THE UNIVERSITY OF ASTON IN BIRMINGHAM

(Department of Chemical Engineering)

MATHEMATICAL MODELLING OF TOWER FERMENTATION PROCESSES

by

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being a thesis submitted in support of an application

for the degree of Doctor of Philosophy.

February 1975

SUMMARY

The Fermentation Industries are currently concerned with a number of large-scale processes, including single cell protein production, effluent treatment, and the production of alcoholic beverages. Successful design and optimisation of these new systems is becoming increasingly dependent upon the availability of mathematical models for use in simulation studies.

The research described in the thesis has been concerned with the analysis and modelling of some of these microbial processes, in particular those involving tower fermenters. Because of the nature of such processes the work has involved considerable interaction between the basic disciplines of chemical engineering and microbiology.

Models of specific aspects of tower fermentation processes are developed in the earlier sections of the thesis, including:

(1) a model to describe liquid-phase mixing in bubble columns and tower fermenters;

(2) an empirical method for predicting mixing parameters for both the backflow stirred tanks model and the axially-dispersed plug flow model;

(3) a theoretical model describing oxygen mass transfer in bubble columns and tower fermentation systems;

(4) a detailed review of deterministic kinetic models for microbial processes;

(5) a brief review of continuous culture theory and mathematical descriptions of transient microbial behaviour; and

(6) analysis and modelling of the batch beer fermentation process.

The application of these concepts to the development of comprehensive models for tower fermentation systems is considered in the latter part of the thesis. First, a model describing a continuous beer fermenter is developed, and second, consideration is given to the design and modelling of aerobic tower systems. These models have been useful in: (1) directing current experimental programmes, and (2) high-lighting areas where more research effort is needed. It is hoped that ultimately the models will be suitable for the scale-up, design and optimisation of tower fermentation systems.

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ACKNOWLEDGMENTS

The author wishes to express his thanks to:

(1) The Science Research Council and the University of Aston in Birmingham for financial assistance in the form of a Research Studentship.

(2) Professor G. V. Jeffreys and all the Staff of the Department of Chemical Engineering for their assistance; in particular, members of the Computer Group.

(3) Dr. E. L. Smith for his friendly and thoughtful supervision of this research project.

(4) Mr. R. A. Spensley for patient and stimulating discussions on microbiological and practical aspects of tower fermentation systems.

(5) Mr. S. D. Pannell, Dr. A. James, Dr. J. Shayegan-Salek, and Dr. S. D. J. Coote for supplying data and for offering constructive criticism of the mathematical models developed in this thesis.

(6) Dr. R. N. Greenshields, Mr. R. Cocker, Mr. A. W. Dowen, and all other members of the Tower Fermentation Research Group for their valuable suggestions throughout the course of this work.

(7) Mrs Kay Davies for typing this thesis.

SECTION 1

INTRODUCTION

Figures

REFERENCES

1.1 FERMENTATION AND THE DEVELOPMENT OF BIOCHEMICAL ENGINEERING

Fermentation can be defined as "any purposeful microbial activity", though GADEN (1955) - from an engineering viewpoint - prefers the definition: "chemical reactions catalysed by enzyme systems which, in turn, are produced during the growth of microorganisms". Since World War II the fermentation industry has assumed great importance due to the discovery and subsequent large-scale manufacture of antibiotics such as penicillin and streptomycin. Other fermentation products of current economic importance include alcoholic beverages, cheeses, yeast, flavouring agents, organic acids, amino acids, bacterial insecticides, steroids, vitamins, and enzymes.

The development of such processes has resulted in the birth of biochemical engineering - a "marriage" of microbiology, biochemistry, and chemical engineering. Biochemical engineering is a technology that applies science to the design, construction, and operation of biological process systems. Within this area of technology, the role of the engineer is becoming increasingly important due to the switch from batch to semi-continuous and fully continuous processes. Biochemical engineering is now being applied to the development of large-scale waste treatment and biomass production systems as well as to traditional fermentation processes. Currently a number of 100,000 t.p.a. Single Cell Protein (S.C.P.) plants are being designed and constructed in many parts of the world to alleviate food shortages. The need for efficient design, control, and optimisation procedures is now greater than ever before.

Concurrently with the progress in the fermentation industry, there

has been a rapid advance in computer technology during the last twenty years. This has resulted in the development of sophisticated design, control, and optimisation procedures for chemical plants. Such techniques are now being applied to biochemical processes, as can be seen by the recent spate of publications on this topic. Current interest in continuous fermentation systems also suggests that computers will play a more important role in the future of the fermentation industry. These developments in biochemical engineering and computer technology have created a need for mathematical models of fermentation processes.

1.2 MATHEMATICAL MODELS

Mathematical models are often used to predict the behaviour of a system. They may be based on either theoretical or empirical knowledge of a process, or a mixture of both. In the case of biochemical systems, complete understanding of the physical, chemical, and biological mechanisms involved is unlikely and, consequently, empirical relationships abound. Such models are therefore simplifications of the systems they represent and have limited applicability since not every factor can be considered. According to BOX and HUNTER (1962): "No model can give a precise description of what happens. A working theoretical model, however, supplies information on the system under study over important ranges of the variables by means of equations which reflect at least the major features of the mechanism".

Models are complementary to experiments and can never be a complete substitute. Fermentation experiments are expensive and time-consuming, and therefore computer simulations of such systems can save both time and

money: but all models contain parameters which must be estimated experimentally. Careful planning (i.e. experimental design) can reduce the number of experiments required, but it must be remembered that a model is only as good as the data employed for parameter estimation.

Simplicity is a key feature of many mathematical models. Whilst recognising that the more complex a model is the more accurate it is likely to be (i.e. more factors are taken into account), it should also be realised that a complex model requires estimation of a greater number of parameters. Most models, therefore, are likely to be "tailor-made" for a particular process and, in general, they are not applicable to a wide range of systems.

Currently there is emphasis upon the use of mathematical models for the design, control, and optimisation of fermentation processes. Models, though, are also useful in research and can provide insight into, for instance, the behaviour of microorganisms. An example of this is the batch beer sugar utilisation model described in Section 6.

In conclusion, mathematical models can be used as a rapid and inexpensive alternative to part of an experimental programme, but before use their limitations must be fully appreciated. Simulation studies are also a useful means for aiding the design and optimisation of fermentation processes, particularly in cases where process control is an integral part of the system.

1.3 SIMULATION, OPTIMISATION, AND CONTROL OF BIOCHEMICAL PROCESSES

Many of the mathematical models developed in the field of biochemical engineering have dealt exclusively with microbial kinetics. These models

will be outlined and discussed in Sections 4 and 5. Currently, though, there is much interest in models encompassing total fermentation systems. Such models are now being used for the design, optimisation, and control of fermentation processes. An excellent review by NYIRI (1972) discusses the applications of computers in biochemical engineering. Many of the publications cited are concerned with simulation of biochemical systems; others refer to both off-line and on-line computer control of fermentation processes. Recent international symposia (4th International Fermentation Symposium, Kyoto, Japan, March 19-25, 1972; 1st International Symposium on Advances in Microbial Engineering, Marianske Lazne, Czechoslovakia, August 28 - September 1, 1972; 4th International Conference on Global Impacts of Applied Microbiology, Sao Paolo, Brazil, July 22-27, 1973) have also reflected the current interest in mathematical analysis of biological processes. Some of the significant publications regarding mathematical applications to biochemical systems are reviewed briefly in the following sections. For clarity the topics of simulation, optimisation, and control are treated separately.

Simulation

Possibly the best paper to date regarding simulation of fermentation systems is that of CALAM et alia (1971), who relate the principles behind the modelling of biochemical processes with particular reference to the griseofulvin fermentation. FREDERICKSON et alia (1970) have also published an excellent paper describing the construction of models of fermentation processes; their review of kinetic models is of particular merit. Systems engineering techniques have been used by

YOUNG et alia (1969, 1970, 1973) to establish a dynamic mathematical model of a chemostat. Other papers of note regarding process simulation are those of MAXON et alia (1966), KOGA et alia (1967), and CHEN et alia (1968).

Optimisation

During recent years many publications have been concerned with the optimisation of fermentation processes. One of the earliest was that of FAN and WAN (1963), who applied the discrete maximum principle to a stagewise biochemical reactor system to optimise metabolite production. WEN et alia (1967) extended FAN'S work to include recycle.

Many papers have been concerned with optimising the penicillin process. CONSTANTINIDES et alia (1970, 1972) determined optimum temperature and pH profiles. CALAM and RUSSELL (1973) and FISHMAN et alia (1972) were concerned with optimising penicillin yield by adjusting the pattern of substrate feed addition.

TOPIWALA (1972) has discussed the optimal design of fermentation systems and BLANCH and ROGERS (1972) have developed an economic function for the optimisation of the continuous Gramicidin S process. Optimum dilution rates for maximum productivity in hydrocarbon fermentations have been studied by ERICKSON et alia (1972c). The paper by SCHRÖDER and WEIDE (1972) concerned with the optimal medium composition for high growth rates of yeast is also worthy of note.

Control

One of the first publications regarding control of fermentation processes was that of FULD (1960), who discussed the use of pH and

temperature controllers in biochemical systems. Extending FULD'S work, HARRISON and HARMES (1972) considered the monitoring of all fermentation parameters and the consequent implications for computer control. A paper by CORRIEU et alia (1972) describes the use of the computer for data acquisition and handling in the fermentation industry. CORSO (1972) has developed an on-line monitoring and control system for a chemostat. Other papers discussing computer control include those of YAMASHITA et alia (1968), MYIRI et alia (1972), and FLYNN (1972).

Many publications have been concerned with the optimal control of fermentation processes, including those of HO and HUMPHREY (1970), MUZYCHENKO et alia (1972), and RYU and HUMPHREY (1973). Further utilisation of modern control theory is made by WEINRICH and LAPIDUS (1972), who describe a parameter adaptation control policy for a biochemical reactor, and by SVRCEK et alia (1974), who apply the Kalman filter state estimation technique to a chemostat system.

1.4 FERMENTER TYPES

The most common type of fermenter is the stirred tank reactor (S.T.R.) - a tank agitated by a suitable mixing device. An alternative to the S.T.R. is the tubular reactor. This can be operated in either a horizontal or vertical position, and may contain baffles or perforated plates. Sometimes a central shaft is fitted with paddles for agitation purposes. Other types of tubular reactors may contain packed or fluidised beds of insolubilised enzymes.

Fermentations may be carried out in either aerobic or anaerobic conditions. Aerobic fermentations require a constant supply of air or

oxygen and in such cases the fermenters must be equipped with aeration devices. In vertical tubular reactors air is generally bubbled into the fermentation broth through a perforated plate or sintered glass distributor. In other types of fermentation vessel the aeration device is often more complex. The amount of oxygen which can be supplied to the microorganisms is frequently a limiting factor in fermentation process systems: consequently, the design of the aeration device is of prime importance.

Fermentation processes may be batch, semi-continuous, or fully continuous. In recent years there has been a trend away from batch processes, even in perhaps the most conservative of the fermentation industries, the brewing industry. The technology resulting from the development of large-scale waste-treatment and biomass processes should benefit the whole of the fermentation industry and speed the switch from costly batch systems to more efficient continuous processes.

Continuous fermenters may be classified as either open or closed systems. An open system is one which allows the microbial phase to escape freely with the effluent medium. A closed system is so-called because the cells are artificially retained by the fermentation system. No system can be truly closed at steady state unless there is no growth of the microbial phase (e.g. insolubilised enzymes attached to porous solid particles in a tubular reactor).

Stirred tank reactors and horizontal tubular reactors are normally open systems. Sometimes, however, S.T.R.'s are fitted with a device which separates microorganisms from the effluent and returns them to the

fermenter: such systems are classified as partially-closed. Vertical tubular reactors also belong to this category: whilst they may appear to be truly open, there is generally a sedimentation effect which prevents some of the solid microbial phase from leaving the fermenter. This facet is an important feature of the tower fermenter - a special type of tubular reactor which is described in more detail below.

1.5 THE TOWER FERMENTER

The simplest form of tower fermenter is a vertical tube; substrate is introduced near the base and removed at the top. For aerobic operation sterile air may be introduced at the bottom through a perforated plate or sintered glass distributor. An expansion section may be fitted near the top of the tower fermenter to (1) reduce foaming, and (2) encourage retention of the microorganisms (i.e. by reducing liquid superficial velocity and thus suppressing fluidisation). To control temperature an attemporator jacket may be fitted around the column.

The tower fermenter was originally developed by the A.P.V. Company (Crawley, Sussex) for continuous beer production (see figure 7.1. p201). Early work with this system is described by KLOPPER et alia (1965). Commercial experience is summarised by AULT et alia (1969), and engineering aspects of the process by ROYSTON (1966a, 1966b) and SHORE and ROYSTON (1968). Publications by GREENSHIELDS and SMITH (1971) and SMITH and GREENSHIELDS (1972, 1974) describe the work carried out at Aston University with tower fermentation systems. A review by GREENSHIELDS and SMITH (1974) assesses commercial applications of the tower fermenter: besides continuous beer production, tower fermenters

have been used to manufacture alcohol, vinegar, citric acid, and biomass.

Systems similar to tower fermenters have been investigated by a number of other workers. KITAI et alia (1969a, 1969b, 1969c, 1969d, 1969e, 1970) and PROKOP et alia (1969) used a tower fermenter containing a number of perforated plates (i.e. compartmentalised). An elementary model of a multi-stage tubular system is reported by ERICKSON et alia (1972a, 1972b). FALCH and GADEN (1969, 1970) have used an agitated, counter-current, multi-stage device and developed a model of the system.

A comprehensive review of tubular fermentation systems is presented by JAMES (1973).

1.6 THE AIMS OF THIS RESEARCH

The Tower Fermentation Research Group (T.F.R.G.) at Aston University consists of two groups of research students: (1) Chemical Engineers working under Dr. E. L. Smith, and (2) Biological Scientists under the direction of Dr. R. N. Greenshields. The aim of the group is to assess and develop the potential of the tower fermenter. Tower fermentation systems have been operated both batchwise and continuously at Aston, and the processes investigated include metabolite production, biomass formation, and waste treatment (see figure 1.1). These systems have involved the use of pure cultures of yeasts, bacteria, and filamentous fungi, and also mixed species of microorganisms.

To date most of the development work with tower fermentation systems has been carried out entirely on an experimental basis with little or no regard paid to theoretical aspects. During the last few years T.F.R.G. has carried out much experimental work relating to various physical and

biochemical aspects of tower fermentation systems (see figures 1.1, 1.2). The aim of the author's work is to utilise the available data to develop mathematical models of fermentation processes, in particular those involving tower fermenters. It is hoped that these models will lead to a better understanding of the behaviour of tower fermentation systems. Ultimately, it is intended that the models developed will be useful for design, control, and optimisation purposes (see figure 1.3).

Research leading to the award of a Ph.D. degree is regarded by the author as training rather than "research for the sake of research". In addition, the thesis has been written with a view to being of immediate use to a chemical engineering graduate with no previous training in microbiology or biochemical engineering. For these reasons the bulk of the thesis is concerned with introducing the reader to various modelling concepts. Later sections in the thesis illustrate applications of these modelling concepts to real fermentation systems, in particular the tower fermenter and the brewing process. A scheme of the thesis is shown in figure 1.4 and explained below.

1.6.1 Description of Thesis Scheme

Individual sections of the thesis are presented as separate modules, each containing a list of contents, tables, figures, symbols, and references. Appendices are collected together and presented at the end of the thesis.

In the first section the reader is introduced to the subjects of fermentation, mathematical models, and simulation of biochemical processes. Sections 2 to 5 describe some of the concepts required to

model fermenter systems. These sections mainly consist of brief literature reviews, although some of the author's modelling work is included.

Section 2 briefly summarises mixing in bubble columns and tower fermenter systems. Two original contributions are included in this section: (1) a model describing backmixing patterns in bubble columns, and (2) a model for predicting backmixing coefficients in such systems.

Mass transfer of oxygen in fermentation systems is considered in Section 3. The author develops a model which describes oxygen mass transfer in tower fermenters with liquid backmixing.

Section 4 contains an extensive literature survey regarding deterministic kinetic models of microbial processes. A comprehensive description of the Monod model and its modifications is included, and means of incorporating process parameters (e.g. pH, temperature) into such models is discussed.

Continuous culture theory is described in Section 5, and publications containing mathematical expressions for transient behaviour in such systems are reviewed briefly. The author shows how a simple model can account for some of these effects.

Sections 6 to 8 describe the application of the modelling concepts to (1) the brewing process, and (2) the tower fermenter system. In Section 6 a model describing sugar utilisation during batch beer fermentation is developed. A system for the continuous production of beer in a tower fermenter is modelled in Section 7. Finally, the design and modelling of an aerobic tower fermenter system is discussed in Section 8.

Conclusions and recommendations for future work are presented in Section 9.

Figure 1.1 Tower Fermenter Applications

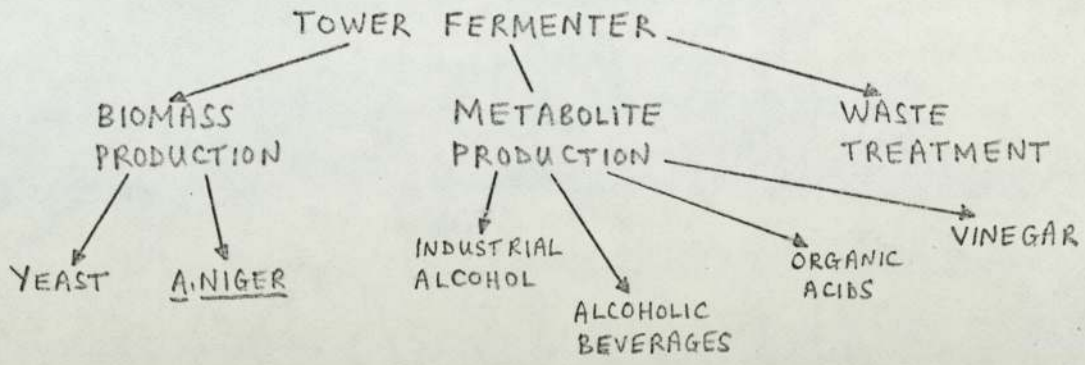


Figure 1.2 Engineering Aspects of Tower Fermenter Systems

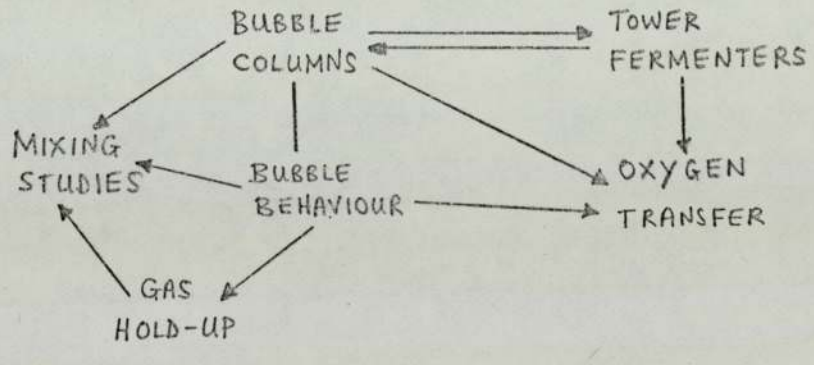
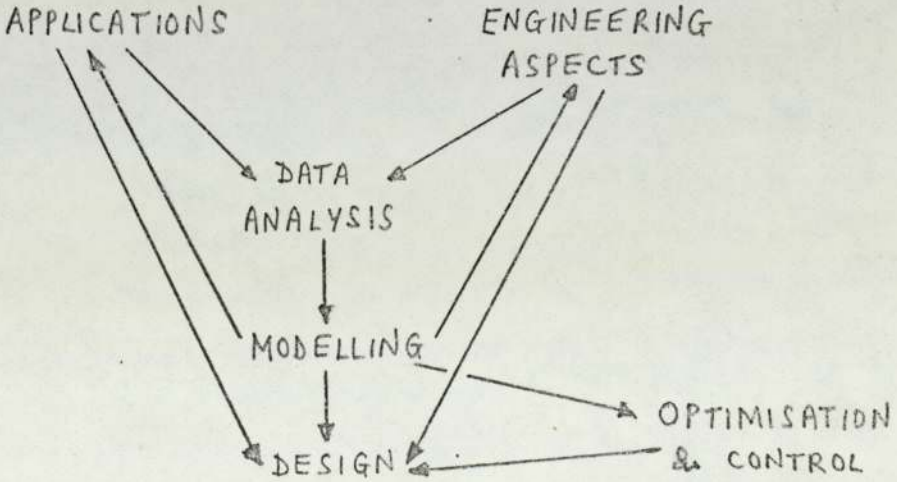


Figure 1.3 Interaction of Experimental and Theoretical Research



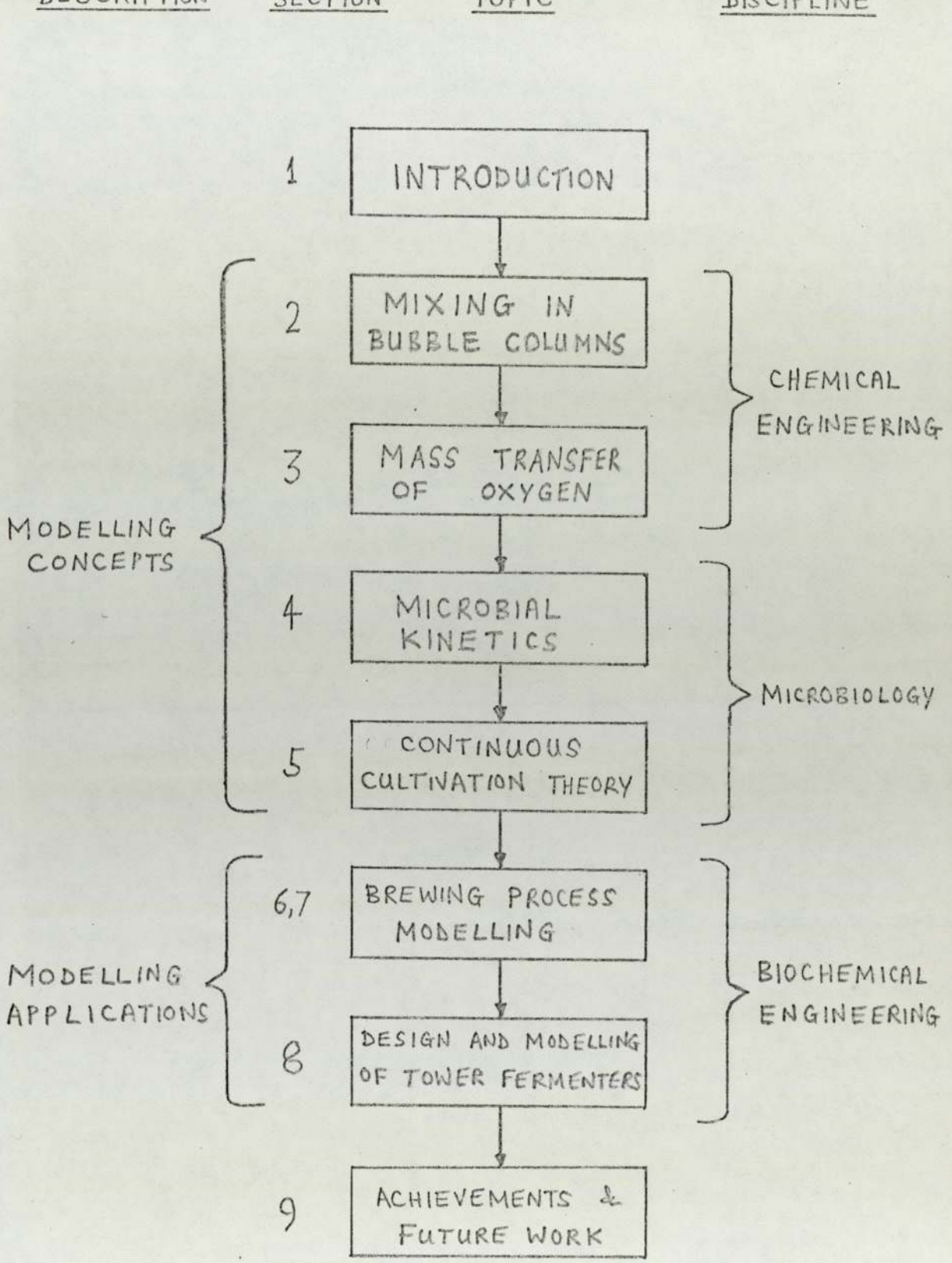


Figure 1.4 Scheme for the Thesis

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SECTION 2

MIXING IN BUBBLE COLUMNS

Figures

Figures

Tables

2.1 INTRODUCTION

Mixing patterns in tubular reactors range between the ideal extremes of perfect mixing and plug flow (i.e. no mixing). The plug flow assumption might be used to describe the behaviour of anaerobic fermentation systems, especially if a high microbial concentration is maintained (e.g. beer fermentation). The assumption of perfect mixing might well be used in the case of highly aerobic systems, such as the vinegar process, where the microorganisms are well-dispersed and can be considered part of the liquid phase. More detailed modelling, however, requires a better description of mixing processes, and the axially-dispersed plug flow model is frequently used in these instances. Solutions to such partial differential equations are awkward and time-consuming, and an alternative model based on a series of perfectly mixed vessels is proposed. Each of the models considered are discussed below.

2.2 DISPERSION MODELS

Dispersion models have frequently been used to describe mixing in chemical engineering processes and have been reviewed in full by SHAYEGAN-SALEK (1974). The simplest, and most commonly used, of these models is the axially dispersed plug flow model, given by the following equation:

$$\frac{\partial c}{\partial t} = \frac{u_L}{h_L} \cdot \frac{\partial c}{\partial x} + D_L \cdot \frac{\partial^2 c}{\partial x^2} \qquad (2.1)$$

Experimental evaluation of the dispersion coefficient may be by either dynamic or steady state process analysis.

Transient Analysis

Dynamic analysis normally involves a pulse input of tracer to a point in the reactor, normally the liquid input, and measurement of the resulting transients downstream of the injection point. The mean and variance (i.e. the first and second moments) of the normalised time-concentration curves are used to calculate the mean superficial liquid velocity, u_L, and axial dispersion coefficient, D_L, between the injection and measurement points.

The practical difficulties involved in generating a perfect delta function resulted in the development of the imperfect tracer pulse method, first by ARIS (1959) and later by BISCHOFF (1960), and BISCHOFF and LEVENSPIEL (1962). This method involves measurement of the transient tracer concentration at two points in the system: and for a system of infinite length the linear velocity and dispersion coefficient are related to the first and second moments of the residence time distribution curve by the following equations:

$$\Delta\mu = \mu_2 - \mu_1 = \frac{L}{\bar{u}_L} = \tau \qquad (2.2)$$

$$\Delta\sigma^2 = \sigma_2^2 - \sigma_1^2 = \left(\frac{L}{\bar{u}_L}\right)^2 \cdot \frac{2}{Pe} \qquad (2.3)$$

where: $$\bar{u}_L = \frac{u_L}{h_L} \qquad (2.4)$$

$$Pe = \frac{\bar{u}_L . L}{D_L} \qquad (2.5)$$

$$\mu = \frac{\int_0^\infty c(t).t.dt}{\int_0^\infty c(t).dt} \qquad (2.6)$$

and:

$$\sigma^2 = \frac{1}{\mu^2} \cdot \frac{\int_0^\infty c(t).t^2.dt}{\int_0^\infty c(t).dt} \qquad (2.7)$$

A frequently encountered problem is that of "tailing" of the residence time distribution. This causes the calculated moments of the curve to be highly sensitive to small errors in the values of c at large values of t. MIXON et alia (1967) and ØSTERGAARD and MICHELSEN (1969, 1970) have discussed this problem at length and devised a method by which the increasing weighting functions t and t^2 are replaced by the decreasing functions e^{-st} and $t.e^{-st}$. The procedure involves the use of the transfer function, F(s), for an axially dispersed plug flow system, and the theory is set out below:

$$F(s) = \frac{C_2(s)}{C_1(s)} = \exp\left[\frac{Pe}{2}\left(1 - \sqrt{1 + \frac{4s\tau}{Pe}}\right)\right] \qquad (2.8)$$

Differentiation yields:

$$-\frac{\dot{F}(s)}{F(s)} = -\left[\frac{\dot{c}_2(s)}{c_2(s)} - \frac{\dot{c}_1(s)}{c_1(s)}\right] = \frac{\tau}{\sqrt{1+\frac{4s\tau}{Pe}}} \qquad (2.9)$$

where: $$c(s) = \frac{\int_0^\infty c(t).\, e^{-st}.\, dt}{\int_0^\infty c(t).\, dt} \qquad (2.10)$$

and $$\dot{c}(s) = \frac{\int_0^\infty c(t).\, t.\, e^{-st}.\, dt}{\int_0^\infty c(t).\, dt} \qquad (2.11)$$

therefore $$\mu_s = -\frac{\dot{c}(s)}{c(s)} = \frac{\int_0^\infty c(t).\, t.\, e^{-st}.\, dt}{\int_0^\infty c(t).\, e^{-st}.\, dt} \qquad (2.12)$$

From equation (2.9),

$$\Delta\mu_s = \mu_{2,s} - \mu_{1,s} = \frac{\tau}{\sqrt{1+\frac{4s\tau}{Pe}}} \qquad (2.13)$$

and on rearrangement, one obtains:

$$\left(\frac{1}{\Delta\mu_s}\right)^2 = \frac{1}{\tau^2} + \frac{4.s}{\tau.Pe} \qquad (2.14)$$

Thus a plot of $\left(\frac{1}{\Delta\mu_s}\right)$ versus s gives a straight line of slope $\left(\frac{4}{\tau \cdot Pe}\right)$ and intercept $\left(\frac{1}{\tau^2}\right)$ on the ordinate. The recommended range of s-values is from $\frac{0.5}{\tau}$ to $\frac{2.0}{\tau}$.

Treatment of Downie's Data

DOWNIE (1972), using a "one-shot" tracer input technique, obtained time-concentration curves at two points in air-water bubble column systems and calculated the mean residence times and dispersion coefficients under a variety of different experimental conditions by utilising equations (2.2) and (2.3). However, the following inaccuracies in his data, arising from poor experimental technique, were noted:

(1) the baseline of the trace curve was not always zero, and

(2) the baseline appeared to shift upwards during the course of some of the experimental runs.

The first error is thought to arise from a background level of electrolyte in the water used; the second was due to recirculation of water from the column via a holding tank, thus causing a gradual increase in the base level of tracer in the system. Adjustments were made to the data to compensate for these errors and the axial dispersion coefficients were recalculated by SHAYEGAN-SALEK (1974). To investigate the effect of "tailing" the dispersion coefficients were calculated using the method of ØSTERGAARD and MICHELSEN (see above). Table 2.1 compares values of dispersion coefficients computed from the first and second moments of the residence time distribution curves using (1) standard increasing weighting functions, and (2) exponentially decreasing weighting functions.

Table 2.1 Comparison of dispersion coefficients and mean residence times calculated by different methods

v_L (cm/s)	h_L	D_L (cm^2/s)			(min)			
		A*	B	C	A	B	C	Actual†
0.5	0	-	0.88	0.88	4.46	4.67	4.67	6.60
0.5	0.05	3.72	17.45	17.48	2.06	2.28	2.28	6.23
0.5	0.10	1.37	16.98	19.08	1.00	1.62	1.80	5.89
0.5	0.20	1.67	14.05	13.86	0.76	0.82	0.82	5.28
1.0	0	-	3.03	3.03	2.36	2.28	2.28	3.30
1.0	0.05	12.87	34.37	34.67	1.47	1.72	1.72	3.14
1.0	0.10	5.16	14.78	15.00	0.77	1.16	1.16	3.00
1.0	0.20	3.04	6.28	6.66	0.50	0.86	0.86	2.64
2.6	0	-	4.32	4.32	1.88	1.43	1.43	1.27
2.6	0.05	144.0	79.57	82.89	1.21	1.33	1.36	1.20
2.6	0.10	36.9	46.74	47.05	0.74	0.99	0.99	1.14

*key: A : DOWNIE (1972) (N.B. 6 inch diameter column; porosity 3 distributor)

B : SHAYEGAN-SALEK (1974)

C : This work

† Actual residence time = $\frac{L.a.h_L}{60\ V_L}$ minutes

DOWNIE'S original values of D_L, calculated from uncorrected data, are also tabulated for comparison. Inspection of the figures in table 2.1 reveals only small differences between approaches (1) and (2), indicating that "tailing" effects are probably not too significant.

A further point of interest arising from DOWNIE'S work is that computed liquid residence times are generally less than the values calculated from volumetric liquid flowrate, column cross sectional area, and gas hold-up. ØSTERGAARD and MICHELSEN (1968) have proposed a hypothesis to explain this phenomenon which suggests that tracer trapped with bubble wakes does not follow the flow patterns of the liquid phase in general. Another cause of error inherent in backmix flow systems is that tracer may flow past measurement probes more than once and thus distort the residence-time distribution curves.

Steady State Analysis

Introduction of tracer at a constant rate into a bubble column eventually results in a steady state concentration profile. Computer simulations suggests that a 99% steady state profile is attained after about fifteen minutes (see Section 2.4.2). Equation (2.1), which describes transient situations, can now be simplified, yielding:

$$D_L \cdot \frac{d^2c}{dx^2} + \frac{u_L}{h_L} \cdot \frac{dc}{dx} = 0 \qquad (2.15)$$

which has the solution:

$$\frac{c(x)}{c(0)} = \exp\left[\frac{-u_L \cdot x}{D_L \cdot h_L}\right] \qquad (2.16)$$

for the boundary conditions derived in Appendix II.

A plot of $\ln\left[\frac{c(x)}{c(0)}\right]$ versus x gives a straight line of slope $\left(\frac{-u_L}{h_L \cdot D_L}\right)$ which should pass through the origin. SHAYEGAN-SALEK (1974) has successfully interpreted data obtained from air-water bubble column systems using this approach, though in some cases the plot of equation (2.16) gave a curve rather than a straight line. There are two possible explanations:

(1) that "end effects" cause different mixing patterns at the top and bottom of the bubble column, and

(2) that the value of the dispersion coefficient changes with axial position in the column.

DECKWER et alia (1973) experienced similar difficulties, and favoured the latter explanation, though inspection of their data suggests that a gently curved line fits the points better than two straight lines.

2.3 TANKS-IN-SERIES MODELS

The conventional tanks-in-series model has long been recognised as an alternative to the dispersion model for dealing with small deviations from plug flow. In this model the reactor is represented by a series of N equal-sized perfectly mixed vessels connected by a forward flow of the liquid phase (see figure 2.1). The time-concentration curve for the output from the n^{th} tank in response to an impulse to the first tank is:

$$\frac{c(n)}{c(0)} = \frac{n^n \cdot t^{n-1}}{(n-1)!} \cdot e^{-nt} \qquad (2.17)$$

Figure 2.1 Conventional Tanks-in-Series Model

Figure 2.2 Backflow Stirred Tanks Model

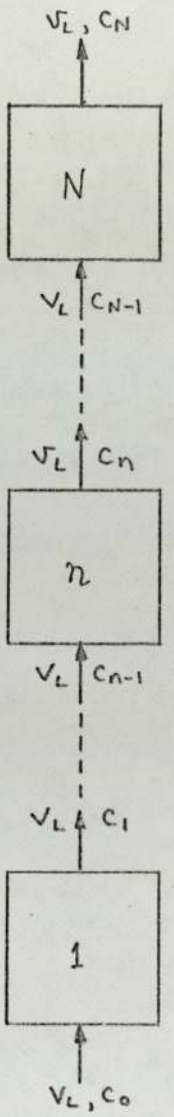


Figure 2.1

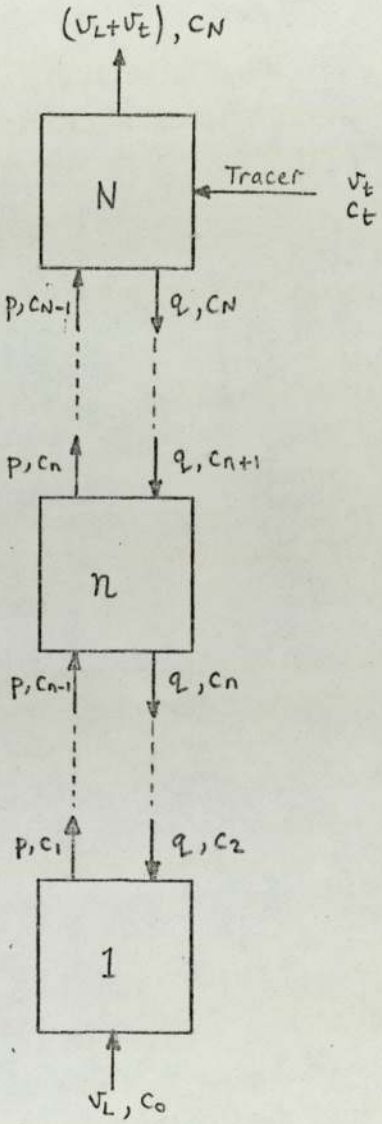


Figure 2.2

with mean $\mu = 1$ (2.18)

and variance $\sigma^2 = \frac{1}{n}$ (2.19)

The tanks-in-series model approximates to the dispersion model at large values of N. However, the time-concentration curves for the two models differ more and more in shape with increasing deviation from plug flow. By matching calculated variances the following correspondence between the two models is obtained (LEVENSPIEL, 1962):

$$\sigma^2 = \frac{1}{N} = \frac{2}{Pe} - \frac{2}{(Pe)^2} \cdot \left(1 - e^{-Pe}\right) \qquad (2.20)$$

2.4 TANKS-IN-SERIES MODELS WITH BACKMIXING

2.4.1 Introduction

Tanks-in-series models incorporating backmix flow have been used to describe two-phase systems by HARTLAND and MECKLENBURGH (1966) who considered counter-current liquid-liquid extraction, and by MIYAUCHI and VERMEULEN (1963). A review by MECKLENBURGH (1974) discusses the various models and relates them to dispersion models.

The author has found that a series of equal-sized perfectly mixed vessels linked by backflow in addition to forward liquid flow (hereafter to be called the "Backflow Stirred Tanks Model") provides an adequate description of liquid-phase mixing patterns within bubble columns at both high and low intensities of mixing. This model offers a better match to

the dispersion model than the conventional tanks-in-series model, especially at low values of N and/or high values of D_L. A flow scheme is presented in figure 2.2, and the relevant equations describing the system are derived below.

Consider a flow of tracer into the N^{th} tank, as shown in figure 2.2. Unsteady state mass balances on component c for each tank can now be written.

For the 1st tank:

$$q \cdot c_2 - p \cdot c_1 = V \cdot \frac{dc_1}{dt} \qquad (2.21)$$

For the n^{th} tank:

$$p \cdot c_{n-1} + q \cdot c_{n+1} - (p+q) \cdot c_n = V \cdot \frac{dc_n}{dt} \qquad (2.22)$$

For the N^{th} tank:

$$p \cdot c_{N-1} + v_t \cdot c_t - (q + v_L + v_t) \cdot c_N = V \cdot \frac{dc_N}{dt} \qquad (2.23)$$

Equations (21)-(23) are conveniently represented in matrix form:

$$\dot{\underline{c}} = \underline{A_1} \cdot \underline{c} + \underline{z} \tag{2.24}$$

where $\underline{A_1} =$

$$\begin{bmatrix} -\frac{p}{v} & \frac{q}{v} & & & \\ \frac{p}{v} & -\frac{(p+q)}{v} & \frac{q}{v} & & \\ & \frac{p}{v} & -\frac{(p+q)}{v} & \frac{q}{v} & \\ & & -\frac{p}{v} & \frac{(q+v_L+v_t)}{v} \end{bmatrix} \tag{2.25}$$

and the forcing function $\underline{z} =$

$$\begin{bmatrix} 0 \\ | \\ 0 \\ v_t . c_t \end{bmatrix} \tag{2.26}$$

For a batch system, with no liquid flow into or out of the column (i.e. $v_L = 0$), we have:

$$\dot{\underline{C}} = \underline{A_2} \cdot \underline{C} \tag{2.27}$$

where the transition matrix $\underline{A_2} =$

$$\begin{bmatrix} -\frac{q}{v} & \frac{q}{v} & & & \\ \frac{q}{v} & -\frac{2q}{v} & \frac{q}{v} & & \\ & \ddots & \ddots & \ddots & \\ & & \frac{q}{v} & -\frac{2q}{v} & \frac{q}{v} \\ & & & \frac{q}{v} & -\frac{q}{v} \end{bmatrix} \tag{2.28}$$

Equation (2.24) can be solved using matrix exponential functions (see Appendix I), yielding:

$$\underline{C}(t_o+h) = \underline{\phi}(h) \cdot \underline{C}(t_o) + \underline{\Delta}(h) \cdot \underline{Z} \tag{2.29}$$

where the exponential matrix $\underline{\phi}(h) = e^{\underline{A}h}$ (2.30)

and $\underline{\Delta}(h) = \underline{A}^{-1}\left[\underline{\phi}(h) - \underline{I}\right]$ (2.31)

2.4.2 Relationship Between the Backflow Stirred Tanks Model and the Dispersion Model

Steady State

It can be shown (see Appendix I) that the steady state solution of equation (2.24) is given by the following equations:

$$C_N = \frac{V_t}{V_L+V_t} \cdot C_t \qquad (2.32)$$

$$C_n = \left(\frac{q}{P}\right)^{N-n} \cdot C_N \qquad (2.33)$$

$$\text{or} \quad \frac{C_n}{C_N} = f^{N-n} \qquad (2.34)$$

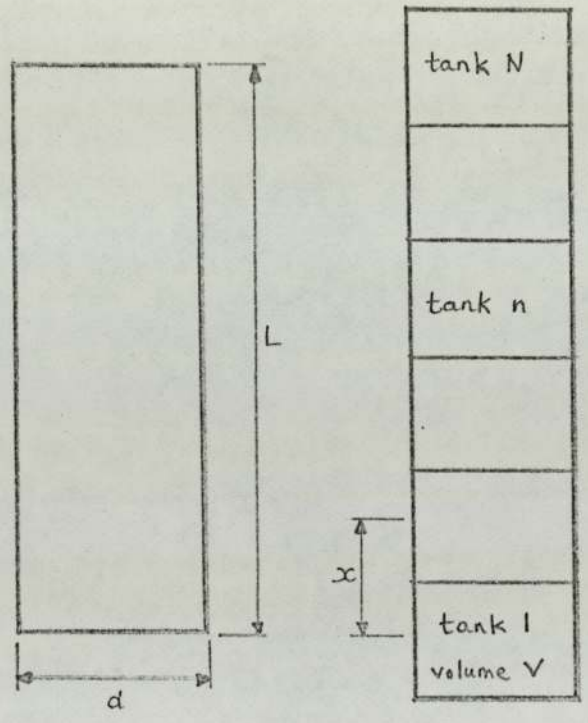
where the backmixing factor $f = \frac{q}{P}$ (2.35)

The corresponding steady state solution to the dispersion model is given by equation (2.16)

$$\frac{C(x)}{C(0)} = \exp\left[\frac{-u_L \cdot x}{D_L \cdot h_L}\right] \qquad (2.16)$$

The solutions given by equations (2.16) and (2.34) are both of a similar form (i.e. both exponential), although (2.16) is continuous whilst (2.34) is discrete. Consider each mixed vessel to represent a specific portion of the column, as in figure 2.3.

Figure 2.3 The Backflow Stirred Tanks Model : Comparison with a Bubble Column.

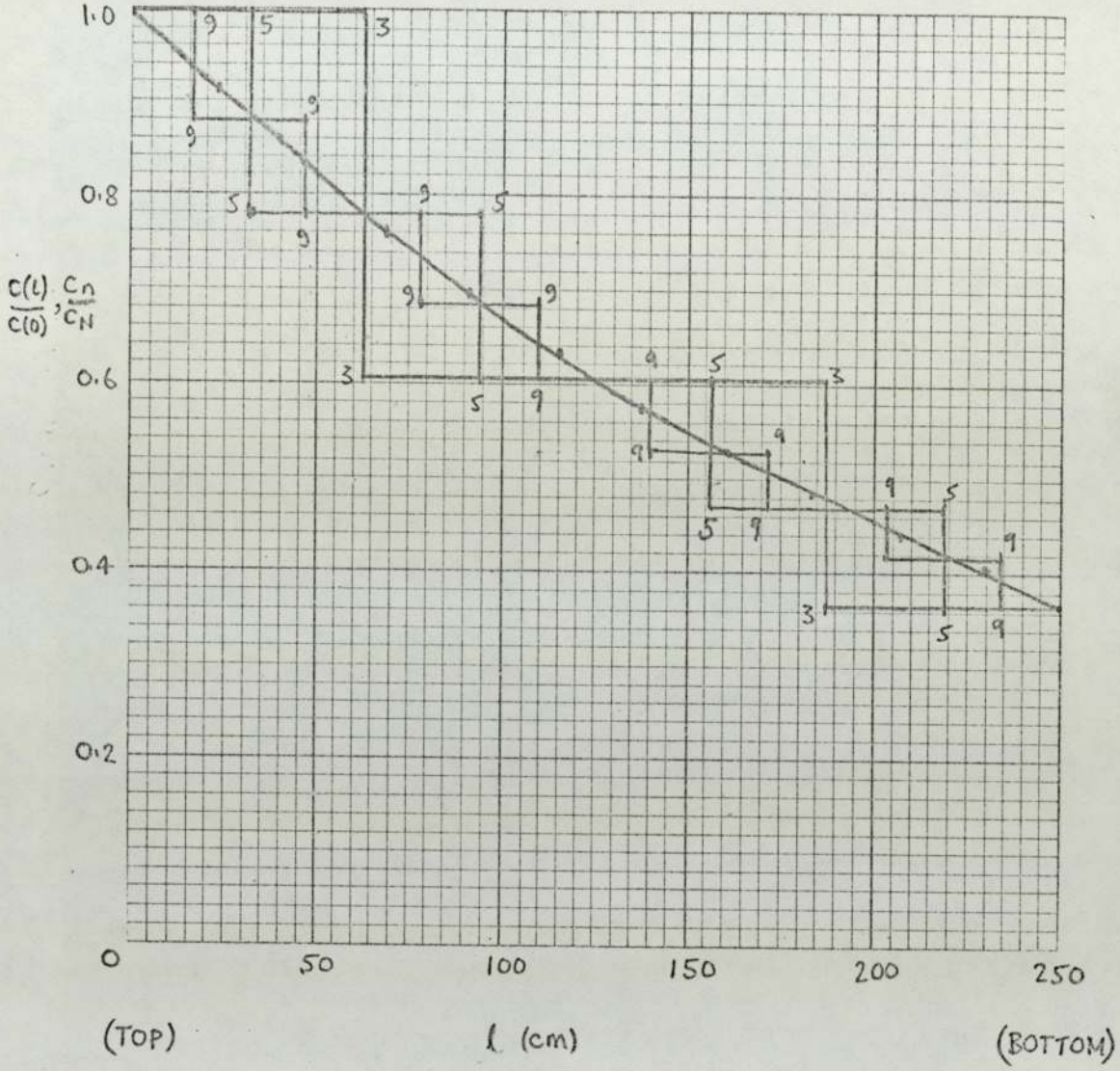


$$x = \frac{L}{(N-1)}$$

$$N.V = L \cdot \pi \cdot \frac{d^2}{4}$$

Bubble Column Model Schematic

At the mid points of the tanks the solutions (2.16) and (2.34) are identical, as can be seen in figure 2.4. Taking the solution for the



N = 3, 5 and 9 tanks

Figure 2.4 Comparison of the Steady-State Solutions to the Dispersion and Backflow Stirred Tanks Models

1st (i.e. bottom) tank we find:

$$\frac{C_1}{C_N} = f^{N-1} \qquad (2.36)$$

Now, the concentration in the $(N-1)^{th}$ tank corresponds to the concentration at the axial position $x_i = \frac{iL}{(N-1)}$ $(i = 1, \ldots, N-1)$ in the column. Equating the solutions of the two models over the length of the column we have:

$$f^{N-1} = \frac{C_i}{C_N} = \frac{C(L)}{C(0)} = \exp\left[\frac{-u_L \cdot L}{D_L \cdot h_L}\right] \qquad (2.37)$$

Equation (2.37) above explains the connection between the two models, i.e. it relates the parameters f, N, and D_L. Thus for any value of dispersion coefficient, D_L, the equivalent backmixing factor, f, can be found assuming a value of N. Table 2.2 indicates typical equivalent values of f, N, and D_L.

The number of perfectly mixed vessels chosen to represent flow patterns in bubble columns is a point of interest. As N tends to infinity the solutions to the two models become identical, and so the greater the large value of N the better the correspondence between the two models. However, large values of N are impractical due to the time required to compute the solutions. It is suggested that a

value of N equal to the aspect ratio* of the column is suitable, thus:

$$N = \frac{L}{d} \qquad (2.38)$$

Visual observations made by SHAYEGAN-SALEK (1974) and SMITH (1973) suggest that this is a reasonable, although admittedly arbitrary, value for N. Further support for the choice is provided by TOWELL et alia (1965), who also observed turbulent eddies with a scale approximately equal to the column diameter.

Unsteady State

Unsteady state solutions of the dispersion model are extremely complex and vary according to the boundary conditions employed. For the purposes of comparing this model with the backflow stirred tanks model a numerical finite difference solution was used, as described in Appendix III. The dispersion model, with suitable boundary conditions, is derived in Appendix II.

Transient solutions for the backflow stirred tanks model require a value for the volume, V, of each perfectly mixed vessel. In order to match the two models the volumes of both systems should be identical, thus:

$$N.V = L.a \qquad (2.39)$$

$$\text{or} \quad V = \frac{L.a}{N} \qquad (2.40)$$

* Aspect ratio = $\frac{\text{length}}{\text{diameter}}$

Results from computer simulations of transient states of the backflow stirred tanks model indicate that such systems closely approximate to the dispersion model, as shown in figure 2.5.

Batch Systems

Where there is no liquid flow into or out of a column the dispersion model, given by equation (2.1), can be simplified, yielding:

$$\frac{\partial c}{\partial t} = D_L \cdot \frac{\partial^2 c}{\partial x^2} \qquad (2.41)$$

The solution of equation (2.41) above is given by OKHI and INOUE (1970) for the following boundary and initial conditions.

Boundary conditions:

$$\frac{\partial c}{\partial x}(0,t) = 0 \qquad (2.42)$$

$$\frac{\partial c}{\partial x}(L,t) = 0 \qquad (2.43)$$

Initial conditions:

$$c(x,0) = C_0 \qquad 0 \leq x \leq \lambda \qquad (2.44)$$

$$c(x,0) = 0 \qquad \lambda \leq x \leq 0 \qquad (2.45)$$

Solution:

$$\frac{c(t)}{c(\infty)} = 1 + \frac{2L}{N\lambda} \cdot \sum_{m=0}^{\infty} \left[\frac{1}{m} \cdot \sin\left(\frac{m\pi}{L} \cdot \lambda\right) \cdot \cos\left(\frac{m\pi}{L} \cdot \lambda\right) \cdot \exp\left\{ -\left(\frac{m\pi}{L}\right)^2 \cdot D_L \cdot t \right\} \right] \qquad (2.46)$$

Note:

$$c(\infty) = C_0 \cdot \frac{\lambda}{L} \qquad (2.47)$$

For $n\lambda \ll L$ the following approximation is valid:

$$\frac{c(t)}{c(\infty)} = 1 + 2 \sum_{m=0}^{\infty} \left[\cos\left(\frac{m\pi}{L} . x\right) . \exp\left\{ -\left(\frac{m\pi}{L}\right)^2 . D_L . t \right\} \right] \qquad (2.48)$$

Equation (2.48) is shown in figure 2.6 as $\left(\frac{c(t)}{c(\infty)}\right)$ plotted against $\left(\frac{\pi^2}{L^2} . D_L . t\right)$ with $\left(\frac{x}{L}\right)$ as a parameter.

The backflow stirred tanks model for a batch system is shown in figure 2.7, and the matrix differential equation describing it is equation (2.27) above, viz:

$$\dot{\underline{c}} = \underline{A_2} . \underline{c} \qquad (2.27)$$

with solution: $$\underline{c}\,(t_o + h) = \underline{\phi}(h) . \underline{c}\,(t_o) \qquad (2.49)$$

Solutions of equation (2.27) are plotted in figure 2.8 as $\frac{c(t)}{c(\infty)}$ versus $\frac{qt}{V}$ with $\frac{x}{L}$ as a parameter for given values of N. It can be seen that the greater the number of stages used the better the fit to the dispersion model solution, i.e. equation (2.48). The precise manner in which N can be incorporated into the dimensionless group $\frac{q.t}{V}$ is not clear, and no conclusions concerning this point can be made at present.

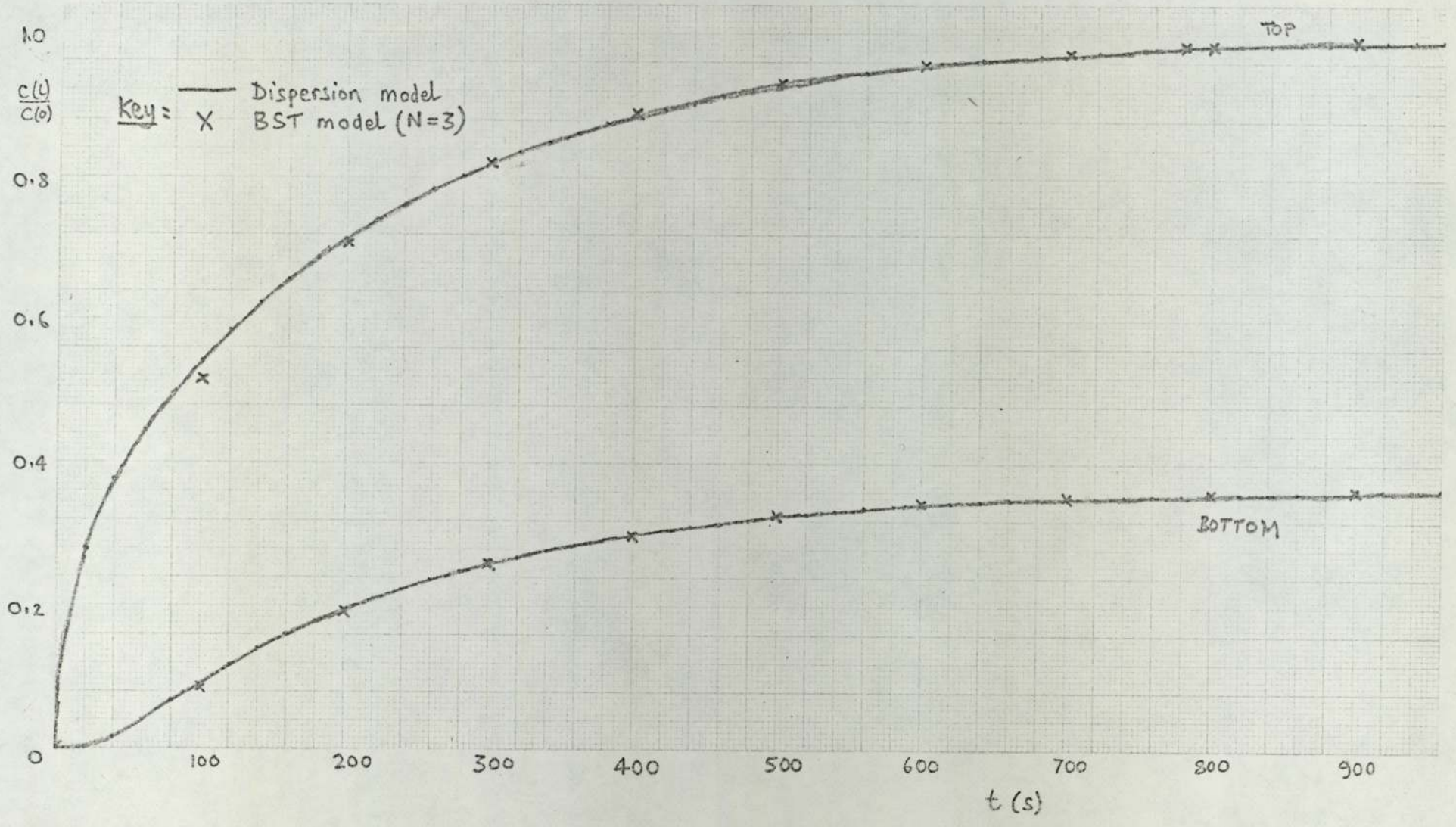
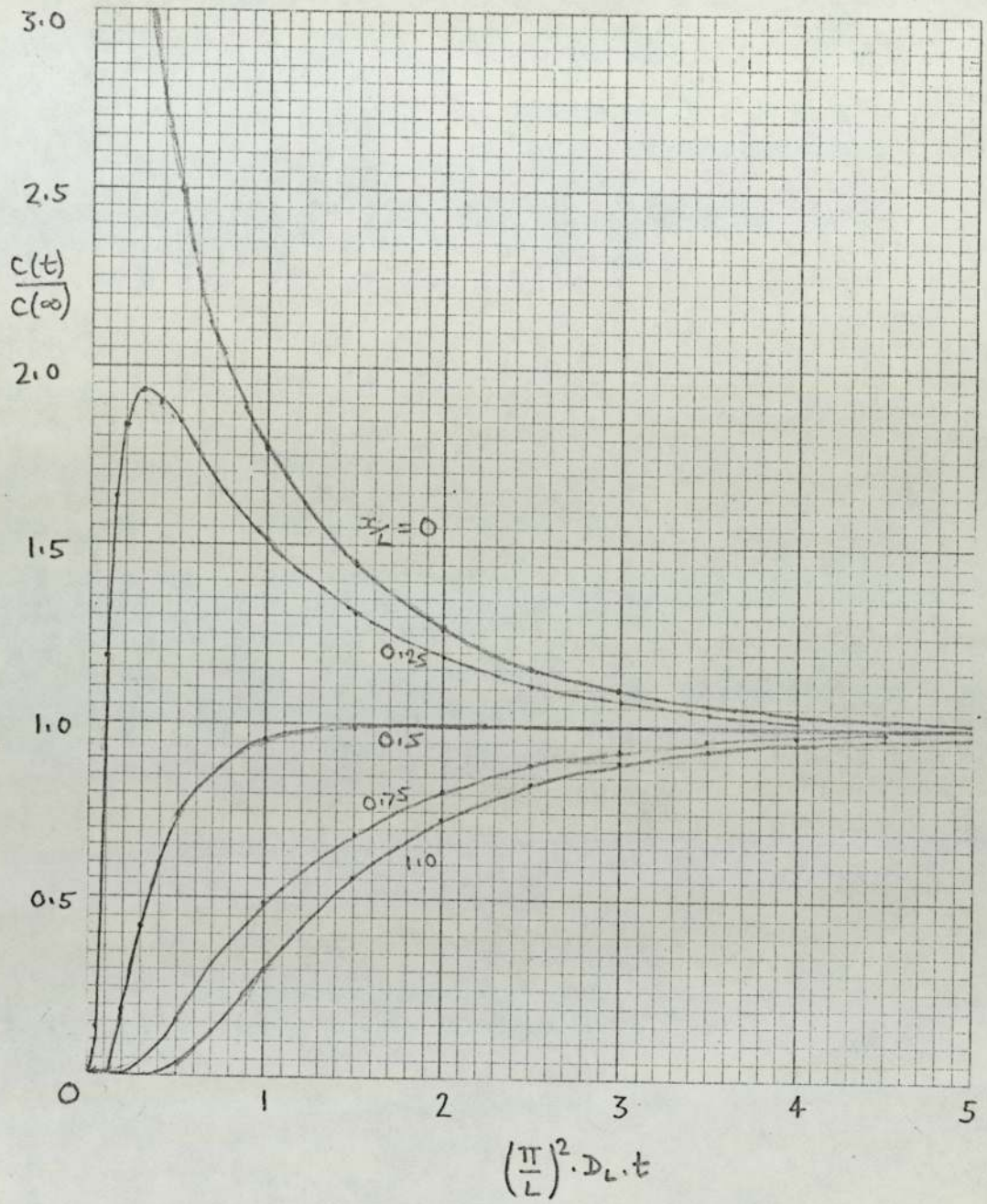


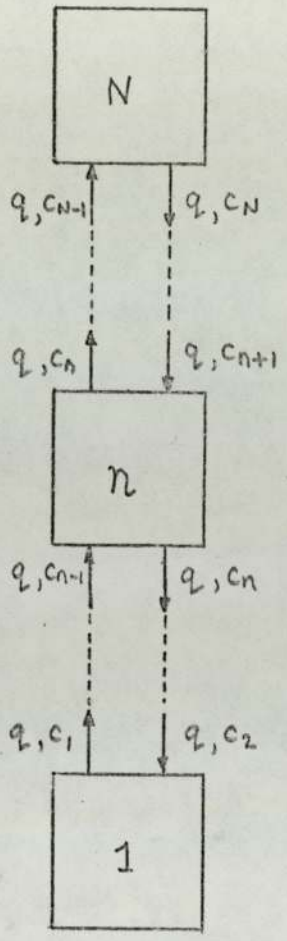
Figure 2.5 Comparison of the Transient Solutions to the Dispersion and Backflow Stirred Tanks Models

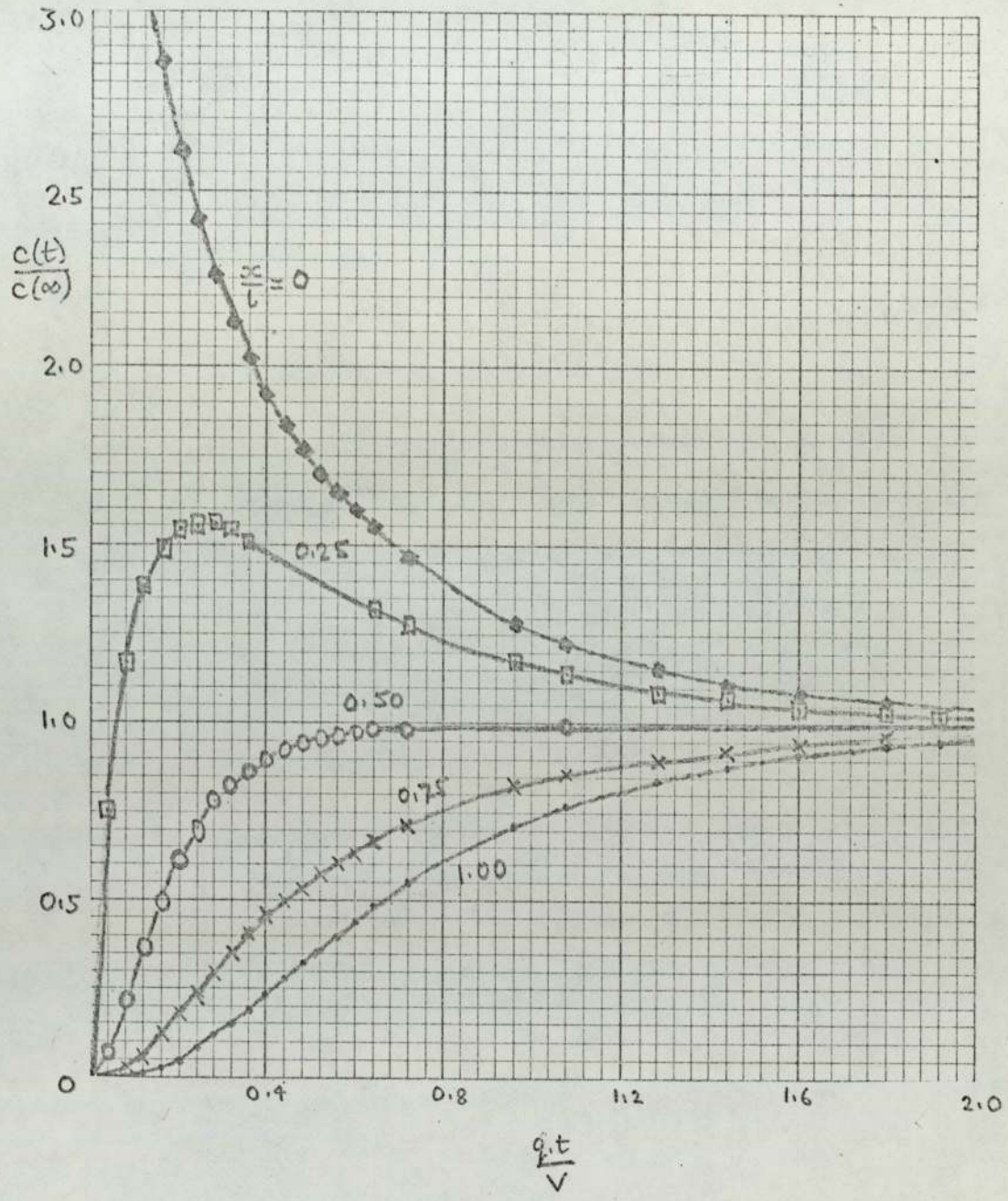


Solution to $\frac{\partial c}{\partial t} = D_L \cdot \frac{\partial^2 c}{\partial x^2}$ with $D_L = 250$ cm/s
$L = 250$ cm
tracer injected at $L = 0$ cm

Figure 2.6 General Solution to the Dispersion Model (batch system)

Figure 2.7 The Backflow Stirred Tanks Model (batch system)





Solution to $\dot{\underline{c}} = \underline{A}.\underline{c}$ for $N = 5$

Figure 2.8 General Solution to the Backflow Stirred Tanks Model (batch system)

2.5 PREDICTION OF BACKMIX FACTORS AND DISPERSION COEFFICIENTS

2.5.1 Introduction

The previous sections in this chapter have described some of the mixing models applicable to tower fermenters. In these models the assumption has been made that values of the mixing parameters (i.e. D_L and f) are known. This is not always the case unless relevant experimental data are available. A further point of note is that when simulating mixing systems using a general computer model it is necessary to store much data relating dispersion coefficients to parameters such as superficial gas and liquid velocities. This is often impractical and inefficient, especially since the graphical relationships between D_L, u_G, and u_L are not simple (SHAYEGAN-SALEK, 1974; DECKWER et alia, 1973; TOWELL et alia, 1972). This section outlines a simple model which predicts the dispersion coefficient and backmix factor based upon the superficial gas and liquid velocities.

2.5.2 The Wake Factor

Assume that liquid mixing is entirely due to upward movement of liquid carried in the wakes of gas bubbles and compensated by an equal volume of liquid moving downwards. Velocity profiles presented by HILLS (1974) indicate that liquid is carried upwards by wakes in the centre of bubble columns and moves downwards near the walls. Visual observations by SHAYEGAN-SALEK (1974) are in agreement with HILL'S results. Thus, it can be postulated that liquid mixing is in some way related to the size of bubble wakes and the rate of replacement of liquid in the wakes.

Consider the backflow stirred tanks model (see figures 2.2 and 2.3, and Section 2.4). The liquid flow due to backmixing between each stage is q (= f.p). Let this liquid flow be equal to $V_G.w$, where w (the wake factor) may be a function of the ratio of the liquid wake volume to the bubble volume, and the rate of change of liquid in the wake. Now, the backmixing factor, f, is defined as the ratio of liquid backflow to forward flow between each mixing stage, or:

$$f = \frac{q}{p} \tag{2.50}$$

but $$p = q + V_L \tag{2.51}$$

since forward flow = backflow + net liquid flow through system,

and $$q = V_G . w \tag{2.52}$$

therefore $$f = \frac{V_G . w}{V_G . w + V_L} \tag{2.53}$$

$$= \frac{u_G . w}{u_G . w + u_L} \tag{2.54}$$

since $$u = \frac{v}{a} \tag{2.55}$$

$$\text{However,} \quad f = \exp\left[\frac{-u_L \cdot L}{D_L (N-1) h_L}\right] \tag{2.37}$$

Therefore, combining and rearranging equations (2.37) and (2.53) we have:

$$D_L = \frac{u_L \cdot L}{h_L (N-1)} \cdot \frac{1}{\ln\left(1 + \frac{u_L}{u_G \cdot w}\right)} \tag{2.56}$$

Since f and N are inter-related, it is necessary to fix N before proceeding. Taking $N = {}^{L}/_{d}$, then D_L can be calculated from equation (2.56) for a series of values of u_L and u_G, provided that the value of w is known. Likewise, f can be computed using equation (2.53).

Calculation of w, The Wake Factor

Using experimental data supplied by SHAYEGAN-SALEK (1974), w was calculated from equation (2.58) below.

Rearranging equation (2.54):

$$w = \frac{f \cdot u_L}{(1-f) u_G} \tag{2.57}$$

Substituting for f from equation (2.37):

$$w = \frac{u_L \cdot \exp\left[\frac{-u_L \cdot L}{D_L (N-1) h_L}\right]}{u_G \cdot \left(1 - \exp\left[\frac{-u_L \cdot L}{D_L (N-1) h_L}\right]\right)} \tag{2.58}$$

$$\text{where} \quad N = \frac{L}{d} \tag{2.38}$$

A plot of w versus u_G, using SHAYEGAN-SALEK'S data, is shown in figure 2.9. In this figure it will be noted that the curves are of an exponentially-declining form with u_L as a parameter. To account for the effect of u_L, and in an attempt to get a better fit, a straight line given by equation (2.59) below was fitted to the data using the "least squares" criterion:

$$\ln w = k_1 + k_2 . u_G + k_3 . u_L \qquad (2.59)$$

It should be pointed out that this equation has no basis in theory and is merely a convenient way of fitting the experimental results. Values of the parameters k_1, k_2, and k_3 for three independent sets of data are presented in table 2.3. It can be seen that SHAYEGAN-SALEK'S data gives the best fit to the line, with a correlation coefficient of 0.975, but it is interesting to note that the data of DECKWER et alia and TOWELL et alia give similar values for the parameters k_1 and k_2. Variations in the value of k_3 are not important since $(k_1 + k_2 . u_G) \gg (k_3 . u_L)$.

Figure 2.10 shows a plot of equation (2.59) including all the data. It is interesting to note that the straight line extends from the bubbly-flow regime (i.e. $u_G < 5$ cm/s) into the coalesced-bubble, slug flow regime ($u_G > 5$ cm/s). Intuitively, one would expect the relationship $q = v_G . w$ to hold at low gas flows in the ordered flow regime (i.e. bubbly flow), but not at higher gas flowrates.

Table 2.2 Typical values of f, the backmixing factor

(for L = 100 cm, h_L = 0.90)

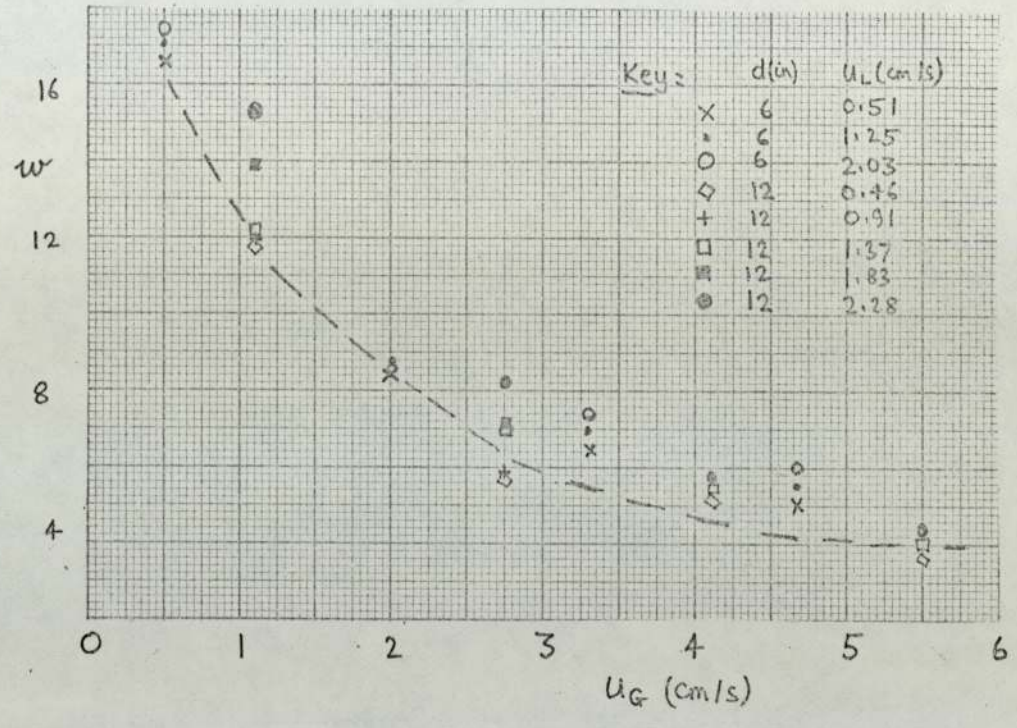
u_L (cm/s)	D_L (cm^2/s)	N	f
0.1	100	10	0.988
0.1	100	5	0.973
1.0	100	10	0.884
1.0	100	5	0.758
1.0	10	10	0.290
1.0	10	5	0.062

Table 2.3 Values of parameters for the wake factor prediction equation

Data (Refs.)	k_1	k_2	k_3	r*
SHAYEGAN-SALEK (1974)	2.74	-0.28	0.10	0.98
TOWELL et alia (1972) / DECKWER et alia (1973)	2.66	-0.30	0.20	0.87
All Data	2.65	-0.29	0.17	0.91

* r = correlation coefficient

Figure 2.9 The Wake Factor as a Function of Superficial Gas Velocity (Shayegan-Salek's data)



$$\ln w = 2.74 - 0.28(u_G - 0.36\,u_L)$$

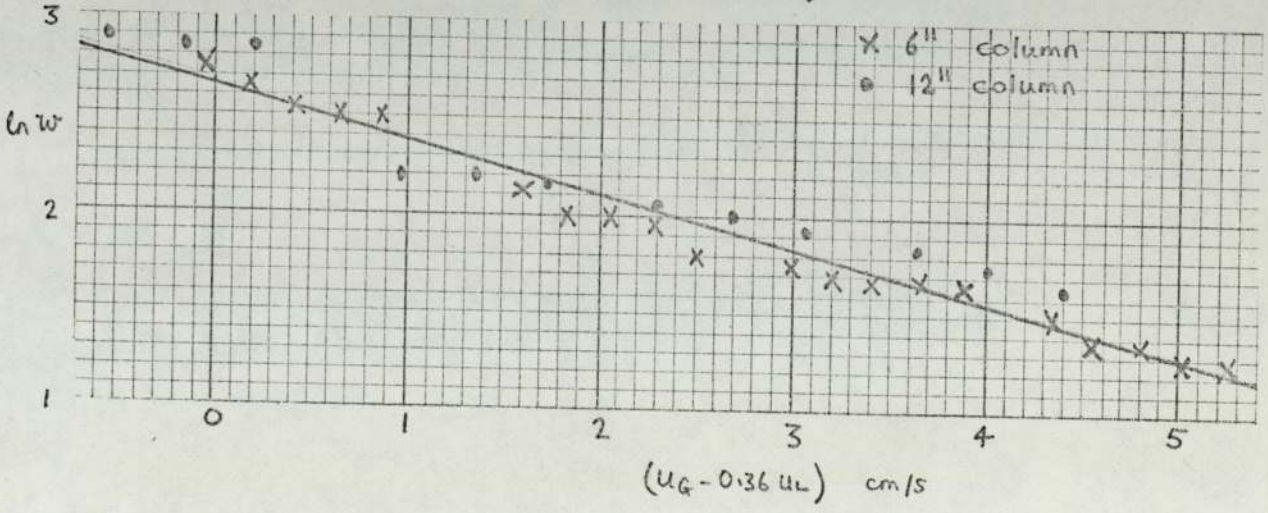


Figure 2.10 (a) Correlation of the Wake Factor (Shayegan-Salek's data)

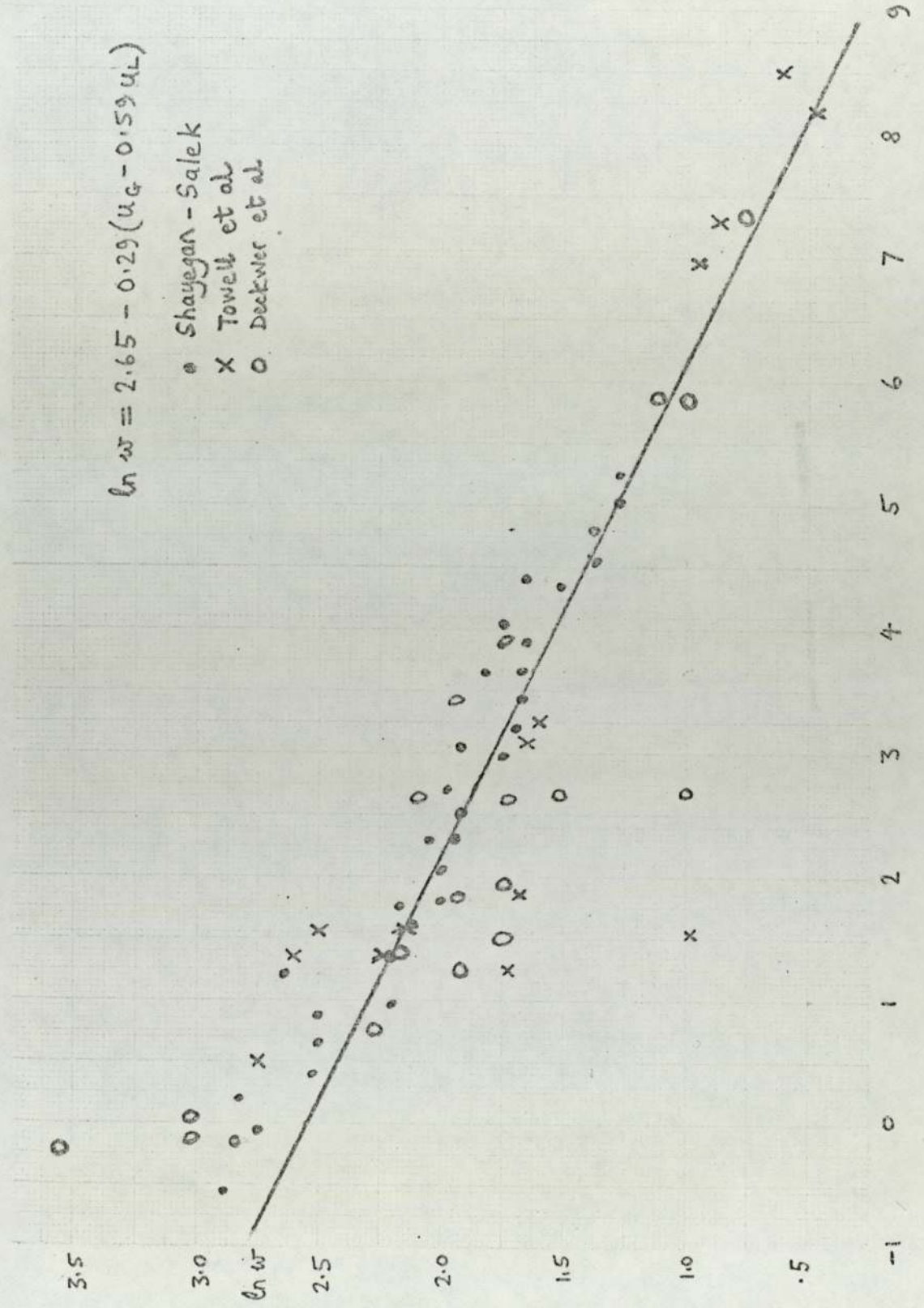


Figure 2.10 (b) Correlation of the Wake Factor (all data)

2.6 CONCLUSIONS

The backflow stirred tanks model has been shown to correspond closely to the dispersion model. It is a useful tool to employ when modelling systems such as bubble columns, because:

(1) it lends itself to a simple mathematical solution, with few of the difficult boundary conditions associated with partial differential equations (e.g. the dispersion model);

(2) it is more directly related to mixing patterns, as observed by TOWELL et alia (1965), SHAYEGAN-SALEK (1974), and SMITH (1973); and

(3) it is relatively easy to incorporate into large and complex models, e.g. the general tower fermenter model (see Section 8).

A disadvantage, however, is that although the solution fits that of the dispersion model, it yields discrete values rather than the continuous solutions of the dispersion model*. Consequently the sum of squared errors between "point" concentrations given by the two solutions increases considerably as N, the number of stages, decreases.

A procedure for predicting backmix factors and dispersion coefficients based on superficial gas and liquid velocities has been developed. The method utilises an intermediate parameter, w, which is computed from u_G and u_L using an empirical correlation, which has been shown to hold for three independent sets of data.

* Although in theory partial differential equations yield continuous solutions, practical methods of solution are based on nodal lattices and, therefore, yield discrete point values only (see Appendix III).

LIST OF SYMBOLS (Section 2)

SYMBOL	EXPLANATION	UNITS
a	cross sectional area of column	cm^2
c	concentration of component 'c'	g/cm^3
d	diameter of column	cm
D_L	axial dispersion coefficient	cm^2/s
f	backmixing factor	-
h	hold-up	-
k_1, k_2, k_3	dimensional constants in equation (2.59)	-
L	length of column	cm
N	number of stirred tanks	-
p	forward liquid flowrate between stirred tanks	cm^3/s
q	backward liquid flowrate between stirred tanks	cm^3/s
t	time	s
u	superficial velocity	cm/s
$\bar{u}$	true velocity	cm/s
v	volumetric flowrate	cm^3/s
V	volume of stirred tanks	cm^3
w	wake factor	-
x	axial position in tower	cm

SYMBOL	EXPLANATION	UNITS
Greek letters		
μ	1st moment (mean) of residence-time distribution curve	s
σ^2	2nd moment (variance) of residence-time distribution curve	s^2
τ	mean residence time	s
Subscripts		
G	gaseous phase	
L	liquid phase	
s	solid phase	
t	tracer	
1,2	tracer measurement points	
Dimensionless Groups		
Pe	Peclet number = $\frac{\bar{u}_L . L}{D_L}$	

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SECTION 3

OXYGEN MASS TRANSFER IN TOWER FERMENTERS

Figures

Tables

3.1 INTRODUCTION

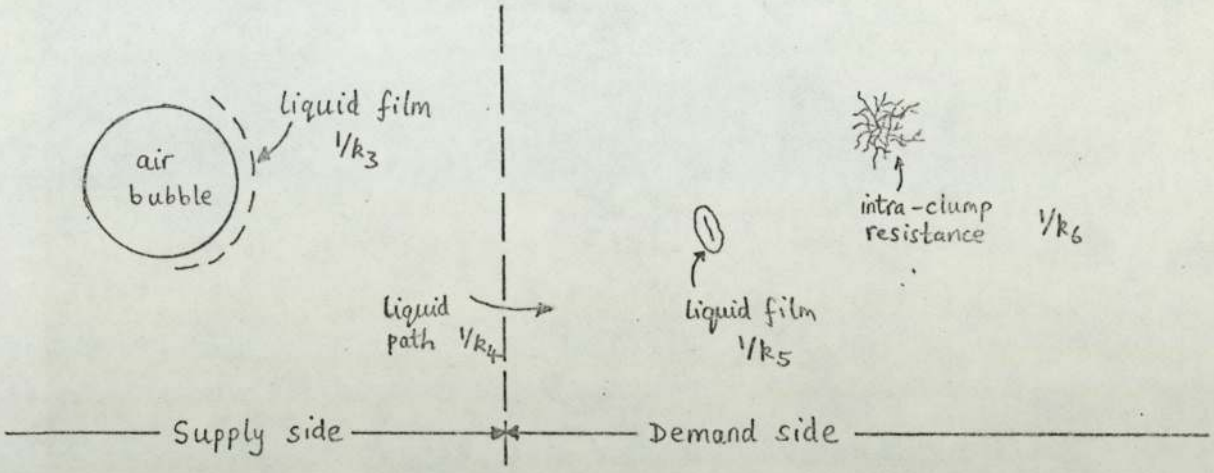
Oxygen transfer is often a limiting factor during aerobic fermentations and, consequently, has received much attention from chemical and biochemical engineers. In tower fermenters aeration results in agitation of the fermentation broth, causing considerable backmixing (see Section 2). Under certain conditions aeration may also result in three-phase fluidisation of the fermenter contents (JAMES, 1973; SMITH and GREENSHIELDS, 1974). The main purpose of aeration, though, is the supply of oxygen to microorganisms to enable them to survive, grow, and synthesize metabolites.

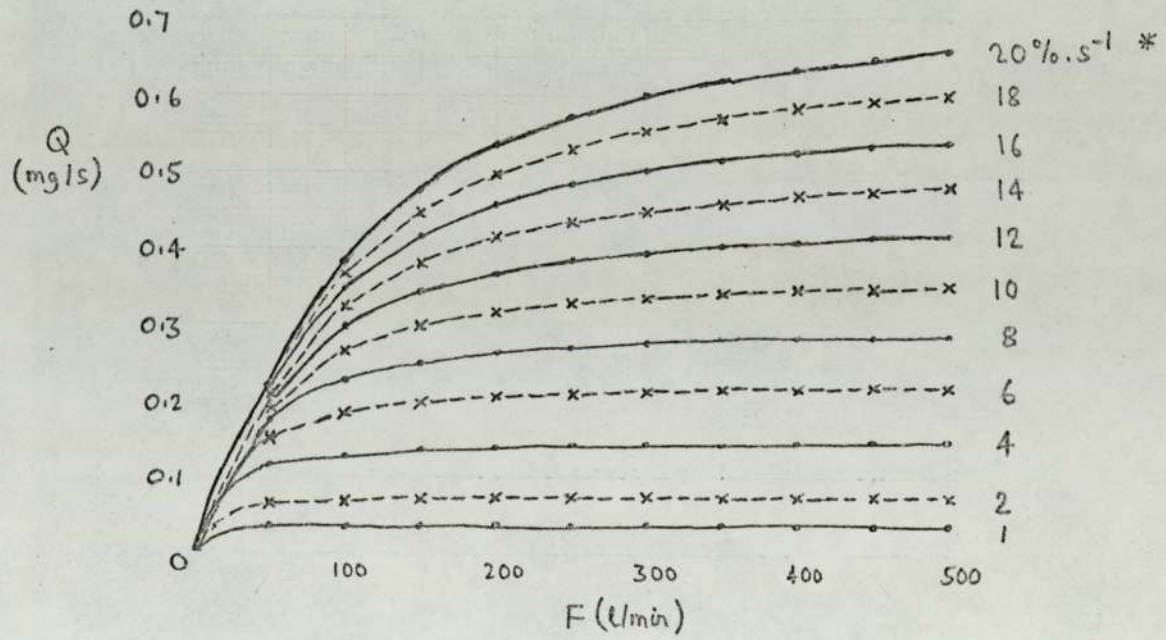
Aeration can be conveniently viewed from two aspects - the demand for oxygen by the microorganisms, and the supply of oxygen from air bubbles to the liquid. Microbial oxygen demand depends upon the conditions and kinetics of the fermentation in question and is covered elsewhere (see Section 4), whereas the rate of oxygen transfer from air bubbles to fermentation media is a purely physical phenomenon. The purpose of this section is to briefly describe some of the more important theoretical and empirical equations relating to oxygen transfer mechanisms. These basic principles are then used to construct a model of oxygen transfer in tower fermenters, assuming that the main resistance lies within the liquid film at the gas-liquid interface.

3.2 THE THEORY OF OXYGEN MASS TRANSFER

The transfer of oxygen from an air bubble submerged in a fermentation broth to a microbial cell involves a number of steps, each of which adds to the overall resistance to transfer (see figure 3.1).

Figure 3.1 Resistances to Oxygen Transfer





* $(100 - [DO]).(k_L a)_{G \to L}$ $\%.s^{-1}$

gas flowrates calculated at 0°C, 1 atm.

assume $\varepsilon_G = 0$ and $\rho_L = 1\ g/cm^3$

$V = 450\ l$; $L = 500\ cm$.

Figure 3.3 Oxygen Transfer Rates as a Function of Gas Flowrate

ARNOLD and STEEL (1958) listed seven resistances in series:

(1) $1/k_1$ gas-film resistance between gas and interface,

(2) $1/k_2$ gas-liquid interfacial resistance,

(3) $1/k_3$ liquid-film resistance between interface and the bulk of the liquid,

(4) $1/k_4$ liquid-path resistance characterised by oxygen gradients in the bulk of the liquid,

(5) $1/k_5$ liquid-film resistance around the cell or cell-clump,

(6) $1/k_6$ intracellular or intraclump resistances, and

(7) $1/k_7$ resistance of reaction of oxygen within the cell respiratory enzymes.

The overall resistance, R, is equal to the sum of the individual resistances, i.e.

$$R = \sum_{i=1}^{7} \frac{1}{k_i} \qquad (3.1)$$

At steady state the rate of oxygen transfer, Q, equals the "driving force" (concentration difference) divided by the resistance. Assuming first order steps we have:

$$Q = \frac{\Delta c_i}{(1/k_i)} \qquad (i = 1, \ldots, 7) \qquad (3.2)$$

It can be seen that the relative magnitude of each resistance determines the concentration difference for the step.

The rate of oxygen transfer is often limited by the liquid-film resistance at the gas-liquid interface (CALDERBANK, 1967), i.e.

$$Q = k_3 \cdot \Delta c_3$$
$$= (k_L a)_{G \to L} (c^* - c_L) \qquad (3.3)$$

or $\left(\begin{matrix}\text{Rate of} \\ \text{oxygen transfer}\end{matrix}\right)$ = constant x $\left(\begin{matrix}\text{specific surface} \\ \text{area of interface}\end{matrix}\right)$ x $\left(\begin{matrix}\text{concentration} \\ \text{difference}\end{matrix}\right)$

TSAO (1968, 1969) and MUCHMORE et alia (1971) have concluded from experimental data that, in addition to the liquid transport pathway, there exists a direct absorption mechanism for oxygen transfer. TSAO (1968) accounts for this phenomenon mathematically by augmenting the value of $(k_L a)_{G \to L}$ in equation (3.3).

Typical $(k_L a)_{G \to L}$ values for various fermentation systems are given in table 3.1. However, most of the values are derived from sulphite oxidation tests and should be treated with caution (BELL and GALLO, 1971).

Oxygen is only sparingly soluble in water, as shown by the empirical equation below (MONTGOMERY et alia, 1964).

$$c^* = \frac{0.468}{31.6 + T} \quad g/l \quad 4^\circ < T < 33^\circ C \qquad (3.4)$$

Fermentation media consist of water plus a variety of nutrients and metabolic products. These components affect the saturation solubility of oxygen (SOLOMONS, 1960) and experimental methods should be used to determine c* (LIU et alia, 1973). The relationship between gas composition and liquid-phase composition is linear, i.e. Henry's Law

is obeyed:

$$P_{O_2} = H.c^* \qquad (3.5)$$

where H = f(temperature, gas liquid composition)

3.3 MEANS OF PREDICTING $k_L a$ VALUES

Values of the volumetric mass transfer coefficient, $(k_L a)_{G \to L}$, can be determined either experimentally (e.g. by sulphite oxidation tests), or theoretically. Estimation of $(k_L a)_{G \to L}$ is normally performed by computing separate values for "k_L" and "a" and then multiplying them together.

Mass Transfer Coefficients

Publications by CALDERBANK (1967), CALDERBANK and MOO-YOUNG (1961), and CALDERBANK and JONES (1961) show that the following correlation can be used to estimate the value of the mass transfer coefficient at both the gas-liquid and liquid-solid interfaces:

$$N_{Sh} = 2.0 + 0.31 \left[N_{Ra}\right]^{1/3} \qquad (3.6)$$

$$\text{or} \quad k_L = \frac{2 D_L}{d} + 0.31 \left[N_{Sc}\right]^{-2/3} \cdot \left[\frac{\Delta\rho \cdot \mu_L \cdot g}{\rho_L^2}\right]^{1/3} \qquad (3.7)$$

$$\text{where} \quad \Delta\rho = (\rho_s - \rho_L) \quad \text{or} \quad (\rho_L - \rho_G)$$

When estimating $(k_L)_{G \to L}$ one may assume that small "rigid-sphere" bubbles are formed and then the first term on the right hand side of equation (3.7) can be neglected; consequently

$$(k_L)_{G \to L} \simeq 0.31 \left[\frac{D_L^2 \cdot \rho_L \cdot g}{\mu_L}\right]^{1/3} \qquad (3.8)$$

For $(k_L)_{L \to S}$ the second term on the right hand side of equation (3.7) can often be neglected, i.e.

$$(k_L)_{L \to S} \simeq \frac{2 D_L}{d} \qquad (3.9)$$

In this case the mass transfer coefficient varies inversely with cell diameter; hence, under conditions where cells clump together the value of the mass transfer coefficient may be reduced considerably.

Interfacial Area in Tower Fermentation Systems

If it is assumed that bubbles and particles are spherical, the specific surface area at the liquid interfaces may be approximated by the following expression:

$$a = \frac{6 h_i}{d_i} \qquad (3.10)$$

where $i = G$ or S

For the liquid - solid interface

$$h_S = \frac{x}{1000 \rho_S} \qquad (3.11)$$

and thus, by combining equations (3.9), (3.10), and (3.11) we have:

$$(k_L a)_{L \to S} \simeq \frac{0.012 \cdot D_L \, x}{\rho_S \cdot d^2} \quad s^{-1} \qquad (3.12)$$

The gas-liquid interfacial area is more difficult to predict since bubble diameter and gas hold-up vary with operating conditions: DOWNIE (1972) and SHAYEGAN-SALEK (1974) have presented literature

reviews and experimental data. In bubble columns and tower fermenters SHAYEGAN-SALEK has shown that

$$h_G \simeq \alpha_1 \cdot u_G \qquad (3.13)$$

over a wide range of superficial gas velocities. Parameter "α_1" depends upon a number of factors, including air distributor type and column diameter.

KLOUD et alia (1973) have presented a computer algorithm capable of predicting (1) bubble size distribution, (2) bubble volume distribution, (3) gas hold-up, and (4) bubble surface area. The correlations used are based on functions of superficial gas velocity and suspension properties, viz: surface tension, viscosity, and density.

MORRIS (1972) and MORRIS et alia (1972) present data for Aspergillus niger fermentations indicating that the following correlation may be of use:

$$h_G = \alpha_2 + \alpha_3 \cdot u_G \qquad (3.14)$$

However, CHAKRAVARTY et alia (1972) and YOSHIDA et alia (1965) prefer correlations of the following form:

$$h_G = \alpha_4 \cdot u_G^{n_1} \qquad (3.15a)$$

$k_L a$ Values in Tower Fermentation Systems

One of the earliest publications regarding aeration in tower-type

Table 3.1 Typical Values of $k_L a$ in Various Fermentation Systems

FERMENTER TYPE	$(k_L a)_{G \rightarrow L}$ (s^{-1})	REFERENCE
1.Laboratory apparatus (shake flasks and unstirred bubbling devices)	0.0025-0.025	FINN (1967)
2.Stirred fermenters	0.005 -0.25	FINN (1967)
3.Specially designed stirred fermenters	up to 0.80	FINN (1967)
4.Tower fermenters	up to 0.12	JONES (1970) MORRIS (1972)

Table 3.2 Typical $k_L a$ Values for Tower Fermentation Processes

PROCESS		k_L(cm/s)	a(cm^2/cm^3)	$k_L a$ (s^{-1})	REFERENCE
1.Vinegar production	(L→S) (G→L)	0.50 0.012	59* 6.5*	29.5 0.08	JONES (1970)
2.Mould growth	(L→S) (G→L)	 0.043*			MORRIS (1972)
3.Yeast growth	(L→S) (G→L)	0.0038*	15*	0.057* 0.25	

* Theoretical values calculated by SMITH and GREENSHIELDS (1974)

fermenters is that of MURPHY et alia (1959). Gas hold-up and $(k_La)_{G\rightarrow L}$ values are presented graphically as a function of a number of parameters. YOSHIDA et alia (1965) present a correlation of the following form:

$$(k_La)_{G\rightarrow L} = \alpha_5 \cdot u_G^{n_2} \qquad (3.15b)$$

A more detailed correlation is that of AIBA et alia (1961), viz:

$$(k_La)_{G\rightarrow L} = \frac{\alpha_6 \cdot L^{2/3} \cdot v}{V \cdot d \cdot N_{Sc}^{1/2}} \qquad (3.16)$$

Typical k_La values for tower fermentation processes are shown in table 3.2.

3.4 FACTORS AFFECTING THE MASS TRANSFER COEFFICIENTS

(1) Temperature

$(k_La)_{G\rightarrow L}$ increases with increasing temperature (O'CONNOR, 1955).

(2) Organic Substances and Surface Active Agents

Addition of small amounts of organic substances or surface active agents can decrease the value of $(k_La)_{G\rightarrow L}$ by up to 60% (FINN, 1967; ECKENFELDER et alia, 1961; MORRIS, 1972). In contrast, EVANS and HALL (1971) claim that silicone antifoams can enhance oxygen diffusion from the gas to liquid phase.

(3) Mycelial Concentration

Liquid viscosity - which is a function of mycelial concentration - affects the value of $(k_L)_{G\rightarrow L}$, as can be seen from

equation (3.8), viz:

$$(k_L a)_{G \to L} = \alpha_7 \cdot \mu_L^{-1/3} \qquad (3.17)$$

The effect of mycelial concentration upon $(k_L a)_{G \to L}$ values has been investigated experimentally by a number of researchers, including FINN (1954), DEINDOERFER and GADEN (1955), BRIERLY and STEEL (1959), and RYU and HUMPHREY (1973).

3.5 A THEORETICAL APPROACH TO OXYGEN TRANSFER IN AN IDEAL BUBBLE COLUMN

In this section a model is developed which describes mass transfer of oxygen in an ideal bubble column. The main assumptions made are that:

(1) the liquid phase is perfectly mixed, and

(2) there is plug flow of gas through the column.

Consider an element, width δL, at position L in a column of height L and cross sectional area A (see figure 3.2).

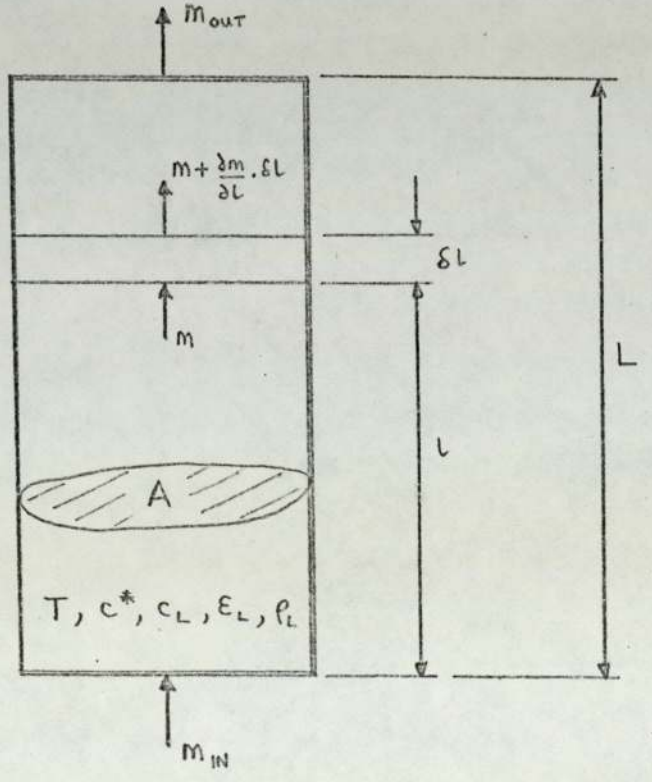


Figure 3.2 Oxygen Mass Transfer in an Ideal Bubble Column.

A steady state oxygen mass balance over the element for the gas phase can be derived as follows:

INPUT RATE : by bulk flow = m

OUTPUT RATE : by bulk flow = $m + \frac{\partial m}{\partial L} . \delta L$

by transfer to liquid phase = $(k_L a)_{G \to L} (c^* - c_L) A \delta L . h_L$

Because of the conservation of mass, INPUT RATE = OUTPUT RATE

$$\text{or} \quad m = m + \frac{\partial m}{\partial L} . \delta L + (k_L a)_{G \to L} (c^* - c_L) . A . \delta L . h_L$$

$$\text{i.e.} \quad -\frac{\partial m}{\partial L} = (k_L a)_{G \to L} (c^* - c_L) . A . h_L \qquad (3.18)$$

Assuming Henry's Law holds, then

$$c^* = \frac{p_{O_2}}{H} \qquad (3.19)$$

$$\text{and} \quad p_{O_2} = \frac{m}{m + \frac{32}{28} n} \cdot P \qquad (3.20)$$

$$\text{where total pressure} \quad P = \left[1 + \frac{(L-l) h_L \cdot \rho_L}{1060} \right] \text{ atm} \qquad (3.21)$$

$$\text{But} \quad c_L = \frac{[DO]}{100} . c^* \qquad (3.22)$$

$$\text{therefore} \quad (c^* - c_L) = \left(\frac{100 - [DO]}{100}\right) . c^* \tag{3.23}$$

For oxygen in water $$c^* = \frac{0.468}{31.6 + T} \quad g/l \tag{3.4}$$

and $$p_{O_2} = H . c^* = 0.21 \ atm \tag{3.24}$$

$$\therefore \quad H = \frac{p_{O_2}}{c^*} = \left(\frac{31.6 + T}{0.468}\right) 0.21 \tag{3.25}$$

Substituting equations (3.4) and (3.19) to (3.25) into (3.18) gives:

$$-\frac{\partial m}{\partial L} = (k_L a)_{G \to L} . A . h_L \left(\frac{100 - [DO]}{100}\right) . \frac{m}{m + \frac{32}{28} n} . \frac{0.468}{0.21(31.6+T)} . \left[1 + \frac{(L-l) h_L . \rho_L}{1060}\right] \tag{3.26}$$

Integrating equation (3.26) we have:

$$\int_{m_{IN}}^{m_{OUT}} \left[\frac{m + \frac{32}{28} n}{m}\right] . dm = \beta . \int_0^L \left[1 + \frac{(L-l) h_L . \rho_L}{1060}\right] . dl \tag{3.27}$$

where $$\beta = (k_L a)_{G \to L} . A . h_L \left[1 - \frac{[DO]}{100}\right] . \left[\frac{0.468}{0.21(31.6+T)}\right] \tag{3.28}$$

$$\text{Then, } \left[m + \frac{32n}{28}\cdot \ln(m)\right]_{m_{OUT}}^{m_{IN}} = \beta\cdot\left[l + \frac{(Ll - \frac{1}{2}l^2)\,h_L\cdot\rho_L}{1060}\right]_0^L \qquad (3.29)$$

$$\text{and} \quad (m_{IN} - m_{OUT}) + \frac{32n}{28}\cdot \ln\left(\frac{m_{IN}}{m_{OUT}}\right) = \beta L\cdot\left[1 + \frac{\frac{1}{2}L\cdot h_L\cdot\rho_L}{1060}\right] \qquad (3.30)$$

$$\text{or} \quad (m_{IN} - m_{OUT}) + \frac{32n}{28}\cdot \ln\left(\frac{m_{IN}}{m_{OUT}}\right) = (k_L a)_{G\to L}\cdot V\cdot h_L\left[1 - \frac{[DO]}{100}\right]\cdot\left[\frac{2.23}{31.6+T}\right]\cdot\left[1 + \frac{L\cdot h_L\cdot\rho_L}{2120}\right] \qquad (3.31)$$

Figure 3.3 shows oxygen transfer rate as a function of air flow rate. (see p. 59)

3.6 A MODEL FOR OXYGEN TRANSFER IN A TOWER FERMENTER

The backflow stirred tanks mixing model (see Section 2.4) is extended here to include oxygen transfer. Figure 3.4 shows a tower fermentation system, divided into N stages; the typical n^{th} stage is depicted in figure 3.5.

It is assumed that:

(1) there is perfect mixing in the liquid phase in each stage, and

(2) the gas is in plug flow throughout the length of the column.

A steady state oxygen mass balance on stage n can be derived as follows:

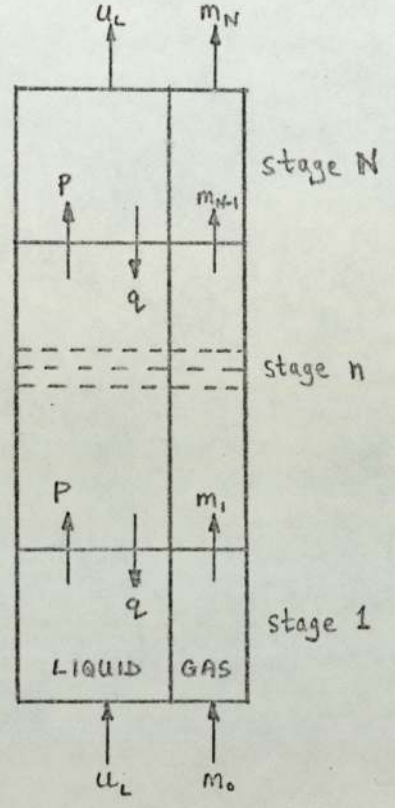
$$\text{INPUT RATE} = m_{n-1} + p\cdot c_{n-1} + q\cdot c_{n+1}$$

$$\text{OUTPUT RATE} = m_n + p\cdot c_n + q\cdot c_n + R_n\cdot V\cdot h_{L,n}$$

Because of the conservation of mass, INPUT RATE = OUTPUT RATE, thus

$$m_{n-1} - m_n = -p\cdot c_{n-1} + (p+q)\,c_n - q\cdot c_{n+1} + R_n\cdot V\cdot h_{L,n} \qquad (3.32)$$

Figure 3.4 Oxygen Mass Transfer in a Tower Fermentation System



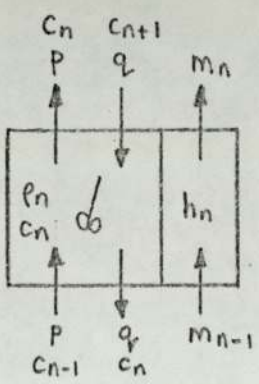


Figure 3.5 Typical n^{th} Stage of a Tower Fermenter Model

or, in matrix form: $\underline{A} \cdot \underline{m} = \underline{B} \cdot \underline{c} + \underline{k_1}$ (3.33)

where $\underline{A} =$

$$\begin{bmatrix} -1 & & & & \\ 1 & -1 & & & \\ & \ddots & \ddots & & \\ & & 1 & -1 & \\ & & & 1 & -1 \end{bmatrix}$$

$\underline{B} =$

$$\begin{bmatrix} P & -q_1 & & & \\ -P & (P+q) & -q & & \\ & \ddots & \ddots & \ddots & \\ & & -P & (P+q) & -q \\ & & & -p & (u+q) \end{bmatrix}$$

and $\underline{k}_1$

$$\begin{bmatrix} R_1.V.h_{L,1} - m_0 - u.c_0 \\ R_2.V.h_{L,2} \\ \vdots \\ R_N.V.h_{L,N} \end{bmatrix}$$

Considering the gas phase only, a steady state mass balance for stage n leads to:

$$m_{n-1} - m_n = (k_L a)_{G \to L} \cdot V \cdot h_{L,n} \cdot (c^* - c_L) \qquad (3.34)$$

or, in matrix form (and assuming $h_{L,n} = h_L$, a constant):

$$\underline{A} \cdot \underline{m} = (k_L a)_{G \to L} \cdot V \cdot h_L (\underline{c}^* - \underline{c}) + \underline{k}_2 \qquad (3.35)$$

where $\underline{k}_2 = \begin{bmatrix} -m_o \\ 0 \\ \vdots \\ 0 \end{bmatrix}$

Combining equations (3.33) and (3.35), and rearranging, we have:

$$\underline{A} \cdot \underline{m} = \underline{B} \cdot \underline{c} + \underline{k}_1$$

$$= (k_L a)_{G \to L} \cdot V \cdot h_L (\underline{c}^* - \underline{c}) + \underline{k}_2$$

Thus $\left(\underline{B} + (k_L a)_{G \to L} \cdot V \cdot h_L \cdot \underline{I}\right) \cdot \underline{c} = (k_L a)_{G \to L} \cdot V \cdot h_L \cdot \underline{c}^* + \underline{k}_2 - \underline{k}_1$

therefore $\underline{c} = \underline{D}^{-1} \cdot \underline{E} \qquad (3.36)$

where $\underline{D} = \left(\underline{B} + (k_L a)_{G \to L} \cdot V_i h_L \cdot \underline{I}\right)$

and $\underline{E} = \left((k_L a)_{G \to L} \cdot V_i h_L \cdot \underline{c}^* + \underline{k}_2 - \underline{k}_1\right)$

Method of Solution

Step 1

Calculate $\underline{c}^*$ from equations (3.19), (3.20), (3.21), and (3.25), or:

$$c_n^* = \left[\frac{\bar{m}_n}{\bar{m}_n + \frac{32}{28}\cdot n}\right] \cdot \left[\frac{2.23}{31.6+T}\right] \cdot \left[1 + \frac{\bar{L}_n \cdot h_{L,n} \cdot \rho_{L,n}}{1060}\right] \qquad (3.37)$$

where $\bar{m}_n = \frac{m_n + m_{n-1}}{2}$

and $\bar{L}_n = \left(N - n + \frac{1}{2}\right) \cdot \frac{L}{N}$

and where, as a first approximation: $m_n = m_o$

Step 2

Substitute $\underline{c}^*$ in equation (3.36) to compute $\underline{c}$.

Step 3

Substitute $\underline{c}$ in equation (3.35) and calculate $\underline{m}$.

Step 4

Substitute m in equation (3.37) and recompute c*.

Step 5

Continue with step 2 in an iterative fashion until values of m and c have converged satisfactorily.

Points of Note

(1) Calculation of m_o and n

m_o and n are calculated from air flowrate (temperature and pressure must be known), assuming 21 mole% oxygen and 79 mole% nitrogen.

(2) Computation of q

q can be calculated from equation (2.52) below:

$$q = v_G . w \qquad (2.52)$$

where v_G is the volumetric gas flowrate and w the wake factor. The wake factor is calculated from equation (2.59) below:

$$\ln(w) = k_1 + k_2 . u_G + k_3 . u_L \qquad (2.59)$$

Suitable values of the constants k_1, k_2, and k_3 can be found in table 2.3 (page 49).

LIST OF SYMBOLS (Section 3)

SYMBOL	EXPLANATION	UNITS
a	specific surface area	cm^2/cm^3
A	cross-sectional area of column	cm^2
c	concentration of dissolved oxygen in medium	g/l
c*	equilibrium concentration of dissolved oxygen in medium	g/l
d	diameter of bubble/microorganism	cm
D_L	diffusivity of oxygen in medium	cm^2/s
[DO]	dissolved oxygen level (percentage of maximum)	%
g	gravitational constant	cm/s^2
h	hold-up	-
H	Henry's Law constant	atm.l/g
k_i	constant (i = 1,2,3...)	-
k_L	mass transfer coefficient	cm/s
l	axial position in column	cm
L	height of column	cm
m	mass flowrate of oxygen	g/s
n	mass flowrate of nitrogen	g/s
Po_2	partial pressure of oxygen	atm
p	forward liquid flowrate	cm^3/s
P	total pressure	atm
q	liquid backflow	cm^3/s
Q	oxygen transfer rate	g/l.s

SYMBOL	EXPLANATION	UNITS
v	volumetric gas flowrate	cm^3/s
V	volume of mixing stage	cm^3
x	biomass concentration	g/l

Greek letters

μ	liquid phase viscosity	g/cm s
ρ	density	g/cm^3

Subscripts

G	gas phase
L	liquid phase
n	stage number
s	solid phase

Dimensionless Groups

N_{Ra}	Raleigh number
N_{Sc}	Schmidt number
N_{Sh}	Sherwood number

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SECTION 4

MICROORGANISMS: GROWTH, SURVIVAL, AND KINETIC BEHAVIOUR

Figures

Figures

Tables

4.1 INTRODUCTION

In the first part of this section the types of organisms most commonly used for industrial fermentation processes are briefly described: the specific examples quoted have all been employed in the T.F.R.G's laboratories at the University of Aston. A short description of the nutritional and energy requirements for maintenance, growth, and metabolite production follows, and then mechanisms of growth and survival, and the roles of enzymes, nucleic acids, and metabolic pathways are introduced and briefly outlined. The second part of the chapter describes patterns of growth and metabolic production, and the third part introduces a number of well-known kinetic models which describe microbial kinetics. Finally, the effects of some of the more important physical and chemical parameters upon microbial kinetics are mentioned.

4.2 MICROBIAL LIFE

4.2.1 Types of Microorganisms

Three main classes of organisms are used in industrial fermentation processes, namely: yeasts, moulds, and bacteria.

Yeasts are single cells of about six microns diameter which, under certain conditions, can agglomerate to form "flocs". Perhaps the best-known yeast is Saccharomyees cerevisiae, which is used for the alcoholic fermentation of brewers' hopped wort to produce beer; different strains of this yeast are used for baking purposes.

Although yeasts are classified as fungi, they are not "true fungi" since they are unable to grow as multicellular chains. Moulds, however, grow in the form of microscopic filaments, called hyphae, which collectively

form the mycelium, and are thus classified as true fungi. The diameter of these filaments is about five microns, and moulds can assume a number of morphological forms depending upon local environmental conditions. Aspergillus niger is a well-known industrial mould which can be utilised to produce a number of substances including oxalic acid, citric acid, gluconic acid, amylases, steroids, or, simply, fungal biomass.

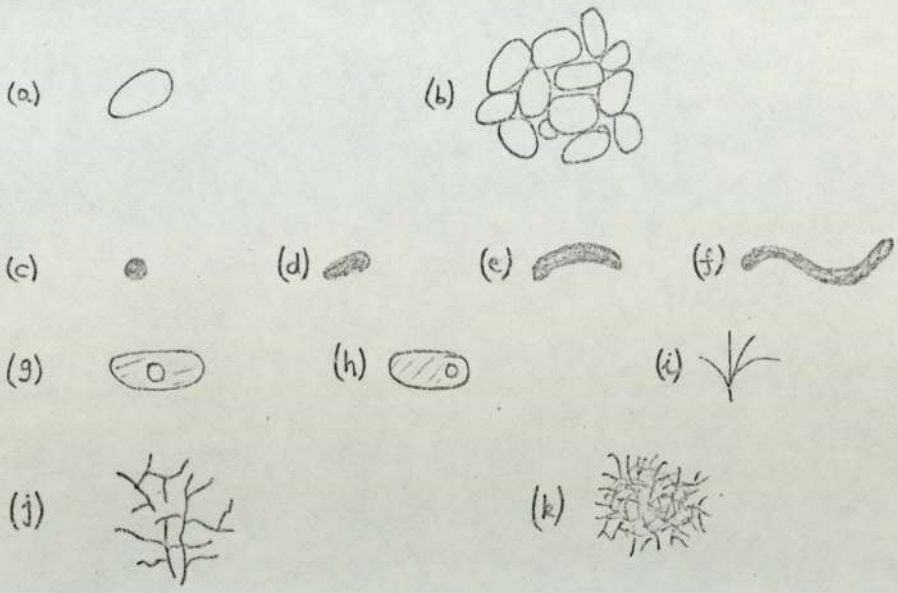
The third main class of microorganism, bacteria, are smaller than yeasts and moulds, and vary greatly in shape. They are normally unicellular, though some are filamentous, and their size is of the order of one micron diameter. The vinegar fermentation is effected by the species Acetobacter, a type of bacteria which can oxidise ethanol to acetic acid. Figure 4.1 shows various types of yeast, bacteria, and moulds.

4.2.2 Microbial Cell Composition

Normally about eighty percent of a microbial cell is water. This figure varies enormously, though, since it is almost impossible to distinguish between water associated with cell dry matter and interstitial fluid in microbial colonies. It is therefore difficult to determine exact "wet" weights of biomass.

The dry matter composition of a cell consists almost entirely of carbon, hydrogen, oxygen, and nitrogen with small amounts of phosphorus, sulphur, iron, potassium, and magnesium, with other elements in trace quantities. Typical elemental compositions of microbial cells are shown in table 4.1. Component elements are present in cells in the form of sugars, organic acids, nucleic acids, nucleotides, nucleoside phosphates,

Figure 4.1 Microbial Species



Key:

Yeast	Bacteria	Moulds
(a) yeast cell	(c) coccus	(j) filamentous form
(b) yeast floc	(d) bacterium	(k) pellet form
	(e) vibrio	
	(f) spirillum	
	(g) bacillus (central spore)	
	(h) bacillus (terminal spore)	
	(i) actinomycete	

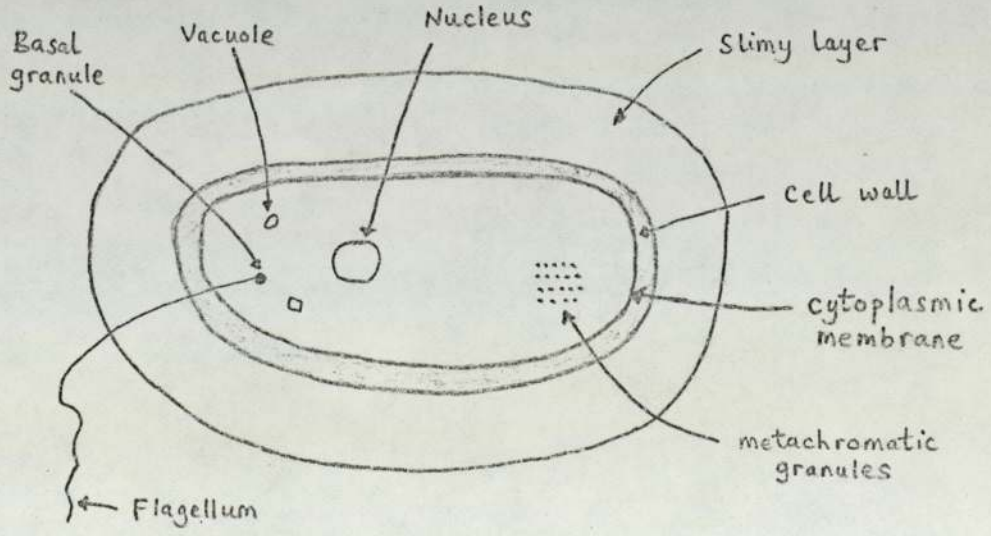


Figure 4.2 Microbial Cell

vitamins, fats, proteins, and polypeptides. Together, these compounds make up the nucleus and "internals" of a cell, the surrounding cytoplasmic membrane, and the cell wall. A typical microbial cell is shown diagrammatically in figure 4.2.

4.2.3 Nutrient Requirements

Microorganisms require a supply of nutrients from the surrounding environment for synthesizing and maintaining their cell substance. Abundant supplies of carbon, nitrogen, water, and energy are required in addition to small quantities of inorganic salts and growth factors. These substances are absorbed into the cells due to the action of a number of transport mechanisms (see Section 6.1.4 for a more detailed description). Once inside the cell, the nutrients are metabolised by any one of a number of routes. Microorganisms are capable of effecting a vast number of biochemical conversions by utilising different metabolic pathways. Control mechanisms within the cells regulate the activity of the organism, and thus it can adapt itself to a number of different environmental conditions.

4.2.4 Energy Transfer

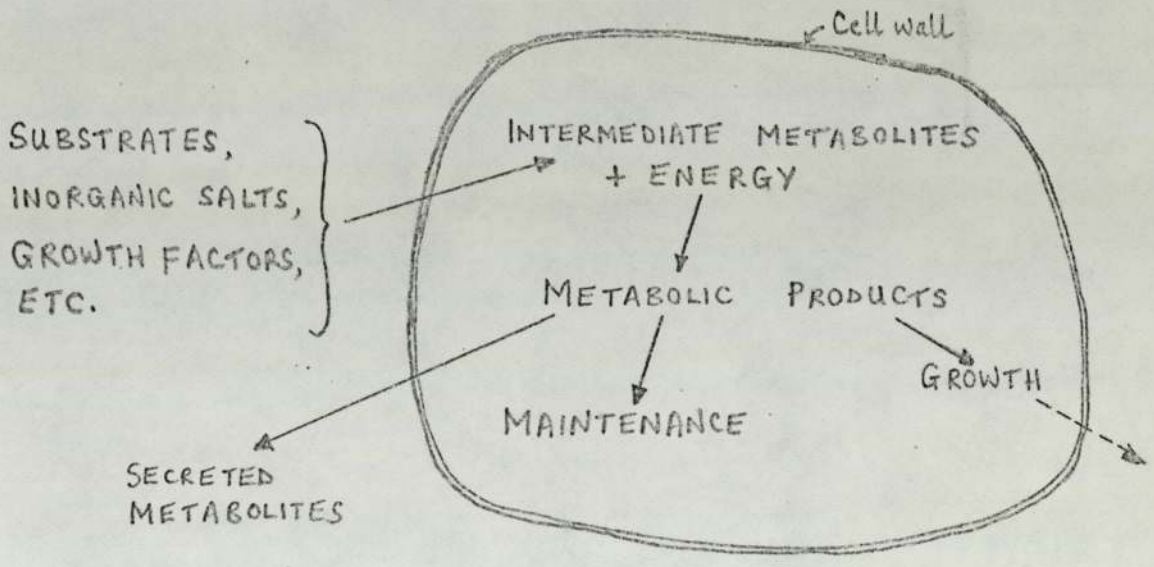
Energy is required to maintain microbial cells in good working order; this is normally supplied by the dissimilation of nutrients (e.g. biological oxidation of carbohydrates to carbon dioxide and water). Energy produced by microorganisms is generally used to generate organic compounds containing high energy sulphur or phosphate bonds. The most important of these compounds is adenosine triphosphate (ATP), and by accumulating this substance the cell is able to "store" energy. Energy

made available in this fashion is utilised for assimilation purposes, i.e. the production of complex, high molecular weight compounds which may be used for maintenance and growth, or secreted as metabolic products. A "general equation" for microbial activity may be written:

SUBSTRATES $\xrightarrow{\text{enzymes}}$ INTERMEDIATE METABOLITES + ENERGY $\xrightarrow{\text{enzymes}}$ METABOLIC PRODUCTS → GROWTH / MAINTENANCE / EXTRA-CELLULAR ENVIRONMENT

The interaction between a microbial cell and its environment can also be shown pictorially, as in figure 4.3.

Figure 4.3 Simplified Microbial System



4.2.5 Growth and Survival

It is often said that the sole objectives of microorganisms is survival. In order to survive microbes must grow and reproduce, since the life of individual cells is finite. Growth of microorganisms can be defined as an increase of total microbial dry mass in a system. This can occur in two ways:-

(1) an increase in the size of individual cells (between narrow limits), and

(2) binary division.

Two mechanisms of yeast growth are shown in figure 4.4.

From another view point, growth is the result of the interaction between the individual cell and its local environment. The laws of thermodynamics and of mass and energy transfer must all apply to the interchange. Consequently, physical and chemical parameters such as termperature, pressure, pH, and nutrient and product concentrations all affect growth kinetics (see Section 4.5).

Like the human body, microbes are able to accumulate fatty substances (for example polysaccharides and polyesters) as a reserve food supply. Under certain unfavourable conditions spores are formed in order to maintain continuity of the species. The microbial spore is capable of withstanding extreme physical and chemical conditions and respires very slowly using its reserve food supplies to provide energy. When conditions become favourable the spore germinates and the growth cycle starts once again.

4.2.6 Enzymes

All microbial reactions are effected by enzymes, which are proteins of high molecular weight and act as "biological catalysts". Reactions must be thermodynamically possible, of course, i.e. involving a loss of free energy. Enzymes are very specific in their action, a given enzyme only catalysing one type of reaction. Thus, a metablic pathway involving a whole series of reactions requires a number of different enzymes. Whilst most enzymes act within the cell, some act on the surface of the cell wall (e.g. hydrolases) and others extra-cellularly (e.g. amylases).

4.2.7 Nucleic Acids

Two types of nucleic acids are present in microbial cells, Deoxyribo-Nucleic Acid (DNA) and Ribo-Nucleic Acid (RNA): both have ability to bind via phosphate groups to form a chain which can act as a code. DNA is the carrier of genetic information in cells - the "code of life". RNA is produced as a representation of the code of DNA; it travels to the ribosomes which are the sites of protein synthesis, where it codes for a specific amino acid sequence. The procedure enables specific proteins to be manufactured.

Increases in microbial growth rate are due to increased proportions of the nucleic acids (RNA and DNA).

4.2.8 Aerobic and Anaerobic Processes

Fermentation processes are normally classified either as aerobic or anaerobic (i.e. with or without air). Yeast, for example, possesses aerobic and anaerobic systems and can function effectively using both

Figure 4.4 Yeast Growth Mechanisms

(a) division by budding
(b) division by fission

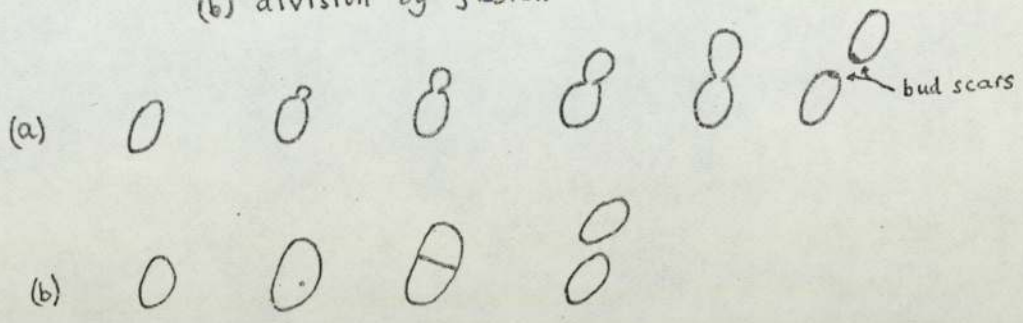
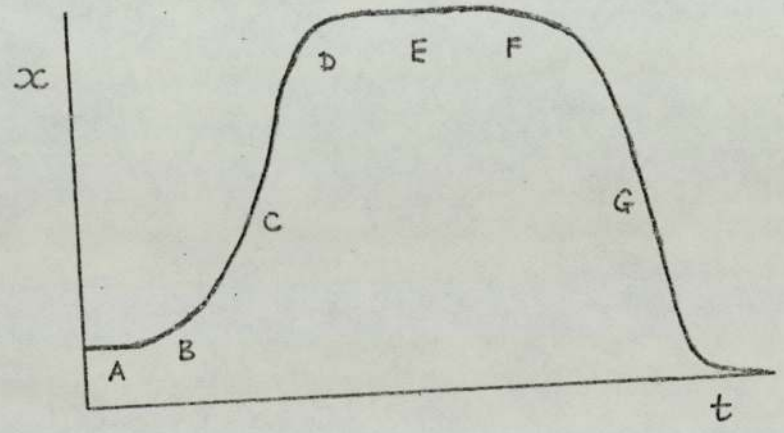


Figure 4.5 Bacterial Growth Curve



Key:
A = lag phase
B = acceleration phase
C = exponential growth
D = declining growth
E = stationary phase
F = accelerating death
G = exponential death

Table 4.1 Elemental Composition of Microbial Cells

Microorganism	Reference	Elemental Composition (%) C	H	O	N	other (S,P,K,etc.)
Yeast	Harrison (1967)	44.6	6.1	31.2	8.5	9.6
"	Olbricht (1956)	47	6	32.5	8.5	6
"	Peppler (1970)	45-47	6-6.5	31-32	7.5-9	8
Bacteria	Abbott (1973)	53	7.3	19	12	8.7
"	Kihlberg (1972)	48	7.2	24	14	6.2

modes, either independently, or simultaneously. Abundant oxygen and low carbohydrate concentrations favour the aerobic system: this releases energy which is normally used for growth purposes. The two modes of operation can be represented by simple "overall" chemical equations. (One must remember, though, that, despite their apparent simplicity, these equations embrace whole chains of reactions along metabolic pathways.)

(1) aerobic: $C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + 37$ ATP*

(2) anaerobic: $C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2 + 2$ ATP*

* See Section 8.6.3 and figure 8.1, p239.

4.3 PATTERNS OF GROWTH AND METABOLITE PRODUCTION

4.3.1 The Growth Curve

The growth cycle of bacteria generally follows an s-shaped curve, which can be split into a number of distinct phases, as shown in figure 4.5.

A. The lag phase

When a bacterial population is transferred to a new medium there is a lag phase during which there is little detectable activity. During this period the organism adjusts to the new extra-cellular environment. The length of this period of adaptation is affected by many factors (DEAN and HINSHELWOOD, 1966).

B. The acceleration phase

This corresponds to the period during which the specific rate of growth of the organism increases to a constant value.

C. The logarithmic growth phase

Under ideal growth conditions, i.e. an excess of all nutrients and growth factors, logarithmic, or exponential, growth occurs. The rate of growth is proportional to the mass of bacteria present, and thus the microbial mass doubles at constant intervals. In practice it is difficult to attain truly exponential growth, except, maybe, for very short periods of time.

D. The retardation phase

During logarithmic growth, nutrients and growth factors must be supplied to the growing microbial mass at an exponentially increasing rate. Eventually, one or more of these substances will become depleted to such a level that growth is limited. Alternatively, growth may become limited by mass transfer or overcrowding problems, or, possibly, by product inhibition.

E. The stationary phase

Here the growth rate is zero, i.e. the rate of formation of biomass equals the death rate. Eventually the nutrients in the medium become completely exhausted.

F. The death phase

As a culture ages the remaining viable cells become increasingly likely to die. Eventually the whole culture dies, since the life of each cell is finite, and the medium is unable to support growth.

Variations in mean cell size

A growth curve based upon the number of cells present in a system differs from one based upon the total biomass. This is because the mean

size of cells changes during the course of the growth cycle. During the lag phases the cells become larger, but their mean size remains constant during the logarithmic growth period. The size of the cells often decreases slightly at the end of the exponential phase.

4.3.2 Types of Fermentation Processes

A number of researchers have attempted to classify fermentation processes.

MAXON (1955) proposed three types, depending upon the growth-product formation relationships:

(1) product formation directly related to growth,

(2) product formation associated with growth, and

(3) product formation not associated with growth.

GADEN (1955, 1959), however, preferred to base his classification upon the substrate utilisation - product formation relationship and distinguished between three types of fermentation process, as follows:

(1) product formation directly related to substrate utilisation,

(2) product formation indirectly related to substrate utilisation, and

(3) product formation apparently not associated with substrate utilisation.

DEINDOERFER (1960), on the other hand, adopted a chemical engineering approach and classified fermentation processes into four types, depending upon the reaction sequences involved, viz:

(1) simple type - nutrients converted to products in a fixed stoichiometry without accumulation of intermediates,

(2) simultaneous type - nutrients converted to products in variable stoichiometric proportions without accumulation of intermediates,

(3) consecutive type - nutrients converted to product with accumulation of an intermediate, and

(4) stepwise type - nutrients going to an intermediate before conversion to product, or nutrients selectively converted to product in preferential order.

4.4 KINETIC MODELS

4.4.1 Introduction

A number of kinetic models for microbial growth and product formation have been proposed since the pioneering work of MICHAELIS and MENTEN (1913) and MONOD (1949, 1950). An excellent mathematical presentation relating a number of these models has been published by TSUCHIYA, FREDERICKSON and ARIS (1966). TSUCHIYA et alia classify kinetic models into a number of groups. The microbial cell population may be either distributed or segregated. A distributed model treats the population as a protoplasmic mass distributed uniformly throughout the population's living space. Alternatively, a segregated model accounts for microbial life in terms of functional units.

Segregated models may be further classified; they can be structured or unstructured. Unstructured models do not account for differences between individual cells. Elements of structure are numerous and may include size, shape, mass, and chemical composition of individual cells.

A further model classification is the distinction between

deterministic and stochastic (or probabilistic) models. TSUCHIYA et alia state that:

"A population of microbial cells is always segregated and structured, and its growth and reproduction should be treated stochastically. On the other hand, the biological knowledge and mathematical tools necessary for the formulation and study of a completely general model do not exist, and a less general approach gives useful results".

It is for this reason that the kinetics used for the mathematical models in this thesis are based upon the Michaelis-Menten equation. This model, later adopted by MONOD, is distributed, and deterministic: although it has its disadvantages, it is the most widely-used, and also one of the simplest kinetic models available.

4.4.2 Michaelis-Menten Kinetics

Kinetic studies of enzyme reactions led to a theory that an intermediate complex is formed between enzyme and substrate. MICHAELIS and MENTEN (1913) further developed this hypothesis by assuming that in an irreversible reaction an equilibrium exists among enzyme (E), substrate (S), and enzyme-substrate complex (E-S) as follows:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E\text{-}S \xrightarrow{k_3} E + P \qquad (4.1)$$

This led to the Michaelis-Menten equation, which can be expressed as:

$$r = -\frac{ds}{dt} = \frac{k_3 S}{K_m + S} \qquad (4.2)$$

where the Michaelis-Menten constant, $K_m = \frac{k_2 + k_3}{k_1}$ (4.3)

Equation (4.2) can be rearranged to a dimensionless, general form as follows:

$$\frac{r}{k_1} = \frac{(S/K_m)}{(1 + S/K_m)} \qquad (4.4)$$

A plot of equation (4.4) is shown in figure (4.6)

In 1935 HOPKINS and ROBERTS successfully applied such kinetics to the fermentations of both fructose and glucose by yeast, and determined values for the constants "k" and "Km".

Expressions of the form of equation (4.1) are often termed "saturation kinetics", and Km in equations (4.2) and (4.3) is known as the saturation constant. At both high and low substrate concentrations the equation reduces to simple zero or first order kinetics, respectively, viz:

$$S \gg K_m \qquad -\frac{dS}{dt} \simeq k \qquad (4.5)$$

$$S \ll K_m \qquad -\frac{dS}{dt} \simeq \frac{k}{K_m} \cdot S \qquad (4.6)$$

Rearrangement of the Michaelis-Menten equation yields a linear relationship between $(1/r)$ and $(1/s)$, viz:

$$\left(\frac{1}{r}\right) = \frac{1}{(dS/dt)} = \frac{K_m}{k} \cdot \left(\frac{1}{S}\right) + \frac{1}{k} \qquad (4.7)$$

Figures 4.6 and 4.7 Dimensionless Plots of the Michaelis-Menten Equation
(constant microbial mass)

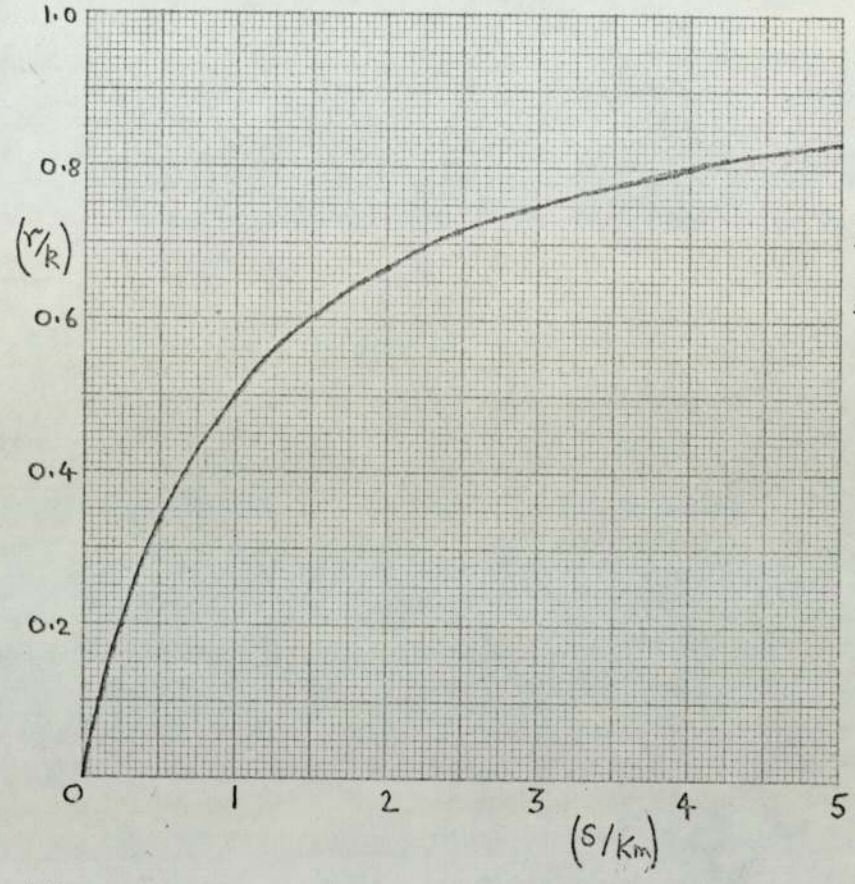


Fig. 4.6
Rate Expression

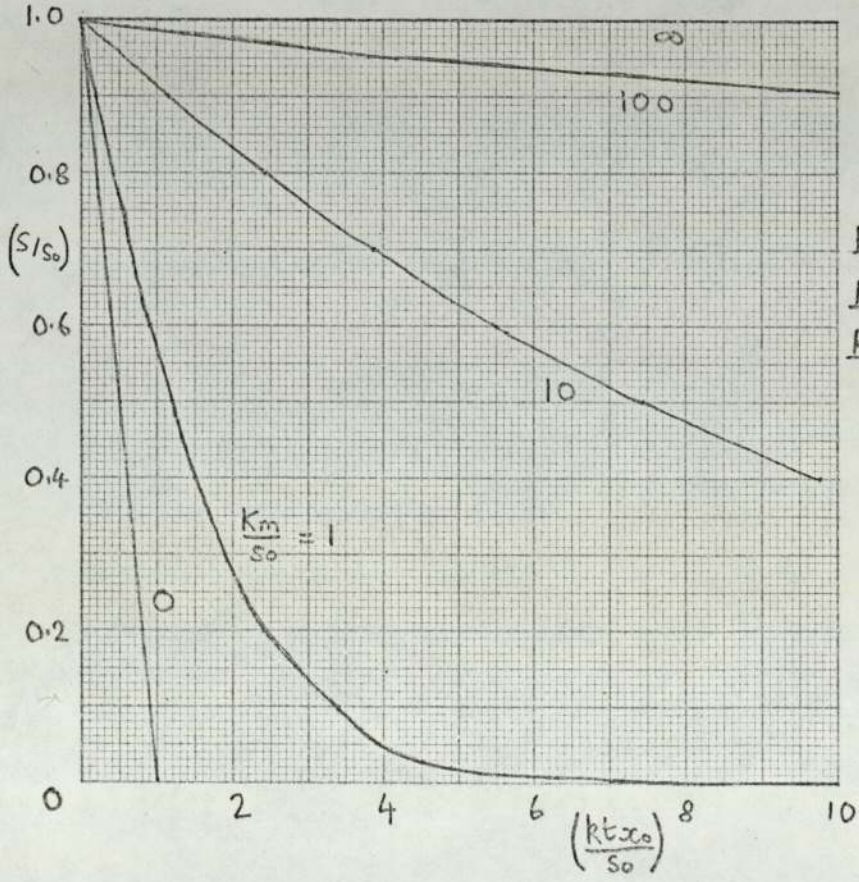


Fig. 4.7
Integrated
Rate Expression

LINEWEAVER and BURK (1934) utilised this equation for testing and interpreting kinetic data.

4.4.3 The Application of Michaelis-Menten Kinetics To Single-Substrate Fermentations

In this section time-concentration expressions are derived mathematically for the ideal conditions of (1) constant microbial mass, and (2) exponentially-increasing microbial mass. In real batch fermentations, though, growth is generally limited by one or more of the nutrients and a complex set of simultaneous, non-linear differential equations is required to describe the system adequately.

(1) constant microbial mass

When biomass concentration remains constant equation (4.2) can be integrated directly (assuming that k and Km are not functions of either s or x), yielding:

$$kt x_o = K_m \cdot \ln\left(\frac{S_o}{S}\right) + (S_o - S) \qquad (4.8)$$

Figure (4.7) shows the effect Km has upon the time-concentration curve derived from equation (4.8).

(2) exponential microbial growth

Under ideal conditions the rate of increase of biomass is proportional to the quantity of biomass present in a system, thus:

$$\frac{dx}{dt} = gx \qquad (4.9)$$

$$\text{or} \quad x = x_o \cdot \exp(gt) \tag{4.10}$$

and "doubling time", $t_d = \frac{\ln 2}{g} = \frac{0.693}{g}$ (4.11)

Combining equations (4.10) and (4.1) gives:

$$-\frac{ds}{dt} = \frac{k_1 s}{K_m + s} \cdot x_o \cdot \exp(gt) \tag{4.12}$$

Assuming k, Km, and g to be constant, direct integration yields:

$$gt = \ln\left\{\left(\frac{g}{kx_o}\right).\left[K_m . \ln\left(\frac{S_o}{S}\right) + (S_o - S)\right] + 1\right\} \tag{4.13}$$

The Effect of Growth Rate Upon Reaction Time

Denoting time by t' in equation (4.8) and by t in equation (4.13) and comparing these two equations we have:

$$gt = \ln(gt' + 1) \tag{4.14}$$

$$\text{or} \quad t' = \left[\frac{\exp(gt) - 1}{g}\right] \tag{4.15}$$

$$\text{Hence:} \quad t' = t + g.\frac{t^2}{2!} + g^2.\frac{t^3}{3!} + \ldots + g^{n-1}.\frac{t^n}{n!} + \ldots \qquad (4.16)$$

Equations (4.14), (4.15), and (4.16) above describe the relationship between "reaction times" for the extremes: constant biomass and exponential microbial growth. The considerable effect of rate of growth, g, upon reaction time is illustrated by figure 4.8 and table 4.2.

4.4.4 Monod's Model

MONOD (1949, 1950) suggested an empirical expression to describe growth of unicellular bacteria in an environment where cessation of growth is due to one limiting nutrient, viz:

$$\frac{1}{x} \cdot \frac{dx}{dt} = \mu = \frac{\mu_m \cdot S}{K_m + S} \qquad (4.17)$$

where μ = specific growth rate, and

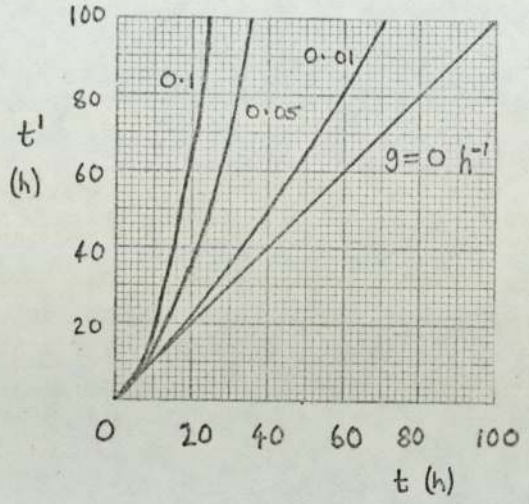
μ_m = maximum specific growth rate

In addition, MONOD showed experimentally that, under certain conditions, carbon-limited growth led to a constant yield factor, Y_T, i.e.

$$\frac{dx}{dt} = -Y_T \cdot \frac{dS}{dt} \qquad (4.18)$$

where Y_T = $\frac{\text{mass of organism formed}}{\text{mass of substrate utilised}}$ (4.19)

Figure 4.8 and Table 4.2 Effect of Growth Rate upon Reaction Time



t (h)	t' (h)			
	g = 0	g = 0·01	g = 0·05	g = 0·10 (h⁻¹)
0	0	0	0	0
10	10	10·5	13·0	17·2
20	20	22·1	34·4	63·9
30	30	35·0	69·6	190·1
40	40	49·2	127·8	536·0
50	50	64·9	233·6	1474·1

4.4.5 Variations of The Monod Equation

A number of kinetic expressions which are similar to the Monod model have been used by other workers, some of which are listed below.

(1) TEISSIER (1942), SPICER (1955)

$$\mu = b_1\left(1 - e^{-S/b_2}\right) \qquad (4.20)$$

For $S \ll b_2$ $\quad \mu \simeq \frac{b_1}{b_2} \cdot S$

and for $S \gg b_2$ $\quad \mu \simeq b_1$

Thus, Teissier's equation approximates to the Monod model when $b_1 = \mu_m$ and $b_2 = Km$.

(2) MOSER (1958)

$$\mu = \left(\frac{b_3}{1 + b_4 \cdot S^{-b_5}}\right) \qquad (4.21)$$

When $b_5 = 1$, Moser's equation becomes identical to the Monod expression with $b_3 = \mu_m$ and $b_4 = Km$.

(3) KONAK (1974)

Konak derived a generalised rate equation relating the specific growth rate of bacteria to the concentration of limiting nutrient in the fermentation medium, viz:

$$\frac{dz}{ds} = b_6 \cdot \mu_m^{b-1} \cdot (1-z)^b \qquad (4.22)$$

where $z = \mu / \mu_m$ (4.23)

Integration yields:

for $b = 1$ $\quad -\ln(1-z) = b_6 \cdot S$ (4.24)

and for $b \neq 1$ $\quad (1-z)^{1-b} - 1 = b_6 \cdot (b-1) \cdot \mu_m^{b-1} \cdot S$ (4.25)

Rearrangement of equation (4.24) gives:

$$\mu = \mu_m \left(1 - e^{-b_6 \cdot S}\right) \qquad (4.26)$$

i.e. Teissier's equation. Substituting $b = 2$ into equation (4.25), and rearranging, we have:

$$\mu = \mu_m \left[\frac{S}{S + \left(\frac{1}{b_6 \cdot \mu_m}\right)}\right] \qquad (4.27)$$

which is Monod's equation with Km = $(\frac{1}{b_6 \cdot \mu m})$. It is interesting to note that the Michaelis-Menten constant is inversely proportional to the maximum specific growth rate.

(4) CONTOIS (1959), FUJIMOTO (1963)

CONTOIS, who studied continuous cultures of bacteria, found that specific growth rate could be related to both biomass concentration and limiting nutrient concentration as follows:

$$\mu = \frac{\mu_m \cdot S}{b_7 \cdot x + S} \qquad (4.28)$$

FUJIMOTO also included biomass concentration in the specific growth rate expression, and derived an equation which, under certain conditions, reduces to equation (4.28).

4.4.6 Deficiencies of The Monod Equation

The Monod equation is purely empirical and is therefore unlikely to provide an accurate description of all fermentation systems under all possible environmental conditions. However, it is extremely simple and has been used by a large number of workers: it is particularly successful when applied to systems utilising unicellular bacteria. Fermentations involving filamentous fungi or aggregates of yeast often require modified kinetics to account for diffusional effects. GREENSHIELDS and SMITH (1971) suggested that this difficulty could be overcome by including an "effectiveness factor" into the kinetic equation, viz:

$$\frac{1}{x} \cdot \frac{dx}{dt} = \mu \cdot \phi \qquad (4.29)$$

Mathematical expressions describing process parameters such as temperature, pH, and concentrations of inhibitory substances may also be combined with the kinetic equation in a similar fashion (see Section 4.5).

A major disadvantage of simple kinetic expressions, such as the Monod equation, is that they are unable to describe the full course of microbial cell growth, as shown in figure 4.5 and described in Section 4.3.1. Whilst steady state fermentations can often be dealt with satisfactorily by Monod kinetics, transient systems cannot. The author, however, has proposed a means of modifying simple kinetics to account for the way in which microorganisms adapt to a multi-substrate environment (see Section 6.2).

A further criticism of the Monod model is that it assumes a constant value for the yield factor, Y_T. In practise Y_T is found to vary due to (1) variations in cellular composition with growth rate, and (2) endogenous respiration required for maintenance energy.

The true yield, Y_T, can be related to the yield based upon the substrate used specifically for growth, Y_G, as follows (assuming no variation in cellular composition):

$$-\frac{ds}{dt} = \frac{1}{Y_G} \cdot \frac{dx}{dt} + mx \qquad (4.30)$$

where m = specific maintenance coefficient

Thus $$\frac{1}{Y_T} = \frac{1}{Y_G} + \frac{m}{\mu} \qquad (4.31)$$

4.4.7 The Luedeking - Piret Model

LUEDEKING and PIRET (1959) examined the kinetics of batch fermentation of glucose to lactic acid and proposed the following equation:

$$\frac{dp}{dt} = \alpha \cdot \frac{dx}{dt} + \beta x \qquad (4.32)$$

$$\text{or} \quad \frac{1}{x} \cdot \frac{dp}{dt} = \alpha \cdot \mu + \beta \qquad (4.33)$$

where p = product concentration

and α, β are dimensional constants

The model combines growth-associated and non-growth-associated product formation expressions, and it has also been applied to the batchwise fermentation of glucose to ethanol (AIYAR and LUEDEKING, 1966). In both systems the constants α and β were found to vary with the pH of the fermenting medium.

4.4.8 Summary of Other Models

Sophisticated deterministic models, such as those of KONO (1968) and HOLZBERG et alia (1967), divide the growth curve into a number of stages, each described by a differential equation. KONO, and later KONO and ASAI (1969a, 1969b), studied the kinetics of microbial cell

growth from a standpoint of chemical reaction kinetics. The model consists of two differential equations - one for cell growth and one for product synthesis. Each equation contains a "growth activity coefficient", a variable expression depending upon the growth phase.

The structured growth models of EAKMAN et alia (1966), TSUCHIYA et alia (1966), RAMKRISHNA et alia (1967), and FREDERICKSON et alia (1970, 1971) are based on cell age distributions and can account for all of the growth phases. Such models, though, are extremely complex, and many of them have not yet been tested experimentally.

4.4.9 Biochemical Pathway Models

In recent years a number of kinetic models involving biochemical pathways have been published. Possibly the first of these investigations was that of FRANZ (1968), who proposed a series of equations to describe the effect of the Pasteur mechanism in yeast cells (i.e. the means by which yeast cells use both the fermentation and respiration mechanisms simultaneously in any possible proportion).

A significant advance was made by CALAM et alia (1971), who simulated the griseofulvin fermentation using a large mathematical model containing a number of equations relating to various biochemical steps.

SHEHATA and MARR (1971) and TANNER and OVERLEY (1974) both suggested models incorporating parallel fermentation pathways. VERHOFF et alia (1972) postulated a model for microbial growth consisting of two mechanisms, one involving a mass transfer or assimilation process, and

the second an ingestion and cell division process.

A comprehensive mathematical model for the growth kinetics of the yeast Saccharomyces cerevisiae has been developed by PERINGER et alia (1972a, 1972b, 1974). The model is based upon an ATP energy balance between the requirements for cellular maintenance, growth, and product synthesis. Kinetic expressions for growth rate, product synthesis rate, and yield are dependant upon substrate concentration, dissolved oxygen tension, and biomass concentration. A number of specific parameters are also required; these must be determined experimentally. Results predicted by the model were verified by experimental data and lead to a number of important physiological parameters. The most important consequence, though, is that the model is thought to be suitable for computer-controlled optimisation of fermentation processes involving yeast growth (LANE and BLACHERE, 1973).

4.4.10 Multiple - Substrate Kinetics

Most published work regarding multiple - substrate kinetics has usually been restricted to situations in which two components are important. A review of fermentation kinetics by DEINDOERFER (1960) includes the double-substrate rate equation derived by LAIDLER and SOCQUET (1950), viz:

$$\mu = \mu_m \left(\frac{S_1}{K_{m_1} + S_1} \right) \cdot \left(\frac{S_2}{K_{m_2} + S_2} \right) \qquad (4.34)$$

Similar expressions to equation (4.34) - the double Michaelis-Menten form of equation - have also been used by MEGEE (1970), FREDERICKSON et alia (1970), and RYDER and SINCLAIR (1972).

Complex expressions, such as those of SEGAL et alia (1952) and HANSON (1969) will not be discussed here.

4.5 THE EFFECT OF PROCESS PARAMETERS UPON FERMENTATION KINETICS

4.5.1 Introduction

Kinetic models generally take no account of important process variables such as temperature, pH, and inhibitory substrate and product concentrations. During batch fermentations many of these variables change appreciably, resulting in significant changes upon the rate of fermentation. This section aims to review some of the work reported in the literature concerning the effect of such parameters upon rate of fermentation.

Consider, for example, the Monod equation:

$$\mu = \frac{1}{x} \cdot \frac{dx}{dt} = \frac{\mu_m \cdot S}{K_m + S} \qquad (4.17)$$

Denoting correction factors for each parameter considered, "ϕ_i", equation (4.17) can be rewritten:

$$\mu = \frac{\mu_m \cdot S}{K_m + S} \cdot \prod_{i=1}^{n} \phi_i \qquad (4.35)$$

Any parameter affecting rate of fermentation may be incorporated into equation (4.35), the "modified kinetic model", providing a mathematical expression describing its effect upon fermentation rate can be derived. The sub-sections below deal with a number of "real" process variables.

4.5.2 Temperature

Temperature is probably the most important variable influencing microbial kinetics; consequently a large number of researchers have investigated this topic. The Arrhenius equation is often used to describe the effect of temperature upon enzymatic reactions, viz:

$$\phi_T = a_1 . \exp\left(\frac{-E}{RT}\right) \qquad (4.36)$$

MONOD (1942), INGRAHAM (1958), ROSE (1968), and RYU and MATELES (1968) are amongst the workers who have used the Arrhenius equation to modify the specific growth rate expression (see figure 4.9). FRANZ (1961) and AIBA et alia (1969) include the Arrhenius equation in the product synthesis rate expression. THORNE (1954), however, suggested that the effect of temperature upon rate of fermentation could be explained by the following expression:

$$\phi_T = a_2 . \exp(c_1 + c_2 T) + a_3 . \exp(c_3 + c_4 T) \qquad (4.37)$$

Suitable choice of the constants (a_2, a_3, c_1, c_2, c_3, c_4) allows equation (4.37) to represent many forms of temperature curve. The first term on the right hand side of the expression represents the stimulatory effect of temperature upon microbial activity, while the second term accounts for heat inactivation of enzymes at higher temperatures. At low temperatures the first term predominates, but as the temperature rises the second term becomes more prominent.

MUZYCHENKO et alia (1972) investigated the influence of temperature on the rates of formation and decay of enzymes. They proposed that the saturation constant, Km, in the Michaelis-Menten equation should be modelled using a double Arrhenius expression, whereas a conventional Arrhenius expression (i.e. equation (4.36)) suffices for the maximum specific growth rate, μ_m. INGRAHAM (1962), however, prefers a complex Arrhenius expression to describe the effect of temperature on μ_m.

TOPIWALA and SINCLAIR (1971) used a double Arrhenius expression to account for changes in the maximum specific growth rate with temperature. In addition they used an Arrhenius expression to model the temperature effects upon the endogenous respiration coefficient and the inverse of the Michaelis-Menten constant. Here it is interesting to note that KONAK (1974) proposed that the Michaelis-Menten constant is inversely proportional to the maximum specific growth rate (see Section 4.4.5). CALAM et alia (1951) used Arrhenius expressions to describe temperature effects upon the rates of mycelial growth, respiration, and penicillin production. MENNETT (1971) found that maintenance and growth rates

gave linear Arrhenius plots over a limited range.

An excellent paper suggesting means of simulating the effect of temperature upon microbial cell propogators is that of MORGAN and EDWARDS (1971). CONSTANTINIDES et alia (1970) and RAI and CONSTANTINIDES (1973) discuss means of including temperature effects in optimisation models.

WHITE and MUNNS (1951), and later WHITE (1966), investigated the effects of temperature upon the specific rates of growth and fermentation of yeast during the exponential phase. They found that both parameter-temperature functions are approximately linear between 15° and 25°C.

MERRITT (1966) noted a reduction in the length of the lag phase for beer fermentation with increasing temperature. HUNTER and ROSE (1972) observed variations in cellular composition and cell volume with temperature. MENNETT (1971), however, found that the heat of combustion of cells was not influenced by their growth temperature.

4.5.3 pH of the Fermentation Broth

LUEDEKING and PIRET (1959), and later AIYAR and LUEDEKING (1966), found variations with pH in the values of both α and β in the Luedeking - Piret kinetic model. RAI and CONSTANTINIDES (1973) also used pH-parameter functions during mathematical modelling and optimisation of the gluconic acid fermentation.

ANDREYEVA and BIRYUKOV (1972) have proposed mathematical models of the effects of pH upon the processes of cell growth and production of secondary metabolites. The models are based upon enzyme kinetics, but

the complexity of them inhibits their usefulness. Another comprehensive paper is that of MUZYCHENKO et alia (1972), who considered microorganisms as two subsystems: (1) the cellular membrane, and (2) the cytoplasm. They developed expressions showing the influence of medium pH upon (1) intracellular pH, and (2) transport mechanisms of substances through biological membranes. Combining these models with Fick's diffusion law, expressions were developed to show Km and μ_M as functions of pH. The effect of pH during batch production of beer is discussed in Section 6.3.3.

4.5.4 Product Inhibition

A number of expressions of widely - differing form have been proposed to account for product inhibition during fermentations. For bacterial systems, HINSHELWOOD (1946) suggested a linear inhibition function, as follows:

$$\mu = \mu_m . d_1 . (1 - d_2 . p) \qquad (4.38)$$

FRIEDMAN and GADEN (1970) and HOLZBERG et alia (1967) also used linear functions, but included a threshold term, viz:

$$\mu = \mu_m . d_3 . [1 - d_4 . (p - d_5)] \qquad (4.39)$$

FRANZ (1961) fitted data for the alcoholic fermentation of molasses by Bakers' yeast using a second order function, i.e.

$$\left.\begin{aligned} \frac{dp}{dt} &= \mu \cdot \left[1-\left(\frac{P}{P_{max}}\right)^2\right] \qquad P < P_{max} \\ \frac{dp}{dt} &= 0 \qquad\qquad\qquad\qquad P \geq P_{max} \end{aligned}\right\} \quad (4.40)$$

AIBA et alia (1968), however, preferred an exponential function to describe the kinetics of product inhibition during alcoholic fermentation viz:

$$\mu = d_6 \cdot \exp\left(-d_7 \cdot P\right) \qquad (4.41)$$

and

$$\frac{dp}{dt} = d_8 \cdot \exp\left(-d_9 \cdot P\right) \qquad (4.42)$$

EGAMBERDIEV and IERUSALIMSKII (1968), on the other hand, suggested an expression of the following form:

$$\mu = \frac{d_{10}}{d_{11} + P} \qquad (4.43)$$

REILLY (1964) and HUMPHREY and REILLY (1965) modified the Luedeking - Piret kinetic model to account for product inhibition, as follows:

$$\frac{1}{x} \cdot \frac{dP}{dt} = \alpha \cdot \mu + \beta - d_{12} \cdot (P - d_{13}) \qquad (4.44)$$

4.5.5 Substrate Inhibition

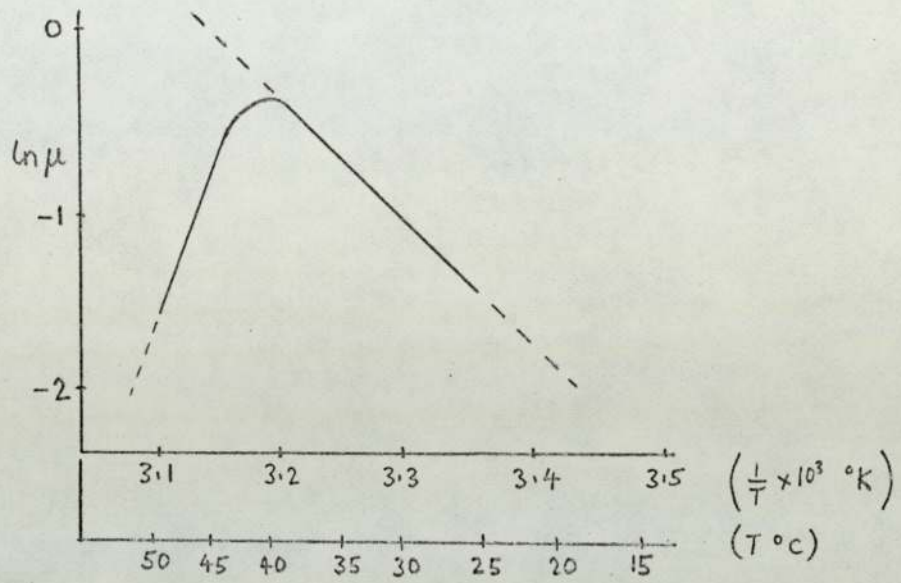
An enzyme kinetic model for substrates which are inhibitory at high concentrations was developed by HALDANE (1930):

$$\mu = \frac{\mu_m}{1 + \frac{K_m}{S} + \frac{S}{K_i}} = \frac{\mu_m \cdot S}{K_m + S + \frac{S^2}{K_i}} \qquad (4.45)$$

where the inhibition constant, K_i, equals the highest substrate concentration at which μ equals ($\mu_M/2$) in the absence of inhibition. This model has been used by a number of workers, including BOON and LAUDELOT (1962), and is described in more detail by DIXON and WEBB (1964) and ANDREWS (1968). A plot of equation (4.45) is shown in figure 4.10. It is worth noting that at low substrate concentrations HALDANE'S expression approximates to Monod kinetics.

For alternative substrate-inhibited kinetic expression the reader is referred to the publications by EDWARDS (1970), BOWSKI et alia (1971), and DABES et alia (1973).

Figure 4.9 An Arrhenius Temperature Plot of Specific Growth Rate



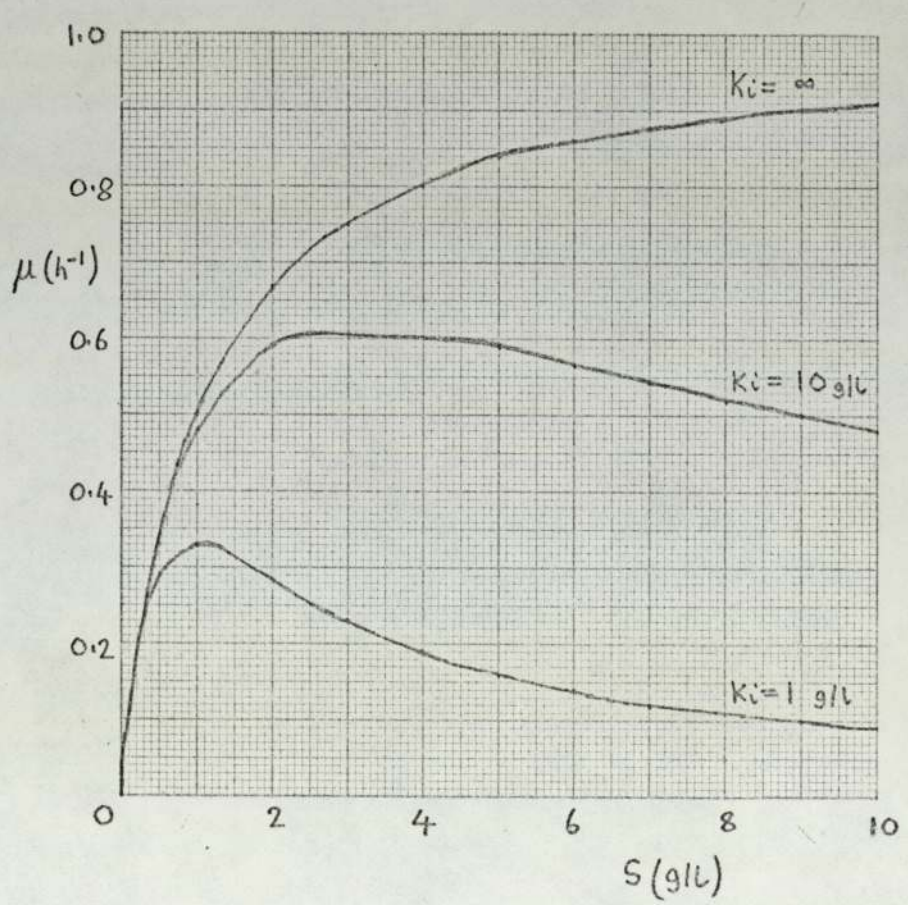


Figure 4.10 Substrate-Inhibited Kinetics ($\mu_m = 1.0\,h^{-1}$; $K_m = 1.0\,g/l$)

4.5.6 Other Factors

Many factors, besides those already mentioned, can affect fermentation kinetics. In particular, the composition of the substrate medium is important, and deficiencies of essential components (such as growth factors), or the presence of toxic substances can seriously disrupt the efficient functioning of microorganisms. MARGALITH (1964) discusses the effect of toxins.

MUZYCHENKO et alia (1972) examined the influence of the oxidation-reduction situation and the effect of the dissolved oxygen concentration upon process kinetics. A number of other factors, which are specific to the batch fermentation of beer, are discussed in Section 6.3.

4.5.7 Concluding Remarks

Examination of the literature reveals that the subject of steady-state fermentation kinetics is well-documented; unfortunately, the same cannot be said about models describing the effects of process parameters upon these kinetic expressions. Real fermentations cannot be carried out under idealised, steady-state laboratory conditions, and, therefore, realistic mathematical models of such systems must include all relevant process variables.

The variety of models proposed by different researchers highlights the difficulties that these workers have faced. For example, there is little similarity between the numerous expressions describing product inhibition, and although many of the parameter - temperature functions

proposed are based upon the Arrhenius expression, it should be noted that even these are inconsistent.

Due to the complexity and unique character of individual fermentation processes it is quite likely that at present the best models are those which include empirical parameter-process variable relationships. Continued research leading to a better knowledge of the workings of the microbial cell should result in better process-parameter models.

LIST OF SYMBOLS (Section 4)

SYMBOL	EXPLANATION	UNITS
a, a_i, b, b_i, c_i, d_i	constants	various
E	activation energy	cal/mole
g	Specific growth rate during exponential phase	h^{-1}
k, k_i	velocity constants	g/l.h
K_i	substrate inhibition constant	g/l
Km	Micahelis-Menten constant	g/l
m	maintenance energy coefficient	g/g.h
p	product concentration	g/l
r	rate of reaction	g/l.h
R	gas constant (= 1.987)	cal/mole.^{o}C
s	substrate concentration	g/l
s_o	initial substrate concentration	g/l
t	time	h
t'	time in equations (4.14) and (4.15)	h
t_d	doubling time	h
T	temperature	^{o}C
x	biomass concentration	g/l
Y_G	yield constant based on substrate used for growth	g/g
Y_T	yield constant based on total substrate used	g/g
z	normalised growth rate (= μ/μ_m)	-

SYMBOL	EXPLANATION	UNITS
Greek Letters		
α	constant in Luedeking-Piret equation	g/g
β	constant in Luedeking-Piret equation	g/g.h
μ	specific growth rate	h^{-1}
μ_m	maximum specific growth rate	h^{-1}

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SECTION 5

CONTINUOUS CULTURE THEORY

Figures

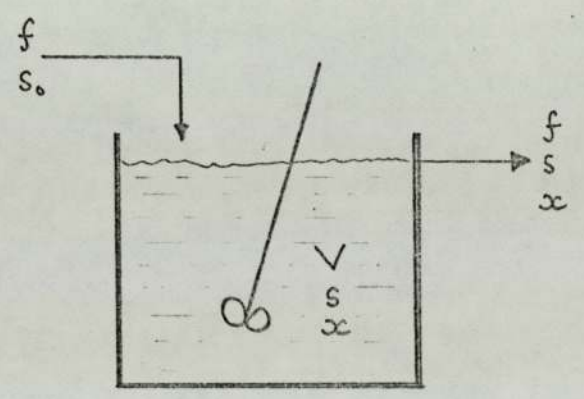
Introduction

Since the publications of MONOD (1950) and NOVICK and SZILARD (1950) much has been written concerning continuous culture theory. This section briefly presents the basic theory of continuous culture for both the steady- and unsteady-state situations.

5.1 BASIC THEORY

Consider a perfectly-mixed vessel of fixed volume V. Substrate is introduced continuously and effluent withdrawn at an equal rate, f (see figure 5.1)

Figure 5.1 Stirred Tank Reactor



Substrate mass balance

IN : by flow = $f.s_o$

OUT : by flow = $f.s$

substrate utilisation: $\frac{V\mu x}{Y_T}$

ACCUMULATION: $V.\frac{ds}{dt}$

Applying the law of conservation of mass, we have:

IN - OUT = ACCUMULATION, therefore:

$$V.\frac{ds}{dt} = f.s_o - f.s - \frac{V.\mu.x}{Y_T} \qquad (5.1)$$

or: $$\frac{ds}{dt} = D(s_o - s) - \frac{\mu.x}{Y_T} \qquad (5.2)$$

where dilution rate , $D = f/V$ (5.3)

Organism mass balance

IN: by flow = 0

from growth= $V_o \mu . x$

OUT: by flow = $f_o x$

ACCUMULATION: $V_o \frac{dx}{dt}$

Conservation of mass, therefore:

$$V.\frac{dx}{dt} = V.\mu.x - f.x \qquad (5.4)$$

or: $$\frac{dx}{dt} = (\mu - D).x \qquad (5.5)$$

At steady state we have $^{ds}/_{dt} = 0$ and $^{dx}/_{dt} = 0$.

Steady state values can be obtained from equations (5.2) and (5.5), viz:

$$\bar{\mu} = D \tag{5.6}$$

$$\bar{x} = Y_T (S_0 - \bar{S}) \tag{5.7}$$

Assuming Monod kinetics (see Section 4.4.4),

$$\mu = \frac{\mu_m . S}{K_m + S} \tag{5.8}$$

and from equation (5.6) we have:

$$\bar{S} = \frac{K_m . D}{\mu_m - D} \tag{5.9}$$

Figure 5.2 shows the effect of D upon $\bar{s}$ and $\bar{x}$.

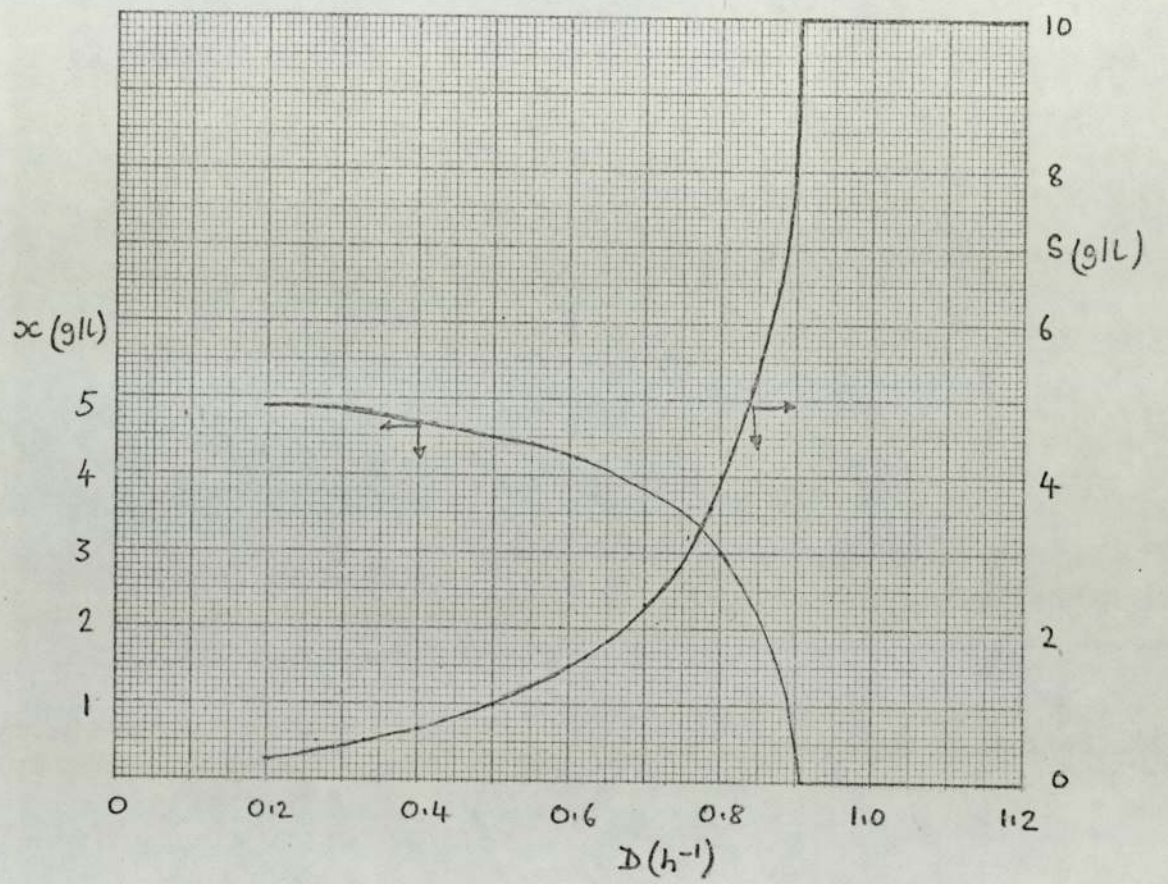
5.2 MODIFICATIONS TO THE BASIC THEORY

5.2.1 Variations in the Value of the Yield Constant

As mentioned in Section 4.4.6, MONOD (1950) assumed that the value of the yield constant is independent of growth rate. However, it is now well-known that the composition of microorganisms varies with growth rate, for example nucleic acid content increases at higher growth rates (see Section 4.2.7). Consequently the yield value for various substrates changes accordingly.

Figure 5.2 Effect of Dilution Rate upon Substrate and Organism Concentrations (steady state)

for: $S_o = 10\ g/l$, $\mu_m = 1\ h^{-1}$
$K_m = 1\ g/l$, $Y_T = 0.5\ g/g$



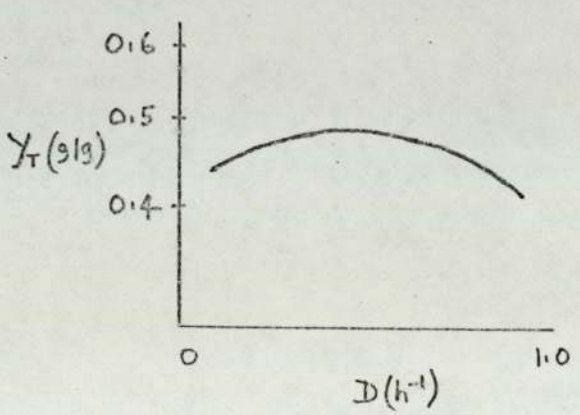


Figure 5.3 Variation of True Yield with Dilution Rate

A further source of error in the value of the yield constant arises from neglect of cellular maintenance energy. This is often accounted for by including an extra term in equation (5.2), as follows (MARR et alia, 1963; DAWES and RIBBONS, 1964; PIRT, 1965):

$$\frac{ds}{dt} = D(s_o - s) - \frac{\mu . x}{Y_G} - mx \qquad (5.10)$$

or

$$\frac{ds}{dt} = D(s_o - s) - \frac{\mu . x}{Y_T} \qquad (5.2)$$

where

$$\frac{1}{Y_T} = \frac{1}{Y_G} + \frac{m}{\mu} \qquad (5.11)$$

Variation of the true yield, Y_T, with dilution rate is shown in (figure (5.3).

An alternative approach is to consider an endogenous metabolism model rather than the maintenance energy modification. In this model equation (5.5) is modified as follows:

$$\frac{dx}{dt} = (\mu - D)x - \beta x \qquad (5.12)$$

Steady state values are now:

$$\bar{\mu} = D + \beta \qquad (5.13)$$

$$\bar{s} = \frac{K_m(D + \beta)}{\mu_m - (D + \beta)} \qquad (5.14)$$

$$\bar{x} = Y_G(s_o - \bar{s}) \qquad (5.15)$$

5.2.2 Consideration of Viability

SINCLAIR and TOPIWALA (1970) proposed a model for the natural death of bacteria in continuous culture at steady state. They found that culture viability becomes significant at low dilution rates. The rate of death of viable cells is assumed to be proportional to the mass of viable cells in the system (x'), and the model equations are as follows:

$$\frac{dx}{dt} = (\mu - D - \beta - \gamma)x \qquad (5.16)$$

$$\frac{dx'}{dt} = \gamma x - Dx' \qquad (5.17)$$

$$\frac{ds}{dt} = D(S_0 - S) - \frac{\mu x}{Y_G} \qquad (5.18)$$

$$\mu = \frac{\mu_m \cdot S}{K_m + S} \qquad (5.8)$$

At steady state we have:

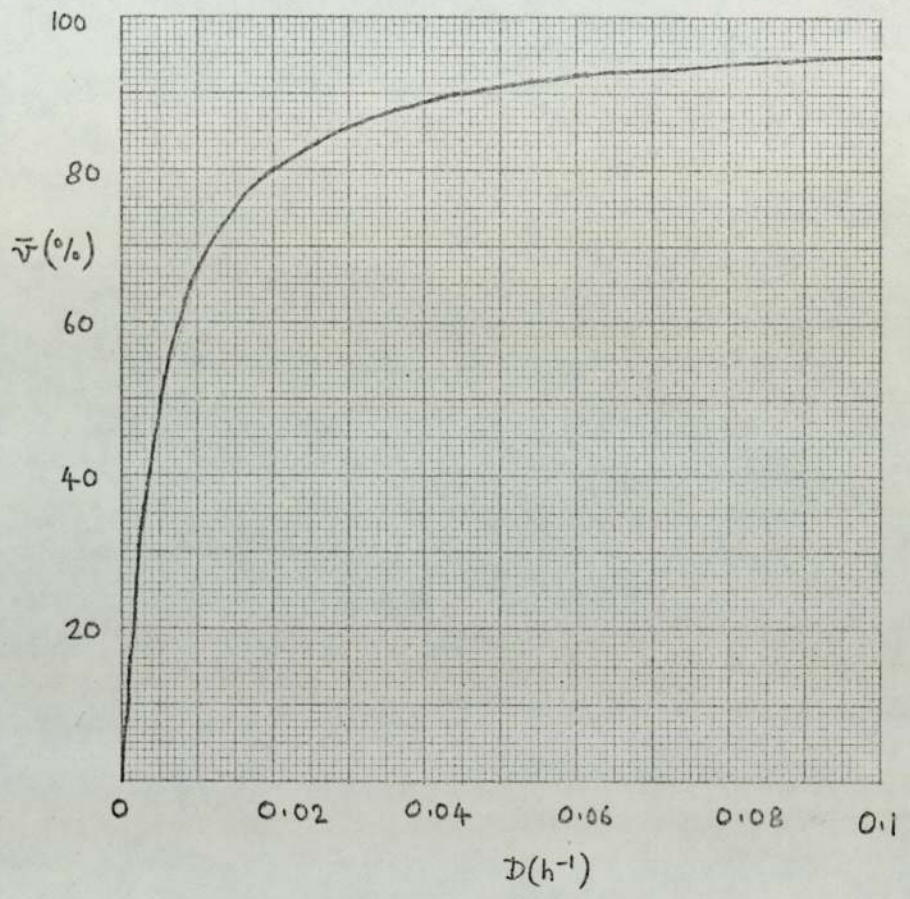
$$\bar{x} = \frac{D \cdot Y_G (S_0 - S)}{D + \gamma + \beta} \qquad (5.19)$$

$$\bar{x}' = \frac{\gamma}{D} \cdot \bar{x} \qquad (5.20)$$

$$\bar{S} = \frac{K_m (D + \beta + \gamma)}{\mu_m - (D + \beta + \gamma)} \qquad (5.21)$$

$$\bar{\mu} = D + \beta + \gamma \qquad (5.22)$$

Figure 5.4 Effect of Dilution Rate Upon Microbial Viability



$$\bar{v} = \frac{D}{D+\gamma}$$

$\gamma = 0.005\ h^{-1}$

and viability, $$\bar{V} = \frac{\bar{x}}{\bar{x}+\bar{x}'} = \frac{D}{D+\gamma} \qquad (5.23)$$

Figure (5.4) shows the effect of D upon $\bar{x}$, $\bar{x}'$, and $\bar{v}$.

5.3 TRANSIENT PHENOMENA

5.3.1 Introduction

Disturbances to continuous fermentation systems result in deviations from the steady state. Continuous culture theory, as derived in Sections 5.1 and 5.2, is not normally applicable to microorganisms functioning in a transient environment. During recent years much experimental work has been aimed at investigating the dynamic behaviour of chemostat systems. This has resulted in some modifications to steady-state continuous culture theory to account for transient changes.

FINN and WILSON (1954) were amongst the first to publicise unsteady-state behaviour when they reported oscillatory characteristics in a pseudo-steady-state continuous culture. They attributed these oscillations to a time lag in the cell's control systems.

A number of workers mounted theoretical investigations into the stability (from the control theory view point) of chemostat systems. KOGA and HUMPHREY (1967) used phase-plane analysis to investigate the kinetics derived in Section 5.1. The simple Monod chemostat model did not produce oscillatory response patterns. YANO and KOGA (1969) came to a similar conclusion when studying the dynamic behaviour of a

substrate-inhibited chemostat system. Further work on the same topic was later published by EDWARDS et alia (1972).

Many researchers (e.g. MATSCHE and ANDREWS, 1972; MATELES et alia, 1965) have attempted to model the transient response of a chemostat system during deviations from the steady state. Possibly the best publications to date are those of YOUNG and BUNGAY (1969, 1970, 1973) who used systems engineering techniques to study the problem. The chemostat system was represented by a block diagram with dilution rate, substrate concentration, temperature, and pH as disturbance variables.

Dynamic changes in specific growth rate are often modelled by incorporating a first order, time-constant term into the growth rate expression, viz:

$$\tau \cdot \frac{d\mu}{dt} + \mu = f(\mu_m, s, pH, T, etc.) \qquad (5.24)$$

or, using Laplace transforms (operator p):

$$\mu(p) = \frac{f(p)}{\tau p + 1} \qquad (5.25)$$

Time constants generally have values in the range 0.5-1.5 hours.

Other workers have used higher order time-constant expressions. ZINES and ROGERS (1970) modelled the effect of ethanol inhibition on continuous culture stability using a second order equation.

GILLEY and BUNGAY (1968) used frequency response analysis to study the effect of dilution rate on yeast growth and proposed a third order expression. SINCLAIR et alia (1971), however, criticised the use of high order dynamic models and suggested that observed oscillatory behaviour was often due to poor experimental technique.

GILLEY and BUNGAY (1967) investigated the transients arising from step changes in dilution rate and substrate feed concentration. They found that the time required for oscillations to damp out seems to be a function of substrate concentration, the magnitude of the dilution rate change, and the range of dilution rates selected. Dilute media give prominent oscillations and large changes in dilution rate tend to give smooth transitions with no peaks or overshooting. MOR and FIECHTER (1968) also found differences in response patterns depending upon dilution rate. At low values of D oscillations were observed, whereas at high values the response was highly damped. YASUDA and MATELES (1964) suggested that cells have the ability to make small, instantaneous adjustments to μ, but large upward changes require time for the synthesis of additional ribosomes. The latter idea has also been put forward by YOUNG and BUNGAY (1973).

TOPIWALA and SINCLAIR (1971) found that the transient response of a culture to a step change in temperature depended upon the nature and direction of the step change. Results were modelled using first order time-lag expressions; values of the time constant were greater for a step-up than for a step-down in temperature. This was attributed to

extra time needed for synthesis of enzymes for increased metabolic activity associated with an increase in temperature. RYU and MATELES (1968) also found dynamic lags in response to temperature changes in continuous culture.

5.3.2 Simulation of Transient Microbial Behaviour

A number of exploratory simulation trials have been made by the author to characterise transient microbial behaviour. The models tested were as follows:

Model 1

$$\frac{dx}{dt} = (\mu - D)x \tag{5.5}$$

$$\frac{ds}{dt} = D(s_0 - s) - \frac{\mu x}{Y_G} - mx \tag{5.10}$$

$$\tau \cdot \frac{d\mu}{dt} + \mu = \frac{\mu_m \cdot S}{K_m + S} \tag{5.26}$$

Model 2

$$\frac{dx}{dt} = (\mu - D - \beta - \gamma)x \tag{5.16}$$

$$\frac{dx'}{dt} = \gamma x - Dx' \tag{5.17}$$

$$\frac{ds}{dt} = D(s_0 - s) - \frac{\mu x}{Y_G} \tag{5.18}$$

$$\tau \cdot \frac{d\mu}{dt} + \mu = \frac{\mu_m \cdot S}{K_m + S} \tag{5.26}$$

Programs were written in BASIC and run using a simulation language, ASP (GAY and PAYNE, 1973). A step length of 0.1 hours was used for the integration procedures. Values of parameters used in the models are listed in table 5.1.

Table 5.1 Parameter Values Used in Transient Microbial Behaviour Models

PARAMETER	VALUE	UNITS
s_o	1.0	g/l
Km	0.0375	g/l
D	0.2	h^{-1}
μ_m	0.5	h^{-1}
Y_G	0.5	g/g
Y_T	0.5	g/g
m	0.1	g/g.h
β	0.07	g/g.h
γ	0.005	h^{-1}
τ	1.0	h

Results

Results for model 1 are shown in figures 5.5 to 5.7, and for model 2 in figures 5.8 and 5.9.

Figure 5.5 Step Change in Dilution Rate (Model 1)

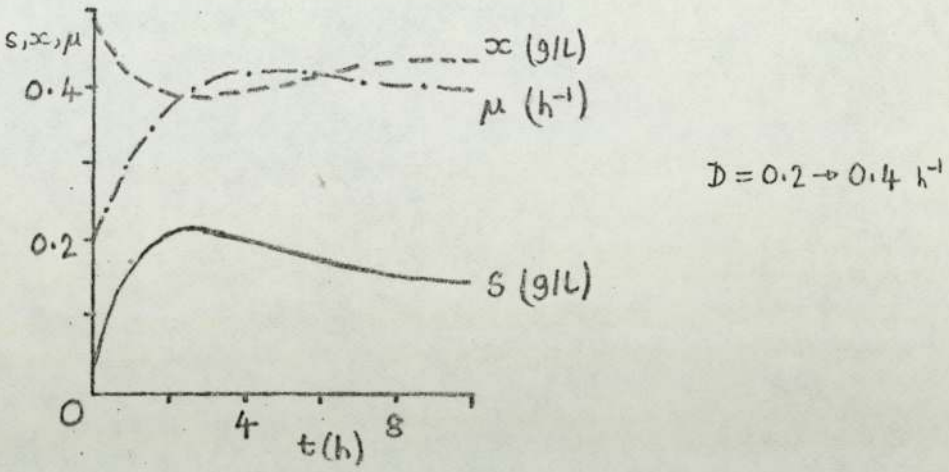


Figure 5.6 Step Change in Substrate Feed Concentration (Model 1)

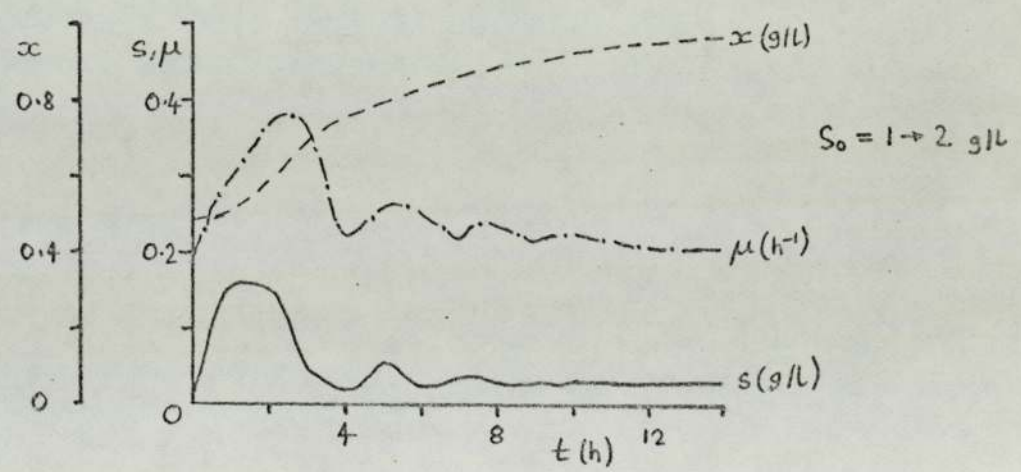
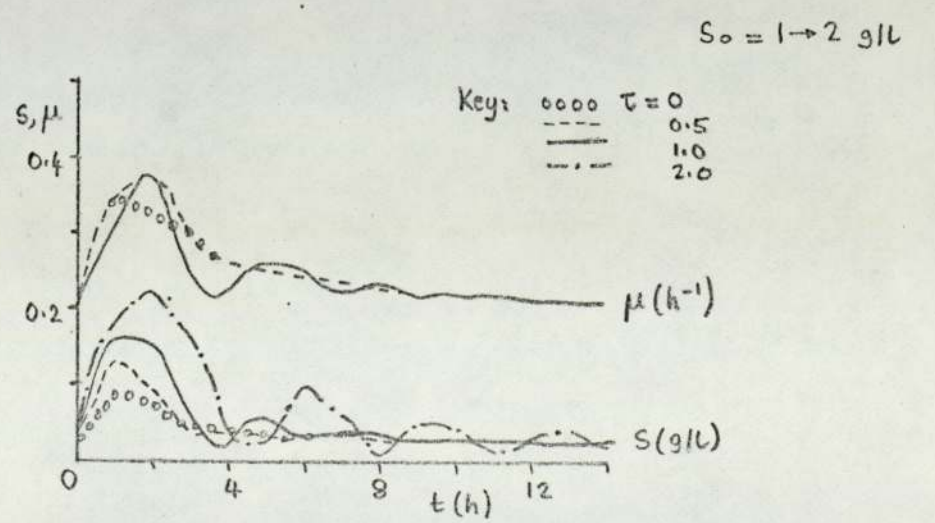


Figure 5.7 Effect of the Time Constant, τ, upon Dynamic Response



Figures 5.8 and 5.9 Simulated Response to a Step Change in Dilution Rate (Model 2)

Figure 5.8 High Dilution Rate

$D = 0.2 \rightarrow 0.4$

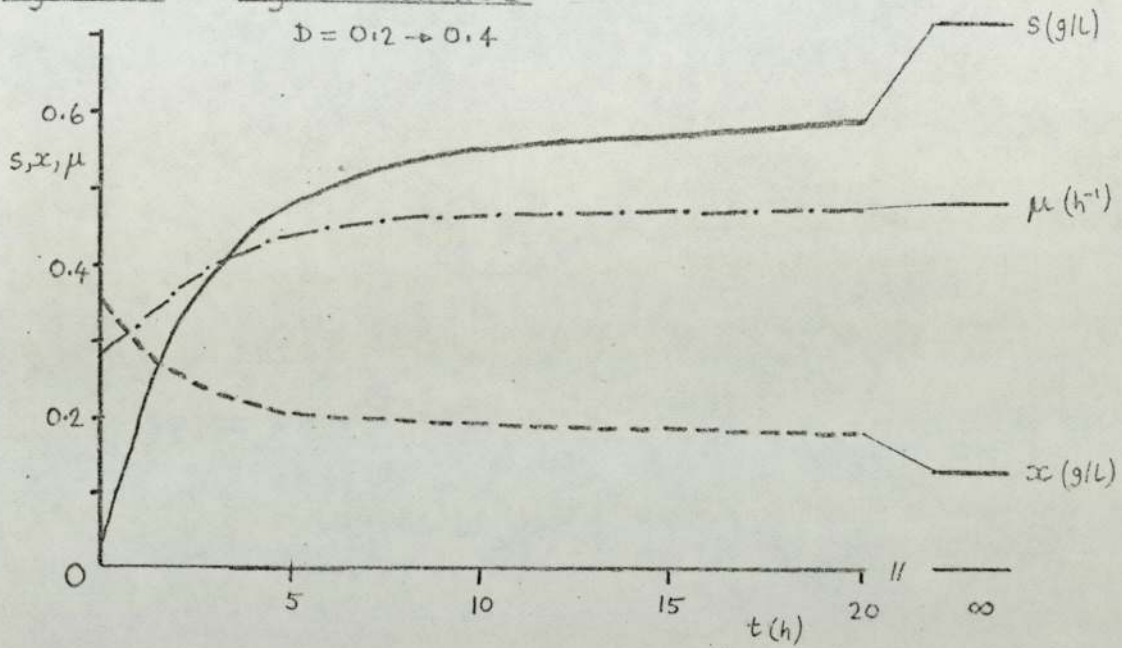
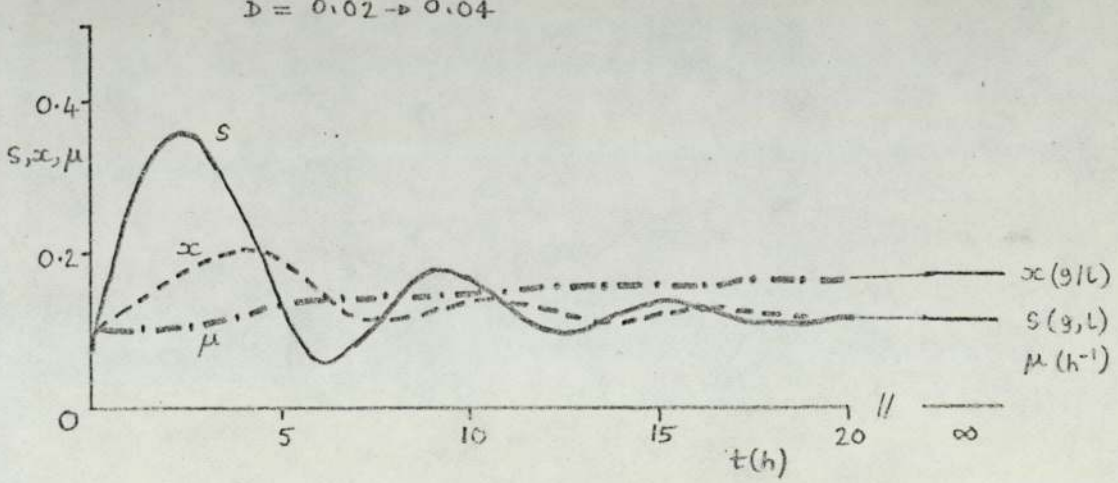


Figure 5.9 Low Dilution Rate

$D = 0.02 \rightarrow 0.04$



Model 1: step changes in dilution rate tend to produce a damped response. Oscillatory response patterns are likely to result from step changes in substrate feed concentration. As expected, the value of the time constant, τ , affects the response; large values lead to highly oscillatory transients.

Model 2: oscillations are apparent at low dilution, whereas at high values of D the response is damped. This is possibly because μ is close to μ_m at the higher dilution rate.

5.3.3 Conclusions

Dynamic behaviour of microorganisms cannot, at present, be modelled satisfactorily. Some researchers, however, have obtained experimental results which give reasonable agreement with predicted theoretical values. In the future it is possible that an improved knowledge of bacterial control mechanisms will lead to the development of better dynamic models. DEAN (1972) has published work on this topic.

LIST OF SYMBOLS (Section 5)

SYMBOL	EXPLANATION	UNITS
D	dilution rate	h^{-1}
f	volumetric flowrate	l/h
Km	Michaelis-Menten constant	g/l
m	maintenance energy coefficient	g/g.h
p	Laplace transform operator	-
s	substrate concentration	g/l
s_o	substrate feed concentration	g/l
t	time	h
T	temperature	^oC
V	volume of fermenter	l
x	biomass concentration (viable - Section 5.2.2)	g/l
x'	non-viable biomass concentration	g/l
Y_G	yield based on substrate used for growth	g/g
Y_T	yield based on total substrate used	g/g

Greek Letters

β	endogenous metabolism coefficient	g/g.h
γ	specific rate of death	h^{-1}
τ	microbial time constant	h
μ	specific growth rate	h^{-1}
μ_m	maximum specific growth rate	h^{-1}

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SECTION 6

ANALYSIS AND MODELLING OF THE BATCH BEER FERMENTATION PROCESS

Figures

Tables

6.1 INTRODUCTION

6.1.1 Batch Beer Fermentation

Batch beer fermentation is an art which has evolved over many centuries. The starting material, brewers' wort, is a complex mixture containing mainly sugars, although many other components, such as amino acids, proteins, and inorganic salts are present. Wort composition is strongly dependent upon the method of preparation, but generally over 90% of wort solids are carbohydrates, approximately three-quarters of this quantity being fermentable. A typical wort carbohydrate analysis is listed in table 6.1.

Table 6.1 Typical wort carbohydrate analysis (HARRIS et alia, 1951) (Pale ale wort, original gravity $1040^{o} = 1.040$)

SUGAR	CONCENTRATION (g/l)	
fructose	3.3	
glucose	10.0	
sucrose	5.3	
maltose	38.9	
maltotriose	11.4	
TOTAL FERMENTABLES		68.9
maltotetraose	2.0	
higher sugars	23.2	
TOTAL NON-FERMENTABLES		25.2
TOTAL SUGARS		94.1
Fermentability = $\frac{68.9}{94.1}$ x 100% = 73.3%		

Wort is fermented in large vessels by pitching (adding) yeast, which initially remains in suspension, thus providing a large surface area through which reactants and products are able to permeate. During fermentation the yeast performs two distinct sets of intra-cellular operations:

(1) production of alcohol, carbon dioxide, and energy, and

(2) utilisation of this energy for cell maintenance and reproduction.

Most of the wort constituents are necessary for the reactions (1) and (2) above. Dissolved oxygen is a further wort ingredient, essential for normal growth of yeast during the early stages of fermentation.

Speed of fermentation is influenced by many factors, notably wort composition, yeast characteristics, and temperature. Much heat is liberated during the fermentation process; temperature patterns are normally controlled by regulating the passage of cooling water through attemperator tubes inside the fermenter.

6.1.2 Yeast Growth During Fermentation

Growth of yeast is essential during fermentation if propagation of the culture (and therefore continuation of the fermentation) is desired. Yeast growth is influenced by the quantity of yeast initially pitched; at high concentrations the individual cells multiply slowly. HOUGH (1961) has studied this aspect in detail and has also produced a relationship between pitching rate and speed of fermentation. Although fermentation velocity is increased, high growth rates are not entirely desirable since new yeast is a by-product of relatively low economic value.

The growth pattern of yeast during batch beer fermentation is

described in detail by BROWN and KIRSOP (1972). Essentially, it is similar to that of bacteria, as described in Section 4.3.1 and shown in figure 4.5.

6.1.3 The Physical Behaviour of Yeast During Fermentation

Rate of fermentation is approximately proportional to the available surface of yeast and, therefore, the physical behaviour of yeast is of prime importance. During fermentation the mass of yeast is distributed between the bottom of the vessel, the bulk of the liquid, and the yeast head on the liquid surface. There is little contact between wort and the yeast sediment or yeast head, so clearly it is the yeast in suspension which is mainly active in reproduction and the synthesis of alcohol.

An ideal yeast remains in suspension during the active part of fermentation, but during the latter stages either sediments (bottom yeast) or ascends to the surface (top yeast), thus leaving a clear, yeast-free beer. Not all yeast strains are ideal, though, and WALKEY and KIRSOP (1969) have characterised six types of flocculation/ sedimentation behaviour exhibited during fermentation.

During the process of active fermentation large volumes of carbon dioxide gas are evolved. Particles of wort-sediment provide nuclei on which bubbles can form, thus ensuring regular liberation of the gas. Ascension of gas bubbles arrests the tendency of yeast flocs to sediment by providing a large upward thrust. Some bubbles become coated with yeast cells which are carried to the surface and form a yeast-head. Mixing patterns within a fermenter are complex and depend upon gas liberation, vessel geometry, additional aeration, and mechanical agitation.

Flocculation occurs when yeast cells clump together, forming "flocs". Bottom yeast flocs sediment to the bottom due to their increased settling velocity, whereas top yeast flocs rise to the surface due to buoyancy exerted by trapped carbon dioxide bubbles. Premature flocculation represses the rate of fermentation as a result of the removal of active (suspended) yeast cells from the wort.

6.1.4 Wort Metabolism

6.1.4.1 Introduction

The removal of nutrients from the wort by yeast, and the subsequent secretion of fermentation products into the mixture yields beer. In addition to new yeast, ethanol, and carbon dioxide, there are many other products of fermentation - for instance, fusel alcohols, esters, and ketones. Although present in minute quantities the balance of these substances is critical since they are largely responsible for the flavour of the final product.

The sets of equations describing the fermentation of beer from wort are extremely complex. However, this fermentation process is frequently represented by "overall" equations expressing the production of ethanol from sugar. For example:

$$C_{12}H_{22}O_{11} + H_2O \longrightarrow 4C_2H_5OH + 4CO_2 + \text{energy} \qquad (6.1)$$

Under optimum conditions about 95% of the theoretical yield of ethanol can be obtained from fermentable sugars, the discrepancy arising due to the production of minor metabolites, and to respiration activities of the

yeast (i.e. oxidation of the sugars to carbon dioxide and water).

It is unrealistic to consider sugar utilisation alone since carbohydrate metabolism is not possible in the absence of many vital constituents of wort. For example, zinc and amino acids are necessary for the synthesis of enzymes which ferment the sugar. It is often useful, though, to characterise the degree of fermentation by the quantity of fermentable sugars remaining in the wort.

6.1.4.2 Absorption of Nutrients By the Yeast

The yeast cell is bounded by a cell wall, known as the cytoplasmic membrane (see figure 4.2, p. 86), through which nutrients must pass to enter the microbial system and become available for metabolism. Entry of fermentable sugars into the yeast cell is believed to be the rate-controlling step in the fermentation process and is, therefore, of prime importance. Two methods of transport are recognised:

(1) simple diffusion, and

(2) carrier mechanisms.

(1) simple diffusion

Simple diffusion arises as a result of a concentration gradient: thermal agitation of the molecules provides energy for the operation. The rate of transport is a function of concentration difference across the membrane, size of the solute molecules, and their lipid solubility. Only a few, low molecular weight substances are able to pass across the cytoplasmic membrane in such a fashion.

(2) carrier mechanisms

Almost all of the transport across the yeast cell membrane is due to

the operation of specific carrier mechanisms. The action of such systems resembles a "ferry-boat", carrier molecules being free to move across the membrane in either direction. Sugar molecules are picked up outside the cell wall, transported into the cell, then released. The carrier molecule then makes the return journey either empty, or transporting a metabolite from the interior to the exterior or the cell. Two main carrier mechanisms exist:

(a) facilitated diffusion, and

(b) active transport.

(a) facilitated diffusion: this process utilises energy derived from thermal agitation of the molecules, but is distinguished from simple diffusion by a number of important differences, notably:

(1) transport takes place at a greater rate than is possible by simple diffusion, and

(2) the rate of transport ceases to increase after the concentration of the environment has risen above a certain value; this is due to saturation of the carrier system.

(b) active transport: this is similar to facilitated diffusion, except that it requires the expenditure of energy by the yeast cell. This mechanism is capable of concentrating substances within the yeast cell against large concentration gradients and is often termed "uphill transport".

Carrier mechanisms may be either constitutive or inducible. A constitutive system is able to function at all times, whereas an inducible mechanism is developed by the cell only in the presence of an inducing

substance. Such systems cease to operate when the inducer is not present in sufficient quantity.

6.1.4.3 Removal of Carbohydrates from Wort

The disappearance of the major fermentable sugars from wort occurs in the following sequence: sucrose, glucose, fructose, maltose, maltotriose. Sucrose is rapidly hydrolysed by invertase at the cell walls of the yeast yielding equal quantities of glucose and fructose. The remaining sugars are then utilised in order of increasing complexity, i.e. mono-saccharides, di-saccharides, tri-saccharides. The order of uptake is due to two factors:

(1) suppression of maltose utilisation by glucose, and

(2) suppression of maltotriose utilisation by both glucose and maltose.

Consequently the glucose concentration must fall below a critical level before induction of the maltose permease mechanism commences. Similarly, when the maltose concentration is below a critical level induction of the maltotriose uptake system commences.

Facilitated diffusion is almost entirely responsible for the entry of mono-saccharides into yeast cells, although free diffusion accounts for about one millionth of the total sugar absorbed. A few yeast strains employ active transport, or both active transport and facilitated diffusion, for the absorption of mono-saccharides. Competition occurs between the individual sugars for the mono-saccharide transport mechanism, which is constitutive.

Entry of maltose and maltotriose into yeast cells is due to active transport systems involving specific permeases. It is believed that some yeast strains exhibit constitutive maltopermeases, but in the majority of strains the mechanisms are inducible (MILLIN, 1963).

6.1.4.4 Carbohydrate Metabolism

Inside the yeast cell maltose and maltotriose are hydrolysed by a single maltase, yielding glucose. The maltase is inducible, although its induction does not necessarily occur simultaneously with the induction of the maltopermease system (HARRIS and MILLIN, 1963).

Fermentation proceeds by the intra-cellular metabolism of a mixture of glucose and a rather smaller amount of fructose. Intra-cellular glucose is supplied by three extra-cellular sources:

(1) glucose - including sucrose hydrolysed to glucose,

(2) maltose, and

(3) maltotriose.

It follows, therefore, that differences in the rates of fermentation of these three sugars are due to variations in their rates of uptake and conversion to intra-cellular glucose.

Glucose and fructose are metabolised along a number of complex pathways culminating in the synthesis of new yeast matter, ethanol, carbon dioxide, and minor metabolic products. Chemical products are then excreted from the yeast cells by carrier mechanisms (see Section 6.1.4.2).

6.1.5 The Course of Brewery Fermentations

Although it is difficult to identify relationships concerning parameters controlling beer quality - i.e. taste, smell, and appearance - the effect of a number of variables upon speed of fermentation is partially understood. A summary and criticism of such information is given in Section 6.3.

HOUGH et alia (1971) have listed the following factors as being important to fermentation performance and beer quality:

(1) choice of yeast strain,

(2) condition of yeast at time of pitching,

(3) amount of yeast added to the wort,

(4) yeast distribution in the fermenting wort throughout the fermentation,

(5) size and geometry of the fermenter,

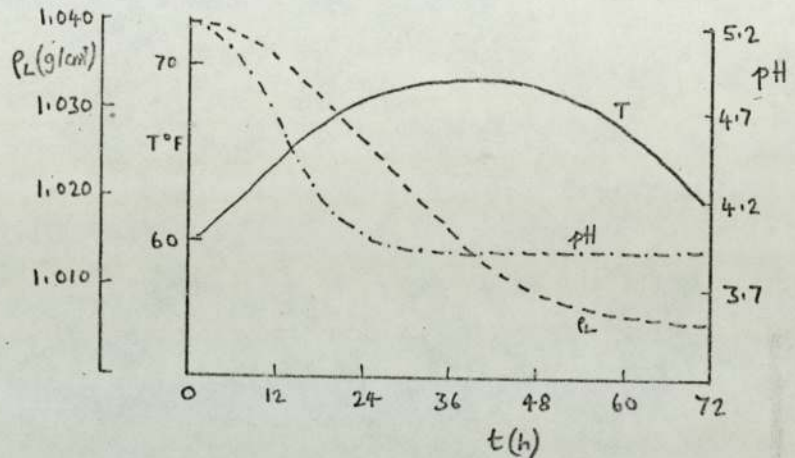
(6) aeration,

(7) wort composition and pH, and

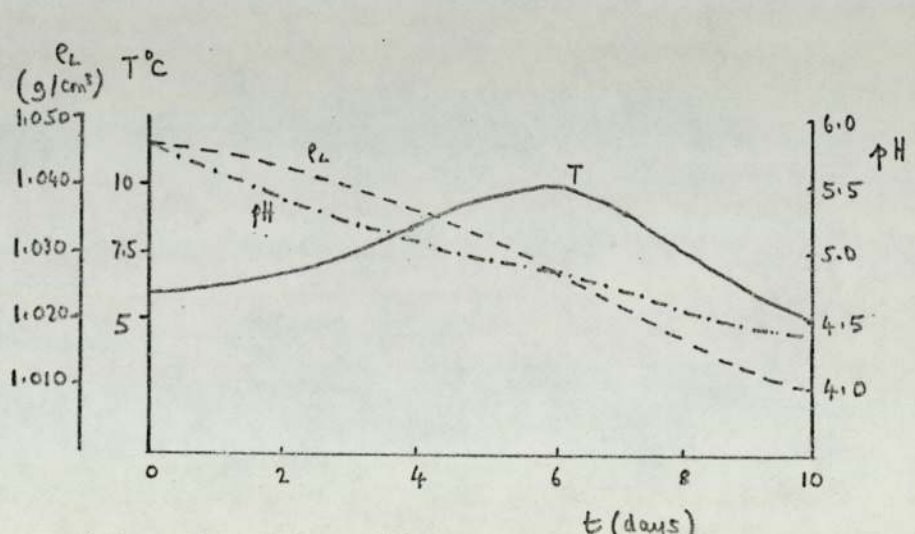
(8) fermentation temperature.

The list above is by no means exhaustive, and the importance of each factor depends upon type of beer, brewery, etc. However, it is significant that every factor, except (4), is under the control of the brewer. Yeast distribution during fermentation has been discussed in Sections 6.1.2 and 6.1.3, and is a function of each of the other seven factors listed above, and many more besides. Consequently it is desirable to possess knowledge of variations in these parameters during the course of fermentation. Figure 6.1 portrays such changes during a typical

Figure 6.1 The Course of a Typical Batch Fermentation
(Hough et alia, 1971)



(a) Top Fermentation



(b) Bottom Fermentation

fermentation. A publication of interest is that of MAULE et alia (1966), who made a simultaneous assessment of changes during a stirred batch fermentation.

6.2 THE DEVELOPMENT OF A MODEL OF SUGAR UTILISATION DURING BATCH BEER FERMENTATION

6.2.1 Introduction

The kinetics of beer fermentation have not previously been modelled in detail, possibly due to the complexity of the series of reactions involved in the conversion of wort to beer. Wort is a complex medium, but may be considered as a multi-substrate system containing five fermentable carbohydrates - glucose, fructose, sucrose, maltose, and maltotriose (see Section 6.1.1). Previously published work about multiple-substrate kinetics is described in Section 4.4.10.

Consideration is first given to the problems of modelling multi-substrate systems in which microorganisms can utilise carbohydrates both simultaneously and sequentially. Ideas outlined in these introductory sections are then used to develop a model of the batch beer fermentation process, and it is shown how the model can be matched to experimental data. Important features of the model are expressions (1) dealing with the effects of certain sugars on the utilisation of other sugars by the yeast, and (2) accounting for adaptation of yeast to changes in the nutritional environment.

6.2.2 Single-Substrate Utilisation Kinetics

The kinetics of single-substrate enzyme reactions are often

adequately described by an equation of the Michaelis-Menten type:

$$\frac{ds}{dt} = \frac{-k.s.x}{Km+s} \qquad (6.2)$$

In the case of batch beer fermentations low yeast concentrations are employed and the effect of diffusion into and out of the yeast flocs can be neglected. FRANZ (1961) found that fermentation rate was proportional to yeast concentration when studying the fermentation of molasses by bakers' yeast and successfully interpreted his results using equation (6.2). However, during fermentations involving high concentrations of flocculent yeasts diffusion can be rate-controlling, and equation (6.2) must then be modified to include an "effectiveness factor" as follows:

$$\frac{ds}{dt} = \frac{-k.s.x}{Km+s} \cdot \phi \qquad (6.3)$$

GREENSHIELDS and SMITH (1971) have previously discussed this problem in more detail.

Adaptation

Immediately after yeast has been pitched into wort there is normally a lag period during which there is little detectable activity. During this lag phase the yeast organises, or adapts, itself to the new extra-cellular environment.

A simple model describing yeast activity during the adaptation, or induction, period can conveniently be explained with the use of basic control theory. Assume that the induction mechanism can be represented by a "black box" with a transfer function equivalent to a single exponential lag (see figure 6.1). When the yeast is pitched into a new medium a step-change in the extra-cellular environment is effected, signified by a step input to the "black box". The response of the "black box" to the step change input is derived as follows:

$$I(p) = G(p).X(p)$$

$$= \left(\frac{A}{p+A}\right).\frac{1}{p}$$

$$= \frac{1}{p} - \left(\frac{1}{p+A}\right)$$

Inversion of the Laplace Transforms from the "p" domain to the "time" domain yields:

$$I(t) = \left[1 - \exp(-At)\right] \qquad (6.4)$$

The response of the "black box" to a step change input is shown in figure 6.2, and it can be seen that large values of A lead to a fast response, whereas small values of this parameter result in a slow response. The value of parameter A thus characterises the speed of adaptation and

Figure 6.2 "Black Box" Representation of the Adaptation Mechanism

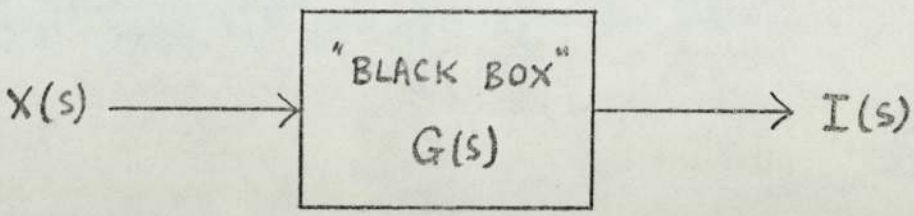
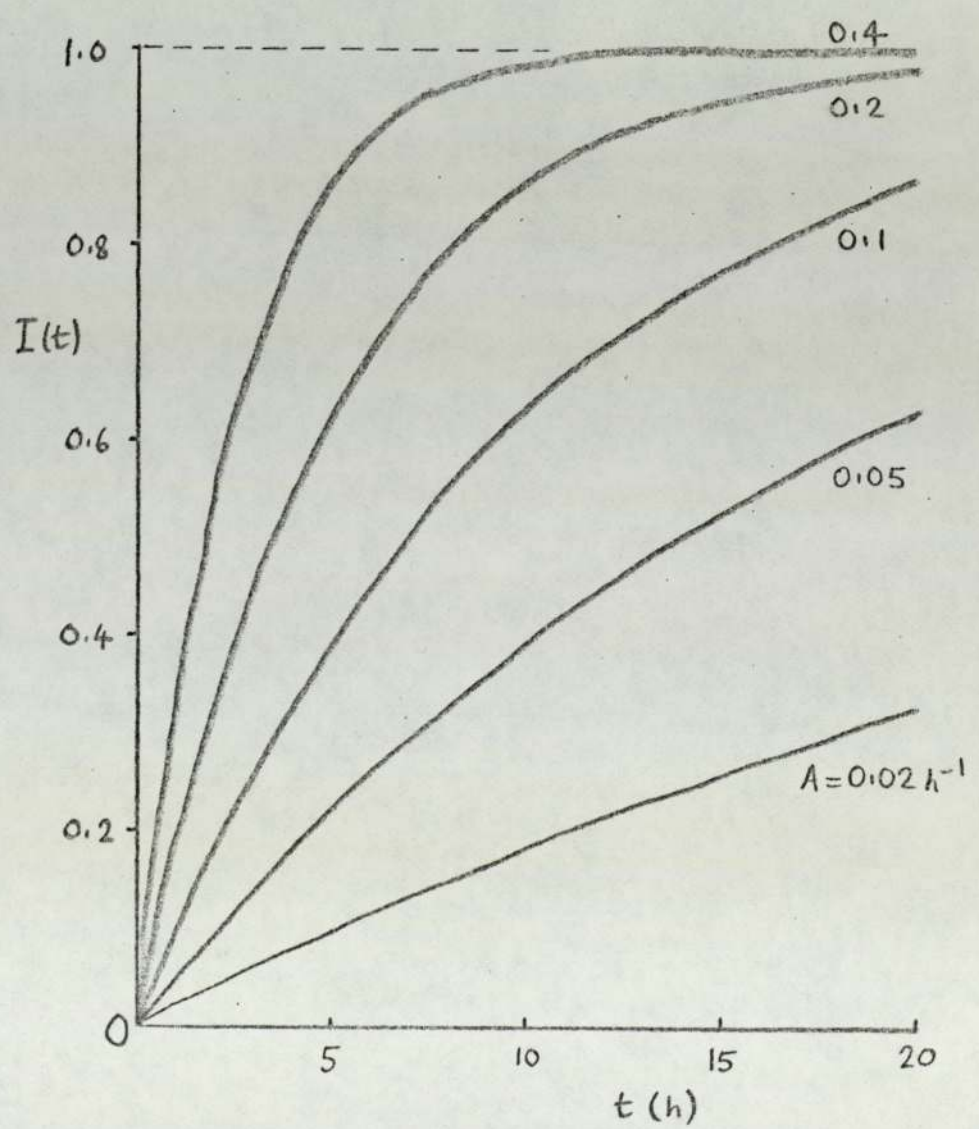


Figure 6.3 Effect of the Adaptation Constant, A, upon Response Speed



is dependent upon a number of factors, notably yeast strain, the previous environment of the yeast, and the new environment of the yeast.

Alternative transfer functions could be considered for the "black box", but since the adaptation process is not fully understood a simple expression such as a single exponential lag is considered appropriate.

By combining equations (6.2) and (6.4) a simple adaptation, or induction, function can be incorporated into the basic Michaelis-Menten form of equation, giving:

$$\frac{ds}{dt} = \frac{-k\varepsilon x}{Km+s} \cdot I(t) \qquad (6.5)$$

6.2.3 Multiple-Substrate Utilisation

The modelling of multiple-substrate systems is rather more complex than that of single-substrate systems since the presence of one substrate may affect the ability of the microorganism to utilise other substrates present in the medium. In addition, one must assess whether or not the microorganism can utilise more than one substrate at any point in time.

Suppression (see Section 6.1.4.3)

During beer fermentation the presence of glucose in the medium suppresses the ability of yeast to utilise maltose, and both glucose and maltose suppress the use of maltotriose (HOUGH et alia, 1971). These phenomena lead to the following ordered sequence of sugar utilisation:

(1) mono-saccharides (glucose, fructose)

(2) maltose, and

(3) maltotriose.

Thus when the glucose concentration has fallen below a critical value, s_1^*, the utilisation of maltose begins. Likewise, maltotriose is not utilised until the level of maltose is below the critical suppression concentration, s_3^*. The results of GRIFFIN (1970a) show that utilisation of maltose begins when there is 0.55 - 0.75% glucose in the medium, the lower figure applying to slowly-fermenting yeasts and the upper figure to rapidly-fermenting strains. However, when using a synthetic medium (glucose, maltose, and other nutrients) GRIFFIN (1970b) found that maltose utilisation commenced when there was 0.3 - 1.0% glucose present, depending upon the composition of the medium and also the strain of yeast employed. Further work by GRIFFIN (1970c) shows that the quantity of maltose present in wort when utilisation of maltotriose commences varies between 1.2 and 3.9% according to the yeast strain. In addition he found that at low pitching rates the "critical" maltose concentration is greater than at high pitching rates.

For the purposes of the mathematical model, and in the absence of more fundamental knowledge, the criterion of "critical" sugar concentration is used to describe factors suppressing the activities of maltose and maltotriose permease systems. A simple "ON-OFF" concept is proposed in the case of maltose, viz.

$$\text{for} \quad s_1 > s_1^* \quad , \quad r_3 = 0 \quad , \text{and}$$

$$\text{for} \quad s_1 < s_1^* \quad , \quad r_3 = -\frac{ds_3}{dt} \quad ,$$

and likewise for maltotriose.

Additive Utilisation Rates

The question of whether substrates are utilised individually or simultaneously is of prime importance when modelling the kinetics of multiple-substrate systems. AMAHA (1966) has carried out experiments to see whether or not the fermenting activities of yeast cells towards individual wort sugars are additive. He found that the fermentation rate (measured in terms of the rate of carbon dioxide evolution) was higher for a combination of 0.5% glucose + 0.5% maltose than for either 1% glucose or 1% maltose. Similar results were obtained for a mixture containing maltose and maltotriose. AMAHA concluded that the fermenting activities of brewery yeasts towards glucose, maltose and maltotriose are additive, thus implying that the sugar transport systems for these three carbohydrates are independent.

It seems feasible to assume that fermenting activity is the sum of the fermentation rates of the individual wort sugars - glucose, maltose, maltotriose, fructose and sucrose. The latter two sugars are present in wort only in minor quantities (as little as 5% of the total fermentable sugars), and they are accounted for in somewhat different ways. Sucrose is known to be rapidly hydrolysed to glucose and fructose on the surfaces of the yeast-cell walls, probably by a mechanism independent of the sugar transport systems. Fructose, on the other hand, is thought to share a common transport system with glucose, and therefore it is probable that these two mono-saccharides are taken up simultaneously. Little is known about the glucose-fructose interaction, and so this facet is

difficult to incorporate into a mathematical model. Since the quantity of fructose present in wort is very small, little accuracy is lost by assuming additive fermentation rates for glucose and fructose.

6.2.4 Active Yeast Concentration

As noted in Section 6.1.3 it can be assumed that the quantity of yeast dry matter suspended in the liquid phase determines the rate of fermentation (see equation (6.2)). The concentration of the suspended, or active, yeast is determined by three factors:

(1) initial yeast concentration (i.e. pitching rate),

(2) growth of new yeast during fermentation, and

(3) flocculation.

Yeast growth occurs during the early and middle stages of fermentation, when the yield of yeast dry matter per unit mass of sugar assimilated, Y_T, is approximately constant (HOUGH et alia, 1971). BROWN and KIRSOP (1972) have shown that variations in Y_T due to yeast strain, wort composition, and fermentation temperature are not normally significant.

An ideal yeast remains in suspension during the active part of fermentation, but not during the latter stages when yeast cells clump together forming "flocs". Because yeast flocculation is complex and not fully understood, it will be assumed that the rate of flocculation, f, is approximately constant (GRIFFIN, 1970a), so that active yeast

concentration is given by the relationships:

$$\frac{dx}{dt} = -Y_T \cdot \frac{ds}{dt} \quad , \; S \gg S_o - S_g^* \qquad (6.6a)$$

$$\frac{dx}{dt} = -f \quad , \; S < S_o - S_g^* \qquad (6.6b)$$

where: s_o = initial sugar concentration, and

s_g^* = sugar used before growth ceases

6.2.5 Assumptions Used in the Model

Assumptions (1) to (4) below are only applicable to the initial simplified kinetic model: means of incorporating the effects of temperature, pH, dissolved oxygen concentration, and mean yeast age into the model are briefly outlined in Section 6.3.

(1) The temperature remains constant.

(2) The pH remains constant.

(3) The system is anaerobic, i.e. the dissolved oxygen concentration is zero.

(4) The ability of the yeast to ferment wort is unchanged throughout the batch process.

(5) The system is well-mixed. (This is not an unreasonable assumption

during the active period of fermentation since large volumes of carbon dioxide are evolved as a result of the fermentation process itself).

(6) The wort is a multiple-substrate system consisting of the five major fermentable sugars - glucose, fructose, sucrose, maltose and maltotriose.

(7) Michaelis-Menten type kinetics are applicable for each of the five sugars over the entire concentration ranges encountered during primary fermentation.

(8) The Michaelis constant, Km_i, and the velocity constant, k_i, possess constant values for a particular sugar, i, and particular yeast strain. (The values of these constants probably vary according to the wort and yeast strain employed).

(9) When utilisation of a sugar commences the yeast gradually adapts itself to the new carbon source according to the adaptation function given by equation (4) and shown in figure 6.2.

(10) Two or more sugars can be utilised simultaneously, the total fermentation rate being equal to the sum of the individual rates of fermentation as defined by equation (6.5).

(11) The suppression of maltose and maltotriose permease systems can be accounted for in terms of critical sugar concentrations and an ON-OFF mechanism (see section headed Suppression).

(12) Inversion of sucrose at the cell wall does not affect the utilisation

rates of other sugars. (Sucrose hydrolysis occurs rapidly during the initial stages of fermentation, probably during the period when the yeast is adapting itself to the utilisation of the mono-saccharides: consequently inaccuracies resulting from this assumption are trivial).

(13) Essential wort nutrients, other than fermentable carbohydrates, are present in normal quantities. (Abnormal concentrations of minor components such as amino-acids, vitamins and inorganic elements are likely to lead to unusual yeast behaviour: such effects cannot be modelled satisfactorily).

(14) "Active" yeast is uniformly suspended in the wort as small aggregates, and diffusion into and out of these aggregates does not affect the overall rate of fermentation.

(15) During "exponential" growth of the yeast, the increase in the total weight of the active yeast mass is equivalent to 12% of the glucose + fructose + maltose + maltotriose utilised during any time increment. (KIRSOP, 1971; BROWN and KIRSOP, 1972). (Sucrose is not included in this calculation since it is hydrolysed to glucose and fructose before utilisation).

6.2.6 The Computer Model

Basically the computer model is a series of mathematical statements corresponding to the assumptions listed above. A block diagram representing the main steps in the program is shown in figure 6.4.

The kinetic relationships for individual wort sugars and for yeast growth are expressed by the non-linear differential equations listed in table 6.2.

Solution of the set of equations in table 6.2 was by Euler's method, a step-by-step "marching" procedure along the time axis. A small step length was used in order to reduce approximation errors to insignificant levels. The computer program was written in FØRTRAN and run on the ICL 1905 E computer at the University of Aston in Birmingham. Compilation time was 1 second and running time approximately 4 seconds for simulation of a 100 hour fermentation.

6.2.7 Data for the Model

Data used to test the model were obtained from the paper published by GRIFFIN (1970a) in which patterns of removal of the sugars - glucose, fructose, maltose, and maltotriose - from wort during the course of fermentation was investigated. GRIFFIN'S data were used because:

(1) similar patterns of sugar utilisation have been observed by other workers (BAVISOTTO, 1958; MONTREUIL, 1956; PHILLIPS, 1955; STOCKLI, 1957; TUNING, 1971);

(2) the results are most comprehensive and include a yeast concentration plot; and

(3) temperature was maintained at a constant value during the course of the experiments.

The last point is of great importance since temperature exerts a profound

Figure 6.4 Outline of Computer Program used for Solving the Model Equations

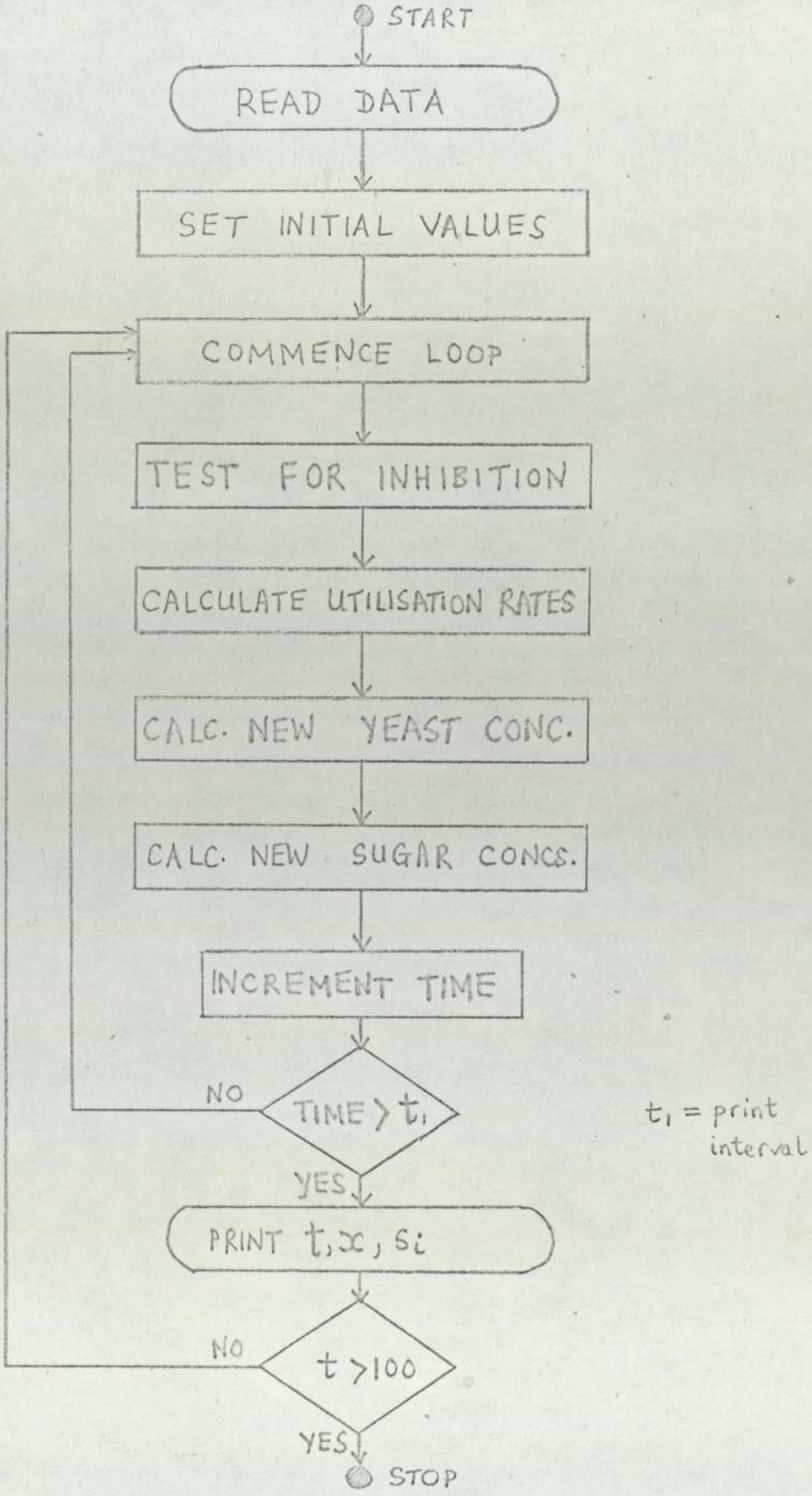


Table 6.2 Equations used to Describe Sugar Utilisation Rates and Active Yeast Concentration

Glucose: $$\frac{ds_1}{dt} = \frac{-k_1 s_1}{K_{m_1}+s_1} \cdot x \cdot I_1 + \frac{180}{342} \cdot \frac{k_5 s_5}{K_{m_5}+s_5} \cdot x \cdot I_5 \qquad (6.7a)$$

Fructose: $$\frac{ds_2}{dt} = \frac{-k_2 s_2}{K_{m_2}+s_2} \cdot x \cdot I_2 + \frac{180}{342} \cdot \frac{k_5 s_5}{K_{m_5}+s_5} \cdot x \cdot I_5 \qquad (6.7b)$$

Maltose: $$\frac{ds_3}{dt} = \frac{-k_3 s_3}{K_{m_3}+s_3} \cdot x \cdot I_3 \quad \text{for } c_1 < c_1^* \qquad (6.7c)$$

Maltotriose: $$\frac{ds_4}{dt} = \frac{-k_4 s_4}{K_{m_4}+s_4} \cdot x \cdot I_4 \quad \text{for } c_3 < c_3^* \qquad (6.7d)$$

Sucrose: $$\frac{ds_5}{dt} = \frac{-k_5 s_5}{K_{m_5}+s_5} \cdot x \cdot I_5 \qquad (6.7e)$$

Yeast: $$\frac{dy}{dt} = -Y_T \cdot \frac{ds}{dt} \quad \text{for } s \geq s^\circ - s_g^* \qquad (6.8a)$$

$$\frac{dy}{dt} = -f \quad \text{for } s < s^\circ - s_g^* \qquad (6.8b)$$

Table 6.3 Data Used in and Obtained from the Computer Simulation

Velocity and Michaelis constants

SUGAR		k_i (g/g.dry yeast.h)	Km_i (g/l)	REFERENCE
i = 1	Glucose	1.0	2	A
	Fructose	0.3	4	A
	Maltose	0.8	5	
	Maltotriose	0.5	20	
	Sucrose	500	28	B

References: A : HOPKINS and ROBERTS (1935)

B : DEMIS et alia (1954)

Adaptation constants

A_i taken as 0.05 h^{-1} for all sugars

Yeast flocculation rate

f = 0.035 g/l.h (see table 6.2)

Critical sugar concentrations

$s_1^* = 6.5$ g/l

$s_3^* = 37$ g/l

$s_g^* = 40$ g/l

effect upon speed of fermentation (see Section 6.3.2; also URION, 1964; HABOUCHA, 1967); indeed a 5°C rise in temperature can double the fermentation rate (WHITE, 1966). It should be noted that temperature is not normally held constant during brewery fermentations and that Griffin's experiments were performed in small-scale apparatus (2 litre capacity).

A number of parameters, specific to yeast strain and experimental conditions, must be estimated for the model. Parameter values were estimated from Griffin's data for yeast strain N.C.Y.C. no. 1018 for the experimental fermentation performed at a temperature of 18°C: the figures are shown in table 6.3 along with Michaelis and velocity constants and other essential information.

6.2.8 Results and Discussion

Results from the computer simulation trials are shown in figures 6.5 and 6.6, and it can be seen that comparison with experimental data is favourable, especially considering the simplicity of the model.

The results are discussed in three sections; the first deals with sugar concentration profiles, the second is concerned with active yeast concentration during fermentation, and the third is about parameter estimation.

Sugar Concentration Profiles

Concentrations of the individual wort sugars predicted by the model and shown in figure 6.5 appear to match Griffin's data quite well during the early and middle stages of fermentation (i.e. 0-40 hours). However,

Figure 6·5 Changes in Sugar Concentrations During Batch Fermentation of Brewers' Wort

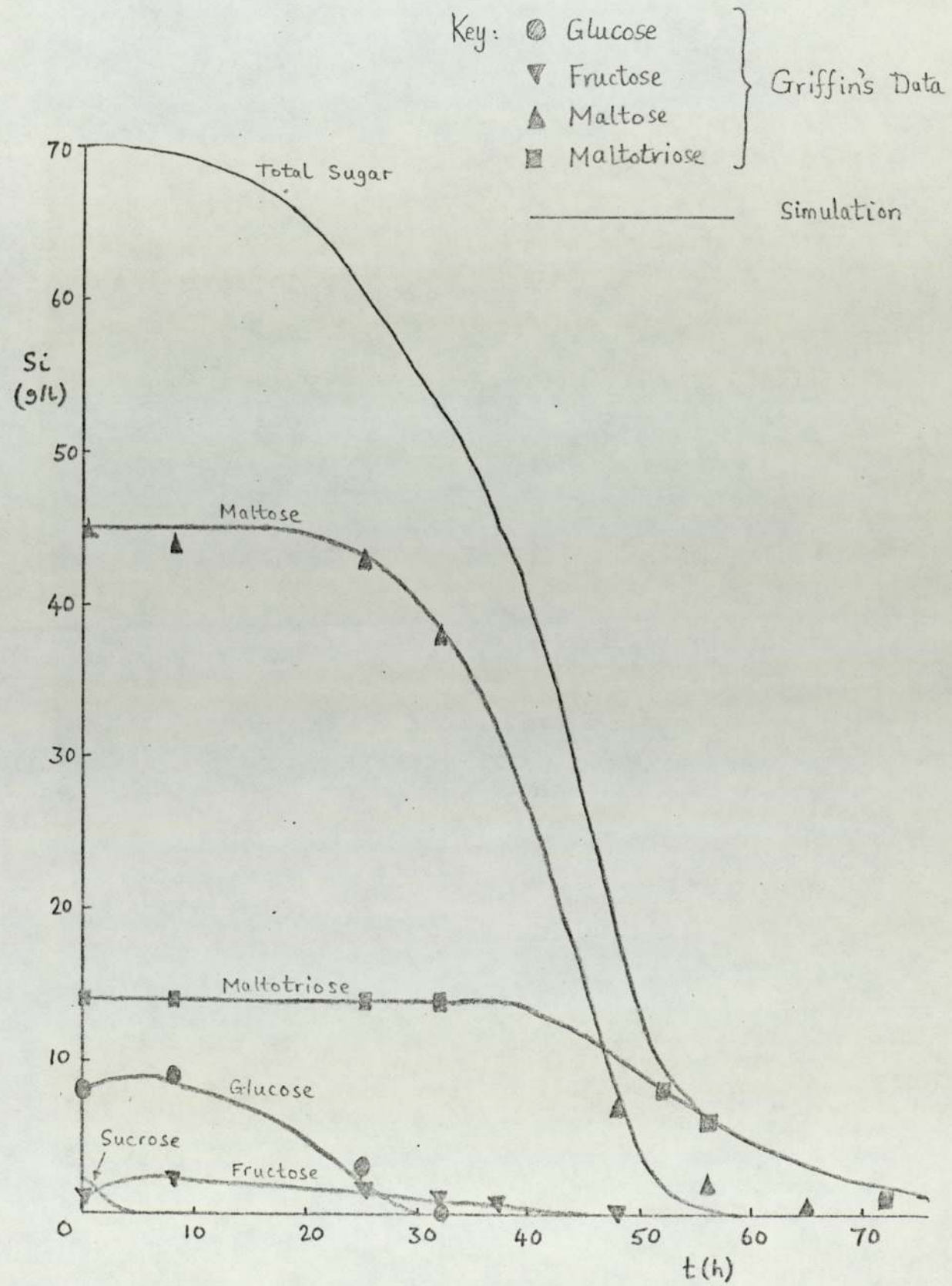
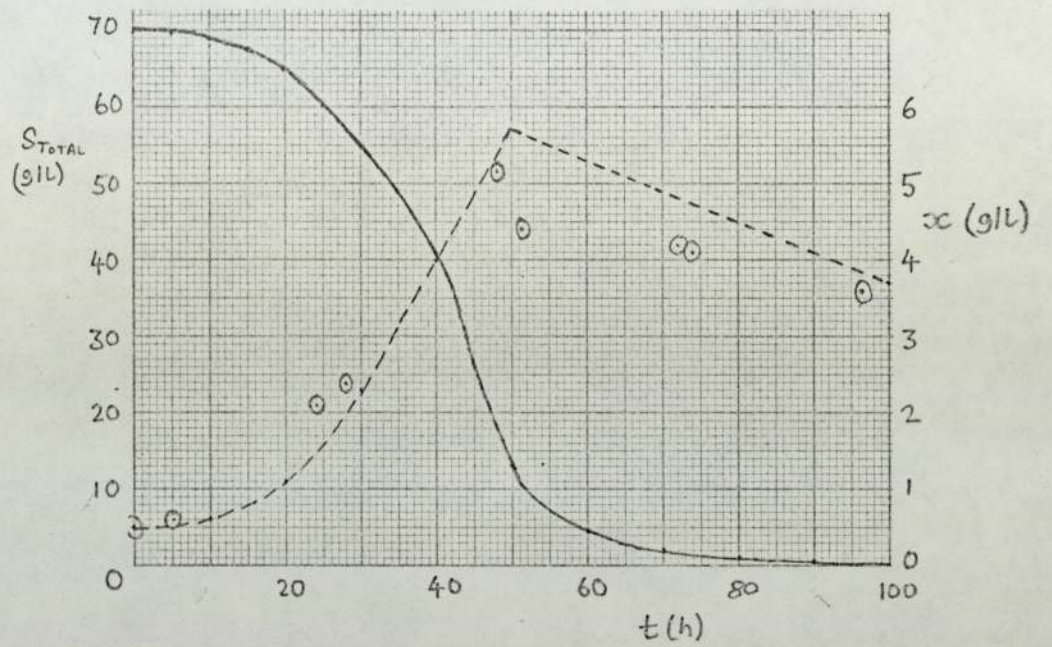


Figure 6.6 Changes in Active Yeast Concentration During Batch Fermentation of Brewers' Wort



Key: ---- dry yeast (simulation)
⊙ dry yeast (Griffin)
—— total sugar (simulation)

towards the end of the fermentation the model predicts that the remainder of the maltose will be utilised too rapidly. This discrepancy is probably due to a number of factors which, when taken together, reduce the activity of the yeast during the later stages of fermentation: the factors thought to be important are considered below.

(1) Inhibitory Effect of Alcohol

As fermentation proceeds alcohol is produced although the concentration remains low until utilisation of the maltose commences. Metabolism of this carbohydrate is rapid and consequently the level of alcohol also rises quickly, producing an increasingly inhibitory effect.

(2) Unfavourable pH

Towards the end of fermentation the pH of the fermenting medium falls steadily from the optimum value for fermentation (see Section 6.3.3).

(3) Age of Yeast Cells

Ageing of yeast reduces the speed of fermentation (THORNE, 1954; HARRIS, 1967). However, the relationship between mean cumulative age of the yeast cell population and time is complex and difficult to quantify since the yeast goes through a number of different growth stages.

(4) De-adaptation of the Yeast

During the later stages of fermentation the concentrations of both maltose and maltotriose fall to low values, and thus it is possible that the yeast's transport mechanisms for these sugars are partially de-activated due to lack of inducing substances.

Active Yeast Concentration

Figure 6.5 shows the concentration of suspended yeast during the course of the fermentation as predicted by the computer model. Comparison with the experimental data reveals that during the first 30 hours of fermentation the predicted level is low, but during the remainder of the growth period the figure is too high. Again the discrepancies are thought to arise from the simplicity of the computer model.

The model is based on a yeast growth figure of 12% of the sugar utilised, an average value during the entire growth phase. This figure could be modified to account for variations in the amounts of oxygen and other components in the wort, thus giving closer agreement between the model predictions and experimental data. For example, at the start of a brewery fermentation the amount of oxygen dissolved in the wort is at a maximum and this encourages yeast growth WHITE (1951, 1952, 1966). As fermentation proceeds this reserve of dissolved oxygen is rapidly depleted resulting in a reduction of growth rate.

A further improvement to the model could be made by incorporating a yeast flocculation term during the whole period of fermentation, rather than after growth has ceased. Such a modification would reduce the maximum concentration of yeast predicted by the model to a level similar to that obtained experimentally.

Estimation of Model Parameters

The shapes of individual sugar utilisation curves can and had to be adjusted to match experimental data by manipulating the parameters

A_i, k_i, and Km_i. Even approximate values of some of the Michaelis and velocity constants could not be found in the literature. CALAM et alia (1971) experienced similar difficulties, and obtained values by performing a series of computer runs and updating the values of these constants after each trial until satisfactory solutions were achieved. A similar technique was used during the present work. The initial values used were, as far as possible, based upon published information. Data regarding anaerobic yeast fermentations are lacking, though there are some concerning the aerobic situation. However, much of this information is of little use since Km values appear to depend upon whether or not the system is aerobic. For example, LEUENBERGER (1972) quotes Km values for glucose utilisation by yeast of 0.02 mM and 3.0 mM respectively for aerobic and anaerobic conditions. In any case, results of different workers are difficult to compare since the values of k_i and Km_i are sensitive to temperature (see Section 4.5.2).

6.3 MODIFICATION OF THE MODEL TO INCLUDE ADDITIONAL PARAMETERS (see also Section 4.5)

6.3.1 Introduction

The basic rate expression, given by equation (6.5),

$$\frac{ds}{dt} = \frac{-k\,s\,x}{Km+s} \cdot I(t) \qquad (6.5)$$

takes no account of important process variables such as temperature, pH, dissolved oxygen level, alcohol concentration, pitching rate, age of yeast,

nitrogen content, etc. During fermentation many of these variables change appreciably (see Section 6.1.5 and figure 6.1) and thus affect the rate of fermentation. The purpose of this section is to survey work reported in the literature concerning the effect of such parameters upon rate of fermentation.

Denoting correction factors for each of the parameters considered by "ϕ_i", equation (6.5) becomes:

$$\frac{ds}{dt} = -\frac{k s x}{K_m + S} \cdot I(t) \cdot \prod_{i=1}^{n} \phi_i \qquad (6.9)$$

Any parameter affecting rate of fermentation may be incorporated into equation (6.9), the "modified rate equation", providing a mathematical expression describing its effect upon fermentation rate can be derived.

6.3.2 Temperature (see also Section 4.5.2)

THORNE (1954) suggested that the effect of temperature upon rate of fermentation could be explained by the following expression:

$$\phi_T = b_1 \exp(a_1 + a_2 \cdot T) + b_2 \exp(a_3 + a_4 \cdot T) \qquad (6.10)$$

Suitable choice of the constants, a_i, allows equation (6.10) to represent many forms of temperature curves.

FRANZ (1961), whilst investigating the rate of fermentation of molasses by Bakers' yeast, chose to represent the effect of temperature by

an expression similar to the Arrhenius equation:

$$\phi_T = a_5 \cdot \exp\left[\frac{E_A \cdot (T_2 - T_1)}{R \cdot T_1 \cdot T_2}\right] \qquad (6.11)$$

where: a_5 = constant

E_A = activation energy, kcal/mole

R = universal gas constant, 2×10^{-3} kcal/mole, ^oK

T_1 = 293 ^oK

T_2 = fermentation temperature, ^oK

WHITE (1951, 1966) investigated the effect of temperature upon the rates of growth and fermentation of yeast during exponential growth: results are illustrated in figure 6.7. It is interesting to note that both curves in figure 6.7 are linear between the temperatures of 15° and 25°C, and can therefore be represented by a simple mathematical expression, such as:

$$\phi_T = a_6 + a_7 \cdot T \qquad (6.12)$$

During a typical top fermentation the temperature is allowed to rise from 15° to 20°C, causing the rate coefficient to rise from 0.08 to 0.16 and the growth coefficient to rise from 0.04 to 0.12 (see figure 6.7). Since a rise in temperature of only 5°C causes the fermentation rate to double and the yeast growth rate to treble, the importance of this parameter can clearly be seen.

Finally, MERRITT (1966) has noted a fall in the duration of the lag phase (after yeast pitching) as temperature is increased - further evidence that yeast is more active at higher temperatures.

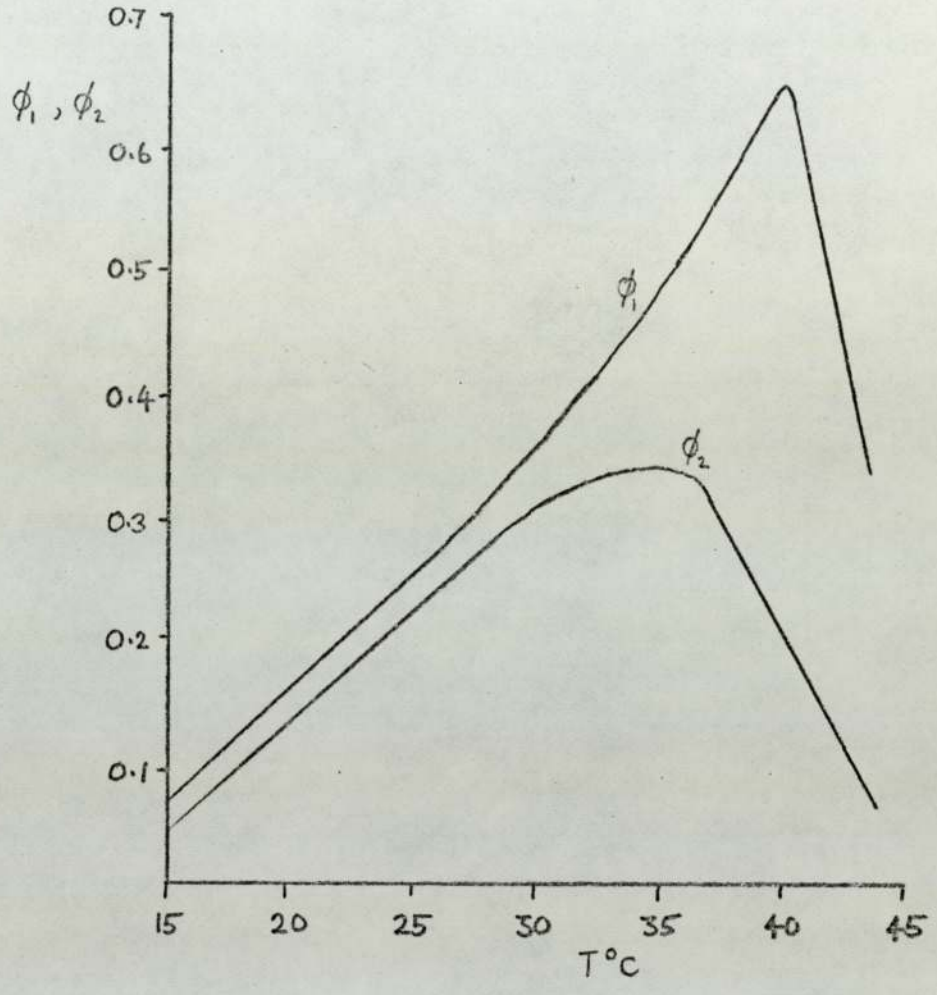
6.3.3 pH of the Fermentation Broth (see also Section 4.5.3)

The findings of various workers regarding the effect of pH on rate of fermentation do not appear to be consistent. FRANZ (1961), investigating the fermentation of molasses by Bakers' yeast, stated that between pH 3.0 and pH 6.5 the fermenting rate is approximately constant, though below pH 3.0 it declines with increasing steepness. THORNE (1954), however, declares that between pH 3.2 and pH 5.3 the rate of fermentation changes by about 15%, whereas VISURI and KIRSOP (1970) found that the fermentation rates of both maltose and maltotriose are strongly influenced by pH (see figure 6.8). During the course of a typical brewery fermentation the pH falls from 5.5 to 4.0 (see Section 6.1.5), and thus the utilisation of both maltose and maltotriose occur at near optimum pH (see figure 6.8). During the latter stages of fermentation, though, the pH value falls progressively away from the optimum for metabolite production.

6.3.4 Dissolved Oxygen Concentration

Oxygen is only sparingly soluble in fermentation media (see Section 3.1) and much of the oxygen dissolved in wort is utilised by the yeast for growth purposes during the early stages of fermentation. According to HUDSON (1967) lack of dissolved oxygen causes degeneracy of yeast, resulting in a loss of fermentative power. PIENDL (1963) states

Figure 6.7 Influence of Temperature upon Growth and Fermentation Rates of Yeast (White, 1966)

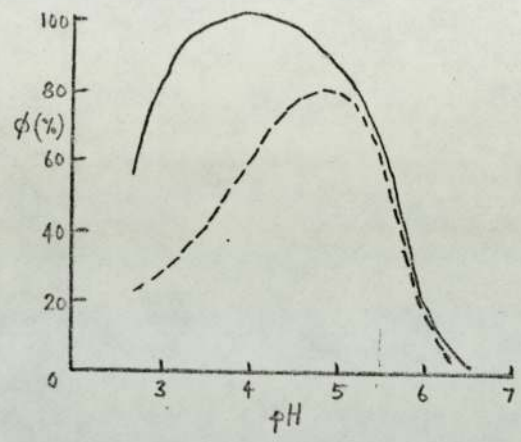


ϕ_1 = sugar utilisation coefficient

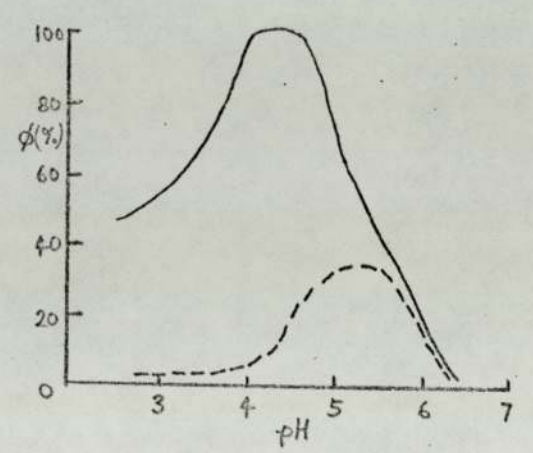
ϕ_2 = specific growth coefficient

Figure 6.8 Influence of pH upon Fermentation Rates of Maltose and Maltotriose by Yeast (Visuri and Kirsop, 1970)

(a) maltose



(b) maltotriose



Key: ——— with 0·25m potassium
------- with no potassium

Figure 6.9 Influence of Wort Oxygenation upon Growth and Fermentation Rates of Yeast (White, 1966)

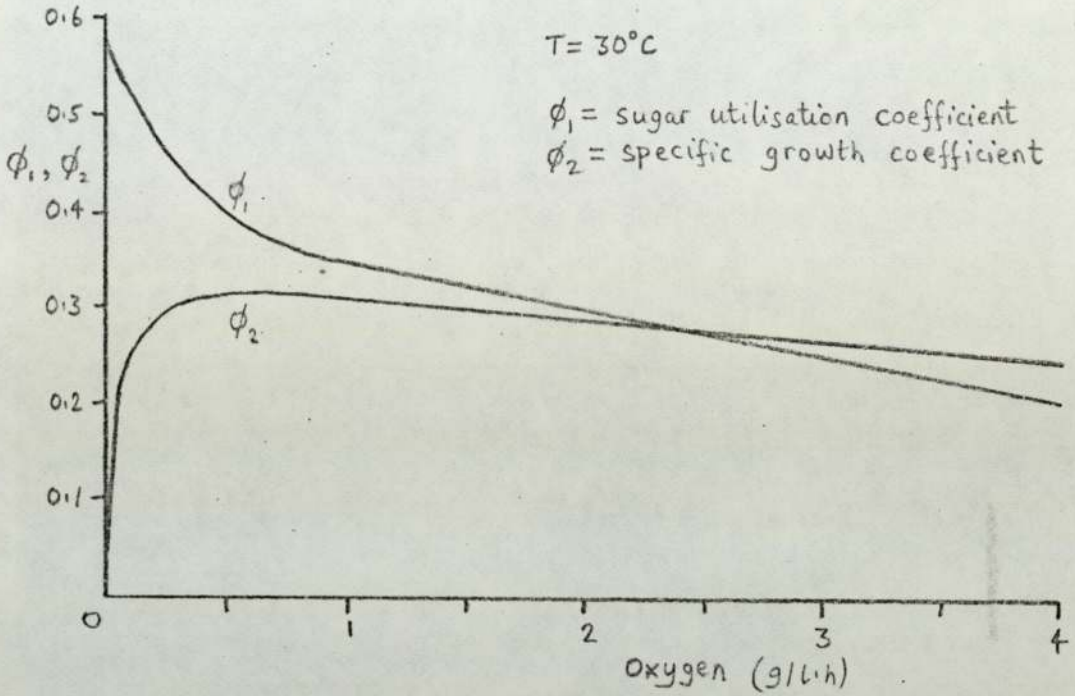
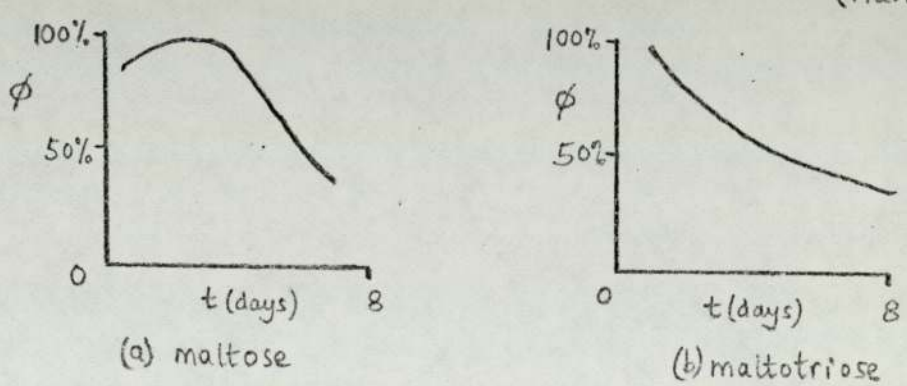


Figure 6.10 Effect of Ageing of a Yeast Culture upon Fermentation Rates of Maltose and Maltotriose (Harris, 1967)



that premature flocculation occurs in both over- and under-oxygenated worts.

WHITE (1951, 1966) studied the effect of artificial oxygenation of the wort on the rates of fermentation and yeast growth; results are presented in figure 6.9 and show that oxygen benefits yeast growth whilst having the opposite effect upon rate of fermentation (the Pasteur effect - see Section 8.2.1).

6.3.5 Alcohol Concentration (see also Section 4.5.4)

It is well-known that towards the end of a batch beer fermentation increasing concentrations of alcohol reduce the rate of fermentation. AIBA et alia (1968) have characterised the kinetics of alcohol inhibition by the following expression:

$$\phi_p = \exp(-a_8 \cdot P) \qquad (6.13)$$

Alternatively, FRANZ (1961) has used as an expression of the following form:

$$\phi_p = \left[1 - \left(\frac{P}{P_{max}}\right)^2\right] \qquad (6.14)$$

where P_{max} = limiting alcohol concentration

FRANZ experimentally determined a limiting alcohol concentration of 120 g/l for the fermentation of molasses by Bakers' yeast.

6.3.6 Pitching Rate of Yeast

High pitching rates of yeast result in reduced fermentation time, but may alter the flavour of the beer. Rate of yeast growth is lower than normal at high pitching rates; HOUGH (1961) found that the yeast crop at the end of fermentation is almost independent of pitching rate in the conventional range of 0.3 - 1.0 lb/brl. This indicates that yeast growth is possibly limited by nutrient or oxygen content of the wort.

According to PIENDL (1969) high pitching rates lead to rapid rates of flocculation, whereas low pitching rates result in more new cell formation which flocculates later. HABOUCHA (1967) states that low pitching rates also lead to rapid degeneracy of the yeast.

6.3.7 Age of Yeast

Ageing of yeast is generally believed to reduce the rate of fermentation; results of THORNE (1954) indicate that speed of fermentation is reduced by 50% over an 8-day period, and also that ageing increases the initial lag period of yeast. In addition, THORNE states that as temperature is increased the effect of yeast age upon fermentation rate becomes more apparent.

HARRIS (1967) performed experiments to determine the effect of ageing of a yeast culture upon the fermentation velocities of glucose and maltose; results are shown in figure 6.10.

6.3.8 Nitrogen Contents of Yeast and Wort

Nitrogenous nutrients are almost entirely removed from wort during the accelerating phase of fermentation (KIRSOP, 1972). THORNE (1954)

measured a 20% increase in speed of fermentation with 0.24 g/l assimilable nitrogen present in wort compared with wort containing no assimilable nitrogen; he suggested a linear relationship between the nitrogen content of yeasts and their fermentation rate.

6.3.9 Other Factors

PIENDL (1969) has found that high mashing temperatures, and over- and under-modified malts produce wort from which yeasts flocculate premately; barley variety is an additional factor influencing flocculation.

WHITE (1955) proposed an inverse relationship between osmotic pressure of the wort and rate of fermentation; at very high osmotic pressures there is no fermentative activity. Elsewhere in the literature WHITE and MUNNS (1952, 1953) declare that sugar concentrations in excess of 100 g/l reduce the rate of fermentation. High substrate concentrations also increase the duration of the lag period and stimulate the yeast growth rate during the exponential phase.

Finally, THORNE (1954) claims that variations in the levels of phosphate, calcium, magnesium, and potassium ions in the wort can effect the rate of fermentation by up to 40%. VISURI and KIRSOP (1970) have also published results on this topic.

6.4 CONCLUSIONS

A mathematical model describing the kinetics of sugar utilisation and changes in active yeast concentration during batch fermentation of brewers' wort has been developed. New ideas for dealing with the kinetics of multi-substrate systems are presented and means of incorporating

important process parameters (such as temperature) into the model are outlined. The model can be fitted to published experimental data, but first a number of parameters must be estimated. Whilst the model is useful for interpreting sugar utilisation data during batch beer fermentation, it could not be used to optimise the production of multi-component alcoholic beverages since it does not account for taste, appearance, or other important selling characteristics of such drinks. Inclusion of these parameters into a mathematical model for fermented beverages would be difficult due to the kinetic complexity of the fermentation process itself (i.e. the conversion of hundreds of wort components into metabolites responsible for flavour, colour, etc., of the alcoholic product).

LIST OF SYMBOLS (Section 6)

SYMBOL	EXPLANATION	UNITS
a_j	constant	various
A, A_i	adaptation constant	h^{-1}
E_A	activation energy	kcal/mole
f	rate of yeast flocculation	g/l.h
G(p)	"black box" transfer function	-
I(p)	adaptation function in the "p" domain	-
I(t)	adaptation function in the "time" domain	-
k, k_i	velocity constant	g/g dry yeast.h
Km, Km_i	Michaelis constant	g/l
p	product (alcohol) concentration	g/l
r, r_i	reaction rate	g/l.h
R	universal gas constant	kcal/mole $.^{o}K$
s, s_i	substrate (sugar) concentration	g/l
s_o	initial substrate concentration	g/l
s_i^*	critical concentration of component "i"	g/l
s_g^*	substrate utilised before growth ceases	g/l
t	time	h
T	temperature	^{o}C
x	biomass (yeast) concentration	g.dry wt./l

SYMBOL	EXPLANATION	UNITS
$X(p)$	system input in the "p" domain	
Y_T	yield of yeast dry matter per unit mass of substrate consumed	g.dry wt./g

Greek Letter

ϕ_m	factor to modify the basic rate equation to account for parameter "m"	-

Subscripts

i = 1	glucose
2	fructose
3	maltose
4	maltotriose
5	sucrose

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SECTION 7

A MODEL OF THE A.P.V. CONTINUOUS BEER TOWER FERMENTATION SYSTEM

Figures

Tables

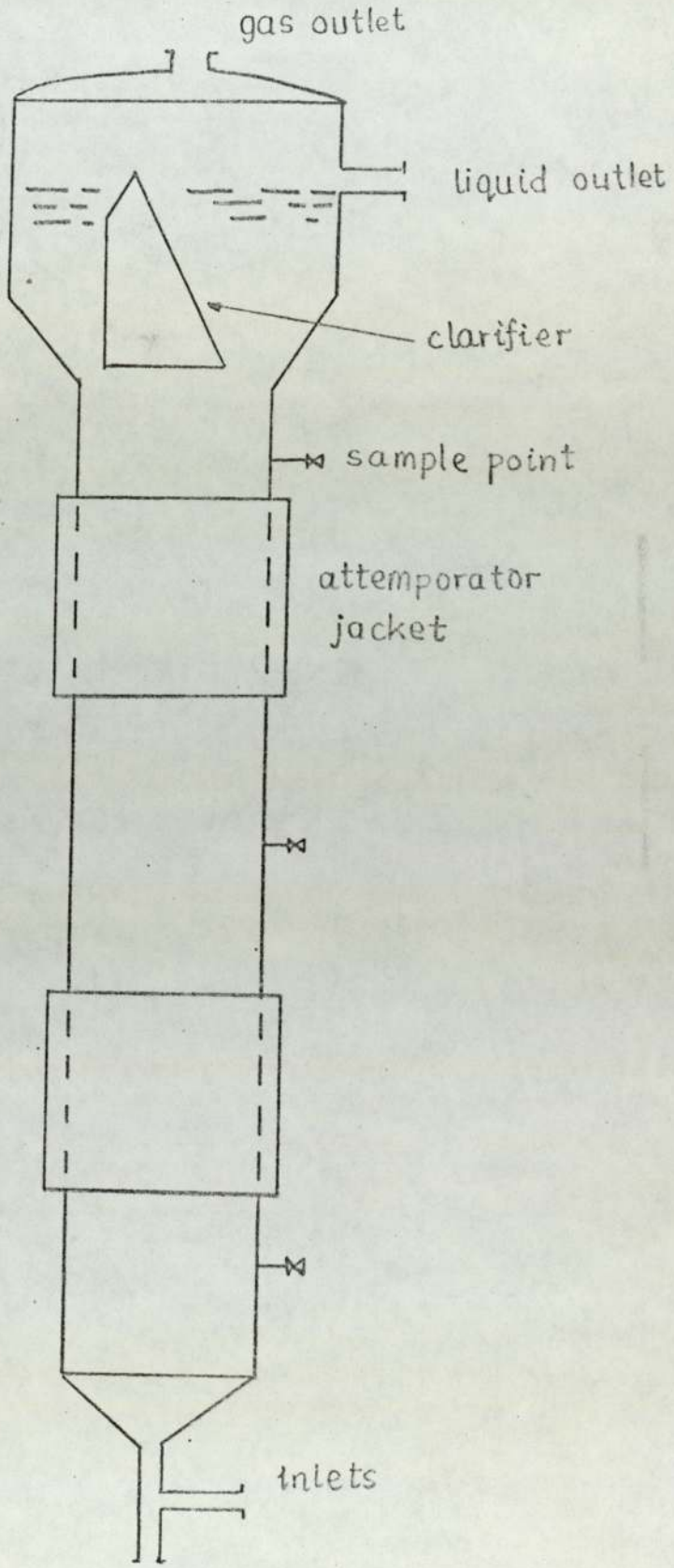
7.1 INTRODUCTION (see also Section 1.5)

The A.P.V. tower fermenter consists, basically, of a vertical cylindrical tower, as shown in figure 7.1. Wort is introduced at the base of the tower through a perforated plate, which ensures even liquid distribution. Further perforated distributor plates may be fixed at intervals up the tower to reduce the tendency towards channelling. At the top of the column the vessel diameter increases considerably, thus providing a natural settling region. This allows the yeast flocs to sediment against the upward flow of liquid and clear beer to overflow through an outlet. Fermentation gas passes through a riser, thus preventing agitation of the still zone.

A high yeast concentration (about 250 g/l wet weight) is maintained in the tower in order to achieve a rapid rate of fermentation. Yeast strains which flocculate to form clumps of about 0.1 cm diameter seem particularly suitable. A classification scheme for continuous tower yeasts is described by GREENSHIELDS and SMITH (1971). At low wort flowrates there is generally a yeast concentration gradient through the tower, the maximum being at the base. However, at high wort flowrates (or high wort sugar concentrations) the yeast concentration gradient alters and a maximum may be observed in the middle or near the top of the tower.

During normal operation there is little mixing in the lower portion of the tower due to the high concentration of compacted yeast. Consequently, there is plug-flow of liquid - with some channelling - in

Figure 7.1 The A.P.V Tower Fermenter



this section. Near the top of the tower, where biomass concentration is much lower, the action of gas bubbles promotes gentle mixing. Much of the wort sugar is utilised near the base of the tower, and, consequently, steep density gradients are apparent in this region.

Heat of reaction from the fermentation process results in a rapid rise in temperature near the base of the column. AULT et alia (1969) observed that wort entering the system at 60°F rose in temperature to between 70° and 74°F by the time it reached the top of the tower. To some extent temperature inside the tower can be controlled using attemperator jackets.

7.2 MODEL BASIS (Developed in collaboration with Dr. A. James)

7.2.1 Introduction

The continuous beer model is based upon the assumption that the tower contains a fluidised bed of yeast flocs. The voidage of the bed is calculated from an expression similar to the RICHARDSON-ZAKI equation (1954), viz:

$$\frac{u_s}{u_T} = \varepsilon^n \qquad (7.1)$$

JAMES (1973) carried out extensive investigations into the characteristics of fluidised yeast flocs and determined values of "n" for various yeasts under different experimental conditions.

In the model, the tower is divided into a number of compartments, each containing an equal quantity of yeast. The volume of each segment,

therefore, depends upon the voidage of the yeast bed, which is computed from equation (7.1). Knowledge of the physical characteristics of the yeast flocs enables the dry weight of yeast to be calculated for each segment. Plug flow of liquid through the tower is assumed, and liquid residence times for each compartment are computed from segment volume, voidage, and wort flowrate.

The fermentation is assumed to be anaerobic, and the gas phase is neglected. The kinetics of sugar utilisation are based upon those developed for the batch beer fermentation model (see Section 6.2). Yeast growth is not taken into account.

Calculation of terminal velocity, U_T, in equation (7.1) depends upon effective yeast floc diameter, and solid and liquid phase densities. Terminal velocity, therefore, varies during the course of fermentation (i.e. with axial position in the tower). Yeast floc size depends upon a number of factors, and simulation of changes in floc size is difficult. Consequently, two alternative approaches have been investigated: (1) constant floc size, and (2) variable floc size, with stratification. Individual sections are discussed in more detail below.

7.2.2 Fluidisation

Fluidisation of yeast flocs can be described by the Richardson-Zaki expression (equation 7.1 above). JAMES (1973) determined values of the exponent "n", but found that the value varied with a number of parameters, including: (1) strain of yeast, and (2) pH and temperature of the liquid medium. It is possible that changes in yeast floc structure result in

apparent variations of n. Such changes could result from alteration of the pH and temperature of the medium. Floc structure is difficult to ascertain experimentally, though, and these effects are more easily interpreted by adjusting the value of n.

James encountered further experimental difficulties which resulted in inconsistent results, such as:

(1) irregularity of size, shape, and structure of the solid microbial phase,

(2) continual growth of the solid phase, and consequent release of gaseous carbon dioxide,

(3) a very small density difference between the solid and liquid phases, and

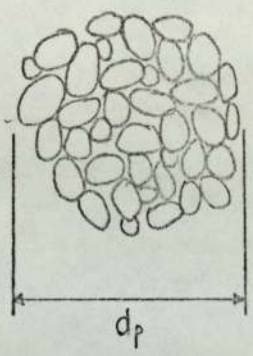
(4) non-homogeneity of both the solid and liquid phases throughout the tower.

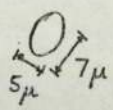
The experiments of James were performed under anaerobic conditions using a liquid medium containing neither fermenting sugars nor metabolic products. Consequently, yeast floc size was not affected by gas evolution or by sugar-induced deflocculation. Further, it is likely that the presence of a gaseous phase would result in three-phase fluidisation. For reasons of simplicity, and lack of suitable data, this phenomenon is not considered in the present work. The reader is referred to ØSTERGAARD (1965, 1966, 1968, 1970, 1972) for further information on this topic.

7.2.3 Yeast Floc Structure

Computation of yeast dry weight and particle density depends upon the structure of the yeast floc. A diagram of an ideal yeast floc is shown in figure 7.2 below.

Figure 7.2 An Ideal Yeast Floc





Individual Yeast Cell

The floc is assumed to be spherical, with characteristic diameter d_p. Individual yeast cells are thought to pack closely together to form a floc. JAMES (1973) assumed a floc voidage of 0.5; this figure has been used in the present model.

Yeast cells consist mainly of water. JAMES (1973) found the volume fraction of liquid in a yeast cell, w_f, to be 0.8, and density of the remaining dry matter, $\rho_y = 1.32$ g/cm^3. Cell density can be calculated as follows:

$$\rho_c = w_f \cdot \rho_L + (1 - w_f)\rho_y \qquad (7.2)$$

$$= 0.8\rho_L + (0.2 \times 1.32)$$

Generally a value of 1.07 g/cm^3 is accepted (AIBA et alia, 1962; ROYSTON, 1966a, 1966b; JAMES, 1973).

Similarly, floc density can be calculated as follows:

$$\rho_F = \varepsilon_F \cdot \rho_L + (1-\varepsilon_F) \cdot \rho_c \qquad (7.3)$$

$$= 0.5\,\rho_L + (0.5 \times 1.07)$$

Here, an assumption must be made about the density of the liquid within the floc. The extreme cases are (JAMES, 1973):

(1) when the density of the interstitial liquid equals the density of the external liquid, and

(2) when the density of the interstitial liquid equals that of water or the final product.

Fermentation involves diffusion of nutrients into the floc, and metabolites out of the floc. James suggested that where diffusion is rate-controlling, case (2) would be appropriate, and where reactions within the cell are rate-controlling, case (1) may hold. Diffusion is not thought to be an important factor for floc diameters of less than 0.2 cm (GREENSHIELDS and SMITH, 1971).

7.2.4 Calculation of Liquid Density

The specific gravity of solutions of sugar can be approximated by the following equation:

$$\text{w/w \% sugar concentration} = \frac{1000}{3.889}\cdot(\rho_L - 1) \qquad (7.4)$$

$$\rho_L > 1.005$$

However, the addition of ethanol to such a solution tends to lower the specific gravity. It seems reasonable to assume that specific gravity of fermenting wort is linearly related to sugar concentration (since alcohol synthesis is almost proportional to sugar utilisation). A suitable expression to fit the data of AULT et alia (1969) is as follows:

$$\rho_L = 0.996 + \frac{0.465}{1000} \cdot \sum_{i=1}^{n} S_i \qquad (7.5)$$

$$\rho_L \geq 1.006$$

7.2.5 Calculation of Floc Terminal Velocity (see COULSON and RICHARDSON, 1968)

For the purpose of calculation of terminal velocity, U_T, the yeast flocs are assumed to be spherical particles with characteristic diameter, d_p. When a spherical particle is allowed to settle in a fluid under the action of gravity, its velocity increases until the accelerating force is exactly balanced by the resistance force. The accelerating force due to gravity is given by:

$$F_A = \frac{1}{6} \pi . d_p^3 . (\rho_F - \rho_L) g \qquad (7.6)$$

Resistance force is given by:

$$F_R = \frac{1}{4} \pi . d_p^2 . R' \qquad (7.7)$$

Expressions for R^1, the drag force per unit projected area of the particle, vary according to the value of the Reynolds number for the particle, N_{Re}. This is given by:

$$N_{Re} = \frac{\rho_L . u_T . d_p}{\mu_L} \qquad (7.8)$$

The drag factor, $\frac{R^1}{\rho_L . u_T^2}$, is given by the following expressions:

(1) $\underline{10^{-4} < N_{Re} < 0.2}$

$$\frac{R^1}{\rho_L . u_T^2} = \frac{12}{N_{Re}} \qquad (7.9)$$

$$\therefore R^1 = \frac{12 \mu_L . u_T}{d_p} \qquad (7.10)$$

substituting (7.10) into (7.7) we have:

$$F_R = 3\pi . \mu_L . u_T . d_p \qquad (7.11)$$

$$= F_A = \frac{1}{6}\pi . d_p^3 . (\rho_F - \rho_L) . g \qquad (7.6)$$

$$\therefore u_T = \frac{d_p^2 (\rho_F - \rho_L) g}{18 \mu_L} \qquad (7.12)$$

i.e. Stoke's Law

(2) $0.2 < N_{Re} < 10^3$

$$\frac{R'}{\rho_L \cdot u_T^2} = \frac{9.25}{N_{Re}^{0.6}} \qquad (7.13)$$

and $$u_T = \frac{0.153 \left[g(\rho_F - \rho_L)\right]^{0.715} \cdot d_p^{1.15}}{\mu_L^{0.43} \cdot \rho_L^{0.29}} \qquad (7.14)$$

i.e. the Allen equation

(3) $10^3 < N_{Re} < 2 \times 10^5$

$$\frac{R'}{\rho_L \cdot u_T^2} = 0.22 \qquad (7.15)$$

and $$u_T = 1.74 \left[\frac{d_p(\rho_F - \rho_L)g}{\rho_L}\right] \qquad (7.16)$$

Generally, the behaviour of yeast flocs during continuous beer fermentation is described by the equations for region (2).

7.2.6 Floc Size Distribution

Floc diameter is an important model parameter, since it determines terminal velocity of the yeast aggregates. Many factors affect floc size and it is a difficult facet to model. At the base of the tower incoming wort tends to deflocculate yeast, due to high sugar concentrations; near the top of the tower the action of gas bubbles breaks up the flocs. As a result of these and other difficulties

(e.g. the effects of pH, temperature, and wort components), it was decided to specify a floc size distribution for the model. Two alternatives were considered: (1) constant floc size, and (2) variable floc size with complete stratification. JAMES (1973) has shown that stratification is likely to occur.

Observations by GREENSHIELDS and SMITH (1971) and JAMES (1973) suggest that a mean floc diameter of 0.1 cm is appropriate. For the case of variable floc size, a normal distribution of floc diameters was assumed: such a distribution, although arbitrary, is easily generated on the computer.

Having fixed the mean floc diameter, a value for the variance of this parameter must be chosen, Points on the standard normal distribution can be represented as follows:

$$P_i = \bar{P} + q_i \cdot \sigma \qquad (7.17)$$

where p_i = point value of parameter p

$\bar{p}$ = mean value of p

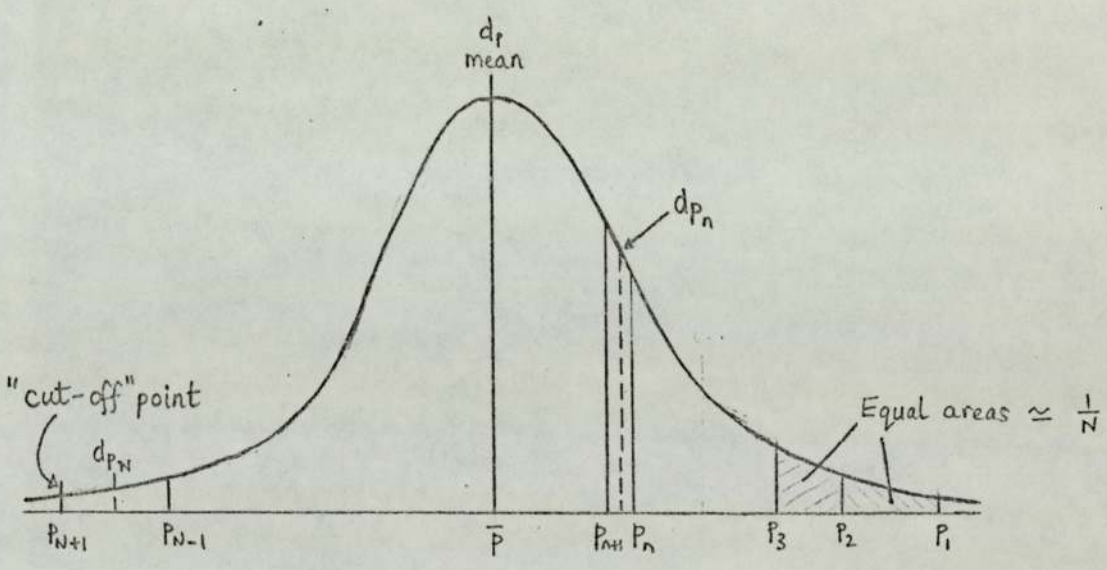
q_i = a variable, and

σ = variance of p

The values q_i represent points on the probability density function for the standard normal distribution; thus 21 points can be chosen which divide the area under this curve into 20 equal portions. However, since

the distribution is symmetrical, only 11 values of q need be chosen and, because the probability function extends to infinity, the end value of q must be chosen arbitrarily - the "cut-off" point (see figure 7.3). Hence, if these 11 values, q_i, are fed into a suitable computer program, it is possible to generate the points, p_i, which define the boundaries of 20 approximately equal portions of the normally-distributed variable, p.

Figure 7.3 The Standard Normal Distribution



7.3 MODEL ASSUMPTIONS

(1) The system is anaerobic and dissolved oxygen concentration is zero.

(2) Temperature and pH remain constant throughout the fermenter.

(3) The wort is a multiple-substrate system consisting of the five major fermentable sugars (i.e. glucose, fructose, sucrose, maltose, and multotriose) and a quantity of unfermentable sugars.

(4) Sugar utilisation kinetics are based on those developed for the batch beer fermentation model (see Section 6.2).

(5) The ability of the yeast to ferment wort remains unchanged throughout the length of the tower.

(6) No gas phase is present.

(7) The liquid phase is in plug flow from segment to segment (i.e. no backmixing and no channelling).

(8) The liquid in each compartment is well-mixed.

(9) Liquid density is calculated using equation (7.5).

(10) The yeast flocs behave as if fluidised, according to equation (7.1).

(11) There is no mixing of the solid phase between segments (i.e. no possibility of yeast in different metabolic states mixing).

(12) Floc terminal velocity is calculated according to Section 7.2.5.

(13) The size distribution of yeast flocs is known.

(14) Yeast floc size is not affected by liquid or gas flow or by any other factors.

(15) Yeast floc structure is defined according to Section 7.2.3.

(16) Interstitial floc liquid has the same composition as the surrounding liquid.

(17) The yeast separation stage at the top of the tower is omitted from the model.

7.4 SINGLE-SEGMENT MODEL

7.4.1 Introduction

In order to investigate the behaviour of the continuous beer tower system a single-segment model (based on the principles described above)

is used for initial computer simulation studies. The sensitivity of this model to a number of key parameters is investigated.

Assumptions are as in Section 7.3 above, except that:

(1) the height of the segment (and thus volume) is fixed, and

(2) the fermentable sugars are lumped together to give a total sugar concentration. Suitable kinetic parameters are inserted into a single Michaelis-Menten expression.

7.4.2 Calculation Sequence

(1) Calculate floc terminal velocity from the Allen equation.

$$u_T = \frac{0.153\,[g(\rho_F-\rho_L)]^{0.715}\,.\,d_p^{1.15}}{\rho_L^{0.29}\,.\,\mu_L^{0.43}} \quad \text{cm/s} \qquad (7.14)$$

(2) Calculate bed voidage from the fluidisation equation.

$$\varepsilon = \left(\frac{u_s}{u_T}\right)^{1/n} \qquad (7.18)$$

(3) Calculate dry weight concentration of yeast.

$$x = 1000\,(1-\varepsilon)(1-\varepsilon_F)(1-w_f)\,\rho_y \quad \text{g/l} \qquad (7.19)$$

(4) Calculate liquid residence time in segment.

$$t = \frac{L.\varepsilon}{3600.u_s} \quad \text{h} \qquad (7.20)$$

(5) Finally, solve the kinetic equation (using the Euler method with step length 0.01h)

$$\frac{ds}{dt} = \frac{-ksx}{K_m + s} \qquad g/l.h \qquad (7.21)$$

7.4.3 Data

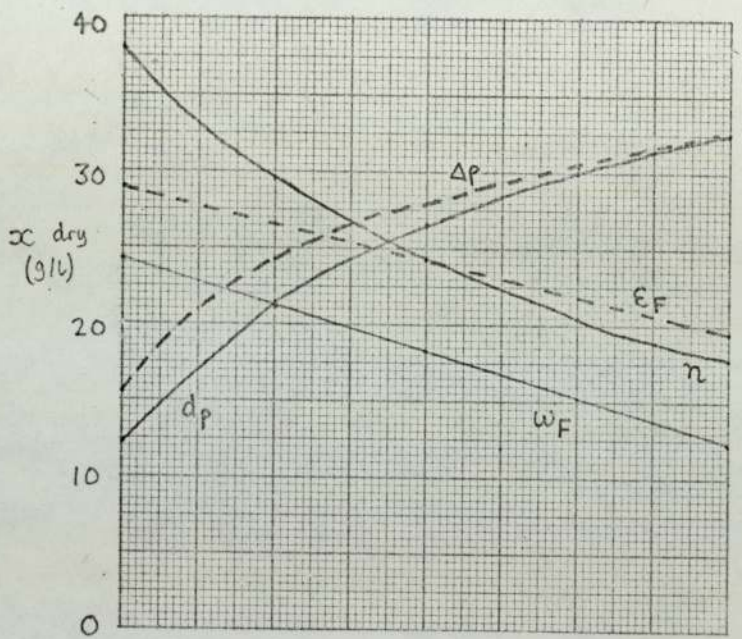
Data is presented in table 7.1 below.

Table 7.1 Data for the Single-Segment Model

Initial sugar concentration, s_o	60 g/l
Velocity constant, k	1 g/g.dry yeast.h
Michaelis constant, Km	5 g/l
Wort viscosity, μ_L	1 cP
Wort gravity, ρ_L	1.035 g/cm^3
Floc density, ρ_F	1.050 g/cm^3
Dry yeast density, ρ_Y	1.32 g/cm^3
Floc diameter, d_p	0.1 cm
Floc liquid fraction, ε_F	0.5
Yeast cell water fraction, w_f	0.8
Fluidisation index, n	10
Segment diameter	91.5 cm
Segment height	30 cm

Figures 7.4 and 7.5 Effect of Key Parameters on the Behaviour of the Single-Segment Model

Figure 7.4 Dry Yeast Concentration



n	6	8	10	12	14
d_p (cm)	·04	·08	·12	·16	·20
$\Delta\rho$ (g/cm³)	·005	·015	·025	·035	·045
ω_F	·8	·825	·85	·875	·9
ε_F	·4	·45	·5	·55	·6

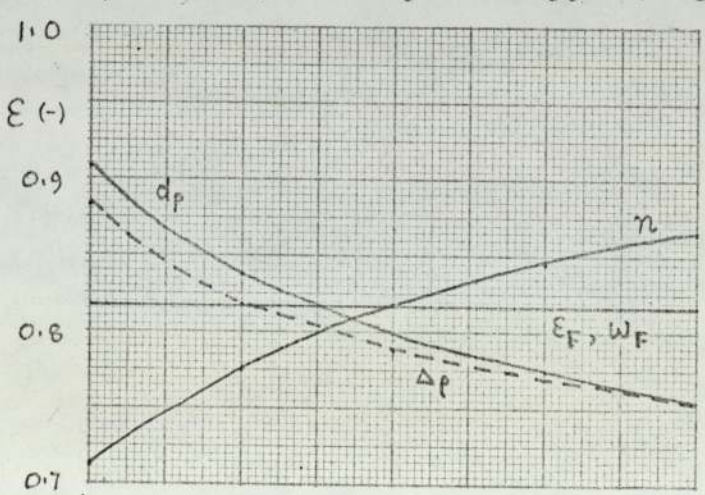


Figure 7.5 Bed Voidage

7.4.4 Results

Results are shown in figures 7.4 and 7.5. It can be seen that bed voidage decreases and dry yeast concentration (expressed as g. dry yeast/litre fermenter volume) increases with:

(1) decreasing n

(2) increasing d_p

(3) increasing $(\rho_p - \rho_L)$

(4) decreasing w_f , and

(5) decreasing ε_F.

These results are useful for interpreting the behaviour of a more complex system, such as the multi-segment model - a better approximation to a real tower fermentation system.

7.5 MULTI-SEGMENT MODEL

7.5.1 Calculation Sequence

(1) The average yeast voidage in the tower is estimated using

$$\varepsilon_{av} = \left(\frac{u_s}{u_{Tav}}\right)^{1/n} \qquad (7.22)$$

where $U_{T_{av}}$ is calculated from the Allen equation using average estimates of the variables d_p, ρ_F, and ρ_L, viz:

$$u_{Tav} = \frac{0.153\left[g(\rho_F - \rho_L)\right]^{0.715} \cdot d_p^{1.15}}{\rho_L^{0.29} \cdot \mu_L^{0.43}} \text{ cm/s} \qquad (7.23)$$

(2) Volume of wet yeast per segment can now be calculated:

$$X_i = \frac{V}{N}(1-\varepsilon_{av}) \qquad g. \qquad (7.24)$$

(3) Dry weight of yeast per segment is, therefore:

$$x_i = X_i(1-\varepsilon_F)(1-w_f)\rho_Y \qquad g. \qquad (7.25)$$

(4) Segment-by-segment calculations now begin, commencing with the 1st (i.e. bottom) compartment. Using physical properties of the inflowing liquid phase, liquid and floc densities for segment "i" can now be calculated from:

$$\rho_{Li} = 0.996 + \frac{0.465}{1000}\cdot\sum_{j=1}^{6} s_{j,i} \qquad g/cm^3 \qquad (7.26)$$

$$\rho_{Fi} = \varepsilon_F \cdot \rho_{Li} + (1-\varepsilon_F)\rho_c \qquad g/cm^3 \qquad (7.27)$$

(5) Floc terminal velocity is then calculated from the Allen equation:

$$u_{Ti} = \frac{0.153\,[g(\rho_{Fi}-\rho_{Li})]^{0.715}\cdot d_{Pi}^{1.15}}{\rho_{Li}^{0.29}\cdot\mu_L^{0.43}} \qquad cm/s \qquad (7.28)$$

(6) Segment voidage can now be computed using the fluidisation equation:

$$\varepsilon_i = \left(\frac{u_s}{u_{Ti}}\right)^{1/n} \qquad (7.29)$$

(7) Volume, height and liquid residence time for segment i are calculated as follows:

$$V_i = \frac{x_i}{1-\varepsilon_i} \quad cm^3 \qquad (7.30)$$

$$h_i = \frac{V_i}{A} \quad cm \qquad (7.31)$$

$$t_i = \frac{h_i.\varepsilon_i}{3600.u_s} \quad h \qquad (7.32)$$

(8) Compartmental liquid composition can now be calculated using the sugar utilisation kinetics described in Section 6.2. Thus for sugar "j" we have:

$$\frac{ds_j}{dt} = -\frac{k_j.s_{j,i}.x_{c_i}}{K_{mj} + s_{j,i}} \qquad (7.33)$$

Equation (7.33) above is integrated between t = 0 and t = t_i using Euler's method with a step length of 0.01 h.

(9) Floc terminal velocity is recalculated using equations (7.26) to (7.28). If the new value of U_{T_i} is not close to the initial value the procedure (i.e. steps 6, 7, 8, 9) is repeated until successive U_{T_i} values have converged.

(10) Next, segment (i + 1) is dealt with in the same fashion as segment i (i.e. steps 4 - 9 are repeated).

(11) When the calculations for the final (i.e. top) compartment have been completed the individual segment heights are summed together and compared with the true height of the tower, L. If the two figures are not in reasonable agreement the estimate of wet yeast volume per segment is adjusted as follows:

$$X_i' = X_i \cdot \frac{L}{\sum_{i=1}^{n} h_i} \qquad 9 \qquad (7.34)$$

The entire procedure is now repeated from step 3 onwards.

A flowchart for a computer program is shown in figure 7.6.

7.5.2 Data

Physical and operating parameters for the A.P.V. tower fermenter are based on the figures quoted by AULT et alia (1969) for the continuous system at Cape Hill Brewery, Birmingham. Data regarding yeast fluidisation and physical characteristics was obtained from Dr. A. JAMES. Model data are listed in table 7.2.

Figure 7.6 Computer Flowsheet for the Continuous Bear Tower Model.

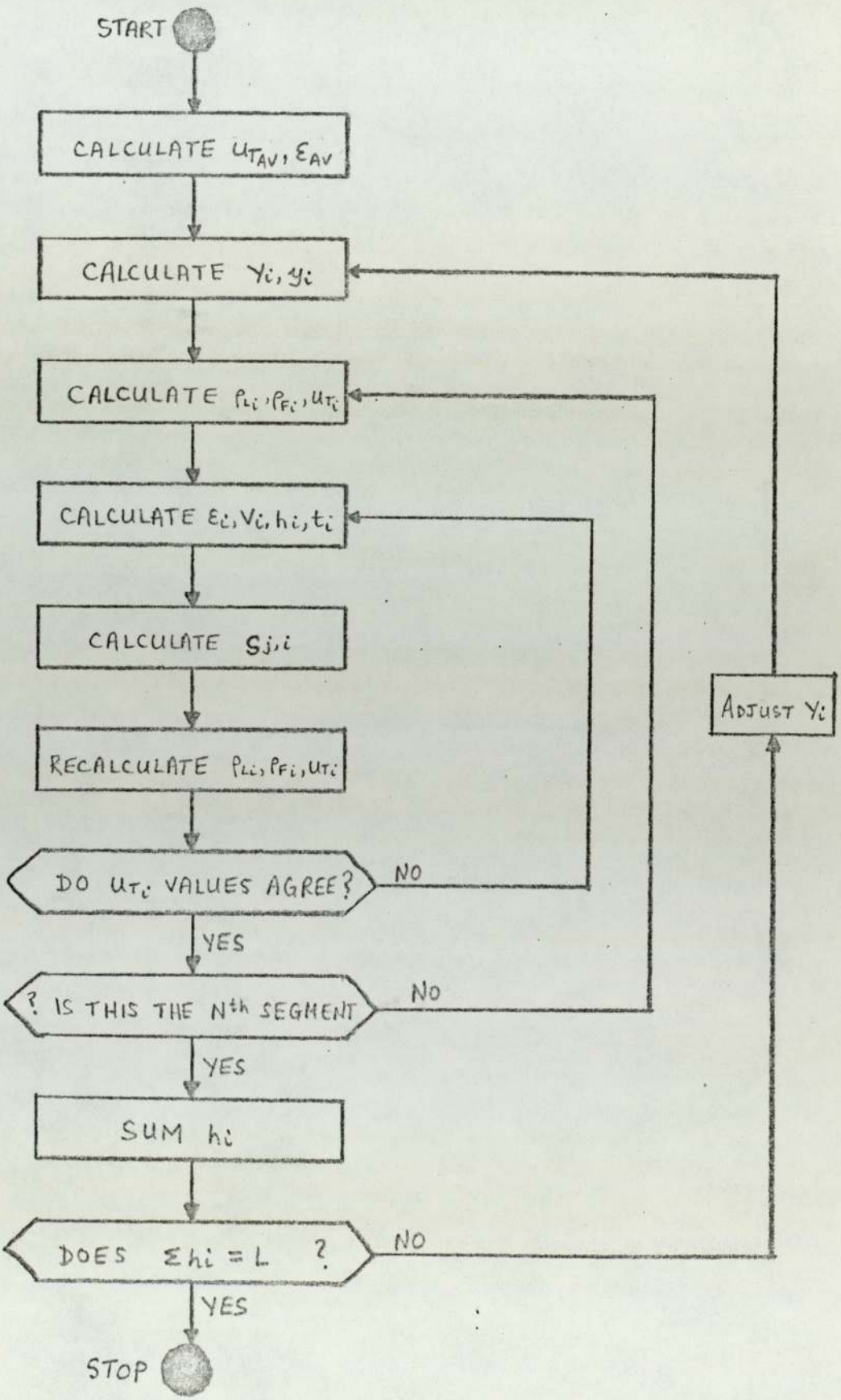


Table 7.2 Data for the Multi-Segment Model (20 Segments)

(a) Tower Characteristics (AULT et alia, 1969)

diameter	91.5 cm
height	750 cm
capacity	30 brl *
separator volume	10 brl
total capacity	40 brl
wort flowrate -min.	5 brl/h
wort flowrate -max.	10 brl/h
wort superficial velocity -min.	0.0346 cm/s
wort superficial velocity -max.	0.0692 cm/s
wort viscosity	1 cP

(b) Carbohydrate Content of Wort and Kinetic Data

sugar	k(g./g.dry yeast.h	Km(g/l)	concentration (g/l) (i)	(ii)
glucose	1.0	2	8.3	20
fructose	0.3	4	2.2	10
sucrose	500	28	12.4	20
maltose	0.8	5	28.8	50
maltotriose	0.5	20	10.7	20
non-fermentables	0		21.6	20
total sugar			84.0	140

wort gravity (i) 1.035 g/cm^3

(ii) 1.061 g/cm^3

(c) Yeast Characteristics (JAMES, 1973)

cell density	1.07 g/cm^3
dry matter density	1.32 g/cm^3
cell water fraction	0.8
floc voidage	0.5
fluidisation index	8
mean floc diameter	0.1 cm
variance of floc diameter	0.03 cm

* 1 barrel = 164 litres

Figure 7.7 Sugar Concentration Profiles in an APV Tower Fermenter (Ault et alia, 1969)

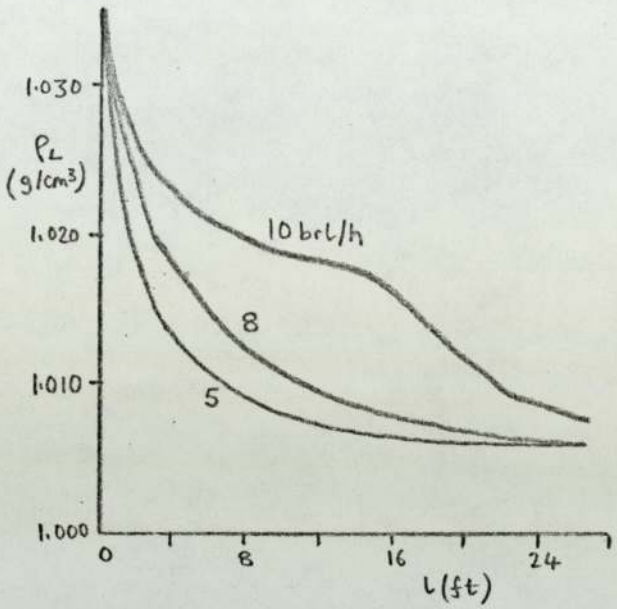


Figure 7.8 Effect of Throughput on the Behaviour of the APV Tower Model ($n = 8$; $d_p = 0.10$ cm ; $\sigma = 0.03$ cm)

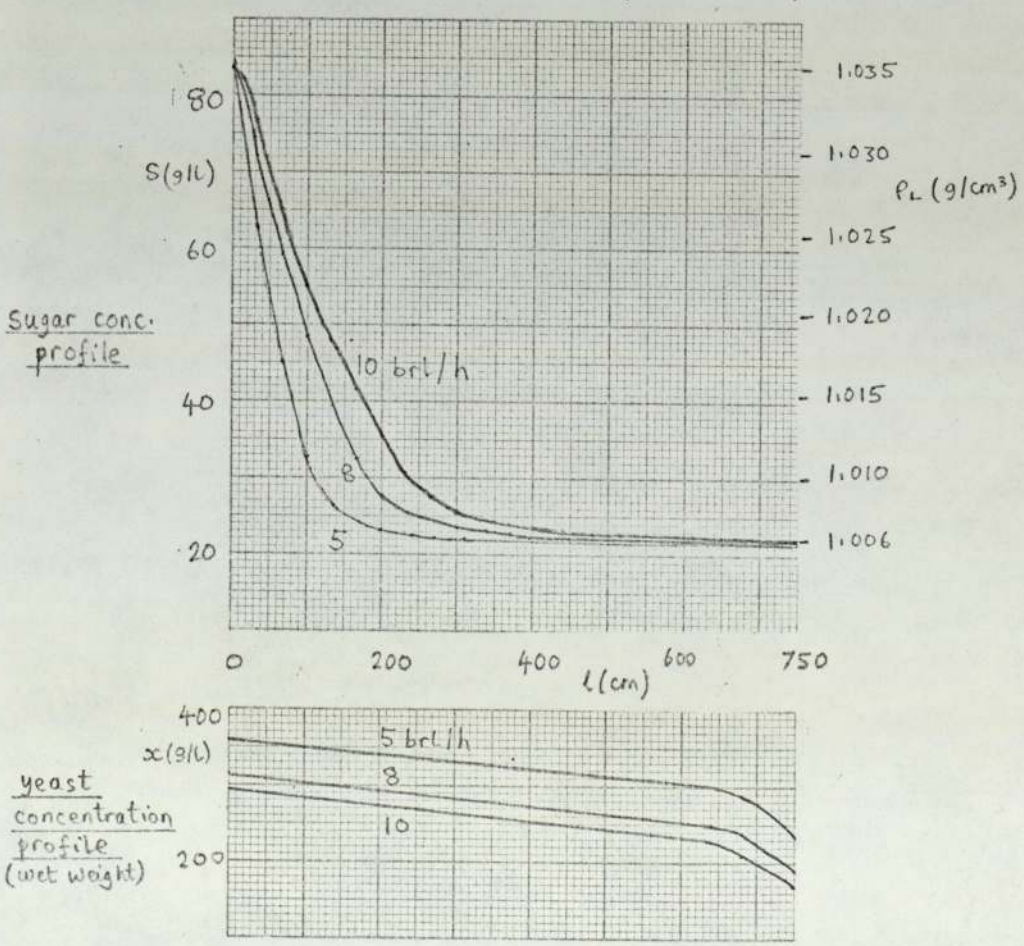
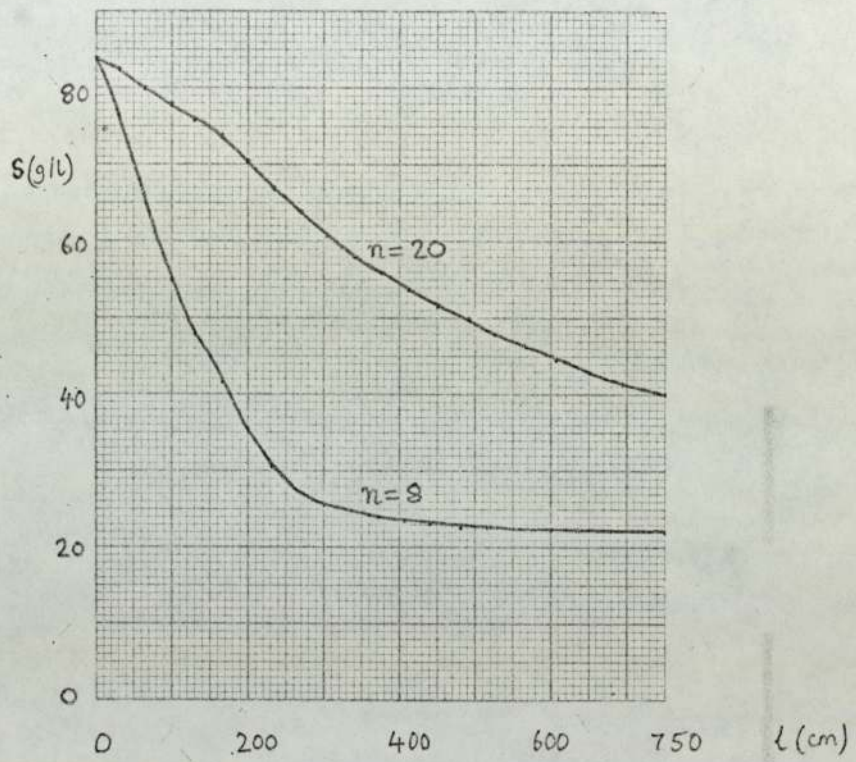
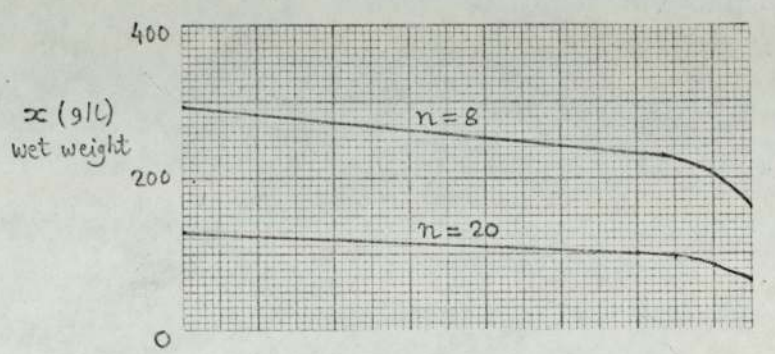


Figure 7·9 Effect of the Fluidisation Index, n, on the Behaviour of the APV Beer Tower Model

(a) Sugar Concentration Profile



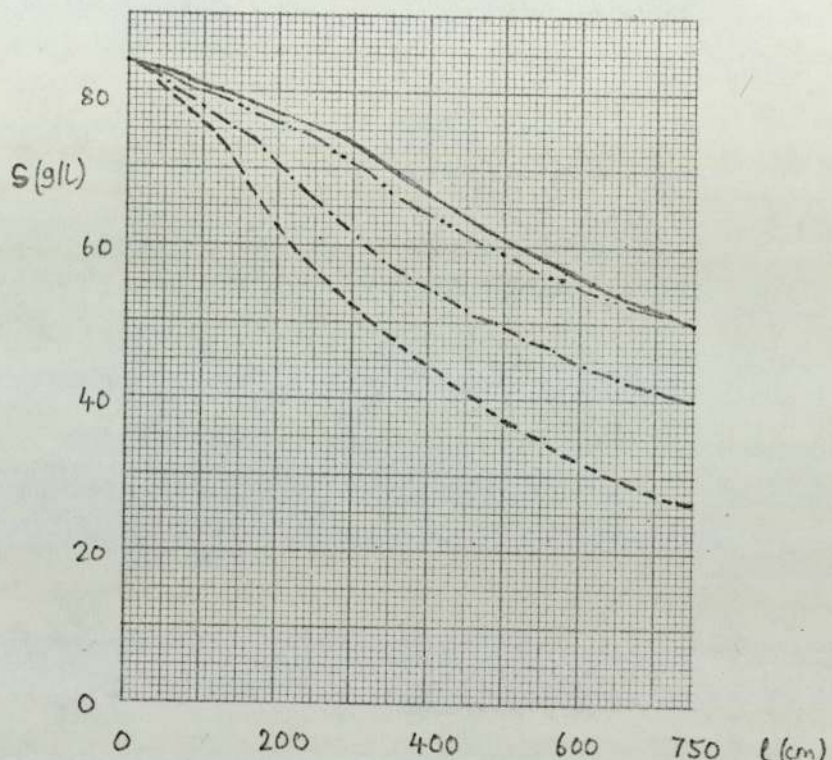


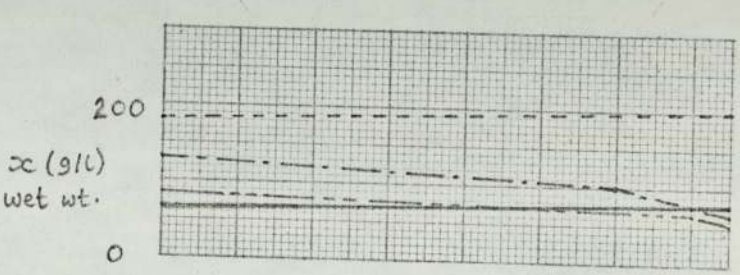
(b) Yeast Concentration Profile

Parameter values: flowrate = 10 brl/h
d_p = 0.10 cm
$\sigma_\cdot$ = 0.03 cm

Figure 7.10 Effect of Yeast Floc Diameter upon the Behaviour of the APV Beer Tower Model

(a) Sugar Concentration Profile





(b) Yeast Concentration Profile

Parameter values: $n = 20$

flowrate = 10 brl/h

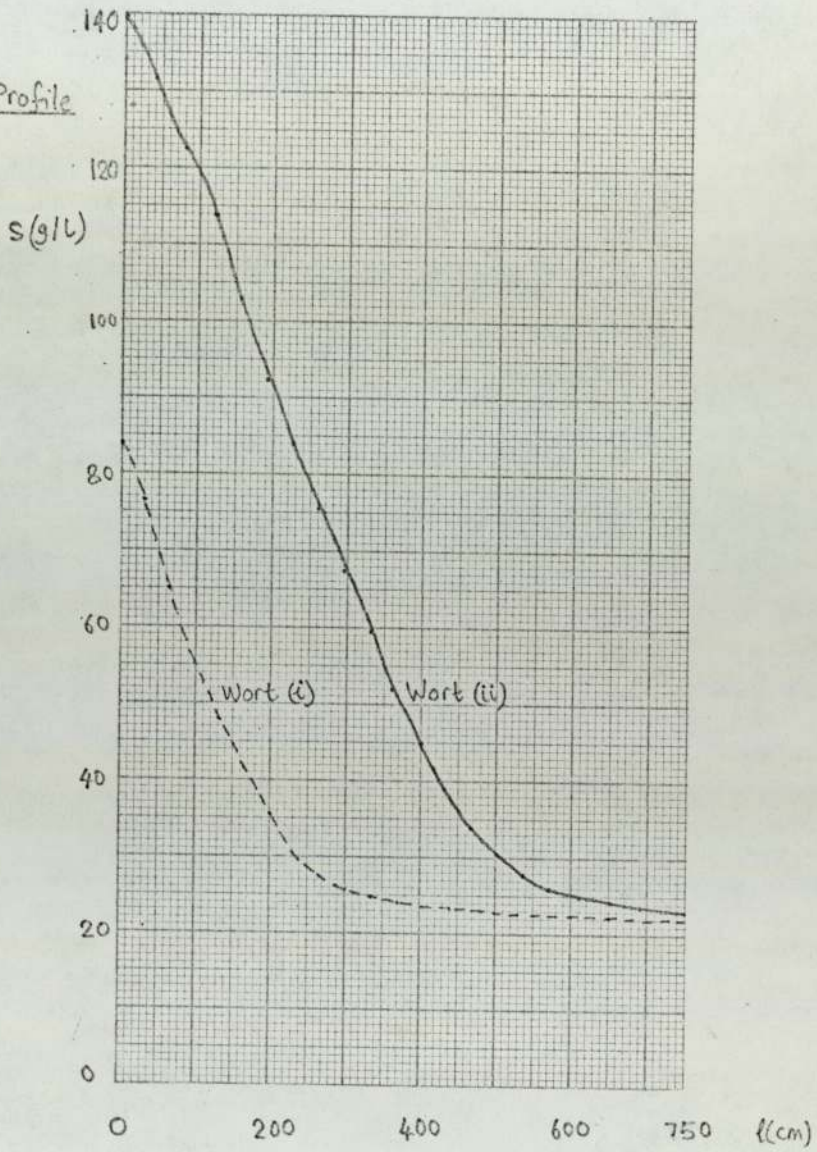
Key:

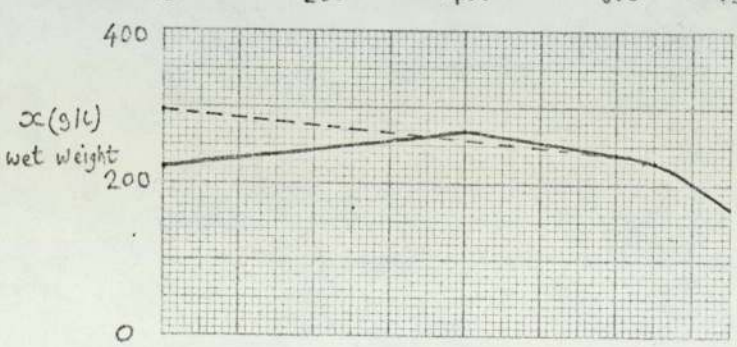
	d_p =	σ =
———	0.05 cm	0 cm
—·—	0.05	0.01
—··—	0.10	0.03
- - - - -	0.50	0

Figure 7.11 Effect of Wort Sugar Concentration on the Behaviour of the APV Beer Tower Model

flowrate = 10 brl/h
d_p = 0.10 cm
σ = 0.03 cm
n = 8

(a) Sugar Conc. Profile





(b) Yeast Concentration Profile

7.5.3 Results and Discussion

The effects of a number of model parameters upon sugar and yeast concentration profiles in the tower fermenter are shown in figures 7.8 to 7.12. Results can be compared with those of AULT et alia (1969), which are presented in figure 7.7. The primary effect of changes in these variables is to alter the yeast concentration; this, in turn, affects the rate of fermentation and, consequently, the sugar concentration profile. The individual effects of each of these variables can be seen more clearly from the results of the single-segment model (see figures 7.4 and 7.5).

The model predicts some of the operational characteristics of continuous beer tower fermenters as discussed by KLOPPER et alia (1965), ROYSTON (1966a, 1966b), and AULT et alia (1969). For instance, at low flowrates and normal wort composition the yeast concentration decreases up the tower. With a high wort sugar concentration there is a maximum yeast concentration in the middle or upper section of the tower. Alternative values of the model parameters lead to an increasing yeast concentration up the tower. Clearly, therefore, the model can account for a number of experimental observations; however, its usefulness at present is limited by a number of factors, some of which are discussed below.

(1) Parameter values

Values of the model parameters should be determined experimentally using techniques such as those developed by JAMES (1973). Unfortunately,

this was not possible during the present work; figures used are typical values obtained by JAMES. The model is, therefore, limited by the accuracy of these "guestimates", and so a number of values for the key parameters were tried in order to determine model sensitivity to inaccuracies in the data.

(2) Size distribution of the yeast flocs (see also Section 7.2.6)

The author believes that this is the weakest aspect of the model since there is little available data concerning the effects of sugar concentration, temperature, pH, agitation, etc., upon floc size and structure. Unfortunately, the model is very sensitive to yeast floc size and so further research on this topic would be beneficial.

(3) Effects of the gas phase

In the present model the presence of a gas phase is not considered. The major effects are (1) interference with two-phase fluidisation, and (2) the promotion of mixing in the system. The effects on fluidisation are complex and the reader is referred to the work of ØSTERGAARD (1965, 1966, 1968, 1970, 1972) on three-phase systems (for a summary of this work see JAMES, 1973).

Approximately 0.25 l. of CO_2 are produced for every gram of sugar utilised, i.e. about 15 l. per litre of wort. This corresponds to a superficial gas velocity of 1 cm/s at a liquid throughput of 10 brl/h. With regard to agitation, the effect of this gas is not significant at high yeast concentrations since the flocs are packed closely together and substantial mixing is not possible. At low yeast concentrations,

however, there is intense backmixing; the present model is not valid under such conditions and the reader is referred to the model described in Section 8.5.

During normal operation of a continuous beer tower fermenter there is a high concentration of yeast in the bottom section of the tower and most of the wort sugars are utilised in this region. Towards the top of the tower there is a lower yeast concentration and the action of the gas bubbles promotes mixing of the small yeast flocs which exist in this region. Most of the fermentable wort sugars are utilised before they reach this point in the tower and so agitation has little effect upon the properties of the liquid medium.

A further effect of gas evolution is that of nucleation of CO_2 bubbles on the surface of yeast aggregates; this may decrease the density of these flocs and cause them to float upwards, thus reducing the yeast concentration in the tower.

(4) Temperature and pH effects

The model does not take into account the effects of changes in temperature and pH of the liquid medium upon fermentation kinetics. Means of incorporating such facets into kinetic models are discussed in Sections 4.5 and 6.3. It is possible that these parameters also affect size and structure of the yeast flocs, though any such effects are not fully understood at present.

SMITH and GREENSHIELDS (1972) estimate that about 0.13 kcal of heat is released for every gram of sugar fermented. This thermal energy is

sufficient to raise the temperature of a system by almost 9°C during a normal brew. KLOPPER et alia (1965) and AULT et alia (1969) have found that wort entering a continuous beer tower fermenter at 15°C rapidly rises in temperature to 21 - 24°C. During normal operation of such a tower cooling is necessary to maintain a steady temperature of 21°C.

(5) The yeast separator

The yeast separation stage at the top of the tower is omitted from the present model. It is thought that the separator has little effect upon the operating characteristics of the tower, except to (1) increase the liquid residence time, and (2) retain a smaller size range of yeast flocs than would otherwise be possible.

(6) Yeast viability

Yeast viability was determined under various experimental conditions by AULT et alia (1969); they found that it varied considerably with the level of oxygen saturation of the wort. Since microbial viability affects speed of fermentation, this is an area in which further work is required.

(7) Diffusion

GREENSHIELDS and SMITH (1971) and SMITH and GREENSHIELDS (1972) studied the diffusion of sugars into yeast flocs. They found that diffusion is not rate-controlling with yeast flocs of less than about 0.2 cm in diameter and so this facet is not incorporated into the model.

7.5.4 Concluding Remarks

A mathematical model describing yeast and sugar concentration profiles in a tower fermenter is presented. Suitable choice of parameters allows the model to predict some of the phenomena observed during the operation of a production-scale A.P.V. tower fermenter and published in the literature. Limitations of the model are discussed in detail and help to highlight areas where further research may be of value. Like the batch beer simulation study (see Section 6.2), the model presented here is not useful for predicting taste or appearance of an alcoholic beverage; however, it is applicable to other anaerobic continuous tower processes involving microbial aggregates. With a better understanding of factors affecting size and structure of microbial flocs, the model will be valuable for predicting maximum throughput, etc., in tower systems.

LIST OF SYMBOLS (Section 7)

SYMBOL	EXPLANATION	UNITS
d_p, d_{pi}	floc diameter (i = segment number)	cm
F_A	accelerating force on particle	$g.cm/s^2$
F_R	resistance force on particle	$g.cm/s^2$
g	gravitational constant = 981	cm/s^2
h, h_i	segment height	cm
k, k_j	reaction velocity constant (j = sugar number)	g/g.dry yeast.h
Km, Km_j	Michaelis constant	g/l
L	height of tower	cm
n	fluidisation index	-
N_{Re}	Reynolds number = $\frac{\rho_L . u_s . d_p}{\mu_L}$	-
R'	drag force per unit projected area of particle	$g/cm.s^2$
s, s_j	sugar concentration	g/l
t	time	h
U_s	superficial liquid velocity	cm/s
U_T, U_{T_i}	particle terminal velocity	cm/s
V_i	segment volume	cm^3
w_F	floc liquid fraction	-
x, x_i	dry weight yeast concentration	g/l
X_i	volume of yeast per segment	cm^3

SYMBOL	EXPLANATION	UNITS
Greek Letters		
ε	voidage	-
ρ	density	g/cm^3
π	pi = 3.142	-
μ	viscosity	g/cm.s
σ	variance of floc diameter	cm
Subscripts		
C	cell	
F	floc	
i	segment number	
j	sugar number	
L	liquid	
Y	yeast	

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SECTION 8

THE DESIGN AND MODELLING OF AEROBIC TOWER FERMENTATION SYSTEMS

Figures

8.1 INTRODUCTION

SMITH and GREENSHIELDS (1974) have reviewed the application of tower fermentation systems to aerobic processes. In particular, work carried out by the T.F.R.G. at the University of Aston on aerobic processes involving yeasts, bacteria, and filamentous fungi is described.

Aerobic processes carried out in tower fermenters require large quantities of air. Consequently, the presence of the gas phase cannot be neglected, as it is in the continuous beer fermenter model (see Section 7). Besides interfering with two-phase liquid-solid fluidisation, the gas phase promotes mixing in tower fermenters. In addition, oxygen mass transfer between phases is frequently the rate-controlling step for fermentation processes.

In designing or modelling a particular process attention must be given to the following topics:

(1) Overall Mass and Energy Balances.

(2) Microbial Kinetics

- substrate utilisation, microbial growth, metabolite production, interaction with diffusion processes.

(3) Mode of Operation

- batch, continuous or semi-batch.

(4) System Geometry

- aspect ratio, distributor designs, outlet section design.

(5) Gas Phase Behaviour

- hold-up, bubble size, break-up and coalescence, mixing patterns.

(6) Liquid Phase Behaviour

- physical properties, mixing patterns.

(7) Solid Phase

- physical properties, hold-up, degree of aggregation, break-up and coalescence of aggregates, mixing patterns.

(8) Rate Processes

- mass-transfer between phases (gas $\rightleftharpoons$ liquid), liquid $\rightleftharpoons$ solid), heat transfer.

Some of these topics are considered briefly below to illustrate how a general model may be developed and to high-light those areas in which further work is required. Reference will be made to systems that have been studied at the University of Aston.

8.2 MASS BALANCE CONSIDERATIONS AND KINETICS

Three processes are considered: (1) yeast growth, (2) vinegar production, and (3) mould pellet growth.

8.2.1 Yeast Growth (This section was developed as a result of discussions with Mr. S. D. J. Coote and Mr. R. A. Spensley at the University of Aston).

8.2.1.1 Previous Work

FRANZ (1968) was probably the first researcher to propose a complex kinetic expression describing yeast behaviour. He distinguished

between sugar utilised for the different metabolic activities: fermentation, respiration, and biomass synthesis. FRANZ was thus able to account for large variations in the yield of yeast dry matter (per unit mass of sugar utilised) resulting from changes in the ratio aerobic/anaerobic metabolism.

KOGA et alia (1968) developed a block-diagram model of a microbial cell based upon an ATP balance. Six key metabolic processes were identified and a balance between energy produced and energy expended was assumed.

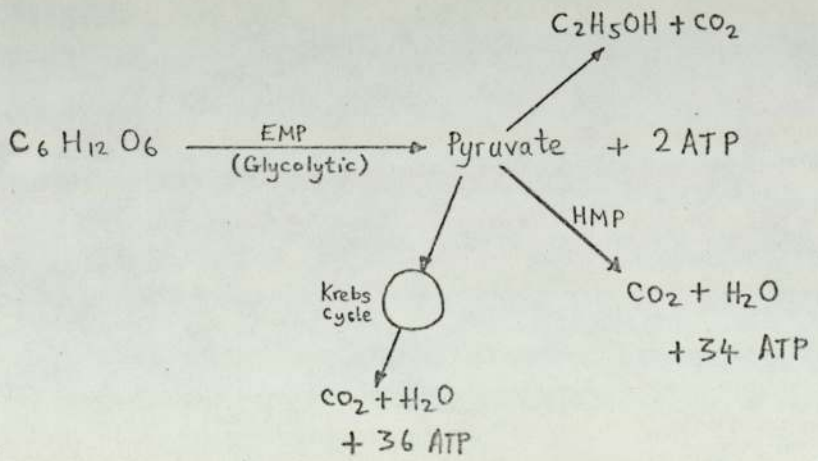
PERINGER et alia (1972a, 1972b, 1974) derived a comprehensive mathematical model of the growth kinetics of the yeast Saccharomyces cerevisae. The model is based upon an ATP balance and expresses analytically the dependence of maximum specific growth rate and growth yield upon dissolved oxygen tension and sugar concentration. The model accounts for a number of important physiological aspects of yeast behaviour and gives good agreement with experimental results. Unfortunately, no account is taken of maintenance energy, but this is not a significant factor at low biomass concentrations. Though complex, PERINGER'S model requires knowledge of only eight parameters - three involving ATP yields and five for kinetic expressions.

Publications regarding mass balance relationships for fermentation processes include those of MATELES (1971) and MINKEVICH and EROSHIN (1972). A later paper by MINKEVICH and EROSHIN (1973) shows how heat generated by fermentation processes can be calculated from the mass balance.

8.2.1.2 A Basic Model

A simple model for yeast growth based upon an ATP energy balance is presented. As mentioned in Section 4.2.8 yeast can function effectively using both aerobic and anaerobic mechanisms, either independently or simultaneously. The two modes of operation yield different quantities of ATP (MAHLER and CORDES, 1968). Under anaerobic conditions one molecule of glucose yields 2 excess ATP molecules via the Embden-Meyerhof-Parnas (EMP) pathway. Under aerobic conditions rather more energy is derived from the sugar; EMP + the Krebs cycle (also known as the TCA - TriCarboxyclic Acid - cycle) yields 38 excess molecules of ATP from one molecule of glucose, and EMP + HMP (Hexose Monophoshate Pathway) yields 36 excess ATP molecules. A reaction scheme is shown in figure 8.1 below. An average value of 37 moles ATP per mole of glucose is used for the aerobic pathway in the present work.

Figure 8.1 Glucose Utilisation by Yeast - Reaction Scheme



Fermentation of glucose by yeast can thus be represented by two simple overall chemical reactions, as follows:

(1) Aerobic mechanism:

$$\underset{180g}{C_6H_{12}O_6} + \underset{192g}{6O_2} \longrightarrow \underset{264g}{6CO_2} + \underset{108g}{6H_2O} + 37\ ATP \qquad (8.1)$$

(2) Anaerobic mechanism:

$$\underset{180g}{C_6H_{12}O_6} \longrightarrow \underset{92g}{2C_2H_5OH} + \underset{88g}{2CO_2} + 2\ ATP \qquad (8.2)$$

The reaction sequence for yeast growth on a mono-saccharide in an aerobic fermenter is represented schematically in figure 8.2.

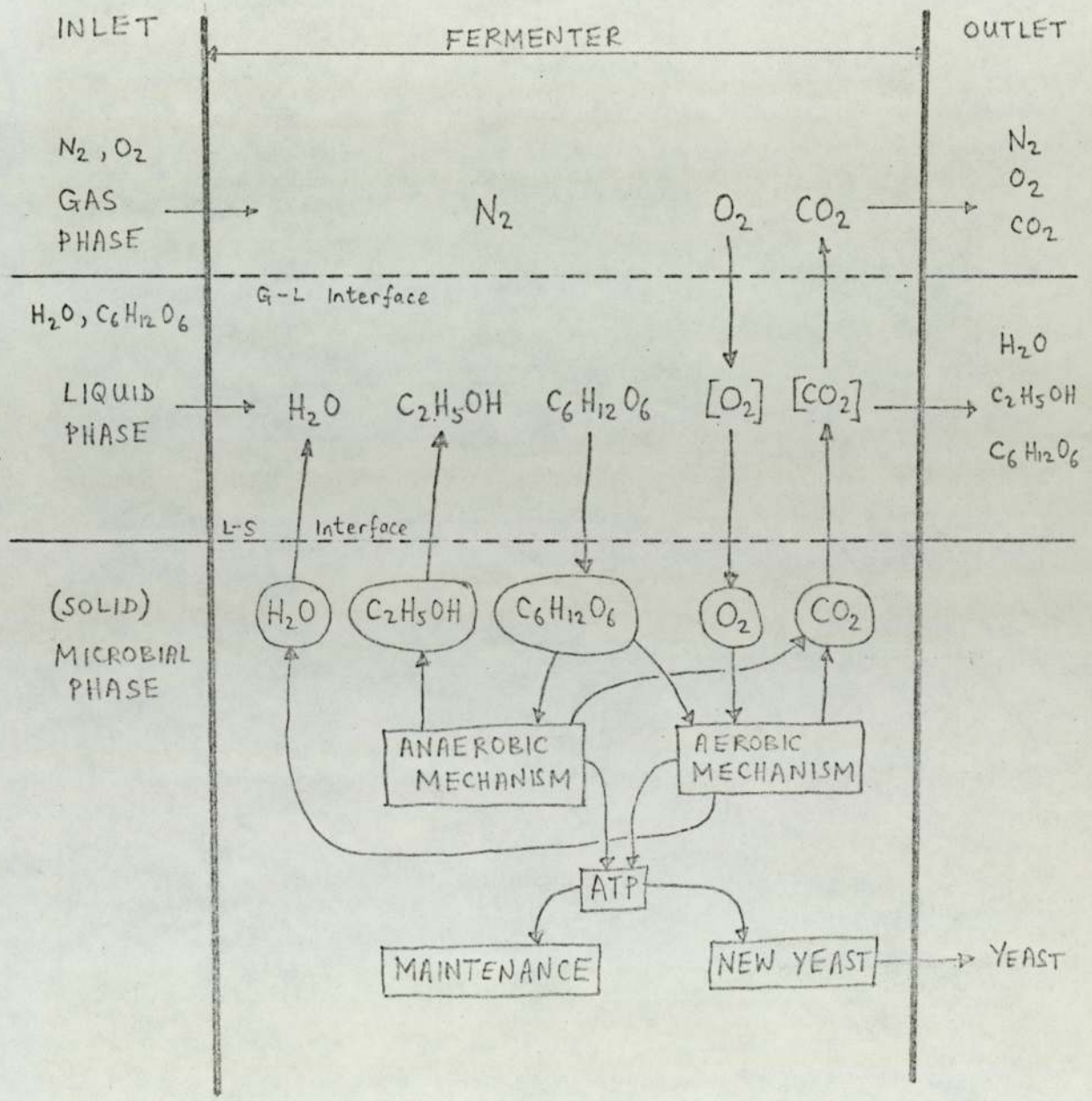
8.2.1.3 Growth

BAUCHOP and ELSDEN (1960) studied the yields of energy-limited cultures grown anaerobically in a complex medium. They expressed yield (Y_{ATP}) as g.dry weight of cells per mole of ATP synthesized during the metabolism of a mole of energy substrate. They found that Y_{ATP} was almost constant, irrespective of the organism or substrate, averaging 10 ± 2 g. For the yeast Saccharomyces cerevisae utilising glucose the yield was 10.5 g.dry weight/mole ATP.

HARRISON (1967) and OLBRICH (1956) determined the composition of yeast dry matter on an elemental basis, as shown in table 4.1 (see p 91). HARRISON suggested the following empirical formula for yeast:

$$C_6H_{10}O_3N$$

Figure 8.2 Schematic Representation of Yeast Growth in an Aerobic Fermenter



Assuming (1) a balance of carbon atoms, and (2) ammonia is the nitrogen source for growth, the overall reaction for the synthesis of yeast can be written as follows:

$$C_6H_{12}O_6 + NH_3 + 15\ ATP \longrightarrow C_6H_{10}O_3N + 2\tfrac{1}{2}H_2O + \tfrac{1}{4}O_2 \qquad (8.3)$$

180g 17g 144g 95g 8g

*157g dry yeast

* Assuming that carbon, hydrogen, oxygen, and nitrogen comprise 92% of the dry weight of yeast (averaged figures of HARRISON (1967) and OLBRICH (1956)), then 144g of $C_6H_{10}O_3N$ is equivalent to 157g yeast (dry weight). This requires 15gmole of ATP.

Equations (8.1), (8.2), and (8.3) can be combined to give the carbon balance for yeast growth. Let the masses of sugar utilised for respiration, alcohol production, and growth be a, b, and c respectively. Overall balances for carbon and ATP can then be drawn up, viz:

<u>carbon balance</u>:

$$(a+b+c)\,C_6H_{12}O_6 + (6a - \tfrac{1}{4}c)\,O_2 + c\,NH_3 \longrightarrow$$

$(a+b+c)$g. $(1.07a - 0.04c)$g. $0.09c$ g.

$$2b\,C_2H_5OH + c\,C_6H_{10}O_3N + (6a+2b)\,CO_2 + (6a+2\tfrac{1}{2}c)\,H_2O \qquad (8.4)$$

$0.51b$ g. $0.80c$ g. $(1.47a + 0.49b)$g. $(0.60a + 0.25c)$ g.

($0.87c$ g. dry wt.)

ATP balance:

$$0.206\,a + 0.011\,b = 0.080\,c + \text{maintenance} \qquad (8.5)$$

Maintenance requirements are often quoted in the form:

g.glucose/g. organism dry wt./h

CARTER et alia (1971) quote 0.018 g./g,h for the mould Aspergillus nidulans at 30°C. Other workers (e.g. BUTTON and GARVER, 1966) quote values close to 0.02 g./g,h for many different types of organisms. Assuming aerobic metabolism of sugar utilised for maintenance purposes, equation (8.1) indicates that 0.2g. of glucose produces 0.004 gmole ATP. Thus, for a system containing X g. of yeast equation (8.5) can be rewritten as follows:

$$0.206\,a + 0.011\,b = 0.080\,c + 0.004.X.t \qquad (8.6)$$

where t is the time period under consideration in hours. Yeast growth is therefore defined by the carbon mass balance (8.4) and the ATP balance (8.6).

8.2.1.4 Discussion

The model is based upon two mass balances - one for carbon and one for ATP. Thus it would be useful for interpreting experimental data, particularly from systems where input and output streams are monitored. Application of the model to yeast growth in a stirred tank reactor is shown in Appendix IV.

At present kinetic relationships are not a feature of the model, but Michaelis-Menten expressions for glucose, oxygen, and nitrogen could easily be incorporated.

Two possible inaccuracies of the model are as follows:

(1) the work of BAUCHOP and ELSDEN (1960) concerned anaerobic systems only, and thus the figure of 10.5 g.biomass/gmole ATP quoted for Y_{ATP} may be inaccurate. PERINGER et alia (1972b) use a figure of 9.01 g.biomass/gmole ATP for a generalised growth model for Saccharomyces cerevisiae. KORMANCIKOVA et alia (1967), however, determined values of 9.4 and 10.3 g.biomass/gmole ATP for the aerobic growth of S.cerevisiae on glucose.

(2) ATP required for maintenance is not known exactly. Although many workers quote a figure of 0.02 g.glucose/g.dry wt.,h, it is likely that maintenance requirements vary with age, metabolic state, and level of metabolic activity of a cell.

In conclusion it should be pointed out that a key feature of the model is its simplicity, and thus its general applicability.

8.2.2 Vinegar Production

Data are presented for the acetification of alcohol to vinegar by HROMATKA and EBNER (1959), JONES (1970), and SMITH and GREENSHIELDS (1974). The conversion is effected by the bacteria Acetobacter and is growth-associated, i.e. acid production is proportional to biomass generation. The overall reaction can be represented by the following equation:

$$\underset{46g}{CH_3CH_2OH} + \underset{32g}{O_2} \longrightarrow \underset{60g}{CH_3COOH} + \underset{18g}{H_2O} + 116\ kcal \qquad (8.7)$$

Oxygen requirements are approximately 10 g/h,g dry wt. bacteria when the fermenter is operating at 20-30°C and an acid concentration of about 50 g/l (HROMATKA and EBNER, 1959; JONES, 1970). Calculations from the data of HROMATKA and EBNER show that the production of 1g of acetic acid is associated with the formation of 0.0016 g. of bacteria (dry weight). Monod kinetics (see Section 4.4.4) are normally used to describe bacterial growth. However, oxygen mass transfer often appears to be the rate-controlling step.

8.2.3 Mould Pellet Growth

Growth of mould pellets is often expressed by cube root kinetics. Assuming that the surface area of a spherical floc increases linearly with time we have:

$$\frac{dx}{dt} = \beta_1 . x^{2/3} \qquad (8.8)$$

Integration yields:

$$x = (x_0^{1/3} + \beta_1 t)^3 \qquad (8.9)$$

This expression was first used by EMERSON (1950). PIRT (1966) proposed a model that can account for such kinetics. He suggested that mould pellets consist of an outer shell of growing hyphae and an inner mass of non-growing mycelium, the thickness of the outer growing layer being limited by nutrient diffusion rates. PIRT'S calculations show that the growth-limiting nutrient is almost certain to be oxygen. He concluded

that growth according to the model may lead to large differences between the metabolism of the outer and inner hyphae of the pellet. Hence the outer surface of the pellet may be generating new biomass whilst the inner layers are producing alternative metabolites, such as organic acids. Near the centre of the pellet autolysis will occur if the carbon substrate is unable to diffuse into this region.

FREDERICKSON et alia (1970) show that diffusion-limited growth can lead to the cube root growth law. TRINCI (1969, 1970a, 1970b) carried out investigations into the growth of mycelial pellets. His experiments confirmed that equation (8.8) can account for pellet growth under certain conditions.

Alternative mould growth models have been proposed by SHU (1961), TERUI et alia (1967), and MEGEE et alia (1970). MEGEE suggested that it may be advantageous to study mould systems from the view point of cellular differentiation.

Oxygen Transfer into Mycelial Pellets and Flocs

Oxygen transfer frequently controls the rate of growth of mycelial flocs and pellets (PIRT, 1966). This topic has, therefore, been of considerable interest to researchers. Early work on this subject was published by YANO et alia (1961), who derived equations accounting for the effects of diffusion and reaction (pellet effectiveness factors). Later work by AIBA and KOBAYASHI (1971) resulted in a more sophisticated model which gave better agreement with YANO'S experimental data. Other publications on this topic include those of PHILLIPS (1966),

KOBAYASHI et alia (1973), and HUANG and BUNGAY (1973). MUELLER et alia (1968) investigated oxygen transfer into floc structures.

8.3 GAS PHASE BEHAVIOUR AND OXYGEN MASS TRANSFER (G→L) (see also Section 3.3)

8.3.1 Gas Hold-up

(1) Air-Water Systems

DOWNIE (1972) and SHAYEGAN-SALEK (1974) investigated gas hold-up in air-water bubble column systems. Their data suggest that the following correlations may be appropriate for column diameters of 15 cm or greater:

$$h_G \simeq 0.04\, u_G \qquad 0 < u_G < 5 \text{ cm/s} \qquad (8.10)$$

$$h_G \simeq 0.20 \qquad u_G > 5 \text{ cm/s} \qquad (8.11)$$

For columns of diameter less than 15 cm gas hold-up fraction may be greater than 0.20 due to slugging.

(2) Fermentation Systems

Gas hold-up in tower fermentation systems appears to vary with: (a) superficial gas velocity, (b) surface tension of the fermentation broth, (c) biomass concentration, and (d) morphology of the microbial phase. At present it is not possible to quantify morphology and it would be difficult to include this facet in a model. The other three parameters, however, are easily measured and could be readily incorporated into a model.

MORRIS (1972) presented data for the batch growth of Aspergillus niger on sugar solutions in tower fermenters. Results can be approximated by a correlation of the following form:

$$h_G \simeq \beta_2 + \beta_3 . u_G \qquad 1 < u_G < 6 \text{ cm/s} \qquad (8.12)$$

Values of the coefficients (β_2, β_3) for different biomass concentrations are given in table 8.1 below.

Table 8.1 Coefficients for the Gas Hold-up Correlation

BIOMASS CONC. (g/l)	MORPHOLOGY (P = pellet; F = filamentous)	β_2	β_3 (s/cm)
0	-	0.06	0.03
5	P	0.05	0.025
7	P	0.04	0.02
7	F	0.03	0.015

8.3.2 Volumetric Mass Transfer Coefficient (G→L)

Values of the volumetric mass transfer coefficient, $(k_L a)_{G \rightarrow L}$, can be related to the gas hold-up fraction, h_G. Such a relationship, however, assumes that k_L remains constant whilst interfacial surface area, a, is proportional to gas hold-up.

KITAI et alia (1969) used the following correlation to predict $k_L a$

values in a perforated plate, column fermenter:

$$k_L a = 0.0095 * u_G^{1.3} \quad s^{-1} \qquad (u_G = cm/s) \qquad (8.13)$$

MORRIS (1972) investigated oxygen transfer in a tower fermenter containing A. niger pellets (8 g/l). The following relationships provide an approximate description of his results:

$$k_L a \simeq 0.039 * u_G \quad s^{-1} \qquad 0 < u_G < 3 \text{ cm/s} \qquad (8.14)$$

$$k_L a \simeq 0.084 + 0.011 * u_G \quad s^{-1} \qquad 3 < u_G < 5 \text{ cm/s} \qquad (8.15)$$

$$k_L a \simeq 0.139 \quad s^{-1} \qquad u_G > 5 \text{ cm/s} \qquad (8.16)$$

$k_L a$ values are also affected by a number of parameters, including broth viscosity and surface tension (see Section 3.4).

8.3.3 Gas-Phase Mixing

Mixing in the gas phase can normally be neglected, i.e. plug flow is assumed.

8.4 THE BEHAVIOUR OF MICROBIAL SUSPENSIONS (Liquid and Solid Phases)

8.4.1 Physical Properties of Microbial Systems

Perhaps the best work to date is that of JAMES (1973), who performed measurements with A. niger and flocculant yeasts. He attempted to quantify the structure and behaviour of microbial aggregates and fermentation broths so that their properties could be predicted in

various situations. Mathematical models of the structures of (1) a fungal pellet, and (2) a microbial floc are presented.

JAMES also investigated the rheological behaviour of microbial suspensions. An excellent literature review is presented, besides results for yeast and A.niger suspensions; an expression of the form;

$$\mu_L = b_3 . x^{b_5} \qquad (8.17)$$

may be appropriate for A.niger (in both the pellet and filamentous forms).

Publications of interest regarding rheology of microbial broths include those of DEINDOERFER and WEST (1960), TAGUCHI (1971), STERBACEK (1972), ROELS et alia (1974), and LEDUY et alia (1974).

Viscosity is an important factor since it affects both mixing and oxygen transfer in fermentation broths. However, it is not possible at present to predict with confidence the effect of changes in physical properties on system behaviour.

8.4.2 Fluidisation-Sedimentation Phenomena

JAMES (1973) has briefly reviewed the literature regarding three-phase fluidisation, and considered the effects of the gas phase on system behaviour. Although he carried out preliminary experimental investigations using suspensions of (1) yeast, and (2) A.niger, the results are descriptive and of little value in predicting the behaviour of general systems.

In the absence of further data, models describing aerobic tower fermentation systems are based on two-phase (i.e. liquid-solid)

fluidisation theory, as described in Section 7.2.2. JAMES (1973) has presented extensive experimental data for two-phase systems containing various yeasts and A.niger pellets.

Mould Growth Systems

Discussions were held with Mr. R. A. Spensley and Mr. S. D. Pannell at the University of Aston regarding mathematical modelling of a continuous system for the growth of A.niger. Traditional continuous culture theory (see Section 5) is not applicable to continuous tower fermentation systems since the biomass concentration in the effluent stream, x_E, is not necessarily the same as the average biomass concentration in the tower, x_T. The relationship $\mu = D$ does not hold and should be replaced by the expression:

$$\mu . x_T = D . x_E \qquad (8.18)$$

Concentration of the microbial phase, x_T, is solely dependent upon fluidisation-sedimentation phenomena, i.e. liquid superficial velocity is an important factor. JAMES (1973) found settling velocities for A.niger pellets of up to 0.71 cm/s. Thus for a column of 10 cm diameter liquid flowrates of up to 55 cm^3/s could be achieved with this morphology before there is complete washout of the culture. For a 10 l fermenter this corresponds to a dilution rate of about 20 h^{-1}. It is therefore apparent that dilution rates well in excess of μ_m can be attained in tower fermentation systems (depending upon the physical characteristics

of the microbial phase). This is not possible in stirred tank fermenters; there is no way that specific growth rate, μ, can be exceeded by the dilution rate, D, without washout occurring (except, of course, by artificial maintenance of a high microbial concentration in the fermenter). Recently PANNELL (1974) has confirmed experimentally that values of D much greater than μ_m are possible in tower fermentation systems containing A.niger.

Biomass Concentrations in Continuous Systems

When applied to tower fermentation systems, the term "dilution rate" is often misused. For example, by halving the height of a column one can double dilution rate and yet microbial concentration remains unaltered (since superficial liquid velocity is unchanged).

The relationship between biomass concentration and superficial liquid velocity is complex. Consider the fluidisation equation:

$$\frac{u_s}{u_T} = \epsilon^n \qquad (8.19)$$

$$\text{or} \quad \epsilon = \left(\frac{u_s}{u_T}\right)^{1/n} \qquad (8.20)$$

$$\text{Now,} \quad x = x_m(1-\epsilon) \qquad (8.21)$$

where x_m = maximum biomass concentration (fixed by morphology)

$$\text{Thus} \quad x = x_m\left[1-\left(\frac{u_s}{u_T}\right)^{1/n}\right] \qquad (8.22)$$

Assuming that floc terminal velocity is independent of superficial liquid velocity (an over-simplification since liquid physical properties, and the size and structure of the flocs may alter) we can predict the relationship between liquid throughput and biomass concentration in a tower. Results are presented in figure 8.3.

8.4.3 Mixing in the Liquid and Solid Phases

Liquid Phase

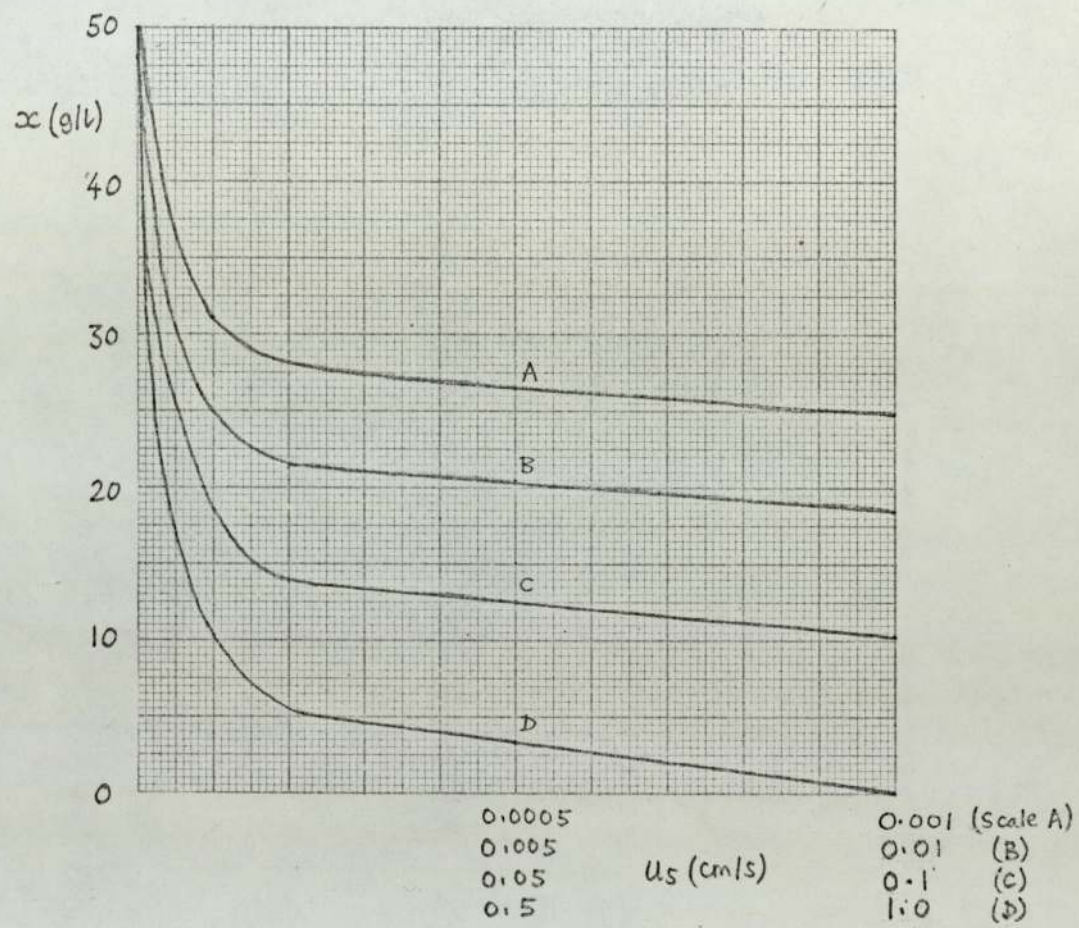
Liquid-phase mixing in tower fermentation systems may be described by the backflow stirred tanks model (see Section 2.4). Backmixing coefficients can be calculated from the volumetric gas flowrate, as in Section 2.5. During some microbial processes there is a change in volumetric gas flowrate; in such systems the backmixing coefficient varies with axial position in the tower.

Solid Phase

In the case of the microbial phase much depends on the size of aggregate. For single cells and small aggregates the suspension can be considered to be pseudo-homogeneous. Such an assumption may, however, introduce error into a model since no account is taken of froth flotation of the microbial phase. JONES (1970) and KITAI et alia (1969) reported this phenomenon when operating tower fermenters for bacterial processes. KITAI et alia have proposed a model incorporating flotation of the microbial phase.

For large aggregates (> 0.1 cm. in size) fluidisation and mixing effects are very difficult to describe. Under some conditions it may be

Figure 8.3 Biomass Concentration in a Continuous Tower Fermenter: The Effect of Liquid Throughput Rate



Plot of $x = x_m \left[1 - \left(\frac{u_S}{u_T} \right)^{1/n} \right]$

parameters: $x_m = 50\ g/l$; $u_T = 1\ cm/s$; $n = 10$

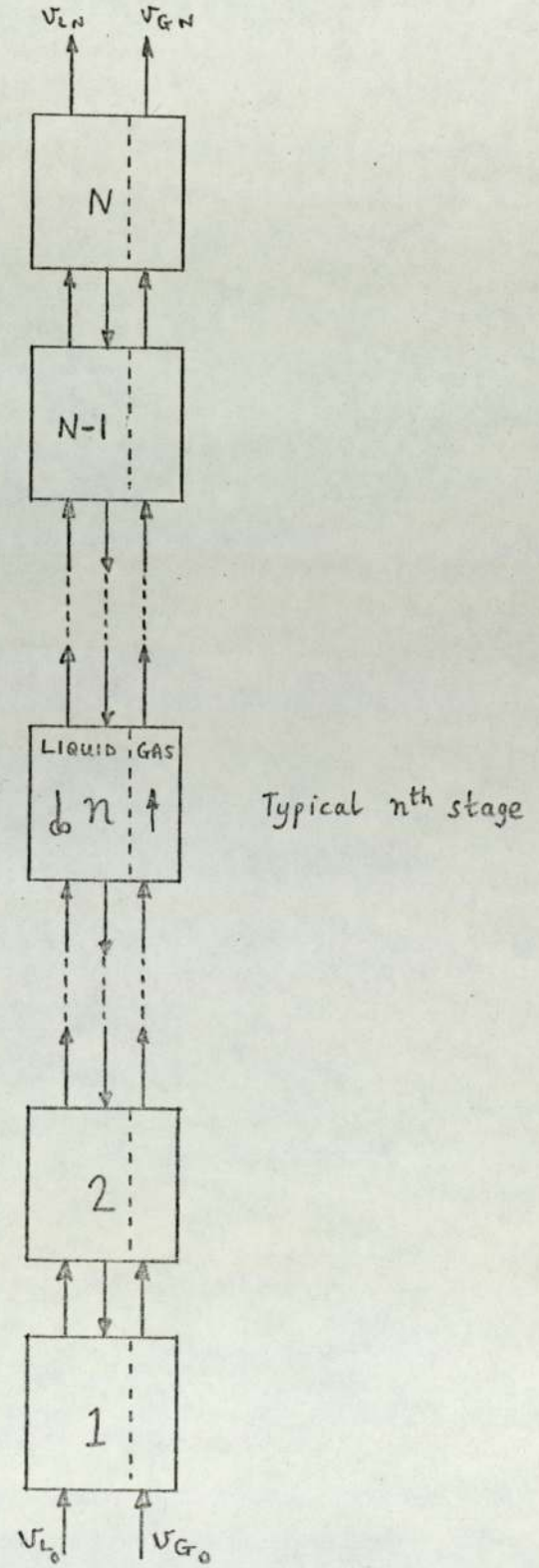
tower dimensions: $A = 100\ cm^2$; $L = 100\ cm$; $V = 10\ l$

thus dilution rates are as follows:

u_S (cm/s)	D (h^{-1})
0.001	0.036
0.01	0.36
0.1	3.6
1.0	36

N.B. assume morphology of floc does not alter with u_S.

Figure 8.4 Schematic Representation of an Aerobic Tower Fermenter



reasonable to assume no mixing of the solid phase: at the other extreme the mixing may be of the same intensity as that in the liquid phase. In the latter case, perfect mixing is approached at low throughputs.

For the case of fluidisation of the microbial phase it would be most difficult to simulate the system unless one assumes no mixing in the solid phase. If microorganisms move from one local environment to another the effects of adaptation and de-adaptation should be considered. In addition, fluidisation calculations become complex due to changes in the physical properties of interstitial floc fluid.

8.5 A GENERALISED MODEL FOR AN AEROBIC TOWER FERMENTER

A generalised model for an aerobic tower fermenter is presented below. Consider the fermenter as a series of perfectly-mixed vessels, as shown in figure 8.4. There is forward flow and backflow of the liquid phase, and forward plug flow of the gas phase. The following calculation sequence is suggested for the n^{th} segment:

(1) Fluidisation-sedimentation phenomena

Calculate solid phase hold-up from fluidisation-sedimentation theory. Some assumption must be made regarding floc/colony structure. Liquid-phase composition and gas flowrate must also be assumed.

(2) Mixing

Liquid backmixing coefficients can be computed from gas and liquid flowrates, as in Section 2.5.

As an approximation, assume no mixing in either the gas or microbial phases.

(3) Rate-limiting step

This determines reaction rate and may be due to:

(a) oxygen transfer from the gas to liquid phase,

(b) liquid substrate concentration, or

(c) mass transfer difficulties into or out of microbial aggregates. Each of these rates should be calculated; the minimum gives the rate-controlling step.

(4) Mass Balances

Mass balances for the liquid, gas, and microbial phases can now be drawn up, assuming the rate-limiting kinetics determined in step (3). New liquid and gas compositions can thus be calculated, and also growth of the microbial phase.

(5) Other considerations

(a) Heat generated may be calculated from the kinetics mass balance. This affects the physiological properties of the microbial phase, and also oxygen solubility in the fermentation broth.

(b) Viscosity of the fermentation broth can be determined from biomass concentration and morphology. This affects mixing, gas hold-up, and oxygen transfer.

(c) Aggregate structure may be affected by changes in volumetric gas flowrate, liquid composition, or biomass concentration. However, changes in morphology are difficult to predict.

It can be seen that solution of such a model requires a complex iteration scheme. There are many interacting variables, and an additional

complication is the backflow of liquid between segments.

Dynamic Modelling (see also Section 5.3)

With the exception of the backflow stirred tanks model (Section 2.4) and the batch beer sugar utilisation model (Section 6.2) the models developed in the present work cannot be used to simulate dynamic responses. The general aerobic tower fermenter model can be modified to account for transient responses by making one of the following assumptions regarding each aspect of the model:

(1) an immediate response to disturbances,

(2) a delay due to the vessel time constant, or

(3) a time lag due to biological adaptation.

All steady-state equations must be modified to include accumulation and/or time constant terms.

LIST OF SYMBOLS (Section 8)

SYMBOL	EXPLANATION	UNITS
a	mass of sugar utilised for respiration	g
b	mass of sugar utilised for alcohol production	g
c	mass of sugar utilised for growth	g
D	dilution rate	h^{-1}
h	hold-up	-
k_La	volumetric mass transfer coefficient	s^{-1}
n	fluidisation index	-
t	time	s,h
u	superficial liquid velocity	cm/s
u_T	floc terminal velocity	cm/s
x	biomass concentration	g/l
x_E	biomass concentration in effluent	g/l
x_T	biomass concentration in tower	g/l
X	total biomass in system	g

SYMBOL	EXPLANATION	UNITS
Greek Letters		
β_i	constant	various
ε	voidage	-
μ	organism specific growth rate	h^{-1}
μ_L	viscosity of fermentation broth	g/cm.s
Subscripts		
G	gas-phase	
L	liquid-phase	
m	maximum	
o	initial	

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SECTION 9

ACHIEVEMENTS AND RECOMMENDATIONS FOR FUTURE WORK

Introduction

The thesis illustrates the value of working with a multi-disciplinary research team. Knowledge, expertise, and experimental results acquired by a number of members of the Tower Fermentation Research Group have been utilised to analyse fermentation processes and to construct mathematical models of these systems. The models developed have been useful in:

(1) suggesting new areas for experimental investigation (e.g. the high flowrate work with continuous A.niger systems),

(2) high-lighting areas where more research effort might be useful (future work - see below), and

(3) the scale-up, design, and optimisation of tower fermentation systems (see Section 8).

Achievements

(1) An alternative to the axial dispersion model has been developed. The new model assumes that a bubble-column can be represented by a series of perfectly-mixed regions linked by both forward- and back-flow of the liquid phase: it closely approximates the axially-dispersed plug flow model and is more amenable to simpler solution techniques. Mixing parameters for both models can be predicted by an empirical procedure which relates a new mixing parameter, w, to changes in process-operating parameters.

(2) A theoretical model has been developed to describe oxygen transfer

in a system in which the liquid phase is well-mixed and the gas is in plug flow. It is shown how this model can be combined with the backflow stirred tanks model to provide a description of oxygen transfer in bubble columns and tower fermenters.

(3) A comprehensive review of deterministic microbial kinetic models has been undertaken.. A particular feature of this section is the critical discussion of means of modifying kinetic models to account for variations in process parameters.

(4) Continuous culture theory has been outlined and discussed: modifications to existing theory to account for transient responses resulting from changes in system parameters are briefly reviewed.

(5) The kinetics of sugar utilisation and the physical behaviour of yeast during batch beer fermentation have been analysed and a model developed. The model incorporates novel features for (a) dealing with multi-component substrates, and (b) accounting for the adaptation of microorganisms to new growth environments.

(6) A model of the A.P.V. continuous beer tower has been developed. Simulation studies with this model show that a number of different yeast and sugar concentration profiles can be generated by choosing different values of the operating parameters.

(7) A general model for aerobic tower fermentation processes has been outlined: consideration is given to a number of design features of such systems. One aspect of this model has been used to demonstrate that it is possible to operate tower fermenters containing microbial aggregates

at much higher flowrates than previously thought possible.

Future Work

(1) This thesis forms a basis for simulation studies of many tower fermentation processes. It should now be possible to develop preliminary models for a number of processes involving yeasts, bacteria, and filamentous fungi.

(2) In order to develop detailed models of tower fermenter systems a greater understanding of factors affecting the size and structure of microbial aggregates is required. The author recommends that further research should be carried out in this area.

(3) Models of fermentation systems are often limited by lack of knowledge about the effects of changes in process parameters (e.g. temperature and pH). The author believes that more fundamental research into the mechanisms which control microbial cells would be beneficial.

APPENDIX I SOLUTION OF MATRIX DIFFERENTIAL EQUATIONS

Consider the equation

$$\dot{\underline{c}} = \underline{A} \cdot \underline{c} + \underline{z} \qquad (2.24)$$

Steady State Solution

Here we have $\underline{A} \cdot \underline{c} + \underline{z} = \underline{0}$

with solution $\underline{c} = -\underline{A}^{-1} \cdot \underline{z}$

If $\underline{z} = \underline{0}$ we have $\underline{A} \cdot \underline{c} = \underline{0}$

For $\underline{c}$ to have a non-trivial solution, the determinant of $\underline{A}$ must equal zero and the solution is given by the eigenvectors of $\underline{A}$.

Consider equation (2.27): $\dot{\underline{c}} = \underline{A}_z \cdot \underline{c}$

Intuitively, the steady state solution for an impulse injection of m units of tracer to any point in the system is given by:

$$C_n = \frac{m}{N.V} \qquad 1 < n < N$$

A steady state solution to equation (2.24) can be obtained for the case where tracer is injected continuously into the top of the column by considering the steady state mass balances around each tank shown in figure 2.2.

For tank 1 we have:

$$V_L \cdot C_0 + q \cdot C_2 = p \cdot C_1$$

For $C_0 = 0$ we have: $C_1 = \frac{q}{p} \cdot C_2 = f \cdot C_2$

For tank 2 we have:

$$p \cdot C_1 + q \cdot C_3 = p \cdot C_2 + q \cdot C_2$$

But $p \cdot C_1 = q \cdot C_2$

therefore $q \cdot C_3 = p \cdot C_2$

or $C_2 = \frac{q}{p} \cdot C_3 = f \cdot C_3$

For tank n we find that $C_n = f \cdot C_{n+1}$

For tank N we have:

$$p \cdot C_{N-1} + V_t \cdot C_t = (V_L + V_t) \cdot C_N + q \cdot C_N$$

But $p \cdot C_{N-1} = q \cdot C_N$

thus $C_N = \frac{V_t}{(V_L + V_t)} \cdot C_t$

Unsteady State Solution

Here we have $\dot{\underline{c}} = \underline{A}.\underline{c} + \underline{z}$ (2.24)

Taking Laplace transforms:

$$S.\underline{c}(s) - \underline{c}(0) = \underline{A}.\underline{c}(s) + \underline{z}(s)$$

Rearranging:

$$\underline{c}(s) = \frac{\underline{c}(0)}{\left[S.\underline{I} - \underline{A}\right]} + \frac{\underline{z}(s)}{\left[s.\underline{I} - \underline{A}\right]}$$

Inverting:

$$\underline{c}(t) = \underline{\phi}(t).\underline{c}(0) + \int_0^t \underline{\phi}(t-\tau).\underline{z}(\tau).d\tau$$

where: $\underline{\phi}(t) = \mathcal{L}^{-1}\left[s.\underline{I} - \underline{A}\right]$

Now:

$$\underline{\phi}(t-t_o) = \exp\left[\underline{A}(t-t_o)\right]$$

$$= \underline{I} + \underline{A}.(t-t_o) + \frac{1}{2!}.\underline{A}^2.(t-t_o)^2 + \ldots + \frac{1}{n!}.\underline{A}^n.(t-t_o)^n + \ldots$$

Let $h = (t-t_o)$, then:

$$\underline{\phi}(h) = \underline{I} + \underline{A}.h + \frac{1}{2!}.\underline{A}^2.h^2 + \ldots + \frac{1}{n!}.\underline{A}^n.h^n + \ldots$$

Provided $\underline{z} \neq f(t)$ then the recursive solution can be written:

$$\underline{c}(t_o+h) = \exp[\underline{A}.h].\underline{c}(t_o) + \int_{t_o}^{t_o+h} \exp[\underline{A}(t-\tau)].d\tau.\underline{z}$$

$$= e^{\underline{A}.h}.\underline{c}(t_o) + \left[-\underline{A}^{-1}.e^{\underline{A}(t-\tau)}\right]_{t_o}^{t_o+h}.\underline{z}$$

$$= e^{\underline{A}.h}.\underline{c}(t_o) + \underline{A}^{-1}.\left[e^{\underline{A}.h} - \underline{I}\right].\underline{z}$$

$$= \underline{\phi}(h).\underline{c}(t_o) + \underline{\Delta}(h).\underline{z} \qquad (2.29)$$

Now $$\underline{\phi}(h) = \underline{I} + \underline{A}.h + \underline{A}^2.\frac{h^2}{2!} + \ldots + \underline{A}^n.\frac{h^n}{n!} + \ldots.$$

and $$\underline{\Delta}(h) = \underline{A}^{-1}\left[\underline{\phi}(h) - \underline{I}\right] \qquad (2.31)$$

$$= h.\left\{\underline{I} + \underline{A}.\frac{h}{2!} + \underline{A}^2.\frac{h^2}{3!} + \ldots + \underline{A}^n.\frac{h^n}{(n+1)!} + \ldots.\right\}$$

Thus the solution of equation (2.24) is given by equation (2.29) and can always be solved, even when there is no inverse of $\underline{A}$, by the following procedure:

(i) generate $\underline{\Delta}(h)$ using equation (2.31)

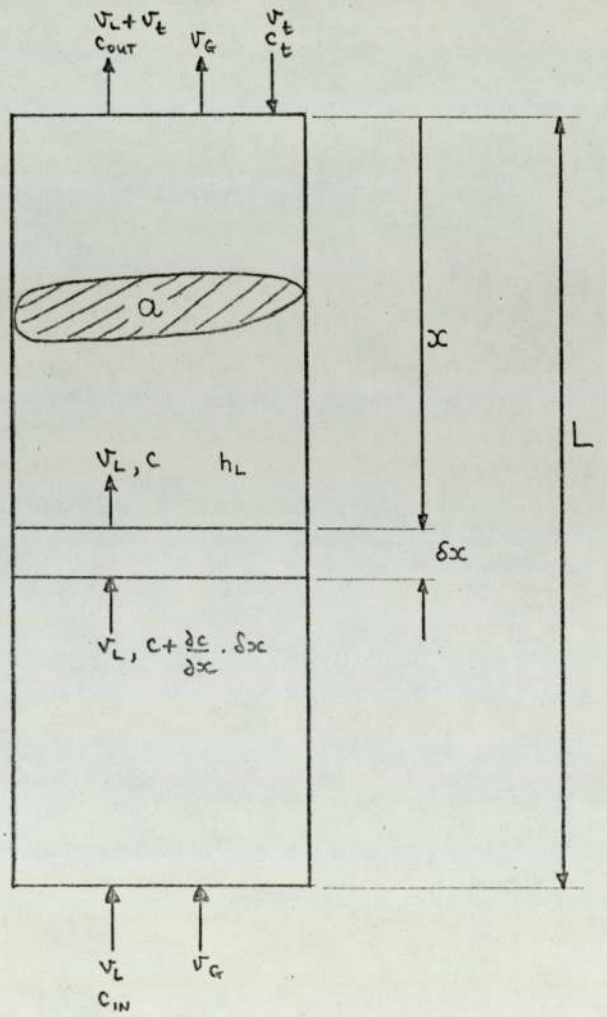
(ii) calculate $\underline{\phi}(h)$ from:

$$\underline{\phi}(h) = \underline{I} + \underline{A}.\underline{\Delta}(h)$$

(iii) apply equation (2.29) recursively, starting at t = to.

APPENDIX II DERIVATION OF THE DISPERSION MODEL AND ITS BOUNDARY CONDITIONS

Consider an element of width δx cm at an axial distance x cm from the top of a bubble column of length L and cross-sectional area a, as shown in the figure below. Let there be upward flows of liquid v_L and gas v_G, entering at the base of the column, and resulting in a uniform liquid hold-up fraction h_L through the column. Consider a constant flow of tracer v_t of concentration c_t entering the top of the column.



Schematic of a Bubble Column

Mass balance on component c over element

IN: by bulk flow = $v_L \cdot \left[c + \frac{\partial c}{\partial x} \cdot \delta x\right]$

by diffusion = $-D_L \cdot a \cdot h_L \cdot \frac{\partial c}{\partial x}$

OUT: by bulk flow = $v_L \cdot c$

by diffusion = $-D_L \cdot a \cdot h_L \cdot \frac{\partial}{\partial x}\left[c + \frac{\partial c}{\partial x} \cdot \delta x\right]$

ACCUMULATION = $h_L \cdot a \cdot \delta x \cdot \frac{\partial c}{\partial t}$

Apply the law of conservation of mass

IN - OUT = ACCUMULATION

$$v_L \cdot \left[c + \frac{\partial c}{\partial x} \cdot \delta x\right] - D_L \cdot a \cdot h_L \cdot \frac{\partial c}{\partial x} - v_L \cdot c +$$

$$+ D_L \cdot a \cdot h_L \cdot \frac{\partial}{\partial x}\left[c + \frac{\partial c}{\partial x} \cdot \delta x\right] = h_L \cdot a \cdot \delta x \cdot \frac{\partial c}{\partial t}$$

Simplifying:

$$v_L \cdot \frac{\partial c}{\partial x} + D_L \cdot a \cdot h_L \cdot \frac{\partial^2 c}{\partial x^2} = h_L \cdot a \cdot \frac{\partial c}{\partial t}$$

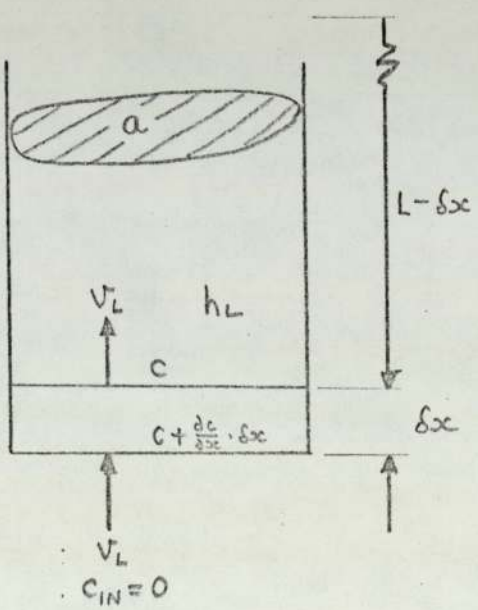
But $u_L = \frac{v_L}{a}$

therefore: $$\frac{u_L}{h_L} \cdot \frac{\partial c}{\partial x} + D_L \cdot \frac{\partial^2 c}{\partial x^2} = \frac{\partial c}{\partial t}$$

Boundary Conditions

(i) $c\,(x,0) = 0$

(ii) Bottom of column



Schematic of Column Base

Mass balance on component c over element

IN: by bulk flow = $v_L \cdot 0$

by diffusion = $-D_L \cdot a \cdot h_L \cdot \frac{\partial c}{\partial x}$

OUT: by bulk flow = $v_L \cdot c$

by diffusion = 0

ACCUMULATION = $h_L \cdot a \cdot \delta x \cdot \frac{\partial c}{\partial t}$

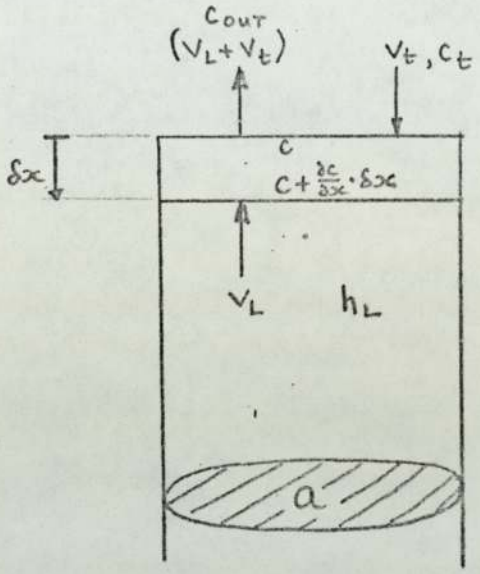
Applying the law of conservation of mass:

$$-D_L \cdot a \cdot h_L \cdot \frac{\partial c}{\partial x} - v_L \cdot c = h_L \cdot a \cdot \delta x \cdot \frac{\partial c}{\partial t}$$

Let $\delta x \to 0$ and simplify, then:

$$\frac{\partial c(L,t)}{\partial x} = -\frac{u_L \cdot c(L,t)}{h_L \cdot D_L}$$

(iii) Top of column



Schematic of Top of Column

Mass balance on component c over element

IN: by bulk flow = $V_L \cdot \left[C + \frac{\partial c}{\partial x} \cdot \delta x\right]$

by diffusion = 0

tracer input = $V_t \cdot C_t$

OUT: by bulk flow = $\left[V_L + V_t\right] \cdot C$

by diffusion = $-D_L \cdot a \cdot h_L \cdot \frac{\partial}{\partial x}\left[C + \frac{\partial c}{\partial x} \cdot \delta x\right]$

ACCUMULATION $= h_L \cdot a \cdot \delta x \cdot \frac{\partial c}{\partial t}$

Applying the law of conservation of mass:

$$v_L \cdot \left[c + \frac{\partial c}{\partial x} \cdot \delta x\right] + v_t \cdot c_t - \left[v_L + v_t\right] \cdot c + D_L \cdot a \cdot h_L \cdot \frac{\partial}{\partial x}\left[c + \frac{\partial c}{\partial x} \cdot \delta x\right]$$

$$= h_L \cdot a \cdot \delta x \cdot \frac{\partial c}{\partial t}$$

Let $\delta x \to 0$ and simplify, then:

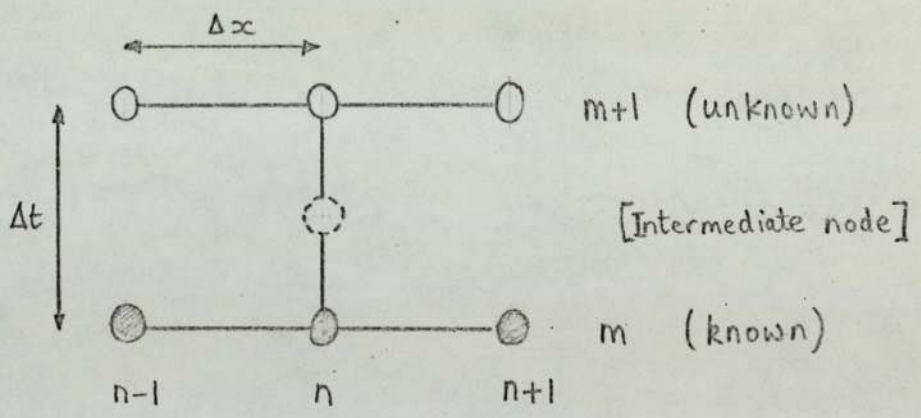
$$\frac{\partial c}{\partial t}(0,t) = \frac{v_t}{D_L \cdot a \cdot h_L} \cdot \left[c(0,t) - c_t\right]$$

APPENDIX III FINITE DIFFERENCE SOLUTION OF THE DISPERSION MODEL

The partial differential equation describing the dispersion model is:

$$\frac{u_L}{h_L} \cdot \frac{\partial c}{\partial x} + D_L \cdot \frac{\partial^2 c}{\partial x^2} = \frac{\partial c}{\partial t} \qquad (2.1)$$

This is a PAROBOLIC equation, which is conveniently solved by the method of CRANK and NICHOLSON (1947), who use the nodal computational molecule pictured below:



Computational Molecule

Using central differences to approximate $\frac{\partial c}{\partial t}$ at the intermediate node yields:

$$\frac{\partial}{\partial t} c(x,t) = \frac{c_{m+1,n} - c_{m,n}}{\Delta t}$$

similarly:

$$\frac{\partial}{\partial x} c(x,t) = \frac{1}{2}\left[\frac{(c_{n+1,m+1} - c_{n-1,m+1})}{2 \cdot \Delta x} + \frac{(c_{n+1,m} - c_{n-1,m})}{2 \cdot \Delta x}\right]$$

and:

$$\frac{\partial^2}{\partial x^2} c(x,t) = \frac{1}{2}\left[\frac{(c_{n+1,m+1} - 2c_{n,m+1} + c_{n-1,m+1})}{(\Delta x)^2} + \right.$$

$$\left. + \frac{(c_{n+1,m} - 2c_{n,m} + c_{n-1,m})}{(\Delta x)^2}\right]$$

Substitution into the dispersion model, equation (2.1), gives:

$$\frac{(C_{n,m+1} - C_{n,m})}{\Delta t} = \frac{u_L}{4.h_L.\Delta x}\left[(C_{n+1,m+1} - C_{n-1,m+1}) + (C_{n+1,m} - C_{n-1,m})\right] +$$

$$+ \frac{D_L}{2(\Delta x)^2}\left[(C_{n+1,m+1} - 2C_{n,m+1} + C_{n-1,m+1}) + (C_{n+1,m} - 2C_{n,m} + C_{n-1,m})\right]$$

Rearranging:

$$C_{n+1,m+1}\left[\frac{u_L}{4.h_L.\Delta x} + \frac{D_L}{2(\Delta x)^2}\right] + C_{n,m+1}\left[\frac{-1}{\Delta t} - \frac{D_L}{(\Delta x)^2}\right] + C_{n-1,m+1}\left[\frac{D_L}{2(\Delta x)^2} - \frac{u_L}{4.h_L.\Delta x}\right]$$

$$= C_{n+1,m}\left[\frac{-u_L}{4.h_L.\Delta x} - \frac{D_L}{2(\Delta x)^2}\right] + C_{n,m}\left[\frac{D_L}{(\Delta x)^2} - \frac{1}{\Delta t}\right] + C_{n-1,m}\left[\frac{u_L}{4.h_L.\Delta x} - \frac{D_L}{2(\Delta x)^2}\right]$$

or: $$p.C_{n+1,m+1} + q.C_{n,m+1} + r.C_{n-1,m+1} = S_{m,n} \qquad (III.1)$$

where $$p = \left[\frac{u_L}{4.h_L.\Delta x} + \frac{D_L}{2(\Delta x)^2}\right]$$

$$q = \left[\frac{-1}{\Delta t} - \frac{D_L}{(\Delta x)^2}\right]$$

$$\gamma = \left[\frac{D_L}{2(\Delta x)^2} - \frac{u_L}{4.h_L.\Delta x}\right]$$

and $$S_m = \alpha . c_{n+1,m} + \beta . c_{n,m} + \gamma . c_{n-1,m}$$

where: $$\alpha = \left[\frac{-u_L}{4.h_L.\Delta x} - \frac{D_L}{2(\Delta x)^2}\right]$$

$$\beta = \left[\frac{D_L}{(\Delta x)^2} - \frac{1}{\Delta t}\right]$$

$$\gamma = \left[\frac{u_L}{4.h_L.\Delta x} - \frac{D_L}{2(\Delta x)^2}\right]$$

Boundary Conditions

(i) $c(x,0) = 0$

(ii) $$\frac{\partial}{\partial x}.c(L,t) = \frac{-u_L}{D_L.h_L}.c(L,t)$$

central difference approximation gives:

$$\frac{C_{N+1} - C_{N-1}}{2.\Delta x} = \frac{-u_L.C_N}{D_L.h_L}$$

or: $$C_{N+1} = C_{N-1} - \left(\frac{2.\Delta x.u_L}{D_L.h_L}\right).C_N$$

(iii) $$\frac{\partial}{\partial x}.c(0,t) = \frac{v_t}{D_L.h_L.a}.\left[c(0,t) - c_t\right]$$

central difference approximation gives:

$$\frac{c_2 - c_0}{2.\Delta x} = \frac{v_t}{D_L.h_L.a}.(c_1 - c_t)$$

$$\text{or:} \quad c_0 = c_2 - \frac{2.\Delta x.v_t}{D_L.h_L.a}.(c_1 - c_t)$$

Method of solution

At t = 0 all $c_{n,m}$ values are known (in fact $c_{n,o} = 0$, $1 < n < N$) and thus $s_{n,m}$ values can be evaluated from equation (III-1). The set of equations described by the left hand side of (III-1) forms a tridiagonal matrix, which can be solved by any of the standard methods for $c_{n,m+1}$ values (i.e. t = Δ t). The method of solution can then be repeated indefinitely using $c_{n,m+1}$ to compute $c_{n,m+2}$ (i.e. t = 2.Δt), etc.

Reference

CRANK, J., and NICHOLSON, P.
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APPENDIX IV MASS BALANCE FOR YEAST GROWTH IN A WELL-MIXED VESSEL

Consider a perfectly-mixed vessel of volume V, as shown in figure AI.1. Substrate feed, concentration s_o and density ρ_o, enters the fermenter and overflows at a volumetric flowrate u. Concentrations of substrate, product, dissolved oxygen, and biomass in the fermenter are s, p, c_L, and x respectively. Density of the overflowing medium is ρ. Air is passed into the vessel at a volumetric flowrate q_1, and exhaust gases leave at a flowrate q_2. Gas hold-up fraction in the vessel is h_G.

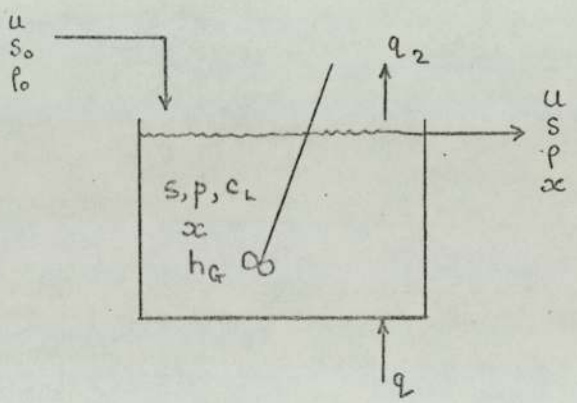

Figure AI.1 Schematic of Yeast Growth Vessel

The following assumptions are made:

(1) there is oxygen limitation,

(2) Michaelis-Menten kinetics can be used for sugar utilisation,

(3) there are no diffusion problems, and

(4) a steady-state exists.

Mass balances for the substrate, oxygen, yeast, and ATP can be drawn up as follows (see Section 8.2.1.3):

(1) substrate mass balances

$$u(s_o - s) = \frac{k s x}{K_m + S} . V . (1 - h_G) \quad g/s \qquad (IV.1)$$

$$u(s_o - s) = a + b + c \quad g/s \qquad (IV.2)$$

(2) oxygen mass balance

$$k_L a . V . (1 - h_G) . (c^* - c_L) = 1.07 a - 0.04 c \quad g/s \qquad (IV.3)$$

(3) yeast mass balance

$$u . x = 0.87 c \quad g/s \qquad (IV.4)$$

(4) ATP energy balance

$$0.206 a + 0.011 b = 0.080 c + \frac{0.004}{3600} . x . V \quad gmol/s \qquad (IV.5)$$

Solution Procedure

(1) Calculate s from equation (IV.1)

(2) Solve equations (IV.2) to (IV.5) simultaneously, yielding a, b, c, x.

Gas and liquid flowrates are summarised by tables IV.1 and IV.2 below

Table IV.1 Liquid Composition Chart for the Yeast Growth Fermenter

	H_2O	$C_6H_{12}O_6$	C_2H_5OH	TOTAL
IN (g/s)	$u\rho_o - us_o$	us_o	0	$u\rho_o$
OUT(g/s)	$u\rho_o - us_o + 0.60_a + 0.25_c$	$us_o - a - b - c$	0.51b	$u\rho = u\rho_o - 0.4_a - 0.49b - 0.75c$

Table IV.2 Gas Composition Chart for the Yeast Growth Fermenter*

	N_2	O_2	CO_2	TOTAL
IN (l/s)	$0.8\ q_1$	$0.2\ q_1$	0	q_1
(g/s)	$0.901q_1$	$0.257q_1$	0	$1.158q_1$
OUT(l/s)	$0.8\ q_1$	$0.2\ q_1 - 0.831_a + 0.03_c$	$0.831_a + 0.277_b$	$q_1 + 0.277_b + 0.03c$
(g/s)	$0.901q_1$	$0.257q_1 - 1.07_a + 0.04_c$	$1.47\ _a + 0.49\ _b$	$1.158q_1 + 0.4_a + 0.49_b + 0.04c$

*Notes For table IV.2

Assume that oxygen, nitrogen, and carbon dioxide behave as ideal gases.

Thus 1 litre of air (80% N_2, 20% O_2 by volume) contains 0.257g. oxygen and 0.901g. nitrogen at 30°C, 760 mm. Hg.

Also, 1g. CO_2 occupies 0.565 l. at 30°C, 760 mm. Hg

1g. O_2 occupies 0.776 l. at 30°C, 760 mm. Hg

1g. N_2 occupies 0.889 l. at 30°C, 760 mm. Hg