MODELLING of BATCH and FED-BATCH ETHANOL FERMENTATION

by

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ABSTRACT

Two series of batch and fed-batch fermentations were carried out using S. cerevisiae in a semi-defined medium containing 200 gl^{-1} glucose as limiting substrate. Growth rates were calculated and the data used to test the applicability of eight empirical kinetic models. The form proposed by Levenspiel, combining the concept of a limiting ethanol concentration with a power-law form, gave the best results with these data. Glucose concentration was found to have a far smaller, though not negligible, effect on growth rate under these conditions.

It was also observed that in fed-batch fermentations the total substrate uptake rate of the broth became constant soon after commencement of feeding, without cessation of growth. It is suggested that ethanol inhibits the synthesis of a rate-controlling enzyme in the glycolytic chain, but no previous work could be found to support or refute this explanation. A quasi-mechanistic model of growth under the condition of constant substrate consumption rate is formulated and discussed.

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NOMENCLATURE.

B	Growth rate parameter - Simplified models	[$lg^{-1}h^{-1}$]
C	Concentration	[gl^{-1}]
F	Feed rate	[lh^{-1}]
K	Substrate saturation or product inhibition constant	[gl^{-1}]
Q	Total uptake (substrate) or production (ethanol) rate	[gh^{-1}]
S	Total substrate present	[g]
T	Percentage transmission of 580 nm light (Beckman colorimeter)	
V	Volume	[l]
X	Total biomass present	[g]
Y	Yield factors	[gg^{-1}]
m	Maintenance coefficient	[$gg^{-1}h^{-1}$]
n	Levenspiel Model exponent	[-]
q	Specific uptake or production rates	[$gg^{-1}h^{-1}$]
t	Time	[h]
μ	Growth rate (specific)	[h^{-1}]
$\hat{\mu}$	Maximum growth rate parameter Levenspiel- and Monod-type models	[h^{-1}]

Subscripts.

0	referring to value at start of applicable period.
M	Monod
L	Levenspiel
cons	consumed
f	feed
p	product (ethanol)
s	substrate (glucose)
t	true value
x	biomass (yeast)
ss	static state

1. INTRODUCTION.

With the search for fuels and chemical feedstocks from renewable resources now some fifteen years old, interest in ethanol from fermentation continues. Concurrent with this is an increasing sophistication in design methods, born of the need to contain capital costs to remain competitive and fed by the rapidly growing power of computers. There is consequently a demand for quantitative models of **yeast fermentation kinetics** that can be used for design purposes. This study was undertaken to assess the usefulness of several growth models that have been proposed in the literature, together with some modifications of these. The assessment was done by carrying out series of batch and fed-batch fermentations, calculating the growth rates obtained and then fitting the various models to the results.

All of these equations relate growth rate to substrate and ethanol concentrations and cannot completely describe yeast behaviour : they say nothing, for example, about product formation or substrate uptake rates. In the course of the fermentations carried out for this work some significant aspects of yeast behaviour were noted which are not catered for in the models. In any practical design work these will have to be taken into account, and hence some analysis of this behaviour was carried out as a separate exercise from the regression work.

In the context of industrial ethanol fermentations, much of the mass of studies done in the field of fermentation kinetics suffers from not having been developed for the sugar and alcohol levels normally encountered in production. Some of the data, notably those used by Rahn[1], Holzberg[2], Egamberdiev[6], Navarro[4], Nagodawithana[5] and Converti[14] cover practical regions, which can be roughly described as 100 to 300 gl^{-1} sugar and 50 to 150 gl^{-1} ethanol. Of these, however, all but one group of workers added ethanol to

their medium in some or all of their experiments, thus casting some doubt on the applicability of their inhibition measurements to normal fermentations. This work partly addresses the limitations by operating in useful concentration regions and using no externally generated ethanol. It is still limited in that, like earlier work, it makes use of bulk ethanol concentrations.

The following section is a resumé of models and parameter values in the literature. The next two deal with the experimental work, the accuracy of the measurements and the methods used in the analysis of the data. Section 5 describes the course of the experiments, the rationale for decisions taken to overcome practical obstacles and some further analysis originally unplanned but prompted by what was observed. Section 6 discusses the results in detail.

2. LITERATURE SURVEY AND SCOPE OF THIS WORK.

This section will review the various models that have been proposed and applied to describe fermentative yeast growth since the early part of the century. This leads into their general limitations and the direction taken in this work.

Growth models in general tend to concentrate on the limiting substrate as the principal factor affecting growth rate, the classical Monod equation being a prime example. In the case of ethanol fermentation, however, it was evident from early on that the influence of the product, namely ethanol, was at least as important, in commercially significant processes at any rate. First proposed was a simple linear relation of the form

$$\mu = \mu_0 \left(1 - \frac{C_p}{C_{p\,max}} \right) \qquad . \; . \; . \; . \; (2.1)$$

where μ is growth rate, [h^{-1}],
μ_o is growth rate at zero ethanol concentration, [h^{-1}],
C_p is ethanol concentration, [gl^{-1}],
$C_{p\,max}$ is a limiting ethanol concentration, [gl^{-1}].

This implied the existence of a maximum ethanol concentration above which no growth took place. Rahn (1) in 1929, re-examining data by an earlier worker, suggested such a model in the form

$$\frac{d}{dt} C_s = -k \left(1 - \frac{C_p}{C_{p\,max}} \right) \qquad . \; . \; . \; . \; (2.2)$$

where k is some constant dependent on the quantity of biomass present. This gave reasonable results both for straight batch fermentations of 20% sucrose and for fermentations in which ethanol was added to the broth initially. Fermentation continued beyond the limiting concentration, however, although growth was negligible at that stage. Strictly speaking this equation describes substrate uptake and not growth rate, unless the biomass yield factor is constant.

Holzberg et al (2), working with S.cerevisiae var ellipsoideus also added ethanol to their 20% grape must. They measured growth rate dynamically in a continuous fermenter using dilution rates above washout so that the increase in ethanol concentration produced by the cells was negligible. They too arrived at a linear model but with a threshold concentration below which no appreciable inhibition occurred.

Ghose and Tyagi (3), using a bagasse hydrolysate measured growth rate in continuous culture for various concentrations of alcohol in the feed. They put forward the same linear relation but applied it separately to both growth rate and product formation :

$$\mu = \mu_m \left(1 - \frac{C_p}{C_{pm}} \right) \qquad \ldots\ldots \quad (2.3)$$

$$q_p = q_{pm} \left(1 - \frac{C_p}{C_{pm}} \right) \qquad \ldots\ldots \quad (2.4)$$

where q_p is specific ethanol formation rate, [$gg^{-1}h^{-1}$]. C_{pm} and C_{pm}' are different maximum ethanol concentrations while μ_m and q_{pm} are maximum growth and product formation rates attained in the absence of ethanol.

Their model was more complete in that it considered both biomass and product formation, while previous ones either ignored this aspect or implied strict proportionality between growth and product formation through the use of yield factors.

Navarro and Durand (4), working with S.carlsbergensis on batch fermentations of 120 gl^{-1} sugar medium, measured both extracellular and intracellular ethanol concentrations, finding that the level inside the cell was much higher than outside it. They did not model growth rate but presented two linear correlations for specific product formation rate based on limiting internal and external ethanol concentrations sufficient to suppress ethanol production, respectively :

$$q_p = k(C_{pc} - C_p) \quad . \quad . \quad . \quad . \quad (2.5)$$

$$q_p = B(C_{pm} - C_p) \quad . \quad . \quad . \quad . \quad (2.6)$$

where k and B are empirical constants and C_{pc} and C_{pm} are the limiting internal and external concentrations.

Equation 2.6 is a variant of the purely empirical linear models. Equation 2.5, however, is more properly classed as part of a mechanistic model : it describes mass transfer at an interface, with the underlying assumption that diffusion rather than reaction is the limiting factor in ethanol fermentation by yeast.

The contrast between internal and external product concentrations was significant because it cast doubt on the validity of inhibition measurements derived from experiments where ethanol was added to the medium. Two years earlier Nagodawithana and Steinkraus (5) had postulated that the high cell mortality in their rapid fermentations was due to the inability of the cells to

excrete the ethanol fast enough and the consequent internal accumulation of alcohol. Thomas and Rose (6) also found higher levels of ethanol inside the cells than outside.

Exponential relationships have also been applied. Aiba, Shoda and Nagatani (7) proposed

$$\mu = \mu_0 \frac{C_s}{(K_s + C_s)} \exp(-k_1 C_p) \quad \ldots\ldots (2.7)$$

where the term $C_s/(K_s + C_s)$ is the Monod relation for dependence of growth rate on substrate concentration, and k_1 is an inhibition constant.

Another group of models that has received considerable attention is the hyperbolic type, of the form

$$\mu = \mu_0 \frac{K_p}{(K_p + C_p)} \quad \ldots\ldots (2.8)$$

where K_p is the inhibition constant and is numerically a concentration of alcohol sufficient to halve the growth rate.

Egamberdiev and Ierusalimskii (8) applied it with some success to their fermentations in which they measured growth rates during the exponential phase at various alcohol levels, set by adding ethanol to the culture. Aiba and Shoda (9) reassessed the data to which Eq. 2.7 had previously fitted and preferred the hyperbolic form. Bazua and Wilke (10) tried both parabolic and hyperbolic functions, respectively,

$$\mu = \hat{\mu}\left(1 + \frac{C_p}{C_{pmax}}\right)^{0.5} \quad \ldots\ldots (2.9)$$

$$\mu = \hat{\mu} - a\frac{C_p}{(b - C_p)} \qquad \ldots\ldots (2.10)$$

They too preferred the hyperbolic equation. In this instance the parameter b, while still having the dimensions of concentration, does not have the clear physical significance of the inhibition constants mentioned above. The constant a, with the same dimensions as growth rate, similarly lacks a physical meaning.

Hoppe (11) combined a hyperbolic function with the Monod expression as in Eq. 2.7 :

$$\mu = \frac{\hat{\mu} C_s K_p}{(K_s + C_s)(K_p + C_p)} \qquad \ldots\ldots (2.11)$$

Levenspiel (12) proposed an extension of the linear model with its growth-limiting ethanol concentration, adding an exponent and, as in Eqs. 2.7 and 2.9, incorporating the Monod relation to account for the influence of substrate. Equation 2a in his paper, when expressed using the nomenclature of this thesis, is

$$\mu = \hat{\mu}\frac{C_s}{(K_s + C_s)}\left(1 - \frac{C_p}{C_p^*}\right)^n \qquad \ldots\ldots (2.12)$$

Luong (13) after reviewing all the work described thus far, selected a variation on this model which has the added advantage that it is not undefined for ethanol concentrations in excess of the limiting value C_p^*, permitting negative growth rates instead :

$$\mu_i = \mu_0\left\{1 - \left(\frac{C_p}{C_{pm}}\right)^a\right\} \qquad \ldots\ldots (2.13)$$

This was also selected for trial in the present work, where it is termed the Modified Levenspiel model. Ethanol production was represented by a similar expression :

$$\nu_i = \nu_0 \left\{ 1 - \left(\frac{C_p}{C'_{pm}} \right)^{\beta} \right\} \quad . \quad . \quad . \quad . \quad (2.14)$$

where C_{pm} and C_{pm}' are limiting ethanol levels for growth and ethanol production, and the effect of substrate has been taken up in μ_o and ν_o respectively.

He estimated C_{pm} and C_{pm}' at 112 gl^{-1} and 115 gl^{-1}, working from batch anaerobic fermentations at an initial substrate concentration of 10 gl^{-1} to which varying amounts of ethanol were added beforehand. He pointed out that for large values of the exponent α the shape of the function represented by Eq. 2.13 could be roughly represented by a threshold alcohol concentration below which inhibition was negligible and above which growth rate decreased linearly to zero.

Converti et al (14) carried out batch fermentations to compare the performance of two Saccharomyces strains at high substrate concentrations. The emphasis of their work was on substrate rather than product inhibition, and they examined the kinetics of sugar metabolism rather than growth rate, using the relation

$$-\frac{d}{dt} C_s = \frac{V_{max}}{(K_M + C_s)} \quad . \quad . \quad . \quad . \quad (2.15)$$

where V_{max} is a maximum rate which is constant for a given biomass concentration,

K_M is a constant akin to the Monod and Michaelis-Menten inhibition constants.

They found significant substrate inhibition of S.cerevisiae at sucrose concentrations above 100 gl^{-1}, and product inhibition above 50 gl^{-1}.

Attempts have also been made to synthesize models that describe what actually happens inside the cell, working from major metabolic and anabolic pathways and considering the control mechanisms governing the rate-controlling steps. These are, however, necessarily very complex : not only is a comparison of such models far beyond the scope of this work but the computing power essential to apply them is only now becoming generally available. Nevertheless, a review of the relationships that have been applied to describe the reaction of the growth rate of yeasts to their environment is not complete without mention of these mechanistic models.

The earliest of this type can be said to have been that of Luedeking and Piret (15), who in essence started out from the assumption that part of the energy obtained from fermentative glycolysis is used to fuel growth and the balance goes towards maintenance of the existing cell mass :

$$q_p = \alpha\mu + m \qquad \ldots \quad (2.16)$$

where α is the mass of ethanol associated with the production of unit mass of cells and is equal to Y_{ps}/Y_{xs}, Y_{ps} and Y_{xs} being true ethanol and biomass yield coefficients respectively.

m is the mass of ethanol associated with the maintenance of unit mass of cells.

The model was successfully applied by Aiyar and Luedeking (16) to batch fermentations using S.cerevisiae in a 20 gl^{-1} glucose medium at 30°C. Evidently this is only a partial model, one which gives a relationship between growth rate and ethanol production but does not help

determine growth rate itself. It can be regarded as complementary to the models already enumerated, replacing the assumption of a constant product yield factor and strictly growth-related product formation.

Bijkerk and Hall (17) proposed and tested with some success a mechanistic model based on the assumption that cell mass can be classified into two portions, one responsible for absorption and processing of substrate for energy, and one which attends to cell reproduction. Growth is regarded as a cycle of accumulation of cell mass followed by division (more accurately in the case of yeasts, budding). It is represented by the sequential interconversion of the two types of cell mass :

Accumulation of cell mass :

$$A_w + a_1 \rightarrow 2B_w + metabolic\ \ products \qquad . \ . \ . \ . \quad (2.17)$$

Cell division :

$$B_w \rightarrow A_w \qquad . \ . \ . \ . \quad (2.18)$$

where A_w represents cell mass devoted to substrate uptake, a_1 is substrate and B_w is the cell mass concerned with replication.

They applied their model to aerobic growth of S.cerevisiae in batch and continuous culture, deriving explanations for the Crabtree Effect, the difference between maximum growth rate and growth rate at the onset of the effect, and behaviour on different substrates. Very good qualitative and fair quantitative agreement were obtained. Even after simplification the equations could not be integrated analytically, numerical methods being used instead.

Peringer et al (18,19) constructed a model in which the demands on the substrate supply of protein, lipid, carbohydrate and other cell materials were allowed for individually. They applied it to aerobic and anaerobic cultures of S.cerevisiae, measuring the actual content of each component. Only the logarithmic growth phase was studied and the initial substrate concentration was 10 gl^{-1}. Modified Monod kinetics were used to describe the dependence of substrate and oxygen on their respective external concentrations. In the notation of this work, substrate uptake was described by

$$q_s = \hat{q}_s \frac{C_s}{(K_s + C_s)(1 + bC_L)} \quad . \quad . \quad . \quad . \quad (2.19)$$

where c_L is dissolved oxygen tension and b is a constant. The expression for oxygen uptake was similar. Mass balances supported the model although no simulation of an actual fermentation was presented.

3. EXPERIMENTAL PROCEDURES.

3.1. Organism Used.

The organism in this investigation was Saccharomyces cerevisiae ATCC 4126. This yeast has been used in a number of studies on the kinetics of ethanol fermentation (10,11,13,20). It was maintained on Wickerham medium, the composition of which is given in Table A8.6 in Appendix 8. The slopes were sub-cultured every two months and stored at room temperature.

3.2. Medium.

3.2.1. General.

The medium was designed to be glucose-limited. A survey of formulations used by previous workers (10, 21-26) was carried out in order to set nitrogen and phosphorus levels. A shortlist of suitable compositions arose from this survey and was screened in a series of shake-flask tests in order to determine which gave the highest biomass yield based on glucose consumed. The resulting formulation is shown in Table A8.1. of Appendix 8. It contains ammonium sulphate and potassium dihydrogen phosphate as sources of nitrogen and phosphorus respectively. Other essential components and trace metals were assumed present in sufficient quantity in the yeast extract supplied and in the tap water used to make up the medium. Sodium citrate and citric acid are present to buffer the pH to 5,0.

Glucose concentrations of 100, 150 and 200 gl^{-1} were used and all other components were varied in proportion. Tables A8.2. to A8.5. of Appendix 8 give the details.

3.2.2. Preparation.

Medium was made up in two portions, one containing the glucose required and the other the remaining constituents. 5-litre aspirators, 2-litre and 1-litre Erlenmeyer flasks

were used depending on the quantities involved. The two portions were autoclaved at 120°C for 20 minutes and then combined while still hot. This procedure avoids the side reactions that take place at higher temperatures between glucose and some of the other components, while minimising the risk of infection during the mixing of the two parts.

In batch fermentations, the glucose portion was sterilised in the fermenter itself : similarly the glucose solution for the initial medium in fed-batch fermentations. All other portions, that is to say glucose for the feed in fed-batch runs and all solutions of other nutrients, were sterilised in a 120 l vertical autoclave.

3.2.3. Inoculum Medium.

Medium for incubation of inocula was drawn from a sterile stock of a similar formulation containing 100 g glucose per litre : Table A8.4 in Appendix 8 gives the composition. Portions drawn were resterilised before use as the procedure used for drawing them was not aseptic.

3.3. Equipment.

Fig. 3.1. is a schematic drawing of the 7-litre CHEMAP fermenter and associated equipment which were used for both batch and fed-batch runs. One end of the fermenter was supported on a load cell, the output of which was plotted on a chart recorder (CR600 by J.J. Instruments). Ammonia, approximately 5N, was used for pH control : it was dosed automatically to keep pH between 5,0 and 5,1. Peristaltic pumps (Watson-Marlow and Verder) were used for all pumping. Evolved gases were led via the reflux condenser through ice and acetone/dry-ice traps to a wet gas meter. The inclusion of a drop of Fermenter Oil B, an antifoam agent, sufficed to control foam. Oxygen concentration, temperature and pH in the broth were logged on a multipoint chart recorder.

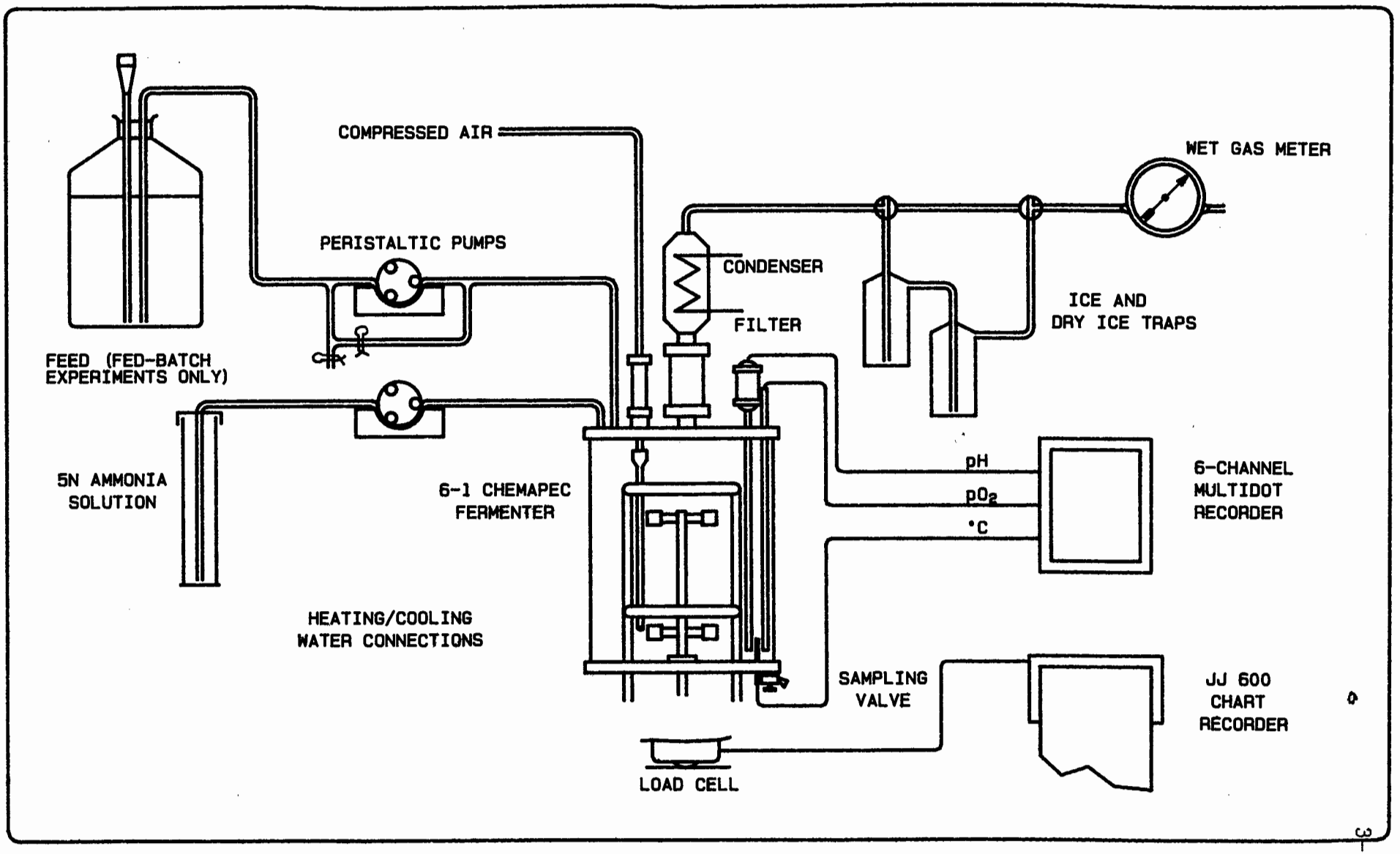


FIG. 3.1 SCHEMATIC DIAGRAM OF CHEMAPEC FERMENTER AND ANCILLARY EQUIPMENT

3.4. Determination of Yeast, Glucose and Ethanol Concentrations.

3.4.1. Yeast.

Biomass concentration was determined by diluting the sample to 80 - 250 mgl^{-1} yeast and then measuring the percentage transmission of 580 nm light. The Beckman 1211 Colorimeter used was standardised to 100% transmission on distilled water before each reading, and readings were as a rule done at two dilutions for each sample.

Transmission readings were converted to concentrations using a calibration curve : in practice a second-order polynomial in log T which represented the data very well was used for the conversion. Details of the calibration are given in Appendix 7.

3.4.2. Glucose.

Samples for glucose analysis were diluted to bring the sugar concentration below 4,5 gl^{-1} (preferably into the range 1 - 2,5 gl^{-1}) and centrifuged free of cells. They were then analysed by a Beckman Glucose Analyser, Model 2. This instrument measures the peak oxygen consumption rate when a 10,0 µl sample of the glucose solution is introduced by micropipette into 1,00 ml of glucose oxidase solution saturated with air. This peak rate is directly proportional to the glucose concentration.

3.4.3. Ethanol.

Samples to be analysed for ethanol were treated immediately with known quantities of 1-butanol, diluted to give suitable alcohol concentrations, centrifuged to remove yeast cells and refrigerated. Batches of samples were subsequently analysed by the internal-standard method on a Varian 1440 Gas Chromatograph linked to a Vista data processor. The nominally 0,5% 1-butanol standard was saturated with benzoic acid, which prevented microbial growth in the standard and retarded growth in the prepared samples.

Quantitative details of sample preparation and chromatograph column and settings are given in Appendix 7.

3.4.4. Accuracy.

Standard B-grade volumetric glassware was used. As sampling appreciable volumes from the broth during fermentation would require complicated adjustments in the subsequent rate calculations, pipetted sample volumes were restricted to 2,00 ml. A study was done to determine what error could be expected in pipetting such small samples, as rate calculations are particularly sensitive to such errors. It was found to be about 0,1%.

Multiple replicates of samples on the glucose analyzer and gas chromatograph indicated standard deviations of 0,5 - 0,7% and better than 2% respectively. The accuracy of biomass readings was more difficult to judge because while two readings at different dilutions sometimes agreed within 0,1%, at other times their difference could reach 15%. Discrepancies of this size were only encountered in fed-batch fermentations and tended to occur in a particular period of several hours after the start of feed. They were therefore probably attributable to the condition of the yeast at the time and could only have been avoided by calibrating with actively growing yeast. The topic is considered further in the Discussion (Section 6.3.4.). Generally the difference between two different dilutions of the same sample was about 1,5%.

3.5. Fermentation Procedure.

3.5.1. General.

Two series of fermentations were carried out, one batch and one fed-batch. In fed-batch fermentations, an initial aerobic batch pre-fermentation at 30°C using 1,5 l of medium was followed by the anaerobic fermentation of 4,5 l feed at 35°C. Biomass yield is higher at 30°C than at 35°C (4), while many studies have found that yeast activity is

greater at 35°C than at 30°C : the procedure followed combined higher initial yeast concentrations with maximum activity, thus attempting to simulate industrially realistic conditions. Glucose concentration in the pre-fermentation was generally 100 gl^{-1} but one run at 150 gl^{-1} and one at 200 gl^{-1} were also done.

Batch fermentations were designed to use the same total quantities (Table A8.5, Appendix 8) as the fed-batch runs so as to facilitate comparisons of product yield and reactor productivity for use in a separate study. They were carried out at 30°C and 35°C and were anaerobic throughout.

3.5.2. Inoculum Preparation.

The quantity and condition of the inoculum were controlled as follows :

150 ml medium was inoculated with a loopful of yeast from a slope and incubated on the shaker at 34°C. When fermentation was complete the yeast was allowed to settle and the clear liquid decanted. The resulting concentrated yeast suspension was homogenised and its concentration measured : this was generally about 23 gl^{-1}. The volume of suspension required to give a concentration of 1,5 gl^{-1} with 150 ml fresh medium could now be calculated. Eight hours before the scheduled inoculation of the CHEMAP fermenter, a fresh 150 ml portion of medium was saturated with air entrained by a magnetic stirrer at high speed, inoculated with the calculated volume of the concentrated suspension and incubated at 34°C on the shaker.

This procedure gave good reproducibility and greatly facilitated scheduling.

3.5.3. Fed-batch Experiments - Inoculation and Aerobic Pre-fermentation.

The fermenter was charged with approximately 1,5 l medium for the pre-fermentation and the agitator was set to 400 rpm. The medium was saturated with air by sparging and brought to 30°C, after which the inoculum was pumped in by a fast peristaltic pump. During the subsequent aerobic fermentation, agitator speed was increased as necessary to maintain the oxygen level above 40% of saturation. Samples were taken at intervals from one to five hours, the frequency increasing towards the end when concentrations were changing more rapidly.

This phase of fermentation lasted until a target value of sugar concentration was reached, when the transition to fed-batch fermentation was made. The target values were varied from 10 - 20 gl^{-1} to 40 - 50 gl^{-1} and were reached 8 - 17 h after inoculation depending on the initial glucose concentration.

3.5.4. Fed-batch Experiments - Anaerobic Fed Fermentation.

Towards the end of the pre-fermentation, when sugar concentrations were approaching the desired level, the following preparations were made :

- The temperature increase from 30°C to 35°C was made in five half-hourly steps of 1°C, the first one hour before and the last one hour after commencement of feeding. This was to avoid any thermal shock to the yeast.

- The feed pump was set to circulating feed around a loop at a preselected rate. The purpose of this was to allow the flow rate to settle down to its precalibrated value, as the delivered flow from a peristaltic pump varies for some time after the pump has been switched on.

- At commencement of feeding, a sample was taken, air was switched off and agitator speed was reduced to 300 rpm.

Subsequent sampling intervals varied from one to four hours depending on how rapidly concentrations were changing. Glucose and yeast concentrations were determined on sampling, while samples prepared for ethanol determination were refrigerated for later analysis in batches.

Whenever sugar concentrations began to fall because of the increasing quantity of yeast present, the feed rate was stepped up. A mild rise in glucose concentration was intended, and the feed rate increase required was estimated from the most recent glucose values.

When the supply of feed was exhausted the time was noted and a sample taken. Sampling continued until the point of exhaustion of glucose could safely be predicted. After the end of the fermentation final samples of the beer were taken for ethanol determination.

3.5.5. Batch Fermentations.

Total quantities used in batch runs were identical with the fed-batch runs. For a batch run, however, the full 6 l of medium was charged to the fermenter at the start. It was initially saturated with air but this supply was shut off at inoculation. Except for the absence of feed, procedures during these anaerobic batch fermentations were the same as during fed-batch runs.

4. DATA ANALYSIS.

Numerical analysis of the data consisted of calculating growth and substrate uptake rates from the raw data, and fitting various growth rate models to the growth rates so determined. In this Section the methods used to calculate growth and substrate uptake rates and to fit growth models to the resultant data will be described. During the analysis an interesting and significant observation was made, namely that the total substrate uptake rate of the broth became constant even though the organism continued to grow. This led to further analysis which is covered not in this Section but in the next two, 5.Results and 6.Discussion.

4.1. Processing of Raw Data to Growth Rates.

The raw data consist of elapsed time, concentrations of yeast, sugar and ethanol and, in the case of fed-batch fermentations, feed rates and broth volume at the start of feed. These were analysed using interactive programs written in BASIC on a Sanyo MBC-555 microcomputer. In the case of batch data growth rates were calculated using

$$\mu = \frac{1}{C_x}\frac{d}{dt}C_x = \frac{d}{dt}\ln C_x \qquad \ldots\ldots (4.1)$$

which holds for constant volume. In fed-batch fermentations the more fundamental relation

$$\mu = \frac{1}{X}\frac{d}{dt}X = \frac{d}{dt}\ln X \qquad \ldots\ldots (4.2)$$

was used. C_x, X and t are biomass concentration, total biomass and time respectively.

In both cases the data were smoothed beforehand : the smoothing process consisted in fitting a third-order polynomial to groups of five points and using this to calculate the smoothed value of the centre point. The polynomials used for the third and third last points were also used to estimate smoothed values for the first two and the last two points in each set respectively.

The actual computation of growth rates involved taking natural logarithms, fitting fourth-order polynomials to the smoothed points and calculating the derivative algebraically. Fourth-order fits were selected after an extensive series of third- to sixth-order regressions showed that they offered the lowest sum of squared deviations in most cases. They also represented a good compromise between the coarseness of lower order fits and the spurious undulations produced by high orders.

4.2. Fitting Yeast Growth Rate Models to the Data.

Fitting of growth rate data to the various models was accomplished using an iterative method which is described in Appendix 9. It does not guarantee a result but can be applied to any algebraic function which is differentiable over the region of interest, and produces a least-squares fit. In practice it is necessary to incorporate checks in the computer program which alert the user when the current values of the parameters move out of allowable range. For instance, the limiting ethanol concentration C_P^* in the Levenspiel model must not drop below the highest ethanol concentration in the data, as this will result in an attempt to take the logarithm of a negative number during the next iteration.

4.3. Models Investigated.

The base models considered in this study were :

- the Monod model with a hyperbolic ethanol inhibition term as applied by Hoppe (11), and

- the Monod model with a power-law inhibition term as proposed by Levenspiel (12).

In addition, another which will be termed the Simplified model suggested itself during the work, and with hybrid models the total number considered came to eight. In detail they are as follows :

1. Monod Model with Product Inhibition Term.
This combines the traditional Monod growth rate expression with a non-competitive product inhibition term

$$\mu = \frac{\hat{\mu}_M}{\left(1 + \frac{K_s}{C_s}\right)\left(1 + \frac{C_p}{K_p}\right)} \quad . \quad . \quad . \quad . \quad (4.3)$$

2. Levenspiel.
This uses the same Monod relation for substrate but ethanol inhibition is expressed by an exponential term :

$$\mu = \hat{\mu}_L \frac{\left(1 - \frac{C_p}{C_p^*}\right)^n}{\left(1 + \frac{K_s}{C_s}\right)} \quad . \quad . \quad . \quad . \quad (4.4)$$

3. Levenspiel - Ethanol Inhibition Only.
The same as No.2 above but without the substrate-dependent term :

$$\mu = \hat{\mu}_L \left(1 - \frac{C_p}{C_p^*}\right)^n \quad . \quad . \quad . \quad . \quad (4.5)$$

4. Modified Levenspiel Model.
Applying the exponent in the Levenspiel model to the term inside the bracket instead of the whole bracket gives a similar expression also suitable as a growth model :

$$\mu = \hat{\mu}_L \frac{\left\{1 - \left(\frac{C_p}{C_p^*}\right)^n\right\}}{\left(1 + \frac{K_s}{C_s}\right)} \quad . \quad . \quad . \quad . \quad (4.6)$$

5. Modified Levenspiel - Ethanol Inhibition Only.
Similar to No.4 above but omitting the substrate-dependent term :

$$\mu = \hat{\mu}_L \left\{1 - \left(\frac{C_p}{C_p^*}\right)^n\right\} \quad . \quad . \quad . \quad . \quad (4.7)$$

6. Simplified Monod Model.
Early attempts to fit the Monod model to experimental data using a variety of methods were uniformly unsuccessful. In the course of work with the iterative methods, however, it was noticed that while the values of the parameters μ and K_s tended to increase without bound during the iterations, their ratio converged to a limit. This observation led to the derivation of a new model formulation as follows :

- o Divide the right-hand side of Eq. 4.3 top and bottom by K_s. There results

$$\mu = \frac{(\hat{\mu}/K_s)}{(1/K_s + 1/C_s)\left(1 + \frac{C_p}{K_p}\right)} \quad . \quad . \quad . \quad . \quad (4.8)$$

- o Now if μ and K_s are allowed to increase without limit while the ratio μ/K_s converges to B, there is obtained

$$\mu = B \frac{C_s}{\left(1 + \frac{C_p}{K_p}\right)} \quad . \quad . \quad . \quad . \quad (4.9)$$

Preliminary work using this model was promising and it was accordingly included in the list of models.

7. Simplified Levenspiel Model.

Derived by analogy with the Simplified Monod model.

$$\mu = BC_s\left(1 - \frac{C_p}{C_p^*}\right)^n \qquad \ldots\ldots (4.10)$$

8. Simplified Modified Levenspiel Model.

Similarly complementing the Modified Levenspiel model :

$$\mu = BC_s\left\{1 - \left(\frac{C_p}{C_p^*}\right)^n\right\} \qquad \ldots\ldots (4.11)$$

4.4. Calculation of Substrate Uptake Rates.

4.4.1. Fed-batch Fermentations.

Total substrate uptake rates during the fed periods of the fed-batch runs were obtained as the difference between sugar feed rate to the broth and accumulation in the broth :

$$Q_s = FC_{sf} - \frac{d}{dt}S \qquad \ldots\ldots (4.12)$$

where Q_s is substrate uptake rate [g glucose h^{-1}],

S is total substrate present [g]

F, C_{sf} are feed rate and concentration respectively.

Substituting dV/dt for feed rate F and $V.C_s$ for S gives

$$Q_s = \frac{d}{dt}\{V(C_{sf} - C_s)\} \qquad \ldots\ldots (4.13)$$

It should be noted that since all feed rate changes were step changes, neither $V.C_{sf}$ nor $V.C_s$ are separately differentiable over the whole time interval from start of

feeding to exhaustion of feed supply. Q_s is, however, continuous and the function $V(C_{s,r} - C_s)$ must accordingly be differentiable at all points.

Specific uptake rate at any moment is obtained by dividing Q_s by the total biomass present.

4.4.2. Batch Data and Pre-fermentations.

For batch experiments, including the pre-fermentations, use was made of the relation

$$q_s = -\frac{1}{C_x}\frac{d}{dt}C_s \qquad \ldots\ldots (4.14)$$

which holds for constant volume, to determine specific uptake rate directly.

4.5. True Biomass Yield and Cell Maintenance.

True cell yield and maintenance coefficients were estimated using the relationship

$$q_s = \frac{\mu}{Y_{xt}} + m \qquad \ldots\ldots (4.15)$$

where m is cell maintenance coefficient, [g glucose g yeast h^{-1}],

Y_{xt} is true cell yield, [g yeast g $glucose^{-1}$]

This expression is akin to the Luedeking-Piret model[15] cited in the literature survey, and can be derived from it given an assumption regarding yield factors.

Yield and maintenance coefficients were then derived by means of linear least squares regressions carried out between specific substrate uptake and growth rates : yield

and maintenance were provided by the reciprocal of the slope and by the intercept respectively, according to Eq. 4.15.

5. RESULTS.

Of the thirty-three fermentations carried out in the course of these studies, eight batch and nine fed-batch runs were selected for inclusion in this work on the basis of practical considerations such as completeness of data and compliance with desired conditions such as average glucose level. One set of batch data from the work of Converti et al[14] was also included in the regressions for growth model parameters. Certain problems encountered in carrying out the fermentations and the analysis of the raw data could affect the results and hence ultimately the conclusions drawn from them, and this Section outlines the decisions taken in the handling of those problems. Owing to the bulkiness of both the raw and the processed data, systematic presentation of the results has been relegated to Appendices, but typical raw and processed results are presented in tabular and graphical form here. Detailed discussion of the results is undertaken in Section 6 after this chapter.

5.1. Time Course of Batch and Fed-batch Fermentations.

The raw data from the eight batch fermentations Nos. B4 to B11 is tabulated in **Appendix 1**. Similarly fed-batches FB13 to FB21 are recorded in **Appendix 2**. **Figs. 5.1** and **5.2** show the typical time profiles of each mode. In the batch runs yeast concentration rose from about 200 mgl^{-1} to 4 gl^{-1} over a 24-hour period. Glucose and ethanol levels showed their greatest rates of change about 10 hours after inoculation. Thereafter they moved at a steady rate, decreasing towards the end, reaching their final values at times varying between 45 and 68 hours. These runs could be regarded as fairly typical batch fermentations with a relatively small inoculum.

Fig.5.1. Typical Batch Fermentation.
Batch No. 10

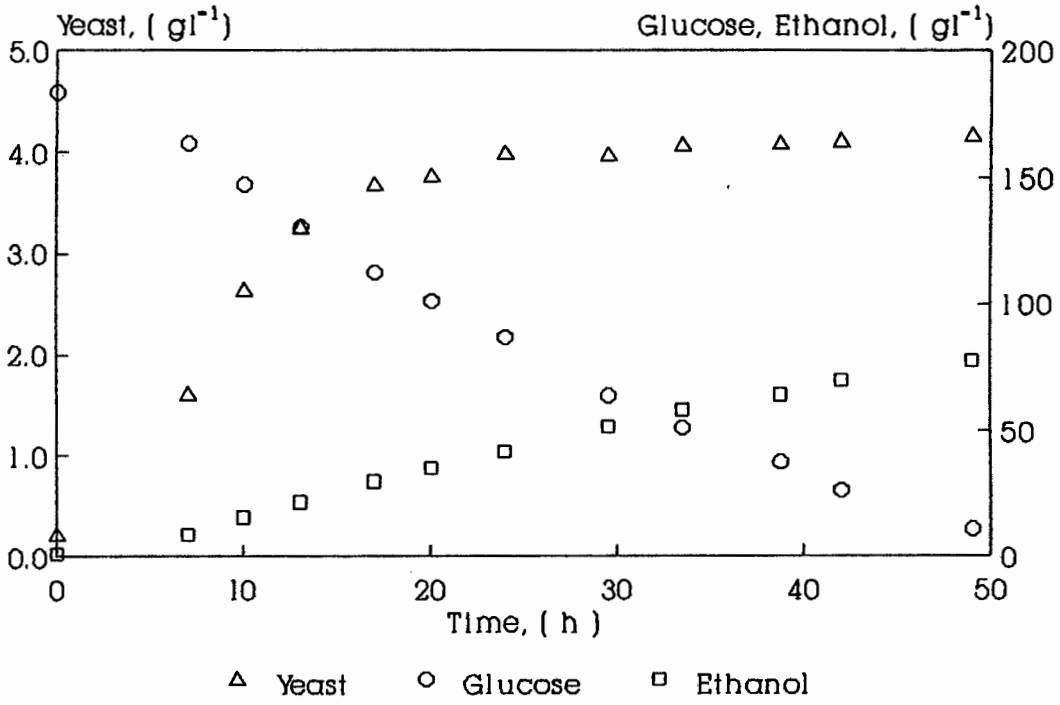
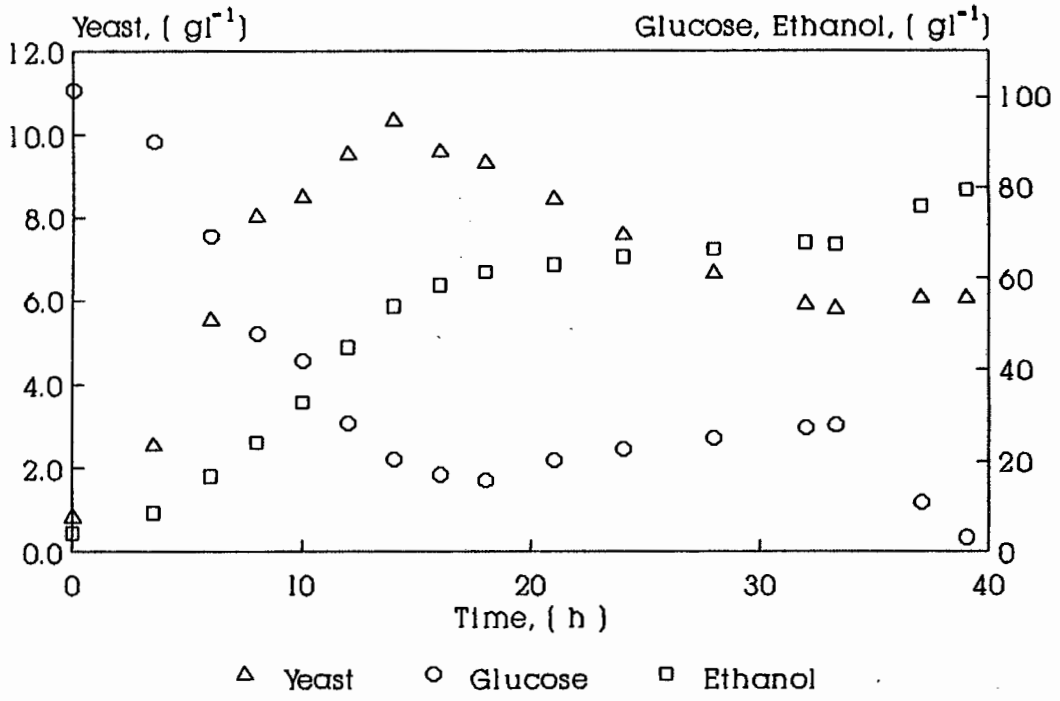


Fig.5.2. Typical Fed-batch Fermentation.
Fed-batch No. 19



In the fed-batch fermentation, the aerobic pre-fermentation using the same size of inoculum but in one quarter of the volume, produced yeast suspensions of 8 gl^{-1} in eight hours. The biomass concentration generally continued to rise after feed was started, as at that stage growth rate exceeded dilution rate, but within a few hours, after increases in the feed rate, it invariably began to fall. Growth continued to the end, however, as was evidenced by the slight increase in concentration recorded in nearly every instance after the end of the fed period. Glucose levels generally rose slowly throughout the fed period as desired, although they were fairly sensitive to feed rate and were particularly difficult to control in the hours immediately following the start of feeding. This was due to the substantial increase in substrate uptake rate on discontinuing the aeration. Ethanol concentration, on the other hand, generally rose sharply for several hours after feed was switched on and then reached a plateau, rising only slowly thereafter until the feed was exhausted.

5.2. Interpolation for Missing Data.

As a result of practical problems such as sample leakage, values were not obtained for several samples in batch fermentations B7 to B9. Where necessary for the purposes of calculating growth rates or carrying out regressions suitable values were interpolated from the time plot of the fermentation. Values estimated in this way are indicated in Appendices 1 to 4 by enclosure in brackets. In many instances the time of exhaustion of substrate was estimated by extrapolation and these are marked in the same way. Another case where it was necessary to take a view was in dealing with the large discrepancies occasionally encountered between measurements of biomass concentration on the same sample carried out at different dilutions. These are discussed in Sec.6.3.4.

5.3. Growth Rate Calculation.

Tables 5.1 and **5.2** contain the growth rates computed from the raw data of Figs. 5.1 and 5.2. They are reproduced from Appendices 3 and 4 - Batch and Fed-batch Growth Rates respectively.

5.3.1. Calculation of Total Biomass.

In the previous chapter there was outlined the method used to determine growth rate for the fed-batch experiments from the total mass of yeast present, this being calculated from its concentration and the total volume at the time. The volume was determined using the chart recorder trace of the load cell output. Preparation for each run included calibration of the cell : despite this, the mass indicated by the chart recording generally did not agree with accurate weighings of the medium and the beer, irrespective of whether or not losses in weight due to evolution of carbon dioxide were taken into account. Between changes in feed rate the trace was straight, indicating a steady feed rate. The procedure used to resolve this obstacle was as follows :

- o accept the traces as giving the relative magnitudes of the feed rates in any particular fermentation ;
- o use the weighings in conjunction with the known density of the medium to determine the total volume of feed and hence actual feed rates during each period between adjustments.

The feed rates quoted in Table 5.2 were calculated in this way. Examination of graphs of total mass of yeast against time did not reveal any discontinuities at the points where feed rate was increased. It was concluded from this that the procedure followed was sound.

Table 5.1. Growth Rates in Batch Fermentation. No.10.

Concentrations [gl^{-1}]		Growth rate
Glucose	Ethanol	[h^{-1}]
183,1	0,836	0,4163
163,0	8,38	0,1894
146,6	15,2	0,1158
130,2	21,7	0,0520
111,8	29,5	0,0130
101,0	34,9	0,0121
86,5	41,6	0,0044

Table 5.2. Growth Rates in Fed-batch Fermentation. No.19.

Concentrations [gl^{-1}]		Growth rate
Glucose	Ethanol	[h^{-1}]
47,5	23,8	0,1486
41,8	32,8	0,1204
28,0	44,7	0,0960
20,2	53,6	0,0753
16,8	58,3	0,0579
15,5	61,1	0,0437
20,0	62,8	0,0275
22,4	64,6	0,0168
24,8	66,1	0,0097

5.3.2. Polynomial Regression to Determine Growth Rate.

Fourth-order polynomials represented the biomass-time data well. In all cases it was necessary to discard results at

the high end of the ethanol concentration range, as the growth rates obtained there were so low as to be of the same order as the residuals : they were sometimes even negative. Growth in fed-batch fermentations after the end of the fed period, referred to in Sec. 5.1, was too low to measure in any run, although yeast concentration consistently showed a slight rise after feed was stopped. In most of the batch fermentations the first growth value was very much larger than the subsequent ones and had to be discarded during the subsequent modelling : Table 5.1 shows a case in point. These first values have, however, been retained in the growth rate tables of Appendix 3.

Fed-batch 18 was an experiment in which the pre-fermentation medium was changed to 200 g glucose l^{-1} from the normal 100 gl^{-1}. Growth was low and no satisfactory rates could be obtained. There is accordingly no entry for this run in Appendix 4.

5.4. Regression to Determine Model Parameters.

The problems encountered in obtaining optimal fits of the various models to the data included those iterative procedures : parameters increasing without bound, oscillation between two unsuitable sets of parameter values and convergence to false solutions with negative or otherwise unacceptable values. Susceptibility to these obstacles increased with the number of parameters in the model under test.

Two other problems also had to be overcome. One occurred in equations using the substrate inhibition parameter K_s, to which the predicted growth rates are not very sensitive. This insensitivity resulted in the calculated adjustments for K_s tending to become and remain large. A great many different starting values had to be tried and even then the method was unsuccessful in most cases involving these

models. In such instances the procedure followed was to adjust parameter values manually until no further improvement was visible.

The second problem arose in work with the three equations which use the expression proposed by Levenspiel (12) to describe inhibition by ethanol. This expression is undefined for ethanol concentrations in excess of the limiting parameter Cp*, and hence it was frequently necessary to exclude from the calculations data in the high-ethanol, low-growth region. This was done interactively between iterations. Successful regressions were ultimately obtained with nearly all sets of data.

Variation between batch fermentations was quite small, the principal sources being the two temperatures used and random differences in the mass of the inoculum. It was accordingly possible to combine growth rate data from batch fermentations done at the same temperature, and the two larger sets of growth rates resulting from this combination were used exclusively during the regression work. **Fig. 5.3** shows growth rates from the batches carried out at 30°C. Sources of variation in the fed-batch experiments were more numerous : in addition to some small differences in the inocula there was a considerable range of feed rates, and the behaviour of the yeast was noticeably influenced by the concentrations prevailing during the first few hours after commencement of feeding. Growth rate data from different runs followed similar trends but did not combine as well : **Fig. 5.4** showing results from three runs illustrates this. Hence each fed-batch fermentation was generally handled separately in carrying out the regressions, although a few results were obtained from combined runs.

The parameter values obtained from the regressions are tabulated by model in **Appendix 5**.

Fig.5.3. Combined 30°C Batch Growth Rate Data.

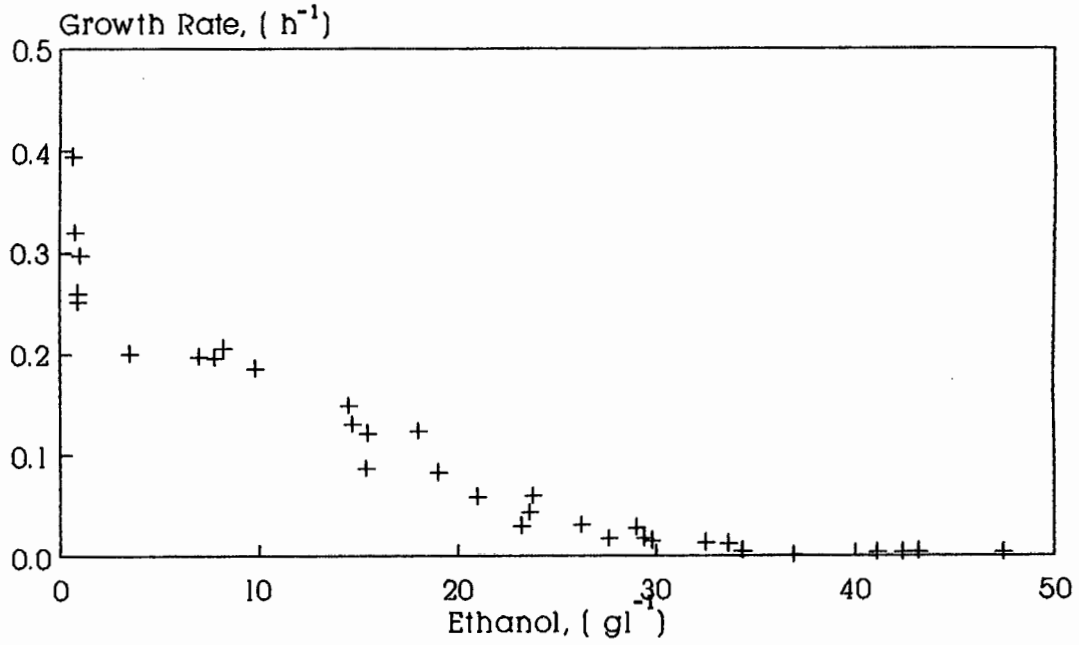
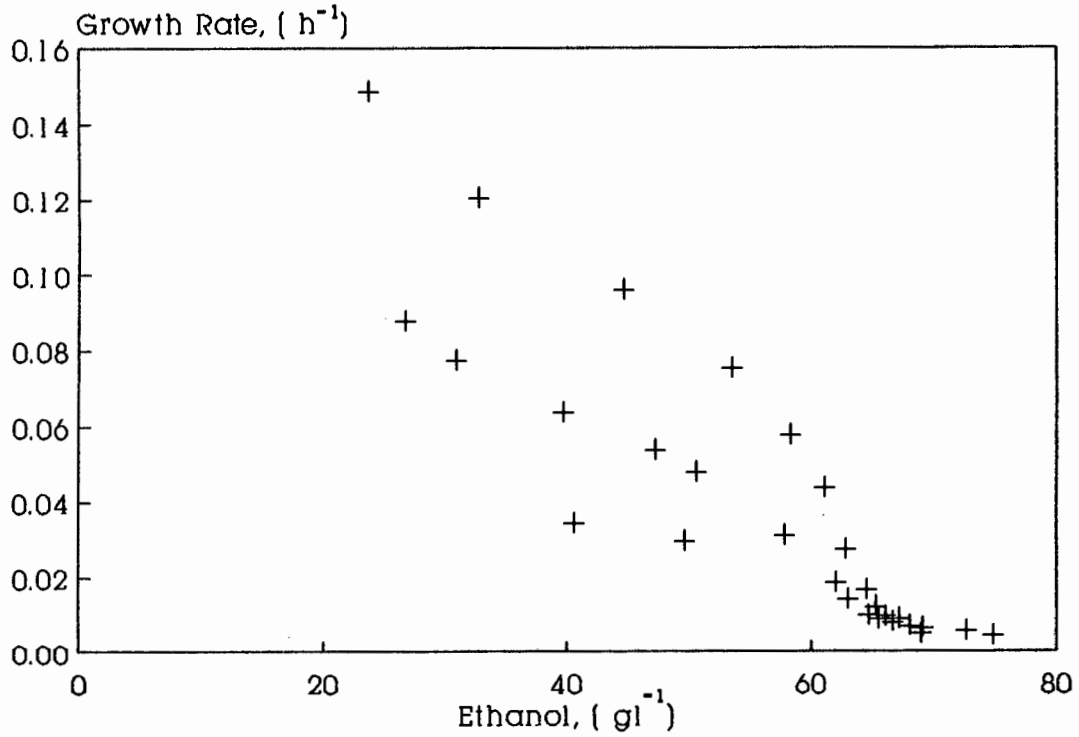


Fig.5.4. Combined Fed-batch Growth Rate Data.



. Fed-batch Nos. 15,16 & 19.

5.5. Substrate Uptake, Biomass Yield and Cell Maintenance.

5.5.1. Fed-batch Fermentations : Anaerobic Period.

Total substrate consumption rates were determined as described in Sec. 4.4., Eq. 4.13 for the anaerobic portions of all fed-batch fermentations, including FB18 for which no other analysis is presented in this work. On plotting the function V(Csf - Cs) in order to apply Eq. 4.13 :

$$Q_s = \frac{d}{dt}\{V(C_{sf} - C_s)\} \qquad \ldots\ldots (4.13)$$

it became apparent in all cases that after a certain point in time the data fell on a straight line. This implied that sugar consumption rate ceased rising at that point and remained constant thereafter irrespective of the quantity of biomass present. This observation was significant and accordingly the constant value attained in each fermentation is tabulated in **Table A6.1** of **Appendix 6** together with the period over which it applied and the average substrate concentration during that time. The phenomenon is discussed in **Sec. 6.2 - Stagnation of Metabolic Rate.**

True cell yield and maintenance coefficients were also estimated as described in Section 4.5 for each anaerobic fed-batch run except No. 18 for which no growth rates were available. The results are given in the second and third columns of **Table 5.3**, which is a copy of Table A6.2 in Appendix 6. In such a sequential calculation, however, considerable computational errors can accumulate, and the ranges of the values in Table 5.3 are wider than would be expected for fermentations run under such uniform conditions. For this reason these crude results were refined making use of the fact that there is an implicit relationship between true cell yield and maintenance

Table 5.3. Biomass Yields and Maintenance Coefficients - Fed-batch Fermentations.

Raw and refined values.

Fed-Batch No.	Raw		Refined	
	Yield gg^{-1}	Maint. $gg^{-1}h^{-1}$	Yield gg^{-1}	Maint. $gg^{-1}h^{-1}$
13	0,097	0,751	0,0815	0,728
14	0,080	0,912	0,0815	0,899
15	0,060	0,746	0,0815	0,774
16	0,089	0,804	0,0815	0,804
17	0,139	0,851	0,0815	0,764
19	0,086	0,635	0,0815	0,700
20	0,051	0,335	0,0815	0,710
21	0,149	0,493	0,0815	0,500

coefficient, based on the mass balance between substrate consumed and biomass produced, which can be expressed for the general case by integrating both sides of Eq. 4.15 :

$$\int_{t_1}^{t_2} q_s(t)\,dt = \frac{1}{Y_{xs}}\int_{t_1}^{t_2} \mu(t)\,dt + m(t_2 - t_1) \qquad . \; . \; . \; . \quad (5.1)$$

In general this is not very useful because the substrate uptake and growth rates must be known as a function of time, but in the case of the fed-batch fermentations it was possible to use it in the form of Eq. 6.2 from Sec. 6.2 :

$$X = \frac{1}{m}\{Q_{ss} - (Q_{ss} - mX_0)\exp(-mY_{xs}t)\}$$

which applies over the period of constant sugar uptake rate described above. There are several different ways in which the correction can be done depending on the assumptions made. In this work it was assumed that true cell yield was less likely to vary from run to run than maintenance coefficient, and accordingly the procedure used was as follows :

- Total biomass present at the end of the period was calculated for each fermentation according to Eq.6.2 using the data of Tables 5.3 and A6.1 and the biomass present at the start of the period.

- This was compared with the known biomass at the end. Where agreement was good, the true yield coefficient value was taken as being a sound estimate and included in an average which was then applied to all the fermentations.

- All maintenance values were then adjusted so that the calculated biomass agreed with the observed quantity at the end of the period.

This work was carried out using a spreadsheet program. The final estimates of true cell yield and maintenance coefficient are presented in the last two columns of Table 5.3.

5.5.2. Batch Data and Pre-fermentations.

Eq. 4.14 was used for the calculation of specific substrate uptake rates for all batch fermentations. In all other respects the procedure was the same as described in the previous Section. Maintenance and yield values for the batch experiments are to be found in **Table 5.4**, reproduced from Appendix 6, Table A6.3. The pre-fermentations were very reproducible and only average values are recorded for them, also in Table A6.3. No consistency check was applied to the batch results.

Table 5.4. Biomass Yields and Maintenance Coefficients - Batch Fermentations.

Batch No.	Biomass Yield gg^{-1}	Maint. Coeff. $gg^{-1}h^{-1}$
4	0,117	0,995
5	0,245	0,717
6	0,148	0,407
7	0,072	0,744
8	0,213	0,729
9	0,237	0,547
10	0,316	0,819
11	0,206	0,503
Pre-ferm	0,325	0,868

The pre-fermentation values are averages of those for Fed-batches Nos. 15,16,19,20 and 21.

6. DISCUSSION.

This chapter comprises four sections. The first covers all aspects of the regression work with the growth models presented in the Chapter on data analysis. In Section 6.2 the observed stagnation in metabolic rate and some implications are considered. Other observations from the experimental work are dealt with in Section 6.3. Some important aspects to consider in the selection of a growth model for any specific application form the topic of the last Section.

6.1. Model Parameters : Significance and Applicability to Ethanol Fermentation by S.cerevisiae.

The growth models applied in this study can be grouped into three overlapping classes - classical Monod, Levenspiel and Simplified - and they share a common set of parameters. Accordingly each parameter is discussed in turn rather than each model, although the most suitable choice of model for these particular fermentations is also considered subsequently.

6.1.1. Maximum Growth Rate $\hat{\mu}$.

The parameter $\hat{\mu}$, occurring in models of both the Monod- and the Levenspiel- types, represents the maximum specific growth rate attainable under ideal conditions and is a natural choice of parameter.

Values of $\hat{\mu}$ obtained in this work varied from as low as 0,04 h^{-1} for one fed-batch fermentation modelled using a Levenspiel variant to 0,75 h^{-1} for combined 35°C batch data using the Monod model with an ethanol inhibition term. **Table 6.1** summarises the results from Appendix 5.

Table 6.1. Summary of Values of $\hat{\mu}$ [h^{-1}].

Model	Batch		Fed-batch	
	30°C	35°C	Average	Range
Monod with product inhibition	0,7	0,751	0,52	0,5 - 0,55
Levenspiel	0,5	0,4	0,45	0,4 - 0,65
Levenspiel without substrate term	0,309	0,320	0,172	0,061 - 0,246
Modified Levenspiel	0,390	0,5	0,46	0,33 - 0,64
Modified Levenspiel without substrate term	0,30	0,5	0,173	0,036 - 0,210

In principle, $\hat{\mu}$ would be expected to be much the same for a given set of data no matter which of the relevant models was used. The values in Table 6.1 do not seem to confirm this. The explanation lies in the difficulty of obtaining good estimates of the theoretical maximum growth rate : while data at near zero product concentration can be obtained easily enough, the correct substrate level to use can be problematic. If it is set too high, substrate inhibition effects may become significant, while if it is too low the term

$$\left(1 + \frac{K_s}{C_s}\right)$$

is not sufficiently close to unity. The value of $\hat{\mu}$ obtained tends to be largely determined by the data taken closest to this region, but it contains the substrate term as well : the value is closer to $\hat{\mu}(1 + K_s/C_{s_o})$, where C_{s_o} is the sugar concentration at low ethanol levels, than to $\hat{\mu}$ itself. This is illustrated by **Table 6.2** :

Table 6.2. Average Values of $\hat{\mu}$ - Influence of Substrate Removed.

$$\hat{\mu}' = \frac{\hat{\mu}}{\left(1 + \frac{K_s}{C_{s0}}\right)}$$

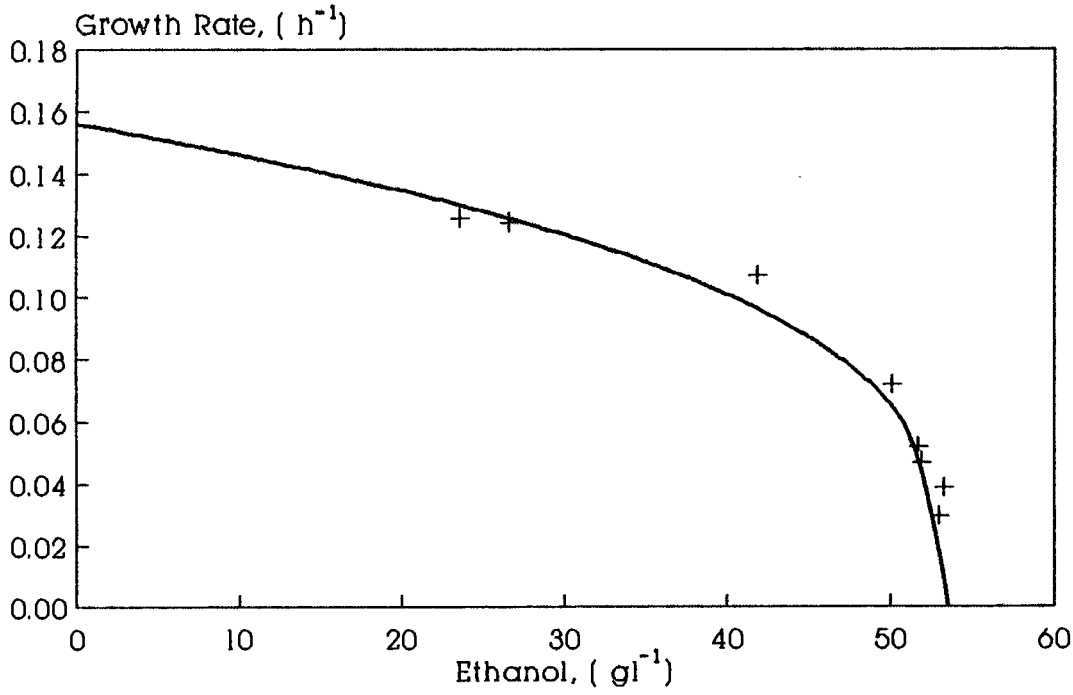
Model	$\hat{\mu}$ h^{-1}
Monod	0,234
Levenspiel	0,257
Levenspiel without substrate term	0,197
Modified Levenspiel	0,226
Modified Levenspiel - no substrate term	0,201

It can be seen that there is much more consistency in these values, and fair agreement between models which have the substrate term and those which do not.

The value of this parameter is largely determined by the data points that fall in the high-substrate, low-product region and this is an important consideration when applying any model which uses it. **Fig. 6.1** is a plot of one of the fed-batch fits and illustrates the effect which such data points have. The value of $\hat{\mu}$ yielded by the regression is the intercept of the curve on the growth-rate axis. It is evident that more data in the low-ethanol region is needed, and for this reason this pattern of fermentation, in which an aerobic period precedes the anaerobic period during which growth is measured, is not ideal for determining $\hat{\mu}$. Ethanol concentration is already relatively high and substrate relatively low when readings are started : in these experiments the broth contained about 35 gl^{-1} at that stage, well above the 26 gl^{-1} threshold found by Holzberg[2]

and the 15 gl^{-1} above which Luong[13] found noticeable effects. Furthermore, the actual transition from aerobic to anaerobic conditions may well affect the growth rate to the extent that the results obtained for the first few hours afterwards do not reflect normal behaviour. The strong increase in substrate uptake transition which normally marks such a transition was observed in these fermentations and is a significant disturbance.

Fig.6.1. Influence of Data at Low Ethanol Levels on the Value of $\hat{\mu}$.



Data and regression from Fed-batch No.20
Levenspiel Model, no substrate term.
u 0,156 h⁻¹, Cp* 53,6 gl⁻¹, n 0,316

The ranges of the values of $\hat{\mu}$ presented in Table 6.1 above are compared with those available from literature in the following table. In the course of analysing the present data for the ethanol inhibition parameter K_P, the direct computer regression was supplemented by some work, discussed in Sec. 6.1.3, using the standard method of reciprocal plots. This also yields estimates of $\hat{\mu}$ and their

range has also been included in Table 6.3. Finally, the 200 gl^{-1} batch data of Converti et al (14) was analysed using the same methods as for this work and these results are incorporated as well.

Table 6.3. Values of Maximum Growth Rate Parameter Relative to Results by Previous Workers.

Worker, and model type	h^{-1}	Comparable values from this work
Egamberdiev & Ierusalimskii (8) Hyperbolic	0,36	Monod/hyperbolic : 0,4 - 0,75
Bazua & Wilke (10) Hyperbolic	0,448	
Hoppe (11) Monod/hyperbolic	0,64	Levenspiel/hyperbolic : 0,4 - 0,65
Converti et al (14) : (analysed by Glyn)		Reciprocal plots :
Levenspiel	0,224	0,35 - 0,49
Monod/hyperbolic	0,63	

The extreme values of 0,64 h^{-1} all arise from models using the Monod expression to account for the influence of substrate, and the preceding discussion applies. Agreement between the literature and other values in the table is satisfactory.

6.1.2. Substrate Saturation Constant K_s.

The saturation constant K_s is a measure of the sensitivity of the organism's growth rate to sugar concentration, a low value implying high sensitivity. Numerically it is equal to the substrate concentration required to attain a growth rate of half the maximum possible, all other influences being held constant.

Three of the models studied in this work have the saturation constant as one of the parameters, viz. Monod with hyperbolic ethanol inhibition term, and the Levenspiel and modified Levenspiel models. The values obtained are tabulated in **Table 6.4** below : they were extracted from Tables A5.1, A5.2 and A5.4 in Appendix 5.

Table 6.4. Values of Saturation Constant K_s.

Data	MODEL		
	Monod	Levenspiel	Modified Levenspiel
Combined :			
35°C Batches	89,1	100	100
30°C Batches	200	100	46,7
Converti et al (14) Batch	> 1000	--	--
Fed-batch :			
15	--	100	100
17	100	--	--
19	57	50	--
20	--	50	75
21	--	50	50

Two of the values are given to one decimal place. These are the only two instances where convergence could be achieved with this model during modelling. The remaining values are estimates obtained by manually adjusting the parameters until the match between the data and the plotted fit could not be visibly improved.

The range of values is between 50 and 100 gl^{-1}, only three results falling outside it. This contrasts with the 3,3 gl^{-1} found by Hoppe (11), while the magnitudes normally encountered in aerobic cultures, not only of yeasts but also of typical procaryotes, are of the order of 500 mgl^{-1}

and lower (10). At the other extreme, a very high value was required to get a reasonable fit to the data of Converti.

Several reasons can be advanced for these results. Agreement with aerobic results was not to be expected. There were several differences between the fermentations carried out by Hoppe and those of this work : he used lower substrate concentrations for most of his experiments, studied the continuous technique rather than batch or fed-batch fermentation and used no aerobic pre-fermentation. What is suggested by the present evidence, however, is not merely that K_s is dependent on the particular fermentation conditions but rather that the form of the function used to account for the reaction of growth rate to substrate concentration, viz.,

$$\frac{C_s}{(K_s + C_s)}$$

is unsuitable for ethanol fermentation by S.cerevisiae in regions of relatively high concentration such as were studied in this work. The form of the above function is such that its greatest influence is at low substrate concentrations : this is precisely when product strength and its repressive effect are highest. Growth rate is at its lowest and experimental uncertainty in it is consequently high. Conversely, at high substrate levels the K_s function is insensitive and approaches 1,0. Any of a wide range of values for K_s can be used without greatly altering the predicted growth rate. This insensitivity contributes largely to the difficulty in obtaining convergence during regression.

Previous workers, inter alia Hoppe (11), and Cysewski and Wilke (20), have found that ethanol exerts a much stronger influence on yeast growth rate than does the substrate. This can be demonstrated semi-quantitatively using one of the models together with parameter values

selected from the appropriate result table. Partial derivatives with respect to substrate and product concentrations of Levenspiel's equation (4.4) are, after simplification,

$$\frac{\partial}{\partial C_s}\mu = \frac{\mu K_s}{C_s(C_s + K_s)}$$

$$\frac{\partial}{\partial C_p}\mu = \frac{\mu n}{(C_p - C_p^*)}$$

Using parameter values from the appropriate Table (A5.2), it can easily be shown that the ratio of the second expression to the first is generally three or greater for alcohol and sugar levels of practical significance. The effect of the glucose level on growth tends to be obscured by that of ethanol and this probably accounts largely for the problems encountered during regression.

6.1.3. Ethanol Inhibition Parameter K_p.

The inhibition constant K_p is a measure of the effect of ethanol on growth and is numerically equal to the concentration of ethanol which halves growth rate at any given substrate level. The form of the inhibition function, viz.,

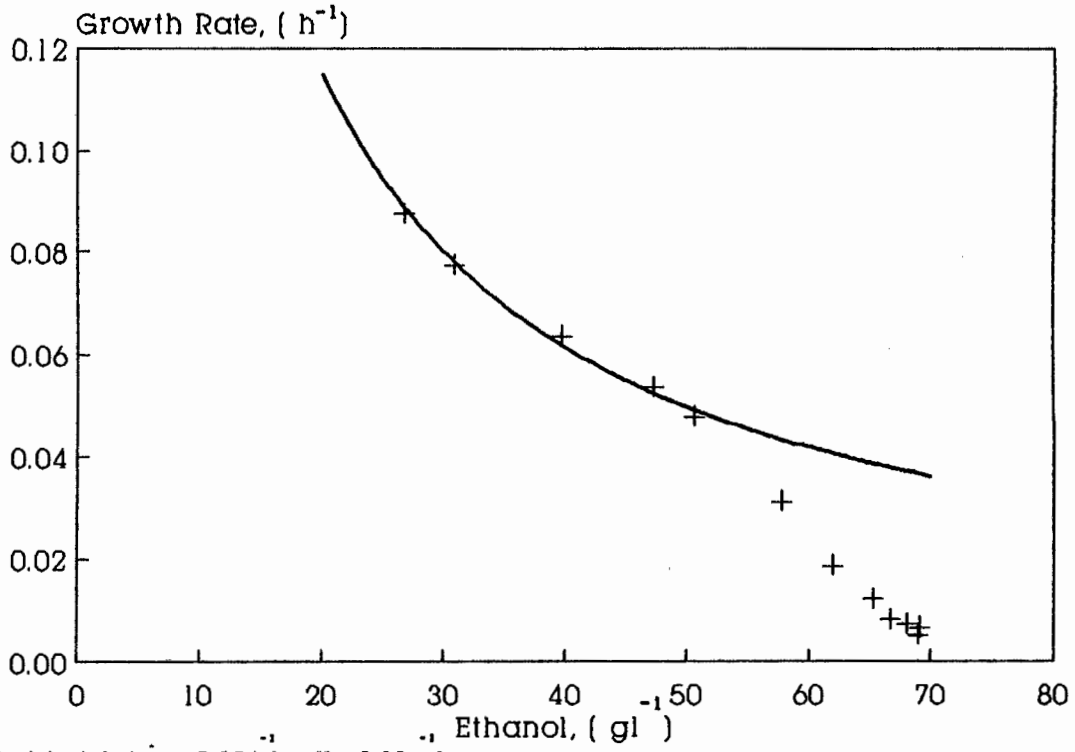
$$\frac{K_p}{(K_p + C_p)}$$

predicts a hyperbolic fall in cell reproduction with increasing alcohol concentration. It also requires, as Luong pointed out, that growth never cease entirely.

Fig. 6.2 illustrates typical growth rate data from one of the fed-batch experiments plotted against alcohol concentration. Also shown is the result of fitting to the data the equation

$$\mu = \frac{\hat{\mu} K_p}{(K_p + C_p)}$$

Fig.6.2. Hyperbolic Inhibition Function. Application to Fed-batch Data.



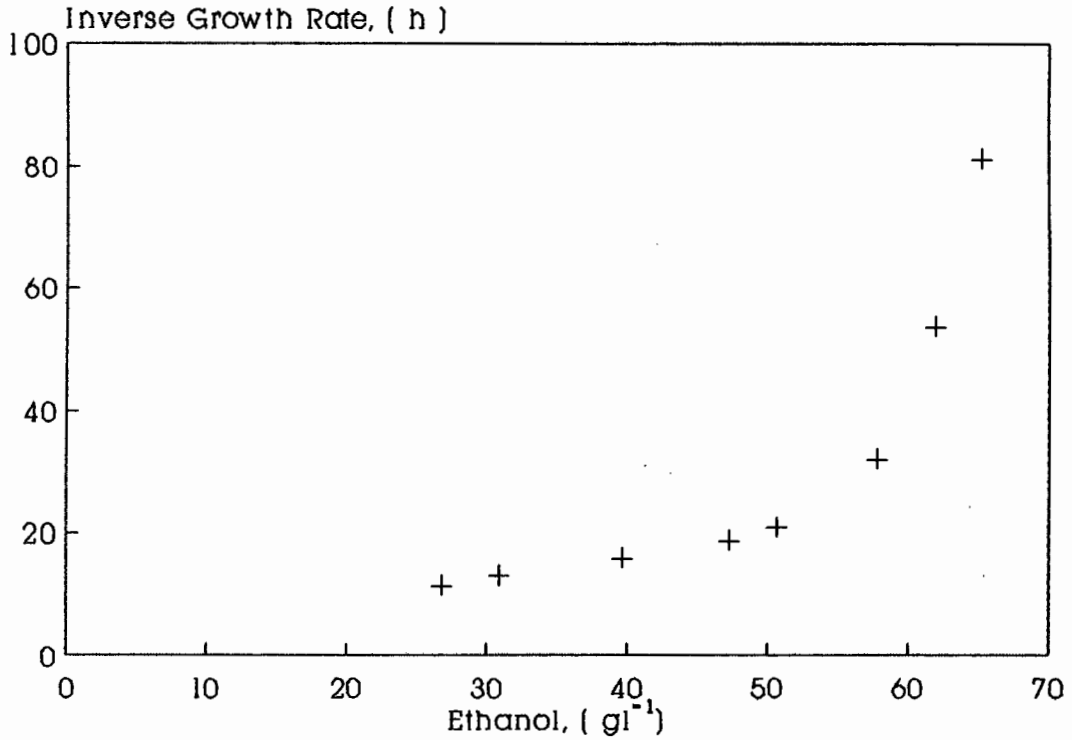
. Fed-batch 16. u 0,904 h^{-1}, Kp 2,92 gl^{-1}

as used by Egamberdiev and Ierusalimskii[8], using the plot of reciprocal growth rate against product concentration (**Fig.6.3**). As using all the points shown resulted in negative values for $\hat{\mu}$, only the first five were used for the line plotted in the diagram. Although the pattern formed by these five points does not suggest a hyperbola, the calculated line passes reasonably close to them. It fails completely, however, to describe the rest of the data. It decays too slowly in the high-concentration range and predicts unrealistically high values at the low end of the range.

It was consistently found that this form of function was incapable of describing the growth pattern encountered in this work, namely an initial rapid fall to very low rates

with increasing alcohol level followed by a gradual further decline. Fig.6.3, the reciprocal plot, provides further illustration.

Fig.6.3. Reciprocal Growth Rate against Ethanol Concentration.



. Fed-batch No.16.

Given the relative insensitivity of growth rate to substrate concentration in the ranges dealt with here, the data would be expected to fall roughly on a straight line were this function suitable for modelling the fermentations. In fact only growth rates at ethanol concentrations below about 55 gl^{-1} obey the pattern : at higher concentrations a strong upward tendency is evident. Hoppe (11) performed a similar analysis in his work and the same pattern is evident in his Fig.5.11.

Results using other fermentations in the fed-batch series were generally not even as good as in the above example. In most instances only the first three points - those with the

lowest alcohol levels - could be included, not only because of the lack of linearity but because the effect of including further data is a negative intercept and hence nonsensical results.

The unsuitability of the function was probably partly responsible for the failure of the regression algorithm in a large proportion of cases involving the Monod and Simplified models. Some success was achieved, however, and **Table 6.5** contains the results, together with values from reciprocal plots where such values could be obtained.

Table 6.5. Values of Ethanol Inhibition Constant K_P.

Data	K_P, gl^{-1}		
	Monod	Simplified	Reciprocal Plot
Batches :			
30°C	7	10,1	7,7
35°C	4,0	5,4	5,0
Fed-batch :			
16	-	-	2,9
17	57	57,2	-
19	35	35,7	6,5

The round figures are estimates obtained by trying different values until no further improvement could be achieved. In the other cases the regression converged successfully.

The outstanding feature of these results is that the inhibition constant is much higher for the fed-batch fermentations than for the batch data, which implies that inhibition is less severe in fed-batch fermentation. Seen from the point of view of the individual yeast cell, the fed-batch fermentations differed from the batch runs in two significant ways : glucose and ethanol concentrations were steadier while feed was running and the main fermentation

was preceded by an aerobic period. It is well established that S.cerevisiae requires a sterol and an unsaturated fatty acid for growth (27), and that its ethanol tolerance increases with the degree of unsaturation of both (6). Under anaerobic conditions these must be supplied from the medium, but if oxygen is present this yeast is capable of synthesising them (27,28,29). The rôle of cell membrane composition in promotion of ethanol tolerance is complex, and seems to involve effects on the transport of various compounds (6), but it seems reasonable to assume that in the case of these fermentations the higher value of K_p found in the fed-batch experiments is attributable to the aerobic pre-fermentation. It is unclear what effect, if any, the steadier concentrations would have on ethanol inhibition.

Another point of note in Table 6.5 is that values of ethanol inhibition constant derived from reciprocal plots are uniformly low. The 30°C batch value of 4,4 gl^{-1} was calculated from fifteen data points and is in fair agreement with the 5,2 gl^{-1} found by Hoppe for anaerobic conditions : the other values in the column used only four to six readings and one cannot place a great deal of confidence in them. Inhibition constants derived from full regression on fed-batch results are much higher than the batch values.

Hoppe also found that the introduction of a small amount of oxygen into the broth markedly improved the alcohol resistance of the yeast, an effect previously investigated by Cyzewski (21). This was reflected in an increase in K_p from 5,2 gl^{-1} to 16 gl^{-1}. Analysis of the 200 gl^{-1} batch data of Converti et al (14), on the other hand, gave an inhibition constant of 15,2 gl^{-1} under anaerobic conditions. Values found in the present work, 35 and 57 gl^{-1}, are much higher still, but quite possible in view of the prolonged and vigorous aeration of the pre-fermentation (at least eight hours at 40% saturation). Egamberdiev and Ierusalimskii (8) also

obtained higher values under aerobic conditions than under anaerobic, but the difference was much smaller - 22,2 and 20,6 gl^{-1} respectively. In their work, however, they added ethanol to the medium in both aerobic and anaerobic fermentations. This could tend to obscure any resistance conferred by aeration, particularly to internally generated ethanol. They were also using a different Saccharomyces strain.

6.1.4. Levenspiel Limiting Concentration Parameter C_p^*.
Models of this type use a two-parameter function to describe product inhibition. Two very similar functions were investigated in this work, viz.,

$$\left(1-\frac{C_p}{C_p^*}\right)^n \quad \text{and} \quad \left\{1-\left(\frac{C_p}{C_p^*}\right)^n\right\}$$

The first is that proposed by Levenspiel (12). The second suggested itself as being worth equal consideration. It was used by Luong in his study quoted in the Literature Survey (13). The practical differences between them will be discussed in the next section dealing with the exponent n : the rôle of C_p^* is the same in both.

The limiting concentration parameter C_p^* represents a product concentration sufficient to stop growth entirely. This is not a new idea : Rahn (1) applied it to substrate uptake rates in 1929 while Holzberg (2) applied it to growth rates twenty years ago. More recently Ghose and Tyagi (3) obtained relationships for growth rate and ethanol production rate containing this parameter. Amongst these workers, however, only Rahn was using data taken under fairly comparable conditions : the others were using relatively low substrate and product concentrations where they might reasonably expect success from the linear relationships they were applying.

In the present study it was found that growth in fed-batch fermentations continued to the end of the period, albeit slowly, no matter how high the ethanol concentration. Despite this the models using C_p^* were quite successfully fitted to both batch and fed-batch data from this work, the only difficulty experienced being with certain combined fed-batch data. These difficulties are attributed to the previously-mentioned (see Sec.5.4) poor conformance between different fed-batch runs in respect of growth rates at given product concentrations. In most instances it was necessary to exclude some of the data at the high end of the ethanol concentration range. Growth rates in this region were low and relative uncertainties accordingly high, but this did not cause their exclusion. Rather it was the mathematical requirement that ethanol concentrations be lower than C_p^*, as otherwise the expression

$$\left(1 - \frac{C_p}{C_p^*}\right)^n$$

in the Levenspiel models is undefined. The corresponding expression in the Modified Levenspiel models does not have this limitation but yields negative growth rates when product concentration exceeds C_p^*.

The success in fitting the Levenspiel-type models to these data contrasts with the difficulty in obtaining results with the functions used to describe ethanol inhibition in the more classical models. A two-parameter model can in general be expected to give a closer fit than a single-parameter expression, but the mathematical properties of the inhibition functions under discussion make them particularly well-suited. Specifically,

- for positive parameter values they are monotonal decreasing ;
- they can mimic asymptotic behaviour, but are not limited to it as is the hyperbolic expression ;

- o suitable choices of exponent cater for either concave or convex inhibition curves, that is to say, for large changes in growth rate at either low or high inhibitor concentrations ;

- o the linear inhibition models of Rahn, Holzberg and others are included as a special case with n = 1,0.

Table 6.6 summarises results from Tables A5.2 - A5.5, A5.7 and A5.8.

Table 6.6. Values of Limiting Ethanol Concentration Parameter Cp* [gl^{-1}].

	Standard	No term in C_e	Simplified
	Levenspiel Models		
Batch - 30°C	40	39,6	36,0
- 35°C	70	51,4	65,6
Fed-batch (average)	78	64,3	66,4
	Modified Levenspiel Models		
Batch - 30°C	34,3	30,6	34,4
- 35°C	37,0	36	39,3
Fed-batch (average)	69,0	71,5	75,8

Values given to the nearest unit denote instances where the regression algorithm did not converge and parameters were estimated by repeated trial. They are therefore somewhat less reliable than the rest of the values in the table.

Significant points arising from this table are that the limiting concentration is higher at 35°C than at 30°C, and is also greater for fed-batch fermentations than for batch, particularly in the case of the Modified Levenspiel models. That it should be higher at the higher temperature is surprising, since the inhibiting effect of ethanol has been shown to be greater at higher temperatures and the limiting

concentration would be expected to be a decreasing function of temperature. Navarro and Durand (4) found significantly lower viabilities and biomass yields in S.carlsbergensis after fermentation at 30°C than at lower temperatures.

The higher limiting concentrations found in the fed-batch fermentations are to be expected given the findings of the previous Section. There the differences between values of ethanol inhibition parameter in batch and fed-batch fermentations are attributed to the aerobic pre-fermentation enjoyed by the yeast in the variable-volume experiments. Another factor requiring mention is that substrate and particularly ethanol concentrations were generally and inherently more stable during fed fermentations than during batch runs. The need to avoid sudden environmental changes when cultivating micro-organisms is mentioned in texts on fermentation, but it is not clear whether the steady change in concentrations that takes place in batch fermentations is significantly less favourable than near-static conditions.

The range of limiting ethanol concentrations found in the literature is approximately 70 to 120 gl^{-1}. Rahn (1) and Holzberg (2) reported values of 10,2% and 6,85% respectively. Bazua and Wilke (3) estimated a limit of close to 93 gl^{-1}, while Levenspiel, applying his model to their data calculated it to be 87,5 gl^{-1} (12). More recently Luong (13) gave 112 gl^{-1}. Analysis of a batch fermentation by Converti et al using 200 gl^{-1} sucrose medium gave an approximate 90 gl^{-1}. The yeasts, media, concentrations and conditions used in these studies differed widely, but a fair assessment is that significant growth of S.cerevisiae stops near 70 gl^{-1} but perceptible growth continues until approximately 110 gl^{-1} has been attained. The results of the fed-batch experiments in this work agree with this but the batch data indicate a lower limit.

6.1.5. Levenspiel Exponent n.

Unlike the parameters discussed in the previous sections, the exponent in the Levenspiel models does not have a simple physical significance. It indicates rather where on the concentration scale growth is most sensitive to ethanol. For n near unity both variants reduce to the linear models proposed by Holzberg (2), Ghose and Tyagi (3) and others. In the original Levenspiel model a high value of n - above 3,0 - indicates that the yeast is resistant to ethanol until the concentration is appreciable, when growth rate begins to fall rapidly. As Luong (13) pointed out, such a pattern is qualitatively similar to Holzberg's combination of a threshold ethanol level below which inhibition is not significant and a linear fall in growth rate with increasing ethanol thereafter. A low exponent value - below 0,7 - characterises an organism that is sensitive to quite low product levels : as alcohol concentration increases from zero, growth initially drops sharply and then continues at a slow pace until the limiting concentration C_p^* is reached. In the modified version the converse applies, with a low n corresponding to an upwardly convex curve when growth rate is plotted against alcohol strength.

The other notable difference between the two Levenspiel functions is that while in the original version, viz.,

$$\left(1 - \frac{C_p}{C_p^*}\right)^n$$

concentrations greater than the limiting one are mathematically unacceptable, in the modified function such concentrations merely yield negative growth rates, thus allowing it in principle to describe death of cells from product poisoning.

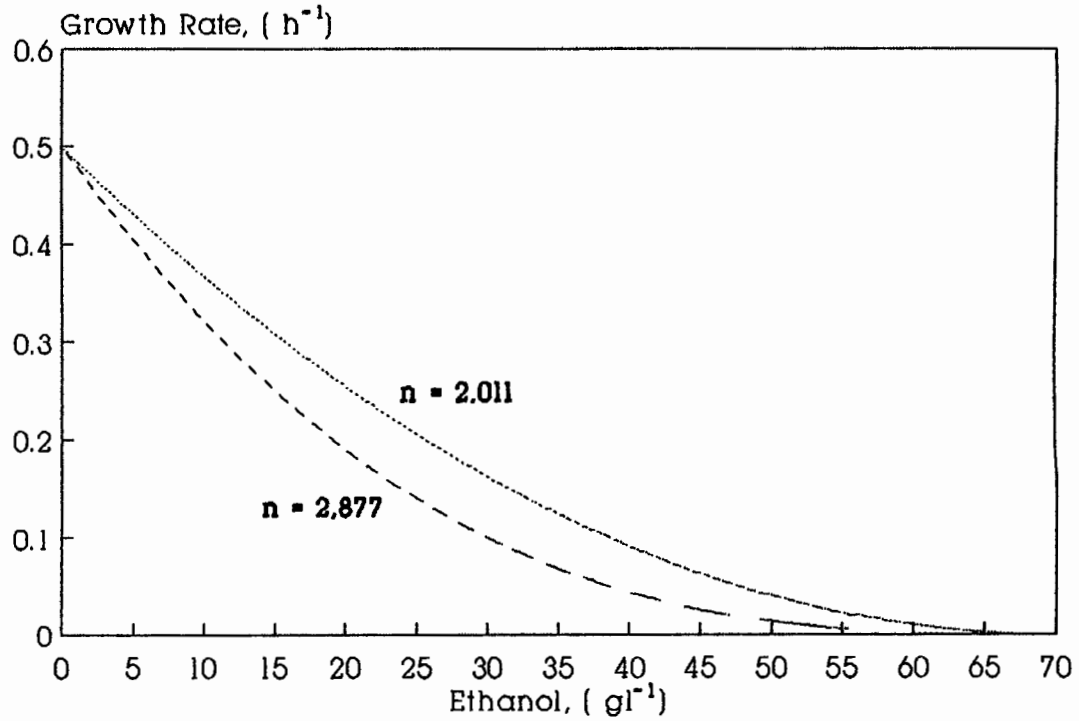
The values of n obtained in this work are presented in **Table 6.7** below, having been extracted from Tables A5.2 - A5.5 and A5.7 in Appendix 5.

Table 6.7. Experimental Values of Levenspiel Exponent n.

Data	Levenspiel			Modified Levenspiel		
	Std.	No C_s term	Simpl.	Std.	No C_s term	Simpl.
Batch :						
30°C	2,2	2,011	1,345	0,629	0,746	0,733
35°C	2,7	2,877	3,484	0,6	0,33	0,239
Fed-batch :						
14	-	-	0,880	-	-	1,213
15	0,7	-	0,97	2,0	0,596	2,457
16	-	0,834	-	-	1,637	-
17	-	0,591	-	-	5,441	-
19	0,4	0,600	-	-	2,649	-
20	1,0	0,316	0,288	9,0	-	-
21	2,2	-	-	0,5	-	1,861

Two points emerge from this table. Firstly, comparing batch data at 30 and 35°C, the parameter values for the unmodified Levenspiel models are all higher at the higher temperature. Conversely, for the modified types the exponent is lower at the higher temperature. This suggests a subtle increase in ethanol inhibition with increasing temperature : while the limiting concentration actually increased between 30°C and 35°C as was discussed in the preceding Section, a higher exponent in the Levenspiel function, or a lower one in the modified Levenspiel function, means a more rapid falloff in growth rate with increasing ethanol concentration. **Fig.6.4** illustrates this using plots of the Levenspiel function for the two exponent values obtained in the batch regressions. For the sake of clear comparison, equal and arbitrary values of $\hat{\mu}$ and Cp* were used for both curves.

Fig.6.4. Effect of Inhibition Pattern on Exponent Value.



. Levenspiel Model, no substrate term.
. u 0,5 h , Cp* 70 gl .

Secondly, whilst the average value of the exponent in batch data is about 2,4 for the normal versions and 0,5 for the modified models, in fed-batch data the corresponding values are about 0,65 and 2,3 respectively. This suggests that the yeast was more resistant to low product concentrations in the fed-batch fermentations than in the batch experiments, and once again points to the postulated improved resistance to ethanol brought about by the aerobic pre-fermentation. Experimental conditions, however, are also relevant : the batch fermentations covered the full range of ethanol concentrations from about 0,80 gl^{-1} to over 80 gl^{-1}, whereas growth rate measurements in fed-batch work started from a point where 20 - 50 gl^{-1} ethanol was already present. Fed-batch data therefore include no growth rates below this concentration range. Furthermore the dilution rates used and the product formation rates

attained were similar, and consequently the ethanol concentration tended to first rise to some level and then remain there until feed ran out. Fig. 5.2 in the Results section, showing the time-concentration profiles of one of the fed-batch experiments, illustrates this. The result was that many of the data points were clustered in a relatively narrow concentration band around this level. This uneven distribution of the data will tend to distort the fed-batch results, giving lower maximum growth rates than expected and lower exponent values in the unmodified Levenspiel model regressions. This could account for some of the observed difference.

Models of the form discussed in this section have only been applied relatively recently, hence values for the exponent in the literature are sparse. Levenspiel in his work on the data of Bazua and Wilke obtained a value of 0,41 (10), while the linear models imply n = 1,00. The fed-batch results in this work fall between these values except for the last two fermentations. Results from the batch experiments cover a range of 2,0 to 3,5. This includes an estimate of 3,0 for the data of Converti and his co-workers (14).

For the modified Levenspiel model, Luong (13), using the same yeast and medium as Bazua and Wilke, obtained an exponent of 1,41. Again the implicit value of 1,00 in the linear models is not to be overlooked. The range from fed-batch runs in the present work is 0,6 to 5,4 excluding extreme results estimated manually. The batch work gave less scattered values but still covered a range of 0,24 to 0,73.

6.1.6. Growth Rate Constant B (Simplified models).

Like the exponent in Levenspiel models, the constant B in the Simplified models lacks the simple physical significance of the other parameters discussed in this work. It is in the nature of a proportionality constant between growth rate and substrate concentration, with units

[$lg^{-1}h^{-1}$] : in view of the way it was derived from the Monod model, B should be comparable to the ratio $\hat{\mu}/K_m$, where $\hat{\mu}$ and K_m are obtained from fitting the same data to the appropriate model containing the classical Monod substrate/growth term.

Table 6.8 contains values of B collected from Appendix 5, together with corresponding values of $\hat{\mu}/K_m$ obtained from the appropriate model.

It can be seen that although B is of the same order of magnitude as $\hat{\mu}/K_m$, the two numbers are not the same. The main source of the discrepancies is probably to be found in uncertainty in the saturation constant K_m. As has been discussed in Section 6.1.2, calculated growth rate is insensitive to the value of K_m, and many of the values used in computing K_m in Table 6.9 are estimates which could be varied considerably without greatly influencing the quality of the fit. Indeed it may well be that better estimates of K_m are to be obtained by indirect calculation from B and $\hat{\mu}$.

Table 6.8. Values of Simplified Parameter B [$lg^{-1}h^{-1}$].

Data	Monod		Levenspiel		Modif. Levenspiel	
	$10^{3}B$	$10^{3}\hat{\mu}/K_m$	$10^{3}B$	$10^{3}\hat{\mu}/K_m$	$10^{3}B$	$10^{3}\hat{\mu}/K_m$
Batch :						
30°C	1,78	3,5	1,64	5	1,72	8,4
35°C	2,51	8,4	1,84	4	3,60	5
Fed-batch :	-	-	4,19	4	2,57	4
15	4,14	5,5	-	-	-	-
17	5,48	8,8	-	-	-	-
19	-	-	3,28	8	-	-
20	-	-	-	-	2,4	12,8
21						

6.1.7. Growth Model of Choice.

On the basis of closeness of fit and similarity between characteristics of the inhibition function and of the data, the most satisfactory model for the present data is the Levenspiel model without the substrate-dependent term. From the considerable variety of values calculated the following are probably most representative :

$\hat{\mu}$	$0,17\ h^{-1}$
C_p^*	$64,3\ gl^{-1}$
n	0,6

Although the influence of substrate on growth rate is not negligible, it is less significant than that of alcohol and is partly taken up in the values of the other parameters. Hence this simple equation should frequently be adequate in practical applications. In circumstances where the separate effect of the substrate cannot be ignored, such as when sugar concentrations are low, the full Levenspiel expression as proposed in his paper (12) may be applicable, suitable values being :

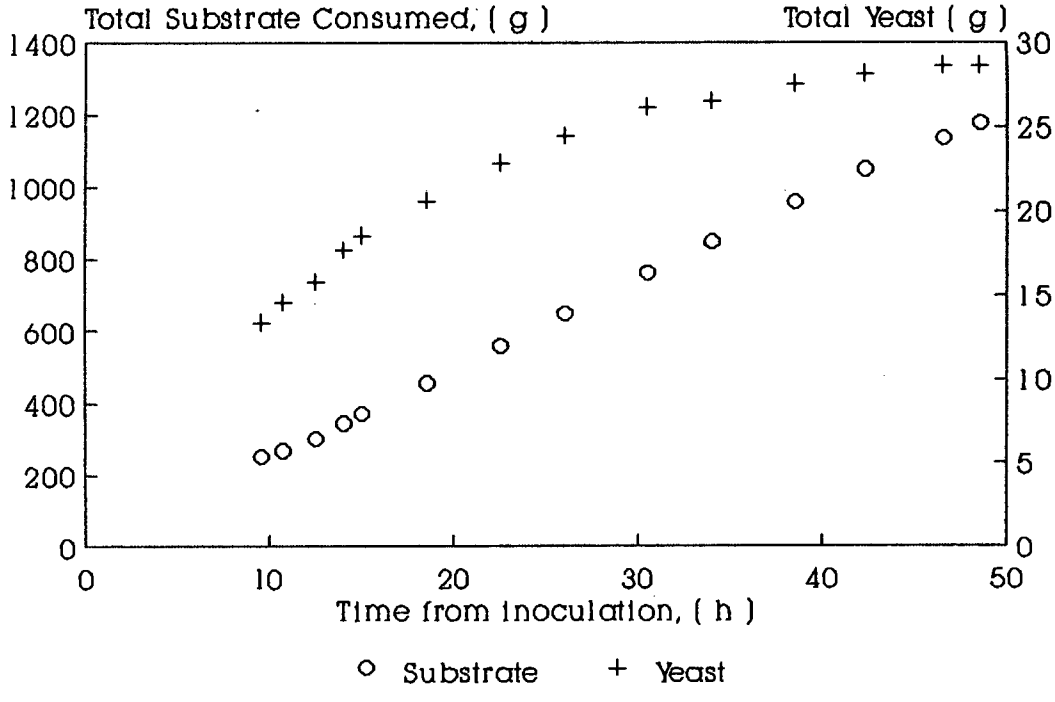
$\hat{\mu}$	$0,45\ h^{-1}$
C_p^*	$64,3\ gl^{-1}$
n	0,7
K_m	$60\ gl^{-1}$

It was stated earlier that low but perceptible growth continued even after exhaustion of feed, when alcohol levels exceeded the above implicit limit. In many instances this will not be of significance but the fact must be considered.

6.2. Stagnation of Metabolic Rate in Fed-batch Fermentation.

It was stated earlier (Sec.5.5) that the substrate uptake rate in fed-batch experiments attained a constant value soon after the start of feeding and remained at that level even though the total mass of yeast present in the broth continued to increase. In this Section possible reasons for this behaviour and its practical applications are explored.

Fig.6.5. Time Course of Substrate Consumption.



. Fed-batch No.16.

6.2.1. Description.

Fig 6.5 is a plot of total substrate consumption as a function of time. From approximately 15 hours elapsed time the increase is linear, indicating that the rate of uptake is steady from then. Also plotted is total biomass, which increases considerably during the fermentation and is still increasing, albeit slowly, at the end, showing that growth

has not stopped. Specific uptake rate (not shown) falls in a roughly hyperbolic curve. This was typical of all the fed-batch runs. The time of onset of the condition varied from immediately on commencement of the fed-batch phase in the case of long pre-fermentations (in excess of 10 hours) to as much as 13 hours after commencement in the case of the last and most successful experiment.

6.2.2. Causative Conditions and Mechanisms.

Neither the conditions which lead to stagnation of the metabolic rate nor the mechanism by which it occurs are clear. No references to such behaviour could be traced in the literature researched for this work, which included the Engineering Index from 1970 to 1987. The following points can be made, however :

- The change, when it occurs, seems to be quick and complete. This contrasts with the gradual levelling-off that would be expected in the case of competitive or non-competitive inhibition.

- No clear correlation could be found between the onset of the condition and any of a number of variables that were investigated, including average glucose and ethanol concentrations during the stagnant period and beforehand, intensity of aeration and inoculum size, but several possibilities exist that merit further investigation. A correlation coefficient of 0,4 was obtained between the time from start of feed to onset and the average sugar level during the same period. This test was prompted by the impression that higher glucose concentrations postponed onset. Another observation was that the average ethanol concentration at the transition lay between 38,5 and 51,5 gl^{-1} for all fermentations with the exception of FB18, which by the end of an exceptionally long pre-fermentation of twice the normal medium strength had attained 55,6 gl^{-1} and had already entered the static condition.

o It is implicit in the work of many earlier investigators, inter alia Ghose and Tyagi (3), and Luong (13), that inhibition by ethanol of growth and of glycolysis have separate mechanisms. The present observation confirms this but suggests a different pattern. The statement by previous workers is that there are limiting concentrations above which growth and glycolysis are completely inhibited and that the limit for glycolysis is higher than that for growth, that is, ethanol production continues beyond the cessation of growth as the alcohol strength increases. What is suggested by the results of the present work is that production of glycolytic capacity is inhibited more severely than either glycolysis itself or growth, being completely curtailed under conditions where growth is still continuing.

One possible mechanism for the effect is inhibition of the synthesis of rate-controlling glycolytic enzyme or substrate-transport systems. If this is accepted it is natural to look for a link between ethanol concentration and the onset of the constant metabolic rate period. The concentration range of 13 gl^{-1} quoted above is too wide to draw definite conclusions. The time of onset was, however, determined only by visual inspection as being at the earliest data point to be included in the regression, and it may be possible to identify a threshold alcohol concentration through further appropriately designed experiments.

Another possibility which must be considered is that continued synthesis of glycolytic or transport capacity is counter-balanced by increasing inhibition such that within experimental error the overall rate measured is unchanged. If this is the case it should be possible to find some dependence between alcohol concentration and specific substrate uptake rate. In fact during the central part of the fermentations beginning some hours after start of feed and continuing to feed exhaustion the ethanol concentration

varies relatively little, while the specific substrate uptake rate falls considerably. The possibility nevertheless cannot be excluded.

6.2.3. Exploitation for Modelling Purposes.

The concept of an culture growing under the condition of static total metabolic rate can be used to construct a potentially useful growth model. Starting from Eq. 4.15 :

$$q_s = \frac{\mu}{Y_{xs}} + m \qquad \ldots\ldots (4.15)$$

and substituting definitions for q_{ss} and μ in terms of total quantities :

$$q_s = \frac{1}{X}\frac{d}{dt}S_{cons} = \frac{1}{X}Q_{ss} \quad : \quad \mu = \frac{1}{X}\frac{d}{dt}X$$

where S_{cons} is total substrate consumed [g],

Q_{ss} is the steady-state substrate metabolic rate [gh^{-1}],

one can obtain

$$\frac{d}{dt}X = Y_{xs}(Q_{ss} - mX) \qquad \ldots\ldots (6.1)$$

If Y_{xs} and m are constant then this can be integrated to give

$$X = \frac{1}{m}\{Q_{ss} - (Q_{ss} - mX_0)\exp(-mY_{xs}t)\} \qquad \ldots\ldots (6.2)$$

where X_0 is the total biomass present at time t = 0.

This equation states that the organism will continue to grow ever more slowly, asymptotically approaching the state where all the energy derived from substrate metabolism is devoted to maintaining the existing biomass and

$$X = \frac{Q_{ss}}{m} \qquad \ldots\ldots (6.3)$$

The approach of the fed-batch fermentations of this study to this ultimate condition was assessed. Dividing Eq.6.2 by Eq.6.3 gives

$$\frac{X}{X_{max}} = 1 - \left(1 - \frac{X_0}{X_{max}}\right)\exp(-mY_{xt}t) \qquad \ldots\ldots (6.4)$$

The data were drawn from Appendix 6 : the refined values of maintenance and yield factor were selected. Fermentations in which the maintenance coefficient was high and the steady substrate period long were estimated to have attained 90 - 97 % of the maximum biomass possible. Fed-batches 20 and 21, in which the average glucose levels were deliberately kept relatively high, had a markedly higher total consumption rate than the other fermentations and were calculated to have attained only 77 and 78 % of their potential maximum biomass.

The application of this model to checking the consistency of yield and maintenance calculations has been described in Section 5. It is clear, however, that it will require considerable refinement before it can find widespread use. Specifically, in any practical application it will be necessary to be able to predict both the onset of a constant rate period and the rate itself, but the conditions giving rise to it have yet to be established. Other media, organisms and methods of operation will need to be tried to determine whether its occurrence is widespread and reproducible. Application to continuous fermentation processes would necessarily be limited, although it could serve to indicate operational bounds.

In nature this model is mechanistic rather than empirical, based on the concepts of constant true biomass yield and maintenance coefficients, together with the static metabolic rate condition.

6.3. Other Observations.

6.3.1. Effect of Temperature.

All fed-batch fermentations were run under the same temperature conditions, and although the pre-fermentations were carried out at 30°C and the main fermentations at 35°C, these periods cannot be compared because only the pre- fermentation was aerobic. Hence a comparison can only be made between batch runs at the two temperatures.

Table 6.9. Comparison of Batch Growth Rates at 30 and 35°C.

Batch Nos.	Total points	Ave. conc. gl^{-1}		Ave growth rate, h^{-1}
		Glucose	Ethanol	
5,6,8,9,11 (30°C)	33	136,2	20,9	0,107
4,7,10 (35°C)	23	131,6	27,2	0,100

Table 6.9 gives the average growth rates for the 30 and 35°C batch runs together with the respective average glucose and ethanol concentrations and the overall figures. Average growth rate at 30°C is actually marginally higher than at 35°C, although the difference is so small that it could easily be attributed to the slightly higher substrate and lower product levels of the 35°C fermentations. It cannot be concluded that temperature had any significant effect in these fermentations.

6.3.2. Biomass Yields.

Table 6.10 summarises the results given in Tables A6.2 and A6.3 on true biomass yields and maintenance coefficients, together with values obtained from the data of other researchers working at sugar concentrations comparable with those used in this work. The data of Converti (14) was analysed using the methods described in Section 4, while the other entries are from Hoppe's analysis of his own and of others' work (11).

Table 6.10. Average True Biomass Yields for Batch and Fed-batch Fermentations.

	True Yield gg^{-1}	Maintenance $gg^{-1}h^{-1}$
BATCH :		
30°C	0,168	0,853
35°C	0,210	0,581
Weighted mean	0,194	0,682
FED-BATCH :		
Aerobic	0,325	0,868
Anaerobic	0,082	0,731
Pironti	0,089	0,33
	0,091	0,39
Cysewski	0,093	0,80
Hoppe	0,094	1,30
	0,104	1,68
Converti	0,122	0,400

Cell yield from the fed-batch work corresponds acceptably with other authors. It is notable that the aerobic pre-fermentation does not seem to have increased yield during the main fermentation, which in fact registers the lowest yield in the table. The yield from the aerobic period, on the other hand is the highest and compensates for the lower subsequent yields. The total quantity of sugar in variable-volume experiments was for practical

purposes identical to that in fixed-volume runs and generally the total mass of yeast at the end was the same as well.

The maintenance coefficients obtained are in agreement with the value reported from Cysewski's work, falling between the 0,4 $gg^{-1}h^{-1}$ of Pironti and Cysewski and the range 1,3 to 1,7 $gg^{-1}h^{-1}$ of Hoppe. Slightly surprising is the value for aerobic conditions. This might reasonably be expected to be lower than for anaerobiosis but in fact it is not substantially different from the other values in this work.

The table also indicates that in batch fermentations yield is higher and maintenance lower at 35°C than at 30°C. This contrasts with the experience of Navarro and Durand (4) who found that biomass yields decreased with increasing temperature. Differences between the studies included the organisms and the temperature ranges : they were using S.carlsbergensis and did not venture above 30°C.

6.3.3. Effect of Aerobic/Anaerobic Transition on Substrate Uptake Rate.

In early fed-batch fermentations the rate of disappearance of the substrate during the last hours of the aerobic period was used as a guide in selecting suitable initial feed rates for the anaerobic portion. It was repeatedly found that the settings so obtained were far too low to maintain the desired substrate levels, suggesting that the uptake rate increased upon transition from aerobic to anaerobic conditions. Just such an increase is observed when a culture growing under fully aerobic, non-fermentative conditions is deprived of further oxygen, this phenomenon being termed the Pasteur Effect, but there was no cogent reason why this should occur under the very different conditions of these experiments. Estimates of specific substrate uptake rate immediately after the switchover were compared with the average rate immediately beforehand. The comparison, tabulated below,

confirms the impression gained during the experimental work, only one fermentation showing a lower value after the change.

Table 6.11. Metabolic Rates Before and After Transition to Anaerobic Conditions [$gg^{-1}h^{-1}$].

	End of aerobic period	Start of anaerobic period
FB15		1,48
FB16		1,83
FB19	1,63	2,58
FB20	(average)	3,08
FB21		2,49

The rates given in the Table are approximate only.

6.3.4. Variation in Light Absorption by S.cerevisiae.

Biomass concentrations were measured generally at two and sometimes at three concentrations. Usually the different values agreed within 1%, but there were periods during fed-batch fermentations when much larger discrepancies - up to 15% - appeared in each of several consecutive readings. These periods generally began towards the end of the pre-fermentation and persisted for six to nine hours.

No reference to such behaviour could be traced in the publications on biotechnology surveyed, which included those in the Engineering Index from 1970 onwards. It is suggested that this phenomenon is due to variation in the absorption characteristics of the yeast with growth conditions. The correlation used in this study was necessarily established using yeast that was not actively growing : the sample was drawn from the broth of a fermentation that had just been completed, containing generally 80 gl^{-1} ethanol and negligible glucose. If absorption of monochromatic light by the cells is due

primarily to certain cell components rather than the organism as a whole, then when the concentration of these substances varies as a result of environmental changes a shift in the calibration curve is to be expected. Identification of the components reponsible for absorption would constitute an interesting and useful study but this was beyond the scope of the work. To be noted in passing is that it is mathematically possible to determine the values of the constants for a polynomial or other correlation which would apply over the troublesome periods encountered in this work, using the readings obtained then. In addition to these readings there would be required one reliable reading of the absorption at a known true biomass concentration.

6.4. Selection and Application of Growth Models.

It was concluded (Sec.6.1.7) at the end of the discussion of the modelling work that the Levenspiel model with or without a substrate-dependent term was the most suitable choice for the data of this study. It is evident, however, from the discussion of the model parameters that the selection of the most suitable system of relations to describe the behaviour of a micro-organism is dependent not only on which one provides the best fit statistically but also on the objective. If the intention is to mimic in detail the response of the cell to its environment, one of the mechanistic models such as those developed by Bijkerk and his co-workers (17) will be chosen in preference to any of those considered here. The mechanistic systems are, however, necessarily complex and although they are probably the systems of the future, the improvement in predictive accuracy obtainable at present will not normally warrant the extra computational effort and attendant cost involved. For a particular design problem the range of conditions likely to be encountered is usually narrow enough to allow the use of the simpler types of model such as have been considered here.

Central to empirical models is the assumed relation between substrate and product concentrations and growth rate. The experience of this study has illustrated the importance of careful experimental design to ensure that the distribution of the data along the concentration axes is suitable before the fermentations which are to provide quantitative results are carried out. If in a particular case the Levenspiel model is under consideration it will be necessary to ensure that sufficient readings at low product concentrations are taken in order to ensure a reliable result. The mode of operation used in the present case, while not a failure, was not ideal from this point of view. If on the other hand the intention is to operate in a region of slow growth, the chosen model must have parameters sensitive to low growth rates, as the volume of a large fermenter may depend on it.

The growth rate relation alone is not sufficient to simulate fermentations. Also required are expressions for substrate consumption and product formation. This may take the form of yield coefficients or specific uptake and excretion rate functions. Ethanol formation by yeast has traditionally been regarded as an instance of growth-related product formation, when two yield coefficients suffice to complete the model. As previous workers have recognised, this does not hold true under all circumstances, but the introduction of an additional parameter in the form of a maintenance coefficient accounts for apparent variation in the biomass yield factor. This is supported by the observation that the straight-line regressions required to calculate the maintenance and true biomass yield factors represented the data well.

In the context of simulation of fermentations using mathematical models, the mathematical formulation can also be important when computer packages based on Runge-Kutta or other numerical integration techniques are being used. In some early work done for this project, appreciably

different results were obtained when two mathematically identical formulations of the same model were run using such a program.

7. CONCLUSIONS.

The principal conclusions from this work are as follows :

7.1. Modelling of Ethanol Fermentation.

The expression proposed by Levenspiel to represent inhibition by ethanol of S.cerevisiae growth was the most successful of those applied to fermentation of a semi-defined, 20% glucose medium. This is attributed to the greater flexibility afforded by having a second parameter and to the inherent ability of the expression to match the particular relationship between growth rate and ethanol concentration. A hyperbolic expression previously proposed did not have this ability. The classical Monod expression was similarly unsuccessful in describing the influence of high substrate concentrations. The best estimates of the parameters in the Levenspiel model were :

$\hat{\mu}$	0,17 h^{-1}
C_p^*	64,3 gl^{-1}
n	0,6

Growth did not entirely cease at ethanol concentrations above the limiting value of 64,3 gl^{-1}.

7.2. Advantages and Disadvantages of the Fed-batch Technique.

In one series of fermentations a fed-batch technique was used whereby the aerobic fermentation of a volume of medium of moderate substrate concentration was followed by the feeding of a further three volumes of a 20% glucose medium under anaerobic conditions. This method gave better reactor productivities than batch fermentations using the identical overall sugar levels, higher biomass yields being

obtained without appreciably lower ethanol yields. For the purposes of modelling ethanol fermentation the method suffers from two drawbacks : the alcohol strength may be too high at the start of the anaerobic phase for reliable estimation of certain parameters, and growth rates must be calculated from measurements of yeast concentration and broth volume instead of being taken equal to the dilution rate as in continuous fermentations.

7.3. Stagnation of Substrate Uptake Rate.

It was found in all fed-batch experiments that after a period of up to 13 hours of fed anaerobic culture, the total rate of substrate metabolism of the broth reached a constant value, even though growth continued. This observation, in combination with the standard concepts of a constant true biomass yield and a maintenance coefficient, allows the formulation of a quasi-mechanistic model. Realisation of its potential for practical application requires that the conditions which bring about the state of constant substrate uptake be identified and the rate itself be quantitatively predictable. The phenomenon is attributed to inhibition by ethanol but the mechanism is unclear.

7.4. Variation in Absorption of Light by S.cerevisiae.

Discrepancies of up to 15% between determinations of yeast concentration carried out on the same sample at different dilutions are attributed to variations in the absorption characteristics of the organism for 580 nm light. The source and precise cause of these variations is unknown.

7.5. Effect of Air on Substrate Consumption Rate.

The observation that substrate uptake rate increased when the supply of air to the culture was discontinued at the start of the anaerobic phase of fed-batch fermentations, is similar to observations of the Pasteur Effect in aerobic, non-fermentative cultures.

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Appendix 1. BATCH FERMENTATION DATA.

Table A1.1. Quantities used in batch fermentations.

Batch	Medium		Inoculum		Ammonia	Final Beer	
Nº	Mass g	Glucose gl^{-1}	Mass g	Yeast gl^{-1}	Mass g	Mass g	Ethanol gl^{-1}
4	6546	177	130,6	10,28	206,6	6334	80,3
5	6327	188	132,4	9,65	165,0	5742	84,2
6	6219	197	145,8	9,45	205,6	5852	88,6
7	5992	229	127,9	9,84	197,1	5523	101,7
8	6676	190	144,0	7,06	137,6	6292	83,5
9	6725	176	122,8	9,96	142,2	6343	78,5
10	6672	187	131,9	9,74	197,8	6320	83,3
11	6554	202	134,8	9,03	157,7	6144	90,4

Table A1.2. BATCH B4. 35°C.

Elapsed time	Concentrations gl^{-1}		
h	Yeast	Glucose	Ethanol
0,0	0,214	174	0,82
8,5	2,34	143	12,9
10,5	3,06	132	18,3
12,5	3,55	121	22,9
15,0	3,78	105	29,0
17,0	4,11	99,0	33,1
24,0	4,40	66,2	47,6
27,5	4,42	52,0	54,6
31,0	4,50	38,9	61,6
33,2	4,53	31,5	65,1
35,8	4,57	22,6	68,8
40,0	4,60	12,2	75,4
44,0	4,68	3,35	79,1

Table A1.3. BATCH B5. 30°C.

Elapsed time	Concentrations gl^{-1}		
h	Yeast	Glucose	Ethanol
0,0	0,214	184	0,88
5,0	0,62	183	3,5
11,0	3,10	150	15,4
14,0	4,26	133	23,2
17,0	4,93	120	29,4
20,0	5,24	103	36,4
23,3	5,56	87,3	43,2
26,5	5,67	75,0	49,3
30,0	5,72	60,8	55,5
33,5	5,71	47,5	62,3
44,8	5,84	13,4	78,6
48,5	5,86	6,06	82,3
50,5	(5,87)	2,82	84,2

Table A1.4. BATCH B6. 30°C.

Elapsed time h	Concentrations gl^{-1}		
	Yeast	Glucose	Ethanol
0,0	0,237	188	1,0
8,5	1,86	171	9,8
12,0	3,40	154	18,0
14,5	4,13	140	23,8
19,0	4,73	118	32,5
23,0	5,04	105	39,8
26,5	5,00	86,2	45,7
30,0	5,00	75,8	51,1
33,0	5,06	66,0	55,4
36,5	5,09	54,5	60,7
40,5	5,13	41,6	66,5
44,0	5,10	32,5	71,4
48,0	5,18	22,2	76,3
59,0	5,22	2,4	85,7

Table A1.5. BATCH B7. 35°C.

Elapsed time	Concentrations gl^{-1}		
h	Yeast	Glucose	Ethanol
0,0	0,225	223	0,91
9,25	2,26	190	12,9
12,0	3,15	174	19,9
15,0	3,62	155	26,6
19,0	4,02	135	34,6
24,0	4,45	116	44,2
28,0	4,41	101	51,3
31,0	4,49	88,8	56,5
34,7	4,63	80,5	63,0
38,1	-	-	68,2
43,25	4,62	50,5	75,9
46,0	4,60	43,2	79,3
50,5	4,59	32,1	84,6
61,0	4,26	10,6	96,2
65,5	-	3,99	99,2

Table A1.6. BATCH B8. 30°C.

Elapsed time	Concentrations gl^{-1}		
h	Yeast	Glucose	Ethanol
0,0	0,161	186	0,65
8,0	1,62		7,8
11,5	3,01		15,5
15,5	3,96	125	23,6
18,6	4,26		29,8
22,5	4,48	96,5	36,9
27,0	4,60		44,2
31,5	4,69	64,5	51,9
36,0	4,74	48,8	58,7
40,0	4,73	38,0	64,1
44,0	4,85	28,5	69,6
54,5	4,84	7,92	79,9
58,5	(4,85)	2,70	83,5

Table A1.7. BATCH B9. 30°C.

Elapsed time	Concentrations gl^{-1}		
h	Yeast	Glucose	Ethanol
0,0	0,201	173	0,77
7,6	1,43	152	7,0
11,2	(2,7)	134	14,7
14,5	3,62	116	21,0
18,0	4,01	100	27,6
22,0	4,12	(86,5)	34,3
26,0	4,36	76,8	41,1
30,0	4,28	62,5	47,5
34,0	4,43	49,8	53,4
38,2	4,59	39,4	59,8
42,75	4,54	26,5	65,0
48,5	4,56	13,4	72,0
(54,5	4,56)	0,0	78,5

Table A1.8. BATCH B10. 35°C.

Elapsed time	Concentrations gl^{-1}		
h	Yeast	Glucose	Ethanol
0,0	0,204	183	0,83
7,0	1,60	163	8,4
10,0	2,63	147	15,2
13,0	3,25	130	21,7
17,0	3,66	112	29,5
20,0	3,76	101	34,9
24,0	3,98	86,5	41,6
29,5	3,96	63,8	51,5
33,5	4,06	51,0	57,9
38,7	(4,08)	37,5	64,0
42,0	4,10	26,0	69,6
49,0	4,16	10,75	77,4
(54,5)	4,2	0,0	83,3

Table A1.9. BATCH B11. 30°C.

Elapsed time	Concentrations gl^{-1}		
h	Yeast	Glucose	Ethanol
0,0	0,197	198	0,87
8,6	1,57	177	8,2
11,5	2,78	162	14,5
13,5	3,37	152	19,0
17,0	4,01	135	26,2
21,0	4,28	121	33,6
26,0	4,57	101	42,4
30,5	4,64	84,2	50,1
34,8	4,80	70,1	57,0
38,5	4,79	59,0	62,9
41,5	4,78	51,2	66,5
46,0	4,79	39,1	72,0
50,5	4,76	29,2	76,9
55,0	4,76	20,4	81,8

Appendix 2. FED-BATCH FERMENTATION DATA.

Table A2.1. Quantities used in Fed-batch Fermentations.

Fed-batch Nº	Pre-ferm. Medium		Feed Medium		Inoculum		Ammonia	Final Beer	
	Mass g	Glucose gl^{-1}	Mass g	Glucose gl^{-1}	Mass g	Yeast gl^{-1}	Mass g	Mass g	Ethanol gl^{-1}
13	1498	108	5128	188	141,3	5,34	137,7	6251	77,5
14	1514	159	5129	204	141,5	4,69	132,0	6226	86,9
15	1492	110	4994	197	127,9	10,13	192,6	6131	78,1
16	1487	106	5081	193	134,6	9,15	189,8	6180	80,2
17	1518	162	5031	198	135,6	10,23	184,2	6128	85,9
18	1579	213	5059	213	129,8	9,85	204,9	6205	88,1
19	1439	111	5080	188	126,6	9,88	136,4	6121	80,6
20	1508	111	5075	200	140,1	10,71	185,6	6279	82,2
21	1474	113	5131	201	145,4	10,90	199,4	6244	77,3

Table A2.2. FB13.

Elapsed time h	Concentrations gl^{-1}		
	Yeast	Glucose	Ethanol
0,0	0,50	100,2	3,5
10,0	7,33	48,0	20,3
11,5	8,45	31,8	25,5
12,5	9,19	21,9	29,0
"	Broth volume : 1,577 l.		
	Feed started : 0,0963 lh^{-1}		
13,5	9,16	22,0	31,0
14,5	Feed rate increased to 0,146 lh^{-1}		
15,0	8,48	23,3	35,3
19,0	8,48	21,1	46,7
22,0	8,48	17,4	53,4
23,0	Feed rate increased to 0,199 lh^{-1}		
27,0	7,71	21,8	58,4
30,0	7,13	24,0	60,5
38,0	5,73	26,8	63,7
39,4	5,55	26,9	64,5
"	End of feed.		
41,0	5,64	19,6	68,1
45,5	5,77	2,72	76,1

Table A2.3. FB14.

Elapsed time	Concentrations gl^{-1}		
h	Yeast	Glucose	Ethanol
0,0	0,44	148	3,25
12,5	9,16	77,0	26,3
14,0	10,57	54,5	32,5
16,0	12,34	28,5	41,1
"	Broth volume: 1,576 l.		
	Feed started : 0,102 lh^{-1}		
18,0	12,29	22,0	47,9
19,0	Feed rate increased to 0,151 lh^{-1}		
22,0	9,83	20,3	57,2
23,0	9,71	20,9	59,2
24,0	Feed rate increased to 0,190 lh^{-1}		
25,0	8,56	25,0	60,6
27,0	7,86	29,5	61,3
30,0	6,89	35,0	63,0
42,8	End of feed.		
43,8	4,77	37,4	68,3
46,0	4,70	27,8	71,7
48,0	4,73	19,7	76,0
51,0	4,76	8,95	82,0
(53,8)	4,92	0,0	86,9

Table A2.4. FB15.

Elapsed time	Concentrations gl^{-1}		
h	Yeast	Glucose	Ethanol
0,0	0,835	100,9	3,30
3,0	1,82	91,9	7,18
6,0	5,19	66,4	15,4
9,0	8,3	34,5	26,6
11,0	10,12	13,16	33,6
"	Broth volume: 1,550 l.		
	Feed started : 0,108 lh^{-1}		
12,0	9,6	17,1	35,0
14,0	9,0	18,4	40,6
17,0	8,76	13,6	49,7
25,0	7,53	8,80	63,0
25,25	Feed rate increased to 0,138 lh^{-1}		
29,0	6,59	12,45	64,7
34,7	5,82	17,6	65,5
39,0	5,29	19,16	67,2
47,0	End of feed.		
48,0	4,67	12,36	72,7
50,0	4,70	6,58	74,9

Table A2.5. FB16.

Elapsed time h	Concentrations gl^{-1} Yeast	Glucose	Ethanol
0,0	0,792	96,7	3,46
3,5	2,19	86,9	7,8
7,0	6,46	58,0	17,7
9,5	8,63	32,0	26,8
"	Broth volume: 1,547 l.		
	Feed started : 0,0543 lh^{-1}		
10,7	9,04	28,2	30,9
12,5	9,23	18,0	39,7
"	Feed rate increased to 0,107 lh^{-1}		
14,0	9,86	10,3	47,3
14,7	Feed rate increased to 0,111 lh^{-1}		
15,0	9,93	6,9	50,7
16,5	Feed rate increased to 0,156 lh^{-1}		
18,5	8,81	8,4	57,8
22,5	7,70	12,3	62,0
26,0	6,97	14,6	65,3
30,5	6,20	17,6	66,7
34,0	5,57	19,4	68,1
38,5	5,05	21,5	69,1
42,3	4,65	23,3	69,0
"	End of feed.		
46,5	4,73	9,25	74,6
48,5	4,73	2,44	77,5

Table A2.6. FB17.

Elapsed time	Concentrations gl^{-1}		
h	Yeast	Glucose	Ethanol
0,0	0,893	148	3,5
4,0	2,51	137	9,5
8,2	7,51	92,9	23,4
11,0	10,85	54,9	36,3
13,0	12,44	29,5	44,8
14,0	12,80	20,1	47,8
"	Broth volume: 1,568 l.		
	Feed started : 0,051 lh^{-1}		
16,0	13,07	7,25	55,7
17,0	13,04	2,00	59,5
"	Feed rate increased to 0,127 lh^{-1}		
19,0	11,83	6,21	62,8
21,0	10,54	9,16	64,7
"	Feed rate increased to 0,155 lh^{-1}		
24,0	8,85	16,3	65,2
27,0	7,63	22,25	66,2
30,5	6,80	27,5	66,4
34,0	6,14	30,1	66,1
38,0	5,52	33,3	66,5
42,0	4,98	35,3	66,6
46,3	4,53	36,6	66,3
"	End of feed.		
52,5	4,55	17,4	76,6

Table A2.7. FB18.

Elapsed time h	Concentrations gl^{-1}		
	Yeast	Glucose	Ethanol
0,0	0,808	195	3,28
4,0	1,87	183	7,2
7,0	5,03	164	13,4
10,0	7,78	130	24,7
12,0	10,05	105	33,8
15,5	13,03	54,3	49,0
17,0	14,01	33,9	55,6
"	Broth volume: 1,604 l.		
	Feed started : 0,108 lh^{-1}		
18,0	13,79	30,8	57,7
19,0	Feed rate increased to 0,132 lh^{-1}		
22,0	11,14	24,7	64,5
25,0	9,70	22,7	68,0
26,0	Feed rate increased to 0,137 lh^{-1}		
29,0	7,98	24,7	69,2
32,0	7,10	27,1	70,0
36,0	6,40	30,3	70,5
40,0	5,80	33,3	70,2
44,2	5,19	36,0	70,6
47,0	4,81	37,5	70,3
51,5	4,37	38,8	70,3
"	End of feed.		
55,5	4,37	27,2	76,0
62,0	4,39	10,27	83,0

Table A2.8. FB19.

Elapsed time	Concentrations gl^{-1}		
h	Yeast	Glucose	Ethanol
0,0	0,83	101,3	3,88
3,5	2,53	89,8	8,3
6,0	5,52	69,1	16,5
8,0	8,02	47,5	23,8
"	Broth volume: 1,462 l.		
	Feed started : 0,129 lh^{-1}		
10,0	8,47	41,8	32,8
11,0	Feed rate increased to 0,165 lh^{-1}		
12,0	9,52	28,0	44,7
14,0	10,33	20,2	53,6
16,0	9,57	16,8	58,3
17,5	Feed rate increased to 0,201 lh^{-1}		
18,0	9,30	15,5	61,1
21,0	8,44	20,0	62,8
24,0	7,58	22,4	64,6
28,0	6,65	24,8	66,1
32,0	5,91	27,1	67,7
33,3	5,80	27,6	67,3
"	End of feed.		
37,0	6,06	10,6	75,5
39,0	6,06	2,92	79,3

Table A2.9. FB20.

Elapsed time	Concentrations gl^{-1}		
h	Yeast	Glucose	Ethanol
0,0	0,948	100,9	4,07
3,5	2,67	91,8	8,5
6,0	5,89	69,0	15,9
8,0	8,35	47,0	23,5
"	Broth volume: 1,567 l.		
	Feed started : 0,183 lh^{-1}		
9,0	8,34	49,6	26,6
12,0	9,19	38,5	41,8
12,8	Feed rate increased to 0,270 lh^{-1}		
15,0	9,03	38,5	50,1
16,0	Feed rate increased to 0,338 lh^{-1}		
16,5	8,65	42,8	51,7
18,5	7,85	49,0	51,9
21,0	7,15	53,5	53,3
24,5	6,58	55,6	53,0
"	End of feed.		
27,0	7,23	38,6	62,2
30,0	7,34	20,7	70,4
(34,5)	7,37	(0,0)	82,2

Table A2.10. FB21.

Elapsed time h	Concentrations gl^{-1}		
	Yeast	Glucose	Ethanol
0,0	1,02	102,5	4,35
3,5	2,84	91,5	8,7
6,2	6,58	68,0	15,8
8,0	8,04	48,8	22,2
"	Broth volume: 1,533 l.		
	Feed started : 0,293 lh^{-1}		
9,0	7,46	60,6	23,2
10,5	7,16	66,5	27,8
11,5	7,49	66,0	31,5
"	Feed rate increased to 0,441 lh^{-1}		
12,5	7,71	70,0	34,1
13,0	7,74	71,5	34,9
"	Feed rate increased to 0,608 lh^{-1}		
14,0	7,48	77,2	35,5
15,5	7,36	81,5	37,5
17,0	7,22	84,0	38,2
17,9	7,20	84,4	39,1
"	End of feed.		
21,0	8,78	55,0	51,5
22,0	8,86	46,0	54,6
24,0	9,34	32,5	61,4
27,0	9,75	14,33	69,7
(29,7)	9,94	0,12	76,7

Appendix 3. GROWTH RATES CALCULATED FROM BATCH DATA.

Batch No.	Concentrations, gl^{-1}		Growth rate h^{-1}
	Glucose	Ethanol	
04	173,7	0,82	0,4331
	142,8	12,9	0,1514
	131,5	18,3	0,1031
	121,0	22,9	0,0588
	102,5	36,4	0,0109
	99,0	33,1	0,0204
	66,2	47,6	0,0018
05	182,6	0,87	0,2589
	182,5	3,47	0,1994
	150,0	15,4	0,0865
	132,5	23,2	0,0303
	120,0	29,4	0,0175
	105,0	29,0	0,0283
	87,3	43,2	0,0046
06	192,3	0,986	0,2966
	171,0	9,78	0,1855
	154,0	18,0	0,1243
	140,0	23,8	0,0603
	118,0	32,5	0,0138
07	223,3	0,907	0,4118
	190,0	12,9	0,1273
	174,0	19,9	0,0807
	155,0	26,6	0,0450
	135,0	34,6	0,0174
	116,0	44,2	0,0054
	101,8	51,3	0,0056
	88,8	56,5	0,0069
	80,5	63,0	0,0053

Batch No.	Concentrations, gl^{-1}		Growth rate h^{-1}
	Glucose	Ethanol	
08	185,7	0,65	0,3943
	(158,5)	7,76	0,1958
	(143,0)	15,5	0,1218
	125,0	23,6	0,0436
	(111,5)	29,8	0,0148
	96,5	36,9	0,0015
09	172,6	0,767	0,3189
	152,0	6,96	0,1972
	134,0	14,7	0,1311
	115,5	21,0	0,0589
	100,0	27,6	0,0177
	86,5	34,3	0,0057
	76,8	41,1	0,0037
	62,5	47,5	0,0034
10	183,1	0,836	0,4163
	163,0	8,38	0,1894
	146,6	15,2	0,1158
	130,2	21,7	0,0520
	111,8	29,5	0,0130
	101,0	34,9	0,0121
	86,5	41,6	0,0044
11	197,8	0,87	0,2505
	177,0	8,19	0,2046
	161,5	14,5	0,1488
	151,7	19,0	0,0832
	134,5	26,2	0,0307
	121,2	33,6	0,0130
	101,0	42,4	0,0040

Appendix 4. GROWTH RATES CALCULATED FROM FED-BATCH DATA

Fed-batch No.	Concentrations, gl^{-1}		Growth rate h^{-1}
	Glucose	Ethanol	
13	21,1	46,7	0,0603
	17,4	53,4	0,0483
	21,8	58,4	0,0277
	24,0	60,5	0,0178
	26,8	63,7	0,0087
	26,9	64,5	0,0081
	19,6	68,1	0,0072
	2,72	76,1	0,0048
14	28,5	41,1	0,0388
	22,0	47,9	0,0307
	20,3	57,2	0,0191
	20,9	59,2	0,0171
	25,0	60,6	0,0138
	29,5	61,3	0,0115
	35,0	63,0	0,0095
	37,4	68,3	0,0061
	27,8	71,7	0,0038
	19,7	76,0	0,0008
15	18,4	40,6	0,0343
	13,6	49,7	0,0296
	8,8	63,0	0,0143
	12,45	64,7	0,0101
	17,6	65,5	0,0091
	19,16	67,2	0,0091
	12,36	72,7	0,0059
	6,58	74,9	0,0048

Fed-batch No.	Concentrations, gl^{-1}		Growth rate h^{-1}
	Glucose	Ethanol	
16	32,0	26,8	0,0876
	28,2	30,9	0,0774
	18,0	39,7	0,0637
	10,3	47,3	0,0538
	6,9	50,7	0,0479
	8,4	57,8	0,0312
	12,3	62,0	0,0187
	14,6	65,3	0,0123
	17,6	66,7	0,0083
	19,4	68,1	0,0073
	21,5	69,1	0,0067
	23,3	69,0	0,0052
17	20,1	47,8	0,0328
	7,3	55,7	0,0249
	2,0	59,5	0,0215
	6,2	62,8	0,0160
	9,2	64,7	0,0119
	16,3	65,2	0,0080
	22,3	66,2	0,0063
	27,5	66,4	0,0061
19	47,5	23,8	0,1486
	41,8	32,8	0,1204
	28,0	44,7	0,0960
	20,2	53,6	0,0753
	16,8	58,3	0,0579
	15,5	61,1	0,0437
	20,0	62,8	0,0275
	22,4	64,6	0,0168
	24,8	66,1	0,0097

Fed-batch No.	Concentrations, gl^{-1}		Growth rate h^{-1}
	Glucose	Ethanol	
20	47,0	23,5	0,1255
	49,6	26,6	0,1242
	38,5	41,8	0,1071
	38,5	50,1	0,0718
	42,8	51,7	0,0517
	49,0	51,9	0,0468
	53,5	53,3	0,0386
	55,6	53,0	0,0295
21	66,5	27,8	0,1608
	66,0	31,5	0,1595
	70,0	34,1	0,1583
	71,5	34,9	0,1325
	77,2	35,5	0,1334
	81,5	37,5	0,1182
	84,0	38,2	0,1010
	84,4	39,1	0,0865
	55,0	51,5	0,0355
	46,0	54,6	0,0266
	32,5	61,4	0,0197
	14,33	69,7	0,0118

Appendix 5. GROWTH PARAMETER VALUES.

Tabulated by growth model.

Table A5.1. Monod Model.

$$\mu = \frac{\hat{\mu}_M}{\left(1 + \frac{K_s}{C_s}\right)\left(1 + \frac{C_p}{K_p}\right)}$$

Data	$\hat{\mu}$ h^{-1}	K_p gl^{-1}	K_m gl^{-1}
COMBINED :			
35°C Batches	0,751	4,0	89,1
30°C Batches	0,7	7	200
Fed-batches	Fits not found or of poor quality.		
INDIVIDUAL :	Generally poor results owing to shape of data.		
Fed-batch 17	0,55	57	100
Fed-batch 19	0,50	35	57
Converti et al*	0,63	15,2	> 1000

* Batch data using 200 gl^{-1} sucrose medium (14).

Table A5.2. Levenspiel Model.

$$\mu = \hat{\mu}_L \frac{\left(1 - \frac{C_p}{C_p^*}\right)^n}{\left(1 + \frac{K_s}{C_s}\right)}$$

Data	$\hat{\mu}$ h^{-1}	C_p^* gl^{-1}	n -	K_s gl^{-1}
COMBINED :				
35°C Batch	0,4	70	2,7	100
30°C Batch	0,5	40	2,2	100
INDIVIDUAL :				
Fed-batch 15	0,4	80	0,7	100
Fed-batch 19	0,4	68	0,4	50
Fed-batch 20	0,4	72	1,0	50
Fed-batch 21	0,65	90	2,2	50

Table A5.3. Levenspiel Model without Substrate-dependent term.

$$\mu = \hat{\mu}_L \left(1 - \frac{C_p}{C_p^*} \right)^n$$

Data	$\hat{\mu}$ h^{-1}	C_p^* gl^{-1}	n -
COMBINED :			
35°C Batch	0,320	51,4	2,877
30°C Batch	0,309	39,6	2,011
Fed-batches 13-16	0,144	74,0	1,202
Fed-batches 19-21	0,246	70,8	1,077
INDIVIDUAL :			
Fed-batch 16	0,130	70,3	0,834
Fed-batch 17	0,061	66,9	0,494
Fed-batch 19	0,190	66,4	0,600
Fed-batch 20	0,156	53,6	0,316
Converti et al*	0,224	90	3,0

* Batch data using 200 gl^{-1} sucrose medium (14).

Table A5.4. Modified Levenspiel Model.

$$\mu = \hat{\mu}_L \frac{\left\{1 - \left(\frac{C_p}{C_p^*}\right)^n\right\}}{\left(1 + \frac{K_s}{C_s}\right)}$$

Data	$\hat{\mu}$ h^{-1}	C_p^* gl^{-1}	n -	K_s gl^{-1}
COMBINED :				
35°C Batch	0,5	37,0	0,6	100
30°C Batch	0,390	34,3	0,629	46,7
Fed-batches	No successful fits.			
INDIVIDUAL :				
Fed-batch 15	0,4	77	2,0	100
Fed-batch 20	0,33	55	9,0	75
Fed-batch 21	0,64	75	0,5	50

Table A5.5. Modified Levenspiel Model without Substrate-dependent term.

$$\mu = \hat{\mu}_L \left\{ 1 - \left(\frac{C_p}{C_p^*} \right)^n \right\}$$

Data	$\hat{\mu}$ h^{-1}	C_p^* gl^{-1}	n -
COMBINED :			
35°C Batch	0,5	36	0,33
30°C Batch	0,30	30,6	0,746
Fed-batches 13-16	0,179	71,9	0,6468
Fed-batches 19-21	0,210	68,3	1,260
INDIVIDUAL :			
Fed-batch 15	0,109	78,8	0,596
Fed-batch 16	0,107	70,9	1,637
Fed-batch 17	0,036	68,3	5,441
Fed-batch 19	0,151	68,3	2,649

Table A5.6. Simplified Model.

$$\mu = B \frac{C_s}{\left(1 + \frac{C_p}{K_p}\right)}$$

Data	B $lg^{-1}h^{-1}$	K_p gl^{-1}
COMBINED :		
35°C Batches	0,00251	5,4
30°C Batches	0,00178	10,1
Fed-batches	Poor or no fits obtained.	
INDIVIDUAL :	Fed-batch fits generally poor owing to shape of data.	
Fed-batch 17	0,00414	57,2
Fed-batch 19	0,00548	35,7

Table A5.7. Levenspiel Model with Simplified Substrate Term.

$$\mu = BC_s\left(1 - \frac{C_p}{C_p^*}\right)^n$$

Data	B h^{-1}	C_p^* gl^{-1}	n -
COMBINED :			
35°C Batch	0,00184	65,6	3,484
30°C Batch	0,00164	36,0	1,345
Fed-batches 13-16	0,00433	69,5	0,769
Fed-batches 19-21	Attempt to fit unsuccessful		
INDIVIDUAL :			
Fed-batch 14	0,00322	69,9	0,880
Fed-batch 15	0,00419	76	0,97
Fed-batch 20	0,00328	53,4	0,288

Table A5.8. Modified Levenspiel Model with Simplified Substrate Term.

$$\mu = BC_s\left\{1-\left(\frac{C_p}{C_p^*}\right)^n\right\}$$

Data	B h^{-1}	C_p^* gl^{-1}	n -
COMBINED :			
35°C Batch	0,00360	39,3	0,239
30°C Batch	0,00172	34,4	0,733
Fed-batches 13-16	0,00306	69,4	2,622
Fed-batches 19-21	attempts to fit unsuccessful		
INDIVIDUAL :			
Fed-batch 14	0,00296	71,8	1,213
	0,0048	77	0,63
Fed-batch 15	0,00257	76,0	2,457
Fed-batch 21	0,0024	79,7	1,861

Appendix 6. SUBSTRATE UPTAKE RATES, BIOMASS YIELDS and MAINTENANCE COEFFICIENTS.

Table A6.1. Substrate Uptake Rates in Fed-batch Fermentations.

Run No.	Constant-uptake-rate Period, h elapsed	Average substrate Concentration, gl^{-1}	Total Substrate Uptake Rate, gh^{-1}
13	19,0 - 45,5	20,0	29,3
14	16,0 - 51,0	25,0	27,3
15	17,0 - 50,0	12,9	23,2
16	12,5 - 48,5	13,7	24,6
17	14,0 - 52,5	20,3	22,0
18	17,0 - 62,0	29,0	19,1
19	8,0 - 39,0	23,5	30,2
20	12,0 - 30,0	42,2	41,6
21	21,0 - 29,5	71,5	39,5

Table A6.2. Biomass Yields and Maintenance Coefficients - Fed-batch Fermentations. Raw and refined values.

Fed-Batch No.	Raw		Refined	
	Yield gg^{-1}	Maint. $gg^{-1}h^{-1}$	Yield gg^{-1}	Maint. $gg^{-1}h^{-1}$
13	0,097	0,751	0,0815	0,728
14	0,080	0,912	0,0815	0,899
15	0,060	0,746	0,0815	0,774
16	0,089	0,804	0,0815	0,804
17	0,139	0,851	0,0815	0,764
19	0,086	0,635	0,0815	0,700
20	0,051	0,335	0,0815	0,710
21	0,149	0,493	0,0815	0,500

Table A6.3. Biomass Yields and Maintenance Coefficients - Batch Fermentations.

Batch No.	Biomass Yield gg^{-1}	Maint. Coeff. $gg^{-1}h^{-1}$
4	0,117	0,995
5	0,245	0,717
6	0,148	0,407
7	0,072	0,744
8	0,213	0,729
9	0,237	0,547
10	0,316	0,819
11	0,206	0,503
Pre-ferm	0,325	0,868

Appendix 7. ANALYTICAL PROCEDURES : BIOMASS and ETHANOL.

1. Correlation of Transmission of 580 nm Light with Yeast Concentration in Broth Samples.

1.1. Procedure.

600 ml of broth containing about 8 gl^{-1} S.cerevisiae was placed on a magnetic stirrer to maintain uniform suspension. The broth came from a fermentation completed several hours previously. Four 25,0 ml portions were pipetted onto 0,8 µm Millipore filters : the yeast cakes were washed with distilled water and dried to constant weight at 105°C. Various dilutions of the broth were prepared by pipetting into volumetric flasks and at each dilution the transmission of 580 nm light was measured on the Beckman 1211 photometer.

Table A7.1. Calibration values : S.cerevisiae ATCC 4126 Suspensions - Concentration vs Transmission of 580 nm light.

March 1980		May 1981	
Transm. %	Conc. mgl^{-1}	Transm. %	Conc. mgl^{-1}
68,0	60,6	75,2	45,9
62,0	75,8	62,7	76,4
56,7	90,9	55,6	95,6
48,5	121,2	50,0	114,7
40,6	151,5	40,7	152,9
35,0	181,8	33,2	191,1
26,3	242,4	28,3	229,3
20,35	305,8	15,2	382,2

1.2. Regression.

Table A7.1. contains the data from two calibrations performed a year apart. A log-linear plot indicated a

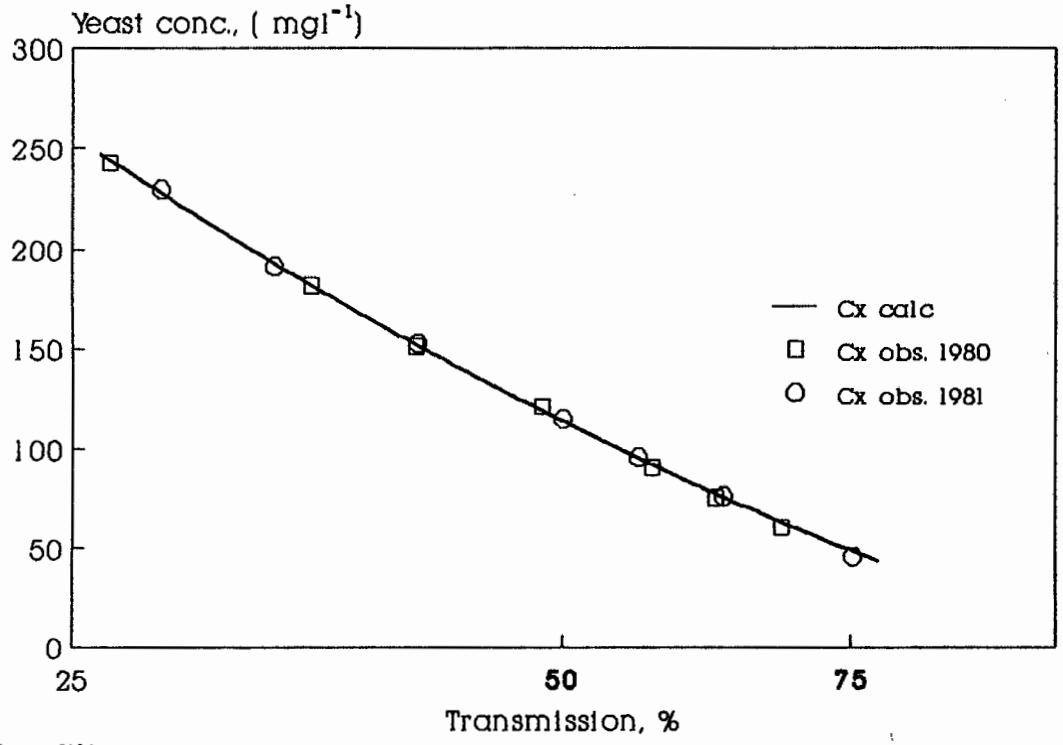
smooth curve, slightly concave upwards (**Fig. A7.1.**), and a quadratic in log T was therefore chosen to represent the data :

$$C_x = 217,4 \log^2 T - 1144,7 \log T + 1431,5 \quad . \; . \; . \; . \quad (A7.1)$$

where T is percentage transmission
C_x is yeast concentration in mgl^{-1}

This is the equation of the curve in Fig. A7.1.

Fig.A7.1. Colorimeter Calibration.
Concentration vs % Transmission.



580nm filter.

It is possible to express the relation in terms of T/100 instead of T. There is obtained :

$$C_x = 217,4 \log^2\left(\frac{T}{100}\right) - 1144,7 \log\left(\frac{T}{100}\right) + 11,7 \quad . \; . \; . \; . \quad (A7.2)$$

With the calibration in this form the constant at the end should be zero, but differs because of experimental error. It was decided not to force the calibration through zero as measurements are not carried out in this region and it would adversely affect accuracy in the central region of 30 - 60 % transmission.

2. Ethanol Analysis.

2.1. Sample Preparation.

The dilution required to bring the ethanol concentration into the range 0,3 - 1,0 gl^{-1} was estimated and a volumetric flask selected accordingly and filled about 80% with distilled water. A known volume of standard butanol, generally 10% of the flask volume, was added and the flask stoppered until needed.

When the sample had been drawn it was cooled and 2,00 ml pipetted into the flask. It was not strictly necessary to make the volume to the line but this was done in any case. The mixture was centrifuged for 20 minutes at 4000 rpm to remove yeast cells and was then refrigerated pending analysis.

2.2. Analysis.

All samples from any one fermentation were analysed in one batch. Injections were done manually. Results were accepted if the first two replicates agreed within about 0,2 gl^{-1} or 0,5%, whichever was smaller. Otherwise further replicates were done until a satisfactory set was obtained. **Table A7.2.** contains details of the chromatograph and the settings used.

Table A7.2. Gas Chromatograph Details and Operating Settings.

Chromatograph	:	Varian 1400 with Vista Data Processor
Column	:	1,5m x 3mm dia. stainless steel
Packing	:	Chromosorb G, HP 100/120, 1,5% OV-101
Detector	:	Flame Ionisation
Carrier gas	:	Medical-grade nitrogen
Temperatures	-	
Injector	:	150°C
Column	:	60°C
Detector	:	250°C
Sample Volume	:	1,0 µl

Appendix 8. MEDIUM FORMULATIONS.

Table A8.1. General Medium Formulation.
Quantity per 100g glucose monohydrate.

Yeast Extract (Difco)	g	7,34
Ammonium Sulphate	g	7,8
Sodium Citrate.5½H_2O	g	4,6
Citric Acid Monohydrate	g	1,4
KH_2PO_4	mmole	5,2
$MgSO_4.7H_2O$	mmole	2,3
$CaCl_2.2H_2O$	mmole	4,6

Table A8.2. Fed-batch Experiments : Feed Medium Quantities.

Volume : 4,80 l Sample : 0,06 l Nett Feed : 4,74 l.

Glucose Monohydrate	g	1046
Yeast Extract	g	77
Ammonium Sulphate	g	82
Sodium Citrate.5½H_2O	g	48
Citric Acid.H_2O	g	13,5
KH_2PO_4	mmole	4,8
$MgSO_4.7H_2O$	mmole	2,4
$CaCl_2.2H_2O$	mmole	4,8
Fermenter Oil B (Antifoam)	1 drop	

Table A8.3. Fed-batch Fermentations : Quantities for Pre-fermentation Medium.

Total Volume : 1,50 l Sample : 0,07 l
Nett charged to fermenter : 1,43 l

		Nominal Concentration, g anhydrous glucose l^{-1} :		
		100	150	200
Glucose Monohydrate	g	181	271	361
Yeast Extract	g	13	20	26,5
Ammonium Sulphate	g	14	21	28
Sodium Citrate.5½H_2O	g	8,3	12,4	16,6
Citric Acid.H_2O	g	2,5	3,7	5,0
KH_2PO_4	mmole	17	25	33
$MgSO_4$.7H_2O	mmole	4	6	8
$CaCl_2$.2H_2O	mmole	8	12	16

Table A8.4. Inoculum Medium Composition.

Quantities for 1 litre.

Glucose Monohydrate	g	109
Yeast Extract	g	8,0
Ammonium Sulphate	g	8,4
Sodium Citrate.5½H_2O	g	10,0
Citric Acid.1H_2O	g	3,0
KH_2PO_4	mmole	10,0
$MgSO_4$.7H_2O	mmole	2,5
$CaCl_2$.2H_2O	mmole	5,0

Table A8.5. Batch Fermentations : Quantities.

Total Volume : 6,25 l Sample : 0,06 l
Nett charged to fermenter : 6,19 l.

Nominal glucose (gl^{-1}) in corresponding fed-batch fermentation :		100	150	200
Glucose Monohydrate	g	1218	1307	1395
Yeast Extract	g	89,4	95,7	102
Ammonium Sulphate	g	95,0	102	109
Sodium Citrate.5½H_2O	g	56	60	64
Citric Acid H_2O	g	17	18	19
KH_2PO_4	mmole	63,5	71	80
$MgSO_4.7H_2O$	mmole	28	30	32
$CaCl_2.2H_2O$	mmole	56	60	64
Fermenter Oil B		1 drop		

Table A8.6. Composition of Wickerham Medium.

In gl^{-1}, made up with distilled water.

Glucose	10
Agar	20
Peptone	5
Malt Extract	3
Yeast Extract	3

Appendix 9. CURVE-FITTING ALGORITHM.

Consider the problem of fitting to m data points an equation of the general form

$$y = f(x, c_1, c_2, \ldots, c_n) \qquad \ldots\ldots \text{(A9.1)}$$

where the c_i are the n parameters whose values for the best fit to the data are to be determined, and x represents one or more independent variables. Suppose that estimates g_i of these parameters have been selected : the true values c_i can be expressed in terms of the g_i and an error or correction term e_i

$$c_i = g_i + e_i, i = 1 \text{ to } n \qquad \ldots\ldots \text{(A9.2)}$$

so that

$$y = f(x, g_1 + e_1, g_2 + e_2, \ldots, g_n + e_n) \qquad \ldots\ldots \text{(A9.3)}$$

An iterative procedure will now be described in which an initial set of estimates g_i is selected and a set of corrections e_i is calculated. The procedure is repeated using the corrected values (g_i + e_i) as new g_i until the corrections become insignificant.

The first step is to expand Eq. A9.3 as a Taylor series in the parameters, retaining only first-order derivatives :

$$y = f(x, g_1, \ldots, g_n) + \sum_{i=1}^{n} e_i \frac{\partial}{\partial c_i} f(x, g_1, \ldots g_n) \qquad \ldots\ldots \text{(A9.4a)}$$

Grouping terms containing the corrections on the LHS and the rest on the RHS,

$$\sum_{i=1}^{n} e_i \frac{\partial}{\partial c_i} f(x, g_1, \ldots g_n) = y - f(x, g_1, \ldots, g_n)$$

. . . . (A9.4b)

which states that the difference between the observed value y and the value calculated using the estimates g_i is to be accounted for by the correction terms e_i multiplied by the corresponding partial derivatives. The e_i are the only unknown quantities in the equation.

Application of Eq A9.4b to each of the m data points gives a system of m equations in n unknowns e_i :

$$D\ E = Y$$

. . . . (A9.5)

where D is the matrix

$$\begin{pmatrix} \frac{\partial}{\partial c_1} f(x_1, g_i) \ldots \frac{\partial}{\partial c_n} f(x_1, g_i) \\ \frac{\partial}{\partial c_1} f(x_m, g_i) \ldots \frac{\partial}{\partial c_n} f(x_m, g_i) \end{pmatrix}$$

$$E = \begin{pmatrix} e_1 \\ \cdot \\ \cdot \\ e_n \end{pmatrix} \quad \text{and} \quad Y = \begin{pmatrix} y_1 - f(x_1, g_i) \\ \cdot \\ \cdot \\ y_m - f(x_m, g_i) \end{pmatrix}$$

Since m is always greater than n (preferably much greater), the system is overdetermined. To obtain best estimates of the e_i, Eq.5 is left-multiplied by the transpose of D :

$$(D^T D)E = D^T Y$$

. . . . (A9.6)

This is a system of n equations in the n unknowns e_i. Solution by Gauss reduction or determinants follows and the e_i are added to the g_i. The process is repeated until the e_i become insignificant. The g_i then give the desired parameter values for a least squares fit of the original equation to the data.

One example will illustrate the method. The Levenspiel growth model without the sugar-dependent term will be used :

$$\mu = \hat{\mu}_L \left(1 - \frac{C_p}{C_p^*} \right)^n \qquad \ldots\ldots \text{(A9.7)}$$

Choosing μ_L, C_p^* and n to be c_1, c_2 and c_3 respectively, the derivative expressions are :

$$\frac{\partial}{\partial c_1}\mu = \left(1 - \frac{C_p}{C_p^*} \right)^n \qquad \ldots\ldots \text{(A9.8)}$$

$$\frac{\partial}{\partial c_2}\mu = \frac{n\hat{\mu}_L}{C_p^*}\left(\frac{C_p}{C_p^*} \right)\left(1 - \frac{C_p}{C_p^*} \right)^{n-1} \qquad \ldots\ldots \text{(A9.9)}$$

$$\frac{\partial}{\partial c_3}\mu = \mu \ln\left(1 - \frac{C_p}{C_p^*} \right) \qquad \ldots\ldots \text{(A9.10)}$$

These three expressions give the three columns of matrix D. Elements of matrix Y are given by

$$\mu_{observed} - \hat{\mu}_L \left(1 - \frac{C_p}{C_p^*} \right)^n$$