utilisation rose to approximately 6.5% of that supplied, but gradually dropped to zero. This coincided with the yeast taking a more familiar pale creamy colour and flocculent morphology, and with a dramatic increase in the yeast concentration in the fermenter. As can be seen from Table 5.14 a number of cases where there was a net loss of nitrogen to the medium were recorded. Similar results were observed in expt. no. 15 (Table 5.15). Coote (1974) also recorded this phenomenon. If his analyses are statistically examined 59% show a net loss of nitrogen from the yeast to the medium; of the remainder 35% show the nitrogen utilised was less than 3% of that supplied.

Shock excretion of nitrogenous compounds from brewers' yeasts has been reported. However, Rainbow (1970) has suggested that yeast cells in high concentrations are likely to be physiologically mature and unlikely to be susceptible to shock excretion. Therefore, the most likely explanation of a net nitrogen gain by the medium is yeast-cell lysis. It is suggested that the tower fermenter was in a pseudo steady-state, there being a delicate balance between cell death and growth. Cyclic changes may have occurred, each cycle lasting several days, with the yeast growing or dying in such small quantities as to be immeasurable by normal methods.

Nitrogen Mass Balance

The yeast cell overflow rate was routinely measured in expt. no. 14. The concentration of cells in the effluent is given in Table 5.16. It was found that 1 g/l of centrifuged yeast was equivalent to 3.58×10^6 cells/cm^3. This is in close agreement with the results of den Blanken (1974), who noted that 1 g/l of centrifuged yeast was equivalent to 3.81×10^6 cells/cm^3.

Considering the fermenter between 240 and 360 hours into the fermentation:

Mean cell overflow = 6.53×10^6 cells/cm^3

= 1.715 g/l of centrifuged yeast

Centrifuged yeast moisture content = 81.0%w/w

Dry yeast nitrogen content = 6.19%w/w

Hence, nitrogen utilisation accounted for by yeast growth

= 1.715 x 0.19 x 0.0619

= 0.0202 g/l.

The mean nitrogen utilisation figure over this period was 0.202 g/l. Therefore, only 10% of the utilised nitrogen can be accounted for by cellular overflow. Even though the yeast concentration in the fermenter increased slightly over this period this can only account for a further 5% of the nitrogen utilised.

This result may be contrasted with figures given by Ault et al. (1969). Using a wort containing 0.60 g/l of nitrogen they recorded a similar nitrogen uptake of 0.205 g/l. However,

TABLE 5.16 Yeast Cell Overflow Rate (Expt. No. 14)

TIME INTO FERMENTATION	CELLS IN EFFLUENT	COMMENTS
Hours	Cells x 10^{-6}/CM^3	
168	29.103	High air flow-rate
216	19.1670	Air flow reduced
240	6.1720	Yeast increase in tower
288	7.4033	Slight yeast increase
312	6.8283	
336	6.6096	Yeast still "sandy". No plug.
360	5.6565	Yeast profile constant
456*	3.3002	Yeast increase
504	2.2187	Dense yeast plug
528	3.4790	Yeast profile constant
576	2.8722	
600	3.9943	
672	0.3317	
696	0.3125	
720	0.3593	
768	0.3345	
816	0.7216	
840	0.4427	

*Unsupplemented medium started at 432 hours into the fermentation.

with a yeast overflow rate of 10.82 g/l (expressed as pressed yeast), approximately 87% of the total nitrogen uptake was accounted for by yeast overflow in the effluent beer.

5.4.6 Vicinal Diketones and Fermentation

Vicinal diketones contribute strongly to the traditional flavour characteristics of both ale and lager. Rainbow (1970) has described the taste and smell of vicinal diketones as buttery, honey-like, or toffee-like. The vicinal diketone (V.D.K.) content of a range of beers is given in Table 5.17.

TABLE 5.17 The V.D.K. Content of Various Beers.

Vicinal Diketones PPM	Comments	Authors
0.20–0.49	A range of beers	West et al. (1952)
0.20–0.25	Various lagers	Drews et al. (1962)
0.03–0.84	A range of ales, lagers stouts, and barley wine	Harrison et al. (1965)

Harrison et al. (1965) demonstrated that diacetyl, the main V.D.K. constituent, was produced in the early stages of a fermentation and decreased in the later stages. This observation was confirmed by Rice and Helbert (1973). Klopper et al. (1965) and Ault et al. (1969) found a similar V.D.K. profile in the tower fermenter.

A number of factors have been said to influence the formation of V.D.K.s. Coote (1974) found that aeration in a small-scale tower fermenter led to an increase in diacetyl levels. Portno (1966) and Wainwright (1973) expressed the view

that aeration was an indirect cause of increased V.D.K. levels: increasing aeration was said to have resulted in increased yeast reproduction which was the direct cause of higher diacetyl levels. This latter view is supported by the work of Rice and Helbert (1973) who observed that an increase in fermentation temperature increased yeast growth which in turn led to a rise in diacetyl concentration. It is interesting to note that increasing temperature and aeration rates in continuous fermentations, where yeast growth was relatively low, had no effect on diacetyl concentration (Klopper et al. (1965), Cowland and Maule (1966), and den Blanken (1974)). Other factors which have been noted as affecting the production of diacetyl are yeast strain (Klopper et al. (1965) and Coote (1974)), carbon dioxide purging (Wainwright (1973)), and bacterial infection of the finished product (West et al. (1952) and Wainwright (1973)).

With tower fermenters Klopper et al. (1965) found equivalent levels of diacetyl in batch and continuously produced beers. A more complex situation was reported by Ault et al. (1969) who found that beer produced continuously had a high level of diacetyl on racking relative to batch beer, but that both beers attenuated to the same diacetyl concentration on storage. den Blanken (1974), when studying lager production, found that a long residence time, (>20 hours), was required in a tower fermenter in order to achieve an acceptable diacetyl concentration. Coote (1974) confirmed this and recommended residence times between 24 and 36 hours for lager production in a tower fermenter.

As V.D.K.s are such an important indicator of beer quality, they were measured routinely in two experiments. The results of

the analyses are given in Tables 5.18 and 5.19 for expt. nos. 12 and 13 respectively.

A strain of Saccharomyces cerevisiae, CFFC1, was used in expt. no. 12. The concentration of V.D.K.s was within the threshold range for ales, apart from when a step increase in the wort flow-rate was made at 384 hours. This increase in flow-rate, from 16.2 cm^3/min to 41.5 cm^3/min, caused some disturbance in the yeast concentration profile and resulted in a rise in the P.G. of the effluent beer. However, the P.G. gradually returned to its former level and the V.D.K concentration followed the same trend. An air flow-rate of approximately 20 cm^3/min at S.T.P. was maintained throughout this run.

A strain of Saccharomyces carlsbergensis, CFCC 39, was used in expt. no. 13, although the wort was not modified to simulate lager production. Prior to 96 hours the yeast was sandy brown and non-flocculent in nature. As the yeast took on its more usual morphology and the organism concentration in the fermenter increased, the V.D.K. concentration decreased to similar levels to those recorded in expt. no. 12. The diacetyl concentration showed little change when alterations in the wort flow-rate were made, though no visual changes occurred in the tower itself.

An air-flow-rate of 10 cm^3/min at S.T.P. was maintained until 528 hours: the air supply was then discontinued and the wort flow-rate reduced. Within 48 hours the effluent P.G. had risen to 15.0 from 8.5, but there was no change in V.D.K. concentration.

TABLE 5.19 Vicinal Diketone Production in the Tower Fermenter (Expt. No. 13)

TIME INTO FERMENTATION	VICINAL DIKETONES	WORT FLOW
Hours	PPM	CM^3/MIN
96	0.836	10.6
120	0.685	10.6
144	-	10.6
168	0.292	10.6
192	0.356	15.9
216	0.420	15.9
240	0.421	15.9
264	-	15.9
288	0.466	33.3
312	0.450	33.3
336	-	33.3
360	0.360	33.3
384	0.362	33.3
408	0.250	42.0
432	-	42.0
456	0.316	42.0
480	0.362	42.0
504	0.332	42.0
528	-	20.2*
552	0.366	20.2
576	-	20.2
600	0.380	20.2
624	0.322	14.7
648	0.286	14.7
672	0.362	14.7

* Air supply discontinued

TABLE 5.18 Vicinal Diketone Production in the Tower Fermenter. (Expt. No. 12)

TIME INTO FERMENTATION	VICINAL DINETONES	WORT FLOW
Hours	PPM	CM^3/MIN
192	0.402	8.7
216	0.363	16.2
240	-	16.2
264	-	16.2
288	0.312	16.2
312	0.344	16.2
336	0.361	16.2
360	0.361	16.2
384	1.000	41.5
408	0.552	41.5
432	-	41.5
456	0.307	41.5
480	0.322	41.5
504	0.326	41.5
528	0.437	41.5
552	0.310	41.5

5.5 Summary

The fermenter was found to have three distinct zones. These were: -

i) the base of the fermenter where a packed bed of yeast (20%-25% of the tower height) existed and there was plug flow of the liquid

ii) the top zone of the fermenter (50%-60% of the tower height) which may be considered as a continuous stirred tank reactor

iii) a transition zone, from plug to C.S.T.R. conditions existed between the two previously mentioned zones.

A low flow of air to the fermenter (approximately 15 cm^3/min at S.T.P.) was found to be necessary both to maintain the physiological well-being of the yeast and to maintain the packed bed of yeast in a stable position at the base of the fermenter.

The tower fermenter was very flexible in operation: wort residence times from 2.5-16 hours were observed, and the fermenter was closed down for 64 hours, the medium re-started, and the fermenter was running normally within 8 hours.

The yeast concentration profile in the fermenter varied greatly from run to run though a mean concentration of approximately 190 g/l was observed in four of the five continuous experiments.

Sugar utilisation rates of 16.8 and 21.4 g/h. l of fermenter volume were readily attainable in the tower with brewers' wort and a molasses-based medium respectively. The majority of the fermentation was found to be complete within the first 20%

of the tower height. These figures are similar to the results of other authors. As ethanol yields of 90% were readily achieved in the tower fermenter the two fore-mentioned sugar utilisation rates correspond to 8.1 and 10.3 g of ethanol/h . l of fermenter volume. There was also evidence that an ethanol production rate of 21.9 g/h . l of fermenter volume would be possible in the tower fermenter.

Previous work by other authors with both continuous and batch fermenters stated nitrogen supplementation of molasses-based media was necessary for efficient ethanol production. Medium supplementation was found to be unnecessary with the tower fermenter. This was probably due to the fact that the tower contained high yeast concentrations and as a result yeast reproduction was low.

The flavour of beer produced in the tower was crudely examined by determining the concentration in the final product of a pair of compounds known as vicinal diketones. A mean concentration of 0.35 ppm was observed which is within the taste threshold for beers.

CHAPTER 6

MODELLING OF THE TOWER FERMENTER

6.1 Introduction

The work in earlier chapters is discussed and drawn together in this penultimate chapter of the thesis. In particular, an attempt is made to integrate the kinetic models, based on batch fermentation studies, with the physical observations and measurements made during operation of the continuous tower fermenter.

The initial sections of the chapter deal briefly with yeast fluidisation/sedimentation, kinetics, floc size and voidage, and floc diffusion. These subjects are followed by proposals for modelling continuous tower fermenters. Results predicted by the model are compared with those obtained by experiment; the differences are discussed although not fully explained.

6.2 Yeast Fluidisation/Sedimentation

The tower fermentation process may be viewed as a three-phase fluidisation operation in which the density of the solid phase (yeast) is close to that of the liquid phase (wort). Such three-phase systems are obviously complex and their behaviour is difficult to describe (see Smith and Greenshields (1973)).

Most of the evolved carbon dioxide is in solution in the lower zones of the fermenter and hence there is little gas flow to affect the structure of the packed bed of yeast. The normal superficial liquid velocities in the tower are of the order of 1.2 cm/min.; this value is such that single yeast cells are readily fluidised whereas flocs greater than about 0.1 cm. diameter settle (see also Table 6.2).

Formation of gas bubbles causes mixing of both the liquid and yeast flocs. Calculations of gas flow-rates, and measurements in expt. no . 13, show that superficial gas velocities of from 0.073 to 0.33 cm/s occurred in the ale fermenter. Smith and Greenshields (1973) estimated a superficial gas velocity of about 0.5 cm/s in a commercial fermenter.

Although gas hold-up in gas-liquid systems with solid particles has been studied by a number of authors (Adlington and Thompson (1965), Viswanathan et al. (1965), and Ostergaard and Michelson (1970)) it is not possible to use their results to predict gas hold-up, which in any case is not more than a few per cent. It is probable that gas hold-up is of less importance than the liquid circulation patterns, which are both difficult to measure and predict. The work of Joshi and Sharma (1979) with air-water systems reveals that there is a strong upward movement of gas and liquid in the centre of the tower and downward flow of liquid near the walls: these flow patterns are very erratic, and circulation cells of height equal to the column diameter may be involved. Despite the complexity of the liquid flow, upward liquid velocities of the order of 20 cm/s are to be expected near the column axis and downward velocities of the same magnitude near the wall. Such flows are sufficient to cause movement of flocs as large as 1 cm in diameter: consequently, relatively rapid mixing of liquid and yeast flocs is to be expected in the upper sections of the tower fermenter.

6.3 Kinetics of Alcohol Fermentation in Tower Systems

The kinetics of the alcohol fermentation have been discussed by a number of authors. Shore and Royston (1968) suggested that the fermentation was essentially zero-order; the rate of fermentation would then be given by

$$\frac{-ds}{dt} = Kx \qquad 6.1$$

where s = sugar concentration (%w/w)

K = a constant, dependent on such factors as yeast type and temperature

x = yeast concentration (volume fraction), assumed to be constant throughout the tower.

With $s = s_I$ at $t = 0$

and $s = s_o$ at $t = t$

integration of eq. 6.1 leads to

$$(s_I - s_o) = Kxt \qquad 6.2$$

where s_I = inlet sugar concentration (%w/w)

s_o = outlet sugar concentration (%w/w)

t = the actual residence time of wort in the fermenter (h).

The yeast and evolved carbon dioxide occupy a significant volume of the tower and so:

$$t = T(1 - V_g - x) \qquad 6.3$$

where T = wort apparent residence time

and V_g = fractional volume of the fermenter occupied by gas .

Shore and Royston (1968) in fact used,

$$t = T(1-x)(1-V_g) \qquad 6.4$$

to describe the relationship between the apparent and actual residence times; however eq. 6.3 is the correct relationship. Hence, from equations 6.2 and 6.3

$$T = \frac{(s_I - s_e)}{Kx(1-V_g-x)} \qquad 6.5$$

The wort apparent residence time, T, calculated from eq. 6.4 is very dependent on the constant, K, which has units of;

$$\left\{\frac{g \text{ sugar}}{100\ g \text{ solution} \cdot h}\right\} \cdot \left\{\frac{\text{Volume of solution}}{\text{volume of yeast floc}}\right\}.$$

Assuming the density of yeast flocs and the solution to be similar then the units of K become

$$\left\{\frac{g \text{ sugar}}{100\ g \text{ solution} \cdot h}\right\} \cdot \left\{\frac{100\ g \text{ solution}}{100\ g \text{ yeast floc}}\right\}$$

or g sugar/100 g yeast floc. h.

Values of K are not given by Shore and Royston (1968). However, the rate of sugar utilisation in the tower fermenter (see section 5.4.3) was approximately 17 g sugar/h.l of fermenter volume and the mean yeast concentration was approximately 200 g/l on a centrifuged wet weight basis (see section 5.4.2). Hence the mean rate of sugar utilisation in the fermenter was 8.5 g sugar/h. 100 g centrifuged wet yeast; if this value is taken as K then Table 6.1 gives typical values of T for various yeast concentrations.

TABLE 6.1 Liquid Residence Time in the Tower Fermenter

Calculated from Eq. 6.5

s_I = 10.0%, s_e = 2.5%, V_g = 0.05, K = 8.5g sugar/h. 100 g yeast floc

VOLUMETRIC YEAST FRACTION	LIQUOR APPARENT RESIDENCE TIME
x	Hours
0.10	10.4
0.20	5.9
0.30	4.5
0.40	4.0
0.50	3.9
0.60	4.2

T is a minimum when x = 0.47 or 0.48

Now it can be shown that for the zero-order situation, given equivalent yeast concentrations, the residence time for a particular wort attenuation will be the same in either a C.S.T.R. or tubular reactor with plug flow: in other words, the degree of attenuation is not affected by the flow or mixing patterns in the fermenter. However, evidence from Royston (1961) does not support the case for zero-order kinetics: when operating a tower fermenter "normally" as a heterogeneous reactor a typical liquor apparent residence time was 4 hours; increasing the level of agitation to create the homogeneous condition required an increase in the apparent residence time

to 7 - 12 hours for the same degree of attenuation. Hence, though the wort apparent residence times presented in Table 6.1 are reasonable they must be treated with caution, both on the basis of the results of Royston's (1961) work and the dependence of T on an "assumed" value of K.

Royston (1966b) has also considered first-order kinetics. As he rightly pointed out, a pseudo first-order reaction arises if the over-all rate at which yeast ferments sugars is first controlled by the rate of entry of the sugars into the cells: Sutton and Lampen (1962) and Griffin (1969) have concluded that this can occur. However, it is unlikely that a simple first-order kinetic model could ever describe the full course of the fermentation. The author must therefore agree with Hough and Button (1972) that the alcohol fermentation cannot readily be described in terms of zero or first-order kinetics.

Smith and Greenshields (1973) stated that ale fermentations might be described by two consecutive zero-order reactions. In general terms the rate of conversion of substrate to product might be of the form:

$$-\frac{ds}{dt} = \left(\frac{k_1 s}{k_2 + s}\right) . \quad x \qquad 6.6$$

Assuming s to be much greater than the constant, k_2, and x to be constant, then the rate of substrate utilisation becomes zero-order. However, the author's batch fermentation model (section 4.6) shows that values of k_2 are of the same order of magnitude as s and that modifications to eq. 6.6, based on endogenous metabolism and alcohol inhibition, are necessary.

Consequently, the use of two consecutive zero-order rate equations must also be abandoned in favour of the more complex model developed by the author.

6.4 Yeast Floc Size and Voidage

The yeast flocs in a tower fermenter may to some extent be considered analagous to porous catalyst particles in a fluidised bed reactor. Therefore, the size, shape, and porosity of the yeast flocs are likely to influence the over-all rate of reaction in the fermenter.

James (1973) estimated that the diameters of flocs in a tower fermenter would vary between 0.15 cm and 0.60 cm, their voidage being of the order of 0.6-0.8 when in an isolated state. The author's observations of the tower fermenter (section 5.4) revealed the existence of a wide range of floc diameters. Small flocs, 0.1 cm in diameter, were predominant in the upper sections of the column, while some large flocs, up to 1 cm in diameter, were evident in the lower zones of the fermenter. However, most of the yeast in the plug was not in the form of flocs; it would be more apt to describe the structure as a "porous mud", consisting of individual yeast cells packed tightly together.

As estimate of the likely minimum voidage of yeast flocs and aggregates may be made by considering yeast cells to be oblate spheroids. Measurements of cells indicate that the major axis, a, = 10×10^{-6} m and the minor axis, b, = 8×10^{-6} m: the cell volume, V_c, can then be estimated from equation 6.7;

$$V_c = \frac{a^2 b}{6} \qquad 6.7.$$

Now, Kunii and Levenspiel (1969) have related the sphericity of particles, ϕ_s, to their packing density or bed voidage. The sphericity is defined by

$$\phi_s = \left[\frac{\text{surface area of sphere}}{\text{surface area of particle}} \right],$$

where both sphere and particle have the same volume. As the sphericity tends to 1 then the correlation of Kunii and Levenspiel (1969) indicates the bed voidage tends to 0.38; if dense packing occurs the voidage may fall to 0.33. However, higher packing densities may be possible, Gratton and Fraser (1935) having observed voidages of as low as 0.2595 for tightly packed spheres.

The surface area of an oblate spheroid, A_s, is defined by;

$$A_s = \frac{\pi a^2}{2} + \frac{\pi b^2}{4e} \cdot \ln\left[\frac{1 + e}{1 - e} \right] \qquad 6.8$$

e = eccentricity (= b/a).

Hence, for the observed yeast cell dimensions $\phi_s = 0.92$, which results in a calculated bed voidage of 0.4.

These calculations are supported by the author's measurements of the yeast plug concentration, which varied between 300 g/l and 700 g/l expressed as centrifuged wet yeast: these figures suggest approximate voidages of 0.72 - 0.35, based on a cell density of 1070 kg/m^3. James (1973) used photomicrographs to estimate the voidage of clumps of yeast cells: he arrived at a figure of 0.4 for the voidage of a small cluster of cells. He also showed, by calculation, that when small flocs aggregate the voidage of the system rapidly increases. Thus, all the

evidence suggests that at the high yeast concentrations (and low voidages) found at the base of the tower fermenter the yeast bed consisted of individual packed cells. It is also likely that even at the relatively high voidage of 0.7 some degree of floc aggregation must have occurred.

Though the above calculations and observations are based on credible assumptions, the following calculations show the caution with which estimates of microbial voidages must be treated. The calculations are introduced after first considering information about the moisture content of centrifuged yeast cakes and yeast cells as well as the densities of hydrated and dry yeast cells.

James (1973) has reported that the moisture content of centrifuged yeast cake is 81.2%w/w. A weighted mean of values from the author's work resulted in a figure of 81.7%w/w. James (1973) also measured the ratio

$$\frac{\text{Cell Wet Wt.}}{\text{Cell Dry Wt.}} \quad ;$$

a value of 4.05 was found for this ratio for a number of yeasts, indicating a cellular moisture content of 75.3%w/w.

Aiba et al. (1962) and Aiba et al. (1964) have measured the density of hydrated yeast cells and give figures of 1.0725 ± 0.0012 g/cm^3 and 1.09 g/cm^3 in their respective papers. James (1973) concluded that the former value was likely to be the more accurate: however, it should be noted that Haddad and Lindegren (1954) have placed values for hydrated cell densities in the wider range of 1.087 ± 0.026 g/cm^3. Dry cell densities are available from the work of Sterbacek (1972)

and James (1973): their values are 1.34 g/cm^3 and 1.32 g/cm^3 respectively.

Now consider 100 g of centrifuged yeast cake having a moisture content of 81.7%w/w and thus a dry mass of 18.3 g. With a cellular moisture content of 75.3%w/w the liquid associated with the cells is 55.8 g, and so the cake interstitial moisture is 25.9 g. If the liquid inside the cells is considered to have a density of 1.0 g/cm^3 and the density of the cell dry matter is 1.32 g/cm^3, then the hydrated cell density is 1.079 g/cm^3. It is now a simple matter to show that the voidage is 0.274. A small change in the assumed value for the cellular moisture content from 75.3%w/w to 72.0%w/w results in an hydrated cell density of 1.0896 g/cm^3 and a cake voidage of 0.366. In other words a 4.4% reduction in the assumed value for the cellular moisture content results in a 25% increase in the calculated cake voidage.

6.5 Floc Diffusion Considerations

Smith and Greenshields (1973) have considered the effects of pore diffusion of reactants/products into and out of yeast flocs. They concluded that surface reactions would be rate controlling in the tower fermenter if the average floc size was 0.1 cm or less. Hence, pore diffusion into and out of flocs observed in the upper zones of the fermenter need not give cause for concern. With flocs larger than 0.1 cm diameter it was calculated that the readily absorbed sugars would be taken up in the outer layers of the floc and pore diffusion could become rate controlling: this also suggests that cell autolysis

might occur in the inner region of such flocs.

However, the above results are based on the concept of a rigid floc. The author's observations revealed that where yeast flocs of large diameter were present they were continually being broken up and reformed, as was also the case with smaller flocs. Thus, it seems unlikely that any cells are deprived of food for sufficiently long periods for damage to occur.

The effects of pore diffusion in the base of the fermenter do not arise if the concept of a yeast plug is accepted. Such a plug can be viewed as individual yeast cells in a packed bed through which there is relatively uniform flow of liquid.

6.6 Modelling of the Tower Fermenter

Fidgett (1975) has developed a theoretical mathematical model of the anaerobic tower fermenter. The model was based on the premise that the fermenter contained yeast flocs in the form of a fluidised bed, the voidage of which could be calculated from an expression similar to the Richardson-Zaki (1954) equation, viz.,

$$\frac{u_S}{u_T} = \varepsilon^n \quad , \qquad 6.9$$

where n = the "fluidisation index", a constant dependent on yeast strain and wort physical and chemical properties.

James (1973) showed that the particle terminal velocity, u_T, could best be calculated using an equation developed by Allen in 1900, viz.,

$$u_T = \frac{0.153\, d_p^{1.142}\, (g(\rho_p - \rho))^{0.714}}{\rho^{0.286}\, \mu^{0.428}} \qquad 6.10$$

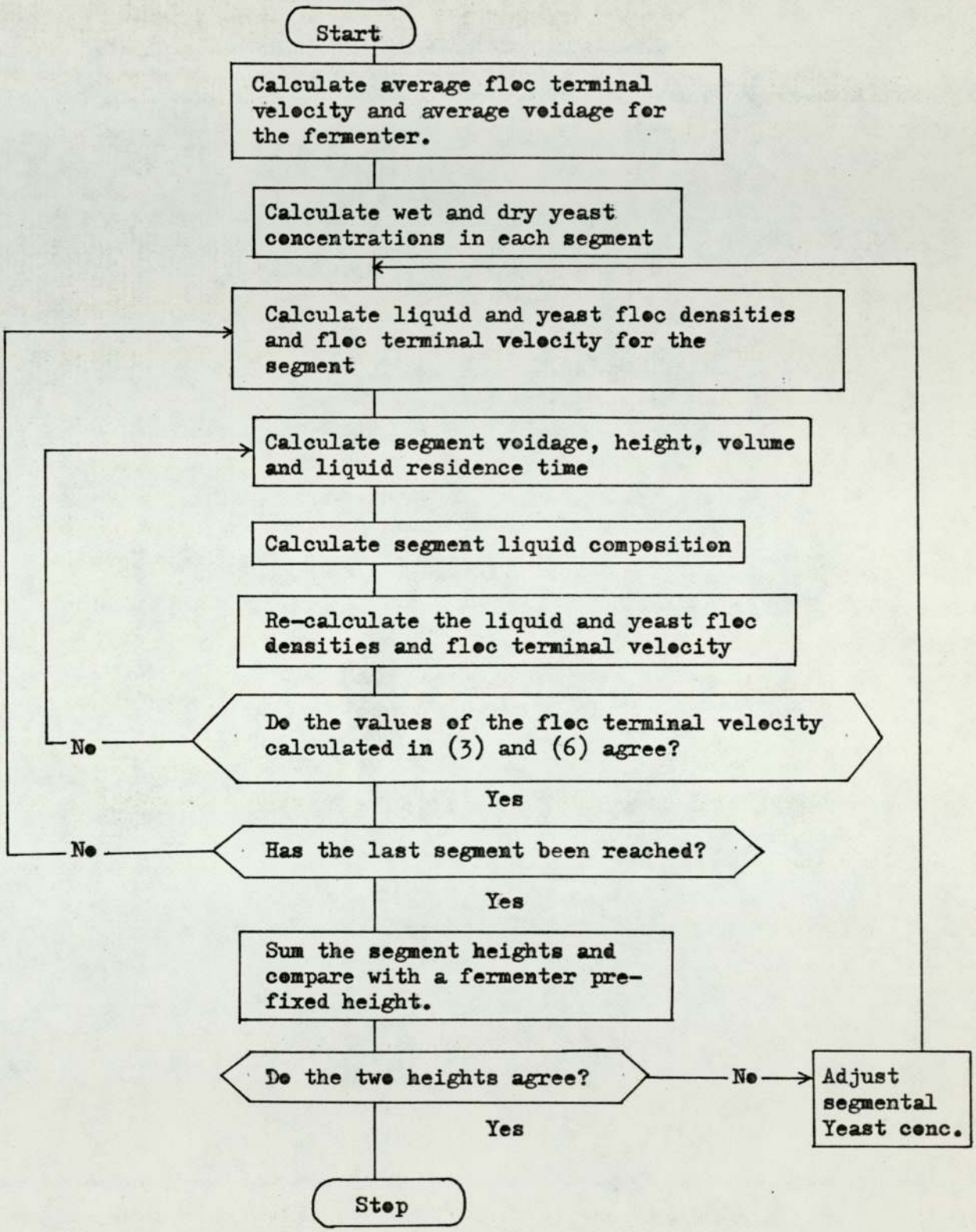
James (1973) also estimated values for the fluidisation factor n, and found it to be in the range 8.0-15.5.

Fidgett's (1975) model involved dividing the fermenter into a number of compartments, say 20, each containing an equal quantity of yeast. Initially estimates of the average floc terminal velocity and average voidage in the tower fermenter were made using equations 6.9 and 6.10. Calculations for each segment of the fermenter were carried out and the sum of the segment heights was compared to a pre-fixed tower height: if these were not in agreement new estimates were made of the yeast concentration in each segment and the procedure repeated. The full calculation sequence is illustrated in Fig. 6.1. Fidgett's (1975) model predicted some of the operational characteristics of continuous tower fermenters observed by Royston (1966b), Klopper et al. (1965), and Ault et al. (1969). For instance, increasing liquid flow-rates gave increased yeast concentrations in the upper sections of the fermenter while the yeast concentration in the base of the fermenter decreased: this in turn affected the rate of fermentation in the tower.

However, a number of observations made during the operation of the pilot-plant fermenter in this work suggests that the model is open to criticism and must be modified: the key points are listed below.

1) The yeast plug in the base of the fermenter could, in no way, be regarded as a fluidised bed of yeast flocs.

Fig. 6.1 Flowsheet for Fidgett's (1975) Tower Fermenter Model



2) Fidgett (1975) used a large number of well mixed tanks - in-series, of variable volume, to describe liquid flow in the fermenter. Observations indicated that there was "good" mixing in the upper sections of the fermenter, suggesting that relatively few well mixed zones could be used for modelling purposes.

3) The estimation of bed voidage by eq. 6.9 is very susceptible to the calculated value of the particle terminal velocity u_T; this in turn is chiefly dependent on the particle diameter, d_p. Table 6.2 shows the effect of varying particle diameter on the parameters u_T, u_s/u_T, and ε .

Table 6.2 The effect of particle diameter on u_T, u_s/u_T, and ε .

g = 980.7 cm/s, ρ_p(cell) = 1.079 g/cm^3, ρ_p(floc) = 1.050 g/cm^3
ρ = 1.010 g/cm^3, μ = 0.0209 g/s.cm, n = 10, u_s = 1.28 x 10^{-2} cm/s,
Θ = 5.4 hours.

d_p	u_T	u_s/u_T	ε
cm	cm/s		
9 x 10^{-4}	5.384 x 10^{-3}	2.377	1.090
0.01	5.705 x 10^{-2}	0.224	0.861
0.10	0.791	1.617 x 10^{-2}	0.662
1.0	10.97	1.166 x 10^{-3}	0.509

Firstly, it is obvious that the model cannot be applied to a bed of individual yeast cells. Secondly, the voidage figures in Table 6.2 suggest that as the floc size increases

then the voidage in the fermenter decreases, i.e. large particles or flocs need to be present to achieve the low voidages observed in the bottom sections of the fermenter. This view is contrary to observations of the fermenter and the discussion (section 6.4) of yeast floc voidages where aggregation of flocs leads to a high system voidage. Also, for the very high voidages observed in the upper zones of the fermenter smaller particles than those observed are required to predict the necessary voidage. There is the further complicating factor that in using eq. 6.10 to estimate u_T, allowances must be made for changes in the wort density and viscosity with height in the fermenter. This is especially significant in the lower zones of the fermenter where fermentation was rapid and plug flow of the liquid, with some channelling, was observed.

4) Equation 6.9 is based on sedimentation/fluidisation measurements made in the absence of gas bubbles, which dominate floc movement in much of the tower.

6.7 Simplified Fermenter Model

6.7.1 Introduction

Observations and measurements of the yeast and specific gravity profiles in the tower fermenter (Chapter 5) indicated that it consisted of three distinct reaction zones:

i) a plug-flow zone

ii) a transition zone

iii) a well mixed or continuous flow stirred tank (C.S.T.R.) region.

The plug-flow zone was the lower section of the fermenter where the medium flowed in plug-flow, with some channelling, through a densely packed bed of yeast. The upper section of the tower was, from yeast and specific gravity measurements, well mixed and therefore assumed to be a C.S.T.R. Between these two sections was the "transition" zone which was assumed to be equivalent to a single well stirred vessel with a parallel region of plug flow.

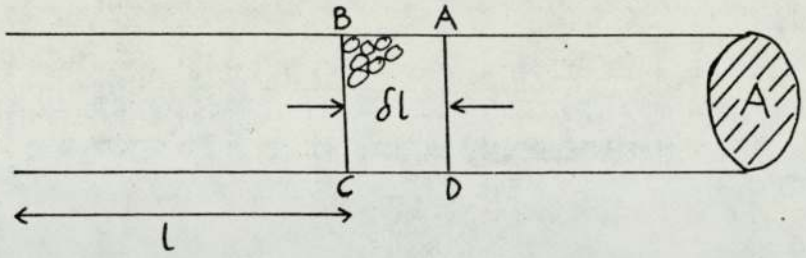
The above approach to modelling is supported by the author's experimental observations and measurements made by other workers. In all cases there was rapid utilisation of the fermentable carbohydrates in the lower 10%-15% of the column. The majority of the yeast was also usually in this region, with yeast concentration decreasing with fermenter height.

6.7.2 Plug-Flow Zone

The liquid is assumed to be flowing through a packed bed of yeast with no back or forward mixing but complete radial mixing. Generally, for a plug-flow reactor used in chemical engineering the volumetric flow-rate will vary through the reactor: this is due to the fact that this type of reactor is usually used for gas-phase reactions. Here, the liquid volumetric flow-rate, Q, can be assumed constant through the segment: hence, the superficial liquid velocity, u_{SL}, will also be constant.

Consider, Fig. 6.2, a small section of this zone of length δl and cross-sectional area, A.

Fig. 6.2 The Plug-Flow Zone



Consider the bed voidage to be ε in the volume ABCD.

The interstitial fluid velocity is then given by

$$u_i = \frac{u_{SL}}{\varepsilon} \qquad 6.11$$

If s is the medium (sugars) concentration (w/v) at length l a sugars balance over ABCD leads to;

$$Au_i s = Au_i\left[s + \frac{ds}{dl}\delta l\right] + (-r_s)A\delta l \qquad 6.12$$

and

$$-u_i\frac{ds}{dl} = (-r_s) \qquad 6.13$$

where $(-r_s)$ = the rate of substrate utilisation, is based on the total bed volume.

Assuming that the limiting sugars concentration, s^*, is not reached, then from Fig. 4.4 (eq. 4.12),

$$(-r_s) = \frac{K1.s.x.}{K2 + s} \qquad 6.14$$

Now $u_{SL} = \frac{Q}{A}$ and $u_i = \frac{Q}{A.\varepsilon}$

Substituting for u_i and $(-r_s)$ in eq. 6.13;

$$\frac{-Q}{A.\varepsilon}\frac{ds}{dl} = \frac{K1.s.x}{K2 + s} \qquad 6.15$$

If x and ε are constant in the plug-zone, then at

$l = l_1$ $\qquad s = s_1$

$l = l_2$ $\qquad s = s_2$

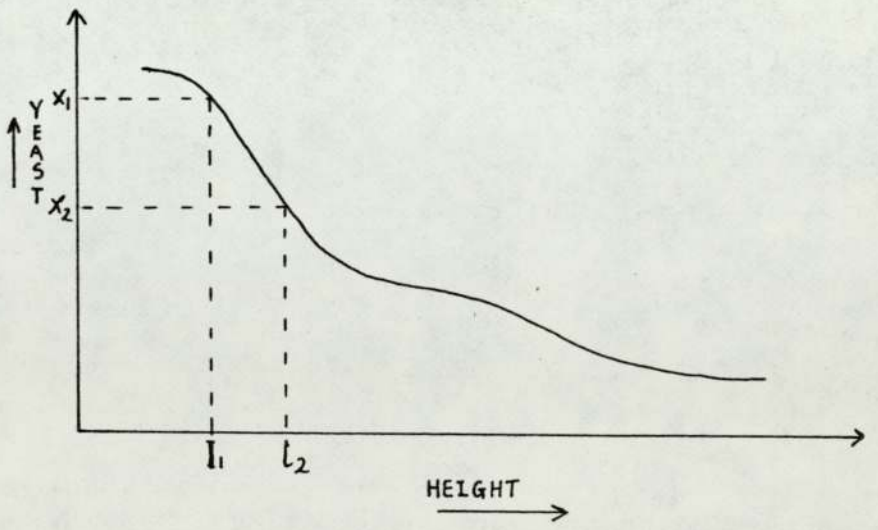
and $$\frac{A.\varepsilon}{Q}\int_{l_1}^{l_2} dl = \frac{1}{x}\int_{s_2}^{s_1}\frac{(K2 + s)}{K1.s} \cdot ds \qquad 6.16$$

which on integration yields

$$\frac{A.\varepsilon}{Q}(l_2 - l_1) = \frac{1}{K1.x}\left[K2 \ln\left(\frac{s_1}{s_2}\right) + (s_1 - s_2)\right] \qquad 6.17.$$

Experimental evidence is that the yeast concentration in the plug-flow zone is not constant. However, the yeast concentration can be related to fermenter height, yeast concentration decreasing linearly with height over sections of the plug (see graphs 5.3-5.8 and Fig. 6.3).

Fig. 6.3 Sketch of Fermenter Yeast Concentration Profile



Over various sections of the column it is assumed that the yeast concentration at a given height, l, is given by:

$$x = x_1 - al \qquad 6.18$$

$$\text{where } a = \frac{(x_1 - x_2)}{(l_2 - l_1)} \qquad 6.19$$

As the yeast concentration profile in the fermenter was not constant then the voidage, ε in the yeast plug will vary. The yeast in the plug-flow zone was assumed to be in the form of individual cells, as opposed to flocs, for reasons discussed in sections 6.4 and 6.6. Hence, the voidage in the zone is calculated from;

$$1 - \frac{x}{\rho} = \left(\frac{\rho - x}{\rho}\right) \qquad 6.20$$

where ρ = the density of a yeast cell

and x = yeast concentration (centrifuged wet weight basis).

However, some allowance must be made for interstitial liquid in the centrifuged yeast cake: if this moisture is not considered then the voidage in the fermenter will appear too low. Calculation, and measurements, of yeast cake voidages (section 6.4) results in a cake voidage of approximately 0.25. Hence, the actual voidage in the plug-flow zone is given by;

$$\varepsilon = \left(\frac{\rho - 0.75x}{\rho}\right) \qquad 6.21$$

As $x = x_1 - al$, then,

$$\varepsilon = \left[\frac{\rho - 0.75(x_1 - al)}{\rho}\right] \qquad 6.22.$$

Substituting for $\mathcal{E}$ (eq. 6.22) and x (eq. 6.18) in equation 6.15 results in,

$$\frac{-Q}{A.K1}\left[\frac{K2 + s}{s}\right].ds = (x_1 - al).\left(\frac{\rho - 0.75x_1 + 0.75\ al}{\rho}\right) \qquad 6.23$$

at $l = l_1$ $\quad s = s_1$

$l = l_2$ $\quad s = s_2$

and

$$\frac{Q.\rho}{A.K1}\int_{s_2}^{s_1}\left(\frac{K2 + s}{s}\right) ds = \int_{l_1}^{l_2}(x_1 - 0.75x_1^2 + 1.5ax_1 l - a\rho l - 0.75a^2 l^2)\, dl$$

$$\frac{Q.\rho}{A.K1}\left[K2 \ln\left(\frac{s_1}{s_2}\right) + (s_1 - s_2)\right] = (l_2 - l_1).(\rho x_1 - 0.75x_1^2) +$$

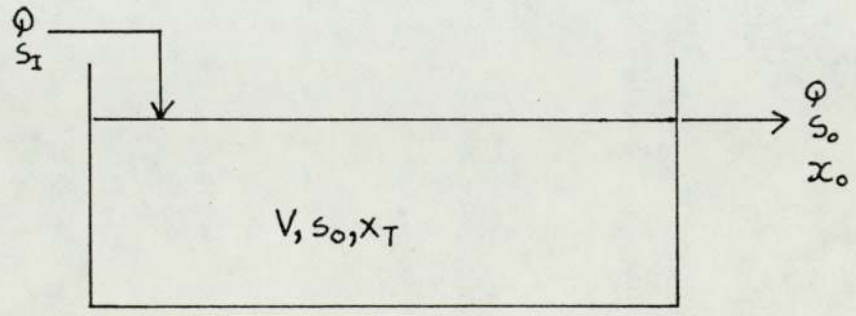
$$(l_2^2 - l_1^2).\left(0.75ax_1 - \frac{a\rho}{2}\right) -$$

$$(l_2^3 - l_1^3).0.25a^2 \qquad 6.24$$

For most practical purposes, the plug-flow zone could be modelled using one or two values of a.

6.7.3 Continuous Stirred Tank Zone

Fig. 6.4 Representation of Fermenter Upper Zone



Inlet sugars concentration	s_I
Outlet sugars concentration	$s_\bullet$
Volumetric flow-rate	Q
Yeast concentration in zone	x_T
Yeast concentration in outlet stream	$x_\bullet$

Due to the expanded separation zone the yeast concentration in the exit stream from the fermenter, $x_\bullet$, is not equal to the yeast concentration in the C.S.T.R. zone, i.e. the system is not well mixed with respect to yeast, only sugars.

A sugars balance leads to,

$$Qs_I = Qs_\bullet + (-r_s)V \qquad 6.25$$

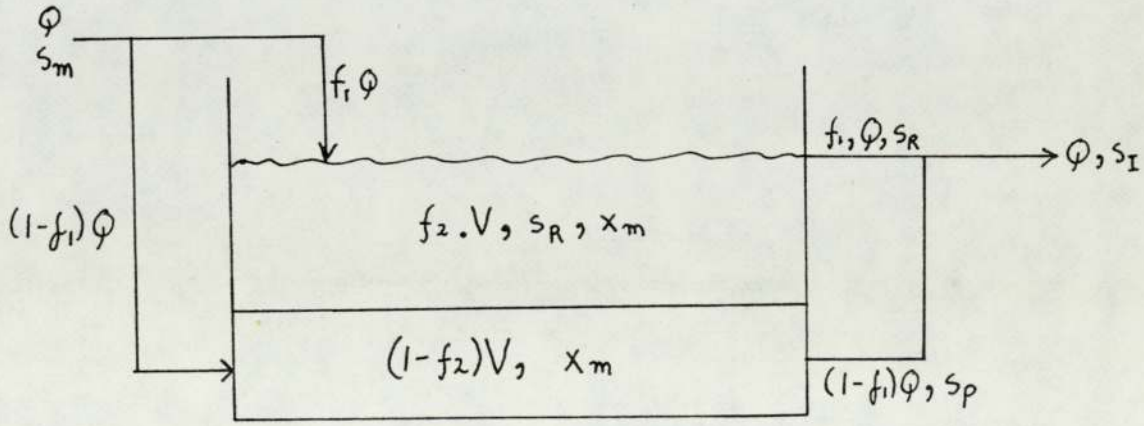
Assuming $s_I \leq s^*$ (See Fig. 4.4),

$$(-r_s) = \frac{K5.s_\bullet.x_T}{K2 + s_\bullet}$$

and so

$$s_I = s_\bullet + \frac{V}{Q}.\frac{K5.s_I.x_T}{(K2 + s_\bullet)} \qquad 6.26$$

6.7.4 <u>Transition Zone</u> - <u>Well Stirred Region with a Parallel Region of Plug Flow</u>



f_1 = the fraction of feed into the Well Stirred Region,

f_2 = the fraction of the vessel which is considered as a Well Stirred Region.

Well Stirred Region

Assuming the limiting sugars concentration, s*, has not been reached a sugars balance leads to,

$$Q.f_1.s_m = Q.f_1.s_R + \frac{Kl.s_r.x_m.f_2.V}{(K2 + s_R)} \qquad 6.27.$$

Plug Flow Region

Assuming the yeast concentration to be constant, and equivalent to the concentration in the stirred zone, a sugars balance leads to,

$$\frac{(1 - f_2).V.\varepsilon}{(1-f_1).Q} = \frac{1}{Kl.x_m}\left[K2\ln\left(\frac{s_m}{s_p}\right) + (s_m - s_p)\right] \qquad 6.28.$$

Exit Stream

A sugars balance gives,

$$Qs_I = f_1 Qs_R + (1-f_1)Qs_p$$

$$s_I = f_1 s_R + (1-f_1)s_p \qquad 6.29.$$

The inlet sugars concentration to this zone, s_m, can be calculated from eq. 6.24, assuming that eq. 6.18 holds over the entire length of the plug-flow zone and that the limiting sugars concentration, s*, is not reached in the plug-flow zone. If the limiting substrate concentration was attained in the plug-flow zone then the rate constant, Kl, in eq. 6.24, would be altered to K5, the rate constant for utilisation of the longer chain sugars: the change would occur at the height

in the plug-zone at which the simpler sugars had been utilised.

With a known value of s_m, by setting f_1 and f_2, which must be done on a purely arbitrary basis, equations 6.27-6.29 may be solved to give a value for s_I. The exit sugars concentration, s_I, from the transition zone may also be calculated from eq. 6.26, knowing the outlet sugars concentration from the fermenter: thus a check is made on the transition zone calculations.

6.8 Model Testing

6.8.1 An Initial Assessment

The sugars feed concentration to the fermenter and the exit stream sugars concentration were known, as was the yeast concentration profile in the tower (see sections 5.4.2 and 5.4.3). Mean values of the constants K1, K2, and K5 (from Table 4.7) were $0.157h^{-1}$, 50.14 g/l, and $0.013h^{-1}$ respectively. Equation 6.24 was used to estimate the amount of sugars metabolised in the plug-flow zone for each experiment, and similarly eq. 6.26 was employed for the C.S.T.R. zone. Tables 6.3 and 6.4 respectively show the calculated values of the exit sugar concentration from the plug-flow zone and the calculated inlet sugars concentration to the C.S.T.R. zone.

The exit sugars concentration from the plug-flow zone is much higher than that observed experimentally: also, the difference between the calculated exit sugars concentration from the plug-flow zone and the inlet sugars concentration to the C.S.T.R. zone is substantial. From the depth of each zone it is evident that the plug-flow zone occupies approximately

Table 6.3 Calculated Sugars Concentration from the Plug-Flow Zone

Expt. No.	Volumetric Flow-rate	Plug Depth	a	Sugars Conc.		Yeast Conc.	
				In	Out	Bottom of Section	Top of Section
	$m^3/h(x10^3)$	m	$kg/m^3.m$	kg/m^3	kg/m^3	kg/m^3	kg/m^3
11	1.68	0.32	172	95	72	385	330
	2.55	0.32	172	95	80	385	330
12	0.97	0-0.25	(x const)	92	53	695	695
		0.25-0.60	757	53	24	695	430
	2.55	0-0.25	(x const)	92	76	695	695
		0.25-0.60	757	76	60	695	430
13	0.95	0-0.4	275	92	41	490	380
		0.4-0.6	725	41	40	380	235
14	2.13	0.55	145	125	97	310	230

Table 6.4 Calculated Sugars Inlet Concentration to the C.S.T.R. Zone

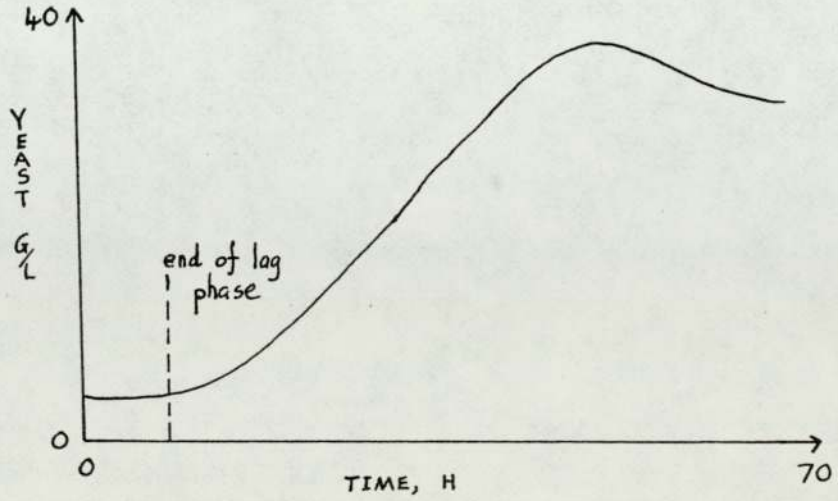
Expt. No.	Volumetric Flow-rate	Zone Depth	Zone Volume	Yeast Concentration	Sugars Conc.	
					In	Out
	$m^3/hr\ (x10^3)$	m	$m^3\ (x10^3)$	kg/m^3	kg/m^3	kg/m^3
11	1.68	0.98	4.47	101	13.2	12.5
		0.60	2.74	136	14.0	13.2
12	0.97	1.08	4.93	12	18.2	18.0
		0.40	1.82	45	18.5	18.2
13	0.95	1.28	5.84	110	19.2	17.0
14	2.13	0.53	2.42	115	12.1	12.0
		0.45	2.05	165	12.2	12.1

25% of the tower volume and the C.S.T.R. zone approximately 50%; this leaves 25% as the transition zone. At low volumetric flow-rates this latter zone could not possibly account for the calculated unused sugars, even if it were considered to be part of the plug-flow zone.

6.8.2 Effect of Ignoring the Lag Phase

In the batch alcohol fermentation there is a definite lag phase during which the yeast acclimatises to its surroundings after pitching: in the continuous fermenter wort is pumped into a vigorously fermenting brew and hence, fermentation could be expected to proceed at a faster rate. For this reason the batch fermentation data were re-examined with the lag phase of the fermentation discounted. This was achieved by examining the yeast growth curves (Appendix 3.1) and determining the points where the lag phase terminated and yeast growth began (see Fig. 6.6).

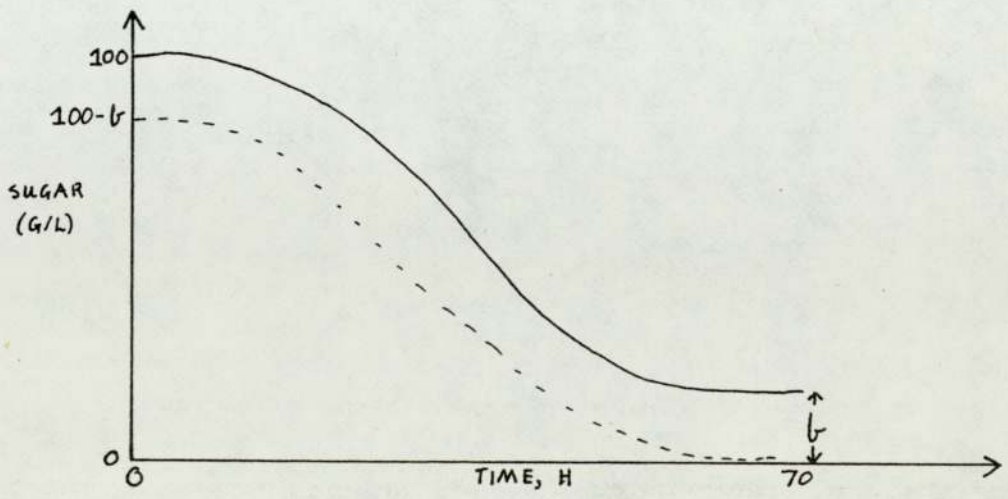
Fig. 6.6 Sketch of a Typical Yeast Growth Curve



Obviously the relatively crude method employed for determining the period of the lag phase does not give a true biological determination of the commencement of growth: however, it is useful to test the concept of ignoring the lag phase on the batch fermentation model.

To determine if the medium sugars concentration affected the batch model estimates of the medium fermentable sugars were made (see Fig. 6.7) and these only were considered available for utilisation by the yeast. The amount of fermentable sugar in the wort was determined by examining experimental reducing sugar utilisation curves (Appendix 3.1), to determine the amount of reducing sugar remaining unfermented at the "end" of the fermentation: this amount was taken to be the non-fermentables (b in Fig. 6.7). The non-fermentable sugar was then subtracted from the initial wort reducing sugars to give an initial amount of fermentable sugars in the medium ((100-b) in Fig. 6.7).

Fig. 6.7 Determination of Wort Fermentable Sugars



Again, though the method of determining fermentable sugars was relatively crude it serves the purpose of model testing.

Finally, the effects of ignoring the lag phase and considering fermentable sugars only were combined.

In the three above described modifications to the batch fermentation model, data from all the batch experiments were considered in each of the three cases; i.e. values for the kinetic constants, K1 $\longrightarrow$ K6, were determined for each experiment with each data base modification. Table 6.5 gives mean values of the constants for the various forms of the batch fermentation data explored. Graphs 6.1 - 6.6 provide some examples. In all cases there is close agreement between the experimental points and the curves generated by the batch fermentation model.

The rate constants for sugar utilisation and yeast growth are also of the same order of magnitude (see Table 6.5) whatever basis is used for data analysis. Consequently, employing these constants in the equations developed to describe the continuous fermenter (eq. 6.24 and 6.26) produced no significant changes in the amount of sugar utilised in either the plug-flow or C.S.T.R. zones.

6.8.3 Effect of Substrate Used for Maintenance

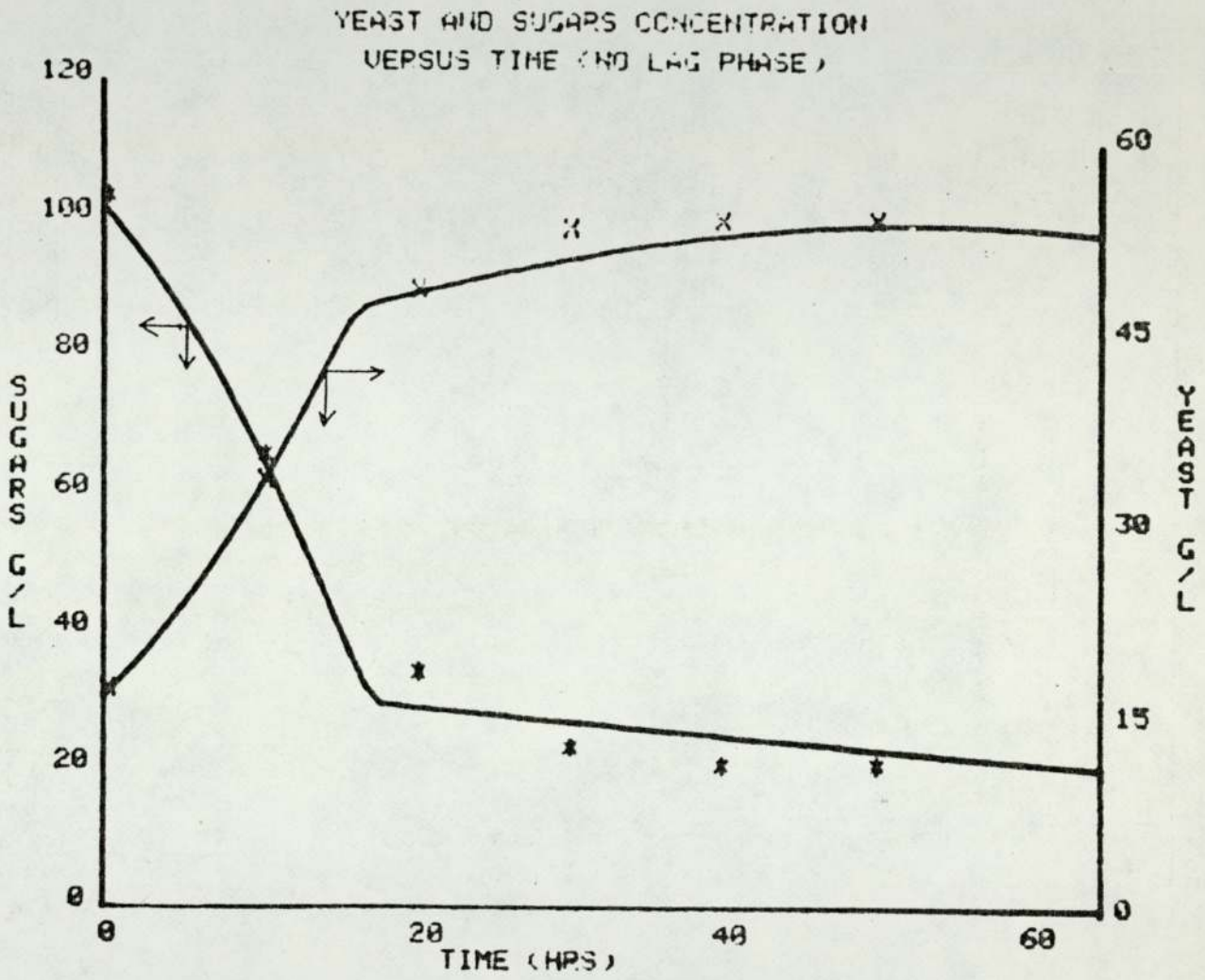
A certain amount of sugar in the feed to the fermenter must be utilised by the yeast for cellular maintenance: Fidgett (1975) has suggested that the rate of sugar utilisation for this purpose is of the order of 1/50 g. of sugar/g. of dry yeast, h. Taking a yeast cell as 24.5%w/w water the maintenance sugar is 1/200 g. of sugar/g. of wet yeast, h. Table 6.6 shows

Table 6.5 Values of Kinetic Constants for Various Forms of the Batch Fermentation

EXPERIMENTAL DATA	K1	K2	K3	K4	K5	K6	X^*	S^*
	H^{-1}	G/L	H^{-1}	G/L	H^{-1}	H^{-1}	G/L	G/L
"Normal" yeast and sugars	0.157	50.14	0.135	31.57	0.013	0.067	30.9	31.4
Lag Phase disregarded	0.184	31.43	0.112	30.00	0.010	0.053	35.5	29.1
Fermentable sugars	0.156	36.43	0.126	33.57	0.018	0.034	29.6	7.8
Fermentable sugars, lag phase disregarded	0.186	31.4	0.106	28.14	0.014	0.025	34.3	7.0

GRAPH 6.1 : EXPT. NO. 2

LAG PHASE OF 20 HOURS DISCOUNTED

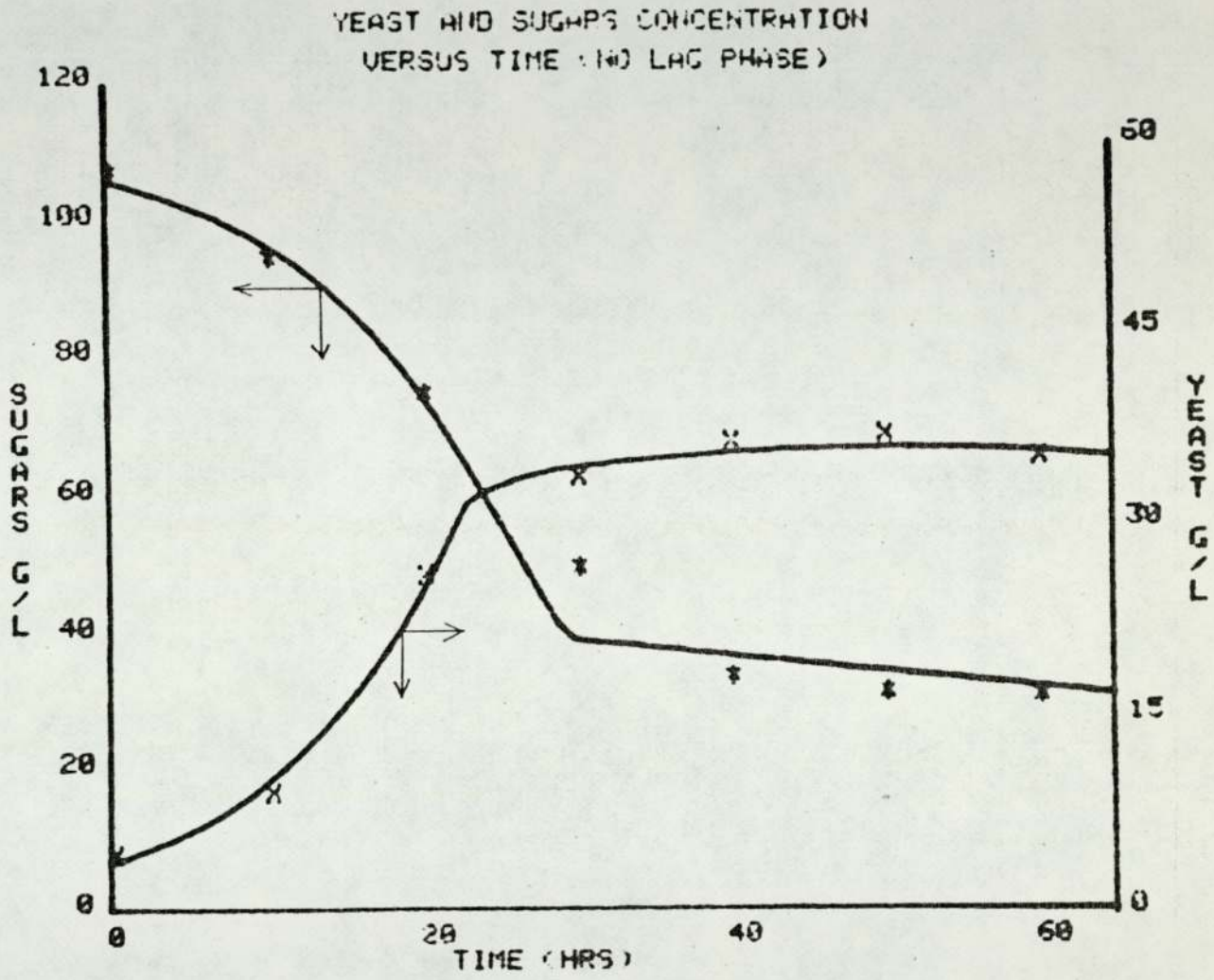


K1 = 0.22
K2 = 40
K3 = 0.10
K4 = 40

K5 = 0.01
K6 = 0.036
X^* = 47
S^* = 30

GRAPH 6.2 : EXPT. NO. 7

LAG PHASE OF 10 HOURS DISCOUNTED

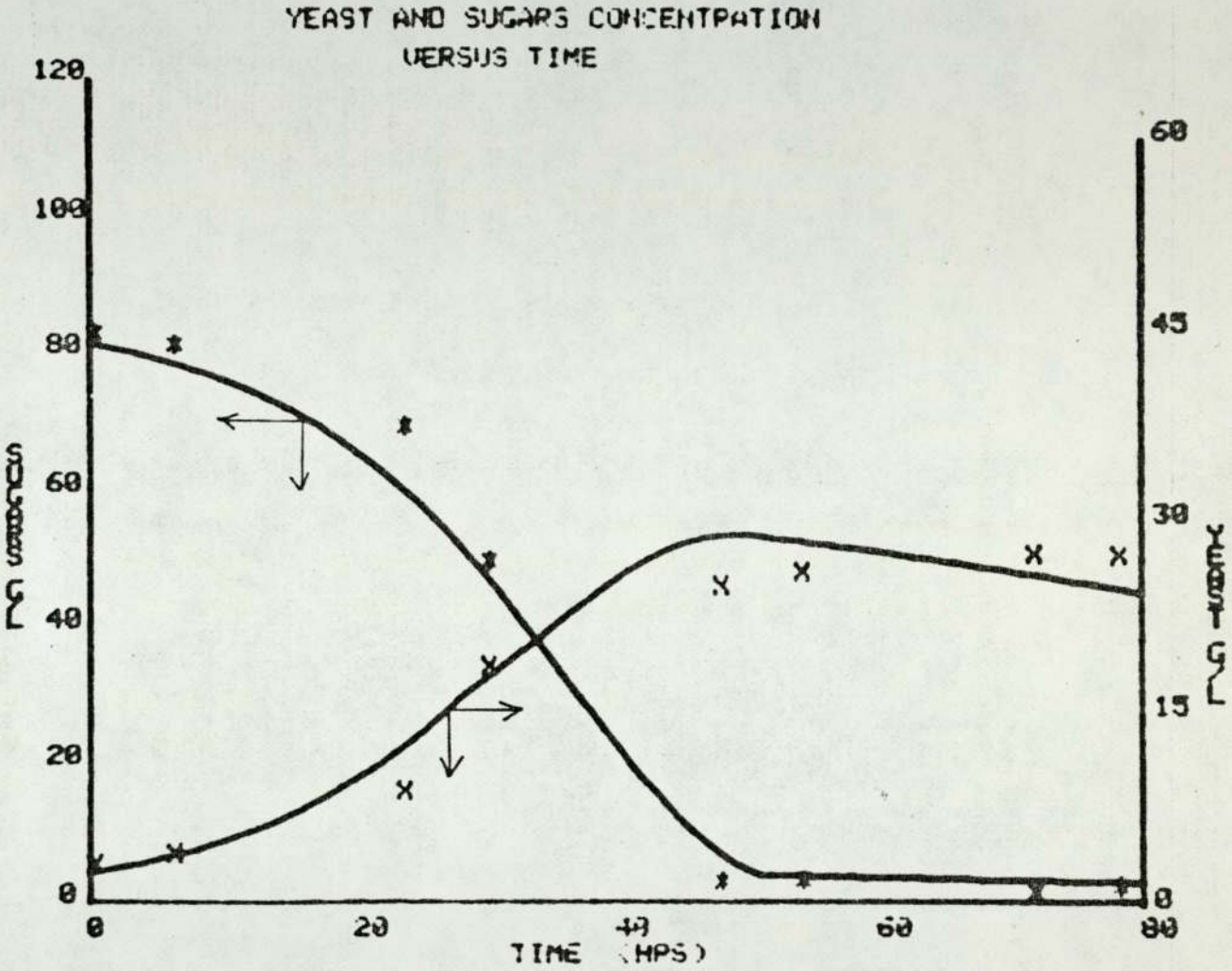


K1 = 0.16
K2 = 20
K3 = 0.12
K4 = 30

K5 = 0.01
K6 = 0.064
X^* = 32
S^* = 40

GRAPH 6.3 : EXPT. NO.1

FERMENTABLE SUGARS ONLY CONSIDERED

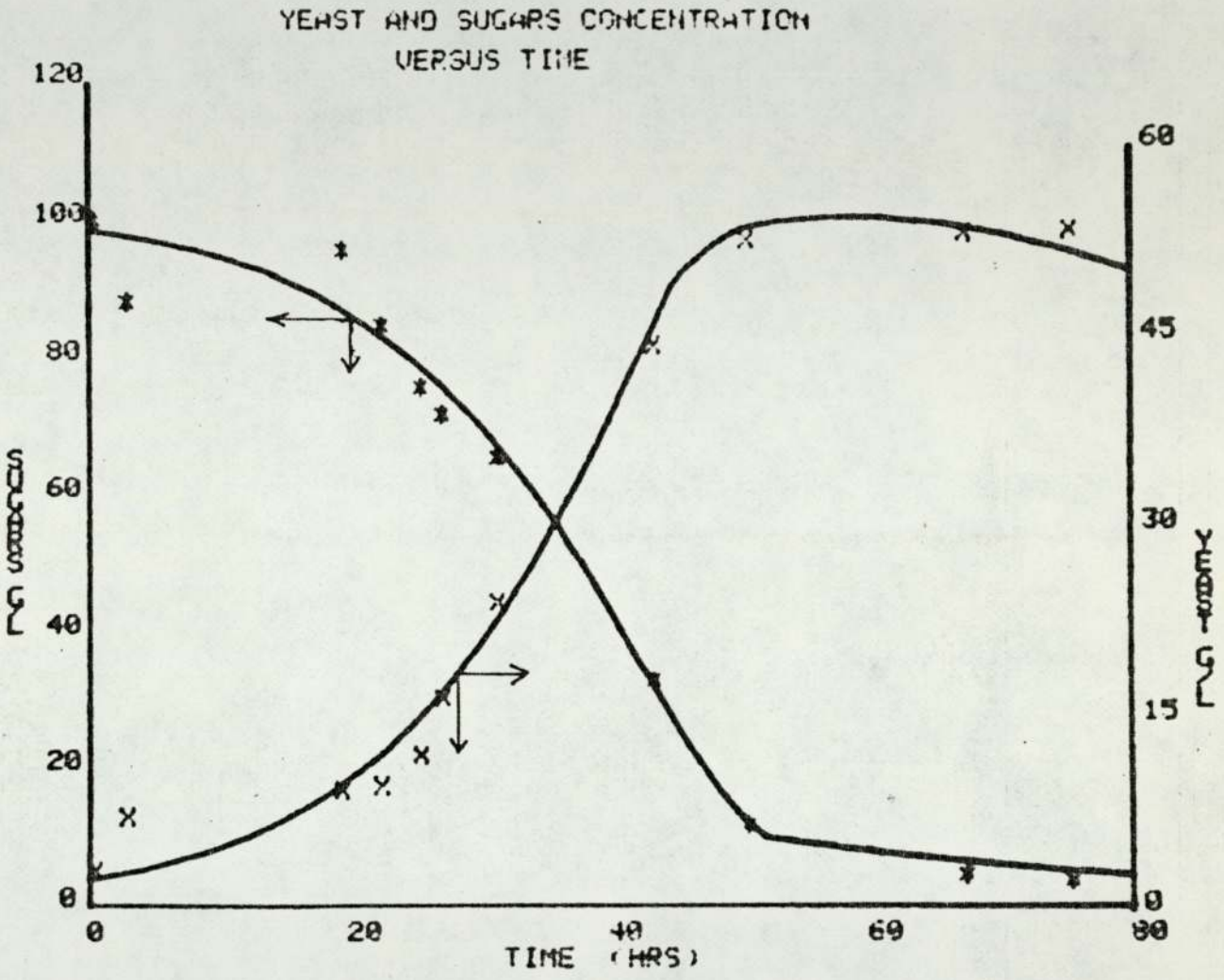


K1 = 0.18
K2 = 20
K3 = 0.09
K4 = 25

K5 = 0.01
K6 = 0.016
X^* = 16
S^* = 4

GRAPH 6.4 : EXPT. NO. 5

FERMENTABLE SUGARS ONLY CONSIDERED

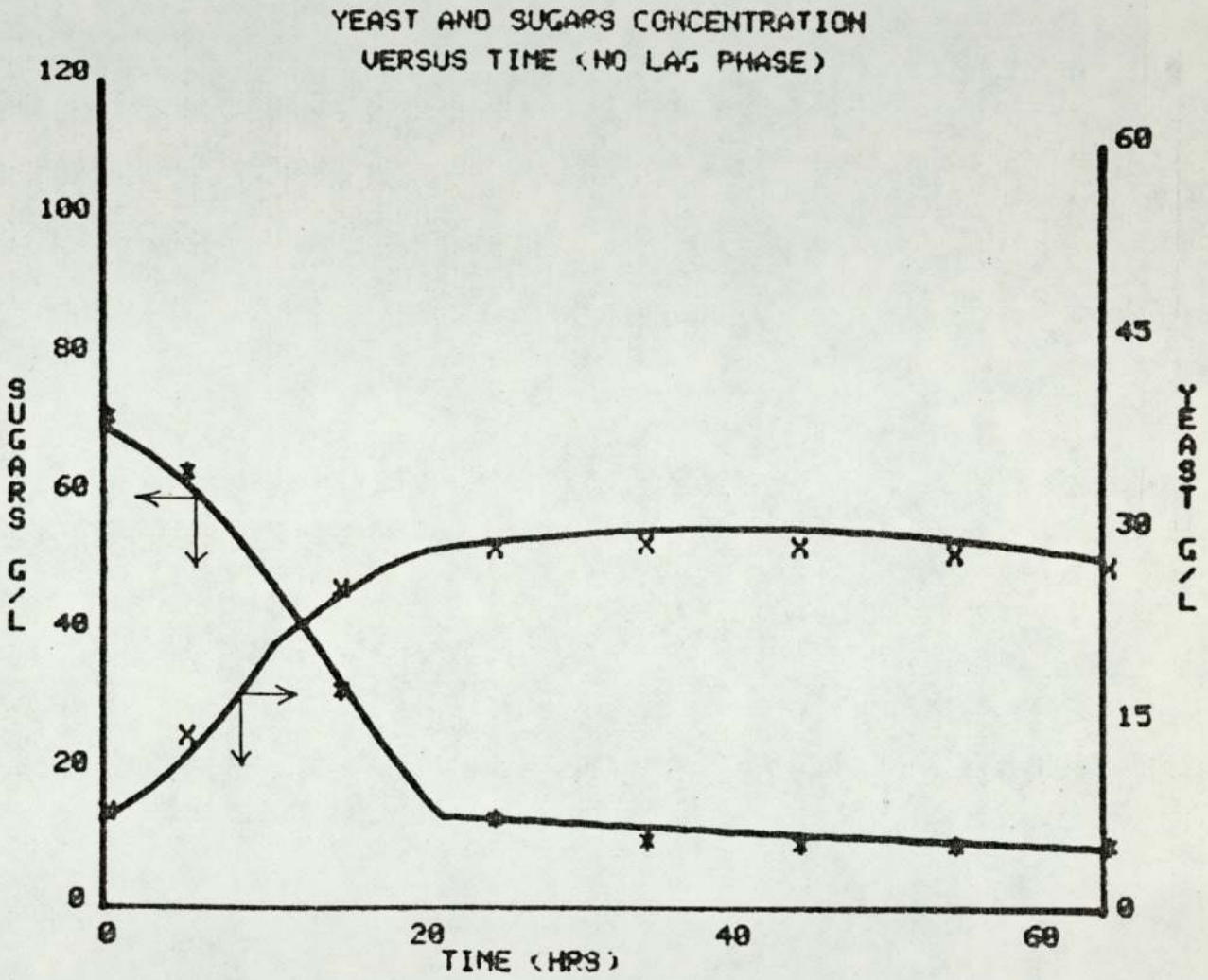


K1 = 0.16
K2 = 40
K3 = 0.11
K4 = 45

K5 = 0.025
K6 = 0.017
X* = 49
S* = 10

GRAPH 6.5 : EXPT. NO. 6

LAG PHASE OF 5 HOURS DISCOUNTED
AND FERMENTABLE SUGARS ONLY CONSIDERED

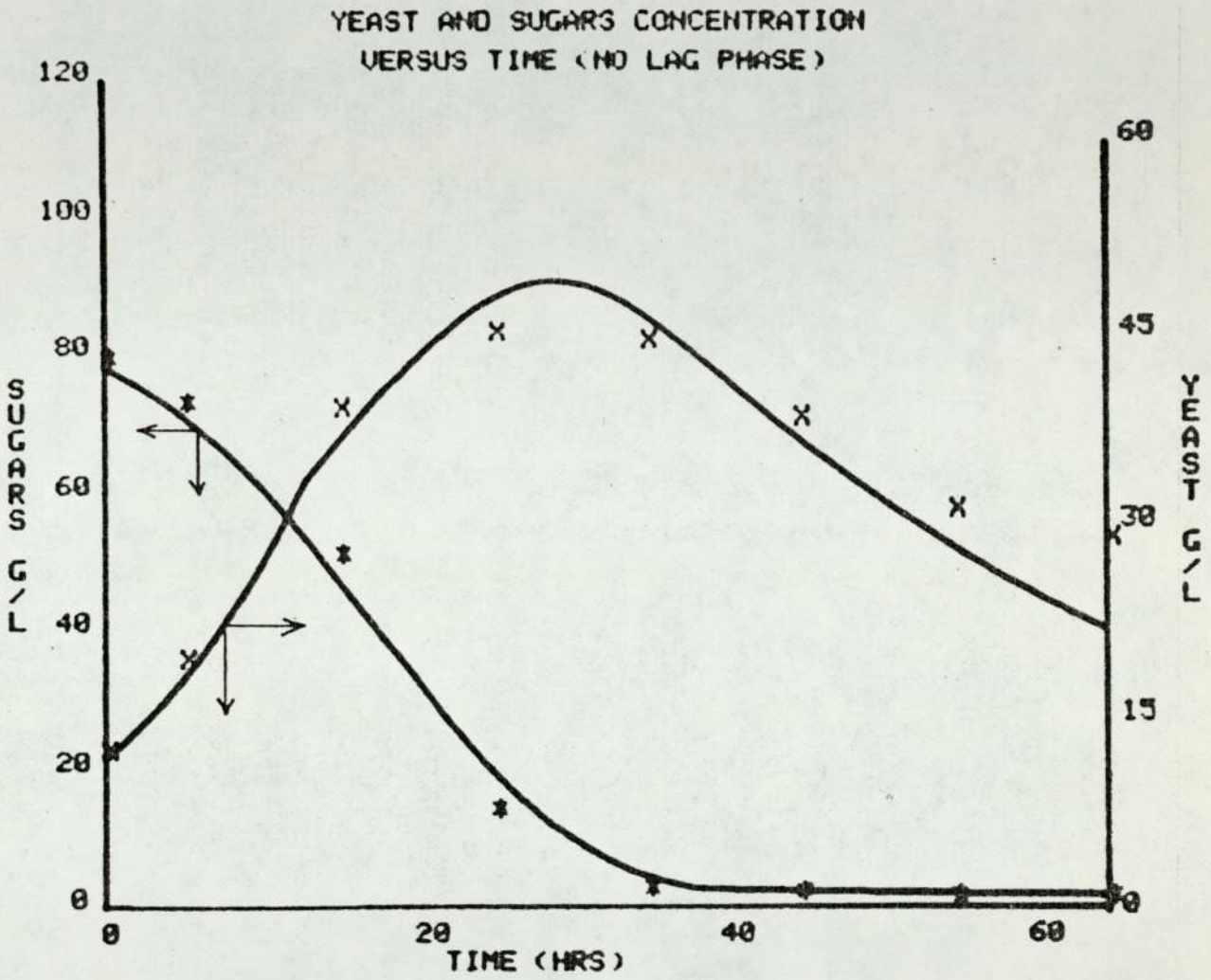


K1 = 0.21
K2 = 20
K3 = 0.13
K4 = 20

K5 = 0.01
K6 = 0.047
X^* = 21
S^* = 14

GRAPH 6.6 : EXPT. NO. 8

LAG PHASE OF 15 HOURS DISCOUNTED AND FERMENTABLE SUGARS ONLY CONSIDERED



K1 = 0.135
K2 = 35
K3 = 0.11
K4 = 25

K5 = 0.015
K6 = 0.035
X^* = 33
S^* = 3

the hourly sugar maintenance requirement for each experiment and the percentage of the input sugar necessary to supply this. All the yeast in the fermenter was assumed to be viable.

Table 6.6 Maintenance Sugar Requirements in the Tower Fermenter

Experiment No.	Mean Yeast Concentration	Maintenance Sugar	Input Sugar	Maintenance Sugar
	G/L	G/H	G/H	% of Input
11	166	9.38	160	5.9
			242	3.9
12	202	11.4	89	12.7
			229	5.0
13	193	10.9	88	12.4
			232	4.7
14	200	11.3	206	5.5
			266	4.2
15	107	6.1	187	3.2

Though the maintenance energy sugar accounts for a significant percentage of the substrate used in the fermenter it does not, by a long way, make up the difference between the observed and calculated sugar utilisation rates.

6.8.4 Yeast Growth

Yeast growth within the tower fermenter, especially within the plug-flow zone, is difficult to express mathematically

and has not been attempted. However, it may be postulated that the majority of yeast growth occurred in the plug-flow zone, as most of the sugar was utilised in this section, and that there was then a net upward flow of yeast cells through the fermenter.

Smith and Greenshields (1973) estimated that during a conventional batch beer fermentation approximately 5% of the fermentable carbohydrate in brewers' wort is utilised for yeast growth, 3.5 g of sugar resulting in 16.5 of centrifuged wet yeast. However, Rainbow (1970), among others, has stated that cellular growth is restricted when yeast is in the high concentrations which occur in the tower fermenter.

Typical organism overflow rates, expressed as centrifuged wet yeast, were 1.7 g/l (see section 5.4.5). This suggests that 0.35 g/l of sugar were utilised for the purposes of growth, which is approximately 0.5% of the fermentable sugar input to the tower fermenter. However, this calculation neglects the cell growth required to balance cell lysis within the fermenter. The fact that there was often a nitrogen "uptake" by the medium as it passed through the fermenter (see section 5.4.5) is evidence of considerable lysis within the fermenter.

Though it was not possible to measure the "in-fermenter" yeast growth, yields of ethanol (see section 5.4.3) indicate that the majority of the sugar feed was converted to alcohol. Therefore, the sugar used for growth was likely to have been negligible compared with the difference between observed and calculated sugar utilisation rates in the tower fermenter.

6.9 Discussion

The values of the kinetic constants, K1 and K2, used to predict sugar utilisation were taken from models developed to account for batch fermentation. It may have to be accepted that sugar utilisation proceeds at faster rates in high concentrations of yeast: Pollock (1961), experimenting with batch fermentations and relatively high concentrations of yeast, found that the increase in the number of cells present was not always itself sufficient to explain massive increases in the rate of fermentation. Values of the constants, K1 and K2, necessary to give complete sugar utilisation in the plug-flow zone of the fermenter are shown in Table 6.7.

Table 6.7 Values of K1 and K2 Necessary to Predict Sugar Utilisation in the Plug-Flow Zone

K1	K2
H^{-1}	G/L
0.7	7.67
0.8	14.8
0.9	21.9
1.0	29.1

The above values may be compared with typical determined values for K1 and K2 as 0.18 h^{-1} and 35 g/l respectively. Though K2 is of the same order as predicted for the batch fermentation it is necessary for K1 to be increased by 4-5 times

to account for sugar utilisation.

Now yeast has a complex enzymic transport system for each of the sugars present in brewers' wort: each system inhibits the others to some extent (see section 3.3.4). The yeast in the tower fermenter, at a particular height, may have been attuned to a particular sugar, and hence the inducement of a particular enzymic transport system may not have been necessary. Weighing against this view is the fact that the yeast in the plug-flow zone was not static. Hence, the argument depends on how quickly particular cell-transport systems within the yeast switch on and off.

An alternative explanation for the increased fermentation rate is that the yeast in the plug-flow zone at a given height had more than one transport system active. As the carbohydrate source was limited this would enable more yeast in the same segment of the fermenter to successfully compete for the feedstock. Evidence supporting the view that individual sugar transport systems may be active simultaneously is provided by Amaha (1966): he found that the fermentation rate of yeast towards a mixture of sugars (e.g. wort) was greater than the rate of utilisation of single sugars, or any dual or triple combination of the sugars present in the mixture. Griffin (1970a) also supported the results of Amaha (1966).

Observations, and mathematical modelling, of the upper or C.S.T.R. zone in the tower fermenter showed that this zone was relatively unimportant with regard to medium attenuation. However, the C.S.T.R. zone is likely to be important in beer

production as a region where the minor components of beer attain their taste threshold-values. This view is supported by the fact that lager production has proved difficult in tower, or stirred continuous systems (Williams and Brady (1965), den Blanken (1974), Coote (1974)). Lager requires a much longer holding period, compared with ale type beers, in the attenuated condition; continuous fermenters probably cannot economically provide the long residence time required.

6.10 Conclusions

Using the kinetic constants developed for the batch fermentation the model of the fermenter as a three-stage system cannot account for the observed sugar utilisation rates. Nevertheless, the results of using this model indicate that the basic model structure is correct as;

i) it predicts that the majority of sugar utilisation will occur within the plug-flow zone

ii) as observed, there is little sugar utilisation in the upper half of the fermenter.

Modifications to the batch fermentation data, considerations of yeast maintenance energy requirements, and allowances for carbohydrate used for yeast growth failed to explain the difference between observed and calculated sugar utilisation rates in the tower fermenter. Reasons for this difference have been discussed. However, for the present it has to be accepted that sugars are metabolised much more rapidly than expected in high concentrations of yeast.

CHAPTER 7

SUGGESTIONS FOR FURTHER WORK

7.1 Non-Sterile Medium

In experiment number 15 the tower fermenter was started-up asceptically and then fed with a non-sterile molasses-based medium. The tower fermenter showed it could adequately cope with this medium: attenuation from a present gravity of 60 to 15 was maintained for approximately 100 hours with no change in either yeast concentration profile or morphology. However, this mode of operation had to be discontinued when the medium became repeatedly contaminated with an organism which converted the medium to a form of muco-polysaccharide.

In future work an in-line mixer for molasses and water would prevent dilute medium becoming contaminated and it seems probable that the tower fermenter, because of its high concentration of yeast, could be operated non-asceptically. Though the ethanol yield may be slightly diminished with this mode of operation, due to competition for feedstock from contaminating micro-organisms, the saving in energy costs for sterilisation should greatly outweigh any minor loss in yield.

7.2 High Medium Flow-Rate

Experimental work showed that liquor apparent residence times of 5 hours were readily attainable in the tower fermenter. During experiment number 14 this apparent residence time was halved for a short period: limitations on sterile medium storage capacity prevented an extended period of low residence time running. However, experimental evidence with the low residence

time showed that after an initial disturbance to the yeast concentration profile the fermenter could operate at this flow-rate: the flow-rate of 75cm^3/min of medium corresponded to sugar utilisation and alcohol production rates of 45.3 g sugar/h.l of fermenter vol. and 29.4 g ethanol/h.l of fermenter vol. respectively.

In future work experiments with high medium throughputs, especially combined with non-sterile medium, would be important in providing an extremely flexible fermentation system, thus significantly reducing the cost of a final product of industrial ethanol.

7.3 Physical Control of the Yeast

The vast majority of the fermentation occurred in the lower 25%-33% of the column height. It is probable that in beer production that the remaining tower volume plays an important role in maturing the end product. However, in industrial ethanol production flavour is unimportant. Future experiments where the yeast was physically retained by, say, distributor plates may prove advantageous in significantly reducing the tower volume. However, some yeast growth and cell escape needs to be allowed for and care should be taken not to complicate the internals of an essentially simple fermentation system.

7.4 Detailed Sugar Analysis

It was not possible to account for the rapid sugar utilisation in the base of the tower fermenter in terms of batch fermentation kinetics. This suggested there may have been homogeneous, as opposed to sequential, uptake of the various

sugars present in brewers' wort. This hypothysis could be investigated by feeding mixtures of "labelled" sugars to the fermenter and noting their rates of utilisation.

APPENDICES

APPENDIX 2.1

L'Hopital's Rule

The expansion factor for orifice plates is given by;

$$Y = \left[r^{2/k} \left(\frac{k}{k-1} \right) \left(\frac{1-r^{(k-1)/k}}{1-r} \right) \left(\frac{1-\beta^4}{1-\beta^4 r^{2/k}} \right) \right]^{1/2}$$

For plates of low free area $\beta \to 0$. Then as $r \to 1$, applying l'Hopitals rule,

$$\text{let } r = 1 - r^{(k-1)/k}$$

$$\therefore \frac{dt}{dr} = - \frac{(k-1)}{k} r^{\left(\left[\frac{(k-1)}{k} \right] - 1 \right)}$$

and let $b = 1-r$

$$\therefore \frac{db}{dr} = -1$$

As $r \to 1$

$$\frac{dt/dr}{db/dr} = \frac{k-1}{k}$$

and $$Y = \left(\frac{k}{k-1} \right) \cdot \left(\frac{k-1}{k} \right) = 1.$$

APPENDIX 2.2

Computer Program to Estimate Distributor Plate Pressure Drops

```
      MASTER
      DIMENSION F(20), ONOR(5)
      FD = 1050
      FV = 0.0020899
      COUNT = 0.0
      READ(1,100) DT
100   FORMAT (F0.0)
      A = (3.142*DT**2)/4
      READ(1,101) N
101   FORMAT(I0)
      READ(1,102) (F(I),I=1,N)
102   FORMAT (6F0.0)
      READ(1,103) K
103   FORMAT (I0)
      READ(1,104) (ONOR(I),I=1,K)
104   FORMAT (4F0.0)
      DO 14 L=1,N
      U=F(L)/A
      RET=DT*FD*U/FV
      IF(RET.LT.2.0) GO TO 2
      IF(RET.GT.2.0.AND.RET.LT.3.0) GO TO 3
      IF(RET.GT.3.0.AND.RET.LT.4.0) GO TO 4
      IF(RET.GT.4.0.AND.RET.LT.7.0) GO TO 5
      IF(RET.GT.7.0.AND.RET.LT.10.0) GO TO 6
      IF(RET.GT.10.0.AND.RET.LT.20.0) GO TO 7
      IF(RET.GT.20.0.AND.RET.LT.40.0) GO TO 8
      IF(RET.GT.40.0.AND.RET.LT.80.0) GO TO 9
      IF(RET.GT.80.0.AND.RET.LT.400.0) GO TO 10
      IF(RET.GT.400.0) GO TO 11
    3 CONTINUE
      CD=0.2
      GO TO 12
    4 CONTINUE
      CD=0.25
      GO TO 12
    5 CONTINUE
      CD=0.35
      GO TO 12
    6 CONTINUE
      CD=0.45
      GO TO 12
    7 CONTINUE
      CD=0.5
      GO TO 12
    8 CONTINUE
      CD=0.6
      GO TO 12
    9 CONTINUE
      CD=0.65
      GO TO 12
```

```
 10  CONTINUE
     CD=0.7
     GO TO 12
 11  CONTINUE
     CD=0.6
 12  CONTINUE
     WRITE(2,200)
200  FORMAT (//22X,1H 16H RE.NO.        CD)
     WRITE(2,201) RET,CD
201  FORMAT(/21X,2F10.4)
     WRITE(2,205)
205  FORMAT(//3X,4H DOR5X,5H ONOR5X,4H RE09X,3HPD10X,6H TPDSI)
     J=4
 22  CONTINUE
     DOR=(1.0/J)*0.0254
     AO=(3.142*DOR**2)/4
     DO 15 M=1,K
     FPH=F(L)/ONOR(M)
     UO=FPH/AO
     PD=UO**2/(CD**2*19.62)
     REO=DOR*UO*FD/FV
     IF(COUNT.GE.3.0) GO TO 23
     TPDSI=PD*2989/0.3048
     GO TO 24
 23  CONTINUE
     TPDSI=(PD*2989)/(0.3048*1000)
 24  CONTINUE
     WRITE(2,206)DOR,ONOR(M),REO,PD,TPDSI
206  FORMAT(/1X,F10.8,2X,F5.1,3X,F8.2,1X,F12.4,3XF13.3)
 15  CONTINUE
     J=J*2
     IF(J.GT.64) GO TO 16
     GO TO 22
  2  CONTINUE
     WRITE(2,202)
202  FORMAT(1H 21H REYNOLDS. NO. TOO SMALL)
 16  CONTINUE
     COUNT=COUNT+1
     IF(COUNT.NE.3.0) GO TO 14
     FD=1.2055
     FV=0.0000178
 14  CONTINUE
     STOP
     END
```

APPENDIX 3.1

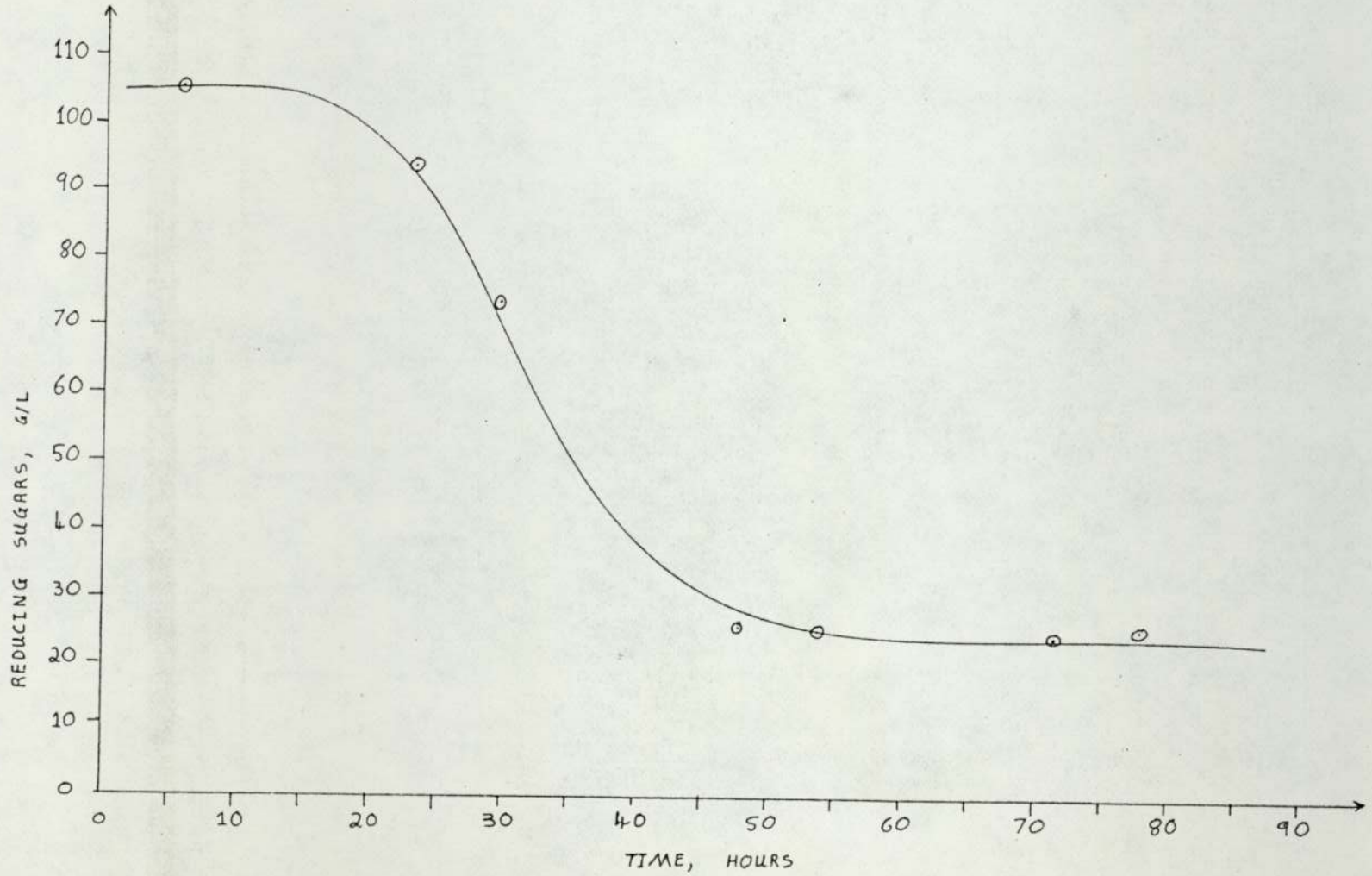
BATCH BEER EXPERIMENTAL RESULTS

Experiment No. 1

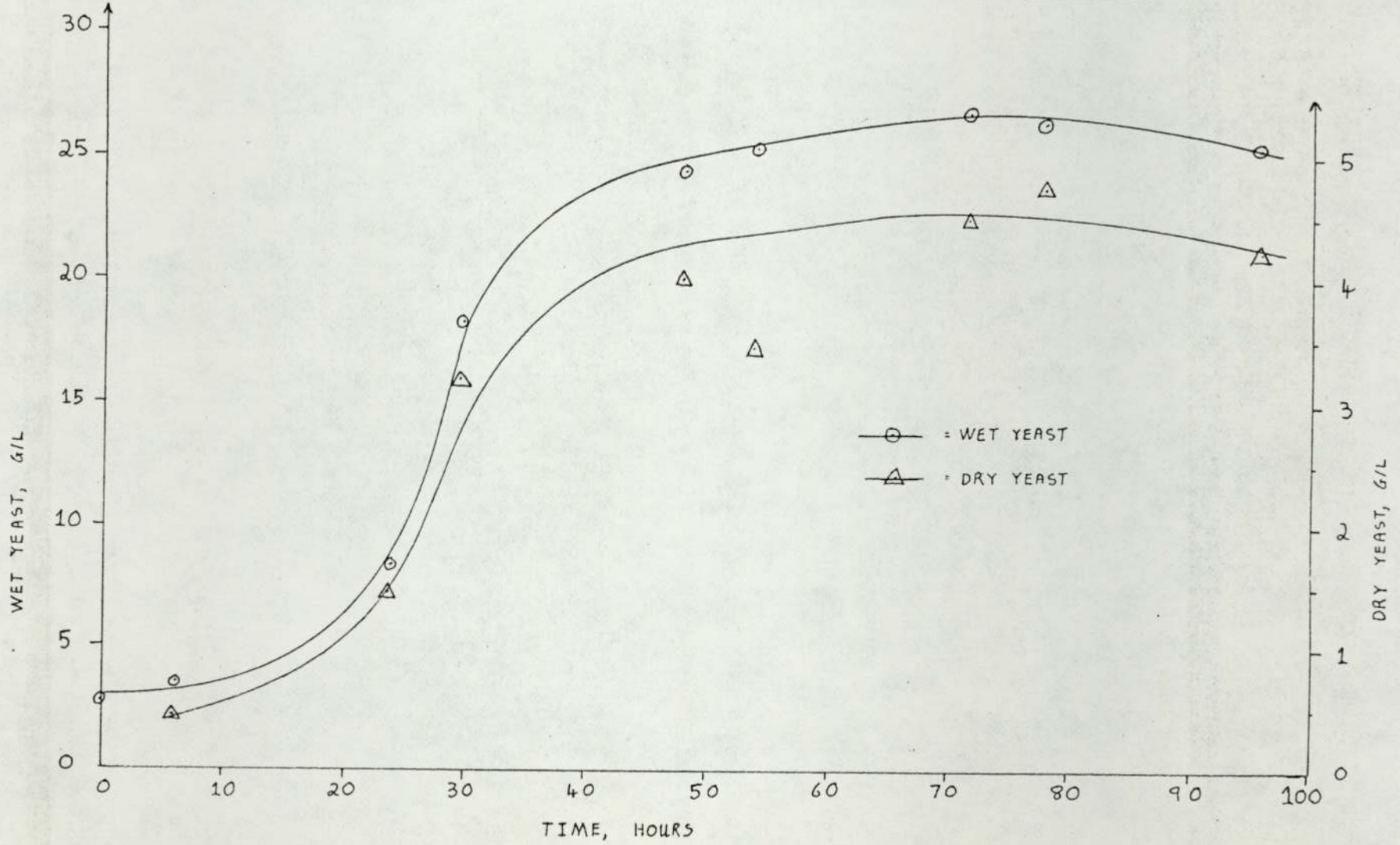
CFCC 54

Time into Fermentation	Present Gravity	Reducing Sugars	Yeast Wet Wt.	Yeast Dry Wt.	Water
Hours		G/L	G/L	G/L	%w/w
0.0	51.7	106.6	2.55	-	-
5.9	50.9	105.0	3.45	0.430	87.5
23.3	44.8	93.1	8.26	1.400	83.1
29.8	34.6	73.3	18.1	3.18	82.5
47.6	10.6	26.6	24.3	3.99	83.6
53.8	10.7	26.8	25.3	3.44	86.4
71.5	9.6	24.6	26.7	4.50	83.2
77.8	10.2	25.8	26.6	4.75	82.1
95.3	1.2	8.3	25.9	4.22	83.7

REDUCING SUGARS CONCENTRATION VERSUS TIME INTO FERMENTATION, EXPT. NO. 1



YEAST CONCENTRATION. VERSUS TIME INTO FERMENTATION, EXPT. NO 1.

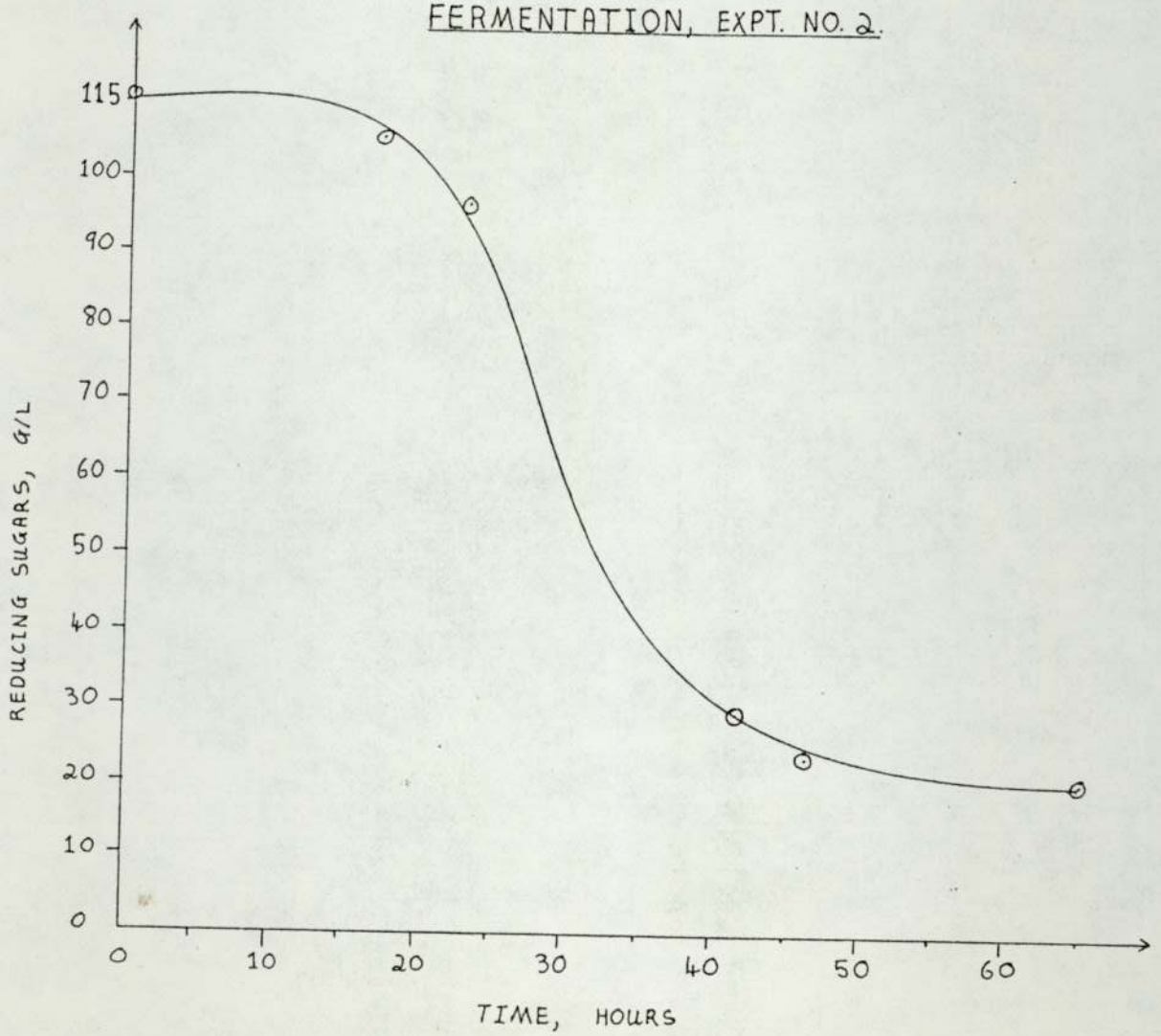


Experiment No. 2

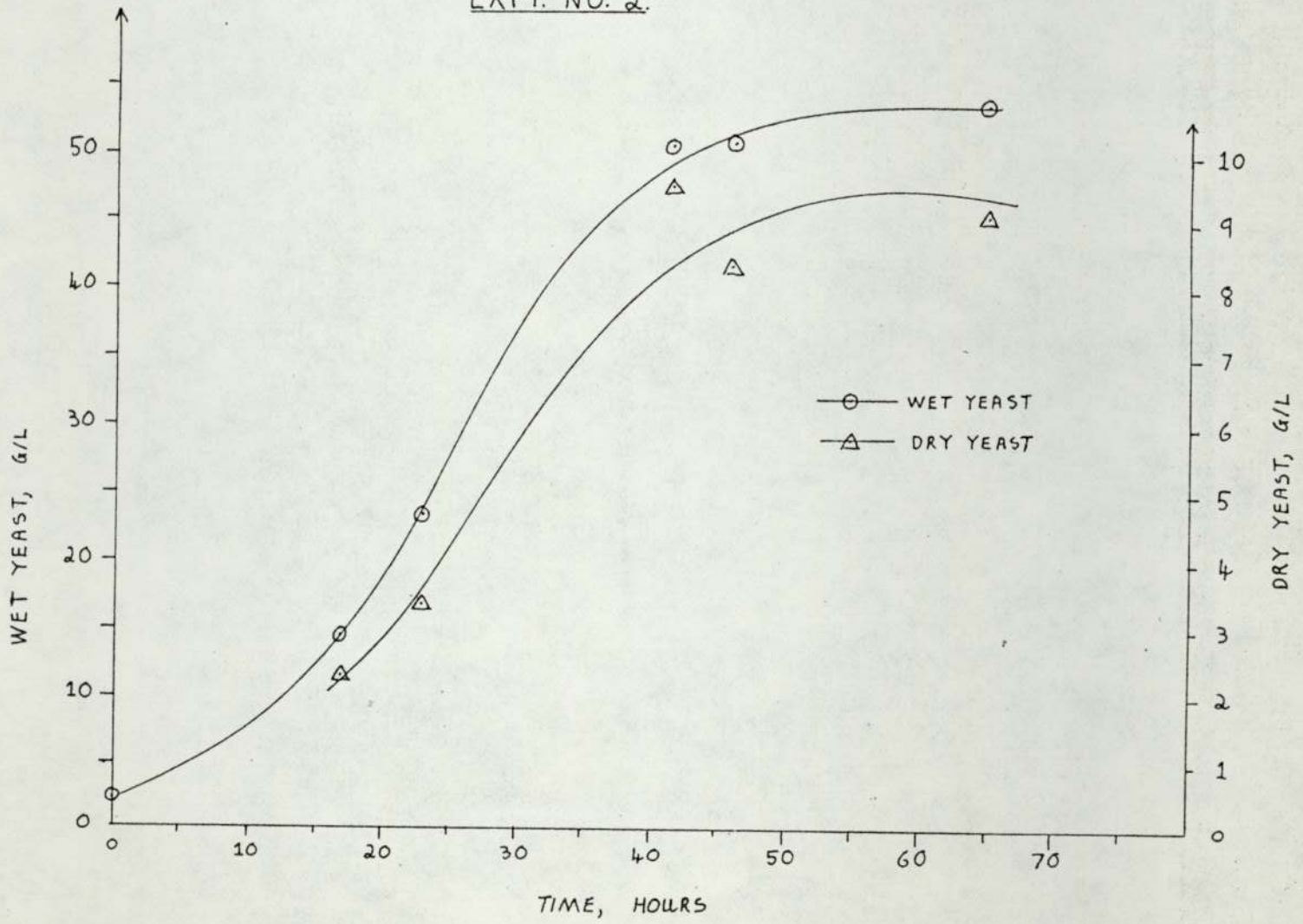
CFCC 54

Time into Fermentation	Present Gravity	Reducing Sugars	Yeast Wet Wt.	Yeast Dry Wt.	Water
Hours		G/L	G/L	G/L	%w/w
0.0	53.2	110.2	2.13	-	-
17.0	51.1	105.4	14.3	2.32	84.4
23.0	48.0	96.3	23.3	3.37	85.6
41.5	11.3	29.4	50.7	9.51	81.2
46.0	9.2	23.9	50.9	8.33	83.6
65.0	7.0	20.2	53.9	9.06	83.2

REDUCING SUGARS CONCENTRATION VERSUS TIME INTO FERMENTATION, EXPT. NO. 2.



YEAST CONCENTRATION VERSUS TIME INTO FERMENTATION,
EXPT. NO. 2.

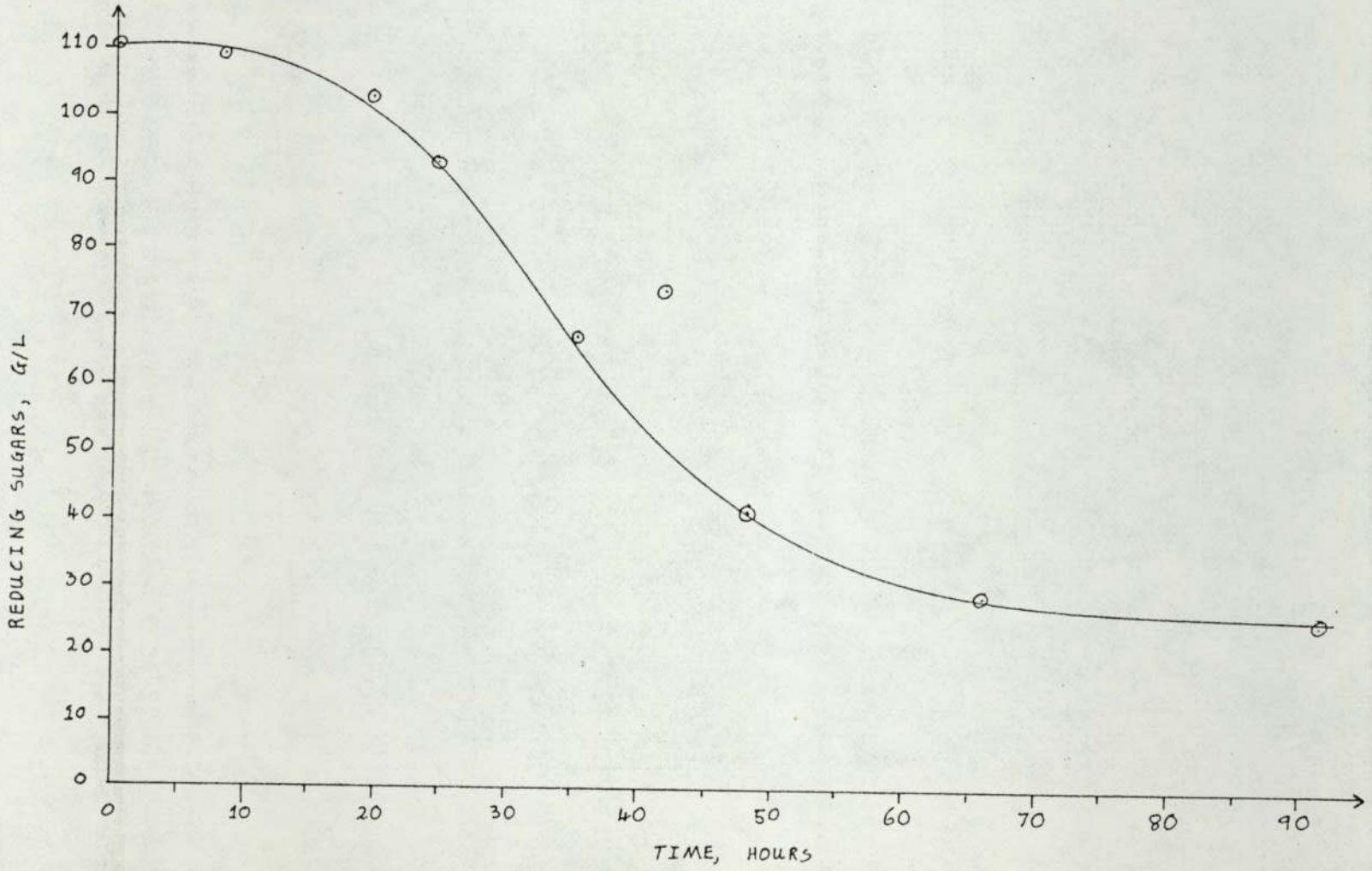


Experiment No. 3

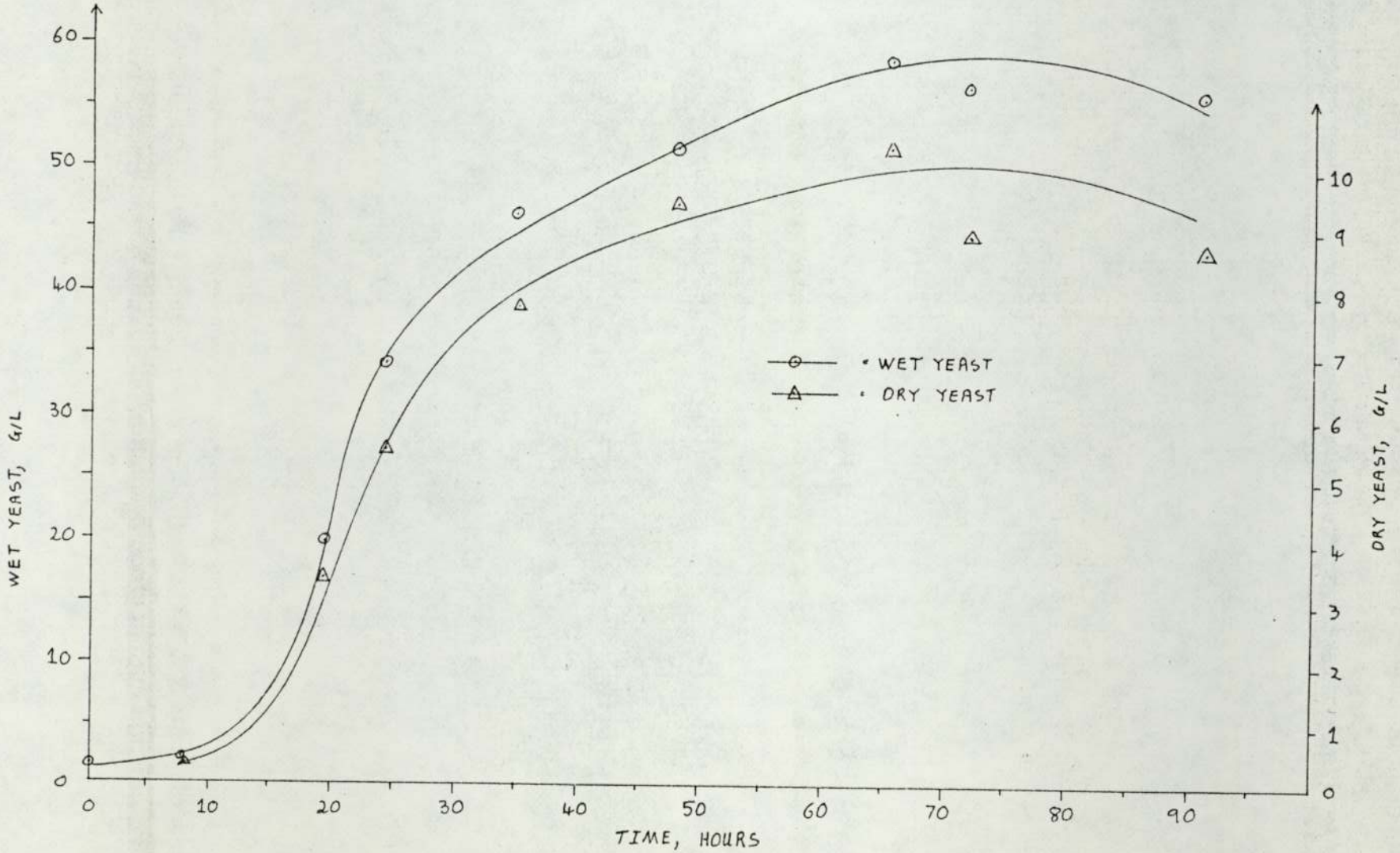
CFCC 1

Time into Fermentation	Present Gravity	Reducing Sugars	Yeast Wet Wt.	Yeast Dry Wt.	Water
Hours		G/L	G/L	G/L	%w/w
0.0	53.5	110.8	1.89	-	-
8.0	53.0	109.1	2.45	0.384	84.3
19.3	50.0	103.3	19.9	3.39	83.0
24.3	45.0	93.6	34.5	5.46	84.2
35.0	31.5	67.3	46.1	7.79	83.1
41.5	35.0	74.1	62.6	10.1	83.9
48.0	18.5	42.0	51.6	9.43	81.7
65.7	12.0	29.3	58.7	10.3	82.4
71.9	10.5	26.4	56.7	8.88	84.3
91.2	10.0	25.4	56.1	8.66	84.6

REDUCING SUGARS CONCENTRATION VERSUS TIME INTO FERMENTATION, EXPT. NO. 3.



YEAST CONCENTRATION VERSUS TIME INTO FERMENTATION, EXPT. NO. 3

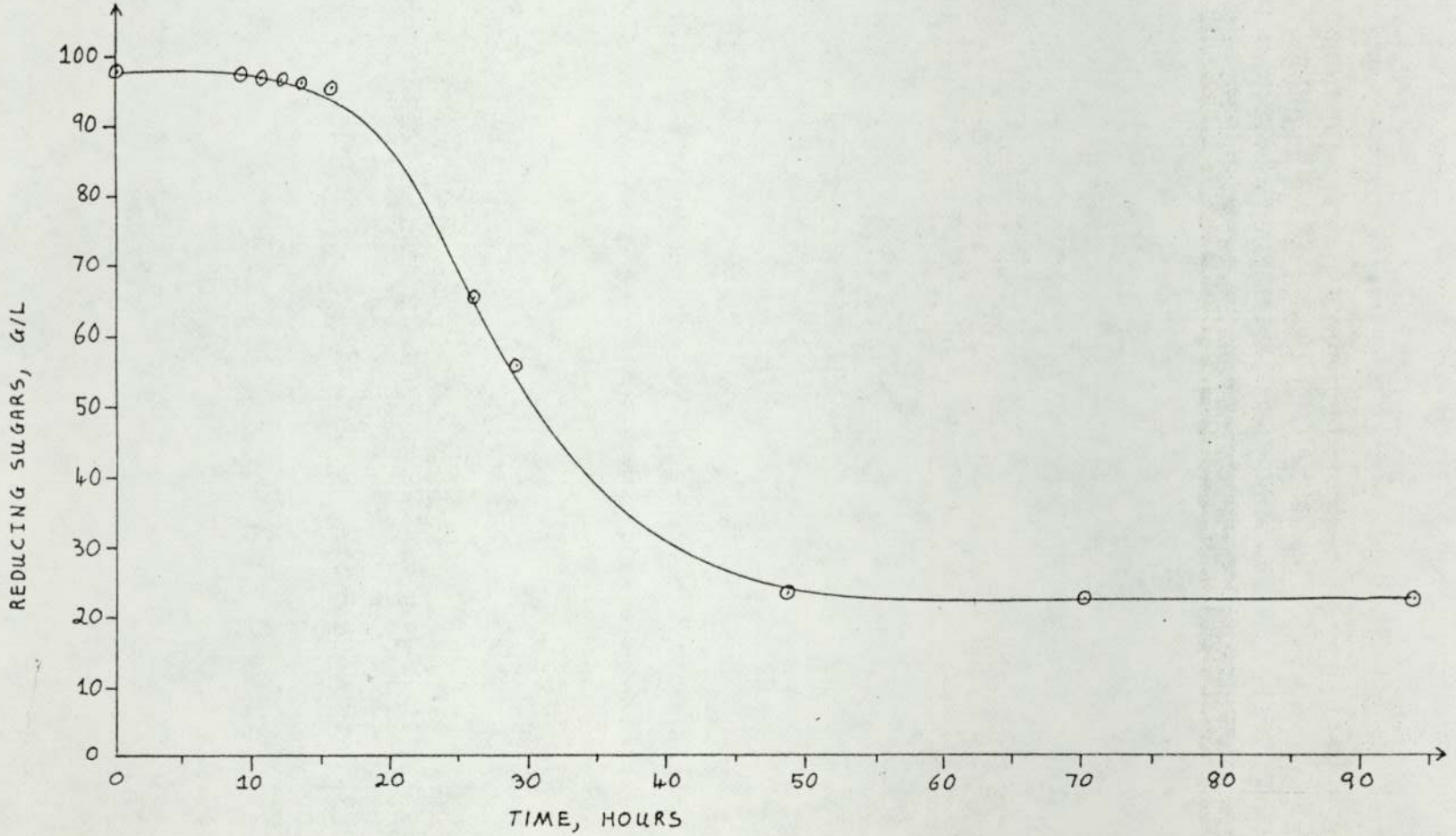


Experiment No. 4

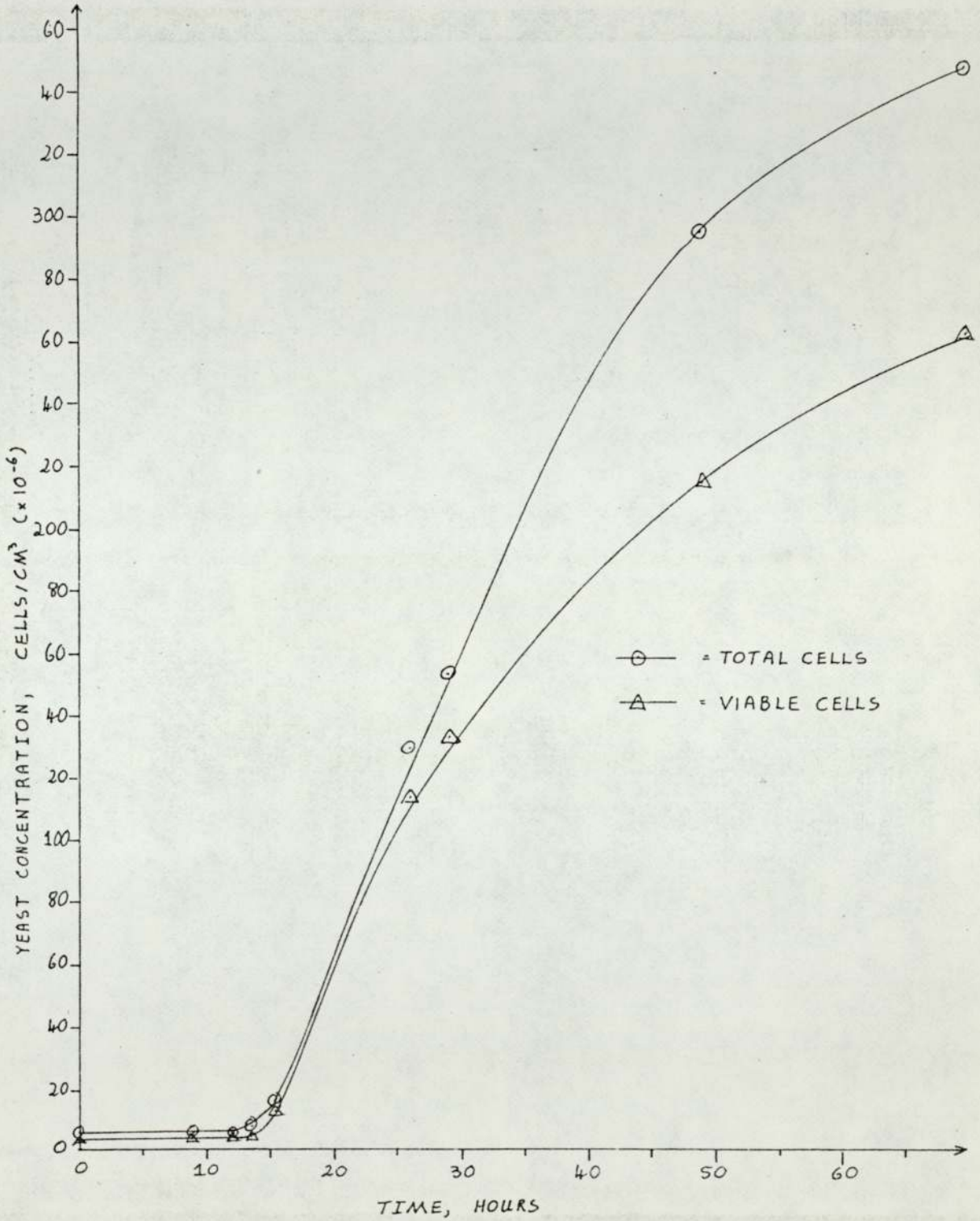
CFCC 54

Time into Fermentation	Present Gravity	Reducing Sugars	pH	Yeast Conc. Cells/$Cm^3 x10^{-6}$		
Hours		G/L		Total	Viable	Viability %
0.0	49.8	98.1	-	5.20	4.22	81.2
9.0	49.7	97.2	-	5.50	4.05	73.6
10.5	49.6	96.8	4.78	-	-	-
12.0	49.4	96.8	4.78	5.70	5.20	91.2
13.5	48.4	96.3	4.69	7.22	5.30	73.4
15.5	47.8	95.2	4.69	15.60	14.00	89.7
26.0	34.3	65.0	4.38	129.00	114.00	88.4
29.0	29.8	56.3	4.30	154.00	134.00	87.0
48.5	12.0	23.8	4.10	296.00	214.00	72.5
70.0	11.2	22.8	4.10	348.00	263.00	75.5
93.5	10.9	22.6	4.10	367.00	-	-

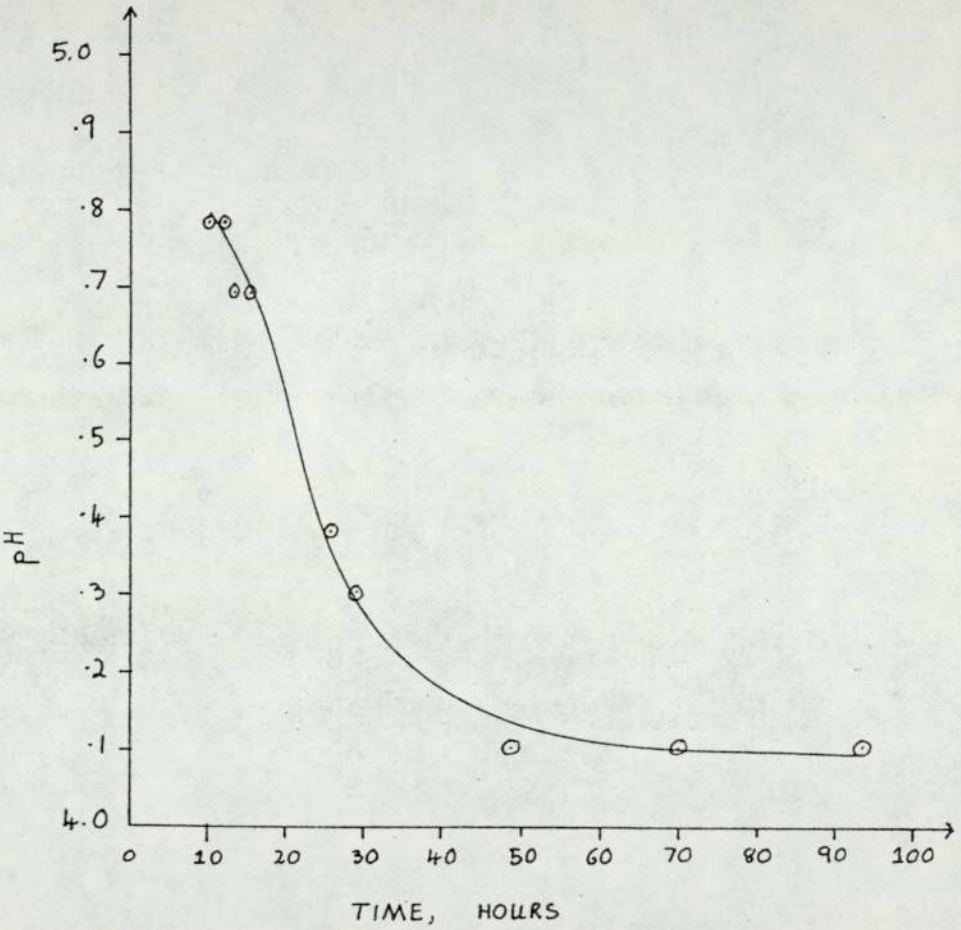
REDUCING SUGARS CONCENTRATION VERSUS TIME INTO FERMENTATION, EXPT. NO. 4.



YEAST CONCENTRATION VERSUS TIME INTO FERMENTATION, EXPT. NO. 4.



MEDIUM pH VERSUS TIME INTO FERMENTATION,
EXPT. NO. 4.

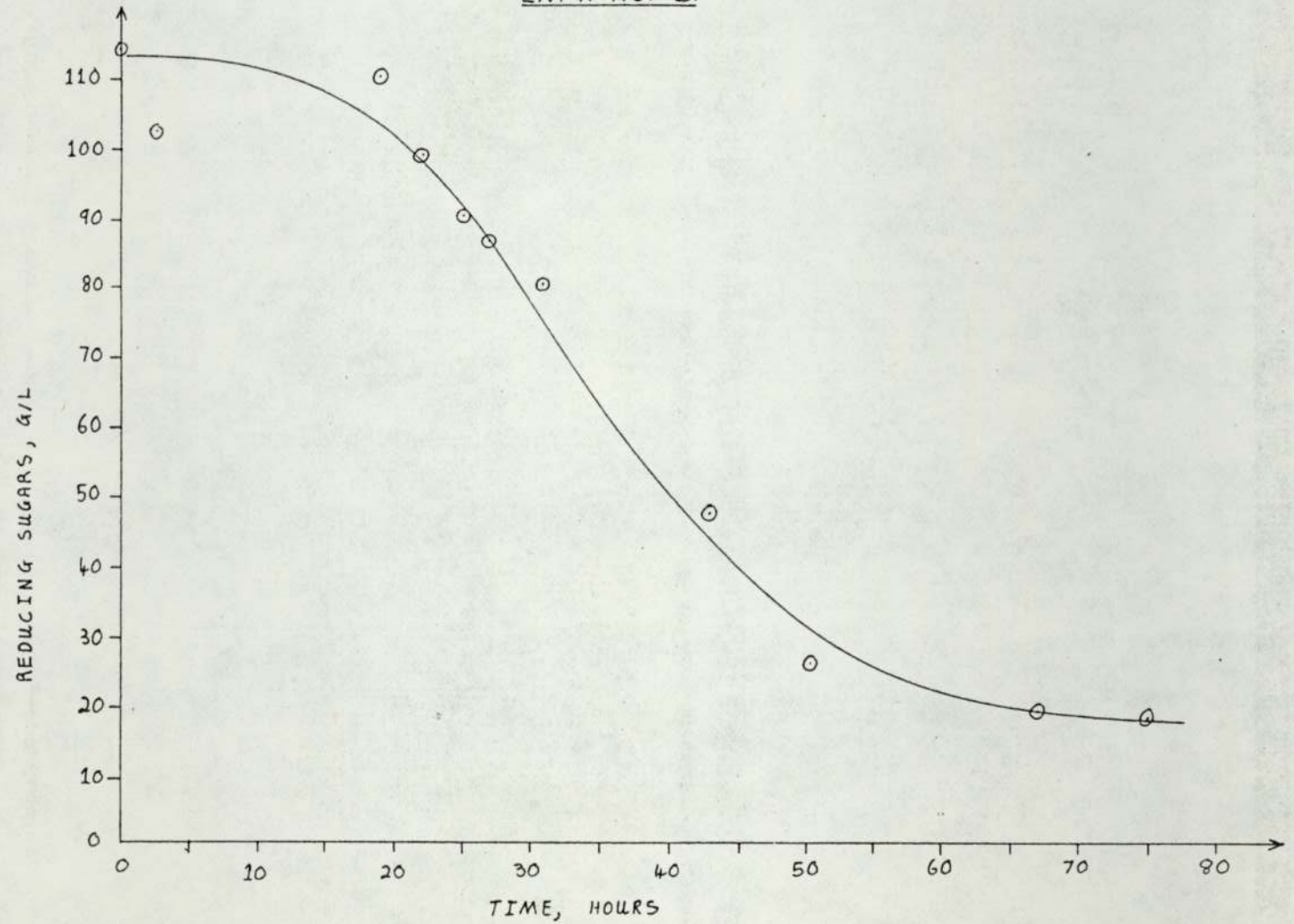


Experiment No. 5

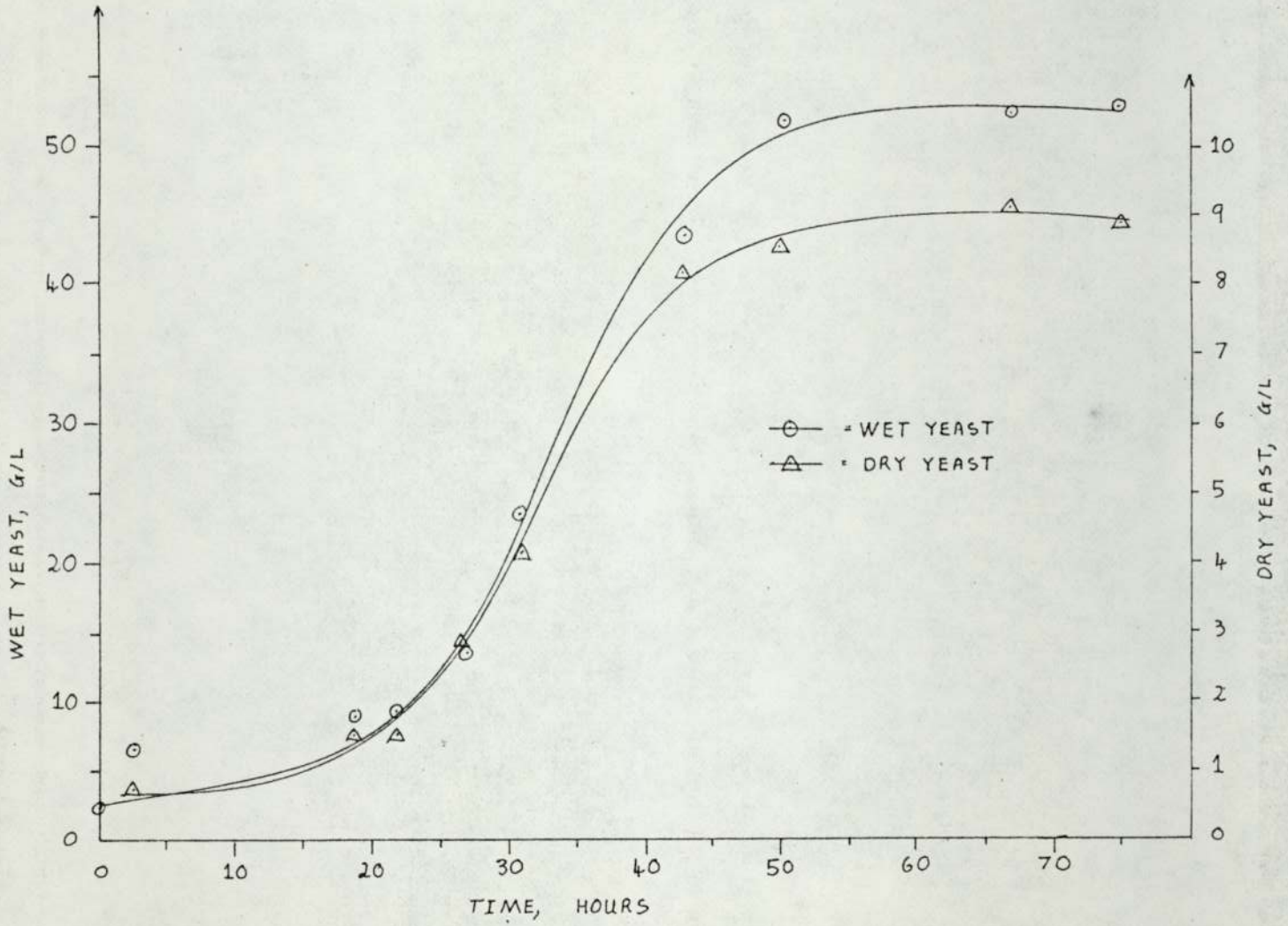
CFCC 1

Time into Fermentation	Present Gravity	Reducing Sugars	YEAST CONCENTRATION				Water	Viability
Hours		G/L	Wet Wt.	Dry Wt.	Cells per Cm^3	Viable Cells per Cm^3	%w/w	%
			G/L	G/L	$\times 10^{-6}$	$\times 10^{-6}$		
0.0	50.0	114.7	2.33	-	4.61	3.82	-	82.9
2.5	50.0	102.8	6.58	0.713	4.25	3.73	89.2	87.7
19.0	47.0	110.4	8.74	1.55	14.40	13.40	82.3	93.1
22.0	45.5	99.6	9.08	1.49	27.80	26.90	83.6	96.7
25.0	41.9	90.7	11.50	1.91	83.90	-	83.5	-
26.8	41.9	86.7	15.90	2.75	91.10	87.10	82.7	95.7
31.0	38.8	80.2	23.50	4.11	260.00	173.00	82.5	66.2
43.0	20.2	47.6	43.60	8.16	511.00	53.50	81.3	10.5
50.3	11.0	26.3	51.70	8.56	-	-	83.4	-
67.0	8.5	19.1	52.30	9.17	-	-	82.5	-
75.0	8.0	18.4	52.60	8.85	-	-	83.2	-

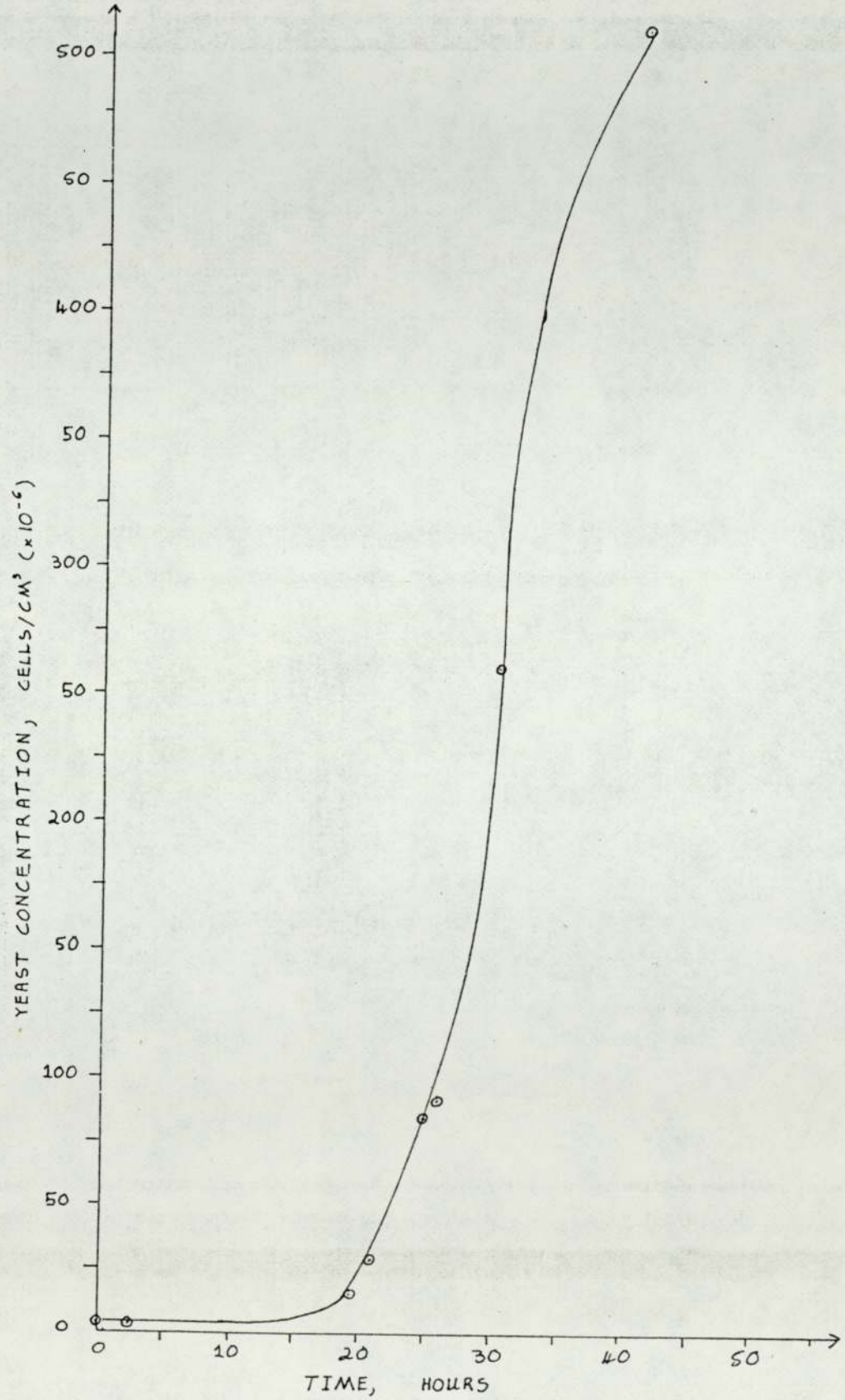
REDUCING SUGARS CONCENTRATION VERSUS TIME INTO FERMENTATION, EXPT. NO. 5.



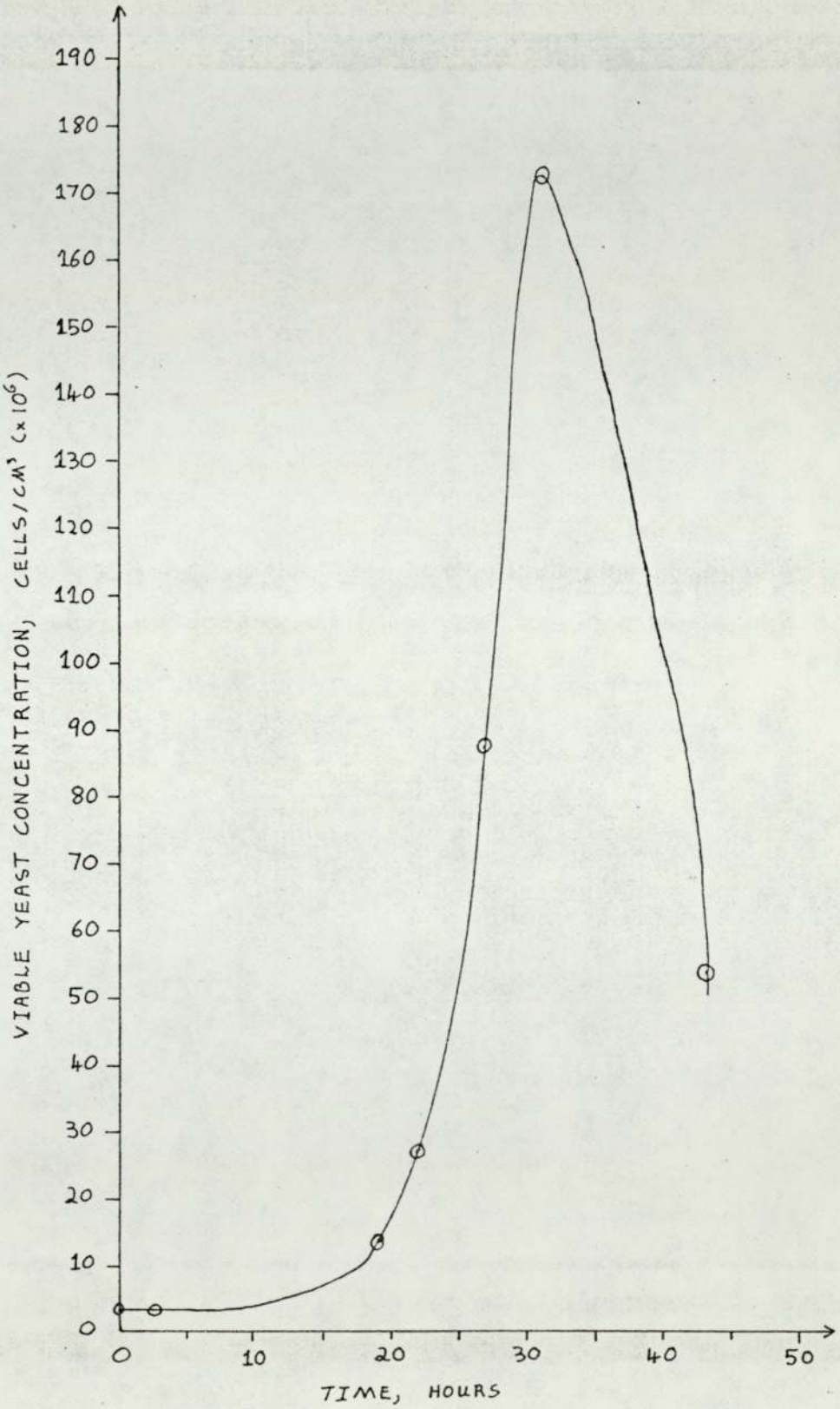
YEAST CONCENTRATION VERSUS TIME INTO FERMENTATION, EXPT. NO. 5.



YEAST CONCENTRATION VERSUS TIME INTO FERMENTATION, EXPT. NO. 5.



YEAST CONCENTRATION VERSUS TIME INTO FERMENTATION, EXPT. NO. 5.

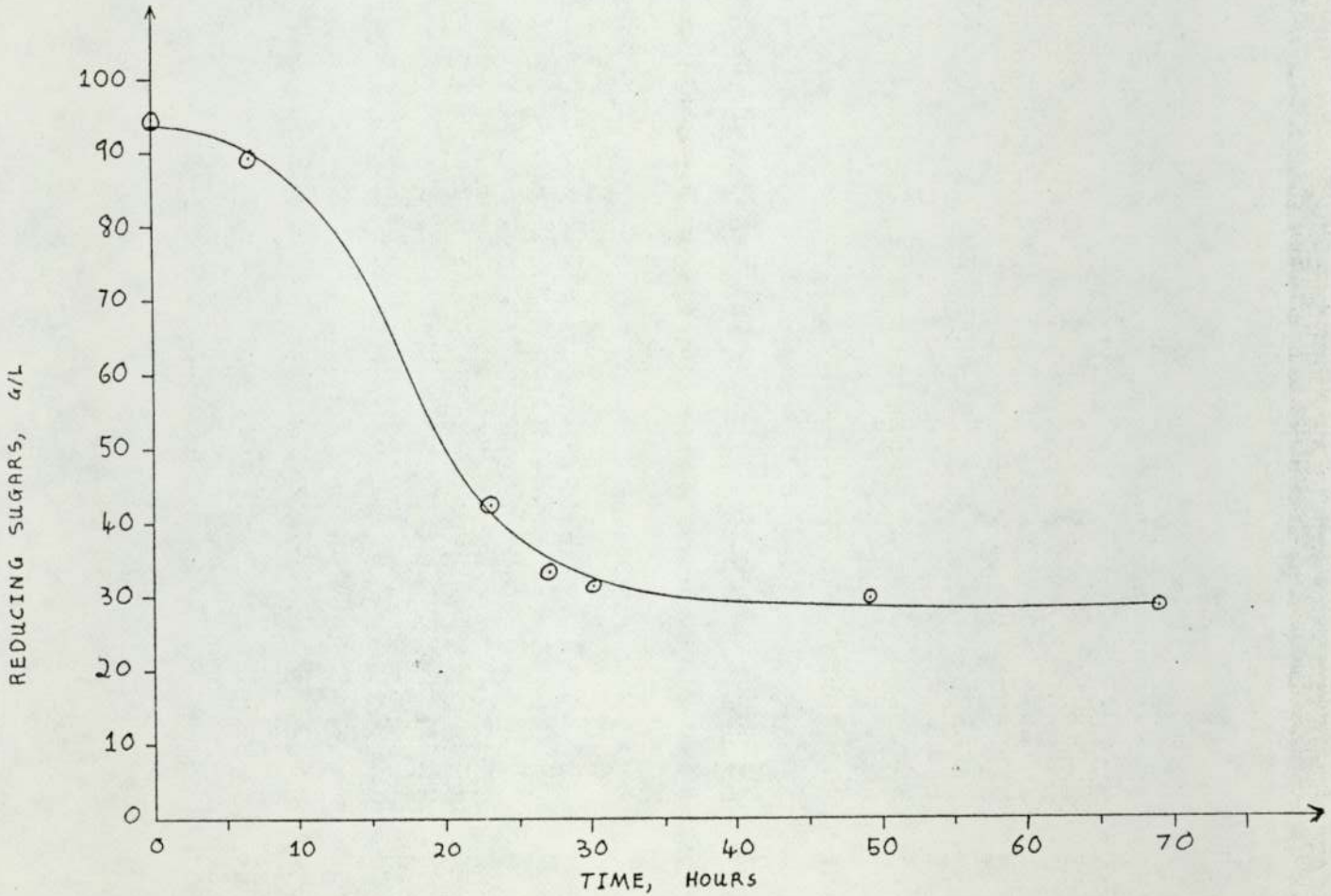


Experiment No. 6

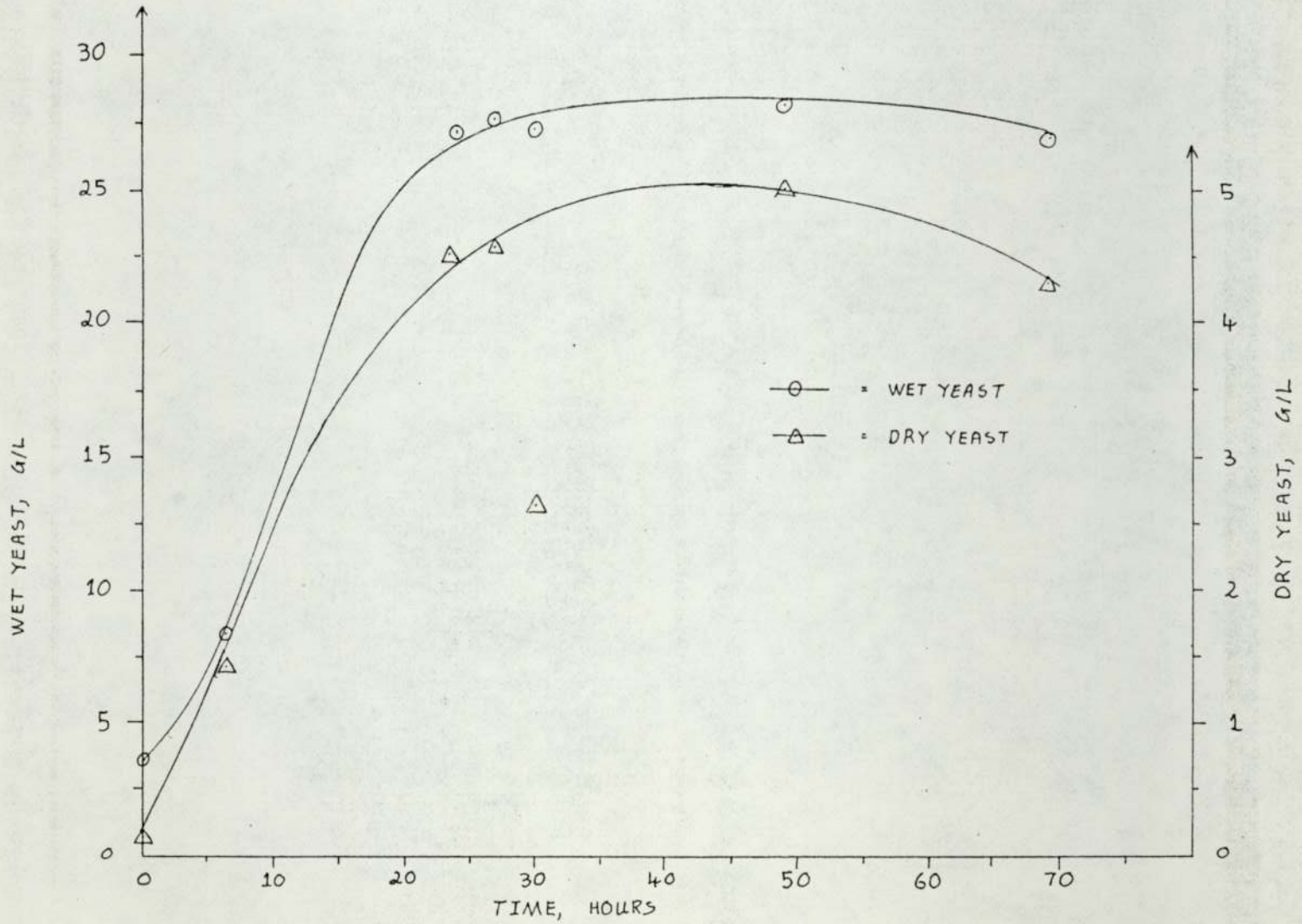
CFCC 39

Time into Fermentation	Present Gravity	Reducing Sugars	Yeast Wet Wt.	Yeast Dry Wt.	Water
Hours		G/L	G/L	G/L	%w/w
0.0	40.0	94.5	3.49	0.540	84.5
6.5	38.0	89.0	8.21	1.44	82.4
23.5	15.0	42.0	27.2	4.52	83.4
27.0	12.0	33.0	27.6	4.58	83.4
30.0	11.0	31.3	27.2	2.64	90.3
49.0	11.0	29.8	28.2	5.02	82.2
69.0	11.0	28.0	26.9	4.29	84.1

REDUCING SUGARS CONCENTRATION VERSUS TIME INTO FERMENTATION,
EXPT. NO. 6.



YEAST CONCENTRATION VERSUS TIME INTO FERMENTATION, EXPT. NO. 6

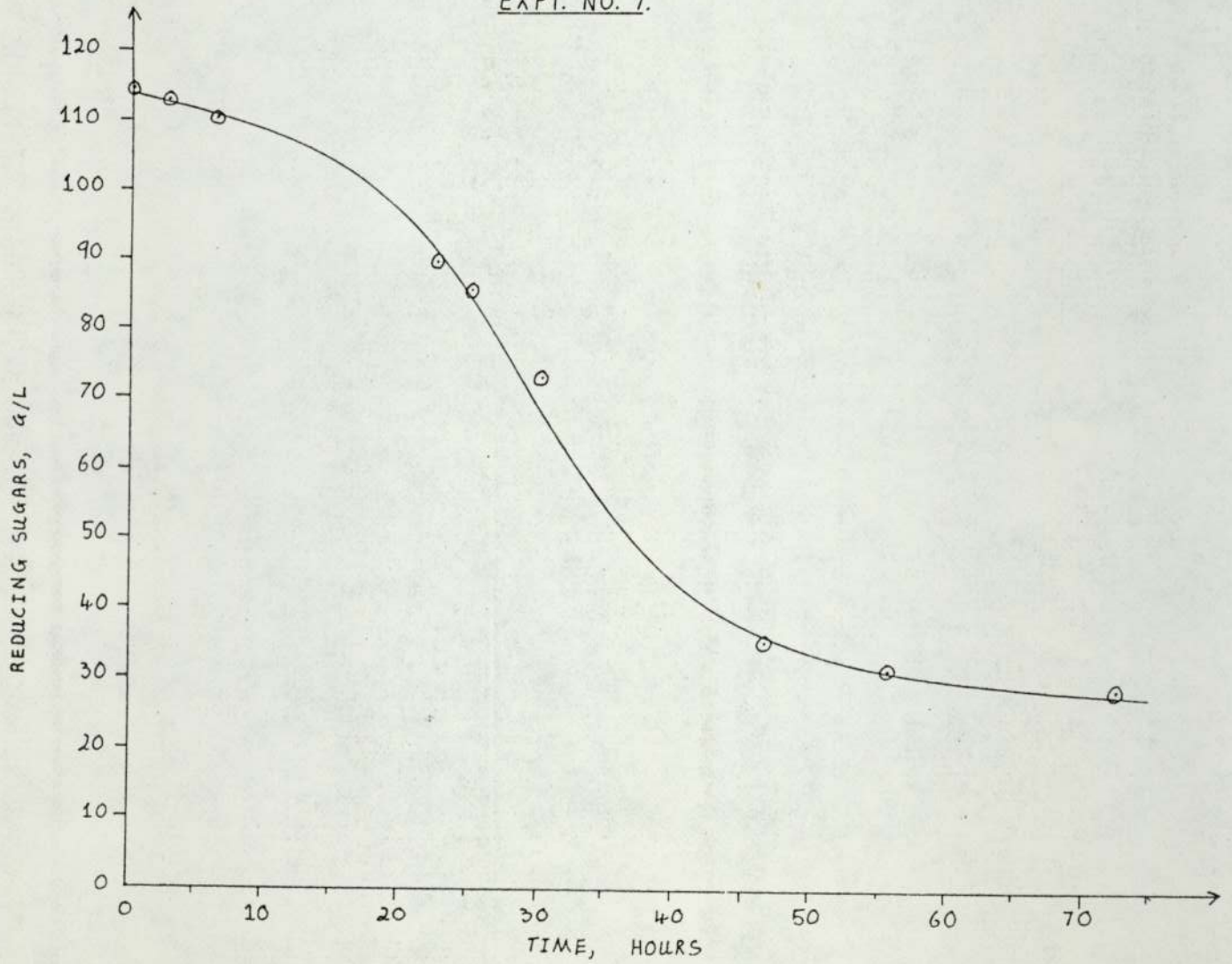


Experiment No. 7

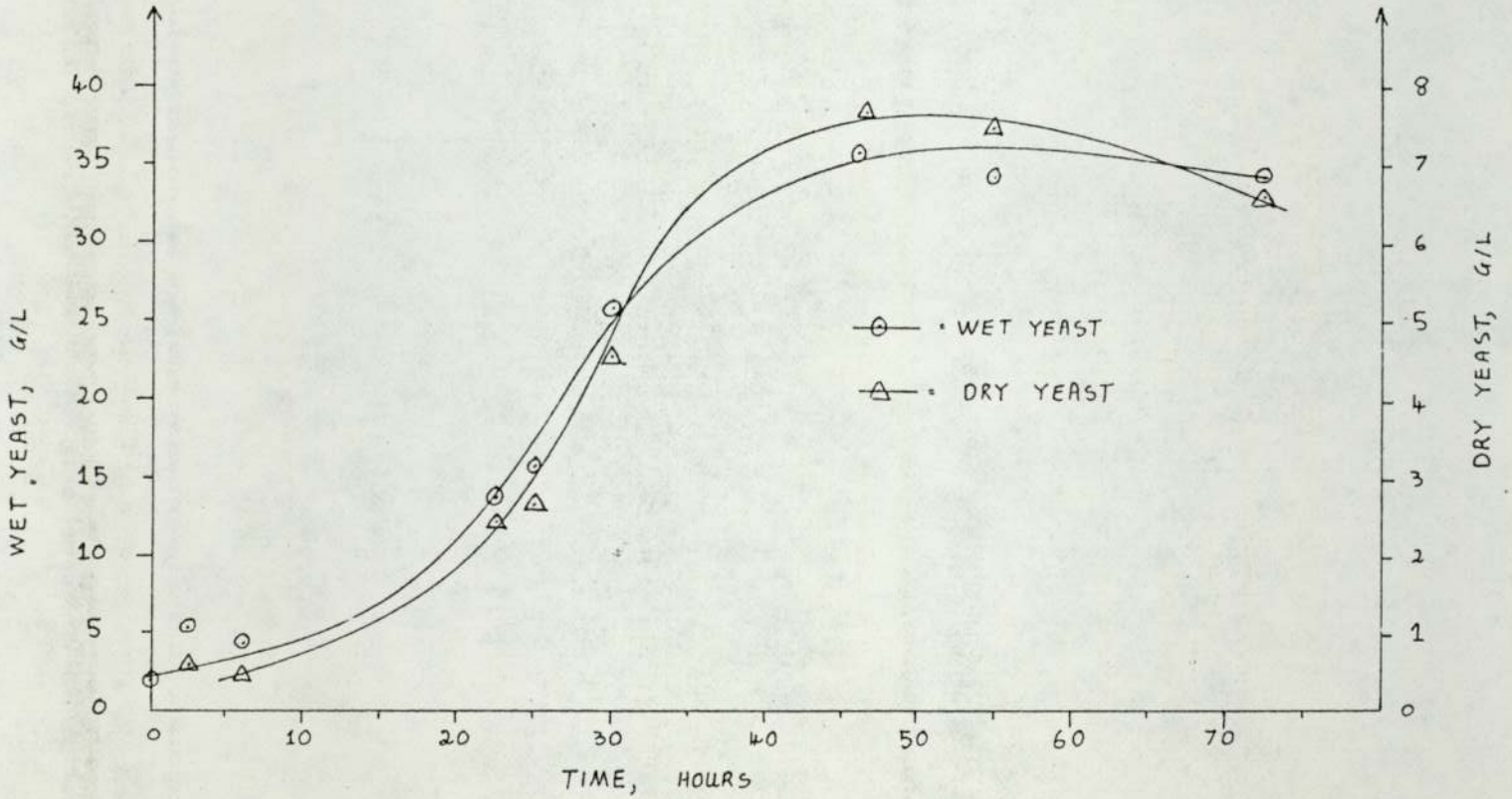
CFCC 39

Time into Fermentation	Present Gravity	Reducing Sugars	Yeast Wet Wt.	Yeast Dry Wt.	Water
Hours		G/L	G/L	G/L	%w/w
0.0	47.0	114.5	1.84	-	-
2.5	47.0	113.0	5.28	0.639	87.9
6.0	46.0	110.0	4.15	0.482	88.4
22.5	36.3	90.3	13.8	2.43	82.4
25.0	32.2	86.0	15.7	2.64	83.1
30.0	24.0	73.0	25.7	4.52	82.4
46.5	13.0	35.5	35.5	7.66	78.4
55.5	12.0	31.5	34.2	7.43	78.3
72.5	12.0	29.0	34.1	6.61	80.6

REDUCING SUGARS CONCENTRATION VERSUS TIME INTO FERMENTATION,
EXPT. NO. 7.



YEAST CONCENTRATION VERSUS TIME INTO FERMENTATION, EXPT. NO. 7

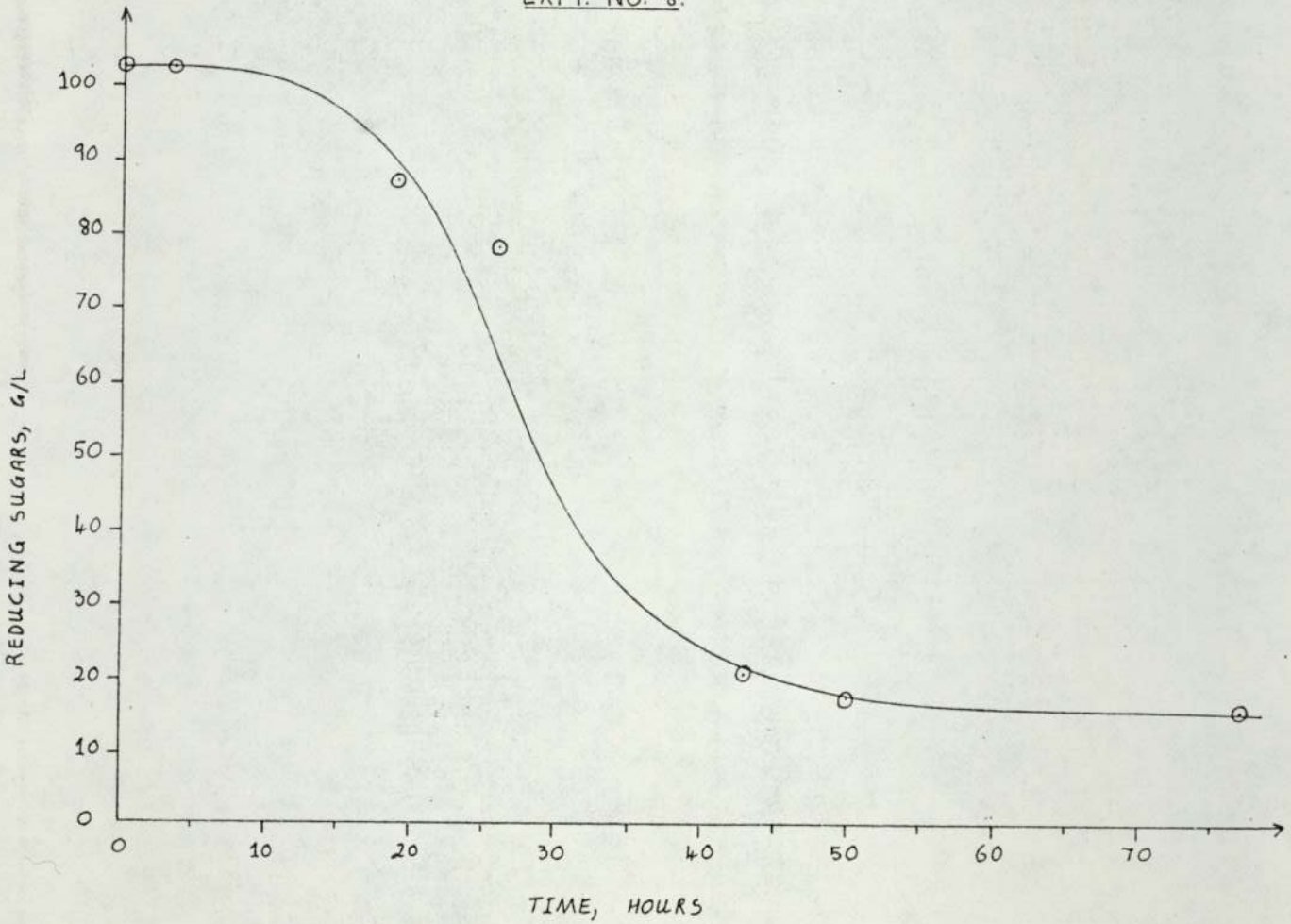


Experiment No. 8

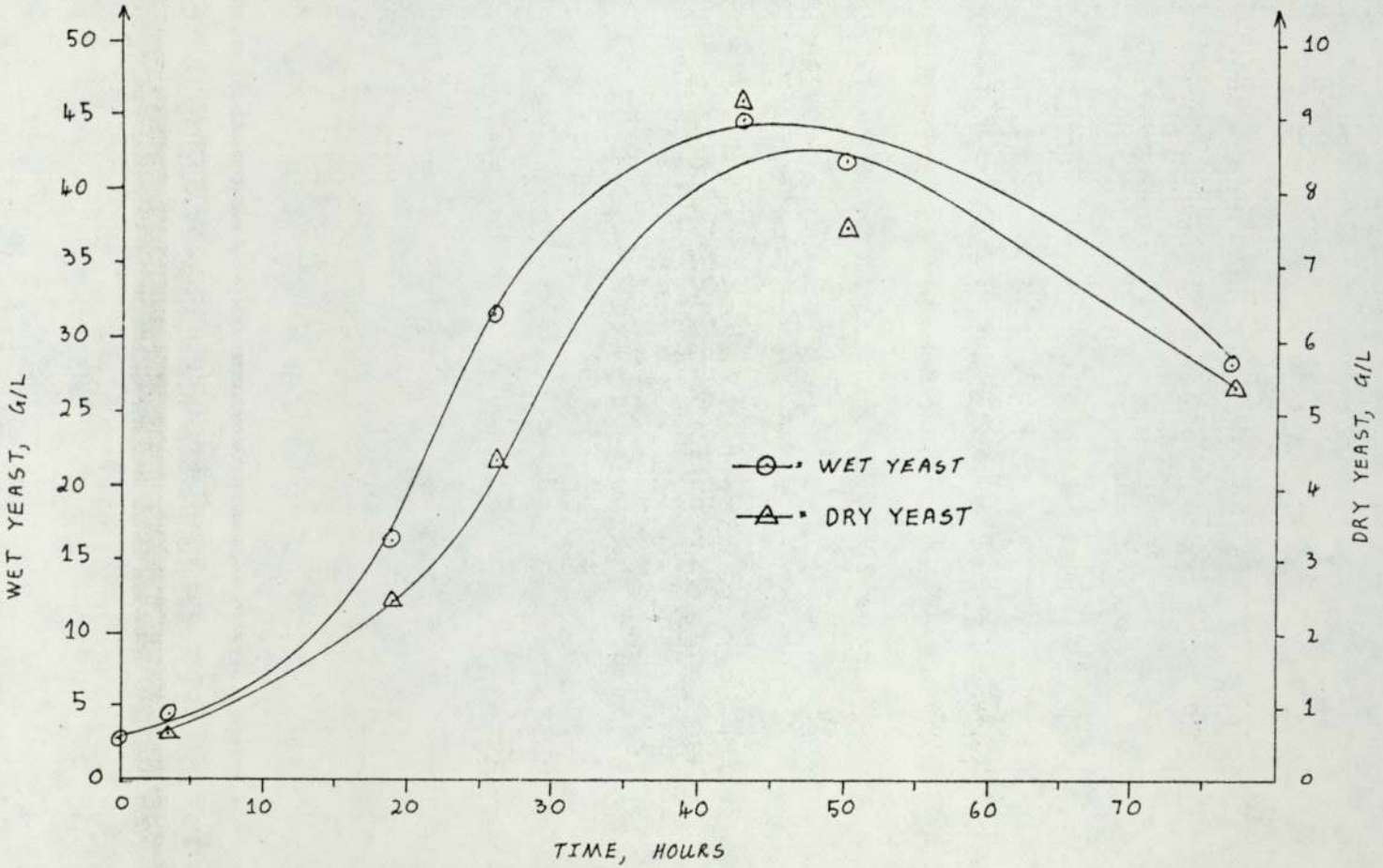
CFCC 1

Time into Fermentation	Present Gravity	Reducing Sugars	Yeast Wet Wt.	Yeast Dry Wt.	Water
Hours		G/L	G/L	G/L	%w/w
0.0	50.5	102.5	2.50	-	-
3.5	50.3	102.4	4.44	0.661	85.1
19.0	48.5	87.3	16.0	2.49	84.5
26.0	42.5	78.3	31.6	4.39	86.1
43.0	8.8	20.9	45.0	9.10	79.8
50.0	8.5	17.7	42.2	7.46	82.3
77.0	8.3	16.9	28.7	5.45	81.0

REDUCING SUGARS CONCENTRATION VERSUS TIME INTO FERMENTATION, EXPT. NO. 8.



YEAST CONCENTRATION VERSUS TIME INTO FERMENTATION, EXPT. NO. 8



APPENDIX 3.2

Back Calculation of Wort Original Gravity

The original gravity of wort may be calculated from;

O.G. = G.R. + D.G.L.

where O.G. = wort original gravity

G.R. = gravity of residue after distillation of the ethanol from beer

D.G.L. = degrees of gravity lost.

The D.G.L. are estimated from brewers' tables by knowing the degrees of spirit indication (D.S.I.).

D.S.I. = 1000 - G.D.

where G.D. = gravity of the distillate from distillation of beer. The D.S.I. need to be corrected to take account of acetic acid in the brew. If acidity above 0.1% is not corrected for the final calculated original gravity will appear too low. 0.1% is subtracted from the acidity found, the corresponding D.S.I. are read from tables, and this figure is then added to the spirit indication already found to give the true spirit indication.

EXPERIMENT NO. 1

TIME INTO FERMENTATION	SPECIFIC GRAVITIES			SPIRIT INDICATION	ACIDITY
HOURS	BEER	DISTILLATE	RESIDUE		%
0.0	1051.7	1000.7	1050.4	0.0	0.137
5.9	1050.9	999.8	1049.3	0.2	0.158
23.3	1044.8	997.9	1041.4	2.2	0.130
29.8	1034.6	997.3	1037.0	2.7	0.180
47.6	1010.6	992.1	1016.9	7.9	0.167
53.8	1010.7	992.7	1017.1	7.3	0.150
71.5	1009.6	992.1	1014.5	7.9	0.187
77.8	1010.2	992.6	1015.5	7.4	0.168
95.3	1001.2	992.3	1015.2	7.7	0.162

EXCESS SPIRIT INDICATION	TRUE SPIRIT INDICATION	D.G.L.	ALCOHOL	ORIGINAL GRAVITY
			%w/w	
0.067	0.067	0.30	0.00	1050.7
0.088	0.288	1.20	0.12	1050.5
0.59	2.259	9.58	1.15	1051.0
0.120	2.820	12.1	1.47	1049.1
0.100	8.000	35.7	4.42	1052.5
0.080	7.380	32.7	4.04	1049.8
0.127	8.027	40.4	4.48	1054.9
0.098	7.498	33.3	4.18	1048.8
0.092	7.792	34.7	4.37	1049.9

EXPERIMENT NO. 2.

TIME INTO FERMENTATION	SPECIFIC GRAVITIES			SPIRIT INDICATION	ACIDITY
HOURS	BEER	DISTILLATE	RESIDUE		%
0.0	1053.2	1000.7	1054.6	-	-
17.0	1051.1	999.7	1053.6	0.34	0.186
23.0	1048.0	999.3	1049.9	0.75	0.254
41.5	1011.3	993.2	1019.8	6.8	0.256
46.0	1009.2	993.1	1017.3	7.0	0.186
65.0	1007.0	991.3	1016.1	8.7	0.139

EXCESS SPIRIT INDICATION	TRUE SPIRIT INDICATION	D.G.L.	ALCOHOL	ORIGINAL GRAVITY
			%w/w	
-	-	-	0.00	1054.6
0.126	0.466	1.94	0.18	1055.5
0.214	0.964	4.07	0.39	1054.0
0.216	7.046	31.2	3.80	1051.0
0.069	8.739	39.1	4.98	1055.2

APPENDIX 4.1

A Computer Program to Evaluate the Maximum Specific Growth Rate and Yield Constant for a Micro-organism

```
      MASTER GC
      DIMENSION T(20),XW (20),XD(20),S (50),A(200),B(200),
                                        C(200),D(200)
      N = 14
      READ(1,100) (T(J),XW(J),XD(J),J=1,N)
  100 FORMAT(3F0.0)
      READ(1,101) ( S(J),J+1,N)
  101 FORMAT(10F0.0)
      WRITE(2,202)
  202 FORMAT(//25X,43H CALCULATIONS USING WET YEAST CONCENTRATION)
      WRITE(2,203)
  203 FORMAT(25X,43H-------------------------------------------)
      WRITE(2,205)
  205 FORMAT(/6X,10H WET YEAST,3X,5H DIFF,7X,7H SUGARS,
                 5X,5H DIFF,6X,5H TIME,5X,5H DIFF,5X,6H YIELD,6X,5H
                 GMAX)
      SGM=0
      SY1=0
      M=0
      DO 10 J=1,N-1
      K=J
      DO 20 I=K,N-1
      M=M+1
      X=XW(I+1)-XW(J)
      TF=T(I+1)-T(J)
      RS=S(J)-S(I+1)
      Y1=X/RS
      A(M)=Y1
      SY1=SY1+Y1
      GM=(ALOG(XW(I+1)/XW(J)))/TF
      B(M)=GM
      SGM=SGM+GM
      WRITE(2,208) XW(I+1),XW(J),X,S(I+1),S(J),RS,T(I+1),T(J),TF,
                                                    Y1,GM
  208 FORMAT(/3X,F5.2,3X,F5.2,4X,F5.2,3X,F5.1,3X,F5.1,3X,5.1,
             3X,F5.2,2X,F5.2,2X,F5.2,4X,F6.3,5X,F6.3)
   20 CONTINUE
   10 CONTINUE
      AVY1=SY1/M
      AVGM=SGM/M
      SY1S=0
      SGMS=0
      DO 50 J=1,M
      SY1S=(A(J)-AVY1)**2+SY1S
      SGMS=(B(J)-AVGM)**2 +SGMS
   50 CONTINUE
```

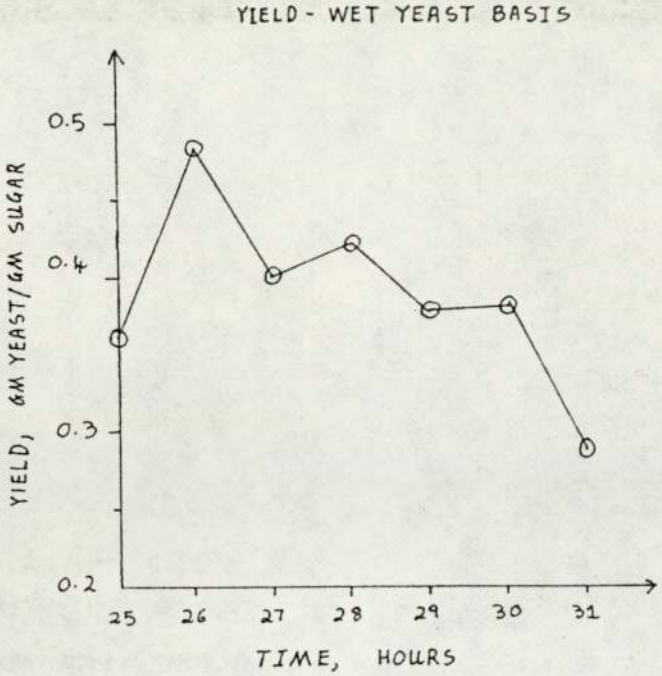
```
      SDY1 =SQRT(SY1S/(M-1))
      SDGM=SQRT(SGMS/(M-1))
      WRITE(2,300)
  300 FORMAT(/23X,6H MEANS,21X,6H ST. DEV)
      WRITE(2,301)
  301 FORMAT(/14X,6H YIELD,7X,5H GMAX,14X,6H YIELD,6X,5H GMAX)
      WRITE(2,302) AVY1,AVGM,SDY1,SDGM
  302 FORMAT(/13X,F7.5,14X,F7.5,5X,F7.5)
      WRITE(2,204)
  204 FORMAT(//25X,43H CALCULATIONS USING DRY YEAST CONCENTRATION)
      WRITE(2,207)
  207 FORMAT(25X,43H-----------------------------------------)
      WRITE(2,206)
  206 FORMAT(/6X,10H DRY YEAST,3X,5H DIFF.7X,7H SUGARS,5X,5H DIFF,6X,5H
     1       TIME,5X,5H DIFF,5X,6H YIELD,6X,5H GMAX)
      SGMD=0
      M=0
      SY2=0
      DO 30 J=1,N-1
      K=J
      DO 40 I=K,N-1
      M=M+1
      DX=XD(I+1)-XD(J)
      TF=T(I+1)-T(J)
      RS=S(J)-S(I+1)
      Y2=DX/RS
      C(M)=Y2
      SY2=SY2+Y2
      GMD=(ALOG(XD(I+1)/XD(J)))/TF
      D(M)=GMD
      SGMD=SGMD+GMD
      WRITE(2,209) XD(I+1),XD(J),X,S(I+1),S(J),RS,T(I+1),T(J),TF,Y2,GMD
  209 FORMAT(/3X,F5.2,3X,F5.2,4X,F5.2,3X,F5.1,3X,F5.1,3X,F5.1,3X,F5.2,2X,
     1       F5.2,4X,F6.3,5X,F6.3)
   40 CONTINUE
   30 CONTINUE
      AVGD=SGMD/M
      AVY2=SY2/M
      SGDS=0
      SY2S=0
      DO60J=1,M
      SGDS=(D(J)-AVGD)**2+SGDS
      SY2S=(C(J)-AVY2)**2+SY2S
   60 CONTINUE
      SDGD=SQRT(SGDS/(M-1))
      SDY2=SQRT(SY2S/(M-1))
      WRITE(2,305)
  305 FORMAT(/23X,6H MEANS,21X,9H ST. DEV.)
      WRITE(2,306)
  306 FORMAT(/14X,6H YIELD,7X,5H GMAX,14X,6H YIELD,6X,5H GMAX)
      WRITE(2,307)AVY2,AVGD,SDY2,SDGD
  307 FORMAT(/13X,F7.5,5X,F7.5,14X,F7.5,5X,F7.5)
      STOP
      END
```

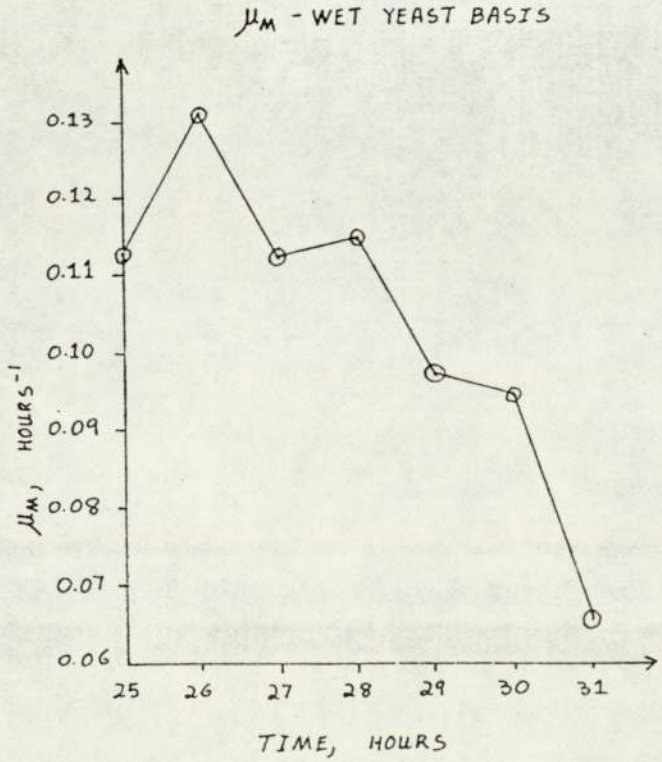
APPENDIX 4.2

Graphs 4.1-4.11

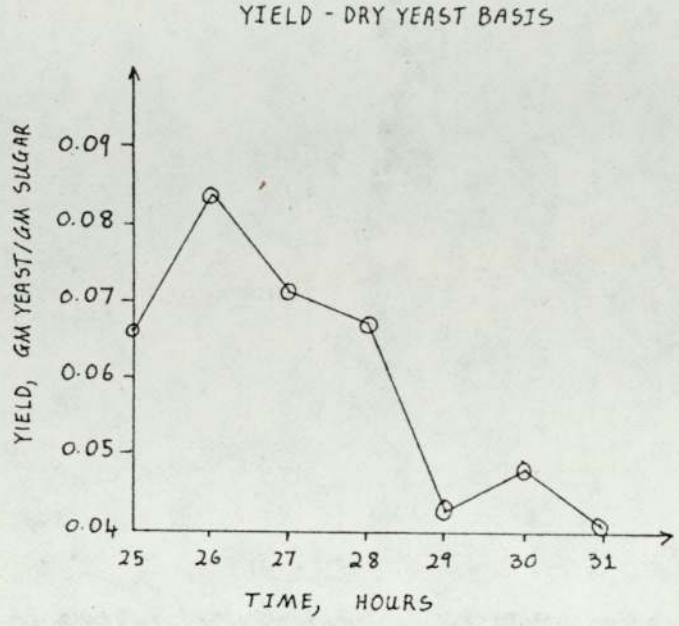
Variation in the maximum specific growth rate and yield constant during the "exponential" growth phase.

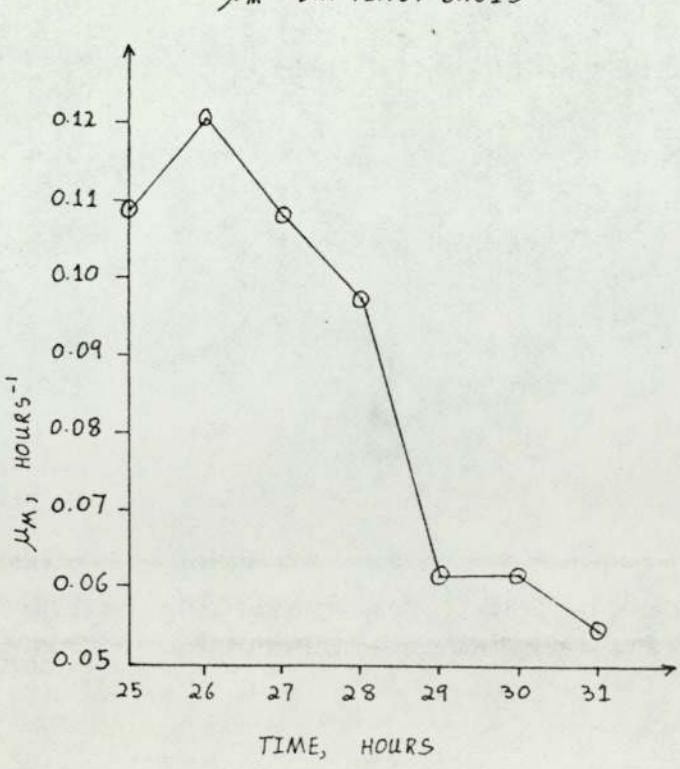
GRAPH 4.1 YIELD AND MAXIMUM SPECIFIC GROWTH RATE VERSUS TIME INTO FERMENTATION, EXPT. NO. 1.



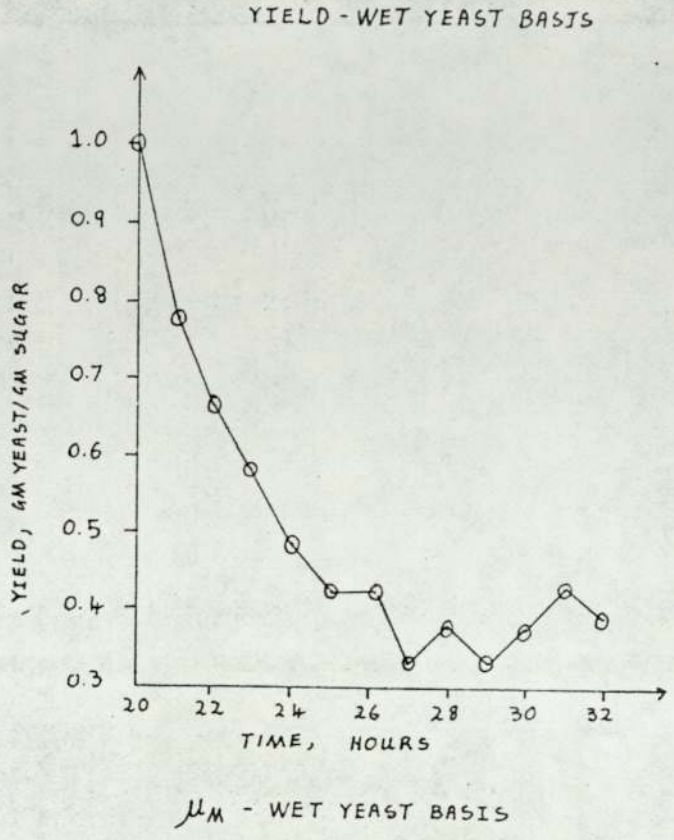


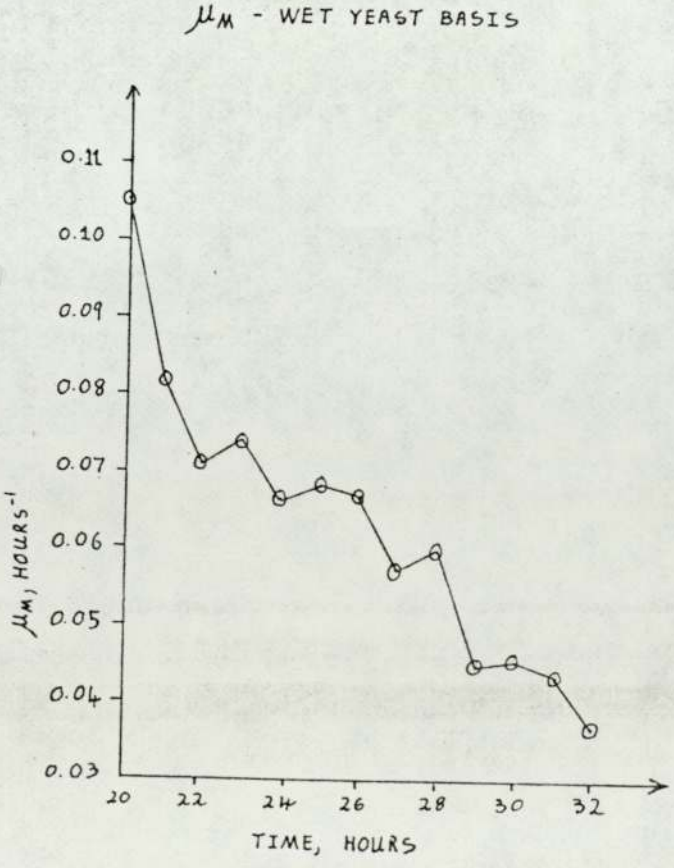
GRAPH 4.2 YIELD AND MAXIMUM SPECIFIC GROWTH RATE VERSUS TIME INTO FERMENTATION, EXPT. NO. 1.



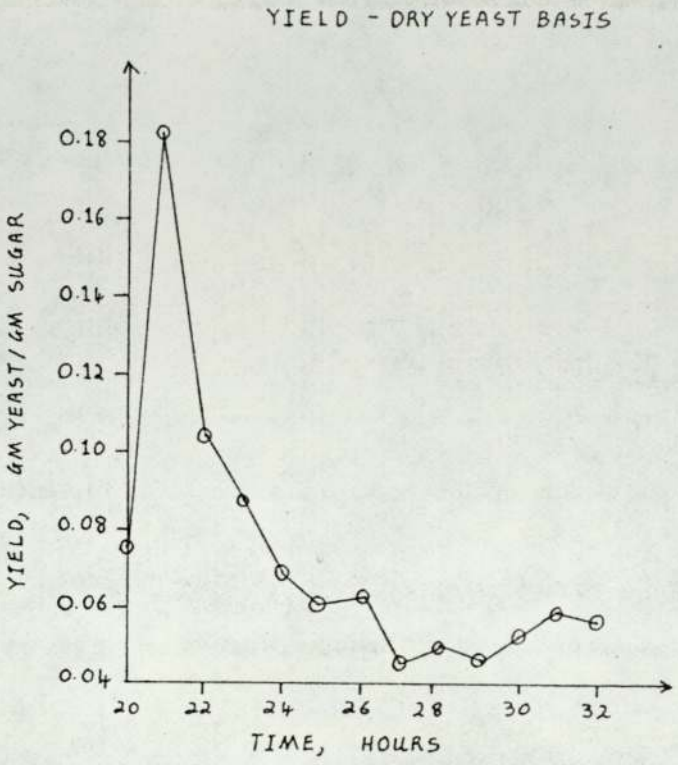


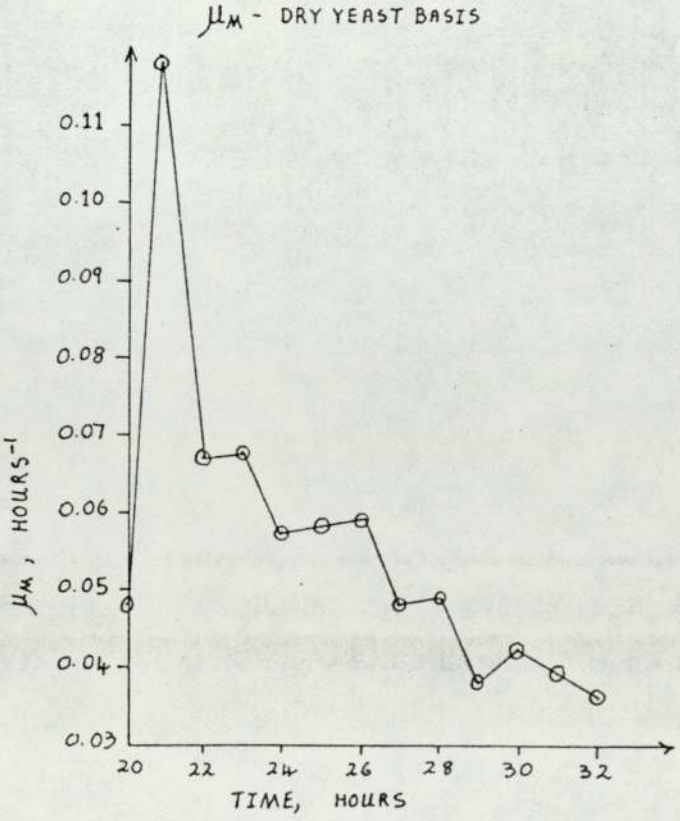
GRAPH 4.3 YIELD AND MAXIMUM SPECIFIC GROWTH RATE VERSUS TIME INTO FERMENTATION, EXPT. NO. 2.



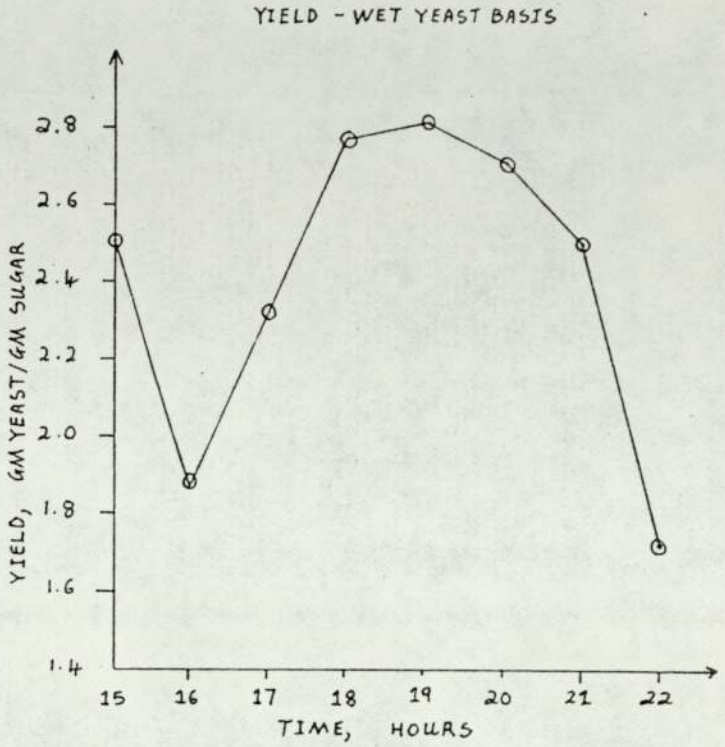


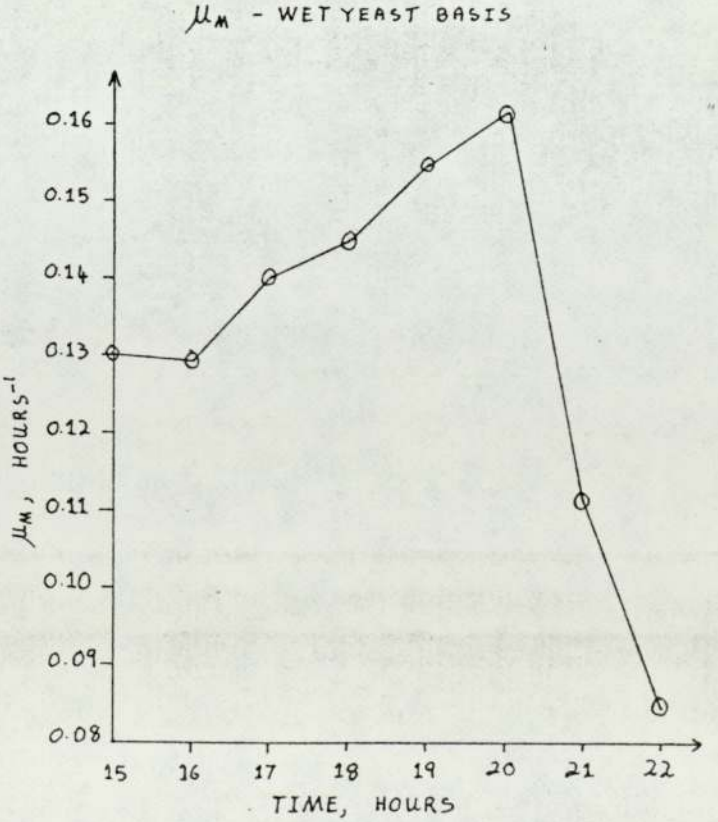
GRAPH 4.4 YIELD AND MAXIMUM SPECIFIC GROWTH RATE VERSUS TIME INTO FERMENTATION, EXPT. NO. 2.



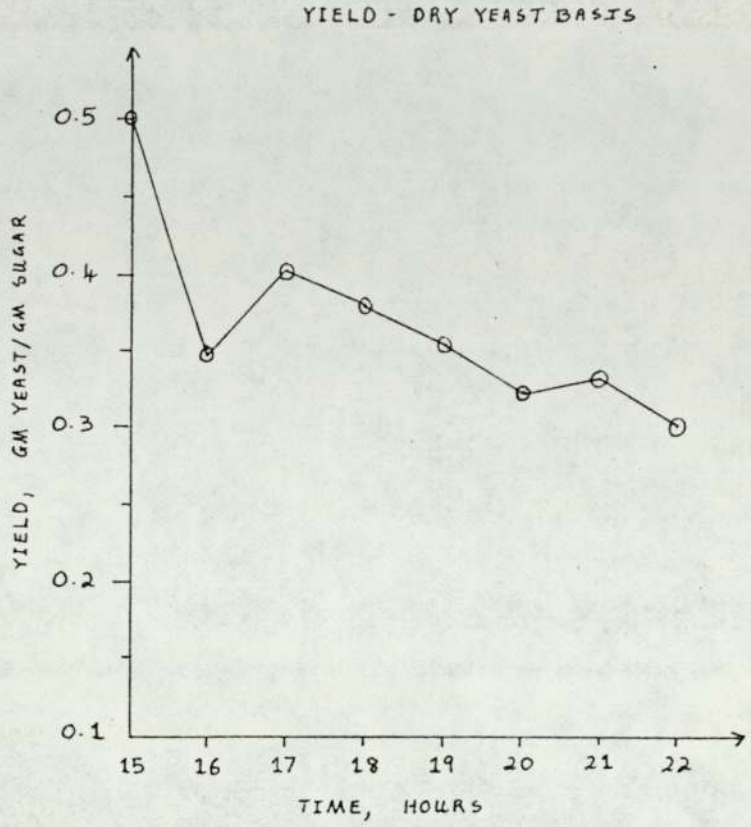


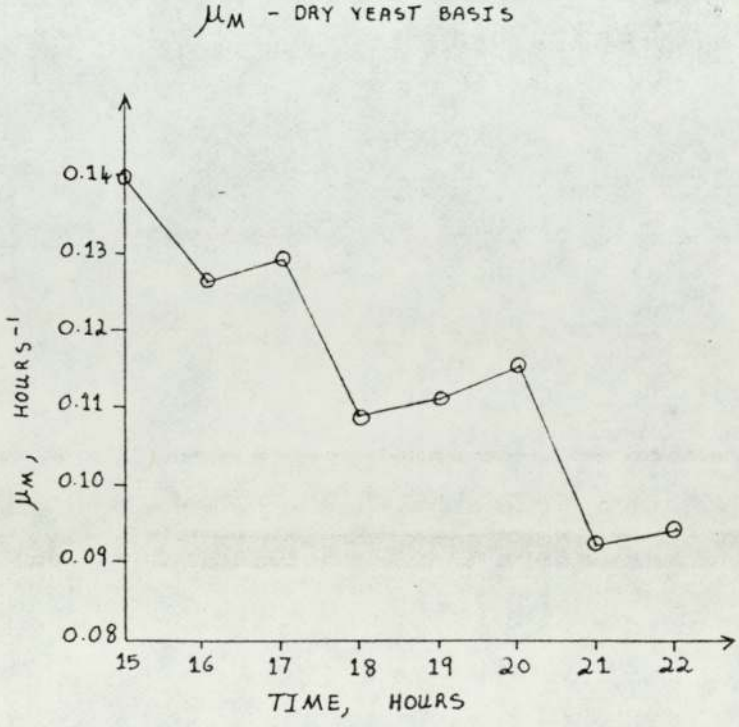
GRAPH 4.5 YIELD AND MAXIMUM SPECIFIC GROWTH RATE VERSUS TIME INTO FERMENTATION, EXPT. NO. 3



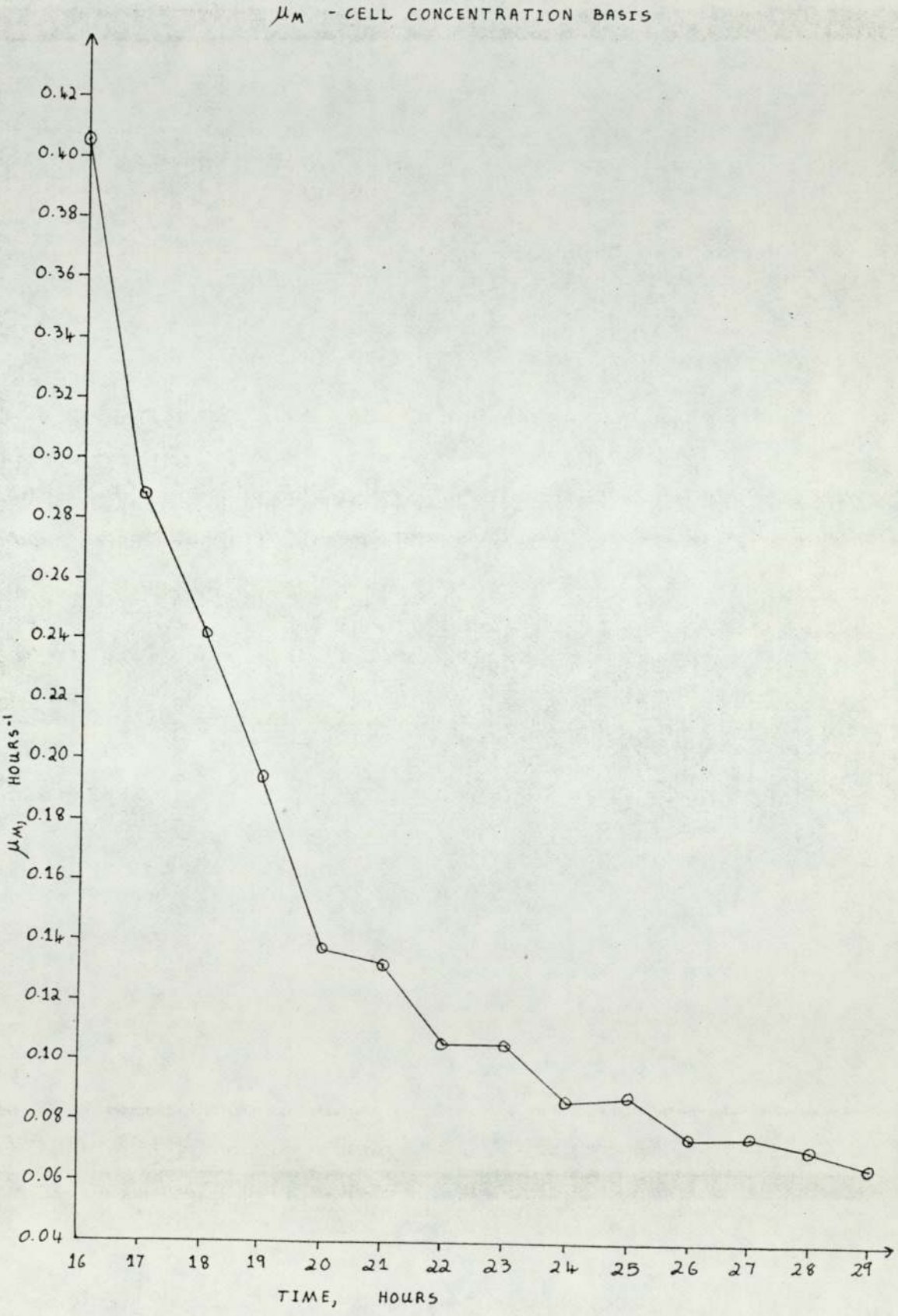


GRAPH 4.6 YIELD AND MAXIMUM SPECIFIC GROWTH RATE VERSUS TIME INTO FERMENTATION, EXPT. NO. 3.



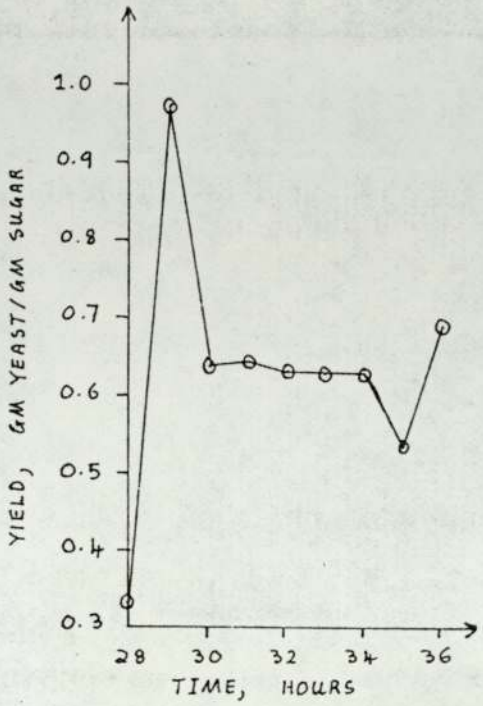


GRAPH 4.7 MAXIMUM SPECIFIC GROWTH RATE VERSUS TIME INTO FERMENTATION, EXPT. NO. 4.

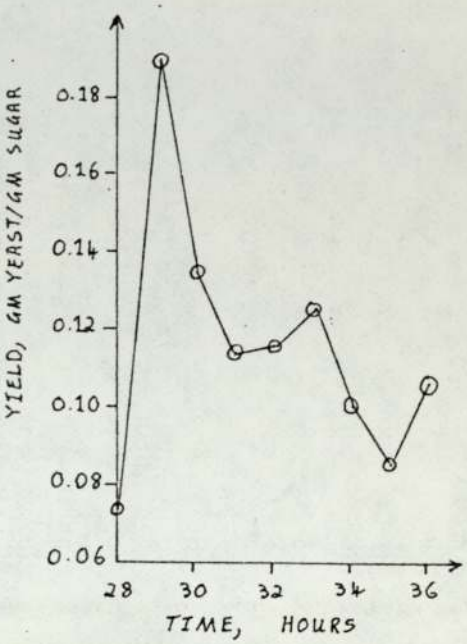


GRAPH 4.8 MAXIMUM SPECIFIC GROWTH RATE AND YIELD VERSUS TIME INTO FERMENTATION, EXPT. NO. 5.

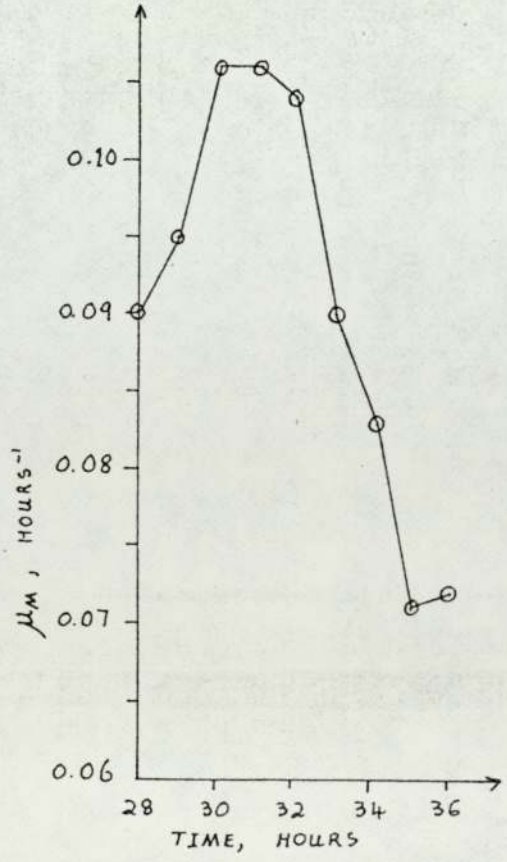
YIELD - WET YEAST BASIS



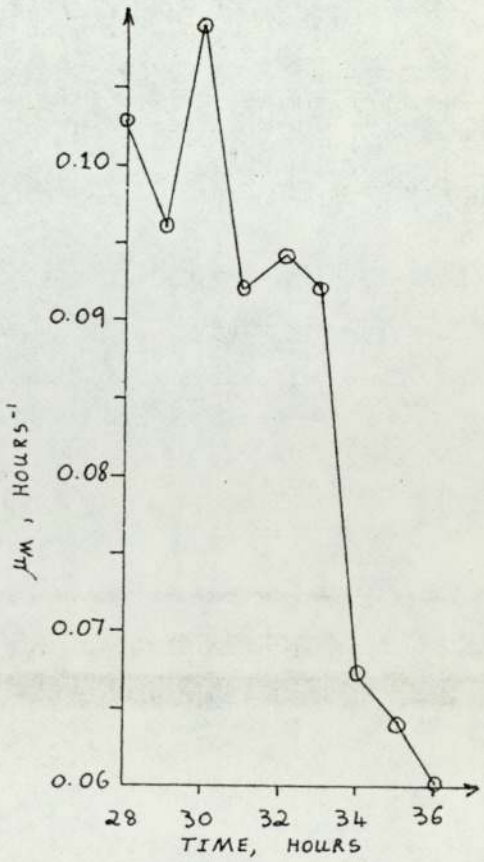
YIELD - DRY YEAST BASIS



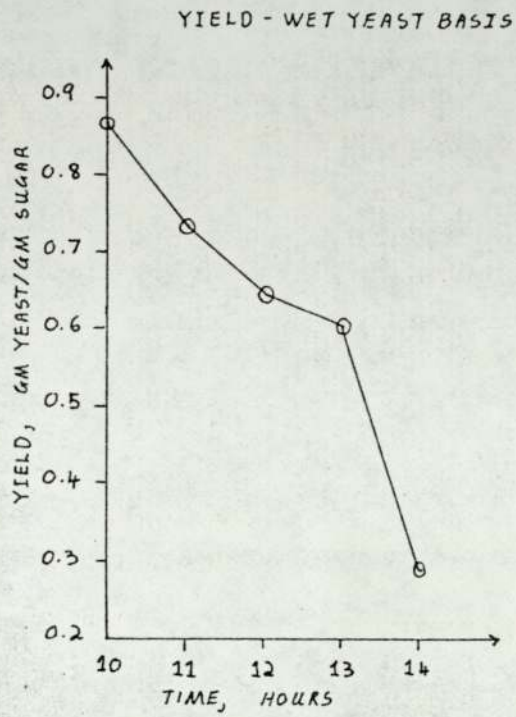
μ_M - WET YEAST BASIS

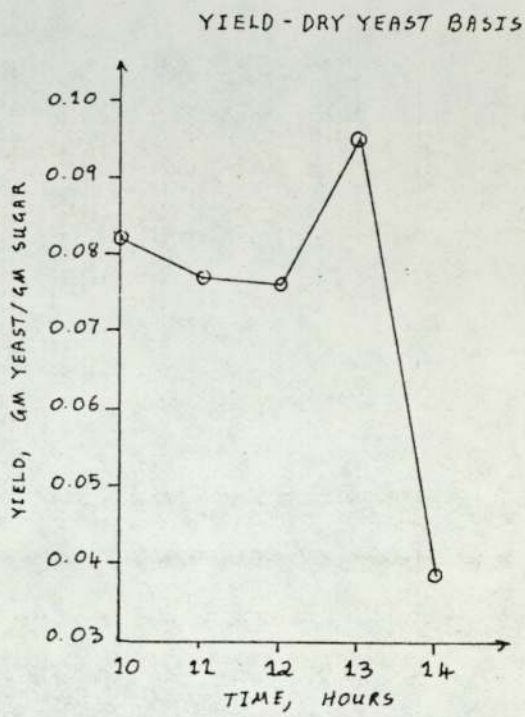


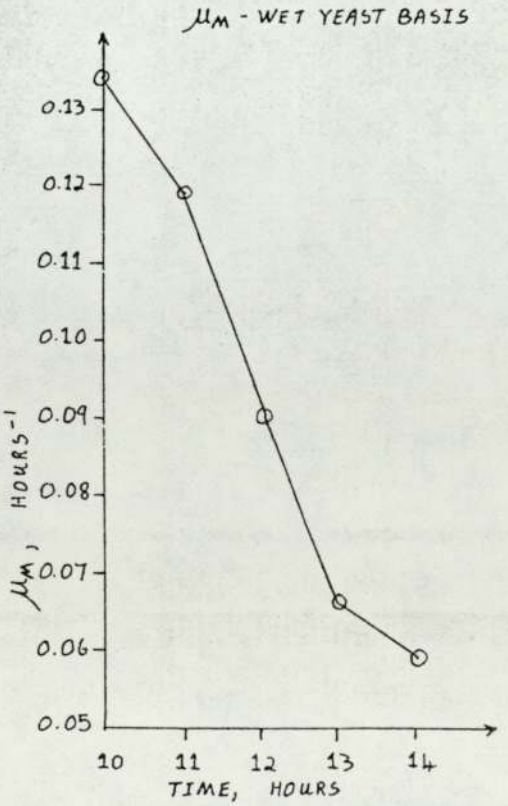
μ_M - DRY YEAST BASIS

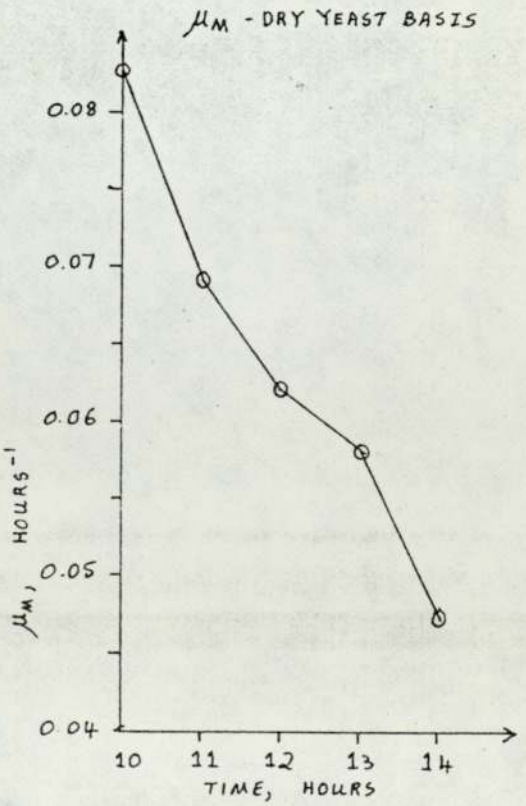


GRAPH 4.9 YIELD AND MAXIMUM SPECIFIC GROWTH RATE VERSUS TIME INTO FERMENTATION, EXPT. NO. 6.

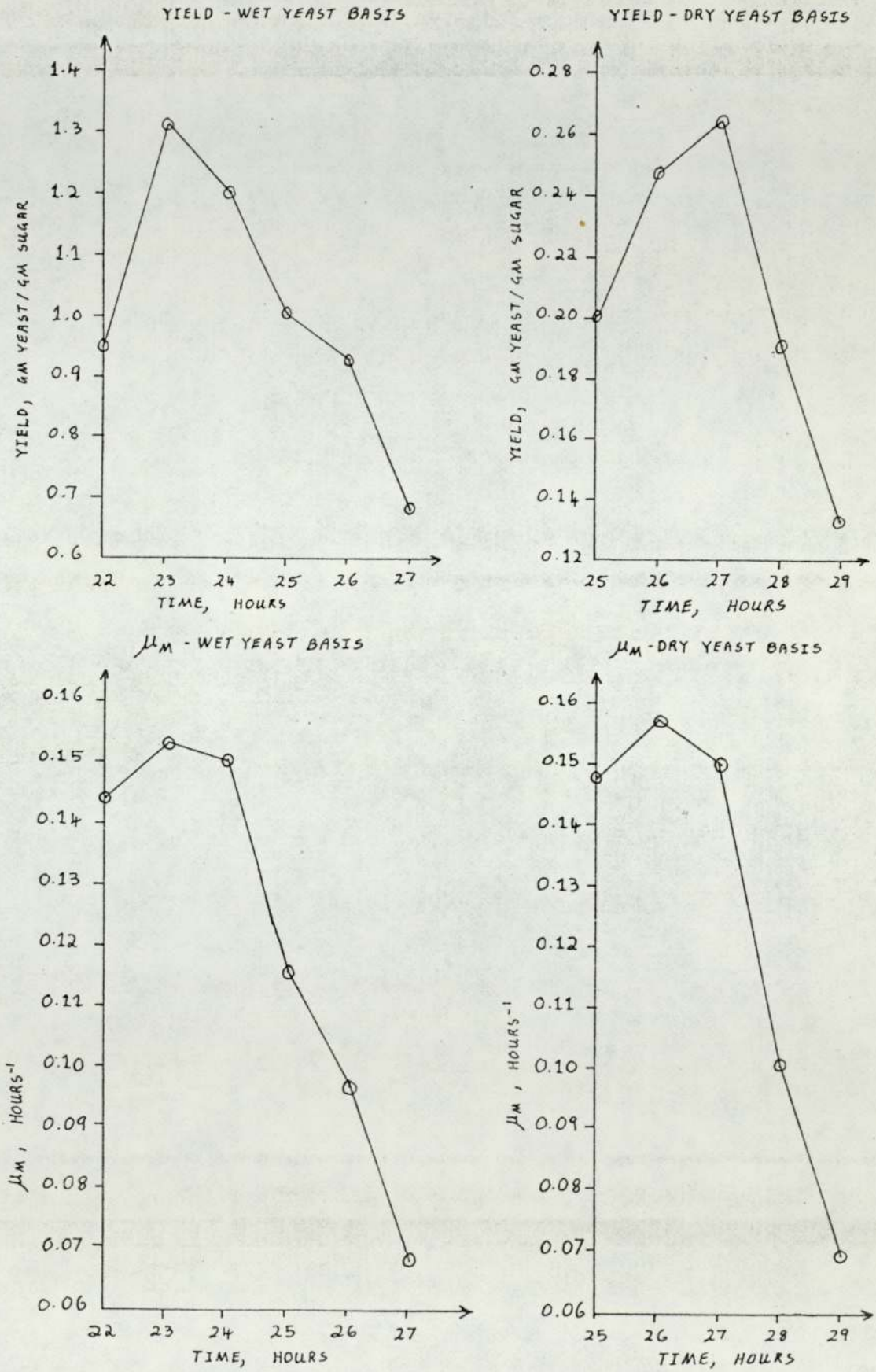






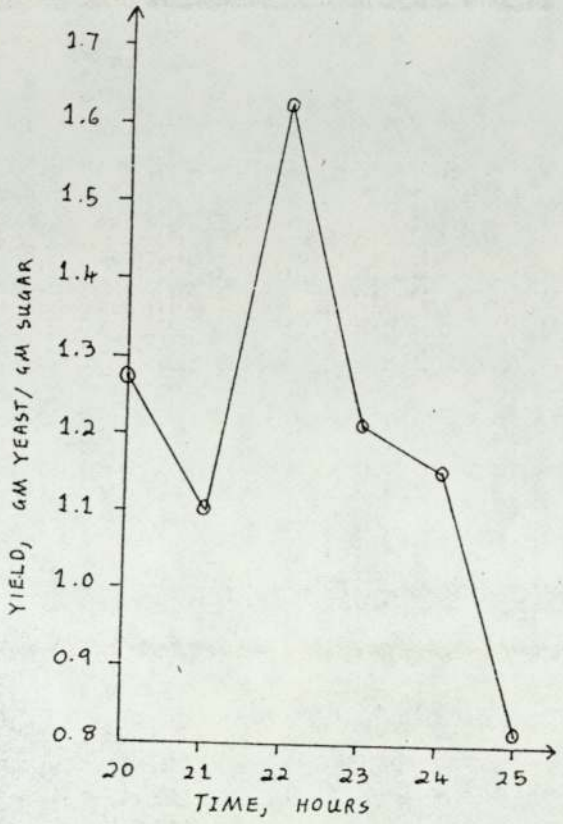


GRAPH 4.10 YIELD AND MAXIMUM SPECIFIC GROWTH RATE VERSUS TIME INTO FERMENTATION, EXPT. NO. 7

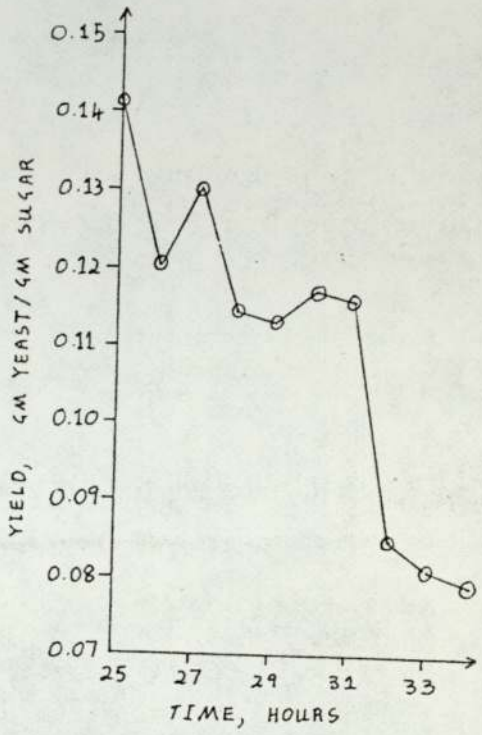


GRAPH 4.11 YIELD AND MAXIMUM SPECIFIC GROWTH RATE VERSUS TIME INTO FERMENTATION, EXPT. NO. 8.

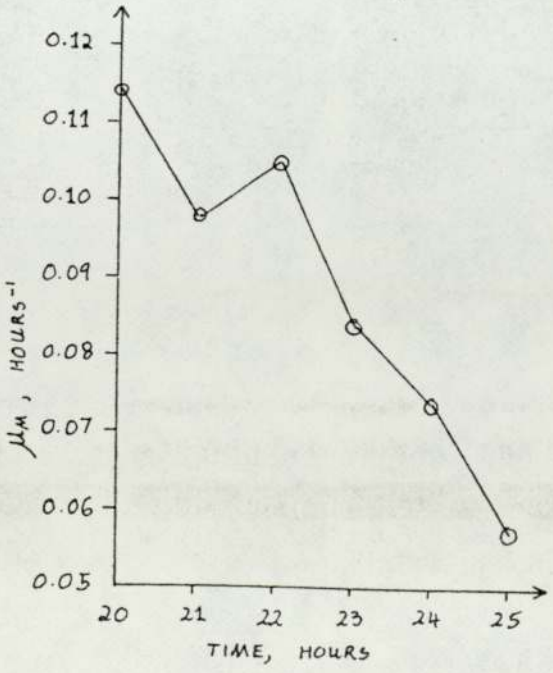
YIELD - WET YEAST BASIS



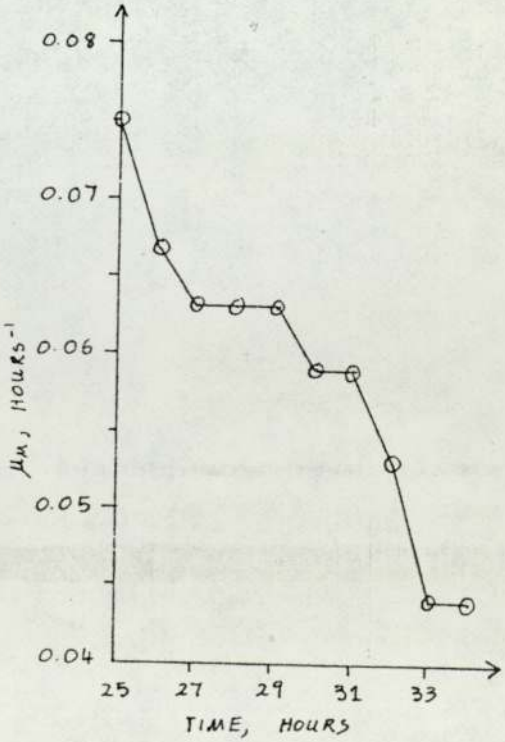
YIELD - DRY YEAST BASIS



μ_M - WET YEAST BASIS



μ_M - DRY YEAST BASIS



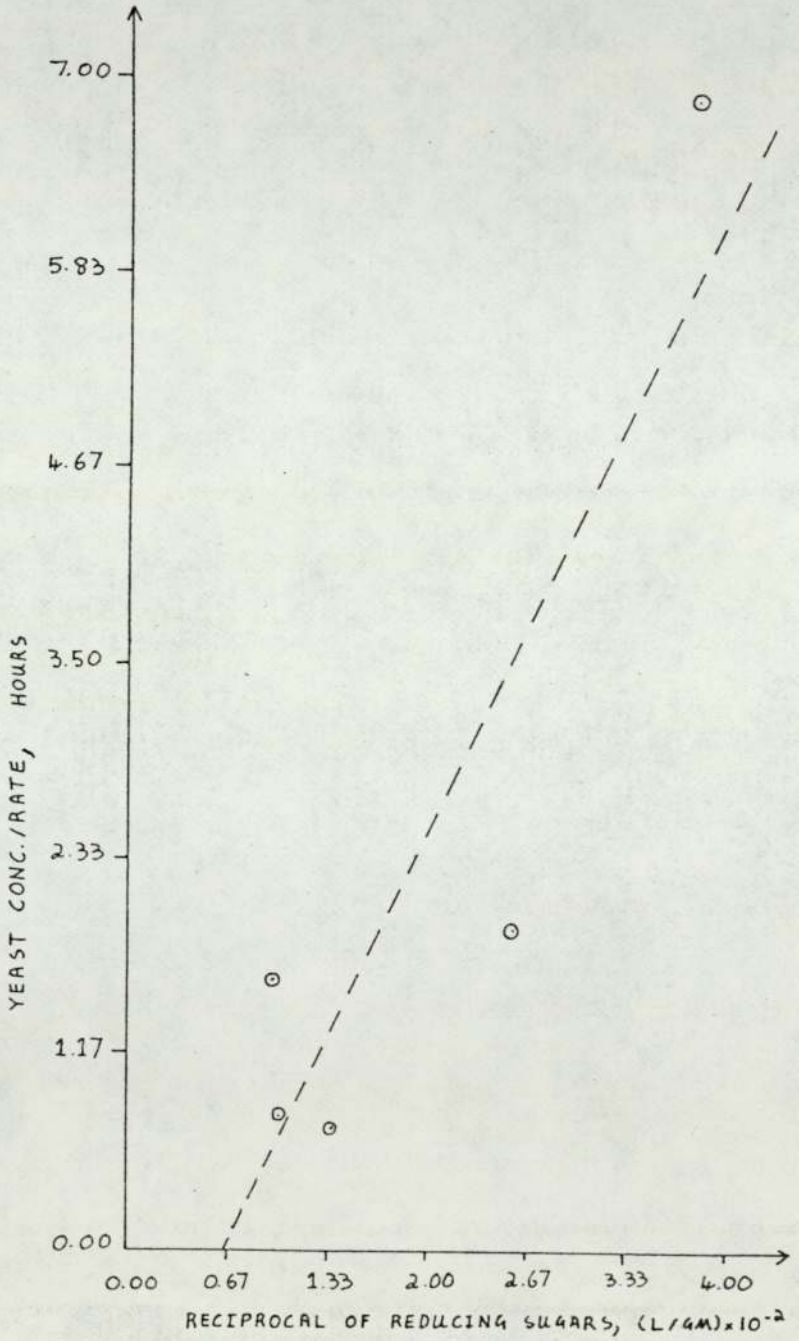
APPENDIX 4.3

Graphs 4.12 - 4.17

Linear testing of the Monod model - some typical graphs

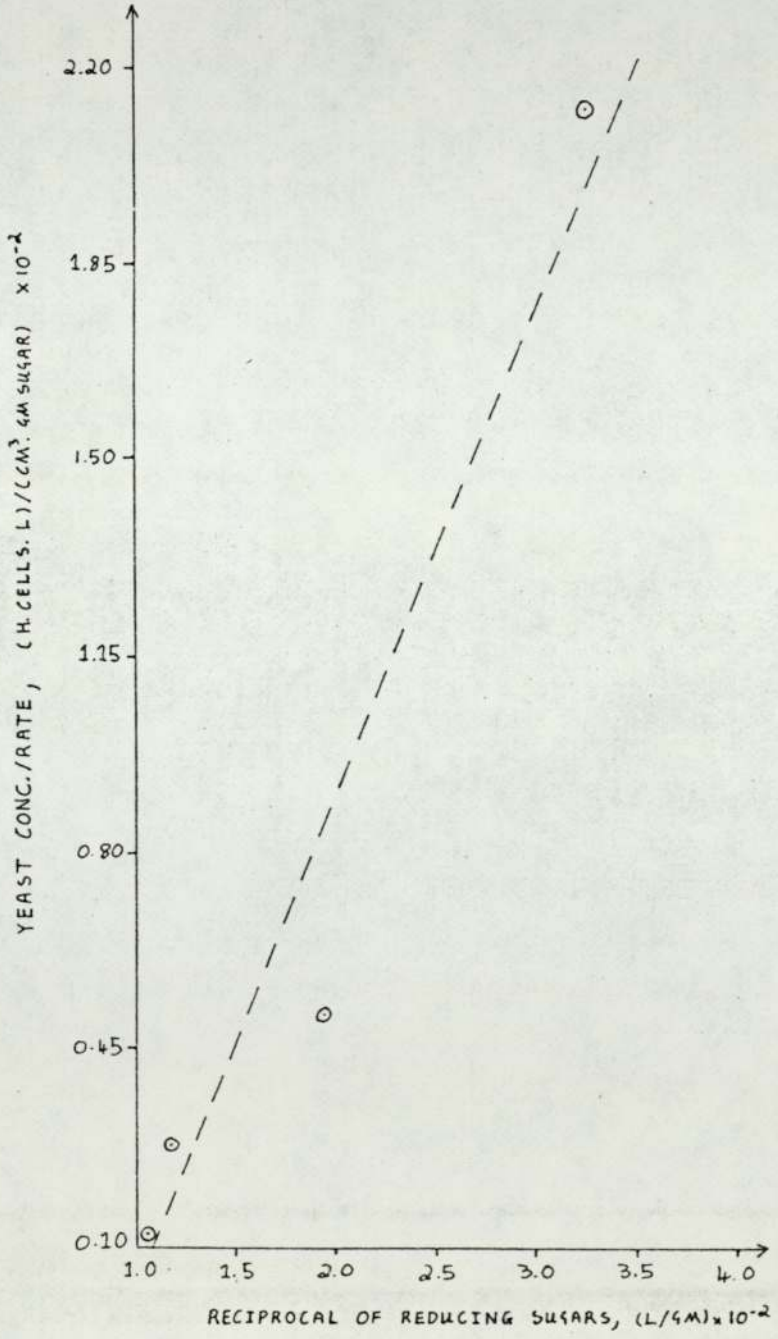
GRAPH 4.12 RATE OF REACTION VERSUS REDUCING SUGARS, EXPT. NO. 1.

REGRESSION COEFFICIENT = 0.737



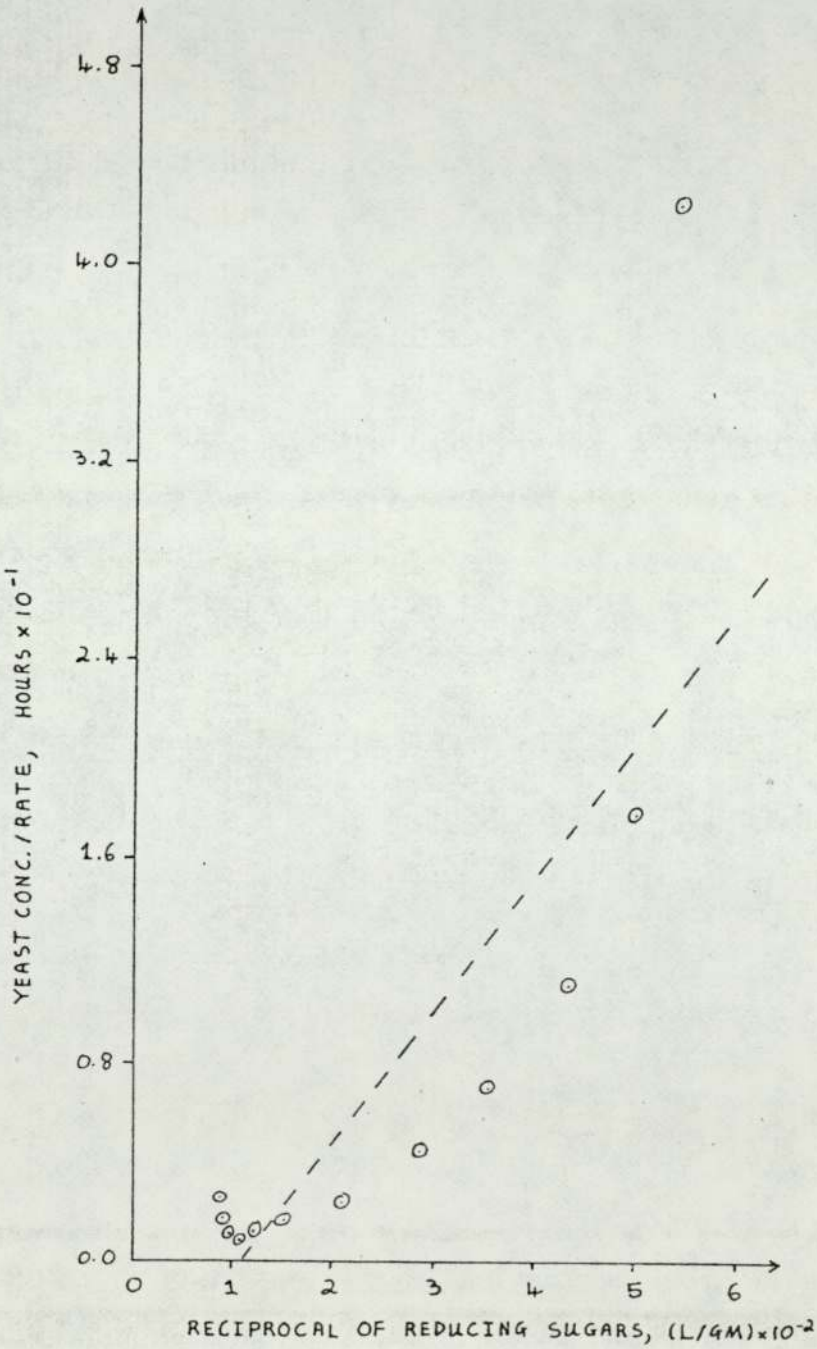
GRAPH 4.13 RATE OF REACTION VERSUS REDUCING SUGARS, EXPT. NO. 4.

REGRESSION COEFFICIENT = 0.917



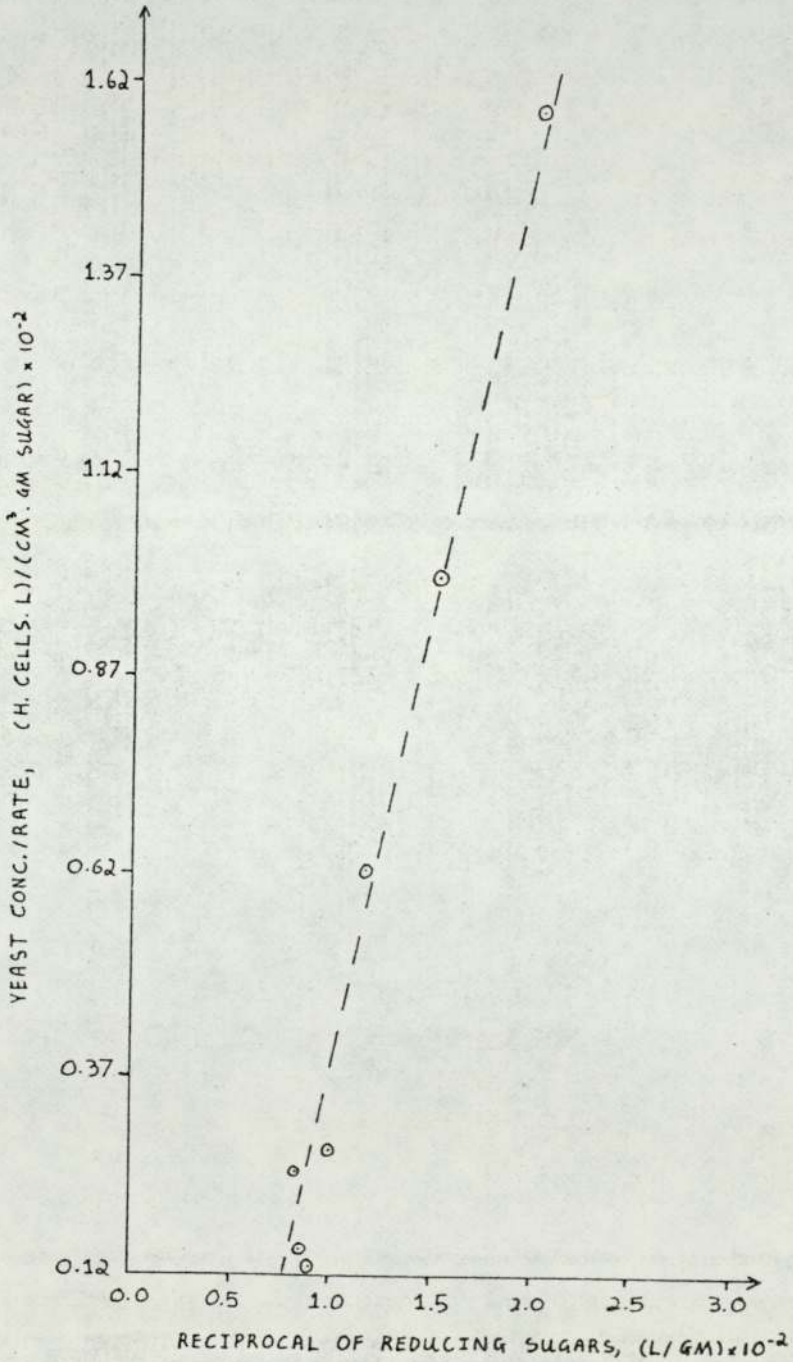
GRAPH 4.14 RATE OF REACTION VERSUS REDUCING SUGARS, EXPT. NO. 5.

REGRESSION COEFFICIENT = 0.654



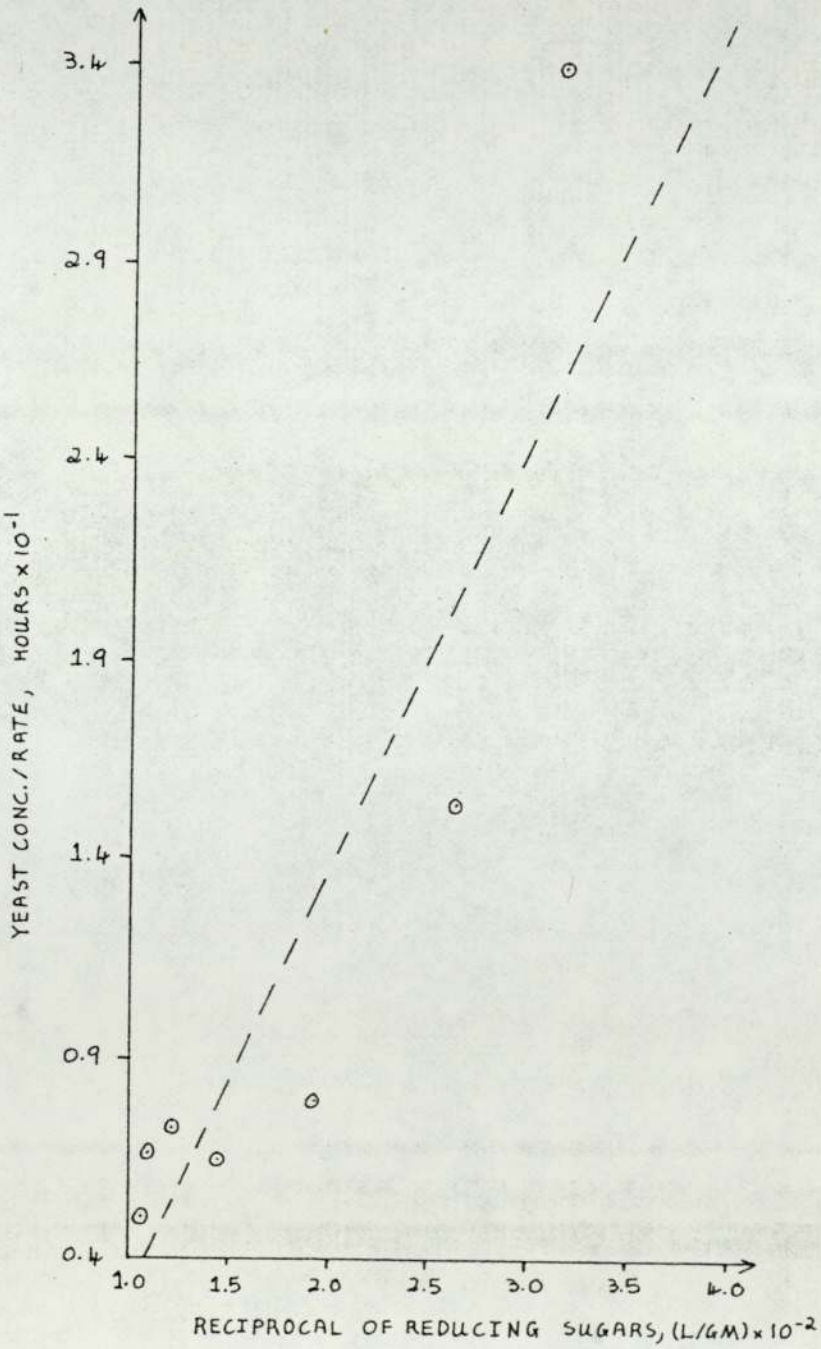
GRAPH 4.15 RATE OF REACTION VERSUS REDUCING SUGARS, EXPT. NO. 5.

REGRESSION COEFFICIENT = 0.974

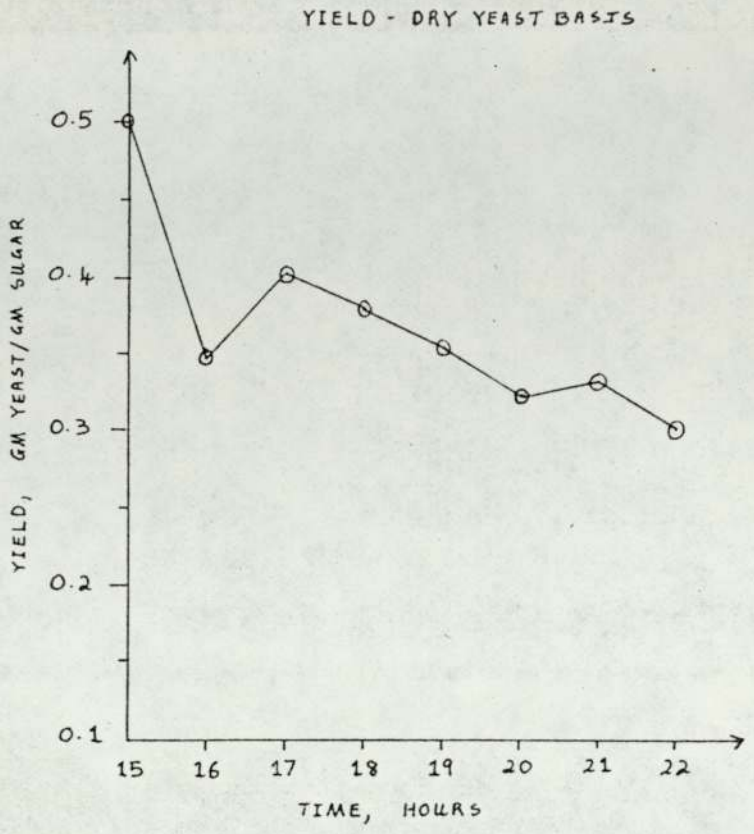


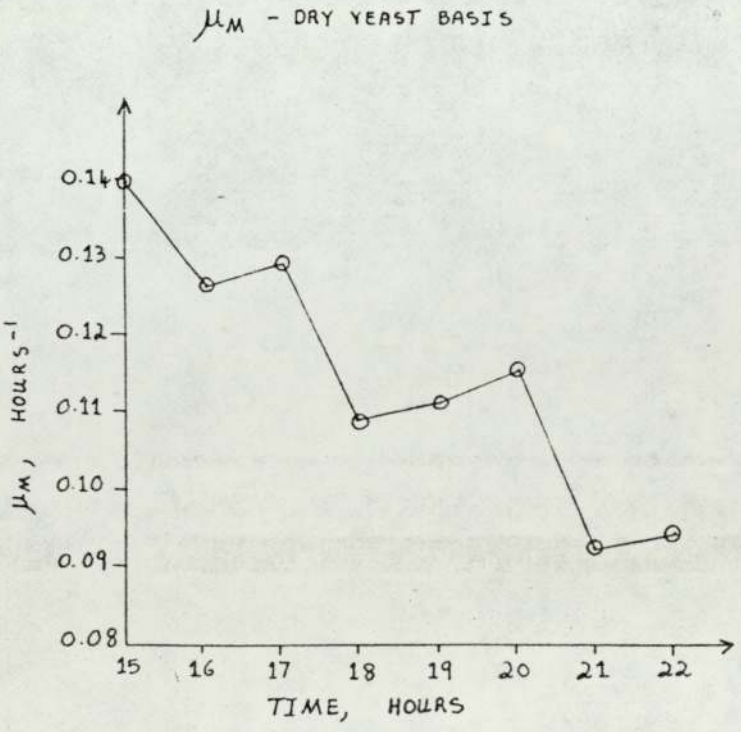
GRAPH 4.16 RATE OF REACTION VERSUS REDUCING SUGARS, EXPT. NO. 6.

REGRESSION COEFFICIENT = 0.764

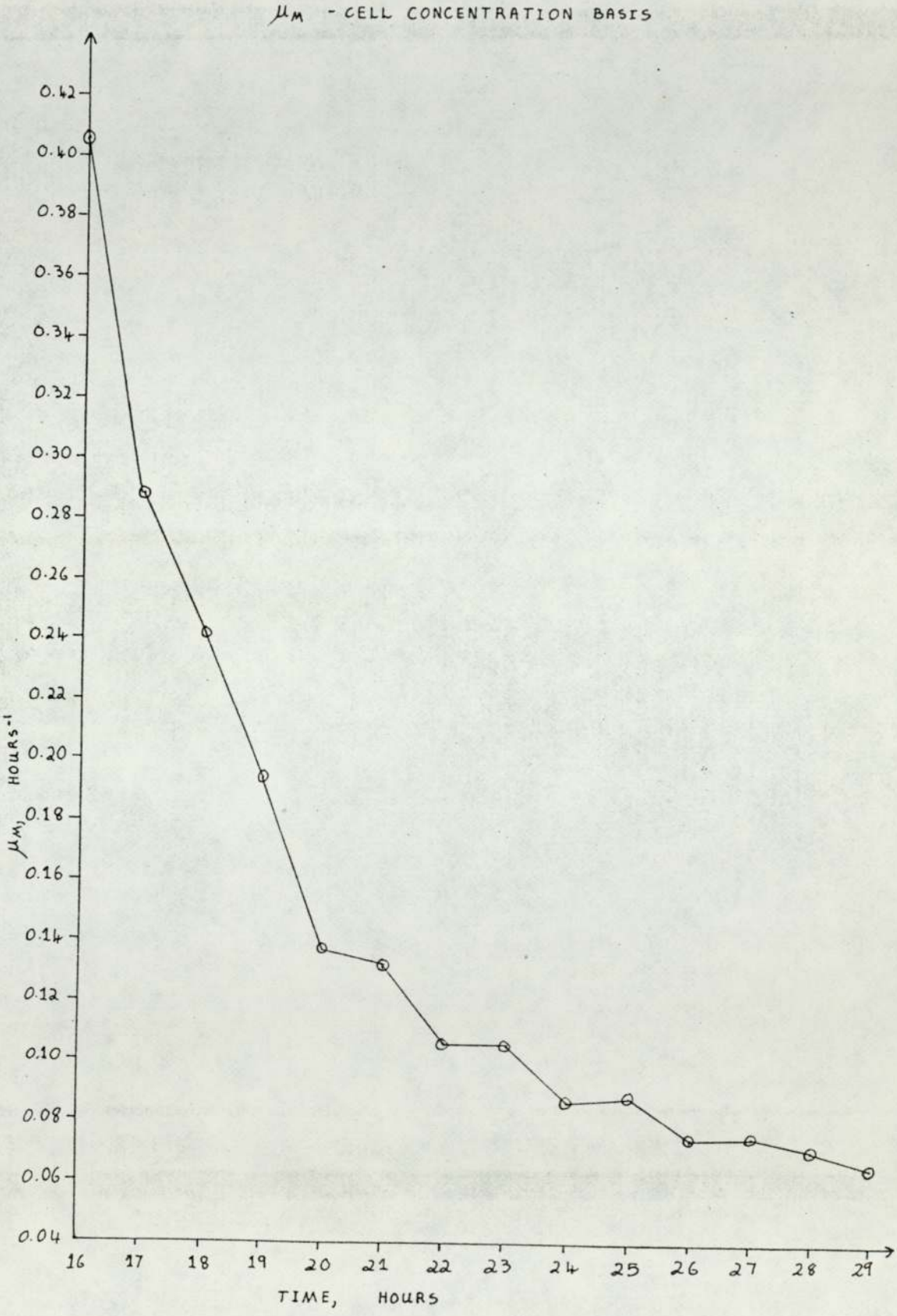


GRAPH 4.6 YIELD AND MAXIMUM SPECIFIC GROWTH RATE VERSUS TIME INTO FERMENTATION, EXPT. NO. 3.



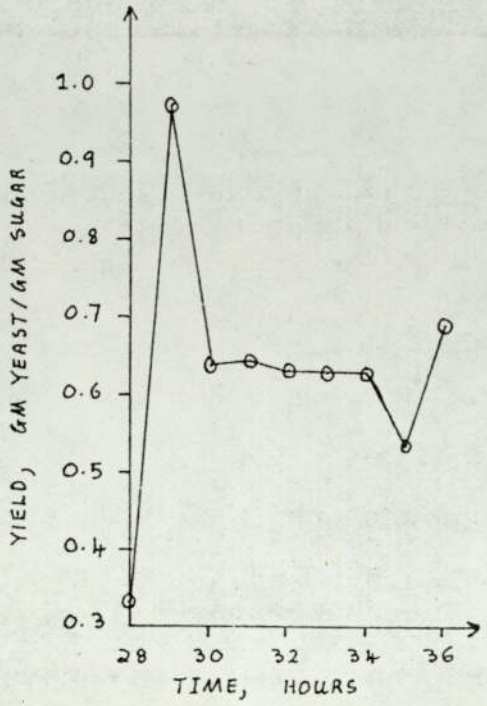


GRAPH 4.7 MAXIMUM SPECIFIC GROWTH RATE VERSUS TIME INTO FERMENTATION, EXPT. NO. 4.

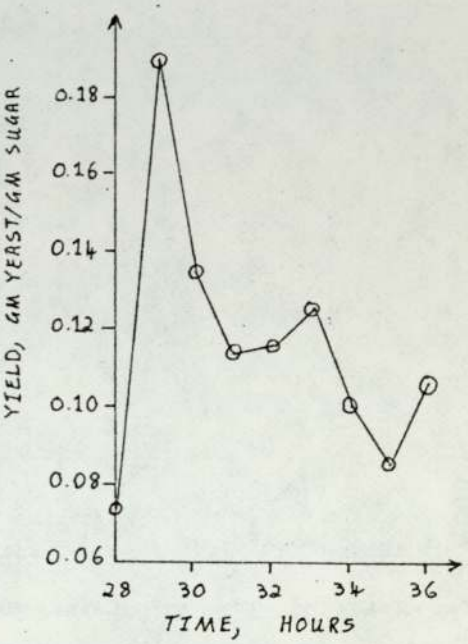


GRAPH 4.8 MAXIMUM SPECIFIC GROWTH RATE AND YIELD VERSUS TIME INTO FERMENTATION, EXPT. NO. 5.

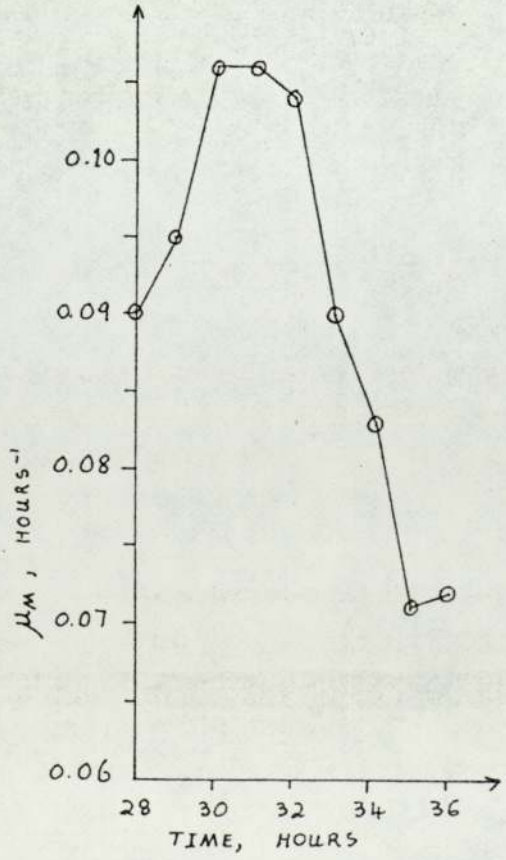
YIELD - WET YEAST BASIS



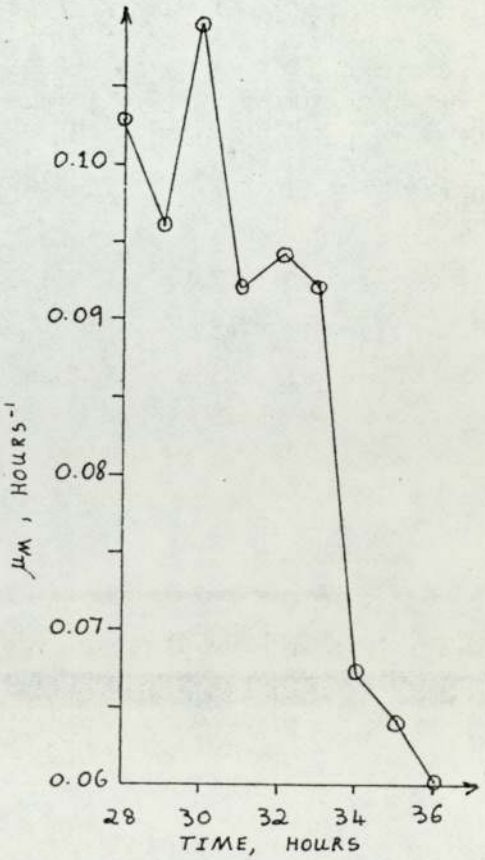
YIELD - DRY YEAST BASIS



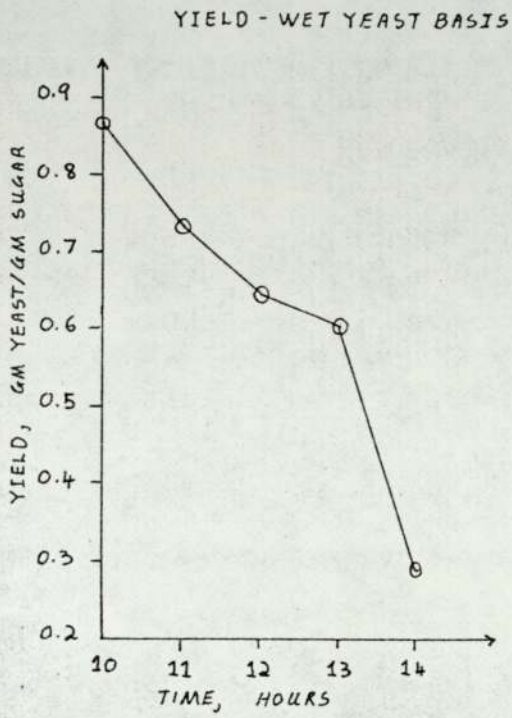
μ_M - WET YEAST BASIS

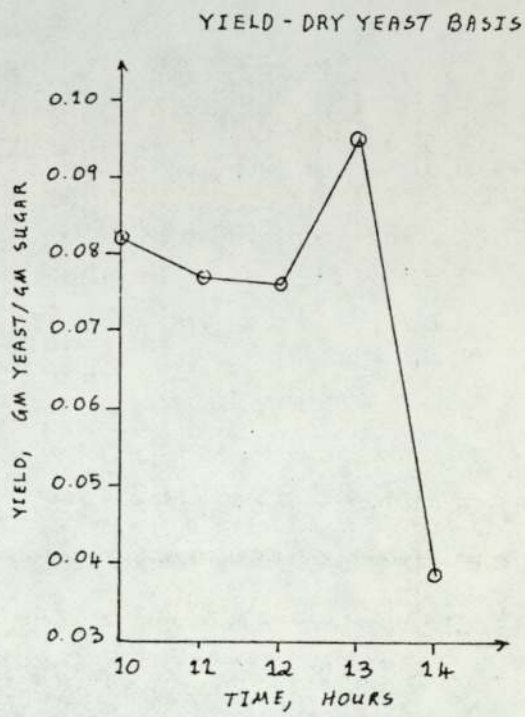


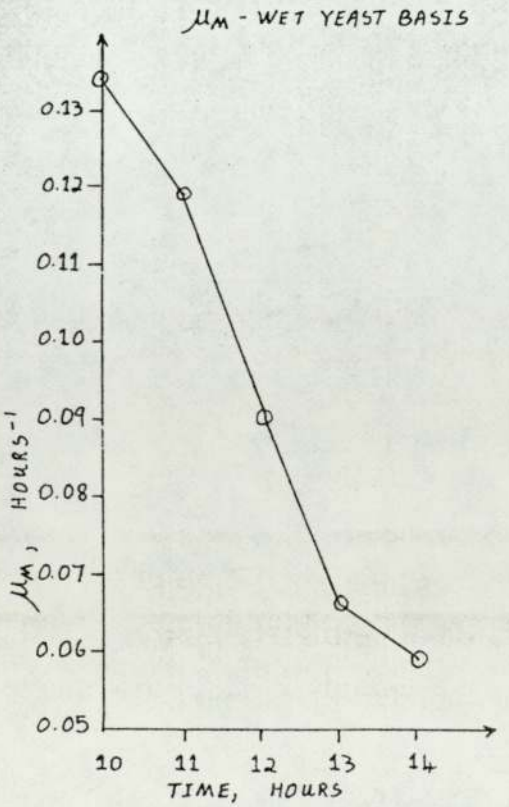
μ_M - DRY YEAST BASIS

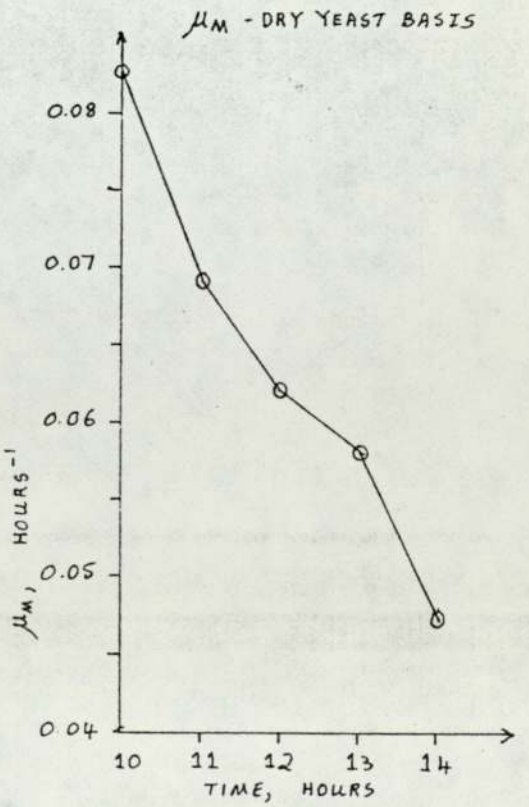


GRAPH 4.9 YIELD AND MAXIMUM SPECIFIC GROWTH RATE VERSUS TIME INTO FERMENTATION, EXPT. NO. 6.

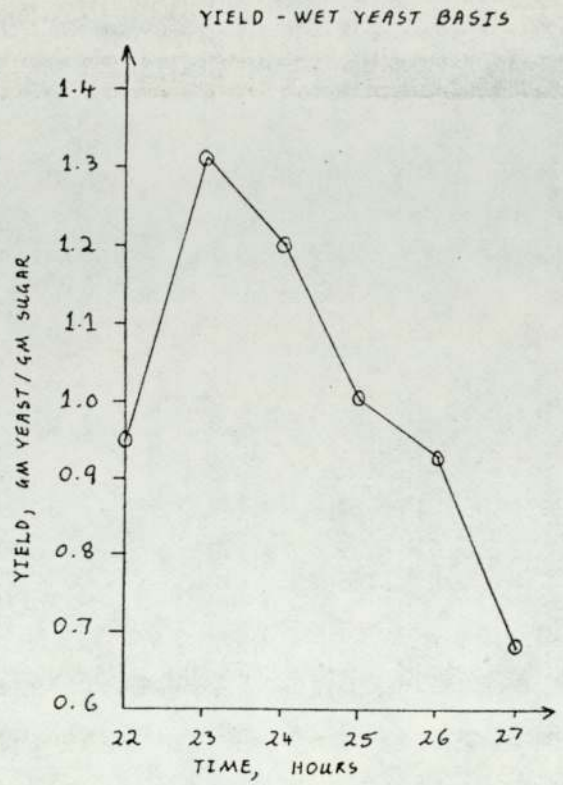


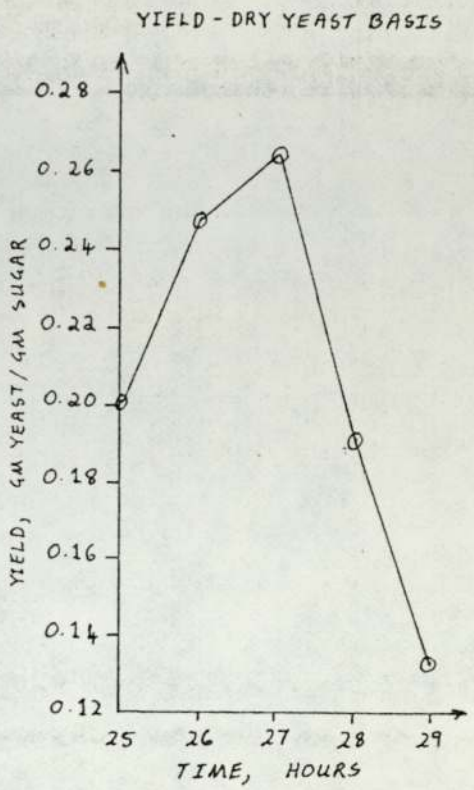


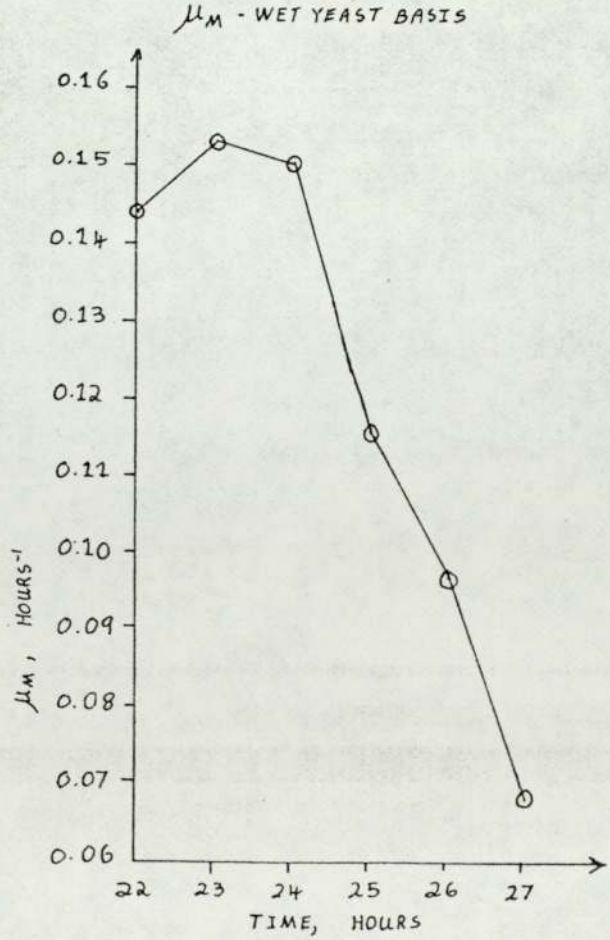


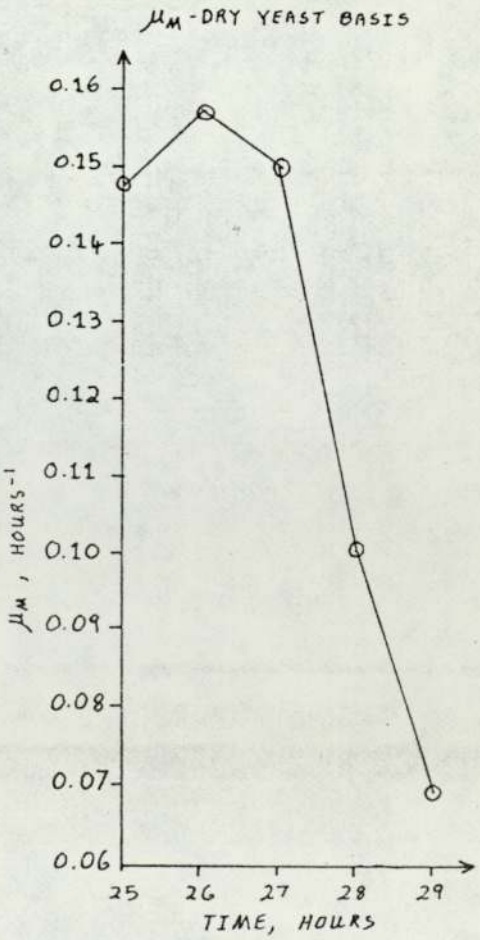


GRAPH 4.10 YIELD AND MAXIMUM SPECIFIC GROWTH RATE VERSUS TIME INTO FERMENTATION, EXPT. NO. 7



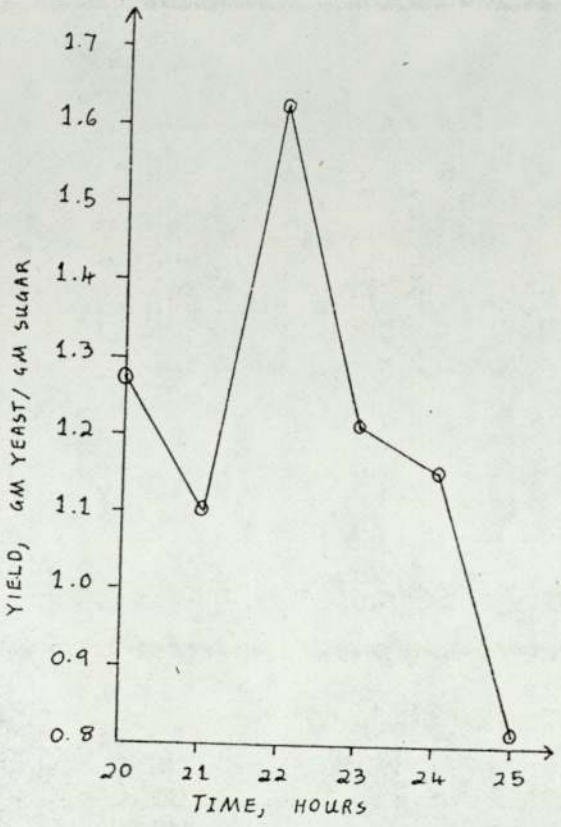




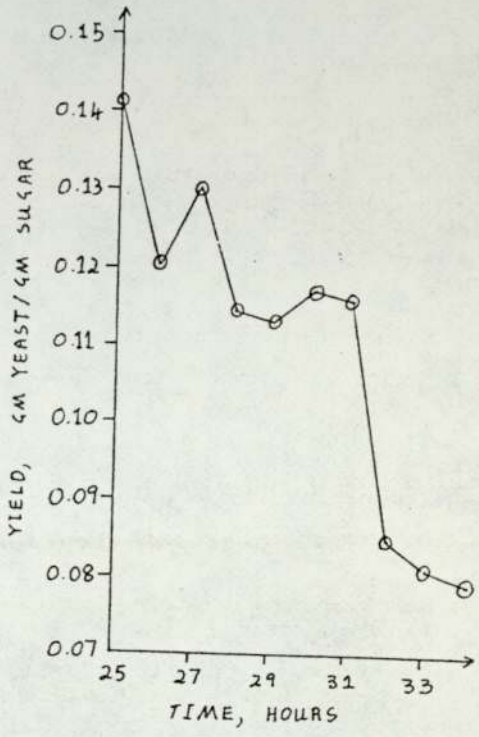


GRAPH 4.11 YIELD AND MAXIMUM SPECIFIC GROWTH RATE VERSUS TIME INTO FERMENTATION, EXPT. NO. 8.

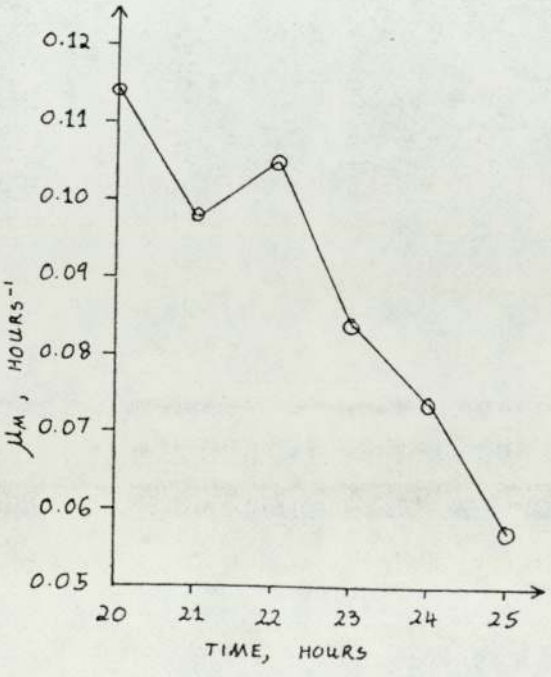
YIELD - WET YEAST BASIS



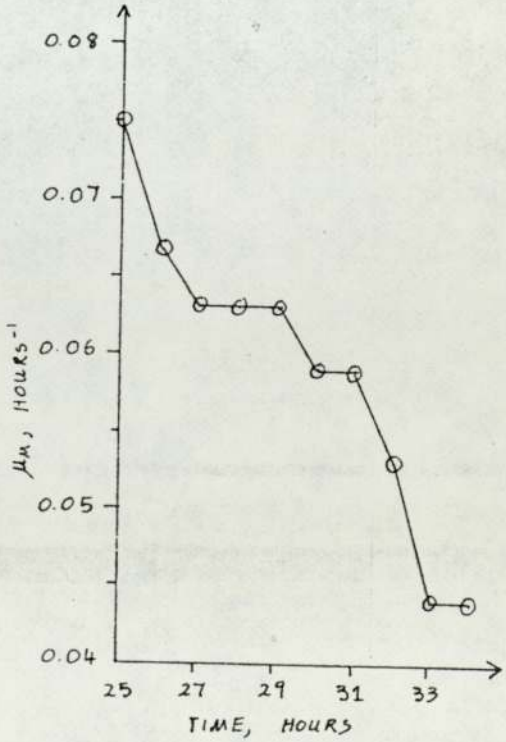
YIELD - DRY YEAST BASIS



μ_M - WET YEAST BASIS



μ_M - DRY YEAST BASIS



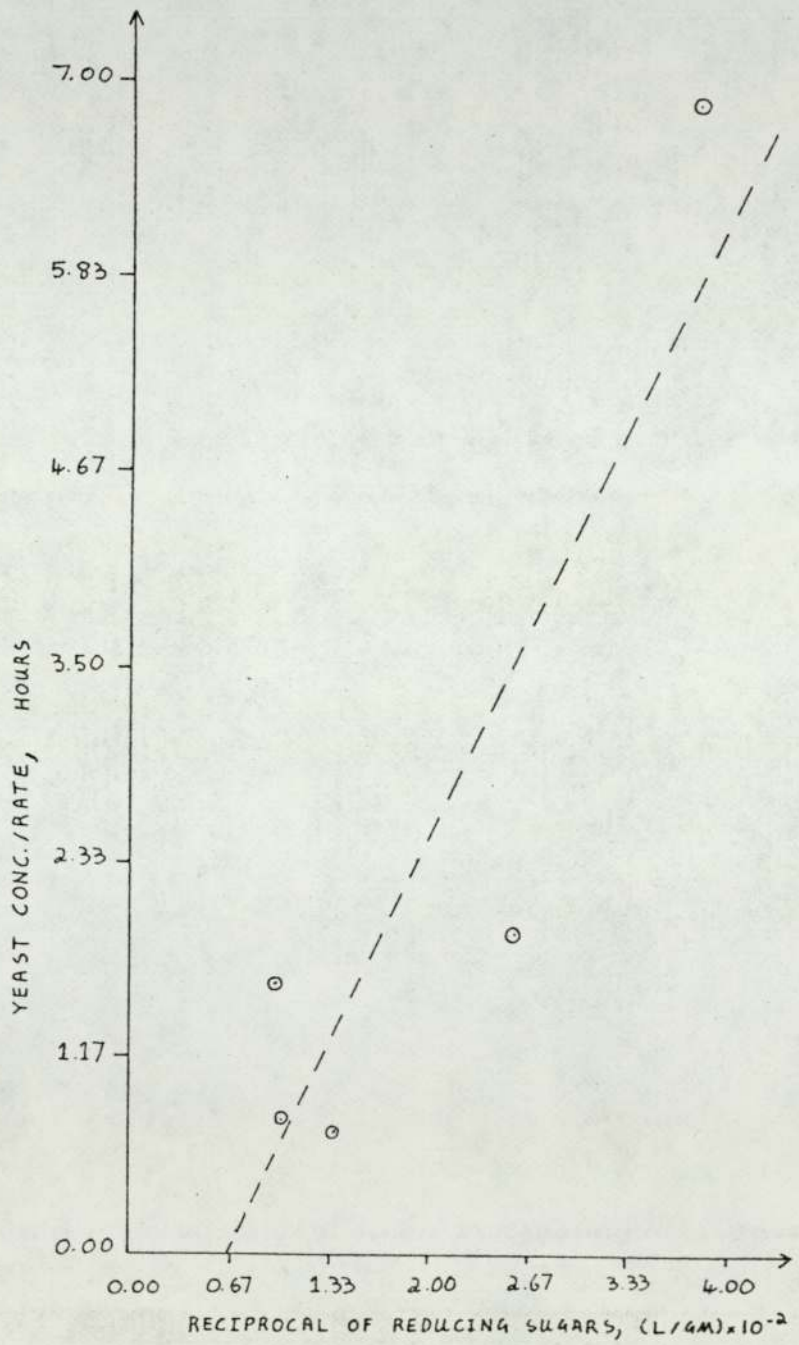
APPENDIX 4.3

Graphs 4.12 - 4.17

Linear testing of the Monod model - some typical graphs

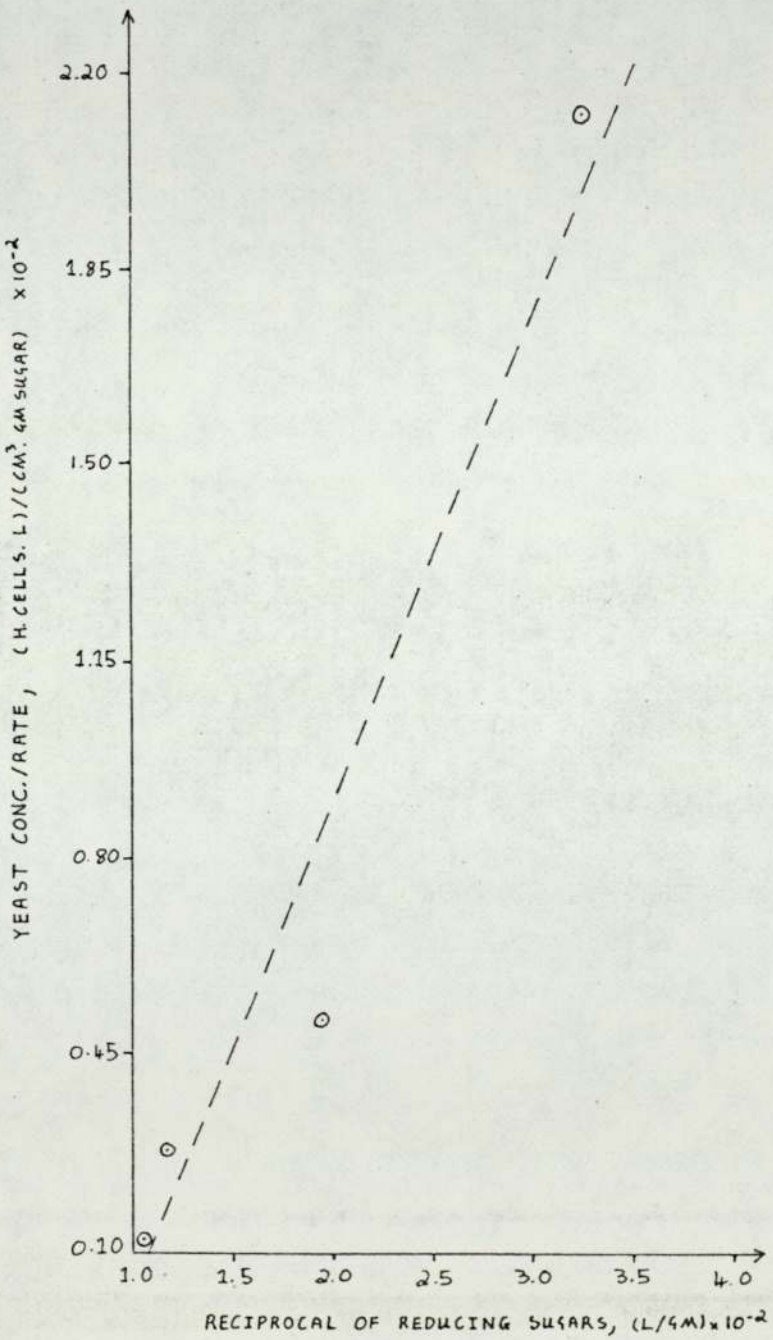
GRAPH 4.12 RATE OF REACTION VERSUS REDUCING SUGARS, EXPT. NO. 1.

REGRESSION COEFFICIENT = 0.737



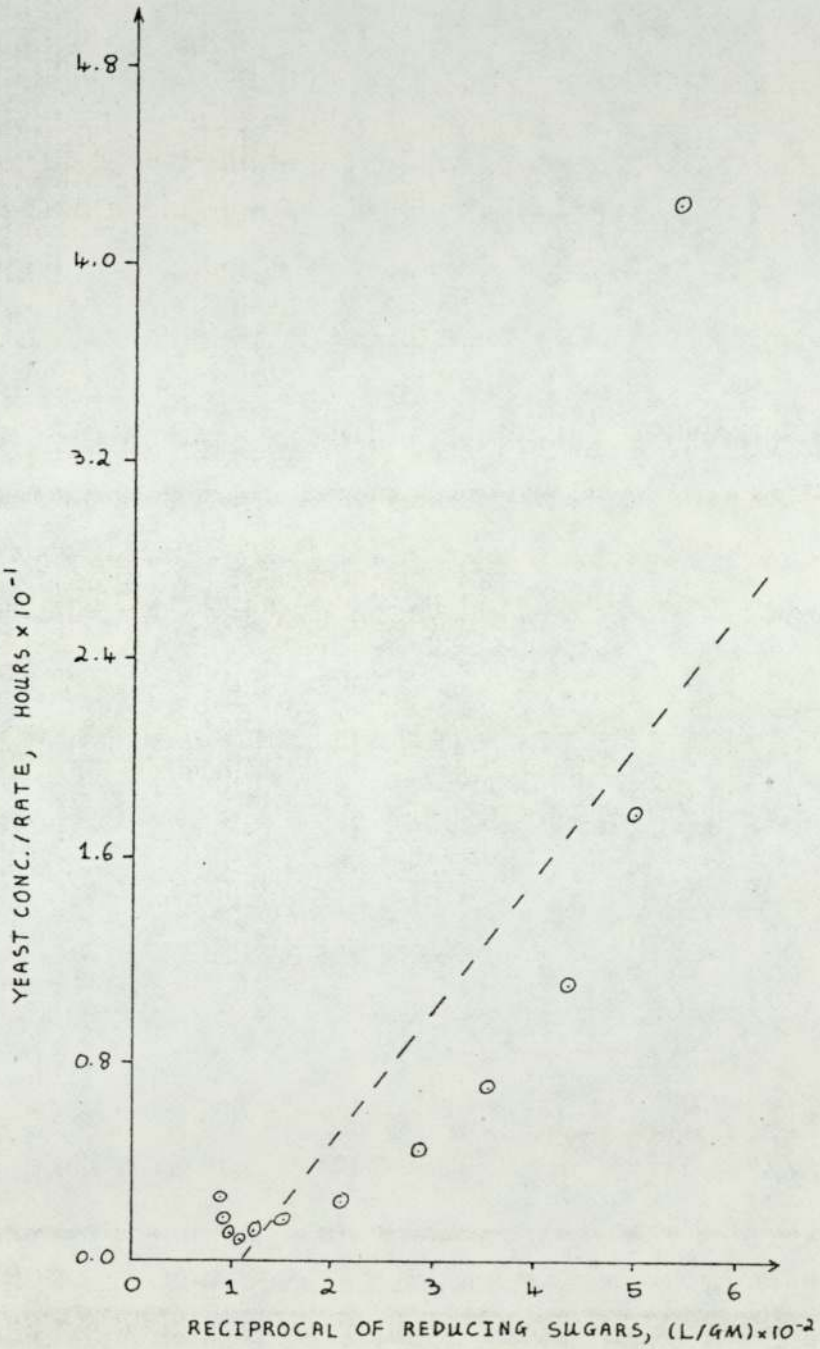
GRAPH 4.13 RATE OF REACTION VERSUS REDUCING SUGARS, EXPT. NO. 4.

REGRESSION COEFFICIENT = 0.917



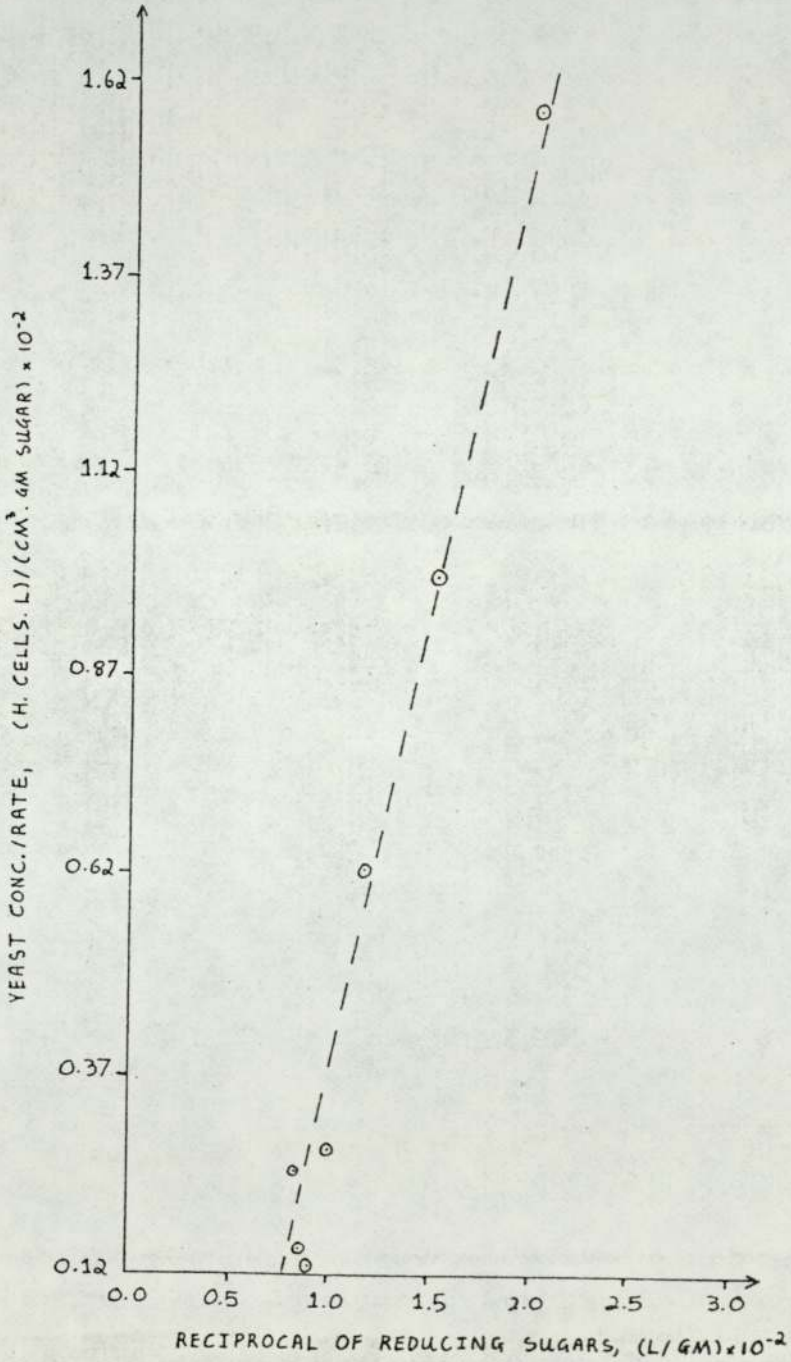
GRAPH 4.14 RATE OF REACTION VERSUS REDUCING SUGARS, EXPT. NO. 5.

REGRESSION COEFFICIENT = 0.654



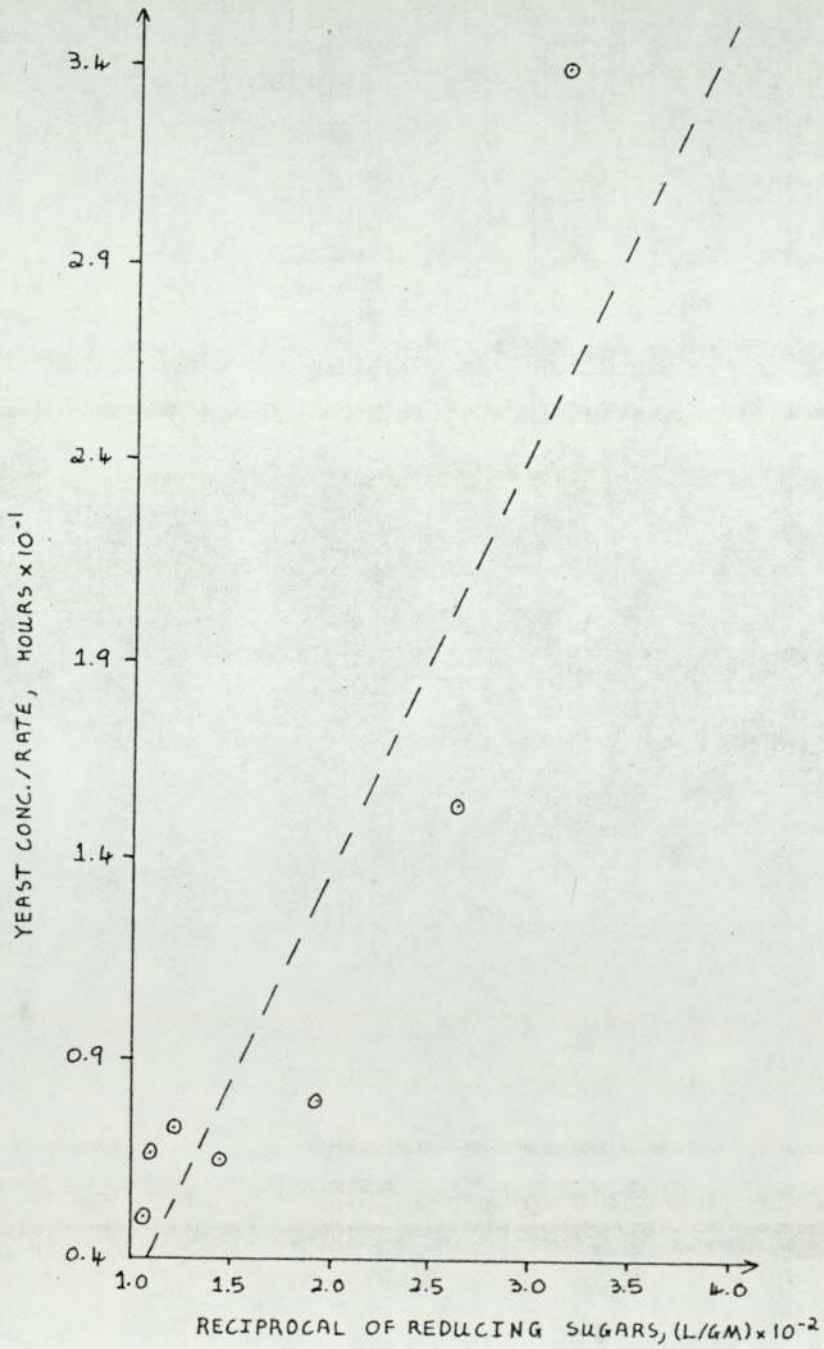
GRAPH 4.15 RATE OF REACTION VERSUS REDUCING SUGARS, EXPT. NO. 5.

REGRESSION COEFFICIENT = 0.974



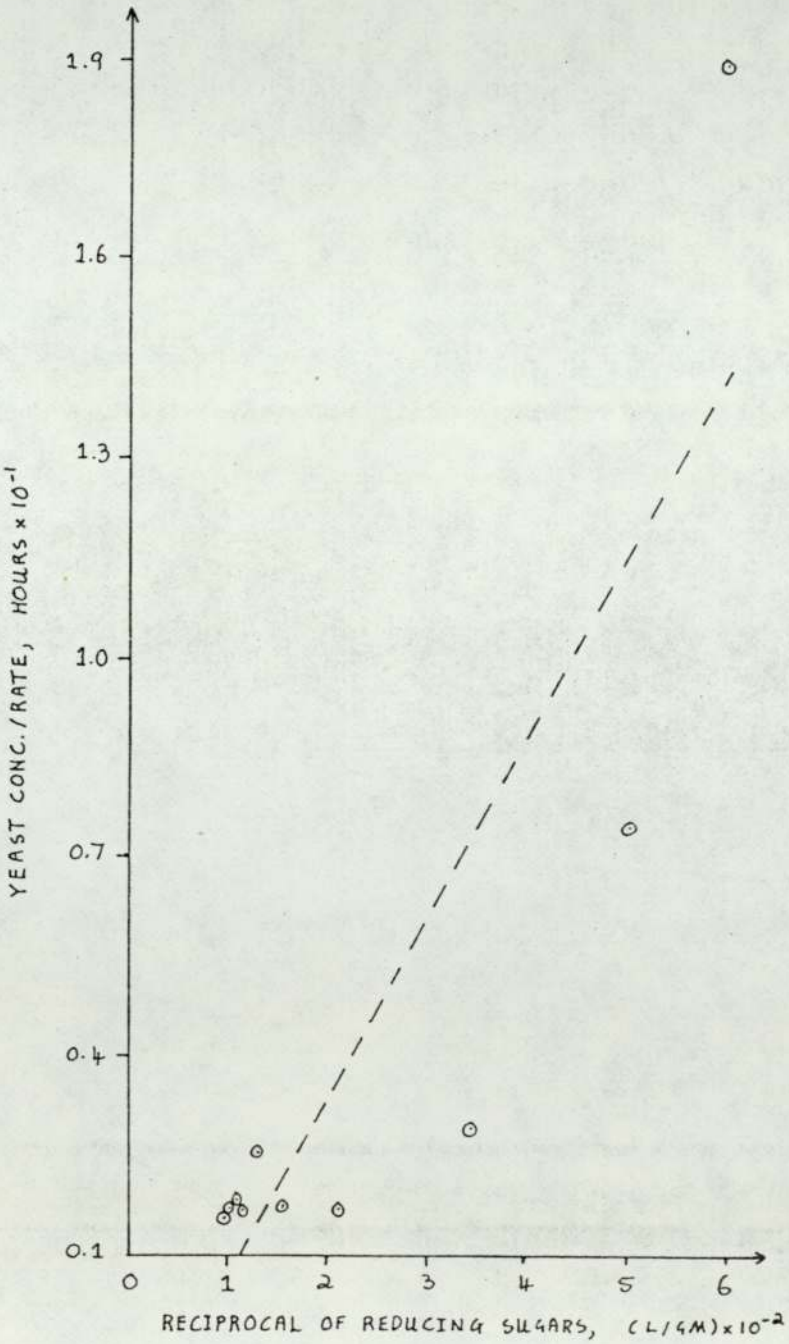
GRAPH 4.16 RATE OF REACTION VERSUS REDUCING SUGARS, EXPT. NO. 6.

REGRESSION COEFFICIENT = 0.764



GRAPH 4.17 RATE OF REACTION VERSUS REDUCING SUGARS, EXPT. NO. 8.

REGRESSION COEFFICIENT = 0.714



APPENDIX 4.4

The Runge-Kutta Stepwise Method of Integration

Given initial values and a specific step length the Runge-Kutta fourth order method calculates a series of constants. A weighted mean of these constants is taken to determine the $n^{th}+1$ value.

$$\frac{dX}{dt} = F(X,S) \qquad \frac{ds}{dt} = G(X,S)$$

$$K_1 = \Delta t F(X_n, S_n) \qquad m_1 = \Delta t G(X_n, S_n)$$

$$K_2 = \Delta t F\left(X_{n+\frac{K_1}{2}}, S_{n+\frac{m_1}{2}}\right) \qquad m_2 = \Delta t G\left(X_{n+\frac{K_1}{2}}, S_{n+\frac{m_1}{2}}\right)$$

$$K_3 = \Delta t F\left(X_{n+\frac{K_2}{2}}, S_{n+\frac{m_2}{2}}\right) \qquad m_3 = \Delta t G\left(X_{n+\frac{K_2}{2}}, S_{n+\frac{m_2}{2}}\right)$$

$$K_4 = \Delta t F(X_{n+K_3}, S_{n+m_3}) \qquad m_4 = \Delta t G(X_{n+K_3}, S_{n+m_3})$$

$$X_{n+1} = X_{n+\frac{1}{6}} \cdot (K_1+2K_2+2K_3+K_4)$$

$$S_{n+1} = S_{n+\frac{1}{6}} \cdot (m_1+2m_2+2m_3+m_4)$$

APPENDIX 4.5

A Computer Program for Mathematical Modelling of the Batch Beer Fermentation

```
  5 DIM A(90),B(90),C( 15),D( 15),F( 15),G( 15)
 10   K1=.16
 15   K2=25
 20   K3=.17
 25   K4=25
 30   K5=. 1E-01
 35   K6=.7E-01
 40   J=1
 45   CALL (1,T)
 50   S=95
 55   B=.9
 60   R=4
 65   H=.1
 70   E=80
 75   P=1
 80   IF B>=40 THEN 95
 85   D2=K2*S*B/(K4+S)
 90   GOTO 100
 95   D2=(K3*S*B/(K4+S))-K6*B
100   CALL (1,P,E,F1,F2)
105   IF F2=1 THEN 130
110   A(J)=T
115   B(J)=B
120   S(J)=S
125   J=J+1
130   IF F1=2 THEN 190
135   CALL (3,T,H,R)
140   CALL (4,B,D2)
145   IF S<=20 THEN 160
150   D1=-K1*S*B/(K2+S)
155   GOTO 165
160   D1=-K5*S*B/(K2+S)
165   CALL (4,S,D1)
170   GOTO 80
190   CALL (5)
195   CALL (6,-10,100,-10,140)
200   CALL (7,2,0,0,E3)
205   CALL (7,1,0,120,E3)
210   CALL (7,2,-2,0,E3)
220   CALL (8,48)
222   CALL (7,2,-3,20,E3)
224   CALL (8,50)
226   CALL (8,48)
228   CALL (7,2,-3,40,E3)
230   CALL (8,52)
232   CALL (8,48)
234   CALL (7,2,-3,60,E3)
236   CALL (8,54)
238   CALL (8,48)
```

```
240  CALL (7,2,-3,80,E3)
242  CALL (8,56)
244  CALL (8,48)
246  CALL (7,2,-4,100,E3)
248  CALL (8,49)
250  CALL (8,48)
252  CALL (8,48)
254  CALL (7,2,-4,120,E3)
256  CALL (8,49)
258  CALL (8,50)
260  CALL (8,48)
270  CALL (7,2,0,0,E3)
300  CALL (7,1,80,0,E3)
310  CALL (7,2,0,-5,E3)
312  CALL (8,48)
314  CALL (7,2,20,-5,E3)
316  CALL (8,50)
318  CALL (8,48)
320  CALL (7,2,40,-5,E3)
322  CALL (8,52)
324  CALL (8,48)
326  CALL (7,2,60,-5,E3)
328  CALL (8,54)
330  CALL (8,48)
332  CALL (7,2,80,-5,E3)
334  CALL (8,56)
336  CALL (8,48)
350  CALL (7,2,0,0,E3)
400  CALL (6,-10,100,-5,75)
410  CALL (7,2,80,0,E3)
420  CALL (7,1,80,60,E3)
450  CALL (7,2,81,0,E3)
452  CALL (8,48)
454  CALL (7,2,81,15,E3)
456  CALL (8,49)
458  CALL (8,53)
460  CALL (7,2,81,30,E3)
462  CALL (8,51)
464  CALL (8,48)
466  CALL (7,2,81,45,E3)
468  CALL (8,52)
470  CALL (8,53)
472  CALL(7,2,81,60,E3)
474  CALL (8,54)
476  CALL (8,48)
500  READ N
505  DATA 7
510  FOR I=1,N
515  READ C(I),D(I)
520  CALL (7,2,C(I),D(I),E3)
522  CALL (8,120)
525  NEXT I
530  DATA 0,.8409,19.25,19.89
535  DATA 24.33,34.477,48.51.614
540  DATA 65.66,58.731,71.91,56.71
545  DATA 80,56.6
570  CALL (6,-10,100,-10,140)
```

```
575  READ M
580  DATA 8
585  FOR I=1,M
590  READ F(I),G(I)
595  CALL (7,2,F(I),G(I),E3)
598  CALL (8,42)
600  NEXT I
605  DATA 0,97,10,95.5,20,90.5
610  DATA 30,75.5,40,29.2,50,16.3
615  DATA 60,14.8,70,14.4
650  FOR J=1,80
655  CALL (6,-10,100,-5,75)
670  CALL (7,2,A(J),B(J),E3)
675  CALL (7,1,A(J+1),B(J+1),E3)
680  CALL (6,-10,100,-10,140)
690  CALL (7,2,A(J),S(J),E3)
695  CALL (7,1,A(J+1),S(J+1),E3)
700  NEXT J
705  FOR K=1,6
710  READ K
715  CALL (8,K)
720  NEXT K
725  DATA 7,7,7,7,7,7
750  CALL (7,2,15,130,E3)
755 FOR J=1,35
760  READ Q
765  CALL (8,Q)
770  NEXT J
775  DATA 89,69,65,83,84
780  DATA 32,65,78,68,32
785  DATA 83,85,71,65,82,83,32
790  DATA 67,79,78,67,69,78
795  DATA 84,82,65,84,73,79,78
800  CALL (7,2,24,122,E3)
805  CALL (8,7)
810  PRINT "VERSUS TIME"
850  FOR J=1,25
855  CALL (7,2,-6,(J+39),E3)
860  READ V
865  CALL (8,V)
870  NEXT J
875  DATA 76,32,47,32,71,32,32
880  DATA 32,32,115,32,32,114,32,32
885  DATA 97,32,32,103,32,32,117
890  DATA 32,32,83
900  CALL (7,2,32,-9,E3)
905  FOR J=1,11
910  READ W
915  CALL (8,W)
920  NEXT J
925  DATA 84,105,109,101,32,32
930  DATA 40,72,82,83,41
950  FOR J=1,24
955  CALL (7,2,85,(J+28),E3
960  READ W1
965  CALL (8,W1)
```

```
970  NEXT J
975  DATA 76,32,32,47,32,32,71
980  DATA 32,32,32,32,116,32,32
985  DATA 115,32,32,97,32,32
986  DATA 101,32,32,89
990  CALL (9)
992  STOP
994  END
```

APPENDIX 5.1

Continuous Fermentations - Experiment Data Sheets

EXPERIMENT NO. 11

TIME INTO FERMENTATION Hours	AIR FLOW AT S.T.P. CM^3/MIN	MEDIUM FLOW CM^3/MIN	COMMENTS
0	56.3	-	Inoculum with CFCC54
24	56.3	-	Slight head of foam
48	56.3	-	Fermentation occurring
72	56.3	-	Vigorous fermentation apparent
96	56.3	-	
120	47.2	34.2	Wort flow started. Effluent P.G.=8.0
144	47.2	34.2	
168	26.6	34.2	Yeast conc. visibly increased
192	26.6	34.2	Yeast plug forming
216	26.6	34.2	Effluent P.G. = 8.0
240	26.6	34.2	Yeast Plug approx. 30 cm deep
264	26.6	-	Fermenter shut-down
288	26.6	-	
312	21.9	28.0	Tower started-up satisfactorily
336	21.9	28.0	
360	21.9	28.0	Effluent P.G. = 5.0
384	21.9	28.0	Yeast plug constant at 30 cm deep
408	19.2	28.0	Yeast plug constant. Effluent P.G. = 5.0
432	18.2	42.5	Medium flow-rate increased
456	18.2	42.5	Yeast plug expanded by approx. 5 cm.
480	18.2	42.5	Wort P.G. = 49. Beer P.G. = 5.0
504	18.2	42.5	
528	18.2	42.5	Yeast plug constant
552	18.2	42.5	Wort P.G. = 48.5 Beer P.G. = 5.0
576	18.5	42.5	
600	18.5	42.5	Yeast conc. profile constant
624	18.5	42.5	
648	17.3	42.5	
672	17.8	42.5	Run terminated

EXPERIMENT NO. 12

TIME INTO FERMENTATION	AIR FLOW AT S.T.P.	MEDIUM FLOW	COMMENTS
Hours	CM^3/MIN	CM^3/MIN	
0	420	-	Innoculation with CFCCl. Excess foaming
24	309	-	Foam stable. Some fermentation
48	309	-	Visible yeast flocs
72	184	-	Yeast stratification
96	63.8	8.7	Medium flow-rate started
120	63.8	8.7	Yeast plug forming
144	29.7	8.7	Larger yeast flocs on air flow reduction
168	29.7	8.7	Yeast plug approx. 20 cm deep
192	29.7	8.7	Yeast plug increasing
216	22.7	16.2	Medium flow-rate increase
240	22.7	16.2	Plug increase. Effluent P.G. to 9.0 from 8.5
264	22.7	16.2	Plug increase. Effluent P.G. = 8.0
288	22.7	16.2	
312	22.7	16.2	Yeast plug approx. 60 cm deep
336	22.7	16.2	Beer P.G. = 7.5. Wort P.G. = 41.0
360	20.4	16.2	
384	17.3	41.5	Medium flow-rate increase
408	17.3	41.5	Effluent P.G. = 100
432	17.3	41.5	Yeast conc. profile constant
456	17.3	41.5	Effluent P.G. = 9.0
480	19.0	41.5	
504	19.0	41.5	
528	19.0	41.5	Beer P.G. = 9.0. Wort P.G. = 40.7
552	19.0	41.5	Run terminated

EXPERIMENT NO. 13

TIME INTO FERMENTATION Hours	AIR FLOW AT S.T.P. CM^3/MIN	MEDIUM FLOW CM^3/MIN	COMMENTS
0	563	-	Inoculation with CFCC39
24	563	-	
48	600	-	Powdery, brown yeast flocs
72	34.4	10.6	Visible yeast flocs on air reduction
96	14.1	10.6	Yeast becoming pale, yellowy from sandy, brown
120	13.9	10.6	Yeast plug forming
144	13.9	10.6	Yeast plug increase
168	13.9	10.6	Yeast plug approx. 40 cm deep
192	11.0	15.9	Medium flow-rate increase
216	11.0	15.9	Wort P.G. = 48.0 Beer P.G. = 8.0
240	11.0	15.9	Yeast plug approx. 60 cm deep
264	11.3	15.9	
288	11.8	33.3	Medium flow-rate increase
312	11.2	33.3	Effluent P.G. rise to 9.5
336	11.2	33.3	Beer P.G. fall back to 8.0
360	11.2	33.3	
384	10.8	33.3	
408	10.8	42.0	Slight medium flow-rate increase
432	8.9	42.0	
456	8.9	42.0	Yeast conc. profile constant
480	9.3	42.0	
504	9.3	42.0	
528	-	20.2	Air discontinued. Yeast plug forced up tower and wort flow reduced because of wash-out. Tower running "bumpy".
552	-	20.2	Tower running more smoothly. Yeast plug risen slightly. Effluent P.G. = 9.5
576	-	20.2	Yeast conc. decreasing and yeast looks unhealthy
600	-	20.2	Effluent P.G. = 15.0. Yeast powdery and brown
624	-	14.7	Medium flow-rate reduced
648	-	14.7	Effluent P.G. = 12.5
672	-	14.7	Effluent P.G. = 16.0. Yeast brown and sludgy. Run terminated.

EXPERIMENT NO. 14

TIME INTO FERMENTATION Hours	AIR FLOW AT S.T.P. CM^3/MIN	MEDIUM FLOW CM^3/MIN	COMMENTS
0	914	-	Inoculation with CFCCl. Excessive foaming
24	600	-	
48	600	-	
72	600	-	Small, sandy, brown yeast flocs
96	524	-	
120	524	-	
144	524	-	Yeast increase, though still "loose"
168	302	15.7	Effluent P.G. = 30.5 on medium start-up
192	189	10.6	Medium flow-rate reduced. Effluent P.G. = 24.0 Medium P.G. = 59.5
216	20.2	10.6	Yeast sandy, brown and no yeast stratification
240	20.2	10.6	Medium P.G. = 60.0. Effluent P.G. = 16.0
264	23.4	15.2	No yeast plug. Effluent P.G. = 16.0
288	23.4	15.2	
312	23.4	20.4	Medium flow-rate increase
336	17.6	20.4	Yeast showed no signs of forming a plug
360	16.8	20.4	Medium P.G. = 59.5. Effluent P.G. = 15.5
384	21.7	24.3	Slight medium flow-rate increase
408	21.7	24.3	
432	21.7	27.5	Unsupplemented medium on-line
456	21.7	27.5	Yeast appears more creamy and flocculent. Medium P.G. = 59. Effluent P.G. = 14.5
480	20.7		Yeast plug forming and larger flocs circulating
504	21.7	27.5	
528	21.7	27.5	Yeast plug approx. 35 cm deep
552	21.7	27.5	Medium P.G. = 60.0. Effluent P.G. = 14.5
576	18.3	27.5	Yeast plug approx. 55 cm deep
600	20.5	27.5	
624	20.5	27.5	
648	20.5	27.5	Yeast conc. profile constant
672	20.5	27.5	Medium P.G. = 60.5. Effluent P.G. = 14.0
696	20.5	35.5	Medium flow-rate increase
720	23.4	35.5	Medium P.G. = 61.0. Effluent P.G. = 14.0
744	23.4	35.5	
768	21.9	35.5	Yeast conc. profile remaining constant
792	21.9	35.5	Medium P.G. = 59.5. Effluent P.G. = 14.0

Continued overleaf

EXPERIMENT NO. 14 (Contd)

TIME INTO FERMENTATION	AIR FLOW AT S.T.P.	MEDIUM FLOW	COMMENTS
Hours	CM^3/MIN	CM^3/MIN	
816	21.9	35.5	
840	21.9	35.5	
864	19.9	75.2	Yeast plug forced approx. 55 cm up the fermenter. Large amount of yeast washed over and effluent P.G. rose to 19.0 before falling back to 14.0

EXPERIMENT NO. 15

TIME INTO FERMENTATION	AIR FLOW AT S.T.P.	MEDIUM FLOW	COMMENTS
Hours	CM^3/MIN	CM^3/MIN	
0	893	-	Inoculation with CFCCl
24	893	-	
48	442	-	Small yeast flocs visible on air reduction
72	442	-	Yeast conc. increase
96	164	15.5	Medium flow-rate started
120	19.4	15.5	Yeast settling on air reduction
144	19.4	15.5	Yeast plug increasing. Medium P.G. = 55.0. Effluent P.G. = 16.0
168	19.4	15.5	
192	18.9	15.5	Yeast plug approx. 25 cm deep
216	18.9	15.5	Yeast conc. increase
240	18.9	15.5	Medium P.G. = 54.5. Effluent P.G. = 15
264	18.9	15.5	Yeast plug approx. 40 cm deep
288	18.9	15.5	
312	18.4	27.3	Medium flow-rate increase
336	18.4	27.3	Medium P.G. = 55.5. Effluent P.G. = 15.0
360	18.4	27.3	
384	18.4	27.3	
408	18.9	27.3	Medium P.G. = 54.5. Effluent P.G. = 15.0
432	18.9	27.3	
456	18.6	24.8	Unautoclaved medium started
480	18.6	24.8	Effluent P.G. = 15.0. Yeast conc. profile stable
504	18.6	24.8	
528	18.6	24.8	Effluent P.G. = 15.0 Yeast stable
552	20.4	-	Medium flow had to be discontinued due to the medium being infected with an organism that caused it to coagulate

APPENDIX 5.2

Analysis of Beet Molasses supplied by British Sugar Corporation Limited.

Gardiner-Farmiloe dry substance,%	81.6
Dry substance (K.F.),%	82.5
Refractometric dry substance,%	81.5
Calculated Brix	85.0
Apparent Purity	61.0
True Purity	60.9
Direct Polarisation	51.9
Sucrose,%	49.7
Raffinose $5H_2O$,%	1.6
Reducing Sugars,%	1.3
Total sweetening matter,%	54.7
Pol/ash ratio	4.5
pH	6.0

Results calculated on dry substance

Total nitrogen,%	2.4
Sulphated ash,%	14.3
Sulphite (SO_2), mg/kg	590
Sulphate (SO_4),%	0.64
Chloride (Cl),%	0.91
Magnesium (Mg),%	0.009
Calcium (Ca),%	0.066
Sodium (Na),%	0.94
Potassium (K),%	4.45
Copper (Cu), mg/kg	15.8
Colour	35900

NOMENCLATURE

a	= a constant	$ML^{-3}.L^{-1}$
A	= cross sectional area of column	L^2
A_f	= total free area of distributor plate	L^2
A_o	= orifice cross sectional area	L^2
C_D	= coefficient of discharge	
d_p	= diameter of a yeast floc	L
D_o	= orifice diameter	L
f	= friction factor	
g	= gravitational acceleration	LT^{-2}
G	= fluid mass flow-rate	MT^{-1}
H	= pressure drop in terms of loss of head of the flowing fluid	L
$K1 \rightarrow K6$	= constants in the batch fermentation model; defined in the text	
l	= reactor length	L
n	= Richardson-Zaki equation index	
N	= number of orifices	
P_1	= pressure below the distributor plate	$MLS^{-2}L^{-2}$
P_2	= pressure above the distributor plate	$MLS^{-2}L^{-2}$
P	= orifice pitch	L
ΔP	= pressure drop	$MLS^{-2}L^{-2}$
Q	= fluid volumetric flow-rate	L^3T^{-1}
r	= ratio of pressures above and below the distributor plate (P_2/P_1)	
Re_o	= orifice Reynolds number	
s, S	= sugar concentration	ML^{-3}
S^*	= limiting sugar concentration	ML^{-3}

t	= time	T
T	= orifice plate thickness (ch. 2)	L
	= wort apparent residence time (ch. 6)	T
u_i	= interstitial fluid velocity	LT^{-1}
u_s	= fluid superficial velocity	LT^{-1}
u_T	= floc terminal velocity	LT^{-1}
V	= fermenter volume	L^3
V_c	= yeast cell volume	L^3
V_g	= fractional volume of the fermenter occupied by gas	L^3
x	= coefficient of adiabatic expansion (ch. 2)	
x, X	= yeast concentration	ML^{-3}
X^*	= limiting yeast concentration	ML^{-3}
Y	= expansion factor (ch. 2)	
	= yield constant (ch. 4)	

Greek Letters

β	= ratio of orifice area to column area (A_o/A)	
ε	= voidage in the fermenter	
μ	= fluid viscosity	$MLTL^{-2}T^{-2}$
μ	= organism specific growth rate	T^{-1}
μ_M	= maximum specific growth rate of an organism	T^{-1}
ρ	= fluid density	ML^{-3}
ρ_p	= density of a yeast floc	ML^{-3}

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